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Optimization of solid-phase microextraction for the gas chromatographic–mass spectrometric determination of synthetic musk fragrances in water samples

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Abstract

Described is a solid-phase microextraction–gas chromatography–mass spectrometric procedure for the determination of three polycyclic musk fragrances (galaxolide, tonalide, celestolide) and a nitro musk fragrance (musk ketone) in natural river water. Both classes of the musk fragrances could be extracted reproducibly from water samples with a recovery in the range of 45–50% and relative standard deviation of 11–18% for fragrances at 25–260 ng/l levels. Detection limits were between 14 and 22 ng/l. To achieve this reproducibility it was necessary to use an internal standard, pentachloronitrobenzene, for all substances. Best recoveries were achieved with polydimethylsiloxane (PDMS)–divinylbenzene fibers (compared to recoveries obtained with PDMS, polyacrylate or carboxen fibers) and extraction times of 45 min at 30°C, with no need for attainment of equilibrium conditions. The latter was achieved at about 2 h. For Elbe River water, in the vicinity of Magdeburg, no matrix effects were observed. While the average levels of celestolide and musk ketone for samples investigated were below the detection limits, 14 and 22 ng/l, respectively, and for tonalide below the limit of quantification, 22 ng/l, the ambient levels of galaxolide in the Elbe River were 117 ng/l.

Keywords: Solid-phase microextraction; Water analysis; Environmental analysis; Musk compounds

1. Introduction

Solid-phase microextraction (SPME) is a solvent-free one-step extraction method for water and gaseous samples that was first introduced by Pawliszyn and coworkers [1–3]. A fused-silica fiber coated with an immobilized polymeric phase is used to extract the analytes from the water or gaseous phases. The fiber is fixed in a syringe and extraction is based on the partitioning of the analytes between the polymeric phase of the fiber and the sample. After exposure of the fiber to the sample for a known time it is retracted in the syringe.

Subsequently, analytes are desorbed and analyzed in chromatographic systems, by exposing the fiber to a hot GC injector or to the liquid mobile phase of a LC. Numerous articles have reported the usefulness of this extraction method for a wide range of compounds in water samples. For example, SPME

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has been used for the extraction of volatiles [4–6], pesticides [7–9], polycyclic aromatic hydrocarbons and polychlorinated biphenyls [10], phenols [11,12] and surfactants [13]. Likewise, this relatively new extraction method has been discussed in reviews [14–16] and books [17,18].

Various parameters must be optimized for SPME to be an effective tool for analyzing organic substances. In particular, careful attention must be given to the extraction time, stirring velocity, polarity of the fiber, temperature, pH and salinity of the sample [16]. The dynamics of the extraction in water is controlled by the diffusion of the analytes into the polymeric phase [3] and thus the stirring velocity and temperature of the sample are the main factors controlling the extraction time. Most substances need more than 1 h to reach equilibrium, defined as the state in which there is a constant level of substance partitioned between the polymeric phase of the fiber and the water phase [16]. However, for use of SPME as a routine extraction procedure, attainment of equilibrium is not necessary if the extraction time is controlled very carefully. Salt content and pH levels are also parameters affecting extraction efficiencies [16]. Likewise, the polarity of the fiber must be similar to the polarity of the analytes to promote partitioning of the analyte to the polymeric phases [19].

A key factor which is known to contribute to poor extraction efficiencies is interference arising from the sample matrix. The magnitude of such interference appears to be dependent on the nature of the organic carbon content. Indeed, SPME has proven useful for the study of the interactions of analytes with large-molecular-mass humic materials, and can thus be a powerful tool for the analysis of organic substances in water.

In recent years several authors have shown that artificial musk fragrances are significant contaminants in the environment and especially in surface water samples [20–22]. These fragrances fall into two main classes: nitro musk compounds which are substituted nitrated aromatic compounds, and polycyclic musk fragrances which consist of substituted indane and tetraline ring systems. Polycyclic musk fragrances are often used in cosmetics and detergents, while nitromusk compounds are nowadays of minor importance. Both classes of fragrances are known to accumulate in fatty tissues in a manner similar to chlorinated pesticides. For example, concentrations up to 63.6 mg/kg lipid of these substances have been reported for fish in some investigations [20,21]. While musk xylene is reported to be a carcinogen [23], it is not established whether such levels are of ecological significance as there is a lack of information on their toxicology, especially for polycyclic musk fragrances [23].

One report has focused on the results of the application of SPME to the determination of musk fragrances [24]. However, full details were not given for the factors affecting the optimization of the extraction procedure. The aim of the current work is to provide a full discussion of the factors which affect the recovery and precision of a SPME–GC–MS procedure for four different fibers and to evaluate the performance of the methods for the determination of three polycyclic musk fragrances (galaxolide, tonalide, celestolide) and a nitro-musk fragrance (musk ketone) in natural river water. The selection of these substances was based on former analytical results from Elbe river water and suspended particulate matter where these four substances were identified [25].

2. Materials and methods

All solvents were of residue analytical grade and purchased from Baker or Merck (Germany). The musk fragrances (structures for which are given in Fig. 1) were purchased from Promochem (Germany). NaCl was analysis grade (Merck). The SPME devices were obtained from Supelco (USA) and utilized: (i) 30 μm polydimethylsiloxane (PDMS), (ii) 65 μm polydimethysiloxane–divinylbenzene (PDMS–DVB), (iii) 85 μm polyacrylate (PA) and (iv) 75 μm Carboxen fiber. These fibers were selected based on their use in our laboratory for extraction of polar, semivolatile, volatile organic or organonitrogen compounds.

2.1. Experimental set-up

A glass vial with a magnetic stirring bar was filled with 3.5 ml Nanopure water (Barnstedt). Stock

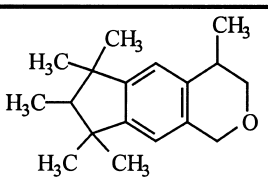
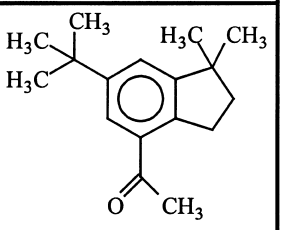
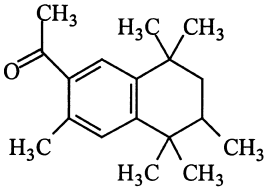
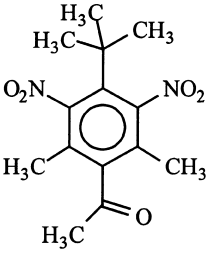
IUPAC-Name Tradename CAS Molecular Mass	Structure	IUPAC-Name Tradename CAS Molecular Mass	Structure
1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexa-methylcyclopenta-(g)-2-benzopyrane **Galaxolide®**, Abbalide® 1222-05-5 258,4 g/mol		4-acetyl-1,1-dimethyl-6-*tert*-butylindan (ADBI) **Celestolide®**, Crysolide® 13171-00-1 244,38 g/mol	
7- acetyl-1,1,3,4,4,6-hexamethyltetraline **Tonalide®**, Fixolide® 1506-02-1 258,4 g/mol		1-*tert*-butyl-3,5-dimethyl-2,6-dinitro-4-acetylbenzene **Musk ketone** 81-14-1 294,3 g/mol	

Fig. 1. Chemical structure of musk fragrances investigated.

solutions (25 ng/ml) of the synthetic musk substances were prepared in methanol. For experiments a volume of 10 μl of the methanolic standards was added to water samples. It was important to keep the volume of solvent low to minimize possible complications arising from sorption of the solvent to the SPME fibers. Once the water sample was spiked, the vial was closed immediately with a PTFE lined septum and placed in a sample holder (Supelco) on a magnetic stirrer.

Upon injection of the SPME syringe with retracted fiber, the fiber was exposed to the water sample for a specific period of time in the range of 10 min to 2 h. After retraction of the fiber back into the syringe the device was withdrawn from the vial and injected immediately in the gas chromatograph. For convenience, selection of an optimum extraction time was based on the results obtained for the PDMS–DVB fiber at two temperatures, 30 and 50°C. This choice was made after selecting the stirring velocity, the GC injector temperature and the injection depth. Comparison of the four fibers was performed after 2 h extraction time.

Specifically, experiments were carried out at (a) stirring velocities: 750, 1000 and 1250 rpm, (b) injection depth: 3.0, 4.0 and 4.5 cm and (c) salt content of NaCl (0, 20 and 200 g/l). In addition, experiments were conducted using two fibres at different injector temperatures: PDMS–DVB at 250 and 270°C, and polyacrylate fiber at 250, 270 and 290°C. Experiments were also performed with and without the addition of a surrogate standard and internal standard, pentachloronitrobenzene (PCNB). For the determination of the recoveries of the musk fragrances, a comparison was made between the levels extracted from water samples with the values measured using direct injection in the splitless mode. The average values of the later were obtained for two runs, one conducted immediately prior to, and the other just after, the SPME experiments. This step was necessary to account for possible differences in the response of the GC–MS over the course of the experiments. All experiments were performed in duplicate. Calibration curve, limit of detection and quantification and precision were determined according to DIN 32645 with ten calibration levels from 28.6 to 286 ng/l by adding 2–20 μl of a 50 ng/ml stock solution of the fragrances to Nanopure water.

2.2. GC–MS analysis

All analyses were performed on a Finnigan GCQ ion-trap mass spectrometer equipped with a HT-8 capillary column (SGE) of 25 m×0.22 mm I.D. and 0.25-μm film thickness. The temperature program was 60°C/5 min, at 30°C/min to 190°C and held for 9 min, followed by 20°C/min to 250°C and held for 3.67 min. The total run time was 25 min. To facilitate quantitative transfer, the fiber was desorbed for 5 min in the hot injector in the splitless mode. To ensure that there was no carryover between runs, the SPME fiber was retained in the GC injector for the duration of the GC run. The injection temperature employed for the four fibers was 260°C. Investigation of the dependencies of the extraction efficiencies on injector temperature were performed at different temperatures (see above). The ion-trap mass spectrometer was operated in the full scan mode, 50–350 u mass range, under positive-ion electron impact conditions, utilizing 70 eV, source temperature of 175°C and transfer line temperature of 250°C. Data acquisition was commenced 5 min after injection on the GC system.

3. Results and discussion

The main findings of this work are that PDMS–DVB fibers provided the best choice for reproducible determination of the musk fragrances and that with careful attention to experimental conditions, it was not necessary to attain equilibrium conditions for the SPME experiments.

A representative chromatogram of the GC–MS extracted ion profile of the four musk fragrances is given in Fig. 2. Under the experimental conditions, the calibrations were linear in the concentration range of 28.6–286 ng/l based on an internal standard method. Calibrations were performed for the whole analytical process taking into account extraction and chromatography. All musk fragrance substances displayed values of $r^2 > 0.993$. Detection limits were 14 ng/l for celestolide, 19 ng/l for tonalide and 22 ng/l for galaxolide and musk ketone. Relative standard deviation of the calibration curves calculated during regression by the software SQS 98 from Perkin-Elmer lie between 2.5 and 3.4%

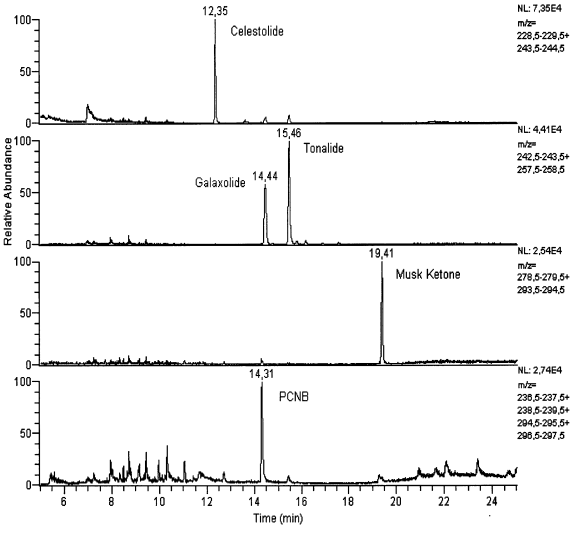


Fig. 2. Extracted chromatograms obtained by GC–MS under full scan conditions of the four musk fragrances (286 ng/l) and PCNB (internal standard) after SPME of Nanopure water.

As illustrated in Fig. 3 a comparison of the extraction efficiencies of the four fibers shows that there is a general trend observed for the relative recoveries of the musk fragrances: namely, PDMS–DVB > polyacrylate ~ Carboxen > PDMS, listed in decreasing order of efficiency. In comparison, the recoveries of PDMS is only 50% of the value obtained for PDMS–DVB fiber for the polycyclic musk fragrances and even lower for the nitro musk fragrance. In other investigations, PDMS–DVB showed good affinity to nitrogen containing analytes like explosives and amines [26,27]. There appears to

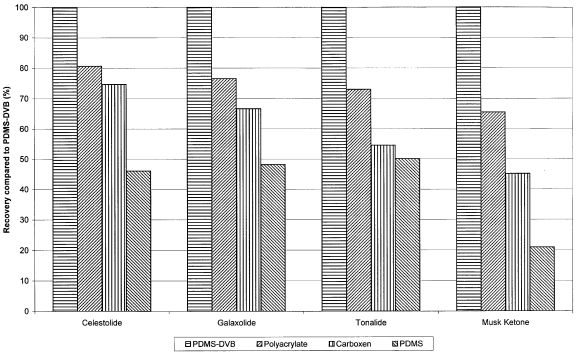


Fig. 3. Comparison of extraction efficiencies of four different fibers (PDMS–DVB was set to 100%).

be a trend for lower extraction efficiency with increasing retention time of the musk fragrances. While this could suggest that the injector temperature was not high enough for some fibers, this is not the case for the polacrylate fiber (see below).

Considering that the best recoveries were obtained for PDMS–DVB fibers, experiments were focused on using PDMS–DVB fibers for further investigation. An evaluation of the results obtained for the optimization of the SPME procedure will now be discussed.

3.1. Stirring velocity

The higher the stirring velocity the better was the extraction efficiency. For example, for PDMS–DVB the extraction efficiency increased from 64 to 100% over the range of stirring velocities from 750 to 1250 rpm with the extraction at 1250 rpm set to 100% (see Table 1). The reason for this observation is that the extraction process is diffusion controlled (3) and stirring enhances the diffusion of the analytes to the fiber, thus resulting in enhancement of the recoveries. In our procedure the upper limit for the stirring velocity was set by the physical constraints of the magnetic stirrer at 1250 rpm.

3.2. Effects of extraction time

Fig. 4 shows that while the maximum recoveries (65–82%) were attained under equilibrium conditions, at ≈90–120 min (for the polycyclic musk fragrances about 90 min and for musk ketone more than 120 min) the four musk fragrances displayed similar recoveries for the various extraction times investigated. It was therefore not necessary to wait until equilibrium was reached. For example, reproducible recoveries were attained at an extraction time of 45 min, in which the recoveries were 45–50%. Since the total run time of the GC–MS analysis was 30 min, we opted to extract the samples for 45 min to facilitate sample throughput in the laboratory. As similar extraction efficiencies were

Table 1
Results of the experiments for the dependencies of extraction efficiencies on salt content, injection depth, stirring velocity and injection temperature

Experiment	Area counts			
	Celestolide	Galaxolide	Tonalide	Musk ketone
Salt content				
0 g/l NaCl	530 028	429 564	575 061	585 501
20 g/l NaCl	265 344	220 183	296 600	322 208
200 g/l NaCl	195 580	170 641	227 712	289 937
Injection depth in the GC injector				
3 cm	1 289 480	2 127 382	2 610 470	1 387 997
4 cm	2 615 231	5 381 359	6 629 687	2 945 289
4.5 cm	3 002 549	7 787 926	9 736 782	5 602 342
Stirring velocity				
750 rpm	2 631 571	2 336 758	2 478 872	2 129 701
1000 rpm	2 820 864	2 748 037	2 726 814	1 692 159
1250 rpm	3 716 014	3 564 712	3 884 021	2 174 221
Injection temperature PDMS–DVB				
250°C	2 420 116	6 480 091	8 522 614	5 257 918
270°C	2 391 417	5 457 379	6 703 830	3 612 586
Injection temperature polyacrylate				
250°C	2 728 876	6 625 467	7 717 285	2 424 478
270°C	2 576 578	6 402 947	8 191 781	2 245 305
290°C	1 730 750	4 189 661	5 477 845	2 268 739

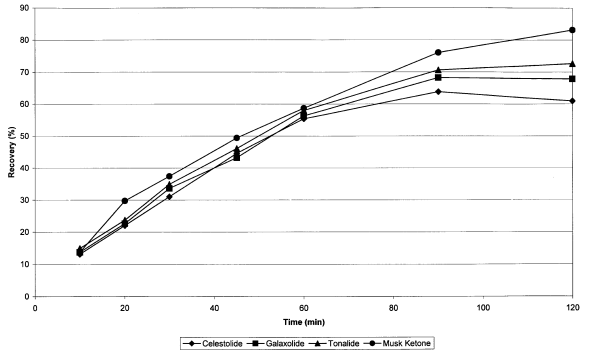


Fig. 4. Time profile for the extraction of four musk fragrances with PDMS–DVB at 30°C.

observed for water samples at 30 and 50°C, all further extractions were performed without heating at 30°C.

3.3. Injection depth: 3.0, 4.0, 4.5 cm

The injection depth into the GC injector of the fiber was a key parameter for which careful attention was needed to ensure reproducible and optimized results. This parameter is dependent on the specific geometry of a given instrument and for the used MS system (with a programmable temperature vaporizer-injector), the response was observed to increase up to a factor of 4 from an injection depth of 3 to 4.5 cm (see Table 1). Thus an injection depth of 4.5 cm was utilized. Injection depth into the water sample of the fiber was not varied.

3.4. Content of NaCl

The addition of salt to the water samples did not promote better extraction efficiencies as is normally found for extractions using organic solvents. In contrast the extraction efficiencies decreased by 60% with increasing salt content over the range investigated (0–200 g/l). This observation is at first surprising. We hypothesized that the addition of salt results in a decrease of the water soluble fraction of the musk fragrances and probably promotes losses through plating out of materials to the walls of the apparatus. This, in turn, limits the aqueous concentration available for partitioning to the SPME fibers. This hypothesis was tested by extracting the walls of the apparatus with two solvents, ethylacetate and *n*-hexane. However, no measurable difference could be found between the concentration of the fragrances washed from the walls for experiments performed with or without salt addition. While an interesting observation, this phenomenon was not investigated further in this work. It is therefore not established whether the decrease in extraction efficiency observed with salt additions could be attributed to wall effects. However, for optimized conditions, SPME procedures were performed using no addition of salt to the water samples.

3.5. Effects of injection temperature

There were no, or only small, detectable temperature effects observed for fibers of PDMS–DVB at 250 and 270°C, and the polyacrylate fiber at 250, 270 and 290°C (see Table 1). As the temperature dependence was low or negligible, all further work was done using PDMS–DVB fibers at a fixed temperature of 260°C, the temperature at which the comparison was conducted for the four fibers as illustrated in Fig. 3.

3.6. Surrogate standard and internal standard, PCNB

For experiments performed without the use of internal standards the precision of the SPME procedure was poor, 25 and 28%, based on the RSD obtained for 8 replicate samples of Elbe River (Germany). The reproducibility, however, was improved significantly (RSD between 11 and 18%) through use of a surrogate standard and internal standard, pentachloronitrobenzene. For example, for internal standard determination of musk ketone, the RSD was 18% while for the three polycyclic musk fragrances better precision was observed with a RSD between 11 and 14%. The choice of standard was based on past experience in our laboratory in which PCNB was used as a surrogate standard for liquid–liquid extractions of musk fragrances (unpublished results). For best reproducibility, PCNB was therefore used as a surrogate standard and internal

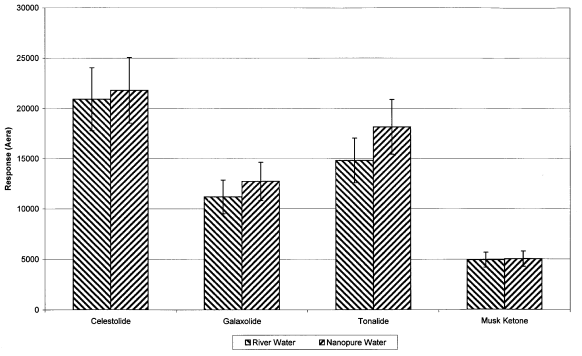


Fig. 5. Comparison of the response of fragrances using river and Nanopure water.

standard for the SPME determination of the musk fragrances.

3.6. Application to real samples — Elbe River

To check whether the SPME was applicable to real river water and not limited to relatively clean laboratory water, the method was applied to River Elbe water. No matrix effects were observed. This point is illustrated in Fig. 5, in which a comparison is given for the mean value of the responses of five extractions of distilled water and 6 Elbe River water samples, both sets of which were spiked with the fragrances. As shown in Fig. 5, there was no significant difference between the mean values for the two sets of samples and thus no matrix effects were detectable for the samples investigated.

While the average levels of celestolide and musk ketone for the samples investigated were below the detection limits and for tonalide below limit of quantification, the ambient levels of the galaxolide in the Elbe River was 117 ng/l. This value falls within the range reported for the Elbe River water [25].

4. Conclusion

Among the SPME fibers investigated, PDMS–DVB fibers proved to be the most efficient for the determination of musk fragrances in natural waters. With careful attention to optimize experimental parameters, reproducible results were achieved based on nonequilibrium conditions with use of an internal standard method for quantification.

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