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Journal of Chromatography A, 932 (2001) 107–118

JOURNAL OF CHROMATOGRAPHY A

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On-site solid-phase extraction and laboratory analysis of ultra-trace synthetic musks in municipal sewage effluent using gas chromatography–mass spectrometry in the full-scan mode

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Received 22 May 2001; received in revised form 17 August 2001; accepted 17 August 2001

Abstract

Fragrance materials such as synthetic musks in aqueous samples, are normally determined by gas chromatography–mass spectrometry in the selected ion monitoring (SIM) mode to provide maximum sensitivity after liquid–liquid extraction of 1-l samples. Full-scan mass spectra are required to verify that a target analyte has been found by comparison with the mass spectra of fragrance compounds in the National Institute of Standards and Technology (NIST) mass spectral library. A 1-l sample usually provides insufficient analyte for full scan data acquisition. This paper describes an on-site extraction method developed at the US Environmental Protection Agency (Las Vegas, NV, USA) for synthetic musks from 60 l of wastewater effluent. Such a large sample volume permits high-quality, full-scan mass spectra to be obtained for a wide array of synthetic musks. Quantification of these compounds was achieved from the full-scan data directly, without the need to acquire SIM data. The detection limits obtained with this method are an order of magnitude lower than those obtained from liquid–liquid and other solid-phase extraction methods. This method is highly reproducible, and recoveries ranged from 80 to 97% in spiked sewage treatment plant effluent. The high rate of sorbent-sample mass transfer eliminated the need for a methanolic activation step, which reduced extraction time, labor, and solvent use. More samples could be extracted in the field at lower cost. After sample extraction, the light-mass cartridges are easily transported and stored. Published by Elsevier Science B.V.

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

1. Introduction

An emerging area of research is the presence of pharmaceuticals and personal care products (PPCPs) in the environment and their possible impact on biota and ecosystems. The long-term effects of PPCPs in the aquatic environment are presently unknown. Some of these compounds have physiological effects on biota (fish, crustaceans) at extremely low concentrations (e.g., estrogen, estrogenic mimics, and certain antidepressants) [1].

Synthetic musks are a group of chemicals possessing chemical structures (Tables 1 and 2) that are not

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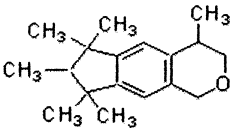
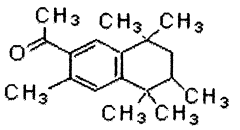
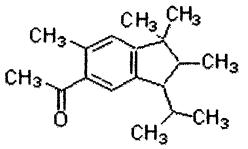
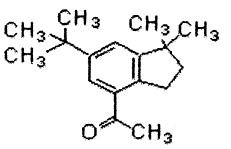
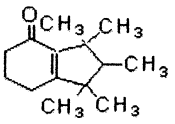
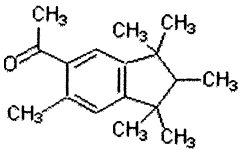
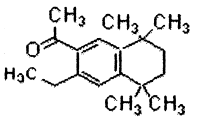
0021-9673/01/$ – see front matter Published by Elsevier Science B.V.
PII: S0021-9673(01)01216-X

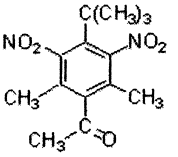
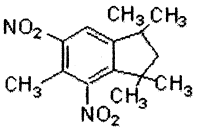
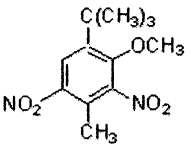
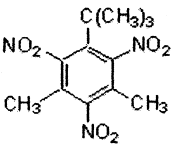
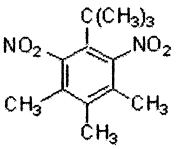
Table 1
Trade and CAS names, structures, log K_{ow}, molecular masses, formulae and Registry Numbers, for seven polycyclic musks

Trade and CAS name (acronym)	Chemical structure	Log K_{ow}	M_r	Molecular formula	CAS no.
Galaxolide, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta-[*g*-2-benzopyran (HHCB)		5.9[a]	258.40	$C_{18}H_{26}O$	1222-0505
Tonalide, 1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthalenyl-ethanone (AHTN)		5.7[a]	258.40	$C_{18}H_{26}O$	1506-02-1
Traseolide, 1-[2,3-dihydro-1,1,2,6-tetramethyl-3-(1-methyl-ethyl)-1H-inden-5-yl]-ethanone (ATII)		6.3[b]	258.40	$C_{18}H_{26}O$	68140-48-7
Celestolide, 1-[6-(1,1-dimethylethyl)-2,3-dihydro-1,1-methyl-1H-inden-4-yl]-ethanone (ADBI)		5.4[b]	244.38	$C_{17}H_{24}O$	13171-00-1
Cashmeran, 1,2,3,5,6,7-hexahydro-1,1,2,3,3-pentamethyl-4H-inden-4-one (DPMI)		5.9[b]	206.32	$C_{14}H_{22}O$	33704-61-9
Phantolide, 1-(2,3-dihydro-1,1,2,3,3,6-hexamethyl-1H-inden-5-yl)-ethanone (AHMI)		5.9[b]	244.38	$C_{17}H_{24}O$	15323-35-0
Versalide, 7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin (AETT)		5.7[b]	258.40	$C_{18}H_{26}O$	88-29-9

[a] Ref. [3].
[b] Estimated values derived by Hansch and Leo fragmentation method in Ref. [2].

readily biodegradable and are capable of being bioconcentrated [2–4] in aquatic organisms. The logarithm of the octanol–water partition coefficient (log K_{ow}) for the parent compounds ranges from 4.3 to 6.3 (Tables 1 and 2) [5,6], and that for some of the metabolites ranges from 4.8 to 5.1 (Table 3) [5]. These synthetic compounds serve as more affordable substitutes for the expensive natural musks (e.g., muscone, civetone, and ambrettolide) contained in many perfumes. Worldwide annual production of nitro musks and polycyclic musks was estimated at $7 \cdot 10^6$ kg in 1988 [7], and $6 \cdot 10^6$ kg in 1999 [8]. Approximately $1 \cdot 10^6$ kg per year of musk xylene are consumed worldwide through the use of detergents [9]. Synthetic musks enter city sewage systems (presumably from bathing, laundry detergents, and

Table 2
Trade and CAS names, structures, log K_{ow}, molecular masses, formulae and Registry Numbers, for five nitro musks

Trade and CAS name (acronym)	Chemical structure	Log K_{ow}	M_r	Molecular formula	CAS No.
Musk ketone, 1-*tert*.-Butyl-3,5-dimethyl-2,6-dinitro-4-acetylbenzene (MK)		4.3[a]	294.31	$C_{14}H_{18}N_2O_5$	81-14-1
Musk moskene, 4,6-Dinitro-1,1,3,3,5-pentamethylindane (MM)		5.8[b]	278.31	$C_{14}H_{18}N_2O_4$	116-66-5
Musk ambrette, 2,6-Dinitro-3-methoxy-4-*tert*.-butyl toluene (MA)		5.7[b]	268.27	$C_{12}H_{16}N_2O_5$	83-66-9
Musk xylene, 1-*tert*.-Butyl-3,5-dimethyl-2,4,6-trinitrobenzene (MX)		4.8[a]	297.27	$C_{12}H_{15}N_3O_6$	81-15-2
Musk tibetene, 1-*tert*.-Butyl-2,6-dinitro-2,4,5-trimethylbenzene (MT)		5.9[b]	266.29	$C_{13}H_{18}N_2O_4$	145-39-1

[a] Ref. [3].
[b] Estimated values derived by Hansch and Leo fragmentation method in Ref. [2].

other washing activities), and then the aquatic ecosystem, where they may potentially bioconcentrate and biomagnify in the tissues of aquatic organisms.

Previously, analysis of musk compounds required that the samples be collected, transported, and preserved before laboratory analysis. Preservatives and contaminants contained in sampling jars are themselves potential sources of analyte interferences [6]. Described in this paper is a simple, rugged, highly sensitive, and reproducible analytical method, developed at the US Environmental Protection Agency (EPA) laboratory in Las Vegas, NV, USA, for quantifying synthetic musk concentrations in water (e.g., effluents from and receiving waters for sewage treatment plants). Such a method would be necessary for assessing human and ecological risks, and for providing data to evaluate and monitor the efficacy of any remedial measures designed to lower the amounts of these compounds that ultimately reach aquatic ecosystems or human drinking water supplies. The method reported here, uses a widely available cartridge containing a mixed polymeric sorbent coupled to a battery-operated peristaltic pump, backed-up by a 1000-W portable generator, an intake tube, and a 5-μm pore-size particulate prefilter to extract synthetic musks from 60-l water samples on-site. Quantification is accomplished by gas chromatographic separation with electron impact ionization mass spectrometric detection (GC–EI-MS) using the positive ionization full-scan mode.

2. Experimental

2.1. Chemicals and materials

The surrogate standards, pentachloronitrobenzene (99.9%) and 2,2′-dinitrobiphenyl (99.9%), and the internal standard, [$^2H_8$]naphthalene (99.9%), were purchased from Absolute Standard (Hamden, CT, USA). All synthetic musks 99% purity (see structures in Figs. 1 and 2) were purchased from Promochem (Wesel, Germany). Musk ketone, musk ambrette, and musk xylene were provided by the Institute of Food Chemistry, University of Hohenheim (Stuttgart, Germany). Musk Versalide (acetyl ethyl tetramethyl tetralin, AETT), was provided by Dr. Peter Spencer of the Oregon Health Sciences University, Portland, OR, USA.

The organic solvents used were 99.9% *n*-hexane and toluene (B&J GC2 grade, Burdick and Jackson, Muskegon, MI, USA). Methylene chloride, ethyl acetate, diethyl ether, light petroleum, acetone, and methanol (all HPLC grade) were purchased from Aldrich (Milwaukee, WI, USA). Hydrazine hydrate and Raney nickel (slurry) were purchased from Aldrich. Anhydrous, granular sodium sulfate (Tracepur) was obtained from EM Science (Gibbstown, NJ, USA) and dimethyldichlorosilane (DMDCS) treated glass wool was purchased from

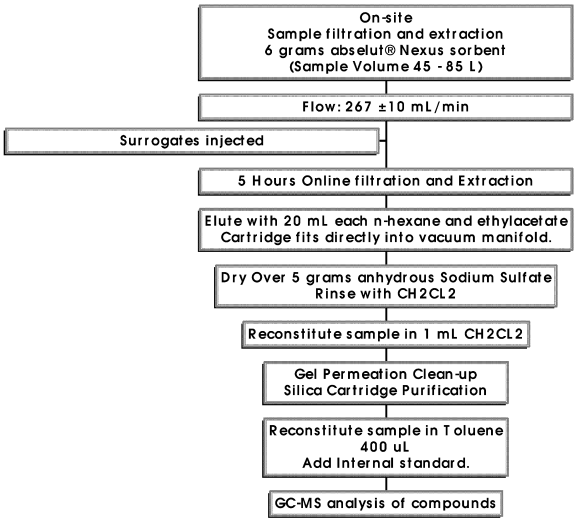


Fig. 2. Schematic representation of on-site solid-phase extraction procedure with Abselut NEXUS sorbent.

Alltech Associates (Arlington Heights, IL, USA). Silica cartridges (2 g each), and polystyrene cross-linked with 50% divinylbenzene and poly(methyl methacrylate) in a polypropylene cartridge (Abselut NEXUS) were provided by Varian (Harbor City, CA, USA). DMDCS (5%) in toluene was obtained from Supelco (Bellefonte, PA, USA) and Amberlite XAD-2 or divinylbenzene styrene copolymer was pur-

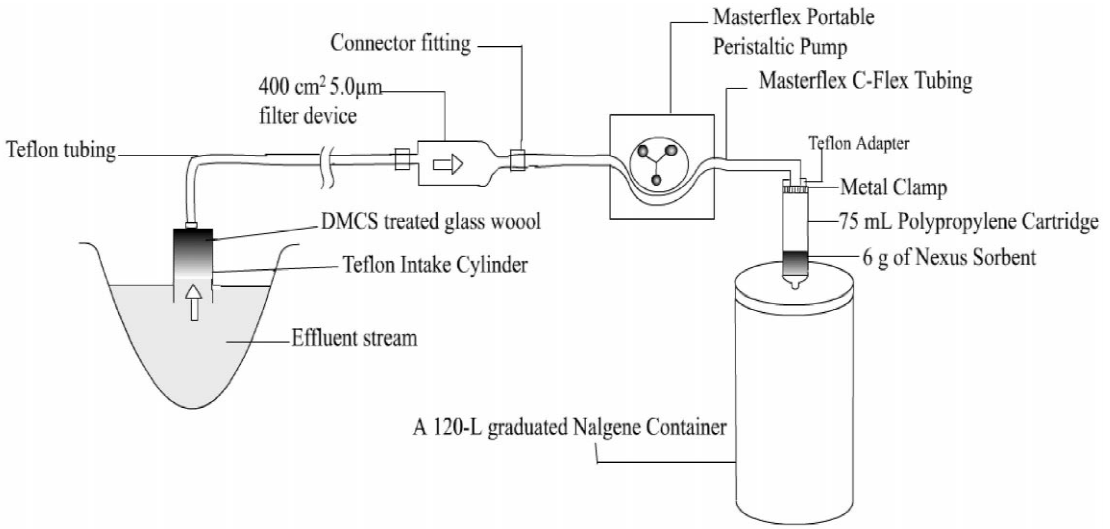


Fig. 1. On-site solid-phase extraction assembly.

chased from Alltech Associates. A Barnstead Nanopure water system (Barnstead/Thermolyne, Dubuque, IA, USA) provided deionized (DI) water with a resistivity of 17.5 Mega Ω cm. Isoclean concentrate, for glassware cleaning, was purchased from Isolab (Akron, OH, USA).

2.2. Filters, pumps, and ancillary supplies

Disposable filter capsules for on-line filtering, were purchased from Whatman (Clifton, NJ, USA). A Masterflex portable sampling pump and medical grade silicone tubing for sampling, were purchased from Cole-Parmer (Vernon Hills, IL, USA). A Turbo-Vap II for concentrating samples was purchased from Zymark (Hopkinton, MA, USA). Gel permeation chromatography (GPC) columns for sample cleanup were purchased from Waters (Milford, MA, USA). A Phenogel 10-μm Linear/Mixed guard column 50×7.8 mm for protecting the expensive GPC columns, was purchased from Phenomenex (Torrance, CA, USA). Nuphase C_{18} fiber solid-phase extraction (SPE) disks (47-mm diameter) for extracting samples were purchased from CPI International (Santa Rosa, CA, USA). A 900 VA Honda electric generator (model EU 1000i) was purchased from American Honda Motor (Apharetta, GA, USA).

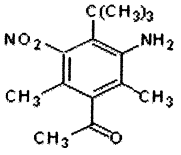
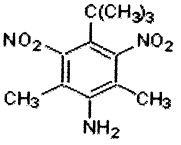
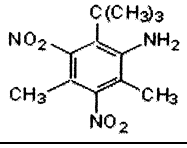
2.3. Synthesis of nitro musk metabolites

Musk ketone and musk xylene metabolites (see Table 3) were synthesized as previously reported by using hydrazine hydrate as a reducing agent in the presence of a small amount of Raney nickel slurry [2]. Purification and separation of 4-amino musk xylene, 2-amino musk xylene, and 2-amino musk ketone from their respective reaction mixtures was done by eluting preparatory thin-layer chromatography plates with a mixture of *n*-hexane and ethyl acetate (1:1, v/v) as described by Zhao and Schwack [10].

2.4. Sample filtration-extraction

Both polar and non-polar organic compounds were extracted from aqueous samples with Varian Abselut NEXUS sorbent [made of a proprietary 1:1 mixture of ultra-pure polystyrene (highly cross-linked with 50% divinylbenzene) and poly(methyl methacrylate) having a surface area of approximately 600 m^2/g] packed between two 20-μm pore size high-purity polyethylene frits contained in a 75-ml ultra-pure medical-grade, polypropylene cartridge. The cartridge was used as received from Varian. Pre-conditioning (''activation'') is not needed. It was connected with PTFE tubing to a 30-cm length of

Table 3
Trade and CAS names, structures, log K_{ow}, molecular masses, formulae and Registry Numbers, for three nitro musk metabolites

Trade and CAS name (acronym)	Chemical structure	Log K_{ow}	M_r	Molecular formula	CAS no.
Amino musk ketone: 2-amino-1-*tert.*-butyl-3,5-dimethyl-6-nitro-4-acetylbenzene (2-AMK)		5.1[a]	264.32	$C_{14}H_{20}N_2O_3$	–
4-Amino musk xylene: 4-amino-1-*tert.*butyl-3,5-dimethyl-2,6-dinitrobenzene (4-AMX)		4.8[a]	267.28	$C_{12}H_{17}N_3O_4$	107342-55-2
2-Amino musk xylene: 2-amino-1-*tert.*-butyl-3,5-dimethyl-4,6-dinitrobenzene (2-AMX)		4.8[b]	267.28	$C_{12}H_{17}N_3O_4$	107342-67-6

[a] Estimated values derived by Hansch and Leo fragmentation method in Ref. [5].

Masterflex medical grade silicone tubing, which passed through a Masterflex sampling pump such that the aqueous sample stream did not make contact with the pump components. Initially, 60 l of sewage effluent were sampled from a dedicated effluent receiving stream (one that receives only sewage effluent and runoff), approximately100 m from a sewage treatment plant's discharge pipe. The 60-l sample was siphoned through a 400-cm^2, 5-μm pore-size, disposable filter device after passing through a PTFE intake cylinder that contained approximately 10 g of DMDCS-treated glass wool. The filtered effluent was peristaltically pumped through the sorbent bed. A diagram of this equipment is shown in Fig. 1. The Masterflex sampling pump was calibrated in the laboratory, and had an average flow-rate under sampling conditions of 267±10 ml/min, which corresponded to approximately 27 bed volumes per minute [11] through a sorbent volume of 10 ml. This high flow-rate was possible because of the large sorbent particle size (65–80 μm average particle diameter) and the average pore size of 10.4 nm in the sorbent bed. Prior to sampling, the PTFE tubing and intake cylinders were flushed overnight with warm tap water and fragrance-free Isoclean soap, then rinsed sequentially with DI water, acetone, methanol and DI water. A new disposable filter capsule was used for each extraction. To prepare a laboratory blank, 60-l of Nanopure water were pumped through the system to simulate on-site sampling. From a surrogate standards mixture, containing 200 μg/ml each of pentachloronitrobenzene [12] and 2,2′-dinitrobiphenyl [13] in methanol, 10 μl were injected into the tubing, just before the 5.0-μm filter, while the pump was operating. This is analogous to spiking surrogates into a 2-l separatory funnel in liquid–liquid extraction techniques. Normally, a surrogate is only added to the entire sample volume prior to extraction. Under this extraction condition, such method of surrogate addition is a limitation. The laboratory blank cartridge was wrapped in methylene chloride-rinsed aluminum foil and transported to the sampling site. The laboratory blank cartridge was later extracted along with the corresponding samples as a laboratory blank. During sampling, the 10-μl surrogate standards mixture was also injected into the tubing in similar fashion. After extraction, the sampling cartridge was detached, wrapped in methylene chloride-rinsed aluminum foil and transported in an ice cooler back to the laboratory for immediate desorption of the adsorbed analytes.

2.5. Solid phase desorption

The first step in desorption was removal of the moisture from the cartridge. Most of the moisture was removed by carbon-filtered air, using a 12-position vacuum manifold (Supelco) to draw the filtered air through the batch of sample cartridges. After drying for about 2 min, the analytes from each sample cartridge were eluted with successive portions of 20 ml of *n*-hexane and 20 ml of ethyl acetate and dried over a column of anhydrous sodium sulfate. The 40-ml eluent was solvent exchanged to methylene chloride and concentrated to 1 ml using a Turbo Vap II solvent evaporator at 30°C under a gentle stream of nitrogen.

2.6. Sample clean-up: GPC

Due to the high volume of water extracted, and to minimize the contamination of the GC–MS system from unexpectedly high concentrations of organic compounds (lipid-like material) that may have been coextracted with the analytes, it was necessary to further clean the sample. Gel permeation chromatography used a Waters GPC system equipped with a 515 HPLC pump, a 717 plus autosampler, a 2487 dual UV detector, and a fraction collector II to remove interferences from the cartridge extracts. The GPC system was fitted with two Envirogel columns in series (300×19 mm and 150×19 mm), preceded by a Phenogel 10-μm Linear/Mixed, 50×7.8-mm guard column. Prior to sample clean-up, the columns were conditioned with 2 l of methylene chloride. To establish collection windows, the instrument was calibrated with a solution containing 1 μg/ml of each of five nitro musks, seven polycyclic musks, three nitro musk metabolites, and two surrogate standards (Table 4). Methylene chloride was used as the eluting solvent (as specified by the column manufacturer), with a flow-rate of 5 ml/min. All 17 compounds eluted from the columns between 12 and 20 min, in a 40-ml volume. This fraction was evaporated to about 400 μl, and reconstituted in 1 ml

Table 4
Characteristic ions used for identification and measurement of musk compounds

Compound	Retention time (min)	Primary ion	Secondary ion (s)
[$^2H_8$]Naphthalene (I.S.)	4.33	136	135, 137, 108
Cashmeran	8.01	191	192, 135, 206
Celestolide	10.46	229	244, 173, 230
Phantolide	10.91	229	244, 187, 230
Pentachloronitorbenzene (surr.)	11.06	237	295, 214, 265
Versalide	11.45	243	244, 258, 259
Musk ambrette	11.69	253	268, 254, 251
Traseolide	11.84	215	216, 173
Galaxolide	11.89	243	258, 213, 244
Tonalide	12.00	243	258, 244, 201
Musk xylene	11.99	282	297, 283
Musk moskene	12.23	263	278, 264, 221
MuskTibetene	12.76	251	266, 252, 115
Musk ketone	13.17	279	294, 128, 280
2,2′-Dinitrobiphenyl (surr.)	13.95	198	168, 139, 115
Amino musk ketone	14.36	264	249, 215, 191
2-Amino musk xylene	14.51	267	252, 218, 160
4-Amino musk xylene	14.92	252	267, 218, 235

I.S., internal standard; Surr., surrogate.

n-hexane for silica gel clean-up if necessary. A preliminary GC–MS run is then performed on a case-by-case basis, to determine if further clean-up is needed. Most of our samples did not need the silica gel clean-up step.

2.7. Silica gel clean-up

For the secondary clean-up procedure, 2 g of silica were placed in each cartridge (Varian Bond-Elut). Each silica cartridge was opened and mounted in a Supelco 12-position vacuum manifold and conditioned sequentially with 5 ml of 2,2,4-trimethylpentane (''isooctane''), 5 ml methylene chloride, and 5 ml *n*-hexane. The approximate 1-ml reconstituted *n*-hexane sample was quantitatively transferred (using *n*-hexane) into each conditioned silica cartridge and eluted sequentially with 8 ml *n*-hexane–ethyl acetate (4:1, v/v), 8 ml dichloromethane, and 8 ml ethyl acetate. Analytes were concentrated as described in Section 2.5, and reconstituted in 400 μl toluene, after which 10 μl of 200 μg/ml [$^2H_8$]naphthalene [14], the internal standard, was added.

2.8. Laboratory contamination with musk compounds

Laundry detergents, dishwashing soap, towels, and other household products may contain musk compounds, and are potential sources of contamination [15]. To avoid contamination of samples through glassware handling, fragrance-free soap (e.g., Isoclean) and nitrile powderless hand gloves were used during extractions and sample analyses. To check equipment for musk contamination, the sampling equipment was purged with 40 l of tap water followed by 10 l of DI water. Subsequently, 60 l of DI water were extracted in the laboratory with 6 g of sorbent within a cartridge (Abselut NEXUS), and the cartridge was taken to the field in an ice cooler. This served as the field blank (Fig. 3). To check for glassware contamination during sample handling in the laboratory, a glassware blank was prepared by adding 10 μl of the surrogate standard to 1 ml *n*-hexane (the same solvent used for reconstituting samples), and processed through the clean-up procedure as described above.

Medical-grade silicone rubber in peristaltic pumps avoids sample contamination by the organic perox-

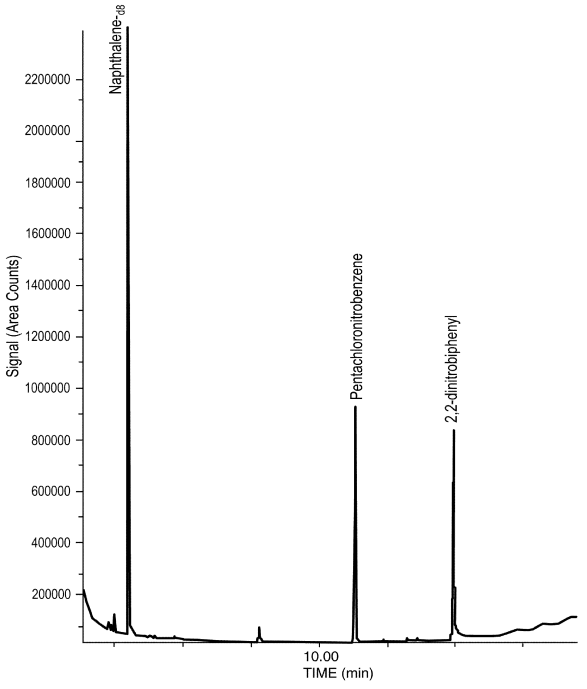


Fig. 3. Total ion chromatogram of field blank.

ides used in the manufacturing of conventional grades of silicone rubber [16]. Short lengths of medical grade silicone rubber at the tube compression in a peristaltic pump reportedly do not alter or contaminate samples [17]. With this in mind, a 30-cm length of Masterflex medical grade silicone tubing was used with the Masterflex peristaltic pump; all of the remaining tubing was PTFE.

2.9. GC–EI-MS quantification using the full-scan mode

Analytes were quantified relative to [$^2H_8$]naphthalene, using positive ionization full-scan mode GC–EI-MS. Data were acquired with an HP 6890 gas chromatograph equipped with an HP 7683 series automatic sampler coupled to an HP 5973 mass spectrometer (Agilent Technologies, San Jose, CA, USA). Baseline separation between most of the compounds was achieved on a 30 m×0.25 mm I.D., 0.25-μm film thickness, HP-5 MS capillary column (Agilent Technologies) using the following temperature program: 90°C, 0 min hold, 10°C/min to 300°C, and 5 min hold with helium carrier gas at a linear velocity of 37 cm/s measured at 90°C. Injections of 2 μl were made in the splitless mode using a splitless time of 1 min and an injection port temperature of 250°C. The transfer line from the GC system to the MS system was maintained at 300°C, the quadrupole within the MS at 150°C, and the ion source at 230°C. The molecular mass range was 35 to 400, energy 70 eV, and 3.94 scan/s were recorded.

Based on a preliminary analysis of the samples, a five-level calibration standard mixture was prepared at concentrations of 0.5, 1.0, 2.0, 4.0, and 6.0 ng/μl of musk compounds in toluene or *n*-hexane, the same solvent used to reconstitute the analytes.

Quantitation was performed by the data system software (HP-Chem acquisition software) using the primary ions indicated in Table 4. The EPA internal standard method 625 for semi-volatile quantitative analysis was used to calculate the concentrations of each identified analyte in the sample [18]. All compounds found were unambiguously identified through comparison of their mass spectra with those in the National Institute of Standards and Technology (NIST) library as well as with mass spectra and GC retention times of authentic standards. The primary characteristic mass spectral m/z peaks and GC retention times produced from laboratory prepared standards were used to quantify the musk compounds.

3. Results and discussion

3.1. Recovery experiment

To determine analyte recoveries prior to environmental sampling, three 60-l volumes of DI water were spiked with 20-, 40- and 60-μl portions of a solution containing 20 μg/ml of each musk compound and three musk metabolites. The spiked water was pumped through a 70-ml disposable polypropylene cartridge that contained 6 g of Abselut NEXUS sorbent at a flow-rate of 267±10 ml/min. Following the extraction scheme in Fig. 2 to GPC step, the final volume of the extract was 400 μl.

The three spiking levels provided the average recoveries presented in Table 5. Initially, Amberlite XAD-2 or divinylbenzene–styrene copolymer was

Table 5
Percent spike recovery data from 60-l sample[a] ($n=3$)

Analytes	Nanopure water (% RSD)	STP effluent (% RSD)	MDL (ng/l)
Musk xylene	102 (4)	97 (6)	0.02
Musk ketone	98 (3)	95 (5)	0.20
Musk ambrette	101 (5)	96 (7)	0.30
Musk moskene	96 (4)	92 (4)	0.03
Musk tibetene	98 (2)	95 (7)	0.02
Versalide (AETT)	99 (5)	96 (6)	0.02
Galaxolide (HHCB)	99 (3)	97 (5)	0.02
Phantolide (AHDI)	97 (3)	91 (5)	0.02
Cashmeran (DPMI)	99 (4)	94 (6)	0.02
Celestolide (ADBI)	98 (3)	96 (8)	0.02
Traseolide (ATII)	95 (5)	90 (8)	0.02
Tonalide (AHTN)	107 (3)	94 (6)	0.02
4-Amino musk xylene	87 (5)	80 (10)	0.30
2-Amino musk xylene	89 (7)	82 (11)	0.25
Amino musk ketone	90 (6)	92 (8)	0.25

[a] Recovery data from extraction through GPC steps.

used as the sorbent material but the requirement of large solvent volumes of 2–3 l for sorbent cleaning made its use impractical. Nuphase 47-mm, C_{18} fiber SPE disks were then used to extract these compounds from 1 l of similarly spiked DI water but the extraction recoveries of only 33–40% for the analytes were unacceptable. The addition of isopropanol in approximately 1:7 (v/v) to the samples [19] improved the recoveries of the analytes to the 67–75% range. This was still not acceptable for three reasons: (1) approximately 150 ml of isopropanol would be required per liter of sample, thus complicating field sampling; (2) isopropanol is a known environmental pollutant; and (3) analyte recoveries were still lower than desired. Polystyrene–divinylbenzene (PS–SDVB) provided good recoveries for non-polar analytes (some nitro and polycyclic musks), but not for more polar compounds (musk ketone and some musk metabolites).

Six grams of a proprietary 1:1 mixture of poly(methyl methacrylate) and polystyrene cross-linked with divinylbenzene (Varian Abselut NEXUS) provided average recoveries of 95–107% for all non-polar analytes and 87–90% for polar analytes without corrections for surrogate recoveries. To achieve such recoveries on-site, it was important to slowly inject very low volumes of the solvent that contained the surrogate and spike standards into the silicone pump tubing. Otherwise the solvent would cause premature elution of the analytes from the SPE particles. Recently, a similar (but in-laboratory) study was conducted by Aistars et al., who compared the use of 10-mg of Abselut NEXUS, LMS, C_{18}, C_{18}HF, C_8, C_2, and CN-E sorbents for solid-phase extraction of naproxen (a human drug) in a 96-well plate format. Abselut NEXUS provided higher recoveries with good cleanup and reproducibility [20].

We believe that our investigation reported here is the first to use Abselut NEXUS for on-site sampling of environmental surface waters, as well as the first to report the application of Abselut NEXUS for trace analysis of musks. Many studies have been conducted in the laboratory with samples collected from the field, preserved and pre-filtered before extraction [2]. One drawback to such an approach is the possible introduction of artifacts during sample collection, preservation, handling, storage, or transport to the laboratory. Water samples are in a chemically dynamic state, and once they are removed from the sample site, chemical, biological, and physical processes can change their compositions [21]. Analyte concentrations can be altered by volatilization, sorption to glass surfaces, diffusion, precipitation, hydrolysis, oxidation, photochemical, and microbiological effects. At best, preservatives and refrigeration will minimize the effects of biological and thermal degradation. Data obtained from such samples may not be reliable.

3.2. Minimum detection limits

Limit of detection (LOD) can be defined as the lowest concentration determined to differ statistically from a blank [22]. The method detection limits for these analytes were estimated for a signal-to-noise ratio of 3 to 1 ($S/N=3$) as described by Kaiser [23] and Deming and Morgan [24]. In this method, LOD ranged from 0.02 to 0.30 ng/l. This is consistent with MDL estimates by Rees and Au in their pesticides experiment with XAD-2 macrorecticular resin [25].

3.3. Method optimization

After extensive laboratory testing and optimization, our method was found to be robust for the fragrance compounds listed in Tables 1–3. The

presence of other organic compounds recognized during preliminary analyses of the extracts suggests that this method should be applicable to other compounds; but laboratory calibration of the pump, and determination of recoveries for each analyte of interest would be necessary.

The volume of aqueous sample that could be extracted with acceptable recoveries of all compounds in Table 5 was determined by measuring the breakthrough volumes, which reflect the analyte-retaining ability of the sorbent. Different volumes (45, 60, 85, and 100 l) of pH 7.5 DI water were each spiked with 50 μl of a solution containing 20 μg/ml each of the 15 musk compounds. The corresponding concentrations in each container were 22.2, 16.7, 11.8, and 10 ng/l, respectively. A 500-mg sorbent cartridge (Abselut NEXUS) was connected in series with a cartridge containing 6 g of Abselut NEXUS sorbent such that the eluent from the larger cartridge discharged into the smaller cartridge. After extraction of a water volume with both cartridges connected in series, the analytes retained on each of the two sorbent beds were eluted as described above. No breakthrough was observed for analytes in spiked volumes up to 85 l. Therefore, 60- and 85-l volumes were considered optimum. The choice of a 60-l sample volume was made to minimize the time spent in the field. Breakthrough of polar compounds occurred for the 100-l volume. The 500-mg Abselut NEXUS cartridge was found to retain (after breakthrough) a concentration equivalent to 0.15 μg/ml, corresponding to 15% of the more water-soluble, amino metabolites that had been present. With more than 6 g of sorbent material, acceptable recoveries could be realized for greater volumes.

The internal standard method was used for quantitation on GC–MS because the surrogate standard corrected for losses during subsequent sample separation and concentration steps, and the internal standard provided a known amount of standard by which to measure the compound of interest. To ensure accurate work, the concentrations of the analyte standards used for calibration were set to bracket the concentration of the analytes of interest in the samples.

From Table 6, the concentrations of polycyclic musks and nitro musks in treated multiple sewage effluents ranged from 0.3 to 152 and 0.5 to 27.5 ng/l, respectively. Those of the nitro musk metabolites ranged from 0.9 to 31.5 ng/l. The results also revealed temporal and spatial variations. The 85- and 65-l[a] samples (Table 6) were taken on the same day and the same sewage stream in southwestern USA, but a few yards apart. The resulting concentrations of traseolide showed considerable variation. The 45- and 85-l[b] samples (Table 6) were taken 2 weeks

Table 6
Concentrations (ng/l) of synthetic musk compounds, and nitro musk metabolites in STP effluent stream

Analytes	85 l[a]	65 l[a]	85 l[b]	45 l[b]	60 l[c] (% RSD)
Musk xylene	1.3	<MDL	<MDL	0.5	<MDL
Musk ketone	27.5	21.5	23.4	21.3	<MDL
Musk ambrette	<MDL	<MDL	<MDL	<MDL	<MDL
Musk moskene	<MDL	<MDL	<MDL	<MDL	<MDL
Musk tibetene	<MDL	<MDL	<MDL	<MDL	<MDL
Versalide	<MDL	<MDL	<MDL	<MDL	<MDL
Galaxolide	138	111	152	35.0	40.8 (1.8)
Phantolide	4.3	3.1	5.0	2.5	2.4 (4.3)
Cashmeran	<MDL	<MDL	<MDL	<MDL	<MDL
Celestolide	2.1	0.3	0.3	0.5	1.4 (7.2)
Traseolide	83.8	34.5	126	6.6	<MDL
Tonalide	67.3	47.1	92.2	26.6	36.8 (2.5)
4-Amino musk xylene	1.4	11.6	<MDL	31.5	<MDL
2-Amino musk xylene	<MDL	<MDL	0.9	<MDL	<MDL
Amino musk ketone	<MDL	<MDL	<MDL	<MDL	<MDL

[a] Effluent sample downstream from a tertiary sewage treatment plant's discharge pipe ($n=1$).
[b] Effluent sample taken 14 days later from same location ($n=1$).
[c] Effluent sample near a different tertiary sewage treatment plant's discharge pipe ($n=3$).

later, at the same location but a few yards apart. Concentration variations were found for most of the compounds. The three 60-l[c] samples were taken from a different sewage stream, approximately 100 yards from a tertiary sewage treatment plant discharge pipe (1 yard = 0.914 m). The absence of AETT and musk ambrette concentrations in Table 6 probably does not reflect an inability to detect them, but rather their absence from commercial fragrance materials due to their being banned in 1980 and 1995, respectively [8,10,26]. Musk tibetene and musk moskene are hardly used in fragrances and are produced in low quantities. Their absence (Table 6) was therefore not unexpected. Sovocool and Osemwengie (G.W. Sovocool, L. Osemwengie, unpublished results) have identified 2,4-di-*tert*.-butylphenol, which elutes in the same retention window as Cashmeran (1,2,3,5,6,7-hexahydro-1,1,2,3,3-pentamethyl-4H-inden-4-one). Identification of musk compounds without the use of a mass spectrometric detector, could potentially lead to the misassignment of Cashmeran instead of 2,4-di-*tert*.-butylphenol.

A possible limitation to the method is the range of suitable matrices. We have evaluated it only for waters relatively low in suspended solids, such as tertiary treatment plant sewage effluent and pristine lakes and rivers; it should also prove useful for groundwater. During subsequent extraction in areas where several treated municipal sewage effluents were discharged into a lake, the presence of an algal bloom clogged the on-line filtration system, and the extraction system performed poorly; only 25 l could be extracted over 6 h. The limitations posed by suspended solids could possibly be overcome by the use of a tangential flow pre-filter.

4. Summary and conclusion

On-site SPE with a mixed sorbent [1:1 poly-(methyl methacrylate) and polystyrene cross-linked with 50% divinylbenzene] provided major sampling and analytical advantages. The sorbent cartridges were compact, easily transported, and required minimal refrigerated storage space. Retention of adequate amounts of ultra-trace levels of synthetic musk analytes from large (e.g., 60-l) aqueous samples and the clean up procedures employed, permitted the use of full-scanning GC–MS rather than selected ion monitoring. Recoveries from extraction and GPC steps ranged from 80 to 97% in spiked sewage treatment plant effluent, and 87 to 107% in spiked DI water. The sorbent does not require the methanolic pre-treatment step normally required for reversed-phase materials. Cartridge preparation and extraction time, labor, and solvent use were reduced. On-site SPE eliminates collection, transport, and storage of large volumes of water samples. Sample vulnerability to cross-contamination from laboratory vapors during extraction is minimized. The temporal variation associated with grab sampling of small water volumes is lessened. Furthermore, large volumes of potentially bio-hazardous aqueous samples (such as treated sewage) may be extracted with less health risk.

We believe this is the first report of Abselut NEXUS sorbent used to extract large volumes of environmental surface water using an on-site sampling device. On-site SPE of large aqueous samples provides a simple, rugged, highly sensitive, reproducible, and less expensive analytical approach for concentrating synthetic musks from treated waste streams or water sources, and has potential for extraction of other micropollutants.

Acknowledgements

The authors acknowledge Dr. Christian G. Daughton for initiating this research on PPCPs and for many helpful discussions with one of the authors (L.I.O). The help of Dr. Jerome Imonigie and Mr. Patrick Ferguson is gratefully acknowledged for assisting in the sampling aspects of this research. Appreciation is extended to Dr. Peter Spencer for the gift of the standard 7-acetyl-6-ethyl-1,1,4,4-tetramethyl-1,2,3,4-tetrahydronaphthalene (AETT), and to Varian, Inc., for providing material and information. Our appreciation is also extended to Dr. Wayne Sovocool, Dr. Shawn Gerstenberger, and Christian Daughton for their helpful and careful reviews of the drafts of the manuscript.

Notice: The US Environmental Protection Agency (EPA), through its Office of Research and Development (ORD), funded and performed the research

described. This manuscript has been subjected to the EPA's peer and administrative review and has been approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation by the EPA.

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