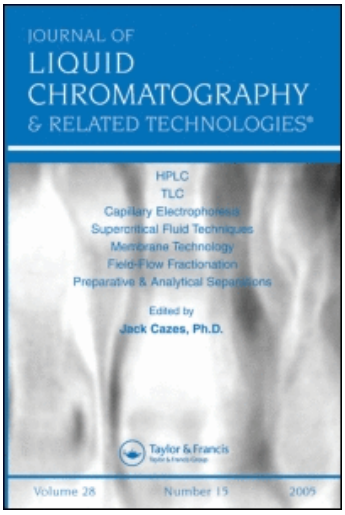

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Determination of Atenolol and Its Related Compounds by Ion Pair High Performance Liquid Chromatography

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DETERMINATION OF ATENOLOL AND ITS RELATED COMPOUNDS BY ION PAIR HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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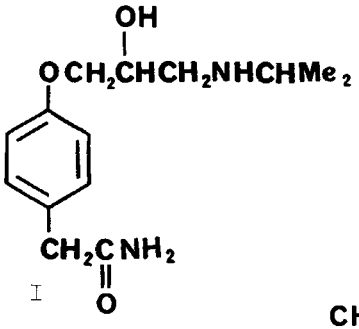
ABSTRACT

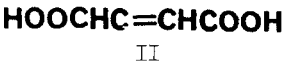
A rapid, simple, accurate, and stability-indicating ion pair high performance liquid chromatography (IP HPLC) procedure is presented for the determination of atenolol in pharmaceutical tablets. The related compounds of atenolol were separated, making the determination specific for atenolol. An aliquot of the sample is dissolved in methanol containing N-butyryl-4-aminophenol as an internal standard and chromatographed on a supelcosil LC-8-DB (5μ) (250mm x 4.6mm i.d.) column using 1.0 mM ammonium acetate and 2.0 mM octanesulfonic acid sodium salt in acetonitrile: water (25:75) solution adjusted to pH 3.5 with glacial acetic acid as the mobile phase. The detection was carried at 225 nm. The relative standard deviations are less than 1.0% for the two commercial products analyzed. The method was tested for linearity, recovery, and specificity.

INTRODUCTION

Atenolol (I), benzeneacetamide, 4-{2-hydroxy-3-{(1-methylethyl)-amino} propoxy}-{29122-68-7}, is a beta-adrenergic blocking agent.



I



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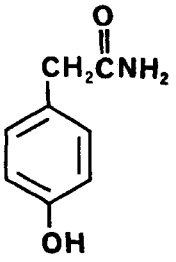

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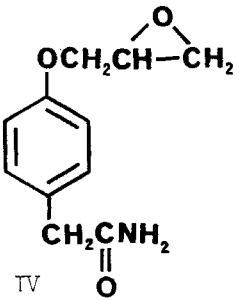

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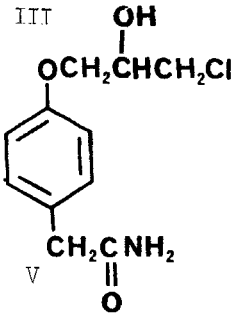

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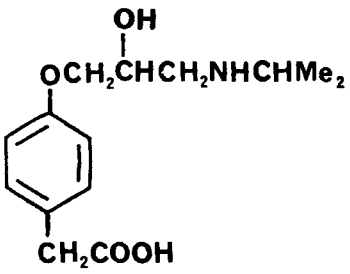

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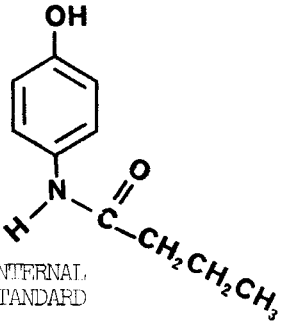

INTERNAL STANDARD

The therapeutic advantages of this drug include selectivity for beta-1 receptors (1) and efficacy in treating hypertension when administered once daily (2).

Previous procedures for the determination of atenolol in plasma and urine employed GLC with electron-capture detection (3,4) or spectrophotofluorometry (5). The spectrophotofluorometry is simple but lacks specificity. Although the GLC methods are highly selective, they are relatively complex and rely on a demanding prechromatography derivatization. The HPLC methods (6,7) with fluorescence detection are selective for the determination of atenolol in plasma but are not stability-indicating.

A high performance liquid chromatographic assay was developed for the determination of atenolol and its related compounds in tablet formulations. The assay is simple, rapid, and precise for the quantitative determination of atenolol tablet formulations. The method is sensitive to the related compounds of I, (fumeric acid (II), 4-hydroxyphenylacetamide (III), 4-(2,3)-epoxypropyloxy) phenylacetamide (IV), 4-(2-hydroxy-3-chloropropyloxy) phenylacetamide (V), and 4-{2-hydroxy-3-{1-methylethyl)amino propoxy}-benzene acetic acid (VI)}.

EXPERIMENTAL

Materials - Atenolol was obtained from the BP (British Pharmacopoeia Commission Laboratory, Middlesex, U.K.).The related compounds were kindly supplied by AMSA (Anonima Materie Sintetiche & Affini S.P.A., Milano, Italy). The internal standard, N-butyryl-4-aminophenol, was

obtained form Sterling Winthrop (England). Acetonitrile-HPLC grade (99.8%), methanol (99.5%), and glacial acid were from May and Baker Ltd. (England), Reidel-de Haen (West Germany), and Koch Light (England), respectively. Ammonium acetate and octanesulfonic acid sodium salt were from Fluka AG (Switzerland). The water used was always distilled and deionized.

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

Apparatus - The apparatus empolyed was a Varian 2010 pump (Varian, California, U.S.A.), equipped with a 10-µL manual Rheodyne loop injector (Rheodyne, California, U.S.A.), a Varian 2050 UV variable wavelength detector, and a Varian 4290 integrator.

Chromatographic Conditions - A reversed phase column (250mm x 4.6mm i.d.), Supelcosil LC-8-DB (5u) (Supelco, Inc. Pennsylvania, U.S.A.) was utilized at ambient temperature. The mobile phase was 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 mobile phase was always filtered using 0.45 µm-membrane filters (Supelco, Inc.), and degassed by vacuum prior to use. The samples also were always filtered. The wavelength was 225 nm, and the sensitivity was set at 0.20 AUFS. The flow rate was 1.5 mL/min. 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-µL injections were made under the chromatographic conditions described.

Preparation of The Standard Solutions:

Internal Standard Solution- Fifty mg of N-butyryl-4-aminophenol was dissolved in 1000 mL methanol.

Standard Solutions For Linearity- One hundred fifty mg of atenolol was dissolved in 25 mL of the internal standard solution. The following concentrations of atenolol in the internal standard solution were prepared: 0.12, 0.10, 0.08, 0.06, 0.04, and 0.02 mg/mL

Atenolol Standard Solution- One hundred mg of atenolol was dissolved in 25 mL internal standard solution, and 0.2 mL was diluted to 10 mL of the internal standard solution to obtain a final concentration of 0.08 mg/mL.

For stability study, the standard solution was prepared by dissolving 100 mg of I in 25 mL internal standard solution.

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 0.08 mg/mL of I. The solutions were filtered through 0.45 μm-membrane filters.

The stability samples were treated in the same manner but without dilution in order to quantitate the related compounds.

Percent Recovery Study- The study was performed by preparing synthetic mixtures identical to the pharmaceutical formulations and were spiked with known amounts of I (25.0, 50.0, 75.0, 100.0, 125.0, and 150.0 mg) spanning the range of 25-150% of the expected assay values. The resulting mixtures were assayed and the results obtained were compared with the expected ones.

Assay Method- Equal volumes (10-μL) and approximately equal concentrations of the standard and sample solutions were injected into the HPLC and chromatographed under the conditions described 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.

In stability study, the external standard method was used for the calculations.

RESULTS AND DISCUSSION

The chromatogram shown in Figure 1 indicates the possibility of separating atenolol from the internal standard, related compounds (IV, V, and VI), and the excipients. These excipients eluted with the solvent peak. Compound II was found to elute with the solvent front and III was detected at 275 nm with a retention time of 2.50 min.

The detection limit based on signal-to-noise of 2 was less than 1.0 ng for I as determined by diluting a standard solution with methanol and injecting 10-μL into the column.

The linearity of the detector response was determined by injecting standard solution of I in the internal standard solution as described previously in the text. The peak area ratios to the internal standard were measured and plotted vs. the ammount injected. The peak area ratios were found linear for the range of 0.02-1.20 μg of I with a correlation coefficient of 0.9997.

The accuracy of the method was demonstrated by the recovery study, each standard was spiked with a placebo and subjected to HPLC analysis. In all cases satisfactory recoveries and reproducible results of peak area were obtained. A linear regression of the data shows excellent linearity over the analysis range studied (Table I).The selectivity of the method is validated by the fact that no interference due to excipients was detected in the chromatograms produced.

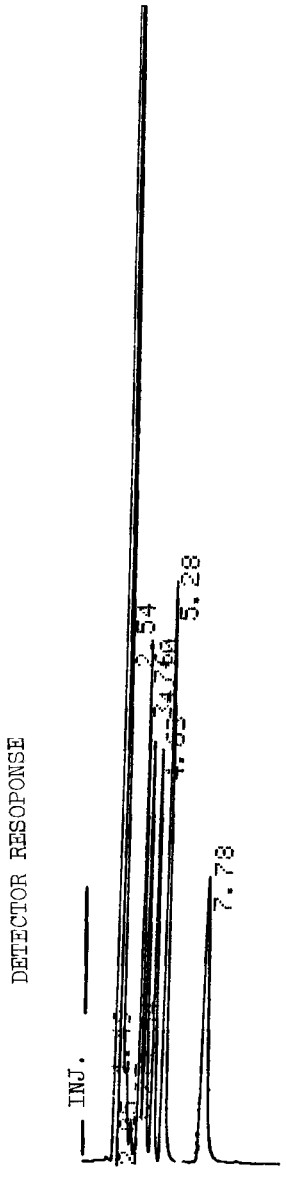

Figure 1. A typical chromatogram of a 10-μL injection of IV (t_R = 8.76 min.), V (t_R = 4.10 min.), internal standard (t_R = 4.63 min.), I (t_R = 5.28 min.), and VI (t_R = 7.78 min.).

TABLE (I)

Recovery Of Atenolol From Spiked Placebo Samples.

mg Added	mg Found[a]	% Recovery[b]
150.00	152.17 ± 1.08	101.51 ± 1.08
125.00	127.58 ± 0.88	102.06 ± 0.88
100.00	100.65 ± 1.89	100.65 ± 1.89
75.00	75.17 ± 1.08	100.24 ± 1.07
50.00	50.91 ± 1.41	101.81 ± 1.41
25.00	24.77 ± 0.35	99.10 ± 0.35

R = 0.9999 Intercept = -0.7073 Slope = 1.0200

[a] Mean ± RSD for 6 determinations

[b] Mean ± RSD for 6 determinations

The results of analysis of the commercial products (Table II) indicate the versatility of the developed assay for the quantitation of I in commercial tablets. Comparison of the results of the HPLC assay with the UV method (Table III) shows that the proposed method is accurate and precise

The specificity of the method is further confirmed by comparing the results of content uniformity test of I which were performed on two commerical tablets (Table IV).The results of content uniformity show compliance to specifications of the tablets and support the specificity of the HPLC results. However, the HPLC method is superior to the UV method since it is faster, simpler, more specific, and more versatile.

TABLE (II)

HPLC Assay Of Atenolol In Commercial Tablets (% Label Claim ± RSD)

Sample	Hypoten (100 mg)[a]	Tenormin (50mg)[b]
1	98.76 ± 1.01	100.73 ± 0.47
2	99.63 ± 0.84	100.62 ± 0.58
3	99.86 ± 0.91	100.68 ± 0.77
Mean	99.42	100.68
RSD	0.58	0.06

(Mean ± RSD for 3x6 determinations).

[a] Lot 2413 (Al-Hikma Pharmaceuticals- Amman - Jordan)

[b] Lot NH 332 (Imperial Chemical Industries PLC, U.K.)

TABLE (III)

Comparison of HPLC with the UV Results of Atenolol.

Product	Percent Label Claim Found ± RSD HPLC[a]	UV[b]
Hypoten-100	99.42 ± 0.58	102.19 ± 2.94
Tenormin -50	100.68 ± 0.06	99.88 ± 2.12

[a] Mean ± RSD for 3x6 determinations (three samples x six injections)

[b] Mean ± RSD for six determinations , UV measurement at 275 nm.

TABLE (IV)

Content Uniformity For Atenolol in Commercial Tablets By HPLC and UV.

Tablet No.	Hypoten-100[a]		Tenormin -50[b]	
	HPLC	UV	HPLC	UV
1	94.44	104.38	95.99	99.22
2	101.53	100.66	98.61	110.85
3	99.87	101.31	95.82	93.41
4	99.17	101.75	102.01	94.19
5	99.20	105.69	98.22	100.78
6	102.64	103.06	106.96	102.23
7	105.89	102.40	102.03	103.49
8	97.72	99.56	97.84	101.94
9	100.10	98.91	103.11	97.67
10	105.56	101.09	99.14	103.10
Mean	100.61	101.88	99.97	100.70
RSD	3.45	2.05	3.50	4.99
High	105.89	105.69	106.96	110.85
Low	94.44	98.91	95.82	93.41

[a] Lot 2413

[b] Lot NH 332

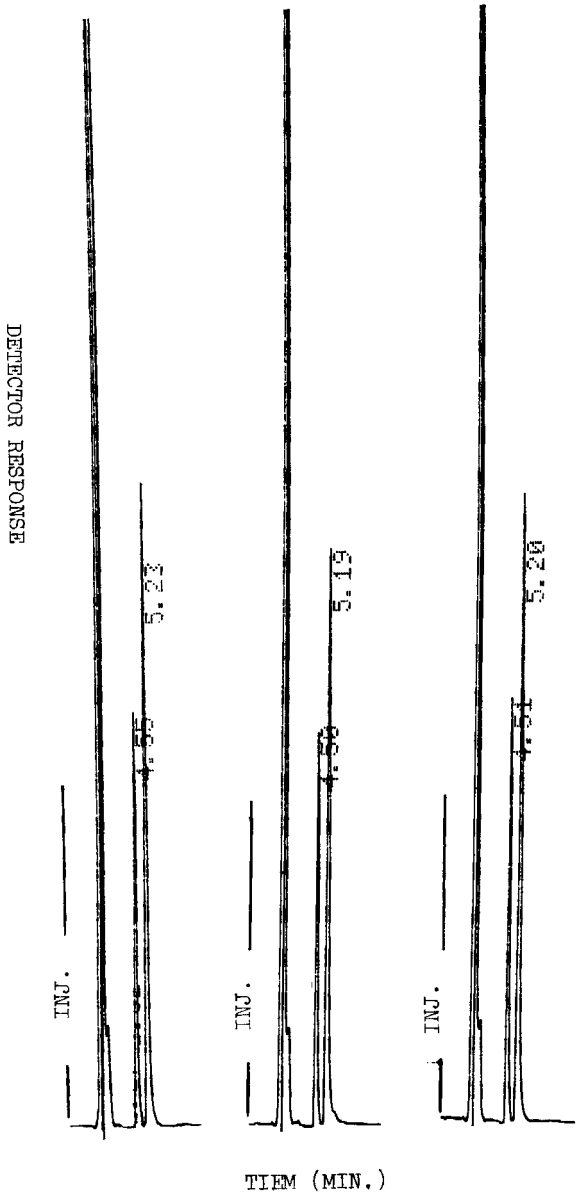

Figure 2. Typical chromatograms of a 10-µL injections containing 0.8 µg of I (t_R = 5.2 min.) and 0.5 µg of the internal standard (t_R = 4.5 min.)

a. Standard solution
b. Sample of Hypoten-100
c. Sample of Tenormine-50

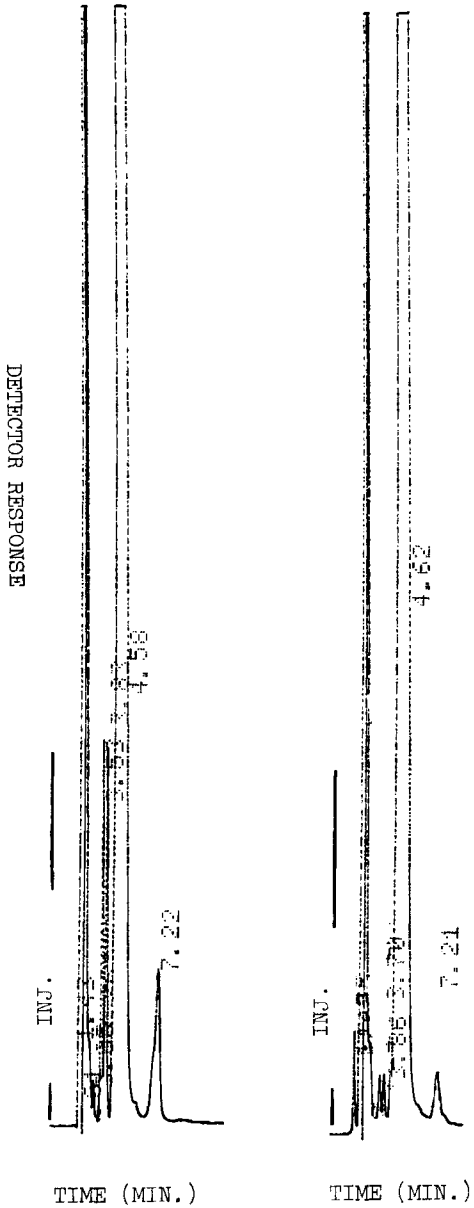

Figure 3. a) A typical chromatogram of a 10-µL injection of a standard containing 2.0µg of IV, 2.0µg of V, and 2.0 µg of VI.

b. Sample chromatogram of a synthetic mixture of standard and placebo excipitents.

Figure 2, shows the chromatograms of the commercial products. This is an additional confirmation to the specificity of the separation and its applicability to drug analysis.

A stability study was performed on atenolol in duplicate by placing samples in dark bottles in glycerin-water bath at 60°C. Samples were taken after one month and assayed in the same manner as the sample procedure without dilutions and it was found that only 0.30mg of IV, 0.44 mg of V, 1.64 mg of VI were formed, and no formation of III (Figure 3).

In conclusion, the assay presented here has been shown to be applicable to commercially available products for the determination of I and its related compounds (IV, V, and VI) quantitatively.

ACKNOWLEDGEMENTS

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