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Novel use of a dual-zone restricted access sorbent: normal-phase solid-phase extraction separation of methyl oleate from polynuclear aromatic hydrocarbons stemming from semi-permeable membrane devices

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Abstract

A normal-phase solid-phase extraction (SPE) method was developed utilizing a dual-zone restricted-access sorbent to separate methyl oleate from the 16 US Environmental Protection Agency priority-pollutant polynuclear aromatic hydrocarbons in a hexane matrix. This technique represents a new development in SPE methodology expanding the limited number of available normal-phase SPE sorbents. While based on a specific application (removal of methyl oleate from semipermeable membrane device extracts), this cleanup method could easily be adapted for other uses requiring the removal/isolation of methyl oleate and potentially related compounds. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Semipermeable membrane devices (SPMDs) have been used extensively as monitors of hydrophobic contaminants in aquatic systems (e.g. [1–6]). They effectively concentrate non-ionic hydrophobic compounds from large volumes of water via passive hydrophobic partitioning into the membrane and its lipid contents from the surrounding water. Use of SPMDs has recently been included in a review of new technologies in solid-phase extraction [7]. These devices, consisting of lay-flat, low-density polyethylene (LDPE) tubing encapsulating 1 ml of a model lipid (triolein), are deployed in aquatic environments for extended periods and have been shown to highly concentrate trace levels of many classes of environmental pollutants. Chemical classes sampled include polynuclear aromatic hydrocarbons (PAHs), organochlorine and organophosphate pesticides, dioxins and polychlorinated biphenyls.

Analyte recovery from SPMDs requires dialysis in hexane and subsequent trace enrichment (including concentration and cleanup). Besides analytes, dialysis coextracts interferences stemming from the

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LDPE tubing (polyethylene oligomers) and triolein impurities. The most problematic interference is methyl oleate, estimated at ca. 2% in a 95% commercially available triolein preparation. Methyl oleate is present in the final dialysate of a standard SPMD concentrated to 1 ml at levels (~18 mg/ml) that produce a substantially overloaded, shark-fin-shaped gas chromatography (GC) peak which obscures co-eluting compounds, shifts analyte retention times, and elevates the baseline. Due to this substantial loading, methyl oleate ghost peaks persist for several subsequent analyses. Currently, an HPLC cleanup procedure based on molecular size-exclusion using gel permeation chromatography (GPC) is employed to separate the methyl oleate from analytes [1,3,4]. This method requires expensive equipment, is time-consuming, labor-intensive, and not entirely effective. An attractive alternative would be a normal-phase (due to the hexane matrix) solid-phase extraction (SPE) method. These adsorption-based methods are widely used in environmental analysis for the separation of analytes from interferences in non-polar matrices [7,8]. The goal of the current work was to develop a cleanup method using a simple and inexpensive normal-phase SPE method to replace conventional techniques for the enrichment of analytes in SPMD extracts.

During method development, several different types of SPE cartridges containing different solid phases were tested to determine the feasibility of separating methyl oleate from polynuclear aromatic hydrocarbons (PAHs). The sixteen United States Environmental Protection Agency (EPA) priority-pollutant PAHs were chosen as model compounds because they are commonly detected in environmental monitoring studies using SPMDs [9–11]. These sixteen PAHs differ in structure only in the number (2–6) and placement of their benzenoid (and occasionally furanoid) rings, but have a wide range in physico-chemical properties. For example, log K_{OW} values (K_{OW} = octanol–water partition coefficient) range from 3.4 for naphthalene to 6.7 for dibenz[*a,h*]anthracene; water solubility for these same compounds ranges from 31 000 to 0.6 μg/l [12]. The aromatic structure (and associated π-electrons) of the PAHs and the ester group in methyl oleate produce a similar degree of polarity and contribute to a polar adsorption mechanism by which these compounds adhere to commonly used solid-phase adsorbents (e.g. silica). Due to their similar degree of polarity, their communal sorption mechanism, and the large differences in physicochemical properties exhibited among PAHs, an effective normal-phase separation scheme was not immediately apparent.

To our knowledge no procedure has so far been described that can selectively remove methyl oleate while retaining PAHs or vice versa. Of the sorbents tested, the only solid-phase providing separation was a restricted-access sorbent [7,8,13,14] commonly used in reversed-phase applications for the cleanup of complex biological matrices, where the bound ligand is used to repel large molecules such as proteins and lipids. Our experiments represent a novel successful use of this sorbent: a normal-phase application where the bound ligand serves as a specific sorbent for the retention of methyl oleate. While based on a specific application, this cleanup method could easily be adapted for other uses requiring the removal/isolation of methyl oleate, other similar fatty acid esters, and perhaps other related compounds.

2. Experimental

2.1. Reagents and materials

The 16-component semi-volatile PAH mixture (purity ≥98% in CH_2Cl_2) and [$^2H_{10}$]anthracene (purity 98% in hexane) used in quantitative analysis and spiking experiments were purchased from Absolute Standards (Hamden, CT, USA). Methyl oleate (99%) was purchased from Sigma (St. Louis, MO, USA). SPMDs (commercially available from EST, St. Joseph, MO, USA) were constructed of LDPE tubing (50 μm wall thickness) and triolein (95% purity from Sigma). Solvents used were Fisher (Fair Lawn, NJ, USA) Optima grade. The SPE cartridges tested are described in Table 1.

2.2. SPMD preparation and processing

Standard SPMDs were prepared: strips 91 cm long of 2.5 cm wide LDPE lay-flat tubing were extracted against hexane for 24 h to remove impurities. After heat-sealing one end of the polyethylene tubing, 1 ml

Table 1
Solid-phase extraction (SPE) cartridge descriptions

Solid phase	Size (mg)	Manufacturer	Part No.
Florisil	1000	J.T. Baker, Phillipsburg, NJ, USA	7213-07
Neutral alumina	500	Alltech, Deerfield, IL, USA	228550
Silica gel	1000	Supelco, Bellefonte, PA, USA	5-7051
Cyano-bound silica[a]	1000	J.T. Baker, Phillipsburg, NJ, USA	7021-07
Diol-bound silica[b]	500	J.T. Baker, Phillipsburg, NJ, USA	7094-03
Amino-bound silica[c]	1000	J.T. Baker, Phillipsburg, NJ, USA	7088-07
Dual-zone silica-based restricted-access sorbent[d]	500	Diazem, Midland, MI, USA	7508-005
	1000		7508-010

[a] $Si–(CH_2)_3CN$
[b] $Si–(CH_2)_3COCH_2CH(OH)CH_2(OH)$
[c] $Si–(CH_2)_3NH_2$
[d] Exterior zone ligands: $Si–(CH_2CH_2O)_nCOCH_3$ and $Si–(CH_2CH_2O)_nH$; interior zone: SiOH

triolein was pipetted into the tubing and the other end of the tube heat-sealed. Spikes were prepared by pipetting a mixture of the 16 PAHs (50 or 100 μl at 100 μg/ml in hexane) into the tubing prior to heat-sealing. SPMDs were stored sealed in precleaned amber glass jars covered with aluminum foil and PTFE-lined caps at −20°C until dialysis. SPMDs were processed according to established protocols [1,11] with minor modifications. Briefly, the exterior membrane surface of each device was cleaned by shaking with hexane for 30 s, rinsed with acetone followed by isopropanol, and dialyzed with 125 ml hexane at 18°C for 18 h. The dialysate was removed and replaced with another 125 ml for an additional 6 h. Hexane dialysates were pooled and concentrated under reduced pressure to approximately 1 ml. These extracts were processed via SPE.

2.3. Solid-phase screening

A generic elution scheme was used for screening the solid phases described in Table 1: SPE cartridges were conditioned with 5 ml (for 500 mg packings) or 10 ml (for 1000 mg packings) hexane; 1.0 ml sample (16 PAHs at 1.6 μg/ml+0.7 mg/ml methyl oleate) in hexane was applied followed by two 1.0-ml hexane aliquots, and the cartridge was evacuated with gentle vacuum until visible solvent was removed (Fraction 1). Subsequently, two 1.5-ml aliquots of a hexane–CH_2Cl_2 (1:1) mixture were added and the cartridge was again evacuated (Fraction 2). Gravity-feed elution was used for all steps except cartridge evacuation where gentle vacuum was applied.

2.4. Optimization of elution protocol

Following the selection of the restricted-access sorbent as the solid-phase, the cartridge size, elution solvent, and elution volume were optimized to provide adequate separation of PAHs and methyl oleate while minimizing solvent volumes and the cartridge size. The solvent volumes for the conditioning and elution steps varied during optimization depending on SPE size (i.e. 500 or 1000 mg packing) and solvent type. However, the basic technique, described below, remained the same.

2.5. PAH recovery determinations

Restricted-access sorbent SPE cartridges were stored in dessicators prior to use. PAH recoveries from simulated SPMD extracts (17.6 mg/ml methyl oleate + 5 μg/ml PAHs in hexane) and spiked SPMDs (at 5 and 10 μg PAHs) were determined using 1000 mg packing size SPE cartridges. The cartridges were conditioned with 12 ml hexane and the sample (1 ml) was added. Prior to exposing the top of the packing material, the cartridge was eluted with 19 ml hexane–CH_2Cl_2 (97:3). The eluent was collected in a concentrator tube via gravity-feed elution and the cartridge evacuated under slight vacuum. Samples were concentrated under a stream of N_2 to <1 ml, quantitatively transferred to a 1.0 ml

volumetric tube and spiked at 4 μg/ml with [$^2H_{10}$]anthracene for GC–MS analysis.

2.6. Analytical

Samples were injected via an autosampler (2 μl injection volume) into a Hewlett-Packard (HP) 6890 GC system (Palo Alto, CA, USA) equipped with a split/splitless inlet operating in splitless mode and an HP-5ms capillary column (30 m×0.25 mm, 0.25 μm film thickness) connected to an HP 6890 series MS detector operating in the full-scan mode — m/z range from 50 to 500. The operating conditions with a helium gas flow of 1 ml/min were: 280°C inlet temperature, 70°C initial column temperature ramped at 5°C/min to 280°C, held for 10 min before ramping at 20°C/min to 300°C which was held for 1 min. Quantitative analysis of the 16 PAHs was achieved by integrating target peaks and calculating concentrations using internal standard calculations with HP Chemstation software.

3. Results and discussion

3.1. Solid-phase screening

SPE cartridges with different solid phases (Table 1) were tested with a PAH–methyl oleate mixture in hexane to screen for potential use in methyl oleate removal. To evaluate the separation of the PAHs from the methyl oleate, qualitative analysis of the gas chromatograms and MS spectra was conducted to determine their presence/absence in both elution fractions. These results are summarized in Table 2. Of the seven phases tested, only the restricted-access sorbent provided the desired separation, eluting the PAHs in the first hexane fraction while retaining the methyl oleate. The methyl oleate was present in the second (hexane–CH_2Cl_2, 1:1) fraction. Other solid phases failed to separate PAHs satisfactorily from methyl oleate. Thus, the restricted-access solid phase was the focus of subsequent experiments.

3.2. Cartridge capacity

The quantity of methyl oleate in these samples (~17.6 mg) was shown to overload 500 mg, but not 1000 mg of restricted-access SPE packing, indicating a sorptive capacity for methyl oleate of ≥1.8%. Higher quantities of packing also provided adequate separation, but larger solvent volumes were required and levels of SPE-derived interferences increased (data not shown).

3.3. Elution optimization

Experiments were conducted using the 1000 mg restricted-access SPE cartridges with simulated SPMD dialysates to determine the most efficient elution scheme. Hexane–CH_2Cl_2 mixtures of different proportions altered elution rates and separation of the methyl oleate from the PAHs: higher-polarity mixtures (with more CH_2Cl_2) eluted the methyl oleate and PAHs simultaneously while lower-polarity solvents provided adequate separation. Of the four eluent mixtures tested (hexane + 0, 3, 4, or 5% CH_2Cl_2) only the 0 and 3% mixtures permitted

Table 2
Presence of PAHs and methyl oleate (MO) in fractions following elution of SPE cartridges[a]

Solid-phase	Fraction 1	Fraction 2
Florisil	PAHs	MO, PAHs
Neutral alumina	MO, PAHs	
Silica gel	PAHs	MO, PAHs
Cyano-bound silica	MO, PAHs	PAHs
Diol-bound silica	MO, PAHs	PAHs
Amino-bound silica	MO, PAHs	MO, PAHs
Dual-zone silica-based restricted-access sorbent	PAHs	MO

[a] SPE cartridges were conditioned with 5 ml (500 mg packing) or 10 ml (1000 mg packing) hexane, 1.0 ml sample (16 PAHs at 1.6 μg/ml+0.7 mg/ml methyl oleate) was applied, and 2.0 ml hexane was added. The cartridge was evacuated and the eluent collected (Fraction 1). Subsequently, 3 ml hexane–CH_2Cl_2 (1:1) was added, the cartridge evacuated, and the eluent collected (Fraction 2).

selective elution of the PAHs while retaining methyl oleate. Because a smaller volume of the 3% mixture recovered all the PAHs from the 1000 mg packing than hexane alone (ca. 19 ml vs. 32 ml) it was selected as the mobile phase of choice (data not shown). The elution scheme is outlined in Section 2.5.

3.4. PAH recoveries

PAH recoveries (%) were determined for simulated SPMD dialysates (17.6 mg/ml methyl oleate + 5 μg/ml PAHs) and spiked SPMDs (SPMDs loaded with 5 and 10 μg of PAHs). The results indicate good recoveries for the simulated dialysates and adequate recoveries from the SPMD samples (Table 3), with losses of the lower-molecular-mass species (PAHs 1–3) likely occurring during nitrogen-blow-down concentration of extracts. The differences between the recoveries from the simulated and actual SPMD dialysates stem from losses occurring during dialysis of and processing of the SPMDs. There is good precision among the data, with standard deviations typically below 5%. These recoveries are similar to those achieved in previous experiments using the HPLC–GPC system conducted by the developers of the SPMD technology [11]. No methyl oleate was found in the post-SPE samples, indicating complete removal. Fig. 1a and b are chromatograms of the pre- and post-cleanup of the simulated SPMD samples, illustrating the extent of this removal. Prior to this cleanup PAHs 7 and 8 were not reliably quantifiable.

The restricted-access sorbent used here consists of an outer and inner zone. The outer zone located solely on the exterior surface and crowded at the pore openings of the silica particle consists of hydrophilic bound ligands [Si–$(CH_2CH_2O)_nCOCH_3$ and Si–$(CH_2CH_2O)_nH$] designed to repel proteins and lipids during reversed-phase chromatography. The inner zone (residing within the pores of the particle) is unmodified reactive silica. The specific removal of methyl oleate by the bound-ligand likely stems from polar interactions between the methyl ester groups on the ligand and methyl oleate by hydrogen bonding, dipole interactions, or a combination. However, other interactions between the ligands and the methyl oleate are not ruled out. The fact that PAHs are not retained (as they are with silica gel—the basis of the cleanup employed in EPA method 610 for the determination of PAHs in water [15]) indicates no interaction with the silica moieties in the

Table 3
Mean recoveries (%) and standard deviations (SD) from SPE cleanup of simulated SPMD extracts and dialysates of spiked SPMDs ($n=3$)[a]

No.	Name	5 μg/ml simulated SPMD		5 μg SPMD spike		10 μg SPMD spike	
		Recovery (%)	SD (%)	Recovery (%)	SD (%)	Recovery (%)	SD (%)
1	Naphthalene	72	2.0	24	13.1	36	1.2
2	Acenaphthylene	83	1.2	54	7.2	56	2.5
3	Acenaphthene	85	1.2	57	7.0	59	2.5
4	Fluorene	90	0.0	67	5.0	65	2.9
5	Phenanthrene	97	2.3	76	4.0	72	2.9
6	Anthracene	99	2.3	76	4.0	72	2.9
7	Fluoranthene	101	4.2	81	4.2	79	2.3
8	Pyrene	101	4.2	81	3.1	79	2.5
9	Benz[*a*]anthracene	101	4.2	83	4.2	82	2.6
10	Chrysene	108	4.0	83	4.2	82	2.6
11	Benzo[*b*]fluoranthene	103	4.2	85	4.2	83	1.7
12	Benzo[*k*]fluoranthene	103	4.2	85	4.2	83	1.7
13	Benzo[*a*]pyrene	103	3.1	85	3.1	81	2.1
14	Indeno[1,2,3-*cd*]pyrene	99	2.3	93	1.2	89	1.5
15	Dibenz[*a,h*]anthracene	101	5.0	90	2.0	88	2.3
16	Benzo[*ghi*]perylene	107	3.1	93	2.3	88	1.5

[a] One ml extract was applied to a conditioned 1000 mg Diazem SPE column and eluted with 19 ml hexane–CH_2Cl_2 (97:3) by gravity feed elution. Extracts: Simulated SPMD, 5 μg PAHs + 17.6 mg methyl oleate in 1 ml hexane. SPMD spikes, 1 ml (in hexane) dialysates of SPMDs containing 5 or 10 μg of PAHs. Eluates were collected, concentrated, and subsequently analyzed by capillary GC–MS.

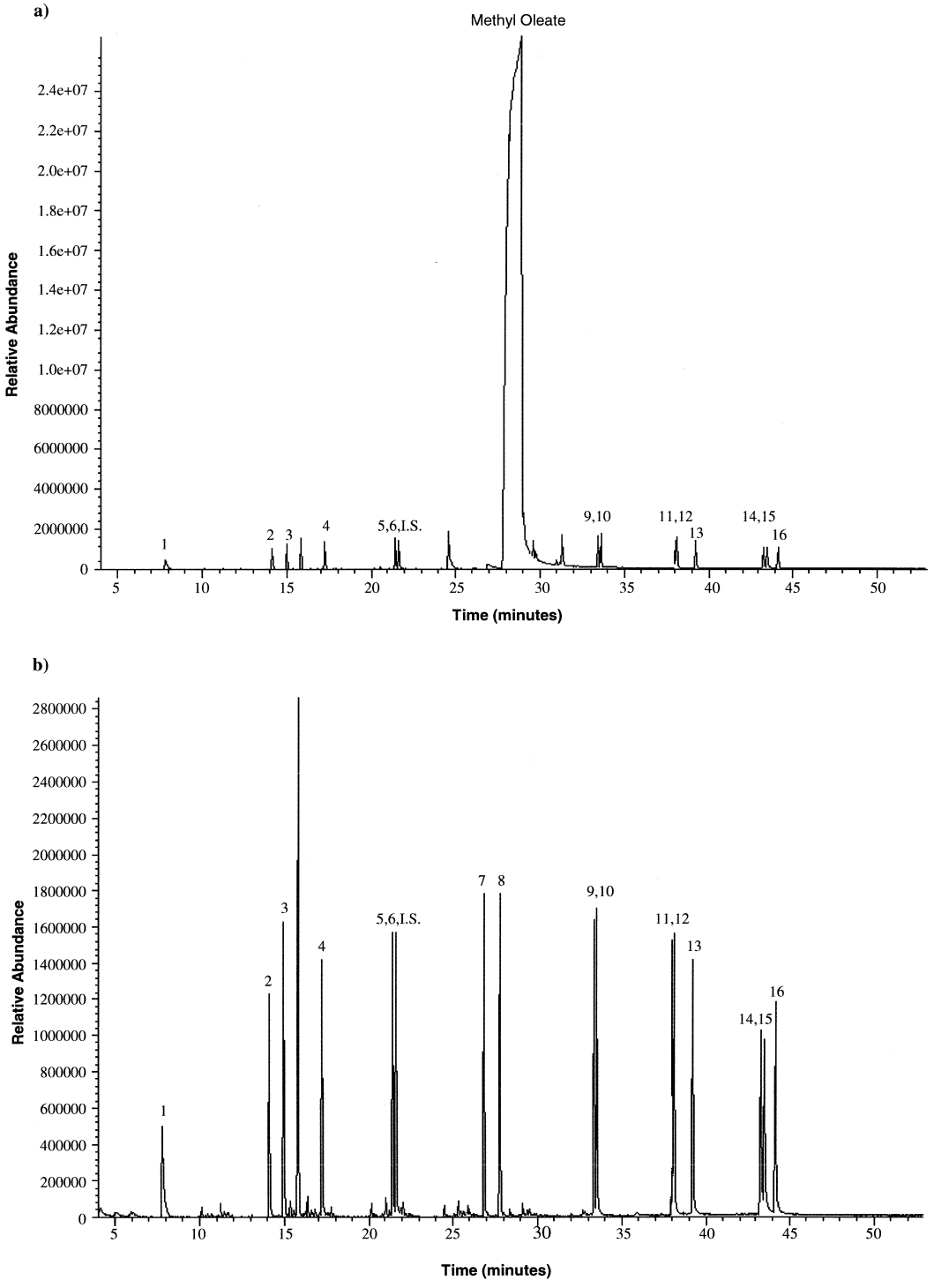

Fig. 1. Chromatograms of a simulated SPMD extract (a) prior to and (b) following cleanup with a 1000 mg dual-zone restricted-access SPE cartridge. Peak numbers correspond to compound numbers in Table 3.

dual-zone restricted-access cartridge. This is possibly because of their inability to diffuse through the exterior ligand into the pores at the flow-rate used.

4. Conclusions

The results have been verified by the developers of the SPMD technology at the United States Geologic Survey (USGS) Columbia Environmental Research Center (CERC) and extended to show separation of polychlorinated biphenyls (PCBs), tetrachlorodibenzodioxin (TCDD), and most of the EPA priority-pollutant organochlorine pesticides (all environmental contaminants of major concern) from methyl oleate using this technique [16].

Overall, the SPE protocol provides a simple, cost-effective method for methyl oleate removal, and allows for further manipulation of samples or direct chromatographic analysis since there is no need for solvent transfer. Further processing requires only concentration of the post-cartridge eluates for analysis via GC–MS. This work represents the first use of this type of sorbent in a normal-phase application which, if adapted to other analytical schemes, could greatly expand the utility of this type of sorbent.

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