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Research Article

Development and validation of RP-HPLC method for simultaneous estimation of Atenolol and Indapamide in combined dosage form

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In the present study a simple, sensitive, accurate and effective Reverse Phase High-Performance liquid chromatographic (RP- HPLC) method was developed for the determination of Atenolol and Indapamide simultaneously in the tablets. The analysis was resolved by using different chromatographic conditions by altering mobile phase and flow rate mechanism and HPLC system consisting of Inertsil ODS (C18, 150*4.6mm), 5nm column at a wavelength of 242 nm. The retention time for the drugs was found for atenolol 2.2 min and for indapamide 4.3 min respectively.

Keywords: HPLC, Atenolol, Indapamide, Zero order, simultaneous estimation.

INTRODUCTION

Atenolol [(4-2 – hydroxy-3 – isopropyl - aminopropoxy) phenylacetamide](Figure 1), is a cardioselective β-blocker. It is reported to lack intrinsic sympathomimetic activity and membrane-stabilising properties. It may be used alone or concomitantly with other antihypertensive agents including thiazide-type diuretics, hydralazine, prazosin and α-methyldopa Besides being one of the most widely used b-blockers clinically, it has often been used as a reference drug in randomized controlled trials of hypertension The elimination half-life of atenolol is 6 to 7 hours and there is no alteration of kinetic profile of drug by chronic administration. Following intravenous administration peak plasma levels are reached within 5 minutes. Declines from peak levels are rapid (5 to 10 fold) during the first 7 hours. Following oral doses of 50 mg or 100 mg both b-blocking and anti-hypertensive effects persist for at least 24 hours. The drug accumulates in patients with renal failure and dosage should be adjusted for patients whose creatinine clearance is less than 35 mL/min/1.73m^2.[1]

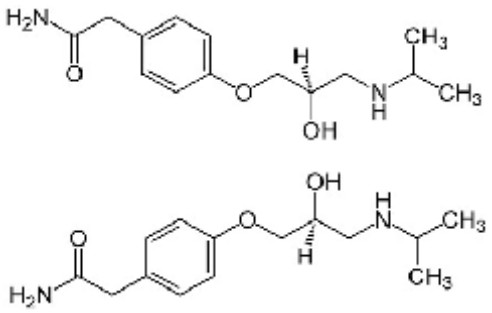

Fig. 1: Structure of Atenolol

Indapamide (thiazide-type diuretics) is

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Indoline derivatives of chlorosulphon-amide (4-Chloro-N-(2-methyl-1-indolinyl) -3- sulfamoyl-benzamide) (Fig. 2). It differs chemically from thiazides and contains only one sulphonamide group and no thiazide ring. Indapamide is an anti-hypertensive diuretic related to the thiazides.

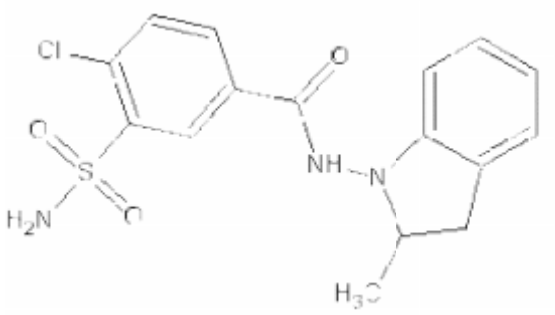

Fig. 2: Structure of Indapamide

The anti-hypertensive effect is associated with an improvement in arterial compliance and a reduction in total and arteriolar peripheral resistance. Indapamide as a first step antihypertensive, has two properties beyond diuresis. First, there is added vasodilation A second unusual property is a high concentration class I and III antiarrhythmic effect Indapamide has a terminal half-life of 14 to 16 hours and effectively lowers the blood pressure over 24 hours. The initial dose is 1.25 mg once daily for 4 weeks, then if needed 2.5 mg daily. Indapamide appears to be more lipid neutral than other thiazides but seems equally likely to cause other metabolic problems such as hypokalemia, hyperglycemia or hyperuricemia. Indapamide (2.5 mg daily) does not adversely affect serum triglycerides, LDL cholesterol, the LDL-HDL cholesterol ratio, or glucose tolerance.[2]

Atenolol and Indapamide combination Tablet was introduced in market for reducing the hypertension by their synergistic effect. Literature survey reveals that many analytical methods are reported for determination of Atenolol [3-6] and Indapamide[7-10] individually or with other combinations.

MATERIALS AND METHODS

Atenolol and Indapamide were obtained from Cadila, Mumbai, India. PVK K-30, a grade of HPMC, was procured from Colorcon Asia Pvt. Ltd., Mumbai. Microcrystalline cellulose (MCC) and AerosilR200 were purchased from Coveral and Company, Chennai. Materials and excipients used in preparing tablets were IP grades. All other ingredients used throughout the study were of analytical grade and The HPLC analysis was performed on reversed-phase high performance liquid chromatographic system with isocratic elution mode using different chromatographic conditions by altering mobile phase and flow rate onsisting of Inertsil ODS (C18, 150*4.6mm), 5nm column at a wavelength of 242 nm using UV detector. Were done from S.G.R.R.I.T.S. Dehradun, Uttarakhand, India.

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Calibration curves for Atenolol and Indapamide: 10 mg of drugs were weighed and transferred to 100 ml volumetric flask. Assed 20 ml of methanol to dissolve and made up the volume to 100 ml with methanol. Pipetted 10 ml of this solution to 100 ml of volumetric flask and made up the volume to 100 ml with methanol. (10µg/ml)

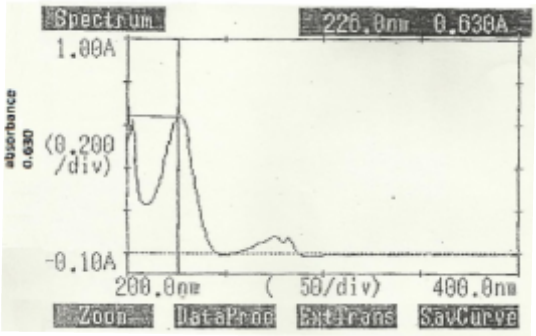

Fig.3:Absorption maxima of indapamide (226 nm)

Measure the absorbance of the solution at 200-400 nm. Results are shown in fig. 3-4

Method Validation

The method of analysis was validated as per the recommendations of ICH [11]for the

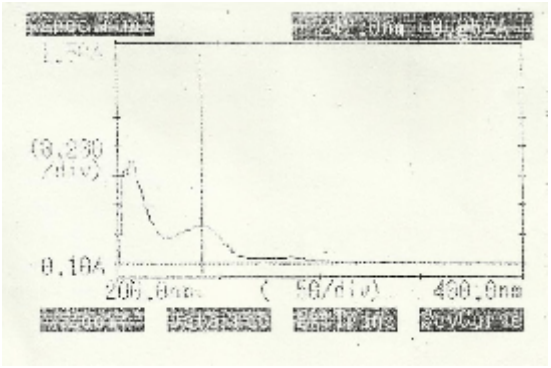

Fig.4: Absorption maxima of atenolol (242 nm)

parameters like accuracy, linearity, precision, and robustness. The accuracy of the method was determined by calculating percentage recovery of Atenolol and Indapamide. For both the drugs, recovery studies were carried out by applying the method to drug sample to which known amount of Atenolol and Indapamide corresponding to 50, to 50, 100 and 120% of label claim had been added (standard addition method).

At each level of the amount 3 determinations were performed and the results obtained were compared. Intraday

Table 1: Various chromatographic conditions of method development for assay

Parameters	**Chromatographic Conditions** **Column name-:** Inertsil ODS(C18,150*4.6mm),5nm						
	Cond.1	**Cond.2**	**Cond.3**	**Cond.4**	**Cond.5**	**Cond.6**	**Cond.7**
Detector	242nm	242nm	242nm	242nm	242nm	242nm	242nm
Flow rate	1.0ml/min	1.0ml/min	1.0ml/min	1.0ml/min	1.0ml/min	**1.5 ml/min**	**1.5 ml/min**
Injection volume	10µL	10µL	10µL	10µL	10µL	10µL	10µL
Temp.	30° C	30° C	30° C	30° C	30° C	30° C	30° C
Mobile phase	Water : acetonitrile (80:20)	Water : methanol (80:20)	Water : methanol (70:30)	Buffer : methanol (70:30)	Buffer : methanol (60:40)	Buffer : methanol (60:40)	Buffer : methanol (50:50)
Run time	12min	12min	12min	12min	12min	12min	12min

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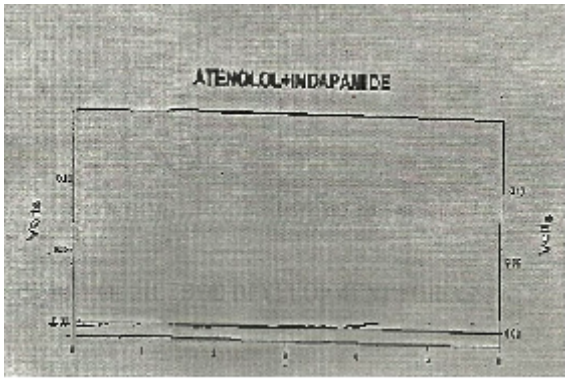

Fig.5: No peak was found in diluents (**Results for diluents (mobile phase)**

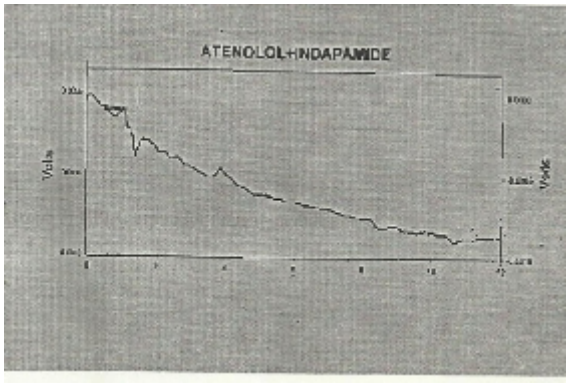

Fig. 6: No peak with the mobile phase was water and acetonitrile (**chromatographic condition 1)**

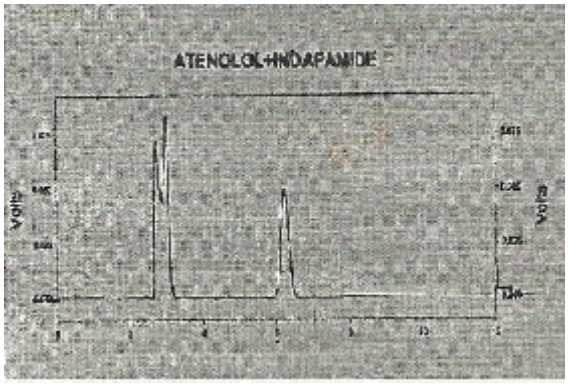

Fig.7: Peak was separated but the first peak showed the merging of two peaks. The mobile phase was water and methanol in the ratio of 80: 20(**Chromatographic condition 2)**

and interday precision study of Atenolol and Indapamide was carried out by estimating the corresponding responses 3 times on the same day and on 3 different days for the concentration of 500_g/ml and 25_g/ml of Atenolol and Indapamide, respectively. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated using following formulae: LOD= 3.3(SD)/S and LOQ= 10 (SD)/S,where SD=standard deviation of response (peak area) and S= average of the slope of the calibration curve.

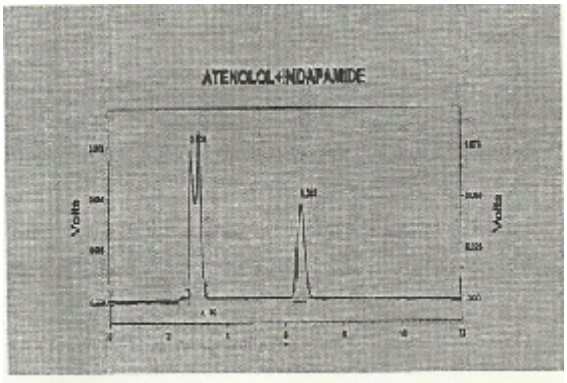

Fig. 8 : Changed ratio of mobile phase. The merging of first peak was found (**Chromatographic condition 3)**

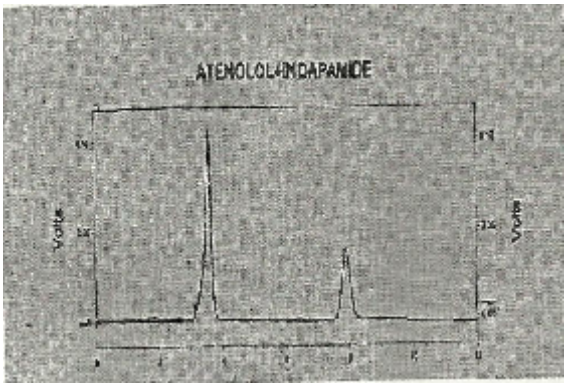

Fig. 9: In this case the buffer and methanol was used as mobile phase. The octane sulfonic acid was used as ion pair chromatographic reagent. Here the peaks were separated but fronting was observed in the first peak (**Chromatographic condition 4)**

System suitability tests are an integral part of chromatographic method which is used

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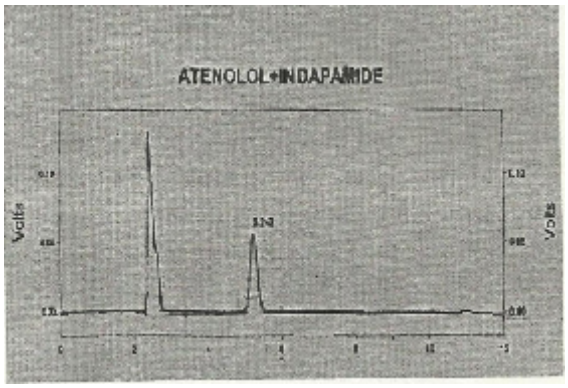

Fig.10: In this ratio of mobile phase was changed, ration of buffer and methanol was 60:40. tailing peak was found (**Chromatographic condition 5)**

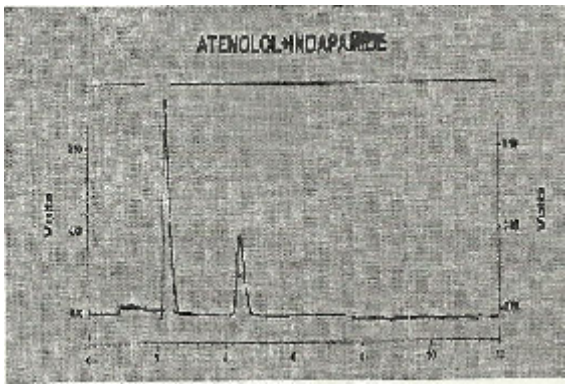

Fig.11:. Here the flow rate was increased to 1.5 ml/min. but problem in first In first peak there was tailing was observed in that peak (**Chromatographic condition 6)**

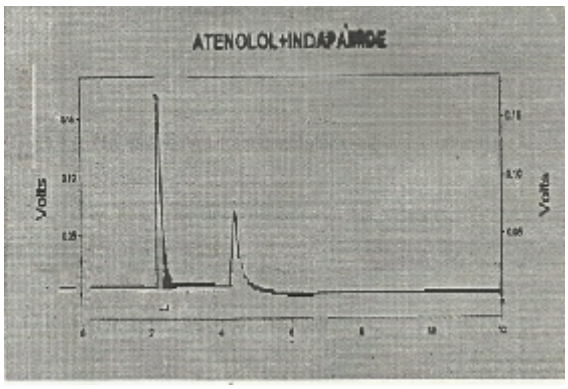

Fig.12: Here in this condition the peak were separated. The ratio of mobile phase was 50:50. The flow rate is1.5ml/min (**Chromatographic condition 7)**

to verify reproducibility of the chromatographic system.

To ascertain its effectiveness, certain system suitability test parameters were checked by repetitively injecting the drug solution at the concentration level 500_g/ml and 25_g/ml for Atenolol and Indapamide, respectively to check the reproducibility of the system and the results are shown in Table 2, 3, 4. For robustness evaluation of HPLC method a few parameters like change in Analysis, Flow rate, percentage of methanol and water in the mobile phase were deliberately changed to estimate the effect at three levels (-5, 0, +5) with respect to optimized parameters.

Results and Discussion:

The observation of above seven graphs that obtained from different chromatographic condition. Showed that Mobile phase Buffer : methanol (50:50) and Flow rate 1.5 ml/min was found to be most suitable for assay of drugs. The retention times for Atenolol and Indapamide drugs were 2.2 min and 4.3 min, respectively. UV overlain spectra of both Atenolol and Indapamide showed that both drugs absorbed appreciably at 242 nm, so this wavelength was selected as the detection wavelength. The calibration curve for atenolol and indapamide was found to be linear respectively. The data of regression analysis of the calibration curves is shown in Table 1.The proposed method was successfully applied to the determination of

Table 2: Assay of tablet

PARAMETERS	OBSERVATIONS			
	ATENOLOL		INDAPAMIDE	
Specificity	No interference was found			
Linearity Correlation coefficient (r)	20 to 120% 0.999		20 to 120% 0.999	
Range	1µg/ml to 6µg/ml		0.3µg/ml to1.8 µg/ml	
Accuracy	Recovery	% RSD	Recovery	% RSD
Recovery from placebo				
50%	100.00%	0.26	100.13%	0.35
100%	100.06%	0.25	99.83%	0.75
150%	100.43%	0.30	100.60%	0.55
Recovery from tablet	100.44%	0.95	99.73%	1.10
Precision	Recovery	% RSD	Recovery	% RSD
Repeatability	100.07%	1.29	100.25%	1.22
Intermediate precision				
Intraday	100.47%	1.18	101.08%	1.07
Interday day-1	100.20%	0.60	100.66%	0.93
day-2	99.88%	0.63	100.00%	0.80
day-3	100.14%	0.41	100.00%	0.94
different equipments	100.02%	0.12	100.00%	0.02
Robustness	Recovery	% RSD	Recovery	% RSD
Change in pH of M.P.	99.76%	0.12	99.44%	0.27
Change in temperature	99.60%	0.47	99.88%	0.98
Change in flow rate	100.22%	1.10	101.55%	0.53
Solution stability	Recovery	% RSD	Recovery	% RSD
	99.90%	0.39	100.44%	0.90

Table 3:Dissolution of atenolol in tablets

PARAMETERS	OBSERVATIONS	
	ATENOLOL	
Specificity	No interference was found	
Linearity Correlation coefficient (r)	20 to 120% 0.9994	
Range	5µg/ml to 60µg/ml	
Accuracy	Recovery	% RSD
Recovery from placebo		
50%	100.30%	0.89
100%	100.73%	0.79
150%	100.43%	1.15
Recovery from tablet	99.32%	1.06
Precision	Recovery	% RSD
Repeatability	100.14%	0.59
Intermediate precision		
Intraday	100.24%	0.59
Interday day-1	100.68%	0.43
day-2	100.90%	0.60
day-3	101.02%	0.61
different equipments	100.90%	0.05
Robustness	Recovery	%RSD
Change in pH of M.P.	100.51%	0.47
Change in temperature	100.19%	0.71
Solution	Recovery	%RSD
	100.33%	1.03

Table 4: Dissolution of indapamide in tablet

PARAMETERS	OBSERVATIONS	
	Indapamide	
Specificity	No interference was found	
Linearity Correlation coefficient (r^2)	20 to 120% 0.9999	
Range	0.15µg/ml to 1.8µg/ml	
Accuracy	Recovery	% RSD
Recovery from placebo		
50%	100.09%	0.51
100%	100.53%	0.69
150%	99.89%	0.68
Recovery from tablet	98.99%	0.70
Precision	Recovery	% RSD
Repeatability	99.33%	0.54
Intermediate precision		
Intraday	100.02%	0.94
Interday day-1	100.11%	0.50
day-2	101.33%	0.36
day-3	99.66%	0.37
different equipments	100.10%	0.15
Robustness	Recovery	%RSD
Change in pH of M.P.	100.44%	0.65
Change in temperature	100.06%	0.98
Solution Stability	Recovery	%RSD
	100.44%	0.53

Table 5: Content uniformity of indapamide in tablet

PARAMETERS	OBSERVATIONS	
	Indapamide	
Specificity	No interference was found	
Linearity Correlation coefficient (r)	20 to 120% 0.9999	
Range	0.15µg/ml to 1.8µg/ml	
Accuracy	Recovery	% RSD
Recovery from placebo		
50%	100.29%	0.75
100%	100.79%	0.82
150%	100.86%	0.65
Recovery from tablet	99.77%	1.09
Precision	Recovery	% RSD
Repeatability	100.10%	1.22
Intermediate precision	100.02%	0.94
Intraday	99.73%	0.09
Interday day-1	100.22%	0.30
day-2	90.06%	0.91
day-3	100.05%	0.39
different equipments		
Robustness	Recovery	%RSD
Change in pH of M.P.	100.40%	0.81
Change in temperature	100.44%	0.99
Solution Stability	Recovery	%RSD
	99.89%	0.59

Atenolol and Indapamide in their Tablet dosage form. The results for the combination were comparable with the corresponding labeled amounts. The developed method was also found to be specific, since it was able to separate other excipients present in tablet from the two drugs.

The LOD for Atenolol and Indapamide were found to be 0.33% and 1.29%, respectively,. The results for validation and system suitability test parameters are summarized in Table 2, 3, 4. Insignificant differences in peak areas and less variability in retention times were observed. In the proposed study, RP-HPLC method was developed for the simultaneous determination of Atenolol and Indapamide and validated as per ICH guidelines. Statistical analysis proved that method was accurate, precise and robust. The developed method was found to be simple, sensitive and selective for analysis of Atenolol and Indapamide in combination without any interference from the excipients. The method was successfully used for determination of drugs in a pharmaceutical formulation. Assay results for combined dosage form using proposed method showed 99.30 ±1.04 % of Atenolol and 100.40±1.09% of Indapamide.

Acknowledgement:

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