NEW VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ATENOLOL AND INDAPAMIDE IN TABLETS

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ABSTRACT

A simple, specific and precise reverse phase high performance liquid chromatographic method was developed and validated for simultaneous estimation of Atenolol and Indapamide in tablets. Quantification was achieved by using an Eclipse XDB plus C18 column (4.6 x 150nm, 5 μm particle size). The mobile phase consisted of a mixture of acetonitrile and water in the ratio of 60:40 v/v at a flow rate of 1.2 ml/min. The retention times of Atenolol and Indapamide were found to be 1.00 min and 2.057 min respectively. The developed method was validated as per ICH Guidelines for linearity, accuracy, precision, detection limit, quantification limit, robustness, rudggedness, specificity and system suitability. The percentage recoveries for both of the drugs from the tablets were found to be 100.2 % and 99.99 % respectively. The method may successfully be employed for the simultaneous determination of Atenolol and Indapamide in pharmaceutical tablet dosage forms.

KEYWORDS: Atenolol, Indapamide, RP-HPLC; tablets.

INTRODUCTION

Atenolol (ATN), 2-(4-{2- hydroxyl-3-[(Propan-2-yl) amino] propoxy} phenyl) acetamide (Fig. 1), is a white crystalline powder with molecular formula is $C_{14}H_{22}N_2O_3$. It is commonly used as antihypertensive agent. Atenolol competes with sympathomimetic neurotransmitters such as catecholamines for binding at β_1- adrenergic receptors in the heart and vascular smooth muscle, inhibiting sympathetic stimulation which results in a reduction in resting heart rate, cardiac output, systolic and diastolic blood pressure, and reflex orthostatic hypotension.[1]
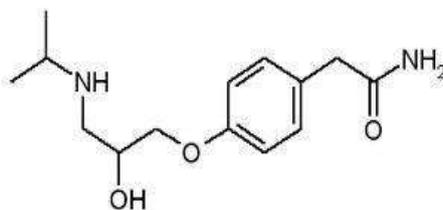
Fig. 1: Chemical structure of Atenolol

Indapamide (IND), chemically 4-chloro-N-(2-methyl-2,3-dihydro-1H-indol-1-yl)-3-sulfomylbenzamide (Fig. 2) is a white crystalline powder having molecular formula $C_{16}H_{16}ClN_3O_3S$. It is commonly used in hypertension and as a diuretic. [2]
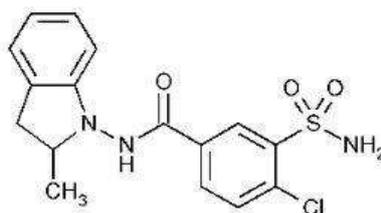

Fig. 2: Chemical structure of Indapamide

The literature survey revealed that very few RP-HPLC [3-8] and spectroscopic methods [9] were reported for the simultaneous estimation of Atenolol and Indapamde in formulations. The authors now propose a new validated, sensitive and reproducible HPLC method for simultaneous determination of Atenolol and Indapamide. The applicability of this method in determining the drugs in commercial dosage forms were also studied.

MATERIALS AND METHODS

Materials

Standard Atenolol and Indapamide were obtained from Yarrow chem. Products, Mumbai, India. Commercial formulation, ATEN-D was purchased from the local market. Each tablet contains 50 mg of Atenolol and 2.5 mg of Indapamide. Other materials required were HPLC grade water and acetonitrile.

Instrumentation

An Agilent model - 1220 Infinity LC – HPLC system with Agilent open LAB CDS (EZ Chrome) software "version A.04.05" equipped with a variable wavelength detector (VWD) and a manual injector was used. It was manufactured by Agilent Technologies, USA. An Eclipse XDB plus C18 Column (4.6 × 150 mm, 5 μm particle size) was used for the analytical separation and quantification of the mixtures. An ELICO (LI 120) pH meter was

used for adjusting the pH of the buffer. The detection was done using a Cary 60 UV-VIS double beam spectrophotometer.

Optimized Chromatographic Conditions

An Eclipse XDB plus C18 (4.6 × 150 mm, 5 µm particle size) column was used for the analytical separation. The mobile phase consisted of a mixture of acetonitrile and water in the ratio of 60:40 v/v with an isocratic elution program. The flow rate was adjusted to 1.2 ml/min and the injection volume was set at 20 µl with a detection wavelength of 235 nm.

Preparation of Standard solutions

Atenolol stock solution: 50 mg of Atenolol was accurately weighed and dissolved in 10ml of the mobile phase. After sonication for 15 min, the volume was made upto 50 ml with the mobile phase to get 1 mg/ ml solution.

Atenolol working standard solutions: The Atenolol stock solution was diluted suitably with mobile phase to get the working standard solutions of concentrations ranging from 10 to 50 µg/ml. 20 µl of each of the dilutions was injected 5 times into the column and the chromatograms were recorded.

Indapamide stock solution: 2.5 mg of Indapmide was weighed accurately and dissolved in 10 ml of the mobile phase. After sonication for 15 min, the volume was made up to the mark with sufficient volume of mobile phase in a 50 ml volumetric flask to get a 50 µg/ml solution.

Indapamide working standard solutions: The Indapamide stock solution was diluted suitably with mobile phase to get the working standard solutions of concentrations ranging from 10 to 50 µg/ml. 20 µl of each of the dilutions was injected 5 times into the column and the chromatograms were recorded.

Preparation of mixed standard stock solution: Atenolol (100 mg) and Indapamide (Equivalent to 5 mg of Indapamide) were weighed accurately into a 100 ml volumetric flask, dissolved in 20ml of diluent, sonicated for 15 min and the volume was finally made up with the mobile phase.

Preparation of mixed working standard solutions: 1mlof the mixed stock solution was taken in a 10 ml volumetric flask and made upto volume with the mobile phase to get a concentration 5 µg/ml of Indapamide and 100 µg/ml of Atenolol. 20 µl of this solution was injected and the chromatogram was recorded.
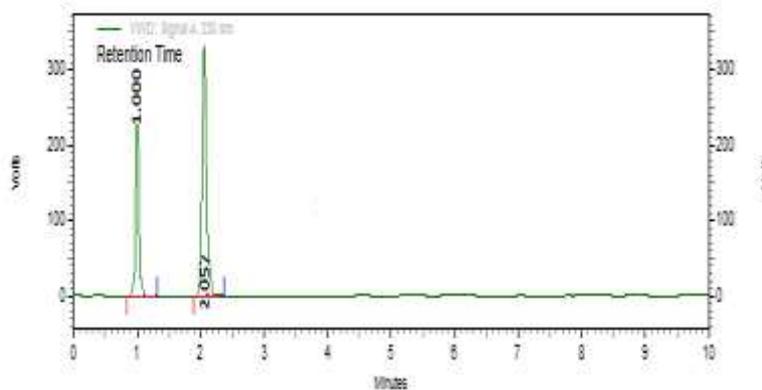
Fig. 3: Typical chromatogram for Atenolol and Indapamide

RESULTS AND DISCUSSION

The analytical method was validated with respect to parameters such as linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) robustness, ruggedness and specificity as per ICH guidelines.

Method validation

The analytical method was developed and validated according to ICH guidelines. Analytical variable parameters such as linearity, precision, accuracy, specificity, and system suitability were tested using the optimized chromatographic conditions and instruments.

System suitability parameters

Five replicates of mixed working standard solutions were injected and the parameters like theoretical plate number (N), tailing factor (K) and resolution are calculated to check the system suitability. The results are presented in Table 1.

Table 1: System suitability test results.

S. No	Parameters	ATN	IND
1	Retention time (min)	1.00	2.057
2	Theoretical plates	2308	4472
3	Asymmetry	1.1	1.1
4	Area	3951465	25898233
5	Resolution	7.1	

The system suitability parameters were found to be within the specified limits for the proposed method

Linearity

Mixed standard stock solution was suitably diluted with the mobile phase to obtain the concentrations ranging from 10-50 µg/ml of Atenolol & 10-50 µg/ml of Indapamide. The solutions were filtered through 0.45 µ nylon membrane filter paper and 20 µl of each of the solutions were injected and the chromatograms were recorded. A good linear relationship (R^2= 0.999 for Atenolol and R^2= 0.999 for Indapamide was observed between the concentrations of the drugs and their corresponding peak areas. The results of linearity studies are shown in Table 2.

Table 2: Linearity study data for Atenolol and Indapamide.

S.No.	Drugs	Slope	Intercept	Correlation coefficient
1	ATN	13070	10258	0.999
2	IND	85327	99987	0.999

Accuracy

The accuracy studies were performed on 80 %, 100 % and 120 % of the analytical method target concentrations of Atenolol and Indapamide. Standard and sample preparations were injected into HPLC system and three determinants for each concentration level were obtained. The percentage recoveries of Atenolol and Indapamide were calculated using standard at the same concentration at each concentration level. The results are presented in Table 3.

Table 3: Recovery study data for Atenolol and Indapamide

S.No.	Drug	%Recovery	%RSD
1	ATN	100.1	0.11
2	IND	99.76	0.38

Precision

System Precision: System precision of the proposed method was checked by injecting five replicate preparations of the standard drug solutions of Atenolol (100 µg/ml) and Indapamide (5 µg/ml). The corresponding peak areas were measured and % RSD calculated.

Method Precision: The method precision study was performed for five replicate sample preparations of marketed formulation containing Atenolol (100 µg/ml) and Indapamide (5 µg/ml). The corresponding peak areas were measured and % RSD calculated as exhibited in Table 4.

Table 4: System and Method Precision of the proposed method

Drug	Spiked level (µg/ml) (n=5)	System precision		Method precision	
		S.D	%RSD	S.D	%RSD
ATN	30	660.5	0.016	914.30	0.023
IND	30	9111.90	0.03	10192.6	0.04

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ of the proposed method were calculated as $3.3\partial/S$ and $10\partial/S$, respectively as per ICH guidelines, where ∂ is the standard deviation of the response and S is the slope of the calibration plot. The results are presented in Table 5.

Table 5: Sensitivity of the proposed method

Drug	LOD (µg/ml)	LOQ (µg/ml)
ATN	0.2680	0.8123
IND	0.3021	0.9156

Robustness

Robustness of the developed analytical method was tested by evaluating the affect of small variations in analytical method parameters such as change in flow rate of 1.2 ml/min by ±0.2 ml/min and change in wavelength by ±2 nm. The results are shown in Table 6.

Table 6: Robustness study data for Atenolol and Indapamide

System suitability Parameters		% RSD of peak area response (n=5)		Mean tailing factor (n=5)		Mean retention time in min. (n=5)	
Variations		ATN	IND	ATN	IND	ATN	IND
Change in Flow rate (ml/min)	+0.2	0.025	0.03	1.36	1.19	1.16	2.68
	0	0.016	0.04	1.1	1.1	1.01	2.06
	-0.2	0.027	0.06	0.65	0.047	1.57	2.74
Change in Wavelength (nm)	+2	0.023	0.027	1.63	1.282	1.05	2.04
	0	0.017	0.04	1.1	1.1	1.00	2.05
	-2	0.035	0.029	1.55	1.026	1.01	2.08

Ruggedness

Ruggedness of the proposed method was determined by spiking five replicates of tablet formulation containing Atenolol (30 µg/ml) and Indapamide (30 µg/ml) prepared by another analyst into another HPLC system. The % recoveries were found to be within the acceptance criteria as shown in Table 7.

Table 7: Ruggedness of the proposed method

S.No.	Parameter	ATN	IND
1	Analyst-01	100.12% w/w	99.80% w/w
2	Analyst-02	100.2% w/w	99.79% w/w
3	Acceptance criteria	98-102% w/w	98-102% w/w

Specificity

The specificity of the proposed method was determined to check whether there is any interference due to presence of excipients, impurities or other components with the retention time of analytical peaks. The HPLC chromatograms were recorded for the drug-matrix (mixture of the drug and excipient) which showed almost no interfering peaks within retention time ranges indicating that the method is quite specific.

Application of the proposed method to commercial formulation

Twenty tablets were weighed accurately and crushed to fine powder. Each tablet contains 50 mg of Atenolol and 2.5 mg of Indapamide. A quantity of powder equivalent to 50 mg of Atenolol and 2.5 mg of Indapamide was weighed and dissolved in 25 ml of the mobile phase in a 50 ml volumetric flask. The volume was made up to give a concentration of 1000 μg/ml of Atenolol and 50 μg/ml of Indapamide. The solution was filtered through 0.45 μ nylon membrane filter. From this filtrate, different dilutions ranging from 10-50 μg/ml of Atenolol & 10-50 μg/ml of Indapamide were prepared in 10 ml volumetric flasks with the mobile phase. 20 μl of each of these solutions were injected 5 times and the chromatograms were recorded. Typical chromatogram obtained for the commercial formulation is presented in Fig. 4. The amount of Atenolol and Indapamide present in each tablet formulation (Table 8) was calculated by comparing the peak area of the tablet solution with that of standard using the given formula:

$$\% \text{ Assay} = \frac{\text{Sample Avg. peak area}}{\text{Standard Avg. peak area}} \times \frac{\text{Wt. of drug (mg)}}{\text{dilution of standard}} \times \frac{\text{dilution of tablet solution}}{\text{wt. of Sample}} \times \frac{\% \text{ Purity}}{100} \times \frac{\text{Avg. wt}}{\text{Lable Claim}} \times 100$$

Table 8: Assay results of the proposed method

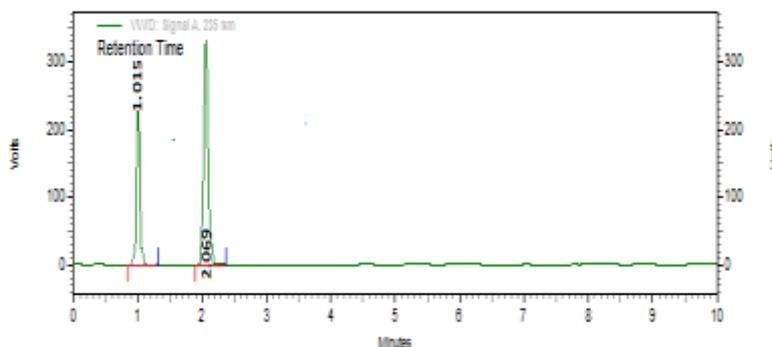| Sr. No	Wt. of std. (mg)		Weight of sample (mg)	Peak area of std		Peak area of sample		%Label claim	
	ATN	IND		ATN	IND	ATN	IND	ATN	IND
1			88			3952770	25878239	100.3	99.96
2	50	2.5	88.4	3951465	25898233	3952475	25888745	100.5	99.96
3			88.6			3950515	25918483	99.97	100.07
Mean								100.25	99.99
S.D								0.267	0.063
%RSD								0.266	0.064

Fig. 4: Typical HPLC chromatogram corresponding to marketed formulation of Atenolol and Indapamide

CONCLUSION

The proposed RP-HPLC method is found to be simple, sensitive, reproducible, less time consuming and is applicable for analysis of Atenolol and Indapamide in bulk and in tablet dosage forms. The validation of the developed method for the required parameters as per ICH guidelines showed that all the parameters were within the acceptance criteria. The proposed method was applied for the simultaneous determination of Atenolol and Indapamide in marketed tablet dosage form. The assay results confirmed with the label claim of the formulation. It can be concluded that the proposed HPLC method is sufficiently sensitive and reproducible for simultaneous estimation of Atenolol and Indapamide in tablet dosage forms.

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