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Note

Hexachlorophene analysis in blood by electron capture gas chromatography

Hexachlorophene has been used as an anti-bacterial agent for many years but until recently satisfactory methods for the determination of hexachlorophene in blood have not been available. The application of electron capture gas chromatography (GC) has permitted development of sensitive methods for the analysis of hexachlorophene in tissues, urine and blood.

Although BACHMANN AND SHETLAR[1] described a method which did not include cleanup of the sample or derivative formation, other methods have employed preparation of a diacetyl derivative (BROWNING *et al.*[2]) and cleanup followed by silylation (PORCARO *et al.*[3]). A sensitive method involving formation of a methyl derivative and cleanup on silica gel micro columns has been described by CURLEY AND HAWK[4]. This method was used by CURLEY *et al.*[5] when they investigated the dermal absorption of hexachlorophene in infants.

We have also examined the blood levels in infants washed with hexachlorophene under two different regimens (ABBOTT *et al.*[6]). The method described here was used in the investigation and it is a modification of that reported by CURLEY AND HAWK[4].

Experimental

Reagents. The following reagents were used: diethyl ether, reagent grade, redistilled; hexane, reagent grade, redistilled over KOH; concentrated H_2SO_4, A.R. grade; Na_2SO_4, reagent grade; diazomethane, ethereal solution prepared from Diazald according to the manufacturer's instructions.

Procedure. All glassware was rinsed with hexane before use to remove any possible contamination which might interfere with the GC analysis.

One millilitre or less of heparinized whole blood, serum, or plasma was placed in a 40-ml glass stoppered centrifuge tube and 3 ml of 0.1 *M* citrate buffer, pH 5, added. Diethyl ether, 10 ml, was then added and the tubes were shaken for 10 min on a Vortex mixer modified to hold four tubes. After centrifuging to separate the phases, the ether was transferred to a 20-ml glass-stoppered tube and the extraction repeated.

The ether extracts were evaporated to less than 1 ml on a 35° water-bath by a stream of dry air. One millilitre of ethereal diazomethane was added and the samples mixed on a Vortex mixer. After leaving for 15 min the ether was evaporated just to dryness. Ten millilitres of hexane were added and the tubes shaken to dissolve the methylated hexachlorophene. Concentrated H_2SO_4, 1 ml, was added and the tubes shaken for 1 min. After phase separation an aliquot was transferred to another tube and shaken with 5 ml of saturated Na_2SO_4 (extracted with hexane before use) for 1 min. An aliquot of the hexane was transferred to another tube and concentrated to an appropriate volume for GC analysis.

Analyses were carried out on a Varian 2100 gas chromatograph fitted with tritium electron capture detectors. The column was a U-shaped Pyrex column 0.5 m × 2.5 mm I.D., packed with 5 % QF-1 on 80–100 mesh Varaport 30. Operating conditions were: injector temperature, 220°; column temperature, 185°; detector block temperature, 276°; nitrogen carrier gas flow-rate, 40 ml/min. The retention time of the methylated derivative was 4.5 min.

Recoveries for the method were determined by adding known amounts of hexachlorophene in small volumes of ethanol to heparinized whole blood, serum, and plasma.

Results and discussion

The recoveries of hexachlorophene added to whole blood, serum or plasma were in the range 90–100 % for samples fortified at levels of 0.01 μg/ml to 1.00 μg/ml. The addition of citrate buffer greatly enhanced the extraction of hexachlorophene. Recoveries as low as 30 % were obtained when citrate buffer was not present. Citrate buffer was found to be more satisfactory than phosphate buffer, pH 7.4, in improving the extraction. Caution must be exercised when adding ethanolic standards of hexachlorophene as too large a volume of ethanol can reduce extraction efficiency. Volumes of less than 0.05 ml did not appear to cause any reduction.

Methylation of the extracted hexachlorophene was apparently instantaneous, but samples were usually left for at least 15 min for convenience. The methylated hexachlorophene was unaffected by contact with concentrated acid during the cleanup step. No change was observed in samples left in contact with acid for periods up to 24 h.

Methylation greatly improved the chromatographic response of hexachloro-

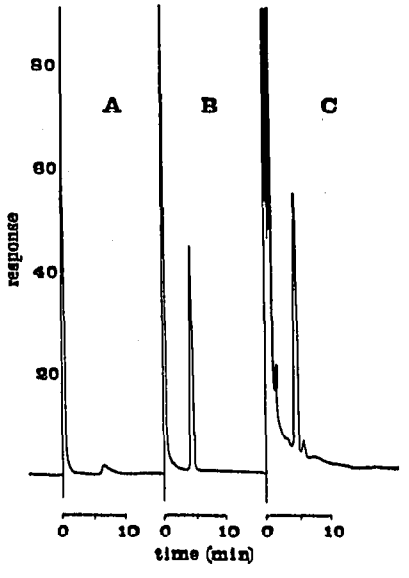

Fig. 1. GC traces of hexachlorophene. (A) 0.25 ng of non-methylated hexachlorophene; (B) 0.1 ng of methylated hexachlorophene; (C) extract of whole blood containing 0.05 μg/ml hexachlorophene.

phene as shown by the chromatograms in Fig. 1. The improved response and clean samples meant that low levels of hexachlorophene could be estimated with ease. The GC response was linear over the range 0.005–0.200 ng of methylated hexachlorophene injected. Injection of 0.005 ng gave a peak height equivalent to 10 cm.

Conclusion

This method has proved to be satisfactory for our investigations into the dermal absorption of hexachlorophene in infants. It is very sensitive, gives quantitative recoveries and requires only small volumes of blood.

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