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To Develop Reverse Phase High Performance Liquid Chromatography Method for Simultaneous Estimation of Atenolol and Indapamide in Pharmaceutical Formulation

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Conflicts of Interest: Nil

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ABSTRACT

An economical RP-HPLC method were developed and validated for the simultaneous estimation of Atenolol (ATL) and Indapamide (INDP) in combined tablet dosage form which has been accurate, precise and simple RP-HPLC methods. The RP-HPLC method uses a Shimadzu LC 10 AT_{VP} system with Luna C_{18} column and Methanol: Acetonitrile and Water (pH 3.5 adjusted with orthophsophoric acid) in the ratio of 45:25:30 (v/v) as the mobile phase. The estimation was performed at 226 nm using a diode array detector as all the two analyte absorbed well at this wavelength. The flow rate was maintained at 1.2 ml min^{-1}. Linearity of chromatographic method was found in the concentration range of 2-10 and 5–25 µg/mL for ATL and INDP respectively. The recoveries were in the range of 99.75 ± 0.0642% and for ATL, 99.71 ± 0.189 % for INDP in HPLC methods respectively. The method was linear in concentration ranges were found to be within the limit and result of accuracy was found to be between 98 to 101%. The method was precise as the % RSD was found that NMT 2.0% and specificity studies reveal no interference in the standard peaks due to placebo. The developed precise method was validated in respect to linearity, range, precision, accuracy, robustness.

Keywords: linearity, validation, Accuracy, Precision

Introduction

Atenolol 2-[4-[2-hydroxy-3-(propan-2-ylamino) propoxy] phenyl] acetamide is an anti hypertensive drug used in treating hypertension competes with sympathomimetic neurotransmitters such as catecholamines for binding at beta(1)-adrenergic receptors in the heart and vascular smooth muscle, inhibiting sympathetic stimulation. This results in a reduction in resting heart rate, cardiac output, systolic and diastolic blood pressure, and reflex orthostatic hypotension. Higher doses of atenolol also competitively block beta(2)-adrenergic responses in the bronchial and vascular smooth muscles.

Indapamide 4-chloro-N-(2-methyl-2,3-dihydroindol-1-yl)-3-sulfamoylbenzamide is an Antihypertensive Agents, diuretics enhances the excretion of sodium, chloride, and water by inhibiting the transport of sodium ions across the renal tubule. The hypovolemic action of indapamide is believed to be responsible for the drug's beneficial cardiovascular effects. Decreased plasma and extracellular fluid volume, along with a decreased peripheral vascular resistance (secondary to loss of

sodium, or to vascular autoregulatory feedback systems), act to lower blood pressure in hypertensive patients who are receiving indapamide. The drug may also produce calcium-channel blockade in smooth muscle cells, thereby causing arteriolar vasodilation.

Literature survey revealed that several analytical methods have been reported for the determination of ATL developed a simple, rapid and precise method for the quantitative simultaneous determination atenolol and amlodipine in combined pharmaceutical doses fom [Barman, B., et al. (2001)] and Developed an accurate, precise an sensitive HPLC assay for the determination of atenolol in human plasma sample to compare the biobility of two atenolol tablet (50mg) formulation in 24 volunteers of both sexes Abreu, [L.R.P.D., et al. (2003)]. Similarly several analytical methods have been reported for the determination of indapamide, in pure form using liquid chromatography (LC). The LC separation achieved on a inertsil ODS 3v, 5 micro in the isocratic mode using mixture of 7v of acetonitrile, 20v of tetra hydro furan and 73v of 1.5g/l solution of triethyle amine adjusted ph 2.8 with ortho phosphoric acid at a flow rate of 1.4 ml/min Barot, T., G., et al. (2009) and indapamide, an indonine derivative of chlorosulfonamide, Since indapamide partition into red blood cell, a whole blood assay in required quantitave analysis and there for hplc/ms/ms method to quantitative inadapamide in human whole blood was developed and validated method clopomide was selected as an internal standard [Foltea.,M.,et al].

This paper describes simple, accurate, precise, and sensitive reversed-phase (RP)-HPLC methods for simultaneous determination of ATL and INDP in a combined tablet dosage form. The proposed methods were optimized and validated according to International Conference on Harmonization (ICH) guidelines**.**

Materials and Methods

Drugs and chemicals

The gift sample of the Atenolol and Indapamide were obtained from Alfa pharmaceutical ltd pigdumber, Rau (M.P.) and Zydus pharmaceutical Ahamdabad (Gujrat) respectively. The tablet dosage dosage form Aten-D Manufactured by Zydus pharmaceuticals ltd. Ahamadabad(Gujrat), India (Label claim: 50mg Atenolol,2.5mg Indapamide).

Instruments

A HPLC system consisting of LC 10 AT_{VP} pump equipped with diode array detector (Shimadzu, Japan) and Luna C_{18} (4.6 mm id) column and class M10A software was used for chromatographic determination. A Rheodyne (Rohnert Park, CA) injector with 20 µL loop was used for injecting the sample.

Method: RP-HPLC method

From the study it was found that best result was obtained in a quality separation in terms of peak symmetry, resolution, reasonable run time and other parameters by use of 45:25:30 (v/v) ratio mixture of methanol, acetonitrile and water (pH 3.5 adjusted with orthophosphoric acid) as mobile phase.

(a) **Standard stock solutions:** 10.05 mg of Atenolol and 10.10mg Indapamide were accurately weighted and dissolved in 10 ml of mobile phase to get solution of 1000mg/ml.

(b) **Preparation of standard solutions for linearity study:** Form the standard stock solutions of 1000 mg/ml different dilutions were prepared for each drug having concentration as shown in Table 1 and Table 2 with mobile phase.

(C) **Assay of mixed standard:** Content of these drugs in tablet dosage form were estimated by RP-HPLC using Methanol: Acetonitrile and Water (pH 3.5 adjusted with orthophsophoric acid) in the ratio of 45:25:30 (v/v) The estimation was performed at 226nm as all the two analyte absorbed well at this wavelength. The flow rate was maintained at 1.2ml min^{-1}

(D) **Validation of method:** The developed method was validated for its accuracy, precision, detection limit, specificity and selectivity.

(E) **Accuracy study**: Accuracy was confirmed by doing recovery study as per ICH norms,

where to a preanalysed sample solution standard solutions of drugs were added equivalent to 80,100 and 120% of its drug content

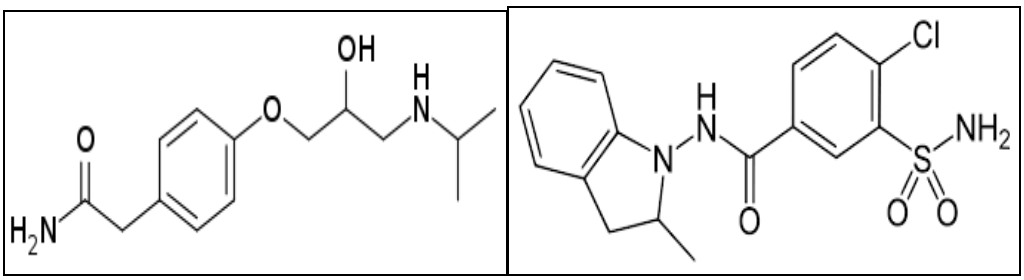
(F) **Precision study:** In intra-day study the sample solutions were analysed on the same day at an interval of 1 hour for 3 hours and measured the total drug content in it. From the result it was observed that Atenolol has drug content of 10.15, 10.13, 10.23 Indapamide has of 30.19, 30.11 and 30.21 in first, second and third hour respectively. The accuracy was expressed as relative error and precision was expressed as the % COV. Here the accuracy ranges from 0.020-0.031 and 0.0188- 0.034 but precision ranges from 0.48 - 0.83 and 0.15 - 0.28 for Atenolol and Indapamide respectively. Both intra and inter day accuracy were within acceptability criteria for relative error, ± 5 % and for precision study the % COV was within acceptability criteria i.e. < 2 indicating the method was précised for quantitative estimation of both drugs.

(G) **LOD and LOQ study:** To find the detection limit of the drugs LOD and LOQ studies were performed. The LOD values were 0.49 and .042 ng mL^{-1} and LOQ values were 1.504 and 3.15ng mL'1 for Atenolol and Indapamide respectively.

(E) **Statistical analysis:** Means, standard deviation (SD), Relative standard deviation (RSD), and linear regression analyses were calculated using Microsoft Excel 2003

Results and Disscusion

In the present work method development for simultaneous estimation of Atenolol and Indapamide in solid tablet dosage form in RP-HPLC were performed by using Methanol:Acetonitrile and Water (pH 3.5 adjusted with orthophsophoric acid) as mobile phase in the ratio of 45:25:30 (v/v) at a flow rate of 1.2 ml min^{-1}. In both the cases the developed method was validated by performing its accuracy, precision, detection limit study, and selectivity and specificity study



Atenolol **Indapamide**

Figure 1: Chemical structures of ATL and INDP Figure 2: Chromatogram of ATL and INDP in tablet dosage form

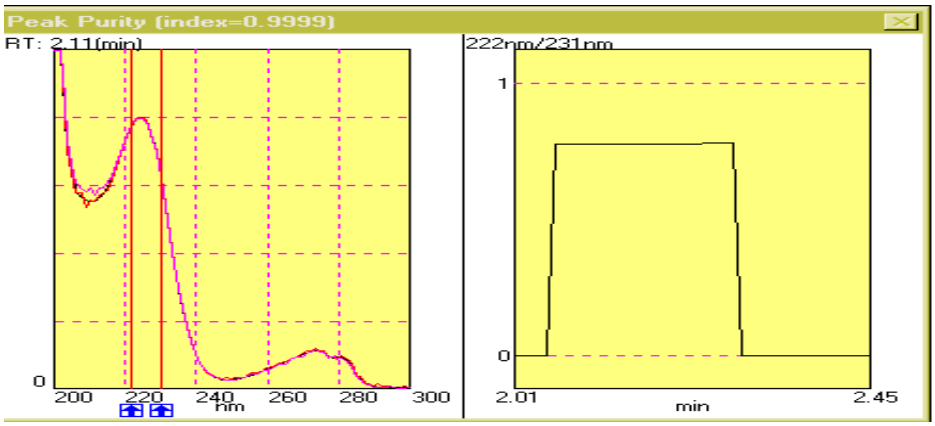


Figure 2: Peak Purity Curve of Atenolol

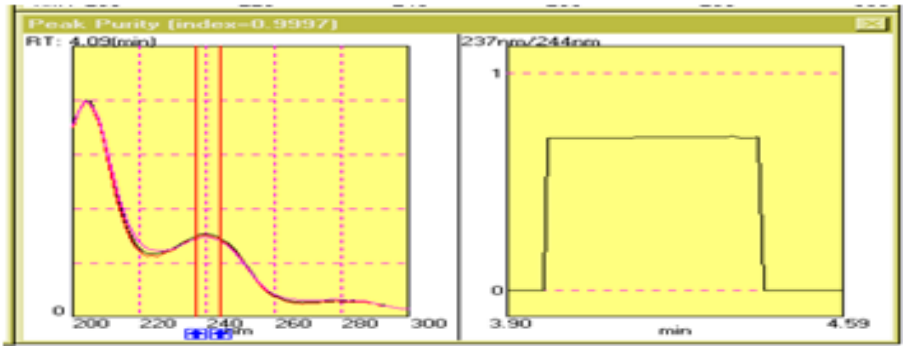


Figure 3: Peak Purity Curve of Indapamide

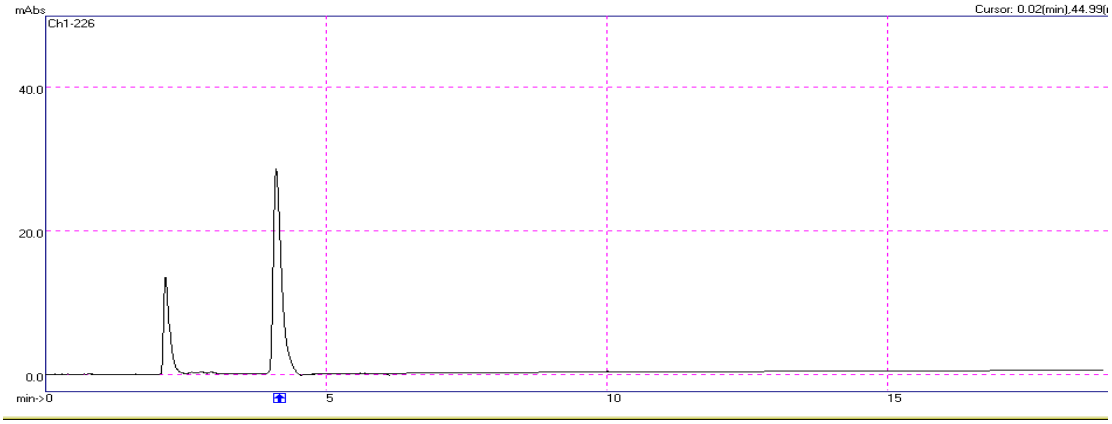


Figure 4: Peak of Atenolol and Indapamide

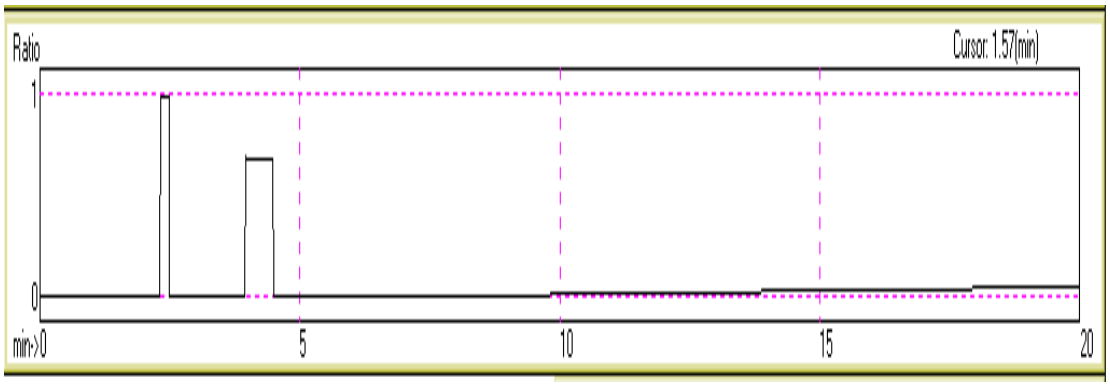


Figure 5: Ratio Chromatogram of Atenolol and Indapamide

Table 1: Analysis of mixed standard

S.No.	Atenolol			Indapamide		
	A.P.	A.F.		A.P.	A.F.	
		(µg/ml)	(%)		(µg/ml)	(%)
1	2	1.90	95	5	4.95	99.00
2	4	4.18	104.5	10	9.81	98.1
3	6	6.28	104.66	15	14.96	99.73
4	8	8.03	100.37	20	20.80	104.0
5	10	9.78	97.8	25	24.46	97.84
Mean	-	-	100.4	-	-	99.73
S.D.	-	-	1.80	-	-	2.1

A.P. Amount present; A.F: Amount found

Table 2: Analysis of Commercial Formulation for RP-HPLC method

S. No.	Atenolol			Indapamide		
	Concentration Present	Concentration Found		Concentration Present	Concentration Found	
		(µg/ml)	(%)		(µg/ml)	(%)
1	20	20.03	100.15	1	1.01	101
2	20	19.98	99.9	1	1.02	102
3	20	19.89	99.45	1	1.01	101
4	20	20.01	100.05	1	0.99	99
5	20	19.96	99.8	1	0.98	98
Mean	-	-	99.68	-	-	100.2
S.D.	-	-	0.517	-	-	1.64
RSD	-	-	0.005	-	-	1.63

Table 3: Recovery study

S. No.	Concentration in mcg/ml		Concentration added in mcg/ml			% Recovery Mean± SD	
	Atenolol	Indapamide	%	Atenolol	Indapamide	Atenolol	Indapamide
Dilution 1	20	1	80	16	0.8	99.75 ± 0.0642	99.71± 0.189
Dilution 2	20	1		16	0.8		
Dilution 3	20	1		16	0.8		
Dilution 1	20	1	100	20	1	100.02± 0.275	100.4 ± 0.669
Dilution 2	20	1		20	1		
Dilution 3	20	1		20	1		
Dilution 1	20	1	120	24	1.2	100.6± 1.00	99.83± 0.354
Dilution 2	20	1		24	1.2		
Dilution 3	20	1		24	1.2		

Table 4: Result of statistical validation of recovery study

%	Drug	Mean± SD	RSD
80	Atenolol	99.75 ± 0.0642	0.0006
	Indapamide	99.71 ± 0.189	0.0018
100	Atenolol	100.02 ± 0.275	0.0027
	Indapamide	100.42 ± 0.669	0.0066
120	Atenolol	100.6 ± 1.00	0.0010
	Indapamide	99.83 ± 0.354	0.0035

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Conclusions

The proposed RP-HPLC methods were found to be simple, fast, accurate, precise, and sensitive. Thus, it may be used for routine analysis of ATL and INDP in combined tablet dosage form.

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