Application Note SI-02107

Analysis of Polycyclic Aromatic Hydrocarbons using Time-Programmed Fluorescence Detection with the Varian 920-LC and Pursuit™ 3 PAH Column

Jeffrey Kibby, Glyn Russell
Varian, Inc.

Introduction

The presence of polycyclic aromatic hydrocarbons (PAHs) is a common environmental concern owing to their inherent carcinogenicity and mutagenicity. PAHs are typically introduced into the environment by both naturally occurring combustion processes such as forest fires, and industrial combustion processes such as the burning of fossil fuels. They may then be carried into rivers, lakes and other water sources[1].

Combustion is not the only source of PAHs, as they also occur naturally. For example, in a diesel fuel with approximately 25% aromatic hydrocarbon content, several of the lower molecular weight PAHs including naphthalene, alkyl-naphthalenes, acenaphthene and anthracene may be present at percent levels. Accidental spillage of diesel fuels may therefore result in serious environmental pollution.

A common analytical method for analysis of PAHs uses HPLC with UV detection at 254 nm because these compounds can often be determined with good sensitivity under these conditions[2]. However, many international standards for the analysis of PAHs, such as ISO 17993:2002[3] use gradient HPLC with a time-programmed fluorescence detection for selectivity and sensitivity. In this paper a gradient HPLC method using time programmed fluorescence detection will be described.

Instrumentation

The method described herein was developed on a Varian 920-LC, comprising a quaternary gradient pump with a built-in four channel degasser unit, an autosampler with 100 µL injection syringe, heated column compartment and time-programmable fluorescence detector.

The Varian 920-LC system was fully controlled by Galaxie™ Chromatography Software.

Materials and Reagents

A PAH standard solution was obtained from Restek (catalog number 31841). The mixture contained the seventeen fluorescent PAHs listed in Table 2, each with a concentration of 500 µg/mL in acetonitrile.

Water (18MΩ, Milli-Q) and acetonitrile (Merck, HPLC grade) were used for preparation of standard solutions and eluents.

Standards Preparation

The 500 µg/mL solution was serially diluted with HPLC grade acetonitrile to give 50, 10 and 1 µg/mL working standards.

Chromatographic Conditions

The conditions for the analysis are summarized in Table 1.

Table 1. Chromatographic conditions

Column	Pursuit 3 PAH, 100 mm x 3 mm ID (Part number A7001100X030)		
Column Temperature	25°C		
Eluent A	Water		
Eluent B	Acetonitrile		
Time Program for Pump	Time (min)	Flow (mL/min)	%B
	0	0.55	55
	3	0.55	55
	9	0.55	100
	15	0.55	100
	15.1	0.55	55
	22	0.55	55
Injection Volume	0.1 µL		
Time Program for Fluorescence Detector	Time (min)	Excitation (nm)	Emission (nm)
	0	225	320
	6.9	250	375
	8.5	270	410
	9.9	265	380
	11.8	280	420
	14.9	300	466
Fluorescence Detector PMT Setting	Medium		

Results and Discussion

The low internal volume of the Pursuit 3 PAH 100 mm x 3 mm ID column, compared with that of commonly used 4.6 mm ID columns, provides greater sensitivity of detection. As an added advantage, solvent consumption is decreased, saving you time and money.

Interestingly, the choice of 225 nm excitation and 320 nm emission wavelengths, instead of the commonly employed 270 nm excitation and 323 nm emission, results in significantly enhanced detection for the lower molecular weight PAHs such as naphthalene, the methyl-naphthalenes and acenaphthene. These can be important markers for contamination due to petroleum-derived species such as diesel fuel.

Typical retention times and estimated limits of detection for individual PAHs are given in Table 2.

Table 2. Retention Times (RT) and Limits of Detection (LOD) for individual PAHs using fluorescence detection

Component	RT(min)	LOD (µg/L)
Naphthalene	3.71	0.01
1-Methyl naphthalene	5.18	0.01
2-Methyl naphthalene	5.57	0.01
Acenaphthene	6.02	0.01
Fluorene	6.44	0.01
Phenanthrene	7.41	0.02
Anthracene	8.16	0.02
Fluoranthene	8.95	0.10
Pyrene	9.42	0.02
Benzo(a)anthracene	10.95	0.01
Chrysene	11.24	0.02
Benzo(b)fluoranthene	12.45	0.01
Benzo(k)fluoranthene	12.99	0.01
Benzo(a)pyrene	13.56	0.01
Dibenzo(ah)anthracene	14.6	0.02
Benzo(ghi)perylene	15.32	0.05
Indeno(123-cd)pyrene	15.82	0.05

Note: Acenaphthylene, eluting between naphthalene and 1-methyl naphthalene, is non-fluorescent.

Conclusion

The Varian 920-LC system, equipped with a time programmable fluorescence detector with a low internal volume and a Varian Pursuit PAH analysis column, offers superior sensitivity and selectivity of detection for polycyclic aromatic hydrocarbons. Limits of detection for the 17 fluorescent PAHs obtained with this system are considerably lower than those specified in the International Organization for Standardization standard method ISO 17993.

Additional Information

For more information on Varian Pursuit™ PAH Columns, please refer to the Varian, Inc. Web site:

www.varianinc.com/products/consum/lccolumns/pursuit/pah.html

Reference

[1]Furata, N., Otsuki, A., Anal. Chem. 1983, 55, 2407-2413.

[2]Truong, Phuong and Russell, Glyn, Varian Application Note SI-00958, "Improved Analysis of Polycyclic Aromatic Hydrocarbons with the Varian 920-LC and Pursuit™ PAH Column".

[3] ISO 17993:2002 Water Quality – Determination of 15 polyaromatic hydrocarbons (PAH) in water by HPLC with fluorescence detection after liquid-liquid extraction.

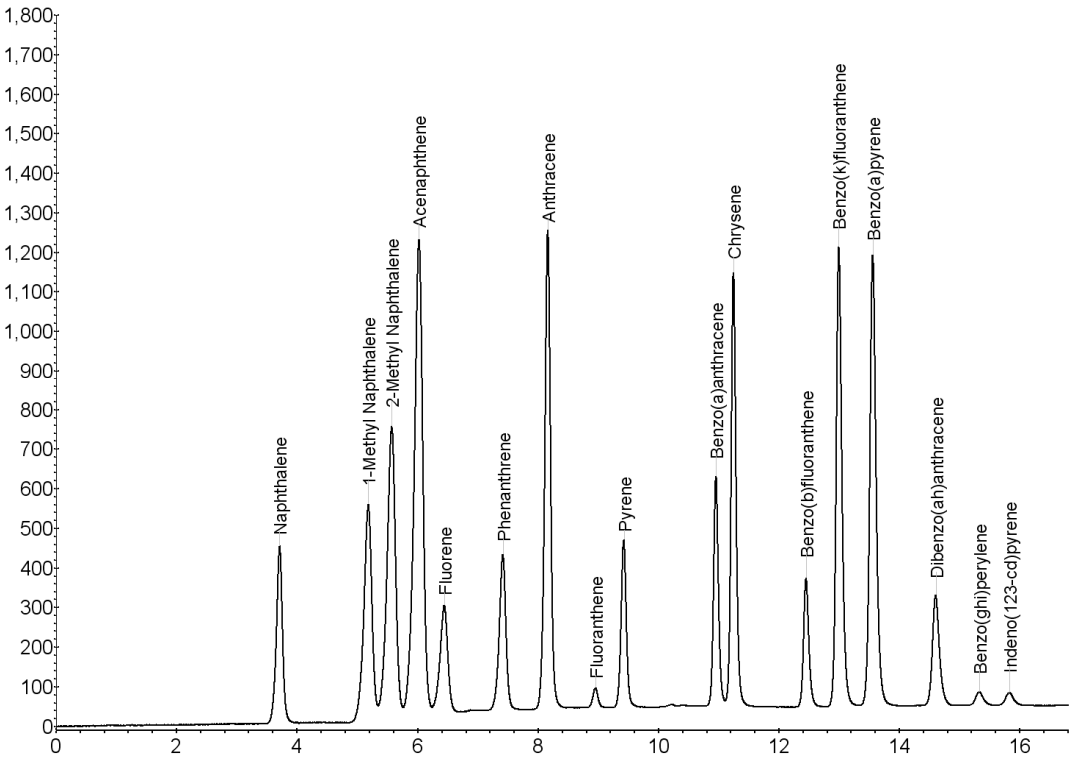

Figure 1. Chromatogram obtained for a 0.1 µL injection of a 1 µg/mL PAH standard (equivalent to 100 pg of each analyte on column) with separation of all the fluorescent PAHs in sixteen minutes.

These data represent typical results.
For further information, contact your local Varian Sales Office.

Varian, Inc.
www.varianinc.com
North America: 800.926.3000 – 925.939.2400
Europe: *The Netherlands*: 31.118.67.1000
Asia Pacific: *Australia*: 613.9560.7133
Latin America: *Brazil*: 55.11.3238.0400

VARIAN