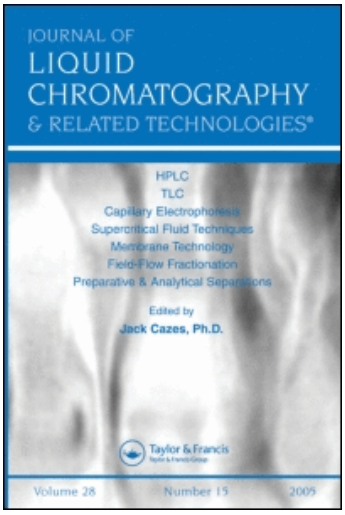

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Validated Reversed-Phase HPLC Method for the Determination of Atenolol in the Presence of Its Major Degradation Product

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Abstract: A reversed-phase liquid chromatographic (RP-LC) assay method, developed for the quantitative determination of atenolol in the presence of its degradation products is described. The assay involved an isocratic elution of atenolol in a Waters μBondapak® C_{18} column using a mobile phase consisting of acetonitrile-sodium phosphate monobasic (0.08 M, pH 3.0) (10:90, v/v). The flow rate was 1.0 mL/min and the analyte monitored at 284 nm. The assay method was found to be linear from 0.4 to 12.8 μg injected. All the validation parameters were within the acceptance range. The developed method was successfully applied to estimate the amount of atenolol in tablets.

Keywords: Atenolol, HPLC, Assay, Stability-indicating method

INTRODUCTION

Atenolol, 4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]-benzeneacetamide, (Figure 1) is a cardioselective β_1-adrenergic receptor blocking agent prescribed for the treatment of hypertension, angina pectoris, and cardiac arrhythmias.[1] Several HPLC methods were reported in the literature for the quantitative determination of atenolol in biological samples,[2–9] with fluorescence detection[10–14] and mass spectrometry.[15–18] Most of the analytical techniques

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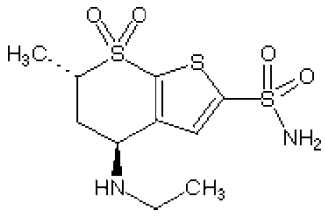

Figure 1. Atenolol.

for atenolol described in the literature are based on the liquid chromatographic determination of this drug in pharmaceutical formulations with another active drug substance.[19–23] Another analytical technique, i.e., derivative spectroscopy, has also been described.[24] An isocratic LC method was developed and reported,[25] the photodecomposition of the drug in aqueous solutions was monitored. A reversed phase LC with UV detection for the quantitation of atenolol in tablets, is described in United States Pharmacopeia and British Pharmacopoeia,.[26,27]

The purpose of this work, was to develop a procedure for the quantitation of atenolol and its separation mainly from its related substances. In addition, forced degradation studies of atenolol were performed to provide an indication of the specificity of the method. The method was validated following the analytical performance parameters suggested by International Conference on Harmonization (ICH).[28]

EXPERIMENTAL

Chemicals and Reagents

Atenolol (99.8% pure) was a gift sample from Andenex-Chemie (Hamburg, Germany). Acetonitrile (HPLC grade) and sodium phosphate monobasic were obtained from J. T. Baker (México). Hydrogen peroxide, hydrochloric acid, sodium hydroxide, were of reagent grade. Water was bidistilled. A commercial local tablet formulation was studied. Its composition was atenolol 50 mg in a matrix of lactose, povidone, magnesium stearate, colloidal silicon dioxide, and pregelatinised starch.

Equipment

The HPLC system consisted of a dual piston reciprocating Spectra Physics pump (model ISO Chrom. LC pump), a UV-Vis Hewlett Packard detector (Model 1050), a Hewlett Packard integrator (Series 3395), and a Rheodyne injector (Model 7125).

Chromatographic Conditions

The analytical column was a Waters μBondapak® C_{18} (3.9 × 300 mm, 5 μm). The mobile phase consists of acetonitrile-sodium phosphate monobasic (0.08 M, pH 3.0) (10:90, v/v). The flow rate was 1.0 mL/min. Detection was performed on a UV detector at 284 nm. The LC was operated at room temperature. The injection volume was 20 μL. In these conditions, atenolol retention time (t_R) was roughly 6.7 minutes.

Preparation of Standard Solutions

A standard stock solution of atenolol (1.0 mg/mL) was prepared by dissolving an appropriate amount in mobile phase. The standard solution was obtained by diluting the standard stock solution with mobile phase to yield a solution containing 0.4 mg/mL.

Sample Preparation

Twenty tablets were weighed and finely powered, and an accurately weighed powder sample equivalent to one tablet was transferred to a 50 mL volumetric flask; 40 mL of mobile phase was added and the flask was kept in an ultrasonic bath for 5 min. The mixture was then diluted to 50 mL with mobile phase. This solution was then diluted with mobile phase to reach a final concentration of 0.4 mg/mL, thoroughly mixed, and filtered through a 0.2 μm nylon membrane (25 mm disposable filter; μicroclar, Argentina, Cat. N° Y02025WPH).

Resolution Solution

A hundred milligrams of atenolol was accurately weighed, transferred to a 50 mL volumetric flask and dissolved in bidistilled water. The solution was placed in an open container in an oven at 110°C for (24 h). The solution was diluted to a concentration of 0.4 mg/mL.

Method Validation

System Suitability

The system suitability was assessed by six replicate injections of the drug at a concentration of 0.4 mg/mL. The acceptance criterion was ± 2% for the percent coefficient of variation (%CV) for the peak area and retention time.

Specificity

Specificity is the ability of the method to measure the analyte in the presence of its potential impurities. Stress testing of the drug substance can help identify the likely degradation products, the stability, and specificity of the analytical procedures.[28] For degradation studies, 100 mg of atenolol was accurately weighed, transferred to a 50 mL volumetric flask, and dissolved in bidistilled water (step 1). To this 5 mL, 5 mL of 0.1 N NaOH (for alkaline degradation) or 0.1 N HCl (for acid degradation), or water, or 15% hydrogen peroxide (for oxidative degradation) was added and refluxed for at least 30 min. The mixture was cooled, transferred to a 25 mL volumetric flask, and diluted with mobile phase. For photolytic degradation, the solution, prepared as in step 1, was exposed to daylight for 24 hr. For thermal degradation, either in the solid state or in solution as in step 1, the sample was placed in an open container in an oven at 110°C for 24 h. Each solution was suitably diluted with mobile phase.

Linearity

Linearity test solutions for the assay method were prepared from stock solution at eight concentration levels, from 5 to 160% of assay analyte concentration (20, 50, 100, 200, 300, 400, 480, 640 μg/mL). The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Precision

Assay precision was evaluated by carrying out six independent sample preparations of a single lot of tablets. The %CV of six assay values obtained was calculated. The intermediate precision of the method was also evaluated by comparing the results obtained by two different analysts.

Accuracy

The accuracy of the assay method was evaluated in triplicate at three concentrations levels of 80, 100, and 120% of atenolol, using tablets from the same lot of a commercial formulation. The percentages of recoveries were calculated.

Robustness

To determine the robustness of the developed method, experimental conditions were purposely altered and retention time and tailing were evaluated. The effect of pH on retention time and tailing was studied by

varying + 0.5 and 1.0 units of final pH buffer. It was also established by changing the proportions of the components in mobile phase.

RESULTS AND DISCUSSION

The described reversed-phase liquid chromatography method was developed to provide a rapid quality control determination of atenolol in tablets. Validation of the method was performed according to ICH. This method uses a simple mobile phase. All samples were analyzed using the assay chromatographic conditions described.

Method Validation

System Suitability

The %CV of peak area and retention time for atenolol is within 2%, indicating the suitability of the system. The resolution between atenolol and its major degradation product was greater than 2.5.

Specificity

No evidence on interactive degradation products was seen during evaluation. However, atenolol showed degradation products after the degradation treatments. Degradation was indicated in the stressed sample by a decrease of the expected value of the drug and increased levels of degradation products. The results of the stress study are presented in Table 1. Selectivity was demonstrated showing that atenolol was free of interference from degradation products and no interference from the sample excipients could be observed

Table 1. Summary of forced degradation results

Condition	Time (h)	% of atenolol	RRT[a] of degradation products
Acid (0.1 N HCl, reflux)	0.5	87.6	0.41, 2.15
Base (0.1 N NaOH, reflux)	0.5	98.7	Non detected
Hydrogen peroxide 15% (reflux)	0.5	97.0	Non detected
Water (reflux)	0.5	97.3	0.41
Heat dry, 105°C (solution)	24	56.6	2.38
Heat dry, 105°C (solid)	24	98.4	Non detected
Daylight exposure	24	101.6	Non detected

[a]RRT, relative retention time.

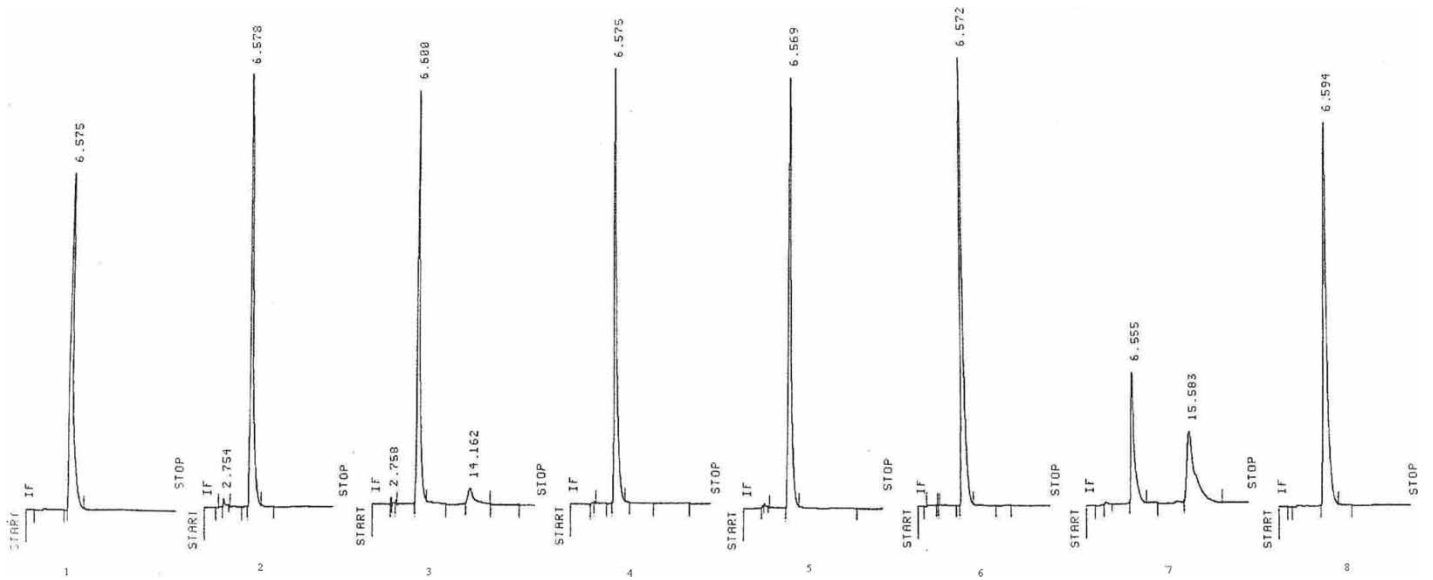

Figure 2. Chromatograms of atenolol: 1-Standard; 2-Hydrolysis (Water, reflux, 0.5 h); 3-Acid hydrolysis (0.1 N HCl, reflux, 0.5 h); 4-Alkaline hydrolysis (0.1 N NaOH, reflux, 0.5 h); 5-Oxidation (Hydrogen peroxide 15%, reflux, 0.5 h); 6-Daylight exposure (24 h); 7-Heat dry, 105°C (solution, 24 h); 8-Heat dry, 105°C (solid, 24 h).

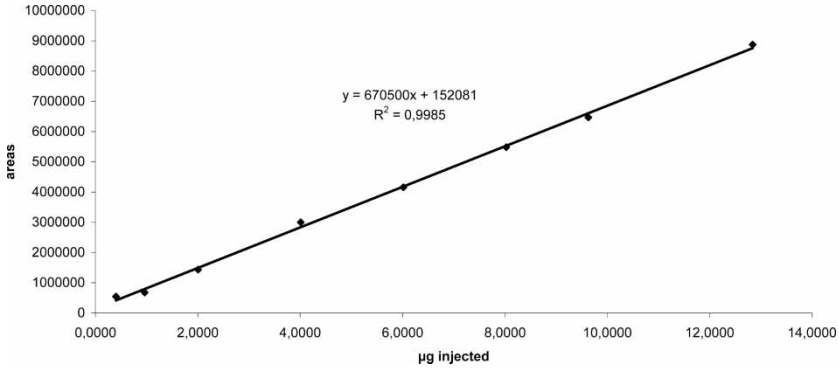

Figure 3. Linearity.

at this detection wavelength, indicating that the proposed method can be used in a stability assay (Figure 2).

Linearity

Linearity of the detector responses was determined by preparing calibration graphs. The linearity of the peak responses versus concentration was studied from 20 to 640 μg/mL (Figure 3). The representative linear equation was $670499.5x + 152081.0$, with a standard error ($S_{x,y}$) of 177908.6 and a correlation coefficient (r) of 0.9992, while intercept was not significantly different from zero ($p = 0.05$) (Table 2).

Table 2. Linearity data of atenolol

% of nominal value	Injected (μg)	Average peak area response	RSD
5	0.4012	546153.0	0.66
12	0.9629	682120.0	0.51
25	2.0060	1434530.0	0.73
50	4.0120	3000030.0	0.75
80	6.0180	4158099.7	0.67
100	8.0240	5480837.3	0.08
120	9.6288	6466884.3	0.66
160	12.8384	8877090.3	0.14
Slope[a]	670499.5 ±	36986.3	2.25
Intercept[b]	152081.0 ±	254691.4	

[a]Confidence limits of the slope (p = 0.05).
[b]Confidence limits of the intercept (p = 0.05).

Precision

The inter-day precision of the assay was performed by analyzing 6 samples and showed a CV% of 1.22%. The intra-day precision was performed by assaying the samples on two different days by two different analysts. The results were given both individually and as the average. For each precision assay, the results were as follows: mean values 51.53 and 52.01 mg per tablet, CV% 1.22 and 1.13. Test "*t*" comparing two samples with 95% confidence for 10 degrees of freedom, disclosed that both results were not significantly different *inter se* ($t_{n-2,\ \alpha:0.05}$) = 2.23 (Table 3).

Accuracy

The results obtained in the accuracy study (recovery test) with 9 samples of one commercial formulation studied (n = 3 for 80%, 100%, and 120%) indicated that the mean recovery was 99.3%. The CV% was 0.91%. Also studied was the experimental *t* of the recovery percentage of which the value was 2.194, it being below the 2.306 established in the tabulated *t* (95% level of probability, 8 d.f) (Table 4).

Robustness

To verify the separation of atenolol and its major impurity under isocratic conditions, the effect of both buffer pH and organic modifier were investigated. Table 5 summarizes the results.

The increase of the buffer pH did not influence, significantly, the retention time of atenolol, but decreased the retention time of its major impurity with a decrease of resolution. The increase of the buffer proportion significantly affected the retention time. Based on these results, a mobile phase composition of acetonitrile-sodium phosphate monobasic (0.08 M) (10:90, v/v) with a buffer pH range of 3.0 ± 0.1 was selected.

Table 3. Precision of the assay method for atenolol

Analyst 1 sample N°	mg per tablet	RSD (%)	Analyst 2 sample N°	mg per tablet	RSD (%)
1	51.77	0.25	1	51.28	0.04
2	51.05	0.36	2	51.53	0.12
3	51.44	0.04	3	52.75	0.05
4	50.78	0.06	4	52.31	0.21
5	52.56	0.05	5	51.61	0.14
6	51.59	0.01	6	52.59	0.12
Mean	51.53	1.22	Mean	52.01	1.13

Table 4. Recovery analysis of atenolol

% of nominal value	Added amount (mg)	Found amount (mg)	Recovery (%)	Average recovery (n = 3)	RSD (%)
80	41.75	41.29	98.91		
	42.48	42.45	99.92	99.69	0.11
	43.60	43.69	100.20		
100	52.62	52.13	99.05		
	52.62	52.38	99.53	99.42	0.11
	51.97	51.80	99.69		
120	62.26	60.55	97.26		
	63.11	62.74	99.41	98.89	0.11
	63.26	63.26	100.00		
Mean (n = 9)				99.33	0.91

Table 5. Robustness of atenolol method

Mobile phase	RT atenolol (min)	RT degradation product (min)	Resolution
Buffer : Acetonitrile (85:15) pH: 3.0	4.0	5.9	1.1
Buffer : Acetonitrile (95:5) pH: 3.0	14.0	37.9	17.0
Buffer : Acetonitrile (90:10) pH: 3.5	6.3	12.6	2.2
Buffer : Acetonitrile (90:10) pH: 4.0	6.4	9.2	1.1

CONCLUSIONS

A simple and rapid stability-indicating LC method has been developed for the determination of atenolol in the presence of its major degradation product, using a UV detector. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The method uses a simple mobile phase composition, easy to prepare with little variation. The method can be used for routine analysis.

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