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Note

Improved method for the electron-capture gas chromatographic determination of trichloroacetic acid in human serum

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Trichloroacetic acid is one of the main metabolites of chloral hydrate and trichloroethylene. It is assumed that this compound may be highly cardiotoxic in man [1]. In several cases of chloral hydrate intoxications, multiple multifocal ventricular extrasystoles have been described. In order to obtain a relationship between the clinical state of an intoxicated patient and the serum concentration of trichloroacetic acid, it is necessary to evaluate an adequate method for determining this metabolite.

Several analytical methods have been reported which can be divided into procedures [2, 3] based on the colorimetric determination according to Fujiwara, and gas chromatographic methods, performed either on a strongly polar phase of the underivatized trichloroacetic acid after extraction [4, 5] or on a non-polar phase after extraction and derivatization [6–8]. The colorimetric methods lack specificity and sensitivity when applied to biological fluids. The reported gas chromatographic methods do not give satisfactory separations between the solvent, water and trichloroacetic acid, or they show tailing of the trichloroacetic acid peaks. Also, various head space techniques for the analysis of trichloroacetic acid after derivatization or decarboxylation have been described [9—12], but these methods are time consuming and inaccurate. Using the above mentioned procedures no reproducible results could be obtained. The purpose of this investigation was to develop a specific and highly sensitive procedure for the determination of trichloroacetic acid in human serum.

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EXPERIMENTAL

Instrumentation

A Tracor Model 550 gas chromatograph equipped with a pulsed mode ^{63}Ni electron-capture detector and a Tekman Model TE 200 1-mV recorder were used. Analyses were performed on a glass column (1.8 m × 4 mm I.D.) packed with 3% OV-17 on Gas-Chrom Q (80—100 mesh). Pre-purified nitrogen was used as the carrier gas at a flow-rate of 35 ml/min. The temperature of the column was maintained at 80°, the injection port at 150° and the detector at 260°.

Procedure

A 0.10-ml serum sample was diluted to 5.0 ml with distilled water using a dispenser/diluter into a disposable glass tube. After mixing for 30 sec on a whirl mixer, 0.20 ml were pipetted into a 10-ml extraction tube. To this solution, 0.2 ml 3 *M* sulphuric acid and 4.0 ml of a solution containing 4 μg/ml *o*-dichlorobenzene in toluene as the internal standard were added. This solution was mixed for 15 min and then centrifuged at 2500 *g* for 10 min.

2.00 ml of the toluene phase were transferred to another extraction tube and 0.5 ml of the methylating agent (14% boron trifluoride in methanol; Sigma, St. Louis, Mo., U.S.A.) was added. The tube was mixed for 3 min and then heated at 80° for 90 min. After cooling the mixture to room temperature, 1.0 ml of distilled water was added to remove excess derivatization reagent. The tube was centrifuged at 3000 rpm for 5 min and 1 μl of the toluene phase was injected into the gas chromatograph.

RESULTS AND DISCUSSION

Chromatograms of serum samples (Fig. 1) demonstrate that no interfering peaks occur. The retention time of trichloroacetic acid methyl ester is 92 sec. The standard curve, prepared by adding known amounts of trichloroacetic acid to blank human serum was linear over the range of 20—200 mg/l ($r = 0.98$, slope = 3.92×10^{-3}, y-intercept = 0.15 mg/l). The inter-assay (day-to-day) variation for the method was investigated by replicate analyses ($n = 5$) of spiked serum samples ranging from 25 -220 mg/l, and showed a coefficient of variation ranging from 10—5% for the respective concentrations. The recovery of various amounts of trichloroacetic acid (20—200 mg/l) added to serum, varied from 90—97%. Using the described method, the lowest trichloroacetic acid serum concentration which can be measured is about 10 mg/l. The amounts of trichloroacetic acid which were found after the intake of 10—20 g chloral hydrate in three patients ranged from 265—120 mg/l over the first 72 h.

Chloral hydrate and trichloroethanol did not interfere in the described gas chromatographic procedure.

Summarizing, it can be concluded that a sensitive, specific and reliable method, which is suitable for routine analysis has been developed to determine trichloroacetic acid in human serum.

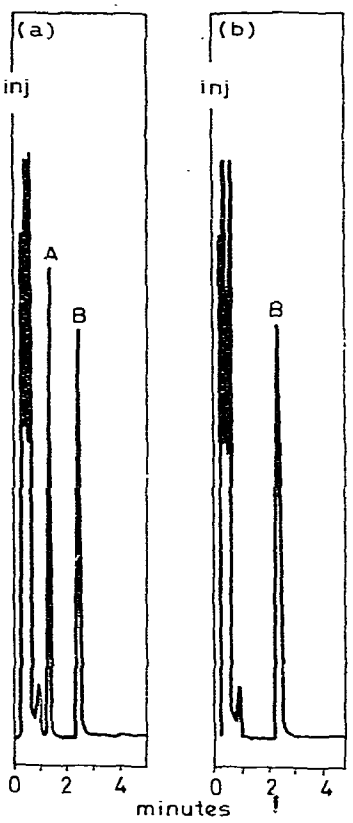

Fig. 1. Gas chromatograms of a serum sample containing 200 mg/l trichloroacetic acid (a) and a blank serum sample (b). Peaks A and B represent 0.5 ng trichloroacetic acid and 4 ng *o*-dichlorobenzene (internal standard), respectively.

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