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Original Research Article

SIMULTANEOUS ESTIMATION OF AZILSARTAN AND CHLORTHALIDONE IN TABLET DOSAGE FORM BY VALIDATED STABILITY INDICATING RP-HPLC METHOD

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Conflict of interest: Nil

Abstract

Objective: To develop and validate a simple, efficient and cost-effective stability indicating RP-HPLC method for simultaneous estimation of Azilsartan and Chlorthalidone in Tablet dosage form. **Methods:** Phosphate buffer (ph4.0)-Methanol (60:40) used as mobile phase and stationary phase (250*4.6mm C18, Hypersil BDS) column, wavelength was selected 236 nm, Flow rate 1.0 ml/ min, injection volume 20 µl. Prepared Standard solution and sample solution at working concentration, used Mobile phase as diluent. **Results:** Elution order of both peaks, Chlorthalidone (Retention time 3.470 min.) eluted first and Azilsartan (Retention time 5.743 min.) second with good resolution and fulfil System suitability parameters. Precision results shows % Relative standard deviation of Azilsartan and Chlorthalidone 0.8 and 1.2 respectively. Linearity results of Azilsartan and Chlorthalidone found acceptable in range 20.0 µg/ml to 120 .0 µg/ml and 12.5 µg/ml to 75.0 µg/ml, respectively. Calibration curve shows good linearity and Correlation coefficient was 0.9999 of Azilsartan and Chlorthalidone. Recovery results of Azilsartan and Chlorthalidone from matrix of tablet formulation were 100.3% and 100.2%, respectively. Robustness and ruggedness results were found well within the acceptance limit. **Conclusion:** The results shows that the proposed simple, precise and accurate method can be successfully applied for simultaneous estimation of Azilsartan and Chlorthalidone in Tablet dosage form.

Keywords: Azilsartan and Chlorthalidone, RP-HPLC, Stability indicating.

Introduction

This Azilsartan Medoxomil and Chlorthalidone fixed-dose combination is found to show superior antihypertensive efficacy in blood pressure reduction in patients with stage 2 hypertension when compared with the maximum approved dose of Olmesartan/hydrochlorothiazide [2]. Azilsartan Medoxomil is an Angiotensin II receptor antagonist which has the chemical name (5 – Methyl – 2 – oxo -1,3 – dioxol -4 – yl) methyl 2 – ethoxy -1 – {[2'- (5 - oxo -4,5 – dihydro - 1, 2, 4 – oxadiazol -3 - yl) biphenyl – 4 – yl] methyl } - 1H – benzimidazole -7 – carboxylate monopotassium salt.It is a white crystalline powder which isfreely soluble in methanol, dimethyl sulfoxide and dimethyl formamide, soluble in acetic acid, slightly soluble in acetone and acetonitrile and very slightly soluble in

tetrahydrofuran and 1-octanol, practically insoluble in water.

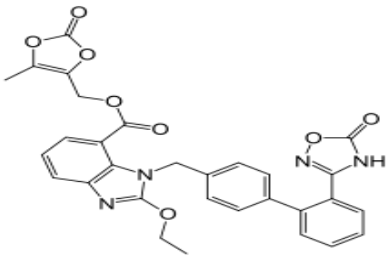

Fig.1: Structure of Azilsartan Medoxomil

Chlorthalidone is used in the treatment of hypertension, it is a thiazide diuretic drug which inhibits Na+ and Cl- ions reabsorption in the distal convoluted tubule by blocking the Na+ /Cl-Symporter. IUPAC name was (RS)-2-Chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1H-isoindol-1-yl) benzene-1sulfonamide with molecular formula C14H11ClN2O4S. Chlorthalidone was soluble in Methanol, water and DMSO.

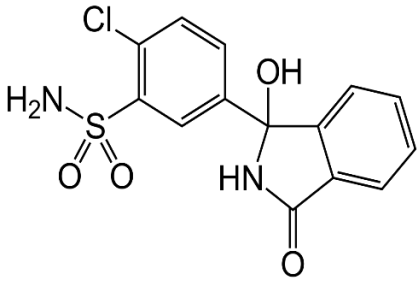

Fig. 2: Structure of Chlorthalidone

According to literature two methods were available in which madhu et al., the retention time for Chlorthalidone and Azilsartan Medoxomil were 3.923min and 7.208 min respectively [3]. Naazneen et al., the retention time for Chlortalidone and Azilsartan Medoxomil were 2.36±0.1 mins and 5.54±0.5 mins respectively. [4]

MATERIAL AND METHODS

Table 1: Instruments details

HPLC	**Make**	Shimadzu
	Pump	LC-20 AT
	Software	SpinchromCFR Software
	Column	HypersilBDS C18
UV-Visible Spectrophotometer	**Make**	Systronic
	Model	119
Analytical Balance	**Make**	Shimadzu
	Model	ATX-224
pH Meter	**Make**	A.L. Scientific Pvt Ltd

Table 2: Chemical and Regents details

Sr. No.	Chemical Name	Make	Grade
1	Water	Milli-Q	HPLC
2	Potassium dihydrogen phosphate	Merck	AR
3	Ortho phosphoric acid	Merck	HPLC
4	Methanol	Rankem	HPLC

Table 3: Drug substances and Drug product details

Name of Drug and Drug Product	Supplier and Manufacturer
Azilsartan Medoxomil	Molecule Laboratory
Chlorthalidone	Molecule Laboratory
Azilsartan Medoxomil and Chlorthalidone Tablets	Emcure Pharmaceuticals Ltd.

METHOD DEVELOPMENT

Wavelength selection

Azilsartan Medoxomil Standard Solution

Weigh equivalent 40 mg of Azilsartan Standard and transferred in to 100 ml volumetric flask. Added 70 ml of methanol and sonicated to dissolve it. Dilute to volume with methanol and mixed well. Further pipette 1.0 ml and dilute up to 10 ml with methanol and mixed well. (40 µg/ml).

Chlorthalidone Standard Solution

Weigh equivalent 25 mg of Chlorthalidone Standard and transferred in to 100 ml volumetric flask. Added 70 ml of methanol and sonicated to dissolve it. Dilute to volume with methanol and mixed well. Further pipette 1.0 ml and dilute up to 10 ml with methanol and mixed well. (25 µg/ml)

Procedure:

Taken UV spectra of above two solutions individually between the range of 200nm-400nm using methanol as a blank. Overlay both the spectra and find iso-absorptive point.

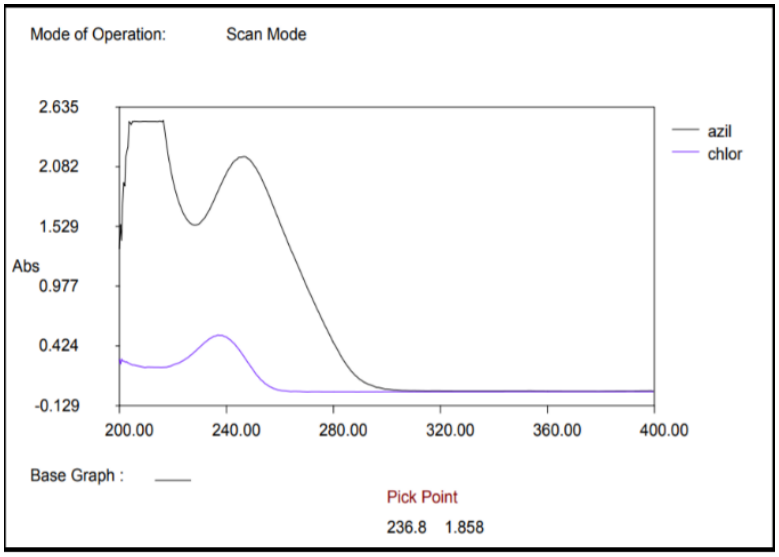

Fig. 3: UV Spectra of Azilsartan Medoxomil and Chlorthalidone

Mobile phase Selection

By the literature survey it is found that Buffer pH 3.00 to 5.00 suitable for the elution of both components.Taken trials by using different pH buffers and methanol mixture to find better resolution between both components. Finally optimized below chromatographic condition.

Table 3: Mobile phase

Mobile Phase	Buffer pH (4.0) and Methanol (60:40)
Column	Hypersil BDS 250*4.6mm C18,
Flow rate	1.0 ml/ min
Column temperature	25°C
Injection volume	20 µl
Wavelength	236 nm
Run Time	10 min
Diluent	Used Mobile Phase

Buffer preparation (0.05M potassium dihydrogen phosphate, ph-4.0)

Taken about 6.8gm potassium dihydrogen ortho phosphate reagent into a 1000ml beaker. Add 800ml water and dissolve. Adjust pH 4.0 of this solution with Orthophosphoric acid. Make up volume up to 1000 ml with water.

Mobile Phase

Prepare a mixture of Buffer and Methanol in the ratio of 60:40 ml (%v/v) and mix well. Degas it by sonication.

Diluent: Used Mobile phase as diluent.

STANDARD PREPARATION

Azilsartan Medoxomil Standard Stock Solution

Weigh equivalent 40 mg of Azilsartan Medoxomil Standard and transferred in to 100 ml volumetric flask. Added 70 ml of methanol and sonicated to dissolve it. Dilute to volume with methanol and mixed well. (400 µg/ml)

Chlorthalidone Standard Stock Solution

Weigh equivalent 25 mg of Chlorthalidone Standard and transferred in to 100 ml volumetric flask. Added 70 ml of methanol and sonicated to dissolve it. Dilute to volume with methanol and mixed well. (250 µg/ml)

Standard Solution

Pipette each 1.0 ml of Azilsartan Medoxomil Standard Stock Solution and Chlorthalidone Standard Stock Solution and transfer in to 10 ml volumetric flask. Dilute up to volume with diluent and mixed well

Sample preparation

Sample stock solution: Weigh and powdered 20 tablets. Take tablet powder equivalent to 40 mg Azilsartan Medoxomil /25 mg Chlorthalidone in to a 100ml volumetric flask. Add 60 ml methanol. Shake for 15 minutes and sonicate for 10 minutes. Make up volume with methanol. Filter this solution with Whatman filter paper no-1. (Chlorthalidone 250 µg/ml, Azilsartan Medoxomil 400 µg/ml)

Working sample preparation

Taken 1.0 ml from sample stock solution into a 10 ml volumetric flask and make up with diluent. (Chlorthalidone 25 µg/ml, Azilsartan Medoxomil 40 µg/ml) Retention time of Azilsartan Medoxomil and Chlorthalidone found 3.470 min and 5.743 min, respectively and % Relative standard deviation found 0.77 and 0.62%, respectively.

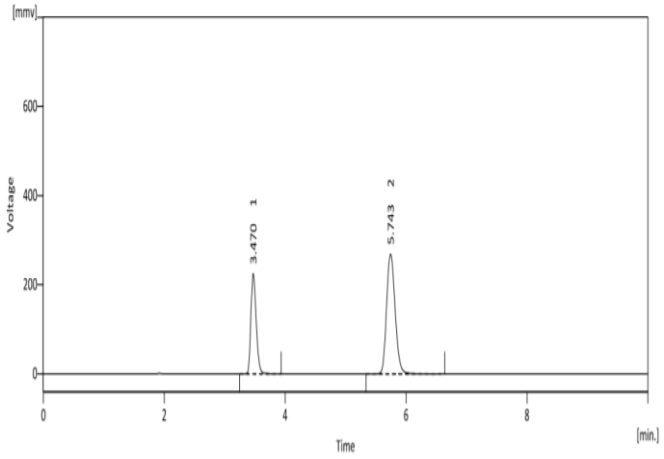

Fig. 4: Chromatogram of Standard

Table 4: System Precision Results

Injection	Chlorthalidone 25 µg/ml Area	Azilsartan Medoxomil 40 µg/ml
1	1365.556	2628.227
2	1355.670	2633.585
3	1375.926	2586.921
4	1378.688	2644.136
5	1371.784	2630.867
6	1374.543	2636.221
Mean	1370.361	2626.660
Std Dev.	8.4704	20.2194
%RSD	0.62	0.77

METHOD VALIDATION

Method validation was carried out by as per ICH guidelines. Parameters included Specificity, Linearity, Method Precision, Intermediate Precision, Accuracy and Robustness.

Forced degradation Study

Forced degradation study performed by Chemical and Physical degradation like Acid stress, Alkali stress, Oxidation stress, Heat stress and Light stress condition.

RESULTS AND DISCUSSION

Specificity

Specificity has been evaluated by assuring no interference observed at the retention time of Azilsartan and chlorthalidone peak in the chromatogram obtained from the Blank solution,Standard solution andSample solution.

Linearity

Linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in Sample. The linearity of Azilsartan Medoxomil and Chlorthalidone are established by analysing linearity solutions of different concentration from 50% to 300% of working concentration of assay method. The linearity curve is plotted of peak area versus concentration. The results are summarized in Table 5. The linearity graph of Azilsartan Medoxomil and Chlorthalidone are shown in Fig. 5 and Fig.6, respectively.

Table 5: Linearity result

L. Level	Azilsartan Medoxomil		Chlorthalidone	
	Con.µg/ml	Area	Con.µg/ml	Area
50 %	20	1306.184	12.5	681.427
80 %	30	1929.307	18.75	1006.184
100 %	40	2638.816	25	1375.925
120 %	50	3250.319	31.25	1694.389
150 %	60	3955.194	37.5	2061.696
250 %	100	6595.817	62.5	3451.063
300 %	120	7862.973	75	4114.731
CC	0.9999		0.9999	
Slope (B)	65.879		55.224	
Y-Intercept	18.632		15.829	

L.Level = Linearity level; CC = Correlation Coefficient

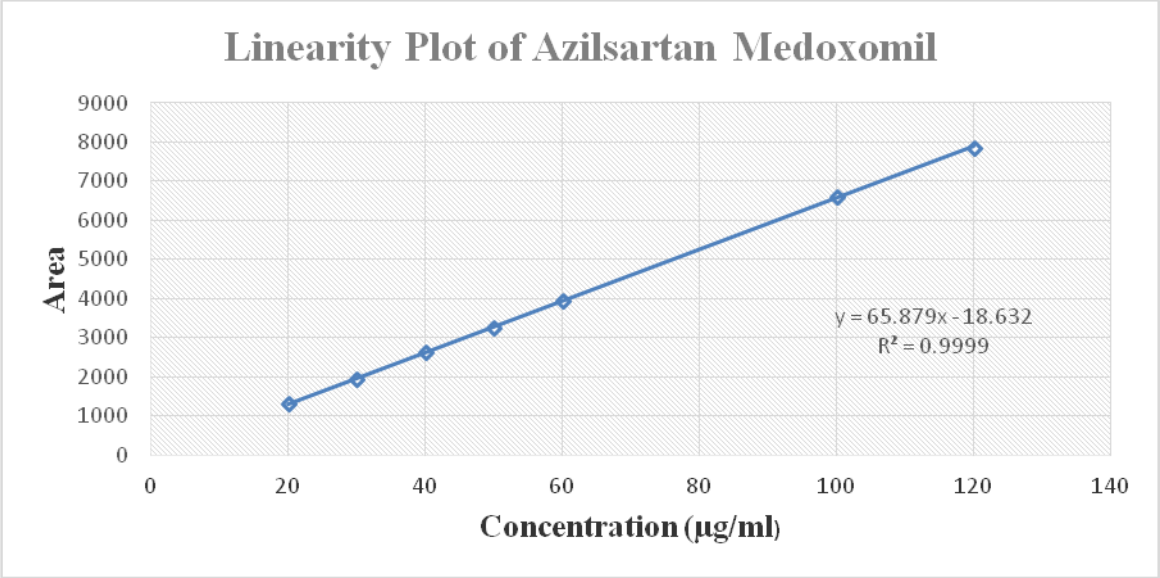

Fig. 5: Linearity curve of Azilsartan Medoxomil

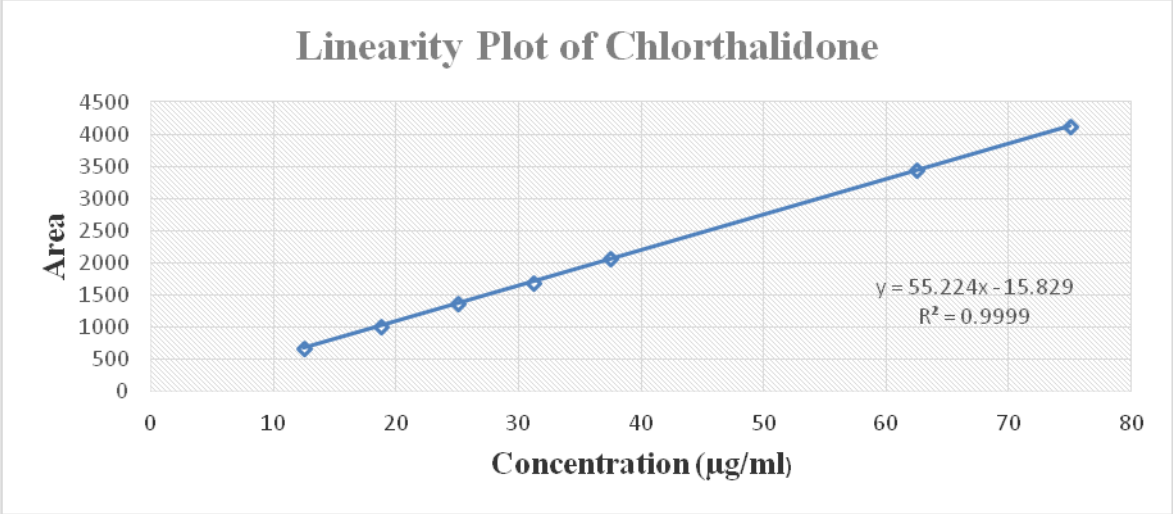

Fig. 6: Linearity curve of Chlorthalidone

Method Precision

The precision of analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of the same sample under the prescribed condition.

Table 6: Results of Method Precision

Sr. No.	Azilsartan Medoxomil		Chlorthalidone	
	RT (Min.)	% Assay	RT (Min.)	% Assay
1	3.457	98.9	5.717	96.7
2	3.460	97.2	5.720	99.1
3	3.467	99.4	5.733	99.4
4	3.453	98.9	5.710	99.9
5	3.457	98.2	5.713	98.8
6	3.450	98.4	5.707	99.6
Mean		98.5		98.9
SD		0.7642		1.1514
% RSD		0.8		1.2

Intermediate Precision

Table 7: Results of Intermediate Precision

Sr. No.	Azilsartan Medoxomil		Chlorthalidone	
	Precision	Intermediate Precision	Precision	Intermediate Precision
1	98.9	99.5	96.7	98.0
2	97.2	97.8	99.1	99.7
3	99.4	99.5	99.4	99.5
4	98.9	97.8	99.9	98.6
5	98.2	99.7	98.8	100.5
6	98.4	101.2	99.6	102.8
Mean	98.5	99.3	98.9	99.9
SD	0.7642	1.2911	1.1514	1.6885
% RSD	0.8	1.3	1.2	1.7
Overall Mean	98.9		99.4	
Overall SD	1.0847		1.4615	
Overall % RSD	1.1		1.5	

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value that is accepted either as a conventional true value or an accepted reference value and the value found. To demonstrate the accuracy of this method by spiking Azilsartan Medoxomil and chlorthalidone standard solution into sample solution are added quantitatively from 50% to 150% for Azilsartan Medoxomil and chlorthalidone of working concentration of this method at each level with triplicate preparation and analysed using the test method. The results are tabulated Table no 8.

Table 8: Accuracy results

Recovery level	Azilsartan Medoxomil			Chlorthalidone		
	% Recovery	Mean % Recovery	%RSD	% Recovery	Mean % Recovery	%RSD
50 % Set-1	101.7	100.3	1.6	101.8	100.2	1.6
50 % Set-2	98.5			98.5		
50 % Set-3	100.6			100.2		
100% Set-1	101.4	100.5	1.1	100.8	101.0	0.6
100% Set-2	100.8			101.7		
100% Set-3	99.3			100.6		
150% Set-1	100.6	100.0	0.7	100.2	99.5	0.8
150% Set-2	99.3			98.6		
150% Set-3	100.1			99.6		
Mean % recovery	100.3			100.2		
% RSD	1.0			1.2		

Robustness

Robustness study was performed by analysing the standard at different conditions the results obtained with altered conditions were compared against results obtained under normal chromatographic condition.

Table 9: Robustness results of Azilsartan Medoxomil

Change in Parameters	Value	Retention time (min.)	Asymmetry factor	Theoretical Plates	% RSD
Control	As Such	5.717	1.270	7376	0.8
Flow rate	+ 0.2 ml/ min	5.573	1.278	7648	1.1
	0.2 ml/min	5.920	1.231	7584	1.0
Mobile Phase Composition	+2 % Solvent	5.633	1.243	7814	1.0
	-2 % Solvent	5.920	1.231	7584	1.1
Buffer pH	+ 0.2 pH	5.460	1.222	7678	1.1
	-0.2 pH	5.863	1.205	7760	1.3

Table 10: Robustness results of Chlorthalidone

Change in Parameters	Value	Retention time (min.)	Asymmetry factor	Theoretical Plates	% RSD
Control	As Such	3.457	1.409	7084	0.6
Flow rate	+ 0.2 ml/ min	3.370	1.364	7223	1.7
	-0.2 ml/min	3.577	1.391	7087	1.5
Mobile Phase Composition	+2 % Solvent	3.403	1.409	6867	1.9
	-2 % Solvent	3.577	1.435	7087	1.8
Buffer pH	+ 0.2 pH	3.300	1.381	6926	1.2
	-0.2 pH	3.543	1.348	6956	1.9

Forced degradation

To demonstrate stability indicating properties of the method force degradation was conducted by applying heat light, acid alkali and oxidation stress to the drug product.

Table 11: Forced degradation study results

	Azilsartan Medoxomil		Chlorthalidone	
	% Assay	% Degradation	% Assay	% Degradation
As such	97.8	-	98.3	-
Acid Stress	75.4	22.4	84.0	14.3
Alkali Stress	74.0	23.8	87.7	10.6
Oxidation Stress	80.9	16.9	77.7	20.6
Heat Stress	96.1	1.7	96.8	1.5
Light Stress	96.5	1.3	97.6	0.7

CONCLUSION:

The results show that the proposed simple, precise and accurate stability indicating method can be successfully applied for simultaneous estimation of Azilsartan and Chlorthalidone in Tablet dosage form. During validation study method was found specific, precise, accurate, rugged and robust for the intended use.

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