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Short communication

Simple method for determination of hydrochlorothiazide in human urine by high performance liquid chromatography utilizing narrowbore chromatography

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Abstract

A simple high performance liquid chromatographic (HPLC) method utilizing narrowbore chromatography was developed for the determination of hydrochlorothiazide in human urine. A mobile phase of 0.1% aqueous acetic acid—acetonitrile (93:7, v/v) pH 3 was used with a C18 analytical column and ultraviolet detection (UV). The method demonstrated linearity from 2 to 50 μg ml^{-1} using 50 μl of urine with a detection limit of 1 μg ml^{-1}. The method was utilized in a study evaluating if racial differences are present in the pharmacokinetic and pharmacodynamic effects of hydrochlorothiazide.

Keywords: Hydrochlorothiazide; Urine; Narrowbore HPLC

1. Introduction

Hydrochlorothiazide (6-Chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide) is a diuretic used in the treatment of hypertension and edematous conditions. A number of methods have been employed for the analysis of hydrochlorothiazide in human urine including high performance liquid chromatography (HPLC) [1–7], gas chromatography (GC) [8], and gas chromatography-mass spectrometry (GC/MS) [9,10]. The HPLC methods published thus far have utilized conventional analytical columns (e.g. 4.6 mm internal diameter), internal standards, several used tedious liquid/liquid extractions, and one used micellar chromatography (e.g. sodium dodecyl sulfate (SDS)) [5]. Published GC and GC/MS methods have employed cumbersome derivatization steps. The method detailed in the present communication utilized a simple sample preparation step and did not require an internal standard or mobile phase modifiers (e.g. SDS). In addition, this method employed current HPLC

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narrowbore column technology which provided adequate sensitivity and significantly reduced mobile phase requirements.

2. Experimental

2.1. Materials

Hydrochlorothiazide (CAS 58-93-5) was purchased from Sigma (St. Louis, MO). Glacial acetic acid was HPLC grade and purchased from Fisher Scientific (Fair Lawn, NJ). Acetonitrile (Burdick and Jackson UV grade) was purchased from Baxter (Columbia, MD). Ultrapure distilled and deionized water was prepared in-house and filtered prior to use.

2.2. Instruments and chromatographic condition

The HPLC equipment consisted of a LKB Model 2150 HPLC pump (Gaithersburg, MD) and LKB Model 2152 LC controller. The analytical column was a Hypersil C_{18}, 150 mm × 2.0 mm I.D., 3 μm packing (Phenomenex, Torrance, CA). The C_{18} guard column, 30 mm × 4.6 mm I.D., 40–50 μm pellicular packing (Alltech, Deerfield, IL) was replaced prior to each analytical run which typically consisted of approximately 50 samples. The mobile phase consisted of 0.1% aqueous acetic acid—acetonitrile (93:7, v/v) with an apparent pH of 3 after mixing. The mobile phase was degassed daily using helium sparging and the flow rate was maintained at 0.30 ml min^{-1}. Typical operating pressure was 22.5 MPa at ambient temperature. An injection volume of 2 μl of the prepared urine sample was accomplished using a WISP Model 712 (Waters, Milford, MA) autosampler. Compound detection was achieved using a Varian 9050 UV-VIS Detector (Walnut Creek, CA) operating at 272 nm with a 1-s response time. A 345-kPa back-pressure regulator (SSI, State College, PA) was coupled to the detector outlet to prevent outgassing. Data acquisition and component computations were performed using Turbochrom (PE Nelson, Norwalk, CT) chromatography software on a Hewlett Packard (Palo Alto, CA) 486 DX-33 personal computer.

2.3. Standard and control preparation

Stock standard of hydrochlorothiazide (1 mg ml^{-1}) was prepared in methanol-deionized water (70/30, v/v) and stored at 4°C. Working standards of 2, 5, 10, 25 and 50 μg ml^{-1} hydrochlorothiazide were prepared using blank human urine as the diluent. Control samples of 3, 15 and 40 μg ml^{-1} hydrochlorothiazide were prepared using blank human urine as the diluent and stored at −30°C along with the patient urine samples.

2.4. Sample conditions

Hypertensive patients off medications for 4 weeks and maintained on a controled sodium and potassium diet received hydrochlorothiazide per mouth at 08:00 on the study morning. Urine samples were collected by spontaneous voiding at specified time intervals post-dosing. Urine samples were measured for volume over each time interval and a 10-ml aliquot frozen at −30°C pending analysis. A second 10-ml aliquot was obtained from each timed urine sample and submitted for analysis of sodium content (Dupont Dimensions ES Chemistry Analyzer, Wilmington, DE). Prior to analysis, urine samples were thawed to ambient temperature, mixed thoroughly by inversion, and allowed to sit 15 min for particulates to settle out.

2.5. Sample preparation

Urine samples were prepared by pipetting 50 μl of urine and 450 μl of deionized water into a 12 × 75-mm polypropylene culture tube and mixing by vortex for 10 s. The diluted urine sample was transferred to a polypropylene autosampler microvial; 2 μl was injected into the HPLC system.

3. Results and discussion

3.1. Chromatography

The method demonstrated excellent chromatographic selectivity with no endogenous interferences at the retention time for

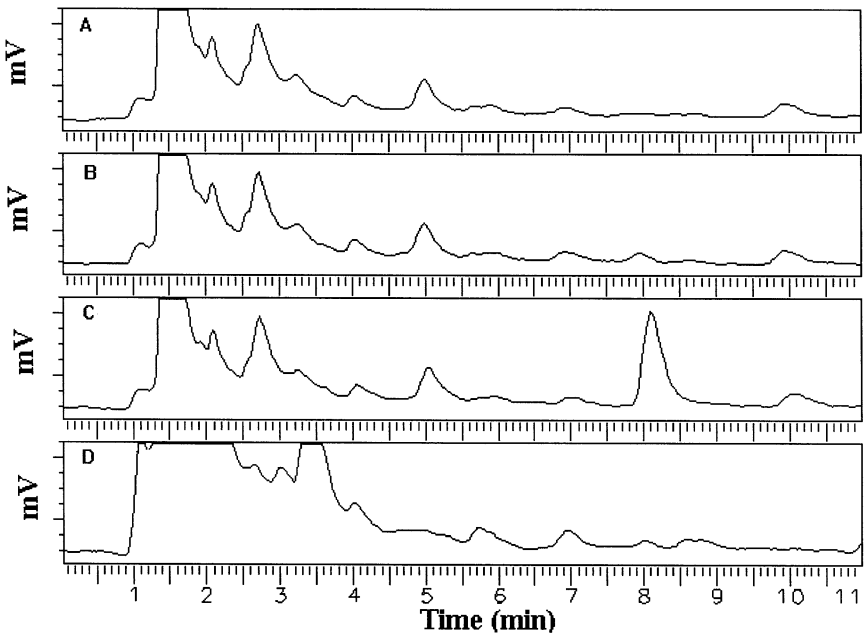

Fig. 1. Chromatograms of (A) prepared blank human urine, (B) prepared blank human urine spiked with 5 μg ml^{-1} hydrochlorothiazide, (C) prepared blank human urine spiked with 50 μg ml^{-1} hydrochlorothiazide, (D) subject dosed with 25 mg hydrochlorothiazide tablet (24–30 h sample—hydrochlorothiazide concentration 3.3 μg ml^{-1}). Peak at 8.1 min = hydrochlorothiazide.

hydrochlorothiazide (8.1 min; Fig. 1A). Chromatograms of prepared blank human urine containing low (5 μg ml^{-1}) and high (50 μg ml^{-1}) concentrations of hydrochlorothiazide (Fig. 1B and C, respectively) indicated good detector response and baseline resolution between hydrochlorothiazide and endogenous substances with an analytical run time of 11.1 min. A typical chromatogram from one subject dosed with hydrochlorothiazide (25 mg tablet) is shown in Fig. 1D. To extend column lifetime, the analytical C_{18} column was flushed after each analytical run for 2 h at 0.3 ml min^{-1} with methanol-deionized water (70/30, v/v) to eliminate retained non-polar substances.

3.2. Linearity, limit of detection and computations

The method was linear throughout the concentration range of 2–50 μg ml^{-1} with a mean correlation coefficient of 0.99963 ($n = 6$ analytical runs). The limit of detection (LOD) for the method was determined by evaluating spiked standards in urine and defined as the lowest concentration which provided a signal-to-noise ratio of three. The method LOD was determined to be 1 μg ml^{-1} ($n = 3$). For all component calculations, normal linear regression using Lotus 1-2-3 with external standardization and peak height was used. The lowest calibration standard (2 μg ml^{-1}) which could accurately and precisely be measured to within 20% of the theoretical concentration was utilized as the limit of quantitation for the method.

3.3. Accuracy, precision and recovery

The accuracy and precision of this HPLC method was determined by evaluation of replicate control samples over the course of all analytical runs at concentrations of 3, 15 and 40 μg ml^{-1}. The accuracy of the method was reported as the percentage error of theoretical versus measured hydrochlorothiazide concentrations and was less than 5.4% for all control samples intra-day (Table 1) and less than 4.8% for all control samples

Table 1
Intraday accuracy and precision

Theoretical concentration (μg ml^{-1})	Measured concentration (μg ml^{-1})	*n*	RSD (%)	Error (%)
3.0	2.9	6	13.3	−3.5
15.0	14.2	6	2.7	−5.4
40.0	38.6	6	1.1	−3.5

Table 2
Interday accuracy and precision

Theoretical concentration (μg ml^{-1})	Measured concentration (μg ml^{-1})	*n*	RSD (%)	Error (%)
3.0	3.1	12	15.9	4.8
15.0	14.9	12	3.0	−1.0
40.0	39.4	9	1.0	−1.6

inter-day (Table 2). The precision of the method was reported as percent relative standard deviation and was less than 13.3% for all control levels intra-day (Table 1) and less than 15.9% inter-day (Table 2). Absolute recovery for the method was not performed as the method does not employ a formal extraction (e.g. liquid–liquid, solid phase). In addition, the standards and controls used for analysis were treated identical to the patient urine samples thus controling for potential errors in micropipetting.

3.4. Pharmacokinetic study

In these studies, nine African-American and nine Caucasian hypertensive patients were age, sex and weight matched. After a 4-week washout period and 7 day controled diet of 150 mEq sodium and 80 mEq potassium, patients received a single dose of hydrochlorothiazide (25 mg) per mouth. The pharmacodynamics of hydrochlorothiazide were assessed by evaluation of its urinary elimination profile relative to sodium excretion

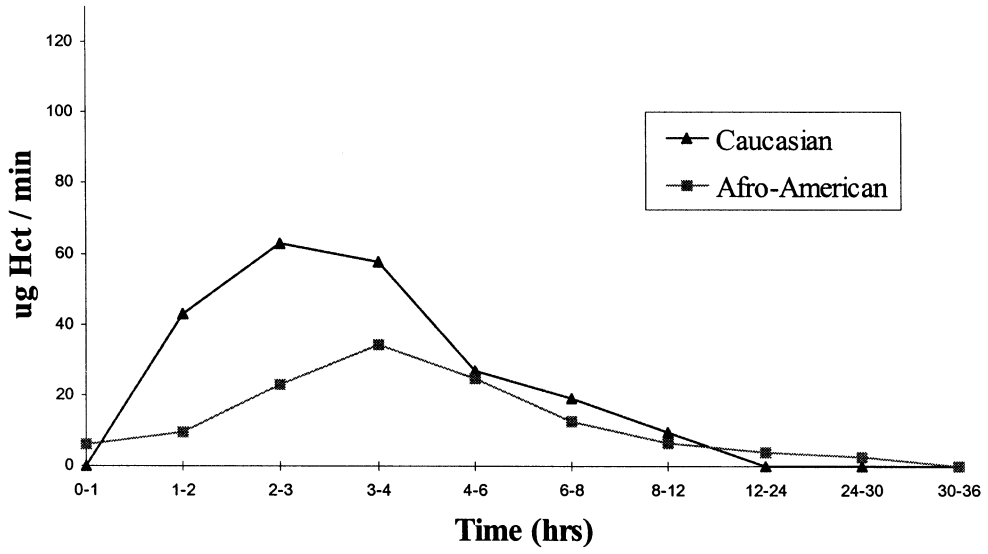

Fig. 2. Pharmacokinetic profile of African-American and Caucasian subject with time (hours) versus urinary excretion rates for hydrochlorothiazide (μg min^{-1}).

patterns. The urinary pharmacokinetic profile for hydrochlorothiazide excretion in two subjects (African-American and Caucasian) is presented in Fig. 2.

4. Conclusions

We developed a simple method for evaluating hydrochlorothiazide in urine that improves upon previously published methods. Whereas previous methods for determining hydrochlorothiazide in bodily fluids highlighted sensitivity, in this case ease of performance for analysis was the sought after objective. For the pharmacokinetic objective of this study, the sensitivity of this method was more than adequate. The authors chose to use a simple dilute and shoot procedure which eliminated both the need for sample extraction and an internal standard, thus making the method cost effective. The method used current narrowbore HPLC column technology which offered adequate sensitivity (e.g. 2 μl injection of a 1:10 diluted urine sample) and significantly reduced mobile phase requirements. This reduced analytical costs particularly as relates to the procurement and subsequent hazardous waste disposal of acetonitrile. It is noteworthy that use of the narrowbore column required two minor modifications to the HPLC system (i.e. small injection volume (2 μl) and detector cell volume (4.5 μl)). The method offers a short analytical run time of 11.1 min and achieved excellent baseline resolution between hydrochlorothiazide and endogenous substances. The method was employed in the evaluation of over 400 urine samples from a pharmacokinetic and pharmacodynamic study without significant methodological problems.

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