KNAUER

Application Note

► Rapid analysis of polycyclic aromatic hydrocarbons

Category	Environmental analysis
Matrix	Environmental samples
Method	UHPLC
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
ID	**VEV1**, 03/09

PLATIN blue by Knauer

Summary

A rapid gradient HPLC method for simultaneous determination of 16 PAH according to EPA 610 method is presented. A baseline resolution for all 16 target analytes in less than 2.50 minutes could be achieved by using a KNAUER BlueOrchid PAH 50 x 2 mm column.

Introduction

Polycyclic aromatic 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 mins. To attain a better selectivity, stationary phases specifically designed for PAH analysis 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 16 PAHs plus 3 additional related aromatic compounds using a PLATINblue system.

Experimental

Preparation of standard solution

All standard solutions were prepared with a mixture of acetonitrile/methanol. A commercially available SUPELCO PAH Standard 49156 solution was diluted with ACN:MeOH (20 – 1000 µg/ml).

Chemical structures

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

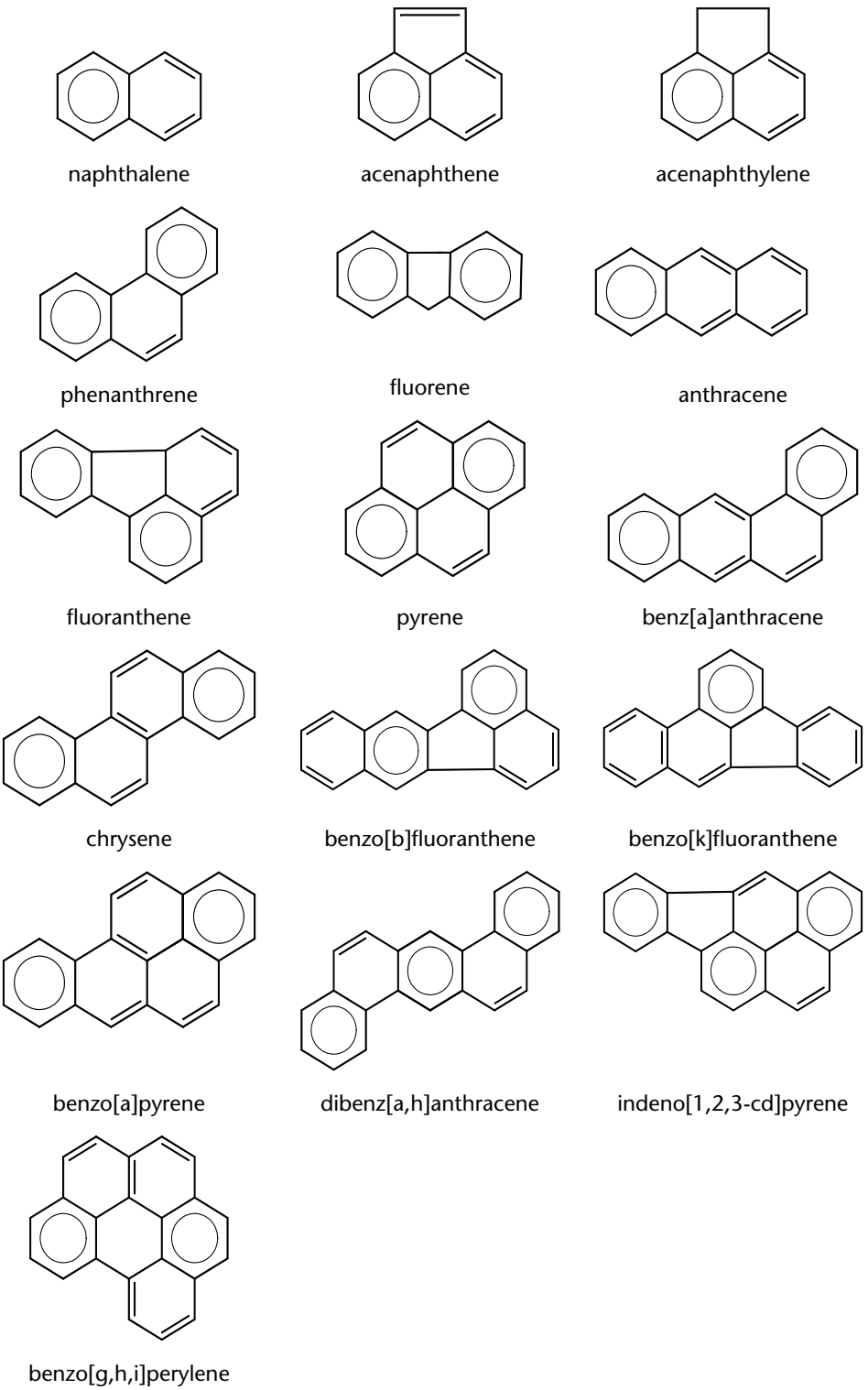

Fig. 1

Method parameters

The analysis was performed using a KNAUER PLATINblue high pressure gradient system equipped with two Pumps P-1, Degasser M-1, Autosampler AS-1, Column Temperature Manager T-1 and Detector PDA-1.

Column	BlueOrchid PAH 50 x 2 mm ID		
Eluent A	MeOH /water 75:25		
Eluent B	ACN		
Gradient	**Time [min]**	**% A**	**% B**
	0.00	90	10
	0.50	90	10
	1.00	0	100
	2.00	0	100
	2.10	90	10
	2.50	90	10
Flow rate	1.0 ml/min (1.5 ml/min for equilibration)		
Injection volume	1 µl		
Column temperature	25 °C		
Detection	UV at 254 nm (100 Hz); 2 µl flow cell		
Run time	3 min		

Results

1 naphthalene
2 acenapthalene
3 acenapthene
4 fluorene
5 phenanthrene
6 anthracene
7 fluoranthene
8 pyrene
9 benzo(a)anthracene
10 chrysene
11 benzo(b)fluoranthene
12 benzo(k)fluoranthene
13 benzo(a)pyrene
14 dibenzo(a,h)anthracene
15 benzo(g,h,i)perylene
16 indeno(1,2,3-cd)pyrene

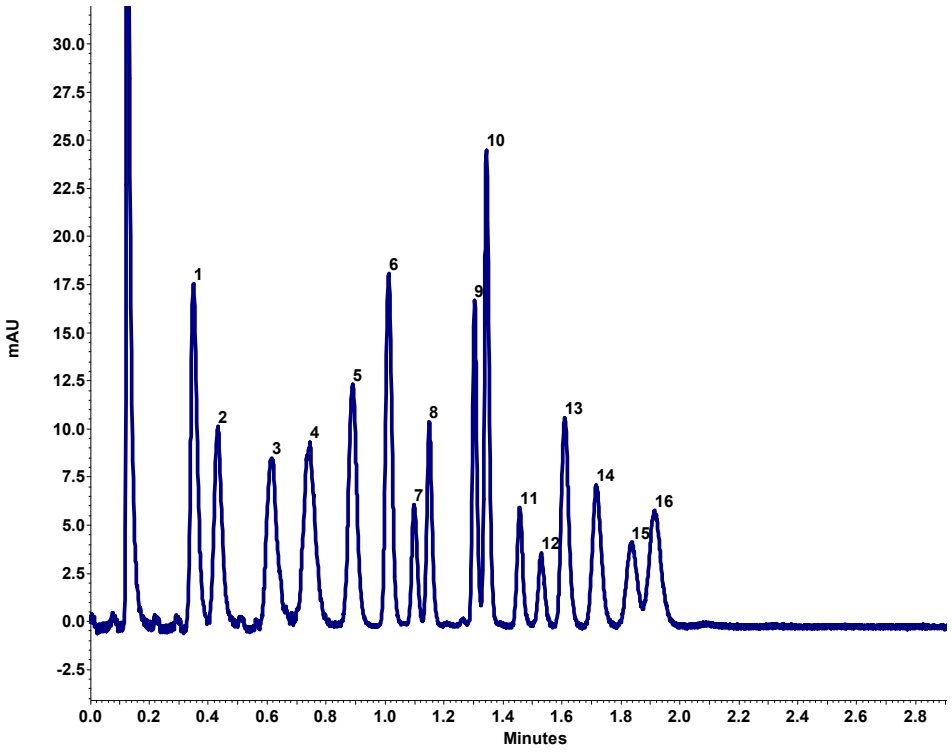

Fig. 2

Separation of a mixture of the 16 EPA-PAH on a BlueOrchid PAH 50 x 2 mm column with a PLATINblue UHPLC System

1	naphthalene
2	acenapthalene
3	1-methylnaphthalene
4	acenapthene
5	fluorene
6	phenanthrene
7	anthracene
8	fluoranthene
9	pyrene
10	triphenylene
11	p-terphenyl
12	benzo(a)anthracene
13	chrysene
14	benzo(b)fluoranthene
15	benzo(k)fluoranthene
16	benzo(a)pyrene
17	dibenzo(a,h)anthracene
18	benzo(g,h,i)perylene
19	indeno(1,2,3-cd)pyrene

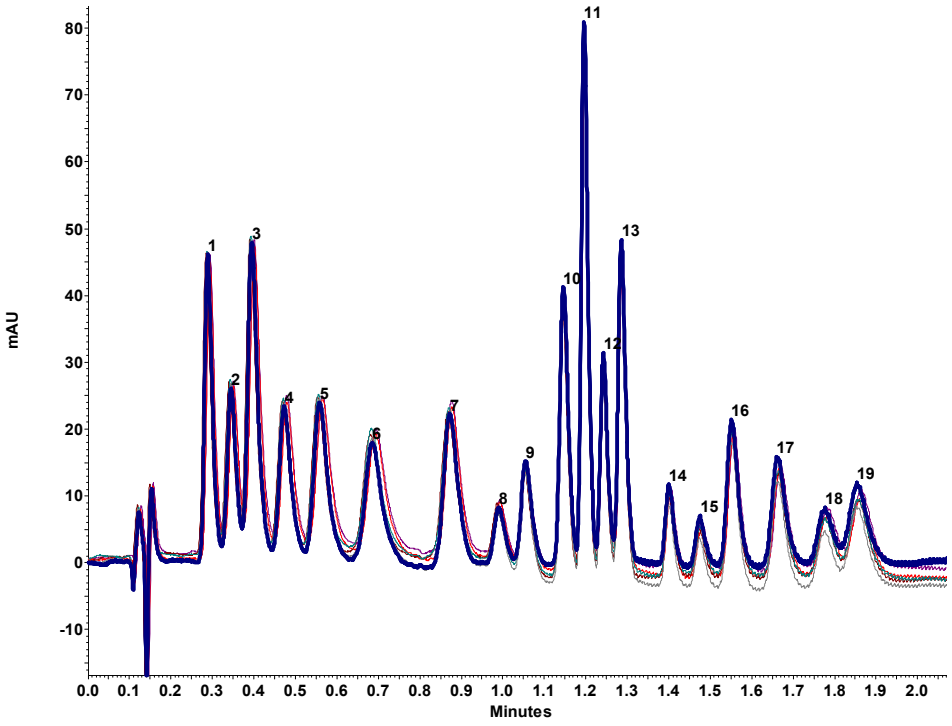

Fig. 3

Separation of 19 PAHs on a BlueOrchid PAH 50 x 2 mm column, system: PLATINblue UHPLC

Results

In previous analyses carried out on a KNAUER Smartline standard high pressure gradient system, the last substance (indeno(1,2,3-cd)pyrene) eluted at 2.83 mins. Those analyses were done using a BlueOrchid PAH column (50 x 4.6 mm) at 2 ml/min flow rate and 380 bar column backpressure, very close to the system's pressure limit of 400 bar. To further decrease the analysis time, we used the same column but with a smaller inner diameter (50 x 2 mm) in combination with the KNAUER PLATINblue UHPLC system using a flow rate of 1 ml/min.

After method optimization for the separation of the standard PAH mixture (see Figure 2) we spiked the PAH mixture with 3 more PAHs to achieve a separation of 19 PAHs in less than 2 min (see Figure 3). The eluent and method parameters were slightly modified to satisfactorily separate all 19 substances in the same time scale. In spite of the fast gradient applied (2% per second), the reproducibility of the method was good with a maximum deviation of Δt_R = 0.012 min (see also Figure 3).

In summary, we would recommend a flow rate of 1 ml/min for an analysis time of 2.5 min, plus an equilibration time of 0.4 min run at a higher flow rate of 1.5 ml/min for a faster overall cycle time.

Method performance

Limit of detection	SUPELCO PAH standard 49156; 20 – 1000 µg/ml in ACN:MeOH; diluted (S/N = 3)
Linearity (r^2)	0.99979 - 0.99990
Linearity range	-
Retention time precision*	better than 1 % RSD
Peak area precision*	better than 3 % RSD

*repeatability calculated over 5 replicate runs

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 EPA PAH mixture can be analyzed with cycle times less than 3 minutes. The high speed separation method can even be easily modified to accommodate additional substances within a similar time scale. Optimizing the speed and resolution of routine analyses can not only save time but can also dramatically decrease eluent costs, particularly important for analyses using acetonitrile

References

[1] M.N. Kayali-Sayadi, S. Rubio-Barroso, C.A. Díaz-Díaz, L.M. Polo-Díez; Fresenius J Anal Chem. Dec 2000;368(7):697-701

[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 EPA Methods TO-13, 550 550.1, 610, 8310 8330

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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 EPA method 610. In this procedure 16 polyaromatic hydrocarbons have to be separated.

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	50 x 4.6 mm / 50 x 2 mm
Order number	05EF420BOG / 05BF420BOG

Recommended instrumentation

The high speed analysis was performed on the KNAUER high pressure gradient PLATINblue UHPLC System, equipped with two pumps P-1, Degasser Unit, Autosampler AS-1, Column Temperature Manager T-1 and Detector PDA-1.

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)	
ChromGate data system + spectra license	
PLATINblue UHPLC method converter	
PLATINblue stainless steel capillary kit	

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