High Throughput Method Development for PAHs using the Agilent 1290 Infinity LC system and a ZORBAX Eclipse PAH column

Application Note

Environmental Analysis

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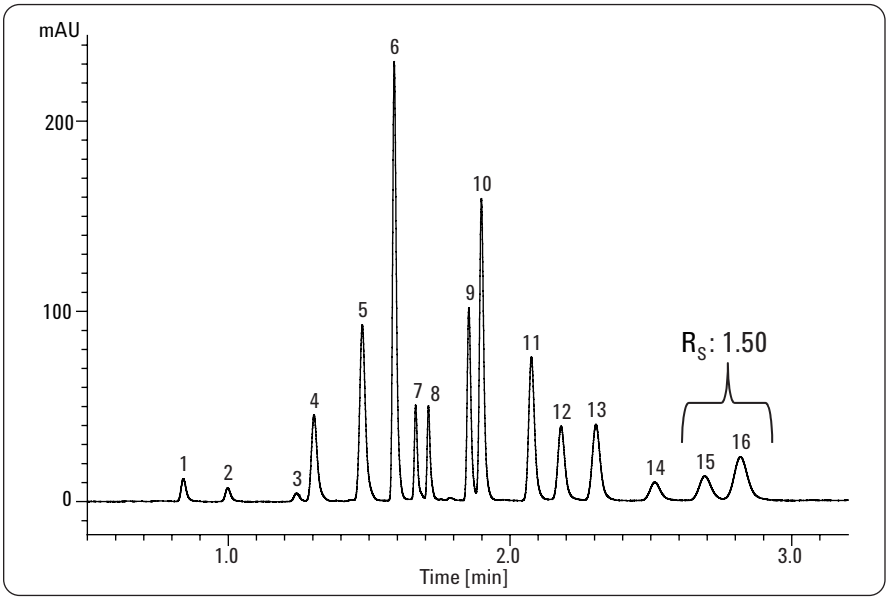

Abstract

This Application Note shows the result of the development of a very fast method for the separation of 16 Polycyclic Aromatic Hydrocarbons (PAHs) with the Agilent 1290 Infinity LC system. The developed method which uses acetonitrile as mobiles phase separates the PAHs in less than 3 minutes. Due to the high price of acetonitrile a method based on methanol for the same separation was developed. Both methods are compared and discussed.

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Introduction

Due to the mutagenic and carcinogenic properties of Polycyclic Aromatic Hydrocarbons (PAHs), the determination of these analytes is an important field in environmental analytical chemistry. PAHs are mainly released through combustion of organic materials, for example, by using fossil fuels in power stations and at home, as well as the exhaust fumes from automobiles. PAHs are determined in solid as well as in liquid and in atmosphere. Environmental laboratories around the world are faced with an ever increasing demand to speed up their methods and increase the sample throughput.

With the introduction of the Agilent 1290 Infinity LC system, a new generation of HPLC systems is available. One of the advantages is the pressure stability up to 1200 bar, but especially the very small dwell volume (volume from the point of mixing solvent A and B up to the column inlet) enables fast HPLC methods. Due to the very small dwell volume of the Agilent 1290 Infinity Binary Pump of 45 up to 135 µL depending on the Jet Weaver mixer, it is possible to use narrow bore columns to shorten analysis time and to reduce organic solvent consumption.

In order to perform a structured method development we used the DryLab® 2000 Plus software to find the optimal method parameters. Therefore, four basic chromatographic runs were performed in order to determine the optimal temperature and solvent gradient. These measurements comprised two linear solvent gradients from 40 to 100 % B in 10 and 30 minutes at 20 °C and the same gradients at a temperature of 50 °C. The measurements were performed on an Agilent ZORBAX Eclipse PAH column (50 x 2.1 mm, 1.8 µm) by using acetonitrile as well as methanol as organic modifier. After that, two methods were developed for acetonitrile and methanol. Afterwards, the computer-optimized methods were confirmed experimentally with high agreement between simulation and experiment.

Experimental

All chromatographic calculations were accomplished by Agilent ChemStation software version B.04.02.

LC system

For PAH method development, an Agilent 1290 Infinity LC system was used. The system is comprised of:

- Agilent 1290 Infinity Binary Pump with integrated degasser (G4220A)
- Agilent 1290 Infinity High Performance Autosampler (G4226A)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316B)
- Agilent 1290 Infinity Diode Array Detector (G4212A)

PAH (EPA) mixture

The EPA mixture of Polycyclic Aromatic Hydrocarbons is a certified reference material from Sigma-Aldrich (Catalog No. 4-7940-U) diluted in acetonitrile. Each PAH has a concentration of 10 µg/mL in the mixture.
The PAHs elution order for all figures:

1. Naphthalene
2. Acenaphthylene
3. Acenaphthene
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

Results and discussion

PAH method development using acetonitrile as organic modifier

Figure 1 shows the optimized separation of sixteen EPA PAHs on an Agilent ZORBAX Eclipse PAH column within three minutes using acetonitrile as organic modifier in the mobile phase. All peaks are baseline separated with a critical resolution of 1.5 between peak pair 15/16. The critical resolution was calculated by the tangent method.

Due to the worldwide shortage of acetonitrile in 2009, the costs increased up to 350 %. Therefore, it is reasonable to use other organic solvents e. g. methanol for the determination of PAHs. Hence, a method using methanol as organic solvent in the mobile phase is described.

PAH method development using methanol as organic modifier

Figure 2 shows the baseline separation of sixteen EPA PAHs on an Agilent ZORBAX Eclipse PAH column within six minutes using methanol as organic modifier in the mobile phase. The critical resolution between peak pair 15/16 is 1.62.

As mentioned before it can be economical to use methanol as organic solvent. If acetonitrile (106 €/L) is used, a volume of 2.0 mL of acetonitrile is necessary to elute the analytes from the column. In comparison, if methanol (21 €/L) is used, a volume of 3.8 mL is needed. On the other hand, the separation using acetonitrile is two times faster compared to methanol. For example, per 1000 PAH samples, either a volume of 2.0 L for acetonitrile and a pure analysis time of 50 hours or a volume of 3.8 L for methanol and a pure analysis time of 100 hours are necessary. In other words, the costs for the organic solvents sum up to 210 € and 80 € for acetonitrile and methanol, respectively.

Chromatographic conditions

Analytes:	PAH (EPA 16) mixture
HPLC:	Agilent 1290 Infinity LC system
Stationary phase:	Agilent ZORBAX Eclipse PAH; 50 x 2.1 mm; 1.8 µm
Mobile phase:	A: water; B: acetonitrile
Flow rate:	0.9 mL/min
Detection:	UV, 254 nm
Injection volume:	1 µL
Temperature:	30.1 °C
Pressure drop:	585 bar

Time [min]	% B
0.00	45
1.26	62
1.52	90
3.00	90

Solvent gradient.

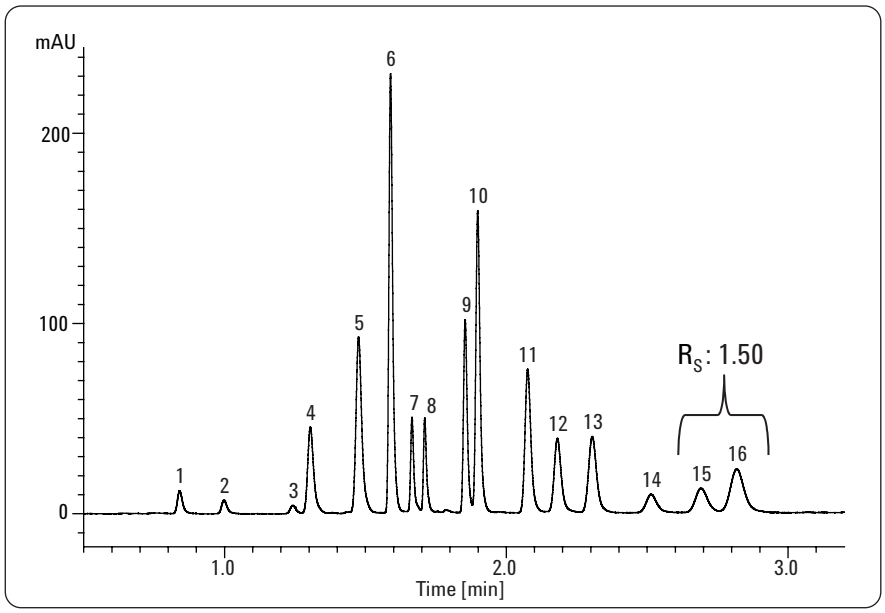

Figure 1
Separation of PAH (EPA 16) mixture on Eclipse PAH 2.1 x 50 mm, 1.8 µm column. Critical resolution (R_S) calculated by tangent method. Pressure limit for the column: 600 bar. Please note that a solvent blank run was subtracted.

Chromatographic conditions

Analytes:	PAH (EPA 16) mixture
HPLC:	Agilent 1290 Infinity LC system
Stationary phase:	Agilent ZORBAX Eclipse PAH; 50 x 2.1 mm; 1.8 µm
Mobile phase:	A: water; B: methanol Flow rate: 0.75 mL/min
Detection:	UV, 254 nm
Injection volume:	1 µL
Temperature:	39 °C
Pressure drop:	593 bar

Time [min]	% B
0.00	75
1.62	85
2.75	85
6.00	90

Solvent gradient.

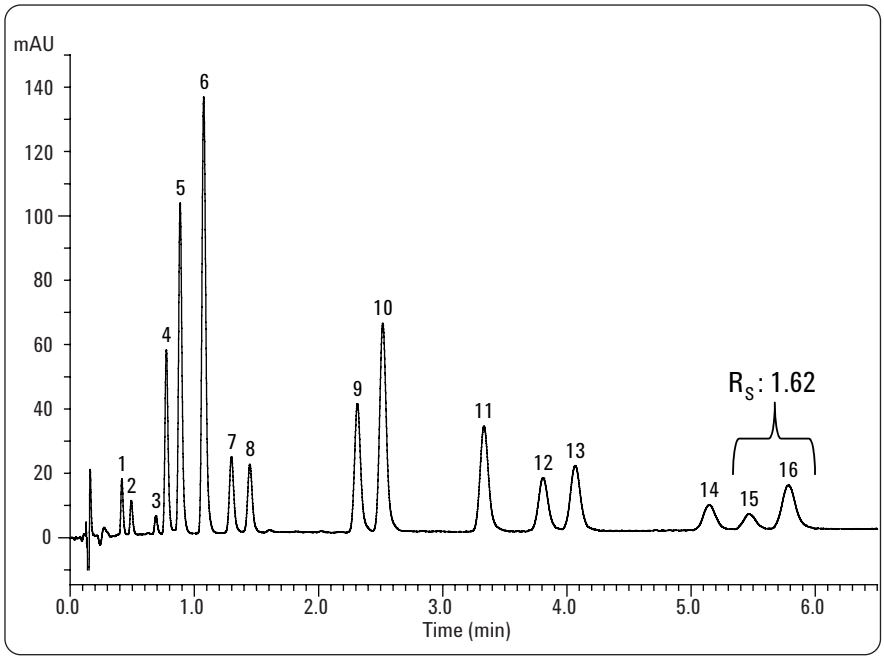

Figure 2
Separation of PAH (EPA 16) mixture on Eclipse PAH 2.1 x 50 mm, 1.8 µm column. Critical resolution (R_S) calculated by tangent method. Pressure limit for the column: 600 bar.

Conclusion

We showed that the Agilent 1290 Infinity LC system is very suited to develop fast HPLC methods. The separation of sixteen EPA PAHs succeeded in three and six minutes by using acetonitrile and methanol as organic modifier in the mobile phase, respectively. Furthermore, the presented methods highlight that fast HPLC separations are only possible using HPLC systems with a very small dwell volume. For example, conventional HPLC systems show a dwell volume of approximately 1000 µL. If a flow rate of 0.75 mL/min is used, the programmed solvent gradient reaches the column inlet with a delay of 1.3 minutes, so that the elution of the early eluting analytes occurs under isocratic conditions. Using the Agilent 1290 Infinity LC system with a pump dwell volume of 45 µL at a flow rate of 0.75 mL/min the programmed solvent gradient reaches the column inlet after 4 seconds and enables fast separations within few minutes. Furthermore, we showed that the Agilent ZORBAX Eclipse PAH column is very suitable for the separation of PAHs.

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