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Note

Simple and selective high-performance liquid chromatographic method for the determination of hydrochlorothiazide in urine

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Hydrochlorothiazide (HCTZ) is a potent, diuretic, antihypertensive agent. Acting alone, or in combination with other natriuretics, it is very effective in the treatment of edema associated with congestive heart failure, hepatic cirrhosis or the nephrotic syndrome [1].

Numerous high-performance liquid chromatographic (HPLC) methods have been reported for the determination of HCTZ in plasma and urine [2–5]. In addition to some limitations of these methods, they all have problems with endogenous interferences at various levels. The most recent method described by Koopmans et al. [6] was a more selective and better method compared to other previously described methods [2–5]. However, this method required a temperature control (38°C) of the analytical column to achieve proper separation. Furthermore, most of these methods were dealing with biological samples containing HCTZ only. Although the method described by Soldin et al. [4] claimed no interference from many other drugs, substantial amounts of endogenous interferences in urine had limited its routine use.

EXPERIMENTAL

Reagents and materials

Pure HCTZ and the internal standard chlorothiazide (CTZ) were obtained from Merck Sharp and Dohme (West Point, PA, U.S.A.) and used without further purification. Dyazide® capsules were obtained from Smith, Kline and French Labs. (Philadelphia, PA, U.S.A.). All chemicals received commercially were reagent grade and solvents were HPLC grade. The extemporaneous

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products of 50 mg triamterene (TRM) and 25 mg HCTZ for the bioavailability study included (A) suspension, (B) pulverized Dyazide capsule in a larger capsule, (C) pure compounds in a capsule and (D) intact Dyazide capsule. Stock solutions of HCTZ and CTZ were prepared in 100% methanol (200 μg/ml) and stored at 4° C.

Chromatographic apparatus and conditions

An HPLC system equipped with a Waters Model 6000A constant-flow pump (Waters Assoc., Milford, MA, U.S.A.), a Model U6K loop-type injector and a Model SF770 Schoeffel spectroflow UV monitor (Schoeffel Instruments, Westwood, NJ, U.S.A.) was used. A prepacked analytical column, μBondapak-phenyl (30 cm × 3.9 mm I.D.), from Waters Assoc. with a guard column (RP-8, 3 cm × 3.9 mm I.D.) from Brownlee Labs. (Santa Clara, CA, U.S.A.) was utilized for HCTZ and CTZ separation. A mobile phase consisting of deionized water–methanol–tetrahydrofuran (THF) (88:10:2) was used to elute the sample at a flow-rate of 2 ml/min and column pressure of 4.2 bar. The eluent was monitored at 272 nm at 0.04 a.u.f.s. and recorded on a Varian Model 9176 strip chart recorder (Varian Assoc., Palo Alto, CA, U.S.A.).

Preparation of urine samples

To 1 ml of urine sample or spiked standard in a 15-ml screw-capped centrifuge tube were added 50 μl of internal standard, 1 ml of water and 1 ml of 0.01 *M* perchloric acid. The mixture was washed with 5 ml of dichloromethane by vigorous mixing using a Vortex Genie mixer (Fisher Scientific, Pittsburgh, PA, U.S.A.) for 30 s. After centrifugation at 500 *g* for 5 min, the aqueous layer was transferred into a clean 15-ml screw-capped tube. The urine was then extracted with 5 ml of ethyl acetate by agitating the tube on a mechanical Burrell shaker (Burrell, Pittsburgh, PA, U.S.A.) for 10 min. After centrifugation, the organic layer was transferred into a conical test tube and evaporated to dryness at 60° C in a water bath under a stream of nitrogen. The residue was reconstituted with 400 μl of methanol and a 20-μl aliquot was injected.

Recoveries of HCTZ and CTZ from urine samples

The recoveries of HCTZ and CTZ from urine samples were determined by comparing the chromatographic peak heights of HCTZ and CTZ obtained after injection of extracted spiked urine samples with peak heights obtained after direct injection of equal amounts of the relevant drugs in solvent.

Validation of the assay method for urine samples

Known amounts of HCTZ were added to aliquots of urine to obtain various final concentrations. The samples were assayed in triplicate and the coefficients of variation and relative percentage errors were calculated.

Bioavailability study

Four oral formulations consisting 50 mg of TRM and 25 mg of HCTZ were given to three healthy male volunteers after overnight fasting in a cross-over design with one week wash-out period between dosage administrations. Urine

samples were collected before drug administration (0 h) and at 0–10, 10–24, 24–34 and 34–48 h intervals after administration. Each of the urine samples was mixed well, the volume was measured and an aliquot was kept frozen until the time of analysis. Urinary HCTZ concentrations were determined as described above. The mean excretion rates and percentage cumulative eliminations of HCTZ at each time interval were then calculated.

RESULTS AND DISCUSSION

Under the assay conditions, baseline resolution between HCTZ and its internal standard CTZ was achieved. In Fig. 1, the chromatogram of the blank urine extract (I) shows that no interfering peaks are present at the retention times of either HCTZ (b) or CTZ (a). Typical chromatograms of a spiked urine extract (II) and a urine sample from a volunteer (III) show that both CTZ and

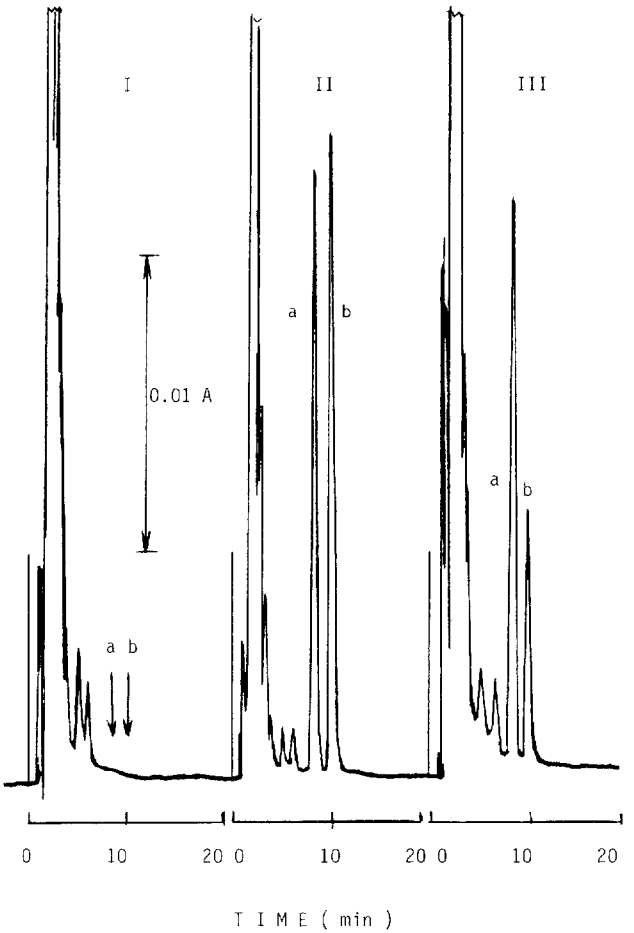

Fig. 1. Chromatograms obtained from the HPLC assay of (I) control urine, (II) urine spiked with (a) 10 μg/ml chlorothiazide and (b) 7.5 μg/ml hydrochlorothiazide and (III) urine from a human volunteer after a single dose of a TRM/HCTZ capsule. Peak b in III corresponds to a concentration of 2.8 μg/ml in urine.

TABLE I

RECOVERIES OF HYDROCHLOROTHIAZIDE (HCTZ) AND CHLOROTHIAZIDE (CTZ) FROM URINE

Concentration (μg/ml)	Recovery (mean ± S.D., n = 4) (%)	
	HCTZ	CTZ
2.00	85.6 ± 4.8	89.6 ± 3.1
10.0	80.7 ± 9.5	80.1 ± 10.2
20.0	92.6 ± 9.0	90.1 ± 12.3

TABLE II

INTRA-DAY PRECISION AND ACCURACY OF THE HPLC ASSAY OF HYDROCHLOROTHIAZIDE IN URINE

Actual concentration (μg/ml)	Experimental concentration (μg/ml)		Coefficient of variation (%)	Error (%)
	Mean (n = 3)	Range		
2.00	2.06	1.98— 2.17	4.8	3.7
5.00	5.03	4.94— 5.07	1.5	1.1
10.0	9.94	9.75—10.2	2.3	1.7
20.0	20.0	19.5 —20.4	2.4	1.8
Overall coefficient of variation			2.8	
Overall error				2.1

TABLE III

DAY-TO-DAY REPRODUCIBILITY OF THE HPLC ASSAY OF HYDROCHLOROTHIAZIDE IN URINE

Actual concentration (μg/ml)	Experimental concentration (μg/ml)		Coefficient of variation (%)
	Mean (n = 6)	Range	
2.00	2.05	1.87— 2.28	7.1
5.00	4.96	4.72— 5.11	3.1
10.0	9.89	9.50- 10.1	2.6
20.0	20.1	19.3 —20.9	2.8
Overall coefficient of variation			3.9

HCTZ appeared as sharp, symmetric peaks with retention times of 8 and 10 min, respectively. Addition of THF as modifier in the mobile phase is essential to obtain a better resolution and symmetric peaks.

No interferences from TRM and its metabolite triamterene sulfate were observed in the volunteer's urine. In a separate study, we found that the presence of drugs such as methyldopa, propranolol, hydralazine, guanethidine, spironolactone, timolol, amiloride and reserpine in urine did not interfere in the assay.

The standard calibration curve of HCTZ/CTZ peak-height ratios versus con-

centrations is linear over the range of 0—20 μg/ml of urine with a correlation coefficient (r) of 0.9993. Owing to the simplicity of the extraction procedure, more than 80% of HCTZ and CTZ were recovered from urine at the various concentrations studied (Table I).

The sensitivity of the assay is about 0.05 μg/ml of urine under the described conditions. The intra-day precision and day-to-day reproducibility of the assay show overall coefficients of variation of 2.8 and 3.9%, respectively (Tables II and III).

The mean excretion rate of HCTZ in urine from three volunteers after administration of four TRM/HCTZ formulations is shown in Table IV and the cumulative percentage excretion of HCTZ is shown in Fig. 2. It is quite evident from our results that the method described here is applicable to quantify HCTZ in combination products as illustrated. The selectivity and sensitivity of this assay achieved for the combination product are equivalent to those of the method described by Koopmans et al. [6] for HCTZ as a single entity. An additional advantage of this procedure by utilizing a less lipophilic stationary phase is that the chromatography is carried out at ambient temperature instead of 38°C which obviates the necessity of special heating equipment to control the temperature. Furthermore, where temperature has to be controlled there is always a possible uncertainty in reproducibility.

In conclusion, the procedure described here has two distinct advantages: (1) the chromatography can be carried out at ambient temperature and (2) the

TABLE IV

MEAN EXCRETION RATES OF HCTZ IN URINE AFTER ADMINISTRATION OF FORMULATIONS OF 50 mg TRM AND 25 mg HCTZ

Formulation*	Time interval (h)	Excretion rate (mean ± S.D., n = 3) (mg/h)
A	0—10	1.275 ± 0.279
	10—24	0.201 ± 0.002
	24—34	0.093 ± 0.013
	34—48	0.072 ± 0.029
B	0—10	1.277 ± 0.398
	10—24	0.233 ± 0.006
	24—34	0.097 ± 0.029
	34—48	0.035 ± 0.021
C	0—10	1.119 ± 0.222
	10—24	0.284 ± 0.010
	24—34	0.104 ± 0.042
	34—48	0.040 ± 0.009
D	0—10	0.703 ± 0.275
	10—24	0.165 ± 0.021
	24—34	0.087 ± 0.018
	34—48	0.040 ± 0.013

*Formulation A is a suspension of 50 mg TRM and 25 mg HCTZ. Formulations B, C and D are capsules with 50 mg TRM and 25 mg HCTZ as described in the text.

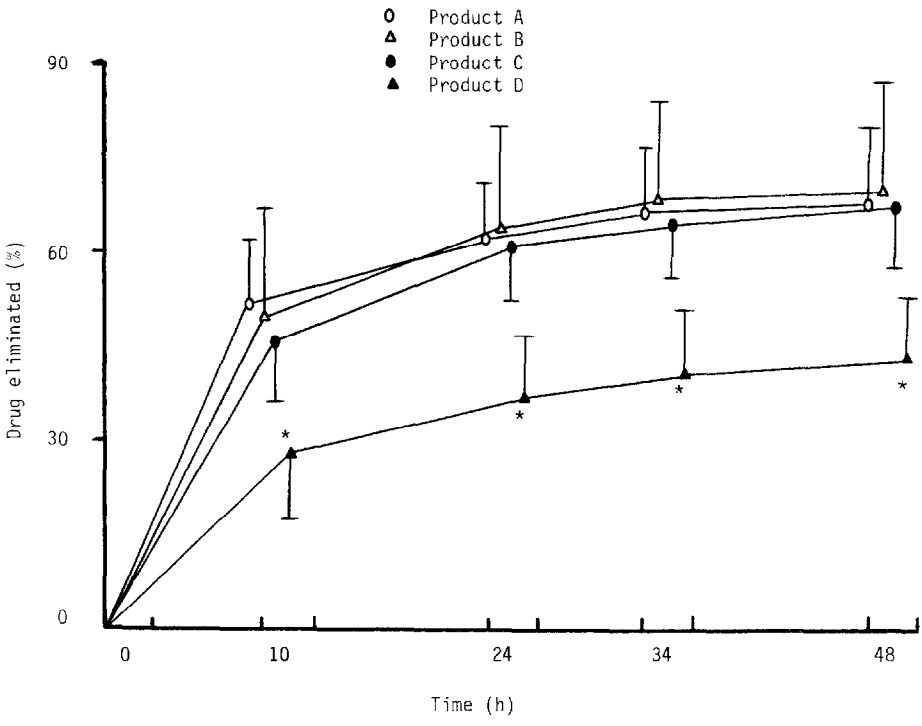

Fig. 2. Percentage cumulative excretion of hydrochlorothiazide (mean ± S.D.). The asterisks represent values significantly different ($P < 0.05$) from those of product A.

method is selective and sensitive to quantify HCTZ in combination with other drugs and is free from interference of endogenous substances in the urine samples.

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