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Note

Rapid assay for triamterene in plasma *

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A great deal of work has been done on the pharmacokinetics and pharmacodynamics of triamterene in animals [1—6]. Although Badinand et al. [7] have measured the serum concentrations of triamterene in humans and obtained profiles up to 5 h post administration in both normal volunteers and patients, no detailed pharmacokinetic studies in humans are available, possibly because of the lack of a rapid, sensitive and selective assay method for the drug in biological fluids.

This paper describes such an assay based on extraction of triamterene as the perchlorate ion pair from plasma and high-performance liquid chromatography (HPLC) of the extract coupled with fluorescence detection.

EXPERIMENTAL

Chemicals and Reagents

Triamterene (USP Reference Standard) was dissolved in water at the concentration of 100 μg/ml and diluted to 1 μg/ml in either blank plasma (for the standard curves) or in methyl isobutyl ketone (for the recovery experiments). Appropriate dilutions in the respective media were made to give the desired concentrations. Outdated plasma, obtained from the Canadian Red Cross, was used for blanks and calibration curves. Dyrenium tablets (50 mg triamterene, Smith, Kline & French, Montreal, Canada) were purchased locally.

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The solvents used for chromatography were from Burdick & Jackson (Muskegon, Mich., U.S.A.). All other chemicals were of reagent grade, purchased locally. Perchloric acid, 3 *M*, was prepared with fluorescence-grade water. Methyl isobutyl ketone was saturated with 1 *M* perchloric acid before use.

HPLC

The chromatographic equipment consisted of a Waters Model 6000A pump and a U6K injector; the column, 250 × 3.2 mm I.D. stainless steel, was slurry packed with LiChrosorb Si 60, 5 μm (BDH, Toronto, Canada). The detector, Schoeffel LS990, was set at 335 nm excitation with a Schoeffel No. 7-54 filter (broad band pass centered at 320 nm) and the emission monitored using a sharp cut filter at 470 nm.

The chromatographic mobile phase consisted of dichloromethane—hexane—methanol—70% perchloric acid (57:35:8:0.1) at a flow-rate of 2 ml/min.

Thin-layer chromatography (TLC)

Concentrated effluents from HPLC, untreated urine or urine extracts were spotted on silica gel plates (Quanta/gram LQ6F, Quantum Ind., Fairfield, N.J., U.S.A.) and developed in ethyl acetate—methanol—25% aqueous ammonia (60:30:10) according to the method of Grebian et al. [5]. Triamterene and its metabolites were located visually by fluorescence under 360 nm light.

Treatment of the human volunteers

Two healthy male volunteers were given triamterene in the form of Dyrenium tablets. Subject 1 was administered one tablet (50 mg) after a normal light breakfast; subject 2 was fasted overnight, then administered four tablets (200 mg total). Venous blood was withdrawn into heparinized evacuated tubes (Vacutainers, Becton-Dickinson, Toronto, Canada) just prior to the dose, and at appropriate intervals thereafter (volunteer 1: 0.5, 1, 2, 3, 5 and 7.7 h; volunteer 2: 0.5, 1, 2, 3, 5, 8, 24 and 32.5 h). The blood samples were immediately centrifuged and the separated plasma kept at −18° until analysis. Urine was collected from subject 2 on the day of the experiment, giving a 7 h combined sample, which was kept at −18° until used.

Procedure

To 0.5 ml plasma or urine in a 10-ml round-bottomed glass tube fitted with a PTFE-lined screw cap, 0.25 ml 3 *M* perchloric acid was added. After mixing briefly on a vortex-type mixer, 1 ml methyl isobutyl ketone was added, and the mixture was shaken vigorously (Evapo-Mix, Buchler Instruments, Fort Lee, N.J., U.S.A.) for 5 min and centrifuged for 5 min at 3000 rpm using a table centrifuge (Model HN-S, International Equipment Co., Needham Heights, Mass., U.S.A.). An aliquot of exactly 50 μl of the supernatant was used for chromatography.

For the verification of the absence of interfering metabolites, plasma or urine extracts were chromatographed, and the eluted fractions corresponding to the peaks for triamterene and a possible metabolite collected separately. The fractions were washed with 0.1 *M* K_2HPO_4, the organic layers evaporated to dryness in a stream of nitrogen at 50° and redissolved in methyl isobutyl

ketone. Aliquots of these solutions, as well as that of the original extract and triamterene standard, were subjected to TLC as described above.

Quantitative analyses

The extraction efficiency was estimated by comparing the peak height obtained from spiked plasma with that from a standard solution of triamterene in methyl isobutyl ketone. The amount of the drug in plasma samples was estimated by comparing the peak height for the sample to a calibration curve, constructed daily, using spiked blank plasma.

RESULTS AND DISCUSSION

Extraction and chromatography

Triamterene was shown previously [8] to chromatograph on a silica gel column using a mobile phase of dichloromethane—hexane—methanol—perchloric acid. From its retention characteristics it was concluded that this drug, extracted from plasma as the perchlorate ion pair, should separate well from endogenous fluorescent materials. The extraction of the ion pair was expected to proceed with high efficiency in view of the three amino functions present in the molecule. Using the mobile phase described for quinidine, however, the retention time for triamterene was excessive (k' = 10.3). In order to reduce the time required for chromatography the methanol content of the mobile phase was increased from the original 5% to 8% at the expense of dichloromethane. Thus, with a mobile phase of dichloromethane—hexane—methanol—70% perchloric acid (57:35:8:0.1) the following k' values were obtained with authentic standards: triamterene, 4.6; 3-hydroxyquinidine, 4.2; quinidine, 2.7; 2′-quinidinone and dihydroquinidine, 2.4.

Chromatograms of plasma extracted and chromatographed as described above are shown in Fig. 1. While the maximum emission of triamterene is probably closer to 430 nm [5], better sensitivities were obtained at 470 nm, due to a quieter baseline. Blank plasma (Fig. 1A) does not appear to contain fluorescent material with retention characteristics similar to triamterene. The baseline noise is approximately 0.15 nA peak-to-peak. The inclusion of 10 ng drug per ml plasma (250 pg actually chromatographed) caused a peak of approximately 6 nA (Fig. 1B), which was well above the noise level.

For the recovery experiments blank plasma spiked with triamterene (100 ng/ml) was chosen. At this concentration the recovery was 88.6%. Since the calibration curve is a straight line passing through the origin (Table I), similar recoveries would be expected at all concentrations studied.

Fig. 1C and D represent traces obtained from plasma extracts from the two subjects 5 h after the 50 mg dose and 3 h after the 200 mg dose of the drug, respectively. Following the higher dose (Fig. 1D) a fluorescent peak appears with a k' value of 8.1. This substance has only been observed in the plasma and urine of subjects who have taken the drug.

Calibration curves

Preliminary experiments have shown the calibration curve to be linear to 1000 ng/ml with good coefficients of variation (C.V., 3.5% or less) between 100 and 1000 ng/ml. To verify the applicability of the method at lower con-

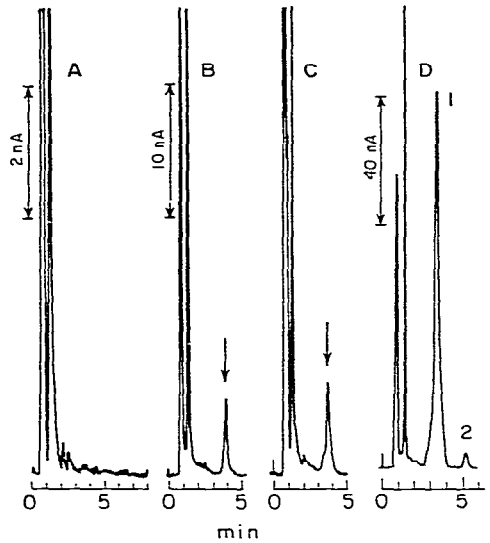

Fig. 1. HPLC of plasma extracts. A, blank plasma; B, blank plasma spiked with triamterene (10 ng/ml); C, plasma of volunteer 1, 5 h after an oral dose of 50 mg drug (estimated concentration 13.9 ng/ml); D, plasma of volunteer 2, 3 h after an oral dose of 200 mg drug (peak 1, triamterene, estimated concentration 350 ng/ml; peak 2, metabolite). Extraction and chromatography as described in the text.

TABLE I

CALIBRATION CURVE FOR TRIAMTERENE IN PLASMA

$n = 4$

Concn. (ng/ml plasma)	Peak height		Ratio peak height : concentration ($nA/ng \cdot ml^{-1}$)
	Mean (nA)	C.V. (%)	
2	0.81	9.26	0.405
4	1.54	6.15	0.385
6	2.29	2.75	0.382
8	3.06	5.73	0.383
10	3.98	3.16	0.398
20	7.39	3.04	0.370
30	11.85	0.84	0.395
40	15.00	1.22	0.375
50	19.15	4.85	0.383

centrations, nine concentrations from 2 to 50 ng/ml were prepared in blank plasma and assayed in quadruplicate (Table I).

Linearity was found to be good down to 2 ng/ml as shown by the steady values for the ratio of peak height to concentration; the coefficients of variation were good down to 4 ng/ml, and acceptable (<10%) at 2 ng/ml.

Interferences

As already mentioned, no endogenous interferences were found in blank plasma (Fig. 1A). However, to verify the identity and purity of the chroma-

tographic peaks, plasma from volunteer 2 (see below), collected between 1 and 3 h following the dose, was pooled, extracted and subjected to HPLC by the method described. Both the triamterene peak and the probable metabolite (peaks 1 and 2, respectively, Fig. 1D) were collected. Chromatography by TLC of these samples revealed a single spot for each peak, but the amounts obtained were judged to be too small to detect lesser contaminants. Since urine was shown by Lehman [4] to contain sizable amounts of the major metabolites of triamterene, the experiment was repeated using the 0—7 h urine of the same volunteer. The TLC of the urine extracts subjected to HPLC, as well as the original urine, urine extract and triamterene standard, gave the R_F values listed in Table II. Urine gave four spots: one unidentified, near the origin, and three

TABLE II

TLC OF TRIAMTERENE AND A METABOLITE EXTRACTED FROM URINE

Sample	R_F			
	Spot 1	Spot 2	Spot 3	Spot 4
Urine	0.09	0.51	0.66	0.77
Urine extract	0.06	—	0.63	0.75
Triamterene	—	—	—	0.77
LC peak 1	—	—	—	0.77
LC peak 2	—	—	0.63	—
Hydroxytriamterene sulphate*	—	0.40	—	—
Hydroxytriamterene *	—	—	0.52	—
Triamterene*	—	—	—	0.65

*Values published by Grebian et al. [5].

others having R_F values in the same order as those found by Grebian et al. [5] for hydroxytriamterene sulphate (spot 2), hydroxytriamterene (spot 3) and triamterene (spot 4). (The difference between the published R_F values and those obtained here may be due to differences in experimental conditions, for example the degree of activation of the TLC plates.) The organic extract gave three spots: the unidentified one near the origin, the presumed hydroxytriamterene and triamterene. Only the fastest-moving spot could be detected when peak 1 (Fig. 1D) from the HPLC was re-run on TLC, and this spot had an R_F value identical to that of authentic triamterene. The second peak from the HPLC also gave a single spot on TLC, with an R_F value similar to that reported for hydroxytriamterene by the above authors.

Quinidine, a commonly used fluorescent drug, did not interfere with the assay. One of its metabolites, 3-hydroxyquinidine, had retention characteristics similar to triamterene [8]. However, because of the less than optimal fluorescence at the wavelengths used and the relatively low amounts expected in plasma, there should be no significant interference from this substance. No other drugs were tested for interference.

Plasma concentrations in volunteers

The method was applied to the analysis of the drug in the plasma of two

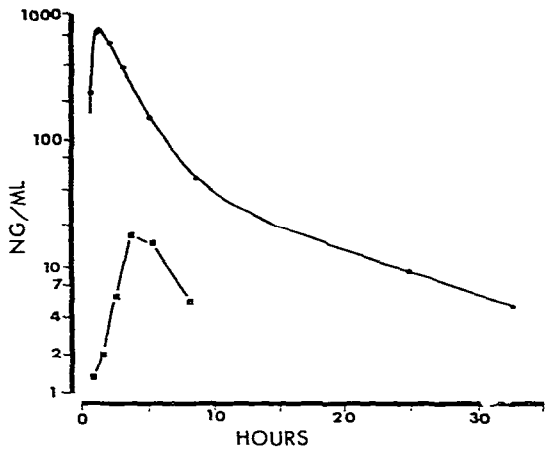

Fig. 2. Plasma concentration profiles of triamterene following a single oral dose of 50 mg (▪) and 200 mg (•) of triamterene to two different volunteers. Upper curve (volunteer 2): non-linear least-square regression fit of the experimental values to a two-compartment open model according to eqn. 2-207 of Wagner [9]. Estimated parameters: R^2, 0.997; K_a, 3.43 h^{-1}; α, 0.58 h^{-1}; β, 0.079 h^{-1}; A_1^*, 998 ng/ml; A_2^*, 61 ng/ml; A_3^*, −1059 ng/ml; lag time, 0.43 h. Assay as described in the text.

volunteers who had taken triamterene orally (Fig. 2). Plasma concentrations of volunteer 1, who was given one 50-mg tablet after a light breakfast, reached a peak of 16.2 ng/ml at 3 h, falling to 6.1 ng/ml at 7.7 h. In volunteer 2, who was given 200 mg of the drug (4 × 50-mg tablet) after overnight fasting, a peak plasma concentration of 706 ng/ml occurred at 1 h, followed by an apparent biphasic decay.

Badinand et al. [7] showed there was a great deal of individual variation in triamterene concentrations in human plasma following the administration of a single dose of 200 mg to fasted normal humans, with peak concentrations of 400—3000 ng/ml occurring at about 1 h. The second subject in our study was well within this range. However, subject 1 showed concentrations well below those expected from dose-independent kinetics. Whether this discrepancy was due to dose dependence, first pass metabolism, dietary interference or malabsorption cannot be ascertained without using a much larger number of volunteers. However, the rapidity and sensitivity of the method should make such studies feasible. Furthermore, the simplicity and selectivity of this method should also make it useful in clinical, toxicological and bioavailability studies.

REFERENCES

1 J.L. McNay and P.G. Dayton, Science, 167 (1970) 988.
2 A.W. Pruitt, J.L. McNay and P.G. Dayton, Drug Metab. Disp., 3 (1975) 30.
3 S.T. Kau and B.V.R. Sastry, J. Pharm. Sci., 66 (1977) 53.
4 K. Lehmann, Arzneim.-Forsch., 15 (1965) 812.
5 B. Grebian, H.E. Geissler and E. Mutschler, Arzneim.-Forsch., 26 (1976) 2125.
6 M. Stopp and H. Bräunlich, Acta Biol. Med. Ger., 30 (1973) 121.
7 A. Badinand, J. Rondelet, J.-J. Vallon and O. Chappuis, J. Med. Lyon, 1047 (1964) 101.
8 S. Sved, I.J. McGilveray and N. Beaudoin, J. Chromatogr., 145 (1978) 437.
9 J.G. Wagner, Fundamentals of Clinical Pharmacokinetics, Drug Intelligence Publications Inc., Hamilton, Ill., 1975.