Keyphrases

Drug accumulation—pharmacokinetics
Pharmacokinetic equations—drug accumulation
Accumulation plateau—drug, multiple doses

Gas Chromatographic Determination of Hexachlorophene in Blood and Urine

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A rapid and convenient gas chromatographic technique has been developed for the determination of traces of hexachlorophene in human blood and urine. After a simple extraction, the hexachlorophene is acetylated. The acetyl derivative chromatographs well on conventional columns at a temperature within the operating range of a tritium foil electron capture detector.

ALTHOUGH HEXACHLOROPHENE (2,2′-methylene bis(3,4,6-trichlorophenol) is widely used in cosmetic preparations and pharmaceuticals, few methods are available for the determination of very low levels in blood or other tissue. Johnston and Porcaro (1) have described a method in which the hexachlorophene, after a relatively complex extraction procedure, is measured by colorimetry with 4-amino antipyrine as reagent. Using their method, as little as 20 mcg. of hexachlorophene can be measured in up to 100 g. of tissue. Wit and Van Genderen (2) used a similar technique in studying the recovery of hexachlorophene from the urine, feces, and milk of cattle dosed with the drug. Recovery data were reported for samples of various sizes, from a fraction of a gram for feces to 25 ml. for milk, containing 10–110 mcg. of drug. In another part of the same study, radiochemical assay methods were used to determine hexachlorophene in the excreta of rats and rabbits dosed with labeled drug.

Porcaro (3) has also described a gas chromatographic procedure for the determination of various bisphenols, including hexachlorophene. The minimum measurable quantity was claimed to be 5–10 mcg. He mentioned, but did not investigate, the potential increase in sensitivity which could result from the use of an electron capture detector. More recently, Wisniewski (4) has reported a gas chromatographic method for the determination of hexachlorophene in soap. In contrast to Porcaro's approach, in which the unmodified phenol was injected on a short, specialized column with a relatively high liquid loading, Wisniewski first prepared the trimethylsilyl derivative in order to reduce tailing and sample loss on the more conventional column he used. He, too, used a flame-ionization detector.

On columns available to the authors, using temperatures and liquid phases compatible with the use of a tritium foil electron capture detector, direct injection of hexachlorophene was unsatisfactory. Instrument response to samples in the subnanogram range was erratic. This variability was largely explained when it was definitely established that fractional amounts of hexachlorophene samples were held in the instrument. Derivatives which could eliminate this problem were therefore investigated. In this laboratory, silyl derivatives were not wholly satisfactory. Detector response at low levels continued to be somewhat erratic, and the retention times for the derivatives were undesirably long. Acetylation, however, proved to be rapid, simple, and reproducible, and the diacetyl derivative chromatographed reliably on several substrates, including a silicone gum (SE-30), a silicone oil (OV-17), and an organosilicone polymer (EGSP-Z).

On the basis of these results, a sensitive gas chromatographic method for the determination of hexachlorophene in extracts from whole blood and urine has now been developed. Using the procedure described here, it is easy to measure hexachlorophene at levels down to 0.05 mcg./ml.

EXPERIMENTAL

Reagents—USP grade hexachlorophene obtained from Winthrop Laboratories was used without further purification. All other materials were reagent grade, obtained from recognized suppliers.

Apparatus—All the glassware used was cleaned before use by soaking in a 1:1 mixture of 6 *N* HCl and methanol for about 0.5 hr., then rinsing with distilled water. Glassware was oven-dried. An F and M (Avondale, Pa.) model 400 gas chromatograph equipped with a tritium foil electron capture detector was used for the chromatography. The 121.9-cm. (4-ft.) long 0.635-cm. (0.25-in.) diameter column was packed with 1% EGSP-Z on 100/120 mesh Gas-Chrom Q (Applied Science Labs, Inc., State College, Pa.). The oven temperature was 205–210°,

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TABLE I—RECOVERY OF HEXACHLOROPHENE FROM BLOOD

Added, p.p.m.	Recovered, p.p.m.
0[a]	0.031
0[a]	0.006
0.050	0.036
0.060	0.051
0.100	0.079
0.120	0.108
0.200	0.162
0.240	0.192
0.300	0.236
0.360	0.238

[a] Blood blanks from two separate donors. All other tabulated values are corrected for the appropriate blank.

the detector temperature 215°, and the injection port temperature 275°. Helium was used as the carrier gas, and the pulse type detector was purged with 10% methane in argon. The pulse setting was 50 μsec.

Procedure—As soon as blood samples were drawn, they were well mixed with finely ground potassium oxalate, 0.7 mg./ml., to prevent clotting. Exactly 3 ml. of the oxalated whole blood was then transferred to a 15-ml. glass-stoppered centrifuge tube, 1 ml. of 0.4*M* pH 7.5 phosphate buffer added (the buffer was prepared by mixing 1 vol. of 0.4 *M* NaH_2PO_4 with 10 vol. of 0.4 *M* Na_2HPO_4), and the contents mixed well. The tube was then immersed at an angle, to prevent breakage, in a dry ice–acetone bath adjusted to about −20°, until the contents were solidly frozen. If the bath temperature is too low, or if tubes are supported vertically, some breakage may result. The tube contents were then allowed to thaw at room temperature. When the contents were liquid, exactly 10 ml. of ethyl acetate was added, the tube stoppered, and the contents mixed vigorously for 3 min. The tube was then centrifuged at moderate speed. Exactly 5 ml. of the clear ethyl acetate layer was transferred to a graduated 15-ml. tube, which was then immersed in a water bath held at 80°. The ethyl acetate was evaporated, under a stream of nitrogen, to 0.5–1.0 ml. The tube was then removed from the bath, 0.1 ml. of a 1:1 mixture of acetic anhydride and pyridine added, and the contents mixed well, after which the tube was placed in a second bath at 60° for 10 min. After removing from the bath, tube and contents were cooled to room temperature and the volume adjusted to 1.1 ml. with ethyl acetate. Aliquot portions of exactly 3 μl. of the well-mixed solution were then injected into the chromatograph.

Urine samples were handled similarly, but were not frozen.

Hexachlorophene standards, containing from 0 to 0.75 mcg. in 5-ml. portions of ethyl acetate, were evaporated to 0.5–1.0 ml. and treated in the same way as the sample extracts.

RESULTS

When amounts of hexachlorophene ranging from 0.05 to 0.36 p.p.m. were added to whole blood, the mean absolute recovery (least squares slope) was 81.6%. The recovery of similar amounts from urine was 84.3%. In no case was the relative standard deviation of the slope greater than 3.1%. The data for blood are reported in Table I and for urine in Table II. In each case the reported value, from which the recoveries above were calculated, is the average of duplicate assays. The estimate of the standard deviation of the assay pairs (blood) was 0.013. Variation in the volume of sample injected into the instrument is undoubtedly the major source of error, and this is not excessive. The relative standard deviation of four replicate injections was 2.3%.

TABLE II—RECOVERY OF HEXACHLOROPHENE FROM URINE

Added, p.p.m.	Recovered, p.p.m.
0	0
0.12	0.101
0.24	0.186
0.48	0.406

DISCUSSION

The peak found in the blank blood samples has not yet been positively identified, but all the present evidence indicates that it is, in fact, caused by hexachlorophene present in the samples. The retention times of hexachlorophene and the unknown peak correspond on both the EGSP-Z and on an SE-30 column, and in each case, before and after acetylation. No such peak was found in monkey, rabbit, or dog blood, nor in human urine.

The procedure has adequate accuracy at the level discussed. Greater precision can be achieved, if necessary, by running additional samples, without greatly increasing analytical time. The total elapsed time for an analysis is approximately 2 hr. Of this, the gas chromatographic step takes about 10 min.

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Keyphrases

Hexachlorophene analysis—blood, urine
GLC—analysis
Tritium foil electron capture detector—GLC analysis