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Note

High-performance semi-preparative liquid chromatography of diesel engine emission particulate extracts

S. P. LEVINE*,* and L. M. SKEWES
Ford Motor Company, Scientific Research Staff, Analytical Sciences Department, Dearborn, MI 48121 (U.S.A.)

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INTRODUCTION

A variety of techniques have been utilized to identify the constituents and assess the biological activity of the soluble organic fraction (SOF) of diesel engine exhaust particulate material[1,2]. Preparative liquid chromatography (PLC) has been used to fractionate and concentrate mixtures containing polycyclic aromatic hydrocarbons (PAHs) prior to analysis[1–8]. PLC techniques such as open-column LC[4–7], analytical (Type 1)[9] high-performance liquid chromatography (HPLC)[3], and automated coupled-column HPLC[8] have been used for the fractionation of SOF and of fuel feedstocks. Recent trends in HPLC column packings have emphasized the use of microparticles which provide 10,000–20,000 theoretical plates per meter for certain analytical and preparative applications[9–11]. In our study, the use of a silica microparticulate packing in conjunction with a large diameter (8 mm) separation column and commercially available HPLC instrumentation has resulted in a maximum allowable sample size of 15–25 mg, with little or no reduction of resolution when compared to analytical HPLC separations. This method fits into the semi-preparative, or Type 2 PLC classification (as defined by Verzele and Geeraert[9]), which is best suited for the fractionation of compounds present in complex mistures. The solvent program used was designed to provide separation of paraffins from PAHs, of nitro-PAHs, and of the oxygenated-PAH sub-fractions.

MATERIALS AND METHODS

All PAH standards were obtained from Aldrich, except for 1-nitropyrene and dinitropyrene which were obtained from C. King (Michigan Cancer Center) and R. Mermelstein (Xerox Corp.), respectively. All solvents were distilled-in-glass and quality-controlled to ensure less than 5 ppb (10^9) phthalate-type impurities (Burdick and Jackson)[12,13]. Chromatography was done with a Varian 5060 HPLC apparatus using Perkin-Elmer 75 AC UV (254 nm) and Schoeffel FS 970 fluorescence (254 nm excitation, 320 nm cut off) detectors. A Spectrum 101 signal amplifier/noise filter was

* Present address: O.H. Materials Company, Box 551, Findlay, OH 45840, U.S.A.

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used to condition the output of the fluorescence detector. Detector monitoring was performed with strip chart recorders and/or with a Varian 401 data system. The chromatographic column was 25 cm × 7.9 mm packed with 10-μm μPorasil (Waters Assoc.)[10]. The solvent gradient was as follows: hold 100% hexane for 5 min after injection, linear change for 5 min to 5% methylene chloride, linear change for 25 min to 100% methylene chloride, hold with 100% methylene chloride for 10 min, linear change for 10 min to 100% acetonitrile, hold with acetonitrile for 5 min, step change to tetrahydrofuran (THF) and hold for 10 min, step change back to acetonitrile and hold for 5 min (end of chromatographic run), step change from acetonitrile to methylene chloride back to hexane at 10-min intervals, re-equilibrate for 15 min before the next injection. The solvent flow-rate was 4.5 ml/min. All preparative injections were made using a Waters U6K injector and 200 μl of methylene chloride solutions containing 50–100 mg of SOF/ml.

RESULTS AND DISCUSSION

The analytical procedure is illustrated in Fig. 1, along with the UV and fluorescence profiles obtained from the PLC of a typical Diesel SOF sample. The aliphatic compounds and alkylbenzenes, which make up 40–80% of a typical SOF sample[2,3], are separated into the hydrocarbon (HC) fraction with 90–95% efficiency (as determined mass spectrometrically). (All fraction designations follow the nomenclature given in refs. 2 and 3, and are illustrated in the Figure.) An injection in which there is column overloading usually by the HC fraction, is characterized by decreased column efficiency which results in reduced detail in the alpha-fraction envelope, and by the pyrene and benzo[*a*]pyrene components eluting at shorter retention times. The efficient separation of the HC and α fractions, which is achieved by the use of the initial hexane hold period, permits an approximately 4- to 5-fold improvement in maximum allowable sample size up to the 20 mg level. Work is underway to increase the maximum allowable sample size to the 50–100 mg range through the use of 20-mm diameter columns.

The γ regions of the fractionation scheme are potentially the most important due to the presence of 1-nitropyrene and related nitro-PAH compounds. These substances characteristically have high mutagenic activity on the Ames bioassay test[2,15], and must therefore be separated cleanly from both the α + β and from the δ fractions, as well as from bis(2-ethylhexyl) phthalate which emerges late in the γ_2 region. (Phthalates are ubiquitous contaminants of solvents and glassware and will therefore be present under even the most carefully controlled conditions.) The Ames-active constituents are resolved from each other and from interfering compounds using this PLC technique.

The retention times of components in the γ_2 and δ regions vary according to the immediate past history of the column. Specifically, the degree of activity of the silica and the silica-solvent interface layers can greatly affect the retentivity of the silica towards polar compounds[14], *e.g.*, 9-hyroxyfluorene. For this reason, a mixture of retention time standards must be chromatographed daily, and the column activity adjusted with dry hexane, THF, or a solution of 1% water in acetonitrile. (The use of water should be kept to an absolute minimum to avoid column degradation.)

The recovery of the sample after PLC fractionation is not readily determined

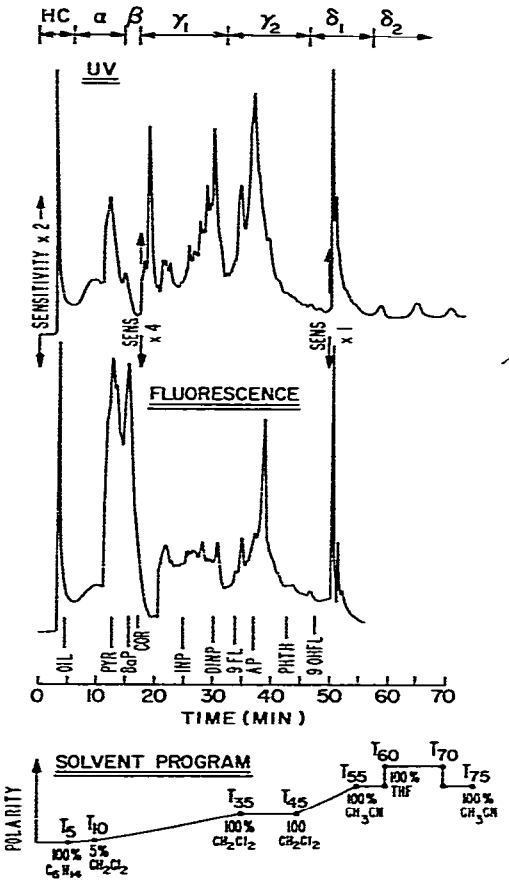

Fig. 1. Typical PLC profile of SOF sample. Top: Fraction designations (HC–δ)[2,3]. Middle: UV and fluorescence detector output tracings with *approximate* retention times of standards noted (PYR = pyrene; BaP = benzo[*a*]pyrene; COR = coronene; 1NP = 1-nitropyrene; DINP = dinitropyrene; 9FL = 9-fluorenone; AP = aminopyrene; PHTH = bis(2-ethylhexyl) phthalate; 9OHFL = 9-hydroxyfluorene). Bottom: Solvent program and time scale (T_5 = 5 min, etc.).

because of the wide variation in sample type, ranging from 75% (w/w) unoxidized hydrocarbons (oil and unburned fuel) in diesel emission samples to 65% (w/w) highly oxygenated PAH compounds in ambient air samples. Furthermore, three separate but related criteria for recovery should be used: recovery as Ames activity[15], recovery of injected mass, and recovery of individual components. Typically, mass recoveries in the range of 60–80% have been achieved without the final THF elution step. THF elution results in mass recoveries of up to 100% probably due to increased recovery of highly polar species[16]. The use of methanol has been discussed with respect to increased recovery of polar species[2]. This solvent must be used with care to avoid significant changes in retention times of polar components in subsequent runs, and to avoid slow degradation of column efficiency. The recoveries of nitropyrene, 9-fluorenone, and 9-hydroxyfluorene were determined through the injection either of a standard or of a standard mixed with engine oil (a simulated SOF matrix), collection of the appropriate peak fraction, and reinjection of that fraction. In all cases, compar-

ison of the peak areas indicated a 100% recovery (within experimental error) for these PAH derivatives. The questions of recovery of other constituents of diesel SOF, and of the Ames activity, are presently being investigated and will be reported at a later date.

The use of simultaneous UV and fluorescence monitoring of HPLC separations of PAH-containing mixtures has been advocated[17]. This procedure is used in these PLC separations, but interpretation is difficult because of the large number of unidentified constituents in the SOF. However, it was observed that typical compounds that elute in the γ and δ regions have strong UV absorbance characteristics but little or no fluorescence emissions. The fluorescence trace shows more individual peaks in the delta region than does the UV trace. Nitro-PAH standards can be monitored using a UV detector, but there is insufficient selectivity and sensitivity to monitor these compounds during an actual analysis.

CONCLUSIONS

Semi-preparative (Type 2) HPLC has been applied successfully to the fractionation of the SOF of Diesel engine exhaust particulate material. A capacity of 15–25 mg has been achieved along with a clean separation of HC, PAHs, mildly oxygenated PAHs, and highly polar oxygenated fractions.

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