Journal of Chromatography, 500 (1990) 655–659
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 740

Note

Determination of trichloroethanol, the active metabolite of chloral hydrate, in plasma by liquid chromatography

RAM N. GUPTA
Department of Laboratory Medicine, St. Joseph's Hospital, Hamilton, ON, L8N 4A6 (Canada)
(First received May 26th, 1989; revised manuscript received July 5th, 1989)

Chloral hydrate has been used as a hypnotic in clinical medicine since 1869, but its use declined after the introduction of barbiturates in 1903. However, there is now a resurgence in the use of chloral hydrate, as barbiturates have been withdrawn as hypnotics in a number of countries because of their high toxicity[1]. Further, psychiatrists prefer chloral hydrate to benzodiazepines because of its lack of anxiolytic properties and its relatively low tendency for addiction. However, chloral hydrate is also a potentially lethal drug when ingested in overdose and hence there is the possibility of an increased incidence of accidental or intentional poisoning. Cases of acute chloral hydrate poisoning can be treated by haemodialysis or haemoperfusion[1].

The plasma half-life of chloral hydrate is extremely short (4–6 min) and it is rapidly oxidized to trichloroethanol, which is responsible for all the pharmacological activity. Trichloroethanol is further oxidized to trichloroacetic acid, which is pharmacologically inactive. Emergency determination of plasma trichloroethanol concentration is required for differential diagnosis and to decide a treatment plan for acute chloral hydrate poisoning.

Tichloroethanol has been determined in body fluids by gas chromatography (GC) with electron-capture detection[2]. However, the use of electron-capture detectors is no longer popular in clinical laboratories. Most drugs contain nitrogen and are detected with nitrogen-selective alkali flame ionization detectors and non-nitrogenous drugs by flame ionization detectors when determined by GC. Chloral hydrate does not contain nitrogen and also shows a poor response with flame ionization detectors. Further, there is an increasing trend to determine toxins by liquid chromatography (LC)[3,4]. However, there does not appear to have been any report of the determination of trichloroethanol by LC because of the lack of suitable properties for its sensitive detection. This paper describes a simple procedure for the preparation of the benzoyl derivative of trichloroethanol, which has good chromatographic properties and allows sensitive detection because of its high absorbance at 237 nm. This approach has been used for the determination of ethylene glycol in plasma[5].

EXPERIMENTAL

All reagents were of analytical-reagent grade. Pentane was glass-distilled by the supplier (Caledon Laboratories, Georgetown, Ontario, Canada). De-ionized water was glass-distilled in the laboratory.

0021-9673/90/$03.50

Standards

Stock trichloroethanol solution, 3 g/l. Dissolve 0.1 ml of 2,2,2-trichloroethanol (Aldrich, Milwaukee, WI, U.S.A.) (sp. gr. 1.5) in 0.2 ml of acetonitrile in a 50-ml volumetric flask and dilute to volume with water. The solution is stable at 4°C for at least 1 month.

Plasma standard, 240 mg/l. Dilute 4 ml of stock trichloroethanol solution to 50 ml with blood-bank plasma. Prepare standards of 120, 60, 30, 15 and 7.5 mg/l by serial dilution. Store the standards frozen at −15°C in 1-ml aliquots.

Stock internal standard (I.S.) solution, 1.5 g/l. Dissolve 50 μl of 4-chloro-1-butanol (Aldrich) in 0.2 ml of acetonitrile in a 5-ml volumetric flask and dilute to volume with water. Store the I.S. solution frozen at −15°C.

Working I.S. solution. Dilute 1 ml of stock I.S. solution to 10 ml with water just prior to use.

Sample preparation

Place 0.25 ml of working I.S. solution in a Teflon screw-capped culture tube (16 × 100 mm) and then 0.25 ml of the sample (standard, control or test plasma). Vortex mix, drop 20 μl of benzoyl chloride on to the liquid surface and vortex mix. To each tube, add 0.25 ml of 4 *M* sodium hydroxide solution, cap the tubes tightly and rotate them on a rotary mixer at a medium speed for 10 min. Add 10 ml of pentane to each tube. Replace the corresponding caps, rotate the tubes in a rotary mixer for 5–7 min and then centrifuge them at 2000 *g* for 5 min. Collect the clear pentane layer in correspondingly labelled disposable glass tubes (16 × 100 mm) taking special care not to collect any aqueous phase or emulsion. Evaporate the pentane in a water-bath at 45–50°C. Dissolve the residue in 100 μl of methanol and inject an aliquot of 5 μl of the resulting solution into the chromatograph. When the concentration of trichloroethanol is less than 10 mg/l, aliquots of 10 μl of this extract are injected.

Chromatography

A Model 100A pump (Beckman, Berkeley, CA, U.S.A.) a 20-μl loop syringe-loading injector (Model 7125; Rheodyne, Cotati, CA, U.S.A.), a 150 × 4.6 mm I.D. Ultrasphere ODS column (5-μm particles) (Beckman) protected by an RP-18 guard cartridge (7-μm particles) (Brownlee Labs., Santa Clara, CA, U.S.A.) and a Model 6-AV UV absorbance detector (Shimadzu, Columbia, MD, U.S.A.). were used. The peaks were recorded with a Model CR601 plotter–integrator (Shimadzu). Chromatography was performed at room temperature. The mobile phase, consisting of 400 ml of water, 300 ml of acetonitrile and 300 ml of methanol, was pumped at a flow-rate of 2 ml/min with an operating pressure of 12.5 MPa. The peaks were monitored at 237 nm.

RESULTS AND DISCUSSION

Fig. 1B shows a representative chromatogram of an extract of plasma spiked with trichloroethanol. The relationship between the ratio of the peak area of trichloroethanol to that of the I.S. and the concentration of trichloroethanol is linear for the range tested (7–240 mg/l), and the graph passes through the origin.

Esterification of alcohols in aqueous medium with the use of acyl halides in the

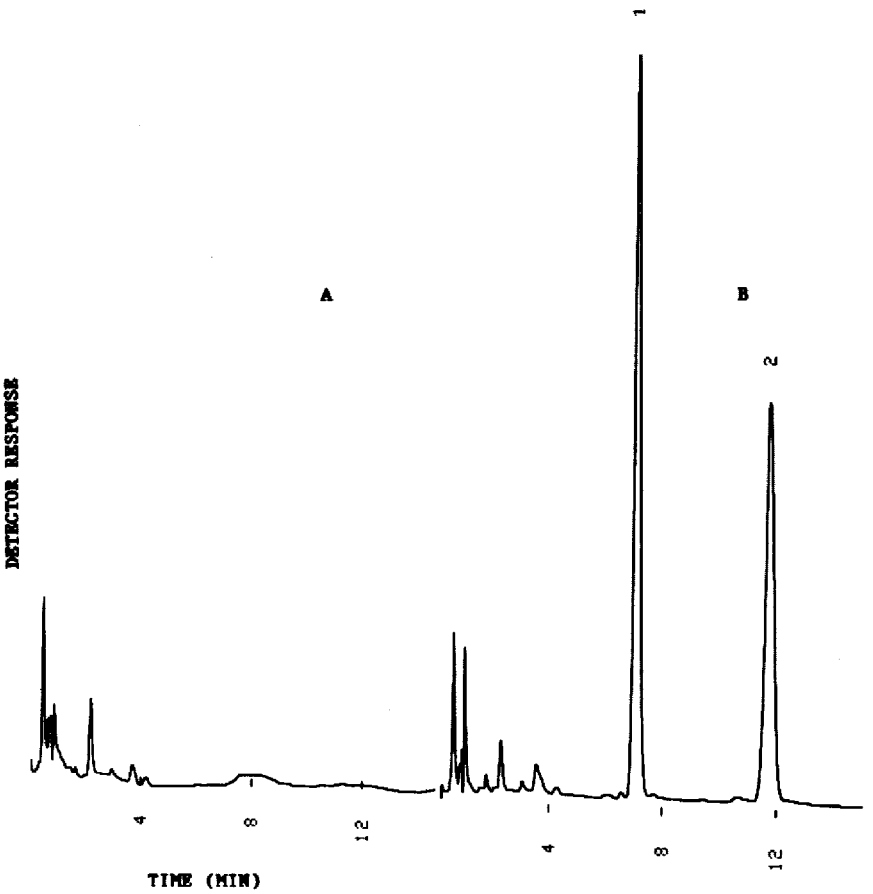

Fig. 1. Chromatograms of plasma extracts. (A) Drug-free plasma; (B) 60 mg/l standard of trichloroethanol in plasma. Peaks: 1 = 4-chloro-1-butyl benzoate; 2 = trichloroethyl benzoate. Detector output = 0.8 V; plotter attenuation = 4; chart speed = 0.5 cm/min.

presence of strong alkali is a well known technique (Schotten–Baumann reaction). It is also known that halogen-substituted compounds can be hydrolysed in aqueous alkali. The aim of this study was to select conditions for benzoylation so as to minimize the hydrolysis of trichloroethanol for adequate sensitivity and precision during the application of the Schotten–Baumann reaction. First 2,2-dichloroethanol (Aldrich) was selected as an internal standard, as it is the closest analogue of trichloroethanol that is available commercially. The benzoyl derivative of dichloroethanol gave a similar detector response to the benzoyl derivative of trichloroethanol and the two compounds were well separated during chromatography. However, in some instances an extraneous peak due to unreacted benzoyl chloride was observed that eluted close to benzoyl derivative of dichloroethanol. For adequate separation chromatographic the run time was more than 20 min or the LC system back-pressure became excessive.

In some tubes, a fraction of a microlitre of benzoyl chloride escapes contact

with alkali during mixing owing to the limited volume of liquid during the esterification step, which is then efficiently extracted by a relatively large volume of pentane. Addition of 0.25–0.5 ml of 1% glycine solution prior to extraction with pentane reduced the incidence of the extraneous peak. However, the presence of this reagent peak in a given tube could not be excluded. A number of chloro- and fluoro-substituted ethanols, propanols and butanols were then screened to select an alternative internal standard for the determination of trichloroethanol. 4-Chloro-1-butanol proved to be the most suitable for this purpose. The benzoyl derivative of this compound was well separated from the reagent peak within a reasonable chromatographic run time. Analysis of plasma supplemented with 60 mg/l of trichloroethanol showed a within-batch relative standard deviation (R.S.D.) of 8.4% (n = 10, mean = 58 mg/l) and a between-batch R.S.D. of 10.7% (n = 6, mean = 57 mg/l) over a 2-week period.

The high detector response and acceptable precision shown by extracts of plasma trichloroethanol standards indicate that alkaline hydrolysis of trichloroethanol is minimal under the conditions described. To check the stability of trichloroethanol further, plasma trichloroethanol standards (60 mg/l) were processed using propylene glycol as the internal standard, which does not contain halogen atoms and is not subject to alkaline hydrolysis. The benzoyl derivative of propylene glycol elutes at 9.8 min under the conditions described and is well separated from the peak of trichloroethanol. The ratio of the peak area of trichloroethanol to that of propylene glycol showed a within-bartch R.S.D. of 8.5% (n = 6). It appears that presence of protein, dilution of alkali by the sample and I.S. solution and the relatively brief contact with alkali during the esterification step reduce the hydrolysis of halogenated alcohols. Changes in the volumes of sample, I.S. solution and alkali or reversal of the order of addition of benzoyl chloride and alkali led to changes in the peak-area ratio of trichloroethanol to I.S.

Pentane is a selective solvent and easy to evaporate. Extracts of plasma from a variety of sources gave clean chromatograms. Alcohols and phenols, if present, will give benzoyl derivatives that will be extracted efficiently by pentane. Benzoyl esters of methanol, ethanol and 2-propanol elute early and do not interfere with the assay of trichloroethanol. The benzoyl derivative of acetaminophen is not extracted into pentane. The benzoyl derivative of ethylene glycol elutes 0.8 min after the elution of the I.S. peak. Primary and secondary amines, if present, will also be benzoylated. However, amides are not efficiently extracted by pentane. Tertiary amines and neutral compounds may be extracted into pentane at alkaline pH. However, most of these compounds are generally present in relatively low concentrations, even under overdose situations, and therefore have a low potential for interference. Trichloroacetic acid, the inactive metabolite of chloral hydrate, salicylic acid, barbiturates and other anticonvulsant drugs, are not extracted.

An ODS-silica column has been used as most toxicological data are being accumulated with this type of column[4]. However, an Ultrasphere octylsilyl-silica column (150 × 4.6 mm I.D., 5-μm particle size) gives a similar performance to an ODS-silica column with respect to the separation and quality of the peaks using a mobile phase consisting of 500 ml of water, 300 ml of acetonitrile and 300 ml of methanol at 2.2 ml/min. To meet an emergency request for the determination of trichloroethanol for the diagnosis of chloral hydrate poisoning, two standards of 60

and 30 mg/ml together with the test sample in duplicate are processed. The result is available in about 2 h. There is no well defined relationship between plasma trichloroethanol concentration and clinical manifestations of toxicity. The plasma concentration of trichloroethanol confirms the ingestion of an overdose of chloral hydrate. In general, plasma trichloroethanol concentrations up to 12 mg/l are in the therapeutic range and concentrations of about 100 mg/l are acutely toxic[6], when special measures for drug removal by haemodialysis or haemoperfusion may be considered.

It is concluded that the procedure described is a convenient alternative to GC with electron-capture detection for the emergency determination of trichloroethanol in plasma.

ACKNOWLEDGEMENTS

Miss Fadia Ros is thanked for her help with the preparation of the manuscript and Mrs. Maria Stefanec for technical assistance.

REFERENCES

1 T. Buur, R. Larsson and B. Norlander, *Acta Med. Scand.*, 223 (1988) 269.
2 B. Levine, J. Park, T. D. Smith and Y. H. Caplan, *J. Anal. Toxicol.*, 9 (1985) 232.
3 E. I. Minder, R. Schaubhut, C. E. Minder and D. J. Vonderschmitt, *J. Chromatogr.*, 419 (1987) 135.
4 K. Jinno, M. Kuwajima, M. Hayashida, T. Watanabe and T. Hondo, *J. Chromatogr.*, 436 (1988) 11.
5 R. N. Gupta, F. Eng and M. L. Gupta, *Clin. Chem.*, 28 (1982) 32.
6 C. L. Winek, *Clin. Chem.*, 22 (1976) 832.