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Original Article

STABILITY-INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF AZILSARTAN MEDOXOMIL AND CHLORTHALIDONE IN SOLID DOSAGE FORMS

S. NAAZNEEN[1], A. SRIDEVI[2]

[1]Assistant Professor, St Mary's College of Pharmacy, Secunderabad, Andhra Pradesh, India. Email: naazneen16_shaik@yahoo.co.in. [2]Associate Professor, IPT, Sri Padmawathi Mahila viswavidyalayam, Tirupathi, Andhra Pradesh, India. Email: naazneen16_shaik@yahoo.co.in

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ABSTRACT

Objective: The main objective of the proposed study was to develop and validate a new stability indicating reverse phase HPLC method for the simultaneous estimation of Azilsartan Medoxomil and Chlorthalidone in solid dosage forms.

Methods: The method was optimized by using BDS C_{18} column (100 x 4.6 mm, 5µ, Hypersil). Phosphate Buffer and Acetonitrile were used as Mobile Phase in the proportion of 90:10. Phosphate buffer pH was adjusted with Orthophosphoric acid. Methanol was used as diluent. The flow rate was 0.9 ml/min and the effluent was monitored at 260nm.

Results: The retention time of Azilsartan Medoxomil and Chlorthalidone was 2.36±0.1 mins and 5.54±0.5 mins respectively. Precision showed that % Relative standard deviation of Azilsartan Medoxomil and Chlorthalidone was about 0.44 and 0.7 respectively. The percentage recoveries of both the drugs Azilsartan Medoxomil and Chlorthalidone from the tablet formulation were 100.16% and 99.88% respectively. Linearity of Azilsartan Medoxomil and Chlorthalidone was in the range of 10.0 to 60.0µg/ml and 6.25 to 37.5µg/ml respectively. Calibration curve showed good linearity and range. The Correlation Coefficient of Azilsartan Medoxomil and Chlorthalidone was 0.999 each. And the results obtained for LOQ, LOD, Robustness and Ruggedness were well within the acceptance criteria.

Conclusion: The proposed method was found to be simple, rapid, accurate and precise. It was found to be economical and suitable for simultaneous determination of Azilsartan Medoxomil and Chlorthalidone in pharmaceutical dosage form.

Keywords: Azilsartan medoxomil, Chlorthalidone, RP-HPLC, Simultaneous estimation, buffer pH 3.2, Acetonitrile, Forced degradation.

INTRODUCTION

This Azilsartan Medoxomil and Chlorthalidone fixed-dose combination [1] 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 is practically insoluble in water, freely soluble in methanol, dimethyl sulfoxide and dimethyl formamide, soluble in acetic acid, slightly soluble in acetone and Acetonitrile and very slightly soluble in Tetra Hydro furan and 1- octanol.

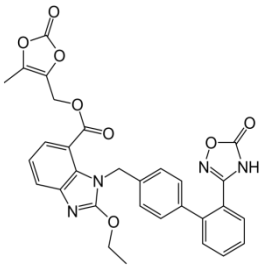

Fig. 1: Structure of Azilsartan Medoxomil

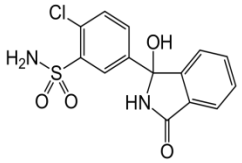

Fig. 2: Structure of Chlorthalidone

It is US FDA approved as Edarby tablets on 25th Feb 2011, to treat hypertension in adults.

It is available in 40mg and 80mg dosages, with the recommended dosage at 80mg once in a day. The active moiety of Azilsartan Medoxomil is released by hydrolysis of medoxomil ester. It is an active ARB (AT1) type and is more effective in lowering blood pressure within 24 hours as compared to other ARBs. Azilsartan Medoxomil an ARB is combined with Chlorthalidone, a thiazide type diuretic in treating hypertension significantly when compared to other fixed dose antihypertensive combination without the difference in safety measurements. Chlorthalidone is practically insoluble in water, ether and chloroform, soluble in methanol and slightly soluble in alcohol. It is a thiazide type diuretic used to treat hypertension. It acts similarly to the thiazides in causing diuresis but does not have benzothiadiazine moiety in it. It acts at the proximal portion of the distal convoluted tubule of the nephron and shows longest duration of action when compared to other thiazide diuretics.

The literature survey shows that spectroscopic and chromatographic methods [3, 4, 5, 6] for individual drugs but there is only a single method available for quantitation of Azilsartan Medoxomil and Chlorthalidone in solid dosage forms simultaneously. Thus it is inevitable to develop [11] such a sensitive, accurate, precise, rapid and economical method for routine analysis of this combination in pharmaceutical dosage form successfully.

MATERIALS AND METHODS

Instrumentation

A high performance liquid chromatography system consisting of Waters 2695 Separation (Alliance)Module with PhotoDiode Array detector was used with data handling system Empower Pro.

Chemicals were weighed using Analytical balance Afcos, ER-180A, Sartorius-M500P, Mettler, AG 104. All pH measurements were done on pH meter Metrohm model 645, Herisau.

Reagents and Chemicals

HPLC grade solvents methanol, orthophosphoric acid, triethylamine, Acetonitrile and water were obtained from Merck Specialities Pvt Ltd, India. Water was deionised and further purified by means of Milli-Q plus water purification system, Millipore Ltd (U.S.A). AR grade Potassium dihydrogen Orthophosphate was obtained from Ranchem Pharmaceuticals India Ltd.

Azilsartan Medoxomil and Chlorthalidone were obtained as pure standards and samples [tablets of Azilsartan Medoxomil (40mg) and Chlorthalidone (25mg)] from Spectrum Labs Pvt Ltd, Hyderabad, India.

Chromatographic conditions and measurement procedure

Preparation of buffer (pH 3.2)

Accurately weighed and transferred 2.72gm of Potassium dihydrogen Orthophosphate in a 1000ml of volumetric flask, about 900ml of Milli-Q water was added along with 1ml of triethylamine and sonicated to degas and finally made up the volume with water. Then pH was adjusted to 3.2 with dil. ortho phosphoric acid solution. The solution was filtered through 0.45 µm membrane filter.

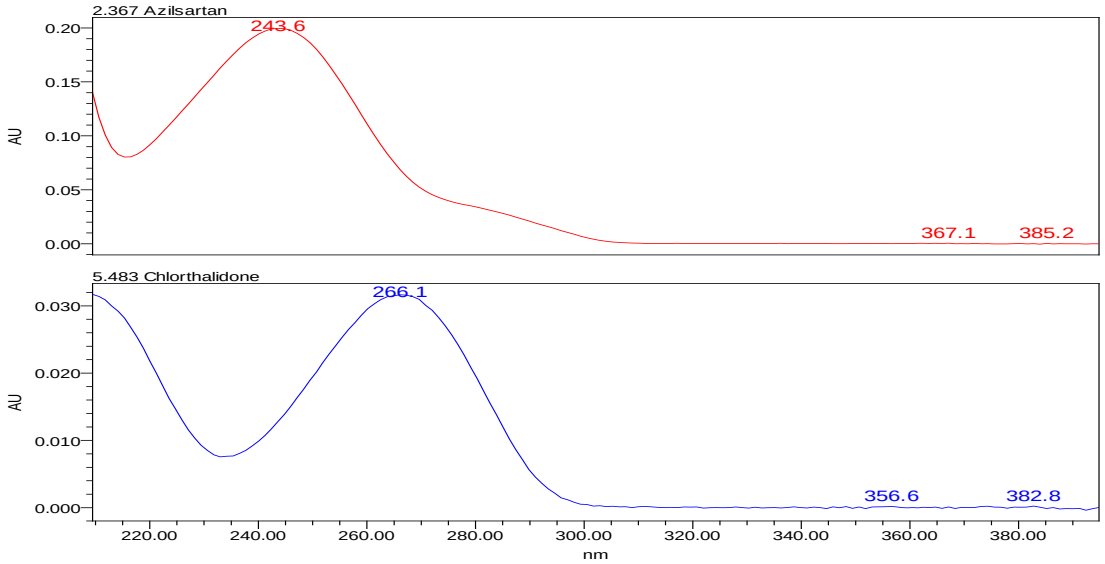

Fig. 3: UV Spectrum of Azilsartan Medoxomil and Chlorthalidone

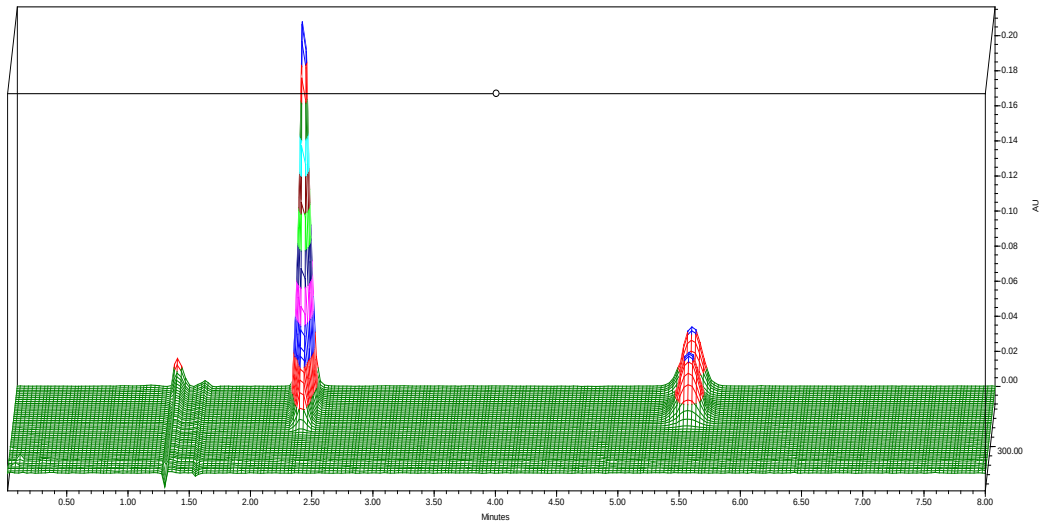

Fig. 4: Representative chromatogram of Azilsartan Medoxomil and Chlorthalidone

Preparation of Mobile phase

A filtered and degased mixture of buffer pH 3.2 and Acetonitrile in the ratio of 90:10 (v/v) was prepared and used as mobile phase.

Standard preparation

Accurately weighed and transferred 40mg of Azilsartan Medoxomil and 25mg of Chlorthalidone working standards into a 100 ml clean dry volumetric flask, 70ml of diluent was added and sonicated to dissolve and the final volume made up with diluent. The solution was filtered through 0.45um PVDF filter. From the filtered solution

0.2ml was pipetted out into a 10 ml volumetric flask and made upto 10.0ml with diluent.

Sample preparation

Twenty tablets were weighed accurately and their average weight calculated. They were ground to fine powder. A quantity of powder equivalent to 200mg of Azilsartan Medoxomil and 125mg of Chlorthalidone was accurately weighed and transferred into 100ml volumetric flask. About 70ml of diluent was added and sonicated for 30 minutes with intermediate shaking. Cooled to room temperature and diluted to volume with diluent. The solution was filtered through 0.45um PVDF filter. From the filtered solution 0.2ml was pipetted out into a 10 ml volumetric flask and made upto 10.0ml with diluent.

Selection of wavelength maxima

Azilsartan Medoxomil showed absorption maxima at 243.6 nm and Chlorthalidone showed at 266.6 nm. For simultaneous estimation of both the drugs Azilsartan Medoxomil and Chlorthalidone a common wavelength was selected as absorption maxima at 260nm. Fig3.

Method Development [7, 12]

By using the chromatographic conditions that were used for assay of Angiotensin- II blocker as reference, various trials were made. Each trial mixture of known components were injected and observed for resolution and tailing factor of the peaks. Various proportions of buffer and Acetonitrile were tried as mobile phase and a ratio of buffer to Acetonitrile as 90:10 gave improved peak symmetry and resolution. Different flow rates of the mobile phase were tried for good resolution. Both the drugs Azilsartan Medoxomil and Chlorthalidone were found to be soluble and stable in a mixture of buffer pH3.2 and Acetonitrile. Finally the chromatographic conditions were optimized at flow rate 0.9ml/min, injection volume of 5 µL, run time of 8 minutes, at column oven temp 30°C with methanol (sonicated and degased) as diluent in a Hypersil BDS, C18, (100mm x 4.6mm), 5 µm column. **Fig 4**

The %RSD for both the drugs Azilsartan Medoxomil and Chlorthalidone were found to be 0.4 and 0.3 respectively and tailing factor was < 1. **Table 1**

The retention time for Azilsartan Medoxomil and Chlorthalidone was found to be 2.3 minutes and 5.5 minutes respectively. Absorption maximum was found to be 260nm. And peaks shape was good.

The method was further validated [8, 9] under the chromatographic conditions.

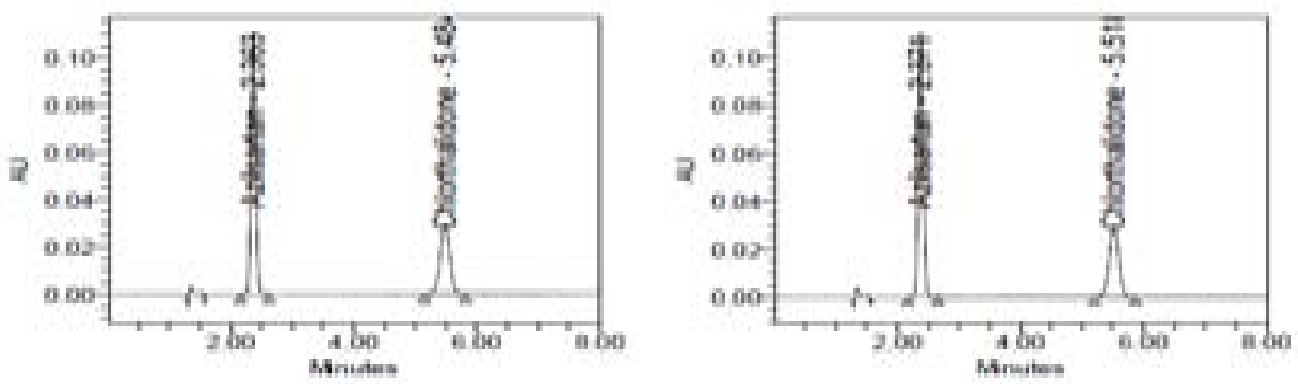

Fig. 5: Chromatograms of standard sample

Table 1: Retention time of Azilsartan Medoxomil and Chlorthalidone

Peak Name: Azilsartan

	Peak Name	RT	Area	USP Plate Count	USP Tailing
1	Azilsartan	2.360	590663	4501	1.09
2	Azilsartan	2.362	591071	4520	1.09
3	Azilsartan	2.363	590333	4493	1.10
4	Azilsartan	2.367	587593	4291	1.10
5	Azilsartan	2.370	589391	4379	1.09
6	Azilsartan	2.371	591960	4445	1.09
Mean			590169		
Std. Dev.			1518.5		
% RSD			0.3		

Peak Name: Chlorthalidone

	Peak Name	RT	Area	USP Plate Count	USP Tailing
1	Chlorthalidone	5.484	318106	5902	0.99
2	Chlorthalidone	5.485	319983	5930	1.00
3	Chlorthalidone	5.504	319049	5861	0.99
4	Chlorthalidone	5.507	317735	5949	0.99
5	Chlorthalidone	5.518	321473	6026	0.99
6	Chlorthalidone	5.533	318691	5797	0.99
Mean			319173		
Std. Dev.			1371.1		
% RSD			0.4		

Method Validation

Once chromatographic conditions were established, the method was validated [8, 9, 10] in compliance with ICH guidelines. The following parameters like system suitability along with specificity, linearity, precision, accuracy, limits of detection and limit of quantification were performed for validation. The specificity of the method was described as the ability to discriminate the analyte from all potential interfering substances (i.e. excipients) in the tablet dosage form. This test was performed by recording chromatograms of placebo blank solution and drug mixture spiked in the placebo solution. The placebo blank solution was prepared by mixing the corresponding tablet excipients such as microcrystalline cellulose, magnesium stearate, starch, lactose, Tween 20, polysorbate 80, and sodium starch glycolate (SSG). It can be seen from the chromatogram, that no peaks were observed in the placebo blank solution and percentage recovery of drugs spiked in placebo blank solution indicating that no interference due the excipients for the recovery of the analytes occurred. A study to evaluate the interference of placebo was conducted. Samples were prepared in duplicate by taking placebo equivalent to the weight present in portion of test preparation as per the test method and injected into the HPLC system. It was observed that there were no peaks interfering with the analyte peak. The chromatogram indicates that the peak is homogeneous, there is no interference from the excipients at the retention time of analyte peak and has no coeluting peaks indicating specificity of the method. For the analytical method, determination of assay specificity was also demonstrated by performing force degradation study of placebo and drug product under various stress conditions like Acid degradation, Alkali degradation, Oxidative degradation, Photolytic degradation and Thermal degradation.

Forced degradation studies

Oxidation

To 1 ml of stock solution of Azilsartan Medoxomil and Chlorthalidone, 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^{0}c. For HPLC study, the resultant solution was diluted to obtain 40µg/ml & 25µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies

To 1ml of stock solution of Azilsartan Medoxomil and Chlorthalidone, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^{0}c .The resultant solution was diluted to obtain 40µg/ml & 25µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies

To 1 ml of stock solution Azilsartan Medoxomil and Chlorthalidone, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^{0}c. The resultant solution was diluted to obtain 40µg/ml & 25µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies

The standard drug solution was placed in oven at 105^{0}c for 6 hours to study dry heat degradation. For HPLC study, the resultant solution was diluted to 40µg/ml & 25µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies

The photochemical stability of the drug was also studied by exposing the 100 µg/ml solution to UV Light by keeping the beaker in UV Chamber. for 7days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 40µg/ml & 25µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

RESULTS AND DISCUSSION

System Suitability

The standard solution was prepared by using working standard as per the method. For six replicate injections system suitability parameters like number of theoretical plates, USP Tailing and % RSD were found to be within specified limits.

Specificity

A study was carried out by spiking all impurities at 1% of sample concentration in sample solution and peak purity was evaluated. It

was observed that there were no peaks interfering with the analyte which was evident from the purity data. The difference between the assay results of spiked sample solution and unspiked sample solution was made as per the test method. The results were found to be within the specified limits. The results are tabulated in **Table 3**. The % assay difference was < 2. It is evident from the data that all the impurities are well resolved from each other and all the peaks are pure. Hence the method is specific.

Table 2: Results of System suitability

S. No.	Drug	Retention Time (min)	Column efficiency(Number of theoretical plates)	USP Tailing	%RSD for replicate injections
1	Azilsartan Medoxomil	2.36	590169	1.09	0.3
2	Chlorthalidone	5.45	319173	0.99	0.4

Table 3: Results of specificity

Specificity Mean % assay	Azilsartan Medoxomil	Chlorthalidone
Control sample, Method precision (n-6)	100.24	99.75
sample spiked with impurities (n-3)	100.16	99.98
% assay difference w.r.t. Method precision	0.08	0.23

Table 4: Results of Linearity

Levels	Concentration (µg/mL)		Average area counts(uV*sec)	
	Azilsartan Medoxomil	Chlorthalidone	Azilsartan Medoxomil	Chlorthalidone
Level-25%	10	6.25	149348	82706
Level-50%	20	12.5	301440	163527
Level-75%	30	18.75	440997	240983
Level-100%	40	25	587726	319173
Level-125%	50	31.25	732719	395048
Level-175%	60	37.5	885502	478767
Correlation Coefficient			0.9999	0.9999
Slope(B)			14677	12666
Y-Intercept			2228.7	2545.8

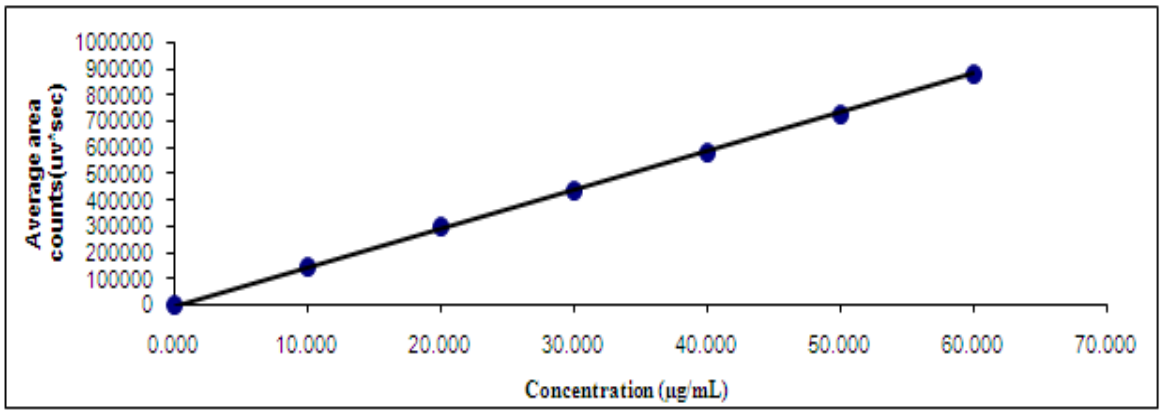

Fig. 5: Linearity Curve of Azilsartan Medoxomil

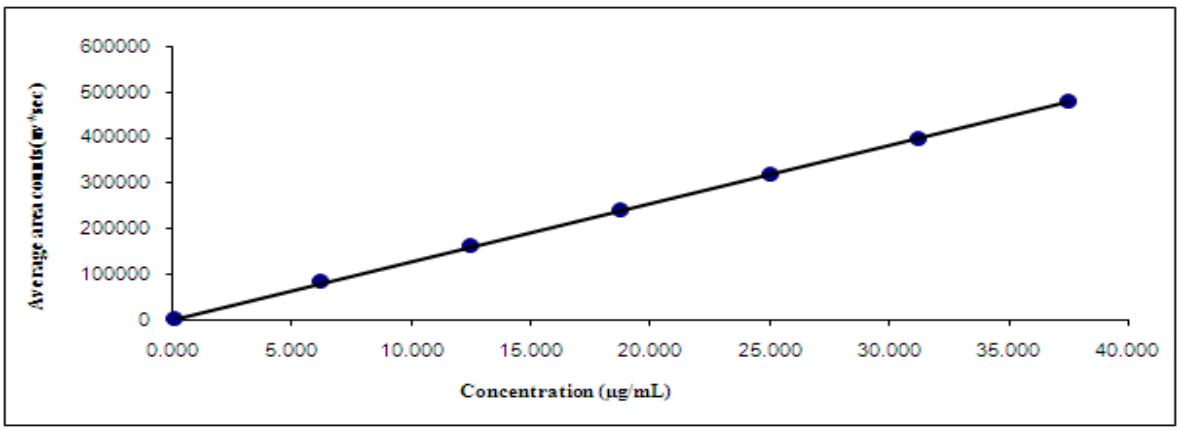

Fig. 6: Linearity Curve Of Chlorthalidone

Table 5: LOD and LOQ

% Level.	Azilsartan Medoxomil		Chlorthalidone	
	Concentration (µg/ml)	**Average area counts (uv*sec)**	**Concentration (µg/ml)**	**Average area counts(uv*sec)**
LOQ	0.080	1176	0.100	1285
	10	149348	6.25	82706
	20	301440	12.5	163527
	30	440997	18.75	240983
	40	587726	25	319173
	50	732719	31.25	395048
	60	885502	37.5	478767
	Slope	14677	**Slope**	12666
	Intercept	2228.7	**Intercept**	2545.8
	Correlation coefficient	0.9999	**Correlation coefficient**	0.9999
LOD(ppm)	0.027		0.033	

Linearity

Linearity of detector response was established by plotting graph between concentrations versus average area counts of the analytes. Data shown in **Table 4** and represented graphically in Graph **Fig 5 and Fig 6** indicate that the response is linear over the specified range.

Accuracy

A study of accuracy (recovery) was performed on known amount of placebo by spiking active pharmaceutical ingredient. Samples were prepared as per the proposed method at 50% to 150% of the sample concentration. Data shown indicate that the method has an acceptable level of accuracy.

Precision

System precision

Six replicate injections of standard solution were injected into the HPLC system. The %RSD for six replicated injections was found to be in the limits.

Method precision

The precision of test method was evaluated by analyzing assay for six individual samples prepared from same batch by the proposed method. The average % Assay and the relative standard deviation for the six sample preparation was found to be in the specified limits.

Table 6: Results of Accuracy (Recovery Studies)

	Azilsartan Chlorthalidone (40 mg)					Chlorthalidone (25 mg)				
Recovery Levels	**Amout added (mg)**	**Amount recovered (mg)**	**% Recoverd**	**Mean % recover**	**% RSD**	**Amount added (mg)**	**Amount recovered (mg)**	**% Recoverd**	**Mean % recovery**	**% RSD**
50% Rec-1	20.58	20.14	97.9	99.3	2.38	12.85	12.68	98.7	99.1	0.40
50% Rec-2	20.64	21.06	102.0			12.64	12.54	99.2		
50% Rec-3	20.95	20.54	98.0			12.94	12.87	99.5		
100% Rec-1	40.28	40.58	100.7	102.1	2.22	25.61	25.14	98.2	100.5	00.1
100% Rec-2	40.31	40.62	100.8			25.34	25.95	102.4		
100% Rec-3	39.12	40.95	104.7			25.07	25.34	101.1		
150% Rec-1	60.85	60.84	100.0	99.9	0.24	37.95	37.64	99.2	99.7	0.53
150% Rec-2	60.24	59.99	99.6			37.64	37.51	99.7		
150% Rec-3	60.84	60.85	100.0			37.02	37.11	100.2		

Table 7: Results of System precision

Injection No	**Azilsartan Medoxomil Area counts (uv*sec)**	**Chlorthalidone Area counts (uv*sec)**
1	590663	318106
2	591071	319983
3	590333	319049
4	587593	317735
5	589391	321473
6	591960	318691
Mean	590169	319173
%RSD	0.26	0.43

Table 8: Results of Method precision

Vessel No	**% Assay**	
	Azilsartan Medoxomil	**Chlorthalidone**
1	99.85	100.95
2	99.84	99.17
3	100.03	99.69
4	100.71	99.22
5	100.86	100.18

6	100.13	99.31
Mean	100.24	99.75
SD	0.4417	0.6985
%RSD	0.44	0.70

Table 9: Results of Ruggedness

S. No.	Azilsartan Medoxomil		Chlorthalidone	
	Assay (mg/tab)		Assay (mg/tab)	
	Analyst-I	Analyst-II	Analyst-I	Analyst-II
1	99.85	99.12	100.95	98.25
2	99.84	100.42	99.17	99.12
3	100.03	99.80	99.69	98.67
4	100.71	99.86	99.22	100.58
5	100.86	100.05	100.18	101.84
6	100.13	99.94	99.31	98.61
Mean	100.24	99.87	99.75	99.5
SD	0.4417	0.43	0.6985	1.40
RSD (%)	0.44	0.43	0.70	1.4
Overall Mean	100.05		99.63	
Overall SD	0.46		1.06	
Overall RSD(%)	0.5		1.1	

Intermediate precision (Ruggedness)

The ruggedness of method was verified by conducting the precision study by using different HPLC, different columns of same make by different analyst on different days. Six samples of same batch were prepared and analysed by the proposed method. The mean, standard deviation, and %RSD for the two sets of data are shown in **Table 9.** .Ruggedness of the method is indicated by the overall RSD between the two sets of data.

Robustness

Robustness of the method was investigated **Table 10** by varying the instrumental conditions such as flow rate (±10%), column oven temperature (±5%), wave length of detection (±5nm), organic content in mobile phase (±2%) and pH of buffer in mobile phase (±0.2 units). Standard solution was prepared and analysed as per the test procedure monitored the system suitability results.

Table 10: Results of Robustness

System suitability Parameters	Flow rate						Column oven Temperature			pH Variation		
	0.81 ml/min		0.9 ml/min		0.99 ml/min		25°C	30°C	35°C	pH 2.7	pH 3.2	pH 3.2
	AZ	CH	AZ	CH	AZ	CH	AZ	CH	AZ	CH	AZ	CH
USP Tailing	1.08	1.1	1.09	1.12	1.06	1.08	1.01	1.2	1.08	1.05	1.06	1.23
USP Plate count	4457	5939	4363	5910	4300	5954	4532	5768	4651	5834	4576	5879
% RSD	0.4	0.7	0.44	0.77	0.44	0.74	0.42	0.77	0.49	0.73	0.45	0.79

System suitability Parameters	Mobile Phase Composition (Buffer: Acetonitrile)					
	85:15		90:10		95:05	
	AZ	CH	AZ	CH	AZ	CH
USP Tailing	0.99	**0.99**	**1.5**	**1.2**	**0.99**	**0.99**
USP Plate count	4457	5939	4363	5910	4300	5958
% RSD	0.44	0.77	0.45	0.76	0.44	0.71

AZ- Azilsartan Medoxomil CH- Chlorthalidone

Table 11: Data of forced degradation

Sample Condition	Component Name	%Assay (w.r.t.Untreated)	% Difference	PDA Peak Purity Purity angle	Purity Threshold
Untreated sample	Azilsartan Medoxomil	100.27	-	0.098	0.295
	Chlorthalidone	98.57	-	0.132	0.696
	Azilsartan Medoxomil	87.1	13.17	0.282	0.371
Acid treated	Chlorthalidone	98.07	0.5	0.480	0.695
	Azilsartan Medoxomil	91.99	8.28	0.150	0.336
Alkali treated	Chlorthalidone	95.10	3.47	0.490	0.697
	Azilsartan Medoxomil	100.06	0.21	0.088	0.290
Peroxide treated	Chlorthalidone	98.06	0.51	0.470	0.692
	Azilsartan Medoxomil	99.95	0.32	0.098	0.292
Thermal exposed	Chlorthalidone	98.3	0.27	0.568	0.698
	Azilsartan	100.07	0.2	0.098	0.292

Photolytic Degradation	Medoxomil Chlorthalidone	93.17	5.4	0.568	0.698

Stability of sample solution [11]

The sample solution was stable up to 24 hours at 5^0C temperature and did not show any appreciable change in sample area.

The Data for Forced degradation are tabulated in **Table 11**. There was no interference of any peak at the retention time of analyte peaks from blank and placebo, Peak purity of all forced degradation treated samples were passed. From this study it has been concluded that the proposed method is specific and stability indicating for the estimation of Azilsartan Medoxomil and Chlorthalidone, in the tablet dosage form.

CONCLUSION

This intended study can be concluded as the proposed method is simple, highly fast, economical, sensitive and reliable and is found to be more precise, accurate, specific, stability indicating, rugged and robust. hence it can be employed for routine estimation of tablets containing Azilsartan Medoxomil and Chlorthalidone.

Conventional reported chromatographic methods may be replaced by the proposed stability indicating HPLC method because of its superiority in cost effectiveness, short analysis time per sample and better detection. For faster samples testing routinely in QC lab the validated method may be used.

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