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VALIDATED SPECTROFLUOROMETRIC METHOD FOR DETERMINATION OF ATENOLOL IN PHARMACEUTICAL PREPARATIONS

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ABSTRACT

In this study, a new and rapid spectrofluorometry method was developed for determination of atenolol in pure and pharmaceutical preparation. The solvent system and wavelength of detection were optimized in order to maximize the sensitivity of the proposed method. Parameters such as linearity, precision, accuracy, specificity, stability, limit of detection and limit of quantification were studied according to the International Conference on Harmonization (ICH) Guidelines. The linearity was established between the concentration range of 50-4000 ng/mL. The intra- and inter-day relative standard deviation (RSD) was less than 2.97%. Limits of detection and quantification were determined as 15.2 and 46.1 ng/mL, respectively. The mean recovery value of atenolol was 99.3% for pharmaceutical preparation. The method was applied for the quality control of commercial atenolol dosage form to quantify the drug and to check the formulation content uniformity.

Key words: Atenolol; Spectrofluorometry; Validation, Tablet.

INTRODUCTION

β-blockers constitute one of the most frequently prescribed groups of cardiovascular drugs. They are competitive antagonists at β-adrenergic receptor sites and are used in the management of cardiovascular disorders, such as hypertension, angina pectoris, cardiac arrhythmias and myocardial infarction[1].

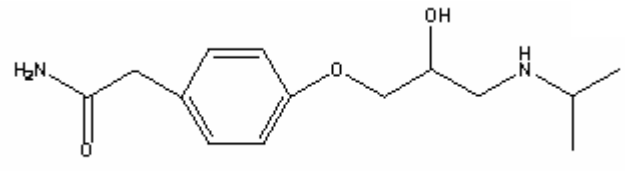
Atenolol (Fig-1), [(4-2–hydroxy-3–isopropyl-aminopropoxy) phenylacetamide], is a cardioselective β-blocker. It is reported to lack intrinsic sympathomimetic activity and membrane-stabilising properties. It may be used alone or concomitantly with other antihypertensive agents including thiazide-type diuretics, hydralazine, prazosin and α-methyldopa[2].

Several methods have been reported for the determination of atenolol including first-order derivative spectrophotometry[3-5], high performance liquid chromatography[6-9], voltametry[10], potentiometry[11] and spectrofluorometry[12] in pharmaceutical preparations.

In this study, we wanted to develop a new spectrofluorometric method for the determination of atenolol in pharmaceutical preparation. The method was aimed at developing an easy and rapid assay method for atenolol without any time consuming sample preparation steps for routine analysis, to be adopted in quality control and drug testing laboratories, and at the same time ensure satisfactory recovery during drug determination from pharmaceutical formulation.

In the proposed method, there is no need to extract the drug from the formulation excipient matrix thereby decreasing the error in quantitation. Formulation sample can be directly used after dissolving and filtration. The developed method was used to determine the total drug content in commercially available tablets of atenolol.

Fig-1 Chemical structure of atenolol



EXPERIMENTAL PROCEDURE

Chemicals:

Atenolol standard and Tensinor tablet (100 mg) were kindly donated from Abdi Ibrahim Pharmaceutical Industry (Istanbul, Turkey).

Instrument:

All fluorescence measurements were done on a SHIMADSU RF-5301 PC spectrofluorometer equipped with a 150 W Xenon lamp. Experimental parameters were slit width 5.0 nm, λ_{exc}=276 nm and λ_{em}=296 nm.

Preparation of the standard and quality control solutions:

The stock solution of atenolol was prepared in methanol to a concentration of 50 ng/mL and kept stored at +4 °C. Standard solutions were prepared as 50-4000 ng/mL (50, 125, 250, 500, 1000, 2000, 3000 and 4000 ng/mL). The quality control (QC) samples were separately prepared at the concentrations of 100, 750 and 3500 ng/mL.

Procedure for pharmaceutical preparation:

The average tablet mass was calculated from the mass of 10 tablets of Tensinor (100 mg atenolol tablet, which was composed of atenolol and some commen excipients). They were then finely ground, homogenized and portion of the powder was weighed accurately, transferred into a 50 mL brown measuring flask and diluted to scale with methanol. The mixture was sonicated for at least 20 min to aid dissolution and then filtered through a Whatman No 42 paper. Approximate dilutions were made at concentrations of 500 and 3000 ng/mL with methanol. Excitation and emission spectra were recorded.

Data analysis:

All statistical calculations were performed with the Statistical Product and Service Solutions (SPSS) for Windows, version 10.0. Correlations were considered statistically significant if calculated P values were 0.05 or less.

RESULTS AND DISCUSSION

METHOD DEVELOPMENT

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For spectrofluorometry method, various solvent systems (water, methanol and acetonitrile) were investigated. The final decision for using methanol as the solvent was based on sensitivity, ease of preparation, suitability for drug content determination and stability studies.

METHOD VALIDATION

The validation was carried out by establishing specifity, linearity, intra- and inter-day precision, accuracy, recovery, limits of detection (LOD) and quantitation (LOQ) according to ICH guidance[13].

Specificity:

All the solutions were scanned from 250 to 350 nm at a slit width of 5.0 nm and checked for change in the emission at respective wavelengths. Comparison of the excitation and emission spectrum of atenolol in standard and drug formulation (Tensinor tablet) solutions show that the wavelength of maximum and minimum emission did not changed (Figs-2 and 3). According to the results obtained, the spectrofluorometric method is able to access the atenolol in presence of excipients and hence the method can be considered specific.

Linearity:

Calibration curves were linear between the range 50-4000 ng/mL. Calibration plots were constructed for atenolol standard by plotting the concentration of atenolol versus spectrum emission intensity response. The calibration curve constructed was evaluated by its correlation coefficient. The correlation coefficient (r) of all the calibration curves were consistently greater than 0.99. The regression equations were calculated from the calibration graphs, along with the standard deviations of the slope and intercept on the ordinate. The results are shown in Table-1.

Precision and accuracy:

The precision of the spectrofluorometry method was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was evaluated by analyzing quality control samples six times per day, at three different concentrations which were quality control samples. The intermediate precision was evaluated by analyzing the same samples once daily for three days. The RSD of the predicted concentrations from the regression equation was taken as precision. The accuracy of this analytic method was assessed as the percentage relative error. For all the concentrations studied, intra- and inter-day RSD values were ≤2.97% and for all concentrations of atenolol the relative errors were ≤3.50%. These results were given in Table-2.

Limits of detection (LOD) and quantification (LOQ):

The LOD and LOQ of atenolol by the proposed method were determined using calibration standards[13]. LOD and LOQ values were calculated as 3.3 σ/S and 10 σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation (n=6) (Table-1).

Stability:

Stability studies indicated that the samples were stable when kept at room temperature, 4 and -20 ^{0}C refrigeration temperature for 6 h (short-term) and refrigerated at 4 and -20 ^{0}C for 48 h (long-term). The results of these stability studies are given in Table-3, where the percent ratios are within the acceptance range of 90-110%.

Recovery:

To determine the accuracy of the spectrofluorometry method and to study the interference of formulation additives, the recovery was checked as three different concentration levels. Analytical recovery experiments were performed by adding known amount of pure drugs to pre-analyzed samples of commercial dosage form. The recovery values were calculated by comparing concentration obtained from the spiked samples with actual added concentrations. These values are also listed in Table-4.

The proposed method was compared with first-order spectrophotometric method in literature[5]. In this study, the method is based on the formation of phenolsulfothaline complex by derivatization. The concentration of atenolol was determined at 558.4 nm by first-order spectrophotometric method. In this study, linearity range was determined as 0.05-0.4 mg/mL. In this present work, developed spectrofluorometric method has small linearity range (50-4000 ng/mL).

Also, the suggested spectrofluorometric method was compared with the official method[14]. There was no significant difference between the two methods with respect to mean values and standard deviations at the 95% confidence level (Table-5). As the LOQ of proposed the method is lower than the earlier reported works[5,14].

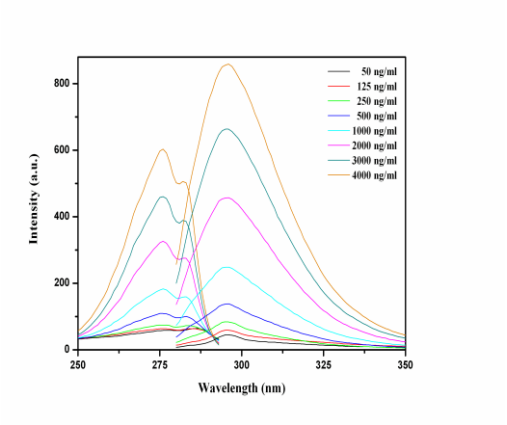

Fig-2 The excitation and emission spectrum of atenolol (50, 125, 250, 500, 1000, 2000, 3000 and 4000 ng/mL)

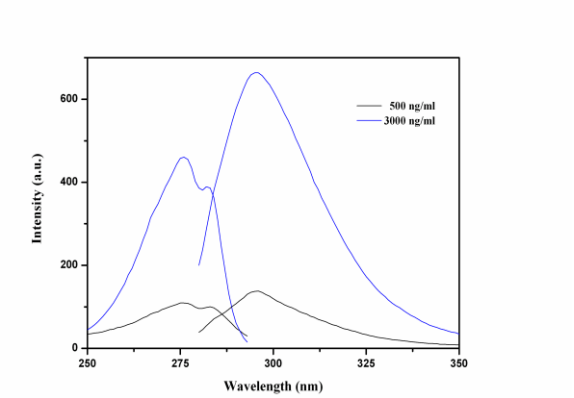

Fig-3Spectrumofsolutions of Tensino tablet containing atenolol (500 and 3000 ng/mL)

TABLE -1

RESULTS OF REGRESSION ANALYSIS OF ATENOLOL

Method	Range (ng/mL)	LR[a]	Sa	Sb	R^2	LOD	LOQ
Spectrofluorometry	50-4000	y=0.2076x+35.894	0.956	0.01	0.9997	15.2	46.1

[a]Based on six calibration curves, LR: Linear regression Sa: Standard deviation of intercept of regression line, Sb: Standard deviation of slope of regression line, R^2: Coefficient of correlation, y: emission intensity, x: atenolol concentration (ng/mL), LOD: Limit of detection, LOQ: Limit of quantification

TABLE-2

PRECISION AND ACCURACY OF ATENOLOL

Method	Added (µg/mL)	Within-day			Between-day		
		Found±SD (ng/mL)	Accuracy	Precision RSD%[a]	Found±SD (ng/mL)	Accuracy	Precision RSD%[a]
Spectrofluorometry	100	97.9 ±2.786	-2.10	2.85	96.5 ± 2.862	-3.50	2.97
	750	741.3 ± 6.532	-1.16	0.88	736.3 ± 11.09	-1.83	1.51
	3500	3481.1 ± 92.16	-0.54	2.65	3509.6 ±84.52	0.27	2.41

SD : Standard deviation of six replicate determinations, RSD: Relative standard derivation, [a]Average of six replicate determinations, Accuracy: (%relative error) (found-added)/addedx100

TABLE 3

STABILITY OF ATENOLOL IN SOLUTION

Stability (%)		Room temperature stability (Recovery % ± SD)		Refrigeratory stability, +4°C (Recovery % ± SD)		Frozen stability, - 20°C (Recovery % ± SD)	
λ (nm)	Added (ng/mL)	6 h	24 h	6 h	48 h	6h	48h
A_{296} nm	100	99.2±0.639	102.3±0.073	98.2±0.709	102.1±0.046	102.2±1.625	101.5±0.227
	1000	102.2±1.961	101.6±0.287	101.0±0.040	100.5±0.087	98.3±0.598	98.7±0.162
	4000	102.3±0.632	98.1±0.017	101.1±3.179	99.1±0.688	101.4±0.187	101.4±0.047

SD: Standard deviation of six replicate determinations, R.S.D: Relative standard derivation, [a]Average of six replicate determinations

TABLE 4

RECOVERY VALUES OF ATENOLOL IN PHARMACEUTICAL PREPARATION

Commercial Preparation	Method	λ(nm)	n	Mean ± SD (mg)	P value
Tensinor (100 mg/tablet)	Official method	-	-	97.87±0.23	0.276
	Spectrofluorometry	$A_{296\ nm}$	6	99.3±4.624	

SD: Standard deviation of six replicate determinations

TABLE 5

STATISTICAL COMPARISON (F-TEST) OF THE RESULTS OBTAINED BY PROPOSED METHODS

Commercial Preparation	Method	λ (nm)	Added (ng/mL)	Found (ng/mL)	Recorvery (%)	RSD[a] (%)
Tensinor tablet (100 ng/mL)	Spectrofluorometry	A_{296} nm	100	98.4 ± 2.832	98.4	2.88
			900	914.6 ± 32.64	101.6	3.57
			3500	3428.0 ±142.3	97.9	4.15

n: number of determination, SD: Standard deviation of six replicate determinations, (P > 0.05)

CONCLUSION

The proposed method was found to be accurate, precise and easy for the determination of atenolol. The sample recovery in a formulation was in good agreement with their respective label claims. No extraction procedure is involved.

The method can be used effectively, without separation and interference, for routine analysis of atenolol in pure form and its formulation and can also be used for dissolution or similar studies. On the other hand, the method is also suitable for analysis of sample during accelerated stability studies, routine analysis of formulations and raw materials.

REFERENCES

1. Al-Ghannam SM, A simple spectrophotometric method for the determination of β-blockers in dosage forms, J. Pharm. Biomed. Anal. 40, 2006, 151-156.
2. Reynolds JEF, In: Martindale, The Extra Pharmacopeia (31st ed.), Royal Pharmaceutical Society, London, 1996, 827.
3. Singh S, Jain R, Simultaneous spectrophotometric determination of atenolol and amlodipine besylate from dosage forms, Indian Drugs, 34, 1997, 678-679.
4. Bonazzi D, Gotti R, Andrisano V, Cavrini V, Derivative UV spectrophotometric determination of atenolol and metoprolol in singleand multi-component pharmaceutical dosage forms, Farmaco, 51, 1996, 733-738.
5. Wehner W, Determination of atenolol and chlorthalidone during dissolution of tablets with UV multicomponent analysis, Pharmazie, 55, 2000, 543-544.
6. Ceresole R, Moyano MA, Pizzorno MT, Segall AI, Validated reversed-phase HPLC method for the determination of atenolol in the presence of its major degradation product. J. Liq. Chrom. Relat. Tech. 2006, 3009-3019.
7. Argekar AP, Powar SG, Simultaneous determination of atenolol and amlodipine in tablets by high-performance thin-layer chromatography, J. Pharm. Biomed. Anal**.** 21, 2000, 1137-1142.
8. Keech AC, Harrison PM, Mclean AJ, Simple extraction of atenolol from urine and its determination by high-performance liquid chromatography, J. Chromatogr. 426, 1988, 234-236.
9. Leloux MS, Dost F, Doping analysis of beta-blocking drugs using high-performance liquid chromatography, Chromatographia, 32, 1991, 429- 435.
10. Goyal RN, Gupta VK, Oyama M, Bachheti N, Diferential pulse voltammetric determination of atenolol in pharmaceutical formulations and urine using nanogold modified indium tin oxide electrode, Electrochem. Commun. 8, 2006, 65-70.
11. Arvand M, Vejdani M, Moghimi M, Construction and performance characterization of an ion selective electrode for potentiometric determination of atenolol in pharmaceutical preparations, Desalination, 225, 2008, 176-184.
12. The European Agency for the Evaluation of Medicinal Products. ICH Topic Q2B Note for Guideline on Validation of Analytical Procedures: Methodology GPMP/ICH/281/95, 1996.
13. The British Pharmacopoeia, Her Majesty Stationary Office, London, 1993.

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