Ultrasound-assisted dispersive liquid–liquid microextraction for the determination of synthetic musk fragrances in aqueous matrices by gas chromatography–mass spectrometry

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A B S T R A C T

A rapid and simple method for the simultaneous determination of twelve synthetic musks in water samples, using ultrasound-assisted dispersive liquid–liquid microextraction (UA-DLLME) coupled with gas chromatography–mass spectrometry (GC–MS) was successfully developed.

The influence of seven factors (volume of the extraction solvent and disperser solvent, sample volume, extraction time, ionic strength, type of extraction and disperser solvent) affecting the UA-DLLME extraction efficiency was investigated using a screening design. The significant factors were selected and optimised employing a central composite design: 80 µL of chloroform, 880 µL of acetonitrile, 6 mL of sample volume, 3.5% (wt) of NaCl and 2 min of extraction time.

Under the optimised conditions, this methodology was successfully validated for the analysis of 12 synthetic musk compounds in different aqueous samples (tap, sea and river water, effluent and influent wastewater). The proposed method showed enrichment factors between 101 and 115 depending on the analyte, limits of detection in the range of 0.004–54 ng L^{-1} and good repeatability (most relative standard deviation values below 10%). No significant matrix effects were found, since recoveries ranged between 71% and 118%. Finally, the method was satisfactorily applied to the analysis of five different aqueous samples. Results demonstrated the existence of a larger amount of synthetic musks in wastewaters than in other water samples (average concentrations of 2800 ng L^{-1} in influent and 850 ng L^{-1} in effluent). Galaxolide, tonalide and exaltolide were the compounds most detected.

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1. Introduction

In recent years, a growing interest in personal care products (PCPs) has emerged. PCPs are chemical products used in consumer products and due to their regular use, they are continuously released to the sewage systems through "down-the-drain" practices, making wastewater effluents the main source of these compounds into the environment [1]. Most of these compounds are lipophilic and tend to accumulate in the environment, affecting negatively the ecosystems.

Within the PCPs are an important class of emerging contaminants, the synthetic musk compounds (SMCs). They are usually divided according to their chemical structure into four classes: nitro, polycyclic, macrocyclic and alicyclic musks [1]. SMCs are usually incorporated in a wide range of consumer products such as detergents, cosmetics and other personal care products both as a fragrance and as a fixative compounds. Their intensive and widespread use combined with their lipophilic nature, made these compounds interesting targets for future research, namely in the environmental and toxicity field [2,3]. In fact, SMCs have been detected in several environmental samples, including air, freshwater, seawater, sediments, marine biota [4–7], and even in human samples [8–10].

SMCs are only partially biodegradable, so they are not completely eliminated by wastewater treatment plants (WWTPs). To date, environmental regulatory limits have not been set for these compounds [6]. Despite these pollutants do not have a regulatory status, it is extremely important to study their occurrence in the environment, in order to determine whether they can be considered hazardous for human health and environment.

For the determination of SMCs in aqueous matrices, several analytical methods have been developed, most of them based on GC–MS analysis. As they occur in the environment at very low concentration levels, extraction techniques to clean up and concentrate are required. The most commonly used are liquid–liquid

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extraction (LLE) [5,11,12] and solid-phase extraction (SPE) [13–15]. However, these techniques are time-consuming and use a relatively high amount of organic solvents. Due to the need of lower solvent consumptions and faster sample preparations, miniaturisation in analytical chemistry has become of interest [16]. Two of the most common miniaturisations of SPE are solid-phase microextraction (SPME) [17,18] and stir-bar sorptive extraction (SBSE) [2,19,20]. Besides reducing the time of sample preparation, they also require low to no solvent consumption. When coupled to thermal desorption (TD), they also reduce the risk of background contamination, since the use of organic solvents is avoided and minimal manipulation of the sample is required [20]. Nevertheless, the main disadvantages of these two techniques are the price, due to the limited lifetime of extracting fibres and stir-bars (degradation with multiple uses) and the carryover problems, which require time-consuming clean-up procedures of the extraction devices.

In 2006, a new microextraction technique called dispersive liquid–liquid microextraction (DLLME) was introduced [21]. It uses an extraction solvent, immiscible in the aqueous phase, and a disperser solvent, miscible in both the extraction solvent (organic phase) and in the sample (aqueous phase). The mixture of solvents is rapidly injected in the sample by the use of a syringe, where the disperser solvent promotes the dispersion of the organic phase (extraction solvent) in the form of microdroplets. This technique uses a very small volume of extraction solvent and the contact surface between phases is infinitely large, leading to high enrichment factors and low extraction times. Rapidity, simplicity, low cost, effectiveness and high enrichment factors are the main advantages of this eco-friendly technique.

Recently, it has been used by few authors for the determination of specific SMCs (polycyclic or nitromusks) in aqueous matrices [22–24]. To the author's best knowledge, DLLME was never simultaneously implemented to different classes of SMCs. Therefore, the aim of this study is to develop and optimise using design of experiments (DoE) an ultrasound assisted-dispersive liquid–liquid microextraction (UA-DLLME) methodology to simultaneously extract twelve SMCs (five polycyclic, five nitro and two macrocyclic musks) from different types of water matrices (river, sea, wastewaters and drinking waters).

2. Materials and methods

2.1. Reagents and materials

Twelve SMCs (five nitro, five polycyclic, and two macrocyclic) were included in this study. Solid standards of synthetic polycyclic musks cashmeran, celestolide, galaxolide, phantolide, and tonalide were obtained from LGC Standards (Barcelona, Spain) with 99% purity, except for galaxolide, which contains approximately 25% of diethyl phthalate. Musk tibetene and musk moskene were also purchased as 10 mg L^{-1} solution in cyclohexane from LGC Standards. Musk ambrette and musk ketone were purchased as solid standards from Dr. Ehrenstorfer (Augsburg, Germany) with 99% and 98% purity, respectively. Musk xylene was obtained from Sigma-Aldrich (St. Louis, MO, USA) as 100 mg L^{-1} solution in acetonitrile and with $\geq$ 95% purity. Exaltolide and ethylene brassylate were also purchased from Sigma-Aldrich with $\geq$ 99% and $\geq$ 95% purity, respectively. Surrogate standards musk xylene-d$_{15}$ and tonalide-d$_3$ were purchased from Dr. Ehrenstorfer (Augsburg, Germany) as 100 mg L^{-1} solutions in acetone and iso-octane, respectively. All the manufacturers used gas chromatography-flame ionisation detection (GC-FID) to check the purity and chemical identity of these compounds.

Acetone, acetonitrile, methanol, chlorobenzene, chloroform

and tetrachloroethylene were purchased from VWR BDH Prolabo (Fontenay-sous-Bois, France), carbon tetrachloride was obtained from Riedel-de Haën (Seelze-Hannover, Germany) and ethanol (96% v/v) was obtained from Panreac (Barcelona, Spain). All solvents used were analytical grade. Sodium chloride, used to adjust the ionic strength, was purchased from Merck (Darmstadt, Germany).

2.2. Standards preparation

Individual stock solutions of polycyclic musks were prepared in cyclohexane at 15 g L^{-1}, while exaltolide, ethylene brassylate, musk ambrette and musk ketone were prepared at 10 g L^{-1} each, also in cyclohexane. A 10 mg L^{-1} intermediate stock solution containing all those previously mentioned synthetic musks was prepared by diluting appropriate amounts in acetonitrile. The final mixed stock solution containing 5 mg L^{-1} of each synthetic musk was prepared by first evaporating an appropriate amount of musk tibetene and moskene solutions under a gentle stream of nitrogen. This step was followed by the addition of the necessary amounts of the former stock solution and of the musk xylene standard and makeup with acetonitrile. A mixed solution of surrogate standards musk xylene-d$_{15}$ and tonalide-d$_3$ was prepared at 10 mg L^{-1}, from which a 225 µg L^{-1} solution was prepared. All solutions were stored and preserved at -20 °C, protected from the light.

2.3. Samples

To evaluate the accuracy and applicability of the proposed method, the extraction of SMCs was performed in different water samples (tap, river and sea waters and wastewaters). Sea water was collected from Matosinhos beach and river water from river Leça (Matosinhos, Portugal). Influent and effluent wastewater was collected from a WWTP of Parada (Maia, Portugal) and each was composed of several samples taken out during a 24 h period. Tap water samples were taken from our laboratory. All the water samples were collected in amber glass bottles and stored in the dark at -20 °C until they were processed.

2.4. Sample extraction

A 6 mL aqueous sample was placed in a 15 mL screw-capped polyethylene centrifuge tube with conical bottom containing 3.5 wt% of sodium chloride. Then, 20 µL of a 225 µg L^{-1} solution of musk xylene-d$_{15}$ and tonalide-d$_3$ (as surrogate standards) were added. A mixture of 880 µL of ACN (as disperser solvent) and 80 µL of chloroform (as extraction solvent) was rapidly injected into the water sample with a syringe. A cloudy suspension was formed and the dispersion was ultrasonicated for 2 min in an ultrasonic bath with 40 kHz (J.P. Selecta, Barcelona, Spain). Phase separation was then performed by centrifugation at 4000 rpm (2400g) for 10 min, and the sedimented phase (65 $\pm$ 6 µL) was collected with a 100 µL syringe and transferred into a 100 µL insert placed into a 1.5 mL amber vial. Then, the extract was analysed by GC–MS.

2.5. Instrumental analysis

The sedimented phase was analysed 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 (MS^n) and an autosampler model CP-8410. The mass spectrometer was operated in the electron ionisation (EI) mode (70 eV) and the system was controlled by Varian MS workstation v. 6.9.3 software. The separation was obtained at a constant flow of 1.0 mL min^{-1} of helium with a purity of 99.999%, in a Varian CP-Sil 8 CB capillary