U.PORTO
FEUP FACULDADE DE ENGENHARIA
UNIVERSIDADE DO PORTO

lepabe
Laboratory for Process Engineering, Environment, Biotechnology and Energy

Emerging contaminants, from waste to taste? – Ultraviolet-Filters and Synthetic Musk compounds as case study

Sara Fernandes Ramos

Dissertation presented to obtain the degree of

Doctor in Environmental Engineering
by the
Faculty of Engineering, University of Porto

Supervision

Doctor Lúcia Maria da Silveira Santos

Doctor Vera Maria Ferreira Cruz Homem

Porto, January 2020

I dedicate this thesis to
my parents, Teresa and Carlos
and my sisters Carla and Marta.

Sara Ramos would like to acknowledge the financial support from:

(i) PhD candidate grant SFRH/BD/110831/2015 of FCT - Fundação para a Ciência e a Tecnologia/MCTES, co-funded by the European Social Fund, under the Human Capital Operational Programme (POCH) from Portugal 2020 Programme; (ii) Project POCI-01-0145-FEDER-006939 006939 (Laboratory for Process Engineering, Environment, Biotechnology and Energy – UID/EQU/00511/2013), funded by European Regional Development Fund (ERDF) through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI), and by national funds (PIDDAC) through FCT – Fundação para a Ciência e a Tecnologia/MCTES; (iii) Project "LEPABE-2-ECO-INNOVATION" – NORTE-01-0145-FEDER-000005, funded by Norte Portugal Regional Operational Programme (NORTE 2020), under PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF); (iv)Project UID/EQU/00511/2019 and UIDB/00511/2020 - Laboratory for Process Engineering, Environment, Biotechnology and Energy – LEPABE funded by national funds through FCT/MCTES (PIDDAC). Dr Vera Homem, co-supervisor of this thesis is responsible for the AGRONAUT project, *Project PTDC/ASP-PLA/29425/2017 - POCI-01-0145-FEDER-029425 - funded by FEDER funds through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES*.

Acknowledgments

I wish to thank the Faculty of Engineering of the University of Porto (FEUP), the Chemical Engineering Department (DEQ) and Laboratory for Process Engineering, Environment, Biotechnology and Energy (LEPABE) for the facilities that allowed me to conduct the scientific experiments.

I am also grateful to "Fundação para a Ciência e a Tecnologia" (FCT) for my PhD grant (SFRH/BD/110831/2015).

I would like to express my deep sense of gratitude to my supervisor Professor Lúcia Santos and co-supervisor Dr. Vera Homem for believing in me and for giving me the opportunity and encouraging me to develop this interesting and challenging work. Thank you for your continuous support and availability, as well as, for the scientific suggestions and recommendations. It has been a great honour and privilege to work and learn with them.

My special thanks to Professor Arminda Alves for helping me every time I needed and for giving me all the wise advices whenever I needed. I have no doubt that I am also writing this thesis today because of her.

My acknowledgement also goes to all the people from 'Centro de Investigação em Biodiversidade e Recursos Genéticos' (CIBIO), namely Professor Mariana Sottomayor for allowing my experiments in their group's 'walk-in chambers', Dr. Humberto Castro for sharing all his knowledge regarding tomatoes growth and plantation as well as helping with all the practical work in the CIBIO facilities, and also, Dr. Sara Freitas for watering my plants and taking good care as if they were hers.

My special thanks to my father, who is also an agronomy engineer, Eng. Carlos Ramos, for all the valuable knowledge shared with me regarding the growth of the tomato plants and helping every time I thought my plants might not survive in my hands.

My gratitude is also towards my lab colleagues for the company, friendship and support during these years: José, Mónica, Nuno, Filipe, Daniela, Fábio, Filipa, Idalina and all the others members of MIA201 that have come and left but somehow contribute to the good environment of the lab and made the hours spent there easier. My special thanks to the technicians Sr. Serafim, Fátima, José Luís, Liliana and Luís Carlos.

Thank you to all my friends for their support, encouragement and for the good times we shared. They are so many that I cannot write all them here. A special thank you to the friends that during this period also pursued a PhD degree and understood all the frustrations and victories: Ana Claudia, Rui Lopes, Helder Xavier, Marta Barbosa e Joana Vilas Boas. Also, to Daniela Capela and Filipe Rocha for being such a good support system.

Also, besides being my co supervisor, I need to give a special thank you to Vera, for all the patience during these years, for pushing me harder, for backing down when I needed space, for supporting me through all and for believing that I could do this. Thank you to always say yes to all my crazy ideas, even when no one else believe possible.

My special acknowledgment to a special person, for all the support, encouragement, and belief in me. For enjoying my conquests as if they were his with the greatest happiness ever possible.

I am grateful to my family, especially to my parents and my sisters, for their love, patience, endless support and encouragement. Thank you for helping me to build a future that I think is always better. I am indebted to you forever.

Contents

Abstract

Emerging contaminants, from waste to taste? Recently, the number of wastewater treatment plants increased and consequently, the sludge/compost production. Sludge retains high organic matter content and minerals, which make them prone to be used as agricultural fertilizer. Although sludge/compost are increasingly used as fertilizer, concerns have emerged on possible risks (e.g. organic pollutants contamination). In fact, after application to soil, contaminants can accumulate, be taken up by plants or leach into groundwater. This work aimed to understand the behaviour of previously selected model compounds of ultraviolet-filters (UVFs) and synthetic musk compounds (SMCs), by determining their presence in environmental matrices such as water, sludge/compost and soil and to study the possible uptake and translocation of UVFs and SMCs by the tomato fruit in composted amended soil.

For that, several analytical methodologies were developed. For water, the analytical method developed consisted on the preconcentration of water samples by dispersive liquid-liquid microextraction (DLLME) and analysis by gas chromatography tandem mass spectrometry detection (GC-MS/MS). This methodology is an easy and fast procedure of detecting a possible contamination, at low levels, in different types of water, namely tap water, river and sea water as well as wastewater. For this methodology, 2-propanol was used as dispersive solvent and 1,1,2-trichloroethane as extractant solvent. The method limit of detection (MDL) ranged from 0.1 ng L^{-1} (octocrylene, OC); celestolide, ADBI) to 20.0 ng L^{-1} (benzophenone, BZ). Recoveries assays were performed at four spike levels (50, 250, 500 and 1500 ng L^{-1}) in all the above mentioned water matrices, and average recoveries of the analytes based on the surrogate correction ranged from 80 to 120%, with a good repeatability (relative standard deviations (RSD) less than 10%). This analytical methodology was necessary for further analysis of leachate samples from the final experiments.

The UVFs and SMCs contamination in sludge/compost samples was already suspected due to literature review. Nevertheless, not all target compounds were mentioned in the peer reviewed papers available. Thus, a Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) methodology followed by gas chromatography – triple quadrupole mass

spectrometry (GC–MS/MS) was developed and optimized by a design of experiments (DoE) approach, applying first a screening design (SD) and then a central composite design (CCD). The best conditions achieved to extract the target UVFs and SMCs simultaneously were: 500 mg freeze dried sludge, 2.5 min of vortex and 15 min ultrasound and the use of a QuEChERS for the dispersive solid-phase extraction (d-SPE) containing 500 mg $MgSO_4$, 410 mg C_{18} and 315 mg PSA. The method detection and quantification limits (MDLs and MQLs) ranged between 0.5 (cashmeran, DPMI) and 1394 (exaltolide, EXA) ng g^{-1} dw and 2 and 4648 ng g^{-1} dw, respectively (DPMI and EXA). High average recoveries were obtained, ranging from 75% (DPMI) to 121% (2-ethylhexyl 4-methoxycinnamate, EMC), as well as good repeatability values (RSD <8%). This methodology was applied either to sludge samples, as well as biosolid compost samples, yielding similar recoveries.

Since soil is the main receiving medium of sludge/compost used as fertilizer, a QuEChERS/GC–MS/MS methodology was optimized and validated for this matrix. Accuracy, assessed by recovery tests, ranged from 81% to 122% and a good precision was achieved, with RSD < 4%. The MDL varied from varied from 0.02 (drometrizole trisiloxane, DTS) to 46.3 (exaltolite, EXA) ng g^{-1} dw. This methodology was also found suitable for the analysis of UVFs and SMCs in commercially substrate as well as in mixtures of soil and substrate.

Due to the deliberate introduction of UVFs and SMCs into soils through the application of sludge or commercially available compost as fertilizers, these pollutants may enter the food chain if crop uptake occurs. To study this behaviour, it was necessary to carry out plant uptake studies and, for that, it is also essential to develop simple, expeditious and reliable analytical methodologies to detect those compounds in tomatoes. Therefore, a sensitive, reliable and fast multiresidue methodology based on a QuEChERS/GC-MS/MS for the determination of six UVFs and thirteen SMCs in tomatoes was developed and validated. Tomatoes were chosen for this study based on European and national production and consumption habits, as well as due to their frequent use in plant uptake trials as they may grow all year in agricultural fields or greenhouses. The proposed methodology was optimized: 2 g of freeze-dried tomato, extracted with 4 mL of water and 10 mL of ethyl acetate, adding 6 g of $MgSO_4$ and 1.5 g of NaCl, then a

dispersive solid-phase extraction was performed using 3 g of $MgSO_4$, 300 mg of primary-secondary amino adsorbent (PSA) and 300 mg of octadecyl-silica (C_{18}). MQLs ranged between 0.4 (celestolide) and 47.9 ng g^{-1} dw (exaltolide) and recoveries between 81 (celestolide, ADBI) and 119% (musk tibetene, MT), with RSD < 10%. Based on the obtained results, primary exposure and risk of human consumption was estimated, suggesting that a potential health risk is unlikely.

The uptake of UVFs and SMCs was assessed in Micro Tom tomatoes, in a 'walk-in chamber' under controlled temperature and humidity conditions and with different types of soil compositions. The results showed that there was an uptake of most of the target compounds. A risk assessment showed that there was no risk in the consumption of tomato fruits from this experiment, based on a tolerable weekly exposure assessment for an adult (body weight of 60 kg), consuming around 0.5 kg of tomato per person per week.

Finally, it can be affirmed that this work is a relevant contribution to the knowledge of the consumption of tomatoes grown in sludge based fertilizers in soils, suggesting that there is no risk associated due to the low levels detected in the fruit, nevertheless, more studies need to be performed.

Resumo

Contaminantes emergentes, dos resíduos ao palato? Recentemente, o número de estações de tratamento de águas residuais aumentou e consequentemente, a produção de lamas/composto. A lama contém um elevado teor de matéria orgânica e de minerais, o que a torna propensa a ser usada como fertilizante agrícola. Embora a lama/composto seja cada vez mais usada como fertilizante, existe uma preocupação crescente sobre os possíveis riscos associados a esta prática (por exemplo, contaminação por poluentes orgânicos). De facto, após a aplicação no solo, os contaminantes podem-se acumular, ser absorvidos pelas plantas neles cultivados ou lixiviar, atingindo águas subterrâneas. Este trabalho teve como objetivo compreender o comportamento de compostos modelo previamente selecionados pertencentes à classe dos filtros ultravioleta (UVFs) e almíscares sintéticos (SMCs), determinando a sua presença em matrizes ambientais como água, lama/composto e solos e estudar a possível absorção dos UVFs e SMCs pelo tomate em solo fertilizado com lama/composto.

Para isso, foram desenvolvidas várias metodologias analíticas. Para as matrizes de água, o método analítico desenvolvido consistiu na pré-concentração das amostras por microextração líquido-líquido dispersiva (DLLME) e na análise por cromatografia em fase gasosa, com deteção em espectrometria de massa (GC-MS/MS). Este pretendia ser um procedimento fácil e rápido para detetar uma possível contaminação a baixos níveis de concentração, em diferentes tipos de água, nomeadamente água da torneira, rio e mar, bem como águas residuais. Para esta metodologia, o 2-propanol foi utilizado como solvente de dispersão e 1,1,2-tricloroetano como solvente de extração. O limite de deteção do método variou entre 0,1 ng L^{-1} (octocrileno, OC; celestolide, ADBI) e 20,0 ng L^{-1} (benzofenona, BZ). Os ensaios de recuperação foram realizados em quatro níveis de fortificação (50, 250, 500 e 1500 ng L^{-1}) em todas as matrizes de água acima mencionadas, e as recuperações médias dos analitos com base na correção dos padrões internos variaram de 80 a 120%, com uma boa repetibilidade (desvios padrão relativos (RSD) inferiores a 10%). Esta metodologia analítica foi necessária para a determinação dos compostos alvo em amostras de lixiviados das experiências finais.

A contaminação por UVFs e SMCs em amostras de lama/composto já era suspeita devido à revisão da literatura. No entanto, nem todos os compostos-alvo foram mencionados nos artigos encontrados. Assim, uma metodologia QuEChERS (siglonimização das palavras "Rápida, Fácil, Barata, Efetiva, Robusta e Segura" em inglês), seguida de análise por GC-MS/MS, foi desenvolvida e otimizada utilizando desenho de experiências (DoE), aplicando primeiro uma etapa *de screening* (SD), seguida de um desenho composto central (CCD). As melhores condições alcançadas para extrair os UVFs e SMCs simultaneamente foram: 500 mg de lama liofilizada, 2,5 min de vórtice e 15 min em banho de ultrassom e o uso de um QuEChERS para a extração em fase sólida dispersiva (d-SPE) contendo 500 mg de $MgSO_4$, 410 mg de C_{18} e 315 mg de PSA. Os limites de deteção e quantificação do método (MDLs e MQLs) variaram entre 0,5 (cashmeran, DPMI) e 1394 (exaltolide, EXA) ng g^{-1} dw e 2 e 4648 ng g^{-1} dw, respectivamente (DPMI e EXA). Além disso, foram obtidas recuperações relativamente elevadas, variando entre 75 (DPMI) e 121% (2-etilhexil-4-metoxicinamato, EMC), com boa repetibilidade (RSD <8%). Esta metodologia foi aplicada tanto nas amostras de lama como nas de composto, obtendo recuperações semelhantes.

Como o solo é o principal local de destino da aplicação da lama/composto como fertilizante, foi desenvolvida, optimizada e validada uma metodologia QuEChERS seguida por deteção por GC-MS/MS para a análise desta matriz. A exatidão, avaliada por testes de recuperação, variou entre 81 e 122% e uma boa precisão foi obtida, com RSD <4%. O limite de deteção do método variou entre 0,02 (drometrizole trisiloxano, DTS) e 73.37 (exaltolite, EXA) ng g^{-1} dw. Esta metodologia também foi considerada adequada para a análise de UVFs e SMCs em substrato comercial, bem como em misturas de solo e substrato.

Devido à introdução deliberada de UVFs e SMCs em solos através da aplicação de lama ou composto comercialmente disponível como fertilizantes, existe a possibilidade de entrada desses poluentes na cadeia alimentar, caso ocorra a sua absorção pelas culturas. Para estudar esse comportamento, foi necessário realizar estudos de *uptake* pelas plantas e, para isso, foi também necessário desenvolver metodologias analíticas simples, rápidas e confiáveis. Assim, foi desenvolvida e validada uma metodologia multirresíduos sensível, confiável e rápida, baseada em QuEChERS/GC-MS/MS para a

determinação de seis UVFs e treze SMCs em tomates. Os tomates foram escolhidos para este estudo com base nos níveis de produção nacionais e nos hábitos de consumo europeus e nacionais, bem como devido ao seu uso frequente em ensaios de *uptake* de plantas, pois podem crescer o ano todo em campos agrícolas ou estufas. A metodologia proposta foi otimizada, consistindo em 2 g de tomate liofilizado, extraído com 4 mL de água e 10 mL de acetato de etilo, adicionando 6 g de $MgSO_4$ e 1,5 g de NaCl; em seguida, foi realizada uma d-SPE usando 3 g de $MgSO_4$, 300 mg de PSA e 300 mg C18. Os limites de quantificação do método variaram entre 0,4 (celestolide, ADBI) e 47,9 ng g^{-1} dw (EXA) e recuperações entre 81 (ADBI) e 119% (musk tibeteno, MT), com RSD <10%. Com base nos resultados obtidos, foi estimada a exposição primária e o risco associado ao consumo humano destes tomates, sugerindo que um potencial risco para a saúde seja pouco provável.

O *uptake* de UVFs e SMCs por tomates Micro-Tom, foi avaliado numa câmara de fitoclima, sob condições controladas de temperatura e humidade e usando diferentes composições de solo. Os resultados mostraram que houve uma absorção da maioria dos compostos alvo. Uma avaliação de risco mostrou que não havia risco no consumo de tomate, com base numa estimativa da exposição semanal tolerável de um adulto (assumindo um peso corporal médio de 60 kg), consumindo cerca de 0,5 kg de tomate por pessoa por semana.

Finalmente, pode-se afirmar que este trabalho constitui uma contribuição relevante para o conhecimento do consumo de tomates cultivados em fertilizantes à base de lama nos solos, sugerindo que não há risco associado devido aos baixos níveis detetados nos frutos. No entanto, será necessário realizar-se mais estudos.

Figure Captions

Annex Figures

Table Captions

Annex Tables

Nomenclature

(MPTS)-Ag-C_{12} – 3-(Mercaptopropyl) trimethoxysilane
[C_4MIM]PF_6 – 1-Butyl-3-methylimidazolium hexafluorophosphate
[HMIM][FAP] – 1-Hexyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate
1-OC – Octanol
Ac – Acetone
ACN – Acetonitrile;
ADI – Acceptable Daily Intake
Al_2O_3 – Aluminium Oxide
BSTFA – N,O-bis(Trimethylsilyl)trifluoroacetamide
C_{18} – Bonded Silica Stationary Phase Column
CB – Chlorobenzene
CCD – Central Composite Design
CHL – Chloroform
CLP – Classification, Labelling and Packaging
Cyhex – Cyclohexane
CYPN – Cyclopentane
DAD – Diode-Array Detector
DCM – Dichloromethane
DE – Diethyl Ether
DI – Direct Immersion
DLLME – Dispersive Liquid–Liquid Microextraction
DOE– Design of Experiments
D-SPE – Dispersive Solid-Phase Extraction
EA – Ethyl Acetate
EED – Estimated Exposure Dose
EI – Electron Ionization
EtOH – Ethanol
eV – Electro Volts
GC-HRMS – Gas Chromatography Coupled with High Resolution Mass Spectrometry
GC-MS – Gas Chromatography Coupled with Mass Spectrometry
GC-MS/MS – Gas Chromatography Triple Quadropole Mass Spectrometry
GPC – Gel Permeation Chromatography
H_2O – Water
HCl – Hydrochloric acid
Hep – Heptane
Hex – Hexane
HPLC-DAD – High Performance Liquid Chromatography Coupled to Diode-Array Detection
HPLC-ESI-MS/MS – High Performance Liquid Chromatography Tandem Mass Spectrometry
HQ – Hazard Quotient
HS – Headspace
HS-SPME – Headspace Solid Phase Microextraction
IDL – Instrumental Detection Limit

IL – SDME - Ionic Liquid-Based Single-Drop Microextraction
IL-USAEME – Ionic Liquid Based Ultrasound-Assisted Emulsification Microextraction
IPA – 2-Propanol
IQL – Instrumental Quantification Limit
LC – Liquid Chromatography
LC-MS/MS – Liquid Chromatography-Tandem Mass Spectrometry
LD – Liquid Desorption
LLE – Liquid–Liquid Extraction
LOD – Limit of Detection
log Koc – Organic Carbon–Water Partitioning Coefficient
log K_{ow} – Partition Coefficient Between Octanol/Water
LOQ – Limit of Quantification
MAE – Microwave Assisted Extraction
MALLE – Membrane-Assisted Liquid–Liquid Extraction
MDL – Method Detection Limit
ME – Matrix effect
MeOH – Methanol
MEPS – Microextraction By Packed Sorbent
MME – Micelle Mediated Extraction
MNPs-based dSPE – Magnetic Nanoparticles Dispersive Solid-Phase Extraction
MQL – Method Quantification Limit
MRM – Multiple Reaction Monitoring
MSTFA – N-Methyl-N-(trimethylsilyl)trifluoroacetamide
Na_2EDTA – Ethylenediamine tetraacetic acid
Na_2SO_4 – Sodium Sulfate
NaCl – Sodium Chloride
NH_4OH – Ammonium hydroxide
NOAEL – No Observed Adverse Effect Level
NOEC – No Observed Effect Concentration
P99 – Percentile of exposure (99^{th})
PCPs – Personal care products
PDMS – Poly(dimethylsiloxane)
PLE – Pressurized liquid extraction
PN – Pentane
PNEC – Predicted no effect concentration
POCIS – Polar organic chemical integrative sampler
PrOH – Propanol
PSA – primary and secondary amine exchange bonded silica sorbent
PTFE – Polytetrafluoroethylene
QA/QC – Quality assurance and control
QuEChERS – Quick, Easy, Cheap, Effective, Rugged and Safe
Rec – Recovery
RQ – Risk quotient
RSD – Relative Standard Deviations
S – Solubility
S/N – Signal-to-noise ratio
SBSE – Stir-bar sorptive extraction

SD – Screening design
SEHSDT – Sequential extraction with high-speed dispersion tool
SF – Safety factor
Si – Silicon
SLE – Solid-liquid extraction
SMCs – Synthetic musk compounds
SPE – Solid-phase extraction
SPF – Sun protection factor
SPLE – Selective pressurized liquid extraction
SPMDs – Semipermeable membrane devices
SPME – Solid-phase microextraction
TC - 1,1,2-Trichloroethane
TCE – Tetrachloroethylene
TD – Thermal desorption
Tol – Toluene
TWI – Tolerable weekly intake
U – Uncertainty
UPLC – Ultra-performance liquid chromatography
USE – Ultrasound extraction
UV – Ultraviolet
UVFs – Ultraviolet-Filters
WWTPs – Wastewater Treatment Plants

Notation

bw – Body weight
g – Gram
h – Hour
kg – Kilogram
L – Litre
min – Minute
mL – Millilitre
ng – Nanogram
^{o}C – Celsius degrees
pg – Picogram
rpm – Rotations per minute
µg – Micrograms
µL – Microliters

UVFs

234THB – 2,3,4-Trihydroxybenzophenone
244THB – 2,4,4’-Trihydroxybenzophenone
2DHB – 2,2’-Dihydroxybenzophenone
2HB – 2-Hydroxybenzophenone
3BC – 3-Benzylidene-camphor
3HB – 3-Hydroxybenzophenone
4DHB – 4,4’-Dihydroxybenzophenone
4HB – 4-Hydroxybenzophenone
4-MBC – 3-(4’-Methylbenzylidene) camphor
4PB – 4-Phenylbenzophenone
BCSA – Benzylidene camphor sulfonic acid
BEMT – Bis-Ethylhexyloxyphenol Methoxyphenyl triazine
BH – (Benzhydrol) Diphenylmethanol
BMDM – 4-*tert*-butil-4’-methoxydibenzoylmethane
BMP – Dimethicodiethylbenzalmalonate
BP – Benzophenone
BP1 – 2,4-Dihydroxybenzophenone
BP10 – 2-Hydroxy-4-methoxy-4'-methylbenzophenone
BP12 – 2-Hydroxy-4-octyloxybenzophenone
BP2 – 2,2’,4,4’-Tetrahydroxybenzophenone
BP3 – 2-Hydroxy-4-methoxybenzophenone
BP4 – 2-Hydroxy-4-methoxy benzophenone-5-sulfonic acid
BP5 – 2-Hydroxy-4-methoxy benzophenone-5-sodium sulfonate
BP8 – 2,2’-Dihydroxy-4-methoxybenzophenone
BZS – Benzylsalicylate
CBM – Camphor benzalkonium methosulfate
DBT – Diethylhexyl butamido triazone
DHHB – Diethylamino hydroxybenzoyl hexyl benzoate
DPDT – Disodium phenyl dibenzimidazole tetrasulfonate
DTS – Drometrizole trisiloxane
EDP – 2-ethylhexyl-4-(dimethylamino)benzoate
EHT – Ethylhexyl triazone
EMC – 2-Ethylhexyl 4 –methoxycinnamate
ES – 2-Ethylhexyl salicylate
Et-PABA – Ethyl 4-aminobenzoate
HMS – 3,3,5-Trimethylciclohexyl salicylate (Homosalate)
IMC – Isoamyl 4-methoxycinnamate
MBBT – Methylene bis-benzotriazolyltetramethyl butylphenol
OC – 2-Ethylhexyl 2-cyano-3,3-diphenylacrylate
ODP – Octyldimethyl-p-aminobenzoic acid
PBC – Polyacrylamidomethyl benzylidene camphor
PBSA – 2-Phenyl-5-benzimidazole sulfonic acid
PEG-25PABA – Ethoxylated ethyl-4-aminobenzoate
TDSA – Terephthalylidene dicamphor sulfonic acid
UV-326 – 2-(3-*tert*-Butyl-2-hydroxy-5-methylphenyl)-5-chlorobenzotriazole
UV-329 – 2-(2’-Hydroxy-5’-octylphenyl)-benzotriazole

SMCs

ADBI – Celestolide
AHMI – Phantolide
AHTN – Tonalide
ATII – Traseolide
DPMI – Cashmeran
EB – Ethylene Brassylate
EXA – Exaltolide
HHCB – Galaxolide
MA – Musk Ambrette
MK – Musk Ketone
MM – Musk Moskene
MT – Musk Tibetene
MX – Musk Xylene

Part I: Framework

In this section, a general overview of the work is presented, as well as their relevance, and the main objectives are defined. The structure of this thesis is also explained, chapter by chapter.

Overview

In the past years, a growing concern about the contaminants commonly known as emerging pollutants has been noticed (Noguera-Oviedo and Aga, 2016). In fact, personal care products (PCPs) have attracted the attention of the scientific community. Although PCPs have been used for over a century, only in the last 20 years their environmental impacts have been considered due to their widespread use and continuous release to the environment (Brausch and Rand, 2011; Pedrouzo et al., 2011). Within the class of PCPs, synthetic musk compounds (SMCs) and UV-filters (UVFs) are examples of compounds that are commonly incorporated in daily life products (e.g. soaps, shampoos, detergents, lotions, perfumes, etc.) (Brooke et al., 2008; Mikkelsen, 2015; Peck, 2006; Vallecillos et al., 2015). SMCs are used as base notes in perfumery and as fragrance fixatives and they are considered environmentally persistent, bioaccumulative and are suspected to be hormone disruptors (Nakata et al., 2015; Witorsch and Thomas, 2010). UVFs are organic chemicals that can absorb UV radiation, attenuating the negative effects of sunlight exposure. Studies have shown that UVFs may also accumulate in the environment through direct and indirect sources and bioaccumulate in living organisms, showing estrogenic and hormonal activities (Molins-Delgado et al., 2016; Osterwalder and Hareng, 2016; Sobek et al., 2013).

Once used, the products containing this kind of PCPs are usually washed down-the-drain and end up in wastewater treatment plants (WWTPs) (Homem et al., 2015a; Ramos et al., 2016). However, WWTPs are not completely efficient in removing these PCPs, which eventually will be discharged through the effluents into the receiving media (rivers, lakes, sea, etc.) (Ramos et al., 2015). Persistent and lipophilic organic pollutants, such as SMCs and some UVFs, tend to preferably accumulate in the produced sewage sludge, which may be applied in farmlands as organic fertilizers (Rigby et al., 2016; Sharma et al., 2017). The application of these biosolids is especially favourable in poor quality soils. In fact, the high amount of organic matter present in those biosolids, as well as essential nutrients for plants, especially nitrogen (N) and phosphorus (P), make these residues good amendment agents, from an agronomic perspective (Banuelos et al., 2004). Nevertheless, the disposal of sludge as fertilizer is only regulated regarding the presence

of heavy metals, pathogens and some organic compounds of concern (linear alkylbenzene sulfonates (LASs), nonylphenols and nonylphenol ethoxylates (NPE), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)), with limits established either in Portugal and Europe (Decree-Law No. 103/2015; Decree-Law No. 276/2009; Regulation (EC) No 2003/2003).

The growing global population has directly resulted in generation of huge amounts of diverse solid wastes worldwide. In Europe, in 2016, around 304,813.9 thousand tonnes of sewage sludge were produced, of which only 3.5%, corresponding to a total amount of 10,674.3 thousand tonnes, were used in agriculture as fertilizers. In Portugal, in the same year, 119.17 thousand tonnes of sludge were produced, from which 13.9 thousand tonnes were used in agriculture and 5.1 thousand tonnes were disposed in landfills (Eurostat, 2019a, 2019b). Landfill disposal of sewage sludge is a worldwide problem due to the associated environmental and social issues. The main environmental problems related to this practice are the risk of nutrient leaching, harmful effects on soil biodiversity and greenhouse gas emissions, these are also problems related to the use of not treated sewage sludge as fertilizers in agricultural fields. Social issues are related to human health problems due to air pollution caused by incineration of the waste and odour or amenity effects by landfills (Przydatek and Wota, 2019). Actually, there is an European Directive that limits sewage sludge storage, called the Landfill Directive (Council Directive 99/31/EC). This document states that the priority over sewage sludge should be prevention in the production, encouragement of recycling and waste recovery, as well as the use of the waste as a source of energy.

However, the potential transfer of PCPs from sewage sludge or fertilizers produced from it to the soils and consequently, to crops, has not been a concern so far. Although, this practice is increasingly encouraged due to the adoption of circular economy policies that promote the reuse of waste generated, emerging contaminants such as UVFs and SMCs have been poorly studied in this context. Nevertheless, the ubiquitous presence of SMCs in the environment and in sewage sludge has been thoroughly described, compiled and compared in the past years (e.g. Homem et al., 2015b; Liu and Wong, 2013). However,

this did not happen for UVFs. Therefore, Part II of this work focuses only on the review of these compounds.

As consequence, the application of sewage sludge as fertilizer may be considered a pathway for the introduction of UVFs and SMCs in the soil and consequently, into the food chain through crop uptake (Lai et al., 2014a; Litz et al., 2007; Macherius et al., 2012; Muller et al., 2006). Only a few studies were found regarding the uptake of UVFs and SMCs by crops grown in amended-soils. In the first case, only studies with benzotriazoles were found (Lai et al., 2014b), while in the latter case, most studies focused on galaxolide (HHCB) and tonalide (AHTN) (Calderón-Preciado et al., 2012; Litz et al., 2007; Macherius et al., 2012), which concluded that these compounds are translocated into the crop under study. Therefore, the development of further studies with emerging compounds in this area is essential to ensure environmental and human health safety.

Different crop plants may be used in this type of study (Christou et al., 2019; Pullagurala et al., 2018). The tomato fruit was the crop chosen for this project. This choice was based on production and consumption statistics in Portugal. According to the '2015 Agricultural Statistics' (Instituto Nacional de Estatística, 2016), 1.8 million tons of tomatoes were produced for industry (reaching a record), but also tomato for fresh consumption reached the highest production volume (around 97 thousand tons). In addition, the tomato plant has a fast growth rate, making this crop the better choice for this study.

Risk assessment approaches for organic compounds in food have already been described in different matrices (e.g. seafood, vegetables and fruits) (Cunha et al., 2015; Hlihor et al., 2019; Prosser and Sibley, 2015). Although there is a lack of human risk studies related to the ingestion of food contaminated by SMCs and UVFs, this situation should be seriously taken into consideration since there are no regulations to prevent the ingestion of these potential hazardous pollutants. Moreover, this work focuses only in UVFs and SMCs, but is fair to say that these are not the only compounds uptake by crops and, therefore, more studies within this field of experiments should be encouraged, either with different compounds and/or crops in order to ensure human safety (Colon and Toor, 2016; Pullagurala et al., 2018; Taylor-Smith, 2015). There is also a lack of information regarding the levels of UVFs and SMCs in sludge/compost and its

partitioning to soil, as well as studies of crop uptake in amended-soils. Therefore, this subject needs to be studied due to the concerning consequences to human health.

Objectives

The main objectives of this project are to understand the behaviour of two distinctive chemical families of PCPs, the UVFs and SMCs, determine their presence in environmental matrices and monitoring their path to the entrance in the food web. Specific objectives include the implementation and validation of analytical methodologies for the determination of SMCs (nitro, poly and macro) and UVFs (six compounds from different families) in environmental matrices such as sludge/compost, soil, leachate water and the tomato fruit; the characterization of soils/sludge/compost to assess the influence of their properties on the behaviour of the target compounds in soil; to investigate the uptake and translocation of PCPs by tomatoes after crop fertilization with compost, using a controlled climatic chamber (evaluation of the effect of the amount of fertilizer, the amount of selected target compounds; analysis of the contamination levels in the tomato fruit; development of partitioning and uptake models; risk assessment).

Outline

For a better understanding of the work developed, this thesis was divided into five parts. The first part is the Framework (Part I), where a general overview of the work is explained, as well as the objectives and the thesis organization.

Part (II), corresponding to the Introduction and State of the Art, comprises two chapters that explain the problematic of UV-Filters (Chapter 1 and 2). An introduction and state of the art devoted only to SMCs were not included in this thesis due to the number of review papers found in the literature on this subject (Balk and Ford, 1999; Clarke and Smith, 2011; Pinkas et al., 2017), including studies developed in the working group (Homem et al., 2015a, 2015b). Also, within each Chapter 3 to 7, a literature overview on musks is present within each topic of research. To complement relevant information, on

Annex 3, Table S3.1, is presented the state of the art regarding treatments, extraction methodologies and concentrations of muks in sewage sludge, and in Annex 5, Table S5.3, is presented hazard identification and NOAEL values for this class also.

Part (III) consists of four chapters devoted to the development of analytical methodologies capable to determine SMCs and UVFs in different matrices. Chapter 3 is related to water matrices, Chapter 4 to sewage sludge, Chapter 5 to soils and Chapter 6 to tomato fruits.

Part IV is the culmination of all the knowledge acquired during this study, and where all methodologies developed have been applied to determine UVFs and SMCs uptake by tomatoes grown in amended-soils (Chapter 7).

Part V is the final section where a general discussion is developed and the main conclusions presented (Chapter 8) and finally, the future work is discussed (Chapter 9).

References

Balk, F., Ford, R.A., 1999. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. I. Fate and exposure assessment. Toxicol. Lett. 11, 57–79.

Banuelos, G.S., Sharmasarkar, S., Pasakdee, S., 2004. Utilization of biosolids as a fertilizer for canola. Compost Sci. Util. 12, 61–68.

Brausch, J.M., Rand, G.M., 2011. A review of personal care products in the aquatic environment: environmental concentrations and toxicity. Chemosphere 82, 1518–1532.

Brooke, D.N., Burns, J.S., Crookes, M.J., 2008. UV-filters in cosmetics – prioritisation for environmental assessment. Environment Agency, Bristol.

Calderón-Preciado, D., Renault, Q., Matamoros, V., Cañameras, N., Bayona, J.M., 2012. Uptake of organic emergent contaminants in spath and lettuce: An in vitro experiment. J. Agric. Food Chem. 60, 2000–2007.

Christou, A., Papadavid, G., Dalias, P., Fotopoulos, V., 2019. Ranking of crop plants according to their potential to uptake and accumulate contaminants of emerging concern. Environ. Res. 170, 422–432.

Clarke, B.O., Smith, S.R., 2011. Review of 'emerging ' organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids. Environ. Int. 37, 226–247.

Colon, B., Toor, G.S., 2016. Chapter Three – A Review of Uptake and Translocation of Pharmaceuticals and Personal Care Products by Food Crops Irrigated with Treated Wastewater. Adv. Agron. 140, 75–100.

Council Directive 99/31/EC, 1999. Council Directive 99/31/EC of 26 April 1999 on the landfill of waste (Landfill Directive). Off. J. Eur. Communities.

Cunha, S.C., Trabalón, L., Jacobs, S., Castro, M., Fernandez-Tejedor, M., Granby, K., Kwadijk, C., Verbeke, W., Ferrari, F., Robbens, J., Sioen, I., Fernandes, J.O., Pocurull, E., Domingo, J.L., Marques, A., 2015. UV-filters and musk fragrances in seafood commercialized in Europe Union: Occurrence, risk and exposure assessment. Environ. Res. 143, 116–122.

Decree-Law No. 103/2015, 2015. MINISTÉRIO DA ECONOMIA. Diário da República, 1.a série — N.o 114 — 15 de junho de 2015.

Decree-Law No. 276/2009, 2009. Ministério do Ambiente, do Ordenamento do Território e do Desenvolvimento Regional. Diário da República, 1.a série - No.192 - 2 de Outubro de 2009 (in Portuguese).

Eurostat, 2019a. Sewage sludge production and disposal [WWW Document]. URL https://appsso.eurostat.ec.europa.eu/nui/submitViewTableAction.do

Eurostat, 2019b. Sewage sludge production and disposal from urban wastewater (in dry substance (d.s)) Thousand tonnes [WWW Document]. URL https://ec.europa.eu/eurostat/databrowser/view/ten00030/default/table?lang=en

Hlihor, R., Olga, M., Rosca, M., Cozma, P., 2019. Modelling the behavior of pesticide residues in tomatoes and their associated long-term exposure risks. J. Environ. Manage. 233, 523–529.

Homem, V., Silva, E., Alves, A., Santos, L., 2015a. Scented traces - Dermal exposure of synthetic musk fragrances in personal care products and environmental input assessment. Chemosphere 139, 276–287.

Homem, V., Silva, A., Ratola, N., Santos, L., Alves, A., 2015b. Long lasting perfume - A review of synthetic musks in WWTPs. J. Environ. Manage. 149, 168–192.

Instituto Nacional de Estatística, I.P., 2016. Estatísticas Agrícolas 2015. Lisboa, Portugal.

Lai, H.J., Ying, G.G., Ma, Y.B., Chen, Z.F., Chen, F., Liu, Y.S., 2014a. Field dissipation and plant uptake of benzotriazole ultraviolet stabilizers in biosolid-amended soils. Environ. Sci. Process. Impacts 16, 558–566.

Lai, H.J., Ying, G.G., Ma, Y.B., Chen, Z.F., Chen, F., Liu, Y.S., 2014b. Occurrence and dissipation of benzotriazoles and benzotriazole ultraviolet stabilizers in biosolid-amended soils. Environ. Toxicol. Chem. 33, 761–767.

Litz, N.T., Mueller, J., Boehmer, W., 2007. Occurrence of polycyclic musks in sewage sludge and their behaviour in soils and plants: Part 2: Investigation of Polycyclic Musks in Soils and Plants. J. Soils Sediments 7, 36–44.

Liu, J.-L., Wong, M.-H., 2013. Pharmaceuticals and personal care products (PPCPs): A review on environmental contamination in China. Environ. Int. 59, 208–224.

Macherius, A., Eggen, T., Lorenz, W.G., Reemtsma, T., Winkler, U., Moeder, M., 2012. Uptake of galaxolide, tonalide, and triclosan by carrot, barley, and meadow fescue plants. J. Agric. Food Chem. 60, 7785–91.

Mikkelsen, S., 2015. Survey and health assessment of UV filters, The Danish Environm. Protection Agency.

Molins-Delgado, D., Díaz-Cruz, M.S., Barceló, D., 2016. Ecological risk assessment associated to the removal of endocrine-disrupting parabens and benzophenone-4 in wastewater treatment. J. Hazard. Mater. 310, 143–151.

Muller, J., Boehmer, W., Litz, N.T., 2006. Occurrence of polycyclic musks in sewage sludge and their behaviour in soils and plants: Part 1: Behaviour of Polycyclic Musks in Sewage Sludge of Different Treatment Plants in Summer and Winter. J. Soils Sediments 6, 231–235.

Nakata, H., Hinosaka, M., Yanagimoto, H., 2015. Macrocyclic-, polycyclic-, and nitro musks in cosmetics, household commodities and indoor dusts collected from Japan: Implications for their human exposure. Ecotoxicol. Environ. Saf. 111, 248–255.

Noguera-Oviedo, K., Aga, D.S., 2016. Lessons learned from more than two decades of research on emerging contaminants in the environment. J. Hazard. Mater. 316, 242–251.

Osterwalder, U., Hareng, L., 2016. Global UV Filters: Current Technologies and Future Innovations, in: Principles and Practice of Photoprotection. Springer International Publishing Switzerland 2016, Ludwigshafen, Germany.

Peck, A.M., 2006. Analytical methods for the determination of persistent ingredients of personal care products in environmental matrices. Anal. Bioanal. Chem. 386, 907–939.

Pedrouzo, M., Borrull, F., Marcé, R.M., Pocurull, E., 2011. Analytical methods for personal-care products in environmental waters. TrAC - Trends Anal. Chem. 30, 749–760.

Pinkas, A., Gonçalves, C.L., Aschner, M., 2017. Neurotoxicity of fragrance compounds: A review. Environ. Res. 158, 342–349.

Prosser, R.S., Sibley, P.K., 2015. Human health risk assessment of pharmaceuticals and personal care products in plant tissue due to biosolids and manure amendments, and wastewater irrigation. Environ. Int. 75, 223–233.

Przydatek, G., Wota, A.K., 2019. Analysis of the comprehensive management of sewage sludge in Poland. J. Mater. Cycles Waste Manag. 1–9.

Pullagurala, V.L.R., Rawat, S., Adisa, I.O., Hernandez-Viezcas, J.A., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2018. Plant uptake and translocation of contaminants of emerging concern in soil. Sci. Total Environ. 636, 158–1596.

Ramos, S., Homem, V., Alves, A., Santos, L., 2016. A review of organic UV-filters in wastewater treatment plants. Environ. Int. 86, 24–44.

Ramos, S., Homem, V., Alves, A., Santos, L., 2015. Advances in analytical methods and occurrence of organic UV-filters in the environment - A review. Sci. Total Environ. 526, 278–311.

Regulation (EC) No 2003/2003, 2003. Regulation (EC) No 2003/2003 of the European Parliament and of the Council of 13 October 2003 relating to fertilisers. The European

Parliament, The Council of The European Union.

Rigby, H., Clarke, B.O., Pritchard, D.L., Meehan, B., Beshah, F., Smith, S.R., Porter, N.A., 2016. A critical review of nitrogen mineralization in biosolids-amended soil, the associated fertilizer value for crop production and potential for emissions to the environment. Sci. Total Environ. 541, 1310–1338.

Sharma, B., Sarkar, A., Singh, P., Singh, R.P., 2017. Agricultural utilization of biosolids: A review on potential effects on soil and plant grown. Waste Manag. 64, 117–132.

Sobek, A., Bejgarn, S., Rudén, C., Molander, L., Breitholtz, M., 2013. In the shadow of the Cosmetic Directive — Inconsistencies in EU environmental hazard classification requirements for UV-filters. Sci. Total Environ. 461–462, 706–711.

Taylor-Smith, A., 2015. Pharmaceutical Compounds in Land-Applied Sludge and Plant Uptake: a Review. Faculty of North Carolina State University.

Vallecillos, L., Borrull, F., Pocurull, E., 2015. Recent approaches for the determination of synthetic musk fragrances in environmental samples. TrAC Trends Anal. Chem. 72, 80–92.

Witorsch, R.J., Thomas, J.A., 2010. Personal care products and endocrine disruption: A critical review of the literature. Crit. Rev. Toxicol. 40, 1–30.

Part II: Introduction and State of the Art

In this section, a review on UVFs is presented in two chapters. The first with a general overview of the compounds properties as wells as the presence of these compounds in environmental matrices and biota. Also, the analytical methods used for the determination of these compounds are presented and discussed. Chapter two describes the presence of UVFs in WWTPs, since they enter in influent wastewater until they exit as effluent wastewater or sewage sludge, discussing treatment methods, concentrations levels and analytical methodologies for their detection.

Chapter 1. Advances in analytical methods and occurrence of organic UV-filters in the environment – A review

Sara Ramos, Vera Homem, Arminda Alves, Lúcia Santos,

Science of the Total Environment, 526, pp. 278–311 (2015)

Abstract

UV-filters are a group of compounds designed mainly to protect skin against UVA and UVB radiation, but they are also included in plastics, furniture, etc., to protect products from light damage. Their massive use in sunscreens for skin protection has been increasing due to the awareness of the chronic and acute effects of UV radiation. Some organic UV-filters have raised significant concerns in the past few years for their continuous usage, persistent input and potential threat to ecological environment and human health. UV-filters end up in wastewater and because wastewater treatment plants are not efficient in removing them, lipophilic compounds tend to sorb onto sludge and hydrophilics end up in river water, contaminating the existing biota. To better understand the risk associated with UV-filters in the environment a thorough review regarding their physicochemical properties, toxicity and environmental degradation, analytical methods and their occurrence was conducted.

Higher UV-filter concentrations were found in rivers, reaching 0.3 mg L^{-1} for the most studied family, the benzophenone derivatives. Concentrations in the ng to µg L^{-1} range were also detected for the *p*-aminobenzoic acid, cinnamate, crylene and benzoyl methane derivatives in lake and sea water. Although at lower levels (few ng L^{-1}), UV-filters were also found in tap and groundwater. Swimming pool water is also a sink for UV-filters and its chlorine by-products, at the µg L^{-1} range, highlighting the benzophenone and benzimidazole derivatives. Soils and sediments are not frequently studied, but concentrations in the µg L^{-1} range have already been found especially for the benzophenone and crylene derivatives. Aquatic biota is frequently studied and UV-filters are found in the ng g^{-1} dw range with higher values for fish and mussels. It has been concluded that more information regarding UV-filter degradation studies both in

water and sediments is necessary and environmental occurrences should be monitored more frequently and deeply.

Keywords: UV-filters; Environment; Occurrence; Analytical methods

1.1 Introduction

In the past few years, concern for sunburns, premature skin aging and the risk of developing skin cancer has raised and ultraviolet (UV) radiation has been considered a public health threat. UV radiation can reach the earth surface in both UVA (315–400 nm) and UVB (280–315 nm) ranges, while solar light UVC (200–280 nm) is absorbed by ozone in the stratosphere (Kim and Choi, 2014).

UV-filters are compounds designed mainly to protect our skin against damage by UVA and UVB radiation. These compounds can either be organic (chemical) absorbers or inorganic (physical) blockers, depending on the basis of their mechanism of action. Organic UV-filters absorb UV radiation and the absorbed energy produces an excited state of the molecule, giving it higher energy content. The excess of energy is dissipated by emission of higher wavelengths or relaxation by photochemical processes, for example isomerisation and heat release (Abdelraheem et al., 2015). Inorganic sunscreens, like titanium dioxide and zinc oxide, protect the skin by reflecting and scattering UV radiation (Crista et al., 2014). In this review, only organic UV-filters are considered because of their frequent use at higher quantities.

Although UV-filters are mainly incorporated in cosmetics (such as sunscreen lotions, skin care, facial makeup and lip care products), they are also included in a wide range of products including plastics, adhesives, paint and rubber in order to protect from UV degradation (Brooke et al., 2008; Gackowska et al., 2014). Personal care products with a high sun protection factor (SPF) values are the most popular among consumers; however, the ‘false’ sense of protection leads to prolonged sun exposure. In order to enhance the SPF values, several combinations of UV-filters are used (both organic UVA and UVB and inorganic) and their total concentration in the final products increased. This results in an increased population exposure to a higher and greater diversity of UV-filters (Chisvert et al., 2001; Manova et al., 2013).

At some point, the majority of cosmetic products will find their way into wastewater (due to bathing and washing activities) and consequently into rivers, lakes and ocean, so it is not surprising that UV-filters are found in the environment (Abdelraheem et al., 2015; Duirk et al., 2013). A schematic of the major pathways of UV-filters in the

environment was presented by Giokas et al. (2007) and can be completed with the understanding of the urban water cycle presented by Pal et al. (2014). UV-filters are very persistent in the environment due to their massive use and physicochemical properties (Liu and Wong, 2013; Rodil et al., 2009a) and their environmental issues are related mainly to their endocrine disrupting potential, systemic circulation and probable exposure of all tissues in the body in humans (Krause et al., 2012), mammals (Schlumpf et al., 2004), amphibian and also fish (Blüthgen et al., 2014).

The first review specifically oriented to UV-filters appeared in 1999 by Daughton and Ternes and the second in 2007 by Giokas et al. However, other reviews regarding specific topics under UV-filters also exist, such as BP3 (Kim and Choi, 2014), UV-filter transformation products (Santos et al., 2012) and UV-filter occurrence in biota (Gago-Ferrero et al., 2012). Overviews of analytical methods for determining UV-filters in cosmetic products (Salvador and Chisvert, 2005), human samples (Jiménez-Díaz et al., 2014) and advanced aspects of current LC–MS/MS methodology (Gago-Ferrero et al., 2013a) were also published, as well as regarding toxicity of few UV-filters in the aquatic environment (Brausch and Rand, 2011).

Therefore, the main objective of this review is to summarize the scattered information about the utilization of UV-filters and to explain why this class of compounds has raised so much concern in the past years. It is also expected to summarize and analyze the UV-filter profiles in several matrices (water, soil, sediments and biota), describe the analytical methods most used and analyze the overall distribution and fate of UV-filters in the environment.

1.2 UV-filter characterization

1.2.1 Chemistry

The most used UV-filters in today's worldwide industry and the most detected in environmental matrices are represented in Figure 1.1, according to their chemical family. Those whose use in cosmetics is currently allowed by European legislation (Regulation (EC) No 1223/2009) (in Annex VI “List of UV-filters allowed in cosmetic

products") are marked in bold. Different abbreviations were found in literature for the same compound. For that reason, the CAS number which unequivocally defines the chemical, an abbreviation and also the chemical structure, were included in Figure 1.1.

The 46 organic UV-filters were grouped in 11 chemical families (Bester, 2007; Crista et al., 2014): benzophenone derivatives (two benzene rings joined by a carbonyl group), *p*-aminobenzoic acid derivatives (one benzene ring substituted with an amino group and a carboxyl group in the para position), camphor derivatives (organic compounds classified as terpenoids), benzotriazole derivatives (composed by a fused benzene and 5 member unsaturated ring structure with 3 nitrogen atoms), salicylate derivatives (containing a monohydroxybenzoic acid group), triazine derivatives (six-membered benzene-like ring, with three carbons replaced by nitrogen atoms), benzimidazole derivatives (heterocyclic aromatic organic compound derivative with a merged benzene and imidazole ring), cinnamate derivatives (unsaturated carboxylic acids), benzylmalonate derivatives (esters of dicarboxylic acids with a benzylic substituent), crylene derivatives (aromatic acrylates) and dibenzoyl methane derivatives (aromatic 1,3-diketone derivative of acetylacetone, where both methyl groups have been substituted by phenyl groups). Another compound, benzhydrol (diphenylmethanol), was not grouped in any family because it is a metabolite that results from benzophenone reduction.

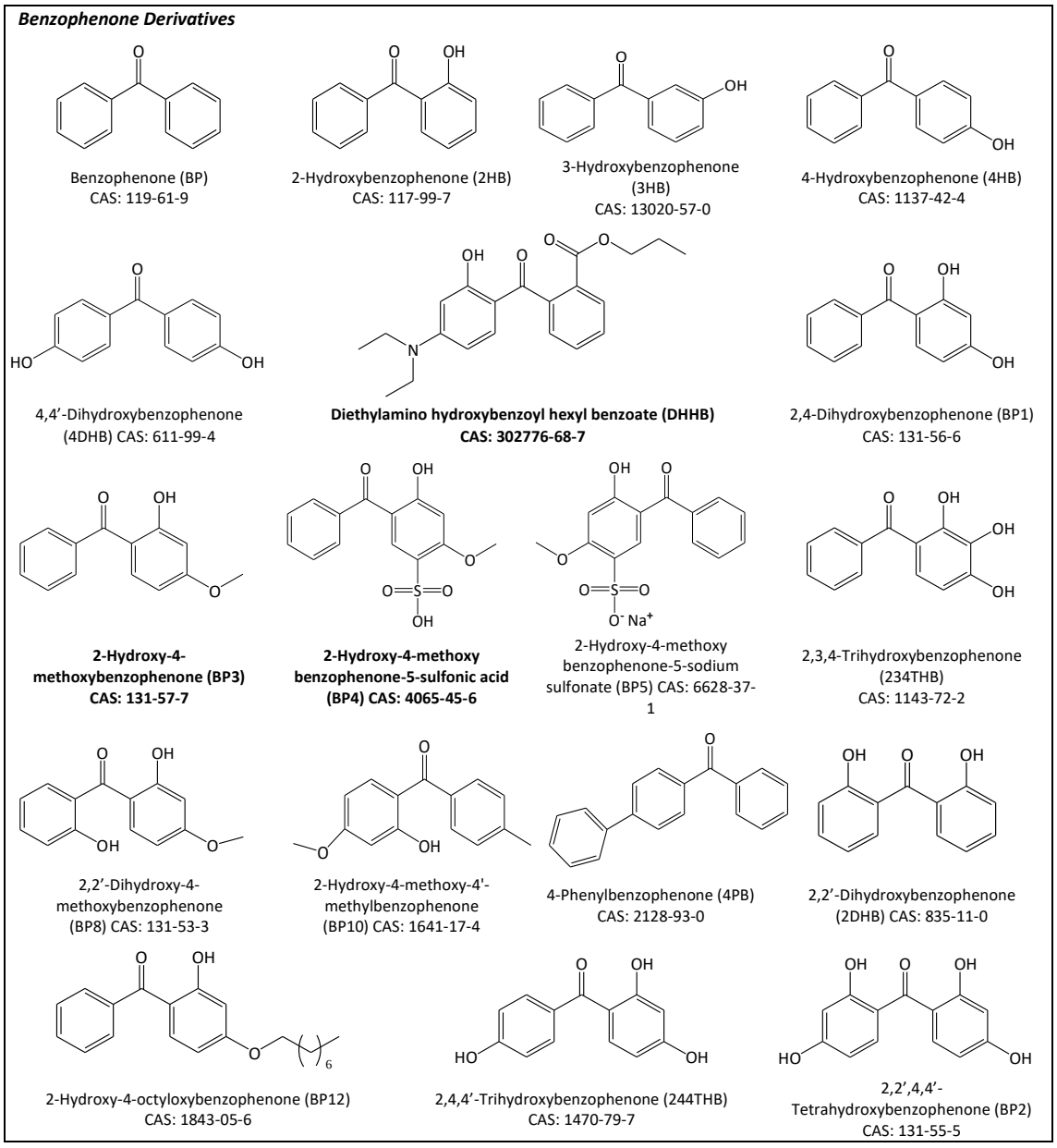
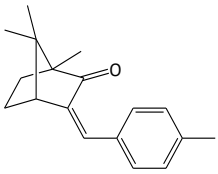
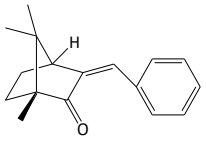


Figure 1.1 Organic UV-filters (in bold the allowed UV-filters in cosmetics)

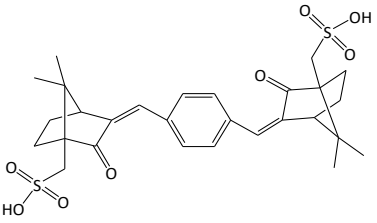
Camphor derivatives



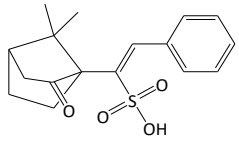
3-(4'-Methylbenzylidene) camphor (4-MBC)
CAS: 36861-47-9



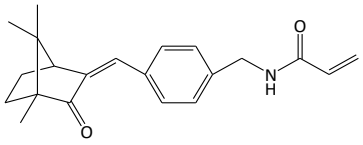
3-Benzylidene-camphor (3BC)
CAS: 15087-24-8



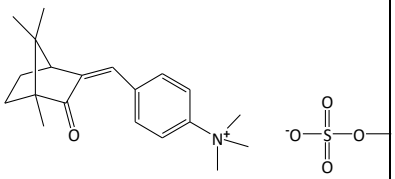
Terephthalylidene dicamphor sulfonic acid (Ecamsule) (TDSA)
CAS: 92761-26-7



Benzylidene camphor sulfonic acid (BCSA)
CAS: 56039-58-8

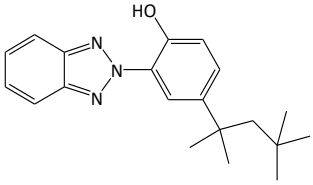


Polyacrylamidomethyl benzylidene camphor (PBC)
CAS: 113783-61-2

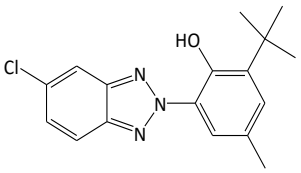


Camphor benzalkonium methosulfate (CBM)
CAS: 52793-97-2

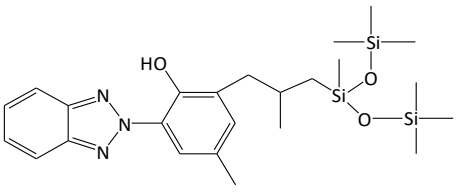
Benzotriazole derivatives



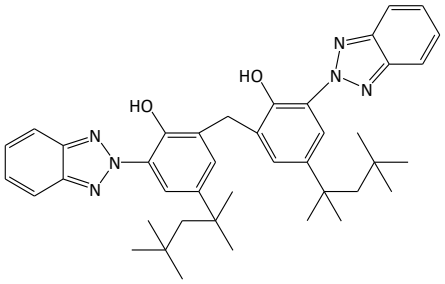
2-(2'-Hydroxy-5'-octylphenyl)-benzotriazole (UV 329)
CAS: 3147-75-9



2-(3-*tert*-Butyl-2-hydroxy-5-methylphenyl)-5-chlorobenzotriazole (UV 326)
CAS: 3896-11-5

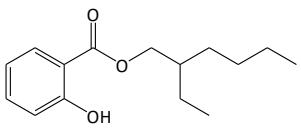


Drometrizole trisiloxane (DTS)
CAS: 155633-54-8

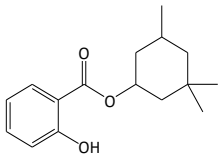


Methylene bis-benzotriazolyltetramethyl butylphenol (MBBT)
CAS: 103597-45-1

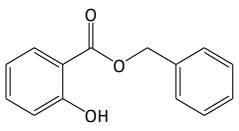
Salicylate derivatives



2-Ethylhexyl salicylate (ES)
CAS: 118-60-5



3,3,5-Trimethylciclohexyl salicylate (Homosalate) (HMS)
CAS: 118-56-9



Benzylsalicylate (BZS)
CAS: 118-58-1

Figure 1.1 Organic UV-filters (in bold the allowed UV-filters in cosmetics) (cont.)

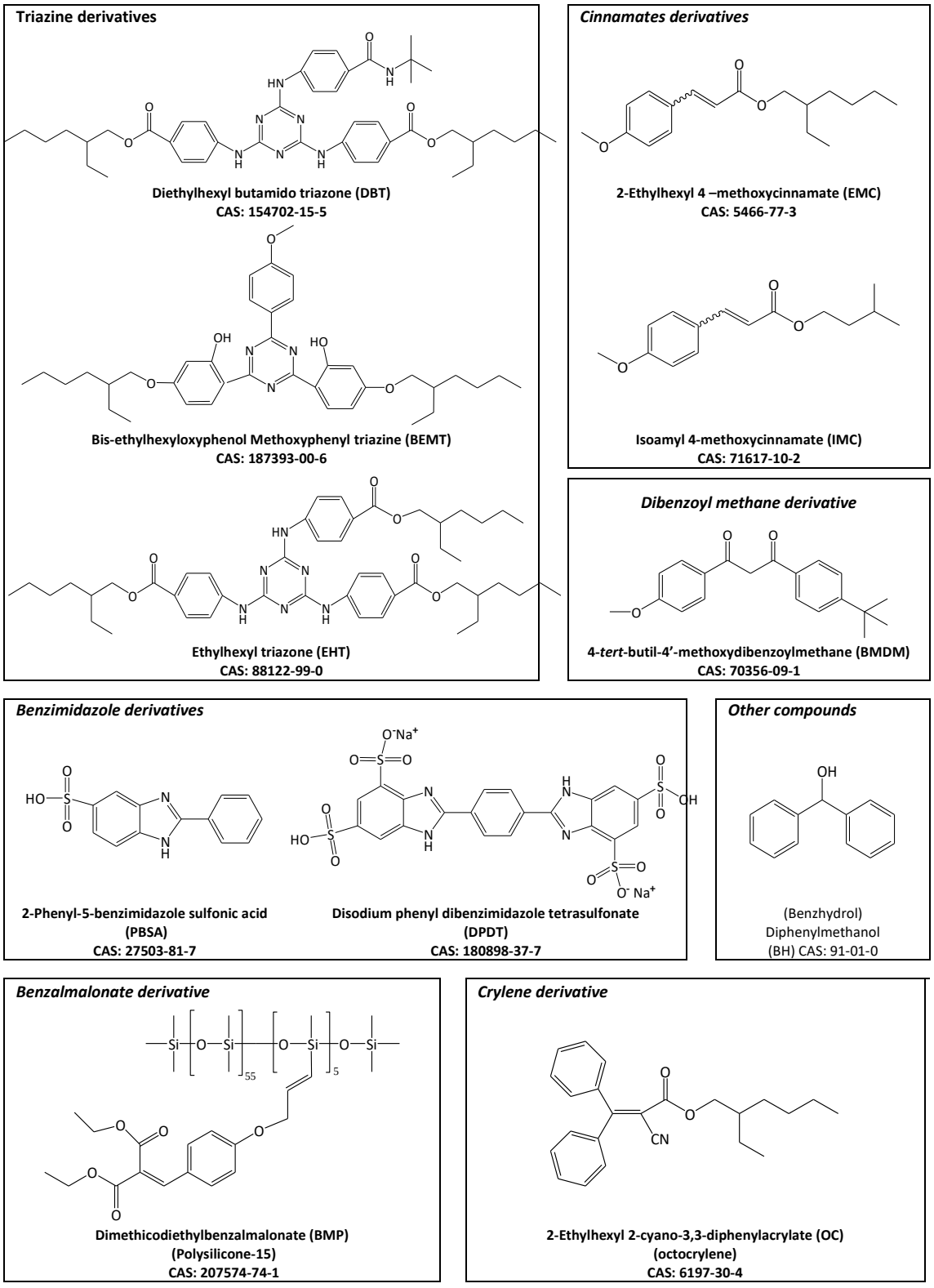


Figure 1.1 Organic UV-filters (in bold the allowed UV-filters in cosmetics) (cont.).

However, this UV-filter is widely used and detected in the environment, therefore is also relevant in this review. A common feature of these compounds is the presence of an aromatic moiety with a side chain, showing different degrees of unsaturation (Díaz-Cruz

et al., 2008). As can be seen in Figure 1.1, some of these compounds are chiral (e.g. EMC, OC and 4-MBC), but their enantiomers are expected to show the same physicochemical properties (Bester, 2007). Among the benzophenone derivatives, BP5 (2-hydroxy-4-methoxybenzophenone-5-sodium sulfonate) is the salt of BP4 (2-hydroxy-4-methoxybenzophenone-5-sulfonic acid), and both are allowed in cosmetics in a maximum concentration of 5% (w/w) (Directive, 1998). The percentage of UV-filter added to cosmetic formulations depends on the degree of protection (SPF) and protection zone desired (UVA, UVB). However, they are usually combined in concentrations that should not exceed 10% in combination with an inorganic UV-filter (Santos et al., 2013). UV-filter drometrizole trisiloxane (DTS) is an exception, whose maximum concentration in the final product is 15% (Regulation (EC) No 1223/2009; Moreta and Tena, 2011).

1.2.2. Physicochemical properties

Physicochemical properties of UV-filters will determine their fate in environment and are also important to understand which analytical methodologies are appropriate to their determination in the different environmental compartments. Figure 1.2 presents their main properties (boiling point (A), water solubility (B) and the octanol–water partition coefficient (C)) grouped by chemical family. Data was not found for most of the compounds presented in Figure 1.1. Therefore, the EPI Suite™ tool was used. This is a screening-level tool that provides either measured and/or estimated physical/chemical property values (EPA, 2012b).

Regarding the boiling points (Figure 1.2(A)) UV-filters are not considered as volatile compounds since they have boiling points with average values of 400 °C (EPA, 2012b). The most volatile compounds are Et-PABA, BP and IMC (around 300–350 °C). The less volatile compounds are benzimidazole and triazine derivatives.

The UV-filters' solubility in water is presented in Figure 1.2(B). Water solubility provides some information on the likely distribution of the chemicals between the different environmental compartments, specially soil/sediment and water and consequently, the potential for environmental or human exposure through release to the aquatic

compartment. Water solubility (S) values were estimated using EPI Suite™ and, accordingly compounds are classified as: highly soluble if $S \geq 1.0 \times 10^4$ mg L^{-1}; soluble if 1.0×10^4 mg $L^{-1} > S \geq 1.0 \times 10^3$ mg L^{-1}; moderately soluble if 1.0×10^3 mg $L^{-1} > S \geq 1.0 \times 10^2$ mg L^{-1}; slightly soluble if 1.0×10^2 mg $L^{-1} > S \geq 1.0 \times 10^{-1}$ mg L^{-1} and negligibly soluble if $S < 1.0 \times 10^{-1}$ mg L^{-1} (EPA, 2012a). According to this classification the benzimidazole group and BP5 and BP4 (benzophenone derivatives) are highly soluble, which was already shown by several studies (Gago-Ferrero et al., 2013b; Fent et al., 2010; Wick et al., 2010; Kasprzyk-Hordern et al., 2009). Most benzophenone derivatives are moderately soluble, as well as BCSA (camphor derivative). Other camphor, benzophenone, *p*-aminobenzoic acid, salicylate and cinnamate derivatives are slightly soluble. On the other hand, compound families of triazine, benzotriazole and crylene derivatives are not soluble, which means that are not likely to be found in water bodies.

The log K_{ow} values for each compound are presented in Figure 1.2(C). This partition coefficient is an indicator of the environmental fate of the UV-filters, translating how they are distributed between octanol (which represents the lipids or fats in biota) and water (the aqueous phase). Values were also estimated with EPI Suite™. Benzimidazole derivatives and benzophenone derivatives BP4 and BP5 with values <1 are considered hydrophilic (highly soluble in water). On the other hand, most compounds with values >4 are hydrophobic like crylene, dibenzoyl methane, cinnamate, *p*-aminobenzoic and salicylate derivatives (Díaz-Cruz and Barceló, 2009). Compounds like BEMT (>8) are considered not readily bioavailable and compounds with values >10, like EHT, DBT, MBBT and DTS, are not bioavailable at all (EPA, 2012a).

The organic carbon–water partitioning coefficient (log K_{oc}), as the log K_{ow}, has a similar distribution. Considering the properties discussed before, the water compartment seems to be the priority matrix for these compounds. Compounds as benzophenone derivatives, BP4 and BP5 and benzimidazole derivatives, PBSA and DPDT, with high solubility in water and low log K_{ow}, are very likely to be found in water. On the other hand, triazine and benzotriazole derivatives with very low solubility in water and high K_{ow} and log K_{oc} are not likely to be detected in that matrix, but in soils/sediments.

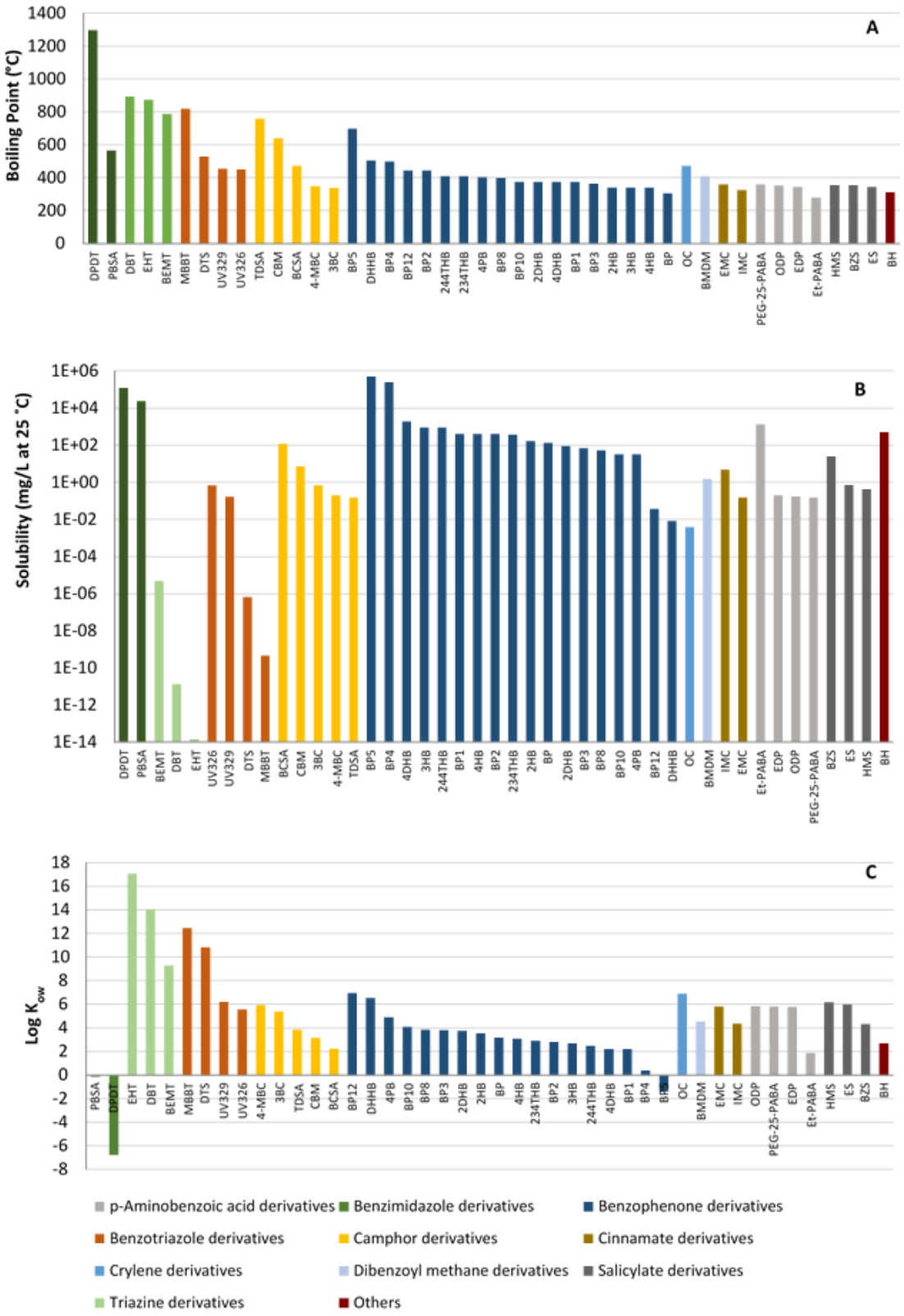


Figure 1.2 UV-filters main properties (A - boiling point, B - water solubility and C - octanol-water partition coefficient) grouped by chemical family.

1.2.3. Toxicity and legislation

For the past years, UV-filters have been detected in trace levels, in different environmental matrices, but mostly in water with values in the μg L^{-1} range. However, the effects and consequences of their presence are a growing subject of discussion. Sobek et al. (2013) presented evidence in inconsistencies in EU environmental hazard classification requirements for UV-filters. Because the Cosmetic Directive (Directive, 1998) does not include any requirements on conducting environmental risk assessments (ERAs), the list of approved UV-filters may include substances with environmentally hazardous properties. In fact, the present review presents evidence of UV-filter detection in surface water, sediments and biota (Kasprzyk-Hordern et al., 2009; Amine et al., 2012; Peng et al., 2015). The European regulation on classification, labelling and packaging (CLP) of substances and mixtures is not used in UV-filters. However, if it was used, 12 of the 26 individual UV-filters approved for use in cosmetics would meet the CLP classification as 'hazardous to the aquatic environment' (Sobek et al., 2013). Of these 12 compounds, 4 would be classified according to the highest toxicity category, and the others would not be classified for lack of information (Sobek et al., 2013).

Regarding water policy, the Council Directive 98/83/EC (Directive, 1998) on the quality of water intended for human consumption makes no reference to UV-filters. Hopefully, with the increasing knowledge about the UV-filter occurrence and ecotoxicity, it will be possible that risk assessment studies may lead to impose legal limits for some compounds of this group in wastewater effluents in the near future. These limits would narrow the amounts of UV-filters discharged from WWTPs to rivers that then accumulate in other matrices.

The reason why these compounds are under scope is related to their toxicity and adverse effects like the known estrogenic effects on biota and humans (Schlumpf et al., 2004; Bester, 2007; Weisbrod et al., 2007; Sieratowicz et al., 2011; Kaiser et al., 2012; Paredes et al., 2014). These effects have already been systematically described by Díaz-Cruz and Barceló (2009) and they include estrogenic activities *in vitro* (Fent et al., 2008), maximum effects on cell proliferation by EMC, ODP, 4-MBC and HMS (Ciszmas et al., 2004), induction of transcriptional activation of human estrogenic receptor α (hERα) and β (hERβ) by BP3, BMDM, EMC, ODP, 4-MBC and HMS (Schreurs et al., 2002). Multiple

hormonal activities have also been demonstrated *in vitro* for estrogenic and antiestrogenic for 4-MBC and also antiandrogenic for BP3 and HMS (Schlumpf et al., 2004). Compounds 4-MBC and EMC, identified as 'endocrine disruptor compounds' (EDCs) are usually compared to estradiol-17β (E2) a chemical that like UV-filters was found to negatively affect reproduction and sometimes detected at environmentally relevant concentrations (Weisbrod et al., 2007).

Although UV-filter estrogenic activity has been widely studied both *in vivo* and *in vitro* test systems, namely in fish and mammals (Schlumpf et al., 2004; Søeborg et al., 2007; Christen et al., 2011; Kim et al., 2014), recent studies have demonstrated that not only estrogens, but also different hormonal targets in mammals and fish are affected by UV-filters (Blüthgen et al., 2014; Ponzo and Silvia, 2013). To date, more attention has been given on the interaction of UV-filters with sex steroid hormones in mammals, because research about the adverse effects of these compounds has been mainly focused on assessing the potential risk to humans. Although significantly less attention has been paid to the effects in invertebrates, there is also some evidence of the toxic effects (Gao et al., 2013) and developmental or reproductive impairments of UV-filters in these organisms (Ozáez et al., 2014).

Information regarding UV-filter toxicity is still very scarce and is not possible to develop adequate aquatic risk assessments. However, preliminary hazard assessments are already available. Brausch and Rand (2011) reviewed some UV-filters in the environment presenting acute (BP, BP3, BP4, 4-MBC and EMC) and chronic toxicity data (BP, BP1, BP2, BP3, BP4, 3BC, 4-MBC and Et-PABA). Rodríguez et al. (2015) presented an approach to environmental risk assessment for BP3, 4-MBC and EMC in waters of monitored beaches and found small potential for adverse effects for BP3 and significant potential for adverse effects for 4-MBC and EMC, whose risk quotient (RQ) values were higher than 10. An ecological risk assessment is available for BP3 and although the levels observed in ambient water are generally an order of magnitude lower than the predicted no effect concentration (PNEC), the authors consider that further studies on environmental monitoring and potential consequences of long-term exposure in aquatic ecosystem are needed (Kim and Choi, 2014).

In Table S1 in the Annex 1, it is available data for some UV-filters regarding their ecotoxicity and assessment of priority. Most of the available data was calculated using the EPI Suit™ tool; however, some information comes from measured experiments (EPA, 2012b). According to information in Table S1, compounds with 'No Observed Effect Concentration' (NOEC) values lower than 0.01 mg L^{-1} are considered high priority for further work. The compounds with this classification are HMS, OC, EMC, IMC, 4-MBC, 3BC, ES and EDP (Brooke et al., 2008).

1.2.4. Environmental degradation and transformation products

Although the main characteristic of UV-filters should be their high stability upon exposure to sunlight, several studies report that some undergo degradation under UV radiation (De Laurentiis et al., 2013; Vione et al., 2013). This happens mainly due to the inability to convert the energy absorbed fast enough, so the molecule stays excited and chemically react (Díaz-Cruz et al., 2008). This compromise the products' efficiency, since the UV-filters lose their photoprotective properties and photodegradation reactions may change their physical properties, namely the maximum absorption wavelength and absorbance coefficient (Díaz-Cruz et al., 2008; Serpone et al., 2002). Considering that these compounds are added to personal care products and applied frequently and in large quantities, it is essential to study their transformation products, since they can accumulate in human skin, posing a threat to human health, and afterwards will end up in the environment (Negreira et al., 2008).

A similar situation occurs in the environment. When these contaminants are released into the ecosystems, they are also susceptible to degradation by sunlight. UV-filter degradation can also happen in chlorine media, like swimming pools, resulting in chlorinated by-products that are often more toxic than the parent UV-filters (Santos et al., 2012).

In fact, photolysis is a chemical process that causes the dissociation of the UV-filters into reactive fragments (free radicals) or reactive intermediates. However, it was shown that the photochemistry of sunscreen products (usually containing different UV-filters) is more complex than the isolated behavior of individual UV-filter (Sayre et al., 2005). It also depends on environmental conditions and on the presence of other compounds,

like dissolved organic matter (Sakkas et al., 2003). UV-filter degradation can also happen in chlorine media, like swimming pools, resulting in chlorinated by-products that are often more toxic than the parent UV-filters (Santos et al., 2012).

As mentioned before, Santos et al. (2012) recently reviewed the transformation products of UV-filters in aqueous and chlorinated aqueous solutions. Although few studies have been found, these authors verified that transformation products of benzophenone, *p*-aminobenzoic acid, camphor, benzimidazole, cinnamate and dibenzoyl methane derivatives were already been identified. Although this topic is beyond the scope of this work, a small overview on these transformation products is presented in Supplementary material.

1.3. Advances in analytical methods for UV-filters in the environment

The major number of analytical methods for UV-filters has been developed for water matrices and therefore this chapter is essentially centered in this matrix. However, other environmental compartments (soil and sediments) are also included. As previously mentioned, the methods regarding biota analysis have been already reviewed by Gago-Ferrero et al. (2012) and, for that reason, it was not included in this study.

An extensive overview of UV-filter publications since 2000 was performed. Information regarding extraction and cleanup procedures, chromatographic analysis, validation parameters (limits of detection and recoveries) and environmental concentrations is presented in Tables 1.1 to 1.3.

1.3.1. Extraction techniques for water analysis

Extraction methods usually follow a common path involving the release of the target components from their matrices to a desirable solvent, followed by removal of the unwanted components. Although several extraction methodologies have been used to determine UV-filters in water, solid-phase extraction (SPE) is the most used (Table 1.1). Considering the extraction recovery yield, this method is often applied using commercial cartridges with monomerically bonded C_{18} silica sorbents (Tsui et al., 2014). This type of

sorbent is able to retain the major organic analytes from aqueous solution, but it is mostly used in the extraction of moderately polar to non-polar analytes from aqueous samples (Giokas et al., 2004; Goksoyr et al., 2009; Li et al., 2007; Tsui et al., 2014). However, polymeric reversed phase sorbents (hydrophobic), with no bonded phase or alkyl ligands, water wettable, were also used (Rodil et al., 2012; Ho and Ding, 2012). This type of sorbents is suitable for applications with target compounds over a wide range of chemical properties like the UV-filters under study (Arukwe et al., 2012; Liu et al., 2011). The high sample volumes in this procedure, usually from 100 mL (Goksoyr et al., 2009; Liu et al., 2010) up to 1.0 L (Kameda et al., 2011; Liu et al., 2011) are the main disadvantage of this technique. Because UV-filters are relatively polar, the great majority of authors use intermediate polarity solvents like dichloromethane (DCM) (Goksoyr et al., 2009; Kameda et al., 2011; Lambropoulou et al., 2002; Tashiro and Kameda, 2013) or ethyl acetate (EA) (Arukwe et al., 2012; Balmer et al., 2005; da Silva et al., 2015; Negreira et al., 2008; Poiger et al., 2004) to extract water samples. However, Rodil et al. (2012) used a more polar solvent, methanol (MeOH), which is justified by the complex mixture analyzed, containing not only very polar UV-filters (like PBSA and BP4), but also other polar compounds like pharmaceuticals and herbicides. SPE is considered a good method, easy to perform and generally yields high recoveries, ranging 60 to 100% (Giokas et al., 2004; Goksoyr et al., 2009; Kameda et al., 2011; Li et al., 2007; Liu et al., 2010; 2011; Tsui et al., 2014). In order to obtain a better cleanup, SPE is also used coupled to other clean-up techniques, as gel permeation chromatography (GPC) (Balmer et al., 2005) with recoveries ranging 78–129%. SPE is usually performed off-line (i.e. prior to separation and detection), however, on-line SPE is emerging as an effective technique, coupled online with an LC system or as a fully-automated system in order to analyze organic UV-filters (Gago-Ferrero et al., 2013b; Grabicova et al., 2013; Jurado et al., 2014) yielding recoveries around 100%.

Besides SPE, other approaches exist, as the dispersive liquid–liquid microextraction (DLLME) (Jeon et al., 2006; Zhang and Lee, 2012b) and solid-phase microextraction (SPME) (Lambropoulou et al., 2002; Zhang and Lee, 2012a). These methods have the benefit of being more environmental-friendly since they use small amounts of organic

solvents, are usually faster and conducts to less matrix effects are less. However, sensitivity and the precision tend to be worse than commonly used SPE techniques.

DLLME is a liquid–liquid extraction (LLE), based on the relative solubility of the analytes in two different immiscible liquids. A small volume of extracting solvent (a high-density solvent) is dispersed by the action of a second solvent, the disperser (a water miscible, polar solvent). Dispersion increases the effective extraction area, obtaining fast extraction rates and high enrichment factors, as well as simplicity of operation and low cost of implementation (Ojeda and Rojas, 2009; Maya et al., 2014). The usual combinations of extractant and disperser used for UV-filter extraction are chloroform/acetone (CHL/Ac) (Benedé et al., 2014b; Tarazona et al., 2010; Tovar-Sánchez et al., 2013) and tetrachloroethylene/Ac (Wu et al., 2013). Ionic liquid-based combinations like 1-hexyl-3-methylimidazolium tris(pentafluoroethyl) trifluorophosphate ([HMIM][FAP])/MeOH (Zhang and Lee, 2012b) or 1-butyl-3-methylimidazolium hexafluorophosphate [C_4MIM]PF_6/MeOH (Ye et al., 2011) are starting to be used, due to their unique physical and chemical properties, such as non-flammability, negligible vapor pressure, good extractability for a wide spectrum of inorganic, organic and organometallic compounds, as well as tunable viscosity and miscibility with water and organic solvents (Zhang and Lee, 2012a). This technique was tested in five classes of UV-filters (benzophenone, camphor, salicylate, crylene and *p*-aminobenzoic acid derivatives) and conducted to high recovery rates (70–118%), using different matrices like river, lake, sea and swimming pool waters (Benedé et al., 2014b; Wu et al., 2013; Tovar-Sánchez et al., 2013; Zhang et al., 2011; Tarazona et al., 2010).

Table 1.1 Overview on analytical methods and occurrence of UV-filters in natural, tap and swimming pool water matrices.

Matrix	Location	Compounds	Extraction method	Instrumental method	Rec. (%)	LOD (ng L^{-1})	Concentration (ng L^{-1})	Reference
River water	Brazil	BP3, EMC, ES, OC	Filtration (glass fiber filter) SPE (500 mL sample, 200 mg polymer-based sorbent cartridges, MeOH + EA)	GC-MS/MS	62 - 107	BP3: 7.1 EMC: 23.5 ES: 12.1 OC: 19.3	<LOD	da Silva et al. (2015)
River water	Bangkok	ODP, 4-MBC, BMDM, EMC, IMC, OC, BP3, ES, BP4, HMS, BP1, BP8	Addition of 5% (w/v) Na_2EDTA SPE (350 mL sample, 500 mg C_{18} cartridges, MeOH/EA (1:1))	HPLC-ESI-MS/MS	63 – 106	0.03 – 1.38	ODP: <LOD 4-MBC: <LOD BMDM: 36 –38 EMC: 88 – 95 IMC: <LOD OC: 153 – 205 BP3: 86 – 116 ES: 28 – 56 BP4: 80 – 95 HMS: 29 – 59 BP1: 127 - 166 BP8: 63 – 71	Tsui et al., (2014a)
River water	Spain	BP3, BP1, 4HB, 4DHB, BP8, BP2, BP4, 4-MBC, Et-PABA	Filtration On line-SPE (5 mL sample, PLRP-s polymer sorbent cartridge, H_2O + ACN, both with 0.1% formic acid)	LC-MS/MS	BP3: 97 - 100 BP1: 100 - 104 4HB: 81 - 84 4DHB: 82 - 83 BP8: 94 - 98 BP2: 90 - 91 BP4: 107 - 111 4-MBC: 100 - 102 Et-PABA: 111 - 113	BP3: 0.7 BP1: 1.0 4HB: 1.1 4DHB: 1.8 BP8: 1.0 BP2: 1.2 BP4: 0.5 4-MBC: 3.5 Et-PABA: 1.5	BP3: n.d. – 37.8 BP1: n.d. – 7.54 BP4: 30.4 - 862 4-MBC: n.d. – 12.6 4HB, 4DHB, BP8, BP2, Et-PABA: n.d.	Gago-Ferrero et al. (2013)
River water	Czech Republic	PBSA, BP4, BP3	Filtration (regenerated cellulose filters) In-line SPE-LC-MS/MS	LC/LC-MS/MS	PBSA: 95 BP4: 97 BP3: 95	LOQ PBSA:2.3 BP4: 1.8 BP3: 3.9	PBSA: 11 - 500 BP4: 4.6 - 390 BP3: 12 - 67	Grabicova et al. (2013)
River water (background sites)	Czech Republic	PBSA, BP4, BP3	Filtration (regenerated cellulose filters) In-line SPE-LC-MS/MS	LC/LC-MS/MS	PBSA: 95 BP4: 97 BP3: 95	LOQ PBSA:2.3 BP4: 1.8 BP3: 3.9	PBSA: 5.1 - 48 BP4: 3.4 - 37 BP3: 14 - 20	Grabicova et al. (2013)
River water	China	BP, BP3, 4PB	DI -SPME (10 mL of sample, MPTS-Ag wires (fiber),60 min, desorption with MeOH for 10 min with 200 mg/mL NaCl)	HPLC-PDA	BP: 94.1 – 102.4 BP3: 69.7 – 87.6 4PB: 82.2 – 92.7	BP: 580 BP3: 1030 4PB: 1860	n.d.	Li et al. (2013)
River water	Taiwan	ES, HMS, BP3, BP1, BP8	UA-DLLME (10 mL sample, 0.5 g NaCl, Ac (dispersant), TCE (extractant), 2 min) Derivatization (20 µL BSFTA)	GC-MS	ES: 70 HMS: 71 - 72 BP3: 78 - 83 BP1: 84 - 90 BP8: 86 - 93	ES: 2 HMS: 2 BP3:1.5 BP1: 1 BP8: 1	ES: n.d. – 10.6 BP3: 12.3 – 15.4 BP1: n.d. – 6.1 HMS, BP8: n.d.	Wu et al. (2013)
River water	Singapore	BP1, BP, BP3, 4-MBC	IL-USAEME (1.5 ml sample, pH 3 (0.1 mol L^{-1} HCl), [HMIM][FAP] (extractant), 12 min)	HPLC-UV	BP1: 98-1 – 102-7 BP: 96.9 – 102.2 BP3: 98.1 – 107.5 4-MBC: 96.4–104.2	BP1, BP3, 4-MBC: 1 BP: 0.5	n.d.	Ge and Lee (2012)

Table 1.1 Overview on analytical methods and occurrence of UV-filters in natural, tap and swimming pool water matrices. (cont).

Matrix	Location	Compounds	Extraction method	Instrumental method	Rec. (%)	LOD (ng L^{-1})	Concentration (ng L^{-1})	Reference
River water	Taiwan	BP3, BP1, BP8	SPE (100 mL sample, 60 mg HLB cartridge, EA, ACN, EA/DCM (1:1 and 2:1)). Derivatization (1 µL MSTFA, 70 °C, 2.5 min)	GC-MS/MS	BP3:72 BP1: 73 BP8: 67	BP3: 0.3 BP1: 0.5 BP8: 1.0	BP3: 3.0 BP1: 1.8 BP8: n.d.	Ho and Ding (2012)
River water	Singapore	BP, ES, HMS, BP3, 4-MBC,	Plunger-in-needle solid-phase microextraction (graphene sorbent, pH 5, 40 min, 25 °C, <1000 rpm, direct immersion mode) Silylation on-fiber (40 µL of MSTFA, 45 °C, 15 min)	GC-MS	BP: 114 ES: 109 HMS: 107 4-MBC: 99 BP3: 102	BP: 6.8 ES: 0.5 HMS: 0.5 4-MBC: 1.6 BP3: 0.7	n.d.	Zhang and Lee, (2012a)
River water	Singapore	BP, BP3, ES, HMS	IL-USA-DLLME (10 mL sample, [HMIM][FAP] (extractant), MeOH (dispersant), 3 min)	HPLC	BP: 94 - 118 BP3: 82 - 114 ES: 81 - 91 HMS: 81 - 86	BP: 200 BP3: 500 ES: 5000 HMS: 1000	n.d.	Zhang and Lee (2012b)
River water (heavily polluted)	Japan	BP3, BZS, 4-MBC, ODP, EMC, ES, HMS, OC, BP	Filtration (glass fiber filter) SPE (1.0 L sample, C_{18} cartridge + DSC-PH (monomerically bonded, phenyl - 7 %C)) cartridge, DCM).	GC-MS	80 – 113	0.1 – 3.0	BP: 21 - 68 BP3: n.d. - 4 EHMC: 125 - 1040 ES: n.d. - 38 BZS, 4-MBC, ODP, HMS, OC: n.d.	Kameda et al. (2011)
River water (moderately polluted)							BP: 2 - 43 BP3: 4 - 12 ODP: 1 -2 EHMC: 12 - 91 ES: n.d. - 6 HMS: n.d. - 22 OC: n.d. - 1 BZS, 4-MBC: n.d.	
River water (background sites)							BP: 1 - 57 BP3: 2 - 10 ODP: n.d. - 5 EHMC: n.d. - 18 OC: n.d. - 1 BZS, 4-MBC, ES, HMS: n.d.	
River water (streams receiving wastewater)							BP: 31 - 82 BP3: 16 - 41 BZS: 107 - 169 4-MBC: n.d. ODP: n.d. - 2 EHMC: 21 - 260 ES: n.d. - 266 HMS: n.d. - 29 OC: 6 - 14	

Table 1.1 Overview on analytical methods and occurrence of UV-filters in natural, tap and swimming pool water matrices. (cont).

Matrix	Location	Compounds	Extraction method	Instrumental method	Rec. (%)	LOD (ng L^{-1})	Concentration (ng L^{-1})	Reference
River water	Spain	ES, HMS, IMC, BP3, 4-MBC, EMC, EDP, OC	MNPs-based dSPE (75 mL sample, pH 3, 30% (w/v) NaCl, 100 mg $CoFe_2O_4$@oleic acid (optimized sorbent), 2 min US, 2 min vortex, 1 min in magnetization system; Hex, 2 min US, 2 min vortex) Derivatization (50 µL BSTFA)	GC-MS	ES, OC: 88 HMS: 74 IMC: 119 4-MBC, EMC: 106 BP3: 113 EDP: 95	ES, BP3: 0.2 HMS: 0.4 IMC: 6.0 4-MBC: 5.8 EDP: 3.1 EMC: 2.5 OC: 1.8	ES: 146 - 586 HMS: 342 - 712 IMC: <LOD - 595 4-MBC: 264 - 794 BP3: 428 - 993 EDP: 56 - 531 EMC: 240 - 770 OC: <LOD - 440	Román et al. (2011)
River water	China	BP3, BP1, BP, 4HB	IL-DLLME (10 µL sample, pH 2.63, 60 mg mL^{-1} NaCl, $[C_4MIM]PF_6$ (extractant solvent), MeOH (disperser solvent))	LC-UV	-	4HB:1900–2200 BP1:5700–6400 BP:800 – 1700 BP3:470 –5300	n.d.	Ye et al.(2011)
River water	Spain	BP8, BP3, OC, ODP	SBSE-LD (50 mL sample; stir bar coated with PDMS; ACN desorption)	UHPLC-MS/MS	BP8: 31 BP3: 67 OC: 59 ODP: 77	2.5	BP3: 6 - 28 BP8, OC, ODP: <LOD	Pedrouzo et al. (2010)
River water	Switzerland	BP4, BP3, 4-MBC, EMC	SPE (1.0 L sample, 500 mg Strata-X-CW polymer-based cartridge, MeOH/DCM)	GC-MS LC–MS/MS	-	-	BP4: n.d. BP3: 56 - 68 4-MBC: 12 - 17 EMC: 6	Fent et al.(2010)
River water (POCIS)			POCIS content washed with HPLC H_2O in empty SPE and dried for 45 min; MeOH and MeOH/TI/DCM for elution				BP4: n.d. - 2402 BP3: n.d. - 178 4-MBC: n.d. - 106 EMC: n.d. - 41	
River water	Spain	BP3, IMC, 4-MBC, OC, EDP, EMC	IL-SDME (20 mL sample with 1% EtOH (v/v), $[C_6MIM][PF_6]$ (extractant), 1300 rpm, 37 min, pH 2, room temperature)	LC-UV	BP3: 96 IMC: 97 4-MBC: 98 OC: 115 EDP: 96 EMC: 101	BP3: 110 IMC: 160 4-MBC: 60 OC: 3000 EDP: 70 EMC: 190	n.d.	Vidal et al. (2010)
River water	China	ES, BP3, 4-MBC, OC	SPME (3 mL sample, PDMS fiber, direct immersion, 90 min, 24 °C, desorption for 7 min at 280 °C)	GC-MS	ES: 75.4 - 112 BP3: 73.6 - 106 4-MBC: 72.5 - 115 OC: 100 - 114	ES: 0.5 BP3: 0.2 4-MBC: 1.3 OC: 2.0	ES: 8 – 5620 BP3:59 – 5390 4-MBC: 10 – 5790 OC: 29 – 5180	Liu et al. (2010)
			SPE (100 mL sample, 60 mg C_8 cartridges, Hex/DCM (6:4))		-	ES: 24.8 BP3: 85.2 4-MBC: 21.0 OC: 42.9	n.d.	
River water	Germany	BP1, BP2, BP3, BP4, PBSA	Filtration SPE (200-500 mL sample, 200 mg HLB cartridges, MeOH)	LC-MS/MS	BP1: 21 - 98 BP2: 6 - 53 BP3: 93 - 130 BP4: 69 - 81 PBSA: 57 - 100	LOQ BP1: 0.5 BP2: 0.5 BP3: 5 BP4: 1 PBSA: 1	BP1: 0.9 - 29 BP2: <LOQ – 6.7 BP3: <LOQ - 47 BP4: 51 - 1980 PBSA: 48 - 3240	Wick et al. (2010)

Table 1.1 Overview on analytical methods and occurrence of UV-filters in natural, tap and swimming pool water matrices. (cont).

Matrix	Location	Compounds	Extraction method	Instrumental method	Rec. (%)	LOD (ng L^{-1})	Concentration (ng L^{-1})	Reference
River water (upstream WWTP)	UK	BP1, BP2, BP3, BP4	Sample acidification (30% HCl to pH 2) Addition 500 mg Na_2EDTA Filtration SPE (1.0 L sample, 60 mg MCX mixed-mode polymeric sorbent cartridge, MeOH, 5% NH_4OH/MeOH)	UPLC-ESI-MS/MS	BP1: 99 BP2: 117 BP3: 97 BP4: 67	LOQ BP1: 0.3 BP2: 0.5 BP3: 15 BP4: 3	BP1: <LOQ - 17000 BP2: <LOQ – 1000 BP3: <LOQ - 43000 BP4: <LOQ - 144000	Kasprzyk-Hordern et al. (2009)
River water (downstream WWTP)							BP1: <LOQ - 13000 BP2: <LOQ - 26000 BP3: <LOQ - 44000 BP4: 32000 - 323000	
River water	Spain	ES, HMS, BP3, BP1, BP8	DI-SPME (10 mL sample, PDMS-DVB fiber, 20 °C, 1200 rpm) Derivatization (20 µL MSTFA, 45 °C, 10 min, headspace)	GC-MS/MS	ES: 110 HMS: 109 BP3: 108 BP1: 99 BP8: 97	LOQ ES: 5 HMS: 5 BP3: 0.5 BP1: 10 BP8: 2	ES: <LOQ BP3: 52 BP1: 37	Negreira et al. (2009)
River water	UK	BP1, BP2, BP3, BP4	Sample acidification (30% HCl to pH 2) Addition 500 mg Na_2EDTA Filtration SPE (1.0 L sample, 60 mg MCX mixed-mode polymeric sorbent cartridge, MeOH, 5% NH_4OH/MeOH)	UPLC-ESI-MS/MS	BP1: 99 BP2: 117 BP3: 97 BP4: 67	BP1, BP2: 0.1 BP3: 5 BP4: 1	BP1: 6 - 9 BP2: <0.5 BP3: 28 - 37 BP4: 10 - 227	Kasprzyk-Hordern et al.(2008a)
River water	UK	BP1, BP2, BP3, BP4	Sample acidification (30% HCl to pH 2) Addition 500 mg Na_2EDTA Filtration SPE (1.0 L sample, 60 mg MCX mixed-mode polymeric sorbent cartridge, MeOH, 5% NH_4OH/MeOH)		BP1: 109.9 BP2: 86.8 BP3: 124.7 BP4: 11.7	BP1: 0.1 BP2: 0.1 BP3: 10 BP4: 1.5	BP1: <0.3 BP2: <0.5 BP3: <30 – 220 BP4: <5	Kasprzyk-Hordern et al. (2008b)
River water	Japan	BP, BP1, BP3, BP10, 2HB, 3HB, 4HB	SBSE (10 mL sample, PDMS coated stir bar, extraction at room temperature, 120 min 1000 rpm, thermodesorption at 250 °C, 5 min) Derivatization (100 µL $C_4H_6O_3$)	TD-GC-MS	BP: 101.0 – 105.2 BP1: 102.0 – 128.0 BP3: 108.7 – 122.0 BP10: 115.3 –126.5 2HB: 101.6 – 117.3 3HB: 112.2 – 128.1 4HB: 114.9 – 125.8	BP: 0.5 BP1: 1 BP3: 0.5 BP10: 2 2HB: 0.5 3HB: 0.5 4HB: 1	BP: 23 BP3: 14 BP10: 12 3HB: 7 4HB: 6 BP1, 2HB: <LOQ	Kawaguchi et al. (2008)
River water	Germany	ES, HMS, IMC, BP3, 4-MBC, EMC, EDP, OC, BMDM	SBSE (20 mL sample, PDMS coated stir bar, extraction time of 3 h at room temperature, 1000 rpm, thermodesorption at 250 °C, 15 min)	TD-GC-MS	ES: 77 HMS: 84 IMC: 106 4-MBC, BP3: 112 EMC: 98 EDP: 87 OC: 89 BMDM: 101	ES, 4-MBC: 4 HMS: 1 IMC: 2 BP3: 11 EMC: 16 EDP: 0.2 OC: 7 BMDM: 63	HMS: <LOD - 5 4-MBC: 5 - 15 BP3: <LOD – 30 EMC: <LOD - 21 EDP: <LOD - 3 OC: <LOD - 16 ES, IMC, BMDM <LOD	Rodil and Moeder (2008a)

Table 1.1 Overview on analytical methods and occurrence of UV-filters in natural, tap and swimming pool water matrices. (cont).

Matrix	Location	Compounds	Extraction method	Instrumental method	Rec. (%)	LOD (ng L^{-1})	Concentration (ng L^{-1})	Reference
River water	Switzerland	EMC, 4-MBC, BP3, 3BC, BP1, BP2, BP3, BP4, Et-PABA	POCIS (exposed for 28 days; PES membranes, 200 mg of triphasic sorbent admixture (80:20 (w/w) ENV+ Ambersorb 572 (dispersed on S-X3 Bio Beads)) SPE (MeOH, MeOH/Tl/DCM (1:1:8))	LC-(ESI)-MS/MS	75 – 100	(ng/POCIS) BP4: 158 4DHB: 465 BP2: 199 Et-PABA: 178 BP1: 389 BP3: 252 3BC: 990 4-MBC: 693 EMC: 621	-	Zenker et al. (2008)
		EMC, 4-MBC, BP3, 3BC		GC-EI-MS/MS		(ng/POCIS) BP3: 77 3BC: 53 4-MBC: 43 EMC: 20	(pg/POCIS) BP3: 432 EMC: 21 3BC, 4-MBC: <LOD	
River water (upstream WWTP)	Switzerland	EMC, 4-MBC, BP3, 3BC	POCIS (exposed for 28 days; PES membranes, 200 mg of triphasic sorbent admixture (80:20 (w/w) ENV+ Ambersorb 572 (dispersed on S-X3 Bio Beads)) SPE (MeOH, MeOH/Tl/DCM (1:1:8))	GC-EI-MS/MS	75 – 100	(ng/POCIS) BP3: 77 3BC: 53 4-MBC: 43 EMC: 20	(ng/POCIS) BP3: 272 EMC: 18 3BC, 4-MBC: <LOD	Zenker et al. (2008)
River water (downstream WWTP)							(ng/POCIS) BP3: 1344 3BC: 96 4-MBC: 64 EMC: 27	
River water	South Korea	BP, BH, 4HB, BP3, BP1, BP8, 234THB	LLE (100 mL sample, EA) Derivatization (50 µL MSTFA, 80 °C, 30 min)	GC-MSD	BP: 77 - 100 BH: 62 - 97 4HB: 65 - 110 BP3: 90 - 113 BP1: 92 – 109 BP8: 76 - 114 234THB: 85 - 112	5 – 100	BP1: 47 BP, BH, 4HB, BP3, BP8: <LOQ 234THB: <LOD	Jeon et al. (2006)
River water (outflow)	Switzerland	4-MBC, BP3, EMC, OC	SPMDs (exposed for 3-6 weeks, depth 1-2 m) Dialysis (CYPN/DCM (95:5)) GPC (10 mL Bio-Beads SM-2, 20-50 mesh absorbent, DCM/CYHex (35:65)) SPE (silica column)	GC-MS	4-MBC: 72 BP3: 51 EMC: 110 OC: 73	(ng/SPMD) 4-MBC, OC: 5 BP3: 25 EMC: 100	(ng/SPMD) 4-MBC: 720 -820 BP3: 50 - 110 EMC: <LOD OC: 510 - 700	Balmer et al., (2005)
River water							(ng/SPMD) 4-MBC: 560 - 1250 BP3: <LOD - 50 EMC: 290 - 320 OC: 130 - 435	
Lake water	China	BP3, BP1, BP, 4HB	IL-DLLME (10 µL sample, pH 2.63, 60 mg mL^{-1} NaCl, $[C_4MIM]PF_6$ (extractant solvent), MeOH (disperser solvent))	LC-UV	-	4HB: 3700 BP1: 6000 BP: 600 BP3: 5300	n.d.	Ye et al. (2011)

Table 1.1 Overview on analytical methods and occurrence of UV-filters in natural, tap and swimming pool water matrices. (cont).

Matrix	Location	Compounds	Extraction method	Instrumental method	Rec. (%)	LOD (ng L^{-1})	Concentration (ng L^{-1})	Reference
Lake water	China	4HB, BP1, BP, BP3	DLLME (20 mL sample, 1-Oc (extractant), no dispersant)	HPLC-DAD	4HB: 92.2 BP1: 91.3 BP: 97.1 BP3: 94.2	4HB: 200 BP1: 400 BP: 500 BP3: 800	n.d.	Zhang et al. (2011)
Lake water	Germany	BP3, 4-MBC, OC, EMC	MEPS (sorbent C_8, MeOH, H_2O (conditioning); 8x 100 µL pump cycles (sample extraction); H_2O (sorbent wash); pressing air (sorbent dry); EA)	PTV-GC-MS	4-MBC: 61 BP3: 95 EMC: 114 OC: 71	4-MBC: 51 BP3: 44 EMC: 34 OC: 81	4-MBC: 2351 BP3: 83 EMC: 150 OC: 274	Moeder et al. (2010)
Lake water	Germany	BP3, IMC, 4-MBC, OC, BMDM, EDP, EMC, ES, HMS	MALLE (15 mL sample, MeOH; 20 mm-membrane bag attached, add 100 µL PrOH; shaking 500 rpm, 40 °C, 120 min)	LC-APPI-MS/MS	BP3: 60 - 78 IMC: 90 - 101 4-MBC: 90 - 97 OC: 80 - 92 BMDM: 76 - 94 EDP: 83 - 97 EMC: 98 - 103 ES: 95 - 104 HMS: 92 - 102	BP3: 0.8 IMC: 0.9 4-MBC: 1.7 OC: 8.5 BMDM: 10 EDP: 0.4 EMC: 16 ES, HMS: 4	BP3: 40 IMC: 146 4-MBC: 1140 BMDM: 2431 OC: 4381 EMC: 3009 ES: 748 EDP, HMS: <LOD	Rodil et al. (2009b)
Lake water	Germany	ES, HMS, IMC, BP3, 4-MBC, EMC, EDP, OC, BMDM	SBSE (20 mL sample, PDMS coated stir bar, extraction time of 3 h at room temperature, 1000 rpm, thermo desorption at 250 °C, 15 min)	TD-GC-MS	ES: 96 HMS: 109 IMC: 95 4-MBC: 100 BP3: 92 EMC: 107 EDP: 78 OC: 93 BMDM: 82	ES, 4-MBC: 4 HMS: 1 IMC: 2 BP3: 11 EMC: 16 EDP: 0.2 OC: 7 BMDM: 63	ES, IMC: <LOD - 51 HMS, EDP: <LOD - 5 4-MBC: <LOD - 148 BP3: <LOD - 55 EMC: <LOD - 33 OC: 10 - 250 BMDM: <LOD	Rodil and Moeder (2008a)
Lake water	South Korea	BP, BH, 4HB, BP3, BP1, BP8, 234THB	LLE (100 mL sample, EA) Derivatization (50 µL MSTFA, 80 °C, 30 min)	GC-MSD	BP: 77 - 100 BH: 62 - 97 4HB: 65 - 110 BP3: 90 - 113 BP1: 92 – 109 BP8: 76 - 114 234THB: 85 - 112	5 – 100	BP: <LOD BH: <LOQ 4HB: 85 BP3:<LOD BP1: <LOD BP8: <LOD 234THB: <LOD	Jeon et al. (2006)
Lake water	Stwitzerland	4-MBC, BP3, EMC, OC	GPC (1 L sample, 10 mL Bio-Beads SM-2, 20-50 mesh absorbent, MeOH/DCM) SPE (silica mini column, EA)	GC-MS	78-129	2	4-MBC: <LOD-28 BP3: <LOD - 35 EMC: <LOD - 7 OC: <LOD - 5	Balmer et al. (2005)
			SPMDs (exposed for 3-6 weeks, depth 1-2 m) Dialysis (CYPN/DCM (95:5)) GPC (10 mL Bio-Beads SM-2, 20-50 mesh absorbent, DCM/CYHex (35:65)) SPE (silica column)		4-MBC: 72 BP3: 51 EMC: 110 OC: 73	(ng/SPMD) 4-MBC, OC: 5 BP3: 25 EMC: 100	(ng/SPMD) 4-MBC: <LOD-2790 BP3, EMC: <LOD - 200 OC: <LOD - 320	

Table 1.1 Overview on analytical methods and occurrence of UV-filters in natural, tap and swimming pool water matrices. (cont).

Matrix	Location	Compounds	Extraction method	Instrumental method	Rec. (%)	LOD (ng L^{-1})	Concentration (ng L^{-1})	Reference
Lake water (midland)	Switzerland	BMDM, 4-MBC, EMC, TDSA, OC, EHT, PBSA, BP3, ES	SPE (1 L sample, 10 mL Bio-Beads SM-2, MeOH, DCM) Silica mini column clean-up (60 mm silica gel 60 + 10 mm Na_2SO_4 in a Pasteur pipette, EA)	GC-MS	MBC: 77 EMC: 90 OC: 57 BP3: 64 BMDM: 42	MBC, EMC, OC, BP3: 2 BMDM: 20	MBC: <LOD - 22 EMC: <LOD - 26 BP3: <LOD - 4 OC, BMDM: <LOD	Poiger et al. (2004)
Lake water (small bathing)							MBC: <LOD - 82 EMC: <LOD - 19 OC: <LOD - 27 BP3: 5 - 125 BMDM: <LOD - 24	
Lake water (midland)	Switzerland	BMDM, 4-MBC, EMC, TDSA, OC, EHT, PBSA, BP3, ES	SPMDs (exposed for 24 – 48 days, depth 1-2 m) Dialysis (CYPN) Purified with high resolution GPC	GC-MS	-	(ng/SPMD) 10	(ng/SPMD) MBC: 430 - 950 EMC: 140 - 360 OC: 85 - 380	Poiger et al. (2004)
Lake water (small mountain)							(ng/SPMD) MBC: <LOD EMC: 63 - 66 OC: <LOD	
Groundwater (urban)	Spain	BP1, BP2, BP3, BP4, 4DHB, 4HB, BP8, Et-PABA, 4-MBC	Filtration (glass fiber filters + nylon membrane filters) On-line SPE (5 mL sample, PLRP-s polymer sorbent cartridge, H_2O + ACN, both with 0.1% formic acid)	LC-MS/MS	-	-	BP1: n.d. – 1.9 BP3: 0.64 – 7.9 BP4: 1.1 – 3.8 4HB: n.d. – 0.38 4DHB: n.d. - 0.58 BP2, BP8, Et-PABA: n.d. 4-MBC: n.d. –6.7	Jurado et al. (2014)
Groundwater	Spain	BP3, BP1, 4HB, 4DHB, BP8, BP2, BP4, 4-MBC, Et-PABA	Filtration On line-SPE (5 mL sample, PLRP-s polymer sorbent cartridge, H_2O + ACN, both with 0.1% formic acid)	LC-MS/MS	BP3: 103 – 107 BP1: 98 - 104 4HB: 89 - 92 4DHB: 90 - 96 BP8: 93 - 100 BP2: 88 - 94 BP4: 110 - 114 4-MBC: 99 - 100 Et-PABA: 109 - 112	BP3: 0.5 Et-PABA, BP1, BP2: 1.0 4HB, BP8: 0.8 4DHB: 1.5 BP4: 0.3 4-MBC: 3.0	BP3: n.d. - 34 BP1: n.d. – 19.4 4HB: n.d. 4DHB: <LOQ BP8: n.d. BP2: n.d. BP4: n.d. – 36.6 4-MBC: <LOQ Et-PABA: n.d	Gago-Ferrero et al. (2013)
Groundwater	Taiwan	BP3, BP1, BP8	SPE (100 mL sample, 60 mg HLB cartridge, EA, ACN, EA/DCM (1:1 and 2:1)) Derivatization (1 µL MSTFA, 70 °C, 2.5 min)	GC-MS/MS	BP3: 82 BP1: 83 BP8: 73	BP3: 0.3 BP1: 0.5 BP8: 1.0	n.d.	Ho and Ding (2012)
Groundwater (well)	Germany	BP1, BP2, BP3, BP4, PBSA	Filtration SPE (200-500 mL sample, 200 mg HLB cartridges, MeOH)	LC-MS/MS	BP1: 69 - 114 BP2: 23 - 142 BP3: 95 - 105 BP4: 92 - 107 PBSA: 101 - 106	-	n.d.	Wick et al. (2010)

Table 1.1 Overview on analytical methods and occurrence of UV-filters in natural, tap and swimming pool water matrices. (cont).

Matrix	Location	Compounds	Extraction method	Instrumental method	Rec. (%)	LOD (ng L^{-1})	Concentration (ng L^{-1})	Reference
Seawater	Spain	BP3, IMC, 4-MBC, OC, EDP, EMC, ES, HMS	SBSE/ DμSPE (25 mL unfiltered samples, 100 mg cobalt ferrite bars coated with oleic acid nanoparticles, desorption with EtOH).	LC-UV	BP3: 84 - 116 IMC: 79 - 116 MBC: 96 - 120 OC: 98 - 103 EDP: 100 - 107 EMC: 97 - 107 ES: 83 - 95 HMS: 87 - 97	(ng mL^{-1}) BP3: 30.6 IMC: 2.4 MBC: 3.2 OC: 2.7 EDP: 3.0 EMC: 2.4 ES: 3.0 HMS:3.2	-	Benedé et al. (2014)
			DLLME (5 ml sample, CHL (extraction solvent), Ac (disperser solvent))	GC-MS	ES: 112 - 117 HMS: 88 - 97 IMC: 97 - 107 4-MBC: 82 - 88 BP3: 111 - 114 EMC: 87 - 99 EDP: 90 - 95 OC: 91 - 104	ES: 26 HMS: 14 IMC: 23 4-MBC: 10 BP3: 30 EMC: 14 EDP: 29 OC: 27	ES: 440 – 880 HMS: 157 - 310 IMC: 118 - 280 4-MBC: <LOD - 220 BP3: <LOQ - 308 EMC: 91 - 260 EDP: 163 - 390 OC: <LOQ - 317	
Seawater (surface)	Hong Kong	ODP, 4-MBC, BMDM, EMC, IMC, OC, BP3, ES, BP4, HMS, BP1, BP8	Addition of 5% (w/v) Na_2EDTA SPE (350 mL sample, 500 mg C_{18} cartridges, MeOH/EA (1:1))	HPLC-ESI-MS/MS	63 – 106	0.03 – 1.38	ODP: 95 – 182 4-MBC: 173 –379 BMDM: 24–721 EMC: 89 – 4043 IMC: 63 – 173 OC: 103 - 6812 BP3: 39 - 5429 ES: 61 – 1030 BP4: 54 – 389 HMS: 66 – 2812 BP1: 82 – 135 BP8: 64 – 117	Tsui et al. (2014a)
	Japan (Tokyo)						ODP, 4-MBC, IMC : <LOD BMDM: 78 – 104 EMC: 46 – 95 OC: 87 – 108 BP3: 24 – 86 ES: 71 - 95 BP4: 71 - 136 HMS: 65 - 110 BP1: 52 - 95 BP8: 76 - 96	

Table 1.1 Overview on analytical methods and occurrence of UV-filters in natural, tap and swimming pool water matrices. (cont).

Matrix	Location	Compounds	Extraction method	Instrumental method	Rec. (%)	LOD (ng L^{-1})	Concentration (ng L^{-1})	Reference
Seawater (surface)	USA (New York)	ODP, 4-MBC, BMDM, EMC, IMC, OC, BP3, ES, BP4, HMS, BP1, BP8	Addition of 5% (w/v) Na_2EDTA SPE (350 mL sample, 500 mg C_{18} cartridges, MeOH/EA (1:1))	HPLC-ESI-MS/MS	63 – 106	0.03 – 1.38	ODP, 4-MBC, IMC: <LOD BMDM: 70 - 87 EMC: 89 - 150 OC: 117 - 128 BP3: 23 - 178 ES: <LOD BP4: 89 - 574 HMS: 91 - 114 BP1: <LOD - 74 BP8: 72 – 92	Tsui et al. (2014a)
	USA (Los Angeles)						ODP, 4-MBC, IMC, BP4: <LOD BMDM: 67 - 109 EMC: 91 - 138 OC: 145 - 377 BP3: 227 - 601 ES: 53 - 120 HMS: 142 - 270 BP1: 100 - 117 BP8: 29 – 96	
	China (Shantou)						ODP, 4-MBC, IMC, ES, BP4, HMS, BP8: <LOD BMDM: 53 - 100 EMC: 52 - 78 OC: 75 - 107 BP3: 55 - 188 BP1: 22 - 58	
Seawater (surface)	China (Chaozhou)	ODP, 4-MBC, BMDM, EMC, IMC, OC, BP3, ES, BP4, HMS, BP1, BP8	Addition of 5% (w/v) Na_2EDTA SPE (350 mL sample, 500 mg C_{18} cartridges, MeOH/EA (1:1))	HPLC-ESI-MS/MS	63 – 106	0.03 – 1.38	ODP, 4-MBC, BMDM, IMC, HMS, BP1, BP8: <LOD EMC: <LOD - 79 OC: 36 - 102 BP3: 37 - 49 ES: 121 - 128 BP4: <LOD - 49	Tsui et al. (2014a)
	Artic						ODP, 4-MBC, IMC, ES, BP4, HMS: <LOD BMDM: 18 - 70 EMC: 25 - 66 OC: 26 - 31 BP3: 17 - 33 BP1: 2.5 - 5 BP8: 2 – 3.3	
Seawater (beach sites)	Japan	BP3, BZS, 4-MBC, ODP, EMC, ES, HMS, OC	Filtration (glass fiber filter) SPE (1 L sample, C_{18} cartridge + DSC-PH cartridge (monomerically bonded, phenyl - 7 %C), DCM)	GC-MS	80 - 113	0.1 – 3.0	BP3: n.d. - 1258 ODP: n.d. – 4.1 EMC: n.d. - 143 ES: n.d. - 10 HMS: n.d. - 214 OC: n.d. - 79 BZS, 4-MBC: n.d.	Tashiro and Kameda (2013)

Table 1.1 Overview on analytical methods and occurrence of UV-filters in natural, tap and swimming pool water matrices. (cont).

Matrix	Location	Compounds	Extraction method	Instrumental method	Rec. (%)	LOD (ng L^{-1})	Concentration (ng L^{-1})	Reference
Seawater (river and reef sites)	Japan	BP3, BZS, 4-MBC, ODP, EMC, ES, HMS, OC	Filtration (glass fiber filter) SPE (1 L sample, C_{18} cartridge + DSC-PH cartridge (monomerically bonded, phenyl - 7 %C), DCM)	GC-MS	80 - 113	0.1 – 3.0	BP3: n.d. – 9.0 ODP, BZS, 4-MBC: n.d. EMC: n.d. – 3.9 ES: n.d. – 1.8 HMS: n.d. – 3.2 OC: n.d. – 8.1	Tashiro and Kameda (2013)
Seawater	Spain	BP3, 4-MBC	DLLME (10 mL sample, Ac (dispersant), CHL (extractant))	GC-MS	-	-	BP3: 15.8 – 314.8 4-MBC: 26.6 – 109.6	Tovar-Sánchez et al. (2013)
Seawater	Italy	BP3, OC, EDP, EMC, ES, HMS	SBSE-LD (10 mL sample, stir bar pre-conditioned in MeOH; desorption in MeOH, 30 min, room temperature)	LC-MS	71 – 100	BP3: 0.08 OC: 0.20 EDP: 0.01 EMC: 0.07 HMS: 1.70 ES: 2.65	BP3: <LOQ - 118 EMC: <LOQ - 83	Nguyen et al. (2011)
Seawater	Spain	ES, HMS, IMC, 4-MBC, BP3, EMC, EDP, OC	MNPs-based dSPE (75 mL sample, pH 3, 30% (w/v) NaCl, 100 mg $CoFe_2O_4$ in oleic acid (optimized sorbent), 2 min US, 2 min vortex, 1 min in magnetization system; Hex, 2 min US, 2 min vortex) Derivatization (50 µL BSTFA)	GC-MS	ES: 86 HMS: 81 IMC, 4-MBC: 80 BP3: 125 EDP: 73 EMC: 101 OC: 88	ES, BP3: 0.2 HMS: 0.4 IMC: 6.0 4-MBC: 5.8 EDP: 3.1 EMC: 2.5 OC: 1.8	ES: 792 - 1222 HMS: 625 - 1030 IMC: 245 - 645 4-MBC: 358 - 758 BP3: 254 - 879 EDP: 409 - 774 EMC: 682 - 1187 OC: <LOQ - 440	Román et al. (2011)
Seawater	Spain	BP3, BP1, BP8, 234THB	DLLME (5 mL sample, Ac (disperser solvent), CHL (extraction solvent)) Derivatization (60 µL BSTFA, 75 °C, 30 min)	GC-MS	BP3: 82 - 126 BP1: 65 - 169 BP8: 99 - 120 234THB: 82 - 222	BP3, BP8: 33 BP1: 32 234THB: 50	BP3: 1340 - 3300 BP1: n.d. - 280 BP8, 234THB: n.d.	Tarazona et al. (2010)
Seawater	Spain	BP3, IMC, 4-MBC, OC, EDP, EMC	IL-SDME (20 mL sample with 1% EtOH (v/v), 10 µL $[C_6MIM][PF_6]$ (extractant), 1300 rpm, no NaCl, 37 min, pH 2, room temperature)	LC-UV	BP3: 99 IMC, OC, EDP: 92 4-MBC: 96 EMC: 107	BP3: 110 IMC: 160 4-MBC: 60 OC: 3000 EDP: 70 EMC: 190	n.d.	Vidal et al. (2010)
Seawater	Pacific Ocean	*E*-EMC, *Z*-EMC, BP3, 4-MBC, 3BC	SPMDs (exposed for 3-6 weeks) Dialysis (CYPN, 18 °C, 24 h)	RP-HPLC	-	(pg/SPMD) 150 – 510	(pg/SPMD) *E*-EMC: 11464 - 27058 *Z*-EMC: 3432 - 8484 4-MBC, 3BC: < LOD BP3: <LOD - 34310	Goksoyr et al. (2009)
Seawater (surface microlayer)	Pacific Ocean	BP1, BP2, BP3, BP4	SPE (100 mL sample, 225 mg HLB cartridge, EtOH, DCM)	GC-MS	-	1 – 5	E-EMC: 7 - 55 Z-EMC: 6 – 37 4-MBC: 18 - 30 BP3: 5 - 6 3BC: 9 - 13	Goksoyr et al. (2009)

Table 1.1 Overview on analytical methods and occurrence of UV-filters in natural, tap and swimming pool water matrices. (cont).

Matrix	Location	Compounds	Extraction method	Instrumental method	Rec. (%)	LOD (ng L^{-1})	Concentration (ng L^{-1})	Reference
Seawater	Greece	PBSA, BP3, 4-MBC, BMDM, EMC	Filtration Micelle mediated extraction (50-100 mL sample, pH 3 (HCl), Na_2SO_4, Triton X-114 (ionic surfactant), 15 min, 60 ºC; re-extraction: MeOH, Hex, US (3 min); upper phase (Hex) analysed)	LC-UV-DAD	PBSA: 98.0 – 99.4 BP3: 98.8 – 99.0 4-MBC: 98.3 – 100.0 BMDM: 96.5 – 98.0 EMC: 97.4 – 98.7	PBSA: 300 BP3: 450 4-MBC: 140 BMDM: 1270 EMC: 560	n.d.	Giokas et al. (2005)
				GC-MS	-	BP3: 6.2 4-MBC: 30.0 EMC: 2.2	BP3: 6.5 – 8.2 4-MBC: 13.1 – 19.7 EMC: 7.4 – 10.7	
Sea water	Greece	BP3, 4-MBC, BMDM, EMC	Filtration (5 min) SPE (500 mL sample, 500 mg C_{18} disks, EA/DCM (1:1); extract reconstitution: MeOH (LC), Hex (GC))	LC-UV-DAD [a] GC-MS [b]	BP3: 95 4-MBC: 96 BMDM: 87[a] EMC: 93	LOQ [a] BP3: 14 4-MBC: 8 BMDM: 24 EMC: 13 LOQ [b] BP3: 1.4 4-MBC: 0.7 BMDM: - EMC: 0.9	[a] BMDM: n.d. [b] BP3: 1.8 4-MBC: <LOD EMC: n.d.	Giokas et al. (2004)
Seawater	Greece	BP3, 4-MBC, BMDM, EMC	DI-SPME (5 mL sample, 100 µm PDMS fibre, 45 min, 960 rpm at room temperature, desorption for 8 min at 240 ºC)	GC-FID [a] GC-MS [b]	BP3:97 ODP: 85	BP3 [a]: 1700 ODP [a] : 870 BP3 [b]: 2470 ODP [b]: 1200	n.d.	Lambropoulou et al. (2002)
			DI-SPME (5 mL sample, 85 µm PA fibre, 45 min, 960 rpm at room temperature, desorption for 8 min at 240 ºC)		BP3: 99 ODP: 82	BP3 [a]: 1170 ODP [a]: 2470 BP3 [b]: 1700 ODP [b]: 2930	-	
Seawater	Greece	BP3, 4-MBC, BMDM, EMC	HS-SPME (100 µm PDMS fibre, 25% g L^{-1} NaCl, 45 min, 90 ºC, desorption for 8 min at 240 ºC)	GC-FID [a] GC-MS [b]	BP3: 91 ODP: 98	BP3[a]: 4100 ODP [a]: 600 BP3[b]: 4430 ODP [b]: 730	-	Lambropoulou et al. (2002)
			HS-SPME (100 µm PDMS fibre, 25% g L^{-1} NaCl, 45 min, 90 ºC, desorption for 8 min at 240 ºC)		BP3: 94 ODP: 95	BP3 [a]: 2530 ODP [a]: 2230 BP3 [b]: 2900 ODP [b]: 3030	-	
Seawater	Greece	BP3, 4-MBC, BMDM, EMC	SPE (10 mL sample, 500 mg C cartridges, DCM)	GC-FID [a] GC-MS [b]	BP3: 94 ODP: 93	BP3 [a]: 3330 ODP [a]: 270 BP3 [b]: 7330 ODP [b]: 8330	-	Lambropoulou et al. (2002)
Tap water	Singapore	BP1, BP3, ES, HMS	TC-IL-DLPME (10 mL sample, [HMIM][FAP] (extractant), 50 ºC until dissolution)	HPLC-UV	BP1: 99 BP3: 97 - 99 ES: 91 - 100 HMS: 96 - 104	BP1: 300 BP3: 800 ES: 5000 HMS: 1000	-	Zhang and Lee (2013)
Tap water	Singapore	BP1, BP, BP3, 4-MBC	IL-USAEME (1.5 ml sample, pH 3 (0.1 mol L^{-1} HCl), [HMIM][FAP] (extractant), 12 min US)	HPLC-UV	BP1: 95.7 – 101.9 BP: 101.1 – 105.1 BP3: 100.4 – 104.9 4-MBC: 98.9 – 100.7	BP1, BP3, 4-MBC: 1 BP: 0.5	n.d.	Ge and Lee (2012)

Table 1.1 Overview on analytical methods and occurrence of UV-filters in natural, tap and swimming pool water matrices. (cont).

Matrix	Location	Compounds	Extraction method	Instrumental method	Rec. (%)	LOD (ng L^{-1})	Concentration (ng L^{-1})	Reference
Tap water	Spain	4-MBC, PBSA, BP3, EMC, OC, ODP, BP4, IMC	Filtration SPE (200-500 mL sample, 200 mg HLB cartridges, MeOH)	LC-ESI-MS/MS	-	-	10000 - 62000	Rodil et al. (2012)
Tap water	Singapore	BP, BP3, ES, HMS	IL-USA-DLLME (10 mL sample, [HMIM][FAP] (extractant), MeOH (dispersant))	HPLC	BP: 91 - 117 BP3: 82 - 105 ES: 82 - 93 HMS: 81 - 87	BP: 200 BP3: 500 ES: 5000 HMS: 1000	n.d.	Zhang and Lee (2012b)
Tap water	Australia	BP3, 4-MBC, EMC, UV-326, UV-329, OC	Filtration (1 L sample, glass fiber filters) Addition of MeOH - pH 2 (4 M H_2SO_4) SPE (1 L sample, 500 mg HLB cartridges, MeOH/DCM (1:1)) Filtration	GC-MS/MS	BP3: 117 - 150 4-MBC: 83 - 126 EMC: 79 - 96 UV-326: 89 - 102 UV-329: 97 - 110 OC: 71 - 88	BP3: 4.3 4-MBC, EMC: 0.3 UV-326: 1.5 UV-329: 5.6 OC: 1.9	< LOQ	Liu et al. (2011)
Tap water	Spain	ES, HMS, IMC, BP3, 4-MBC, EMC, EDP, OC	MNPs-based dSPE (75 mL sample, pH 3, 30% (w/v) NaCl, 100 mg $CoFe_2O_4$ in oleic acid (optimized sorbent), 2 min US, 2 min vortex, 1 min in magnetization system; Hex, 2 min US, 2 min vortex) Derivatization (50 µL BSTFA)	GC-MS	ES: 91 HMS: 103 IMC: 63 4-MBC: 101 BP3: 90 EDP: 99 EMC: 86 OC: 110	ES, BP3: 0.2 HMS: 0.4 IMC: 6.0 4-MBC: 5.8 EDP: 3.1 EMC: 2.5 OC: 1.8	ES: 160 - 615 HMS: <LOD - 515 IMC: 65 - 315 4-MBC: <LOQ - 505 BP3: <LOQ - 450 EDP: 126 - 621 EMC: <LOD - 430 OC: <LOQ - 550	Román et al. (2011)
Tap water	Spain	BP3, BP1	SPE (500 mL sample, 60 mg HLB cartridge, EA) Derivatization (20 µL MTB-STFA)	GC-MS	BP3: 98 BP1: 89	LOQ BP1, BP3: 8	-	Negreira et al. (2008)
		ES, EDP	SPE (500 mL sample, 60 mg HLB cartridge, EA) Derivatization (20 µL MTB-STFA)		ES: 84 EDP: 82	LOQ ES: 24 EDP: 25	-	
Swimming pool water (outdoor)	Czech Republic	PBSA, BP4, BP3	Filtration (regenerated cellulose filters) In-line SPE-LC-MS/MS	LC/LC-MS/MS	PBSA, BP3: 95 BP4: 97	LOQ PBSA: 2.3 BP4: 1.8 BP3: 3.9	PBSA: 240 -13000 BP4: 3.3 - 35 BP3: 26 - 620	Grabicova et al. (2013)
Swimming pool water	Singapore	BP1, BP3, ES, HMS	TC-IL-DLPME (10 mL sample, [HMIM][FAP] (extractant), 50 ºC until dissolution)	HPLC-UV	BP1: 88 - 102 BP3: 90 - 100 ES: 110 - 111 HMS: 109 - 116	BP1: 300 BP3: 800 ES: 5000 HMS: 1000	-	Zhang and Lee (2013)
Swimming pool water	Singapore	BP, BP3, ES, HMS	IL-USA-DLLME (10 mL sample, [HMIM][FAP] (extractant), MeOH (dispersant))	HPLC	BP: 98 - 117 BP3: 87 - 106 ES: 71 - 75 HMS: 71 - 77	BP: 200 BP3: 500 ES: 5000 HMS: 1000	n.d.	Zhang and Lee (2012b)
Swimming pool (seawater)	Italy	BP3, OC, EDP, EMC, ES, HMS	SBSE-LD (10 mL sample; desorption in MeOH, 30 min, room temperature)	LC-MS	71 – 100	BP3: 0.08 OC: 0.20 EDP: 0.01 EMC: 0.07 HMS: 1.70 ES: 2.65	BP3: 25 - 216 EMC: 53 - 86	Nguyen et al. (2011)

Table 1.1 Overview on analytical methods and occurrence of UV-filters in natural, tap and swimming pool water matrices. (cont).

Matrix	Location	Compounds	Extraction method	Instrumental method	Rec. (%)	LOD (ng L^{-1})	Concentration (ng L^{-1})	Reference
Swimming pool	China	BP3, BP1, BP, 4HB	IL-DLLME (10 µL sample, pH 2.63, 60 mg mL^{-1} NaCl, $[C_4MIM]PF_6$ (extractant solvent), MeOH (disperser solvent))	LC-UV	-	-	4HB: 15400 BP1: 8700 BP: 18800 BP3: 4500	Ye et al. (2011)
Swimming pool water	Spain	BP3, IMC, 4-MBC, OC, EDP, EMC	IL-SDME (20 mL sample with 1% EtOH (v/v), 10 µL $[C_6MIM][PF_6]$ (extractant), 1300 rpm, 37 min, pH 2, room temperature)	LC-UV	BP3: 99 IMC: 100 4-MBC: 100 OC: 110 EDP: 103 EMC: 110	BP3: 110 IMC: 160 4-MBC: 60 OC: 3000 EDP: 70 EMC: 190	IMC: 700 4-MBC: <LOQ	Vidal et al., (2010)
Swimming pool water	Greece	BP3, 4-MBC, BMDM, EMC	Filtration (5 min) SPE (500 mL sample, 500 mg C_{18} disks, EA/DCM (1:1); extract reconstitution: MeOH (LC), Hex (GC))	LC-UV-DAD [a] GC-MS[b]	BP3: 97 4-MBC: 99 BMDM: 88 [a] EMC: 96	LOQ [a] BP3: 14 4-MBC: 8 BMDM: 24 EMC: 13 LOQ [b] BP3: 1.4 4-MBC: 0.7 BMDM: - EMC: 0.9	[a] BMDM: n.d. [b] BP3: 4.2 4-MBC: 6.9 EMC: 4.5	Giokas et al. (2004)
Swimming pool water (game pool)	Greece	BP3, 4-MBC, BMDM, EMC	Filtration (5 min) SPE (500 mL sample, 500 mg C_{18} disks, EA/DCM (1:1); extract reconstitution: MeOH (LC), Hex (GC))	LC-UV-DAD [a] GC-MS [b]	-	LOQ [a] BP3: 14 4-MBC: 8 BMDM: 24 EMC: 13 LOQ [b] BP3: 1.4 4-MBC: 0.7 BMDM: - EMC: 0.9	[a] BMDM: n.d. [b] BP3: 5.7 4-MBC: 5.4 EMC: 3.0	Giokas et al. (2004)
Swimming pool water	Greece	BP3, 4-MBC, BMDM, EMC	DI-SPME (5 mL sample, 85 µm PA fiber, 45 min, 960 rpm at room temperature, desorption for 8 min at 240 °C)	GC-FID [a] GC-MS [b]	BP3: 95 ODP: 94	BP3 [a]: 1700 ODP [a]: 870 BP3 [b]: 2470 ODP [b]: 1200	BP3: 2400 – 3300 ODP: n.d.	Lambropoulou et al. (2002)
Swimming pool water	Greece	BP3, 4-MBC, BMDM, EMC	DI-SPME (5 mL sample, 85 µm PA fiber, 45 min, 960 rpm at room temperature, desorption for 8 min at 240 °C)	GC-FID [a] GC-MS [b]	BP3: 99 ODP: 98	BP3[a]: 1170 ODP [a]: 2470 BP3[b]: 1700 ODP [b]: 2930	-	Lambropoulou et al. (2002)
Swimming pool water	Greece	BP3, 4-MBC, BMDM, EMC	HS-SPME (100 µm PDMS fiber, 25% g L^{-1} NaCl, 45 min, 90 °C, desorption for 8 min at 240 °C)	GC-FID [a] GC-MS [b]	BP3: 89 ODP: 97	BP3 [a]: 4100 ODP [a]: 600 BP3 [b]: 4430 ODP [b]: 730	-	Lambropoulou et al. (2002)

Table 1.1 Overview on analytical methods and occurrence of UV-filters in natural, tap and swimming pool water matrices. (cont).

Matrix	Location	Compounds	Extraction method	Instrumental method	Rec. (%)	LOD (ng L^{-1})	Concentration (ng L^{-1})	Reference
Swimming pool water	Greece	BP3, 4-MBC, BMDM, EMC	HS-SPME (100 µm PDMS fiber, 25% g L^{-1} NaCl, 45 min, 90 °C, desorption for 8 min at 240 °C)	GC-FID [a] GC-MS [b]	BP3: 90 ODP: 95	BP3 [a]: 2530 ODP [a]: 2230 BP3 [b]: 2900 ODP [b]: 3030	-	Lambropoulou et al. (2002)
Swimming pool water	Greece	BP3, 4-MBC, BMDM, EMC	SPE (10 mL sample, 500-mg C cartridges, DCM)	GC-FID [a] GC-MS [b]	BP3: 97 ODP: 95	BP3 [a]: 3330 ODP [a]: 270 BP3 [b]: 7330 ODP [b]: 8330	-	Lambropoulou et al. (2002)
Recreational ponds water	Czech Republic	PBSA, BP4, BP3	Filtration (regenerated cellulose filters) In-line SPE-LC-MS/MS	LC/LC-MS/MS	PBSA: 95 BP4: 97 BP3: 95	LOQ PBSA: 2.3 BP4: 1.8 BP3: 3.9	PBSA: 24 - 930 BP4: 4.0 - 46 BP3: 21 - 550	Grabicova et al. (2013)
Run-off water	Nigeria	EMC, OC	Filtration (glass fiber filter) SPE (820 mL sample, HLB cartridge, EA)	GC-MS	-	-	EMC: n.d. OC: 3	Arukwe et al. (2012)
Shower wastes water	Greece	BP3, 4-MBC, BMDM, EMC	Filtration (5 min) SPE (500 mL sample, 500 mg C_{18} disks, EA/DCM (1:1); extract reconstitution: MeOH (LC), Hex (GC))	LC-UV-DAD [a] GC-MS [b]	BP3: 97 4-MBC: 95 BMDM [a]: 86 EMC: 92	LOQ [a] BP3: 14 4-MBC: 8 BMDM: 24 EMC: 13 LOQ [b] BP3: 1.4 4-MBC: 0.7 BMDM: - EMC: 0.9	[a] BMDM: n.d. [b] BP3: 10.0 4-MBC: 3.8 EMC: 4.1	Giokas et al. (2004)
Shower wastes water	Greece	BP3, 4-MBC, BMDM, EMC	DI-SPME (5 mL sample, 85 µm PA fiber, 45 min, 960 rpm at room temperature, desorption for 8 min at 240 °C)	GC-FID [a] GC-MS [b]	-	BP3 [a]: 1700 ODP [a]: 870 BP3 [b]: 2470 ODP [b]: 1200	BP3: 8200 – 9900 ODP: 5300 – 6200	Lambropoulou et al. (2002)
Pure water	Germany	ES, HMS, IMC, BP3, 4-MBC, EMC, EDP, OC, BMDM	SBSE (20 mL sample, PDMS coated stir bar, extraction time of 3 h at room temperature, 1000 rpm, thermodesorption at 250 °C, 15 min)	TD-GC-MS	ES: 90 HMS: 93 IMC: 111 4-MBC: 123 BP3: 107 EMC: 125 EDP: 108 OC: 106 BMDM: 114	ES, 4-MBC: 4 HMS: 1 IMC: 2 BP3: 11 EMC: 16 EDP: 0.2 OC: 7 BMDM: 63	=	Rodil and Moeder (2008a)
Ultrapure water	UK	BP1, BP2, BP3, BP4	Sample acidification with 30% HCl to pH 2 and addition of 500 mg Na_2EDTA Filtration SPE (1.0 L sample, Oasis MCX mixed-mode polymeric sorbent cartridge, MeOH, 5% NH_4OH/MeOH)	UPLC-ESI-MS/MS	BP1: 109.6 BP2: 114.4 BP3: 79.7 BP4: 16.0	BP1, BP2: 0.1 BP3: 10 BP4: 1.5	-	Kasprzyk-Hordern et al. (2008b)

Table 1.2 Overview on analytical methods and occurrence of UV-filters in sediments and soils.

Matrix	Location	Compounds	Extraction method	Analysis method	Rec. (%)	LOD (ng g^{-1} dw)	Concentration (ng g^{-1} dw)	Reference
River sediments (estuarine)	Chile	BP3, 4-MBC, OC, EMC, ODP, 4HB, BP1, 4DHB	SPLE (1 g sample, 1 g Al_2O_3, MeOH) Filtration (syringe filter)	UPLC-MS/MS	4- MBC: 89 OC: 85 EMC: 90 ODP, 4DHB: 120 BP3: 125 BP1: 58 4HB: 80	4- MBC: 1.1 OC: 9.9 EMC:4.1 ODP, 4HB: 0.7 BP3: 0.4 BP1: 4.6 4DHB: 0.8	BP3: n.d. - 2.96 4-MBC, EMC: n.d.	Barón et al. (2013)
River sediments (estuarine)	Colombia	BP3, 4-MBC, OC, EMC, ODP, 4HB, BP1, 4DHB	SPLE (1 g sample, 1 g Al_2O_3, MeOH) Filtration (syringe filter)	UPLC-MS/MS	4- MBC: 89 OC: 85 EMC: 90 ODP, 4DHB: 120 BP3: 125 BP1: 58 4HB: 80	4- MBC: 1.1 OC: 9.9 EMC:4.1 ODP, 4HB: 0.7 BP3: 0.4 BP1: 4.6 4DHB: 0.8	BP3: n.d. – 5.38 4-MBC: n.d. – 17.2 EMC: n.d. – 47.1	Barón et al. (2013)
River sediments	Lebanon	EMC, OC, ODP	MAE (5 g sample, Ac/Hep (1:1), 115 °C, 15 min)	GC-MS/MS	EMC: 99-113 OC: 97 – 115 ODP: 98-104	EMC: 1.5 OC: 2.0 ODP: 1.5	EMC: 35.8 OC: 90.0 ODP: 11.0	Amine et al. (2012)
River sediments	Spain	4DHB, 4HB, BP1, BP3, 4-MBC, OC, EMC, ODP	ASE (1 g sample, 1 g Al_2O_3, 2 x MeOH + 2x MeOH/H_2O (1:1)) Filtration (syringe filter)	UPLC-MS/MS	4-MBC: 89 OC: 85 EMC: 90 ODP, 4DHB: 120 BP3: 125 BP1: 58 4HB: 80	4-MBC: 8.0 OC: 2.2 EMC: 1.6 ODP: 0.5 BP3: 0.8 BP1: 15.5 4HB, 4DHB: 2.4	OC: n.d. - 2400 EMC: n.d. - 42 ODP: n.d. – 5.2 BP3: n.d. - 27 4HB: n.d. - 21 4DHB, BP1, 4-MBC: n.d.	Gago-Ferrero et al. (2011)
River sediments (heavily polluted river)	Japan	BP3, BZS, 4-MBC, ODPABA, EHMC, OS, HMS, OC, BP	Freeze-drying USE (4 g sample, 2 x DCM + 2 x Ac, 40 min) Centrifugation (3000 rpm, 10 min) SPE (5 g florisil cartridge, Hex -1st, Hex/Ac (19:1) - 2nd, Hex/Ac (1:1) - 3rd) SPE (3rd fraction into graphite column, Ac/Tol (70:30)) SPE (2nd and 4th fraction combined into NH_2 cartridge, Hex + Ac/Hex (4:96))	GC-MS	70 - 125	0.05 – 1.00	BP3, BZS, 4-MBC, ODP: n.d. EMC: 2.2 – 9.6 HMS: 0.8 – 6 OC: 2.7 - 50	Kameda et al. (2011)
River sediments (moderately polluted river)							BP3, BZS, 4-MBC, ODP: n.d. EMC: 3.8 – 30 HMS: 0.5 – 0.8 OC: 0.4 – 8.1	
River sediments (background sites)							BP3, BZS, 4-MBC, ODP, HMS: n.d. EMC: 2.0 – 8.0 OC: 1.0 - 12	
River sediments	Spain	4HB, BP1, BP3, BP8, BP6, ES, HMS	SPE (2 g sample, 1.5 g C_{18} + 1 g Na_2SO_4, 2x EA/MeOH (9:1), 15 min USE)	GC-MS/MS	4HB: 102-105.3 BP1: 94.3-101.9 BP3: 98.9-101.3 BP8: 88.4-91.4 BP6: 89.9-92.4 ES: 99.4-102 HMS: 97.4-101.3	4HB: 0.23 BP1: 0.21 BP3: 0.28 BP8: 0.14 BP6: 0.15 ES: 0.11 HMS:0.12	4HB, BP1, BP3, BP8, HMS: n.d. BP6: 6.1 ES: 20	Sánchez-Brunete et al. (2011)

Table 1.2 Overview on analytical methods and occurrence of UV-filters in sediments and soils. (cont.).

Matrix	Location	Compounds	Extraction method	Analysis method	Rec. (%)	LOD (ng g^{-1} dw)	Concentration (ng g^{-1} dw)	Reference
River sediments	Korea	BP, BH, 4HB, BP3, BP1, BP8, 234THB	SLE (10 g sample, 10 g Na_2SO_4, MeOH, 20 min) Centrifugation (1660 x g, 15 min) Evaporation + 5% NaCl solution + EA Derivatization (50 µL MSTFA, 80 °C, 30 min)	GC-MS	BP: 102-125 BH:76-111 4HB:73-115 BP3:71-81 BP1:62-84 BP8:60-78 234THB: 81-92	0.1	BP:1520-9730 BH:530 4HB:18380 BP1:500-2140 BP3, BP8, 234THB: n.d.	Jeon et al. (2006)
River sediments	Germany	BP, 4-MBC	SEHSDT (200-400 g sample, Ac + Ac/Hex (1:1) + Hex) Centrifugation (4000 rpm, 5 min per 20 g) USE (organic layer, Na_2SO_4, copper powder) Filtration	GC-MS	BP:90 4-MBC: 75	-	BP: n.d.- 4 4-MBC: n.d.- 4	Ricking et al. (2003)
Lake sediments	Germany	ES, HMS, IMC, 4-MBC, BP3, EMC, ODP, OC	PLE (4-5 g sample, 2 g silica gel, 2 g copper powder, 1 g Na_2SO_4, 4x 5 min, 160 °C, 100 bar, EA/Hex (80:20)) Derivatization (50 µL BSTFA, 1 h)	GC-MS	ES: 99 HMS: 98-103 IMC: 113-128 4-MBC: 108-111 BP3: 88-98 EMC: 97-121 ODP: 97-121 OC: 73-120	ES:2-20 HMS: 3-55 IMC:4-21 4-MBC: 6-30 BP3: 1-51 EMC: 5-12 ODP: 2-14 OC:2-15	ES, HMS, IMC, 4-MBC, BP3, ODP: n.d. EMC: 14-34 OC: 61-93	Rodil et al. (2008)
Coastal sediments	Chile	BP3, 4-MBC, OC, EMC, ODP, 4HB, BP1, 4DHB	SPLE (1 g sample, 1 g Al_2O_3, MeOH) Filtration (syringe filter)	UPLC-MS/MS	4- MBC: 89 OC: 85 EMC: 90 ODP, 4DHB: 120 BP3: 125 BP1: 58 4HB: 80	4- MBC: 1.1 OC: 9.9 EMC:4.1 ODP, 4HB: 0.7 BP3: 0.4 BP1: 4.6 4DHB: 0.8	BP3: n.d. - 1.42 4-MBC, EMC: n.d.	Barón et al. (2013)
Coastal sediments	Colombia	BP3, 4-MBC, OC, EMC, ODP, 4HB, BP1, 4DHB	SPLE (1 g sample, 1 g Al_2O_3, MeOH) Filtration (syringe filter)	UPLC-MS/MS	4- MBC: 89 OC: 85 EMC: 90 ODP, 4DHB: 120 BP3: 125 BP1: 58 4HB: 80	4- MBC: 1.1 OC: 9.9 EMC:4.1 ODP, 4HB: 0.7 BP3: 0.4 BP1: 4.6 4DHB: 0.8	BP3: n.d. – 2.52 4-MBC: n.d. – 7.90 EMC: n.d. – 17.8	Barón et al. (2013)
Coastal sediments	Lebanon	EMC, OC, ODP	MAE (5 g sample, Ac/Hep (1:1), 115 °C, 15 min)	GC-MS/MS	EMC: 99-113 OC: 97 – 115 ODP: 98-104	EMC: 1.5 OC: 2.0 ODP: 1.5	EMC: 9.0 OC: 79.0 ODP: 9.0	Amine et al. (2012)
Coastal sediments	Spain	4HB, BP1, BP3, BP8, BP6, ES, HMS	SPE (2 g sample, 1.5 g C_{18} + 1 g Na_2SO_4, EA/MeOH (9:1), 15 min USE)	GC-MS/MS	4HB: 102-105.3 BP1: 94.3-101.9 BP3: 98.9-101.3 BP8: 88.4-91.4 BP6: 89.9-92.4 ES: 99.4-102 HMS: 97.4-101.3	4HB: 0.23 BP1: 0.21 BP3: 0.28 BP8: 0.14 BP6: 0.15 ES: 0.11 HMS:0.12	4HB, BP1, BP3, BP8, BP6, HMS: n.d. ES: 13.3	Sánchez-Brunete et al. (2011)

Table 1.2 Overview on analytical methods and occurrence of UV-filters in sediments and soils. (cont.).

Matrix	Location	Compounds	Extraction method	Analysis method	Rec. (%)	LOD (ng g^{-1} dw)	Concentration (ng g^{-1} dw)	Reference
Streams sediments (receiving wastewater)	Japan	BP3, BZS, 4-MBC, ODPABA, EHMC, OS, HMS, OC, BP	Freeze-drying USE (4 g sample, DCM + Ac, 40 min) Centrifugation (3000 rpm, 10 min) SPE (5 g florisil cartridge, Hex -1st, Hex/Ac (19:1) - 2nd, Hex/Ac (1:1) - 3rd) SPE (3rd fraction into graphite column, Ac/Tol (70:30)) SPE (2nd and 4th fraction combined into NH_2 cartridge, Hex + Ac/Hex (4:96))	GC-MS	70 - 125	0.05 – 1.00	BP3, BZS, 4-MBC, ODP: n.d. EMC: 3-101 HMS: 26 OC: 3.0 - 635	Kameda et al. (2011)
STP effluents sediments	Japan	BP3, BZS, 4-MBC, ODPABA, EHMC, OS, HMS, OC, BP	Freeze-drying USE (4 g sample, DCM + Ac, 40 min) Centrifugation (3000 rpm, 10 min) SPE (5 g florisil cartridge, Hex -1st, Hex/Ac (19:1) - 2nd, Hex/Ac (1:1) - 3rd) SPE (3rd fraction into graphite column, Ac/Tol (70:30)) SPE (2nd and 4th fraction combined into NH_2 cartridge, Hex + Ac/Hex (4:96))	GC-MS	70 - 125	0.05 – 1.00	BP3, BZS, 4-MBC, ODP: n.d. EMC: 3-101 HMS: 26 OC: 3.0 - 635	Kameda et al. (2011)
Agricultural soil (fertilized with sewage sludge)	Spain	4HB, BP1, BP3, BP8, BP6, ES, HMS	SPE (2 g sample, 1.5 g C_{18} + 1 g Na_2SO_4, 2x EA/MeOH (9:1), 15 min USE)	GC-MS/MS	4HB: 97.5-101.1 BP1: 93.6-103.8 BP3: 92.8-96.9 BP8: 89.8-93.7 BP6: 91.9-98.9 ES: 97.9-104.4 HMS: 91.3-97.4	4HB: 0.07 BP1: 0.10 BP3: 0.10 BP8: 0.07 BP6: 0.09 ES: 0.08 HMS:0.07	4HB, BP1, BP3, BP8, ES, HMS: n.d. BP6: 0.6	Sánchez-Brunete et al. (2011)
Industrial soil (fertilized with sewage sludge)	Spain	4HB, BP1, BP3, BP8, BP6, ES, HMS	SPE (2 g sample, 1.5 g C_{18} + 1 g Na_2SO_4, 2x EA/MeOH (9:1), 15 min USE)	GC-MS/MS	4HB: 97.5-101.1 BP1: 93.6-103.8 BP3: 92.8-96.9 BP8: 89.8-93.7 BP6: 91.9-98.9 ES: 97.9-104.4 HMS: 91.3-97.4	4HB: 0.07 BP1: 0.10 BP3: 0.10 BP8: 0.07 BP6: 0.09 ES: 0.08 HMS:0.07	BP1: 5.7 4HB, BP3, BP8, BP6, ES, HMS: n.d	Sánchez-Brunete et al. (2011)
Ground soil	Korea	BP, BH, 4HB, BP3, BP1, BP8, 234THB	SLE (10 g sample, 10 g Na_2SO_4, MeOH, 20 min) Centrifugation (1660 x g, 15 min) Evaporation + 5% NaCl solution + EA Derivatization (50 µL MSTFA, 80 °C, 30 min)	GC-MS	BP: 102-125 BH:76-111 4HB: 73-115 BP3: 71-81 BP1: 62-84 BP8: 60-78 234THB: 81-92	0.1	BP: 820-16550 BH: 510-6950 4HB: 1060-4910 BP3: 730-3880 BP1: n.q. BP8: 500-4170 234THB: n.d.	Jeon et al. (2006)

In SPME, a fiber coated with a stationary phase is exposed to the sample, typically until equilibrium is reached either by direct immersion (DI) or headspace (HS) (Doong et al., 2000). Usually sampling in the headspace presents a significant advantage in terms of selectivity because only volatile and semi-volatile organic compounds can be released into the headspace. Because the fiber is not in contact with the sample, background adsorption and the matrix effect are reduced, which also enhances the life expectancy of SPME fibers (Doong et al., 2000). These fibers are reusable, unlike single-use SPE cartridges, resulting in cost savings (Wong and MacLeod, 2009). However, few types of fiber are available (e.g. PDMS, PDMS-DVB, polyacrylate (PA) and carboxen) narrowing the users' choice. In order to extract the most volatile UV-filters (salicylate derivatives ES and HMS and benzophenone derivatives BP1, BP3 and BP8), Negreira et al. (2009) verified that PDMS-DVB coated fibers were the most appropriate, using a headspace assembly (relative recoveries ranging from 89 to 115%). Note that recoveries in microextraction techniques (e.g. SPME) are usually referred as relative recoveries. Liu et al. (2010) used PDMS coated fiber for the extraction of salicylate ES, benzophenone BP3, camphor 4-MBC and crylene OC, obtaining relative recoveries of 72.5–115%. 3-(Mercaptopropyl) trimethoxysilane (MPTS)-Ag-C_{12} wire was also used in a SPME approach (Li et al., 2013), conducting to relative recoveries of 69.7 to 102.4% for benzophenone derivatives BP, BP3 and 4 PB. This technique seems to be appropriate for the extraction of the most volatile UV-filters. However, when compared with SPE, the SPME procedure is limited in the method manipulation and presents a restriction in the choice of fibers.

Stir-bar sorptive extraction (SBSE), a technique related to SPME, is based on the extraction of the analytes from the liquid matrix onto a thick film coated on a magnetic stir bar. This technique is usually followed by liquid or thermal desorption (LD or TD) (Wong and MacLeod, 2009). This technique was successfully applied for the extraction of UV-filters, using stir bars externally coated with poly(dimethylsiloxane) (PDMS) (Kawaguchi et al., 2008; Pedrouzo et al., 2010; Rodil and Moeder, 2008a). This methodology has the advantage of using small sample volumes (10 to 50 mL) with extraction times from 120 to 180 min and 800 to 1000 rpm stirring at room temperature. Recoveries varied from 80 to 130% when thermal desorption was used (Kawaguchi et

al., 2008; Rodil and Moeder, 2008a) and ranging 30 to 80% using liquid desorption (Pedrouzo et al., 2010).

Other less conventional methodologies were also applied to water samples in order to extract the organic compounds, like ionic liquid based ultrasound-assisted emulsification microextraction (IL-USAEME) (Ge and Lee, 2012) with around 100% recovery, ionic liquid-based single-drop microextraction (IL-SDME) (Vidal et al., 2010) with average recovery of 100%, non-porous membrane-assisted liquid–liquid extraction (MALLE) (Rodil et al., 2009b) yielding 60 to 100% recovery, magnetic nanoparticles dispersive solid-phase extraction (MNPs-based dSPE) (Román et al., 2011) with about 70–125% recovery, microextraction by packed sorbent (MEPS) (Moeder et al., 2010) with 60 to 115% and micelle mediated extraction (MME) (Giokas et al., 2005) with average recoveries of 100%. These techniques are relatively new and their applicability to extract UV-filters from water samples has been poorly investigated. However, the results obtained so far are very promising. It is also important to remember that some of these extraction techniques have disadvantages. For example, most ionic liquids are not commercially available, being necessary to synthesize them. A similar situation can be verified with the specific sorbents used in MEPs or MNPs-based dSPE.

Water may also be indirectly analyzed (passive sampling) using semipermeable membrane devices (SPMDs), which support the presence of UV-filters in lakes and rivers (Goksoyr et al., 2009). SPMDs are usually used for integrative *in situ* concentration of more lipophilic contaminants and measure time-weighted average concentrations of the dissolved (bioavailable) compounds (Balmer et al., 2005). These devices consist of a thin lay flat tube made from semipermeable polyethylene membranes. They are mounted on assemblies to give a spread configuration in perforated stainless steel containers and are exposed for 3 to 6 weeks at a 1 to 2 m depth. The UV-filter concentrations in the SPMDs (C_{SPMD}, ng/SPMD) can be used to estimate the respective concentrations in water (C_W, ng L^{-1}) (Poiger et al., 2004). Extractions from these devices are usually by dialysis for 24 h and the solvents used are cyclopentane/DCM (95:5) (CYPN/DCM), CYPN or hexane (Hex) (Goksoyr et al., 2009; Balmer et al., 2005; Poiger et al., 2004). These authors studied mid-polar UV-filters (BP3, OC, 4-MBC, EMC, BMDM,

3BC) and obtained average recoveries of 42 to 110%, with the lowest values for the more polar compounds.

Fent et al. (2010) reported a similar passive sampling device, a polar organic chemical integrative sampler (POCIS) which is used with the same purpose as SMPDs. However, they are used to *in situ* collection of hydrophilic organic contaminants. The POCIS sampler consists of several sampling disks mounted on a support rod. Each disk consists of a solid sorbent sandwiched between two microporous membranes. For the analysis, the sorbent is removed and placed into a SPE column or empty cartridge. The UV-filters are usually extracted using more polar solvents or mixtures as MeOH and MeOH/toluene/DCM. In this specific case, Fent et al. (2010) studied four polar to mid-polar UV-filters (BP3, BP4, 4-MBC and EMC). This sampler yields good recoveries, ranging from 70 to 100%.

1.3.2. Extraction techniques for sediments and soil analysis

Information regarding occurrence and method development of UV-filters in soils and sediments is rather scarce, unlike water samples. It is worth noticing that sediments and soils are extracted with similar methodologies. Usually, prior to extraction, samples are either frozen (Sánchez-Brunete et al., 2011) or freeze-dried (Gago-Ferrero et al., 2011) and homogenized. Samples are then extracted by solid–liquid techniques and sometimes clean-up with solid-phase extraction. Solid-phase techniques usually require small amounts of sample (1 to 10 g) and in these specific cases, small volumes of extraction solvents (8 to 120 mL). The extraction solvents vary in polarity from polar MeOH, intermediate polarity like DCM and Ac, to apolar Hex, depending on the target compounds. Mixtures with intermediate polarity are also used and in different proportions like Ac/heptane (Hep) (1:1) (Amine et al., 2012), Ac/toluene (Tl) (7:3) (Kameda et al., 2011), EA/Hex (8:2) (Rodil and Moeder, 2008b), EA/MeOH (9:1) (Sánchez-Brunete et al, 2011).

These extraction techniques yielded high recoveries like conventional solid–liquid extraction (SLE) using ultrasounds with 65 (BP8) to 125% (BP) (Jeon et al., 2006), pressurized liquid extraction (PLE) with 58 (BP1) to 128% (IMC) (Gago-Ferrero et al.,

2011; Rodil and Moeder, 2008b), and selective pressurized liquid extraction (SPLE) from 85 (OC) to 125% (BP3) (Barón et al., 2013). Solid-phase extraction (SPE) used as a cleanup also yields high recoveries, ranging from 70 to 125% (Kameda et al., 2011), applied to sediments, and 88 to 105% applied to soils (Sánchez-Brunete et al., 2011). Other techniques were also used in sediment extraction like microwave assisted extraction (MAE) which is a fast, reliable method, with high recoveries from 97 to 115% (OC) and sequential extraction with high-speed dispersion tool (SEHSDT), used to analyze camphor 4-MBC (75% recovery) and benzophenone BP (90% recovery) (Ricking et al., 2003).

1.3.3. Chromatographic analysis

Usually, after extraction and clean-up of UV-filters, chromatographic methods are employed to both identify and quantify several components in a single analysis. Those have to be sensitive enough to detect trace levels of the potential contaminants.

Peck (2006) already described the most common analytical methods for the determination of persistent ingredients of personal care products in environmental matrices, dedicating a section to the UV-filter extraction from water, sewage sludge and fish tissue samples and also to specific details related with detection and quantification. Pietrogrande and Basaglia (2007) reviewed in deep detail the GC–MS analytical methods for the determination of PCPs (UV-filters included) in water matrices. Regarding liquid chromatography, Gago-Ferrero et al. (2013a) thoroughly reviewed the liquid chromatography–tandem mass spectrometry for the multi-residue analysis of organic UV-filters and their transformation products in the aquatic environment. Therefore, in this section a small comparison and discussion of the most commonly used chromatographic methods to determine UV-filters will be presented.

The most used chromatographic method is liquid chromatography (LC) (Oliveira et al., 2010; Rodil and Moeder, 2008b; Giokas et al., 2004, 2005), since UV-filters are generally non-volatile compounds (Figure 1.2). Reversed-phase chromatography with octadecyl-based stationary phase is the normally used, combined with mobile phases consisting of mixtures of acetonitrile (ACN), MeOH and water (H_2O), with phase modifiers to improve

peak shape, retention, and resolution (Zenker et al., 2008). Good chromatographic separations are desirable, even with sophisticated detectors like mass spectrometers. Diode-array (DAD) (Giokas et al., 2005; Zhang et al., 2011) or photodiode array detectors (PDAs) (Li et al., 2013) are also used coupled to HPLC. Recently, ultra-performance liquid chromatography (UPLC) has been explored for this type of analysis since it uses less solvent and provides improved speed, resolution, and sensitivity from narrower and sharper chromatographic peaks and also reduction of matrix effects during MS/MS detection (Wong and MacLeod, 2009).

When choosing the analytical method, the physiochemical properties of the target analyte must be taken into consideration. More polar and less volatile compounds are usually analyzed by LC–MS, while to identify and quantify volatile or volatilizable compounds and transformation products, gas chromatography coupled with mass spectrometry (GC–MS) is the choice (Jurado et al., 2014; Rodil andMoeder, 2008a,b), especially when resolution is essential to separate isomers or congeners (Goksoyr et al., 2009). Most studies using GC–MS method present a significant improvement in limits of detection (LODs) in the low-ng L^{-1} level (Kawaguchi et al., 2008; Li et al., 2007; Lambropoulou et al., 2002).However, an additional derivatisation step is needed as UV-filters with polar groups are not easily analyzed by GC–MS due to their low sensitivity and volatility for GC (Jeon et al., 2006). In general, derivatisation reduces the polarity of the analyte, which prevents co-elution with high polar endogenous materials in complex matrices. Furthermore, the derivatisation increases the molecular weight of relatively low weight molecules. As a result, the interference of endogenous materials is prevented by increasing the retention times during the reversed-phase chromatographic run (Ho and Ding, 2012). Several reagents have been steadily developed for this purpose, such as N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) (Jeon et al., 2006; Negreira et al., 2009; Zhang and Lee, 2012a), N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (Román et al., 2011; Tarazona et al., 2010) and N-methyl-N-(tert-butyldimethylsilyl)trifluoroacetamide (MTB-STFA) (Negreira et al., 2008). These studies were conducted under high temperatures (60 °C) and for at least 30 min of reaction time. Some authors couple extraction methods like DLLME (Wu et al.,

2013) and SBSE (Kawaguchi et al., 2008) with in situ derivatisation, which seems to increase the throughput of sample analysis.

1.4. Occurrence in the environment

1.4.1. Occurrence in water matrices

The presence of UV-filters in water has been verified in tap water, natural waters (lake, river, groundwater and sea water) and swimming pool water (Table 1.1). Probably the increasing number of analytical methods for UV-filters enhanced the incidence of occurrence in water matrices (Figure 1.3).

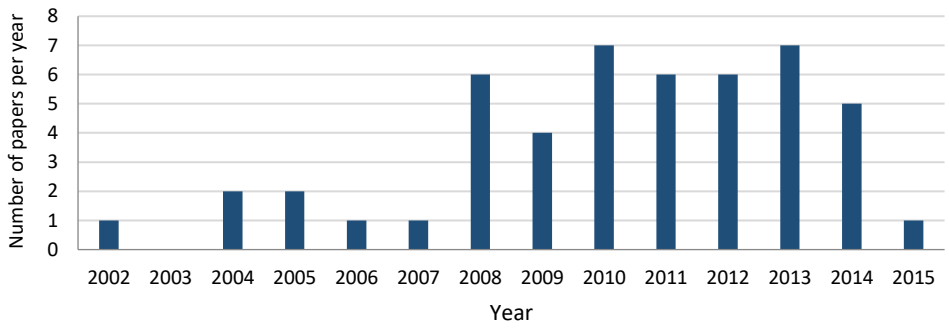


Figure 1.3 Evolution of articles numbers dedicated to the detection of UV-filters in water matrices.

1.4.1.1. Natural and tap waters

A detailed overview about the occurrence of UV-filters in natural and tap water is shown in Table 1.1. Distribution is analyzed by type of water and per family of UV-filters.

In water bodies, UV-filter compounds can be separated into two groups since they present different mobility depending on their physicochemical properties: the less mobile molecules have significant log K_{ow}, since they might exhibit sorption affinity with organic matter present in aquifer sediments; the more mobile molecules might have a more hydrophilic character (Jurado et al., 2014). In fact, UV-filters like BP4, BP5, PBSA and DPDT are expected predominantly in aqueous matrices (Figure 1.2).

1.4.1.1.1. *River water*

River water is the matrix that shows higher concentrations and different types of UV-filters (Figure 1.4). The highest detected concentration was in the UK with concentrations up to 0.3 mg L^{-1} (BP4). Kasprzyk-Hordern et al. (2009) examined the presence of four UV-filters (BP1, BP2, BP3, and BP4) in water from two rivers, upstream and downstream of a WWTP. Among the studied benzophenones, BP4 was found at higher concentrations (32–323 µg L^{-1}) followed by BP3 (n.d.–44 µg L^{-1}), BP2 (n.d.–26 µg L^{-1}) and BP1 (9–17 µg L^{-1}). In Spain Gago-Ferrero et al. (2013b) determined BP3, BP1, 4HB, 4DHB, BP8, BP2, BP4, 4-MBC, Et-PABA. Out of the 9 compounds, only four (BP1, BP3, BP4, 4-MBC) were found in river water, where BP4 had the highest concentration (21.3–862 ng L^{-1}). The above results could be explained with the high polarity and solubility in water of BP4 compared with other benzophenones (Figure 1.2).

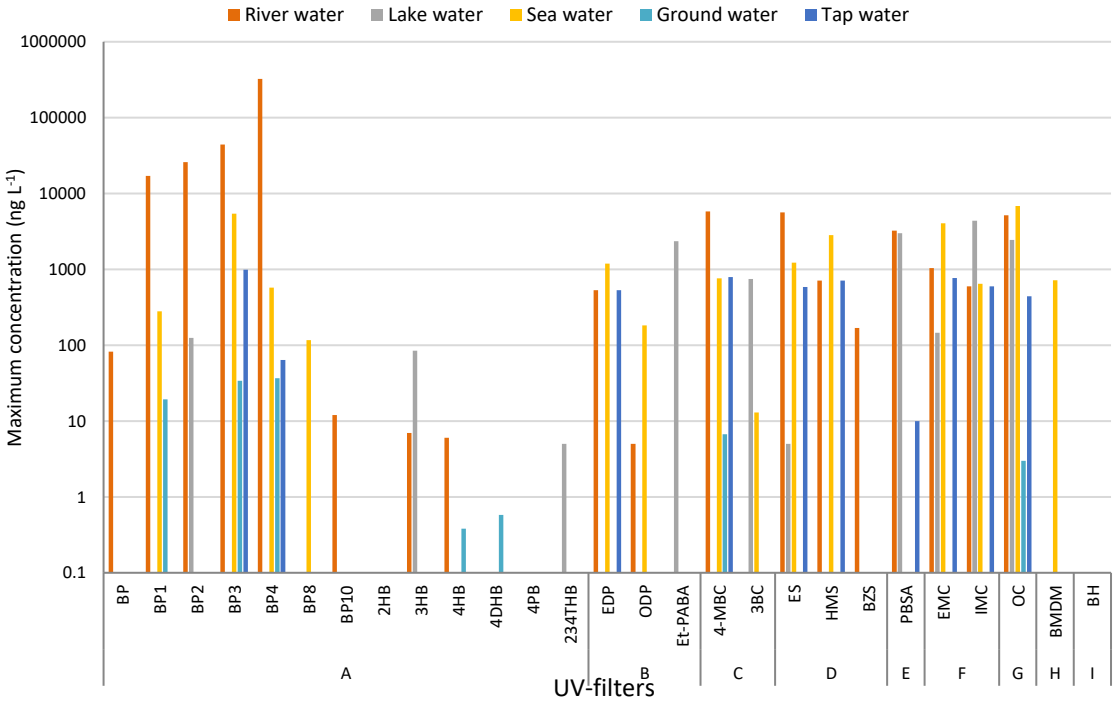


Figure 1.4 UV-filters maximum concentration found in tap, river, lake, sea and groundwater (A – benzophenone derivatives, B – p-aminobenzoic acid derivatives, C – camphor derivatives, D – salicylate derivatives, E – benzimidazole derivatives, F – cinnamate derivatives, G – crylene derivative, H – dibenzoyl methane derivatives, I – other).

In Japan, BP4 has not been studied. However, the most dominant sun-blocking agent in heavily polluted rivers is EMC, with concentrations from 125 to 1040 ng L^{-1}. This profile was consistent with those found in moderately polluted river (12–91 ng L^{-1}) and background river sites (18 ng L^{-1}), but not with smaller river streams where OS was up

to 266 ng L^{-1}. In addition to BP4 being found in high concentrations (Gago-Ferrero et al., 2013b; Kasprzyk-Hordern et al., 2008a; Kasprzyk-Hordern et al., 2009), it is possible to point other UV-filters, such as PBSA ranging from 5.1 to 500 ng L^{-1} in Czech Republic (Grabicova et al., 2013) and 48–3240 ng L^{-1} in Germany (Wick et al., 2010).

BP3 is also a UV-filter frequently studied and found in high concentration in rivers. It was found in Taiwan with concentrations from 12.3 to 15.4 ng L^{-1} (Wu et al., 2013) and 3 ng L^{-1} (Ho and Ding, 2012), in Switzerland from 56 to 68 ng L^{-1} (Fent et al., 2010), in the UK up to 220 ng L^{-1} (Kawaguchi et al., 2008), and in Germany with 30 ng L^{-1} (Rodil and Moeder, 2008a). In Spain concentrations were found from 28 ng L^{-1} in a background river to 993 ng L^{-1} in a heavily polluted river (Negreira et al., 2009; Pedrouzo et al., 2010; Román et al., 2011).

Salicylate derivatives ES, HMS and BZS were frequently detected and in high concentrations. Román et al. (2011) found 586 ng L^{-1} of ES and 712 ng L^{-1} of HMS in samples from Spain, and Liu et al. (2010) found concentrations of ES ten times higher (5620 ng L^{-1}) in samples from China. On the other hand, BZS was only found in samples from Japan (169 ng L^{-1}) (Kameda et al., 2011). The crylene derivative OC was also often under study and higher concentrations (5180 ng L^{-1}) were found in China (Liu et al., 2010). The cinnamate derivatives EMC and IMC were also found in high concentrations ranging 21–1040 ng L^{-1} and 595 ng L^{-1}, respectively (Kameda et al., 2011; Román et al., 2011).

The most detected UV-filter families in river water were the benzophenone derivatives (BP, BP1, BP2, BP3 and BP4) with concentrations up to 0.4 mg L^{-1}, *p*-aminobenzoic acids (EDP) with 531 ng L^{-1}, camphor derivatives (4-MBC) up to 5.8 µg L^{-1}, salicylate derivatives (ES, HMS and BZS) from 169 ng L^{-1} to 5.6 µg L^{-1}, benzimidazole derivatives (PBSA) up to 3.3 µg L^{-1}, cinnamate derivatives (EMC and IMC) from 595 ng L^{-1} to 1.1 µg L^{-1} and crylene derivatives (OC) with 5.2 µg L^{-1}. Although the maximum values for benzophenones (0.4 mg L^{-1} for BP4) were found after a WWTP discharge point in the UK (Kasprzyk-Hordern et al., 2009), most authors don't mention this pollution source. Kameda et al. (2011) studied a heavily polluted river in Japan where BZS and EMC were found at high concentrations. It's worth mentioning that EMC is allowed in Japan at a concentration limit in sunscreens of 20% (only 10% allowed in EU) (Jansen et al, 2013). In Spain, Román

et al. (2011) found the higher concentrations for IMC, EDP and HMS, whereas in Germany Wick et al. (2010) found higher concentrations for PBSA. In the µg L^{-1} range were found 4-MBC and OC in river samples from China (Liu et al., 2010).

1.4.1.1.2. *Lake water*

As previously mentioned, UV-filters enter the environment in two ways, either indirectly via WWTP effluent or directly from swimming and other recreational activities (Pal et al., 2014). Studies performed in lake water reported higher UV-filter content in samples collected during the summer. In fact, recreational activities like bathing and swimming occur most frequently in summer months, which may create seasonal variations (Rodil and Moeder, 2008a). Moeder et al. (2010) presented a study from a lake intensively used for swimming and bathing in Germany and detected 4-MBC (2351 ng L^{-1}), BP3 (83 ng L^{-1}), EMC (150 ng L^{-1}) and OC (274 ng L^{-1}). In the same conditions, but with higher range of concentrations, Rodil et al. (2009a,b) detected 7 of the 9 compounds under study (BP3, IMC, 4-MBC, BMDM, OC, EMC and ES). OC was found in the highest concentration (4381 ng L^{-1}) and the dibenzoyl methane derivative BMDM was found in 2431 ng L^{-1}, the highest concentration among all types of water studied.

Besides the previous studies, benzophenone-type UV-filters were determined in lakes of South Korea, whose main pollution sources are indirect inputs (contaminated rivers) (Jeon et al., 2006). Out of 7 compounds under study, only 4HB showed concentrations above the limit of quantification (85 ng L^{-1}).

Balmer et al. (2005) analyzed UV-filters in lakes with direct and indirect inputs and in remote locations. UV-filters were detected in several lakes, but concentrations were lower than expected, even during summer when direct inputs are supposedly higher. In fact, maximum concentrations were detected for 4-MBC and BP3 (28 and 35 ng L^{-1}, respectively). The same concentration levels were reported by Poiger et al. (2004) in midland lakes, where BP3 showed the highest concentration (125 ng L^{-1}) followed by 4-MBC (82 ng L^{-1}). In both studies UV-filters were determined using SPMD systems. In the latest study, the concentrations measured in the SPMDs exposed during summer were generally higher than in spring, again reflecting an increased use of UV-filters (sunscreens) during this season. In the remote mountain lake, Balmer et al. (2005)

detected no compounds above blank levels. However, Poiger et al. (2004) reported levels around 60 ng/SPMD for EMC in a small mountain lake.

Compared to river water, fewer studies were performed in lakes and again, lower concentrations were found, which is expectable considering that sources are mainly swimming and other recreational activities. Higher concentrations were found in Germany, for camphor 4-MBC (2.4 µg L^{-1}) (Moeder et al., 2010), salicylate ES (0.8 µg L^{-1}), cinnamate derivatives EMC (3.01 µg L^{-1}) and IMC (146 ng L^{-1}), crylene OC (4.4 µg L^{-1}) and dibenzoyl methane BMDM (2.4 µg L^{-1}) (Rodil et al., 2009a,b). It is worth mentioning that BMDM was not detected in any river water. Benzophenone derivatives were only found in Switzerland (BP3 at 125 ng L^{-1}) (Poiger et al., 2004) and South Korea (4HB at 85 ng L^{-1}) (Jeon et al., 2006).

1.4.1.1.3. *Groundwater*

Surface and groundwater bodies, used sometimes for water supply purposes, are the endpoint for some UV-filters (Gago-Ferrero et al., 2013b). The presence of UV-filters in groundwater may be due to water leaks in the plumbing systems that collect wastewater (Gago-Ferrero et al., 2013b). Climate conditions can also affect UV-filter entrance in groundwater since intense sun irradiation, high temperatures and high microbial activity can accelerate material decomposition. Then, heavy rain might be able to leach chemicals and transport them directly or adsorbed on particles into groundwater (Arukwe et al., 2012). UV-filters are either not found in groundwater (Ho and Ding, 2012; Wick et al., 2010) or found at very low concentrations (0.38–36.6 ng L^{-1}) (Arukwe et al., 2012; Gago-Ferrero et al., 2013b; Jurado et al., 2014). Considering the mixing of the different sources that contribute to the occurrence of the UV-filters in groundwater, these concentrations are below the expected. Jurado et al. (2014) suggest that in groundwater, UV-filters might be removed under different redox conditions.

The UV-filter found in higher concentrations in groundwater was BP4, whose major inputs could be explained by its highly solubility in water and frequent use in cosmetics and as color protector in products with translucent package (Gago-Ferrero et al., 2013b).

4.1.1.4. *Sea water*

In sea water, apart from recreational activities and surface runoff, the major contributor to UV-filter occurrence is probably wastewater release into the ocean (incomplete removal of organic UV-filters in WWTPs) (Tsui et al., 2014).

Most sea water data come from method development studies and takes particular emphasis in Southern Europe (Spain and Greece) and Japan. Among these studies, BP3, ES and OC appear in higher concentrations, all part of 'the Allowed UV-filter in cosmetics list'. Tarazona et al. (2010) obtained the higher concentrations for BP3 (3300 ng L^{-1}) in samples from Alicante and BP1 (280 ng L^{-1}) in samples from Murcia, Spain. Other UV-filters such as BP8 and 234THB, which are not part of the allowed compounds in cosmetics, were also studied but they were not detected.

In a comparison study between water collected in the ocean and collected in a natural swimming pool, higher concentrations were found in the latest matrix, with BP3 ranging from 25 to 216 ng L^{-1} and EMC ranging 53 to 86 ng L^{-1}, opposed to 118 ng L^{-1} and 83 ng L^{-1} respectively from the seawater samples (Tovar-Sánchez et al., 2013). Although the difference was expected, since the dilution factor is much higher at sea, the reported concentrations are in lower concentration levels than in other studies (Román et al., 2011; Tarazona et al., 2010).

The UV-filter ES was found in high concentrations in Alicante, Spain, ranging 792–1222 ng L^{-1} (Román et al., 2011), while in Majorca (Spain) concentrations were 440–880 ng L^{-1} (Benedé et al., 2014a,b). This compound, although relatively weak UV absorber has an excellent safety record, is easily incorporated into cosmetic formulations due to its aesthetics, stability, emollience and non-water-solubility, so it is widely used in many sunscreen products (Lowe et al., 1996).

Román et al. (2011) detected other 7 UV-filters in high concentrations: HMS (625–1030 ng L^{-1}), IMS (245–645 ng L^{-1}), 4-MBC (358–758 ng L^{-1}), BP3 (254–879 ng L^{-1}), EDP (409–774 ng L^{-1}), EMC (682–1187 ng L^{-1}) and OC (440 ng L^{-1}). The same compounds were detected by Benedé et al. (2014a,b), but in a lower concentration range (220 to 390 ng L^{-1}). Vidal et al. (2010) also detected OC at 3000 ng L^{-1}, while BP3, IMC, 4-MBC, EDP and EMC were detected between 60 and 190 ng L^{-1}.

Sea water collected from beach sites revealed higher UV-filter concentration (e.g. BP3: 1258 ng L^{-1}) than reef sites (BP3: 9 ng L^{-1}). In Japan, concentrations ranged from 4.1 ng L^{-1} (ODP) to 1258 ng L^{-1} (BP3) in beach sites, while in reef sites they varied between 1.8 ng L^{-1} (ES) and 9.0 ng L^{-1} (BP3). Compounds like BZS and 4-MBC were not detected in either site. In Greece, UV-filters were not detected (Lambropoulou et al., 2002) or detected at low concentrations — 8.2 (BP3) to 19.7 ng L^{-1} (4-MBC) (Giokas et al., 2005) and 1.8 ng L^{-1} (BP3) (Giokas et al., 2004).

SPMDs were used to determine UV-filters in the middle of the Pacific Ocean and these levels were compared to those found in the collected sea water. Compounds were detected in concentrations between 6 and 55 ng L^{-1} in water and below LOD (0.15–0.51 ng/SPMD) and 34.3 ng/SPMD in the devices (Goksoyr et al., 2009). Due to the fact that UV-filters were found far away from the coastal area, where direct inputs are predominant, it may indicate that they are transported via ocean currents or atmospheric transport, either long-range or short-range.

Tsui et al. (2014) determined the concentrations and spatial occurrence of twelve commonly consumed UV-filters, including BP1, BP3, BP4 and BP8, ES, IMC, ODP, BMDM, EMC,HMS, 4-MBC and OC in surface sea water samples collected in different countries, including China (Hong Kong, Shantou and Chaozhou), United States (New York City and Los Angeles), Japan (Tokyo Bay), Thailand (Bangkok) and the Arctic region. Hong Kong showed the higher concentrations (117 (BP8) to 6812 (OC) ng L^{-1}). OC was the compound found in higher concentrations among all Chinese cities. Tokyo's highest concentration was BP4 (136 ng L^{-1}), while in Los Angeles and Shantou it was the BP3 (601 and 188 ng L^{-1}, respectively). In Chaozhou the ES was detected in higher concentration levels (128 ng L^{-1}) and in Arctic was the BMDM (70 ng L^{-1}). This is the only report of the occurrence and distribution of organic UV-filters in the Arctic, for which there possible pathways are the same as for the middle of the Pacific Ocean (ocean currents or atmospheric transport).

1.4.1.1.5. *Tap water*

The presence of UV-filters in tap/drinking water has been poorly studied. Therefore, few conclusions can be drawn.

da Silva et al. (2015) investigated drinking water samples from a water treatment plant (WTP), while Ge and Lee (2012), Rodil et al. (2012) and Zhang and Lee (2013) studied samples collected in their research labs. In fact, if treatment plants are efficient, no UV-filters should be found in tap water. This was verified by da Silva et al. (2015) in a Brazilian WTP. This showed good treatment efficiencies, since reported UV-filter concentrations were below the limit of detection (7.6–24.1 ng L^{-1}). Regarding the tap water analyzed in Singapore (Ge and Lee, 2012; Zhang and Lee, 2013) and in Japan (Kameda et al., 2011), UV-filters were not found in the collected samples. However, Rodil et al. (2012) and Román et al. (2011) from Spain found high amounts of these compounds in tap water samples — around 10 ng L^{-1} for PBSA and 4-MBC and 62 ng L^{-1} for BP4 in the first case and below LOQ (0.5–20 ng L^{-1}) to 160 ng L^{-1} for ES, EMC and IMC. Also in Spain, Díaz-Cruz et al. (2012) found in a slight higher range the following UV-filters: BP3, ODP, EMC and OC ranging from 110 to 290 ng L^{-1} and 4-MBC at 35 ng L^{-1}.

1.4.1.2. Swimming pool water

Swimming pools, as lakes, are widely used in summer for recreational activities, where UV-filter entrance is a direct input since sunscreens are often used. Higher UV-filter concentrations were found in swimming pool water samples. The maximum concentrations detected are shown in Figure 1.5.

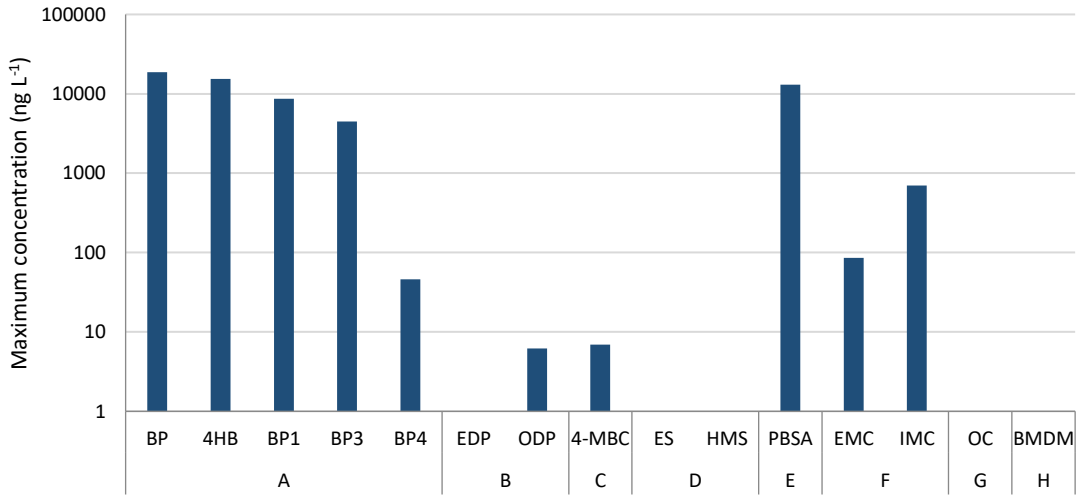


Figure 1.5 UV-filters maximum concentration found in swimming pool water (A – benzophenone derivatives, B – p-aminobenzoic acid derivatives, C – camphor derivatives, D – salicylate derivatives, E – benzimidazole derivatives, F – cinnamate derivatives, G – crylene derivative, H – dibenzoyl methane derivatives).

Chlorination disinfection is still one of the most widely used techniques in water treatment practices, and often associated to swimming pools, because of its strong oxidation ability, lower cost when comparing to other techniques and effective persistence. However, the free available chlorine does not only kill the harmful pathogens but may also react with some chemical pollutants that enter or already exist in the water and may possibly create poisonous and harmful by-products (Liu et al., 2014).

Ye et al. (2011) analysed four benzophenone-type UV-filters in a swimming pool water sample from China and detected concentrations as high as 4500 (BP3), 8700 (BP1), 15,400 (4HB) and 18,800 (BP) ng L^{-1}. BP and 4HB are not part of 'the compounds allowed in cosmetics list (Annex VI)' so their presence in swimming pool water is not very well understood. It's known that BPs are usually not directly incorporated in personal care products, but no information regarding 4HB utilization was found among literature. Therefore, their presence in swimming pools may be due to its presence in the tap or other source of water that is used to fill the pool (PROGRAM, 2006).

UV-filters were also detected in Czech Republic by Grabicova et al. (2013). PBSA (24–13,000 ng L^{-1}), BP3 (21–620 ng L^{-1}) and BP4 (3.3–46 ng L^{-1}) were detected in the collected samples. Higher concentration levels of PBSA could be explained by its massive use as an UV-filter in cosmetic products (maximum concentration of 8% (expressed as acid) in Europe and 4% in the USA (SCCP/1056/06)). On the other hand, PBSA is highly water-soluble and chlorine plays a negligible role in PBSA degradation (Ji et al., 2013).

Nguyen et al. (2011) reported concentrations of BP3 ranging 25–216 ng L^{-1} and EMC from 53 to 86 ng L^{-1} in a seawater swimming pool in Italy. Other compounds were also investigated (OC, EDP, HMS and ES), but they were not detected. Limits of detection were considerably low (0.01 ng L^{-1} for EDP to 2.65 ng L^{-1} for ES) and the method combined SBSE-LD with LC–MS.

Giokas et al. (2004) obtained concentrations of 4.2–5.7 ng L^{-1} for BP3, 5.4–6.9 ng L^{-1} for 4-MBC and 3.0–4.5 ng L^{-1} for EMC, by SPE–GC–MS, in Greece. However, BMDM was not detected using LC–UV–DAD. Zhang and Lee (2012a,b) did not detect UV-filters in swimming pool water, either because the analytes (BP, BP3, ES, HM) were not present

or due to the high LOD of the IL-SDME–LC–UV method (200–5000 ng L^{-1}). The UV-filter IMC was the only compound found in samples from Spain at 700 ng L^{-1}, among OC, BP3, EDP, EMC and 4-MBC, using IL-SDME and LC–UV (Vidal et al., 2010).

UV-filters in shower waste water samples were compared with swimming pool water samples by Lambropoulou et al. (2002), where BP3 and ODP levels were higher in shower waste (8200–9900 ng L^{-1} and 5300–6200 ng L^{-1} respectively) than in swimming pool water (2400–3300 ng L^{-1} for BP3 and ODP was not found). In the same country (Greece), shower waste water UV-filter levels were in a lower range for BP3 (10.0 ng L^{-1}), 4-MBC (3.8 ng L^{-1}) and EMC (4.1 ng L^{-1}) (Giokas et al., 2004).

The detection range of UV-filters in swimming pool water is different from the other water matrices probably because of the different contamination sources and degradation processes. The most frequently detected compounds are benzophenones BP (18.8 µg L^{-1}), 4HB (15.4 µg L^{-1}), BP1 (8.7 µg L^{-1}) and BP3 (4.5 µg L^{-1}) (Ye et al., 2011), however, benzophenone BP4 was also found at lower concentrations by Grabicova et al. (2013) at 46 ng L^{-1}. Other compounds such as benzimidazole PBSA (13 µg L^{-1}) (Grabicova et al., 2013) and cinnamate derivatives EMC and IMC were also found at high concentrations, 86 and 700 ng L^{-1} respectively. Compounds such as ODP, EDP and BMDM were either not detected or detected at low concentrations, which may be due to the degradation processes they suffer upon contact with chlorine. Although UV-filter EMC and BP3 were found at relatively high concentrations, they also suffer degradation with chlorine (Supplementary material).

1.4.2. Occurrence in sediments and soils

Although the occurrence of UV-filters in water samples has been well documented, the information regarding soil and sediments is rather scarce. So far only 8 papers regarding this subject were published since 2000 (Table 1.2).

UV-filters' maximum concentration found in soil and sediments is shown in Figure 1.6. For lipophilic organic UV-filters, these matrices constitute a trapping compartment (Amine et al., 2012). Most UV-filters found in these matrices can be called hydrophobic once their log K_{ow} values are higher than 4, and their affinity to the matrices in study can

be proven by their high log K_{oc} up to 5.5, which translates into moderate to very strong sorption to soil/sediments. Among all the UV-filters found in these solid compartments, the crylene derivative OC presented the higher frequency of detection and one of the highest concentrations. Although it was not studied in soil samples, it was found in sediments with concentrations ranging 79 and 2400 ng g^{-1} dw (Amine et al., 2012; Gago-Ferrero et al., 2011; Kameda et al., 2011; Rodil and Moeder, 2008b). This compound is highly lipophilic with log K_{ow} 6.9 (Fig. 2), therefore with tendency to adsorb upon sediment organic matter. It also has very low water solubility (0.0038 mg L^{-1} at 25 °C), which makes lixiviation not possible, and is highly stable and resistant to sunlight degradation (Gago-Ferrero et al., 2011). These high concentrations in sediments can be associated with its extensive use in personal care products, especially sunscreens (Amine et al., 2012; Gago-Ferrero et al., 2011). However, OC was not found in all studies, which suggests that the production and use profiles of UV-filters are different among countries (Barón et al., 2013).

Like the UV-filter OC, the cinnamate derivative EMC (log K_{ow}=5.8) was frequently studied and detected in sediments with concentrations between 9 (Amine et al., 2012) and 101 ng g^{-1} dw (Kameda et al., 2011). The average range concentrations of UV-filters in river/lake sediments are similar on different impacted environments: river transition zones (11–90 ng g^{-1} dw) (Amine et al., 2012), moderately polluted rivers (0.4–30.0 ng g^{-1} dw), highly polluted rivers (0.8– 50 ng g^{-1} dw) (Kameda et al., 2011), slightly polluted rivers (5.2–42 ng g^{-1} dw, exception for OC found at 2400 ng g^{-1} dw) (Gago- Ferrero et al., 2011) and recreational lakes (14–93 ng g^{-1} dw) (Rodil and Moeder, 2008b). River sediment samples from Korea present high concentration levels, which constitutes an exception to the tendency presented above: benzophenone derivatives BP (1520–9730 ng g^{-1} dw), 4HB (18,380 ng g^{-1} dw), BP1 (500–2140 ng g^{-1} dw) and benzophenone metabolite BH (530 ng g^{-1} dw). UV-filters BP3 and BP8 were not detected in these samples (Jeon et al., 2006).

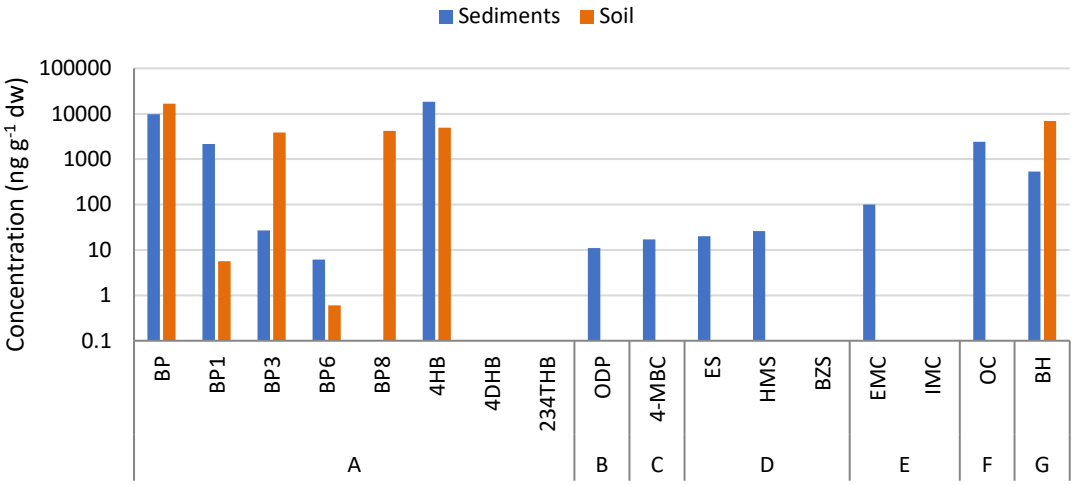


Figure 1.6 UV-filters maximum concentration found in sediments and soils (A – benzophenone derivatives, B – p-aminobenzoic acid derivatives, C – camphor derivatives, D – salicylate derivatives, E – cinnamate derivatives, F – crylene derivative, G – other).

A temporal trend in sediment contamination was also shown by Amine et al. (2012), who explain that higher concentrations of UV-filters can be found in low flow conditions like the ones in the dry season, where simultaneously happens an increase in UV-filter consumption. Also, Gago-Ferrero et al. (2011) tried to correlate UV-filter concentrations with total organic carbon (TOC) values of the sediments, however, no direct correlation was found.

Regarding soil samples, Jeon et al. (2006) detected really high concentrations of UV-filters in soil collected from residential, park, commercial and industrial areas with dense population. Concentrations found were around 820–16,550 ng g^{-1} dw (BP), 510–6950 ng g^{-1} dw (BH), 1060–4910 ng g^{-1} dw (4HB), 730–3880 ng g^{-1} dw (BP3) and 500–4170 ng g^{-1} dw (BP8). UV-filters BP1 and 234THB were not detected.

On the other hand, Sánchez-Brunete et al. (2011) studied salicylate and benzophenone-type UV-filters in agricultural soils amended with sewage sludge. Compounds 4HB, BP3, BP8, ES and HMS were not detected. BP1 (5.7 ng g^{-1} dw) and BP6 (0.6 ng g^{-1} dw) were detected at lower concentration levels.

1.4.3. Occurrence in biota

UV-filter occurrence in biota has been widely studied throughout the past years. In fact, an overview of UV-filters in aquatic biota by Gago-Ferrero et al. (2012) synthesizes the

scattered information in this subject by discussing the analytical methods and levels. This section, however, intends to compile the latest results published in the past 3 years (since 2012). An overview on the occurrence for the UV-filters is presented in Table 1.3. Since the last review several fish species have been investigated together with, although to a lesser extent, clams, urchins, prawns, crabs and mussels.

A study carried out by Peng et al. (2015) compared the levels of wild and farmed fish species from China, detecting BP3 in both at low ng g^{-1} dw. 4-MBC and EDP were detected at 41.5 and 52 ng g^{-1} dw, respectively in the farmed fish species, opposed to the wild species (2.3 ng g^{-1} dw and not detected, respectively). Similar concentrations were found in Taiwan for ES, HMS, BP3, BP1 and BP8 ranging 0.5 and 6.9 ng g^{-1} dw for wild fish (Tsai et al., 2014). Higher levels were found in wild fish, in samples from Spain for EMC at 241.7 ng g^{-1} dw (Gago-Ferrero et al., 2013c, 2015) and from Norway for BP3 at 1037 ng g^{-1} dw and OC at 11,875 ng g^{-1} dw (Langford et al., 2015). Besides the high concentrations of UV-filters in codfish, Langford et al. (2015) also detected levels of BP3, EMC and OC in prawns *Pandalus borealis* (BP3 at 68.4, OC at 23.1 ng g^{-1} dw). Crabs (*Carcinus maenas*) were also under studied, but values were below the limit of detection for all compounds. In New Zealand, BP3 was detected in samples of clams (108 ng g^{-1} dw), urchins (8.6 ng g^{-1} dw) and fish (14.1 in the filet and 41.0 ng g^{-1} dw in the liver), but BP1 was not detected in either sample (Emnet et al., 2015). Samples of wild mussels (*Mytilus galloprovincialis*) from Portugal were studied by Groz et al. (2014) and high levels were detected for EMC (1765 ng g^{-1} dw), ODP (833 ng g^{-1} dw) and OC (3992 ng g^{-1} dw); however, compound UV-326 was not detected. These results are in a lower range of that detected by Bachelot et al. (2012), except OC which was found at higher concentrations (7112 ng g^{-1} dw).

As shown, of all the UV-filters under study, BP3 is the most frequently found and in all type of biota (except crab, where no compounds were detected) at concentrations ranging 68.9 (urchins) to 1037 ng g^{-1} dw (fish). However, the UV-filter OC was found at higher concentrations ranging 23.1 (prawns) and 11875 ng g^{-1} dw (fish).

Table 1.3 UV-filters concentration and analytical method overview in biota (from 2012).

Country	Matrix Species	Compounds	Concentration (ng g^{-1} dw)	Method	Recovery (%)	LOD (ng g^{-1} dw)	Ref
China	Wild Fish	BP3, 4-MBC, OC, ODP, BMDM, EMC, UV-326, UV-329	BP3: 0.106 – 1.52 4-MBC: 0.2 – 2.3 UV-329: 0.105 – 0.225 ODP, BMDM, UV-326: n.d.	Samples were freeze-dried, ground, and homogenized. USA (4 g sample, MeOH (extraction solvent), vortex, ultrasonic bath 15 min, 300W, 3 times); GPC (Biobeads S-X3 (200–400 mesh), Acetate/CYHex (1:1) (elution solvent)) SPE (Silica column, DCM/EA (1:1) (elution solvent)) UHPLC-MS/MS	BP3: 88.3 – 102.0 4-MBC: 80.8 – 102.4 OC: 87.9 – 115.6 ODP: 64.2 – 102.3 BMDM: 41.1 – 82.8 EMC: 81.1 – 109.5 UV-326: 42.2 – 95.8 UV-329: 54.8 – 101.6	MQL BP3: 0.08 4-MBC: 0.2 OC: 0.1 ODP: 0.005 BMDM: 1 EMC: 10 UV-326:0.01 UV-329: 0.003	Peng et al., 2015
	Farmed red snapper		BP3: 0.59 – 0.80 4-MBC: 14.7 – 41.5 ODP: 0.239 – 0.36 BMDM: 33 – 52 UV-326: 7.95 – 11.38 OC, EMC, UV-329: n.d.				
New Zealand	Clams *Laternula elliptica*	BP1, BP3	BP3: 9.2 - 108 BP1: n.d.	Samples homogenized PLE (ASE, 1st [2 cycles, 5 min, 120 °C, 1450 psi, H_2O/IPA (1:1) (extraction solvent)], 2nd [2 cycles, 10 min, 180 °C, 1450 psi, H_2O/IPA (20:80) (extraction solvent)]) SPE (Oasis HLB (1g/20mL) cartridges, 2x MeOH + 2x H_2O (elution solvents)) SPE (Florisil (Isolute 2g/15 mL) cartridges, 3x DCM/MeOH (95:5) + DCM/MeOH (95:5) (elution solvents)) GPC (SX8 Biobeads, DCM/MeOH (95:5) (elution solvent)) Derivatization (BSTFA/TMSI (98:2)) GC-MS	BP3: 53 BP1:47.9	MQL BP3: 6.6 BP1: 2.0	Emnet et al., 2015
	Urchin *Sterechinus neumayeri*		BP3: 8.6 BP1: n.d.				
	Fish (fillet) Trematomus bernachi		BP3: <6.6 – 14.1 BP1: n.d.		BP3: 67.4 BP1: 52.2		
	Fish (liver) Trematomus bernachi		BP3: 41.0 BP1: n.d.				
Spain	River fish *Luciobarbus sclateri*	BP3, EMC, 4-MBC, OC	BP3: < 4.0 – 24.3 EMC: <16.7 – 241.7 4-MBC: n.d. OC: <20.0 – 30.4	Freeze-dried samples; PLE (ASE, 1 g Florisil, 1 g sample, 4 cycles, 5 min, 100 °C, 1500 psi, AcEt/DCM (1:1) (extraction solvent)) SPE (C_{18} (500 mg, 3 mL) cartridges, EA/DCM (1:1) + DCM (elution solvent) LC-MS/MS	-	BP3: 1.2 EMC: 5.0 4-MBC: 0.7 OC: 6.0	Gago-Ferrero et al., 2015
	Barbus graellsii		BP3: 2.2 4-MBC: <2.3 – 2.7 EMC, OC: n.d.				

Table 1.3 UV-filters concentration and analytical method overview in biota (from 2012). (cont.).

Country	Matrix Species	Compounds	Concentration (ng g^{-1} dw)	Method	Recovery (%)	LOD (ng g^{-1} dw)	Ref
Norway	Codfish (liver) *Gadus morhua*	BP3, EDP, EMC, OC, UV-329	BP3: <30 – 1037 EDP: <20 – 21.3 EMC: <30 – 36.9 OC: <20 – 11875 UV-329: <25	PLE (ASE, 3 cycles, 5 min, 100 °C, 1500 psi, Hex/DCM (1:1) (extraction solvent)) GPC (DCM (elution solvent)) LC-HRMS GC-HRMS	BP3: 75 EDP: 51 EMC: 85 OC: 75 UV-329: 72	BP3: 30 EDP: 20 EMC: 30 OC: 20 UV-329: 25	Langford et al., 2015
	Prawn *Pandalus borealis*		BP3: <30 – 68.9 EDP: <20 EMC: <20 OC: <10 – 23.1 UV-329: <25				
	Crab Carcinua meanas		BP3: <30 EDP: <20 EMC: <10 OC: <10 UV-329: <25				
	Fish (fillet) *Lota lota, Perca fluviatilis, Coregonus lavaretrus*		BP3: <5 – 182 EDP: <20 EMC: <5 OC: <2 – 2.1 UV-329: <25				
Portugal	Wild mussels *M. galloprovincialis*	EMC, ODP, OC, UV-326	EMC: 1765 ODP: 833 OC: 3992 UV-326: n.d.	Freeze-dried samples; QuEChERS (2 g sample, H_2O, vortex, ACN, shaking; add Q1 (4 g Na_2SO_4, 1 g NaCl, 1 g $Na_3Cit{:}2H_2O$, 0.5 g $Na_2HCit{:}3H_2O$), shake 1 min, centrifuge 5 min x 3500 rpm, upper layer transferred, add Q2 (750 mg Na_2SO_4, 125 mg C_{18}, 125 mg PSA silica), add 50 µL formic acid, shake 1 min, Centrifuge 5 min x 5000 rpm) GC-MS	EMC: 93 - 106 ODP: 90 - 93 OC: 99 - 126 UV-326: 33 - 50	EMC: 1.0 ODP: 2.5 OC: 5.0 UV-326: 12.5	Groz et al., 2014

Table 1.3 UV-filters concentration and analytical method overview in biota (from 2012). (cont.).

Country	Matrix Species	Compounds	Concentration (ng g^{-1} dw)	Method	Recovery (%)	LOD (ng g^{-1} dw)	Ref
Taiwan	Striped bass fish	ES, HMS, BP3, BP1, BP8	ES: 2.9 HMS: n.d. BP3:5.7 BP1:1.7 BP8: 1.7	Freeze-dried samples; MSPD (matrix solid-phase dispersion) (0.5 g sample, 0.5 g Na_2SO_4, 1.0 g Florisil) SPE (C_{18} cartridge, 7 mL ACN) GC-MS/MS	ES: 75 - 79 HMS: 78 - 83 BP3: 90 - 102 BP1: 84 - 88 BP8: 71 - 72	ES: 0.02 HMS: 0.02 BP3: 0.03 BP1: 0.02 BP8: 0.03	Tsai et al., 2014
	Tilapia fish		ES: 1.8 HMS:n.d. BP3:5.4 BP1: 0.7 BP8:1.5				
	Codfish		ES: 0.8 HMS :n.d. BP3:3.3 BP1: 1.0 BP8: 0.5				
	Salmon (fish)		ES: 3.9 HMS: 0.7 BP3: 6.9 BP1: 3.6 BP8: 2.4				
Spain	Fish *Luctobarbus sclateri*	BP3, BP1, 4HB, 4DHB, 4-MBC, EMC, OC, ODP	BP3: 16.5 – 24.3 EMC: 19.0 – 241.7 OC: <20.0 – 30.4 BP1, 4HB, 4DHB, 4-MBC, ODP: n.d.	Freeze-dried samples; PLE (ASE, 1 g Florisil, 1 g sample, 2 cycles, 5 min, 100 °C, 1500 psi, EA/DCM (1:1) (extraction solvent)) SPE (C_{18} (500 mg, 3 mL) cartridges, EA/DCM (1:1) + DCM (elution solvent) LC-MS/MS	BP3: 106 - 112 BP1: 90 - 92 4HB: 110 - 112 4DHB: 92 - 96 4-MBC: 95 - 109 EMC: 66 - 72 OC: 70 - 80 ODP: 36 - 42	BP3: 1.2 BP1: 4.0 4HB: 6.0 4DHB: 5.0 4-MBC: 0.7 EMC: 5.0 OC: 6.0 ODP: 0.1	Gago-Ferrero et al., 2013
	Fish *Cyprinus carpto*		BP3: 11.2 EMC: <16.7 OC, BP1, 4HB, 4DHB, 4-MBC, ODP: n.d.				

1.5. Conclusions

The present review provided comprehensive information about the occurrence and fate of UV-filters in the environment, as well as the main analytical methods to detect them. The widespread use of UV-filters in several personal care products, including sunscreens and cosmetics, household products or as industrial additives and its frequent detection in both water and sediments have raised multiple concerns. Their multiple endocrine disruptive activities make them a threat both to biota and humans.

Based in the available in vitro and in vivo toxicity studies and the levels at which they occur in the environment, UV-filters may pose a risk to freshwater ecosystems, with higher risk incidence in some hotspot areas. However, much more information is needed in order to establish a temporal effect in water and long-term exposure in biota. Also, it is known that under certain conditions UV-filters can degrade and form, in some cases, unknown by-products. These by-products may be more toxic than the parent compounds.

Due to the wide dimension of the UV-filter class and the different physico-chemical properties of these compounds, several analytical procedures have been developed so far in order to obtain a reliable multi-residue method to determine different UV-filters in a single extraction. Solid-phase extraction (SPE) has been the favorite procedure and yield to high recoveries, probably due to its simple procedure and versatility in the sorbents and solvents that can be used. However, this technique when compared with microextraction methodologies is not environmentally friendly, considering the great amounts of solvents used and can be time consuming. On the other hand, techniques like DLLME and SPME, that are often used, need small amounts of solvents and sample and often deliver good results. Passive sampling using either SPMDs or POCIS was found to be a good method to indirectly analyze UV-filters in water, more specifically for lipophilic compounds. Extraction from these devices is mostly by dialysis and recoveries are usually high.

UV-filters were found, to date, in water bodies, soil and sediments. However, most studies have focused on the occurrence in water. In natural waters they are detected in higher concentrations in river water and are especially detected benzophenones BP1,

BP2, BP3 and BP3 with concentrations up to 0.3 mg L^{-1}. There are few studies on sediments and soils, but those that exist show that benzophenones 4HB and BP were found at higher concentrations, up to 0.02 mg g^{-1} dw. Studies on biota had already been extensively reviewed in 2012. However, a small overview was performed since then. These latest studies showed that fish presents concentration levels up to 11.9 µg g^{-1} dw for crylene OC and around 1 µg g^{-1} dw for BP3. Other compounds such as 4-MBC, BMDM, UV-326, EMC and EDP were detected at relevant concentrations (from 10 to 200 ng g^{-1} dw). Relevant concentrations of UV-filters were also detected in mussels for EMC (1765 ng g^{-1} dw), ODP (833 ng g^{-1} dw) and OC (3992 ng g^{-1} dw). Clams, urchins and prawns also showed the presence of BP3 (up to 100 ng g^{-1} dw). Although different type of marine biota is being studied there's a lack of information in terrestrial biota in order to evaluate the potential bioaccumulation and biomagnification of these compounds.

Attending to the massive use of these compounds and their occurrence in the environment, new approaches should be developed in order to reduce discharges into the environment and/or submit them under new legislation.

Acknowledgments

This work was funded by FEDER funds through the Operational Programme for Competitiveness Factors — COMPETE, ON.2 — O Novo Norte — North Portugal Regional Operational Programme and National Funds through FCT — Foundation for Science and Technology under the projects: PEst-C/EQB/UI0511, NORTE-07-0124-FEDER-000025 — RL2_Environment & Health. Vera Homem would like to thank Fundação para a Ciência e a Tecnologia (FCT — Portugal) for the post-doctoral grant SFRH/BPD/76974/2011 co-funded by the QREN-POPH.

Supplementary Information

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2015.04.055 or in Annex 1.

References

Abdelraheem, W.H.M., He, X., Duan, X., Dionysiou, D.D., 2015. Degradation and mineralization of organic UV absorber compound 2-phenylbenzimidazole-5-sulfonic acid (PBSA) using UV-254 nm/H2O2. J. Hazard. Mater. 282, 233–240.

Amine, H., Gomez, E., Halwani, J., Casellas, C., Fenet, H., 2012. UV filters, ethylhexyl methoxycinnamate, octocrylene and ethylhexyl dimethyl PABA from untreated wastewater in sediment from eastern Mediterranean river transition and coastal zones. Mar. Pollut. Bull. 64, 2435–2442.

Arukwe, A., Eggen, T., Moder, M., 2012. Solid waste deposits as a significant source of contaminants of emerging concern to the aquatic and terrestrial environments — a developing country case study from Owerri, Nigeria. Sci. Total Environ. 438, 94–102.

Bachelot, M., Li, Z., Munaron, D., Gall, P.L., Casellas, C., Fenet, H., Gomez, E., 2012. Organic UV filter concentrations in marine mussels from French coastal regions. Sci. Total Environ. 420, 273–279.

Balmer, M.E., Buser, H.-R., Müller, M.D., Poiger, T., 2005. Occurrence of some organic UV filters in wastewater, in surface waters, and in fish from Swiss Lakes. Environ. Sci. Technol. 39, 953–962.

Barón, E., Gago-Ferrero, P., Gorga,M., Rudolph, I., Mendoza, G., Zapata, A.M., Díaz-Cruz, S., Barra, R., Ocampo-Duque, W., Páez, M., Darbra, R.M., Eljarrat, E., Barceló, D., 2013. Occurrence of hydrophobic organic pollutants (BFRs and UV-filters) in sediments from South America. Chemosphere 92, 309–316.

Benedé, J.L., Chisvert, A., Giokas, D.L., Salvador, A., 2014a. Development of stir bar sorptive–dispersive microextraction mediated by magnetic nanoparticles and its analytical application to the determination of hydrophobic organic compounds in aqueous media. J. Chromatogr. A 1362, 25–33.

Benedé, J.L., Chisvert, A., Salvador, A., Sánchez-Quiles, D., Tovar-Sánchez, A., 2014b. Determination of UV filters in both soluble and particulate fractions of seawaters by dispersive liquid–liquid microextraction followed by gas chromatography–mass spectrometry. Anal. Chim. Acta 812, 50–58.

Bester, K., 2007. Personal Care Compounds in the Environment: Pathways, Fate and Methods for Determination. Wiley — John Wiley & Sons.

Blüthgen, N., Meili, N., Chew, G., Odermatt, A., Fent, K., 2014. Accumulation and effects of the UV-filter octocrylene in adult and embryonic zebrafish (Danio rerio). Sci. Total Environ. 476–477, 207–217.

Brausch, J.M., Rand, G.M., 2011. A reviewof personal care products in the aquatic environment: environmental concentrations and toxicity. Chemosphere 82, 1518–1532.

Brooke, D.N., Burns, J.S., Crookes, M.J., 2008. UV-filters in Cosmetics — Prioritisation for Environmental Assessment. Environment Agency.

Chisvert, A., Pascual-Martí, M.C., Salvador, A., 2001. Determination of the UV filters worldwide authorised in sunscreens by high-performance liquid chromatography: use of cyclodextrins as mobile phase modifier. J. Chromatogr. A 921, 207–215.

Christen, V., Zucchi, S., Fent, K., 2011. Effects of the UV-filter 2-ethyl-hexyl-4-trimethoxycinnamate (EHMC) on expression of genes involved in hormonal pathways in fathead minnows (*Pimephales promelas*) and link to vitellogenin induction and histology. Aquat. Toxicol. 102, 167–176.

Ciszmas, L., McDonald, T.J., Phillips, T.D., Guillespie, A.M., Lingenfelter, R.A., Kubena, L.F., Phillips, T.D., Donnelly, K.C., 2004. Toxicity characterization of complex mixtures using biological and chemical analysis in preparation for assessment of mixture similarity. Environ. Sci. Technol. 38, 5127–5133.

Crista, D.M.A., Miranda, M.S., Esteves da Silva, J.C.G., 2014. Degradation in chlorinated water of the UV filter 4-tert-butyl-4'-methoxydibenzoylmethane present in commercial sunscreens. Environ. Technol. 9, 1–8.

da Silva, C.P., Emídio, E.S., de Marchi, M.R.R., 2015. Method validation using weighted linear regression models for quantification of UV filters in water samples. Talanta 131, 221–227.

De Laurentiis, E., Minella, M., Sarakha, M., Marrese, A., Minero, C., Mailhot, G., Brigante, M., Vione, D., 2013. Photochemical processes involving the UV absorber benzophenone-4 (2-hydroxy-4-methoxybenzophenone-5-sulphonic acid) in aqueous solution: reaction pathways and implications for surface waters. Water Res. 47, 5943–5953.

Díaz-Cruz, M.S., Barceló, D., 2009. Chemical analysis and ecotoxicological effects of organic UV-absorbing compounds in aquatic ecosystems. TrAC 28, 708–717.

Díaz-Cruz, M.S., Llorca, M., Barceló, D., Barceló, D., 2008. Organic UV filters and their photodegradates, metabolites and disinfection by-products in the aquatic environment. TrAC Trends Anal. Chem. 27, 873–887.

Díaz-Cruz, M.S., Gago-Ferrero, P., Llorca, M., Barceló, D., 2012. Analysis of UV filters in tap water and other clean waters in Spain. Anal. Bioanal. Chem 402, 2325–2333.

Directive C, 1998. COUNCIL DIRECTIVE 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. Off. J. Eur. Communities 32–53.

Doong, R., Chang, S., Sun, Y., 2000. Solid-phase microextraction and headspace solid phase microextraction for the determination of high molecular-weight polycyclic aromatic hydrocarbons in water and soil samples. J. Chromatogr. Sci. 38, 528–534.

Duirk, S.E., Bridenstine, D.R., Leslie, D.C., 2013. Reaction of benzophenone UV filters in the presence of aqueous chlorine: kinetics and chloroform formation. Water Res. 47, 579–587.

Emnet, P., Gaw, S., Northcott, G., Storey, B., Graham, L., 2015. Personal care products and steroid hormones in the Antarctic coastal environment associated with two Antarctic research stations, McMurdo Station and Scott Base. Environ. Res. 136, 331–342.

EPA, 2012a. 5. Estimating Physical/Chemical and Environmental Fate Properties with EPI Suite™. Sustainable Futures/Pollution Prevention (P2) Framework Manual. EPA-748-B12-001. U.S. Environmental Protection Agency, OCSPP.

EPA, 2012b. EPI Suite TM. EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC), United States.

Fent, K., Kunz, P.Y., Goméz-Hernandez, E.M., 2008. UV filters in the aquatic environment induce hormonal effects and affect fertility and reproduction in fish. Chimia 5, 368–375.

Fent, K., Zenker, A., Rapp, M., 2010. Widespread occurrence of estrogenic UV-filters in aquatic ecosystems in Switzerland. Environ. Pollut. 158, 1817–1824.

Gackowska, A., Przybyłek, M., Studziński,W., Gaca, J., 2014. Experimental and theoretical studies on the photodegradation of 2-ethylhexyl 4-methoxycinnamate in the presence of reactive oxygen and chlorine species. Cent. Eur. J. Chem. 12, 612–623.

Gago-Ferrero, P., Diaz-Cruz, M.S., Barceló, D., 2011. Fast pressurized liquid extraction with in-cell purification and analysis by liquid chromatography tandem mass spectrometry for the determination of UV filters and their degradation products in sediments. Anal. Bioanal. Chem. 400, 2195–2204.

Gago-Ferrero, P., Diaz-Cruz, M.S., Barceló, D., 2012. An overview of UV-absorbing compounds (organic UV filters) in aquatic biota. Anal. Bioanal. Chem. 404, 2597–2610.

Gago-Ferrero, P., Diaz-Cruz, M.S., Barceló, D., 2013a. Liquid chromatography–tandem mass spectrometry for themulti-residue analysis of organic UV filters and their transformation products in the aquatic environment. Anal. Methods 5, 355–366.

Gago-Ferrero, P., Mastroianni, N., Díaz-Cruz, M.S., Barceló, D., 2013b. Fully automated determination of nine ultraviolet filters and transformation products in natural waters and wastewaters by on-line solid phase extraction–liquid chromatography–tandem mass spectrometry. J. Chromatogr. A 1294, 106–116.

Gago-Ferrero, P., Díaz-Cruz, M.S., Barceló, D., 2013c. Multi-residue method for trace level determination of UV filters in fish based on pressurized liquid extraction and liquid chromatography–quadrupole-linear ion trap-mass spectrometry. J. Chromatogr. A 1286, 93–101.

Gago-Ferrero, P., Díaz-Cruz, M.S., Barceló, D., 2015. UV filters bioaccumulation in fish from Iberian river basins. Sci. Total Environ. 518–519, 518–525.

Gao, L., Yuan, T., Zhou, C., Cheng, P., Bai, Q., Ao, J., Wang, W., Zhang, H., 2013. Effects of four commonly used UV filters on the growth, cell viability and oxidative stress responses of the *Tetrahymena thermophila*. Chemosphere 93, 2507–2513.

Ge, D., Lee, H.K., 2012. A new 1-hexyl-3-methylimidazolium tris(pentafluoroethyl) trifluorophosphate ionic liquid based ultrasound-assisted emulsification microextraction for the determination of organic ultraviolet filters in environmental water samples. J. Chromatogr. A 1251, 27–32.

Giokas, D.L., Sakkas, V.A., Albanis, T.A., 2004. Determination of residues of UV filters in natural waters by solid-phase extraction coupled to liquid chromatography–photodiode array detection and gas chromatography–mass spectrometry. J. Chromatogr. A 1026, 289–293.

Giokas, D.L., Sakkas, V.A., Albanis, T.A., Lampropoulou, D.A., 2005. Determination of UVfilter residues in bathing waters by liquid chromatography UV-diode array and gas chromatography–mass spectrometry after micelle mediated extraction–solvent back extraction. J. Chromatogr. A 1077, 19–27.

Giokas, D.L., Salvador, A., Chisvert, A., 2007. UV filters: from sunscreens to human body and the environment. TrAC 26, 360–374.

Goksoyr, A., Tollefsen, K.E., Grung, M., Loken, K., Lie, E., Zenker, A., Fent, K., Schlabach, M., Huber, S., 2009. Balsa raft crossing the Pacific finds low contaminant levels. Environ. Sci. Technol. 43, 4783–4790.

Grabicova, K., Fedorova, G., Burkina, V., Steinbach, C., Schmidt-Posthaus, H., Zlabek, V., Kocour, K.H., Grabic, R., Randak, T., 2013. Presence of UV filters in surface water and the effects of phenylbenzimidazole sulfonic acid on rainbow trout (*Oncorhynchus mykiss*) following a chronic toxicity test. Ecotoxicol. Environ. Saf. 96, 41–47.

Groz, M.P., Bueno, M.J.M., Rosain, D., Fenet, H., Casellas, C., Pereira, C., Maria, V., Bebianno, M.J., Gomez, E., 2014. Detection of emerging contaminants (UV filters, UV stabilizers and musks) in marine mussels from Portuguese coast by QuEChERS extraction and GC–MS/MS. Sci. Total Environ. 493, 162–169.

Ho, Y.-C., Ding,W.-H., 2012. Solid-phase extraction coupled simple on-line derivatization gas chromatography–tandem mass spectrometry for the determination of benzophenone-type UV filters in aqueous samples. J. Chin. Chem. Soc. 59, 107–113.

Jansen, R., Osterwalder, U.,Wang, S.Q., Burnett,M., Lim, H.W., 2013. Photoprotection: part II. Sunscreen: development, efficacy, and controversies. J. Am. Acad. Dermatol. 69, 867, 1-14.

Jeon, H.-K., Chung, Y., Ryu, J.-C., 2006. Simultaneous determination of benzophenone type UV filters in water and soil by gas chromatography–mass spectrometry. J. Chromatogr. A 1131, 192–202.

Ji, Y., Zhou, L., Zhang, Y., Ferronato, C., Brigante, M., Mailhot, G., Yang, X., Chovelon, J.M., 2013. Photochemical degradation of sunscreen agent 2-phenylbenzimidazole-5-sulfonic acid in different water matrices. Water Res. 47, 5865–5875.

Jiménez-Díaz, I., Zafra-Gómez, A., Ballesteros, O., Navalón, A., 2014. Analytical methods for the determination of personal care products in human samples: an overview. Talanta 129, 448–458.

Jurado, A., Gago-Ferrero, P., Vàzquez-Suñé, E., Carrera, J., Pujades, E., Díaz-Cruz, M.S., Barceló, D., 2014. Urban groundwater contamination by residues of UV filters. J. Hazard. Mater. 271, 141–149.

Kaiser, D., Sieratowicz, A., Zielke, H., Oetken,M., Hollert, H., Oehlmann, J., 2012. Ecotoxicological effect characterisation of widely used organic UV filters. Environ. Pollut. 163, 84–90.

Kameda, Y., Kimura, K., Miyazaki, M., 2011. Occurrence and profiles of organic sun blocking agents in surface waters and sediments in Japanese rivers and lakes. Environ. Pollut. 159, 1570–1576.

Kasprzyk-Hordern, B., Dinsdale, R., Guwy, A., 2008a. Multiresidue methods for the analysis of pharmaceuticals, personal care products and illicit drugs in surface water and wastewater by solid-phase extraction and ultra performance liquid chromatography–electrospray tandem mass spectrometry. Anal. Bioanal. Chem. 391, 1293–1308.

Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2008b. The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. Water Res. 42, 3498–3518.

Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2009. The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters.Water Res. 43, 363–380.

Kawaguchi, M., Ito, R., Honda, H., Endo, N., Okanouchi, N., Saito, K., Seto, Y., Nakazawa, H., 2008. Simultaneous analysis of benzophenone sunscreen compounds in water sample by stir bar sorptive extraction with *in situ* derivatization and thermal desorption–gas chromatography–mass spectrometry. J. Chromatogr. A 1200, 260–263.

Kim, S., Choi, K., 2014. Occurrences, toxicities, and ecological risks of benzophenone-3, a common component of organic sunscreen products: a mini-review. Environ. Int. 70, 143–157.

Kim, S., Jung, D., Kho, Y., Choi, K., 2014. Effects of benzophenone-3 exposure on endocrine disruption and reproduction of Japanese medaka (Oryzias latipes)—a two generation exposure study. Aquat. Toxicol. 155, 244–252.

Krause, M., Klit, A., Blomberg Jensen, M., Søeborg, T., Frederiksen, H., Schlumpf, M., Lichtensteiger, W., Skakkebaek, N.E., Drzewiecki, K.T., 2012. Sunscreens: are they beneficial for health? An overview of endocrine disrupting properties of UV-filters. Int. J. Androl. 35, 424–436.

Lambropoulou, D.A., Giokas, D.L., Sakkas, V.A., Albanis, T.A., Karayannis, M.I., 2002. Gas chromatographic determination of 2-hydroxy-4-methoxybenzophenone and octyldimethyl-p-aminobenzoic acid sunscreen agents in swimming pool and bathing waters by solid-phase microextraction. J. Chromatogr. A 967, 243–253.

Langford, K.H., Reid, M.J., Fjeld, E., Øxnevad, S., Thomas, K.V., 2015. Environmental occurrence and risk of organic UV filters and stabilizers in multiple matrices in Norway. Environ. Int. 80, 1–7.

Li, W., Ma, Y., Guo, C., Hu, W., Liu, K., Wang, Y., Zhu, T., 2007. Occurrence and behavior of four of the most used sunscreen UV filters in a wastewater reclamation plant. Water Res. 41, 3506–3512.

Li, J., Ma, L., Tang, M., Xu, L., 2013. C12-Agwire as solid-phase microextraction fiber for determination of benzophenone ultraviolet filters in river water. J. Chromatogr. A 1298, 1–8.

Liu, J.-L., Wong, M.-H., 2013. Pharmaceuticals and personal care products (PPCPs): a review on environmental contamination in China. Environ. Int. 59, 208–224.

Liu, H., Liu, L., Xiong, Y., Yang, X., Luan, T., 2010. Simultaneous determination of UV filters and polycyclic musks in aqueous samples by solid-phase microextraction and gas chromatography–mass spectrometry. J. Chromatogr. A 1217, 6747–6753.

Liu, Y.-S., Ying, G.-G., Shareef, A., Kookana, R.S., 2011. Simultaneous determination of benzotriazoles and ultraviolet filters in ground water, effluent and biosolid samples using gas chromatography–tandem mass spectrometry. J. Chromatogr. A 1218, 5328–5335.

Liu, Q., Chen, Z., Wei, D., Du, Y., 2014. Acute toxicity formation potential of benzophenone type UV filters in chlorination disinfection process. J. Environ. Sci. 26, 440–447.

Lowe, N.J., Shaath, N.A., Pathak, M.A., 1996. Sunscreens: Development: Evaluation, and Regulatory Aspects. Second edition. CRC Press, p. 744.

Manova, E., von Goetz, N., Hauri, U., Bogdal, C., Hungerbuhler, K., 2013. Organic UV filters in personal care products in Switzerland: a survey of occurrence and concentrations. Int. J. Hyg. Environ. Health 216, 508–514.

Maya, F., Horstkotte, B., Estela, J.M., Cerdà, V., 2014. Automated in-syringe dispersive liquid–liquid microextraction. TrAC Trends Anal. Chem. 59, 1–8.

Moeder, M., Schrader, S., Winkler, U., Rodil, R., 2010. At-line microextraction by packed sorbent-gas chromatography–mass spectrometry for the determination of UV filter and polycyclic musk compounds in water samples. J. Chromatogr. A 1217, 2925–2932.

Moreta, C., Tena, M.T., 2011. Determination of UV filters in packaging by focused ultrasonic solid–liquid extraction and liquid chromatography. J. Chromatogr. A 1218, 3392–3399.

Negreira, N., Canosa, P., Rodríguez, I., Ramil, M., Rubí, E., Cela, R., 2008. Study of some UV filters stability in chlorinated water and identification of halogenated by-products by gas chromatography–mass spectrometry. J. Chromatogr. A 1178, 206–214.

Negreira, N., Rodríguez, I., Ramil, M., Rubí, E., Cela, R., 2009. Sensitive determination of salicylate and benzophenone type UV filters in water samples using solid-phase microextraction, derivatization and gas chromatography tandem mass spectrometry. Anal. Chim. Acta 638, 36–44.

Nguyen, K.T., Scapolla, C., Di Carro, M., Magi, E., 2011. Rapid and selective determination of UV filters in seawater by liquid chromatography–tandem mass spectrometry combined with stir bar sorptive extraction. Talanta 85, 2375–2384.

Ojeda, C.B., Rojas, F.S., 2009. Separation and preconcentration by dispersive liquid–liquid microextraction procedure: a review. Chromatographia 69, 1149–1159.

Oliveira, H.M., Segundo, M.A., Lima, J.L.F.C., Miró, M., Cerdà, V., 2010. On-line renewable solid-phase extraction hyphenated to liquid chromatography for the determination of UV filters using bead injection and multi syringe-lab-on-valve approach. J Chromatogr A 1217, 3575–3582.

Ozáez, I., Martínez-Guitarte, J.L., Morcillo, G., 2014. The UV filter benzophenone 3 (BP-3) activates hormonal genesmimicking the action of ecdysone and alters embryo development in the insect *Chironomus riparius* (Diptera). Environ. Pollut. 192, 19–26.

Pal, A., He, Y., Jekel, M., Reinhard, M., Gin, K.Y.-H., 2014. Emerging contaminants of public health significance as water quality indicator compounds in the urban water cycle. Environ. Int. 71, 46–62.

Paredes, E., Perez, S., Rodil, R., Quintana, J.B., Beiras, R., 2014. Ecotoxicological evaluation of four UV filters using marine organisms from different trophic levels *Isochrysis galbana, Mytilus galloprovincialis, Paracentrotus lividus,* and *Siriella armata*. Chemosphere 104, 44–50.

Peck, A.M., 2006. Analytical methods for the determination of persistent ingredients of personal care products in environmental matrices. Anal. Bioanal. Chem. 386, 907–939.

Pedrouzo, M., Borrull, F., Marcé, R., Pocurull, E., 2010. Stir-bar-sorptive extraction and ultra-high-performance liquid chromatography–tandem mass spectrometry for simultaneous analysis of UV filters and antimicrobial agents in water samples. Anal. Bioanal. Chem. 397, 2833–2839.

Peng, X., Jin, J., Wang, C., Ou, W., Tang, C., 2015. Multi-target determination of organic ultraviolet absorbents in organism tissues by ultrasonic assisted extraction and ultra-high performance liquid chromatography–tandem mass spectrometry. J. Chromatogr. A 1384, 97–106.

Pietrogrande, M.C., Basaglia, G., 2007. GC–MS analytical methods for the determination of personal-care products in water matrices. Trends Anal. Chem 26, 1086–1094.

Poiger, T., Buser, H.-R., Balmer, M.E., Bergqvist, P.-A., Müller, M.D., 2004. Occurrence of UV filter compounds from sunscreens in surface waters: regional mass balance in two Swiss lakes. Chemosphere 55, 951–963.

Ponzo, O.J., Silvia, C., 2013. Evidence of reproductive disruption associated with neuroendocrine changes induced by UV-B filters, phtalates and nonylphenol during sexual maturation in rats of both gender. Toxicology 311, 41–51.

PROGRAM N.T., 2006. Toxicology and carcinogenesis studies of benzophenone (CAS No. 119-61-9) in F344/N rats and B6C3F1 mice (feed studies). Natl. Toxicol. Program Tech. Rep. Ser. 1–264.

Regulation (EC) No 1223/2009, 2009. Cosmetic ingredient database (Cosing) - List of UV filters allowed in cosmetic products. The Official Journal of the European Union.

Ricking, M., Schwarzbauer, J., Franke, S., 2003. Molecular markers of anthropogenic activity in sediments of the Havel and Spree Rivers (Germany).Water Res. 37, 2607–2617.

Rodil, R., Moeder, M., 2008a. Development of a method for the determination of UV filters in water samples using stir bar sorptive extraction and thermal desorption–gas chromatography–mass spectrometry. J. Chromatogr. A 1179, 81–88.

Rodil, R., Moeder, M., 2008b. Development of a simultaneous pressurised-liquid extraction and clean-up procedure for the determination of UV filters in sediments. Anal. Chim. Acta 612, 152–159.

Rodil, R., Moeder, M., Altenburger, R., Schmitt-Jansen, M., 2009a. Photostability and phytotoxicity of selected sunscreen agents and their degradation mixtures in water. Anal. Bioanal. Chem. 395, 1513–1524.

Rodil, R., Schrader, S., Moeder, M., 2009b. Non-porous membrane-assisted liquid–liquid extraction of UV filter compounds from water samples. J. Chromatogr. A 1216, 4887–4894.

Rodil, R., Quintana, J.B., Concha-Graña, E., López-Mahía, P., Muniategui-Lorenzo, S., Prada-Rodríguez, D., 2012. Emerging pollutants in sewage, surface and drinking water in Galicia (NW Spain). Chemosphere 86, 1040–1049.

Rodríguez, A.S., Sanz, M.R., Rodríguez, J.R.B., 2015. Occurrence of eight UV filters in beaches of Gran Canaria (Canary Islands). An approach to environmental risk assessment. Chemosphere 131, 85–90.

Román, I.P., Chisvert, A., Canals, A., 2011. Dispersive solid-phase extraction based on oleic acid-coated magnetic nanoparticles followed by gas chromatography–mass spectrometry for UV-filter determination in water samples. J. Chromatogr. A 1218, 2467–2475.

Sakkas, V.A., Giokas, D.L., Lambropoulou, D.A., Albanis, T.A., 2003. Aqueous photolysis of the sunscreen agent octyl-dimethyl-*p*-aminobenzoic acid: formation of disinfection by products in chlorinated swimming pool water. J. Chromatogr. A 1016, 211–222.

Salvador, A., Chisvert, A., 2005. Sunscreen analysis: a critical survey on UV filters determination. Anal. Chim. Acta 537, 1–14.

Sánchez-Brunete, C., Miguel, E., Albero, B., Tadeo, J.L., 2011. Analysis of salicylate and benzophenone-type UV filters in soils and sediments by simultaneous extraction cleanup and gas chromatography–mass spectrometry. J. Chromatogr. A 1218, 4291–4298.

Santos, A.J., Miranda, M.S., Esteves da Silva, J.C., 2012. The degradation products of UV filters in aqueous and chlorinated aqueous solutions. Water Res. 46, 3167–3176.

Santos, A.J.M., Crista, D.M.A., Miranda, M.S., Almeida, I.F., Sousa e Silva, J.P., Costa, P.C., Amaral, M.H., Lobão, P.A.L., Lobão, P.A.L., Sousa Lobo, J.M., Esteves da Silva, J.C.G., 2013. Degradation of UV filters 2-ethylhexyl-4-methoxycinnamate and 4-tertbutyl-4'-methoxydibenzoylmethane in chlorinated water. Environ. Chem. 10, 127–134.

Sayre, R.M., Dowdy, J.C., Gerwig, A.J., Shields,W.J., Lloyd, R.V., 2005. Unexpected photolysis of the sunscreen octinoxate in the presence of the sunscreen avobenzone. Photochem. Photobiol. 81, 452–456.

Schlumpf,M., Schmid, P., Durrer, S., Conscience,M., Maerkel, K., Henseler, M., Gruetter,M., Herzog, I., Reolon, S., Ceccatelli, R., Faass, O., Stutz, E., Jarry, H., Wuttke, W., Lichtensteiger, W., 2004. Endocrine activity and developmental toxicity of cosmetic UV filters — an update. Toxicology 205, 113–122.

Schreurs, R., Lanser, P., Seinen,W., van der Burg, B., 2002. Estrogenic activity of UV filters determined by an in vitro reporter gene assay and an in vivo transgenic zebrafish assay. Arch. Toxicol. 76, 257–261.

Serpone, N., Salinaro, A., Emeline, A.V., Horikoshi, S., Hidaka, H., Zhao, J., 2002. An in vitro systematic spectroscopic examination of the photostabilities of a random set of commercial sunscreen lotions and their chemical UVB/UVA active agents. Photochem. Photobiol. Sci. 1, 970–981.

Sieratowicz, A., Kaiser, D., Behr, M., Oetken, M., Oehlmann, J., 2011. Acute and chronic toxicity of four frequently used UV filter substances for *Desmodesmus subspicatus* and *Daphnia magna*. J. Environ. Sci. Health A 46, 1311–1319.

Sobek, A., Bejgarn, S., Rudén, C., Molander, L., c, 2013. In the shadow of the Cosmetic Directive — inconsistencies in EU environmental hazard classification requirements for UV-filters. Sci. Total Environ. 461–462, 706–711.

Søeborg, T., Basse, L.H., Halling-Sørensen, B., 2007. Risk assessment of topically applied products. Toxicology 236, 140–148.

Tarazona, I., Chisvert, A., León, Z., Salvador, A., 2010. Determination of hydroxylated benzophenone UV filters in sea water samples by dispersive liquid–liquid microextraction followed by gas chromatography–mass spectrometry. J. Chromatogr. A 1217, 4771–4778.

Tashiro, Y., Kameda, Y., 2013. Concentration of organic sun-blocking agents in seawater of beaches and coral reefs of Okinawa Island, Japan. Mar. Pollut. Bull. 77, 333–340.

Tovar-Sánchez, A., Sánchez-Quiles, D., Basterretxea, G., Benedé, J.L., Chisvert, A., Salvador, A., Moreno-Garrido, I., Blasco, J., 2013. Sunscreen products as emerging pollutants to coastal waters. PLoS One 8, e65451.

Tsai, D.-Y., Chen, C.-L., Ding, W.-H., 2014. Optimization of matrix solid-phase dispersion for the rapid determination of salicylate and benzophenone-type UV absorbing substances in marketed fish. Food Chem. 154, 211–216.

Tsui, M.M., Leung, H.W., Wai, T.C., Yamashita, N., Taniyasu, S., Liu, W., Lam, P.K., Murphy, M.B., 2014. Occurrence, distribution and ecological risk assessment of multiple classes of UV filters in surface waters from different countries. Water Res. 67c, 55–65.

Vidal, L., Chisvert, A., Canals, A., Salvador, A., 2010. Ionic liquid-based single-drop microextraction followed by liquid chromatography–ultraviolet spectrophotometry detection to determine typical UV filters in surface water samples. Talanta 81, 549–555.

Vione, D., Caringella, R., De Laurentiis, E., Pazzi, M., Minero, C., 2013. Phototransformation of the sunlight filter benzophenone-3 (2-hydroxy-4-methoxybenzophenone) under conditions relevant to surface waters. Sci. Total Environ. 463–464, 243–251.

Weisbrod, C.J., Kunz, P.Y., Zenker, A.K., Fent, K., 2007. Effects of the UV filter benzophenone-2 on reproduction in fish. Toxicol. Appl. Pharmacol. 225, 255–266.

Wick, A., Fink, G., Ternes, T.A., 2010. Comparison of electrospray ionization and atmospheric pressure chemical ionization for multi-residue analysis of biocides, UV-filters and benzothiazoles in aqueous matrices and activated sludge by liquid chromatography–tandem mass spectrometry. J. Chromatogr. A 1217, 2088–2103.

Wong, C.S., MacLeod, S.L., 2009. Recent advances in analysis of pharmaceuticals in the aquatic environment. J. Environ. Monit. 11, 923–936.

Wu, J.-W., Chen, H.-C., Ding, W.-H., 2013. Ultrasound-assisted dispersive liquid–liquid microextraction plus simultaneous silylation for rapid determination of salicylate and benzophenone-type ultraviolet filters in aqueous samples. J. Chromatogr. A 1302, 20–27.

Ye, L., Liu, J., Yang, X., Peng, Y., Xu, L., 2011. Orthogonal array design for the optimization of ionic liquid-based dispersive liquid–liquid microextraction of benzophenone-type UV filters. J. Sep. Sci. 34, 700–706.

Zenker, A., Schmutz, H., Fent, K., 2008. Simultaneous trace determination of nine organic UV-absorbing compounds (UV filters) in environmental samples. J. Chromatogr. A 1202, 64–74.

Zhang, H., Lee, H.K., 2012a. Simultaneous determination of ultraviolet filters in aqueous samples by plunger-in-needle solid-phase microextraction with graphene-based sol–gel coating as sorbent coupled with gas chromatography–mass spectrometry. Anal. Chim. Acta 742, 67–73.

Zhang, Y., Lee, H.K., 2012b. Ionic liquid-based ultrasound-assisted dispersive liquid–liquid microextraction followed high-performance liquid chromatography for the determination of ultraviolet filters in environmental water samples. Anal. Chim. Acta 750, 120–126.

Zhang, Y., Lee, H.K., 2013. Determination of ultraviolet filters in environmental water samples by temperature-controlled ionic liquid dispersive liquid-phase microextraction. J. Chromatogr. A 1271, 56–61.

Zhang, P.-P., Shi, Z.-G., Yu, Q.-W., Feng, Y.-Q., 2011. A new device for magnetic stirring assisted dispersive liquid–liquid microextraction of UV filters in environmental water samples. Talanta 83, 1711–1715.

Chapter 2. A review of organic UV-filters in wastewater treatment plants

Sara Ramos, Vera Homem, Arminda Alves, Lúcia Santos,

Environment International, 86, pp. 24–44 (2016)

Abstract

UV-filters are a group of compounds which have been massively used in the past years due to the recent concerns with sunburns, premature skin ageing and the risk of developing skin cancer, related to sun exposure. At the moment, these compounds have been identified by the scientific community as emerging pollutants, due to their persistence in the environment, potential to accumulate in biota and potential threat as endocrine disruptors. At some point, the majority of sunscreens will find their way into wastewater (due to bathing and washing activities) and because wastewater treatment plants (WWTPs) are not able to remove and/or degrade them, consequently they find their way into rivers, lakes and ocean, so it is not surprising that UV-filters are found in the environment. Therefore, wastewater treatment plants should be the focus of the scientific community aiming to better understand the fate of the UV-filters and develop new technologies to remove them from wastewater and sludge. This review aims to provide the current state of the art in the occurrence and fate of UV-filters in wastewater treatment plants and how the technologies that are being used are successfully removing these compounds from both wastewater and sludge.

Keywords: UV-filters; Wastewater treatment plants; Occurrence; Fate

2.1. Introduction

Personal care products (PCPs) have gained growing interest in recent years due to their increased production and consumption (Giokas et al., 2007). Since the 1920s with the new wave of Coco Chanel known as the 'Chanel look', the bronzed look became fashionable (Wolf et al., 2001). However, overexposure to ultraviolet (UV) radiation has been the main cause of several skin disorders such as sunburn, photo-ageing and even skin cancer. The growth in public awareness of these hazards led to an increase in the availability of UV protection products containing organic UV-filters (Langford et al., 2015). Organic UV-filters are compounds used to absorb UVA and/or UVB radiation (also called 'UV-absorbers'), while inorganic UV-filters mainly scatter and reflect the radiation (Serpone et al., 2007). They are present in sunscreens and cosmetics to prevent skin damage under sunlight irradiation, but they are also used as sun blocking agents for the protection of several materials such as plastics, adhesives and rubber (Brooke et al., 2008; Gackowska et al., 2014; Kupper et al., 2006). The amount and type of UV-filters incorporated into cosmetic formulations depend on the desired degree of sun protection factor (SPF) and protection wavelength range (UVA, UVB). However, there combined concentrations should not exceed 10% with other organic or inorganic UV-filters (Santos et al., 2013).

Nowadays, UV-filters are considered emerging contaminants due to their widespread presence in the environment and because of the unknown risks associated with their presence (Liu and Wong, 2013; Ramos et al., 2015). UV-filters' occurrence have been reported in several matrices such as river water (Gago-Ferrero et al., 2013a,b; Grabicova et al., 2013; Tsui et al., 2014a,b), lake water (Moeder et al., 2010; Rodil et al., 2009; Zhang et al., 2011), sea water (Benedé et al., 2014; Tsui et al., 2014a,b), groundwater (Gago-Ferrero et al., 2013a; Ho and Ding, 2012; Jurado et al., 2014), sediments (Amine et al., 2012; Barón et al., 2013; Gago-Ferrero et al., 2011a) and even biota (Blüthgen et al., 2014; Christen et al., 2011; Kim et al., 2014). The main issue of concern of these compounds are their potential toxicity and adverse effects, namely as xenohormone affecting reproductive activity (Bester, 2007; Kaiser et al., 2012; Paredes et al., 2014; Schlumpf et al., 2004; Sieratowicz et al., 2011; Weisbrod et al., 2007). Camphor derivative 4-MBC has shown multiple hormonal activities in vitro (estrogenic and

antiestrogenic), as well as 2-hydroxy-4-methoxybenzophenone (BP3) and HMS (antiandrogenic) (Schlumpf et al., 2004). An extensive description on these effects has been provided by Díaz-Cruz and Barceló (2009).

Although recreational activities are direct input pathways of UV-filters into the environment, the major source of contamination are the effluents from the wastewater treatment plants (WWTPs) and also sewage sludge, which is frequently used as fertilizer in agriculture or simply disposed in landfills. It is, therefore, essential to understand the UV-filters' behaviour in WWTPs and define the best removal mechanisms, in order to reduce the quantities released into the environment. For that reason, the aim of this work is to systematize the main results and conclusions obtained on the levels and fate of organic UV-filters in WWTPs.

2.2. UV-filters occurrence and fate in wastewater treatment plants

The number of papers studying UV-filters in WWTPs has been growing in the past couple of years (Figure 2.1). Improvements in analytical methodologies, allowing lower detection limits, helped the scientific community to became aware that these compounds are not easily degraded in WWTPs and their physicochemical properties allows them to persist in the environment which may cause long-term negative impacts on biota and human health. Scientific publications regarding the occurrence and fate of UV-filters in WWTPs between 2000 and 2015 were retrieved and the information compiled in Table 2.1, in order to facilitate the analysis. A brief description of the used extraction and clean-up methods was also complimentary included, although this topic has already been systematically reviewed by Zuloaga et al. (2012) and Ramos et al. (2015).

To better understand the influence of physicochemical properties on the fate of the organic UV-filters within this review, the most important properties (such as log K_{OW}, solubility and boiling points) are presented in Annex (Annex 2). Additionally, an overview of current legislation regarding incorporation of these compounds in cosmetics in Europe (Cosmetics Directive, 2009), USA (21CFR352.10, 2014) and Japan (MHW, 2000) was also included.

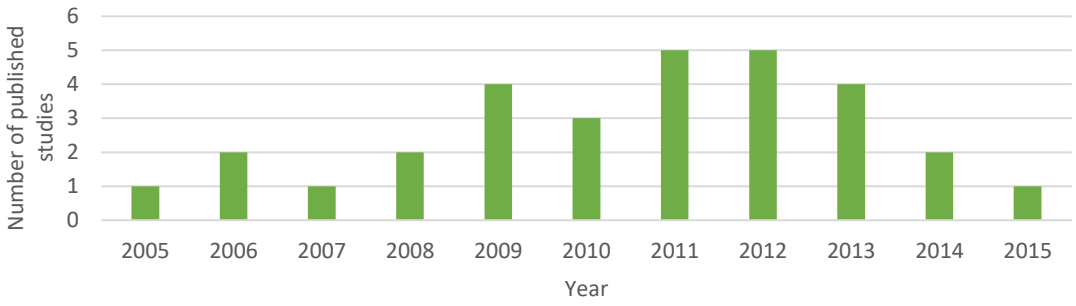


Figure 2.1 Number of published studies per year studying UV-filters in WWTPs.

Table 2.1 Overview occurrence and fate of UV-filters in wastewater treatment plants.

Compounds	Country	Method overview	LOD (ng L^{-1})	Recovery (%)	Type of WWTP/inhabitants/type of treatment	Influent (ng L^{-1})	Effluent (ng L^{-1})	Sludge (ng g^{-1} dw)	Reference
ES, HMS, IMS, 4-MBC, BP3, BP1, BP8, EDP, 234THB, EMC, OC, BMDM	Portugal	**Extraction method:** DLLME (10 mL sample, pH 3, Ac (dispersant), TCE (extractant)) Derivatization (Microwave, 600W, 5 min, 40 µL BSTFA, 1% TMCS) **Instrumental method:** GC-MS	ES, IMS, 4-MBC, BP8, EDP: 6 HMS, BP3, EMC: 2 BP1, OC: 3 234THB: 23 BMDM: 26	ES: 91 - 103 HMS: 76 - 89 IMS: 89 - 101 4-MBC: 93 - 99 BP3: 93 - 105 BP1: 59 - 99 BP8: 60 - 78 EDP: 69 - 79 234THB: 55 - 68 EMC: 61 - 101 OC: 75 - 104 BMDM: 69 - 105	Domestic, industrial (residual) (41955 inhabitants) -Secondary treatment - Activated Sludge with conventional aeration)	4-MBC, BP1: n.d. BP3: 71.3 – 234.2 EDP: 51.5 - 197 EMC: 47.9 OC, BMDM: <LOQ	4-MBC, BP1, OC, BMDM: n.d. BP3: <LOQ – 22.2 EDP: < LOQ EMC: 57.8	-	(Cunha et al., 2015)
					Domestic, industrial (mainly) (10000 inhabitants) -Tertiary treatment - Activated Sludge with extended aeration	4-MBC, BP1: n.d. BP3: 6.6 – 273.3 EDP: 28.2 EMC: 37.9 OC: <LOQ BMDM: 495	4-MBC, BP1, EDP, EMC, OC: n.d. BP3: <LOQ - 136 BMDM: <LOQ		
					Domestic, industrial (residual) (57748 inhabitants) - Secondary treatment with UV disinfection - Activated Sludge with extended aeration	4-MBC, BP1: n.d. BP3: 5.4 – 98.1 EDP: 24.9 EMC: 41.6 OC: <LOQ BMDM: 195	4-MBC, BP1, EDP, OC: n.d. BP3: <LOQ – 16.4 EMC: 35.1 BMDM: <LOQ		
					Domestic, hospital and industrial (45257 inhabitants) - Secondary treatment with UV disinfection - Activated Sludge with medium load aeration	4-MBC: 45.8 BP3: 29.5 - 237 BP1: n.d. EDP: 38.3 EMC: 133.3 OC: <LOQ – 689.6 BMDM: 312.2	4-MBC: <LOQ BP3: <LOQ – 32.5 BP1, EDP: n.d. EMC: 54 OC: 154.3 BMDM: 93.2		
					Domestic and industrial (255557 inhabitants) -Tertiary treatment with UV disinfection -Activated Sludge with conventional and extended aeration	4-MBC, BP1, BMDM: n.d. BP3: 31.2 – 188 EDP: 22 – 139.9 EMC: 48.7 OC: <LOQ	4-MBC, BP1, EMC, OC, BMDM: n.d. BP3: <LOQ – 36.1 EDP: <LOQ		
					Domestic (300000 inhabitants) -Tertiary treatment with UV disinfection -Activated Sludge with extended aeration	4-MBC, BMDM: n.d. BP3: 61.2 BP1: 88.5 – 184.4 EDP: 34.8 – 87.2 EMC: 222.5 OC: 88.1 – 247.6	4-MBC, BP1, EDP, OC, BMDM: n.d. BP3: <LOQ EMC: 49.5		
					Domestic and industrial (213000 inhabitants) -Secondary treatment -Trickling Filters	4-MBC: 84.6 BP3: 16.9 - 126 BP1, EDP, BMDM: n.d. EMC: 32.8 OC: <LOQ	4-MBC: <LOQ BP3: <LOQ - 60 BP1, EDP, OC, BMDM: n.d. EMC: 42.7		
					Domestic, hospital and industrial (6850 inhabitants) -Secondary treatment -Activated Sludge	4-MBC, BP1: n.d. BP3:15.8 - 60 EDP: 12.2 – 23.2 EMC: 46.1; OC: <LOQ BMDM: 71.1 – 1247.5	4-MBC, BP1, EDP, OC: n.d. BP3: <LOQ – 13.1 EMC: 37.4 BMDM: 44.5 – 62.6		

Table 2.1 Overview occurrence and fate of UV-filters in wastewater treatment plants (cont).

Compounds	Country	Method overview	LOD (ng L^{-1})	Recovery (%)	Type of WWTP/inhabitants/type of treatment	Influent (ng L^{-1})	Effluent (ng L^{-1})	Sludge (ng g^{-1} dw)	Reference
ES, HMS, IMS, 4-MBC, BP3, BP1, BP8, EDP, 234THB, EMC, OC, BMDM	Portugal	**Extraction method:** DLLME (10 mL sample, pH 3, Ac (dispersant), TCE (extractant)) Derivatization (Microwave, 600W, 5 min, 40 µL BSTFA, 1% TMCS) **Instrumental method:** GC-MS	ES, IMS, 4-MBC, BP8, EDP: 6 HMS, BP3, EMC: 2 BP1, OC: 3 234THB: 23 BMDM: 26	ES: 91 - 103 HMS: 76 - 89 IMS: 89 - 101 4-MBC: 93 - 99 BP3: 93 - 105 BP1: 59 - 99 BP8: 60 - 78 EDP: 69 - 79 234THB: 55 - 68 EMC: 61 - 101 OC: 75 - 104 BMDM: 69 - 105	Domestic and industrial -Secondary treatment with biofiltration -Activated Sludge	4-MBC, BP1, EMC, BMDM: n.d. BP3: 48.8 – 152.5 EDP: 12.9 OC: <LOQ	4-MBC, BP1, EDP, EMC, OC, BMDM: n.d. BP3: <LOQ	-	(Cunha et al., 2015)
					Domestic and industrial -Tertiary treatment with disinfection -Activated Sludge	4-MBC, BP1, EDP, BMDM: n.d. BP3: 32.9 – 323.3 EMC: 689.5 OC: <LOQ	4-MBC, BP1, EDP, OC, BMDM: n.d. BP3: <LOQ EMC: 483.4		
					Domestic -Secondary treatment with disinfection -Biofiltration	4-MBC, BP1, EDP: n.d. BP3: 80.8 - 171 EMC: 159.3 OC: 49.1 – 687 BMDM: 2935	4-MBC, BP1, EDP: n.d. BP3: 17.2 – 68.2 EMC: 153.9 OC: 353 – 357.4 BMDM: 168.1		
					Domestic, hospital and industrial (60000 inhabitants) -Secondary treatment with disinfection -Activated Sludge with medium load aeration	4-MBC, BP1, EMC, BMDM: n.d. BP3: 57.6 - 150 EDP: 66.4 OC: <LOQ	4-MBC, BP1, EDP, EMC, OC, BMDM: n.d. BP3: 16.6 – 36.5		
					Domestic (8700 inhabitants) -Secondary treatment with disinfection -Activated Sludge with extended aeration	4-MBC, BP1, EMC, BMDM: n.d. BP3: 40 - 90 EDP: 92.8 – 315.3 OC: <LOQ	4-MBC, BP1, EDP, EMC, OC, BMDM: n.d. BP3: <LOQ – 60.1		
					Domestic (49547 inhabitants) -Secondary treatment with UV disinfection -Activated Sludge with extended aeration	4-MBC: 154.9 BP3: 31.8 – 178.6 BP1: n.d. EDP: 32.2 - 418 EMC: 147.7 OC: <LOQ – 785.5 BMDM: 507	4-MBC: <LOQ BP3: 21.6 – 31.3 BP1, EDP: n.d. EMC: 46.1 OC: 124.6 BMDM: <LOQ		
					Domestic (30766 inhabitants) -Secondary treatment with UV disinfection -Lagoons with extended aeration	4-MBC: 48.6 BP3: 84 – 256.7 BP1: 408.5 EDP, BMDM: n.d. EMC: 93.7 OC: <LOQ - 546	4-MBC: <LOQ BP3: <LOQ – 35.7 BP1, EDP, EMC, OC, BMDM: n.d.		

Table 2.1 Overview occurrence and fate of UV-filters in wastewater treatment plants (cont).

Compounds	Country	Method overview	LOD (ng L^{-1})	Recovery (%)	Type of WWTP/inhabitants/type of treatment	Influent (ng L^{-1})	Effluent (ng L^{-1})	Sludge (ng g^{-1} dw)	Reference
BP3, EDP, EMC, OC, UV-234, UV-327, UV-328, UV-329	Norway	**Extraction method for wastewater:** SPE (1 L sample, 200 mg, 6 mL HLB cartridges, EA/DCM (1:1)) **Extraction method for sludge:** Freeze-dried samples PLE (1.0 g PSA, Hex/DCM (1:1) (extraction solvent), 100 °C, 5 min, 3 cycles) GPC (Alliance 2695 system, DCM (mobile phase), 12.1 – 20.0 min collected fractions) Additional cleaning (100 mg PSA, centrifuge 21,000 g, 10 min) **Instrumental method:** LC-HRMS GC-HRMS	Wastewater BP3, UV-327: 10 EDP, EMC, OC, UV-234, UV-328, UV-329: 5 Sludge BP3, UV-234, UV-327, UV-328, UV-329: 10 EDP: 4 EMC, OC: 5	Wastewater BP3: 78 EDP: 49 EMC: 64 OC: 78 UV-234: 14 UV-327: 34 UV-328: 76 UV-329: 67 Sludge BP3: 72 EDP: 81 EMC: 98 OC: 102 UV-234: 78 UV-327: 114 UV-328: 89 UV-329: 100	WWTP1 – Urban (domestic) (580 000 inhabitants) - Treatments: mechanical, chemical, biological (pos-denitrification) - Sludge: anaerobic digestion and drying WWTP2 – Urban (domestic) (52 000 inhabitants) - Treatments: mechanical, biological (no nitrogen removal), chemical - Sludge: thermal hydrolysis, 160 °C, prior to anaerobic digestion at 38 °C. WWTP3 – Urban (domestic) (38 400 inhabitants) - Primary treatment	-	WWTP1 BP3: 81 – 598 OC: 181 - 538 EDP, EMC, UV-234, UV-328, UV-329: <5 UV-327: <10 WWTP2 BP3: 10 - 438 OC: 7-227 EDP, EMC UV-234, UV-328, UV-329: <5 UV-327: <10 WWTP3 BP3: 374-1915 EMC: 4.3 - 37 OC: 1701 - 6969 UV-234: 4.5 – 5.6 UV-327: <10 EDP, UV-328, UV-329: <5	WWTP1 EMC: 551 - 793 OC: 3449 - 12661 UV-327: 30.4 – 77.1 UV-329: 1172 – 3075 BP3: < 10 EDP: < 4 UV-234, UV-328: <11 WWTP2 BP3:824-2116 EDP: <10 EMC: 2501 - 4689 OC: 26823 -41610 UV-234: <14 UV-327: 83.3 – 159.9 UV-328: <25 UV-329: 1493 - 3303	(Langford et al., 2015)
BMDM, BP1, BP3, BP4, EMC, ODP, OC, BP8, IMC, ES, 4-MBC, HMS	China	**Extraction method:** Filtration (glass fiber filters) SPE (250 mL sample, 500 mg C_{18} cartridges, MeOH/EA (1:1)) **Instrumental method:** HPLC-ESI-MS/MS	Influent ODP: 0.31 4-MBC: 3.46 BMDM: 0.97 EMC: 1.63 IMC: 14.09 OC: 66.62 BP3: 1.61 ES: 63.87 BP4: 0.59 HMS: 38.95 BP1: 15.41 BP8: 4.97 Effluent ODP: 0.11 4-MBC: 1.58 BMDM: 0.44 EMC: 0.85 IMC: 11.64 OC: 5.91 BP3: 0.60 ES: 4.18 BP4: 0.60 HMS: 3.75 BP1: 7.54 BP8: 2.32	Influent ODP: 64 - 76 4-MBC: 82 - 85 BMDM: 67 - 76 EMC: 77 - 78 IMC: 85 - 93 OC: 72 - 77 BP3: 90 - 92 ES: 69 - 70 BP4: 98 - 99 HMS: 73 - 74 BP1: 94 - 103 BP8: 98 - 102 Effluent ODP: 66 - 69 4-MBC: 81 BMDM: 75 - 76 EMC: 73 - 79 IMC: 80 - 82 OC: 72 - 79 BP3: 94 - 95 ES: 71 BP4: 101 - 102 HMS: 70 - 72 BP1: 93 - 97 BP8: 90 - 94	Urban (domestic) (3 500 000 inhabitants) - Primary treatment (Flocculation and sedimentation with ferric (III) chloride and polymers; Chlorination) Urban (domestic) (600 000 inhabitants) - Primary treatment (primary sedimentation) - Secondary treatment (Modified Ludzack-Ettinger system with UV-disinfection)	ODP: 39.2 – 258.9 4-MBC: <LOD – 288.6 BMDM: 93.9 – 169.4 EMC: 295.3 – 1134.4 IMC: <LOD – 111.1 BP3: 159.5 – 371.3 OC, ES: <LOD BP4: 620.1 – 945.7 HMS: 61.4 – 262.3 BP1: 23.3 – 168.9 BP8: <LOD – 121.7 ODP: 43.3 – 136.9 4-MBC: 67.1 – 350.0 BMDM:58.1 – 257.1 EMC: 119.5 – 558.6 IMC, OC: <LOD BP3: 141.1 – 374.1 ES: <LOD – 1188.3 BP4: 601.1 – 904.7 HMS: 75.6 – 1650.4 BP1: 114.8 – 240.1 BP8: 61 – 174.2	ODP: 47.7 – 140.5 4-MBC: <LOD – 181.8 BMDM: 17.5 – 59.4 EMC: 86.9 – 492.1 IMC: <LOD – 56.7 BP3: 34.3 – 115.8 OC, ES: <LOD BP4: 374.5 – 457.1 HMS: <LOD – 153.9 BP1: 19.6 – 146.4 BP8: <LOD – 83.5 ODP: <LOD – 77.7 4-MBC: <LOD – 118.0 BMDM: 27.8 – 99.3 EMC: 90.0 – 174.4 IMC, OC: <LOD BP3: 18.4 – 67.5 ES: <LOD – 128.9 BP4: 343.3 – 496.8 HMS: <LOD – 154.2 BP1: <LOD – 122.0 BP8: 10.0 – 60.4	-	(Tsui et al., 2014)

Table 2.1 Overview occurrence and fate of UV-filters in wastewater treatment plants (cont).

Compounds	Country	Method overview	LOD (ng L^{-1})	Recovery (%)	Type of WWTP/inhabitants/type of treatment	Influent (ng L^{-1})	Effluent (ng L^{-1})	Sludge (ng g^{-1} dw)	Reference
BMDM, BP1, BP3, BP4, EMC, ODP, OC, BP8, IMC, ES, 4-MBC, HMS	China	**Extraction method:** Filtration (glass fiber filters) SPE (250 mL sample, 500 mg C_{18} cartridges, MeOH/EA (1:1)) **Instrumental method:** HPLC-ESI-MS/MS	Influent ODP: 0.31 4-MBC: 3.46 BMDM: 0.97 EMC: 1.63 IMC: 14.09 OC: 66.62 BP3: 1.61 ES: 63.87 BP4: 0.59 HMS: 38.95 BP1: 15.41 BP8: 4.97 Effluent ODP: 0.11 4-MBC: 1.58 BMDM: 0.44 EMC: 0.85 IMC: 11.64 OC: 5.91 BP3: 0.60 ES: 4.18 BP4: 0.60 HMS: 3.75 BP1: 7.54 BP8: 2.32	Influent ODP: 64 - 76 4-MBC: 82 - 85 BMDM: 67 - 76 EMC: 77 - 78 IMC: 85 - 93 OC: 72 - 77 BP3: 90 - 92 ES: 69 - 70 BP4: 98 - 99 HMS: 73 - 74 BP1: 94 - 103 BP8: 98 - 102 Effluent ODP: 66 - 69 4-MBC: 81 BMDM: 75 - 76 EMC: 73 - 79 IMC: 80 - 82 OC: 72 - 79 BP3: 94 - 95 ES: 71 BP4: 101 - 102 HMS: 70 - 72 BP1: 93 - 97 BP8: 90 – 94	Urban (domestic) (300 000 inhabitants) - Primary treatment - Secondary treatment (SBR) - Tertiary treatment (fine suspended solids filtration by dual-media filter containing carbon and sand; UV-disinfection; chlorination	ODP: <LOD 4-MBC: <LOD BMDM: 256.6 EMC: 104.7 IMC: <LOD OC: <LOD BP3: 113.8 ES: <LOD BP4: <LOD HMS: <LOD BP1: 37.0 BP8: <LOD	ODP: <LOD 4-MBC: <LOD BMDM: <LOD EMC: <LOD IMC: <LOD OC: <LOD BP3: 19.3 ES: <LOD BP4: <LOD HMS: <LOD BP1: <LOD BP8: <LOD	-	(Tsui et al., 2014)
					Urban (domestic) (110 000 inhabitants) - Primary treatment - Secondary treatment (Modified Ludzack-Ettinger system with UV-disinfection) - Additional treatments (Reverse-osmosis)	ODP: 73.8 – 346.4 4-MBC: 101.9 – 320.8 BMDM: 44.4 – 194.8 EMC: 249.3 – 755.9 IMC: <LOD – 71.2 OC: <LOD – 131.5 BP3: 155.7 – 450.7 ES: <LOD – 218.3 BP4: 426.5 – 872.8 HMS: 93.7 – 404.8 BP1: 204.9 – 281.3 BP8: <LOD – 89.3	ODP: <LOD – 94.4 4-MBC, IMC, OC, ES, BP8: <LOD BMDM: 15.5 – 92.7 EMC: <LOD – 105.8 BP3: 25.6 – 55.2 BP4:218.9 – 466.4 HMS: <LOD – 21.0 BP1: 64.1 – 89.8	-	
					Urban (domestic) (40 000 inhabitants) - Primary treatment (screening grit with diameter> 6 mm)	ODP: <LOD – 376.9 4-MBC: 70.5 – 335.4 BMDM: 35.0 – 1290.2 EMC: 50.2 – 989.8 IMC: 29.4 – 226 OC, ES, BP8: <LOD BP3: 116.3 – 576.5 BP4: 389.2 – 576.5 HMS: <LOD – 149.8 BP1: 85.7 – 172.9	ODP: <LOD – 224.3 4-MBC:<LOD–207.2 BMDM:27.9–1018.3 EMC: 36.1 – 505.2 IMC: <LOD – 165.5 OC, ES, BP8: <LOD BP3: 91.0 – 541.1 BP4: 312.4 – 409.3 HMS: <LOD – 93.3 BP1: 56.1 – 155.0	-	
PBSA, BP1, BP3, BP4	Czech Republic	**Extraction method:** Samples frozen until analysis Filtration (0.45 um, regenerated cellulose filters) Internal Standard added (10 ng to 10 mL sample) **Instrumental method:** LC-MS/MS	LOQ PBSA: 20 BP1: 30 BP3, BP4: 40	-	Urban (domestic) (>95%) + Industrial (<5%) (112 000 inhabitants) -Secondary treatment (biological activated sludge with partial nitrification and thermophile anaerobic sludge stabiliozation)	-	-	-	(Golovko et al., 2014)

Table 2.1 Overview occurrence and fate of UV-filters in wastewater treatment plants (cont).

Compounds	Country	Method overview	LOD (ng L^{-1})	Recovery (%)	Type of WWTP/inhabitants/type of treatment	Influent (ng L^{-1})	Effluent (ng L^{-1})	Sludge (ng g^{-1} dw)	Reference
BP3, BP1, 4HB, 4DHB, BP8, BP2, BP4, 4-MBC, Et-PABA	Spain	**Extraction method:** Filtration On line-SPE (5 mL sample, PLRP-s polymer cartridge, H_2O + ACN, both with 0.1% formic acid) **Instrumental method:** LC-MS/MS	Influent BP3: 5.0 BP1: 8.0 4HB: 8.0 4DHB: 9.0 BP8: 7.0 BP2: 7.0 BP4: 6.0 4-MBC: 10.0 Et-PABA: 5.0 Effluent BP3: 1.5 BP1: 2.5 4HB: 1.5 4DHB: 3.5 BP8: 1.5 BP2: 3.0 BP4: 1.0 4-MBC: 4.0 Et-PABA: 2.5	Influent BP3: 96 - 106 BP1: 101 - 105 4HB: 70 - 76 4DHB: 70 - 88 BP8: 81 - 87 BP2: 70 - 80 BP4: 102 - 105 4-MBC: 98 - 102 Et-PABA: 110 - 112 Effluent BP3: 101 - 111 BP1:86 - 100 4HB: 77 - 92 4DHB: 70 - 80 BP8: 74 - 78 BP2: 84 - 85 BP4: 108 - 111 4-MBC:99 - 103 Et-PABA:105 - 110	Urban (domestic) (154 103 – 1 142 103 inhabitants) - Primary treatment - Secondary treatment (conventional activated sludge) - Tertiary treatment	BP3: 75.6 – 306 BP1: 152.4 - 722 BP4: 738 - 1548 4-MBC: <LOQ – 48.3 Et-PABA: 17 –120.9 4HB, 4DHB, BP8, BP2: n.d.	BP3: 7.71 - 34 BP1: <LOQ – 31.1 BP4: n.d. - 1420 4-MBC: <LOQ – 23.8 Et-PABA, 4HB, 4DHB, BP8, BP2: n.d.	-	(Gago-Ferrero et al., 2013)
ES, HMS, BP3,BP1, BP8	Taiwan	**Extraction method:** UA-DLLME (10 mL sample, pH 7, 0.5 g NaCl, Ac (dispersant), TCE (extractant)) Derivatization (20 µL BSTFA, 15 min) **Instrumental method:** GC-MS	ES: 2 HMS: 2 BP3: 1.5 BP1: 1 BP8: 1	ES: 73 - 75 HMS: 73 - 74 BP3: 82 BP1: 85 - 87 BP8: 89 - 91	Urban (domestic) (380 000 inhabitants)	-	ES: n.d. – 6.1 BP3: 12.5 – 21.4 BP1: 7.7 – 16.8 BP8: 9.8 – 10.1 HMS: n.d.	-	(Wu et al., 2013)
BP3, OC, ODP, EMC, ES, HMS	Italy	**Extraction method:** SBSE-LD (50 mL sample, pH 6, room temperature, 800 rpm, 5h; MeOH pre-conditioned stir bar; desorption: MeOH, 30 min, room temperature, 800 rpm) **Instrumental method:** LC-MS/MS	BP3: 1.5 OC: 1.6 ODP: 1.2 EMC: 2.6 HMS: 152 ES: 199	BP3: 61 OC: 71 ODP: 97 EMC: 105 HMS: 108 ES: 90	Urban (domestic) (631 000 inhabitants)	BP3: 6 - 163 OC: 12 - 390 ODP: <LOD - 4 EMC: <LOD 48 HMS, ES: -	BP3: 5 - 28 OC: 4 - 126 ODP, EMC, HMS, ES: -	-	(Magi et al., 2013)

Table 2.1 Overview occurrence and fate of UV-filters in wastewater treatment plants (cont).

Compounds	Country	Method overview	LOD (ng L^{-1})	Recovery (%)	Type of WWTP/inhabitants/type of treatment	Influent (ng L^{-1})	Effluent (ng L^{-1})	Sludge (ng g^{-1} dw)	Reference
BP, BP3, OC	Australia	**Extraction method for wastewater:** SPE **Extraction method for sludge:** Freeze-dried samples Dried samples turned into dust Clean-up (vortex 3 min, ultrasound 10 min at 40 °C (2x); DCM + MeOH) **Instrumental method:** GC-MS/MS	-	-	Syntetic wastewater: -Membrane bioreactor (MBR)	-	-	-	(Wijekoon et al., 2013)
BP1, 4DHB, 4HB, BP3, 4-MBC, OC, ODP, EMC	Spain	**Extraction method:** Freeze-dried and grinded samples PLE (1 g sample, Al_2O_3, preheating of 5 min, 2 static cycles of 5 min with MeOH, 2 static cycles of 5 min using MeOH/H_2O (1:1) at 100 °C, 10,000 kPa) **Instrumental method:** UPLC–ESI-MS/MS	4-MBC: 12 OC: 18 EMC: 19 ODP: 0.2 BP3: 1.0 BP1: 60 4HB: 5.0 4DHB: 5.0	4-MBC: 102 OC: 70 EMC: 90 ODP: 85 BP3: 70 BP1: 30 4HB: 95 4DHB: 96	Urban (domestic) (2 000 000 inhabitants) -Secondary treatment: biological activated sludge plant with sludge anaerobic digestion, 26 days (HRT) and thermal dehydration -Stabilized sewage sludge used in agriculture	-	-	Raw sludge 4DHB: 70 BP3: 60 4-MBC: 3100 OC: 8000 EMC: 2200 BP1, 4HB, ODP: n.d. Treated sludge 4-MBC: 250 OC: 570 EMC: 100 BP1, 4DHB, 4HB, BP3, ODP: n.d.	(Badia-Fabregat et al., 2012)
BP3, BP1, BP8	Taiwan	**Extraction method:** SPE (100 mL sample, 60 mg HLB cartridges, EA, ACN, EA/DCM (1:1; 2:1)) Derivatization (1 µL MSTFA, 70 °C, 2.5 min) **Instrumental method:** GC-MS/MS	BP3: 0.3 BP1: 0.5 BP8: 1.0	BP3: 98 – 103 BP1: 81 – 96 BP8: 77 – 108	Urban (domestic) (380 000 inhabitants) - Primary treatment (mechanical clarification; flocculation filtration)	-	BP3: n.d. – 3.6 BP1: 1.5 – 1.7 BP8: n.d.	-	(Ho and Ding, 2012)
4-MBC, PBSA, BP3, EMC, OC, ODP, BP4, IMC	Spain	**Extraction method:** Filtration SPE (200-500 mL sample, 200 mg HLB cartridges, MeOH) **Instrumental method:** LC-ESI-MS/MS	-	-	WWTP1: Urban (domestic) (1 000 inhabitants): -Pre-treatment -Secondary activated Sludge Treatment WWTP2: Urban (domestic) (500 000 inhabitants): -Pre-treatment WWTP 3: Rural (domestic) (4 000 inhabitants): -Pre-treatment -Secondary activated Sludge Treatment	BP4:2100 PBSA: 200 4-MBC, BP3: < 90 OC, ODP, IMC, EMC: n.d.	BP4: 1200 PBSA: 240 4-MBC: 5 BP3, OC, ODP, IMC, EMC: n.d.	-	(Rodil et al., 2012)

Table 2.1 Overview occurrence and fate of UV-filters in wastewater treatment plants (cont).

Compounds	Country	Method overview	LOD (ng L^{-1})	Recovery (%)	Type of WWTP/inhabitants/type of treatment	Influent (ng L^{-1})	Effluent (ng L^{-1})	Sludge (ng g^{-1} dw)	Reference
BP3, 4-MBC, EMC, UV-326, UV-329, OC	Australia	**Extraction method for wastewater:** Sample acidification (MeOH, pH 2, 4 M H_2SO_4) Filtration (glass fiber filters) SPE (1 L sample, 500 mg HLB cartridges, MeOH/DCM (1:1)) **Extraction method for sludge:** 1 g sodium azide added Freeze-dried samples PLE (1.0 g, Na_2SO_4, Si, copper powder, acid washed sand, Hex/DCM (1:1) (extraction solvent), 120 ◦C, 5 min, 2 cycles) **Instrumental method:** GC-MS/MS	Influent BP3: 3.2 4-MBC: 0.7 EMC: 0.5 UV-326: 4.1 UV-329: 5.6 OC: 2.8 Effluent BP-3: 6.5 4-MBC: 0.5 EMC: 0.7 UV-326: 3.3 UV-329: 4.8 OC: 3.4 Sludge(ng g^{-1}) BP3: 7.3 4-MBC: 2.8 EMC: 0.7 UV-326: 0.3 UV-329: 8.2 OC: 3.6	Influent BP3: 121 - 133 4-MBC: 82 - 119 EMC: 75 - 98 UV-326: 97 - 105 UV-329: 96 - 108 OC: 85 - 96 Effluent BP3: 119 - 127 4-MBC: 88 - 96 EMC: 82 - 91 UV-326: 95 - 110 UV-329: 97 - 101 OC: 84 - 93 Sludge BP3: 94 - 130 4-MBC: 68 - 81 EMC: 76 - 90 UV-326: 81 - 96 UV-329: 125 - 152 OC: 82 - 91	Urban (domestic) (75%) + Industrial (25%) (1 300 000 inhabitants): - Primary treatment (sedimentation) - Secondary treatment (activated sludge; stabilization lagoons; dissolved air flotation /filtration)	BP-3: 1059 – 3112 4-MBC: 394 – 406 EMC: 106 – 319 UV-326: 15 – 35 UV-329: 227 – 414 OC: 88 – 89	Primary effluent: BP3: 1053 – 2469 4-MBC: 368 – 404 EMC: 11 – 384 UV-326: 5 – 39 UV-329: 52 – 125 OC: 65 – 141 Secondary effluent: BP3: 54 – 488 4-MBC: 17 – 140 EMC: <LOD – 53 UV-326: <LOD – 55 UV-329: <LOD – 98 OC: <LOD – 73 Lagoon effluent: BP3: 36 – 363 4-MBC: <LOD – 91 EMC, UV-326, UV-329, OC: <LOD Final effluent: BP3: 32 – 273 4-MBC: n.d. – 90 EMC, UV-326, UV-329, OC: n.d.	Influent: BP3: 104 – 111 4-MBC: 341 - 403 EMC: 218 - 229 UV-326: 81 - 90 UV-329: 91 - 93 OC: 303 - 326 Primary sludge: BP3: 120 - 201 4-MBC: 743 - 1031 EMC: 207 - 312 UV-326: 101 - 111 UV-329: 71 - 96 OC: 438 - 561 Secondary sludge: BP3: 189 – 1785 4-MBC: 64 – 171 EMC: 50 – 62 UV-326: 48 – 114 UV-329: 79 – 27 OC: 281 – 443 Digested sludge: BP3: 149 – 303 4-MBC: 958 – 2020 EMC: 385 – 401 UV-326: 52 – 60 UV-329: 64 – 74 OC: 1147 – 1838 Lagoon sludge: BP3: 18 4-MBC: 701 EMC: 53 UV-326: 89 UV-329: 72 OC: 317 Biosolid: BP3: 16 4-MBC: 962 EMC: 30 UV-326: 88 UV-329: 27 OC: 465	(Liu et al., 2012)

Table 2.1 Overview occurrence and fate of UV-filters in wastewater treatment plants (cont).

Compounds	Country	Method overview	LOD (ng L^{-1})	Recovery (%)	Type of WWTP/inhabitants/type of treatment	Influent (ng L^{-1})	Effluent (ng L^{-1})	Sludge (ng g^{-1} dw)	Reference
BP1, 4DHB, BP3, 4-MBC, OC, ODP, EMC	Spain	**Extraction method:** Freeze-dried and grinded samples PLE (1 g sample, Al_2O_3 (purification step); MeOH (extraction solvent)) Filtration **Instrumental method:** UPLC-TQD	-	-	Urban (domestic) (2 000 000 inhabitants) Biological activated sludge (sludge anaerobic digestion; thermal dehydration) Bioslurry reactor with *T. versicolor*	-	-	Raw sludge: BP1: 0.08 4DHB: 0.051 BP3: 0.034 4-MBC: 0.520 OC: 7.71 ODP: 0.012 EMC: 1.031 Treated sludge: BP1: n.d. 4DHB: 0.050 BP3: 0.019 4-MBC: 0.205 OC: 3.214 ODP: 0.004 EMC: 0.211	(Rodríguez-Rodríguez et al., 2012)
BP1, 4DHB, 4HB, BP3, 4-MBC, OC, ODP, EMC	Spain	**Extraction method:** Freeze-dried and grinded samples PLE (1 g sample, Al_2O_3, preheating of 5 min, 2 static cycles of 5 min with MeOH, 2 static cycles of 5 min using MeOH/H_2O (1:1) at 100 ◦C, 10,000 kPa) **Instrumental method:** UPLC–ESI-MS/MS	4-MBC: 12 OC: 18 EMC: 19 ODP: 0.2 BP3: 1.0 BP1: 60 4HB: 5.0 4DHB: 5.0	4-MBC: 102 OC: 70 EMC: 90 ODP: 85 BP3: 70 BP1: 30 4HB: 95 4DHB: 96	Urban (domestic) (147 000 inhabitants) 1: Biological with P and N removal; Tertiary treatment; Sludge Treatments: -Anaerobic digestion/centrifuge.	-	-	BP1, 4HB, BP3, ODP, EMC: n.d. 4DHB: <LOQ 4-MBC: 1630 OC: 2600	(Gago-Ferrero et al., 2011)
					Urban (domestic) (198 000 inhabitants) 2: Biological; Tertiary treatment; Sludge Treatments: -Anaerobic digestion/centrifuge.			BP1, 4DHB, 4HB, BP3, ODP: n.d. 4-MBC: 1610 OC: 2870 EMC: 750	
					Urban (domestic) (142 000 inhabitants) 3: Biological with N removal; Sludge Treatments: -Anaerobic digestion.			BP1, 4HB, BP3, ODP: n.d. 4DHB: 70 4-MBC: 3830 OC: 9170 EMC: 1220	
					Urban (domestic) (182 000 inhabitants) 4: Biological with P and N removal; Sludge Treatments: -Anaerobic digestion/centrifuge.			BP1, BP3, ODP: n.d. 4DHB: 40 4HB:150 4-MBC: 1520 OC: 2610 EMC: 780	
					Urban(domestic) (124 000 inhabitants) 5: Biological; Sludge Treatments: -Anaerobic digestion/filter press.			BP1, 4DHB, 4HB, BP3, ODP: n.d. 4-MBC: 2980 OC: 5390 EMC: 1910	
					Urban (domestic) (229 000 inhabitants) 6: Biological;			BP1, 4DHB, 4HB, BP3, ODP: n.d. 4-MBC:3170 OC: 4150; EMC: 1090	

Table 2.1 Overview occurrence and fate of UV-filters in wastewater treatment plants (cont).

Compounds	Country	Method overview	LOD (ng L^{-1})	Recovery (%)	Type of WWTP/inhabitants/type of treatment	Influent (ng L^{-1})	Effluent (ng L^{-1})	Sludge (ng g^{-1} dw)	Reference
BP1, 4DHB, 4HB, BP3, 4-MBC, OC, ODP, EMC	Spain	**Extraction method:** Freeze-dried and grinded samples PLE (1 g sample, Al_2O_3, preheating of 5 min, 2 static cycles of 5 min with MeOH, 2 static cycles of 5 min using MeOH/H_2O (1:1) at 100 ◦C, 10,000 kPa) **Instrumental method:** UPLC–ESI-MS/MS	4-MBC: 12 OC: 18 EMC: 19 ODP: 0.2 BP3: 1.0 BP1: 60 4HB: 5.0 4DHB: 5.0	4-MBC: 102 OC: 70 EMC: 90 ODP: 85 BP3: 70 BP1: 30 4HB: 95 4DHB: 96	Urban (domestic) (205 000 inhabitants) 7: Biological with P removal; Sludge Treatments: -Anaerobic digestion/centrifuge.	-	-	BP1, 4HB, BP3, ODP, EMC: n.d. 4DHB: 620 4-MBC: 1790 OC: 4490	(Gago-Ferrero et al., 2011)
					Urban(177 000 inhabitants) 8: Biological; Sludge Treatments: -Anaerobic digestion/centrifuge.			BP1, 4DHB, 4HB, BP3, ODP, EMC: n.d. 4-MBC: 730 OC: 2860	
					Urban domestic) (87 000 inhabitants) 9: Biological with P and N removal; Tertiary treatment; Sludge Treatments: -Anaerobic digestion/centrifuge.			BP1, 4DHB, 4HB, BP3, ODP: n.d. 4-MBC: 1530 OC: 2160 EMC: 610	
					Urban (domestic) (154 000 inhabitants) 10: Biological; Sludge Treatments: -Anaerobic digestion/centrifuge.			BP1, 4DHB, 4HB, BP3, ODP: n.d. 4-MBC: 1760 OC: 3630 EMC: 1080	
					Urban (domestic) (1 142 000 inhabitants) 11: Biological; Sludge Treatments: -Anaerobic digestion/centrifuge.			BP1, 4DHB, 4HB, BP3, ODP: n.d. 4-MBC: 1840 OC: 6600 EMC: 3350	
					Urban (domestic) (165 000 inhabitants) 12: Biological; Sludge Treatments: -Anaerobic digestion/centrifuge.			BP1, 4DHB, 4HB, BP3, ODP, EMC: n.d. 4-MBC: 1340 OC: 2250	
					Urban (domestic) (272 000 inhabitants) 13: Biological; Sludge Treatments: -Anaerobic digestion/centrifuge.			BP1, 4DHB, 4HB, BP3, ODP: n.d. 4-MBC: 2840 OC: 3860 EMC: 2090	
					Urban (domestic) (84 000 inhabitants) 14: Biological: Sludge Treatments: -Centrifuge.			BP1, 4DHB, 4HB, ODP: n.d. BP3: 790 4-MBC: 810 OC: 3000 EMC: 2010	
					Urban (domestic) 399 000 inhabitants) 15: Biological with P and N removal Sludge Treatments: -Anaerobic digestion/filter press.			BP1, 4HB, BP3, ODP, EMC: n.d. 4DHB: <LOQ 4-MBC: 890 OC: 1060	

Table 2.1 Overview occurrence and fate of UV-filters in wastewater treatment plants (cont).

Compounds	Country	Method overview	LOD (ng L^{-1})	Recovery (%)	Type of WWTP/inhabitants/type of treatment	Influent (ng L^{-1})	Effluent (ng L^{-1})	Sludge (ng g^{-1} dw)	Reference
BP3, BZS, 4-MBC, ODP, EHMC, ES, HMS, OC, BP , UV-326, UV-329, UV-327, UV-328,	Japan	**Extraction method:** Filtration (glass fiber filter) SPE (1.0 L sample, C_{18} silica and Polymerically bonded cartridges, DCM) **Instrumental method:** GC-MS	BP, ODP, UV-326, UV-328: 0.2 HMS: 0.4 BZS: 3.0 4-MBC, BP3, EMC, UV-327: 0.1 UV-329, OC: 0.3	BP: 88.5 HMS: 95.6 BZS: 106.9 4-MBC: 111.2 BP3: 113.2 ODP: 97.4 EMC: 92.5 UV-326: 92.6 UV-329: 102.5 OC: 94.8 UV-327: 88.8 UV-328: 85.7	Urban (domestic) - Primary treatment - Secondary treatment (conventional activated sludge) Sewage Treatment Plant Efflents: 1 - 47 000 inhabitants 2 – 30 000 inhabitants 3 – 300 000 inhabitants 4 – 700 000 inhabitants	-	BP: 8 - 74 BP3: 29 - 164 BZS: 107 - 169 EHMC: n.d. - 12 ES: n.d. – 77 UV-326: 13 UV-327: 2 UV-328: 47 - 88 HMS, 4-MBC, ODP, OC, UV-329: n.d.	-	(Kameda et al., 2011)
BP3, 4-MBC, EMC, UV-326, UV-329, OC	Australia	**Extraction method for wastewater:** Filtration (1 L sample, glass fiber filters) SPE (1 L sample, 500 mg HLB cartridges, MeOH/DCM (1:1)) Filtration (membrane filter) **Extraction method for biosolid:** Freeze-dried samples PLE (1 g sample, Na_2SO_4, silica, copper powder, 120 °C, Hex/DCM (1:1) (extraction solvent), 2 cycles 5 min) Filtration (membrane filter) **Instrumental method:** GC-MS/MS	Effluent BP3: 6.5 4-MBC: 0.5 EMC: 0.7 UV-326: 3.3 UV-329: 4.8 OC: 3.4 Biosolid (ng g^{-1}) BP3: 7.3 4-MBC: 2.8 EMC: 0.7 UV-326: 0.3 UV-329: 8.2 OC: 3.6	Effluent BP3: 119 - 127 4-MBC:88 - 96 EMC: 82 - 91 UV-326: 95 - 110 UV-329: 97 - 101 OC:84 - 93 Biosolid BP3: 94 - 130 4-MBC: 68 - 81 EMC: 76 - 90 UV-326: 81 - 96 UV-329: 125 - 152 OC: 82 – 91	Urban (domestic) (75%) + Industrial (25%) (700 000 inhabitants) - Biosolid production	-	BP3: 32.7 4-MBC, EMC, UV-326, UV-329, OC: < LOQ	BP3: 74.0 4-MBC: 250 EMC: 31.9 UV-326: 49.9 UV-329: 122.9 OC: 138.4	(Liu et al., 2011)
BP1, BP2, BP3, BP4, PBSA	Germany	**Extraction method for wastewater:** Filtration (glass fiber filters) SPE (100 mL sample influent or 200 mL sample effluent, 200 mg HLB cartridge, MeOH/Ac (60:40)) **Extraction method for sludge:** **Freeze-dried samples** Centrifugation (4000 rpm, 15 min) PLE (200 mg sample, baked out sea sand; H_2O/MeOH (1:1), 4 static cycles, 80 ◦C) SPE (200 mg HLB cartridge; MeOH/Ac (3:2)) **Instrumental method:** LC-MS/MS (ESI mode)	Effluent BP1, BP2: 2.5 BP3: 25 BP4, PBSA: 5 Sludge BP1, BP2: 2.5 BP3: 25 BP4, PBSA: 5	Effluent BP1: 105 BP2: 113 BP3: 90 BP4: 106 PBSA: 110 Sludge BP1: 83 BP2: 99 BP3: 108 BP4: 103 PBSA: 118	Urban (domestic) (300 000 inhabitants) - Mechanical treatment (screen, grit removal and primary clarifier) - Trickling filter - Activated sludge treatment (nitrification and denitrification, phosphate removal and a final clarification)	Sludge-water distribution coefficients ($K_{d, sec}$) PBSA: 9 BP1: 260 BP2: 300 BP3: 720 BP4: - (L $kg_{dw\ sludge}^{-1}$)			(Wick et al., 2011)

Table 2.1 Overview occurrence and fate of UV-filters in wastewater treatment plants (cont).

Compounds	Country	Method overview	LOD (ng L^{-1})	Recovery (%)	Type of WWTP/inhabitants/type of treatment	Influent (ng L^{-1})	Effluent (ng L^{-1})	Sludge (ng g^{-1} dw)	Reference
ES, HMS, BP3, 4-MBC, EMC, OC, IMC, ODP	Spain	**Extraction method:** **Freeze-dried samples** PLE (0.5 g sample, diatomaceous earth, graphitized carbon, Hex/DCM (80:20) (extraction solvent), 75 ◦C, 1 cycle, 5 min, 1500 psi) SPE (0.5 g PSA cartridge, Hex/Eth (1:1) (elution); isooctane (keeper)) **Instrumental method:** GC-MS	LOQ (ng g^{-1}) ES: 17 HMS: 34 IMC:34 BP3: 61 4-MBC: 26 EDP:22 EMC: 24 OC: 33	Primary sludge ES: 95 - 101 HMS: 78 - 96 IMC: 80 - 107 BP3: 89 - 106 4-MBC: 79 - 86 EDP: 88 - 93 EMC: 73 - 90 OC: 84 - 85 Biological sludge ES, HMS: 100 - 103 IMC: 90 - 98 BP3: 112 - 100 4-MBC, OC: 91 - 112 EDP: 83 - 104 EMC: 88 - 90	Urban (domestic) Non-digested sludge (primary, secondary and mixtures of both)	-	-	Primary sludge ES, HMS, IMC, BP3, EDP: n.d. 4-MBC: 106 - 1543 EMC: 213 - 3287 OC: 1039 - 2242 Biological sludge ES: 133 - 270 HMS: 110 - 207 IMC, EDP: n.d. BP3: 93 4-MBC: 97 - 1439 EMC: 104 - 856 OC: 377 - 3263	(Negreira et al., 2011)
BP3, 4-MBC, OC, EMC	Germany	**Extraction method:** MEPS (C_{18}, 8x 100 µL pump cycles (sample extraction); H_2O (sorbent wash); pressing air (dry); EA (elution)) **Instrumental method:** PTV-GC-MS	4-MBC: 61 BP3: 53 EMC: 35 OC: 87	4-MBC: 77 BP3: 109 EMC: 107 OC: 65	Urban (domestic) (10 000 inhabitants)	-	4-MBC: 102 BP3: 431 EMC: 332 OC: 461	-	(Moeder et al., 2010)
BP8, BP3, OC, ODP	Spain	**Extraction method:** SBSE-LD (50 mL sample, pH 5, room temperature, 900 rpm, 180 min; PDMS stir bar; desorption: ACN, 15 min, 30 ºC) **Instrumental method:** UHPLC-MS/MS	5	Influent BP8: 25 BP3: 59 OC: 48 ODP: 68 Effluent BP8: 28 BP3: 64 OC: 43 ODP: 72	Urban (domestic) (120 000 inhabitants)	BP8: <LOD - 185 BP3: <LOD - 127 OC: <LOD - 129 ODP: <LOD - 55	BP8: <LOD - 55 BP3: <LOD - <LOQ OC: <LOD - <LOQ ODP: <LOD - 25	-	(Pedrouzo et al., 2010)

Table 2.1 Overview occurrence and fate of UV-filters in wastewater treatment plants (cont).

Compounds	Country	Method overview	LOD (ng L^{-1})	Recovery (%)	Type of WWTP/inhabitants/type of treatment	Influent (ng L^{-1})	Effluent (ng L^{-1})	Sludge (ng g^{-1} dw)	Reference
BP1, BP2, BP3, BP4, PBSA	Germany	**Extraction method for wastewater:** Filtration (glass fiber filters) SPE (100 mL sample influent or 200 mL sample effluent, 200 mg HLB cartridge, MeOH/Ac (60:40)) **Extraction method for sludge:** Freeze-dried samples Centrifugation (4000 rpm, 15 min) PLE (200 mg sample, baked out sea sand; H_2O/MeOH (1:1), 4 static cycles, 80 ◦C) SPE (200 mg HLB cartridge; MeOH/Ac (3:2)) **Instrumental method:** LC-MS/MS (ESI mode)	LOQ Influent BP1, BP2: 5 BP3: 50 BP4, PBSA: 10 Effluent BP1, BP2: 2.5 BP3: 25 BP4, PBSA: 5 Sludge BP1, BP2: 2.5 BP3: 25 BP4, PBSA: 5	Influent BP1: 19 - 180 BP2: 9 - 111 BP3: 42 - 100 BP4: 89 - 105 PBSA: 34 - 96 Effluent BP1: 11 - 93 BP2: 6 - 93 BP3: 46 - 102 BP4: 89 - 105 PBSA: 26 - 66 Sludge BP1: 74 BP2: 99 BP3: 104 BP4: 114 PBSA: 118	Urban (domestic) (320 000 inhabitants) - Mechanical treatment (screen, grit removal and primary clarifier) - Trickling filter - Activated sludge treatment (nitrification and denitrification, phosphate removal and a final clarification)	BP1: 43 BP2: 35 BP3: 195 BP4: 2120 PBSA: 275	BP1: 12 BP2: 14 BP3: 96 BP4: 572 PBSA: 316	BP1: 5.1 BP2: 11 BP3: 132 BP4: 29 PBSA: <LOQ	(Wick et al., 2010)
					Urban (domestic) (307 000 inhabitants) - Mechanical treatment (screen, grit removal and primary clarifier) - Activated sludge treatment (nitrification and denitrification, phosphate removal and a final clarification)	BP1: 488 BP2: 93 BP3: 518 BP4: 55130 PBSA: 3890	BP1, BP2, BP3: <LOQ BP4: 105 PBSA: 1820	-	
BP1, BP2, BP3, BP4	UK	**Extraction method:** Sample acidification (31% HCl to pH 2) + addition of 500 mg Na_2EDTA Filtration SPE (250 mL sample, 5% NH_4OH/MeOH) **Instrumental method:** UPLC-ESI-MS/MS	LOQ Influent BP1: 3 BP2: 18 BP3: 104 BP4: 35 Effluent BP1: 2 BP2: 13 BP3: 80 BP4: 10	Influent BP1: 31 - 43 BP2: 17 - 20 BP3: 39 - 49 BP4: 17 - 50 Effluent BP1: 48 - 54 BP2: 24 - 37 BP3: 50 - 81 BP4: 59 - 118	Urban (domestic) and Industrial (230 000 inhabitants) - Primary treatment - Secondary treatment (activated sludge)	BP1: 51000 - 700000 BP2: 61000 - 403000 BP3: <LOQ - 3975000 BP4: 2218000 - 6084000	BP1: <LOQ - 38000 BP2: <LOQ - 13000 BP3: <LOQ - 223000 BP4: <LOQ - 6325000	-	(Kasprzyk-Hordern et al., 2009)
					Urban (domestic) (500 000 inhabitants) - Primary treatment - Secondary treatment (trickling filter beds)	BP1: 46000 - 400000 BP2: 9000 - 247000 BP3: <LOQ - 1068000 BP4: 1425000 - 13248000	BP1: <LOQ - 41000 BP2: <LOQ - 17000 BP3: <LOQ - 2196000 BP4: 818000 - 4309000		
ES, HMS, BP3, BP1, BP8	Spain	**Extraction method:** HS-SPME (10 mL sample, 65 µm PDMS-DVB fibre, 30 min, room temperature, 1200 rpm, desorption for 3 min at 270 ◦C) Derivatization (20 µL MSTFA, 45 ◦C, 10 min) **Instrumental method:** GC-MS/MS	LOQ ES: 5 HMS: 5 BP3: 0.5 BP1: 10 BP8: 2	Influent ES: 53 HMS: 48 BP1, BP3: 92 BP8: 80 Effluent ES: 95 HMS: 89 BP3: 115 BP1: 97 BP8: 108	Urban (domestic): - Primary treatment; - Activated sludge units	ES: n.d. - 28 BP3: 216 - 462 BP1: 131 - 245	ES: n.d. – 7.5 BP3: n.d. - 44 BP1: n.d. - 41	-	(Negreira et al., 2009)

Table 2.1 Overview occurrence and fate of UV-filters in wastewater treatment plants (cont).

Compounds	Country	Method overview	LOD (ng L^{-1})	Recovery (%)	Type of WWTP/inhabitants/type of treatment	Influent (ng L^{-1})	Effluent (ng L^{-1})	Sludge (ng g^{-1} dw)	Reference
BP3, IMC, 4-MBC, OC, BMDM, EDP, EMC, ES, HMS	Germany	**Extraction method:** MALLE (15 mL sample, MeOH in a bottle,20 mm - membrane bag, PrOH, 500 rpm, 40 °C, 120 min) **Instrumental method:** LC-APPI-MS/MS	BP3: 0.8 IMC: 0.9 4-MBC: 1.7 OC: 8.5 BMDM: 10 EDP: 0.4 EMC: 16 ES, HMS: 4	BP3: 60 - 78 IMC: 90 - 101 4-MBC: 90 - 97 OC: 80 - 92 BMDM: 76 - 94 EDP: 83 - 97 EMC: 98 - 103 ES: 95 - 104 HMS: 92 - 102	Urban (domestic): WWTP1 - Ultrafiltration WWTP2 - Soil filtration WWTP3 - Trickle filter WWTP4 - Sequential batch reactor	BP3: 234 IMC: 66 4-MBC: 278 BMDM: 407 OC: 5322 EDP: <LOD EMC: 1732 ES: 753 HMS: <LOD	WWTP1 BP3: 3 WWTP4 BP3: 19 WWTP2 BP3: 18 4-MBC: 30 WWTP3 BP3: 45 4-MBC: 62 BMDM: 29 OC: 179	-	(Rosario Rodil et al., 2009)
BP3, IMC, 4-MBC, OC, BMDM, ODP, EMC, ES, HMS, DBT, EHT	Belgium	**Extraction method:** PLE (75 g sample, EA) **Instrumental method:** LC-MS/MS	BP3: 8 IMC, EMC: 12 4-MBC: 10 OC: 18 BMDM: 17 ODP: 9 ES, HMS:>1000 DBT: 200 EHT: 52	BP3, IMC: 120 4-MBC: 106 OC: 108 BMDM, ODP: 113 EMC: 105 ES: 95 HMS: 96 DBT: 107 EHT: 124	Dried test material from the Institute for Reference Materials and Measurements	-	-	BP3: 6.6 IMC: 5.0 4-MBC: 3893 OC: 2479 BMDM:144 ODP: 1.4 EMC: 127 ES: 49 HMS: 22 DBT: 136 EHT: 928	(R Rodil et al., 2009)
BP3, IMC, 4-MBC, OC, BMDM, ODP, EMC, ES, HMS, DBT, EHT	Germany	**Extraction method:** PLE (75 g sample, EA) **Instrumental method:** LC-MS/MS	BP3: 8 IMC, EMC: 12 4-MBC: 10 OC: 18 BMDM: 17 ODP: 9 ES, HMS:>1000 DBT: 200 EHT: 52	BP3, IMC: 120 4-MBC: 106 OC: 108 BMDM, ODP: 113 EMC: 105 ES: 95 HMS: 96 DBT: 107 EHT: 124	Urban(domestic) (100 000 inhabitants)	-	-	BP3: 29 IMC: 20.0 4-MBC: 73 OC: 585 BMDM: 517 ODP: 1.9 EMC: 35 ES: 280 HMS: 331 DBT: 54 EHT: 1433	(R Rodil et al., 2009)
BP1, BP2, BP3, BP4	UK	**Extraction method:** Sample acidification (31% HCl to pH 2) + addition of 500 mg Na_2EDTA Filtration SPE (250 mL sample, 5% NH_4OH/MeOH) **Instrumental method:** UPLC-ESI-MS/MS	LOQ Influent BP1: 3 BP2: 18 BP3: 104 BP4: 35 Effluent BP1: 2 BP2: 13 BP3: 80 BP4: 10	Influent BP1: 31 - 43 BP2: 17 - 20 BP3: 39 - 49 BP4: 17 - 50 Effluent BP1: 48 - 54 BP2: 24 - 37 BP3: 50 - 81 BP4: 59 - 118	Urban (domestic) (500 000 inhabitants) - Primary treatment - Secondary treatment (trickling filters)	BP1: 306 BP2: 25 BP3: 971 BP4: 5790	BP1: 32 BP2: 1 BP3: 143 BP4: 4309	-	(Kasprzyk-Hordern et al., 2008)

Table 2.1 Overview occurrence and fate of UV-filters in wastewater treatment plants (cont).

Compounds	Country	Method overview	LOD (ng L^{-1})	Recovery (%)	Type of WWTP/inhabitants/type of treatment	Influent (ng L^{-1})	Effluent (ng L^{-1})	Sludge (ng g^{-1} dw)	Reference
ES, HMS, IMC, 4-MBC, BP3, EMC, EDP, OC, BMDM	Germany	**Extraction method:** SBSE (20 mL sample, PDMS coated stir bar, extraction time of 3 h at room temperature, 1000 rpm, thermos desorption at 250 °C, 15 min) **Instrumental method:** TD-GC-MS	ES, 4-MBC: 4 HMS: 1 IMC: 2 BP3: 11 EMC: 16 EDP: 0.2 OC: 7 BMDM: 63	ES: 86 HMS: 93 IMC: 115 4-MBC: 112 BP3: 108 EMC: 89 EDP: 83 OC: 81 BMDM: 75	Urban (domestic) (500 000 inhabitants)	-	HMS: 8 - 9 IMC: <LOD - 3 4-MBC: 38 BP3: 42 - 54 EMC: 11 - 23 EDP: 2 - 7 OC: 10 - 18 BMDM, ES: <LOD	-	(Rodil and Moeder, 2008)
BP3, 4-MBC, EMC, OC	China	**Extraction method:** Filtration SPE (1.0 L sample, 200 mg C_{18} cartridge, EA/DCM (1:1)) **Instrumental method:** GC-MS	10	67 - 118	Urban (domestic) (9 000 000 inhabitants) - Primary treatment - Secondary treatment - Tertiary treatment (coagulation–flocculation; continuous microfiltration; ozonation)	BP3: 97 - 722 4-MBC:475-2128 EMC: 54 - 116 OC: 34 – 153	After coagulation treatment: BP3: 88 - 664 4-MBC:418-1851 EMC: 45-100 OC: 27-121 After continuous microfiltration: BP3: 88 - 664 4-MBC:418-1851 EMC: 45 - 100 OC: 27 – 121 After ozonation treatment: BP3: 68 - 506 4-MBC:299-1287 EMC: 30 - 67 OC: 21 - 95	-	(Li et al., 2007)
4-MBC, OMC, OC, EHT	Switzerland	**Extraction method for wastewater:** LLE (700 mL sample, 50 g NaCl, PN + PN/DE (1:1) + DE) SPE (silica gel activated during 15 h at 180 °C (H_2O added to 1.5% by weight); Hex/DE (9:1)) **Extraction method for sludge:** LLE (50 g sample + Na_2SO_4, Hex, DCM/Hex (1:1)) GPC (100 g Bio-Beads S-X3, Hex/DCM (1:1)) SPE (4 g florisil activated at 650 °C for 2 h, 1 cm Na_2SO_4, Hex/DE (9:1) (elution discarded), DE (elution solvent)) **Instrumental method:** GC-MS (4-MBC, OMC, OC) HPLC-DAD (EHT)	Wastewater 4-MBC: 14 EMC: 3 OC: 5 EHT: 34 Sludge 4-MBC: 4 EMC: 3 OC: 6 EHT: 57	Wastewater 4-MBC: 91 EMC: 75 OC: 84 EHT: 74 Sludge 4-MBC: 95 EMC: 101 OC: 87 EHT: 75	Rural (domestic) and Industry (23 000 inhabitants) - Primary treatment (screen, aerated grit removal tank, clarifier) - Secondary treatment (aeration tank, secondary clarifier, nitrification, $FeClSO_4$ precipitation, thickeners, disinfection, two-stage mesophilic anaerobic stabilization, storage tanks)	4-MBC: 680 –1410 EMC:10400-49740 OC: 950 - 3060 EHT: 550 - 980	Primary effluent: 4-MBC: 380 - 920 EMC: 3250 - 15960 OC: 410 - 1490 EHT: 280 - 770 Secondary effluent: 4-MBC: 50 - 110 EMC: 20 – 40 OC: <LOQ - 20 EHT: <LOQ	Raw sludge: 4-MBC: 210 – 1830 EMC: 920 – 14450 OC: 1200 – 4680 EHT: 1700 – 2700 Excess sludge: 4-MBC: 340 – 500 EMC: 150 – 440 OC: 1010 – 1320 EHT: 1000 – 1300 Digestion sludge: 4-MBC: 1260 – 2290 EMC: 1020 – 1500 OC: 3040 – 4950 EHT: 2600 – 2700 Storage tank sludge: 4-MBC: 1900 – 2970 EMC: 30 – 370 OC: 1980 – 9520 EHT: 1500 – 8100	(Kupper et al., 2006)

Table 2.1 Overview occurrence and fate of UV-filters in wastewater treatment plants (cont).

Compounds	Country	Method overview	LOD (ng L^{-1})	Recovery (%)	Type of WWTP/inhabitants/type of treatment	Influent (ng L^{-1})	Effluent (ng L^{-1})	Sludge (ng g^{-1} dw)	Reference
4-MBC, EMC, OC, EHT	Switzerland	**Extraction method:** LLE (60 g sample, NaCl, PN/Ac (1:1), 30 min; PN/DE (1:1) + DE/DCM (4:1)) SPE (5 g silica gel activated during 15 h at 180 °C (H_2O added to 1.5% by weight), Hex/DE (9:1)) **Reconstitution:** EA (4-MBC, EMC, OC), EtOH (EHT) **Instrumental method:** GC-MS (4-MBC, EMC, OC) HPLC/DAD, LC-ES-MS-MS (EHT)	4-MBC: 4 OMC: 3 OC: 6 EHT: 57	4-MBC: 94.6 OMC: 101.2 OC: 87.5 EHT: 75.0	Rural (domestic) (210 – 514 inhabitants): - Primary treatment - Secondary treatment Rural (domestic)and Industrial (674 – 8 460 inhabitants): - Primary treatment - Secondary treatment Urban (domestic) and Industrial (10 000 – 30 000 inhabitants): - Primary treatment - Secondary treatment	-	-	4-MBC: 150 - 1000 EMC: 30 - 95 OC: 320 - 2480 EHT: 700 – 6300 4-MBC: 250 - 3340 EMC: 70 – 390 OC: 2580 – 7860 EHT: 1000 – 11000 4-MBC: 610 – 4980 EMC: 10 – 295 OC: 1600 – 18740 EHT: 3300 - 27700	(Plagellat et al., 2006)
4-MBC, BP3, EMC, OC	Switzerland	**Extraction method:** SPE g-1PC (100-200 mL sample, 10 mL Biobeads SM-2, MeOH/DCM) SPE (silica mini column clean-up, EA/MeOH (95:5)) **Instrumental method:** GC-MS	10	78 - 12	Urban (domestic) (10 000 – 30 000 inhabitants): - mechanical treatment - biological treatment - chemical treatment - sand filtration	4-MBC:600–6500 BP3:700 – 7800 EMC:500-19000 OC: 100 - 12000	4-MBC: 60 – 2700 BP3: <LOD – 700 EMC: <LOD – 100 OC: <LOD – 2700	-	(Balmer et al., 2005)

Table 2.1 abbreviations: Ac - acetone; ACN – acetonitrile; Al_2O_3 - Aluminium oxide; BSTFA - N,O-bis(trimethylsilyl)trifluoroacetamide; C_{18} - bonded silica stationary phase column; DCM – dichloromethane; DE – diethyl ether; DLLME, dispersive liquid–liquid microextraction; EA - ethyl acetate; GC-HRMS - gas chromatography coupled with high resolution mass spectrometry; GC-MS - gas chromatography coupled with mass spectrometry; GPC – gel permeation chromatography; H_2O – water; HCl - hydrochloric acid; Hex – hexane; HPLC-DAD - high performance liquid chromatography coupled with diode-array detection; HS-SPME – headspace solid phase microextraction; LC-MS/MS – Liquid chromatography-tandem mass spectrometry; LLE – liquid liquid extraction; LOD – limit of detection; LOQ – limit of quantification; MALLE - membrane-assisted liquid–liquid extraction; MeOH – methanol; MEPS - microextraction by packed sorbent; MSTFA - N-methyl-N-(trimethylsilyl)trifluoroacetamide; Na_2EDTA - Ethylenediamine tetraacetic acid; Na_2SO_4 - sodium sulfate; NaCl - sodium chloride; NH_4OH - Ammonium hydroxide; PDMS - poly(dimethylsiloxane); PLE - pressurized liquid extraction; PN – Pentane; PrOH, propanol; PSA - Primary-secondary amine sorbent; Rec – recovery; SBSE - stir bar sorptive extraction; Si – Silica; SPE – solid-phase extraction; TCE – tetrachloroethylene

2.2.1. Occurrence in wastewater

Monitoring programmes, where river samples are collected up and downstream of a selected WWTP along with influent and effluent wastewater, revealed that effluents are the main contributors to UV-filters contamination in the aquatic ecosystem (Gago-Ferrero et al., 2013b). In order to understand the dimension of the problem, the concentration ranges of UV-filters found in influent and effluent wastewater were represented in Figure 2.2.

At first sight, the benzophenone derivatives BP1, BP2, BP3 and BP4, stand out due to their high concentrations both in influent and effluent. This means a poor removal efficiency of the WWTP treatments and a high tendency to remain in water (higher solubility in water and relatively low log K_{OW}; Table S2.1, Annex 2). Although these values seem inflated due to a single study performed by Kasprzyk-Hordern et al. (2009), which reports extremely high concentrations, namely for BP4 (concentrations reaching 13.3 mg L^{-1} in influent and 6.3 mg L^{-1} in effluent), other studies confirm this tendency. For example, Gago-Ferrero et al. (2013b) reported concentrations up to 1420 ng L^{-1} for BP4, while Wick et al. (2010) mentioned levels up to 572 ng L^{-1}. Similarly, Balmer et al. (2005) and Tsui et al. (2014a,b) detected maximum concentrations of 541 and 700 ng L^{-1} for BP3, respectively. In fact, BP4 was the compound detected in higher concentration levels, probably due to its very low octanol–water partition coefficient (log K_{OW}=0.37; Table S2.1, Annex 2). Another benzophenone which appear in fairly high concentrations is BP8. This compound is less frequently studied than other benzophenones and its incorporation in cosmetics is prohibited in Europe and Japan. However, it was already found in effluents at concentration levels up to 10 ng L^{-1} in Taiwan (Wu et al., 2013), 84 ng L^{-1} in China (Tsui et al., 2014a,b) and up to 55 ng L^{-1} in Spain (Pedrouzo et al., 2010).

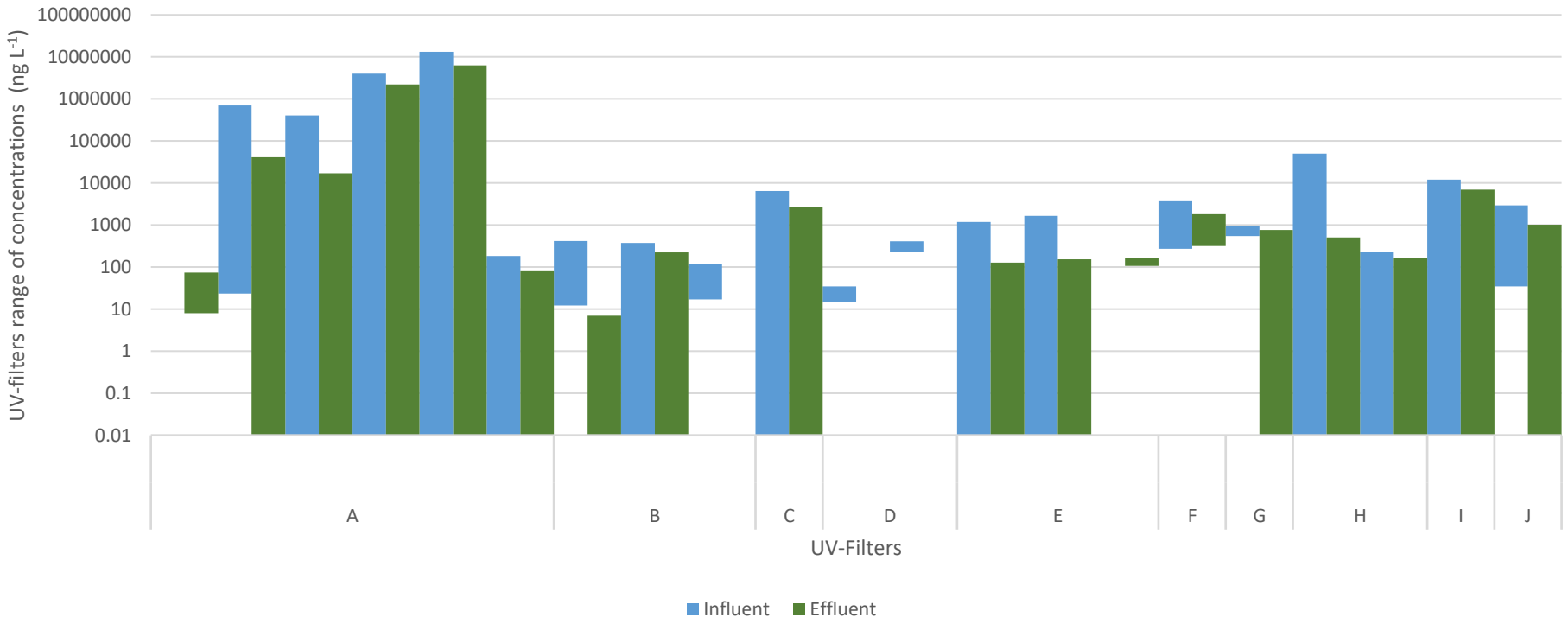


Figure 2.2 UV-filters range of concentration (between minimum and maximum) in influent and effluent wastewaters (A – benzophenone derivatives, B – p-aminobenzoic acid derivatives, C – camphor derivatives, D – benzotriazole derivatives, E – salicylate derivatives, F – benzimidazole derivatives, G – triazine derivative, H – cinnamate derivatives, I – crylene derivative, J – dibenzoyl methane derivatives).

The crylene derivative OC also stands out due to the high concentrations found both in influent (around 12,000 ng L^{-1}; Balmer et al., 2005) and effluent wastewater (near 7000 ng L^{-1}; Langford et al., 2015). However, most studies present lower concentration ranges, i.e. from 18 to 461 ng L^{-1} (Cunha et al., 2015; Kupper et al., 2006; Li et al., 2007; Magi et al., 2013; Moeder et al., 2010; Rodil and Moeder, 2008a; Rodil et al., 2009a,b,c). In a few articles OC was not even detected (Liu et al., 2011, 2012; Pedrouzo et al., 2010; Tsui et al., 2014a,b). This wide concentration range deeply depends on the type of population that the WWTP serves (influent concentrations), and on the employed technology (effluent concentrations). This high frequency of detection in wastewaters is probably related to its widespread use in cosmetics, since its use is allowed both in USA, Europe and Japan. However, the lipophilic nature of OC (log K_{OW} of 6.88) suggests that it should be predominantly found in sludge due to its sorption capacity.

According to Table 2.1 and Figure 2.2, the camphor derivative 4-MBC, whose incorporation into cosmetic products is only allowed in Europe, is often found in high concentration levels. 4-MBC has been detected in WWTPs influents in concentrations ranging from 394 to 406 ng L^{-1} in Australia (Liu et al., 2012), 3 ng L^{-1} (Tsui et al., 2014a,b) to 2128 ng L^{-1} (Li et al., 2007) in China and from 600 ng L^{-1} (Kupper et al., 2006) to 6500 ng L^{-1} (Balmer et al., 2005) in Switzerland and ranging 45.8 to 154.9 ng L^{-1} (Cunha et al., 2015) in Portugal. Lower concentrations were detected in samples from Spain (10–90 ng L^{-1}) (Gago-Ferrero et al., 2013b). Effluent wastewater shows a similar geographical pattern, with maximum concentrations ranging from 207 ng L^{-1} (Tsui et al., 2014a,b) to 1851 ng L^{-1} (Li et al., 2007) in China and 110 ng L^{-1} (Kupper et al., 2006) to 2700 ng L^{-1} (Balmer et al., 2005) in Switzerland. Also, other authors present lower concentrations in effluent wastewater from Spain (24 ng L^{-1}) (Gago-Ferrero et al., 2013b) and Germany (30–60 ng L^{-1}) (Rodil and Moeder, 2008b; Rodil et al., 2009a,b,c), while 4-MCB was not detected in Japan (Kameda et al., 2011).

The benzimidazole PBSA, allowed in Europe, USA and Japan, was found, both in the influent (from 275 to 3615 ng L^{-1}) and effluent (from 316 to 1504 ng L^{-1}) wastewater in a single study performed by Wick et al. (2010). Although this compound has not been frequently studied, Ji et al. (2013) have shown that a fast photochemical transformation via direct photolysis is expected to be an important degradation pathway, compared to

other processes such as sorption or biological degradation. This indicates that monitoring studies should focus on the photoproducts of this compound, since the half-live of the PBSA will be short. The dibenzoyl methane derivative BMDM was found in a screening study from Tsui et al. (2014a,b), at relatively high concentrations in both influent (35–1290 ng L^{-1}) and effluent (18–1018 ng L^{-1}) wastewater. However, in a WWTP that employs advanced treatments, BMDM was not detected (this will be further discussed in 3.1). The dibenzoyl methane derivative BMDM was also found in Portugal in June at high concentrations, 2935 ng L^{-1} (Cunha et al., 2015) in a WWTP which employs a secondary treatment followed by disinfection by biofiltration. The salicylate derivatives ES were found in the same range at 1188 ng L^{-1} and 129 ng L^{-1} and HMS at 1650 ng L^{-1} and 154 ng L^{-1}, in influent and effluent respectively (Tsui et al., 2014a,b). The widely used EHT (triazine derivative) was found at concentrations ranging from 550 to 980 ng L^{-1} in influent wastewater, and 770 ng L^{-1} in effluent wastewater (Kupper et al., 2006). However, due to its high log K_{OW} (>10), it is expected that EHT has more tendency to adhere on organic matter and therefore, be present at higher concentrations in sludge.

The cinnamate derivatives EMC (allowed in European, American and Japanese cosmetics) and IMC (allowed in USA and Japan) are also compounds that were frequently studied and detected in both influent and effluent. In influents, EMC and IMC concentrations range from not detected to 50,000 ng L^{-1} (Kupper et al., 2006) and 226 ng L^{-1} (Tsui et al., 2014a,b), respectively. In effluents, maximum concentrations of 505 ng L^{-1} for EMC and 165 ng L^{-1} for IMC (Tsui et al., 2014a,b) were detected. The benzotriazoles found in wastewater are not usually used in cosmetics as UV-filters, but in technical products such as plastics and paints as UV stabilizers. Since the allowed benzotriazoles in cosmetics have never been studied in WWTPs, these following compounds (UV-234, UV-326, UV-327, UV-328 and UV-329) may be used as representatives. Langford et al. (2015) studied the occurrence of benzotriazoles UV-234, UV-327, UV-328 and UV-329 in WWTPs, but the authors verified that they were not detected either in influent or effluent. Still, Liu et al. (2012) detected UV-326 and UV-329 in influent wastewater at 35 and 414 ng L^{-1}, respectively, but did not detect them in

effluent wastewater. Because of the lipophilic character of these compounds (log KOW N 5), they may be probably sorbed onto sludge (Liu et al., 2012).

The *p*-aminobenzoic acid derivatives EDP, ODP and Et-PABA are present in lower concentrations at WWTPs in comparison to the other UV-filter classes. On the one hand Gago-Ferrero et al. (2013a,b) found Et-PABA at 120 ng L^{-1} in the influent, but not in the effluent. On the other hand, the compound ODP was detected by Tsui et al. (2014a,b) at 376 ng L^{-1} in influent and 225 ng L^{-1} in effluent wastewater. Some other authors also found these *p*-aminobenzoic acid derivatives, but in lower concentration ranges. Magi et al. (2013) detected ODP at 4 ng L^{-1} in an influent, but did not detect this compound in the effluent, while Pedrouzo et al. (2010) detected 55 ng L^{-1} in influent and 25 ng L^{-1} in effluent. The derivative EDP was found in Portugal at 418 ng L^{-1} in influent wastewater from a WWTP near a beach area in June (Cunha et al., 2015) but was not detected in the treated effluents (Rodil et al., 2009a,b,c).

Although values presented in Table 2.1 show an overview, UV-filters concentrations differ with geographical location, treatment scheme, type of sewage (urban, rural or industrial), flow conditions and even seasonally (Balmer et al., 2005; Kasprzyk-Hordern et al., 2009).

Data suggests some variability of UV-filters inputs to WWTPs, with higher loads during warmer months (Balmer et al., 2005; Cunha et al., 2015; Li et al., 2007; Magi et al., 2013; Pedrouzo et al., 2010). In fact this seasonal trend is not surprising as during warmer months care with skin is more relevant among population, with consequent increase in use of sunscreens, lotions and other type of cosmetics aimed to protect against for sunburns. Predominantly in these products, some common UV-filters are used (mentioned by their trade name): avobenzone (BMDM), octocrylene (OC), ensulizole (PBSA), oxybenzone (BP3) or sulisobenzone (BP4) (21CFR352.10, 2014; CosmeticsDirective, 2009; MHW, 2000; Richardson and Ternes, 2014; Salvador and Chisvert, 2005). For this reason, these are some of the compounds that appear in higher concentrations in warmer months. However, a considerable amount of UV-filters can be released from people' skin during showering and washed off from towels and clothes during laundering, representing an indirect input into the environment throughout the year. Moreover, UV-filters can be absorbed by the human body through the skin

(Chisvert et al., 2012; Giokas et al., 2007) and then, they may be excreted in urine and/or faeces as parent compounds or metabolites, also constituting a continuous indirect input during the year (Giokas et al., 2007; Magi et al., 2013). As mentioned above, UV-filters are added not only to cosmetics specific for sun protection (sunscreens), but also in common products that are used throughout the year. A recent study by Chisvert et al. (2013) showed that UV-filters such as BP3, 4-MBC, OC, EMC, BMDM, DBT and MBP were detected in daily routine personal care products (moisturizers, after shave products, firming facial creams and lip balms) in concentrations between 0.55 and 7.80% (w/w). It's also worth to notice that during snowing periods sunscreens are also frequently used and should be considered as an input source in remote locations.

Most studies related to the occurrence of UV-filters in wastewaters are focused on municipal WWTPs located in urban areas and those receiving only domestic sewage (Gago-Ferrero et al., 2013b; Langford et al., 2015; Tsui et al., 2014a,b). However, a couple of studies have focused on urban WWTPs receiving domestic and industrial sewage (usually with an average ratio of 75:25) (Kasprzyk-Hordern et al., 2008a,b, 2009; Liu et al., 2011, 2012) and only one investigated the UV-filters' behaviour in a rural WWTP that treats domestic/industrial sewage (Kupper et al., 2006). Besides the scarce number of available studies, information is not always clear regarding the type of influent being studied. Furthermore, information regarding type of employed treatments in the WWTP, the amount of served population and in case of industrial influents, the type of industry in its surroundings is not always evident. This lack of information may be related to the fact that most of the studies are more focused in method development rather sample analysis. Therefore, making the analysis and comparison of results is a quite complex undertaking.

An analysis of total concentrations in effluents from rural (domestic)/industrial, urban (domestic) and urban (domestic)/industrial WWTPs showed that the last group presented the major load of UV-filters, in opposition to the rural (domestic)/industrial ones. This could be expected if the mentioned industry was somehow related to the cosmetics industry. Otherwise, concentrations would be lower due to the dilution effect.

There is a general lack of information regarding the presence of UV-filters in WWTPs worldwide. Considering that these compounds may be incorporated in different

concentrations in daily life products depending on the regulation in force in the specific country (e.g. European, USA and Japanese legislation), their levels should be monitored more often than they already are. Results showed maximum average concentration of UV-filters in effluents of 3300 μg L^{-1} in UK and between 0.15 and 3.57 μg L^{-1} for Italy < Spain < Switzerland < Germany < Norway (European countries). The high levels of UV-filters detected in the UK reflect only the results of a single study (Kasprzyk-Hordern et al., 2009) and therefore, they should be analysed with caution and should not be used as representative of that country. European countries present concentrations in the same range, as they are due to comply to the same legislation, and small differences may be due to type of treatment employed in the different facilities. In Asia, average concentrations are in a lower range (0.03 and 1.74 μg L^{-1}) for Taiwan < Japan < China. In Japan, BZS was the only compound found in effluent wastewater that is not allowed in cosmetics. Regarding China and Taiwan, it was not possible to find reliable information regarding UV-filters legislation in cosmetics to compare. In Oceania only data from Australia is available (0.20 μg L^{-1}) and in this country most compounds are allowed in cosmetics (Shaath, 2010).

Although the amount of UV-filters in the effluent is in average lower than in influent, it does not mean that they are effectively degraded. Most probably they migrate to sludge or originate new by-products (Barón et al., 2013; Santos et al., 2012), but this situation will be discussed in more detail in Section 2.3.

2.2.2. Occurrence in sludge

As mentioned before, UV-filters' fate in WWTP is not yet well known. However, taking into account the lipophilic nature of some UV-filters (Table S2.1 from Annex 2), they may tend to sorb onto sludge. Therefore, sewage sludge may contain multiple UV-filters, representing an appropriated matrix to characterize the release of environmentally relevant substances (Plagellat et al., 2006). One of the main use of sewage sludge is as fertilizer in agriculture, which pose a contamination pathway to soils, animals and even humans. The concentration range of UV-filters per compound in sludge is shown in Figure 2.3.

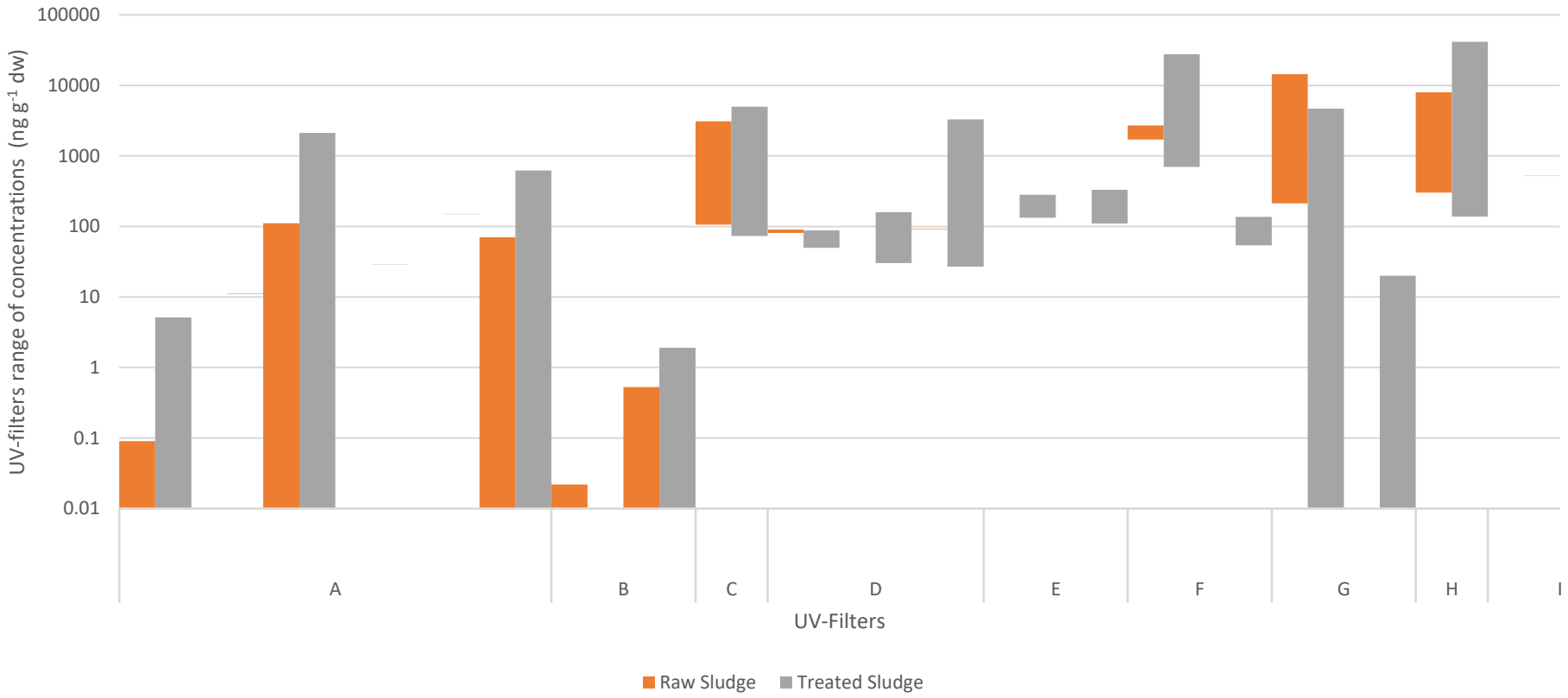


Figure 2.3 UV-filters range of concentration found in raw and treated sludge (A – benzophenone derivatives, B – p-aminobenzoic acid derivatives, C – camphor derivatives, D – benzotriazole derivatives, E – salicylate derivatives, F – triazine derivative, G – cinnamate derivatives, H – crylene derivative, I – dibenzoyl methane derivatives)

Unlike wastewaters, a different scenario is presented regarding UV-filters in sludge (Figure 2.3). Generally, it seems that the number of UV-filters detected in sludge is lower than in wastewaters and the range of concentration levels also appears to be narrower.

Among benzophenone derivatives, BP3 seems to be the predominant compound reaching concentrations around 2100 ng g^{-1} dw in treated sludge (Langford et al., 2015). However, lower concentrations were also reported, ranging from 16 to 790 ng g^{-1} dw (Gago-Ferrero et al., 2011b; Liu et al., 2011, 2012; Negreira et al., 2011; Rodil et al., 2009a,b,c; Wick et al., 2010). Benzophenone BP1 was detected in treated sludge by Wick et al. (2010) at 5.1 ng g^{-1} dw, but BP2 and BP4 were only found in raw sludge (in the same study). Compounds like benzophenones 4HB and 4DHB that were not found in effluent wastewater were detected in sludge. The benzophenones 4HB and 4DHB were found at maximum concentrations of 150 and 620 ng g^{-1} dw, respectively, in three WWTPs in Spain by Gago-Ferrero et al. (2011a,b) and 4DHB also at lower concentrations (0.05 ng g^{-1} dw) by Rodríguez-Rodríguez et al. (2012). However, this behaviour was not totally expected, since both 4HB and 4DHB are not considered very lipophilic compounds due to their log K_{OW} values of 3.1 and 2.2, respectively. In fact, these compounds, besides being used by industry as UV-filters, are also major BP3 degradation products. Considering that they were not found in wastewater, it is possible to assume that BP3 is mostly degraded during biological treatment.

As seen in Figure 2.3, compounds like benzotriazoles UV-326 and UV-329 that were not found in effluent wastewater were detected in sludge. This is already expected for these compounds since they present a lipophilic character (log $K_{OW} > 5$). Concentrations of 88 and 123 ng g^{-1} dw were found for UV-326 and UV-329, respectively in Australia (Liu et al., 2011, 2012) and higher levels in Norway for UV-327 and UV-329 — 160 and 3303 ng g^{-1} dw, respectively (Langford et al., 2015). The *p*-aminobenzoic acid derivatives detected in effluent wastewater showed small concentrations in treated sludge. EDP was not found at all and ODP was found at 2 ng g^{-1} dw by Rodil et al. (2009a,b,c) and 0.004 ng g^{-1} dw by Rodríguez-Rodríguez et al. (2012).

Camphor derivative 4-MBC, crylene derivative OC and triazine derivatives EHT and DBT showed the highest concentrations in treated sludge. Actually, these compounds are the most lipophilic ones under study, as the partition coefficients (log K_{OW}) are 6, 8, 17 and

14, respectively, so they were expected to sorb easily onto sludge. 4-MBC was found in raw and treated sludge at concentrations ranging from 73 to 4980 ng g^{-1} dw and 106 to 3100 ng g^{-1} dw, respectively (Badia-Fabregat et al., 2012; Gago-Ferrero et al., 2011b; Kupper et al., 2006; Liu et al., 2011, 2012; Negreira et al., 2011; Plagellat et al., 2006; Rodil et al., 2009a,b,c). OC was found at higher concentrations levels: 303–8000 ng g^{-1} dw in raw sludge and 138–41,610 ng g^{-1} dw in treated sludge (Badia-Fabregat et al., 2012; Gago-Ferrero et al., 2011b; Kupper et al., 2006; Langford et al., 2015; Liu et al., 2011, 2012; Negreira et al., 2011; Plagellat et al., 2006; Rodil et al., 2009a,b,c). Regarding the triazine derivatives, EHT was found at concentrations ranging 700 to 27,700 ng g^{-1} dw in raw sludge and 1700 to 2700 ng g^{-1} dw in treated sludge (Kupper et al., 2006; Plagellat et al., 2006; Rodil et al., 2009a,b,c), while DBT was only found in raw sludge, ranging 54 to 136 ng g^{-1} dw (Rodil et al., 2009a,b,c). The high concentrations reported for 4-MBC and OC are in agreement with the high sorption coefficients reported by Kupper et al. (2006). Similar concentrations of salicylate derivatives ES (133–280 ng g^{-1} dw) and HMS (110–331 ng g^{-1} dw) and dibenzoyl methane derivative BMDM (517 ng g^{-1} dw) were found in treated sludge (Negreira et al., 2011; Rodil et al., 2009a,b,c); however they were not studied in raw sludge. Perhaps due to the frequent incorporation of the cinnamate derivative EMC in cosmetic formulations (Manova et al., 2013), this compound was monitored in all studies focused on sludge analysis. Concentrations from 213 to 14,450 ng g^{-1} dw (Kupper et al., 2006; Liu et al., 2012) in raw sludge and in treated sludge up to 4689 ng g^{-1} dw (Langford et al., 2015) were detected.

Like wastewaters, a seasonal variation was also observed in sludge, with maximum concentration peaks during the summer period (Plagellat et al., 2006).

Except for a couple of studies performed in Australia (Liu et al., 2011, 2012), most UV-filter studies in sludge are performed in Europe. Therefore, a comprehensive knowledge about levels on global scale is still lacking and therefore scientific community should widen and intensify studies regarding on these compounds of emerging concern to better understand their behaviour and consequences in the environment.

2.3. Treatment of UV-filters in WWTPs

WWTPs' main goal is to treat wastewater, removing contaminants (Spellman, 2013a). However, due to the great number and diversity of known and unknown contaminants treatment plants are not prepared to remove all these compounds. For example, in a study involving several emerging contaminants, Kasprzyk-Hordern et al. (2009) proves that WWTP effluent discharges into rivers are the main source of environmental contamination. Nevertheless, some WWTPs are able to remove UV-filters from water and sludge at some extent and their removal strongly depends on the technology implemented in the WWTP.

Generally, biological or chemical degradation and volatilization are the possible removal mechanisms from WWTPs. Since UV-filters have a relatively high boiling point (Table S2.1, Annex 2), it is expected that volatilization is not a key removal step. However, compounds relatively stable to bio or chemical degradation can also be removed from the water line by sorption onto sludge. Compounds with low water solubility and high octanol–water partitioning coefficient are especially prone to this phenomenon. Nevertheless, the efficiency of conventional sludge treatment technologies should be investigated since sewage sludge is frequently applied in agriculture as a fertilizer.

2.3.1. Water line treatments

In a WWTP, the primary treatment is mechanical and/or physicochemical aimed to remove at least half of the suspended material and at least 20% of biochemical oxygen demand (BOD) through processes such as decantation/sedimentation/clarification, flotation and coagulation. Usually, these kind of treatments are not capable enough to remove UV-filters from wastewaters (Spellman, 2013c; Tsui et al., 2014a,b; Wang et al., 2010). Langford et al. (2015) showed that a WWTP, which employs only a primary treatment, discharges 7 to 13 times more UV-filters to the environment than facilities that employ a secondary treatment (activated sludge), although served population is considerably smaller (Table 2.1). Tsui et al. (2014a,b) also showed that a WWTP that employs only a treatment to screen grit with diameter exceeding 6 mm and with a hydraulic retention time(HRT) of less than 1 h is not efficient in the removal of UV-filters.

Detectable UV-filters removal efficiencies were low (0–30%) to moderate (30.1–70%) after this type of treatment, except for ODP which showed 75% removal. These results prove that physical screening is not an effective treatment for UV-filters in wastewater, compromising the aquatic environment through their continuous release.

During primary sedimentation solids are removed by gravity and water moves slowly through the sedimentation tank or basin with a minimum turbulence at entry and exit points, and sludge keeps accumulating at the bottom of the tank (Spellman, 2013b). The removal of contaminants due to sedimentation of primary sludge usually depends on the sorption coefficient of compounds as well as the amount of suspended solids present in the wastewater. Compounds with higher log K_{OW} values will likely sorb onto primary sludge, which means that UV-filters with low coefficients like benzophenones derivatives (e.g. BP9 (−2.78) and BP3 (3.79)) will be less removed. Moderate removal (between 30 and 70%) was observed in this stage of treatment for compounds like BMDM, HMS, 4-MBC, ODP and EMC, whose log K_{OW} >4 (Li et al., 2007; Tsui et al., 2014a, b). Also Liu et al. (2012) showed that 4-MBC, UV-326 and OC were removed from the primary influent in a range between 44 and 82% by sorption onto sludge, while EMC and UV-329 were removed to a lesser extent (about 45%). Lower removal rates (<5%) were determined for BP3.

Another primary treatment referred in literature is coagulation–flocculation (CF). This methodology is usually applied before sedimentation and is used to enhance not only the removal of suspended solid particles, but also the biochemical oxygen demand (BOD), chemical oxygen demand (COD), and bacterial population in primary settling facilities (Semerjian and Ayoub, 2003). Coagulants are added and the wastewater is turbulently stirred. This process is usually followed by flocculation to agglomerate small particles into well-defined flocs by gentle agitation for a much longer time (Spellman, 2013b; Wang et al., 2010). For example, the coagulant ferric (III) chloride neutralizes the surface charges on suspended particles and colloidal material and allows their aggregation into larger and heavier flocs for sedimentation (Tsui et al., 2014a,b). This improved the removal efficiencies for BMDM (from 26 to 75% in dry season and 26 to 69% in wet season), BP3 (23 to 52% in dry season and 28 to 67% in wet season) and EMC (32 to 52% in dry season). However, this methodology showed not to be effective in the

removal of UV-filters with low log KOW like benzophenones BP1 and BP4 (Tsui et al., 2014a,b). Li et al. (2007) studied the use of polyaluminum chloride as a coagulant in a CF treatment; however, the removal efficiencies achieved for BP3 (7.6–9.7%), 4-MBC (12–15%), EMC (13–16%) and OC (19–21%) were relatively low. Authors correlate the different behaviour obtained in the CF treatment with the potential to adsorb onto the particulate organic matter in suspension. This adsorption potential can be characterized by the organic matter/organic partition coefficient (log K_{OM}) that were estimated by the authors as 3.1 for BP3, 4.3 for 4-MBC, 5.3 for EMC and 6.1 for OC. In fact, the removal efficiencies obtained by this study follow this tendency (Li et al., 2007). Overall, although the primary treatment is essential in a WWTP, its contribution to the removal of UV-filters is not great, except for those whose log K_{OW} is high enough to allow them to rapidly sorb into primary sludge.

The main purpose of secondary treatment (sometimes referred to as biological treatment) is to provide biochemical oxygen demand (BOD) removal beyond what is achievable by primary treatment. Some common approaches are activated sludge and the trickling filter, and they take advantage of the ability of microorganisms to convert organic wastes (via biological treatment) into stabilized, low-energy compounds. Usually, it follows the primary treatment (Spellman, 2013b).

Balmer et al. (2005) consistently showed lower concentrations in the effluent wastewater (after mechanical, biological, chemical treatment and also sand filtration) with elimination rates quite high for most compounds (18–82% for 4-MBC, 68–96% for BP3, 88–99% for OC and 97–99% for EMC). Still, authors point out that it is not clear whether compounds are actually degraded or just removed from wastewater by sorption onto sewage sludge. Also, Kasprzyk-Hordern et al. (2008a,b) showed a 85% removal for BP3, 89% for BP1, 96% for BP2, but only a 30% removal of BP4 (primary treatment followed by trickling filter beds). This is probably due to BP4 higher solubility in water (2.50×10^5 mg L^{-1}), opposed to the other benzophenones (see Annex 2 Table S2.1). Kasprzyk-Hordern et al. (2009) studied two different secondary WWTPs systems (trickling filter and traditional activated sludge). Both systems conducted to similar removal rates for the studied benzophenone derivatives (about 90% for BP1, 98% for BP2, 70–95% for BP3 and < 15% for BP4). The UV-filter BP4 also showed lower removal

rates, which may be explained by its high polarity, when compared to the other benzophenone derivatives studied. The removal of BP3 and BP1 from WWTP is also corroborated by Negreira et al. (2009) (83% removal for BP1 and 90% removal for BP3 after activated sludge treatment).

Although several studies mention the average removal of the UV-filters in the WWTPs, it is not always clear if they are actually degraded or removed from wastewater by sorption to sewage sludge. Nevertheless, few authors opt to investigate the fate and removal of these compounds in different wastewater treatment steps (Kupper et al., 2006; Liu et al., 2012).

Kupper et al. (2006) studied four UV-filters (4-MBC, EMC, OC and EHT) along a conventional activated sludge treatment plant, which also has a sludge treatment line (Switzerland), performing mass balances for both water and sludge lines. They verify that 50–80% of OC and EHT and less than 35% of 4-MBC and EMC present in the influent were removed by sorption onto sludge in the primary treatment (residence time: 3 h). Also, the remaining of those last two compounds were found to be degraded (30 and 50%, respectively) in this step. In the biological unit (residence time: 8 h), it was verified a greater degradation: 99% for EMC, 90% for 4-MBC and 80% for OC. Only, EHT presented a lower degradation, being sorbed in more extensively onto sludge (more than 30%). On overall, less than 25% of UV-filters were detected in the final effluent and OC and EHT were mainly removed by sorption.

Liu et al. (2012) investigated six UV-filters (BP3, 4-MBC, EMC, UV-326, UV-329, and OC) in a full-scale municipal wastewater treatment in South Australia. The WWTP consists of primary sedimentation, secondary activated sludge treatment (hydraulic retention time: 18 h), stabilization lagoons (hydraulic retention time: 27 days), dissolved air flotation/filtration and also a sludge line (will be further discussed). For 4-MBC, UV-326 and OC, the authors concluded that 44–82% of the influent load partitioned to sludge in the primary treatment, which was expected considering their high log KOW values (Annex 2). Sorption was low for BP3 and UV-329 (<5%), which was expected for BP3 (log K_{OW} of 3.79) but not for UV-329 (log K_{OW} of 6.21). Higher degradation rates were reported in primary treatment for EMC (≈50%) and UV-329 (≈40–80%). For all the target compounds, except for OC, a considerable degradation was achieved during secondary

treatment (60–95%). Only 14% of degradation was verified for OC, which is not consistent with previous results from (Kupper et al., 2006). Mass balances were used to understand the UV-filters' behaviour during WWTP (sorption to sludge and degradation), however, authors point out that some mass balance errors occurred and that they could be explained by the long hydraulic retention times. It is also important to mention that there were two sampling campaigns and that a different behaviour among UV-filters was verified. However, a consistent variation was not detected, which prevents a deeper analysis. The mass balance analysis for the entire water line showed that more than 54% of 4-MBC, UV-326 and OC were sorbed onto sludge (hydrophobic compounds according Table S2.1, Annex 2). Significant degradation occurred for BP3, EMC and UV-329 (86%–97%). The proportions in the final effluent were <16% for all target compounds.

Tsui et al. (2014a,b) investigated five different wastewater treatment methods for 12 organic UV-filters (Table 1). Compounds like BMDM, BP1, BP3, BP4 and EMC were frequently detected (> 80%) in both influent and effluent, with mean concentrations ranging from 23 to 1290 ng L^{-1} and 18–1018 ng L^{-1}, respectively. Overall, the UV-filters have been degraded in a greater extent during the biological treatment: >99% for ES, OC and BP8, >70% for BP1, BP3 and HMS, 38–77% for 4-MBC, 30–55% for EMC and 10–50% for BMDM, IMC and ODP in the modified Ludzack–Ettinger (MLE) system. The compounds BMDM, IMC and ODP were considered by the authors less biodegradable in the given aeration time (not shown). Nevertheless, the sequential batch reactor (SBR) presented higher removal efficiencies for UV-filters considering most compounds were not detected and BMDM, EMC and BP1 were removed between 77–99% and BP3 54%. The SBR system treats a portion of the day's total wastewater flow in a batch-type process thereby becoming more efficient than the MLE process (Spellman, 2013b; Wang et al., 2010) These values were consistent with those observed in the previously mentioned studies (Kupper et al., 2006; Liu et al., 2012).

Because primary and secondary treatments are not able to remove the majority of the organic contaminant compounds, more sophisticated WWTP already own tertiary treatments, also called advanced wastewater treatments. They follow secondary treatment and usually include phosphorus removal or nitrification (Spellman, 2013b).

Disinfection is an important step of wastewater treatment, especially when the secondary effluent is discharged into a body of water used for swimming or as a downstream water supply. The most commonly used disinfectants and oxidants are chlorine, chlorine dioxide, ozone, and potassium permanganate (Spellman, 2013b). Li et al. (2007) studied the effect of ozonation (advanced oxidation process) in the removal of UV-filters. Ozone is a strong oxidizing gas that reacts with most organic and many inorganic molecules. However, this treatment conducted to poor removal efficiencies, around 20% of BP3, 25% of 4-MBC, 28% of EMC and 17% of OC. The lower removal efficiencies than expected may be due to low ozone dose (5–6 mg L^{-1}), short hydraulic residence time (3 h), as well as the competitive reactions with other organic contaminants.

Tsui et al. (2014a,b) studied different WWTP with tertiary treatments and concluded that the chlorination process is not efficient in removing most compounds from wastewater (<30%), except for HMS which showed removals of 46% in the dry season and 76% in the wet season. In contrast, the UV-disinfection (short UV-irradiation) showed good results for 4-MBC and IMC (>90%), HMS (71%) and EDP (61%). UV-disinfection can oxidize organic contaminants through direct photolysis or formation of reactive free radical from water to attack other organic compounds. During this process (UV-disinfection), the removal efficiencies of BP1, BP3, BP4, BMDM and EMC were lower than 30% (lower than 60% for EMC in the wet season), which can be explained by the previously mentioned photostability of these compounds (Liu et al., 2013; Rodil et al., 2009a; Santos et al., 2012, 2013). Also, according to Kockler et al. (2012) the presence of suspended particles during the treatment can also scatter and absorb UV-irradiation, while several UV-filters may enhance the photostability effect of compounds and consequently diminish the removal efficiency of the process. It is important to mention that by-products with higher toxicity than their parent compounds can be formed during these processes, which is also an important issue (Santos et al., 2012, 2013).

In the conventional water treatment process, filtration usually follows coagulation, flocculation, and sedimentation. However, this technique can also be used as disinfection in tertiary treatment (Wang et al., 2010). Wastewater filtration is a physical process employed in order to separate the suspended and colloidal particles from water

through a granular material (Spellman, 2013a). It can also be used to some extent for the removal of some microorganisms and viruses. Li et al. (2007) studied the effect of a continuous microfiltration (CMF) treatment (0.2 µm pore size), which besides the removal of particles, is also effective for absolute removal of Giardia cysts (responsible for giardiasis, an intestinal infection) with 9 to 21 µm length (EPA, 1999) and partial removal of bacteria and viruses (Wang et al., 2007). Nevertheless, removal efficiencies of the target compounds (BP3, 4-MBC, EMC and OC) were lower than 10%, which means that CMF process has minimal effect in the removal of UV-filters. Authors admit that the minimal removal efficiencies obtained during this process are due to UV-filters' adsorption onto the particles retained on the membranes.

Besides disinfection purposes, other technologies are employed in tertiary treatment. Tsui et al. (2014a,b) evaluated the ability of reverse osmosis in removing UV-filters from water and concluded that this technology showed the highest removal efficiencies for 4-MBC, IMC, HMS and EDP (>99% removal) so far. These high removal efficiencies can be attributed to the molecular weights of these compounds (ranging between 100 and 300), which are in the range of values for membrane exclusion. However, the operational costs of a large-scale reverse osmosis treatment are very high and need to be taken into consideration (Tsui et al., 2014a,b).

Solutions like membrane bioreactors (MBR) treatments with long solid retention time (SRT) have also been suggested for the removal of this type of compounds from WWTPs. Longer SRT also allows the MBR system to produce less waste sludge than an SBR system (as an SBR can't operate at longer SRTs due to the negative impact on the settling of sludge). It favours the proliferation of slowly growing bacteria, improving microbial diversity in the reactor and achieving better biodegradation of compounds. One of the benefits of MBR technology over other activated sludge processes is its ability to operate at high biomass concentrations, leading to much smaller process basins (Spellman, 2013b; Wijekoon et al., 2013). This type of reactor improves the removal of some compounds via adsorption onto sludge and subsequent biodegradation. Because of the prolonged SRT, the MBR yields a higher biodegradation rate. In fact, Wijekoon et al. (2013) obtained high biodegradation/transformation rates for some UV-filters like BP

and BP3 (>96%) and OC (67–96%) with MBR working conditions of HRT of 26 h, temperature of 26 °C, dissolved oxygen concentration of 2.4 mg L^{-1} and SRT of 88 days.

As mentioned before, nitrification is often applied after secondary treatment in WWTPs. In nitrification, the ammonia nitrogen is converted to nitrate nitrogen, producing a nitrified effluent. At this point, nitrogen has actually not been removed, only converted to a form that is not toxic to aquatic life and that does not cause an additional oxygen demand. Biological denitrification removes nitrogen from the wastewater. When bacteria come in contact with a nitrified element in the absence of oxygen, they reduce the nitrates to nitrogen gas, which escapes from the wastewater (Spellman, 2013b). Some authors mention biological nitrification and denitrification (Gago-Ferrero et al., 2011b; Golovko et al., 2014; Wick et al., 2010, 2011). However, it is not possible to conclude if this treatment influences the removal of UV-filters since no information is given regarding the compounds concentration.

Because of the limitations of the primary and secondary treatments, the advanced treatments should overcome the gaps that still exist in WWTPs. However, so far, few studies have been made in that area and many other potentially effective treatments need to be studied.

2.3.2. Solid line treatments

The typical sewage sludge is classified as primary, biological, and chemical, according to its origin (Wang, 2007). The removal of UV-filters in sludge treatment is barely discussed in literature, even though most WWTP have a specific line dedicated to sludge treatment.

Langford et al. (2015) presented the concentrations of UV-filters in sludge from WWTP 1, which undergoes anaerobic digestion of sludge and a drying process and from a WWTP 2, which consists in thermal hydrolysis at 160 °C prior to anaerobic digestion at 38 °C. Although plants may have different inputs due to the different population they serve (580,000 the first and 52,000 the second), total concentrations in treated sludge were higher in the second plant (16.6 µg g^{-1} dw and 51.9 µg g^{-1} dw respectively) for which authors conclude that the differences are due to the more advanced treatments in

WWTP 1. However, a wider discussion about the reasons and underlying phenomena that may explain these results were not given by the authors.

Liu et al. (2012) studied the distribution of UV-filters in sludge /biosolids from the primary sludge, through the different stages of sewage treatment (anaerobic digestion–sludge retention time of 7 days and sludge stabilization lagoons) until the final biosolid. They observed that the profiles of the detected UV-filters in the sludge were very variable. The highest concentrations were found in the digested sludge for 4-MBC and OC (2020 ng g^{-1} and 1838 ng g^{-1} respectively), which were much higher than the primary sludge (1031 ng g^{-1} and 561 ng g^{-1} respectively), probably due to water loss during the process. This indicates recalcitrance of 4-MBC and OC to anaerobic degradation in mesophilic anaerobic digestion process. However, because concentrations of these compounds are reduced in sludge stabilization lagoon samples, it may suggest biodegradation occurred in the stabilization process. The final biosolid still presents high concentrations of 4-MBC and OC (962 ng g^{-1} and 465 ng g^{-1} respectively), but nevertheless, lower than the raw sludge.

Kupper et al. (2006) analysed the sludge line, which comprises three thickeners (RT: 2 days), a disinfection unit (pasteurization), a two-stage mesophilic anaerobic stabilization (RT: 20 days; 34 °C) and two storage tanks. Results show that during sludge treatment only EMC suffers degradation (>90%). The other compounds studied, 4-MBC, OC and EHT primarily migrate to the stabilized sludge of the storage tank, which is expected considering that these compounds are some of the most lipophilic known UV-filters (Table S2.1 in Annex 2). Also, 13% of 4-MBC was found in the supernatants from the thickener that was considered negligible for mass balances in the authors' point of view.

The use of ligninolytic fungi is a promising alternative for pollutant treatment because of their ability to degrade a broad spectrum of compounds such as pharmaceuticals, textiles, dyes, oestrogens and PAHs in both liquid and solid media. Also, the use of a bioslurry approach is considered an advantage as the sludge can be treated directly from the effluent of the anaerobic digester. UV-filters were studied in a bio-slurry reactor with fungus *Trametes versicolor*. Compounds presented different behaviours, BP3 showed low removal (22%), 4-MBC, OC and ODP showed moderate removals (58–70%) and EMC and BP1 high removals (79 and 100% respectively). Only 4DHB remained unchanged

after the treatment with this fungus. This treatment showed good results to some compounds, however, the different profiles in the overall content of pollutants often present in the sludge may play a role in synergistic or antagonistic interactions in terms of removal (Rodríguez-Rodríguez et al., 2012). Also, Badia-Fabregat et al. (2012) showed that the use of ligninolytic fungi is a promising alternative for pollutants treatment in sludge due to their ability to remove from 87 to 100% of several UV-filters (BP1, 4DHB, 4HB, BP3, 4-MBC, OC, ODP and EMC). It was noticed that UV-filters were detected at strikingly higher concentrations than pharmaceuticals in the same sampled sludge, putting these compounds in an alarming position compared to some drugs that have been studied for a long time (Rodríguez-Rodríguez et al., 2012). Also, it is worth to notice the presence of BP3 metabolite and 4DHB in sludge, before fungal treatment. Its origin may be related to its transformations and excretion by the human body or partial degradation in the WWTP by microorganisms in the activated sludge treatment. This fact stresses the need for identifying and establish methods to detect not only the parent compounds, but also their intermediate metabolites (Badia-Fabregat et al., 2012).

The sludge line has still been poorly studied, even though concentrations of UV-filters in treated sludge have been published. However, because this issue is becoming of great importance, development of technologies which are capable to remove these contaminants from sludge should be an objective of research in the future.

Treated sludge has been used as an agricultural fertilizer for decades, in fact, data from the European Commission shows that as far from 2010, 'some 42% of Europe's municipal sewage sludge was treated and used on farmland, 27% was incinerated, 14% was disposed of by landfilling and about 17% was disposed of in other ways' (EC, 2015). This means that all the contaminants present in the sewage sludge, which include the UV-filters previously described, are continuously released into the environment.

So far, UV-filters have been found from the low ng g^{-1} to some µg g^{-1} in treated sludge, like EHT (27.7 µg g^{-1} dw) and OC (41.6 µg g^{-1} dw). However, if we compare with other emerging contaminants, such as synthetic musks which have been massively used for decades and are not successfully removed from WWTP and end up in agricultural soils and eventually are taken up by crops, this could also be the reality for UV-filters

(Macherius et al., 2012). In fact, there are already some evidences that benzotriazoles (UV-326, UV-327, UV-328 and UV-329) can accumulate and persist in biosolid-amended soils, being already found from low ng g^{-1} up to 3.3 µg g^{-1} (UV-329). Preliminary results have shown that they have slow dissipation in soils, and compounds were not found in the crop plants collected from the trial plots, however, more studies are already in motion.

Given the magnitude of the situation, the high concentrations found and diversity of compounds, more information is needed. There is a great lack of studies related to UV-filters behaviour in soils, their uptake and accumulation by plants, namely crops. However, these studies are essential to correctly asses their potential threat to environment, and most important to food safety.

2.4. Conclusion

This review summarized the available information to date on occurrence and removal of UV-filters on WWTPs. Due to the recent concern to sun exposure, UV-filters have been massively used in cosmetics, namely in sunscreens, translating into higher loads being discharged in WWTPs. Although most studies are focused in method development for the determination of UV-filters in wastewater and sludge, information regarding occurrence is rather large. However, information is geographically restricted to some European and Asian countries, as well as Australia, whereas data from other regions, namely America is missing. Therefore, there is indeed a need to expand and intensify studies in different regions of the globe to better understand the magnitude of this issue. Studies performed in WWTPs should specify in more detail operative parameters and employed treatments in order to better understand fate of the contaminants and to point out most potentially successful approaches for their removal.

Regarding the occurrence, the benzophenone derivatives BP3 and BP4 are the compounds found at higher concentrations both at influent and effluent wastewater in the mg L^{-1} range, probably because of being the most hydrophilic ones. On the other hand, compounds like EHT, 4-MBC and OC that are more lipophilic tend to sorb onto sludge and appear at high concentration in treated sludge in the µg g^{-1} dw range.

The high concentrations found among literature show that UV-filters may pose a potentially relevant threat to aquatic ecosystems, due to adverse effects UV-filters have shown in vitro and in vivo. Seasonal variation in usage and release for some compounds can also endanger sensitive species, especially during breeding periods.

The efficiency of the removal of UV-filters strongly depends on the employed wastewater treatment technology in WWTPs. Primary treatment is poor in removing UV-filters from wastewater, its contribution is mainly based on the fast sorption onto sludge by the more lipophilic compounds. During secondary treatment – a commonly used treatment – it's not always clear if compounds are degraded or removed by sorption, showing some potential for further clarification of this quest. Tertiary treatment is still uncommon in WWTPs and only available at the more sophisticated ones. So far, reverse osmosis presented the best removal rates for all compounds, however, its high operational costs and other technical limitations can be limiting to a large-scale implementation.

Acknowledgements

This work was financially supported by: Project UID/EQU/00511/2013-LEPABE, by the FCT/MEC with national funds and when applicable co-funded by FEDER in the scope of the P2020 Partnership Agreement; Project NORTE-07-0124-FEDER-000025 — RL2_ Environment & Health, by FEDER funds through Programa Operacional Factores de Competitividade — COMPETE, by the Programa Operacional do Norte (ON2) and by national funds through FCT — Fundação para a Ciência e a Tecnologia (Scholarships SFRH/BPD/76974/2011 and SFRH/BD/110831/2015).

Supplementary Information

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2015.10.004 or in Annex 2.

References

21CFR352.10, 2014. Code of Federal Regulations , Title 21, Volume 5. Sec. 352.10 Sunscreen active ingredients, USA directive (web: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=352.10).

Amine, H., Gomez, E., Halwani, J., Casellas, C., Fenet, H., 2012. UV filters, ethylhexyl methoxycinnamate, octocrylene and ethylhexyl dimethyl PABA from untreated wastewater in sediment from Eastern Mediterranean river transition and coastal zones. Mar. Pollut. Bull. 64, 2435–2442.

Badia-Fabregat, M., Rodríguez-Rodríguez, C.E., Gago-Ferrero, P., Olivares, A., Piña, B., Díaz-Cruz, M.S., Vicent, T., Barceló, D., Caminal, G., 2012. Degradation of UV filters in sewage sludge and 4-MBC in liquid medium by the ligninolytic fungus *Trametes versicolor*. J. Environ. Manag. 104, 114–120.

Balmer, M.E., Buser, H.R., Müller, M.D., Poiger, T., 2005. Occurrence of some organic UV filters in wastewater, in surface waters, and in fish from Swiss lakes. Environ. Sci. Technol. 39, 953–962.

Barón, E., Gago-Ferrero, P., Gorga, M., Rudolph, I., Mendoza, G., Zapata, A.M., Díaz-Cruz, S., Barra, R., Ocampo-Duque, W., Páez, M., Darbra, R.M., Eljarrat, E., Barceló, D., 2013. Occurrence of hydrophobic organic pollutants (BFRs and UV-filters) in sediments from South America. Chemosphere 92, 309–316.

Benedé, J.L., Chisvert, A., Salvador, A., Sánchez-Quiles, D., Tovar-Sánchez, A., 2014. Determination of UV filters in both soluble and particulate fractions of seawaters by dispersive liquid–liquid microextraction followed by gas chromatography–mass spectrometry. Anal. Chim. Acta 812, 50–58.

Bester, K., 2007. Personal Care Compounds in the Environment: Pathways, Fate and Methods for Determination. Wiley — John Wiley & Sons.

Blüthgen, N., Meili, N., Chew, G., Odermatt, A., Fent, K., 2014. Accumulation and effects of the UV-filter octocrylene in adult and embryonic zebrafish (Danio rerio). Sci. Total Environ. 476–477, 207–217.

Brooke, D.N., Burns, J.S., Crookes, M.J., 2008. UV-filters in Cosmetics — Prioritisation for Environmental Assessment.

Chisvert, A., León-González, Z., Tarazona, I., Salvador, A., Giokas, D., 2012. An overview of the analytical methods for the determination of organic ultraviolet filters in biological fluids and tissues. Anal. Chim. Acta 752, 11–29.

Chisvert, A., Tarazona, I., Salvador, A., 2013. A reliable and environmentally-friendly liquid-chromatographic method for multi-class determination of fat-soluble UV filters in cosmetic products. Anal. Chim. Acta 790, 61–67.

Christen, V., Zucchi, S., Fent, K., 2011. Effects of the UV-filter 2-ethyl-hexyl-4-trimethoxycinnamate (EHMC) on expression of genes involved in hormonal pathways in fathead minnows (*Pimephales promelas*) and link to vitellogenin induction and histology. Aquat. Toxicol. 102, 167–176.

Cosmetics Directive, 2009. Annex VI: list of UV filters allowed in cosmetic products in Europe. Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products (web: http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.results&annex_v2=VI&search).

Cunha, S.C., Pena, A., Fernandes, J.O., 2015. Dispersive liquid–liquid microextraction followed by microwave-assisted silylation and GC–MS analysis for simultaneous trace quantification of bisphenol A and 13 UV filters in wastewaters. J. Chromatogr. A 1414, 10–21.

Díaz-Cruz, M.S., Barceló, D., 2009. Chemical analysis and ecotoxicological effects of organic UV-absorbing compounds in aquatic ecosystems. TrAC Trends Anal. Chem. 28, 708–717.

EC, 2015. European Commission — new life for sewage sludge [WWW document], 06/05/2015. (web: http://ec.europa.eu/programmes/horizon2020/en/news/new-lifesewage-sludge) (accessed 7.3.15).

EPA, 1999. Giardia: drinking water health advisory [WWW document]. EPA-822-R-99-008.
http://water.epa.gov/action/advisories/drinking/upload/2009_02_03_criteria_human health_microbial_giardiaha.pdf (accessed 9.8.15).

Gackowska, A., Przybyłek, M., Studziński,W., Gaca, J., 2014. Experimental and theoretical studies on the photodegradation of 2-ethylhexyl 4-methoxycinnamate in the presence of reactive oxygen and chlorine species. Cent. Eur. J. Chem. 12, 612–623.

Gago-Ferrero, P., Alonso, M.B., Bertozzi, C.P., Marigo, J., Barbosa, L., Cremer, M., Secchi, E.R., Azevedo, A., Lailson-Brito, J., Torres, J.P.M., Malm, O., Eljarrat, E., Díaz-Cruz, M.S., Barceló, D., 2013a. First determination of UV filters in marine mammals. Octocrylene levels in Franciscana dolphins. Environ. Sci. Technol. 47, 5619–5625.

Gago-Ferrero, P., Diaz-Cruz, M.S., Barcelo, D., 2011a. Fast pressurized liquid extraction with in cell purification and analysis by liquid chromatography tandem mass spectrometry for the determination of UV filters and their degradation products in sediments. Anal. Bioanal. Chem. 400, 2195–2204.

Gago-Ferrero, P., Diaz-Cruz, M.S., Barcelo, D., 2011b. Occurrence ofmulticlass UV filters in treated sewage sludge from wastewater treatment plants. Chemosphere 84, 1158–1165.

Gago-Ferrero, P., Mastroianni, N., Díaz-Cruz, M.S., Barceló, D., 2013b. Fully automated determination of nine ultraviolet filters and transformation products in natural waters and wastewaters by on-line solid phase extraction–liquid chromatography–tandem mass spectrometry. J. Chromatogr. A 1294, 106–116.

Giokas, D.L., Salvador, A., Chisvert, A., 2007. UV filters: from sunscreens to human body and the environment. TrAC Trends Anal. Chem. 26, 360–374.

Golovko, O., Kumar, V., Fedorova, G., Randak, T., Grabic, R., 2014. Removal and seasonal variability of selected analgesics/anti-inflammatory, anti-hypertensive/cardiovascular

pharmaceuticals and UV filters in wastewater treatment plant. Environ. Sci. Pollut. Res. 21, 7578–7585.

Grabicova, K., Fedorova, G., Burkina, V., Steinbach, C., Schmidt-Posthaus, H., Zlabek, V., Kocour Kroupova, H., Grabic, R., Randak, T., 2013. Presence of UV filters in surface water and the effects of phenylbenzimidazole sulfonic acid on rainbow trout (*Oncorhynchus mykiss*) following a chronic toxicity test. Ecotoxicol. Environ. Saf. 96, 41–47.

Ho, Y.-C., Ding,W.-H., 2012. Solid-phase extraction coupled simple on-line derivatization gas chromatography–tandem mass spectrometry for the determination of benzophenone type UV filters in aqueous samples. J. Chin. Chem. Soc. 59, 107–113.

Ji, Y., Zhou, L., Zhang, Y., Ferronato, C., Brigante, M., Mailhot, G., Yang, X., Chovelon, J.-M.,2013. Photochemical degradation of sunscreen agent 2-phenylbenzimidazole-5-sulfonic acid in different water matrices. Water Res. 47, 5865–5875.

Jurado, A., Gago-Ferrero, P., Vàzquez-Suñé, E., Carrera, J., Pujades, E., Díaz-Cruz, M.S., Barceló, D., 2014. Urban groundwater contamination by residues of UV filters. J. Hazard. Mater. 271, 141–149.

Kaiser, D., Sieratowicz, A., Zielke, H., Oetken,M., Hollert, H., Oehlmann, J., 2012. Ecotoxicological effect characterisation of widely used organic UV filters. Environ. Pollut. 163, 84–90.

Kameda, Y., Kimura, K., Miyazaki, M., 2011. Occurrence and profiles of organic sunblocking agents in surface waters and sediments in Japanese rivers and lakes. Environ. Pollut. 159, 1570–1576.

Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2008a. Multiresidue methods for the analysis of pharmaceuticals, personal care products and illicit drugs in surface water and wastewater by solid-phase extraction and ultra performance liquid chromatography–electrospray tandem mass spectrometry. Anal. Bioanal. Chem. 391, 1293–1308.

Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2008b. The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. Water Res. 42, 3498–3518.

Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2009. The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. Water Res. 43, 363–380.

Kim, S., Jung, D., Kho, Y., Choi, K., 2014. Effects of benzophenone-3 exposure on endocrine disruption and reproduction of Japanese medaka (*Oryzias latipes*)—a two generation exposure study. Aquat. Toxicol. 155, 244–252.

Kockler, J., Oelgemöller, M., Robertson, S., Glass, B.D., 2012. Photostability of sunscreens. J. Photochem. Photobiol. C Photochem. Rev. 13, 91–110.

Kupper, T., Plagellat, C., Brändli, R.C., de Alencastro, L.F., Grandjean, D., Tarradellas, J., 2006. Fate and removal of polycyclic musks, UV filters and biocides during wastewater treatment. Water Res. 40, 2603–2612.

Langford, K.H., Reid, M.J., Fjeld, E., Øxnevad, S., Thomas, K.V., 2015. Environmental occurrence and risk of organic UV filters and stabilizers in multiple matrices in Norway. Environ. Int. 80, 1–7.

Li,W., Ma, Y., Guo, C., Hu,W., Liu, K., Wang, Y., Zhu, T., 2007. Occurrence and behavior of four of the most used sunscreen UV filters in a wastewater reclamation plant. Water Res. 41, 3506–3512.

Liu, J.-L., Wong, M.-H., 2013. Pharmaceuticals and personal care products (PPCPs): a review on environmental contamination in China. Environ. Int. 59, 208–224.

Liu, Y.-S., Ying, G.-G., Shareef, A., Kookana, R.S., 2011. Simultaneous determination of benzotriazoles and ultraviolet filters in ground water, effluent and biosolid samples using gas chromatography–tandem mass spectrometry. J. Chromatogr. A 1218, 5328–5335.

Liu, Y.-S.S., Ying, G.-G.G., Shareef, A., Kookana, R.S., 2012. Occurrence and removal of benzotriazoles and ultraviolet filters in a municipal wastewater treatment plant. Environ. Pollut. 165, 225–232.

Liu, Y.-S., Ying, G.-G., Shareef, A., Kookana, R.S., 2013. Degradation of six selected ultraviolet filters in aquifer materials under various redox conditions. Groundw. Monit. Remediat. 33, 79–88.

Macherius, A., Eggen, T., Lorenz, W.G., Reemtsma, T., Winkler, U., Moeder, M., 2012. Uptake of galaxolide, tonalide, and triclosan by carrot, barley, and meadow fescue plants. J. Agric. Food Chem. 60, 7785–7791.

Magi, E., Scapolla, C., Di Carro, M., Rivaro, P., Ngoc Nguyen, K.T., 2013. Emerging pollutants in aquatic environments: monitoring of UV filters in urban wastewater treatment plants. Anal. Methods 5, 428.

Manova, E., von Goetz, N., Hauri, U., Bogdal, C., Hungerbuhler, K., 2013. Organic UV filters in personal care products in Switzerland: a survey of occurrence and concentrations. Int. J. Hyg. Environ. Health 216, 508–514.

MHW, 2000. Standards for cosmetics in Japan (web: http://www.mhlw.go.jp/file/06-Seisakujouhou-11120000-Iyakushokuhinkyoku/0000032704.pdf).

Moeder, M., Schrader, S., Winkler, U., Rodil, R., 2010. At-line microextraction by packed sorbent-gas chromatography-mass spectrometry for the determination of UV filter and polycyclic musk compounds in water samples. J Chromatogr A 1217, 2925–2932.

Negreira, N., Rodríguez, I., Ramil, M., Rubí, E., Cela, R., 2009. Sensitive determination of salicylate and benzophenone type UV filters in water samples using solid-phase microextraction, derivatization and gas chromatography tandem mass spectrometry. Anal. Chim. Acta 638, 36–44.

Negreira, N., Rodríguez, I., Rubí, E., Cela, R., 2011. Optimization of pressurized liquid extraction and purification conditions for gas chromatography–mass spectrometry determination of UV filters in sludge. J. Chromatogr. A 1218, 211–217.

Paredes, E., Perez, S., Rodil, R., Quintana, J.B., Beiras, R., 2014. Ecotoxicological evaluation of four UV filters using marine organisms from different trophic levels *Isochrysis galbana*, *Mytilus galloprovincialis*, *Paracentrotus lividus*, and *Siriella armata*. Chemosphere 104, 44–50.

Pedrouzo, M., Borrull, F., Marcé, R., Pocurull, E., 2010. Stir-bar-sorptive extraction and ultra-high-performance liquid chromatography–tandem mass spectrometry for simultaneous analysis of UV filters and antimicrobial agents in water samples. Anal. Bioanal. Chem. 397, 2833–2839.

Plagellat, C., Kupper, T., Furrer, R., de Alencastro, L.F., Grandjean, D., Tarradellas, J., 2006. Concentrations and specific loads of UV filters in sewage sludge originating from a monitoring network in Switzerland. Chemosphere 62, 915–925.

Ramos, S., Homem, V., Alves, A., Santos, L., 2015. Advances in analytical methods and occurrence of organic UV-filters in the environment — a review. Sci. Total Environ. 526, 278–311.

Richardson, S.D., Ternes, T.A., 2014. Water analysis: emerging contaminants and current issues. Anal. Chem. 86, 2813–2848.

Rodil, R., Moeder, M., 2008a. Development of a method for the determination of UV filters in water samples using stir bar sorptive extraction and thermal desorption–gas chromatography–mass spectrometry. J. Chromatogr. A 1179, 81–88.

Rodil, R., Moeder, M., 2008b. Development of a simultaneous pressurised-liquid extraction and clean-up procedure for the determination of UV filters in sediments. Anal. Chim. Acta 612, 152–159.

Rodil, R., Moeder, M., Altenburger, R., Schmitt-Jansen, M., 2009b. Photostability and phytotoxicity of selected sunscreen agents and their degradation mixtures in water. Anal. Bioanal. Chem. 395, 1513–1524.

Rodil, R., Schrader, S., Moeder, M., 2009a. Non-porous membrane-assisted liquid–liquid extraction of UV filter compounds from water samples. J. Chromatogr. A 1216, 4887–4894.

Rodil, R., Schrader, S., Moeder, M., 2009c. Pressurised membrane-assisted liquid extraction of UV filters from sludge. J Chromatogr A 1216, 8851–8858.

Rodil, R., Quintana, J.B., Concha-Graña, E., López-Mahía, P., Muniategui-Lorenzo, S., Prada-Rodríguez, D., 2012. Emerging pollutants in sewage, surface and drinking water in Galicia (NW Spain). Chemosphere 86, 1040–1049.

Rodríguez-Rodríguez, C.E., Barón, E., Gago-Ferrero, P., Jelić, A., Llorca, M., Farré, M., Díaz-Cruz, M.S., Eljarrat, E., Petrović, M., Caminal, G., Barceló, D., Vicent, T., 2012. Removal of pharmaceuticals, polybrominated flame retardants and UV-filters from sludge by the fungus *Trametes versicolor* in bioslurry reactor. J. Hazard. Mater. 233-234, 235–243.

Salvador, A., Chisvert, A., 2005. Sunscreen analysis: a critical survey on UV filters determination. Anal. Chim. Acta 537, 1–14.

Santos, A.J.M., Crista, D.M.A., Miranda, M.S., Almeida, I.F., Sousa e Silva, J.P., Costa, P.C., Amaral, M.H., Lobão, P.A.L., Sousa Lobo, J.M., Esteves da Silva, J.C.G., 2013. Degradation of UV filters 2-ethylhexyl-4-methoxycinnamate and 4-tert-butyl-4'-methoxydibenzoylmethane in chlorinated water. Environ. Chem. 10, 127–134.

Santos, A.J., Miranda, M.S., Esteves da Silva, J.C., 2012. The degradation products of UV filters in aqueous and chlorinated aqueous solutions. Water Res. 46, 3167–3176.

Schlumpf, M., Schmid, P., Durrer, S., Conscience,M., Maerkel, K., Henseler, M., Gruetter,M., Herzog, I., Reolon, S., Ceccatelli, R., Faass, O., Stutz, E., Jarry, H., Wuttke, W., Lichtensteiger, W., 2004. Endocrine activity and developmental toxicity of cosmetic UV filters–an update. Toxicology 205, 113–122.

Semerjian, L., Ayoub, G.M., 2003. High-pH–magnesium coagulation–flocculation in wastewater treatment. Adv. Environ. Res. 7, 389–403.

Serpone, N., Dondi, D., Albini, A., 2007. Inorganic and organic UV filters: their role and efficacy in sunscreens and suncare products. Inorg. Chim. Acta 360, 794–802.

Shaath, N., 2010. Ultraviolet filters. Photochem. Photobiol. Sci. 9, 464–469.

Sieratowicz, A., Kaiser, D., Behr, M., Oetken, M., Oehlmann, J., 2011. Acute and chronic toxicity of four frequently used UV filter substances for *Desmodesmus subspicatus* and *Daphnia magna*. J. Environ. Sci. Health., Part A 46, 1311–1319.

Spellman, F.R., 2013a. Wastewater and wastewater treatment. Handbook of Water and Wastewater Treatment Plant Operations, Third ed. CRC Press, pp. 679–680

Spellman, F.R., 2013b. Current issues in water and wastewater treatment operations. Handbook of Water and Wastewater Treatment Plant Operations, Third ed. CRC Press, pp. 3–22

Spellman, F.R., 2013c. Wastewater treatment operations. Handbook of Water and Wastewater Treatment Plant Operations, Third ed. CRC Press, pp. 681–834

Tsui, M.M.P., Leung, H.W., Lam, P.K.S., Murphy, M.B., 2014b. Seasonal occurrence, removal efficiencies and preliminary risk assessment of multiple classes of organic UV filters in wastewater treatment plants. Water Res. 53, 58–67.

Tsui, M.M., Leung, H.W., Wai, T.C., Yamashita, N., Taniyasu, S., Liu, W., Lam, P.K., Murphy, M.B., 2014a. Occurrence, distribution and ecological risk assessment of multiple classes of UV filters in surface waters from different countries. Water Res. 67c, 55–65.

Wang, L.K., Shammas, N.K., Hung, Y.-T. (Eds.), 2007. Biosolids treatment processes, In: handbook of environmental engineering. Humana Press, Totowa, NJ.

Wang, L.K., Hung, Y.-T., Shammas, N.K. (Eds.), 2007. Advanced physicochemical treatment technologies, In: handbook of environmental engineering. Humana Press, Totowa, NJ.

Wang, L., Tay, J., Hung, Y., Tay, S., 2010. Environmental bioengineering. Handbooks of Environmental Engineering. Springer.

Weisbrod, C.J., Kunz, P.Y., Zenker, A.K., Fent, K., 2007. Effects of the UV filter benzophenone-2 on reproduction in fish. Toxicol. Appl. Pharmacol. 225, 255–266.

Wick, A., Fink, G., Ternes, T.A., 2010. J. Chromatogr. A 1217, 2088–2103.

Wick, A., Marincas, O., Moldovan, Z., Ternes, T.A., 2011. Sorption of biocides, triazine and phenylurea herbicides, and UV-filters onto secondary sludge. Water Res. 45, 3638–3652.

Wijekoon, K.C., Hai, F.I., Kang, J., Price, W.E., Guo, W., Ngo, H.H., Nghiem, L.D., 2013. The fate of pharmaceuticals, steroid hormones, phytoestrogens, UV-filters and pesticides during MBR treatment. Bioresour. Technol. 144, 247–254.

Wolf, R.,Wolf, D., Morganti, P., Ruocco, V., 2001. Sunscreens. Clin. Dermatol. 19, 452–459.

Wu, J.-W., Chen, H.-C., Ding, W.-H., 2013. Ultrasound-assisted dispersive liquid–liquid microextraction plus simultaneous silylation for rapid determination of salicylate and benzophenone-type ultraviolet filters in aqueous samples. J. Chromatogr. A 1302, 20–27.

Zhang, P.-P., Shi, Z.-G., Yu, Q.-W., Feng, Y.-Q., 2011. Talanta 83, 1711–1715, A new device for magnetic stirring-assisted dispersive liquid–liquid microextraction of UV filters in environmental water samples.

Zuloaga, O., Navarro, P., Bizkarguenaga, E., Iparraguirre, A., Vallejo, A., Olivares, M., Prieto, A., 2012. Overview of extraction, clean-up and detection techniques for the determination of organic pollutants in sewage sludge: A review. Anal. Chim. Acta 736, 7–29.

Part III: UV-Filters and Synthetic Musk Compounds – analytical methods for quantification in environmental matrices

In this section the analytical methodologies necessary to fulfil the aim of this work are presented. In Chapters 3, 4 and 5 the development and optimization of methodologies for the determination of the target compounds in environmental matrices (water, sludge and soil) are described. In Chapter 6 the methodology used for the analysis of the tomato is described. Within every methodology developed and validated, different real samples were analysed to test the applicability of each method.

Chapter 3. Simultaneous determination of synthetic musks and ultraviolet filters in water matrices by dispersive liquid-liquid microextraction followed by gas chromatography tandem mass-spectrometry

Sara Ramos, Vera Homem, Lúcia Santos,

Journal of Chromatography A, 1590, pp. 47-57 (2019)

Abstract

An analytical method was developed for the simultaneous analysis of 19 emergent compounds in water matrices, six UV-filters (UVFs), five nitro, six polycyclic and two macrocyclic musks. The target compounds were extracted by a dispersive liquid-liquid microextraction (DLLME) approach, using 2-propanol as the dispersive solvent and 1,1,2-trichloroethane as the extractant solvent. The extracts were then analysed by gas chromatography tandem mass-spectrometry (GC–MS/MS). This methodology was successfully validated for the analyses of the target compounds in five types of aqueous samples (tap, river and seawater and influent and effluent wastewater). Recoveries of the analytes based on the surrogate correction ranged from 80 to 120%, with a good repeatability (relative standard deviations less than 10%). The method limit of detection ranges from 0.1 ng L^{-1} (octocrylene (OC), celestolide (ADBI)) to 20.0 ng L^{-1} (benzophenone (BZ)). Both UVFs and synthetic musk compounds (SMCs) were detected in all matrices. Higher concentrations were found in wastewaters (total mean concentration in influents of 6248 ng L^{-1} and 3856 ng L^{-1} in effluents), followed by river water (159 ng L^{-1}). Only BZ was detected in one of the analysed seawater samples and none of the compounds were detected in tap water. The most detected UVFs among all matrices were BZ and drometrizole (DTS) and tonalide (AHTN) and galaxolide (HHCB) within the SMCs class. Among all matrices, wastewater was the one with higher number of compounds found per sample (both UVFs and SMCs).

Keywords: UV-filters; Synthetic musk compounds; Aqueous matrices; Dispersive liquid-liquid microextraction; GC–MS/MS.

3.1. Introduction

Personal care products (PCPs) are compounds that can be found in a variety of toiletries and household products (Buchberger, 2011; Gracia-Lor et al., 2012). Synthetic musk compounds (SMCs) are a multiclass group of semi-volatile organic compounds used as fragrances and fixative compounds in a variety of applications (perfumes, lotions, deodorants, soaps, textiles, washing agents, softeners, etc.) (Peck and Hornbuckle, 2006; Pinkas et al., 2017). They can be divided according to their chemical structure into four groups: nitro, polycyclic, macrocyclic and alicyclic musks (Vallecillos et al., 2015). Most of these compounds are freely used in cosmetics, however, due to toxicologic concerns, some nitro musks (musk ambrette (MA), musk moskene (MM) and musk tibetene (MT)) were prohibited and others (musk xylene (MX) and musk ketone (MK)) were restricted (Commission, 2011; European Parliament, 2009). UV-Filters (UVFs) are compounds used as active ingredients in sunscreens in order to protect the skin from deteriorating when exposed to ultraviolet radiation (Salvador and Chisvert, 2005). UVFs compounds protect either from UV-A (320-400 nm) or UV-B (280-320 nm) radiation or both, and can be used alone or combined in order to achieve the sun protector factor (SPF) desired (González et al., 2008). Their use is restricted to concentrations between 0.1 and 10% (%w/w) and, according to the European legislation, only 26 organic compounds and 2 inorganic are allowed in cosmetic products (CosmeticsDirective, 2009).

Due to the massive and continuous use of SMCs and UVFs, they are considered emerging contaminants (Sauvé and Desrosiers, 2014), having direct and indirect pathways to the environment (Ramos et al., 2016; Richardson and Ternes, 2014; Wang and Wang, 2016). The source and type of these PCPs found in environmental matrices vary according to their physicochemical properties. However, many PCPs are frequently found in wastewater treatment plants (WWTPs) since they are disposed down-the-drain daily (Prosser and Sibley, 2015). Although lipophilic compounds tend to accumulate in the sewage sludge, some of these remain in water phase. In fact, most conventional WWTPs are not capable to remove these compounds and therefore, they are often detected in effluent wastewaters at relatively high concentrations, eventually being discharged into the environment(to rivers or even directly to the sea) (Homem et al., 2015; Ramos et al.,

2015). Nevertheless, these compounds can also end up in lake, river or sea water due to direct contamination, through wash-off from the skin and cloth during recreational activities like aquatic sports or swimming (Fent et al., 2010; Tashiro and Kameda, 2013).

Several critical and updated reviews of the literature regarding analytical methods used for synthetic musks and UV-filter determination in water matrices have recently appeared (Albero et al., 2015; Pedrouzo et al., 2011; Pietrogrande and Basaglia, 2007; Ramos et al., 2016, 2015; Richardson and Ternes, 2014). Microextraction techniques stand out due to the lower sample volume required and low organic solvent consumption, simpler equipment and handling, such as dispersive liquid–liquid microextraction (DLLME) (Cunha et al., 2015; Homem et al., 2016). So far, different types and amounts of extraction and dispersion solvents have been used both for UVF and SMCs. Carbon tetrachloride (TCC) has been used as an extractant for SMCs, where five polycyclic musks (ADBI, Phantolide (AHMI), Traseolide (ATII), HHCB, AHTN) were extracted yielding recoveries between 77 and 93% when methanol (MeOH) was used as a dispersive solvent (Panagiotou et al., 2009) and six polycyclic musks (Cashmeran (DPMI), ADBI, AHMI, ATII, HHCB, AHTN) were extracted yielding recoveries between 70 and 95% for surface water and wastewater with 2-propanol (IPA) as the dispersive solvent (Yang and Ding, 2012). On the other hand, chloroform (CF) has been used both for SMCs (Homem et al., 2016; López-Nogueroles et al., 2011) and UVFs (Benedé et al., 2014; Tarazona et al., 2010; Tovar-Sánchez et al., 2013). For SMCs, CF was combined either with acetonitrile (ACN) (dispersive solvent), yielding recoveries between 71 and 118% for 5 nitro, 5 poly and 2 macrocyclic musks (Homem et al., 2016), and with acetone (Ac) resulting in recoveries of 87 to 93% both in surface water and wastewater, but only for 5 nitro musks (López-Nogueroles et al., 2011). For UVFs, CF was combined with Ac and different compounds were extracted: benzophenone-3 (BP3), Isoamyl *p*-methoxycinnamate (IMC), 4-Methyl benzylidene camphor (4MBC), OC, Ethylhexyl dimethyl PABA (EDP), 2-ethylhexyl 4 –methoxycinnamate (EMC), 2-Ethylhexyl salicylate (ES) and Homosalate (HMS) with recoveries ranging 82 and 117% (Benedé et al., 2014); BP3 and 4MBC (Tovar-Sánchez et al., 2013) and benzophenones: benzophenone-1 (BP1), BP3, benzophenone-8 (BP8) and 2,3,4-Trihydroxybenzophenone (234THB) with recoveries between 65 and 169% (Tarazona et al., 2010). Other extraction solvents have

also been used for UVFs like tetrachloroethylene (TCE), combined with Ac, to extract ES, HMS, BP3, BP1, BP8 (70-93% recovery range) (Wu et al., 2013); 1-octanol (Zhang et al., 2011) with no dispersive combined (for 4-Hydroxybenzophenone (4HB), BP1, BP, BP3) yielding recoveries around 90%; and ionic liquid based solvents like [C4MIM]PF6 (Ye et al., 2011) and [HMIM][FAP] (Zhang and Lee, 2012), both combined with MeOH as the dispersive solvent, but only benzophenone compounds, ES and HMS were analysed.

Until now, most studies regarding the detection of UVFs in aqueous samples apply a final step of derivatization before analysis in GC-MS/MS (in order to convert these compounds in more volatile ones) or UVFs compounds are analysed by HPLC or LC-MS. However, it is possible to analyse some UV-filters by gas chromatography without a derivatization step and develop an analytical method to extract and analyse both SMs and UVFs simultaneously.

Therefore, the aim of the present work was to optimize and validate a simple methodology based on ultrasound-assisted dispersive liquid-liquid microextraction followed by gas chromatography tandem mass-spectrometry (USA-DLLME-GC-MS/MS), for the simultaneous analyses of different classes of UV-filters and synthetic musks in different aqueous matrices. The method performance was assessed in terms of linearity, limits of detections (LODs), accuracy and precision. To demonstrate the applicability of the proposed methodology, several types of water samples, including wastewater, tap water, sea water and river water, were analysed.

3.2. Materials and methods

3.2.1 Standards, reagents and materials

The polycyclic musks were obtained as solid standards from LGC Standards (Barcelona, Spain) with 99% purity for cashmeran (DPMI), celestolide (ADBI), phantolide (AHMI), traseolide (ATII), and tonalide (AHTN), except for galaxolide, which contains approximately 25% of diethyl phthalate. The nitro musks tibetene (MT) and musk moskene (MM) were also obtained as a 10 mg L^{-1} in cyclohexane from LGC Standards. Musk ambrette (MA) and musk ketone (MK) were purchased as solid standards from Dr. Ehrenstorfer (Augsburg, Germany) with 99% and 98% purity, respectively. Musk xylene

(MX) was obtained from Sigma-Aldrich (St. Louis, MO, USA) as a 100 mg L^{-1} solution in acetonitrile. Solid standards of exaltolide (EXA) and ethylene brassylate (EB) were also purchased from Sigma-Aldrich with 99% and 95% purity, respectively. The surrogate standards musk xylene-d15 (MX-d15) and tonalide-d3 (AHTN-d3) were purchased from Dr. Ehrenstorfer (Augsburg, Germany) as 100 mg L^{-1} solutions in acetone and iso-octane, respectively. UV-filters 2-Ethylhexyl 4-dimethylaminobenzoate (EDP) and 3-(4'-Methylbenzylidene) camphor (4-MBC) were purchased from Alfa Aesar (Karlsruhe, Germany), both with 99% purity. 2-Ethylhexyl 4 –methoxycinnamate (EMC), 2-ethylhexyl 2-cyano-3,3-diphenylacrylate (OC) and benzophenone (BZ) were purchased from Sigma-Aldrich (St. Louis, MO, USA) with 98%, 97% and 99% purity, respectively. Drometrizole trisiloxane (DTS) was purchased from Fluka (Saint Louis, MO, USA) with 98% purity. Surrogate (±)-3-(-4-Methylbenzylidine-d4)camphor (4-MBC-d4) was purchased with 99% purity from CDN Isotopes (Pointe-Claire, Quebec, Canada).

Individual stock solutions of each compound were prepared in both acetonitrile and hexane. Those stock solutions in acetonitrile were used to prepare the spike mix solution and those in hexane to prepare the analytical control standard. Stock and working solutions were stored and preserved in a freezer at -20 °C, protected from the light.

Acetone (Ac), acetonitrile (ACN), methanol (MeOH), chlorobenzene (CB), chloroform (CF), 2-propanol (IPA) and tetrachloroethylene (TCE) were purchased from VWR BDH Prolabo (Fontenay-sous-Bois, France), ethanol (EtOH) (96% v/v) was obtained from Panreac (Barcelona, Spain) and 1,1,2-trichloroethane (TC) was purchased from Merck (Darmstadt, Germany). All solvents used were analytical grade. Sodium chloride (NaCl), used to adjust the ionic strength, was purchased from Merck (Darmstadt, Germany) as well as ultrapure water, with LC-MS grade.

3.2.2 Samples

To demonstrate the applicability of the proposed methodology, several water samples were analysed - tap, river and sea waters and influent and effluent wastewaters. Tap water was collected in the laboratory on the day of the analysis, while seawater was collected in June 2017 from Angeiras Sul beach (Matosinhos, Portugal) and Carneiro

beach (Porto, Portugal) and river samples from river Leça (Matosinhos, Portugal) and Ave (Vila do Conde, Portugal). 24 h composite samples from influent and effluent wastewater were collected from a WWTP with secondary treatment (Porto, Portugal). All samples were collected in amber glass bottles and stored in the freezer at -20 °C until they were processed.

3.2.3 USA-DLLME procedure

Before the extraction, all samples were centrifuged (Hettich® Rotofix 32A, Tuttlingen, Germany) for 15 min at 2670 × g to remove suspended particles. Then, 6 mL of the centrifuged aqueous sample was placed into a 15 mL polypropylene tube with conical bottom, containing 3.5% wt of NaCl and 50 µL of a 100 µg L^{-1} surrogate solution (MX-d15, AHTN-d3 and 4MBC-d4) in acetonitrile. Subsequently, 880 µL of 2-propanol and 80 µL of 1,1,2-trichloroethane were mixed and rapidly injected into the sample, forming a cloudy solution. The sample was ultrasonicated for 2 minutes in a 420 W ultrasonic bath (J.P. Selecta, Barcelona, Spain) and the organic phase separated by centrifugation at 2670 x g for 15 minutes. The sedimented phase was transferred to a vial with insert, dried under a gentle stream of nitrogen and reconstituted with 50 µL of hexane before GC-MS/MS analysis. Samples were analysed in duplicate.

3.2.4 GC-MS/MS analysis

Analysis were performed on a gas chromatograph coupled to triple quadrupole mass spectrometer from Bruker (Massachusetts, EUA). This GC-MS system was equipped with a 436 gas chromatograph, a mass spectrometer EVOQ triple quadrupole, an injector CP-1177 split/splitless and an autosampler CP8410 from Bruker. For the separation of the target compounds, a J&W CP-Sil 8 CB capillary column (50 m x 0.25 mm I.D. x 0.12 µm) from Agilent Technologies (Santa Clara, California, EUA) was used. Helium (99.999%) was used as a carrier at a constant flowrate of 1.0 mL min^{-1}. 2 µL sample were injected in splitless mode (split ratio of 1:100 after 1 min for injector purging) at 280 ^{o}C. The GC oven temperature program starts at 70 ^{o}C for 1 min, raise to 180 ^{o}C at 25 ^{o}C min^{-1}, then 10 ^{o}C min^{-1} until 240 ^{o}C and finally 25 ^{o}C min^{-1} until 300 ^{o}C (for 5 min).

The MS/MS analysis was carried out in electron ionization (EI) mode, using the multiple reaction monitoring (MRM) mode. Two specific MRM transitions were chosen *per* compound (one for quantifying and one as qualifier), except for the nitro musks and surrogates, where two qualifiers were used for better identification. The ion source was operated at 280 ^{o}C with electron energy of -70 eV and filament current of 40 μA. The temperature of the transfer line was set at 270 ^{o}C. Ultra-pure argon was used as collision gas and its pressure was set at 2.00 mTorr. The MRM transitions and collision energies optimized for each compound are presented in Table 3.1.

Table 3.1 Optimized transitions for the analysis of the target compounds, UV-Filters, Synthetic Musk Compounds and Surrogates. Quantifier transition presented in bold.

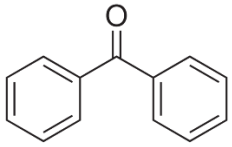
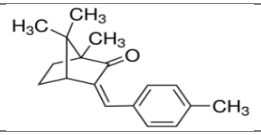
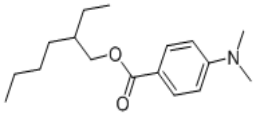
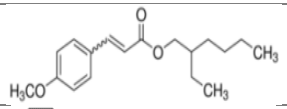
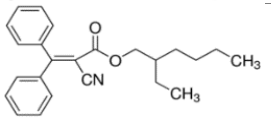
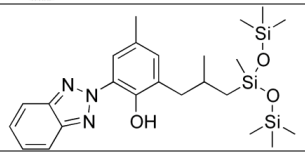
Class	Compound	Abbreviation	Structure	CAS	Molecular weight	Log K_{ow}	Retention time (min)	MRM transition	Collision Energy (eV)	Surrogate
UV-Filters	Benzophenone	BZ		119-61-9	182.22	3.2	7.71	**105>51** 182>105	(25) (10)	AHTN-d3
	3-(4'-methylbenzylidene) camphor	4-MBC		36861-47-9	254.37	5.9	11.23	**128>77** 254>149	(25) (10)	4MBC-d4
	Ethylhexyl dimethyl PABA	EDP		21245-02-3	277.4	5.8	12.63	**165>119** 165>149	(20) (10)	4MBC-d4
	2-ethylhexyl 4 – methoxycinnamate	EMC		5466-77-3	290.4	5.8	12.91	**178>161** 178>132	(10) (15)	4MBC-d4
	Octocrylene	OC		6197-30-4	361.48	6.9	14.49	**204>176** 360>276	(25) (20)	4MBC-d4
	Drometrizole trisiloxane	DTS		155633-54-8	501.85	10.8	15.71	**221>73** 221>221	(15) (5)	4MBC-d4

*Table 3.1 Optimized transitions for the analysis of the target compounds, UV-Filters, Synthetic Musk Compounds and Surrogates. Quantifier transition presented in bold (*cont.).

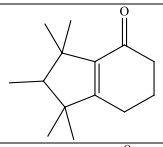
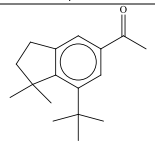
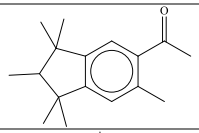
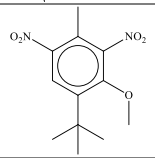
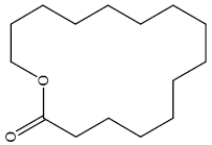
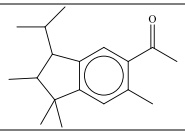
Class	Compound	Abbreviation	Structure	CAS	Molecular weight	Log K_{ow}	Retention time (min)	MRM transition	Collision Energy (eV)	Surrogate
Synthetic Musks	Cashmeran	DPMI		33704-61-9	206.3	4.9	6.77	**191>135** 206>192	(10) (10)	AHTN-d3
	Celestolide	ADBI		13171-00-1	244.3	6.6	8.26	**229>173** 244>229	(5) (10)	AHTN-d3
	Phantolide	AHMI		15323-35-0	244.3	6.7	8.59	**244>229** 229>187	(5) (5)	AHTN-d3
	Musk ambrette	MA		83-66-9	268.3	5.7	9.18	**253>106** 253>121 268>253	(10) (5) (5)	MX-d15
	Exaltolide	EXA		106-02-5	240.4	6.0	9.35	**83>55** 69>68	(5) (5)	AHTN-d3
	Traseolide	ATII		68140-48-7	258.4	8.1	9.32	**215>173** 258>215	(10) (5)	AHTN-d3

*Table 3.1 Optimized transitions for the analysis of the target compounds, UV-Filters, Synthetic Musk Compounds and Surrogates. Quantifier transition presented in bold (*cont.).

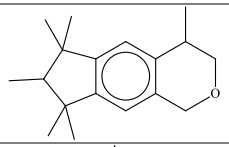
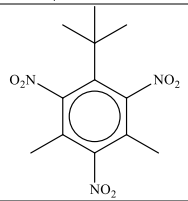
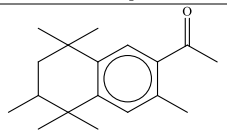
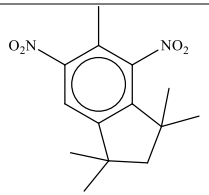
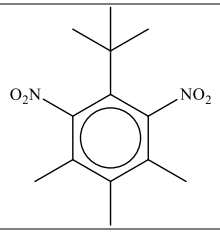
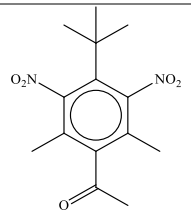
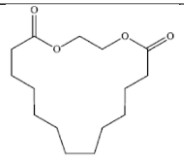
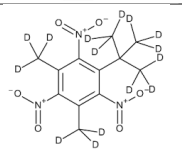
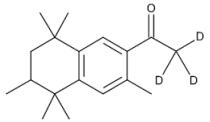
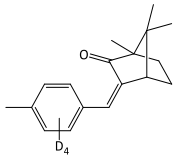
Class	Compound	Abbreviation	Structure	CAS	Molecular weight	Log K_{ow}	Retention time (min)	MRM transition	Collision Energy (eV)	Surrogate
Synthetic Musks	Galaxolide	HHCB		1222-05-5	258.4	5.9	9.39	**243>213** 213>171	(10) (5)	AHTN-d3
	Musk xylene	MX		81-15-2	297.2	4.8	9.40	**282>119** 282>160 282>265	(10) (10) (5)	MX-d15
	Tonalide	AHTN		1506-02-1	258.4	5.7	9.40	**258>243** 243>128	(10) (40)	AHTN-d3
	Musk moskene	MM		116-66-5	278.3	5.8	9.63	**263>156** 263>144 263>211	(20) (25) (5)	MX-d15
	Musk tibetene	MT		145-39-1	266.3	5.9	10.04	**266>251** 251>132 251>160	(5) (10) (15)	MX-d15

*Table 3.1 Optimized transitions for the analysis of the target compounds, UV-Filters, Synthetic Musk Compounds and Surrogates. Quantifier transition presented in bold (*cont.).

Class	Compound	Abbreviation	Structure	CAS	Molecular weight	Log K_{ow}	Retention time (min)	MRM transition	Collision Energy (eV)	Surrogate
Synthetic Musks	Musk ketone	MK		81-14-1	294.3	4.3	10.38	**279>118** 279>191 294>279	(20) (10) (5)	MX-d15
	Ethylene brassylate	EB		105-95-3	270.4	4.7	10.83	**98>83** 227>113	(5) (10)	AHTN-d3
Surrogates	Xylene-d15	MX-d15		877119-10-3	312.36		9.28	**294>294** 294>122 294>276	(5) (15) (10)	
	Tonalide-d3	AHTN-d3			261.40		9.42	**246>190** 246>204 261>246	(5) (10) (5)	
	(±)-3-(4-Methylbenzylidine-d4) camphor	4-MBC-d4		1219806-41-3	258.40		10.22	**132>105** 258>150 258>108	(15) (5) (10)	

3.2.5. Quality assurance and control

UVFs and SMCs are present in most of the personal care products and for that reason some precautions were taken to avoid sample contaminations. Analysts avoided the use of scented cosmetics such as perfume, lotions and hand creams, as well as personal care products containing UV protection. All the glass material was rinsed with acetone and distilled water and the non-calibrated material was further subject to heating at 400 ^{o}C for at least 1 hour. Additionally, procedural blanks were extracted and analysed in order to identify eventual contaminations and correct samples concentration.

3.3. Results and discussion

3.3.1. Optimization of USA-DLLME conditions

The extraction methodology was developed based on a previously one implemented by Homem et al. (2016) for SMCs. This method was tested (880 µL of ACN as disperser solvent and 80 µL of CF as extraction solvent) and results were quite satisfactory for both SMs and UVFs when matrix free ultrapure water was used, with recoveries varying between 80 and 120%. Apparent recoveries of the analytes were calculated based on the response factor (RF). This RF is based on the peak area of the compound/ peak area of corresponding surrogate x 100. However, when this methodology was implemented to real water samples, like wastewaters, recoveries for UVFs were low (<50%). Trying to improve these recovery rates, different dispersing and extraction solvents were tested: acetonitrile (ACN), methanol (MeOH), ethanol (EtOH), acetone (Ac) and 2-propanol (IPA) were used as disperser solvents, while chlorobenzene (CB), chloroform (CF), tetrachloroethylene (TCE) and 1,1,2-trichloroethane (TC) as extraction solvents. Those were selected based on the physicochemical properties of the target compounds as well as information available in literature (Cacho et al., 2016; Cunha et al., 2015; Homem et al., 2016; Maya et al., 2014; Yang and Ding, 2012; Zhang et al., 2011). The target compounds present a low polarity, although most selected UVFs are slightly more polar than SMCs. For this reason, non- to low polar extraction solvents were chosen for this work. Among the extraction solvents, TCE is a non-polar solvent, which may not favour the extraction procedure since the selected SMCs and UVFs are not completely non-

polar. CF is considered a slightly polar solvent (1.15 D), followed by TC (1.36 D) and CB (1.54 D) (Stenutz, n.d.). The water interfacial tension is another important parameter that may influence the extraction efficiency. In fact, the lower the water interfacial tension of the extraction solvents, the greater the efficiency in the emulsion formation and, consequently, the greater the extraction of the compounds into the organic phase. The extraction solvent that presents the lowest value is the TC (29.6 mN m^{-1}), followed by CF (31.6 mN m^{-1}), CB (37.4 mN m^{-1}) and TCE (47.5 mN m^{-1}) (Montgomery, 2007)

Regarding the disperser solvent, it plays an important role in the DLLME process, decreasing the interfacial tension between water and extracting solvent. In fact, this will enhance the extraction yields through the production of droplets with a smaller size (increase of the contact surface areas)(Al-Said and Emara, 2014). From the selected solvents, IPA has the lowest surface tension (20.9 mN m^{-1}), followed by EtOH (21.9 mN m^{-1}), MeOH (22.2 mN m^{-1}) and Ac (22.7 mN m^{-1}) and ACN (28.7 mN m^{-1})(Rumble, 2017).

In this stage, the different combinations of the previously mentioned solvents were tested (Figure 3.1), using the original methodology (Homem et al., 2016) (volume of sample, volumes of solvents, extraction time and ionic strength were maintained). At this time, tap water was used since it can be considered a more challenging matrix than ultrapure water due to the presence of residual chlorine, a powerful oxidizing agent employed during water disinfection.

In general, a combination of high recoveries (>70%) and low RSD values (<15%) were found for all the pairs of solvents tested (Figure 3.1). However, analysing in more detail the results, it can be seen that the UV-filter EMC was not recovered when CF was used as extraction solvent or when the pair TC-ACN was applied (Figure 3.1B and 3.1C). EMC is one of the most commonly used UVFs in Europe and has often been detected in environmental matrices at high concentration ranges(Kameda et al., 2011; Ramos et al., 2015). For that reason, EMC should be maintained as one of the target compounds of this study and authors decided to discard the use of CF as extraction solvent, as well as the pair TC-ACN. Comparing the remaining results, it can also be seen that for certain compounds, especially nitro musks, macrocyclic musk EB and UV-filter BZ and DTS, some pairs of solvents conducted to considerable matrix effects (Rec > 120%). This behaviour is particularly noticeable when combinations like CB-ACN, CB-MeOH, CB-EtOH, CB-IPA

(Figure 3.1A) and TCE-Ac and TCE-EtOH (Figure 3.1D) were used (recoveries reached the 147%). Then, the recoveries of surrogates were analysed for the other pairs of solvents (*data not shown)*.

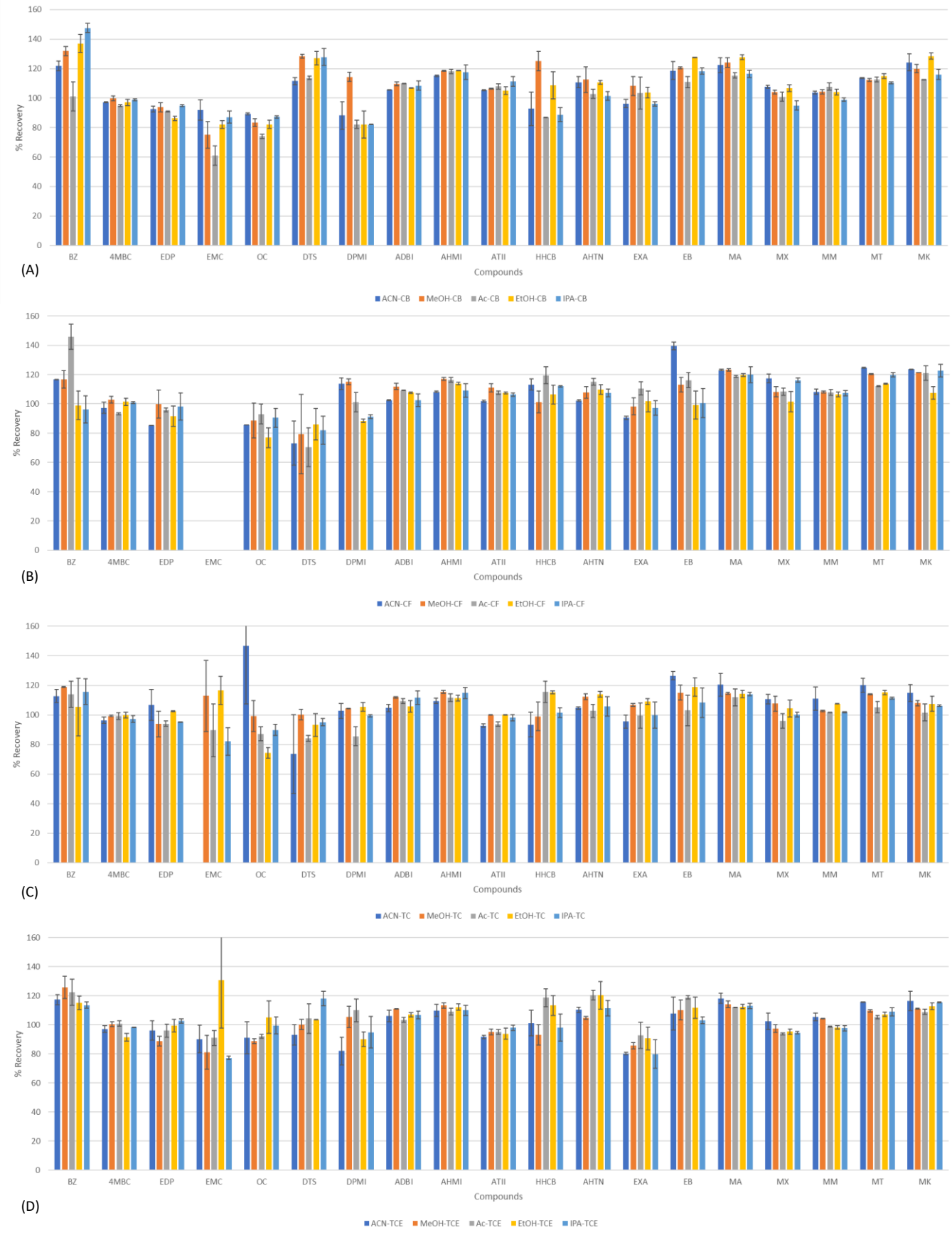


Figure 3.1 Mean recoveries (n=3) of the target compounds from tap water samples, spiked at 1500 ng L^{-1} using as extraction solvents: (A) CB, (B) CF, (C) TC and (D) TCE.

Relatively low values were found for the CB-Ac, TCE-ACN, TCE-MeOH and TCE-IPA (around 50%). The most promising pairs of solvents were subsequently tested for the extraction of wastewater, a matrix particularly difficult to work due to the high number of interfering compounds and organic matter present (Figure 3.2). The best results were obtained for the TC-IPA pair, considering the high recoveries (between 80 and 119%) and precision (RSD between 1 and 10%). In fact, this is in accordance with the DLLME theory. TC present a low polarity and, among the extraction solvents tested, it is the compound with the lowest water interfacial tension, promoting the conditions for the formation of an emulsion. Besides that, the combined use of IPA as disperser solvent may increases the extraction rates. As explained before, IPA has the lowest surface tension and, for that reason, it may promote the formation of smaller droplets. This pair of solvents was also tested for river and seawater (*data not shown*) and results were similar to those obtained with wastewater. Since results were acceptable, validation was performed for these four matrices.

The influence of adding salt on the efficiency of DLLME, was studied and optimized by Homem et al. (2016). Nevertheless, int his study, the ionic strength of the samples was also tested adding NaCl (0%, 1.5%, 3.5%, 5.5% and 7.5%). The addition of NaCl intends to stimulate the transport of the target analytes to the extraction solvent. This occurs because water molecules have the tendency to form hydration spheres around the salt ions. This reduces the water availability to dissolve molecules, thus forcing them into the extraction phase (Vallecillos et al., 2012). Higher recoveries were obtained with 3.5% NaCl, which coincides with that reported by Homem et al. (2016) (*data not shown*).

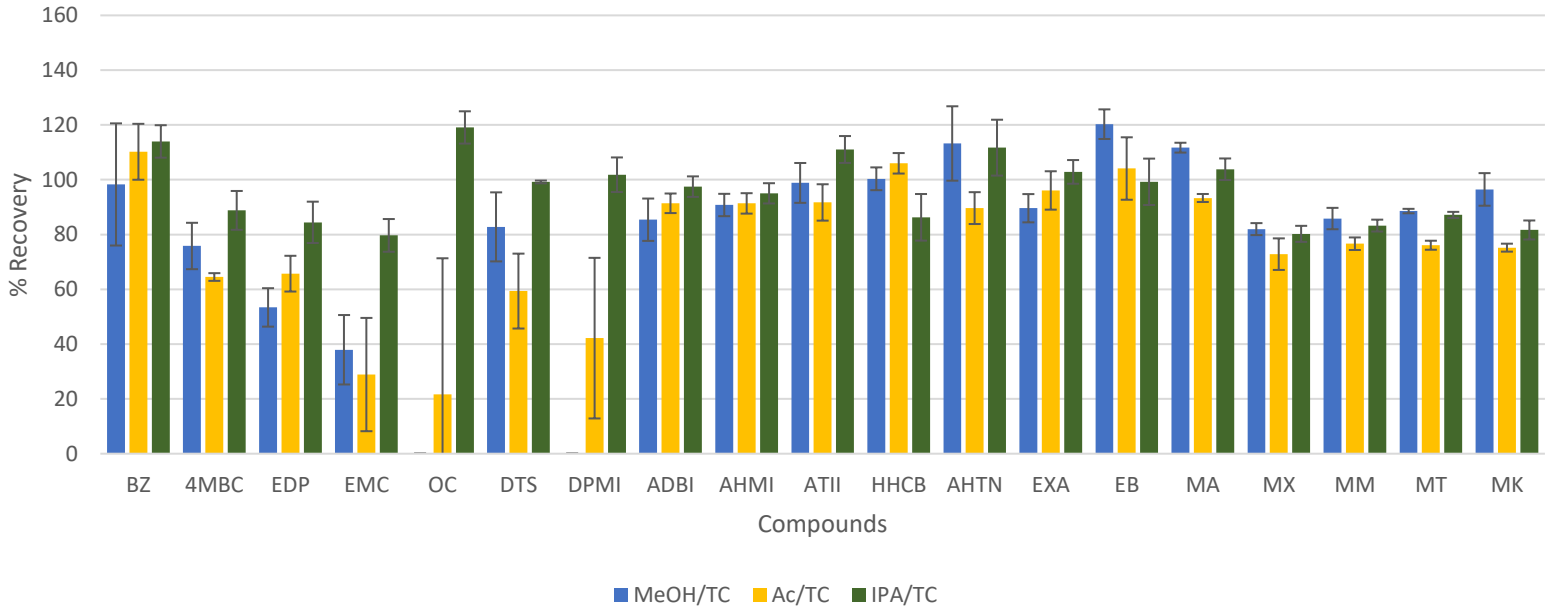


Figure 3.2 Mean recoveries (n= 3) of the target compounds from influent wastewater samples, spiked at 1500 ng L^{-1}, using TC as extraction solvent and ACN, MEOH, Ac and IPA as dispersive solvents.

3.3.2. Method validation

The method developed was validated regarding the linearity ranges, coefficients of determination, limits of detection (LODs) and quantification (LOQs), accuracy and precision (intra and inter-day).

For SMs and UVFs a good linear behaviour was obtained ($R > 0.975$) for all target compounds within the linearity range (4 to 1500 ng L^{-1}) (Table 3.2). The LODs and LOQs were estimated based on a signal-to-noise (S/N) ratio of 3 and 10, respectively. The LODs ranged from 0.1 ng L^{-1} (OC and ADBI) to 20.0 ng L^{-1} (BZ) (Table 3.2). The accuracy was assessed by recovery tests using spiked water samples (influent and effluent wastewater, tap, river and sea water) at three different levels (250, 500 and 1500 ng L^{-1}). The recoveries obtained under the optimized conditions are summarized in Table 3.3. Those are quite satisfactory, varying between 83 and 120%, which is well acceptable for this type of analysis. Mean absolute recovery of the surrogates in water samples was about 70%, in tap water (78% for 4-MBC-d4, 69% for MX-d15, 64% for AHTN-d3), sea water (97% for 4-MBC-d4, 74% for MX-d15, 69% for AHTN-d3), river water (75% for 4-MBC-d4, 60% for MX-d15, 67% for AHTN-d3), influent wastewater (70% for 4-MBC-d4, 75% for MX-d15, 62% for AHTN-d3) and effluent wastewater (62% for 4-MBC-d4, 62% for MX-d15, 61% for AHTN-d3). These recovery values are comparable to those found in literature using more complicate and time-consuming methodologies (Fent et al., 2010; Hu et al., 2011a; Kasprzyk-Hordern et al., 2009; Lee et al., 2010; M. M. P. Tsui et al., 2014). The precision of the method (intra and inter-day) was studied assessing the relative standard deviations (%RSD) of three replicate spiked samples at different concentrations (250, 500 and 1500 ng L^{-1}). Either for intra- and inter-day precision, the RSDs were always below 10% (Table 3.2 and 3.3), indicating that the proposed methodology is precise.

Considering the distinct types of aqueous matrices tested and the proposed multi-residue approach, results are quite satisfactory. Overall recoveries and LODs are in the same order of magnitude of those reported in literature for the analysis of UVFs or SMs in water matrices using more complex and time-consuming methodologies. For example, Rodil and Moeder (2008) developed a method for the determination of UVFs in water samples (lake water, river water and treated wastewater) using stir-bar sorptive

extraction followed by thermal desorption–gas chromatography–mass spectrometry. The authors achieved similar recoveries for the studied compounds (EMC, 4MBC, EDP and OC) and LODs ranging from 0.2 to 63 ng L^{-1}. Pedrouzo et al. (2010), also using a stir-bar sorptive extraction and ultra-high-performance liquid chromatography–tandem mass spectrometry for simultaneous analysis of UVFs in water samples, achieved lower recovery percentages (20-97%), but similar LODs (2.5-10 ng L^{-1}). Yang and Ding (2012) determined several synthetic polycyclic musks in aqueous samples, using a similar analytical methodology, i.e. ultrasound-assisted dispersive liquid–liquid microextraction coupled to gas chromatography–mass spectrometry. They used a different pair of solvents (carbon tetrachloride as extractant solvent and IPA as disperser solvent) and achieved similar LODs (0.2 ng L^{-1}), RSD values below 11% (both intra- and inter-day precision) and recoveries between 71 and 104%. Both da Silva et al. (2015), Kameda et al. (2011) and Tsui et al. (2014) used solid phase extraction (SPE) as the extraction method coupled to GC-MS analysis. In the first case, recoveries ranged from 62 to 107%, which are accordant to our study, however LOD were 19.3 ng L^{-1} for OC and 23.5 ng L^{-1} for EMC (da Silva et al., 2015), a bit higher than our study. The second one (Kameda et al., 2011) obtained recoveries ranging 80-113 for 4-MBC, EDP, EMC and OC and lower LOD (0.1 to 3.0 ng L^{-1}). As for the study of Tsui et al. (2014), several UVFs were studied, among them EDP, 4-MBC, EMC and OC, with recoveries ranging 63 and 106 and low method limits (0.03 to 1.38 ng L^{-1}). Regarding SMCs, Hu et al. (2011b) studied several nitro and poly musks in water (AHTN, HHCB; ADBI, AHMI, ATII, MK, MX) yielding recoveries from 78.6 to 106.3% using SPE with C_{18} disks followed by GC-MS analysis.

Table 3.2 Limits of detection (LOD) and quantification (LOQ) and interday precision for different aqueous matrices.

	Compounds																		
	BZ	4MBC	EDP	EMC	OC	DTS	DPMI	ADBI	AHMI	ATII	HHCB	AHTN	EXA	EB	MA	MX	MM	MT	MK
LOD (ng L^{-1})[a]	20.0	2.9	3.9	0.4	0.1	8.6	0.9	0.1	0.3	0.2	0.6	0.5	10.9	1.5	8.7	5.3	7.3	3.5	3.2
LOQ (ng L^{-1})[b]	66.7	9.5	12.9	1.2	0.4	28.6	3.1	0.2	1.0	0.8	1.9	1.6	36.4	5.0	28.9	17.8	24.2	11.8	10.5
Intreray precision (%RSD) Influent wastewater																			
1500 ng L^{-1}	6	4	9	6	9	6	5	5	9	3	8	7	8	6	6	5	10	10	10
500 ng L^{-1}	8	4	10	6	6	4	8	1	2	9	4	6	9	6	10	1	3	8	10
250 ng L^{-1}	9	8	6	4	9	1	9	2	5	4	4	9	7	7	2	10	1	3	10
50 ng L^{-1}	1	2	0	4	1	3	3	3	2	5	5	3	3	3	3	1	4	2	2
Interday precision (%RSD) Effluent wastewater																			
1500 ng L^{-1}	7	1	6	7	9	8	5	3	2	2	2	7	1	3	3	6	7	6	6
500 ng L^{-1}	8	2	6	6	1	4	9	9	7	7	8	9	1	9	1	7	4	1	4
250 ng L^{-1}	9	3	5	8	3	3	10	6	5	5	9	0	5	6	4	6	3	2	5
50 ng L^{-1}	2	1	2	2	1	1	2	1	1	1	2	3	4	4	1	4	1	1	2
Interday precision (%RSD) Tap water																			
1500 ng L^{-1}	9	7	7	4	5	4	4	7	3	2	2	8	3	7	4	4	4	7	2
500 ng L^{-1}	2	4	6	2	6	5	3	2	3	2	2	2	1	1	1	8	4	0	2
250 ng L^{-1}	4	3	10	3	2	5	2	4	3	4	3	6	2	5	4	6	2	3	2
50 ng L^{-1}	2	1	4	5	0	5	2	3	1	1	1	2	3	3	3	2	2	2	5
Interday precision (%RSD) Sea water																			
1500 ng L^{-1}	2	3	8	3	4	7	8	6	4	2	2	5	4	7	2	6	3	0	1
500 ng L^{-1}	1	6	3	5	10	2	2	4	10	3	6	5	5	4	1	4	4	5	7
250 ng L^{-1}	3	4	5	3	5	10	8	3	4	5	7	3	5	9	4	3	4	3	2
50 ng L^{-1}	1	0	2	2	4	2	3	1	1	5	1	1	2	3	1	2	2	3	1
Interday precision (%RSD) River water																			
1500 ng L^{-1}	8	1	9	9	7	6	7	8	3	3	7	2	4	3	1	7	5	2	3
500 ng L^{-1}	9	3	9	6	9	2	3	8	7	6	3	6	3	6	4	5	4	1	1
250 ng L^{-1}	5	5	6	8	5	8	7	3	2	3	7	6	4	8	3	7	6	1	1
50 ng L^{-1}	2	2	2	3	3	2	7	3	1	12	3	0	1	2	3	2	2	3	1

[a] Limit of detection calculated based on S/N = 3

[b] Limit of detection calculated based on S/N = 10

Table 3.3 Accuracy and intraday precision for influent and effluent wastewater, tap, sea and river water (n=3).

	Compounds																		
	BZ	4MBC	EDP	EMC	OC	DTS	DPMI	ADBI	AHMI	ATII	HHCB	AHTN	EXA	EB	MA	MX	MM	MT	MK
Accuracy (%Mean recovery ±RSD)																			
Influent wastewater																			
1500 ng L^{-1}	100±11	81±2	81±1	86±3	105±3	115±4	99±10	98±3	100±3	104±7	102±9	114±6	97±4	91±2	105±5	83±6	84±1	87±3	83±3
500 ng L^{-1}	105±2	90±5	100±5	94±4	88±5	118±3	100±7	96±2	102±4	109±6	102±7	98±6	103±11	84±3	114±5	95±1	93±2	116±6	90±6
250 ng L^{-1}	116±10	84±4	83±1	91±4	95±8	121±10	89±4	85±10	95±6	94±5	111±1	99±4	97±4	97±10	114±1	88±12	91±4	118±2	120±13
50 ng L^{-1}	114±5	109±3	111±4	90±5	104±0	93±4	86±0	87±1	80±0	101±0	113±5	112±2	86±1	99±2	114±1	114±0	105±3	83±3	83±4
Effluent wastewater																			
1500 ng L^{-1}	102±5	88±4	86±2	85±5	91±9	102±6	90±7	103±3	115±1	119±5	88±7	94±4	117±3	120±1	119±1	102±4	118±3	122±2	123±4
500 ng L^{-1}	97±9	81±3	107±4	80±3	83±5	123±3	101±10	85±5	93±3	99±4	111±8	84±4	109±4	112±6	122±5	97±9	93±6	120±4	116±3
250 ng L^{-1}	97±11	80±8	98±4	81±7	121±3	125±6	121±3	99±4	100±3	104±2	96±7	106±7	108±5	101±7	117±6	86±5	94±5	117±3	111±10
50 ng L^{-1}	96±1	104±5	107±3	111±3	84±1	93±2	96±3	83±2	83±3	83±2	103±1	88±6	114±2	106±1	98±8	95±3	93±1	100±7	101±7
Tap water																			
1500 ng L^{-1}	86±0	104±3	112±3	102±6	94±4	118±3	97±10	98±7	108±6	114±1	97±2	106±1	116±2	100±4	109±2	99±6	107±2	110±3	119±6
500 ng L^{-1}	91±8	82±6	90±4	83±5	99±4	99±7	110±10	93±1	106±4	109±3	98±2	91±3	100±4	91±5	107±2	98±2	83±9	109±7	108±6
250 ng L^{-1}	93±5	89±3	100±2	105±1	86±6	97±5	105±7	90±3	97±3	100±5	106±1	93±7	100±4	83±3	116±2	87±4	94±11	112±10	104±5
50 ng L^{-1}	103±3	106±5	115±1	96±4	87±3	99±1	86±3	108±2	83±3	105±3	93±4	94±3	87±6	96±4	88±9	86±6	95±1	102±2	110±0
Sea water																			
1500 ng L^{-1}	80±1	87±3	93±6	95±5	90±5	103±10	99±8	106±6	111±6	118±5	91±2	112±2	119±4	114±9	112±1	107±9	111±3	118±3	117±6
500 ng L^{-1}	87±4	87±2	90±2	90±6	90±10	120±0	88±3	92±0	104±2	118±1	95±9	104±1	110±1	97±2	114±2	107±7	98±3	118±2	114±1
250 ng L^{-1}	93±7	93±5	94±2	87±3	94±8	86±6	97±3	89±4	94±4	113±3	112±1	102±3	104±1	97±9	108±6	109±5	101±9	113±9	112±10
50 ng L^{-1}	84±2	105±5	105±0	98±0	97±1	100±1	88±1	81±0	88±6	106±0	115±1	112±4	81±8	93±3	101±1	87±4	92±0	102±1	106±1
River water																			
1500 ng L^{-1}	85±6	83±1	93±4	87±5	80±2	120±2	82±7	106±6	117±5	119±2	86±4	116±4	113±6	108±5	114±3	109±2	109±3	120±2	114±5
500 ng L^{-1}	100±7	85±7	91±7	105±10	81±2	118±6	109±5	103±3	111±2	119±1	116±1	115±2	110±1	97±4	107±4	115±6	115±6	118±4	118±2
250 ng L^{-1}	114±6	84±2	98±1	82±3	90±5	111±6	102±10	94±4	97±6	97±8	117±4	118±9	98±1	103±3	119±2	118±4	120±4	113±7	118±2
50 ng L^{-1}	111±4	84±1	113±2	110±3	89±3	117±3	97±4	86±3	93±2	105±2	110±3	85±5	89±6	91±5	94±2	86±1	93±4	94±1	105±3

3.3.3. Application to real samples

In order to assess the methodology's performance, naturally contaminated samples of different aqueous matrices were analysed (river, sea and tap water and influent and effluent wastewater). Results are summarized in Table 3.4.

Overall, polycyclic musks were the most abundant compounds in all water matrices, followed by UVFs. Regarding the SMCs, HHCB was found in higher levels and from UVFs, it was the BZ.

Both UVFs and SMCs were not detected in tap water and only BZ was found in seawater. However, UVFs have already been determined in tap water before, in concentrations ranging the ng L^{-1} (Ge and Lee, 2012; Rodil et al., 2012; Román et al., 2011), with EMC, EDP and 4-MBC being detected in higher concentrations. SMCs were also detected before in this matrix, with 228 ng L^{-1} for EB (Homem et al., 2016). Regarding seawater, higher concentrations of UVFs were found before, either in Spain (up to 880 ng L^{-1} for ES) (Benedé et al., 2014), Hong Kong (up to 6812 ng L^{-1} for OC) (Tsui et al., 2014) or even in the Artic sea with concentrations up to 128 ng L^{-1} (Tsui et al., 2014).

River samples from two different locations were analysed in this study. A different concentration profile was found at each sampling point, with a total of 86.9 ± 3.5 ng L^{-1} in point 1 and 230.9 ± 5.7 ng L^{-1} for point 2. UVFs have been found within the ng L^{-1} range by other authors, as well. For example, UVFs were detected in river samples from Switzerland (6-2402 ng L^{-1}) (Fent et al., 2010), China (8 – 5790 ng L^{-1}) (Liu et al., 2010), the UK 6-227 ng L^{-1} (Kasprzyk-Hordern et al., 2008) or even Spain (6-28 ng L^{-1}) (Pedrouzo et al., 2010). As for SMCs detection in river water samples, the average concentrations of HHCB, AHTN and HHCB-lactone were 260, 60 and 1000 ng L^{-1}, respectively (Lange et al., 2015) in a German river, and the concentrations of HHCB, AHTN were in the ranges of 3.5–32.0, 2.3–26.7 ng L^{-1}, respectively, in a river in China (Hu et al., 2011b), which are more similar to those found in this study.

Table 3.4 Levels of UV-filters and synthetic musks in naturally contaminated aqueous matrices - river, sea and tap water, influent (in) and effluent (out) wastewater (WW). Results are presented in ng L^{-1} ± SD.

	River 1	River 2	Sea water 1	Sea water 2	Tap water	WW in 1	WW out 1	WW in 2	WW out 2
UV filters									
BZ	81.4 ± 3.5	156.7 ± 3.1	68.5 ± 0.5	< LOQ	<LOQ	793.1 ± 31.2	803.1 ± 55.2	478.7 ± 21.5	69.5 ± 2.0
4MBC	nd	nd	nd	nd	<LOQ	<LOQ	<LOQ	13.6 ± 1.9	<LOQ
EMC	nd	nd	nd	nd	nd	nd	nd	2.1 ± 0.8	nd
OC	nd	nd	nd	nd	nd	554.3 ± 47.4	101.3 ± 7.2	671.0 ± 79.4	nd
DTS	nd	nd	nd	nd	nd	104.9 ± 0.3	50.5 ± 0.1	176.6 ± 2.2	42.8 ± 0.5
Policyclic Musks									
DPMI	nd	nd	nd	nd	nd	361.4 ± 98.4	515.6 ± 4.6	797.8 ± 99.9	739.6 ± 75.1
ADBI	nd	nd	nd	nd	nd	nd	nd	2.7 ± 0.7	2.1 ± 0.2
HHCB	nd	60.4 ± 4.7	nd	nd	nd	1618 ± 18	867.7 ± 39.0	5801 ± 177	3799 ± 56
AHTN	5.5 ± 0.3	13.8 ± 0.6	nd	nd	nd	152.8 ± 2.1	89.8 ± 2.1	537.4 ± 12.3	281.5 ± 1.0
Nitro Musks									
MK	nd	nd	nd	nd	nd	171.2 ± 3.8	105.6 ± 0.5	250.0 ± 14.5	242.1 ± 8.6
Σ/ location	86.9 ± 3.5	230.9± 5.7	68.5 ± 0.5	nd	nd	3756 ± 115	2534 ± 68	8740 ± 220	5177 ± 94

Wastewater samples were taken from the same WWTP, but different periods. Within influent wastewater results vary from 2.1 ± 0.8 ng L^{-1} (EMC) to 5.8 ± 0.2 µg L^{-1} (HHCB) and for effluent wastewater from 2.1 ± 0.2 ng L^{-1} (ADBI) to 3.80 ± 0.05 µg L^{-1} (HHCB). Concentrations in these levels have been detected before in Switzerland (Balmer et al., 2005; Kupper et al., 2006) and in the UK (Kasprzyk-Hordern et al., 2008).

3.4. Conclusion

A method based on dispersive liquid-liquid microextraction (DLLME) followed by gas-chromatography tandem mass spectrometry (GC-MS/MS) was optimized for the rapid analysis of 19 UVFs and SMs compounds in water matrices. To the authors' best knowledge, this is the first method based on DLLME and GC-MS/MS for the analysis of all compounds without a derivatization process. Several types of extraction and dispersion solvents were tested to achieve the best results for tap, river, sea water and influent and effluent wastewater. The chosen solvents were IPA and TC, with recoveries from 83 to 123%. The performance of the method has been demonstrated in terms of linearity, accuracy and precision (RSD<10%). Real samples were analysed, and concentrations found at the ng L^{-1} range, with higher concentration in wastewater samples, in the µg L^{-1} range.

Acknowledgements

This work was the result of the project:(i) POCI-01-0145-FEDER-006939 (Laboratory for Process Engineering, Environment, Biotechnology and Energy –UID/EQU/00511/2013) funded by the European Regional Development Fund (ERDF), through COMPETE2020-Programa Operational Competitividade e Internacionalização (POCI) and by national funds, through FCT - Fundação para a Ciência e a Tecnologia. (ii) Project UID/EQU/00511/2019 - Laboratory for Process Engineering, Environment, Biotechnology and Energy – LEPABE funded by national funds through FCT/MCTES (PIDDAC).(iii) NORTE-01-0145-FEDER-000005 – LEPABE-2-ECO-INNOVATION, supported by North Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development

Fund(ERDF).(iv) Post-doctoral Grant SFRH/BPD/76974/2011-Vera Homem.(v) Doctoral Grant SFRH/BD/110831/2015 – Sara Ramos.

References

Al-Said, H.M., Emara, A.A.A., 2014. The recent developments in dispersive liquid–liquid microextraction for preconcentration and determination of inorganic analytes. J. Saudi Chem. Soc. 18, 745–761.

Albero, B., Sánchez-Brunete, C., García-Valcárcel, A.I., Pérez, R.A., Tadeo, J.L., 2015. Ultrasound-assisted extraction of emerging contaminants from environmental samples. TrAC - Trends Anal. Chem. 71, 110–118.

Balmer, M.E., Buser, H.R., Müller, M.D., Poiger, T., 2005. Occurrence of some organic UV filters in wastewater, in surface waters, and in fish from Swiss lakes. Environ. Sci. Technol. 39, 953–962.

Benedé, J.L., Chisvert, A., Salvador, A., Sánchez-Quiles, D., Tovar-Sánchez, A., 2014. Determination of UV filters in both soluble and particulate fractions of seawaters by dispersive liquid–liquid microextraction followed by gas chromatography–mass spectrometry. Anal. Chim. Acta 812, 50–58.

Buchberger, W.W., 2011. Current approaches to trace analysis of pharmaceuticals and personal care products in the environment. J. Chromatogr. A 1218, 603–18.

Cacho, J.I., Campillo, N., Viñas, P., Hernández-Córdoba, M., 2016. Improved sensitivity gas chromatography-mass spectrometry determination of parabens in waters using ionic liquids. Talanta 146, 568–574.

Commission, E., 2011. Commision regulation (EU) N.o 143/2011 of 17 February 2011 amending Annex XIV to regulation (EC) No 1907/2006 of the european parliament and of the Council on the registration, evaluation, authorisation and restriction of chemicals ('REACH'). Off. J. Eur. Union 2-6. L44.

CosmeticsDirective, 2009. Annex VI: LIST OF UV FILTERS ALLOWED IN COSMETIC PRODUCTS in Europe. REGULATION (EC) No 1223/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 30 November 2009 on cosmetic products (web: http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.results&annex_v2=VI&search).

Cunha, S.C., Pena, A., Fernandes, J.O., 2015. Dispersive liquid–liquid microextraction followed by microwave-assisted silylation and GC-MS analysis for simultaneous trace quantification of bisphenol A and 13 UV filters in wastewaters. J. Chromatogr. A.

da Silva, C.P., Emídio, E.S., de Marchi, M.R.R., 2015. Method validation using weighted linear regression models for quantification of UV filters in water samples. Talanta 131, 221–227.

European Parliament, 2009. Regulation (EC) N.o 1223/2009 of the european parliament and of the Council of 30 November 2009 on cosmetic products. Off. J. Eur. Union 59-209. L 342.

Fent, K., Zenker, A., Rapp, M., 2010. Widespread occurrence of estrogenic UV-filters in aquatic ecosystems in Switzerland. Environ. Pollut. 158, 1817–1824.

Ge, D., Lee, H.K., 2012. A new 1-hexyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate ionic liquid based ultrasound-assisted emulsification microextraction for the determination of organic ultraviolet filters in environmental water samples. J Chromatogr A 1251, 27–32.

González, S., Fernández-Lorente, M., Gilaberte-Calzada, Y., 2008. The latest on skin photoprotection. Clin. Dermatol. 26, 614–26.

Gracia-Lor, E., Martínez, M., Sancho, J. V, Peñuela, G., Hernández, F., 2012. Multi-class determination of personal care products and pharmaceuticals in environmental and wastewater samples by ultra-high performance liquid-chromatography-tandem mass spectrometry. Talanta 99, 1011–23.

Homem, V., Alves, Alice, Alves, Arminda, Santos, L., 2016. Ultrasound-assisted dispersive liquid-liquid microextraction for the determination of synthetic musk fragrances in aqueous matrices by gas chromatography-mass spectrometry. Talanta 148, 84–93.

Homem, V., Silva, A., Ratola, N., Santos, L., Alves, A., 2015. Long lasting perfume e A review of synthetic musks in WWTPs. J. Environ. Manage. 149, 168–192.

Hu, Z., Shi, Y., Cai, Y., 2011. Concentrations, distribution, and bioaccumulation of synthetic musks in the Haihe River of China. Chemosphere 85, 262–267.

Kameda, Y., Kimura, K., Miyazaki, M., 2011. Occurrence and profiles of organic sun-blocking agents in surface waters and sediments in Japanese rivers and lakes. Env. Pollut 159, 1570–1576.

Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2009. The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. Water Res. 43, 363–380.

Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2008. The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. Water Res. 42, 3498–3518.

Kupper, T., Plagellat, C., Brändli, R.C., de Alencastro, L.F., Grandjean, D., Tarradellas, J.,

2006. Fate and removal of polycyclic musks, UV filters and biocides during wastewater treatment. Water Res. 40, 2603–2612.

Lange, C., Kuch, B., Metzger, J.W., 2015. Occurrence and fate of synthetic musk fragrances in a small German river. J. Hazard. Mater. 282, 34–40.

Lee, I., Lee, S., Oh, J., 2010. Occurrence and fate of synthetic musk compounds in water environment. Water Res. 44, 214–222.

Liu, H., Liu, L., Xiong, Y., Yang, X., Luan, T., 2010. Simultaneous determination of UV filters and polycyclic musks in aqueous samples by solid-phase microextraction and gas chromatography–mass spectrometry. J. Chromatogr. A 1217, 6747–6753.

López-Nogueroles, M., Chisvert, A., Salvador, A., Carretero, A., 2011. Dispersive liquid – liquid microextraction followed by gas chromatography – mass spectrometry for the determination of nitro musks in surface water and wastewater samples. Talanta 85, 1990–1995.

Maya, F., Horstkotte, B., Estela, J.M., Cerdà, V., 2014. Automated in-syringe dispersive liquid-liquid microextraction. TrAC Trends Anal. Chem. 59, 1–8.

Montgomery, J.H., 2007. Groundwater chemicals desk reference, 4th ed. CRC Press.

Panagiotou, A.N., Sakkas, V.A., Albanis, T.A., 2009. Application of chemometric assisted dispersive liquid-liquid microextraction to the determination of personal care products in natural waters. Anal. Chim. Acta 649, 135–140.

Peck, A., Hornbuckle, K., 2006. Synthetic musk fragrances in urban and rural air of Iowa and the Great Lakes. Atmos. Environ. 40, 6101–6111.

Pedrouzo, M., Borrull, F., Marcé, R., Pocurull, E., 2010. Stir-bar-sorptive extraction and ultra-high-performance liquid chromatography–tandem mass spectrometry for simultaneous analysis of UV filters and antimicrobial agents in water samples. Anal. Bioanal. Chem. 397, 2833–2839.

Pedrouzo, M., Borrull, F., Marcé, R.M., Pocurull, E., 2011. Analytical methods for personal-care products in environmental waters. TrAC - Trends Anal. Chem. 30, 749–760.

Pietrogrande, M.C., Basaglia, G., 2007. GC-MS analytical methods for the determination of personal-care products in water matrices. TrAC - Trends Anal. Chem. 26, 1086–1094.

Pinkas, A., Gonçalves, C.L., Aschner, M., 2017. Neurotoxicity of fragrance compounds: A review. Environ. Res. 158, 342–349.

Prosser, R.S., Sibley, P.K., 2015. Human health risk assessment of pharmaceuticals and personal care products in plant tissue due to biosolids and manure amendments and wastewater irrigation. Environ. Int. 75, 223–233.

Ramos, S., Homem, V., Alves, A., Santos, L., 2016. A review of organic UV-filters in wastewater treatment plants. Environ. Int. 86, 24–44.

Ramos, S., Homem, V., Alves, A., Santos, L., 2015. Advances in analytical methods and occurrence of organic UV-filters in the environment - A review. Sci. Total Environ. 526, 278–311.

Richardson, S.D., Ternes, T.A., 2014. Water analysis: emerging contaminants and current issues. Anal. Chem. 86, 2813–48.

Rodil, R., Moeder, M., 2008. Development of a method for the determination of UV filters in water samples using stir bar sorptive extraction and thermal desorption–gas chromatography–mass spectrometry. J. Chromatogr. A 1179, 81–88.

Rodil, R., Quintana, J.B., Concha-Graña, E., López-Mahía, P., Muniategui-Lorenzo, S., Prada-Rodríguez, D., 2012. Emerging pollutants in sewage, surface and drinking water in Galicia (NW Spain). Chemosphere 86, 1040–1049.

Román, I.P., Chisvert, A., Canals, A., 2011. Dispersive solid-phase extraction based on oleic acid-coated magnetic nanoparticles followed by gas chromatography–mass spectrometry for UV-filter determination in water samples. J. Chromatogr. A 1218, 2467–2475.

Rumble, J.R., 2017. Handbook of Chemistry and Physics, 98th ed. CRC Press.

Salvador, A., Chisvert, A., 2005. Sunscreen analysis: A critical survey on UV filters determination. Anal. Chim. Acta 537, 1–14.

Sauvé, S., Desrosiers, M., 2014. A review of what is an emerging contaminant. Chem. Cent. J. 8.

Stenutz, R., n.d. Tables for Organic Chemistry [WWW Document]. URL http://www.stenutz.eu/ (accessed 8.21.17).

Tarazona, I., Chisvert, A., León, Z., Salvador, A., 2010. Determination of hydroxylated benzophenone UV filters in sea water samples by dispersive liquid–liquid microextraction followed by gas chromatography–mass spectrometry. J. Chromatogr. A 1217, 4771–4778.

Tashiro, Y., Kameda, Y., 2013. Concentration of organic sun-blocking agents in seawater of beaches and coral reefs of Okinawa Island, Japan. Mar. Pollut. Bull. 77, 333–340.

Tovar-Sánchez, A., Sánchez-Quiles, D., Basterretxea, G., Benedé, J.L., Chisvert, A., Salvador, A., Moreno-Garrido, I., Blasco, J., 2013. Sunscreen Products as Emerging Pollutants to Coastal Waters. PLoS One 8, e65451.

Tsui, M.M., Leung, H.W., Wai, T.C., Yamashita, N., Taniyasu, S., Liu, W., Lam, P.K., Murphy, M.B., 2014. Occurrence, distribution and ecological risk assessment of multiple

classes of UV filters in surface waters from different countries. Water Res 67c, 55–65.

Tsui, M.M.P., Leung, H.W., Lam, P.K.S., Murphy, M.B., 2014. Seasonal occurrence, removal efficiencies and preliminary risk assessment of multiple classes of organic UV filters in wastewater treatment plants. Water Res. 53, 58–67.

Vallecillos, L., Borrull, F., Pocurull, E., 2015. Recent approaches for the determination of synthetic musk fragrances in environmental samples. TrAC Trends Anal. Chem. 72, 80–92.

Vallecillos, L., Pocurull, E., Borrull, F., 2012. Fully automated ionic liquid- based head space singledrop microextraction coupled to GC–MS/MS to determine musk fragrances in environmental water samples. Talanta 99, 824–832.

Wang, J., Wang, S., 2016. Removal of pharmaceuticals and personal care products (PPCPs) from wastewater: A review. J. Environ. Manage. 182, 620–640.

Wu, J.-W., Chen, H.-C., Ding, W.-H., 2013. Ultrasound-assisted dispersive liquid–liquid microextraction plus simultaneous silylation for rapid determination of salicylate and benzophenone-type ultraviolet filters in aqueous samples. J. Chromatogr. A 1302, 20–27.

Yang, C.Y., Ding, W.H., 2012. Determination of synthetic polycyclic musks in aqueous samples by ultrasound-assisted dispersive liquid-liquid microextraction and gas chromatography-mass spectrometry. Anal. Bioanal. Chem. 402, 1723–1730.

Ye, L., Liu, J., Yang, X., Peng, Y., Xu, L., 2011. Orthogonal array design for the optimization of ionic liquid-based dispersive liquid-liquid microextraction of benzophenone-type UV filters. J Sep Sci 34, 700–706.

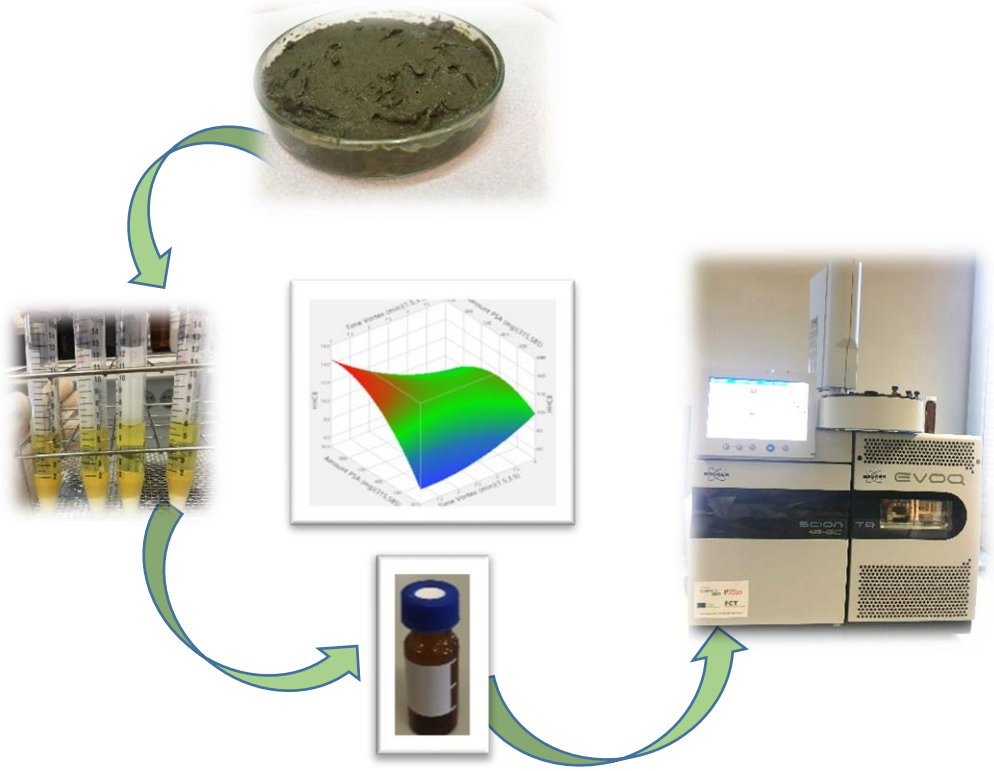
Zhang, P.-P., Shi, Z.-G., Yu, Q.-W., Feng, Y.-Q., 2011. A new device for magnetic stirring-assisted dispersive liquid–liquid microextraction of UV filters in environmental water samples. Talanta 83, 1711–1715.

Zhang, Y., Lee, H.K., 2012. Ionic liquid-based ultrasound-assisted dispersive liquid-liquid microextraction followed high-performance liquid chromatography for the determination of ultraviolet filters in environmental water samples. Anal Chim Acta 750, 120–126.

Chapter 4. Development and optimization of a QuEChERS-GC–MS/MS methodology to analyze ultraviolet-filters and synthetic musks in sewage sludge

Sara Ramos, Vera Homem, Lúcia Santos,

Science of The Total Environment, Vol. 651, Part 2, pp. 2606-2614 (2019)



Abstract

A Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) methodology followed by gas chromatography–triple quadrupole mass spectrometry (GC–MS/MS) analysis was developed to extract synthetic musk compounds (SMCs) (6 polycyclic, 2 macrocyclic and 5 nitro musks) and ultraviolet-filters (UVFs) (6 compounds) from sludge. This methodology fills a gap in the literature, since the proposed technique does not require specific equipment, nor large amounts of solvents, sorbents and time to extract SMCs and UVFs from sludge. To optimize this new methodology, a design of experiments (DoE) approach was used, applying first a screening design (SD) and then a central composite design (CCD). The best conditions achieved to extract these 19 compounds simultaneously were: 500 mg freeze dried sludge, 2.5 min of vortex and 15 min ultrasound and the use of a QuEChERS for the dispersive solid-phase extraction (d-SPE) containing 500 mg $MgSO_4$, 410 mg C_{18} and 315 mg PSA. Then, this methodology was successfully validated. Recoveries of the target compounds ranged from 75% (cashmeran, DPMI) to 122% (2-ethylhexyl 4-methoxycinnamate, EHMC), with good repeatability (relative standard deviation < 10%). The instrumental detection limits (IDLs) and quantification (IQLs) varied from 0.001 pg (musk moskene, MM) to 7.5 pg (musk xylene, MX) and from 0.003 (MM) to 25 pg (MX), respectively. The method detection and quantification limits (MDLs and MQLs) ranged between 0.5 (DPMI) and 1394 (exaltolide, EXA) ng g^{-1} dw and 2 and 4648 ng g^{-1} dw, respectively. Both SMCs and UVFs were detected in all sludge samples analysed. Higher concentrations were found for octocrylene (OC: maximum value of 115,486 ng g^{-1} dw) followed by galaxolide (HHCB: 81,771 ng g^{-1} dw). Only the nitro musks ambrette, xylene, moskene and tibetene and macrocyclic musk ethylene brassylate (EB) were not detected in any sample.

Keyworks: UV-filters; Synthetic musk compounds; Sludge; QuEChERS; GC–MS/MS; Design of experiments

4.1. Introduction

The presence of personal care products (PCPs), namely synthetic musk compounds (SMCs) and UV-filters (UVFs) in sewage sludge is unquestionable. So far, several studies have shown their presence in this matrix in concentrations ranging from a few to thousands of μg g^{-1} dw (Homem et al., 2015; Ramos et al., 2015). Although these compounds are legislated in cosmetics (Cosmetics Directive, 2009), they are not regulated in the environment, nor in wastewaters or sludge.

The application of sludge in agricultural fields as fertilizer has raised a growing concern due to the potential existence of non-legislated contaminants in the sludge, which may bioaccumulate in the environment and when present in agricultural soils can migrate to crops (Prosser and Sibley, 2015). Although the European Union has defined specific rules for its use and maximum allowed levels for some metals and organic compounds (e.g. polycyclic aromatic hydrocarbons, polychlorinated biphenyls, etc.) (Decreto-Lei N.276/2009), the effects produced by the presence of emerging contaminants in the sludge, such as those studied in this work, are not clearly defined.

So far, the methodologies developed for the extraction of these compounds from sewage sludge are often expensive and time consuming, like pressurized liquid extraction (PLE), Soxhlet or solid phase extraction (SPE). A compilation of the methodologies used so far to determine SMCs and UVFs in sewage sludge is presented in Tables S3.1 and S3.2 of Annex 3. Methodologies like Soxhlet (Horii et al., 2007; Reiner et al., 2007) or Soxhlet followed by SPE (Chen et al., 2007; Shek et al., 2008; Zeng et al., 2005) or Soxhlet followed by SPE and gel permeation chromatography (GPC) (Bester, 2004; Stevens et al., 2003) show good recoveries for galaxolide (HHCB), but lower values for the other polycyclic musks. These techniques also have the disadvantages of being time consuming and use high amounts of solvents and/or sorbents. Liquid-liquid extraction (LLE) followed by SPE provided good recoveries for both UVFs (75–101%) (Plagellat et al., 2006) and SMCs (98.5–111%) (Muller et al., 2006), as well as the combination of LLE with SPE and GPC (75–103%) (Kupper et al., 2006). Pressurized liquid extraction depends on the capacity of the solvent to extract the target analytes. Despite the advantage of using less solvent volume, it requires a specific equipment. This method is often used by itself (Badia-Fabregat et al., 2012; Gago-Ferrero et al., 2011; Liu

et al., 2011, 2012; Rodil et al., 2009; Rodríguez-Rodríguez et al., 2012) or combined with SPE or GPC (Langford et al., 2015; Liu et al., 2014; Negreira et al., 2011; Ternes et al., 2005; Wick et al., 2010). In general, PLE enables high recoveries for both classes (around 100%) and low limits of detection (around 10 ng g^{-1} dw). Accelerated solvent extraction (ASE) methodology was also used to extract poly- and nitro musks from sludge, yielding recoveries around 70 to 100% and limits of detection below 10 ng g^{-1} dw (Guo et al., 2010; Hu et al., 2011; Yang and Metcalfe, 2006). The instrumental methodology most used for the analysis of SMCs is either gas chromatography–mass spectrometry (GC–MS) or GC–MS/MS. Regarding UVFs, either gas or liquid chromatography followed by mass spectrometry are equally used. Considering that most methodologies described in the literature are time consuming, expensive, use large amount of solvents and require specialized equipment, it is crucial to develop a quick, inexpensive and environmentally friendly approach. A Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) methodology has already been described to the extraction of UVFs in other matrices such as milk (Vela-Soria et al., 2018), coastal vegetation (Ribeiro et al., 2017) and marine mussels (Picot Groz et al., 2014) and in SMCs such as beach sands (Homem et al., 2017) and seafood products (Saraiva et al., 2016; Vallecillos et al., 2015). Except the study of coastal vegetation, which used a mixture of dichloromethane and n-hexane (1:1) as extraction solvent, the other studies used acetonitrile. C_{18} and PSA seem to be the most commonly used sorbents for the dispersive solid-phase extraction step. Thus, in this study, the authors proposed to develop and optimize a QuEChERS methodology followed by GC–MS/MS analysis to simultaneously extract 13 SMCs and 6 UVFs from sewage sludge (Table S3.3 in Annex 3).

To develop this new methodology, a design of experiments (DoE) approach was used to optimize the extraction parameters, as the type and amount of solvents and sorbents, time of vortex and time of extraction. First, a screening design (SD) was applied to define the most important extraction parameters, then a central composite design (CCD) was used to obtain the optimized conditions. Finally, the optimized method was validated using the procedure proposed by EuraChem (Magnusson and Örnemark, 2014).

4.2. Materials and methods

4.2.1. Standards, reagents and materials

Acetone (Ac), acetonitrile (ACN), n-hexane (Hex), dichloromethane (DCM) and ethyl acetate (EA) were of analytical grade and were purchased from VWR BDH Prolabo (Fontenay-sous-Bois, France). For the QuEChERS preparation, anhydrous magnesium sulphate ($MgSO_4$) was obtained from Panreac AppliChem (Barcelona, Spain), while primary and secondary amine exchange bonded silica sorbent (PSA) and octadecyl-silica (C_{18}) from Supelco (Bellefonte, PA, USA). Florisil (magnesium silicate, particle size 0.150–0.250 mm) and alumina (neutral aluminium oxide 90, particle size 0.063–0.200 mm) were acquired from Merck (Darmstadt, Germany). Both florisil, alumina and $MgSO_4$ were activated overnight at 450 °C before use.

Helium (99.9%) used in the GC–MS/MS and nitrogen (99.9%) used in the evaporation step were supplied by Air Liquide (Maia, Portugal).

The polycyclic musks were obtained as solid standards from LGC Standards (Barcelona, Spain) with 99% purity for cashmeran (DPMI), celestolide (ADBI), phantolide (AHMI), traseolide (ATII), and tonalide (AHTN), except for galaxolide, which contained approximately 25% of diethyl phthalate. The nitro musks tibetene (MT) and moskene (MM) were also obtained from LGC Standards, as a 10 mg L^{-1} solution in cyclohexane. Musk ambrette (MA) and musk ketone (MK) were purchased as solid standards from Dr. Ehrenstorfer (Augsburg, Germany) with 99% and 98% purity, respectively. Musk xylene (MX) was obtained from Sigma-Aldrich (St. Louis, MO, USA) as a 100 mg L^{-1} solution in acetonitrile. Solid standards of exaltolide (EXA) and ethylene brassylate (EB) were also purchased from Sigma-Aldrich with 99% and 95% purity, respectively. The surrogate standards musk xylene-d15 (MX-d15) and tonalide-d3 (AHTN-d3) were purchased from Dr. Ehrenstorfer (Augsburg, Germany) as 100 mg L^{-1} solutions in acetone and isooctane, respectively. UV-filters 2-ethylhexyl 4-dimethylaminobenzoate (EDP) and 3-(4'-methylbenzylidene) camphor (4-MBC) were purchased from Alfa Aesar (Karlsruhe, Germany), both with 99% purity. 2-Ethylhexyl 4-methoxycinnamate (EHMC), 2-ethylhexyl 2-cyano-3,3-diphenylacrylate (OC) and benzophenone (BZ) were purchased from Sigma-Aldrich (St. Louis, MO, USA) with 98%, 97% and 99% purity,

respectively. Drometrizole trisiloxane (DTS) was purchased from Fluka (Saint Louis, MO, USA) with 98% purity. Surrogate (±)-3-(−4-methylbenzylidine-d4) camphor (4-MBC-d4) was purchased with 99% purity from CDN Isotopes (Pointe-Claire, Quebec, Canada).

Individual stock solutions of each compound (10 mg L^{-1}) were prepared in both acetonitrile and n-hexane. The stock solutions in acetonitrile were used to prepare the spike mix solution due to miscibility with the water sample and those in n-hexane to prepare the analytical control standard, since all extracts are evaporated to dryness and reconstituted in n-hexane for injection in the GC–MS/MS. Stock and working solutions (50, 250 and 2500 µg L^{-1}) were stored and preserved in a freezer at −20 °C, protected from the light. The stability of the solutions was tested, and results revealed that they were stable about 3 months.

4.2.2. Sample collection and pre-treatment

Sludge samples were collected from a wastewater treatment plant (WWTP) with secondary treatment (activated sludge process) that serves a population of 80,000 inhabitants, treating an average flow of 18,000 m^3 day^{-1}, in Oporto (Portugal). Grab samples were collected (December 2013–December 2017) from the thickening tank in wide mouth 5 L polyethylene containers, transported to the lab in a cooler with ice packs and then around 10 g of sample was freeze-dried in a Virtis Benchtop K Freeze Dryer (SP Scientific, New York, USA) for 72 h. Then, it was stored in the dark at −20 °C until analysis. These samples were used to prove the method applicability and to compare the levels with the literature.

4.2.3. Sample extraction

Samples were extracted using a QuEChERS methodology. In short, 500 mg of freeze-dried sludge were weighted into a 15 mL polypropylene tube with conical bottom, containing 10 mL of ACN and 250 ng g^{-1} surrogate solution (MX-d15, AHTN-d3 and 4MBC-d4) in acetonitrile. The sample was vortexed for 2.5 min and then, ultrasonicated for 15 min in a 420 W ultrasonic bath (J.P. Selecta, Barcelona, Spain). The organic phase was separated by centrifugation at 2670 ×g for 15 min and it was added to a 15 mL

polypropylene tube with conical bottom containing 500 mg $MgSO_4$, 410 mg C_{18} and 315 mg PSA. The extract was then vortexed for 2.5 more minutes and centrifuged for 15 min. The supernatant was transferred to a 12 mL amber vial, evaporated to dryness under a gentle N_2 stream and reconstituted in 500 µL of n-hexane. Finally, the extract was transferred to a 1.5 mL amber vial for instrumental analysis. Samples were analysed in triplicate (Figure 4.1).

4.2.4. GC–MS/MS analysis

Analysis was performed on a gas chromatograph coupled to triple quadrupole mass spectrometer from Bruker (Massachusetts, USA). This GC–MS system was equipped with a 436-gas chromatograph, a mass spectrometer EVOQ triple quadrupole, an injector CP-1177 split/splitless and an autosampler CP8410. For the separation of the target compounds, a J&W CP-Sil 8 CB capillary column (50 m × 0.25 mm I.D. × 0.12 mm) from Agilent Technologies (Santa Clara, California, USA) was used. Helium (99.999%) was used as a carrier at a constant flowrate of 1.0 mL min^{-1}. 2 µL sample was injected in splitless mode (split ratio of 1:100 after 1 min for injector purging) at 280 °C. The GC oven temperature program started at 70 °C for 1 min, raising to 180 °C at 25 °C min^{-1}, then 10 °C min^{-1} until 240 °C and finally 25 °C min^{-1} until 300 °C (for 5 min).

The MS/MS analysis was carried out in electron ionization (EI)mode, using the multiple reaction monitoring (MRM) mode. Two specific MRM transitions were chosen per compound (one for quantifying and one as qualifier), except for the nitro musks and surrogates, where two qualifiers were used for better identification. The ion source was operated at 280 °C with electron energy of 70 eV and filament current of 50 µA. The temperature of the transfer line was set at 270 °C. Ultrapure argon was used as collision gas and its pressure was set at 2.00 mTorr. The MRM transitions and collision energies optimized for each compound are presented in Table S3.3 in Annex 3.

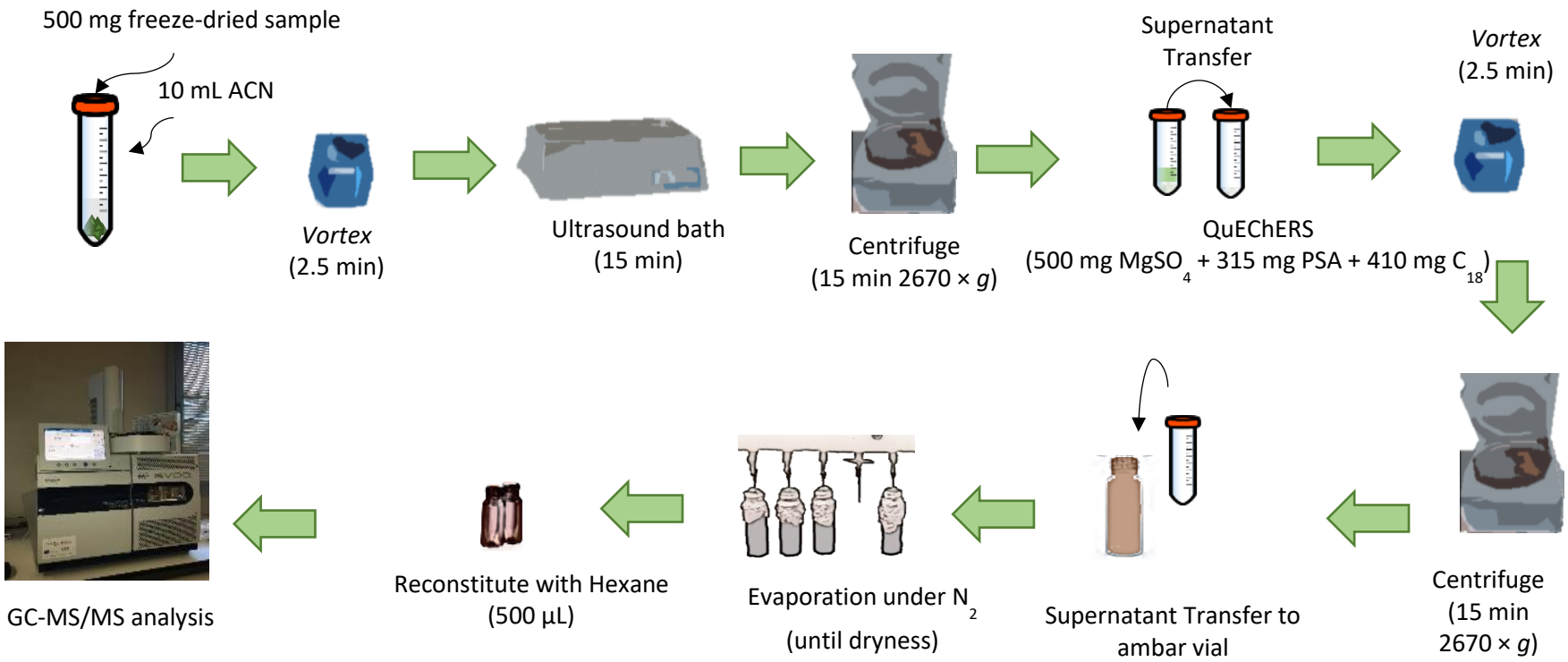


Figure 4.1 Scheme of the QuEChERS procedure proposed.

4.2.5. Quality assurance and control (QA/QC)

As mentioned before, UVFs and SMCs are present in most personal care products. Therefore, some precautions were taken to avoid sample contamination. The analyst did not use scented cosmetics such as perfume, lotions and hand creams, as well as other personal care products containing UV protection. All the glass material was pre-rinsed with acetone and distilled water and the non-calibrated material was further subject to heating at 400 °C for at least 1 h. Additionally, procedural blanks (where no sample is used, only solvent and sorbents subjected to the same conditions as the samples) were analysed with every extraction batch to identify eventual contaminations and to correct samples concentration. Chromatographic blanks were also performed, but no memory effects were observed.

4.3. Results and discussion

4.3.1. Preliminary tests

Since few QuEChERS methodologies were reported for the extraction of emerging contaminants from sludge, and none for the simultaneous determination of UVFs and SMCs, some preliminary tests were performed. Several aspects have been considered, such as the type and amount of solvent, the time of extraction, the number of extractions needed for the complete extraction of the target compounds and the processing of the sample.

4.3.1.1. Solvents and extraction time

Regarding the type and amount of solvent and the number of extractions needed, several experiences were performed. First, the sludge (containing 6–8% of dry solids) was centrifuged (20 min at 2670 ×g) and the supernatant was removed. The remaining sludge, considered the ‘wet’ sludge, was used for subsequent experiments.

In the first approach, the extraction of 500 mg of wet sludge was tested using different solvents - ethyl acetate (EA), acetonitrile (ACN), n-hexane (Hex) and acetone (Ac). The sample was subjected to successive ultrasonic extractions using fresh solvent to

evaluate the amount of solvent and if multiple extractions would be required to extract the target compounds. For each extraction step, 2 mL of solvent, 2 min of vortex, 15 min in an ultrasound bath and 15 min of centrifugation for phase's separation were used. Each fraction was then evaporated to dryness, under a N_2 stream, and reconstituted in 100 µL of Hex for GC–MS/MS analysis. Although no clean-up step was added to this procedure, the chromatographic analyses of these extracts proved that only one extraction step was sufficient to extract >90% of the target compounds from the sample.

Regarding the type of solvent used, ACN seemed to be the one that led to higher recoveries for most target compounds (11/19 compounds with recoveries between 47 and 132%), with only a single extraction step. Ac also appeared to be a solvent with potential, with 9/19 compounds extracted with recoveries between 28 and 119%. However, the chromatograms present many interferences. The Hex and EA do not seem to be suitable solvents for the extraction of the target compounds (3/19 and 5/19 compounds extracted, respectively). The resultant chromatograms have also demonstrated the presence of different interferences Figure S3.1 in Annex 3.

4.3.1.2. Sample processing

Through the various experiments carried out, it was noticed that the results obtained were affected by a great variability (relative standard deviation (RSD) > 50%). Such situation could be related to the fact that the analysed sample is not homogeneous (e.g. variable water content). Thus, it was decided to test the samples lyophilization. All tests with lyophilized sludge presented a relatively small relative standard deviation (RSD < 5%). Therefore, in the subsequent assays the collected samples were centrifuged and lyophilized. After lyophilization, samples were homogenized (by mortar and pestle followed by sieving) and kept in the freezer at −20 °C until analysis.

4.3.1.3. Clean-up procedure

In order to obtain better results by decreasing the presence of interferences, a clean-up step using dispersive solid-phase sorbents were tested. Only C_{18} and PSA were tested as sorbents, and the results were quite satisfactory, but not for all the target compounds.

In fact, PSA sorbent is usually used to remove polar interferences, while C_{18} sorbent to remove nonpolar interferences.

Tests were performed using either 250 mg of C_{18} or PSA combined with 500 mg $MgSO_4$ (drying agent). In both cases, recoveries of most target compounds were improved adding this clean-up procedure (mean: 89±20% for C_{18} and 82±20% for PSA). However, some matrix effects were still detected in the quantification of OC, DTS, HHCB or AHTN (recoveries ≈ 500%). To overcome this problem, more assays should be performed to optimize the clean-up procedure (amounts and type of sorbents).

Considering these preliminary results, the authors opted to perform an experimental design (screening design followed by a central composite design) to optimize the main parameters of the extraction procedure more quickly and systematically and considering the possible interaction between factors.

4.3.2. Optimization of the extraction procedure

The optimization of the extraction procedure was done using a design of experiments (DoE), previously described by Homem et al. (2016), where multiple extraction parameters were tested in order to determine their effect on a desired response (recovery of the target analytes).Thus, a screening design (SD) was initially performed to understand which variables affect the response. After that, a second order model central composite design (CCD) was used to optimize the extraction methodology. This design of experiments was performed using the JMP14 Statistical Software.

4.3.2.1. Screening design

Several parameters may influence the QuEChERS extraction. Therefore, in this study, a screening design considering eight factors was implemented: volume (V_E) and type (S_E) of extraction solvent, ultrasound time (t_E), time of vortex (t_V), amount of different sorbents - C_{18} (m_{C18}), PSA (m_{PSA}), florisil (m_{FLO}) and aluminium oxide (m_{AL}). All extractions were tested using a sludge sample spiked with a concentration of 2500 ng g^{-1} dw (poly/macro musks and UVFs) and 500 ng g^{-1} dw for nitro musks. The values proposed for each factor are presented in Table 4.1.

The experimental screening design consisted in a total of twelve experiences (Table S3.4 – Annex 3) and the average recovery of each compound analysed (n = 3) was selected as a response. Overall, the experiences were considered reproducible once the relative standard deviation were generally under 10%. All responses were adjusted to a quadratic model with a r^2 higher than 0.82. The main effects were determined by the F-probability, calculated for each factor (Figure 4.2). A F-probability ≤0.05 represents a significant effect on the selected response, whereas 0.05 < F-probability ≤ 0.10 indicates a relative effect on the extraction. The factors that significantly affect the responses were the volume of the extraction solvent, time of vortex, amount of C_{18}, amount of PSA and type of solvent, since their F-probability stands below 0.10 for the different target compounds, namely the nitro musks. The other investigated factors (time of ultrasound extraction, amount of aluminium oxide and florisil) that do not affect significantly the responses need to be defined, as well as the discrete variable – type of solvent. To define those factors, the desirability function was used to maximize the response of each target compound to achieve recovery values of 100 ±20% (values defined by user). The optimal desirability was obtained for ACN as extraction solvent, 15 min for US extraction and no amount of either aluminium oxide or florisil. The solvent ACN, as proven before, seems to be the best, probably due to its physicochemical properties and the need of a US extraction also helps the transference of the target compounds from the sludge into the solvent phase. These conditions were defined based on the results from the JMP software. The other four factors were further evaluated in the CCD optimization step (volume of the extraction solvent, time of vortex, amount of C_{18} and amount of PSA).

Table 4.1 Experimental factors and coded levels for the proposed screening approach.

i	Factors	Coded values (x_i)	
		Low (-1)	High (+1)
1	V_E - Volume of extraction solvent(mL)	5	12
2	t_E - Extraction time in US (min)	0	15
3	t_V - Vortex time (min)	1	5
4	m_{C18} - Amount of C_{18} (mg)	0	500
5	m_{PSA} - Amount of PSA (mg)	0	500
6	m_{Flo} - Amount of florisil (mg)	0	500
7	m_{AL} - Amount of aluminium oxide (mg)	0	500
8	S_E – Type of extraction solvent	ACN; EA; Ac; DCM/Hex (1:1, v/v)	

Obs.: ACN – Acetonitrile; EA – Ethyl Acetate; Ac – Acetone; DCM – Dichloromethane; Hex – Hexane.

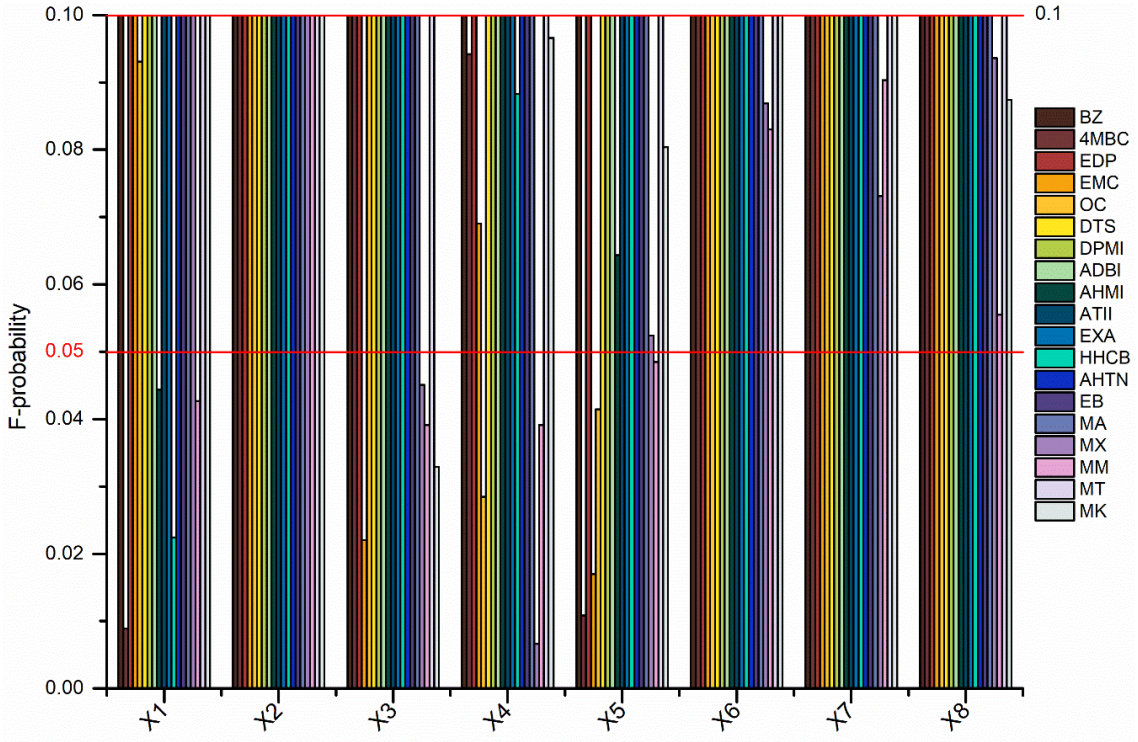


Figure 4.2 F-probability obtained in the screening design for each factor (X1: Volume of extraction solvent (mL); X2: Extraction time in US (min); X3: Vortex time (min); X4: Amount of C_{18} (mg); X5: Amount of PSA (mg); X6: Amount of florisil (mg); X7 - Amount of aluminium oxide (mg); X8: Type of extraction solvent).

4.3.2.2. Central composite design

After selecting the most important factors using a screening design, those selected parameters were optimized by a central composite design (CCD) (Table 4.2). A total of thirty experimental runs were carried out, including six assays performed at the center of the cubic domain for the repeatability determination. The conditions set in each experiment are listed in Table S3.5 in Annex 3.

Table 4.2 Factors and values for the CCD.

i	Factors	Coded levels (x_i)				
		-1.483	-1	0	1	1.483
1	V_E - Volume of extraction solvent (mL)	5.0	6.0	8.5	11.0	12.0
2	t_V - Vortex time (min)	1.0	1.5	2.5	3.5	4.0
3	m_{C18} - Amount of C_{18} (mg)	250	315	450	585	650
4	m_{PSA} - Amount of PSA (mg)	250	315	450	585	650

Using the response surface methodology (RSM), a mathematical relationship between dependent and independent variables was determined. As mentioned above, a second-order polynomial equation (Eq. 1) was fitted to the experimental data and the model coefficients were calculated by a least-square regression analysis. These results are summarized in Table S3.6 (Annex 3).

$$Y = b_0 + \sum_{i=1}^{k} b_i x_i + \sum_{i=1}^{k} b_{ii} x_i^2 + \sum_{j>1}^{k} \sum_{i=1}^{k} b_{ij} x_i \, x_j \qquad \text{(Eq. 1)}$$

where Y represents the response, x is the codified variables, b_0 is the interception term, b_i is the influence of the variable i in the response, b_{ii} is the parameter that determines the shape of the curve and b_{ij} corresponds to the effect of the interaction among variable i and j. The equations found for each compound, containing only the factors that most affect the response, are presented in Table 4.3.

The model was applied to all compounds and its suitability determined by an ANOVA approach after the assumptions for ANOVA and Student's t-test are met. A good relationship between the experimental data and the fitted model (r^2 > 0.83) was achieved (Table 4.3). All models indicate a F-probability <0.001, which means that variations that occur in the responses are associated to the model and not to experimental errors. The relevant variables and interactions were identified by the Student's t-test and results are also presented in Table 4.3. It is also possible to conclude that all variables and interactions have significant effect (Prob > |t| is <0.05) on at least one of the responses (bold values in Table 4.3).

Table 4.3 Results from the Student's t-test for the main and quadratic effects, and intercept and the interaction; And model suitability parameters for the response functions.

	Prob>|t|																		
Compounds	Intercept	X1	X2	X3	X4	X1X2	X1X3	X2X3	X1X4	X2X4	X3X4	$X1^2$	$X2^2$	$X3^2$	$X4^2$	R^2	F-ratio	Prob>F	LOF Prob > F
BZ	**<.0001**	0.6795	0.2056	0.7575	**0.0244**	**0.0002**	0.1366	0.7274	0.1148	**0.0091**	0.3504	0.091	**0.0002**	0.4723	**0.0192**	0.85	5.8956	0.0008	0.852
4MBC	**<.0001**	**<.0001**	0.1575	0.2806	0.3093	**<.0001**	**0.0262**	**0.0001**	0.2365	0.2365	0.5037	0.408	**0.0034**	0.1768	0.8193	0.9	10.0885	<.0001	0.3822
EDP	**<.0001**	**0.0267**	0.7494	<.0001	0.0666	0.9318	**0.0002**	0.4972	0.1024	0.2117	**0.0014**	**0.023**	0.1961	0.7321	0.9655	0.86	6.7146	0.0004	0.0458
EMC	**<.0001**	**<.0001**	**0.0183**	**0.0002**	0.8388	**0.023**	**0.0013**	0.5313	0.9761	**0.0036**	**0.0007**	**0.0036**	0.1727	0.1113	0.1484	0.93	14.2988	<.0001	0.0411
OC	**<.0001**	**0.0171**	0.5289	0.2557	0.3546	**0.0011**	**0.0007**	**0.0025**	**0.0019**	0.8373	**0.0241**	0.1946	0.9138	**0.0044**	**0.0054**	0.88	7.9086	<.0001	0.0991
DTS	**<.0001**	**<.0001**	0.0728	0.0536	**0.0089**	0.4235	0.5594	0.099	**0.0215**	0.2572	0.6022	0.1563	0.0868	**0.0057**	0.9344	0.88	7.7152	0.0002	0.0324
DPMI	**<.0001**	**<.0001**	0.1419	**0.0103**	0.508	0.4287	0.2127	0.2127	**0.0088**	**0.0045**	**0.0017**	**0.0112**	**0.0009**	**0.0053**	0.2234	0.9	9.954	<.0001	0.1388
ADBI	**<.0001**	0.2534	0.8343	0.2904	0.9197	0.3219	0.4708	0.616	0.0981	**0.0005**	0.438	**0.0031**	**0.0022**	**0.0125**	0.7299	0.84	5.6248	0.001	0.0966
AHMI	**<.0001**	0.4467	**0.0133**	**0.0311**	**0.0098**	**0.0086**	0.1306	**0.006**	**0.005**	**0.0422**	0.3439	**0.0499**	**0.0003**	**0.0223**	0.518	0.86	6.7749	0.0003	0.0619
ATII	**<.0001**	**<.0001**	0.1773	**0.0134**	0.1935	**0.0009**	0.619	**0.0021**	0.1021	0.2113	0.0788	0.0646	**0.0314**	0.2593	**0.0025**	0.9	9.3512	<.0001	0.1898
EXA	**<.0001**	**0.0412**	**0.0007**	0.9919	**0.0069**	0.8232	0.0675	**<.0001**	**0.0114**	0.0775	**<.0001**	0.1516	**0.0032**	**<.0001**	0.1129	0.94	15.4349	<.0001	0.0115
HHCB	**<.0001**	**0.0007**	**0.0325**	0.0791	**0.0028**	**0.0103**	**0.0005**	0.4364	**<.0001**	**<.0001**	**0.0128**	0.53	**0.0112**	0.1292	**0.0003**	0.93	13.4317	<.0001	0.5619
AHTN	**<.0001**	0.0548	**0.0302**	**0.0118**	0.0839	0.8455	**0.0048**	0.8455	0.4039	0.5188	**0.0162**	**0.0013**	0.418	**0.0006**	0.2033	0.83	5.3397	0.0013	0.0039
EB	**<.0001**	0.3874	0.661	0.0833	**0.0001**	**0.0217**	0.085	**0.0002**	**0.0003**	0.102	0.6877	**0.0439**	**<.0001**	0.3157	**0.0037**	0.91	10.2362	<.0001	0.0766
MA	**<.0001**	0.4714	0.4175	0.767	0.177	**0.0001**	**0.0009**	**0.0002**	**0.009**	0.241	**0.0004**	**0.0001**	**<.0001**	0.0701	**0.0005**	0.94	16.387	<.0001	0.2963
MX	**<.0001**	**0.0425**	**0.044**	**0.0099**	0.7786	**0.0013**	0.1781	**0.0119**	**0.0025**	**0.0137**	**0.0137**	**0.0403**	0.8096	0.8675	0.7883	0.84	5.6317	0.001	0.8127
MM	**<.0001**	0.2858	0.4248	0.0521	**0.0426**	**0.0215**	0.2745	**0.0004**	**0.0004**	**0.0015**	**0.0019**	**<.0001**	**0.0187**	0.0533	0.1026	0.92	13.0058	<.0001	0.0035
MT	**<.0001**	**<.0001**	0.0658	0.1402	0.1479	**0.025**	0.1124	**0.0007**	**0.025**	**<.0001**	**0.001**	**<.0001**	0.0902	0.4118	0.5559	0.94	16.3158	<.0001	0.0012
MK	**<.0001**	**0.0193**	**<.0001**	**<.0001**	**0.0409**	0.8679	**<.0001**	**0.0003**	0.6191	**0.0001**	0.3261	**<.0001**	**0.002**	0.5028	0.7444	0.95	19.5173	<.0001	0.1031

Obs.: X1 - Solvent Volume; X2 - Time of Vortex; X3 - Amount C_{18}; X4 - Amount PSA.

Due to the high number of responses, the optimal conditions were determined using a desirability function. Once again, this function was used to maximize the target recoveries (recovery = 100 ± 20%). The optimal conditions were obtained with 10 mL of ACN, 2.5 min of vortex, 410 mg of C_{18} and 315 mg of PSA. The predicted recovery values using these conditions ranged between 75% (DPMI) and 122% (EHMC). The software also allows the visualization of three-dimensional response surfaces of the predicted responses. As an example, the three-dimensional response surface plot for galaxolide (HHCB) is represented in Figure 4.3.

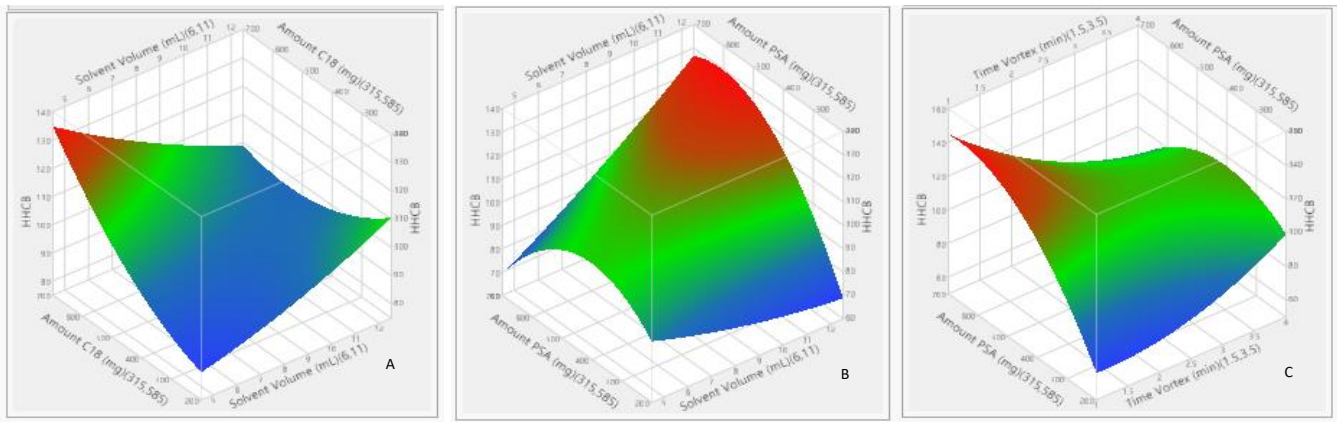


Figure 4.3 Example of a response surface plot for HHCB (response: recovery). (A) Solvent volume (X1) vs. Amount of C_{18} (X3); (B)Solvent volume (X1) vs. Amount of PSA (X4); (C) Time of vortex (X2) vs. amount of PSA (X4).

This optimization indicates that a higher volume of extraction solvent (10 mL instead of 5 mL) allows the maximization of the target's extraction. In fact, a higher amount of solvent will probably help in the transference of the target compounds from the sample to the solvent. The optimal time of vortex is in the middle of the studied range (2.5 min), which may indicate that long time of vortex will extract not only the target compounds, but other interferences, and short times are not enough to transfer all the target compounds from the sludge to the solvent. Regarding the clean-up sorbents C_{18} and PSA, seems that is necessary quite more C_{18} than PSA, probably because of the amount of lipophilic/non-polar interferences found in sludge.

To prove the applicability of this empirical model, five additional tests were performed, aiming to confirm the prediction viability of the model, where random patterns of the coded levels (xi) were selected (Table S3.7 in Annex 3). Results obtained were in the

range of the predicted values by the proposed model. Deviations between the experimental and predicted extraction recoveries were low (<5%).

4.3.3. Method validation

To evaluate the performance of the developed methodology (QuEChERS-GC–MS/MS), validation tests were carried out – assessment of the linearity range, instrumental and methodologic limits of detection and quantification as well as precision and accuracy. The main results are presented in Table 4.4.

The calibration curve was constructed directly injecting thirteen calibration standards in n-hexane with all target compounds at concentrations from 0.5 to 5000 ng g^{-1} dw, since preliminary tests revealed not significant matrix effects (< 20%). All target compounds showed a linear behavior within the studied range, except for DTS, MX and MM (5–5000 ng g^{-1} dw) and DPMI and MA (1–5000 ng g^{-1} dw). The r^2 ranged between 0.991 and 0.999. The instrumental detection limits (IDLs) were determined injecting solvent solutions, whereas both detection and quantification limits of the method (MDL and MQL, respectively) were assessed by the injection of spiked sludge samples. They were calculated based on a signal-to-noise ratio (S/N) of 3 and 10, respectively. IDLs varied between 0.001 pg (MM) to 7.5 pg (MX). These values are generally in the same range as those reported in the literature (e.g. Wu and Ding (2010)). The MDLs were calculated based on the matrix effect and values ranged between 0.5 (DPMI) and 1394 (EXA)ng g^{-1} dw. These results are in accordance, for example, with those published by Gago-Ferrero et al. (2011) in the analysis of UVFs in sludge.

The precision was evaluated by the inter- and intra-day precision at different spiking levels (50, 250 and 2500 ng g^{-1} dw). This method can be considered precise once the values obtained vary between 1 and 8% (expressed as %RSD), which are quite acceptable considering this type of matrix.

Table 4.4 Main validation parameters for the QuEChERS/ GC-MS/MS methodology.

Compounds	Linearity Range (ng/g-dw)	R^2	ILOD (pg)	ILOQ (pg)	MDL (ng/d-dw)	MQL (ng/g-dw)	Interday precision (%RSD)			Accuracy ± Intraday precision (%Mean recovery ±RSD)		
							50 ng/g-dw	250 ng/g-dw	2,500 ng/g-dw	50 ng/g-dw	250 ng/g-dw	2,500 ng/g-dw
BZ	0.5-5,000	0.998	0.11	0.36	26	86	2	4	6	100±2	101±4	92±5
4MBC	0.5-5,000	0.998	0.03	0.11	59	196	7	2	2	88±8	87±1	85±5
EDP	0.5-5,000	0.999	1.00	3.33	31	102	2	1	1	86±3	82±3	85±2
EHMC	0.5-5,000	0.998	0.02	0.07	5	18	1	2	5	122±3	125±2	113±8
OC	0.5-5,000	0.999	0.04	0.12	6	19	3	4	5	94±2	81±2	88±7
DTS	5 -5,000	0.991	5.00	16.67	2	7	2	3	3	118±2	113±6	114±1
DPMI	1 -5,000	0.999	0.08	0.27	0.5	2	1	1	7	75±4	85±2	77±9
ADBI	0.5-5,000	0.994	0.01	0.02	2	7	8	5	1	111±3	103±3	82±4
AHMI	0.5-5,000	0.999	0.06	0.21	4	13	2	7	2	78±8	82±1	88±5
ATII	0.5-5,000	0.998	0.16	0.53	4	13	1	2	0	97±5	97±1	87±3
HHCB	10-5,000	0.993	4.29	14.29	1394	4648	3	1	1	98±6	94±4	94±1
AHTN	0.5-5,000	0.995	0.01	0.05	16	55	2	5	4	97±1	103±4	105±8
EXA	0.5-5,000	0.995	0.03	0.11	5	18	2	3	1	97±3	103±6	93±8
EB	0.5-5,000	0.990	0.60	2.00	126	421	5	2	3	74±8	86±3	99±2
MA	1-5,000	0.995	1.20	4.00	21	70	5	2	4	118±2	112±6	111±3
MX	5 -5,000	0.999	7.50	25.00	5	15	2	2	4	97±6	95±3	90±2
MM	5 -5,000	0.999	0.000050	0.00017	5	16	1	2	2	111±6	115±1	115±2
MT	0.5-5,000	0.999	0.60	2.00	15	50	3	2	2	112±2	108±5	103±3
MK	50 -5,000	0.991	0.000022	0.000072	0.5	2	6	5	2	103±10	87±8	92±2

Accuracy was determined by recovery tests, using three replicate spiked samples at the same concentration levels as before. Recoveries vary from 75% (DPMI) to 122% (EHMC), which are similar to those found in the literature, using more time-consuming and expensive techniques (Shek et al., 2008; Herren and Berset, 2000; Langford et al., 2015; Rodil et al., 2009). To the authors' best knowledge, the proposed methodology is the first one based on QuEChERS followed by GC–MS/MS that can be applied for the simultaneous extraction of 6 polycyclic, 2 macrocyclic and 5 nitro musks and 6 UVFs.

4.3.4. Real samples analysis

The applicability of the proposed methodology was proved by the analyses of real samples. Thus, the occurrence of the target contaminants was determined in seven sewage sludge samples. The samples were analysed in triplicate and the results are summarized in Table 4.5.

SMCs and UVFs were found in all samples. Exception for, the nitro musks MA, MM and MT, which were not detected in any sample. In fact, this was already expected since their use in personal care products and cosmetics is prohibited in Europe. From the restricted nitro musks (MX and MK), only MK, which is mainly used in cosmetics (Reiner et al., 2007; Yang and Metcalfe, 2006), was detected. This compound is usually found in low concentrations in literature, ranging from 7 ng g^{-1} dw (Herren and Berset, 2000) to 359 ng g^{-1} dw (Liu et al., 2014), whether in this study values ranged from 25 to 471 ng g^{-1} dw. Both polycyclic musks HHCB and AHTN show the highest concentrations and were also the predominant SMCs in sewage sludge. The low variability of results with seasonality in the SMCs class may be due to their continuous use in personal care and household products (average total values of 92,488 ± 4912 ng g^{-1} dw in summer and 77,126 ± 10,186 ng g^{-1} dw in winter). Despite the small number of samples analysed, these values are in the same range than those found in the literature for SMCs. The season variation is more clear in UVFs class, with concentrations of the most detected compounds as OC and DTS (Gilbert et al., 2013) being higher in the summer. Mean levels of OC varied from 59,557 ±8054 ng g^{-1} dw in winter to 87,999±12,428 ng g^{-1} dw in summer and DTS from 8662 ± 664 ng g^{-1} dw to 17,139 ± 2203 ng g^{-1} dw in the same

seasons. Other UVFs' compounds are present in relatively lower concentrations throughout the year, as 4MBC (246 to 538 ng g^{-1} dw), EDP (13 ng g^{-1} dw), EHMC (726 to 3448 ng g^{-1} dw) and BZ (665 to 2471 ng g^{-1} dw). These results are in the same order of magnitude as those found in the literature (Langford et al., 2015; Kupper et al., 2006; Plagellat et al., 2006). It is also important to mention that DTS has never been studied in sewage sludge and so these are the first results published in this matrix. Also, it is worth to notice that DTS is only used in products owned by the L'Oreal brand (Manova et al., 2013), which can indicate a high consume of these products in the region where sludge samples were collected. Overall, these results reported prove that the developed QuEChERS methodology coupled to GC–MS/MS analysis is suitable to determine both SMCs and UVFs in sewage sludge.

Despite the small sampling campaign, the results showed that SMCs and UVFs end up in sludge at concentrations ranging from few ng g^{-1} dw to hundreds of µg g^{-1} dw. This is particularly worrisome, since sludge is often used as fertilizer in agricultural fields due to their high nutrients content (Molla et al., 2005). So far, few studies have been conducted with UVFs and SMCs, however, their potential to migrate from soils to crops needs to be considered (Calderón-Preciado et al., 2012; Lai et al., 2014; Macherius et al., 2012).

Table 4.5 Concentration (ng g^{-1} dw) of UVFs and SMCs in the analysed sludge samples. (number of replicates=3; n.d. – not detected; <MQL - below method quantification limit).

		Winter 13'	Spring 14'	Summer 14'	Summer 14'	Winter 14'	Winter 17'	Winter 17'
				Sample 1	Sample 2		Sample 1	Sample 2
UV-Filters	BZ	1,665±74	1,893±56	2,471±72	2,389±109	1,918±83	2,043±373	665±38
	4MBC	268±2	246±0	532±1	462±3	254±0	538±9	<MQL
	EDP	13±1	< MQL	<MQL	<MQL	<MQL	<MQL	<MQL
	EHMC	822±16	1,661±17	726±1	863±9	1,145±8	3,448±77	3,420±480
	OC	48,879±1,249	38,324±2,158	110,186±8,485	115,486±8,821	59,607±306	95,334±4,398	34,406±6,624
	DTS	4,424±7	4,461±83	22,326±1,686	24,631±1,415	6,324±229	17,428±607	6,472±143
Policyclic Musks	DPMI	285±12	210±9	346±10	299±8	271±6	60±2	228±15
	ADBI	n.d.	n.d.	14±2	<MQL	<MQL	n.d.	n.d.
	AHMI	13±1	<MQL	20±2	15±1	15±1	n.d.	<MQL
	ATII	84±6	306±13	231±25	185±23	151±28	n.d.	n.d.
	HHCB	59,985±1,924	64,741±1945	81,771±3,432	66,519±2,717	77,618±2,821	41,315±6,006	41,132±2,216
	AHTN	6,637±146	7,292±173	10,293±384	9,004±430	8,051±199	6,766±13	3,722±94
Macro cyclic Musks	EXA	9,737±127	14,939±468	10,497±180	10,662±753	12,684±1,250	36,705±7,030	1,461±180
	EB	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
Nitro Musks	MA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	MX	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	MM	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	MT	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	MK	n.d.	25±1	n.d.	n.d.	n.d.	189±17	471±22

4.4. Conclusions

In this work, a QuEChERS methodology coupled to GC–MS/MS analysis was successfully developed to extract simultaneously SMCs and UVFs from sludge samples. Method development was performed using a Design of Experiments approach. Firstly, a Screening Design was used to understand which parameters influence the most the compounds in the extraction, and then a Central Composite Design was performed to optimize the proposed parameters. QuEChERS procedure proved to be a useful extraction method to the selected 19 compounds among SMCs and UVFs, using low volume of organic solvents and low amount of sorbents. The optimal conditions achieved were: 10 mL ACN, 2.5 min vortex, 15 min ultrasound extraction, 500 mg $MgSO_4$, 315 mg PSA and 410 mg C_{18} for the QuEChERS. These conditions lead to compound recoveries between 75% (DPMI) to 122% (EHMC). The method was validated, achieving low %RSD in the inter- and intra-day precision (<10%) and accuracy was proven fit to this methodology with good %recoveries and low limits of detection. The method was applied to real sewage sludge samples, with higher concentrations found in the most used UVFs (OC and DTS) and SMCs (HHCB and AHTN). The target compounds were detected in all samples. Overall, concentrations of polycyclic musks varied from 12.6 (AHMI) to 81,771 ng g^{-1} dw (HHCB), macro musk from 1461 to 36,705 ng g^{-1} dw (EB) and UVFs from 12.5 (EDP) to 115,486 ng g^{-1} dw (OC). Among nitro musks, only MK was detected in the samples, with concentrations between 25 and 471 ng g^{-1} dw.

Acknowledgements

This work was the result of the project:

(i) POCI-01-0145-FEDER-006939 (Laboratory for Process Engineering, Environment, Biotechnology and Energy – UID/EQU/00511/2013) funded by the European Regional Development Fund (ERDF), through COMPETE2020-Programa Operational Competitividade e Internacionalização (POCI) and by national funds, through FCT - Fundação para a Ciência e a Tecnologia. (ii) NORTE-01-0145-FEDER-000005 – LEPABE-2-ECO-INNOVATION, supported by North Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European

Regional Development Fund (ERDF). (iii) Post-doctoral Grant SFRH/BPD/76974/2011-Vera Homem. (iv) Doctoral Grant SFRH/BD/110831/2015 – Sara Ramos.

Supplementary Information

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.10.143 or in Annex 3.

References

Badia-Fabregat, M., Rodríguez-Rodríguez, C.E., Gago-Ferrero, P., Olivares, A., Piña, B., Díaz-Cruz, M.S., Vicent, T., Barceló, D., Caminal, G., 2012. Degradation of UV filters in sewage sludge and 4-MBC in liquid medium by the ligninolytic fungus Trametes versicolor. J. Environ. Manag. 104, 114–120.

Bester, K., 2004. Retention characteristics and balance assessment for two polycyclic musk fragrances (HHCB and AHTN) in a typical German sewage treatment plant. Chemosphere 57, 863–870.

Calderón-Preciado, D., Renault, Q., Matamoros, V., Cañameras, N., Bayona, J.M., 2012. Uptake of organic emergent contaminants in spath and lettuce: an in vitro experiment. J. Agric. Food Chem. 60, 2000–2007.

Chen, D., Zeng, X., Sheng, Y., Bi, X., Gui, H., Sheng, G., Fu, J., 2007. The concentrations and distribution of polycyclic musks in a typical cosmetic plant. Chemosphere 66, 252–258.

Cosmetics Directive, 2009. Annex VI: list of UV filters allowed in cosmetic products in Europe. Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. Web:. http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.results&annex_v2=VI&search.

Decreto-Lei N.276/2009, 2009. Regime jurídico de utilização agrícola das lamas de depuração em solos agrícolas. Diário da República no 192, Série I de 2 de Outubro de 2009.

Gago-Ferrero, P., Diaz-Cruz, M.S., Barcelo, D., 2011. Occurrence of multiclass UV filters in treated sewage sludge from wastewater treatment plants. Chemosphere 84, 1158–1165.

Gilbert, E., Pirot, F., Bertholle, V., Roussel, L., Falson, F., Padois, K., 2013. Commonly used UV filter toxicity on biological functions: review of last decade studies. Int. J. Cosmet. Sci. 35, 208–219.

Guo, R., Lee, I.S., Kim, U.J., Oh, J.E., 2010. Occurrence of synthetic musks in Korean sewage sludges. Sci. Total Environ. 408, 1634–1639.

Herren, D., Berset, J.D., 2000. Nitro musks, nitro musk amino metabolites and polycyclic musks in sewage sludges. Quantitative determination by HRGC-ion-trap-MS/MS and mass spectral characterization of the amino metabolites. Chemosphere 40, 565–574.

Homem, V., Silva, A., Ratola, N., Santos, L., Alves, A., 2015. Long lasting perfume e a review of synthetic musks in WWTPs. J. Environ. Manag. 149, 168–192.

Homem, V., Alves, A., Alves, A., Santos, L., 2016. Ultrasound-assisted dispersive liquid liquid microextraction for the determination of synthetic musk fragrances in aqueous matrices by gas chromatography-mass spectrometry. Talanta 148, 84–93.

Homem, V., Magalhães, I., Alves, A., Santos, L., 2017. Assessing seasonal variation of synthetic musks in beach sands from Oporto coastal area: a case study. Environ. Pollut. 226, 190–197.

Horii, Y., Reiner, J.L., Loganathan, B.G., Senthil Kumar, K., Sajwan, K., Kannan, K., 2007. Occurrence and fate of polycyclic musks in wastewater treatment plants in Kentucky and Georgia, USA. Chemosphere 68, 2011–2020.

Hu, Z., Shi, Y., Cai, Y., 2011. Concentrations, distribution, and bioaccumulation of synthetic musks in the Haihe River of China. Chemosphere 85, 262–267.

Kupper, T., Plagellat, C., Brändli, R.C., de Alencastro, L.F., Grandjean, D., Tarradellas, J., 2006. Fate and removal of polycyclic musks, UV filters and biocides during wastewater treatment. Water Res. 40, 2603–2612.

Lai, H.J., Ying, G.G., Ma, Y.B., Chen, Z.F., Chen, F., Liu, Y.S., 2014. Field dissipation and plant uptake of benzotriazole ultraviolet stabilizers in biosolid-amended soils. Environ. Sci. Processes Impacts 16, 558–566.

Langford, K.H., Reid, M.J., Fjeld, E., Øxnevad, S., Thomas, K.V., 2015. Environmental occurrence and risk of organic UV filters and stabilizers in multiple matrices in Norway. Environ. Int. 80, 1–7.

Liu, Y.-S., Ying, G.-G., Shareef, A., Kookana, R.S., 2011. Simultaneous determination of benzotriazoles and ultraviolet filters in ground water, effluent and biosolid samples using gas chromatography–tandem mass spectrometry. J. Chromatogr. A 1218, 5328–5335.

Liu, Y.-S.S., Ying, G.-G.G., Shareef, A., Kookana, R.S., 2012. Occurrence and removal of benzotriazoles and ultraviolet filters in a municipal wastewater treatment plant. Environ. Pollut. 165, 225–232.

Liu, N., Shi, Y., Li, W., Xu, L., Cai, Y., 2014. Concentrations and distribution of synthetic musks and siloxanes in sewage sludge of wastewater treatment plants in China. Sci. Total Environ. 476–477, 65–72.

Macherius, A., Eggen, T., Lorenz, W.G., Reemtsma, T., Winkler, U., Moeder, M., 2012. Uptake of galaxolide, tonalide, and triclosan by carrot, barley, and meadow fescue plants. J. Agric. Food Chem. 60, 7785–7791.

Magnusson, B., Örnemark, U., 2014. EurachemGuide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics. 2nd ed. Eurachem.

Manova, E., von Goetz, N., Hauri, U., Bogdal, C., Hungerbuhler, K., 2013. Organic UV filters in personal care products in Switzerland: a survey of occurrence and concentrations. Int. J. Hyg. Environ. Health 216, 508–514.

Molla, A.H., Fakhru'l-Razi, A., Hanafi, M.M., Alam, M.Z., 2005. Compost produced by solid state bioconversion of biosolids: a potential resource for plant growth and environmental friendly disposal. Commun. Soil Sci. Plant Anal. 36, 1435–1447.

Muller, J., Boehmer,W., Litz, N.T., 2006. Occurrence of polycyclic musks in sewage sludge and their behaviour in soils and plants: part 1: behaviour of polycyclic musks in sewage sludge of different treatment plants in summer and winter. J. Soils Sediments 6, 231–235.

Negreira, N., Rodríguez, I., Rubí, E., Cela, R., 2011. Optimization of pressurized liquid extraction and purification conditions for gas chromatography–mass spectrometry determination of UV filters in sludge. J. Chromatogr. A 1218, 211–217.

Picot Groz, M., Martinez Bueno, M.J., Rosain, D., Fenet, H., Casellas, C., Pereira, C., Maria, V., Bebianno, M.J., Gomez, E., 2014. Detection of emerging contaminants (UV filters, UV stabilizers and musks) in marine mussels from Portuguese coast by QuEChERS extraction and GC-MS/MS. Sci. Total Environ. 493, 162–169.

Plagellat, C., Kupper, T., Furrer, R., de Alencastro, L.F., Grandjean, D., Tarradellas, J., 2006. Concentrations and specific loads of UV filters in sewage sludge originating from a monitoring network in Switzerland. Chemosphere 62, 915–925.

Prosser, R.S., Sibley, P.K., 2015. Human health risk assessment of pharmaceuticals and personal care products in plant tissue due to biosolids and manure amendments, and wastewater irrigation. Environ. Int. 75, 223–233.

Ramos, S., Homem, V., Alves, A., Santos, L., 2015. A review of organic UV-filters in wastewater treatment plants. Environ. Int. 86, 24–44.

Reiner, J.L., Berset, J.D., Kannan, K., 2007. Mass flow of polycyclic musks in two wastewater treatment plants. Arch. Environ. Contam. Toxicol. 52, 451–457.

Ribeiro, H., Ramos, S., Homem, V., Santos, L., 2017. Can coastline plant species be used as biosamplers of emerging contaminants? - UV-filters and synthetic musks as case studies. Chemosphere 184.

Rodil, R., Schrader, S., Moeder, M., 2009. Pressurised membrane-assisted liquid extraction of UV filters from sludge. J. Chromatogr. A 1216, 8851–8858.

Rodríguez-Rodríguez, C.E., Barón, E., Gago-Ferrero, P., Jelić, A., Llorca, M., Farré, M., Díaz-Cruz, M.S., Eljarrat, E., Petrović, M., Caminal, G., Barceló, D., Vicent, T., 2012. Removal of pharmaceuticals, polybrominated flame retardants and UV-filters from sludge by the fungus *Trametes versicolor* in bioslurry reactor. J. Hazard. Mater. 233–234, 235–243.

Saraiva, M., Cavalheiro, J., Lanceleur, L., Monperrus, M., 2016. Synthetic musk in seafood products from South Europe using a quick, easy, cheap, effective, rugged and safe extraction method. Food Chem. 200, 330–335.

Shek, W.M., Murphy, M.B., Lam, J.C.W., Lam, P.K.S., 2008. Synthetic polycyclic musks in Hong Kong sewage sludge. Chemosphere 71, 1241–1250.

Stevens, J.L., Northcott, G.L., Stern, G.A., Tomy, G.T., Jones, K.C., 2003. Pesticides, synthetic musks, and polychlorinated n -alkanes in U.K. Sewage sludge: survey results and implications. Environ. Sci. Technol. 37, 462–467.

Ternes, T.A., Bonerz, M., Herrmann, N., Löffler, D., Keller, E., Lacida, B.B., Alder, A.C., 2005. Determination of pharmaceuticals, iodinated contrast media and musk fragrances in sludge by LC tandem MS and GC/MS. J. Chromatogr. A 1067, 213–223.

Vallecillos, L., Pocurull, E., Borrull, F., 2015. Influence of pre-treatment process on matrix effect for the determination of musk fragrances in fish and mussel. Talanta 134, 690–698.

Vela-Soria, F., Iribarne-Durán, L.M., Mustieles, V., Jiménez-Díaz, I., Fernández, M.F., Olea, N., 2018. QuEChERS and ultra-high performance liquid chromatography–tandem mass spectrometry method for the determination of parabens and ultraviolet filters in human milk samples. J. Chromatogr. A 1546, 1–9.

Wick, A., Fink, G., Ternes, T.A., 2010. Comparison of electrospray ionization and atmospheric pressure chemical ionization for multi-residue analysis of biocides, UVfilters and benzothiazoles in aqueous matrices and activated sludge by liquid chromatography–tandem mass spectrometry. J. Chromatogr. A 1217, 2088–2103.

Wu, S.-F., Ding, W.-H., 2010. Fast determination of synthetic polycyclic musks in sewage sludge and sediments by microwave-assisted headspace solid-phase microextraction and gas chromatography-mass spectometry. J. Chromatogr. A 1217, 2776–2781.

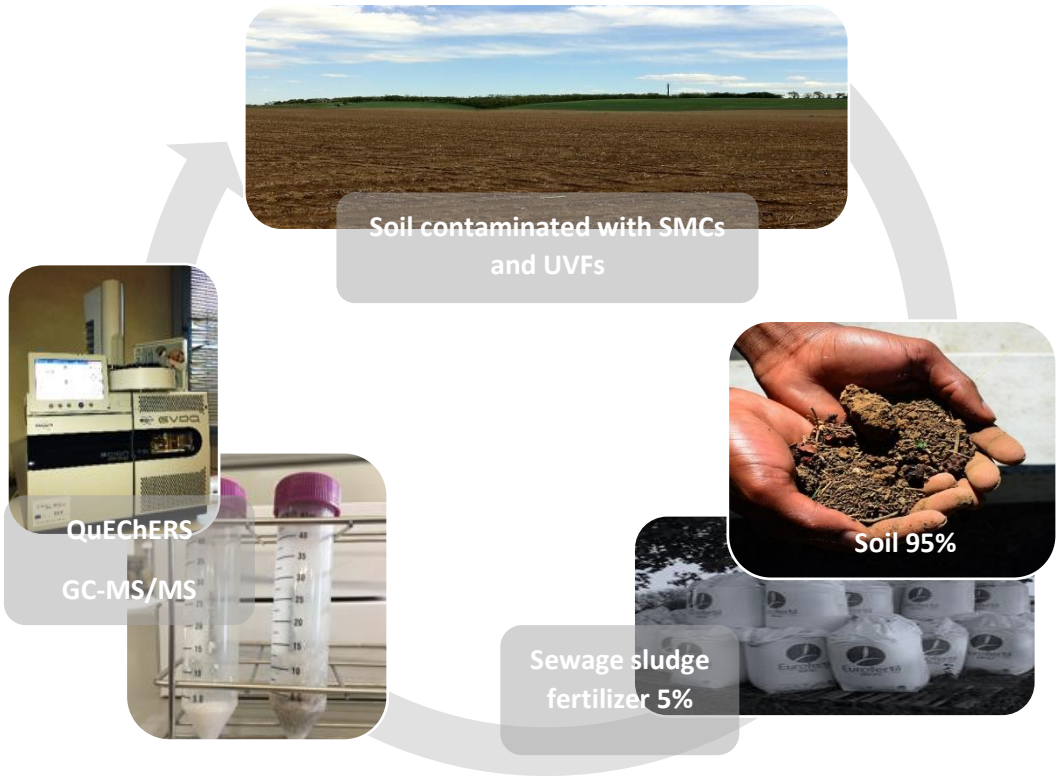
Yang, J.J., Metcalfe, C.D., 2006. Fate of synthetic musks in a domestic wastewater treatment plant and in an agricultural field amended with biosolids. Sci. Total Environ. 363, 149–165.

Zeng, X., Sheng, G., Xiong, Y., Fu, J., 2005. Determination of polycyclic musks in sewage sludge from Guangdong, China using GC-EI-MS. Chemosphere 60, 817–823.

Chapter 5. A feasible analytical methodology using a QuEChERS-GC-MS/MS approach to quantify ultraviolet filters and synthetic musk compounds in soil samples

Sara Ramos, Vera Homem*, Lúcia Santos

Submitted



Abstract

A simple method for the analysis of thirteen synthetic musk compounds (SMCs) and six UV-filters (UVFs) in soil samples was developed using a Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) technique followed by gas chromatography – triple quadrupole mass spectrometry (GC-MS/MS).

The QuEChERS procedure included an initial ultrasound-assisted extraction step with organic solvents (acetone/hexane 1:1, v/v) and the addition of sodium chloride. Then, in the second phase, a clean-up based on dispersive solid-phase extraction, using C_{18} as sorbent, was performed. Accuracy, assessed by recovery tests, ranged from 81% to 122% and a good precision was achieved, with relative standard deviation less than 4%. The instrumental limit of detection (ILOD) varied from 0.01 pg to 5.00 pg, while the method detection limit (MDL) ranged between 0.01 and 10.00 ng g^{-1} dw. The applicability of the proposed methodology was tested using different types of soils. Both SMCs and UVFs were detected in all soil samples. The most frequently detected compounds were benzophenone (BZ), octocrylene (OC), 2-ethylhexyl 4-dimethylaminobenzoate (EDP), 2-ethylhexyl 4–methoxycinnamate (EMC) and galaxolide (HHCB). Higher levels were detected for benzophenone (maximum value of 158 ng g^{-1} dw) and octocrylene (137 ng g^{-1} dw).

The proposed method compared to conventional techniques uses lower amounts of solvents and sorbents, thus producing little waste (“green” technique). Also, it is a faster and easier to perform methodology, does not need sophisticated equipment, and in the end, the cost of each analysis is cheaper.

Keywords: UV-filters, Synthetic musk compounds, Soils, QuEChERS, GC-MS/MS, Sewage sludge-based fertilized soils.

5.1 Introduction

Soils could be considered as reservoirs for lipophilic compounds since organic matter is a key sorption medium for contaminants (Qin et al., 2017). Whereas highly mobile hydrophilic compounds can easily leach into groundwater, lipophilic compounds may accumulate in the upper soil layer (Chefetz et al., 2008). The accumulation in soils is influenced by both the soil properties, such as organic matter content, type and quantity of clay, ion exchange capacity and pH and the physicochemical properties of the target compounds, such as their water solubility, octanol–water partition coefficient (K_{OW}) or organic carbon-water partition coefficient (K_{OC}) (Drillia et al., 2005; Tolls, 2001).

Irrigation with contaminated water (Wang et al., 2013; Xu et al., 2009) or fertilization with biosolids from wastewater treatment plants (WWTPs) (Clarke et al., 2016; Dodgen et al., 2014) may be possible origins of soil contamination. Nevertheless, literature has shown that over the past decades several organic contaminants like pharmaceutical and personal care products (PCPs), are present either in biosolids or in treated wastewater (Homem et al., 2015; Ramos et al., 2016; Sharma et al., 2017). Few works have already been published on the presence of PCPs, and more specifically on ultraviolet filters (UVFs) and synthetic musk compounds (SMCs). UVFs and SMCs are two classes of organic compounds used in a broad range of products, such as plastics, adhesives, rubber, cosmetics, toiletries, etc. UVFs were created to protect products or skin from ultraviolet (UV) radiation, whereas SMCs are used as base notes in perfumed products and as fragrance fixatives (Witorsch and Thomas, 2010). Several reviews have proven that these compounds are environmentally persistent, biologically active, with bioaccumulation capability and some are considered potential endocrine disrupters.

To the authors' best knowledge, the UVFs under scrutiny in this paper have never been studied before in soils and few studies are available regarding SMCs. Analysing the available works on SMCs, soil samples were generally extracted and cleaned up either by pressurized liquid extraction (PLE) (Albero et al., 2012; Chen et al., 2014; Kinney et al., 2008; Lai et al., 2014; Wang et al., 2013; Yang and Metcalfe, 2006), stir-bar sorptive extraction (SBSE) (Aguirre et al., 2014), solid-phase extraction (SPE) (Sánchez-Brunete et al., 2011) or using a solid-liquid extraction approach (SLE) (Camino-Sánchez et al., 2016; Chase et al., 2012). Although PLE and SBSE are methods with good performances, the

equipment required is not always available in all laboratories. SPE and SLE are often time-consuming techniques that use large amounts of solvent and sample. Therefore, it is essential to develop quick, efficient and reliable methodologies to extract contaminants from soils. In this work a Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) methodology was proposed to simultaneously extract SMCs and UVFs. This technique is a very appealing approach by combining extraction with clean-up, using low amounts of solvents and sorbents and producing little waste. On the other hand, the procedure is quite fast, easy to perform, needs few equipment and the reagents' cost is low, when compared to conventional techniques (Perestrelo et al., 2019). This methodology has also the advantage of generating potentially high recoveries and low detection limits (Bragança et al., 2012). It has already been employed to extract other compounds, especially pesticides, from soils and it is easy to adapt and optimize.

Thus, the purpose of this work was to optimize and validate a QuEChERS methodology followed by GC-MS/MS analysis for the determination of 19 compounds (6 UVFs and 13 SMCs) in different types of soil, providing references for future research on soil contamination. To the author's best knowledge this technique has never been tested for the simultaneous extraction of these compounds in soils.

5.2 Materials and Methods

5.2.1 Standards, reagents and materials

In this study, the solvents used to optimize the extraction method were all analytical grade and purchased from VWR BDH Prolabo (Fontenay-sous-Bois, France): acetonitrile (ACN), acetone (Ac), ethyl acetate (EA) and hexane (Hex). Ultrapure water was achieved using a combined equipment Millipore Reverse Osmosis System Elix® coupled to a Millipore® Synergy® with UV from Merck (Germany). The salts and sorbents used in the extraction were the sodium chloride (NaCl) from Merck (Germany), anhydrous magnesium sulphate ($MgSO_4$) from Panreac AppliChem (Barcelona, Spain) and octadecyl-silica (C_{18}) from Supelco (Bellefonte, PA, USA). To ensure that $MgSO_4$ was in an anhydrous form, it was heated at 450 °C for 12 hours before use. Extracts were

filtered using a 0.2 µm polytetrafluoroethylene (PTFE) syringe filters from VWR BDH Prolabo (Fontenay-sous-Bois, France), using a 2.5 mL sterile luer lock tip syringes from Terumo (Leuven, Belgium) and sterile disposable needles from Sterican, Braun (Melsungen, Germany). Helium, nitrogen and argon (all with 99.9999% purity) were supplied by Linde (Porto, Portugal) or by Air Liquid (Maia, Portugal). More information regarding analytical standards used in this work is provided in the Annex 4.

5.2.2 Soil samples

Different soil samples were collected (near a beach, on agricultural lands, garden, industrial area and school yard). Roots and large stones were removed, and soil samples were collected from the top layer (0–30 cm) with a stainless-steel shovel and carried to the lab in polypropylene bags. Then, samples were dried in an oven at 50 ^{o}C for 3 h (to avoid the loss of the target compounds), sieved through a 2 mm sieve opening (mesh n.^{o} 10), thoroughly mixed and kept frozen (-20 °C) in amber glass containers until analysis.

5.2.3 Sample characterization

Different types of soils were selected to represent diverse contents of organic carbon and clay (Table 5.1). The organic carbon (OC) was determined in a Primacs^{SNC} Carbon-Nitrogen/Protein Analyzer from Skalar (Breda, The Netherlands) and content varied between 0.8 and 14.7%. The particle size distributions for determination of the clay content variation were measured with the COULTER LS 230 laser diffraction analyser from Beckman (West Hialeah, USA).

A fertilizer bought in a local agricultural cooperative was also used in this study. This fertilizer is produced by the composting of a sewage sludge from an urban WWTP, which is subsequently sanitized. It has a grain size between 1 and 10 mm, a 42% humidity and a bulk density of 0.4 kg dm^{-3}. The organic content is about 75±9%, but total carbon is 42±5%. Regarding inorganic nutrients, this fertilizer has a total phosphorous content (P_2O_5) of 0.4%, potassium (K_2O) of 0.2%, calcium (CaO) of 0.9%, magnesium (MgO) of

0.1% and total boron (B) content of 8.6 mg kg^{-1} dw. Levels of heavy metals are all below the maximum legal limits allowed for this type of product (*information collected from the label*).

Table 5.1 Characterization of soils and fertilizer, regarding percentage of organic carbon (OC), clay, silt and sand.

Soil	%OC	Clay [%] (0-2 µm)	Silt [%] (2-63 µm)	Sand [%] (63-2000 µm)	Soil texture
Agricultural (A1)	1.7	2.4	33.7	63.9	Sandy loam
Agricultural (A2)	1.5	4.8	64.4	30.7	Silt loam
Beach (B)	0.8	1.0	11.8	87.3	Loamy sand
Garden (G)	8.1	0.6	9.7	89.7	Sand
Industrial (I)	1.1	1.0	12.1	87.0	Loamy sand
School yard (SY)	14.7	0.9	12.2	86.9	Loamy sand
Fertilizer (F)	42.0	-	-	-	-

In order to assess the effect of a sewage sludge-based fertilizer in a soil, preliminary experiments were carried out, applying 5% of sewage sludge-based fertilized (w/w) to the agricultural soil A2. The sewage sludge-based fertilizer was mixed uniformly with soil and pots were filled with this mixture. Samples were collected for analyses after four days of initial mixing. These assays were performed in six independent pots and an extra pot was prepared with unamended soil A2 as control. The fertilizer was analysed by the method proposed by Ramos et al. (2019), while the amended-soil was analysed using the methodology developed in this study.

Considering an efficient mix of the fertilizer with the agricultural soil, it is possible to estimate the maximum expected concentration accumulated of the target compounds in the treated soil (worst-case scenario), according to Eq. 1:

$$Maximum\ concentration_{\ i} = C_{soil\ i} \times f_{soil} + C_{fertilizer\ i} \times f_{fertilizer} \qquad \text{(Eq. 1)}$$

where *i* is the target compound, $C_{soil\ i}$ is the concentration of the target in the soil (ng g^{-1} dw), $C_{fertilizer\ i}$ is the concentration of the target compound in the fertilizer (ng g^{-1} dw), f_{soil} is the fraction of agricultural soil used in the experiment (w/w) and $f_{fertilizer}$ is fraction of fertilizer (w/w).

5.2.4 QuEChERS approach

The methodology optimized and validated was based on that proposed by Pang et al. (2016) to study the extraction of four herbicides from soil. Briefly, 5 g of previously air-dried and sieved soil (d < 2 mm) were put into a 50 mL polypropylene tube with conical bottom and then, 0.25 ng of surrogate compounds (MX-d15, AHTN-d3 and 4MBC-d4) was added. After vortexing, samples were kept at 4 ^{o}C overnight. Then, 4 mL of ultrapure water was added and vortexed to completely mix with the soil. After that, 10 mL of Ac/Hex (1:1) was added, vortexed for 1 min and then the tube was placed in an ultrasound bath (420 W) for 15 minutes (J.P. Selecta, Barcelona, Spain). Afterwards, 6 g of $MgSO_4$ and 1.5 g of NaCl were added. Samples were vortexed again for 1 min and then, the organic supernatant was separated by 15 min of centrifugation at 2670 x *g*. The organic phase was added to a dispersive solid-phase (d-SPE) mixture containing 3 g of $MgSO_4$ and 300 mg of C_{18}. It was then vortexed for 1 min and centrifuged once again for 15 min. The supernatant was filtered with a 0.2 µm PTFE syringe filter to a 15 mL amber vial, completely evaporated under a N_2 stream and resuspended in 1 mL of Hex. The sample was transferred to an amber chromatographic vial for further instrumental analysis.

5.2.5 GC-MS/MS analysis

Analyses were performed in a GC–MS/MS system from Bruker (Massachusetts, EUA), using multiple reaction monitoring (MRM) mode. A J&W CP-Sil 8 CB capillary column (50 m x 0.25 mm I.D. x 0.12 µm) from Agilent Technologies (Santa Clara, California, EUA) was used and the chromatographic separation was achieved using the following temperature program: 70 ^{o}C for 1 min, then raised at 25 ^{o}C min^{-1} to 180 ^{o}C, then a second ramp was applied at 10 ^{o}C min^{-1} to 240 ^{o}C and finally the temperature raised at a rate of 25 ^{o}C min^{-1} until 300 ^{o}C, holding for 5 min. The temperature of the injector was set at 280 ^{o}C, as well as the ion source, while the temperature of the transfer line at 270 ^{o}C. 2 µL of sample were injected in splitless mode, the electron energy was set at 70 eV and the filament current at 40 µA. The collision gas was ultra-pure argon at 2.00 mTorr. The

MRM transitions and collision energies, optimized for each compound, are presented in Table S4.1 (Annex 4).

5.2.6 Quality Control and Assurance (QC/QA)

UVFs and SMCs are incorporated in most personal care and household products. Therefore, safety measures need to be taken to avoid external sample contamination. In the laboratory, no scented products were allowed for washing purposes and analysts could not use scented toiletries. To guarantee the proper decontamination of the material, the glass calibrated material was previously rinsed with an appropriated solvent and the other glass material was subjected to 1 hour heating at 400 ^{o}C. Laboratory blanks were performed in each sample batch and were used to implement appropriate corrections to the results. Chromatographic blanks were also performed, but no memory effects or system contamination were detected.

5.3. Results and discussion

5.3.1 Extraction and clean-up optimization

The extraction of compounds from complex matrices, such as industrial or amended soils, is seen as a major challenge because of the wide variety of compounds and high organic matter content that these matrices may contain, making the analysis difficult and requiring time-consuming cleaning procedures. QuEChERS methodology emerges as a possible solution. This is a technique that combines extraction with clean-up, it is considered an environmentally friendly methodology (due to the small amount of solvent used and with low toxicity) and it is fast and non-labour intensive. Since no QuEChERS methodologies were reported for SMCs and UVFs extraction from soils, the methodology proposed by Pang et al. (2016) and developed for the extraction of pesticides was used as a starting point.

In that study, 5 g of homogenized soil was extracted in an ultrasonic bath with a mixture of ACN, acetic acid and water. In the new proposed approach, 4 mL of ultrapure water was added to the soil sample at the beginning of the process, allowing soil particles to

be hydrated more easily. This is crucial to allow greater penetration of the extraction solvent, which should be added later. SMCs and UVFs are compounds structurally different from the pesticides, therefore, the addition step of acetic acid was eliminated, and several extraction solvents were tested: ACN, Ac, Ac/Hex (1:1, v/v), EA and EA/Hex (1:1, v/v). Due to the water addition, NaCl and $MgSO_4$ were added in the first step of the QuEChERS methodology. The NaCl was used to promote a salting-out effect, while $MgSO_4$ was added to remove the aqueous phase by hydration, promoting the partitioning of the target analytes into the organic layer. After centrifugation, instead of a fraction of the supernatant being transferred to the tube containing the sorbents for the dispersive solid-phase extraction (d-SPE), all supernatant volume was transferred in order to increase the mass of compounds present in the final extract. In the d-SPE, $MgSO_4$ and C_{18} were used and their quantity set based on the volume increase of supernatant. Finally, the samples were evaporated to dryness and reconstituted in 1 mL of Hex before analysis.

The main results from the extraction solvent tests are presented in Figure 5.1. Recoveries obtained from samples extracted with ACN ranged between 63 and 138%, with relative standard deviations (RSD) below 7%. The solvents EA and EA/Hex (1:1) showed lower recoveries for exaltolide (EXA; 48% and 21%, respectively) and for all UVFs in general (around 70%), with RSD values between 1 and 26%. Ac and Ac/Hex (1:1) presented the best results in terms of recoveries (73-119% and 85-129%, respectively) and RSD values (<8%). Combining this information with chromatographic issues (peak resolution, presence of other interfering contaminants, which hinder chromatographic quantification), the Ac/Hex (1:1) proved to be the best solvent for extracting SMCs and UVs. An example of chromatograms of blanks, soils, spiked soils and standard is presented in Annex 4 (Figures S4.1 and S4.2).

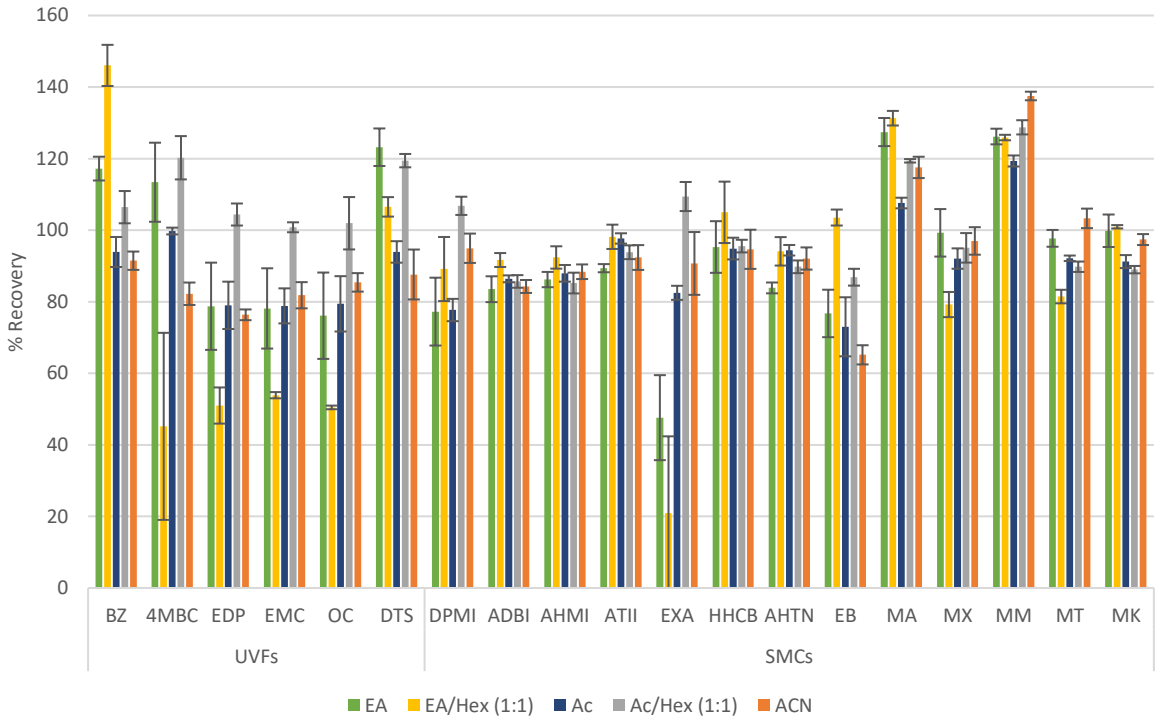


Figure 5.1 Evaluation of the % Recovery of UVFs and SMCs from soils using different extraction solvents (EA; EA/Hex (1:1); Ac; Ac/Hex (1:1); ACN).

5.3.2 Method Validation

5.3.2.1 Linearity, Precision and Accuracy

Results concerning the method validation are shown in Table 5.2. Calibration curves were built injecting directly calibration standards prepared in Hex at concentrations ranging from 1 to 1000 µg L^{-1}. All target compounds showed a linear behaviour within the studied range, with R^2 values greater than 0.996. Instrumental limits of detection (ILODs) and quantification (ILOQs) were calculated based on a signal-to-noise ratio (S/N) of 3 and 10, respectively. ILODs varied between 0.01 pg (MK) to 5.00 pg (MT). Also, the method limits of detection (MDLs) were assessed by the injection of spiked soil samples and vary between 0.01 (ADBI) to 10.00 ng g^{-1} dw (EXA). These values are generally in the same order of magnitude than those reported in the literature, although there is not much information available for SMCs (0.01 - 1.58 ng g^{-1} dw) (Aguirre et al., 2014; Chen et al., 2014) and none for UVFs.

The precision was assessed either by inter- or intra-day precision assays (relative standard deviation, %RSD) at different spiking levels (10, 50 and 200 μg g^{-1} dw), in triplicate. The proposed methodology can be considered precise, since the RSD values vary between 1 and 4%, which are quite acceptable considering this type of complex matrix. Accuracy was measured by recovery tests, using three replicate spiked samples at the same spiking levels mentioned before (10, 50 and 200 μg g^{-1} dw). Recoveries vary from 81±2% (ATII and AHTN) to 122±4% (MM) and are analogous to those found in the literature, using more time-consuming and expensive techniques (Aguirre et al., 2014; Chen et al., 2014; Wang et al., 2013). The surrogates presented recoveries of 87±10% for 4MBC-d4, 64±5% for AHTN-d3 and 78±5% MX-d15. All data found in the literature on UVFs are related to compounds not studied in this paper, such as salicylates, benzophenones (BP-1, -2, -3, -6, -8), homosalates and benzotriazoles (Albero et al., 2012; Camino-Sánchez et al., 2016; Lai et al., 2014; Sánchez-Brunete et al., 2011). This means that the information presented in this paper is a novelty for the scientific community. To the authors' best knowledge, the proposed method is the first one that can be applied for the simultaneously determination of polycyclic, macrocyclic and nitro musks and UVFs from soils.

Table 5.2 Main validation parameters for the QuEChERS-GC–MS/MS methodology proposed.

Compounds	Linearity Range (μg L^{-1})	R^2	ILOD (pg)	ILOQ (pg)	MDL (μg g^{-1} dw)	MQL (μg g^{-1} dw)	Inter-day precision (%RSD)			Accuracy ± Intra-day precision (%mean recovery ± RSD)		
							10 μg g^{-1} dw	50 μg g^{-1} dw	200 μg g^{-1} dw	10 μg g^{-1} dw	50 μg g^{-1} dw	200 μg g^{-1} dw
BZ	1 - 1000	0.996	0.27	0.91	0.09	0.31	3	2	1	100±4	106±2	102±1
4MBC	1 - 1000	1.000	0.68	2.27	1.16	3.87	1	2	1	120±3	119±1	119±2
EDP	1 - 1000	1.000	0.60	2.00	0.15	0.49	1	1	1	101±3	102±2	106±3
EMC	1 - 1000	1.000	0.05	0.17	0.05	0.15	3	1	1	94±3	98±2	94±1
OC	1 - 1000	1.000	0.29	0.98	0.21	0.69	2	1	2	96±6	101±2	97±1
DTS	1 - 1000	1.000	1.50	5.00	0.02	0.08	3	2	1	119±3	119±2	114±2
DPMI	1 - 1000	0.999	0.75	2.50	0.12	0.41	3	1	2	109±4	105±6	98±1
ADBI	1 - 1000	1.000	0.04	0.15	0.01	0.02	1	3	1	97±2	83±1	86±1
AHMI	1 - 1000	1.000	0.54	1.79	0.02	0.06	3	3	1	113±2	84±2	89±4
ATII	1 - 1000	1.000	1.00	3.33	0.26	0.88	5	3	3	109±5	94±3	81±1
EXA	1 - 1000	0.999	3.75	12.50	10.00	33.34	2	2	1	106±3	105±5	107±2
HHCB	1 - 1000	1.000	0.58	0.10	0.10	0.34	1	4	1	98±2	94±2	93±2
AHTN	1 - 1000	1.000	0.33	1.11	0.08	0.27	1	2	3	97±5	89±2	81±2
EB	1 - 1000	1.000	3.00	10.00	3.85	12.83	2	4	2	84±1	87±2	95±2
MA	1 - 1000	1.000	1.88	6.25	0.13	0.42	3	4	1	101±2	110±2	106±3
MX	1 - 1000	1.000	2.14	7.14	0.15	0.50	1	4	1	94±3	95±3	93±4
MM	1 - 1000	1.000	1.14	3.79	0.06	0.19	2	2	1	122±4	118±2	120±2
MT	1 - 1000	1.000	5.00	16.67	0.10	0.32	2	2	2	91±5	86±1	89±2
MK	1 - 1000	1.000	0.01	0.03	0.02	0.05	2	3	2	83±3	86±2	83±1

5.3.3 Applicability studies

The suitability of the developed method was assessed by the study of the occurrence of the target compounds in different soil samples, in a sewage sludge-based fertilizer and in an amended-soil. Each sample was processed in triplicate as described above. The concentrations of the studied contaminants are summarized in Table 5.3.

Table 5.3 . Contaminants detected in different soils, fertilizer and amended-soil. Mean results are expressed in ng g^{-1} dw (n=3). The prediction of Maximum Expected Concentration (M.E.C.) in the amended-soil is also presented.

UVFs/ SMCs	A1	A2	B	G	I	SY	F	AS	M.E.C.
BZ	5.59±0.04	2.8±0.3	4.8±0.2	158±3	20±1	19±1	205±13	8.7±0.3	30
4MBC	nd	<MQL	Nd	<MQL	nd	12.8±0.8	49±2	13.8±0.4	7
EDP	4.23±0.06	nd	4.8±0.2	nd	4.23±0.06	4.40±0.06	nd	nd	-
EMC	nd	4.09±0.02	Nd	1.8±0.1	1.59±0.01	6±3	1.7±0.6	4.31±0.01	2
OC	nd	5.21±0.01	6±1	3.3±0.5	3±1	137±8	1519±22	84±2	212
DTS	nd	nd	Nd	nd	nd	7±1	1629±53	56±2	225
DPMI	nd	nd	Nd	nd	nd	Nd	58±6	1.36±0.05	8
ADBI	nd	nd	Nd	0.46±0.03	nd	Nd	33±1	1.62±0.01	5
AHMI	nd	0.92±0.01	Nd	nd	nd	Nd	6.8±0.1	1.04±0.01	1
ATII	nd	nd	Nd	9.0±0.8	nd	Nd	nd	2.52±0.02	-
EXA	nd	<MQL	Nd	nd	nd	Nd	nd	nd	-
HHCB	nd	5.3±0.2	4.8±0.8	<MQL	<MQL	6.8±0.9	17,140±594	341±5	2367
AHTN	nd	2.2±0.1	Nd	nd	nd	3.7±0.4	4416±198	88±1	610
EB	nd	nd	<MQL	nd	<MQL	<MQL	nd	nd	-
MA	nd	nd	nd	nd	nd	Nd	nd	nd	-
MX	nd	nd	nd	nd	nd	Nd	nd	nd	-
MM	nd	nd	nd	nd	nd	Nd	nd	nd	-
MT	nd	nd	nd	nd	nd	Nd	nd	nd	-
MK	nd	nd	nd	nd	nd	Nd	15.07±0.04	nd	2
Total per site	9.8±0.1	20.5±0.4	20±1	172±3	29±1	196±9	25,073±890	602±11	3,469

Obs.: A1 and A2 (Agricultural), B (Beach), G (Garden), I (Industrial), SY (School yard); F (Fertilizer); AS (Amended-soil); M.E.C. (Maximum Expected Concentration in the amended-soil); <MQL (below method quantification limit): nd – not detected.

The soil presenting most compounds of all classes is the school yard (SY) soil, where 9 out of 19 compounds were detected. This soil also presented the highest total concentration of UVFs and SMCs, reaching a total of 196±9 ng g^{-1} dw. It was collected in an area where cleaning waters, containing residues of household products, are often disposed and students hang for long periods, which means that these PCPs may accumulate in this matrix (i.e. through particle deposition). Also, the garden soil (G) is one of the collected samples with a larger number of compounds and these were detected at high levels (total of 172±3 ng g^{-1} dw. It presented the higher amount of BZ, which may be explained by the utilization of plastic flowerpots and, for example,

agricultural chemicals. In fact, BZ may be used with different purposes. It is usually applied as an ultraviolet filter in sunscreens and cosmetics and UV blocker in plastic packaging, coatings and adhesive formulations. However, it can also be used as an aroma ingredient, a fragrance enhancer and fixative, but it also can be used in the production of insecticides and agricultural chemicals, some pharmaceuticals and laundry and household cleaning products (Working Group on the Evaluation of Carcinogenic Risk to Humans, 2013). All these applications lead to a greater likelihood of environmental contamination, since its availability is greater in a wide range of products and consequently its disposal. In this specific case, the use in agricultural chemicals may be a likely source of this contamination. In the beach soil (B), most compounds detected were UVFs, apart from the HHCB (a very common SMC), which could indicate than the main source of contamination is the use of sunscreens and other personal care products by people who go to the beach. In this case, the total concentration found reached 20±1 ng g^{-1} dw. The industrial soil (I) showed the presence of 4 UVFs (BZ, EDP, EMC and OC – total concentration of 29±1 ng g^{-1} dw). Although the industry is not related to the production of these compounds, it is located near the beach, which may explain the obtained results. In fact, the total levels found in I and B soil are similar. Among agricultural soils, A2 present the higher number of compounds and total UVFs and SMCs concentration (20.5±0.4 ng g^{-1} dw). This soil was collected in an agricultural area near a construction site. In addition, a music festival took place near the location where the sample A2 was collected, which can also help to explain the high levels found. On the other hand, the agricultural soil A1 has been fallow, which also may explain the lowest levels found.

As previously mentioned, soil A2 has the highest percentage of clay (near 5%), with a range of particles varying between 0 and 2 μm (Table 5.1). Since one of the properties that leads to the compounds' adsorption to the soil is the amount of clay - smaller particles have a larger surface area (Drillia et al., 2005), it is normal that soil A2 may present a slightly higher adsorption capacity. Nevertheless, the soil SY has the highest percentage of organic carbon available (around 15%), followed by the soil G (around 8%), which is the main property to the compounds adsorption to soils (Drillia et al.,

2005). This may explain why these soils present the higher total concentration of the contaminants.

To the authors' best knowledge, no information regarding the UVFs detection in soils (at least the ones under scrutiny) is available in the literature, but only some information on SMCs can be found (Domínguez-Morueco et al., 2018). SMCs have been detected in industrial soils in concentrations ranging from 0.05 and 5.24 ng g^{-1} (Muller et al., 2006), in urban areas from not detected to 2.87 ng g^{-1} (Federle et al., 2014) and in agricultural soils in concentrations similar to this study ranging from <0.07- 7.22 ng g^{-1} (Zheng et al., 2019). The concentrations found in these studies are of the same order of magnitude as those found in the present study. Nevertheless in biosolid-amended soils, these compounds were found in higher concentrations, ranging from 2.4 to 67.5 ng g^{-1} and from 0.7 to 29.0 ng g^{-1} (HHCB and AHTN, respectively) (Chen et al., 2014) after repeated applications. MK was also detected (6.5 – 7.8 ng g^{-1}) (Aguirre et al., 2014).

In this study, sewage sludge-based fertilizer was applied to agricultural soil (A2). The fertilizer presented levels of the target compounds ranging from 2 to 17,140 ng g^{-1} dw. After analysis of the amended-soil, concentrations between 1 and 341 ng g^{-1} dw were found, which are higher than those found in the initial soil. By analysing the Maximum Expected Concentrations calculated by Eq.1, higher concentrations were found for HHCB (341 ng g^{-1}), AHTN (88 ng g^{-1}), OC (84 ng g^{-1}) and DTS (56 ng g^{-1}) in the amended-soil. However, in general, lower levels (80%) than expected were found. This may indicate that part of the target compounds may volatilize or biodegrade. Nevertheless, the presence of target compounds increased in the amended-soil for all compounds, especially for those whose concentration in the fertilizer was considerably high. Therefore, it is possible to confirm an accumulation potential of the target compounds in the soil. Accumulation in soils is somewhat expected given their lipophilic nature and their high log K_{oc}, which means that they are strongly adsorbed onto soil and organic matter.

The presence of these compounds in the soils (amended or not) may be considered an environmental and public health problem. Thus, it is essential to create or update the existing regulations, regarding this kind of emerging pollutants and to develop alternative management systems.

5.4. Conclusions

A QuEChERS methodology was successfully validated for the extraction of 13 SMCs and 6 UVFs from soils. The analysis was performed by GC-MS/MS. This methodology conducted to good recoveries (81-122%) and precision (RSD<10%) and low detection limits (ILOD: 0.01- 5.00 pg; MDL: 0.01-10.0 ng g^{-1} dw). The analysis of seven soil samples confirmed the applicability of this method, showing that soils from different areas present distinct levels of UVFs and SMCs. BZ was the compound detected at higher concentrations (2.8-158 ng g^{-1} dw). The study in which sewage sludge-based fertilizer was applied to soil (amended-soil) revealed concentrations from 1.04 (AHMI) to 341 ng g^{-1} dw (HHCB), showing that there is an accumulation potential of the target contaminants. This study proves that an environmentally friendly methodology as QuEChERS can be easily implemented in all laboratories to routinely analyse UVFs and SMCs in soils.

Declaration of interest

The authors declare no competing interests.

Supporting Information

All the supporting information can be found in Annex 4.

Acknowledgements

This work was the result of the project: (i) Project UID/EQU/00511/2019 - Laboratory for Process Engineering, Environment, Biotechnology and Energy – LEPABE funded by national funds through FCT/MCTES (PIDDAC); (ii) Project POCI-01-0145-FEDER-029425 - AGRONAUT - Agronomic Impact of Sludge Amendment Using a Comprehensive Exposure Viewpoint, funded by FEDER funds through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI) and by national funds

(PIDDAC) through FCT/MCTES; (iii) Project “LEPABE-2-ECO-INNOVATION” – NORTE-01-0145-FEDER-000005, funded by Norte Portugal Regional Operational Programme (NORTE 2020), under PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF); (iv) Doctoral Grant SFRH/BD/110831/2015 – Sara Ramos.

References

Aguirre, J., Bizkarguenaga, E., Iparraguirre, A., Fernández, L.Á., Zuloaga, O., Prieto, A., 2014. Development of stir-bar sorptive extraction-thermal desorption-gas chromatography-mass spectrometry for the analysis of musks in vegetables and amended soils. Anal. Chim. Acta 812, 74–82.

Albero, B., Sánchez-Brunete, C., Miguel, E., Pérez, R.A., Tadeo, J.L., 2012. Determination of selected organic contaminants in soil by pressurized liquid extraction and gas chromatography tandem mass spectrometry with in situ derivatization. J. Chromatogr. A 1248, 9–17.

Bragança, I., Plácido, A., Paíga, P., Domingues, V.F., Delerue-Matos, C., 2012. QuEChERS: A new sample preparation approach for the determination of ibuprofen and its metabolites in soils. Sci. Total Environ. 433, 281–289.

Camino-Sánchez, F.J., Zafra-Gómez, A., Dorival-García, N., Juárez-Jiménez, B., Vílchez, J.L., 2016. Determination of selected parabens, benzophenones, triclosan and triclocarban in agricultural soils after and before treatment with compost from sewage sludge: A lixiviation study. Talanta 150, 415–24.

Chase, D.A., Karnjanapiboonwong, A., Fang, Y., Cobb, G.P., Morse, A.N., Anderson, T.A., 2012. Occurrence of synthetic musk fragrances in effluent and non-effluent impacted environments. Sci. Total Environ. 416, 253–260.

Chefetz, B., Mualem, T., Ben-Ari, J., 2008. Sorption and mobility of pharmaceutical compounds in soil irrigated with reclaimed wastewater. Chemosphere 73, 1335–1343.

Chen, F., Ying, G.G., Ma, Y.B., Chen, Z.F., Lai, H.J., Peng, F.J., 2014. Field dissipation and risk assessment of typical personal care products TCC, TCS, AHTN and HHCB in biosolid-amended soils. Sci. Total Environ. 470–471, 1078–1086.

Clarke, R., Healy, M.G., Fenton, O., Cummins, E., 2016. A quantitative risk ranking model to evaluate emerging organic contaminants in biosolid amended land and potential transport to drinking water. Hum. Ecol. Risk Assess. 22, 958–990.

Dodgen, L.K., Li, J., Wu, X., Lu, Z., Gan, J.J., 2014. Transformation and removal pathways of four common PPCP/EDCs in soil. Environ. Pollut. 193, 29–36.

Domínguez-Morueco, N., Carvalho, M., Sierra, J., Schuhmacher, M., Luis, J., Ratola, N., Nadal, M., 2018. Multi-component determination of atmospheric semi-volatile organic compounds in soils and vegetation from Tarragona County ,. Sci. Total Environ. 631–632, 1138–1152.

Drillia, P., Stamatelatou, K., Lyberatos, G., 2005. Fate and mobility of pharmaceuticals in solid matrices. Chemosphere 60, 1034–1044.

Federle, T., Sun, P., Dyer, S., Kiel, B., 2014. Probabilistic assessment of environmental exposure to the polycyclic musk, HHCB and associated risks in wastewater treatment plant mixing zones and sludge amended soils in the United States. Sci. Total Environ.

Homem, V., Silva, A., Ratola, N., Santos, L., Alves, A., 2015. Long lasting perfume e A review of synthetic musks in WWTPs. J. Environ. Manage. 149, 168–192.

Kinney, C.A., Furlong, E.T., Kolpin, D.W., Burkhardt, M.R., Zaugg, S.D., Werner, S.L., Bossio, J.P., Benotti, M.J., 2008. Bioaccumulation of pharmaceuticals and other anthropogenic waste indicators in earthworms from agricultural soil amended with biosolid or swine manure. Environ. Sci. Technol. 42, 1863–1870.

Lai, H.J., Ying, G.G., Ma, Y.B., Chen, Z.F., Chen, F., Liu, Y.S., 2014. Occurrence and dissipation of benzotriazoles and benzotriazole ultraviolet stabilizers in biosolid-amended soils. Environ. Toxicol. Chem. 33, 761–767.

Muller, J., Boehmer, W., Litz, N.T., 2006. Occurrence of polycyclic musks in sewage sludge and their behaviour in soils and plants: Part 1: Behaviour of Polycyclic Musks in Sewage Sludge of Different Treatment Plants in Summer and Winter. J. Soils Sediments 6, 231–235.

Pang, N., Wang, T., Hu, J., 2016. Method validation and dissipation kinetics of four herbicides in maize and soil using QuEChERS sample preparation and liquid chromatography tandem mass spectrometry. Food Chem. 190, 793–800.

Perestrelo, R., Silva, P., Porto-Figueira, P., Pereira, J.A.M., Silva, C., Medina, S., Câmara, J.S., 2019. QuEChERS - fundamentals, relevant improvements, applications and future trends. Anal. Chim. Acta. (in press)

Qin, Q., Chen, X., Zhuang, J., 2017. The surface-pore integrated effect of soil organic matter on retention and transport of pharmaceuticals and personal care products in soils. Sci. Total Environ. 599–600, 42–49.

Ramos, S., Homem, V., Alves, A., Santos, L., 2016. A review of organic UV-filters in wastewater treatment plants. Environ. Int. 86, 24–44.

Ramos, S., Homem, V., Santos, L., 2019. Development and optimization of a QuEChERS-GC–MS/MS methodology to analyse ultraviolet-filters and synthetic musks in sewage sludge. Sci. Total Environ. 651, 2606–2614.

Sánchez-Brunete, C., Miguel, E., Albero, B., Tadeo, J.L., 2011. Analysis of salicylate and benzophenone-type UV filters in soils and sediments by simultaneous extraction cleanup and gas chromatography–mass spectrometry. J. Chromatogr. A 1218, 4291–4298.

Sharma, B., Sarkar, A., Singh, P., Singh, R.P., 2017. Agricultural utilization of biosolids: A review on potential effects on soil and plant grown. Waste Manag. 64, 117–132.

Tolls, J., 2001. Sorption of veterinary pharmaceuticals in soils: A review. Environ. Sci. Technol. 35, 3397–3406.

Wang, M., Peng, C., Chen, W., Markert, B., 2013. Ecological risks of polycyclic musk in soils irrigated with reclaimed municipal wastewater. Ecotoxicol. Environ. Saf. 97, 242–247.

Witorsch, R.J., Thomas, J.A., 2010. Personal care products and endocrine disruption: A critical review of the literature. Crit. Rev. Toxicol. 40, 1–30.

Working Group on the Evaluation of Carcinogenic Risk to Humans, I., 2013. Some Chemicals Present in Industrial and Consumer Products, Food and Drinking-Water: IARC

Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 101.) BENZOPHENONE [WWW Document]. Int. Agency Res. Cancer. URL https://www.ncbi.nlm.nih.gov/books/NBK373188/ (accessed 4.24.19).

Xu, J., Wu, L., Chang, A.C., 2009. Degradation and adsorption of selected pharmaceuticals and personal care products (PPCPs) in agricultural soils. Chemosphere 77, 1299–1305.

Yang, J.J., Metcalfe, C.D., 2006. Fate of synthetic musks in a domestic wastewater treatment plant and in an agricultural field amended with biosolids. Sci. Total Environ. 363, 149–165.

Zheng, M., Hu, S., Liu, X., Wang, W., Yin, X., Zheng, L., Wang, L., Lou, Y., 2019. Levels and distribution of synthetic musks in farmland soils from the Three Northeast Provinces of China. Ecotoxicol. Environ. Saf. 172, 303–307.

Chapter 6. Analytical methodology to screen UV-filters and synthetic musk compounds in market tomatoes

Sara Ramos, Vera Homem, Lúcia Santos

Chemosphere, 238 (2020)

Abstract

A Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) methodology followed by gas chromatography-tandem mass spectrometry (GC-MS/MS) analysis was developed to extract thirteen synthetic musk compounds (SMCs: cashmeran, celestolide, phantolide, traseolide, galaxolide, tonalide, musk ambrette, musk xylene, musk ketone, musk tibetene, musk moskene, ethylene brassylate and exaltolide) and six ultraviolet-filters (UVFs: 2-ethylhexyl 4-dimethylaminobenzoate, 3-(40-methylbenzylidene) camphor, 2-ethylhexyl 4-methoxycinnamate, 2-ethylhexyl 2-cyano-3,3-diphenylacrylate, benzophenone and drometrizole trisiloxane) from tomatoes. The proposed methodology was optimized: 2 g of freeze-dried tomato was extracted with 4 mL of water and 10 mL of ethyl acetate, adding 6 g of $MgSO_4$ and 1.5 g of NaCl, then a dispersive solid-phase extraction was performed using 3 g of $MgSO_4$, 300 mg of primary-secondary amino adsorbent (PSA) and 300 mg of octadecyl-silica (C_{18}). Validation delivered recoveries between 81 (celestolide) and 119% (musk tibetene), with relative standard deviations <10%. The instrumental limit of detection varied from 0.02 (2-ethylhexyl 4-methoxycinnamate) to 3.00 pg (exaltolide and musk xylene). Regarding the method quantification limits, it ranged between 0.4 (celestolide) and 47.9 ng g^{-1} dw (exaltolide). The method was applied to different varieties of tomatoes (*Solanum lycopersicum*), revealing UVFs and SMCs between 1 and 210 ng g^{-1} dw. Higher concentrations were found for benzophenone (29-210 ng g^{-1} dw) and galaxolide (9-53 ng g^{-1} dw). The risk associated to the ingestion of contaminated tomatoes has also been estimated, showing that a potential health risk is unlikely.

Keywords: UV-Filters; Synthetic musk compounds; Tomatoes; QuEChERS; GC-MS/MS; Daily intake.

6.1. Introduction

In recent years, a growing concern has arisen in the scientific community with a new class of pollutants, the so-called “emerging pollutants”. They are massively used and the wastewater treatment plants (WWTPs) are ineffective in their removal, which leads to (bio)accumulation in the environment, with evidence of accumulation in the trophic chains in aquatic environments (Ramos et al., 2016; Sauvé and Desrosiers, 2014).

Regarding emerging pollutants, different classes are considered of concern, such as pesticides, pharmaceuticals, personal care products (PCPs), plasticizers, hormones, flame retardants, nanoparticles, perfluoroalkyl compounds, chlorinated paraffins and various trace elements, including radionuclides, etc. (Caldéron-Preciado et al., 2011; Sauvé and Desrosiers, 2014).

Among PCPs, there are two sub-classes of compounds that have been poorly studied but are largely used in several cosmetics, toiletries and household products as key ingredients in their formulation - ultraviolet-filters (UVFs) and synthetic musk compounds (SMCs). Due to their physicochemical properties, such as Log K_{ow}, they are considered lipophilic, (bio)accumulative and not biodegradable (Homem et al., 2015; Ramos et al., 2015; Zuloaga et al., 2012). Some of the compounds within these sub-classes are also known to have carcinogenic and endocrine disrupting activity, are human respiratory toxicant and cause dermal irritation (Burnett, 2008; ECHA, 2019).

These compounds can reach the environment either by direct contact or due to down-the-drain practices, reaching WWTPs, where they are not completely degraded. Thus, a fraction may be discharged into the rivers while another part may accumulate in the sludge. The sludge disposal may be another route of contamination due to its application in agricultural soils as fertilizers, given their high content of macro and micronutrients, such as N and P. However, this practice can introduce these pollutants into the food chain if crop uptake occurs. To study this behaviour, it is necessary to carry out plant uptake studies and, for this, it is essential to develop simple, expeditious and reliable analytical methodologies to determine the target pollutants in the studied matrix.

Notwithstanding that in recent years the analysis of UFVs and SMCs has gained more attention (Homem et al., 2015; Ramos et al., 2015), there is still a gap in the literature,

regarding analytical methodologies able to determine them in edible crops. In fact, this may be explained by the matrix complexity and the low concentrations expected in those samples. In fact, the low water solubility of many UVFs and the volatility of SMCs make their uptake via roots less important, which results in lower concentrations in plants. To overcome these issues, gas chromatography (GC) or liquid chromatography (LC) coupled to mass spectrometry (MS) should be the preferable instrumental methodologies used to quantify these compounds (Sabourin et al., 2012; Vallecillos et al., 2015).

As mentioned before, less data is available in the literature, regarding methodologies to extract and quantify SMCs in edible crops (vegetables and fruits) and no information is available for UVFs. Sample preparation usually consists in chopping the sample and mixing with several salts/buffers and sorbents like Na_2SO_4, NaCl, citrates, Florisil, etc., performing a matrix solid-phase dispersion (MSPD) (Calderon-Preciado et al., 2011; Hurtado et al., 2016). Samples may also be frozen and sonicated or freeze-dried. Regarding extraction and clean-up methodologies, pressurized liquid extraction (PLE), either alone (Hurtado et al., 2016) or combined with solid-phase extraction (SPE) (Calderón-Preciado et al., 2012, 2009; Calderón-Preciado et al., 2011; Calderón-Preciado et al., 2011), was the more frequently used analytical method to determine SMCs in crops. Alternative employed methodologies were stir-bar sorptive extraction (SBSE) (Aguirre et al., 2014), solid-liquid extraction (SLE) (Litz et al., 2007), a Quick, Easy, Cheap, Effective, Rugged, and Safe method (QuEChERS) (Macherius et al., 2012) and sonication by ultraturrex followed by ultrasound bath and SPE (Fussell et al., 2013). Of all the presented methodologies, the QuEChERS technique seemed to be the most appealing, once it is as a rather “green” analytical approach, combining the extraction (using low amounts of solvents) with the clean-up step (which also employs low amounts of sorbents). Moreover, this procedure is rather quick (hence the name) when compared to conventional techniques, such as SPE or Soxhlet, and consequently produces smaller amount of waste (Perestrelo et al., 2019). Due to its simplicity and easiness to perform, a person with little training or technical skill can perform this method. Moreover, few equipment is needed and cost with reagents is low. This methodology has also the advantage of potentially high recoveries and low detection limits (Bragança et al., 2012).

Although this technique has mainly been used for the analysis of pesticides in vegetables and fruits (Li et al., 2014), more recent studies suggest the use of QuEChERS for the determination of other environmentally relevant compounds, such as pharmaceuticals (Cerqueira et al., 2014; Kim et al., 2019; Zhang et al., 2019) and PCPs (e.g. UVFs and SMCs) (Macherius et al., 2012).

Thus, the purpose of this work was to develop and validate a sensitive, reliable and fast multiresidue methodology based on a QuEChERS/GC-MS/MS for the determination of six UVFs and thirteen SMCs in tomatoes. Subsequently, the developed methodology was used to analyse several tomato samples from supermarkets. Based on the obtained results, primary exposure and risk of human consumption was estimated. Tomatoes were chosen for this study based on European and national production and consumption habits, as well as due to their frequent use in plant uptake trials as they may grow all year in agricultural fields or greenhouses.

6.2. Materials and methods

6.2.1. Standards and reagents

The description of standards preparation has been described in a previous publication (Ramos et al., 2019). Briefly, the compounds under study were SMCs, divided in: polycyclic musks – cashmeran (DPMI), celestolide (ADBI), phantolide (AHMI), traseolide (ATII), tonalide (AHTN) and galaxolide (HHCB) from LGC Standards (Barcelona, Spain); nitro musks - tibetene (MT) and moskene (MM), from LGC Standards, ambrette (MA) and ketone (MK) from Dr. Ehrenstorfer (Augsburg, Germany) and xylene (MX) from Sigma- Aldrich (St. Louis, MO, USA); macrocyclic musks - exaltolide (EXA) and ethylene brassylate (EB) from Sigma-Aldrich. Surrogate standards used were musk xylene-d15 (MX-d15) and tonalide-d3 (AHTN-d3) from Dr. Ehrenstorfer (Augsburg, Germany) and (±)-3-(-4-methylbenzylidene-d4) camphor (4MBC-d4) from CDN Isotopes (Pointe-Claire, Quebec, Canada). The UVFs in this study were: 2-ethylhexyl 4-dimethylaminobenzoate (EDP), 3-(40-methylbenzylidene) camphor (4MBC) from Alfa Aesar (Karlsruhe, Germany), 2-ethylhexyl 4-methoxycinnamate (EMC), 2-ethylhexyl 2-cyano-3,3-

diphenylacrylate (OC), benzophenone (BZ) from Sigma-Aldrich (St. Louis, MO, USA) and drometrizole trisiloxane (DTS) from Fluka (Saint Louis, MO, USA).

To optimize the extraction method, several solvents (all analytical grade) and sorbents were used: acetone (Ac), acetonitrile (ACN), hexane (Hex) and ethyl acetate (EA) from VWR BDH Prolabo (Fontenay-sous-Bois, France), sodium chloride (NaCl), ultrapure water from Merck (Darmstadt, Germany), anhydrous magnesium sulphate ($MgSO_4$), dried at 450 ^oC for 12 h, from Panreac AppliChem (Barcelona, Spain), primary and secondary amine exchange bonded silica sorbent (PSA), octadecyl-silica (C_{18}) and Supelclean Envicarb from Supelco (Bellefonte, PA, USA).

Stock solutions of individual compounds were prepared in both Hex and ACN in concentrations between 1 and 5 g L^{-1}. Stock solution in ACN were used to prepare spike mix solutions in concentrations of 15, 75 and 150 µg L^{-1} in ACN and the ones in Hex to prepare the analytical control and calibration standards (1 - 1000 µg L^{-1}). All standards were preserved at -20 ^oC and protected from the light.

6.2.2. Sample collection and pre-treatment

Six different varieties of tomato samples were purchased in 2018 at local supermarkets (Table S5.1 in Annex 5). Samples were packed in polypropylene bags and washed with tap water. Then, samples were cut in small pieces and chopped in a blender (Ergo Mix, Bosch, 600 W). After this process, samples were frozen and freeze-dried in a Virtis Benchtop K Freeze Dryer (SP Scientific, New York, USA) for 3 days (until constant weight). Water content each variety was determined by this procedure.

6.2.3. Sample extraction

The QuEChERS methodology was chosen to extract the samples, which were analysed in triplicate. Briefly, 2 g of freeze-dried sample was placed in a 50 mL Falcon tube (polypropylene tube with conical bottom), and 125 ng of surrogate solution (MX-d15, AHTN-d3 and 4MBC-d4) was added. Then, 4 mL of ultrapure water was mixed with the sample and vortexed for 1 min. Ten mL of EA (chosen solvent) was also added to the tube, and vortexed again for 1 min. After this, the mixture was ultrasonicated for 15 min

at room temperature in a 420 W ultrasonic bath (J.P. Selecta, Barcelona, Spain). Then, 6 g of $MgSO_4$ and 1.5 g of NaCl (used to adjust the ionic strength) were added to the sample tube. The extract was rapidly vortexed for 1 min and then, the organic phase was separated by centrifugation at 2670 x g for 15 min. The organic phase was transferred to a conical polypropylene tube containing 3 g of $MgSO_4$, 300 mg of PSA and 300 mg of C_{18}. The tube was vortexed again for 1 min and centrifuged in the same conditions mentioned above. The supernatant was carefully removed to a 12 mL amber vial, evaporated to dryness under a gentle N_2 stream and solubilized in 500 µL of Hex. In the end, the final extract was shifted to an amber vial for instrumental analysis.

6.2.4. GC-MS/MS analysis

The description of the instrumental analysis is detailed in Ramos et al. (2019). Briefly, the analysis was conducted on a gas chromatograph coupled to a triple quadrupole mass spectrometer from Bruker (Massachusetts, EUA). Used column was a J&W CP-Sil 8 CB capillary column (50m x 0.25mm I.D. x 0.12 µm) from Agilent Technologies (Santa Clara, California, EUA). Helium (99.999%) was used as a carrier at a constant flowrate of 1.0 mL min^{-1}. Injector was set to 280 ^oC and 2 µL of sample were injected in splitless mode. GC oven temperature program started at 70 ^oC for 1 min, raised to 180 ^oC at 25 ^oC min^{-1}, then 10 ^oC min^{-1} until 240 ^oC and finally 25 ^oC min^{-1} until 300 ^oC (for 5 min). Further parameters are listed in Table S5.2 in Annex 5.

6.2.5. Validation procedure

The analytical method was validated using the procedure suggested by Eurachem (Magnusson and Ornemark, 2014). Therefore, linearity, limits of detection and quantification, precision, accuracy and the global uncertainty were assessed.

Linearity was evaluated by the direct injection of calibration standards prepared in Hex, containing all target compounds of UVFs and SMCs at different levels (1 - 1000 µg L^{-1}). Response factors were calculated ($RF=Area_{compound}/Area_{surrogate\ standard}$), using 4MBC-d4, AHTN-d3 and MX-d15 as surrogate standard (250 µg L^{-1}), and then correlated to standards concentrations.

Both instrumental and method detection/quantification limits (IDLs/IQLs, MDLs/MQLs) were calculated. The IDLs were determined by the injection of calibration standards, while MDLs were determined through spiked tomato samples. Both limits were calculated based on a signal-to-noise ratio (S/N) of 3 and 10, respectively. Nevertheless, the MDLs also consider the standard deviation of the replicates of the spikes and a t-student factor.

The method's accuracy was assessed by performing recovery tests, extracting tomato samples spiked at three concentrations (4, 19 and 38 ng g^{-1} dw). The precision was assessed both by the repeatability and intermediate precision (intra-day and inter-day precision, respectively). The repeatability was evaluated by the relative standard deviation (%RSD) of three replicates at different levels of spike (4, 19 and 38 ng g^{-1} dw), while inter-day precision was determined in a similar way, for the same levels of spike, but in three different days.

6.2.6. Quality assurance and control (QA/QC)

UVFs and SMCs are incorporated in almost every personal care and household product. Therefore, preventive measures needed to be taken to avoid external sample contamination. In the laboratory, no scented products were allowed for cleaning purposes. Also, the analysts performing the experiments took extra precautions related to the daily routine care products they use. To guarantee proper decontamination of the material, calibrated glassware was rinsed with an appropriated solvent, other glassware was further subject to 1-h bakeout at 400 ^{o}C. Laboratory blanks (where no sample was used, but the procedure followed was the same) were performed and analysed in order to identify background levels of the target analytes and implement appropriate corrections to the results. Chromatographic column blanks were regularly performed, but no carry-over or system contamination was detected.

6.2.7. Exposure assessment and risk characterization

Based on the no observed adverse effect level (NOAEL) values, tolerable weekly intake (TWI) was established through application of an uncertainty factor, in this case a factor of 100, the most described one (factor used to adjust human variability and different

species). In fact, TWI for each compound was calculated, dividing the NOAEL values by 100 and then multiplying by 7 (to access the weekly intake). TWI is usually expressed in mg kg^{-1} bw $week^{-1}$, translated in the concentration of the target compound in mg per kg of sample multiplied by the body weight (bw) of the consumer (standard of 60 kg) and per week (Zarn et al., 2015). Whenever the calculated exposure levels exceed the established TWI, risk management mechanisms will have to be triggered (Barlow and Schlatter, 2010; Cunha et al., 2015).

6.3. Results and discussion

6.3.1. QuEChERS optimization procedure

Different parameters that affect QuEChERS extraction, such as extraction solvent, type and amount of sorbents were studied. The method was based on a work previously described by Macherius et al. (2012) to extract galaxolide, tonalide and triclosan from carrot, barley, and meadow fescue plants.

Preliminary tests were performed using 2 g of freeze-dried sample, which was extracted with 10 mL of EA/Ac (1:1, v/v). Resulting extracts were cleaned using 1.8 g of $MgSO_4$, 90 mg of Supelclean ENVI-Carb (graphitized carbon to remove pigments and nonpolar interferences) and 300 mg of PSA (removal of fatty acids, organic acids, and some polar pigments and sugars). Obtained results were not satisfactory (Figure 6.1 A), with low recoveries for most compounds and a huge matrix effect for 4MBC, OC, DPMI, HHCB and EXA (recoveries ≥ 200%). Since tests with Supelclean ENVI-Carb did not provide good results, a different procedure was chosen. To improve extraction performance, 4 mL of ultrapure water was added to the sample, followed by the addition of 10 mL of EA/Ac (1:1, v/v). Then, a drying agent (6 g of $MgSO_4$) and a salt (1.5 g of NaCl) were added. The clean-up procedure consisted in the addition of 3 g of $MgSO_4$ (to remove vestigial water) and 600 mg of PSA to the extract (Figure 6.1 A). Although a slight improvement was observed, a pronounced matrix effect for some compounds was still noticeable (e.g. BZ, 4MBC, DPMI, HHCB, EXA, EB). Therefore, the addition of a new sorbent in the clean-up step was tested. The adopted procedure was similar to that described in the previous test, but the dispersive solid-phase (d-SPE) step was performed using 3 g of $MgSO_4$, 300

mg of PSA and 300 mg of C_{18} (to remove lipids and nonpolar interferences). The main results are presented in Figure 6.1 B. The results were satisfactory, with recoveries varying between 83% (EXA) and 175% (DTS), with an average value of 107% and deviations below 10%.

A new set of experiments was performed to test the effect of the extraction solvent (EA, ACN and EA/Ac (1:1, v/v)) and the final reconstitution volume (500 µL and 1000 µL). In fact, not only the type of extraction solvent can affect the results, but also the final volume of the extract. In a smaller final volume, target compounds will be more concentrated, but also interferents that may have not been eliminated. In Figure 6.1 B, are presented the results for the previously mentioned experiments. Similar results were achieved for the three tested solvents, as well as for the reconstitution volumes. Therefore, based on the required time to evaporate with N_2, EA was chosen as solvent and 500 µL as the final reconstitution volume. The smaller volume was chosen because lower detection limits may be reached, with no significant matrix effect.

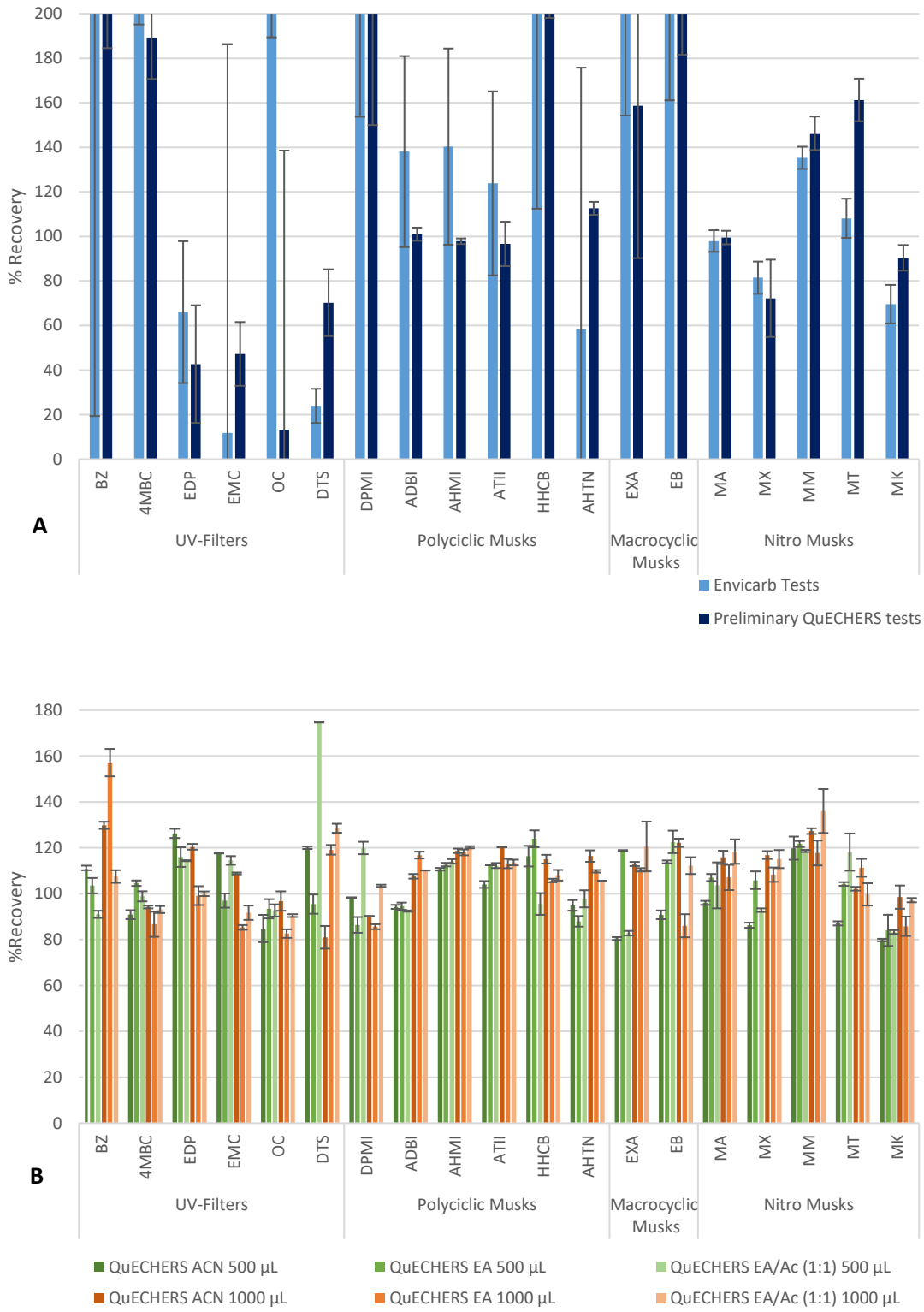


Figure 6.1 Optimization procedure: effect of different sorbents, solvents and final volume of sample reconstitution on the recovery and repeatability (error bars) of target compounds from tomato samples spiked at 18.8 ng g^{-1} (n = 3).

6.3.2. Method validation

As mentioned before, validation of the optimized method was done based on linearity, precision, accuracy, detection and quantification limits, matrix effects and global uncertainty.

6.3.2.1. Linearity, detection and quantification limits, precision and accuracy

A good linearity was obtained (1-1000 µg L^{-1}), with correlation coefficients (R^2) equal or higher than 0.997 for all compounds. Results are presented in Table 6.1.

IDLs varied between 0.02 pg (EMC) and 3.0 pg (MX and EXA). These values are satisfactory since the matrix is very complex. MDLs values ranged between 0.1 (ADBI and MK) and 47.89 (EXA) ng g^{-1} dw. Available information for comparison is scarce, but according to the existing studies, it is possible to conclude that the achieved limits in this work are lower than those found in the literature (Fussell et al., 2013; Hurtado et al., 2016). For that reason, this validated methodology is an enhancement in this area, as it enables the identification of lower levels of the studied compounds, namely the UVFs that were for the first time determined in tomatoes.

Regarding the method's accuracy, the recovery results obtained were satisfactory, ranging between 81 and 115% (Table 6.1). Considering this complex type of matrix and intra- and inter-day precision, with RSD values below 10% (average of 5%), this method may be considered precise.

Table 6.1 Main validation parameters for the QuEChERS-GC–MS/MS methodology.

Compounds	R^2	Interday precision (%RSD)			Accuracy ± intraday precision (%mean recovery ± RSD)			IDL (pg)	IQL (pg)	MDL (ng g^{-1} dw)	MQL (ng g^{-1} dw)
		4 ng g^{-1} dw	19 ng g^{-1} dw	38 ng g^{-1} dw	4 ng g^{-1} dw	19 ng g^{-1} dw	38 ng g^{-1} dw				
BZ	0.998	4	0	8	97 ± 6	96 ± 4	105 ± 6	0.11	0.36	1.12	8.75
4MBC	0.999	2	2	2	109 ± 8	90 ± 8	87 ± 1	0.22	0.74	0.17	4.78
EDP	0.999	2	2	0	107 ± 9	103 ± 6	113 ± 4	1.00	3.33	0.47	3.74
EMC	1.000	4	5	1	106 ± 8	108 ± 9	105 ± 6	0.02	0.07	0.13	1.17
OC	0.999	7	7	1	106 ± 8	101 ± 8	115 ± 2	0.12	0.39	0.30	2.46
DTS	0.995	6	5	2	116 ± 6	112 ± 3	97 ± 1	2.00	6.67	0.15	2.86
DPMI	0.999	4	1	4	111 ± 4	103 ± 6	96 ± 4	0.30	1.00	0.49	2.77
ADBI	0.999	1	1	4	96 ± 7	81 ± 1	83 ± 3	0.02	0.07	0.06	0.40
AHMI	1.000	2	1	4	114 ± 2	84 ± 4	84 ± 4	0.11	0.37	0.17	1.22
ATII	1.000	0	1	2	104 ± 7	90 ± 1	86 ± 5	0.50	1.67	0.05	0.99
EXA	1.000	2	2	5	101 ± 9	83 ± 5	85 ± 5	3.00	10.00	6.41	47.89
HHCB	0.999	1	2	1	111 ± 7	106 ± 4	105 ± 4	0.25	0.83	0.40	1.41
AHTN	1.000	5	2	3	95 ± 6	92 ± 0	86 ± 3	0.30	1.00	0.09	0.87
EB	0.999	4	8	3	99 ± 7	105 ± 3	90 ± 8	1.00	3.33	0.91	4.21
MA	0.999	3	2	4	106 ± 3	87 ± 3	92 ± 7	2.00	6.67	0.25	2.22
MX	0.998	8	1	1	97 ± 2	87 ± 2	85 ± 2	3.00	10.00	0.17	1.97
MM	0.997	2	2	3	86 ± 4	88 ± 3	91 ± 2	0.15	0.52	0.50	1.70
MT	0.999	3	2	6	119 ±2	103 ± 6	94 ± 4	2.00	6.67	0.18	1.31
MK	0.999	4	2	1	89 ± 3	86± 2	83 ± 1	0.04	0.15	0.09	0.65

6.3.2.2. Matrix effect

The utilization of an instrumental technique such as GC-MS/MS is of great help when analysing samples with many interferents. Nevertheless, this technique is equally vulnerable to matrix effects, able to influence the quantification of compounds. Typically, in these cases, matrix-matched calibration is performed. Samples are spiked with increasing amounts of the target compounds and the obtained calibration curves are used for quantification. However, preparation of this type of calibration curves is more labour and time intensive. Therefore, in order to avoid the above mention procedure, a matrix effect study was performed. For each compound, the percentage of matrix effect (%ME) was calculated accordingly to the following equation: ME (%) ¼ [(peak area (spiked extract) - peak area (extract)) - peak area (standard solution)]/peak area (standard solution) (Stremel et al., 2018). Results are presented in Figure 6.2, and no relevant matrix effects were found, since ME values vary between -20% and 20% (Costa et al., 2014; Kaczýnski et al., 2016; Matuszewski et al., 2003; Rutkowska et al., 2019; Sante, 2015). Therefore, it is not necessary to use matrix-matched calibration and quantification of UVFs and SMCs should be performed by direct injection of calibration standards in Hex.

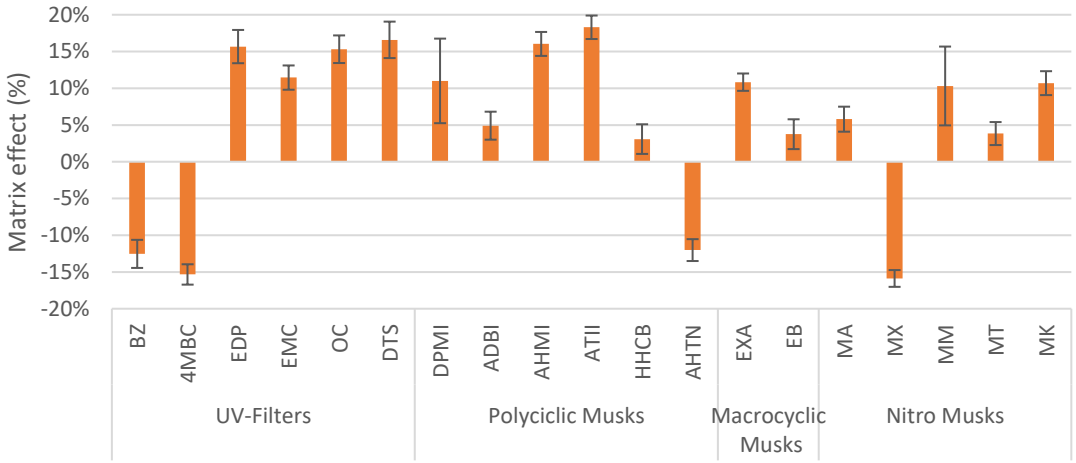


Figure 6.2 Matrix effects (%) for each compound analysed in the tomato sample (sample spiked at 5 ng g^{-1} dw).

6.3.2.3. Global uncertainty

Global uncertainty evaluated was performed by assessing the main factors of uncertainty that affect the whole process. The identification of all sources of error and

the estimation and combination of all those factors of uncertainty allow the analysis of single contributions and determination of the most meaningful one. Global uncertainty arises from four major sources of uncertainty: (U1) the error associated to the calibration standards preparation, (U2) the calibration curve, (U3) the precision and (U4) the accuracy (Ratola et al., 2006).

Figure 6.3 A shows the global uncertainty (Uglobal) and how the relative weight of each individual source of uncertainty varies for drometrizole trisiloxane (DTS) and Figure 6.3 B for galaxolide (HHCB), as representative compounds from UVFs and SMCs, respectively.

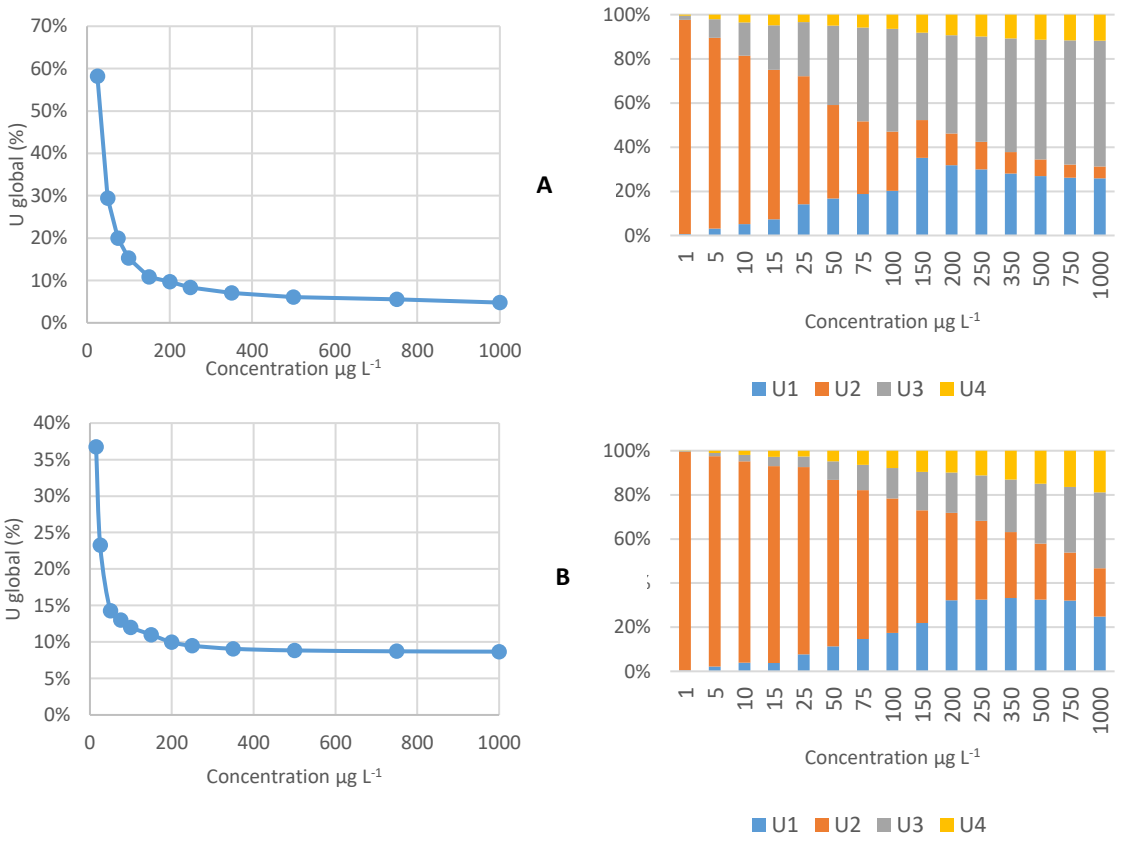


Figure 6.3 Global uncertainty on the left and variation of the relative weight of each individual source of uncertainty on the right, for DTS (A) and HHCB (B), as representative compounds from UVFs and SMCs respectively.

For UVFs (example DTS), the percentage of Uglobal at the high and middle calibration levels was 5%; nevertheless, the global uncertainty increases significantly for lower concentrations, particularly as they approach the limit of detection. For SMCs (example HHCB), the scenario is similar, with percentages of Uglobal being about 10% for the

higher concentration levels, increasing exponentially for lower concentrations, as found for UVFs.

Regarding the variation of the relative weight of each individual source, results are very similar. In both cases, the relative contribution of U1 decreases with the decrease of concentrations. However, with U2 and U3, results are slightly different. In both cases, the weight of U2 increases as concentrations decreases, reaching almost 100% at the lowest concentration and the contribution of the U3 decreases as lower concentrations are reached. However, U2 has more weight for SMCs, while U3 for UVFs. Regarding U4, it does not show a very important input for the Uglobal over all the range of concentrations.

6.3.3. Supermarket samples

Once the proposed methodology has been validated, several samples of tomato were analysed in order to assess the fitness of the methodology. The occurrence of the target compounds was studied in tomato samples of different varieties purchased at local supermarkets. Each sample was processed in triplicate as described above. The concentrations of the studied contaminants found in the analysed vegetables are summarized in Table 6.2.

Compounds AHMI, MA, MM, MT and MK were not detected in any sample. Overall, UVFs account to maximum levels detected in all samples, with values reaching 210 ng g^{-1} dw for BZ, 45 ng g^{-1} dw for OC and 22 ng g^{-1} dw for DTS. Regarding SMCs, maximum levels were found in HHCB (53 ng g^{-1} dw) and MX (15 ng g^{-1} dw). The levels found for MX are concerning, since legislation limits its use in cosmetic products (European Parliament, 2009).

Since there are no published studies on the presence of these compounds in tomatoes, these values may only be compared with studies in other supermarket fruit/vegetables.

Table 6.2 Analytes detected in tomatoes. Results are expressed as ng g^{-1} dw, mean ± SD (n = 3).

Compounds		P_Pt	C_Es	C_Pt	B_Pt	LR_Pt	A_N	O_Pt	B_Sp	B_Pt	LR_Sp
UFVs	BZ	66 ± 4	30 ± 3	58 ± 4	49 ± 5	40 ± 5	150 ± 10	76 ± 9	29 ± 2	135 ± 13	210 ± 22
	4MBC	nd	nd	nd	nd	nd	nd	nd	6 ± 1	nd	<MQL
	EDP	nd	nd	nd	nd	nd	<MQL	nd	nd	nd	5 0 ±1
	EMC	7 ± 1	3.1 ± 0.4	3.9 ± 0.4	6 ± 1	7 ± 1	14 ± 2	13 ± 1	7.0 ± 0.5	6 ± 1	2.3 ± 0.2
	OC	45 ± 5	8 ± 1	10 ± 1	24 ± 2	35 ± 3	11 ± 2	24 ± 1	27 ± 1	19 ± 2	10 ± 1
	DTS	18 ± 3	nd	22 ± 1	9 ± 1	11 ± 1	15 ± 1	nd	nd	nd	nd
SMCs	DPMI	<MQL	<MQL	<MQL	<MQL	<MQL	2.5 ± 0.1	2.9 ± 0.1	2.6 ± 0.2	2.6 ± 0.1	3.0 ± 0.2
	ADBI	nd	Nd	nd	nd	nd	0.72 ± 0.01	nd	nd	nd	nd
	ATII	2.2 ± 0.3	4.5 ± 0.2	2.6 ± 0.1	2.4 ± 0.1	2.2 ± 0.3	2.1 ± 0.2	1.9 ± 0.2	1.9 ± 0.4	1.9 ± 0.2	2.1 ± 0.2
	HHCB	36 ± 1	9 ± 1	31 ± 4	11.1 ± 0.1	17 ± 2	53 ± 4	19 ± 1	12 ± 1	13 ± 1	10 ± 1
	AHTN	3.9 ± 0.1	1.3 ± 0.5	3.4 ± 0.3	1.1 ± 0.1	1.9 ± 0.2	6 ± 1	3.2 ± 0.4	1.4 ± 0.1	1.6 ± 0.1	2.5 ± 0.1
	EXA	<MQL	<MQL	nd	nd	<MQL	<MQL	<MQL	nd	nd	nd
	EB	<MQL	6 ± 2	8 ± 1	14 ± 3	<MQL	5 ± 1	8 ± 1	9 ± 1	6.3 ± 0.3	11 ± 1
	MX	15 ± 2	nd	13 ± 1	9 ± 1	10 ± 1	nd	nd	nd	nd	nd

OBS.: nd - not detected; Tomato varieties: LR: loose round; B: Bunch; C: Cherry; P: Plum; A: Anairis; O: Oxheart; Countries: Pt- Portugal; Es- Spain; N – Netherlands; <MQL: below method quantification limit.

The only available study is from Aguirre et al. (2014), which studied the presence of SMCs in lettuce, carrots and pepper. MK (0.03 ng g^{-1} dw), HHCB (4.6 ng g^{-1} dw) and AHMI (0.0652 ng g^{-1} dw) were detected in lettuce, ADBI (0.04 ng g^{-1} dw) in carrots and MA (0.07 ng g^{-1} dw) and AHTN (3.7 ng g^{-1} dw) in peppers. The results of this study differ significantly as Aguirre et al. (2014) detected AHMI, MK and MA. Also, although detected levels of ADBI are very similar, higher concentrations of HHCB were found. The great variability of these results is not surprising due to the different uptake and translocation mechanism among vegetable species. In addition, conditions of agricultural practice (e.g. concentration of the components in the irrigation water, fertilizers or sludge e if the latter are applied) are unknown when tomatoes are bought in supermarkets.

However, some authors studied the behaviour of certain vegetables irrigated with effluent wastewater or fertilized with sewage sludge, proving the uptake of some compounds (Sablayrolles et al., 2006; Shenker et al., 2011; Wu et al., 2012, 2015). For instance, in a field study where sewage sludge was applied, HHCB and AHTN were detected in lettuce at levels of 198 and 738 ng g^{-1} dw, respectively.

6.3.4. Exposure and risk assessment

So far, there are few studies on the determination of the levels of certain contaminants, particularly emerging pollutants, to which population is daily exposed through various routes. One of these pathways is the consumption of vegetables and fruits. To this day, there is no type of guideline that establishes maximum residue levels of the target contaminants in these food matrices. Thus, values of NOAEL should be used to estimate risk. Though NOAEL are not available for all compounds under study, for most detected compounds in the tested samples they could be found in published literature (Burnett, 2008; Christian et al., 1999; ECHA, 2019; NTP, 2006) and are summarized in Table S5.3 in Annex 5.

The possibility of risk to human health due to exposure to UVFs and SMCs through ingestion of tomatoes was estimated using wet weight concentrations. Unfortunately, some compounds that were detected in the samples (ADBI and ATII) could not be included in this analysis due to the lack of NOAEL data. Table 6.3 shows the weekly

exposure and the relative contribution of each variety and target compound, for both, average concentration and percentile of exposure (P99). Average exposure (mg kg^{-1} bw $week^{-1}$) were determined in this work and reflect the human consumption of each compound per week, due to ingestion of different varieties of tomato (0.5 kg tomato per week). The TWI (Tolerable Weekly Intake) was calculated based on the NOAEL presented in Table S5.3 (Annex 5), as explained before.

Table 6.3 Results of the exposure assessment for SMCs and UVFs for different tomato samples. Results of Mean exposure (Av. Conc.) are reflected in (µg kg^{-1} bw $week^{-1}$) as well as the P99 exposure for each compound and tomato variety.

	TWI_cal (µg kg^{-1} bw $week^{-1}$)	Cherry		Loose round		Bunch		Plum		Anairis		Oxheart	
		Av. Conc.	P99	Av. Conc.	P99	Av. Conc.	P99	Av. Conc.	P99	Av. Conc.	P99	Av. Conc.	P99
BZ	1,750	193.6	29.8	550.3	107.1	313.6	68.8	292.7	34.1	664.0	77.5	334.3	39.0
4MBC	1,750			10.7	1.2	25.1	2.9						
EDP	7,000			21.1	2.4					3.8	0.4		
EMC	31,500	15.5	2.0	21.1	3.7	27.7	3.6	29.6	3.4	60.5	7.1	57.6	6.7
OC	12,250	39.0	5.1	99.4	18.1	103.4	14.1	199.8	23.3	50.4	5.9	104.9	12.2
DTS	22,223	95.6	11.0	47.3	5.5	41.0	4.7	81.3	9.5	68.3	8.0		
DPMI	700	3.4	0.5	8.1	1.5	9.0	1.4	5.0	0.6	11.1	1.3	12.6	1.5
HHCB	3,500	89.7	16.0	61.6	9.0	51.7	6.5	159.9	18.7	235.4	27.5	85.5	10.0
AHTN	350	10.5	1.7	9.8	1.3	6.0	0.8	17.2	2.0	28.2	3.3	14.0	1.6
EXA	70,000	19.7	2.3	20.3	2.3			20.7	2.4	38.7	4.5	40.7	4.7
EB	70,000	29.8	3.9	33.0	5.6	44.1	7.3	10.9	1.3	22.0	2.6	34.3	4.0
MX	1,400	57.3	6.6	45.2	5.2	37.9	4.3	67.0	7.8				

According to the performed risk assessment, a potential health risk is not likely, as exposure is considerably less than established TWI values. These values are in accordance with a study of exposure to SMCs through seafood consumption carried out in Catalonia (Spain) (Trabalón et al., 2015) and a study of UVFs and SMCs in seafood marketed in Europe (Cunha et al., 2015).

Regarding risk assessments in tomatoes, only studies with pesticides were found (Bhandari et al., 2019; Hlihor et al., 2019; Li et al., 2014; Loughlin et al., 2018; Malhat et al., 2017; Yu et al., 2016; Reiler et al., 2015). Pesticides have been studied more often than UVFs and SMCs, therefore risk assessment studies have been performed based on short-term intake and acute reference doses available in literature. Due to the detected levels of pesticides in tomatoes, for some compounds a hazard quotient (HQ) >1 was determined, meaning there is risk for some of the studied compounds found in tomatoes. In fact, pesticides are commonly applied chemicals in agricultural crops, which may explain the risk to human consumption found in some situations.

The methodology developed, QuEChERS/GC-MS/MS, has already been described for the analysis of environmentally relevant compounds in vegetables and fruits, such as pesticides (Li et al., 2014). This methodology, due to its simplicity and easiness to perform, is a powerful tool for extracting emerging compounds, such as PCPs.

In this study, the methodology revealed a good performance, with a high accuracy and precision. Detection limits were also a key point of this method, being reliable for the quantification of UVFs and SMCs in tomatoes, since they were as low as necessary to quantify the target compounds in this matrix (usually lower than 1 ng g^{-1} dw). The method's uncertainty was also appropriate for this kind of analysis. In fact, this methodology presented a performance similar to other time-consuming and more complex techniques, such as SPE. Therefore, the proposed method presents as mains strengths its speed, low waste production and use of solvents ("green" method), low cost and high performance when compared to other conventional methodologies. The proposed methodology may also be adapted for the determination of the target compounds in other crops, which may be considered other main advantage.

To the authors' best knowledge, this was the first approach to quantify both UVFs and SMCs in market tomatoes and, the first study in which UVFs were quantified in fruits/vegetables. Relevant concentrations of UVFs and SMCs could be found in fruits/vegetables, when agricultural practices introduce these compounds to their growing environment (e.g. irrigation with contaminated waters, addition of sewage sludge as fertilizers, etc.). Uptake of these compounds can occur via different pathways and move either to the leaves or fruits by translocation mechanisms. There are many research publications that prove this uptake of several emerging contaminants by plants and indicate that these compounds can be absorbed and introduced into the food chain (Karnjanapiboonwong et al., 2011; Sablayrolles et al., 2006; Shenker et al., 2011; Wu et al., 2012). Nevertheless, few of these studies analyse market vegetables or fruits, being the crops in these studies subjected to specific environmental conditions and agricultural practices (e.g. controlled conditions as temperature, humidity and light exposure) that may influence their exposure to the target contaminants.

Therefore, the analysis of market fruit/vegetables is important to understand the levels of contamination the population is usually exposed to, while studies in controlled

environment may be performed to study the uptake extension and its relationship with the levels present in the soil/irrigation waters and the mechanisms involved.

6.4. Conclusions

In this study, a QuEChERS methodology combined with GC-MS/MS analysis has been optimized and validated and proved to be an efficient and reliable method for the simultaneous analysis of SMCs (6 polycyclic, 2 macrocyclic and 5 nitro musks) and UVFs (6 compounds) from tomatoes.

The validated method yielded good recoveries for the sample matrix tested (81 - 119%), with RSD values typically below 10%. Minor matrix effects were found, which did not affect quantification. The developed extraction procedure was successfully applied to analyse of different varieties of tomatoes (e.g. cherry, loose round, bunch, plum, *Anairis* and oxheart). Among all analysed samples, concentrations varied between 1 (AHTN) and 210 (BZ) ng g^{-1} dw. The most frequently detected compounds were UVFs BZ, EMC and OC, whereas for SMCS were ATII, HHCB and AHTN.

Relevant concentrations of UVFs and SMCs can be found in fruit/vegetables, such as tomatoes, when agricultural practices introduce these compounds to their growing environment. Therefore, sampling and analysis of market tomatoes is important to better understand the levels of contamination to which populations are exposed.

To the authors' best knowledge, this is the first attempt focused on the analysis of UVFs and SMCs in tomato samples and on the assessment of human populations exposure to these compounds through an integrated approach. Although most UVFs and SMCs were detected in the analysed samples, human exposure levels estimated based on detected concentration levels in raw tomato samples were far below the estimated toxicological reference values. These findings and conclusions were based on the available data and should be interpreted with attention as variability and restrictions are involved.

Although this sampling was only restricted to local supermarkets in Portugal, some analysed tomato species were imported from other countries, showing that this is not a geographically restricted problem. Nevertheless, these results must be considered as a

"first screening" of UVFs and SMCs in vegetables. Future research in this specific area is recommended to account for multiple exposure routes.

Acknowledgements

This work was the result of the project: (i) Project UID/EQU/00511/2019 - Laboratory for Process Engineering, Environment, Biotechnology and Energy e LEPABE funded by national funds through FCT/MCTES (PIDDAC); (ii) Project POCI-01-0145-FEDER-029425 - AGRONAUT - Agronomic Impact of Sludge Amendment Using a Comprehensive Exposure Viewpoint, funded by FEDER funds through COMPETE2020 e Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES; (iii) Project "LEPABE-2-ECO-INNOVATION" e NORTE-01-0145-FEDER-000005, funded by Norte Portugal Regional Operational Programme (NORTE 2020), under PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF); (iv) Doctoral Grant SFRH/BD/110831/2015 e Sara Ramos.

Supplementary Information

Supplementary data to this article can be found online at:
https://doi.org/10.1016/j.chemosphere.2019.124605 or in Annex 5.

References

Aguirre, J., Bizkarguenaga, E., Iparraguirre, A., Fernandez, L.A., Zuloaga, O., Prieto, A., 2014. Development of stir-bar sorptive extraction-thermal desorption-gas chromatography-mass spectrometry for the analysis of musks in vegetables and amended soils. Anal. Chim. Acta 812, 74-82.

Barlow, S., Schlatter, J., 2010. Risk assessment of carcinogens in food. Toxicol. Appl. Pharmacol. 243, 180-190.

Bhandari, G., Zomer, P., Atreya, K., Mol, H.G.J., Yang, X., 2019. Pesticide residues in Nepalese vegetables and potential health risks. Environ. Res. 172, 511-521.

Bragança, I., Placido, A., Paíga, P., Domingues, V.F., Delerue-Matos, C., 2012. QuEChERS: a new sample preparation approach for the determination of ibuprofen and its metabolites in soils. Sci. Total Environ. 433, 281-289.

Burnett, C.L., 2008. Amended final report of the safety assessment of Drometrizole as used in cosmetics. Int. J. Toxicol. 27, 63-75.

Calderon-Preciado, D., Jimenez-Cartagena, C., Pe~nuela, G., Bayona, J.M., 2009. Development of an analytical procedure for the determination of emerging and priority organic pollutants in leafy vegetables by pressurized solvent extraction followed by GC-MS determination. Anal. Bioanal. Chem. 394, 1319-1327.

Calderon-Preciado, D., Jimenez-Cartagena, C., Matamoros, V., Bayona, J.M., 2011. Screening of 47 organic microcontaminants in agricultural irrigation waters and their soil loading. Water Res. 45, 221-231.

Calderon-Preciado, D., Matamoros, V., Bayona, J.M., 2011. Occurrence and potential crop uptake of emerging contaminants and related compounds in an agricultural irrigation network. Sci. Total Environ. 412,413, 14-19.

Calderon-Preciado, D., Renault, Q., Matamoros, V., Ca~nameras, N., Bayona, J.M., 2012. Uptake of organic emergent contaminants in spath and lettuce: an in vitro experiment. J. Agric. Food Chem. 60, 2000-2007.

Cerqueira, M.B.R., Guilherme, J.R., Caldas, S.S., Martins, M.L., Zanella, R., Primel, E.G., 2014. Evaluation of the QuEChERS method for the extraction of pharmaceuticals and personal care products from drinking-water treatment sludge with determination by UPLC-ESI-MS/MS. Chemosphere 107, 74-82.

Christian, M.S., Parker, R.M., Hoberman, A.M., Diener, R.M., Api, A.M., 1999. Developmental toxicity studies of four fragrances in rats. Toxicol. Lett. 111, 169-174.

Costa, F.P., Caldas, S.S., Primel, E.G., 2014. Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in canned and fresh peach. Food Chem. 165, 587-593.

Cunha, S.C., Trabalon, L., Jacobs, S., Castro, M., Fernandez-Tejedor, M., Granby, K., Kwadijk, C., Verbeke, W., Ferrari, F., Robbens, J., Sioen, I., Fernandes, J.O., Pocurull, E., Domingo, J.L., Marques, A., 2015. UV-filters and musk fragrances in seafood commercialized in Europe Union: occurrence, risk and exposure assessment. Environ. Res. 143, 116-122.

ECHA. URL. https://echa.europa.eu/. accessed 3.25.19.

European Parliament, 2009. Regulation (EC) N.o 1223/2009 of the European parliament and of the Council of 30 November 2009 on cosmetic products. Off. J. Eur. Union L 342, 59-209.

Fussell, R.J., Garcia Lopez, M., Mortimer, D.N., Wright, S., Sehnalova, M., Sinclair, C.J., Fernandes, A., Sharman, M., 2013. Investigation into the Occurrence in Food of Veterinary Medicines, Pharmaceuticals, and Chemicals Used in Personal Care Products. Report to the Food Standards Agency.

Hlihor, R., Olga, M., Rosca, M., Cozma, P., 2019. Modelling the behavior of pesticide residues in tomatoes and their associated long-term exposure risks. J. Environ. Manag. 233, 523-529.

Homem, V., Silva, A., Ratola, N., Santos, L., Alves, A., 2015. Long lasting perfume - A review of synthetic musks in WWTPs. J. Environ. Manag. 149, 168-192.

Hurtado, C., Domínguez, C., Perez-Babace, L., Cañameras, N., Comas, J., Bayona, J.M., 2016. Estimate of uptake and translocation of emerging organic contaminants from irrigation water concentration in lettuce grown under controlled conditions. J. Hazard Mater. 305, 139-148.

Kaczynski, P., Łozowicka, B., Jankowska, M., Hrynko, I., 2016. Rapid determination of acid herbicides in soil by liquid chromatography with tandem mass spectrometric detection based on dispersive solid phase extraction. Talanta 152, 127-136.

Karnjanapiboonwong, A., Chase, D.A., Cañas, J.E., Jackson, W.A., Maul, J.D., Morse, A.N., Anderson, T.A., 2011. Uptake of 17a-ethynylestradiol and triclosan in pinto bean, Phaseolus vulgaris. Ecotoxicol. Environ. Saf. 74, 1336-1342.

Kim, L., Lee, D., Cho, H., Choi, S., 2019. Review of the QuEChERS method for the analysis of organic pollutants : Persistent organic pollutants, polycyclic aromatic hydrocarbons, and pharmaceuticals. Trends Environ. Anal. 22, e00063.

Li, Y., Qiao, L., Li, F., Ding, Y., Yang, Z., Wang, M., 2014. Determination of multiple pesticides in fruits and vegetables using a modified quick, easy, cheap, effective, rugged and safe method with magnetic nanoparticles and gas chromatography tandem mass spectrometry. J. Chromatogr. A 1361, 77-87.

Litz, N.T., Mueller, J., Boehmer, W., 2007. Occurrence of polycyclic musks in sewage sludge and their behaviour in soils and plants: Part 2: Investigation of Polycyclic Musks in Soils and Plants. J. Soils Sediments 7, 36-44.

Loughlin, T.M.M., Peluso, M.L., Etchegoyen, M.A., Alonso, L.L., de Castro, M.C., Percudani, M.C., Marino, D.J.G., 2018. Pesticide residues in fruits and vegetables of the argentine domestic market: occurrence and quality. Food Control 93, 129-138.

Macherius, A., Eggen, T., Lorenz, W.G., Reemtsma, T., Winkler, U., Moeder, M., 2012. Uptake of galaxolide, tonalide, and triclosan by carrot, barley, and meadow fescue plants. J. Agric. Food Chem. 60, 7785-7791.

Magnusson, B., Ornemark, U., 2014. Eurachem Guide: the Fitness for Purpose of Analytical Methods e A Laboratory Guide to Method Validation and Related Topics, second ed. (Eurachem).

Malhat, F., Boulange, J., Abdelraheem, E., Abd, O., El-hamid, R.A., El-salam, S.A., 2017. Validation of QuEChERS based method for determination of fenitrothion residues in tomatoes by gas chromatography e flame photometric detector: Decline pattern and risk assessment. Food Chem. 229, 814-819.

Matuszewski, B.K., Constanzer, M.L., Chavez-Eng, C.M., 2003. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. Anal. Chem. 75, 3019-3030.

NTP, 2006. Toxicology and carcinogenesis studies of benzophenone (CAS No. 119-61-9) in F344/N rats and B6C3F1 mice (feed studies). Natl. Toxicol. Program Tech. Rep. Ser. 1-264.

Perestrelo, R., Silva, P., Porto-Figueira, P., Pereira, J.A.M., Silva, C., Medina, S., Câmara, J.S., 2019. QuEChERS - fundamentals, relevant improvements, applications and future trends. Anal. Chim. Acta 1070, 1-28.

Ramos, S., Homem, V., Alves, A., Santos, L., 2015. Advances in analytical methods and occurrence of organic UV-filters in the environment - a review. Sci. Total Environ. 526, 278-311.

Ramos, S., Homem, V., Alves, A., Santos, L., 2016. A review of organic UV-filters in wastewater treatment plants. Environ. Int. 86, 24-44.

Ramos, S., Homem, V., Santos, L., 2019. Simultaneous determination of synthetic musks and UV-filters in water matrices by dispersive liquid-liquid microextraction followed by gas chromatography tandem mass-spectrometry. J. Chromatogr. A 1590, 47-57.

Ratola, N., Santos, L., Herbert, P., Alves, A., 2006. Uncertainty associated to the analysis of organochlorine pesticides in water by solid-phase microextraction/gas chromatography-electron capture detection-Evaluation using two different approaches. Anal. Chim. Acta 573,574, 202-208.

Reiler, E., Jørs, E., Bælum, J., Huici, O., Mercedes, M., Caero, A., Cedergreen, N., 2015. The influence of tomato processing on residues of organochlorine and organophosphate insecticides and their associated dietary risk. Sci. Total Environ. 527,528, 262-269.

Rutkowska, E., Łozowicka, B., Kaczynski, P., 2019. Three approaches to minimize matrix effects in residue analysis of multiclass pesticides in dried complex matrices using gas chromatography tandem mass spectrometry. Food Chem. 279, 20-29.

Sablayrolles, C., Patria, L., Montrejaud-Vignoles, M., Silvestre, J., 2006. Sand to root transfer of PAHs and PCBs by carrots grown on sand with pure substances and biosolids amended sand. In: Acta Horticulturae.

Sabourin, L., Duenk, P., Bonte-Gelok, S., Payne, M., Lapen, D.R., Topp, E., 2012. Uptake of pharmaceuticals, hormones and parabens into vegetables grown in soil fertilized with municipal biosolids. Sci. Total Environ. 431, 233-236.

Sante, 2015. SANTE Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticides Residues Analysis in Food and Feed European Commission Document No SANTE/11945/2015 2015.

Sauve, S., Desrosiers, M., 2014. A review of what is an emerging contaminant. Chem. Cent. J. 8, 15-22.

Shenker, M., Harush, D., Ben-Ari, J., Chefetz, B., 2011. Uptake of carbamazepine by cucumber plants - a case study related to irrigation with reclaimed wastewater. Chemosphere 82, 905-910.

Stremel, T.R.D.O., Domingues, C.E., Zittel, R., Silva, C.P., Weinert, P.L., Monteiro, F.C., Campos, S.X., 2018. Development, validation and matrix effect of a QuEChERS method for the analysis of organochlorine pesticides in fish tissue. J. Environ. Sci. Health Part B - Pestic. Food Contam. Agric. Wastes 53, 246-254.

Trabalon, L., Cano-Sancho, G., Pocurull, E., Nadal, M., Domingo, J.L., Borrull, F., 2015. Exposure of the population of Catalonia (Spain) to musk fragrances through seafood consumption: risk assessment. Environ. Res. 143, 116-122.

Vallecillos, L., Borrull, F., Pocurull, E., 2015. Recent approaches for the determination of synthetic musk fragrances in environmental samples. TrAC Trends Anal. Chem. 72, 80-92.

Wu, C., Spongberg, A.L., Witter, J.D., Sridhar, B.B.M., 2012. Transfer of wastewater associated pharmaceuticals and personal care products to crop plants from biosolids treated soil. Ecotoxicol. Environ. Saf. 85, 104-109.

Wu, X., Dodgen, L.K., Conkle, J.L., Gan, J., 2015. Plant uptake of pharmaceutical and personal care products from recycled water and biosolids: a review. Sci. Total Environ. 536, 655-666.

Yu, R., Liu, Q., Liu, J., Wang, Q., Wang, Y., 2016. Concentrations of organophosphorus pesticides in fresh vegetables and related human health risk assessment in Changchun , Northeast China. Food Control 60, 353-360.

Zarn, J.A., H€anggi, E., Engeli, B.E., 2015. Impact of study design and database parameters on NOAEL distributions used for toxicological concern (TTC) values. Regul. Toxicol. Pharmacol. 72, 491-500.

Zhang, C., Deng, Y., Zheng, J., Zhang, Y., Yang, L., 2019. The application of the QuEChERS methodology in the determination of antibiotics in food: a review. Trends Anal. Chem. 118, 517-537.

Zuloaga, O., Navarro, P., Bizkarguenaga, E., Iparraguirre, A., Vallejo, A., Olivares, M., Prieto, A., 2012. Overview of extraction, clean-up and detection techniques for the determination of organic pollutants in sewage sludge: a review. Anal. Chim. Acta 736, 7-29.

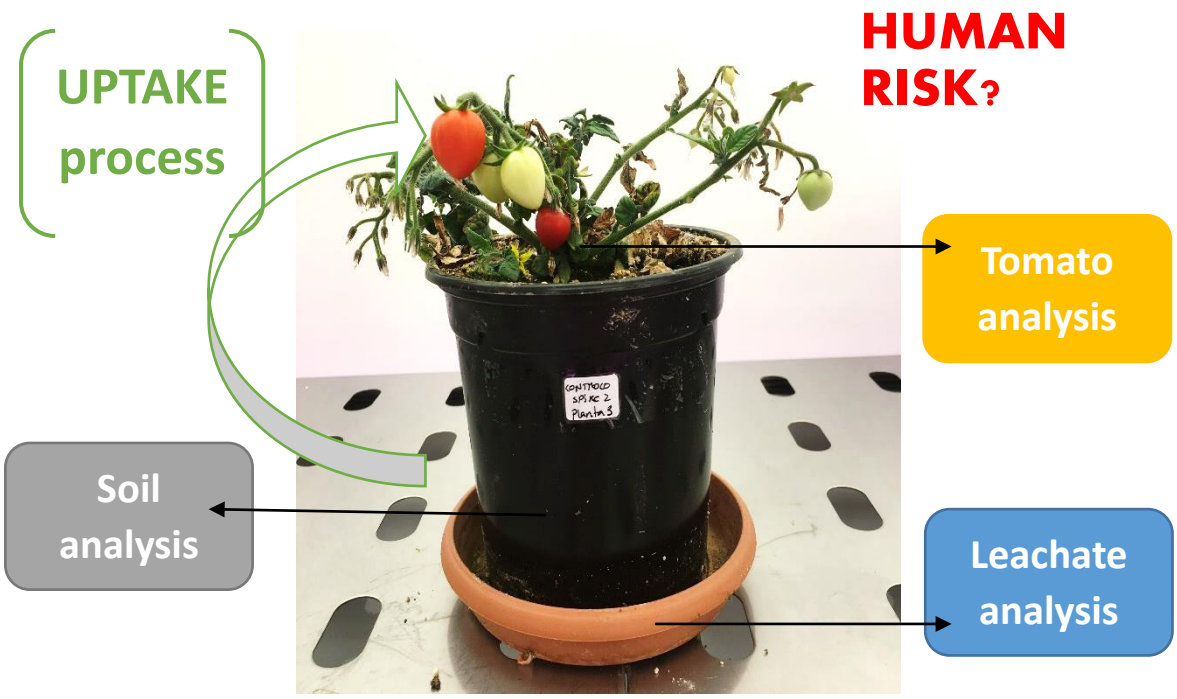
Part IV: Plant Uptake of UV-filters and Synthetic Musk Compounds

In this section, studies of plant uptake and translocation grown in amended soils are presented. The analytical methodologies developed in Part III are essential tools to understand the uptake mechanism of UVFs and SMCs in a pot system, between amended soil and tomato plants.

Chapter 7. Uptake and Translocation of UV-Filters and Synthetic Musk Compounds into Edible Parts of Tomato Grown in Amended Soils

Sara Ramos, Vera Homem, Lúcia Santos

Submitted



Abstract

In the last years, the number of wastewater treatment plants (WWTPs) has increased and consequently, the sewage sludge production. This residue is very rich in crop nutrients, which make it prone to be used as organic fertilizer/conditioner in agricultural fields. However, the presence of numerous emerging pollutants in these fertilizers, such as personal care products (PCPs), has raised concern, namely their potential accumulation in the soil and then their transference to food crops. Therefore, the main goal of this study was to study the potential plant uptake and translocation of ultraviolet-filters (UVFs) and synthetic musk compounds (SMCs). A total of 6 UVFs and 13 SMCs were analysed in the Micro-Tom tomato fruit grown in soil amended with a commercial sewage sludge-based organic fertilizer. Most of the studied compounds were detected in the tomato fruit, in concentrations ranging from 4.0 to 95.8 ng g^{-1} dw for UVFs, 2.6 to 64.7 ng g^{-1} dw for polycyclic SMCs, 22.4 to 187.0 ng g^{-1} dw for macrocyclic SMCs and 1.4 ng g^{-1} dw for nitro SMCs (musk ketone). This indicates a potential uptake of these emerging pollutants and a subsequent translocation to the fruits. Nevertheless, no risk was observed by the estimation of the weekly exposure dose and hazard quotients (HQ < 0.02).

Keywords*:* UV-filters, Synthetic musk compounds, Tomatoes, Uptake

7.1 Introduction

Wastewater treatment has evolved into an important mechanism used to protect public health. However, during conventional wastewater treatment, solid residues are produced along the process and may accumulate pathogens, micropollutants, heavy metals and other hazard substances (Evgenidou et al., 2015). The solids produced are typically processed in an anaerobic digester, in which the organic matter suffers bacterial breakdown in the absence of oxygen. The resulting solids, commonly known as biosolids, may be used in agriculture because they contain high concentrations of essential crop nutrients, especially nitrogen (N), phosphorous (P) and potassium (K). The nutrients are recycled into crop production and are used instead of inorganic fertilizers (Apedaile, 2001). Concerns related to the safety of these biosolids led wastewater treatment plants (WWTPs) to sanitize the final product through aerobic/anaerobic stabilization and composting, in order to be marketable (composted biosolids). Nevertheless, sewage sludge and sewage sludge-based fertilizers are known to contain a wide range of different classes of emerging contaminants (Prosser and Sibley, 2015; Rodríguez-Rodríguez et al., 2012; Sablayrolles et al., 2006; Sabourin et al., 2009; Sharma et al., 2017). Although the use of organic fertilizers is beneficial for soil and crops, it may be contributing to increase the amount of emerging contaminants in the receiving soil and then, be potentially taken up and translocated to crops, entering into the food chain (Qin et al., 2015; Shenker et al., 2011).

In recent years, a growing concern with personal care products (PCPs) has arisen since this class of compounds is continuous and massively used. Among PCPs, there are two classes that have been poorly studied, but are extensively used as ingredients in the formulation of a wide range of cosmetics, toiletries and household products - the ultraviolet-filters (UVFs) and the synthetic musk compounds (SMCs). The UVFs have as main function block or absorb ultraviolet radiation, while SMCs are fixative aroma compounds. Due to their physicochemical properties, they are considered lipophilic, bioaccumulative and are not readily biodegradable. Thus, when they reach WWTPs are not effectively removed, accumulating in the sewage sludge.

Notwithstanding that in recent years the analysis of UVFs and SMCs has gain more attention (Aguirre et al., 2014; Calderón-Preciado et al., 2013, 2012; Diana Calderón-Preciado et al., 2011; Fussell et al., 2013; Hurtado et al., 2016; Lai et al., 2014; Litz et al., 2007; Macherius et al., 2012), there is still a gap in the literature regarding their potential translocation and partitioning behaviour in the amended-soil-plant system. In fact, few uptake experiments were found in the literature and most focused on the study of SMCs, namely galaxolide (HHCB) and tonalide (AHTN) (Calderón-Preciado et al., 2012; D. Calderón-Preciado et al., 2011; Diana Calderón-Preciado et al., 2011; Hurtado et al., 2016; Litz et al., 2007; Macherius et al., 2012). From those, some were dedicated to plant uptake by irrigation with contaminated water (Calderón-Preciado et al., 2013; D. Calderón-Preciado et al., 2011; Diana Calderón-Preciado et al., 2011; Hurtado et al., 2016) and others to biosolid-amended soil (Litz et al., 2007; Macherius et al., 2012). Several empirical and process-based models have been developed to try to predict the concentration of compounds in plants. Litz et al. (2007) (Litz et al., 2007) performed *in vitro* and field experiments to determine the uptake of the musk fragrances by lettuce and carrots, applying sewage sludge to soils. The authors concluded that considerable amounts of HHCB and AHTN were taken up only by the carrot roots and the selected polycyclic musk compounds showed high adsorption to soil. Macherius et al. (2012) (Macherius et al., 2012) also studied the uptake of HHCB and AHTN by barley, meadow fescue, and four carrot cultivars in spiked soils (10 mg kg^{-1} dw) under greenhouse conditions. The authors concluded that different crops can uptake different amounts of the target compounds. For example, barley and meadow fescue roots incorporated higher amounts of the target substances than carrots, but translocation into the leaves was negligible. However, the authors concluded that the introduction of HHCB and AHTN into the food chain via edible plants of carrots could be of certain relevance when sludge is applied as fertilizer.

Therefore, the aim of this work was to evaluate the uptake and translocation of six UVFs and thirteen SMCs to edible portions of tomato (*Solanum lycopersicum L.* cv. Micro-Tom) grown in composted biosolids-amended soils, using climate chambers (controlled conditions of temperature, humidity and light exposure). Tomato was selected because it represents an edible fruit crop, being one of the most produced and consumed

nationally. In 2018, Portugal produced 1.2 million tons for the tomato industry and tomato for fresh consumption was the largest crop production (104,000 tonnes) (INE, 2019). Results from this study may be used as a first step in prioritizing emerging contaminants for future evaluations and to improve the understanding of human exposure to PCPs. To our knowledge, this study is the first to investigate the uptake and translocation of UVFs and SMCs in tomato from compost-amended soils.

7.2. Materials and Methods

7.2.1 Chemicals

Native synthetic musks (cashmeran, celestolide, phantolide, traseolide, galaxolide, tonalide, musk ambrette, musk xylene, musks moskene, musk tibetene, musk ketone, exaltolide and ethylene brassylate) and UV-filters standards (benzophenone, 3-(4'-methylbenzylidene) camphor, ethylhexyl dimethyl PABA, 2-ethylhexyl 4-methoxycinnamate, octocrylene and drometrizole trisiloxane) and isotopes were used in this study and prepared as described in Table S6.1 of the Annex 6. Ultrapure water used for the extractions was obtained from Merck (Darmstadt, Germany), while all the other analytical grade solvents were purchased from VWR BDH Prolabo (Fontenay-sous-Bois, France). Sodium chloride (NaCl) from Merck, anhydrous magnesium sulphate ($MgSO_4$) from Panreac AppliChem (Barcelona, Spain), primary and secondary amine exchange bonded silica sorbent (PSA) and octadecyl-silica (C_{18}) from Supelco (Bellefonte, PA, USA) were also used in the extraction and clean-up of the samples. To remove residual water from the $MgSO_4$, it was baked-out for 12 hours at 450 °C.

Tomato seeds were provided by the Research Centre in Biodiversity and Genetic Resources (CIBIO, Porto, Portugal) seed bank. The commercially available composted biosolid was bought in a local agriculture store, as well as the substrate (SIRO Interiors, Mira, Portugal), the limestone (UCA Norte, Asturias, Spain) and the superphosphate 26% (ADP, Vila Nova de Gaia, Portugal) and the perlite (Flower, Alicante, Spain).

7.2.2 Plant uptake study

The experiments of plant uptake and translocation were performed under controlled conditions, in a climatic chamber by Aralab Fitoclima PLH "walk-in" (Rio de Mouro, Portugal). Conditions were previously established to be optimal for tomato Micro-Tom cultivar growth and resulted in 16 h of photoperiod with 100 µmol m^{-2} s^{-1} of light intensity, ambient temperature of 22 ^{o}C and 65% humidity (Shikata and Ezura, 2016).

Agricultural soil collected from Vila Real (Portugal) was used for these experiments. Initially, it was sieved through a 2 cm opening screen to remove larger stones, roots and possible branches. Due to the poor soil quality (*cf. Sample characterization*, Table 7.1), some soil improvements had to be performed. 0.5 kg of soil was added to each pot and was mixture with 2 g of limestone, 0.5 g of superphosphate 26% (recommended addition of 4 g kg^{-1} and 30 g m^{-2}, respectively, based on the previous soil analysis) and 100 g of a commercially organic substrate. Then, about 3/4 of each pot was filled with perlite to increase the drainage and aeration of the soil. In fact, perlite has a very porous surface that retains both water and nutrients but allows excess water to drain away. This also prevents the soil from becoming clogged by water, providing root aeration (Gürsoy and Karaman, 2018).

The plant uptake experiences were divided into four groups (Figure 7.1): i) control (unamended control soil); ii) composted biosolids-amended soil (addition of 30 g of a commercially available composted biosolid to the soil mixture indicated above; according to the label of the product, 3 L m^{-2} should be added for tomato growth); iii) spike control (unamended soil spiked with 125 ng g^{-1} dw (S1) or 500 ng g^{-1} dw (S2) of selected target compounds OC, DTS, HHCB and AHTN); iv) spiked composted biosolids-amended soil (amended-soils to which 125 ng g^{-1} dw (S1) or 500 ng g^{-1} dw (S2) of OC, DTS, HHCB and AHTN was added).

Seeds of tomato cultivar Micro-Tom (*Solanum lycopersicum L.*) were previously grown in a substrate free of UVFs and SMCs (around 25 seeds *per* pot). When plants reached 3-5 cm, one tomato plant was transplanted for each pot, according to the scheme of Figure 7.1. Plants were watered every 2 days and leachates collected during the trial (6 months). Each uptake experience (Figure 7.1) was performed using six pot replicates.

Tomato fruits (edible part) from each pot were combined as one replicate and harvested at maturation. Soil samples were collected from each pot in the beginning of the experiment and in the end. All samples were stored at -20 °C until analysis.

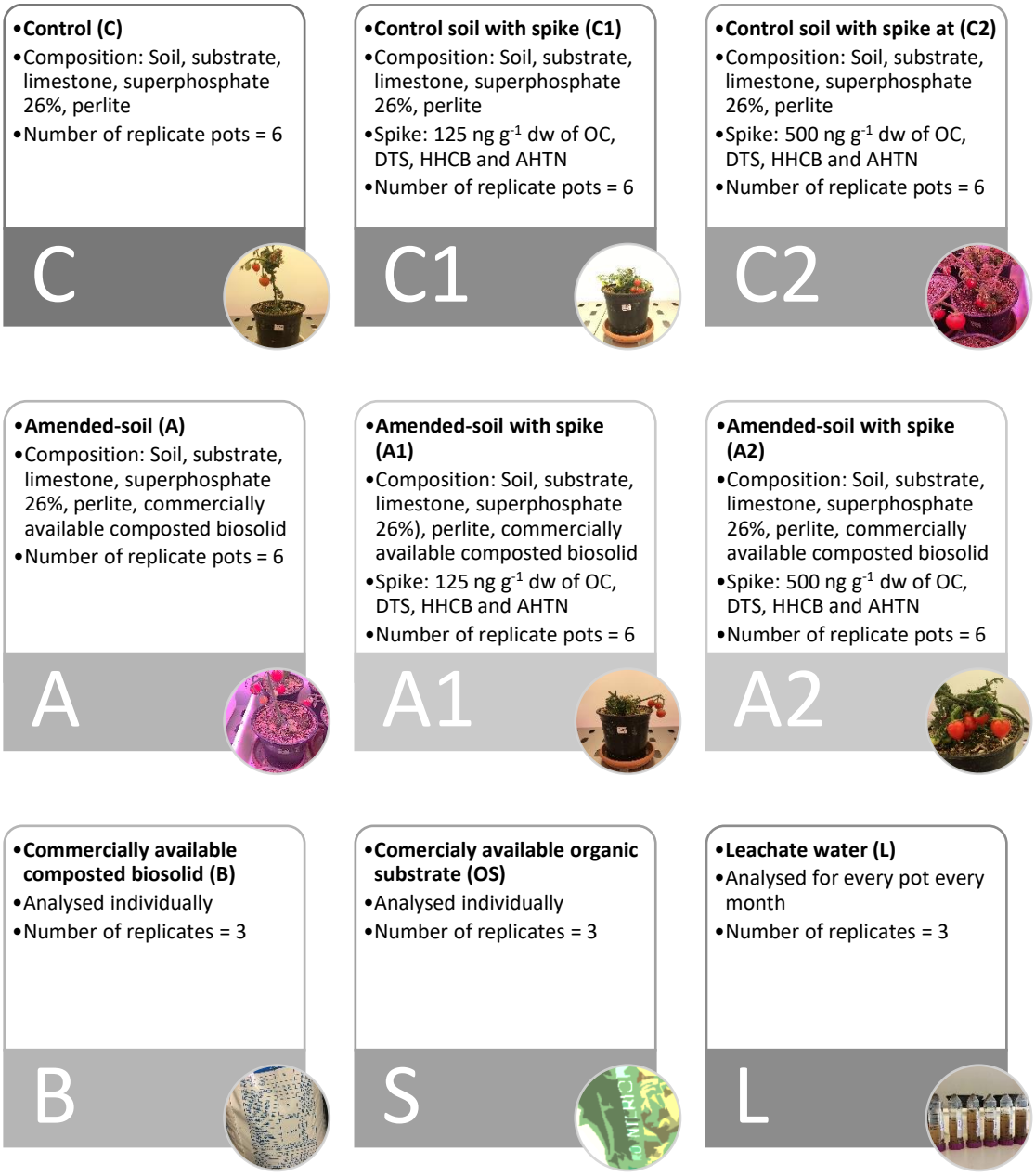


Figure 7.1. Plant uptake experiences layout.

7.2.3 Sample extraction and instrumental analysis

The collected samples (soil, tomato and leachates), as well as the composted biosolid and the organic substrate used in these experiments were analysed using methodologies described elsewhere (Ramos et al., 2020, 2019a, 2019b). More information about the extraction methodologies used could be found in the Annex 6.

The obtained extracts were analysed using a SCION 436-Gas Chromatograph (GC) coupled to an EVOQ Triple Quadrupole Mass Spectrometer from Bruker (Massachusetts, EUA), equipped with a J&W CP-Sil 8 CB capillary column (50 m x 0.25 mm I.D. x 0.12 µm) from Agilent Technologies (Santa Clara, California, EUA). The column operated in the following conditions: helium (99.999%) as carrier gas at 1.0 mL min^{-1}; injection in splitless mode (2 µL) at 280 ^{o}C; GC oven temperature program - 70 ^{o}C for 1 min, raised to 180 ^{o}C at 25 ^{o}C min^{-1}, then raised to 240 ^{o}C at 10 ^{o}C min^{-1} and finally raised to 300 ^{o}C at 25 ^{o}C min^{-1} (hold 5 min); transfer line at 270 ^{o}C. The parameters used in the mass spectrometer were as follows: electron energy of 70 eV; ion source temperature of 280 °C; filament current of 40 µA; ultra-pure argon at 2.00 mTorr as collision gas. The MS/MS analysis was performed in electron ionization (EI) mode, using the multiple reaction monitoring (MRM) method. Two transitions were chosen for each compound (quantifier/qualifier ion transitions), except for the nitro musks and surrogates, where two qualifiers were used for better identification (Table S6.2, Annex 6).

7.2.4 Quality Assurance and Control (QA/QC)

As mentioned before, UVFs and SMCs are incorporated in most personal care and household products and therefore, precautions were taken to avoid external contamination of the samples. No scented products or containing UV-filters were allowed in the laboratory. Calibrated glassware was decontaminated by rinsing with hexane, while other glassware was further subjected to thermal decontamination (1 h at 400 ^{o}C). Lab and analytical blanks were performed to identify background levels of the target analytes and implement appropriate corrections to the results.

In the QA/QC analysis, method quantification limits (MQLs), precision (assessed by relative standard deviation, RSD) and accuracy (assessed by recovery tests) were also assessed for each matrix studied. For soils, MQLs ranged between 0.03 and 46 ng g^{-1} dw and average recoveries from 81 to 122% (samples fortified at 10, 50 and 200 ng g^{-1} dw), while for biosolids, MQLs varied from 2 and 4648 ng g^{-1} dw and average recoveries between 75 and 122% (samples fortified at 50, 250 and 2500 ng g^{-1} dw) (Ramos et al., 2019a). For the tomato, recoveries ranged between 81 and 119%, and the MQLs between 0.4 and 47.9 ng g^{-1} dw (samples fortified at 4, 19 and 38 ng g^{-1} dw) (Ramos et al., 2020). For water samples, the leachates, recoveries ranged between 80 and 120%, and the MDLs between 0.1 and 20.0 ng L^{-1} (samples fortified at 50, 250, 500 and 1500 ng L^{-1}) (Ramos et al., 2019b) . All samples were analysed in triplicate and the calculated relative standard deviation was less than 10%.

7.2.5 Exposure assessment and risk characterization

Exposure to UVFs and SMCs through the ingestion of the tested tomatoes was assessed and risk characterization was estimated based on the no-adverse-effect level (NOAEL) values, for each studied compound (Table S6.1, Annex 6). For the compounds where no NOAEL value was found, LD_{50} (lethal dose required to kill 50% of the test population) or NOEL (no-observed-effect level) was considered. Then, an acceptable daily intake (ADI) was calculated by dividing the NOAEL or LD_{50} by a safety factor (SF). This SF should consider human variability (10x), extrapolation of toxicological data from animals to humans (10x) and, if toxicity data used are based on acute tests, an extra factor of 10 should still be considered (Commission, 2003; EPA, 1993). Then, the tolerable weekly intake (TWI) was calculated by multiplying the ADI by 7 (number of days in a week) (Zarn et al., 2015). The estimated exposure dose (EED) was determined by multiplying the concentration found for each compound in tomato (in wet weight) by its intake (466.2 g per person per week) (WHO, 2003), assuming 60 kg as the weight of an adult consumer. Whenever calculated exposure levels exceeds the established TWI, it means that the compound is present at levels considered hazardous to human health and therefore, risk management mechanisms should be triggered (Barlow and Schlatter,

2010; Cunha et al., 2015). Therefore, hazard quotients (HQ) were calculated as the ratio of the EED and the TWI, and risk is considered if HQ>1 (IGHRC, 2009).

The UVFs and SMCs accumulation in the tomato fruit was also estimated, using the bioconcentration factor (BCF) (Al-Farsi et al., 2017; Trapp and Legind, 2011). The BCF of compounds in plant tissues was calculated as the ratio of the chemical concentration in the plant tissue (the tomato fruit) by the nominal concentration in the growth medium (in this case the soil) at harvest point.

7.3. Results and Discussion

7.3.1 Samples characterization

The properties of both soil and commercially available fertilizer are described in Table 7.1. An agricultural soil poor in organic carbon (OC) and classified as silt loam (64.4% of silt) was selected to these experiments. Regarding the available nutrients, this soil is also poor in phosphorus (P_2O_5) and nitrogen (N). On the other hand, the fertilizer produced from sewage sludge has a considerable amount of OC (42%) and is rich in nutrients, being appropriate to correct the nutritional deficiencies of the soil used.

Table 7.1 Physicochemical properties of the soil and fertilizer used

	Agricultural Soil	Fertilizer
Location	Vila Real (Portugal)	Porto
OC	1.5%	42%
Clay (0-2 µm)	4.8%	
Silt (2-63 µm)	64.4%	99% of the particles have a diameter between 1 and 10 mm
Sand (63-2000 µm)	30.7%	
Soil texture	Silt loam	
pH H_2O	4.9	6.0
pH KCl	4.3	6.0
P_2O_5 (mg kg^{-1})	5	6897
K_2O (mg kg^{-1})	83	3448
Ca (mg kg^{-1})	174	15,517
Mg (mg kg^{-1})	74	1724
B (mg kg^{-1})	0.05	8.6
N (mg kg^{-1})	980	24,138

Prior to soil-amendment, background contamination by UVFs and SMCs was determined for control soil (S), the substrate (OS) and commercially available composted biosolid (B). The main results are presented in Table 7.2.

Table 7.2 UVFs and SMCs contamination (n= 6) in agricultural soil (S), substrate (OS) and commercially available composted biosolid (B), presented in ng g^{-1} dw (n.d.- not detected)

Compounds	S	OS	B
BZ	2.8±0.3	67±12	205±13
4MBC	2.4±0.6	n.d.	49±2
EDP	n.d.	6±1	n.d.
EMC	4.09±0.02	7±2	1.7±0.5
OC	5.21±0.01	13±4	1519±22
DTS	n.d.	n.d.	1629±54
Total UVFs	**14.4±0.9**	**93±19**	**3404±92**
DPMI	n.d.	n.d.	58±6
ADBI	n.d.	n.d.	32.9±0.8
AHMI	0.92±0.01	n.d.	6.8±0.1
ATII	n.d.	n.d.	21.3±0.8
HHCB	5.3±0.2	37±6	17,141±594
AHTN	2.2±0.1	8±1	4417±198
Total Polycyclic SMCs	**8.4±0.3**	**46±8**	**21,676±800**
EXA	6±1	20±18	n.d.
EB	n.d.	76±19	14.5±0.3
Total Macrocyclic SMCs	**6±1**	**96±36**	**14.5±0.3**
MA	n.d.	n.d.	n.d.
MX	n.d.	n.d.	n.d.
MM	n.d.	n.d.	17.6±0.1
MT	n.d.	n.d.	n.d.
MK	n.d.	n.d.	15.07±0.04
Total Nitro SMCs	**-**	**-**	**32.7±0.2**
Total per sample	***29±2***	***235±63***	***25,127±892***

The agricultural soil present UVFs, polycyclic and macrocyclic SMCs in concentrations ranging from n.d. to 6±1 ng g^{-1} dw (total concentration of 29±2 ng g^{-1} dw). The organic substrate shows higher amounts of these emerging pollutants (total concentration of 235±63 ng g^{-1} dw), being the UVFs and the macrocyclic SMCs the predominant compounds. As expected, the commercially available composted biosolid presented higher levels of the target compounds, with concentrations varying between n.d. and 17,141±594 ng g^{-1} dw (total concentration of 25,127±892 ng g^{-1} dw). The main contributors for this result were the polycyclic SMCs (around 86%). Nevertheless, the UVFs were also present at higher concentrations the other matrices. These results are

consisted with previously reported levels of these compounds in WWTPs sludge and biosolids (Homem et al., 2015; Ramos et al., 2016).

As mentioned before, Micro-Tom (*Solanum lycopersicum L.*) plant tomatoes were used in this study. This plant is often used in research due to its high growth rate under controlled conditions, producing small cherry-like tomatoes in large quantities and very quickly (70-90 days from sowing to fruit-ripening) (Gonzalez et al., 2015). The harvested tomatoes (about 47±12 g per pot) presented a high water content (91.3±0.8%).

7.3.2 Uptake and translocation of UVFs and SMCs by tomatoes

The uptake and translocation of UVFs and SMCs were assessed by the determination of these compounds in the tomato fruit, after growing plants of Micro-Tom tomatoes in pots under different conditions (Figure 7.1) for 6 months. Tomatoes were collected whenever considered mature for consume. The interest of this study lies in the compounds themselves due to their daily and massive use, which leads to their presence in high concentrations in the sewage sludge and, consequently, in commercial sewage sludge-based organic fertilizers (Table 7.2) that can be used by any farmer. As explained before, the tomato was chosen due to their high consumption rate in Europe and particularly in Portugal. Assays in which soils (amended or not) were fortified at different concentrations were performed to evaluate the maximum uptake and translocation of the target compounds to the tomato fruit. There was no significant difference in the biomass of plants grown in amended-soil and controlled soil, neither in the spiked experiments, indicating that there was no phytotoxicity or other effects from the added compounds (DTS, OC, HHCB and AHTN) at concentrations of 125 and 500 ng g^{-1}. To understand the uptake of the target compounds, concentrations of UVFs and SMCs were determined in the soil in the beginning of the experiment (t_0) and in the end (t_f) and in the tomato fruits. As explained before, the leachates were also collected along the experiments but did not show any of the compounds. In Table 7.3, those results are summarized.

Table 7.3 Average concentrations for each compound found in soil (t_0 and t_f) and in tomato fruits (ng g^{-1} dw), in control and amended-soil (n=6). (n.d.- not detected, MQL – method quantification limit)

Compounds	Soil t_0	Soil t_f	Tomato
BZ			
Control	3.8±0.5	1.0±0.4	33±10
Amended-soil	8.7±0.3	2.6±0.4	52±20
4MBC			
Control	n.d.	<MQL	19±5
Amended-soil	13.8±0.4	<MQL	74±43
EDP			
Control	n.d.	n.d.	n.d.
Amended-soil	n.d.	n.d.	5±3
EMC			
Control	4.16±0.07	n.d.	10±6
Amended-soil	4.31±0.01	n.d.	21±18
OC			
Control	5.0±0.7	8±5	33±5
Amended-soil	84±2	9±2	158±63
DTS			
Control	n.d.	n.d.	n.d.
Amended-soil	56±2	15±2	45±3
∑UVFs			
Control	13±1	9±7	94±26
Amended-soil	111±3	26±5	311±148
DPMI			
Control	n.d.	n.d.	n.d.
Amended-soil	1.36±0.05	n.d.	5±3
ADPI			
Control	n.d.	n.d.	n.d.
Amended-soil	1.62±0.01	0.28±0.02	n.d.
AHMI			
Control	n.d.	n.d.	n.d.
Amended-soil	1.04±0.01	n.d.	n.d.
ATII			
Control	n.d.	n.d.	2.4±0.2
Amended-soil	2.52±0.02	<MQL	2.6±0.2
HHCB			
Control	2.0±0.6	1.5±0.8	35±11
Amended-soil	341±5	56±19	65±29
AHTN			
Control	1.5±0.1	n.d.	2±1
Amended-soil	88±1	21±3	4±2
∑Polycyclic SMCs			
Control	3.5±0.7	1.5±0.8	39±12
Amended-soil	435±6	77±23	76±61
EXA			
Control	n.d.	n.d.	<MQL
Amended-soil	n.d.	n.d.	187±52
EB			
Control	n.d.	<MQL	n.d.
Amended-soil	n.d.	<MQL	22±14
∑Macrocyclic SMCs			
Control	n.d.	n.d.	n.d.
Amended-soil	n.d.	n.d.	209±65
MX			
Control	n.d.	n.d.	n.d.
Amended-soil	n.d.	23±4	n.d.
MK			
Control	n.d.	n.d.	n.d.
Amended-soil	n.d.	n.d.	1±1
∑Nitro SMCs			
Control	n.d.	n.d.	n.d.
Amended-soil	n.d.	23±4	1±1

In general, the results show that in soil the concentrations of the target compounds decreased throughout the experiments. As expected, due to the previous characterization of the fertilizer, amended-soil had higher levels of contaminants. Higher concentrations were also found in tomato samples that grew up in amended-soil than in the controls. At the beginning of the study, polycyclic musks were the predominant pollutants in the amended-soil (total concentration of 435±6 ng g^{-1} dw), but UVFs were the compounds that seem to be more easily uptake and translocated to the tomatoes (total concentration of 311±148 ng g^{-1} dw).

In this study, nitro musks were not found in any of the controls (soil, substrate or fertilizer). However, MX was detected in the amended soil t_f, but not in t_0 (at the beginning) nor in tomatoes. A similar behaviour was found in the spiked amended-soil experiments. This may be explained by external contamination, since the camera is a walk-in type and the high organic matter content present in amended-soil may favour the retention of this contaminant. The macrocyclic musks EB and EXA were both found in the tomato fruit in concentrations of 187 and 22 ng g^{-1} dw, respectively, but could not be detected in soil. Nevertheless, both compounds were detected in the substrate (OS) and commercially available composted biosolid (B), which could mean that although they were not detected in the control and in the amended-soil, they were present in the mixture at levels lower than those detected by the method and could somehow translocate to the tomato and be detected there due to the pre-concentration during extraction.

To the authors' best knowledge, there is only one study where UVFs and SMCs were determined in different species of tomato. In that study, concentrations ranged from 1 (AHTN) to 210 (BZ) ng g^{-1} dw (Ramos et al., 2020), which are slightly higher than those found in tomatoes from controls (1.7 - 35.1 ng g^{-1} dw), but similar to the concentrations found in the tomatoes grown in amended-soil (2-180.0 ng g^{-1} dw). Different studies have also investigated the uptake of some SMCs in other vegetables and fruits, such Litz et al. (2007). Experiments were conducted in greenhouses to study the uptake of HHCB and AHTN by lettuce and carrots. These compounds were found in carrot leaves in concentrations of 107 – 1070 ng g^{-1} dw and 115 – 902 ng g^{-1} dw, and in lettuce in 78 – 110 ng g^{-1} dw and 209 – 275 ng g^{-1} dw, respectively. The levels found were much higher

than those found in the current study. Another study performed in a field, studied the presence of different SMCs in wheat and sugar beet leaves. DPMI (0.26 ng g^{-1} dw), ADBI (0.02 – 0.05 ng g^{-1} dw), HHCB (3.65 – 4.6 ng g^{-1} dw), AHTN (0.44 – 0.62 ng g^{-1} dw) and MX (0.09 ng g^{-1} dw) were detected in wheat, while DPMI (0.16-0.22 ng g^{-1} dw) in sugar beet leaves (Fussell et al., 2013). Also field experiments with alfalfa, HHCB was found at 16.9 ng g^{-1} dw (D. Calderón-Preciado et al., 2011) and in concentrations ranging 0.032 and 67.6 ng g^{-1} dw (Diana Calderón-Preciado et al., 2011). In literature, most uptake studies using tomato plants investigated the presence of pesticides (e.g. (Bidari et al., 2011), (Martins et al., 2017)).

The partition of the target compounds was analysed by a mass balance to better understand their fate and presence in each matrix. It is important to realize that if the target compounds could not be identified either in the soil at the end of the experiment (t_f) or in the tomato fruit, then they may have accumulated in other parts of the tomato plant that were not analysed (e.g. roots, stem, leaf), degraded or even volatilized. Table 7.4 shows the results of partition in soil-tomato plant system only for OC, DTS, HHCB and AHTN (the spiked compounds) in the different experiments.

Table 7.4 - Mass balance of the target compounds spiked in the controls (C1 and C2) and amended-soil (A1 and A2).

		Mass of target compounds (ng)			Partition in soil-tomato plant system		
Compounds	**Exp.**	**Soil t_0**	**Soil t_f**	**Tomato fruit**	**% Soil**	**% Tomato**	**%Loss**
OC	C1	78009	9273	261	12%	0.335%	88%
	A1	127778	27906	61	22%	0.047%	78%
	C2	303009	25092	167	8%	0.055%	92%
	A2	352778	41332	82	12%	0.023%	88%
				Average OC%	**13%**	**0.115%**	**86%**
DTS	C1	75000	14800		20%	0.000%	80%
	A1	110165	41166	80	37%	0.073%	63%
	C2	300000	47279		16%	0.000%	84%
	A2	335165	83915	129	25%	0.038%	75%
				Average DTS%	**24%**	**0.028%**	**75%**
HHCB	C1	76182	7517	131	10%	0.171%	90%
	A1	289630	50331	63	17%	0.022%	83%
	C2	301182	20822	110	7%	0.037%	93%
	A2	514630	53548	91	10%	0.018%	90%
				AverageHHCB%	**11%**	**0.062%**	**89%**
AHTN	C1	75788	10846	6	14%	0.008%	86%
	A1	130489	37853	5	29%	0.004%	71%
	C2	300788	33928	10	11%	0.003%	89%
	A2	355489	63306	18	18%	0.005%	82%
				Average AHTN%	**18%**	**0.005%**	**82%**

Regarding the mass results for OC, HHCB and AHTN in tomato, uptake and translocation was found in every tested condition, but always in percentages lower than 1% (determined based on the initial mass of the target compounds available for plant uptake in the soil). In average, 17% of the compounds remained in soil. As explained before, the mass of compounds not accountable in the soil-plant system is considered lost. This loss accounts for about 83% of the total target compounds mass available in the beginning of the experiment (leachates were also considered, but as mentioned before, the target compounds were not found in the collected water.

The initial mass of the compounds in the soil t_0 is higher in the amended-soil (A1 and A2) than in the controls (C1 and C2). However, that trend does not occur in the uptake and translocation to the tomato fruit and varies between compounds. In the case of the compound DTS, there is no uptake in any control experiment. This compound is the less volatile and the one with higher partition to soil, which could be explained by its high log K_{ow} (10.8). The partition between soil and fruit is smaller the larger the log K_{ow}. Although variations are very small, the compound AHTN appears to be the one with

lowest uptake for tomato, when compared to the other target compounds. And, even though, differences are not very high, HHCB, shows a higher % of partition from soil to the tomato plant system.

Differences in the target compounds partition between C1-A1 and C2-A2 do not appear to be significant. The increased organic matter in the amended-soil (A1, A2) (addition of a fertilizer with 42% of OC) when compared with a poor soil such as the control (C1, C2) with only 1.5% OC, does not appear to have an effect on the uptake and translocation of the compounds to the tomato fruit.

The bioconcentration factors (BCF) of UVFs and SMCs detected in the tomato were also calculated. Results are presented in Table S6.5 in the Annex 6. As mentioned before, for most UVFs and SMCs, a decline in concentrations in the soil samples was observed both in the controls and the amended-soils, indicating that the plant uptake the target compounds or they suffered soil microbial transformation or volatilized. The BCF values in this study are in the range of 0.2 to 44.4. The UVFs show BCFs of 3.1 (DTS) to 44.4 (BZ) and for SMCs, BCFs could only be calculated for HHCB (24.8 in control and 1.1 in the amended-soil), and AHTN (0.2 for the amended-soil). The calculations of BCF take into account the concentration of the target compounds in soil in the harvest period (Soil t_f), which means for most cases that UVFs and SMCs are not detected in the final soil. In these cases, it does not mean that the compounds did not bioconcentrate in the tomato fruit. In fact, if concentration in soil is tending to 0, the concentration ratio BCF is tending to infinite ($C_{plant}/C_{soil\ tf} \rightarrow \infty$) (Trapp and Legind, 2011).

7.3.3 Human exposure implications

Due to the demonstrated accumulation of UVFs and SMCs in the tomatoes, a fruit commonly incorporated in the Mediterranean diet, an exploratory assessment of the potential human risk due to their dietary intake was carried out. Main results are presented in Table 7.5.

Table 7.5 Results of the exposure assessment for SMCs and UVFs for different experiments, including the tolerable weekly intake (TWI) for each compound (µg kg^{-1} $_{bw}$ $week^{-1}$), estimated exposure dose (EED) (µg kg^{-1} $_{bw}$ $week^{-1}$), adult intake to exceed the TWI (AI) (kg $week^{-1}$) and hazard quotient (HQ).

Compounds	TWI	C			C1			C2			A			A1			A2		
		EED	AI	HQ	EED	AI	HQ	EED	AI	HQ	EED	AI	HQ	EED	AI	HQ	EED	AI	HQ
BZ	1750	3.0	40	**0.002**	4.2	27	**0.002**	4.5	26	**0.003**	4.6	25	**0.003**	2.3	51	**0.001**	2.0	57	**0.001**
4MBC	1750	1.7	70	**0.001**	-	-	**-**	-	-	**-**	6.6	18	**0.004**	8.8	13	**0.005**	5.5	21	**0.003**
EDP	7000	-	-	**-**	0.4	1209	**<0.001**	0.4	1134	**<0.001**	0.4	1069	**<0.001**	0.8	558	**<0.001**	0.8	567	**<0.001**
EMC	31500	0.8	2473	**<0.001**	1.1	1943	**<0.001**	0.8	2734	**<0.001**	1.9	1101	**<0.001**	1.4	1510	**<0.001**	0.6	3266	**<0.001**
OC	12250	3.0	277	**<0.001**	8.8	93	**<0.001**	6.1	134	**<0.001**	14.1	58	**0.001**	3.1	266	**<0.001**	2.4	336	**<0.001**
DTS	2222	-	-	**-**	-	-	**-**	-	-	**-**	4.0	37	**0.002**	3.6	41	**0.002**	3.7	40	**0.002**
DPMI	700	-	-	**-**	-	-	**-**	-	-	**-**	0.4	107	**<0.001**	-	-	**-**	0.001	51901	**<0.001**
ADBI	35000	-	-	**-**	-	-	**-**	-	-	**-**	-	-	**-**	-	-	**-**	-	-	**-**
AHMI	350	-	-	**-**	-	-	**-**	-	-	**-**	-	-	**-**	-	-	**-**	-	-	**-**
ATII	12	0.2	4	**0.020**	0.3	3	**0.020**	0.3	3	**0.02**	0.2	3	**0.020**	0.2	3	**0.020**	0.2	3	**0.020**
HHCB	3500	1.7	139	**<0.001**	1.7	138	**<0.001**	0.3	677	**<0.001**	16.7	14	**0.005**	10.9	21	**0.003**	10.1	23	**0.003**
AHTN	350	3.1	7	**0.010**	4.5	5	**0.010**	3.4	7	**0.010**	5.8	4	**0.020**	2.6	9	**0.007**	2.6	9	**0.007**
EXA	70000	0.2	28060	**<0.001**	0.2	29450	**<0.001**	0.3	14883	**<0.001**	0.3	13416	**<0.001**	0.2	21496	**<0.001**	0.5	9363	**<0.001**
EB	70000	-	-	**-**	-	-	**-**	-	-	**-**	2.0	2329	**<0.001**	5.8	800	**<0.001**	1.7	2743	**<0.001**
MX	1400	-	-	**-**	-	-	**-**	-	-	**-**	-	-	**-**	-	-	**-**	-	-	**-**
MK	1050	-	-	**-**	-	-	**-**	-	-	**-**	0.1	576	**<0.001**	0.04	1653	**<0.001**	0.04	1637	**<0.001**

Health risks associated to the consumption of biosolid-amended tomato fruits, as well as tomato fruits with no treatment or spiked with selected compounds during the period of 6 months were estimated by using the HQ approach. The estimated EED values demonstrate that the weekly consumption of 0.44 kg of tomatoes per person used in this study does not pose a health threat. Only a consumption of tomato ranging from 3 to 51,901 kg per week (AI, Table 7.5) reach the TWI for an adult. The conclusions regarding the risks of the studied compounds are similar to those found in other studies, namely a study in seafood with UVFs and SMCs (Cunha et al., 2015) and other only with SMCs (Trabalón et al., 2015). The absence of health implications due to the consumption of biosolid-amended grew tomatoes is further corroborated by the estimated low values (<0.02) of HQ (Table 7.5).

Although the preliminary results from this work suggest that the consumption of tomatoes grown in biosolid-amended soils should be considered safe, further studies are needed to reach a definite conclusion that the use of these organic fertilizers is considered a safe practice for the human health. Such studies should take into account the consumption of different foods (namely fruits and vegetables) that may bioaccumulate UVFs and SMCs in similar agricultural practices, the potential additivity or synergetic behaviour of the mixture of these and other compounds, potential toxic metabolites that may be present in plant tissues, the potential sensitivity of subgroups of the population (i.e. pregnant, infants, elderly people, and chronic sufferers) and the dietary habits of the population studied.

7.4. Conclusions

The application of biosolids or commercially available fertilizers produced based on biosolids to agricultural soils may facilitate the uptake of UVFs and SMCs by plants and consequently, their entrance into the food web. This represents an important pathway for the human's exposure to these compounds with potential implications. The present work intends to be the beginning of a study of two major classes of emerging contaminants. This 'in chamber' study yields the first results to understand the process of uptake and translocation of these compounds from biosolid-amended soil to the

tomato fruit, a widely consumed crop, during the period of six months. The concentration of UVFs and SMCs were also determined in soil, substrate, biosolid-amended commercial fertilizer and leachates.

Results showed that the concentration of the target compounds in soil decreased along the experiments, showing that these compounds accumulate in the tomato fruit. In addition, a mass balance to each experiment showed that there was a loss of compounds that could not be identified and should be explained by the accumulation of the target compounds in other parts of the tomato plant that were not analysed, by potential degradation or volatilization.

Based on the risk assessment performed, a potential health risk it is not likely to exist because the EED values are considerably lower than TWI calculated for each compound, meaning HQ values lower than 0.02. Nevertheless, further studies need to be performed to ensure that these commercially available fertilizers are safe and do not compromise public health.

Declaration of interest

The authors declare no competing interests.

Supporting Information

All the supporting information can be found in Annex 6.

Acknowledgements

This work was financially supported by: (i) Project UID/EQU/00511/2019 - Laboratory for Process Engineering, Environment, Biotechnology and Energy – LEPABE funded by national funds through FCT/MCTES (PIDDAC); (ii) Project PTDC/ASP-PLA/29425/2017 - POCI-01-0145-FEDER-029425 - funded by FEDER funds through COMPETE2020 -

Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES; (iii) Doctoral Grant SFRH/BD/110831/2015 – Sara Ramos.

Authors would like to acknowledge the Research Centre in Biodiversity and Genetic Resources (CIBIO, Porto, Portugal) and specially Dra. Mariana Sottomayor, Dr. Humberto Castro and Dr. Sara Freitas for the knowledge shared in the experimental work and for lending us the 'walk-in' chamber for six months. Also, the contribution of Eng. Carlos Ramos for all technical assistance.

References

Aguirre, J., Bizkarguenaga, E., Iparraguirre, A., Fernández, L.Á., Zuloaga, O., Prieto, A., 2014. Development of stir-bar sorptive extraction-thermal desorption-gas chromatography-mass spectrometry for the analysis of musks in vegetables and amended soils. Anal. Chim. Acta 812, 74–82.

Al-Farsi, R.S., Ahmed, M., Al-Busaidi, A., Choudri, B.S., 2017. Translocation of pharmaceuticals and personal care products (PPCPs) into plant tissues : A review. Emerg. Contam. 3, 132–137.

Apedaile, E., 2001. A perspective on biosolids management. Can. J. Infect. Dis. 12, 202–204.

Barlow, S., Schlatter, J., 2010. Risk assessment of carcinogens in food. Toxicol. Appl. Pharmacol. 243, 180–190.

Bidari, A., Reza, M., Norouzi, P., Reza, M., Hosseini, M., 2011. Sample preparation method for the analysis of some organophosphorus pesticides residues in tomato by ultrasound-assisted solvent extraction followed by dispersive liquid – liquid microextraction. Food Chem. 126, 1840–1844.

Calderón-Preciado, D., Jiménez-Cartagena, C., Matamoros, V., Bayona, J.M., 2011. Screening of 47 organic microcontaminants in agricultural irrigation waters and their soil loading. Water Res. 45, 221–231.

Calderón-Preciado, Diana, Matamoros, V., Bayona, J.M., 2011. Occurrence and potential crop uptake of emerging contaminants and related compounds in an agricultural irrigation network. Sci. Total Environ. 412–413, 14–19.

Calderón-Preciado, D., Matamoros, V., Savé, R., Muñoz, P., Biel, C., Bayona, J.M., 2013. Uptake of microcontaminants by crops irrigated with reclaimed water and groundwater under real field greenhouse conditions. Environ. Sci. Pollut. Res. 20, 3629–3638.

Calderón-Preciado, D., Renault, Q., Matamoros, V., Cañameras, N., Bayona, J.M., 2012. Uptake of organic emergent contaminants in spath and lettuce: An in vitro experiment. J. Agric. Food Chem. 60, 2000–2007.

Commission, E., 2003. Introduction to Risk Assessment.

Cunha, S.C., Trabalón, L., Jacobs, S., Castro, M., Fernandez-Tejedor, M., Granby, K., Kwadijk, C., Verbeke, W., Ferrari, F., Robbens, J., Sioen, I., Fernandes, J.O., Pocurull, E., Domingo, J.L., Marques, A., 2015. UV-filters and musk fragrances in seafood commercialized in Europe Union: Occurrence, risk and exposure assessment. Environ. Res. 143, 116–122.

EPA, 1993. Reference Dose (RfD): Description and Use in Health Risk Assessments.

Evgenidou, E.N., Konstantinou, I.K., Lambropoulou, D.A., 2015. Occurrence and removal of transformation products of PPCPs and illicit drugs in wastewaters: A review. Sci. Total Environ.

Fussell, R.J., Garcia Lopez, M., Mortimer, D.N., Wright, S., Sehnalova, M., Sinclair, C.J., Fernandes, A., Sharman, M., 2013. Investigation into the occurrence in food of veterinary medicines, pharmaceuticals, and chemicals used in personal care products, Report to the Food Standards Agency.

Gonzalez, C., Ré, M.D., Sossi, M.L., Valle, E.M., Boggio, S.B., 2015. Tomato cv. 'Micro-Tom' as a model system to study postharvest chilling tolerance. Sci. Hortic. (Amsterdam). 184, 63–69.

Gürsoy, M., Karaman, M., 2018. Improvement of wetting properties of expanded perlite particles by an organic conformal coating. Prog. Org. Coatings.

Homem, V., Silva, A., Ratola, N., Santos, L., Alves, A., 2015. Long lasting perfume - A review of synthetic musks in WWTPs. J. Environ. Manage. 149, 168–192.

Hurtado, C., Domínguez, C., Pérez-Babace, L., Cañameras, N., Comas, J., Bayona, J.M., 2016. Estimate of uptake and translocation of emerging organic contaminants from irrigation water concentration in lettuce grown under controlled conditions. J. Hazard. Mater. 305, 139–148.

IGHRC, 2009. Chemical Mixtures: A framework for assessing risks to human health (CR14).

INE, I.N. de E., 2019. Estatísticas Agrícolas 2018.

Lai, H.J., Ying, G.G., Ma, Y.B., Chen, Z.F., Chen, F., Liu, Y.S., 2014. Field dissipation and plant uptake of benzotriazole ultraviolet stabilizers in biosolid-amended soils. Environ. Sci. Process. Impacts 16, 558–566.

Litz, N.T., Mueller, J., Boehmer, W., 2007. Occurrence of polycyclic musks in sewage sludge and their behaviour in soils and plants: Part 2: Investigation of Polycyclic Musks in Soils and Plants. J. Soils Sediments 7, 36–44.

Macherius, A., Eggen, T., Lorenz, W.G., Reemtsma, T., Winkler, U., Moeder, M., 2012. Uptake of galaxolide, tonalide, and triclosan by carrot, barley, and meadow fescue plants. J. Agric. Food Chem. 60, 7785–91.

Martins, M.L., Kemmerich, M., Prestes, O.D., Maldaner, L., Jardim, I.C.S.F., Zanella, R., 2017. Evaluation of an alternative fluorinated sorbent for dispersive solid-phase

extraction clean-up of the quick , easy , cheap , effective , rugged , and safe method for pesticide residues analysis. J. Chromatogr. A 1514, 36–43.

Prosser, R.S., Sibley, P.K., 2015. Human health risk assessment of pharmaceuticals and personal care products in plant tissue due to biosolids and manure amendments, and wastewater irrigation. Environ. Int. 75, 223–233.

Qin, Q., Chen, X., Zhuang, J., 2015. The Fate and Impact of Pharmaceuticals and Personal Care Products in Agricultural Soils Irrigated With Reclaimed Water. Crit. Rev. Environ. Sci. Technol. 45, 1379–1408.

Ramos, S., Homem, V., Alves, A., Santos, L., 2016. A review of organic UV-filters in wastewater treatment plants. Environ. Int. 86, 24–44.

Ramos, S., Homem, V., Santos, L., 2020. Analytical methodology to screen UV- filters and synthetic musk compounds in market tomatoes. Chemosphere 238, 124605.

Ramos, S., Homem, V., Santos, L., 2019a. Development and optimization of a QuEChERS-GC–MS/MS methodology to analyse ultraviolet-filters and synthetic musks in sewage sludge. Sci. Total Environ. 651, 2606–2614.

Ramos, S., Homem, V., Santos, L., 2019b. Simultaneous determination of synthetic musks and UV-filters in water matrices by dispersive liquid-liquid microextraction followed by gas chromatography tandem mass-spectrometry. J. Chromatogr. A 1590, 47–57.

Rodríguez-Rodríguez, C.E., Barón, E., Gago-Ferrero, P., Jelić, A., Llorca, M., Farré, M., Díaz-Cruz, M.S., Eljarrat, E., Petrović, M., Caminal, G., Barceló, D., Vicent, T., 2012. Removal of pharmaceuticals, polybrominated flame retardants and UV-filters from sludge by the fungus Trametes versicolor in bioslurry reactor. J. Hazard. Mater. 233–234, 235–243.

Sablayrolles, C., Patria, L., Montréjaud-Vignoles, M., Silvestre, J., 2006. Sand to root transfer of PAHs and PCBs by carrots grown on sand with pure substances and biosolids amended sand, in: *Acta Horticulturae*.

Sabourin, L., Beck, A., Duenk, P.W., Kleywegt, S., Lapen, D.R., Li, H., Metcalfe, C.D., Payne, M., Topp, E., 2009. Runoff of pharmaceuticals and personal care products following application of dewatered municipal biosolids to an agricultural field. Sci. Total Environ. 407, 4596–4604.

Sharma, B., Sarkar, A., Singh, P., Singh, R.P., 2017. Agricultural utilization of biosolids: A review on potential effects on soil and plant grown. Waste Manag. 64, 117–132.

Shenker, M., Harush, D., Ben-Ari, J., Chefetz, B., 2011. Uptake of carbamazepine by cucumber plants - A case study related to irrigation with reclaimed wastewater. Chemosphere 82, 905–910.

Shikata, M., Ezura, H., 2016. Micro-Tom Tomato as an Alternative Plant Model System: Mutant Collection and Efficient Transformation, in: Botella, J.R., Botella, M.A. (Eds.), Plant Signal Transduction: Methods and Protocols. Springer New York, New York, NY, pp. 47–55.

Trabalón, L., Cano-Sancho, G., Pocurull, E., Nadal, M., Domingo, J.L., Borrull, F., 2015. Exposure of the population of Catalonia (Spain) to musk fragrances through seafood consumption: Risk assessment. Environ. Res. 143, 116–122.

Trapp, S., Legind, C.N., 2011. Uptake of Organic Contaminants from Soil into Vegetables and Fruits, in: Dealing with Contaminated Sites. Springer, pp. 369–408.

WHO, W.H.O., 2003. GEMS/Food regional diets : regional per capita consumption of raw and semi-processed agricultural commodities / prepared by the Global Environment Monitoring System/Food Contamination Monitoring and Assessment Programme (GEMS/Food). Food safety issues.

Zarn, J.A., Hänggi, E., Engeli, B.E., 2015. Impact of study design and database parameters on NOAEL distributions used for toxicological concern (TTC) values. Regul. Toxicol. Pharmacol. 72, 491–500.

Part V: Final Remarks

In this section, final considerations and conclusions are discussed as well as proposals for future work to be developed.

Chapter 8. General discussion and conclusions

The work developed during this project aimed to contribute to increase the knowledge regarding the potential risk to the soil of using commercially available sewage sludge-based fertilizers. It also aimed to study a possible uptake of UVFs and SMCs by the tomato fruit (Micro-Tom crop) when grown in amended soil and to assess the human risk based on the weekly intake of this crop.

This work was focused on two classes of PCPs, which are considered emerging pollutants due to their massive use, widespread presence in the environment, they are not regulated and commonly monitored and have the potential to cause adverse ecological and/or human health effects even at low levels. The classes chosen were the UVFs and SMCs, due to their frequent use in daily life products and the recent concerns regarding some of the compounds belonging to these classes.

In order to understand the cycle of these compounds since they reach the WWTPs until their potential application in crops, several analytical methodologies were developed and validated. The instrumental analysis was performed in a GC-MS/MS system, due to the high sensitivity of the electron ionization source, high selectivity and ability to analyse complex matrices and work at low levels of quantitation. This is particularly important because of the different type and complex matrices used and the need to detect the target compounds at trace levels. The disadvantage of using GC when compared to LC, for example, is the difficulty in analysing less volatile compounds, like most UVFs. Therefore, several compounds with potential interest could not be included in this study. Nevertheless, the compounds chosen for this work, are representative of the most used compounds incorporated in personal care products, and the fact that we were able to analyse them without derivatization, with 'green methodologies', and validate the methodologies with good accuracy and precision, means that, in the end, the instrumental equipment was well chosen.

A method was developed for water matrices, consisting on a DLLME technique using 880 µL IPA as dispersive solvent and 80 µL TC as extractant solvent to extract 6 mL of water. To the authors' best knowledge, this is the first method based on DLLME and GC–MS/MS for the analysis of all compounds without a derivatization process, which allows a faster and more reliable methodology. The performance of the method was demonstrated in terms of linearity, accuracy and precision (RSD < 10%). Recoveries varied between 83 and 123% for different water matrices such as river, lake, sea, tap water and wastewater. Wastewater samples showed that UVFs and SMCs were present in the ng to µg L^{-1} levels. Influent wastewater concentrations varied from 2.1 ± 0.8 ng L^{-1} (EMC) to 5.8 ± 0.2 µg L^{-1} (HHCB) and effluent wastewater from 2.1 ± 0.2 ng L^{-1} (ADBI) to 3.80 ± 0.05 µg L^{-1} (HHCB), showing a decreasing trend from the influent to the effluent. This methodology was developed with the final purpose of leachate analysis in the final experiment, nevertheless, it was found that a single method, could be used for more than one water matrix. Samples for each matrix were analysed from two different locations and in triplicate. For example, river 1 showed a total concentration of 86.9±3.5 ng L^{-1}, whereas river 2 has more than double (230.9±5.7 ng L^{-1}). No contaminants where detected in tap water, which is a good indicator.

The method developed for sludge and compost samples was based in a QuEChERS methodology followed by GC–MS/MS analysis. It was successfully developed and optimized using a design of experiments approach. Firstly, a screening design was used to understand which parameters influence the most the extraction, and then a central composite design was performed to optimize the selected parameters. The optimal conditions for this QuEChERS procedure were: 10 mL ACN, 2.5 min vortex, 15 min ultrasound extraction, 500 mg $MgSO_4$, 315 mg PSA and 410 mg C_{18} for the QuEChERS. These conditions lead to compound recoveries between 75% (DPMI) and 122% (EMC). The method achieved low %RSD in the inter- and intra-day precision (<10%) and low limits of detection. The application of the method to real samples showed that the most used UVFs (OC and DTS) and SMCs (HHCB and AHTN) presented the higher concentrations. Overall, concentrations of polycyclic musks varied from 12.6 (AHMI) to 81,771 ng g^{-1} dw (HHCB), macro musk from 1461 to 36,705 ng g^{-1} dw (EB) and UVFs from 12.5 (EDP) to 115,486 ng g^{-1} dw (OC). Among

nitro musks, only MK was detected in the samples, with concentrations between 25 and 471 ng g^{-1} dw. Real sludge samples were analysed from summer and winter sampling campaigns, the results showed that SMCs and UVFs end up in sludge at concentrations ranging from few ng g^{-1} dw to hundreds of μg g^{-1} dw without a significant difference between the two seasons. This study was the first to analyse the DTS (drometrizole trisiloxane) in sludge, revealing concentrations in the order of the μg g^{-1} dw. And this UVF is especially interesting, since it is only used in products owned by the L'Óreal brand, which can indicate a high consume of these products in the region where samples presented the highest concentrations.

The method developed for soils was also based on a QuEChERS approach followed by GC–MS/MS analysis, which was adapted from a published methodology used for the extraction of pesticides. In the developed methodology, the d-SPE only contained 3 g of $MgSO_4$ and 300 mg of C_{18}. The use of water in the beginning of the procedure was also very important, allowing the greatest penetration of the extraction solvent, which was added later. The characterization of soils is also very important, namely the organic carbon content, and particle size (clay, silt and sand). The method was applied to soils with different % of organic carbon and particle sizes. The low % of organic carbon is an important pattern to understand the quality of the soil. Accuracy, assessed by recovery tests, ranged from 81% to 122% and a good precision was achieved, with RSD<10%. The instrumental limit of detection (ILOD) varied from 0.01 pg to 3.75 pg. Regarding the method quantification limits (MQLs), they ranged between 0.03 and 46 ng g^{-1} dw. Both SMCs and UVFs were detected in soils samples from different origins (agricultural, industrial, garden, beach and school yard), but higher levels were found for BZ (maximum value of 158 ng g^{-1} dw), OC (137 ng g^{-1} dw) and ATII (9.0 ng g^{-1} dw). Studies where agricultural soil was mixed with sewage sludge-based fertilizer in a proportion of 95% soil and 5% fertilizer, showed an increase of the UVFs and SMCs concentrations. Overall, concentration ranged between 1.04 (AHMI) and 341 ng g^{-1} dw (HHCB) in the amended soil. The analysis of the amended-soil showed that there was an accumulation of the target contaminants. Therefore, in the final study, further experiments should be conducted with amended-soils, testing different initial concentrations of some

target compounds by spiking the soil at relevant concentrations, and monitoring contaminant concentrations over time. This study among others, recognizes the importance of including ubiquitous environmental pollutants in sludge disposal regulations.

After developing analytical methodologies for the determination of the target analytes in environmental matrices, the work proceeded to the analysis in plants. The plant chosen for this study was the tomato, due to the availability throughout the year, consumption per capita and Portugal's biggest crop production.

The methodology developed to extract UVFs and SMCs from tomatoes was also based on a QuEChERS approach followed by GC–MS/MS analysis. Considering the experience optimizing this type of methodology, the easiness to perform it, the low amounts of solvent and sorbents required as well as sample needed for the extraction, this was the preferable methodology. The validated method yielded good recoveries for the sample matrix tested (81 - 119%), with RSD<10%. The developed extraction procedure was also applied to different varieties of tomatoes bought in supermarkets (e.g. cherry, loose round, bunch, plum, Anairis and oxheart). Concentrations varied between 1 (AHTN) and 210 (BZ) ng g^{-1} dw. To the authors' best knowledge, this was the first attempt focused on the analysis of UVFs and SMCs in tomato samples and on the assessment of human populations exposure to these compounds through an integrated approach. Although most UVFs and SMCs were detected in the analysed samples, human exposure levels estimated based on detected concentration levels in raw tomato samples were far below the estimated toxicological reference values. This was the first approach to understand the tomato fruit potential to uptake UVFs and SMCs, at which levels they could be found and if at these levels ('supermarket levels') were considered a risk for human consumption.

A final experiment was performed integrating all the previously studied matrices. The uptake and translocation of UVFs and SMCs into edible parts of the tomato fruit grown in amended soils was assess by a six month experiment in a 'walk-in' chamber with controlled conditions of temperature, humidity and light. Micro-Tom cultures of tomato plants were grown in pots with different types of soil mixtures. Results showed that the concentration

of the studied compounds in soil decreased from the beginning of the experiment to the end and that there was an accumulation of UVFs and SMCs in the tomato fruit. A mass balance to each experiment showed that there was a loss of the target compounds in the soil-tomato plant system that could not be directly quantified. This loss in the system analysed means that the target compounds could either be in the parts of the tomato plant that were not analysed (roots, leaves or stems), they could be degraded in soil or volatized. This volatilization probably happened, since the 'walk-in' chamber had a ventilation system to maintain the controlled conditions inside the room. This mass balance was performed using the experiments with fortification, analysing the behaviour of OC, DTS, HHCB and AHTN. It considers the initial amount of these compounds in soil available (t_0) to be taken-up by plants, their partition to the tomato fruit and their remaining amount in soil at the end of the experiment (t_f). The partition in the soil-tomato plant system was estimated based on the ratios between the mass of compounds in the tomato fruit or soil (t_f) and the initial mass present in the soil available for the uptake. Results showed that less than 1% of the available mass in soil (t_0) was uptake and translocated into the tomato fruit, either in the control and the amended-soil experiments. Although there are some variations between compounds, 0.005% (AHTN) to 0.115% (OC), they are not very significative. The similarities found in the partitions to the tomato in the control and amended-soil experiments were very similar. This may be explained by the maximum capacity of accumulation of these compounds in the fruit. Further studies needed to be performed in order to prove this conjecture. Also, the log K_{ow} of the target compounds could have some role in the ability to uptake and translocate to the tomato fruit, since the higher the value, the lower is expected the partition between soil and fruit.

Based on the risk assessment performed, a potential health risk was not likely to exist because the estimated exposure dose (EED) values were considerably lower than the tolerable weekly intake (TWI) value calculated for each compound. Also, the hazard coefficient (HQ) values were lower than 0.02. Nevertheless, more studies need to be performed in order to be perfectly sure that these biosolid commercially available fertilizers are safe for soil amendment without endangering public health.

Chapter 9. Future work

To improve the knowledge regarding the uptake and translocation of contaminants of concern, further studies need to be performed.

So, some suggestions for future work are:

- To study the PCPs sorption behaviour in soils and mixtures of sludge or compost/soil - laboratory studies of adsorption/desorption (evaluation of the effect of some parameters like initial pollutants concentration, temperature, pH, etc.; kinetics; isotherms);
- To perform dissipation studies (to assess if microbial attack might have any effect in PCPs degradation/removal from amended soils) and leaching (to understand if PCPs are washed away in soils and how deep they can get);
- To investigate the uptake of PCPs by vegetables after crop fertilization with sludge/compost - studies developed in farmlands and greenhouses (evaluation of the effect of operating parameters such as humidity, temperature, irrigation level, amount of fertilizer;
- To analyse the contamination levels in different parts of the plant - root, leaf, fruit, seed) and in different types of crops;
- To develop partitioning and uptake models;
- To develop a risk assessment based on more data;
- To assess the fate of PCPs in amended soils after fertilization during a long period of time (6 months, 1 year, 2 year), to understand what happens;
- To identify possible degradation by-products resulting from the degradation of the studied compounds either in soil or metabolites in the plant.

Annex

Annex 1. Supporting Information Chapter 1

1. Toxicity information

Table S1.1 - UV-filters ecotoxicity data and assessment of priority (Brook et al., 2008).

Compound	Organism	Endpoint	Predicted value (mg L^{-1})	NOEC (mg L^{-1})	Priority
CBM	Neutral Organic (SAR)	14 d LC_{50}	57*	>>0.01	Low priority for further work
	Fish	96 h LC_{50} Chv	50* 2.7		
	Invertebrate	48 h LC_{50} Chv	23* 3.1		
	Algae	96 h EC_{50} Chv	11* 5.0		
HMS	Neutral Organic (SAR)	14 d LC_{50}	0.084	0.005	High priority for further work
	Fish	96 h LC_{50} Chv	0.240 0.005		
	Invertebrate	48 h LC_{50} 21 d Chv	0.034 -		
	Algae	96 h EC_{50} Chv	0.022 0.019		
BP3	Neutral Organic (SAR)	14 d LC_{50}	14.538	>0.01	Low priority for further work
	Fish	96 h LC_{50} (*L. idus*) 96 h LC_{50} 30 d Chv	100-220* 3.80 0.57		
	Invertebrate	48 h LC_{50} 21 d Chv	2.90 0.42		
	Algae	96 h EC_{50} 96 h Chv	5.10 1.20		
PBSA	Neutral Organic (SAR)	14 d LC_{50}	28027*	>> 0.01	Low priority for further work
	Fish	48 h LC_{50} (*L. idus)* 96 h LC_{50}	4250* 18223*		
	Invertebrate	EC_{50} (*D. magna*) 48 h Chv	>10000* 1018*		
	Algae	96 h EC_{50} 96 h Chv	911* 124*		

**above the expected water solubility of the substance; SAR – structure activity relationship; NOEC - No Observed Effect Level; LC_{50} – lethal concentration 50%; EC_{50} – half maximal effective concentration; Chv – chronic toxicity level (used for NOECs prediction)*

Table S1.1 - UV-filters ecotoxicity data and assessment of priority (Brook et al., 2008). (cont.).

Compound	Organism	Endpoint	Predicted value (mg L^{-1})	NOEC (mg L^{-1})	Priority
BMDM	Neutral Organic (SAR)	14 d LC_{50}	2.7*	>0.01	Low priority for further work
	Fish	96 h LC_{50}	2.4*		
	Invertebrate	48 h LC_{50} 21 d Chv	0.830 0.030		
	Algae	Chv	0.067		
BCSA	Neutral Organic (SAR)	14 d LC_{50}	277*	>>0.01	Low priority for further work
	Fish	96 h LC_{50} Chv	1516* 117		
	Invertebrate	48 h LC_{50} Chv	903* 112		
	Algae	96 h LC_{50} Chv	446 157		
BP4	Neutral Organic (SAR)	14 d LC_{50}	10882	>>0.01	Low priority for further work
	Fish	96 h LC_{50} 30 d Chv	4572 719		
	Invertebrate	48 h LC_{50} 21 d Chv	770 488		
	Algae	96 h EC_{50} 96 h Chv	42416 16554		
OC	Neutral Organic (SAR)	14 d LC_{50}	0.027	0.00089	High priority for further work
	Fish	96 h LC_{50} (*L. idus*) 96 h LC_{50} (Acrylates) 32 d Chv (Acrylates) 96 h LC_{50} (Allylic/vinyl nitrates)	>10000* 0.720* 0.00089 0.330*		
	Invertebrate	48 h EC_{50} *D. magna* 48 h LC_{50} (Acrylates)	100* 0.110*		
	Algae	96 h EC_{50}	0.015*		
EMC	Neutral Organic (SAR)	14 d LC_{50}	0.191	0.003	High priority for further work
	Fish	96 h LC_{50} (*B. rerio*) 96 h LC_{50} 32 d Chv	>10000* 0.91 0.003		
	Invertebrate	48 h EC_{50} (*D. magna*) 48 h LC_{50}	1 0.32		
	Algae	96 h EC_{50}	0.040		
3BC	Neutral Organic (SAR)	14 d LC_{50}	0.38	0.022	High priority for further work
	Fish	96 h LC_{50} Chv	1.09* 0.022		
	Invertebrate	48 h LC_{50} Chv	0.28 0.041		
	Algal	96 h EC_{50} Chv	0.12 0.10		

**above the expected water solubility of the substance; SAR – structure activity relationship; NOEC - No Observed Effect Level; LC_{50} – lethal concentration 50%; EC_{50} – half maximal effective concentration; Chv – chronic toxicity level (used for NOECs prediction)*

Table S1.1 - UV-filters ecotoxicity data and assessment of priority (Brook et al., 2008). (cont.).

Compound	Organism	Endpoint	Predicted value (mg L^{-1})	NOEC (mg L^{-1})	Priority
IMC	Neutral Organic (SAR)	14 d LC_{50}	3.1	0.013	High priority for further work
	Fish	96 h LC_{50} 32 d Chv	1.4 0.013		
	Invertebrate	48 h LC_{50}	1.5		
	Algae	96 h EC_{50}	0.18		
4-MBC	Neutral Organic (SAR)	14 d LC_{50}	0.13	0.008	High priority for further work
	Fish	96 h LC_{50} Chv	0.510* 0.008		
	Invertebrate	48 h EC_{50} 48 h LC_{50} Chv	<0.800* 0.110 0.047		
	Algae	96 h EC_{50} Chv	0.048 0.017		
ES	Neutral Organic (SAR)	14 d LC_{50}	0.117	0.008	High priority for further work
	Fish	LC_{50} (*B. rerio*) 96 h LC_{50} Chv	613* 0.29[a]/0.13[b] 0.008[a]/0.018[b]		
	Invertebrate	EC_{50} (*D. magna*) 48 h LC_{50} 21 d Chv	10[a]* 0.049[a]/0.32[b] 0.014[b]		
	Algae	96 h EC_{50} Chv	0.026[a]/0.038[b] 0.022[a]/0.038[b]		
EDP	Neutral Organic (SAR)	14 d LC_{50}	0.190	0.012	High priority for further work
	Fish	96 h LC_{50} Chv	0.400* 0.012		
	Invertebrate	48 h LC_{50}	0.082		
	Algae	96 h EC_{50} Chv	0.037 0.031		

*[a] Estimate based on the substance behaving like an ester; [b]Estimate based on the substance behaving like a phenol; *above the expected water solubility of the substance; SAR – structure activity relationship; NOEC - No Observed Effect Level; LC_{50} – lethal concentration 50%; EC_{50} – half maximal effective concentration; Chv – chronic toxicity level (used for NOECs prediction)*

2. Additional information about UV-filters environmental transformation products

Rodil et al. (2009) showed that BP3 has a high photostability, since only 20% degraded after 72 h of irradiation in water. In fact, this compound presents a half-life in water in the range of weeks to a couple of months (higher in winter months). Its stability, however, is compromised when it interacts with other UV-filters, increasing BP3 degradation percentage (Rodil et al., 2009; Vione et al., 2013). Liu et al. (2014) determined 2,4-dimethylanisole as a photoproduct of BP3, produced through the loss of hydroxyl and benzoyl functional groups. Also, BP3 under oxic conditions (i.e. media in which oxygen is present) was found to produce BP1 as a biodegradation product (Kim and Choi, 2014; Liu et al., 2012). BP1 and 4-cresol were detected as transformation products under anoxic conditions (Liu et al., 2012) (Figure S1.1).

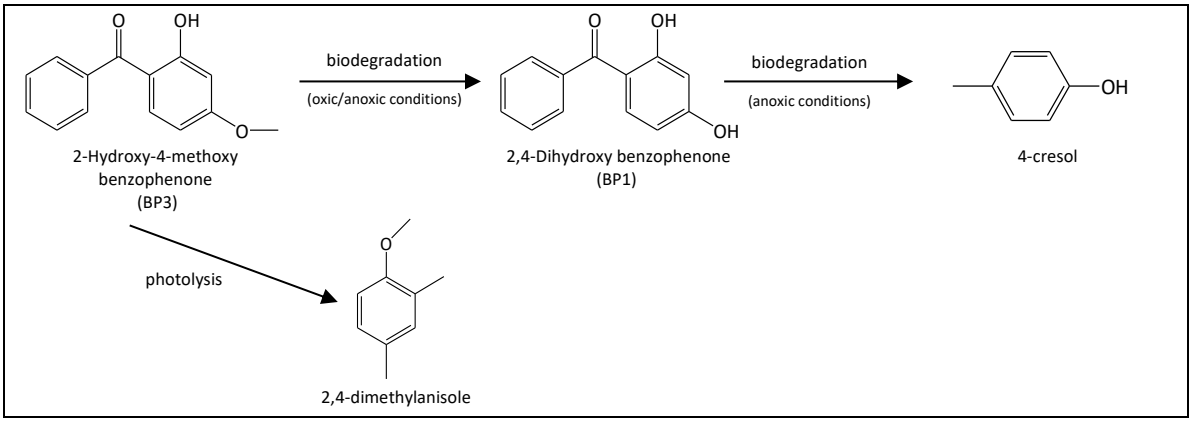


Figure S1.1 - Main BP3 transformation products.

In addition to direct photolysis, indirect photolysis with hydroxyl radicals (•OH) and the photochemically excited triplet of the coloured dissolved organic matter (^{3}CDOM*) are an important path in BP3 degradation (Vione et al., 2013). Other benzophenone, BP4, is present in surface waters in two prevailing forms, the singly deprotonated (HA^-) and the doubly deprotonated one (A^{2-}) and like BP3 undergoes degradation by reaction with •OH and direct photolysis (De Laurentiis et al., 2013).

Sakkas et al. (2003) found that the transformation products were strongly dependent on the constitution of the media irradiated. However, they determined that sea water transformation rate is lower than distilled water because of the dissolved organic matter that slows the photodegradation. Also, three transformation products were detected as

a result from dealkylation and hydroxylation reactions of ODP (Figure S1.2) (Rodil et al., 2009; Sakkas et al., 2003).

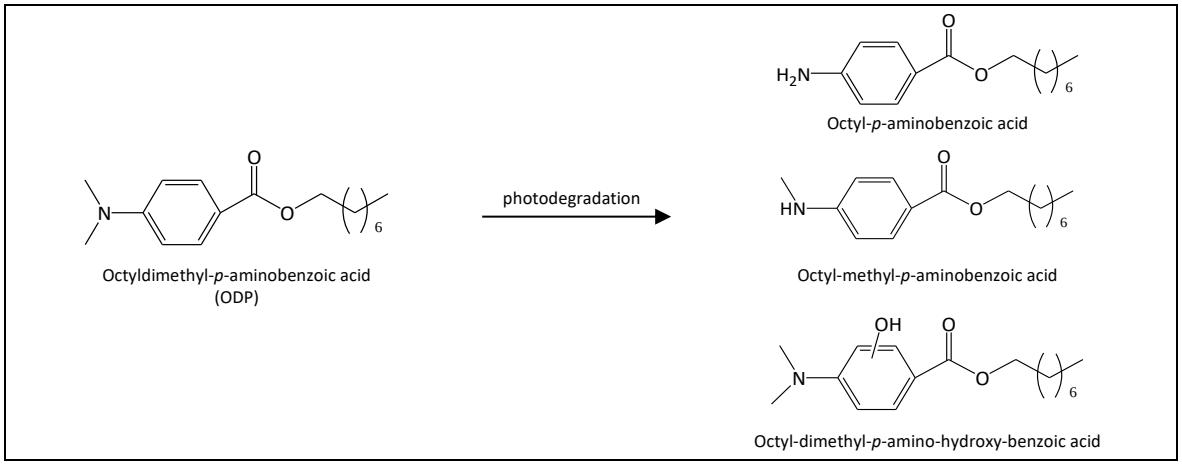


Figure S1.2 - Main ODP phototransformation products in sea water.

Serpone et al. (2002) showed that the filtering ability of *p*-aminobenzoic acid EDP as reduced by 35% after 60 min of UV exposure in aqueous media. Rodil et al. (2009) also found that upon continuous radiation, EDP half-life ($t_{1/2}$) is about 20 h. They also found 3 transformation products in water solution as the result of a dealkylation and methylation (Figure S1.3) (Rodil et al., 2009).

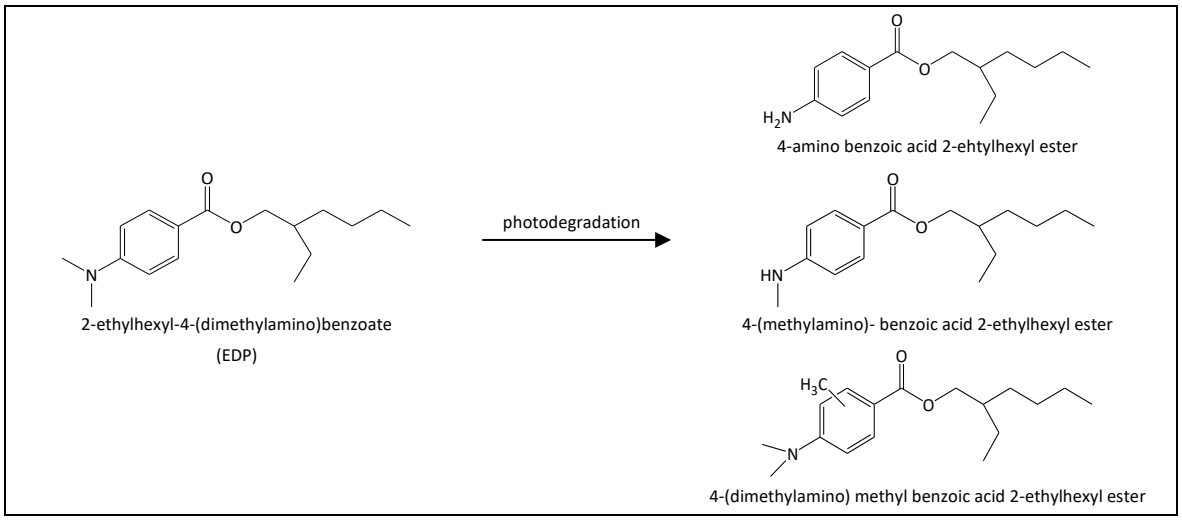


Figure S1.3 - Main EDP phototransformation products in distilled water.

The PBSA, benzimidazole derivative, was found to photodegrade upon irradiation and particularly fast and extensively in water (Serpone et al., 2002; Zhang et al., 2010). Its degradation is associated with the production of free radicals and reactive oxygen species (ROS) (Inbaraj et al., 2002). Its photolysis pathways includes desulfonation and benzimidazole ring cleavage, which are probably initiated by the excited triplet state ($^3PBSA^{\bullet}$) and radical cation ($PBSA^{\bullet+}$) (Ji et al., 2013).

The camphor derivative 4-MBC was studied in groundwater under aerobic and anaerobic conditions during 77 days and was found more effectively degraded in the aquifer materials under the aerobic (half-life of 33 days) than anaerobic (half-life of 75 days) conditions with a removal of 42 to 69%. Reducing conditions were also under scope and 4-MBC showed higher half-life with Fe (III), nitrate and sulphate conditions (Liu et al., 2013). In ultrapure water, 4-MBC was exposed to continuous artificial sunlight for 72 h but only 20% degradation was obtained (Rodil et al., 2009). Regarding its biological degradation was observed in sterile sludge treated with fungus *Trametes versicolor* an 87% reduction in 4-MBC content. The main metabolites identified were hydroxylated and pentose-conjugated compounds as degradation was complete in less than 24 h (Badia-Fabregat et al., 2012).

Some cinnamates, salicylates, camphor and dibenzoyl methane derivatives when irradiated by UV undergo photoisomerisation that may yield species that absorb less UV light than the parent species (Díaz-Cruz et al., 2008). Some of these compounds exist in the environment as geometrical isomers (*E/Z*) due to the presence of an exocyclic C=C double bond adjacent to the aromatic ring (Nguyen et al., 2011). Generally, (*E*)-isomers come from commercial substances and isomerise to the (*Z*)-form under the influence of light. Isomerisation rate depends on several factors: the compound, spectrum of light source and matrix (solvent) (Li et al., 2007). The compounds, IMC, 4-MBC and EMC consist of geometrical (*Z*)- and (*E*)-isomers and, HMS, consists of *cis*- and *trans*- isomers (Rodil and Moeder, 2008). Also, BMDM occurs in the *keto* and *enol* form (Cantrell and McGarvey, 2001).

The cinnamate derivative EMC is widely used in sunscreens and its conversion from (*E*)- to (*Z*)- isomer in water upon irradiation was verified by Huong et al. (2008). Although both isomers maximum wavelength are similar (λ_{max}, 310 nm) (Alves et al., 2011), *Z*-isomer has a lower molar absorption coefficient, which results in a decrease in the efficiency of EMC as a UVB absorber. In fact, this compound is difficult to degrade by photolysis. After 24 h of irradiation about 24% of EMC was degraded. Only when the right conditions are provided, like addition of an acid (HCl) or an oxidizer (H_2O_2), a higher conversion rate is observed (higher than 50%) (Gackowska et al., 2014). As a result of experimental studies, some EMC transformation products were identified: 4-

methoxycinnamic acid (4-MCA), 4-methoxybenzaldehyde (4-MBA) and 4-methoxyphenol (4-MP) (Gackowska et al., 2014). These compounds also were proven to react with chlorination and oxidant agents, yielding 6 other degradation compounds (Gackowska et al., 2014). Also, Rodil et al. (2009) showed that cinnamate EMC and IMC can transform in cyclobutane dimeric structures probably via [2+2] cycloaddition reaction. The resulting compounds, dEMC and dIMC (Figure S1.4) present higher log K_{ow} values than the parent compounds (11.3 and 8.3, respectively), which means that they could be easily found in sediments rather than in water. MacManus-Spencer et al. (2011) also found that the photolysis of EMC also produces 4-methoxybenzaldehyde and 2-ethylhexyl alcohol (Figure S1.4).

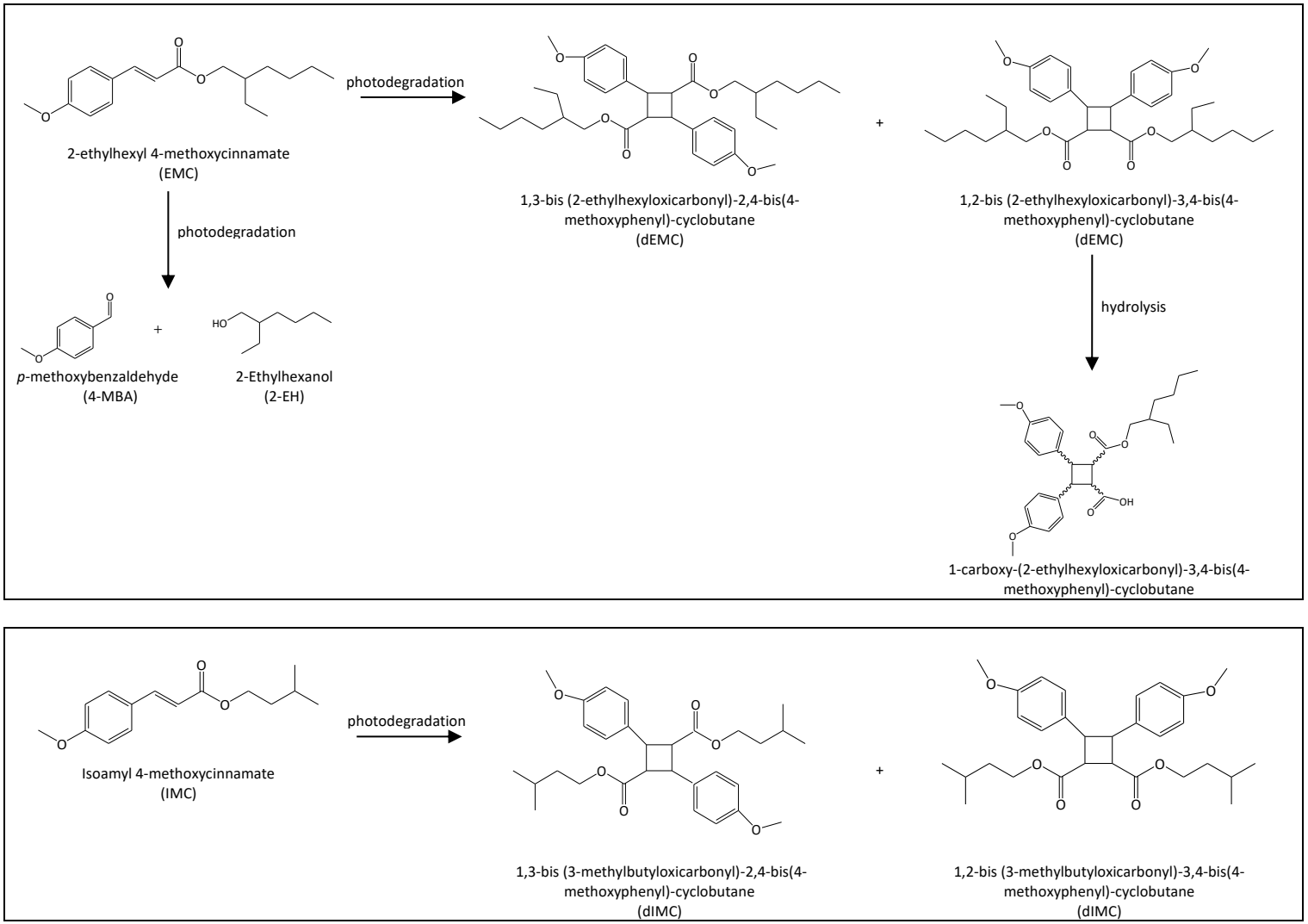


Figure S1.4 - Main EMC and IMC phototransformation products.

.

The dibenzoyl methane derivative, BMDM, under irradiation in aqueous solution tautomerises from the *enol* to the *keto* form. Under laser flash photolysis, BMDM tends also to transform into non-chelated forms (Cantrell and McGarvey, 2001). Huong et al. (2008) also verified that BMDM in aqueous medium is fully degraded by light and identified several photoproducts like substituted benzoic acids, benzyls, dibenzoylmethanes and dibenzoyl ethanes (Figure S1.5).

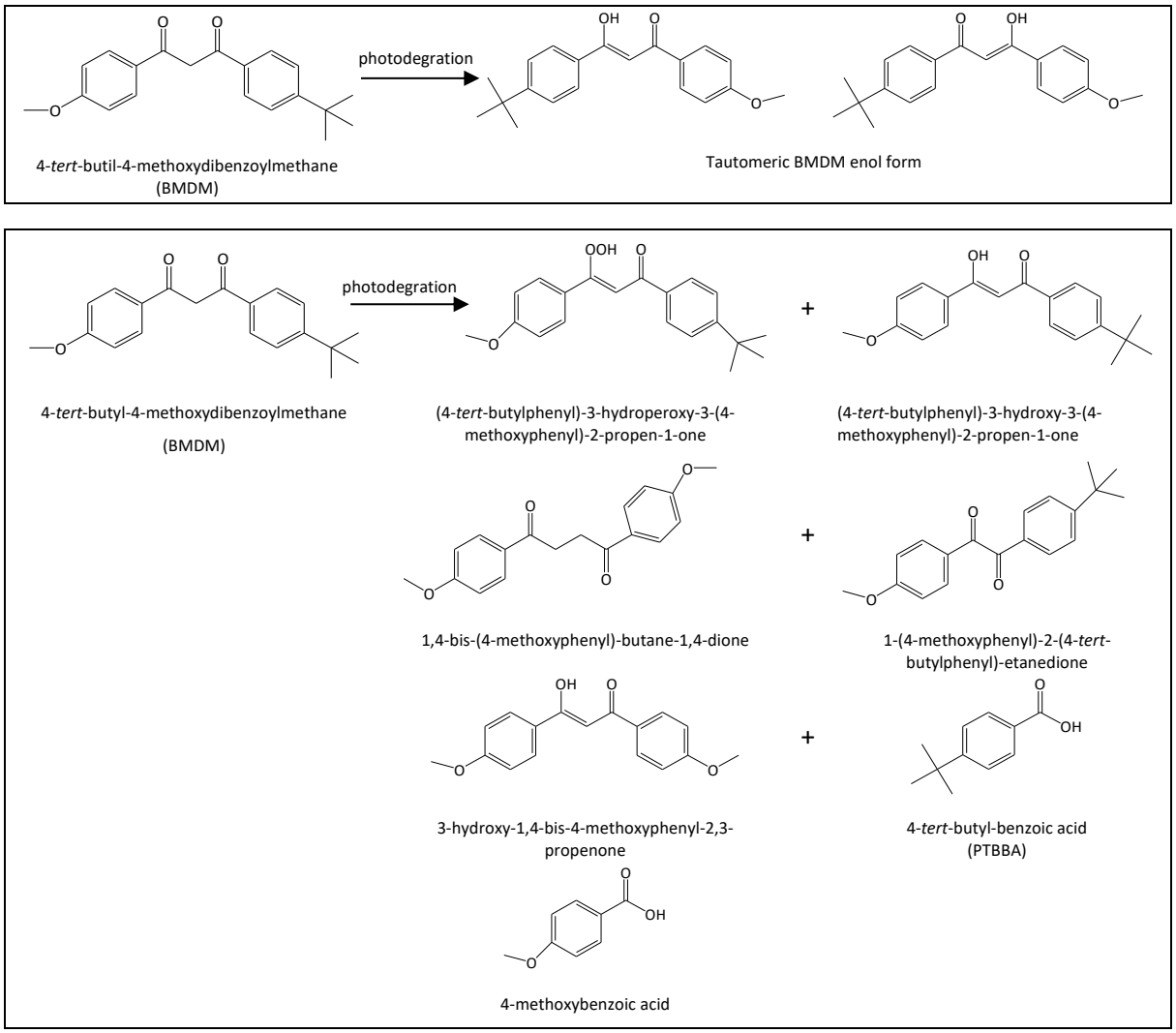


Figure S1.5 - Main BMDM phototransformation products.

UV-filter degradation resulting from chlorine reactions is very common in swimming pools. Since aqueous chlorine is not a strong enough oxidant to mineralize anthropogenic compounds, numerous transformation products may be formed due to oxidation/substitution reactions (Duirk et al., 2013). Chlorine reacts with the organic matter

present in water, producing a variety of chlorinated organic compounds known as disinfection by-products. It reacts with compounds containing aromatic rings, mostly by electrophilic substitutions mainly in the *ortho* and *para* positions to a substituent (Santos et al., 2013).

As an example, Duirk et al. (2013) showed that BP3 and BP8 were rapidly transformed by aqueous chlorine resulting in the formation of chloroform as a stable transformation product. Liu et al. (2014) also found that 13 of 14 BP-type UV-filters exhibited lower toxicity after the chlorination disinfection process (due to the ready cleavage of the aromatic ring), while 2-hydroxy-4-methoxy benzophenone-5-sulfonic acid showed a higher toxicity.

Also, ODP reacted quickly with free chlorine and the rate of chlorine consumption increased with the pH decrease. Concerning the photoproduct analysis in swimming pool water, five intermediates were detected (Figure S1.6) (Sakkas et al., 2003). Negreira et al. (2008) observed the production of EDP by-products and di-halogenated BP3 forms in a significant extension in under quasi real-life conditions of chlorinated bath waters, like swimming pools (Figure S1.6).

The UV-filters EMC and BMDM react with chlorine and monochloro and dichloro transformation products are formed (Figure S1.6). In the first case, there is a hydrogen replacement by chlorine in the benzene ring of EMC; on the other hand, BMDM substitution should only occur in the benzene ring containing the methoxy group (Santos et al., 2013).

In addition to the detection of the main UV-filters, it is important to also consider the detection and toxicological effects of their transformation products, which sometimes are more toxic and not frequently put under the scope.

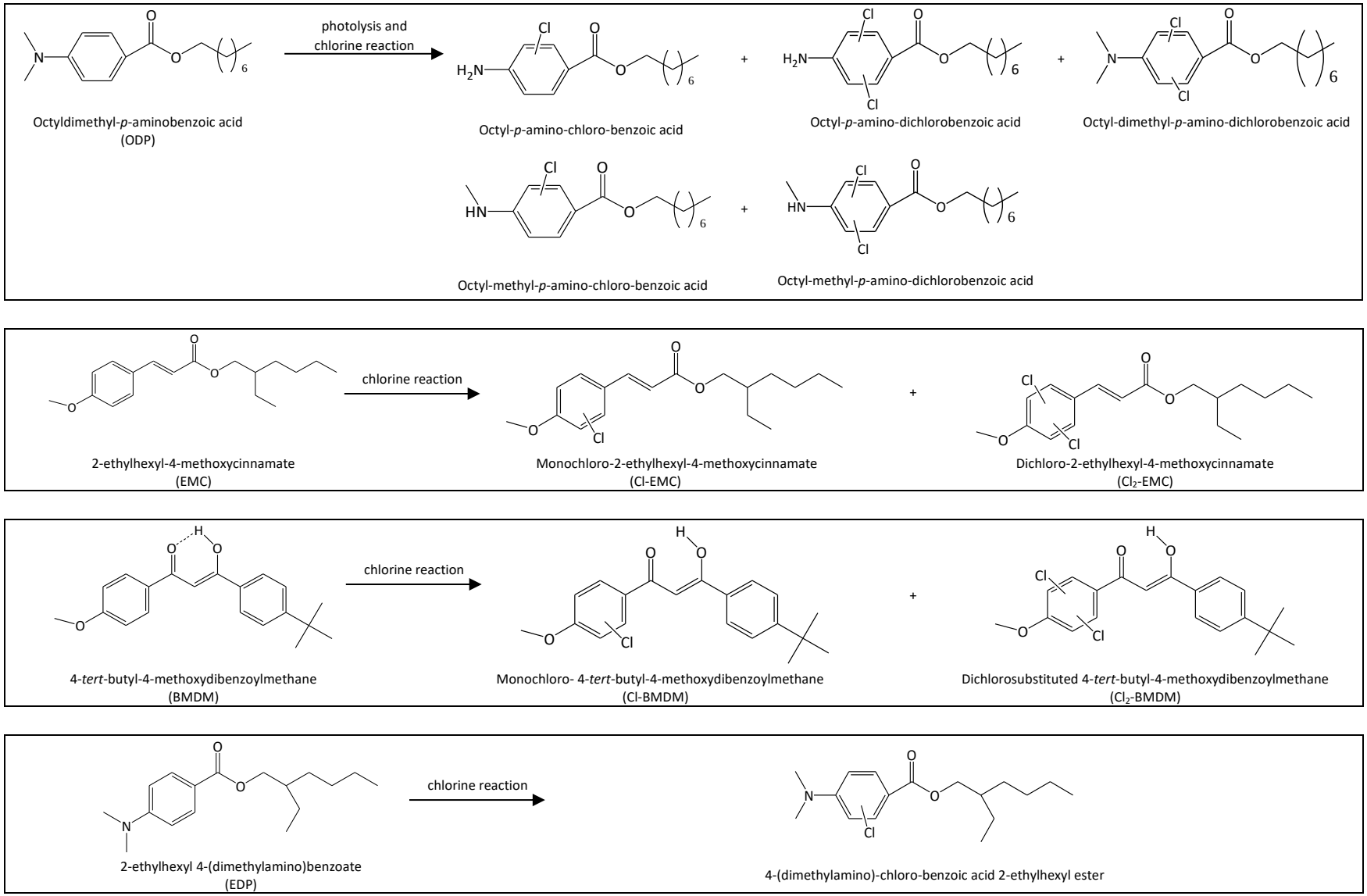


Figure S1.6 - Main transformation products with chlorine agents in swimming pools.

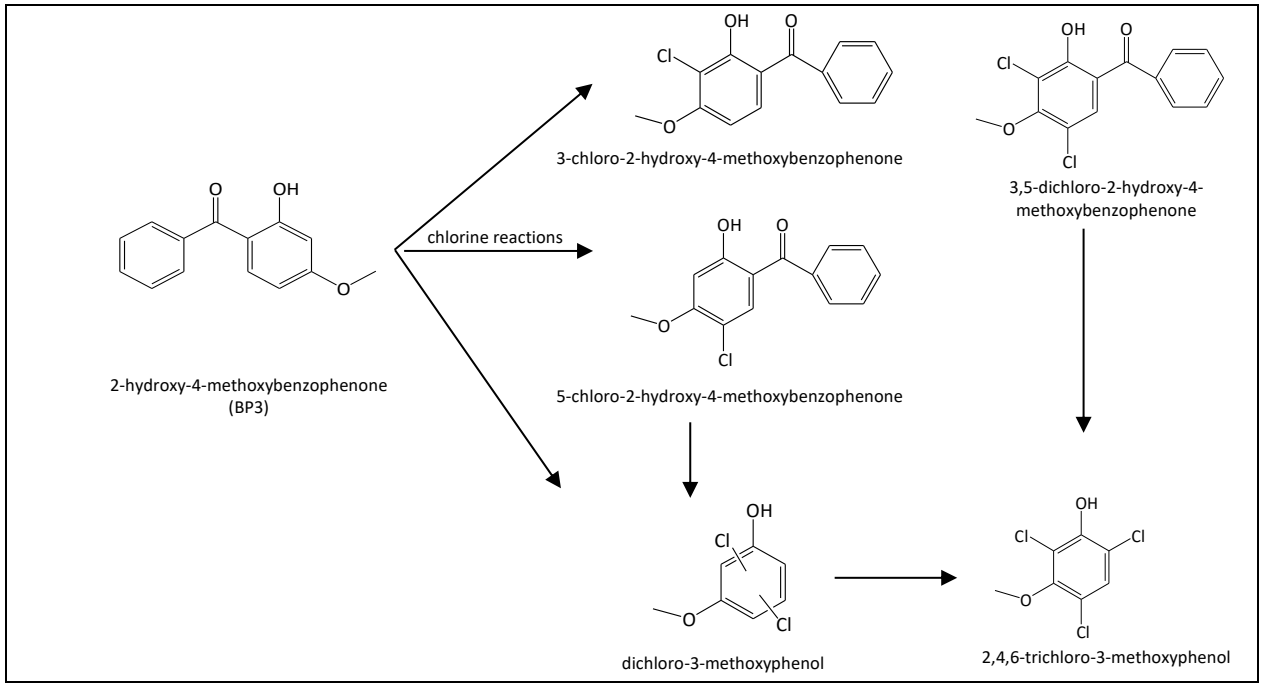


Figure S1.6 - Main transformation products with chlorine agents in swimming pools. *(cont.)*.

References

Alves LF, Gargano R, Alcanfor SKB, Romeiro LAS, Martins JBL. A chromophoric study of 2-ethylhexyl *p*-methoxycinnamate. Chem Phys Lett 2011; 516: 162-165.

Badia-Fabregat M, Rodriguez-Rodriguez CE, Gago-Ferrero P, Olivares A, Pina B, Diaz-Cruz MS, Vicent T, Barceló D., Caminal G. Degradation of UV filters in sewage sludge and 4-MBC in liquid medium by the ligninolytic fungus *Trametes versicolor*. J Environ Manage 2012; 104: 114-20.

Brooke DN, Burns JS, Crookes MJ, UV-filters in cosmetics – prioritisation for environmental assessment, Environment Agency, 2008.

Cantrell A, McGarvey DJ. Photochemical studies of 4-tert-butyl-4'-methoxydibenzoylmethane (BM-DBM). J Photochem Photobiol B 2001; 64: 117-122.

De Laurentiis E, Minella M, Sarakha M, Marrese A, Minero C, Mailhot G, Brigante M, Vione D. Photochemical processes involving the UV absorber benzophenone-4 (2-hydroxy-4-methoxybenzophenone-5-sulphonic acid) in aqueous solution: reaction pathways and implications for surface waters. Water Res 2013; 47: 5943-53.

Díaz-Cruz MS, Llorca M, Barceló D, Barceló D. Organic UV filters and their photodegradates, metabolites and disinfection by-products in the aquatic environment. TrAC 2008; 27: 873-887.

Duirk SE, Bridenstine DR, Leslie DC. Reaction of benzophenone UV filters in the presence of aqueous chlorine: Kinetics and chloroform formation. Water Res 2013; 47: 579-587.

Gackowska A, Przybyłek M, Studziński W, Gaca J. Experimental and theoretical studies on the photodegradation of 2-ethylhexyl 4-methoxycinnamate in the presence of reactive oxygen and chlorine species. Cent Eur J Chem 2014; 12: 612-623.

Huong SP, Rocher E, Fourneron J-D, Charles L, Monnier V, Bun H, Andrieu V. Photoreactivity of the sunscreen butylmethoxydibenzoylmethane (DBM) under various experimental conditions. J Photochem Photobiol A 2008; 196: 106-112.

Inbaraj JJ, Bilski P, Chignell CF. Photophysical and photochemical studies of 2-phenylbenzimidazole and UVB sunscreen 2-phenylbenzimidazole-5-sulfonic acid. Photochem Photobiol 2002; 75: 107-16.

Ji Y, Zhou L, Zhang Y, Ferronato C, Brigante M, Mailhot G, Yang X, Chovelon J-M. Photochemical degradation of sunscreen agent 2-phenylbenzimidazole-5-sulfonic acid in different water matrices. Water Res 2013; 47: 5865-5875.

Kim S, Choi K. Occurrences, toxicities, and ecological risks of benzophenone-3, a common component of organic sunscreen products: A mini-review. Environ Int 2014; 70: 143-157.

Li W, Ma Y, Guo C, Hu W, Liu K, Wang Y, Zhu T. Occurrence and behavior of four of the most used sunscreen UV filters in a wastewater reclamation plant. Water Res 2007; 41: 3506-3512.

Liu Q, Chen Z, Wei D, Du Y. Acute toxicity formation potential of benzophenone-type UV filters in chlorination disinfection process. J Environ Sci 2014; 26: 440-447.

Liu YS, Ying GG, Shareef A, Kookana RS. Biodegradation of the ultraviolet filter benzophenone-3 under different redox conditions. Environ Toxicol Chem 2012; 31: 289-95.

Liu J-L, Wong M-H. Pharmaceuticals and personal care products (PPCPs): A review on environmental contamination in China. Environ Int 2013; 59: 208-224.

MacManus-Spencer LA, Tse ML, Klein JL, Kracunas AE. Aqueous photolysis of the organic ultraviolet filter chemical octyl methoxycinnamate. Environ Sci Technol 2011; 45: 3931-7.

Negreira N, Canosa P, Rodríguez I, Ramil M, Rubí E, Cela R. Study of some UV filters stability in chlorinated water and identification of halogenated by-products by gas chromatography–mass spectrometry. J. Chromatogr A 2008; 1178: 206-214.

Nguyen KT, Scapolla C, Di Carro M, Magi E. Rapid and selective determination of UV filters in seawater by liquid chromatography-tandem mass spectrometry combined with stir bar sorptive extraction. Talanta 2011; 85: 2375-84.

Rodil R, Moeder M. Development of a method for the determination of UV filters in water samples using stir bar sorptive extraction and thermal desorption–gas chromatography–mass spectrometry. J Chromatogr A 2008; 1179: 81-88.

Rodil R, Moeder M, Altenburger R, Schmitt-Jansen M. Photostability and phytotoxicity of selected sunscreen agents and their degradation mixtures in water. Anal Bioanal Chem 2009; 395: 1513-24.

Sakkas VA, Giokas DL, Lambropoulou DA, Albanis TA. Aqueous photolysis of the sunscreen agent octyl-dimethyl-*p*-aminobenzoic acid. Formation of disinfection byproducts in chlorinated swimming pool water. J Chromatogr A 2003; 1016: 211-22.

Sakkas VA, Calza P, Islam MA, Medana C, Baiocchi C, Panagiotou K, Albanis T. TiO_2/H_2O_2 mediated photocatalytic transformation of UV filter 4-methylbenzylidene camphor (4-MBC) in aqueous phase: Statistical optimization and photoproduct analysis. Appl Catal B 2009; 90: 526-534.

Santos AJM, Crista DMA, Miranda MS, Almeida IF, Sousa e Silva JP, Costa PC, Amaral MH, Lobão PAL, Lobo JMS, Silva JCGE. Degradation of UV filters 2-ethylhexyl-4-methoxycinnamate and 4-tert-butyl-4'-methoxydibenzoylmethane in chlorinated water. Environ Chem 2013; 10: 127-134.

Serpone N, Salinaro A, Emeline AV, Horikoshi S, Hidaka H, Zhao J. An in vitro systematic spectroscopic examination of the photostabilities of a random set of commercial sunscreen lotions and their chemical UVB/UVA active agents. Photochem Photobiol Sci 2002; 1: 970-81.

Vione D, Caringella R, De Laurentiis E, Pazzi M, Minero C. Phototransformation of the sunlight filter benzophenone-3 (2-hydroxy-4-methoxybenzophenone) under conditions relevant to surface waters. Sci Tot Environ 2013; 463–464: 243-251.

Zhang S, Chen J, Qiao X, Ge L, Cai X, Na G. Quantum chemical investigation and experimental verification on the aquatic photochemistry of the sunscreen 2-phenylbenzimidazole-5-sulfonic acid. Environ Sci Technol 2010; 44: 7484-90.

Annex 2. Supporting Information Chapter 2

1. Relevant physicochemical properties for the behaviour of UV-filters in WWTPs

To better understand the fate of organic UV-filters in WWTPs, it is important to know the main physicochemical properties of these compounds. Therefore, in Table S1 are presented the organic-UV filters currently allowed in Europe (CosmeticsDirective, 2009), USA (21CFR352.10, 2014) and Japan (MHW, 2000) to be incorporated in cosmetics. Although these compounds may be grouped in different chemical families, they present a common feature, i.e. the presence of an aromatic moiety with a side chain, showing different degrees of unsaturation (Díaz-Cruz et al., 2008). Some of these compounds are chiral (e.g. EMC, OC and 4-MBC), but their enantiomers should present identical physicochemical properties (Bester, 2007).

Organic UV-filters are not considered volatile because boiling points are around 400 ^{o}C. Their solubility in water varies from $1.45x10^{-14}$ mg L-1 for triazine derivative EHT to $2.5x10^{5}$ mg L-1 for benzophenone BP4, resulting in a wide spectrum from not soluble to highly soluble. Most compounds are considered lipophilic (log K_{ow} >4), which indicates that they have higher affinity to sludge rather than dissolve in the aqueous phase. However, compounds like benzophenones BP4, BP5, BP9 and benzimidazole PBSA present negative log K_{ow} values, which indicates a strong tendency to water rather than sludge.

Table S2.1 - Structure and some physicochemical properties (boiling point, water solubility and octanol-water partition coefficient) of organic UV-filters.

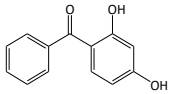
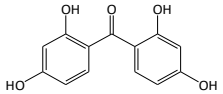
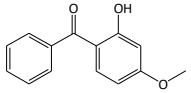
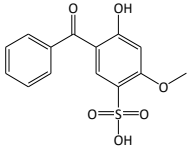
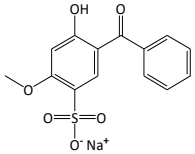
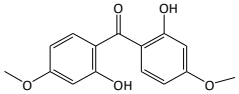
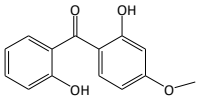
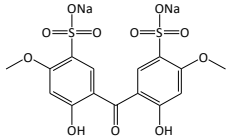
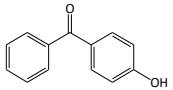
Family	Compound (Abbreviation) CAS number	Chemical Structure	UVA	UVB	Boiling Point (°C)	Log K_{ow}	Solubility in water at 25 °C (mg L-1)	UV-filters allowed by:		
								Europe	USA	Japan
Benzophenone derivatives	2,4-Dihydroxybenzophenone (BP1) CAS: 131-56-6		X	X	374.6	2.19	$4.13x10^3$			X
	2,2',4,4'-Tetrahydroxybenzophenone (BP2) CAS: 131-55-5		X	X	444.6	2.78	$3.99x10^2$			X
	2-Hydroxy-4-methoxybenzophenone (BP3) CAS: 131-57-7		X	X	363.4	3.79	$6.90x10^1$	X	X	X
	2-Hydroxy-4-methoxy benzophenone-5-sulfonic acid (BP4) CAS: 4065-45-6		X	X	497.6	0.37	$2.50x10^5$	X	X	X
	2-Hydroxy-4-methoxy benzophenone-5-sodium sulfonate (BP5) CAS: 6628-37-1		X	X	698.3	-1.42	$5.05x10^5$	X		X
	2,2'-Dihydroxy-4,4'-dimethoxybenzophenone (BP6) CAS: 131-54-4		X	X	421.9	3.90	$3.10x10^1$			X
	2,2'-Dihydroxy-4-methoxybenzophenone (BP8) CAS: 131-53-3		X	X	398.2	3.82	$5.30x10^1$		X	
	2,2'-Dihydroxy-4,4'-dimethoxybenzophenone-5,5'-disulfonic acid disodium salt (BP9) CAS: 76656-36-5		X	X	852.1	-2.78	$8.89x10^5$			X
	4-Hydroxybenzophenone (4HB) CAS: 1137-42-4		X	X	339.6	3.07	$4.06x10^2$			

Table S2.1 - Structure and some physicochemical properties (boiling point, water solubility and octanol-water partition coefficient) of organic UV-filters (cont.).

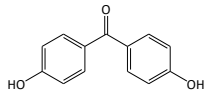
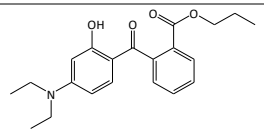
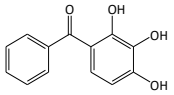
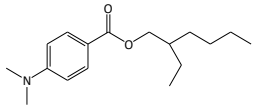
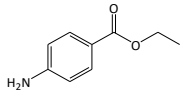
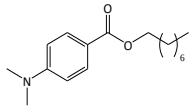
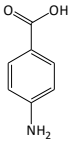
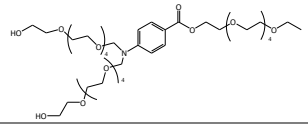
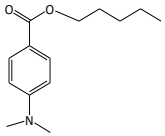
Family	Compound (Abbreviation) CAS number	Chemical Structure	UVA	UVB	Boiling Point (°C)	Log K_{ow}	Solubility in water at 25 °C (mg L-1)	UV-filters allowed by:		
								Europe	USA	Japan
Benzophenone derivatives	4,4'-Dihydroxybenzophenone (4DHB) CAS: 611-99-4		X	X	374.6	2.19	$1.91x10^{3}$			X
	Diethylamino hydroxybenzoyl hexyl benzoate (DHHB) CAS: 302776-68-7		X		504.8	6.54	$8.19x10^{-3}$	X		X
	2,3,4-Trihydroxybenzophenone (234THB) CAS: 1143-72-2		X	X	409.4	2.91	$3.811x10^{2}$			
p-Aminobenzoic acids derivatives	2-Ethylhexyl 4-(dimethylamino)benzoate (EDP) CAS: 21245-02-3			X	344.5	5.77	$2.00x10^{-1}$	X	X	X
	Ethyl *p*-aminobenzoate (Et-PABA) CAS: 94-09-7			X	278.9	1.86	$1.31x10^{3}$			
	Octyldimethyl-*p*-aminobenzoic acid (ODP) CAS: 58817-05-3			X	351.6	5.84	$1.70x10^{-1}$			
	p-Aminobenzoic acid (PABA) CAS: 150-13-0			X	307.7	0.83	$6.11x10^{3}$		X	
	Ethoxylated ethyl-*p*-aminobenzoate (PEG-25 PABA) CAS: 116242-27-4			X	?	?	?	X		
	Amyl *p*-dimethylaminobenzoate (APABA) CAS: 14779-78-3			X	314.1	4.37	$5.33x10^{0}$			X

Table S2.1 - Structure and some physicochemical properties (boiling point, water solubility and octanol-water partition coefficient) of organic UV-filters (cont.).

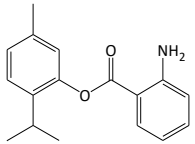
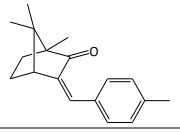
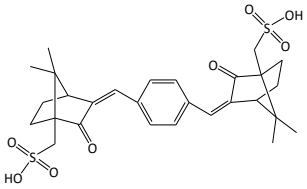
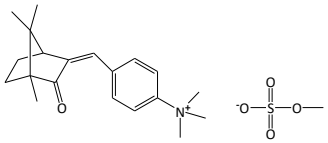
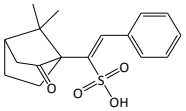
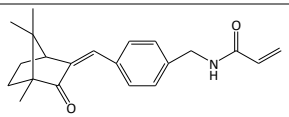
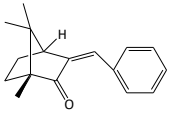
Family	Compound (Abbreviation) CAS number	Chemical Structure	UVA	UVB	Boiling Point (°C)	Log K_{ow}	Solubility in water at 25 °C (mg L-1)	UV-filters allowed by:		
								Europe	USA	Japan
o-Aminobenzoic acids derivatives	Menthyl *o*-aminobenzoate (MOABA) CAS: 134-09-8		X		374.3	6.28	$7.40x10^{-2}$		X	
Camphor derivatives	3-(4'-Methylbenzylidene) camphor (4-MBC) CAS: 36861-47-9			X	349.4	5.92	$2.00\ x10^{-1}$	X		
	Terephthalylidene dicamphor sulfonic acid (Ecamsule) (TDSA) CAS: 92761-26-7		X		757.5	3.83	$1.50\ x10^{-1}$	X	X	X
	Camphor benzalkonium methosulfate (CBM) CAS: 52793-97-2			X	638.2	3.11	$7.34\ x10^{0}$	X		
	Benzylidene camphor sulfonic acid (BCSA) CAS: 56039-58-8			X	472.0	2.22	$1.20\ x10^{2}$	X		
	Polyacrylamidomethyl benzylidene camphor (PBC) CAS: 113783-61-2			X	?	?	?	X		
	3-Benzylidene-camphor (3BC) CAS: 15087-24-8			X	337.6	5.37	$6.90\ x10^{-1}$	X		

Table S2.1 - Structure and some physicochemical properties (boiling point, water solubility and octanol-water partition coefficient) of organic UV-filters (cont.).

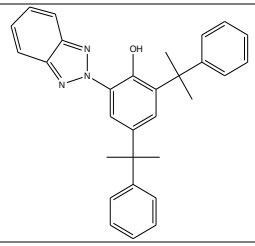
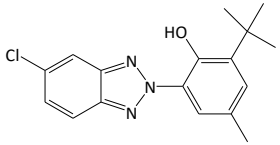
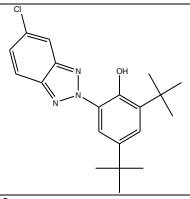
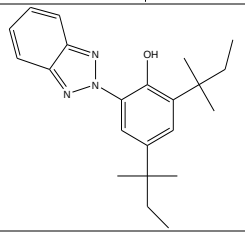
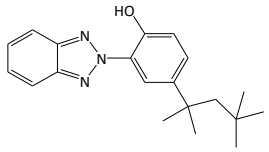
Family	Compound (Abbreviation) CAS number	Chemical Structure	UVA	UVB	Boiling Point (°C)	Log K_{ow}	Solubility in water at 25 °C (mg L-1)	UV-filters allowed by:		
								Europe	USA	Japan
Benzotriazole derivatives	2-(2H-Benzotriazol-2-yl)-4,6-bis(2-phenyl-2-propanyl)phenol (UV-234) CAS: 70321-86-7				599.7	7.67	1.65×10^{-3}			
	2-(3-*tert*-butyl-2-hydroxy-5-methylphenyl)-5-chlorobenzotriazole (UV 326) CAS: 3896-11-5		X	X	450.1	5.55	6.80×10^{-1}			
	2-(5-Chloro-2H-benzotriazol-2-yl)-4,6-bis(2-methyl-2-propanyl)phenol (UV-327) CAS: 3864-99-1				473.3	6.91	2.63×10^{-2}			
	2-(2H-Benzotriazol-2-yl)-4,6-bis(2-methyl-2-butanyl)phenol (UV-328) CAS: 25973-55-1				477.8	7.25	1.48×10^{-2}			
	2-(2'-hydroxy-5'-octylphenyl)-benzotriazole (UV 329) CAS: 3147-75-9		X	X	454.6	6.21	1.70×10^{-1}			

Table S2.1 - Structure and some physicochemical properties (boiling point, water solubility and octanol-water partition coefficient) of organic UV-filters (cont.).

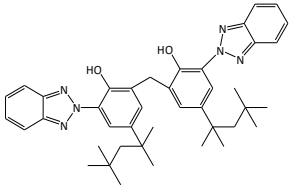
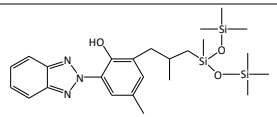
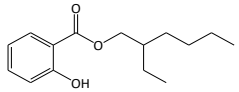
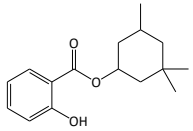
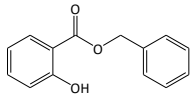
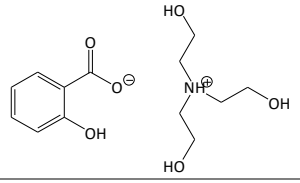
Family	Compound (Abbreviation) CAS number	Chemical Structure	UVA	UVB	Boiling Point (°C)	Log K_{ow}	Solubility in water at 25 °C (mg L-1)	UV-filters allowed by:		
								Europe	USA	Japan
Benzotriazole derivatives	Methylene bis-benzotriazolyltetramethyl butylphenol (MBBT) CAS: 103597-45-1		X	X	818.5	12.46	$4.47x10^{-10}$	X		X
	Drometrizole Trisiloxane (DTS) CAS: 155633-54-8		X	X	528.6	10.82	$6.40x10^{-7}$	X		X
Salicylate derivatives	2-Ethylhexyl salicylate (ES) CAS: 118-60-5			X	344.9	5.97	$7.20\ x10^{-1}$	X	X	X
	3,3,5-trimethylciclohexyl salicylate (Homosalate) (HMS) CAS: 118-56-9			X	355.9	6.16	$4.20\ x10^{-1}$	X	X	X
	Benzylsalicylate (BZS) CAS: 118-58-1			X	354.9	4.31	$2.46\ x10^{1}$			
	Triethanolaminium salicylate (TAS) CAS: 2174-16-5			X	460.9	-0.53	$4.24x10^{4}$	X		

Table S2.1 - Structure and some physicochemical properties (boiling point, water solubility and octanol-water partition coefficient) of organic UV-filters (cont.).

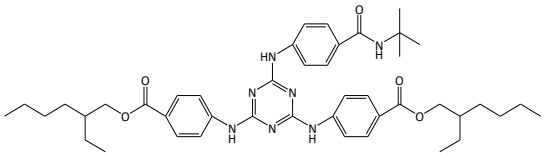
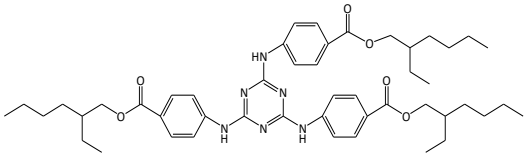
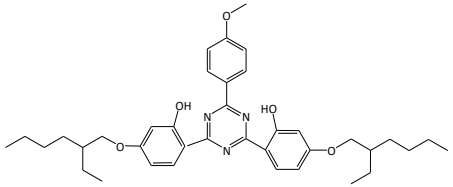
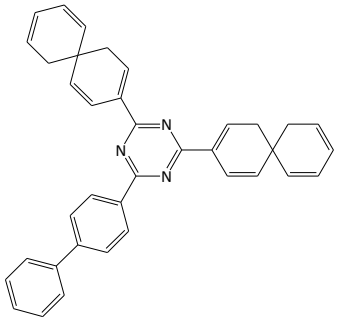
Family	Compound (Abbreviation) CAS number	Chemical Structure	UVA	UVB	Boiling Point (°C)	Log K_{ow}	Solubility in water at 25 °C (mg L-1)	UV-filters allowed by:		
								Europe	USA	Japan
Triazine derivatives	Diethylhexyl butamido triazone (DBT) CAS: 154702-15-5			X	893.5	14.03	$1.33x10^{-11}$	X		
	Ethylhexyl triazone (EHT) CAS: 88122-99-0			X	874.4	17.05	$1.45x10^{-14}$	X		X
	Bis-ethylhexyloxyphenol Methoxyphenyl triazine (BEMT) CAS: 187393-00-6		X	X	786.6	9.29	$1.45x10^{-16}$	X		X
	Tris-biphenyl triazine (TBPT) CAS: 31274-51-8		X	X	792.2	10.38	$5.56x10^{-7}$	X		

Table S2.1 - Structure and some physicochemical properties (boiling point, water solubility and octanol-water partition coefficient) of organic UV-filters (cont.).

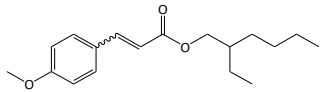
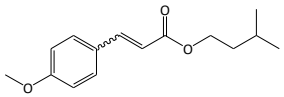
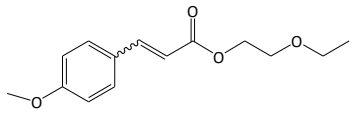
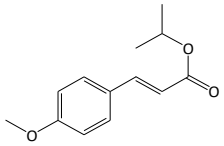
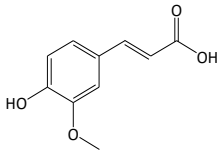
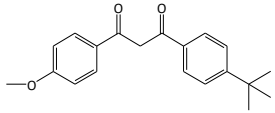
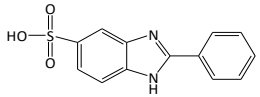
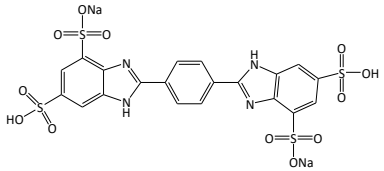
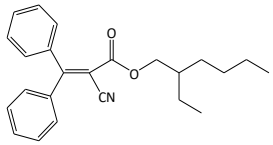
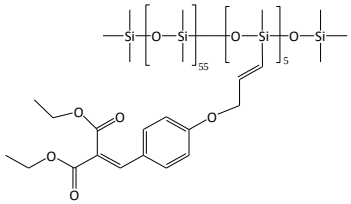
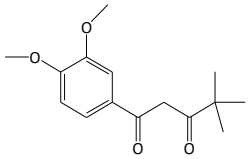
Family	Compound (Abbreviation) CAS number	Chemical Structure	UVA	UVB	Boiling Point (°C)	Log K_{ow}	Solubility in water at 25 °C (mg L-1)	UV-filters allowed by:		
								Europe	USA	Japan
Cinnamates derivatives	2-Ethylhexyl 4-methoxycinnamate (EMC) CAS: 5466-77-3			X	360.5	5.80	1.50×10^{-1}	X	X	X
	Isoamyl 4-methoxycinnamate (IMC) CAS: 71617-10-2			X	324.4	4.33	4.86×10^{0}	X		X
	2-Ethoxyethyl 4-methoxycinnamate (Cinoxate) (EOMC) CAS: 104-28-9			X	185.5	2.65	5.00×10^{2}		X	X
	Isopropyl 4-methoxycinnamate (IPMC) CAS: 5466-76-2			X	296.4	3.35	4.80×10^{1}			X
	4-Hydroxy-3-methoxycinnamic acid (ferulic acid) (FA) CAS: 1135-24-6			X	354.2	1.51	5.97×10^{3}		X	
Dibenzoyl methane derivative	4-*tert*-butil-4'-methoxydibenzoylmethane (Avobenzone) (BMDM) CAS: 70356-09-1		X		409.3	4.51	1.52×10^{0}	X	X	X
Benzimidazole derivative	2-Phenyl-5-benzimidazole sulfonic acid (PBSA) CAS: 27503-81-7			X	566.1	-0.16	2.36×10^{4}	X	X	X
	Disodium phenyl dibenzimidazole tetrasulfonate (DPDT) CAS: 180898-3-7		X		?	-2.00	2.00×10^{1}	X		

Table S2.1 - Structure and some physicochemical properties (boiling point, water solubility and octanol-water partition coefficient) of organic UV-filters (cont.).

Family	Compound (Abbreviation) CAS number	Chemical Structure	UVA	UVB	Boiling Point (°C)	Log K_{ow}	Solubility in water at 25 °C (mg L-1)	UV-filters allowed by:		
								Europe	USA	Japan
Crylene derivative	2-Ethylhexyl 2-cyano-3,3-diphenylacrylate (OC) CAS: 6197-30-4			X	472.9	6.88	$3.81x10^{-3}$	X	X	X
Benzalmalonate derivative	Dimethicodiethylbenzalmalonate (Polysilicone-15) (BMP) CAS: 207574-74-1			X	?	?	?	X		X
Other	1-(3,4-dimethoxyphenyl)-4,4-dimethyl-1,3-pentanedione (PD) CAS: 135099-97-7			X	348.8	1.61	$8.24 x10^{2}$			X

References

21CFR352.10, 2014. Sec. 352.10 Sunscreen active ingredients in USA (web: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=352.10).

Bester, K., 2007. Personal Care Compounds in the Environment: Pathways, Fate and Methods for Determination. Wiley - John Wiley & Sons.

CosmeticsDirective, 2009. Annex VI: LIST OF UV FILTERS ALLOWED IN COSMETIC PRODUCTS in Europe. REGULATION (EC) No 1223/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 30 November 2009 on cosmetic products (web: http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.results&annex_v2=VI&search).

Díaz-Cruz, M.S., Llorca, M., Barceló, D., Barceló, D., 2008. Organic UV filters and their photodegradates, metabolites and disinfection by-products in the aquatic environment. TrAC Trends Anal. Chem. 27, 873–887.

EPA. 5. Estimating Physical / Chemical and Environmental Fate Properties with EPI Suite™. Sustainable Futures / Pollution Prevention (P2) Framework Manual. EPA-748-B12-001. U.S. Environmental Protection Agency, OCSPP, 2012.

MHW, 2000. Standards for Cosmetics in Japan (web: http://www.mhlw.go.jp/file/06-Seisakujouhou-11120000-Iyakushokuhinkyoku/0000032704.pdf).

Shaath N.A. Ultraviolet filters. Photochem. Photobiol. Sci. 2010; 9; 464–469.

Annex 3. Supporting Information Chapter 4

1. Abbreviations

4DHB - 4,4'-Dihydroxybenzophenone; 4HB - 4-Hydroxybenzophenone; 4-MBC - 3-(4'-Methylbenzylidene) camphor; Ac- Acetone; ADBI – Celestolide; AHMI – Phantolide; AHTN – Tonalide; Al_2O_3 – Aluminium oxide; ASE – Accelerated solvent extraction; ATII – Traseolide; BMDM – Avobenzone; BP1 - 2,4-Dihydroxybenzophenone; BP2 - 2,2',4,4'-Tetrahydroxybenzophenone; BP3 - 2-Hydroxy-4-methoxybenzophenone; BP4 - 2-Hydroxy-4-methoxy benzophenone-5-sulfonic acid; CYHex – cyclohexane; DBT: Diethylhexyl butamido triazone; DCM – Dichloromethane; DE - Diethyl ether; DPMI – Cashmeran; EA – Ethyl Acetate; EDP - 2-Ethylhexyl 4-(dimethylamino)benzoate; EHT – Ethylhexyl triazone; EMC - 2-Ethylhexyl 4-methoxycinnamate; ES - 2-Ethylhexyl salicylate; GC-MS – gas chromatography – mass spectrometry; GPC – gel permeation chromatography; Hex – Hexane; HHCB – galaxolide; HMS - Homosalate; IMC - Isoamyl 4-methoxycinnamate; LC-MS – liquid chromatography – mass spectrometry; LLE – liquid liquid extraction; LOD – Limit of detection; LOQ - Limit of quantification; MA – Musk Ambrette; MAE - Microwave-assisted extraction; MA-HS-SPME - Microwave-assisted headspace solid-phase microextraction; MeOH – Methanol; MK – Musk Ketone; MM – Musk Moskene; MT – Musk Tibetene; MX – Musk Xylene; N_2 – Nitrogen; Na_2SO_4 – Sodium Sulfate; NaCL – Sodium Chloride; OC – Octocrylene; ODP - Octyldimethyl-p-aminobenzoic acid; PBSA - 2-Phenyl-5-benzimidazole sulfonic acid; PLE – Pressurize liquid extraction; PN – Pentane; RT – Room temperature; SFE - Supercritical fluid extraction; SPE – Solid phase extraction; SPME - Solid Phase Micro Extraction; US – Ultrasound; UV-234 - 2-(2H-Benzotriazol-2-yl)-4,6-bis(2-phenyl-2-propanyl)phenol; UV-326 - 2-(3-tert-butyl-2-hydroxy-5-methylphenyl)-5-chlorobenzotriazole; UV-327 - 2-(5-Chloro-2H-benzotriazol-2-yl)-4,6-bis(2-methyl-2-propanyl)phenol; UV-328 - 2-(2H-Benzotriazol-2-yl)-4,6-bis(2-methyl-2-butanyl)phenol; UV-329 - 2-(2'-hydroxy-5'-octylphenyl)-benzotriazole;

2. State of the Art

Table S3.1 - Overview on analytical methods for determinations of SMCs in sewage sludge.

Compounds	Country	Extraction/Clean-up method	Instrumental method	% REC	LOD (ng g^{-1})	LOQ (ng g^{-1})	Concentration (ng g^{-1} dw)	References
MK, MX, HHCB, AHTN, ADBI, AHMI, ATII	China	0.1 g of freeze-dried sample added 20 µL (100 µg/L) IS), 4 g Activated silica and 28 g Na_2SO_4. **PLE**: 34 mL Hex/DCM (1:1), 60 ºC, 1,500 psi, 2 cycles, 15 min. **SPE**: 3 g Activated silica gel, 0.5 g Na_2SO_4, 8 mL Hex (conditioning); 5 mL Hex + 15 mL Hex/DCM (2:1) + 15 mL Hex/DCM (1:2) + 20 mL Hex/DCM (1:3) elution). Concentration to 1 mL.	GC-MS	55 - 107	-	0.4 – 2.2	HHCB: <2.2 – 41,400 AHTN: <1.5 – 22,000 MX: 382 MK: 359	(Liu et al., 2014)
AHTN, HHCB	USA	1 g wet sample added to 20 mL MeOH, vortex 30 min; MeOH is recovered and process repeated 2 more times. Samples of combined extracts are diluted 10-fold with MeOH/H_2O (1:1).	LC-MS/MS	AHTN: 111 HHCB: 112	-	AHTN: 0.1 HHCB: 0.5	AHTN: 649 – 14,971 HHCB: 4079 – 91,018	(Sun et al., 2014)
Exaltone, Exaltolide, Muscone, Habanolide, Ambrettolide, Musk MC4, Civetone, Musk-NN	Spain	0.25 g freeze-dried sample; **HS-SPME**: 0.5 mL H_2O, 80 ºC, 1 min. Introduced a PDMS/DVB 65 µm fiber, 45 min, stirred at 750 rpm. Desorption at 250 ºC for 3 min.	GC-MS		Exaltone, Exaltolide, Musk-NN: 0.005 Muscone, Habanolide, Musk MC4, Civetone: 0.025 Ambrettolide: 0.0075	-	Exaltone: 0.05-0.08 Exaltolide: nd-0.13 Muscone: nd-2.0 Habanolide: nd-0.50 Ambrettolide: 0.025-0.85 Musk MC4: nd - 0.19 Civetone: nd - 0.13 Musk-NN: 0.025 - 1.45	(Vallecillos et al., 2013)
HHCB, AHTN, DPMI, Ambrettolide	Denmark	0.5 g freeze-dried sample added to 5 mL Hex, 15 min sonication, 15 min centrifugation at 2,000 rpm. Liquid phase recovered, process repeated 2 more times. Evaporation to 300 µL under N_2 and 186 ng of triphenylamine added as IS.	GC-MS	-	2 – 10	3 - 30	HHCB: 250 – 2,500 AHTN: 1900 – 5,000 DPMI: 10 - 50 Ambrettolide: 10 - 150	(Matamoros et al., 2012)

Table S3.1 - Overview on analytical methods for determinations of SMCs in sewage sludge. (cont.).

Compounds	Country	Extraction/Clean-up method	Instrumental method	% REC	LOD (ng g^{-1})	LOQ (ng g^{-1})	Concentration (ng g^{-1} dw)	References
AHTN, HHCB, ADBI, AHMI, ATII, MK, MX	China	Freeze-dried sludge. **ASE**: 4 g activated silica and 28 g of Na_2SO_4/0.1 g sludge mixture; C_6H_{14}:CH_2Cl_2 (1:1), 60 °C, 1500 psi, static mode, 15 min, twice. **Column clean-up**: activated copper and 6 g Na_2SO_4; **SPE**: 2 g Al_2O_3 and 0.5 g Na_2SO_4, 5 mL Hex (conditionate), 5 mL Hex + 20 mL Hex/DCM (2:1) + 30 mL Hex/DCM (1:2) + 20 mL Hex/DCM (1:3) (elution). Concentration of the eluates to 1 mL.	GC-MS	83.6 – 105.1	3.3	-	HHCB: 260 – 12,590 AHTN:10 – 2,560 MK: 130 – 530 ATII: 15 – 300 MX: <3.3 AHMI, ADBI: nd	(Hu et al., 2011)
HHCB, AHTN, ADBI, AHMI, ATII, DPMI, MA, MX, MM, MT, MK	Korea	Freeze-dried sludge. **ASE**: 1 g sample, Ac/Hex (1:1), 100 °C. Pre-heat, heat and static times of 5, 5 and 10 min; 14.1 MPa, 100% flush volume, 60 s purge, 2 cycles. Volume reduce to 2 mL and dilution to 10 mL with Hex. **SPE:** 2 g Na_2SO_4, 8 g 5% deactivated silica gel, 2 g Na_2SO_4; 100 mL Hex (washing). Sample loading (2 mL extract). Elution: 50 mL Ac/Hex (1/99) (discarded), 100 mL Ac/Hex (5/95) (collected, evaporated, exchanged into DCM and adjusted to 200 µl for analysis).	GC-MS	DPMI: 79 ADBI: 82 AHMI: 80 ATII: 71 HHCB: 85 AHTN: 82 MA:77 MX: 79 MM: 73 MT: 71 MK: 80	DPMI: 10 ADBI: 3 AHMI: 3 ATII: 10 HHCB: 7 AHTN: 7 MA: 7 MX: 3 MM: 10 MT: 10 MK: 3	DPMI: 30 ADBI: 10 AHMI: 10 ATII: 30 HHCB: 20 AHTN: 20 MA: 20 MX: 10 MM: 30 MT: 30 MK: 10	ADBI: <3 – 250 AHMI: <3 – 235 HHCB: 15,900 – 82,100 AHTN: 4,480 – 28,800 MK: <3 – 1,900	(Guo et al., 2010)
HHCB, AHTN, MX, MK	China	**Phase separation:** centrifugation at 10,000 rpm for 5 min. **Horizontal mechanical shaker extraction:** solid phase is extracted with Hex, 3 times, by vigorous stirring for 2 h (270 rounds per min). **Concentration**: extracts evaporation by rotary evaporation at 40 °C and 40 Pa to dryness and reconstituted to 1 mL with Hex.	GC-MS	-	-	-	**Primary sludge** HHCB: 2,605.6 – 3,293.3 AHTN: 676.6 – 1,188.9 MX: 1.1 - 7.1 MK: 4.4- 7.1 **Treated dewatered sludge** HHCB: 3,281.3 – 3,560.2 AHTN: 793.1 – 1,529.2 MX: nd MK: nd - 28.8	(Lv et al., 2010)

Table S3.1 - Overview on analytical methods for determinations of SMCs in sewage sludge. (cont.).

Compounds	Country	Extraction/Clean-up method	Instrumental method	% REC	LOD (ng g^{-1})	LOQ (ng g^{-1})	Concentration (ng g^{-1} dw)	References
AHTN, HHCB, DPMI, ADBI, AHDI, ATII	Austria	**Sample**: 1 g of sewage sludge dry matter was used. **Extraction**: 20 mL EtOH and 30 mg diethylammoniumdiethyldithiocarbamate added to sample; 150 min extraction on a rotation-shaker, 3 min in ultrasound (US). Added 20 mL of sodiumacetate buffer (pH = 3.4) and 20 mL Hex; Extration on a rotation-shaker for 150 min and 3 min US. Centrifugation at 3,000 rpm, to separate Hex layer. Extraction repeated with 5 mL Hex. Combined Hex extracted evaporated under N_2 to 5 mL. **SPE**: aluminium oxide column; 35 mL Hex/EA (90:10) (elution).	GC-MS	80 - 105	AHTN: 5 HHCB: 25 DPMI: 5 ADBI: 7.5 AHDI: 5 ATII: 5	AHTN: 10 HHCB: 50 DPMI: 10 ADBI: 15 AHDI: 10 ATII: 10	**WWTP A** Excess sludge: AHTN: 400 HHCB: 4,200 DPMI: 22 ATII: 290 ADBI, AHDI: nd Digested sludge: AHTN: 1,100 HHCB: 8,500 DPMI: 79 ADBI: 23 AHDI: 10 ATII: 510 **WWTP B** Excess sludge: AHTN: 910 HHCB: 11,000 DPMI: 68 ADBI: 23 AHDI: <10 ATII: 870 Digested sludge: AHTN: 1,800 HHCB: 14,000 DPMI: 120 ADBI: 32 AHDI: 11 ATII: 680 **WWTP C and D** (data not shown)	(Clara et al., 2011)

Table S3.1 - Overview on analytical methods for determinations of SMCs in sewage sludge. (cont.).

Compounds	Country	Extraction/Clean-up method	Instrumental method	% REC	LOD (ng g^{-1})	LOQ (ng g^{-1})	Concentration (ng g^{-1} dw)	References
DPMI, ADBI, AHMI, ATII, HHCB, AHTN	China (Taiwan)	Samples homogenized by stirring and dewatered by filtration through glass fiber papers. The filter cake is collected. **MA-HS-SPME**: 5 g sample, 20 mL H_2O 3 g NaCl (pH=1). Microwave irradiation at 80 W for 5 min, adsorbed in situ on the PDMS-DVB fiber. Desorption at 270 ^oC for 2 min.	GC-MS	DPMI: 76-79 ADBI: 80-89 AHMI: 68-71 ATII: 76-80 HHCB: 83-87 AHTN: 70-75	DPMI: 0.1 ADBI, AHMI, ATII, HHCB, AHTN: 0.04	DPMI: 0.3 ADBI, AHMI, ATII, HHCB, AHTN: 0.1	DPMI: 1.2 - 2.5 ADBI, AHMI, ATII: nd HHCB: 1.4 - 2.8 AHTN: 0.5 - 0.7	(Wu and Ding, 2010)
HHCB, AHTN	China	**Sample pre-treatment:** filtration through a GF/B glass fiber filter. The filter cake was collected and freeze-dried, ground in a mortar, mixed thoroughly and stored until analysis. **Extraction:** 0.02 g sample extracted with 5 mL MeOH/H_2O (5:3), then 3 times with 5 mL Hex/Ac (85/15). In each extraction, sample is ultrasonicated for 10 min. Extract filtered through glass fiber filter and solvent evaporated to 200 µL. Dilution with 500 mL H_2O. **SPE:** Oasis HLB cartridge, 2 times 5 mL Methyl tert-butyl ether (MTBE), and then rinsed three times with 5 mL MeOH and 5 mL H_2O. Elution with 10 mL EA **SPE:** Silica gel cartridge. Extract evaporated to near dryness under N_2 and reconstituted in 100 µl Hex.	GC-MS	HHCB: 74.8 AHTN: 60.7	HHCB: 200 AHTN: 180	HHCB: 670 AHTN: 590	**WWTP A** Anaerobic: HHCB: 3,700 AHTN: 1,100 Anoxic: HHCB: 2,500 AHTN: 700 Oxic: HHCB: 4,800 AHTN: 1,000 **WWTP B** Anoxic: HHCB: 5,300 AHTN: 6,100 Oxic: HHCB: 9,000 AHTN: 13,100 **WWTP C** Bioseletion: HHCB: 16,800 AHTN: 2,900 Oxidized ditch: HHCB: 17,000 AHTN: 6,100 Anaerobic: HHCB: 10,900 AHTN: 2,100	(Zhou et al., 2009)

Table S3.1 - Overview on analytical methods for determinations of SMCs in sewage sludge. (cont.).

Compounds	Country	Extraction/Clean-up method	Instrumental method	% REC	LOD (ng g^{-1})	LOQ (ng g^{-1})	Concentration (ng g^{-1} dw)	References
DPMI, ADBI, AHMI, ATII, AHTN, HHCB	China	**Sample treatment:** samples were freeze-dried, grounded finely into powder and thoroughly homogenized. **Soxhlet:** 3 g sample, 2 g activated copper (activated with 10% hydrochloric acid), extracted for 10 h with 400 ml DCM/Hex (3:1). Concentration with a rotary evaporator to approximately 1 ml for clean-up. **SPE:** 3 g silica gel, 2 g neutral alumina, 0.5 Na_2SO_4, 3 g activated copper and 0.5 g Na_2SO_4; 70 ml Hex, 70 ml Hex/DCM (1:1), 70 ml DCM. Concentration to near-dryness and reconstituted 1 ml with Hex.	GC-MS	DPMI:75 ADBI: 95 AHMI:93 ATII:77 AHTN:97 HHCB:101	ng L^{-1}* DPMI:0.15 ADBI:0.05 AHMI:0.06 ATII:0.13 AHTN:0.14 HHCB:0.09	-	HHCB: 3,580-78,600 AHTN: 475-13,900 AHMI: <LOD – 670 ADBI: <LOD – 351	(Shek et al., 2008)
DPMI, ADBI, AHMI, ATII, AHTN, HHCB	China	**Soxhlet:** extracted for 72 h with DCM (with copper). Concentration with a rotary evaporator. **SPE:** silica-alumina column (2:1). Elution with Hex, Hex/DCM, DCM.	GC-MS	DPMI: 57 ADBI: 62 AHMI: 68 ATII: 73 AHTN: 78 HHCB: 107	*µg/mL ADBI, AHMI: 0.06 DPMI: 0.11 ATII, HHCB, AHTN: 0.12	-	**Primary sludge** DPMI: 40,750 – 50,430 ADBI: 1,460 – 2,590 AHMI: 1,380 – 2,120 HHCB: 479,730 – 545,170 AHTN: 49,690 – 68,130 ATII: nd **Secondary sludge** DPMI: 42,640- 52,380 ADBI: 2,120- 4,010 AHMI: 2,130- 3,650 HHCB: 530,080 – 601,270 AHTN: 82,870 – 107,610 ATII: nd	(Chen et al., 2007)
HHCB, AHTN, HHCB-lactone	USA	**Sample treatment:** moisture was removed from sludge samples (1-5 g) by homogenization of each sample with approximately 75 g of Na_2SO_4. **Soxhlet:** 400 ml Hex/DCM (1:3) for 16 h. Concentration to 1 mL.	GC-MS	HHCB: 87 AHTN: 85 HHCB-lactone: 86	-	HHCB: 20 AHTN: 20 HHCB-lactone: 50	**WWTP A** HHCB: 2,000 – 21,000 AHTN: 1,200 – 6,700 HHCB-lactone: 5,600 – 10,000 **WWTP B** HHCB:<20 AHTN: <20-21 HHCB-lactone: <50	(Horii et al., 2007)

Table S3.1 - Overview on analytical methods for determinations of SMCs in sewage sludge. (cont.).

Compounds	Country	Extraction/Clean-up method	Instrumental method	% REC	LOD (ng g^{-1})	LOQ (ng g^{-1})	Concentration (ng g^{-1} dw)	References
HHCB, AHTN, HHCB-lactone	USA	**Sample treatment:** moisture was removed from sludge samples (1-5 g) by homogenization of each sample with approximately 75 g of Na_2SO_4. **Soxhlet:** 400 ml Hex/DCM (1:3) for 16 h. Concentration to 1 mL.	GC-MS	HHCB: 87 AHTN: 85 HHCB-lactone: 86	-	HHCB: 20 AHTN: 20 HHCB-lactone: 50	**WWTP A** HHCB: 63,400 – 117,000 AHTN: 10,400- 16,800 HHCB-lactone: 12,900 – 22,000 **WWTP B** HHCB: 7,230 – 46,100 AHTN: 809 – 3,250 HHCB-lactone: 3,160 – 19,400	(Reiner et al., 2007)
DPMI, ADBI, AHMI, ATII, HHCB, AHTN, MA, MX, MM, MT, MK	Canada	**SFE:** Not described. Followed by SPE. SPE: silica gel column, 10 mL Ac/Hex (5:95). **MAE:** Ac and Hex.	GC-MS	89 (MAE) 88 (SFE)	DPMI: 27 ADBI: 36 AHMI: 27 ATII: 39 HHCB: 41 AHTN: 32 MA, MX, MM, MK: 4 MT: 3	-	DPMI: nd ADBI: 173 - 278 ATII: 416 - 495 HHCB: 17,500 – 25,600 AHTN: 4,240 – 7,040 MX: nd MK: 170-196	(Smyth et al., 2007)
HHCB, AHTN, ADBI, AHDI, DPMI, ATII	Switzerland	**LLE:** 50 g sludge and 2.5 g NaCl, 20 mL Hex, shaking for 20 min with a horizontal mechanical shaker, 3 times and centrifugation thereafter; Hexane phases were dried over Na_2SO_4. Isooctane (0.5 mL) was added as a keeper. Concentration to 0.5 mL by rotary evaporation at 40 ^oC and 33,000 Pa. The extracts were diluted with DCM/Hex (1:1) up to 5 mL. **GPC:** 100 g of Bio-Beads S-X3, Hex/DCM (1:1), flow rate: 5 mL min^{-1}, sampling window: 25–45 min. Volume reduction by rotary evaporator to 0.5 mL.	GC-MS	HHCB:92 AHTN:101 ADBI:103 AHDI:95 ATII:102 DPMI:84	HHCB:6 AHTN:4 ADBI:1 AHDI:1 ATII:1 DPMI:1	HHCB:20 AHTN:13 ADBI:3 AHDI:3 ATII:3 DPMI:3	**Raw sludge** HHCB: 9,420 – 11,670 AHTN: 2,950 – 3,870 ADBI: 180 - 440 AHDI: 90 - 260 ATII: 150 - 210 DPMI: 10 - 20 **Digested sludge** HHCB: 9,390 AHTN: 3,220 ADBI: 250 AHDI: 110 ATII: 180 DPMI: 30	(Kupper et al., 2006)

Table S3.1 - Overview on analytical methods for determinations of SMCs in sewage sludge. (cont.).

Compounds	Country	Extraction/Clean-up method	Instrumental method	% REC	LOD (ng g^{-1})	LOQ (ng g^{-1})	Concentration (ng g^{-1} dw)	References
HHCB, AHTN, ADBI, AHDI, ATII, ATTN	Germany	**LLE:** Dried sludge was extracted with Ac, H_2O and Hex. Centrifugation for 10–30 min at 3,500 rpm. **SPE:** Alumina column	GC-MS/MS	HHCB: 98.5 AHTN: 105.3 ADBI: 102.5 AHDI:100.7 ATII: 105.9 ATTN:111	25	-	**Activated sludge** HHCB: 2,700 – 14,400 AHTN: 800 – 4,700 ADBI: 30 - 180 AHDI: 80 - 310 ATII: 110 - 360 ATTN: 30 - 460 **Dried sludge** HHCB: 6,020 – 23,000 AHTN: 1,970 – 6,940 ADBI: 80 - 340 AHDI: 150 - 320 ATII: 150 - 590	(Muller et al., 2006)
DPMI, ADBI, AHMI, ATII, HHCB, AHTN, MA, MX, MM, MT, MK	Canada	**ASE:** 4–6 g sample of centrifuged biosolids, 16 g of pre-cleaned Na_2SO_4, three 5 min cycles of Hex/EA (1:1), 1500 psi, 80 ^oC. **Column:** Na_2SO_4 and concentration to 2 mL. **GPC:** Bio-Beads S-X, Hex/EA (1:1), 3–4 $mLmin^{-1}$. Fractions: A (40 mL), B (35 mL) and C (75 mL). Fraction C was collected. **SPE:** 5 g silica gel column, 2 g Na_2SO_4, 60 mL EA, 50 mL Ac. Volume concentrated to 0.25 mL.	GC-MS	80 *DPMI: 70	*ng L^{-1} 0.4-4.0	-	**Raw sludge** DPMI: 12.8 - 48.4 ADBI: 16.1 - 29.3 AHMI: 11.3-30.0 ATII: 92.8-244.3 HHCB: 2,482.4 – 4,514.1 AHTN: 408.6 - 929.4 MX: nd - 83.7 MK: nd - 48.1 MA, MM, MT: nd **Digested sludge** DPMI: 38.8 - 68.4 ADBI: 44.5 - 61.4 AHMI: 21.2 - 43.0 ATII: 501.9 - 259.4 HHCB: 5,772.7 – 7,896.7 AHTN: 1,040.2 – 1,569.0 MX: 62.0 - 133.5 MK: nd - 71.6 MA, MM, MT: nd	(Yang and Metcalfe, 2006)

ATTN - versalide

Table S3.1 - Overview on analytical methods for determinations of SMCs in sewage sludge. (cont.).

Compounds	Country	Extraction/Clean-up method	Instrumental method	% REC	LOD (ng g^{-1})	LOQ (ng g^{-1})	Concentration (ng g^{-1} dw)	References
AHTN, HHCB	Switzerland	0.2 g of freeze-dried samples **PLE:** 100 °C, 100 bar, 2 x 5 min, MeOH, final volume 20 mL **US:** 4 x 5 min; MeOH 4 and 2 mL, Ac 3 x 2 mL. **Centrifugation:** Supernatants combine and evaporate to about 0.2 mL under N_2. **SPE**: RP-C_{18}, MeOH; evaporation close to dryness and add 200 µL Hex; Clean-up with Silica gel, Hex/Ac (85/15). Evaporation to 300 µL.	GC-MS	**Activated sludge** AHTN: 78 HHCB: 87 **Digested sludge** AHTN: 74 HHCB:64	-	AHTN: 250 HHCB: 250	**Activated sludge** AHTN: 2,300 – 4,300 HHCB: 4,500 - 8,500 **Digested sludge** AHTN: 6,600 HHCB:15,000	(Ternes et al., 2005)
AHTN, HHCB	Germany						**Activated sludge** AHTN: 1,400 – 2,600 HHCB: 5,200 – 6,500	
DPMI, AHDI, ATII, AHTN, HHCB, HHCB-lactone	Switzerland	**Extraction:** 500 ml of homogenized sample (dry weight 1–1.5%), 20 g of NaCl, 200 ml Hex, stirred for 2 h at RT. The Hex was decanted, dried over Na_2SO_4 and 50–100 ml taken for the final analysis. Concentration to 5-10 mL by rotary evaporator and further concentration under N_2.	GC-MS	HHCB: 78 AHTN: 83 ADBI: 92 AHDI: 82 ATII: 87 DPMI: 79 HHCB-lactone: 94	-	*ng L^{-1} HHCB, AHTN, ADBI: 20 AHDI, DPMI: 10 ATII: 15	HHCB: 3,170 – 5,270 AHTN: 1,250 – 2,060 ADBI: 50 - 70 AHDI: 170 - 240 ATII: 120 - 210 DPMI: nd HHCB-lactone: 1,280 – 1,570	(Berset et al., 2004)
HHCB, AHTN	Germany	**US:** dried sludge, 4 x 5 min; 4 and 2 mL MeOH, 2 x 2 mL Ac. Supernatants combine and evaporate to about 0.2 mL under N_2. **SPE**: RP-C_{18} (pH=7)	GC-MS	-	-	-	Primary sludge HHCB: 187,000 AHTN: 183,000 Secondary sludge HHCB: 13,100 AHTN: 10,200	(Ternes et al., 2004)
AHTN, HHCB, HHCB-lactone	Germany	**Soxhlet:** 10 g lyophilized sample, extracted for 6 h, 100 ml EA. Extracts exchanged into toluene (by rotary evaporator). **SPE**: 1 g silica cartridges, 6 mL EA. **GPC:** Biorad SX-3, 2.5 ml min^{-1}, CYHex/EA (1:1), collected fraction (21.30–32 min). Samples transferred to toluene by rotary evaporator.	GC-MS	AHTN: 76 HHCB: 100	-	AHTN: 6 HHCB: 5	AHTN: 1,343 – 1,746 HHCB: 2,709 – 3,342 HHCB-lactone: nd	(Bester, 2004)

Table S3.1 - Overview on analytical methods for determinations of SMCs in sewage sludge. (cont.).

Compounds	Country	Extraction/Clean-up method	Instrumental method	% REC	LOD (ng g^{-1})	LOQ (ng g^{-1})	Concentration (ng g^{-1} dw)	References
DPMI, ADBI, AHMI, ATII, HHCB, AHTN	China	**Soxhlet:** 1 g sample, extracted for 72 h with DCM (with copper). Concentration in rotary evaporator to 1 mL, solvent changed to Hex and reduced again. **SPE:** 12 cm silica, 6 cm neutral activated alumina, 2 cm Na_2SO_4. Elution with Hex, Hex/DCM, DCM. Concentration to 0.5 mL.	GC-MS	DPMI: 58.13 ADBI: 63.08 AHMI:67.35 ATII:71.96 HHCB: 108.95 AHTN:77.73	*µg/ml DPMI, ATII, HHCB, AHTN:0.1 ADBI, AHMI: 0.05	-	**WWTP A** DPMI: 599 – 1,004 ADBI: 192 - 210 AHMI: <LOD - 227 ATII: nd HHCB: 5656 – 21,214 AHTN: 768 – 6,195 **WWTP B** DPMI: 1,004 ADBI: 207 AHMI: 112 ATII: nd HHCB: 5,416 AHTN: 715 **WWTP C, D** Data not shown	(Zeng et al., 2005)
AHTN, HHCB	Netherlands	**SPME**	GC-MS	85-106	-	-	**Primary sludge** AHTN: 310 – 470 HHCB: 1,080 – 1,590 **Primary sludge** AHTN: 15,260 – 92,250 HHCB: 39,300 – 257,730 **Waste sludge** AHTN: 380 – 460 HHCB: nd – 1,800	(Artola-Garicano et al., 2003)
MA, MX, MM, MT, MK, DPMI, ADBI, AHMI, ATII, HHCB, AHTN	UK	**Sample treatment:** 2.5 g of centrifuged sewage sludge was dried with Na_2SO_4 **Soxhlet:** extracted for 18 h, 280 mL DCM (with copper). **SPE:** 5 g alumina, 10 g silica, 1 cm Na_2SO_4; 100 mL of DCM/pentane (1:1). **GPC:** Bio-Beads SX-3, 25 cm bed height, 120 mL Hex/DCM (1:1). **SPE:** 3 g silica gel 60, 2 g neutral alumina, 0.5 Na_2SO_4, 20 mL DCM.	GC-MS	-	-	-	**Digested sludge** ADBI: 10 - 260 AHMI: 32 – 1,100 ATII: 44 – 1,100 HHCB: 1,900 – 81,000 AHTN: 120- 16,000 MA, MX, MM, MT, MK, DPMI: nd	(Stevens et al., 2003)

Table S3.1 - Overview on analytical methods for determinations of SMCs in sewage sludge. (cont.).

Compounds	Country	Extraction/Clean-up method	Instrumental method	% REC	LOD (ng g^{-1})	LOQ (ng g^{-1})	Concentration (ng g^{-1} dw)	References
HHCN, AHTN, MX, MK, 4-AMX, 2-AMK	Germany	**GPC** **SPE**	GC-MS	-	-	-	HHCB: 4,800 AHTN: 2,000 MX: <10 MK: <10 4-AMX: <10 2-AMK: nd	(Gatermann et al., 2002)
DPMI, ADBI, AHMI, MA, MX, HHCB, AHTN, MM, AMA, MT, AMM, MK, AMT, AMK, AMX	Switzerland	**Extraction:** 1 L of wet sewage sludge extracted with 600 ml Hex, RT, 2 h, by agitating vigorously. Decantation of the organic phase dried over Na_2SO_4 and concentrated at 35 °C under vacuum to 5 ml. **GPC:** 30 g of Bio-Beads SX3, CYHex/EA (1:1), 5 mL min^{-1}. **SPE:** silica cartridge, 8 ml DCM.	GC-MS/MS	DPMI: 73 ADBI: 108 AHMI: 106 MA: 112 MX:118 MM:102 MT:89 MK: 107	-	-	Wet sludge **WWTP A** MA, MX, MM, MT, AMA, AMT, AMK: nd MK: nd - 6.9 AMX: nd - 49.1 AMM: nd - 7.9 DPMI: 47.2 - 332 ADBI: 61.6 - 245 AHMI: 103 - 843 HHCB: 2,347 – 12,157 AHTN: 973 – 4,161 **WWTP B** MA, MM, MT, AMA, AMT: nd MX: nd - 32.5 MK: nd - 7.0 AMX: nd - 31.5 AMM: nd - 36.2 DPMI: 38.4 - 147 ADBI: 41 - 330 AHMI: 64.9 - 266 HHCB: 2,293 – 4,074 AHTN: 741 – 1,418	(Herren and Berset, 2000)

Table S3.2 - Overview on analytical methods for determinations of UVFs in sewage sludge.

Compounds	Location	Method overview	Instrumental method	Recovery (%)	LOD (ng g^{-1})	Concentration (ng g^{-1} dw)	Reference
BP3	Xiamen, China	Freeze-dried samples. MSPD (0.1 g sample, 0.4 g C_{18}), Extraction solvents: 12 mL MeOH, 6 ml MeOH/Ac (1/1), 10 mL ACN/5% oxalic acid (8/2), Elute evaporated to dryness under N_2 at 40 °C; Dissolved in 1 mL ACN/H_2O (1/1) and filtered through a 0.45 um filter	LC-QqQ-MS	44	0.2	6.9 (mean)	(Sun et al., 2016)
BP3, EDP, EMC, OC, UV-234, UV-327, UV-328, UV-329	Norway	Freeze-dried samples **PLE:** 1.0 g PSA, Hex/DCM (1:1) (extraction solvent), 100 ◦C, 5 min, 3 cycles. **GPC:** Alliance 2695 system, DCM (mobile phase), 12.1 – 20.0 min collected fractions) Additional cleaning (100 mg PSA, centrifuge 21,000 g, 10 min.	LC-HRMS GC-HRMS	BP3: 72 EDP: 81 EMC: 98 OC: 102 UV-234: 78 UV-327: 114 UV-328: 89 UV-329: 100	BP3, UV-234, UV-327, UV-328, UV-329: 10 EDP: 4 EMC, OC: 5	WWTP1 EMC: 551 - 793 OC: 3,449 – 12,661 UV-327: 30.4 – 77.1 UV-329: 1,172 – 3,075 BP3: < 10 EDP: < 4 UV-234, UV-328: <11 WWTP2 BP3:824-2,116 EDP: <10 EMC: 2,501 – 4,689 OC: 26,823 -41,610 UV-234: <14 UV-327: 83.3 – 159.9 UV-328: <25 UV-329: 1,493 – 3,303	(Langford et al., 2015)
BP1, 4DHB, 4HB, BP3, 4-MBC, OC, ODP, EMC	Spain	Freeze-dried and grinded samples **PLE:** 1 g sample, Al_2O_3, preheating of 5 min, 2 static cycles of 5 min with MeOH, 2 static cycles of 5 min using MeOH/H_2O (1:1) at 100 ◦C, 10 000 kPa)	UPLC–ESI-MS/MS	4-MBC: 102 OC: 70 EMC: 90 ODP: 85 BP3: 70 BP1: 30 4HB: 95 4DHB: 96	4-MBC: 12 OC: 18 EMC: 19 ODP: 0.2 BP3: 1.0 BP1: 60 4HB: 5.0 4DHB: 5.0	Raw sludge 4DHB: 70 BP3: 60 4-MBC: 3,100 OC: 8,000 EMC: 2,200 BP1, 4HB, ODP: n.d. Treated sludge 4-MBC: 250 OC: 570 EMC: 100 BP1, 4DHB, 4HB, BP3, ODP: n.d.	(Badia-Fabregat et al., 2012)

Table S3.2 - Overview on analytical methods for determinations of UVFs in sewage sludge (Cont.)

Compounds	Location	Method overview	Instrumental method	Recovery (%)	LOD (ng g^{-1})	Concentration (ng g^{-1} dw)	Reference
BP3, 4-MBC, EMC, UV-326, UV-329, OC	Australia	1 g sodium azide added Freeze-dried samples **PLE:** 1.0 g, Na_2SO_4, Si, copper powder, acid washed sand, Hex/DCM (1:1) (extraction solvent), 120 ◦C, 5 min, 2 cycles.	GC-MS/MS	BP3: 94 - 130 4-MBC: 68 - 81 EMC: 76 - 90 UV-326: 81 - 96 UV-329: 125 - 152 OC: 82 - 91	BP3: 7.3 4-MBC: 2.8 EMC: 0.7 UV-326: 0.3 UV-329: 8.2 OC: 3.6	Influent: BP3: 104 – 111 4-MBC: 341 - 403 EMC: 218 - 229 UV-326: 81 - 90 UV-329: 91 - 93 OC: 303 - 326 Digested sludge: BP3: 149 – 303 4-MBC: 958 – 2,020 EMC: 385 – 401 UV-326: 52 – 60 UV-329: 64 – 74 OC: 1,147 – 1,838 Biosolid: BP3: 16 4-MBC: 962 EMC: 30 UV-326: 88 UV-329: 27 OC: 465	(Liu et al., 2012)
BP1, 4DHB, 4HB, BP3, 4-MBC, OC, ODP, EMC	Spain	Freeze-dried and grinded samples **PLE:** 1 g sample, Al_2O_3, preheating of 5 min, 2 static cycles of 5 min with MeOH, 2 static cycles of 5 min using MeOH/H_2O (1:1) at 100 ◦C, 10,000 kPa.	UPLC–ESI-MS/MS	4-MBC: 102 OC: 70 EMC: 90 ODP: 85 BP3: 70 BP1: 30 4HB: 95 4DHB: 96	4-MBC: 12 OC: 18 EMC: 19 ODP: 0.2 BP3: 1.0 BP1: 60 4HB: 5.0 4DHB: 5.0	BP1, 4HB, BP3, ODP, EMC: n.d. 4DHB: <LOQ 4-MBC: 1,630 OC: 2,600	(Gago-Ferrero et al., 2011)

Table S3.2 - Overview on analytical methods for determinations of UVFs in sewage sludge (Cont.)

Compounds	Location	Method overview	Instrumental method	Recovery (%)	LOD (ng g^{-1})	Concentration (ng g^{-1} dw)	Reference
BP1, 4DHB, BP3, 4-MBC, OC, ODP, EMC	Spain	Freeze-dried and grinded samples **PLE:** 1 g sample, Al_2O_3 (purification step); MeOH (extraction solvent). Filtration	UPLC-TQD	-	-	Raw sludge: BP1: 0.08 4DHB: 0.051 BP3: 0.034 4-MBC: 0.520 OC: 7.71 ODP: 0.012 EMC: 1.031 Treated sludge: BP1: n.d. 4DHB: 0.050 BP3: 0.019 4-MBC: 0.205 OC: 3.214 ODP: 0.004 EMC: 0.211	(Rodríguez-Rodríguez et al., 2012)
BP3, 4-MBC, EMC, UV-326, UV-329, OC	Australia	Freeze-dried samples **PLE**: 1 g sample, Na_2SO_4, silica, copper powder, 120 °C, Hex/DCM (1:1) (extraction solvent), 2 cycles 5 min. **Filtration** (membrane filter)	GC-MS/MS	BP3: 94 - 130 4-MBC: 68 - 81 EMC: 76 - 90 UV-326: 81 - 96 UV-329: 125 - 152 OC: 82 – 91	BP3: 7.3 4-MBC: 2.8 EMC: 0.7 UV-326: 0.3 UV-329: 8.2 OC: 3.6	BP3: 74.0 4-MBC: 250 EMC: 31.9 UV-326: 49.9 UV-329: 122.9 OC: 138.4	(Liu et al., 2011)
BP1, BP2, BP3, BP4, PBSA	Germany	Freeze-dried samples **Centrifugation:** 4,000 rpm, 15 min. **PLE:** 200 mg sample, baked out sea sand; H_2O/MeOH (1:1), 4 static cycles, 80 °C. **SPE:** 200 mg HLB cartridge; MeOH/Ac (3:2)	LC-MS/MS	BP1: 74 BP2: 99 BP3: 104 BP4: 114 PBSA: 118	BP1, BP2: 2.5 BP3: 25 BP4, PBSA: 5	BP1: 5.1 BP2: 11 BP3: 132 BP4: 29 PBSA: <LOQ	(Wick et al., 2010)

Table S3.2 - Overview on analytical methods for determinations of UVFs in sewage sludge (Cont.)

Compounds	Location	Method overview	Instrumental method	Recovery (%)	LOD (ng g^{-1})	Concentration (ng g^{-1} dw)	Reference
ES, HMS, BP3, 4-MBC, EMC, OC, IMC, ODP	Spain	Freeze-dried samples **PLE:** 0.5 g sample, diatomaceous earth, graphitized carbon, Hex/DCM (80:20) (extraction solvent), 75 ◦C, 1 cycle, 5 min, 1500 psi. **SPE:** 0.5 g PSA cartridge, Hex/Eth (1:1) (elution); isooctane (keeper))	GC-MS	**Primary sludge** ES: 95 – 101; HMS: 78 - 96 IMC: 80 – 107; BP3: 89 - 106 4-MBC: 79 – 86; EDP: 88 - 93 EMC: 73 – 90; OC: 84 - 85 **Biological sludge** ES, HMS: 100 - 103 IMC: 90 – 98; BP3: 112 - 100 4-MBC, OC: 91 - 112 EDP: 83 – 104; EMC: 88 - 90	ES: 17 HMS: 34 IMC:34 BP3: 61 4-MBC: 26 EDP:22 EMC: 24 OC: 33	**Primary sludge** ES, HMS, IMC, BP3, EDP: n.d. 4-MBC: 106 – 1,543 EMC: 213 – 3,287 OC: 1,039 – 2,242 **Biological sludge** ES: 133 - 270 HMS: 110 - 207 IMC, EDP: n.d. BP3: 93 4-MBC: 97 – 1,439 EMC: 104 - 856 OC: 377 – 3,263	(Negreira et al., 2011)
BP3, IMC, 4-MBC, OC, BMDM, ODP, EMC, ES, HMS, DBT, EHT	Belgium	**PLE:** 75 g sample, EA	LC-MS/MS	BP3, IMC: 120 4-MBC: 106 OC: 108 BMDM, ODP: 113 EMC: 105 ES: 95 HMS: 96 DBT: 107 EHT: 124	BP3: 8 IMC, EMC: 12 4-MBC: 10 OC: 18 BMDM: 17 ODP: 9 ES, HMS:>1000 DBT: 200 EHT: 52	BP3: 6.6 IMC: 5.0 4-MBC: 3,893 OC: 2,479 BMDM:144 ODP: 1.4 EMC: 127 ES: 49 HMS: 22 DBT: 136 EHT: 928	(Rodil et al., 2009)
	Germany					BP3: 29 IMC: 20.0 4-MBC: 73 OC: 585 BMDM: 517 ODP: 1.9 EMC: 35 ES: 280 HMS: 331 DBT: 54 EHT: 1,433	

Table S3.2 - Overview on analytical methods for determinations of UVFs in sewage sludge (Cont.)

Compounds	Location	Method overview	Instrumental method	Recovery (%)	LOD (ng g^{-1})	Concentration (ng g^{-1} dw)	Reference
4-MBC, EMC, OC, EHT	Switzerland	**LLE:** 50 g sample + Na_2SO_4, Hex, DCM/Hex (1:1). **GPC:** 100 g Bio-Beads S-X3, Hex/DCM (1:1). **SPE:** 4 g florisil activated at 650 °C for 2 h, 1 cm Na_2SO_4, Hex/DE (9:1) (elution discarded), DE (elution solvent).	GC-MS (4-MBC, OMC, OC) HPLC-DAD (EHT)	4-MBC: 95 EMC: 101 OC: 87 EHT: 75	4-MBC: 4 EMC: 3 OC: 6 EHT: 57	Raw sludge: 4-MBC: 210 – 1,830 EMC: 920 – 14,450 OC: 1,200 – 4,680 EHT: 1,700 – 2,700 Excess sludge: 4-MBC: 340 – 500 EMC: 150 – 440 OC: 1,010 – 1,320 EHT: 1,000 – 1,300 Digestion sludge: 4-MBC: 1,260 – 2,290 EMC: 1,020 – 1,500 OC: 3,040 – 4,950 EHT: 2,600 – 27,000 Storage tank sludge: 4-MBC: 1,900 – 2,970 EMC: 30 – 370 OC: 1,980 – 9,520 EHT: 1,500 – 8,100	(Kupper et al., 2006)
4-MBC, EMC, OC, EHT	Switzerland	**LLE:** 60 g sample, NaCl, PN/Ac (1:1), 30 min; PN/DE (1:1) + DE/DCM (4:1). **SPE:** 5 g silica gel activated during 15 h at 180 °C (H_2O added to 1.5% by weight), Hex/DE (9:1). **Reconstitution:** EA (4-MBC, EMC, OC), EtOH (EHT)	GC-MS (4-MBC, EMC, OC) HPLC/DAD, LC-ES-MS-MS (EHT)	4-MBC: 94.6 OMC: 101.2 OC: 87.5 EHT: 75.0	4-MBC: 4 OMC: 3 OC: 6 EHT: 57	WWTP A 4-MBC: 150 – 1,000 EMC: 30 - 95 OC: 320 – 2,480 EHT: 700 – 6,300 WWTP B 4-MBC: 250 – 3,340 EMC: 70 – 390 OC: 2,580 – 7,860 EHT: 1,000 – 11,000 WWTP C 4-MBC: 610 – 4,980 EMC: 10 – 295 OC: 1,600 – 18,740 EHT: 3,300 – 27,700	(Plagellat et al., 2006)

3. Compound table

Table S3.3 - Optimized transitions for the analysis of the target compounds, UV-Filters, Synthetic Musk Compounds and Internal Standards. Quantifier transition presented in bold.

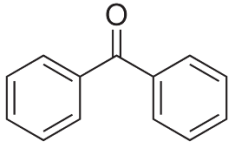
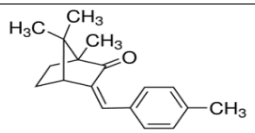
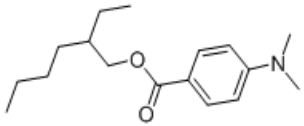
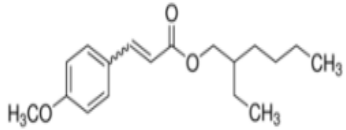
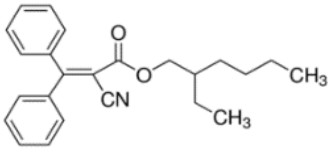
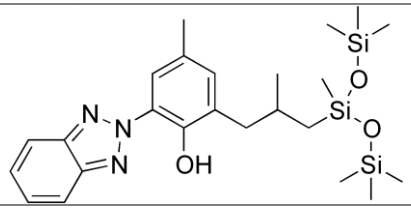
Class	Compound	Abbreviation	Structure	Chemical name/CAS	Molecular weight	Retention time (min)	MRM transition	Collision Energy (eV)	Internal Standard
UV-Filters	Benzophenone	BZ		119-61-9	182.22	7.71	**105>51** 182>105	(25) (10)	AHTN-d3
	3-(4'-methylbenzylidene) camphor	4-MBC		36861-47-9	254.37	11.23	**128>77** 254>149	(25) (10)	4MBC-d4
	Ethylhexyl dimethyl PABA	EDP		21245-02-3	277.4	12.63	**165>119** 165>149	(20) (10)	4MBC-d4
	2-ethylhexyl 4 – methoxycinnamate	EMC		5466-77-3	290.4	12.91	**178>161** 178>132	(10) (15)	4MBC-d4
	Octocrylene	OC		6197-30-4	361.48	14.49	**204>176** 360>276	(25) (20)	4MBC-d4
	Drometrizole trisiloxane	DTS		155633-54-8	501.85	15.71	**221>73** 221>221	(15) (5)	4MBC-d4

Table S3.3 - Optimized transitions for the analysis of the target compounds, UV-Filters, Synthetic Musk Compounds and Internal Standards. Quantifier transition presented in bold. (cont.)

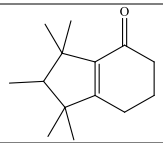
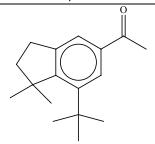
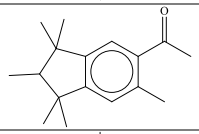
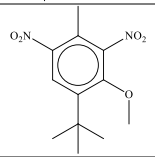
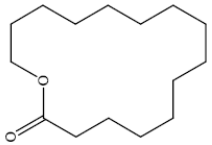
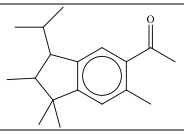
Class	Compound	Abbreviation	Structure	Chemical name/CAS	Molecular weight	Retention time (min)	MRM transition	Collision Energy (eV)	Internal Standard
Synthetic Musks	Cashmeran	DPMI		33704-61-9	206.3	6.77	**191>135** 206>192	(10) (10)	AHTN-d3
	Celestolide	ADBI		13171-00-1	244.3	8.26	**229>173** 244>229	(5) (10)	AHTN-d3
	Phantolide	AHMI		15323-35-0	244.3	8.59	**244>229** 229>187	(5) (5)	AHTN-d3
	Musk ambrette	MA		83-66-9	268.3	9.18	**253>106** 253>121 268>253	(10) (5) (5)	MX-d15
	Exaltolide	EXA		106-02-5	240.4	9.35	**83>55** 69>68	(5) (5)	AHTN-d3
	Traseolide	ATII		68140-48-7	258.4	9.32	**215>173** 258>215	(10) (5)	AHTN-d3

Table S3.3 - Optimized transitions for the analysis of the target compounds, UV-Filters, Synthetic Musk Compounds and Internal Standards. Quantifier transition presented in bold. (cont.)

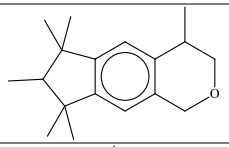
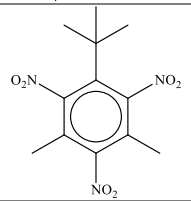
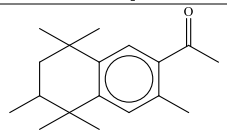
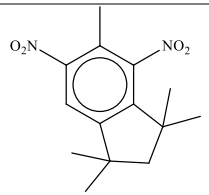
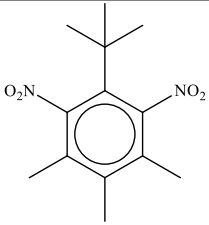
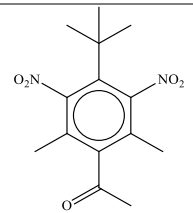
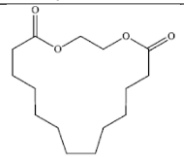
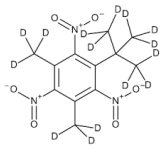
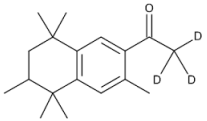
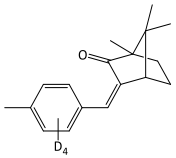
Class	Compound	Abbreviation	Structure	CAS	Molecular weight	Retention time (min)	MRM transition	Collision Energy (eV)	Internal Standard
Synthetic Musks	Galaxolide	HHCB		1222-05-5	258.4	9.39	**243>213** 213>171	(10) (5)	AHTN-d3
	Musk xylene	MX		81-15-2	297.2	9.40	**282>119** 282>160 282>265	(10) (10) (5)	MX-d15
	Tonalide	AHTN		1506-02-1	258.4	9.40	**258>243** 243>128	(10) (40)	AHTN-d3
	Musk moskene	MM		116-66-5	278.3	9.63	**263>156** 263>144 263>211	(20) (25) (5)	MX-d15
	Musk tibetene	MT		145-39-1	266.3	10.04	**266>251** 251>132 251>160	(5) (10) (15)	MX-d15

Table S3.3 - Optimized transitions for the analysis of the target compounds, UV-Filters, Synthetic Musk Compounds and Internal Standards. Quantifier transition presented in bold. (cont.)

Class	Compound	Abbreviation	Structure	CAS	Molecular weight	Retention time (min)	MRM transition	Collision Energy (eV)	Internal Standard
Synthetic Musks	Musk ketone	MK		81-14-1	294.3	10.38	**279>118** 279>191 294>279	(20) (10) (5)	MX-d15
	Ethylene brassylate	EB		105-95-3	270.4	10.83	**98>83** 227>113	(5) (10)	AHTN-d3
Internal Standards	Xylene-d15	MX-d15		877119-10-3	312.36	9.28	**294>294** 294>122 294>276	(5) (15) (10)	
	Tonalide-d3	AHTN-d3			261.40	9.42	**246>190** 246>204 261>246	(5) (10) (5)	
	(±)-3-(4-Methylbenzylidine-d4) camphor	4-MBC-d4		1219806-41-3	258.40	10.22	**132>105** 258>150 258>108	(15) (5) (10)	

4. Chromatogram

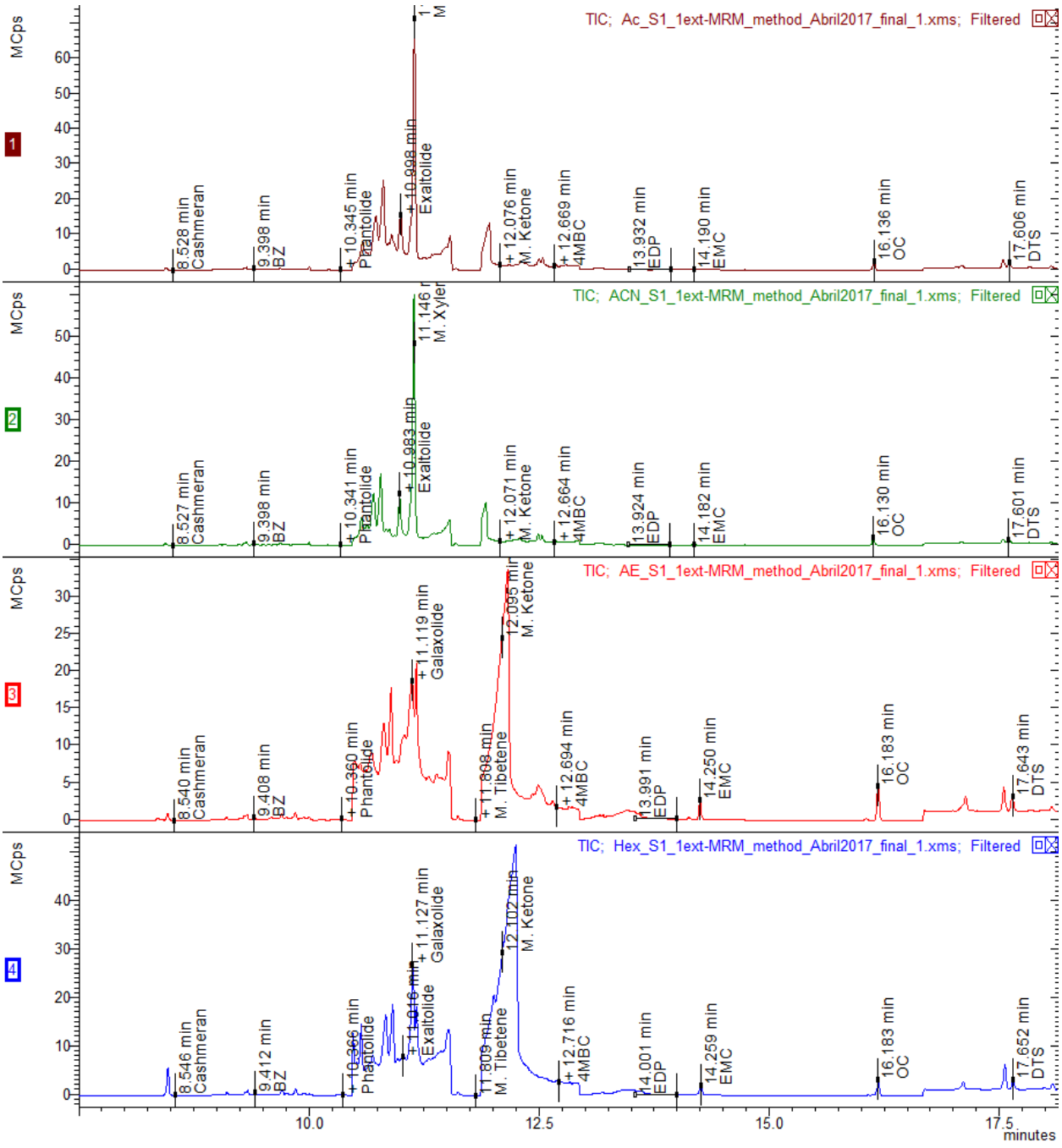


Figure S3.1 - Total Ion Chromatograms resultant of the extraction of SMCs and UVFs using different solvents: Acetone (dark red), Acetonitrile (green), Ethyl Acetate (red) and Hexane (blue). Conditions described in manuscript.

5. Conditions for the Screening Design

Table S3.4 - Conditions set for the screening design

Exp #	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8
1	12	0	1	0	0	500	500	Ac
2	5	0	1	0	500	0	500	ACN
3	12	0	5	0	500	500	0	Hex/DCM (1:1)
4	5	0	1	500	0	0	0	Hex/DCM (1:1)
5	5	15	5	0	0	0	500	Hex/DCM (1:1)
6	5	15	1	500	500	500	0	ACN
7	12	15	1	500	0	500	500	EA
8	12	15	1	0	500	0	0	EA
9	12	15	5	500	500	0	500	Ac
10	5	0	5	500	500	500	500	EA
11	5	15	5	0	0	500	0	Ac
12	12	0	5	500	0	0	0	ACN

Obs. X1 - Solvent Volume (mL); X2 - US extraction (min); X3 - Time Vortex (min); X4 - Amount of C_{18} (mg); X5 - Amount of PSA (mg); X6 - Amount of Florisil (mg); X7 - Amount of Alumina (mg); X8 - Type of solvent.

Table S3.5 - Conditions set for the CCD

Exp. #	Pattern	X_1	X_2	X_3	X_4
1	0000	8.5	2.5	450	450
2	++−+	11.0	3.5	315	585
3	+−−−	11.0	1.5	315	315
4	++++	11.0	3.5	585	585
5	++−−	11.0	3.5	315	315
6	0000	8.5	2.5	450	450
7	00A0	8.5	2.5	650	450
8	+−+−	11.0	1.5	585	315
9	+−−+	11.0	1.5	315	585
10	−+++	6.0	3.5	585	585
11	−++−	6.0	3.5	585	315
12	0A00	8.5	4.0	450	450
13	0000	8.5	2.5	450	450
14	0a00	8.5	1.0	450	450
15	00a0	8.5	2.5	250	450
16	−−−−	6.0	1.5	315	315
17	a000	5.0	2.5	450	450
18	+−++	11.0	1.5	585	585
19	0000	8.5	2.5	450	450
20	+++−	11.0	3.5	585	315
21	0000	8.5	2.5	450	450
22	−−++	6.0	1.5	585	585
23	−+−+	6.0	3.5	315	585
24	000A	8.5	2.5	450	650
25	−−−+	6.0	1.5	315	585
26	−+−−	6.0	3.5	315	315
27	000a	8.5	2.5	450	250
28	−−+−	6.0	1.5	585	315
29	A000	12.0	2.5	450	450
30	0000	8.5	2.5	450	450

Obs. X1 - Solvent Volume (mL); X2 - Time Vortex (min); X3 - Amount of C_{18} (mg); X4 - Amount of PSA (mg).

Table S3.6 - Second-order polynomial equation obtained for each target compound

Compound	Equation
BZ	$y = 92.51 - 2.73x_4 + 6.06x_1x_2 - 3.69x_2x_4 - 2.69x_1^2 - 7.24x_2^2 + 3.91x_4^2$
4MBC	$y = 88.56 - 5.5x_1x_2 - 2.25x_1x_3 - 4.75x_2x_3 - 3.84x_2^2$
EDP	$y = 75.06 + 3.13x_1 - 7.05x_3 - 2.52x_4 - 6.88x_1x_3 + 5.62x_3x_4 - 4.40x_1^2$
EHMC	$y = 116.67 + 17.66x_1 + 4.80x_2 - 9.04x_3 + 5.19x_1x_2 - 8.06x_1x_3 - 7.06x_2x_4 + 8.69x_3x_4 - 8.55x_1^2$
OC	$y = 82.86 - 4.26x_1 + 7.25x_1x_2 - 7.62x_1x_3 + 6.5x_2x_3 + 6.75x_1x_4 + 4.5x_3x_4 + 7.27x_3^2 + 7.04x_4^2$
DTS	$y = 88.73 + 27.54x_1 + 6.62x_2 - 7.19x_3 - 10.30x_4 - 6.81x_2x_3 - 9.94x_1x_4 - 8.58x_2^2 + 15.08x_3^2$
DPMI	$y = 75.37 - 8.62x_1 + 3.99x_3 - 4.63x_1x_4 - 5.13x_2x_4 - 5.88x_3x_4 - 5.37x_1^2 - 7.65x_2^2 - 6.06x_3^2$
ADBI	$y = 103.15 + 3.88x_1x_4 - 9.63x_2x_4 - 9.35x_1^2 - 9.81x_2^2 - 7.53x_3^2$
AHMI	$y = 76.63 + 3.49x_2 - 2.96x_3 + 3.69x_4 + 4.25x_1x_2 - 4.5x_2x_3 - 4.63x_1x_4 - 3.13x_2x_4 + 3.63x_1^2 - 7.97x_2^2 - 4.33x_3^2$
ATII	$y = 72.55 - 4.28x_3 + 7.13x_1x_2 - 6.58x_2x_3 + 3.25x_3x_4 + 4.15x_1^2 - 4.94x_2^2 + 7.57x_4^2$
EXA	$y = 105.72 - 3.26x_1 + 6.24x_2 + 4.57x_4 - 3.25x_1x_3 + 4.75x_1x_4 - 3.13x_2x_4 + 8.75x_3x_4 - 6.99x_2^2 - 15.64x_3^2$
HHCB	$y = 108.00 + 4.40x_1 - 2.45x_2 + 1.96x_3 + 3.70x_4 - 3.44x_1x_2 - 5.19x_1x_3 + 6.31x_1x_4 - 8.56x_2x_4 - 3.31x_3x_4 + 4.10x_2^2 - 6.59x_4^2$
AHTN	$y = 97.61 - 3.49x_1 + 4.01x_2 - 4.80x_3 + 3.10x_4 - 6.25x_1x_3 + 5.13x_3x_4 + 9.01x_1^2 - 9.87x_3^2$
EB	$y = 71.70 - 2.01x_3 + 5.46x_4 + 3.13x_1x_2 - 2.25x_1x_3 - 6x_2x_3 - 5.63x_1x_4 + 3.24x_1^2 - 9.72x_2^2 + 5.06x_4^2$
MA	$y = 114.72 - 2.94x_1x_2 + 2.32x_1x_3 + 2.81x_2x_3 + 1.69x_1x_4 - 2.56x_3x_4 - 3.45x_1^2 + 6.56x_2^2 + 1.33x_3^2 - 2.99x_4^2$
MX	$y = 103.64 + 3.56x_1 - 3.53x_2 + 4.74x_3 - 7.19x_1x_2 + 5.19x_2x_3 + 6.56x_1x_4 - 5.06x_2x_4 - 5.06x_3x_4 - 4.92x_1^2$
MM	$y = 122.44 + 1.96x_3 - 2.05x_4 - 2.69x_1x_2 + 4.69x_2x_3 + 4.69x_1x_4 - 4.06x_2x_4 - 3.94x_3x_4 - 9.02x_1^2 - 3.34x_2^2 - 2.66x_3^2$
MT	$y = 116.93 - 6.26x_1 - 1.37x_2 - 1.94x_1x_2 + 3.31x_2x_3 - 1.94x_1x_4 - 4.44x_2x_4 - 3.19x_3x_4 - 5.80x_1^2 - 1.70x_2^2$
MK	$y = 101.56 - 1.71x_1 - 4.16x_2 + 3.48x_3 - 1.46x_4 + 4.38x_1x_3 + 3.5x_2x_3 - 3.75x_2x_4 - 8.94x_1^2 + 3.34x_2^2$

Table S3.7 - Additional testing to prove the applicability of the proposed empirical model

	A *(Pattern: AAAA)*		B *(Pattern: aaaa)*		C *(Pattern: aa-a)*		D *(Pattern: a-+A)*		E *(Pattern: aAA-)*	
	%Rec	%Rec Pred.	%Rec	%Rec Pred.	%Rec	%Rec Pred.	%Rec	%Rec Pred.	%Rec	%Rec Pred.
BZ	85±5	70±17	89±9	73±17	88±2	76±15	89±3	100±13	82±10	73±15
4MBC	53±1	41±13	69±3	61±13	94±3	67±11	98±6	95±9	95±2	86±11
EDP	66±2	52±20	65±3	73±22	66±3	66±18	62±6	68±15	64±2	53±18
EMC	116±3	105±29	89±8	69±29	93±9	60±25	78±9	96±21	73±6	67±25
OC	131±4	154±25	127±10	162±25	128±5	151±22	110±3	122±19	149±5	140±22
DTS	54±8	81±54	47±10	36±54	45±6	16±47	23±3	52±40	27±3	23±47
DPMI	95±7	47±71	43±6	19±21	46±2	32±19	76±9	83±16	62±3	51±19
ADBI	69±10	40±31	70±4	40±31	76±8	47±27	69±3	72±23	70±5	45±27
AHMI	62±10	47±20	50±3	32±20	54±10	39±17	89±9	101±15	42±4	35±17
ATII	93±5	96±24	87±6	76±24	88±4	79±21	76±2	89±18	33±2	15±21
EXA	74±10	104±23	93±3	81±23	81±5	88±20	88±2	88±17	85±3	98±20
HHCB	80±3	91±16	82±2	70±16	88±3	73±14	91±4	102±12	157±3	153±14
AHTN	93±5	84±26	111±4	87±26	82±3	97±23	116±8	108±20	116±2	93±23
EB	56±6	40±17	50±2	34±17	42±2	41±15	106±2	115±13	40±1	29±15
MA	118±6	125±8	120±8	122±8	115±3	119±7	91±5	92±6	132±6	134±7
MX	87±10	92±25	87±8	80±25	79±3	81±22	83±3	63±19	124±6	137±22
MM	101±3	85±15	99±5	86±15	100±6	89±13	86±7	74±11	118±9	129±13
MT	87±4	76±11	101±1	92±11	102±3	92±10	104±2	113±8	114±5	124±10
MK	98±4	94±10	109±3	105±10	113±6	101±9	99±9	89±8	105±5	102±9

References

Artola-Garicano, E., Borkent, I., Hermens, J.L.M., Vaes, W.H.J., 2003. Removal of two polycyclic musks in sewage treatment plants: Freely dissolved and total concentrations. Environ. Sci. Technol. 37, 3111–3116.

Badia-Fabregat, M., Rodríguez-Rodríguez, C.E., Gago-Ferrero, P., Olivares, A., Piña, B., Díaz-Cruz, M.S., Vicent, T., Barceló, D., Caminal, G., 2012. Degradation of UV filters in sewage sludge and 4-MBC in liquid medium by the ligninolytic fungus Trametes versicolor. J. Environ. Manage. 104, 114–120.

Berset, J.D., Kupper, T., Etter, R., Tarradellas, J., 2004. Considerations about the enantioselective transformation of polycyclic musks in wastewater, treated wastewater and sewage sludge and analysis of their fate in a sequencing batch reactor plant. Chemosphere 57, 987–996.

Bester, K., 2004. Retention characteristics and balance assessment for two polycyclic musk fragrances (HHCB and AHTN) in a typical German sewage treatment plant. Chemosphere 57, 863–870.

Chen, D., Zeng, X., Sheng, Y., Bi, X., Gui, H., Sheng, G., Fu, J., 2007. The concentrations and distribution of polycyclic musks in a typical cosmetic plant. Chemosphere 66, 252–258.

Clara, M., Gans, O., Windhofer, G., Krenn, U., Hartl, W., Braun, K., Scharf, S., Scheffknecht, C., 2011. Occurrence of polycyclic musks in wastewater and receiving water bodies and fate during wastewater treatment. Chemosphere 82, 1116–1123.

Gago-Ferrero, P., Diaz-Cruz, M.S., Barcelo, D., 2011. Occurrence of multiclass UV filters in treated sewage sludge from wastewater treatment plants. Chemosphere 84, 1158–1165.

Gatermann, R., Biselli, S., Hühnerfuss, H., Rimkus, G.G., Hecker, M., Karbe, L., 2002. Synthetic musks in the environment. Part 1: Species-dependent bioaccumulation of polycyclic and nitro musk fragrances in freshwater fish and mussels. Arch. Environ. Contam. Toxicol. 42, 437–446.

Guo, R., Lee, I.S., Kim, U.J., Oh, J.E., 2010. Occurrence of synthetic musks in Korean sewage sludges. Sci. Total Environ. 408, 1634–1639.

Herren, D., Berset, J.D., 2000. Nitro musks, nitro musk amino metabolites and polycyclic musks in sewage sludges. Quantitative determination by HRGC-ion-trap-MS/MS and mass spectral characterization of the amino metabolites. Chemosphere 40, 565–574.

Horii, Y., Reiner, J.L., Loganathan, B.G., Senthil Kumar, K., Sajwan, K., Kannan, K., 2007. Occurrence and fate of polycyclic musks in wastewater treatment plants in Kentucky and Georgia, USA. Chemosphere 68, 2011–2020.

Hu, Z., Shi, Y., Zhang, S., Niu, H., Cai, Y., 2011. Assessment of synthetic musk fragrances in seven wastewater treatment plants of Beijing, China. Bull. Environ. Contam. Toxicol. 86, 302–306.

Kupper, T., Plagellat, C., Brändli, R.C., de Alencastro, L.F., Grandjean, D., Tarradellas, J., 2006. Fate and removal of polycyclic musks, UV filters and biocides during wastewater treatment. Water Res. 40, 2603–2612.

Langford, K.H., Reid, M.J., Fjeld, E., Øxnevad, S., Thomas, K. V., 2015. Environmental occurrence and risk of organic UV filters and stabilizers in multiple matrices in Norway. Environ. Int. 80, 1–7.

Liu, N., Shi, Y., Li, W., Xu, L., Cai, Y., 2014. Concentrations and distribution of synthetic musks and siloxanes in sewage sludge of wastewater treatment plants in china. Sci. Total Environ. 476–477, 65–72.

Liu, Y.-S., Ying, G.-G., Shareef, A., Kookana, R.S., 2011. Simultaneous determination of benzotriazoles and ultraviolet filters in ground water, effluent and biosolid samples using gas chromatography–tandem mass spectrometry. J. Chromatogr. A 1218, 5328–5335.

Liu, Y.-S.S., Ying, G.-G.G., Shareef, A., Kookana, R.S., 2012. Occurrence and removal of benzotriazoles and ultraviolet filters in a municipal wastewater treatment plant. Environ. Pollut. 165, 225–232.

Lv, Y., Yuan, T., Hu, J., Wang, W., 2010. Seasonal occurrence and behavior of synthetic musks (SMs) during wastewater treatment process in Shanghai, China. Sci. Total Environ. 408, 4170–4176.

Matamoros, V., Nguyen, L.X., Arias, C.A., Nielsen, S., Laugen, M.M., Brix, H., 2012. Musk fragrances, DEHP and heavy metals in a 20 years old sludge treatment reed bed system. Water Res. 46, 3889–3896.

Muller, J., Boehmer, W., Litz, N.T., 2006. Occurrence of polycyclic musks in sewage sludge and their behaviour in soils and plants: Part 1: Behaviour of Polycyclic Musks in Sewage Sludge of Different Treatment Plants in Summer and Winter. J. Soils Sediments 6, 231–235.

Negreira, N., Rodríguez, I., Rubí, E., Cela, R., 2011. Optimization of pressurized liquid extraction and purification conditions for gas chromatography–mass spectrometry determination of UV filters in sludge. J. Chromatogr. A 1218, 211–217.

Plagellat, C., Kupper, T., Furrer, R., de Alencastro, L.F., Grandjean, D., Tarradellas, J., 2006. Concentrations and specific loads of UV filters in sewage sludge originating from a monitoring network in Switzerland. Chemosphere 62, 915–925.

Reiner, J.L., Berset, J.D., Kannan, K., 2007. Mass flow of polycyclic musks in two wastewater treatment plants. Arch. Environ. Contam. Toxicol. 52, 451–457.

Rodil, R., Schrader, S., Moeder, M., 2009. Pressurised membrane-assisted liquid extraction of UV filters from sludge. J Chromatogr A 1216, 8851–8858.

Rodríguez-Rodríguez, C.E., Barón, E., Gago-Ferrero, P., Jelić, A., Llorca, M., Farré, M., Díaz-Cruz, M.S., Eljarrat, E., Petrović, M., Caminal, G., Barceló, D., Vicent, T., 2012. Removal of pharmaceuticals, polybrominated flame retardants and UV-filters from sludge by the fungus Trametes versicolor in bioslurry reactor. J. Hazard. Mater. 233–234, 235–243.

Shek, W.M., Murphy, M.B., Lam, J.C.W., Lam, P.K.S., 2008. Synthetic polycyclic musks in Hong Kong sewage sludge. Chemosphere 71, 1241–1250.

Smyth, S.A., Lishman, L., Alaee, M., Kleywegt, S., Svoboda, L., Yang, J.J., Lee, H.B., Seto, P., 2007. Sample storage and extraction efficiencies in determination of polycyclic and nitro musks in sewage sludge. Chemosphere 67, 267–275.

Stevens, J.L., Northcott, G.L., Stern, G.A., Tomy, G.T., Jones, K.C., 2003. Pesticides , Synthetic Musks , and Polychlorinated n -Alkanes in U . K . Sewage Sludge : Survey Results and Implications. Environ. Sci. Technol. 37, 462–467.

Sun, P., Casteel, K., Dai, H., Wehmeyer, K.R., Kiel, B., Federle, T., 2014. Distributions of polycyclic musk fragrance in wastewater treatment plant (WWTP) effluents and sludges in the United States. Sci. Total Environ. 493, 1073–1078.

Sun, Q., Li, M., Ma, C., Chen, X., Xie, X., Yu, C.P., 2016. Seasonal and spatial variations of PPCP occurrence, removal and mass loading in three wastewater treatment plants located in different urbanization areas in Xiamen, China. Environ. Pollut. 208, 371–381.

Ternes, T.A., Bonerz, M., Herrmann, N., Löffler, D., Keller, E., Lacida, B.B., Alder, A.C., 2005. Determination of pharmaceuticals, iodinated contrast media and musk fragrances in sludge by LC tandem MS and GC/MS. J. Chromatogr. A 1067, 213–223.

Ternes, T.A., Herrmann, N., Bonerz, M., Knacker, T., Siegrist, H., Joss, A., 2004. A rapid method to measure the solid-water distribution coefficient (Kd) for pharmaceuticals and musk fragrances in sewage sludge. Water Res. 38, 4075–4084.

Vallecillos, L., Pocurull, E., Borrull, F., 2013. A simple and automated method to determine macrocyclic musk fragrances in sewage sludge samples by headspace solid-phase microextraction and gas chromatography-mass spectrometry. J. Chromatogr. A 1314, 38–43.

Wick, A., Fink, G., Ternes, T.A., 2010. Comparison of electrospray ionization and atmospheric pressure chemical ionization for multi-residue analysis of biocides, UV-filters and benzothiazoles in aqueous matrices and activated sludge by liquid chromatography–tandem mass spectrometry. J. Chromatogr. A 1217, 2088–2103.

Wu, S.F., Ding, W.H., 2010. Fast determination of synthetic polycyclic musks in sewage sludge and sediments by microwave-assisted headspace solid-phase microextraction and gas chromatography-mass spectrometry. J. Chromatogr. A 1217, 2776–2781.

Yang, J.J., Metcalfe, C.D., 2006. Fate of synthetic musks in a domestic wastewater treatment plant and in an agricultural field amended with biosolids. Sci. Total Environ. 363, 149–165.

Zeng, X., Sheng, G., Xiong, Y., Fu, J., 2005. Determination of polycyclic musks in sewage sludge from Guangdong, China using GC-EI-MS. Chemosphere 60, 817–823.

Zhou, H., Huang, X., Gao, M., Wang, X., Wen, X., 2009. Distribution and elimination of polycyclic musks in three sewage treatment plants of Beijing, China. J. Environ. Sci. 21, 561–567.

Annex 4. Supporting Information Chapter 5

1. Standards and Solutions preparation

All synthetic musk compounds were obtained from LGC Standards (Barcelona, Spain) with 99% purity, except galaxolide (HHCB), which contains approximately 25% of diethyl phthalate. The polycyclic musks were available as solid standards: cashmeran (DPMI), celestolide (ADBI), phantolide (AHMI), traseolide (ATII), tonalide (AHTN) and galaxolide (HHCB). The nitro musks tibetene (MT) and moskene (MM) were obtained as a 10 mg L^{-1} solution in cyclohexane. Musk ambrette (MA) and musk ketone (MK) were purchased as solid standards from Dr. Ehrenstorfer (Augsburg, Germany) with 99% and 98% purity, respectively. From Sigma-Aldrich (St. Louis, MO, USA) were obtained musk xylene (MX) as a 100 mg L^{-1} solution in acetonitrile, and solid standards of exaltolide (EXA) and ethylene brassylate (EB) with 99% and 95% purity, respectively. The surrogate standards musk xylene-d15 (MX-d15) and tonalide-d3 (AHTN-d3) were purchased from Dr. Ehrenstorfer (Augsburg, Germany) as 100 mg L^{-1} solutions in acetone and iso-octane, respectively. UV-filters 2-ethylhexyl 4-dimethylaminobenzoate (EDP) and 3-(4'-methylbenzylidene) camphor (4-MBC) were purchased from Alfa Aesar (Karlsruhe, Germany), both with 99% purity. 2-ethylhexyl 4–methoxycinnamate (EMC), 2-ethylhexyl 2-cyano-3,3-diphenylacrylate (OC) and benzophenone (BZ) were purchased from Sigma-Aldrich (St. Louis, MO, USA) with 98%, 97% and 99% purity, respectively. Drometrizole trisiloxane (DTS) was purchased from Fluka (Saint Louis, MO, USA) with 98% purity. Surrogate (±)-3-(-4-methylbenzylidine-d4) camphor (4-MBC-d4) was purchased with 99% purity from CDN Isotopes (Pointe-Claire, Quebec, Canada).

Individual stock solutions of each compound were prepared between 1 and 5 g L^{-1}, both in acetonitrile and hexane. Using the individual stock solutions, working solutions were prepared in acetonitrile for spiking purposes (50, 250 and 1000 µg L^{-1}). Mix solutions in hexane were prepared for the construction of the calibration curves (1-1000 µg L^{-1}). Stock

and working solutions were stored and preserved in a freezer at -20 °C, protected from the light.

2. Instrumental Analyses

Table S4.1 - Optimized transitions for the analysis of the target compounds. Quantifier transition presented in bold.

Class	Compound	t_R (min)	MRM Transition	Collision Energy (eV)	Internal Standard
UVFs	BZ	7.71	**105>51;** 182>105	(25); (10)	AHTN-d3
	4MBC	11.23	**128>77;** 254>149	(25); (10)	4MBC-d4
	EDP	12.63	**165>119;** 65>149	(20); (10)	4MBC-d4
	EMC	12.91	**178>161;** 78>132	(10); (15)	4MBC-d4
	OC	14.49	**204>176;** 360>276	(25); (20)	4MBC-d4
	DTS	15.71	**221>73;** 221>221	(15); (5)	4MBC-d4
SMCs	DPMI	6.77	**191>135;** 206>192	(10); (10)	AHTN-d3
	ADBI	8.26	**229>173;** 244>229	(5); (10)	AHTN-d3
	AHMI	8.59	**244>229;** 229>187	(5); (5)	AHTN-d3
	MA	9.18	**253>106;** 253>121; 268>253	(10); (5); (5)	MX-d15
	EXA	9.35	**83>55;** 69>68	(5); (5)	AHTN-d3
	ATII	9.32	**215>173;** 258>215	(10); (5)	AHTN-d3
	HHCB	9.39	**243>213;** 213>171	(10); (5)	AHTN-d3
	MX	9.40	**282>119;** 282>160; 282>265	(10); (10); (5)	MX-d15
	AHTN	9.40	**258>243;** 243>128	(10); (40)	AHTN-d3
	MM	9.63	**263>156;** 263>144; 263>211	(20); (25); (5)	MX-d15
	MT	10.04	**266>251;** 251>132; 251>160	(5); (10); (15)	MX-d15
	MK	10.38	**279>118;** 279>191; 294>279	(20); (10); (5)	MX-d15
	EB	10.83	**98>83;** 227>113	(5); (10)	AHTN-d3
IS	MX-d15	9.28	**294>294;** 294>122; 294>276	(5); (15); (10)	
	AHTN-d3	9.42	**246>190;** 246>204; 261>246	(5); (10); (5)	
	4MBC-d4	10.22	**132>105;** 258>150; 258>108	(15); (5); (10)	

3. Chromatograms

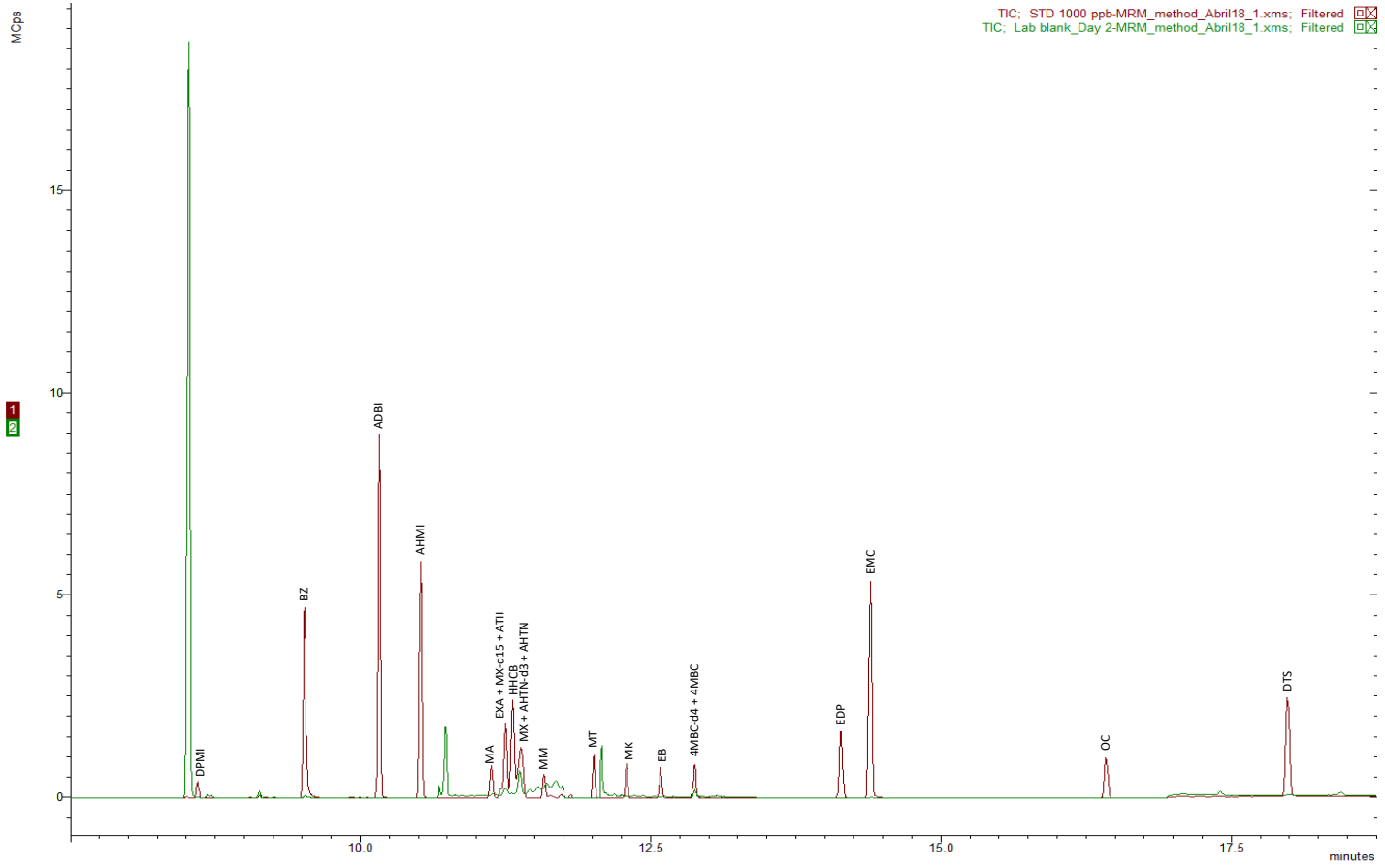


Figure S4.1 - Chromatogram of a 1000 µg L^{-1} direct injection standard (red) and a lab blank (green), overlaid.

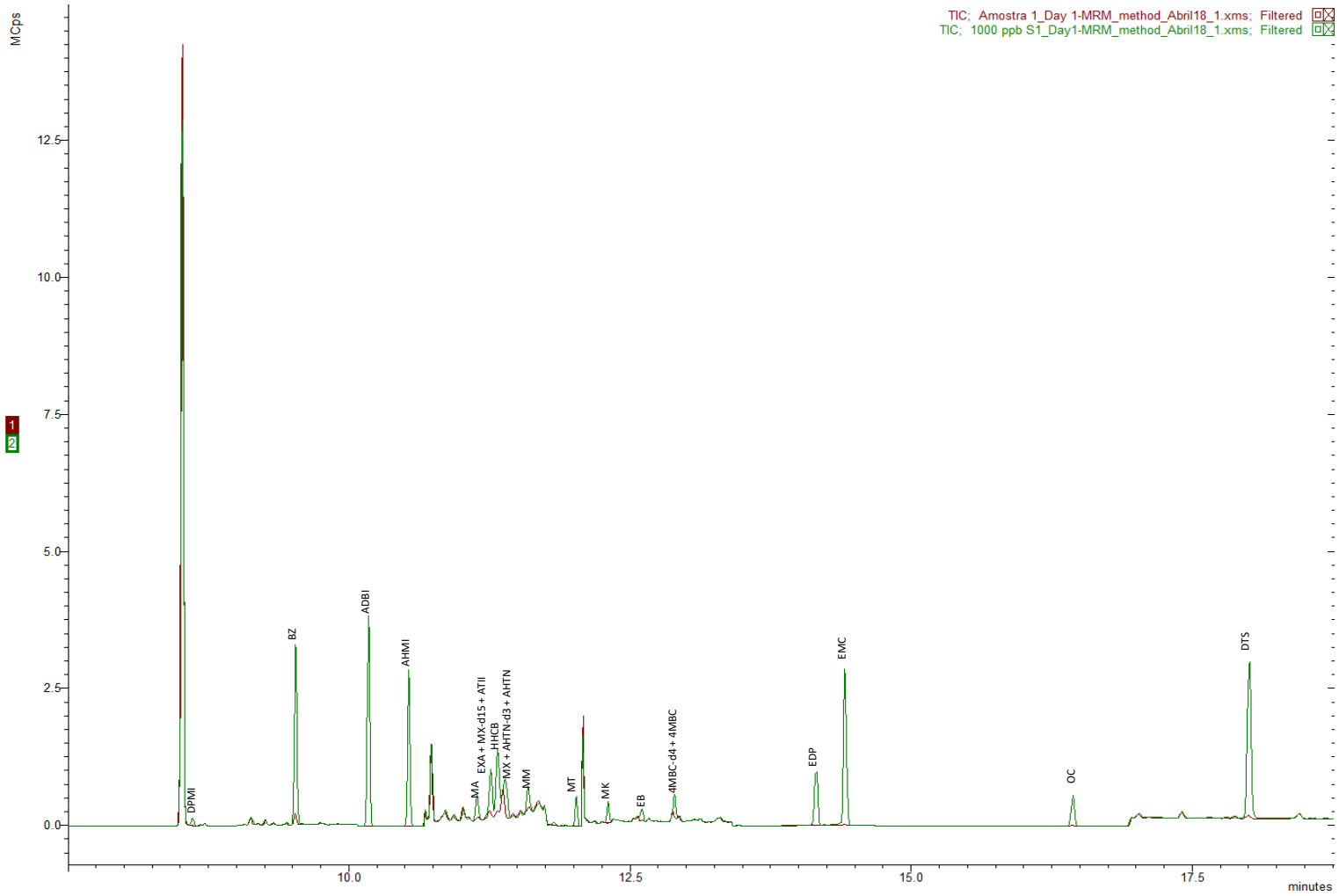


Figure S4.2 - Chromatogram of a soil sample (red) and a soil sample spiked at 1000 µg L^{-1} (green), overlaid.

Annex 5. Supporting Information Chapter 6

1. Supermarket tomatoes information

Table S5.1 - Supermarket tomatoes (origins, varieties, %H_2O, descriptive image for each sample and collection year).

Code	Varieties	Origin	% H_2O	Description	Collection Year
LR_Pt	Loose round	Portugal	94.3		2018
LR_Sp	Loose round	Spain	94.4		2018
B_N	Bunch	Netherlands	94.5		2018
B_Pt	Bunch	Portugal	94.5		2018
B_Sp	Bunch	Spain	93.3		2018
C_Sp	Cherry	Spain	93.5		2018
C_Pt	Cherry	Portugal	93.4		2018
P_Pt	Plum	Portugal	94.1		2018
A_Pt	*Anairis*	Portugal	94.8		2018
O_Pt	Oxheart	Portugal	95.9		2018

2. Compound Table

Table S5.2 - Optimized transitions for the analysis of the target compounds (UV-Filters, Synthetic Musk Compounds and Internal Standards). Quantifier transition presented in bold.

Class	Compound	t_R (min)	MRM transition and collision energy (eV)	Internal Standard
UVFs	BZ	7.71	**105>51** (25); 182>105 (10)	AHTN-d3
	4MBC	11.23	**128>77** (25); 254>149 (10)	4MBC-d4
	EDP	12.63	**165>119** (20); 65>149 (10)	4MBC-d4
	EMC	12.91	**178>161** (10); 78>132 (15)	4MBC-d4
	OC	14.49	**204>176** (25); 360>276 (20)	4MBC-d4
	DTS	15.71	**221>73** (15); 221>221 (5)	4MBC-d4
SMCs	DPMI	6.77	**191>135** (10); 206>192 (10)	AHTN-d3
	ADBI	8.26	**229>173** (5); 244>229 (10)	AHTN-d3
	AHMI	8.59	**244>229** (5); 229>187 (5)	AHTN-d3
	MA	9.18	**253>106** (10); 253>121 (5); 268>253 (5)	MX-d15
	EXA	9.35	**83>55** (5); 69>68 (5)	AHTN-d3
	ATII	9.32	**215>173** (10); 258>215 (5)	AHTN-d3
	HHCB	9.39	**243>213** (10); 213>171 (5)	AHTN-d3
	MX	9.40	**282>119** (10); 282>160 (10); 282>265 (5)	MX-d15
	AHTN	9.40	**258>243** (10); 243>128 (40)	AHTN-d3
	MM	9.63	**263>156** (20); 263>144 (25); 263>211 (5)	MX-d15
	MT	10.04	**266>251** (5); 251>132 (10); 251>160 (15)	MX-d15
	MK	10.38	**279>118** (20); 279>191 (10); 294>279 (5)	MX-d15
	EB	10.83	**98>83** (5); 227>113 (10)	AHTN-d3
IS	MX-d15	9.28	**294>294** (5); 294>122 (15); 294>276 (10)	
	AHTN-d3	9.42	**246>190** (5); 246>204 (10); 261>246 (5)	
	4MBC-d4	10.22	**132>105** (15); 258>150 (5); 258>108 (10)	

The MS/MS analysis was carried out in electron ionization (EI) mode, using the multiple reaction monitoring (MRM) mode. Two specific MRM transitions were chosen *per* compound (one for quantifying and one as qualifier), except for the nitro musks and surrogates, where two qualifiers were used for better identification. The ion source was operated at 280 ^{o}C with electron energy of 70 eV and filament current of 40 µA. The temperature of the transfer line was set at 270 ^{o}C. Ultra-pure argon was used as collision gas and its pressure was set at 2.00 mTorr.

3. Hazard identification and NOAEL values

Table S5.3 - Hazard identification and NOAEL values for UVFs and SMCs.

Group	Compound	Hazard identification	NOAEL value (mg kg^{-1} bw d^{-1})	Ref.
UVFs	BZ	Carcinogenic activity	25	NTP, 2006
	4MBC	Repeated dose toxicity rats (oral), thyroid effects	25	ECHA, 2019
	EDP	Caused skin irritation including burning, stinging, pruritus, and erythema on rare occasions	100	ECHA, 2019
	EMC	Sub chronic oral toxicity rat, effects on liver, kidney	450	ECHA, 2019
	OC	Eyes: May cause irritation, tearing and mild temporary pain. Inhalation: May cause irritation of the respiratory tract. Skin: May cause skin irritation Ingestion: Not an intended route of exposure. May be harmful if swallowed. Symptoms include: gastrointestinal tract upset and diarrhoea	175	ECHA, 2019
	DTS	Known human respiratory toxicant Limited or incomplete evidence of cancer according to safety/hazard data Dermal irritation.	32*	Burnett, 2008
SMCs	DPMI	Acute toxicity, oral Category 5 Skin corrosion/irritation Category 2 Serious eye damage/eye irritation Cat 2A Sensitization, skin Cat 1B Specific target organ toxicity, repeated exposure Cat 2	10	ECHA, 2019
	HHCB	Developmental toxicity/teratogenicity	50	ECHA, 2019
	AHTN	Haematological effects (based on 90-day repeated dose study with rats)	5	ECHA, 2019
	EXA	Skin corrosion/ irritation Cat 3 Skin sensitization cat 1	1000	ECHA, 2019
	EB	Skin corrosion/irritation Cat 2	1000	ECHA, 2019
	MX	Carcinogenic activity	20	Christian et al., 1999

***** For DTS the value is not a NOAEL, but a NOEL.

References

Burnett, C. L. (2008). Amended final report of the safety assessment of Drometrizole as used in cosmetics. International Journal of Toxicology, 27(SUPPL. 1), 63–75.

Christian, M. S., Parker, R. M., Hoberman, A. M., Diener, R. M., & Api, A. M. (1999). Developmental toxicity studies of four fragrances in rats. Toxicology Letters, 111, 169–174.

ECHA. (2019). Retrieved March 25, 2019, from https://echa.europa.eu/

NTP. (2006). Toxicology and carcinogenesis studies of benzophenone (CAS No. 119-61-9) in F344/N rats and B6C3F1 mice (feed studies). Natl Toxicol Program Tech Rep Ser, (533), 1–264.

Annex 6. Supporting Information Chapter 7

1. Experimental

1.1 Chemicals

Cashmeran (DPMI), celestolide (ADBI), phantolide (AHMI), traseolide (ATII) tonalide (AHTN) and galaxolide (HHCB), from LGC Standards (Barcelona, Spain); Nitro musks: tibetene (MT) and moskene (MM), from LGC Standards, ambrette (MA) and ketone (MK), from Dr. Ehrenstorfer (Augsburg, Germany) and xylene (MX) from Sigma-Aldrich (St. Louis, MO, USA); Macrocyclic musks: exaltolide (EXA) and ethylene brassylate (EB) from Sigma-Aldrich. Surrogate standards used were musk xylene-d15 (MX-d15) and tonalide-d3 (AHTN-d3) from Dr. Ehrenstorfer (Augsburg, Germany) and (±)-3-(-4-methylbenzylidene-d4) camphor (4-MBC-d4) from CDN Isotopes (Pointe-Claire, Quebec, Canada). The UVFs in this study are: 2-ethylhexyl 4-dimethylaminobenzoate (EDP), 3-(4'-methylbenzylidene) camphor (4MBC) from Alfa Aesar (Karlsruhe, Germany), 2-ethylhexyl 4-methoxycinnamate (EMC), 2-ethylhexyl 2-cyano-3,3-diphenylacrylate (OC), benzophenone (BZ) from Sigma-Aldrich (St. Louis, MO, USA) and drometrizole trisiloxane (DTS) from Fluka (Saint Louis, MO, USA).

1.2 Compounds Table

Table S6.1 - Compounds analysed in this study and organized by class (abbreviation and CAS number), log K_{ow} and NOAEL values for risk assessment calculations.

Class	Compounds	Abbreviation CAS number	log K_{ow}	NOAEL value (mg kg^{-1}, bw day^{-1})	Reference
UVFs	Benzophenone	BZ 119-61-9	3.2	25	(NTP, 2006)
	3-(4'-Methylbenzylidene) camphor	4MBC 36861-47-9	5.1	25	(ECHA, 2019a)
	Ethylhexyl dimethyl PABA	EDP 21245-02-3	6.2	100	(ECHA, 2019b)
	2-Ethylhexyl 4-methoxycinnamate	EMC 5466-77-3	5.8	450	(EC, 1991)
	Octocrylene	OC 6197-30-4	6.9	175	(ECHA, 2019c)
	Drometrizole trisiloxane	DTS 155633-54-8	10.8	31.75*	(Burnett, 2008)
SMCs	Cashmeran	DPMI 33704-61-9	4.2	10	(ECHA, 2019d)
	Celestolide	ADBI 13171-00-1	5.7	5000**	(ECHA, 2019e)
	Phantolide	AHMI 15323-35-0	5.59	5	(ECHA, 2019f)
	Musk ambrette	MA 83-66-9	5.7	-	
	Exaltolide	EXA 106-02-5	6.0	1000	(ECHA, 2019g)
	Traseolide	ATII 68140-48-7	8.1	1.68**	(ECHA, 2019h)
	Galaxolide	HHCB 1222-05-5	5.3	50	(ECHA, 2019i)
	Musk xylene	MX 81-15-2	4.8	20	(Christian et al., 1999)
	Tonalide	AHTN 1506-02-1	5.7	5	(ECHA, 2019j)
	Musk moskene	MM 116-66-5	5.8	-	
	Musk tibetene	MT 145-39-1	5.9	-	
	Musk ketone	MK 81-14-1	4.3	15	(Christian et al., 1999)
	Ethylene brassylate	EB 105-95-3	4.7	1000	(ECHA, 2019k)

Obs.: log K_{ow} values obtained from EPI Suite™ (EPA, 2012); for the compounds were no NOAEL value was available, was used the NOEL (*) and the LD_{50} (**).

1.3 Standards Preparation

Stock solutions of individual compounds were prepared in both hexane and acetonitrile in concentrations between 1 and 5 g L^{-1}. Stock solution in acetonitrile were used to prepare spike mix solutions and the ones in hexane to prepare the analytical control and calibration standards. All standards were preserved by keeping them covered from the light and kept at -20 °C.

1.4 Extraction Procedures

All samples were extracted in triplicate.

1.4.1 Tomatoes extraction

This methodology was already described elsewhere (Ramos et al., 2020). 2 g of freeze-dried sample was put in a 50 mL conical polypropylene tube and 125 ng of the surrogate solution (MX-d15, AHTN-d3 and 4MBC-d4) was added. 4 mL of ultrapure water was mixed with the sample and vortexed for 1 min and then, 10 mL of ethyl acetate was also added to the tube, and vortexed again for 1 min. The mixture was ultrasonicated for 15 min at room temperature in a 420 W ultrasonic bath (J.P. Selecta, Barcelona, Spain). Then, 6 g of $MgSO_4$ and 1.5 g of NaCl (used to adjust the ionic strength) was added to the sample tube. The extract was rapidly vortexed for 1 min and then, the organic phase was separated by centrifugation at 2670 x *g* for 15 min. The organic phase was transferred to a conical polypropylene tube containing 3 g of $MgSO_4$, 300 mg of PSA and 300 mg of C_{18} and the tube was vortexed again for 1 min and centrifuged in the same conditions mentioned above. The supernatant was evaporated to dryness under a gentle N_2 stream and reconstituted in 500 µL of hexane before GC-MS/MS analysis.

1.4.2 Soils and organic substrate extraction

5 g of previously air-dried and sieved soil (d < 2 mm) was put into a 50 mL conical polypropylene tube with 0.25 ng of surrogate solution in acetonitrile (MX-d15, AHTN-d3 and 4MBC-d4). After vortexing, samples were kept at 4 ^{o}C overnight. Then, 4 mL of

ultrapure water was added and vortexed to completely mix with the soil. After that, 10 mL of acetone/hexane (1:1, v/v) was added, vortexed for 1 min and then placed in an ultrasound bath (420 W) for 15 minutes (J.P. Selecta, Barcelona, Spain). Afterwards, 6 g of $MgSO_4$ and 1.5 g of NaCl were added. Samples were vortexed again for 1 min and then, the organic supernatant was separated by 15 min of centrifugation at 2670 x *g*. The organic phase was added to a dispersive solid-phase (d-SPE) mixture containing 3 g of $MgSO_4$ and 300 mg of C_{18}. It was then vortexed for 1 min and centrifuged once again. The supernatant was filtered with a 0.2 µm polytetrafluoroethylene (PTFE) syringe filter to a 15 mL amber vial, completely evaporated under a N_2 stream and resuspended in 1 mL of Hex before GC-MS/MS analysis.

1.4.3 Composted biosolids

500 mg of freeze-dried composted biosolids were weighted into a 15 mL polypropylene tube with conical bottom, containing 10 mL of acetonitrile and 125 ng of surrogate solution (MX-d15, AHTN-d3 and 4MBC-d4) in acetonitrile. The sample was vortexed for 2.5 min and then, ultrasonicated for 15 minutes in a 420 W ultrasonic bath (J.P. Selecta, Barcelona, Spain). The organic phase was separated by centrifugation at 2,670 x g for 15 minutes and it was added to a polypropylene tube containing 500 mg $MgSO_4$, 410 mg C_{18} and 315 mg PSA. The extract was then vortexed for 2.5 min and centrifuged for another 15 min. The supernatant was transferred to an amber vial, evaporated to dryness under a gentle N_2 stream and reconstituted in 500 µL of hexane before GC-MS/MS analysis. This methodology was already described in Ramos et al. (2019) (Ramos et al., 2019a).

1.4.4 Soil leachates

Before the extraction, all samples were centrifuged (Hettich® Rotofix 32A, Tuttlingen, Germany) for 15 min at 2670 × g to remove suspended particles. Then, 6 mL of the centrifuged aqueous sample was placed into a 15 mL polypropylene tube with conical bottom, containing 3.5% wt of NaCl and 50 µL of a 100 µg L^{-1} surrogate solution (MX-d15, AHTN-d3 and 4MBC-d4) in acetonitrile. Subsequently, 880 µL of 2-propanol and 80

µL of 1,1,2-trichloroethane were mixed and rapidly injected into the sample, forming a cloudy solution. The sample was ultrasonicated for 2 min in a 420 W ultrasonic bath (J.P. Selecta, Barcelona, Spain) and the organic phase separated by centrifugation at 2670 x g for 15 minutes. The sedimented phase was transferred to a vial with insert, dried under a gentle stream of N_2 and reconstituted with 50 µL of hexane before GC-MS/MS analysis. This methodology was described in Ramos et al. (2019) (Ramos et al., 2019b).

1.5 Mass spectrometer parameters

Table S6.2 - Compounds analysed in this study and organized by class (abbreviation (Abb), CAS number, log Kow and chromatographic parameters such as retention time tR, Multiple Reaction Monitoring (MRM) transition and internal standard used).

Class	Compounds	t_R (min)	MRM transition (Collision Energy, eV)	Internal Standard
UVFs	BZ	7.71	**105>51** (25) 182>105 (10)	AHTN-d3
	4MBC	11.23	**128>77** (25) 254>149 (10)	4MBC-d4
	EDP	12.63	**165>119** (20) 65>149 (10)	4MBC-d4
	EMC	12.91	**178>161** (10) 78>132 (15)	4MBC-d4
	OC	14.49	**204>176** (25) 360>276 (20)	4MBC-d4
	DTS	15.71	**221>73** (15) 221>221 (5)	4MBC-d4
SMCs	DPMI	6.77	**191>135** (10) 206>192 (10)	AHTN-d3
	ADBI	8.26	**229>173** (5) 244>229 (10)	AHTN-d3
	AHMI	8.59	**244>229** (5) 229>187 (5)	AHTN-d3
	MA	9.18	**253>106** (10) 253>121 (5) 268>253 (5)	MX-d15
	EXA	9.35	**83>55** (5) 69>68 (5)	AHTN-d3
	ATII	9.32	**215>173** (10) 258>215 (5)	AHTN-d3
	HHCB	9.39	**243>213** (10) 213>171 (5)	AHTN-d3
	MX	9.40	**282>119** (10) 282>160 (10) 282>265 (5)	MX-d15
	AHTN	9.40	**258>243** (10) 243>128 (40)	AHTN-d3
	MM	9.63	**263>156** (20) 263>144 (25) 263>211 (5)	MX-d15
	MT	10.04	**266>251** (5) 251>13 (10) 251>160 (15)	MX-d15
	MK	10.38	**279>118** (20) 279>191 (10) 294>279 (5)	MX-d15
	EB	10.83	**98>83** (5) 227>113 (10)	AHTN-d3
IS	MX-d15	9.28	**294>294** (5) 294>122 (15) 294>276 (10)	
	AHTN-d3	9.42	**246>190** (5) 246>204 (10) 261>246 (5)	
	4MBC-d4	10.22	**132>105** (15) 258>150 (5) 258>108 (10)	

Obs.: In bold are presented the transitions used for quantification. Only 3 transitions were used for the IS and the nitro musks for better identification.

2. Bioconcentration Factors (BCF)

Table S6.3 - Bioconcentration factors for tomato in the control and amended soil experiments

Compounds	BCF	
	Control	Amended soil
BZ	44.4	19.7
OC	5.2	18.1
DTS	-	3.1
HHCB	24.8	1.1
AHTN	-	0.2

References

Burnett, C.L., 2008. Amended final report of the safety assessment of Drometrizole as used in cosmetics. Int. J. Toxicol. 27, 63–75.

Christian, M.S., Parker, R.M., Hoberman, A.M., Diener, R.M., Api, A.M., 1999. Developmental toxicity studies of four fragrances in rats. Toxicol. Lett. 111, 169–174.

EC, E.C., 1991. Reports of the Scientific Committee on Cosmetology (ninth series), p. 67, 2-Ethylhexyl-4-Methoxycinnamate.

ECHA, 2019a. (±)-1,7,7-trimethyl-3-[(4-methylphenyl)methylene]bicyclo[2.2.1]heptan-2-one - Substance description.

ECHA, 2019b. 2-ethylhexyl 4-(dimethylamino)benzoate - Substance description.

ECHA, 2019c. Octocrilene - Substance description.

ECHA, 2019d. 1,2,3,5,6,7-hexahydro-1,1,2,3,3-pentamethyl-4H-inden-4-one information.

ECHA, 2019e. 6-tert-butyl-1,1-dimethylindan-4-yl methyl ketone - Substance description.

ECHA, 2019f. 1,1,2,3,3,6-hexamethylindan-5-yl methyl ketone - Substance description.

ECHA, 2019g. Pentadecan-15-olide - Substance Information.

ECHA, 2019h. 1-[2,3-dihydro-1,1,2,6-tetramethyl-3-(1-methylethyl)-1H-inden-5-yl]ethan-1-one - Substance description.

ECHA, 2019i. 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylindeno[5,6-c]pyran - Substance Information.

ECHA, 2019j. 1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthyl)ethan-1-one - Substance description.

ECHA, 2019k. 1,4-dioxacycloheptadecane-5,17-dione - Substance description.

EPA, 2012. EPI Suite TM.

NTP, 2006. Toxicology and carcinogenesis studies of benzophenone (CAS No. 119-61-9) in F344/N rats and B6C3F1 mice (feed studies). Natl Toxicol Progr. Tech Rep Ser 1–264.

Ramos, S., Homem, V., Santos, L., 2020. Analytical methodology to screen UV- filters and synthetic musk compounds in market tomatoes. Chemosphere 238, 124605.

Ramos, S., Homem, V., Santos, L., 2019a. Development and optimization of a QuEChERS-GC–MS/MS methodology to analyse ultraviolet-filters and synthetic musks in sewage sludge. Sci. Total Environ. 651, 2606–2614.

Ramos, S., Homem, V., Santos, L., 2019b. Simultaneous determination of synthetic musks and UV-filters in water matrices by dispersive liquid-liquid microextraction followed by gas chromatography tandem mass-spectrometry. J. Chromatogr. A 1590, 47–57.