Journal of Chromatography, 241 (1982) 419–422
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,800

Note

Efficient clean-up of fat samples by Sep-Pak cartridges for polybrominated biphenyl analysis

L. ALICE HU*, G. A. S. ANSARI, MARY TREINEN MOSLEN and EDWARD S. REYNOLDS
Chemical Pathology Laboratory, The University of Texas Medical Branch, Galveston, TX 77550 (U.S.A.)
(Received January 26th, 1982)

Measurement of polybrominated biphenyl (PBB) contamination in fat samples from humans or animals by gas chromatography generally involves preliminary column chromatography on Florisil for removal of neutral fat[1–4]. This clean-up step is time consuming and requires large volumes of solvents, but in the end allows the PBBs in the sample to be concentrated. We have found that sequential extraction of fat samples through Florisil and C_{18} disposable cartridges (Sep-Pak) provides a rapid and efficient method to remove neutral fat prior to PBB analysis.

EXPERIMENTAL

Materials

All solvents were pesticide grade (Burdick and Jackson, Muskegon, MI, U.S.A.) [^{14}C]2,4,5,2′,4′,5′-hexabromobiphenyl (HBB) (10.2 mCi/mmole) of 98+% purity was obtained from New England Nuclear, Boston, MA, U.S.A. Aldrin (99% purity) was obtained from Analabs, North Haven, CT, U.S.A. Florisil and C_{18} Sep-Pak cartridges were obtained from Waters Assoc., Milford, MA, U.S.A. Human fat samples were obtained at autopsy.

Fat preparation

Fat samples containing 10 or 1000 ppb* were prepared by combining 0.5 g fat with 5.0 or 500 ng [^{14}C]HBB. A 5-ml volume of hexane and 0.5 g anhydrous Na_2SO_4 were added and the mixture was homogenized for 5 min. The hexane layer was removed and the residue reextracted by homogenization two more times with hexane (5 ml each time). The hexane fractions were pooled and evaporated to dryness under vacuum at room temperature.

Sep-Pak chromatography

Dried fat was dissolved in 1 ml of acetonitrile or hexane containing 0.1 μg aldrin as an internal standard for C_{18} or Florisil Sep-Pak chromatography, respectively. The dissolved sample was placed on the Sep-Pak cartridge with a syringe. The cartridge was eluted with 40 ml of additional acetonitrile or 10 ml of hexane. Preliminary

* Throughout this article, the American billion (10^9) and trillion (10^{12}) is meant.

0021-9673/82/0000–0000/$02.75

studies of the needed elution volume of C_{18} and Florisil Sep-Pak cartridges demonstrated that the [^{14}C]HBB radioactivity was eluted with the first 40 ml of acetonitrile or the first 10 ml of hexane.

If the fat was to be chromatographed through a second Sep-Pak cartridge, the eluate was dried under vacuum at room temperature, the residue redissolved in 1 ml of the appropriate solvent, placed on the second cartridge and eluted with 40 ml of acetonitrile or 10 ml of hexane.

[^{14}C]HBB and fat recoveries

The final Sep-Pak eluate was dried under vacuum at room temperature in a tared vial and weighed to determine fat recovery. [^{14}C]HBB recovery was determined by dissolving the residue in 1 ml hexane, suspending an aliquot in Scintiverse (Fisher, Fair Lawn, NJ, U.S.A.) and measuring the radioactivity levels with a Packard Mark III liquid scintillation counter (Searle Analytics, Des Plains, IL, U.S.A.). Quench correction was made according to an external standard. Aliquots of the suspended residue were also analyzed by gas chromatography.

Gas chromatography

Gas chromatography was carried out on a Varian Model 3700 equipped with a ^{63}Ni electron-capture detector, a Varian CDS 111 chromatography data system and a fused-silica capillary column (30 m × 0.25 mm) coated with 0.25 μm DB-1 phase (J & W Scientific, Rancho Cordova, CA, U.S.A.). Nitrogen carrier flow-rate was 1 ml/min, and make-up rate was 20 ml/min. Split ratio was 1:100. Injector temperature was 270°C. Detector temperature was 350°C. Oven temperature was programmed to increase from 230°C to 300°C at a rate of 1°C/min.

RESULTS AND DISCUSSION

Recovery of [^{14}C]HBB after homogenization and hexane extraction averaged 98%. As detailed in Table I, fat removal was four times higher with C_{18} Sep-Pak elution than with Florisil Sep-Pak elution. Sequential elution through both types of Sep-Pak cartridges in either sequence removed all but 4 to 6% of the fat. Recoveries of 1000 ppb [^{14}C]HBB after single or sequential Sep-Pak clean-up ranged from 96 and 99% (Table I). [^{14}C]HBB recoveries from samples containing 10 ppb HBB were similarly high and ranged from 97 to 99%.

TABLE I

RECOVERY OF 1000 ppb [^{14}C]HBB AND FAT FOLLOWING SEP-PAK CHROMATOGRAPHY

Extraction	*[^{14}C]PBB recovery (%)*	*Fat recovery (%)*
C_{18}	96.5 ± 0.8*	19.8 ± 0.6
Florisil	99.7 ± 0.3	78.2 ± 1.0
C_{18}–Florisil	96.3 ± 0.4	3.1 ± 0.3
Florisil–C_{18}	97.2 ± 1.1	5.5 ± 0.4

* Mean ± S.E.M. of 3–5 determinations.

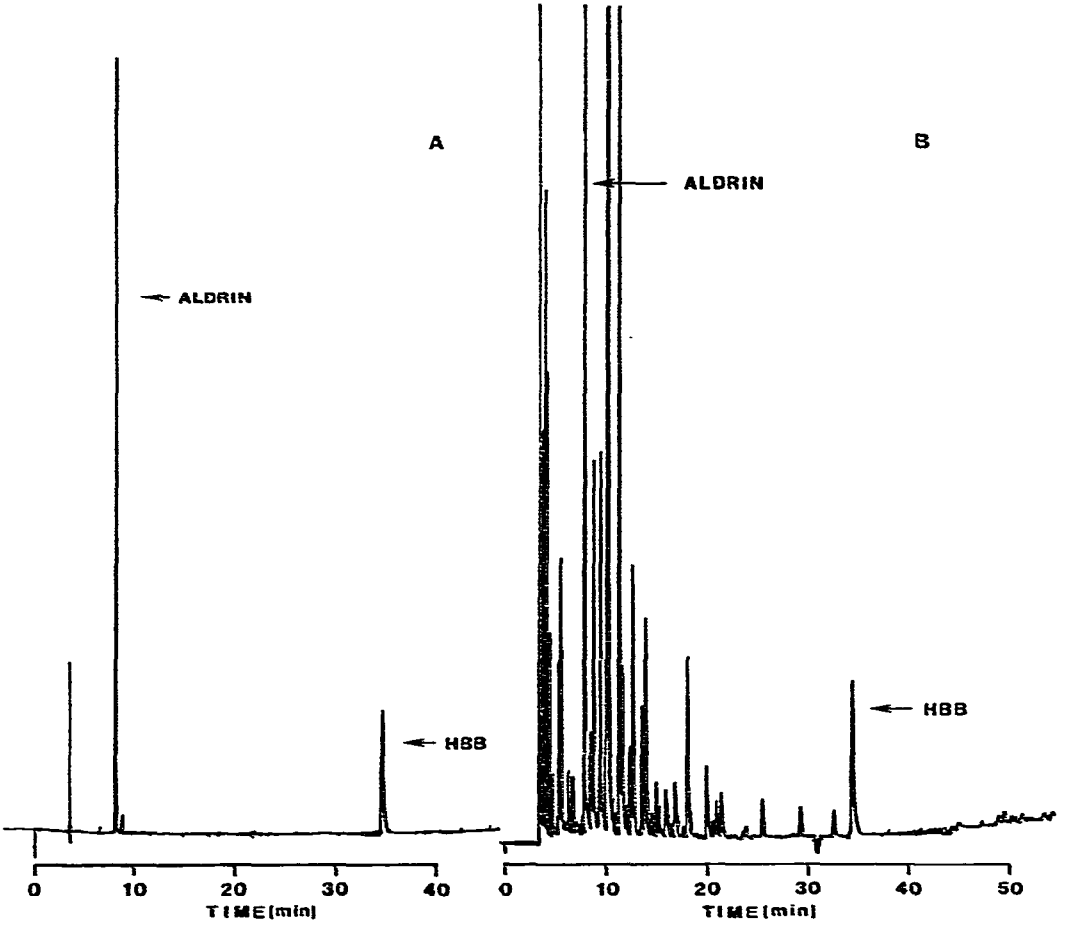

Fig. 1. Gas chromatograms of HBB and aldrin: A, Sep-Pak eluate of standard mixture of 100 ng aldrin and 30 ng HBB in 1 ml hexane; B, Sep-Pak eluate of human body fat (0.5 g) plus 100 ng aldrin and 30 ng HBB in 1 ml hexane.

The sequential Sep-Pak elution procedure provided a clean extract as illustrated by the relatively flat base lines of the chromatograms in Fig. 1A and B. In other studies to be described elsewhere[5] we have identified some of the other peaks in the human fat sample (Fig. 1B) as polyhalogenated aromatics.

In conclusion, sequential elution through C_{18} and Florisil Sep-Pak cartridges provides a convenient and rapid method to clean-up fat samples prior to gas chromatographic analysis of PBB contents. The achieved fat removal of 94% and HBB recovery of 96% are equivalent or better than that achieved with other methods[1–3]. The method is equally efficient for samples containing low (10 ppb) levels such as have been found in eagle carcasses in multiple states[4] or for samples containing higher (1000 ppb) levels such as were found in PBB exposed workers[3]. We estimate that fat samples could be concentrated using this efficient clean-up procedure to allow detection of PBBs at ppt levels.

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