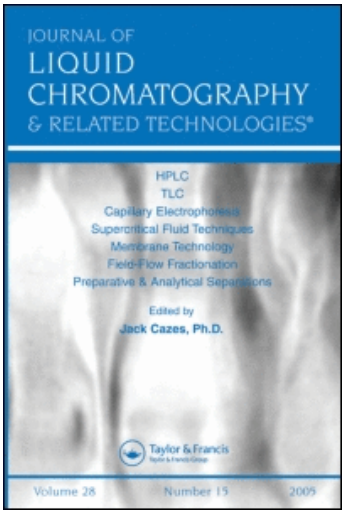

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SIMPLE AND FAST HPLC METHOD FOR THE DETERMINATION OF TRIAMTERENE AND HYDROXYTRIAMTERENESULPHATE IN PLASMA AND URINE

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ABSTRACT

Determination of triamterene (T) and hydroxytriamterenesulphate (HTS) in plasma and urine requires a very sensitive and selective method. As both of the substances are highly hydrophilic, it is hardly feasible to carry out an extraction. The method described obviates the need for pre-cleansing of samples. Plasma and urine samples, diluted with water, are directly injected into the HPLC-column and analysed. For the detection of both of the substances fluorescence was used. The use of a Spherisorb-NH2-column with a mobile phase, consisting only of a buffer solution in water, makes it possible to dispense with protein precipitation of plasma. Both substances were analysed within 2 minutes in a single run. Detection limits of 1 ng T and 20 ng HTS/ml plasma as required in practice, were obtained without any difficulties. Referring to the precision of this method with plasma samples, the variation coefficient was below 3 % with HTS in the range of 20 to 1100 ng HTS/ml. Day to day variation showed with T in plasma values of smaller than 7 % in the range of 1 to 100 ng/ml.

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INTRODUCTION

Triamterene is often administered with combined diuretic drugs, because it efficaciously preserves potassium in the body. As the main metabolite HTS is also effective (1), it should also be determined in bioavailability studies or clinical controls (Fig. 1). Several studies on the determination of T and/or HTS (2) have been published, some of them determine the T only (3) or HTS under varying chromatographical conditions (4,5).

DC is frequently used with fluorodensitometry, a method based upon a study dating back to the year 1976 (2). However there are also HPLC methods using fluorescence detection (3,4,5,6). Most of these studies use extraction steps, which requires a longer period of time.

This study describes the determination of T and HTS by HPLC during a single analytical run. Except for dilution, no other preparation of the urine and plasma samples is necessary. Besides, only very small quantities of as little as 50 mcl of plasma are required.

Both substances were analysed within 2 minutes, thus permitting a high number of samples to be analysed per day.

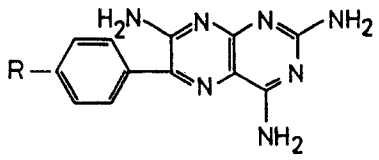
EXPERIMENTAL

Chemicals

Triamterene was supplied by Sagitta (Feldkirchen, FRG). Hydroxytriamterenesulphate and some Hydroxytriamterene was isolated from human urine in our laboratory. Reagents of GR quality were supplied by E. Merck (Darmstadt, FRG).

Apparatus and Chromatographic Equipment

The chromatographic system consisted of a LC 420 pump (Kontron, Zürich, CH), a Rheodyne injection valve type 7125 with a 20 μl loop (Cotati, CA, USA) and a fluorescence detector F 1000 (Merck-Hitachi, Darmstadt, FRG) (excitation 360 nm/



Triamterene: - R = -H

Hydroxytriamterenesulphate - R = $-OSO_3H$

FIGURE 1: Structure of Triamterene and Hydroxytriamterenesulphate

emission 436 nm). The analytical column 125 x 4 mm i.d., (SRD-Pannosch, Vienna, Austria) was filled with Spherisorb-Amino 5 μ (Phase Separation, USA). The mobile phase consisted of water with 0.01 M perchloric acid, 0.002 M triethylamin and 0.1 M ammoniumacetate. Chromatography was carried out at ambient temperature. The flowrate was 3.0 ml/min.

Methods

Preparation of plasma-samples

0.2 ml plasma was diluted with 0.6 ml water. 20 μl was injected with a loop.

Preparation of urine-samples

10 μl urine was diluted with 2 ml water. 20 μl was injected with a loop.

Preparation of Calibration Samples

Plasma: Pool-plasma was spiked with T in the range of 1-100 ng/ml and with HTS in the range of 20 - 1100 ng/ml. Dilution was carried out as indicated above.

Urine: Pool-urine was spiked with T in the range of 0.1 - 1.7 μg/ml and with HTS in the range of 5-54 μg/ml urine. Dilution was carried out as above.

RESULTS

Recovery

As only a single dilution process was carried out it amounts to 100 %.

Reproducibility and accuracy for plasma

The within-day standard deviation (Precision) for HTS in calibration samples was in the range of 0.2 - 2.2% (CV) and the day to day variation for T in calibration samples was between 1.8 - 6.8% (CV) (Table 1 and Table 2).

REPRODUCIBILITY of volunteer-samples showed the following results:

T in plasma concentration range	n	CV % range	median (CV %)
1- 10 ng/ml	7	0,0 - 17,9	3,8
20- 40 ng/ml	13	0,0 - 8,8	2,4
50-160 ng/ml	17	0,1 - 9,6	1,6

HTS in plasma concentration range	n	CV % range	median (CV %)
5- 100 ng/ml	6	0,1 - 18,5	3,2
100- 500 ng/ml	16	0,3 - 8,8	1,3
500-1000 ng/ml	17	0,0 - 5,8	2,0

Linearity

In the calibration series, the linear regression between the spiked plasma concentrations and the peak areas was determined after analysis of the calibration samples. When multiple analysis was carried out over the range from 1-100 ng T/ml plasma a typical correlation coefficient value of 0,9983 was obtained. With HTS in the range of 20-1100 ng/ml plasma a typical value of r was 0,9999. In practice, a detection limit of 1 ng/ml T and 5 ng/ml HTS was fixed, but this could be lowered without difficulty.

Proof of Method Specificity

Hydrochlorothiazide, Furosemide, Chlorthalidone, Bendroflumethiazide, Butizide,various ß-blocking agents, Nifedipine and Hydroxytriamterene within therapeutical ranges did not influence the determination of T and HTS in plasma and

TABLE 1

REPRODUCIBILITY and ACCURACY of plasma T determinations (day to day variation over 3 days)

Spiked value (ng/ml)	Number of samples	Assay value (ng/ml)	CV %	Accuracy day to day (3 days)
1.0	6	0.87	4.2	- 12.6 %
5.0	6	4.91	6.8	- 1.8 %
30.0	6	28.90	4.3	- 3.7 %
100.0	6	99.90	1.8	- 0.1 %

TABLE 2

PRECISION and ACCURACY of plasma HTS determinations (within day)

Spiked value (ng/ml)	Number of samples	Assay Value (μg/ml)	CV %	Accuracy within day
21.4	3	18.7	2.2	- 12.5 %
107.0	3	107.0	0.8	0.0 %
428.0	3	435.7	1.1	+ 1.8 %
1070.0	3	1067.0	0.2	- 0.3 %

urine. Some drugs have no fluorescence (EX 360 nm/EM 436 nm) and/or different retention times. Amiloride might interfere but was not tested in this pharmacokinetic study on volunteers.

Chromatography

Different types of columns were tested. On C18 and C8 the elution order of T and HTS was changed and both substances could not be determined within one run. On the other hand a certain percentage of methanol is necessary for elution in reversed phase, as ion-pairs or as free bases. For this reason plasma may not be injected without protein precipitation.

The retention times for HTS and T on Spherisorb-Amino were relatively stable but after injection of about 100 samples (plasma or urine), the column was cleaned with 60% methanol.

After injection of plasma and urine samples taken from 12 different volunteers before drug administration no interfering peaks were observed. Also after injection of volunteer-samples following oral administration of T, there were no subsequent interfering eluting peaks.

Precision for urine

Table 3 shows the results for HTS (0.9 - 2.1% CV) for calibraton samples and Table 4 for T (1.2 - 4.1% CV) for calibration samples.

In volunteer samples the reproducibility was lower than 5 % for HTS and for T..

TABLE 3

PRECISION and ACCURACY of urine HTS determinations (within day)

Spiked value (μg/ml)	Number of samples	Assay Value (μg/ml)	CV%	Accuracy
5.35	3	5.40	1.0	+ 0.1 %
21.4	3	21.02	2.1	- 1.8 %
53.5	3	53.53	0.9	+ 0.1 %

TABLE 4

PRECISION and ACCURACY of urine T determinations (within day)

Spiked value (μg/ml)	Number of samples	Assay Value (μg/ml)	CV%	Accuracy
0.109	3	0.112	2.5	+ 2.8 %
0.436	3	0.432	4.1	- 1.0 %
1.744	3	1.745	1.2	0.0 %

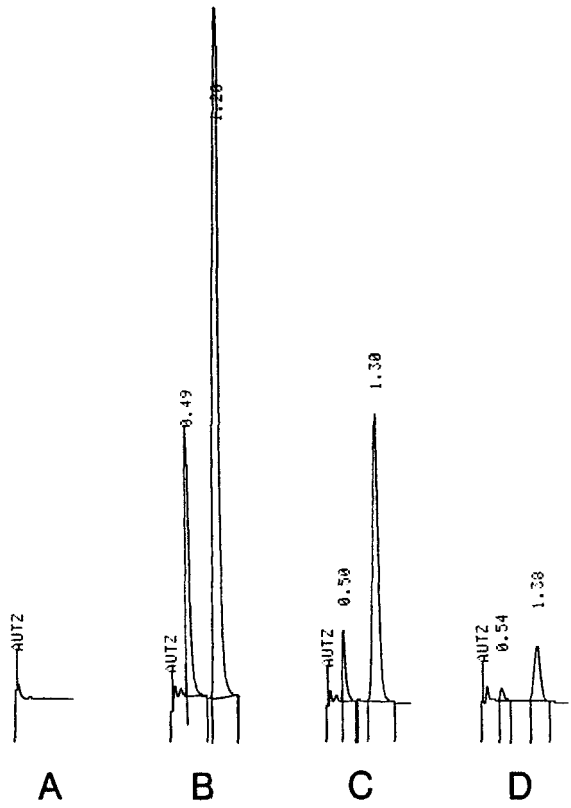


FIGURE 2:

Plasma levels after oral administration of 50 mg Triamterene (in a combination with Hydrochlorothiazide) are shown:

- A: before drug administration
- B: 20 min after drug administration (69.9 ng T and 376 ng HTS resp./ ml plasma)
- C: 4 hours after drug administration (19.4 ng T and 169 ng HTS resp./ ml plasma)
- D: 12 hours after drug administration (3.7 ng T and 31 ng HTS resp./ ml plasma)

HPLC conditions:

mobile phase: 0.01 M perchloric acid/ 0.004 M triethylamine/ 0.1 M ammoniumacetate

column: Spherisorb-Amino, 5 μm, 125 x 4 mm ID

detection: Fluorescence 360 nm excitation, 436 nm emission

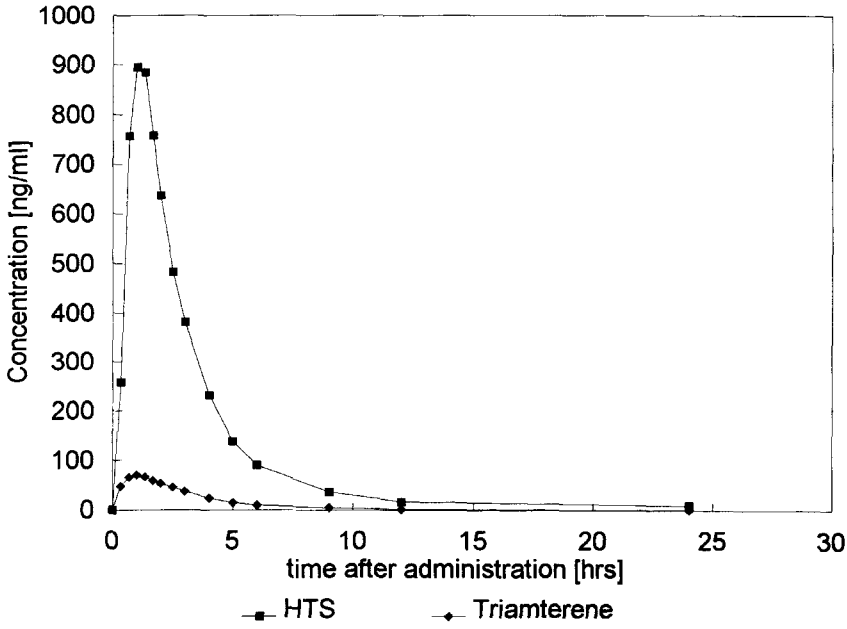


FIGURE 3:

Mean plasma levels of Triamterene and HTS from 12 volunteers. Oral dosage was 50 mg Triamterene (in combination with 25 mg Hydrochlorothiazide)

Chromatography

Figure 2 shows that the retention time for T is approx. 0.5 minutes and for HTS approx. 1.3 minutes. Determination limits as required in practice can be easily obtained.

Plasma levels after oral administration

Figure 3 shows mean plasma levels of T and HTS from 12 volunteers after oral administration of 50 mg T.

CONCLUSION

The method of analysis described above shows fast and accurate determination of T and HTS in plasma and urine, with high precision, reproducibility and accuracy. As most of the published papers analyse only one of the substances concerned, in a single chromatographic run, and/or require preliminary cleansing of the samples, the method described in this paper may be regarded as a real improvement.
The method described was used in a bioavailability study on 12 volunteers with 2 different oral galenic formulations.

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