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Stability-indicating HPLC Method for the Determination of Atenolol in Pharmaceutical Preparations

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Abstract

A stability-indicating reversed-phase liquid chromatographic method for the determination of atenolol was developed. Different chromatographic conditions were carefully studied to optimize the parameters for the evaluation of the studied drug. The chromatographic assay involved the use of a C8 Column (250 mm×4.6 mm i.d., 5 μm) with a simple mobile phase composed of acetonitrile:methanol:0.02 M phosphate buffer, pH 5 (20:20:60) at a flow rate of 1 ml/min and UV detection at 226 nm. Pindolol was used as an internal standard. The method showed good linearity over the range of 0.05-10 μg/mL with a detection limit of 0.01 μg/mL and a quantitation limit of 0.03 μg/mL. The proposed method was successfully applied for the analysis of atenolol in three commercial tablets with average percent recoveries of 100.14 ± 1.04, 100.20 ± 0.92, 100.00 ± 0.91 and 100.75 ± 0.67, respectively. Several co-formulated and co-administered drugs did not interfere with the proposed method. The results were statistically compared with those obtained by the official method and were found to be in good agreement. The stability-indicating capability of the method was also tested after accelerated degradation of atenolol in acidic and basic media and after freezing and heating treatments.

Keywords: Atenolol; HPLC; Pharmaceutical preparations; Stability study

Introduction

Atenolol, (2-[4-[(2RS)-2-hydroxy-3-[(1-methylethyl)amino]propoxy] phenyl]acetamide [1] (Figure 1), is a cardioselective beta blocker and reported to lack intrinsic sympathomimetic activity and membrane-stabilizing properties. It is used in the management of hypertension, angina pectoris, cardiac arrhythmias, and myocardial infarction. It may also be used in the prophylactic treatment of migraine. The usual oral dose for hypertension or angina pectoris is 50-100 mg daily. Atenolol is co-formulated with chlorthalidone, hydrochlorothiazide, nifedipine, amiloride HCl, hydralazine HCl, indapamide, and bendrofluazide [2].

Atenolol is an official drug in the British Pharmacopoeia (BP) [1]. Different analytical methods were developed for the determination of atenolol. A good guide for the work published on atenolol up to 1984 is presented as a comprehensive monograph in analytical profiles of drug substances [3]. The most recent articles concerning the analysis of this drug include spectroscopic methods [1,4-10], chromatographic methods including GC [11-13] and HPLC [14-18].

Atenolol is extensively prescribed in the market and so there is a great need to develop a rapid simple analytical method for the determination of atenolol in pharmaceutical preparation to be used in routine quality control laboratories. In the present work an accurate, sensitive and selective reversed-phase liquid chromatographic method for the determination of atenolol in presence of some co-formulated or co-administered drugs was developed. Different chromatographic conditions were carefully studied in an attempt to optimize the parameters for the evaluation of the studied compound in pure form and in tablets. The stability-indicating capability of the method was also investigated.

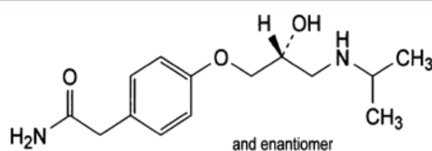

Figure 1: Chemical structure of atenolol.

Experimental

Materials and reagents

All the chemicals used were of Analytical Reagents grade, and the solvents were of HPLC grade.

- Atenolol was kindly provided by (Egyptian International Pharmaceutical Industries Company, (EIPICO), 10th of Ramadan City, Egypt). The purity of the sample was found to be 99.78 ± 0.55 according to the BP [1] method.
- Pindolol (Sigma-Aldrich Co Ltd., UK).
- Ateno® tablets (Egyptian International Pharmaceutical Industries Company (EIPICO), 10th of Ramadan City, Egypt), Blokium® 100 tablets (Medical Union Pharmaceuticals Co., Abu-Sultan, Ismailia, Egypt; under license of Almirall Prodesfarma, Spain), and Tenormin® tablets (Kahira Pharmaceutical & Chemical Industries Co., Cairo, Egypt; under license of AstraZeneca, UK) were purchased from commercial sources.
- Acetonitrile (Sigma-Aldrich Chemie GmbH, Germany).

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- Methanol (Prolabo, France).

- Potassium dihydrogen phosphate (Winlab, England). 0.02 M Phosphate buffer of pH 5, prepared according to the BP [1] method.

Instrumentation and chromatographic system

Chromatographic analyses were carried out using a Merck Hitachi Chromatograph model L-7100 equipped with a Rheodyne injector valve with a 20 µl loop, and a Merck Hitachi L-7400 UV detector.

The chromatograms were recorded on a Merck Hitachi D-7500 integrator. Mobile phase was degassed using Merck solvent L-7612 degasser. A Consort P-901 pH-meter was used for pH measurements. A Hibar® pre-packed column RT 250-4 LiChrospher® 100-RP8 (250 mm×4.0 mm i.d., 5 µm particle size) was used in this study. The mobile phase was acetonitrile:methanol:0.02 M phosphate buffer, pH 5 [20:20:60].

Standard solutions

Stock solutions (0.20 mg/mL) of atenolol and pindolol [internal standard (I.S.)] were prepared by transferring 0.02 g of each, accurately weighed, to 100-mL volumetric flasks, adding about 50 mL of methanol, and dissolving by swirling and with the aid of sonication. The solution was then diluted with methanol to volume, and mixed. This solution was stable for at least 7 days when kept in the refrigerator.

Procedures

Study of experimental parameters

Different experimental conditions including type of column, mobile phase composition, detection wavelength, flow rate, and nature of internal standard were extensively studied in order to determine the optimal conditions for the assay procedure. Variables were optimized by changing each in turn, while, keeping all others constant. Chromatographic parameters are calculated according to the USP [14] and BP [1] guidelines.

Construction of the calibration graph

Working standard solutions (20.0 µg/mL) and (1.0 µg/mL) of atenolol and (20.0 µg/mL) of pindolol were prepared from the previous stock solutions by dilution with the mobile phase. Aliquots of the suitable atenolol stock or working standard solutions were transferred into a series of 10-mL volumetric flasks so that the final concentration was in the range of 0.05-10 µg/mL. To each flask, 1.0 mL of pindolol working standard solution was added so that its final concentration was 2.0 µg/mL. Then the flasks were completed to volume with the mobile phase. 20 µL aliquots were injected (triplicate) and eluted with the mobile phase under the optimum chromatographic conditions. A plot of the average peak area ratio (drug/I.S.) versus the final concentration in µg/mL was then constructed to obtain the standard calibration graph. Alternatively, the linear regression equation was derived.

Stability studies

The stability-indicating capability of the method was demonstrated by accelerated degradation of atenolol. Samples of atenolol were exposed to acidic and basic conditions, frozen, and heated adopting the reported [19,20] degradation conditions as follows:

A solution of atenolol was adjusted to pH 0.7 with hydrochloric acid and stored for 2.5 hours at room temperature. A second solution of atenolol was alkalinized to pH 12.0 with potassium hydroxide solution and stored for 2.5 hours at room temperature. A third solution was frozen at -10°C for 14 hours. A fourth solution of atenolol was heated in an oven at 100°C for 3 hours.

Application of the proposed method to the analysis of atenolol in its tablets

Twenty tablets were weighed and then powdered. An accurately weighed amount of the powder equivalent to 20.0 mg of atenolol was transferred into a 100-mL volumetric flask and diluted to the mark with methanol. The flask was sonicated for 30 min, filtered and then analyzed as described under Construction of the calibration graph. The concentration of the drug was determined using, either the calibration curve or the corresponding regression equation. The results obtained were compared to those given with the official method [1].

Results and Discussion

The conditions affecting the chromatographic performance of atenolol were carefully studied in order to recognize the most suitable chromatographic system. The choice was based on the highest number of theoretical plates and the best resolution.

Optimization of the chromatographic performance and system suitability

A well-defined symmetrical peak was obtained after thorough experimental trials. These trials can be summarized as follows:

Choice of column: Different columns were used for performance investigations, including Hibar® pre-packed column RT 250-4 LiChrospher® 100-RP8 (250 mm×4.0 mm i.d., 5 µm particle size), LiChroCART® 250-4 LiChrospher® 100-RP18 (250 mm×4.0 mm i.d., 5 µm particle size), and Beckman Ultrasphere ODS (150 mm×4.6 mm i.d., 5 µm particle size). The experimental studies revealed that, the first column was the most suitable one since a good resolution of peaks was obtained, as atenolol was eluted after 3.0 min while pindolol (I.S.) was eluted after 5.3 min. The second two columns were not suitable due to the overlapping of the drug peak with the solvent front.

Choice of detection wavelength: The UV absorption spectrum of atenolol in methanol showed three maxima at 226, 275, and 282 nm. Therefore, the three wavelengths in addition to 254 nm were tried to detect the peak of atenolol. The most suitable wavelength was 226 nm showing the highest sensitivity with a reasonable response.

Mobile phase composition: Several modifications in the mobile phase composition were performed in order to study the possibilities of changing the selectivity of the chromatographic system. These modifications included changing the proportions of acetonitrile, methanol, and phosphate buffer, and changing the pH and concentration of phosphate buffer, the flow rate, and the nature of internal standard. The optimum chromatographic performances were achieved when using a mobile phase composed of acetonitrile:methanol:0.02 M phosphate buffer adjusted to pH 5 [20:20:60]. A summary of the study

Ratio (Acetonitrile:methanol:buffer)	D_m	R_r	R	T
10:10:80	1.41	2.41	10.73	1.40
15:15:70	0.86	1.86	8.38	1.23
20:20:60	0.66	1.66	8.73	1.07
25:25:50	0.57	1.57	5.65	1.30
30:30:40	0.57	1.57	4.36	1.33

D_m is the mass distribution ratio [also known as the capacity factor (K') or the retention factor (K)].
R_r is the relative retention times [also known as the unadjusted relative retention (*rG*)].
R is the resolution.
T is the tailing factor [also known as the symmetry factor (*As*)].

Table 1: Effect of the ratio of mobile phase components on the chromatographic performance of the HPLC method.

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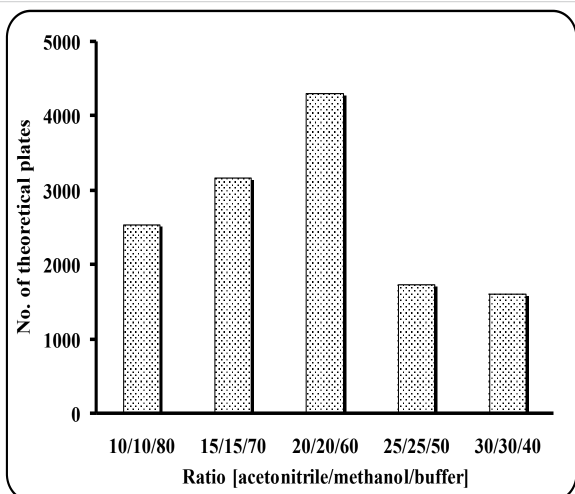

Figure 2: Effect of the ratio of mobile phase components on the number of theoretical plates for atenolol.

pH	D_m	R_r	R	T
3	0.35	1.35	5.69	1.17
4	0.28	1.28	4.62	1.36
5	0.46	1.46	6.71	1.07
6	0.51	1.51	7.07	1.27

D_m is the mass distribution ratio [also known as the capacity factor (K') or the retention factor (K)].
R_r is the relative retention times [also known as the unadjusted relative retention (*rG*)].
R is the resolution.
T is the tailing factor [also known as the symmetry factor (*As*)].

Table 2: Effect of pH of phosphate buffer on the chromatographic performance of the HPLC method.

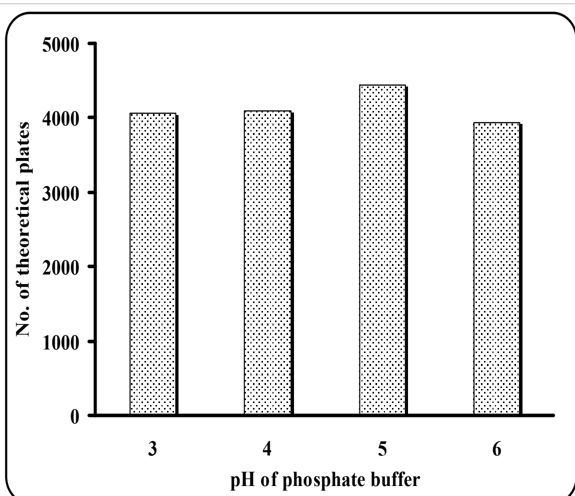

Figure 3: Effect of pH of phosphate buffer on the number of theoretical plates for atenolol.

done for the optimization of the mobile phase composition can be presented as follows:

Ratio of mobile phase components: As shown in table 1 and figure 2, different ratios were tried and the optimum ratio was 20:20:60 of acetonitrile, methanol, and phosphate buffer, respectively.

pH of phosphate buffer: The pH of phosphate buffer was changed over the range of 3-6 using sodium hydroxide or phosphoric acid. Above pH 6, the peak shape was not suitable for measurements. The study revealed that the effect of pH of phosphate buffer was not critical over the studied pH range. The optimum pH was found to be 5 considering the different chromatographic parameters. The results are presented in table 2 and figure 3.

The concentration of phosphate buffer: The effect of changing the concentration of phosphate buffer on the chromatographic performance of atenolol was investigated. Different concentrations in the range of 0.005 to 0.06 M were studied. The study revealed that the optimum chromatographic performance was achieved when using 0.02 M phosphate buffer. Although 0.005 and 0.01 M gave the highest numbers of theoretical plates and the highest resolution values, yet they were not selected due to the very bad reproducibility of retention times observed when using these concentrations as well as the relatively high values of the tailing factors (Table 3 and figure 4).

Choice of flow rate: The effect of flow rate was studied to optimize the chromatographic efficiency of the proposed method and improve the resolution of the eluted peaks. The flow rate was changed over the range of 0.5-1.5 mL/min and a flow rate of 1 mL/min was optimal for good separation in a reasonable time. The results are shown in table 4 and figure 5.

Choice of internal standard: The use of different internal standards

Buffer concentration	D_m	R_r	R	T
0.005 M	0.56	1.68	7.52	1.32
0.01 M	0.44	1.47	6.60	1.33
0.02 M	0.20	1.03	3.64	1.11
0.03 M	0.18	1.01	3.18	1.18
0.04 M	0.14	0.94	2.54	1.15
0.05 M	0.14	0.93	2.72	1.19
0.06 M	0.13	0.91	2.54	1.19

D_m is the mass distribution ratio [also known as the capacity factor (K') or the retention factor (K)].
R_r is the relative retention times [also known as the unadjusted relative retention (r_G)].
R is the resolution.
T is the tailing factor [also known as the symmetry factor (A_s)].

Table 3: Effect of concentration of phosphate buffer on the chromatographic performance of the HPLC method.

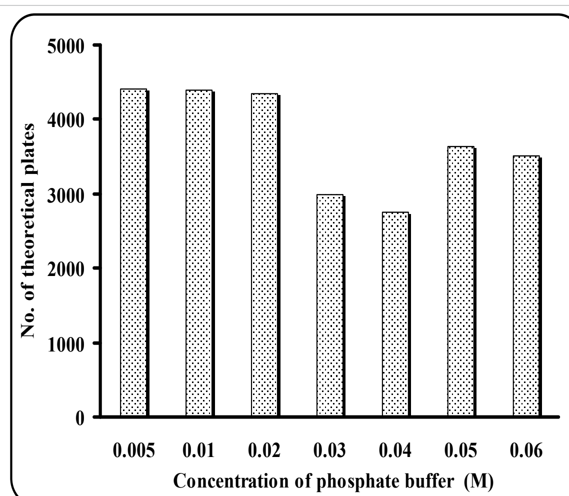

Figure 4: Effect of concentration of phosphate buffer on the number of theoretical plates for atenolol.

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Flow rate (mL/min)	D_m	R_r	R	T
0.5	0.20	1.03	3.41	1.18
0.8	0.21	1.03	3.81	1.15
1.0	0.21	1.03	3.65	1.11
1.2	0.20	1.02	3.27	1.09
1.5	0.19	0.97	2.79	1.05

D_m is the mass distribution ratio [also known as the capacity factor ($K^`$) or the retention factor (K)].
R_r is the relative retention times [also known as the unadjusted relative retention (r_G)].
R is the resolution.
T is the tailing factor [also known as the symmetry factor (A_s)].

Table 4: Effect of flow rate on the chromatographic performance of the HPLC method.

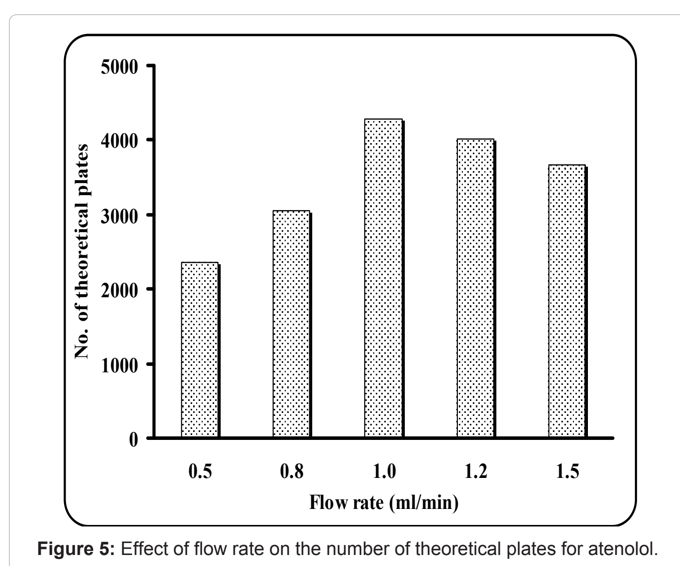

Figure 5: Effect of flow rate on the number of theoretical plates for atenolol.

Column	Stainless Steel Merck 250 C8 Column, 5 µm (250 mm×4.6 mm i.d.)
Mobile phase	Acetonitrile:Methanol:0.02 M Phosphate buffer (pH 5) [20:20:60]
Flow rate	1 mL/min.
Detector	UV-detection at 226 nm
Injection volume	20 µL
Temp.	Ambient
Internal Standard	Pindolol

Table 5: Typical chromatographic conditions for the HPLC determination of atenolol.

such as propanol hydrochloride, labetalol hydrochloride and pindolol was studied. Pindolol was the internal standard of choice and it was used in 2 µg/mL concentration.

The optimum chromatographic conditions for the HPLC determination of atenolol are summarized in table 5. Typical chromatographic parameters of the developed HPLC method and the chromatogram of atenolol under the described chromatographic conditions are shown in table 6 and figure 6, respectively.

Stability indication of the method

The stability-indicating capability of the proposed method was tested after accelerated degradation of atenolol using acidic conditions, basic conditions, freezing, and heating. In all cases, degradation products did not interfere with the intact atenolol peak as shown in figures 7 and 8. These results demonstrated the ability of the proposed method to be used as a stability-indicating HPLC method for the analysis of atenolol.

Validation:

Linearity and range: The calibration graph for the determination of atenolol by the proposed method was constructed by plotting the peak area ratio [drug/I.S.] against the concentration of atenolol. The graph was found to be rectilinear over the concentration range cited in table 7.

Statistical analysis [21] of the data gave high value of the correlation coefficient (r) of the regression equation, small values of the standard deviation of residuals ($S_{y/x}$), of intercept (S_a), and of slope (S_b), and small value of the percentage relative standard deviation and the percentage relative error (Table 7). These data proved the linearity of the calibration graph.

Accuracy and precision: To prove the accuracy of the proposed method, the results of the assay of atenolol, both in pure form and in

Parameter	Value
Mass distribution ratio for atenolol (Dm1)	0.65
Mass distribution ratio for pindolol [I.S.] (Dm2)	1.91
Relative retention time (Rr)	1.76
Number of theoretical plates for atenolol (N1)	2319
Number of theoretical plates for pindolol [I.S.] (N2)	3669
Resolution (R)	7.63
Tailing factors for atenolol (T1)	1.06
Tailing factors for pindolol [I.S.] (T2)	1.13

Table 6: Typical chromatographic parameters for the HPLC determination of atenolol.

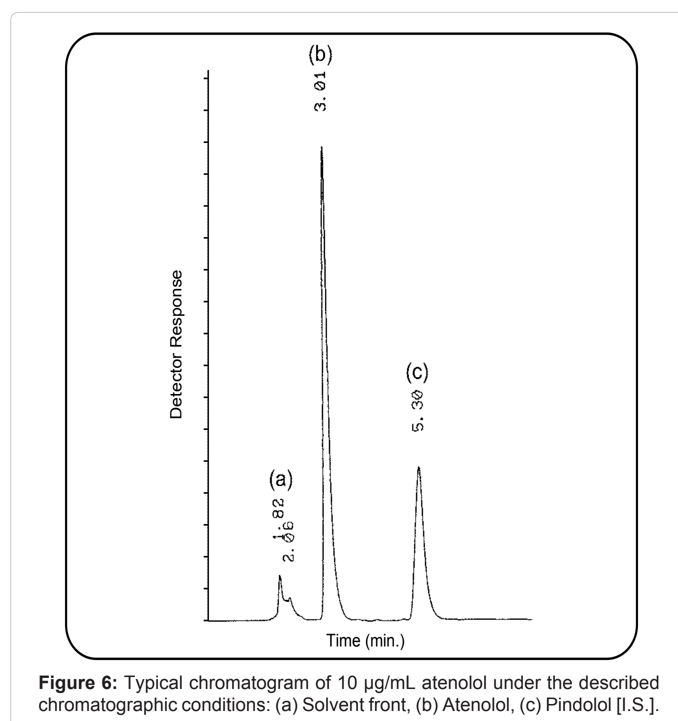

Figure 6: Typical chromatogram of 10 µg/mL atenolol under the described chromatographic conditions: (a) Solvent front, (b) Atenolol, (c) Pindolol [I.S.].

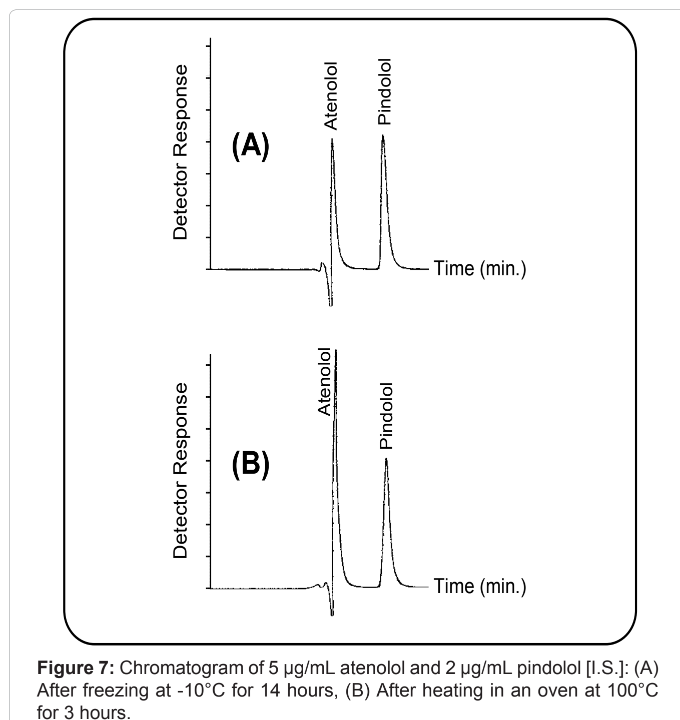

Figure 7: Chromatogram of 5 µg/mL atenolol and 2 µg/mL pindolol [I.S.]: (A) After freezing at -10°C for 14 hours, (B) After heating in an oven at 100°C for 3 hours.

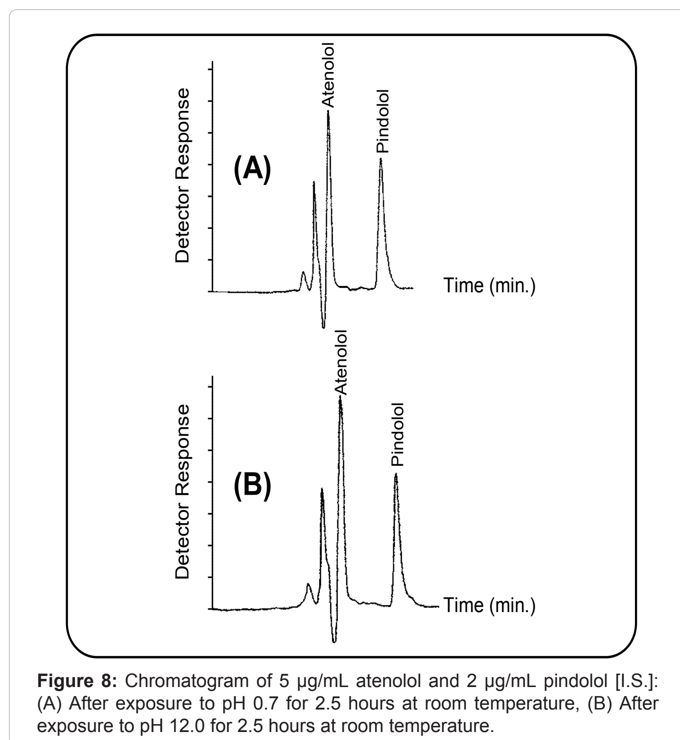

Figure 8: Chromatogram of 5 µg/mL atenolol and 2 µg/mL pindolol [I.S.]: (A) After exposure to pH 0.7 for 2.5 hours at room temperature, (B) After exposure to pH 12.0 for 2.5 hours at room temperature.

pharmaceutical preparations were compared with those of the official methods [1].

Statistical analysis [21] of the results obtained by the proposed and official methods using Student's t-test and variance ratio F-test showed no significant difference between them regarding accuracy and precision, respectively (Tables 8 and 9).

Intraday and interday precisions were assessed using three concentrations and three replicates of each concentration. The relative standard deviations were found to be very small indicating reasonable repeatability and intermediate precision of the proposed method (Table 10).

Specificity: The specificity of the method was investigated by observing any interference encountered from common tablet excipients. It was shown that these compounds did not interfere with the results of the proposed method (Table 10).

Interferences likely to be introduced from co-formulated or co-administered drugs, such as chlorthalidone, nifedipine, hydrochlorothiazide, enalapril maleate or from other related drugs, such as labetalol hydrochloride, alprenolol hydrochloride, celiprolol hydrochloride, and propranolol hydrochloride were studied under the same experimental conditions using a methanolic stock solution of each of the above mentioned drugs. None of the above mentioned drugs interfered with the HPLC assay of atenolol except enalapril maleate which showed a marked overlap with atenolol peak.

Limit of detection (LOD) and limit of quantitation (LOQ): LOD and LOQ were determined according to the USP [14] guidelines. LOD was determined by establishing the minimum level at which the analyte can reliably be detected (signal-to-noise ratio is 3:1) while LOQ was determined by establishing the lowest concentration of analyte that can be determined with acceptable precision and accuracy (signal-to-noise ratio is 10:1). The results are shown in table 7.

Ruggedness: To examine the ruggedness of the procedure, the

Parameter	Value
Linearity range (µg/mL)	0.05-10.00
Intercept (*a*)	0.004
Slope (*b*)	0.195
Correlation coefficient (*r*)	0.9999
S.D. of residuals ($S_{y/x}$)	8.070×10^{-3}
S.D. of intercept (S_a)	4.706×10^{-3}
S.D. of slope (S_b)	8.563×10^{-4}
% RSD[a]	1.03
% Error[b]	0.42
LOD (µg/mL)[c]	0.01
LOQ (µg/mL)[d]	0.03

[a]Percentage relative standard deviation for six replicate samples.
[b]Percentage relative error for six replicate samples.
[c]Limit of detection.
[d]Limit of quantitation

Table 7: Analytical performance data for the HPLC determination of atenolol.

Parameter	Proposed method	Official Method [1]
% Found[a]	102.00	99.16
	99.40	100.32
	100.00	99.48
	99.48	100.15
	100.64	
	99.33	
Mean ± S.D.	100.14 ± 1.04	99.78 ± 0.55
t	0.64 (2.31)[b]	
F	3.568 (9.013)	

[a]The average of three separate determinations.
[b]The figures between parentheses are the tabulated values of t and F at P=0.05.

Table 8: Assay results for the determination of atenolol in pure form by the HPLC and official methods.

Dosage form	% Found[a] of atenolol	
	Proposed method	Official method [1]
Ateno® tablets[b]	100.86	98.78
	100.59	99.48
	99.15	99.47
Mean ± S.D.	100.20 ± 0.92	99.24 ± 0.40
t	1.65	
F	5.25	
Blokium 100® tablets[c]	99.69	100.18
	101.02	100.02
	99.28	99.59
Mean ± S.D.	100.00 ± 0.91	99.93 ± 0.31
t	0.12	
F	8.89	
Tenormin® tablets[d]	101.43	100.52
	100.09	100.60
	100.73	100.95
Mean ± S.D.	100.75 ± 0.67	100.69 ± 0.23
t	0.15	
F	8.59	

[a]The average of three separate determinations.

[b]Labeled to contain 50 mg atenolol per tablet; manufactured by Egyptian International Pharmaceutical Industries Co. (EIPICO), 10th of Ramadan City, Egypt; batch number 053901.

[c]Labeled to contain 100 mg atenolol per tablet; manufactured by Medical Union Pharmaceuticals Co., Abu-Sultan, Ismailia, Egypt; under license of Almirall Prodesfarma, Spain; batch number 042281.

[d]Labeled to contain 50 mg atenolol per tablet; manufactured by Kahira Pharmaceutical & Chemical Industries Co., Cairo, Egypt; under license of AstraZeneca, UK; batch number 0510116.

N.B. Tabulated *t*-value at *P*=0.05 is 2.78, tabulated *F*-value at *P*=0.05 is 19.00.

Table 9: Assay results for the determination of atenolol in commercial tablets by the HPLC and official methods.

Parameter		Atenolol Concentration (µg/mL)		
		0.50	4.00	8.00
Intraday	% Found	99.4	99.48	100.64
		99.02	100.52	100.23
		100.61	98.99	99.76
	Mean	99.68	99.66	100.21
	S.D.	0.83	0.78	0.44
	% RSD	0.83	0.78	0.44
	% Error	0.48	0.45	0.25
Interday	% Found	99.4	99.48	100.64
		101.11	99.62	101.05
		99.44	100.7	99.8
	Mean	99.98	99.93	100.50
	S.D.	0.98	0.67	0.64
	% RSD	0.98	0.67	0.63
	% Error	0.56	0.39	0.37

N.B. Each result is the average of three separate determinations

Table 10: Accuracy and precision data for the determination of atenolol by the HPLC method.

intraday and interday precisions were evaluated as shown in table 10. The precision of the proposed method was fairly high, as indicated by the low values of the percentage relative standard deviation (%RSD).

Applications

Dosage forms analysis: The proposed method was successfully applied to the assay of atenolol in its tablets. Three commercial tablet preparations produced by different manufacturers were used. The average percent recoveries of different concentrations were based on the average of three replicate determinations. The results obtained were in good agreement with those obtained with the official method [1] as shown in table 9.

Conclusion

The proposed method for the determination of atenolol based on the use of liquid chromatography with spectrophotometric detection was shown to be reliable, simple, accurate, sensitive and precise. Moreover, the method is fast and feasible. It could be successfully applied for the determination of atenolol in pharmaceutical preparations without interference from co-formulated drugs. The good validation criteria of the proposed method allow its use in quality control laboratories as an alternative to the official methods. The detection limit of the proposed method was found to be 0.01 µg/ml while the quantitation limit was 0.03 µg/mL. The results demonstrated the ability of the proposed method to be used as a stability-indicating HPLC method for the analysis of atenolol.

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