Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences

Master's thesis in
European Master in Environmental Science – Soil, Water, and Biodiversity

Identification of new emerging pollutants in surface water using suspect screening analysis and prioritisation strategies based on regulatory databases

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Section for Organic Environmental Chemistry and Ecotoxicology
Department of Aquatic Science and Assessment
Master thesis, European Master in Environmental ScienceUppsala, 2017

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Swedish University of Agricultural Sciences

Credits: 30 ECTS
Level: A2E
Course title: Independent Project in Environmental Science – Master's thesis
Course code: EX0431
Program/education: European Master in Environmental Science – Soil, Water, and Biodiversity

Place of publication: Uppsala
Year of publication: 2017
Online publication: http://stud.epsilon.slu.se

Keywords: emerging pollutants, suspect screening, LC-HRMS, regulatory databases, surface water

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List of abbreviations

AcN	Acetonitrile
APCI	Atmospheric-pressure chemical ionisation
APPI	Atmospheric-pressure photoionisation
BT	benzothiazole
BTSA	benzothiazole-2-sulfonic acid
Da	Dalton
DEP	diethyl phthalate
°C	Degree Celsius
CAS	Chemical abstracts service
EA	Ethyl acetate
Eff	Effluent
EI	Exposure Index
EP	Emerging pollutants
ESI	Electrospray ionisation
FOSA	Perfluorooctane sulphonamide
FWHM	Full width and half maximum
h	Hour
HE	High energy
HRMS	High resolution mass spectrometry
Inf	Influent
IP	Identification point
IS	Internal standard
JRC	Joint Research Centre
KemI	Kemikalieinspectionen (Swedish Chemicals Agency)
kV	Kilovolt
L	Liter
LE	Low energy
LC	Liquid Chromatography
Lk	Lake
Log K_{ow}	Octanol-water partition coefficient
LTQ	Linear ion trap
mDa	Millidalton
MBT	2-mercaptobenzothiazole
MeOH	Methanol
MLOD	Method limits of detection
MP	Monobenzyl phthalate

MS/MS	Tandem Mass spectrometry
NI	Negative electrospray ionisation mode
NORMAN	Network of reference laboratories, research centres, and related organisations for monitoring of emerging environmental substances
NSAID	Nonsteroidal anti-inflammatory drug
OHBT	2-Hydroxybenzothiazole
PE	Population equivalent
PI	positive electrospray ionisation mode
PFAS	poly- and perfluoroalkyl substances
PFBS	Perfluorobutane sulfonic acid
PFBA	Perfluorobutanoic acid
PFDA	Perfluorodecanoic acid
PFDoDA	Perfluorododecanoic acid
PFHpA	Perfluoroheptanoic acid
PFHxA	Perfluorohexanoic acid
PFHxS	Perfluorohexane sulfonic acid
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonic acid
PFPeA	Perfluoropentanoic acid
PFTeDA	Perfluorotetradecanoic acid
PFUnDA	Perfluoroundecanoic acid
QSRR	Quantitative structure-retention relationships
QTOF	quadrupole-time-of-flight
REACH	Regulation concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals
Riv	River
Rt	Retention time
SLU	Sveriges lantbruksuniversitetet (Swedisch University of Agricultural Sciences)
SPE	Solid phase extraction
STP	Sewage treatment plant
TP	Transformation products
UI	Use Index
UPLC	Ultra-performance liquid chromatography
WW	Waste water
WWTP	Waste water treatment plan

Abstract

Emerging pollutants (EP) have the potential to enter the water system and cause adverse ecological and human health effects while simultaneously not being covered by existing water-quality regulations. However, the existing target analysis methodology only allows the detection of a very small fraction of the substances present in wastewater samples. The new advances in high resolution mass spectrometry (HRMS) and the application of suspect screening, with a suspected screening list based on prior information but with no reference standard, greatly increases the list of substances that can be identified. The present study aims to detect and identify new, potentially hazardous pollutants based on the hypothesis that regulatory databases can assist in the prioritisation of relevant substances.

Data from the Swedish Chemical Agency was used to prioritise compounds based on the occurrence on the market, the consumer tonnages, and the use pattern, among other factors. Out of the approximately 20 000 chemicals present in the database, 143 potential organic pollutants were prioritised and a screening was performed in surface water from different locations in Sweden using a LC-HRMS-based analytical approach. 21 tentative identifications were successfully performed with most substances being formerly out of the focus for environmental scientists (also not included in regulations nor monitoring programs). 16 of those substances were further confirmed with reference standard (the highest number in a study of this nature) showing the efficiency of both, the prioritisation strategy, and the suspect screening approach. Results indicate that the use of regulatory databases is a promising way to enhance identification rates as well as to identify new, potentially hazardous compounds.

Popular Science Summary

Emerging pollutants present a new global water quality challenge. Over the last decades, the occurrence of emerging pollutants in natural waters has increased the worldwide concerns about potential negative effects on aquatic ecosystems and human health. Meanwhile, those substances are not covered by existing water-quality regulations nor included in monitoring programs.

Emerging pollutants refer to residues of substances used every day in modern society, including, for example, pharmaceuticals, personal care products, hormones, pesticides, and industrial chemicals. Since they are neither completely biodegradable nor entirely removed by conventional wastewater treatment technologies, emerging pollutants are considered as persistent and bioactive. Their enduring release with wastewater effluents is believed to cause long-term hazards as the contaminants are bioaccumulating and even forming new mixtures in our waters. At the same time, the exact effects are not completely understood. Especially, low concentrations and the diversity of emerging pollutants not only puzzle the associated detection and analysis procedures but also creates challenges for water and wastewater processes. Advances in analytical technologies, such as high-resolution mass spectrometry, are helping in the battle against those potentially hazardous compounds.

The collaboration with the Swedish Chemicals Agency KemI provided a chemicals registry database on all chemicals produced and used in Sweden which greatly increased the list of substance that can be identified. Applying criteria on the substance's occurrence on the market including user tonnages, market availability and use pattern, relevant substances were prioritised according to the probability of exposure for surface waters. As a result, 143 potential organic pollutants were selected for the application of suspect screening strategies. Samples from different locations in mid-western Sweden were investigated. A high ratio of substances has been identified with a large part formerly out of the focus for environmental scientists, neither included in regulations nor monitoring programs.

Currently, there are 100 000 commercially registered compounds in Europe and residues from the majority of these will eventually end up in the water cycle. Furthermore, the production of chemicals is predicted to increase. The present study demonstrates that the inclusion of commercial use and exposure data of chemicals is an essential key feature in the screening of emerging pollutants. Results indicate that the collaboration with governmental authorities and the availability of regulatory databases is a promising way to enhance identification rates of new, potentially hazardous compounds.

1 Introduction

With more than 100.000 substances in commercial use globally, the world of chemicals is very complex (Swedish EPA 2011). The impacts on the environment or human health as well as the combined effects of various of these compounds are thereby not at all or only partially investigated (EEA 2010). Substances of increasing interest are emerging pollutants (EP) comprising a wide range of physiochemical properties. Although they may have been present in the environment for some considerable time, their presence and significance has been elucidated only recently (NORMAN 2017). Potentially hazardous EP can enter natural waters through urban and industrial sewage, erosional runoff, leaching from agricultural areas and wastewater treatment plant effluents (Chiaia-Hernandez et al. 2013). Simultaneously, they are not yet covered by existing water-quality regulations nor included in environmental screening programs (Farré et al. 2008). After their first release into, inter alia, the aquatic environment, EP can reach several environmental compartments including soil, air, biota, or groundwater due to their persistent and bioaccumulative properties (Zedda and Zwiener 2012). Some of these are, furthermore, carcinogenic, mutagenic, toxic for reproduction or endocrine-disruptive (Richardson 2003; Daughton 2004; Loos et al. 2010; Richardson and Ternes 2011). These notably include high-performing chemicals, such as waterproofing agents and flame retardants, as well as substances developed to affect the biological system as pesticides and pharmaceuticals (Swedish consumption). In an overview of Zedda and Zwiener (2012) the variety of newly detected contaminants mainly comprise artificial sweeteners, poly- and perfluoroalkyl substances (PFASs), pharmaceuticals, hormones, disinfection by-products, UV filters, brominated flame retardants, benzotriazoles, naphthenic acids, siloxanes and musk fragrances. Additionally, biological, chemical, and photochemical degradation in the environment or through water treatment such as chlorination or ozonation, produce numerous transformation products (TP), respectively metabolites with unknown properties and consequences to the environment (Zedda and Zwiener 2012).

A growing number of researches are emphasising on the occurrence and risks of EP in the environment (cf. Küster and Adler 2014, Daneshvar 2012, Fabbri 2015, Cooper et al. 2008). Roos et al. (2013) conducted a study on liver samples from Swedish otters, discovering that the concentration of 9 out of 11 investigated PFASs increased in the range of 5.5 – 13 % every year between 1972 and 2011. Beyond that, Ahrens et al. (2015) provided evidence of a potential effect of PFAS to the physiological function of European perch (*Perca fluviatilis*) by comparing the concentration of the pollutant with the individual tissue weight and, thus, drawing conclusions on the body burden. Examples like these made the EU ban many substances including special brominated flame retardants, several PFASs as well as certain pesticides (Swedish EPA). However, national, and international regulations merely cover a

small excerpt of the broad range of known and yet unknown chemical pollutants occurring in the environment. In fact, it is assumed that those selective lists of well-known priority substances pose a significant share of risk to the environment and human health (Daughton 2004). Thus, the Water Framework Directive determines 33 priority pollutants as predominantly hazardous to the aquatic environment (EC 2013). The efficiency and comprehensiveness of such actions remains questionable as those regulated substances are not representative of the entire range of chemical stressors, the multitude of yet unknown EP or the large number of TP. Consequently, data collected from water monitoring is biased to lists of preselected analytes ("target" analysis), ignoring a major part of potentially harmful substances. Hence, the aim of this study is to detect and identify new pollutants by means of the last advances in high resolution mass spectrometry (HRMS) that are present in surface waters but have been so far dedicated with only little or none attention. By developing strategies that allow the identification of new EP, the project also seeks to contribute to obtain a broader picture regarding the presence of EP in the environment

1.1 Trends in the analysis of EP

Within the last years, polar organic EP became an increasing area of focus for environmental scientists and regulatory authorities offered by the advances in LC-MS technologies. Existing target screening methods are based on the preselection of chemicals which can only cover a relatively small proportion of organic contaminants missing important site-specific and potentially ecotoxicologically relevant compounds (Hug et al. 2014). For a holistic risk assessment, target-based environmental monitoring should be accompanied by non-target analysis. When coping with the analysis of various known and unknown substances at low concentrations and within complex matrices, the coupling of LC to HRMS has emerged as a reliable and effective instrument (Krauss et al. 2010). To achieve high selectivity, resolution as well as sensitivity in full-scan mode, hybrid instruments consisting of two different mass spectrometers such as quadrupole/TOF (QTOF) or linear ion trap/orbitrap (LTQ Orbitrap) are used for identifying low molecular weight compounds (<1000 Da) in environmental matrices. Electrospray ionisation (ESI) is by far the most commonly used ionisation technique since it provides good performance for a much wider range of substances than other techniques like atmospheric-pressure chemical ionisation (APCI) or atmospheric-pressure photoionisation (APPI; ibid.).

As seen in Fig.1 three conceptually different analytical approaches can be distinguished in the identification of compounds. Target analysis, which involves a reference standard, is the most commonly used approach and the regular procedure. In recent years, however, target analysis became more frequently complemented with non-target acquisition methods (Schymanski et al. 2015). Those methods include, among others, suspect screening analysis which is

performed with preliminary information on exact mass and isotope pattern from the molecular formula plus or minus the expected adduct(s) but without reference standard. In this way, the structure of a compound that might be present in the sample is suggested, while leaving the final allocation more open (Schymanski et al. 2015). In case that no well-founded database is at hand providing candidates and, thus, prior information on exact masses, isotopes, adducts, and fragmentation, pure non-target screening can offer a plausible alternative (ibid.). When using LC-HRMS-based techniques suspect compounds are treated as subset of a group of exact masses (adduct and isotopologues) associated with one compound (Schyanski et al. 2015). In order to perform tentative identifications, the isotope patterns, the presence of additional adducts as well as the predicted RTs are beneficial. Especially, the gathering of fragmentation information through tandem mass spectrometry (MS-MS) supports the identification procedure (ibid.). To confirm the identification, the use of the corresponding native standard is necessary.

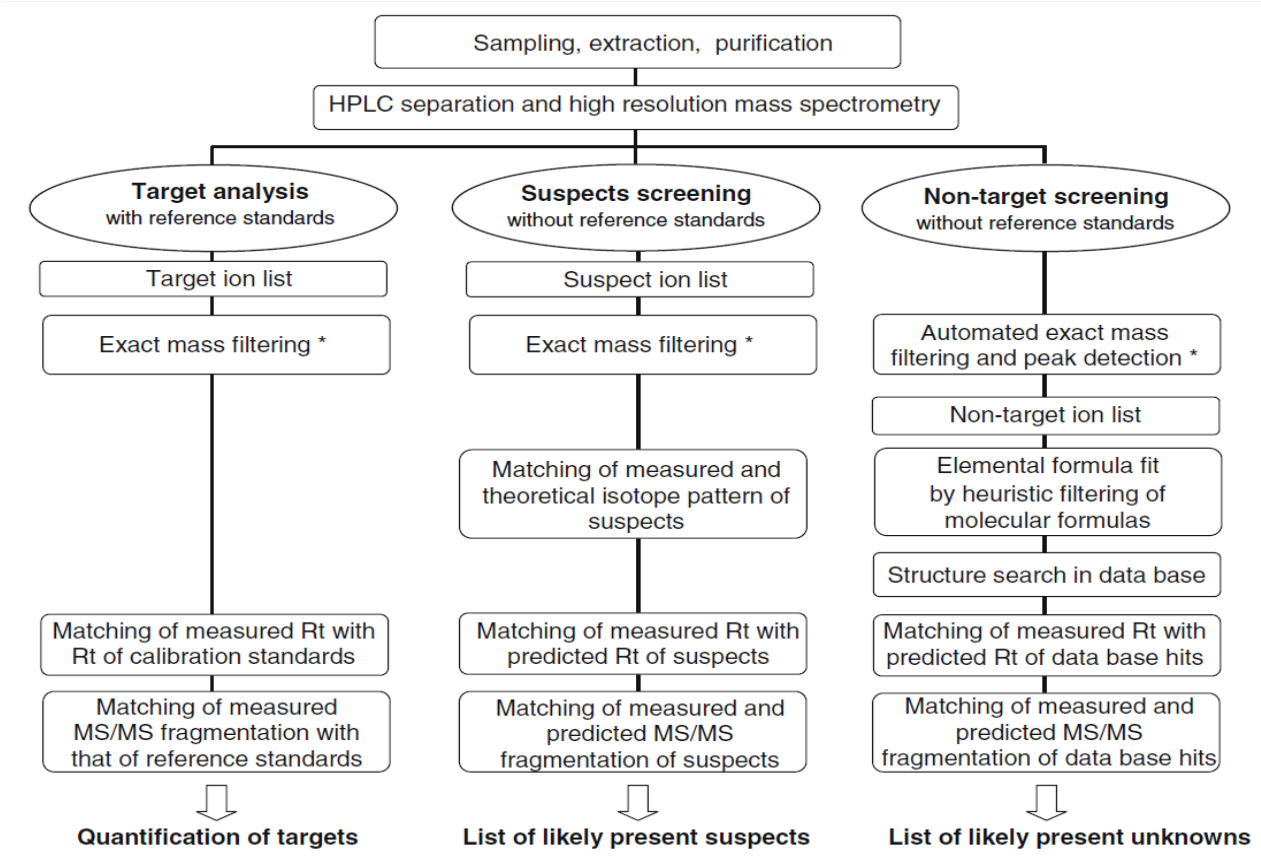


Fig.1: Matrix of identification
Source: Schymanski et al. 2015

With an increasing interest in suspect and non-target workflows, there is a need to communicate the confidence in the identification in a way that reflects the evidence available (Gago-Ferrero et al. 2015). The EU Guideline 2002/657/EC offered the concept of identification points (IPs) in order to guarantee a consistent identification framework where reference

standards, thus a RT is available (EC 2002). However, this system does not take into account the new capabilities of HRMS instruments and should be re-evaluated. The idea of identification levels was, however, just recently thematised relating to HRMS analysis to deal with the varying confidence levels among the three approaches for identifying substances (Schymanski et al. 2015). By definition, target, suspect and non-target analysis start at different confidence levels assuming that the certainty that assigned compounds are sought ones is differing. Schymanski et al. (2015) introduced a matrix of identification approach distinguishing five levels of identification confidence (Fig.2).

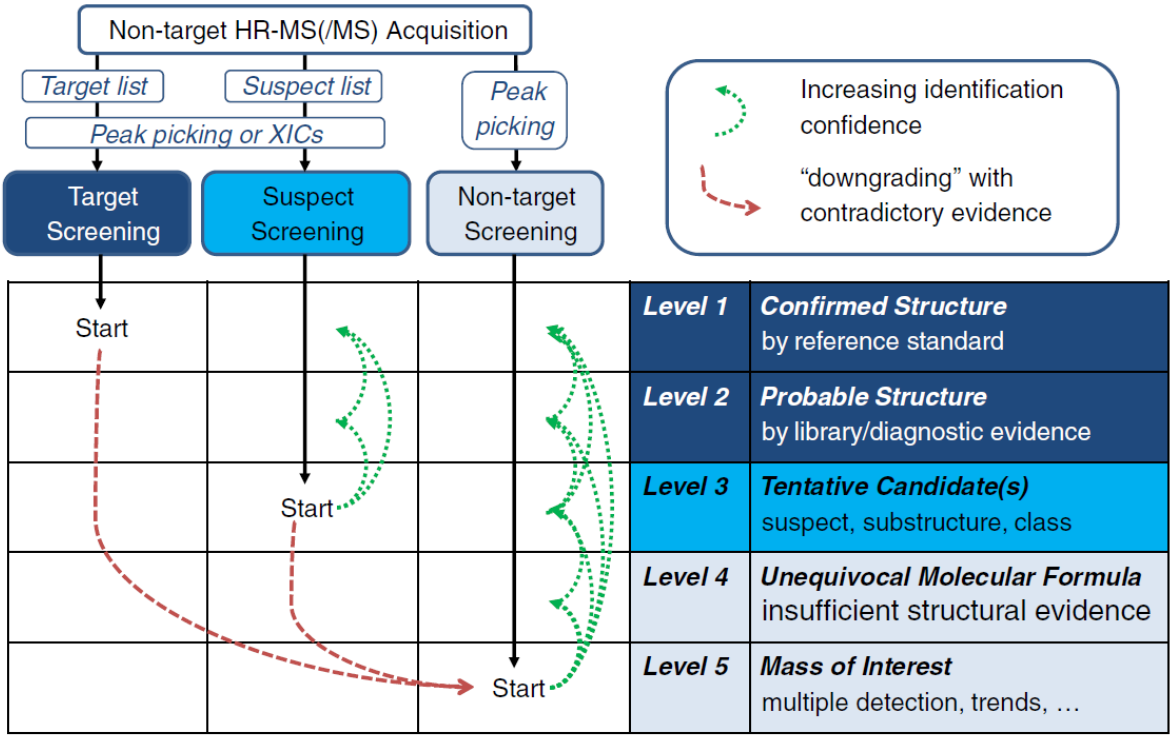


Fig.2: Comparison of systematical workflows for different analytical approaches
Source: Krauss et al. 2010

This level system is not intended to replace guidance documents (e.g., EU Guideline 2002/657/EG), but specifically covers the new possibilities in HRMS-based analysis. Normally, target analysis starts at confidence level 1 as the identification can be proved with the available reference standard. At the same time, suspect screening begins at level 3 where one or several tentative candidates can be allocated, while non-target analysis assumes the absence of any information positioning this approach at the lowest level of the matrix. Through the analysing performance, additional information for MS (exact mass, isotope, adduct), fragmentation and retention behaviour can be acquired to set up the confidence level of suspect or non-target components. The green arrows in Fig.2 represent this increase in confidence up to level 2 indicating the probable allocation of the compounds exact structure. Schymanski et al.

(2014(1)) suggest the differentiation between level 2a where matching literature or library spectrum data is available, and level 2b where diagnostic fragments and other evidences fit the tentative structure but no standard or literature information is accessible. If the identity can be approved with a corresponding standard the level of confidence can, thus, even improve up to level 1. In future analysis, those confirmed compounds will then serve as target ones. On the contrary, if experimental evidence doesn't match the reference standard or target nor the tentative or suspected candidate, the level of confidence for those components decreases to level 5 making them a non-target of interest which is indicated by the red arrows.

1.2 Prioritisation of potentially hazardous EP based on regulatory database

Chemical monitoring and analysis are commonly realised with target screening methods. Nonetheless, a preselection of compounds can only cover a comparably small fraction of contaminants as most organic constitutes of environmental samples are not yet identified (Gago-Ferrero et al. 2015). In this sense, only the 'tip of the iceberg', namely a small proportion of information is visible, whereas the bulk of data is hidden. Besides, there is the possibility of bias due to the initial selection such that potential chemical stressors are insufficiently covered or completely omitted. As described in the previous section, for a holistic analysis of complex samples, a balance between an extensive target-based environmental monitoring and suspect screening methods assisting in the tentative identification of additional potentially relevant compounds is necessary (Gago-Ferrero et al. 2015). To cope with the challenge of the numerous EP and their TP, occurrence and toxicity data are yet the most promising indicators for the preselection of those substances (Zedda and Zwiener 2011). Hence, the basic prerequisite to conduct a reasonable suspect analysis is the availability of a profound database. Through the ongoing collaboration between the Swedish Chemicals Agency (KemI) and the Department for Aquatic Science and Assessment at SLU, an extensive database of the Swedish product register was available for the study at hand.

The Swedish chemicals legislation requires manufacturers and importers to register chemical substances and products to a national product register. Its obligations apply, for instance, to pesticides while other products as foodstuff, cosmetics, medicines, and hygiene products are not considered in the legislation. This was no detriment for the results at hand as the scope of this study didn't emphasis on those substances (cf. Chapter 1.1). However, it is worth mentioning that also quantities less than 100 kg / year / company are not included, thus, those chemicals are not reported. Registered chemicals and products enclose information on, inter alia, the area of use, the composition and the quantities that are on the market. The product register is supervised and enforced by KemI, which uses the provided information in a second step to calculate statistical estimates. About 70-75% of the information in the national register

are classified as confidential which makes a majority not available to the general public (SPIN n.d.).

With the implementation of the Use Index (UI) a basic method was established to make use and exposure information publicly accessible. In doing so, the UI uses a worst-case methodology only presenting those products with the highest UI if a substance is contained in several products. This can result in an insufficient representation of certain product types while, simultaneously, overestimating real exposure of other products. Despite all difficulties, the UI was an essential first step of providing information to the public, but also to meet the demands by the REACH (Regulation concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals) regulation (EC 1907/2006) of the European Union which aims to fill the information gaps on chemical substances to ensure a proper hazards and risks assessment to human health and the environment in Europe (EC 2016). Nonetheless, it inhibits an exact quantification on exposure, serving rather as an indicator tool for screening. In order to thoroughly assess hazards and risks of a wide range of substances, the scope of the UI was insufficient. For this purpose, KemI introduced the Exposure Index (EI), a tool calculated for all substances appearing in the Swedish product register (see Chapter 2.3). Through the inclusion of market availability, consumer tonnage and use patterns, the EI is indicative for the highly-promising potential regulatory databases offer in the prioritisation of environmentally relevant substances.

1.3 Objectives

The main objectives of the present project are the identification of new, potentially hazardous, pollutants in surface waters by using a UPLC-HRMS-based approach and advanced suspect screening strategies as well as the assessment of the practical feasibility of regulatory databases in the prioritisation of relevant substances.

The attainment of these main objectives implies other specific objectives:

- Development of a new generic prioritisation method for the screening of chemicals with a broad range of physiochemical properties in surface water samples.
- Determination of whether potentially affecting WWTP effluents are the presumed major source of the identified pollutants in surface waters.
- Assessment of how the cooperation with governmental agencies and the availability of regulatory databases can support the prioritisation performance.

2 Material and Methodology

The methodological approach is divided into the sampling acquisition and preparation, the development of a prioritisation approach for the Swedish registration database on chemicals and the subsequent combination of the two. The entire study was conducted in the period from January to June 2017.

2.1 Chemicals and reagents

In total, 143 suspect compounds were evaluated with a systematic suspect screening workflow (cf. Chapter 2.6). Suspect analyte names, molecular formulas, log K_{OW} values and their corresponding SMILES are shown in Annex A1 Tab.A1.1. All substances used in the isotopically labelled standards (IS) mixture can be found in Annex A1 Tab.A1.2 and were acquired from Wellington Laboratories (Canada), Sigma-Aldrich and Toronto Research Chemicals (Toronto, Canada) and were exclusively used for quality control purposes.

For the target analysis, 82 substances were selected comprising compounds that were available in our analytical target methodologies, with a broad range of physiochemical properties and a high probability to be present in surface waters according to the literature. The evaluation including 44 pharmaceuticals of different therapeutic groups (viz. antibiotics, analgesics, anaesthetics, antidepressants, antiepileptics antihypertensives, antilipidemics, antiulcers, antifungals, benzodiazepines, β-blocking agents, diuretics, antidiabetics and NSAIDs); 14 per- and polyfluoroalkyl substances (PFASs), 5 personal care products, 5 flame retardants, 3 pesticides; 2 artificial sweeteners, 2 phthalates, 3 of the group opiates, opioids and metabolites, 2 UV filter, one illicit drug and one stimulant. Target analyte names, CAS numbers, molecular formulas, exact masses, molecular weights and log K_{OW} values are presented in Annex A1 Tab.A1.3.

For the sample preparation, glass fibre filters from Whatman™ (1.2 µm and 0.7 µm) were purchased from Sigma-Aldrich (Sweden). Consumable supplies for the solid phase extraction (SPE), namely, empty polypropylene tubes (6 mL), regenerated cellulose filters of 15 mm diameter and 0,2 µm pore size and the cartridge sorbent materials Sepra ZT (Strata-X), Sepra ZT-WCX (Strata-X-CW), Sepra ZT-WAX (Strata-X-AW) and Isolute ENVI+ were obtained from Phenomenex (Torrance, USA).

The chemical analysis was conducted with gradient grade methanol (MeOH), acetonitrile (AcN) and ethyl acetate (EA) purchased from Merck (Darmstadt, Germany), while formic acid 98%, ammonium formate, 25% ammonia solution and ammonium acetate were obtained from Sigma-Aldrich (Sweden). Distilled water was acquired through a Milli-Q Advantage Ultrapure Water purification system (Millipore, Billercia, MA).

For the confirmation of tentative identified compounds, high purity grade (>95%) analytical standards for Dibutyl phosphate, Stearic acid, Di-(2-ethylhexyl)phosphoric acid, 4-Dodecylbenzenesulfonic acid, Laurilsulfate, Benzoic acid, Sulisobenzone, Dazomet, Diisobutyl phthalate, Oleic acid, Ricinoleic acid, Tolytriazole, Sebacic acid, (9E)-9-Octadecenamide, 2,4,7,9-Tetramethyl-5-Decyn-4,7-Diol, Butyl glycolate, Tetraethyleneglycol, Tributyl citrate acetate, Tris(2-butoxyethyl) phosphate, 2,2'-Dimorpholinyldiethyl-ether, Sorbitol were purchased from Sigma-Aldrich (Sweden).

2.2 Sample collection

Surface water samples were collected in 3 different catchments in mid-eastern Sweden (Fig.3). At each sampling point, a mount onto which a polypropylene bottle with a volume of 1 L was fixed served for the water withdrawal.

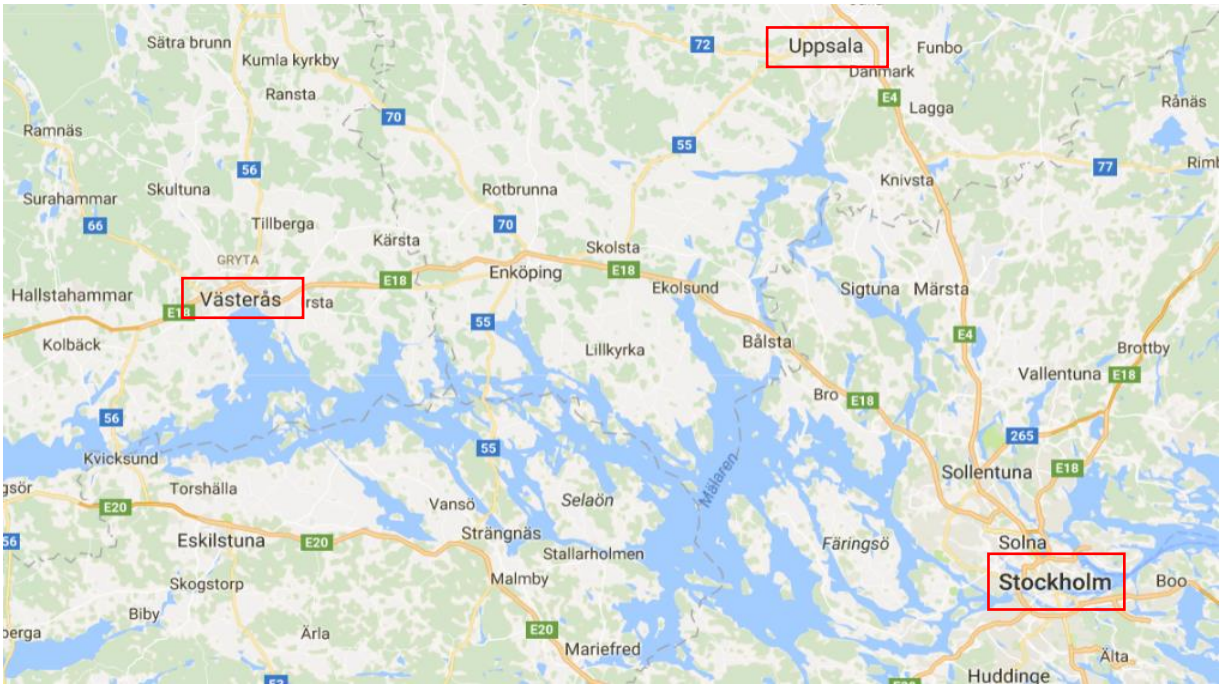


Fig.3: Overview map of the sampling locations

On the 27th of January, the sample for Uppsala was taken from the river Fyris approximately three kilometres south of the city centre (59°49.544'N 17°39.398'E; Fig.4). The Fyris river basin has a population of about 150 000 of which more than 85% live in Uppsala (http://www.peer.eu/fileadmin/user_upload/projects/flagship_projects/PEER_Euraqua/Fyris%20Sweden%20%282%29.pdf). Due to its comparatively high pressure related to urban areas, the sampling point was located just downstream of the large-scale WWTP Kungsängsverket of Uppsala. The applied treatment steps for wastewater at this plant comprise mechanical treatment and primary sedimentation as well as biological treatment for nitrogen removal using activated sewage sludge. In a chemical processing step iron chloride is added before a final

lamella sedimentation treatment for the removal of particular matter. With a population equivalent (PE) of 172 000, the discharge of sewage water constitutes a substantial share of the flow (ibid.), making the site a suitable point of reference for studies coping with potential problems.

The surface water sample for Stockholm was drawn on the 14th February from river Bällsta (59°22.0765'N 17°56.0554'E). Most water in the river originates from settlements, industrial areas, streets, and other infrastructure. The water quality of river Bällsta is, accordingly, low with high nutrient levels, relatively high metal contents and a great bacteria count (Stockholm Vatten och Avfall 2015). Again, sampling was conducted at a river section influenced by the discharge of a WWTP. With 780 000 PE, the plant is the biggest of the three investigated sites. Accordingly, river Bällsta receives the highest amount of effluent among the surface waters. The applied treatment at the plant includes a mechanical treatment as well as an activated sludge sedimentation. Moreover, a chemical processing step is followed by a sand filtration (reference?).

Thirdly, river Svart (59°36.545'N16°32.649'E) in Västerås was sampled on the 24th February 2017. The catchment area of river Svart is a tributary to Lake Mälaren, Sweden's third largest lake (Ekstrand et al. 2010). The WWTP in Västerås constitutes the smallest plant with approximately 120 000 PE. The specific treatment used, is comprised by an active sludge process through nitrification and denitrification with a pre- and post-sedimentation. Since the discharge of the plant goes into lake Mälaren (ibid.) and to gain a better overview of interdependencies in the aquatic environment around Västerås, a further sample within the lake has been taken for comparison (59°36.139'N 16°33.642'E).

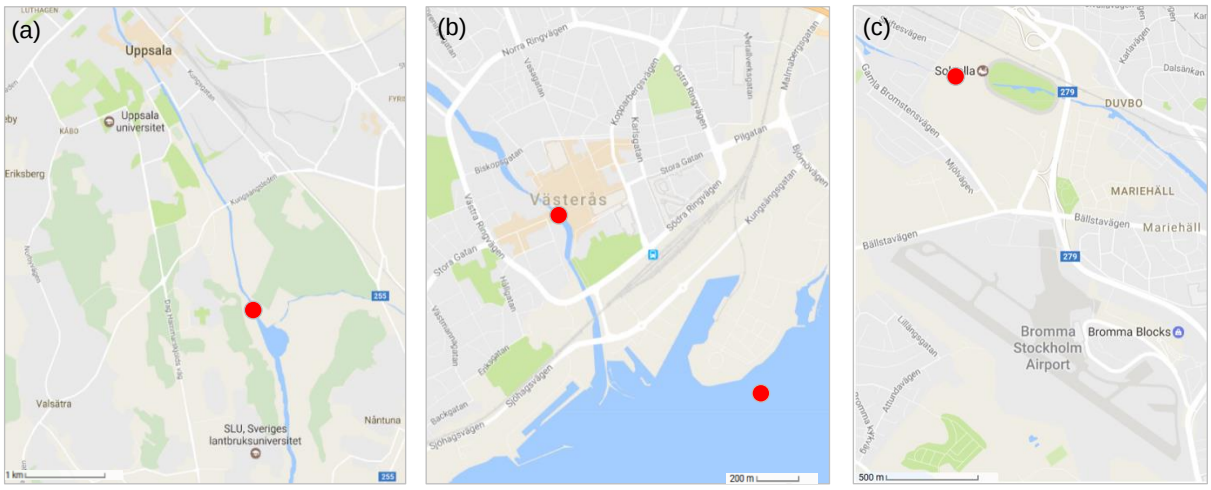


Fig.4: Sampling points for (a) Uppsala, (b) Västerås and (c) Stockholm
Source: google.maps.com

Additionally, 24 hours composite influent, and effluent wastewater samples from the WWTPs of Stockholm, Uppsala and Västerås were collected for comparison and backtracking of

positive detections in the surface waters. Next to WWTP effluents, discharges from surround industrial areas or boat traffic may represent a potential alternative source of emerging pollutants of the investigated sites. Two method blanks, consisting of 1 L Milli-Q water were analysed to check for any background levels of the detected analytes.

2.3 Prioritisation

A prioritisation strategy was developed based on the national chemical substances and products register supervised and enforced by KemI. In general, most information listed in national registers are subject to confidentiality obligation. Through aggregation and categorisation of information into general exposure indices, confidentiality can, however, be circumvented. Along with an informative overview comprising the area of use, the composition or the quantities of a chemical, KemI provided a self-developed Exposure Index (EI), a tool calculated for all substances appearing in the Swedish Product Register (SPIN n.d.). All specific product uses were weighted according to product tonnages and added up to one single value between 0 and 7. The EI focuses on diffuse end product uses, while industrial point source releases are not considered. In addition, it was applied to six primary recipients; soil, air, surface water, sewage treatment plant (STP), consumer and occupational (ibid.). It is referred to those recipients as the immediate surrounding of a potential discharge. Further dispersal can be only estimated as not all necessary data can be acquired for a large number of substances due to cost-intensity and knowledge gaps (Swedish EPA 2008). For the prioritisation in this study, the only category of interest was surface water referring to the direct discharge to surface waters (SPIN n.d.).

At the starting point, the entire database comprised approximately 20 000 substances (Fig.5). Substances for which information on the range of use, consumer availability and the use of article production were lacking were initially rejected by which reduced the database by half.

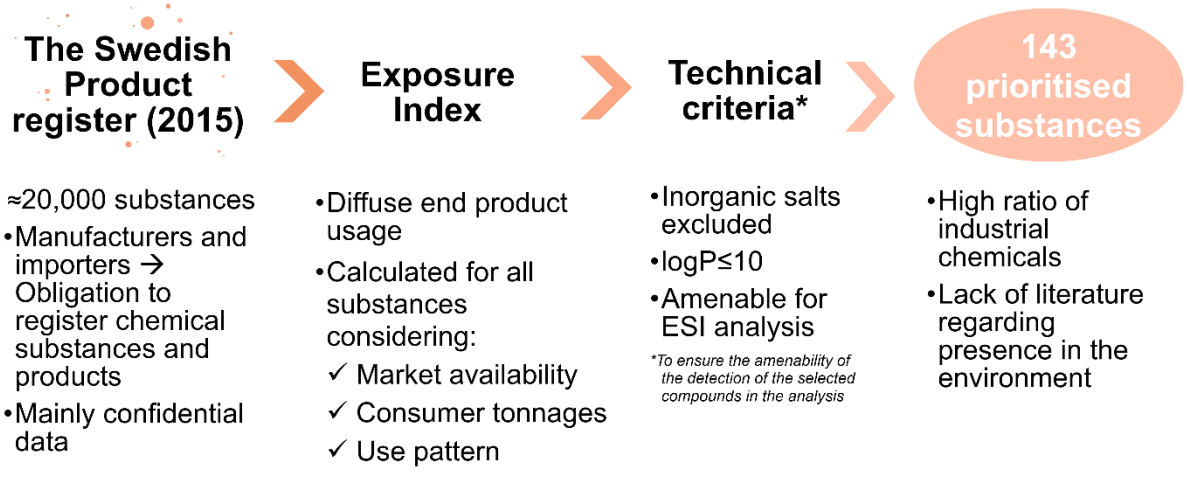


Fig.5: Prioritisation workflow

The most important precondition applied to the remaining list of compounds was the EI which is calculated in several steps. As general input, a chemicals potential to be dispersed from a certain type of chemical plant is calculated. Each individual quantity of contained chemicals is then added up to lay the base for the EI (Swedish EPA 2008). With the information considered in the calculation, it serves as a crude measure of a recipient's exposure to a specific substance. The higher the index, the more likely does a substance function as pollutant (ibid.). The prioritisation strategy used, limited the database to the three highest exposure indices 5 to 7 to screen for those compounds surface waters are exposed the most. The use of a certain target or suspect list clearly shows the dependency of a hypothetical detection on this very database (Schymanski et al. 2015). A further reduction of the compound's list simultaneously decreases the options for detection. By adopting that certain substances contribute to the highest exposure to the investigated environmental sphere, however, a reduced number of exercisable options benefit the applicability of the method.

Next to the EI framework condition, some technical criteria has been applied to increase the probability of detection of the selected substances in the samples. As the investigation was restricted to organic chemicals, inorganic salts were excluded in a first step. Thereafter, the n-octanol/water partition coefficient given in the form of its logarithm to base ten (Log K_{ow}) as a generally inverse indicator of water solubility was taken into account (OECD 2004). In recent years, Log K_{ow} has become an essential parameter for predicting the fate of chemical substances and its distribution in various environmental compartments as soil, water, air, or biota. Compounds with high log K_{ow} (> 4.5) values have a low affinity to water and, thus, tend to adsorb more readily to organic matter in soils or sediments (ibid.). Moreover, those substances have the potential to bioaccumulate in living organism. Due to its increasing use in the estimation of soil/sediment adsorption coefficients and bioconcentration factors for aquatic life, Log K_{ow} is considered indispensable in studies of EP. The tendency of chemicals to partition themselves between an organic phase, respectively a fish or soil, and an aqueous phase, furthermore, was used for prioritisation. Moschet et al. (2013) considered a Log K_{OW} value ≤ 5 as potentially water relevant. However, for the present study this range was increased since (I) approximately one third of the substances with high EI had a Log K_{OW} between 5 and 10, (II) it has been demonstrated that similar treatments can retain substances with Log K_{OW} > 5 (Gago-Ferrero et al. 2015; Schymanski et al. 2014(2)) and (III) compounds with a Log K_{OW} between 5 and 10, showing similar properties, have been previously detected in wastewater samples (Gago-Ferrero et al. 2015; Lara-Martín et al. 2011). Therefore, only chemicals with Log K_{ow} values >10 were excluded, as compounds above this threshold were considered as too hydrophobic and, thus, not detectable in the surface water samples investigated. Substances with a higher water solubility were, hence, favoured. To guarantee optimum performance for suspect screening and increase the likelihood of detection, the amenability for

electrospray ionisation mass spectrometry (ESI-MS) analysis was another criterion considered. Analytes that are not ionisable, cannot be detected by the HRMS and will remain unnoticed (Krauss et al. 2010). Complementary, benzoic acid, Di-(2-ethylhexyl)phosphoric acid, dibutyl phosphate and sulisobenzone were selectively added to the suspect list due to their detection in WW samples within a previous on-going study at the Department of Aquatic Sciences and Assessment.

2.4 Sample Preparation

Samples were filtered through regenerated cellulose filters which were discarded afterwards as the focus of the analysis was on compounds present in the dissolved phase. Sample triplicates of 500 mL were prepared, spiked with 100 µl of an internal standard mix with a concentration of 1 µg mL^{-1} for quality control (cf. Chapter 2.1) and adjusted to pH 6.5.

2.4.1 SPE

In order to cover a very broad range of compounds during the extraction, a SPE method using mixed-bed cartridges with four sorbent materials was applied. For this, cartridges were filled with a mixture of 150 mg Isolute ENVI+, 100 mg Strata-X-AW and 100 mg Strata-X-CV and, secondly, with 200 mg Strata X resulting in two compartments separated with a cellulose filter. The cartridges got preconditioned with 6 mL methanol followed by 6 mL Milli-Q water. With a flow rate of approximately 2 mL/min, sample aliquots of 500 mL were passed through the cartridges and dried under vacuum for 20 min. The elution was conducted with 4 mL of methanol / ethyl acetate (v:v 50:50) containing 2 % ammonia followed by 2 mL of methanol / ethyl acetate (v:v 50:50) containing 1.7 % formic acid. Extracts were collected in glass tubes and gently evaporated under a nitrogen stream to a volume of 100 µL. In a next step, they were transferred to chromatographic vials and reconstituted to 0.5 mL with a final proportion of MeOH / water (v:v 2:3).

2.4.2 Instrumental analysis

The instrumental analysis was conducted with an Acquity Ultra-Performance Liquid Chromatography (UPLC) system (Waters Corporation, USA) coupled to a quadrupole-time-of-flight (QTOF) mass spectrometer (QTOF Xevo G2S, Waters Corporation, Manchester, UK). Extracts were analysed in positive (PI) and negative (NI) electrospray ionisation mode. The chromatographic separation was carried out on an Acquity HSS T3 column (100 mm x 2.1 mm, 1.8 µm) in PI mode and on an Acquity BEH C18 column (50 mm x 2.1 mm, 1.7 µm) in NI mode which were both purchased from Waters Corporation (Manchester, UK). For PI mode, the aqueous phase consisted of 5 mM ammonium formate buffer with 0.01% formic acid and the organic phase with acetonitrile and 0.01% formic acid. For NI mode, the aqueous phase was

composed of 5 mM ammonium acetate buffer with 0.01% ammonia and the organic phase consisting of acetonitrile with 0.01% ammonia.

The adopted eluction gradient for both ionization modes started with 5% of organic phase for 0.5 minutes, increasing to 95% by 16 min, and then to 99% in the following 0.1 min. These almost pure organic conditions were kept constant for 3 min, and then initial conditions were restored and kept for 2 min. The total run time was 21 min in both modes. The chromatographic flow rate was 0.5 mL min^{-1} and the injection volume was 5 µL. The column temperature was set to 40 °C and the sample manager temperature was 15 °C. The resolution of the TOF mass spectrometer was 30 000 at full width and half maximum (FWHM) at m/z 556. MS data were acquired over an m/z range of 100-1200 in a scan time of 0.25 s. Capillary voltages of 0.35 kV were used in PI and 0.4 kV in NI. A cone voltage of 30 V was applied, the desolvation gas flow rate was set at 700 L h^{-1} and the cone gas flow was set to 25 L h^{-1}. The desolvatio temperature was set to 450 °C and the source temperature to 120 °C. Two acquisition functions with different collision energies were created: the low energy (LE) function with a collision energy of 4 eV, and the high energy (HE) function with a collision energy ramp ranging from 10 to 45 eV. Calibration of the mass axis from m/z 100 to 1200 was conducted daily with a 0.5 mM sodium formate solution prepared in 90:10 (v/v) 2-propanolol/water. For automated accurate mass measurement, the lock spray probe was employed (10 µL min^{-1}), using a lock mass leucine encephalin solution (2 mg mL^{-1}) in ACN/water (50:50) with 0.1% formic acid.

2.5 Target Screening Performance

In a first step, a target screening methodology was applied including EP from different categories such as pharmaceuticals, pesticides, phthalates, flame retardants and artificial sweeteners. The complete list containing the target analyte names, CAS number, molecular formula, exact masses, molecular weights, and Log K_{OW} is presented in Annex A1, Tab.A1.3. Substances were selected based on available expert knowledge concerning their usage, physiochemical properties and the occurrence of those compounds in waters. Altogether, the screening comprised 82 compounds. The target screening performance was conducted using Waters UNIFI scientific information system, a software platform merging LC and MS data and displaying base peak chromatograms for masses above the given intensity threshold, excluding the isotopic peaks. For the identification, mass accuracy and given RT were sufficient. Nonetheless, the presence of characteristic fragments in the MS/MS served as additional indicator for the assignment of target compounds.

2.6 Suspect Screening Performance

Suspect screening was performed using Waters UNIFI, the same software used for the target screening performance. The strategy applied for tentative identification included different

criteria that can be divided into two categories illustrated in Fig.6. Firstly, the objective of the green conditions was a substantial reduction of features with regards to the molecular formula. The thresholds were determined in the software settings and performed automatically. In addition, the predicted Rt was utilised and constituted as the only criteria in this first step which is related to the compounds structure. Secondly, the objective of the evaluation of evidences was the confirmation or rejection of tentative identifications. Potential evidences and the comparison with reference data served as indicators for a compound's structure which was examined manually.

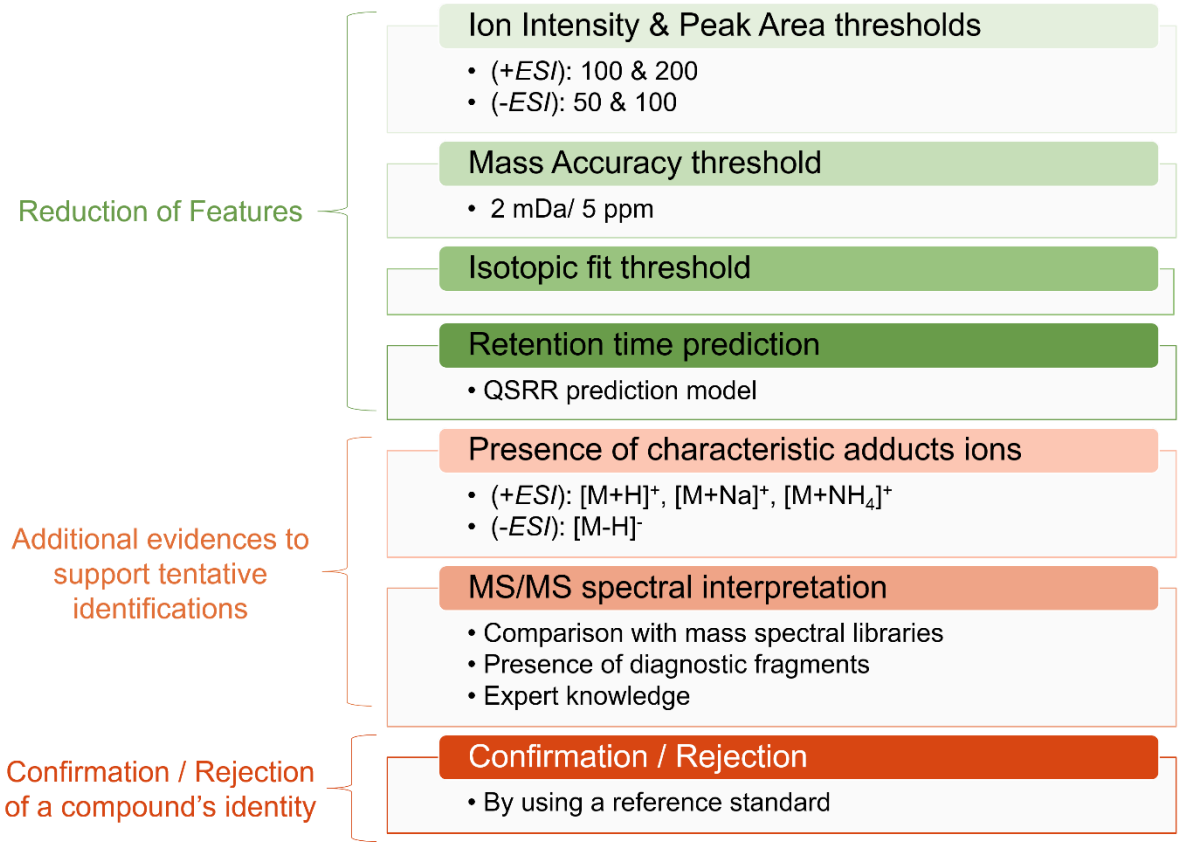


Fig.6: Suspect screening workflow

As a basic step, those compounds below a threshold of 100 and 200 in positive mode (+*ESI*) for ion intensity and peak area, respectively were discarded. In negative mode (-ESI) those thresholds were set to 50 and 100, respectively. By specifying this in the initial settings, UNIFI displayed solely base peak chromatograms for masses above the given intensity threshold. Furthermore, the settings considered a mass accuracy threshold of 2 mDa and 5 ppm on the monoisotopic peaks, and the isotopic pattern fit, and the chromatographic retention time plausibility, using a quantitative structure-retention relationship (QSRR) retention time prediction model (Aalizadeh et al. 2016). It relates a chemicals' structure to predict the chromatographic behaviour and proposes a probable Rt. This predicted Rt was applied with a

deviation of +/- 2,5 min. Following this first reduction of features, a compound's tentative identity was either rejected or confirmed. The evaluation of evidences, as the second step, included the revision of the presence of characteristic adduct ions and the comparison with spectral libraries. In addition, information on the fragmentation of suspects was gathered through the MS/MS spectral interpretation, the presence of diagnostic fragments and expert knowledge. In this way, a compound's identity could be classified at different identification confidence levels (cf. Chapter 1.1). For tentatively identified substances that were commercially available, the corresponding standard was purchased to confirm the identity of the compound.

2.7 Toxicity prediction model ECOSAR

Toxicity values for the tentatively identified and confirmed suspect compounds were calculated based on the ecological structure-activity relationships (ECOSAR) predictive model (US EPA 2016). Based on the similarity of structures to chemicals for which the aquatic toxicity has been previously reported, the model predicts the respective aquatic toxicity. The model estimates LC50 (Median concentration in mg/L associated with 50% mortality), EC50 (Median concentration associated with effects on 50% of the organisms) and ChV (Chronic toxicity value) after 96h, respectively 48h of exposure, applied to fish, daphnia magna and green algae representing the entire aquatic environment (ibid.). If one of those estimates (provided concern concentration divided by an uncertainty factor of 10) was < 1Ble mg/L an acute toxicity of the corresponding compounds is expected. Annex 3 Tab.A3.1 shows all described estimates for all tentatively identified and confirmed substances. Since data is missing on the majority of EP, ECOSAR serves as a good alternative to experimentally derive toxicity data.

2.8 Quality assurance and quality control

Background contamination in the laboratory represents a frequent problem in the determination of EP (Moschet et al. 2013). To reduce those errors, several measures were taken into account when preparing and processing the samples. All glassware used was previously washed and heated overnight at 450 °C. Furthermore, gloves were worn during sample preparation. Since many of the compounds analysed undergo photodegradation and the samples may suffer the exposure to light during the procedure, all samples and stock standard solutions were in amber glass bottles and stored in the dark. Blanks were prepared to avoid a false determination of compounds coming from a different source than the surface water, respectively the WW samples.

The present work followed the same protocol and used the same materials for sample pre-treatment and SPE as Bletsou et al. (2017). The applied methodology for obtaining recoveries included the spiking of a known concentration of target analytes and comparing the

concentrations before and after the entire SPE-UPLC-MS/MS process. The approach demonstrated good recoveries for 2327 target compounds with a very wide range of physiochemical properties (Bletsou et al. 2017). It has already been used in other suspect and non-target screening studies showing very good results (Gago-Ferrero et al. 2017; Schymanski et al. 2014(2)). This indicated the decent performance of the approach and its applicability for the study at hand.

Apart from that, method limits of detection (MLODs) have been recently determined in the used LC-MS/MS system for several target EP in surface and wastewater samples (Gago-Ferrero et al. 2017). They were calculated by using method blanks to evaluate potential background levels of target analytes and to determine MLODs. While compounds detected in the blank samples were calculated from those (average of the concentrations detected in blanks + 3 x standard deviation or the lowest calibration point when compounds were not detected in the blanks), all other compounds were obtained with the signal-to-noise ration of real samples (ibid.). Although, the extraction process followed in this study was different, the instrumental analysis was identical and, thus, it can be assumed that great variations stay out. A summary of the quality parameters for the analytical method comprising MLODs can be found in Annex A1, Tab.A1.4. Since the acquisition of MLODs and recoveries was beyond this study, information for those target compounds are missing where no data was available in the literature. However, as the objectives focus on the suspect screening performance, there is no impairment of the works' quality.

3 Results

3.1 Target screening of selected EP

In total, 52 out of the 82 investigated target compounds were detected in at least one of the evaluated samples (n =11) (Annex A2 Tab.A2.1 and Tab.A2.2). 12 compounds (15% of the total) were detected in all the samples and 36 (44%) in at least one of the surface water samples. WW samples contained larger counts of detected compounds (66%) in comparison to the surface water samples from the three evaluated rivers and lake Mälaren.

As expected, all influent samples exhibited the highest contaminant counts. With 48 compounds, the urban influent sample from Uppsala contained the highest number of detected targets, followed by the Västerås' (n =1) and Stockholm's influent (n =1) (Fig.7). Simultaneously, the influent from Uppsala's industrial area comprised considerably less detected target substances. Since target compounds are associated to a direct human consumption rather than to an industrial usage profile, a higher count in urban wastewater had to be expected. However, for drawing any definite conclusions on this, a quantification of levels would be necessary.

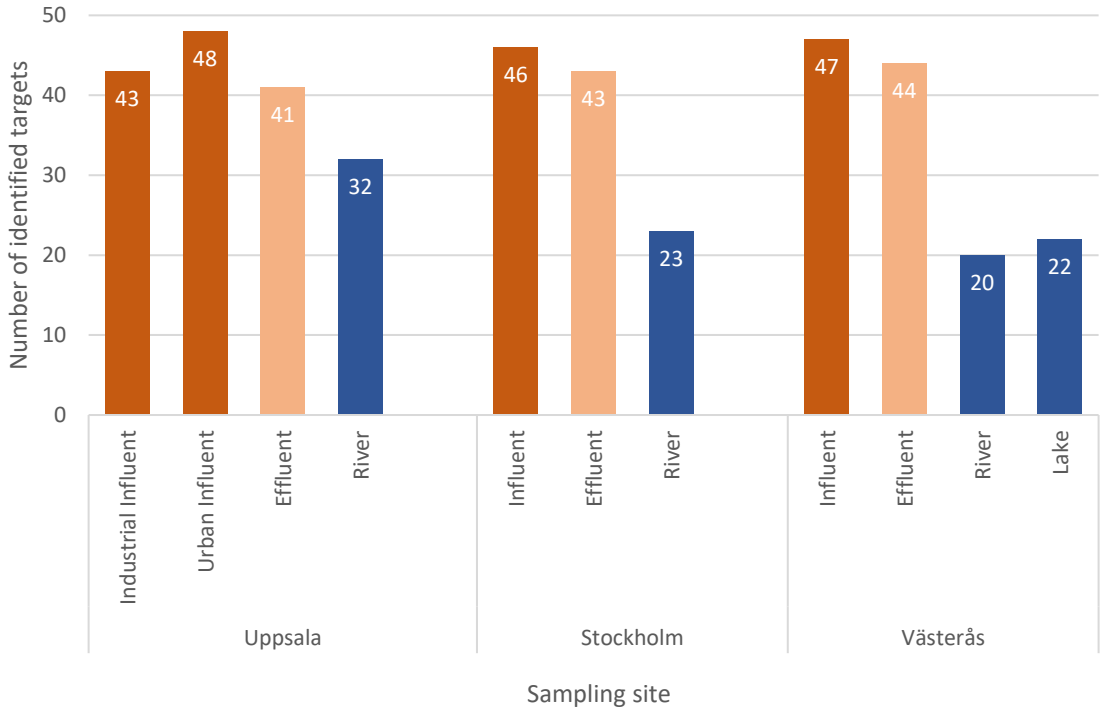


Fig.7: Number of detected target analytes in WW and surface water samples

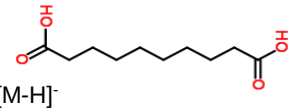
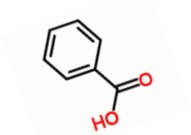
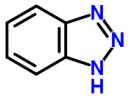
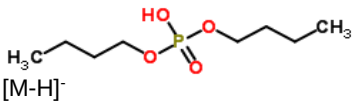
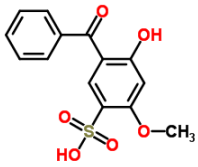
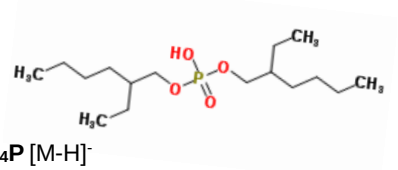
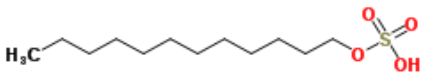
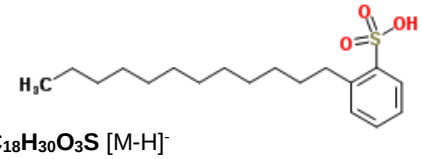
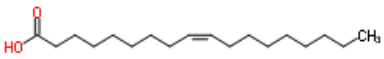
Among the effluent samples, Västerås features the highest detection count (n =3). Despite this comparably little higher contamination, its samples from river Svart and lake Mälaren have the lowest count among the surface water samples. Particularly noticeable is, thus, the higher contamination with investigated target analytes of lake Mälaren (n =1) compared to river Svart (n =1). This fact can be possibly attributed to the discharges of the WWTP which is located within the lake while the river is not influenced by this large facility (Chapter 2.2). With 32 detected target compounds river Fyris in Uppsala exhibits a relatively high number of compounds with approximately 50% higher detection counts than all other surface water samples.

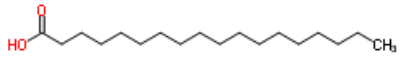
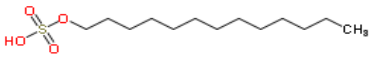
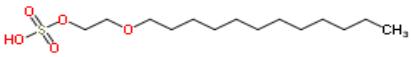
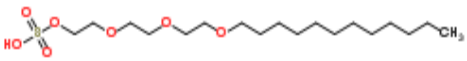
The most ubiquitous substances in surface water were, inter alia, Acesulfame, Caffeine, Carbamazepine, Desvenlafaxine PFBS and several other PFASs and Metoprolol, showing frequencies of detection of 100%. Except of perfluorodecanoic acid (PFDA), featured in river samples of Stockholm and Västerås and the single lake sample, all targets that are proved in the surface water were also identified in the WW samples. Several pollutants proved in the WW samples could, however, not be determined in the surface waters. These include Amitryptilline, Atorvastatin, Ciprofloxacin, Clarithromycin, Climbazole, Cocaine, Codeine, Diethyl phthalate (DEP), Diltiazem, Ethylparaben, Fluconazole, Irbesartan, Methylparaben, Metronidazole, Octocrylene, Oxycodone, Perfluorobutane sulfonic acid (PFBS), Perfluorohexanoic acid (PFHxA) and others.

3.2 Suspect screening: Identification of prioritised suspect analytes

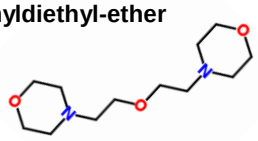
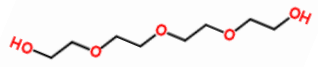
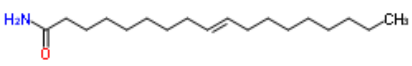
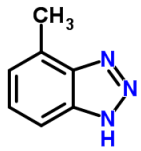
After the reduction of features by applying thresholds for ion intensity, peak area, mass accuracy, isotopic fit and chromatographic retention time (Fig.6), the screening of surface waters for the 143 suspects resulted in 31 hits in NI and 22 hits in PI with an overlap of 9 compounds detected in both modes. Note that the only a priori information was the exact mass of the protonated and deprotonated ion ($[M+H]^+$, PI and $[M-H]^-$, NI) calculated from the chemical formula. The presence of characteristic fragments supported the assessment of data. A QSRR prediction model served as further assistance in the identification of suspects. A match of experimental (Rt_{exp}) and predicted retention time (Rt_{pred}) increased the likelihood of an identified peak to belong to an assigned compound. All compounds showing feasible chromatographic retention times in accordance with the model were further investigated. However, the prediction model was not available in NI. Thus, it is noteworthy that in PI where the Rt prediction model could be applied, the number of hits was reduced by more than 25% by rejecting those substances where the experimental Rt did not match the predicted Rt with a deviation of 2 min. Hence, the use of a reliable Rt prediction model increases the accuracy and is time- and effort-saving. Those positive matches accomplishing all the thresholds were, simultaneously, in accordance with the mass of interest and reached in almost all cases not less than confidence level 4 of identification (unequivocal molecular formula). However, the eventual identity of a substance is not guaranteed because a multitude of compounds (from one to several thousands) can share a given molecular formula. In a next step, the deep evaluation of the MS/MS spectra and the investigation of additional evidences helped to increase the identification confidence. The comparison of the obtained MS/MS spectra those found in spectral libraries (MassBank), the use of in-silico fragmentation prediction tools (Metfrag) and the use of expert knowledge in the evaluation of the fragments served as further positive indication in the identification of suspects. Additional evidences included the presence of characteristic adducts and also the comparison with the rest of compounds with the same molecular formula in terms of usage and consumption (using the number of references and data sources as indicator (Hug et al. 2014)). Following this workflow, the 31 in NI and 22 in PI, respectively 53 substances in total, could be reduced significantly to 21 tentatively identified compounds. In the process, the allocation of corresponding fragments proved to be an important evidence. For those compounds were no additional evidences could be endorsed (Annex 2, Tab.A2.3 and Tab.A2.4), no further investigation was conducted within this study remaining at level 4 or 5 (not tentatively identified). Tab.1 shows the 13 compounds tentatively identified or confirmed in NI, their experimental retention time (Rt), the list of previously discussed evidences for each compound as well as the level of identification confidence. Tab.2 shows the respective results in PI further including the comparison of experimental Rt (Rt_{exp}) and predicted Rt (Rt_{pred}).

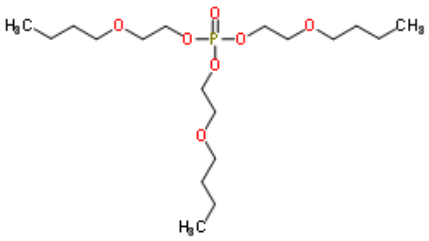
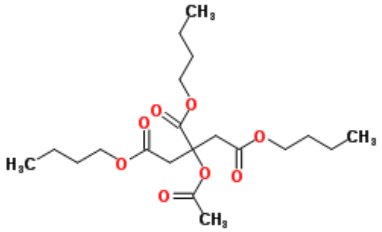
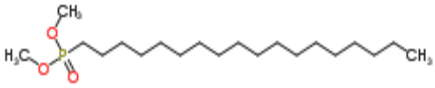
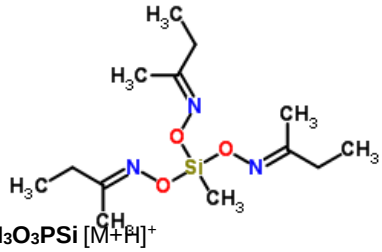
Tab.1: Details on the 13 tentatively identified and confirmed suspect analytes in NI

Suspect analyte	Rt	Additional Evidences	Level*
Sebacic acid  $C_{10}H_{18}O_4$ [M-H]$^-$	0.78	▪ Presence of characteristic fragments 99.0445 [C5H7O2]; 109.0655 [C7H9O]; 165.0916 [C10H13O2] ▪ Similarity with MassBank [record PR100605] ▪ CONFIRMED with reference standard	1
Benzoic acid  $C_7H_6O_2$ [M-H]$^-$	0.87	▪ CONFIRMED with reference standard	1
1,2,3-Benzotriazole  $C_6H_5N_3$ [M-H]$^-$	1.88	▪ Plausible MS/MS spectra also in PI ▪ Plausible Rt in PI (3.82) according to the QSRR model ▪ CONFIRMED with reference standard	1
Dibutyl phosphate  $C_8H_{19}O_4P$ [M-H]$^-$	4.38	▪ Presence of characteristic fragments 78.9583 [O3P]; 96.9691 [H2O4P]; 153.0317 [C4H10O4P] ▪ CONFIRMED with reference standard	1
Sulisobenzone  $C_{14}H_{12}O_6S$ [M-H]$^-$	4.63	▪ Presence of characteristic fragments 79.9568 [O3S]; 210.0321 [C13H6O3]; 228.9809 [C8H5O6S] ▪ Similarity with MassBank [record TUE00147] ▪ CONFIRMED with reference standard	1
Di-(2-ethylhexyl)phosphoric acid  $C_{16}H_{35}O_4P$ [M-H]$^-$	9.38	▪ Presence of characteristic fragments 78.9584 [O3P]; 123.9923 [C2H5O4P]; 209.0945 [C8H18O4P] ▪ CONFIRMED with reference standard	1
Laurilsulfate  $C_{12}H_{26}O_4S$ [M-H]$^-$	9.71	▪ Presence of characteristic fragments 79.9567 [O3S]; 96.9688 [HO_4S]; 122.9746 [C2H3O4S] ▪ CONFIRMED with reference standard	1
2-Dodecylbenzenesulfonic acid  $C_{18}H_{30}O_3S$ [M-H]$^-$	10.77 / 10.9	▪ Presence of characteristic fragments 183.01196 [$C_8H_7O_3S$]; 198.0357 [$C_9H_{10}O_3S$]; 79.9560 [O_3S] ▪ Plausible MS/MS spectra also in PI ▪ Plausible Rt in PI (12.49) according to the QSRR model ▪ CONFIRMED with reference standard	1
Oleic acid  $C_{18}H_{34}O_2$ [M-H]$^-$	12.51	▪ Presence of characteristic fragment 263.2379 [C18H31O] ▪ CONFIRMED with reference standard	1

Stearic acid  **$C_{18}H_{36}O_2$** [M-H]$^-$	12.53	▪ Presence of characteristic fragments 83.0494 [C5H7O]; 255.2317 [C16H31O2]; 265.2535 [C18H33O] ▪ Similarity with MassBank [record MT000015] ▪ CONFIRMED with reference standard	1
Tridecyl hydrogen sulfate  **$C_{13}H_{28}O_4S$** [M-H]$^-$	9.47	▪ Presence of characteristic fragments 96.9596 [HO_4S] ▪ Best match with Metfrag	2b
2-(Dodecyloxy)ethyl hydrogen sulfate  **$C_{14}H_{30}O_5S$** [M-H]$^-$	10.13	▪ Presence of characteristic fragment 96.9590 [HO4S] ▪ Similarity with MassBank [record ETS00008] ▪ Good match with Metfrag	2a
2-{2-[2-(Dodecyloxy)ethoxy]ethoxy}ethyl hydrogen sulfate  **$C_{18}H_{38}O_7S$** [M-H]$^-$	10.72	- Presence of characteristic fragments 96.9594 [HO_4S]; 79.9564 [O_3S]; 213.1851 [$C_{13}H_{25}O_2$] ▪ Best match in Metfrag	2b

Tab.2: Details on the 8 tentatively identified and confirmed suspect analytes in PI

Suspect analyte	Rt_{exp} (Rt_{pred})	Additional Evidences	Level*
2,2'-Dimorpholinyldiethyl-ether  **$C_{12}H_{24}N_2O_3$** [M+H]$^+$	0.82 (2.14)	▪ Presence of characteristic fragments 130.0859 [$C_6H_{12}NO_2$]; 102.0912 [$C_5H_{12}NO$]; 86.0599 [C_4H_8NO] ▪ CONFIRMED with reference standard	1
Tetraetheleneglycol  **$C_8H_{23}N_5$** [M+H]$^+$, [M+Na]$^+$	1.72 (-0.89)	▪ Presence of characteristic fragments 133.0855 [$C_6H_{13}O_3$]; 89.0594 [$C_4H_9O_2$]; 103.0387 [$C_4H_7O_3$] ▪ CONFIRMED with reference standard	1
(9E)-9-Octadecenamide  **$C_8H_{19}O_4P$** [M+H]$^+$	15.33 (13.96)	▪ Presence of characteristic fragments 212.1996 [$C_{13}H_{26}NO$]; 86.0601 [C_4H_8NO]; 139.1113 [$C_9H_{15}O$] ▪ CONFIRMED with reference standard	1
Tolytriazole  **$C_7H_7N_3$** [M+H]$^+$	5.14 (5.15)	▪ Presence of characteristic fragment 108.0798 [$C_7H_{10}N$] ▪ Plausible MS/MS spectra also in NI ▪ Plausible Rt in NI (3.48) ▪ CONFIRMED with reference standard	1

Tris(2-butoxyethyl) phosphate  **$C_{18}H_{39}O_7P$** $[M+H]^+$, $[M+Na]^+$	12.87 (13.64)	▪ Presence of characteristic fragments 98.9837 [H4O4P; 143.0096 [C2H8O5P]; 199.0714 [C6H16O5P]; 299.1607 [C12H28O6P] ▪ Similarity with MassBank [record SM880602] ▪ CONFIRMED with reference standard	1
Tributyl citrate acetate  **$C_{20}H_{34}O_8$** $[M+H]^+$, $[M+Na]^+$	14.43 (12.48)	▪ Presence of characteristic fragments 101.0588 [$C_5H_9O_2$]; 259.1536 [$C_{13}H_{23}O_5$] ▪ Plausible MS/MS spectra also in NI ▪ Plausible Rt in NI (8.02) ▪ CONFIRMED with reference standard	1
Dimethyl octadecylphosphonate  **$C_{20}H_{43}O_3P$** $[M+H]^+$	14.20 (13.23)	▪ Presence of characteristic fragments 219.1152 [$C_{10}H_{20}O_3P$]; 209.1335 [$C_9H_{22}O_3P$]; 104.0420 [C_4H_9OP] ▪ Good match with Metfag	2b
butan-2-one O,O',O''-(methylsilanetriyl)oxime  **$C_{13}H_{27}N_3O_3PSi$** $[M+H]^+$	15.78 (13.69)	▪ Presence of characteristic fragments 86.0595 [C_4H_8NO]; 287.1630 [$C_{12}H_{25}N_3O_3Si$] ▪ Best match with Metfrag	2b

** Levels of Confidence: 1=Confirmed structure 2a=Probable structure by library 2b=Probable structure by diagnostic evidence 3=Tentative Candidate 4=Unequivocal Molecular Formula 5=Mass of Interest*

The complete identification methodology (including the confirmation step) for the suspect screening performance is demonstrated in Fig.8-10 through the example of tris(2-butoxyethyl) phosphate. The chromatographic peak associated to this substance accomplished all threshold condition applied in the feature reduction steps, including a plausible Rt (12.87 min) according to the QSRR model. These facts make the suspect a suitable candidate for further investigation. The fragments at *m/z*: 98.9837, 199.0714 and 299.1607 are characteristic for the investigated substance corresponding to [H4O4P], [C6H16O5P] and [C12H28O6P], respectively. Additionally, the MS/MS spectrum of tris(2-butoxyethyl) phosphate matched well

with the MassBank spectrum record SM880602 (Fig.9). Accordingly, there have been strong evidences of the identity of the compound.

After the purchase of the commercial reference standard, the identification of tris(2-butoxethyl) phosphate was confirmed via MS/MS and Rt comparison, reaching level 1. For this purpose, the samples were re-analysed; first without any spike and subsequently after adding small aliquots of the reference standard. The tentatively identified compound could be eventually verified, visualised in the gradually increasing peak intensity seen in Fig.11.

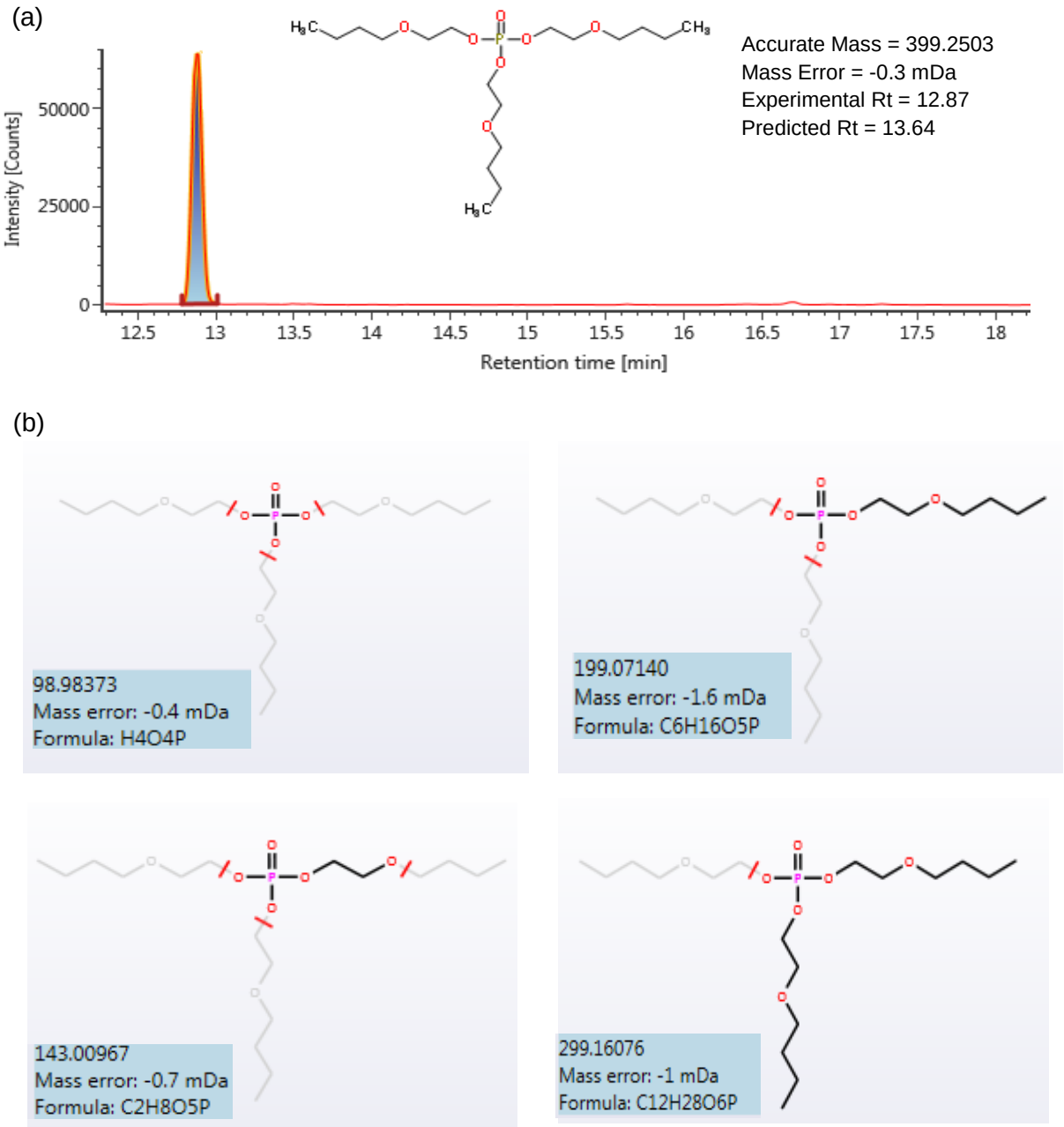


Fig.8:Example of tris(2-butoxyethyl) phosphate with (a) MS spectra and applied qualitative reference values and (2) characteristic fragments of the MS/MS

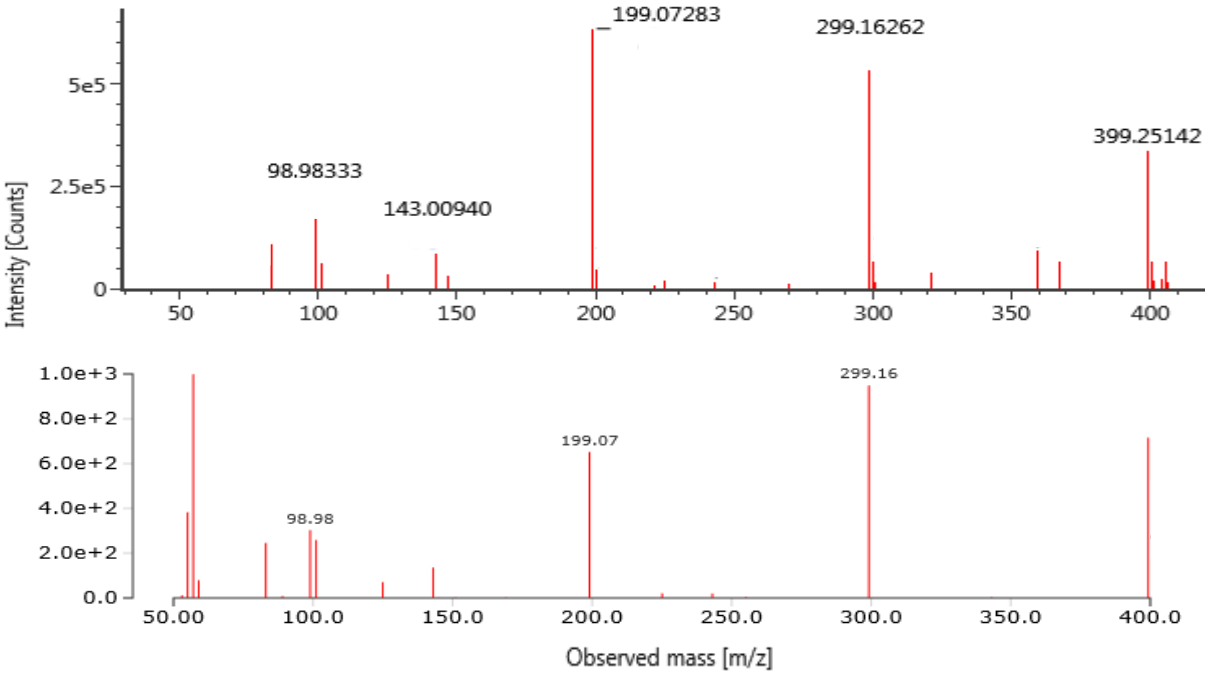


Fig.9: Spectra of Tris(2-butoxyethyl) phosphate and MassBank spectra SM880602 in comparison

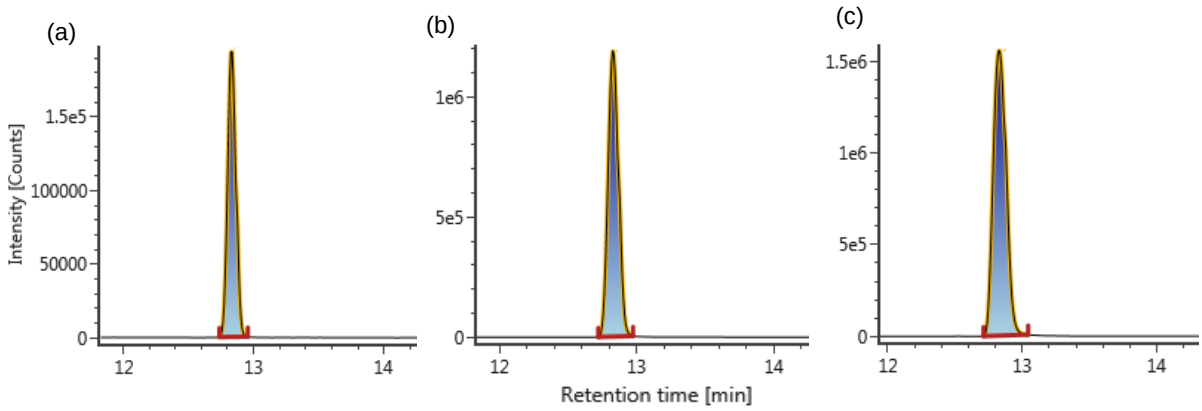


Fig.10: Confirmation of tris(2-butosyethyl) phosphate with reference standard (a) sample with no spike (b) first spike with 10 µl (c) second spike with 10 µl

Another example for this procedure is shown in Annex 2 Fig.A.2.1 by the suspect analyte 2,2'-Dimorpholinyldiethyl-ether. Although no spectral library entry was obtainable, the good mass accuracy and isotopic fit, the plausible Rt according to the QSRR model and the presence of characteristic fragments *m/z:* 84.0805 [C5H10N], 102.0914 [C5H12NO] and 114.0908 [C6H12NO] sufficed in order to tentatively identify the compound (level 2b) and further purchase the reference standard to confirm it (level 1). The unavailability of proper spectra in libraries was the general case, as mass spectral libraries are not yet adequately developed for LC-HRMS-based analysis but cover only a minor fraction of compounds. However, where library entries were available and coincided with measured spectra, an identification

confidence of level 2a was assigned initially. Otherwise, characteristic fragments were necessary to categorise a compounds identification confidence at level 2b (Annex 2 Fig.A2.2). Those substances where reference standards were not available, remained on this level of confidence. For all other compounds reaching level 2, reference standards served for the confirmation, respectively a rejection of the identity of 44,5% in PI and 23,1% in NI. Although a Rt prediction model was used in PI, the rejection comprised almost double the compounds in PI than in NI. A possible explanation is that molecular formulas in PI are generally more widespread and, thus, more options for potential substances exist. Simultaneously, NI provides a higher number of characteristic fragments (e.g. SO3) which makes a tentative identification more likely resulting in a lower ratio of rejections in the confirmation step. The confirmation was conducted through the successive injection of standard resulting in an increase of either the tentative or a different peaks' intensity. Fig.11 shows the confirmation procedure for 2-dodecylbenzenesulfonic acid. The gradual raising of several peaks is due to the mixture of isomers which are simultaneously used in the reference standard. Nonetheless, this and the initial evidences gives proof that different isotopes are present in the sample and are therefore confirmed. It is remarkable that the intensity profile for the different isotopes is identical in the commercial standard mixture to the environmental samples showing a common origin. In comparison, the spectra for sebacic acid also exhibits two indistinguishable peaks in first instance (Fig.12). However, after adding the reference standard of sebacic acid to the sample, the peak at 0.98 min remained at its initial intensity. Thus, the Rt at 0.78 min could be confirmed for sebacic acid, proofing its presence in the investigated surface sample. In this regard, the case of dibutyl phosphate once again illustrated the importance of the confirmation step. All evidences indicated the peak at Rt=4.20 min to be the suspected compound. However, through the spiking with the corresponding reference standard, the compound was confirmed at Rt=4.38 min while the initially assumed peak complied with an isotope having the same fragments (Fig.13).

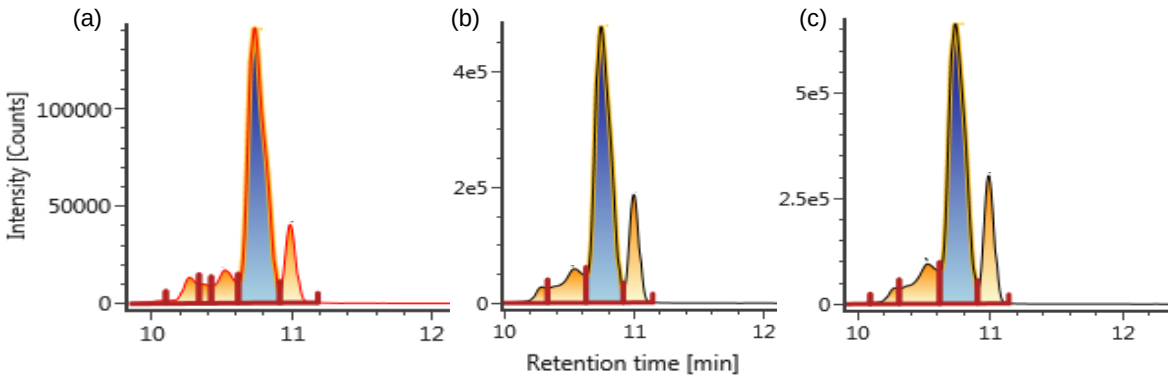


Fig.11: Confirmation of 2-Dodecylbenzenesulfonic acid standard (a) sample with no spike (b) first spike with 10 µl (c) second spike with 10 µl

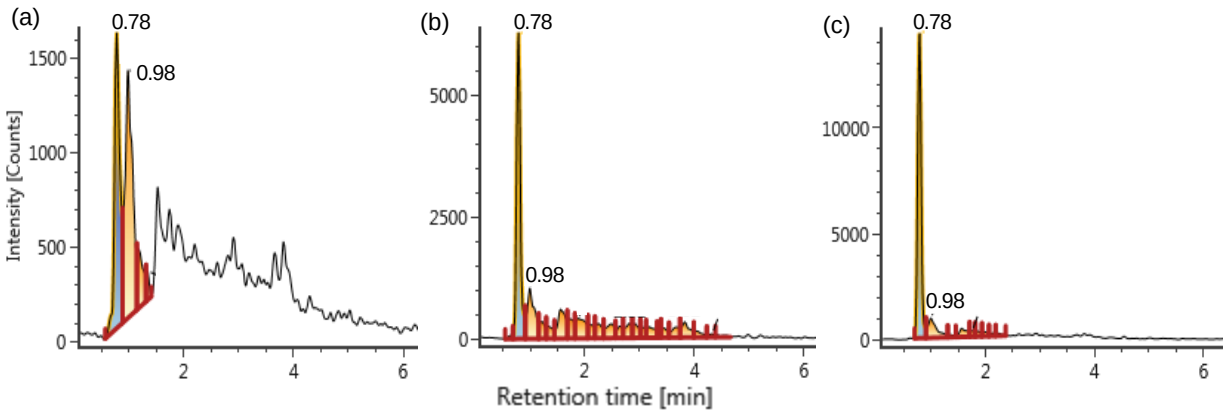


Fig.12: Confirmation of sebacic acid standard (a) sample with no spike (b) first spike with 10 µl (c) second spike with 10 µl

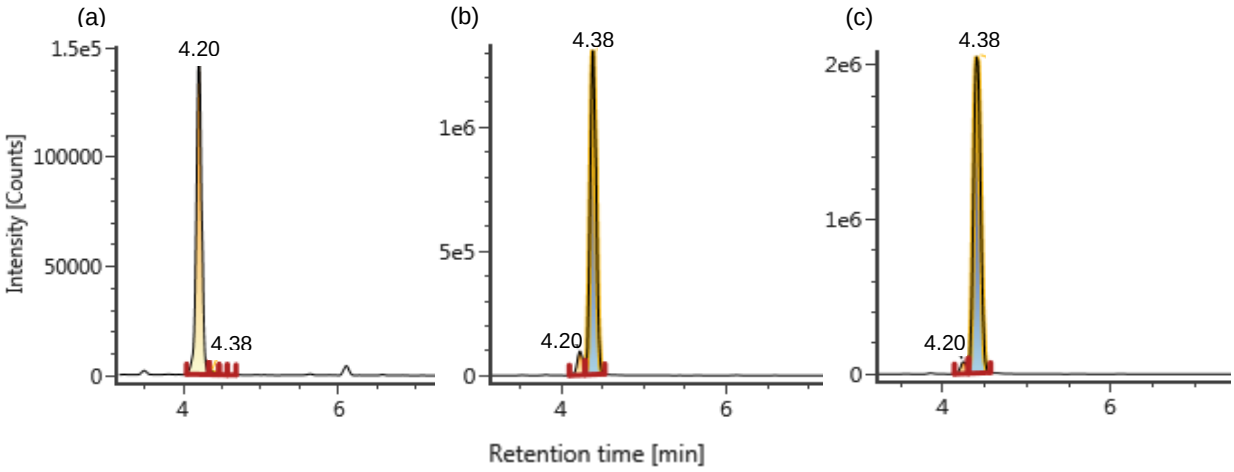


Fig.13: Confirmation of dibutyl phosphate standard (a) sample with no spike (b) first spike with 10 µl (c) second spike with 10 µl

3.3 Distribution of identified suspect analytes

Tab.3 shows the distribution of all tentatively identified or confirmed suspect pollutants (Chapter 3.2) in influents and effluents of the WWTPs and surface water for all three research areas. In total, 14 compounds that were determined in the surface water samples could also be found in the WW, while 5 substances couldn't evidently be ascribed to those. Although, there are minor differences for the single sites, all the pollutants that can't be traced back to the WW were contained in all investigated surface waters

Tab.3: Distribution of tentatively identified or confirmed suspects in the WW influents, WW effluents and surface water (river, lake) for Uppsala, Stockholm and Västerås

Suspect analyte	Uppsala			Stockholm			Västerås			
	Inf	Eff	Riv	Inf	Eff	Riv	Inf	Eff	Riv	Lk
(9E)-9-Octadecenamide										
1,2,3-Benzotriazole										
2-{2-[2-(Dodecyloxy)ethoxy]ethoxy} ethyl hydrogen sulfate										
2-(Dodecyloxy)ethyl hydrogen sulfate										
2,2'-Dimorpholinyldiethyl-ether										
2-Dodecylbenzenesulfonic acid										
Benzoic acid										
butan-2-one O,O',O''-(methylsilanetriyl)oxime										
Di-(2-ethylhexyl)phosphoric acid										
Dibutyl phosphate										
Dimethyl octadecylphosphonate										
Laurilsulfate										
Oleic acid										
Sebacic acid										
Stearic acid										
Sulisobenzone										
Tetraethyleneglycol										
Tributyl citrate acetate										
Tridecyl hydrogen sulfate										
Tris(2-butoxyethyl) phosphate										
Tolytriazole										

Inf Influent
Eff Effluent
Riv River
Lk Lake

Not identified
In influent
In effluent
In surface water

Despite compounds were not identified in the WW, they may, however, be contained but at intensities not relevant for the presence in the surface water. This is illustrated by the case of (9E)-9-Octadecenamide in Västerås where the peak at Rt=15.29min could be clearly determined in all three samples (Fig.14). The conspicuous difference is the comparably low intensity in the influent. Although the effluent features a little higher intensity, it is only about one third of the one in the surface water sample. The matrix effect is an important objection; however, it is not probable when considering the delusion effect in the lake. Hence, the compound was omitted to be present in the WW as it is of little significance and obviously not the main source for the presence in the river. In other cases, the evaluation of the different intensities was not necessary as the corresponding peak was not detected in wastewater (Fig.15).

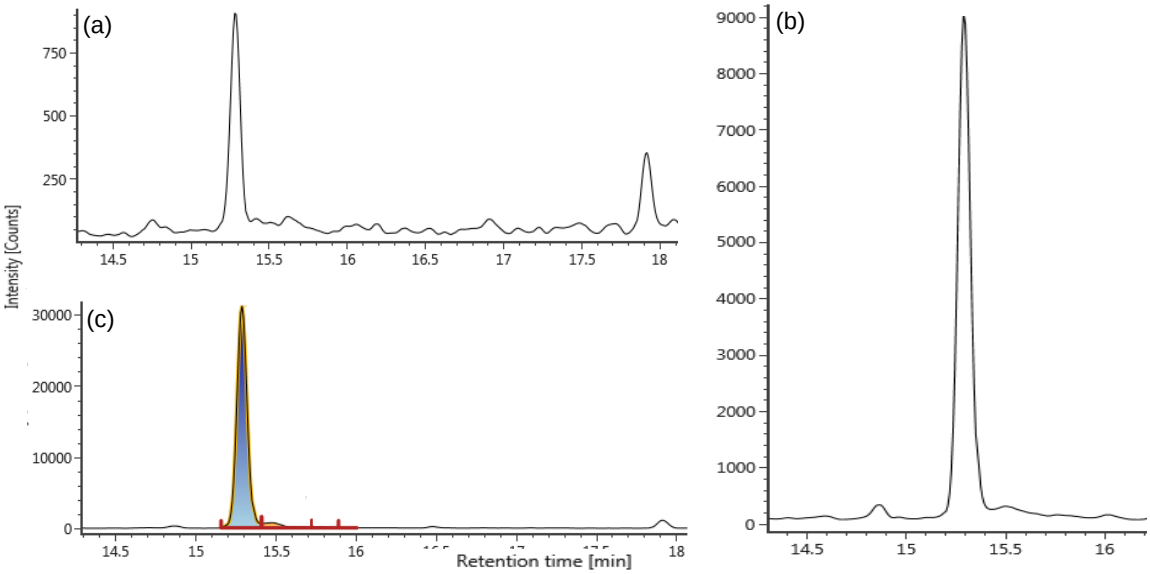


Fig.14: Comparison of (a) influent, (b) effluent and (c) surface water for (9E)-9-Octadecenamide in Västerås

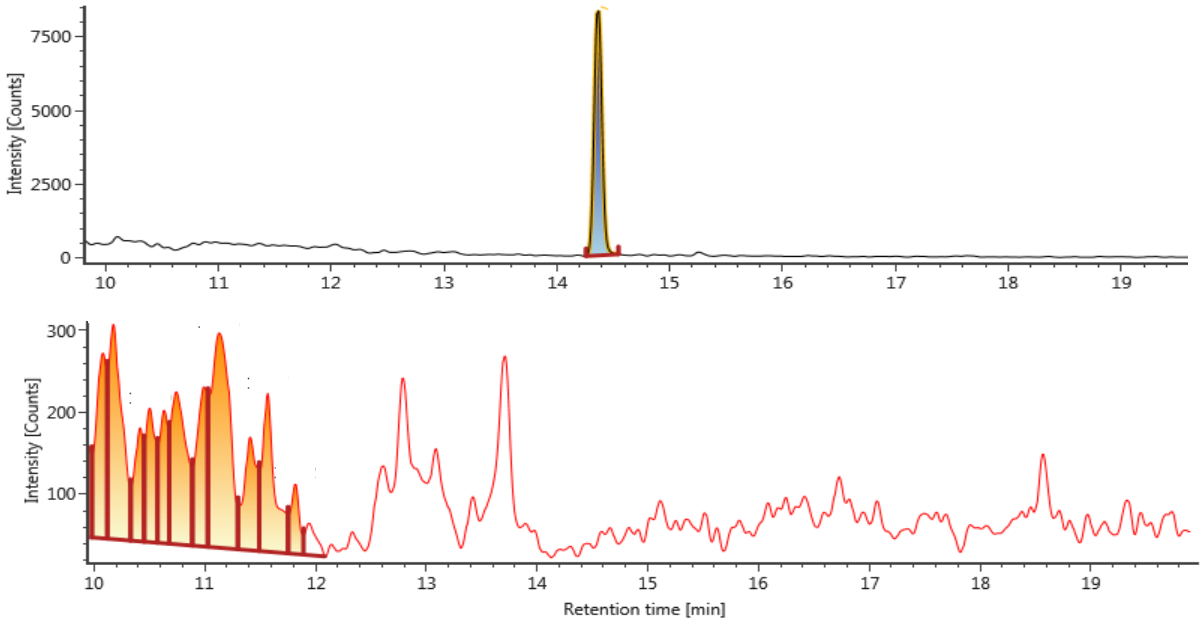


Fig.15: Comparison of (a) surface water and (b) WW for Tributyl citrate acetate in Stockholm

4 Discussion

4.1 Efficiency of the prioritisation strategy based on regulatory databases in the selection of suspects

In view of the multitude of chemicals produced, used, and released into the environment, it is impossible to experimentally assess all the hazards and risks due to existing time and budget constraints. Thus, prioritisation approaches should be used to focus monitoring and research resources and to identify those substances likely to pose the greatest risk in a particular situation. One objective of the study at hand, was to develop a new generic prioritisation method for the screening of compounds with a wide range of physiochemical properties in surface waters which allows the identification of new EP that are not on the radar of environmental scientists. Thereby, the inclusion of market data served as highly-promising indicator to contribute to obtain a broader picture regarding the presence of EP in the environment. Through the cooperation with a governmental agency and the possible availability of the Swedish chemicals registry database comprising the whole range of chemicals circulating on the Swedish market, an unbiased selection of suspects could be guaranteed.

The prioritisation workflow has been successful in the reduction of the large registry database and in the generation of a suspect list comprising relevant substances. However, the different prioritisation steps turned out to have varying importance. The initial database of 20000 compounds, could be significantly reduced to 2239 by the application of the EI considering only those compounds with the three highest exposure indices. A further reduction to the final 143 substances was achieved through the implementation of technical criteria functioning as second main step in the prioritisation. Conclusively, the application of the EI threshold had the highest impact in the prioritisation approach.

Several prioritisation approaches for organic substances can be found in literature most of which compare modelled or measured occurrence concentrations and toxicological impacts (Guillén et al. 2012). In this way, a first insight of potential risks is provided. A majority of approaches focus on the occurrence in surface waters by assessing monitoring data (ibid.) which results in a set of known compounds (Denzer et al. 1990; Daginnus et al. 2010; Guillèn et al. 2012). One example is provided by Von der Ohe et al. (2011) examining 500 classical and emerging pollutants in four European river basins considering the frequency and the extent of exceedance with respect to the WFD. Both indicators were obtained by measured environmental concentrations (Von der Ohe et al. 2011). Also, the procedure applied to obtain the list of 33 priority pollutants of the WFD was compiled by a prioritisation strategy. In general,

especially EP and their TP are not taken into account in official monitoring due to limited knowledge of their occurrence and fate.

The Joint Research Centre (JRC) of the EU has made a first step to integrate various inputs from different sources in their prioritisation strategy. Among them, certain Member States of the EU, the NORMAN network, the European Parliament, and other stakeholders provided lists of substances of possible concern. Although no effort has been spared to include a broad variety of lists, EP were solely derived from scientific literature, expert knowledge, and monitoring databases (Daginnus et al. 2010). The inclusion of completely unknown compounds was, thus, not considered. In general, most prioritisation approaches emphasis on a compounds' environmental concentration and on the establishment of toxicity rankings for already known suspect compounds. This gets especially obvious in view of the wide range of prioritisation methods that have been proposed for pharmaceuticals (Batt et al. 2015; Berninger et al. 2015; Sangion and Gramatica 2016; Aubakirova et al. 2017). All those approaches use either exposure or toxicological prediction methods. In the study of Sangion and Gramatica (2016), for instance, the potential hazard of existing pharmaceuticals was modelled using structural molecular descriptors. Although this approach is advantageous as toxicity indices can be predicted without experimental data (Sangion and Gramatica 2016), prioritisation is merely applied to known substances and does, ones again, not consider real unknowns.

Sjerps et al. (2016) attributed more significance to databases of chemicals authorized on the market, considering European regulatory frameworks under the REACH legislation and, additionally, obtaining information of the Dutch chemicals market. Using reference standards, a proportion of 15,2% was confirmed corresponding to confidence level 1. However, only well know compounds were confirmed (e.g. caffeine or tramadol) and they did not consider tentative identifications at all. No new knowledge regarding new EPs was generated. Whereas, Chiaia-Hernandez et al. (2014) compiled a suspect list based on consumption data and confirm the presence of 3 relevant new substances in sediments samples.

In the suspect screening performance, the reduction of features step considering only the molecular formula of a compound minimised the suspect list to 53 hits (31 in NI and 22 in PI) of which a high ratio of tentative identifications (including confirmations) has been obtained (42% in Ni and 36% in PI). Thus, the efficiency of the applied prioritisation strategy has been approved. In comparison, Schymanski et al. (2014(2)) achieved 35% in NI and 3% in PI in the tentative identification of suspects using a similar suspect screening performance including a variety of evidences. However, their suspect list was compiled from compounds previously found in the literature (Schymanski et al. 2014(2)). Confirmations with reference standards were not carried out due to difficulties in obtaining (ibid.) which distorts a direct comparison. As seen in this example, identification ratios in all the studies are usually higher in NI. This

trend is attributable to the smaller number of ionisable compounds and the easier ionisation of characteristic ions in NI (e.g. SO3). However, it is in discordance with the present study where the rate of identification in both modes is almost equal. This can be explained by a good preselection of compounds and the high number of confirmation that were proceeded.

The present study is one of the first using market data for prioritisation purposes. In this regard, it is the study with the highest number of identifications with most substances that were up to now out of the focus for environmental scientists. Results clearly show that a promising way to enhance identification rates is the collaboration with authorities as aimed in the applied strategy. The use of a Rt prediction did not support the selection of suspect compounds, but was introduced to assign peaks to potential substances in a more efficient way. Furthermore, it has turned out to be positive to include additional compounds, in this study namely Dibutyl phosphate, Sulisobenzone, Di-(2-ethylhexyl)phosphoric acid and Benzoic acid which were prioritised in a preliminary study focusing on the “sewage treatment plant (STP)” recipient. While those substances were not prioritised in the scope of the present study due to the limitation to “surface water” (cf. Chapter 2.3), they yielded additional valuable results. This shows that despite the outstanding results that could be yielded, future improvements could be achieved in the prioritisation steps by considering other recipients, decreasing the threshold for the EI or including toxicity data when complying the substances that are environmentally relevant. It is, moreover, noteworthy that next to various identifications, a high number of confirmations could be obtained. Thus, the study constitutes the highest number of confirmations in this way. 21 substances were purchased and 16 confirmed, showing the good performance of both, the prioritisation approach, and the suspect screening performance. As will be discussed in the next section, the occurrence of some of those substances in the environment is not at all or only partially studied in the literature.

4.2 Identified compounds: Usage, sources and, distribution

As a first step, a target screening performance was integrated into the study at hand to characterise the investigated waste and surface water. With this aim 82 substances were investigated including pharmaceuticals, PFAS, personal care products, pesticides, phthalates, flame retardants and artificial sweeteners. The percentage and identity of detected target compounds in all the samples of all sites were similar compared to other studies carried out in the immediate surroundings (Gago-Ferrero et al. 2017; Gros et al. 2017) and is, furthermore, in accordance to other regions in Europe (Archer et al. 2017; Nikolaou et al. 2007; Schymanski et al. 2014(2)) showing a comparable pattern. Since the focus of this study is the application and development of suspect screening strategies, no further research was conducted with regards to the target screening performance and no further discussion will be made in the following sections.

All substances identified by the suspect screening performance, their main uses, their presence in previous studies with regards to the environment, their toxicity as well as their presence in the potentially influencing WW effluents are shown in Tab.4. The high ratio of identifications and confirmations comprised compounds of different awareness levels. First, the identification contained few compounds that are already widely mentioned in the literature as EP. Those include 1,2,3-benzotriazole, tolytriazole, benzoic acid and sulisobenzone. Although, no new findings could be acquired with regards to the compounds occurrence and source, their confirmation supported the valuation of the prioritisation and suspect screening performance. 1,2,3-benzotriazole and tolytriazole were prioritised due to their legitimately high EI for the recipient “surface water”. Both substances are complexing agents applied as corrosion inhibitor, e.g. in aircraft deicer (Giger et al 2006). Their widespread use serves as explanation for their presence in all waste water and surface water samples. Other studies confirmed their presence in waste water (Voutsa et al. 2006; Reemtsma et al. 2010) and surface water (Giger et al. 2006; Kiss et al. 2009). The conclusion of Giger et al. (2006) that both substances are ubiquitous contaminants in the aquatic environment could be substantiated in this study. The polar UV filter sulisobenzone and the food preservative benzoic acid were not prioritised in the applied prioritisation method but have been included due to their presence in the WW samples used for comparison in a preliminary study in the Department of Aquatic Sciences and Assessment. It appears justified, that the compounds’ EI didn’t meet the threshold in the applied prioritisation as they were not widely spread in the surface water samples. However, indications of the ecotoxicological effects of sulisobenzone (Molins-Delgado et al. 2016(1); Molins-Delgado et al. 2016(2)) and its occurrence in surface waters (Liu et al. 2016) made it a reasonable candidate to be included into the suspect list. Sulisobenzone could in fact be proved in the surface water of Uppsala, which should be further investigated due to its potential endocrine disruptive effects (Molins-Delgado et al. 2016(1)). A similar interest applied to benzoic acid which, generally, occurs in almost all environmental compartments (WHO 2000). Thus, its presence in WW, especially from wood production in Scandinavia (Lindström and Österberg 1986; Carlberg et al. 1986) and surface water (Schou and Krane 1981) was proved in the literature. Schou and Krane (1981) conclude a specific industrial effluent to be the dominating source for benzoic acid in the investigated water-course in Norway. Since surface water samples in the present study were taken in areas potentially affected by WWTP effluents, those findings seem applicable. It is noteworthy, that intensities of benzoic acid were, in general, very low; even after spiking the samples with the reference standard. The amount of the substance in the surface water must be accordingly high if it has been, nonetheless, detected. The awareness towards all four compounds is high with extensive research efforts. However, as all of them were detected in all WW and almost all surface water samples, a continuous monitoring of the substances seems recommendable.

Tab.4: Main uses, presence reported in previous literature, toxicity and detection in potentially influencing WW effluents for all tentatively identified or confirmed compounds from the suspect screening.

Identified suspect analyte	Main Usages	Presence reported in the environment[1]	Toxicity of high concern[2]	Presence in WW[3]
(9E)-9-Octadecenamide	Lubricant, corrosion inhibitor	No	Yes	No
1,2,3-Benzotriazole	Corrosion inhibitor, deicing fluids for aircrafts	Yes (Giger et al. 2006; Kiss et al. 2009; Voutsa et al. 2006; Reemtsma et al. 2010)	Yes	Yes
2-{2-[2-(Dodecyloxy)ethoxy]ethoxy} ethyl hydrogen sulfate	Anionic surfactant	Yes (Schymanski et al. 2014(2))	No	No
2-(Dodecyloxy)ethyl hydrogen sulfate	Anionic surfactant	Yes (Schymanski et al. 2014(2))	No	Yes
2,2'-Dimorpholinyldiethyl-ether	Catalyst for flexible foam, coating	No	No	Yes
2-Dodecylbenzenesulfonic acid	Anionic surfactant	Yes (Pérez-Carrera et al. 2010; Qv et al. 2013)	Yes	Yes
Benzoic acid	Preservative in food, beverages, cosmetics, pharmaceuticals	Yes (Schou and Krane 1981; Lindström et al. 1986; Carlberg et al. 1981)	No	Yes
butan-2-one O,O',O''-(methylsilanetriyl)oxime	Adhesive, sealant	No	No	No
Di-(2-ethylhexyl)phosphoric acid	Solvent extraction	No	No	Yes
Dibutyl phosphate	Lubricant, paint, coating	No	No	Yes
Dimethyl octadecylphosphonate	Lubricant in hydraulic fluids	No	Yes	Yes
Laurilsulfate	Anionic surfactant	Yes (Cserháti et al. 2002)	Yes	Yes
Oleic acid	Surfactant, soap, plasticiser,	No	No	Yes
Sebacic acid	Plasticiser, lubricant, hydraulic fluid, cosmetics, candles	Yes (Siotto et al. 2012)	No	Yes
Stearic acid	Detergent, cosmetics, lubricant	No	No	Yes
Sulisobenzone	Polar UV filter	Yes (Liu et al. 2016; Molins-Delgado et al. 2016(1))	No	Yes
Tetraethyleneglycol	Plasticiser, hydraulic fluids	Yes (Schymanski et al. 2014(2); Gago-Ferrero et al. 2015)	No	Yes
Tributyl citrate acetate	Plasticiser	No	Yes	No

Tridecyl hydrogen sulfate	Anionic surfactant	Yes (Schymanski et al. 2014(2); Gago-Ferrero et al. 2015)	No	No
Tris(2-butoxyethyl) phosphate	Flame retardant, plasticiser	Yes (Bendz et al. 2005)	Yes	No
Tolytriazole	Corrosion inhibitor, deicing fluids for aircrafts	Yes (Giger et al. 2006)	Yes	Yes

[1] Only literature explicitly reporting the occurrence in WW or the aquatic environment were considered (Scopus, Web of science)

[2] Compounds of high concern regarding the toxicity according to ECOSAR (cf. Chapter 2.7). Acquired data is shown in Annex 3 Tab.A3.1

[3] Presence in wastewater effluents that are potentially affecting the studied surface waters

Besides, many substances were identified for which the availability of literature on usage, toxicity and occurrence in aquatic environments varied widely from none to few but without any extensive investigations. Those compounds without any accessible information included the plasticisers tributyl citrate acetate and tetraethyleneglycolate, dibutyl phosphate used as lubricant and in coatings, butan-2-one O,O',O''-(methylsilanetriyl)oxime applied as adhesive and sealant and, finally, di-(2-ethylhexyl)phosphoric acid for the extraction of solvents. The three anionic surfactants tridecyl hydrogen sulfate, 2-{2-[2-(dodecyloxy)ethoxy] ethoxy}ethyl hydrogen sulfate and 2-(dodecyloxy)ethyl hydrogen sulfate were previously detected in natural waters (Schymanski et al. 2014(2), Gago-Ferrero et al. 2015). The prioritisation and detection of oleic acid and stearic acid seemed to present an ambiguous case. Both are widely used in the manufacturing of detergents, soaps, cosmetics or as plasticisers (HMDB, n.d.(1); HMDB, n.d.(2)). Due to their wide and abundant presence in nature, both substances are not environmentally toxic (Annex 3, Tab.A3.1). However, the applied prioritisation approach was based on market availability, consumer tonnage and use pattern while toxicity was not included due to the unavailability of reliable data for the initial 20 000 substances and, thus, did not exclude the prioritisation of oleic acid and stearic acid. The naturally occurring metabolite of oleic acid, (9E)-9-octadecenamide, was likewise prioritised and detected. Although, no explicit literature could be found on the occurrence in the aquatic environment, the substance is highly toxic according to ECOSAR (ibid.). A study by McDonald et al. (2008) found the substance leaking out of polypropylene plastics. Since (9E)-9-octadecenamide was also detected in the method blanks a contamination through the used 1L polypropylene bottles used for sampling is probable. Furthermore, no explicit literature exists for dimethyl octadecylphosphonate and 2,2'-dimorpholinyldiethyl-ether. However, there is a suspicion of harmful effects to the aquatic environment. Dimethyl octadecylphosphonate, used as lubricant in automotive suspensions, motor oils, break fluids and cooling liquid in refrigerators, is classified as having long lasting harmful effects to aquatic life (ECHA 2017) and was equally assigned high toxicity by the ECOSAR model (Annex 3 Tab.A3.1). A high likelihood to occur from industrial use, matches

its detection in the investigated surface waters which are located close to industrial effluents (cf. Chapter 2.2). 2,2'-dimorpholinyldiethyl-ether is an amine catalyst in the production of flexible foam, high-resilient molded foam, coatings and warm melt adhesives. It is an industrial intermediate and does not occur naturally (NCI n.d.). Information on its presence in waste or surface waters was not found in the available literature. Since the presence of 2,2'-dimorpholinyldiethyl-ether was, next to the surface water samples, predominantly in the effluents, it would be interesting to investigate whether the substance is a transformation product. No evidence could be found in the literature for this statement.

The two identified anionic surfactants 2-dodecylbenzenesufonic acid and laurilsulfate are in widespread commercial use (HSDB 2002a, Schymanski et al. 2014(2)). Although their removal is highly successful in WWTPs, those compounds are TP of other, more complex surfactants and a release to the environment is probable with unknown effects (Ivanković and Hrenović 2009). The plasticiser sebacic acid, is likewise widely used in industry, but due to a high biodegradability in soils its impacts on the environment seem minor (Siotto et al. 2012) which could be substantiated by the ECOSAR prediction assigning no toxicity. However, no study was available on its presence in water and the transferability of literature coping with soil samples is questionable. The fact that it was detected in the surface water, despite its good biodegradation might require some deeper investigations. Finally, the flame retardant tris(2-butoxyethyl) phosphate was exclusively detected in the surface water samples. Due to its additional use as plasticiser in rubber and plastics, the substance might origin from a different source than the WWTP passing in plumbing washers with synthetic rubbers (WHO 2000). Tris(2-butoxyethyl) phosphate has been described as pharmaceutically active compounds and was earlier detected in surface waters in Sweden (Bendz et al. 2005). In a study by Han et al. (2014) the substance has been found to be toxic in developing zebrafish by inhibiting the degradation and utilization of nutrients. Tris(2-butoxyethyl) phosphate was predicted to be highly toxic according to the ECOSAR prediction model. Although the compound is expected to partition in sediments and to degrade rapidly (WHO 2000), it could clearly be identified in all surface water samples.

Most suspect compounds that were prioritised and tentatively identified or confirmed, are not included in regulations, or monitoring programs. This study constitutes the first evidence of the presence of 6 substances in environmental samples. These substances include tributyl citrate acetate, butan-2-one O,O',O''-(methylsilanetriyl)oxime, dimethyl octadecyl phosphonate and 2,2'-dimorpholinyldiethyl-ether, di-(2-ethylhexyl)phosphoric acid, dibutyl phosphate. Two of them (dimethyl octadecylphosphonate and tributyl citrate acetate) with indications for being toxic. This fact proves the efficiency of the prioritisation approach. It is of paramount environmental relevance since it can be included in the design of future monitoring programs to gain more insights in the distribution and concentrations of EP.

Applying the suspect screening performance to waste and surface water samples proved as successful tool in the investigation of whether the major source of identified pollutants in surface waters are effluents of WWTPs. In 29% suspect analytes were merely detected in the surface water sample which means that alternative sources have to be investigated in the future. Particular attention should be given to tributyl citrate acetate and butan-2-one O,O',O''-(methylsilanetriyl)oxime which were only detected in the surface water samples indicating an alternative source than the WWTPs in all three locations. Since this study was based on a qualitative investigation which excluded the involvement of quantitative levels, those interrelations could only be assessed in a relative matter. Thus, the acquisition of concentration levels would benefit further deductions. Information gabs are to be clarified in a follow up consultation with the authority KemI. At the moment, no further conclusions can be drawn upon those suspect compounds.

5 Conclusion and Outlook

The present study demonstrated that the inclusion of commercial use and exposure data of chemicals is an essential key feature in the screening of EP. This combined with the application of suspect screening strategies (where the standard is not necessary in a first step) allowed the determination of several substances that have been out of the radar of environmental chemists. Target-based approaches only cover a minor part of the universe of pollutants and smart strategies that take advantage of the last advances in HRMS, like the one applied in this study, are necessary in order to expand knowledge on occurrence and distribution of EP and to find new substances that are potentially triggering the quality of the water. The collaboration with governmental authorities and the availability of regulatory databases proved to have a beneficial impact on the identification ratio of previously not sufficiently or not at all considered substances.

As exemplified in this study, the application of reference standards is indispensable for the confirmation step to achieve unconditional confidence in the identification of unknown compounds. Furthermore, it has been demonstrated that the inclusion of WWTP effluents as potential influencers of aquatic environments is a recommendable way of proceeding for the assessment of tracing back positive findings and the consideration regarding necessary alternative sources. Thus, some substances are present in surface water without being present in the potential affecting effluents from WWTP suggesting other sources (e.g. industrial discharge). The preceding results helped to underline existing knowledge, draw new conclusions, and reveal the need for further investigations. The assessment of interrelations of findings from waste and surface water could be substantiated by the quantification of target and suspect analytes. To improve this ratio of identifications, available MS/MS libraries have to be expanded for LC-HRMS to facilitate suspect screening performances. Moreover, further

information on the usage and toxicity of compounds would ease the categorisation of the identified compounds and enable fast responses in the case of harmful impacts of certain substances with regards to the aquatic environment. The integration into existing monitoring programs could be accelerated if necessary. Using the collaboration with governmental agencies those pursuing information could be obtained through the registry database. For the confirmation step, the application of reference standards showed to be an inevitable strategy for the unequivocal identification of suspect compounds. In general, the emphasise on market-based data is an efficient approach in the preceding prioritisation of those suspect compounds for which reference standards were purchased. This study is of paramount environmental relevance and it will be considered in the design of future monitoring programs to gain deeper insights in the distribution and concentrations of EP.

Acknowledgements

I would like to express my gratitude to my supervisor Dr. Pablo Gago-Ferrero at the Department for Aquatic Sciences and Assessment at the Swedish University for Agricultural Sciences (SLU), Uppsala, for the useful comments, remarks and engagement throughout the learning process of this master thesis. The door to Dr. Gago-Ferreros' office was always open whenever I ran into a trouble spot or had a question about my research or writing. He consistently allowed this paper to be my own work, but steered me in the right direction whenever he thought I needed it.

Furthermore, I like to thank a number of contributing partners.

> Stellan Fischer from the Swedish Chemicals Agency (Kemikalieninspektionen, KemI) for providing data and quick responses to all upcoming questions.
>
> Nikolaos Thomaidis and Reza Aalizadeh from the University of Athens for contributing with the QSRR prediction model for the retention times.
>
> Eric Cato from the Kungsängsverket in Uppsala as well as Agnieszka Juszkiewicz from Kungsängsverket in Västerås who were available for explanations and dialogue when acquiring information on the waste water treatment plants.

Without their participation and input, this study could not have been successfully conducted.

I would also like to acknowledge Dr. Lutz Ahrens of the Department of Aquatic Sciences and Assessment at the Swedish University for Agricultural Sciences (SLU), Uppsala, as the examiner of this thesis, and I am gratefully indebted to his very valuable comments on this thesis.

A great thank you also goes to Ande Rostvall for her patience when introducing me to the routines in the lab and for answering all upcoming questions.

Finally, I want to express my very profound gratitude to my parents Lidia and Peter Krettek for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis. This accomplishment would not have been possible without them.

Thank you.

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Annex

A1: Materials and Methods

A2: Results

A3: Discussion

A1: Materials and Methods

Tab.A1.1: Prioritised suspect analyte names, molecular formulas, and corresponding SMILES

Compound name	SMILE	Formula
Diuron	CN(C)C(=O)Nc1cc(Cl)c(Cl)cc1	C9H10Cl2N2O
2-(4-Methyl-3-cyclohexen-1-yl)-2-propanol	CC1=CCC(CC1)C(C)(C)O	C10H18O
laurilsulfate	CCCCCCCCCCCCOS(=O)(=O)O	C12H26O4S
propylsulfonic acid	CCCS(=O)(=O)O	C9H14O4S
3,6-Anhydro-1-O-dodecanoyl-D-galactitol	CCCCCCCCCCCC(=O)OCC([C@@H]1[C@H]([C@@H](CO1)O)O)O	C18H34O6
Oleic acid	CCCCCCCC/C=C\CCCCCCCC(=O)O	C66H130O18
Sorbitol	C([C@H]([C@H]([C@@H]([C@H](CO)O)O)O)O)O	C66H130O18
Methyltriacetoxysilane	CC(=O)O[Si](C)(OC(=O)C)OC(=O)C	C7H12O6Si
Ethylsilanetriyl triacetate	CC[Si](OC(=O)C)(OC(=O)C)OC(=O)C	C8H14O6Si
Methyltrimethoxysilane: Silane, trimethoxymethyl-	CO[Si](C)(OC)OC	C4H12O3Si
1-Phenyl-3,5-diethyl-2-propyl-1,2-dihydropyridine	CCCC1C(=CC(=CN1c2ccccc2)CC)CC	C18H25N
Dipropylene glycol dibenzoate	c1ccc(cc1)C(=O)OCCCOCCCOC(=O)c2ccccc2	C20H22O5
Oxybispropanol	CCC(O)OC(O)CC	C6H14O3
1-(3-Butoxypropoxy)-1-propanol	CCCCOCCCOC(O)CC	C10H22O3
2,2,4-TRIMETHYL-1,3-PENTANEDIOL 1-ISOBUTYRATE	CC(C)C(O)C(C)(C)COC(=O)C(C)C	C12H24O3
DI-T-BUTYLSULFIDE	CC(C)(C)SC(C)(C)C	C8H18S
Kitazin	CC(C)OP(=O)(OC(C)C)SCc1ccccc1	C13H21O3PS
O,O-diheptyl hydrogen dithiophosphate	CCCCCCCOP(=S)(OCCCCCCC)S	C14H30O2PS2
Phosphorodithioic O,S,S-acid	OP(=S)(O)S	H3O2PS2
O-sec-butyl O-(1,3-dimethylbutyl) hydrogen dithiophosphate	CCC(C)OP(=S)(OC(C)CC(C)C)S	C10H22O2PS2
O,O-Diisobutyl hydrogen phosphorodithioate	CC(C)COP(=S)(OCC(C)C)S	C8H18O2PS2
Dimethyl octadecylphosphonate	CCCCCCCCCCCCCCCCCCP(=O)(OC)OC	C20H43O3P
Dibutyl phosphite (VAN)	CCCCOP(O)OCCCC	C8H19O3P
Aminotrimethylene phosphonic acid	C(N(CP(=O)(O)O)CP(=O)(O)O)P(=O)(O)O	C3H12NO10P3
Phenol, tetrapropylene-	CCCC(C)C(CCC)C(C)Cc1ccc(cc1)O	C18H30O
dodecylphenol	CCCCCCCCCCCCc1c(O)cccc1	C18H30O
Tris(4-isocyanatophenyl)thiophosphate	c1cc(ccc1N=C=O)OP(=S)(Oc2ccc(cc2)N=C=O)Oc3ccc(cc3)N=C=O	C21H12N3O6PS
4-Dodecylphenol	CCCCCCCCCCCCc1ccc(cc1)O	C18H30O

p-Chlorocresol	Cc1cc(O)ccc1Cl	C7H7ClO
2,4-Di-tert-butyl-6-(5-chlorobenzotriazol-2-yl) phenol	CC(C)(C)c1cc(c(c(c1)n2nc3ccc(cc3n2)Cl)O)C(C)(C)C	C20H24ClN3O
Decan-1-ol	CCCCCCCCCCO	C10H22O
2,2'-[1,4-Cyclohexanediylbis(methyleneoxymethylene)]dioxirane	C1CC(CCC1COCC2CO2)COCC3CO3	C14H24O4
Vinyl 7,7-dimethyloctanoate	CC(C)(C)CCCCCC(=O)OC=C	C12H22O2
Stearic acid	CCCCCCCCCCCCCCCCCC(=O)O	C18H36O2
2-Naphthoic acid	c1ccc2cc(ccc2c1)C(=O)O	C22H14O4
2,2'-Dimorpholinyldiethyl-ether	C1COCCN1CCOCCN2CCOCC2	C12H24N2O3
3-(2-Hydroxy-3-methoxyphenyl)-2-[2-methoxy-4-(3-sulfopropyl)phenoxy]-1-propanesulfonic acid	COc1cccc(c1O)CC(CS(=O)(=O)O)Oc2ccc(cc2OC)CCCS(=O)(=O)O	C20H26O10S2
tetraethylenepentamine	C(CNCCNCCNCCN)N	C18H36O2
1,3,4,6-Tetrakis(hydroxymethyl)tetrahydroimidazo[4,5-d]imidazole-2,5(1H,3H)-dione	C(N1C2C(N(C1=O)CO)N(C(=O)N2CO)CO)O	C8H14N4O6
2,2,4,6,6-PENTAMETHYLHEPTANE	CC(CC(C)(C)C)CC(C)(C)C	C12H26
2-Ethylcaproic acid	CCCCC(CC)C(=O)O	C8H16O2
Adipic acid	C(CCC(=O)O)CC(=O)O	C6H10O4
Octyl adipate	CCCCCCCCOC(=O)CCCCC(=O)OCCCCCCCC	C22H42O4
Bis(6-methylheptyl) adipate	CC(C)CCCCCOC(=O)CCCCC(=O)OCCCCCC(C)C	C22H42O4
Methyltrioxitol	COCCOCCOCCO	C7H16O4
2-[2-(2-Ethoxyethoxy)ethoxy]ethanol	CCOCCOCCOCCO	C8H18O4
Tris(2-butoxyethyl) phosphate	CCCCOCCOP(=O)(OCCOCCCC)OCCOCCCC	C18H39O7P
2-{2-[2-(Dodecyloxy)ethoxy]ethoxy}ethyl hydrogen sulfate	CCCCCCCCCCCCOCCOCCOCCOS(=O)(=O)O	C18H38O7S
Butoxytriglycol	CCCCOCCOCCOCCO	C10H22O4
Diethanolamine	C(CO)NCCO	C4H11NO2
N-(m-Tolyl)-diethanolamine	Cc1cccc(c1)N(CCO)CCO	C11H17NO2
Diethylene glycol	OCCOCCO	C4H10O3
hexyl cellosolve	CCCCCCOCCO	C8H18O2
tetraethyleneglycol	OCCOCCOCCOCCO	C8H18O5
Triethylamine	CCN(CC)CC	C6H15N
2-(Dodecyloxy)ethyl hydrogen sulfate	CCCCCCCCCCCCOCCOS(=O)(=O)O	C14H30O5S
Anavenol	c1ccc2cc(ccc2c1)OCCO	C12H12O2
Hexonic acid	C(C(C(C(C(C(=O)O)O)O)O)O)O	C6H12O7
Hexyl laurate	CCCCCCCCCCCC(=O)OCCCCCC	C18H36O2
Caprylic acid	CCCCCCCC(=O)O	C24H50O7

Trimethylolpropane	CCC(CO)(CO)CO	C24H50O7
Sebacic acid	C(CCCCC(=O)O)CCCC(=O)O	C10H18O4
5-Benzyl 3-ethyl 2-methyl-6-phenyl-4-(phenylethynyl)-1,4-dihydro-3,5-pyridinedicarboxylate	CCOC(=O)C1=C(NC(=C(C1C#Cc2ccccc2)C(=O)OCc3ccccc3)c4ccccc4)C	C81H125N22O39P
Methylene bis(dibutylcarbamodithioate)	CCCCN(CCCC)C(=S)SCSC(=S)N(CCCC)CCCC	C19H38N2S4
Iodopropynyl butylcarbamate	CCCCNC(=O)OCC#CI	C8H12INO2
4,4'-{[4-(Methylimino)-2,5-cyclohexadien-1-ylidene]methylene}bis(N,N-dimethylaniline)	CN(C)C3=CC=C(C=C3)/C(C2=CC=C(N(C)C)C=C2)=C(C=C1)/C=C/C1=N/C	C24H27N3
Ethyl acetoacetate	CCOC(=O)CC(=O)C	C6H10O3
(±)-Tartaric acid	OC(C(O)C(=O)O)C(=O)O	C4H6O6
N-(Carboxymethyl)-N,N-dimethyl-1-dodecanaminium	O=C(O)C[N+](C)(C)CCCCCCCCCCCC	C16H33NO2
2-[(E)-(4-Chloro-2-nitrophenyl)diazenyl]-N-(2-chlorophenyl)-3-oxobutanamide	CC(=O)C(N=Nc1ccc(Cl)cc1[N+](=O)[O-])C(=O)Nc1ccccc1Cl	C16H12Cl2N4O4
4-Icosylbenzenesulfonic acid	CCCCCCCCCCCCCCCCCCCCc1ccc(cc1)S(=O)(=O)O	C52H90CaO6S2
Gallic acid	OC(=O)c1cc(O)c(O)c(O)c1	C7H6O5
2-Dodecylbenzenesulfonic acid	CCCCCCCCCCCCc1ccccc1S(=O)(=O)O	C18H30O3S
Toluene	Cc1ccccc1	C7H8
3-Methyl-4-[(2E)-2-(2-oxo-1(2H)-naphthalenylidene)hydrazino]benzenesulfonic acid	Cc1cc(ccc1N/N=C/2\c3ccccc3C=CC2=O)S(=O)(=O)O	C17H14N2O4S
4-[(2Z)-2-(2-Oxo-1(2H)-naphthalenylidene)hydrazino]benzenesulfonic acid	c1ccc\2c(c1)C=CC(=O)/C2=N\Nc3ccc(cc3)S(=O)(=O)O	C16H12N2O4S
3,3'-[(9,10-Dioxo-9,10-dihydroanthracene-1,4-diyl)diimino]bis(2,4,6-trimethylbenzenesulfonic acid)	Cc1cc(c(c(c1Nc2ccc(c3c2C(=O)c4ccccc4C3=O)Nc5c(cc(c(c5C)S(=O)(=O)O)C)C)C)S(=O)(=O)O)C	C32H30N2O8S2
Chlorobenzene	Clc1ccccc1	C6H5Cl
Undecylbenzene	CCCCCCCCCCCc1ccccc1	C17H28
4,4'-Methylenediphenylene diisocyanate	O=C=Nc1ccc(Cc2ccc(cc2)N=C=O)cc1	C15H10N2O2
Benzanilide	c1ccc(cc1)/C(=N/c2ccccc2)/O	C13H11NO
2-Methyldecane	CCCCCCCCC(C)C	C11H24
3,5,7-Trimethyldecane	CCCC(C)CC(C)CC(C)CC	C13H28
Dibromoacetonitrile	BrC(Br)C#N	C2HBr2N
Thioglycolic acid	C(C(=O)O)S	C2H4O2S
Glycolic acid	C(C(=O)O)O	C2H4O3
Isobutyl acetate	CC(C)COC(=O)C	C6H12O2
Butyl glycolate	CCCCOC(=O)CO	C6H12O3
Ricinoleic Acid	CCCCCC[C@@H](O)C/C=C\CCCCCCCC(=O)O	C18H34O3

N-Oleyl-1,3-propanediamine	CCCCCCCC/C=C\CCCCCCCCNCCCN	C21H44N2
Elaidic Acid	CCCCCCCC/C=C/CCCCCCCC(=O)O	C18H34O2
(9E)-9-Octadecenamide	CCCCCCCC/C=C/CCCCCCCC(=O)N	C18H35NO
1-Oleoyl-rac-glycerol	CCCCCCCC/C=C\CCCCCCCC(=O)OCC(O)CO	C21H40O4
Glycerol	C(C(CO)O)O	C21H42O5
2,4,7,9-Tetramethyl-5-Decyn-4,7-Diol	CC(C)CC(C)(C#CC(C)(CC(C)C)O)O	C14H26O2
3,6,9,12-Tetraoxatetradecan-1-ol	CCOCCOCCOCCOCCO	C10H22O5
Dcoit	CCCCCCCCn1c(=O)c(c(s1)Cl)Cl	C11H17Cl2NOS
methylisothiazolinone	Cn1c(=O)ccs1	C4H5NOS
Pentadecyl methacrylate	CCCCCCCCCCCCCCCOC(=O)C(=C)C	C19H36O2
Isobutyl methacrylate	CC(C)COC(=O)C(=C)C	C8H14O2
2-Ethylhexyl methacrylate	CCCCC(CC)COC(=O)C(=C)C	C12H22O2
2,2-Propanediylbis(4,1-phenyleneoxy-2,1-ethanediyl) bis(2-methylacrylate)	CC(=C)C(=O)OCCOc1ccc(cc1)C(C)(C)c1ccc(OCCOC(=O)C(=C)C)cc1	C27H32O6
1-Butoxy-2-propanol	CCCCOCC(C)O	C7H16O2
2-Propanol, 1-(tert-dodecylthio)-	CC(O)CSCCCCCCCCC(C)(C)C	C15H32OS
Tris(1-chloro-2-propanyl) phosphate	CC(CCl)OP(=O)(OC(C)CCl)OC(C)CCl	C9H18Cl3O4P
Dazomet	CN1CSC(=S)N(C)C1	C5H10N2S2
2-BUTANONE OXIME	CC/C(=N/O)/C	C4H9NO
butan-2-one O,O',O''-(methylsilanetriyl)oxime	N(/O[Si](O\N=C(\CC)C)(O\N=C(/C)CC)C)=C(/C)CC	C13H27N3O3Si
Maleic anhydride	O=C1OC(=O)C=C1	C4H2O3
3-(Trimethoxysilyl)-1-propanamine	CO[Si](CCCN)(OC)OC	C6H17NO3Si
3-[(8-Methylnonyl)oxy]-1-propanamine	CC(C)CCCCCCCOCCCN	C13H29NO
dehydroabietic acid	CC(C)c1cc2c(cc1)[C@@]1(C)CCC[C@](C)([C@@H]1CC2)C(=O)O	C20H28O2
5-Oxo-1-(4-sulfophenyl)-4-[(E)-(4-sulfophenyl)diazenyl]-4,5-dihydro-1H-pyrazole-3-carboxylic acid	c1cc(ccc1/N=N/C2C(=NN(C2=O)c3ccc(cc3)S(=O)(=O)O)C(=O)O)S(=O)(=O)O	C16H12N4O9S2
N-Phenyl-6-(2,4,4-trimethyl-2-pentanyl)-1-naphthalenamine	CC(C)(C)CC(C)(C)c1cc2c(cc1)c(Nc1ccccc1)ccc2	C24H29N
2-(2-heptadec-1-enyl-2-imidazolin-1-yl)ethanol	CCCCCCCCCCCCCCC/C=C/C1=NCCN1CCO	C22H42N2O
3,4,5,6-Tetrachloro-N-(2-(4,5,6,7-tetrachloro-2,3-dihydro-1,3-dioxo-1H-inden-2-yl)-8-quinolyl)phthalimide	Clc1c(Cl)c(Cl)c(Cl)c2c1C(=O)C(C2=O)c1nc2c(cccc2N2C(=O)c3c(C2=O)c(Cl)c(Cl)c(Cl)c3Cl)cc1	C26H6Cl8N2O4
(Z)-2-(8-Heptadecenyl)-2-imidazoline-1-ethanol	CCCCCCCC/C=C\CCCCCCCC1=NCCN1CCO	C22H42N2O
tolytriazole	Cc1cccc2n[nH]nc12	C7H7N3
1,2,3-Benzotriazole	[nH]1nc2ccccc2n1	C6H5N3
2-(Hydroxymethyl)-2-nitropropan-1,3-diol	OCC(CO)(CO)[N+](=O)[O-]	C4H9NO5

8-Amino-1,3,6-naphthalenetrisulfonic acid	c1c2cc(cc(c2c(cc1S(=O)(=O)O)N)S(=O)(=O)O)S(=O)(=O)O	C10H9NO9S3
3-Methoxybutyl acetate	COC(C)CCOC(=O)C	C7H14O3
Irgarol	CSc1nc(NC(C)(C)C)nc(NC2CC2)n1	C11H19N5S
Propylene carbonate	CC1COC(=O)O1	C4H6O3
5-[(2-Methyl-2-undecanyl)disulfanyl]-1,3,4-thiadiazole-2(3H)-thione	CCCCCCCCCC(C)(C)SSc1n[nH]c(=S)s1	C14H26N2S4
N-{3-[Dimethoxy(methyl)silyl]propyl}-1,2-ethanediamine	CO[Si](C)(CCCNCCN)OC	C8H22N2O2Si
glycol diacetate	CC(=O)OCCOC(=O)C	C6H10O4
(3-Trimethoxysilylpropyl)diethylenetriamine	CO[Si](CCCNCCNCCN)(OC)OC	C10H27N3O3Si
Benzisothiazolone	O=c1[nH]sc2c1cccc2	C7H5NOS
Diisononylphthalate	CC(C)CCCCCCOC(=O)c1c(cccc1)C(=O)OCCCCCCC(C)C	C26H42O4
Bis(5-methylhexyl) phthalate	CC(C)CCCCOC(=O)c1ccccc1C(=O)OCCCCC(C)C	C22H34O4
Benzyl butyl phthalate	CCCCOC(=O)c1c(cccc1)C(=O)OCc1ccccc1	C19H20O4
Diisobutyl phthalate	CC(C)COC(=O)c1c(cccc1)C(=O)OCC(C)C	C16H22O4
Tributyl citrate acetate	CCCCOC(=O)CC(CC(=O)OCCCC)(OC(=O)C)C(=O)OCCCC	C20H34O8
Tridecyl hydrogen sulfate	CCCCCCCCCCCCCOS(=O)(=O)O	C14H31O4S
n-butylamine	CCCCN	C17H14N2O4S
2-Ethylpentyl 3-[4-hydroxy-3,5-bis(2-methyl-2-propanyl)phenyl]propanoate	CCCC(CC)COC(=O)CCc1cc(c(c(c1)C(C)(C)C)O)C(C)(C)C	C25H42O3
4,4'-Diamino-1,1'-bianthracene-9,9',10,10'-tetrone	c1ccc2c(c1)C(=O)c3c(ccc(c3C2=O)N)c4ccc(c5c4C(=O)c6ccccc6C5=O)N	C28H16N2O4
Octyltriethoxysilane	CCCCCCCC[Si](OCC)(OCC)OCC	C14H32O3Si
O-(1,3-dimethylbutyl) O-isopropyl hydrogen dithiophosphate	CC(C)CC(C)OP(=S)(OC(C)C)S	C9H20O2PS2
2-(2-Methoxyethoxy)ethanol	COCCOCCO	C5H12O3
Benzyl benzoate	c1ccc(cc1)COC(=O)c2ccccc2	C14H12O2
p-Toluenesulfonyl isocyanate	Cc1ccc(cc1)S(=O)(=O)N=C=O	C8H7NO3S

Tab.A1.2: List of substances in the internal standard (IS) mixture with a concentration of 1 µg mL-1

1H-Benzotriazole-d4
4-BP-d4
4-Nonylphenol-d4
Acetaminophen-d4
Atenolol-d7
Atorvastatin-d5
Azithromycin-d3
Benzophenone-d10
Benzyl butyl phthalate-d4
Bezafibrate-d4
Bisphenol A 13C12
Caffeine-13C3
Ciprofloxacin-d8
Citalopram-d4
Codeine-d3
Cyclophosphamide-d4
DEET-d10
Diazepam-d5
Dibutyl phthalate-d4
Diclofenac-13C6
Diethyl phthalate-d4
Diltiazem-d4
EHMC-d15
Erythromycin-d3-13C
Fluoxetine-d5
Furosemide-d5
Heroine-d9
Hydrochlorothiazide-d2-13C
Ibuprofen-d3
Irbesartan-d7
Isoproturon-d3
Lidocaine-d10
Losartan-d4
Mefenamic Acid 13C6
Metronidazole-d4
Morphine-d3
Naproxen-d3
Octorylene-d10
Ofloxacin-d3
Oxazepam-d5
Oxybenzone-d5
Propylparaben-d7
Ranitidine-d6
Sertraline-d3

Sucralose-d6
Sulfamethoxazole-d4
Tamoxifen-13C2,15N
TCEP-d12
TEP-d15
TPHP-d15
TPP-d21
Tramadol-13C,d3
Trimethoprim-d9
Valproic acid d6
Venlafaxine-d6
EtFOSAA-d5
MeFOSAA-d3
perfluorobutanoic acid (PFBA)-13C4
perfluorodecanoic acid (PFDA)-13C2
perfluorododecanoic acid (PFDoDA)-13C2
perfluorohexane sulfonic acid (PFHxS)-18O2
perfluorohexanoic acid (PFHxA)-13C2
perfluorononanoic acid (PFNA)-13C5
perfluorooctane sulfonamide (FOSA)-13C8
perfluorooctane sulfonic acid (PFOS)-13C4
perfluorooctanoic acid (PFOA)-13C4
perfluoroundecanoic acid (PFUnDA)-13C2

Tab.A1.3: Target analyte names, CAS numbers, molecular formulas, molecular weights, and log K_{ow} values

Category	Compound	CAS number	Molecular formula	MW (g mol $^{-1}$)	log K_{ow}[a]
Pharmaceuticals (Antibiotics)	Azithromycin	83905-01-5	C38H72N2O12	748.5	4.02
	Ciprofloxacin	85721-33-1	C17H18FN3O3	331.1	0.28
	Clarithromycin	81103-11-9	C38H69NO13	747.5	3.16
	Erythromycin	114-07-8	C37H67NO13	733.4	-
	Metronidazole	443-48-1	C6H9N3O3	171.1	0.02
	Norfloxacinirb	70458-96-7	C16H18FN3O3	319.1	-1.03
	Ofloxacin	82419-36-1	C18H20FN3O4	361.1	-2.00
	Roxithromycin	80214-83-1	C41H76N2O15	836.5	-
	Sulfamethoxazole	723-46-6	C10H11N3O3S	253.0	0.89
	Tetracycline	64-75-5	C22H24N2O8	480.8	-1.47
	Trimethoprim	738-70-5	C14H18N4O3	290.1	0.91
Pharmaceuticals (analgesics)	Acetaminophen (Paracetamol)	103-90-2	C8H9NO2	151.1	0.46
Pharmaceuticals (Anesthetics)	Lidocaine	137-58-7	C14H22N2O	234.3	1.66
Pharmaceuticals (Antidepressants)	Amitriptylline	50-48-7	C20H23N	277.4	3.95
	Citalopram	59729-33-9	C20H21FN2O	324.4	3.74
	Desvenlafaxine	93413-62-8	C16H25NO2	263.1	2.72
	Fluoxetine	54910-89-4	C17H18F3NO	309.3	4.65
	Sertraline	79617-96-3	C17H17Cl2N	306.2	5.29
	Venlafaxine	93413-69-6	C17H27NO2	277.4	3.28
Pharmaceuticals (Antiepileptics)	Carbamazepine	298-46-5	C15H12N2O	236.3	2.25
	Lamotrigine	84057-84-2	C9H7Cl2N5	256.1	0.99
Pharmaceuticals (Antihypertensives)	Atenolol	29122-68-7	C14H22N2O3	266.2	0.16
	Diltiazem	42399-41-7	C22H26N2O4S	414.1	2.79
	Irbesartan	138402-11-6	C25H28N6O	428.2	5.31
	Losartan	114798-26-4	C22H23ClN6O	422.2	4.01
	Metoprolol	51384-51-1	C15H25NO3	267.2	1.88

	Valsartan	137862-53-4	C24H29N5O3	435.2	3.65
Pharmaceuticals (Antilipidemic agents)	Atorvastatin	134523-00-5	C33H35FN2O5	558.2	4.13
Pharmaceuticals (Antiulcers drugs)	Omeprazole	73590-58-6	C17H19N3O3S	345.1	2.23
	Ranitidine	66357-35-5	C13H22N4O3S	314.1	0.27
Pharmaceuticals (Antifungal)	Climbazole	38083-17-9	C15H17ClN2O2	292.0	3.76
	Fluconazole	86386-73-4	C13H12F2N6O	306.1	0.25
	2-mercapto benzothiazole (MBT)	149-30-4	C7H5NS2	167.2	2.38
Pharmaceuticals (Benzodiazepines)	Diazepam	439-14-6	C16H13ClN2O	284.7	2.70
	Oxazepam	604-75-2	C15H11ClN2O2	286.7	3.34
Pharmaceuticals (Beta blocking agents)	Propranolol	525-66-6	C16H21NO2	259.1	2.60
	Sotalol	3930-20-9	C12H20N2O3S	272.1	0.37
Pharmaceuticals (Diuretics)	Furosemide	54-31-9	C12H11ClN2O5S	330.0	2.03
	Hydrochlorothiazide	58-93-5	C7H8ClN3O4S2	297.0	-0.07
Pharmaceuticals (Lipid lowering agent)	Bezafibrate	41859-67-0	C19H20ClNO4	361.1	4.25
Pharmaceuticals (NSAIDs)	Diclofenac	15307-86-5	C14H11Cl2NO2	295.0	4.51
	Meclofenamic acid	644-62-2	C14H11Cl2NO2	295.0	6.02
	Mefenamic acid	61-68-7	C15H15NO2	241.1	5.12
	Niflumic acid	4394-00-7	C13H9F3N2O2	282.1	4.43
Artificial sweetener	Sucralose	56038-13-2	C12H19Cl3O8	396.0	-1.00
	Acesulfame	33665-90-6	C4H5NO4S	163.1	-0.32
Illicit drugs	Cocaine (COC)	50-36-3	C17H21NO4	303.2	2.17
Personal care products (Insect repellents)	DEET (diethyltoluamide)	134-62-3	C12H17NO	191.1	2.26
Personal care products (Parabens)	Ethylparaben	120-47-8	C9H10O3	166.0	2.49
	Methylparaben	99-76-3	C8H8O3	152.0	2.00
	Propylparaben	94-13-3	C10H12O3	180.0	2.98

Personal care products (Sunscreens)	Octocrylene	6197-30-4	C24H27NO2	361.2	6.88
Pesticides	Isoproturon	34123-59-6	C12H18N2O	206.1	2.84
	Terbutryn	886-50-0	C10H19N5S	241.1	3.77
	BAM (Dichlorobenzamide)	2008-58-4	C7H5Cl2NO	189.0	0.90
PFAS	perfluorobutane sulfonic acid (PFBS)	375-73-5	C4HF9O3S	300.1	2.41
	perfluorobutanoic acid (PFBA)	375-22-4	C4HF7O2	214.0	2.43
	perfluorodecanoic acid (PFDA)	335-76-2	C10HF19O2	514.0	-
	perfluorododecanoic acid (PFDoDA)	307-55-1	C12HF23O2	614.0	-
	perfluoroheptanoic acid (PFHpA)	375-85-9	C7HF13O2	364.0	5.33
	perfluorohexane sulfonic acid (PFHxS)	355-46-4	C6HF13O3S	400.0	4.34
	perfluorohexanoic acid (PFHxA)	307-24-4	C6HF11O2	314.0	4.37
	perfluorononanoic acid (PFNA)	375-95-1	C9HF17O2	464.1	7.27
	perfluorooctane sulfonamide (FOSA)	754-91-6	C8H2F17NO2S	500.0	7.58
	perfluorooctane sulfonic acid (PFOS)	1763-23-1	C8HF17O3S	500.0	-
	perfluorooctanoic acid (PFOA)	335-67-1	C8HF15O2	414.0	6.30
	perfluoropentanoic acid (PFPeA)	2706-90-3	C5HF9O2	264.0	3.40
	perfluorotetradecanoic acid (PFTeDA)	376-06-7	C14HF27O2	714.0	-
	perfluoroundecanoic acid (PFUnDA)	2058-94-8	C11HF21O2	564.0	-
Opiates, opioids, and metabolites	Codeine (COD)	76-57-4	C18H21NO3	299.4	1.28
	Oxycodone (OC)	76-42-7	C18H21NO4	315.4	0.66

	Tramadol	46941-76-8	C16H25NO2	263.4	-
Stimulants	Caffeine	8/2/1958	C8H10N4O2	194.1	-0.07
Flame retardants	2-hydroxybenzothiazole (OHBT)	934-34-9	C7H5NOS	151.2	2.28
	4-Bromophenol	106-41-2	C6H5BrO	173.0	2.49
	α-HBCD	678970-15-5	C12H18Br6	641.7	6.63
	β-HBCD	134237-51-7	C12H18Br6	641.7	6.63
	γ-HBCD	134237-52-8	C12H18Br6	641.7	6.63
Phthalate	diethyl phthalate (DEP)	84-66-2	C12H14O4	222.237	2.70
	monobenzyl phthalate (MP)	2528-16-7	C15H12O4	256.253	2.90
UV filters	benzothiazole (BT)	95-16-9	C7H5NS	135.2	2.01
	benzothiazole-2-sulfonic acid (BTSA)		C7H5NO3S2	215.2	1.67

Tab.A1.4: Method performance with target compounds, their corresponding Internal Standard (IS), electrospray mode and methodological Limit of Detection (MLOD)

Compound	Corresponding Internal Standard (IS)	ESI	MLOD [ng L^{-1}]
2-hydroxybenzothiazole (OHBT)		-	
2-mercaptobenzothiazole (MBT)		-	
4-Bromophenol		-	
Acesulfame		-	
Atorvastatin	Atorvastatin-D5	-	1.0
benzothiazole (BT)		-	
benzothiazole-2-sulfonic acid (BTSA)		-	
Bezafibrate	Bezafibrate-D4	-	1.0
Diclofenac	Diclofenac-13C6	-	2.0
diethyl phthalate (DEP)		-	
Ethylparaben	Propylparaben-D7	-	5.0
FOSA	FOSA-M8	-	0.1
Furosemide	Furosemide-D5	-	6.0
Hydrochlorothiazide	Hydrochlorothiazide-D2-13C	-	1.0
Irbesartan	Irbesartan-D7	-	5.0
Losartan	Losartan-D4	-	10
Meclofenamic acid	Mefenamic Acid 13C6	-	5.0
Mefenamic Acid	Mefenamic Acid 13C6	-	10
Methylparaben	Propylparaben-D7	-	5.0
monobenzyl phthalate (MP)		-	
Niflumic acid	Mefenamic Acid 13C6	-	1.0
PFBA	PFBA-13C4	-	10
PFBS	PFHxS-18O2	-	1.0
PFDA	PFDA-13C2	-	1.0
PFDoDA	PFDoDA-13C2	-	0.2
PFHpA	PFOA-13C4	-	1.0
PFHxA	PFHxA-13C2	-	1.0
PFHxS	PFHxS-18O2	-	1.0
PFNA	PFNA-13C5	-	1.0

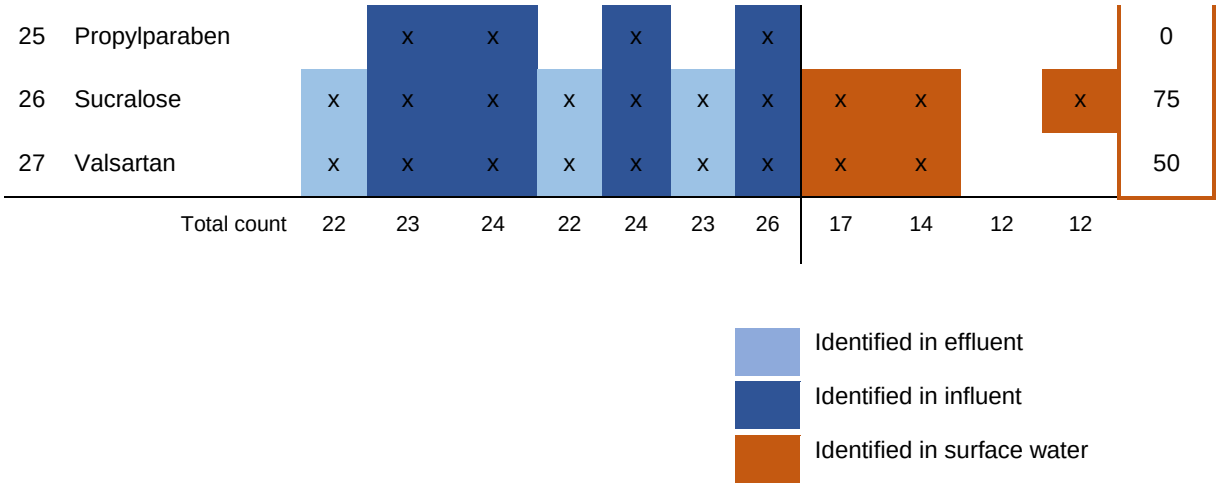
PFOA	PFOA-13C4	-	1.0
PFOS	PFOS-13C4	-	1.0
PFPeA	PFHxA-13C2	-	10
PFTeDA	PFDoDA-13C2)	-	1.0
PFUnDA	PFUnDA-13C2	-	1.0
Propylparaben	Propylparaben-D7	-	5.0
Sucralose	Sucralose-D6	-	10
Valsartan	Irbesartan-D7	-	20
α-HBCD		-	
β-HBCD		-	
γ-HBCD		-	
Acetaminophen	Acetaminophen-D4	+	3.0
Amitriptyline	Carbamazepine-(carboxamide-13C,15N)	+	0.5
Atenolol	Atenolol-D7	+	0.1
Azithromycin	Erythromycin-D3-13C	+	5.0
BAM	DEET-D10	+	10
Caffeine	Caffeine-13C3	+	1.0
Carbamazepine	Carbamazepine-(carboxamide-13C,15N)	+	0.5
Citalopram	Oxazepam-D5	+	1.0
Ciprofloxacin	Ciprofloxacin-D8	+	5.0
Clarithromycin	Erythromycin-D3-13C	+	1.0
Climbazole	Metronidazole-D4	+	0.5
Cocaine	Codeine-D3	+	1.0
Codeine	Codeine-D3	+	1.0
DEET	DEET-D10	+	0.2
Desvenlafaxine	Venlafaxine-D6	+	1.0
Diazepam	Diazepam-D5	+	1.0
Diltiazem	Diltiazem-D4	+	0.5
Erythromycin	Erythromycin-D3-13C	+	1.0
Fluconazole	Metronidazole-D4	+	2.0
Fluoxetine	Fluoxetine-D5	+	0.5

Isoproturon	Isoproturon-D3	+	1.0
Lamotrigine	Lidocaine-(diethyl)-D10	+	0.3
Lidocaine	Lidocaine-(diethyl)-D10	+	0.2
Metoprolol	Atenolol-D7	+	0.1
Metronidazole	Metronidazole-D4	+	5.0
Norfloxacin	Ofloxacin-D3	+	5.0
Octocrylene		+	
Ofloxacin	Ofloxacin-D3	+	5.0
Omeprazole	Metronidazole-D4	+	5.0
Oxazepam	Oxazepam-D5	+	2.0
Oxycodone	Codeine-D3	+	0.5
Propranolol	Atenolol-D7	+	0.1
Ranitidine	Ranitidine-D6	+	2.5
Roxithromycin	Erythromycin-D3-13C	+	1.0
Sertraline	Cis-Sertraline-D3	+	1.0
Sotalol	Atenolol-D7	+	0.5
Sulfamethoxazole	Sulfamethoxazole-D4	+	5.0
Terbutryn	Isoproturon-D3	+	0.3
Tetracycline		+	
Tramadol	Tramadol-D3-13C	+	0.5
Trimethoprim	Trimethoprim-D9	+	0.1
Venlafaxine	Venlafaxine-D6	+	1.0

A2: Results

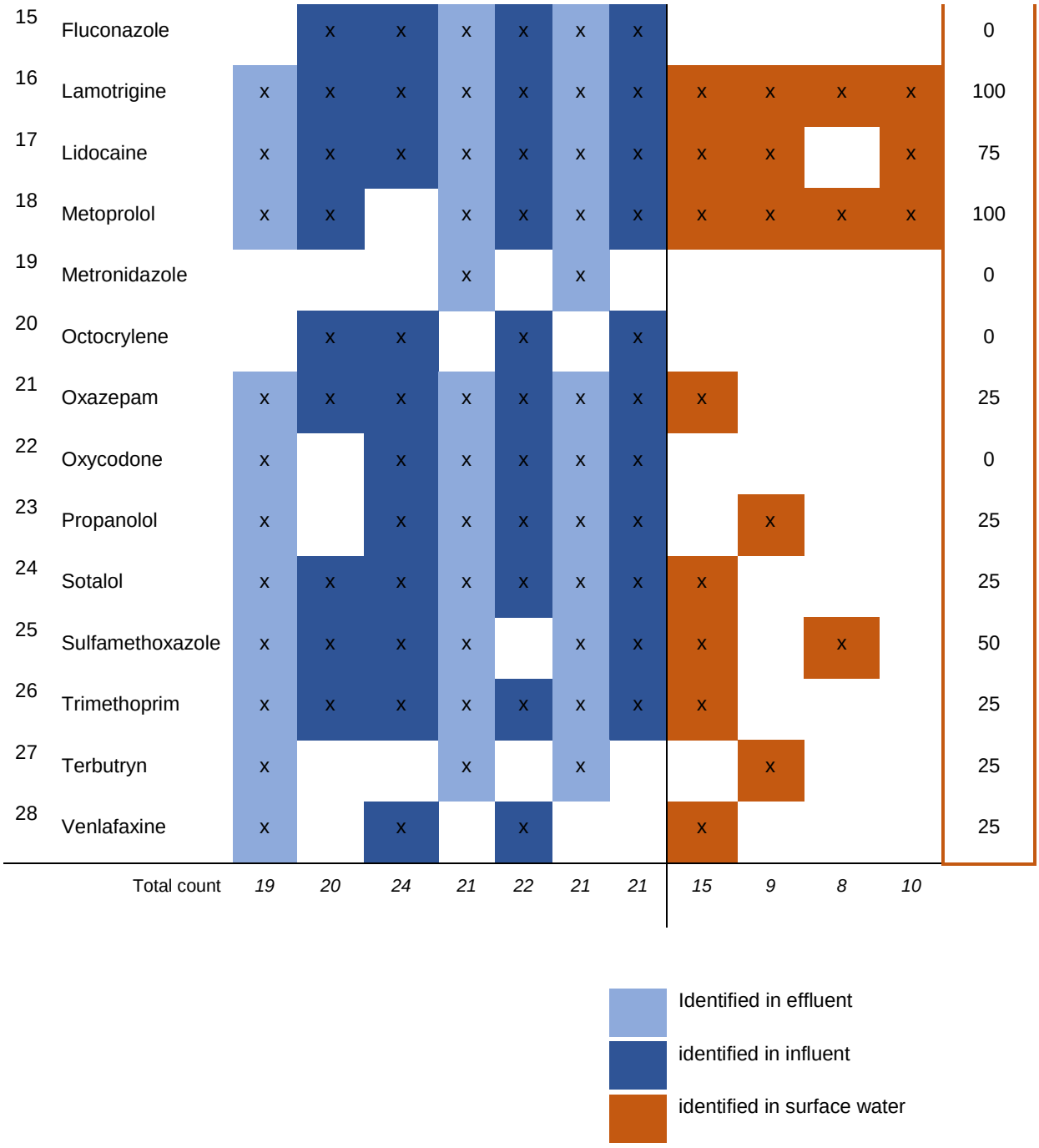
Tab.A2.1: Identified target analytes in WW and surface water samples in NI with total count of compounds in each sample and the proportion [%] of identification within the surface water samples

	Target Compounds	U_{eff}	$U_{inf(I)}$	$U_{inf(U)}$	S_{eff}	S_{inf}	V_{eff}	V_{inf}	U_{river}	S_{river}	V_{river}	V_{lake}	Share [%]
1	Acesulfame	x	x	x	x	x	x	x	x	x	x	x	100
2	Atorvastatin	x	x	x	x	x	x	x					0
3	Bezafibrate	x	x	x	x	x	x	x	x				25
4	BT	x	x	x	x	x	x	x	x	x	x	x	100
5	BTSA	x	x	x	x	x	x	x	x	x	x	x	100
6	DEP						x	x					0
7	Diclofenac	x	x	x	x	x	x	x	x				25
8	Ethylparaben		x	x		x		x					0
9	Furosemide	x	x	x	x	x	x	x	x				25
10	Hydrochlorothiazide	x	x	x	x	x	x	x	x				25
11	Irbesartan	x	x	x	x	x	x	x					0
12	Losartan	x	x	x	x	x	x	x	x				25
13	Methylparaben		x	x		x		x					0
14	OHBT	x	x	x	x	x	x	x	x	x	x	x	100
15	PFBS	x	x	x	x	x	x	x	x	x	x	x	100
16	PFBA	x	x	x	x	x	x	x					0
17	PFDA									x	x	x	75
18	PFHpA	x	x	x	x	x	x	x	x	x	x	x	100
19	PFHxS	x	x	x	x	x	x	x	x	x	x	x	100
20	PFHxA	x		x	x	x	x	x					0
21	PFNA	x			x		x	x	x	x	x	x	100
22	PFOS	x	x	x	x	x	x	x	x	x	x	x	100
23	PFOA	x	x	x	x	x	x	x	x	x	x	x	100
24	PFPeA	x	x	x	x	x	x	x		x	x		50



Tab.A2.2: Identified target analytes in WW and surface water samples in PI with total count of compounds in each sample and the proportion [%] of identification among the surface water samples

	Target compounds	U_{eff}	$U_{inf(I)}$	$U_{inf(U)}$	S_{eff}	S_{inf}	V_{eff}	V_{inf}	U_{river}	S_{river}	V_{river}	V_{lake}	Share [%]
1	Acetaminophen		x	x		x		x	x	x	x	x	100
2	Amitryptilline	x	x	x	x	x	x	x					0
3	Atenolol	x	x	x	x	x	x	x	x		x	x	75
4	Caffeine		x	x	x	x	x	x	x	x	x	x	100
5	Carbamazepine	x	x	x	x	x	x	x	x	x	x	x	100
6	Ciprofloxacin					x							0
7	Citalopram	x	x	x	x	x	x	x	x			x	50
8	Clarithromycin			x									0
9	Climbazole	x	x	x	x	x	x	x					0
10	Cocaine			x		x		x					0
11	Codeine	x	x	x	x	x	x	x					0
12	DEET	x	x	x	x		x		x			x	50
13	Desvenlafaxine	x	x	x	x	x	x	x	x	x	x	x	100
14	Diltiazem		x	x									0


15 Fluconazole 0
16 Lamotrigine 100
17 Lidocaine 75
18 Metoprolol 100
19 Metronidazole 0
20 Octocrylene 0
21 Oxazepam 25
22 Oxycodone 0
23 Propanolol 25
24 Sotalol 25
25 Sulfamethoxazole 50
26 Trimethoprim 25
27 Terbutryn 25
28 Venlafaxine 25
Total count 19 20 24 21 22 21 21 15 9 8 10
Identified in effluent
identified in influent
identified in surface water

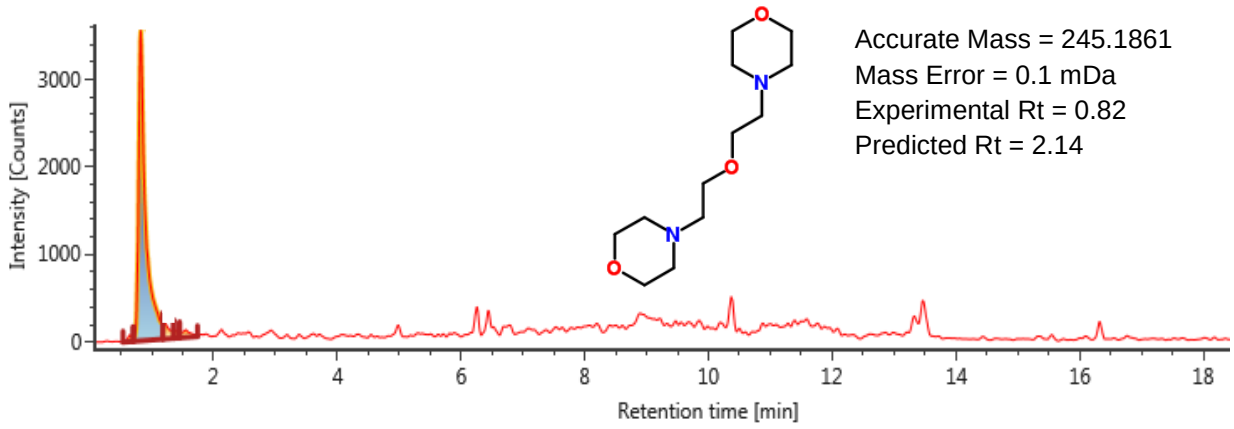
Tab.A2.3: Suspect analytes in surface water samples complying with the mass of interest (confidence level 5) but with no additional evidences, NI.

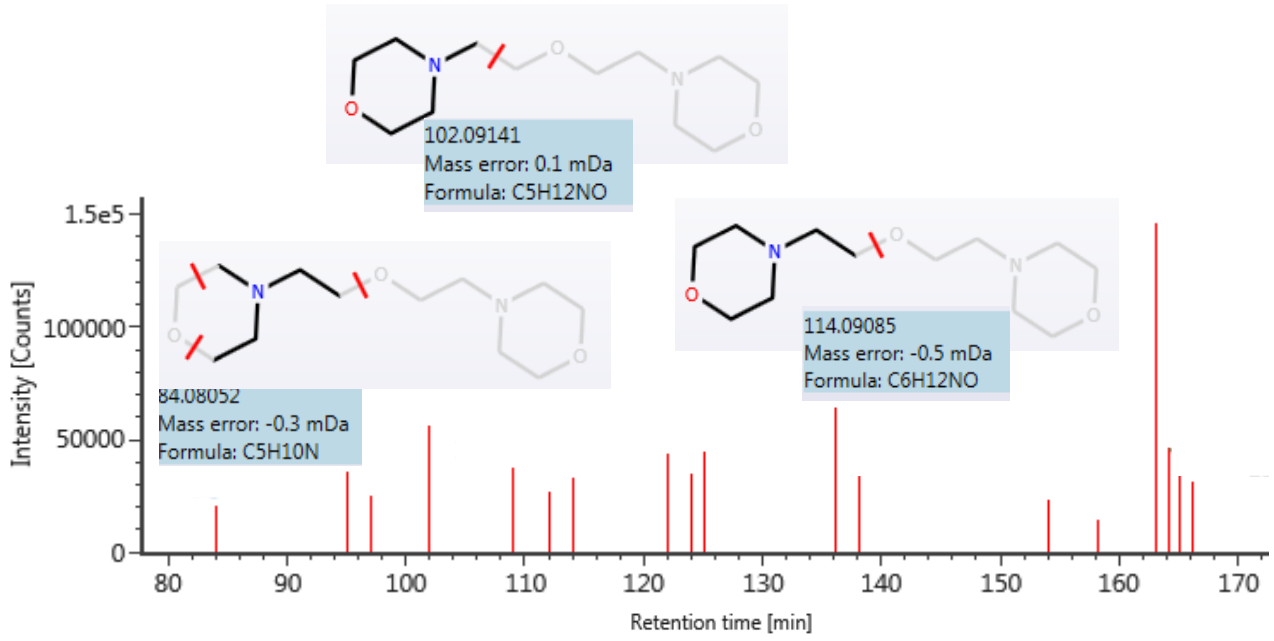
Compound Name	Molecular formula
(±)-Tartaric acid	C4H6O6
2-[(E)-(4-Chloro-2-nitrophenyl)diazenyl]-N-(2-chlorophenyl)-3-oxobutanamide	C16H12Cl2N4O4
2,2'-[1,4-Cyclohexanediylbis(methyleneoxymethylene)] dioxirane	C14H24O4
2,2,4-Trimethyl-1,3-Pentanediol 1-Isobutyrate	C12H24O3
2,4,7,9-Tetramethyl-5-Decyn-4,7-Diol	C14H26O2
5-[(2-Methyl-2-undecanyl)disulfanyl]-1,3,4-thiadiazole-2(3H)-thione	C14H26N2S4
Adipic Acid	C6H10O4
Benzisothiazolone	C7H5NOS
Bis(6-methylheptyl) adipate	C22H42O4
Dazomet	C5H10N2S2
Dehydroabietic acid	C20H28O2
Diisobutyl phthalate	C16H22O4
Dipropylene glycol dibenzoate	C20H22O5
Ethylsilanetriyl triacetate	C8H14O6Si
Methylene bis(dibutylcarbamodithioate)	C19H38N2S4
O-(1,3-dimethylbutyl) O-isopropyl hydrogen dithiophosphate	C9H21O2PS2
O-sec-butyl O-(1,3-dimethylbutyl) hydrogen dithiophosphate	C10H23O2PS2
Pentadecyl methacrylate	C19H36O2
Ricinoleic Acid	C18H34O3
Tris(2-butoxyethyl) phosphate	C18H39O7P
Vinyl 7,7-dimethyloctanoate	C12H22O2

Tab.A2.4: Suspect analytes in surface water samples complying with the mass of interest (confidence level 5) but with no additional evidences, PI.

Compound Name	**Molecular formula**
2,4,7,9-Tetramethyl-5-Decyn-4,7-Diol	C14H26O2
2-BUTANONE OXIME	C4H9NO
Adipic Acid	C6H10O4
Butyl glycolate	C6H12O3
3-(Trimethoxysilyl)-1-propanamine	C6H17NO3Si
3,3'-[(9,10-Dioxo-9,10-dihydroanthracene-1,4-diyl)diimino]bis(2,4,6-trimethylbenzenesulfonic acid)	C32H30N2O8S2
Diisobutyl phthalate	C16H22O4
Diuron	C9H10Cl2N2O
Kitazin	C13H21O3PS
O,O-diheptyl hydrogen dithiophosphate	C14H31O2PS2
Pentadecyl methacrylate	C19H36O2
Sorbitol	C6H14O6

Fig.A2.1: Complete identification methodology including (a) MS spectra according to applied reference values (b) MS/MS spectra and corresponding fragments and (c) confirmation step with reference standard for 2,2-Dimorpholinyldiethyl-ether.





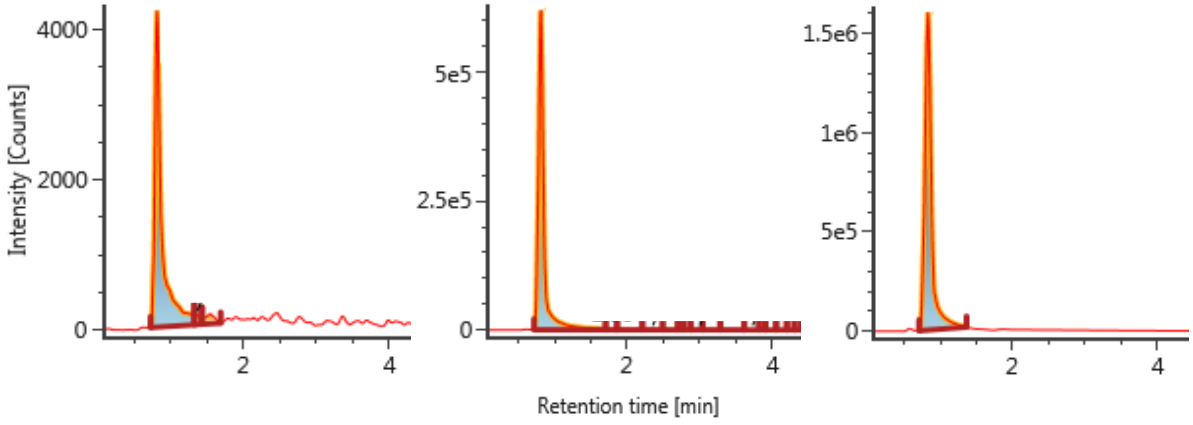
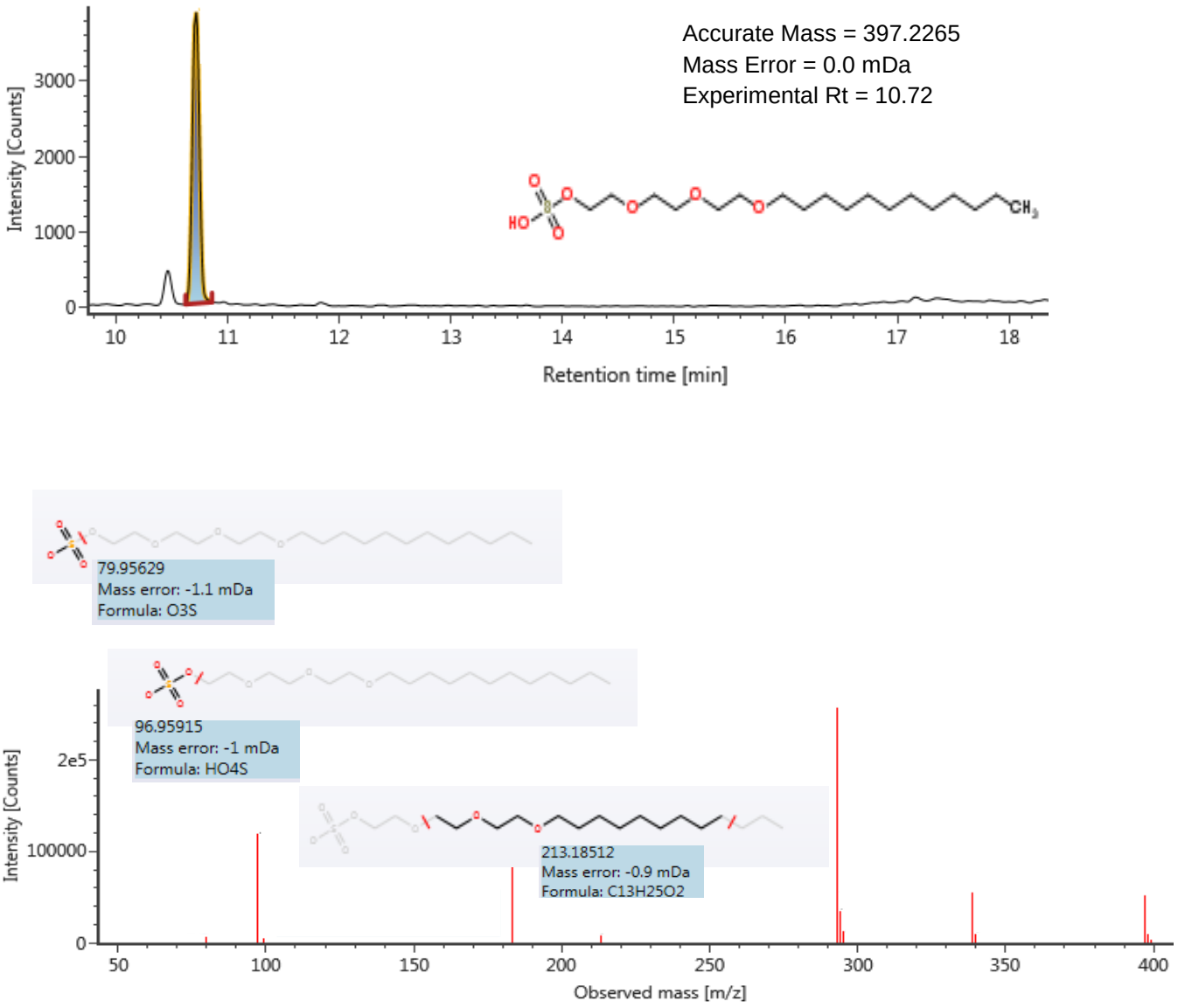


Fig.A2.2: Complete identification methodology including MS spectra, applied reference values and fragments of 2-{2-[2-(Dodecyloxy)ethoxy]ethoxy}ethyl hydrogen sulfate. No reference standard was available leaving the compound at level 2 of identification confidence.



A3: Discussion

Tab.A3.1: Toxicity values calculated based on the ECOSAR prediction model. Estimates are given for fish, daphnia magna and green algae at 96h, respectively 48h of exposure.

Compounds' name	Recipient	Reference unit[1]	Toxicity value [mg/L][2]
(9E)-9-Octadecenamide	Fish	LC50 (96h)	0.053
	Daphnia	LC50 (48h)	0.007
	Green Algae	EC50 (96h)	0.004
	Fish	ChV	0.00129
	Daphnia	ChV	0.008
	Green Algae	ChV	0.027
1,2,3-Benzotriazole	Fish	LC50 (96h)	28.321
	Daphnia	LC50 (48h)	66.766
	Green Algae	EC50 (96h)	5.904
	Fish	ChV	4.615
	Daphnia	ChV	3.859
	Green Algae	ChV	2.715
2-{2-[2-(Dodecyloxy)ethoxy]ethoxy} ethyl hydrogen sulfate	Fish	LC50 (96h)	750.915
	Daphnia	LC50 (48h)	410.861
	Green Algae	EC50 (96h)	262.568
	Fish	ChV	70.259
	Daphnia	ChV	36.141
	Green Algae	ChV	63.314
2-(Dodecyloxy)ethyl hydrogen sulfate	Fish	LC50 (96h)	188.025
	Daphnia	LC50 (48h)	108.225
	Green Algae	EC50 (96h)	85.283
	Fish	ChV	18.676
	Daphnia	ChV	10.962
	Green Algae	ChV	23.021
2,2'-Dimorpholinyldiethyl-ether	Fish	LC50 (96h)	7040.513
	Daphnia	LC50 (48h)	571.003
	Green Algae	EC50 (96h)	1009.315
	Fish	ChV	1325.720
	Daphnia	ChV	32.256
	Green Algae	ChV	253.842
2-Dodecylbenzenesulfonic acid	Fish	LC50 (96h)	8.469
	Daphnia	LC50 (48h)	6.218
	Green Algae	EC50 (96h)	13.410
	Fish	ChV	1.121
	Daphnia	ChV	1.240
	Green Algae	ChV	6.225
Benzoic acid	Fish	LC50 (96h)	1300.781
	Daphnia	LC50 (48h)	730.075
	Green Algae	EC50 (96h)	518.374
	Fish	ChV	125.419
	Daphnia	ChV	68.937
	Green Algae	ChV	132.290
butan-2-one O,O',O''-(methylsilanetriyl)oxime	Neutral Organics*		
Di-(2-ethylhexyl)phosphoric acid	Neutral Organics*		
Dibutyl phosphate	Neutral Organics*		
Dimethyl octadecylphosphonate	Fish	LC50 (96h)	0.0021
	Daphnia	LC50 (48h)	0.023

	Green Algae	EC50 (96h)	0.004
	Fish	ChV	0.000468
	Daphnia	ChV	0.003
	Green Algae	ChV	0.007
Laurilsulfate	Fish	LC50 (96h)	0.0021
	Daphnia	LC50 (48h)	0.023
	Green Algae	EC50 (96h)	0.004
	Fish	ChV	0.000468
	Daphnia	ChV	0.003
	Green Algae	ChV	0.007
Oleic acid	Neutral Organics*		
Sebacic acid	Neutral Organics*		
Stearic acid	Neutral Organics*		
Sulisobenzone	Fish	LC50 (96h)	9336.408
	Daphnia	LC50 (48h)	1358.373
	Green Algae	EC50 (96h)	7322.173
	Fish	ChV	742.008
	Daphnia	ChV	259.097
	Green Algae	ChV	3511.742
Tetraethyleneglycol	Neutral Organics*		
Tributyl citrate acetate	Fish	LC50 (96h)	2.488
	Daphnia	LC50 (48h)	4.049
	Green Algae	EC50 (96h)	1.200
	Fish	ChV	0.116
	Daphnia	ChV	1.436
	Green Algae	ChV	0.646
Tridecyl hydrogen sulfate	Neutral Organics*		
Tris(2-butoxyethyl) phosphate	Fish	LC50 (96h)	13.976
	Daphnia	LC50 (48h)	26.115
	Green Algae	EC50 (96h)	9.494
	Fish	ChV	0.853
	Daphnia	ChV	13.412
	Green Algae	ChV	3.362
Tolytriazole	Fish	LC50 (96h)	16.386
	Daphnia	LC50 (48h)	36.053
	Green Algae	EC50 (96h)	3.851
	Fish	ChV	2.133
	Daphnia	ChV	1.941
	Green Algae	ChV	1.763

[1] LC50=Median concentration associated with 50% mortality after the given exposure
EC50=Median concentration associated with effects on 50% of the organisms
ChV=Chronic toxicity value

[2] Acute toxicity concern concentration is the lowest toxicity value divided by an uncertainty factor of 10. Highlighted in red are the toxicity values of high concern corresponds to an estimate < 1mg/L

* Estimates provided below the Neutral Organics QSAR equations which represents the baseline toxicity potential (minimum toxicity) assuming a simple non-polar narcosis model. Without empirical data on structurally similar chemicals, it is uncertain if this substance will present significantly higher toxicity above baseline estimates.