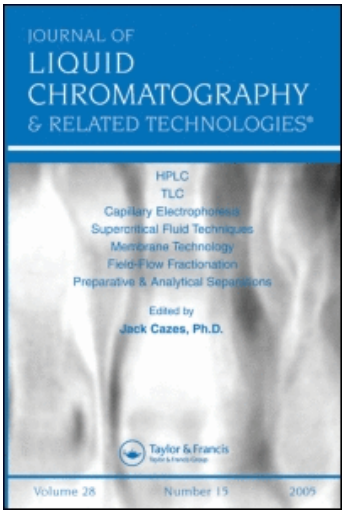

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SIMULTANEOUS DETERMINATION OF HYDROCHLOROTHIAZIDE AND PROPRANOLOL HYDROCHLORIDE IN TABLETS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A simple and stability indicating HPLC procedure is described for the simultaneous determination of hydrochlorothiazide and propranolol hydrochloride in tablet formulations. Potential degradation products of both drugs and synthesis impurities of hydrochlorothiazide were separated, making the determination stability indicating for both drugs and selective for hydrochlorothiazide. All compounds were chromatographed on a cyanopropylsilane column, eluted with a 15:85 mixture of acetonitrile:0.05 M ammonium

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phosphate (pH 3.0) and detected at 290 nm. Excellent interlaboratory precision and recovery data were obtained. Linearity studies were carried out using peak area measurements. Detector response to the concentration of each drug was confirmed. The method was applied to dosage forms containing 25 mg of hydrochlorothiazide and 40 or 80 mg of propranolol hydrochloride.

INTRODUCTION

Hydrochlorothiazide, 6-chloro-3,4-dihydro-1,2,4-benzothiazine-7-sulfonamide-1,1-dioxide, is a widely used diuretic. Propranolol hydrochloride, 1-(isopropylamino)-3-(inapthyloxy)-2-propanol hydrochloride, is a beta-adrenergic receptor blocking agent. These compounds are included in the listings of prescribed drugs used singly or in combination for the treatment of hypertension. A rapid, accurate and stability indicating procedure was required for the simultaneous determination of both drugs in tablet formulations.

Numerous methods for the determination of hydrochlorothiazide in dosage forms as a single entity and in combination with other drugs have been reported in the literature. Traditional procedures such as spectrophotometry (1), fluorescence (2,3), colorimetry (4) and titrimetry (5) are subject to interferences from the components present in the samples. High pressure liquid chromatographic procedures have been successfully applied to the determination of the diuretic drug in dosage forms (6,7,8). Several HPLC procedures have been reported for the assay of propranolol, mainly in biological materials (9-12). Patel (13) has described an HPLC procedure for nadolol in tablets and recommends it for other beta-adrenergic blocking drugs, however only retention times are reported. Conventional

analyses, such as pharmacopeial procedures for the individual drugs, are not suited for simultaneous determination. The methods described in the literature are not applicable to the simultaneous assay of the antihypertensive combination of hydrochlorothiazide and propranolol hydrochloride.

This report presents a simple and rapid HPLC method for the quantitative determination of both substances in tablet formulations. The sample preparation is simple and analysis of the two drugs can be performed in less than 20 minutes. The procedure eliminates interferences due to formulation excipients and chromatographically separates impurities such as 4-amino-6-chloro-1,3-benzenedisulfonamide and chlorothiazide as well as possible degradation products from both drugs.

MATERIALS AND METHODS

Apparatus - The liquid chromatograph included a pump[1], an automatic injector[2] with a precise 20 mcl loop, a 4.6 mm x 25 cm column packed with cyanopropylsilane on silica (5 mcm particles)[3], a variable wavelength detector set at 290 nm[4] and a computing integrator[5]. The attenuation and chart speed on the integrator were set at AT = 128 and 0.5 cm per minute, respectively.

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1 Waters Associates Model 501

2 Perkin Elmer LC-420B

3 Altex Ultrasphere - Cyano

4 Perkin Elmer LC-95

5 Spectra Physics SP-4270

Materials - All reagents and solvents were of analytical grade and used as received. Hydrochlorothiazide (I), 4-amino-6-chloro-1,3-benzenedisulfonamide (II), chlorothiazide (III) and propranolol hydrochloride (IV) were all USP reference standards.

Mobile phase - Mix 15 ml of acetonitrile with 85 ml of 0.05 M ammonium phosphate monobasic, adjust to pH 3.0 with phosphoric acid, filter through a 0.45 mcm solvent resistant filter and degas.

System suitability - When 20 mcl of a solution containing 50 mcg of hydrochlorothiazide and 20 mcg of p-dimethyl-aminobenzoic acid (this compound can be used as an internal standard if so desired) in 1 ml of mobile phase are injected into the chromatograph using the parameters described under apparatus, and a flow rate of 2 ml per minute, the resolution factor (R) should not be less than 12 and the theoretical plates (N) should not be less than 6250. Six successive injections of the standard preparation should provide a relative standard deviation of less than 2.0%. Plots of peak area versus hydrochlorothiazide concentration (0.010 - 0.092 mg/ml) and peak area versus propranolol hydrochloride concentration (0.016 - 0.160 mg/ml) result in straight lines.

Standard preparation - Weigh accurately about 40 mg of Propranolol Hydrochloride USP Reference Standard (80 mg for tablets with 80 mg propranolol hydrochloride label claim) and 25 mg of Hydrochlorothiazide USP Reference Standard, transfer to a 100 ml volumetric flask, dissolve in and dilute to the mark with methanol. Quantitatively dilute with mobile phase to a concentration 50 mcg/ml of hydrochlorothiazide.

Assay preparation -

a. Tablets - Weigh and finely powder not less than 20 tablets. Weigh accurately a portion of the powder equivalent to about 25 mg of hydrochlorothiazide and transfer to a 100 ml volumetric flask. To the flask, add 5 ml of 0.1 N hydrochloric acid and mix to disperse the powder. Add about 50 ml of methanol and sonicate for about 10 minutes. Cool, dilute to the mark with methanol and mix to form a uniform suspension. Filter a portion through a 0.45 mcm solvent resistant filter. Quantitatively dilute with mobile phase to an approximate concentration of 50 mcg/ml of hydrochlorothiazide.

b. Solution phase stability of propranolol hydrochloride (thermal stress) - Prepare solutions of propranolol hydrochloride in 1.0 N hydrochloric acid, distilled water and 0.1 N methanolic potassium hydroxide, respectively, at a concentration of about 1 mg/ml. Protect from light. Transfer a volume of 50 ml of each of the solutions to separate round bottom flasks and reflux for 24 hours. Allow the contents to cool to room temperature, then transfer exactly 4.0 ml of each solution to separate 50 ml volumetric flasks and dilute to volume with mobile phase.

Procedure - Pump the mobile phase through the column at a flow rate of 2.0 ml/min. until a stable baseline is obtained. Alternately inject 20 mcl volumes of the assay preparation and the standard preparation by means of a precise loop injector and allow the chromatogram to develop for about 20 minutes. The peaks corresponding to hydrochlorothiazide and propranolol hydrochloride elute at 4.5 and 14.2 minutes, respectively.

a. Tablets - Calculate the quantity of each drug, in mg per tablet, by the formula: $(R_u/R_s) \times (S/SW) \times TW$, where R_u and R_s are the peak areas obtained from the chromatograms of the assay preparation and the standard preparation, respectively, S equals the respective standard weight in mg, SW equals the sample weight, in mg and TW equals the average tablet weight, in mg.

b. Solution phase stability of propranolol hydrochloride (thermal stress) - Calculate the quantity, in mg of propranolol hydrochloride per ml, by the formula: $0.025 (R_u/R_s) \times S$, where R_u and R_s are the peak areas obtained from the chromatographs of the assay preparation and the standard preparation, respectively, and S equals the standard weight of propranolol hydrochloride, in mg.

RESULTS AND DISCUSSION

System suitability: Baseline separation of the peaks corresponding to hydrochlorothiazide and p-dimethylamino-benzoic acid was obtained. The elution times were 4.5 and 7.5 minutes, respectively. The resolution factor (R) and the number of theoretical plates (N) were found to be 12.1 and 8962, respectively. A standard preparation containing 0.080 mg/ml of propranolol hydrochloride and 0.051 mg/ml of hydrochlorothiazide was injected into the chromatographic system. The relative standard deviation of six replicate injections was 0.06% for both drugs.

Linearity: Typical standard curves obtained by assaying samples containing 0, 10.3, 30.8, 51.4, 71.9, 92.4 mcg/ml of hydrochlorothiazide and 0, 16.0, 48.1, 80.2, 112.3, 144.4 and 160.5 mcg/ml of propranolol hydrochloride had linear regression coefficients of 0.99999 and 0.99986, respectively.

Recovery: The recovery of the two drugs was determined by adding known amounts of hydrochlorothiazide and propranolol hydrochloride to placebo powder and assaying by the described procedure. An average recovery of 100.5% for hydrochlorothiazide and 100.2% for propranolol hydrochloride with relative standard deviations of 0.3% and 0.7%, respectively, was obtained. The method was demonstrated to be linear at 80% and 120% of label claim. These data are presented in Table I.

Sensitivity - Peak responses of hydrochlorothiazide (I) and propranolol hydrochloride (IV) exceeding about 4 times the instrumental noise level were obtained when 20 mcl of a standard solution containing 0.1 mcg/ml of (I) and 0.4 mcg/ml of (IV) were injected into the chromatograph. The integrator sensitivity was increased to AT = 4.

Assay of tablet formulations - Composite samples of five tablet formulations, claiming 25 mg hydrochlorothiazide/40 mg propranolol hydrochloride and 25 mg hydrochlorothiazide/80 mg propranolol hydrochloride per tablet, were assayed by the proposed procedure. The results are summarized in Table II.

Separation of hydrochlorothiazide from process impurities and potential degradation products - Hydrochlorothiazide (I), tr = 4.5 min., was well separated from its hydrolysis product and synthesis precursor 4-amino-6-chloro-1,3-benzenedisulfonamide (II), tr = 3.6 min., its synthesis impurity chlorothiazide, tr = 4.0 min. and propranolol hydrochloride (IV), tr = 14.8 min. A typical chromatogram obtained from a standard solution containing about 50 mcg (I), 80 mcg (IV), 0.4 mcg (II) and 0.5 mcg (III) in 1 ml of mobile phase is shown in Figure 1.

TABLE I

RECOVERY OF HYDROCHLOROTHIAZIDE AND PROPRANOLOL HYDROCHLORIDE AT 80%, 100% AND 120% OF LABEL CLAIM

% OF LABEL CLAIM	% RECOVERED HYDROCHLORO-THIAZIDE	% RECOVERED PROPRANOLOL HYDROCHLORIDE
80	100.8	100.4
80	99.6	98.6
100	100.1	99.3
100	100.3	101.1
100	100.6	99.7
100	100.3	100.8
100	101.0	100.7
100	100.4	99.8
AVERAGE	100.5	100.2
% RSD	0.3	0.7
120	99.7	99.6
120	99.8	100.0

TABLE II

ASSAY OF HYDROCHLOROTHIAZIDE/ PROPRANOLOL HYDROCHLORIDE TABLET FORMULATIONS

SAMPLE	HYDRO-CHLORO-THIAZIDE (mg/TAB.)	% LABEL	PROPRANO-LOL HYDRO-CHLORIDE (mg/TAB.)	% LABEL
25/40	25.1	100.4	40.1	100.3
25/80	24.6	98.4	78.8	98.5
25/80	25.5	102.0	78.5	98.1
25/80(a)	24.9	99.6	78.2	97.8
25/80(a)	24.5	98.0	40.1	100.3

Average of three trials

(a) Inderide; Ayerst Laboratories Inc.

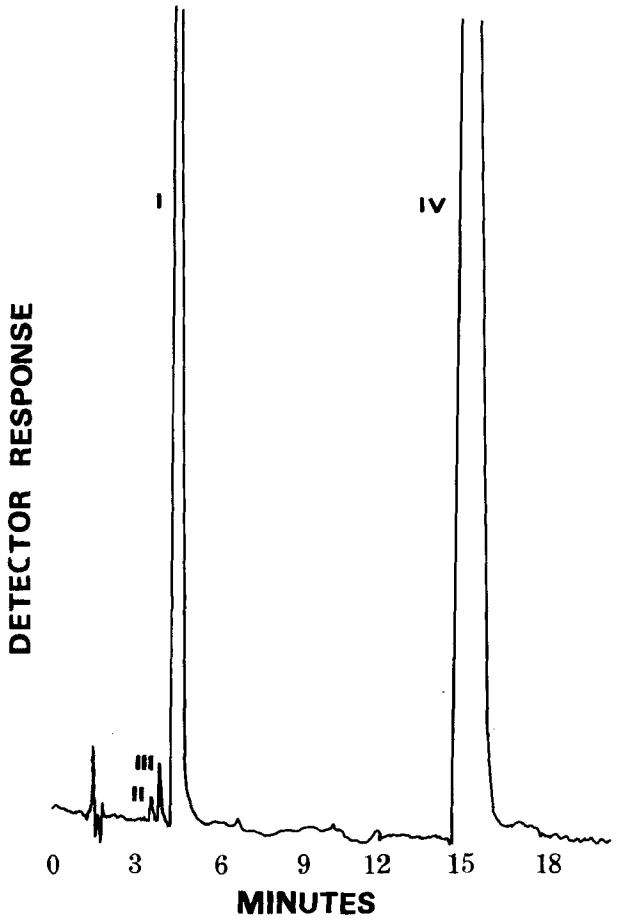

FIGURE 1 Liquid chromatogram of hydrochlorothiazide (I), 4-amino-6-chloro-1,3-benzenedisulfonamide (II), chlorothiazide (III) and propranolol hydrochloride (IV)

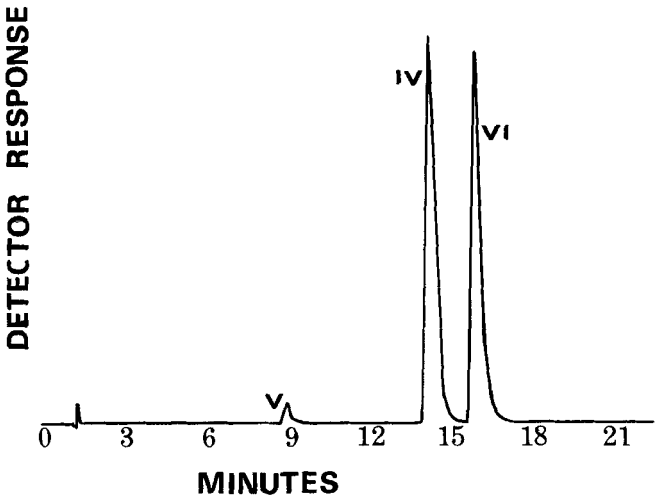

FIGURE 2 Liquid chromatogram of thermally stressed propranolol hydrochloride (IV) in 0.1 N hydrochloric acid [more polar product (V) and less polar product (VI)].

Separation of propranolol hydrochloride from possible degradation products - Propranolol hydrochloride is, as expected from its structure, a stable molecule. The drug degraded only under severe stress conditions, e.g. refluxing for 24 hours in 1.0 N hydrochloric acid, to a more polar product, tr = 9.0 min., and to a less polar unidentified product, tr = 15.9 min. A typical chromatogram of (IV) is shown in Figure 2. No degradation was observed when solutions of (IV) in distilled water and 0.1 N methanolic potassium hydroxide were heated at reflux for 24 hours.

SUMMARY

A simple stability indicating assay for the simultaneous determination of propranolol hydrochloride and

hydrochlorothiazide has been successfully developed and applied to both drugs in tablet formulations. The assay is selective for hydrochlorothiazide, separating the drug from chlorothiazide, a process contaminant and 4-amino-6-chloro-1,3-benzenedisulfonamide, a synthesis precursor and hydrolysis product.

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