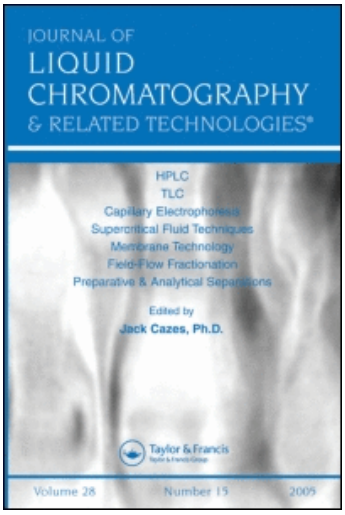

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Detectors for Liquid Chromatographic Analysis for Polynuclear Aromatic Hydrocarbons

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DETECTORS FOR LIQUID CHROMATOGRAPHIC ANALYSIS FOR POLYNUCLEAR AROMATIC HYDROCARBONS

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ABSTRACT

A number of liquid chromatographic detectors of various types have been evaluated for both selectivity and sensitivity for the detection of polynuclear aromatic hydrocarbons (PAH). Detection limits for fixed and variable wavelength UV photometers, filter fluorimeters, and spectrofluorimeters have been determined. The utility of each of these types of detectors for use in the reversed-phase HPLC analysis of environmental extracts containing trace levels of PAH's is discussed.

INTRODUCTION

Polynuclear aromatic hydrocarbons (PAH) have been of increasing interest to analytical chemists in the last few years. In addition to the general toxicity exhibited by aromatic compounds, several of the PAH's have been discovered to be carcinogenic. These materials are found in petroleum, are products of combustion, and are frequently found as trace contaminants in

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water. From water, the PAH's find their way into aquatic organisms or are adsorbed onto sediments. Since the solubilities of most PAH's in water range from one part in 10^6 to one part in 10^9 (1), analytical techniques must be quite sensitive to identify and quantify these materials.

The extraction, separation, and analysis of PAH's have been extensively investigated. (2-12) Most early investigators used ultraviolet absorption (UV) or fluorescence spectroscopy for quantitative analysis of extracts. Later, gas chromatography (GC) became the analytical technique of choice since it allowed further isolation of the PAH of interest. Some qualitative information can be obtained from the retention time. Recently, the use of capillary GC has become very popular, and has been used for the analysis of PAH's in engine exhausts (13), anthracene oil (10), cigarette smoke (11), soot samples (12), and sediments (5). Janini et al (14) have discussed some of the difficulties associated with separating complex mixtures of PAH's by GC and suggested the use of a nematic liquid crystal column for such analysis. Assuming that the necessary separations can be made, the individual components must be identified before they can be usefully quantified.

Gas chromatography - mass spectrometry is generally recognized as being the most powerful tool available to the analyst for performing trace organic analysis. The technique has been used for the analysis of complex PAH mixtures (6-8,15) but does have certain limitations. Isomeric PAH's are not readily distinguishable from their mass spectra alone, and are often not resolved chromatographically. For example, 1,2-benzanthracene, chrysene, and naphthacene give essentially the same mass spectrum and are difficult to separate by GC.

With the advent of high performance liquid chromatography (HPLC) the analyst has a powerful tool for solving these problems. Larger sample sizes can be accommodated and less sample pretreatment is necessary. A great advantage is afforded by the use of UV and fluorescence detectors, both of which are more selective (in PAH analysis) than mass spectrometry.

In this paper we discuss the merits of several types of commercially available HPLC detectors in terms of both their sensitivity and their selectivity for PAH's. (21)

EXPERIMENTAL

Liquid Chromatography:

(a) Pumps. The liquid chromatography described here was done using either a Waters Model 6000 solvent delivery system (Waters Associates, Milford, Mass.), or a Familic-100 chromatograph (Jasco, Ltd, Bay Street, Easton, Md.). (22)

(b) Columns. The column used with the Waters unit was μBondapak C-18 300 x 4 mm. A micro-scale C-18 bonded-phase column 150 x 0.5 mm packed in our laboratory by the procedure described by Ishii (16) was used with the Familic-100 unit.

(c) Solvents. The solvents were water and acetonitrile. The water was distilled from an alkaline potassium permanganate solution and passed through a column of XAD-2 resin (Rohm & Haas) to remove organic contaminants. The acetonitrile was spectroscopic grade. For the detection limit tests, methanol solutions of the PAH's were injected. In the oyster analysis, a cyclohexane extract of an alkaline homogenate was injected.

Detectors:

The detectors used in this study were: Waters Associates' Model 440, a fixed wavelength filter photometer; the Perkin-Elmer Model LC-55, Schoeffel Model 770, Varian Vari-Chrom, and JASCO Uvidec-100 UV spectrophotometric detectors; the Aminco Fluoromonitor, Schoeffel FS 970, and Varian Fluorichrom filter fluorometric detectors; and the JASCO FP-4 spectrofluorometric detector.

The Uvidec-100 was fitted with an 0.1 µL cell for use with the micro-scale column. The spectrofluorometer was originally equipped with a cylindrical cell of approximately 9 µL, and with large apertures in both the emission and excitation monochromators. The spectral band pass was about 10 mm. The instrument was modified by reducing both slits of the emission monochromator by one-half, which provided sufficient resolution to obtain useful emission spectra. The four-fold loss in sensitivity was offset by the use of a larger cell. The new cell is square in cross-section and has a volume of about 36 µL, most of which is illuminated. The square shape also appears to suffer less loss of light by reflection and scattering than did the cylindrical cell.

The Spectra-Physics Minigrator was used in conjunction with the detectors to obtain heights and areas of peaks.

RESULTS AND DISCUSSION

A number of performance characteristics are desired in a detector for quantitative and qualitative analysis of PAH's. One would like high sensitivity because often only trace amounts are present. High selectivity

would obviate making some separations. Indeed, the ideal selective detector is one that could be made completely specific for one compound. This ideal is seldom realized, however. The degree to which the detectors meet these criteria of high sensitivity and selectivity determines their usefulness.

Sensitivity:

Detector sensitivity is usually expressed in terms of detection limits. In the literature, we find detection limits reported in two ways. One method defines the detection limit as the minimum amount of material that must be injected into the chromatograph in order to produce an observable peak. This limit obviously depends on the chromatographic process which is used, as well as upon the sensitivity of the detector, and is useful if the sensitivity of the entire chromatographic process is of major interest. If the sensitivity of the detector alone is desired, one should report the concentration of material which must be present in the cell in order that a peak be observed. Baker *et al* advocate reporting detector sensitivities in this manner. (17) We report limits measured by both methods to enable the reader to judge the sensitivity of the analytical method as well as the sensitivity of the detectors.

In considering detection limits, a usual criterion is that for a peak to be observed, it must be "twice as great as the background noise". If the noise is random, twice the range of the noise might be as many as six times the standard deviation about the mean. This would imply that the probability that the peak is real is greater than .9999. It might seem that this criterion is too stringent.

A better way to specify the noise level is to characterize it by the standard deviation of the signal about the mean or baseline. If one adopts the criterion of accepting all peaks greater than three times the standard deviation, the probability that these peaks are real is greater than .997. This is the method suggested by Parsons. (18)

Still another means of specifying the noise level has become practical with the advent of the electronic integrator. This is to report the noise level as the RMS average of the peak areas given by the noise excursions. The RMS average is easy to obtain, and a peak three times greater than the RMS average should also have a probability of .997 of being a real peak. A graphic representation of these three methods is given in Figure 1, and a tabulation of the results obtained

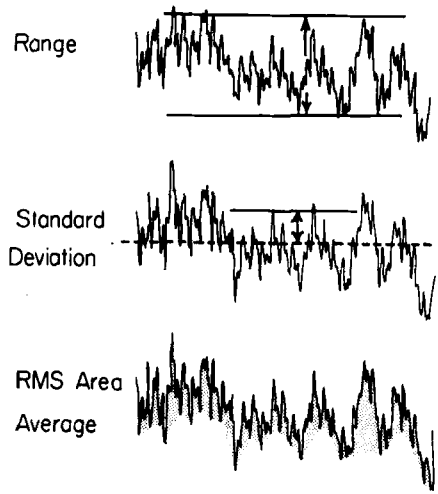

FIGURE 1

Various criteria for expressing background noise level

using each of these methods is given in Table I. It is readily apparent that the three methods are comparable. In making our detection limit determinations, we used one or more of the above criteria.

The results of the detection limit tests are given in Tables II and III. Note that the limits reported in Table II are in terms of the amount of material that must be injected into our system for detection to occur, and in Table III in terms of the concentration which must be present in the cell of the detector for detection to occur. The latter was calculated to be the concentration at the tip of the peak, knowing the width and assuming a triangular shape. The latter will be the more useful of the two in assessing the sensitivity of the detectors in other chromatographic systems.

Fluorescence photometry has long been a valuable tool for analytical chemists. Its enhanced sensitivity over conventional absorption techniques makes it ideal for trace analysis. PAH's, however, have large extinction coefficients at 254 nm (19) and the fixed wavelength filter photometric detector provides sensitivities that are comparable to those provided by fluorimetric detectors.

Variable wavelength detectors are not as sensitive for PAH's as the 254 nm fixed wavelength detector. The data presented in Tables II and III show that the sensitivity of the variable wavelength detectors was often less than that offered by the fixed wavelength detector by an order of magnitude even when the optimum wavelength for each PAH was selected. Therefore, the fixed wavelength UV detector is ideal for the universal sensitive detection of trace levels of PAH's.

TABLE I

Comparison of Methods for Determining Detection Limit

UV Absorbance Detector at 254 nm.
Detection Limit in ng Injected.

Criterion for Determining Limit	Napthalene	Phenanthrene	Pyrene	Chrysene	3,4-Benzpyrene
2X Range	.16	.031	.10	.056	.039
3X Std. Dev.	.12	.024	.07	.042	.029
3X RMS Area	.19	.021	.085	.042	.025

TABLE II

Detection Limits in Terms of Nanograms Injected into Chromatograph

Detector	Naphthalene	Phenanthrene	Pyrene	Chrysene	3,4-Benzpyrene
Fixed-Wavelength at 254 nm	.16	.025	.085	.046	.031
Variable-Wavelength Detectors					
A at 254 nm	4.7	.33	1.29	.42	.28
B at 254 nm	.8	.06	.2	.1	.05
C at 254 nm	4.0	.26	.9	.38	.5
A at optimum	.14	.20	.26	.19	---
C at optimum	.21	---	.3	.2	---
D at optimum	.32	---	.07	.12	---
Fluorimeters:					
E	---	---	---	---	.02
F	---	---	---	---	.007
G	---	---	---	---	.05
Spectrofluorimeter	6.8	.5	.11	.16	.012

TABLE III

Detection Limits in Terms of Concentration Present in the Cell, ng/mL

Detector	Naphthalene	Phenanthrene	Pyrene	Chrysene	3,4-Benzpyrene
Fixed-Wavelength at 254 nm	.34	.035	.14	.054	.022
Variable-Wavelength Detectors:					
A at 254 nm	7.8	.55	2.2	.47	.19
B at 254 nm	140	10	40	10	4
C at 254 nm	6.6	.43	1.5	.45	.35
A at optimum	.23	.33	.43	.21	---
C at optimum	.35	---	.50	.23	---
D at optimum	.53	---	.11	.13	---
Fluorimeters:					
E	---	---	---	---	.014
F	---	---	---	---	.005
G	---	---	---	---	.003
Spectrofluorimeter	.23	.8	.18	.18	.008

Selectivity:

Selectivity is an advantage afforded by the variable wavelength UV absorbance detectors. Brown *et al* (20) have shown that such detectors may be used to enhance detection signals for selected compounds while reducing or eliminating interferences. They also recommend the simultaneous monitoring of the effluent at two separate wavelengths so that peak area ratios may be utilized as a method of identification. This technique has only limited practical application because a high degree of resolution of each peak is necessary. For example, anthracene and phenanthrene will not be completely resolved in most LC separations. We have found that identification of phenanthrene by absorbance ratios is not possible when it is contaminated with as little as 10 percent of anthracene. Anthracene could be identified from a mixture of 10 percent phenanthrene and 90 percent anthracene, but identification of anthracene was not possible when 50 percent phenanthrene was present.

Fluorescence detectors offer enhanced selectivity over absorption detectors. Selectivity is obtained by the adjustment of both excitation and emission wavelengths. While many compounds will absorb energy at a given excitation wavelength, only a select few will emit radiation at a chosen emission wavelength. Therefore, signals from compounds which would interfere with absorption detectors can often be attenuated or eliminated with a properly tuned fluorimetric detector. Alternatively, it is sometimes possible to make use of selective excitation to obtain a similar result.

A spectrofluorimeter is more useful as a selective detector than is a filter fluorimeter, since the tuning of the filter fluorimeter is limited by the extent of

one's assortment of filters. From the fluorescence spectra shown in Figures 2 and 3, it can be deduced that phenanthrene can be monitored in an effluent containing ten times as much anthracene; or, conversely, anthracene can be detected in the presence of a large excess of phenanthrene.

Since the spectrofluorimeter can also be used to obtain fluorescence spectra, we have connected it in series with a UV absorbance detector. A low dead volume switching valve allows us to trap some of the effluent in the flow cell of the spectrofluorimeter. This allows us to use the UV detector as a universal monitor and either to use the spectrofluorimeter as a selective monitor or to use it to obtain fluorescence spectra of certain peaks without stopping flow and

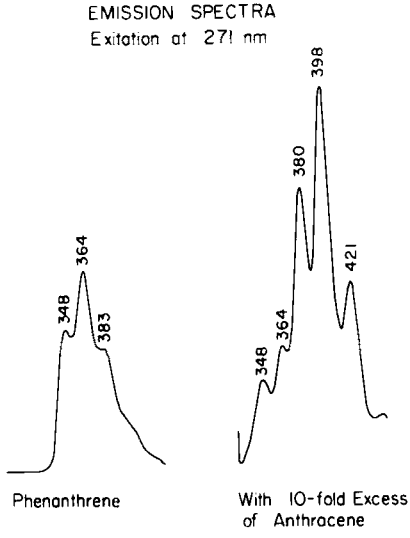

FIGURE 2

Fluorescence detection of phenanthrene in the presence of anthracene

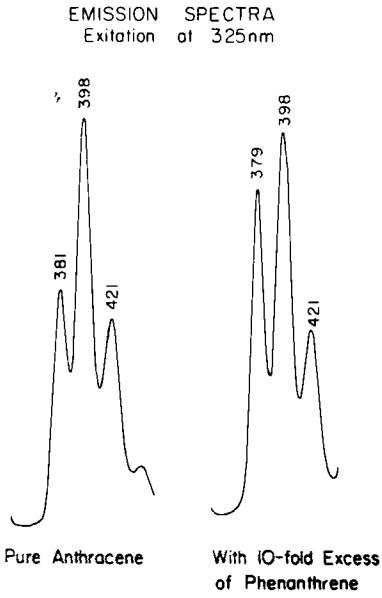

FIGURE 3

Fluorescence detection of anthracene
in the presence of phenanthrene

disturbing the UV trace. An example of this use is presented in Figure 4. The UV trace (A) and the fluorescence trace (C) were obtained from the first injection. From a second injection, we obtained a duplicate UV trace and the two emission spectra (B). The spectra shown confirm that peaks 1 and 2 are chrysene and benz(a)pyrene, respectively.

From the data presented, we conclude that the most powerful detector system for analysis of trace level PAH's is the combination of the fixed-wavelength 254 nm UV detector for universal detection and the spectrofluorimeter for selective monitoring or identification of individual PAH's.

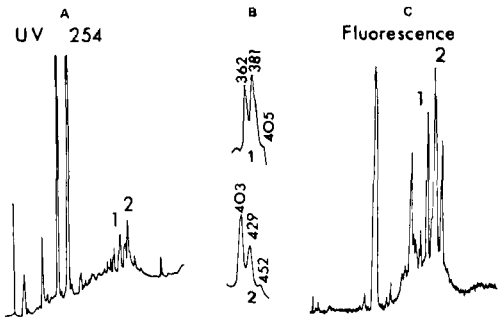

FIGURE 4

LC analysis of Elizabeth River Oysters. A, chromatogram by UV absorption at 254 nm; B, emission spectra of peaks 1 and 2 with excitation at 290 nm; C, chromatogram by fluorescence emission at 400 nm with excitation at 290 nm.

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(21) The authors wish to emphasize that the instruments used in this study were chosen because they were available for testing. Further, we would like to point out that the detectors of similar type gave comparable results with regard to the detection limits attainable.
(22) Certain commercial instruments and materials are identified in this paper in order to adequately specify the experimental procedure. In no case does such identification imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the material or equipment identified is necessarily the best available for the purpose.