Integrated Masters in Bioengineering

Synthetic musks in personal care products: method development and exposure assessment

Master's Thesis

of

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Laboratory for Process, Environmental and Energy Engineering,
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Abstract

Synthetic musks are organic ingredients used as an alternative for the natural musks in a wide variety of personal care products. They have a pleasant odour and can be divided in four different musk families: nitro, polycyclic, macrocyclic and alicyclic musks.

Due to their widespread use, these synthetic compounds turned up in different environmental compartments, such as water, sludge, sediments, human and animal tissues. However, little is known about their distribution and occurrence in personal care and household products. This information would enable an assessment of the source of exposure to the environment.

The concentrations and distributions of 12 synthetic musks (musks xylene, ketone, moskene, tibetene, ambrette, galaxolide, tonalide, cashmeran, celestolide, phantolide, exaltolide and ethylene brassylate) were investigated in five different product categories: skin moisturisers, toothpastes, deodorants, toilet soaps, body and hair washes. To extract the musk compounds from the personal care products, an innovative methodology was used and optimized. QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method, which combines extraction/isolation with cleanup, was first developed to extract pesticides from food matrices. To the author's knowledge, musks extraction from cosmetics has never been performed using this method. The final extraction conditions used were: 0.5 g of sample amount, 3 mL of acetonitrile as extraction solvent, a stream of nitrogen was used to pre-concentrate de extracts, as well as, masses of 2400 mg $MgSO_4$ + 750 mg $NaCH_3COO$, and 180 mg $MgSO_4$ + 60 mg PSA + 30 mg C18. The extraction using this method takes 45 minutes. Samples were analysed in GC-MS using SIS mode.

LODs varied between 2.15 x 10^{-5} (galaxolide) and 5.00 x 10^{-3} µg/g (tonalide), while LOQs varied between 7.17 x 10^{-5} (galaxolide) and 1.67 x 10^{-2} µg/g (tonalide). Average recovery values of musks were 99.2%, 74.5%, 56.9% and 87.6%, for skin moisturisers, toothpastes, toilet soaps, and body and hair washes, respectively.

Especially skin moisturisers and body and hair washes contained high levels of synthetic musks (302 and 113 µg/g, respectively). Nitro musks were not found in the samples analysed. Maximum concentrations of cashmeran, celestolide, phantolide, exaltolide, galaxolide, tonalide and ethylene brassylate were 15, 0.3, 0.3, 78, 882, 204 and 0.7 µg/g, respectively. Galaxolide accounted for 75% of the total musk concentrations and exaltolide was the second most abundant compound (12%).

Exposure profiles through dermal application were also estimated. In the present study, the daily exposure rate to total synthetic musks from the use of personal care products was estimated to be 6652 µg/d for a person in Portugal; the highest contributor to exposure amounts was shampoo (2492 µg/d).

Key Words: Synthetic musks; Personal care products; QuEChERS method; Human exposure

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Notation and Glossary

List of Acronyms

3-D	Three-dimensional
ACN	Acetonitrile
ADBI	Celestolide
AHMI	Phantolide
AHTN	Tonalide
ASE	Accelerated solvent extraction
ATD	Automated thermal desorption
ATII	Traseolide
ChV	Chronic value
CY	Cyclohexane
DLLME	Dispersive liquid–liquid microextraction
DPMI	Cashmeran
d-SPE	Dispersive solid-phase extraction
EC	Effective concentration
FID	Flame ionization detector
GC	Gas chromatography
GCB	Graphitized carbon black
GFF	Glass fiber filter
GLC	Gas-liquid chromatography
GSC	Gas-solid chromatography
HHCB	Galaxolide
HS	Headspace
LLE	Liquid-liquid extraction
LC	Lethal concentration
LOD	Limit of detection
LOQ	Limit of quantification
LVI	Large volume injection
MA	Musk ambrette, microwave assisted
MAE	Microwave assisted extraction
MASE	Membrane assisted solvent extraction
MK	Musk ketone
MM	Musk moskene
MS	Mass spectrometry
MT	Musk tibetene
MX	Musk xylene
PCP	Personal care product
PDMS	Polydimethylsiloxane polymer
PLE	Pressurized liquid extraction
PSA	Primary-secondary amine
PTV	Programmable temperature vaporization
PUF	Polyurethane foam
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
RTL	Retention time locked
SBSE	Stir-bar sorptive extraction
SCOT	Support-coated open tubular
SFE	Supercritical fluid extraction
SIS	Selected ion storage
Sol.	Solubility
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
TOF	Time-of-flight
TD	Thermal desorption
TIC	Total ion chromatography
USE	Ultrasonic solvent extraction
WCOT	Wall coated open tubular
WWTP	Wastewater treatment plant

1 Introduction

1.1 Organization of the Thesis

This thesis is organized in several sections. In Section 1 an Introduction to the project is presented. The structural composition and properties of synthetic musks, as well as the basics of the most used extraction methodologies and GC-MS are introduced. Section 2 is the State of the Art, where all the advances in musk monitoring and extraction and quantitative analysis methods are presented. The importance, interest and purpose of the work conducted is also presented. Section 3 is the Technical Description of the work conducted, where all the methods and protocols used are described in detail. Results and Discussion are presented in Section 4, which is one of the most important sections of this thesis, as it is where the results obtained from the experiments conducted are presented and discussed.

The main conclusions of this project are then presented in Section 5, followed by a general description of the limitations as well as some suggestions for future work to be performed under the scope of this project. In the appendix additional information can be found. In this last section, the calibration curves for each musk compound studied are presented.

1.2 Background and Presentation of the Project

In this first part, the principles outlined in the thesis are discussed and the state of the art will be only presented later. Furthermore, both the introduction and the state of the art are focused on the incidence of musks in environmental matrices, since there are more available studies on this theme. However, this thesis is directed towards the study of musks in personal care products.

1.2.1 Personal-Care Products in the Environment

Personal-care products (PCPs) are synthetic organic chemicals used in a wide variety of consumer products such as soaps, lotions, toothpaste, cosmetics, etc. They represent an important class of “emerging” or ‘‘unrecognized’’ pollutants (Daughton and Jones-Lepp, 2001, Daughton, 2004, Ellis, 2006, Barceló and Petrovic, 2007, Brausch and Rand, 2011). Some PCPs were identified by the EU Water Framework Directive as future emerging priority candidates for regulation and monitoring (EPA, 2012, European Comission, 2012).

PCPs enter the environment mainly by disposal in urban receiving waters from individual households, after bathing and showering. Traditional wastewater treatment plants (WWTP) are usually

not prepared to remove these compounds from sewage water and, therefore, PCPs can reach the natural waterways (Carballa et al., 2004, Daughton, 2004, Ternes et al., 2004, Ellis, 2006). In fact, PCPs have been found in high concentration levels near WWTP discharge points (Chase et al., 2012). The application of slurry as fertilizer in agricultural fields and their irrigation with contaminated waters are direct entry routes of PCPs in soil. Surface and groundwater contamination may occur through run-off and leaching (Chase et al., 2012). It is important to mention that once present in the surface waters, they can be eliminated naturally through volatilization, photolysis, biodegradation and/or sorption (Buerge et al., 2003, Chase et al., 2012). Rainwater due to atmospheric deposition may also be considered another point of contamination (Peters et al., 2008). Legal limits have not yet been established for these compounds in environmental matrices.

The incidence of PCPs in low concentrations in environmental samples may have a negative impact on humans and ecosystems either by their accumulation and persistence or by long-term chronic exposure of aquatic organisms to these compounds (Daughton and Jones-Lepp, 2001, Daughton, 2004, Mackay and Barnthouse, 2010). Furthermore, there is some evidence that these low doses of PCPs may guide to synergic toxicity effects and cumulative stress in exposed organisms (Daughton and Jones-Lepp, 2001, Daughton, 2004, Grung et al., 2007). A number of PCPs (e.g., insect repellents, some synthetic musk fragrances, parabens, and ultraviolet screens) have been suspected to imitate the natural hormones of animals, also referred to as endocrine-disruptors (Daughton and Jones-Lepp, 2001, Ternes et al., 2004, Brausch and Rand, 2011).

Emerging contaminants and the analytical capabilities of monitoring their incidence in a variety of environments are mutually connected. Along with the progress of the extraction techniques and the detectors, an increasingly number of PCPs can be detected at trace levels in the environment (Daughton and Jones-Lepp, 2001, Peck, 2006). As a result, a number of new or previously unrecognized or ignored contaminants are now being analyzed. Amongst them, musks are perhaps one of the classes that raised most concern. These compounds belong to a class of aromatic substances regularly used as base notes in perfumery. Due to the high cost and the uncertainty of supply of the natural musks, synthetic musks have emerged as an alternative. Nitromusks dominated the market for many years but declined significantly in the 90s (Rimkus, 1999) due to their bioaccumulative and toxicological properties, as well as high stability against biological and chemical degradation, which led to the prohibition of musk tibetene, musk moskene and musk ambrette. However, musk ketone and musk xylene can still be used in personal care products with some legal restrictions (Decree-Law Nº 189/2008). With the decreasing use of nitromusks a second group of synthetic musks emerged, the so-called polycyclic musks. A large portion of worldwide musk production is focused on galaxolide (HHCB) and tonalide (AHTN), two compounds from this class. However, reports on the presence of these compounds in different environmental matrices, aquatic organisms and human samples caused a slowdown in the use of musks

and a decrease in the production volumes (Roosens et al., 2007). Nonetheless, these compounds are still largely used in personal care and sanitation products (Reiner and Kannan, 2006). Polycyclic musks have been tested in the past and showed no toxicological and dermatological effects. However, due to their chemical stability, low biodegradability and their widespread use, this group of musks has been gradually replaced by a new group of partially artificial and partially nature-identical compound called macrocyclic musks (Peters et al., 2008).

Regarding the legislation, there seems to be a concern of the cosmetic industry on the safety of fragrance ingredients used, namely musks. The free circulation of cosmetics products in the market and the safety of cosmetics placed on it have to be ensured by the respective governments. Recently, on 30 November 2009, in the European Union context, a new Cosmetic Products Regulation (Regulation (EC) N.º 1223/2009) has been adopted in order to reinforce the regulatory framework for cosmetics, such as in-market control to ensure consumer safety, public health and the environmental protection (under the new European chemicals legislation REACH). This Regulation is already into force and it will be implemented from 11th July 2013. Although the use of some musks have been prohibited based on their toxicological and environmental effects, legal limits in environmental matrices have not been created.

1.2.2 Natural Musks

Since antiquity, the odour musk has been of high importance. Compounds which present a scent are essential in life processes. These compounds were initially used in religious ceremonies and have been applied for years as odorants and pharmaceutical ingredients (Ravi et al., 2001, Yang et al., 2003). Castoreum, civet and ambergist musk represent some of the most precious perfumery ingredients until today. Located in the skin of the abdomen in the proximity of the male genitalia of the musk deer (*Moschus moschiferus*) (Fig. 1a) are the exocrine odour glands also named pods (Ravi et al., 2001, Yang et al., 2003). The exocrine glands of the male musk deer comprise about 30 grams of natural musk (Schmeiser et al., 2001). Other source of odorous compounds is the American musk rat (*Ondarta zibethicus rivalicius*) (Fig. 1b) (Ravi et al., 2001). The odorous components of natural musk extracts are macrocyclic ketones, lactones or alcohols and pyridine derivatives (Schmeiser et al., 2001), such as muscone, civetone, dihydrocivetone and exaltone.

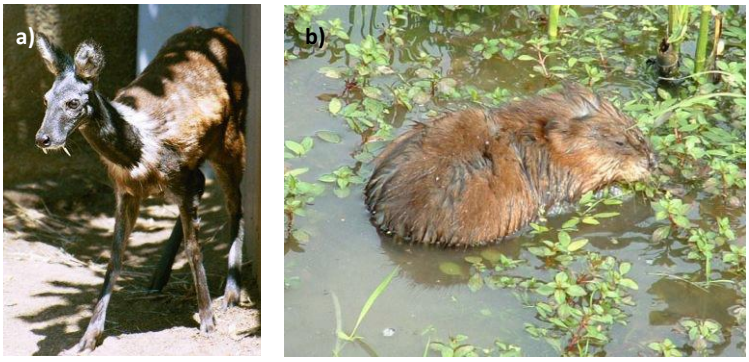


Fig. 1 - a) Musk deer (*Moschus moschiferus)* (Brent Huffman, 2001) and b) Musk rat (*Ondarta zibethicus rivalicius) (Nature Photo, 2012)*.

Two very well known musks of vegetable origin are exaltolide from angelica root (*Angelica archangelica*) (Fig. 2a) and ambrettolide from ambrette seeds (*Abelmoschus moschatus, Hibiscus abelmoschus)* (Fig. 2b) (Ravi et al., 2001). Nonetheless, these compounds are found in small quantities and as a complicated mixture in nature.

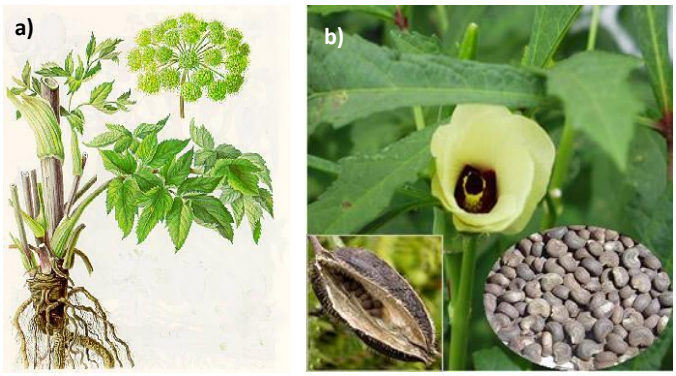


Fig. 2 – a) Angelica root (*Angelica archangelica)* and b) Ambrette seeds (*Abelmoschus moschatus, Hibiscus abelmoschus)* (GFMER, 2008, Bitterroot Restoration, 2012).

The perfumery industry has a constant demand for these musks in bulk. Due to their high cost and uncertainty of supply, synthetic musks have been developed (Lu et al., 2011a).

1.2.3 Synthetic Musks

Synthetic musks are widely incorporated in fragrances as fixative compounds, in several consumer products such as cosmetics and hygienic products (perfumes, body moisturisers, deodorants, soaps, shampoo, etc), as well as in household products (detergents, fabric softeners and air fresheners) (Mottaleb et al., 2012). They include four main chemical groups: nitro, polycyclic, macrocyclic and alicyclic musks (Arbulu et al., 2011).

1.2.3.1 Nitro musks

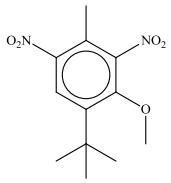
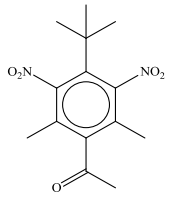
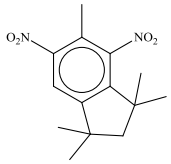
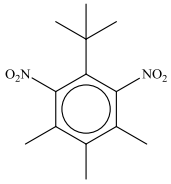
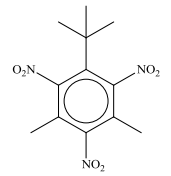
The group of nitro musks was first discovered by A. Bauer at the end of the 19th century. These synthetic compounds consist of dinitro- and trinitro- substituted benzene derivatives. Despite being structurally distinct from the compounds that occur in natural musk extracts, these musks hold fragrance properties comparable to those found in musks of animal and plant origin (Schmeiser et al., 2001). This group is composed of five most common synthetic alkylated nitro benzenes with a typical musk odour (Herren and Berset, 2000, Chase et al., 2012) and hereby shown in Table 1.

Nitro musks have been used in high volume as artificial fragrances in the industrial production of personal care products due to the ease of preparation and low production costs. Additionally some nitro musks are used as room fragrances, as food additives, in technical products such as herbicide formulations and explosives, in chewing tobacco, and in fish baits (Schmeiser et al., 2001). These compounds began their major environmental exposure after sewage discharges. This is when they become a part of the environment and reach detectable and likely harmful concentration levels (Mottaleb et al., 2012).

The use in cosmetic products of musk ambrette (MA), musk tibetene (MT) and musk moskene (MM) was banned in the European Union, while the use of musk xylene (MX) and musk ketone (MK) is restricted. Nevertheless, its use is allowed in North America. In Portugal, MX is restricted to 1% in perfumes, 0.4% in eau de toilette and 0.03% in the other cosmetic products. MK is restricted to 1.4% in perfumes, 0.56% in eau de toilette and 0.042% in the other cosmetic products. Oral hygiene products can not contain these compounds (Decree-Law Nº 189/2008).

In Table 1 are presented the most relevant characteristics of nitro musks, such as K_{ow}, solubility, boiling point/range, vapour pressure and data related to the toxicity. LC_{50} refers to the lethal concentration and is defined as the concentration of a toxicant that kills 50% of a test population for a given exposure duration. The Chronic Value (ChV) is defined as the geometric mean of the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC). EC_{50} is the effective concentration and it is defined as the concentration of a given compound that reduces the specified effect to half that of the original response.

Table 1 – Chemical structures and characteristics of the nitro musks class

Common Name (Chemical abbr.) CAS n.º Relative molecular mass	Chemical structure	Log K_{ow}	Sol. in water (mg/L)	Boiling point/range (°C)	Vapour Pressure (Pa)	Toxicity data[f]		
						Organism	Endpoint	Dose (mg/L)
Musk ambrette (MA) 83-66-9 268.3		5.7[a]	Slightly soluble in ethanol; soluble in ethyl ether, chloroform[b]	185 (16 mmHg)[c]	0.00333[c]	Fish	LC_{50} (96 h) ChV	>Sol. 0.275
						Daphnia	LC_{50} (48 h) ChV	>Sol. 0.308
						Green algae	EC_{50} (96 h) ChV	>Sol. 1.283
Musk ketone (MK) 81-14-1 294.3		4.3[d]	1.90[d]	395 (760 mmHg)[c]	0.00004[d]	Fish	LC_{50} (96 h) ChV	>Sol. 0.226
						Daphnia	LC_{50} (48 h) ChV	>Sol. 0.265
						Green algae	EC_{50} (96 h) ChV	>Sol. 1.174
Musk moskene (MM) 116-66-5 278.3		5.8[a]	0.17[c]	350-353 (760 mmHg)[c]	0.01132[e]	Fish	LC_{50} (96 h) ChV	>Sol. 0.025
						Daphnia	LC_{50} (48 h) ChV	>Sol. 0.042
						Green algae	EC_{50} (96 h) ChV	>Sol. >Sol.
Musk tibetene (MT) 145-39-1 266.3		5.9[a]	0.29[c]	391 (760 mmHg)[e]	0.00076[e]	Fish	LC_{50} (96 h) ChV	>Sol. 0.037
						Daphnia	LC_{50} (48 h) ChV	>Sol. 0.057
						Green algae	EC_{50} (96 h) ChV	>Sol. >Sol.
Musk xylene (MX) 81-15-2 297.2		4.9[d]	0.49[d]	200-202[c]	0.00003[d]	Fish	LC_{50} (96 h) ChV	>Sol. 0.174
						Daphnia	LC_{50} (48 h) ChV	>Sol. 0.214
						Green algae	EC_{50} (96 h) ChV	>Sol. >Sol.

[a]Osemwengie and Steinberg (2001); [b]NIST Chemistry WebBook (2011); [c]The Good Scents Company (2012); [d]Chase et al. (2012); [e]LookChem (2008), [f]Ecosar Database (2009).

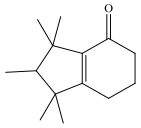
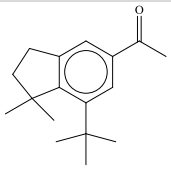
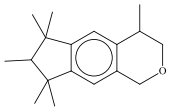
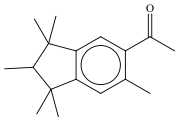
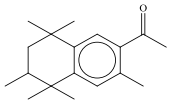
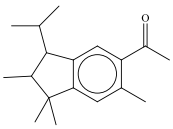
In this class of compounds, musk tibetene is the compound having the highest K_{ow} (5.9) and it is therefore the most lipophilic compound. Musk ketone is the most soluble nitro musk and it has the highest boiling point (395 °C) among all musks from this class.

1.2.3.2 Polycyclic musks

The polycyclic musk fragrances, other important group, were introduced in the 1950s. These musks are indane and tetraline derivatives substituted mainly by methyl groups. They are not related, neither chemically or structurally, with the natural musk compounds (Rimkus, 1999). The industrial synthesis of polycyclic musks is moderately difficult, which make them more expensive than the nitro musks. However, they are considered very important ingredients in fragrances for a series of consumer products due to their substantive property to bind fragrances to fabrics and their typical musky scent

(Rimkus, 1999). Galaxolide (HHCB), tonalide (AHTN), cashmeran (DPMI), celestolide (ADBI), phantolide (AHMI) and traseolide (ATII) (Table 2) are the main components of the polycyclic musk group (Herren and Berset, 2000).

Table 2 - Chemical structures and characteristics of the polycyclic musks class (Chase et al., 2012)

Common Name (Chemical abbr.) CAS n.º Relative molecular mass	Chemical structure	Log K_{ow}	Sol. in water (mg/L)	Boiling point/range (°C)	Vapour pressure (Pa)	Toxicity data[a]		
						Organism	Endpoint	Dose (mg/L)
Cashmeran (DPMI) 33704-61-9 206.3		4.9	0.17	285-286 (760 mmHg)[b]	5.20	Fish	LC_{50} (96 h) ChV	1.241 0.112
						Daphnia	LC_{50} (48 h) ChV	0.968 0.139
						Green algae	EC_{50} (96 h) ChV	1.159 0.661
Celestolide (ADBI) 13171-00-1 244.3		6.6	0.02	308-309 (760 mmHg)[b]	0.02	Fish	LC_{50} (96 h) ChV (30 d)	0.066 0.009
						Daphnia	LC_{50} (48 h) ChV	0.066 0.014
						Green algae	EC_{50} (96 h) ChV	0.174 0.128
Galaxolide (HHCB) 1222-05-5 258.4		5.9	1.75	127-136 (2 mmHg)[b]	0.07	Fish	LC_{50} (96 h) ChV (30 d)	0.036 0.005
						Daphnia	LC_{50} (48 h) ChV	0.038 0.009
						Green algae	EC_{50} (96 h) ChV	0.114 0.088
Phantolide (AHMI) 15323-35-0 244.3		6.7	0.03	393 (760 mmHg)[b]	0.02	Fish	LC_{50} (96 h) ChV (30 d)	0.077 0.010
						Daphnia	LC_{50} (48 h) ChV	0.076 0.016
						Green algae	EC_{50} (96 h) ChV	0.193 0.140
Tonalide (AHTN) 1506-02-1 258.4		5.7	1.25	393(760 mmHg)[b]	0.06	Fish	LC_{50} (96 h) ChV (30 d)	0.030 0.004
						Daphnia	LC_{50} (48 h) ChV	0.032 0.007
						Green algae	EC_{50} (96 h) ChV	0.100 0.079
Traseolide (ATII) 68140-48-7 258.4		8.1	0.09	178 (5 mmHg)[b]	1.20	Fish	LC_{50} (96 h) ChV (30 d)	0.032 0.004
						Daphnia	LC_{50} (48 h) ChV	0.034 0.008
						Green algae	EC_{50} (96 h) ChV	>Sol. 0.083

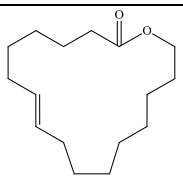
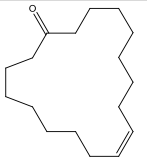
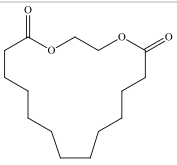
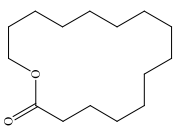
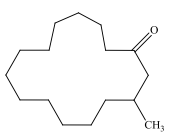
[a]Ecosar Database (2009); [b]The Good Scents Company (2012).

As can be seen from Table 2, traseolide is the musk with the highest K_{ow} (8.1). The most soluble polycyclic musk is galaxolide. This characteristic associated with a high volatility (127-136 °C) justifies why this musk is the most commonly found in personal care products. Phantolide and tonalide are the two musks with the highest boiling point (393 °C). Both galaxolide and tonalide comprise about 95% of the European market and 90% of the USA market among all polycyclic musks (Zhang et al., 2011). These musks did not show mutagenic potential in different tests (Hutter et al., 2010).

1.2.3.3 Macrocyclic musks

Macrocyclic musks (Table 3), more recently introduced in the market, are chemically similar to animal and plant musk odorants. They are large ringed ketones and lactones (10-15 carbons) (Sumner et al., 2010). Because of relatively high production cost they still represent only a minor part (3-4%) of the musks on the market and are almost exclusively used in perfumes. Nonetheless, due to their fragrance characteristics and excellent fixative properties, they are highly regarded by the industry (Abramsson-Zetterberg and Slanina, 2002). These compounds show higher degradability in the environment than the polycylic musks. Macrocyclic musks frequently pass unnoticed while analyzing environmental samples because their mass spectra resemble those of natural fatty acids. In addition, their chemical properties are similar to those of natural products making the separation from these more complicated (Bester, 2009).

Table 3 – Chemical structures and characteristics of some macrocyclic musks

Common Name(s) (Chemical abbr.) CAS n.º Relative molecular mass	Chemical structure	Log K_{ow}	Sol. in water (mg/L)	Boiling point/range (°C)	Vapor pressure (Pa)	Toxicity data[f]		
						Organism	Endpoint	Dose (mg/L)
Ambrettolide 123-69-3 252.4		5.37[a]	0.593[a]	379[a]	0.0030[a]	Fish	LC_{50} (96 h) ChV (33 d)	0.405 0.016
						Daphnia	LC_{50} (48 h) ChV (21 d)	0.539 0.143
						Green algae	EC_{50} (96 h) ChV	0.160 0.121
Civetone 542-46-1 250.4		6.31[b]	0.095[b]	344[b]	0.0452[b]	Fish	LC_{50} (96 h) ChV (30 d)	0.031 0.004
						Daphnia	LC_{50} (48 h) ChV	0.033 0.008
						Green algae	EC_{50} (96 h) ChV	>Sol. 0.080
Ethylene brassylate 105-95-3 270.4		4.70[c]	1.719[c]	434[c]	0.0438[c]	Fish	LC_{50} (96 h) ChV	1.056 0.094
						Daphnia	LC_{50} (48 h) ChV	0.845 0.126
						Green algae	EC_{50} (96 h) ChV	1.109 0.659
Exaltolide, Thibetolide 106-02-5 240.4		6.00[d]	0.148[d]	364[d]	0.0069[d]	Fish	LC_{50} (96 h) ChV	0.057 0.005
						Daphnia	LC_{50} (48 h) ChV	0.054 0.010
						Green algae	EC_{50} (96 h) ChV	0.128 0.099
Muscone 541-91-3 238.4		5.96[e]	0.221[e]	320-324 (730 mmHg)[e]	0.0625[e]	Fish	LC_{50} (96 h) ChV (30 d)	0.060 0.008
						Daphnia	LC_{50} (48 h) ChV	0.061 0.013
						Green algae	EC_{50} (96 h) ChV	0.161 0.119

[a]McGinty et al. (2011e), [b]McGinty et al. (2011b), [c]McGinty et al. (2011d), [d]McGinty et al. (2011f), [e]McGinty et al. (2011a), [f]Ecosar Database (2009).

In this class of compounds, civetone presents the highest K_{ow} (6.31). The most soluble macrocyclic musk is ethylene brassylate (1.719 mg/L). Additionally, this compound also shows the highest boiling point (434 ^{o}C).

1.2.3.4 Alicyclic musks

Alicyclic musks (Fig. 3) are a novel class of musk compounds. They were first introduced in 1975 with the trisubstituted cyclopentene derivative Cyclomusk. Alicyclic musks differ considerably in structure from nitro, polycyclic and macrocyclic musks because they are modified akyl esters. It was only in 1990 with the discovery and introduction of Helvetolide at Firmenich (a private Swiss company in the perfume and flavor business) that a compound of this class was produced at a commercial scale. Romandolide, a more ambrette-like and less fruity alicyclic musk compared to Helvetolide, was discovered ten years later (Eh, 2004). Until today there is no study on the incidence of alicyclic musks in the environment.

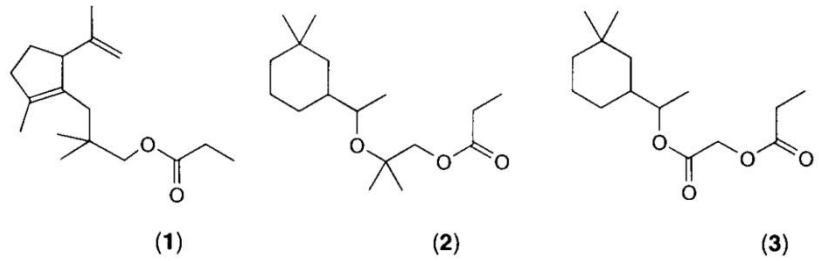


Fig. 3 – Examples of alicyclic musks: (1) Cyclomusk, (2) Helvetolide and (3) Romandolide (Eh, 2004).

Synthetic musks are lipophilic chemicals, as can be seen from the high K_{ow} shown in the tables. Chemicals with high K_{ow} values are lipophilic, i.e., they tend to have low water solubility, high soil/sediment adsorption and high bioconcentration factors for aquatic life. Synthetic musks have high bioaccumulation potential and are not readily biodegraded. Detection of musks in environmental matrices will be explained with greater detail in the state of the art.

1.2.4 Analytical methods to determine synthetic musks

Numerous analytical methodologies have been developed for the determination of synthetic musks in environmental samples, most of them based on GC-MS analysis. Usually, prior to GC-MS analysis, sample preparation is required to remove some compounds that may interfere with the detection of the musks of interest, reducing the separation efficiency or the column life. The preliminary extraction step also enables the pre-concentration of the sample before the chromatographic analysis. Depending on the type of matrix and characteristics of the compounds of interest, different types of extraction may be used.

1.2.4.1 Extraction techniques

There are several studies of musks on environmental matrices using extraction steps. This section will only present the principles of the extraction methods. A more detailed description of the methods will be given in the state of the art, according to the type of matrix, which musks exist in environmental matrices and personal care products, what methods were used by each author to determine musks, as well as some results.

In liquid-liquid extraction (LLE) the mixture containing the analyte of interest is treated with an immiscible solvent in which those components are more readily soluble. The separation occurs because two immiscible phases are brought into contact allowing the mass transfer of the analyte. The raffinate is defined as the layer from which the solute is removed and the extract is the solvent layer with the extracted solute (Skoog et al., 2007).

When the matrix is solid (for example, sediments) a solid-liquid extraction may be used. In this case, a liquid solvent is used to remove a solute or solutes from the solid. It is usually applied when thermal and mechanical methods of separations are not possible or practical (Luque de Castro and Priego-Capote, 2010). The simplest method is the Soxhlet extraction. In this technique, the solvent is heated to reflux contacting with the sample contained in a chamber. When the chamber is almost full, it is automatically emptied through a siphon restarting the next reflux cycle.

Solid-phase extraction (SPE) is faster and usually more efficient than LLE. In SPE the solute is extracted from the liquid phase to a solid phase, typically constituted by silica-based porous particles of small diameters. The extracted analytes can be removed from the solid phase using a suitable solvent. Therefore, the regular procedure of SPE begins with the conditioning (activation of the sorbent ligands and equilibrium of the sorbent bed), followed by loading the sample onto the SPE cartridge. Then an appropriate solvent passes through the solid phase, eluting weakly bound contaminants. Finally, in the elution a medium strength solvent is used to remove the product of interest (Fig. 4) (Skoog et al., 2007).

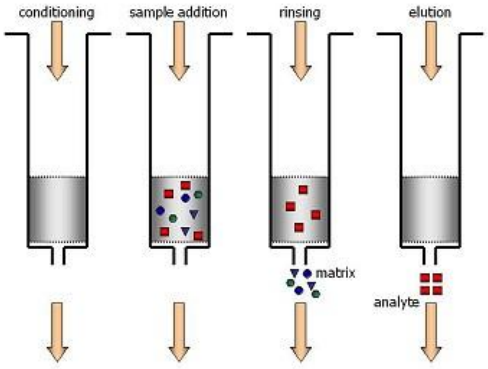


Fig. 4 – Schematic illustration of SPE steps (Leaderhead Food Research, 2012).

The procedures described previously have the disadvantage of being time-consuming and of using large amounts of organic solvents, involving high cost and increasing the environmental pollution (Klinsunthorn et al., 2011).

Solid-phase microextraction (SPME) is a sample preparation technique based on an equilibrium process in which the analyte partitions between the SPME coating (that can be a polymeric "liquid" or a solid sorbent porous material with a high surface area) and the sample matrix. After the extraction procedure, the SPME fiber is transferred to the injection part of a gas chromatograph (Mester and Sturgeon, 2005), where thermal desorption occurs. However, SPME has the following disadvantages: being relatively expensive, the polymer coating is fragile and easily broken, low recovery of analytes and high variability in results. In the same way as SPME, stir-bar sorptive extraction (SBSE) is a sample preparation method that does not involve the use of solvents. In this method, the solutes are extracted into a polymer coating on a magnetic stirring rod through a sorptive process. The extraction is controlled by two factors: the phase ratio between the polymer coating and the sample volume and by the partitioning coefficient of the solutes between the polymer coating and the sample matrix (David and Sandra, 2007).

New environmentally friendly alternative methodologies are being developed based on miniaturization. Dispersive liquid–liquid microextraction (DLLME) is one of these techniques. A mixture of extraction and disperser solvents is injected into the aqueous sample forming a cloudy solution. The extraction occurs in a short time due to the large contact surface between the extractant and the sample. After this step, the organic extract is separated from the aqueous phase by centrifugation. DLLME has several advantages such as simplicity of operation, rapidity, low cost, high recovery and high enrichment factor (Li et al., 2012). The membrane assisted solvent extraction (MASE) is another technique that has been exploited for the musks extraction from aqueous matrices. In MASE, a non-porous polypropylene membrane bag filled with an organic solvent is placed into a vial containing the aqueous sample. The organic compounds are transferred from the aqueous phase through the membrane into the organic solvent through agitation. This technique is suitable for a wide polarity range of organic compounds since it can be applied a series of different organic solvents as acceptor phase (such as cyclohexane for non-polar and ethyl acetate for polar compounds) (Schellin and Popp, 2006).

Other modern techniques used for the extraction of musks from different matrices are ultrasonic solvent extraction (USE), pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), microwave assisted extraction (MAE) and accelerated solvent extraction (ASE). USE presents high extraction efficiencies of organic compounds due to a phenomenon named cavitation, which is produced in the solvent by the passage of an ultrasonic wave. The bubbles produced and compressed during this process allow higher penetration of the solvent into the raw samples. The ultrasound has

been shown to aid extraction by significantly reducing extraction time and increasing maximum extraction yield (Lee and Lin, 2007). PLE uses an organic solvent at a temperature above its boiling point as well as high pressures to extract substances from a solid matrix. It offers several advantages such as the possibility of automation, low solvent volumes and reduced extraction time. This technique can be accomplished in the static mode (sample and solvent are maintained for a particular time at constant pressure and temperature), the dynamic mode (the solvent flows through the sample in a continuous manner) or a combination of both (Delgado-Zamarreño et al., 2006). SFE is usually performed with a relatively inert and nontoxic solvent such as carbon dioxide. The extraction can be fine-tuned to selectively extract certain analytes while leaving others behind due to the capability of changing the solvating power of the fluid as a function of pressure and temperature. Extraction rates are usually much higher than those verified for liquid-solid extractions due to higher diffusivity of a supercritical fluid when compared with a liquid. The major advantage of using carbon dioxide for SFE is the fact that the removal of this solvent and waste disposal do not represent a problem or an expense (McDaniel et al., 2001). MAE allows the heating of the sample in a very short time and accelerates the extraction. It has the advantage of enabling a significant reduction in the consumption of organic solvent. However, this technique tends to cause inhomogeneous heating (Cheng et al., 2011). ASE promotes the acceleration of the kinetic processes involved in the analytes desorption from the matrix. In addition, it takes advantage of the increased analyte solubility at temperatures higher than the boiling points of common solvents (He et al., 2009). Anastassiades et al. (2003) developed an original extraction method called QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe), which combines extraction/isolation with cleanup. This method was originally developed for the extraction of pesticides from food matrices (Matamoros et al., 2012a), but has suffered several modifications and enhancements over the years, being nowadays applied to different matrices and compounds.

The QuEChERS method involves two simple steps (Lehotay et al., 2010, Cieślik et al., 2011, Lee et al., 2011). First, the sample is extracted with an organic solvent, usually acetonitrile, in the presence of anhydrous salts (e.g. magnesium sulphate, sodium chloride) and optionally buffers (Fig. 5). Then, an easy purification is carried out using dispersive solid-phase extraction (d-SPE) sorbents in a centrifuge tube. Both steps are effortlessly performed by vortexing and centrifuging for a few minutes (Klinsunthorn et al., 2011, Wilkowska and Biziuk, 2011). Using the d-SPE approach, the quantity and sorbents type can be easily optimized for different matrix interferences and analytes.

The addition of inorganic salts during the partitioning stage usually promotes the extraction of polar analytes, since its solubility in the aqueous phase decreases, as well as the water amount in the organic phase. In some instances, the pH of the extraction must be controlled using buffers (e.g. sodium acetate, sodium citrate) (Lee et al., 2011). For the d-SPE the most frequently used sorbent is primary-secondary amine (PSA), but graphitized carbon black (GCB) and C18 can also be incorporated as an

additional clean-up step. The main function of PSA is to remove fatty acids, sugars, organic acids and some ionic lipids. However, PSA sorbent is not capable of adsorbing non-polar pigments, such as carotenoids and chlorophylls. Conversely, GCB is a very retentive sorbent and highly effective for removing these pigments (Wilkowska and Biziuk, 2011). The C18 sorbent may be used to remove non-polar interfering substances like lipids (Lee et al., 2011). Thus, the choice of the sorbent should be based on matrix composition and the target analyte.

Although QuEChERS has been extensively employed for sample preparation in the determination of pesticides in food (Klinsunthorn et al., 2011, Wilkowska and Biziuk, 2011), no study have used this method for the determination of musks in PCPs.

Fig. 5 – QuEChERS kit (UCT, 2007).

The QuEChERS method is a quick and inexpensive procedure which provides reliable results, whilst reducing the quantities of reagents and laboratory glassware, as well as the number of analytical steps. This is noteworthy, since every additional analytical step complicates the procedure and is also a potential source of systematic and random errors (Cieślik et al., 2011, Wilkowska and Biziuk, 2011). Due to the ubiquity of musks and therefore increased risk of contamination, QuEChERS are more advantageous owing to the use of a reduced number of containers which additionally are disposable. This methodology simplifies the extraction of analytes and extract cleanup without adversely affecting the magnitude of analyte recoveries.

The choice of the analytical instrumentation for QuEChERS method extracts is dependent upon the properties of the analytes being tested. As mentioned above, several analytical methods for determining synthetic musks in environmental matrices and personal care products have been developed in the last years, most of them based on GC-MS analysis (Ramirez et al., 2011). In this work, this will also be the analytical technique used. For that reason, the basics related to the GC-MS analysis are presented in the following section.

1.2.4.2 Basic GC-MS principles

The gas chromatography-mass spectrometry (GC-MS) is a hyphenated analytical technique that combines the separation of the components of a mixture in time (GC) with the characterization of the components (MS). It is widely used in several fields such as environmental science, forensics, health care, medical and biological research, flavour and fragrances industry, food safety, and others. Fig. 6 shows a scheme of a typical system.

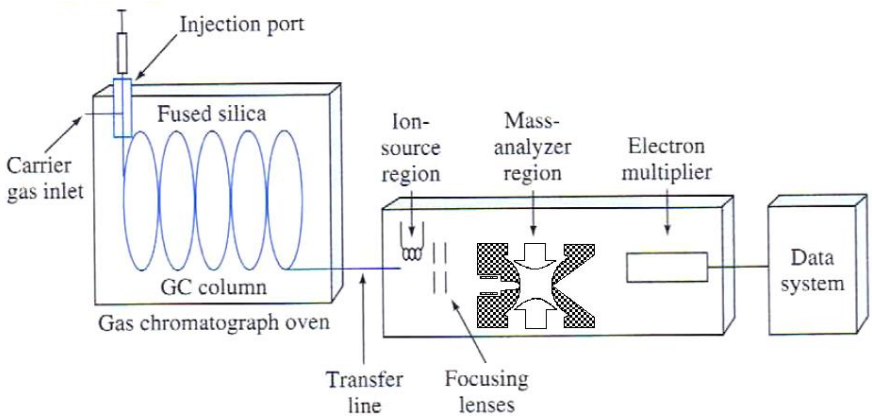


Fig. 6 - Scheme of a typical GC-MS system (adapted from Skoog et al., 2007).

In the gas chromatography the components of a vaporized sample are fractionated as a result of being partitioned between a liquid or a solid stationary phase and an inert gaseous mobile phase. Gas-liquid chromatography (GLC) and gas-solid chromatography (GSC) are the two types of gas chromatography encountered. GLC is widely used in all fields of science, where it is commonly called gas chromatography (GC) (McMaster, 2011). Each component of the GC-MS system will be briefly described in the next sections.

1.2.4.2.1 Carrier Gas

The carrier gas is the gaseous mobile phase in GC and it must be chemically inert. Contrary to other types of chromatography, the carrier gas does not interact with the sample molecules and its only function is to transport the sample species through the column (Hubschmann, 2008). The most common carrier gas is helium, although argon, nitrogen, and hydrogen are also used. Pressures at the column inlet range from 10 to 50 psi (0.7-3.4 atm) and provide flow rates up to 25 mL/min for open tubular capillary columns (McMaster, 2011). Modern commercial chromatographs are equipped with electronic flow meters that are computer controlled to maintain the flow rate at any desired level.

1.2.4.2.2 Sample Injection

The injector is a device which allows the introduction of the sample into the column (or GC system). The sample may be in the liquid state or adsorbed on a support (SPME). Column efficiency is influenced by the size of the sample and by the way it is introduced. Oversized samples or slow injections cause band spreading and poor resolution. Liquid samples are injected with calibrated microsyringes through a rubber or silicone diaphragm or septum into a heated sample port located at the head of the column (McMaster, 2011). The temperature of the sample port (Fig. 7) is set to 50 ^{o}C above the boiling point of the least volatile component of the sample, so that it can be analyzed without being subjected to complete degradation. Commercial gas chromatographs using capillary columns, usually incorporate sample splitters to release only a small fraction of the injected sample into the column.

Most of the higher-end gas chromatographs use automatic sampling trays which improve significantly the precision of the injected volume over manual syringe, besides allowing the processing of samples without a technician's assistance.

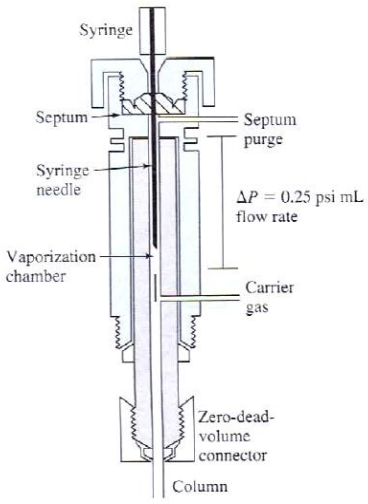


Fig. 7 – Layout of a microflash vaporizer direct injector (Skoog et al., 2007).

1.2.4.2.3 Chromatographic column

GC uses two types of columns: packed and open tubular or capillary. For most applications, capillary columns are preferably used. These columns can range from a few meters to 100 m. They are typically a fused silica or stainless steel coil, although glass and Teflon are also used (Hubschmann, 2008). Capillary columns are of two types: wall coated open tubular (WCOT) and support-coated open tubular (SCOT) columns. WCOT columns are coated with a thin layer of the stationary phase. In SCOT columns, the inner surface of the capillary is lined with a thin film of a support material, such as

diatomaceous earth. SCOT columns have a greater sample capacity, holding greater amounts of stationary phase than a WCOT column. Usually, a WCOT column is more efficient.

The column is placed in a thermostated oven so that the temperature can be controlled. The boiling point of the target compounds and solvent, together with the degree of separation define the optimal column temperature. Most of the times, temperature programming is employed for samples containing analytes with a broad boiling range. In this method the column temperature is increased either continuously or in steps as the separation proceeds (Hubschmann, 2008, McMaster, 2011).

The choice of the most appropriate stationary phase is essential to achieve satisfactory separation of the sample. An analyte must demonstrate some degree of compatibility with the stationary phase, in order to have a reasonable residence time in the column. Polar stationary phases are characterized for containing functional groups such as -CN, -CO, and -OH. Polyester stationary phases are highly polar, while dialkyl siloxanes and hydrocarbon phases are nonpolar. Polar analytes include alcohols, acids, and amines. Ethers, ketones, and aldehydes represent solutes of medium polarity and saturated hydrocarbons are nonpolar. Usually, the polarity of the stationary phase should match that of the sample components. When a good match is achieved, the order of elution is obtained by the boiling point of the analytes (McMaster, 2011).

1.2.4.2.4 Detector

Detectors for GC must respond rapidly to small concentrations of solutes as they exit the column, have stability, and uniform response for a wide variety of chemical species or, instead, a predictable and selective response toward one or more classes of solutes. Till this day, no single detector has fulfilled all these requirements (Skoog et al., 2007). One of the most powerful detectors for GC is the mass spectrometer (MS), which is an instrument that produces gas phase-ions and separates them according to their mass-to-charge ratios (m/z).

The flow rate from capillary columns is generally low enough that the column output can be fed directly into the ion source of the mass spectrometer. There the components of the sample are converted into ions by bombardment with electrons. The output of the ion source is a stream of positive ions that are then accelerated into the mass analyzer. The dispersion in the mass analyzer depends on the mass-to-charge ratio of analyte ions. A mass spectrometer also contains a transducer that converts the beam of ions into an electrical signal that can then be processed and stored in the memory of a computer, and after that displayed. This equipment requires a vacuum system to avoid collision in the mass spectrometer so that free ions and electrons are maintained (Rossi and Sinz, 2002). A brief description of each component of the mass spectrometer will be presented.

Ion Source

Ion sources can be divided into two types: hard and soft sources. Hard ionization sources transfer the necessary quantity of energy to bring the analyte molecules to a highly excited state. Then, breaking of chemical bonds in the molecule occurs, producing fragments. Soft ionization sources (e.g. chemical ionization) use less energy, producing low fragmentation (Rossi and Sinz, 2002, McMaster, 2011). Fig. 8 illustrates the difference in spectra obtained from a hard and a soft ionization source.

The data obtained from both hard- and soft-source spectra is useful for analysis. Hard-source spectra provide many peaks which supply information about the functional groups and the structure of the analytes, while soft-source spectra give information about the molecular mass.

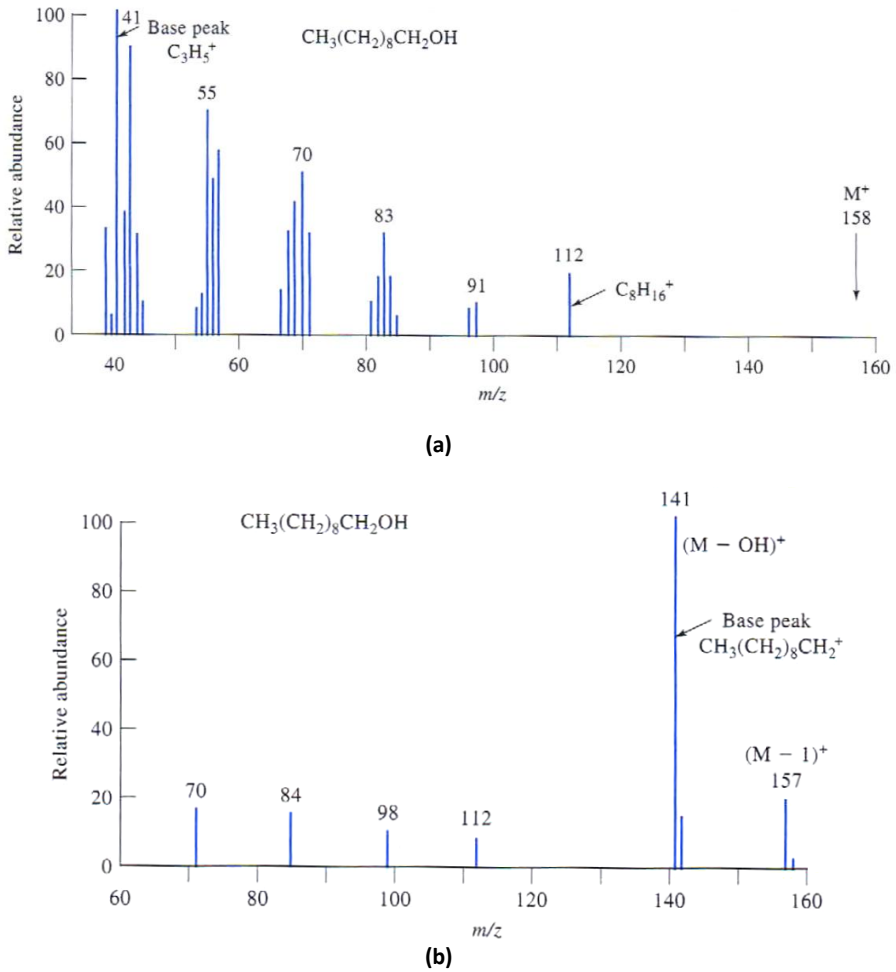


Fig. 8 – Mass spectrum of 1-decanol from (a) electron impact (a hard ionization source) and (b) chemical ionization (a soft ionization source) (Skoog et al., 2007).

The most common ion sources used in GC-MS are electron-impact and chemical ionization. It will only be described the ion source used in the equipment available to do this work, the electron-

impact ionization. In this process, the molecules are ionized by bombarding them with a beam of energetic electrons (70 eV). Fig. 9 shows a scheme of a basic electron-impact ion source.

A heated tungsten or rhenium filament emits electrons which are accelerated by a current of 70 V between the filament and the anode. Collision of the electrons and molecule ionization occur near the center of the source. The energetic electrons collide with gaseous molecules of the injected sample and cause the loss of electrons by electrostatic repulsion, producing singly charged positive ions (Hubschmann, 2008). These ions are attracted to the slit in the first accelerating plate by a small potential difference that is applied between this plate and the repellers. The accelerator plates, which have voltages that range between 10^3 and 10^4 V, give the ions their final velocities before they enter the mass analyzer (Skoog et al., 2007, McMaster, 2011).

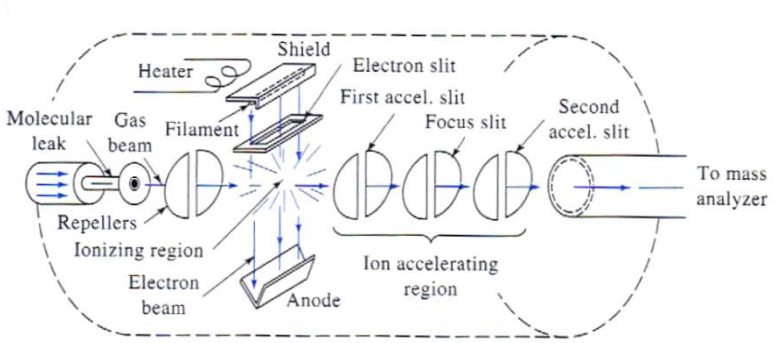


Fig. 9 – Diagram of an electron-impact ion source (Skoog et al., 2007).

Electron-impact sources make unambiguous identification of analytes, providing good sensitivity and high ion currents. However, the extensive fragmentation produced in this technique can also be a disadvantage, as it might cause de disappearance of the molecular ion peak making it difficult to establish the molecular mass of analytes (McMaster, 2011). Electron-impact sources are applicable only to analytes having molecular masses smaller than about 10^3 Da (Rossi and Sinz, 2002).

Mass Analyzer

Double-focusing spectrometers, quadrupole, time-of-flight (TOF) and ion-trap mass analysers are some devices available for separating ions with different mass-to-charge ratios. The most common mass analyzers are quadrupoles and ion traps. In this work, it will only be explained the operation of the ion trap mass analyzer, which is used to obtain mass spectra of a series of analytes. Fig. 10 is a cross-sectional view of a simple ion trap.

It is composed of a central ring electrode subjected to a variable radio-frequency voltage and two grounded end-cap electrodes. Ions produced by electron-impact source enter through the upper end cap and circulate within the cavity due to electric and magnetic fields. The ions over a large mass range of interest are trapped simultaneously. Then, mass-selective ejection is used to eject the trapped

ions in order of increasing mass. These ions pass into a transducer such as the electron multiplier. Ion trap mass analyzers are rugged, compact and less costly than other mass analyzers and have the potential for achieving low detection limits (Rossi and Sinz, 2002, McMaster, 2011).

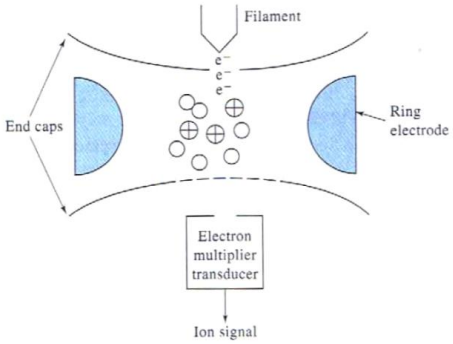


Fig. 10 – Sketch of an ion trap mass analyzer (Skoog et al., 2007).

Electron Multiplier

Several types of transducers are commercially available for mass spectrometers. The electron multiplier is the transducer of choice for most routine experiments. Fig. 11 illustrates a continuous-dynode electron-multiplier which is made of glass heavily doped with lead, to give the material a small conductivity (Hubschmann, 2008). A voltage gradient is produced from one end to the other of the transducer. By hitting the surface, ions eject electrons which are then attracted to higher-voltage points farther along the tube. As these secondary electrons hit along the surface, more electrons are ejected. Electron multipliers provide high-current gains and short response times, and they are consistent and rugged (Rossi and Sinz, 2002).

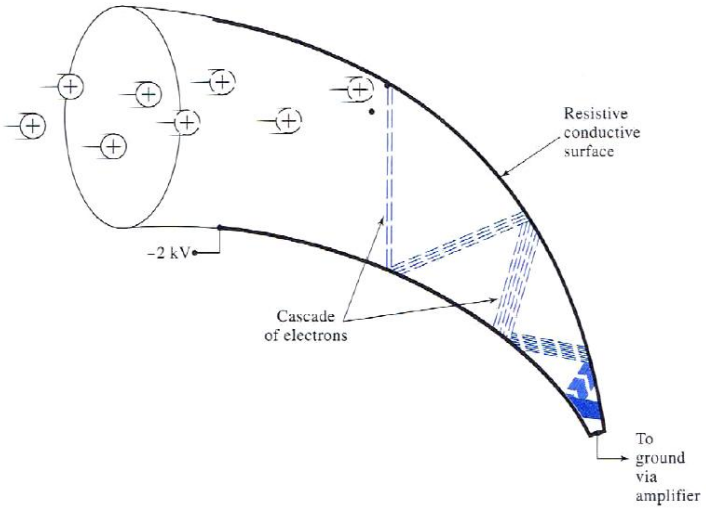


Fig. 11 – Representation of a continuous-dynode electron multiplier (Skoog et al., 2007).

1.2.4.2.5 Data analysis

The combination of gas chromatography (GC) with mass spectrometry (MS) generates 3-D data, which provide qualitative and quantitative information. Two of these dimensions are the signal intensity *vs.* GC retention time (so-called mass chromatogram). The representation of signal intensity *vs.* mass-to-charge ratio, the third dimension, is designated by mass spectrum. Fig. 12 illustrates an example of a mass spectrum.

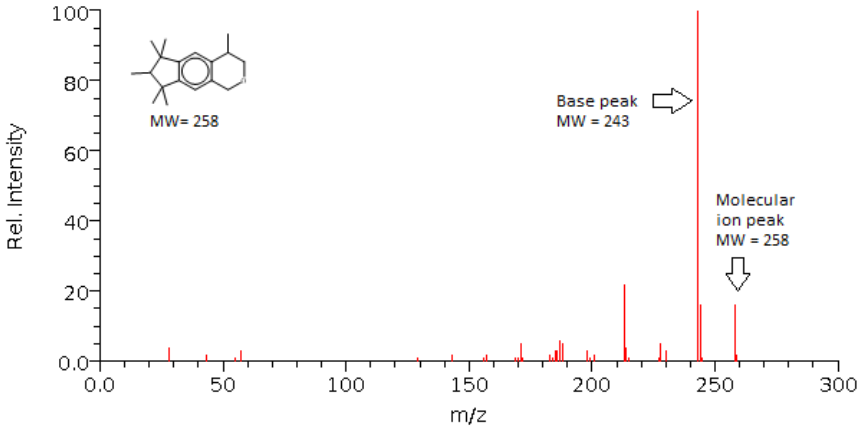


Fig. 12 – Mass spectrum of galaxolide (adapted from NIST, 2012).

In the mass spectra, the molecular ion is a radical cation ($M^{\bullet+}$) formed during electron ionization by the loss of an electron from the molecule. Therefore, in the spectrum of a pure compound, the molecular ion appears at the highest value of *m/z* (followed by ions containing heavier isotopes) and has the same molecular mass as the initial molecule. The peak with the highest intensity is called the base peak (Hubschmann, 2008). The collision between analyte molecules and energetic electrons typically transmits enough energy to the molecules, leaving them in an excited state. Relaxation occurs by fragmentation of part of the molecular ions to produce ions of lower masses (the fragments). Many pathways exist for fragmentation, but only cations or radical cations will show up in the mass spectrum, not radical or neutral fragments. As can be seen in Fig. 10, peaks with *m/z* ratios larger than the molecular ion peak are visible. They are called isotope peaks and result from fragments with the same chemical formula but different isotopic compositions (e.g. ^{2}H or ^{13}C for [M+1] peaks and ^{37}Cl for [M+2]) (McMaster, 2011).

During a chromatographic experiment, the masses are scanned continually by the MS. For example, if the duration of the chromatographic run is 10 minutes, and the MS scans every second, it counts for a total of 600 mass spectra (Hubschmann, 2008). There are two major methods to analyze the data obtained: full-scan total ion chromatography and selected ion storage (SIS). A full scan ion chromatogram is a plot of the sum of the signals across the entire mass range detected during the analysis as a function of chromatographic time (Rossi and Sinz, 2002). It comprehends the *m/z* of the

smallest fragment ion of any compound detected through the highest m/z. In the SIS mode, only the selected *m/z* ranges are detected by the instrument during the analysis. Usually, the ion range selected for scanning using SIS includes the key ions in the spectra of a compound of interest. This operation mode usually increases the sensitivity for the selection ions and conducts to lower detection limits. However, it provides limited qualitative information because a characteristic mass spectrum for the target compounds is not produced.

The mass spectrometry can also be applied to obtain information even when the compounds are not completely separated. In fact, the nature of the MS detector allows the quantification of two co-eluting compounds as long as their mass fragments are different (McMaster, 2011).

2 State of the Art

This section summarizes the methods used for the determination of musk compounds in environmental matrices and in personal care products. It includes instrumental aspects, procedures for extraction and clean-up, as well as musk concentration data.

Although synthetic musks have been used in large scale in the last decades, it was only in recent years they became a topic of great concern, representing a broad class of new micropollutants. Their lipophilic nature along with the vast use in consumer products, make them an interesting subject to study. In Tables 4-7 some studies conducted in recent years are summarized, both in environmental matrices and in consumer products.

2.1 Water matrices

Different extraction methodologies have been used to determine synthetic musks in water samples. The main difficulty in analyzing these compounds in water samples is the concentration at which they may be present (ng/L-µg/L), turning pre-concentration of the sample necessary. Additionally, there is a great risk of sample contamination due to the widespread use of these compounds in daily life. Nevertheless, this issue may be overcome by using blank samples (Bester, 2009). Furthermore, the nature of water (surface, drinking, groundwater and wastewater) also determines the type of methodology to be applied due to the coexistence of other substances which may interfere in the analysis, such as organic matter, surfactants, inorganic salts, etc. An overview of the analytical methods for musks in water matrices is given in Table 4.

Synthetic musk fragrances tend to adsorb on suspended particles in water samples because of their hydrophobicity. So, filtration of samples prior to the extraction may cause the lost of the analytes. For that reason, musks present in waters containing suspended material should be extracted using a methodology that does not require filtration. Therefore, traditional liquid-liquid extraction (LLE) without sample filtration has been used to measure the total adsorbed and dissolved analytes. The extraction of musks ketone, xylene, galaxolide and tonalide has typically been performed using dichloromethane and n-hexane (solvents for which musks have greater affinity) (Lee et al., 2010, Reiner and Kannan, 2010). The studies found in literature presented recoveries of 70-100% and quantification limits between 0.4 and 50 ng/L (LOQ slightly higher for this type of matrices). Unlike the previous authors, Yang and Metcalfe (2006) chose to filter the samples before the LLE procedure. The author explains that the detected levels were lower in comparison to other works possibly due to this procedure. Musks ambrette, xylene, moskene, tibetene, ketone, cashmeran, celestolide, phantolide, traseolide, galaxolide and tonalide were the musks analysed by these two authors. It is important to notice that LLE is difficult

to automate, requires large volumes of organic solvents and is time-consuming. Therefore, other alternative methodologies have emerged in order to overcome these disadvantages.

Another classic approach is the application of solid phase extraction (SPE). Actually, it is the most commonly used technique to extract musks from water matrices. Authors prefer this technique because it is well suited for extracting and concentrating many compounds that display a wide range of polarities and physico-chemical properties. This technique has been used to analyse nitro musks (musks ketone and xylene), polycyclic musks (galaxolide, tonalide, phantolide and celestolide), as well as, one macrocyclic musk (pentadecanolide). The main drawback of this technique is the use of large amounts of solvents, although lower than those used so far in LLE.

Two types of SPE configurations are usually used: disks and cartridges. SPE disks combine the advantages of membranes and solid-phase extraction and therefore, pre-filtration of the samples is no longer necessary. Most studies applied C_{18} disks to determine musks in surface water (Zhang et al., 2008, Hu et al., 2011), groundwater (Chase et al., 2012) and even wastewater (Chen et al., 2007, Zhang et al., 2008, Chase et al., 2012). When C_{18} disks were applied to extract musks, the authors used to elute mixture of n-hexane and dichloromethane (1:1) (Chen et al., 2007, Zhang et al., 2008) or acetone and n-hexane (1:1) (Chase et al., 2012) (solvents similar to those applied in LLE).

All authors who report the use of cartridges, have previously filtered the samples through paper or glass fiber filters to remove coarse particles and suspended solids. As mentioned before, this can be disadvantageous since musks tend to adhere to particles, leading to an erroneous measurement of musks sample levels. In case of using SPE cartridges, HLB, appropriate for either hydrophilic or lipophilic compounds (Moldovan, 2006, Sumner et al., 2010, Villa et al., 2012) and functionalized styrene divinylbenzene appropriate for non-polar compounds (Quednow and Püttmann, 2008) are the most employed sorbents. The choice of the elution solvents depends on the interactions of the compounds with the stationary phase – for example the polarity of compounds. Elution solvent mixtures varied within authors, but n-hexane/ethyl acetate (2:1), ethyl acetate/dichloromethane/methanol (2:2:1) and acetonitrile/dichloromethane (1:1) were the most used solvents.

The limits of detection found for surface water are usually low (0.5-5 ng/L) and the recovery rates did not differ significantly within the studies (62-106%). For wastewaters, the detection limits are low, but higher than those obtained for surface waters (1-120 ng/L). For example, Chen et al. (2007) reported a range of 60-120 ng/L for this parameter. It is important to notice that only one study was found about musks extraction from groundwater by SPE. In that case, the results were also similar.

Apart from SPE, other techniques have been reported for the extraction of musk fragrances in water. Solid-phase microextraction (SPME) is also applied. SPME uses a polymer-coated fiber to extract volatile and semi-volatile compounds in just one extraction step. This technique minimizes the disadvantages of the former mentioned conventional methods, such as high solvent amount and time

consumption (as it can be automated), and at the same time it allows to achieve low detection limits. One drawback, however, is related to low reproducibility in some cases. To the author's knowledge, only one SPME study has been published for the extraction of musks from aqueous matrices. Wang and Ding (2009) developed a microwave-assisted solid-phase microextraction method (MA-HS-SPME) to determine the concentrations of polycyclic musks in an industrial wastewater in Taiwan. Obtained LODs ranged 0.05-0.1 ng/L and recoveries were 64-102%. The results were similar to those reported for SPE based extraction methods.

SBSE is also based on sorptive extraction, i.e. in equilibrium processes. This technique has the advantage of being easily applicable, robust and reproducible, allowing the use of high sample volumes, which improves the extraction efficiency. The major disadvantage of SBSE is that commercial stir bars are coated with polydimethylsiloxane polymer (PDMS), which is specific for non-polar compounds. Therefore, when the goal is to extract polar compounds, other phases have to be developed. Another handicap is the fact that only a few solvents are compatible with PDMS and can be adopted for analyte desorption.

Two studies with SBSE extraction were found for surface and groundwater. Chase et al. (2012) filtered the samples and performed the extraction with acetone/hexane. Arbulu et al. (2011) analyzed the highest number of compounds of interest (5 nitro, 6 polycyclic, 5 macrocyclic, and 2 alicyclic musks) using SBSE followed by automated thermal desorption (ATD). This last study also examined the musks content in wastewaters, along with Ramirez et al. (2011) who also carried the extraction with PDMS coated stir bars and reached low LODs (0.02-0.3 ng/L), with recoveries ranging between 87-95%. This technique also conducts to low detection limits and high recovery levels.

Environmental friendly techniques are in vogue, therefore novel alternatives are being developed with focus on reducing environmental impact of the analytical procedure. Miniaturization is such an alternative. It allows the reduction in organic solvent volumes and consequently minimizes the environmental impact. A typical example of miniaturization for sample preparation is dispersive liquid-liquid microextraction (DLLME). In this technique, the extraction efficiency is maximized by the high contact area between organic extraction solvent and aqueous sample provided by fine droplets dispersed in the medium. This improves the transition of analytes from the aqueous to the organic phase. In addition, the extraction solvent volume is reduced as well as the time required for analysis. The major disadvantage of DLLME is the use of a disperser solvent, which typically decreases the partition coefficient of analytes into the extraction solvent. According to the literature review carried out in this work, the limits of detection of this method for nitro (musks tibetene, moskene, xylene, ambrette and ketone) and polycyclic musks (celestolide, phantolide, traseolide, galaxolide and tonalide) ranged between 4–63 ng/L for surface water. The recoveries obtained for different water samples (sea, river, irrigation channel and water treatment plant) ranged between 77 and 116% (Panagiotou et al.,

2009, Lopez-Nogueroles et al., 2011). To extract musks from water samples Lopez-Nogueroles et al. (2011) used acetone and chloroform while Panagiotou et al. (2009) used methanol and carbon tetrachloride. The first author also analyzed wastewaters and verified LODs of 4-33 ng/L and recoveries of 87-116% for musks tibetene, moskene, xylene, ambrette and ketone. In general, the detection limits were superior to those obtained by SPE and SPME.

Membrane-assisted solvent extraction (MASE) was another alternative technique tested for musks extraction. MASE is based on small-scale LLE, where a non porous polymeric membrane containing an organic phase, act as a selective barrier between two extraction phases (De Jager et al., 2009). This technique allows handling very complex matrices especially for the pre-concentration of trace organic compounds from water matrices. Besides, MASE requires low volumes of organic solvents (400–1000 µL) and medium sample volumes (10–150 mL) for achieving a high sensitivity at the ng/L level. Posada-Ureta et al. (2012) used MASE together with a large volume injection (LVI) in a programmable temperature vaporization (PTV) injector for the quantification of ten synthetic musks (musks ambrette, ketone, moskene, xylene, celestolide, phantolide, tonalide, traseolide, cashmeran and galaxolide) in surface water samples. Low density polyethylene membrane bags (LDPE) filled with 200 µL of n-hexane, were used to extract the musk fragrances from the matrix. Low detection limits (between 4 and 25 ng/L) and good recoveries (83-108%) were achieved using this technique. However, these results may be misleading since the membrane functions as a filter retaining particles, not allowing the quantification of musks adhered to them. The former mentioned variety of analytical techniques allows obtaining an overview of typical levels in different aqueous matrices which are summarized in Table 4.

According to the table, synthetic musks are commonly found in high levels in sewage waters (8-549680 ng/L) and wastewaters (1-32060 ng/L), which indicates that treatment processes in most WWTPs are not efficient to remove such compounds. In surface waters are also detected moderate levels of musks (0.03-2544 ng/L), particularly in sampling points near WWTP discharges (Chase et al., 2012). These compounds have been found in a lower concentration levels in groundwater. Therefore, musks seem to migrate along the entire water cycle. To the author's best knowledge, there are no studies on the existence of musks in drinking waters.

Table 4 - Overview on analytical methods for determination of synthetic musks in water

Matrix	*Analytes*	*Extraction method*	*Analytical method*	*LOD (ng/L)*	*% Rec*	*Concentration (ng/L)*	*Ref.*
Surface water	MK, MX, HHCB and AHTN	LLE	GC-MS	5	86-88	0.03-2.72	Lee et al. (2010)
	HHCB and AHTN	LLE	GC-MS	LOQ: 1	85-98	3.95-25.8	Reiner and Kannan (2010)
	HHCB, AHTN and ADBI	SPE	GC–MS	0.05-0.25	>80	0.025-1141	Villa et al. (2012)
	AHTN, HHCB, ADBI, AHMI, ATII, MK and MX	SPE	GC-MS	1.0-1.2	79-106	2.3-120.6	Hu et al. (2011)
	HHCB, AHTN, AHMI, ADBI, MX, MK and Pentadecanolide	SPE	PTV-GC-MS	0.3-1.2	92-105	3-28	Sumner et al. (2010)
	AHTN and HHCB	SPE	GC-MS	3-5	n.a.	46-141	Quednow and Püttmann (2008)
	HHCB, AHTN, MX, MK, DPMI, ATII, AHMI and ADBI	SPE	GC-MS	1-2	62-83	8-93	Zhang et al. (2008)
	HHCB and AHTN	SPE	GC-MS	LOQ: 30	87-91	81-314	Moldovan (2006)
	HHCB, AHTN, DPMI, ADBI, AHMI, ATII, MX and MK	SPE SBSE	GC–MS	1	>50	112-794	Chase et al. (2012)
	DPMI, AHMI, ADBI, MA, ATII, HHCB, MX, AHTN, MM, MT, MK, Helvetolide, Globalide, Romandolide, Thibetolide, Muscone, Ambrettolide and Ethylene brassylate	SBSE–ATD	RTL–GC–MS	LOQ: 5-80	n.a.	41-2544	Arbulu et al. (2011)
	MT, MM, MX, MA and MK	DLLME	GC-MS	4–33	87-116	n.a.	Lopez-Nogueroles et al. (2011)
	ADBI, AHMI, ATII, HHCB and AHTN	DLLME	GC-MS	28–63	77-98	n.a.	Panagiotou et al. (2009)
	MA, MK, MM, MX, ADBI, AHMI, AHTN, ATII, DPMI, HHCB	MASE	LVI-PTV-GC–MS	3-25	83-108	41	Posada-Ureta et al. (2012)
Groundwater	HHCB, AHTN, DPMI, ADBI, AHMI, ATII, MX and MK	SPE SBSE	GC–MS	1	>50	72	Chase et al. (2012)
	DPMI, AHMI, ADBI, MA, ATII, HHCB, MX, AHTN, MM, MT, MK, Helvetolide, Globalide, Romandolide, Thibetolide, Muscone, Ambrettolide and Ethylene brassylate	SBSE–ATD	RTL–GC–MS	LOQ: 5-80	n.a.	270-573	Arbulu et al. (2011)

PTV - Programmable temperature vaporization.

Table 4 - Overview on analytical methods for determination of synthetic musks in water *(cont.)*

Matrix	*Analytes*	*Extraction method*	*Analytical method*	*LOD (ng/L)*	*% Rec*	*Concentration (ng/L)*	*Ref.*
Wastewater	MK, MX, HHCB and AHTN	LLE	GC-MS	10	86-88	Influent: 3690-7330 Effluent: 960-2690	Lee et al. (2010)
	HHCB and AHTN	LLE	GC-MS	LOQ: 50	85-87	Influent: 43-7000 Effluent: 10-230	Horii et al. (2007)
	MA, MX, MM, MT, MK, PDMI, ADBI, AHMI, ATII, HHCB and AHTN	LLE Clean-up: silica gel column	GC-MS	LOQ: 0.4-4.0	70-100	Influent: 4.8-390.2 Effluent: 2.7-173.1	Yang and Metcalfe (2006)
	HHCB, AHTN, DPMI, ADBI, AHMI, ATII, MX and MK	SPE	GC–MS	4	>50	Influent: 45-13399 Effluent: 129-10525	Chase et al. (2012)
	HHCB, AHTN, AHMI, ADBI, MX, MK and Pentadecanolide	SPE	PTV-GC-MS	1.1-8.0	92-105	Effluent: 4-2098	Sumner et al. (2010)
	HHCB, AHTN, MX, MK, DPMI, ATII, AHMI and ADBI	SPE	GC-MS	1-2	62-83	Influent: 2300 Effluent: 300	Zhang et al. (2008)
	DPMI, ADBI, AHMI, ATII, AHTN and HHCB	SPE	GC-MS	60-120	57-108	Influent: 4700-549680 Effluent: 60 - 32060	Chen et al. (2007)
	HHCB, AHTN, ADBI, ATII, DPMI and AHMI	MA-HS-SPME	GC-MS	0.05-0.1	64-102	Effluent: 1.2-37.3	Wang and Ding (2009)
	DPMI, AHMI, ADBI, MA, ATII, HHCB, MX, AHTN, MM, MT, MK, Helvetolide, Globalide, Romandolide, Thibetolide, Muscone, Ambrettolide and Ethylene brassylate	SBSE–ATD	RTL–GC–MS	LOQ: 5-80	n.a.	Influent: 40-3568 Effluent: 21-3021	Arbulu et al. (2011)
	DPMI, ADBI, AHMI, ATII, HHCB, AHTN, MX, MM and MK	SBSE	TD–GC–MS	0.02-0.3	87-95	Influent: 8-2069 Effluent: 4-1432	Ramirez et al. (2011)
	MT, MM, MX, MA and MK	DLLME	GC-MS	4–33	87-116	n.a.	Lopez-Nogueroles et al. (2011)

PTV - Programmable temperature vaporization; RTL - Retention time locked systems; TD - Thermal desorption; n.a. - Not available.

2.2 Sludge, sediments, dust and biota

As mentioned before, synthetic musk compounds are very lipophilic, tending to accumulate in sludge, sediments, dust and biota (Bester, 2009). Analysis of these matrices are more challenging because of its greater complexity, containing more potential interferents and due to necessity to extract the better retained musks.

Most studies found in literature focus on the determination of musks in sludge from WWTPs, where the highest levels are expected. The most traditional technique is soxhlet extraction. This method was used to extract polycyclic musks with dichloromethane, hexane or mixtures of these two solvents, followed by a clean-up in a silica and alumina column (Zeng et al., 2005, Chen et al., 2007, Shek et al., 2008). The limits of detection varied considerably (0.05-120 ng/mL) as well as the recovery rates (48-109%). It should be noted that soxhlet extraction has a high consumption of solvents and heating may degrade more thermolabile compounds. In addition, this method might co-extract interferents, which is disadvantageous to the analysis.

Modern extraction technologies, such as ultrasonic solvent extraction (USE), microwave assisted extraction (MAE), supercritical fluid extraction (SFE) and accelerated solvent extraction (ASE), can improve compound recovery by enhancing the kinetics of compound extraction, in comparison with soxhlet extraction techniques. However, the high cost makes the acquisition of this equipment very difficult for the majority of laboratories that continue to use traditional based solvent extraction methods.

USE was used to extract galaxolide and tonalide from sludge samples. The detection limits found are high (180-250 ng/g) and this is mainly due to the large amount of interferents present in the matrix. The recovery rates did not differ significantly (61-87%). Zhou et al. (2009) opted to extract the samples with methanol/H_2O and then hexane/acetone, and sludge samples were previously filtered with glass fiber filters. Other authors chose to use only methanol and acetone to extract unfiltered samples (Ternes et al., 2005, Carballa et al., 2008). These last two studies made the best decision, by not filtering the samples, and may well account for musks adsorbed to coarse particles. USE has some disadvantages, as it might cause de degradation of sample compounds and has limited extraction efficiency.

MAE, as well as SFE, have been used by Smyth et al. (2007) to determine polycyclic and nitro musks in sludge samples. LODs of 3-41 ng/g and recoveries of 70-113% were obtained. Heating is one of the drawbacks of MAE, as it might be inhomogeneous and cause degradation of more thermolabile compounds. In addition, the samples are subjected to interferences of microwave energy absorbing materials and it requires filtration after extraction.

Guo et al. (2010) used ASE to analyze six polycyclic musks and five nitro musks in dewatered sludge samples from municipal, livestock and pharmaceutical WWTPs. Whereas, Hu et al. (2011) used

this technique to measure tonalide, galaxolide, celestolide, phantolide, traseolide, musks ketone and xylene in samples from sediments. The occurrence of synthetic musks in indoor dust was also investigated (Fromme et al., 2004). LODs for this method varied between 0.004 and 10 ng/g. High recoveries were obtained, ranging between 71-105%. As other techniques, it also has its drawbacks. Usually, the extraction temperature performed in ASE is high, which may lead to degradation of thermolabile compounds.

Apart from soxhlet and the more modern extraction techniques other methods have been applied. Wu and Ding (2010) determined six polycyclic musks in sewage sludge and sediments by microwave-assisted headspace solid-phase microextraction (MA-HS-SPME). The LODs found for this method (0.04-0.1 ng/g) were low and high recoveries were assured (85-96%). HS-SPME technique presents some disadvantages. This method is limited to semi-volatile compounds and requires relatively long sampling times with low sensitivity and reproducibility.

Matamoros et al. (2012b) used a traditional extraction with hexane to determine the concentrations of galaxolide, tonalide, cashmeran and celestolide in biosolids in a Sludge Treatment Reed Bed system. Lu et al. (2011b) also used extraction with hexane to study two polycyclic musks and three nitro musks in indoor-dust samples from homes, dormitories, offices, and laboratories. The LODs were higher than those obtained by SPME.

Chase et al. (2012) also analyzed sediments samples through SBSE. Stir-bars were extracted with acetone/hexane and samples were filtered prior to extraction. For sediments, a relatively high LOD and low recovery rate were obtained. SBSE is a very simple, solvent-free, fast technique with high sensitivity. Unfortunately, until now, the stir bar, which is only available in PDMS coating, offers a limited enrichment capability for polar compounds.

Synthetic musks were detected at high concentrations in sewage sludge (0.326-512450 ng/g), especially in samples from WWTPs attached to cosmetic plants (Chen et al., 2007). The levels detected in sediments were much lower (0.049-32.3 ng/g). In the case of dust, no correlation was found between these samples and those of sludge or sediments (4.42-11400). In Table 5 are summarized some of the most recent studies.

Besides the environmental matrices previously referred, several studies have also reported the occurrence of synthetic musks in aquatic organisms (Gooding et al., 2006, Pedersen et al., 2009) but also in human blood (Hutter et al., 2009, Hutter et al., 2010), fat (Schiavone et al., 2010, Moon et al., 2012), and breast milk (Wang et al., 2011, Zhang et al., 2011). Due to their ubiquitous occurrence, bioaccumulation, and toxic potential, synthetic musks are of concern from human exposure point of view. Therefore, a small overview is also present in Table 5.

Table 5 - Overview on analytical methods for determination of synthetic musks in solid environmental samples

Matrix	*Analytes*	*Extraction method*	*Analytical method*	*LOD (ng/g)*	*% Rec*	*Concentration (ng/g)*	*Ref.*
Sludge	DPMI, ADBI, AHMI, ATII, AHTN and HHCB	Soxhlet extraction Clean-up: silica and alumina column	GC-MS	0.05-0.15 ng/mL	75-101	351-78600	Shek et al. (2008)
	DPMI, ADBI, AHMI, ATII, AHTN and HHCB	Soxhlet extraction Clean-up: silica and alumina column	GC-MS	60-120 ng/mL	48-108	1780-512450	Chen et al. (2007)
	HHCB and AHTN	Soxhlet extraction	GC-MS	20-50	72-98	20–36000	Horii et al. (2007)
	DPMI, ADBI, AHMI, ATII, AHTN and HHCB	Soxhlet extraction Clean-up: silica and alumina column	GC-FID GC-MS	50-100 ng/mL	49-109	192-21214	Zeng et al. (2005)
	HHCB and AHTN	USE	GC-MS	180-200	61-75	700-17000	Zhou et al. (2009)
	HHCB and AHTN	USE	GC-MS	250	64-74	6600-22800	Carballa et al. (2008)
	HHCB and AHTN	USE PLE Clean-up: silica gel	GC-MS	250	78-87	2300-15000	Ternes et al. (2005)
	DPMI, ADBI, AHMI, ATII, HHCB, AHTN, MA, MX, MM, MT and MK	SFE or MAE Clean-up: LLE	GC-MS	3-41	70-113	126-19800	Smyth et al. (2007)
	HHCB, AHTN, ADBI, ATII, DPMI and AHMI	MA-HS-SPME	GC–MS	0.04-0.1	85-96	300 - 10900	Wu and Ding (2010)
	HHCB, AHTN, ADBI, AHMI, ATII, DPMI, MA, MX, MM, MT and MK	ASE Clean-up: silica gel column	GC-MS	3-10	71-97	250-82000	Guo et al. (2010)
	HHCB, AHTN, DPMI and ADBI	Extraction with hexane	GC-MS	2-10	>90	n.a.	Matamoros et al. (2012b)
Sediments	HHCB, AHTN, ATII, ADBI, AHMI, MX and MK	Soxhlet extraction Clean-up: silica gel	GC-MS	0.025-5.1	63-86	0.049-16	Peck et al. (2006)
	HHCB, AHTN, DPMI, ADBI, AHMI, ATII, MX and MK	SBSE	GC–MS	1	>50	10.24-24.12	Chase et al. (2012)
	AHTN, HHCB, ADBI, AHMI, ATII, MK and MX	ASE	GC–MS	0.25-0.33	84-105	1.5–32.3	Hu et al. (2011)
	HHCB, AHTN, ADBI, ATII, DPMI and AHMI	MA-HS-SPME	GC–MS	0.04-0.1	85-96	0.3 - 10.9	Wu and Ding (2010)
Dust	HHCB, AHTN, MK, MM and MX	Extraction with hexane	GC-MS	0.6-2	80-97	4.42 – 688	Lu et al. (2011b)
	HHCB, AHTN, ADBI, ATII, AHMI, DPMI, MK and MX	ASE	GC-MS	0.004-0.30	78-96	3100-11400	Fromme et al. (2004)
Biota	AHTN and HHCB Freshwater mussel *Lampsilis cardium*	Extraction with cyclohexane	GC-MS	n.a.	n.a.	281-1750 µg/L	Gooding et al. (2006)
	HHCB Gastropod *Potamopyrgus antipodarum*	Extraction with dichloromethane	GC-MS	n.a.	64-86	86-64200	Pedersen et al. (2009)
	HHCB Polychaete *Capitella* species I	ASE - Extraction with dichloromethane	GC-MS	n.a.	n.a.	1.46-168.19 mg/kg	Ramskov et al. (2009)

LOD- Limit of detection; FID - Flame ionization detector; USE – Ultrasonic solvent extraction; PLE - Pressurized liquid extraction; n.a. - Not available.

2.3 Air samples

Musks volatilization from large contaminated water basins proved to be an important source of these compounds to the atmosphere. However, volatilization during use and production of personal care products, from landfills and from wastewater treatment plants are other sources of these compounds to the atmosphere (Peck, 2006). In the last years, some studies have been developed about this issue. A small overview is present in Table 6.

To analyse air it is typically necessary to do an active sampling, using polyurethane foam (PUF) impregnated with polymeric resins (e.g. XAD-2). In active sampling, a ventilator is used to force a known amount of air through an adsorbent, whereas in passive sampling a suitable sampler containing an adsorbent is placed at the site for a period of time. Passive sampling allows obtaining overall pollution data while active sampling is typically used to obtain data regarding a specific period.

Usually, prior to the PUF is a glass fiber filter (GFF) to collect the particulate phase, whereas PUF adsorb the contaminants from the gaseous phase (Kallenborn and Gatermann, 2004). However, this process requires the removal of the target analytes from the adsorbent materials before the sample analysis. To perform this task, it is usual to do Soxhlet extraction using dichloromethane, mixtures of hexane/acetone or hexane/diethyl ether. After that, a clean-up step is most of the times required, in order to remove interferences.

Six polycyclic (cashmeran, celestolide, phantolide, traseolide, tonalide and galaxolide) and two nitro (musks xylene and moskene) musks have been collected from wastewater aeration basins and outdoor and indoor air by active sampling through PUF (Chen et al., 2007, Upadhyay et al., 2011). According to data present in Table 6, synthetic musks seem to be in lower concentrations in the outdoor air (dilution effect, UV or ozone degradation, etc.).

Table 6 – Some studies about synthetic musk fragrances in air samples

Matrix	*Analytes*	*Extraction method*	*Analytical method*	*LOD (ng/m^3)*	*% Rec*	*Concentration (ng/m^3)*	*Ref.*
Wastewater aeration basins	HHCB, MX, MK, AHTN and ADBI	GFF/PUF and solvent extraction	GC-MS	0.03-0.6	n.a.	2 - 344306	Upadhyay et al. (2011)
Indoor air	DPMI, ADBI, AHMI, ATII, HHCB, MX, MM and MK	Active sampling SPE-SPME	GC-MS	0.029-0.380	85-103	2.6 – 1129	Regueiro et al. (2009)
Industrial air	DPMI, ADBI, AHMI, ATII, AHTN and HHCB	GFF/PUF and Soxhlet extraction Clean-up: silica-alumina column	GC–EI-MS	0.06-0.12 µg/mL	48-108	Inside the cosmetic plant: 5416.07 Outside the cosmetic plant: 14.89	Chen et al. (2007)
Urban and rural air	HHCB, AHTN, ATII, AHMI, ADBI, DPMI, MX and MK	PUF and Soxhlet extraction Clean-up: florisil column	GC-EI-MS	0.00029-0.056	n.a.	10000 - 800000	Peck and Hornbuckle (2006)
Indoor and outdoor air	HHCB, AHTN, ATII, MX, MK	GFF/PUF and Soxhlet extraction	GC-EI-MS GC-NCI-MS	0.004-0.045	n.a.	Indoor air: 0.3 - 44 Outdoor air: 0.006 – 0.119	Kallenborn and Gatermann (2004)

n.a. - Not available.

2.4 Personal care products

As mentioned before, personal care products are the main source of synthetic musks. Therefore, it became essential to study the concentrations and distributions of these compounds in personal care products and household commodities in order to evaluate exposure to musks. Although in-house analytical methods for manufacturers' quality control may exist, few studies regarding the analysis of musks in PCPs were published. In Table 7 the studies found about extraction and analysis of musks in consumer products are summarized.

Lu et al. (2011a) analyzed personal care products (toothpastes, hair care products, body washes, toilet soaps, skin moisturisers, and makeup products) in China and followed the procedure reported by Reiner and Kannan (2006) with some modifications. Briefly, weighted samples were extracted using ultrasonic assisted extraction and n-hexane, followed by ethyl acetate/n-hexane. Clean-up was performed using silica gel SPE cartridges and n-hexane and dichloromethane were used as eluents. It was observed that more than 80% of the samples contained at least one of the synthetic musks, and their total concentrations were as high as 1.02 mg/g. The authors concluded that galaxolide was the predominant musk in all of the samples analyzed. Recoveries ranged between 81.7 ± 7.0% and 91.6 ± 10.3%. Reiner and Kannan (2006) used a similar procedure, but have not applied the last clean-up step with SPE. It was found that galaxolide and tonalide concentrations ranged from <5 ng/g to over 4000 μg/g and <5 ng/g to 451 μg/g, respectively. The authors also concluded that the highest concentrations of musks were in perfumes, body creams and lotions, and deodorants. Roosens et al. (2007) followed a similar procedure, but apart from n-hexane it was also used water to extract the samples. Besides that, the samples were vortexed rather than being sonicated. The organic phase was passed through a clean-up silica column and the musks were eluted with dichloromethane. Maximum concentrations of galaxolide, tonalide, musks xylene and ketone were 22 mg/g, 8 mg/g, 26 μg/g and 0.5 μg/g, respectively. It was also concluded that synthetic musks were majority present in perfumes, lotions, sanitation products and deodorants. Zhang et al. (2008) extracted the samples with n-hexane and then the combined organic layers were concentrated and cleaned up by a silica/alumina column. It was verified that the highest mean concentrations ($8.04x10^5$ ng/g) of galaxolide was found in perfume, while the highest tonalide concentration ($4.69x10^4$ ng/g) was detected in shampoo. The average concentrations in body washes, shampoos and laundry detergents were $5.9x10^4$, $5.2x10^4$, $2.4x10^4$ ng/g for galaxolide and $2.5x10^3$, $1.2x10^4$, $8.8x10^3$ ng/g for tonalide, respectively.

Sanchez-Prado et al. (2011) investigated commercial perfumes and colognes with the purpose of verifying if these products were in accordance with the recent changes in European legislation, regarding the maximum allowed concentrations of the ingredients. All samples were clear liquids and no special pre-treatment was applied apart from homogenization and dilution with ethyl acetate. The data

obtained allowed the authors to confirm the trend about the replacement of nitromusks by polycyclic musks, as well as the introduction of macrocyclic musks in the perfumes composition. It was also noticed that the prohibited musk moskene was present in one sample in an appreciable concentration. Recoveries were satisfactory in all cases, with a minimum of 88.6% and a maximum of 110%.

Unlike other authors who used GC-MS as analytical technique, Martinez-Giron et al. (2010) developed a capillary electrophoresis method to separate and quantify chiral polycyclic musks in perfumes samples. However, the authors only injected a perfume sample containing 14.5 mg/g galaxolide and 9.6 mg/g tonalide. LODs and LOQs obtained for the three musks which could be simultaneously analyzed, galaxolide, tonalide and traseolide, ranged between 8-49 mg/L.

A thorough bibliographic research of the state of the art regarding the presence of musks in PCPs was performed. Based on this research it was verified that none of the mentioned methods allows the simultaneous analysis of the three classes of musks chosen: nitro (musks xylene, ketone, tibetene, moskene and ambrette), polycyclic (galaxolide, tonalide, cashmeran, celestolide and phantolide), and macrocyclic musks (ethylene brassylate and exaltolide). All this, led to the development of a new method.

Initially, the QuEChERS method was developed for the analysis of pesticides in food. Therefore, application of this kind of technique to other matrices and analytes may widen the application field of this convenient extraction and clean-up technique. QuEChERS is advantageous due to a series of reasons: it involves low amounts of solvent, which compared to former mentioned solvents (chloroform, dichloromethane, n-hexane, etc.) is less toxic and less pollutant, there is a reduced risk of sample contamination as most labware is disposable, it is easier to handle allowing the development of a high throughput method. Additionally, the method presents comparable lower costs of analysis, which reduces overall cost of analyzing a large sample pool. Those were the main reasons that conducted our choice to the extraction methodology of QuEChERS, which is completely new regarding their application to the analysis of musks.

Table 7 - Overview on analytical methods for determination of synthetic musks in cosmetics

Matrix	*Analytes*	*Extraction method*	*Analytical method*	*LOD (ng/g)*	*% Rec*	*Concentration (µg/g)*	*Ref.*
Personal care products *(Toothpastes, hair care products, body washes, toilet soaps, skin lotions, and makeup products)*	HHCB, AHTN,MX and MK	Extraction with hexane SPE	GC-MS	LOQ: 0.5-3.01	81.7-91.6	5×10^{-4} – 1020	Lu et al. (2011a)
Household commodities *(Perfume, bath gel, liquid hand soap, liquid facial soap, shampoo, fabric softener and detergent, toothpaste and facial/body cream)*	HHCB, AHTN, MX, MK, DPMI, ATII, AHMI and ADBI	Extraction with hexane	GC-MS	2-3	62-83	2.5 – 804	Zhang et al. (2008)
Personal care products *(Body lotions, perfumes, deodorants, hair care, shower and sanitation products)*	AHTN, HHCB, MK and MX	Extraction with hexane and water	GC-MS	3-17	74-138	1000 - 22000	Roosens et al. (2007)
Household commodities *(Perfumes, lotions, hair care products and household cleaners)*	HHCB and AHTN	Extraction with hexane	GC-MS	5	123	5 - 4000	Reiner and Kannan (2006)
Perfumes	DPMI, ADBI, AHMI, MA, ATII, HHCB, MX, AHTN, MM, MT, MK and Ambrettolide	Homogenization Dilution with ethyl acetate	GC-MS	1.9×10^{-7} - 1.1×10^{-6} % (w/v)	93-110	6.5×10^{-4}-1.6% (w/v)	Sanchez-Prado et al. (2011)
	HHCB, AHTN, ATII and AHMI	Extraction with hexane SPE	Capillary electro-phoresis	8-49 mg/L	n.a.	9600-14500	Martinez-Giron et al. (2010)

LOQ – Limit of quantification; n.a. - Not available.

2.5 Aim of the thesis

This research was divided into four different objectives:

- Analytical method development and optimization
  - Development of a GC-MS analysis method suitable for the three classes of musks studied (nitro, polycyclic and macrocyclic musks). The selected musks for this work were musks xylene, ketone, moskene, tibetene and ambrette, galaxolide, tonalide, cashmeran, celestolide, phantolide, exaltolide and ethylene brassylate.
  - Testing and optimization of a novelty application of the QuEChERS extraction method to musks in personal care products.

- Method validation - through quantification parameters like linearity, limits of detection and quantification, and sensitivity and reliability parameters as precision and accuracy.

- Musks monitoring in personal care products - Analysis of the concentration levels and distribution of the 12 synthetic musk compounds in variety of personal care products. These products were divided into five categories: skin moisturisers, toothpastes, deodorants, toilet soaps, body and hair washes.

- Assessment of human exposure to these contaminants through dermal application. For this purpose, distribution data of musks in the investigated personal care products will be combined with the daily usage data (obtained by a literature review) of these products.

3 Technical Description

3.1 Chemicals and reagents

Acetonitrile and cyclohexane, used to prepare stock solutions and as organic solvents in the QuEChERS procedure, were purchased from VWR (Fontenay-sous-Bois, France). Magnesium sulphate and sodium acetate from Sigma-Aldrich (St. Louis, MO, USA), PSA bonded silica and DSC-18 from Supelco (Bellefonte, PA, USA) were also used for the QuEChERS procedure. All organic solvents and reagents used in this study were analytical grade. Commercial QuEChERS were obtained from UCT with the references ECMSSA50CT and ECMPSC1815CT.

The solid standards of synthetic fragrances 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[g]-2-benzopyran (galaxolide) (75% purity), 7-acetyl-1,1,3,4,4,6-hexamethyltetraline (tonalide), 6,7-dihydro-1,1,2,3,3-pentamethyl-4(5H)-indanone (cashmeran), 4-acetyl-1,1-dimethyl-6-tert-butylindane (celestolide), 6-acetyl-1,1,2,3,3,5-hexamethylindane (phantolide) were obtained from LGC Standards (Barcelona, Spain) with 99% purity, except for galaxolide which contains approximately 25% of diethyl *phthalate* (*DEP*). 1-(tert-butyl)-3,4,5-trimethyl-2,6-dinitrobenzene (musk tibetene) and 1,1,3,3,5 pentamethyl-4,6-dinitroindane (musk moskene) were purchased as 10 mg/L solution in cyclohexane from the same company. 2,6-dinitro-3-methoxy-4-tert-butyltoluene (musk ambrette) and 4-aceto-3,5-dimethyl-2,6-dinitro-tert-butylbenzene (musk ketone) were purchased as solid standards from Dr. Ehrenstorfer GmbH (Augsburg, Germany) with 99% and 98% purity, respectively. 2,4,6-trinitro-1,3-dimethyl-5-tert-butylbenzene (musk xylene) was obtained from Sigma-Aldrich (St. Louis, MO, USA) as 100 mg/L solution in acetonitrile. Oxacyclohexadecan-2-one (exaltolide) and 1,4-dioxacycloheptadecane-5,17-dione (ethylene brassylate) were also purchased from Sigma-Aldrich with ≥99% and ≥95% purity, respectively.

3.2 Standards preparation

For each polycyclic musk, an individual stock solution containing 13.3 g/L was prepared in cyclohexane. Similarly individual stock solutions of exaltolide and ethylene brassylate containing 10 g/L were prepared as well as 6.7 g/L musk ambrette and musk ketone solutions.

A 10 mg/L intermediate stock solution containing all polycyclic and macrocyclic musks, and musk ambrette and ketone was prepared by diluting appropriate amounts in acetonitrile. The final mixed stock solution was prepared by first evaporating an appropriate amount of musk tibetene and moskene solutions under a gentle stream of nitrogen. This step was followed by the addition of the necessary amounts of the former stock solution and of the musk xylene standard and makeup with

acetronitrile. Calibration standards (0.005 - 4 mg/L) were prepared in acetonitrile from the previously described stock solution. All solutions were stored and preserved at -20 ^{o}C.

3.3 Samples

A total of 41 personal care products were purchased from retail stores in Porto, Portugal, in 2012. The samples were divided into five different categories according to their overall composition: skin moisturisers (n = 12), toothpastes (n = 3), deodorants (n = 6), toilet soaps (n = 3), body and hair washes (n = 17). For each category, were selected branded products, considered to be the most used by the Portuguese population. A detailed description is given in Table 8.

Table 8 – Overview of the investigated personal care products, where "n" is the number of samples in each category

Categories		*n*	*Categories*		*n*
Skin moisturisers (n = 12)	Facial cream	3	Toilet soaps (n = 3)	Solid soap	3
	Body lotion	9			
Toothpastes (n = 3)	Paste tubes	3	Body and hair washes (n = 17)	Liquid hand soap	3
				Shower gel	8
				Shampoo	6
Deodorants (n = 6)	Roll-on deodorants	6			

3.4 Extraction and clean-up

The conditions provided below refer to optimal conditions. In the optimization phase, several conditions were tested, which will be introduced and discussed throughout the discussion of the results for reasons of better understanding of the text.

Sample extraction method consisted of weighing five hundred milligrams of each sample in a polypropylene tube and adding 3 mL of acetonitrile. Then, the samples were vortexed for 3 min and sonicated for 10 min. The first QuEChERS (2400 mg $MgSO_4$ and 750 mg $NaCH_3COO$) was added and the mixture was vortex for another 3 min. Samples were centrifuged at 3700 rpm for 10 min, and the solvent layer was transferred into the tube containing the second QuEChERS (180 mg $MgSO_4$, 60 mg PSA and 30 mg C_{18}). Once again, the samples were vortexed for 3 min and centrifuged at 3700 rpm for 10 min. The supernatant was collected into 1.5 mL amber glass vials. The extracts were concentrated to 1 mL under a gentle stream of nitrogen before GC-MS analysis. After the first round of analysis, some extracts were further diluted to an appropriate volume and reanalysed.

A simplified scheme of the procedure is shown in Fig. 13.

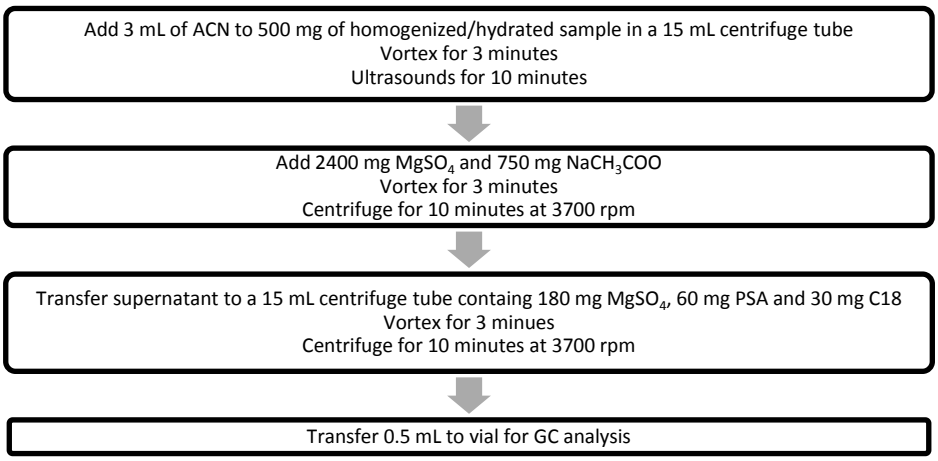


Fig. 13 – QuEChERS method flow chart.

3.5 Instrumental analysis

Synthetic musks were analysed by a Varian Ion Trap GC-MS system (Walnut Creek, CA, USA), equipped with a 450-GC gas chromatograph, a 240-MS ion trap mass spectrometer, a CP-1177 split/splitless injector, a waveboard for multiple MS (MS^n) analysis and an autosampler model CP-8410. The system was operated by Varian MS workstation v. 6.9.3 software.

The separation was carried out using a Varian CP-Sil 8 CB capillary column (50 m × 0.25 mm i.d., 0.12 µm) in combination with a FS deactivated pre-column (5 m × 0.530 mm i.d.) from Agilent Technologies (Palo Alto, CA, USA). The ion trap mass spectrometer was operated in the electron impact (EI) ionization mode (70 eV). For quantitative analysis of target compounds, selected ion monitoring (SIM) mode was applied. The temperatures of manifold, ion trap, and transfer line were maintained at 50, 250, and 250 ^oC, respectively. The filament emission current was 50 µA.

The carrier gas employed was helium with a purity of 99.999%, at a constant column flow of 1.0 mL/min. The GC oven temperature was programmed from 60 ^oC (hold for 1 min) to 150 ^oC at 6 ^oC/min (hold for 10 min), to 225 ^oC at 6 ^oC/min and, to 300 ^oC at 20 ^oC/min (total analysis time = 45 min). Splitless mode (hold for 5 min) was used for injection, and the injector temperature was kept at 250 ^oC. The injection volume was 1 µL.

The identification of the analytes was achieved by comparing their mass spectra and retention times to those of standards. The retention times as well as the identification and quantification ions for each target compound are listed in Table 9. Quantification ions are marked in bold.

Table 9 – Retention times, quantification and identification ions of the targets

Compound	*Retention time (min)*	*Target ions for GC–MS analysis*
Cashmeran	20.60	**191.2** + 163.2 + 135.2
Celestolide	30.04	**229.4** + 173.2 + 244.3
Phantolide	31.42	**229.4** + 187.2 + 171.6
Exaltolide	33.43	**81.1** + 83.1 + 67.2 + 69.2
Musk ambrette	33.58	**253.1** + 91.0 + 77.1
Galaxolide	34.06	**243.3** + 213.3 + 128.3
Musk xylene	34.29	**128.2** + 115.4 + 117.3
Tonalide	34.40	**243.3** + 159.2 + 128.3
Musk moskene	34.91	**263.2** + 128.4 + 115.2
Musk tibetene	36.05	**251.2** + 115.4 + 128.4
Musk ketone	37.07	**279.2** + 128.4 + 115.4
Ethylene brassylate	37.70	**83.1** + 98.1 + 227.2 + 125.5

3.6 Quality assurance and control

Synthetic musks are present in a large number of PCPs and the analysis of these compounds asks some discipline from the laboratory personnel in order to prevent contamination of samples. Cosmetics were avoided whenever possible for labcoats, and detergents used in the laboratories as well as in the personal use of the laboratory personnel. Room cleaning agents, such as floor polishers, window cleaners, etc. must also be taken into account. Other sources of contamination are sunscreen protection creams and lotions.

Procedural blanks were analyzed with every extraction batch. Trace levels of HHCB, DPMI and exaltolide were detected in procedural blanks. Blank values were subtracted for all of the concentrations reported. Limits of detection (LODs) and limits of quantification (LOQs) were calculated as the concentration giving a signal-to-noise ratio of three (S/N = 3) and of 10 (S/N = 10), respectively. Recovery tests were performed by spiking with a standard mixture containing the synthetic musks into selected products (one of each category) at three levels (100, 200 and 400 ng).

In this GC-MS methodology, an external standard calibration curve was used for musks quantification. In order to assess the response stability over time, a 400 $\mu g.L^{-1}$ standard control was injected daily.

3.7 Waste treatment

The waste generated in this work consisted in organic solutions containing acetonitrile and traces of musks and mixtures of different sorbents ($MgSO_4$, $NaCH_3COO$, PSA and C_{18}) contaminated with musks. All these residues were collected in closed containers, properly labelled, and they were stored protected from light and from ignition sources for further treatment by the Environmental Management System of FEUP - EcoFEUP.

4 Results and Discussion

4.1 Development of the chromatographic method

In this study, a chromatographic method to separate and identify 12 analytes belonging to the cosmetic additive family of synthetic musks was developed. The aim is to make possible the application of the chromatographic method to the analysis of this important group of regulated cosmetic ingredients in a single run.

Any chromatographic separation may be optimized by varying experimental conditions, such as the stationary phase, the injection temperature or the column temperature, until the components of a mixture are completely resolved in a minimum amount of time. In this work, the optimization of the chromatographic method by finding a more suitable chromatographic column was not an option, since the GC-MS apparatus was shared with other researchers; changing the injection temperature does not have such a significant effect on the separation of compounds, as the variation of the temperature of the oven. Temperature programming allows the effective separation of closely related compounds and it is based on an initial oven temperature, a rate of temperature variation (the temperature “ramp”) and a final temperature. An increase in temperature will increase the vapour pressure of the analyte reducing its interaction with the stationary phase of the capillary column. Therefore, during a temperature ramp, separation of compounds is mainly based on differences of boiling temperatures/vapour pressures. A temperature program shortens the required time for late-eluting analytes to pass through the column, while allowing the adequate separation of analytes that elute early in the analysis. This study, started from a temperature program which had been previously developed for a multi-residue analysis of micropollutants in which most of the musks were included.

Fig. 14 shows a chromatogram of the compounds analysed using the temperature program previously mentioned (program 1).

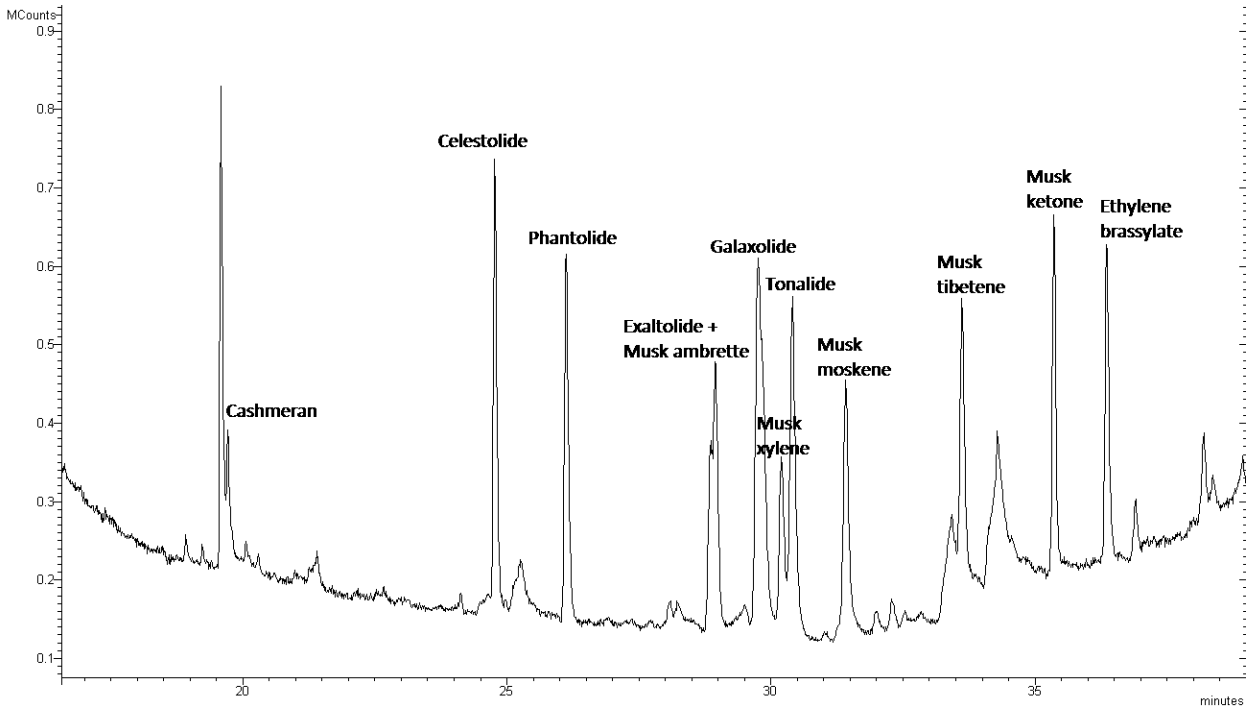


Fig. 14 – Full scan chromatogram of a standard solution of musks (1 mg/L in acetonitrile) by program 1.

However, this program was not developed for the analysis of macrocyclic musks and when tested, it was found that exaltolide co-eluted with musk ambrette. Thus, it was necessary to make modifications to the original program. Different temperature programs were tested to achieve the best possible separation conditions. The most relevant programs are displayed in Fig. 15.

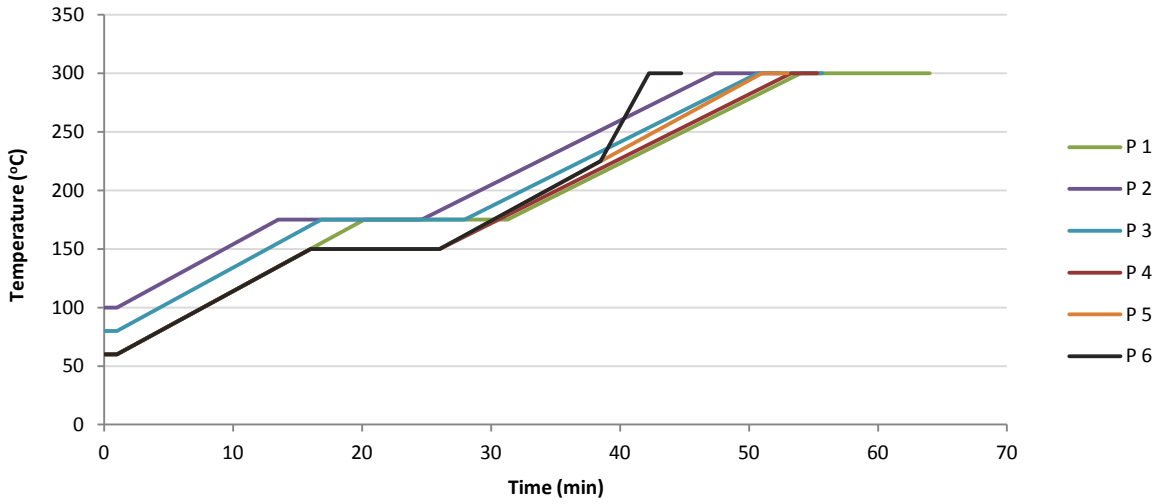


Fig. 15 – Graphical display of the most relevant temperature programs tested.

In programs 2 and 3, the initial temperature was increased and the retention times of the compounds were reduced, leading to the consequent decrease in analysis time. A higher initial temperature accelerates the output of the more volatile compounds, which allows achieving a better

separation. Still, the co-elution of exaltolide and musk ambrette was verified. Fig. 16 shows the chromatogram using program 3.

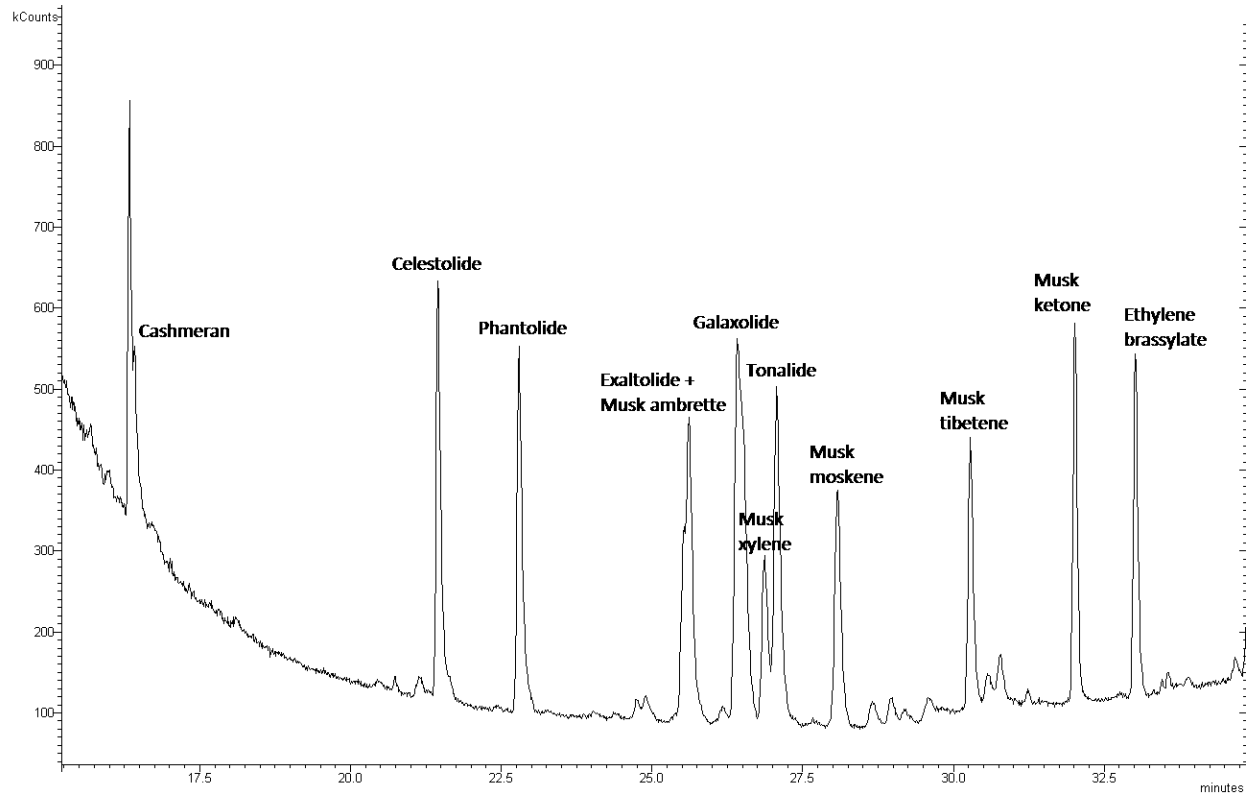


Fig. 16 – Full scan chromatogram of a standard solution of musks (1 mg/L in acetonitrile) by program 3, showing exaltolide and musk ambrette co-elution.

Starting again from the initial conditions, program 4 was developed, in which the *plateau* of temperature was reduced to 150 ^{o}C and consequently, retention times were increased. Under isothermal conditions, separation is mostly caused by distinct interaction behaviour between analyte and stationary phase. Polar stationary phases will retain more effectively polar compounds while nonpolar stationary phases will retain more easily nonpolar compounds. In this case, the used capillary column is nearly nonpolar and musks are in general slightly polar. Therefore, separation during a temperature *plateau* will be due to a combination between vapour pressure and polarity differences of the compounds. These *plateaus* also affect the separation of musks with higher boiling points. The reduction in the *plateau* of temperature resulted in the separation of the former mentioned co-eluting compounds. Program 5 was created by increasing the heating rate from 4.5 ^{o}C/min (program 4) to 6 ^{o}C/min, which improved the compounds resolution. In program 6, after the last compound was eluted (which occurred at 225 ^{o}C) heating rate was significantly increased to 20 ^{o}C/min in order to achieve the clean-up temperature sooner. The resulting chromatogram of this program is shown in Fig. 17.

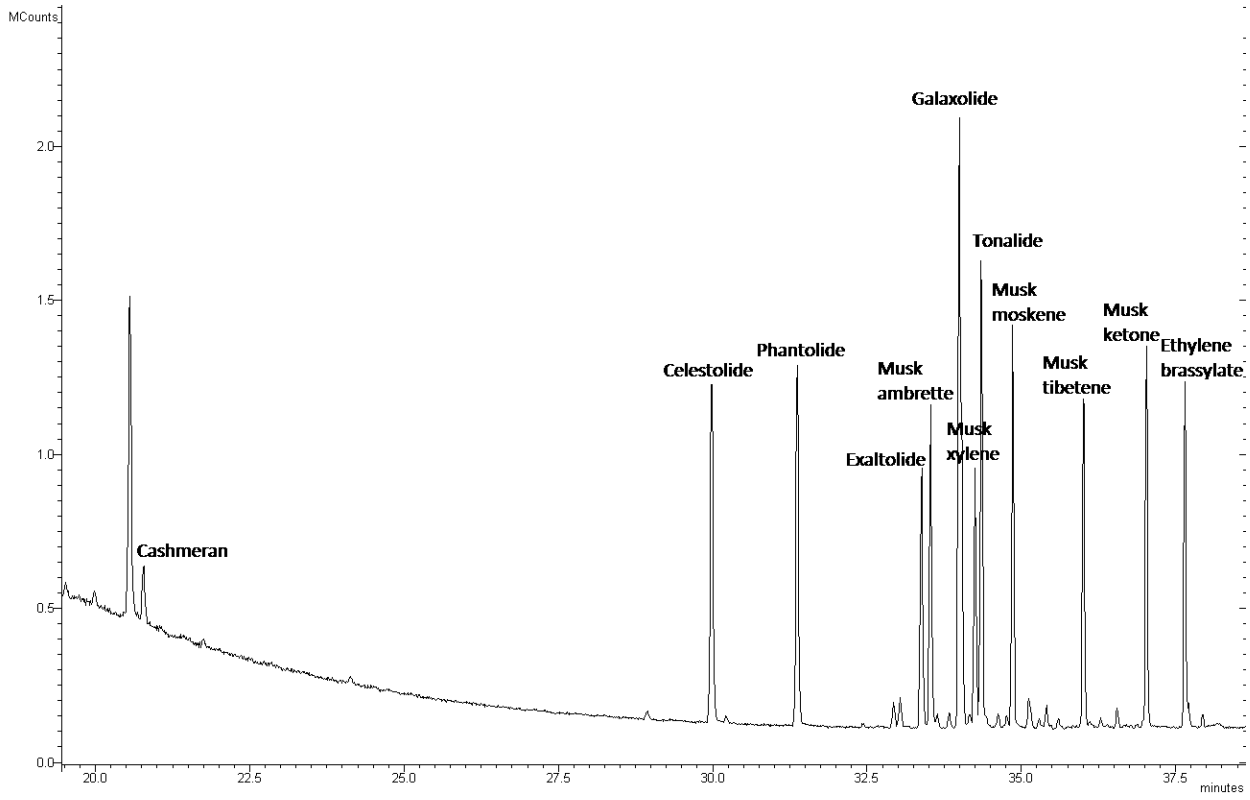


Fig. 17 – Full scan chromatogram of a standard solution of musks (1 mg/L in acetonitrile) by program 6, exhibiting all musks resolved.

An emission current of 50 µA was used for all the programs. As mention before, the filaments located in the ion source, when heated by an electric current, are responsible for the emission of electrons, which will ionize the sample molecules. Therefore, the number of ions produced by unit time will be proportional to the emission current. In order to verify the effect of this parameter, a 70 µA current was tested using the temperature program 6. No significant changes were found in the peak areas, and therefore the initial option was kept for the musk analysis.

As mentioned before, there are two methods available to analyze the data obtained: full scan and selected ion storage (SIS). In full scan mode, a plot of the sum of the signals across the entire mass range detected during the analysis as a function of chromatographic time is formed. It includes the *m/z* of the smallest fragment ion of any compound detected through the highest m/z. In this study, the m/z varied between 50 and 1000. Widespread computer libraries containing mass-spectra of numerous different compounds to compare to the unknown analyte spectrum are available.

The SIS mode increases sensitivity for the selection ions relatively to full scan mode. In this mode, instead of looking for all masses over a wide range, the MS collects data for masses of interest. This allows the instrument to be specific for a particular analyte of interest. Usually two to four ions are monitored per compound and the ratios of those ions will be unique to the analyte of interest. The mass

scan rate and dwell times, i.e., the time spent looking at each mass, are adjusted in order to increase sensitivity. Table 10 refers to the ion ranges used in SIS mode.

Table 10 – Ion ranges used in SIS mode

	t_r (min)	*Ion range (m/z)*
Cashmeran	19.00-27.00	134-136, 162-164, 190-192, 205-208
Celestolide and Phantolide	27.00-32.50	150-153, 172-189, 228-231, 243-247
Exaltolide and Musk ambrette	32.50-33.75	54-56, 65-95, 239-255, 268-270
Galaxolide, Musk xylene and Tonalide	33.75-34.60	114-135, 152-160, 212-216, 242-245, 257-261
Musk moskene	34.60-35.50	114-116, 126-132, 261-265, 277-280
Tibetene and Musk ketone	35.50-37.30	114-119, 126-136, 140-149, 155-165, 250-253, 278-282
Ethylene brassylate	37.30-38.00	54-56, 79-89, 96-100, 124-126, 225-229, 270-273

The full scan mode was used first to determine the retention times of the compounds as well as the quantification ions. In SIS mode, because unwanted ions are being filtered, the selectivity is greatly enhanced providing an additional tool to eliminate difficult matrix interferences. The comparison between the detector response in full scan and SIS modes is presented in Table 11. These results are related to the injection of a standard of 1 mg/L in acetonitrile.

Table 11 – Comparison between retention times and peak areas in full scan and SIS mode

	Full Scan		*SIS*	
	tr (min)	Area (UA)	tr (min)	Area (UA)
Cashmeran	20.560	1.164×10^6	20.561	3.144×10^6
Celestolide	29.978	1.543×10^6	19.991	4.380×10^6
Phantolide	31.372	9.602×10^5	31.373	3.810×10^6
Exaltolide	33.387	2.354×10^5	33.373	2.983×10^6
Musk ambrette	33.530	2.251×10^5	33.530	2.180×10^6
Galaxolide	34.029	9.102×10^5	33.997	9.044×10^6
Musk xylene	34.255	2.768×10^5	34.249	1.173×10^6
Tonalide	34.349	1.205×10^6	34.355	4.649×10^6
Musk moskene	34.861	4.832×10^5	34.864	2.016×10^6
Musk tibetene	36.005	4.331×10^5	35.999	3.110×10^6
Musk ketone	37.030	3.130×10^5	37.022	2.967×10^6
Ehylene brassylate	37.649	1.544×10^5	37.645	2.730×10^6

As can be seen from Table 11, peak areas are higher in SIS mode due to, above mentioned, increased selectivity of this data acquisition method. Fig. 18 refers to a chromatogram of all compounds analysed in SIS mode.

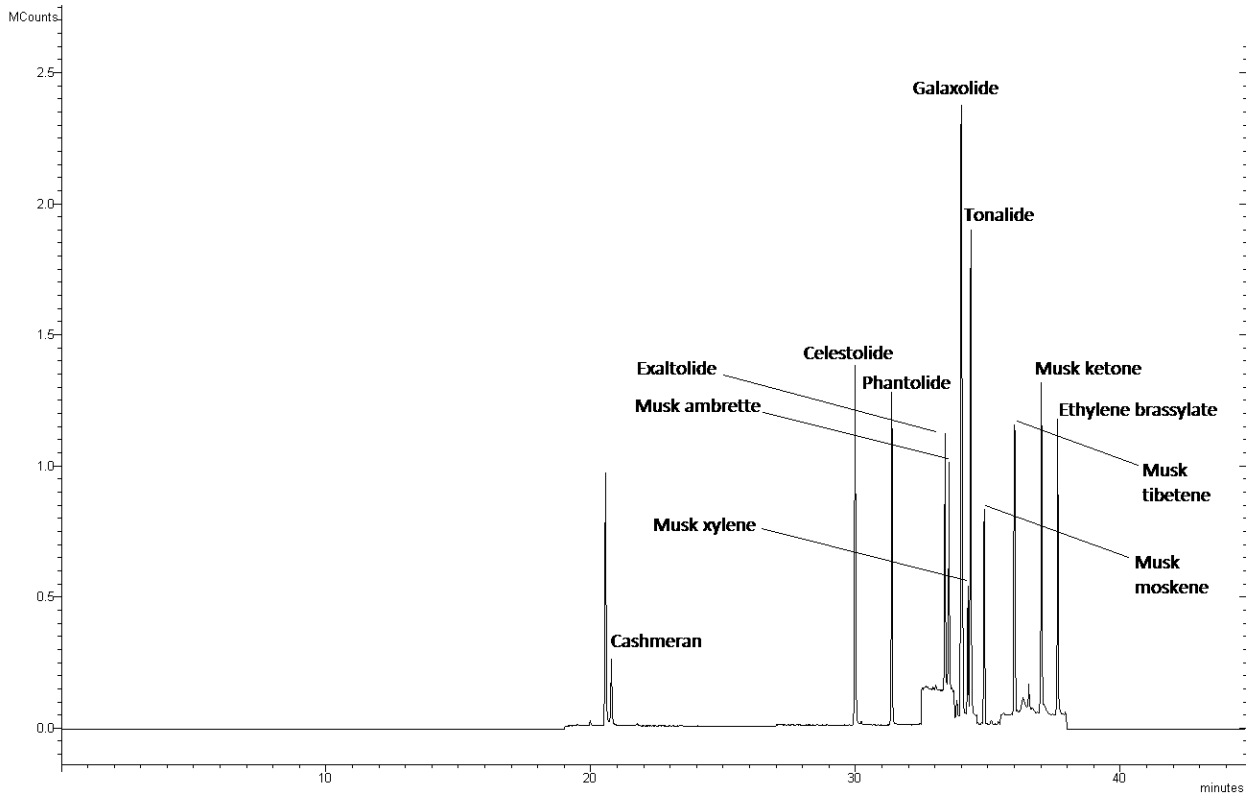


Fig. 18 – SIS mode chromatogram of a standard solution of musks (1 mg/L in acetonitrile) by program 6, exhibiting all musks resolved.

4.2 Development of the extraction methodology

The main extraction parameters as the sample quantity, the type or volume of extraction solvent, the evaporation step and the amount of sorbents of QuEChERS were optimised. A skin moisturiser was chosen to carry the optimisation process. This decision was taken based on the complexity of the matrix and it was expected that the extraction method would work for the other matrices since they are less complex. Due to the high cost of standards and the small amount available, standard additions were not made in some parameters, such as, the sample quantity, the type or volume of extraction solvent and the evaporation step. A scheme of the extraction process optimisation is presented in Fig. 19.

Sample amount	
Variables	0.5, 1.0, 2.0 g
Experimental conditions	Extraction solvent: Acetonitrile Solvent volume: 15 mL QuEChERS 1: 6000 mg $MgSO_4$ + 1500 mg $NaCH_3COO$ QuEChERS 2: 900 mg $MgSO_4$ + 300 mg PSA + 150 mg C18

0.5 g of sample

Extraction solvent	
Variables	Acetonitrile Cyclohexane
Experimental conditions	Sample amount: 0.5 g Solvent volume: 15 mL QuEChERS 1: 6000 mg $MgSO_4$ + 1500 mg $NaCH_3COO$ QuEChERS 2: 900 mg $MgSO_4$ + 300 mg PSA + 150 mg C18

Acetonitrile

Solvent volume	
Variables	15 mL 3 mL
Experimental conditions	Sample amount: 0.5 g Extraction solvent: Acetonitrile For 15 mL QuEChERS 1: 6000 mg $MgSO_4$ + 1500 mg $NaCH_3COO$ QuEChERS 2: 900 mg $MgSO_4$ + 300 mg PSA + 150 mg C18 For 3 mL QuEChERS 1: 1200 mg $MgSO_4$ + 300 mg $NaCH_3COO$ QuEChERS 2: 180 mg $MgSO_4$ + 60 mg PSA + 30 mg C18

3 mL

Evaporation step	
Variables	Rotary evaporator Stream of nitrogen
Experimental conditions	Sample amount: 0.5 g Extraction solvent: Acetonitrile Solvent volume: 3 mL QuEChERS 1: 1200 mg $MgSO_4$ + 300 mg $NaCH_3COO$ QuEChERS 2: 180 mg $MgSO_4$ + 60 mg PSA + 30 mg C18

Stream of nitrogen

QuEChERS 1	
Variables	$MgSO_4$: 1200, 1800, 2400, 3000 mg $NaCH_3COO$: 300, 450, 600, 750 mg
Experimental conditions	Sample amount: 0.5 g Extraction solvent: Acetonitrile Solvent volume: 3 mL Evaporation step: Stream of nitrogen QuEChERS 2: 180 mg $MgSO_4$ + 60 mg PSA + 30 mg C18

2400 mg $MgSO_4$
750 mg $NaCH_3COO$

QuEChERS 2	
Variables	$MgSO_4$: 180 mg PSA: 0, 60, 120 mg C18: 0, 30, 60 mg
Experimental conditions	Sample amount: 0.5 g Extraction solvent: Acetonitrile Solvent volume: 3 mL Evaporation step: Stream of nitrogen QuEChERS 1: 2400 mg $MgSO_4$ + 750 mg $NaCH_3COO$

60 mg PSA
30 mg C18

Sample amount: 0.5 g
Extraction solvent: Acetonitrile
Solvent volume: 3 mL
Evaporation step: Stream of nitrogen
QuEChERS 1: 2400 mg $MgSO_4$ + 750 mg $NaCH_3COO$
QuEChERS 2: 180 mg $MgSO_4$ + 60 mg PSA + 30 mg C18

Fig. 19 – Scheme of the extraction process optimisation (variables and experimental conditions).

4.2.1 Effect of the sample amount

The first step of the QuEChERS method is weighing the sample. The original method of Anastassiades et al. (2003) states that the quantity of sample weighed is 10 g. However, it was decided to reduce the amount of sample, because a greater amount of sample means higher amount of analytes but, at a cost of having also a higher amount of interferents. Analytes transfer from the sample to the solvent is also an important issue, as solvent may be saturated if high amounts of sample are used.

Masses of 0.5, 1.0 and 2.0 g of skin moisturiser were tested (Fig. 20). The first tests were performed using 15 mL of acetonitrile and commercial QuEChERS (QuEChERS 1: 6000 mg $MgSO_4$ + 1500 mg $NaCH_3COO$; QuEChERS 2: 900 mg $MgSO_4$ + 300 mg PSA + 150 mg C18).

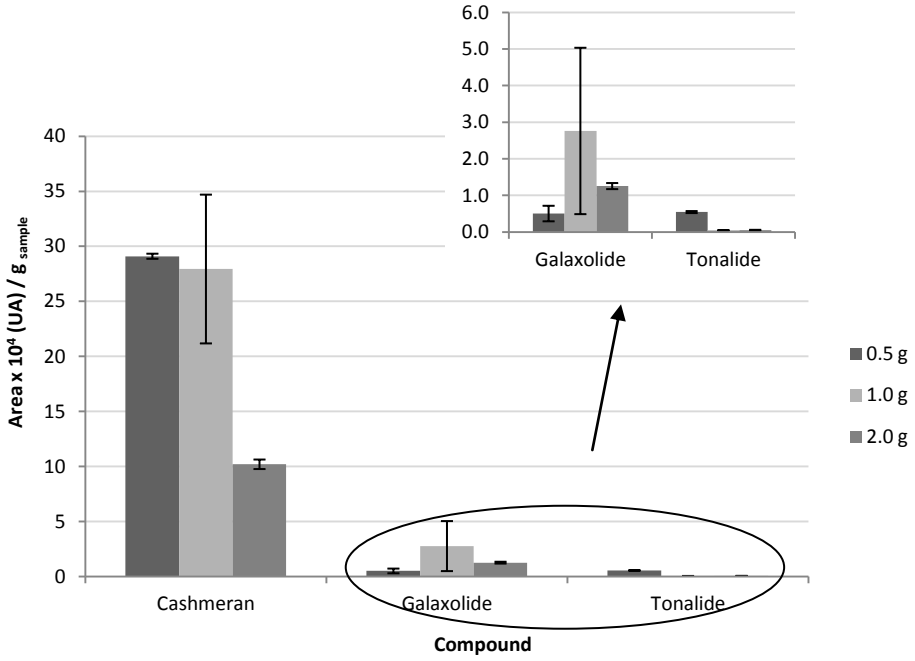


Fig. 20 – Comparison of peak areas of musks for different sample amounts

(Extraction solvent: Acetonitrile; Solvent volume: 15 mL; QuEChERS 1: 6000 mg $MgSO_4$ + 1500 mg $NaCH_3COO$; QuEChERS 2: 900 mg $MgSO_4$ + 300 mg PSA + 150 mg C18. Error bars represent the standard deviation).

A greater extraction of analytes per gram of sample was verified for 0.5 g. In spite of the amount of galaxolide extracted per gram of sample being greater in 1.0 g, the standard deviation associated to this result is very high. In 2.0 g of the sample, probably saturation of the solvent was achieved which leads to a lower extraction of analytes per gram of sample. It was concluded that the best choice among those tested was 0.5 g of sample.

4.2.2 Effect of the extraction solvent

Two extraction solvents were tested in the QuEChERS method: acetonitrile (ACN) and cyclohexane (CY). ACN is widely used as a medium-polarity organic solvent in extractive processes. It is miscible with water and extracts fatty acids from animal and vegetable oils (Feng and Lee, 2009). CY is used as a nonpolar organic solvent in analytical chemistry. Analytes are soluble in both solvents and ACN is immiscible in CY. ACN and CY are costly solvents which have similar boiling points and are relatively easy to evaporate if necessary. The same volume of both solvents (15 mL) was tested with commercial QuEChERS. As can be seen in Fig. 21, peak areas are different for both solvents.

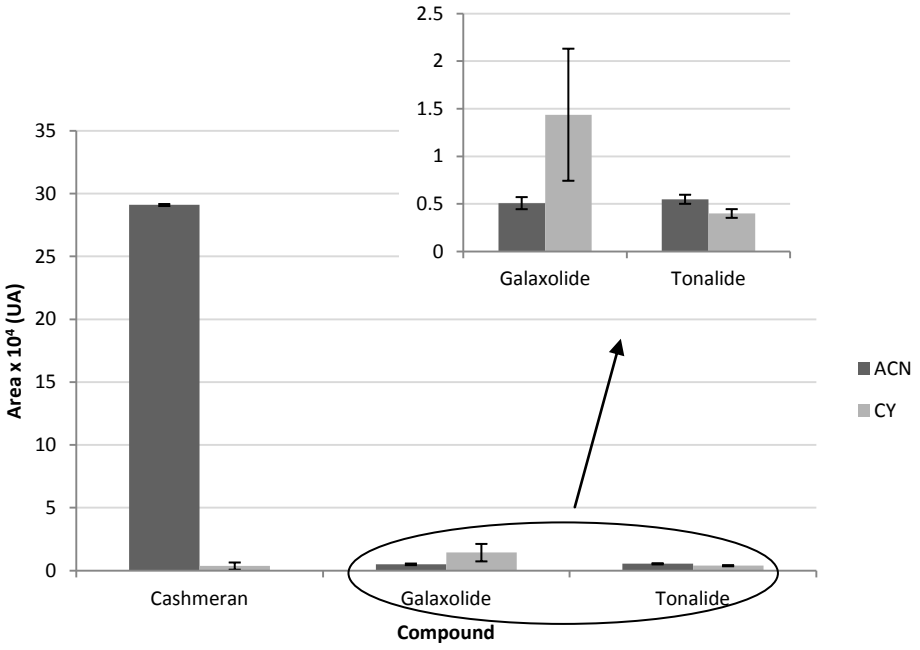


Fig. 21 - Comparison of peak areas of musks for two different extraction solvents

(Sample amount: 0.5 g; Solvent volume: 15 mL; QuEChERS 1: 6000 mg $MgSO_4$ + 1500 mg $NaCH_3COO$; QuEChERS 2: 900 mg $MgSO_4$ + 300 mg PSA + 150 mg C18. Error bars represent the standard deviation).

ACN showed better extraction properties with regard to cashmeran and tonalide. The same behaviour was not verified for galaxolide which showed higher signal in CY; however this result has a great standard deviation associated to it. In addition, problems occurred in the extraction with CY. It was found that this solvent eroded the polypropylene centrifuge tubes. Therefore, ACN was chosen for this study.

4.2.3 Effect of the solvent volume

Initially, 15 mL of acetonitrile were used to extract the samples, as it was the volume used in traditional methods. However, in view of more environmentally friendly options, the volume of the extraction solvent was reduced. Additionally, in 15 mL of solvent some analytes were too diluted to be properly detected. Therefore, a pre-concentration step, such as solvent evaporation, would be necessary, which may increase losses. So, a reduction of the extraction volume to 3 mL was performed, keeping the QuEChERS sorbent masses ratio. Each extract was injected into the GC-MS and the results are shown in Fig. 22.

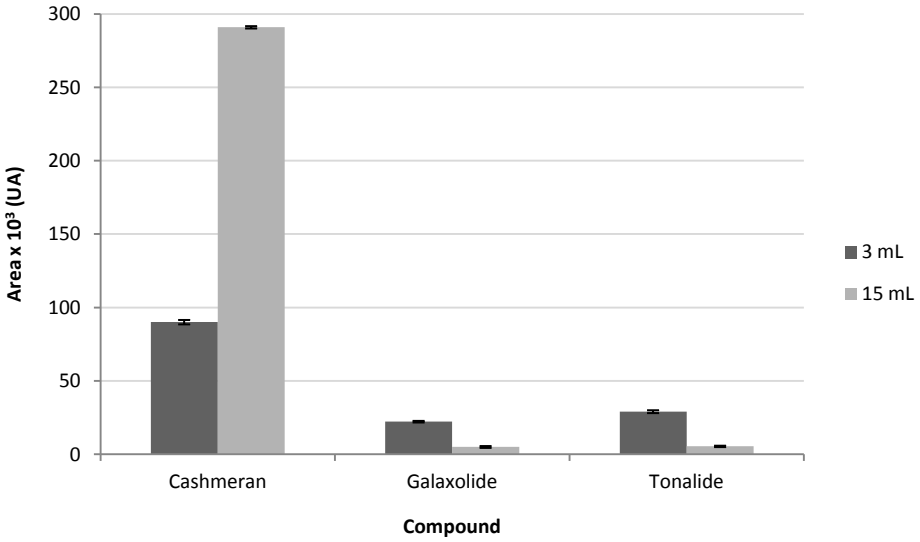


Fig. 22 - Comparison of peak areas of musks for two different solvent volumes

(Sample amount: 0.5 g; Extraction solvent: Acetonitrile; For 15 mL - QuEChERS 1: 6000 mg $MgSO_4$ + 1500 mg $NaCH_3COO$, QuEChERS 2: 900 mg $MgSO_4$ + 300 mg PSA + 150 mg C18; For 3 mL - QuEChERS 1: 1200 mg $MgSO_4$ + 300 mg $NaCH_3COO$, QuEChERS 2: 180 mg $MgSO_4$ + 60 mg PSA + 30 mg C18; error bars represent the standard deviation).

As can be seen in the previous display, peak area of cashmeran is higher for 15 mL of extraction solvent. However, galaxolide and tonalide peak areas are greater when the extraction was carried out with smaller solvent volume (3 mL). Since these two compounds are more relevant, due to the frequency that they are found, and since the reduction in the quantity of residues is a goal in the development of this extraction method, it was decided to proceed with 3 mL of extraction solvent.

4.2.4 Effect of the evaporation step

As mentioned before, some analytes were too diluted to be properly detected. So it was necessary to concentrate the samples. Two different evaporation methods were tested: rotary evaporator and nitrogen blow down. The rotary evaporator is a device for gently and efficiently evaporating solvents from a mixture. Reduced pressure allows the evaporation of the solvent at more moderate temperatures. Rotation will create a thin film of liquid on the walls of the flask, increasing mass transfer from the liquid into the vapour phase. Additionally, by this an effective mixing of the bulk solution will be ensured while avoiding overheating.

Nitrogen is commonly used during sample preparation procedures for chemical analysis. It is used to concentrate and reduce the volume of liquid samples. Directing a pressurised stream of nitrogen gas perpendicular to the surface of the liquid allows the solvent to be removed by forced convection while leaving the solutes and un-evaporated solvent behind. Advantages rely on the fact that this can be done at environmental temperature, leaving eventually to the cooling of the solution as latent heat is removed by vaporisation, which is beneficial in case of volatile compounds. The comparison between the two evaporation methods tests is presented in Fig. 23.

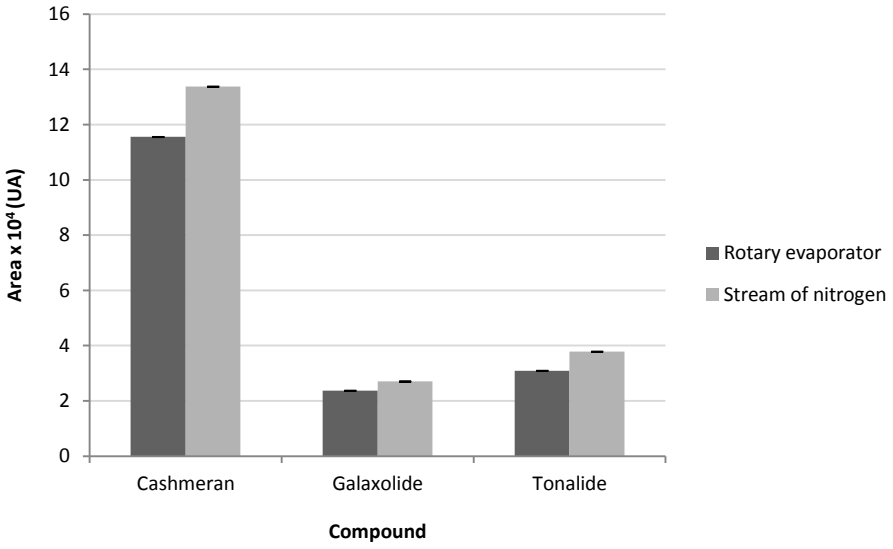


Fig. 23 - Comparison of peak areas of musks for two different evaporation steps

(Sample amount: 0.5 g; Extraction solvent: Acetonitrile; Solvent volume: 3 mL; QuEChERS 1: 1200 mg $MgSO_4$ + 300 mg $NaCH_3COO$; QuEChERS 2: 180 mg $MgSO_4$ + 60 mg PSA + 30 mg C18. Error bars represent the standard deviation).

As can be seen in the previous display, the concentration process by nitrogen stream has peak areas similar to those of evaporation by rotary evaporator. However, evaporation through nitrogen stream is simpler to handle and allows better control. Thus, this method was chosen to concentrate the extracts.

4.2.5 QuEChERS 1 optimisation

As mentioned before, in the first step of the QuEChERS method, the sample is extracted with an organic solvent, in the presence of anhydrous salts as drying agents (in this case, magnesium sulphate) and optionally buffers (sodium acetate). The amount of salts and buffers was optimised for different matrix interferences and wanted analytes (Table 12).

Table 12 - Study of the effect of magnesium sulphate (1a-d) and sodium acetate (1e-h) masses

Hypothesis	*Mass of $MgSO_4$ (mg)*	*Mass of $NaCH_3COO$ (mg)*	*Weight ratio $NaCH_3COO$: $MgSO_4$*
1a	1200	300	4:1
1b	1800	450	4:1
1c	2400	600	4:1
1d	3000	750	4:1
1e	3000	300	10:1
1f	2400	300	8:1
1g	2400	450	5:1
1h	2400	750	3:1

The addition of inorganic salts during the partitioning stage usually promotes the extraction of polar analytes, as well as the reduction of the water amount in the organic phase. Sodium acetate is used as buffer to control the pH of the extraction. Four different combinations of magnesium sulphate and sodium acetate quantities were tested, keeping the weight ratio of 4:1 (Fig. 24).

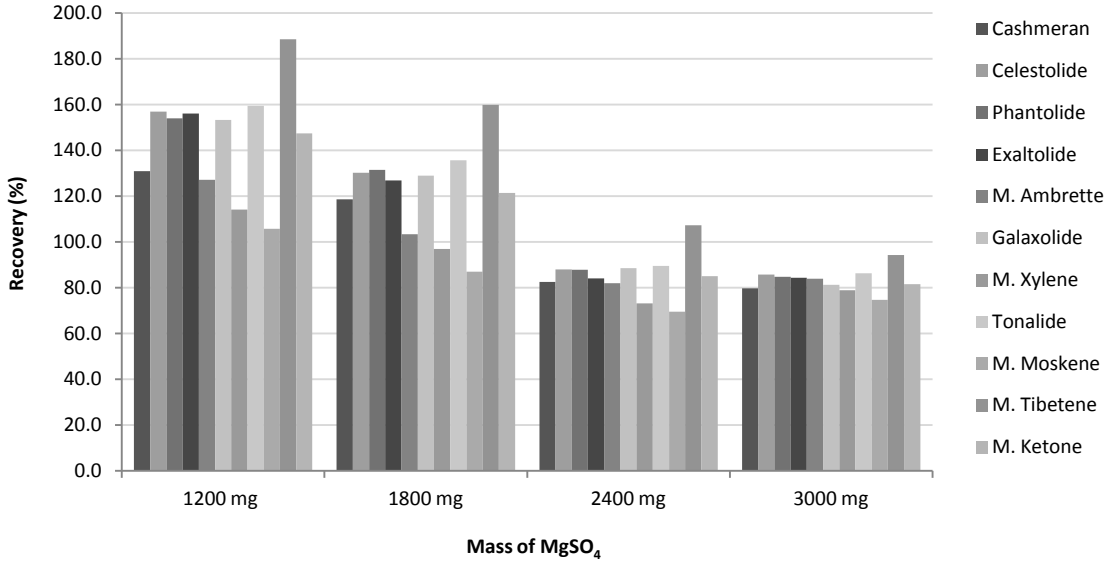


Fig. 24 - Study of the effect of magnesium sulphate mass (weight ratio $NaCH_3COO$: $MgSO_4$ = 0.25) in the musks extraction
(Sample amount: 0.5 g; Extraction solvent: Acetonitrile; Solvent volume: 3 mL; Evaporation step: Stream of nitrogen; QuEChERS 2: 180 mg $MgSO_4$ + 60 mg PSA + 30 mg C18).

The average recoveries were calculated for hypothesis 1a to 1d, and the results were 145, 122, 85 and 83%, respectively. For lower masses of magnesium sulphate (1a - 1200 mg and 1b - 1800 mg), recoveries were higher, although they were superior to 100%. Since recoveries of hypothesis 1c and 1d

are relatively similar, it was decided to maintain these two quantities of magnesium sulphate (1c - 2400 mg, 1d - 3000 mg) and vary the amount of sodium acetate. To study the effect of sodium acetate in the musks extraction, four different weight ratios (10:1, 8:1, 5:1 and 3:1) of magnesium sulphate and sodium acetate quantities were tested (Fig. 25).

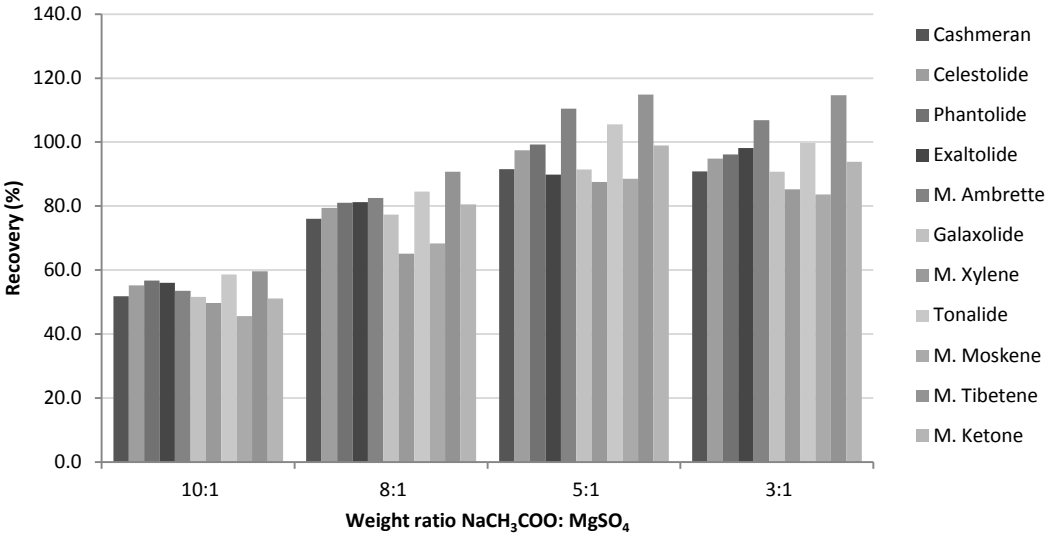


Fig. 25 - Study of the effect of sodium acetate mass in the musks extraction

(Sample amount: 0.5 g; Extraction solvent: Acetonitrile; Solvent volume: 3 mL; Evaporation step: Stream of nitrogen; QuEChERS 2: 180 mg $MgSO_4$ + 60 mg PSA + 30 mg C18).

In hypothesis 1e (weight ratio 10:1), 3000 mg of magnesium sulphate and 300 mg of sodium acetate were used and there was a reduction in recovery. In hypotheses 1f (weight ratio 8:1), 1g (weight ratio 5:1) and 1h (weight ratio 3:1) the amount of magnesium sulphate was 2400 mg and the amounts of sodium acetate were 300, 450 and 750 mg, respectively. Comparing all three, hypothesis 1h (weight ratio 3:1) shows best results, since most musks have recoveries near 100%. This shows that sodium acetate is critical in the extraction process. Therefore the combination of sorbents of choice for the first step of the QuEChERS method was hypothesis 1h, i.e., 2400 mg of magnesium sulphate and 750 mg of sodium acetate.

4.2.6 QuEChERS 2 optimisation

In the second step of QuEChERS, purification is carried out using dispersive solid-phase extraction (d-SPE) sorbents. The most frequently used sorbent is primary-secondary amine (PSA). Its main function is to remove fatty acids, sugars, organic acids and some ionic lipids, which are compounds present in the matrices of PCPs. C18 can also be incorporated as an additional clean-up step to remove non-polar interfering substances like lipids. Thus, the combinations of these sorbents were tested (Table

13). In this step, the amounts of magnesium sulphate were not tested because its function is to absorb water which was not removed previously in the first step.

Table 13 - Study of the effect of PSA and C18 masses

Hypothesis	*Mass of $MgSO_4$ (mg)*	*Mass of PSA (mg)*	*Mass of C18 (mg)*	*Weight ratio $MgSO_4$: PSA: C18*
2a	180	0	30	6:0:1
2b	180	60	30	6:3:1
2c	180	120	30	6:2:1
2d	180	0	60	6:0:3
2e	180	60	60	6:3:3
2f	180	120	60	6:2:3
2g	180	60	0	6:3:0
2h	180	120	0	6:2:0

Fig. 26 shows the recoveries of musks for the combinations of sorbents listed above.

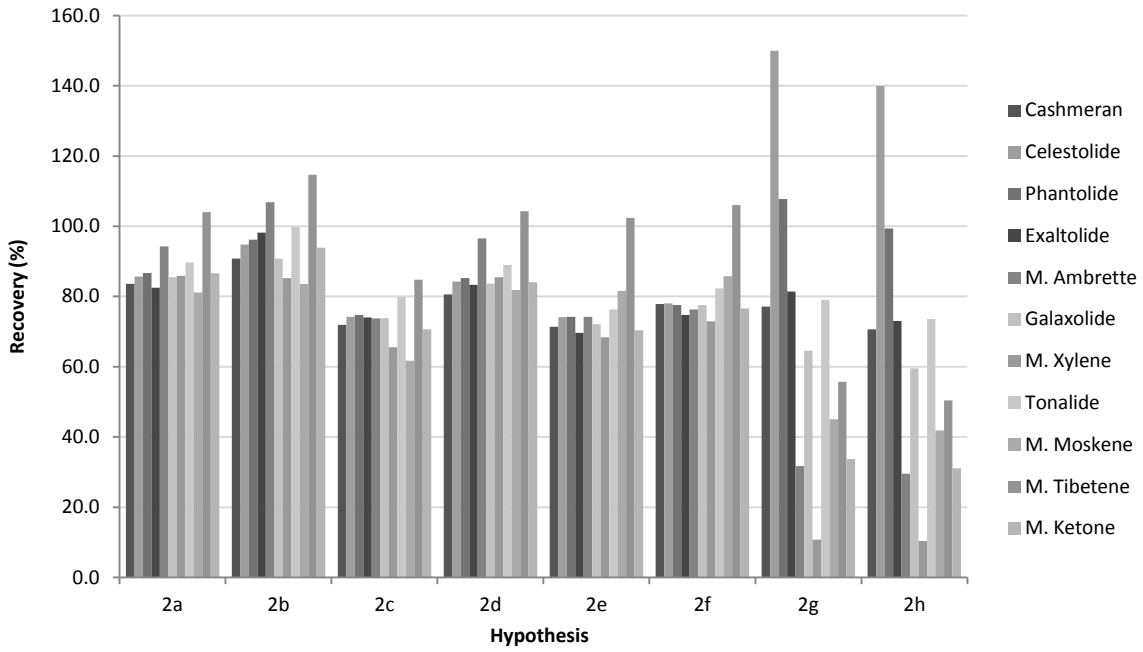


Fig. 26 - Study of the effect of PSA and C18 masses in the musks extraction

(Sample amount: 0.5 g; Extraction solvent: Acetonitrile; Solvent volume: 3 mL; Evaporation step: Stream of nitrogen; QuEChERS 1: 2400 mg $MgSO_4$ + 750 mg $NaCH_3COO$).

As can be seen in Fig. 26, the hypothesis which had the best recoveries was 2b, i.e., 180 mg of magnesium sulphate, 120 mg of PSA and 30 mg of C18. Furthermore, it must be noted the effect of the C18 removal in the recovery of some musks. PSA removal did not produce any significant alteration to the recoveries, as can be seen in hypothesis 2a and 2d. Hypothesis 2g and 2h, show an increase in the recovery of celestolide and phantolide, and a drastic decrease in the recovery of musks ambrette and xylene. The extraction method final conditions are presented in Table 14.

Table 14 – Extraction method final conditions

Extraction method final conditions	
Sample amount	0.5 g
Extraction solvent	Acetonitrile
Solvent volume	3 mL
Evaporation step	Stream of nitrogen
QuEChERS 1	2400 mg $MgSO_4$ + 750 mg $NaCH_3COO$
QuEChERS 2	180 mg $MgSO_4$ + 60 mg PSA + 30 mg C18
Total extraction time	45 minutes

4.3 Method validation

Fig. 27 shows a chromatogram in SIS mode of a spiked skin lotion sample (400 µg/L final concentration). As expected, the skin lotion matrix presented other compounds besides the musks studied. Comparing this chromatogram with the chromatogram of the standard alone, there is the occurrence of interferents at 21, 27.3, 28.5, 30.5 and 32.5 minutes. It was observed that the signal of all musks was slightly smaller in the extracted samples. This might be due to interactions with the skin lotion matrix or it might be related to the intrinsic aspects of the extraction process.

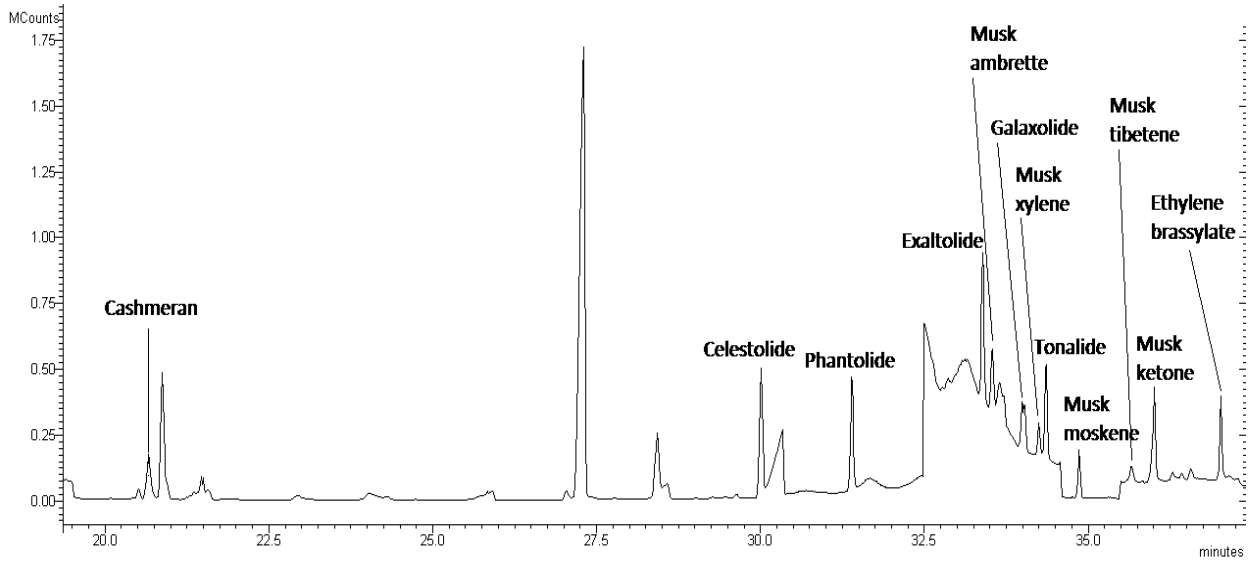


Fig. 27 – SIS mode chromatogram of the extraction of a skin lotion with a final concentration of 400 µg/L.

To verify that the developed GC-MS method was suitable for the quantitative determination of the selected group of ingredients in cosmetic products, method quality parameters were evaluated for each compound.

4.3.1 Quantification parameters (linearity, limits of detection and quantification and sensitivity)

A calibration curve was constructed for each musk compound, by duplicate injections, at ten calibration levels (5, 10, 50, 100, 200, 400, 1000, 2000, 3000 and 4000 µg/L). As can be seen further ahead, the products analysed contain musks in very different concentration ranges, which requires diluting. Musks at very high concentrations, when diluted, will be within the linearity range. On the other hand, musks which have concentrations near the calibration's curve upper limit, will be outside this range after dilution. Therefore, some dilutions result in concentration values which are in close proximity to the lower limit of the calibration curve. To avoid negative concentration values, curves were forced to pass through the origin of the graph (Appendix 1).

Regarding the linear response of the detector, which ranged between the 5 and 4000 µg/L, the method exhibited a direct proportional relationship between the amount of each analyte and the peak area with correlation coefficients $R \geq 0.989$ (Table 15). Limits of detection (LODs) and limits of quantification (LOQs) were calculated as the concentration giving a signal-to-noise ratio of three (S/N = 3) and of 10 (S/N = 10), respectively. Recoveries were assumed to be 100% so that units could be converted from µg/L to µg/g. The sensitivity of the method, expressed as the slope of the calibration curve, is also included in Table 15.

Table 15 – Linearity of the method for the target compounds

	Linearity R	LOD (µg/L)	LOQ (µg/L)	LOD (µg/g)	LOQ (µg/g)	Sensitivity
Cashmeran	0.9982	0.43	1.45	8.70×10^{-4}	2.90×10^{-3}	1389
Celestolide	0.9960	0.07	0.22	1.33×10^{-4}	4.42×10^{-4}	2736
Phantolide	0.9949	0.08	0.27	1.62×10^{-4}	5.39×10^{-4}	1923
Exaltolide	0.9933	0.34	1.15	6.90×10^{-4}	2.30×10^{-3}	1409
Musk ambrette	0.9921	1.58	5.26	3.16×10^{-3}	1.05×10^{-2}	443
Galaxolide	0.9896	0.01	0.04	2.15×10^{-5}	7.17×10^{-5}	1090
Musk xylene	0.9970	0.17	0.58	3.47×10^{-4}	1.16×10^{-3}	220
Tonalide	0.9929	2.50	8.33	5.00×10^{-3}	1.67×10^{-2}	1218
Musk moskene	0.9963	1.25	4.17	2.50×10^{-3}	8.33×10^{-3}	803
Musk tibetene	0.9964	1.03	3.45	2.01×10^{-3}	6.90×10^{-3}	809
Musk ketone	0.9961	1.11	3.70	2.22×10^{-3}	7.41×10^{-3}	553
Ethylene brassylate	0.9968	0.02	0.06	3.88×10^{-5}	1.29×10^{-4}	769

LODs varied between 0.01 and 2.50 µg/L (2.15×10^{-5} and 5.00×10^{-3} µg/g) while LOQs varied between 0.04 and 8.33 µg/L (7.17×10^{-5} and 1.67×10^{-2} µg/g). The method showed higher sensitivity to celestolide and proved to be less sensitive to musk xylene. As can be seen from the table presented above, LODs and the majority of the LOQs are below the first point (the lowest concentration, 5 µg/L) of the calibration curves. To be more accurate, calibration curves with a higher amount of points near the LOD should have been drawn. However, the musks whose concentrations are between the LOD and the first standard were quantified.

4.3.2 Reliability parameters (precision and accuracy)

Method precision was studied within a day and among days at three concentration levels, 100, 200, and 400 µg/L (Table 16). For intra-day precision, spiked extracts were used. A product was selected from each of the four classes: skin moisturisers, toothpastes, toilet soaps, body and hair washes. Precision studies were not carried for deodorants because the analysis of these samples coincided with the breakdown of GC-MS and the lack of time was another constraint. Inter-day precision was evaluated for the injection of standards alone on three different days.

Table 16 – Precision (% RSD) of the method for the target compounds

	Intra-day precision (n = 4)												Inter-day precision (n = 3)		
	Skin Moisturisers			Toothpastes			Toilet soaps			Body and hair washes					
Concentration (µg/L)	100	200	400	100	200	400	100	200	400	100	200	400	100	200	400
Cashmeran	15.8	14.7	9.4	5.3	7.5	8.4	5.7	7.1	2.4	0.4	5.2	0.3	14.1	16.1	20.8
Celestolide	14.4	14.8	10.0	4.7	6.9	8.3	8.8	9.2	3.2	7.6	4.1	4.4	11.2	19.2	15.6
Phantolide	13.9	13.9	9.5	6.3	6.6	8.0	8.9	8.5	3.1	6.9	4.4	4.1	15.2	19.1	15.5
Exaltolide	18.5	11.7	8.5	9.6	10.2	6.9	10.3	8.5	1.6	8.8	7.3	2.0	8.5	9.5	13.6
Musk ambrette	25.5	19.5	11.8	68.2	4.4	13.4	8.7	6.6	8.0	4.2	2.2	9.7	10.0	24.2	38.4
Galaxolide	14.6	15.0	9.7	5.1	6.4	7.9	8.7	8.5	3.1	5.2	3.5	4.4	8.9	21.7	14.6
Musk xylene	27.1	17.0	7.4	45.3	8.0	17.4	2.4	0.7	0.4	7.3	15.8	1.9	27.5	34.9	53.9
Tonalide	19.5	15.0	9.4	6.8	5.8	7.3	9.1	8.3	3.2	8.1	5.0	4.3	15.8	18.6	12.3
Musk moskene	20.0	19.8	11.1	12.5	4.4	12.0	12.6	6.9	3.7	4.8	6.0	6.6	19.6	25.9	29.5
Musk tibetene	21.8	14.4	6.8	4.6	7.3	8.0	11.1	9.9	4.4	0.8	2.3	4.5	12.9	16.9	15.8
Musk ketone	18.6	15.2	9.8	10.7	9.5	13.0	37.8	6.4	4.7	3.7	69.5	1.4	9.5	15.5	18.8
Ethylene brassylate	17.8	12.8	7.3	13.5	3.3	9.2	16.2	11.7	6.5	3.7	3.9	7.9	20.0	23.1	7.1

Relative standard deviation (RSD) values of musks for skin moisturisers ranged from 6.8 to 27.1% (intra-day precision, average 14.6%). It was noted that the higher the concentration of standard, better the precision. Toothpastes presented RSD values that ranged between 3.3 and 68.2% (the average was 10.9%). In general there is a decrease in precision with increasing concentration of standard. Additionally, musks ambrette and xylene exhibited RSD values way above the average, 68.2 and 45.3%, respectively. RSDs for toilet soaps varied from 0.4 to 37.8% (average 7.8%). In this case, an increased precision was observed with increased concentration of standard. The highest RSD value was observed for musk ketone (37.8%). For body and hair washes, RSDs for the intra-day precision ranged from 0.3 to 69.5% with an average value of 6.7%. There was as increase in precision with increased concentration of standard. The most discrepant RSD value was 69.5% for musk ketone. RSDs for the inter-day precision ranged from 7.1 to 53.9% with an average value of 18.7%. Inter-day RSD values of musk xylene were high for the three concentration levels (27.5, 34.9 and 53.9% for 100, 200 and 400 µg/L, respectively). This may be due to problems related with musk xylene's peak integration as this musks presents a very low signal.

In general, the best way to reduce the variation is through the use of an internal standard because the uncertainties introduced by sample injection are avoided. In this procedure, a carefully measured quantity of an internal-standard substance is introduced into each standard and sample, and the ratio of analyte to internal standard peak areas serves as the analytical variable. The internal-standard peak must be well separated from the peaks of all other components of the sample in order to be successful. However, due to lack of funds, it was not possible to use this method.

Method accuracy was also studied for one product of each of the four classes of products previously mentioned, at three concentration levels, 100, 200, and 400 µg/L (Table 17).

Table 17 - Accuracy (% Rec) of the method for the target compounds

	Accuracy (% Rec)											
	Skin Moisturisers			Toothpastes			Toilet soaps			Body and hair washes		
Concentration (µg/L)	100	200	400	100	200	400	100	200	400	100	200	400
Cashmeran	75.7	95.2	87.7	116.5	78.9	89.1	89.8	82.1	105.2	113.3	95.2	103.4
Celestolide	89.3	103.2	90.1	69.5	58.5	72.5	49.4	42.8	48.1	59.7	56.8	59.0
Phantolide	87.0	103.7	91.3	64.6	55.7	68.4	45.8	41.8	47.5	60.9	63.6	69.5
Exaltolide	79.2	102.3	91.8	56.6	66.8	73.0	61.3	54.9	48.4	123.9	104.0	94.4
Musk ambrette	121.8	137.4	97.6	48.1	79.0	85.1	125.6	85.5	78.4	103.4	99.7	107.8
Galaxolide	73.5	97.1	87.8	58.1	49.0	66.3	58.6	38.1	45.9	58.5	62.4	66.4
Musk xylene	119.4	118.7	82.2	68.9	70.0	117.9	80.3	62.6	90.9	111.6	111.2	112.1
Tonalide	88.4	115.3	95.0	64.4	56.3	70.5	45.2	39.5	47.4	81.9	76.2	78.7
Musk moskene	86.8	95.4	90.6	50.2	60.5	91.8	39.8	41.4	49.2	71.5	68.4	110.6
Musk tibetene	123.8	127.8	115.8	71.4	61.6	95.8	48.1	40.1	62.0	70.5	72.7	102.8
Musk ketone	100.2	113.5	90.1	123.1	117.7	97.8	43.2	38.7	44.2	99.1	103.8	118.4
Ethylene brassylate	**-8366.7**	**-1122.7**	**-1123.7**	60.8	60.2	85.5	40.2	37.7	50.4	**1572.1**	**794.6**	**409.7**

The recoveries of all musks extracted from skin moisturisers, with the exception of ethylene brassylate, were high and ranged from 73.5 to 137.4% with an average of 99.2%. The intermediate concentration (200 µg/L) presented better recoveries. For toothpastes, recoveries varied between 48.1 and 123.1% (average 74.5%). The best recovery results were observed for the samples spiked with 400 µg/L of standard. In the case of toilet soaps, musks showed reasonable recoveries, ranging between 37.7 and 125.6% (average 56.9%). The highest concentration of standard (400 µg/L) exhibited higher recoveries. Body and hair washes' recoveries varied from 56.8 to 123.9%, with an average of 87.6% for all musk except for ethylene brassylate. One reason that might explain the recovery values of ethylene brassylate in skin moisturisers and body and hair washes is the similarity between these musks' fragments and the fatty acids present in the samples. Since it was not possible to distinguish between them, the resulting signal was suppressed (recovery is negative) or exaggerated and did not match ethylene brassylate but instead it represented this musk together with fatty acids. Ethylene brassylate will only be taken into account in toothpaste and solid soap.

Skin moisturisers may have in their composition emulsifiers, fatty acid esters and/or sorbitan fatty acid esters (Umbach, 1991). The constituent ingredients of this type of matrix did not appear to have affected negatively musks recovery, since the highest mean value (99.2%) was observed for this class of products. Basic ingredients for body and hair washes preparations are surfactants which are composed of one hydrophilic and one hydrophobic portion. Foaming, wetting, dirt and grease emulsification, and rinsability are essential properties attributable to the surfactants molecular composition (Umbach, 1991). The mean recovery observed for this class of products was high (87.6%).

Toothpastes, on the other hand, are essentially made of abrasives, moisturisers, bonding and foaming agents and surfactants (Umbach, 1991). The ingredients that may have caused the decrease of the average recovery for this class of products, which came down to 74.5%, might have been abrasives. The other ingredients are present in skin moisturisers, and body and hair washes and did not have a negative effect on the mean recovery.

Coconut oil represents approximately 20 to 50% of the fat component in a bar of toilet soap. They contain superfatting additives (up to 5%) such as fatty acids, fatty alcohols, lanolin, lecithin, vegetable oils, partial glycerides, and other fat-like (lipophilic) compounds (Umbach, 1991). The musks are lipophilic, as can be seen by their K_{ow} values (Table 2 and 3), and when placed in a lipid matrix, as is the case of toilet soaps, they will be retained. This retention results in a reduction of recoveries to a mean value of 56.9%.

4.4 Synthetic musks in personal care products

Synthetic musks were detected in all of the 41 samples analysed. Galaxolide and cashmeran were the most commonly detected musks in personal care products, found in 93 and 83% of the samples, respectively. Nitro musks were not found in the samples analysed. The concentrations and frequency of occurrence of polycyclic and macrocyclic musks are shown in Table 18.

Table 18 - Concentrations (μg/g) and frequency of occurrence (%) of synthetic musks in personal care products analysed

Personal care products	*N*	Cashmeran	Celestolide	Phantolide	Exaltolide	Galaxolide	Tonalide	Ethylene brassylate
		Mean (Range) Frequency						
Skin moisturisers	12	2.040	0.210	0.177	14.621	11.290	71.331	---
		(0.168-7.579)	(0.093-0.328)	(0.054-0.301)	(3.741-58.303)	(0.052-882.340)	(5.026-203.660)	---
		92	17	17	83	75	25	---
Toothpastes	3	NA	NA	NA	NA	0.003	NA	NA
		NA	NA	NA	NA	(0.002-0.004)	NA	NA
		0	0	0	0	100	0	0
Deodorants	6	6.508	0.134	0.028	22.423	66.903	3.684	---
		(1.708-14.721)	(0.134)	(0.028)	(0.084-77.774)	(0.007-400.446)	(3.684)	---
		100	17	17	83	100	17	---
Toilet soaps	3	2.790	0.086	NA	5.683	17.812	0.169	0.736
		(1.881-3.906)	(0.086)	NA	(0.213-14.811)	(0.057-53.134)	(0.012-0.326)	(0.736)
		100	33	0	100	100	67	33
Body and hair washes	17	3.308	0.132	0.057	10.899	112.906	2.460	---
		(0.041-8.697)	(0.047-0.217)	(0.008-0.280)	(0.198-39.841)	(0.004-730.142)	(1.166-5.410)	---
		82	24	41	71	100	29	---
All products	41	3.662	0.141	0.088	13.406	61.783	19.411	0.736
		(0.041-14.721)	(0.047-0.328)	(0.008-0.301)	(0.084-77.774)	(0.002-882.340)	(0.012-203.660)	(0.736)
		83	20	24	73	93	27	2

NA = not available.

Polycyclic musks were found in 98% of the analysed samples. The lowest frequency of occurrence for polycyclic musks was found in toothpaste and the highest was found in body and hair washes. The highest percentage of occurrence for cashmeran was in toilet soaps and deodorants (100% of the samples) and the highest concentration was found in deodorants (approximately 15 μg/g). The highest occurrence of celestolide was also in toilet soaps while the highest concentration was found in skin moisturisers (0.328 μg/g). Phantolide occurred mostly in body and hair washes and, just like celestolide, showed the highest concentration in skin moisturisers. Galaxolide was present in every toothpaste, deodorant, toilet soap and body and hair wash sample. The highest concentration for this musk was observed in skin moisturisers (882 μg/g) followed by body and hair washes (730 μg/g). Tonalide's highest percentage of occurrence was in toilet soaps (67% of samples) and the highest concentration was found for skin moisturisers.

Macrocyclic musks were not detected in toothpastes. The highest percentage of occurrence for exaltolide was found in toilet soaps (100% of samples analysed), followed by skin moisturisers and deodorants (both in 83% of samples). Toilet soaps were the only class of products where ethylene brassylate was identified with a concentration of 0.736 μg/g. As mentioned earlier there is no extraction method for skin moisturisers and body and hair washes.

Although concentrations of musks varied from sample to sample, galaxolide was the predominant compound found in all the classes of products studied (Fig. 28).

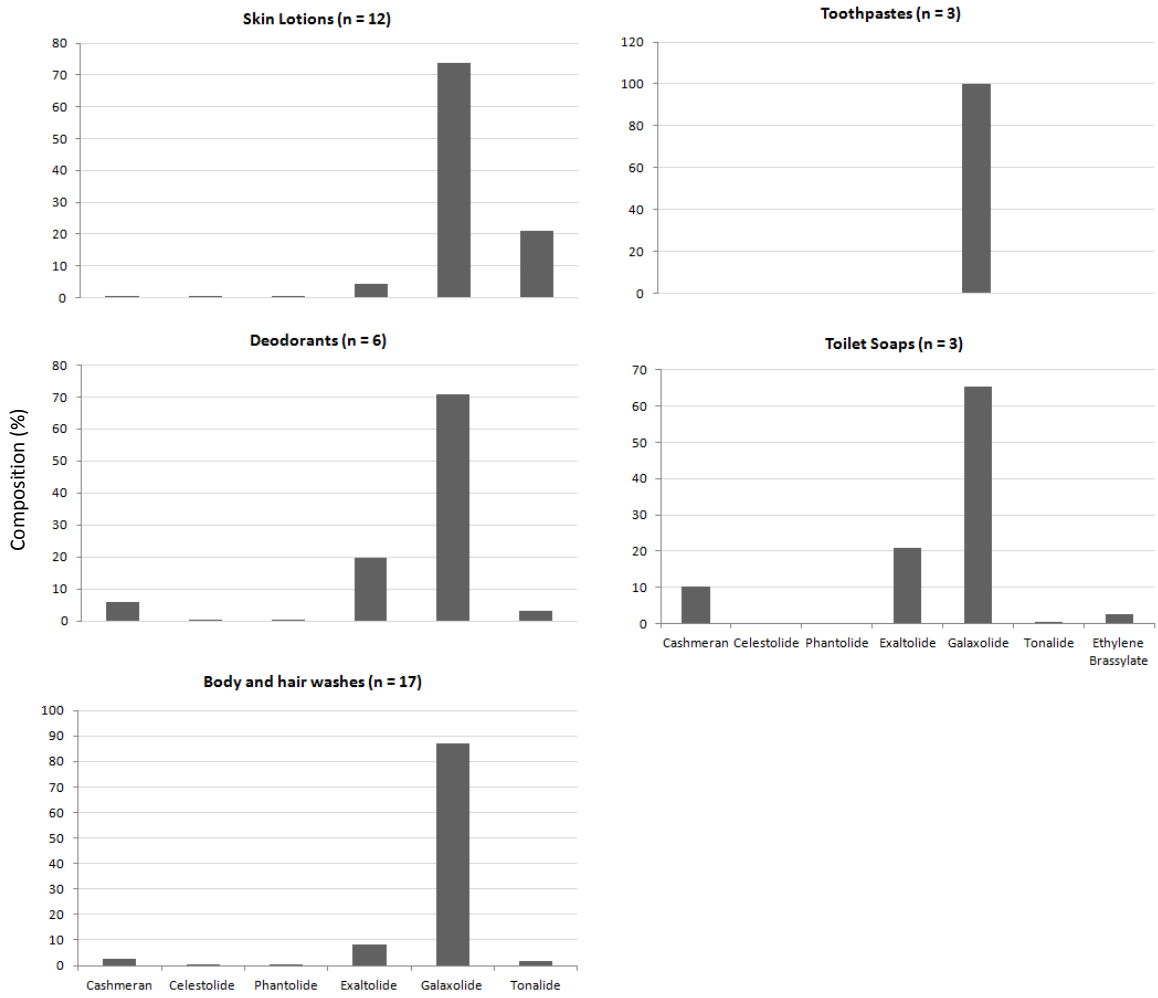


Fig. 28 – Composition (%) of synthetic musks in skin moisturisers, deodorants, body and hair washes, toothpastes and toilet soaps.

Galaxolide accounted for, on average, 75% of the total musk concentrations. Exaltolide was the second most abundant compound (12%). The use of nitro musks has been dramatically reduced in the U.S. and European countries and it is visible by the results previously shown as these compounds were not detected in the several products analysed.

Lu et al. (2011a) analysed galaxolide, tonalide, musks xylene and ketone in 158 personal care products marketed in China. Galaxolide was the most frequently found musk (73% of samples) followed by tonalide (65%). The authors identified musk ketone in 57% of the products. All toilet soaps contained the three previously mentioned musks. This class of products had also the highest percentage of occurrence for musk xylene (50%). The highest concentrations of galaxolide, tonalide and musk ketone were found in hair care products which also presented high concentrations of total synthetic musks with a median value of 50.3 µg/g. Just like for this study, galaxolide was the predominant compound found in each category of products analysed (on average, 52% of the total musk concentrations). The authors verified that tonalide was the second most abundant compound (24%) which would be in accordance

with this study if exaltolide had not been analysed. Musk ketone was found in more than 50% of the products analysed suggesting ongoing production and use of this compound as a fragrance ingredient in China.

Roosens et al. (2007) analysed 82 personal care and sanitation products in Belgium to determine the concentration of galaxolide, tonalide, musks ketone and xylene. Tonalide was present in 70% and galaxolide in 55% of the samples, whereas musks xylene and ketone could be detected in only 10% and 9% of the samples, respectively. Deodorants contained high concentrations of synthetic musks with levels up to 1000 μg/g sample galaxolide. Considerable levels of synthetic musks were found in most body lotions. Only 40% of shower products contained galaxolide, but the median value is rather high. Tonalide was more frequently detected in shower samples (60%), but at lower concentration. The same trend was observed for hair care products, but to a lesser extent.

Reiner and Kannan (2006) measured 60 household commodities in USA for polycyclic musks galaxolide and tonalide. Galaxolide was detected in 72% of the samples analysed, tonalide was detected in 32% of the samples. All deodorants analysed contained galaxolide and this category of products had the highest percentage of occurrence of this musk as well as tonalide (75%). None of the shower gels analysed showed measurable concentrations of galaxolide and tonalide. The highest concentrations of galaxolide were found body lotions and deodorants. Concentrations of polycyclic musks in products such as shower gels and shampoos were lower. The frequency of occurrence and concentrations of tonalide were lower than those of galaxolide. The highest concentration of tonalide (438 μg/g) was detected in an antiperspirant sample. A few body lotions and body creams also contained tonalide concentrations on the order of a few hundred parts per million. Shampoos contained tonalide at a few tens of parts per million.

Zhang et al. (2008) studied 31 household commodities for the distribution characteristics of galaxolide, tonalide, cashmeran, traseolide, phantolide, celestolide, musks xylene and ketone. Galaxolide was found in 61% of the total samples analysed while tonalide was detected in 36%. A few samples contained nitro musks, the occurrence frequency of musks ketone and xylene was 16% and 6%. The highest mean concentration (804 μg/g) of galaxolide was found in perfume, while the highest tonalide concentration (46.9 μg/g) was detected in shampoo. The concentrations of musks ketone and xylene did not exceed the levels established by the European Union, but high concentrations were determined in some of the products. The average concentrations in body washes and shampoos were 59 and 52 μg/g for galaxolide, and 2.5 and 12 μg/g for tonalide, respectively. Zhang et al. (2008) study presented average concentrations of galaxolide and tonalide much lower than those found in USA and Belgium.

To the author's knowledge, there are no studies on cosmetic products which include macrocyclic musks. As with the other studies, galaxolide was the most detected musk compound. In this research, nitro musks were not detected in cosmetic samples, whereas these were observed by Lu et al.

(2011a), Roosens et al. (2007) and Zhang et al. (2008). It is reported that the production of nitro musks has been dramatically reduced in the U.S. and European countries (Kallenborn and Gatermann, 2004), however, these compounds are still sparsely produced in India and China (Schmeiser et al., 2001), and frequently detected in various environmental matrices from China (Zhang et al., 2008).

4.5 Exposure assessment

In the present work, the exposure amounts through application of seven categories of personal care products (e.g. body lotion, facial cream, toothpaste, deodorant, toilet soap, shampoo, shower gel) were estimated. The user is exposed to the synthetic musks coming from each of these categories of products due to direct application to the skin. It represents the major source of exposure of humans to these compounds, particularly if the products are used on a daily basis. The behaviour of the consumer such as choice of product, frequency of application, as well as amount applied, have a consequence on individual exposure to these compounds. Depending on which products a consumer uses, the exposure to synthetic musks varies and a person can be exposed to several musks at the same time from the use of a particular product.

Body moisturisers are the most important source of dermal exposure due to their retention in the skin, followed by deodorants which have a high concentration of musk-containing fragrances. On the other hand, shower gels and shampoos, which are applied over a larger area, are rinsed off after a short period of time. These classes of products are probably a less important dermal source of musk compounds. Consequently, there are two exposure situations: human and environmental. The environmental exposure is expected to be due to down-the-drain rinsing of musks, through the sewer system and ending in wastewater treatment plants. The removal of fragrance materials in several countries wastewater treatment plants was determined (Table 4). The effluent concentrations can vary, as a result of the different types of removal processes that wastewater treatment plants employ.

The human exposure amount was obtained using the sum of the mean concentrations measured in this study multiplied by the daily usage rates of the products reported earlier (Bickers et al., 2003, Loretz et al., 2005, Loretz et al., 2006, Hall et al., 2007). Mean daily usage rate, except for toilet soap and toothpaste, were from the exposure profiles reported by Loretz et al. (2005) and Loretz et al. (2006), and the values for toilet soap and toothpaste were from Bickers et al. (2003) and Hall et al. (2007), respectively. Estimated exposure profiles of synthetic musks (µg/d) through dermal application of personal care products are shown in Table 19.

Table 19 – Daily exposure rates (µg/d) to total musks from different categories of personal care products. A retention of 100% was considered.

Product	*Daily usage (g/d)*	*Total musks (µg/g)*	*Synthetic musks (µg/d)*
Body lotion	8.70	259.900	2261.129
Facial cream	0.90	42.302	38.072
Toothpaste	2.09	0.003	0.007
Deodorant	0.90	99.681	89.712
Toilet soap	4.80	27.277	130.931
Shampoo	12.8	194.716	2492.359
Shower gel	14.5	113.063	1639.418
Total	-	-	6651.629

In present study, the daily exposure rate to total synthetic musks from the use of personal care products for a person in Portugal was estimated to be 6652 µg/d; the highest contributor to exposure amounts was shampoo (2492 µg/d). Among all of the musk compounds, galaxolide contributed most to total musk exposure. Relatively high exposure amounts to polycyclic musks were from the use of body lotion, facial cream and deodorants. Skin moisturisers and deodorants were the major contributors of exposure to macrocyclic musks, with their highest daily exposure rates of individual musks up to several hundreds of microgram per day.

Few studies have investigated the dermal exposure rates to musks in developed countries. Based on the sum of average concentrations of musks in this study, the daily exposure amounts through application of personal care products were higher than those reported by Lu et al. (2011a) (3380 µg/d) and similar to those observed by Roosens et al. (2007) (7493 µg/d – medium exposure). However, the number of samples used in this study was lower than those of other studies. So it would be necessary to consider more samples in order to get a more robust result.

5 Conclusions

In this study, a new analysis method suitable to separate and identify 12 synthetic musk compounds was developed, optimised and validated. The QuEChERS method is quick and inexpensive, it provides reliable results, whilst reducing the quantities of reagents and laboratory glassware, as well as the number of analytical steps. Due to the ubiquity of musks and therefore increased risk of contamination, QuEChERS are more advantageous owing to the use of a reduced number of containers which are disposable. This method simplifies the extraction of analytes and extract cleanup without adversely affecting the magnitude of analyte recoveries. Several parameters such as the sample quantity, the type or volume of extraction solvent, the evaporation step and the amount of sorbents of QuEChERS were manipulated in order to achieve the optimal conditions. The developed extraction method was validated through quantification and reliability parameters.

The concentrations and distribution of the synthetic musks were determined in the five categories selected: skin moisturisers, toothpaste, deodorants, toilet soaps, and body and hair washes. Synthetic musks were detected in all of the 41 samples analysed. Galaxolide (93% of samples) and cashmeran (83 % of samples) were the most commonly detected musks in personal care products. Nitro musks were not found in the samples analysed. Polycyclic musks were found in 98% of the analysed samples. The highest concentration for galaxolide was observed in skin moisturisers (882 μg/g) followed by body and hair washes (730 μg/g). Tonalide's highest percentage of occurrence was in toilet soaps (67% of samples) and the highest concentration was found for skin moisturisers (204 μg/g). The highest percentage of occurrence for exaltolide was found in toilet soaps (100% of samples analysed), followed by skin moisturisers and deodorants (both in 83% of samples). Toilet soaps were the only class of products where ethylene brassylate was identified with a concentration of 0.736 μg/g.

Human exposure assessment to musk through dermal application was assessed. It was observed that the daily exposure rate to total synthetic musks from the use of personal care products for a person in Portugal was 6652 μg/d. Shampoo was the highest contributor to exposure amounts. Galaxolide was the highest contributor to the total amount of synthetic musks.

6 Limitations and Future Work

Although good results were achieved, some limitations were faced while conducting this work. The capillary column installed on the used GC-MS was chosen based on other works being developed by other researchers. A substitution by a more appropriate column (more polar) was not possible as this equipment was shared with other technicians. This led also to longer time for each analysis (about 60 min) and as this equipment was shared, time to perform further experiments was limited.

A failure of a vital component of the GC-MS (turbomolecular pump) occurred during the work and repair took some time as spare parts had to be imported. Therefore, analysis of spiked deodorant samples was not possible and for this no precision and accuracy could be evaluated. In future, missing analysis to obtain these results will be performed.

Due to the wide concentration range of musks contained in the analysed products, dilutions of some of the extracts had to be done. In some samples, concentrations of musks were quite disperse, while the level of some compounds were low, others were beyond the calibration curves linearity range. Therefore, a compromise in the dilution had to be made resulting in concentration values which were in close proximity to the lower limit of the calibration curve. To overcome this, an extension of the calibration curve nearer to the detection limit may be tested in future, in order to allow the analysis of samples with lower levels of musks (after dilution).

The developed extraction method did not yield acceptable results for sunscreen samples. Furthermore, distinction between ethylene brassylate and fatty acids in the skin moisturisers and body and hair washes was challenging as this musk has lipid-like structure (lactone). Therefore, further experiments have to be done in order to adapt the existing method to these issues.

Perfumes were initially planned to be analysed as last samples, as no extraction seems to be necessary. However, lack of time due to the above mentioned equipment reasons, didn’t allow the processing of this samples, which will be done in near future.

In order to present a more representative exposure assessment, a bigger sampling pool of personal care products would be necessary. However, due to time and funding limitations only a reduced number of branded products were analysed. In future, a more representative choice of products including white label and cleaning products will be included.

The obtained results have an uncertainty associated to them. In practical terms, the uncertainty of the result may derive from many possible sources, including examples such as inadequate definition, sampling, matrix effects and interference, experimental conditions, uncertainties of the scales, reference values, approaches and conventions incorporated in the measurement method and procedure and random error. In the future, global uncertainty should be evaluated.

As future work, it is expected to carry out the extraction of synthetic musks by QuEChERS or other appropriate methods (liquid-liquid extraction, etc.) method in environmental samples, such as surface water, wastewater, sludge and sediments.

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Appendix 1 Calibration curves

For each synthetic musk compound, a calibration curve was drawn, by injecting ten calibration levels (5, 10, 50, 100, 200, 400, 1000, 2000, 3000 and 4000 µg/L) in duplicate. As explained before, the personal care products selected have musks in very different concentration ranges. This requires a dilution. When diluted, musks at very high concentrations will be within the linearity range. However, musks whose concentrations are near the calibration's curve upper limit, will be outside this range after dilution. Therefore, some dilutions result in concentration values which are in close proximity to the lower limit of the calibration curve. To avoid negative concentration values, curves were forced to pass through the origin of the graph (Fig. A 1 – A 12).

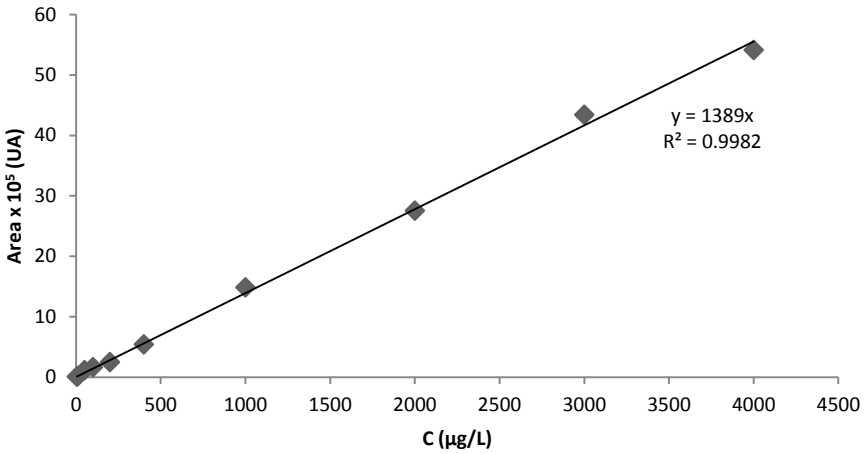

Fig. A 1 - Calibration curve of cashmeran.

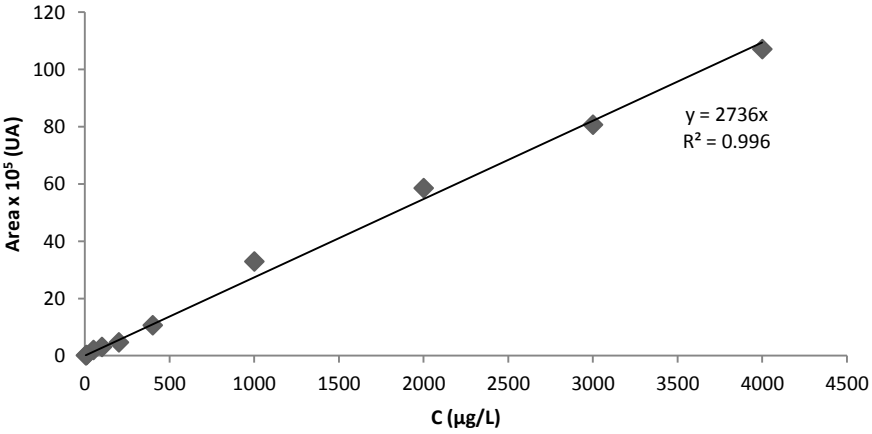

Fig. A 2 – Calibration curve of celestolide.

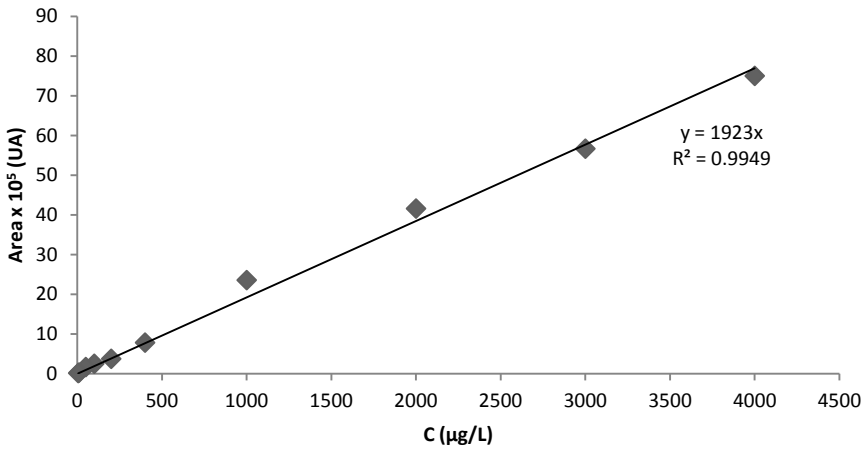

Fig. A 3 – Calibration curve of phantolide.

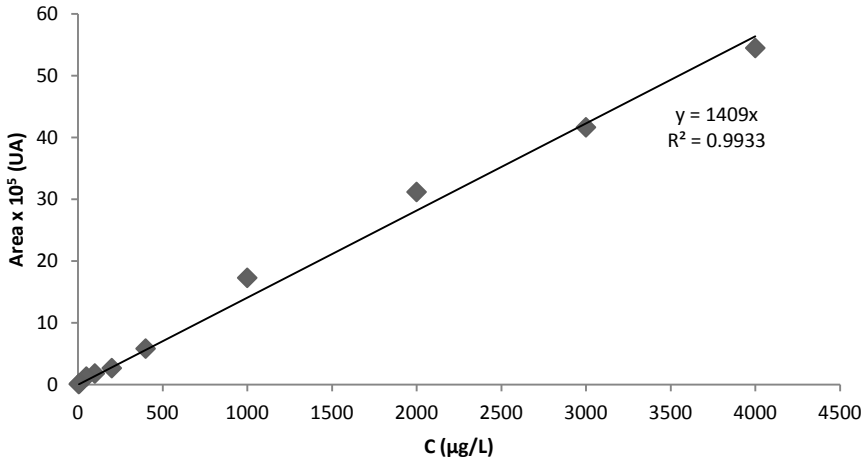

Fig. A 4 – Calibration curve of exaltolide.

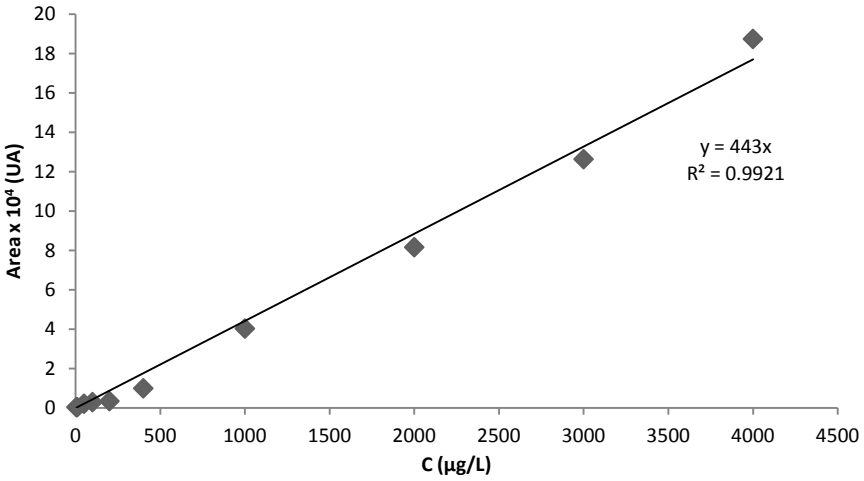

Fig. A 5 – Calibration curve of musk ambrette.

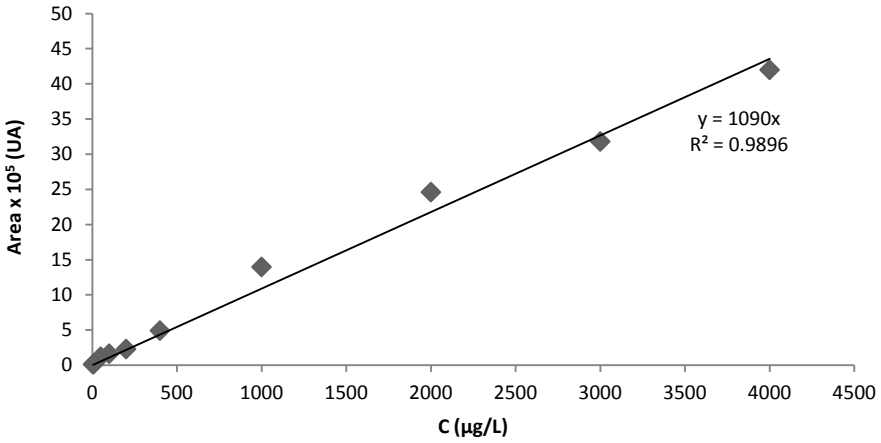

Fig. A 6 – Calibration curve of galaxolide.

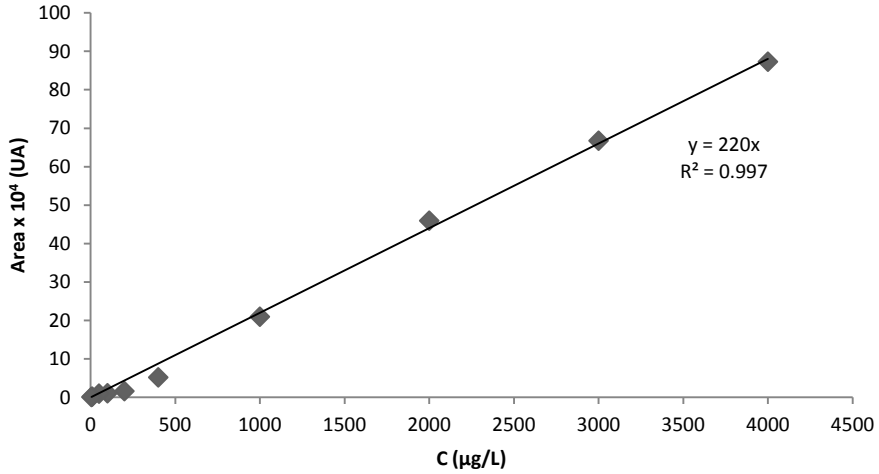

Fig. A 7 – Calibration curve of musk xylene.

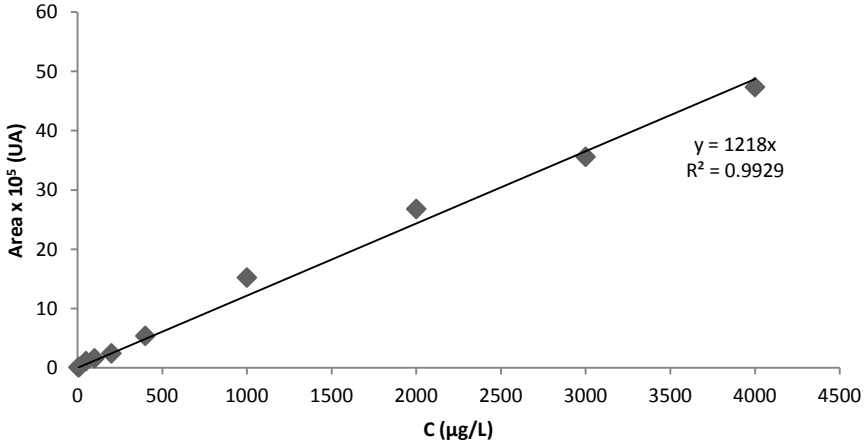

Fig. A 8 – Calibration curve of tonalide.

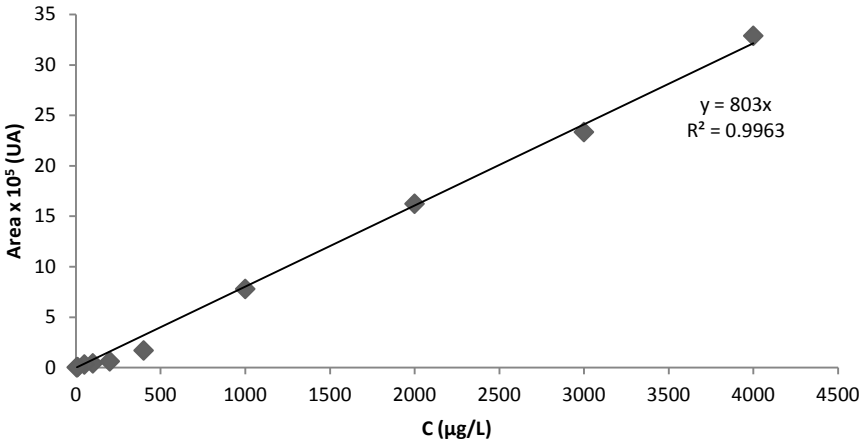

Fig. A 9 – Calibration curve of musk moskene.

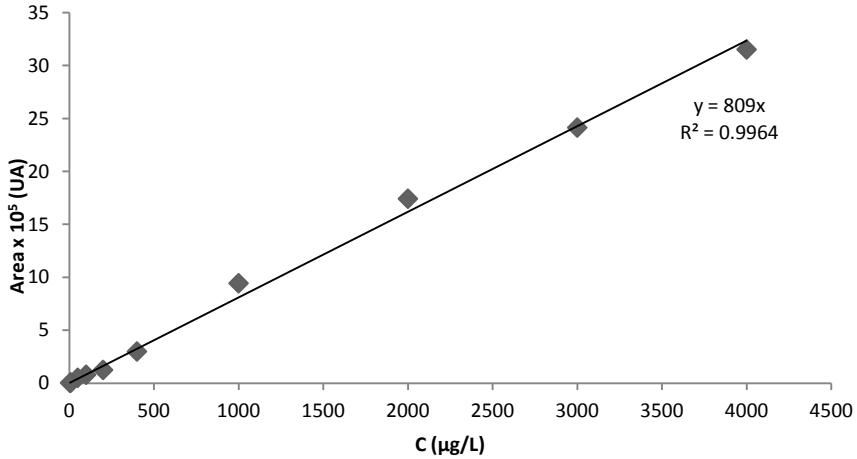

Fig. A 10 – Calibration curve of musk tibetene.

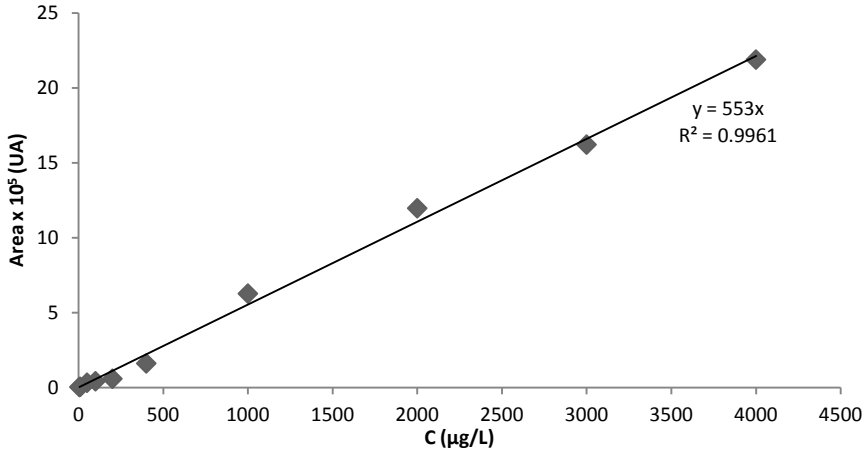

Fig. A 11 – Calibration curve of musk ketone.

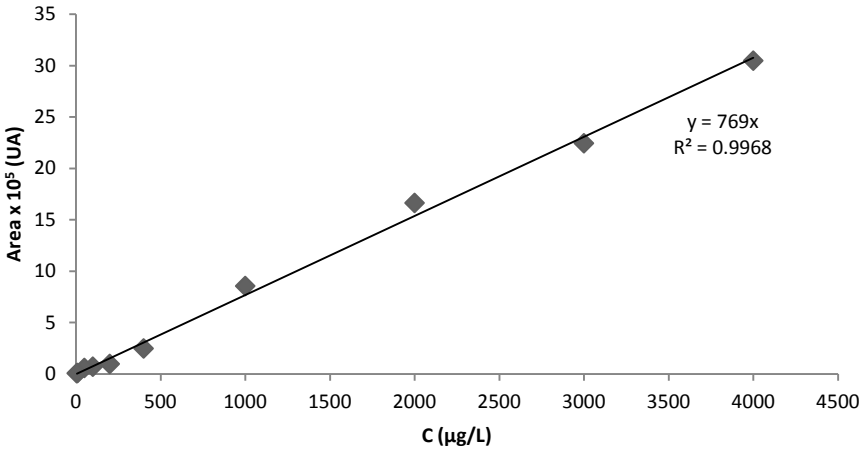

Fig. A 12 – Calibration curve of ethylene brassylate.

Appendix 2 Precision and Accuracy

Method precision was studied within a day and among days at three concentration levels, 100, 200, and 400 µg/L. For intra-day precision, spiked extracts were used. A product was selected from each of the four classes: skin moisturisers, toothpastes, toilet soaps, body and hair washes (Tables A 1 – A 16). Precision studies were not carried for deodorants because the analysis of these samples coincided with the breakdown of GC-MS and the lack of time was another constraint. Inter-day precision was evaluated for the injection of standards alone on three different days (Tables A 1 – A 19).

Table A 1 – Musks areas (UA) in blanks, standards and a skin lotion sample

Compound	*Blank 1*	*Blank 2*	*Mean*	*100 µg/L Std*	*200 µg/L Std*	*400 µg/L Std*	*Sample*
Cashmeran	0	0	0	90915	203647	435698	9982.5
Celestolide	0	0	0	187300	407680	882123	0
Phantolide	0	0	0	140594	298791	626640	0
Exaltolide	1669	1879	1774	100856	210410	440616	60689
M. Ambrette	0	0	0	35201	65417	187046	0
Galaxolide	3118	2988	3053	79476	165862	376119	3198.5
M. Xylene	0	0	0	12384	29215	73245	0
Tonalide	543	751	647	98606	190930	423615	5986.5
M. Moskene	0	0	0	55942	124080	241401	0
M. Tibetene	0	0	0	64019	133633	233496	0
M. Ketone	0	0	0	36156	79212	201203	0
Ethylene Brassylate	2937	4448	3692.5	52843	101647	230445	22775000

Table A 2 – Musk areas (UA) for four injections of a spiked skin moisturiser with a final concentration of 400 µg/L, intra-day precision (%CV) and accuracy (%Rec)

Compound	*1*	*2*	*3*	*4*	*Mean*	*%CV*	*Rec (%)*
Cashmeran	368177	436962	407433	356382	392238	9.42	87.7
Celestolide	771949	901093	797422	709985	795112	10.01	90.1
Phantolide	559621	645528	570575	513760	572371	9.54	91.3
Exaltolide	439187	512933	486299	428791	466803	8.49	91.8
M. Ambrette	189599	209946	166408	164147	182525	11.83	97.6
Galaxolide	315479	376055	350006	304767	336577	9.70	87.8
M. Xylene	57991	66888	57796	58048	60181	7.43	82.2
Tonalide	392194	461965	409594	372685	409110	9.37	95.0
M. Moskene	184363	219187	239140	231961	218663	11.12	90.6
M. Tibetene	246698	288868	280297	265576	270360	6.83	115.8
M. Ketone	170771	206871	180240	167652	181384	9.82	90.1
Ethylene Brassylate	18784105	20932287	2.19E+07	1.92E+07	20189098	7.29	-1123.7

Table A 3 - Musk areas (UA) for four injections of a spiked skin moisturiser with a final concentration of 200 µg/L, intra-day precision (%CV) and accuracy (%Rec)

Compound	*1*	*2*	*3*	*4*	*Mean*	*%CV*	*Rec (%)*
Cashmeran	172687	204278	244320	194260	203886	14.72	95.2
Celestolide	354152	438607	498382	391550	420673	14.80	103.2
Phantolide	262719	314045	366269	296769	309951	13.93	103.7
Exaltolide	245676	284264	320174	260631	277686	11.70	102.3
M. Ambrette	78128	90558	114463	76377	89882	19.54	137.4
Galaxolide	138906	183576	192691	154216	167347	14.99	97.1
M. Xylene	30547	34649	43017	30452	34666	17.02	118.7
Tonalide	186378	234948	267847	217653	226707	15.00	115.3
M. Moskene	96447	125839	148100	103296	118421	19.79	95.4
M. Tibetene	141048	173774	210710	157462	170749	17.45	127.8
M. Ketone	75249	92673	107509	84315	89937	15.24	113.5
Ethylene Brassylate	1.83E+07	2.23E+07	2.50E+07	2.10E+07	21637500	12.79	-1122.7

Table A 4 - Musk areas (UA) for four injections of a spiked skin moisturiser with a final concentration of 100 µg/L, intra-day precision (%CV) and accuracy (%Rec)

Compound	*1*	*2*	*3*	*4*	*Mean*	*%CV*	*Rec (%)*
Cashmeran	61703	77616	87020	88920	78815	15.77	75.7
Celestolide	132288	170822	179875	185875	167215	14.41	89.3
Phantolide	97175	127543	129948	134752	122355	13.94	87.0
Exaltolide	105443	141702	157348	164751	142311	18.54	79.2
M. Ambrette	26592	48025	49963	46877	42864	25.48	121.8
Galaxolide	50640	68041	70378	69682	64685	14.55	73.5
M. Xylene	10123	16860	13006	19138	14782	27.10	119.4
Tonalide	69333	96483	95897	113600	93828	19.48	88.4
M. Moskene	34296	53537	55555	50901	48572	19.98	86.8
M. Tibetene	55380	96560	81946	83073	79240	21.75	123.8
M. Ketone	26393	37251	40938	40305	36222	18.63	100.2
Ethylene Brassylate	1.35E+07	1.97E+07	1.98E+07	2.05E+07	18357500	17.78	-8366.7

Table A 5 - Musks areas (UA) in blanks, standards and toothpaste sample

Compound	*Blank 1*	*Blank 2*	*Mean*	*100 µg/L Std*	*200 µg/L Std*	*400 µg/L Std*	*Sample*
Cashmeran	0	0	0	73202	174379	322128	0
Celestolide	0	0	0	174723	415047	789189	0
Phantolide	0	0	0	151759	343305	620990	0
Exaltolide	0	0	0	118620	193809	444130	0
M. Ambrette	0	0	0	28869	49121	111598	0
Galaxolide	921	966	943.5	88988	211628	376505	2237
M. Xylene	0	0	0	7183	14022	21196	0
Tonalide	0	0	0	101721	235466	416575	0
M. Moskene	0	0	0	37579	73346	140683	0
M. Tibetene	0	0	0	58970	143899	214724	0
M. Ketone	0	0	0	33912	79602	168461	0
Ethylene Brassylate	0	0	0	69540	144257	239447	0

Table A 6 – Musk areas (UA) for four injections of a spiked toothpaste with a final concentration of 100 µg/L, intra-day precision (%CV) and accuracy (%Rec)

Compound	*1*	*2*	*3*	*4*	*Mean*	*%CV*	*Rec (%)*
Cashmeran	91960	82242	82740	84254	8.53E+04	5.3	116.5
Celestolide	126702	117913	115173	125655	1.21E+05	4.7	69.5
Phantolide	103122	95559	90290	102948	9.80E+04	6.3	64.6
Exaltolide	73130	58920	65187	71355	6.71E+04	9.6	56.6
M. Ambrette	17403	16817	0	21359	1.39E+04	68.2	48.1
Galaxolide	57201	53454	51735	57296	5.49E+04	5.1	58.1
M. Xylene	1706	6875	5558	5666	4.95E+03	45.3	68.9
Tonalide	70160	62430	61090	68553	6.56E+04	6.8	64.4
M. Moskene	17938	16847	18397	22266	1.89E+04	12.5	50.2
M. Tibetene	43400	41406	39666	43887	4.21E+04	4.6	71.4
M. Ketone	38843	39419	40330	48387	4.17E+04	10.7	123.1
Ethylene Brassylate	35028	41702	43558	48832	4.23E+04	13.5	60.8

Table A 7 - Musk areas (UA) for four injections of a spiked toothpaste with a final concentration of 200 µg/L, intra-day precision (%CV) and accuracy (%Rec)

Compound	*1*	*2*	*3*	*3*	*Mean*	*%CV*	*Rec (%)*
Cashmeran	150248	139536	125245	135398	1.38E+05	7.5	78.9
Celestolide	265442	241891	225066	239479	2.43E+05	6.9	58.5
Phantolide	207534	192259	176802	188777	1.91E+05	6.6	55.7
Exaltolide	148091	129873	119485	120493	1.29E+05	10.2	66.8
M. Ambrette	41354	37819	38042	37914	3.88E+04	4.4	79.0
Galaxolide	115987	106057	99288	106516	1.07E+05	6.4	49.0
M. Xylene	9948	8740	9957	10621	9.82E+03	8.0	70.0
Tonalide	143037	132629	124500	130325	1.33E+05	5.8	56.3
M. Moskene	46705	43279	42391	45176	4.44E+04	4.4	60.5
M. Tibetene	97296	87406	81649	87991	8.86E+04	7.3	61.6
M. Ketone	82121	91159	101095	100260	9.37E+04	9.5	117.7
Ethylene Brassylate	89468	87169	82776	88215	8.69E+04	3.3	60.2

Table A 8 - Musk areas (UA) for four injections of a spiked toothpaste with a final concentration of 400 µg/L, intra-day precision (%CV) and accuracy (%Rec)

Compound	*1*	*2*	*3*	*4*	*Mean*	*%CV*	*Rec (%)*
Cashmeran	307130	252868	287787	300226	2.87E+05	8.4	89.1
Celestolide	623709	509217	570103	584848	5.72E+05	8.3	72.5
Phantolide	465854	383114	421403	429728	4.25E+05	8.0	68.4
Exaltolide	342604	291947	328536	333928	3.24E+05	6.9	73.0
M. Ambrette	98492	76252	100808	104489	9.50E+04	13.4	85.1
Galaxolide	276650	227630	254244	253017	2.53E+05	7.9	66.3
M. Xylene	21245	21244	28169	29269	2.50E+04	17.4	117.9
Tonalide	317584	265658	295858	295394	2.94E+05	7.3	70.5
M. Moskene	138864	106819	130746	140064	1.29E+05	12.0	91.8
M. Tibetene	225255	184930	206393	206553	2.06E+05	8.0	95.8
M. Ketone	181834	134545	177974	164880	1.65E+05	13.0	97.8
Ethylene Brassylate	221919	178093	212536	206043	2.05E+05	9.2	85.5

Table A 9 – Musks areas (UA) in blanks, standards and a toilet soap sample

Compound	*Blank 1*	*Blank 2*	*Mean*	*100 µg/L Std*	*200 µg/L Std*	*400 µg/L Std*	*Sample*
Cashmeran	0	0	0	73202	174379	322128	1306000
Celestolide	0	0	0	174723	415047	789189	0
Phantolide	0	0	0	151759	343305	620990	0
Exaltolide	0	0	0	118620	193809	444130	150427.5
M. Ambrette	0	0	0	28869	49121	111598	158371
Galaxolide	921	966	943.5	88988	211628	376505	134507.5
M. Xylene	0	0	0	7183	14022	21196	0
Tonalide	0	0	0	101721	235466	416575	0
M. Moskene	0	0	0	37579	73346	140683	0
M. Tibetene	0	0	0	58970	143899	214724	0
M. Ketone	0	0	0	33912	79602	168461	0
Ethylene Brassylate	0	0	0	69540	144257	239447	0

Table A 10 – Musk areas (UA) for four injections of a spiked toilet soap with a final concentration of 100 µg/L, intra-day precision (%CV) and accuracy (%Rec)

Compound	*1*	*2*	*3*	*4*	*Mean*	*%CV*	*Rec (%)*
Cashmeran	1.46E+06	1.28E+06	1.41E+06	1.33E+06	1.37E+06	5.7	89.8
Celestolide	94869	76633	85215	88595	8.63E+04	8.8	49.4
Phantolide	74950	60581	71076	71509	6.95E+04	8.9	45.8
Exaltolide	250109	194057	224153	224101	2.23E+05	10.3	61.3
M. Ambrette	202622	172299	191844	211798	1.95E+05	8.7	125.6
Galaxolide	204376	165923	185304	194623	1.88E+05	8.7	58.6
M. Xylene	5723	5625	5762	5958	5.77E+03	2.4	80.3
Tonalide	49453	39906	47194	47530	4.60E+04	9.1	45.2
M. Moskene	16909	12974	13770	16192	1.50E+04	12.6	39.8
M. Tibetene	31559	24144	28129	29652	2.84E+04	11.1	48.1
M. Ketone	22365	9867	11604	14724	1.46E+04	37.8	43.2
Ethylene Brassylate	33182	22170	28910	27608	2.80E+04	16.2	40.2

Table A 11 - Musk areas (UA) for four injections of a spiked toilet soap with a final concentration of 200 µg/L, intra-day precision (%CV) and accuracy (%Rec)

Compound	*1*	*2*	*3*	*4*	*Mean*	*%CV*	*Rec (%)*
Cashmeran	1.31E+06	1.51E+06	1.54E+06	1.43E+06	1.45E+06	7.1	82.1
Celestolide	154493	191486	186258	178119	1.78E+05	9.2	42.8
Phantolide	127088	155290	149775	141864	1.44E+05	8.5	41.8
Exaltolide	229487	260442	282619	254867	2.57E+05	8.5	54.9
M. Ambrette	180857	207268	209928	203471	2.00E+05	6.6	85.5
Galaxolide	191433	232616	227034	212889	2.16E+05	8.5	38.1
M. Xylene	8718	8732	8784	8858	8.77E+03	0.7	62.6
Tonalide	84134	102167	95567	89893	9.29E+04	8.3	39.5
M. Moskene	27250	31125	31723	31297	3.03E+04	6.9	41.4
M. Tibetene	50340	63845	60147	56706	5.78E+04	9.9	40.1
M. Ketone	29901	31784	32902	28479	3.08E+04	6.4	38.7
Ethylene Brassylate	46927	60583	58728	51363	5.44E+04	11.7	37.7

Table A 12 - Musk areas (UA) for four injections of a spiked toilet soap with a final concentration of 400 µg/L, intra-day precision (%CV) and accuracy (%Rec)

Composto	*1*	*2*	*3*	*4*	*Mean*	*%CV*	*Rec (%)*
Cashmeran	1.61E+06	1.69E+06	1.61E+06	1.67E+06	1.65E+06	2.4	105.2
Celestolide	371745	396009	380597	369374	3.79E+05	3.2	48.1
Phantolide	285657	306553	297367	290843	2.95E+05	3.1	47.5
Exaltolide	364694	372595	358651	366025	3.65E+05	1.6	48.4
M. Ambrette	228575	274027	238905	241852	2.46E+05	8.0	78.4
Galaxolide	300750	320703	300705	310494	3.08E+05	3.1	45.9
M. Xylene	19297	19361	19210	19185	1.93E+04	0.4	90.9
Tonalide	192516	206633	197117	193913	1.98E+05	3.2	47.4
M. Moskene	66766	72220	70333	67438	6.92E+04	3.7	49.2
M. Tibetene	130387	141895	130907	129409	1.33E+05	4.4	62.0
M. Ketone	71946	79546	72521	73691	7.44E+04	4.7	44.2
Ethylene Brassylate	113396	131710	119313	117906	1.21E+05	6.5	50.4

Table A 13 – Musks areas (UA) in blanks, standards and a shower gel sample

Compound	*Blank 1*	*Blank 2*	*Mean*	*100 µg/L Std*	*200 µg/L Std*	*400 µg/L Std*	*Sample*
Cashmeran	2886	2698	2792	70707	147196	299086	4860000
Celestolide	0	0	0	149645	288510	643359	0
Phantolide	0	0	0	112135	232735	469822	0
Exaltolide	5146	5045	5095.5	105628	173814	345593	0
M. Ambrette	0	0	0	31590	40807	92358	0
Galaxolide	1313	1773	1543	74841	137837	288280	18963
M. Xylene	0	0	0	11985	26648	50935	0
Tonalide	0	0	0	75160	162826	336981	0
M. Moskene	0	0	0	49913	96369	160809	0
M. Tibetene	0	0	0	49450	102732	170041	0
M. Ketone	0	0	0	29928	59858	137691	45733.5
Ethylene Brassylate	0	0	0	47877	96105	208184	0

Table A 14 - Musk areas (UA) for four injections of a spiked shower gel with a final concentration of 100 µg/L, intra-day precision (%CV) and accuracy (%Rec)

Compound	*1*	*2*	*3*	*4*	*Mean*	*%CV*	*Rec (%)*
Cashmeran	4.92E+06	4.95E+06	4.96E+06	4.94E+06	4.94E+06	0.4	113.3
Celestolide	79977	94975	88599	93792	8.93E+04	7.6	59.7
Phantolide	63860	74745	65898	68478	6.82E+04	6.9	60.9
Exaltolide	120833	149919	135358	137923	1.36E+05	8.8	123.9
M. Ambrette	34134	32363	33185	30927	3.27E+04	4.2	103.4
Galaxolide	60697	67752	62312	66326	6.43E+04	5.2	58.5
M. Xylene	14675	13163	12314	13343	1.34E+04	7.3	111.6
Tonalide	54242	62335	64588	64993	6.15E+04	8.1	81.9
M. Moskene	33250	36845	35702	36995	3.57E+04	4.8	71.5
M. Tibetene	35140	34999	34604	34619	3.48E+04	0.8	70.5
M. Ketone	77055	78214	72026	74246	7.54E+04	3.7	99.1
Ethylene Brassylate	731197	790759	733131	755597	7.53E+05	3.7	1572.1

Table A 15 - Musk areas (UA) for four injections of a spiked shower gel with a final concentration of 200 µg/L, intra-day precision (%CV) and accuracy (%Rec)

Compound	*1*	*2*	*3*	*4*	*Mean*	*%CV*	*Rec (%)*
Cashmeran	5236456	5088392	4629517	5057181	5002886.5	5.215145	95.2
Celestolide	168325	155216	170118	162217	1.64E+05	4.113347	56.8
Phantolide	152171	140368	154305	145005	1.48E+05	4.351433	63.6
Exaltolide	175170	174590	202542	191345	1.86E+05	7.28076	104.0
M. Ambrette	41794	40190	39811	41006	4.07E+04	2.170366	99.7
Galaxolide	110077	103094	109142	103478	1.06E+05	3.451572	62.4
M. Xylene	36580	28116	27401	26469	2.96E+04	15.77032	111.2
Tonalide	132371	119912	125643	118686	1.24E+05	5.043602	76.2
M. Moskene	70010	61074	68156	64600	6.60E+04	5.997428	68.4
M. Tibetene	74377	74403	77039	72936	7.47E+04	2.289808	72.7
M. Ketone	115328	165804	150289	0	1.08E+05	69.4805	103.8
Ethylene Brassylate	734773	749245	804200	766449	7.64E+05	3.923699	794.6

Table A 16 - Musk areas (UA) for three injections of a spiked shower gel with a final concentration of 400 µg/L, intra-day precision (%CV) and accuracy (%Rec)

Compound	*1*	*2*	*3*	*Mean*	*%CV*	*Rec (%)*
Cashmeran	5.16E+06	5165633	5187189	5.17E+06	0.25	103.4
Celestolide	395089	362025	381151	3.79E+05	4.37	59.0
Phantolide	329790	311819	338013	3.27E+05	4.10	69.5
Exaltolide	337700	324336	331769	3.31E+05	2.02	94.4
M. Ambrette	107521	88757	102396	9.96E+04	9.74	107.8
Galaxolide	216722	201012	217771	2.12E+05	4.43	66.4
M. Xylene	58170	56047	57064	5.71E+04	1.86	112.1
Tonalide	274103	252295	269182	2.65E+05	4.31	78.7
M. Moskene	184190	164424	185004	1.78E+05	6.55	110.6
M. Tibetene	180296	165834	178260	1.75E+05	4.48	102.8
M. Ketone	209478	205479	211124	2.09E+05	1.39	118.4
Ethylene Brassylate	924927	792031	841650	8.53E+05	7.87	409.7

Table A 17 – Musk areas (UA) for the injection of a spiked shower gel with a final concentration of 100 µg/L in three different days and inter-day precision (%CV)

Compound	*Day 1*	*Day 2*	*Day 3*	*Mean*	*%CV*
Cashmeran	90915	73202	70707	78274.67	14.1
Celestolide	187300	174723	149645	170556	11.2
Phantolide	140594	151759	112135	134829.3	15.2
Exaltolide	100856	118620	105628	108368	8.5
M. Ambrette	35201	28869	31590	31886.67	10.0
Galaxolide	79476	88988	74841	81101.67	8.9
M. Xylene	12384	7183	11985	10517.33	27.5
Tonalide	98606	101721	75160	91829	15.8
M. Moskene	55942	37579	49913	47811.33	19.6
M. Tibetene	64019	58970	49450	57479.67	12.9
M. Ketone	36156	33912	29928	33332	9.5
Ethylene Brassylate	52843	69540	47877	56753.33	20.0

Table A 18 – Musk areas (UA) for the injection of a spiked shower gel with a final concentration of 200 µg/L in three different days and inter-day precision (%CV)

Compound	*Day 1*	*Day 2*	*Day 3*	*Mean*	*%CV*
Cashmeran	203647	174379	147196	175074	16.1
Celestolide	407680	415047	288510	370412.3	19.2
Phantolide	298791	343305	232735	291610.3	19.1
Exaltolide	210410	193809	173814	192677.7	9.5
M. Ambrette	65417	49121	40807	51781.67	24.2
Galaxolide	165862	211628	137837	171775.7	21.7
M. Xylene	29215	14022	26648	23295	34.9
Tonalide	190930	235466	162826	196407.3	18.6
M. Moskene	124080	73346	96369	97931.67	25.9
M. Tibetene	133633	143899	102732	126754.7	16.9
M. Ketone	79212	79602	59858	72890.67	15.5
Ethylene Brassylate	101647	144257	96105	114003	23.1

Table A 19 – Musk areas (UA) for the injection of a spiked shower gel with a final concentration of 400 µg/L in three different days and inter-day precision (%CV)

Compound	*Day 1*	*Day 2*	*Day 3*	*Mean*	*%CV*
Cashmeran	435698	322128	299086	352304	20.8
Celestolide	882123	789189	643359	771557	15.6
Phantolide	626640	620990	469822	572484	15.5
Exaltolide	440616	444130	345593	410113	13.6
M. Ambrette	187046	111598	92358	130334	38.4
Galaxolide	376119	376505	288280	346968	14.6
M. Xylene	73245	21196	50935	48458.67	53.9
Tonalide	423615	416575	336981	392390.3	12.3
M. Moskene	241401	140683	160809	180964.3	29.5
M. Tibetene	233496	214724	170041	206087	15.8
M. Ketone	201203	168461	137691	169118.3	18.8
Ethylene Brassylate	230445	239447	208184	226025.3	7.1

Appendix 3 Synthetic musks in personal care products

Distribution and concentrations of the studied synthetic musks among the five classes: skin moisturisers, toothpastes, deodorants, toilet soaps, body and hair washes are presented in Tables A 20 – A 24.

Table A 20 - Toothpastes (n = 3) mean, minimum and maximum concentrations (µg/g), n, and frequency of occurrence (%)

Compound	TP 1	TP 2	TP 3	**Mean**	**n**	**Frequency**	**Min.**	**Max.**
Cashmeran	--	--	--	--	0	0	--	--
Celestolide	--	--	--	--	0	0	--	--
Phantolide	--	--	--	--	0	0	--	--
Exaltolide	--	--	--	--	0	0	--	--
M. Ambrette	--	--	--	--	0	0	--	--
Galaxolide	0.002	0.003	0.004	0.003	3	100	0.002	0.004
M. Xylene	--	--	--	--	0	0	--	--
Tonalide	--	--	--	--	0	0	--	--
M. Moskene	--	--	--	--	0	0	--	--
M. Tibetene	--	--	--	--	0	0	--	--
M. Ketone	--	--	--	--	0	0	--	--
Ethylene Brassylate	--	--	--	--	0	0	--	--
			Total	**0.003**				

Table A 21 - Deodorants (n = 6) mean, minimum and maximum concentrations (µg/g), n, and frequency of occurrence (%)

Compound	D 1	D 2	D 3	D 4	D 5	D 6	**Mean**	**n**	**Frequency**	**Min.**	**Max.**
Cashmeran	2.110	4.520	4.630	11.359	1.708	14.721	6.508	6	100	1.708	14.721
Celestolide	--	--	--	--	--	0.134	0.134	1	17	0.134	0.134
Phantolide	--	--	--	--	--	0.028	0.028	1	17	0.028	0.028
Exaltolide	18.507	0.094	--	15.654	0.084	77.774	22.423	5	83	0.084	77.774
M. Ambrette	--	--	--	--	--	--	--	0	0	--	--
Galaxolide	0.897	0.007	0.042	0.018	0.007	400.446	66.903	6	100	0.007	400.446
M. Xylene	--	--	--	--	--	--	--	0	0	--	--
Tonalide	--	--	--	--	--	3.684	3.684	1	17	3.684	3.684
M. Moskene	--	--	--	--	--	--	--	0	0	--	--
M. Tibetene	--	--	--	--	--	--	--	0	0	--	--
M. Ketone	--	--	--	--	--	--	--	0	0	--	--
						Total	**99.681**				

Table A 22 - Toilet Soaps (n = 3) mean, minimum and maximum concentrations (µg/g), n, and frequency of occurrence (%)

Compound	TS 1	TS 2	TS 3	**Mean**	**n**	**Frequency**	**Min.**	**Max.**
Cashmeran	3.906	2.584	1.881	2.790	3	100	1.881	3.906
Celestolide	--	0.086	--	0.086	1	33	0.086	0.086
Phantolide	--	--	--	--	0	0	--	--
Exaltolide	2.025	14.811	0.213	5.683	3	100	0.213	14.811
M. Ambrette	--	--	--	--	0	0	--	--
Galaxolide	0.057	53.134	0.245	17.812	3	100	0.057	53.134
M. Xylene	--	--	--	--	0	0	--	--
Tonalide	0.012	0.326	--	0.169	2	67	0.012	0.326
M. Moskene	--	--	--	--	0	0	--	--
M. Tibetene	--	--	--	--	0	0	--	--
M. Ketone	--	--	--	--	0	0	--	--
Ethylene Brassylate	--	0.736	--	0.736	1	33	0.736	0.736
			Total	**27.277**				

Table A 23 - Skin Moisturisers (n = 12) mean, minimum and maximum concentrations (µg/g), n, and frequency of occurrence (%)

	Body Moisturisers									*Facial creams*							
Compound	BL 1	BL 2	BL 3	BL 4	BL 5	BL 6	BL 7	BL 8	BL 9	FC 1	FC 2	FC 3	**Mean**	**n**	**Frequency**	**Min.**	**Max.**
Cashmeran	3.430	0.168	0.930	0.397	7.519	1.712	0.849	3.898	0.958	2.133	0.451	--	2.040	11	92	0.168	7.519
Celestolide	0.328	--	--	--	--	--	--	--	--	0.093	--	--	0.210	2	17	0.093	0.328
Phantolide	--	--	--	0.054	--	--	--	--	--	0.301	--	--	0.177	2	17	0.054	0.301
Exaltolide	--	--	11.681	7.266	8.369	19.917	14.329	5.806	58.303	3.741	11.907	4.897	14.621	10	83	3.741	58.303
M. Ambrette	--	--	--	--	--	--	--	--	--	--	--	--	--	0	0	--	--
Galaxolide	882.340	--	0.698	58.170	0.960	0.888	1.011	--	0.052	56.900	0.586	--	111.291	9	75	0.052	882.340
M. Xylene	--	--	--	--	--	--	--	--	--	--	--	--	--	0	0	--	--
Tonalide	203.660	5.308	--	--	--	--	--	--	--	5.026	--	--	71.331	3	25	5.026	203.660
M. Moskene	--	--	--	--	--	--	--	--	--	--	--	--	--	0	0	--	--
M. Tibetene	--	--	--	--	--	--	--	--	--	--	--	--	--	0	0	--	--
M. Ketone	--	--	--	--	--	--	--	--	--	--	--	--	--	0	0	--	--
												Total	**199.670**				

Table A 24 - Body and hair washes (n = 17) mean, minimum and maximum concentrations (µg/g), n, and frequency of occurrence (%)

	Liquid soaps			*Shampoos*						*Shower gels*												
Compound	LS 1	LS 2	LS 3	SH 1	SH 2	SH 3	SH 4	SH 5	SH 6	SG 1	SG 2	SG 3	SG 4	SG 5	SG 6	SG 7	SG8	**Mean**	**n**	**Frequency**	**Min.**	**Max.**
Cashmeran	5.142	0.916	1.179	0.180	7.385	--	0.764	1.713	0.041	6.995	8.697	6.261	--	2.551	2.126	--	2.364	3.308	14	82	0.041	8.697
Celestolide	--	0.124	--	--	0.047	--	0.217	0.139	--	--	--	--	--	--	--	--	--	0.132	4	24	0.047	0.217
Phantolide	--	0.021	0.008	--	0.008	--	0.033	0.019	--	--	0.280	0.032	--	--	--	--	--	0.057	7	41	0.008	0.280
Exaltolide	0.254	6.256	4.061	--	1.552	1.502	39.841	29.062	2.824	--	7.258	--	36.864	1.112	--	--	0.198	10.899	12	71	0.198	39.841
M. Ambrette	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0	0	--	--
Galaxolide	0.018	134.185	0.014	0.293	192.381	0.015	451.652	410.383	0.004	0.032	0.034	730.142	0.025	0.006	0.144	0.072	0.004	112.906	17	100	0.004	730.142
M. Xylene	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0	0	--	--
Tonalide	--	1.486	--	--	1.166	--	1.668	2.567	--	--	--	5.410	--	--	--	--	--	2.460	5	29	1.166	5.410
M. Moskene	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0	0	--	--
M. Tibetene	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0	0	--	--
M. Ketone	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0	0	--	--
																	Total	**129.761**				

Appendix 3 Exposure assessment

Mean and total concentrations of the studied synthetic musks, necessary to calculate the exposure assessment to skin moisturisers, toothpastes, deodorants, toilet soaps, body and hair washes are presented in Table A 25.

Table A 25 – Mean and total concentrations (μg/g) of musks in different categories of personal care products

	Body lotions	*Facial creams*	*Toothpastes*	*Deodorants*	*Toilet soaps*	*Liquid soaps*	*Shampoos*	*Shower gels*
Cashmeran	2.207	1.292	--	6.508	2.790	2.412	2.016	4.832
Celestolide	0.328	0.093	--	0.134	0.086	0.124	0.134	--
Phantolide	0.054	0.301	--	0.028	--	0.014	0.020	0.156
Exaltolide	17.953	6.848	--	22.423	5.683	3.524	14.956	11.358
M. Ambrette	--	--	--	--	--	--	--	--
Galaxolide	134.875	28.743	0.00329081	66.903	17.812	44.739	175.788	91.307
M. Xylene	--	--	--	--	--	--	--	--
Tonalide	104.484	5.026		3.684	0.169	1.486	1.800	5.410
M. Moskene	--	--	--	--	--	--	--	--
M. Tibetene	--	--	--	--	--	--	--	--
M. Ketone	--	--	--	--	--	--	--	--
Ethylene Brassylate	--	--	--	--	0.736	--	--	--
Total	**259.900**	**42.302**	**0.003**	**99.6819**	**27.277**	**52.300**	**194.716**	**113.063**