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Research Article

A NEW RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE DISSOLUTION STUDIES OF ATENOLOL AND CHLORTHALIDONE IN IMMEDIATE RELEASE TABLET DOSAGE FORMS

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ABSTRACT

A simple, precise, rapid, specific and accurate reverse phase high performance liquid chromatography method was developed for dissolution studies of Atenolol (ATN) and Chlorthalidone (CTN) in immediate release tablet dosage forms. Dissolution sample was obtained by placing tablets into the USP2 dissolution apparatus (Paddle Type) rotating with 50rpm speed. Water was used as medium and a volume of 900ml of dissolution medium was used. The bath temperature was 38°C and bowl temperature was 37°C. The samples were collected after 45 minutes. Chromatographic separation was performed Agilent C8, 150X4.6, 5µm column, with mobile phase comprising of ammonium acetate buffer: pH 7.00 and methanol in the ratio of 60:40v/v, at the flow rate 1.0ml/min. The detection was carried out at 228 nm. The retention times of ATN and CTN were found to be 2.18 and 3.32 min respectively with a run time of 5 min, theoretical plate counts for ATN and CTN were 5366 and 6689 respectively, with a resolution of 7.79. As per ICH guidelines the method was validated for linearity, accuracy, precision, and robustness. The correlation coefficient for ATN and CTN were 0.999 and 1 respectively. This demonstrates that the developed method is simple, precise, rapid, selective, accurate and reproducible for dissolution studies of ATN and CTN in immediate release tablet dosage form by RP-HPLC.

Keywords: Atenolol, Chlorthalidone, Dissolution, RP-HPLC, Validation.

INTRODUCTION

Atenolol is chemically 2-(4-{2-hydroxy-3-[(propan-2-yl)amino] propoxy} phenyl) acetamide.It is a β_1 selective (cardio selective) adrenergic receptor blocking agent. It does not have membrane stabilizing and intrinsic sympathomimetic (partial agonist) activities. Atenelol (free base) is a white or almost white powder and is soluble in ethanol, soluble in water, slightly soluble in dichloromethane and practically insoluble in ether.

.Chlorthalidone is chemically 2-chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1H-isoindol-1-yl)benzene-1-sulfonamide.It is considered a thiazide-like diuretic. It has a water solubility of 12mg/100mL at 20° C.It inhibits sodium ion transport across the renal tubular epithelium in the cortical diluting segment of the ascending limb of the loop of Henle. By increasing the delivery of sodium to the distal renal tubule, indirectly increases potassium excretion via the sodium-potassium exchange mechanism.

MATERIALS AND METHODS

Equipment

Chromatographic separationwas performed on HPLCsystem–Waters e2695 model, PDA Detector, equipped with a solvent delivery pump, sample injector and column thermostats. Empower system software was applied for data collecting and processing.

Chemicals and Reagents

1. Methanol - HPLC grade
2. Ortho phosphoric acid – HPLC grade
3. Potassium dihydrogen phosphate – AR grade
4. Ammonium acetate – AR grade
5. Water for HPLC

OPA, Methanol, Potassium dihydrogen Phosphate and Ammonium acetate were obtained from Merck manufactures and Water was obtained from Rakem Chemicals.

Atecard D tablets (50+12.5) manufactured by Alembic Chemical Works, were procured from local market. Reference standards of Atenolol and Chlorthalidone were obtained from Rainbow Labs.

Preparation of Standard Solution

Weigh accurately 50mg of atenolol and 12.5mg of chlorthalidone. Transfer them into a100ml volumetric flasks respectively. Then it was dissolved in HPLC grade water, made upto mark and sonicated. Then 1ml of above solution was diluted to 10 ml with HPLC grade water.

Preparation of Buffer

Prepare a 0.1M ammonium acetate solution by dissolving 7.7g ammonium acetate in 1000 mL water.

Preparation of Mobile Phase

A mixture of buffer 600ml (60%) and 400ml (40%) of methanol were mixed and degassed in ultrasonic water bath for 5 minutes. The mobile phase was filtered through 0.45µ membrane filter under vaccum.

Preparation of Sample

Tablets were placed in the USP2 dissolution apparatus (Paddle Type) rotating with 50rpm speed. Water was used as medium and a volume of 900ml of dissolution medium was used. The bath temperature was 38^{o}C and bowl temperature was 37^{o}C. The samples were collected after 45 minutes. Then 1ml was diluted to 10ml.

CALCULATION

Dissolution % =

$$\frac{AT}{AS} \times \frac{WS}{100} \times \frac{1}{10} \times \frac{900}{1} \times \frac{P}{100} \times \frac{100}{LC} \times 100$$

Where, AT & AS= absorbance of test and standard respectively,
WS= Weight of Standard
LC= Labeled Claim
P= Potency

Procedure

Dissolution Parameters

USP Type : Type 2 (Paddle)
Bowl Temperature : 38^{o}C
Bath Temperature : 37^{o}C
Speed : 50rpm
Medium : Water
Volume of Medium : 900ml
Sample collection time : 45min

Chromatographic Conditions

HPLC System : Waters HPLC
PDA detector done by using Empower 2 software package
Stationary phase: Agilent C8, 150X4.6, 5µm
Mobile phase : 60:40 (Ammonium acetate Buffer : Methanol)
Detection wavelength: 228 nm (PDA Detector)
Flow rate: 1.0 ml/min
Temperature : Room temperature
Column temperature :30°C
Mode : Isocratic
Injection volume : 10µl
Run time : 5 min
Retention Time : 2.1 and 3.3 for Atenolol and Chlorthalidone respectively.

METHOD VALIDATION

1. System suitability

By analyzing the standard solution 6 times, calculate the theoretical plates, tailing, resolution and % RSD for each component from standard solution. Results are shown in the Table 2.

2. Accuracy

Accuracy was determined by the recovery studies at three different concentrations (corresponding to 50,100 and 150% of the test solution concentration by addition of known amounts of standard to pre-analysed sample preparation. For each concentration, three sets were prepared and median concentration six sets were prepared and injected. Results are shown in the Table 3.

3. Precision

The precision of an analytical method is a measure of the random error and is defined as the agreement between replicate measurements of the same sample. It is expressed as the percentage coefficient of variation (%CV) or relative standard deviation (RSD) of the replicate measurements.The solution was injected for six times and measured the area for all six injections in HPLC.The %RSD for the area of six replicate injections was found to be within the specified limits. Results are shown in the Table 4.

4. Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

5. Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Five levels of solutions were prepared (50,75,100,125&150%) respectively. Each level of solution was injected into the chromatographic system and the peak areas were measured. Results are shownTable 5 & Table 6. Plotted a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and the correlation coefficient was calculated.Linearity graphs are shown in Fig. 5 & Fig. 6.

6. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Results are shown in the Table 7.

Details of Robustness

Variation	Actual	Low	High
Flow(ml)	1.0	0.8	1.2
Temperature(^{o}C)	30	28	32

RESULTS AND DISCUSSIONS

RP-HPLC method was developed for the dissolution studies of Atenolol and Chlorthalidone in immediate release tablet dosage forms. Dissolution was run to obtain the sample. Chromatographic separation was performed Agilent C8, 150mm X4.6mm, 5µmcolumn, with mobile phase comprising of ammonium acetate buffer and methanol in the ratio of 60:40v/v, at the flow rate 1.0ml/min. The detection was carried out at 228 nm. The detection was carried out at 228 nm.The retention times ofand CTN were found to be 2.18 and 3.32 min respectively with a run time of 5 min, theoretical plate counts for ATN and CTN were 5366and 6689 respectively, with a resolution of 7.79. Robustness was performed by varying experimental conditions i.e, flow rate and temperature. As per ICH guidelines the method was validated for linearity, accuracy, precision, and robustness. The correlation coefficient for ATN and CTN were 0.999 and 1 respectively.

CONCLUSION

The proposed HPLC method was found to be precise, specific, accurate, rapid and economical for dissolution studies of Atenolol and Chlorthalidone in Immediate release tablet dosage forms. The method development and validation process for the analysis of Atenolol and Chlorthalidone by RP-HPLC has been investigated in the study. The mobile phase is simple to prepare and it's economical. The optimized method showed good resolution with appropriate retention time for the respective peaks and good system suitability. The parameters which are validated for the developed method offered satisfactory results within acceptable limits, which reveal that developed method is validatable, transferable, robust, reliable, accurate and precise.

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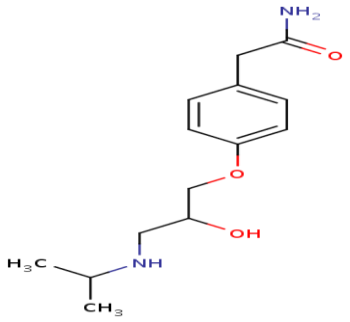

Fig. 1: The chemical structure of Atenolol

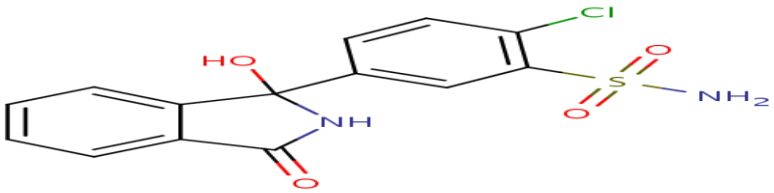

Fig 2: The chemical structure of Chlorthalidone

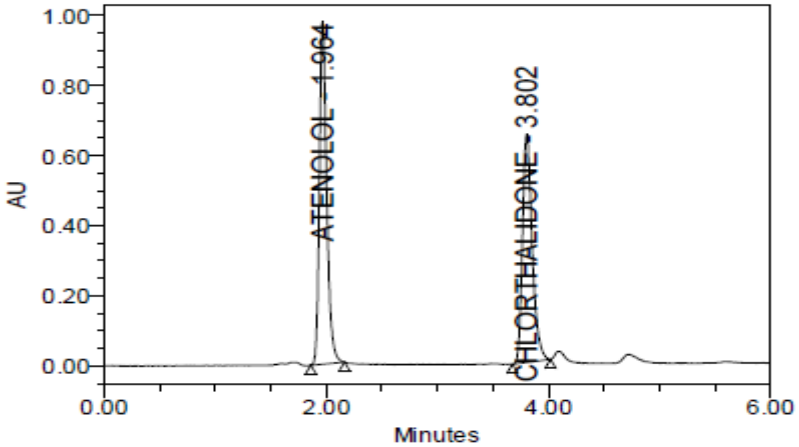

Fig. 3: Chromatogram of Atenolol and Chlorthalidone standard

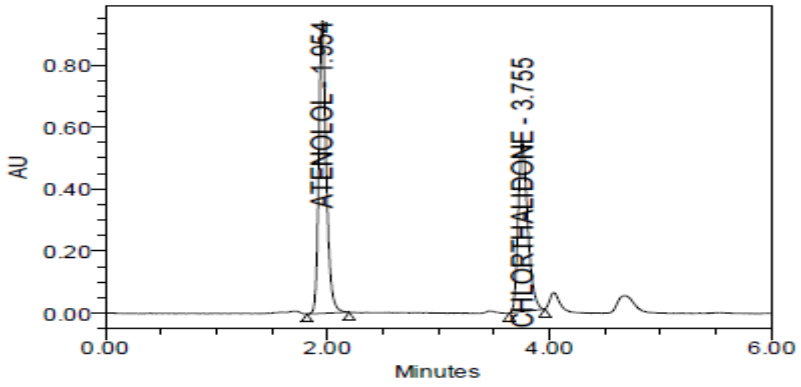

Fig. 4: Chromatogram of Atenolol and Chlorthalidone sample

Table1: Analysis data of formulation (Atecard D)

Injection	Label claim(mg)	Assay (%)
Atenolol	50mg	98.88%
Chlorthalidone	12.5mg	99.80%

Table 2: System suitability studies

Parameters	Atenolol	Chlorthalidone	Acceptance criteria
Theoretical plates	5366	6689	Not less than 2000
Tailing factor	1.56	1.41	Not more than 2
Resolution	-	7.79	Not less than 2

Table 3: Recovery studies for Atenolol and Chlorthalidone

DRUG	Spiked level%	Amount taken (µg/ml)	Amount found (µg/ml)	Percent recovery	Mean recovery
Atenolol	50	27.528	27.496	99.794	99.794
	100	55.056	54.922	99.800	
	150	82.583	82.483	99.893	
Chlorthalidone	50	6.924	6.922	99.869	99.869
	100	13.847	13.824	101.230	
	150	20.771	20.786	99.73	

Table 4: Precision Values of Atenolol and Chlorthalidone

S.No	Sample Weight	Sample Area-1	Sample Area-2	% Assay	% Assay
1	1.00	4443224	3917635	98.69	99.71
2	1.00	4453801	3917389	98.92	99.71
3	1.00	4467848	3918410	99.24	99.73
4	1.00	4450592	3915045	98.85	99.65
5	1.00	4452059	3921109	98.88	99.80
6	1.00	4449522	3913635	98.83	99.61
Average Assay:				98.80	99.70
STD				0.18	0.07
%RSD				0.18	0.07

Table 5: Linearity Values of Atenolol

CONC%	Area	ug/mL
50	2219492	27.75
75	3340138	41.63
100	4452136	55.50
125	5574343	69.375
150	6684173	83.25

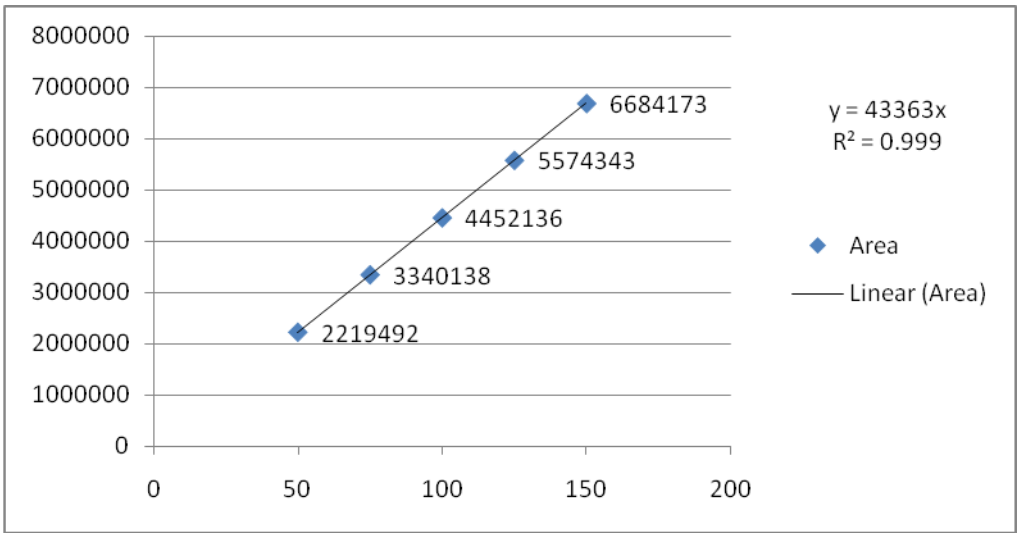

Fig. 5: Linearity Graph for Atenolol

Table 6: Linearity Values of Chlorthalidone

CONC%	Area	ug/mL
50	1953542	6.9445
75	2935138	10.41675
100	3914271	13.889
125	4895220	17.36125
150	5879423	20.83

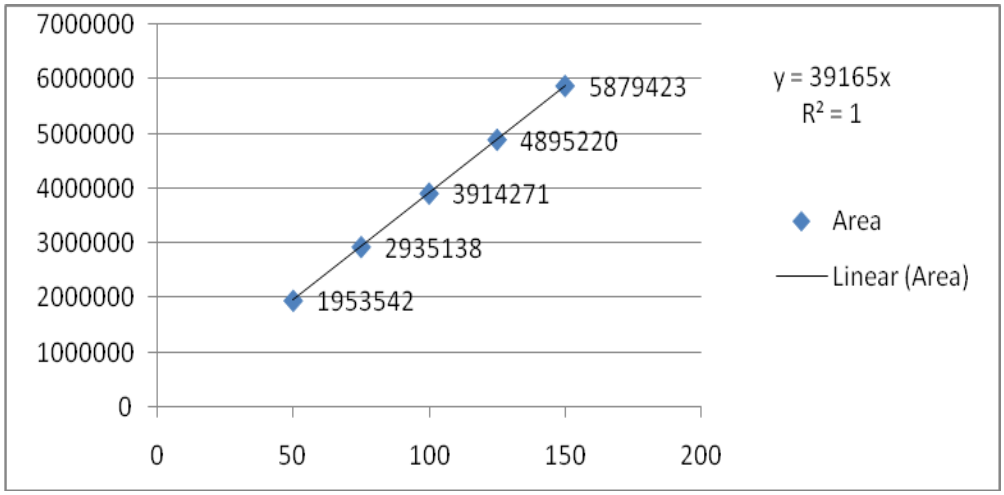

Fig. 6: Linearity Graph for Chlorthalidone

Table 7: Results of Robustnessstudy

S. no	Parameter	Condition	Theoretical plates		Tailing factor		Retention time	
			Atenolol	Chlorthalidone	Atenolol	Chlorthalidone	Atenolol	Chlorthalidone
1	Flow rate	0.8min/ml	4587	10273	1.37	1.33	2.610	4.895
		1.2min/ml	5165	9441	1.39	1.26	1.575	2.966
2	Temperature	28^{0}C	5103	9608	1.40	1.30	1.962	3.765
		32^{0}C	5280	10121	1.40	1.28	1.964	3.688

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