Waters Lab Highlights

An Internal Communication of Applications and Techniques

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Fluorescence Wavelength Programming for Detection of Polynuclear Aromatic Hydrocarbons

An important feature of the new Waters™ 470 Scanning Fluorescence Detector is the ability to program changes in excitation wavelength, emission wavelength, gain, and attenuation to occur at selected points in time. This makes it possible to analyze mixtures of fluorescent analytes whose spectral properties vary from compound to compound.

Polynuclear Aromatic Hydrocarbons (PAHs) represent a class of analytes where programmed changes in fluorescence detection parameters are useful for obtaining maximum analytical sensitivity. PAHs are common combustion products, and many are carcinogens. Accordingly, there is demand for detecting them at low levels in environmental samples,which requires the most sensitive detection possible.

Recently, Marina Mientjes of Waters Holland and Sven Edlund, formerly of the San Francisco sales region, have developed wavelength programs for the analysis of PAHs using the 470 detector. The programs are outlined in Table 1. Good results have been obtained with both programs. Since the time at which the programmed changes must occur will vary according to the column used and the instrument configuration, the change points are noted by

TABLE 1

Program Segment	Ex/Em Mientjes	Ex/Em Edlund	Change to Next Segment Between Peaks
Initial	270/323	275/321	4 and 5*
1	248/374	248/386	6 and 7
2	270/400	272/410	10 and 11
3	290/418	292/410	15 and 16
4	270/490	300/462	

*Peak 2 (acenaphthylene) is not fluorescent

arrows in Figure 1, a typical chromatogram of PAHs obtained with UV detection. Even if a different column is used, the peaks will elute in the same order, and the proper times for wavelength switching can be determined with ease. Because the detector executes an auto-zero when wavelength is switched, it is important to switch wavelengths only at times when the detector is at baseline.

The results of using such a program are seen in Figure 2, a programmed fluorescence chromatogram. While the overall appearance is similar to the UVchromatogram with the exception of acenaphthylene (peak 2), which is not

Waters
Division of MILLIPORE 34 Maple Street Milford, MA 01757 508-478-2000

Craig Dorschel/Marina Mientjes/Sven Edlund

fluorescent, the amount of material injected has been reduced by a factor of 50. While picogram quantities of most of the compounds were injected, the analysis is far above the limit of detection, given the lack of baseline noise.

With creative use of the programming feature of the 470 detector, the analyst is able to tailor fluorescence detection to fulfill needs for both sensitivity and selectivity in the analysis of mixtures of fluorescent compounds.

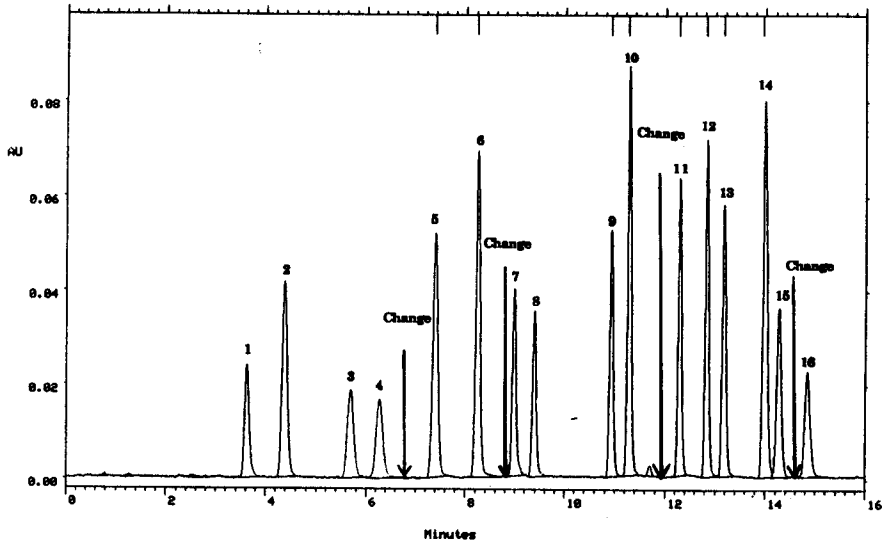

Figure 1. **Column, Vydac™ 201TP5415; Gradient, 50% ACN/Water for 3min., then linear to 100% ACN 3 to 10 min. and hold; Flow 1.5mL/min; Sample, Standard Reference Material 1647 (NIST, formerly NBS), 5 µL; Detector M490, Maxplot 254, 307, 297 nm; Peaks - 1, naphthalene; 2, acenaphthylene; 3, acenaphthene; 4, fluorene; 5, phenanthrene; 6, anthracene; 7, fluoranthene; 8, pyrene; 9, benz[a]anthracene; 10, chrysene; 11, benzo[b]fluoranthene; 12, benzo[k]fluoranthene; 13, benzo[a]pyrene; 14, dibenz[a,h]anthracene; 15, benzo[ghi]perylene; 16, indeno[1,2,3-cd]pyrene.**

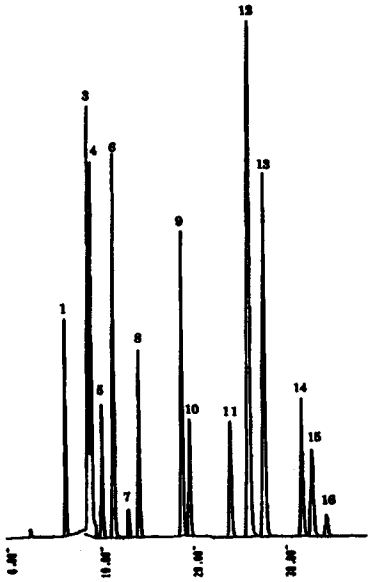

Figure 2. **Column, Vydac 25 cm RVS; Gradient, 55 to 85 % ACN in water, 30min.; Flow, 1 mL/min; Sample, Standard Reference Material 1647 diluted 100 x, 10 µL; Detector, M470, At. 4, Gain 100, Em bandwidth 30, T.C. 1.5 sec, Cell Vol. 5 µL, program as in Table 1; Peaks same as Figure 1.**

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