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Liquid Chromatographic Analysis of Triamterene and Its Major Metabolite, Hydroxytriamterene Sulfate, in Blood, Plasma, and Urine

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Abstract □ The first rapid and highly sensitive high-performance liquid chromatographic (HPLC) assay for triamterene, hydroxytriamterene, and hydroxytriamterene sulfate is reported. Plasma samples were processed by protein precipitation, while urine was used untreated. Three different solvent systems were used to analyze (*a*) triamterene in plasma (30% acetonitrile, pH 4.0; internal standard: furosemide; sensitivity limit: 1 ng/mL); (*b*) hydroxytriamterene and hydroxytriamterene sulfate in plasma (12% acetonitrile, pH 5.5; internal standard: cefamandole; sensitivity limits: 20 and 2 ng/mL, respectively) and (*c*) triamterene, hydroxytriamterene, and hydroxytriamterene sulfate in urine (13% acetonitrile, pH 5.3; internal standard: hydroflumethiazide; sensitivity limits: 0.04 μg/mL, 0.5 μg/mL, and 0.1 μg/mL, respectively). Fluorescence detection of compounds was performed at 365-nm excitation and 440-nm emission wavelengths. Recovery of triamterene and its metabolites from plasma was complete, and calibration curves were linear. Intraday variation was <6% except for the lowest plasma concentration. The assay procedure has already been used in several pharmacokinetic studies.

Keyphrases □ Triamterene—HPLC analysis, major metabolites, hydroxytriamterene sulfate, blood, plasma, and urine □ Hydroxytriamterene sulfate—HPLC analysis, major metabolite of triamterene, blood, plasma, and urine

Although triamterene has been clinically available since the 1960's, the metabolic pathways for this drug have been a matter of controversy, with conflicting reports appearing up through 1982. Early work by Lehmann (1) indicated that triamterene undergoes hydroxylation and subsequent sulfation to hydroxytriamterene sulfate, which he found in urine. Pruitt *et al.* (2), using TLC separation, could only detect hydroxytriamterene, whereas Grebian *et al.* (3) and our group (4, 5) did find the sulfate ester of hydroxytriamterene using TLC techniques.

More recently, three HPLC techniques for triamterene analysis have been published (6-8). One of these research groups (6) assumed, on the basis of the work of Pruitt *et al.* (2), that hydroxytriamterene was the major metabolite of triamterene and did not develop a method to specifically measure the sulfate conjugate. The other two groups did not measure any metabolites. The first HPLC assay by which the specific measurement of hydroxytriamterene sulfate can be obtained and by which very low concentrations of unchanged triamterene and hydroxytriamterene sulfate can be quantitated is reported herein. The method is simple, fast, and accurate.

EXPERIMENTAL

Reagents—Triamterene[1], hydroxytriamterene[2], hydroxytriamterene sulfate[2], and the internal standards hydroflumethiazide[3], furosemide[4], and cefamandole[5] were used as received. Acetonitrile[6] for the HPLC measurements was glass-distilled; water was redistilled and stored in glass.

Apparatus—A high-performance liquid chromatograph[7] equipped with a sample processor[8], a fluorescence spectrophotometer[9], and a UV detector[10] was used. The assay was carried out on a 10-μm particle size, 4 × 300-mm reverse-phase column[11] at a solvent flow rate of 2 mL/min.

Mobile Phase and Retention Times—Three different solvent systems were used for the measurement of triamterene, hydroxytriamterene, and hydroxytriamterene sulfate. Each solvent system (A-C, as indicated in Table I) was used for a particular quantitation problem.

Detection Wavelengths—Triamterene, hydroxytriamterene, and hydroxytriamterene sulfate as well as the internal standards furosemide and hydroflumethiazide were analyzed using their native fluorescence at an excitation wavelength of 365 nm and an emission wavelength of 440 nm. Cefamandole, which was used as an internal standard in mobile phase B, was measured by UV detection at 254 nm. For that purpose the HPLC system was equipped with two detector systems, one for fluorescence and another for UV detection.

Methods—Each plasma or total blood sample (0.1 mL) was deproteinated by adding 0.4 mL of acetonitrile containing both furosemide (3.5 μg/mL) and cefamandole (60 μg/mL) internal standards. After mixing[12] for 1 min, the sample was centrifuged[13] for 10 min at 3200 rpm. The supernatant was transferred to a clean test tube and, if necessary, evaporated at room temperature to 0.2 mL under nitrogen when very low concentrations had to be measured. However, most of the samples could be analyzed by injection of a volume as little as 5 μL.

Urine samples ranging from 0.02 to 0.1 mL were added to 1 mL of an acetonitrile solution containing hydroflumethiazide as an internal standard (500 μg/mL). After mixing[12] and centrifugation[13], 2 μL was injected onto the HPLC system.

Human plasma, blood, and urine were stored at −20°C. Blank plasma and blood for assay development was obtained from healthy volunteers using 143 IU/10 mL of lithium heparin as anticoagulant. To test the stability of tri-

[1] Mylan Pharmaceuticals Inc., Morgantown, W.Va.
[2] Röhm-Pharma, Darmstadt, West Germany.
[3] Le Pharmaceutical Products, 2750 Ballerup, Denmark.
[4] Hoechst Pharmaceuticals Inc., Somerville, N.J.
[5] Eli Lilly & Co., Indianapolis, Ind.
[6] J. T. Baker Chemical Co., Phillipsburg, N.J.
[7] Perkin-Elmer Series 3, Norwalk, Conn.
[8] WISP Model 710A; Waters Associates, Milford, Mass.
[9] 204 S; Perkin-Elmer, Norwalk, Conn.
[10] LC 65 T; Perkin-Elmer, Norwalk, Conn.
[11] Micro Pak MCH; Varian, Los Altos, Calif.
[12] Vortex-Genie Mixer; Scientific Industries Inc., Bohemia, N.J.
[13] IEC HN-SII Centrifuge, Damon IEC Div.; Needham Heights, Mass.

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Table I—Retention Times (min) for Triamterene, Its Metabolites, and the Internal Standards in Three Different Solvent Systems

Compound	Mobile Phase A[a]	Mobile Phase B[b]	Mobile Phase C[c]
Triamterene	5.7	—	8.6
Hydroxytriamterene	—[d]	9.0	4.8
Hydroxytriamterene sulfate	—	5.7	3.2
Furosemide (I.S.)[e]	8.1	—	—
Cefamandole (I.S.)	—	8.7	—
Hydroflumethiazide (I.S.)	—	—	7.0

[a] 30% Acetonitrile in 0.02% H_3PO_4, adjusted to pH 4 with NaOH (for plasma or blood). [b] 12% Acetonitrile in 0.02% H_3PO_4, adjusted to pH 5.5 with NaOH (for plasma or blood). [c] 13% Acetonitrile in 0.02% H_3PO_4, adjusted to pH 5.3 with NaOH (for urine). [d] — = Not measured. [e] I.S. = internal standard.

amterene and its primary metabolite over time, biological samples (over the concentrations measured) were analyzed after 6 and 12 months storage at −20°C. They did not show any degradation. On the basis of our work it appears that samples can be stored for more than 1 year at −20°C without significant degradation.

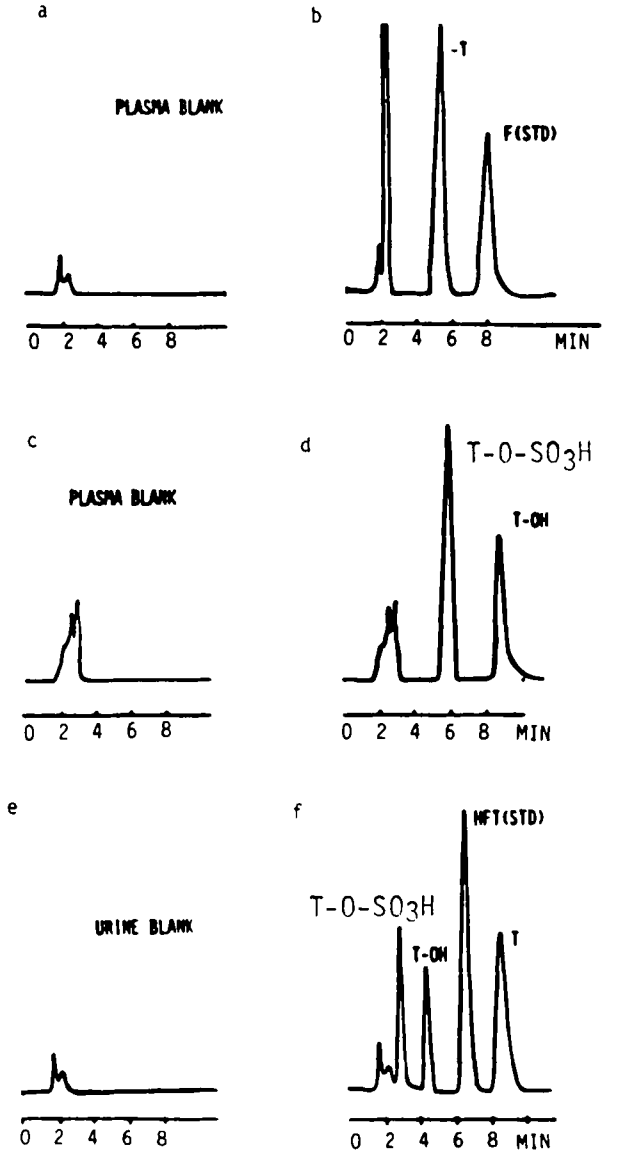

Figure 1– *Chromatograms developed using methods A–C. (Cefamandole was measured by UV and is not shown here.) Key: (a) blank plasma (method A); (b) actual plasma sample (method A); (c) blank plasma sample (method B); (d) actual plasma sample (method B); (e) blank urine (method C); (f) actual urine sample (method C); (STD) internal standard; (T) triamterene; (T-OH) hydroxytriamterene; (T-O-SO$_3$H) hydroxytriamterene sulfate; (F) furosemide; (HFT) hydroflumethiazide.*

Table II—Recovery of Triamterene and Hydroxytriamterene Sulfate from Human Plasma

Compound	Plasma Conc., ng/mL	Recovery, %
Triamterene	1.21	118.1
	3.27	100.0
	5.24	103.4
	9.83	97.6
	19.7	101.9
	32.8	98.6
	49.2	99.3
Hydroxytriamterene sulfate	10.8	96.6
	21.6	101.8
	32.4	100.7
	54.0	103.4
	86.4	103.3
	108.0	100.0
	215.0	97.4

RESULTS

Three different solvent systems were used to analyze triamterene, hydroxytriamterene, and hydroxytriamterene sulfate in plasma and urine (Table I).

Triamterene (Mobile Phase A)—Triamterene was analyzed using a 30% acetonitrile in 0.02% H_3PO_4 solvent system, adjusted to pH 4.0 with 0.5 M NaOH. As can be seen in Fig. 1a and b these conditions allowed excellent separation of triamterene (retention time 5.7 min) from endogenous materials. The internal standard, furosemide, has a retention time of 8.1 min, which means that the total run time for one sample is <10 min, allowing a large number of samples to be measured each day. Standard curves for triamterene obtained using mobile phase A exhibited linearity over the concentration range of 1–50 ng/mL. The limit of quantitation was 1 ng/mL.

Hydroxytriamterene (Mobile Phases B and C)—Hydroxytriamterene, had it been present in actual plasma or urine samples, could have been detected with solvent systems B and C. Since the retention times for hydroxytriamterene and the internal standard, cefamandole, were so close with mobile phase B (Table I), plasma samples with and without the internal standard were injected onto the column. Hydroxytriamterene peaks were not detected in either plasma or urine from healthy volunteers even when the instruments were set to the highest sensitivity. Therefore, no further attempts were made to fully establish a method of quantitation. The lower limit of quantitation was 20 ng/mL in plasma and 0.5 μg/mL in urine.

Hydroxytriamterene Sulfate (Mobile Phase B)—Hydroxytriamterene sulfate was separated from hydroxytriamterene and from endogenous compounds in solvents B and C with retention times of 5.7 and 3.2 min, respectively, in each solvent. Cefamandole used as an internal standard for mobile phase B had a retention time of 8.7 min, maintaining the total run time for hydroxytriamterene sulfate below 10 min (see representative chromatograms, Fig. 1c and d). Standard curves for hydroxytriamterene sulfate were shown to be linear over a 10–215-ng/mL concentration range. The lower limit of quantitation was 2 ng/mL.

Urine Assay (Mobile Phase C)—In urine, all three triamterene compounds can be measured simultaneously with the internal standard hydroflumethi-

Table III—Within-Day Assay Precision for Triamterene and Hydroxytriamterene Sulfate in Plasma

Plasma Conc., ng/mL	Peak Height Ratio[a]	*CV*, %
	Triamterine	
1.21	0.080 ± 0.010	12.6
3.27	0.180 ± 0.003	1.4
5.24	0.279 ± 0.013	4.7
9.83	0.537 ± 0.018	3.4
19.7	1.087 ± 0.044	4.0
32.8	1.800 ± 0.050	2.8
49.2	2.745 ± 0.094	3.4
	Hydroxytriamterene Sulfate	
10.8	0.295 ± 0.019	6.4
21.6	0.615 ± 0.011	1.9
32.4	0.861 ± 0.024	2.8
54.0	1.62 ± 0.10	5.9
86.4	2.52 ± 0.13	5.0
108.0	3.07 ± 0.12	4.0
215.0	6.17 ± 0.04	0.6

[a] Mean ± *SD*; *n* = 4.

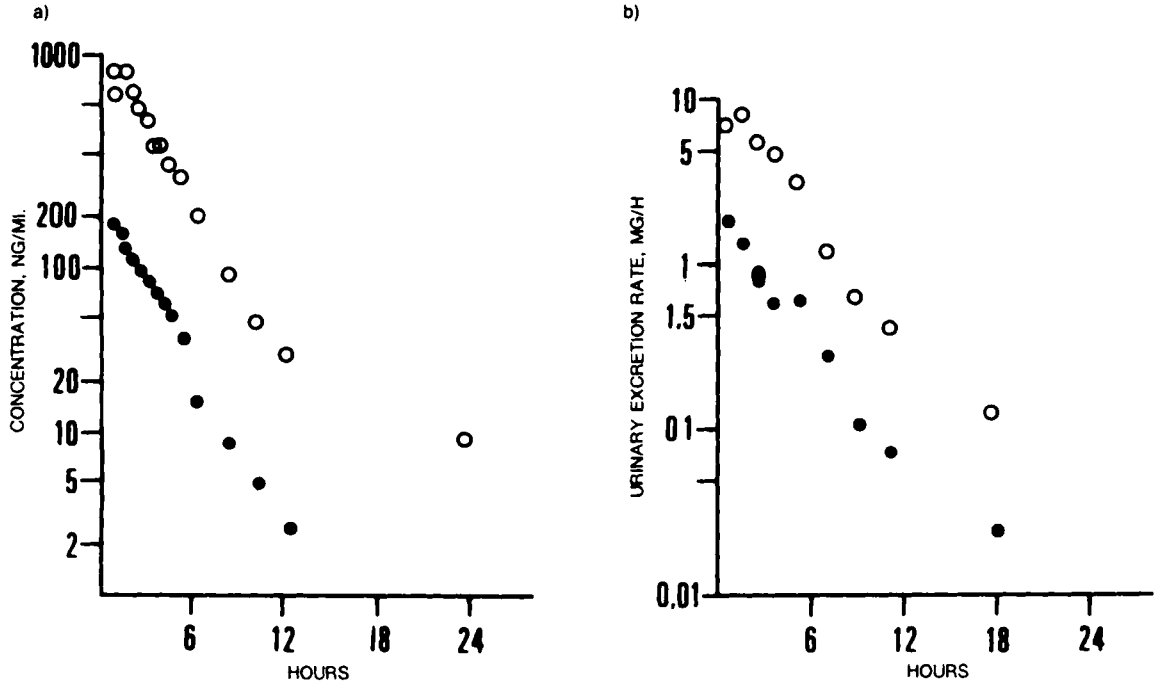

Figure 2—*Semilogarithmic plasma concentration* versus *time plot (a) and urinary excretion rate* versus *midpoint time (b) following a 100-mg oral suspension dose of triamterene to a healthy volunteer. Key: (●) triamterine; (○) hydroxytriamterine sulfate.*

azide (Fig. 1e,f); the limits of quantitation of triamterene and hydroxytriamterene sulfate were 0.04 μg/mL and 0.1 μg/mL, respectively. Standard curves for these two compounds were prepared over the following concentration ranges: triamterene, 0.15–2.35 μg/mL; hydroxytriamterene sulfate, 0.75–57.0 μg/mL.

Recovery—The recoveries of triamterene and hydroxytriamterene sulfate from plasma were determined by acetonitrile precipitation. The peak height ratios of samples prepared with plasma and water were compared. As shown in Table II, the recovery of triamterene and hydroxytriamterene sulfate was complete.

Within-Day Precision—The within-day precision was assessed by conducting replicate analyses. As shown in Table III, the coefficients of variation ranged from 0.5–6.4 for all concentrations except the lowest plasma concentration (12.6% *CV*), indicating good precision for the assay.

Blood Sample Assays—Analysis of triamterene and hydroxytriamterene sulfate were undertaken so as to determine the blood-to-plasma ratios of these compounds. The blood–plasma ratios were examined in two subjects and found to be constant over the concentration range measured following a 100-mg oral dose of triamterene (9). The ratio was 1.03 ± 0.14 for triamterene and 0.60 ± 0.07 for hydroxytriamterene sulfate.

DISCUSSION

There have been several reports in the literature regarding the quantitation of triamterene and its metabolites in humans. On the basis of a probably inappropriate TLC procedure, Pruitt *et al* (2) concluded that the major metabolite of triamterene was hydroxytriamterene rather than the sulfate conjugate (1, 3, 4).

Following the detection of hydroxytriamterene sulfate, but not hydroxytriamterene, in actual biological specimens and by using synthetic hydroxytriamterene sulfate as a reference compound, it was possible to develop a procedure to quantitatively measure hydroxytriamterene sulfate in urine and plasma. Hydroxytriamterene, the intermediate metabolite of triamterene, was not detected in any study. Had it been present, it could have been detected with our HPLC method. The most recent report on the measurement of triamterene and metabolites of Yakatan *et al.* (6) only provides information about hydroxytriamterene measurements, not mentioning the sulfate conjugate. The techniques described here have been used successfully in several pharmacokinetic studies in both humans and dogs (4, 5, 9) and are suitable to quantify triamterene and its metabolite, hydroxytriamterene sulfate.

The results of a representative experiment in a healthy volunteer receiving a 100-mg oral suspension dose of triamterene are shown in Fig. 2a and b. As indicated earlier no hydroxytriamterene could be detected in either plasma or urine. The concentration of hydroxytriamterene sulfate exceeded that of unchanged triameterene ~10-fold in plasma and ~5-fold in urine, indicating that hydroxytriamterene sulfate is the major metabolite of triamterene. Further analysis of the pharmacokinetic data in humans using the analytical techniques described here has been recently reported (9).

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