KNAUER

Application Note

PLATIN blue

Rapid analysis of 17 polycyclic aromatic hydrocarbons with UV- and FL-detection according to DIN EN 17993:2002

Category	Environmental analysis
Matrix	Environmental samples
Method	UHPLC UV/FLD
Keywords	Environmental monitoring
Analytes	Naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene. Benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene, indeno(1,2,3-cd)pyrene, benzo(j)flouranthene
ID	**VEV0054**, 11/11

Summary

A rapid gradient HPLC method for simultaneous determination of 17 polyaromatic hydrocarbons (PAH) according to EN ISO 17993:2002 is presented in this application note. A baseline resolution for all 17 target analytes in less than 10 minutes could be developed using a 150 x 2 mm BlueOrchid PAH column. Fluorescence and UV detection in combination with the powerful PLATINblue high pressure gradient UHPLC system allows for limits of detection below 1 µg/L for all PAH, with the exception of acenaphthylene and benzo(J)flouranthene. (< 10 µg/L) which are detected using the PDA-1 detector.

Introduction

Polyaromatic hydrocarbons (PAHs) are particularly relevant in the analysis of environmental pollution because of their ubiquity, toxicity and persistence. In the 1980's, the American Environmental Protection Agency (US EPA) compiled a list of the 16 most relevant PAHs in terms of environmental pollution. Consequently these PAHs have become the most intensively studied pollutants in environmental analysis.[1,2] Standard and official methods for their analysis are available in guidelines for air, water, solid waste and food analysis.[3] These methods generally specify HPLC, usually with UV and fluorescence detection, with run times in excess of 30 min. To attain the required selectivity, stationary phases specifically designed for PAH analyses are required. High speed chromatography systems like KNAUER's PLATINblue UHPLC system make it possible to work at pressures up to 1000 bars and therefore enable the use of very short columns packed with small particles. The aim of this study was to investigate the resolution limit and analysis time for 17 PAHs using a PLATINblue UHPLC system in combination with the very sensitive fluorescence detection according to the EN ISO 17993 method.

Chemical structures

Polycyclic aromatic hydrocarbons are chemical compounds that consist of fused aromatic rings and do not contain heteroatoms or carry substituents (Fig. 1).

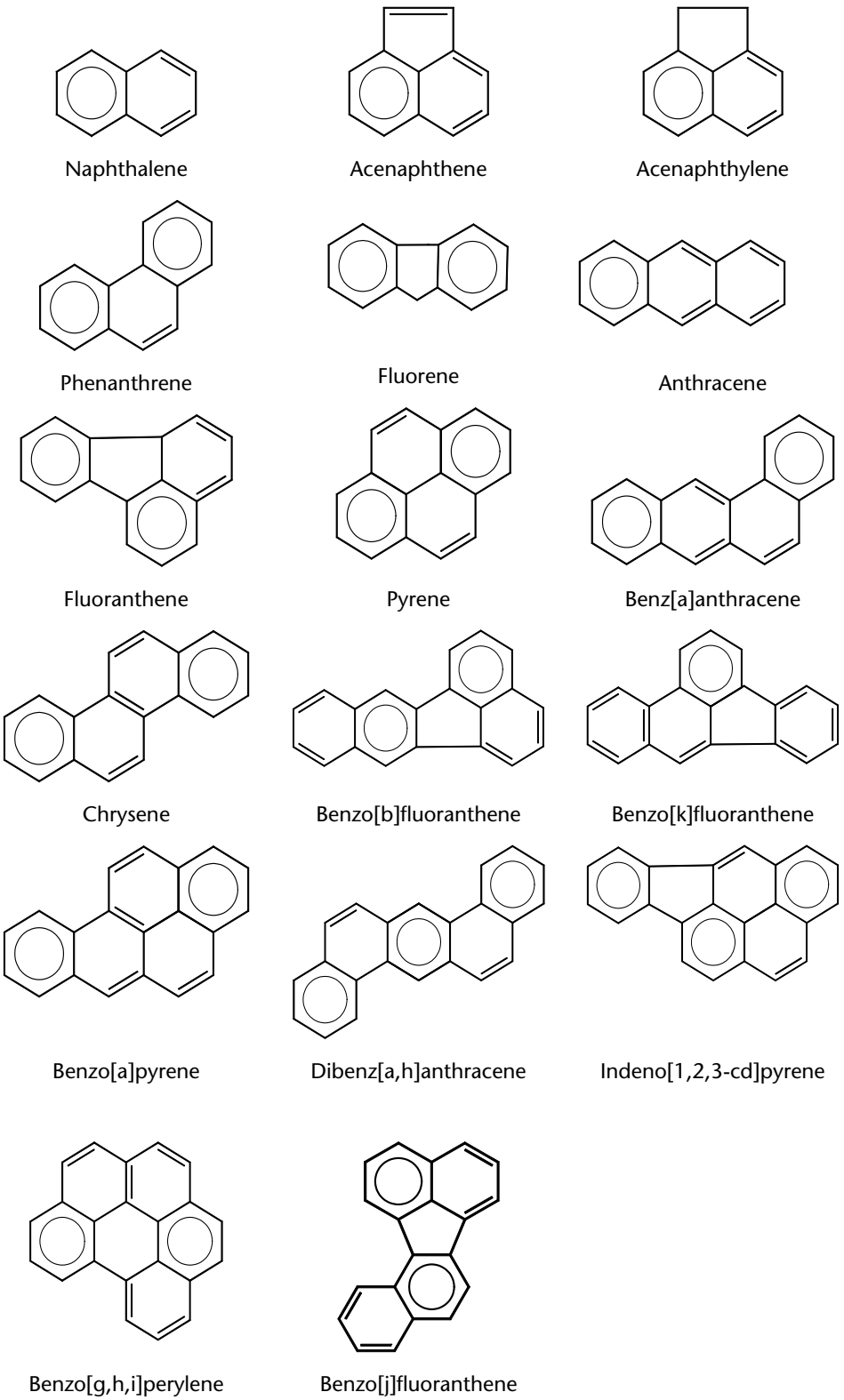

Fig. 1

Chemical structures of the analyzed PAHs

Experimental preparation of standard solution

A standard mixture (stock solution) of all 17 PAHs was prepared with a concentration of 1 mg/L for every single substance using acetonitrile as the diluting solvent. With this stock solution two calibration series were realized. The first calibration range lay in the range of 1 - 10 and the second one in the range of 10 – 100 µg/L. Concentrations of 1, 3, 5, 7 and 10 µg/L were prepared for the first calibration range and concentrations of 10, 30, 50, 70 and 100 µg/L for the second calibration range using the eluent A (MeOH / H2O 75:25 v/v) as the solvent. These 5 concentrations were in each case used according to the EN ISO 17993 method. Splitting of the calibrated range into two calibrations is necessary because of the sensitivity of the FL-detector. Its sensitivity has to be set down to medium in the higher calibration range otherwise the signals will be cut off. Furthermore, evaluation by the EN ISO 17993 method is much easier if only one of the two calibration ranges is analyzed. In this case, the homogeneity of variance does not have to be regarded.
Additionally, for the calibration according to the EN ISO 17993 method, an appropriate blank must be prepared. In this work, the blank was prepared by diluting the lowest calibrated concentration of 1 µg/L for the first calibration range and 10 µg/L for the second calibration range 1:1 so that blank values of 0.5 µg/L and 5 µg/L were reached. For the analysis according to the EN ISO 17993 method, it is important to prepare a synthetic blank with reproducible peak heights below the determination limit and far enough above zero.

Method parameters

The analysis was performed using a KNAUER PLATINblue high pressure gradient system equipped with two pumps P-1 with degasser, autosampler AS-1, column thermostat T-1, detector PDA-1and detector FLD.

Column	BlueOrchid PAH 150 x 2 mm ID		
Eluent A	MeOH / H_2O 75:25 v/v		
Eluent B	Acetonitrile		
Gradient	**Time [min]**	**% A**	**% B**
	0.00	90	10
	2.50	90	10
	6.00	0	100
	10.00	0	100
Flow rate	0.6 ml/min		
Injection volume	10 µl		
Column temperature	25 °C		
Detection	FLD: with wavelength switching program:		
	0.0 – 3.0 min 270 Ex, 330 Em		
	3.0 – 4.6 min 250 Ex 370 Em		
	4.6 – 5.8 min 330 Ex 430 Em		
	5.8 – 7.0 min 270 Ex 390 Em		
	7.0 – 8.9 min 290 Ex 430 Em		
	8.9 – 12.0 min 370 Ex 460 Em		
	12.0 – 15.0 min 270 Ex 330 Em		
	UV (50 Hz, 0.02 s); 50 mm 10 µl cell: Channel 1 (Trace 1) : 239nm, bandwidth 8nm Channel 2 (Trace 2): 229 nm, bandwidth 8nm, with reference correction* at 250nm, bandwidth 8nm * The reference wavelength for Trace 2 must be defined under the "Advanced" settings in the ChromGate instrument setup window. The reference signal at 250nm will be in effect subtracted from the signal produced at 229nm, resulting in negative peaks for some substances		
Run time	15 min (inclusive equilibration time)		

Results

1 Naphthalene
2 Acenapthene
3 Fluorene
4 Phenanthrene
5 Anthracene
6 Fluoranthene
7 Pyrene
8 Benzo(a)anthracene
9 Chrysene
10 Benzo(b)fluoranthene
11 Benzo(k)fluoranthene
12 Benzo(a)pyrene
13 Dibenzo(a,h)anthracene
14 Benzo(g,h,i)perylene
15 Indeno(1,2,3-cd)pyrene

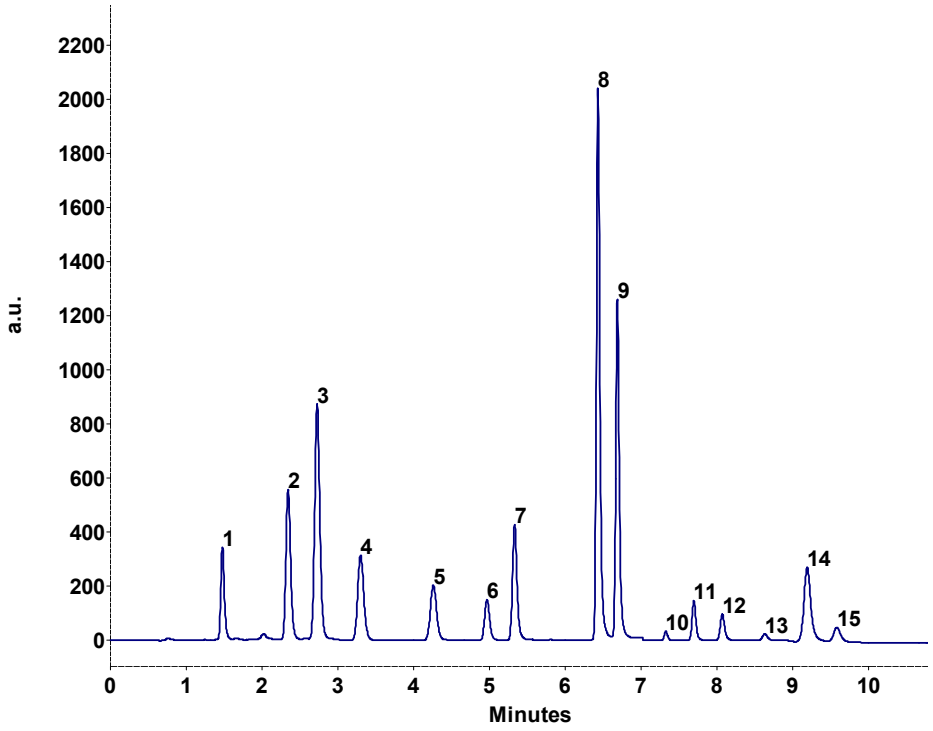

Fig. 2
Fluorescence trace of the PAH separation (10 µg/L)

1 Acenaphthylene
2 Benzo(j)fluoranthene

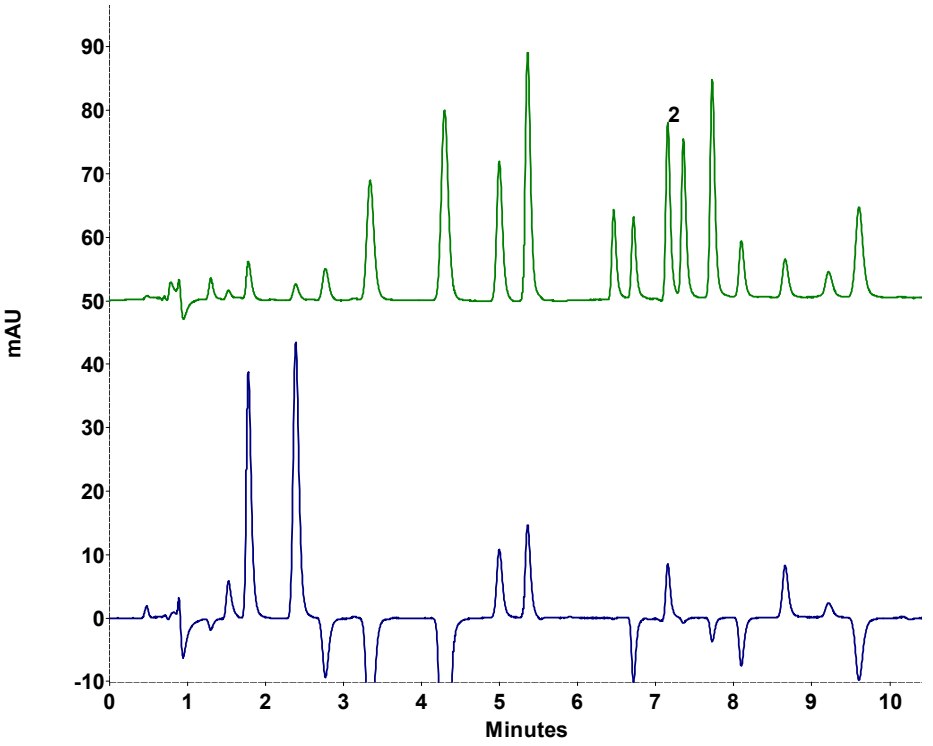

Fig. 3
PDA-1 UV-traces of the PAH seaparation (100 µg/L)
green (Trace 1): 239 nm
blue (Trace 2): 229 nm with reference wavelength 250 nm

All peaks were baseline separated with high resolution values. According to EN ISO 17993, resolution has to be at least 1 for the critical pair Acenaphthene/Fluorene and 1.5 for all other substances. If the FLD wavelength is switched, resolution has to be at least 2.5. These values were easily reached using the presented method and column.

Two compounds (Acenaphthylene/Benzo(j)flouranthene) of the PAH mixture must be detected via UV trace. Regarding the UV-PDA traces, it can be seen that not only the requested peak could be detected with the chosen wavelength but most of the peaks detected using the FLD trace can also be seen in the PDA trace just with lower intensities. Negative peaks also occur caused by the chosen UV-PDA reference wavelength for 229 nm trace that was set to improve the detection limit of the target compound Acenaphthylene.

Calibration

Calibration was done according to EN ISO 17993 and DIN 38402 and the decision limit, detection limit and determination limit under repeatability conditions were evaluated according to the DIN 32645 blank value method. We chose to evaluate peak heights instead of peak areas. The blank value arranged as described above has to be analyzed in 10 replicate runs for the evaluation according to DIN 32645. The aim was to reach a determination limit of < 1 µg/L for the PAHs detected with the FL detector and < 10 µg/L for the PAHs detected with the PDA detector, because the UV detection is not as sensitive as the fluorescence detection in this case.
Some example calibration curves are shown in fig. 4, 5 and 6 for the fluorescence detected substances Naphthalene, Chrysene and Indeno(1,2,3-c,d)pyrene represented by peaks 1, 9 and 15 in the FLD chromatogram shown above. The confidence interval according to EN ISO 17993 is also shown in red.

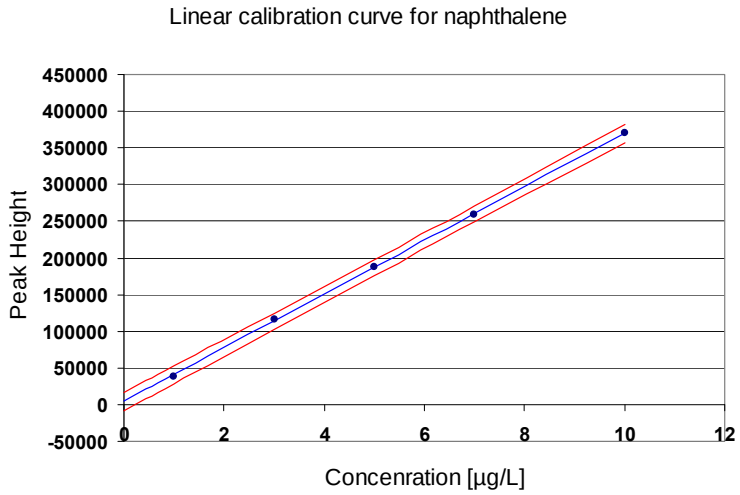

Fig. 4
Calibration curve and confidence interval for Naphthalene for the first calibration range (1-10 µg/L) detected with FLD

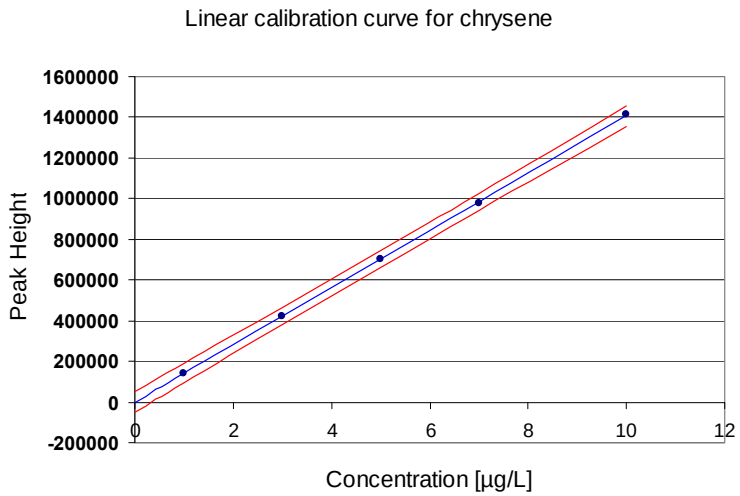

Fig. 5
Calibration curve and confidence interval for Chrysene for the first calibration range (1-10 µg/L) detected with FLD

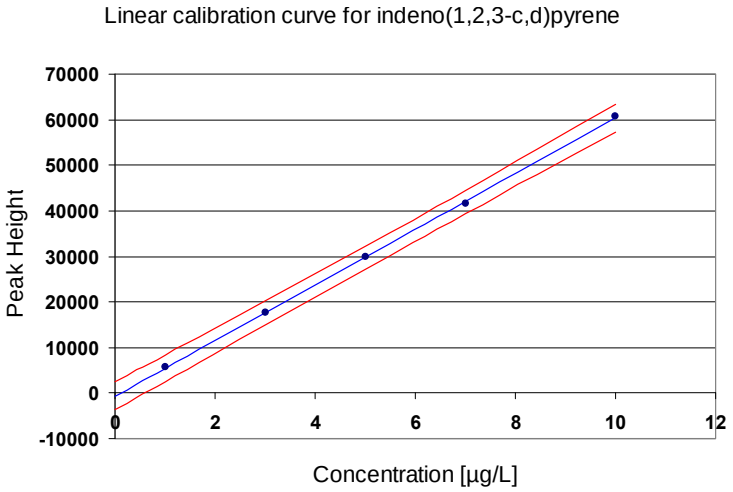

Fig. 6

Calibration curve and confidence interval for Indeno(1,2,3-cd)pyrene for the first calibration range (1-10 µg/L) detected with FLD

Some example calibration curves are shown in fig. 7 and 8 for the UV detected substances acenaphthylene, and benzo(j)fluoranthene represented by peaks 1 and 2 in the PDA chromatograms shown above. The confidence interval according to EN ISO 17993 is also shown in red.

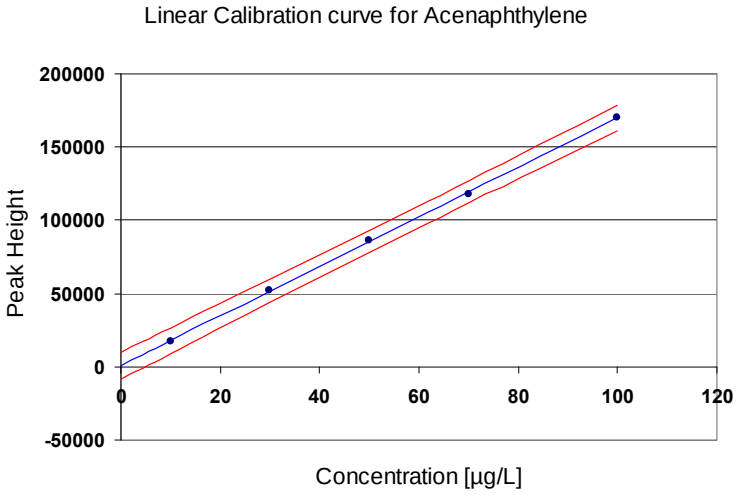

Fig. 7

Calibration curve and confidence interval for Acenaphthylene for the second calibration range (10-100 µg/L) detected with PDA

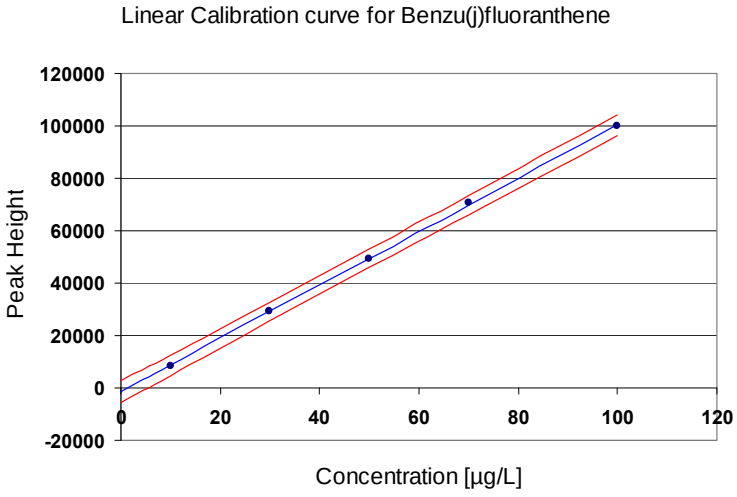

Fig. 8

Calibration curve and confidence interval for Benzo(j)fluoranthene for the second calibration range (10-100 µg/L) detected with PDA

The following table shows the calculated specific values of the method according to the blank value method described in DIN 32645 with a level of significance of 99 % and a relative uncertainty of <= 33 %. All PAHs that could be detected with the FL detector in the first calibration range and with the PDA in the second calibration range are shown.

Table 1
Specific values of the method according to DIN 32645 for all PAHs detected with the FL and the PDA detector.

PAH detected with FLD	decision limit [µg/L]	detection limit [µg/L]	determination limit [µg/L]
Acenaphthene	0.02	0.04	0.08
Anthracene	0.01	0.02	0.05
Benzo(a)anthracene	0.01	0.03	0.06
Benzo(a)pyrene	0.004	0.01	0.02
Benzo(b)fluoranthene	0.04	0.08	0.17
Benzo(k)fluoranthene	0.01	0.02	0.05
Benzo(g,h,i)perylene	0.04	0.09	0.20
Chrysene	0.01	0.01	0.03
Dibenz(a,h)anthracene	0.15	0.30	0.65
Fluoranthene	0.01	0.03	0.06
Fluorene	0.03	0.05	0.12
Indeno(1.2.3-cd)pyrene	0.07	0.14	0.31
Naphthalene	0.15	0.31	0.67
Phenanthrene	0.02	0.04	0.08
Pyrene	0.02	0.04	0.08
PAH detected with PDA			
Acenaphthylene	0.60	1.21	2.65
Benzo(j)fluoranthene	0.68	1.35	2.97

The linearity coefficient r^2 lies in the range of > 0.999 for all calibrated substances. With the chosen blank value, the limit of quantification lies in the range of 1 µg/L for all PAHs detected with the FLD and in the range of 10 µg/L for Acenaphthylene and Benzo(j)fluoranthene detected with the PDA-1 detector. The limit of detection naturally lies under it and can be named as below 10 µg/L for Acenaphthylene and Benzo(j)fluoranthene and below 1 µg/L for all other PAHs as requested. Even for the very volatile substance naphthalene which is the most critical to detect, the requested limit of quantification of 1 µg/L could easily be reached.

Method performance

Limit of detection	< 1 µg/L (detected with FLD) < 10 µg/L (Acenaphthylene, Benzo(j)fluoranthene; detected with UV)
Linearity (r^2)	> 0.999 for all 17 PAH
Linearity range	1 – 10 µg/L for the first calibration range 10 – 100 µg/L for the second calibration range

Conclusion

Using standard HPLC for PAH analysis, injection to injection cycle times can often exceed 30 mins. By using a shorter column with a smaller inner diameter and a KNAUER PLATINblue UHPLC system with a very low system dead volume, complex mixtures such as the PAH mixture used in this application can be analyzed with a short cycle time. Most of the polycyclic aromatic compounds are fluorescent and can therefore be detected with high selectivity and sensitivity using a fluorescence detector. In the presented application note we analyzed a mixture of 15 PAH´s via fluorescence detection with time programmed excitation and emission wavelength switching. Acenaphthylene and Benzo(j)fluoranthene which does not display fluorescence were analysed via UV detection at different wavelength. The high speed separation method can even be easily modified to accommodate additional substances within a similar time scale. There is also the possibility to speed up the method, if the high resolution of 2.5 between the peaks is not needed.

References

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2 T. Wenzl, R. Simon, E. Anklam and J. Kleiner; Trends in Analytical Chemistry, Volume 25, Issue 7, July-August 2006, 716-725

3 AOAC 973.30; Deutsche DIN TVO; UK ISBN 0 11 752032 2; US EPS Methods TO-13, 550 550.1, 610, 8310 8330

Physical properties of recommended column

BlueOrchid PAH is a special stationary phase for the determination of polyaromatic hydrocarbons in environmental analysis. This stationary phase was designed to meet the requirements of EPS method 610.

Stationary phase	BlueOrchid PAH
USP code	L1
Form	spherical
Surface area	220 m^2/g
Special phase	designed for the determination of 16 PAH
Endcapping	no
Dimensions	150 x 2 mm
Order number	15BF420BOG

Recommended instrumentation

The high speed analysis was performed on the KNAUER high pressure gradient PLATINblue System, equipped with two pumps P-1 incl. Degasser Unit, Autosampler AS-1, Column Thermostat T-1, Detector PDA-1 and Fluorescence detector RF-20 Axs.

Description	Order No.
PLATINblue UHPLC-System	A69420
PLATINblue Pump P-1	
PLATINblue Pump P-1 with Degasser	
PLATINblue Autosampler AS-1	
PLATINblue Column Thermostat T-1 Basic	
PLATINblue Detector PDA-1	
PDA-1 flow cell (10 mm, 2 µl)	
PLATINblue ChromGate Data system	
PLATINblue ChromGate spectra license	
PLATINblue UHPLC method converter	
PLATINblue stainless steel capillary kit	
PDA-1 flow cell (50 mm, 10 µl)	A64151
Fluorescence detector RF-20 Axs	A59201

Author

Dr. Silvia Marten, Head of Columns and Applications Department, KNAUER

Mareike Naguschewski, Columns and Applications Department, KNAUER

Contact information

Wissenschaftliche Gerätebau
Dr. Ing. Herbert Knauer GmbH
Hegauer Weg 38 · D-14163
Berlin, Germany

Tel: +49 (0)30 / 809727-0
Fax: +49 (0)30 / 8015010
E-Mail: info@knauer.net
Internet: www.knauer.net