*Journal of Chromatography*, 413 (1987) 315—319
*Biomedical Applications*
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3381

Note

# Rapid method for the determination of the diuretic triamterene and its metabolites in plasma and urine by high-performance liquid chromatography

KENNETH JOHN SWART* and HANLIE BOTHA

*Department of Pharmacology, Faculty of Medicine, University of the Orange Free State, Bloemfontein 9301 (South Africa)*

(First received February 24th, 1986; revised manuscript received August 11th, 1986)

Triamterene (2,4,7-triamino-6-phenylpteridine) is a potassium sparing diuretic which is widely used. The main metabolites, *p*-hydroxytriamterene sulphate ester (conjugated metabolite) and *p*-hydroxytriamterene (unconjugated metabolite) are also active [1, 2]. We have previously found that the concentration of the unconjugated metabolite was negligible compared to the concentrations of the other two and therefore can be ignored. Although a number of articles have been published dealing with the determination of triamterene, most of them required extractions [3—5] or special applicators for thin-layer chromatography [6].

In this paper we describe a rapid, sensitive high-performance liquid chromatographic (HPLC) method for the determination of triamterene and its main conjugated metabolite which requires no extraction of body fluids. The unconjugated metabolite can, if necessary, also be determined without any change to the method.

## EXPERIMENTAL

### *Reagents*

Triamterene, *p*-hydroxytriamterene and *p*-hydroxytriamterene sulphate ester were obtained from Smith Kline and French Labs. (Philadelphia, PA, U.S.A.). All the reagents were of guaranteed reagent grade (Merck, Darmstadt, F.R.G.) and were used as received.

The buffer for the mobile phase was prepared by adding 6.75 ml of concentrated phosphoric acid (89%) to approximately 900 ml of double-distilled water. The pH was adjusted to 2.8 by the dropwise addition of triethylamine and the volume adjusted to 1 l with distilled water.

0378-4347/87/$03.50 

*Apparatus*

An M-49 pump and a WISP autosampler (Waters Assoc., Milford, MA. U.S.A.) were coupled to a Waters Nova-Pak $C_{18}$ (5 μm) stainless-steel column (15 cm x 4.6 mm). A Fluorichrom fluorescence detector (Varian, Walnut Creek, CA, U.S.A.) was used to measure fluorescence of the eluate. The results were processed on a Waters 730 data module in the peak-height mode.

*Plasma and urine standards*

An accurately weighed amount of triamterene and the conjugated metabolite were dissolved in methanol and 0.05 *M* sodium hydroxide, respectively. Plasma standards were prepared by spiking drug-free plasma with triamterene up to 100 ng/ml and the conjugate up to 400 ng/ml. These plasma standards were divided into 300-μl aliquots and stored at − 20°C in Venoject tubes.

The maximum concentrations for urine standards which were similarly prepared and stored were 3 μg/ml for triamterene and 10 μg/ml for the conjugate.

*Procedure*

Plasma samples (150 μl) were transfered to 400-μl Microfuge tubes (Beckman EET 23), and the protein was precipitated with 10 μl of a 70% perchloric acid solution. After shaking for 15 min on a Wig-L-Bug (Crescent Dental MFG, Chicago, IL, U.S.A.) the tubes were centrifuged for 10 min at 900 *g*, and 30 μl of the supernatant injected for analysis.

Aliquots of freshly collected urine were diluted with volumes of methanol twice that of the urine samples and stored at − 20°C until analysis could be performed. This procedure was necessary since it was observed that storage of untreated urine resulted in the formation of an insoluble complex between triamterene and urinary components leading to inaccurate and misleading results. The urine samples were centrifuged in the same way as the plasma samples, and 5 μl injected for analysis.

*Chromatography*

The mobile phase was buffer — acetonitrile — methanol (70:14:8). A constant flow-rate of 0.8 ml/min was maintained at ambient temperature (24°C) through the column. Excitation energy was obtained through glass band filters 7-60 and 7-54 giving an excitation band maximum at 340—380 nm while emission energy was monitored above 400 nm by using glass cut-off emission filter 3-73, which cut off energy below 400 nm, combined with a wide-band filter 4-76, which prevents transmission of long-wavelength red leakage above 600 nm. Retention times for the conjugated metabolite, unconjugated metabolite and triamterene were 1.9, 2.4 and 3.3 min, respectively.

## RESULTS AND DISCUSSION

Figs. 1 and 2 show representative chromatograms obtained for plasma and urine determinations and demonstrate the lack of interfering endogenous compounds.

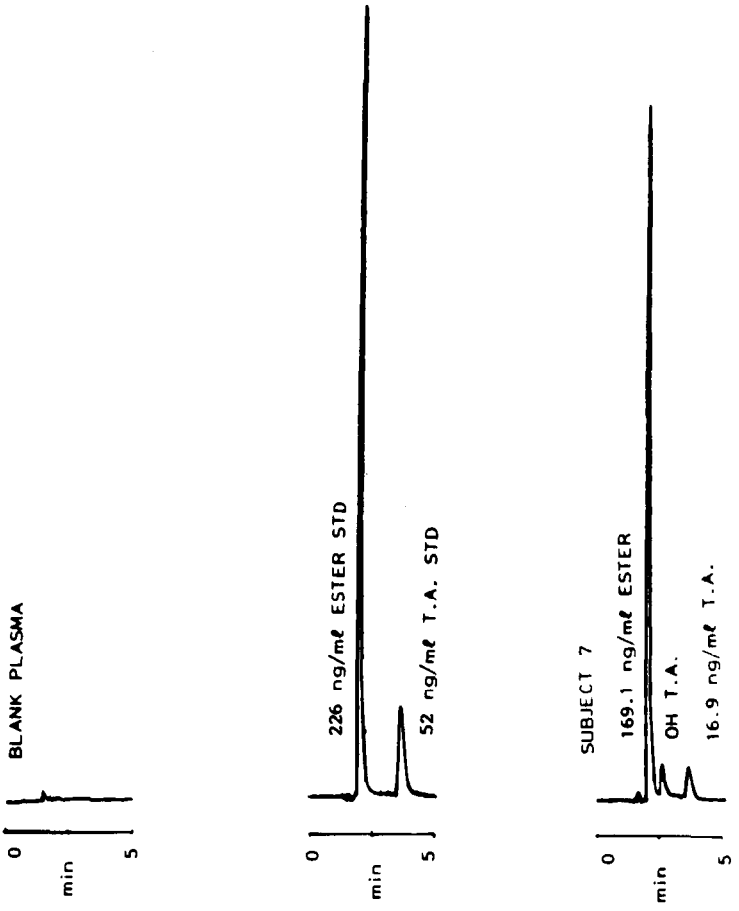


Fig. 1. Typical chromatograms of plasma samples obtained 4 h after a 50-mg triamterene oral dosing procedure in a human volunteer. Peaks: T.A. = triamterene; ESTER = *p*-hydroxytriamterene sulphate ester. For HPLC conditions see text.

All the plasma calibration curves were shown to be linear over a wide concentration range with the curves almost passing through the origin. For ten replicates of five different concentrations the regression equations for triamterene between 4 and 100 ng/ml are $y = 5.1709 + 0.026046\,x$ ($r^2 = 0.9984$) and for the conjugated metabolite between 10 and 400 ng/ml $y = 0.1722 + 1.802\,x$ ($r^2 = 0.9981$) where $y$ is the concentration in ng/ml and $x$ the peak height in mV.

For the urine a power curve fitted best. For triamterene concentrations between 0.09 and 3.1 μg/ml the regression equations are $y = 0.000122\,x^{0.93032}$ ($r^2 = 0.9998$) and for the conjugated metabolite between 0.3 and 9.7 μg/ml $y = 0.000089\,x^{0.97892}$ ($r^2 = 0.9989$) where $y$ is the concentration in μg/ml and $x$ the peak height in mV.

The precision of the method is indicated in Tables I and II and is expressed as the coefficient of variation (%). A maximal coefficient of variation of 4.50%

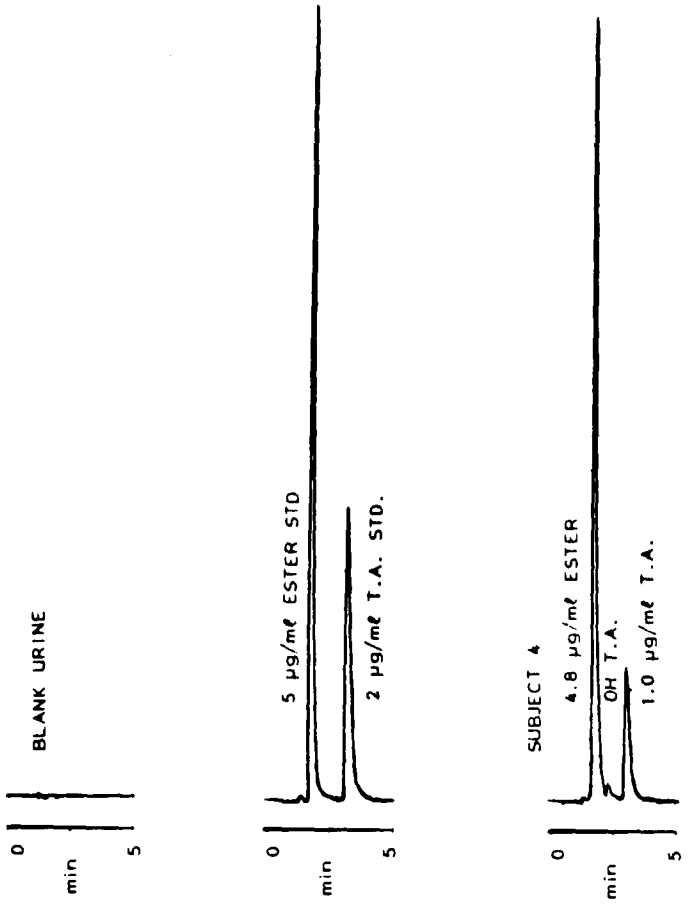


Fig. 2. Typical chromatograms of urine samples obtained 8 h after a 50-mg triamterene oral dosing procedure in a human volunteer. Peaks: T.A. = triamterene; ESTER = *p*-hydroxytriamterene sulphate ester. For HPLC conditions see text.

TABLE I

COEFFICIENTS OF VARIATION OF THE PEAK HEIGHTS AT VARIOUS CONCENTRATIONS TO INDICATE THE PRECISION OF THE METHOD IN PLASMA ($n = 10$)

| Triamterene | | Conjugated metabolite | |
|---|---|---|---|
| Concentration (ng/ml) | Coefficient of variation (%) | Concentration (ng/ml) | Coefficient of variation (%) |
| 4.88 | 4.50 | 10.26 | 4.50 |
| 9.76 | 3.71 | 51.30 | 3.90 |
| 19.52 | 4.35 | 102.60 | 3.93 |
| 48.80 | 2.62 | 205.20 | 2.12 |
| 97.60 | 2.31 | 410.40 | 3.20 |

TABLE II

COEFFICIENTS OF VARIATION OF THE PEAK HEIGHTS AT VARIOUS CONCENTRATIONS TO INDICATE THE PRECISION OF THE METHOD IN URINE ($n = 10$)

| Triamterene | | Conjugated metabolite | |
|---|---|---|---|
| Concentration (ng/ml) | Coefficient of variation (%) | Concentration (ng/ml) | Coefficient of variation (%) |
| 3.16 | 1.62 | 9.76 | 2.79 |
| 1.50 | 1.65 | 4.88 | 2.28 |
| 0.79 | 0.64 | 2.44 | 2.33 |
| 0.395 | 1.29 | 1.22 | 2.31 |
| 0.1975 | 1.17 | 0.61 | 2.62 |
| 0.0988 | 1.81 | 0.35 | 1.94 |

for both triamterene and the conjugated metabolite were found in plasma and 0.81% for triamterene and 2.79% for the conjugated metabolite in urine.

The samples were accurately determined and a mean difference from the spiked values of 1.70 ± 1.26% and 2.38 ± 2.07% in plasma and 2.36 ± 1.6% and 3.8 ± 1.45% in urine for triamterene and the conjugated metabolite, respectively, were obtained.

The limit of quantitation was 1 ng/ml at a signal-to-noise ratio of 2:1.

As mentioned before the unconjugated metabolite can also be determined if necessary without any change in the method since it has a retention time of 2.4 min compared to the 1.9 min of the conjugated metabolite, but the concentration is normally too low to be of any significance.

No interfering peaks were present in predose (blank) plasma samples taken from subjects during a clinical trial where they received 500 mg triamterene pure substance suspended in 2 ml polyethylene glycol 400.

The advantage of this method is that it requires no extraction, is very precise and accurate so that many samples may be analyzed in a relatively short time.

REFERENCES

1 L.S. Goodman and A. Gilman, The Pharmacological Basis of Therapeutics, Macmillan, New York, 7th ed., 1985, p. 902.
2 H. Knauf, E. Mutschler, K.-D. Völger and U. Wais, Arzneim.-Forsch., 28 (1978) 1417.
3 G.J. Yakatan and J.E. Cruz, J. Pharm. Sci., 70 (1981) 949.
4 R.R. Brodie, L.F. Chasseaud, T. Taylor and L.M. Walmsley, J. Chromatogr., 164 (1979) 527.
5 S.Sved, J.A.A. Sertié and I.J. McGilveray, J. Chromatogr., 162 (1979) 474.
6 B. Grebian, H.E. Geissler and E. Mutschler, Arzneim.-Forsch., 26 (1976) 2125.