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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF AZILSARTAN AND CHLORTALIDONE IN BULK AND ITS PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A new simple, accurate, rapid and precise isocratic RP-HPLC was developed and validated for the determination of Azilsartan and Chlortalidone in Pharmaceutical tablet dosage form. The Method employs waters LC system on Hypersil ODS column (4.6 x 250 mm, 5 µm) and flow rate of 1.0ml/min with an injection volume 10µl. pH 4.2 phosphate Buffer, and acetonitrile was used as mobile phase in the composition of 55:45 v/v. The detection was carried out at 220nm. Linearity ranges for Azilsartan and Chlortalidone were 40-240 µg/ml, 25-150µg/ml respectively for HPLC. Retention time of Azilsartan and Chlortalidone were found to be 2.6 and 4.2 minutes respectively. Percent Recovery study values of Azilsartan and Chlortalidone were found 98.7-100.1% and 98.9-100.3% respectively. This newly developed method was successfully utilized for the Quantitative estimation of Azilsartan and Chlortalidone in tablet dosage form. This method was validated for selectivity, accuracy, precision, linearity and Robustness as per ICH guidelines.

KEYWORDS: Liquid Chromatography, Azilsartan, Chlortalidone, Simultaneous estimation, Validation.

1.0 INTRODUCTION

Azilsartan Figure: 1.01 is an angiotensin II receptor antagonist used in the treatment of hypertension, chemical name is 2-Ethoxy-1-{[2'-(5-oxo-2,5-dihydro-1,2,4-oxadiazol-3-yl)-4-biphenylyl] methyl}-1H-enzimidazole-7-carboxylic acid. It is marketed in tablet form under the brand name Edarbi as the prodrug Azilsartan medoxomil.

The most common adverse reaction in adults is diarrhea. It is also sold as a combination drug with Chlortalidone under the brand name Edarbyclor. It is used for the treatment of hypertension in adults. Azilsartan medoxomil lowers blood pressure by blocking the action of angiotensin II at the AT1 receptor, a hormone that contracts blood vessels and reduces water excretion through the kidneys. Literature survey shows various analytical methods, either alone or in combination with other drugs includes UV spectrophotometric,[1,2] HPLC,[3-6] LC-MS,[7-10] HPTLC[11] in pure drug, pharmaceutical formulations as well as biological fluids.

Chlortalidone Figure:1.02 is chemically 2-Chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1H-isoindol-1-yl) benzene sulfonamide. It is a thiazide type diuretic used to treat hypertension. It acts in a similar way like the thiazides in causing diuresis but do not possess a benzothiadiazine moiety in it. At the proximal portion of the distal convoluted tubule of the nephron, it shows its action with longest duration of action as compared to other thiazide diuretics. Based on extensive evaluation in clinical trials, the combination of Azilsartan Medoxomil with Chlortalidone has greater efficacy to other ARBs alone or in combination with hydrochlorothiazide. This advanced efficacy is not offset by a large imbalance in clinically important adverse events.[12] A number of analytical methods have been reported so far for the determination of Chlortalidone either alone or in combination with other drugs in pure drug, pharmaceutical dosage forms and also in biological samples using spectrophotometry,[13-14] GC,[15-16] and SFC,[17-18] HPLC.[19-26]

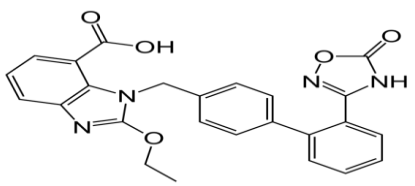

Figure 1.01: Chemical structure of Azilsartan.

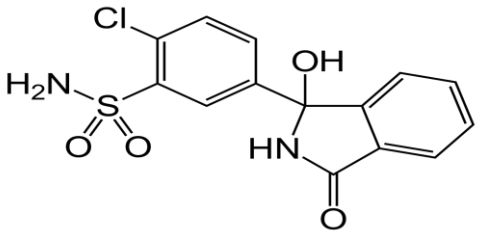

Figure 1.02: Chemical structure of Chlortalidone.

2.0 Experimental

2.1. *Chemicals and Reagents*

Analytical-grade potassium dihydrogen orthophosphate, orthophosphoric acid, Methanol, Acetonitrile and Water HPLC-grade, were from Merck Chemicals. Mumbai, India. Millex syringe filters (0.45 µm) were from Millex-HN, Millipore Mumbai, and India. All dilutions were performed in standard class-A, volumetric glassware.

2.2. Instrumentation and Chromatographic Conditions

Instrumentation: Waters 2690/2695 separation module with empower[3] software, Bandelin ultrasonic bath, pH Meter (Thermo Orion Model), Analytical Balance (Sartorius) and Micro balance (Metller Toledo Model) were use in the present assay.

pH 4.2 phosphate buffer

Accurately weighed 2.72gm of Potassium dihydrogen Ortho phosphate in a 1000ml of milli-Q water added and then pH was adjusted to 4.20 with diluted orthophosphoric acid solution. Filtered through 0.45µ membrane filter paper.

Mobile phase preparation

Mixed accurately pH 4.2 phosphate buffer and acetonitrile in the ratio of 55:45 v/v, filtered and degassed.

Diluent preparation

Mixed accurately water and acetonitrile in the ratio of 80:20 v/v, filtered and degassed.

Standard preparation

Weighed accurately 40mg of Azilsartan standard and 25mg of Chlortalidone standard in 25ml volumetric flask and added 10 ml of diluent and sonicated for 10minutes to dissolved and make up to the volume with diluent and mixed well. Further diluted 5ml of above solution was transfer in to 50ml volumetric flask and diluted with diluent and mixed well.

Sample preparation

Weighed and transfer 10 tablets in a 50ml volumetric flask, added 20ml diluent and sonicated for 20minutes, after make up to the volume with diluent. Further diluted 1ml of this solution to 50ml with diluent and mixed well.

Chromatographic conditions

Hypersil ODS column (250 x 4.6 mm, 5µ) Column was used for analysis at ambient column temperature. The mobile phase was pH 4.2 phosphate Buffer and acetonitrile was used in the composition of 55:45 v/v pumped through the column at a flow rate of 1.0mL/min. The sample injection volume was 10 µL. The photodiode array detector was set to a wavelength of 220 nm for the detection and Chromatographic runtime was 7minutes.

3.0 *Method development*

To develop a suitable and robust LC method for the determination of Azilsartan and Chlortalidone, different mobile phases were employed to achieve the best separation and resolution. The method development was started with Inertsil ODS-3V Column (250×4.6mm, 5µm). Mobile phase was used 0.1% OPA Buffer: Acetonitrile in the ratio of 55:45 v/v. Detector wavelength 220nm, column temperature ambient, Injection volume 10 µL and Flow rate 1.0 ml/min used. Peak shapes were not satisfactory more tailing was observed for both Azilsartan and Chlortalidone.

For next trial the Column was changed Hypersil ODS column (250×4.6mm, 5µm) Column was used. Mobile phase was used 0.1% OPA Buffer: Acetonitrile in the ratio of 55:45 v/v. Detector wavelength 220nm, column temperature ambient, Injection volume 10 µL and Flow rate 1.0 ml/min used. More retention time for Chlortalidone was observed and also the plate count was not within the limits. For next trial mobile phase composition was changed pH 4.2 phosphate Buffer: Acetonitrile: 55:45v/v. Detector wavelength 220 nm, column temperature ambient, Injection volume 10 µL and Flow rate 1.0 ml/min used. Run time 7minutes. Peak shape was satisfactory in both standard and sample preparations. Retention time of Azilsartan and Chlortalidone were found to be 2.59 minutes and 4.02 minutes. The chromatogram of Azilsartan Calcium and Chlortalidone standard using the proposed method is shown in Figure:1.03 System suitability results of the method are presented in Table:1.02.

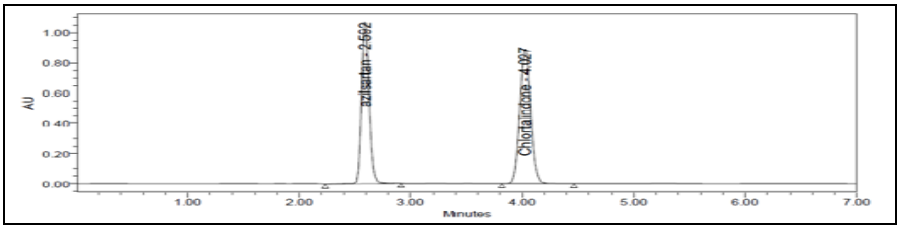

Figure 1.03: Typical Chromatogram of Standard.

4.0 Analytical Method validation

The developed RP-LC method extensively validated for assay of Azilsartan and Chlortalidone using the following Parameters.

4.1 *Specificity & System suitability*

Blank and Placebo interference

A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of Blank solution Figure: 1.04 showed no peaks at the retention time of Azilsartan and Chlortalidone peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of Azilsartan and Chlortalidone in tablets. Similarly Chromatogram of Placebo solution Figure: 1.05 showed no peaks at the retention time of Azilsartan and Chlortalidone peak.

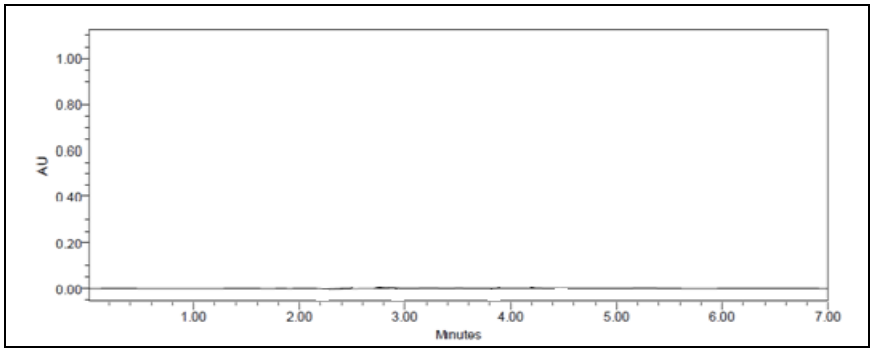

Figure: 1.04: Typical Chromatogram of Blank.

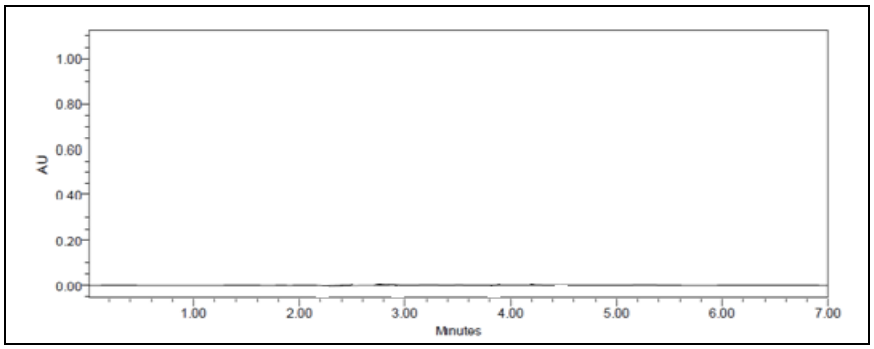

Figure 1.05: Typical Chromatogram of Placebo.

Table: 1.01: Specificity results.

S.No	Name	Retention Time (min)	
Component name		Azilsartan	Chlortalidone
1	Blank	ND	ND
2	Placebo solution	ND	ND
3	Standard solution	2.60	4.2
4	Sample solution	2.65	4.10

The chromatogram of blank and placebo are not showing any peak at the retention time of Azilsartan and Chlortalidone peak

Table 1.02: System suitability results.

Parameters	Azilsartan	Chlortalidone
Retention time (min)	2.6	4.2
No. of Theoretical plates	6956	7860
Tailing factor	1.10	1.06

4.2 *Method Precision*

The precision of test method was evaluated by doing assay for six samples of Azilsartan and Chlortalidone as per test method. The % assay for Azilsartan and Chlortalidone for each of the test preparation was calculated. The average %assay and % RSD of the six preparations were calculated. The chromatogram was

shown in Figure: 1.06 and data were shown in Table: 1.03.

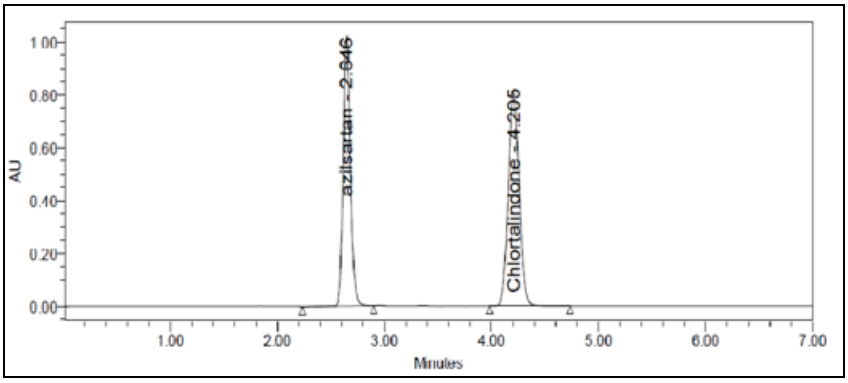

Figure 1.06 Typical Chromatogram of Precision sample

Table: 1.03: Method Precision results.

No.of Preparations	%Assay of Azilsartan	%Assay of Chlortalidone
Preparation-1	99.83	100.93
Preparation -2	99.82	99.15
Preparation -3	100.81	99.67
Preparation -4	100.69	99
Preparation -5	100.84	100.16
Preparation -6	100.11	99.29
Mean	**100.4**	**99.7**
SD	**0.49**	**0.73**
% RSD	**0.48**	**0.74**

4.3 *Accuracy*

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on composite blend collected from 20 tablets of Azilsartan and Chlortalidone, analyzed as per the proposed method. The amount of the each drug present, percentage recovery, percentage relative standard deviation % RSD was calculated. The accuracy studies showed % recovery of the Azilsartan and Chlortalidonein the range 98.7-100.1%, 98.9-100.3%. From the data obtained which given in Table: 1.04 and 1.05 the method was found to be accurate.

Table 1.04: Recovery studies results of Azilsartan.

Recovery levels	Amount added mg	Amount recovered in mg	%Recovery	% Mean recovery	%RSD
50% Level	20.56	20.12	98.2	98.8	2.36
	20.62	21.02	100.0		
	20.93	20.52	98.1		
100% Level	40.26	40.56	98.7	100.1	2.2
	40.29	40.60	98.8		
	39.10	40.93	102.7		
150% Level	60.83	60.82	98.0	98.7	0.22
	60.22	59.97	99.4		
	60.40	60.84	98.6		

Table 1.05: Recovery studies results of Chlortalidone.

Recovery levels	Amount added mg	Amount recovered in mg	% Recovery	% Mean recovery	%RSD
50% Level	12.83	12.66	98.5	98.9	0.38
	12.62	12.52	99.0		
	12.92	12.85	99.3		
100% Level	25.59	25.12	98.0	100.3	0.01
	25.32	25.93	102.2		
	25.05	25.32	99.0		

150% Level	39.93	37.62	109.0	99.5	0.51
	37.62	37.49	99.0		
	37.0	37.09	100.0		

4.4 *Linearity*

The standard curve was obtained in the concentration range of 40-240 µg/ml for Azilsartan and 25-150 µg/ml for Chlortalidone. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r2] of standard curve were calculated and given in Figure:1.06 for Azilsartan and Figure:1.07 for Chlortalidone to demonstrate the linearity of the proposed method. From the data obtained which given in Table: 1.06 and 1.07. For Azilsartan and Chlortalidone the method was found to be linear within the proposed range.

Table 1.06: Linearity data for Azilsartan.

S.No	Linearity Level	Azilsartan	
		Conc. (µg/ml)	Peak area
1	25	40	1117522
2	50	80	2498725
3	75	100	3042961
4	100	160	4914908
5	125	200	6015394
6	150	240	7219408
Slope		48378.725	
Y Intercept		1.681.267	
Correlation co-efficient		0.999	

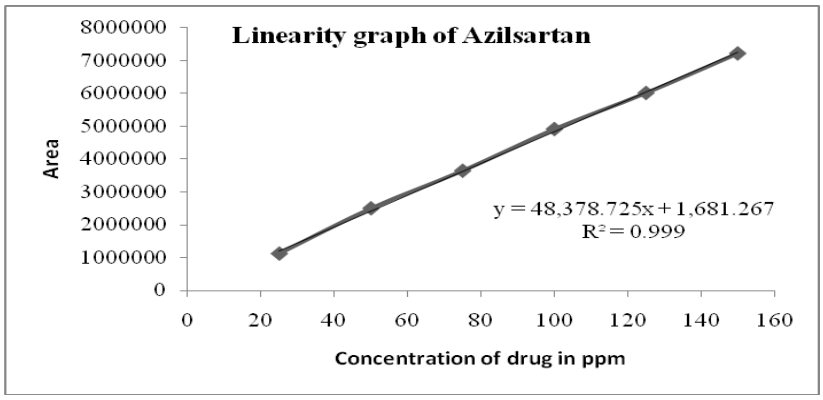

Figure 1.06: Calibration curve for Azilsartan.

Table 1.07: Linearity data for Chlortalidone.

S.No	Linearity Level	Chlortalidone	
		Conc. (µg/ml)	Peak area
1	25	25	1415981
2	50	50	2894082
3	75	75	4352007
4	100	100	5822702
5	125	125	7117463
6	150	150	8670352
Slope		57614.506	
Y Intercept		4161.867	
Correlation co-efficient		1.000	

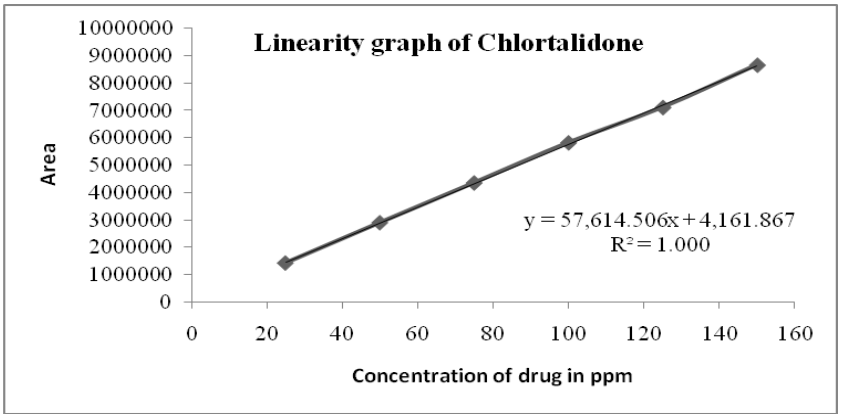

Figure: 1.07 Calibration curve for Chlortalidone.

4.5 *Robustness studies*

To validate the method robustness the chromatographic performance at changed conditions was evaluated compared to nominal conditions of the method. Standard solution was injected at each of the following changed conditions:

Table 1.08: Robustness studies Results.

Parameter		Azilsartan		Chlortalidone		Resolution
		Theoretical plates	Tailing factor	Theoretical plates	Tailing factor	
Flow variation ±10%	10%	6850	1.14	7725	1.07	8.16
	-10%	7054	1.13	7778	1.06	8.92
Temperature variation ±5°C	+5°C	6814	1.12	8087	1.06	8.86
	-5°C	7084	1.15	8374	1.07	8.76
Mobile phase Variation± 10%	10	7186	1.15	7749	1.06	8.56
	-10	7284	1.14	8130	1.06	8.65

Method is robust for changes like column oven temperature, flow rate and organic phase of mobile phase.

4.7 *Solution stability of analytical solutions*

Standard and sample solutions were kept for about 48 hrs at room temperature in transparent bottles in auto sampler and in refrigerator 2-8°C. The response of these was compared with respect Initial standard solution and sample solution.

Table 1.09: Results for solution stability of standard at room temperature.

Component Name	Azilsartan	Chlortalidone
Time Interval	similarity factor	similarity factor
Initial	NA	NA
24hrs	1.0	0.99
48hrs	0.98	0.98

Table 1.10: Results for solution stability of standard in Refrigerator.

Component Name	Azilsartan	Chlortalidone
Time Interval	similarity factor	similarity factor
Initial	NA	NA
24hrs	0.99	1.01
48hrs	1.01	1.02

Table: 1.11 Results for solution stability of standard at room temperature.

Component Name	Azilsartan		Chlortalidone	
Time Interval	%Assay	% of Assay difference	%Assay	% of Assay difference
Initial	100.4	NA	99.7	NA
24hrs	100.1	0.3	99.8	0.1
48hrs	99.8	0.6	99.5	0.2

Table: 1.12 Results for solution stability of sample in Refrigerator.

Component Name	Azilsartan		Chlortalidone	
Time Interval	%Assay	% of Assay difference	%Assay	% of Assay difference
Initial	100.4	NA	99.7	NA
24hrs	100.3	0.1	100.0	0.3
48hrs	100.1	0.3	99.9	0.2

Standard and sample solutions are stable for 48 hours when stored at room temperature (RT) and 2-8°C in refrigerator.

5.0 *CONCLUSION*

An RP-HPLC method for simultaneous estimation of Azilsartan Calcium and Chlortalidone was developed and validated as per ICH guidelines. The results obtained indicate that the proposed method is rapid, accurate, selective, and reproducible.

As there is no interference of blank and placebo at the retention time of Azilsartan Calcium and Chlortalidone. Retention time of Azilsartan and Chlortalidone were found to be 2.6 and 4.2 minutes respectively. Relative standard deviation (%RSD) for method precision was found to be 0.48% for Azilsartan and 0.74% for Chlortalidone. Linearity ranges for Azilsartan and Chlortalidone were 40-240 µg/ml, 25-150µg/ml respectively for HPLC. Percent Recovery study values of Azilsartan and Chlortalidone were found 98.7-100.1% and 98.9-100.3% respectively. This newly developed method was successfully utilized for the Quantitative estimation of Azilsartan and Chlortalidone in tablet dosage form. This method was validated for selectivity, accuracy, precision, linearity and Robustness as per ICH guidelines.

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Conflict of interests

The authors claim that there is no conflict of interest.

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