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Determination of Chloral Hydrate and Trichloroethanol in Biological Tissue

A number of methods for the determination of chloral hydrate and trichloroethanol in biological tissue have been described [*1–5*]. The most recent of these procedures, that of Jain et al [*5*], involved direct injection of an aqueous sample on to a gas chromatographic (GC) column. Both chloral hydrate and trichloroethanol exhibited relatively broad peaks with the column used and the separation of the chloral hydrate and water was not always satisfactory.

A more convenient method is described below.

Experimental

Instrumentation

A Varian 1200 gas chromatograph (Varian Associates, Walnut Creek, Calif.) equipped with an electron capture detector (H^3 type) and a Varian 0–1 mV Model 20 recorder was employed. An 8-ft coiled aluminum (¼ in. outside diameter) column packed with 10 percent OV-225 on 80-100 mesh acid washed chromasorb W (Chromatographic Specialites Ltd.) was used with a glass liner in the injection port. The operating conditions were as follows:

Injection port temperature 170 C;

Column temperature 80 C for chloral hydrate alone, 112 C for chloral hydrate and trichloroethanol, and 130 C for trichloroethanol alone;

Detector temperature 220 C;

Carrier gas (nitrogen) flow rate 120 ml/min;

Vortex-Genie Mixer (Fisher Scientific), used for mixing the solutions.

Material

Reference standard aqueous solution of chloral hydrate (B.D.H.) and trichloroethanol (Aldrich Chemical Co.) were used for the preparation of calibration curves. Solutions of these compounds were also added to blood, liver, and urine for recovery studies. Diethyl ether (Burdick & Jackson Labs., Inc.) containing 2 percent ethanol was used for extraction A commercial buffer (pH 5.8) was used as a diluent (Micro Essential Labs., N.Y.).

Procedure

0.5 ml of a liquid sample (blood, urine, etc.) was diluted with 0.5 ml of buffer in a 15-ml centrifuge glass stoppered centrifuge tube and 1.0 ml of ether is added. If solid tissue is

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used 1.0 gm of tissue is homogenized with 1.0 ml of buffer and the resulting mixture, together with 1 ml of washings are transferred to a centrifuge tube and 2.0 ml of ether added. The tube is tightly stoppered and the mixture agitated on a Vortex mixer for 3 min before being centrifuged. One to three microliters of the ether layer are injected directly into the GC. The area of the peak obtained for either chloral hydrate or trichloroethanol is compared directly with a calibration curve made up by treating reference standard aqueous solutions in a similar manner.

Results and Discussion

Attempts at obtaining satisfactory separation of water, chloral hydrate, and trichloroethanol using an aluminum (¼ in. outside diameter) column of varying lengths with a liquid phase of 15 percent F.F.A.P. proved disappointing. It would appear that the ⅛ in. outside diameter column used by Jain et al [5] is essential if this liquid phase is to be used. In our laboratory ¼ in. outside diameter columns only, are used because column fabrication is much easier and column life is considerably longer than for an equivalent ⅛ in. outside diameter column.

The use of an 8 ft (¼ in. outside diameter) aluminum column packed with 10 percent OV-225 as the liquid phase gave good results with ether solutions of chloral hydrate and trichloroethanol, but on direct injection of aqueous solutions the water and chloral hydrate peaks were not resolved.

The extraction procedure using the Vortex mixer proved very convenient. The use of ether as the extracting solvent has disadvantages such as its solubility for water but consistent results were obtained and the calibration curves obtained are similar to those of Jain et al [5].

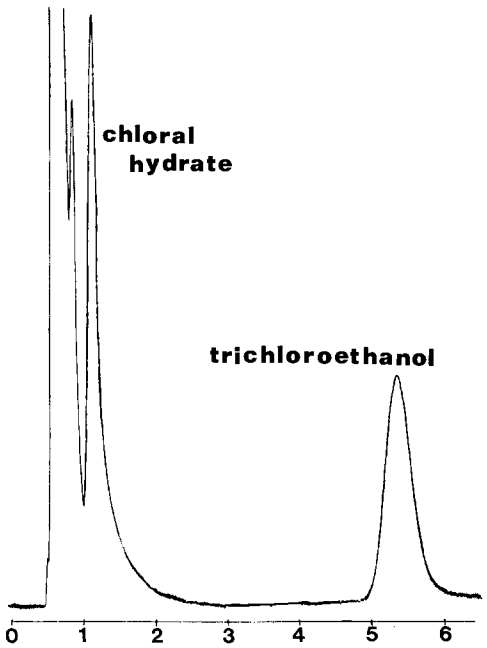

FIG. 1—*Ether extract of blood spiked with a mixture of chloral hydrate and trichloroethanol. Column temperature 112 C, retention time in minutes.*

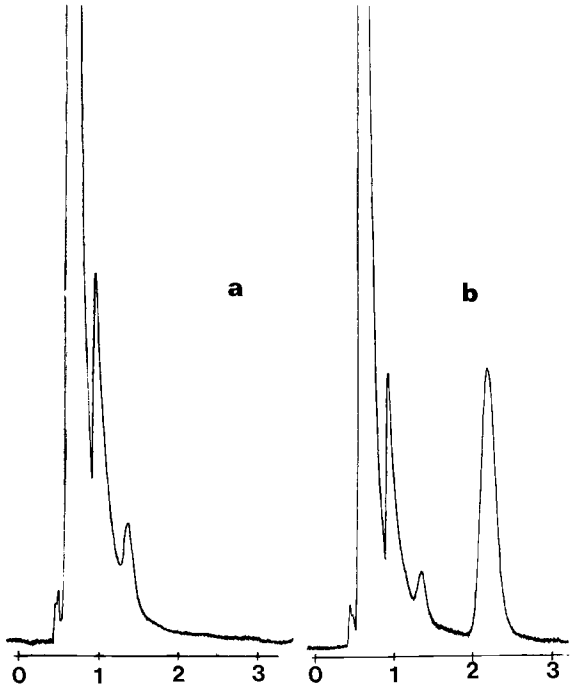

FIG. 2—(a) *Ether extract of blank blood;* (b) *Ether extract of blood spiked with chloral hydrate. Column temperature 80 C, retention time in minutes.*

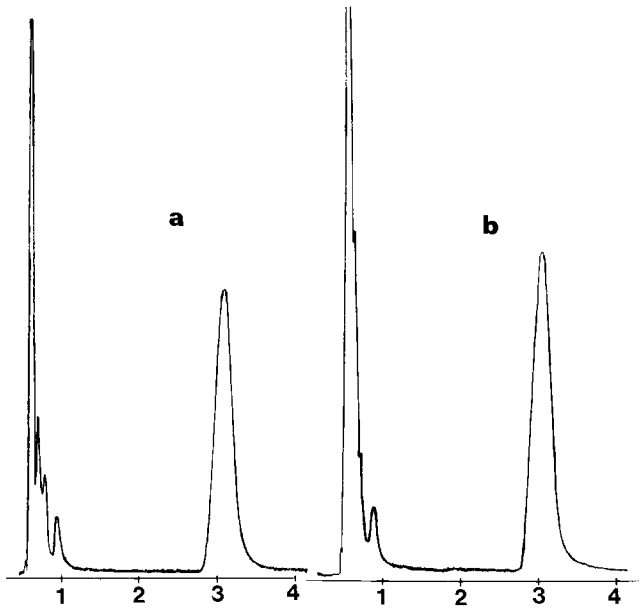

FIG. 3—(a) *Ether extract of blood from case 75-72-BY;* (b) *Ether extract of blood spiked with trichloroethanol. Column temperature 130 C, retention time in minutes.*

A sensitivity of 0.5 μg/ml is obtainable using the extraction procedure as described. Recoveries from blood, liver, and urine were excellent (95–100 percent) and to date no interfering substances have been detected in a number of blank tissue extractions.

Figures 1 to 3 illustrate the results obtained. For screening a sample the column temperature was set at 112 C (Fig. 1) but for quantitative analysis a column temperature of 80 C for chloral hydrate (Fig. 2) and 130 C for trichloroethanol (Fig. 3) was used. Specimens from a number of cases in which the presence of chloral hydrate was suspected have been analyzed using this method. No chloral hydrate has been detected in blood (only trichloroethanol) even though in one case chloral hydrate was detected in the stomach contents.

Summary

A sensitive and relatively specific method for the detection of chloral hydrate and trichloroethanol in biological tissue is described. An ether extract of the tissue is injected into a gas chromatograph equipped with an electron capture detector. The sensitivity of the method is 0.5 μg/ml using the extraction procedure described. The method has been successfully applied in routine toxicological work.

Acknowledgments

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