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Development and Validation of a Novel Isocratic RP-HPLC Method For Simultaneous Determination of Atenolol and Aspirin in Fixed dose combinations

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ABSTRACT

In this study, reverse phase high performance liquid chromatographic method have been developed and validated for the simultaneous determination of Atenolol and Aspirin in combined pharmaceutical formulation. HPLC separation was achieved with a Phenomenex – Luna, C18 (250 x 4.6 mm i.d., 5µ) as stationary phase and phosphate buffer (pH adjusted to 4.5 with ortho phosphoric acid): Methanol (85:15 v/v) as eluent, at a flow rate of 0.8 ml/min. UV detection was performed at 239.5 nm. The retention time of Atenolol and Aspirin was found to be 5.1 and 12.5 min respectively. Results of the analysis were validated statistically and by recovery studies. Linearity, accuracy, and precision were acceptable in the ranges (20-100 µg/ml) for Aspirin and (10-50 µg/ml) for Atenolol. The calibration curves were linear (r^2 > 0.999) in the range for each analyte. The % recovery for Atenolol and Aspirin is 99.17 and 99.75 respectively. No spectral or chromatographic interferences from the tablet excipients were found. The result of the studies showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which can be used for the routine determination of Atenolol and Aspirin in bulk and in its pharmaceutical dosage forms.

Key words: Atenolol; Aspirin; RP-HPLC; Assay.

INTRODUCTION

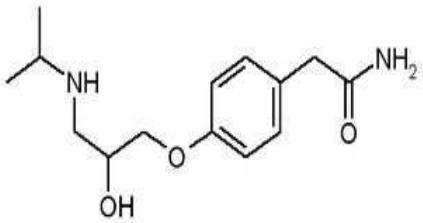
Monotherapy with various antihypertensive agents is not always sufficient to control the blood pressure, and concomitant use of two or more drugs is necessary in 50% of the hypertensive patient [1-4]. The primary goal of any antihypertensive therapy is therefore achievement of normotension, without the addition of intolerable side effects, which can be accomplished by combining drug with different mechanism of action.

Atenolol is a synthetic, $\beta_1$selective (cardio selective), adrenoreceptor blocking agent. It is chemically described as (*R*, *S*)-4-(2-hydroxy-3-isopropyl-aminopropoxy) phenyl acetamide (fig.1). It is indicated in the management of hypertension. The analytical methods available for the estimation of Atenolol are official in IP, BP, USP where as the reported methods for the estimation of Atenolol in the literature are its estimation by high performance liquid chromatography (HPLC) [5-10] ,gas chromatography (GC) [11-12] and UV spectrophotometry methods [13].

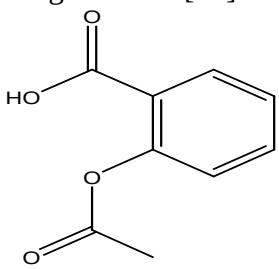
Aspirin, chemically 2-(acetyloxybenzoic acid), it is used as an analgesic and antipyretic, anti-inflammatory, anti-arthritic and anti-platelet drug (fig.1).There are various analytical methods like spectrometric determination of Aspirin in biological fluids [14], RP-HPLC methods for the simultaneous estimation of Aspirin, Paracetamol, Caffeine [15] and Aspirin with Atorvastatin [16] reported . Spectrofluromertric method is also reported for the estimation of Aspirin and Dipyridamole [17].

A combination of Atenolol and aspirin is used in the treatment and prevention of cardiovascular conditions such as stroke and to regulate blood pressure.

Literature survey reveals that there is no RP-HPLC method available for the deter- mination of these analytes in combination; therefore the aim of the study was to develop simple, rapid, accurate, reproducible and precise HPLC method for simultaneous estimation of Aspirin and Atenolol in its formulation. The proposed method was validated as per the International Conference on Harmonization (ICH) analytical method validation guidelines [18].



Atenolol



Aspirin

Fig.1 Chemical structure of Atenolol and Aspirin

MATERIALS AND METHODS

2.1 Materials: Working standards of Aspirin (Purity 100.99%) and Atenolol (Purity 99.81%) were provided as a gift sample by Alembic limited, Vadodara, India and used without further purification. Acetonitrile and orthophosphoric acid procured from Merck and methanol from Rankem. Highly pure water was prepared by using Millipore system. Potassium di hydrogen phosphate and sodium hydroxide were also procured from Merck.

The pharmaceutical preparation of combination of Aspirin and Atenolol is ATO-PRIN (Perk pharma)**.** The commercial formulation of Aspirin and Atenolol is available in the ratio of 2:1 (100/50 mg).

2.2 Instrumentation:
A High performance liquid chromatography system Adept series CECIL CE 4201 with UV/Visible detector was used for analysis. The data was recorded by using the software Power stream. The column used for separation was octadecyl silane (C_{18}) with length 250mm and internal diameter 4.6mm (Phenomenex) as well as particle size 5µ.

2.3 Solubility determination:
Solubility of Aspirin and Atenolol was determined in different solvents. Both the drugs were found to be soluble in Methanol.

2.4. Selection of Detection Wavelength:
The λmax for Atenolol was 239 nm and for Aspirin it was 240 nm in methanol. The isoabsorptive point for both the drugs was found to be 239.5 nm so it was selected as detection wavelength.(fig. no.2)

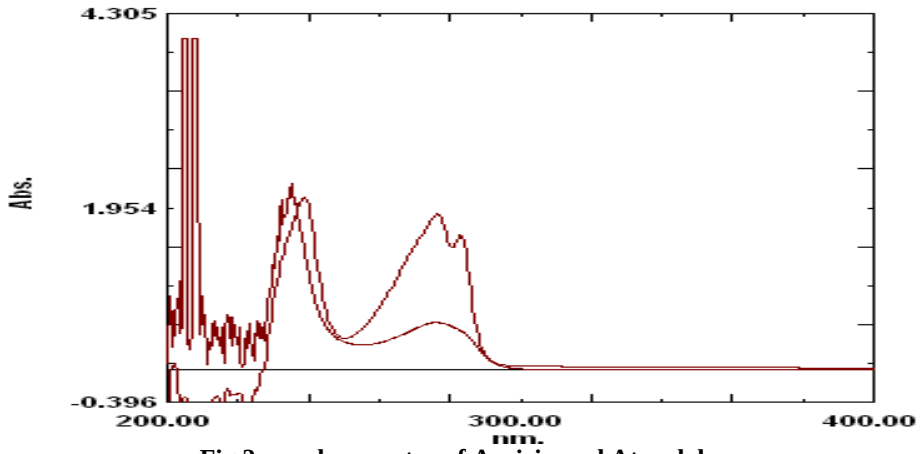


Fig.2. overlay spectra of Aspirin and Atenolol

2.5. Mobile phase selection:
Taking into consideration the System Suitability Parameter like R.T., peak symmetry, No. of Theoretical Plates , the Mobile Phase found to be most suitable for analysis was phosphate buffer (pH adjusted to 4.5 with ortho phosphoric acid): Methanol (85:15 v/v). The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 0.8 ml/min.

2.6. Preparation of Standard Solution:
For Aspirin : - 10 mg of Aspirin was accurately weighed and transferred to a 10 ml volumetric flask and volume was made up to 10 ml with methanol (Stock A; 1000 µg/ml). From stock A, 5.0 ml was taken in to a 50 ml volumetric flask and volume was made up to 50 ml with methanol (Stock B; 100 µg/ml),

For Atenolol : - 10 mg of Atenolol was accurately weighed, transferred to a 10 ml volumetric flask and the volume was made up to 10 ml with methanol(Stock C 1000 µg/ml). From stock C, 1.0 ml was taken in to a 10 ml volumetric flask and volume was made up to 10 ml with methanol (Stock D; 100 µg/ml).

For Mix standard: - From the stock solutions B and D dilutions of different concentration were prepared in the ratio of 2:1 for Aspirin and Atenolol as mentioned in the table. No.1.

Table no.1 Preparation of mix standard

Stock Solution→ Volume Taken (ml)		Total Vol.(ml)	Concentration in µg/ml	
B	D		(Aspir	(Atenolol)
2	1	10	20	10
4	2	10	40	20
6	3	10	60	30
8	4	10	80	40
10	5	10	100	50

2.7. Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from 20-100 µg/ml for Aspirin and 10-50 µg/ml for Atenolol was prepared in the same manner described in the table no.1. All the solution were filtered through 0.22 µm membrane filter and injected, as well as the chromatograms were recorded (fig.3, fig. 4, fig. 5).

A calibration graph was plotted between the mean peak area vs. respective concentration and the regression equation was derived. The correlation coefficient for Aspirin was 0.9998 and for Atenolol 0.9995.

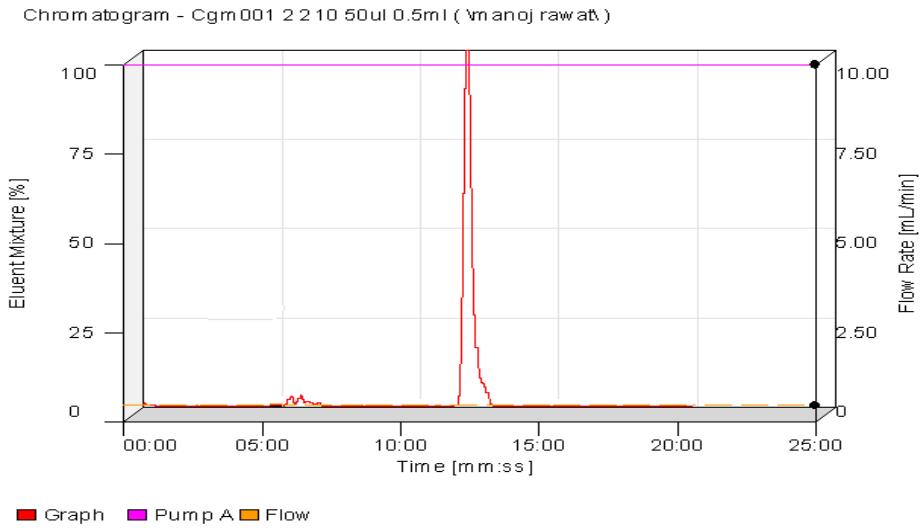


Fig.3. 2D Chromatogram of Aspirin with R.T. 12.55 minute

2.8 Analysis of Commercial Dosage Forms

The pharmaceutical preparation of combination of Aspirin and Atenolol is **ATO-PRIN** (Perk pharma), labelled to contain 100mg of Aspirin and 50mg of Atenolol.

Twenty tablets were weighed accurately and powdered finely. A quantity of tablet powder equivalent to 25 mg of Aspirin and 12.5 mg of Atenolol was accurately weighed and transferred into a 25 ml calibrated volumetric flask, 10 ml of diluent solution was added and the content was

ultrasonicated for 20 min; the volume was then diluted to the mark and mixed well. A small portion was withdrawn and filtered through a 0.22 µm filter to ensure the absence of particulate matter. The filtered solution was appropriately diluted with the diluent used for analysis as already described. The amount of drug present in the sample solution was determined using the calibration curve of standard drug. The results are shown in Table no.4.

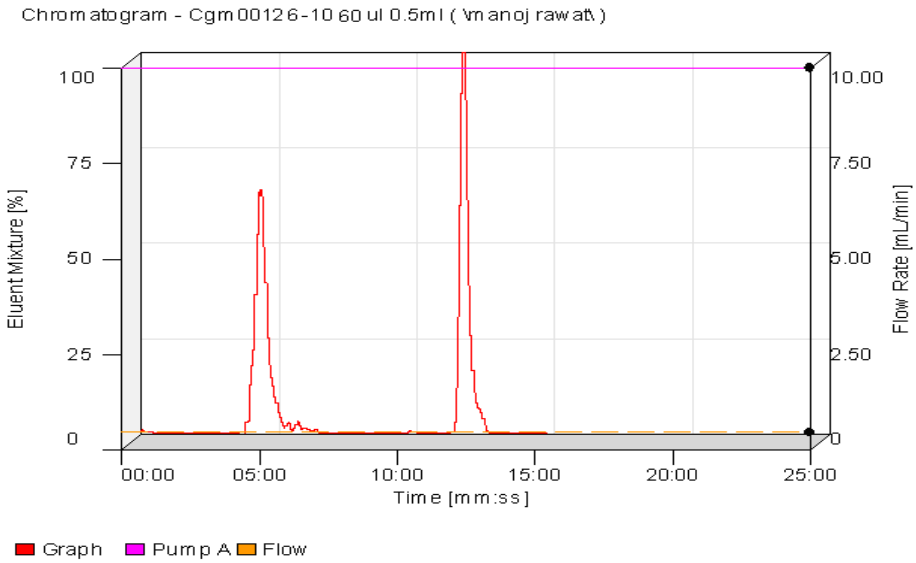


Fig.4. 2D Chromatogram of Atenolol and Aspirin with R.T. 5.11 & 12.57 minute respectively

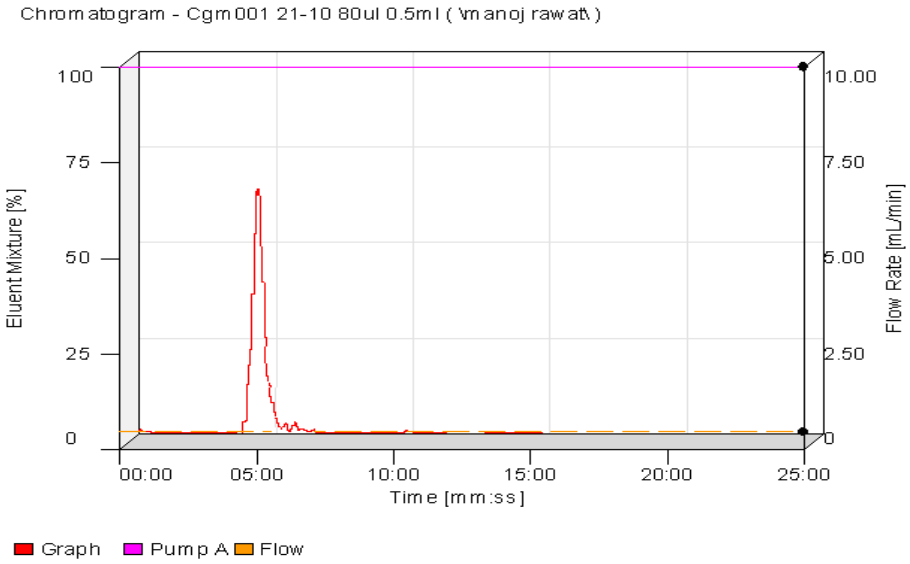


Fig.5. 2D Chromatogram of Atenolol with R.T. 5.12 minute

RESULT AND DISCUSSION

Proposed method for simultaneous estimation of Aspirin and Atenolol in combined dosage form was found to be accurate, simple and rapid.

This work was focused on optimization of the conditions for the simple and rapid as well as low cost effective analysis including a selection of the proper column and mobile phase to obtain satisfactory results. To obtain satisfactory resolution and to avoid peak tailing of compounds, optimization of the proposed method was carried by using the different mobile phases (Table no.2). The best results were obtained using the mobile phase phosphate buffer (pH adjusted to 4.5 with ortho phosphoric acid): Methanol (85:15 v/v), at a flow rate of 0.8 ml/min. The proposed chromatographic conditions were found to be appropriate for the quantitative determination. (Table.no.3)

The results obtained by the assay of marketed formulation are summarized in Table.no.4. System suitability tests were carried out as per USP XXIV and parameters are summarized in Table.no.5**.**

3.1 Validation: The proposed HPLC method was validated as per ICH guidelines .

3.1.1 Linearity:
The drugs followed a linearity of peak area *versus* concentrations ranging from 20-100 µg/ml for Aspirin and 10-50 µg/ml for Atenolol respectively. A linear response was observed over the examined concentration range. The slope, intercept were found to be 108564, 151222 and 96812, 38555 for Aspirin and Atenolol respectively. The correlation coefficients for Aspirin and Atenolol were found to be 0.9998 and 0.9995 respectively.

3.1.2 Accuracy:
Recovery studies were performed to validate the accuracy of developed method. To the preanalysed sample solution, a definite concentration of standard drug was added and then its recovery was analyzed. The percent recovery for Aspirin was found to be 99.75 % and for Atenolol it was 99.17 %.(Table no.6)

3.1.3 Precision

A. Repeatability Dilutions of different concentrations were prepared and triplicates of each dilution was analyzed in same day for repeatability and the results were subjected to statistical analysis. The %RSD for Aspirin was 0.46 and for Atenolol it was 0.89 which is according to ICH norms.

B. Intermediate Precision: In this triplicate of each dilution was analyzed in different days and by different analysts. In all the condition %RSD was near to1 which shows method is precise.(Table no.7)

3.1.4 Robustness
As per ICH norms, small, but deliberate variations, by altering the pH, concentration of the mobile phase or flow rate or detection wavelength were made to check the method's capacity to remain unaffected The robustness was performed by changing the detection wavelength ± 5nm, the flow rate ± 10 % and pH ± 0.5 unit , the results were interpreted by statistical analysis by calculating RSD values. All the results were with in the acceptance criteria i.e. RSD is not more than 2 %.(Table no.8)

Table No.2: Selection of suitable Mobile Phase.

Mobile Phase Components	Solvent Ratio.	Flow Rate ml/min.	Retention Time (min)		Remark.
			Aspirin	Atenolol	
Methanol: Water.	50:50	1.0	2.8	-	Not Suitable.
Methanol: Water.	75:25	0.9	3.2	-	Not Suitable
Acetonitrile: Water.	60:40	0.5	3.8	4.3	Poor resolution Not Suitable.
Acetonitrile: Water.	75:25	0.5	3.4	3.8	Peak splitting of Atenolol Not suitable.
Acetonitrile: Water pH 4 (pH adjusted with 0.1% OPA)	30:70	0.8	8.6	4.8	Due to Peak broadening of Atenolol
phosphate buffer: Methanol pH 4.5 (adjusted with Glacial Acetic acid)	75:25	1.0	9.4	5.8	Solvent peak comes along with Asp. Not suitable
phosphate buffer: Methanol pH 4.5 (adjusted with ortho phosphoric acid):	85:15	0.8	12.57	5.11	Suitable Mobile Phase.

Table no.3 Optimized chromatographic conditions

Seperation Variable	**Optimized condition**
Chromatography	Cecil CE 4201
Column	$C_{18.}$ 5µ, (250mm $_X$4.6mm)
Mobile phase	Phosphate buffer: Methanol (85:15) pH 4.5 (adjusted with ortho phosphoric acid)
Diluent	Methanol
Flow rate	0.8 ml/min
Temperature	Ambient
Detection wavelength	239.5 nm
Retention time – Atenolol	5.11 ± 0.2 min
Retention time – Aspirin	12.57 ± 0.2 min

Table no.4 Result of Aspirin and Atenolol in marketed formulation (n=6).

Brand Name	Labeled amount (mg)		Observed amount ± S.D.		% R.S.D.	
ATO-PRIN	Aspirin	Atenolol	Aspirin	Atenolol	Aspirin	Atenolol
	100	50	98.7 % ± 2.0	95.0 % ± 1.8	1.99	1.85

Table No.5 System suitability parameters

Parameter	**Aspirin**	**Atenolol**
Linearity range (µg/ml)	20-100	10-50
Correlation Coefficient (r^2)	0.9998	0.9995
Slope (m)	108564	96812
Tailing factor	1.22	1.93
No. of theoretical plates	5567	4315
Retention time(min)	12.57	5.11

3.1.5 Stability

Stability of the drugs in the proposed mobile phase was checked at ambient conditions by storing it up to 48hrs. No significant loss of the active constituents was monitored also no interfering peaks of degraded products were observed at the retention times of the drugs.

Table no.6. Statistical data for accuracy

Statistical data	Aspirin	Atenolol
% Recovery	99.75	99.17
SD	1.18	1.73
%R.S.D.	1.179	1.75

Table no.7. Statistical data for precision

Statistical parameter	**Aspirin**			**Atenolol**		
Repeatability	**% Recovery**	**SD**	**%RSD**	**% Recovery**	**SD**	**%RSD**
	99.02	0.45	0.46	98.98	0.88	0.89
Intermediate Precision						
a. Day to day	98.93	0.59	0.60	98.38	0.98	0.99
b. Analyst to Analyst	99.06	0.72	0.73	99.20	0.58	0.59

Table no.8. Statistical data for Robustness

Parameters	**Aspirin**	**Atenolol**
Change in pH of Mobile Phase ± 0.5 unit		
S.D.	0.30	0.87
% R.S.D.	0.29	0.88
Change in Ratio of Mobile Phase ± 10 %		
S.D.	0.48	1.55
% R.S.D.	0.47	1.56
Change in the detection wavelength ± 5 nm		
S.D.	0.24	0.39
% R.S.D.	0.243	0.40

CONCLUSION

A reversed-phase High performance Liquid chromatographic method was developed for the simultaneous determination of Aspirin and Atenolol, and validated. The regression co efficient (r^2) for each analyte is not less than 0.999 which shows good linearity. The good % recovery in tablet dosage forms suggests that the excipients present in the dosage forms have no interference in the determination. The %RSD was also less than 2% showing high degree of precision of the proposed method. As the precision accuracy and robustness are concern the maximum %RSD found is 1.75 % which is with in range of ICH guidelines. Since the method does not require use of expensive reagent and also less time consuming, it can be performed routinely in industry for analysis of drug.

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