International Journal for Advanced Review and Research in Pharmacy (IJARRP)

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

K.Madhavi*, B.Harika & K.Deepti
M.Pharmacy, Department of Pharmaceutical Analysis & Quality Assurance, Smt. Sarojini Ramulamma College of Pharmacy, Mahabubnagar(509001), A.P. INDIA
corresponding author email id: madhavi.kori @gmail.com

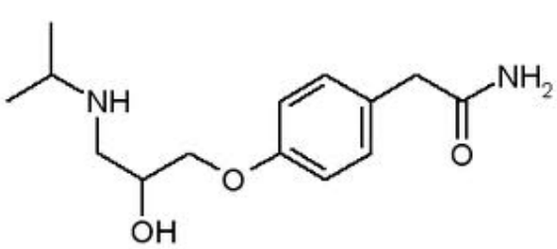
ABSTRACT
A simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for simultaneous determination of Atenolol and Indapamide in pharmaceutical tablet dosage form. Chromatographic analysis was performed on a Symmetry X-terra C8 (4.6mm x 150mm, 5µm)column at ambient temperature with a mixture of mixed Potassium di hydrogen phosphate and Acetonitrile in the ratio 40:60 v/v (Potassium di hydrogen phosphate preparation; 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. as mobile phase, at a flow rate of 0.7 mL min-1. UV detection was performed at 240 nm. The method was validated for accuracy, precision, linearity and sensitivity. The retention times of Atenolol and Indapamide were 2.1 and 3.6 min, respectively.
Calibration plots were linear over the concentration ranges 20-100µg mL-1 and 1-5 µg mL-1 for Atenolol and Indapamide, respectively. The Limit of detection was 0.223 and 0.286µg mL-1 and the quantification limit was 0.677 µg mL-1 and 0.867µg 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%. 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.

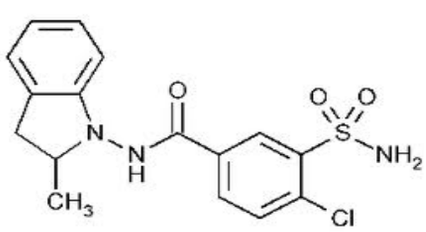
INTRODUCTION:

Atenolol is chemically (RS)-2-{4-[2-hydroxy-3-(propan-2-ylamino)propoxy]phenyl}acetamide [Figure 1] and its molecular formula is C14H22N2O3 hypertension, and molecularweight is 266.34 gm/mole. Atenolol can be used to treat cardiovascular diseases andconditions such as , coronary heart disease, arrhythmias, angina and to treat and reduce the risk of heart complications following myocardial infarction.

Fig No. 1 Structure of Atenolol & Indapamide



Atenolol



Indapamide

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.

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

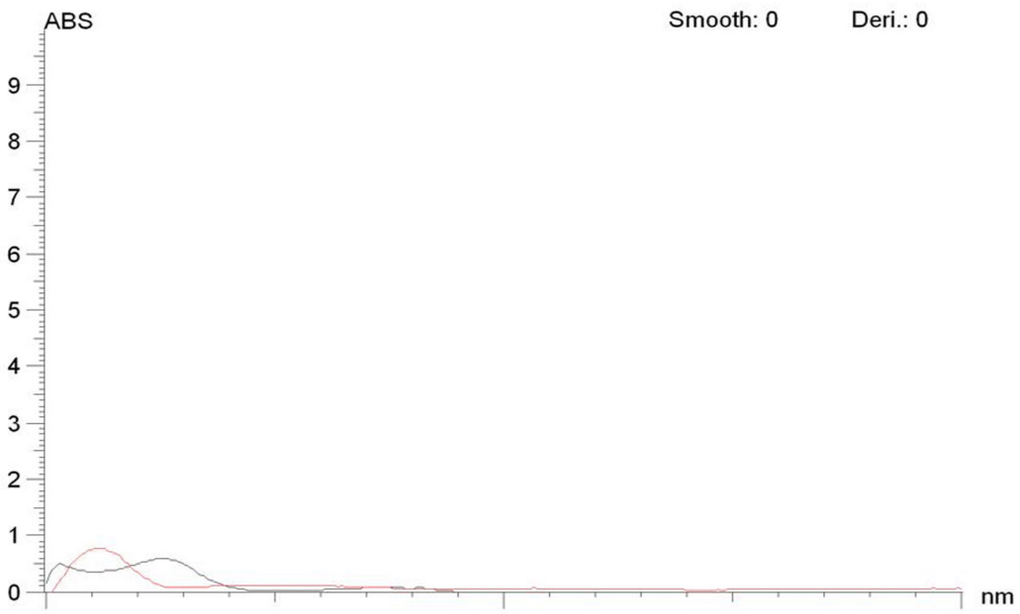
high pressure liquid chromatograph outfitted with 515 reciprocating dual column HPLC pump, a manually operating Rheodyne injector with 20µL sample loop, X-terra C8 4.6mm x 150mm analytical column reversed-phase material of 5µ size and a 2487 model UV-Visible detector. All the parameters of HPLC were 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 C8 (4.6mm x 150mm, 5µm) was used for chromatographic separation . The mobile phase composed of pH3Buffer(Orthophosphoricacid):Acetonitrile (40:60) at flow rate 0.7 mL/min with run time 5mins. Mobile phase and sample solution were filtered through a 0.45µm membrane filter and degassed.The detection of both drugs ws carried out at 240nm.

Figure-1.Isobestic point of Atenolol and Indapamide



Optimized Method Parameters

MobilePhase : Phosphate buffer (3.0 pH): Acetonitrile(40:60)

Column (Stationary Phase): X-terra(C8) (4.6mm x 150mm, 5µm)

Flow rate (ml/min) 0.7:

Column temperature (°C): Ambient

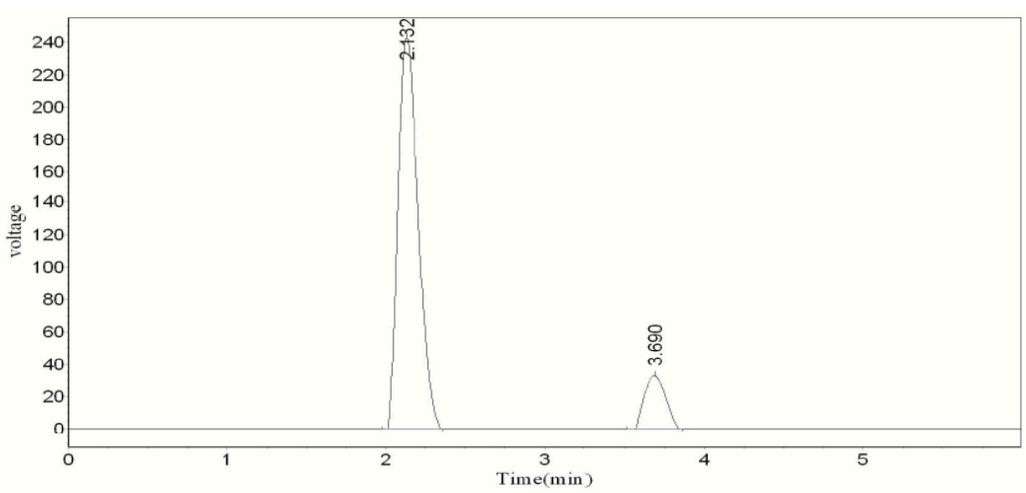
Volume of injection loop (µl): 20

Detection wavelength (nm):240

Drug RT (min): Atenolol – 2.1

Indapamide – 3.6

Figure no.3 optimized chromatogram



Results

Peak No.	Peak ID	Ret Time	Height	Area	Conc.
1	Atenolol	2.132	242462.563	2131048.500	0.0000
2	Indapamide	3.690	33008.902	304502.406	0.0000
Total			275471.465	2435550.906	0.0000

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 Mobile phase Diluent Phase

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 a solution was pipetted into a 5 0ml 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:

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

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 = Lable 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:

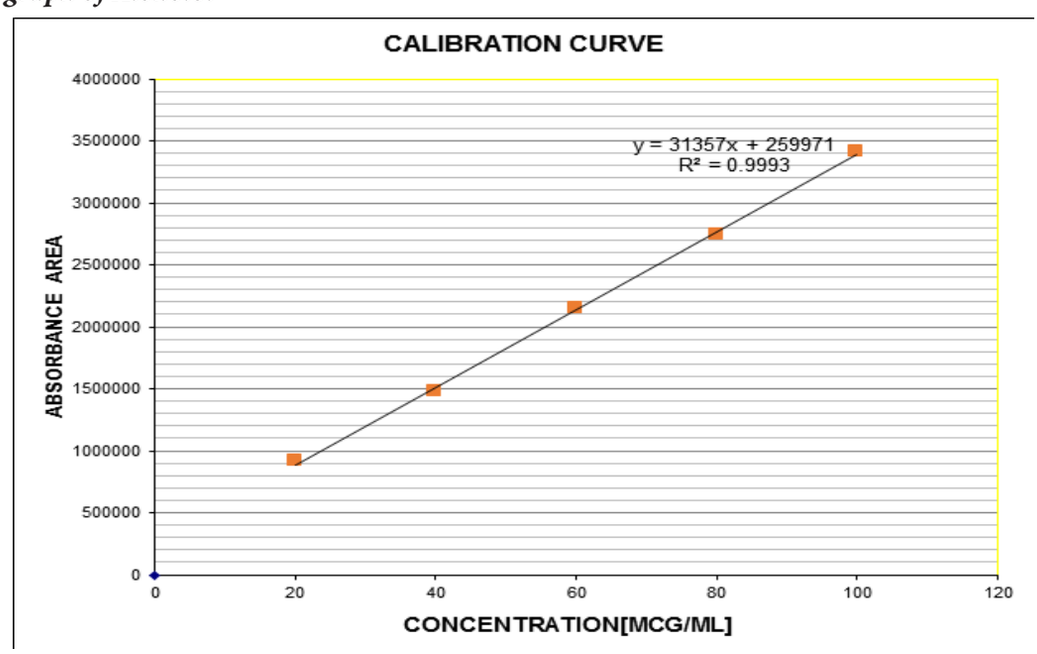
Mobile phase was optimized to separate ATEN and INDA using Symmetry C8 column (150 mm x 4.6

mm i.d., 5µm). 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 3).

Table 1: 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

Linearity graph of Atenolol



Linearity graph of Indapamide

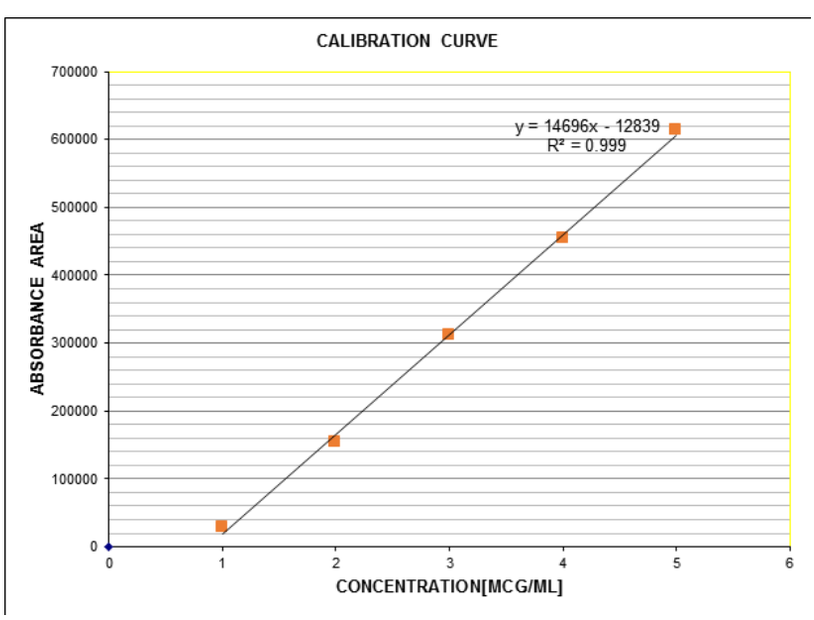


Table2: PRECISION

	ATENOLOL		INDAPAMIDE	
S.NO	RT	AREA	RT	AREA
1	2.132	2015090	3.673	1030445
2	2.132	2100046	3.673	1028130
3	2.107	2065369	3.648	1001212
4	2.132	2096138	3.673	1017377
5	2.132	2103317	3.673	1031363
Average		2075992		1021705.4
Standard Deviation		37259.27		12744.02
% RSD		1.79		1.27

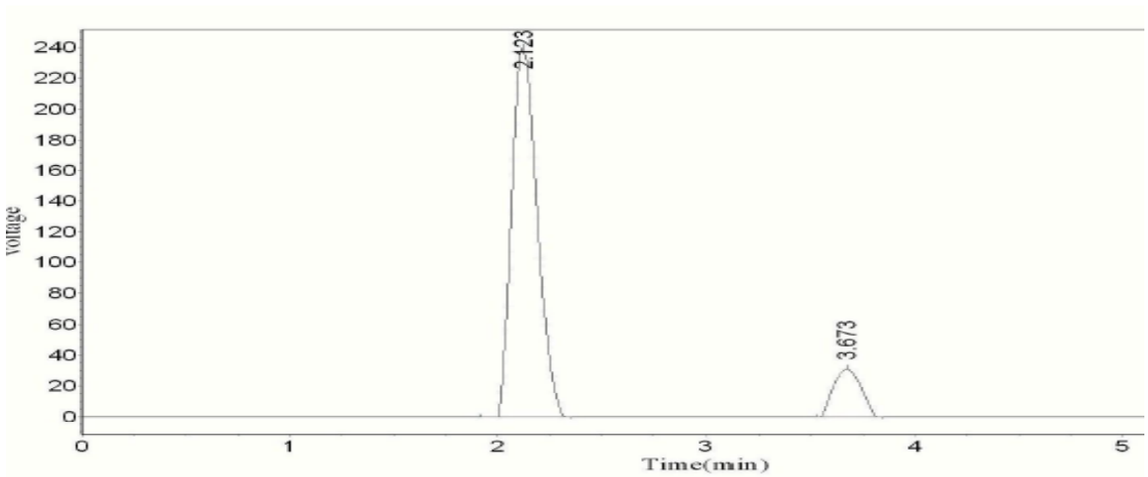
Table3: INTERMEDIATE PRECISION

	ATENOLOL		INDAPAMIDE	
S.NO	RT	AREA	RT	AREA
1	2.123	2038600	3.665	1086110
2	2.140	2069689	3.682	1066922
3	2.123	2086267	3.665	1095482
4	2.123	2147805	3.665	1085921
5	2.107	2075926	3.657	1083763
Average		2083657.4		1083640
Standard Deviation		40021.19		10380.79
% RSD		1.92		0.96

Table-4: ACCURACY DATA

Drugs	*Amount Added (mg)*	*Amount Found (mg)*	*% Recovery*	*% of mean recovery*
Atenolol	90	89.8	99.23	100.74 %
	120	121.2	102	
	150	150.9	101	
Indapamide	4.50	4.524	101.33	99.93%
	6	5.98	99.33	
	7.50	7.47	99.33	

Figure no.4



Results

Peak No.	Peak ID	Ret Time	Height	Area	Conc.
1	Atenolol	2.123	239071.234	2042942.250	0.0000
2	Indapamide	3.673	30869.615	306592.938	0.0000
Total			269940.850	2329535.188	0.0000

System Evaluation

Peak No.	Peak ID	Ret. Time	Half-Peak Width	Theoretical levels	Resolution	Tail Factor	Asymmetry
1	Atenolol	2.123	0.137	2337.275	0.000	1.273	1.450
2	Indapamide	3.673	0.160	2920.052	5.225	1.070	1.132

Table No.5.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(0.8ml/min)	1341	1.319	-	3057	1.143	5.291
2	More flow(0.98 ml/min)	1170	1.357	-	2751	1.178	5.000
3	%10 Less organic	1271	1.348	-	3001	1.160	5.396
4	%10 More organic	1188	1.304	-	2750	1.081	5.094

Table 6: LOD AND LOQ RESULTS

S.No	Drug name	Standard deviation	Slope	LOD	LOQ
1	Atenolol	21246	313566	0.223595	0.677561
2	Indapamide	12744	146966	0.286155	0.86713

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% ATEN and 99.93% INDA. The results are shown in Table 1.

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. Results of precision study are shown in Table 2 & 3

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. The results are shown in Table 4..

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. The results are shown in Table 6.

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. The results are shown in Table 5.

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. The results are shown in Table 7.

System Suitability Test:
The system suitability parameters such as resolution, number of theoretical plates and tailing factor were studied and were summarized in Table 8.

Table 7: ASSAY RESULTS

Assay Results Drug	Amount present/tablet	% of Assay
Atenolol	50mg	100.2
Indapamide	2.5mg	99.11

Table 8: SYSTEM SUITABILITY PARAMETERS

System suitability parameters	Atenolol	Indapamide
Tailing Factor	1.332	1.1405
Theoretical plates	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.

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. 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.