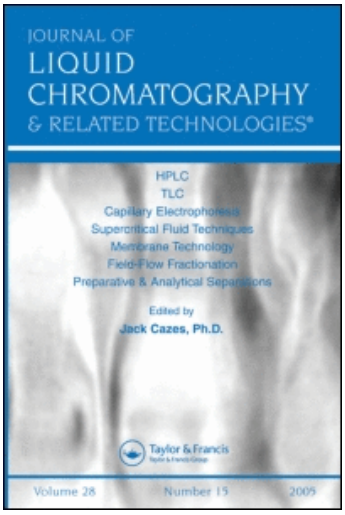

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EXTRACTION AND QUANTIFICATION OF BENZO[a]PYRENE IN SOIL BY REVERSED PHASE THIN LAYER CHROMATOGRAPHY

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ABSTRACT

As research on the biodegradation of benzo[a]pyrene (B[a]P) shifts from laboratory to field based studies, a need has arisen for reproducible, economical, and accurate methods for extraction and quantification of B[a]P. This study describes a method for the analysis of B[a]P using batch extraction techniques with quantification by reversed phase high performance thin layer chromatography (HPTLC) and densitometry. A loam soil was fortified with B[a]P at a level of 100 mg B[a]P kg^{-1} soil. Several solvents were evaluated as potential soil extraction systems including: acetone, acetonitrile, ethyl acetate, methanol, and methylene chloride:acetone (1:1). Reverse phase thin layer chromatography of B[a]P was performed on hydrocarbon impregnated, C18 reverse phase plates using methanol:acetonitrile (1:1) as the developing solvent. The Rf for B[a]P in this system was found to be 0.52. Recovery of B[a]P from soil ranged from 11% with methanol to 84% with ethyl acetate.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs), formed during the incomplete combustion of petroleum products are persistent and potentially dangerous contaminants of soils.[1] Benzo[a]pyrene (B[a]P), a known carcinogen, has become the focus of several bioremediation efforts.[2,3,4,5] The majority of these studies on microbial biodegradation of B[a]P have been conducted in liquid culture. However, several recent studies have focused on biodegradation in soil systems.[6,7] As more research shifts from the laboratory to field-based studies, it is apparent that a reliable and efficient method is needed for the extraction and quantification of B[a]P in soils.

A number of solvents have been used to extract B[a]P from liquid cultures including ethyl acetate,[3,8,9,10] acetonitrile:H_2O (2:1),[11] methylene chloride,[12] and methylene chloride:acetone (1:1).[13] Methylene chloride was used to extract B[a]P in several recently published soil studies.[6,7] Quantification of B[a]P has been routinely performed by high performance liquid chromatography (HPLC)[12,13] and GC-MS.[12] Both of these procedures usually require substantial sample preparation and also involve a significant investment in equipment and supplies. Thin layer chromatography, (TLC) provides a low-cost, rapid alternative to these methods.[14] The objective of this paper was to develop a procedure to extract and quantify B[a]P in soil extracts. This method, which is based on reverse phase thin layer chromatography method (RPTLC), is particularly well suited for screening soil samples from B[a]P microbial biodegradation experiments.

EXPERIMENTAL

Soil and Chemicals

The soil used in this study was an Ap surface horizon from a Commerce silt loam (fine-silty, mixed, nonacid, thermic Aeric Fluvaquent) from Iberville Parish, LA, USA[15] and was composed of 11% clay, 38% silt, and 51% sand. The benzo[a]pyrene used in this study was obtained from Sigma Chemical, St. Louis, MO, USA. The stated purity of the compound was ≥ 98% and it was used without further purification. All solvents used for extraction, reconstitution, and RPTLC were HPLC grade.

Soil Extraction Procedures

Fifty gram samples of soil were placed in a 250 mL Erlenmeyer flask that had been covered with aluminum foil to prevent exposure to light. The soil samples were inoculated with 5 mL of a stock solution of B[a]P in acetone (1000 mg L^{-1}) to yield a soil concentration of 100 mg kg^{-1}. All pipette tips were

exposed to the B[a]P solution (by successively drawing and expelling the solution into the tip) five times prior to final delivery, to minimize differential sorption of the B[a]P to the pipette tips. After complete evaporation of the acetone (approx. 12 hrs.) in a chemical hood, 100 mL of each solvent system (ethyl acetate, methylene chloride, methylene chloride:acetone 1:1 or acetonitrile:H_2O 1:2) were added to the soil. The resultant soil slurries were shaken overnight on a rotary shaker at 150 rpm, in the dark, at room temperature. The solvent-soil slurry was passed through a 100 mm top diameter glass funnel lined with Whatman No. 2 filter paper and containing 5 g sodium sulfate. The flask and soil were washed with an additional 2 x 50 mL aliquots of the appropriate solvent system. The pooled solvent extracts were dried in a chemical hood, in the dark, and re-suspended in 10 mL acetone. All trials were performed in triplicate.

Photodegradation

One mL samples of 100 ppm and 1000 ppm B[a]P in acetone were prepared and pipetted into 10 mL scintillation vials. The vials were placed under a fume hood and exposed to ambient light as well as a 40 watt GE soft white incandescent bulb (distance = 20 cm). Duplicate samples were exposed for 1, 2, 3, and 4 days, and were covered in lab tape and kept in the dark until analysis by thin layer chromatography.

Reversed Phase Thin Layer Chromatography

Reversed phase thin layer chromatography was performed on reversed phase hydrocarbon impregnated uniplates (10 x 20 cm, 250 micron thickness, Analtech Inc., Newark, DE). Standards and extracts were spotted, in duplicate, on each plate using 1 μL microcapillary pipettes and a Nanomat III sample positioner (Camag Scientific, Wilmington, NC). Standards of 125, 250, and 500 mg L^{-1} B[a]P were included on all plates analyzed. Several mobile phase combinations were initially evaluated, including; hexane:acetone (1:1), acetonitrile:methanol (60:40), and acetonitrile:methanol (50:50). The optimum mobile phase for this plate was determined to be acetonitrile:methanol (50:50). Spotted plates were developed in a vertical chamber containing the mobile phase. Developed plates were air-dried and then scanned using a Shimadzu Densitometer CS9000U Dual Wavelength Flying Spot Scanner.

To determine the optimum analytical wavelength, one μL of a 250 mg L^{-1} B[a]P solution was spotted and its absorbance spectra determined (Figure 1). The spectra exhibited several distinct maxima, most notably at 250, 280, and 360-370 nm. It was determined that analysis at 370 nm minimized the influence of interfering compounds present in soil extracts, while maximizing sen-

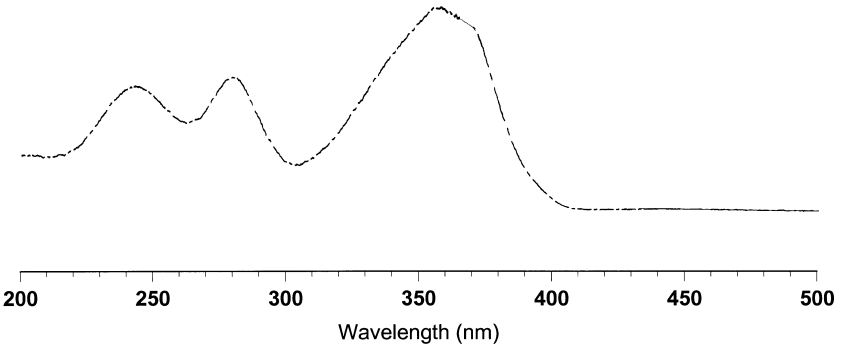

Figure 1. Absorbance spectrum for Benzo[a]pyrene.

sitivity. B[a]P concentrations were determined by comparison to standard curves, which were evaluated using linear and polynomial regression analysis.[16]

RESULTS AND DISCUSSION

The retention factors (R_f) for B[a]P varied slightly between experimental systems with Rf values of 0.52 for standard curves of pure chemicals and 0.55 for fortified soil samples. The observed variation is most likely related to the presence of interfering compounds in the soil extracts. It should be noted that B[a]P was clearly resolved in all systems (Figure 2). To account for any variation in the developing of the RPTLC plates, a genuine standard of B[a]P was included on all plates analyzed. Standard curves for B[a]P were initially prepared from 12.5 to 1000 mg L^{-1}. This range yielded curvilinear results at higher concentrations (Figure 3). Although a linear equation did fit the data very well ($r^2 = 0.987$), a quadratic equation ($r^2 = 0.999$) significantly improved the overall description of the standard curve.

To simplify routine analysis, standards were selected to encompass the lower, linear range (0 - 500 mg L^{-1}) and the final sample was reconstituted in a solvent volume suitable to assure inclusion in this range (10 mL). The detection limit of this method was 12.5 ng for the spotted standards.

Several reports have indicated that B[a]P is very susceptible to photodegradation.[17,6] In our study, concern was raised as to the stability of our extracted samples. It was also our goal to separate the effects of photo- from biodegradation. Results from our evaluations indicated that minimal degradation of acetone solutions of B[a]P occurred at time periods up to 4 days (data not shown).

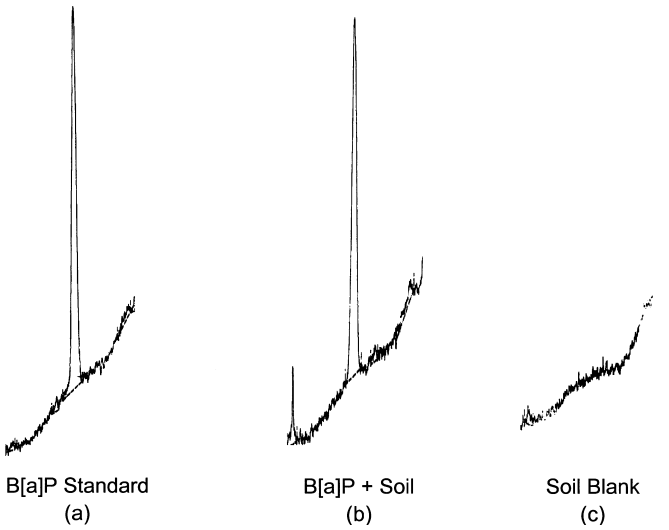

Figure 2. Densitometer chromatograms of a) Benzo[a]pyrene standard, b) Benzo[a]pyrene in a fortified Commerce soil sample, and c) Commerce soil blank.

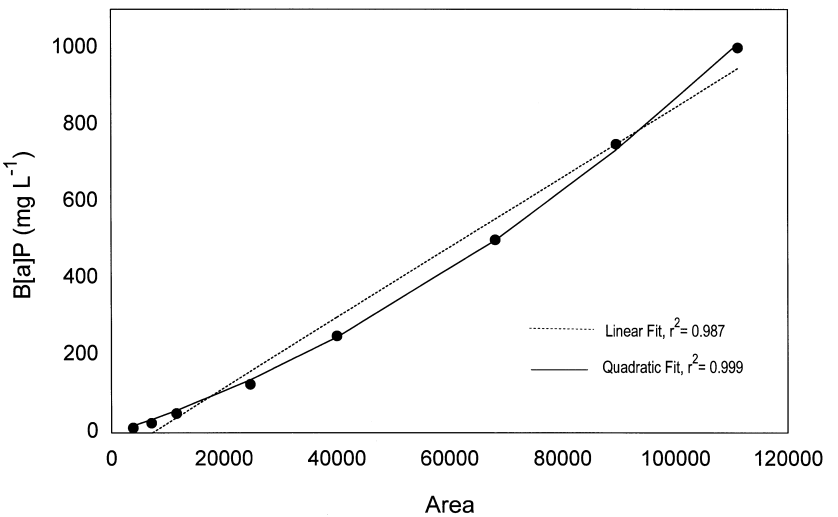

Figure 3. Standard curve for Benzo[a]pyrene.

Table 1

Effect of Solvent System on Recovery of Benzo[a]pyrene from Fortified Commerce Soil Samples

Solvent System	Recovery (%)[a] Mean	Standard Deviation	Coefficient of Variation
Acetone	57.5	10.3	17.9
Methanol	11.2	3.2	28.6
Ethyl Alcohol	83.9	9.5	11.3
Methylene Chloride:Acetone (1:1)	63.8	9.6	15.0
Acetonitrile	54.9	0.74	1.3

[a] All determinations performed in triplicate.

It should be noted, however, that B[a]P samples were rapidly oxidized on RPTLC plates. In an attempt to minimize this loss, all samples were analyzed immediately after developing and B[a]P control standards were included on each TLC plate analyzed.

Soil extraction results of parent B[a]P from fortified soil samples are presented in Table 1. Clear differences were noted between the solvent systems investigated. The greatest recovery was obtained with ethyl acetate (84%) and the lowest with methanol (11%). Methylene chloride:acetone (1:1), acetone, and acetonitrile yielded intermediate results with recoveries of 64, 58, and 54%, respectively. Ethyl acetate also resulted in the second lowest coefficient of variation for the extracted samples (11.3%), compared to 13.5, 15, 17.9, and 28.8% for acetonitrile, methylene chloride:acetone (1:1), acetone and methanol, respectively. Although acetonitrile did possess an extremely low CV (1.3%), the recovery (54.9%) was the second lowest obtained. Finally, ethyl acetate offers several advantages as a solvent for extraction including; lower cost, and carcinogenicity.

Several other methods to extract B[a]P from soil have been described in the literature. A soxhlet apparatus with benzene was used to extract soil samples (250 g) taken from the vicinity of a coke plant.[18] Recovery data was not reported, but TLC results were compared favorably with gas chromatography. In another report,[19] B[a]P was extracted from two soils using methylene chloride in a blender. This method was found to be superior to standard soxhlet procedures, although significant variation in recovery was noted at low concentrations.

Finally, in a recent study, the influence of three extraction procedures (batch, sonication, and soxhlet) on the recovery of PAH from coal tar was performed.[20] There was not a consistent difference reported in PAH recovery between the three methods.

CONCLUSIONS

The method described in this communication to analyze B[a]P in soils is a simple procedure that minimizes cost of analysis and does not rely on chlorinated solvents. In the proposed method, B[a]P is batch extracted with ethyl acetate. Extracted samples are filtered, dried, and reconstituted and finally analyzed by RPTLC. This system yielded B[a]P recoveries of 84% for fortified soil samples. The limit of detection of the method was 12.5 ng. This procedure is particularly well suited for screening soil samples from B[a]P microbial biodegradation experiments.

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