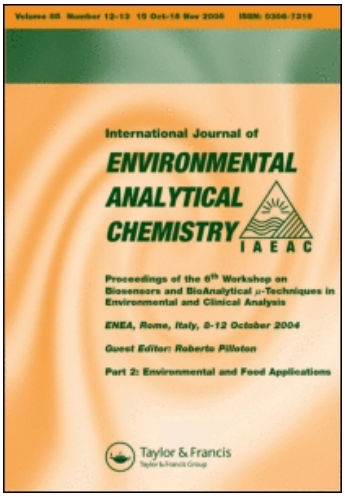

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S. N. Chesler[a]; H. S. Hertz[a]; W. E. May[a]; S. A. Wise[a]; F. R. Guenther[a]
[a] Analytical Chemistry Division, National Bureau of Standards, Washington, D.C., U.S.A.

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Organic Marker Compounds for Environmental Analysis

S. N. CHESLER, H. S. HERTZ, W. E. MAY, S. A. WISE and F. R. GUENTHER

Analytical Chemistry Division, National Bureau of Standards Washington, D.C. 20234, U.S.A.

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Marker compounds have been used extensively in fields such as clinical chemistry, but their use in environmental analyses has been minimal. Two definitions of marker compounds and their use in environmental assessment will be discussed in this paper. The first definition is the use of marker compounds as internal reference compounds for quantitative analysis of pollutants. The second definition is their use as qualitative indicators of certain types of chemical pollution.

KEY WORDS: Environmental pollution, trace organic analysis, liquid chromatography, gas chromatography, gas chromatography–mass spectrometry, marker compounds, internal standard.

INTRODUCTION

Individual organic compounds or compound classes may be used as markers for quantitative and qualitative determination of environmental pollution. Quantitative markers are compounds which are added to a sample at an appropriate point in the analytical procedure to facilitate the measurement of the concentration of the analyte. Generally, the compounds selected as quantitative markers are not naturally present in the sample. Conversely, qualitative markers are compounds which are found in the sample and indicate, by their presence, a qualitative property of the sample. For example, the presence of phytane in environmental samples has been used to indicate petroleum pollution.[1,2]

QUANTITATIVE MARKER COMPOUNDS

A common and effective use of markers for quantitation is illustrated in the internal reference compound (IRC) method (also called the internal standard method). In the IRC method, an accurately known amount of a marker compound is added to the sample at the beginning of the analysis.

The concentration of the analyte is calculated from the relative responses of the analyte and the IRC and from the known amount of IRC added. The compound selected as the IRC is usually similar in chemical and physical properties to the compounds to be measured so that any factors which affect the analyte during the analysis should affect the IRC to the same extent. Thus, errors resulting from losses due to volatility, adsorption on surfaces, or minor variations in analysis conditions are minimized. The assumption that the IRC behaves exactly as the analyte is not completely valid, as will be discussed later, but the utilization of the IRC method is still the most powerful quantitative analysis technique available for trace organic analysis.

The majority of the trace organic environmental analyses employ some form of chromatography for separation and quantitation; thus the following discussion focuses on criteria for and examples of IRC's employed in chromatographic analyses. The concentration of the analyte, C_A, is given by

$$C_A = \frac{K_{IRC} C_{IRC} R_A}{R_{IRC} K_A}$$

where C_{IRC} is the concentration of the IRC, K_{IRC} and K_A are detector response factors (signal/g), and R_{IRC} and R_A are the measured detector signals for the IRC and analyte, respectively. If response factors cannot be determined, as is commonly the case in multicomponent analyses, the IRC should have a detector response similar to that of the analyte.

The characteristics of an ideal IRC are as follows:

1) It should yield a completely resolved chromatographic elution profile.
2) It should have an elution time (or volume, R_f, etc) near to that of the analyte being measured.
3) The ratio of its peak height or area to that of the analyte should be close to unity.
4) It should have a response factor similar to the compound being measured.
5) It should not occur in the original sample.
6) It should act chemically like the analyte being measured.
7) It should be in equilibrium with the matrix which contains the analyte.

While the above discussion outlines the requirements of an ideal IRC, these requirements are never realized in the analyses of real samples (with the possible exception of an isotope dilution experiment). Therefore, one

must make certain compromises when choosing an IRC. Even an isotopic diluent is an ideal IRC for only one compound, and therefore, not very useful when quantitating such complex mixtures as petroleum or PCB's in an environmental sample. Furthermore, even with isotope dilution experiments one must still prove (or else assume) that the IRC has equilibrated with the sample matrix.

There are occasions when several IRC's are required in the same analysis. If dissimilar analytes are to be analyzed simultaneously, an IRC combination should be added to represent each class of compounds to be determined. Multiple IRC's are also needed to compensate for physical properties which affect the efficiency of the analysis. For example, in the

TABLE I

Recovery of aliphatic and aromatic hydrocarbons from water by headspace sampling

Compound	Average percent recovery from water	
	4-hour sampling (6)[a]	18-hour sampling (3)
mesitylene	8±2[b]	6±1
naphthalene	29±4	52±9
2,3,6-trimethylnaphthalene	—	95±5
phenanthrene	12±4	92±4
2-methylundecane	17±5	31±8
5-methyltetradecane	62±4	84±3
7-methylhexadecane	74±8	97±3
2-methyloctadecane	57±8	94±3

[a]( ) denotes number of samples.

[b]data reported as the standard deviation (1s) of a set of replicate values from the mean of the replicate values.

determination of petroleum hydrocarbons in water using a headspace sampling technique,[3] the extraction efficiency of any hydrocarbon is a function of both its volatility and water solubility. This effect is illustrated by the headspace sampling extraction efficiencies for several aliphatic and aromatic hydrocarbons given in Table I. Since petroleum hydrocarbon determinations are complex multicomponent analyses, it was impractical to attempt to mimic each component with an IRC, yet it would have been unsatisfactory to use only one IRC. Instead, a set of representative compounds was selected to compensate for differences in the extraction efficiencies of the analytes. The analyte concentrations were determined by

reference to two IRC's as follows:

$$C_A = S\frac{C_{IRC_1}R_A}{R_{IRC_1}} + (1-S)\frac{C_{IRC_2}R_A}{R_{IRC_2}}$$

and

$$S = \frac{T_{IRC_2} - T_A}{T_{IRC_2} - T_{IRC_1}}$$

where C_A, C_{IRC_1} and C_{IRC_2} are the concentrations of the analyte and the internal reference compounds which elute before and after the analyte, respectively; R_A, R_{IRC} and R_{IRC_2} are the measured responses; and T_A, T_{IRC_1} and T_{IRC_2} are the elution times, volumes or R_f values.

IRC's and external standards can be used together to put separate analyses on a comparable or absolute basis. This procedure was employed for the determination of the petroleum content in environmental samples (sediment, water, and tissue) from an oil spill area.[4] The samples from the oil spill area and a sample of pure spill oil were independently extracted by headspace sampling after adding the same IRC to each sample. The addition of the IRC allows one to compensate for differing recoveries during the extraction. The results of the analyses of the environmental samples and the pure spill oil were then compared and the weight percent oil in the environmental sample was calculated using the following equation:

$$\frac{\sum_{n=a}^{b} h_n \cdot Wc \cdot hc_{IRC}}{\sum_{n=a}^{b} hc_n \cdot W \cdot h_{IRC}} \cdot 100 = \text{wt \% oil in sample}$$

where h_n is the peak height of the normal aliphatic hydrocarbon of carbon number n in the sample chromatogram; hc_n is the corresponding peak height from the pure spill oil chromatogram; h_{IRC} is the peak height of the internal reference compound in the sample chromatogram; hc_{IRC} is the corresponding internal reference compound peak height in the pure spill oil chromatogram; and W and Wc are the weights of the sample and the pure spill oil, respectively. This combination of an IRC and an external standard (the pure spill oil) allows one to compensate for differing extraction efficiencies for the two matrices analyzed (environmental sample and spill oil) and to directly quantitate the oil levels in the various samples.

QUALITATIVE MARKER COMPOUNDS

Complex mixtures of the same general type can often be differentiated by subtle differences in their chemical composition. The specific compounds or classes of compounds used to identify or differentiate complex samples are qualitative marker compounds. The application of qualitative markers has been particularly useful in the identification of petroleum products and crude oils[5] and in the differentiation of hydrocarbons of petroleum origin from those of biogenic origin.[2]

The use of qualitative markers relies on the ability of the analyst to selectively identify the marker compound.[5] Fortunately, several chromatographic detectors exist which can be utilized to selectively monitor certain classes of organic compounds. Some of these detectors and their specificities are summarized in Table II. The techniques of gas chromatography (GC), high-performance liquid chromatography (HPLC), and mass spectrometry (MS) can provide considerable information regarding the composition of a complex sample.

The chromatographic analysis of crude oil samples using selective detectors illustrates the use of qualitative marker compounds to differentiate between oil samples. Samples of three crude oils (Southern Louisiana, Kuwait, and a crude oil collected at the site of a tanker spill) were analyzed by GC utilizing both non-specific detection, the flame ionization detector (FID), and sulfur-specific detection, the flame photometric detector (FPD) (see Figure 1). The very similar FID chromatograms for all three samples made positive identification difficult; whereas the FPD chromatograms revealed that the spill oil was closely related to the Kuwait crude. These samples were also analyzed by HPLC with selective UV absorption detection of the aromatic and polynuclear aromatic compounds in the sample (see Figure 2). The HPLC analysis of the crude oils again indicated similarity between the Kuwait oil and the spill oil. Petroleum also contains nitrogen heterocyclic compounds which could be used as qualitative markers using a nitrogen selective detector.

Combined gas chromatography–mass spectrometry (GC–MS) is currently the most powerful technique for the identification of organic compounds in complex mixtures. In addition to providing a mass spectrum of each peak eluting from the GC, GC–MS data can be plotted in the form of mass chromatograms as an additional interpretive aid. Mass chromatograms are computer-generated, mass-specific gas chromatograms which are used in locating particular compounds or classes of compounds containing a characteristic m/e peak in their mass spectra. The use of GC–MS for selective identification of qualitative markers is illustrated by the analysis of a sediment sample that was collected near a

TABLE II

Selective chromatographic detectors

GC detectors	Specificity	Type	Identification of individual compounds	Sensitivity
Flame photometric	Sulfur, phosphorous	Spectroscopic	No	10^{-10}g
Electron capture	Halogens	Ionization	No	10^{-13}g
Alkali flame (thermionic)	Nitrogen, phosphorous	Ionization	No	10^{-11}g
Coulometric	Nitrogen, phosphorous, halogens	Coulometric	No	10^{-13}g
Fourier transform infrared	All organic compounds	Spectroscopic	Yes	10^{-9}–10^{-6}g
Mass spectrometer	All organic compounds	Mass fragment	Yes	10^{-11}–10^{-8}g
LC detectors				
Ultraviolet	UV absorbing compounds	Spectroscopic	Yes	10^{-8}–10^{-11}g
Fluorescence	Fluorescent compounds	Spectroscopic	Yes	10^{-8}–10^{-12}g
Electrochemical	Electroactive compounds	Potentiometric	No	10^{-12}g
Mass spectrometer	All organic compounds	Mass fragment	Yes	10^{-14}g

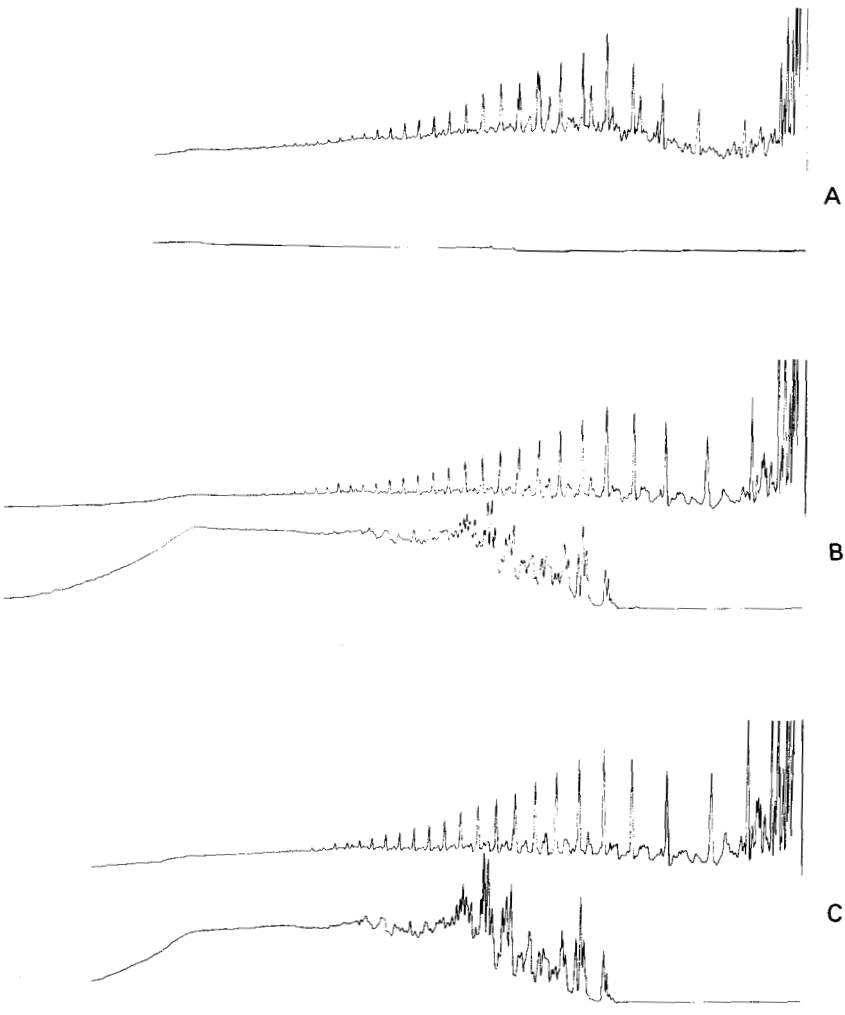

FIGURE 1 Gas chromatograms of three crude oils using non-specific and sulfur-specific detection; (A) Southern Louisiana Crude, (B) Kuwait crude, and (C) tanker spill oil. The upper trace for each sample is flame ionization detection (FID) and the lower trace is flame photometric detection (FPD).

Column: 10 ft × 2 mm i.d. glass packed with 1% SE-30 on Chromasorb WHP 100/120. Conditions: Helium at 30 ml/min; 50 °C for 4 min, then temperature program to 350 °C at 8 °C/min.

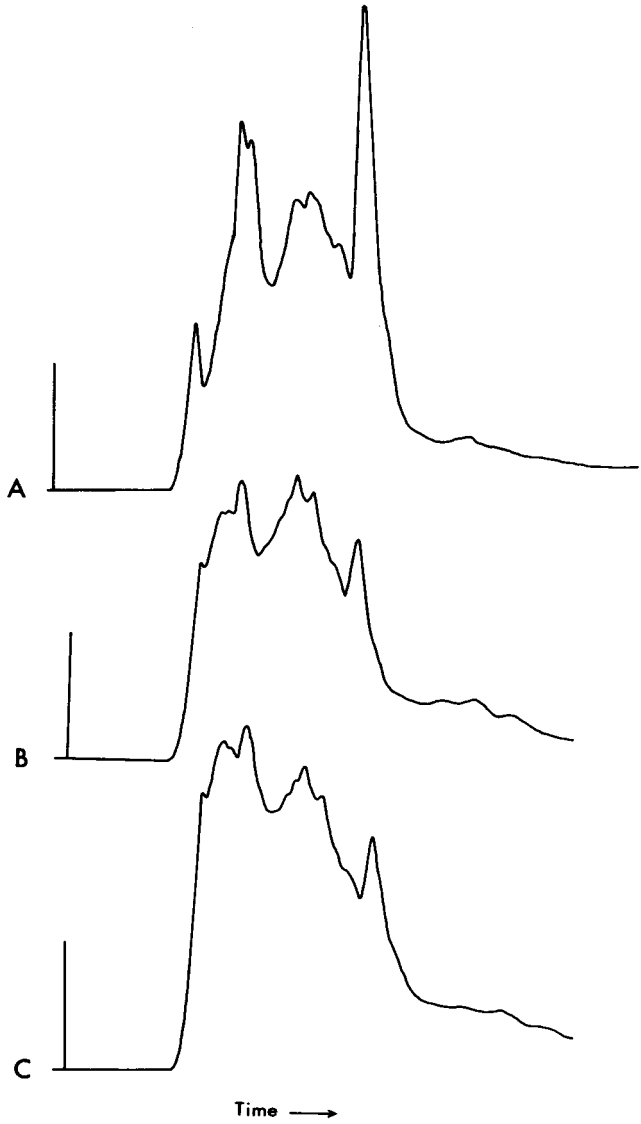

FIGURE 2 Liquid chromatograms of three crude oils using UV absorption detection; (A) Southern Louisiana crude, (B) Kuwait crude, and (C) tanker spill oil.

Column: μBondapak NH_2; Mobile phase: *n*-pentane.

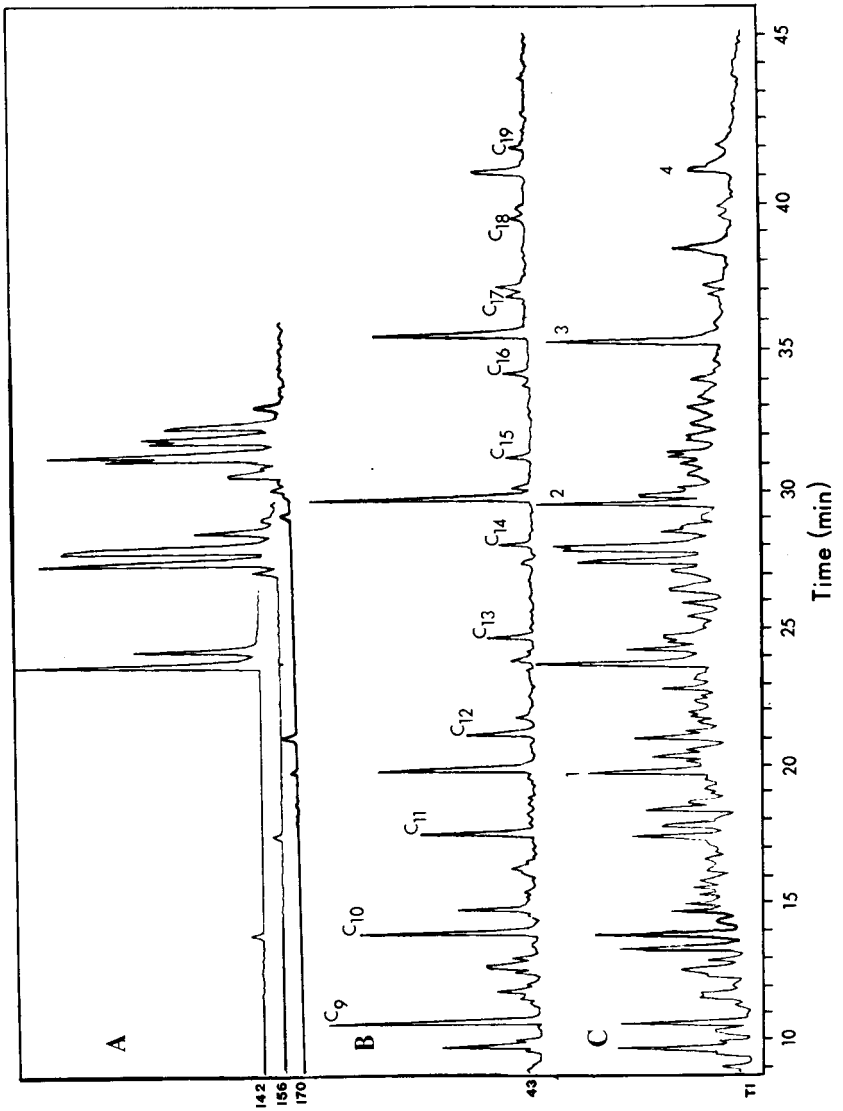

FIGURE 3 GC/MS analysis of sediment from an oil seep area: (A) mass chromatogram for m/e 142, 156 and 170; (B) mass chromatogram for m/e 43; and (C) total ion chromatogram.

Column: 100 m × 0.65 mm i.d. glass SE-30 coated SCOT. Conditions: Helium at 6 ml/min: 80 °C for 4 min then temperature program to 270 °C at 8 °C/min.

natural oil seep (see Figure 3). Figure 3C is the non-specific total ion chromatogram (sum of all the ions produced) in which very little qualitative information is readily available because of the complexity introduced by co-eluting components. However, the mass chromatogram for m/e 43 (Figure 3B) is indicative of aliphatic hydrocarbons. A homologous series of *n*-alkanes, which could not have been otherwise visually identified in the complex chromatogram, is apparent in this mass chromatogram. The presence of a homologous series of *n*-alkanes with adjacent odd-number and even-number carbon alkanes in nearly equal concentrations is an indication of hydrocarbons of petroleum origin.[2] Figure 3A is a composite mass chromatogram for m/e 142, 156 and 170 which indicate the presence of C_1-, C_2-, and C_1-substituted naphthalenes, respectively. Alkyl naphthalenes and alkyl homologs of larger polynuclear aromatic hydrocarbons can also serve as qualitative markers of petroleum.[6]

The results of GC–MS analysis of a sediment sample from a pristine environment are shown in Figure 4. The mass chromatogram at m/e 43 (Figure 4A) indicates a homologous series of *n*-alkanes. Figure 4A exhibits a predominance of odd-carbon hydrocarbons in the pristine sediment, while no such condition is evident for the oil-seep sediment (Figure 3B). In samples with *n*-alkanes of recent biogenic origin, the odd-carbon hydrocarbons predominate over the even-carbon hydrocarbons.[7] Thus, the odd/even-carbon number ratio can be used as a qualitative marker of the origin of the *n*-alkanes.

HPLC is an extremely valuable technique for the analysis of labile or non-volatile compounds. Examination of the previously discussed oil-seep sediment by HPLC (with selective UV absorption and fluorescence emission detection) yielded qualitative information about the polynuclear aromatic hydrocarbons (PAH's) in the sample. The PAH's in the sediment were chromatographically separated according to the number of condensed rings using normal-phase HPLC.[8] Each fraction was subsequently analyzed using reversed-phase HPLC to separate out the alkyl-substituted homologs within each fraction. The reversed-phase liquid chromatogram of one PAH fraction is shown in Figure 5. The HPLC effluent was monitored simultaneously with UV absorption and fluorescence emission detectors. The fluorescence emission spectra obtained for each chromatographic peak were utilized for compound identification. Compound *a* in Figure 5 was identified as phenanthrene; the fluorescence spectra of components *b*, *c* and *d* were tentatively identified as alkyl substituted phenanthrenes. In addition, GC–MS analysis of this fraction identified the compounds as C_1-, C_2-, and C_3-substituted phenanthrenes or anthracenes. The presence of these phenanthrenes suggests petroleum contamination of

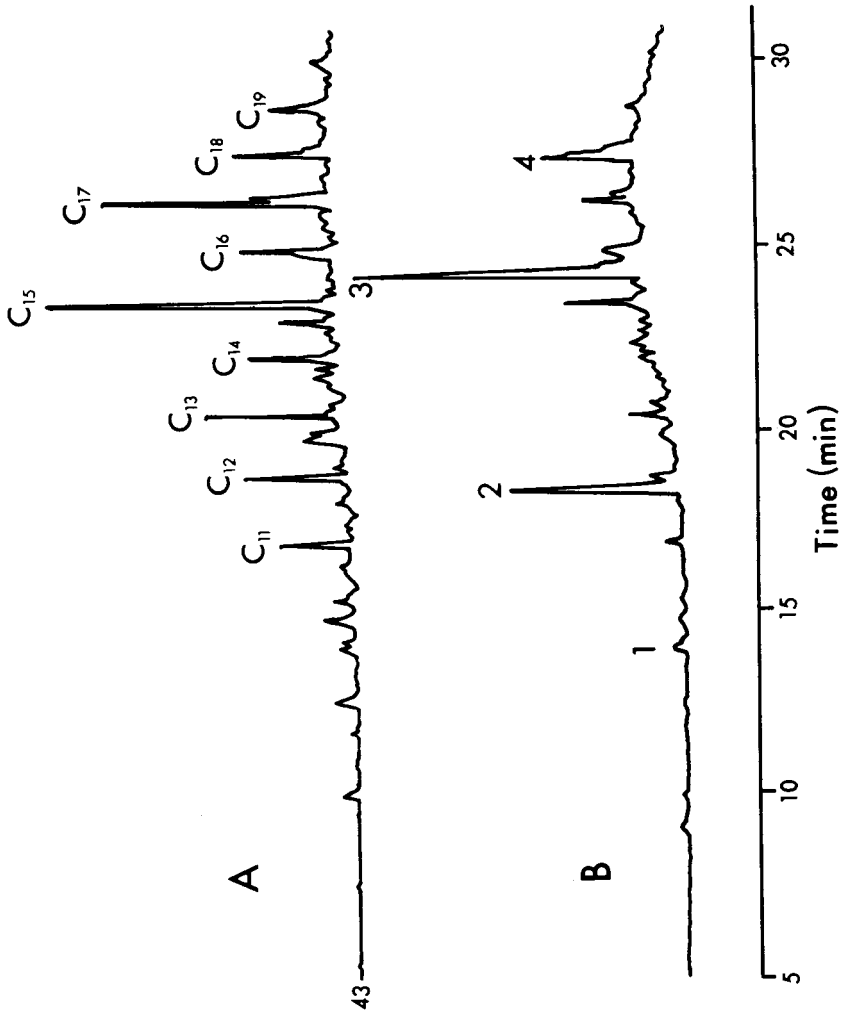

FIGURE 4 GC/MS analysis of sediment from pristine area: (A) mass chromatogram for m/e 43, and (B) total ion chromatogram.
Column: 200 ft × 0.030 in i.d. stainless steel SE-30 coated SCOT. Conditions: Helium at 6 ml/min, 50 °C for 4 min then temperature program to 270 °C at 8 °C/min.

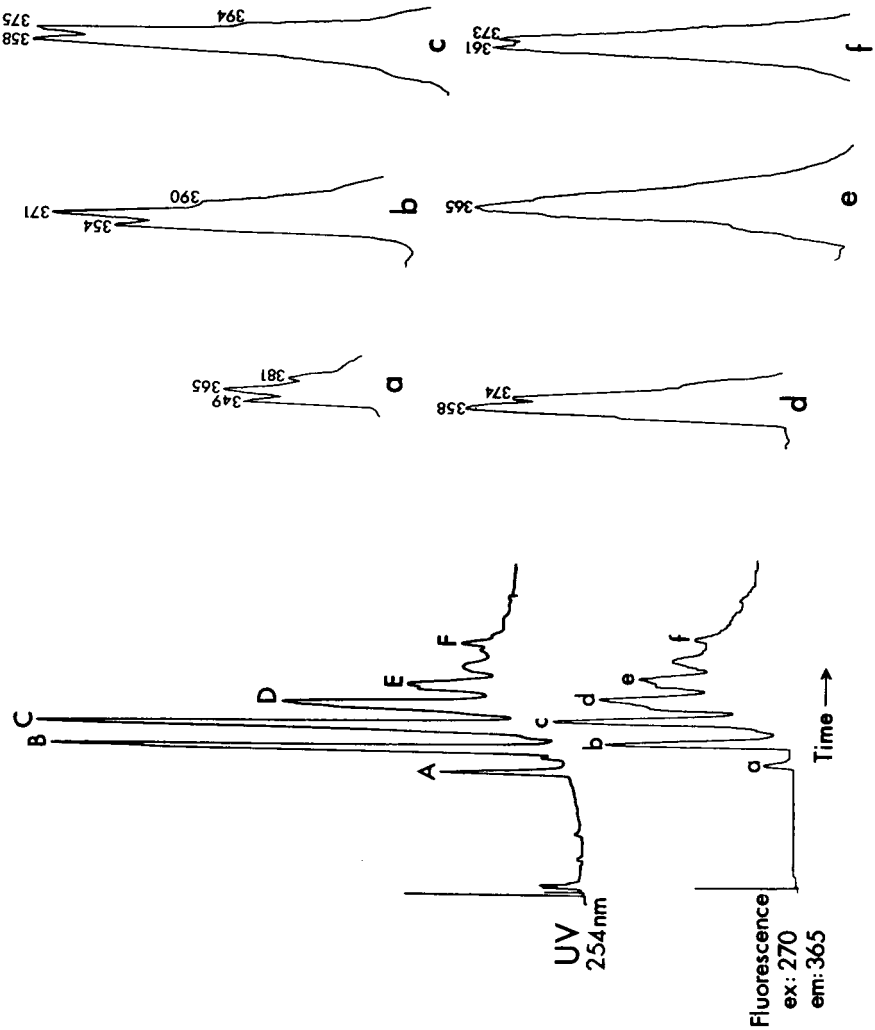

FIGURE 5 Liquid chromatogram of 3-condensed ring fraction from oil-seep sediment sample with fluorescence emission detection. Compound *a* is phenanthrene; compounds *b–f* are tentatively identified as substituted phenanthrenes.
Column: μBondapak C_{18}, Mobile phase: 50–100% acetonitrile-H_2O linear gradient in 30 min.

the sediment since other investigators[9] have reported that phenanthrene and methylphenanthrene are the most abundant PAH's in many crude oils.

CONCLUSIONS

The purpose of this paper is to illustrate the advantages and uses of marker compounds in environmental analytical chemistry. Through the use of marker compounds, analytical procedures may be simplified and quantitative results should be improved. Although the authors have drawn only upon their experience in environmental petroleum analyses, the same techniques are also adaptable to the analysis of other classes of compounds.

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