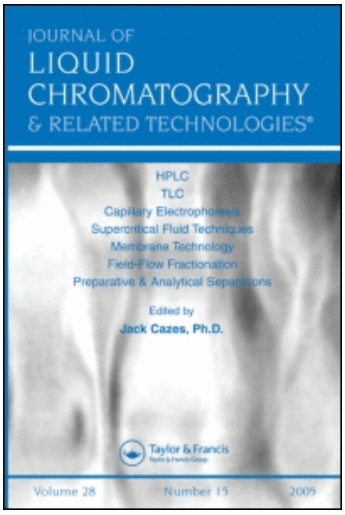

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SIMPLE AND SENSITIVE REVERSED-PHASE LIQUID CHROMATOGRAPHIC ASSAY FOR ANALYSIS OF CHLORTHALIDONE IN URINE

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ABSTRACT

This study describes a rapid method for the determination of chlorthalidone in human urine by reversed-phase liquid chromatography and UV detection at 230 nm, after clean-up over a C8 solid-phase extraction column. Liquid chromatography was carried out on a C18-bonded phase using acetonitrile-phosphate buffer (pH=3) gradient elution. Triamterene was used as internal standard. The system has been applied to the determination of chlorthalidone in the 0.10-10.0 µg/mL concentration interval; the limit of detection was 6 ng/mL.

INTRODUCTION

Chlorthalidone, 2-chloro-5-(1-hydroxy-3-oxo-1-isoindolinyl) benzene sulphonamide, is an oral diuretic widely used in the

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treatment of hypertension and eodema, both alone and in combination with other compounds. Chlorthalidone is commonly administered in a single dose of 25-100 mg daily, and it has been reported to provide a prolonged action. The recent increase in the use of chlorthalidone in combination with other antihypertensive agents, have resulted in lower doses [1]. Therefore sensitive methods to measure chlorthalidone levels in biological samples are required for pharmacokinetic studies.

Although most of the reported assays are only applied to pharmaceutical preparations [2-5], liquid chromatography is the most useful technique for the separation and the determination of chlorthalidone in biological samples. Guelen et al. [6] first described a chromatographic procedure which allows for the determination of chlorthalidone from different biological fluids with a sensitivity of 30 ng/mL. This sensitivity has been improved for blood samples [1, 7]; Lin [8] proposed an assay for this diuretic in blood and urine which was applied to the 4-190 μg/mL concentration range. However, chlorthalidone levels in urine samples after normal administration of drug are in the low μg/mL range [9, 10], and therefore such procedure cannot be suitable for real samples.

This work describes a simple and rapid HPLC method for the quantification of chlorthalidone in urine samples. A validation of the usefulness of the proposed procedure was accomplished by analyzing urine extracts obtained after the administration of the lowest recommended dose of chlorthalidone.

MATERIALS

Apparatus

A Hewlett-Packard model 1040A liquid-chromatography, equipped with a diode-array detector linked to a data system (Hewlett-Packard HPLC Chem Station) was used for data acquisition and storage. The system was coupled to a quaternary pump (Hewlett-Packard, 1050 Series) with a 25 µL sample loop injector.

The column was an HP-LiChrospher 100 RP 18 (5 µm, 125 mm x 4 mm ID). The chromatographic signal was monitored at 230 nm and, all the assays were performed at room temperature.

Reagents

All the reagents were of analytical-reagent grade. Methanol and acetonitrile were of HPLC grade (Scharlau). Water was distilled, deionized and filtered through 0.45 µm nylon membranes (Teknokroma). Chlorthalidone and internal standard (triamterene) stock solutions were prepared by dissolving in methanol pure compounds: chlorthalidone (ICI-Pharma) and triamterene (Sigma). Propylamine hydrochloride (Fluka), sodium dihydrogen phosphate monohydrate (Merk), and phosphoric acid (Probus) were also used.

METHODS

Standard Solutions

The standard solution of chlorthalidone was prepared by dissolving 50 mg of the pure compound in 25 mL of methanol (2000

µg/mL). The internal standard stock solution was prepared by dissolving 50 mg of pure compound in 100 mL of methanol (500 mg/mL). All the solutions were stored in the dark at 2^0C. Working standard solutions were prepared daily by dilution of the stock standard solutions with the appropriate volumes of methanol.

Mobile Phase

A gradient of phosphate buffer-acetonitrile, with an increasing acetonitrile content from 15% at zero min to 30 % at min 5, was used as the mobile phase. After 5 min, the acetonitrile content was kept constant. The phosphate buffer was prepared by dissolving 3.45 g of sodium dihydrogen phosphate monohydrate in 500 mL of distilled and deionized water; 0.7 mL of propylamine hydrochloride were added to this solution, and then, the pH was adjusted to 3 by adding the minimum amount of concentrated phosphoric acid (c. a. 0.5 mL). The solution was prepared daily, filtrated trough a 0.45 µm nylon membrane (Teknokroma) and degassed with helium before use. The flow was set to 1 mL/min.

Sample Treatment

Solid-phase extraction columns Bond-Elut C8, 100 mg/1 mL, (Teknokroma) for sample treatment were previously conditioned by drawing through 0.5 mL of methanol, followed by 0.5 mL of distilled water. A 300 µL volume of a methanolic solution of internal standard (1 µg/mL) was added to 2.0 mL of urine samples.

The samples were then drawn through the columns, and washed with 0.5 mL of distilled water to eliminate the biological matrix. The analyte and the internal standard were eluted from the column with 0.5 mL of methanol. The resulting solutions were finally filtered through 0.45 µm nylon filters (Teknokroma) and 5 µL of these solutions were then injected into the analytical column.

Recovery Studies

Free of drug urine samples (2.0 mL) were spiked with chlorthalidone standard solutions producing different concentrations in the therapeutic range (0.10 - 10 µg/mL). These samples were subjected to the previously described extraction procedure. The percentage of drug recovered for a particular extraction was calculated comparing the peak-area ratio chlorthalidone to internal standard in the spiked samples, with the peak-area ratio obtained for the direct injection 5 µL of methanolic solutions containing an equivalent amount of drugs. Each concentration was assayed in duplicate.

Preparation of Standards for Calibration

Standards for calibration were prepared by spiking 2.0 mL of urine samples with the appropriate volumes of chlorthalidone methanolic solution to give different concentrations in the 0.10 - 10.0 µg/mL range. These samples were extracted and chromatographed as described above. Peak area ratios of chlorthalidone to triamterene, were plotted against

chlorthalidone concentration, and the resulting calibration curves were used to calculate the chlorthalidone concentration in the unknown samples. Each concentration was assayed in duplicate.

Human Studies

Urinary excretion studies were performed with a human healthy volunteer after a minimum single dose administration of chlorthalidone (25 mg). Urine samples were collected at appropriate time intervals post-dose, and analyzed as described above.

RESULTS AND DISCUSSION

Figure 1 shows the chromatograms of blank (1a) and spiked with chlorthalidone (1b) urine samples. As can be seen from this figure, under the selected conditions the analyte and internal standard are eluted at 6.1 and 3.8 min, respectively. By comparing Figures 1a and 1b it can be derived that with the proposed sample treatment and elution profile, the chromatograms are free from endogenous compounds which may interfere with the identification or quantification of chlorthalidone, as the urinary endogenous compounds are primarily eluted in times shorter than 3 min.

The efficiency and precision obtained in the sample clean-up step is adequate, with mean recoveries for chlorthalidone of (98

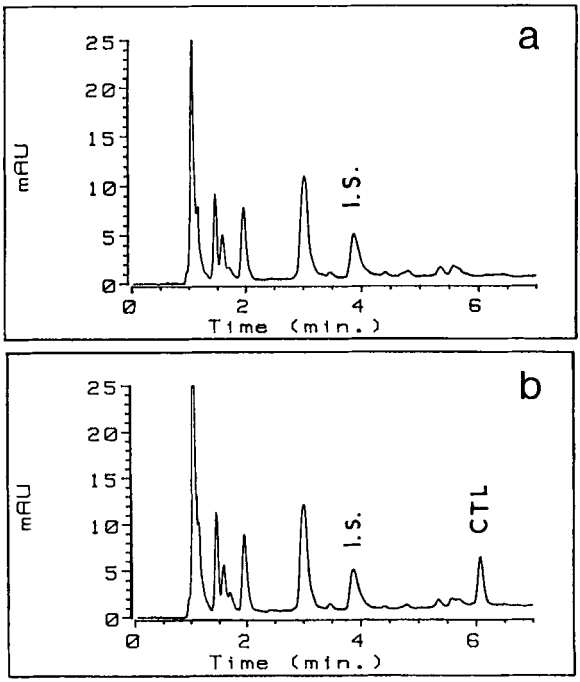

FIGURE 1

Chromatograms at 230 nm of (1a) blank and (1b) spiked with 0.50 µg/mL of chlorthalidone (CTL) urine samples. Peak at 3.8 min corresponds to the internal standard (I.S.).

± 3) % (n = 15) in the concentration range 0.10 - 10.0 µg/mL. For the internal standard the mean recovery obtained was (95 ± 3) % (n = 15), similar to the value obtained for the analyte. The time required for the sample clean-up is very short as the samples can be directly injected into the analytical column without any previous evaporation step.

The calibration curves obtained were linear over the working range 0.10 - 10.0 µg/mL, the intercept being essentially zero.

TABLE 1

Precision and Accuracy for Chlorthalidone in Urine (n=3).

Subject number	Added concentration (µg/mL)	Determined concentration (µg/mL)
1	0.50	(0.50 ± 0.06)
	1.0	(0.99 ± 0.08)
	5.0	(4.9 ± 0.1)
2	0.50	(0.49 ± 0.02)
	1.0	(1.07 ± 0.02)
	5.0	(5.1 ± 0.1)
3	0.50	(0.49 ± 0.05)
	1.0	(1.04 ± 0.03)
	5.0	(5.1 ± 0.1)
4	0.50	(0.52 ± 0.02)
	1.0	(1.06 ± 0.07)
	5.0	(5.06 ± 0.06)

The mean correlation coefficient was 0.9995. In order to evaluate the precision and accuracy of the method, control urine samples from different volunteers were spiked with chlorthalidone, and tested by triplicate to determine chlorthalidone levels. The results obtained are summarized in Table 1. The concentrations found were close to the actual concentrations in all cases tested. From these results it can be derived that the accuracy and precision of the method is suitable, with relative standard deviations ranging between 1 % and 12 % for concentrations of 5.0 and 0.50 µg/mL, respectively.

The limit of detection (established to generate a signal-to-noise ratio of 3) corresponds to a chlorthalidone concentration

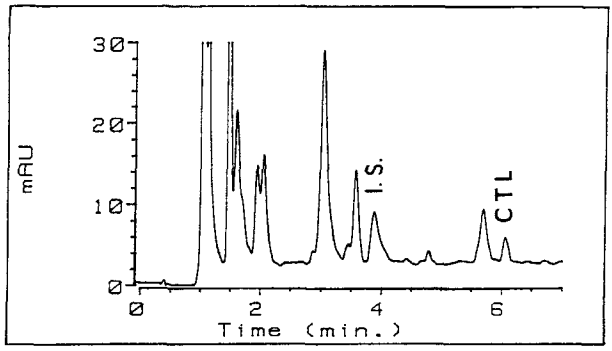

FIGURE 2

Chromatogram at 230 nm of an urine sample obtained 52 hours after a single dose administration of 25 mg of chlorthalidone (CTL). Peak at 3.8 min corresponds to the internal standard (I.S.).

in urine of 6 ng/mL, indicating improved sensitivity over previous HPLC methods [1, 6-8].

The described assay has been applied to the measurement of urine levels of chlorthalidone after a single dose administration of 25 mg to a human volunteer. Figure 2 shows a chromatogram obtained 52 hours after the administration of drug. Chlorthalidone is primarily excreted unchanged, and its metabolites do not interfere with the analyte and internal standard peaks, the concentration of drug being (0.54 ± 0.04) µg/mL (n = 3).

The accuracy, precision and sensitivity of the described assays, allow for the determination of chlorthalidone at least four days after its minimum single dose administration. However, the drug can be detected at least five days after dosing.

TABLE 2

Retention Times of Different Compounds Commonly Administered with Chlorthalidone.

Compound	Retention time (min)
Atenolol	1.5
Oxprenolol	6.7
Reserpine	7.1
Spironolactone	10.6

Table 2 lists the retention times obtained for different compounds which are usually coadministered with chlorthalidone. Interference by these compounds have been tested by extracting a commercial control containing therapeutic levels of drugs. This table indicates that these drugs should not interfere with the quantification of chlorthalidone using the proposed method.

In conclusion, a sensitive and selective assay for analysis of chlorthalidone in urine is described. The determination drug can be achieved in a few minutes, the precision and accuracy of the assay being suitable at therapeutical levels.

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