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Fast and sensitive environmental analysis utilizing microextraction in packed syringe online with gas chromatography–mass spectrometry Determination of polycyclic aromatic hydrocarbons in water

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Abstract

A new sensitive, selective, fast and accurate technique for online sample preparation was developed. Microextraction in a packed syringe (MEPS) is a new miniaturised, solid-phase extraction (SPE) technique that can be connected online to GC or LC without any modifications. In MEPS approximately 1 mg of the solid packing material is inserted into a syringe (100–250 ml) as a plug. Sample preparation takes place on the packed bed. The bed can be coated to provide selective and suitable sampling conditions. The new method is very promising. It is very easy to use, fully automated, of low cost and rapid in comparison with previously used methods. The determination of polycyclic hydrocarbons (PAHs) in water was performed using MEPS as sample preparation method online with gas chromatography and mass spectrometry (MEPS–GC–MS). The results from MEPS as sample preparation were compared with other techniques such as stir bar sorptive extraction (SBSE) and solid-phase microextraction (SPME). The method was validated and the standard curves were evaluated by the means of quadratic regression and weighted by inverse of the concentration: $1/x$ for the calibration range 5–1000 ng/L. The MEPS applied polymer (silica-C8) could be used more than 400 times before the syringe was discarded. The extraction recovery was about 70%. The results showed close correlation coefficients ($R > 0.998$) for all analytes in the calibration range studied. The accuracy of MEPS–GC–MS was between 90 and 113% and the inter-day precision ($n = 3$ days), expressed as the relative standard deviation (RSD%), was 8–16%. MEPS reduced the handling time by 30 and 100 times compared to SPME and SBSE, respectively.

Keywords: Online sample preparation; Microextraction in packed syringe; Gas chromatography/mass spectrometry; Polycyclic aromatic hydrocarbons; Validation

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a large group of organic compounds that are formed by the incomplete combustion of organic matter by natural (e.g. carbonisation) and anthropogenic processes. The carcinogenic and genotoxic potential of these compounds has attracted most attention. A number of PAHs as well as coal-tar and some occupational exposures to combustion emissions containing these compounds have shown carcinogenicity in experimental animals and genotoxicity and mutagenicity in vitro and in vivo [1]. Several analytical procedures have been developed for PAHs analysis in water, soil, air and food matrices. Common extraction methods of PAHs from wastewater samples involve liquid–liquid extraction (LLE) [2] using non-polar solvents or solid-phase extraction (SPE) [3]. Solid-phase microextraction (SPME) [4] and stir bar sorptive extraction (SBSE) were also used to extract PAHs from aqueous samples [5,6]. In addition, there is a need for development of more selective sorbents for sample clean-up and enrichment [7–10].

Microextraction in packed syringe (MEPS) is a new technique for miniaturized solid-phase extraction that can be connected online to gas chromatography (GC) or liquid chromatography (LC) without any modifications [11–14]. In MEPS, approximately 1 mg of the solid packing material is inserted into a syringe (100–250 μL) as a plug. The sample (10–250 μL) is withdrawn through the syringe by an autosampler. When the

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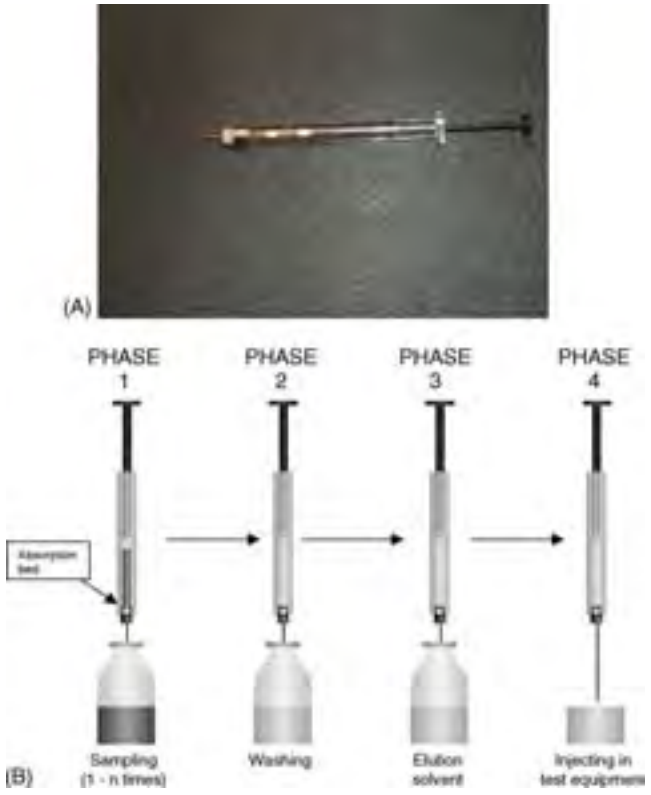

Fig. 1. (A) Packed syringe and (B) a schematic drawing of MEPS (the process is fully automated).

sample has passed through the solid support, the analytes are adsorbed to the solid phase. The solid phase is then washed once by water to remove the interfering material. The analytes are then eluted with an organic solvent such as methanol or the LC mobile phase (20–50 μL) directly into the instrument's injector. The process is fully automated. Any adsorption material such silica based (C2, C8, C18), restricted access material (RAM) or molecular imprinted polymers (MIPs) can be used (Fig. 1).

In this study, we describe a new automated method utilizing MEPS as sample preparation online with GC–MS for the analysis of PAHs in water. Compared to liquid–liquid extraction and commercial solid phase extraction, it saves time and reduces solvent waste significantly.

2. Experimental

2.1. Reagents and materials

Fluorene, anthracene, fluoranthene, pyrene and chrysene were delivered by Sigma–Aldrich (Stockholm, Sweden). [$^2H_7$]Ropivacaine was used as internal standard (I.S.) supplied by the Department of Medicinal Chemistry, AstraZeneca (Södertälje, Sweden) and methanol LiChrosolv grade by Merck (Darmstadt, Germany). Milli-Q water (Millipore, Billerica, MA, USA) was used to prepare the samples.

2.2. Instrumentation

The GC–MS system consisted of an HP 6890-Plus gas chromatograph and a mass selective detector model 5973 (Agilent, Palo Alto, CA, USA) equipped with a programmed temperature vaporiser (PTV) and a Combi Pal autosampler (Crelab, Uppsala, Sweden). The PTV system was an OPTIC 2 (ATAS International, Veldhoven, The Netherlands). The PTV conditions were: vent flow 100 mL/min, vent time 3 min (evaporation time), purge flow 2 mL min^{-1} (purge pressure 5 p.s.i.), split flow 50 mL/min and split open time 2 min. The injector temperature was set at 45 °C and after the evaporation period the temperature was raised by 5 °C/s to 300 °C. Helium was used as carrier gas and was obtained from ScanGas (Stockholm, Sweden).

2.3. GC conditions

The column used was an HP50 (50% phenyl dimethylpolysiloxane) fused-silica capillary column (25 m × 0.25 mm I.D., 0.31 μm film thickness) obtained from Agilent (Palo Alto, CA, USA). The gas flow rates were 2 ml/min. The GC oven temperature was programmed for an initial hold of 3 min at 90 °C; the temperature was increased at 50 °C/min to 280 °C.

Conditions for MS measurements were: MS transfer line at 280 °C, ion source at 230 °C, electron impact ionisation (EI) at 70 eV, SIM mode with dwell time 50 ms, solvent delay: 4.5 min. The ions corresponding to anthracene, chrysene, fluoranthene, fluorine and pyrene *m/z*: 178, 228, 202, 166 and 202, respectively. A MSD ChemStation data system (version B.01.00) was used for data processing.

2.4. Preparation of samples

Stock solutions of the analytes in methanol were prepared. From the stock solution a stepwise dilution series were made in water. Spiked water samples were prepared by adding few microliters of stock solution to 1.0 mL water, after which 50 μL of the internal standard (1000 ng/L) was added. The concentration range of standard curves was between 5 and 1000 ng/L.

2.5. MEPS conditions

MEPS was performed using 1 mg of solid-phase material (silica-C8) inserted into a 250 μL gas-tight syringe. The solid-phase material is fixed in the syringe as a plug with filters (polyethylene, 0.2 μm) on both sides. The packed syringe was conditioned first with methanol and then with water (50 μL) before being used for the first time. The water sample (50 μL each) is drawn through the syringe 60 times by the autosampler. The analytes are then eluted with 30 μL methanol directly into the GC injector. The multiple pulling/pushing of the sample by the syringe increase the extraction recovery. Also, using a small amount of the adsorbing (1 mg) make it easy to wash it and use the same syringe many times. In MEPS standard syringe with removable needle was used and no modifications were needed either for autosampler or GC.

Table 1
Regression parameters, limit of detection (LOD) and limit of quantification (LOQ)

	Anthracene	Chrysene	Fluoranthene	Fluorene	Pyrene
R^2 ($n = 6$)	0.9990	0.9981	0.9992	0.9982	0.9989
LOD (ng/L)	5	5	5	1	1
LOQ (ng/L)	5	5	5	5	5

3. Result and discussion

3.1. MEPS method development

The method using microextraction in packed syringe was used with silica-C8 (1 mg) as sorbent material. The recovery was measured as the response of a processed spiked sample expressed as peak area and compared to that of pure standard solution (1000 ng/L). To increase the sensitivity of the method, the sample was pumped up and down through the syringe 60 times by the autosampler with a speed of 20 μL/s and the syringe was warmed up to 40 °C under the elution step using methanol as elution solution. The extraction time was only about 2 min and the recovery was 70%. To avoid carry over the syringe was washed five times by methanol after each run.

3.2. Calibrations

Calibration curves of water samples spiked with PAH standards were performed in the range 10–1000 ng/L, with [$^2H_7$]ropivacaine as internal standard. Table 1 shows the correlation coefficients of between 0.9981 and 0.9992 for the different analytes. The results are shown in Table 1. In addition, data from calibration curves of spiked water samples with back calculated values, accuracy and precision data are shown in Table 2.

3.3. Selectivity accuracy and precision

The developed method using MEPS with silica-C8 material followed by GC–MS showed good selectivity for the studied compound. Typical chromatograms presented in Fig. 2, from a water sample spiked with PAHs (100 ng/L) and from a blank water sample.

The intra-day precisions (relative standard deviation, RSD) at three different concentrations for quality control (QC) samples were about 5.6–14% ($n = 6$) and the accuracy −11 to 16% ($n = 6$). The inter-day precisions (RSD) were 8.3–16% ($n = 18$). The accuracy varied from 10 to 13% ($n = 18$). The accuracy and precision data are summarized in Table 3.

3.4. Limit of quantification (LOQ) and carry-over

The limit of detection (LOD), defined as the lowest detectable concentration (S/N ≥ 3), was in the range:1–5 ng/L and the limit of quantification (LOQ) defined as the lowest measurable concentration to 5 ng/L for the different PAH analytes. The carry-over was tested by injecting heptane after the highest standard concentration. To eliminate the memory effect, the MEPS is washed four times by methanol and five times by water after every injection. The carry-over was about 0.2–1%.

Table 2
Back-calculated values of the calibration samples

Compound	Concentration (ng/L)	Mean calculated concentration ($n = 6$)	Accuracy (%)	RSD (%)
Anthracene	10	11	110	5.3
	50	42	84	12
	100	115	115	9.8
	200	173	86	10
	400	403	101	10
	500	524	105	8.8
	800	777	97	7.0
	1000	1019	102	3.7
Chrysene	10	12	121	1.4
	50	53	107	1.2
	100	87	87	16
	200	196	98	5.2
	400	448	112	13
	500	526	105	13
	800	789	99	5.3
	1000	968	97	4.0
Fluoranthene	10	11	107	8.9
	50	50	100	21
	100	90	91	13
	200	185	93	13
	400	415	104	12
	500	517	103	13
	800	755	94	4.8
	1000	975	98	3.7
Fluorene	10	9.0	86	7.6
	50	51	103	4.5
	100	105	105	2.7
	200	225	112	4.8
	400	373	93	7.6
	500	480	96	11
	800	765	96	5.9
	1000	952	95	7.8
Pyrene	10	9.0	85	8.3
	50	57	115	7.3
	100	102	102	13
	200	192	96	7.0
	400	408	102	7.4
	500	532	106	6.0
	800	769	96	8.6
	1000	1004	100	1.9

3.5. Comparison of extraction methods

The results of the present study were compared with the results from other sample preparation techniques such as SBSE and SPME (Table 4). The present method (MEPS) showed comparable values of the accuracy and precision with the other methods for the analysis of PAH in water samples. In addition MEPS reduced the extraction time for the analytes studied by 30–100 times compared to earlier studies using SPME and SBSE. Compared with SPME, MEPS is more sensitive and more robust. Furthermore, the present method used a low sample volume of

Table 3
The accuracy and precision at various concentrations in water

Compound	Concentration (ng/L)	Intra-assay accuracy (%, $n = 6$)	RSD (%)	Inter-assay accuracy (%, $n = 18$)	RSD (%)
Anthracene	150	116	14	90	16
	300	97	11	103	11
	600	89	11	98	12
Chrysene	150	105	8.5	101	13
	300	98	9.1	106	11
	600	98	12	95	13
Fluoranthene	150	95	14	99	11
	300	98	7.1	104	10
	600	91	5.6	99	9.1
Fluorene	150	108	8.2	113	8.3
	300	110	12	107	11
	600	96	9.9	92	10
Pyrene	150	112	12	107	15
	300	101	6.6	105	8.3
	600	94	13	100	9.6

Table 4
Comparison of accuracy and precision using MEPS, SPME and SBSE

Compound (50ng/L)	Accuracy (%)			Precision RSD (%)			Limit of detection (ng/L)			Extraction time (min)		
	MEPS	SPME [4]	SBSE [6]	MEPS	SPME [4]	SBSE [6]	MEPS	SPME [4]	SBSE [6]	MEPS	SPME [4]	SBSE [6]
Anthracene	84	81	99	12	3	6	5	100	1.2	2	30	200
Chrysene	107	81	100	1	4	5	5	90	0.2	2	30	200
Fluoranthene	100	84	100	9	4	4	5	100	1.2	2	30	200
Fluorene	103	96	97	5	5	4	1	40	0.7	2	30	200
Pyrene	115	86	100	7	3	3	1	40	0.7	2	30	200

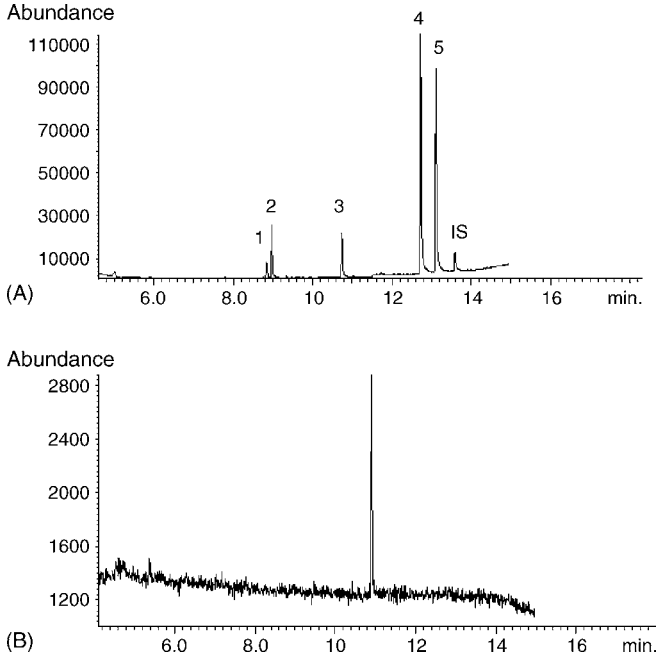

Fig. 2. GC–MS chromatograms obtained from (A) spiked water sample with 1: anthracene; 2 chrysene; 3: fluoranthene; 4: fluorine; 5: pyrene (50–100 ng/L) and [$^2H_7$]ropivacaine (I.S.) and (B) blank water.

1.0 mL, compared to 10 and 30 mL using SBSE and SPME, respectively.

4. Conclusions

A new sensitive, selective and accurate online sample preparation technique was developed and validated for the determination of PAH traces in aqueous samples. The reproducibility of the applied method for PAH analysis is below 10% at 50 ng/L. Detection limits are between 1 and 5 ng/L. Compared with SBSE, MEPS reduced sample preparation time by about 100 times and it is fully automated. Compared with SPME, the new technique is more robust. In SPME the sampling fibre of SPME is quite sensitive to the nature of the sample matrix. Also, much higher extraction recovery (higher sensitivity) can be obtained using MEPS compared to SPME. The packed syringe can also be used several times, more than 400 times for water samples. This study demonstrated that the present method could be attractive for the analysis of other groups of compounds, e.g. pesticides, herbicides, and phenols. This approach to sample analysis is very promising for many reasons: (1) it is easy to use; (2) fully automated online procedure; (3) rapid; and (4) the cost of analysis is minimal when compared to conventional solid-phase extraction. The method can be used for small sample volumes (50 μL) as well as large volumes (>1 mL).

References

[1] European Commission, SCF/CS/CNTM/PAH/29 Final, Opinion of the Scientific Committee on Food on the risks to human health of polycyclic aromatic hydrocarbons in food, 2002.
[2] D. Lin, Y. Tu, L. Zhu, Food Chem. Toxicol. 43 (2005) 41.
[3] M.S. García-Falcón, B. Cancho-Grande, J. Simal-Gándara, Food Chem. 90 (2005) 643.
[4] E. Cortazar, O. Zuloaga, J. Sanz, J.C. Raposo, N. Etxebarria, L.A. Fernandez, J. Chromatogr. A 978 (2002) 165.
[5] V.G. Zuin, L. Montero, C. Bauer, P. Popp, J. Chromatogr. A 1091 (2005) 2.
[6] B. Kolahgar, A. Hoffmann, A.C. Heiden, J. Chromatogr. A 963 (2002) 225.
[7] J. O'Reilly, Q. Wang, L. Setkova, J.P. Hutchinson, Y. Chen, H.L. Lord, C.M. Linton, J. Pawliszyn, J. Sep. Sci. 28 (2005) 2010.
[8] H. Kataoka, Curr. Pharm. Anal. 1 (2005) 65.
[9] H. Kataoka, Trends Anal. Chem. 22 (2003) 232.
[10] E.M. Thurman, K. Snavely, Trends Anal. Chem. 19 (2000) 18.
[11] M. Abdel-Rehim, J. Chromatogr. B 801 (2004) 317.
[12] M. Abdel-Rehim, Z. Altun, L. Blomberg, J. Mass Spectrom. 39 (2004) 1488.
[13] Z. Altun, M. Abdel-Rehim, L. Blomberg, J. Chromatogr. B 813 (2004) 129.
[14] M. Vita, P. Skansen, M. Hassan, M. Abdel-Rehim, J. Chromatogr. B 817 (2005) 303.