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Note

Combined ultraviolet absorbance and fluorescence monitoring: an aid to identification of polycyclic aromatic hydrocarbon metabolites by high-pressure liquid chromatography

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High-pressure liquid chromatography (HPLC) has been increasingly utilized to separate various polycyclic aromatic hydrocarbons (PAH), as they occur in the environment or as metabolic products[1–6]. The basis for such studies is that PAH are environmental pollutants and potential human carcinogens[7]. Most investigations have used primarily UV absorbance for monitoring of the eluent[1–5]. However, many compounds can be resolved by HPLC and it has been demonstrated that, when applicable, fluorescence monitoring, due to increased sensitivity, can be more useful than absorbance[8,9]. In addition, it was recently shown that simultaneous monitoring of absorbance and fluorescence can be used to more fully interpret chromatograms involving incomplete resolution of eluting components[6,10].

While investigating the metabolism of various PAH using HPLC the question arose, during difficult separations, of whether total resolution of the eluting components was necessary while monitoring the eluent by fluorescence and absorbance.

MATERIALS AND METHODS*

Purified, authentic samples of 7-CH_3-BA, 7-CHO-BA, 7-CH_2OH-BA, MC, MC-1-one, MC-2-one, MC-1-OH, MC-2-OH and MCL were synthesized and/or purified and kindly supplied by Drs. E. Cavalieri and R. Roth (Eppley Institute, Omaha, Nebr., U.S.A.). Purity of all compounds was checked by HPLC. All solvents were spectro-grade (Burdick & Jackson).

Chromatography was done with a Spectra Physics 3500 B system using combined absorbance (Schoeffel SF-770) and fluorescence (American Optical Fluoro-Monitor) detection. Reverse-phase chromatography was performed using a 50 × 0.9 cm column of 10 μm Partisil-ODS (Whatman) with a 45–99% methanol–water linear gradient of 40 min and column temperature of 46°. Adsorption chromatography was performed using a 25 × 0.32 cm column of 5 μm LiChrosorb (Altex Assoc.) run

* Abbreviations used: 7-CH_3-BA = 7-methylbenz(*a*)anthracene; 7-CHO-BA = benz(*a*)anthracene-7-carboxaldehyde; 7-CH_2OH-BA, = 7-hydroxymethylbenz(*a*)anthracene; 7-CH_2OAc-BA = 7-acetoxymethylbenz(*a*)anthracene; MC = 3-methylcholanthrene; MC-1-OH = 1-hydroxy-MC; MC-2-OH = 2-hydroxy-MC; MC-1-one = 1-keto-MC; MC-2-one = 2-keto-MC; MCL = 3-methylcholanthrylene.

10 min isocratically at 10% methylene chloride, followed by a 10–99% methylene chloride–hexane gradient of 15 min, with a column temperature of 21°.

RESULTS AND DISCUSSION

While chromatographing authentic samples of MC and some of its derivatives, some difficulty was experienced in separating MC-1-one from MC-2-one under these conditions (Fig. 1). As shown, base-line resolution of the two compounds was not achieved. However, this was not essential because it was noted that the ratio of UV absorbance/fluorescence was different for the two compounds. This ratio allowed us to readily identify either compound in a relatively complex chromatogram of metabolites. A more pronounced difference in this ratio is shown in Fig. 2 for two different derivatives of 7-CH_3-BA, namely 7-CHO-BA and 7-CH_2OAc-BA. The interpretation of a complex chromatogram involving these two compounds is facilitated greatly because of the almost total lack of fluorescence of the 7-CHO-BA. Lindner concluded that combined UV–fluorescence detection facilitated evaluation of mixtures of incompletely resolved compounds, as long as only one of the two compounds fluoresced[10]. This article shows how combined UV–fluorescence detection can be further exploited to include compounds which fluoresce using the ratio of UV/fluorescence.

Retention times in HPLC systems tend to vary from injection to injection, and variances of 1% are not unusual. In a chromatogram, such as shown in Fig. 1, if only the MC-1-one or MC-2-one were present, a 1% variation could lead to con-

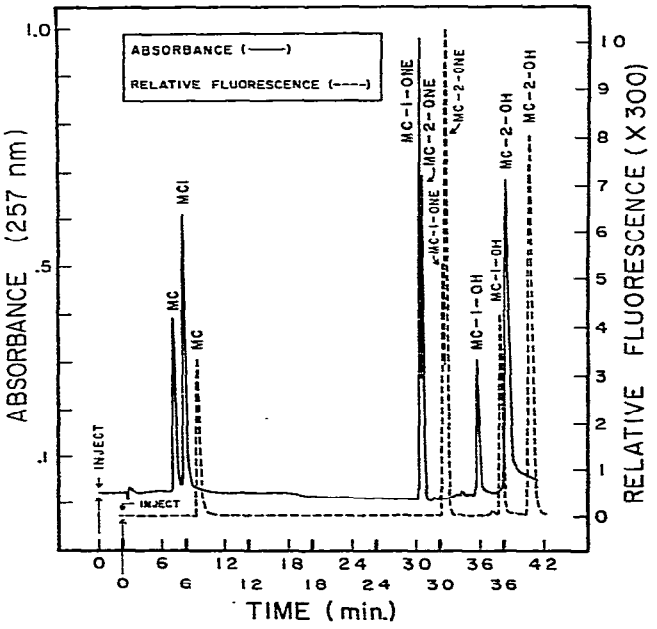

Fig. 1. Mixture of 3-methylcholanthrene derivatives; 10 min at 10% dichloromethane followed by 15 min linear gradient 10–99% dichloromethane–hexane. Flow-rate, 0.8 ml/min; column, 25 × 0.32 cm 5 μm LiChrosorb; column temperature, 21°.

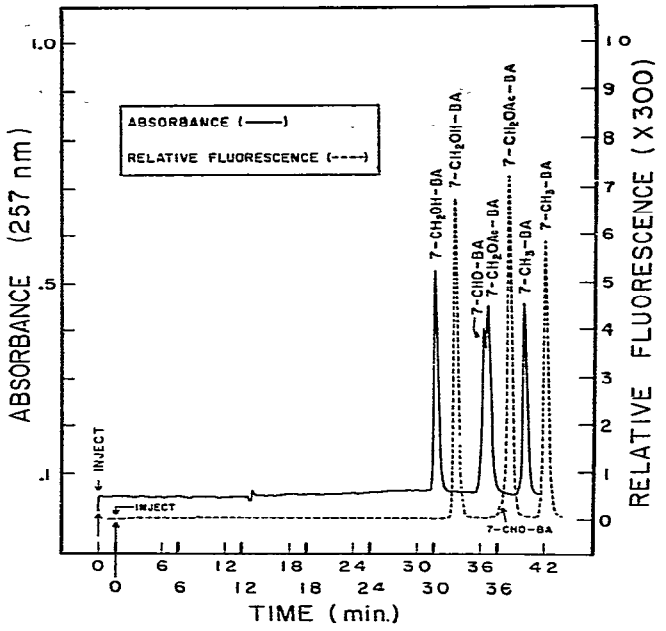

Fig. 2. Mixture of 7-methylbenz(*a*)anthracene derivatives; 40 min linear gradient 45–99%. methanol–water. Flow-rate, 2.6 ml/min; column, 50 × 0.9 cm 10 μm Partisil ODS; column temperature, 46°.

fusion between the two isomers, if the ratio of UV/fluorescence were not available. Admittedly, unknown samples should also be co-chromatographed with known standards for positive identification; however, by making use of UV/fluorescence ratio information, selection of the appropriate authentic standard can be facilitated. It would also be possible, by means of a longer, shallower gradient or recycling, to further separate the compounds. Both of these alternatives would require appreciably more time for each chromatographic run. As shown, further evaluation of complex chromatograms can be made by also comparing the combined fluorescence and absorbance response of poorly resolved components.

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