

## High Sensitivity Detection of Polycyclic Aromatic Hydrocarbons using Fluorescence Detection in SFC

### Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) are produced by the incomplete combustion of organic compounds from various sources including diesel exhaust, coal dust, and cigarette smoke; many are strongly carcinogenic. PAHs are subject to regulation by various organizations including the EPA (United States Environmental Protection Agency). These regulations strictly control the impact of PAHs on environmental pollution and their effects on human health.

PAHs exhibit strong natural fluorescence due to the aromatic structure with many conjugated double bonds. This makes the high sensitivity of fluorescence detection necessary for the low levels often found in the environment.

In this application note, 16 PAHs were separated by supercritical fluid chromatography (SFC) and detected using a FP-4020 fluorescence detector (FL detector) with a high pressure flow cell (previously introduced in LC application data No.032006U). Data was collected using both UV-Vis and fluorescence detectors for comparative purposes.

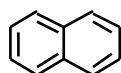
### Keywords

Polycyclic Aromatic Hydrocarbons, PAHs, Ethylpyridine column, SFC, Fluorescence detector, carcinogenicity, UV-Vis, absorbance detector

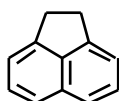
## Experimental

Equipment		Conditions	
CO <sub>2</sub> Pump	PU-4380	Column	2-Ethylpyridine (Princeton Chromatography) (4.6 mm I.D. x 250 mm L, 5 μm)
Modifier Pump	PU-4180	Eluent A	CO <sub>2</sub>
Modifier Pump Option	SV Unit MX Unit	Eluent B	Acetonitrile
Autosampler	AS-4350	Gradient	(A/B), 0 min (95/5) → 1.5 min (95/5) → 12 min (80/20) → 14 min (80/20) → 15 min (95/5) → 20 min (95/5)
Column Oven	CO-4060	Flow Rate	3.0 mL/min
UV/Vis Detector	UV-4070	Column Temp.	40°C
UV/Vis Detector Flow Cell	Analytical High Pressure	Wavelength	Refer to Figure 1
FL Detector	FP-4020	Back Pressure	15 MPa
FL Detector Flow Cell	Analytical High Pressure	Injection Volume	5 μL
Back Pressure Regulator	BP-4340	Standard Sample	Mixture of 16 PAHs standards in acetonitrile

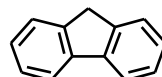
## Structure of PAHs



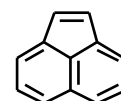
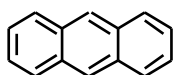
1. Naphthalene



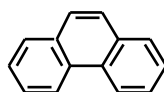
2. Acenaphthene



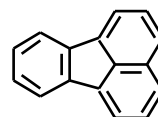
3. Fluorene

4. Acenaphthylene  
(non-fluorescent)

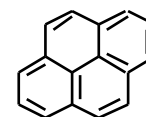
5. Anthracene



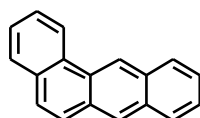
6. Phenanthrene



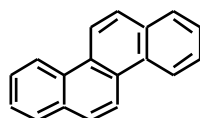
7. Fluoranthene



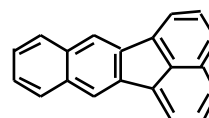
8. Pyrene



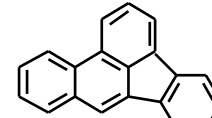
9. Benzo[a]anthracene



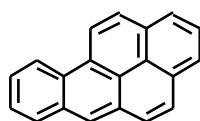
10. Chrysene



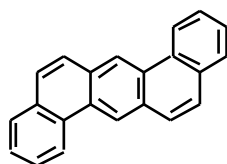
11. Benzo[k]fluoranthene



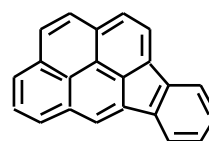
12. Benzo[b]fluoranthene



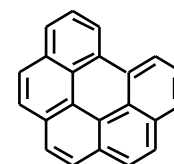
13. Benzo[a]pyrene



14. Dibenz[a,h]anthracene



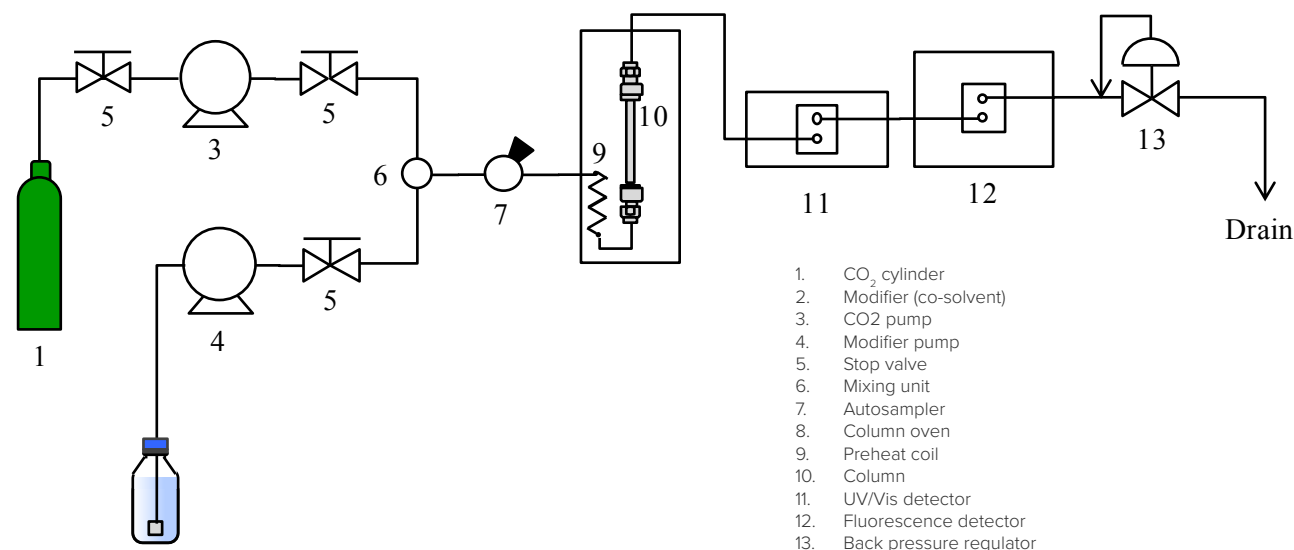
15. Indeno[1,2,3-cd]pyrene



16. Benzo[g,h,i]perylene

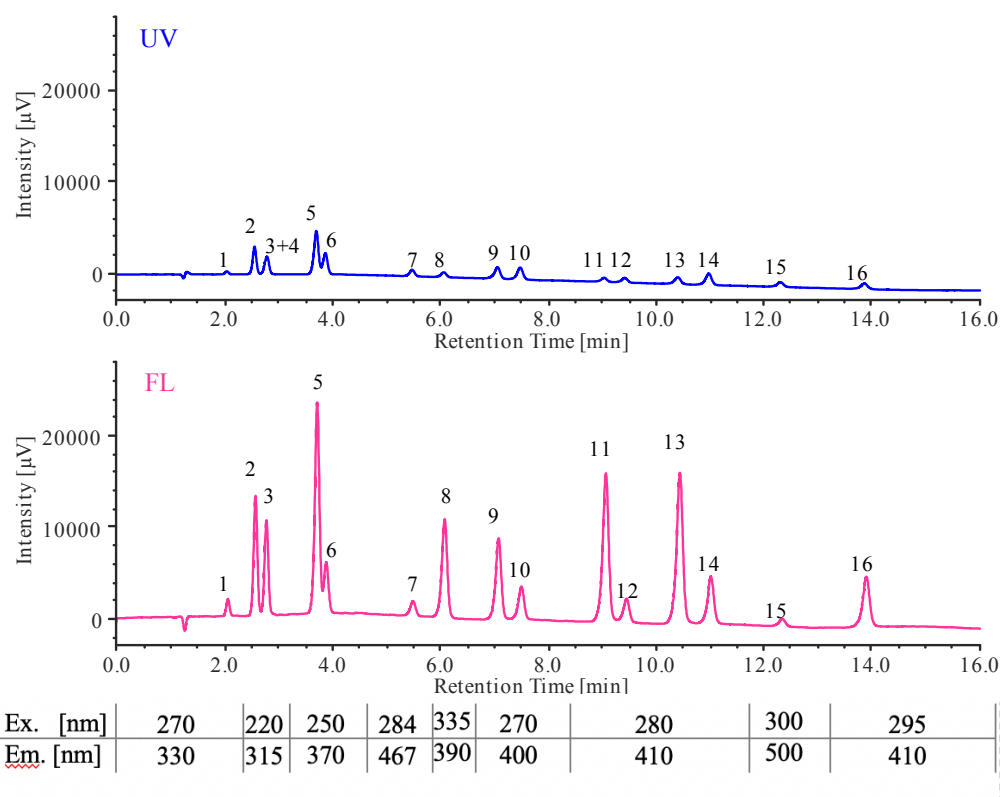
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SFC System Schematic



## Result

Figure 1 shows the separation of a 16-component PAHs standard mixture with UV-visible detection (500 pg/ $\mu$ L each) and fluorescence detection (5 pg/ $\mu$ L each). Since Acenaphthylene does not fluoresce, it was not detected in the fluorescence chromatogram and was co-eluted with Fluorene (peak No. 3) overlapping in the UV chromatogram. Table 1 shows the detection limits of each component.



**Figure 1.** UV and FL chromatogram of PAHs 16-component (UV: 500 pg/ $\mu$ L, FL: 5 pg/ $\mu$ L)

1: Naphthalene, 2: Acenaphthene, 3: Fluorene, 4: Acenaphthylene (non-fluorescent), 5: Anthracene, 6: Phenanthrene, 7: Fluoranthene, 8: Pyrene, 9: Benzo[a]anthracene, 10: Chrysene, 11: Benzo[k]fluoranthene, 12: Benzo[b]fluoranthene, 13: Benzo[a]pyrene, 14: Dibenzo[a,h]anthracene, 15: Indeno[1,2,3-cd]pyrene, 16: Benzo[g,h,i]perylene  
 The measurement wavelength used for the UV/Visible detector is the same as the excitation wavelength of the fluorescence detector. The gain of the fluorescence detector is x100.

Using the fluorescence detector, the PAHs were detected at the low detection limit of 0.17 pg to 4.6 pg, and the sensitivity ratio compared with the UV detector is approx. 20 to 400 times. Figure 2 shows a calibration curve for the standard PAH mixture at concentrations from 1 to 100 pg/ $\mu$ L measured with the fluorescence detector. From these results (Table 1), excellent linearity with a correlation coefficient of 0.9997 or higher was confirmed for all components.

**Table 1.** Lower limit of detection of PAHs in each detector and linearity in FL detector

Peak No.	Compound	Detection limit [pg]		Sensitivity Ratio* <sup>3</sup>	Correlation coefficient (FL)* <sup>4</sup>
		UV* <sup>1</sup>	FL* <sup>2</sup>		
1	Naphthalene	120	2.04	59	1.0000
2	Acenaphthene	13.6	0.293	46	0.9998
3	Fluorene* <sup>5</sup>	21.3	0.369	58	0.9999
5	Anthracene	8.67	0.166	52	0.9998
6	Phenanthrene	17.8	0.676	26	1.0000
7	Fluoranthene	58.8	2.34	25	0.9999
8	Pyrene	75.2	0.357	211	0.9998
9	Benzo[a]anthracene	32.5	0.432	75	0.9999
10	Chrysene	31.5	1.06	30	1.0000
11	Benzo[k]fluoranthene	93.0	0.236	393	0.9997
12	Benzo[b]fluoranthene	81.9	1.46	56	0.9999
13	Benzo[a]pyrene	53.6	0.233	230	0.9998
14	Dibenz[a,h]anthracene	32.6	0.737	44	1.0000
15	Indeno[1,2,3-cd]pyrene	79.2	4.61	17	0.9998
16	Benzo[g,h,i]perylene	65.5	0.697	94	0.9998

\*1 Calculated from the measurement of 500 pg/mL standard sample (S/N=3)

\*2 Calculated from the measurement of 5 pg/mL standard sample (S/N=3)

\*3 Lower limit of detection of UV detector/Lower limit of detection of FL detector

\*4 Calibration curve was made using standards at 1, 5, 10, 50, 100 pg/mL

\*5 The value of the Fluorene peak using UV detection includes Acenaphthylene which is co-eluted.

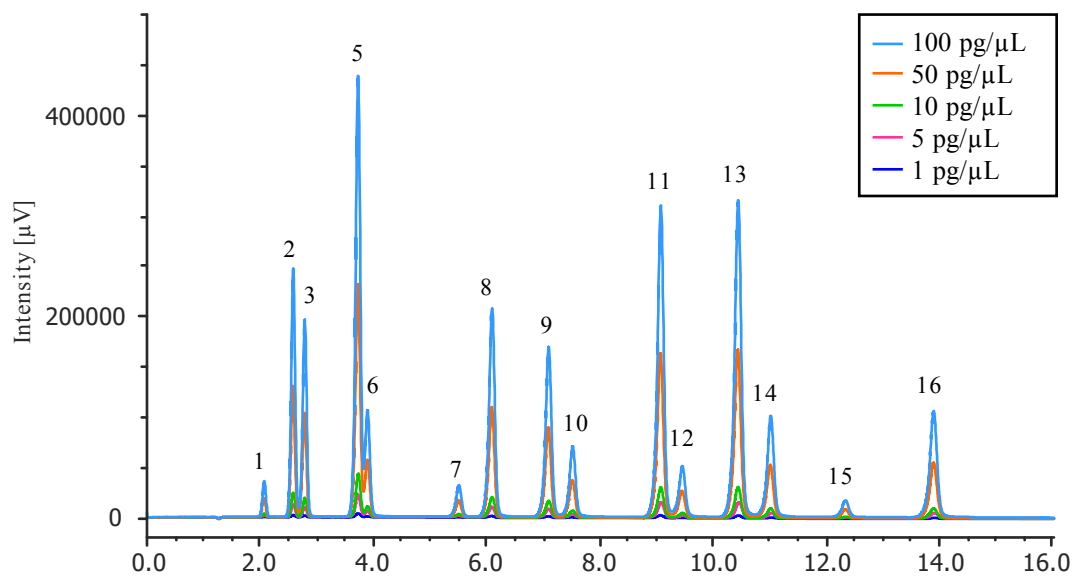


Figure 2. FL chromatogram of PAHs 16-components

1: Naphthalene, 2: Acenaphthene, 3: Fluorene, 4: Acenaphthylene (non-fluorescent), 5: Anthracene, 6: Phenanthrene, 7: Fluoranthene, 8: Pyrene, 9: Benzo[a]anthracene, 10: Chrysene, 11: Benzo[k]fluoranthene, 12: Benzo[b]fluoranthene, 13: Benzo[a]pyrene, 14: Dibenzo[a,h]anthracene, 15: Indeno[1,2,3-cd]pyrene, 16: Benzo[g,h,i]perylene  
Measurement wavelength and gain set for the fluorescence detector is the same as Figure 1.

Part Number	Model	Description
7032-J002A	PU-4380	CO <sub>2</sub> Delivery Pump
7002-J004A	PU-4180	RHPLC Pump (Base Unit)
7008-H350A	SV-4300	Stop valve unit
7007-H063A	MX-4300-NC	Dynamic Mixing Unit (for SFC/E)
7007-H146B		Mixing chamber, 1.5 mL, for Analytical
7038-J002A	AS-4350	SFC Autosampler
7021-J002A	CO-4060	Column Oven
6990-J002A	BP-4340	Automatic Back Pressure Regulator
7025-J002A	UV-4070	UV/VIS detector
7025-H201B		Analytical high pressure flow cell
6996-J001A	FP-4020	Fluorescence detector
7052-H201A		Analytical high pressure flow cell
7139-J002A	BS-4500-4	Bottle stand
Custom order		Princeton SFC 2-Ethylpyridine 60 Å (4.6 mm I.D. x 250 mm L, 5 µm)
6688-H564A	LC-NetCG	CG cable (x6)
6925-H262A		Preheat coil 1/16", 0.8 µm I.D. x 5 m L
7059-J012A	ChromNAV V2	ChromNAV 2.0 Chromatography Data System
		SFC tubing kit
7001-H405B		Maintenance tool kit