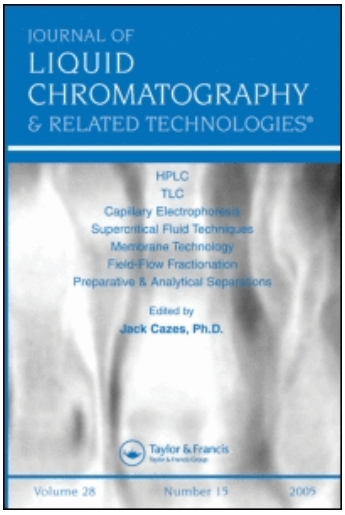

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Selective Quenchofluorometric Detection of Fluoranthenic Polycyclic Aromatic Hydrocarbons in High-Performance Liquid Chromatography

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SELECTIVE QUENCHOFLUOROMETRIC DETECTION OF FLUORANTHENIC POLYCYCLIC AROMATIC HYDROCARBONS IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

The phenomenon of fluorescence quenching was used for selective HPLC detection of fluoranthenic polycyclic aromatic hydrocarbons (PAH). Termed a "Quenchofluorometric" detection system, it employs a filter fluorimeter or spectrofluorimeter and nitromethane in the mobile phase as the fluorescence quenching reagent. Chromatograms obtained with and without the quenching reagent are compared for PAH standards, a coal tar extract, and a shale oil sample. The quenchofluorometric detection system provides an inexpensive method to achieve selective detection for fluoranthenic PAH as a group.

INTRODUCTION

Since the advent of modern high-performance liquid chromatography (HPLC), a variety of detection systems have been used to enhance both sensitivity and selectivity. Recently, efforts have focused on the development and use of selective detectors, which

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are specific for various groups of compounds or individual compounds, rather than a "universal" detector. The need for selective detectors in HPLC is often the result of the inability of the chromatographic column to separate the compound(s) of interest from the other constituents in the complex mixture. This is particularly true for the analysis of mixtures of polycyclic aromatic hydrocarbons (PAH) from air particulates, coal tar, petroleum, coal liquids, tobacco smoke condensate, etc.

Due to the complexity of such mixtures, i.e., a large number of both unsubstituted and alkyl-substituted PAH, co-elution of compounds is a major problem. Wise *et al.* (1,2) have advocated the use of multi-dimensional chromatographic techniques, i.e., separation on normal-phase followed by reverse-phase columns, to separate such complex mixtures. The use of UV absorption and fluorescence detection for the HPLC determination of PAH also improves the selectivity.

In 1964 Sawicki *et al.* (3) described the use of selective fluorescence quenching of certain PAH in the presence of nitromethane and termed this phenomenon "quenchofluorometric" analysis. They found that in the presence of nitromethane, the fluorescence spectra of non-fluoranthenic PAH (i.e., those not containing the fluoranthene structure) were quenched and the spectra of fluoranthenic PAH were not quenched. Sawicki *et al.* (3) employed this quenchofluorometric technique, following column chromatography and directly on thin-layer chromatographic (TLC) plates, in characterizing fluoranthene and benzo[*k*]fluoranthene in air particulate extracts. Later, Dreeskamp *et al.* (4) studied the nitromethane fluorescence quenching of 22 PAH and observed some exceptions to Sawicki's rule (3), i.e., some fluoranthenic PAH (particularly fluoranthene, benzo[*b*]fluoranthene, and benzo[*k*]fluoranthene exhibited some quenching. In a brief note, Blumer and Zander (5) reported the application of nitromethane fluorescence quenching as a selective detection system for PAH in HPLC. The selectivity achieved by the addition of 5 percent nitromethane to the mobile

phase was illustrated for the analysis of a technical pyrene fraction from coal tar.

In this paper further application of the use of nitromethane as a selective quenchofluorometric HPLC detection system for PAH is described, particularly for the determination of the benzofluoranthenes in the presence of perylene and the benzopyrene isomers in coal tar and shale oil. The selectivity achieved in the HPLC determination of PAH with the quenchofluorometric detection method is compared to normal UV and fluorescence detection.

EXPERIMENTAL

Experiments were conducted on an HPLC system with gradient elution capability, a loop injector, and a fixed-wavelength UV detector (254 nm). Reverse-phase C_{18} columns (Vydac 201TP and Zorbax ODS) were used for the chromatographic separations.

The quenching reagent (a solution of 2.5 percent or 5 percent nitromethane in acetonitrile) was pumped into the system after UV detection via a "T", followed by a 3 m x 0.51 mm i.d. mixing cell. The mobile phase flow was 2.0 mL/min and the reagent flow was 0.5 mL/min. After mixing, the eluent and reagent entered a filter fluorimeter with an excitation filter passing $\sim$ 250-380 nm (color specification number 7-54) and an emission filter passing > 380 nm (color specification number 0-52). For comparison, a spectrofluorimeter was used in place of the filter instrument to evaluate the selectivity achieved by varying the excitation and emission wavelengths. For the analysis of the shale oil sample, a fluorimeter was employed with an excitation monochromator set at 300 nm and an emission filter passing > 400 nm. For this application the nitromethane was added directly to the mobile phase instead of after the UV detection. This small amount of nitromethane in the mobile phase did not affect the chromatographic separation and produced only a small shift in baseline due to its UV absorbance.

The percent of the PAH fluorescence quenched was determined by injecting the PAH standard in the presence and absence of the

nitromethane reagent. The peak heights were measured and used in the following equation:

$$\frac{\text{Percent Quenched}}{100} = 1 - \frac{\text{peak height in presence of } CH_3NO_2}{\text{peak height in absence of } CH_3NO_2}$$

RESULTS AND DISCUSSION

The structures of the PAH used in this study are shown in Figure 1. These compounds are found in most naturally occurring PAH mixtures. The six PAH isomers of molecular weight 252, i.e., the three benzofluoranthenes, benzo[*e*]pyrene, benzo[*a*]pyrene, and perylene, are generally isolated as a group using normal-phase liquid chromatography or TLC on silica or polar bonded phases (1,2,6). In addition, baseline resolution of all six of these isomers is difficult to achieve on reverse-phase C_{18} columns (2).

The use of the quenchofluorometric technique for the selective HPLC determination of fluoranthenic PAH was investigated. The effects of nitromethane on the fluorescence of the PAH shown in Figure 1 are summarized in Table 1.

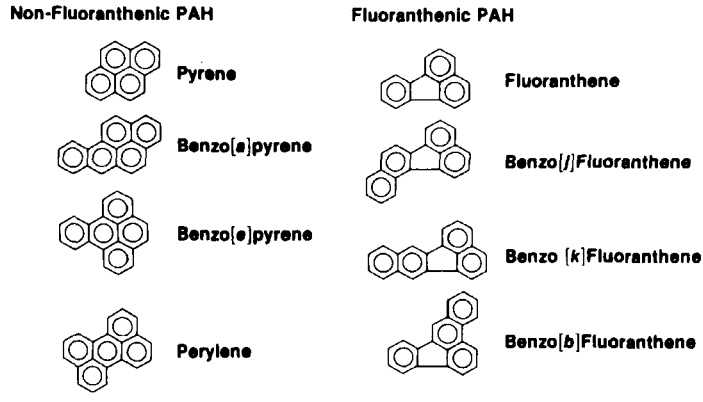

FIGURE 1 Structures of polycyclic aromatic hydrocarbons used in this study.

TABLE 1

Fluorescence Quenching of PAH by Nitromethane

CH_3NO_2 concentration in the mobile phase (volume/volume):	0.5 percent	1 percent
	Percent Quenched	
Fluoranthenic PAH:		
Fluoranthene	14	27
Benzo[*b*]fluoranthene	28	37
Benzo[*j*]fluoranthene	18	27
Benzo[*k*]fluoranthene	42	56
Non-fluoranthenic PAH:		
Pyrene	98	100
Benzo[*a*]pyrene	92	96
Benzo[*e*]pyrene	88	96
Perylene	71	86

Mobile phase: 90% CH_3CN/10% H_2O
Detector: Filter fluorimeter

With 1 percent nitromethane present in the mobile phase, the non-fluoranthenic PAH are almost completely quenched, whereas less quenching is observed for the fluoranthenic PAH. These results agree with Dreeskamp *et al.* (4) indicating that some quenching does occur even with the fluoranthenic PAH. At 0.5 percent nitromethane in the mobile phase, greater selectivity was achieved between the non-fluoranthenic and fluoranthenic PAH than at 1 percent. Thus, the samples were analyzed using the lower percentage of nitromethane in the mobile phase.

The selectivity of quenchofluorometric detection is illustrated in Figure 2 using a sample of benzo[*b*]fluoranthene (B[*b*]F), benzo-[*j*]fluoranthene (B[*j*]F), benzo[*k*]fluoranthene (B[*k*]F), and perylene in acetonitrile. Chromatogram (A) is without nitromethane; chromatogram (B), at the same attenuation, is with 0.5 percent nitromethane present. In chromatogram B the nitromethane quenches the B[*a*]P, almost all of the perylene, and presumably the B[*e*]P (since it coelutes with B[*j*]F, it is not observable).

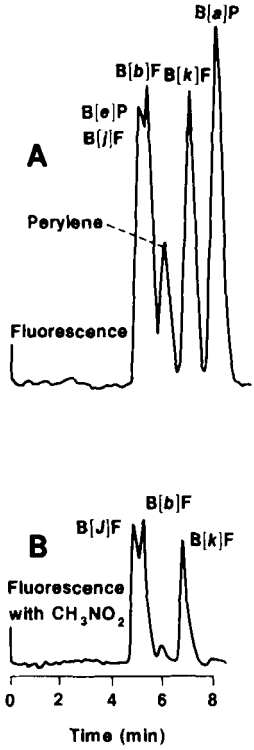

FIGURE 2 Reverse-phase C_{18} HPLC separation of six isomeric PAH standards with (A) fluorescence detection and (B) quenchofluorometric detection with 0.5 percent nitromethane in the mobile phase. Column: Vydac 201TP, mobile phase: 90 percent acetonitrile in water at 2 mL/min, detector: filter fluorimeter.

In Figure 3, chromatograms of a naturally occurring sample, a coal tar extract, are shown using three different detection methods: (A) UV at 254 nm, (B) filter fluorimeter, and (C) filter fluorimeter with nitromethane as the quenching reagent at the same attenuation as in (B). Both the UV and fluorescence chromatograms are quite complex; however, excellent selectivity is obtained for the fluoranthenic PAH using the nitromethane quenchofluorometric detection scheme as shown in chromatogram (C). Due to the large

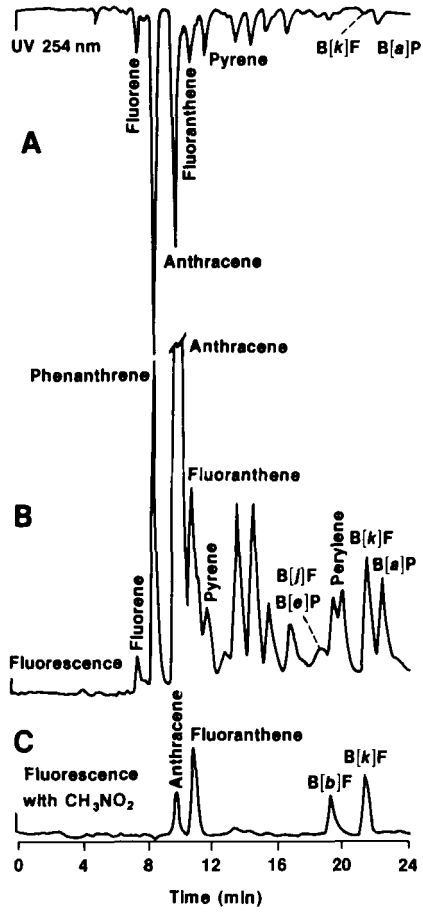

FIGURE 3 Reverse-phase C_{18} HPLC separation of a coal tar extract with (A) UV detection at 254 nm, (B) fluorescence detection, and (C) quenchofluorometric detection with 0.5 percent nitromethane in the mobile phase. Column: Vydac 201TP, mobile phase: linear gradient from 50 to 100 percent acetonitrile in water in 25 min at 2 mL/min, detector: filter fluorimeter.

fluorescence response of anthracene in this sample, a small peak for anthracene is observed in chromatogram (C) even though the great majority of its fluorescence is quenched.

The chromatograms in Figure 4 are of the same coal tar extract as in Figure 3. A spectrofluorimeter was used with the excitation and emission monochrometers set to optimize the response for fluoranthene in chromatogram (A) and benzo[*k*]fluoranthene in chromatogram (B). The selectivity for individual compounds using a spectrofluorimeter (e.g., fluoranthene in Figure 4A) is far greater than using a filter fluorimeter. Recently, May *et al.* (7) described the use of a spectrofluorimeter as an HPLC detector to achieve optimum selectivity for the determination of individual PAH in shale oil. The nitromethane quenchofluorometric method, however, provides a

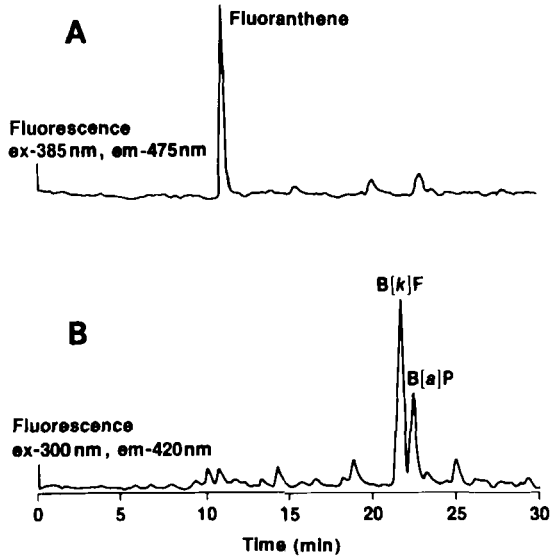

FIGURE 4 Reverse-phase C_{18} HPLC separation of a coal tar extract with selective fluorescence detection (A) λ_{ex} = 385 nm, λ_{em} = 475 nm and (B) λ_{ex} = 300 nm, λ_{em} = 420 nm. Column and mobile phase same as Figure 3, detector: spectrofluorimeter.

"fluoranthenic group specific" detector using a relatively inexpensive filter fluorimeter.

The nitromethane quenchofluorometric HPLC detection system was employed to quantitate three fluoranthenic PAH (i.e., fluoranthene, benzo[*b*]fluoranthene, and benzo[*k*]fluoranthene) in a shale oil sample [National Bureau of Standards (NBS) Standard Reference Material (SRM) 1580, "Organics in Shale Oil"]. The chromatograms in Figure 5 compare the reverse-phase C_{18} separations

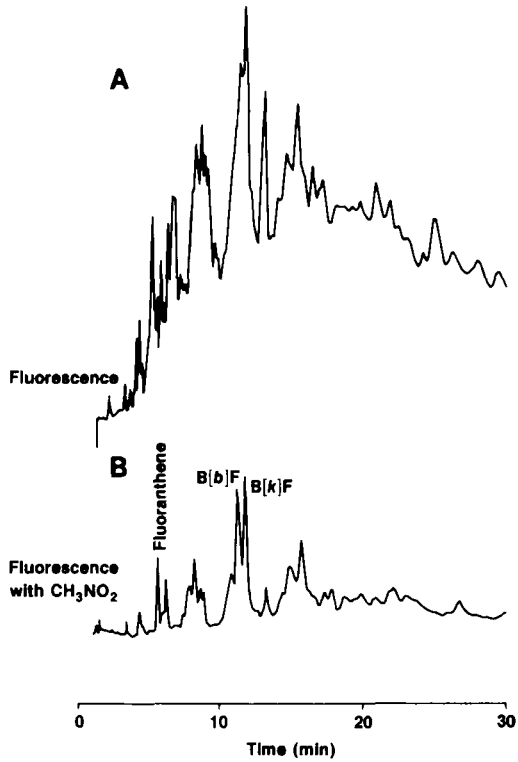

FIGURE 5 Reverse-phase C_{18} HPLC separation of PAH in shale oil (A) with fluorescence detection and (B) quenchofluorometric detection with 0.5 percent nitromethane in the mobile phase. Column: Zorbax ODS, mobile phase: 80 percent acetonitrile in water at 3 mL/min, detection: filter fluorimeter, λ_{ex} = 300 nm, λ_{em} > 400 nm.

of a total PAH fraction, which was collected from a normal-phase HPLC separation on a bonded amine column (8), (A) without the addition of a quenching reagent and (B) with the addition of nitromethane. The fluoranthene, benzo[*b*]fluoranthene, and benzo[*k*]-fluoranthene are easily quantitated from the chromatogram using the quenchofluorometric detection. Concentrations (μg/g) of these fluoranthenic PAH in the shale oil sample were found to be fluoranthene (48 ± 4), benzo[*b*]fluoranthene (12 ± 2), and benzo[*k*]-fluoranthene (5 ± 1) (uncertainty is one standard deviation of the mean). The concentrations of the fluoranthene in NBS SRM 1580, as determined by direct injection GC/MS with single ion monitoring and sequential normal- and reverse-phase HPLC with fluorescence detection, are 55 ± 5 and 53 ± 2 μg/g, respectively. The certified concentration is 54 ± 10 μg/g (9).

In summary, the quenchofluorometric HPLC detection system provides an inexpensive method to achieve selectivity for fluoranthenic PAH as a group. A spectrofluorimeter has the capability of providing a high degree of specificity for a single compound (e.g., fluoranthene in Figure 4A), however, excitation and emission wavelengths cannot be selected to provide a "group specific" chromatogram for the fluoranthenic PAH such as obtained with the nitromethane quenchofluorometric system and the filter fluorimeter.

ACKNOWLEDGMENT

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