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Note

High-performance liquid chromatographic determination of hydrochlorothiazide in plasma and urine

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Hydrochlorothiazide (HCTZ: 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide) is a potent diuretic widely used in the treatment of systemic hypertension.

Several methods to determine HCTZ in plasma and urine have been described, e.g. in patient compliance studies. These methods include thin-layer chromatography [1], colorimetry [2], gas—liquid chromatography [3,4] and high-performance liquid chromatography (HPLC) [5—10].

The method described by Tisdall et al. [8] is suitable only for qualitative determination of thiazide diuretics in urine. Other recent papers on HPLC procedures [9,10] point out limitations of previously available methods. Each of the more recent methods, however, has disadvantages as well: in the method described by Soldin et al. [9], substantial amounts of interfering substances appear in the chromatograms of both serum and urine; the method described by Barbhaiya et al. [10] requires different procedures for plasma and urine.

For these reasons and because we needed to determine plasma and urinary HCTZ levels in a clinical trial, we developed a new, simple, rapid and sensitive HPLC method to measure HCTZ in plasma and urine, using chlorothiazide (CTZ) as an internal standard.

MATERIALS AND METHODS

Reagents

Ethyl acetate, acetic acid, sodium bicarbonate, tris(hydroxymethyl)aminomethane and methanol were obtained from Merck (Darmstadt, F.R.G.). Anhydrous sodium acetate and tetrabutylammonium hydrogen sulphate were obtained from Baker Chemicals (Deventer, The Netherlands) and Janssen Chimica (Beerse, Belgium), respectively. All reagents were of analytical grade quality. HCTZ and CTZ were kindly supplied by Merck, Sharp and Dohme (Haarlem, The Netherlands).

Biological fluids

Human blood plasma stored at −20°C was obtained from the local blood bank. Human urine (pH adjusted to 5.0 ± 0.5) was collected from a male volunteer shortly before analysis.

Apparatus and chromatographic conditions

We used a Hewlett-Packard HP 1084B liquid chromatograph equipped with a variable-wavelength detector and autosampler. The detection wavelength was 272 nm. The stainless-steel column (15 cm × 4.6 mm I.D.) was packed with LiChrosorb RP-18, particle size 5 μm (Merck). The oven temperature was 38°C and the injection volume was 10 μl for urine and 30 μl for plasma.

The mobile phase was a mixture of methanol and twice-distilled water (20:80) containing 0.01024 *M* tetrabutylammonium hydrogen sulphate and 0.00976 *M* tris(hydroxymethyl)aminomethane (pH 5.5). This was delivered at a rate of 1.2 ml/min, producing a pressure of 178 bars.

The mobile phase was prepared as follows: 3.820 g of tetrabutylammonium hydrogen sulphate (mol. wt. = 339.54) were dissolved in 450 ml of twice-distilled water, and 1.363 g of tris(hydroxymethyl)aminomethane (mol. wt. = 121.14) were dissolved in 450 ml of twice-distilled water. The latter solution was added to the tetrabutylammonium hydrogen sulphate until pH 5.5 was attained (429 ml were required). Next, the mixture was filtered through a 0.45-μm Millipore filter and 200 ml of methanol were subsequently added to 800 ml of the filtered mixture.

Procedure

We pipetted 100 μl of a solution of CTZ in methanol (50 mg per 100 ml methanol for urine, and 1 mg per 100 ml methanol for plasma) into a screw-capped extraction tube. The methanol was evaporated with a gentle stream of dry-filtered air, whereupon 0.5 ml of plasma (or urine), 0.5 ml of 0.1 *M* acetate buffer (pH 3.8 for plasma and pH 5.0 for urine) and 5 ml of ethyl acetate were successively pipetted into the tube. The tube was then closed and shaken mechanically for 30 min; after centrifugation at 1300 *g* for 15 min the organic layer was transferred to a clean extraction tube containing 400 mg of sodium bicarbonate.

The tube was closed again and placed on a whirl mixer for 20 sec. After centrifugation at 1300 *g* for 10 min the organic layer was then pipetted into a clean tube and evaporated to dryness at 30°C with dry filtered air. Subsequently the residue was dissolved in 0.5 ml of the mobile phase (1 ml for urine sample), and 30 μl (for plasma) or 10 μl (for urine) were injected onto the column.

RESULTS

Figs. 1 and 2 show typical chromatograms for blank plasma and blank urine samples with or without sodium bicarbonate treatment, for plasma containing 0.106 μg/ml HCTZ and 2.024 μg/ml CTZ and for urine containing 2.66 μg/ml HCTZ and 1.012 μg/ml CTZ (both treated with sodium bicarbonate). The retention time of HCTZ is about 7.0 min, and that of CTZ about 9.9 min.

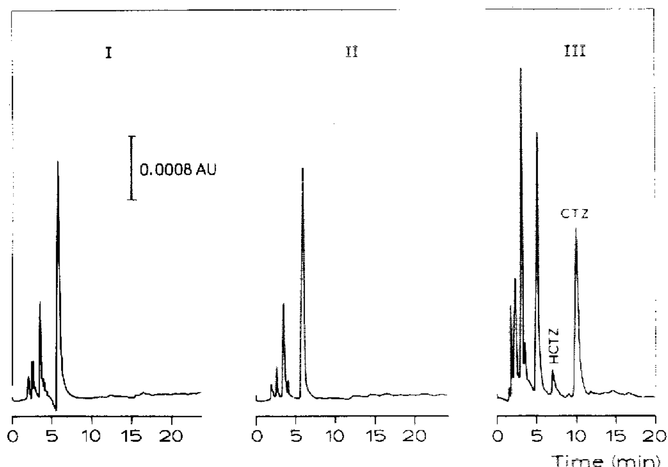

Fig. 1. Chromatograms obtained from a blank plasma sample not treated with sodium bicarbonate (I), a blank sample treated with 200 mg of sodium bicarbonate (II), and a sample containing 0.106 μg/ml HCTZ and 2.024 μg/ml CTZ, treated with sodium bicarbonate (III).

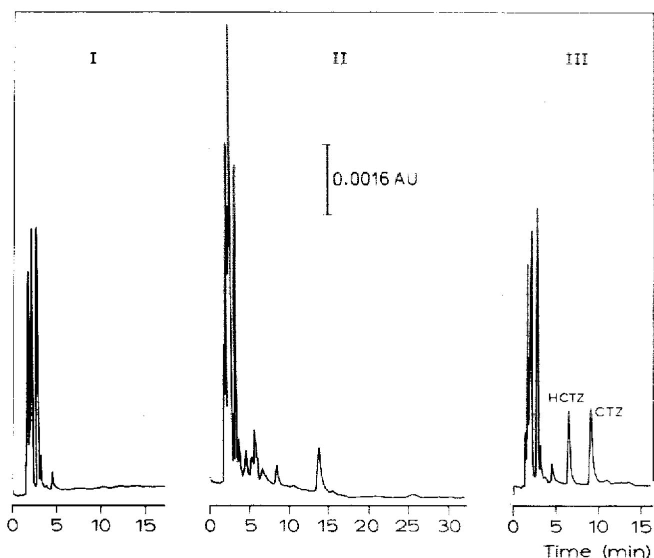

Fig. 2. Chromatograms obtained from a blank urine sample treated with 400 mg of sodium bicarbonate (I), a blank sample not treated with sodium bicarbonate (II), and a sample containing 2.66 μg/ml HCTZ and 10.12 μg/ml CTZ, treated with sodium bicarbonate (III).

Calculation

The HCTZ concentration in a sample was determined by comparing the peak height ratio (HCTZ/internal standard) with a standard curve of peak height ratio versus HCTZ concentration. Whenever a sample containing HCTZ was measured, a standard curve was generated by adding different amounts of HCTZ to blank plasma or blank urine and analyzing them by the method already described.

A linear relationship was found between the peak height ratio (PHR) of HCTZ to CTZ (Y) and the plasma HCTZ concentration (X), as given by the equation $Y = 1.4117\ X + 0.007$ ($r = 0.9997$, $n = 6$) for the plasma HCTZ concentration range 0.025 — 1.00 μg/ml. For urine the equation was $Y = 0.03930\ X + 0.00047$ ($r = 0.9998$, $n = 6$) for the range 2.66 — 53.20 μg/ml (internal standard concentration 101.2 μg/ml), and $Y = 0.3703\ X - 0.00183$ ($r = 0.9999$, $n = 5$) for the range 0.25 — 5.39 μg/ml (internal standard concentration 10.12 μg/ml).

Recovery

Overall recovery was determined by comparing the peak heights of HCTZ and CTZ obtained after injection of standard solutions with peak heights obtained after injection of extracted standard solutions (Table I).

CTZ recovery from plasma was 80 ± 4% at a concentration of 2.0 μg/ml and that from urine was 64 ± 2% at 50 μg/ml.

TABLE I

RECOVERY OF HCTZ IN PLASMA AND URINE

n = 10 for all concentrations.

Plasma			Urine		
HCTZ concentration (μg/ml)	Recovery (%)	C.V. (%)	HCTZ concentration (μg/ml)	Recovery (%)	C.V. (%)
0.1	92	2	0.5	95	3
0.3	95	4	5.0	92	3
1.0	96	4	15.0	88	2
			50.0	87	1

Sensitivity and precision

The detection limit with the analysis described was 0.025 μg/ml for plasma and 0.5 μg/ml for urine. Tables II and III show the within-day precision of HCTZ analysis in plasma and urine, respectively. The between-day precision for spiked HCTZ plasma samples (in the range 0.05 — 1.00 μg/ml) was 2.8% (n = 9), and that for spiked HCTZ urine samples (in the range 5 — 50 μg/ml) was 1.6% (n = 6).

DISCUSSION

As pointed out, none of the published methods to determine HCTZ met our requirements. Our method is simple and rapid, and does not require different procedures to measure HCTZ in plasma and in urine.

TABLE II

ANALYSIS OF SPIKED HCTZ PLASMA SAMPLES

Sample	Concentration (μg/ml)	n	C.V. (%)
1	0.021	10	8.3
2	0.107	10	2.4
3	0.320	10	3.7
4	1.080	10	2.7

TABLE III

ANALYSIS OF SPIKED HCTZ URINE SAMPLES

Sample	Concentration (μg/ml)	n	C.V. (%)
1	0.51	10	2.2
2	5.23	10	3.3
3	15.46	10	2.0
4	51.20	10	2.0

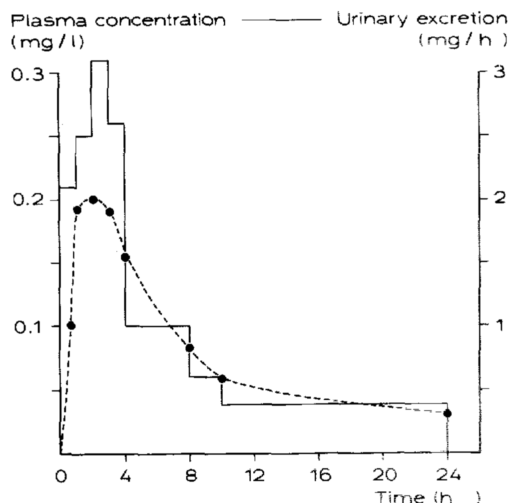

Fig. 3. Plasma concentration (- - -) and urinary excretion (——) of HCTZ in a healthy adult male volunteer given 50 mg of HCTZ orally.

Recovery of our internal standard, CTZ, is admittedly only about 70%; however, it is very constant. Like Cooper et al. [5], we used sodium bicarbonate. We found the HCTZ retention time in plasma to be constant after addition of sodium bicarbonate. For urine, we used sodium bicarbonate to eliminate interfering substances.

Our method was applied in pharmacokinetic studies. As a typical example Fig. 3 shows the plasma concentration and urinary excretion of HCTZ in a healthy adult male volunteer after ingestion of one 50-mg tablet at 08.00 a.m.

The fall in plasma concentration was biphasic, confirming results reported by others [11,12]. The elimination half-life is 4 h, and a peak plasma level is reached at 2 h. The other kinetic parameters were also within the reported range.

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