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DEVELOPMENT AND VALIDATION OF RP- HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ATENOLOL AND INDAPAMIDE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, sensitive, precise and specific Reverse Phase High Performance Liquid Chromatographic method was developed and validated for the determination of Atenolol and Indapamide in bulk and tablet dosage forms. It was found that the excipient in the tablet dosage forms does not interfere in the quantification of active drug by proposed method. The HPLC separation was carried out by reverse phase chromatography by Intelligent C_{18} column (200×4.6mm) with a mobile phase composed of Methanol: Water: Diethylamine: Glacial Acetic Acid, (70:30:0.12:0.08) in isocratic mode at a flow rate of 1.2ml/min. The detection was monitored at 240 nm. The calibration curve for Atenolol and Indapamide was linear from 20-100 µg/ml and 1-5 µg/ml respectively. The inter-day and intra-day precision was found to be within limits. The proposed method has adequate sensitivity, reproducibility and specificity for the determination of Atenolol and Indapamide in bulk and its tablet dosage forms. Accuracy (recoveries: 99.07-101.44%) and reproducibility were found to satisfactory.

KEYWORDS: Atenolol, Indapamide, Bulk and Tablet dosage form, Reverse Phase, High Performance Liquid Chromatography.

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INTRODUCTION

Atenolol (ATL), chemically (R, S)-4-(2-hydroxy-3-isopropyl-aminopropoxy) phenylacetamide, is a beta-adrenoceptor antagonist. It is official in the Indian Pharmacopoeia.[1] Literature survey reveals, HPLC and HPTLC methods have also been reported for estimation of ATL in Pharmaceutical dosage forms[2-4] and also there are various methods such as UV spectrophotometry for Atenolol.[5-7]

Indapamide (IND), Benzamide, 3-(aminosulphonyl)-4 chloro-N-(2,3-dihydro-2-methyl-1H-indol-1-yl trihydrate is β-blocking agent, that lowers blood pressure and used for control and management of edema and widely used in treatment of hypertension.[8] Few spectroscopic methods have been reported for determination of IND as single drug or in combination with other drugs in blood and urine sample.[9] Indapamide can determine by the use of spectrophotometer[10,11] and also chromatographic methods.[12,13] Extensive literature survey reveals, none of the method is available that is based on estimation of Atenolol and Indapamide simultaneously by high performance liquid chromatographic method. The proposed work was to develop simple, precise, accurate and economical chromatographic methods for simultaneous determination of binary drug formulation. The proposed method was optimized and validated in accordance with International Conference on Harmonization (ICH) guidelines.[14]

Combination of Atenolol and Indapamide in tablet formulation has been recently launched in market by Cadila Health Ltd., as Brand name 'ATEN-D'. It is used in the management of hypertension.

In literature, very few methods are reported for determination of Atenolol and Indapamide in Pharmaceutical formulations. Also, no HPLC methods were found to our knowledge for the determination of Atenolol and Indapamide in their combined dosage form.

Therefore, it was thought meaningful to develop simple, precise and rapid RP-HPLC methods for analysis of Atenolol and Indapamide individually as well as in combined tablet dosage form.

MATERIALS AND METHODS

Chromatographic analysis was carried out on JASCO HPLC system with JASCO TU 1580 Intelligent HPLC Pump and JASCO UV- 1575 Intelligent UV/CIS detector by using Intelligent C_{18} (5 µm, 200 mm X 4.6 mm i.d) as a column. The Borwin Chromatographic software system of the instrument was used for obtaining the peak. Pure drug samples of ATL and IND were kindly gifted by Suchem Lab, Ahmadabad and Supra chemicals, Thane respectively. Methanol was procured from Merck Chemical Corporation, Mumbai. Commercial Pharmaceutical preparation (ATEN-D, Cadila Health Care, Ahmadabad) was procured from commercial source. All the reagents used were of analytical grade.

Preparation of Standard Stock Solution

Standard stock solutions were prepared by dissolving 50 mg of ATL and 2.5 mg of IND in 50 ml of mobile phase to obtained concentration of 1000 µg/ml of ATL and 50 µg/ml of IND.

Study of Optimization of Chromatographic Parameters

Mobile phases such as varying proportions of Methanol:Water and Methanol:Water: Diethylamine:Glacial Acetic Acid were tried to achieve separation of ATL and IND. A mobile phase consisted of Methanol:Water:Diethylamine:Glacial Acetic Acid, pH 7 (70:30:0.12:0.08 v/v) was selected to achieve symmetrical peaks and sensitivity. The effects of flow rates in the range of 0.9-1.1 ml/min were examined. A flow rate of 1.2 ml/min showed reasonable retention time of ATL 1.858 min and IND 2.758 min and total time of analysis was less than 10 min.

Optimization of Detection Wavelength

Wavelength 240 nm was selected as per the response of analyte.

Linearity Study

Different aliquots 0.2, 0.4, 0.6, 0.8 and 1.0 ml of standard stock were transferred into 10 ml volumetric flasks and volume was adjusted to mark to obtain concentration in the range of 20-100 µg/ml for ATL and 1-5 µg/ml for IND. An appropriate volume 20 µL of each solution was injected with the help of Hamilton Syringe. All measurements were repeated five times for each concentration and calibration curve was constructed by plotting the peak area Vs the drug concentration. The observations and calibration curve is shown in **Table 1 and 2** and **Fig. 1 and 2.**

Analysis of Laboratory Mixture by Proposed Method

Accurately weighed 50 mg of ATL and 2.5 mg of IND were transferred to 100 ml volumetric flask, dissolved in mobile phase and volume was adjusted up to the mark with same solvent. Appropriate aliquot is taken for the concentration of 50 µg/ml of ATL and 2.5 µg/ml of IND. The procedure was repeated for five times, the results are shown in **Table 3.**

Application of Proposed Method to Tablet Formulations

Twenty tablets **ATEN-D** (containing 50 mg of ATL and 2.5 mg of IND**)** were weighed and ground to fine powder. A quantity of sample equivalent to 50 mg of ATL and 2.5 mg of IND was transferred into 100 ml volumetric flask containing mobile phase, sonicated for 30 min, the volume was made up to the mark and filtered through 0.45 µ membrane filter. An appropriate aliquot is taken for the concentration of 50 µg/ml and 2.5 µg/ml. The assay procedure was repeated for five times, the results are shown in **Table 4.**

Validation of Proposed Method

The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment.

Accuracy

The accuracy of the method was assessed by recovery experiment performed at 80, 100 and 120 % levels. A known amount of standard ATL and IND were added to pre-analyzed sample and subjected to the proposed HPLC method. Results are shown in **Table 5.**

Precision

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions.

Repeatability

It was measured by multiple injections of a homogenous sample containing 60 µg/ml of ATL and 3 µg/ml of IND that indicates the performance of the HPLC instrument under chromatographic conditions. Results are shown in **Table 6.**

Intra-Day and Inter-Day Precision

Intra-day precision was determined by analyzing the three different concentrations 40, 60 and 80 µg/ml of ATL and 2, 3 and 4 µg/ml of IND, for three times in the same day. Day to day variability was assessed using above mentioned three concentrations analyzed on three different days. The results are shown in shown in **Table 7.**

Robustness

Robustness of the proposed method was assessed by making deliberate changes in flow rate and proportion of

methanol which was performed by injecting sample solution containing 40 µg/ml of ATL and 2 µg/ml of IND, the results are shown in **Table 8.**

Sensitivity

The sensitivity of proposed method was estimated in terms of the Limit of Quantitation (LOQ) and Limit of Detection (LOD).

The LOQ and LOD were calculated using following equations,

LOQ = 10 x N/B

LOD = 3.3 x N/B

Where,

N is standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise,

B is the slope of the corresponding calibration curve.

The linearity equations were found to be Y=6983.9X+6035.9 for ATL and Y=52559X+3243.7 for IND. The LOD and LOQ for ATL were found to be 0.70 µg and 2.12 µg, respectively. For IND, the LOD and LOQ were found to be 0.21 µg and 0.62 µg, respectively.

Ruggedness

From stock solution, solutions containing 40 µg/ml of ATL and 2 µg/ml of IND were prepared and analyzed by two different analysts using same operational and environmental conditions. Peak area was measured for same concentration solutions, three times. The results are shown in **Table 9.**

System Suitability Test

System suitability testing is essential for the assurance of the quality performance of the chromatographic system. Earlier prepared solutions for chromatographic conditions were tested for system suitability testing. Results are shown in **Table 10.**

RESULT AND DISCUSSION

RP-HPLC method has been developed for simultaneous determination of ATL and IND in tablet dosage form. Chromatographic separation was performed on Intelligent C_{18} (200 mm X 4.6 mm i.d.), 5µm particle size in isocratic mode, at 25ºC temperature using a mobile phase consisting of Methanol:Water:Diethylamine:GAA (70:30:0.12:0.08 v/v), pH 7, at a flow rate of 1.2 ml/min. Detection was carried out at 240 nm. The RT of ATL and IND were 1.858 and 2.758, respectively. ATL and IND obeyed linearity range in the concentration of 20-100 µg/ml (r^2 = 0.9996) and 1-5 µg/ml (r^2 = 0.9995), respectively. As the correlation coefficient values of ATL and IND were found to be greater than 0.999, indicates good linear response. The LOD and LOQ for ATL were found to be 0.70 µg and 2.12 µg, respectively. For IND, the LOD and LOQ were found to be 0.21 µg and 0.62 µg, respectively. The observations proved adequate sensitivity of the method. The proposed method was applied to Pharmaceutical formulation and % label claim for ATL and IND was found to be 99.99 % and 98.48 %, respectively. Amount of drugs estimated by proposed method was in good agreement with the label claim. The recovery studies were carried out at 80, 100 and 120 % level and mean percentage recovery for ATL and IND was found to be 99.07-101.44 % and 99.45-100.73%, respectively. The % R.S.D. values less than 2 indicate proposed method is accurate. The method was found to be precise as indicated by the inter-day, intra-day and repeatability analysis. Low % R.S.D. indicates high precision and reproducibility of the proposed method. Robustness of the method was assessed by making deliberate variations in mobile phase composition and flow rate and effects on the results were examined. The method was successively applied to Pharmaceutical formulation, no chromatographic interferences from the tablet excipients were found. The suitability of this RP-HPLC method was proved by validation in accordance with the requirements of ICH guidelines.

CONCLUSION

RP-HPLC for determination of ATL and IND in bulk and tablet dosage form. RP-HPLC method is simple, economical and rapid as compared to other method, also it was found to be more precise, accurate, rugged and robust All these developed methods can be used for routine analysis of ATL and IND in Pharmaceutical formulation.

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Table 1 Linearity Study of ATL at 240 nm for HPLC

Sr. No.	Concentration of ATL in [µg/ml]	Peak area Mean ± S.D. [n = 5]	% R.S.D.
1	20	150479.3 ± 745.58	0.4954
2	40	290761.8 ± 1321.55	0.4545
3	60	420363.4 ± 601.37	0.1430
4	80	569157.4 ± 693.12	0.1217
5	100	700613.2 ± 546.02	0.0779

Table 2. Linearity Study of IND at 240 nm for HPLC

Sr. No.	Concentration of IND in [µg/ml]	Peak area Mean ± S.D. [n = 5]	% R.S.D.
1	1	58302.6 ± 73.68	0.1263
2	2	109046.4 ± 518.22	0.4752
3	3	162611.1 ± 965.65	0.5938
4	4	214259.4 ± 909.37	0.4244
5	5	263623.4±2602.74	0.9872

µg/ml = Microgram per milliliter
S.D. = Standard Deviation
R.S.D. = Relative Standard Deviation
ATL = Atenolol
IND = Indapamide

Table 3. Analysis of ATL and IND in Laboratory Mixture for HPLC

Drugs	Amount taken [µg/ml]	Amount found [µg/ml]	Amount found [%]
ATL	50	50.70	101.41
	50	50.88	101.76
	50	49.31	98.63
	50	49.80	99.61
	50	49.74	99.49
	Mean ± S.D.	50.09 ± 0.6712	100.18 ± 1.3424
	%R.S.D.	1.3399	1.3399
IND	2.5	2.48	99.33
	2.5	2.50	100.20
	2.5	2.50	100.31
	2.5	2.53	101.57
	2.5	2.52	100.82
	Mean ± S.D.	2.51 ± 0.0207	100.45 ± 0.8284
	%R.S.D.	0.8247	0.8247

µg/ml = Microgram per milliliter
S.D. = Standard Deviation
R.S.D. = Relative Standard Deviation
ATL = Atenolol
IND = Indapamide

Table 4. Application of Proposed Method for Analysis of Tablet Formulation of ATL and IND for HPLC

Drugs	Label claim [mg]	Amount found [mg]	Amount found [%]
ATL	50	49.57	99.15
	50	49.69	99.38
	50	49.82	99.64
	50	50.02	100.05
	50	50.86	101.72
	Mean ± S.D.	49.99 ± 0.5126	99.99 ± 1.0253
	% R.S.D.	1.0253	1.0253
IND	2.5	2.43	97.43
	2.5	2.44	97.65
	2.5	2.48	98.65
	2.5	2.48	99.28
	2.5	2.48	99.38
	Mean ± S.D.	2.46± 0.0226	98.48 ± 0.9041
	% R.S.D.	0.9180	0.9180

mg = Milligram
S.D. = Standard Deviation
R.S.D. = Relative Standard Deviation
ATL = Atenolol
IND = Indapamide

Table 5. Results of Recovery Studies of ATL and IND for HPLC

Pre-analysed sample solution [µg/ml]	Excess drug added [µg/ml] [n = 3]	Amount recovered [µg/ml]	% Recovery	% R.S.D.
ATL 50	0	49.65	99.31	0.1805
	40	40.57	101.44	0.7768
	50	50.16	100.33	1.0495
	60	59.44	99.07	0.0218
IND 2.5	0	2.51	100.56	0.9022
	2	1.98	99.45	0.2898
	2.5	2.51	100.73	1.2797
	3	3.00	99.66	1.3321

µg/ml = Microgram per mililitre
R.S.D. = Relative Standard Deviation
ATL = Atenolol
IND = Indapamide

Table 6. Results of Repeatability Studies of ATL and IND for HPLC

Drug	Amount taken [µg/ml] [n = 5]	Amount found [µg/ml] ± S.D.	% R.S.D.
ATL	60	59.89 ± 0.1348	0.2252
IND	3	2.99 ± 0.0341	1.1403

µg/ml = Microgram per milliliter
S.D. = Standard Deviation
R.S.D. = Relative Standard Deviation
ATL = Atenolol
IND = Indapamide

Table 7. Results of Intra-day and Inter-day Precision of ATL and IND for HPLC

Drug	Conc. [µg/ml]	Intra-day Amount found [µg/ml]		Inter-day Amount found [µg/ml]	
		Mean ± S.D [*n* = 3]	% R.S.D.	Mean ± S.D. [*n* = 3]	% R.S.D.
ATL	40	40.32± 0.5199	1.2893	40.07 ± 0.6358	1.5866
	60	59.58 ± 0.5971	1.0021	59.55± 0.1074	0.1805
	80	79.86 ± 0.3321	0.4158	79.94 ± 0.1809	0.2263
IND	2	1.98 ± 0.0132	0.6659	2.00 ± 0.0205	1.0295
	3	3.02 ± 0.0072	0.2384	3.00 ± 0.0265	0.8823
	4	4.02 ± 0.0432	1.0723	4.00 ± 0.0557	1.3918

µg/ml = Microgram per milliliter
S.D. = Standard Deviation
R.S.D. = Relative Standard Deviation
ATL = Atenolol
IND = Indapamide

Table 8. Results Robustness Evaluation of ATL and IND for HPLC

Chromatographic conditions	RT		K'		TF	
	ATL	IND	ATL	IND	ATL	IND
A: Flow Rate (ml/min)						
1.10	2.06	2.97	39.32	45.34	1.24	1.21
1.20	1.90	2.77	36.12	50.55	1.12	1.32
1.30	1.72	2.51	36.11	58.32	1.42	1.24
Mean ± S.D.	**1.89±0.17**	**2.75± 0.23**	**37.18±1.85**	**51.40± 6.53**	**1.26±0.15**	**1.25± 0.05**
B: Percentage Methanol in Mobile Phase (v/v)						
69	2.00	2.80	57.44	63.00	1.20	1.42
68	2.14	3.01	60.62	55.23	1.34	1.47
67	1.50	2.97	61.00	61.58	1.23	1.16
Mean ± S.D.	**1.88±0.33**	**2.92± 0.11**	**69.68±1.95**	**59.93±4.13**	**1.25±0.07**	**1.35± 0.16**

ml/min = milliliter per minutes
S.D. = Standard Deviation
RT = Relative Time
K` = Capacity Factor
TF = Tailing Factor
S.D. = Standard Deviation
v/v = Volume by Volume
ATL = Atenolol
IND = Indapamide

Table 9. Result of Ruggedness of ATL and IND for HPLC

Drug	Amount taken [µg/ml] [n = 3]	Analyst I	% R.S.D.	Analyst II	% R.S.D.
ATL	40	100.73	0.4729	100.14	0.8717
IND	2	100.10	0.4653	99.98	1.2417

µg/ml = Microgram per mililitre
R.S.D. = Relative Standard Deviation
ATL = Atenolol
IND = Indapamide

Table 10. Results of System Suitability Test of ATL and IND for HPLC

System suitability parameters	Proposed method	
	ATL	IND
Retention time	1.87	2.82
Capacity factor	56.34	61.34
Theoretical plate	1034.56	2834.4
Tailing factor	1.12	1.67

ATL = Atenolol
IND = Indapamide

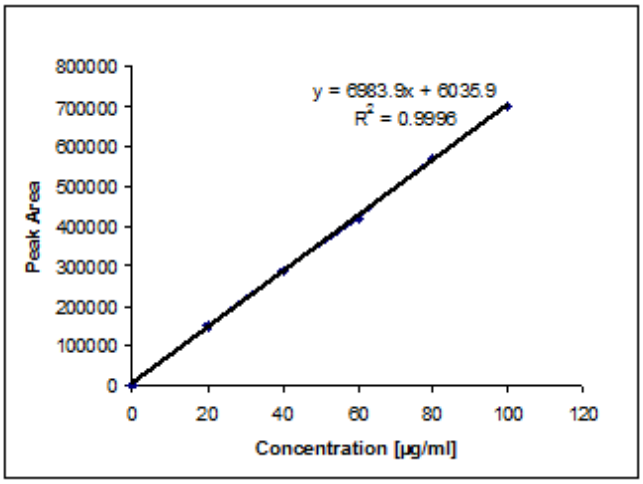

Fig. 1 Calibration Curve of ATL for HPLC at 240 nm

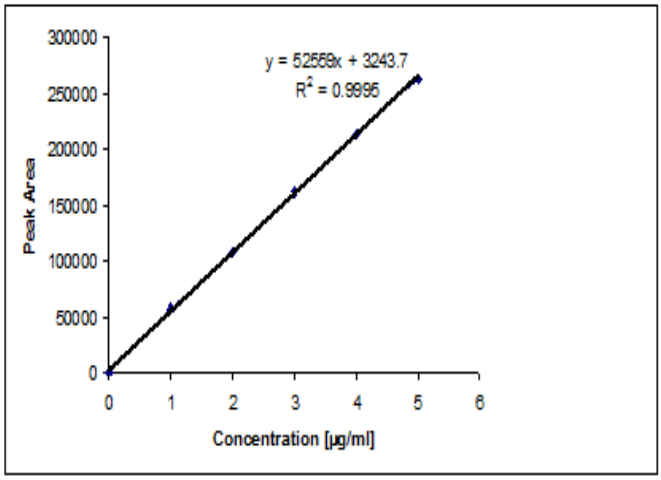

Fig. 2 Calibration Curve of IND for HPLC at 240 nm

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