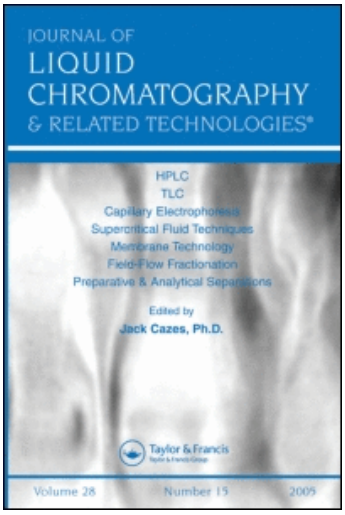

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Fast and Precise SBSE-HPTLC/FLD Method for Quantification of Six Polycyclic Aromatic Hydrocarbons Frequently Found in Water

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Fast and Precise SBSE-HPTLC/FLD Method for Quantification of Six Polycyclic Aromatic Hydrocarbons Frequently Found in Water

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Abstract: The newly developed SBSE-HPTLC/FLD method for analysis of PAH was focused on rapidness, cost-effectiveness, and the usage of more environmentally friendly, chlorine-free solvents. The sample preparation was streamlined and allowed 30 water samples to be extracted and analyzed within a routine working day. The validation showed that this method was reliable for control of the limit levels for benzo[a]pyrene (10 ng/L) and for the sum of benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[ghi]perylene and indeno[1,2,3-cd]pyrene (100 ng/L) in drinking, mineral, ground, or slightly to moderately contaminated surface water. Starting around the LOQ (0.08–0.44 ng/band depending on the PAH) the linearity showed coefficients of correlation $\geq$0.9920. The repeatabilities (%RSD, $n = 3$ at 10–58 ng/L depending on the PAH) using the same twister ranged between 0.4 and 6.3% and using 3 different twisters between 0.8 and 7.0%. Respective recoveries ($n = 3$) using the same twister were between 87–100% depending on the PAHs, using 3 different twisters between 77–103%. Moreover digital quantification proved to be a rapid and reliable alternative to conventional scanning.

Keywords: Drinking water, Food, HPTLC, PAH, Planar chromatography, SBSE, Quantification

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INTRODUCTION

Food can be contaminated with polycyclic aromatic hydrocarbons (PAHs) due to processing practices, like heating, drying, smoking, roasting, or charcoal grilling, besides packaging of food or environmental contamination by particles from the air. Although PAHs are a large group of about 10,000 compounds and are showing half-lives up to decades, only few occur in considerable amounts in the food. The current EU legislation on food contaminants (Regulation (EC) 208/2005)[1] follows a recommendation by the Scientific Committee on Food (SCF)[2] to use the carcinogen (group A2) benzo[a]pyrene (BaP) as a marker. The maximum allowed concentrations for BaP in various food products are set in the range of 1–10 μg/kg with the exception of liquid smoke flavoring primary products for which also a limit value for benz[a]anthracene is set at 20 μg/kg.[3]

Some PAHs, especially the lighter ones, are water-soluble or can be adsorbed on particles and thus be found in rivers and groundwater.[4] Hence, PAH have to be monitored, besides other priority pollutants, by the EU Member States in surface waters, ground waters, and coastal waters according to the Water Framework Directive (2000/60/EC).[5] The Drinking Water Directive (98/83/EC)[6] sets the maximum level for BaP at 0.010 μg/L and for the sum of benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[ghi]perylene (BgP) and indeno[1,2,3-cd]pyrene (IcP) at 0.10 μg/L. These limit values in the ultra trace level require enrichment techniques, like liquid liquid extraction (LLE), solid-phase extraction (SPE), membrane extraction, solid-phase microextraction (SPME) or stir-bar sorptive extraction (SBSE).[7–9] The latter is generally used for extraction of a variety of middle polar to unpolar analytes (log $K_{O/W}$−values >2.7) from the liquid or gaseous phase of different kind of matrices.[10,11] In the field of food analysis SBSE was used, e.g., for extraction of strawberry flavor-giving components (ethyl-3-methyl butyrate, *y*-decalactone) from respective yoghurts,[12] of artificial and authentic flavor-giving components from strawberry products,[13] preservatives (benzoic acid) from soft drinks,[14] pesticides from wine and orange juice,[12,15] off-flavor (geosmin, 2-methylisoborneol and trichloroanisol) from mineral water,[16,17] and PAHs from mate tea.[18] For analysis of PAH in water, SBSE turned out to be less discriminative against polar PAHs and more sensitive due to the by a factor of 100 higher phase ratio if compared to SPME.[19]

An impressive review[20] about the current legislative situation of PAH analysis in the European Union (EU) was given in 2006. Therein, the needs were highlighted for further investigations and analytical research for new EU legislation of food, as well as of the environment. It was reported that fast and precise methods covering

a large range of concentrations and, at the same time, a high sample throughput were necessary to obtain sufficient data for risk assessment and implementation of legislation. The analysis of multiple PAHs is considered necessary to get a clearer picture of levels of different PAHs in food and to verify the use of BaP as a marker. 15 EU-priority PAHs were discussed, together with benzo[c]fluorene (BcF) highlighted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). 13 of the 15 EU-priority PAHs are also recommended by JECFA and eight of them are known to be mutagenic or carcinogenic and also listed by the US Environmental Protection Agency (EPA).

Analytical methods reported were based on column chromatography (HPLC and GC). So far, in proficiency tests, mostly BaP, BaA, BbF, and IcP have been analyzed – additionally chrysene, fluorine, fluoranthene (FLT) and BgP in some studies. A recent proficiency test organized for column chromatographic analysis of the EU-priority PAHs showed that there is still a great need for improvement, as very few of the laboratories were able to analyze the PAHs satisfactorily.[21]

Considering the needs for a fast, precise, and high throughput method, also planar chromatography (HPTLC) is an optimum tool. As reported in a recent review about the contribution of HPTLC to food analysis[22] it has decisive benefits, e.g., regarding minimized sample preparation and high matrix tolerance, high dynamic range of the sample application volume, cost-effective targeted coupling with mass spectrometry, or parallel chromatography under identical environmental conditions. It was shown that, for quantification of sucralose in dietetic products,[23] a throughput of 1,000 runs per day can be performed and that, at the same time, highly reliable results can be obtained. The parallel chromatography of 44 runs within 15 min with 15 mL mobile phase allowed 20-seconds runs with 350 μL mobile phase consumption. The staggered offline system of planar chromatography supported the high throughput because, in this case, the automated instruments of the single steps can work independently from each other. Due to the fixation of the matrix, mainly at the origin, and selective post-chromatographic derivatization, the sample preparation was kept very simple. Despite such clear facts, it was noticed that for food analysis the planar chromatographic usage generally stopped at the screening state and only 10% of the users apply the advanced, high performance mode of the method (HPTLC). This might explain the general misunderstanding of the real potential of this method.

In the following study, the current ISO standard for drinking, mineral, ground, and slightly to moderately contaminated surface water[24] was used as basis and optimized regarding sample preparation and the chromatographic system. The LLE described was tried to be replaced by a more effective sample preparation method regarding

costs, solvent consumption, and time. To the best of our knowledge, it is the first time using SBSE prior to HPTLC analysis. The whole analytical system was investigated regarding the use of chlorine-free (not-halogenated) solvents, as already shown for the mobile phase,[25] and the respective plate impregnation needed for the charge transfer complex formation because pre-impregnated plates are only available from one manufacturer. The current progress in digital detection systems (homogeneous illumination, highly efficient 12-bit cameras) encouraged us to evaluate such rapid quantification tools for this kind of analysis as well as the employment of bio-activity-based detection of PAHs. Hence, the intention was to streamline the current official method to enable a cost-effective, more environmental friendly, rapid, but precise method for routine analysis.

EXPERIMENTAL

Chemicals and Materials

2-Methylanthracene (98.6%) was purchased by Riedel-de Haen (Sigma-Aldrich, Seelze, Germany), benzo(g,h,i)perylene (99%) by Radian (Austin, USA), indeno(1,2,3-cd)pyrene (99%) by Cerilliant (Round Rock, Texas, USA), benzo[b]fluoranthene (98%), fluoranthene (98%), benzo[a]pyrene (97%), benzo[k]fluoranthene (puris.) and caffeine (>99%) by Fluka (Sigma-Aldrich). Acetonitrile (≥99%) was obtained from VWR (Darmstadt, Germany), dichloromethane (>99.5%) from Carl Roth (Karlsruhe, Germany), isopropyl acetate (≥99%) from Fluka and paraffin subliquidum (Ph.Eur.) from Carl Roth. Methanol and n-hexane, both technical grade (BASF, Ludwigshafen, Germany), were distilled prior to use. Ultrapure water ($18\,M\Omega/cm^2$) was generated from a Synergy Ultrapure Water System (Millipore, Schwalbach, Germany).

Twisters (10 mm long) coated with polydimethylsiloxane (PDMS, layer thickness 0.5 mm) were purchased from Gerstel (Mühlheim, Germany). For preparation of handmade stir bars silicone tubes (for analysis No. 14244, 3.5×4.3 mm (ID × OD) and No. 14248, 4.0×6.0 mm, and for high temperature No. 28731, 3.5×4.5 mm) were supplied by Reichelt Chemietechnik (Heidelberg, Germany). Flasks with nitrogen gas 5.0 (99.999%) were delivered by Sauerstoffwerke (Friedrichshafen, Germany).

Chromatography was performed on HPTLC plates silica gel 60 (Merck, Darmstadt, Germany), 20 cm × 10 cm, 0.2 mm in thickness. Alternatively HPTLC plates LiChrospher 60 F_{254s} (Merck), HPTLC plates silica gel 60 $WRF_{254}s$, layer thickness 0.1 mm (Merck), and HPTLC plates Nano-SIL-PAH, 10 cm × 10 cm (Machery-Nagel, Düren,

Germany) were used. For caffeine-impregnation, the silica gel plates (Merck) were dipped in a caffeine solution (2 g caffeine in 120 mL acetonitrile) for 20 min, followed by drying for 15 min at 120°C. The impregnated plates were stored in a desiccator until use.

Standard and Spiking Solutions

PAH standard solutions (Table 1) in methanol were mixed resulting in PAH concentrations in the standard mixture A ranging from 5 to 29 pg/μL. Application of 10, 15, 20, 25, and 30 μL let to calibration ranges of 290–870 pg/band for FLT, 95–285 pg/band for BkF, 108–324 pg/band for BbF, 51–153 pg/band for BaP, 110–330 pg/band for IcP, and 200–600 pg/band for BgP (Table 2). The control standard (IS) 2-methylanthracene was employed in a higher concentration (142 pg/μL, 1.42–4.26 ng/band) due to its lower response. The standard mixture A was diluted 1:10 with methanol (standard mixture B) which was used for

Table 1. Six PAHs frequently found in water plus control standard (IS)

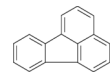
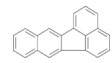
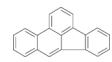
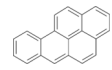
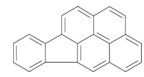
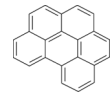
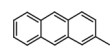
Substance	CAS No.	Structure formula	Marker in water	Carcino-genity
Fluoranthene (FLT)	206-44-0		+	
Benzo[k]fluoranthene (BkF)	207-08-9		+	+
Benzo[b]fluoranthene (BbF)	205-99-22		+	+
Benzo[a]pyrene (BaP)	50-32-8		+	+
Indeno[1,2,3-cd]pyrene (IcP)	193-39-5		+	+
Benzo[g,h,i]perylene (BgP)	191-24-2		+	+
2-Methylanthracene (IS)	613-12-7			

Table 2. Standard and spiking levels (ng/L) of PAHs in water

Substance	Standard mix A (pg/μL methanol) for application of 10, 15, 20, 25 and 30 μL	Standard mix B (pg/μL methanol) diluted 1:10 for spiking	Addition of 200 μL B to water sample (ng/10 mL)	Resulting spiking level in water (ng/L)
FLT	29.0	2.90	0.58	58
BkF	9.5	0.95	0.19	19
BbF	10.8	1.08	0.22	22
BaP	5.1	0.51	0.10	10
IcP	11.0	1.10	0.22	22
BgP	20.0	2.00	0.40	40
IS	141.9	14.19	2.84	284

spiking. Therefore, 200 μL standard mixture B (dissolved in methanol) was added to 10 mL water sample with which also the modifier (2% methanol) was added necessary to prevent glass wall adsorption of PAHs. This resulted in a final spiking level in water in the range from 10 to 58 ng/L. The respective spiking level of the IS was 284 ng/L. All solutions stored refrigerated and protected from light were at least stable for 6 months.

Sample Preparation by SBSE

Cleaning of new or used twisters was performed in a four-step procedure lasting 30 min. First, the twister was cleaned with 1.5 mL dichloromethane-methanol 3:2 (v/v) in a 1.8 mL vial which was placed in an ultrasonic bath for 10 min, followed by 3 min N_2-drying. Then, the cleaning step was repeated with acetonitrile, again followed by drying. Later on dichloromethane was substituted by isopropyl acetate to obtain a complete chlorine-free analytical system. Clean twisters can be stored in a tight vial under nitrogen atmosphere for up to 3 days until usage.

A representative 10 mL aliquot of drinking, mineral, ground, or moderately contaminated surface water (with potential suspended particles) was placed in a 25 mL amber Erlenmeyer flask. For recovery, repeatability, and handmade stir bar studies, 10 mL ultrapure water were spiked with 200 μL of the PAH standard mixture B to obtain spiking levels of the single PAHs between 10 to 58 ng/L, respectively 284 ng/L for the IS. For SBSE a cleaned PDMS twister was added (Figure 1) and the sample was extracted at room temperature at 800 rpm for 140 min. For liquid micro desorption the twister were transferred by a magnetic tool onto a clean, lint-free tissue and then into the 300 μL micro-insert of a amber 1.8 mL vial filled with 150 μL acetonitrile. Desorption took

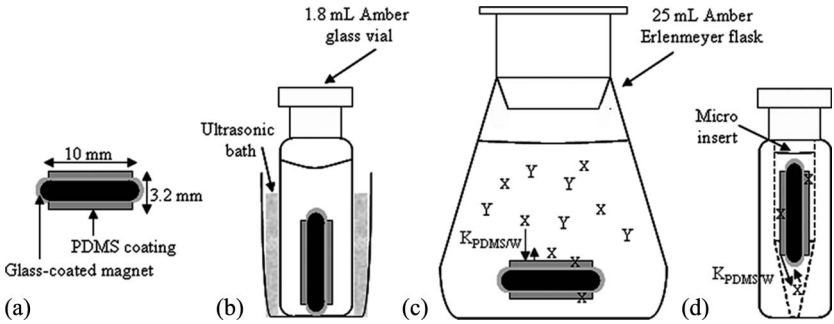


Figure 1. Stir bar sorptive extraction (SBSE) and its workflow (a) layers of the twister with polydimethylsiloxane (PDMS) coating, (b) cleaning of the twister with first isopropyl acetate – methanol 3:2 (v/v) and then acetonitrile, both for 10 min in a vial placed in an ultrasonic bath followed by 3 min N_2-drying, (c) extraction process of 10 mL water sample, and (d) liquid micro desorption in a 300 μL micro-insert filled with 150 μL acetonitrile.

25 min on a heating plate at 50°C. Thereafter, the twister was removed by a magnetic tool and the solution in the vial was used for application.

For the preparation of handmade stir bars, the commercially available silicone tubes were cut into 15 mm pieces, if necessary cut on the long side, and put on respective cleaned magnetic stir bars. Cleaning was performed with 8 mL dichloromethane-methanol 3:2 (v/v) in a beaker for 15 min at 200 rpm, then in a 1.8 mL vial filled with fresh solvent and extracted in an ultrasonic bath for 10 min, followed by 3 min N_2-drying. The cleaning steps were repeated with acetonitrile. Extraction was performed as mentioned above; just for extraction again 200 rpm (instead of 800 rpm) were used to prevent dismantling of the tube from the magnetic stir bar. The silicone tube was removed from the magnetic bar and desorption was performed with 900 μL acetonitrile in an amber 1.8 mL vial under the same conditions as for the twisters, followed by concentration to 250 μL under a stream of nitrogen.

Chromatography

Sample and standard solutions were applied on the caffeine-impregnated HPTLC plate with the Automatic TLC Sampler 4 (ATS 4, CAMAG, Muttenz, Switzerland) using the following settings: area application (band length 7 mm, band width 3 mm), track distance 11 mm, distance from left plate edge 23 mm and from the lower plate edge 9 mm, resulting in 15 tracks per plate, dosage velocity 300 nL/s, as application volumes 10–30 μL of the standard mixture A and 150 μL of the sample extracts

were applied (respectively 250 μL of the handmade stir bar extracts). For determination of the LOQ, 10 μL standard mixture A and 150 μL sample extracts, spiked at 10–58 ng/L depending on the PAH, were applied. Due to area application, a short focusing of the PAHs with methanol up to the upper edge of the area was performed (4 s-run), followed by 10 min-drying at 100°C on the TLC plate heater (CAMAG).

Chromatography was performed at −20°C in a 20 × 10 cm twin trough chamber (CAMAG) placed in a deep freezer up to a migration distance of 80 mm (from the lower plate edge) using a mixture of isopropyl acetate – acetonitrile 7:3 (v/v) as mobile phase. Alternatively, also mixtures of 9:1 and 6:4 or just isopropyl acetate were investigated. Then, the plate was dried for 3 min in a stream of warm air.

For bioactivity-based detection, the Bioluminex assay (Chromadex, Boulder, USA) was used according to its protocol. After application, the plate was dipped in the bacteria solution using the Chromatogram Immersion Device (CAMAG) with a dipping speed of 4.5 cm/s and a dipping time of 1 s. The luminescent images (30 images per plate) were recorded with the BioLuminizer (CAMAG) using an exposure time of 60 s.

For fluorescence enhancement, by a factor of up to 6 for BaP and BgP, the plates were dipped with the Chromatogram Immersion Device (dipping speed 4.5 cm/s, dipping time 1s), in a solution of paraffin-n-hexane 1:1 (v/v) and dried for 1 min in a stream of cold air. Fluorescence measurement at 366/>400 nm was performed by TLC Scanner 3 (CAMAG) with a slit dimension of 5 mm × 0.2 mm and a scanning speed of 20 mm/s. For digital documentation the DigiStore 2 Documentation System (CAMAG) consisting of illuminator Reprostar 3 with digital camera Baumer optronic DXA252 was applied. The image taken was quantified by VideoScan software (CAMAG). All other instrumentation was controlled via the software platform winCats 1.4.2 Planar Chromatography Manager (Camag).

RESULTS AND DISCUSSION

Stir Bar Sorptive Extraction (SBSE)

Our initial study focused on the design of experiments in order to obtain the optimal SBSE conditions for PAHs frequently found in drinking water (Table 1).[24] However, this study was crossed by an excellent paper.[26] Therein, many open questions were clarified especially regarding the liquid desorption. The optimal extraction time for 10 mL-samples was established to be 140 min to ensure extraction under equilibrium conditions and the desorption time 25 min at 50°C. The use

of a modifier to prevent adsorption of PAH on the glass vessel was fixed at 2% acetonitrile addition. In contrast, higher modifier additions (10% methanol)[8,9] were also considered as suitable, however, the lower percentage (2%) was chosen in this study to avoid a reduced partition to the PDMS phase. Despite this, some basic questions were still open. SBSE-HPLC publications for PAH analysis,[18,27] except,[26] are using a thermal desorption for cleaning of the coated stir bars. For subsequent gas chromatographic analysis, cleaning the twisters by thermal desorption at 300°C for one to four hours is a reasonable practice,[8,9] however, not duly necessary for subsequent liquid chromatographic techniques which use a liquid desorption step. Thus, the cleaning of the twisters by thermal desorption using the tube conditioner (Gerstel) at 300°C for 1 h was compared with drying at room temperature, both using nitrogen gas, to prove if any impurities are left or recoveries are influenced if the thermal cleaning or conditioning step was omitted. The work flow is depicted in Figure 1. The recoveries (Table 3) confirmed that the use of the thermal cleaning was not justified for liquid chromatographic PAH analysis, as well as a conditioning of the twister. As expected, additional impurities were not observed and the cleaning of the twisters by N_2 drying just at room temperature was highly sufficient. Thus, it was proven that the thermal cleaning can be omitted, which avoided additional 2.5 hours-cooling down time.

For cleaning of the twisters, a 7-step cleaning procedure[26] was employed first. For cleaning validation the extract of each rinsing solvent was investigated indicating that the complete rinsing procedure could

Table 3. Comparison of the drying step (after rinsing with each solvent, see Figure 1) using the tube conditioner (N_2) at 300°C or just a stream of N_2 at room temperature (22°C) for 3 different twisters (10 mm long, 0.5 mm PDMS layer)

Recoveries (%) of	BgP	IcP	BaP	BbF	BkF	FLT	IS
N_2 at 300°C in tube conditioner (1 h, 4 bar)							
Stir bar 1	81	77	103	99	100	98	91
Stir bar 2	73	81	96	99	99	97	92
Stir bar 3	65	94	95	98	99	91	89
X	**73**	**84**	**98**	**99**	**100**	**95**	**91**
S	**6**	**7**	**4**	**0.2**	**0.5**	**3**	**1**
N_2 at 22°C (3 min, 2 bar)							
Stir bar 1	82	85	98	96	100	98	87
Stir bar 2	71	79	101	98	102	96	89
Stir bar 3	76	87	102	102	107	96	96
X	**77**	**84**	**100**	**99**	**103**	**97**	**91**
S	**5**	**4**	**2**	**3**	**3**	**0.8**	**5**

be reduced to a 4-step procedure which was still highly adequate, but reduced the time needed by a factor of 60%. Dichloromethane used, besides methanol and acetonitrile, for cleaning of the twisters was substituted by the chlorine-free solvent isopropyl acetate. Using this 4 step chlorine-free solvent procedure a carry-over was not observed for the samples investigated. The desorption volume of 150 μL acetonitrile proved to be sufficiently. In a second 150 μL-desorption aliquot, only PAH traces were found slightly higher as the noise level, not justifying the additional effort for a second desorption.

The usage of commercially available, inexpensive silicone tube pieces put on a magnetic stir bar as a kind of twisters or respectively PDMS coating substitute was reported for extraction of organic contaminants from aqueous samples.[28] Its transfer to PAH analysis was investigated. Besides manufacturing, also handling of the SBSE stir bars was much more effort. Even the 7 step-cleaning procedure[26] was not sufficient for proper cleaning of the silicone tubes, which was evident in the higher background noise and three additional impurities in the upper hR_F range (>53, Figure 2). The purity of the silicone tubes was reported to be 70% PDMS.[28] Consequently, potential extractable substances have to be removed by extra extraction steps first. Additionally, a by a factor of 9 increased desorption volume (900 μL instead of 150 μL) was necessary to guarantee wetting of the complete tube piece with the desorption liquid. However, Table 4 shows that, especially, one silicone tube led to

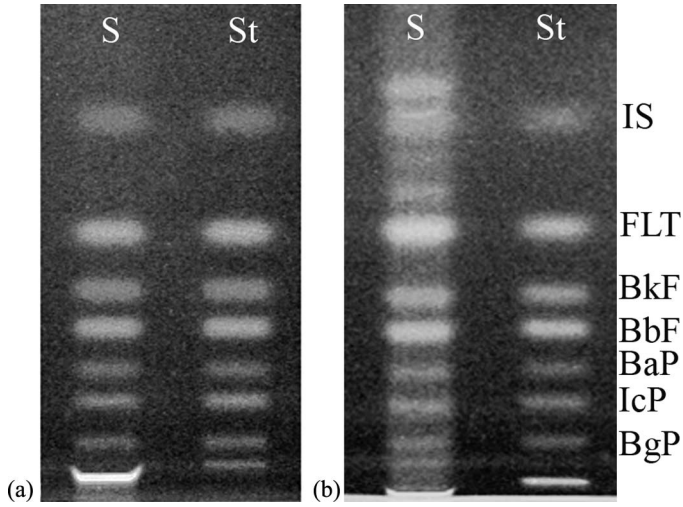


Figure 2. Comparison of the extraction of a spiked ultrapure water sample (S) related to a standard track (St) by a (a) commercially available twister and (b) hand-made stir bar; for substance abbreviations see Table 1.

Table 4. Comparison of the recoveries of 3 different commercially available silicone tubes for preparation of handmade stir bars

Recoveries (%) for	BgP	IcP	BaP	BbF	BkF	FLT
Tube 1 No. 14244	28	33	31	39	35	104
Tube 2 No. 14248	44	40	39	34	32	105
Tube 3 No. 28731	59	61	71	76	75	105

satisfying recovery rates. Its usage required additional effort regarding cleaning, especially cleaning validation, as well as an additional final concentration step. Concluding the usage of the twisters was much more comfortable and, additionally, the manufacturer guaranteed their purity.

Finally, to ensure the proper working of the whole sample preparation procedure,[25,29] 2-methylanthracene (hR_F value 67, blue fluorescence, linearity $r = 0.9967$, precision %RSD = 2.5%) was used as a kind of control standard which was not listed in the 15 EU-priority and 16 US EPA PAHs.

Optimization of the Chromatographic System

The chromatographic system was optimized regarding the plate impregnation (loading with caffeine). The best chromatographic separation was obtained on HPTLC plates silica gel 60 impregnated with a solution of 1.7% caffeine in acetonitrile. Higher concentrations required the transfer to dichloromethane (used in Ref.[24]) to avoid caffeine crystallization which, however, did not improve the separation of the 6 PAHs. Caffeine concentrations >7% led to crystallization on the plate. The transfer to special layers (HPTLC plates LiChrospher 60 F_{254s}or silica gel 60 WRF_{254}s with a layer thickness of 100 μm, HPTLC plates Nano-SIL-PAH) did not improve the separation. The mobile phase (dichloromethane) according to ISO[24] was substituted by the chlorine-free mixture of isopropyl acetate-acetonitrile 7:3 (Figure 3) with which the best separation was obtained, if the ratio was varied between 6:4 and 9:1 or just isopropyl acetate was used.

Validation of the Method

The optimized method was validated (Table 5) regarding LOQ (S/N 10), linearity, recovery and repeatability. The LOQ in matrix (pg/band) was established to be between 84 and 520 pg/band, depending on the PAH, respectively, between 80 and 435 pg/band for standards. Using a

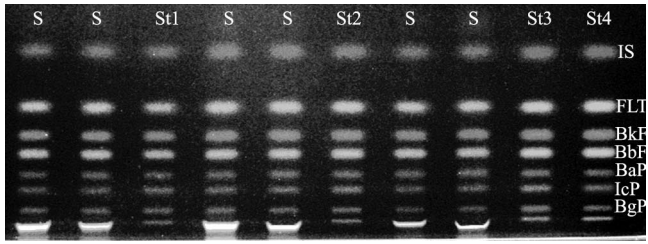


Figure 3. Digital documentation at UV 366 nm of the analysis of six PAH (substance abbreviations see Table 1) in drinking water: different drinking water samples (S, spiked with PAHs) and standards (St) between 0.1 and 0.9 ng/band depending on the PAH.

10 mL-water sample volume, LOQs in the range of 8 and 52 ng/L were obtained. Hence, with this 10 mL-sample volume, the limit levels for BaP (10 ng/L) and for the sum of BbF, BkF, BgP, and IcP (100 ng/L) can be controlled. If necessary, the LOQ can be decreased by a factor of 2 using 20 mL sample volumes and a prolonged extraction time of 180 min.[26] Linearity was starting with a standard level around the LOQ. Even in this very low calibration range (pg/band range) satisfying

Table 5. Validation data of the SBSE-HPTLC/FLD method

PAHs	BgP	IcP	BaP	BbF	BkF	FLT
Migration distance (mm)	22	27	31	36	40	47
hR_F value	14	21	27	34	41	51
Fluorescence color	Blue	Yellow	Blue	Light blue	Blue	Light blue
LOQ (S/N10)						
-pg/band for standards	300	170	80	162	143	435
-pg/band in matrix	351	170	84	197	162	520
-ng/L for a 10 mL-sample[a]	35	17	8	19	16	52
Linearity[b]						
-Coefficient of correlation r	0.9950	0.9920	0.9974	0.9983	0.9983	0.9967
-Standard deviation (*%RSD*)	4.8	6.3	3.5	3.0	2.8	3.3
Spiking level (ng/L)	40	22	10	22	19	58
Repeatabilities (*%RSD*, $n = 3$)						
-using the same stir bar	2.2	6.3	1.7	3.8	0.7	0.4
-using 3 different stir bars	7.0	4.5	2.3	2.9	3.2	0.8
Recoveries $\underline{x} \pm s$ (%, $n = 3$)						
-using the same stir bar	87 ± 2	94 ± 6	99 ± 2	95 ± 4	100 ± 1	99 ± 0.4
-using 3 different stir bars	77 ± 5	84 ± 4	100 ± 2	99 ± 3	103 ± 3	97 ± 1

[a]Using 20 mL sample volume can decrease the LOQ by a factor of 2.
[b]Lowest level starts around the LOQ.

coefficients of correlation $\geq$0.9920 and respectively standard deviation (%RSD) $\leq$6.3% were obtained. The recoveries ($n = 3$) using the same twister were between 87–100% $\pm$ 0.4–6% depending on the PAHs, and using 3 different twisters between 77–103% $\pm$ 1–5%. The repeatabilities (%RSD, $n = 3$ at 10–58 ng/L depending on the PAH) using the same twister were between 0.4 and 6.3% and, using 3 different twisters between 0.8 and 7.0%.

Digital Evaluation

Digital detection and quantification can be employed after documentation of the plate as an image. It is a very fast way of evaluation if compared to scanning with a TLC scanner (Figure 4). However, scanning offers the utmost spectral range and resolution. For PAH analysis, digital evaluation by VideoScan was performed after documentation of the plate at 366 nm. The color image (fluorescent zones) was automatically transferred into a grey-scale image and the pixels within a track line were summed up and generated one data point (Figure 5a). Thus, an analogue curve was obtained. Digital evaluation was compared to conventional scanning (Figure 5b) and both quantification methods showed a comparable quality of the calibration

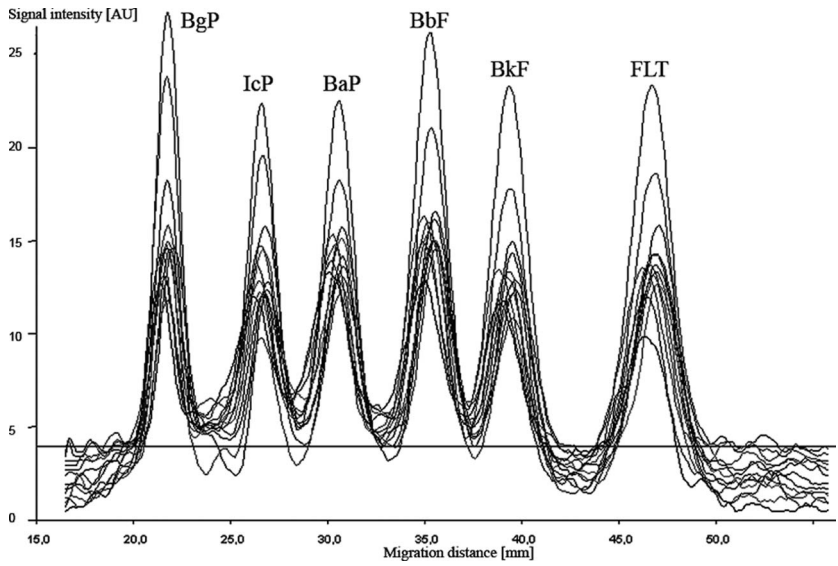


Figure 4. Parallel chromatography (3D-overview of all standard and sample tracks on the plate) contributes to the rapid analysis of PAHs.

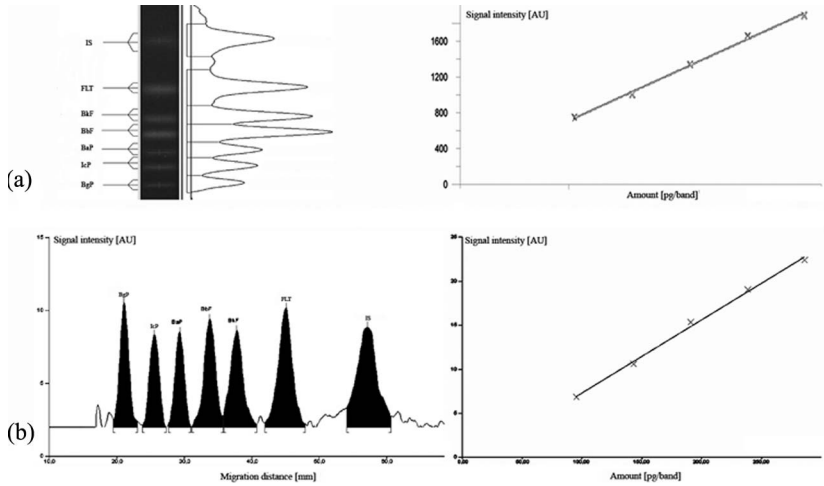


Figure 5. Comparison of the BkF calibration curves of the same plate obtained by (a) digital evaluation by DigiStore2/VideoScan ($\%RSD = 2.4\%$) and (b) conventional scanning by TLC Scanner 3 ($\%RSD = 2.8\%$). The lowest standard level of the respective calibration (right) is depicted as track (left), see also Table 6.

function (Table 6). In the case of PAH analysis, digital evaluation proved to be a very rapid, reliable tool.

Bioactivity-Based Detection

In water analysis, detection of toxic compounds with the marine luminescent bacteria *Vibrio Fischeri* (NRRL B-11177) is a standardized

Table 6. Digital evaluation by DigiStore2/VideoScan versus conventional scanning by TLC Scanner 3 of the same HPTLC plate

	Calibration range	Linearity* obtained by TLC Scanner 3		DigiStore 2/VideoScan	
PAH	(pg/band)	Equation	%RSD	Equation	%RSD
FLT	290–870	$y = 0.025x + 1.608$	3.3	$y = 1.84x + 184$	3.5
BkF	95–285	$y = 0.083x$–1.031	2.8	$y = 6.13x + 159$	2.4
BbF	108–324	$y = 0.092x$–1.937	3.0	$y = 6.91x + 125$	2.0
BaP	51–153	$y = 0.163x$–0.891	3.5	$y = 7.64x + 27$	3.1
IcP	110–330	$y = 0.068x$–1.18	6.3	$y = 2.00x + 190$	3.0
BgP	200–600	$y = 0.046$–0.896	4.8	$y = 1.72x + 46$	2.5

*Lowest level starts around the LOQ for scanning.

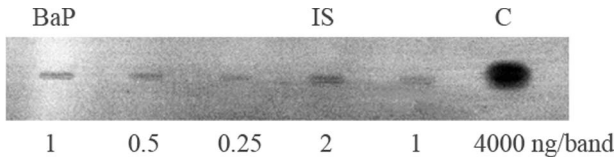


Figure 6. Detection of benzo[a]pyrene (BaP) with the luminescent bacteria *Vibrio Fischeri* down to 0.25 ng/band and of 2-methylanthracene (IS) down to 1 ng/band; without chromatography, caffeine used as control standard C (4 μg/band) for the bio-assay.

procedure.[30] However, the benefit of combining this kind of bioactivity-based detection with chromatography is evident: instead of a sum parameter, the detection and assignment of individual zones are possible and the coincidence of contrary effects (luminescence enhancement versus inhibition) can clearly be differentiated and thus may not lead to false results. Hence, the response of PAHs to Vibrio Fischeri was investigated. The visual LODs were established to be 300 ng/band for BgP, IcP and BbF, 260 ng/band for BkF, 80 ng/band for FLT, 1 ng/band for IS and, very sensitive, 0.25 ng/band for BaP (Figure 6). Consequently, the sensitivities obtained were suitable for the employment of this detection, especially for the carcinogenic marker BaP. However, the usage of caffeine-impregnated plates made this combination, per se, impossible because the amount of caffeine, calculated per PAH zone, was in the range of 30 μg on the silica gel plate and already some μg-amounts of it inhibit the luminescence as well. In contrast, the usage of RP18 plates[24] could be an alternative, but there, the wetting with the aqueous bacteria solution was not possible, even when special water-wettable plates were used or modifier (e.g., up to 2% i-propanol) were added for reduction of the surface tension.

CONCLUSION

The whole method was tried to be kept cost-effective, rapid and minimized in effort. Sample preparation was streamlined by omitting the thermal cleaning and reducing the solvent cleaning cycles. The usage of a control standard guaranteed its proper working. The extraction of 10-mL sample aliquots enabled a reduced extraction time compared to 20-mL samples (140 min versus 180 min). The avoidance of chlorine-free solvents for cleaning of the twister, plate impregnation and the mobile phase allowed a more environmentally friendly analytical system. Thus the simplified sample preparation and parallel chromatography allowed 30 water samples to be extracted and analyzed within a routine working

day using one working station and 10 twisters in parallel. Thereby the labor time needed, all in all about 1.5 h for sample preparation and 20 min for HPTLC transfer steps, is minor. Further work must focus on the employment of bioactivity-based detection, the inclusion of the residual EU-priority PAHs and clarify the contribution of HPTLC to cost-effective, fast and reliable quantification of PAHs in other food matrices.

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