Application Note

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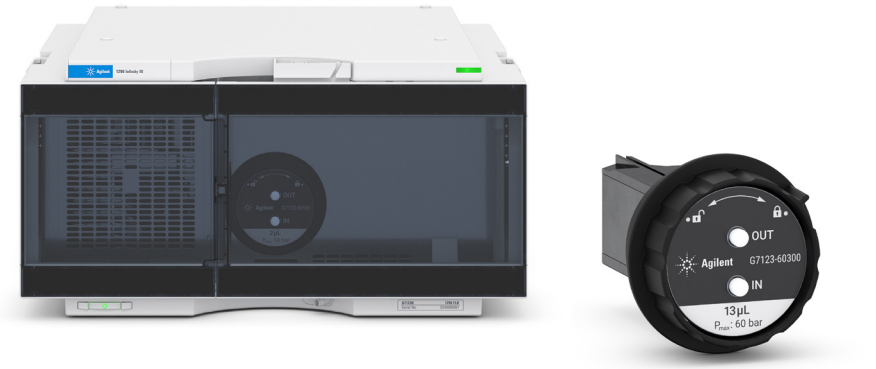
Sensitive Detection of PAHs Using the Agilent 1290 Infinity III Fluorescence Detector



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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are environmentally persistent organic contaminants whose chemical stability and toxicity make them critical targets in environmental monitoring, industrial process control, and health protection. This application note demonstrates that the Agilent 1290 Infinity III Fluorescence Detector (FLD) offers a high linear dynamic range and can measure PAHs at the highest sensitivity, enabling lowest limits of detection (LODs) and limits of quantification (LOQs). The 1290 Infinity III FLD also offers a low dispersion volume cell to be used under UHPLC conditions, providing lowest peak dispersion. In addition, fast multiple wavelength switching can be used for detection of compounds at their individual wavelength optimum in UHPLC runs. For fast and easy method development, online scan mode can be used to determine the most suitable excitation and emission wavelength of target analytes.

Introduction

Polyaromatic hydrocarbon compounds occur as pollution in environments such as soil and water, and in foodstuffs and feed stock. PAHs are of significant scientific and regulatory interest due to their persistence, bioaccumulation potential, and toxicological properties. Several PAHs exhibit mutagenic or carcinogenic behavior, with compounds such as benzo(a) pyrene serving as reference markers for assessing PAH contamination and associated health risks. Their hydrophobic nature allows them to bind strongly to particulate matter and organic-rich environmental matrices, enabling long-range transport and long-term environmental residence. Due to their biological impact, even at very low concentration, a highly sensitive measurement method is required. PAHs are typically actively fluorescent, which offers a sensitive and selective measurement method by means of a fluorescent detection after HPLC separation. Fluorescent behavior offers the generation of an optimized fluorescent method for each compound by using individual excitation and emission wavelengths. Additionally, matrix compounds which are typically not fluorescent will not disturb detection.

The Agilent 1290 Infinity III Fluorescence Detector is designed to allow lowest LODs as well as high linear dynamic range. It enables superior high-speed wavelength switching and rapid spectral scans for method development, allowing scanning over a broad wavelength range using a low step size which can still record a sufficient number of spectra per peak. The FLD detector's high data rate, combined with fast wavelength switching capability ensure effective highspeed analysis of multiple compounds.

This application note will describe a method for the determination of 16 PAH compounds by means of the 1290 Infinity III FLD. The optimization of the fluorescence detection method will be described by means of the spectral tool for determination of optimum excitation and emission wavelengths. The separation will be done under UHPLC conditions with the optimum excitation and emission in the elution time window of each compound. Determination of the LOD of anthracene is shown with a separate method using the large volume 13 µL flow cell and high PMT gain to obtain best sensitivity performance.

Experimental

Instrument setup

- Agilent 1290 Infinity III High-Speed Pump (G7120A)
- Agilent 1290 Infinity III Multisampler (G7167B)
- Agilent 1290 Infinity III Multicolumn Thermostat (G7116B)
- Agilent 1290 Infinity III Fluorescence Detector (G7123B)

Software

Agilent OpenLab CDS, version 2.8

Columns

- Agilent ZORBAX RRHD Eclipse Plus PAH Column (2.1 × 50 mm, 1.8 µm, 1200 bar) (part number 959757-918)
- Agilent ZORBAX RRHD Eclipse Plus PAH Column (2.1 × 100 mm, 1.8 µm, 1200 bar) (part number 959758-918)

Standards

- PAH mixture (part number 8500-6035), comprising 16 PAH compounds (see Table 1) at a concentration of 500 µg/mL in acetonitrile:acetone:toluene (6:3:1).
- Anthracene 10 µg/mL in Acetonitrile (obtained from Dr. Ehrenstorfer GmbH)

Calibration

- To determine the linear dynamic range, the PAH mixture was diluted in a 1:1 pattern from 50 µg/mL to 1.49 ng/mL.
- For LOD measurements, the anthracene standard was diluted two times 1:1000 and further on in 1:1 pattern. 3 µL were injected to reach 15 to 0.468 fg on column.

Solvents

- Agilent InfinityLab Acetonitrile for HPLC (part number 5191-5100-002)
- Agilent InfinityLab Water for HPLC (part number 5191-5120-002)

Method

Method Settings	
Binary Pump	
Parameter	Value
Mobile Phase A	Water
Mobile Phase B	Acetonitrile
Stop Time	4 min
Flow Rate	0.9 mL/min
Gradient	Time (min) %B 0 45 1.26 62 1.52 90 3.10 55
Multisampler	
Injection Volume	0.5 µL
Multiwash	Solvent S2: acetone, 15 s, needle wash Solvent S1: acetone/water 65/35, 8 s, needle wash
Column Oven	
Column Temperature	30 °C
Fluorescence Detector	
Excitation/Emission Wavelength (nm)	See Table 1
Wavelength Switching	See Table 1
PMT Gain	Standard
Peak Width	0.125 s response time (80 Hz)

Results and discussion

Optimization of excitation and emission wavelength

To discover the most suitable excitation and emission wavelengths for all PAHs, online scans were performed using a 20-minute gradient method with the Agilent ZORBAX RRHD Eclipse Plus PAH Column (2.1 × 100 mm, 1.8 µm, 1200 bar, part number 959758-918) at 0.42 mL/min as described in the column data sheet.[1] As an example for an emission scan, the results for the PAH mixture (part number 8500-6035) are displayed in Figure 1. In the first step, an emission scan was performed using 250 nm as an excitation wavelength, emission scan range from 270 to 800 nm, and a 2 nm step size (Figure 1A). The emission wavelength yielding the maximum response was determined for each compound by moving the vertical dashed line to the region of interest in the heatmap. The emission spectrum of anthracene is presented in Figure 1B, showing the optimal wavelength of 402 nm.

Table 1. Optimized excitation and emission wavelength and switching times for the 16 PAH compounds inherent in the standard PAH mixture used for this study.

Compound Name	Excitation Wavelength (nm)	Emission Wavelength (nm)	Switching Time (min)
Naphthalene	246	294	0.00
Acenaphthylene	280	328	0.73
Acenaphthene	298	328	1.00
Fluorene	272	310	1.27
Phenanthrene	247	364	1.40
Anthracene	246	402	1.54
Fluoranthene	364	456	1.66
Pyrene	312	392	1.73
Benzo(a)anthracene	283	390	1.84
Chrysene	266	382	1.95
Benzo(b)fluoranthene	299	440	2.05
Benzo(k)fluoranthene	302	412	2.18
Benzo(a)pyrene	365	408	2.29
Dibenzo(a,h)anthracene	296	398	2.44
Benzo(g,h,i)perylene	298	414	2.61
Indeno(1,2,3-cd)pyrene	304	502	2.74

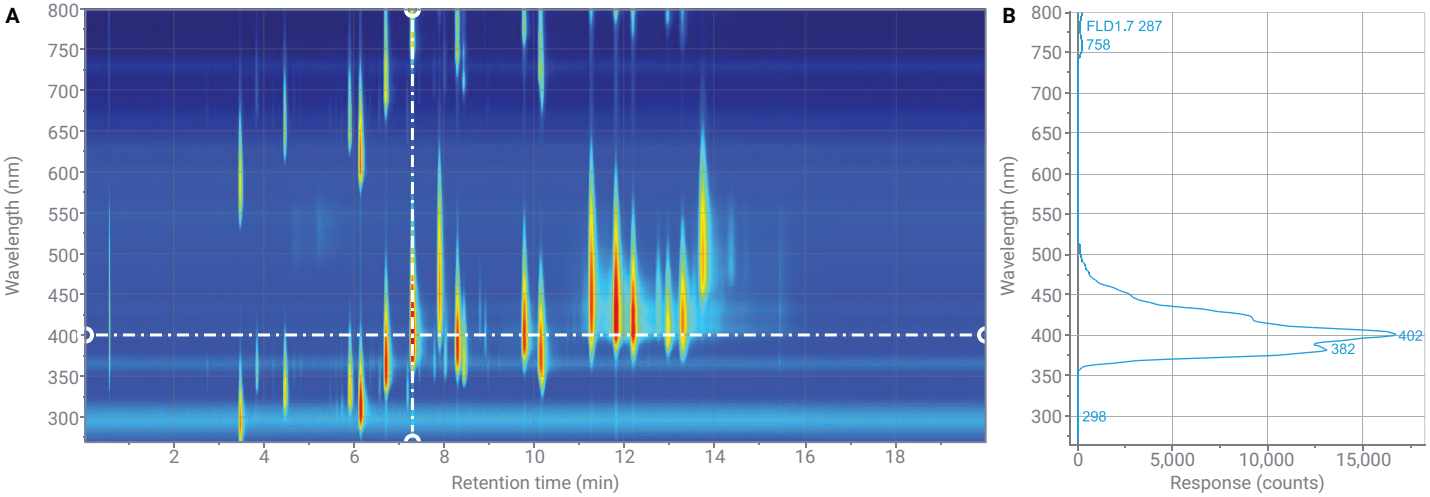

Figure 1. (A) Emission scan of the PAH mixture using 250 nm as excitation wavelength and emission wavelength ranging from 270 to 800 nm. (B) Emission spectrum of anthracene at 7.287 minutes.

Using the optimum emission wavelength, an excitation scan was used to identify the optimum excitation wavelength (Figure 2). Therefore, an excitation wavelength range from 200 to 380 nm was used (Figure 2A). This experiment identified the optimum excitation wavelength for anthracene at 246 nm in the excitation spectrum (Figure 2B). The complete results obtained for the optimized excitation and emission wavelengths are displayed in Table 1.

Fast wavelength switching

Because each PAH compound requires different optimal excitation and emission wavelengths (Table 1), fast wavelength switching is necessary. In fast multiwavelength analyses, precise switching, as well as reproducible chromatographic conditions are critical due to closely eluting peaks. The standard HPLC method applied for the optimization of the excitation and emission wavelength was transferred to a fast UHPLC method by means of a short UHPLC column (ZORBAX RRHD Eclipse Plus PAH Column, 2.1 × 50 mm, 1.8 µm, 1200 bar part number 959757-918) and increasing the flow rate. Following a previous application note[2], a short gradient using water (A) against acetonitrile (B) was executed at 0.9 mL/min and 30 °C column temperature. The FLD was equipped with the standard 2 µL flow cell, which is designed for minimal peak dispersion required in UHPLC analysis. The robust flow cell design also allows pressures up to 60 bar and can easily handle higher flow rates used in UHPLC applications. The system was also

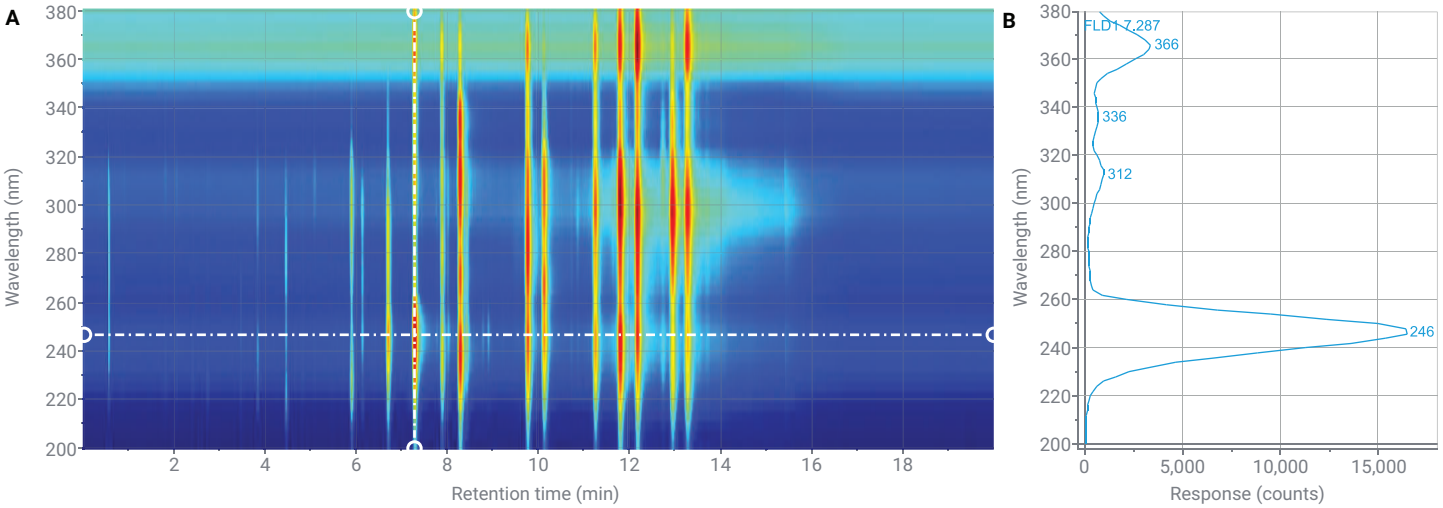

Figure 2. (A) Excitation scan of the PAH mixture using 402 nm as emission wavelength and excitation wavelength ranging from 200 to 380 nm. (B) Excitation spectrum of anthracene at 7.287 minutes.

equipped with the Agilent 1290 Infinity III Low Dispersion Kit (part number 5067-5963) to allow a further reduction of the extra column volume. The FLD provides a broad data rate range from 1.25 to 160 Hz. The used data rate of 80 Hz delivered enough data points for the presented UHPLC runs. The total run time was four minutes for the separation of the 16 PAH compounds (Figure 3) including re-equilibration of the column. From the separation done under optimized wavelength conditions, the resolution, retention time, RSDs, and the peak area RSDs were calculated. Retention time RSDs were typically below 0.05% and peak area RSDs below 0.9% (Table 2).

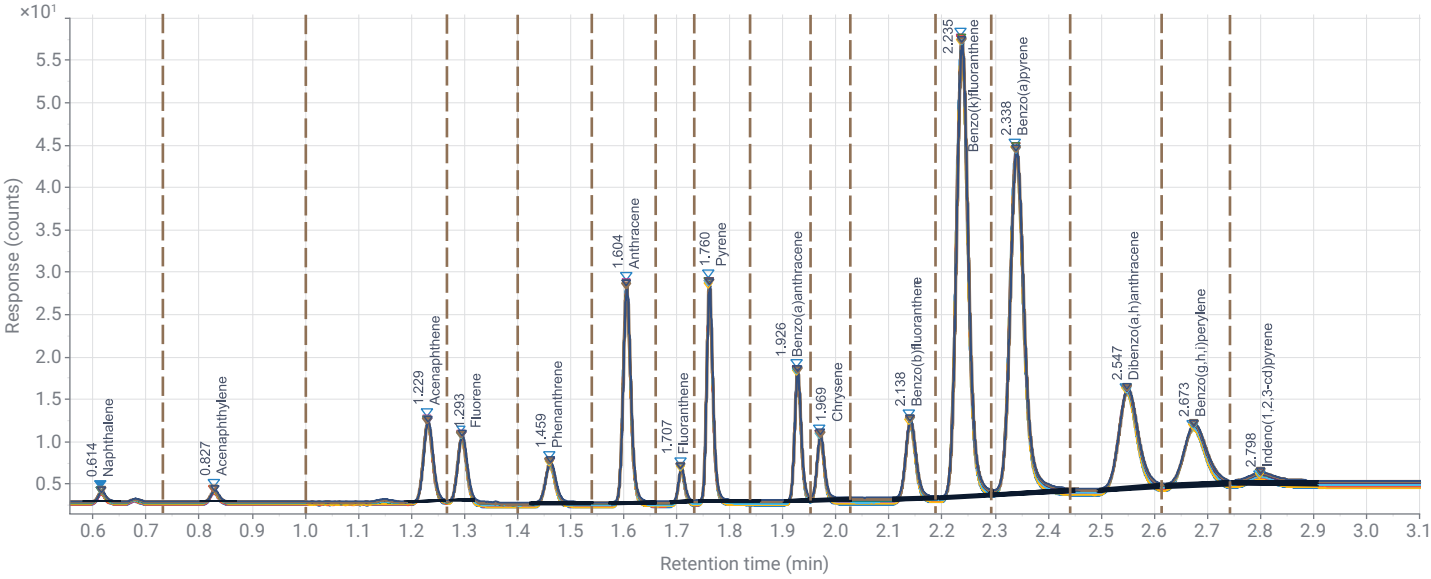

Figure 3. Overlay of 10 consecutive UHPLC analyses of the PAH mixture using fast wavelength switching.

Table 2. Resolution, retention time, and peak area RSDs of the 16 separated standard compounds used in this study.

Compound Name	Retention Time (min)	Retention Time RSD (%)	Area RSD (%)	Average Resolution
Naphthalene	0.614	0.11	0.59	–
Acenaphthylene	0.827	0.09	0.94	7.9
Acenaphthene	1.229	0.05	0.73	13.1
Fluorene	1.293	0.05	0.98	2.0
Phenanthrene	1.459	0.04	0.69	4.9
Anthracene	1.604	0.02	0.52	4.7
Fluoranthene	1.707	0.02	0.68	4.3
Pyrene	1.760	0.02	0.46	2.5
Benzo(a)anthracene	1.926	0.01	0.59	7.6
Chrysene	1.969	0.02	0.62	1.8
Benzo(b)fluoranthene	2.138	0.02	0.47	5.6
Benzo(k)fluoranthene	2.235	0.02	0.42	2.6
Benzo(a)pyrene	2.338	0.03	0.55	2.3
Dibenzo(a,h)anthracene	2.547	0.03	0.69	3.7
Benzo(g,h,i)perylene	2.673	0.03	0.99	1.8
Indeno(1,2,3-cd)pyrene	2.798	0.04	0.40	1.6

The time necessary for fast wavelength switching was examined for benzo(a)anthracene and chrysene peaks, showing that the switching time of approximately 120 ms is fast enough to ensure analysis of both compounds at their individual wavelength optimum (Figure 4). Thus, the FLD's 80 Hz data rate combined with fast wavelength switching capability ensure effective highspeed analysis of multiple compounds.

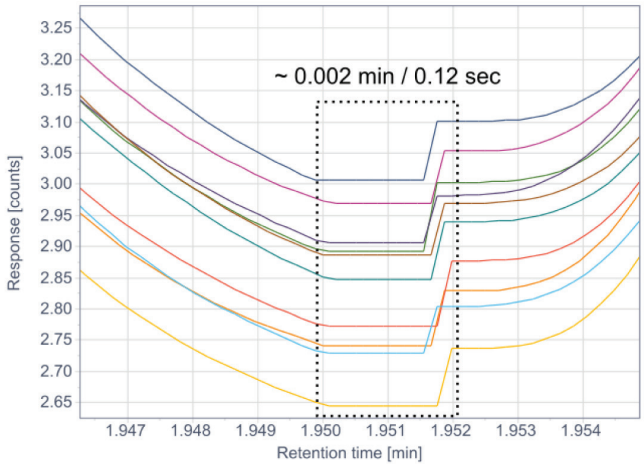

Figure 4. Overlay of 10 consecutive UHPLC runs highlighting the required wavelength switching time between the benzo(a)anthracene and chrysene peak, showing an average resolution of 1.8.

High sensitivity analysis of anthracene

For determination of the lowest achievable sensitivity, anthracene has been analyzed at Ex 246 and Em 402 nm using an isocratic method at 0.6 mL/min with the ZORBAX RRHD Eclipse Plus PAH Column, 2.1 × 50 mm, 1.8 µm (part number 959757-918). As the 1290 Infinity III FLD is designed to allow quick and easy cell exchange between applications, the flow cell could be rapidly exchanged with the 13 µL version without the need for recalibration. To achieve lowest LODs, the PMT gain was set to High. The standard solution was diluted in a 1:1 pattern from 15 to 0.47 fg on column to generate a calibration curve showing the linearity at lowest concentration levels (Figure 5). The achieved R^2 value was 0.99971, and the LOD (calculated for S/N = 3) was below 1 fg on column.

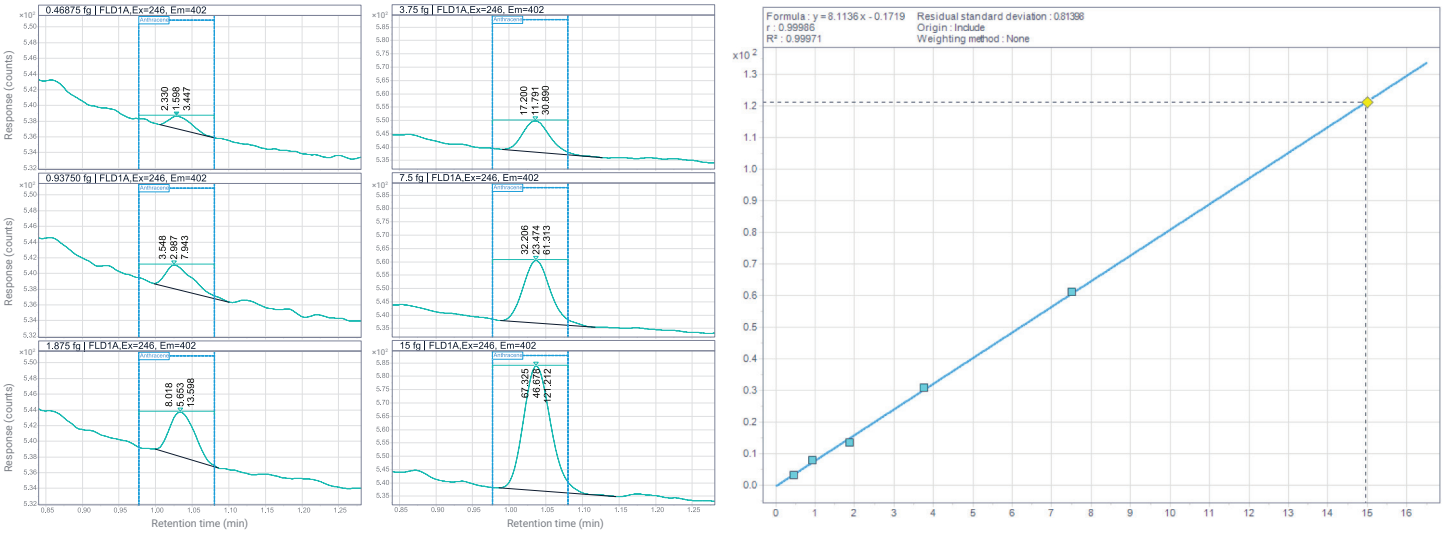

Figure 5. High-sensitivity analysis of anthracene showing 1:1 dilutions ranging from 0.46875 to 15 fg on column using isocratic elution at 35:65 water:acetonitrile, PMT Gain High, and the 13 µL FLD flow cell. Peak annotations show S/N, height, and area (left to right).

Linear dynamic range

To determine the linear dynamic range (LDR) for all PAHs, gradient elution from 40 to 100% B in 12 minutes using water (A) and acetonitrile (B) at 0.42 mL/min was executed as described in the ZORBAX RRHD Eclipse Plus PAH column data sheet.[1] The PMT Gain was set to Standard and the FLD was equipped with the standard 2 µL flow cell. The obtained calibration ranges, LOQs (S/N = 10), LDR, and correlation coefficient of linear calibration curves for all compounds are listed in Table 3. Typically, a linear dynamic range above five decades and LOQs in the lower ng/L region could be achieved. For benzo(a)pyrene, which is—due to its carcinogenicity, toxicity, and widespread occurrence—one of the most extensively studied members of the PAHs, an LDR of 5.5 decades showing an R^2 of 0.99987 and a LOQ of 19 ng/L could be achieved.

Table 3. Calibration range in fg on column, LOQ in ng/mL, Linear dynamic range, and correlation coefficients of linear calibration.

Compound Name	Calibration Range (fg on Column)	LOQ (ng/L)	LDR (Decades)	R^2
Naphthalene	381.469–50,000,000	253.0	5.13	0.99931
Acenaphthylene	381.469–50,000,000	112.0	5.13	0.99994
Acenaphthene	95.367–50,000,000	21.3	5.52	0.99992
Fluorene	47.683–50,000,000	27.2	6.1	0.99992
Phenanthrene	95.367–50,000,000	59.3	5.52	0.99982
Anthracene	23.842–50,000,000	17.9	6.21	0.99938
Fluoranthene	190.734–50,000,000	96.6	5.26	0.99995
Pyrene	23.842–50,000,000	12.5	6.21	0.99989
Benzo(a)anthracene	95.367–50000000	71.1	5.52	0.99991
Chrysene	190.734–50,000,000	117.7	5.26	0.99982
Benzo(b)fluoranthene	95.367–50,000,000	82.1	5.52	0.99996
Benzo(k)fluoranthene	11.920–25,000,000	10.2	6.21	0.9998
Benzo(a)pyrene	47.683–25,000,000	19.1	5.52	0.99987
Dibenzo(a,h)anthracene	95.367–50,000,000	58.4	5.52	0.99987
Benzo(g,h,i)perylene	95.367–25,000,000	28.1	5.26	0.99947
Indeno(1,2,3-cd)pyrene	762.939–50,000,000	649.9	4.66	0.99421

Conclusion

This application note describes the results obtained for the measurement of a multicompound PAH sample by means of the Agilent 1290 Infinity III Fluorescence Detector. The comprehensive optimization of excitation and emission wavelengths, combined with the implementation of fast wavelength switching and UHPLC-compatible low dispersion hardware, demonstrate that the Agilent 1290 Infinity III FLD provides highly sensitive, rapid, and reliable analysis of PAHs. The method achieved excellent chromatographic performance, reproducible retention times and peak areas, and robust separation of closely eluting PAHs at high speed. Additionally, high sensitivity analysis of anthracene using the 13 µL flow cell and high PMT settings resulted in an LOD below 1 fg on column. Additionally, the Agilent 1290 Infinity III FLD showed a linear dynamic range of up to more than six decades with R^2 values typically better than 0.999.

References

1. Agilent ZORBAX Rapid Resolution High Definition Eclipse PAH Threaded Columns. *Agilent Technologies data sheet*, publication number 820210-018, **2011**.
2. Wiese, S.; Teutenberg, T.; Hoffmann, B.; Naegele, E. High Throughput Method Development for PAHs using the Agilent 1290 Infinity LC system and a ZORBAX Eclipse PAH column. *Agilent Technologies application note*, publication number 5990-5007EN, **2009**.

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