Integrated Masters in Environmental Engineering

Detection of synthetic musks in beach sands by QuEChERS and GC-MS analysis

Master's Thesis

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Abstract

Synthetic musks are compounds used as fragrance fixatives in several personal care products. They can be divided into four classes, depending on their physicochemical properties: nitro, polycyclic, macrocyclic and alicyclic musks. Despite being considered emerging pollutants, to the author's best knowledge, no studies have been published to date regarding their presence on beach sands.

In this work, five nitro musks (musk ambrette, ketone, moskene, tibetene and xylene), five polycyclic musks (cashmeran, celestolide, galaxolide, phantolide and tonalide) and two macrocyclic musks (exaltolide and ethylene brassylate) were quantified in 45 beach sand samples. These samples were collected in beaches from Porto's metropolitan area, in the summer and in the winter.

Sample extraction was carried out using a QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) methodology that was previously developed in this research group, which combines both extraction and clean-up procedures. Conditions were as follows: 5 g of sample, extraction with 3 mL acetonitrile, first QuEChERS (2400 mg $MgSO_4$ + 750 mg $NaCH_3COOH$), second QuEChERS (180 mg $MgSO_4$ + 60 mg PSA + 30 mg C_{18}). The samples were then analyzed by GC-MS.

The analytical method was validated and calibration curves were elaborated (5 – 1000 μg/L). Limits of detection (LODs) and quantification (LOQs) were calculated based on signal-to-noise ratio; LODs ranged between 7.85 x 10^{-4} (tonalide) to 3.75 x 10^{-2} ng/g (musk tibetene). Recoveries ranged from 49.9 to 127.2%. The method's precision was proven, with values of relative standard deviation ranging between 1.9% (musk moskene) and 16.6 % (ethylene brassylate).

Musks were detected in all samples, however, musk ambrette, moskene, tibetene and xylene were not detected. Musk ketone was only detected in 31% of the samples, tonalide and exaltolide were the most detected musks (93% e 89% respectively).

Higher concentration of musks was found in the summer sample from Valadares Sul (32.59 ng/g dw) and lower concentration in the summer sample from Aguda (0.58 ng/g dw). Galaxolide was the musk present in higher concentration (0.04 – 26.93 ng/g dw); phantolide (0.03 – 0.09 ng/g dw) and celestolide (0.01 – 0.05 ng/g dw) presented the lowest concentrations.

Resumo

Os musks sintéticos são compostos utilizados como fixadores de fragrâncias em diversos produtos de higiene e cuidado pessoais. Podem ser divididos em quatro classes, de acordo com as suas propriedades físico-químicas: nitro musks, musks policíclicos, musks macrocíclicos e musks alicíclicos. Apesar de serem considerados poluentes emergentes, tanto quanto é do conhecimento do autor, nenhum estudo foi publicado até à data visando a sua presença em areias de praia.

Neste trabalho, foram quantificados, em 45 amostras de areias de praia, cinco nitro musks (musk ambrette, ketone, moskene, tibetene e xylene), cinco musks policíclicos (cashmeran, celestolide, galaxolide, phantolide e tonalide) e dois musks macrocícliclos (exaltolide e ethylene brassylate) Estas amostras foram recolhidas em praias da área metropolitana do Porto, no verão e no inverno.

A extração das amostras foi realizada utilizando uma metodologia QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) já previamente desenvolvida no grupo de investigação, e que combina extração e *clean-up* das amostras. As condições foram as seguintes: 5 g de amostra, extração com 3 mL de acetonitrilo, primeiro QuEChERS (2400 mg $MgSO_4$ + 750 mg $NaCH_3COOH$), segundo QuEChERS (180 mg $MgSO_4$ + 60 mg PSA + 30 mg C_{18}). As amostras foram depois analisadas por GC-MS.

O método analítico foi validado e as retas de calibração elaboradas (5 – 1000 μg/L). Os limites de deteção (LOD) e de quantificação (LOQ) foram calculados com base na razão sinal-ruído; os valores de LOD variaram entre 7,85 x 10^{-4} (tonalide) e 3,75 x 10^{-2} ng/g (musk tibetene). As recuperações variaram entre 49,9 e 127,2%. A precisão do método foi comprovada, com os valores do desvio padrão relativo a variar entre 1,9 % (musk moskene) e 16,6 % (ethylene brassylate).

Foram detetados musks em todas as amostras, no entanto, os musks ambrette, moskene, tibetene e xylene não foram detetados. O musk ketone só foi detetado em 31% das amostras, o tonalide e o exaltolide foram os musks mais detetados (93% e 89% respetivamente).

Foram encontrados musks em concentrações mais elevadas na amostra recolhida no verão, em Valadares Sul (32,59 ng/g dw) e em concentrações mais baixas na amostra no verão em Aguda (0,58 ng/g dw). O galaxolide foi o musk presente em maior concentração (0,04 – 26,93 ng/g dw); o phantolide (0,03 – 0,09 ng/g dw) e o celestolide (0,01 – 0,05 ng/g dw) apresentaram as concentrações mais baixas.

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Notation and Glossary

ACN	Acetonitrile
ADBI	Celestolide
Agla	Agudela
Agu	Aguda
AHMI	Phantolide
AHTN	Tonalide
AngN	Angeiras Norte
ASE	Accelerated solvent extraction
Ate	Aterro
ATII	Traseolide
Car	Carneiro
CAS	Chemical abstract service
CM	Cabo do Mundo
CN	Canide Norte
CQ	Castelo do Queijo
DCM	Diclhoromethane
DEP	Diethyl phthalate
DLLME	Dispersive liquid-liquid microextraction
DPMI	Cashmeran
d-SPE	Dispersive solid-phase microextraction
DVB	Divinylbenzene
dw	Dry weight
EcoFEUP	Environmental Management System of FEUP
EI	Electron impact
ETH	Ethylene brassylate
EXA	Exaltolide
Fra	Francelos
Fun	Funtão
GC	Gas chromatography
GPC	Gel permeation chromatography
Hex	Hexane
HHCB	Galaxolide
HL	Homem do Leme
HS	Headspace
Ing	Ingleses
Koc	Organic carbon-water partition coefficient
Kow	Octanol-water partition coefficient
Lav	Lavadores
LLE	Liquid-liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
LVI	Large volume injection
MA	Musk ambrette; microwave assisted
Mat	Matosinhos
Mem	Memória
MeOH	Metanol

Mir	Miramar
MK	Musk ketone
MM	Musk moskene
MN	Madalena Norte
MS	Mass spectrometry
MT	Musk tibetene
MW	Microwave
MX	Musk xylene
PB	Pedras Brancas
PCP	Personal care products
PDMS	Polydimethylsiloxane
PSA	Primary-secondary amine
PTV	Programmable temperature vaporization
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
Rec	Recovery
rpm	Rotations per minute
RSD	Relative standard deviation
São	Sãozinha
SCOT	Support-coated open tubular
SDE	Simultaneous distillation-extraction
SFM	São Félix da Marinha
SIS	Selected ion storage
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
USE	Ultrasonic extraction
UV	Ultraviolet
VS	Valadares Sul
WCOT	Wall coated open tubular
WWTP	Wastewater treatment plant

1. Background

The growing research regarding the environment and its contamination and the development of more sophisticated analytical methods have brought to light a new class of chemicals pollutants, called emerging contaminants. These chemical compounds are present in numerous everyday use products, such as detergents, personal care products, pharmaceuticals, disinfectants and agro-chemicals. Many are not new and have been present in the environment for many decades, however, they are only now being recognized as potentially hazardous (Sumner et al., 2010), and there is a lack of adequate data to assess their potential risk.

In this group of emerging contaminants are included the synthetic musks, a heterogeneous group of chemicals (Kallenborn et al., 2001), usually applied in personal care products (PCPs) as fragrance fixatives. Synthetic musks are difficult to degrade, and they are easily bioaccumulated through the food chain (Che et al., 2011). In general, human use is the main pathway of synthetic musks in to the environment (Lee et al., 2014). After these products are consumed, about 77% of synthetic musks are drained into the sewer system and reach the wastewater treatment plants (Hu et al., 2011), being widely dispersed in the environment. Despite the high production and use, the levels of synthetic musk compounds in the environment and their impact on ecosystems have only recently been addressed by the scientific community (Osemwengie and Gerstenberger, 2004).

Due to the persistent and lipophilic properties of synthetic musks, there is a strong accumulation of these compounds in soils, sediments and even biota. However, few studies have been conducted to assess the occurrence of synthetic musks in sediment samples, and there is a lack of global monitoring data on sedimentary concentrations of these compounds (Lee et al., 2014).

Therefore, the main objective of this work is to determine the concentration of synthetic musks in beach sands from Oporto region, using a QuEChERS methodology to extract and clean-up the samples and GC-MS for the analysis.

2. Introduction

2.1. Personal-care products

Personal care products (PCPs) are a diverse group of synthetic organic chemical compounds used in a wide variety of products such as soaps, lotions, toothpastes, perfumes, sunscreens, detergents, fabric conditioners, air fresheners, etc. The primary classes of PCPs include antiseptics (e.g. triclosan, triclocarban), fragrances (e.g. musks), insect repellants (e.g. DEET), preservatives (e.g. parabens) and UV filters (e.g. benzophenone, octocrylene, methylbenzylidene camphor)(Brausch and Rand, 2011). Due to the continuous usage of PCPs in daily-life products, associated to their unknown long term effects, the environmental impact of these compounds is an emerging area of research (Patterson et al., 2000).

These products are intended to be used externally, and so they are generally not subjected to metabolic alterations. The fraction that is not absorbed by the organism enters the environment unaltered (Brausch and Rand, 2011). PCPs may enter wastewater treatment plants (WWTPs) and the aquatic environment directly from showering, bathing or cleaning and from industrial wastes, which is considered the primary environmental pathway and indirectly from leaching at landfill sites (Sumner et al., 2010). In the wastewater treatment process, sorption (to solid particles and sludge) and biodegradation are the main processes involved in the removal of some PCPs (Lee et al., 2010); some may be degraded during treatment whereas others may pass through the process largely unchanged. The fraction of these compounds removed by WWTPs depends on the nature and level of treatments used, the size of the treatment plant, the size of the population served and the type of waste (domestic, industrial and/or commercial) (Hedgespeth et al., 2012; Lee et al., 2010; Osemwengie and Gerstenberger, 2004). It has been shown that PCPs occur in surface water and groundwater, with concentrations peaking near effluent discharge points (Chase et al., 2012). During the wastewater treatment process, an adsorption of PCPs to sludge may occur. This sludge will then be treated, and the resulting biosolids are commonly applied to agricultural land, becoming a route through which PCPs that did not degrade during the sludge digestion process are released into the soil environment (DiFrancesco et al., 2003).

One of the ingredients of PCPs is musks, important synthetic chemicals used as ingredients in fragrances applied in several consumer products, due to their typical musky scent and fixative properties, once their low volatility helps to retard the release of the fragrances and helps maintain the desired scent of the products

(Correia et al., 2013; HERA, 2004). Natural musks such as floral and animal extracts have been used since antiquity to improve attractiveness of persons and items; around 1950 several synthetic fragrances became available (Bester, 2009). The study of musks in environmental samples takes growing importance due to their accumulative and persistent nature, as well as its possible harmful effects on humans, animals and the environment.

2.2. Natural musks

Several materials presenting peculiar and pleasant odour, such as flowers, plants and spices, were used by the Egyptians as fragrance ingredients primarily for religious ceremonies, and it is believed that they were the first to incorporate perfume into their culture. These compounds have been used for years as pharmaceutical ingredients and odorants (Ravi et al., 2001; Reiner, 2007).

Natural musks can be obtained both from animal and vegetable sources. There are several animals from which these compounds can be obtained, such has the muskrat (*Ondatra zibethicus*) or the musk duck (*Biziura lobata*), but the most common animal source is the secretions of the exocrine gland of the musk deer, which contains only around 30 grams of it (Fromme et al., 2001; Schmeiser et al., 2001). Several species of musk deer were hunted and killed for their abdominal gland, including the siberian (*Moschus moschiferus),* himalayan (*Moschus chrysogaster),* forest (*Moschus berezovskii)* and black musk deer (*Moschus fuscus*) (Reiner, 2007). These animal sources originate mixtures of macrocyclic musks such as muscone, civetone, dihydrocivetone and exaltone, among others (Ravi et al., 2001).

The most common musks of vegetable origin are exaltolide, obtained from angelica root (*Angelica archangelica*) and ambrettolide, obtained from ambrette seeds (*Abelmoschus moschatus, Hibiscus abelmoschus)*, which are found in small quantities and as a complicated mixture in nature (Ravi et al., 2001).

Harvesting these musks usually implies the killing of the animals they are obtained from, making these species endangered ones. Therefore, the cost of using these compounds is considered too high, and so, natural musks have widely been replaced by synthetic ones, thus conserving animal populations (Heberer, 2002).

2.3. Synthetic musks

Musk fragrances have been synthesized for more than 100 years; the "Musk Baur" [2-(1,1-dimethylethyl)-4-methyl-1,3,5-trinitro-benzene] was the first artificial compound with fragrance odour (Che et al., 2011; Nakata et al., 2007). It was discovered by Dr. Albert Baur in 1888 as a result of his attempts at producing a more effective form of trinitrotoluene (TNT) (Reiner, 2007). Since then, several kinds of musks have been synthesized around the 1950s. Synthetic musks are structurally and chemically different from the natural musk compounds they are designed to replace. Some of their physical and chemical properties are more similar to those of man-made chemicals such as polychlorinated biphenyls and organochlorine pesticides, which are known to biomagnify through the food chain (Kubwabo et al., 2012). Since these compounds are stable ingredients in personal care products, they have been considered to be useful municipal anthropogenic markers (Kronimus et al., 2004).

According to their physicochemical properties, musks are usually divided into four main groups: nitro, polycyclic, macrocyclic and alicyclic musks (Boethling, 2011).

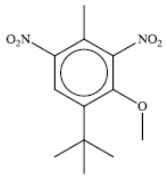
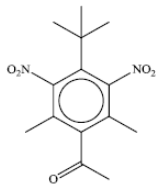
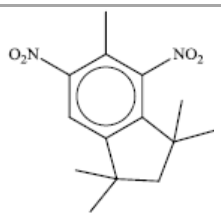
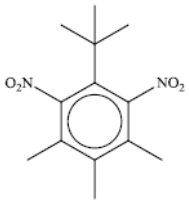
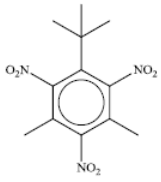
2.3.1. Nitro musks

Nitro musks were the first musk compounds to be synthesized; they consist of dinitro- and trinitro- substituted benzene derivatives, and comprise methylated nitrates and acetylated benzene rings (Rimkus, 1999; Sumner et al., 2010). The main compounds in this group are musk ambrette (MA), musk ketone (MK) musk moskene (MM), musk tibetene (MT) and musk xylene (MX), whose properties are presented in Table 1. Amongst these, musk xylene and musk ketone are the most widely used, since their production is relatively inexpensive and they are fairly easy to manufacture. The first is mainly used in detergents and soaps, whilst the second is primarily incorporated in cosmetics (Reiner, 2007; Yang and Metcalfe, 2006). Nitro musks are also utilized as food additives, room fragrances, in chewing tobacco, in fish baits and in products such as herbicides and explosives (Schmeiser et al., 2001).

Nitro musks were first detected in biological samples and river water in 1981 by Japanese scientist and since then, they were found in a wide variety of environmental samples, such as air (0.014 – 0.14 ng/m^3) (Aaron M. Peck and Hornbuckle, 2003), natural waters (0.08 – 0.24 µg/L) (Lee et al., 2010), aquatic organisms (0.1 – 178 ng/g) (Kallenborn et al., 2001), and even in human blood (2 – 67 ng/L) (Hutter et al., 2009), adipose tissue and breast milk (15.1 – 23.6 ng/g fat) (Duedahl-Olesen et al., 2005). As a result of this concern regarding its toxicity, and despite having a long history, the usage of nitro musk in cosmetic has been

decreasing in recent years, and in some countries they were restricted or even banned (Che et al., 2011; Rubinfeld and Luthy, 2008; Yang and Metcalfe, 2006). Musk ambrette (MA), musk tibetene (MT) and musk moskene (MM) were banned in the European Union, while the use of musk xylene (MX) and musk ketone (MK) is restricted (Cosmetics Directive – 76/768/EEC).

Table 1 - Chemical properties of nitro musks (US EPA 2012).

Name CAS number Chemical formula	Chemical structure	Molecular weight	Log K_{ow}	Solubility in water (mg/L)	Boiling point (°C)	Henry constant ($atm.m^3/mol$)	Vapour pressure (Pa)	Log K_{oc}
Musk ambrette (MA) **83-66-9** $C_{12}H_{16}N_2O_5$		268.3	4.17	1.67	367.9	$1.41x10^{-8}$	$1.75x10^{-3}$	3.73
Musk ketone (MK) **81-14-1** $C_{14}H_{18}N_2O_5$		294.3	4.30	1.90	401.8	$4.80x10^{-10}$	$5.33x10^{-3}$	3.94
Musk moskene (MM) **116-66-5** $C_{14}H_{18}N_2O_4$		278.3	5.39	0.17	377.0	$2.05x10^{-7}$	$3.32x10^{-4}$	4.35
Musk tibetene (MT) **145-39-1** $C_{13}H_{18}N_2O_4$		266.3	5.18	0.30	367.5	$2.19x10^{-7}$	$5.22x10^{-4}$	4.23
Musk xylene (MX) **81-15-2** $C_{12}H_{15}N_3O_6$		297.3	4.45	0.47	411.6	$1.04x10^{-9}$	$8.47x10^{-5}$	3.83

Analysing the values presented on Table 1, it is possible to verify that musk moskene and tibetene are the compounds with the highest value of log K_{ow} and the

lowest values of solubility in water - most lipophilic compounds. Musk ketone and musk xylene have the highest boiling points.

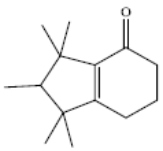
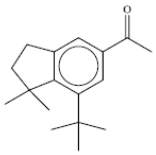
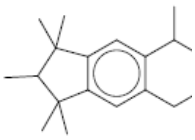
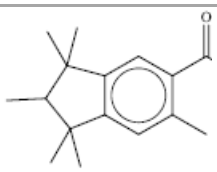
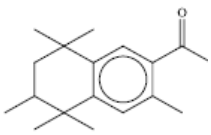
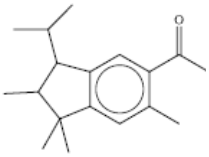
2.3.2. Polycyclic musks

Polycyclic musk compounds are another group of synthetic fragrance materials. They were developed in the 1950s and are widely used in many household products, currently dominating the market of synthetic musks (Che et al., 2011; Lee et al., 2010). These compounds are acetylated and highly methylated pyran, tetralin and indane skeletons (Sumner et al., 2010). They present a high degree of resistance towards alkali and light, good molecular stability, due to the fact that their chemical structure has more than one ring, and excellent fixative properties (Osemwengie and Gerstenberger, 2004; Roosens et al., 2007).

The main representatives of the polycyclic musk are galaxolide (HHCB), tonalide (AHTN), cashmeran (DPMI), celestolide (ADBI), phantolide (AHMI) and traseolide (AITI). Its chemical structures and properties are presented in Table 2. Galaxolide and tonalide represent the highest production volume within this group; galaxolide is the most used since the 90th decade, being listed as a high production-volume chemical (production greater than 4,500 tons per year) by the United States Environmental Protection Agency (Herren and Berset, 2000; Reiner, 2007). In Europe, about 8000 tons of synthetic musks were produced in 1996, from which about 90% are galaxolide and tonalide (Kiymet, 2009).

Polycyclic musks were detected for the first time in 1994 in Europe, and since then their occurrence has been proven in several environmental and biological matrices, including in natural waters (0.06 – 13.92 µg/L) (Lee et al., 2010), wastewaters (45 – 13399 ng/L) (Chase et al., 2012), sewage sludge (0.1 – 36.0 mg/kg) (Kupper et al., 2004), aquatic organisms (0.4 – 1510 ng/g) (Kallenborn et al., 2001), human tissues (22-170 ng/g lipid weight) (Kannan et al., 2005) and blood (17 – 4100 ng/L) (Hutter et al., 2009) Therefore, concerns have been raised recently regarding their persistence, bioaccumulation ability and toxicity to aquatic organisms and humans (Che et al., 2011; Correia et al., 2013; Zeng et al., 2008).

Table 2 – Chemical properties of polycyclic musks (US EPA 2012).

Name CAS number Chemical formula	Chemical structure	Molecular weight	Log K_{ow}	Solubility in water (mg/L)	Boiling point (ºC)	Henry Constant ($atm.m^3/mol$)	Vapour pressure (Pa)	Log K_{oc}
Cashmeran (DPMI) 33704-61-9 $C_{14}H_{22}O$		206.3	4.50	5.94	277.9	$1.42x10^{-4}$	$5.37x10^{-1}$	3.60
Celestolide (ADBI) 13171-00-1 $C_{17}H_{24}O$		244.4	5.93	0.22	319.1	$3.18x10^{-5}$	$1.92x10^{-2}$	4.40
Galaxolide (HHCB) 1222-05-5 $C_{18}H_{26}O$		258.4	5.90	1.75	325.0	$1.31x10^{-4}$	$7.27x10^{-2}$	4.01
Phantolide (AHMI) 15323-35-0 $C_{17}H_{24}O$		244.4	5.85	0.25	317.6	$3.18x10^{-5}$	$1.95x10^{-2}$	4.36
Tonalide (AHTN) 1506-02-1 $C_{18}H_{26}O$		258.4	5.70	1.25	331.9	$1.39x10^{-4}$	$6.83x10^{-2}$	4.27
Traseolide (ATII) 68140-48-7 $C_{18}H_{26}O$		258.4	6.31	0.09	329.8	$4.22x10^{-5}$	$9.11x10^{-3}$	4.61

Amongst the compounds presented in Table 2, traseolide has the highest value of log K_{ow} and also the lowest value of solubility in water, making this the most lipophilic of the polycyclic musks. Cashmeran presents the lowest boiling point, whilst the rest of the compounds present similar values for this property.

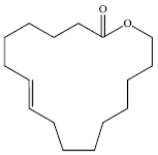
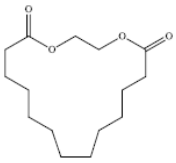
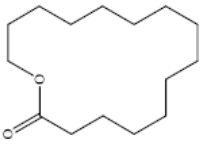
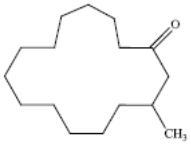
2.3.3. Macrocyclic musks

Macrocyclic musks are chemically similar to natural musks; they are large ringed ketones or lactones, often comprising 10-15 carbons (Reiner, 2007; Sumner et al., 2010; Yang and Metcalfe, 2006) (Table 3). The production is rather costly and

intricate, which explains the low market share (3-4%) ergo they are mainly used in perfumery. However, they seem to have a more intense odour, meaning that smaller quantities are needed to gain the same performance as other synthetic musks; also, they seem to be more easily degradable in the environment, which makes this group more environmentally beneficial (Bester, 2009; Roosens et al., 2007).

Macrocyclic musks' mass spectra have great resemblance with those of natural fatty acids or their derivatives, and thus there is a possibility to escape attention in analysis of environmental samples. Also their chemical properties are similar to those of natural products, so separation from these is more difficult (Bester, 2009).

Table 3 - Chemical properties of macrocyclic musks (US EPA 2012).

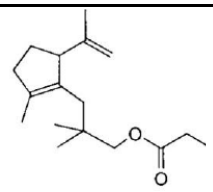
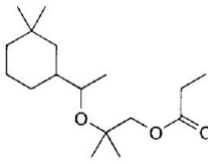
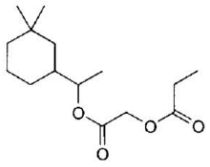
Name CAS number Chemical formula	Chemical structure	Molecular weight	Log K_{ow}	Solubility in water (mg/L)	Boiling point (°C)	Henry Constant ($atm.m^3/mol$)	Vapour pressure (Pa)	Log K_{oc}
Ambrettolide **123-69-3** **$C_{16}H_{28}O_2$**		252.4	5.37	0.59	378.7	$2.70x10^{-3}$	$2.99x10^{-3}$	3.83
Civetone **542-46-1** **$C_{17}H_{30}O$**		250.4	6.31	0.10	354.1	$1.01x10^{-3}$	$4.52x10^{-2}$	4.61
Ethylene Brassylate **105-95-3** **$C_{15}H_{26}O_4$**		270.4	4.71	1.72	434.4	$3.13x10^{-6}$	$5.85x10^{-5}$	3.40
Exaltolide **106-02-5** **$C_{15}H_{28}O_2$**		240.4	6.15	0.15	364.5	$2.32x10^{-3}$	$6.89x10^{-3}$	4.26
Muscone **541-91-3** **$C_{16}H_{30}O$**		238.4	5.96	0.22	335.1	$8.69x10^{-4}$	$6.25x10^{-2}$	4.42

Civetone and exaltolide have the highest values of log K_{ow} and lowest values of solubility in water, thus being the most lipophilic compounds of this class. Ethylene brassylate presents the highest boiling point.

2.3.4. Alicyclic musks

Alicyclic musks, also known as linear musk, are a new class of musk compounds, considerably different from the other synthetic musks (Table 4). They were first introduced in 1975, with the trisubstituted cyclopentene derivative. However, they were only introduced in the market in 1990 with the discovery and introduction of Helvetolide (Eh, 2004; Kraft, 2004).

Table 4 - Chemical properties of alicyclic musks (US EPA 2012).

Name CAS number Chemical formula	Chemical structure	Molecular weight	Log K_{ow}	Solubility in water (mg/L)	Boiling point (ºC)	Henry Constant ($atm.m^3/mol$)	Vapour pressure (Pa)	Log K_{oc}
Cyclomusk **84012-64-6** $C_{17}H_{28}O_2$		264.4	6.67	0.03	310.5	$4.40x10^{-3}$	$5.51x10^{-2}$	4.60
Helvetolide **141773-73-1** $C_{17}H_{32}O_3$		284.4	5.51	0.30	311.9	$3.60x10^{-5}$	$1.40x10^{-1}$	2.92
Romandolide **236391-76-7** $C_{15}H_{26}O_4$		270.4	4.45	2.86	294.0	$2.22x10^{-5}$	$3.60x10^{-1}$	2.71

Cyclomusk is the compound that presents higher value of log K_{ow} and lowest value of solubility in water. The boiling points of these compounds are very similar.

2.4. Analytical methods to determine synthetic musks in environmental samples

The screening of environmental samples for synthetic musks requires a preparation of the sample in order to facilitate its analysis. Due to the properties and characteristics of synthetic musks and the range of concentrations they are usually found in environmental matrices, it is very important to choose the most suitable extraction technique. In this section, the most common techniques applied to environmental samples will be briefly described.

2.4.1. Extraction techniques

The analysis of an environmental sample generally requires a preparation step before applying the analytical method itself, due to the complexity of the matrices. In this section, several techniques designed for pre-concentration and clean-up of environmental samples are mentioned and briefly described, with especial focus on the method used in this study – the QuEChERS methodology.

Liquid-liquid extraction is a technology applied to aqueous samples, based on the contact between the aqueous phase containing the sample and an organic immiscible phase; once the analytes present higher degree of affinity to the organic phase, the analytes will be transferred for this phase. The most common organic solvents used when extracting musks are dichloromethane and n-hexane. Despite being a simple method that can use a wide range of solvents, it requires a high volume of solvents, which can sometimes be toxic, making this technique a rather costly one, and not environmental friendly.

One of the most commonly used techniques is the solid-phase extraction (SPE), which is applied for extraction and for clean-up. It is based on the partitioning between a liquid phase – liquid sample or extract containing analytes – and a solid phase – the sorbent, which can be constituted by silica, alumina, florisil, amongst others. It is intended that, because the analytes have greater affinity to the sorbent phase, they will be transferred from the sample to the solid phase. After this step, the solid can be washed with a weak solvent to remove some interferences, and finally an appropriate solvent comes in to contact with the solid phase, causing the elution of the analytes of interest (they will be transferred from the sorbent to the washing solvent) (Ribeiro et al., 2014; Zwir-Ferenc and Biziuk, 2006). The most commonly used elution solvents for the determination of synthetic musks are dichloromethane, hexane, acetonitrile and methanol. In comparison to LLE, SPE

requires less time and smaller amount of solvents and it can handle smaller samples (Berrueta et al., 1995).

The extraction of a solid sample may be carried out by a solid-liquid technique such as the Soxhlet extraction, which is applied to non-volatile or semivolatile organic compounds. In this method, a liquid solvent placed in a flask is heated, the vapours are distilled and contact the solid sample that is placed in a chamber, thus promoting the transfer of analytes to the solvent. When this chamber is almost full, it is emptied through a siphon and the solvent returns to the flask, restarting the next cycle. For the determination of synthetic musks, the most commonly used extraction solvents are acetone, hexane, dichloromethane and methanol, or a mixture of these. Solid samples may also be extracted using an ultrasonic extraction technique, which is also intended for non-volatile or semivolatile organic compounds. In this technique, the solid sample contacts with a solvent, and this system is placed in an ultrasonic bath. As the ultrasound waves pass through the extraction solvent, a phenomenon called cavitation occurs, that is a formation of bubbles and its implosion. The collapse of these bubbles near the surface of the sample promotes the transfer of analytes to the liquid phase (Zuo et al., 2004). Using this technique it is possible to achieve higher extraction efficiency with lower extraction time and consumption of solvents.

In solid-phase microextraction (SPME), the sample contacts with a small portion of an extracting phase associated with a solid support for certain period of time, until equilibrium between the two phases is established. This contact may occur either by immersing the fibre in the sample, which is applied for liquid samples, or by placing it in the headspace above the sample, which is applied to gaseous samples (Vas and Vékey, 2004). When extraction is complete, the analytes are desorbed by placing the extracting phase directly in the GC injector. This method does not require the use of solvents, but it is proven to be quite expensive, due to the costs of the extracting phase, and produce highly variable results (Pawliszyn, 2000).

Accelerated solvent extraction (ASE) is a technique designed to extract solid and semi-solid samples using organic solvents at high pressure and temperature. The sample is placed in a cell that is then filled with the solvent and submitted to high pressure and temperature for short periods of time (5-10 minutes). This technique allows the reduction of both extraction time and volume of solvents used (Richter et al., 1996).

Some extraction methods have been developed recently that aim to offer high enrichment values from small quantities of sample. One of these methods is

dispersive liquid-liquid microextraction (DLLME), which consists in the extraction of analytes in a dispersion of the extracting solvent. This dispersion is achieved using a second solvent, called the dispersing solvent. It comprises two steps: first, the rapid injection of a mixture containing the extracting and dispersing solvents into a water sample, forming a dispersion that promotes fast extraction of analytes; then, the dispersion is removed by centrifugation and the extracting solvent containing the analytes is analyzed. Some of the advantages of this method are its low cost, rapidity and simplicity (Zgoła-Grześkowiak and Grześkowiak, 2011).

The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) methodology was first introduced by Anastassiades et al. (2003) for the determination of pesticide residues, as a fast and easy multiresidue method. This method comprises three basic steps: a microscale extraction using an organic solvent, usually acetonitrile; the addition of anhydrous salts and/or buffers (such as magnesium sulphate, sodium chloride and sodium acetate) that will promote phase separation and extraction of the analytes to the organic phase; purification of the obtained supernatant using dispersive solid-phase extraction (d-SPE). This purification consists of a clean-up step that uses appropriate sorbents to remove undesired components. Shaking by ultrasounds and centrifuging are the only procedures necessary to perform in between these steps (Homem et al., 2013; Wilkowska and Biziuk, 2011). The schematic representation of the QuEChERS methodology is presented in Figure 1.

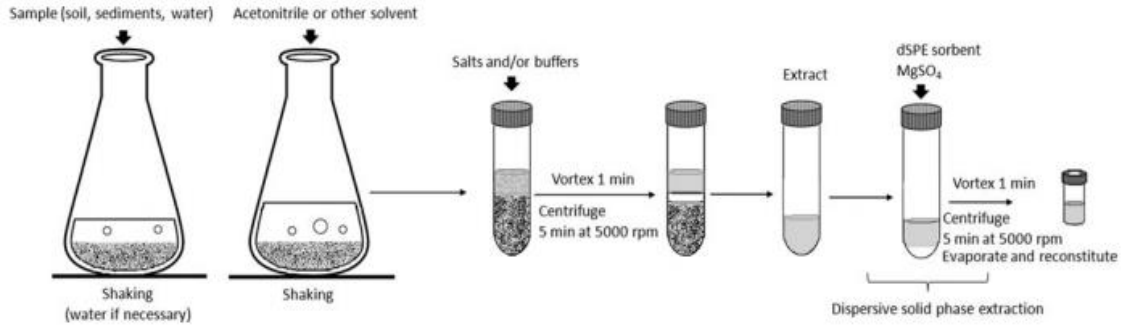


Figure 1 – Schematic representation of the QuEChERS procedure (Ribeiro et al., 2014).

This method presents several advantages in comparison to other techniques. First, a reduced amount of non-chlorinated solvents and glassware is required, thus reducing the waste generated. Also, it implies a small number of steps and less handling of extracts, meaning only one technician can perform the method, which eliminates potential sources of systematic and random errors (Lehotay, 2006). Finally, this technique implies a lower cost and is less time-consuming, when compared to other more conventional methods (Cieślik et al., 2011).

Other solvents can be used in the first step instead of acetonitrile, such as ethyl acetate or acetone. The disadvantage of using acetonitrile resides on the expansion volume during vaporization in GC vaporization (Ribeiro et al., 2014). The supernatant resulting from the last step (d-SPE) of the QuEChERS method can either be analyzed directly or submitted processes of concentration or solvent exchange.

The extraction techniques described in this section are employed in order to prepare the sample for analysis. There are several analytical instruments that can be used for the quantification of synthetic musk compounds in environmental samples. One of the most common and powerful method is the gas chromatography – mass spectrometry (GC-MS). In this work, this was the analytical technique chosen to perform the analysis of the extracts resulting from the QuEChERS method, and so it will be described in the next section.

2.4.2. GC-MS principles

Gas chromatography – mass spectrometry (GC-MS) is an analytical technique consisting in the combination of the separation of the components of a mixture in time by chromatographic methods (GC) with the characterization of those components (MS).

Basically, the analytical process consists of the vaporization of a sample, which is then fractionated in the chromatographic column as a result of a differential separation between the inert gaseous mobile phase and the solid or liquid stationary phase. The components will then enter the detector, were they will be identified and quantified (McMaster, 2011).

This method is widely used in several fields of research, such as medical and biological research, environmental science, forensics, food safety, amongst many others. It is one of the most important tools in investigative work of this nature, due to its simplicity, sensitivity and effectiveness. In the next sections the components of the GC-MS system will be briefly described.

2.4.2.1. Carrier gas

The carrier gas is the gaseous mobile phase, and its only function is to transport the sample and its components through the column. There for, it must be carefully selected according to the type of detector used. It must be chemically inert, not reacting with the sample, and it must be free of any oxygen or moisture. The most

commonly used gases are helium, hydrogen, nitrogen and argon (Hubschmann, 2008; McMaster, 2011).

2.4.2.2. Injector

The introduction of the sample into the GC system is made through the injector, usually in liquid state, but it can also be adsorbed on a support (SPME). Liquid samples are injected using a calibrated microsyringe into a heated sample port, through a rubber septum, where the sample is volatilized (McMaster, 2011). The temperature of this sample port is set to 50 °C above the boiling point of the least volatile component of the sample. The most common injectors used are the split/splitless injectors, which allow the selection of the injection mode, whether it is desired that the entire sample enters the column (splitless) or only a portion of it, discarding the rest (split) (Hubschmann, 2008). Nowadays, most of the systems use automatic sampling trays, called autosamplers, thus significantly improving the precision of the injected volume, besides allowing the processing of samples without a technician's assistance (Skoog et al., 2007).

2.4.2.3. Chromatographic column

The separation of the components of a sample occurs in the chromatographic column, which is placed in a thermostated oven so that its temperature can be controlled. This is usually achieved through a program that increases the temperature either continuously or in steps as the separation proceeds. The separation occurs due to the differences in boiling points of the components, and the degree of affinity between the analytes and the coating of the column – the stationary phase. (Hubschmann, 2008; McMaster, 2011).

There are several types of columns, being that the most commonly used nowadays is the capillary column. These can be of two types: wall coated open tubular (WCOT), which consists of a column coated with a thin layer of the stationary phase or support-coated open tubular (SCOT), in which the inner surface of the capillary is lined with a thin film of a support material. SCOT columns have a greater sample capacity, but usually a WCOT column is more efficient (McMaster, 2011; Skoog et al., 2007).The selection of the most suitable column, with the most appropriate stationary phase is essential to obtain a satisfactory separation of the sample. It must have some degree of compatibility with the analytes, in order to achieve reasonable residence time. Generally the polarity of the column must match that of the sample

components; polar compounds require polar columns, whilst non-polar compounds must be analyzed with non-polar columns. In the first case, for similar retention times, elution occurs by ascending order of polarity; in the second case the elution occurs by ascending order of boiling points (Hubschmann, 2008).

2.4.2.4. Ion Trap Mass Detector

One of the most powerful and most commonly used detectors for GC is the mass spectrometer (MS). This detector is a highly versatile, precise and sensitive instrumental technique, largely used in several areas of chemistry, environmental science, forensics, pharmaceutical science, etc (Dass, 2007). This instrument ionizes the chemical compounds to generate charged molecules or fragments that are subsequently separated and detected based on their mass-to-charge ratios (m/z).

The MS main components are an ionization source, a mass analyzer, a detector and a data recorder/processor (Rossi and Sinz, 2002). After exiting the chromatographic column, the components of the sample are fed to the ionization source, where they suffer an electron ionization. In this process, a heated filament emits electrons, which are accelerated by a 70 eV current in the region between the filament and the entrance to the ion source. The accelerated electrons are then concentrated into a beam and the sample, containing neutral molecules, is introduced in a perpendicular direction. The close passage of highly energetic electrons causes large fluctuations in the electric field around the neutral molecules and induces ionization and fragmentation. The singly charged positive ions formed (radical cations) are attracted to the slits of accelerator plates, gaining the required velocity to enter the cavity of the ion trap mass analyzer (McMaster, 2011; Skoog et al., 2007), formed by a three-electrode structure, where they circulate due to electric and magnetic fields. Then, changing the applied radio-frequency field will cause the trapped ions to eject sequentially in order of increasing mass (Hubschmann, 2008; Rossi and Sinz, 2002). The ions will then pass through a transducer called electron multiplier, which is submitted to a voltage gradient. As these ions hit the surface of the electron multiplier, one or more electrons are ejected and subsequently accelerated further into the multiplier, ultimately creating an amplification cascade of electrons (Rossi and Sinz, 2002). Finally, the resulting electrical signal is processed, stored and later displayed by a computer. This entire process is required to take place under vacuum, which allows ions to move freely in space without colliding or interacting with other species (Dass, 2007).

3. State of the art

In this section, studies published regarding the detection of synthetic musks in sediments from rivers, lakes and coastal environments will be presented and discussed, focusing on the extraction and analytical techniques used, as well as the results obtained. Recently, there has been a growing concern towards understanding the presence of synthetic musks in several environmental matrices, and the effects these compounds have in human health and in the environment. Therefore, several environmental matrices have been investigated, such as water and wastewater, air, sewage sludge, soil, biota, among others. However, to the author's best knowledge, nothing has been published to date about detection of synthetic musks in beach sands. Beach sands are a type of sediment, constituted mainly by quartz particles, though with different characteristics from other types of sediments, such as considerable size pores and low organic matter content. This analysis focuses on studies conducted in river, lake and coastal sediments, since this matrix is the most similar to beach sands. In Table 5 these studies are summarized, including instrumental aspects, procedures for extraction and clean-up and musk concentrations.

Analyzing Table 5, it can be verified that the most commonly used extraction technique is the Soxhlet extraction, usually followed by a SPE clean-up. Peck et al. (2006) studied the presence of five polycyclic musks (HHCB, AHTN, ATII, AHMI and ADBI) and 2 nitro musks (MX and MK) in 23 sediment samples from Lake Ontario, in Canada. A Soxhlet extraction was carried with dichloromethane for 24 hours, and the extract was exchanged into hexane with a rotary evaporator and cleaned-up using a SPE column packed with 3% deactivated silica gel, which is a stationary phase suitable for compounds with intermediate polarity such as synthetic musks. Three eluent fractions were collected in series, according to their eluting power (from least to most powerful) and to the increasing polarity – hexane, dichloromethane and methanol. The final extract was analyzed by GC-MS with electron ionization and quadrupole mass detector. The recoveries achieved ranged from 63% for HHCB to 86% for MK and the limits of detection from 0.06 ng/g for ATII to 5.1 ng/g for HHCB. From the compounds considered, only MK was not detected in the samples. For most musks, concentrations were below 1 ng/g, except for HHCB, which presented a much higher concentration (16 ng/g), probably because it is the most used synthetic musk in personal care products, and therefore released in higher amounts to the environment. Zhang et al. (2008) used a similar procedure to analyze 8 sediment samples from Suzhou Creek, in Shanghai. Soxhlet extraction was carried for 72 hours,

and the extract was cleaned-up with a silica gel and alumina SPE column, using the following eluents: n-hexane, a mixture of n-hexane and dichloromethane and dichloromethane. The combined use of silica and alumina in the SPE column slightly increases the polarity of the stationary phase, in comparison to using only silica. The eluents are also used in order of increasing polarity. The analytes investigated were also the same, with the addition of DPMI. The range of recoveries obtained in this case was very similar to the previous one, ranging from 62% to 83%. For ADBI and AHMI, the limit of detection was 0.3 ng/g and for the remaining synthetic musks was 0.5 ng/g. In this study only HHCB and AHTN, the most commonly used synthetic musks, were detected, with concentrations ranging between 3 ng/g to 78 ng/g (dry weight) and 2 ng/g to 31 ng/g (dry weight), respectively. In the study performed by Zeng et al. (2008), the same extraction, clean-up and analysis techniques were employed to determine the presence of six polycyclic musks (DPMI, ADBI, AHMI, ATII, HHCB and AHTN) in 20 sediment samples from the Pearl River Delta (China) and 3 sediment samples from the mouth of the Pearl River, in Macao's coastal region . Recoveries obtained in this case presented a wider range, from 57% to 109%, and the limits of detection presented a very similar range, varying from 0.30 ng/g to 0.67 ng/g. Both in river and coastal environment sediments, highest concentrations were obtained for ADBI, HHCB and AHTN, while for the other three analytes, concentrations where below the detection limit. In the river sediments, concentrations ranged between not detected to 121 ng/g, while for coastal sediments concentrations ranged from 2.49 to 44.5 ng/g. Sediments from estuarine and coastal environments from Tamar Valley (UK) were examined by Sumner et al. (2010) to determine the concentration of HHCB and AHTN. Five sediment samples were submitted to Soxhlet extraction with a mixture of hexane and dichloromethane (1:1) for 12 hours. The clean-up step was performed eluting the SPE neutral alumina cartridges with ethyl acetate. Since the stationary phase used in this case is more polar than silica, it was also necessary to use a more polar elution solvent such as ethyl acetate. The final extract was analyzed by GC-MS using a large volume injection technique. Recoveries obtained for this method were 62% for HHCB and 38% for AHTN, and the limits of detection ranged from 1.1 ng/g to 8.0 ng/g. Samples presented a higher concentration of HHCB (11-17 ng/g) then AHTN (2-10 ng/g). Once again, this can be explained by the fact that these two synthetic musks are the most used in consumer products, and so it is expected that they are more widely spread in the environment. Reiner and Kannan (2010) analyzed three sediment samples collected from the upper Hudson River (New York, USA) to determine the presence of

polycyclic musks. The samples were Soxhlet extracted with a hexane and dichloromethane mixture (1:3) for 16 hours and then, injected onto the GC-MS. Average recoveries ranged from 85% to 98%, with relative standard deviations below 15%, and the limit of quantification was 5 ng/g. HHCB and AHTN were detected in all sediments, with concentrations ranging from 72.8 ng/g to 388 ng/g and from 113 ng/g to 544 ng/g (dry weight), respectively. Lee et al. (2014) also tested Soxhlet to extract AHTN and HHCB from 25 sediment samples from Nakdong River and coastal areas of Korea. Prior to the GC-MS analysis, the extracts suffer a clean-up with a SPE column packed with 3% deactivated silica gel and the compounds were eluted with a mixture of dichloromethane and hexane (1:1). Since dichloromethane presents some level of polarity and hexane is nonpolar, the resulting solvent mixture acquires an intermediate polarity, matching that of synthetic musks. Similar recoveries (about 85%) and detection limits (0.2 ng/g) were determined for both polycyclic musks. Concentrations for AHTN and HHCB ranged from not detected to 1.0 ng/g and to 2.7 ng/g, respectively.

From the studies presented in the previous paragraph, it is possible to verify that all recoveries were very similar, since the extraction and clean-up of the samples were performed using the same techniques, as well as solvents and sorbents.

Sapozhnikova et al. (2010) analyzed 39 sediment samples collected from three tidal tributaries of the Chesapeake Bay (USA) to determine the presence of two polycyclic musks – HHCB and AHTN. Samples were extracted using accelerated solvent extraction (ASE) with a mixture of dichloromethane and acetone (1:1) at 100 ºC and 1500 psi. Since in this technique the extraction is carried out under pressure, there is a better penetration of the solvent into the pores of the matrix, thus promoting a higher degree of contact and removal of the target analytes. Also, the usage of acetone in the solvent mixture aids this mechanism, given the higher wettability of this solvent. The extract was then subjected to a clean-up procedure that included a gel permeation chromatography (GPC) with dichloromethane, from which the first fraction was discarded, and a SPE step on a silica column applied to the second fraction, using as eluents mixtures of hexane and dichloromethane (9:1) and hexane:ethyl acetate (1:1) in order of increasing polarity. Finally, the resulting extracts were analyzed by GC-MS, using a large volume injection (LVI) with programmable temperature vaporization (PTV). The recovery achieved for HHCB was very satisfactory (96%); however a very low recovery was obtained for AHTN (6–9%). Concentrations of HHCB and AHTN found in the sediment samples ranged from not detected to 9.2 ng/g and from not detected to 8.0 ng/g (dry weight), respectively. In

the study performed by Hu et al. (2011) the extraction technique employed to 13 sediment samples from the Haihe River (China) was also ASE. A mixture of hexane and dichloromethane (1:1) at 60 ºC and 1500 psi, in two 15 minute cycles were used to extract synthetic musks. SPE was employed for cleaning-up of the extract, using as eluents hexane and mixtures of hexane and dichloromethane in different proportions, in order to gradually increase the polarity, presumably to separate compounds from interferences. The extract was analyzed by GC-MS with electron ionization and a quadrupole mass detector. The limit of detection obtained ranged from 0.25 to 0.33 ng/g, whilst recoveries ranged from 83.6% to 105.1%. The concentrations obtained for HHCB, AHTN, ATII, ADBI, MK and MX varied from 1.5 to 47.5 ng/g (dry weight). AHMI was not detected in any of the samples. In similarity to the previous two studies, Che et al. (2011) used ASE as extraction technique to analyze 15 sediment samples collected from the Taihu Lake (China). In this study the method was optimized and the final conditions were: mixture of hexane and acetone (1:1) as extraction solvent in two 5 minute cycles at 100 ºC and 1500 psi and the addition of florisil to perform an in-cell clean-up. Acetone is more polar than hexane and presents a higher wettability, thus promoting a better penetration of the solvent into the pores of the matrix. Regarding its polarity, florisil is located somewhere in the middle of the previously mentioned sorbents silica and alumina. Analysis of the extract was carried out with a GC-MS system. This method reported very satisfactory limits of detection, varying between 0.03 ng/g for AHTN and HHCB and 0.05 ng/g for DPMI, ADBI, AHMI, ATII, MX and MK. Recoveries obtained were also satisfactory, being higher than 80% for all analytes. Concentrations of HHCB, AHTN, MX and MK ranged from 0.156 to 3.10 ng/g, 0.184 to 1.21 ng/g, not detected to 0.349 ng/g and not detected to 0.0768 ng/g, respectively. DPMI, ADBI, AHMI and ATII were not detected in any of the samples. As expected, concentrations of HHCB and AHTN were significantly higher than MK and MX, which can be explained by the considerably higher usage and subsequent discharge into the environment of the polycyclic musks.

Fromme et al. (2001), in order to determine the presence of five polycyclic musks (HHCB, AHTN, ADBI, ATII and AHMI) in 59 sediment samples from rivers of Berlin (Germany) used simultaneous steam-distillation/solvent extraction (SDE) with water and cyclohexane, which also includes a clean-up step, and GC-MS to analyze the samples. The limits of detection obtained were as low as 0.030 ng/g for HHCB, 0.020 ng/g for AHTN and 0.004 ng/g for ADBI, ATII and AHMI; recoveries ranged from 89 to 96%. The concentrations of synthetic musks in the samples were determined to be relatively high: 220 to 920 ng/g for HHCB, 20 to 1100 ng/g for AHTN, 10 to 25 ng/g

for ADBI, 21 to 101 ng/g for ATII and 11 to 36 ng/g to AHMI. These values were substantially higher than all the other studies mentioned in this section.

Dsikowitzky et al. (2002) collected 17 sediment samples from the Lippe River (Germany) in order to screen four polycyclic musks (HHCB, AHTN, AHMI and ADBI) using a high-speed dispersion with acetone and hexane as extraction technique. Clean-up was achieved with an activated silica gel SPE column, eluted with mixtures of pentane, dichloromethane and methanol, in order to obtain six fractions. The analysis was performed by GC-MS. This produced a relatively high limit of quantification of 2 ng/g, and low recoveries ranging from 43% (HHCB and AHMI) to 49% (ADBI). ADBI was not detected in any of the samples. As for the remaining analytes, their concentrations varied significantly from sample to sample, ranging from not detected to 1399 ng/g for AHTN. Kronimus et al. (2004) also analyzed sediments from the Lippe River, collecting a total of 27 samples from 9 different locations, divided in three sampling campaigns. Extraction and clean-up procedures were identical to those used by Dsikowitzky et al. (2002). The resulting extract was then analyzed by GC-MS. The limit of detection obtained was 0.1 ng/g and recoveries were 46% for AHTN and 43% for HHCB. Concentrations of AHTN and HHCB were determined to be ranging from not detected to 90 ng/g (dry weight) and from not detected to 56 ng/g (dry weight), respectively.

Rubinfeld and Luthy (2008) collected 14 sediment samples from a coastal environment in the San Francisco Bay area (USA) in order to determine the presence of two nitromusks, MK and MX. An ultrasonic extraction (USE) technique was carried out, using a mixture of hexane and acetone (1:1) in 6 minute pulses, alternating between 15 seconds on and 15 seconds off. Clean-up was conducted through a SPE florisil cartridge eluted with hexane and dichloromethane, in order to obtain two separate fractions (only the second was analyzed). Average recoveries ranged from 64 to 125%, while limits of detection were 0.018 ng/g for MK and 0.021 ng/g for MX. The samples presented a small concentration of MK and MX, with values of 0.038 and 0.034 ng/g, respectively. These low values may be explained by the fact that nitro musks were proven to be prejudicial, and therefore their use was restricted in many countries. Wu and Ding (2010) collected 3 sediment samples from a river in Taiwan to monitor 6 polycyclic musks (DPMI, ADBI, AHMI, ATII, HHCB and AHTN). A microwave-assisted headspace solid-phase microextraction (MA-HS-SPME) technique was employed for both the extraction and clean-up of the samples. The microwave system operated on 300 rpm and 80 W for 5 minutes, using a polydimethylsiloxane/divinylbenzene (PDMS/DVB) fibre, which was then inserted in

the GC injection port at 270 ºC for 2 minutes. The limits of detection were 0.04 ng/g for all musks, with the exception of DPMI (0.1 ng/g). Recoveries ranged from 67% to 88%. Only AHMI, AHTN and HHCB were detected in the samples, with concentrations ranging from not detected to 5.9 ng/g, 0.2 to 4.0 ng/g and 0.1 to 1.0 ng/g, respectively.

Overall, concentrations of synthetic musks determined in these studies ranged from 0.1 to 1399 ng/g in river sediments and from 0.034 to 44.5 ng/g in sediments from coastal environments. Comparing the studies presented in this section, it is possible to conclude that the lowest limit of detection was obtained by Fromme et al. (2001), that resorted to a SDE technique to extract and clean-up the samples. They also obtained very satisfactory recovery rates. This technique has the advantage of coupling both extraction and clean-up in just one procedure, which allows to reduce the number of steps and minimize errors. However, it has the disadvantage of being time-consuming and using high solvent amount. On the other hand, the lowest recoveries were presented by Dsikowitzky et al. (2002) and Kronimus et al. (2004). In both studies, a dispersion technique coupled with silica gel SPE clean-up was selected. It is also verified that Soxhlet followed by a SPE column was the most common combination used for extraction and clean-up, obtaining satisfactory limits of detection and/or quantification and recovery rates. The main advantages associated with these methodologies reside on the fact that they are simple and well established techniques, carried out with common and relatively inexpensive equipment and chemicals. However, comparing to other techniques previously referred, these configurations are extremely time consuming and require the use of a large volume of solvents, which results in the production of a considerable amount of waste. Therefore, in the authors' opinion, it is essential to develop an alternative methodology to overcome drawbacks mentioned above. In this study, a QuEChERS methodology will be employed for the extraction and clean-up of beach sands. This will allow to significantly reduce the amount of solvent used (consequently reducing the amount of waste produced), as well as the risk of cross-contamination since this technique involves less sample manipulation and the majority of labware is disposable. Furthermore, the method has a lower cost compared to some of the previously presented methodologies, which reduces overall cost of the analysis of a large sample pool.

Table 5 - Summary of analytical methods for the determination of synthetic musks in sediment samples.

Matrix	Analyte	Extraction	Clean-up	Analysis	LOD (ng/g)	LOQ (ng/g)	Rec (%)	Conc. (ng/g)	Site	Reference
Sediments (river) n=59	HHCB	SDE (10 g sample; water, cyclohexane)		GC-MS	0.030	-	96 ± 4	220–920 (dw)	Berlin, Germany	(Fromme et al., 2001)
	AHTN				0.020		89 ± 5	20–1100 (dw)		
	ADBI				0.004		92 ± 5	10–25 (dw)		
	ATII				0.004		95 ± 6	21–101 (dw)		
	AHMI				0.004		89 ± 6	11–36 (dw)		
Sediments (river) n=17	HHCB	High-speed dispersion (10 g sample; acetone, hex:acetone (1:1) (2x), hex (2x))	SPE column (?? mg silica gel; pentane, pentane:DCM (9.5:0.5), pentane:DCM (9:1), pentane:DCM (4:6), DCM, MeOH)	GC-MS	-	2	43 ± 16	<2–191 (dw)	Lippe River, Germany	(Dsikowitzky et al., 2002)
	AHTN						46 ± 17	<2–1399 (dw)		
	AHMI						43 ± 18	<2–60 (dw)		
	ADBI						49 ± 16	nd		
Sediments (river) n=27	AHTN	High-speed dispersion (?? g sample; acetone, hex:acetone (1:1) (2x), hex (2x), acetone, hex)	SPE column (?? mg silica gel; pentane, pentane:DCM (9.5:0.5), pentane:DCM (9:1), pentane:DCM (4:6), DCM, MeOH)	GC-MS	0.1 (dw)	0.5 (dw)	46 ± 17	nd–90	Lippe River, Germany	(Kronimus et al., 2004)
	HHCB						43 ± 16	nd–56		
Sediments (river) n=20	DPMI	Soxhlet (20 g sample; DCM; 72 h)	SPE column (?? mg silica-alumina; hex, hex:DCM (3:1), DCM)	GC-MS	0.30-0.67	-	56 ± 6	<0.3 (dw)	Pearl River Delta, China	(Zeng et al., 2008)
	ADBI						62 ± 2	<0.3–8.95 (dw)		
	AHMI						68 ± 2	<0.3 (dw)		
	ATII						72 ± 2	<0.67 (dw)		
	HHCB						109 ± 1	2.58–121 (dw)		
	AHTN						78 ± 2	3.14–42.5 (dw)		
Sediments (river) n=8	HHCB	Soxhlet (1 g sample; DCM; 72 h)	SPE column (?? mg silica gel-alumina (2:1); hex, hex:DCM, DCM)	GC-MS	0.5	1	78 ± 14	3–78 (dw)	Suzhou Creek, Shanghai, China	(Zhang et al., 2008)
	AHTN				0.5	1	83 ± 12	2–31 (dw)		
	DPMI				0.5	1	79 ± 13	nd		
	ADBI				0.3	0.6	74 ± 16	nd		
	AHMI				0.3	0.6	75 ± 16	nd		
	ATII				0.5	1	71 ± 15	nd		
	MK				0.5	1	69 ± 23	nd		
	MX				0.5	1	62 ± 20	nd		

n – number of samples; LOD – limit of detection; LOQ – limit of quantification; Rec – recovery; Conc. – concentration; GC-MS - gas chromatography-mass spectrometry; nd – not detected; dw – dry weight; SDE- simultaneous distillation extraction; SPE - solid-phase extraction; Hex - hexane; DCM - dichloromethane; MeOH - methanol

Table 5 - Summary of analytical methods for the determination of synthetic musks in sediment samples (*cont.*).

Matrix	Analyte	Extraction	Clean-up	Analysis	LOD (ng/g)	LOQ (ng/g)	Rec (%)	Conc. (ng/g)	Site	Reference
Sediments (river) n=3	DPMI	MA-HS-SPME (5 g dewatered sample; MW system: 300 rpm, 80 W, 5 min; PDMS-DVB fibre; Desorption: 270 ºC, 2 min)		GC-MS	0.10	0.3	80	nd	Taiwan	(Wu and Ding, 2010)
	ADBI				0.04	0.1	77 – 80	nd		
	AHMI				0.04	0.1	67 – 81	nd–5.9		
	ATII				0.04	0.1	68 – 79	nd		
	HHCB				0.04	0.1	81 – 88	0.2–4.0		
	AHTN				0.04	0.1	69 – 70	0.1–1.0		
Sediments (river) n=39	HHCB	ASE (?? g sample; DCM:acetone (1:1); 100 ºC, 2000 psi)	GPC (?? mg BioBeads; DCM) + SPE (500 mg silica; hex:DCM (9:1), hex:ethyl acetate (1:1))	GC-MS	-	-	96 ± 12	nd–9.2 (dw)	Chesapeake Bay, USA	(Sapozhnikova et al., 2010)
	AHTN						(6 – 9) ± 1	nd–8.0 (dw)		
Sediments (river) n=3	HHCB	Soxhlet (40 g sample; hex:DCM (1:3); 16 h)	-	GC-MS	-	5	(85 – 98) ± 15	72.8–388 (dw)	Hudson River, New York, USA	(Reiner and Kannan, 2010)
	AHTN							113–54 (dw)		
Sediments (river) n=13	HHCB	ASE (?? g sample; hex:DCM (1:1), 60 ºC, 1500 psi (static mode), 15 min, 2 cycles)	SPE column (4000 mg silica-alumina (1:1); hex, hex:DCM (2:1), hex:DCM (1:2), hex:DCM (1:3))	GC-MS	0.25– 0.33	-	84–105	1.5–32.3 (dw)	Haihe River, China	(Hu et al., 2011)
	AHTN							nd–21.9 (dw)		
	ATII							nd–7.4 (dw)		
	ADBI							nd–13.9 (dw)		
	AHMI							nd		
	MK							nd–22.6 (dw)		
	MX							nd–47.5 (dw)		

n – number of samples; LOD – limit of detection; LOQ – limit of quantification; Rec – recovery; Conc. – concentration; GC-MS - gas chromatography-mass spectrometry; nd – not detected; dw – dry weight; MA-HS-SPME - microwave-assisted headspace solid-phase microextraction; MW - microwave; ASE- accelerated solvent extraction; GPC - gel permeation chromatography; SPE - solid-phase extraction; Hex - hexane; DCM - dichloromethane

Table 5 - Summary of analytical methods for the determination of synthetic musks in sediment samples (*cont.*).

Matrix	Analyte	Extraction	Clean-up	Analysis	LOD (ng/g)	LOQ (ng/g)	Rec (%)	Conc. (ng/g)	Site	Reference
Sediments (river) n=22	AHTN	Soxhlet (10 g sample; DCM:hex (3:1); 24 h)	SPE column (10 g 3% deactivated silica gel; DCM:hex (1:1))	GC-MS	0.2	-	84 ± 6	nd–2.3 (dw)	Nakdong River, Korea	(Lee et al., 2014)
	HHCB				0.2	-	85 ± 5	nd–6.3 (dw)		
Sediments (lake) n=23	HHCB	Soxhlet (?? g sample; DCM; 24 h)	SPE column (?? mg 3% deactivated silica gel; hex, DCM, MeOH)	GC-MS	0.06–5.1	-	63 - 86	16	Lake Ontario, Canada	(A. M. Peck et al., 2006)
	AHTN							0.96		
	ATII							0.27		
	AHMI							<0.049		
	ADBI							0.10		
	MX							<0.068		
	MK							nd		
Sediments (lake) n=15	DPMI	ASE (5 g powdered sample; hex:acetone (1:1), 100 ºC, 1500 psi, 5 min, 2 cycles)	"In cell clean-up" (2 g Florisil)	GC-MS	0.050	0.16	81	nd	Taihu Lake, China	(Che et al., 2011)
	ADBI				0.050	0.16	90	nd		
	AHMI				0.050	0.16	89	nd		
	HHCB				0.030	0.16	104	0.156–3.10		
	ATII				0.050	0.16	82	nd		
	AHTN				0.030	0.16	92	0.184–1.21		
	MX				0.050	0.16	93	nd–0.349		
	MK				0.050	0.16	86	nd–0.0768		
Sediments (coastal) n=3	DPMI	Soxhlet (20 g sample; DCM; 72 h)	SPE column (?? mg silica-alumina; hex, hex:DCM (3:1), DCM)	GC-MS	0.30–0.67	-	56 ± 6	< 0.3 (dw)	Macao coastal region	(Zeng et al., 2008)
	ADBI						62 ± 2	2.49–2.90 (dw)		
	AHMI						68 ± 2	<0.3 (dw)		
	ATII						72 ± 2	<0.67 (dw)		
	HHCB						109 ± 1	3.67–44.5 (dw)		
	AHTN						78 ± 2	3.56–17.3 (dw)		

n – number of samples; LOD – limit of detection; LOQ – limit of quantification; Rec – recovery; Conc. – concentration; GC-MS - gas chromatography-mass spectrometry; nd – not detected; dw – dry weight; ASE- accelerated solvent extraction; SPE - solid-phase extraction; Hex - hexane; DCM - dichloromethane; MeOH - methanol

Table 5 - Summary of analytical methods for the determination of synthetic musks in sediment samples (*cont.*).

Matrix	Analyte	Extraction	Clean-up	Analysis	LOD (ng/g)	LOQ (ng/g)	Rec (%)	Conc. (ng/g)	Site	Reference
Sediments (coastal) n=14	MK	USE (5 g sediment; hex:acetone (1:1); 6 min-pulses 15 s on and 15 s off)	SPE cartridge (?? mg florisil; hex,DCM)	GC-MS	0.018	-	64 – 125	0.038±0.026	San Francisco Bay, USA	(Rubinfeld and Luthy, 2008)
	MX				0.021			0.034±0.021		
Sediments (coastal) n=5	HHCB	Soxhlet (?? g sample; hex:DCM (1:1); 12 h)	SPE cartridge (?? mg neutral alumina; ethyl acetate)	GC-MS	1.1–8.0 (dw)	-	62	11–17 (dw)	Tamar Valley, UK	(Sumner et al., 2010)
	AHTN						38	2–10 (dw)		
Sediments (coastal) n=25	AHTN	Soxhlet (10 g sample; DCM:hex (3:1); 24 h)	SPE column (10 g of 3% deactivated silica gel; DCM:hex (1:1))	GC-MS	0.2	-	84 ± 6	nd–1.0 (dw)	Korean coasts	(Lee et al., 2014)
	HHCB			GC-MS	0.2	-	85 ± 5	nd–2.7 (dw)		

n – number of samples; LOD – limit of detection; LOQ – limit of quantification; Rec – recovery; Conc. – concentration; GC-MS - gas chromatography-mass spectrometry; nd – not detected; dw – dry weight; USE- ultrasound extraction; SPE - solid-phase extraction; Hex - hexane; DCM – dichloromethane

4. Technical description

4.1. Chemicals

Solid standards of the synthetic polycyclic musks cashmeran, celestolide, galaxolide, phantolide, and tonalide were purchased from LGC Standards (Barcelona, Spain) with a purity of 99%, except for galaxolide, which contains approximately 25% of dietyl phthalate (DEP). Musk tibetene and musk moskene were purchased from the same company as 10 mg/L solutions in cyclohexane. Musk ambrette and musk ketone were obtained as solid standards from Dr. Ehrenstorfer GmbH (Augsburg, Germany) with 99% e 98% purity, respectively. Musk xylene was acquired as 100 mg/L solution in acetonitrile from Sigma-Aldrich (St. Louis, MO, EUA). Also from Sigma-Aldrich exaltolide and ethylene brassylate were obtained with respective purities of ≥95% and ≥98%. Musk xylene-d_{15} and tonalide-d_3 were also purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany) as individual 100 ng/µL solution in acetone and iso-octane, respectively.

The compounds necessary to carry out the QuEChERS methodology, magnesium sulphate and sodium acetate were purchased from Sigma-Aldrich, and PSA and DSC-18 from Supelco (Bellefonte, PA, USA). All organic solvents used, namely acetonitrile and cyclohexane, were acquired from VWR (Fontenay-sous-Bois, France) in analytical grade.

4.2. Standard preparation

Solutions containing the solid standards were prepared in cyclohexane, obtaining solutions with a concentration of 15 g/L for each of the five polycyclic musks, 7 g/L for musk ambrette and musk ketone and 10 g/L for exaltolide and ethylene brassylate.

A 10 mg/L intermediate stock solution containing the musks listed above was prepared by diluting the appropriate quantities in acetonitrile. The final stock solution was obtained by evaporation under a stream of nitrogen of a determined quantity of musk tibetene and musk moskene followed by the addition of the musk xylene solution and the intermediate stock solution. Finally, the volume was made up with acetonitrile. This solution was used to prepare calibration standards (5 – 1000 µg/L) in acetonitrile. All solutions were stored at -20ºC and protected from light.

Commercial solutions of musk xylene-d_{15} and tonalide-d_3 were used to prepare a surrogate standard with a concentration of 500 µg/L.

4.3. Sampling

In this study, a total of 45 sand samples were analyzed, collected from 23 different beaches from Porto's metropolitan area (Portugal) in 2013/2014. The samples were collected in two sampling campaigns, one in the end of Summer (September 2013) and the other in the end of Winter (March 2014). Dry sand samples were collected in three equidistant points along the beach (about 5 m above the high tide level) at a 5 cm depth, after removing impurities such as stones and gravels. Sand was placed in sterile plastic bags in a cooler for transport to the laboratory, where they were kept at -20 ºC until extraction.

These beaches are located in three cities – Matosinhos, Porto (the district's capital) and Vila Nova de Gaia. A map showing the relative location of these three cities is represented in Figure 2. Figure 3 shows the location of all 23 beaches from where the samples were collected; Figures 4a, b and c are magnifications of the three cities and its beaches.

Figure 2 - Relative location of the three cities.

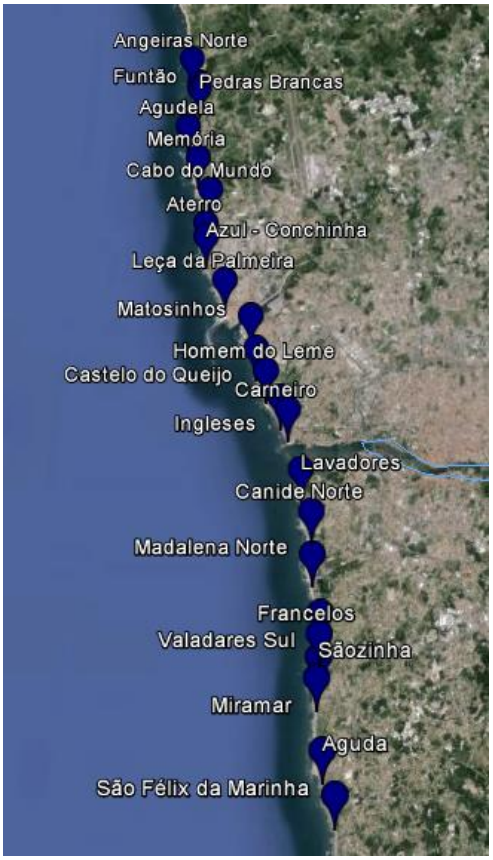


Figure 3 - Location of the 23 beaches.

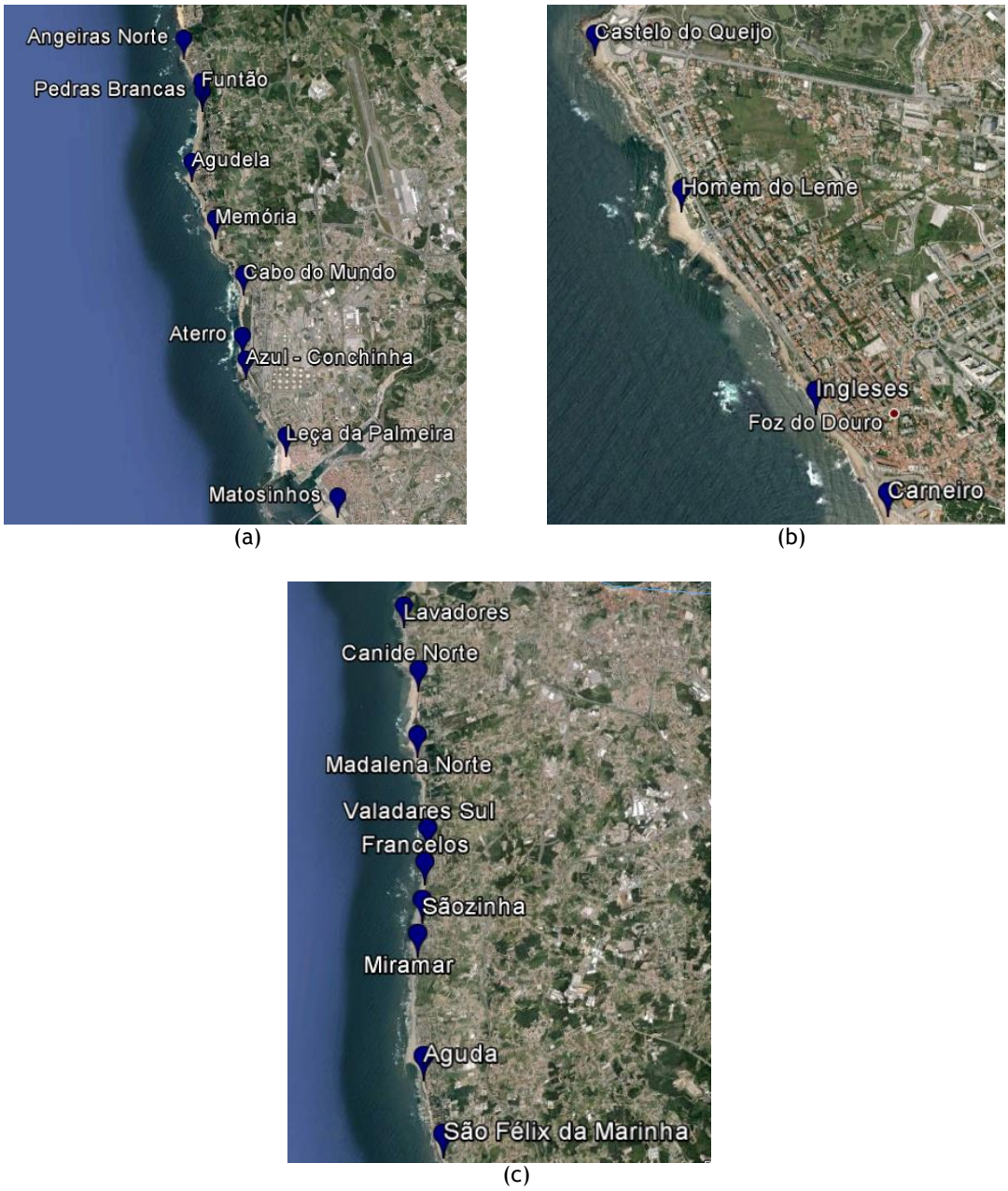


Figure 2 – Magnification of the three cities: Matosinhos (a), Porto (b) and Vila Nova de Gaia (c).

Sampling conditions as well as some characteristics of the beaches are given in Table 6. Sample collection from Carneiro beach in Porto in March 2014 (2nd Campaign) was not possible, because access to the beach was forbidden, due to bad weather.

Table 6 – Details of sample collection and characteristics of the beaches.

	Location	1st campaign		2nd campaign		*Blue Flag* (a)	N.º users in the bathing season (a)	Extension of the beach line (m) (a)	Distance to WWTP (m) (a)
		Date	Temp. (ºC)	Date	Temp. (ºC)				
Matosinhos	Angeiras Norte (AngN)	23-09-13	25	07-03-14	15	X	?	730	5400
	Funtão (Fun)	23-09-13	25	07-03-14	16	✓	150	320	4400
	Pedras Brancas (PB)	23-09-13	27	07-03-14	17	X	?	330	4200
	Agudela (Agla)	23-09-13	26	07-03-14	15	✓	800	800	2700
	Memória (Mem)	23-09-13	25	07-03-14	14	✓	500	510	1400
	Cabo Mundo (CM)	23-09-13	25	07-03-14	14	✓	200	560	200
	Aterro (Ate)	23-09-13	25	07-03-14	15	✓	300	550	800
	Azul	23-09-13	27	07-03-14	14	X	?	50	1600
	Leça	23-09-13	25	07-03-14	13	X	?	846	3300
	Matosinhos (Mat)	23-09-13	27	07-03-14	14	X	?	734	5000
Porto	Castelo do Queijo (CQ)	23-09-13	28	07-03-14	14	X	?	140	4400
	Homem do Leme (HL)	23-09-13	28	07-03-14	14	✓	120000	374	3500
	Ingleses (Ing)	23-09-13	27	07-03-14	14	✓	3200	86	2400
	Carneiro (Car)	23-09-13	26	-	-	✓	3200	187	1900
Vila Nova de Gaia	Lavadores (Lav)	24-09-13	22	07-03-14	13	✓	277500	580	2000
	Canide Norte (CN)	24-09-13	24	07-03-14	13	✓	311250	505	600
	Madalena Norte (MN)	24-09-13	24	07-03-14	13	✓	345000	300	800
	Valadares Sul (VS)	24-09-13	23	07-03-14	12	✓	277500	735	2800
	Francelos (Fra)	24-09-13	24	07-03-14	12	✓	277500	355	3400
	Sãozinha (São)	24-09-13	24	07-03-14	13	✓	345000	190	4100
	Miramar (Mir)	24-09-13	24	07-03-14	13	✓	345000	250	4800
	Aguda (Agu)	24-09-13	24	07-03-14	12	✓	378750	420	7100
	S. Félix Marinha (SFM)	24-09-13	23	07-03-14	13	X	?	660	8500

(a) – (Associação Bandeira Azul da Europa, 2009)

The parameter Blue Flag refers to a classification given to beaches that certifies its quality as a bathing area. The average number of users is referent to the bathing season, between June 15 and September 15. Regarding the distance to WWTP, three different WWTPs were considered, depending on the city – Leça WWTP for beaches of Matosinhos, Sobreiras WWTP for beaches of Porto and Gaia Litoral WWTP for beaches

of Vila Nova de Gaia. These WWTPs were chosen given the fact that they were the ones located closer to the beaches. Distances were measured approximately based on the maps presented above.

4.4. Moisture content determination

The moisture content of each sample was determined. Approximately 5 g of each sample was weighed, registering the exact mass, and placed in a thermostated oven at 80 ºC. Samples were weighed every 24 hours, until constant mass was verified.

4.5. Extraction and clean-up

The selected method for the extraction and clean-up of the samples was the QuEChERS methodology, previously developed by Homem et al. (2013), and optimized by Cunha (2012) and Silva (2013).

The method consisted of weighing 5 grams of each sample in a polypropylene tube and adding 3 mL of acetonitrile. The samples are then vortexed for 3 minutes and sonicated for 10 minutes. The first QuEChERS, consisting of 2400 mg of $MgSO_4$ and 750 mg of $NaCH_3COO$, is added to the sample and the mixture is vortexed again for 3 minutes and centrifuged at 3700 rpm for 10 minutes. The solvent layer is transferred into the tube containing the second QuEChERS (180 mg of $MgSO_4$, 60 mg of PSA and 30 mg of C_{18}), which is once again submitted to vortex mixing for 3 minutes and centrifugation for 10 minutes at 3700 rpm. The supernatant is removed and placed in a conical vial, and dried under a gentle nitrogen stream. Finally, the dried extract is reconstituted with 50 µL of acetonitrile before GC-MS analysis. This methodology is summarized in the flowchart of Figure 5.

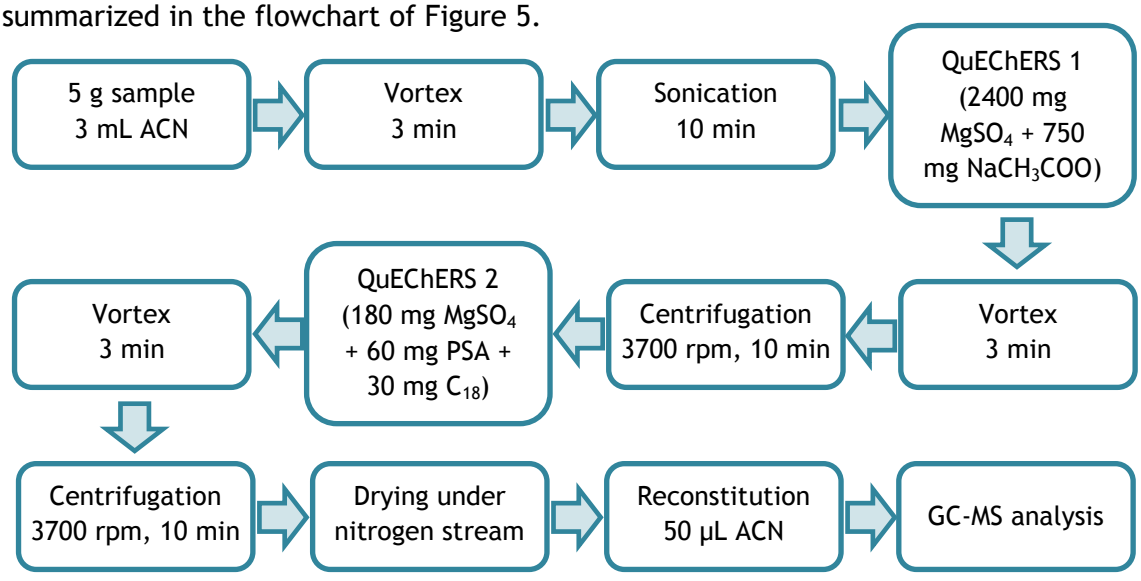


Figure 3 - QuEChERS method schematic representation.

4.6. Instrumentation

Musks analysis was carried out using 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 analysis (MSn) and an autosampler model CP-8410. The mass spectrometer was operated in the electron ionization (EI) mode (70 eV) and the system was controlled 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 id, 0.12 μm). The oven temperature was programmed as follows: 60 ºC hold for 1 min, raised at 6 ºC/min to 150 ºC (hold for 10 min), then 6 ºC/min to 225 ºC and finally 20 ºC/min to 300 ºC (hold for 2.5 min). The carrier gas employed was helium with a purity of 99.999%, at a constant column flow of 1.0 mL/min. Injection (1 μL) was in splitless mode, with the split valve closed for 5 min. Temperatures of manifold, ion trap, and transfer line were maintained at 50, 250, and 250 ºC, respectively. The filament emission current was 50 μA. For quantitative analysis of target compounds, selected ion storage (SIS) mode was applied. Table 7 shows the retention times and the quantifier and qualifier ions used for the SIS detection.

Table 7 – Retention time and quantifier and qualifier ions for each musk.

Compound	Retention time (min)	Quantifier ion (m/z)	Qualifier ions (m/z)
Cashmeran	19.998	191	135, 163
Celestolide	29.410	229	173, 244
Phantolide	30.856	229	173, 187
Exaltolide	32.918	67	55, 83
Musk ambrette	33.095	253	91, 77
Musk xylene-d_{15}	33.455	294	122, 154
Galaxolide	33.566	243	157, 213
Musk xylene	33.822	282	115, 128
Musk tonalide-d_3	33.860	246	128, 160
Tonalide	33.918	243	128, 159
Musk moskene	34.444	263	115, 128
Musk tibetene	35.616	251	115, 128
Musk ketone	36.650	279	128, 160
Ethylene brassylate	37.285	227	98, 125

4.7. Quality control

Musks are present in many daily use products, thus being extremely important to take some specific measures in order to avoid contamination during sample extraction. The usage of detergents in the laboratory and cosmetics of personal use were avoided by all personnel during handling of the samples. Procedural blanks were analyzed in every batch of extractions. Trace levels of musk ketone, cashmeran, galaxolide, tonalide, exaltolide and ethylene brassylate were found. Its values were subtracted to every concentration reported. Chromatographic blanks were also performed, in order to verify the existence of a memory effect.

4.8. Waste management

Waste generated in this work consisted mainly on liquid residues of organic solutions containing acetonitrile and trace amounts of musks, and on sand and sorbents used during the extraction and clean-up ($MgSO_4$, $NaCH_3COO$, PSA and C_{18}). All residues were collected in labeled closed containers and kept away from light and ignition sources for further treatment by the Environmental Management System of FEUP – EcoFEUP.

5. Results and Discussion

5.1. Method Validation

In order to evaluate the method used for sample analysis, several parameters were studied. An internal standard method was employed to quantify all musks. Calibration curves were constructed (Appendix 1) by direct injection of 9 calibration standards containing all musks at different levels (5 – 1000 μg/L). Correlation coefficients obtained varied from 0.9881 to 0.9987. Limits of detection (LOD) and limits of quantification (LOQ) were calculated as the concentration giving a signal-to-noise ratio of 3 (S/N=3) and 10 (S/N=10), respectively. LODs varied between 7.85 x 10^{-4} and 3.75 x 10^{-2} ng/g while LOQs varied between 2.62 x 10^{-3} and 1.25 x 10^{-1} ng/g. The lowest LOD was obtained for tonalide. Overall, these values are lower than those obtained in the studies presented in the State of the Art section. Table 8 includes the values obtained for these parameters; sensitivity of the method is expressed as the slope of the calibration curve. The method proved to be more sensitive to musk tibetene and less sensitive to ethylene brassylate.

Table 8 - Linearity parameters of the method for the determination of synthetic musks.

Compound	Sensitivity	Correlation R^2	LOD (μg/L)	LOQ (μg/L)	LOD (ng/g)	LOQ (ng/g)
Cashmeran	0.0025	0.9921	0.91	3.03	9.09×10^{-3}	3.03×10^{-2}
Celestolide	0.0040	0.9950	0.16	0.55	1.65×10^{-3}	5.49×10^{-3}
Phantolide	0.0030	0.9969	0.11	0.38	1.14×10^{-3}	3.80×10^{-3}
Exaltolide	0.0016	0.9950	0.88	2.94	8.82×10^{-3}	2.94×10^{-2}
Musk ambrette	0.0021	0.9987	1.43	4.76	1.43×10^{-2}	4.76×10^{-2}
Galaxolide	0.0019	0.9944	0.43	1.45	4.35×10^{-3}	1.45×10^{-2}
Musk xylene	0.0029	0.9889	1.43	4.76	1.43×10^{-2}	4.76×10^{-2}
Tonalide	0.0020	0.9983	0.08	0.26	7.85×10^{-4}	2.62×10^{-3}
Musk moskene	0.0043	0.9912	3.33	11.11	3.33×10^{-2}	1.11×10^{-1}
Musk tibetene	0.0060	0.9909	3.75	12.50	3.75×10^{-2}	1.25×10^{-1}
Musk ketone	0.0034	0.9881	1.30	4.35	1.30×10^{-2}	4.35×10^{-2}
Ethylene Brassylate	0.0001	0.9985	2.50	8.33	2.50×10^{-2}	8.33×10^{-2}

Method precision was evaluated by the relative standard deviation (%RSD) of three independent extractions, whilst accuracy was studied by recovery tests for each musk (%Rec) (Table 9). Values for these parameters were obtained using spiked samples at three concentration levels (10, 500 and 1000 μg/L). In most cases, precision levels were below 15%, with the exceptions of exaltolide (15.6%) and

ethylyne brassylate (16.6%). Recoveries obtained ranged from 49.9 to 127.2%, being almost always higher than 80%; these results were generally better than those presented by other authors.

Table 9 - Method precision and accuracy.

	Precision (%RSD)			Accuracy (%Rec)		
Compound	**10 µg/L**	**500 µg/L**	**1000 µg/L**	**10 µg/L**	**500 µg/L**	**1000 µg/L**
Cashmeran	2.2	14.5	9.8	95.5	59.5	102.1
Celestolide	7.7	7.7	5.8	104.8	85.5	97.1
Phantolide	2.9	7.5	5.3	96.1	93.7	99.7
Exaltolide	7.4	15.6	8.9	87.9	87.1	81.9
Musk ambrette	10.6	2.3	2.3	112.0	99.5	102.5
Galaxolide	4.5	11.3	5.1	100.7	88.3	103.4
Musk xylene	10.4	9.9	9.8	98.9	98.6	89.7
Tonalide	7.3	5.8	6.5	103.3	87.4	52.5
Musk moskene	1.9	5.1	6.1	106.1	102.4	89.9
Musk tibetene	6.9	9.2	4.7	101.2	115.3	109.0
Musk ketone	8.1	5.5	8.2	101.7	127.2	106.4
Ethylene Brassylate	*	16.6	8.7	*	102.7	49.9

* The response for ethylene brassylate shows some problems for lower concentrations.

Three chromatograms (SIS mode) referring to synthetic musk mix standard (1000 µg/L), a beach sand sample and a spiked sample (1000 µg/L) are presented in Appendix 2. It is possibly to verify that in the spiked sample, the peaks referent to the target analytes have increased, in comparison to those of the sample. This contamination aids in the location and identification of each compound, thus allowing to determine their existence in the sample.

5.2. Synthetic musks in beach sands

After validation, the analytical method was employed to a total of 45 samples, as described above. Synthetic musks were found in all the samples analyzed. All musk concentrations are reported on a dry basis (average moisture content was 0.04% for summer samples and 0.55% for winter samples).

In Figure 6 are represented the total concentrations of synthetic musk compounds found in each analyzed sample. As can be seen, higher synthetic musks concentrations were detected in summer (1st campaign). Valadares Sul (32.59 ng/g dw), Castelo do Queijo (29.58 ng/g dw) and Francelos (25.85 ng/g dw) showed the

highest levels in Summer, while Aguda (0.58 ng/g dw) and Pedras Brancas (0.76 ng/g dw) the lowest. During Winter, higher levels were detected in the samples from Francelos (32.21 ng/g dw), Miramar (20.03 ng/g dw) and Valadares Sul (19.38 ng/g dw), whilst the lowest values were detected in Azul (1.21 ng/g dw) and Castelo do Queijo (1.28 ng/g dw). It was expected that musk concentration in the summer samples was higher than in the inter samples, due to the use of personal care products by the users of the beach. However, as can be observed, this did not occur in some of the beaches, where higher levels of musks were detected in the winter samples. This leads to the assumption that, during the winter, there are other means of contamination that contribute to the levels of synthetic musks. One possible explanation is the fact that, despite the points from where the samples were collected were above the high tide level, thus being generally dry in the summer, in the winter the tide level may be higher, especially during storms. Therefore, the presence of musks in seawater, strongly influenced by WWTP discharges, may be a significant source of musk contamination to sand in the winter, which does not occur in the summer.

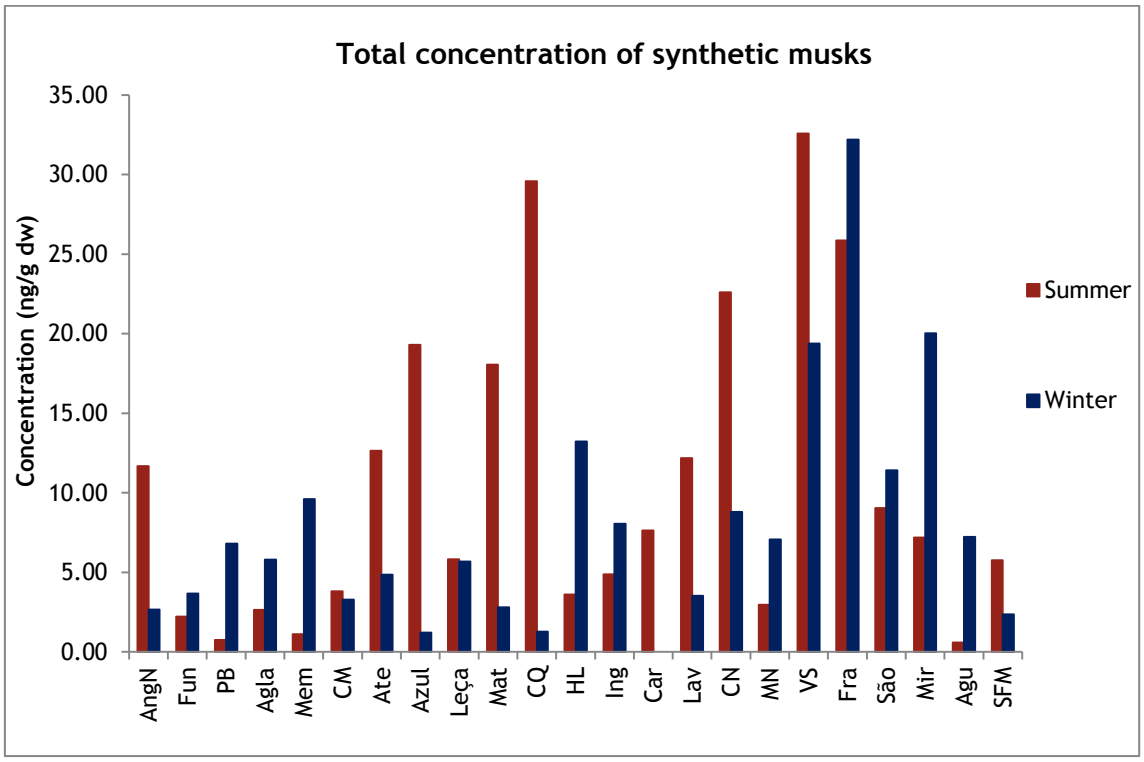


Figure 4 - Total concentration levels of synthetic musks in the beach sand samples.

In these samples, the most commonly detected synthetic musk was tonalide, found in 93% of the samples (42 samples), closely followed by exaltolide, which was found in 89% of the samples (40). Galaxolide and ethylene brassylate were found in

76% (34) and 71% (32) of the samples, respectively. All the other musks were found in less than 40% of the samples analyzed. Musk ambrette, musk tibetene and musk moskene were not detected in any of the samples, as it was expected, since the use of these nitro musks in the European Union has been banned. From the restricted nitro musks (musk ketone and musk xylene) only the first was detected. These values are in agreement with those found in personal care products, the main source of synthetic musks to the environment, in which galaxolide and exaltolide are the most commonly found compounds. A schematic representation of these frequencies of detection is showed in Figure 7.

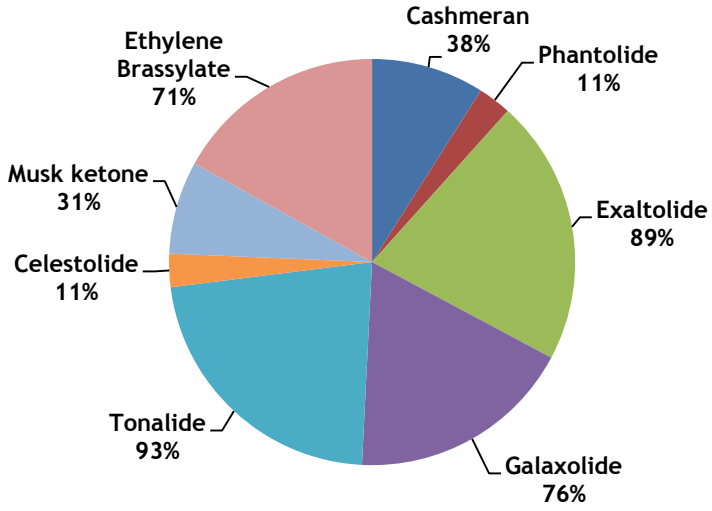


Figure 5 - Frequency of detection of musks in all beach sand samples.

Regarding the samples from the first sampling campaign (summer), the musks presenting higher frequency of detection were also tonalide and exaltolide, detected in 91% and 83% of the 23 samples, respectively. Also detected in a large number of samples were galaxolide (78%) and ethylene brassylate (74%). Nitromusks were not detected in any of the samples collected in September 2013.

Samples from the second sampling campaign (winter) followed the same trends, however in this case musk ketone was detected in 64% of the samples. The presence of this compound only in winter samples may be explained by the fact that, during summer, the high levels of sun radiation may promote its photodegradation (National Center for Biotechnology Information, 2014), which is not significant during winter. These results are illustrated in Figures 8a and 8b.

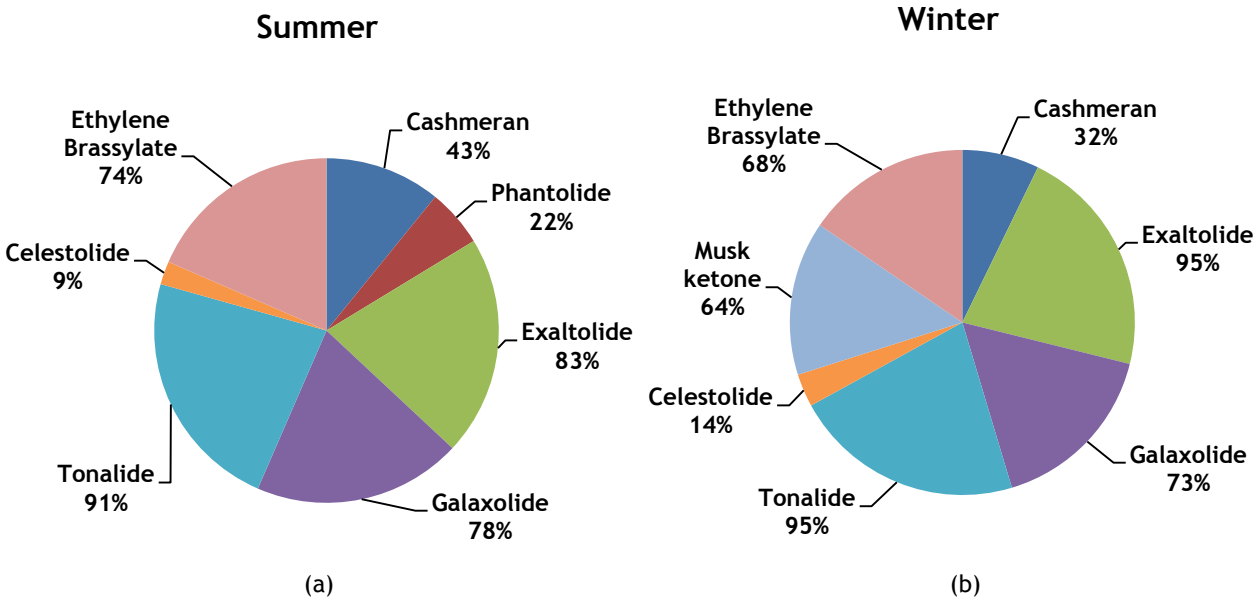


Figure 6 - Frequencies of detection of synthetic musks in sands in Summer (a) and in Winter (b).

As mentioned before, from the nitro musks group only musk ketone was found, with concentrations ranging from 0.06 (Homem do Leme) to 0.70 ng/g dw (Miramar), and only in sands from the second sampling campaign. All the other musks were detected in the beach sand samples.

The results of the analysis to the beach sand samples are shown in Table 10 to 12. As it can be seen in Table 10, the samples from the summer campaign in Matosinhos city contained the five polycyclic and the two macrocyclic musks analyzed in this study, but nitromusks were not detected. Galaxolide was the compound detected in higher concentration levels (0.04 - 15.61 ng/g dw). In the sands from the winter sampling campaign, celestolide and phantolide were not detected, but musk ketone was found in a total concentration of 0.97 ng/g dw. Similarly, galaxolide was the synthetic musk detected in higher concentration (0.52- 8.30 ng/g dw). In general, all musks presented lower concentration when compared to those of the summer sampling campaign, with the exception of exaltolide (17.26 ng/g).

Table 10 – Concentrations of synthetic musks in sands from Matosinhos city in ng/g dw.

Compound	Beach									
	Angeiras Norte	Funtão	Pedras Brancas	Agudela	Memória	Cabo do Mundo	Aterro	Azul	Leça	Matosinhos
Summer sampling campaign										
Cashmeran	0.64	0.48	0.63	0.23	0.07	0.26	nd	Nd	nd	nd
Celestolide	nd	nd	nd	nd	0.05	nd	nd	Nd	nd	nd
Phantolide	nd	nd	nd	nd	0.09	nd	0.04	Nd	nd	nd
Exaltolide	0.92	nd	nd	nd	nd	1.94	1.70	1.39	1.16	0.63
Musk ambrette	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Galaxolide	6.22	nd	nd	0.98	nd	0.04	8.87	15.61	4.48	10.28
Musk xylene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Tonalide	0.39	0.31	0.13	1.21	1.00	1.58	1.23	0.83	0.17	3.12
Musk moskene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Musk tibetene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Musk ketone	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ethylene Brassylate	3.51	1.42	nd	0.24	nd	nd	0.79	1.48	nd	4.02
Σ Musks	**11.68**	**2.21**	**0.76**	**2.65**	**1.11**	**3.82**	**12.64**	**19.31**	**5.82**	**18.05**
Winter sampling campaign										
Cashmeran	0.10	nd	nd	0.14	nd	nd	0.11	<LOQ	nd	nd
Celestolide	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Phantolide	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Exaltolide	1.53	1.80	2.05	1.74	1.02	1.75	2.18	0.79	2.06	2.34
Musk ambrette	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Galaxolide	nd	0.93	2.93	nd	8.30	0.52	1.50	nd	nd	nd
Musk xylene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Tonalide	0.16	0.45	0.19	nd	0.22	0.22	0.11	0.75	0.33	0.46
Musk moskene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Musk tibetene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Musk ketone	nd	nd	0.40	0.11	0.08	nd	0.19	nd	0.19	nd
Ethylene Brassylate	0.88	0.50	1.23	3.81	nd	0.80	0.78	0.61	3.11	<LOQ
Σ Musks	**2.67**	**3.67**	**6.81**	**5.79**	**9.61**	**3.29**	**4.87**	**1.21**	**5.69**	**2.80**

nd – not detected; LOQ - limit of quantification

In the summer sampling campaign from Porto (Table 11), only five musks were detected. Once again, the highest values were obtained for galaxolide, in a total concentration of 28.59 ng/g dw, followed by exaltolide (7.57 ng/g dw), ethylene brassylate (7.02 ng/g dw), tonalide (2.42 ng/g dw) and phantolide (0.11 ng/g dw). In samples from the winter campaign, cashmeran and musk ketone were detected in

one sample each. Total values obtained were lower than those obtained in the first campaign, with the exception of the galaxolide levels detected in Homem do Leme and Ingleses beach, which were significantly higher (11.42 and 6.62 ng/g dw).

Table 11 - Concentrations of synthetic musks in sands from Porto city in ng/g dw.

Compound	Beach			
	Castelo do Queijo	**Homem do Leme**	**Ingleses**	**Carneiro**
Summer sampling campaign				
Cashmeran	nd	nd	nd	nd
Celestolide	nd	nd	nd	nd
Phantolide	0.03	0.05	0.04	nd
Exaltolide	2.02	1.25	1.79	2.51
Musk ambrette	nd	nd	nd	nd
Galaxolide	22.39	1.31	2.36	2.53
Musk xylene	nd	nd	nd	nd
Tonalide	0.80	0.35	0.69	0.59
Musk moskene	nd	nd	nd	nd
Musk tibetene	nd	nd	nd	nd
Musk ketone	nd	nd	nd	nd
Ethylene Brassylate	4.35	0.65	nd	2.02
∑ Musks	**29.58**	**3.61**	**4.87**	**7.64**
Winter sampling campaign				
Cashmeran	0.07	nd	nd	*
Celestolide	nd	nd	0.01	*
Phantolide	nd	nd	nd	*
Exaltolide	0.66	1.12	1.22	*
Musk ambrette	nd	nd	nd	*
Galaxolide	0.09	11.42	6.62	*
Musk xylene	nd	nd	nd	*
Tonalide	0.20	0.63	0.22	*
Musk moskene	nd	nd	nd	*
Musk tibetene	nd	nd	nd	*
Musk ketone	nd	0.06	nd	*
Ethylene Brassylate	0.25	nd	nd	*
∑ Musks	**1.28**	**13.23**	**8.07**	**---**

* Sample collection was not possible because access to the beach was forbidden due to bad weather; nd – not detected

In the samples collected in summer in Vila Nova de Gaia, six synthetic musks were found. Galaxolide presented the highest values (1.80 - 24.58 ng/g dw), mainly distributed between Canide Norte, Valadares Sul and Francelos followed by exaltolide (0.58 - 3.79 ng/g dw). These were in fact the highest values found for these two synthetic musks among all the groups of samples.

Table 12 – Concentrations of synthetic musks in sands from Vila Nova de Gaia city in ng/g dw.

Compound	Beach								
	Lavadores	Canide Norte	Madalena Norte	Valadares Sul	Francelos	Sãozinha	Miramar	Aguda	São Félix da Marinha
Summer sampling campaign									
Cashmeran	nd	nd	nd	0.15	0.15	nd	0.15	nd	0.10
Celestolide	nd	nd	0.03	nd	nd	nd	nd	nd	nd
Phantolide	nd	nd	nd	nd	nd	nd	nd	nd	nd
Exaltolide	3.79	1.39	2.72	3.56	2.41	1.86	1.70	0.58	2.79
Musk ambrette	nd	nd	nd	nd	nd	nd	nd	nd	nd
Galaxolide	4.81	18.21	nd	24.58	22.22	4.71	3.85	nd	1.80
Musk xylene	nd	nd	nd	nd	nd	nd	nd	nd	nd
Tonalide	0.91	0.88	0.21	1.06	0.61	0.56	nd	nd	0.66
Musk moskene	nd	nd	nd	nd	nd	nd	nd	nd	nd
Musk tibetene	nd	nd	nd	nd	nd	nd	nd	nd	nd
Musk ketone	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ethylene Brassylate	2.66	2.13	nd	3.25	0.47	1.90	1.49	<LOQ	0.41
Σ Musks	**12.17**	**22.61**	**2.96**	**32.59**	**25.85**	**9.04**	**7.19**	**0.58**	**5.77**
Winter sampling campaign									
Cashmeran	0.05	nd	nd	nd	0.24	nd	nd	nd	nd
Celestolide	0.01	nd	nd	nd	nd	0.03	nd	nd	nd
Phantolide	nd	nd	nd	nd	nd	nd	nd	nd	nd
Exaltolide	1.38	0.55	2.00	2.34	2.47	0.48	0.43	0.65	nd
Musk ambrette	nd	nd	nd	nd	nd	nd	nd	nd	nd
Galaxolide	1.30	nd	3.76	12.57	26.93	9.56	15.01	4.71	0.40
Musk xylene	nd	nd	nd	nd	nd	nd	nd	nd	nd
Tonalide	0.68	0.43	0.85	1.10	1.97	1.05	1.33	0.74	1.47
Musk moskene	nd	nd	nd	nd	nd	nd	nd	nd	nd
Musk tibetene	nd	nd	nd	nd	nd	nd	nd	nd	nd
Musk ketone	0.10	0.14	0.47	0.53	0.59	nd	0.70	0.08	0.50
Ethylene Brassylate	nd	7.68	nd	2.83	nd	0.30	2.56	1.04	nd
Σ Musks	3.52	8.80	7.08	19.38	32.21	11.41	20.03	7.22	2.36

nd – not detected; LOQ - limit of quantification

In the samples from the winter campaign, the same musks were found, with the addition of musk ketone. Total concentration of galaxolide (74.25 ng/g dw), ethylene brassylate (14.42 ng/g dw), cashmeran (0.29 ng/g dw) and celestolide (0.04 ng/g dw) were very similar to those of the first sampling campaign. While the concentration of

exaltolide was about half (10.29 ng/g dw), value obtained for tonalide was roughly two times higher than that found in the first campaign.

To sum up, the highest values were obtained for galaxolide, with concentrations up to 26.93 ng/g dw (Francelos), followed by ethylene brassylate, with values ranging from 0.24 (Agudela) to 7.68 ng/g dw (Canide Norte). Concentrations of exaltolide and tonalide were very similar, between 0.43 (Miramar) and 3.79 ng/g dw (Lavadores) and between 0.11 (Aterro) and 3.12 ng/g dw (Matosinhos), respectively. Lowest levels of concentration were obtained for cashmeran, phantolide and celestolide, ranging from 0.05 (Lavadores) to 0.64 ng/g dw (Angeiras Norte), 0.03 (Castelo do Queijo) to 0.09 ng/g dw (Memória) and 0.01 (Lavadores and Ingleses) to 0.05 ng/g dw (Memória), respectively. The levels of synthetic musks found in this study are consistent with the concentration of these compounds in personal care products. A study conducted by Homem et al. (2013) showed that galaxolide (2 – 882340 ng/g) and tonalide (960 – 203660 ng/g) were the compounds found with higher concentrations in several products, such as body and hair washes, toothpaste and skin lotions.

Several factors may influence the concentration of synthetic musks found in these samples. Atmospheric deposition is one of the main mean of transport of these compounds into beach sands. The production and use of personal care products releases musks into air, which are then transported, mainly by wind, until they are deposited in several environmental sinks, such as beach sands. Therefore, the direction of the wind, which is different in summer and in winter, strongly influences the amount of synthetic musks that reach a determinate location. Moreover, the temperature differences between air and sea cause the dislocation of air masses, which transport these contaminants into their final sinks. A much higher occurrence of rain in the winter also promotes wet deposition of the contaminants that are adsorbed into air particles. On the other hand, during summer solar radiation is much more intense, thus possibly promoting the photodegradation of these compounds.

As mentioned before, synthetic musks are widely dispersed in the environment through the effluents from WWTPs. Therefore, in order to assess to what extent these discharges may influence musk concentration in beach sands, the results obtained for each sample were organized and presented according to the distance of the beaches to the nearest WWTP. For each city, a different WWTP was considered, as explained in the "Sampling" subsection of the "Technical Description" (Matosinhos city - Leça WWTP, Porto - Sobreiras WWTP, Vila Nova de Gaia - Gaia Litoral WWTP). These results are presented in Figure 9. Observing the results, it is easily understood

that there is not a direct proportionality between the distance of the sampling locations to the nearest WWTP and the total musk concentration found in the samples.

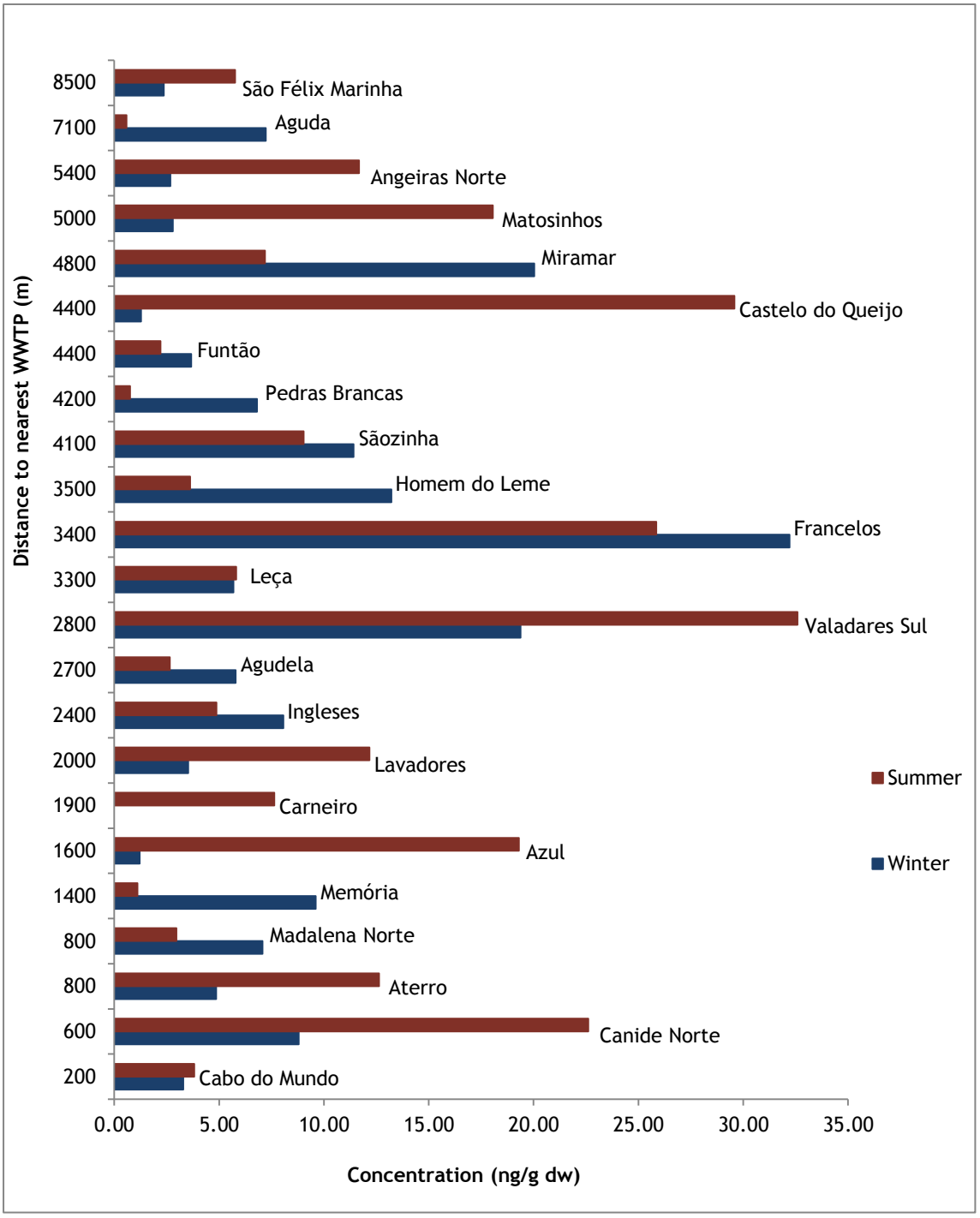


Figure 7 - Graphical representation of total musk concentration as a function of distance to the nearest WWTP.

In order to determine if the number of users of the beaches in question has significant influence in the contamination of beach sands by synthetic musks, the results obtained in the sample analysis (summer campaign) were presented according to estimations of average number of users of the beaches in the bathing season (Figure 10). As mentioned above, this information is not available for every site, therefore not all beaches were considered.

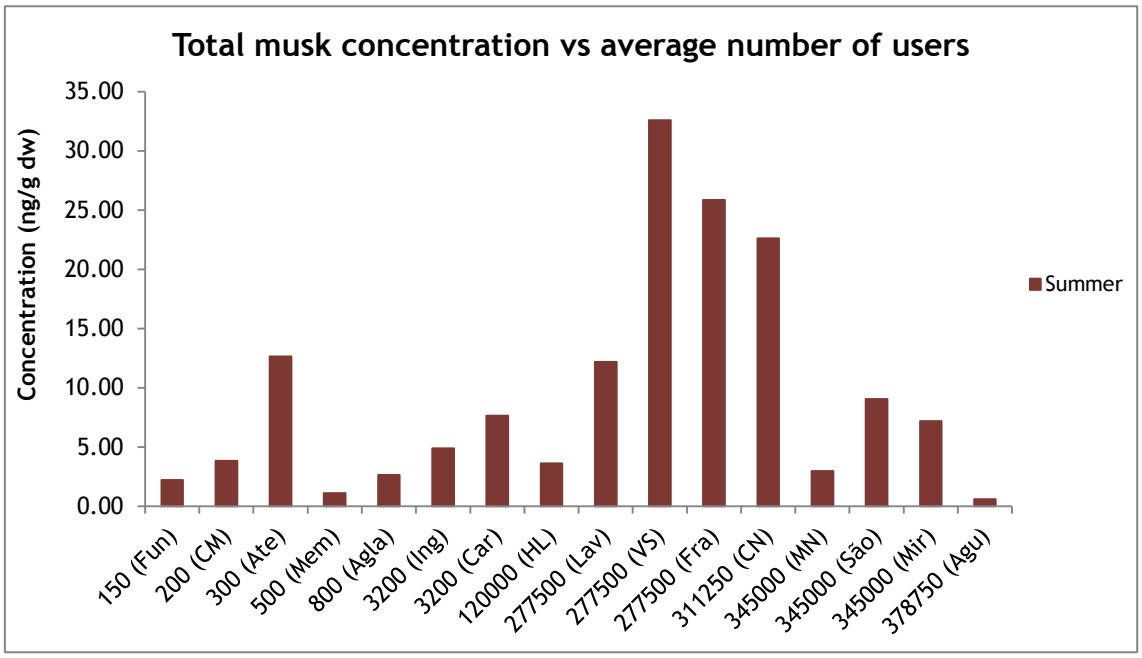


Figure 8 - Graphical representation of musk concentration as a function of the beaches' average number of users.

Once again, there is not a direct relation between the number of users of the beaches in question and the synthetic musks found on the respective samples.

It is important to notice that this is a very simplified analysis, which does not allow establishing a realistic relation between these factors and the total concentration of synthetic musks in beach sands. In order to fully comprehend the concentrations found on beach sands, a combination of a considerably higher number of factors should be taken into account (number of users, population habits, influence of the nearest WWTPs, tidal influence in the spreading and dispersion of contaminants, degradation by solar radiation, wind effect, etc.). The use of advanced multivariate statistics like principal component analysis (PCA) would help in the analysis and interpretation of this large multivariate data generated from environmental monitoring schemes.

The results from the monitoring scheme presented above are compiled in Table 14, including the average concentration found for each synthetic musk, the range of concentrations obtained and the frequency of detection of the compounds.

Table 13 – Overview of the results obtained in this study.

Compound	Average concentration (ng/g dw)	Concentration range (ng/g dw)	Frequency of detection
Cashmeran	0.22	nd - 0.64	17
Celestolide	0.03	nd - 0.05	5
Phantolide	0.05	nd - 0.09	5
Exaltolide	1.67	nd - 3.79	40
Musk ambrette		nd	
Galaxolide	7.70	nd - 26.93	34
Musk xylene		nd	
Tonalide	0.73	nd - 3.12	42
Musk moskene		nd	
Musk tibetene		nd	
Musk ketone	0.30	nd - 0.70	14
Ethylene Brassylate	1.91	nd - 7.68	32

nd – not detected

To the author's best knowledge, that are no studies regarding the detection of synthetic musks in beach sands; therefore, it is not possible to compare the obtained results with published data.

Production and use of personal care products is the main pathway of synthetic musks into the environment. A study conducted in this laboratory by Homem et al. (2013) analyzed the concentration of synthetic musks in personal care products; the most frequently detected musks were galaxolide, exaltolide and cashmeran, with galaxolide presenting the highest concentrations, which is in agreement with the results of this work. This reinforces the assumption that these products are a primary source of synthetic musks to the environment, either by direct volatilization to air and subsequent atmospheric deposition, by disposal to the sewage system or, in the case of beach sands, by contact with the personal care products applied directly in the skin of the users.

Fromme et al. (2001) found galaxolide, tonalide, celestolide and phantolide in river sediments. However, the concentrations obtained were much higher than those found in this work, with values up to 1100 ng/g dw of tonalide and 920 ng/g dw of galaxolide. Hu et al. (2011) also analyzed river sediments, but found significantly

smaller concentrations of synthetic musks, with values ranging from 1.5 to 47.5 ng/g dw. However, the results were still higher than those determined in this study; musk xylene presented a much higher concentration of up to 47.5 ng/g dw. The analysis of lake sediments by Peck et al. (2006) demonstrated similarities with this study in the results. Galaxolide was the compound that presented higher concentration (16 ng/g dw), whilst other musks were detected in smaller concentrations: tonalide (0.96 ng/g dw), celestolide (0.10 ng/g dw), phantolide (<0.049 ng/g dw) and musk xylene (<0.068 ng/g dw). Musk ketone was not detected.

Rubinfeld and Luthy (2008) studied the presence of nitro musks in coastal sediments from San Francisco Bay (USA), and found small concentrations of musk ketone (0.038 ng/g) and musk xylene (0.034 ng/g). Sumner et al. (2010) found galaxolide (11 – 17 ng/g dw) and tonalide (2 – 10 ng/g dw) in the same type of sediments from Tamar Valey (UK), values that resemble those found in this work. However, in both cases, the samples refer to submerged sediments collected near wastewater discharge points. Therefore, these effluents are considered the main contributors to sediment contaminant by synthetic musks. In these studies, the values of musk concentration are reported as having a direct correlation with the WWTP discharges, and consequently to the distances from the sampling points to WWTPs outfalls. Other factors such as tidal regimes are suggested as possible explanations for the differences in concentration of synthetic musks found in the analyzed samples.

6. Conclusions

In this study, a QuEChERS methodology was employed to extract samples of beach sand for further GC-MS analysis. A total of 45 samples were analyzed, collected from beaches located in three cities from the district of Porto, Portugal, in the summer (September 2013) and in the winter (March 2014).

The chosen method proved to be suitable for the matrix and target analytes in question, obtaining satisfactory results for precision – relative standard deviation values between 1.9% (musk moskene) and 16.6% (ethylene brassylate) – and accuracy – recoveries ranging between 49.9 and 127.2%. The values for LOD ranged from 7.85 x 10^{-4} (tonalide) to 3.75 x 10^{-2} ng/g (musk tibetene).

Synthetic musks were detected in all samples. Musk ambrette, musk moskene, musk tibetene and musk xylene were not detected in any of the samples; musk ketone was only detected in samples collected during the winter. Tonalide and exaltolide were the most commonly detected compounds.

The highest concentration of synthetic musks was found in the sample from Valadares Sul (32.59 ng/g dw), whilst the lowest refers to the sample from Aguda (0.58 ng/g dw), both collected in the summer. Galaxolide was the compound that presented highest concentration, ranging from 0.04 to 26.93 ng/g dw. On the other hand, phantolide (0.03 – 0.09 ng/g dw) and celestolide (0.01 – 0.05 ng/g dw) were the musks found in lower concentrations.

An attempt was made to try to relate the results obtained for each beach with its distance to a WWTP, as well as with the average number of users. A direct proportionality was not observed in either case. However, the analysis performed was extremely simplistic, not allowing to realistically determine causality between the different parameters and the musk concentrations.

This study performed the quantification of synthetic musk in an important environmental matrix, thus enlarging our understanding on the occurrence and fate of these compounds in the environment.

7. Limitations and Future Work

The difficulties experienced in this work resided mainly in time and equipment restraints, due to the fact that the GC-MS is used by other researches, and so it was not always available.

The study of the samples should have included the determination of more parameters, such as the total organic carbon content, for example. Given the lipophilic character of synthetic musks, this determination could aid in a better understanding of the results obtained for the compounds concentration. Also, the analysis of more samples, both from the same places but different seasons (spring and autumn) and from other locations, would allow a more complete study of synthetic musks in this environmental sample.

Finally, in order to perform a complete investigation to justify and explain the different concentrations detected in each sample, several parameters could be taken into account. The analysis of other environmental samples, such as seawater and air, mainly in the surrounding areas of the beaches, could shed some light on the results. Furthermore, the determination of synthetic musks in the effluent discharges from the WWTPs in the area could allow assessing what is the contribution of this source in the contamination of the samples. The comprehension of tidal movements along the area of study could subsequently aid to understand how the contaminants released in the WWTP's discharges are dispersed in the environment.

Some of the results of this study will be presented as a poster ("Determination of Synthetic Musks in Beach Sands by QuEChERS extraction and GC-MS analysis", Vera Homem, Inês Magalhães, Arminda Alves and Lúcia Santos) in an internacional conference, ICEH 2014 – 3rd Internacional Conference on Environmental Health, that will take place in Porto, 24 – 26 September 2014. The abstract submitted to the conference is presented in Appendix 3.

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Appendix 1 – Calibration Curves

The calibrations curves elaborated for the 12 synthetic musks are presented in igures A1 through A12. To avoid negative concentration values, curves were forced to pass through the origin of the graph. The equations and respective errors and the parameters used to evaluate the quality of the curve are presented in table A1.

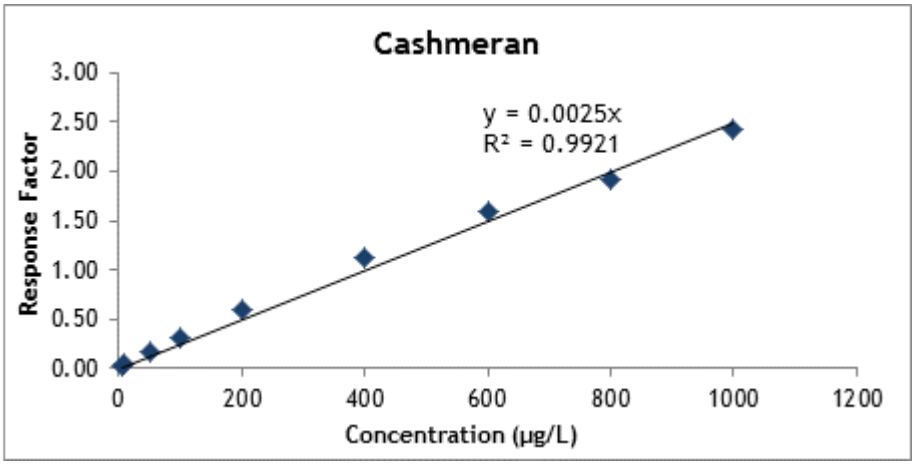

Figure A1 – Calibration curve of cashmeran.

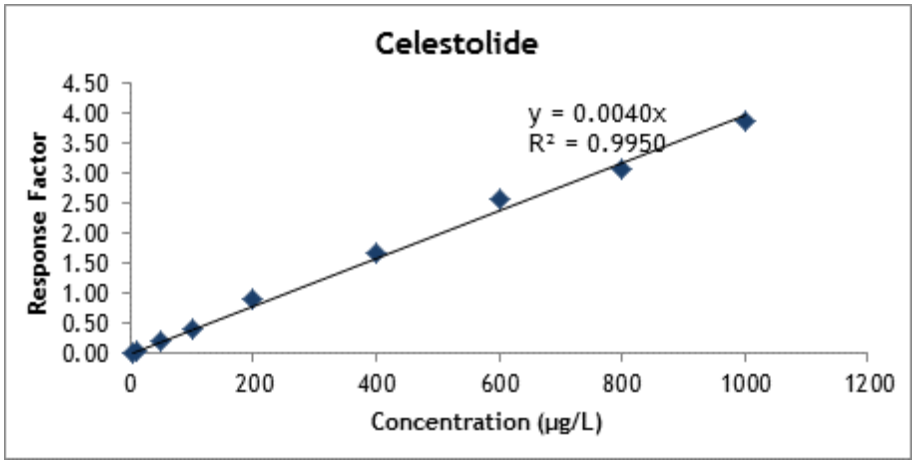

Figure A2 – Calibration curve of celestolide.

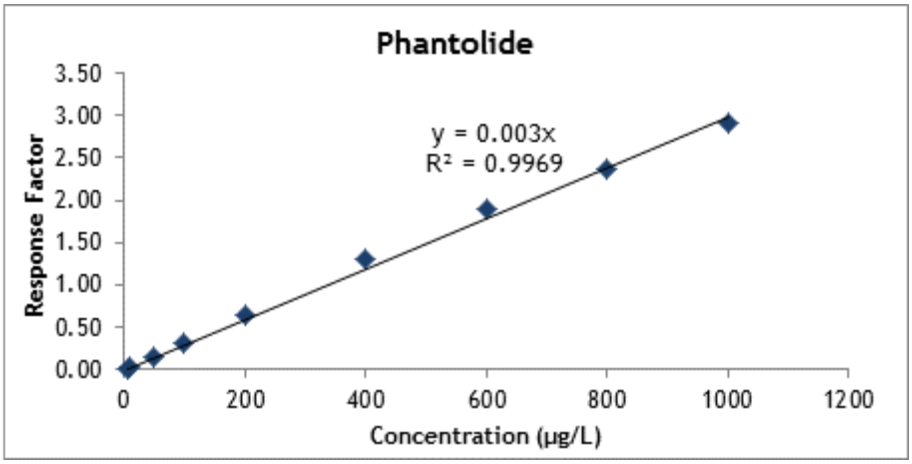

Figure A3 – Calibration curve of phantolide.

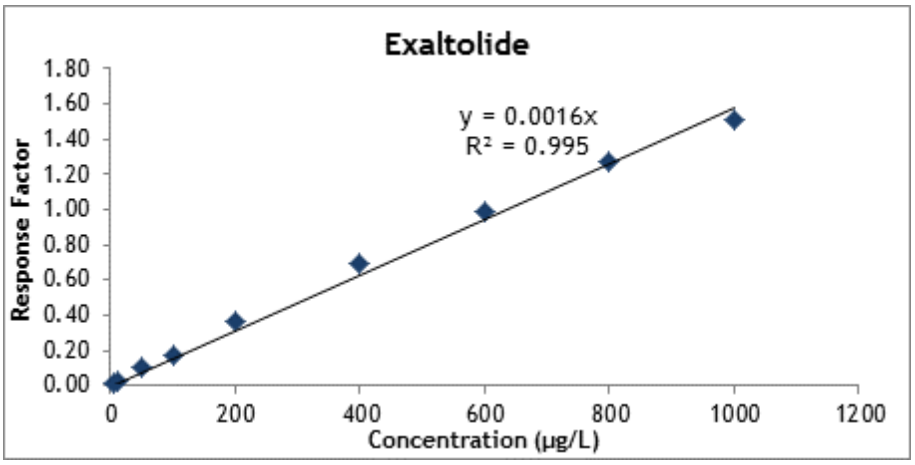

Figure A4 – Calibration curve of exaltolide.

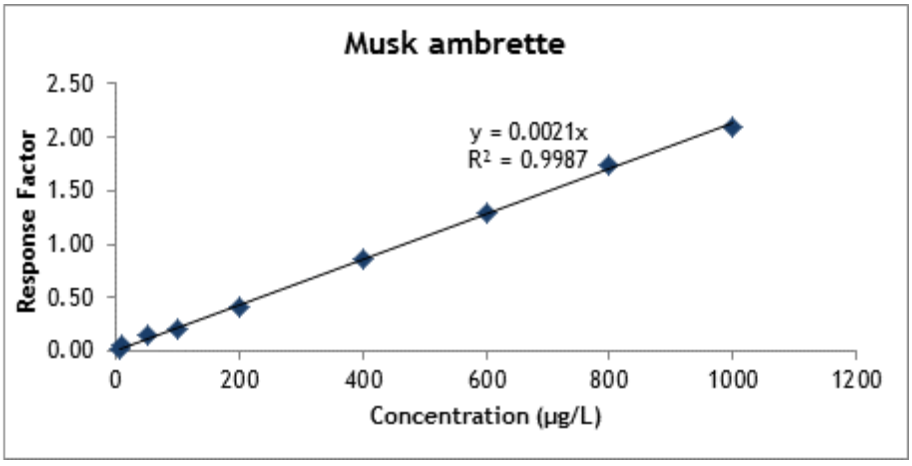

Figure A5 – Calibration curve of musk ambrette.

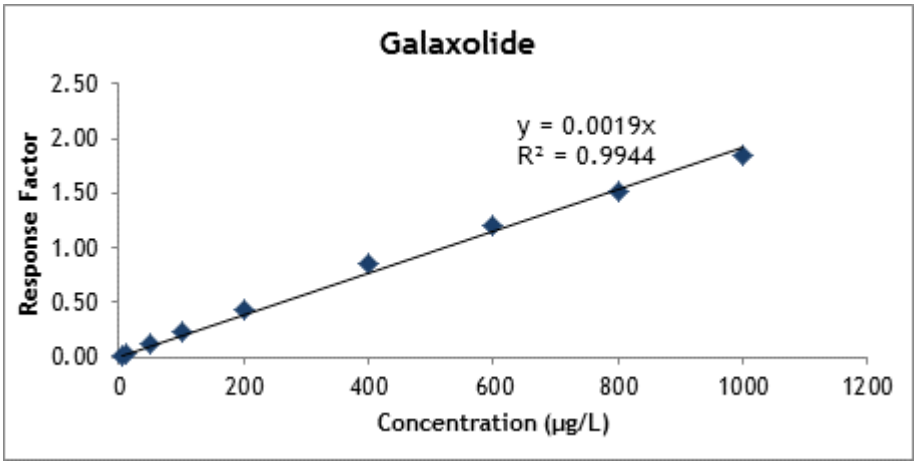

Figure A6 – Calibration curve of galaxolide.

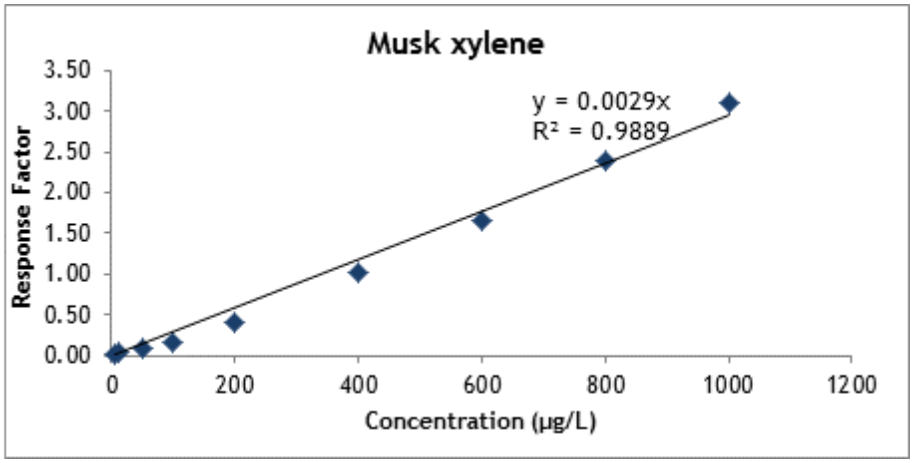

Figure A7 – Calibration curve of musk xylene.

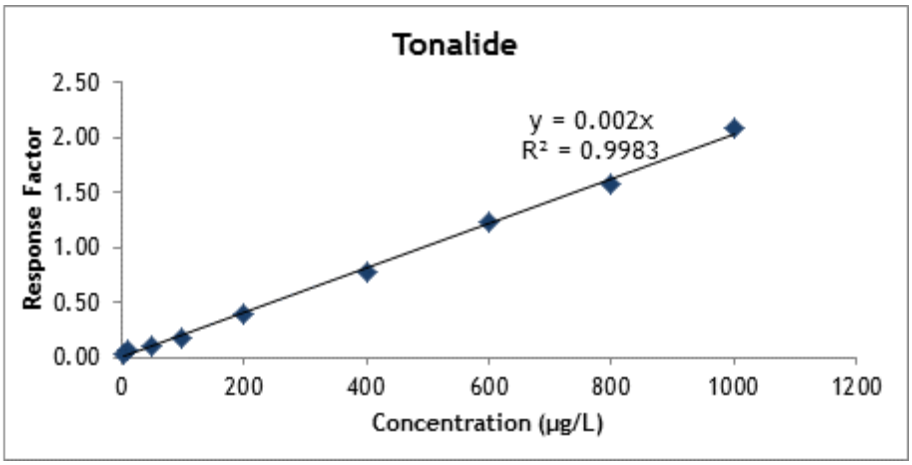

Figure A8 – Calibration curve of tonalide.

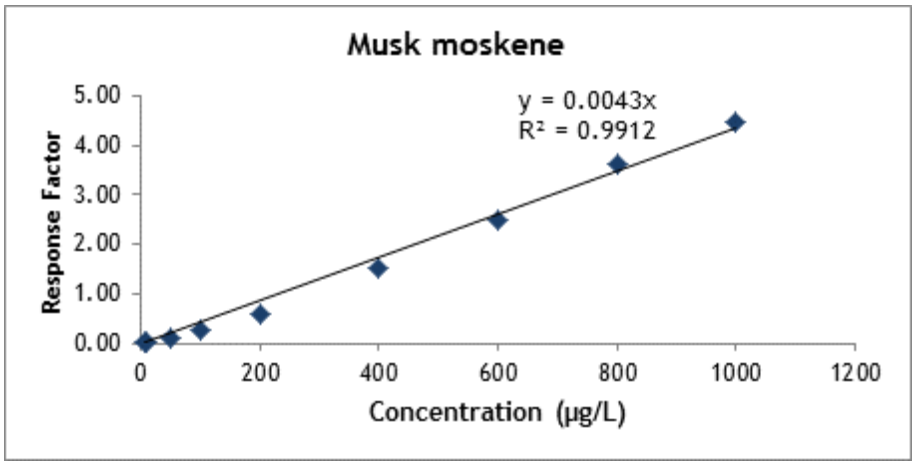

Figure A9 – Calibration curve of musk moskene.

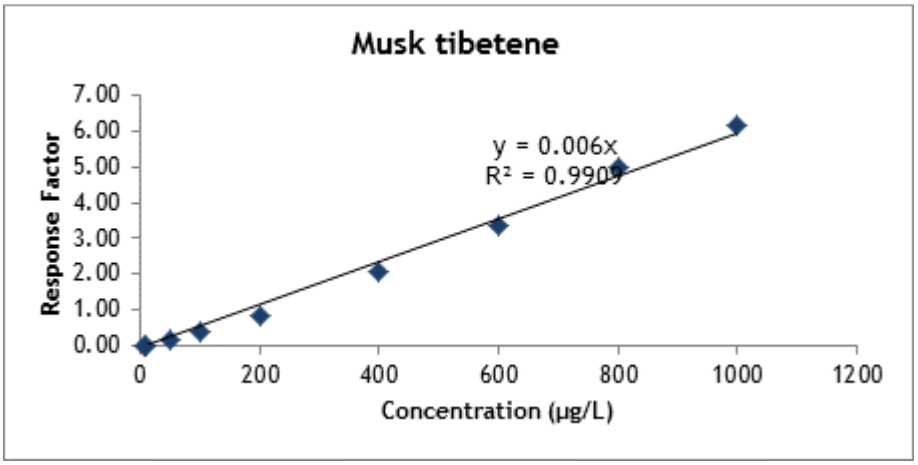

Figure A10 – Calibration curve of musk tibetene.

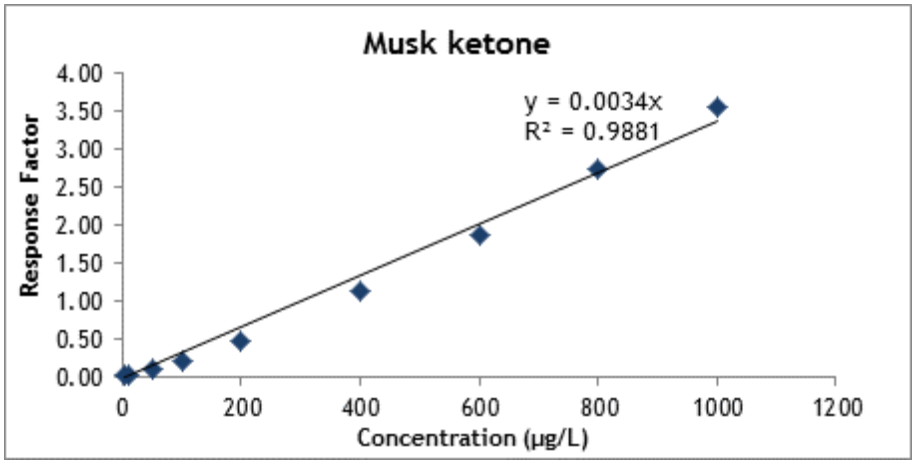

Figure A11 – Calibration curve of musk ketone.

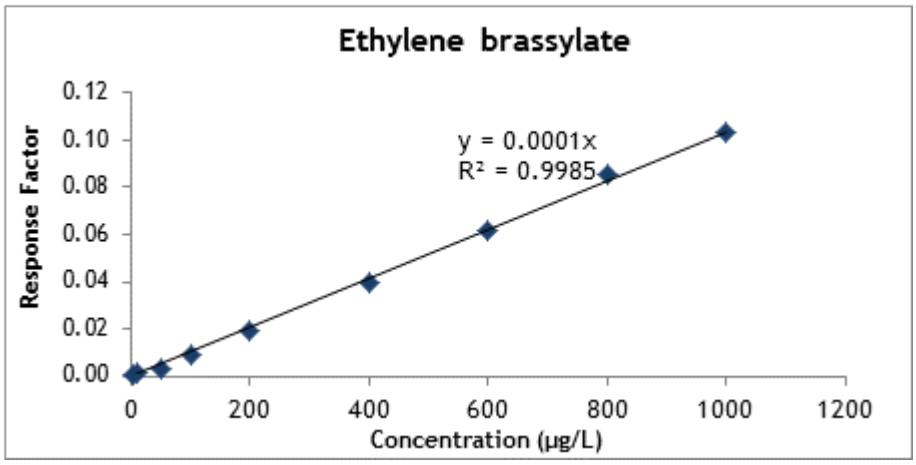

Figure A12 – Calibration curve of ethylene brassylate.

Table A1 – Evaluation parameters of the calibration curves.

Compound	Equation ($y = (a \pm S_a)\ x$)	R (>0.995)	S_a/a (<5%)
Cashmeran	y = (0.00250 ± 0.00008)x	0.996	3.24%
Celestolide	y = (0.0040 ± 0.0001)x	0.997	2.65%
Phnatolide	y = (0.00299 ± 0.00006)x	0.998	2.08%
Exaltolide	y = (0.00158 ± 0.00004)x	0.997	2.67%
Musk ambrette	y = (0.00213 ± 0.00003)x	0.999	1.60%
Galaxolide	y = (0.00191 ± 0.00005)x	0.997	2.79%
Musk xylene	y = (0.0029 ± 0.0001)x	0.994	4.34%
Tonalide	y = (0.00203 ± 0.00004)x	0.999	1.80%
Musk moskene	y = (0.0043 ± 0.0002)x	0.996	3.79%
Musk tibetene	y = (0.0060 ± 0.0002)x	0.995	3.78%
Musk ketone	y = (0.0034 ± 0.0001)x	0.994	4.33%
Ethylene Brassylate	y = (0.000104 ± 0.000003)x	0.999	2.51%

y – response factor; x – concentration; a – slope; S_a – standard deviation of the slope; S_a/a – relative standard deviation of the slope; R – correlation coefficient.

Appendix 2 – Chromatograms (SIS mode)

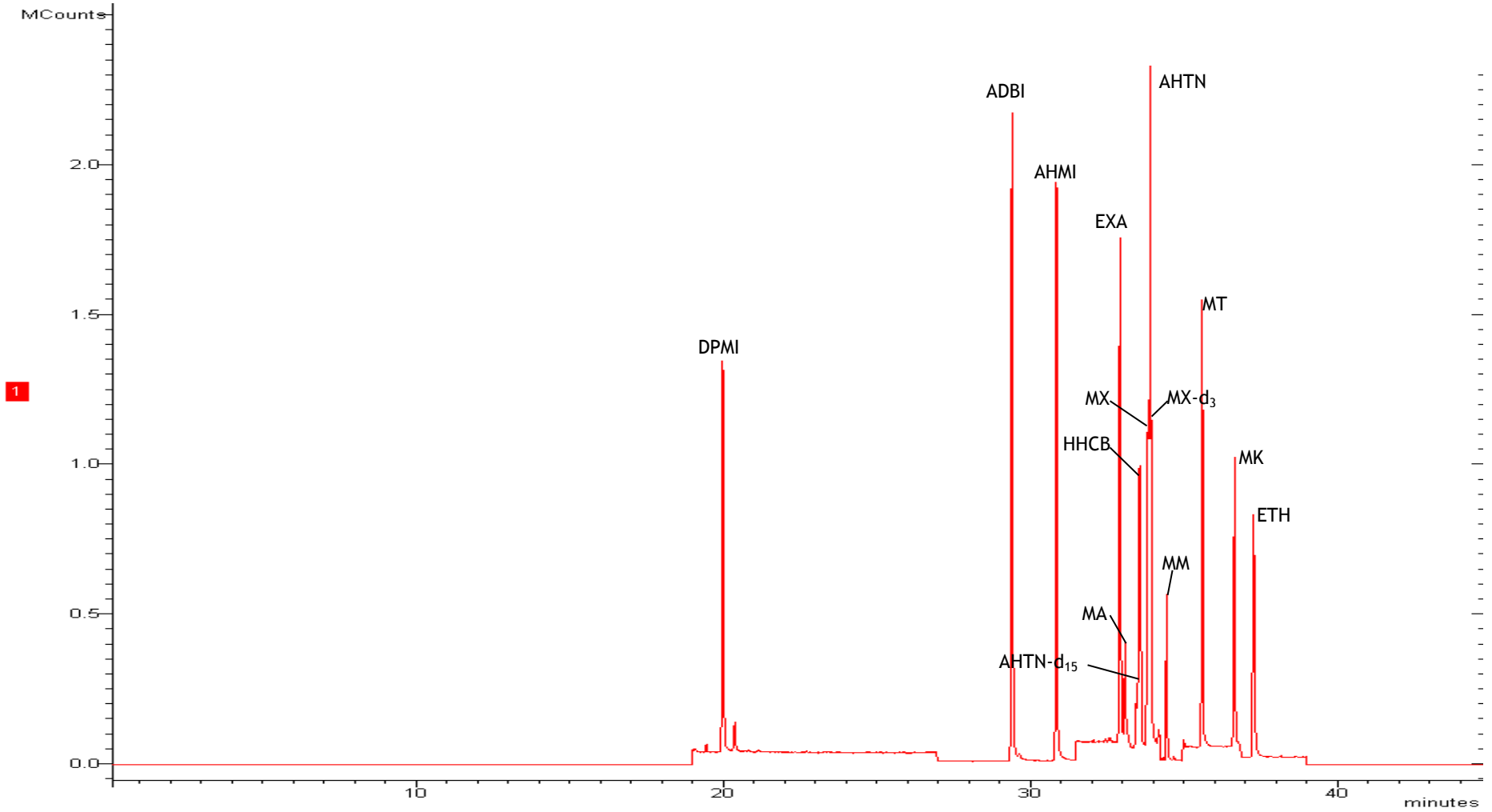

Figure A13 – Chromatogram in SIS mode of a synthetic musk mix standard (1 mg/L).

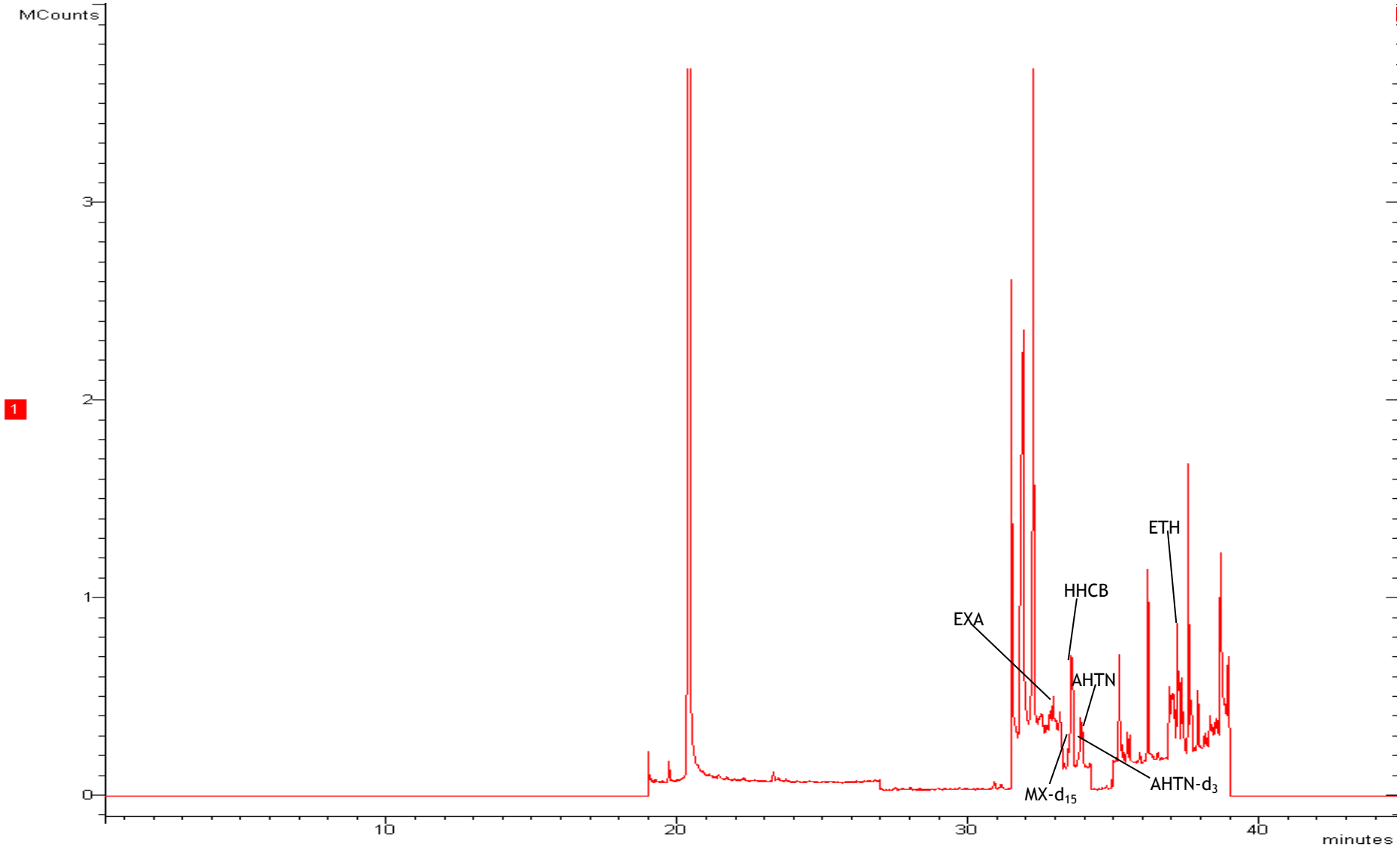

Figure A2 – Chromatogram in SIS mode of a beach sand sample.

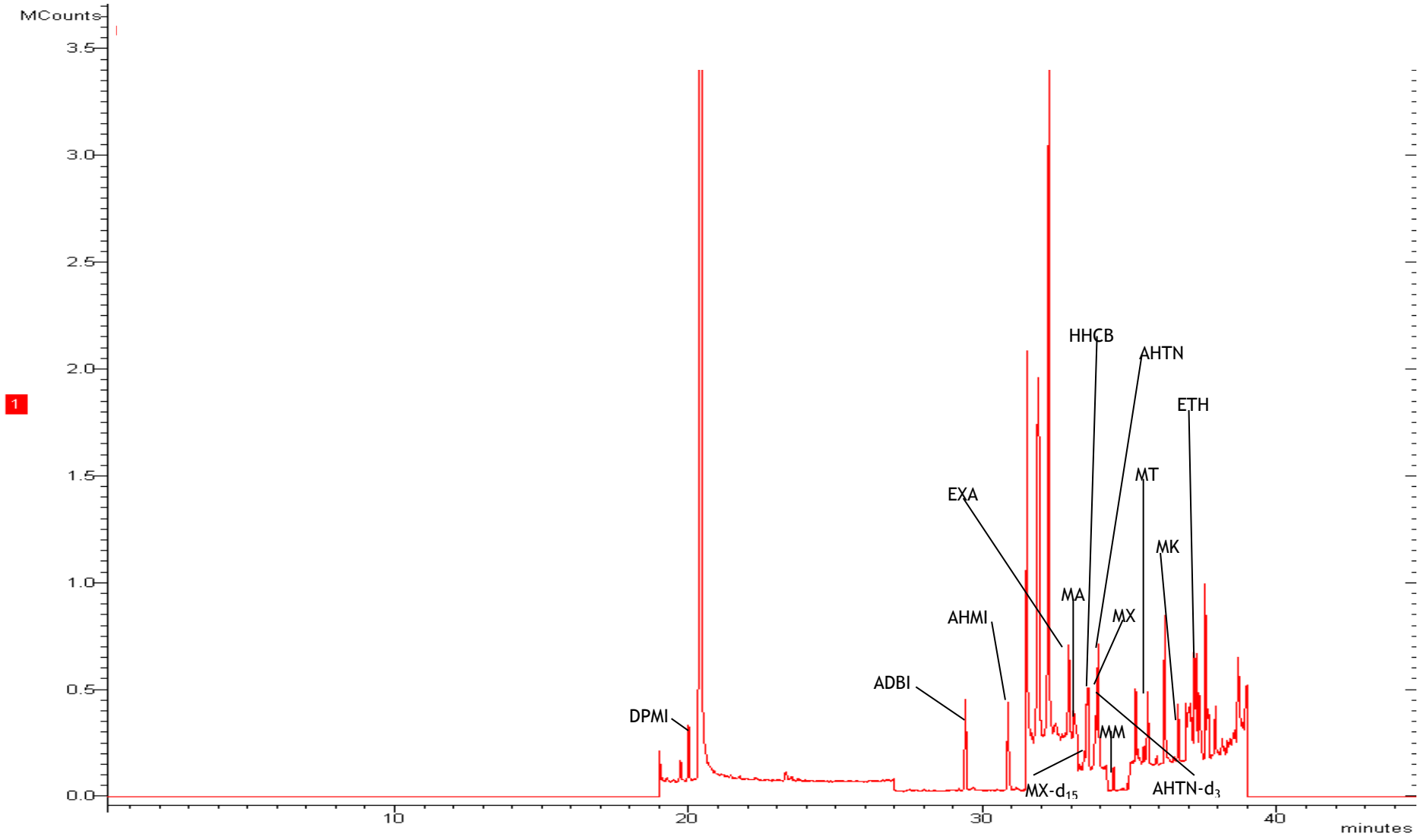

Figure A3 – Chromatogram of a spiked beach sand sample with (1000 µg/L).

Appendix 3 – Abstract submitted for presentation

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Determination of Synthetic Musks in Beach Sands by QuEChERS extraction followed by GC-MS analysis

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INTRODUCTION: Synthetic musks are organic compounds produced in large quantities and extensively used in daily human life in a wide variety of cosmetics, personal care products and household products (Mottaleb et al., 2012). They are continuously released into the environment through a variety of direct and indirect routes as swimming and bathing in rivers or sea, from showering, washing/cleaning and via wastewater treatment plants (WWTPs). Due to their lipophilic, persistent and bioaccumulative nature, synthetic musks are easily adsorbed by particular organic matter and accumulate in sediments (Martinez-Giron et al., 2010) that become another potential contamination source. Few studies have been conducted to assess the occurrence of synthetic musks in sediment samples, namely beach sands (marine sediment) and, for that reason, there is a lack of global monitoring data on sedimentary concentrations of these compounds.

OBJECTIVES: The aims of the present research were: to investigate the application of QuEChERS extraction coupled to GC-MS analysis to the determination of synthetic musks in beach sands, to characterize the musk residues in this matrix and to understand their distribution in the Oporto coastal area.

MATERIALS AND METHODS: Sand samples were collected from 23 beaches on the Oporto coastal area. Samples were spiked with AHTN-d_3 and MX-d_{15} (surrogate standards) and extracted using the following procedure: 3 mL of acetonitrile was added to the sample and then, the mixture was vortexed for 3 min and sonicated for 10 min. The first QuEChERS ($MgSO_4$ and $NaCH_3COO$) were added and the mixture was vortexed 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 ($MgSO_4$, PSA bonded silica and C_{18}). Once again, the samples were vortexed and centrifuged. The supernatant was collected and evaporated to dryness under a gentle stream of N_2. Then, the extract was reconstituted in 50 µL of acetonitrile before GC-MS analysis. Chromatographic analyses were performed by a Varian Ion Trap GC-MS system, using an electron impact ionization mode. The separation was carried out using a CP-Sil 8 CB capillary column (50 m × 0.25 mm i.d., 0.12 µm) from Agilent Technologies. For these analyses, the GC oven 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). For quantitative analysis of target compounds, selected ion storage (SIS) mode was applied.

RESULTS AND DISCUSSION: The performance of a previously validated QuEChERS-GC-MS methodology (Homem et al., 2013) for the determination of musks was assessed for this new matrix - beach sands. Low detection limits and high recoveries (>80%) were determined. Galaxolide and tonalide were the most frequently detected musk fragrances. Nitromusks were rarely present and, when detected, their levels were low.

CONCLUSION: In this work, the overall contamination status of synthetic musk fragrances in Oporto coastal area (beach sands) was investigated. Different musk levels were detected, but polycyclic musks were the most common group found. In general, concentrations of galaxolide were the highest, followed by tonalide.

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