

## SHIMADZU APPLICATION NEWS

## HIGH PERFORMANCE LIQUID CHROMATOGRAPHY No. L211

## Analysis of Polycyclic Aromatic Hydrocarbone with a Multi-functional Fluorescence Detector

Polycyclic aromatic hydrocarbons contained in airborne particulates and emissions from internal-combustion engine are an important monitoring item in the environmental regulation field because of their suspected carcinogenicity. They have an intensive fluorescence, but the optimum excitation and fluorescence wavelength vary with each component. Therefore, by selecting the wavelength optimal to each polycyclic aromatic compound, it is expected to enhance the sensitivity notably. The multi-functional fluorescence detector RF-550 capable of setting or varying the excitation and fluorescence wavelength by the time program is a new tool useful in simultaneous analysis of plural components differing in the detection optimum wavelength. Fig. 1 compares the chromatogram of analysis of extraction of airborne particulates at fixed wavelength (B) and wavelength changeover by time program (A).

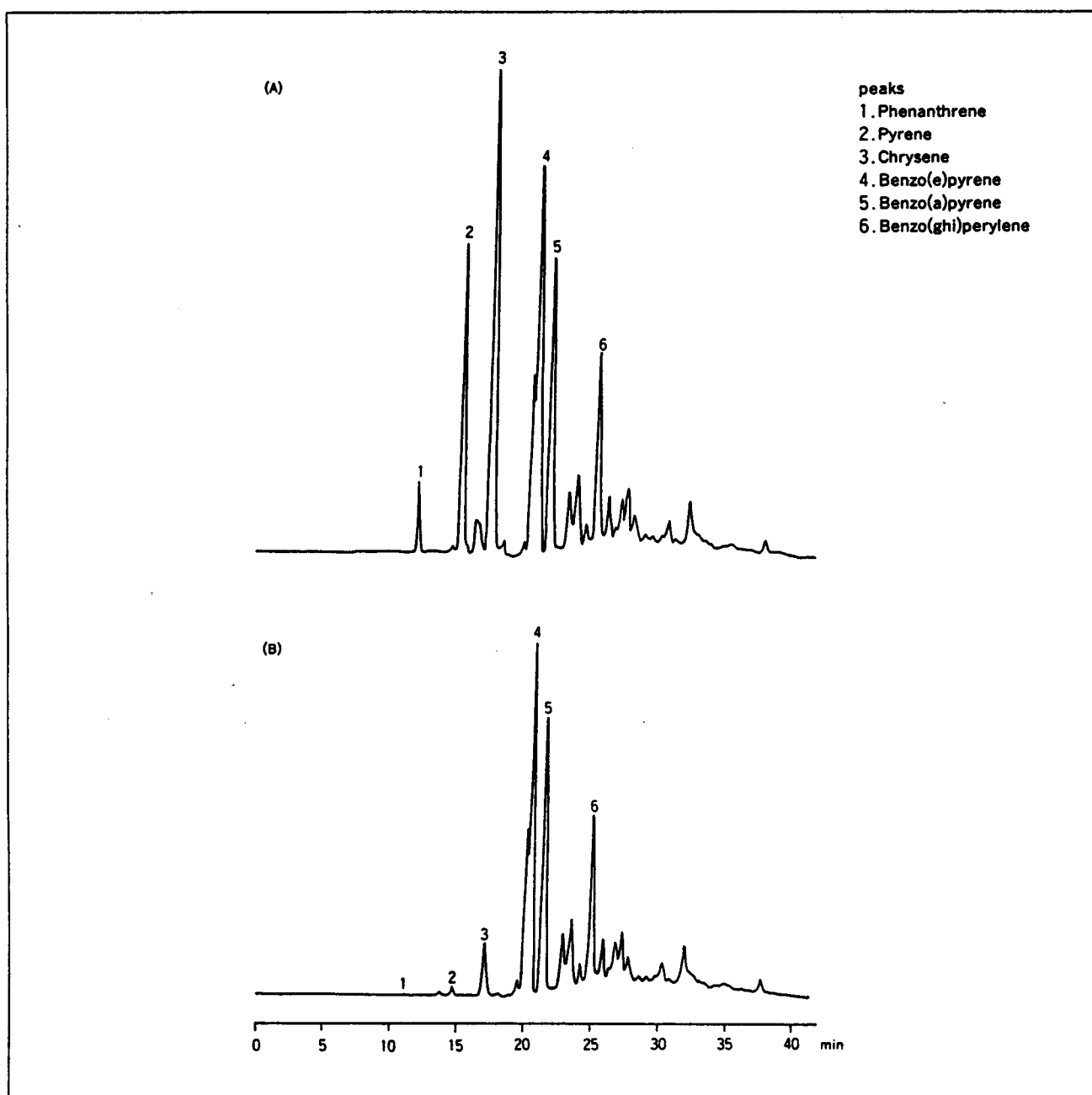
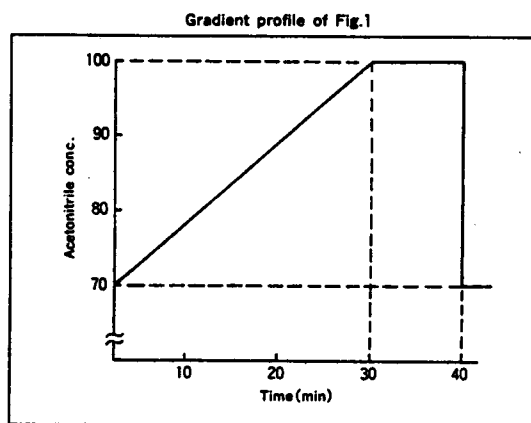


Fig. 1 Chromatogram of an Extraction of Airborne Particulates

Table 1 Analytical Conditions of Fig. 1

Column : Shim-pack CLC-ODS  
(6.0mm I.D. × 150mm L.)  
Mobile phase : Water/Acetonitrile  
gradient elution  
Flow rate : 1.0ml/min.  
Temp. : 40°C  
Detection : RF-550  
(B) Ex.295nm, Em.420nm  
(A) as shown below

Time	Ex	Em
0	295	420
7	280	360
13.5	240	380
16	265	385
18.5	295	420



When analyzing by changing over the wavelength as shown in Fig. 1, it is important to find out the detection optimum wavelength of each component. Accordingly, the functions of scanning of peak spectrum of target component and correction of background of mobile phase or reaction reagent should be required. In the RF-550, these functions of spectrum scanning of excitation and fluorescence, memory, and background correction are usually done on the built-in display screen, but external outputs are also possible. Fig. 2 shows the spectra of fluorescence of anthracene measured by the spectrophotometer RF-5000 and by the RF-550 by the stopped flow technique. By accelerating the scanning speed, it is possible to measure the spectra directly in the on-flow state without applying the stopped flow method.

Finally an example of high sensitivity detection by the RF-550 is shown. The sample is benzo(a)pyrene, of which absolute load is 100 fg. Regarding S/N = 2 as the detection limit, it nearly corresponds to 300 fg.

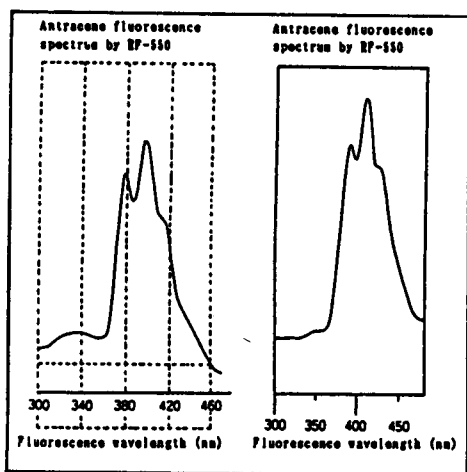


Fig. 2 Comparison of Fluorescence Spectra of Anthracene

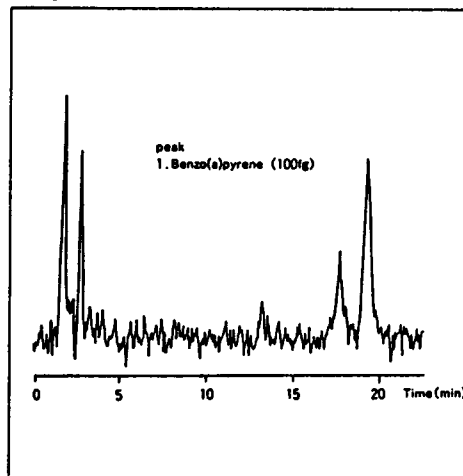


Fig. 3 Trace Analysis of Benzo(a)pyrene

Table 2 Analytical Conditions of Fig. 2

Column : Shim-pack CLC-ODS  
(6.0mm I.D. × 150mm L.)  
Mobile phase : water/Acetonitrile = 2/8  
Flow rate : 1.0ml/min.  
Temp. : 40°C  
Detection : RF-550  
Ex.295nm, Em.403nm



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