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A Facile and Rapid HPLC Method for the Determination of Atenolol in Pharmaceutical Formulations

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ABSTRACT

A new high performance liquid chromatography method is developed and validated to quantify atenolol in pharmaceutical preparations. The high performance liquid chromatography determination is performed on a reversed phase column, Atlantis dC_{18} (250×4.6 mm, 5 μm; internal diameter) using a mobile phase (1.0 mL min^{-1}) with ultra violet detection at 225 nm. A rectilinear relationship between mean peak area and concentration of atenolol is observed in the range 1-100 μg mL^{-1} with a detection limit of 0.4 μg mL^{-1} and a quantitation limit of 1.0 μg mL^{-1}. The validation parameters such as specificity, precision, linearity and range, ruggedness and robustness have been established according to the current ICH guidelines. The results were statistically compared with those of the reference/literature method by applying Student's t-test and F-test. Accuracy, evaluated by means of the spike recovery method was in the range 98.3-102.5%. The method was characterized by a shorter retention time (3.39 min) and a wide linear dynamic range (1-100 μg mL^{-1}) of concentration over which it is applicable. The method was demonstrated to be both accurate and precise which qualify it to be adopted for routine use in pharmaceutical quality control laboratories.

Key words: Atenolol, HPLC, pharmaceuticals, determination, UV-visible spectroscopy, β-blockers

INTRODUCTION

Atenolol is a cardio selective β-adrenergic receptor-blocking agent without membrane-stabilizing or intrinsic sympathomimetic activities (Medical Economics, 1999). It is also used to treat myocardial infarction, arrhythmias, angina, disorders arising from decreased circulation and vascular constriction, including migraine (Wadworth *et al*., 1991). It is prescribed for high blood pressure and regulating tachycardia. It works by relaxing blood vessels and slowing heart rate to improve blood flow and decrease blood pressure (Fig. 1).

Symptoms of overdose may include unusually slow heartbeat, severe dizziness, shallow breathing, weakness or fainting. Atenolol is excreted in breast milk and may cause adverse effects in the breastfed infant (El-Gindy *et al*., 2008).

Many methods for the determination of atenolol have been reported such as Ultraviolet spectrophotometry (Ferraro *et al*., 2003; Wehner, 2000), high-performance liquid chromatography

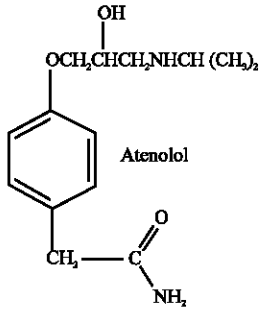

Fig. 1: Structure of Atenolol - (RS)-4-(2-hydroxy-3-isopropylaminopropoxy) phenylacetamide

(Sasa *et al.*, 1988; Ruiz-Angel *et al.*, 2002), Capillary electrophoresis (Coragem Briguenti and Bonato, 2005; Shafaati and Clark, 1996), Ultra performance liquid chromatography (Wren and Tchelitcheff, 2006).

Other known methods include spectrofluorimetry (Gajewska *et al.*, 1992), kinetic methods using ammonium vanadate as oxidant (Sultan, 1992) differential scanning calorimetry and thermogravimetry (Phyramides *et al.*, 1995), electrophoresis (Ferraro *et al.*, 2004), NMR spectroscopy (Agarwal *et al.*, 1992; Zakhari *et al.*, 1991) and chemo- metric assisted spectrophotometric methods (El-Gindi *et al.*, 2005).

Most of these reported methods are complicated and do require expensive instruments. Normal HPLC with UV or fluorescence detection need extraction steps and complicated clean up processes (Modamio *et al.*, 1998; Zarapkar *et al.*, 1997; Jain and Jain, 1991; Pawlak and Clark, 1992; Radulovic *et al.*, 1991). The kinetic method is less sensitive and involves a heating step where as thermal methods require expensive experimental setup and also are less sensitive (Basavaiah *et al.*, 2006).

This study deals with development and validation of a sensitive method for the assay of ATN in pharmaceuticals, based on HPLC technique. The separation and determination were caused on a reversed phase Atlantis dC_{18} column and UV-detection at 225 nm. The method was demonstrated to be both accurate and precise which qualify it to be adopted for routine use in pharmaceutical quality control laboratories.

MATERIALS AND METHODS

This study was conducted in 2010.

Apparatus: The chromatographic system consisted of an Agilent 1100 series chromatograph equipped with an in built solvent degasser, quaternary pump, photo diode array detector with variable injector and auto sampler and a reversed phase 5 μm Atlantis dC18 column (250×4.6 mm, internal diameter).

Reagents and standards: All chemicals used were of analytical reagent grade, Ammonium acetate (S.D. Fine Chem. Ltd, India) and HPLC grade methanol (Merck. Ltd, Mumbai) was used. Distilled water filtered through 0.45 μm filter (Millipore) was used to prepare solutions. Methanol and water in the ratio of 50:50 was used as a diluent for the sample preparations.

- Mobile phase A: 20 mM ammonium acetate
- Mobile phase B: Methanol

Pharmaceutical grade ATN, certified to be 99.85% pure was procured from Cipla India Ltd, Mumbai, India and was used as received. For the study, an accurately weighed 50 mg of ATN was dissolved in and diluted to volume with the diluent solution in a 100 mL calibrated flask to obtain a concentration of 500 μg mL^{-1} ATN.

Procedures

Chromatographic conditions: The separation was achieved at 30°C on the column using the mobile phase of 60:40 (20 mM ammonium acetate: methanol) at a flow rate of 1.0 mL min^{-1}. The detector wavelength was set at 225 nm with a sensitivity of 0.2 a.u.f.s.

Calibration graph: Working standard solutions equivalent to 1 to 100 μg mL^{-1} of ATN were prepared by appropriate dilution of stock standard solution (500 μg mL^{-1}) with the diluent solution. Ten μl aliquot of each solution was injected automatically on to the column in duplicate and the chromatograms were recorded. Calibration graph was prepared by plotting the mean peak area versus concentration of ATN.

The concentration of the unknown was read from the calibration graph or computed from the regression equation derived from the mean peak area-concentration data.

Assay in dosage forms: A quantity of tablet powder equivalent to 50 mg of ATN was accurately weighed into a 100 mL calibrated flask and 60 mL of diluent solution was added. The content was shaken for 20 min and then the volume was diluted to the mark and mixed well. A small portion of the extract (say 10 mL) was withdrawn and filtered through 0.2 μm filter to ensure the absence of particulate matter. The filtered solution was appropriately diluted with the diluent solution for analysis as described already.

RESULTS AND DISCUSSION

Method development: A solution of ATN was injected in duplicate on to the column and was monitored by UV-detection at 225 nm. An isocratic method was selected rather than gradient to get the lesser retention time. At a flow rate of 1.0 mL min^{-1}, the retention time was 3.39 min (Fig. 2).

Under the described experimental conditions, the peak was well defined and free from tailing. ATN was determined by measuring the peak area. A plot of mean peak area against concentration gave a linear relationship (correlation coefficient, r = 0.9993, n = 5) over the concentration range 1-100 μg mL^{-1}. Using the regression analysis, the linear equation, Y = (-2328) + (14857) X was obtained, where; Y is the mean peak area and X concentration in μg mL^{-1}. The limits of detection and quantification calculated as per ICH guidelines (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use., 2005) were 0.4 and 1.0 μg mL^{-1}, respectively.

Method validation

Specificity: The specificity of the assay was examined by the parameters obtained in the chromatogram. The theoretical plates and tailing factor were found to be 5547 and 1.62, respectively. This indicates the system suitability of the method.

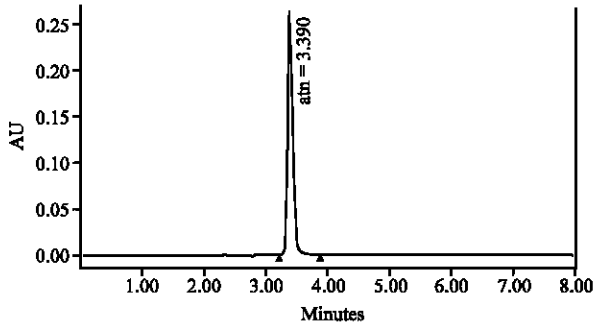

Fig. 2: HPLC chromatogram of atenolol performed on a reversed phase column, Atlantis dC_{18} (250×4.6 mm, 5 μm; internal diameter) using 20 mM ammonium acetate and methanol as mobile phase (1.0 mL min^{-1}) with ultra violet detection at 225 nm

Table 1: Accuracy and intra-day precision

ATN taken (μg mL^{-1})	ATN found[a] (μg mL^{-1})	RE (%)	RSD[b] (%)	RSD[c] (%)
25	24.8	0.80	0.15	0.21
50	50.2	0.40	0.20	0.42
75	74.8	0.28	0.26	0.18

RE: Relative error and RSD- Relative standard deviation. [a]Mean value of seven determinations. [b]Based on peak area. [c]Based on retention time

Accuracy and precision: To determine the accuracy and intra-day precision, pure ATN solutions at three different concentrations was analysed in seven replicates. The percent relative error, which is an index of accuracy, is <1.0% and indicates good accuracy. The relative standard deviation (<2%) at the 95% confidence level can be considered to be satisfactory. The inter-day precision was established by performing analyses over a period of five days on solutions prepared afresh each day (Table 1). The peak-area based and retention-time-based RSD values were <2.0 and <1%, respectively.

Linearity and range: Linearity was assessed in the range of 50 to 150% of the working level concentration including working level concentration. First and last level of linearity was carried out in six replicates and other levels in duplicates. The Linearity co-efficient of mean response of replicate determination plotted against respective concentration was found to be 0.9993. The percent y-intercept as obtained from the linearity data was less than 2%. The% RSD for peak area response of six replicates of first and last level was less than 2.0 and 1.0% for retention time.

Ruggedness (Intermediate Precision): Intermediate precision of six replicate determination of assay of a sample was analysed by different analyst with different instrument in different day after specifying the system suitability of the method. The % RSD of assay was less than 2.0% and the cumulative % RSD of assay of precision study and intermediate precision was also less than 2.0%.

Robustness: Robustness was done by altering deliberately two critical parameters by minor variation:

- Flow rate was changed to 1.1 mL min^{-1}
- Column temperature changed to 32°C

The % RSD for peak area response was less than 2.0 and 1.0% for retention time. The cumulative % RSD of assay of precision study and Robustness was also less than 2.0%.

Limit of quantitation and limit of detection: LOQ and LOD were established based on signal-to-noise ratio, performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples. LOQ and LOD were found to be 1.0 μg mL^{-1} and 0.4 μg mL^{-1}, respectively.

Application: The results obtained are presented in Table 2 and found to be comparable, well with label claim. The results were also compared statistically with those obtained by a literature/reference method (Basavaiah and Chandrashekhar, 2006) by applying Student's t-test for accuracy and F-test for precision. The proposed method was found to be superior as it has lower LOD (0.4 μg mL^{-1} against 0.625 μg mL^{-1}) and lower Beer's law limit suitable for trace level detection.

At the 95% confidence level, the calculated t- and F-values did not exceed the tabulated values (t = 2.77 and F = 6.39) suggesting that the proposed methods are as accurate and precise as the reference method.

The accuracy and validity of the proposed methods were further ascertained by performing recovery experiments. Pre-analysed tablet powder was spiked with pure ATN at three different levels and the total was found by the proposed methods. Each determination was repeated three times. The recovery of pure drug added was quantitative (Table 3) and revealed that co-formulated substances such as talc, starch, gelatin, gum acacia, calcium carbonate, calcium gluconate, calcium dihydrogen orthophosphate, sodium alginate and magnesium stearate did not interfere in the determination.

Table 2: Results of determination of Atenolol in formulations and statistical comparison with the reference method

Formulation brand name#	Nominal amount (mg)	% Found[d]			
		Literature method	Proposed method	t-value	F-value
TENORMIN[a]	100	102.3±0.62	101.5±1.29	1.32	4.33
ATENOLOL[b]	50	100.2±1.06	100.9±1.48	0.87	1.95
ATENOLOL[c]	25	101.3±0.62	100.2±1.32	1.79	4.53

[d]Mean±SD, n = 5; #Marketed by: [a]Nicolas pirmal; [b]Zydus cedilla; [c]Blue cross. Tabulated t-value at 95% confidence level is 2.77; Tabulated F-value at 95% confidence level is 6.39

Table 3: Recovery study

Formulation studied	ATN in formulation (μg mL^{-1})	ATN added (μg mL^{-1})	Total found (μg mL^{-1})	Pure ATN recovered[c] (%)
Tenormin 100[a]	10.2	20	30.24	100.2±1.8
	10.2	50	59.95	99.5±1.7
	10.2	80	90.44	100.3±2.2
Atenolol 50[b]	10.1	20	29.80	98.5±1.9
	10.1	50	58.95	97.7±1.5
	10.1	80	91.06	101.2±2.1

[c]Mean±SD, n = 3; Marketed by: [a]Nicolas pirmal; [b]*Zydus cedilla*

Table 4: Comparison of proposed method with literature methods

Methods	Retention time (min)	LOQ	LOD	Remarks
Proposed method	3.39	1.0 (μg mL^{-1})	0.4 (μg mL^{-1})	Beers law range: 1-100 (μg mL^{-1})
Basavaiah and Chandrashekhar (2006)	4.07	-	0.62 (μg mL^{-1})	Beer's law range: 6.25-200 (μg mL^{-1})
Radulovic *et al.* (1991)	5.07	-	-	pH sensitive, uses internal standard
Venkatesh *et al.* (2007)	4.30	15 (μg mL^{-1})	-	pH sensitive
Ranta *et al.* (2002)	7.0	-	0.7-1.3 nM	Fluorescence detection
Elshanawane *et al.* (2009)	4.36	0.06 to 0.14 (μg mL^{-1})	0.02 to 0.04 (μg mL^{-1})	Beer's law range: 20-200 (μg mL^{-1})
Anelise Weich *et al.* (2007)	2.7	0.4 (μg mL^{-1})	0.2 (μg mL^{-1})	Higher Beer's law range (125-375 μg mL^{-1}), Hplc performed at temperature over 40°C
Ceresole *et al.* (2006)	6.7	-	-	Narrow Beers Law range (0.4 to 12.8 μg mL^{-1}); pH sensitive
Argekar and Sawant (1999)	Retention Factor 0.17	0.140	0.046 (μg mL^{-1})	Lower retention factor, Narrow Beers Law range (4 to 10 μg mL^{-1})

The alternative HPLC method described here is compared with other previously published methodologies. However, these methods are pH sensitive and have relatively high retention times. (Basavaiah and Chandrashekhar, 2006; Radulovic *et al.*, 1991; Venkatesh *et al.*, 2007; Ranta *et al.*, 2002; Elshanawane *et al.*, 2009; Weich *et al.*, 2007; Cersole *et al.*, 2006; Argekar and Sawant, 1999) (Table 4).

CONCLUSION

The method is simple, rapid and convenient since it doesn't need any internal standard. The method is characterized by shorter retention time and a wide linear dynamic range (1-100 μg mL^{-1}) of concentration over which it is applicable. There was no interference from matrix sources. Through the statistical analysis and recovery studies (98.3-102.5%.) it is found that the proposed method can be effectively employed for quantification of atenolol in pharmaceutical formulations and real samples.

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