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Analytical RP- HPLC Method Development and Validation for Simultaneous Estimation of Azilsartan Medoxomil and Chlorthalidone In Pharmaceutical Dosage Form

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ABSTRACT

A reverse phase HPLC method is developed for the determination of Azilsartan medoximil and chlorthalidone in pharmaceutical dosage forms. Chromatography was carried out on a C_{18} column 4.6 x 100mm, 5µm, Make: BDS using a mixture of potassium Di hydrogen ortho phosphate buffer and acetonitrile (35:65 v/v) as the mobile phase at a flow rate of 1.0 ml/min. Detection was carried out at 273 nm . The retention time of Azilsartan Medoxomil and Chlorthalidone was 2.59±0.1 mins and 3.85±0.5 min respectively. The linearity was observed In range of 2.5-15 µg/ml and 10-60 µg/ml with a correlation coefficient of Azilsartan medoximil and chlorthalidone were 0.996 and 0.999.the proposed method was validated for its linearity, accuracy, precision and robustness. 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: HPLC, Azilsartan medoximil and Chlorthalidone, Estimation, Tablets.

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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. 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.

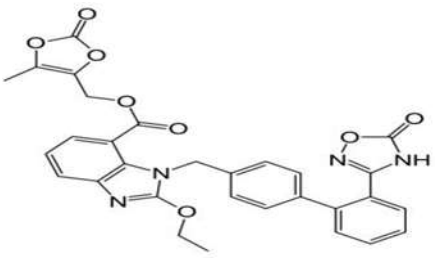

Figure 1: Structure of Azilsartan Medoxomil

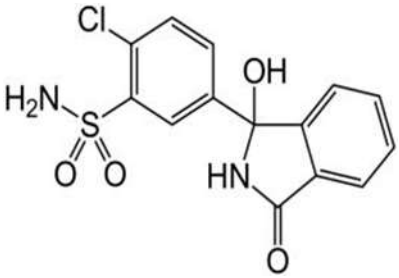

Figure 2: Structure of Chlorthalidone

MATERIALS AND METHOD

Instrumentation

A high performance liquid chromatography system consisting of Waters 2695 Separation (Alliance)Module with Photo diode Array detector was used with data handling system Empower Pro. Chemicals were weighed using Analytical balance Afcos, ER-180A. 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.

EXPERIMENTAL WORK

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.

Preparation of mobile phase

Mix a mixture of potassium di hydrogen phosphate buffer 650 ml (65%) and 350 ml of Acetonitrile HPLC (35%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration

Diluent Preparation

Use the Mobile phase as Diluent.

Preparation of standard solution

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.

Preparation of sample solution

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. 20µLof this solution was injected for HPLC analysis. The analyte peaks were identified by comparisons with those of respective standard for their retention time. The peak areas were used to calculate the drugs. The assay results, expressed as % of the label claim, are in table.1.

METHOD VALIDATION

The method was validated as per International Conference on Harmonization (ICH) guidelines.

Selection of wavelength maxima

Azilsartan Medoxomil showed absorption maxima at 243.6 nm and Chlorthalidone showed at 249 nm. For simultaneous estimation of both the drugs Azilsartan Medoxomil and Chlorthalidone a common wavelength was selected as absorption maxima at 243nm. Figure3.

Linearity

Under the experimental conditions described above, linear calibration curves for the drugs were obtained throughout the concentration ranges studied. Regression analysis was done on the peak areas of the drugs (y) Vs concentration (x). The linear ranges of Azilsartan medoximil and Chlorthalidone are 2.5-15 µg/ml and 10-60 µg/ml.

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.

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.

Robustness

Robustness of the method was investigated 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.

RESULTS AND DISCUSSION

In the present work, an attempt was made to provide a newer, sensitive, simple, accurate, and less time consuming HPLC new method. It is successfully applied for the determination of Domperidone and Esomeprazolein pharmaceutical preparations.

Table 1: Method application (Assay)

S.No	Parameters	Drug	
		Azilsartan medoximil	Chlorthalidone
1	Label claim (mg)	40mg	25mg
2	Drug content (%)	100.8%	100.1%
3	%RSD	0.27	0.31

Table 2: Linearity Regression data for Azilsartan medoximil and chlorthalidone

S.No	Parameters	Azilsartan medoximil	Chlorthalidone
1	Linear Range(µg/ml)	2.5-15 µg/ml	10-60 µg/ml
2	Correlation coefficient (r^2)	0.996	0.999
3	LOD	0.02	0.06
4	LOQ	0.04	1.2
5	Tailing Factor	1.4	1.3

Table 3: Precision of Azilsartan medoximil and Chlorthalidone

S.No	Azilsartan		Chlorthalidone	
	AREA	RT	AREA	RT
1	3983572	2.571302869		3.85
2	3985214	2.581302586		3.80
3	3990228	2.591318521		3.83
4	3985261	2.561302569		3.85
5	3996512	2.591302896		3.85
AVG	3988157		1305888	
SD	5295.407		7063.605	
%RSD	0.13		0.54	

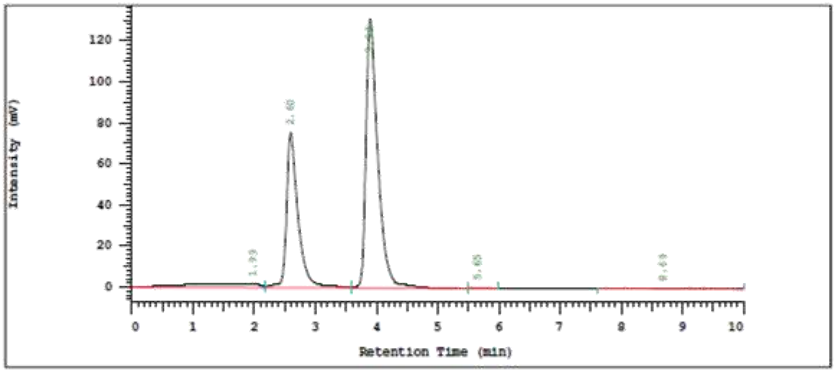

Figure 3: Azilsartan medoximil and Chlorthalidone standard chromatogram

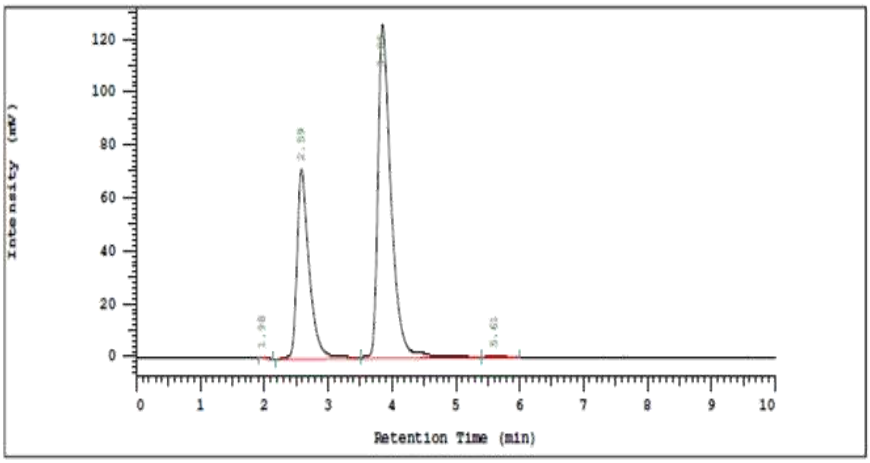

Figure 4: Azilsartan medoximil and Chlorthalidone sample chromatogram

Table 4: Results of Accuracy (Recovery Studies)

Recovery Levels	Azilsartan medoximil(40 mg) Amount added (mg)	Amount recovered (mg)	% Recovered	Mean % recover	% RSD	Chlorthalidone (25 mg) Amount added (mg)	Amount recovered (mg)	% Recovered	Mean % recovery	% RSD
80%	32.58	32.14	97.9	99.3	2.38	20.85	20.68	98.7	99.1	0.40
80%	32.64	32.06	102.0			20.64	20.54	99.2		
80%	32.95	32.54	98.0			20.94	20.87	99.5		
100%	40.28	40.58	100.7	102.1	2.22	25.61	25.14	98.2	100.5	00.1
100%	40.31	40.62	100.8			25.34	25.95	102.4		
100%	39.12	40.95	104.7			25.07	25.34	101.1		
120%	48.85	48.84	100.0	99.9	0.24	30.95	30.64	99.2	99.7	0.53
120%	48.24	47.99	99.6			30.64	30.51	99.7		
120%	48.84	48.85	100.0			30.02	30.11	100.2		

Table 5: System suitability results for Azilsartan medoximil

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	4326	1.06
2	0.9	4456	1.08
3	1.0*	4364	1.09

Table 6: System suitability results for Chlorthalidone

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	5937	0.71
2	0.9	5956	0.74
3	1.0*	5910	0.77

Table 7: System suitability results for Azilsartan medoximil

S.No	Change in Composition of the Mobile Phase	System Suitability Results USP Plate Count	USP Tailing
1	10% less	4455	0.99
2	*Actual	4362	1.50
3	10% more	4349	0.99

Table 8: System suitability results for Chlorthalidone

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results USP Plate Count	USP Tailing
1	10% less	5936	0.99
2	*Actual	5924	1.21
3	10% more	5956	0.99

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. 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. The proposed method was validated in accordance with ICH parameters Thus it was show that proposed methods could be successfully applied to estimate commercial pharmaceutical products containing Azilsartan medoximil and Chlorthalidone.

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