Journal of Chromatography, 164 (1979) 527—533
Biomedical Applications

CHROMBIO. 409

Note

Determination of the diuretic triamterene in the plasma and urine of humans by high-performance liquid chromatography

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(First received May 16th, 1979; revised manuscript received August 6th, 1979)

Triamterene is a potassium-sparing diuretic which increases sodium, chloride, and to a lesser extent bicarbonate, excretion [1].

Earlier studies of triamterene concentrations in the plasma and urine of humans were carried out using somewhat non-specific fluorimetric methods [2—4] which determined the parent drug and its metabolites together. A more specific assay, involving fluorimetry has also been described [5].

Because the reported procedures were not considered sufficiently sensitive for bioavailability studies of triamterene-containing formulations, a high-performance liquid chromatographic (HPLC) method utilising fluorimetric detection has been developed and is described in this paper. Since these studies were completed, a dissimilar high-performance liquid chromatography—fluorescence detection assay has been reported [6] which requires extraction of triamterene from plasma as the perchlorate ion-pair, a silica column and does not include an internal standard. Its sensitivity appears to be marginally less than the procedure described below.

EXPERIMENTAL

Materials

Triamterene, 6-*p*-hydroxytriamterene and 6-*p*-methoxytriamterene (Fig. 1) were kindly provided by Pharma Schwarz (Monheim, G.F.R.). Standard solutions of triamterene and the internal standard *p*-methoxytriamterene were prepared at a concentration of 1 μg/ml in methanol and stored at 4°. Reagents were of analytical grade and inorganic reagents were prepared in freshly glass-distilled water.

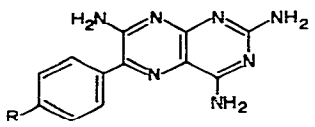

Fig. 1. Chemical structure of triamterene (R = H), 6-*p*-hydroxytriamterene (R = OH) and 6-*p*-methoxytriamterene (R = OCH_3).

Extraction procedure

Plasma samples (1 ml) were transferred into 10-ml pointed centrifuge tubes and spiked with a solution of the internal standard (40 μl, containing 40 ng of *p*-methoxytriamterene). Sodium bicarbonate (200 mg) was added and the mixture extracted by shaking it for 1 min with ethyl acetate (5 ml). After centrifugation for 10 min, the organic layer was carefully transferred into a 10-ml pointed centrifuge tube and evaporated to dryness under a stream of nitrogen at 37°. The sides of the tube were washed with ethyl acetate to ensure that all the residue was washed to the bottom of the tube, and the ethyl acetate evaporated to dryness. The residue was dissolved in methanol (20—50 μl) and an aliquot (5—12 μl) injected into the chromatograph.

Urine samples (100 μl) were diluted with distilled water (1 ml), spiked with internal standard (40 ng) and taken through the same extraction procedure as described for plasma.

High-performance liquid chromatography

The chromatograph consisted of a Waters M6000A pump (Waters Assoc., Northwich, Great Britain) coupled to a Perkin-Elmer LC 1000 fluorescence detector (Perkin-Elmer, Beaconsfield, Great Britain) operated at an excitation wavelength of 365 nm and an emission wavelength of 440 nm. Peak area measurements were obtained using a Hewlett-Packard 3380A Advanced Reporting Integrator (Hewlett-Packard, Hitchin, Great Britain). Injection was performed by syringe (25 μl, Precision Sampling, Baton Rouge, La., U.S.A.) via a U6K universal injector (Waters Assoc.). The column was stainless steel (300 × 4 mm I.D.), prepacked with μBondapak C_{18} (mean particle diameter, 10 μm) (Waters Assoc.).

Chromatography was performed in a reversed-phase mode using a solvent system of 45% (v/v) methanol in aqueous 0.1% (w/v) potassium dihydrogen phosphate with the final pH adjusted to 3.8 with phosphoric acid. The mobile phase flow-rate was 2 ml/min. The retention times (t_R) of triamterene and internal standard (*p*-methoxytriamterene) were 2.6 min and 3.1 min, respectively (Fig. 2), and the metabolite *p*-hydroxytriamterene (t_R 1.9 min) did not interfere with the assay.

Calibration curves

Calibration curves were constructed of peak area ratio measurements of triamterene to internal standard against concentration over concentration ranges of up to 40 ng/ml in plasma and 800 ng/ml in urine. Samples of blank plasma (1 ml) were spiked with triamterene at concentrations of 1, 10, 20, 30

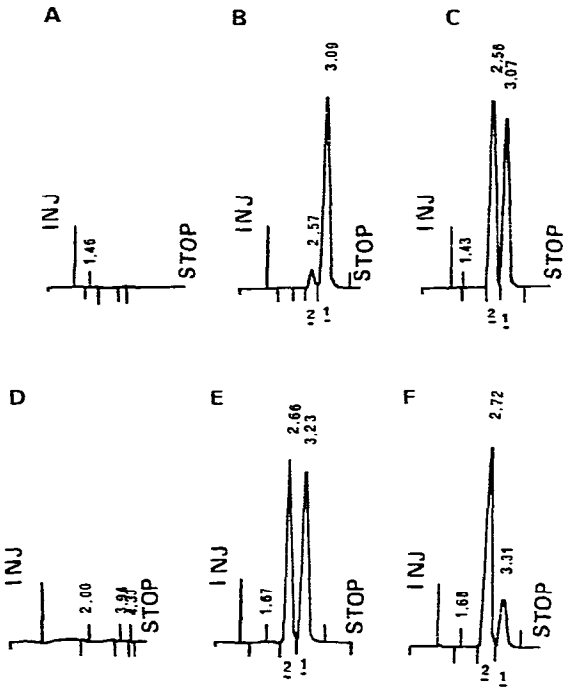

Fig. 2. Chromatograms of (A) predose control plasma; (B) and (C) plasma samples containing 1 and 10 ng/ml of triamterene, respectively; (D) predose control urine; and (E) and (F) urine samples containing 100 and 400 ng/ml of triamterene, respectively. Column (300 × 4 mm I.D.) prepacked with μBondapak C_{18}; flow-rate, 2 ml/min; solvent system, 45% (v/v) methanol—aqueous 0.1% (w/v) potassium dihydrogen orthophosphate; excitation and emission wavelengths, 365 and 440 nm, respectively; scale expansion, ×50; integrator attenuation, 32. Peaks: 1 = internal standard (*p*-methoxytriamterene, 40 ng/ml); 2 = triamterene.

and 40 ng/ml and with internal standard at 40 ng/ml. Samples of predose urine (100 μl) were spiked with 10, 20, 30, 40 and 80 ng of triamterene and 40 ng of internal standard. The samples were taken through the extraction procedure described previously.

Studies in humans

Plasma and urine samples obtained from human volunteer subjects dosed with a commercially available triamterene formulation (50-mg capsule) were analysed by the foregoing procedures. The studies in volunteers were carried out under conditions similar to those described by Brodie et al. [7].

RESULTS AND DISCUSSION

Precision

Extraction and measurement at each concentration were repeated on fourteen occasions from plasma and on six occasions from urine. The precision of the method for the measurement of triamterene was indicated by the coefficient of variation of peak area ratios which was ± 11%, ± 3% and ± 2% at

1 ng/ml, 20 ng/ml and 40 ng/ml, respectively, when extracted from plasma (1 ml) and ± 3%, ± 2% and ± 2% at 100 ng/ml, 300 ng/ml and 800 ng/ml, respectively, when extracted from urine (100 μl).

The coefficient of variation of peak area ratio measurements of a non-extracted mixture of triamterene and internal standard was ± 1% throughout the analysis of all plasma and urine samples.

Accuracy

The calibration curve for the measurement of triamterene in plasma was constructed from fourteen replicates at five concentrations over the range, and plots of peak area ratios against concentration were linear ($y = a + bx$, where $a = 0.0119$, $b = 0.0864$, correlation coefficient $r = 0.9991$) and the value of the intercept was not significantly different from zero ($P > 0.05$). The equation for the line forced through the origin was $y = 0.0868$ (± 0.0003 S.D.)x, where y is the peak area ratio and x is the concentration of triamterene (ng/ml). The accuracy of the method as defined by the 95% confidence limits of the least squares regression line forced through the origin, i.e. taking the calibration line as an estimate of the concentration of triamterene in plasma, was ± 122%, ± 6% and ± 3% at 1 ng/ml, 20 ng/ml and 40 ng/ml, respectively.

The calibration curve for the measurement of triamterene in urine was constructed from six replicates at five concentrations over the range, and plots of peak area ratios against concentration were linear ($y = a + bx$, where $a = 0.1214$, $b = 0.0802$, correlation coefficient $r = 0.9984$, and y is the peak area ratio and x is the amount of triamterene in 100 μl of urine). The value of the intercept was shown to be significantly different from zero. The 95% confidence limits for the curve were ± 25%, ± 7% and ± 4% at 100 ng/ml, 400 ng/ml and 800 ng/ml, respectively.

Recovery

The recovery of internal standard (40 ng/ml) from plasma (1 ml) was determined by comparison of peak area ratio measurements of internal standard to triamterene of standards taken through the extraction procedure to those injected into the chromatograph without extraction. The mean recovery of internal standard was 80.3% ± 2.8 S.D. ($n = 6$).

TABLE I

RECOVERY OF TRIAMTERENE FROM PLASMA AND URINE

Concentration added to plasma (ng/ml)	Recovery* (%)	Concentration added to urine (ng/ml)	Recovery* (%)
1	90.3	100	79.4
10	69.9	200	88.3
20	78.9	300	87.0
30	78.5	400	85.9
40	76.4	800	86.2

*Means of 14 determinations (plasma) and 6 determinations (urine) at each concentration.

The mean recovery of internal standard from urine (100 μl) at a concentration of 40 ng/100 μl was determined similarly and was 90.4% ± 1.3 S.D. (n = 6).

The mean recovery of triamterene from plasma was determined by comparison of peak area ratio measurements of non-extracted standards to those of extracted standards corrected for 100% recovery of internal standard, and was 78.8% ± 7.4 S.D. (Table I).

The mean recovery of triamterene from urine was determined similarly and was 85.4% ± 3.5 S.D. (Table I).

Stability of triamterene

The stability of triamterene in plasma and urine under the storage conditions used was tested by storing spiked plasma and urine samples for one week at −20° before analysis and then comparing the results obtained with freshly spiked standards. No decomposition was detected in either plasma or urine. Similarly results of samples re-analysed after three weeks storage at −20° were in good agreement with those attained initially and showed no decomposition.

Limit of detection

No interfering peaks were present in predose (blank) plasma samples taken from each subject prior to the start of the study. The limit of detection based on instrumental noise was set at 1 ng/ml (Fig. 2), but levels in the higher pg/ml range could be detected. The limit of detection using an ultraviolet absorption detector was poorer (λ_{max} 230 nm) and was about 10 ng/ml.

Concentrations of triamterene in plasma

After single oral doses of triamterene, a peak of mean concentrations of

TABLE II

CONCENTRATIONS OF TRIAMTERENE (ng/ml) IN THE PLASMA OF SIX HUMAN SUBJECTS AFTER SINGLE ORAL DOSES OF 50 mg

Time (h)	Subject No.						Mean ± S.D.
	1	2	3	4	5	6	
0.25	4	1	1	1	<1	<1	–
0.5	8	3	6	20	1	5	7.2 ± 6.7
0.75	13	7	8	20	2	10	10.0 ± 6.1
1	14	13	10	23	5	16	13.5 ± 6.0
1.5	13	14	15	17	8	26	15.5 ± 6.0
2	12	11	15	16	10	20	14.0 ± 3.7
3	8	10	8	12	14	16	11.3 ± 3.3
4	6	10	11	9	19	15	11.7 ± 4.6
6	4	6	10	8	10	13	8.5 ± 3.2
8	3	4	6	6	5	14	6.3 ± 3.9
12	2	6	6	3	2	3	3.7 ± 1.9
16	1	5	6	2	1	3	3.0 ± 2.1
24	1	3	3	1	2	2	2.0 ± 0.9
30	1	1	2	2	3	2	1.8 ± 0.8
36	1	3	1	2	2	2	1.8 ± 0.8

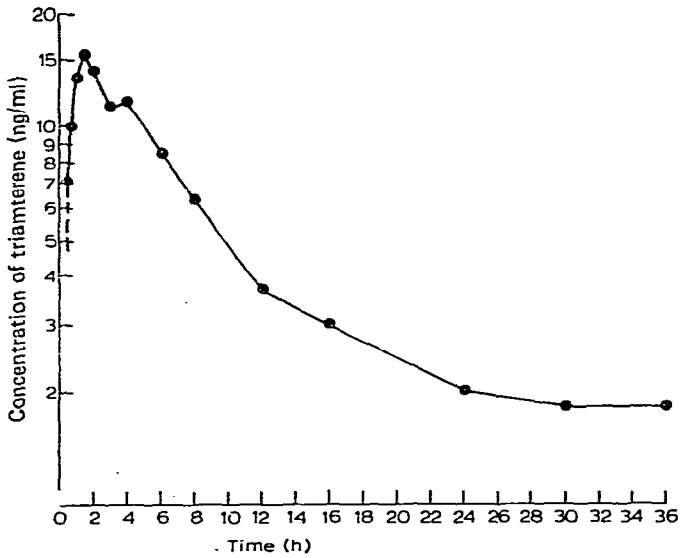

Fig. 3. Mean plasma concentrations of triamterene during 36 h after an oral dose of 50 mg of drug in a capsule. Semilogarithmic scale.

parent drug in plasma of 15.5 ng/ml was reached at 1.5 h after dosing (Table II) and was disproportionately lower than peak levels reported after oral doses of 100 mg of [^{14}C]-triamterene [8]. Thereafter mean concentrations declined to 8.5 ng/ml at 6 h, to 3.7 ng/ml at 12 h and to low but maintained levels of about 2 ng/ml between 24 and 36 h. Between 1.5 and 12 h, mean plasma concentrations declined with a half-life of about 5 h (Fig. 3), a value about twice that reported by Pruitt et al. [8] but the data indicate that this may not be the terminal elimination phase (Fig. 3).

Concentrations of triamterene in urine

During 24 h after single oral doses of triamterene, a mean of 1.6% dose was excreted in the urine (Table III) as parent drug, which is somewhat lower than

TABLE III

EXCRETION OF UNCHANGED TRIAMTERENE IN URINE (0—24 h) AFTER SINGLE ORAL DOSES OF 50 mg

Subject No.	Concentration (ng/ml)	Amount excreted (mg)	Dose excreted (%)
1	530	0.76	1.52
2	410	0.71	1.42
3	720	1.37	2.74
4	390	0.36	0.72
5	180	0.26	0.52
6	1300	1.43	2.86
Mean ± S.D.	588 ± 391	0.82 ± 0.49	1.63 ± 0.99

was reported after higher doses of the drug [8]. There was a six-fold variation in the proportion of triamterene excreted unchanged in 0—24 h urine.

ACKNOWLEDGEMENT

We are grateful to Dr. R. Bonn of Pharma Schwarz, Monheim, G.F.R. for helpful discussions and for supply of the analytical standards.

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