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Semi-Automated Stir Bar Sorptive Extraction (SBSE) in Combination with HPLC - Fluorescence Detection for the Determination of Polycyclic Aromatic Hydrocarbons in Water

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Introduction

Stir bar sorptive extraction (SBSE) was introduced 1999 by Baltussen et al. [1]. This new sorptive extraction technique bases on the same principles than solid phase microextraction (SPME), but instead of a polymer-coated fibre, stir bars 10 mm long coated with 24 µl PDMS (Twister, GERSTEL) are used for enrichment of organic contaminants from aqueous matrices. Due to the much lower phase ratio (volume of water phase divided by the volume of the PDMS phase) compared to SPME, higher recoveries can be obtained by SBSE. Analogous to SPME, coated stir bars can be desorbed thermally with subsequent gas chromatographic analysis.

An approach of interfacing SBSE to liquid chromatography has been described recently [2]. In this case, the desorption of the extracted analytes was performed using a small volume of acetonitrile (ACN) or an ACN-water mixture. An aliquot of this desorption solution was then analyzed by HPLC.

In this study we describe the possibility of automation of the SBSE desorption step with direct transfer of the liquid extract to HPLC by use of the GERSTEL- MultiPurposeSampler MPS 3 (Twister Back-Extraction). For this purpose the MPS 3 was equipped with a temperature-programmable agitator and a special software. Using this procedure, after extraction of water samples by SBSE, the only manual step is the removal of stir bars from the sample vial and transfer to 250 µl glass inserts placed inside 2 ml autosampler vials. The MPS 3 then adds the ACN-water mixture to the stir bar, transports the vial to the agitator, where it is agitated at a defined temperature during the preset desorption time and finally withdraws an aliquot of the extract, which is injected into the HPLC.

Experimental

Reagents. The PAH calibration mix (10 µg of each compound/ml ACN) was obtained from Supelco (Bellefonte, PA, USA). HPLC grade water and ACN (HPLC, Ultra Gradient Grade) were supplied by Baker (Deventer, The Netherlands). The HPLC method was optimized and validated for 15 of the 16 EPA-PAHs. Acenaphthylene was omitted, because this compound is not detectable with fluorescence detection. Working standards were prepared with ACN. Aqueous standards were prepared by diluting 10 µl organic standard with 10 ml HPLC grade water.

Environmental water sample. TA precipitation water taken from a bulk sampler mounted near a traffic artery in Halle/Saale was used for determination of PAHs.

Stir bar sorptive extraction - pretreatment and extraction of samples. Before use, Twisters were conditioned as follows: They were placed into a vial containing 1 ml of a 1:1 mixture of methylene chloride and methanol for 5 - 10 minutes. Then the solvent mixture was rejected and the procedure was repeated. Afterwards Twisters were dried in a desiccator for at least 30 minutes and then heated to 280 °C in a nitrogen stream of about 100 ml/min for 90 min. Alternatively, a nitrogen stream of 250 °C was applied overnight.

To enrich PAHs, a 10 ml water sample was placed in a 10 ml glass vial and extracted with a preconditioned Twister for 60 min at a stirring speed of 1000 rpm at room temperature. After extraction, the Twister was removed with clean tweezers and dried with a lint-free tissue. Then the Twister was placed into a conical glass insert (250 µl, Agilent Technologies, Waldbronn, Germany) of a 2 ml autosampler vial, which was closed with a metallic cap. For closure of the vials, either metallic crimp caps (GERSTEL, Mülheim an der Ruhr, Germany) or plastic screw caps with a metall ring glued on top were used.

Twister back-extraction and injection of the extract by use of the MPS 3. The MultiPurposeSampler MPS 3 (Gerstel, Mülheim, Germany) used for this study was equipped with a temperature programmable agitator, a 98 well sample tray for 2 ml vials, a 100 µl syringe, a fast wash station and a standard six port injection valve equipped with a 20 µl sample loop, which was connected to the column of the HPLC and the mobile phase line. The MPS 3 was operated with a special version of the GERSTEL MASter Software, MASter Prep Station. With this software version it is possible to combine for one sample preparation different steps of the sampler, for example like moving one vial from a tray to the agitator and adding an amount of liquid from a solvent reservoir to a vial, in one sequence loop. For each step special methods have to be created. These methods can be different add-, move-, mix-, inject- and wait-methods. The vials with loaded Twisters were placed into the sample tray, subsequently the addition of 200 µl desorption solvent, mixing of the vials and injection of the extract was performed automatically.

A volume of 200 µl desorption solvent was chosen, in order to enable the Twister to be completely immersed with a supernatant of solvent of about 5 mm. The sample preparation procedure (sequence loop) which was carried out by the sampler automatically was as follows:

1) Add method 1: The syringe is filled with 100 µl desorption solvent (70:30/ACN:H2O) at the fast wash station with a fill speed of 5 µl/min; the solvent is disposed into the vial containing the Twister using a vial penetration of 14.3 mm (plastic screw cap) or 12.1 mm (metallic crimp cap). This procedure is repeated once.

2) Move method 1: The vial with Twister and desorption mixture is transferred from the sample tray to the agitator.

3) Mix method 1: The vial is agitated at a defined temperature (24-45°C - set at the keypad terminal) with a stirring speed of 0-750 rpm for 2-10 minutes.

4) Move method 2: The vial is transferred from the agitator to the sample tray.

5) Inject method 1: An aliquot of 35 µl desorption mixture is withdrawn above the Twister at a fill speed of 5 µl/min (vial penetration 14.3/12.1 mm) using 5

syringe pumps. This aliquot is injected into the six port valve of the MPS 3 at an injection penetration of 21.9 mm which starts the HPLC run. The sample loop is overloaded to ensure small air bubbles to be purged out before the injection. The injection volume corresponded to the volume of the sample loop (20 µl). Afterwards the syringe is cleaned at the fast wash station.

6) Wait method 1: The MPS 3 waits for a preset time - depending on the lenght of the HPLC run and the desorption time chosen (for example 25 min including desorption time of 8 min)- before starting the add-method again with the next vial containing another Twister.

HPLC - fluorescence detection. Analysis of the Twister extracts was carried out with an HP 1100 system equipped with a programmable fluorescence detector (HP 1046A). The PAHs were separated on a Vydac 201 TP 52 (250 mm x 2.1 mm i.d.) column. ACN and HPLC grade water were used as mobile phase. The composition gradient started with 50% water and 50% ACN, then the ACN content was increased to 60% (0-2 min), 90% (2-13.5 min) and 95% (13.5-19 min). This level was held constant for 7 min until the end of the analysis. The column oven temperature was 22°C. The excitation (ex) and emission (em) wavelength program used is given in Table 1.

Table 1. Fluorescence wavelength programme.

Compound	Excitation [nm]	Emission [nm]
Naphthalene	277	337
Acenaphthene	227	315
Fluorene	227	315
Phenanthrene	252	372
Anthracene	252	372
Fluoranthene	237	440
Pyrene	237	440
Benzo(a)anthracene	277	393
Chrysene	277	393
Benzo(b)fluoranthene	258	442
Benzo(k)fluoranthene	266	415
Benzo(a)pyrene	266	415
Dibenz(a,h)anthracene	295	425
Benzo(g,h,i)perylene	295	425
Indeno(1,2,3)pyrene	251	510

Although the selected column has the advantage of a very low solvent consumption, the sample volume which can be injected without deterioration of the peak shapes is limited. With ACN as solvent, only 2 µl of the extract could be injected, whereas the use of an ACN-water mixture of 70:30 (v/v) for desorption of the Twister enables the injection of 20 µl (volume of the sample loop) of the total extract of 200 µl. Therefore, in order to improve detection limits, an ACN-water mixture of this composition was chosen.

Results and Discussion

Reproducibility. At the beginning of the study the reproducibility of the injection of the MPS 3 into the HPLC using a composite standard of 1.4 µg/l of each compound in ACN: water (70:30) and an injection volume of 20 µl was checked. The relative standard deviation of four consecutive injections was between 0.9 - 4.6%. Then, the reproducibility of the Twister Back-Extraction was examined under similar conditions, which were found to be optimal for the off-line desorption [2]. Results were comparable to those obtained by off-line desorption; the relative standard deviation of 8 consecutive automatic Twister Back-Extractions ranged from 4.5 to 14.3%. Details for each compound are given in Table 2.

Table 2. Reproducibility of Twister Back-Extraction (water spiked to 0.1 µg/l each compound, 1 h extraction; desorption time 10 min at 35°C and 250 rpm).

Compound	RSD (n = 8) [%]
Naphthalene	8.3
Acenaphthene	6.6
Fluorene	9.3
Phenanthrene	7.3
Anthracene	6.5
Fluoranthene	7.2
Pyrene	6.4
Benzo(a)anthracene	6.7
Chrysene	6.0
Benzo(b)fluoranthene	6.2
Benzo(k)fluoranthene	6.2
Benzo(a)pyrene	5.4
Dibenz(a,h)anthracene	14.3
Benzo(g,h,i)perylene	4.6
Indeno(1,2,3)pyrene	11.2

Thus, the automatic Twister Back-Extraction procedure can be considered equivalent to the off-line method.

Optimization of desorption parameters. As, for PAHs, the extraction equilibrium of SBSE in spiked water was found to be approached after 1 hour stirring [2], this extraction time was chosen for all investigations presented in this application. Every variation of desorption parameters was applied twice on different Twisters. For desorption of loaded Twisters by use of the MPS 3, the first parameter to be optimized was the agitation speed. The efficiency of desorption was compared for 0, 250, 500 and 750 rpm at 35°C using a desorption time of 2 minutes. As shown in Figure 1, the acceleration of orbital shaking led to an increase of desorbed PAHs from 0 to 750 rpm; this increase was most pronounced from 0 to 500 rpm and weakened when further enhancing the agitation speed to 750 rpm.

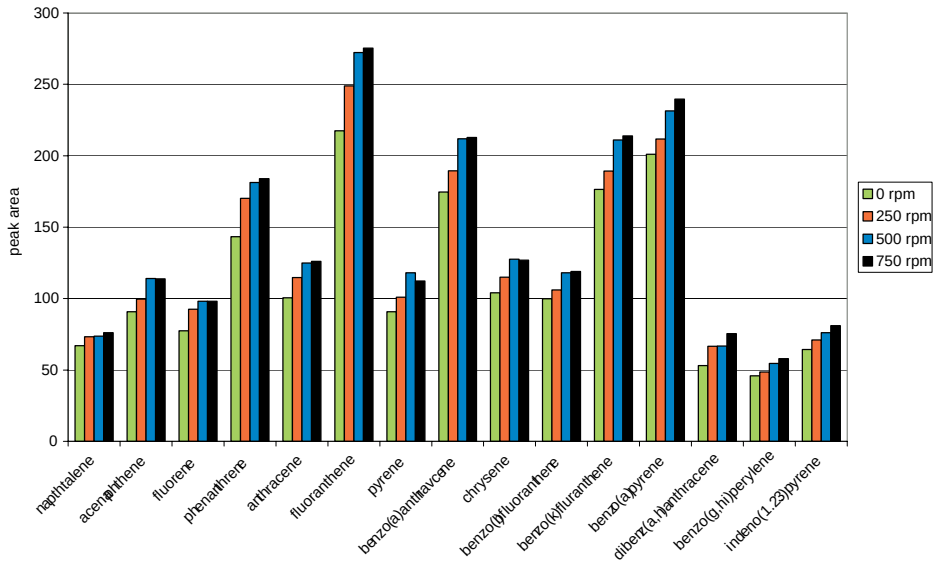

Figure 1. Optimization of agitation speed for Twister Back-Extraction (water spiked to 0.1 µg/l each compound, extraction time 1 h; desorption time 2 min at 35 °C).

In the next step, the desorption time of the Twister Back-Extraction was varied from 2 to 10 minutes (Figure 2) using an agitation speed of 750 rpm and a temperature of 35°C. The amount of PAHs desorbed from the Twister increased when extending the desorption time from 2 to 8 minutes; a further prolonging of desorption led to a decrease of recovery. Thus, 8 minutes of desorption were chosen for the remaining investigations.

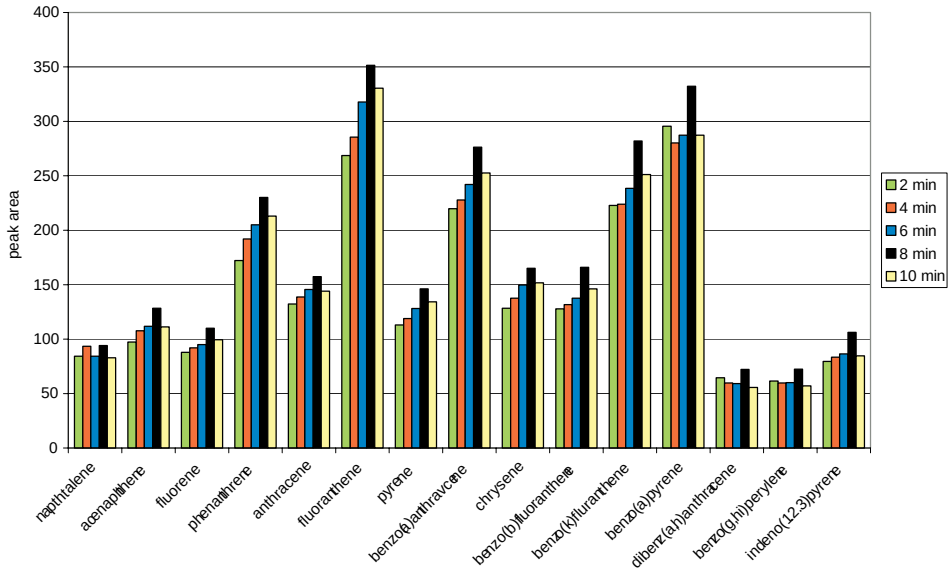

Figure 2. Optimization of desorption time for Twister Back-Extraction (water spiked to 0.1 µg/l each compound, extraction time 1 h; agitation speed 750 rpm at 35 °C).

Finally, the temperature of the agitator was increased, in order to promote the desorption of PAHs from the Twister during agitation. The results of temperature variation are presented in Figure 3. Enhancing the temperature from 24 to 38 °C led to a small increase of the peak area of desorbed PAHs. A further rise of temperature only improved recoveries for some PAHs, whereas for most higher boiling PAHs 38°C represented the optimal desorption temperature. According to these results detection limits and linear dynamic range of the method were evaluated under the optimized conditions of 38 °C, 750 rpm and 8 min desorption.

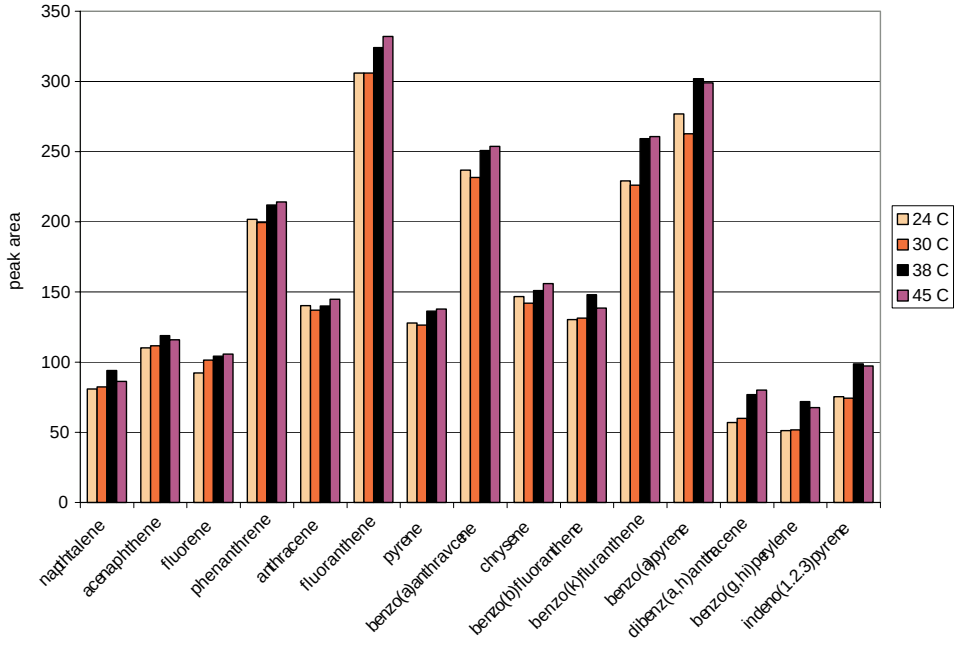

Figure 3. Effect of elevated temperatures on the desorption efficiency of Twister Back-Extraction (water spiked to 0.1 µg/l each compound, extraction time 1 h; desorption time 8 min at 750 rpm).

Detection limits, linear dynamic range and recovery. Calibration and the determination of detection limits was performed by extracting spiked HPLC water samples at nine different calibration levels (1, 2, 4, 6, 10, 20, 50, 100 and 200 ng/l). Each level was measured in duplicate. The limit of detection (LOD) was defined as the concentration corresponding to a signal-to-noise-ratio of 3. The results are listed in Table 3.

Table 3. Calibration data obtained for SBSE - Twister Back-Extraction under optimized desorption conditions (1 h extraction; desorption time 8 min at 38 °C and 750 rpm; [a]water spiked to 0.1 µg/l).

Compound	LOD [ng/l]	Linear range [ng/l]	R^2	Recovery [%][a]
Naphthalene	1.0	1.0 - 200	0.998	54.8
Acenaphthene	2.0	2.0 - 200	1.000	67.2
Fluorene	1.0	1.0 - 200	0.999	77.6
Phenanthrene	0.5	1.0 - 200	1.000	84.2
Anthracene	1.0	1.0 - 200	0.999	72.4
Fluoranthene	1.0	1.0 - 200	1.000	78.2
Pyrene	2.0	2.0 - 200	1.000	75.7
Benzo(a)anthracene	0.5	0.5 - 200	0.999	74.3
Chrysene	0.8	0.8 - 200	0.999	75.8
Benzo(b)fluoranthene	0.8	0.8 - 200	1.000	69.8
Benzo(k)fluoranthene	0.4	0.4 - 200	0.999	67.0
Benzo(a)pyrene	0.4	0.4 - 200	0.998	58.0
Dibenz(a,h)anthracene	1.0	1.0 - 200	0.979	33.5
Benzo(g,h,i)perylene	1.5	1.5 - 200	0.988	45.7
Indeno(1,2,3)pyrene	2.5	2.5 - 200	0.989	49.4

The LODs estimated for spiked HPLC water were very low and ranged from 0.4 to 2 ng/l. Compared to the off-line procedure [2] LODs were about two times higher due to the larger volume of desorption solvent, which had to be applied for Twister Back-Extraction. Linearity was found between the detection limit and the highest calibration level of 200 ng/l with correlation coefficients between 0.979 and 1.000.

Recovery of Twister Back-Extraction was calculated by spiking the same amount of methanolic PAH-standard used for preparation of 10 ml aqueous standard directly into 200 µl desorption solvent. A chromatogram of a precipitation water analyzed by the optimized SBSE Twister Back-Extraction procedure is shown in Figure 4, the concentration of PAHs in this sample were calculated using the calibration data in Table 3.

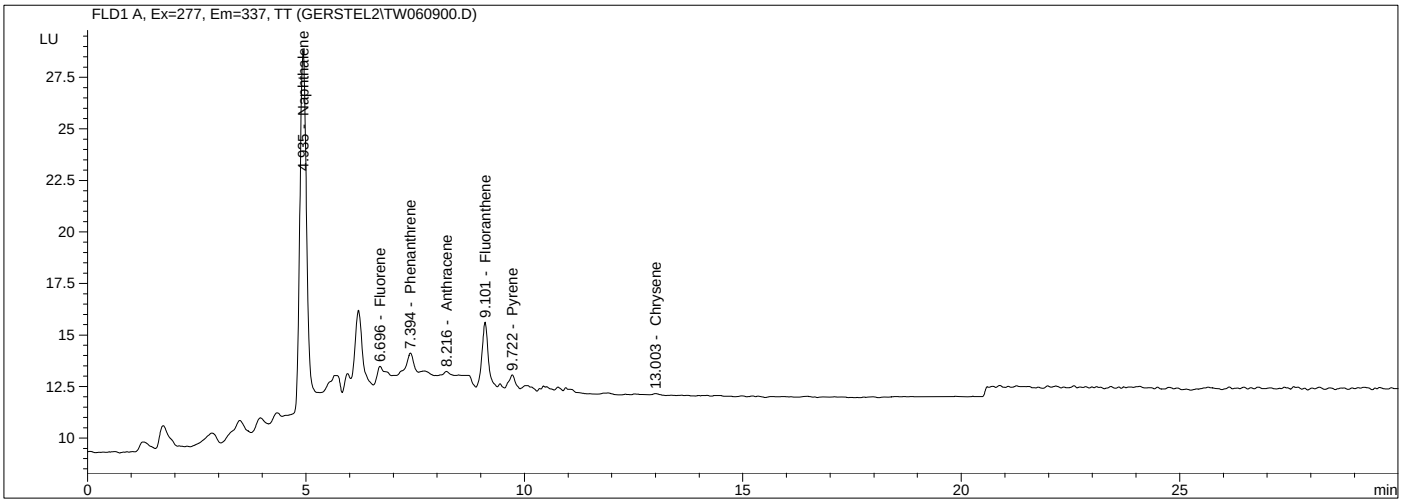

Figure 4. Chromatogram of a precipitation water analyzed by SBSE Twister Back-Extraction (extraction time 1 h; desorption time 8 min at 38 °C and 750 rpm; PAH concentrations determined by calibration of SBSE: naphthalene: 225 ng/l, fluorene 3.0 ng/l, phenanthrene 3.5 ng/l, anthracene 2.5 ng/l, fluoranthene 10 ng/l, pyrene 5.6 ng/l, chrysene 2.3 ng/l).

Conclusions

The combination of stir bar sorptive extraction (SBSE) with solvent desorption followed by LC-fluorescence detection enables the sensitive and reproducible determination of PAHs in aqueous samples. By use of the GERSTEL-MultiPurposeSampler MPS 3 for automatic solvent desorption and subsequent injection of the extract, manual operations can be reduced to a minimum. The procedure is robust and easy to handle. Due to the only small deviations between different Twisters many samples can be extracted in parallel, in this way a high sample throughput can be realized. The application range of this method can probably be extended to other groups of compounds, e.g. pesticides, herbicides and phenols.

References

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