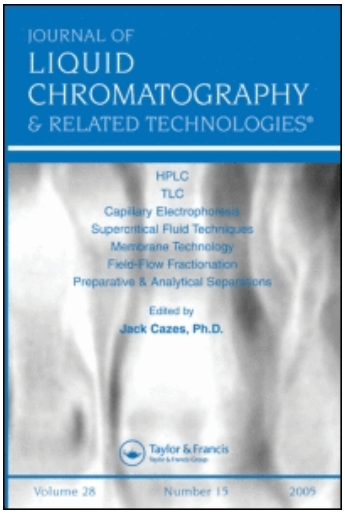

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Determination of Bendroflumethiazide in Plasma by High Performance Liquid Chromatography and Fluorescence Detection

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DETERMINATION OF BENDROFLUMETHIAZIDE IN PLASMA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND FLUORESCENCE DETECTION

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ABSTRACT

An accurate, precise, and specific assay is described for the determination of bendroflumethiazide (BFTZ) in plasma. The procedure employs a C18 column, a mobile phase consisting of 35% acetonitrile in 0.015M phosphoric acid, and a fluorescence detector with a 254nm excitation filter and a 400nm emission filter. Furosemide is used as the internal standard. Using 1ml of plasma, this method can detect 10ng/ml of BFTZ.

INTRODUCTION

Bendroflumethiazide (BFTZ) is a potent diuretic that has been used extensively for over 15 years for treatment of hypertension and edema. Several methods have been proposed for the analysis of BFTZ including nonaqueous titrimetry (1), photometry (2), gas chromatography (3,4), semiaqueous potentiometry (5), polarography (6), and TLC with fluorescence scanning (7). The present work describes a sensitive procedure

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for the determination of BFTZ in plasma, which can be applied to routine analysis in pharmacokinetic studies using therapeutic doses.

EXPERIMENTAL

Chemicals and Reagents

BFTZ was supplied by The Squibb Institute for Medical Research (Princeton, New Jersey), and used as obtained, acetonitrile (Burdick & Jackson Laboratories, Inc., Muskegon, Michigan) was HPLC grade, furosemide (Sigma Chemical Co., St. Louis, Missouri) and phosphoric acid (Mallinckrodt Chemical Works, St. Louis, Missouri) were used as supplied.

Instrumentation

The instrument used consisted of a Model 6000A solvent delivery system, a Model U6K injector, a Model 420AC fluorescence detector (all Waters Associates, Milford, Massachusetts), and a Model 3380A integrator (Hewlett Packard, Avondale, Pennsylvania). The column was 30cm x 4mm, i.d., reverse phase (μBondapak C18) with a guard column, 25mm x 4mm, i.d., packed with Bondapak C18 Corasil (both Waters Associates) attached to the inlet end of the analytical column. The mobile phase was 35% acetonitrile in 0.015M phosphoric acid, pH = 2.4±0.1.

Conditions for Quantitation

The analysis was conducted at room temperature. Solvent flow rate was 1ml/min. A 254nm band filter was used for excitation, and a 400nm cut-off filter was used for emission. Detector settings were: gain, 64; span, maximum; and recorder attenuation was set at 64. The mobile phase was filtered through a membrane filter and degassed before use.

Standard Solutions

Solutions of BFTZ were prepared in methanol at concentrations of 0.1, 0.5, 1, 10, 50, 100, 200, and 500μg/ml. A solution of the internal standard, furosemide, was prepared in acetonitrile at 10μg/ml.

Treatment of Plasma

Two procedures were followed; one procedure was for plasma concentrations of 0.01 to 1.0μg/ml, and the other for concentrations of 1 to 50μg/ml. For the lower concentration range, 900μl of plasma was spiked with 100μl of a standard solution containing 0.1, 0.5, or 1.0μg/ml, to yield a final plasma concentration of 0.01, 0.05, and 0.1μg/ml, and extracted as follows. The spiked plasma was transferred to a screw-capped centrifuge tube and treated with 100μl of 0.1N HCl, 1g sodium chloride, and 5ml ether. The mixture was shaken for 15min and centrifuged for 30min. The ether layer was transferred to another tube, and evaporated to dryness under a stream of nitrogen at 40°. The residue was redissolved in 50μl of acetonitrile containing the internal standard, and 30μl was injected onto the column.

In the range of concentration 1-50μg/ml, no extraction was needed. Ninety microliters of plasma was spiked with 10μl of a standard BFTZ solution to yield a final concentration of 1, 5, 10, 20, and 50μg/ml. The spiked plasma was treated with an equal volume of the internal standard solution in acetonitrile, vortexed for 1min, then centrifuged for 15min. An aliquot of the clear supernate was injected on the column. The volume injected varied from 6 to 30 μl, depending on the concentration, and adjusted to keep the peak on scale.

RESULTS AND DISCUSSION

Figures 1 and 2 show typical chromatograms obtained from blank plasma and plasma spiked with BFTZ, respectively. Under present experimental conditions, BFTZ had a retention time of about 9.5min, while the internal standard had a retention time of approximately 6.2min. The relationship between peak area ratio and concentration was linear with a correlation coefficient of better than 0.99. A linear relationship was also observed

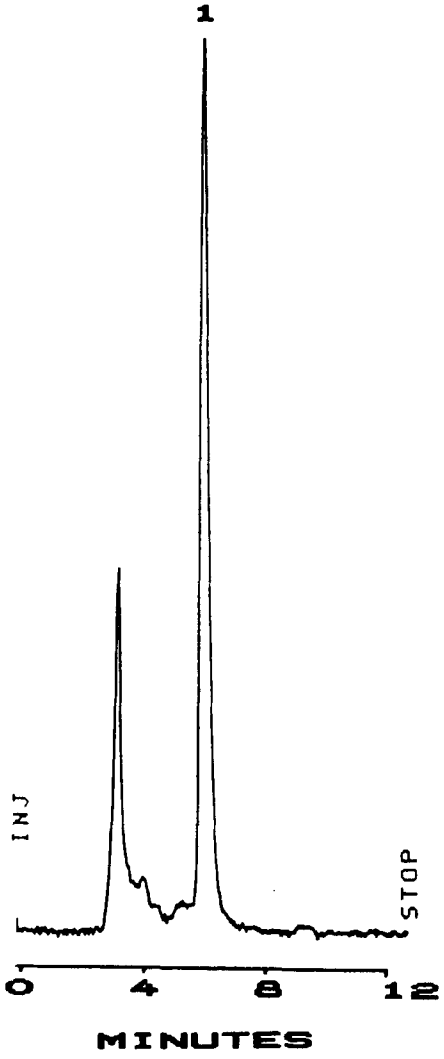

FIGURE 1

Chromatogram obtained from blank plasma. Peak 1, internal standard.

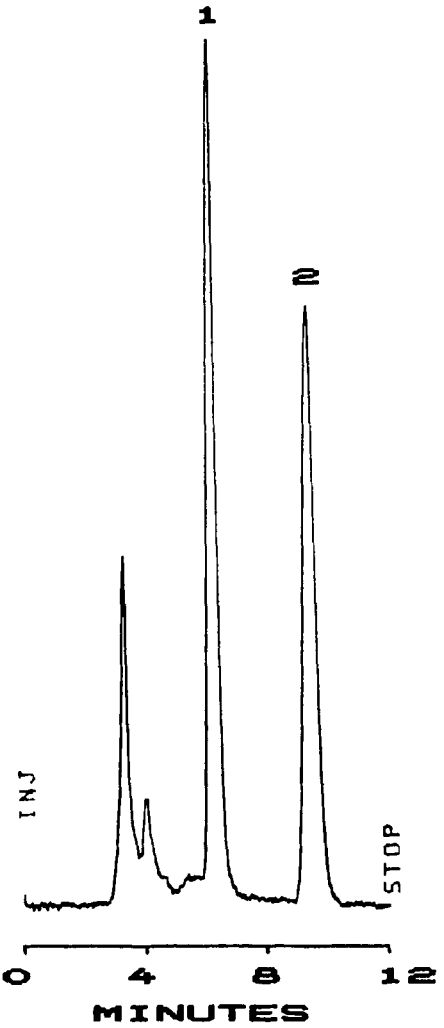

FIGURE 2

Chromatogram obtained from plasma spiked with BFTZ. Peak 1, internal standard, peak 2, BFTZ.

between absolute peak area of BFTZ and concentration, indicating that the use of an internal standard may be avoided. There was no interference from normal plasma constituents, nor was there any interference from propranolol, atenolol, or nadolol, which may be administered concomitantly with BFTZ.

Table 1 shows the day-to-day precision of the assay, calculated in terms of peak area ratio for the concentration range 1 to 50μg/ml. Table 2 shows day-to-day precision using absolute peak area units. The coefficient of variation obtained

TABLE 1
Day-To-Day Variation Using Peak Area Ratio

C, μg/ml	1	2	3	4	5
0	0.0	0.0	0.0	0.0	0.0
1	0.1275	0.1481	0.0861	0.1009	0.0859
5	0.6835	0.5174	0.5173	0.5439	0.5111
10	1.1185	1.0777	0.9610	1.0330	0.9900
20	2.5925	2.8996	1.9356	2.0530	1.9732
50	4.8650	4.9667	5.0031	5.0430	4.7411
Y intercept	0.1635	0.1461	-0.0157	0.0188	0.0242
Slope	0.0977	0.1015	0.0999	0.1007	0.0948
r	0.9910	0.9830	0.9999	0.9999	0.9998

TABLE 2
Day-To-Day Variation Using Absolute Peak Area Units

C, μg/ml	1	2	3	4	5
0	0000	0000	0000	0000	0000
1	5221	5984	5300	5370	5243
5	27847	27051	28176	29250	27063
10	66334	63670	57370	60493	60632
20	110549	117528	113880	117192	118406
50	308850	297357	276210	303967	309293
Y intercept	-1652	36	913	-1064	-2082
Slope	6148	5946	5529	6077	6199
r	0.9987	0.9998	0.9999	0.9999	0.9998

from repeated injections of the same solution varied from 9.8% at the lowest concentration to 3.1% at the highest concentration. The rate of recovery following the extraction procedure described for concentrations of 0.01 to 1μg/ml averaged 85.4±2.5%.

In summary, the procedure described herein was shown to be accurate, precise, specific, and applicable to concentrations encountered in pharmacokinetic studies using therapeutic doses of BFTZ.

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