IJPAR |Volume 3 | Issue 1 | Jan-Mar-2014 **ISSN: 2320-2831**

INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

Available Online at: www.ijpar.com

[Research article]

RP-HPLC Method Development and Validation for the Simultaneous Estimation of Atenolol and Indapamide in Pharmaceutical Tablet Dosage Form

*** K.Madhavi, [2]K.Deepti, [3]B.Harika**

Department of Pharmaceutical Analysis and Quality Assurance, Smt. Sarojini Ramulamma College of Pharmacy, Sheshadrinagar, Mahabubnagar - 509001, Andhrapradesh, India.

ABSTRACT

A simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for simultaneous determination of ATENOLOL and INDAPAMIDE in pharmaceutical tablet dosage form. Chromatographic analysis was performed on a Symmetry X-terra C8 (4.6mm x 100mm, 5µm) column at ambient temperature with a mixture of Potassium di hydrogen phosphate buffer and Acetonitrile in the ratio 40:60 v/v as mobile phase, at a flow rate of 0.7 mL min^{-1}. UV detection was performed at 240 nm. The retention times of Atenolol and Indapamide were 2.1 and 3.6 min, respectively. The correlation coefficient of Atenolol and Indapamide was found to be 0.999. Calibration plots were linear over the concentration ranges 20–100 µg mL^{-1} and 1-5 µg mL^{-} for Atenolol and Indapamide respectively. The Limit of detection was 0.223 and 0.286µg mL^{-1} and the quantification limit were 0.677 µg mL^{-1} and 0.86µg mL^{-1} for Atenolol and Indapamide, respectively. The accuracy of the proposed method was determined by recovery studies and found to be 100.74% to 99.93%.The method was validated for accuracy, linearity, sensitivity, precision, robustness, system suitability Commercial tablet formulation was successfully analyzed using the developed method and the proposed method is applicable to routine analysis of determination of Atenolol and Indapamide in pharmaceutical tablet dosage form.

Keywords: Atenolol, Indapamide, RP-HPLC, Validation.

INTRODUCTION

Atenolol is chemically (RS)-2-{4-[2-hydroxy-3-(propan-2-yl amino) propoxy] phenyl} acetamide [Figure 1] and its molecular formula is C14H22N2O3 hypertension, and molecular weight is 266.34 gm/mole. Atenolol can be used to treat cardiovascular diseases and conditions such as, coronary heart disease, arrhythmias, angina and to treat and reduce the risk of heart complications following myocardial infarction. Indapamide is chemically 4-chloro-N-(2-methyl-2,3-dihydroindol-1-yl)-3-sulfamoyl-benzamide [Figure-2]. Its molecular formula is C16H16ClN3O3S having molecular weight 365.84 gm/mole. Indapamide is a non-thiazide sulphonamide diuretic drug. generally used in the treatment of hypertension, as well as decompensated cardiac failure.

***Corresponding author: K.Madhavi**
E-mail address: madhavi.kori@gmail.com

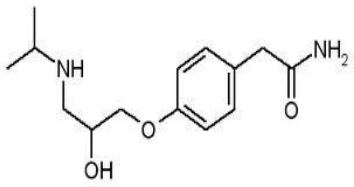


Fig:1 Atenolol

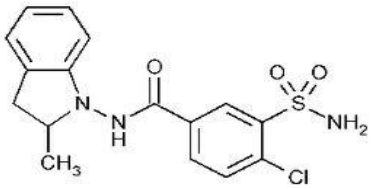


Fig:2 lndapamide

MATERIALS AND METHODS

Chemicals/ Reagents and Solvents

Atenolol-25 mg and Indapamide-2.5mg were obtained from, ZYDUS MEDICA Health Care. Ltd. Ahmedabad, Double Distilled Water (HPLC grade), Methanol (HPLC grade), Acetonitrile (HPLC grade), orthophosphoric acid and Potassium-dihydrogen phosphate were of reagent grade. The pharmaceutical preparations of combination of Atenolol & Indapamide that is ATEN-D tablet (ZYDUS MEDICA Health Care. Ltd. Ahmedabad).

Instrumentation and Equipments

The HPLC analysis was accomplished on WATERS high pressure liquid chromatograph outfitted with 515 reciprocating dual column HPLC pump, a manually operating Rheodyne injector with 20µL sample loop, X-terra C_8 4.6mm x 150mm analytical column reversed-phase material of 5µ size and a 2487 model UV-Visible detector. All the parameters of HPLC were controlled by N 2000 chromatographic system software. Other instruments used were TECHCOMP UV-Vis spectrophotometer of model 2310, Shimadzu electronic balance of model XEX-200, ADWA of model AD102U digital pH meter and ENERTECH of model SE60US ultrasonic bath sonicator

ANALYTICAL METHOD DEVELOPMENT

Optimization of UV conditions

A waters symmetry X-terra C_8 (4.6mm x 150mm, 5µm) was used for chromatographic separation. Mobile phase and sample solution were filtered through a 0.45µm membrane filter and degassed. The mobile phase composed of pH3 Buffer (Potassium di hydrogen phosphate):Acetonitrile (40:60) at flow rate 0.7 mL/min with run time 5mins detection of both drugs was carried out at 240nm.

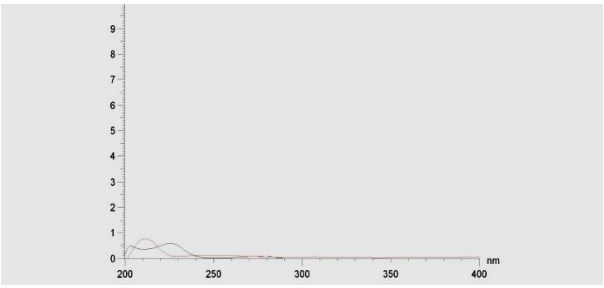


Fig.3. Isobestic point of Atenolol and indapamide

Optimized Method Parameters

Mobile Phase : Phosphate buffer (3.0 pH): Acetonitrile(40:60)
Column (Stationary Phase) : X-terra(C_8) (4.6mm x 150mm, 5µm)
Flow rate (ml/min) :0.7
Column temperature (°C) : Ambient
Volume of injection loop (µl) : 20
Detection wavelength (nm) :240
Drug RT (min) : Atenolol – 2.1
Indapamide-3.6

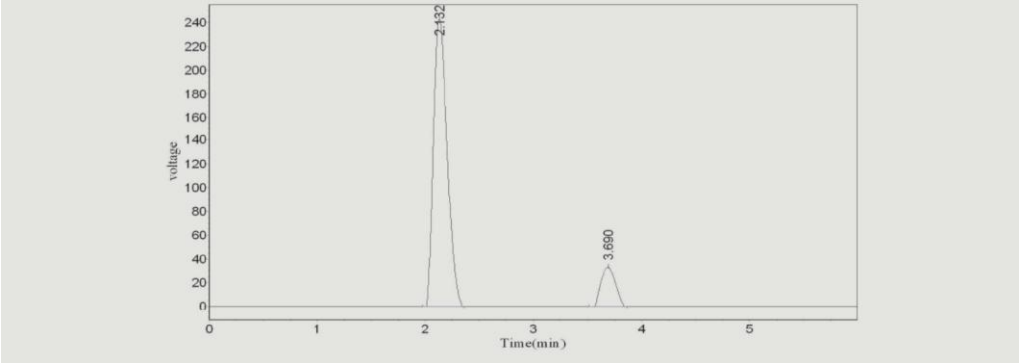


Figure- 4 Optimized chromatogram

Procedure for preparation of solution

Preparation of buffer

Take 1000ml of HPLC grade water. Dissolve 2.72 grams of Potassium di hydrogen phosphate salt and Adjusted the pH to 3.0 with orthophosphoric acid.

Preparation of mobile phase

A mixture of above prepared buffer 400 ml (40%), and 600 ml of HPLC grade Acetonitrile (60%) were mixed and degassed in ultrasonic water bath for 5 minutes. The mobile phase was filterred through 0.45 µ filter under vacuum.

Diluent Preparation

Use the Mobile phase as Diluent.

ASSAY

Preparation of the Atenolol and Indapamide standard & sample solution

Preparation of Standard Solution

Accurately weighed and transferred 58.8mg of atenolol and indapamide working standard into a 50ml clean dry volumetric flask and added about 30ml of diluent. It was sonicated to dissolve completely and made volume up to the mark with the same diluent. (Stock solution)

From the above stock solution, 10ml of the solution was pipetted into a 50 ml volumetric flask and diluted up to the mark with diluent. . From this, 3 ml of the solution was pipetted into another 10ml volumetric flask and diluted up to the mark with diluent..

Sample Solution Preparation

Accurately weighed and transferred 58.8 mg of Atenolol and Indapamide tablet powder into a 100ml clean dry volumetric flask and added about 70 ml of diluent. It was sonicated to dissolve it completely and made volume up to the mark with the same diluent. (Stock solution).

From the above stock solution, 3 ml of the solution was pipetted into a 10 ml volumetric flask and diluted up to the mark with diluent.

Procedure

20 µL of the standard and sample solutions were injected into the chromatographic system and areas for the Atenolol and Indapamide peaks were measured. %Assay was calculated by using the formulae.

Calculation

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.\ Wt}{Label\ Claim} \times 100$$

Where:

AT = Average area counts of sample preparation.
AS = Average area counts of standard preparation.
WS = Weight of working standard taken in mg.
P = Percentage purity of working standard
LC = LABEL CLAIM mg/ml.

ANALYTICAL METHOD VALIDATION

The HPLC method was validated in accordance with ICH guidelines.

Accuracy

Accuracy was carried out by % recovery studies at three different concentration levels. To the pre-analyzed sample solution of ATEN and INDA a known amount of standard drug powder of ATEN and INDA were added at 50, 100 and 150 % level.

Precision

The system precision of the method was verified by five replicate injections of standard solution containing ATEN and INDA. The method precision was carried out the analyte five times using the proposed method. Repeatability was measured by multiple injections of a homogenous sample of ATEN and INDA.

Linearity

The linearity was determined separately for ATEN and INDA. Linearity of the method was studied by injecting 5 concentrations of both drugs prepared in methanol and calibration curves were constructed by plotting peak area against the respective concentrations

Limit of detection and Limit of quantitation

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD = 3.3 x ASD/S and LOQ = 10 x ASD/S, Where, 'ASD' is the average standard deviation and 'S' is the slope of the line.

Robustness

Robustness was evaluated by making deliberate variations in few method parameters such as variation of wave length; flow rate and change in mobile phase composition. The robustness of the method was studied for ATEN and INDA.

RESULTS

Selection of Chromatographic Conditions and Optimization of Mobile Phase

Mobile phase was optimized to separate ATEN and INDA using Symmetry C8 column (150 mm x 4.6 mm i.d.,5 µm). Initially, ACN and phosphate buffer in the equal proportions were tried as mobile phase but the splitting of the peaks for both these drugs was observed. Therefore, after adjustment of pH of mixed phosphate buffer to 3.0 with ortho-phosphoric acid, and mobile phase composition (ACN and phosphate buffer in 40:60 % v/v*)* was tried for resolution of both drugs. Good resolution and symmetric peaks were obtained for both drugs when the pH of the mobile phase (buffer) was adjusted to 3.0. The flow rate of the mobile phase was 0.7 mL min-1. Under optimum chromatographic conditions, the retention time for ATEN and INDA was found to be 2.1 and 3.6 min, respectively when the detection was carried out at 240 nm

A typical chromatogram of two drugs is shown in (Figure -4).

Table-1 Accuracy data for Atenolol and Indapamide:

	Atenolol			**Indapamide**		
Injection	50%	100%	150%	50%	100%	150%
Inj-1	3058153	4141888	5193545	464090	614631	751157
Inj-2	3086875	4139942	5991256	460733	617416	764616
Inj-3	3039485	4128889	5134625	463249	602389	774998
AVG	3061504	4136906	5151290	462690.7	611478.7	763590.3
S.D	23872.09	7011.059	36865.34	1746.758	7994.097	11953.55
%R.S.D	0.78	0.17	0.72	0.38	1.31	1.57

Table-2 Accuracy(Recovery) result for Atenolol and Indapamide :

Drug Name	Spike level	Area	Amount Added(mg)	Amount Found(mg)	% Recovery	% of mean recovery
Atenolol	50%	3028171	90	89.8	99.23	100.74
	100%	4136906	120	121.2	102	
	150%	5151290	150	150.9	101	
Indapamide	50%	462690	4.50	4.524	101.33	99.93
	100%	611478	6	5.98	99.33	
	150%	763590	7.50	7.47	99.33	

Table-3 System Precision Result for Atenolol and Indapamide:

S.No	Injections	Area of Atenolol	Area of Indapamide
1	Injection-1	2015090	1030445
2	Injection-2	2100046	1028130
3	Injection-3	2065369	1001212
4	Injection-4	2096138	1017377
5	Injection-5	2103317	1031363
	Average	2075992	1021705.4
	Standard deviation	37259.27	12744.02
	%RSD	1.79	1.27

Table-4 Method precision (reproducibility) of Atenolol and Indapamide:

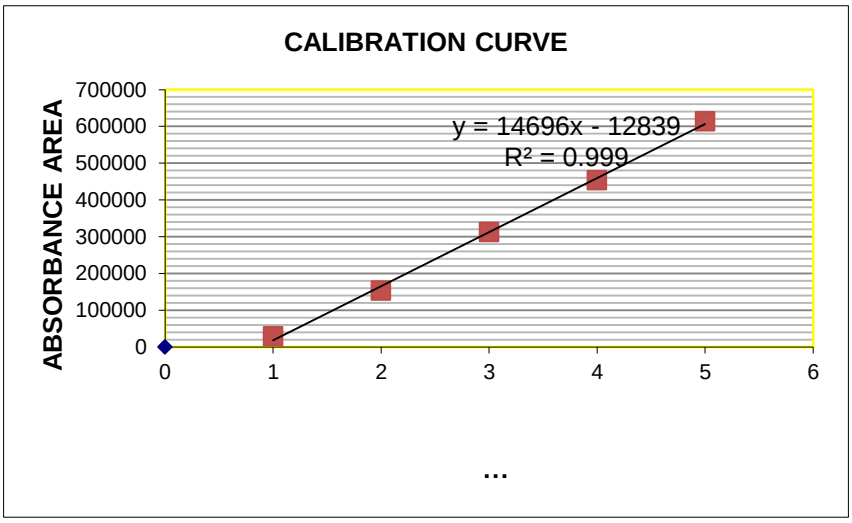
S.No	Injections	Area of Atenolol	Area of Indapamide
1	Injection-1	2038600	1086110
2	Injection-2	2069689	1066922
3	Injection-3	2086267	1095482
4	Injection-4	2147805	1085921
5	Injection-5	2075926	1083763
	Average	2083657.4	1083640
	Standard deviation	40021.19	10380.79
	%RSD	1.92	0.96

Table-5 LINEARITY RESULTS OF Atenolol and Indapamide:

ATENOLOL		INDAPAMIDE	
Conc(mcg/ml)	**Area**	**Conc(mcg/ml)**	**Area**
20	913226	1	29279
40	1482271	2	153131
60	2147805	3	312399
80	2747059	4	454118
100	3416501	5	613618

Figure-5: Linearity Graphs of Atenolol and Indapamide

Linearity graph of Atenolol



Linearity graph of Indapamide

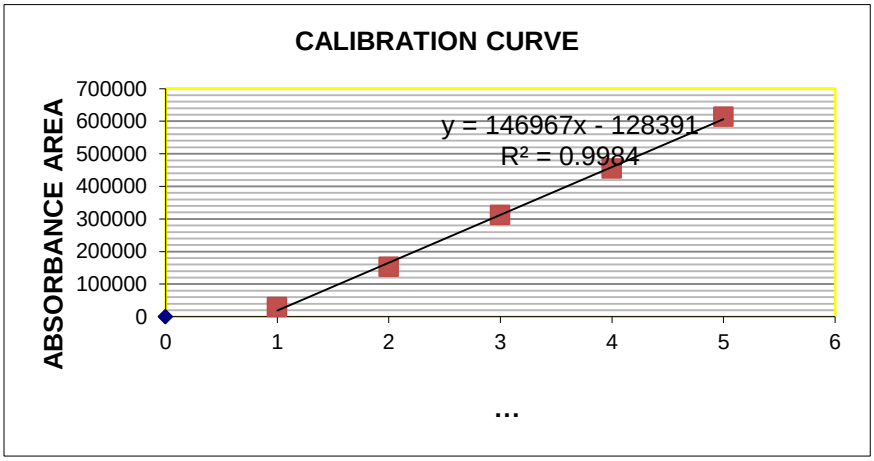


Table-6 Results of LOD and LOQ

S.No	Drug name	Standard deviation	Slope	LOD	LOQ
1	Atenolol	21246	313566	0.223595	0.677561
2	Indapamide	12744	146966	0.286155	0.867139

Table -7 Robustness Result For Atenolol and Indapamide At Different Condition

S.No	Parameter	Atenolol			Indapamide		
		Theoretical plates per column	Tailing factor	Resolution	Theoretical plates per column	Tailing factor	Resolution
1	Less flow(0.63ml/min)	1341	1.319	-	3057	1.143	5.291
2	Accuracy std flow rate(1.0 ml/min)	2215	1.265	-	2776	1.069	5.109
3	More flow(0.77 ml/min)	1170	1.357	-	2751	1.178	5.000
4	%10 Less organic	1271	1.348	-	3001	1.160	5.396
5	Accuracy std (100% organic)	2215	1.265	-	2776	1.069	5.109
6	%10 More organic	1188	1.304	-	2750	1.081	5.094

RESULTS AND DISCUSSION

Accuracy

The accuracy of the method studied at three different concentration levels i.e. 50 %, 100 % and 150 % showed acceptable % recoveries in the range of 100.74 % for atenolol and 99.93 % for indapamide.

Precision

The precision study of ATEN and INDA was evaluated on the basis of % RSD value was found to be in the range 0.7 – 1.8%respectively. As the RSD values were < 2% therefore developed method was precise.

Linearity

The linearity was determined separately for ATEN and INDA. Linearity of the method was studied by injecting five concentrations of both drugs prepared in methanol and calibration curves were constructed by plotting peak area against the respective concentrations. The ATEN and INDA followed linearity in the concentration range of 20-100 µg mL-1 and 1-5 µg mL-1; respectively.

Limit of detection and Limit of quantitation

The LOD was found to be 0.223595 and 0.286155 µg, respectively. The LOQ for ATEN and INDA was found to be and 0.677561 and 0.867139 µg, respectively. The low values of LOD and LOQ indicates high sensitivity of the method.

Robustness study

Robustness of the method was studied by making deliberate changes in the chromatographic conditions and the effects on the results were examined. The low

value changes of theoretical plates, tailing factor indicating robustness of the method. When the method was performed by two different analysts under the same experimental and environmental conditions it was found to be rugged and % RSD (less than 2 %) indicating ruggedness of the method.

Analysis of marketed tablet formulation

3 replicates of the samples solutions (20 µL) were injected for quantitative analysis. The amounts of ATEN and INDA estimated were found to 100.74 % and 99.93 %, respectively. A good separation and resolution of both drugs indicates that there was no interference from the excipients commonly present in pharmaceutical formulations.

System Suitability Test

The system suitability parameters such as resolution, number of theoretical plates and tailing factor were studied..

Table- 8 ASSAY RESULTS

Assay Results Drug	Amount present/tablet	% of Assay
Atenolol	25 mg	100.2
Indapamide	2.5mg	99.11

Table-9 System Suitability parameter

System suitability parameters	Atenolol	Indapamide
Retention time(min	2.1	3.6
Tailing factor	1.332	1.1405
Theoretical plates number	1242	2889
Resolution	-	5.195

CONCLUSION

The developed RP-HPLC method is simple, precise, accurate, selective and reproducible. The method has been found to be adequately rugged and robust and can be used for simultaneous determination of Atenolol and Indapamidel in tablet formulation. The method was validated as per ICH guidelines

ACKNOWLEDGEMENTS

I like thankful to Pharmatech research labotatories., Hyderabad, India for providing the gift samples of Atenolol and Indapamide. And also to the principal Dr.K.Rajeshwar Dutt, Smt. Sarojini Ramulamma College of Pharmacy, Mahabubnagar, Andhra pradesh, India and special thanks for Ms.K.Deepti madam & Ms.Ramathilagam madam as well as my friends who helped during the project work.

REFERENCES

[1] Indian pharmacopeia 2007.

[2] British pharmacopeia 2007 vol-I .

[3] Martindale the complete drug reference, thirty sixth edition.

[4] Merck index, 12th edition

[5] ICH, Q1A (R2) Stability testing of new drug substances and products, in: International Conference on Harmonization, IFPMA, Geneva, (2003).

[6] Practical HPLC method development Lloyd R.Snyder, Joseph J. Kirkland, Joseph L. Glajch, second edition

[7] http://www.rxlist.com/lozol-drug.htm

[8] MeSH *Indapamide*

[9] K. Basavaiah, U. Chandrashekar, *Bulgarian Chem. Comm.* **38(2)** (2006), 104-111.

[10] L. Dong, J. Huang, *Chromatographia* **64(9-10)** (2006), 583-586.

[11] R. Ceresole, M. A. Moyano, M. T. Pizzorno, A. I. Segall, *J. Liq. Chromatography Related Techno.* **29(20)** (2006), 3009-3019.

[12] L. Tong, Dan-dan Qi, Kai-shun Bi, Xiao-hui Chen, *Zhongguo Xinyao Yu Linchuang Zazhi* **25(11)** (2006), 851-855.

[13] A. Mohammadi, I. Haririan, N. Rezanour, L. Ghiasi, R. B. Walker, *J. Chromatogr.A* **1116** (2006) 153-157.

[14] A. Weich, D. Carvalho de Oliveira, J. de Melo, K. Goebel, C.M.B. Rolim, *Latin Am. J. Pharm.* **26(5)** (2007), 765-770).

[15] T. Jiang, W. Wang, X. Zhang, *Zhongguo Yaoshi (Wuhan, China)* **11(9)** (2008), 1068-1069.

[16] S. Saraf, S. Saraf, G. Garg, *Indian Pat.* Appl. IN 2007MU00559 A 20081121, (2008).

[17] Yan-lin Zhu, Jing Zhang, Dan-bi Tian, *Huaxue Shiji* **30(7)** (2008), 512-514.

[18] L. Li, X. Zhang, *Yiyao Daobao* **27(6)** (2008), 714.

[19] Y. Tang, L.Gu, *Zhongnan Yaoxue* **7(5)** (2009), 341-343

[20] M. J. Legorburu, R. M. Alonso, R. M. Jimenez, E. Ortiz, *J. Chromatographic Sci.* **37(8)** (1999), 283-287.

[21] K. Abdussaleem, D. Boopathy, P. Perumal, *Int. J. PharmTech Res.* **2(1)** (2010), 471-474.

[22] A. B. Thomas, U. B. Chavan, R. K. Nanda, L. P. Kothapalli, S. N. Jagdale, S. B. Dighe, A. D. Deshpande, *Acta Chromatographica* **22(2)** (2010), 219-226.

[23] H. Jogia, U. Khandelwal, T. Gandhi, S. Singh, D. Modi, *J. AOAC Int.* **93(1)** (2010), 108-115.

****