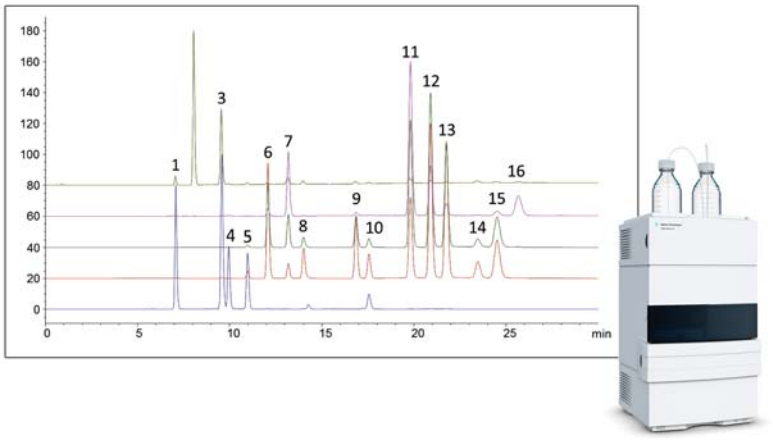
Proof of Performance

Enhancing the capabilities of the Agilent 1220 Infinity LC system with the Agilent 1260 Infinity Fluorescence Detector

Technical Overview



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Abstract

The Agilent 1220 Infinity LC is an integrated, robust and easy-to-use system for HPLC and UHPLC. Capable of handling back pressures up to 600 bar at flow rates up to 5 mL/min, the 1220 Infinity LC is ideal for UHPLC applications that deploy sub-2-µm or superficially porous columns with an id of 3 – 4.6 mm. In addition, instrument-to-instrument method transfer has been implemented by design in the 1220 Infinity LC, enabling the system to run legacy methods from any Agilent 1100 Series, Agilent 1200 Series, or Agilent 1200 Infinity Series instrument. In this Technical Overview, we demonstrate how the integrated 1220 Infinity LC can be enhanced with an external Agilent 1260 Infinity Fluorescence Detector and how a method for the analysis of polynuclear aromatic hydrocarbons can be transferred easily from an Agilent 1260 Infinity LC system.

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Introduction

Polynuclear aromatic hydrocarbons (PAHs) are hydrocarbons with multiple ring structures. This class of compounds is suspected to be mutagenic and/or contain toxic compounds. As a result, many countries have introduced maximum permission levels, which has led to the need for suitable monitoring methods. Such HPLC and UHPLC methods were described in a previous Agilent Application Note[1]. Detection using a fluorescence detector (FLD) with its high selectivity and sensitivity has proven to be ideal for the quantitative analysis of PAHs.

The Agilent 1220 Infinity LC system is ideally suited for this type of analyses but does not have an integrated FLD. Using the Controller Area Network (CAN) it is easy to connect any other Agilent module to the 1220 Infinity LC system. In this Technical Overview, the CAN connection was used to connect an FLD to the 1220 Infinity LC system for the analysis of PAHs. To demonstrate the instrument-to-instrument method transfer by design, the retention times of the PAH compounds measured on the 1220 LC system and on a 1260 Infinity LC system from a previous Application Note[1] were compared.

Experimental

Instruments and software

An Agilent 1220 Infinity LC Gradient System (G4290B), including a gradient pump (max. pressure 600 bar) with integrated degasser, autosampler, column oven, and variable wavelength detector with standard flow cell (10 mm path length) was used.

The Agilent 1200 Infinity Series Fluorescence Detector (G1321B) was equipped with the standard flow cell (8 µL) and connected via CAN to the 1220 Infinity LC.

The system was controlled using the Agilent OpenLAB CDS ChemStation Edition Rev. C.01.03.

Samples and solvents

SS EPA 610 PAH Mix in Methanol/ Methylene Chloride (1:1), (Supelco Analytical), containing the following PAHs: Naphtalene, Acenaphtylene, Acenaphtene, 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 and Indeno(1,2,3-cd)pyrene

Acetonitrile was LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak).

Chromatographic conditions

Parameter	Setting
Column:	Agilent ZORBAX Eclipse PAH 4.6 x 150 mm, 5 µm
Mobile phase:	A = Water, B = Acetonitrile
Gradient:	at 0 min 40% B at 20 min 95% B at 30 min 95% B
Stop time:	30 min
Post time:	10 min
Flow rate:	1.5 mL/min
Injection volume:	3 µL
Column temp.:	25 °C
VWD:	230 nm
FLD:	Emission: 260 nm Excitation: 350, 420, 440, and 500 nm (A, B, C, D)

Results and Discussion

The PAH sample was analyzed on a dedicated PAH column applying a gradient using water and acetonitrile as mobile phase. Since the PAHs have their emission maximums at different wavelengths, the multi-signal acquisition feature of the Agilent 1260 Infinity FLD was applied. In addition, the UV signal of the integrated variable wavelength detector (VWD) of the Agilent 1220 Infinity LC system was used for the analysis of acenaphthylene.

Figure 1 shows the multisignal chromatogram for the analysis of PAHs using fluorescence detection.

A major advantage of the 1220 Infinity LC system is the "instrument-to-instrument method transfer by design", which means that methods from any Agilent 1100 Series, Agilent 1200 Series, or Agilent 1200 Infinity LC system can be transferred without modifications and lead to the same results, for example, the same retention time. In this Technical Overview, the transfer was even done from an Agilent 1260 Infinity Series Binary Pump, which is a high-pressure mixing pump, to the 1220 Infinity System, with a built-in low-pressure mixing pump. But even when the transfer is done from a pump using a different operation and mixing principle[2], the retention time differences are 2.2 % or less as shown in Table 1. While the experiments on the 1260 Infinity LC and the 1220 Infinity LC system were done on the identical column, several weeks passed and many injections were meanwhile done on that column. Therefore, this value lies well within the limits that would be expected from column aging over this time.

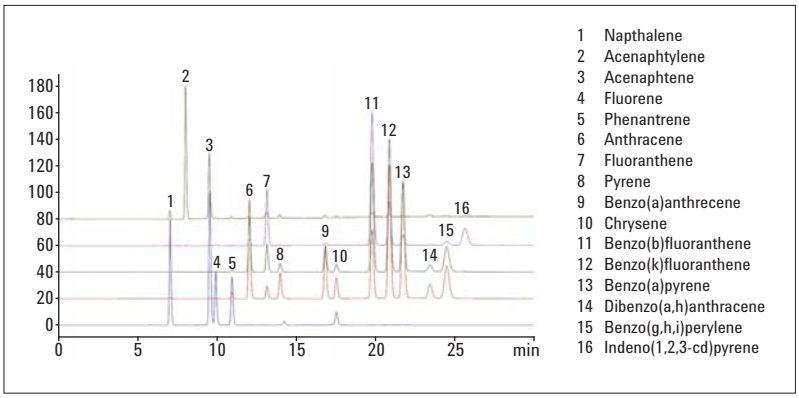

Figure 1
Multi-signal chromatogram for the analysis of PAHs using fluorescence detection (acenaphthylene was determined using UV detection).

Compound	Signal	RT (1260 Infinity)	RT (1220 Infinity)	Δ (%)
Naphthalene	FLD A	7.24	7.08	2.2
Acenaphthylene	VWD	8.24	8.06	2.2
Acenaphthene	FLD A	9.72	9.57	1.5
Fluorene	FLD A	10.1	9.95	1.5
Phenanthrene	FLD A	11.11	10.97	1.3
Anthracene	FLD B	12.22	12.07	1.2
Fluoranthene	FLD C	13.34	13.17	1.3
Pyrene	FLD B	14.16	14.00	1.1
Benzo(a)anthracene	FLD B	16.95	16.86	0.5
Chrysene	FLD B	17.63	17.56	0.4
Benzo(b)fluoranthene	FLD C	19.82	19.82	0.0
Benzo(k)fluoranthene	FLD B	20.86	20.90	0.2
Benzo(a)pyrene	FLD B	21.68	21.76	0.4
Dibenzo(a,h)anthracene	FLD B	23.37	23.46	0.4
Benzo(ghi)perylene	FLD B	24.47	24.50	0.1
Indeno(1,2,3-cd)pyrene	FLD D	25.75	25.66	0.3

Table 1
Retention time differences between the Agilent 1260 Infinity Binary LC System and the Agilent 1220 Infinity LC System.

Conclusion

In this Technical Overview, we have shown the enhancement of an Agilent 1220 Infinity LC system by simply adding an Agilent 1260 Infinity FLD through CAN bus connection. Using the analysis of PAHs as an example, we could also demonstrate the simple method transfer between systems leading to identical results.

References

1
"Agilent Application Solution: Analysis of PAHs in soil according to EPA 8310 method with UV and fluorescence detection" Agilent Technologies Application Note, **2011**, publication number 5990-8414EN

2
"Scope of low and high pressure mixing Comparing the Agilent 1260 Infinity Quaternary and Binary Pumps" Agilent Technologies Technical Overview, **2011**, publication number 5990-7143EN

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