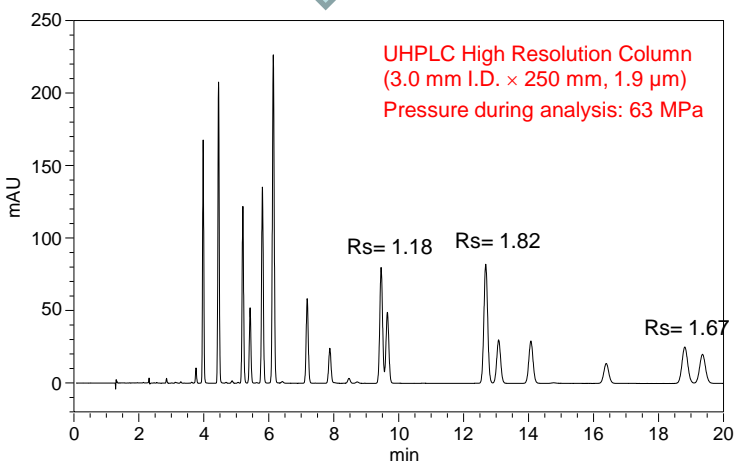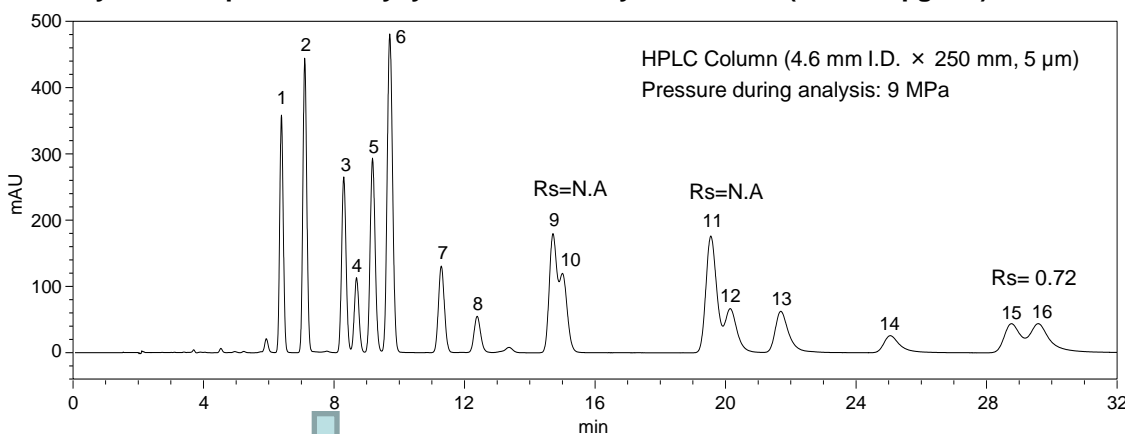In AS/LC-039, the example of the analysis in which 16 polycyclic aromatic hydrocarbons (PAHs) were analyzed by connecting 6440 fluorescence detector to Hitachi Ultra High Performance Liquid Chromatograph ChromasterUltra Rs was introduced.
This time, the analysis method in which DAD is used as the detector is introduced here.
For the columns, highly versatile LaChromUltra II series and LaChrom II series were used and the differences in the resolutions depending on the packing material particle sizes and column sizes were compared. In addition, the example of the analysis for a non-fluorescence component by DAD detection is introduced.
PAHs is the general term to described a group of compounds consisting of multiple benzene rings and a part of the PAH components are known as suspected carcinogens. The range for the analysis is also wide and covers air, water, food, etc. Thus, the fact-finding surveys are conducted all over the world and the regulations are being established.

ChromasterUltraR_S
(Example with DAD Connection)
(Includes optional parts and those prepared by a customer)

■ Analysis Example of 16 Polycyclic Aromatic Hydrocarbons (10 - 200 μg/mL)

HPLC Column (4.6 mm I.D. × 250 mm, 5 μm)
Pressure during analysis: 9 MPa

UHPLC High Resolution Column
(3.0 mm I.D. × 250 mm, 1.9 μm)
Pressure during analysis: 63 MPa

<Standard Samples>

No.	Component	No.	Component
1	Naphthalene	9	Benzo(a)anthracene
2	Acenaphthylene	10	Chrysene
3	Acenaphthene	11	Benzo(b)fluoranthene
4	Fluorene	12	Benzo(k)fluoranthene
5	Phenanthrene	13	Benzo(a)pyrene
6	Anthracene	14	Dibenzo(a,h)anthracene
7	Fluoranthene	15	Benzo(ghi)perylene
8	Pyrene	16	Indenol(1,2,3,-cd)pyrene

SS EPA 610 PAH Mix(SIGMA) is used for the standard samples.

LaChromUltra II C18 column realizes high-pressure resistance by using inorganic-organic complex silica material with the higher physical and chemical resistance than conventional silica gel. As a result, the UHPLC high resolution column (1.9 μm, length of 250 mm) achieving the theoretical plate number of 50000/column (specification conditions) is added to the product lineup. By using this column, the analysis time can be shortened by about 40% compared to that by a HPLC column and the resolution can also be greatly improved.
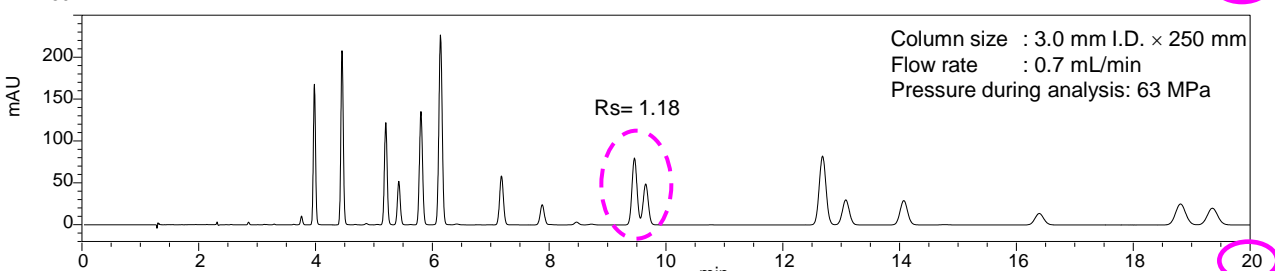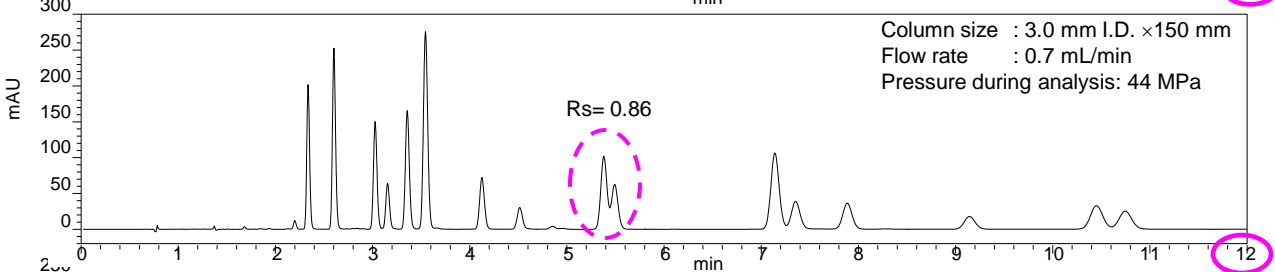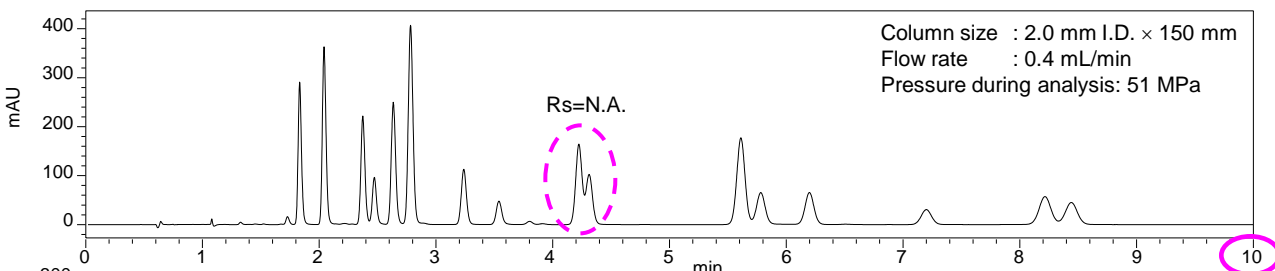
<Analytical Conditions for HPLC Column>
Column : LaChrom II C18 (5 μm) 4.6 mm I.D. × 250 mm
Eluents : CH_3CN / H_2O = 80 / 20 (v/v)
Flow rate : 1.0 mL/min
Column temperature : 30 °C
Detection wavelength : UV 254 nm (DAD)
Injection vol : 20 μL

<Analytical Conditions for UHPLC High Resolution Column >
Column : LaChromUltra II C18 (1.9 μm) 3.0 mm I.D. × 250 mm
Eluents : CH_3CN / H_2O = 80 / 20 (v/v)
Flow rate : 0.70 mL/min
Column temperature : 30 °C
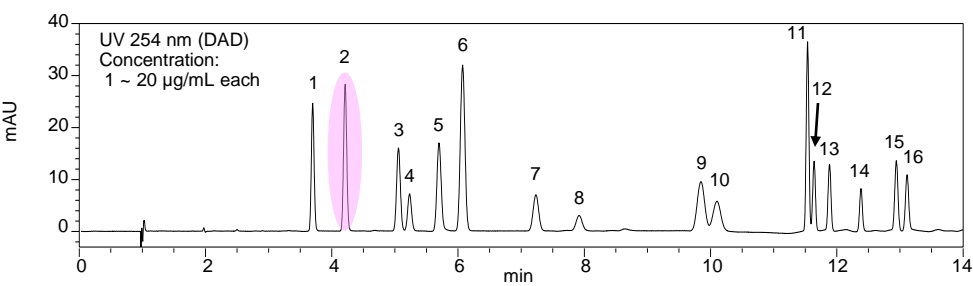Detection wavelength : UV 254 nm (DAD)
Injection vol. : 2 μL

■ Analysis Example of 16 Polycyclic Aromatic Hydrocarbons (10 - 200 μg/mL each) (Comparison of Resolutions for Different Column Sizes)


Column size : 2.0 mm I.D. × 150 mm
Flow rate : 0.4 mL/min
Pressure during analysis: 51 MPa

Rs=N.A.

Column size : 3.0 mm I.D. ×150 mm
Flow rate : 0.7 mL/min
Pressure during analysis: 44 MPa

Rs= 0.86

Column size : 3.0 mm I.D. × 250 mm
Flow rate : 0.7 mL/min
Pressure during analysis: 63 MPa

Rs= 1.18

<Analytical Conditions for UHPLC High Resolution Column>
Column : LaChromUltra IIC18 (1.9 μm)
Eluents : CH_3CN / H_2O = 80 / 20 (v/v)
Flow rate : Described in chromatogram
Column temperature : 30 °C
Detection wavelength : UV 254 nm (DAD)
Injection Vol. : 2 μL

The resolution generally is improved as the column size increases, but the analysis time also increases. LaChromUltra II C18 columns are available in various column sizes, and thus the column size suitable for the purpose can be used.

■ Analysis Example of 16 Polycyclic Aromatic Hydrocarbons (Comparison of DAD and Fluorescence Detector)

UV 254 nm (DAD)
Concentration:
1 ~ 20 μg/mL each

FL (with wavelength program)
Concentration: 0.2 ~ 4 μg/mL each

<Analytical Conditions>
Column : LaChromUltra II C18 (1.9 μm)
3.0 mm I.D. × 250 mm
Eluents : (A) H_2O (B) CH_3CN
75 % B (0 - 9min)
→ 95 % B (10 - 15min)
Flow rate : 0.9 mL/min
Column temperature : 30 °C
Detection wavelength : UV 254 nm (DAD)
FL (with wavelength program)
Injection vol. : 5 μL
Max. pressure during analysis: 88 MPa
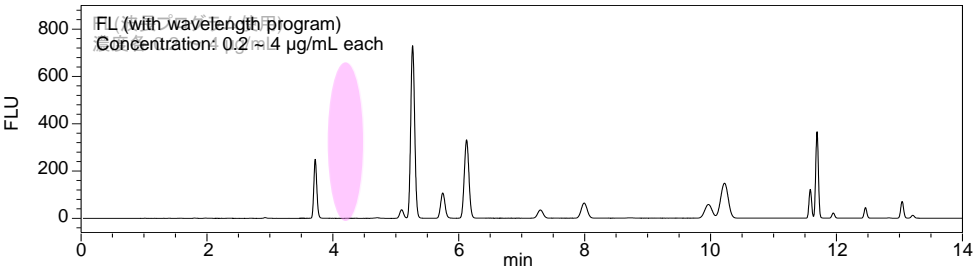
Refer to AS/LC-039 for the wavelength program of the fluorescence detector.

6440 Fluorescence Detector

A fluorescence detector is generally used for the analysis of PAHs. However, (2) Acenaphthylene, one of the standard samples used this time, cannot be detected by a fluorescence detector. By using a DAD for the analysis, non-fluorescence components can also be detected.

Main system configuration: ChromasterUltra Rs DAD system (6170 Binary Pump, 6270 Autosampler, 6310 Column Oven, 6430 Diode Array Detector, Organizer) 6440 Fluorescence Detector

NOTE: These data are an example of measurement; the individual values cannot be guaranteed.