Journal of Chromatography, 114 (1975) 459–462

CHROM. 8567

Note

Gas chromatographic determination of hydrochlorothiazide in plasma, blood corpuscles and urine using an extractive alkylation technique

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(First received May 7th, 1975; revised manuscript received June 23rd, 1975)

The diuretic drug hydrochlorothiazide can be determined by gas chromatography (GC) after an extractive alkylation procedure which has been described previously for the diuretics chlorthalidone[1] and furosemide[2]. Hydrochlorothiazide is converted into its tetramethyl derivative by this technique. Chlorthalidone is added as an internal standard to the (plasma or urine) samples before the extractions are performed. In the case of samples prepared from blood corpuscles, the internal standard is added after one extraction. The method provides the sensitivity and specificity necessary to determine the low concentrations of hydrochlorothiazide resulting in plasma and blood corpuscles after normal administration of the drug. Earlier spectroscopic methods did not possess these qualities[3].

EXPERIMENTAL

Plasma and blood-corpuscle analyses were made on a Varian 1400 gas chromatograph equipped with a ^{63}Ni electron-capture detector (ECD). Urine analysis was performed on a Packard-Becker 409 gas chromatograph equipped with a model 706 flame-ionization detector (FID). The oven temperature was set at 225° and the injector temperature at 230°. The detectors were operated at 300° (ECD) and 270° (FID). The carrier-gas (nitrogen) flow-rate was 30–40 ml/min. The silanized glass column (1.5 × 0.002 m I.D.) was packed with 1% Sephadex SE-30 stationary phase (Supelco, Bellefonte, Pa., U.S.A.) coated on Gas-Chrom Q (80–100 mesh). Peak areas were measured with a Spectraphysics minigrator. The hydrochlorothiazide derivative was identified on an LKB 2091 gas chromatograph–mass spectrometer. Radioactivity was measured on a Packard-Tricarb 2405 instrument.

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Method

Plasma sample. To 1 ml of plasma (2 or 3 ml can also be used when the drug concentration is very low) were added 100 μl of a 3.6 μM (1.20 μg/ml) solution of chlorthalidone and 1 ml of 0.05 *M* phosphate buffer at pH 6. The mixture was extracted with 5 ml of 4-methyl-2-pentanone. The organic phase was removed and extracted with 2.5 ml of 0.2 *M* sodium hydroxide solution. The aqueous alkaline phase was transferred to a screw-capped tube and 50 μl of a 0.1 *M* solution of tetrahexylammonium hydrogen sulphate were added followed by 5 ml of a 0.5 *M* solution of methyl iodide in dichloromethane. The tube was shaken for 20 min in a water-bath at 50°. After the mixture had been cooled and centrifuged, the dichloromethane phase was transferred to a second tube and the solvent was then evaporated at room temperature in a stream of nitrogen. The residue was dissolved in 0.2 ml of a hexane–toluene mixture (3:1) and 1–4 μl of the resulting solution were injected into the gas chromatograph (ECD). The areas under the hydrochlorothiazide and chlorthalidone peaks were recorded. Fig. 1 shows the chromatograms obtained.

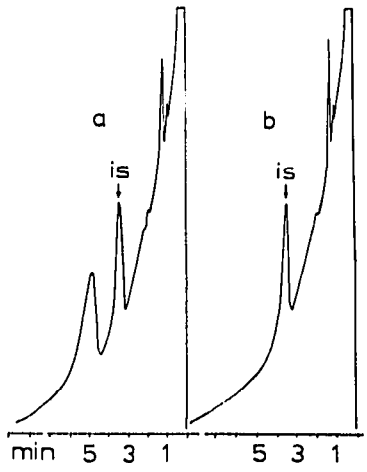

Fig. 1. GC determination of hydrochlorothiazide in plasma. Detector, ECD. (a) Plasma sample containing hydrochlorothiazide (92 ng/ml) and the internal standard (is = chlorthalidone); (b) blank containing the internal standard.

Blood-corpuscle sample. The blood corpuscles were separated from the plasma and washed twice with an isotonic saline solution. The corpuscles were centrifuged (Wifug doctor, 3700 rpm for 10 min and 0.5 ml of the centrifugate were lyzed with 1 ml of distilled water and exposed to ultrasonic treatment for *ca.* 2 min. The resulting mixture was extracted with 5 ml of 4-methyl-2-pentanone. 100 μl of a 3.6 μM (1.20 μg/ml) solution of chlorthalidone were added to 4 ml of the organic phase, which was then extracted with 2.5 ml of a 0.2 *M* sodium hydroxide solution. The alkaline solution was then treated as described above. The chromatograms obtained are similar to those from the plasma samples.

Urine sample. To 1 ml of urine were added 2 ml of 0.5 *M* phosphate buffer at pH 6 and 200 μl of a 0.15 μM (50 μg/ml) solution of chlorthalidone. The resulting

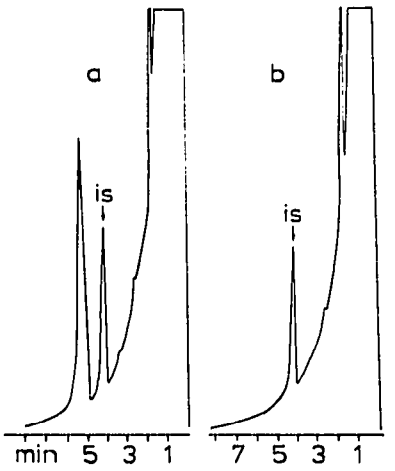

Fig. 2. GC determination of hydrochlorothiazide in urine. Detector, FID. (a) Urine sample containing hydrochlorothiazide (14.5 μg/ml) and chlorthalidone (is); (b) blank containing chlorthalidone.

mixture was extracted with 5 ml of 4-methyl-2-pentanone and the extract was then treated as described above. After dissolution in 100 μl of chloroform, the sample was injected into a gas chromatograph equipped with an FID. Fig. 2 shows the chromatograms obtained.

RESULTS AND DISCUSSION

Extraction and alkylation

Hydrochlorothiazide and chlorthalidone were extracted to approximately the same extent from an aqueous phase into 4-methyl-2-pentanone. The effectiveness of the extraction was checked by using ^{14}C-labelled hydrochlorothiazide (Merck, Sharp and Dohme, Rahway, N.J., U.S.A.). At pH 6, 91% of the hydrochlorothiazide was found in the organic phase after one extraction. After extraction at pH 5 and 7.8 (aqueous phase saturated with sodium hydrogen carbonate), 96 and 86% of the hydrochlorothiazide, respectively, were found in the organic phase. Samples of blood corpuscles to which chlorthalidone had been added before the first extraction (with 4-methyl-2-pentanone) gave a much higher ratio of hydrochlorothiazide to chlorthalidone than plasma samples to which the same amounts of the two substances had been added. This is probably due to the affinity for chlorthalidone of carbonic anhydrase[4] present in the corpuscles. Variations in the analytical results caused by different affinities for chlorthalidone of different samples of blood corpuscles are excluded by the addition of the standard after the first extraction.

An investigation analogous to that made *in vitro* with chlorthalidone[4] showed that red blood corpuscles had a much lower affinity for hydrochlorothiazide than for chlorthalidone (corpuscles to plasma ratio of *ca.* 5 compared with 50 for chlorthalidone). Further, hydrochlorothiazide did not show any affinity for carbonic anhydrase (bovine) as chlorthalidone did under the same conditions, *i.e.*, when chromatographed together with this enzyme on a Sephadex column. No difference was found in the extractabilities of blood-corpuscle and plasma samples for hydrochlorothiazide under the conditions described.

Gas chromatography

A mixture of 25% toluene in hexane instead of pure hexane is used as solvent for the sample injection since the tetramethyl derivative of hydrochlorothiazide is insoluble in the aliphatic hydrocarbon. In order to obtain optimum sensitivity and a stable system, 4–5 injections of a sample were necessary before the determination was started.

The calibration graphs were constructed by plotting the peak area ratio (hydrochlorothiazide to internal standard) against the concentration of hydrochlorothiazide as determined from samples (serum, blood corpuscles and urine) to which known amounts of hydrochlorothiazide had been added. Hydrochlorothiazide concentrations of 20–250 ng/ml and 2–30 μg/ml were used for the blood and urine samples, respectively. The graphs were linear and passed through the origin. Two series of six samples containing 90 ng (serum) and 10 μg (urine) of hydrochlorothiazide per ml, respectively, were used to determine the coefficient of variation which was found to be just above 3% for the plasma and urine samples. The minimum amount of hydrochlorothiazide that could be quantitatively determined was just below 10 ng/ml for plasma (2–3 ng/ml if 3 ml of plasma were extracted).

ACKNOWLEDGEMENT

The authors gratefully acknowledge the technical assistance of M. Andersson.

REFERENCES

1 M. Ervik and K. Gustavii, *Anal. Chem.*, 46 (1974) 39.
2 B. Lindström and M. Molander, *J. Chromatogr.*, 101 (1974) 219.
3 H. Sheppard, T. F. Mowles and A. J. Plummer, *J. Amer. Pharm. Ass., Sci. Ed.*, 49 (1960) 722.
4 B. Beermann, K. Hellström, B. Lindström and A. Rosén, *Clin. Pharmacol. Ther.*, 17 (1975) 424.