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Size-exclusion chromatography on zeolites in the trace analysis of polyaromatic hydrocarbons and organochlorine pesticides

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ABSTRACT

In analysis of polyaromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs) difficulties can arise with interference from naturally occurring compounds which behave chromatographically similarly to the target analytes. In this paper we report that much of this material (approximately 98%) can be removed simply and rapidly by passing the isolate through a short column of powdered zeolite ZSM-5. No losses of PAHs or OCPs were discernible in this procedure, which provides substantial improvements in terms of analytical performance.

INTRODUCTION

In some circumstances the analysis of polyaromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs) can be confounded by the presence in isolates of long-chain oxygen-containing material which behaves chromatographically in a manner similar to the target analytes [1]. This difficulty is exacerbated in situations where the concentrations of target analytes are very low and the burden of interfering material is high, for example in biota such as oysters. To some extent the interference can be overcome by the use of size-exclusion chromatography (SEC) using gels, *i.e.* gel permeation chromatography (GPC) [2–5] and the use of selective detector systems such as in GC–MS, GC–electron-capture detection, and HPLC–ultraviolet fluorescence detection (UVF) in the final analysis stage. A disadvantage of the GPC step is that it is slow and requires the use of comparatively large volumes of solvent with the attendant risk of contamination [5,6]. In the present paper we describe a simple and rapid procedure involving SEC using a commercially available zeolite, which while not affecting the target PAH and OCP analytes which are excluded from the zeolite pores, removes most of the interfering material.

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EXPERIMENTAL

Materials

Analytical-grade *n*-pentane, dichloromethane, *n*-hexane and benzene all purchased from Ajax (Sydney, Australia), were purified by fractional distillation. Water obtained with a Millipore Milli-Q ultrapure water system fitted with an organic free kit was further purified by refluxing with potassium permanganate (approximately 0.01%, w/w) followed by fractional distillation.

Analytical-grade potassium hydroxide was finely ground, washed with dichloromethane, dried, then stored in a desiccator. Laboratory-grade anhydrous magnesium sulphate was heated to 650°C for 1 h prior to use. Both were purchased from May & Baker (Melbourne, Australia).

Ajax laboratory-reagent basic alumina (100

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mesh) was extracted with purified dichloromethane using a Soxhlet apparatus, air dried, then activated at 150°C overnight. The alumina was deactivated by the addition of purified water (1.5%, w/w) immediately before use.

ZSM-5 powder was obtained from Conteka (Delfzijl, Netherlands) as CBV 2802, a low aluminium content material (SiO_2:Al_2O_3 = 280:1) in the H^+ form. This zeolite was heated at 500°C in air for 2 h to yield organic-free material.

Standards

The PAH standard reference mixture M-610A was obtained from AccuStandard (New Haven, CT, USA). This mixture contains each of the sixteen PAHs designated as priority pollutants by the United States Environmental Protection Agency dissolved in 1 ml dichloromethane–methanol (1:1) at concentrations ranging from 0.1 to 2.0 mg ml^{-1}. This 1-ml sample was diluted to 50 ml with *n*-hexane to yield a solution ranging in concentrations of individual PAHS from 2 to 40 ng μl^{-1}.

The deuterated phenanthrene standard was purchased from the US National Institute of Standards and Technology as a solution (1.5 ml) containing 5 mg ml^{-1} [$^2H_{10}$]phenanthrene. This was diluted to 100 ml with *n*-hexane to yield a solution of 75 ng μl^{-1}

1,8-Dimethylnaphthalene (1,8-DMN) was synthesised from 1,8-naphthalic anhydride (Fluka, Buchs, Switzerland) as described by Mitchell *et al.* [7]. A standard solution of 1,8-DMN (20 mg) in 100 ml *n*-hexane (200 ng ml^{-1}) was similarly prepared.

The OCPs aldrin, dieldrin, α-chlordane, γ-chlordane, *p,p*-DDT and heptachlor were purchased from PolyScience (Evanston, IL, USA) and were similarly diluted to prepare a standard solution comprising each of the 6 OCPs from 3.2 to 18.5 ng μl^{-1}.

Isolation of PAH fractions from oysters

Approximately 100 *g* of oyster tissue (wet mass) was blended with purified water (60 ml) and potassium hydroxide (8 g) until a homogeneous mixture was obtained. The internal standard 1,8-DMN (400 ng) was added and the mixture refluxed under a nitrogen atmosphere for 4 h. After cooling, the digest was acidified to pH 3 with 3 *M* hydrochloric acid, then extracted with four successive portions of dichloromethane (4 × 50 ml). Where necessary, the resulting emulsion was broken by the addition of sodium chloride (*ca.* 2 g). The combined organic extract was washed with purified water (50 ml), back-extracted with a 4% potassium hydroxide solution (3 × 50 ml) then finally washed with purified water (2 × 50 ml). The organic extract was dried with anhydrous magnesium sulphate and filtered. The solvent was evaporated to leave a residue of approximately 5 ml which was further concentrated to approximately 250 μl using a Kuderna–Danish apparatus. This residue was sorbed onto deactivated basic alumina (0.5 g) and the solvent removed in a gentle stream of nitrogen. The alumina was transferred to the head of a column of basic alumina (6 g) deactivated with 1.5% (w/w) water and a fraction containing the saturated hydrocarbons and PAHs was collected by eluting the column with benzene–*n*-pentane (30:70) (50 ml). The solvent was removed as before, then the residue was dissolved in approximately 100 μl of *n*-pentane. Medium-pressure liquid chromatography (MPLC) was performed using a Merck LiChroprep Si 60 (40–63 μm) column attached to a Waters Millipore Model 510 double piston pump and a Model 440 ultraviolet absorbence detector (254 nm). The PAH fraction was collected between 6 and 20 min using a flow-rate of 4 ml min^{-1} of dichloromethane–*n*-hexane (5:95). The volume of eluate was reduced to 100 μl as described above.

ZSM-5 liquid chromatography

ZSM-5 powder (1 g) was dry packed into a Pasteur pipette which had been plugged at one end with a wad of cotton wool which had previously been washed with purified dichloromethane.

The behaviour of organochlorine pesticides was evaluated by adding a 10-μl aliquot of the mixture of pesticides containing 32–185 ng of individual OCPs to 100 μl *n*-pentane and placing this solution onto the column. This solution remained in contact with the ZSM-5 for 10 min after which the column was eluted with *n*-pentane. A fraction collected between 0 and 5 ml was concentrated to 100 μl and a 3-μl aliquot (225 ng) of the deuterated phenanthrene solution was added as a normalisation standard. The fraction was analysed by GC–MS and found to contain all of the pesticides in the mixture by comparison with an identical untreated mixture

spiked with the normalisation standard. The procedure was repeated by applying a 1-μl aliquot (2–40 ng) of the diluted M-610A mixture and spiking with a 1 μl (75 ng) aliquot of normalisation standard. Again, analysis by GC–MS showed the first 5 ml of pentane contained the PAHs.

The procedure was repeated using a PAH isolate from oyster tissue spiked with a 1 μl aliquot of the M-610A diluted solution. Analysis by GC–MS using the selected ion monitoring (SIM) mode yielded results which were identical to those obtained from analysis of the standard mixture in the absence of the oyster matrix. Another oyster tissue PAH isolate was spiked with a 2 μl aliquot (400 ng) of the 1,8-DMN as an internal standard. The isolate was divided into two with one half subjected to ZSM-5 liquid chromatography and the other remaining untreated. To each portion an aliquot (2 μl, 150 ng) of the deuterated phenanthrene solution was added as a normalisation standard prior to analysis by GC–flame ionisation detection.

Gas chromatography

Capillary GC was carried out on a 1 μl aliquot using a Varian Model 3500 gas chromatograph equipped with an on-column injector (OCI-3, SGE, Australia) and a flame ionisation detector. A 25 m × 0.22 mm I.D. cross-linked methylsilicone fused-silica column (BP 1, SGE, Australia) was used with a temperature programme of 50 to 300°C at 4°C min^{-1} then held isothermally for 15 min. Hydrogen was the carrier gas at a linear flow velocity of 40 cm s^{-1}.

Gas chromatography–mass spectrometry

GC–MS analysis was performed using a Hewlett-Packard (HP) 5970 mass-selective detector with a HP RTE/A data system and a HP 5890A gas chromatograph, fitted with a 60 m × 0.25 mm I.D. fused-silica column coated with crossed-linked 5% phenylmethylsilicone (DB-5, J&W Scientific, USA). In a typical analysis, helium was used as a carrier gas at a linear flow velocity of 30 cm s^{-1}. With the transfer line temperature held at 280°C, a 1 μl aliquot of the sample was injected into a HP on-column injection system using a HP 7673A automatic liquid sampler. The oven temperature was programmed from 70 to 300°C at 3°C min^{-1} then held isothermally at 300°C for 20 min. For the analysis of OCPs, the mass-selective detector was operated in the total ion monitoring mode with an electron ionising voltage of 70 eV. The detector was operated in the SIM mode for PAH analysis where the parent ion for each of the PAHs present in M-610A was monitored with a dwell time of 50 ms.

RESULTS AND DISCUSSION

PAH fractions were isolated from tissue of oysters collected from comparatively uncontaminated sites. Similar methods have been published previously [8], and an improved version was reported in 1988 [9]. A recent modification to our procedure has been to employ the alumina filtration step only to remove large quantities of interfering biogenic lipids and to defer the isolation of separate saturate and aromatic fractions until the silica gel MPLC stage.

Typically, GC traces similar to that shown in Fig. 1a were obtained [9]. This chromatogram is dominated by peaks with retention times between 25 and 35 min, revealing the presence of non-target analytes in amounts of approximately ten to several hundred nanograms. In view of the fact that in a typical analysis, target analytes may only be present in sub-nanogram quantities, the presence of these compounds represents a potentially serious source of interference.

After a pentane solution of this PAH isolate had been passed through a short column of ZSM-5 loosely packed in a Pasteur pipette, the gas chromatogram of the eluent, measured at both the same and at × 50 sensitivity was as shown in Fig. 1b (note the height of the internal standard 1,8-DMN). Comparing the traces in Fig. 1a and b it is clear that each of the major peaks of the trace in Fig. 1a has been removed or very substantially diminished. On the basis of total integral counts, approximately 98% of the material is sorbed by the zeolite. In fact, measured at the sensitivity used to obtain the chromatogram shown in Fig. 1a, the purified PAH sample showed an almost smooth baseline.

Recoveries of polyaromatic hydrocarbons (PAHs)

Recoveries of PAHs were estimated by spiking the PAH isolate with a portion of a commercial PAH standard mixture (M-610A) containing 2–40 ng of sixteen individual PAHs. The sample was ex-

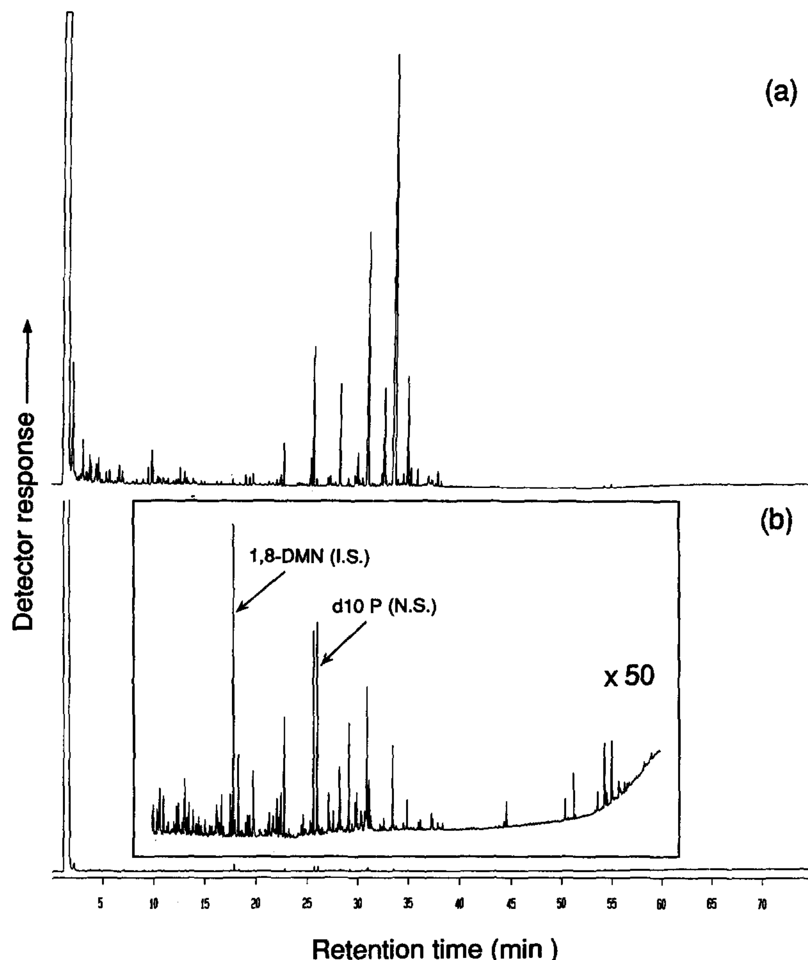

Fig. 1. Capillary gas chromatograms of a PAH isolate from oyster tissue (a) before and (b) after treatment with ZSM-5. The inset in (b) shows the chromatogram after treatment with ZSM-5 at a fifty-fold increase in sensitivity. Labelled peaks are: 1,8-DMN (I.S.) = 1,8-dimethylnaphthalene internal standard; d10 P (N.S.) = $[^{2}H_{10}]$phenanthrene normalisation standard.

amined by GC–MS before and after the treatment comparing peak areas relative to the normalisation standard in each case. These analyses were performed in triplicate and indicated recoveries of 98–104%.

Recoveries of organochlorine pesticides (OCPs)

Similar recovery trials to those described above were conducted by preparing a pentane solution containing 32–185 ng of the OCPs heptachlor, aldrin, γ-chlordane, α-chlordane, dieldrin and DDT and passing it through the ZSM-5 column. Analysis by GC–MS indicated that the recoveries of each of these compounds was in the range 98–104%. This is an encouraging observation, indicating that the zeolite treatment procedure may be a useful prospect for analysis of this group of compounds.

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