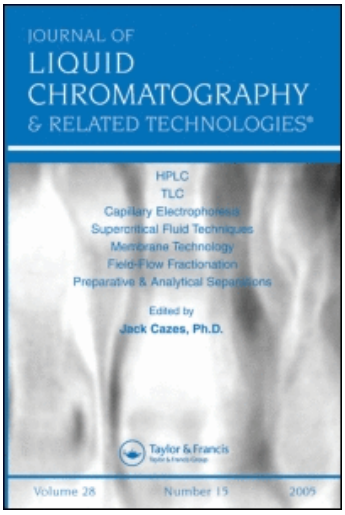

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DETERMINATION OF ATENOLOL COMBINATIONS WITH HYDROCHLOROTHIAZIDE AND CHLORTHALIDONE IN TABLET FORMULATIONS BY REVERSE-PHASE HPLC

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ABSTRACT

A high performance liquid chromatographic procedure is presented for the simultaneous determination of atenolol, hydrochlorothiazide, and chlorthalidone in pharmaceutical tablets combinations. An aliquot of the sample is dissolved in methanol containing methyl p-hydroxybenzoate as an internal standard and chromatographed on a supelcosil (5 um) LC-8-DB (250mm x 4.6mm i.d.) column using a mobile phase of 1.0 mM ammonium acetate and 2.0 mM octanesulfonic acid sodium salt in acetonitrile: water (25 : 75) solution, the pH was adjusted to 3.5 with glacial acetic acid. The relative standard deviations are less than 1 % for the five commercial tablets analyzed. The method was tested for linearity, recovery, and specificity and was found to be fast, stability-indicating, and free from interferences.

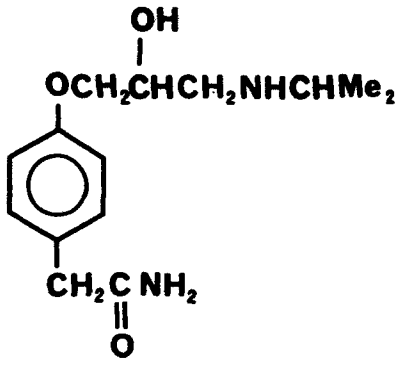
INTRODUCTION

Combination tablet forms of atenolol (I) with hydrochlorothiazide (II), and atenolol with chlorthalidone (III) are marketed by several manufacturers as diuretic and antihypertensive drugs.

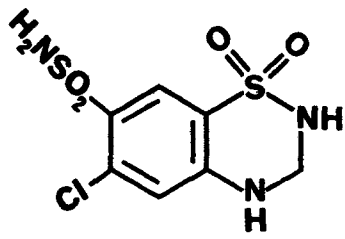
Atenolol is a beta-adrenergic receptor antagonist. Analysis of atenolol has previously been described by spectrofluorometric method(1). The method is simple but could not be applied to its combinations with hydrochlorothiazide and chlorthalidone. The GLC determination of I is specific and sensitive but requires a lengthy derivatization step (2).

Hydrochlorothiazide and chlorthalidone are widely used as diuretics in the treatment of hypertension and in the treatment of congestive heart failure. Several methods are available for the assay of II alone (3-5), or in combination with other drugs such as methyldopa(6), hydralazine(7), reserpine(7,8), furosemide(9), trimaterene(10), and other-antihypertensive drugs of the thiazide family (11). However, none of the methods is applicable to the simultaneous determination of these compounds in combination with I.

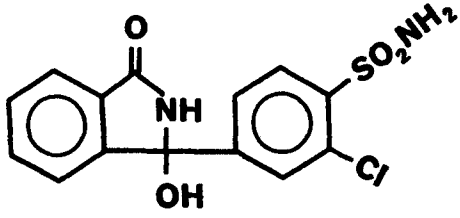
This paper describes a reverse phase HPLC assay for the quantitative determination of I, II and III and some of their



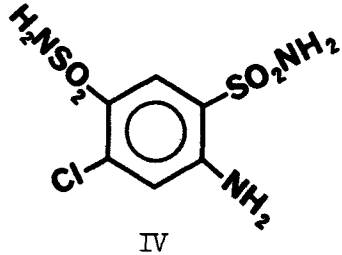
I



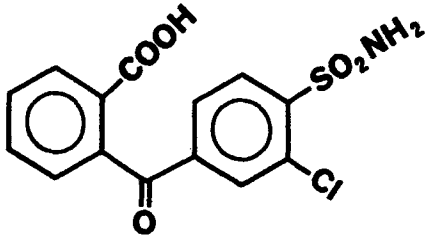
II



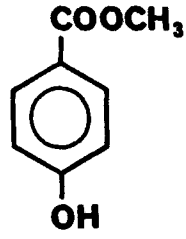
III



IV



V



Internal Standard

ralated compounds [4-amino-6-chloro-1,3-benzenedisulfonamide (IV) and 2-(4-chloro-3-sulphamoylbenzoyl) benzoic acid (V)]. The assay was applied successfully to five commercial products and proved to be free of interferences from excipients normally used in tablet formulations. The assay is fast since it requires only little sample manipulation.

It was also validated for precision and accuracy. The parameters investigated in this assay included acetonitrile percentage, pH, and ionic strength of the mobile phase.

EXPERIMENTAL

Apparatus

The apparatus employed was a Varian 2010 pump (Varian Associates, Inc. Palo Alto, CA, U.S.A.) equipped with a 10-uL loop injector model 7125 (Rheodyne, Cotati, Ca, USA) connected to a Varian 2050 spectrophotometric detector and a Varian 4290 integrator. A reverse phase column(250mm x 4.6mm i.d.) Supelcosil LC-8-DB (5 um) (supelco, Inc., Pennsylvania, USA) was used at ambient temperature.

Materials

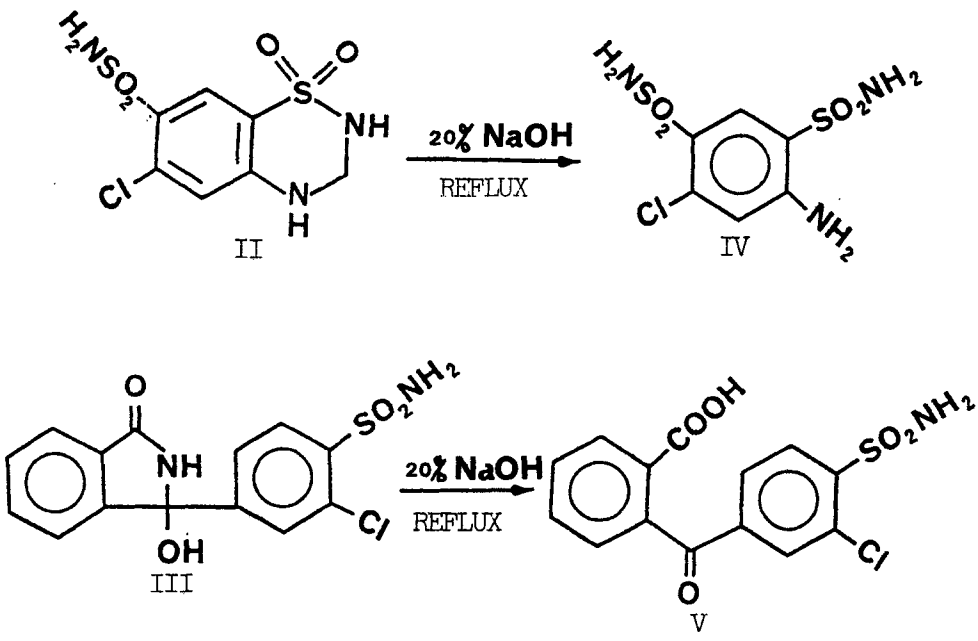
The reference standards, and the internal standard (methyl p-hydroxybenzoate, were from BP (British

Pharmacopoeia Commission Laboratory, Middlesex, U.K.). Acetonitrile-HPLC, methanol, and glacial acetic acid were from May & Baker Ltd (Dagenham, U.K.), Riedel-de Haen AG (West Germany) and Koch-Light Ltd (Haverhill, U.K.), respectively. Ammonium acetate purum grade and octanesulfonic acid sodium salt (99.0 %) were obtained from Fluka (Switzerland). Water was always distilled and deionized.

Excipients usually used in the tablet formulations: magnesium carbonate, potato starch, sodium lauryl sulfate, povidone microcrystalline cellulose, magnesium stearate, and coloring agents were supplied by Al-Hikma Pharmaceuticals, Amman-Jordan. Commercial tablets were purchased locally.

Preparation of Reference Decomposition Products:

The decomposition products of hydrochlorothiazide and chlorthalidone (IV and V) were prepared (Scheme I) by dissolving 1.0 gm of the parent compound in 30 ml of 20 % NaOH solution. The mixtures were refluxed for 2 hrs, allowed to stand for 20 hrs, and then acidified to pH 4.0 with 6 N HCl. The resulting precipitates were separated by vacuum filtration, washed with distilled water, and recrystallized from water for IV and methanol: water for V to a constant



Scheme 1

melting point. The identity of the products (IV and V) was confirmed by IR and NMR.

Chromatographic Conditions:

The mobile phase consists of 1.0 mM ammonium acetate and 2.0 mM sodium octanesulfonate in acetonitrile: water (25:75); the pH was adjusted to 3.5 with glacial acetic acid. The mobile phase was always filtered using 0.45 um-membrane filters (Supelco, Inc.), and degassed by vacuum prior to use. The flow rate was 1.5 mL/min. The wavelength was 254 nm and

the sensitivity was set at 0.2 AUFS. The chart speed was 0.25 cm/min.

Study of The Interferences of Placebo Excipients - A mixture of the excipients was dissolved and treated in the same manner as the sample solution. Ten-ul injections were made under the chromatographic conditions described.

Preparation of the Standard Solutions:

Internal Standard Solution - The internal standard solution was prepared by dissolving 10mg of methyl p-hydroxy-benzoate in 1.0 L of methanol.

Standard Solutions For Linearity - Standard stock solution containing 150.0 mg of Atenolol, 37.5 mg of hydrochlorothiazide, and 37.5 mg of chlorthalidone was prepared in 25 ml of the internal standard solution. The following concentrations of atenolol, hydrochlorothiazide, and chlorthalidone in the internal standard solution were prepared by dilution: 5.00, 7.00, 8.00, 10.00 and 15.00, 1.25, 1.50, 2.00, 2.50, and 3.75 ; and 1.25, 1.50, 2.00, 2.50, and 3.75 ug/10uL, respectively.

Atenolol and Chlorthalidone Standard Solution- One hundred mg of atenolol and 25.0 mg of chlorthalidone were

dissolved in 25.0 mL internal standard solution. This was further diluted with the internal standard solution to obtain a final concentration of 1.0 mg/mL and 0.25 mg/mL, respectively.

Hydrochlorothiazide Standard Solution - Twenty five mg hydrochlorothiazide was dissolved in 25 mL internal standard solution. This was further diluted with the internal standard solution to obtain a final concentration of 0.25 mg/mL.

Chlorthalidone Standard Solution - Twenty five mg of chlorthalidone was dissolved in 25 mL internal standard solution. This was further diluted with the internal standard solution to obtain a final concentration of 0.25 mg/mL.

Preparation of The Sample Solution:

Twenty tablets (one tablet if content uniformity was to be determined) were weighed and powdered. Accurately weighed portions of the powder (each equivalent to the weight of one tablet) were placed in 25 mL-volumetric flask. Each sample was sonicated for three minutes with 20 mL of the internal standard solution, then completed to volume with the internal standard solution. Samples were further diluted with the internal standard solution to obtain a concentration of 1.00,

0.25, and 0.25 mg/mL of atenolol, hydrochlorothiazide, and chlorthalidone. The solutions were filtered through 0.45 um-membrane filters.

Percent Recovery Study - The study was performed by preparing sythetic mixutres identical to the pharmaceutical formulations and were spiked with known amounts of atenolol 50.0, 70.0, 80.0, 100.0, 125.0, and 150.0 mg and the following amounts of hydrochlorothiazide and chlorothalidone: 12.50, 15.00, 20.00, 25.00, 30.00, and 37.50mg spanning the range of 50-150% of the expected assay values. The resulting mixtures were assayed and the results obtained were compared with the expected one.

Assay Method - Equal volumes (10-uL) and approximately equal concentrations of the standard and sample solutions were injected into the HPLC and chromatographed under the conditions descdribed above. The standard and the sample solutions contained the same concentration of the internal standard. The quantity of each component injected was always within the linearity range

Calculations - The results were calculated using response ratios (RR) relative to internal standard based on peak areas:

$$\text{Percent of the label claim found} = \frac{RRx}{RRs} \times 100$$

Where RRx = sample response ratio; RRs = standard response ratio.

RESULTS AND DISCUSSION

In order to optimize the chromatographic parameters, the effects of acetoitrile composition, pH, and ionic strength on the capacity factor (k') were conducted (Fig. 1-3). The capacity factor (k') values for I, III, V, and the internal standard were substantially affected by the variation of acetonitrile composition in the mobile phase (Fig. 1). At low acetonitrile concentration, k' values were high and the peaks were broadened. At high acetonitrile concentrations k' values were lower with sharper peaks. Values of k' for II and IV were slightly affected by acetonitrile composition in the mobile phase with interference at higher than 30 %. Thererfore, a mobile phase of 25 % acetonitrile was selected since it provided completely resolved sharp peaks within a reasonable elution time.

Variation of pH (Fig. 2) yielded considerable change in k' values of V while it had no effect on k' values of the other compounds. compound V, being the only carboxylic acid, explains the considerable increase in k' upon increasing the pH value. This behaviour could be attributed to the

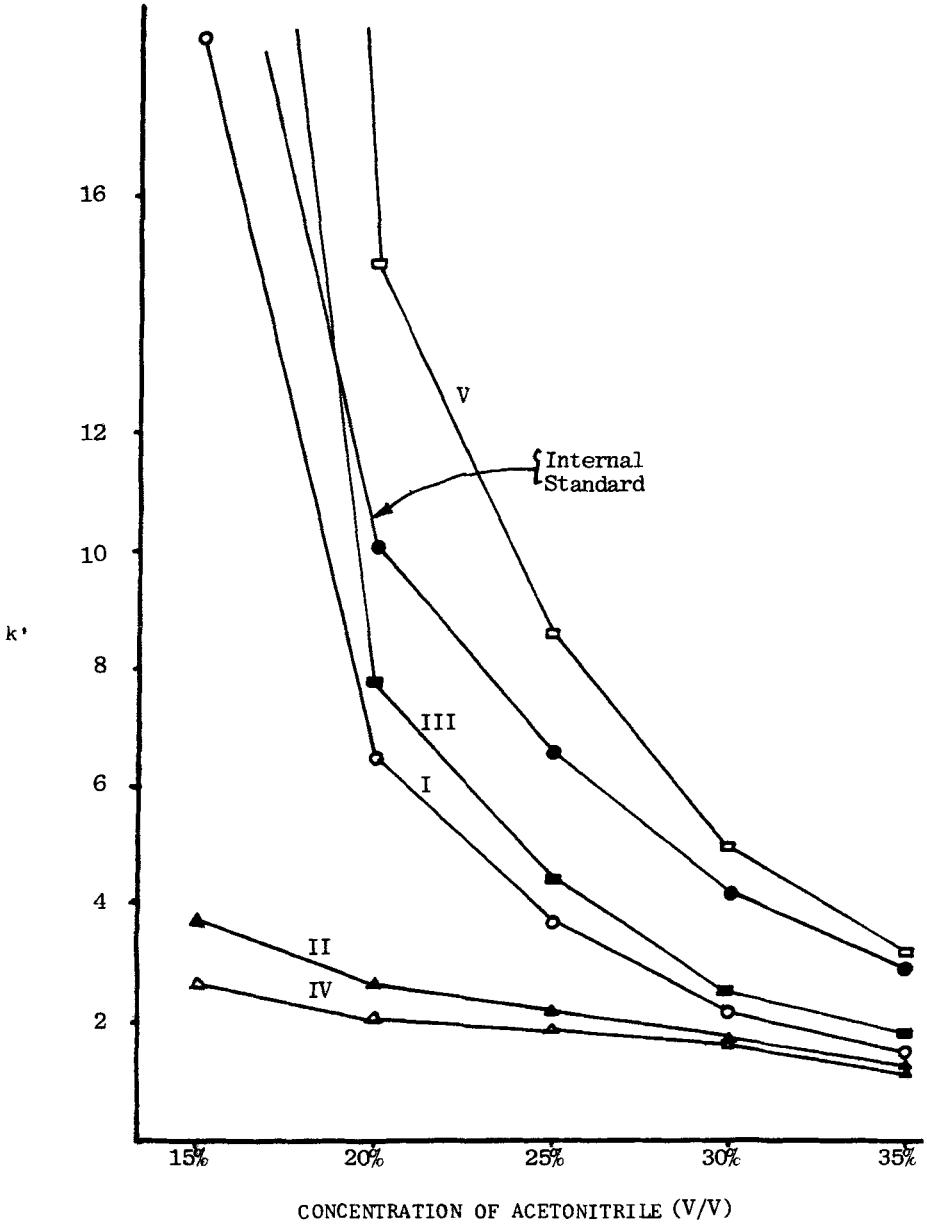


Figure (1) : Plots of the capacity factor versus the acetonitrile compostion in the mobile phase.

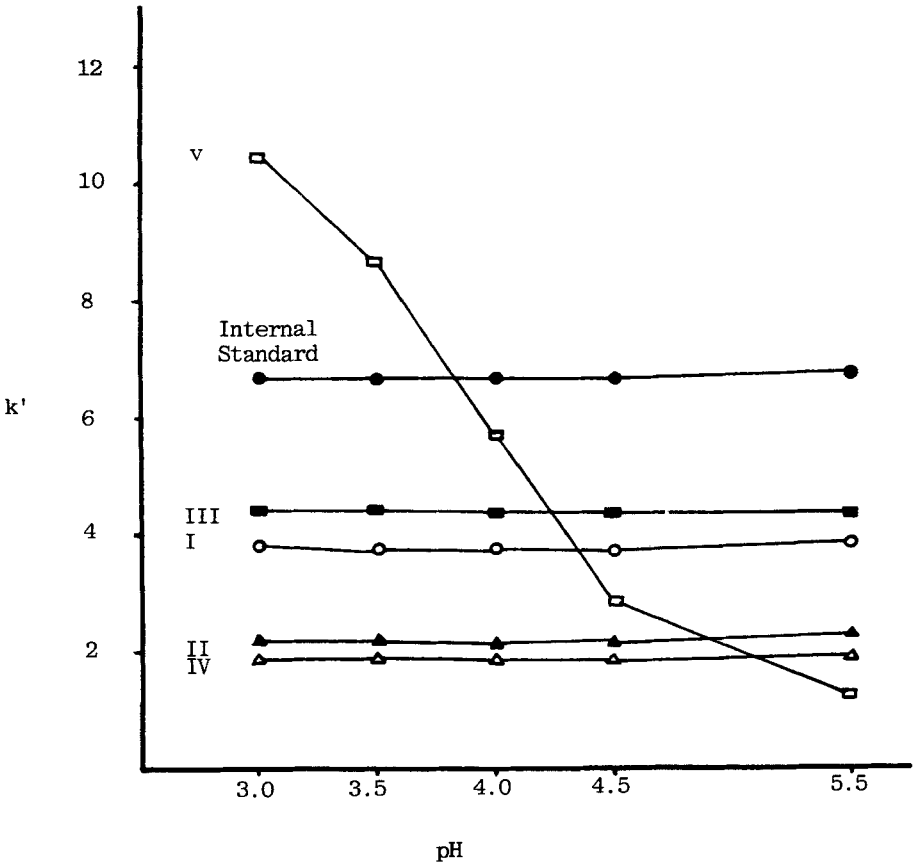


Figure (2) : Plots of the capacity factor versus pH.

increased solubility of acid at higher pH values. pH 3.5 - 4.5 could be chosen as optimum values for the analysis. However, pH 3.5 was selected at which the internal standard elutes before compound V. There was, however, no variation in the sharpness of the peaks between pH 3.5 - 4.5 and no considerable difference in the elution time.

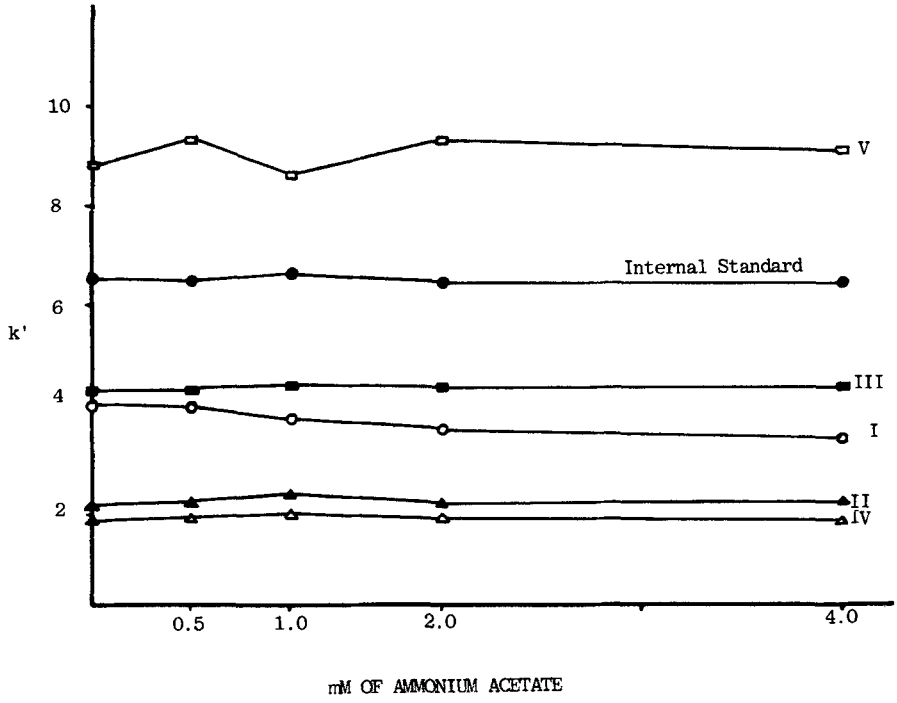


Figure (3) : Plots of the capacity factor versus the molarity of ammonium acetate.

The variation of the ionic strength slightly affected the k' values (Fig. 3). Satisfactory k' values were obtained at 1.0 mM ammonium acetate. Although the effect of ammonium acetate on k' values is insignificant, yet it has a considerable effect on reducing the peak tailing of atenolol. Also, the addition of 2.0 mM of sodium octanesulfonate has been found to reduce the peak tailing of atenolol.

To determine the linearity of the detector response, calibration standard solutions of I, II and III were prepared as described in the text. A plot of peak area ratios versus amounts injected was linear up to 15.00, 3.75, and 3.75 ug, respectively with correlation coefficients of 0.999 or better for each compound.

To determine the accuracy of the method, each standard was spiked with a placebo and subjected to HPLC analysis. In all cases, satisfactory recoveries and reproducibility of peak areas were obtained. A linear regression analysis of the data shows excellent correlation over the analysis range studied (Table I). No interferences due to excipients were detected in the chromatograms produced. The detection limits were 200, 25, and 50 pg for I, II, and III, respectively, as determined by diluting the standard solutions with methanol and injecting 10-uL of each solution into the column.

The specificity of the method is illustrated in (Fig. 4) where complete separation was noticed for a synthetic mixture of I, II, III, IV, V, and the internal standard. The results of analysis of five commercial products (Table II, Figures 5-8) indicate that the proposed assay can be used for the quantitation of I, II, and III in commercial samples. The accuracy of the method was supported by the closeness of the results to the label claim. The precision of the HPLC method

TABLE (I)

RECOVERY OF I, II, AND III FROM SPIKED PLACEBO SAMPLES FOR 6 DETERMINATIONS

I ± RSD			II±RSD			III±RSD		
Added/Tablet (mg)	Found/Tablet (mg)	Recovery (%)	Added/Tablet (mg)	Found/Tablet (mg)	Recovery (%)	Added/Tablet (mg)	Found/Tablet (mg)	Recovery (%)
50.00	50.01±1.76	100.02±1.76	12.50	12.77±1.06	102.15±1.06	12.50	12.47±0.37	99.76±0.37
70.00	69.81±0.34	99.73±0.34	15.00	15.10±0.73	100.67±0.73	15.00	15.32±0.27	102.13±0.27
80.00	80.72±0.82	100.90±0.82	20.00	20.18±1.81	100.90±1.81	20.00	20.07±0.60	100.35±0.60
100.00	100.20±0.55	100.20±0.55	25.00	25.01±0.62	100.04±0.62	25.00	24.60±0.20	98.40±0.20
125.00	124.46±0.29	99.57±0.29	30.00	29.68±0.70	98.93±0.70	30.00	29.96±0.32	99.87±0.32
150.00	148.86±0.27	99.24±0.27	37.50	37.31±0.96	99.49±0.96	37.50	37.81±0.43	100.83±0.43
Intercept :	1.0182		0.4881			- 0.0079		
Slope :	0.9877		0.9794			1.0020		
R :	0.9999		0.9999			0.9996		

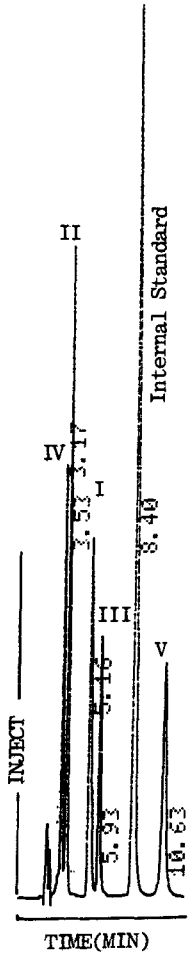


Figure (4) : A typical chromatogram of a 10-uL injection of a synthetic mixture of I - V and the internal standard.

TABLE (II)

HPLC ASSAY RESULTS FOR COMMERCIAL PRODUCTS

PERCENT OF THE LABEL CLAIM FOUND

PRODUCT	ATENOLOL [f]	HYDROCHLOROTHIAZIDE [f]	CHLORTHALIDONE [f]
Hypozide [a]	100.3 ± 0.04	100.82 ± 0.03	——
Tenoretic [b]	100.41 ± 0.55	——	99.27 ± 0.81
Esidrex [c]	——	100.06 ± 0.59	——
Hygroton-50 [b]	——	——	99.83 ± 0.46
Hygroton-100 [e]	——	——	99.87 ± 0.99

a Lot 634 (Al-Hikma Pharmaceuticals - Amman - Jordan).
b Lot 1 H 323 (Imperial Chemical Industries PLC, U.K.)
c Lot 006000 (CIBA - GEIGY Limited, Basle, Switzerland)
d Lot 011500 (CIBA - GEIGY)
e Lot 745587 (CIBA - GEIGY)
f Mean ± RSD for 3 x 6 determinations (three samples, six injections each).

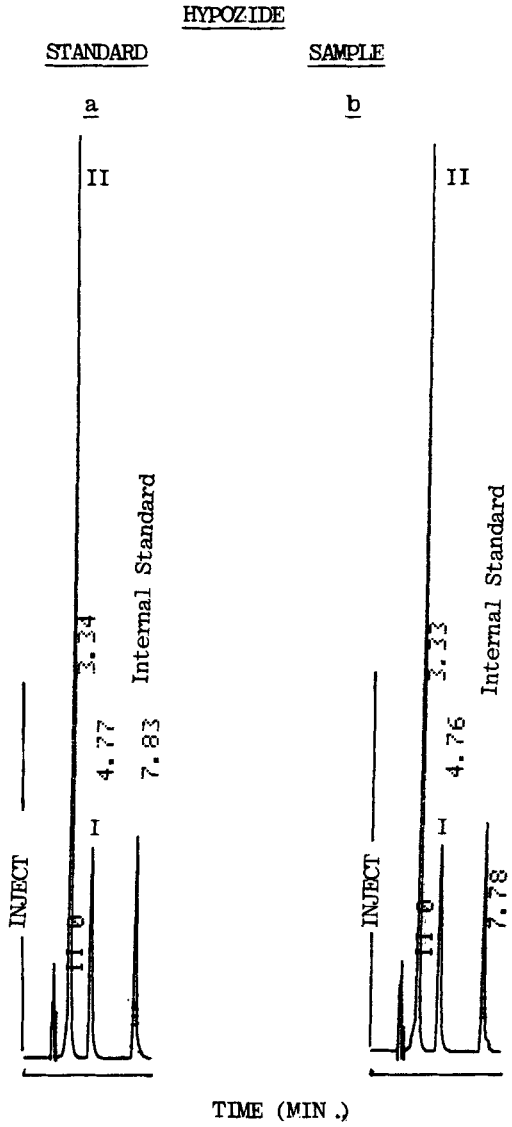


Figure (5) : a) A typical chromatogram of a 10-uL injection of a standard containing 2.5 ug of II, 10 ug of I, and 0.1 ug of the interal standard (the assay method procedure).

b) A typical chromatogrtam of a 10-uL injection of a commercial product (Hypozide).

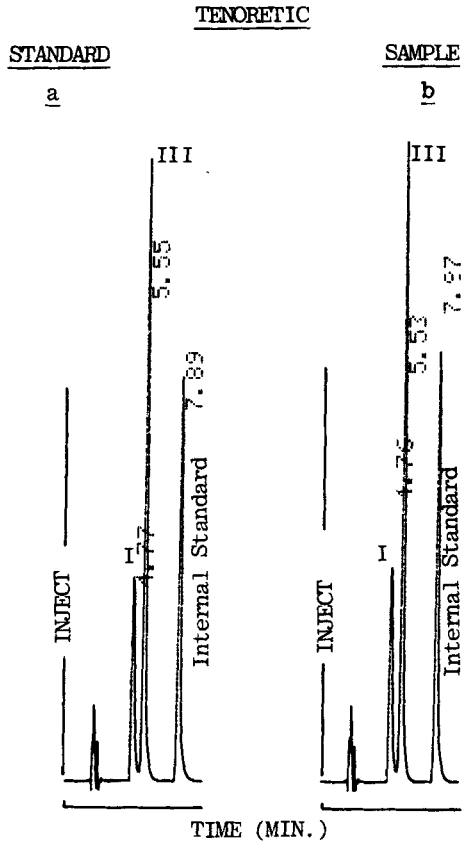


Figure (6) : a) A typical chromatogram of a 10-uL injection of a standard containing 10 ug of I , 2.5 ug of III, and 0.125ug of the internal standard.

b) A chromatogram for a 10-uL injection of a solution made of one tablet of Tenoretic (the assay method procedure).

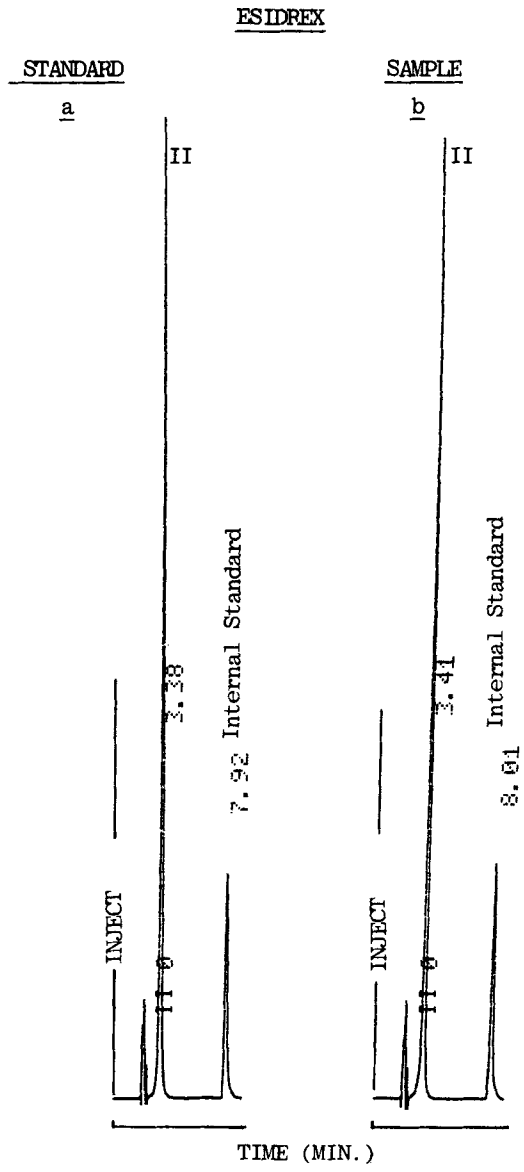


Figure (7) : a) A typical chromatogram of a 10-uL injection of a standard containing 2.5 ug of II and 0.1 ug of the internal standard.

b) A chromatogram for a 10-uL injection of a solution made of one tablet of Esidrex (the assay method procedure).

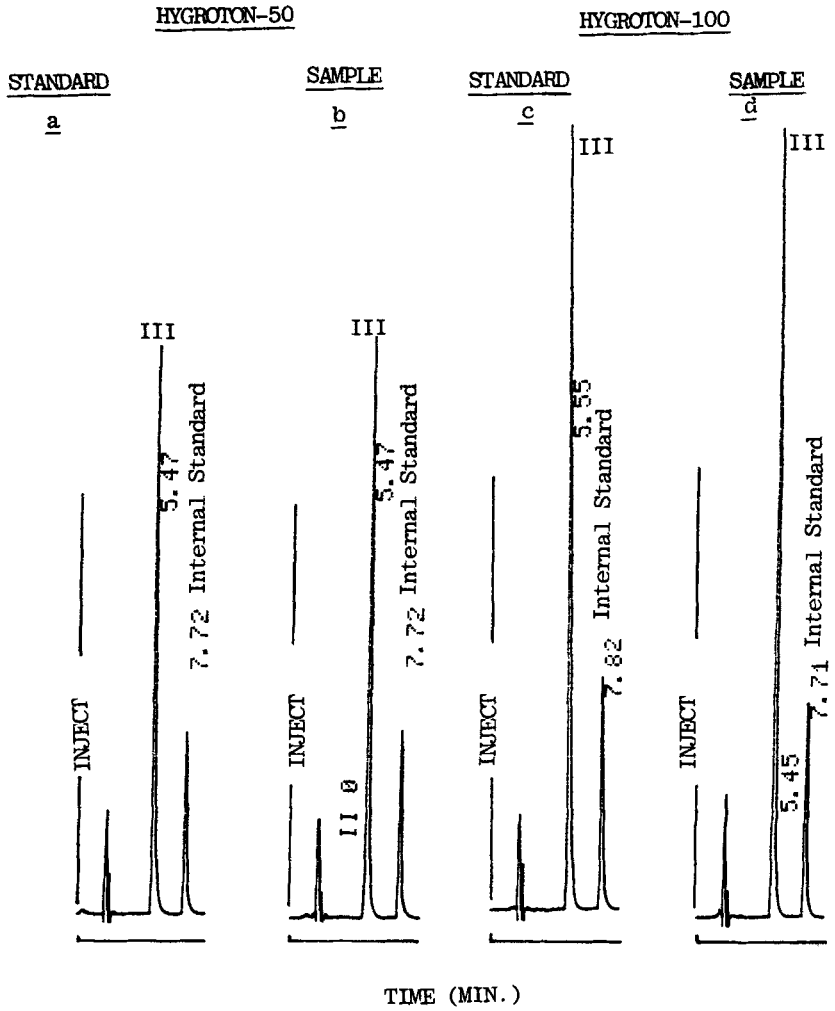


Figure (8) : A typical chromatogram of a 10-uL injections for:

a) a standard contining 2.0 ug of III and 0.1 ug of the internal standard.

b) sample of Hygroton-50.

c) a standard containing 2.5 ug of III and 0.1 ug of the internal standard.

d) sample of Hygroton-100.

TABLE (III)

CONTENT UNIFORMITY FOR I, II, AND III IN COMMERCIAL TABLETS BY HPLC.

	PERCENT LABEL CLAIM FOUND (*)						
TABLET No.	HYPOZIDE		TENORETIC		ESIDREX	HYGROTON-50	HYGROTON-100
	I	II	I	III	II	I	I
1	96.42	97.86	98.98	96.32	100.78	95.21	100.16
2	104.62	104.28	100.00	97.10	105.25	102.46	95.11
3	99.58	97.89	103.48	100.32	103.50	101.79	102.91
4	106.56	103.75	99.86	97.49	91.63	96.53	95.11
5	98.05	99.85	100.67	98.12	97.14	94.48	97.05
6	100.49	99.21	97.97	97.55	98.11	100.22	103.01
7	104.04	100.63	100.12	98.31	100.12	97.76	100.86
8	98.66	99.84	96.50	96.32	94.58	101.47	98.50
9	94.56	100.59	96.76	96.68	105.50	97.45	95.11
10	103.03	100.84	102.12	100.83	97.07	97.81	94.89
Mean	100.30	100.47	99.65	97.90	99.37	98.51	98.27
RSD	3.44	2.13	2.21	1.60	4.58	2.86	3.34
High	104.65	104.28	103.48	100.83	105.50	102.46	103.01
Low	97.86	94.56	96.50	96.32	91.63	94.48	94.89

(*) Each Data point is the average of two injections).

is supported by the very small relative standard deviation (RSD) based on 3 x 6 readings.

The specificity of the method is furhter confirmed by the results of content uniformity which show compliance to specifications of all dosage forms and support the specificity of the HPLC results (Table III).

A stability study was performed on standards of I, II, and III by placing samples in water-glycerin bath at 60°C for one mounth, the results show that, compound II was stable in this peroid without any measurable degradation to IV. Compound III degraded to give traces of V(0.06±5.33% mg).

In conclusion, the HPLC assay described here has been shown to be of general applicability to commercially available products. The method is quite simple, accurate, precise, rapid and easy to perform.

ACKNOWLEDGEMENTS

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