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Fast isolation of hydrophobic organic environmental contaminants from exposed semipermeable membrane devices (SPMDs) prior to GC analysis

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Abstract

Semipermeable membrane devices (SPMD) represent a passive sampling technology that is becoming widely used for monitoring of surface waters pollution. While "classic" procedures employ dialysis to recover target compounds from exposed SPMDs, in the present study analytes were isolated from cut membrane together with sequestering medium (triolein) using hexane as an extraction solvent. This approach allowed us to reduce the time needed for accomplishment of isolation step from 48 h to only 1 h. Automated gel permeation chromatography (GPC) clean-up is employed in the following step to separate triolein from analytes fraction. Musk compounds (MCs), polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs) and several other persistent organochlorine compounds (OCs) were determined in the respective fraction by GC method employing selective detectors (MSD, ECD). As shown in a series of analyses of SPMDs deployed in various aquatic ecosystems, high recoveries and good repeatability of results together with a possibility to obtain the information on the pollution of sampling site at the day of sample arrival to laboratory make this newly implemented procedure an interesting alternative to time consuming dialysis.

Keywords: Semipermeable membrane devices; SPMDs; Sampling; Aquatic ecosystem; Musk compounds; Polychlorinated biphenyls; Brominated flame retardants; Polybrominated diphenyl ethers; Hexabromocyclododecane; Organochlorine pesticides; Octachlorostyrene

1. Introduction

Monitoring of aquatic ecosystem pollution represents one of the major activities involved in measures aimed at environment protection. The choice of a relevant sampling strategy depends on information to be obtained in a particular case. Active sampling methods based on a one-shot sample collection reflect only the situation at the moment of sampling hence episodic short-time pollutions might remain unregistered. Utilization of a long-term passive sampling technique is a preferred approach whenever information on typical pollution levels in respective sampling site is of an interest.

In general, passive sampling techniques can employ two approaches. Adsorption process involves the transfer and the accumulation of analyte molecules at a phase interface resulting in their equilibrium distribution between the liquid phase and the adsorbent [1]. The liquid phase/adsorbent partition coefficients depend mainly on the temperature during adsorption process, thickness and porosity of the adsorbent and stereochemical character of the analyte [2]. Alternatively, phenomena being under way can be characterized as absorption: the analyte is not only adsorbed onto the surface but it also penetrates through the wall of a natural or synthetic membrane [1]. Under these conditions selective sampling due to discriminative penetration of molecules differing in physico-chemical properties can take a place. SPMD, passive absorption sampling technology employing the above

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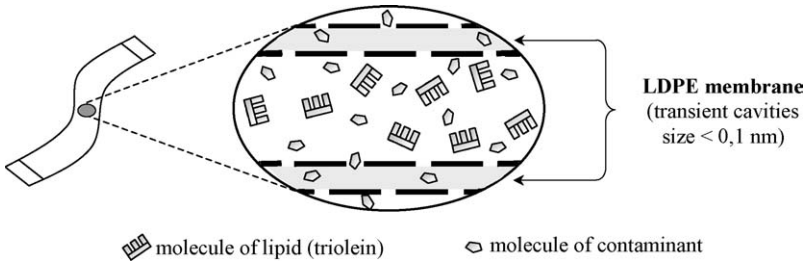

Fig. 1. Detailed scheme of contaminant molecules penetration into the SPMD.

principles, was developed and patented at the Columbia Environmental Research Centre (CERC, USA) by Huckins et al. [3–6] in early nineties. SPMD was shown to be suitable for in situ monitoring of a wide range of semi- and non-polar organic compounds that may be present in various environmental compartments.

Fish and/or other common aquatic biota that were traditionally employed in many monitoring studies as bioindicators of occurrence of persistent organic pollutants (POPs) [7,8] have been replaced in recent years successfully by SPMDs. Some authors classify this technique as a "virtual fish" since when deployed in aquatic ecosystem, SPMD simulates the main mechanism of hydrophobic contaminants bioconcentration, i.e. passive diffusion of bioavailable substances through biomembranes into the aquatic organism (this occurs in fish mostly through the branchial epithel) [9]. Using SPMD more realistic contamination pattern of the respective locality is obtained since the sampling mechanism is independent on stress and other factors affecting fish during exposure (age, sex, momentary conditions, water quality etc.) under field conditions. In addition, changes caused by metabolisation of chemicals and/or their excretion due to spawning are absent. As regards SPMDs use, device is placed into the sampling medium for a period ranging from a few days to several weeks, depending on the type of target analyte (*integrative* or *equilibrium* approach). In this way, the extent of pollution is much better reflected as compared to grab sampling due to the time weighted average (TWA) concept. Thanks to the accumulation of analytes during the exposure period, even very low pollution levels can be determined. Generally, passive sampling techniques such as SPMD may also play an important role in confirmation of results acquired by other sampling methods or may provide data completing of existing information.

SPMD (in field applications it is placed into protective stainless steel cage) typically consists of a non-porous, additive-free low-density polyethylene (LDPE) membrane filled with sequestrant [9]. The thin film of sequestrant sealed inside the membrane is typically a synthetic neutral lipid of high purity, mostly triolein (1,2,3-tri-[*cis*-9-octadecenoyl]glycerol) that is a major neutral triacyl glycerol found in fat tissue of many organisms [9]. There are no fixed pores in the membrane, only transient cavities, the size of which is similar to those occurring in biomembranes, i.e. ranges up to 10 Å (1 nm), while the size limit for gill membranes of fish is typically 9.8 Å [9]. Most of environmental contaminant molecules are small enough (molecular weights <600) to be able to penetrate into the membrane while the release of large molecules of triolein to the external environment is not possible (shown in Fig. 1). It should be noted that SPMDs trap only dissolved non-ionic compounds because charged particles are rather hydrophilic and essentially insoluble in non-polar LDPE [9].

Extraction of compounds sequestered inside the membrane is commonly realised by dialysis utilizing organic solvent as the receiving phase. Hexane [5,10–17], cyclohexane [18–21] or cyclopentane [4,21,22] are the extraction solvents used in most of applications. It should be noted that isolation of target analytes employing dialysis is very time-consuming (usually takes 48 h) and the consumption of extraction solvents is relatively high. In addition, such a long extraction procedure realized at the laboratory temperature may lead under certain circumstances to losses of some analytes due to their (photo)degradation (e.g. for polycyclic aromatic hydrocarbons [23]), and/or volatilisation. Intralaboratory contamination is another problem that might be encountered when realizing dialysis for a long time period in practically open system (only aluminium foil is usually used for covering of the dialysis column). Microwave-assisted extraction of intact (exposed) mebranes in closed PTFE cartridge was described recently [24] as an alternative to dialysis.

Prior to quantitative analysis, purification and fractionation of concentrated dialysate is typically carried out to remove interferences, which may originate either from membranes (release of polyethylene oligomers) or occasionally from triolein (trace impurities such as oleic acid and methyl oleate are often present in commercial chemicals) and interfere with chromatography of target analytes [9,12]. The overview of reported strategies employed for isolation and identification/quantification of compounds sequestered in SPMDs from the external aquatic environment is shown in Table 1.

The potential of lipid-containing membranes to concentrate trace amounts of a wide range both persistent and biodegradable compounds has been confirmed for various groups of organic pollutants such as: polychlorinated biphenyls (PCBs) [4,9,12,13,22,25,26], polychlorinated dibenzodioxins/dibenzofurans (PCDD/Fs) [4,9,13], polycyclic

Table 1
Overview of strategies used for analysis of various pollutants in exposed SPMDs

Target compounds	Sampling media	Isolation of analytes	Clean-up	Identification and quantification	Ref.
PCBs	Indoor air (National Fisheries Contaminant Research Center, USA)	Dialysis for 48 h, 250 ml of hexane	(1) GPC (Bio-Beads® S-X3, 100 ml of hexane:dichlormethane, 80:20, v/v), (2) adsorption fractionation (Florisil, 20 ml of hexane)	GC/ECD, DB-5 column (30 m × 0.25 mm × 0.25 μm)	[11]
OCPs	Freshwater (The Mississippi River, USA)	Dialysis with 250 ml of hexane (dialysis time not specified)	GPC (15 g of Bio-Beads® S-X3, 150 ml 20% methylene chloride/hexane, v/v)	GC/MSD, Rtx-5 column (30 m × 0.25 mm × 0,25 μm)	[15]
PCBs	Air (meteorological field station, Lancaster, UK)	Dialysis for 2 × 24 h, 2 × 130 ml of hexane	(1) Adsorption fractionation (silica gel, dichlormethane), (2) size exclusion chromatography (HPLC/DAD with Phenomenex Phenogel column, dichlormethane), (3) adsorption fractionation (silica gel, hexane)	GC/MSD, CP-Sil 8 column (50 m × 0.18 mm)	[17]
Nitroaromatics	Water in laboratory exposure chamber (Beijing, China)	Dialysis for 24 h (13 °C), 40 ml of cyclohexane	Filtration through anhydrous Na_2SO_4	GC/ECD, OV-17 column (2.1 m × 3.2 mm)	[18]
OCPs	Water in laboratory exposure chamber (Beijing, China)	Dialysis for 48 h (20 °C), 50 ml of cyclohexane	–	GC/ECD, HP-5 column (30 m × 0.32 mm × 0.18 μm)	[19]
PAHs	Urban air (Southern Italy)	Dialysis for 48 h (13 °C), 100 ml of cyclohexane	Filtration (nylon filter, 0.22 μm)	GC/FID, ZB-5 column (30 m × 0.25 mm × 0.25 μm)	[20]
PCBs, OCPs	Indoor household composts (Umeå, Sweden)	Dialysis for 2 h × 24 h, 2 ml × 150 ml of cyclohexane	(1) Filtration through anhydrous Na_2SO_4, (2) GPC (two tandem columns with PL gel, dichlormethane:hexane, 35:65, v/v)	GC/MSD, PTE-5 column (60 m × 0.32 mm × 0.25 μm)	[21]
PCBs, HCB	Air, sea-surface microlayer, seawater (Western Wadden Sea, The Netherlands)	Dialysis for 2 h × 24 h, 2 ml × 60 ml hexane:DCM (80:20, v/v)	(1) NP-HPLC (Waters 8SI5 μ Radial Pack column, hexane:dichlormethane gradient), (2) organic sulphur removed with tetrabutyl ammonium sulphite	GC/ECD, CP-Sil 19 column (60 m × 0.15 mm × 0.20 μm)	[28]
MCs	Influent/effluent of sewage treatment plant (Germany)	Dialysis for 48 h, 250 ml of hexane	(1) GPC (Bio-Beads® S-X3, 100 ml of hexane:dichlormethane, 80:20, v/v), (2) adsorption fractionation (Florisil®, 20 ml of hexane)	Nitro-MCs: GC/FID, SE-54 column (25 m × 0.20 mm × 0,2 μm), polycyclic MCs: GC/MSD, DB-5MS column (30 m × 0.25 mm × 0,25 μm)	[29]
PAHs	Coastal waters (Hong Kong, China)	Membrane cut, internal cavity washed three times with 10 ml of hexane	Adsorption fractionation (silica gel, methylene chloride:hexane, 20:80, v/v)	GC/MSD, Ultra-2 column (30 m × 0.2 mm × 0.33 μm)	[30]
OCPs, PCBs, PAHs	Sewage water (Valencia, Spain)	Microwave-assisted extraction in the PTFE reactor with 33 ml of hexane-water (10:1, v/v), 3 min	GPC (two tandem columns with Envirogel, methylene chloride)	GC/MSD BPX-5 (30 m × 0.25 mm × 0.25 μm)	[24]

"Standard" membranes defined in Section 2.2 used in all studies.

aromatic hydrocarbons (PAHs) [4,9,12,22], organochlorine pesticides (OCPs) [4,9,22,25–27], pyrethroids [9], non-ionic organometallic chemicals [9], etc. Environmental Protection Agency (EPA) also used SPMD technique for its monitoring purposes: the results using SPMD were compared to those obtained by EPA method 610 for determination of PAHs in water and it was shown that the SPMD approach was equivalent to this EPA-approved method [9].

The aim of the presented study was to investigate the possibility to replace the time-consuming isolation step based on dialysis of exposed membranes by faster extraction procedure enabling more flexible solution of potential contamination problem. The target analytes consisted of persistent industrial halogenated contaminants represented by PCBs, brominated flame retardants (BFRs), OCPs including their isomers/metabolites and related compounds like octachlorostyrene (OCS). In addition, synthetic musk compounds (MCs)-both nitro-musk and polycyclic substances, relatively persistent pollutants typically contained in waste-waters from municipal sewage treatment plants, were involved in our study.

2. Experimental

2.1. Standards and chemicals

The overview of suppliers of all analytical standards used in this study together with their purities is given in Table 2, log K_{OW} values (*n*-octanol/water distribution constants) illustrating hydrophobicity of target analytes are shown here too. All solvents were of analytical grade: hexane and cyclohexane for gas chromatography, isooctane for spectroscopy (all Merck, Germany), ethyl acetate for pesticide residue analysis (Scharlau, Spain). Technical gases used for GC analyses were helium 4.6, nitrogen 5.0 and methane 4.5 (all Linde, Czech Republic). Sodium sulphate, anhydrous (Penta, Czech Republic) used for filtration was heated up to 600 °C for 7 h and stored in a dark vessel in desiccator before use.

2.2. Materials and technical equipment

"Standard" low-density polyethylene (LDPE) semipermeable membranes (length 91.4 cm, width 2.54 cm with wall thickness in the range from 70 to 95 μm) filled with 1 ml of high-purity triolein, specific density 0.91 g per cm^3 (25 °C) [31], were purchased from ExposMeter AB (Tavelsjő, Sweden). Sampling device for SPMD deployment consists of a stainless steel box with Teflon plates and a support rope (Labicom, Czech Republic).

Ultrasonic water bath Sonorex Super RK 510 (Bandelin Electronic, Germany) was used for extraction supported by sonication and rotary vacuum vaporiser Rotavapor R-114 with Waterbath B-480 (Bűchi, Switzerland) for evaporation of solvents and sample concentrating.

Glass columns for dialysis (length 250 mm, internal diameter 42 mm, wall thickness 2 mm) and all other laboratory glassware were obtained from Simax (Czech Republic).

The GPC system employed for clean-up of primary extracts consisted of a piston-type 305 Master Pump with manometric module 805, dilutor 402 and programmable fraction collector 231 XL (all Gilson, France), 6-way injection valve Rheodyne 7010 and 2ml injection loop (Rheodyne, USA). Stainless steel column (length 500 mm, internal diameter 8 mm, Tessek, Czech Republic) was filled with Bio-Beads® S-X3 (Bio-Rad Laboratories, USA), styrene-divinylbenzene copolymer with particle size 200–400 mesh (0.104–0.127 mm), cyclohexane:ethyl acetate mixture (1:1, v/v) was used as a mobile phase (mobile phase flow rate 0.6 ml per min).

Identification and quantification of MCs and BFRs was carried out using a gas chromatograph HP 6890 (Agilent Technologies, USA) equipped with autosampler (HP 6890 Series split/splitless injector). The gas chromatograph was coupled to a quadrupole (Q) mass selective detector (MSD HP 5973) operated in an electron impact (EI) ionization mode for MCs and negative chemical ionization (NCI, methane as the reagent gas) mode for BFRs. DB-5MS column (60 m × 0.25 mm × 0.25 μm, J&W Scientific, USA) was used for separation of MCs, DB-XLB column (30 m × 0.25 mm × 0.1 μm, J&W Scientific, USA) for BFRs separation.

GC analyses of PCBs, OCPs and OCS were performed using a gas chromatograph HP 5890, series II (Agilent Technologies, USA) with autosampler (HP 7673 GC/SFC split/splitless injector) and a system of two parallel columns (DB-5 and DB-17, both 60 m × 0.25 mm × 0.1 μm, J&W Scientific, USA) in combination with two ECD ^{63}Ni detectors. GC conditions used for the determination of particular analytes are summarized in Section 2.3.4.

2.3. Methods

2.3.1. SPMD sampling procedures

Two sampling sites on the Moldau river (see Fig. 2) were chosen for SPMDs deployment: (i) Podoli located upstream

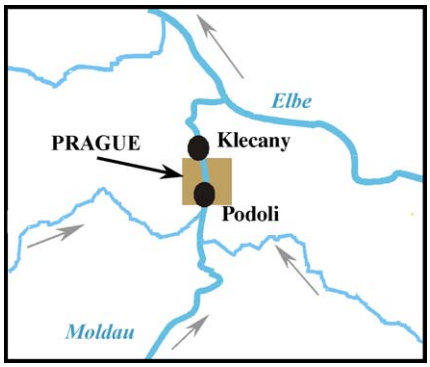

Fig. 2. Location of sampling sites, Moldau river basin.

Table 2
Pollutants monitored within the study -characterization of analytical standards, characteristic ions in EI spectra of MCs and NCI spectra of BFRs

Group of compounds	Supplier	Analyte	Purity (%)	M_r (g/mol)	log K_{OW}	*m/z*
MCs	LGC Promochem, Germany	Galaxolide (HHCB)	75.0	258.4	5.9 [32]	**243**, 213, 258
		Tonalide (AHTN)	98.0	258.4	5.7 [32]	**243**, 258, 201
		Celestolide (ADBI)	98.0	244.4	5.4 [32]	**229**, 244, 173
		Phantolide (AHDI)	94.5	244.4	5.9 [32]	**229**, 244, 187
		Traseolide (ATII)	90.0	258.4	6.3 [32]	**215**, 258, 173
		Musk xylene (MX)	99.0	297.3	4.8 [32]	**279**, 294, 264
		Musk ketone (MK)	99.0	294.3	4.3 [32]	**282**, 297, 283
	Dr. Ehrenstorfer, Germany	Tonalide D3[a]	99.0	261.4	See AHTN	***246***, 261, 204
		Musk xylene D15[a]	97.5	312.3	see MX	**294**, 312, 295
PCBs	Dr. Ehrenstorfer, Germany	CB 28	96.0	257.6	5.6–5.7 [9,33,34]	–
		CB 52	98.5	292.0	5.8–5.9 [9,33,34]	–
		CB 101	99.0	326.4	6.3–6.4 [9,33,34]	–
		CB 118	99.5	326.4	6.5–6.7 [9,33,34]	–
		CB 138	99.5	360.9	6.7–6.8 [9,33,34]	–
		CB 153	97.0	360.9	6.8–6.9 [9,33,34]	–
		CB 180	99.0	395.3	7.2–7.4 [9,32,33]	–
		CB 112[a]	99.8	326.4	6.2 [34]	–
OCPs and related compounds	Dr. Ehrenstorfer, Germany	α-Hexachlorcyclohexane (α-HCH)	97.5	290.8	3.7–3.9 [9,35]	–
		β-Hexachlorcyclohexane (β-HCH)	97.0	290.8	3.9 [9,35]	–
		Lindane (γ-HCH)	98.5	290.8	3.7–3.9 [9,19,35]	–
		Hexachlorbenzene (HCB)	99.5	284.8	5.7–6.2 [9,19,35]	–
		o,p′-DDE	97.5	318.0	5.6 [9]	–
		p,p′-DDE	99.0	318.0	5.8–6.1 [9,35]	–
		o,p′-DDD	99.5	320.1	6.1 [9]	–
		p,p′-DDD	97.5	320.1	5.8–6.0 [9,35]	–
		o,p′-DDT	98.0	354.5	5.6 [9]	–
		p,p′-DDT	98.5	354.5	5.5–6.2 [9,19,35]	–
		Octachlorostyrene (OCS)	99.1	379.7	6.9–7.7 [36]	–
BFRs	Cambridge Isotope Laboratory, USA	Brominated diphenyl ethers (PBDEs):				
		BDE 28	99.0	406.9	5.5–5.6 [37]	**79**,81,159,161
		BDE 47	99.0	485.8	5.9–6.2 [37]	(all congeners)
		BDE 49	99.0	485.8	5.9–6.2 [37]	
		BDE 66	99.0	485.8	5.9–6.2 [37]	
		BDE 85	99.0	564.7	6.6–7.0 [37]	
		BDE 99	99.0	564.7	6.6–7.0 [37]	
		BDE 100	99.0	564.7	6.6–7.0 [37]	
		BDE 153	99.0	643.5	6.9–7.9 [37]	
		BDE 154	99.0	643.5	6.9–7.9 [37]	
		BDE 183	99.0	722.4	not available	
		Hexabromocyclododecane (HBCD)	98.0	641.7	5.6 [38]	**158**, 160, 79, 71

m/z values in bold were used for quantification.
[a] Deuterated musk xylene-D15 and tonalide-D3 were used as syringe internal standards for compensation of potential matrix effects in hot GC injection area, congener CB 112 was applied to verify GPC clean-up step recovery.

and (ii) Klecany located downstream of Prague, the largest industrial and urban area in the Czech Republic. In each locality, six SPMDs were deployed for a 21-day period (from May 21 to June 11, 2002). The membranes were then transported to the laboratory in tightly closed clean aluminium cans and kept deeply frozen (−20 °C) until the analysis. Field blank experiments were carried out in both localities.

2.3.2. Isolation of analytes

Before analysis, the sealed loops were removed and exposed membranes were carefully mechanically cleaned from biofouling using cold tap water. The surface of membranes was then rinsed with small amounts of hexane, 1M HCl and distilled water and dried. Two alterative extraction approaches, both employing hexane as an extraction solvent, were used to isolate analytes:

(i) routinely used dialysis procedure,
(ii) extraction of cut membrane by sonication.

2.3.2.1. Dialysis. Membrane was rolled, inserted into the glass column and after addition of 100 ml of hexane was

left to dialyse for 24 h. The dialysate was collected passing through the layer of anhydrous sodium sulphate, 100 ml portion of hexane was added again and left to dialyse for another 24 h. Combined dialysates were evaporated to a small volume (approximately 0.5 ml) and the remaining solvent was removed using a gentle nitrogen stream.

2.3.2.2. Sonication. Membrane was lengthwise cut using sharp scissors (pre-cleaned in acetone) and then placed into Erlenmeyer flask. Three extraction cycles enhanced by sonication (100 ml hexane, 20 min each) followed. The traces of moisture were removed from combined extract by passing through the layer of anhydrous sodium sulphate. The bulk solvent was vacuum evaporated, the last drop was removed using a gentle nitrogen stream. The residue composed mainly from triolein was then weighted.

2.3.3. Clean-up

The residue left after removing of extraction solvent (see isolation procedures described in Section 2.3.2) was dissolved in 10 ml of GPC mobile phase (cyclohexane:ethyl acetate mixture, 1:1, v/v) containing 5 ng per ml of PCB congener no. 112, a recovery internal standard. The aliquot of 2 ml of this solution was loaded onto the Bio-Beads® S-X3 column to separate triolein and other interferences from analytes (the capacity of the GPC column used in this study was 200 mg of lipids per injection). Under applied experimental conditions, all the target analytes were eluted in 16 ml fraction corresponding to elution volume 14–30 ml at a flow rate 0.6 ml per min.

The repeatability of a clean-up step expressed as RSD was obtained by repeated injection ($n = 6$) of a standard mixture containing 25 ng of each target analyte.

2.3.4. Identification and quantification of analytes

Eluate collected from GPC column was vacuum evaporated, the remaining solvent was removed using a gentle nitrogen stream and then 1 ml of syringe internal standard mixture containing 40 ng per ml of musk xylene D15 and tonalide D3 was added. Approximately 500 μl of sample were transferred into GC vials for analysis of musk compounds. Remaining part of the sample was treated with concentrated H_2SO_4 and then the upper layer was carefully transferred into GC vials for PCB, OCP and BFR analyses. Sample equivalent of 1 μl injected for GC analysis corresponded to 0.02% of total SPMD extract. Musk compounds content was determined by GC/Q-MS method with EI ionization in SIM (selected ion monitoring) mode. Internal standard calibration technique was used for quantitation of these analytes (concentrations of syringe internal standards tonalide D3 and musk xylene D15 in calibration standards were 40 ng per ml isooctane). The solutions used for calibration were in the range 0.2–100 ng of ADBI and AHDI, 0.5–100 ng of ATII and MX, 1–100 ng of MK, and 0.5–1000 ng of major MCs (HHCB and AHTN) per ml of isooctane.

Analysis of BFRs was carried out by GC/Q-MS operated in NCI mode. External calibration technique was applied with standard solutions range 0.2–10 ng of BDE 183, 0.05–10 ng of other BDE congeners (28, 47, 49, 66, 85, 99, 100, 153 and 154) and 2–10 ng of HBCD per ml (all in isooctane).

The characteristic fragments used for selected ion monitoring of musks and brominated flame retardants are shown in Table 2.

GC/ECD system equipped with two parallel columns (different stationary phases) inserted into common injection port was used for identification and quantification of PCBs, OCPs and related compounds. External standard calibration technique was applied with solutions range 0.2–200 ng of PCBs, 0.1–200 ng of HCB, α-, β-, γ-HCH and OCS and 0.5–200 ng of DDEs, DDDs and DDTs per ml of isooctane.

The GC conditions used for determination all the above groups of target analytes are summarized in Table 3.

Repeatabilities (expressed as RSD, %) of the GC quantification step were calculated using data obtained by repeated injection ($n = 6$) of standard solution at concentration levels corresponding to the first calibration point (see the text above).

Two reagent blanks consisting of aliquots of all solvents used during the SPMD processing were performed too.

2.4. Validation of the procedure

New, non-deployed membranes were immersed in ultrapure water for 1 week to wash off most of the methyl oleate (common impurity in triolein). 100 μl of spiking mixture with all the target compounds in hexane (concentration 1 μg per ml each) were added into the solvent used for extraction. In case of cutting/sonication approach, the spiking mixture was added to the first portion of extraction solvent (100 ml) before sonication, after the membrane had been cut. Similarly, in case of dialysis, the same amount of spiking solution was added into the solvent (first portion of 100 ml) after the membrane had been rolled down to the bottom of the glass column. Two spiking experiments for each of the extraction approaches tested were realized.

2.5. Statistical analysis of data

Statistical apparatus was applied on the whole set of obtained data [39]. Supposing Gaussian normal distribution of results, arithmetic mean ($\bar{x}$) and standard deviation (SD) were calculated.

Dean-Dixon range (R) was used for calculations using following equation (this type of statistical apparatus employing range was found to be the most suitable for statistical assessment of the data set consisting of only three parallel experiments):

$$R = x_{\max} - x_{\min} \quad (1)$$

Table 3
GC conditions used in the determinative step

Target analytes	MCs	PCBs, OCPs and related compounds	BFRs
Identification and quantification	GC/Q-MS	GC/ECD (2 parallel columns)	GC/Q-MS
Injector temperature	250 °C	250 °C	275 °C
Injection technique	Pulsed splitless (pulse 50 psi)	Splitless	Pulsed splitless (pulse 60 psi)
Injection volume	1 μl	1 μl	1 μl
Splitless time	2 min	2.5 min	2 min
Carrier gas	Helium	Helium	Helium
Linear velocity of carrier gas	33 cm/s (flow-rate 1.5 ml/min)	36 cm/s (flow-rate 1.7 ml/min)	34 cm/s (flow-rate 1.5 ml/min)
Temperature program	60 °C (2 min), 10 °C/min to 180 °C, 1.5 °C/min to 220 °C, 30 °C/min do 280 °C (3 min)	60 °C (2.5 min), 30 °C/min to 220 °C, 0.5 °C/min to 240 °C, 2.5 °C/min to 280 °C (10 min)	105 °C (2 min), 50 °C/min to 280 °C, 5 °C/min to 300 °C (5 min)
Auxiliary gas	–	Nitrogen (make-up gas)	Methane (reaction gas)
Interface temperature	280 °C	–	300 °C
Ionization type	EI	–	NCI
Ionization energy	70 eV	–	220 eV
Source temperature	230 °C	–	150 °C
Total analysis time	25 min	73 min	16 min

Standard deviation using range (S_R) was then calculated employing equation:

$$S_R = k_n R, \quad (2)$$

where coefficient k_n for $n = 3$ is 0.5908. Relative standard deviation (RSD_R) can be calculated:

$$RSD_R = \frac{S_R}{\bar{x}} 100 \quad (3)$$

The confidence interval ($L_{1,2}$) is defined:

$$L_{1,2} = \bar{x} \pm K_n R, \quad (4)$$

where coefficient K_n is *1.3* ($n = 3$, the significance level $\alpha = 0.05$).

3. Results and discussion

Dialysis of exposed SPMDs is currently almost exclusively used way for recovering the target compounds from sequestrant media (see overview in Table 1). To our knowledge, there have been published only few studies [30,40,41] reporting another option of SPMD handling prior to determinative step: isolation of analytes from cut membrane was for instance obtained using its triplicate rinsing with organic solvent. Regarding the first study by Richardson et al. [30], PAHs and petroleum hydrocarbons were determined in SPMDs and mussels. Hexane was used to isolate these analytes from cut membranes by simple rinsing the membrane interior (no validation data for overall procedure provided). It should be noted that under such conditions, the portion of analytes dissolved in membrane cannot be, contrary to our sonication strategy or dialysis-based procedure, efficiently recovered. Considering the fact that up to 50% of the total content of analytes concentrated in SPMDs may be present in the membrane wall [9], biased (underestimated) results can be obtained in this way provided membrane is not analysed separately in an additional step. In the other mentioned study, Huckins et al. [40] conducted intralaboratory experiments with radiolabelled compounds (PCBs, Mirex, etc.); pieces of membrane and sequestering lipids were analysed separately. Almost 50% of the sequestered analytes were shown to be dissolved in the membrane; combined recoveries ranged from 79.1 to 95.0%. The study performed by Prest et al. [40] described even more complex isolation procedure: lipids were flushed out of the PE tube using hexane and membrane was then soaked for three days in cyclopentane. The combined extracts were transferred into empty PE tube and dialysed in cyclopentane for 2 h × 48 h. Recoveries of all monitored OCPs exceeded 85%. To overcome the time-limiting drawback and employ one-step, faster "cutting" approach, comparative study based on critical assessment of both alternatives, classic (dialysis-based) and novel (sonication-based) one, was realized.

Dialysis, the former option, is a separation process of compounds in solution directed by concentration difference. While small molecules diffuse through the membrane, the larger ones are excluded from this transport process. In this process the flow of small molecules is independent on the solvent flux and may also occur in the opposite direction from the solvent flux [42]. It is obvious that the exchange of target pollutants (these are smaller molecules as compared to triolein) is a slow process; from practical point of view its speed is a limiting factor in the procedure aimed at obtaining information on the concentration of analytes – in case of SPMDs completion of dialysis usually takes 48 h. Searching

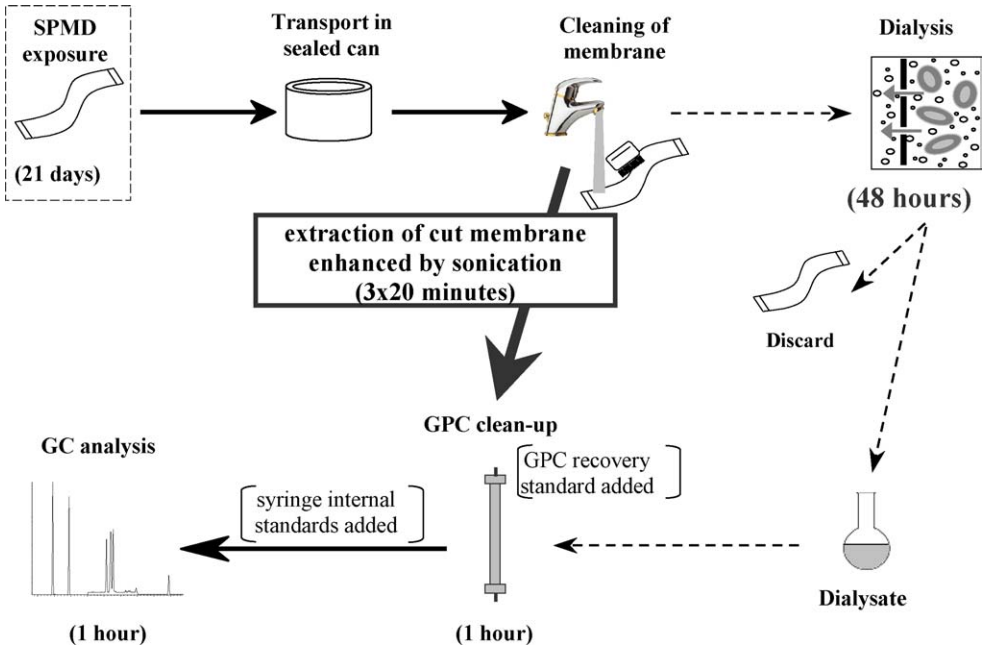

Fig. 3. Novel procedure simplifying SPMD processing prior to identification/quantification step.

practical solution that would enable obtaining the results in a more rapid way, extraction of cut membranes enhanced by sonication (ultrasound applied to obtain efficient extraction of analytes also from polyethylene part of SPMD) was selected as a conceivable strategy. As shown in Fig. 3, the procedure developed in our study enabled significant reduction of total isolation time, as compared to dialysis.

While saving 2 days (time needed for dialysis isolation of the target analytes) within monitoring programs that employ typically 3-week SPMD exposure might not seem to be so important, under certain circumstances, fast response of analytical laboratory is a crucial prerequisite for adopting effective measures to manage pollution problem indicated by membranes.

Actually, handling of membranes does not have necessarily to be preceded by several-week exposure; scenarios working with significantly shorter exposure times can be encountered. For instance, when evaluating performance of technical process on the basis of measured TWA values (5–7 days of sampling might be sufficient obtaining indicative data), delivery of results within one working day attainable by introduced cutting/sonication approach would facilitate flexible validation and, if needed, redirection of the process. With the cutting/sonication approach, the laboratory could deliver both analytical and for example toxicity results during one working day.

Comparing the time demands of both approaches, it was found that the interval between the arrival of the sample to laboratory and obtaining final results could be reduced approximately 3.5times – considering processing of three SPMDs by cutting/sonication strategy (the novel approach takes approx. 19 h versus 65 h needed for dialysis-based isolation). Although the labor intensity is slightly higher by the new procedure, additional approximately 30 min of manual work (analyst in contact with the ongoing sonication procedure) may compensate for 48 waiting period associated with older approach whenever fast generation of results is required. With respect to technical possibilities, only one isolation approach can be applied for processing of a single exposed membrane prior to quantification step, therefore, data needed for comparison of alternative sample handling strategies had to be obtained via realization of two sets of measurements. For this purpose six membranes were deployed simultaneously in each locality hence the same amount of analytes was assumed to be trapped in each membrane. Three membranes were afterwards processed using “classic” dialysis and the rest by a new procedure developed in this study. Hexane was chosen as an extraction solvent both for dialysis and sonication because it has been the most frequently used solvent in up-to-now conducted studies (see overview in Table 1). In the next step, undesirable components (mainly oligomers released from polyethylene membrane and oleic acid and methyl oleate, typical impurities from synthetic triolein [9]) present in primary (crude) extract both after dialysis and sonication together with triolein have to be separated from analytes. Automated GPC was found to be a suitable tool for removing of most of these interferences.

Recoveries of all the target compounds were in the range 89–103% (PCB 112 was added to the GPC mobile phase mixture prior to clean-up step as an internal standard to control GPC performance, see Section 2.3.3). The repeatability of a clean-up step expressed as RSD (for experimental conditions see also Section 2.3.3) was in the range 2.9–8.1%.

Repeatabilities of the GC step (obtained by repeated injection of standard solutions at concentration levels corresponding to the first calibration point into the GC system, see Section 2.3.4) were in the range 1.5–5.7%. One of the general problems we have encountered within validation process was determination of overall recovery of isolation procedures. One of the strategies conceivable for accuracy measurement (both isolation procedures) is processing of membranes containing target analytes. However, PRCs membranes were not commercially available at the time of our experiments, moreover e.g. musk compounds are not on the list of chemicals used for this purpose. It should be also noted that analysis of non-exposed SPMDs does not necessarily reflect the real-life sample since exposure may, due to many reasons, change its character and hence extraction efficiency (no bioufouling layer on the membrane surface, lack of chemical noise in a GC run caused by other, non-target compounds, potentially interfering methyl oleate not washed off the membrane present etc.). In-laboratory spiking of SPMD does not also represent a viable alternative since puncturing of commercial membrane would have to be carried out and, on such conditions, dialysis of SPMD is not practicable. Even if trying to heat-seal the punctured membrane, uniform distribution of internal standards in triolein phase would be problematic; moreover, incorporation of analytes into membrane wall would be impossible. As a compromise, within recovery experiments spiking was carried out into solvent used for dialysis or sonication (for detail description of experimental conditions see Section 2.4). No significant differences in recoveries of target analytes were found between isolation procedures tested, their values ranged from 89.8 to 100.5% (the results shown in Fig. 5 were not corrected for recoveries).

The performance characteristics of analytical procedures employed in this study are summarized in Table 4. It should be noted that, contrary to dialysis approach, in which the isolate from the whole membrane is available for analysis, the experimental set-up of purification step involved in the cutting/sonication approach allowed, due to the limited capacity of GPC column, to process only 200 mg of lipids extracted from membrane (i.e. approx. 20% of crude extract is taken for examination, the rest can be left for toxicity tests of for archiving). In spite of this fact, thanks to low detection limits (see Table 4), we were still able to determine reliably almost all the target analytes even in SPMDs deployed in a locality with a low extent of pollution. The only target compounds not occurring above LODs were some of the BFRs, steric effects probably inhibit their penetration through membrane cavities. Provided the LODs are not sufficient in particular case, we would suggest increase the size of GPC column, onto which 1 ml of triolein could be loaded; this would, however, result in evaporation of larger volume of solvent.

The example of GC/MS of MCs analysis occurring in the real-life sample is shown in Fig. 4. Due to a good efficiency of a clean-up step and, accordingly low chemical noise in chromatographic record, even further enhancement of detection sensitivity is feasible. This can be achieved by increased amount of sample equivalent injected onto GC column, e.g. by using large volume injection (LVI) technique or by more intensive pre-concentration of sample (repeated GPC separation of lipids isolated from SPMD and following combining of eluates is possible too).

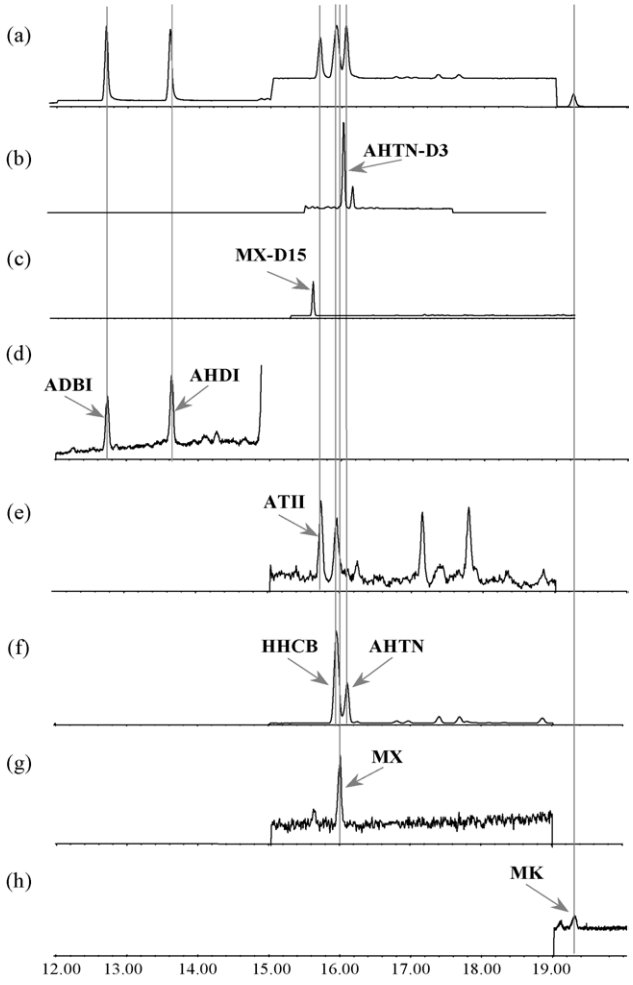

Fig. 4. GC–MS analysis of musk compounds in SPMD deployed in locality Podoli: standard (total ion current, TIC) (a) standard solution containing 100 ng/ml of MCs mixture, 40 ng/ml of tonalide D3 and musk xylene D15 in isooctane; Extract obtained from SPMD by sonication (selected ion monitoring, SIM) (b) tonalide D3, (c) musk xylene D15, (d) celestolide and phantolide, (e) traseolide, (f) galaxolide and tonalide, (g) musk xylene, (h) musk ketone. For concentrations of MCs determined in SPMD see Fig. 5.

The mean concentrations of individual pollutants determined in SPMDs deployed at two sampling sites are shown in Fig. 5. As documented here, the procedure employing batch extraction of cut membrane enhanced by sonication provided, with a few exceptions, higher results than that based on dialysis approach. As shown in Table 4, repeatability of results expressed as RSD values was comparable for both sample handling approaches. In accordance with higher content of analytes, lower values of RSDs were observed for samples from the more polluted locality Moldau-Klecany.

Table 4
The performance characteristics of the current method

Target analytes		LOD[a] (pg injected)	RSD (%)			
			Podoli		Klecany	
			Sonication	Dialysis	Sonication	Dialysis
MCs	HHCB	0.15	5.4	2.7	3.4	3.5
	AHTN	0.16	4.3	9.1	6.7	12.3
	ADBI	0.06	9.9	7.4	4.5	12.0
	AHDI	0.06	9.7	15.9	2.1	6.8
	ATII	0.10	7.0	3.8	5.2	7.1
	MX	0.18	6.0	3.7	6.9	8.5
	MK	0.27	6.0	5.0	4.4	12.3
PCBs	PCB 28	0.05	10.9	9.3	4.3	5.7
	PCB 52	0.08	5.4	15.1	7.6	13.6
	PCB 101	0.07	7.2	12.7	16.0	14.0
	PCB 118	0.07	15.9	21.2	9.2	9.5
	PCB 138	0.06	10.4	21.0	11.5	16.8
	PCB 153	0.08	12.6	19.2	0.6	12.2
	PCB 180	0.08	20.0	22.2	6.8	19.9
OCPs and related compounds	HCB	0.02	5.5	7.3	2.3	2.9
	α-HCH	0.03	2.2	14.0	14.6	14.3
	β-HCH	0.04	11.0	11.3	7.5	1.5
	γ-HCH	0.03	1.2	12.4	1.1	11.6
	o,p′-DDE	0.10	7.3	12.7	1.2	12.9
	p,p′-DDE	0.10	5.7	4.5	1.5	4.0
	o,p′-DDD	0.10	13.2	14.8	1.4	8.4
	p,p′-DDD	0.09	1.6	9.3	4.7	6.8
	o,p′-DDT	0.11	10.3	11.1	9.4	9.5
	p,p′-DDT	0.12	14.4	15.4	6.4	9.6
	OCS	0.03	18.5	10.8	6.5	9.8
BFRs	BDE 28	0.02	–	–	–	–
	BDE 47	0.02	4.6	4.6	5.4	7.1
	BDE 49	0.02	–	–	–	–
	BDE 66	0.02	–	–	–	–
	BDE 85	0.02	–	–	–	–
	BDE 99	0.02	4.0	5.6	1.3	5.2
	BDE 100	0.02	–	–	1.7	8.7
	BDE 153	0.02	–	–	–	–
	BDE 154	0.02	–	–	–	–
	BDE 183	0.07	–	–	–	–
	HBCD	0.69	–	–	–	–

[a] Limit of detection, S/N = 3.

The data obtained within the present study were subjected to a comprehensive statistical analysis (see Section 2.5) to ascertain whether significant differences between the results obtained by the two alternative isolation procedures exist. Dean-Dixon range (R) was chosen as the most suitable tool for comparison of results since only three parallel experiments ($n = 3$) for each isolation approach in both localities were performed. As can be seen in Fig. 5, statistically significant differences based on the determination of confidence intervals were found between most of the relevant pairs within the particular data set. Generally, the concentrations (means) of pollutants found in SPMDs treated with sonication were higher than those obtained by dialysis, this trend was not found only for β-HCH in locality Klecany, where results were significantly higher employing dialysis (see Fig. 5).

Relationship between the values of the measured sampling rates and extraction procedure used for analytes isolation obviously exists. It should be noted that the published sampling rates can be used only for approximate determination of ambient concentration of the respective pollutant. Temperature variations and other factors specific for the particular sampling site (e.g. water-flow rates, specific flow profiles such as turbulences, etc.) influence the sampling rates. Small systemic errors in laboratory data associated with different extraction procedures described in our paper might be of minor importance. It should also be emphasized that sampling rates for musk compounds, which are involved in the current study, have not been reported yet, R_S data have to be produced, supposing monitoring of these analytes is of interest.

As far as comparison between the two above discussed sample handling methods is required, correction factors

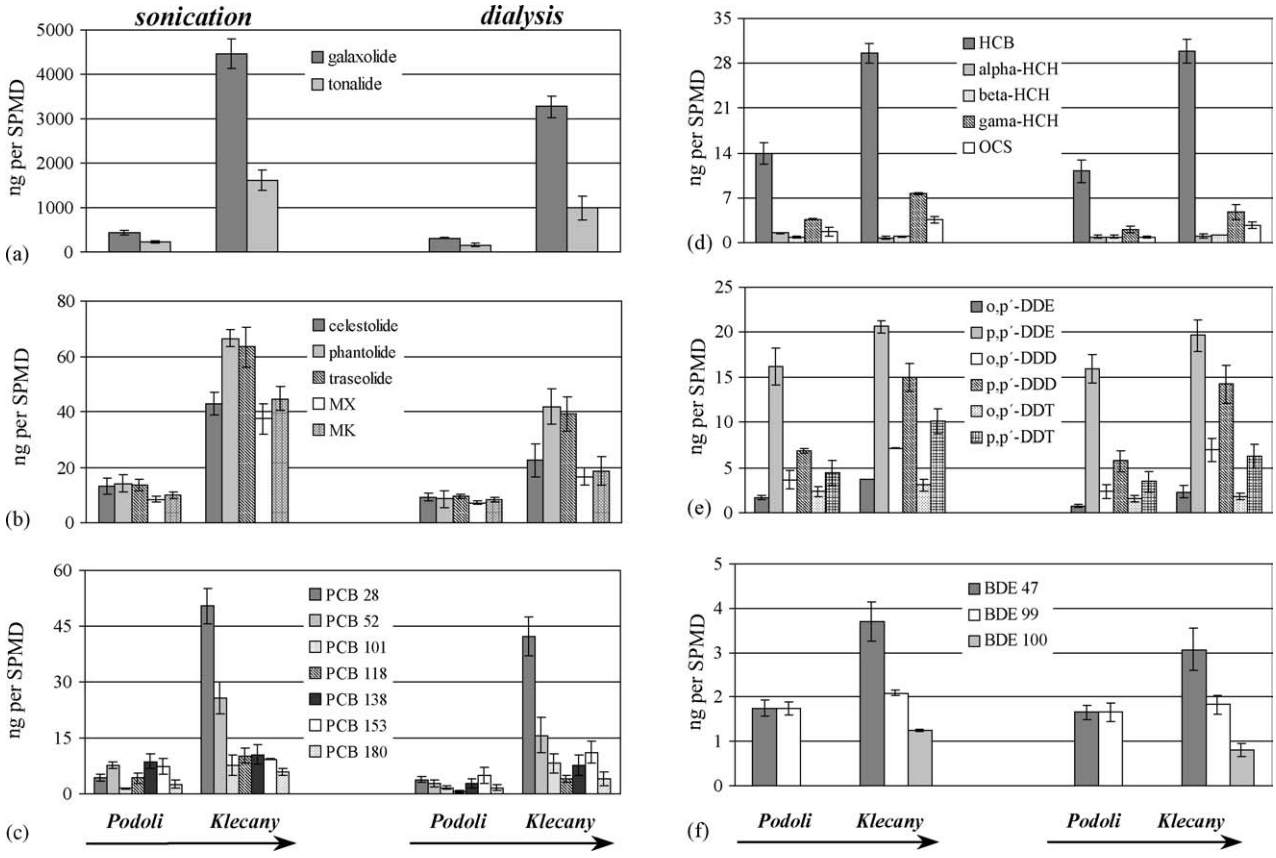

Fig. 5. Data (mean values, $n = 3$) obtained by examination of SPMDs by two alternative isolation techniques (the arrows indicate the Moldau river flow direction, the error bars indicate calculated confidence intervals). (a) Major MCs, (b) minor MCs, (c) PCBs, (d) and (e) OCPs and related compounds and (f) BFRs.

should be established for this purpose. As regards compounds for which R_S data are not available, their values can be estimated by the use of relevant chemical model employing published data for similar compounds. As already emphasized, introduction of PRCs, the calibration based on R_S values is not necessary anymore.

4. Conclusions

A novel procedure applicable for isolation of organic pollutants from exposed SPMDs has been developed. Extraction of analytes from cut membrane supported by sonication allows substantial reduction of the time needed for finalizing of sample analysis. Increased sample turnover and, consequently, the possibility of fast obtaining the results make this approach challenging alternative to the time-consuming dialysis. In addition to improved flexibility, higher recoveries of many of the target pollutants together with comparable variance of results document good performance of this procedure. It is believed that this study contributes to further expansion of SPMDs use. As shown by our experiments, a wide range of environmental pollutants can be monitored by this technology whenever information on their bioconcentration potential is required.

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