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LIQUID CHROMATOGRAPHIC DETERMINATION OF BENZO[*a*]PYRENE AT PART-PER-BILLION CONCENTRATIONS IN HIGHLY REFINED COAL- AND PETROLEUM-DERIVED FUELS*

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SUMMARY

Benzo[*a*]pyrene (BaP), a well-known carcinogen, is frequently measured as an "indicator" of the potential dermal tumorigenicity of a sample. The present sequential high-performance liquid chromatography–high-performance liquid chromatography method overcomes problems in trace-level sample enrichment and recovery corrections encountered earlier. An amount of 5 g of naphtha or fuel oil is diluted to 10 ml with dichloromethane and spiked with a small quantity (*ca.* 0.25 μg) of ^{14}C-labeled BaP tracer. A BaP-enriched fraction is obtained from a 1-ml aliquot of this sample by semipreparative chromatography on a Partisil PAC 10 column with dichloromethane–hexane (10:90) as the eluent, and concentrated to exactly 0.3, 0.5, or 1.0 ml in acetonitrile. Quantitation is performed using a reversed-phase Vydac 201 TP 5415 column with acetonitrile–water (75:25) as eluent and a Waters Model 420 E/420 AC fluorescence detector, employing an excitation/emission filter pair of 360/425 nm. The recovery of the radiolabeled tracer is evaluated by combustion of 50 μl of the final isolate in pure oxygen, collection of the liberated $^{14}CO_2$ in an alkaline desorber, and liquid scintillation counting.

The recovery of BaP normally exceeded 90%, but values as low as *ca.* 50% were occasionally observed. Potential matrix interferences in the recovery determination were eliminated by sample combustion. The nominal precision of the overall method is approximately ±30% relative standard deviation at a BaP concentration of 30 ppb. The nominal analysis time for a single sample is approximately 4 h.

INTRODUCTION

Sequential high-performance liquid chromatography–high-performance liquid chromatography (HPLC–HPLC) consists of a semipreparative-scale HPLC fractionation, coupled either on-line or off-line with an analytical-scale HPLC determination. HPLC–HPLC has been estasblished[1–5] as an accurate and efficient method for the

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determination of toxic compounds, such as benzo[*a*]pyrene (BaP) at ppm (μg/g) and sub-ppm concentration levels in a variety of complex sample matrices. These applications have included crude petroleum[1,3,4], shale oil[2,4], coal liquids[4], fresh and spent lubricating oils[3], and cigarette smoke particulate matter[5].

The determination of BaP at ppb (ng/g) concentration levels in highly refined fuels presents problems that are somewhat different from those encountered in analyses conducted at much higher levels on less refined samples. The much lower BaP concentration levels in refined fuels require that a larger amount of fuel sample be fractionated and concentrated for use in the measurement step. Also, analyte losses incurred during sample processing must be individually corrected for each aliquot because of the potentially greater adsorptive losses of trace level analyte from the relatively "clean" sample matrix.

This paper describes the successful modification of the HPLC–HPLC method to accommodate these requirements for BaP determinations at the ppb level in fuels refined from petroleum and coal liquids. Larger fuel sample volumes can be processed in the sample fractionation step owing to the low concentrations of polar constituents in these refined fuels. The use of radiolabeled tracers coupled with sample matrix oxidation and liquid scintillation counting allow analyte recovery corrections to be determined for individual samples.

EXPERIMENTAL

Samples, solutions, and standards

The refined coal liquid and petroleum samples were supplied by the U.S. Department of Energy Synthetic Fuels Repository[6]. The samples included API No. 2 fuel oil (repository sample No. 975) obtained from the American Petroleum Institute (Washington, DC, U.S.A.); H-Coal home-heating oil (No. 978), and H-Coal reformed naphtha (No. 936). Blending and hydrogenation of H-Coal heavy and light oils for samples No. 978 and 936 were performed by the Chevron Research Company (Richmond, CA, U.S.A.). Further refining of the sample No. 936 was accomplished by Universal Oil Products (now Signal Research Center, Des Plains, IL, U.S.A.). These samples do not necessarily reflect the properties of future commercially available coal-derived products. A sample of Standard Reference Material (SRM) No. 1582, petroleum crude oil, was purchased from the National Bureau of Standards, Office of Standard Reference Materials (Washington, DC, U.S.A.).

A 2–5 g portion of each oil was dissolved in dichloromethane, spiked with radiotracer as described below, and diluted to exactly 10 ml.

BaP was obtained from the National Cancer Institute Carcinogen Repository at the Illinois Institute of Technology Research Institute (Chicago, IL, U.S.A.). Naphthalene and pyrene were purchased in 99% or greater purity from Aldrich (Milwaukee, WI, U.S.A.). These standards were used as received.

Solvents

All solvents were "distilled-in-glass" HPLC grade, purchased from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.), and were used as received.

Radiotracer solution

A 250 μCi lot of [7,10-$^{14}C_2$]BaP of 58.5 mCi/mmol specific activity was purchased from Amersham (Arlington Heights, IL, U.S.A.), and diluted to exactly 250 ml with toluene. A 50-μl aliquot, representing 1.125 (10^5) dpm or 0.278 μg of BaP, of tracer solution was added to each dichloromethane solution of oil.

Three-component standard

A solution containing *ca.* 100 μg/ml each of naphthalene, pyrene, and BaP in dichloromethane–hexane (1:1) was used to locate the cutpoints for BaP, as described below.

BaP standard solutions

Five BaP standards ranging in concentration from 0.1 to 1.0 μg/ml were prepared in acetonitrile. A calibration curve was constructed daily from HPLC analysis of the five standards.

Instrumentation

Isolation HPLC. The semipreprative-scale HPLC system used for isolating a BaP-enriched fraction was adapted from two systems described elsewhere[4,5,7]. Briefly, a 1-ml portion of the sample or a three-component standard solution was injected into a semipreparative scale Partisil PAC-10 column (25 cm × 10 mm I.D., custom-packed by Alltech Assoc., Deerfield, IL, U.S.A.) and MPLC "AMINO" guard column (3 cm × 4.6 mm I.D., Brownlee Labs., Santa Clara, CA, U.S.A.) by means of a Valco six-port valve (Model CV-6-UHD-a-N60, Houston, TX, U.S.A.).

The dichloromethane–hexane (1:10), neat dichloromethane, or acetonitrile–dichloromethane (66:34) eluent was selected from one of three reservoirs, using a digital programmer (Model DP 810) and the associated automatic stream selection valve (Model SSV-6), both purchased from Glenco Scientific (Houston, TX, U.S.A.). The digital programmer also operated a battery of four three-way miniature PTFE valves, ganged in series, which were used to collect the BaP-enriched fraction (General Valve, East Hanover, NJ, U.S.A.). The eluent was pumped at 2 ml/min by a single Model 2396 Duplex reciprocating pump [Laboratory Data Control (LDC), Riviera Beach, FL, U.S.A.]*. The eluent was monitored at 254 nm with a Model 1285 UV monitor (LDC). The resulting chromatogram was displayed on a stripchart recorder.

Analytical HPLC. The analytical HPLC system used to quantitate BaP was described elsewhere[4,5]. Briefly, a 20-μl aliquot of the final isolate was– injected onto a Vydac 201 TP 5415 reversed-phase octadecylsilane column (15 cm × 4.6 mm I.D., The Separations Group, Hesperia, CA, U.S.A.) backed by an Uptight guard column, packed with Perisorb RP-18 (Upchurch Scientific, Oak Harbor, WA, U.S.A.), using a Rheodyne Model 7125 high-pressure valve (Rheodyne, Cotati, CA, U.S.A.). BaP was eluted from the column with acetonitrile–water (75:25), continuously sparged

* The pump had to be primed daily. Inserting a three-way priming valve (*e.g.*, part no. 02-0125, Scientific Systems, State College, PA, U.S.A.) into the system allows this to be accomplished quickly and easily.

with nitrogen, and pumped at 1.5 ml/min with a single LDC Model 2396 Duplex mini-pump. The analyte was quantitated using a filter fluorescence detector (Model 420-E/420-AC, Waters Assoc., Milford, MA, U.S.A.) with 360/425 excitation/emission filters.

Sample oxidizer and liquid scintillation counter. The recovery of the radiolabeled tracer was determined by adding a 50-μl aliquot of the final isolate to an ashless gauze pad and combustion cone, and burning it in pure oxygen in a Model B306 Tri-Carb sample oxidizer (Packard Instrument, Downers Grove, IL, U.S.A.). The instrument was operated under the following conditions: nitrogen, 36 p.s.i.; oxygen, 46 p.s.i.; Carbosorb (alkaline organic carbon dioxide desorber), 7 ml; Permafluor V (liquid scintillation cocktail), 13 ml; burn time, 30 s. The carbon dioxide collected in the desorber and cocktail was subjected to liquid scintillation counting (10 min/sample, five or six cycles per set of samples) in a Tri-Carb Model C-2425 liquid scintillation counter (Packard Instrument). All sample counts were corrected for scintillation quenching with the automatic external standard option of the instrument.

Procedure

Isolation of the BaP-enriched fraction. A 1-ml aliquot of the three-component standard was injected into the semipreparative-scale HPLC column and eluted with dichloromethane–hexane (1:10). The resulting chromatogram resembles that shown by the dotted line in Fig. 1. The BaP-enriched fraction is defined by the volume eluted within ± 7 min of the retention time of BaP. A 1-ml aliquot of sample solution in dichloromethane was then injected, and the BaP fraction was collected. The solvent was removed under a flow of dry nitrogen and vacuum. The residue was redissolved in 0.3, 0.5, or 1.0 ml of acetonitrile.

The column was then washed sequentially with neat dichloromethane and acetonitrile–dichloromethane (66:34) (30 min each) and flushed sequentially with neat dichloromethane and dichloromethane–hexane (1:10) (30 min each) before injection of the next sample.

Quantitation of BaP. The BaP peak heights from the analytical-scale HPLC of the five BaP standard solutions in acetonitrile were fitted to a least-squares linear regression curve (the correlation coefficient typically exceeded 0.99). The concentration of BaP in the sample was then calculated by the method of external standards. The mass of [^{14}C]BaP added as tracer is not negligible with respect to the native BaP, and a correction must be applied. Therefore,

$$\text{BaP } (\mu\text{g/g}) = 10\ Cv/Wy - w/W$$

where C is the concentration of BaP in the final solution in μg/ml; v is the volume of the final solution in ml; W is the mass of sample in g; y is the recovery of the radiolabeled tracer, determined by sample combustion; and w is the mass of tracer, in μg, added to the sample.

RESULTS AND DISCUSSION

Our previous analyses of BaP in less refined sample materials required[4] only *ca.* 25 mg of sample for fractionation and measurement because of their generally

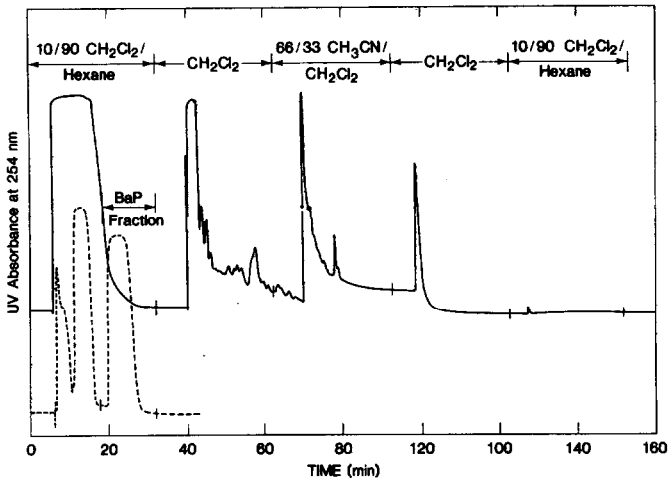

Fig. 1. Isolation of the BaP-enriched fraction from API No. 2 fuel oil. Three-component standard (100 μg/ml each of naphthalene, pyrene, BaP) shown by dotted line. Chromatographic conditions described in the text.

much higher BaP concentration levels compared to those in the highly refined fuels reported here. The conditions described for isolating BaP in the present work required up to 500 mg of fuel sample per analysis. Such an increase in sample size was required because the present samples contained ppb- or low-ppm-levels of BaP. This increase was due to the fact that these fuels were highly refined, and contained only low levels of polar contaminants, which otherwise would foul the isolation column.

Fig. 1 shows that a considerable amount of the UV-absorbing material in the sample was eliminated by the solvent program of the fractionation procedure. Only a small portion of the tail end of the aliphatic/aromatic hydrocarbons peak was collected in the BaP-enriched fraction. The reproducibility of the BaP cutpoints in-

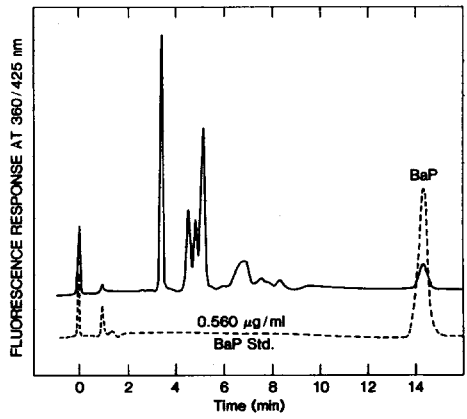

Fig. 2. Chromatogram of the BaP-enriched fraction from API No. 2 fuel oil. BaP standard (0.560 μg/ml) shown by dotted line. Chromatographic conditions described in the text.

dicated that the stepwise washing and flushing procedure depicted by Fig. 1 was also effective in removing contaminants from the column, and restoring it to its original activity. The cutpoints in the fractionation procedure did not vary appreciably over the course of this work from the 18- and 32-min cutpoints initially established with the three-component standard.

Fig. 2 demonstrates the effectiveness of the analytical-scale reversed-phase separation of BaP from various components eluted in the same fraction of the semipreparative-scale isolation procedure. The BaP peak is eluted in a chromatographic region free of obvious interferences. Typically, few interfering compounds from a refined petroleum product have retention times comparable to BaP, and the separation and measurement is made without difficulty. However, in refined coal-derived products or petroleum crudes, compounds such as benzo[*e*]pyrene, benzo[*b*, *j*, or *k*]fluoranthenes, perylene, and alkylated homologues of BaP may be present in the final isolate. These potential interferences normally pose no problem, because they are either chromatographically separated from BaP, discriminated against by the fluorescence detector, or both. The detection of BaP is at least 100-fold more sensitive than that of either benzo[*e*]pyrene or benzo[*j*]fluoranthene. In addition, both are chromatographically separated from BaP. The detector does respond well to the remaining compounds. However, the benzo[*b* or *k*]fluoranthenes and perylene are eluted before BaP, and alkylated homologues of BaP are eluted later. Thus, none of them interfere with the measurement of BaP.

Recovery measurements with radiolabeled BaP were complicated by several competing factors. First sufficient [^{14}C]BaP tracer activity had to be added to the sample to permit accurate final measurement, but not so large a mass as to "swamp" the native BaP in the sample. Secondly, our initial measurements demonstrated that contaminating species in the final isolate seemed to provide a highly fluorescent medium, which yielded spurious recovery values (significantly exceeding 100%), when the isolate was directly subjected to liquid scintillation counting.

We overcame these problems by adding an amount of radiolabeled BaP tracer which would yield approximately 1000 dpm of ^{14}C (sufficient for confident recovery measurements), yet which would not contribute more than 50–60 ppb of BaP to the sample. This value is not more than twice the level of BaP already present in most refined petroleum products, and was considered a satisfactory compromise between activity and added mass. The problems with the fluorescent matrix were easily remedied by combustion of an aliquot of the final isolate in pure oxygen, collection of the ^{14}C released as $^{14}CO_2$, and subjecting the resulting solution to liquid scintillation spectroscopy. In this manner, unbiased recovery values were obtained for each sample aliquot. The necessity for individual recovery measurements is illustrated (Table I) by the variability of recoveries. Although recoveries typically ranged between 70 and 100%, values as low as *ca.* 50% were occasionally observed.

Validation of this method was difficult, because no refined petroleum or coal-derived oils are available that have certified BaP values at the ppb level. Triplicate determinations of BaP for NBS SRM 1582 (petroleum crude oil, certified value of 1.1 ± 0.3 μg BaP/g oil) yielded a result of 1.7 ± 0.2 μg BaP/g oil. The reasonable (albeit not perfect) agreement between the two values was considered sufficient.

Table I presents results for determinations for BaP in two coal-derived refined products and a refined petroleum oil product. In all cases, the mean concentration

TABLE I

DETERMINATION OF BaP IN REFINED PRODUCTS

Bottle aliquot	*Recovery (%)*	*BaP (μg/g)*	$\bar{x} \pm S.D.$*
H-Coal home heating oil			
A	77	0.71	
	77	0.66	
	74	0.75	0.71 ± 0.45
B	83	0.65	
	66	0.98	
	88	0.56	0.73 ± 0.20
C	57	0.70	
	54	0.67	0.68**
H-Coal reformed naphtha			
A	87	1.37	
	94	1.43	
	91	1.46	1.42 ± 0.046
B	94	1.51	
	94	1.35	
	93	1.32	1.35 ± 0.087
C	100	1.35	
	100	1.33	
	100	1.37	1.35 ± 0.020
API No. 2 fuel oil			
A	100	0.059	
	96	0.022	
	42	0.012	0.031 ± 0.025
B	100	0.035	
	94	0.042	
	96	0.039	0.039 ± 0.003
C	100	0.026	
	100	0.026	
	81	0.052	0.035 ± 0.015
D	79	0.027	
	57	0.034	
	74	0.033	0.031 ± 0.0035

* Represents mean ± standard deviation of triplicate determination.
** Represents mean of two values.

of BaP was at or below 1 ppm. For the API No. 2 fuel oil, the mean value was approximately 30 ppb. The results for the different bottled aliquots of this sample showed good reproducibility. The precision achieved for each data set did vary; however, a typical value would be *ca.* ±30% R.S.D. at the 30-ppb level, and *ca.* 10–20% at the low-ppm level. This precision is similar to that reported elsewhere for trace-level BaP determinations in complex combustion sample matrices.

The detection limit of this procedure is determined not by the detection of BaP

itself (which can be less than 25 ng/ml with current instrumentation), but rather by the presence of the radiolabeled tracer itself. However, the variability in the individual measurements requires the presence of the tracer in each sample and speaks against using a previously-determined figure from a spiked sample as an overall recovery factor for each aliquot. If typical values for these BaP determinations are chosen (*e.g.* 5 g sample, 75% recovery, 10 ng BaP/ml final isolate concentration, 0.3 ml final isolate volume, and 0.278 μg BaP tracer added to the sample), a detection limit of *ca.* 20 ppb is readily calculated. This estimated detection limit, coupled with a reasonable analysis time (*ca.* 4 h per sample), indicates that the present method is practical for determining trace and ultratrace quantities of BaP in refined fossil fuel samples.

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