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Short communication

Isolation and recovery of 9,10-dimethyl-1,2-benzanthracene from animal bedding by reversed-phase solid-phase extraction combined with high-performance liquid chromatography

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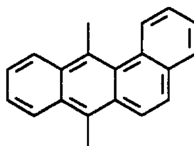
Abstract

Animal bedding which may contain toxic substances represents a new type of hazardous waste. The conventional technique of liquid–liquid extraction and acid–base cleanup was found to be unsuitable due to the high lipophilic content of the sample matrix. Liquid–solid bath sonication combined with reversed-phase solid-phase extraction and reversed-phase high-performance liquid chromatography (RP-HPLC) as the instrumental determinative step resulted in high recovery and good precision among triplicate samples of the bedding material.

Keywords: Polynuclear aromatic hydrocarbons; Dimethylbenzanthracene

1. Introduction

Animal bedding represents a novel environmental sample matrix and could be categorized as a "toxic waste" in which trace concentration levels of priority pollutants might be present. The polycyclic aromatic hydrocarbon (PAH), 9,10-dimethyl-1,2-benzanthracene (DBA) was introduced into the diet of laboratory rats and the analytical challenge presented was to isolate and recover this PAH from bedding material. This specific PAH is a carcinogen and has been assigned a RCRA Waste Number while giving numerous positives on EPA Genetox programs according to a recent materials safety data sheet [1]. It becomes important to know the residue level of this PAH since the animal bedding itself is more than likely to be considered as non-hazardous waste. The bedding consisted of rat feces and rat urine mixed with a finely divided cellulosic substrate. The molecular structure of DBA is shown below



The bedding is a complex matrix and it was anticipated that it might be difficult to achieve adequate recoveries. A number of EPA and related methods could be applied to this challenging sample matrix since DBA is amenable to both gas chromatographic and liquid chromatographic determinative techniques [2–4]. Reversed-phase solid-phase extraction (RP-SPE) is today a viable alternative to the more conventional liquid–liquid extraction (LLE) technique [5]. RP-SPE eliminates any possibility of emulsion formation within the non-polar extract in

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contrast to LLE techniques. In our laboratory, attempts to isolate polychlorinated biphenyls from sample matrices high in lipids results in formation of an intractable emulsion and our success with RP-SPE led us to consider this technique and apply it to the animal bedding [6].

This paper discusses our experience in first using a conventional LLE/acid–base cleanup approach to sample preparation using capillary gas chromatography with flame ionization detection (cGC–FID) followed by adopting a RP-SPE strategy using reversed-phase high-performance liquid chromatography with UV absorbance detection (RP-HPLC–UV). This is a preliminary study with the objective of assessing the feasibility of isolating DBA from a complex matrix and falls short of any conclusions based on exhaustive studies.

2. Experimental

2.1. Apparatus

A VacMaster-10 (International Sorbent Technology) was used to perform the SPE reported here. The stainless steel rack openings were enlarged slightly to accommodate a 1.0 ml volumetric flask. This enables the eluent from SPE to directly enter the receiver and the final volume is easily adjusted to the mark without the need to transfer liquid and eliminates a possible loss in analyte recovery. Polypropylene SPE cartridges were manually packed with 200 mg of Bondesil C_{18} (Varian Sample Preparation Products). A Model 2200 Bath Sonicatoror (Branson) was used to maximize the contact between the solid matrix and the solvent. A Compact II Centrifuge (Becton-Dickinson) was used to conduct all centrifugation. This mini-centrifuge operates at 3200 revolutions per minute and when a conical centrifuge tube is used, the supernatant is easily separated from the solid residue. The use of this mini-centrifuge was essential in developing good precision among replicate SPEs performed in related method development earlier [6].

2.2. Instrumentation

The HPLC used consisted of a 510 dual pump, a 710B WISP autosampler, a Lambda Max 481 uv absorbance detector set at 254 nm and an automated gradient controller (all from Waters). The detector and autosampler contact closure were connected to a 900 Interface (PE-Nelson) which in terms was connected to a 2000 4SX-33 (Gateway). Turbochrom processing software (PE-Nelson) was used to acquire data and methods within this environment were written. The column used was a 150×4.6 mm Adsorbosphere, 3 μm C_{18} (Alltech) while the mobile phase consisted of acetonitrile–distilled, deionized water (DDI) (90:10) at a flow-rate of 1.0 ml/min.

2.3. Sample preparation

Approximately 20 ml of acetonitrile is added to a weighed portion of a representative sample of bedding. The mixture is bath sonicated at 100% of full power for 5 min. The heterogeneous mixture is spun for 30 s on a mini-centrifuge. The supernatant liquid is quantitatively transferred to a reservoir which contains about 65 ml of distilled, deionized water. The aqueous sample is passed through a C_{18} chemically bonded sorbent which was conditioned for use with methanol prior to passage of the sample. The sorbent was washed with distilled, deionized water (DDI). The retained analyte is eluted twice with 500 μl portions of methylene chloride. Since methylene chloride is immiscible with the HPLC mobile phase, the eluent from SPE is evaporated to dryness then reconstituted with acetonitrile to a precise final extract volume of 2 ml. 10 μl of this extract is injected via autosampler into the liquid chromatograph. The RP-HPLC–UV was previously optimized to retain DBA.

The conventional method utilizing 24 h Soxhlet extraction, extract acid–base back extractions, concentration of the extract via rotary evaporation and determination via cGC–FID resulted in a thick, red-orange colored gel. Spiking the bedding with DBA resulted in a 0% recovery and no further attempt was made to isolate and recover DBA using Soxhlet extraction.

3. Results and discussion

DBA is easily retained and detected using either

cGC–FID or RP-HPLC with UV absorption detection. Our initial approach was to use cGC–FID but we were not satisfied with the instrument detection limits (IDLs). We then considered HPLC–UV and realized a ten-fold increase in IDLs and proceed to construct a four-point calibration which covered the range of concentrations from 4 to 20 ppm. We anticipated that the residue level of DBA in the bedding would be quite low so we narrowed the range of concentration for calibration from 1 to 5 ppm. A series of standards prepared by dilution of a stock solution containing DBA dissolved in methanol was used to establish the calibration curve. The curve is quite linear within this range of DBA concentrations with a correlation coefficient of 0.9986. A mobile phase of relatively high solvent strength composed of acetonitrile–DDI (90:10) was used. Since our analytical objective was to measure DBA, a mobile phase with high solvent strength enabled the capacity factor k' to fall between the 2 and 10 criteria which is deemed optimal for HPLC [7].

We proceeded initially with a conventional LLE approach which consisted of a 24 h Soxhlet extraction with methylene chloride followed by an alkaline–aqueous extraction using 1 M NaOH to remove phenolics. The organic phase was then extracted with 1.25 M sulfuric acid to remove N-

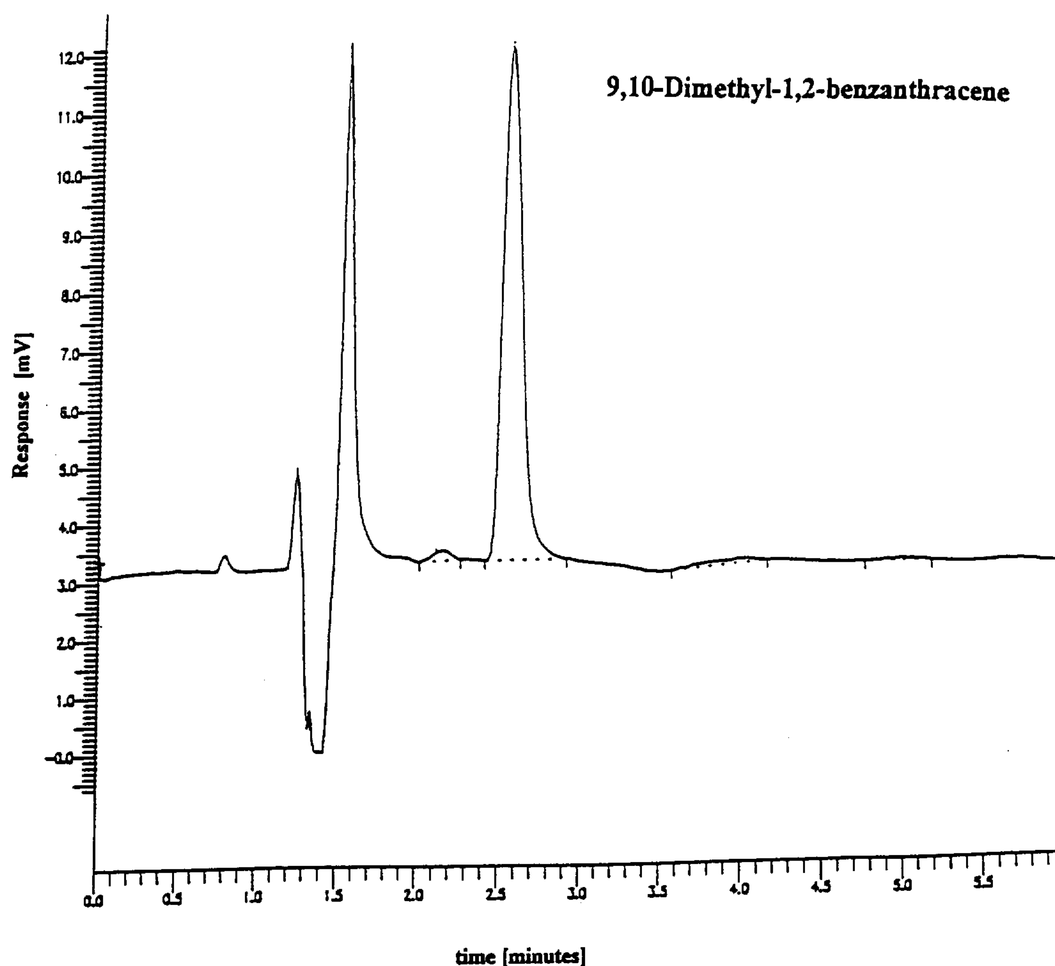

Fig. 1. RP-HPLC–UV chromatogram for the recovery of 9,10-dimethyl-1,2-benzanthracene from a spiked blank using bath sonication/RP-SPE. Mobile phase: acetonitrile–DDI (90:10); column: 150×4.6 mm Adsorbosphere, 3 μm, C_{18} (Alltech); detection at 254 nm.

heterocycles. The remaining organic phase is dried with anhydrous sodium sulfate. This procedure is satisfactory for the solvent spiked with DBA but resulted in a gelatinous material when applied to 10 g of bedding. This finding reflects perhaps the lipophilic fat and oil content which has been concentrated into the methylene chloride extractant. In other words, the application of a conventional LLE approach did not prove to be fruitful. We abandoned this sample preparation method in favor of approaching the problem from the perspective of RP-SPE.

Fig. 1 shows a HPLC chromatogram obtained from the addition of 100 μl of a methanolic solution containing DBA at a concentration of 208 ppm to 20 ml of acetonitrile. This "spiked blank" was then taken through the SPE procedure as discussed previously. The peak at 2.56 min corresponds to DBA. Fig. 2 is a HPLC chromatogram obtained from spiking the dry bedding with DBA prior to adding the acetonitrile. It is evident from the relatively large peak which is present in the sample at about 2.1 min perhaps corresponds to a PAH metabolite which might be present and might be considered as more polar than DBA. The identity of this earlier eluting peak was not pursued. It is evident that the sample contains not only the metabolite but the analyte and

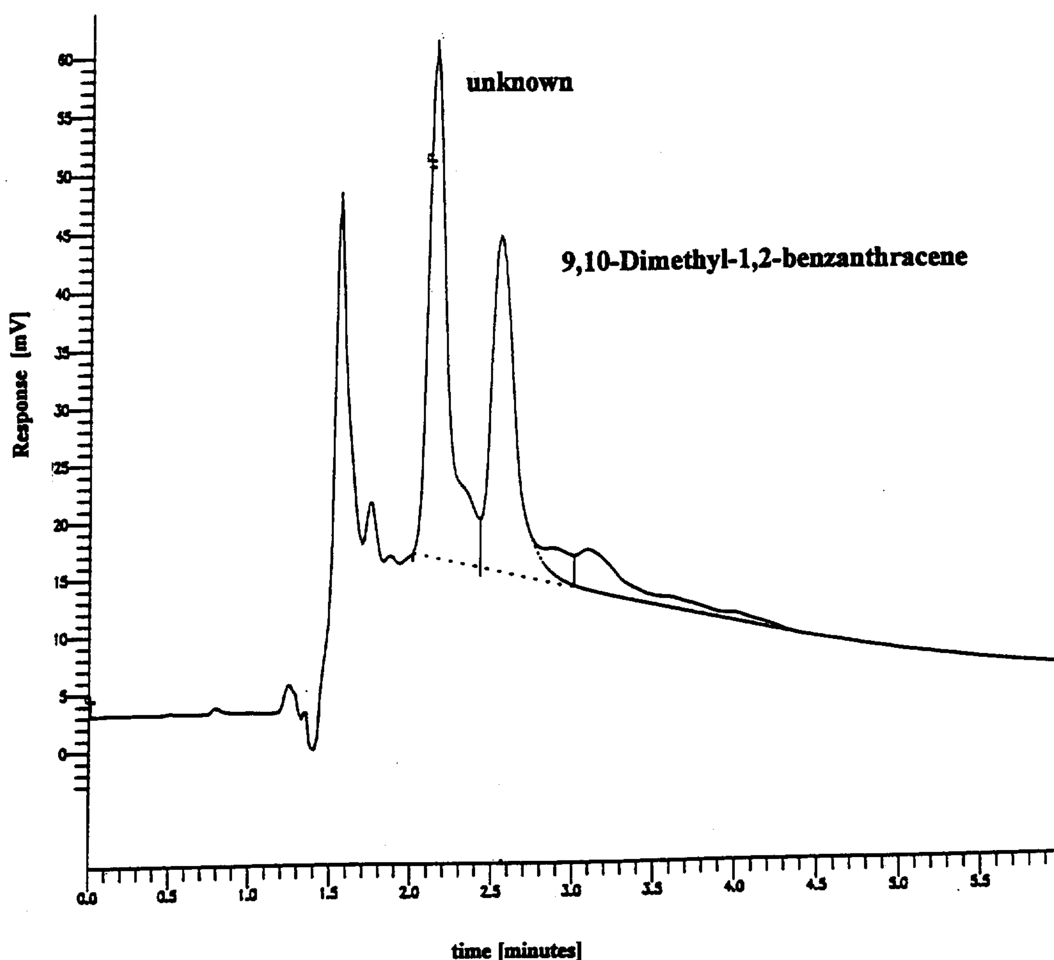

Fig. 2. RP-HPLC–UV chromatogram for the recovery of 9,10-dimethyl-1,2-benzanthracene from a spiked bedding sample using bath sonication/RP-SPE. Mobile phase: acetonitrile–DDI (90:10); column: 150×4.6 mm Adsorbosphere, 3 μm, C_{18} (Alltech); detection at 254 nm.

Table 1
Analytical results for the determination of 9,10-dimethyl-1,2-benzanthracene in unspiked animal bedding

Sample	Mass (g)	Extract concentration (μg/ml)[a]	Concentration in sample (μg/g)
S-1	3.16	5.1	3.2
S-2	2.51	4.4	3.5
S-3	2.88	4.3	3.0

[a] Volume of extract is 2 ml.

that the chromatogram from the sample is a relatively clean one despite given the complex nature of the sample matrix. Duplicate spiked blanks gave recoveries of 127 and 100%, respectively while for the spiked sample, a recovery of 89% was obtained. Three portions of the unspiked bedding were then taken and DBA was isolated and recovered using the combined bath sonication/RP-SPE technique. Analytical results for these unspiked bedding samples are shown in Table 1. These results reflect the actual DBA residue level that was found in the bedding. Approximately equal numbers of rat feces were taken and included in the mass reported for each of the samples. The precision in the result among the three samples of about 2 lb (1 lb=0.45359237 kg) of bedding material is very good.

4. Conclusion

A preliminary study has demonstrated that a high recovery of the toxic DBA from animal bedding is possible using the combination of liquid–solid bath sonication/extraction using acetonitrile and RP-SPE with the determinative step accomplished by RP-HPLC with UV absorption detection.

Acknowledgments

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References

[1] Material Safety and Data Sheet obtained from Sigma, St. Louis, MO.
[2] Gas Chromatography–Mass Spectrometry for Semivolatile Organics: Capillary Column Technique, Method 8270, Test Methods for Evaluating Solid Wastes, SW-846, Office of Solid Waste, EPA, Washington, DC, 3rd ed., 1986.
[3] Polynuclear Aromatic Hydrocarbons, Method 610, Fed. Reg., Vol. 44, No. 223, December 3 (1979).
[4] M. Dong, J. Duggan and S. Stefanou, LC·GC, 11 (1993) 802–810.
[5] P.R. Loconto, LC·GC, 9 (1991) 460–465 and 752–760.
[6] P.R. Loconto, presented at FACSS 1993, (Federation of Analytical Chemistry and Spectrocopy Societies) Detroit, MI, 1993, Abstract 467.
[7] L. Snyder, J. Glajch and J. Kirkland, Practical HPLC Method Development, Wiley, 1988, pp. 27–30.