Application Note

032003H

Improvement in Fluorescence Sensitivity by changing the Emission Slit Width of the FP-4020 Fluorescence Detector

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are comprised of aromatic rings, and are produced by the incomplete combustion of hydro-carbon containing materials such as diesel oil and coal. Some PAHs are known to be carcinogenic and so the detection and quantitative determination of environmental PAHs is extremely important.

When using fluorescence detection, the sensitivity can be increased by narrowing the emission slit width; when the excitation wavelength is close to fluorescence wavelength, narrowing the slit width increases the signal-to-noise (S/N) ratio. On the other hand, broadening the slit width can be an effective way to increase the S/N when the excitation wavelength is far from fluorescence emission wavelength.

In this application note, the fluorescence measurement of PAHs was performed using an HPLC with FP-4020 fluorescence detector to compare the effects of adjusting the emission slit width from 20 nm to 40 nm.

Keyword: polyaromatic hydrocarbons, HPLC, fluorescence detection, LC-4000 Series, FP-4020, PAHs, CrestPak C18S, FP-4020, slit width, signal to noise

Experimental Condition

Column:	CrestPak C18S (4.6 mmI.D. x 150 mmL, 5 µm)
Eluent:	Acetonitrile/Water (78/22)
Flow rate:	1.0 mL/min
Column temp.:	40℃
Wave length:	Time program (see Table 1)
Cell temp.:	20℃
Injection volume:	1 µL
Standard:	Mixture of 10 ng/mL naphthalene, 1 ng/mL fluorene, anthracene, pyrene chrysene and benzo[a]pyrene in methanol

Table 1 Time program

Time (min)	Ex (nm)	Em (nm)
0.0	276	334
3.5	266	310
4.1	250	402
5.0	336	392
6.0	268	384
8.5	262	408

Results

Figure 1 Chromatograms of 6 PAHs at each slit width.

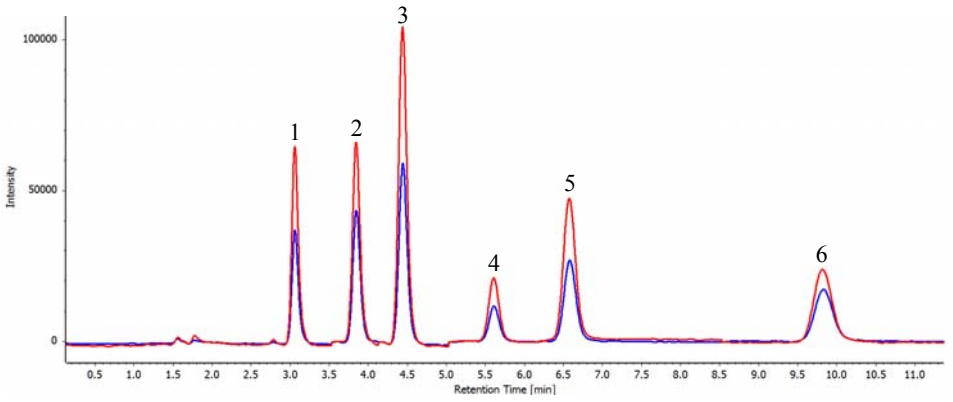

Fig. 1 Chromatograms of PAHs 6 components (Blue: 20 nm, Red: 40 nm)
1: Naphthalene, 2: Fluorene, 3: Anthracene, 4: Pyrene, 5: Chrysene, 6: Benzo[a]pyrene

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Table 2 Detection sensitivity at each slit width. An increased S/N ratio was observed with a wider 40nm slit width for the compounds (Anthracene, Chrysene, Benzo[a]pyrene) whose excitation wavelength is more than 100 nm away from the fluorescence emission wavelength. For the compounds whose Ex and Em maxima are closer together, the narrower 20nm slit width yields greater detection limits.

The results indicate that setting an appropriate wavelength and slit width for each component is important in increasing the detection sensitivity.

Table 2 Detection sensitivity at each slit width (S/N=3)

S/N=3 (pg)

Slit Width	Naphthalene	Fluorene	Anthracene	Pyrene	Chrysene	Benzo[a]pyrene
20 nm	0.143	0.0277	0.0160	0.0398	0.0206	0.0431
40 nm	0.145	0.0428	0.0117	0.0420	0.0178	0.0359

Wavelength	Naphthalene	Fluorene	Anthracene	Pyrene	Chrysene	Benzo[a]pyrene
Ex (nm)	276	266	250	336	268	262
Em (nm)	334	310	402	392	384	408
Em-Ex (nm)	58	44	152	56	116	146

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