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Increased sample loading capacity
for online SPE-HPLC analysis of PAHs in water

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SUMMARY

Polycyclic aromatic hydrocarbons (PAHs) are environmental pollutants and are widespread across the globe mainly due to long-term anthropogenic sources of pollution. Due to their high mutagenic and carcinogenic potential a sensitive determination is mandatory. The used system configuration makes it possible to switch between direct injection via autosampler and injection with an auxiliary pump (feed pump) without changing/replumbing the system. The use of autosampler injection or injection via feed pump enables the user to inject a broad variety of different volumes from µl to ml scale.

INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAHs), also known as polyaromatic hydrocarbons are environmental pollutants and are widespread across the globe mainly due to long-term anthropogenic sources of pollution [1]. These organic compounds contain only carbon and hydrogen and are composed of multiple aromatic rings. These compounds are formed when oxygen is limited, and incomplete combustion of organic matter such as wood, tobacco products, or fossil fuels occurs. These pollutants do not break down and are very persistent in the environment [2]. Due to their high mutagenic and carcinogenic potential a sensitive determination is mandatory. Solid phase extraction (SPE) is a useful technique to enhance detection limits to analyse even very small amounts of these compounds. Most often SPE is carried out manually but coupled to an HPLC system, the so called online SPE technique allows an automated sample analysis. Generally, the following developed method is suitable for the determination of 16 PAHs according to EPA 610 method [3]. During the further measurements, the focus was set only on the following analytes: fluoranthene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[g,h,i]perylene, and indeno[1,2,3-cd]pyrene.

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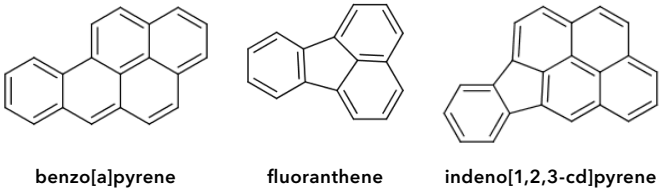

benzo[a]pyrene fluoranthene indeno[1,2,3-cd]pyrene

Fig. 1 Exemplary structures of selected PAHs

SAMPLE PREPARATION

EPA 610 PAH mixed standard (Sigma Aldrich, product number: 4S8743) came as a ready to use solution. The mixed standard was diluted with acetonitrile:water 60:40 (v/v) to different concentrations.

Tab. 1 Concentrations of PAHs in EPA 610 PAH mixed standard and calibration solutions

Peak	Analyte	Stock solution (µg/l)	Level 1 (µg/l)	Level 2 (µg/l)	Level 3 (µg/l)	Level 4 (µg/l)	Level 5 (µg/l)
1	Fluoranthene	20 0000	2	20	40	200	1 000
2	Benzo[b] fluoranthene	20 0000	2	20	40	200	1 000
3	Benzo[k] fluoranthene	10 0000	1	10	20	100	500
4	Benzo[a]pyrene	10 0000	1	10	20	100	500
5	Benzo[g,h,i] perylene	20 0000	2	20	40	200	1 000
6	Indeno[1,2,3-cd] pyrene	10 0000	1	10	20	100	500

RESULTS

To determine the method precision for all PAHs **(Tab. 1)** and online SPE, seven consecutive runs with the mixed standard in a dilution 1:10^3 were injected (5 µl injection volume). To determine the recovery of the trapping process on the SPE cartridge, the same volume (5 µl) of the mixed standard (dilution 1:10^3) solution was injected directly onto the analytical column and afterwards onto the SPE cartridge. The seven replicates of each method were averaged and compared.

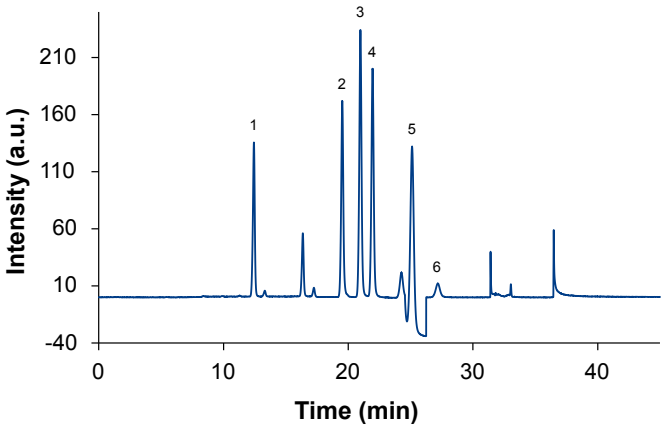

Fig. 2 Direct injection via autosampler of mixed standard, (1) fluoranthene, (2) benzo[b]fluoranthene, (3) benzo[k]fluoranthene, (4) benzo[a]pyrene, (5) benzo[g,h,i]perylene, (6) indeno[1,2,3-cd]pyrene

The drop of baseline **(peak 5, Fig. 2)** is a result of the fluorescence wavelength switching. Although an autozero was performed after switching, the baseline was not set to zero properly. Nevertheless, a determination of benzo[g,h,i]perylene is possible in high resolution. The precision of the direct injection method was calculated from 7 replicates.

Tab. 2 Precision of direct injection

Peak	Analyte	Retention time (%RSD)	Area (%RSD)	Height (%RSD)
1	Fluoranthene	0.01	0.87	0.77
2	Benzo[b] fluoranthene	0.01	0.88	0.60
3	Benzo[k] fluoranthene	0.03	0.89	0.82
4	Benzo[a]pyrene	0.02	0.69	0.38
5	Benzo[g,h,i] perylene	0.02	0.75	0.79
6	Indeno[1,2,3-cd] pyrene	0.03	0.52	0.94

The relative standard deviation for retention time is below 0.05 % for all analytes. Values for area and peak height are below 1.00 % for all substances.
The precision was again determined by injecting the same concentration of mixed standard (dilution 1:10^3) onto the SPE column.

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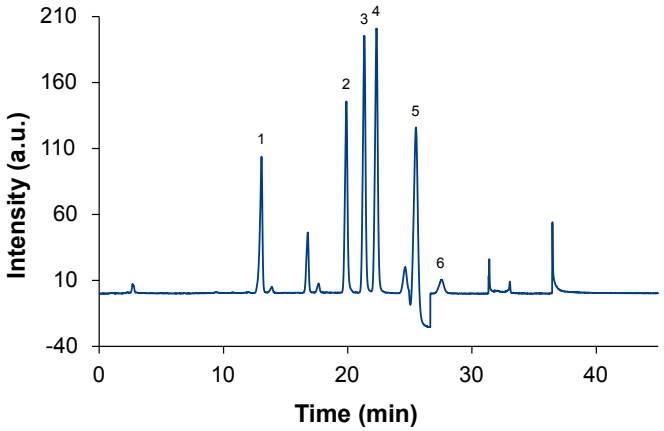

Fig. 3 Direct injection on SPE via autosampler, (1) fluoranthene, (2) benzo[b] fluoranthene, (3) benzo[k]fluoranthene, (4) benzo[a]pyrene, (5) benzo[g,h,i] perylene, (6) indeno[1,2,3-cd]pyrene

Tab. 3 Precision of SPE injection

Peak	Analyte	Retention time (%RSD)	Area (%RSD)	Height (%RSD)
1	Fluoranthene	0.06	0.89	1.08
2	Benzo[b] fluoranthene	0.04	0.90	1.15
3	Benzo[k] fluoranthene	0.03	0.88	1.10
4	Benzo[a]pyrene	0.03	1.00	1.99
5	Benzo[g,h,i] perylene	0.02	1.13	2.10
6	Indeno[1,2,3-cd] pyrene	0.03	0.78	1.15

The relative standard deviation for retention time using SPE is comparable to the direct injection and is below 0.07 % for all analytes. Values for area are below/equal 1 %RSD, except for benzo[g,h,i]perylene. Peak height is below 2 %RSD, again the value for benzo[g,h,i]perylene is slightly higher.

The averaged values for peak area over the seven replicates are compared to determine the recovery of the SPE injection method.

Tab. 4 Recovery

Peak	Analyte	Area (a.u./s) direct	Area (a.u./s) SPE	Recovery (%)
1	Fluoranthene	1457.01	1455.06	99.87
2	Benzo[b] fluoranthene	2098.73	2088.37	99.51
3	Benzo[k] fluoranthene	2975.11	2936.93	98.72
4	Benzo[a]pyrene	2619.43	3019.53	115.27
5	Benzo[g,h,i] perylene	3069.36	3135.30	102.15
6	Indeno[1,2,3-cd] pyrene	282.92	285.31	100.84

A five-point calibration was set up, with three replicates for each concentration level. The concentrations were listed in **Tab. 1**. To consider the varying injection volumes of direct injection and/or SPE injection, the calibrated concentrations were calculated as absolute values **(Tab. 5)**.

For the direct injection the limit of detection (LOD) and limit of quantification (LOQ) were calculated. For LOD a signal to noise ratio (S/N) of S/N=3 and for LOQ a S/N=10 was taken as basis for calculation. The noise was determined using the software integrated ASTM calculation.

Tab. 5 Linearity range, correlation coefficient, LOD and LOQ

Peak	Analyte	Linearity range (ng)	R^2	LOD (ng)	LOQ (ng)
1	Fluoranthene	0.02–10	0.99999	0.15	0.50
2	Benzo[b] fluoranthene	0.02–10	0.99999	0.12	0.41
3	Benzo[k] fluoranthene	0.01–5	0.99999	0.04	0.15
4	Benzo[a]pyrene	0.01–5	0.99999	0.04	0.14
5	Benzo[g,h,i] perylene	0.02–10	0.99985	0.12	0.40
6	Indeno[1,2,3-cd] pyrene	0.01–5	0.99994	1.42	4.70

The next step was to increase the injected volume onto the SPE column using the feed pump to enhance the detection limits. The mixed standard in a dilution 1:105 (equal to concentration of lowest calibration level) was used. A volume of 1800 µl was loaded on the SPE cartridge. This volume is equal to 1 column volume of the analytical column.

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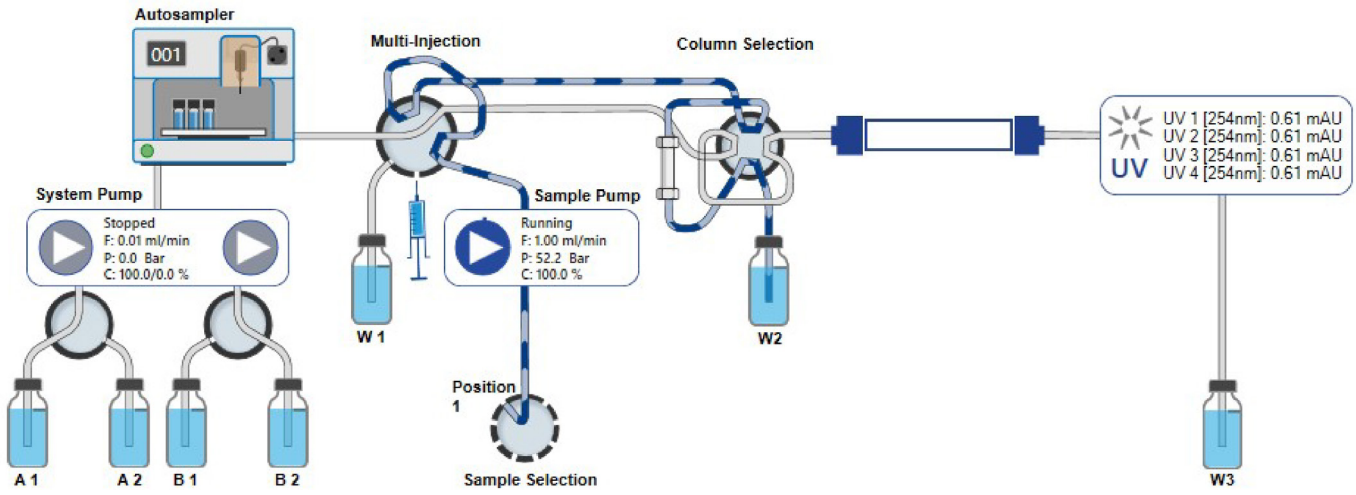

Fig. 4 Exemplary flow scheme for SPE load via sample/feed pump

Tab. 6 Enhanced LOD, feed pump vs. direct injection

Peak	Analyte	Peak height direct (a.u.)	LOD (µg/l)	Peak height SPE (a.u.)	LOD (µg/l)	Increase (%)
1	Fluoranthene	2.307	0.15	10.590	0.04	75
2	Benzo[b] fluoranthene	2.434	0.12	8.633	0.04	64
3	Benzo[k] fluoranthene	3.754	0.04	16.208	0.01	73
4	Benzo[a]pyrene	3.595	0.04	11.427	0.02	58
5	Benzo[g,h,i] perylene	2.105	0.12	35.744	0.01	90
6	Indeno[1,2,3-cd] pyrene	0.235	1.42	0.954	0.40	72

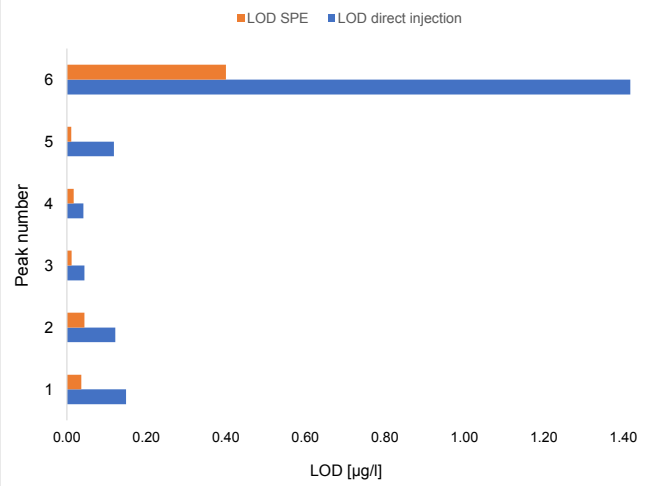

Fig. 5 Comparison of LOD values for direct injection (blue) and SPE injection (orange)

Using the feed pump for the SPE injection results in a lower limit of detection, which means that with the SPE method it is possible to detect lower concentrations of PAHs in the samples. The LOD was increased in a range from 50–90 %, still using a relatively low injection volume. These values are specific for an injection volume of 1800 µl and a 1:10^5 dilution **(see Tab. 1, level 1)**.

CONCLUSION

The used system configuration makes it possible to switch between direct injection via autosampler and injection with an auxiliary pump (feed pump) without changing/replumbing the system. The use of autosampler injection or injection via feed pump enables the user to inject a broad variety of different volumes from µl to ml scale. It was shown that the recovery of the SPE injection was in range from 80 %–120 % and all calibrations showed excellent linearity. The approach of injecting 1800 µl via the auxiliary feed pump has enhanced the detection limits. As seen in **Tab. 6**, the LOD was improved in a range from 50–90 %, still using a relatively low injection volume. Loading higher volumes on the SPE can lead to a further enhancement of the method sensitivity. Although, only six PAHs were investigated in detail, the method can also be used for the determination of all 16 PAHs according to EPA method 610.

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MATERIAL AND METHODS

Tab. 7 Analytical method parameters for direct injection via autosampler and SPE method

Parameter	Description		
Eluent A	Water		
Eluent B	Acetonitrile		
Flow rate	0.9 ml/min		
Temperature	20 °C		
Gradient	Time [min]	% A	% B
	0.00	40	60
	5.00	40	60
	15.00	10	90
	20.00	0	100
	35.00	0	100
	35.02	40	60
	45.00	40	60
Detection UV	230 nm, 254 nm		
Data rate UV	20 Hz		
Time constant UV	0.05 s		
Detection FLD	Wavelength switching		
Data rate FLD	5 Hz		
Time constant FLD	0.2 s		
Sensitivity	HIGH		
Gain	x 16		
Recorder range	x 1		
Output mode	Emission not corrected		
Autozero	At 0.00 min		
Injection mode	µl pick up		
Injection volume	5 µl		
Analysis time AS	30 min		
Transport vial	Wash		
Transport volume	4		

Tab. 8 Autosampler advanced wash program, after 30 min analysis time

Position	Volume (µl)	Wash loop
Transport	250	-
Transport	500	+
Wash	500	+
Wash	250	-

Tab. 9 Excitation/emission wavelength and switching times for PAHs, direct injection via autosampler

Analyte	Excitation (nm)	Emission (nm)	Switching time (min)
Fluoranthene	281	453	0.00
Benzo[b]fluoranthene	260	440	17.00
Benzo[k]fluoranthene	243	412	20.40
Benzo[a]pyrene	260	408	21.50
Benzo[g,h,i]perylene	292	415	24.50
Indeno[1,2,3-cd]pyrene	293	485	26.20

Tab. 10 Valve switching program, SPE loading/elution/cleaning

Time (min)	Multi-injection valve	8port-2position valve	Multiposition valve 8port, 1/8"	Comment
0.00	4	1	1*	-
0.02	3	1	1	SPE load
0.20	4	1	1	SPE wash
1.00	4	2	1	SPE elution
30.00	4	1	1	SPE cleaning and conditioning
39.00	4	1	2**	
42.00	4	1	1	

* H_2O, ** acetonitrile

Tab. 11 Pump program for ASM 2.2L (aux pump), SPE washing/cleaning/conditioning

Time	Flow rate (ml/min)
0.00	0.00
0.20	0.00
0.22	3.00
1.00	3.00
1.02–39.00	0.00
39.02–44.00	3.00
45.00	0.00

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System configuration

Component	Description	Article No.
Pump	AZURA P 6.1L HPG, 10 ml, 862 bar	APH35EA
Autosampler	AZURA AS 6.1L, Standard	AAA50AA
Detector UV	AZURA DAD 2.1L	ADC01
Flow cell	High Sensitivity KNAUER LightGuide UV Flow Cell Cartridge	AMD59XA
Detector FLD	Shimadzu RF-20A	A59200
Thermostat	AZURA CT 2.1	ATC00
Valve	AZURA Valve Unifier VU 4.1	AWA01XA
Valve	Multiposition Valve, 8 port, 1/8"	AVT34AE
Assistant	AZURA Assistant ASM 2.2L	AY03231
	Left module: Pump P 4.1S, 10 ml, stainless steel	DPG22EA
	Middle module: Valve drive VU 4.1	EWA04
	Right module: Valve drive VU 4.1	EWA04
Valve	High-pressure injection Valve, 8 Port 2-position	AVC38AC
Valve	Multi-injection Valve, DLC stainless steel	AVN96AE
Column	NUCLEOSIL 100-5 C18 PAH, 5 µm, 150×4 mm	
SPE column	Eurosil Bioselect 300-5 C8. 30×4 mm	03DK081EBJ
Software	ClarityChrom 8.5 – Workstation, autosampler control included	A1670
Software	ClarityChrom 8.5 – PDA extension	A1676

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