Research Article

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REVERSE PHASE-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF AZILSARTAN MEDOXOMIL AND CHLORTALIDONE IN PHARMACEUTICAL DOSAGE FORMS

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Abstract:

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for the simultaneous estimation of Azilsartan Medoxomil and Chlortalidone in pharmaceutical dosage forms. Isocratic elution at a flow rate of 0.9ml min^{-1} was employed on a symmetry C18 column at ambient temperature. The mobile phase consisted of Methonal: Water: Acetonitrile : 0.1% Ortho phosphoric acid 30:35:15:5(v/v/v/v). The UV detection wavelength was at 251 nm. The retention time for Chlortalidone was 3.923min and Azilsartan Medoxomil was 7.208 min. The method was validated as per the ICH guidelines. The proposed method can be successfully applied for the estimation of Azilsartan Medoxomil and Chlortalidone in pharmaceutical dosage forms.

Key Words:

Azilsartan Medoxomil and Chlortalidone, HPLC Development, Validation, 251nm.

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Introduction:

Chlortalidone or chlorthalidone is a diuretic drug used to treat hypertension, originally marketed as Hygroton in the USA. It is described as a thiazide diuretic (or, rather, a thiazide-like diuretic because it acts similarly to the thiazides but does not contain the benzothiadiazine molecular structure). Compared with other medications of the thiazide class, chlorthalidone has the longest

duration of action but a similar diuretic effect at maximal therapeutic doses. It is often used in the management of hypertension and oedema.

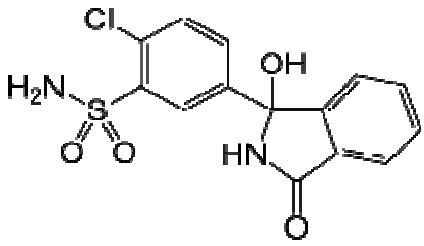


Figure 1: Stricture of Chlortalidone

Chlortalidone works on the kidney. It is used to treat fluid retention caused by liver or kidney conditions, *hypertension* (high blood pressure), *heart failure* (a condition where the pumping action of the heart is reduced) and *diabetes insipidus* (a condition in which an individual produces large amounts of dilute urine). It removes excess water from the body by increasing how much and how often you pass urine. This removal of fluid reduces blood pressure and helps reduce the work of the heart. How it works in diabetes insipidus however is not fully understood.

Chlortalidone is used to treat high blood pressure (hypertension), heart failure (the heart is unable to properly pump blood around the body), fluid collection in the stomach (ascites) due to cirrhosis of the liver, fluid retention due to kidney disease (nephrotic syndrome) and a type of diabetes called diabetes insipidus that causes you to be excessively thirsty and pass large amounts of urine. It is a type of diuretic, sometimes knows as water tablets. It is used to remove excess fluid from the body by increasing your production of urine. The reduction in fluid in your body causes your blood pressure to drop. In diabetes insipidus, the drug stops the over production of urine. In general this drug is used to reduce high blood pressure (hypertension) and remove excess fluid in the body (oedema) in certain conditions. It is also used to treat a type of diabetes called diabetes insipidus.

Azilsartan is an angiotensin II receptor antagonist used in the treatment of hypertension that was developed by Takeda.

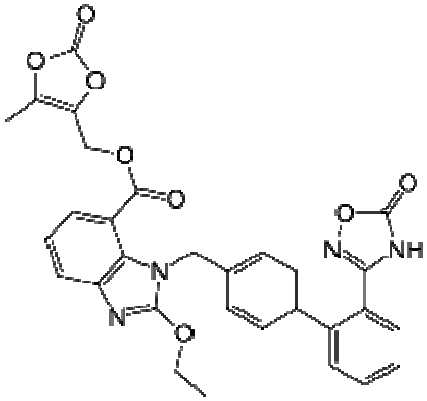


Figure 2: Stricture of Azilsartan

Azilsartan is an angiotensin II receptor blocker (ARB) that lowers blood pressure by blocking the action of angiotensin II, a vasopressor hormone. Azilsartan is used to treat high blood pressure (hypertension). Lowering high blood pressure helps prevent strokes, heart attacks, and kidney problems. Azilsartan belongs to a class of drugs called angiotensin receptor blockers (or ARBs). It works by relaxing blood vessels so that blood can flow more easily.

Azilsartan has a boxed warning that says the use of the drug should be avoided in pregnant women because use of the drug during the second or third trimester can cause injury and even death in the developing fetus. If a woman becomes pregnant while using the drug, it should be discontinued as soon as possible.

Experimental

Chemicals and reagents

All solvents used like Methanol, Water, Acetonitrile, and Ortho Phosphoric Acid (OPA) which are of HPLC grade were purchased from E.Merck, Mumbai.

Instrumentation and analytical conditions

The analysis of the drug was carried out on Shimadzu HPLC model (VP series) containing LC-10AT (VP series) pump, variable wave length programmable UV/visible detector SPD-10AVP and Rheodyne injector (7725i) with 20µl fixed loop. Chromatographic analysis was performed using Gemini C-18 column with 250 x 4.6mm internal diameter and 5µm particle size. Shimadzu electronic balance (AX-200) was used for weighing. Isocratic elution with Methonal : Water : Acetonitrile : 0.1% Ortho phosphoric acid 30:35:15:5(v/v/v/v). was selected with a flow rate of 0.9ml min^{-1}. The detection wavelength was set at 251nm with a runtime of 10 min. The mobile phase was prepared freshly and it was degassed by sonicating for 5 min before use. The column was equilibrated for at least 30min with the mobile phase flowing through the system. The column and the HPLC system were kept at ambient temperature.

Preparation of Stock, working standard solutions and Sample solutions

100mg of Azilsartan Medoxomil and Chlorthalidone was weighed separately and transferred (working standard) into a 100ml volumetric flask. The diluent Methanol was added and sonicated to dissolve it completely and made up to the mark with the same solvent. Further 1ml of the above stock solution was pipetted into a 10ml volumetric flask and diluted up to the mark with diluent. The contents were mixed well and filtered through Ultipor N_{66} Nylon 6, 6 membrane sample filter paper. The calibration curve was plotted with the concentrations of the 10 to 80 ppm working standard solutions. Calibration solutions were prepared and analyzed immediately after preparation.

The formulation tablets of Azilsartan Medoxomil and Chlorthalidone were crushed to give finely powdered material. Powder equivalent to10 mg of drug was taken in 10 ml of volumetric flask containing 5 ml of mobile phase and was shaken to dissolve the drug and then filtered through Ultipor N_{66} Nylon 6,6 membrane sample filter paper. Volume of the filtrate was adjusted to the mark with the same solvent to obtain concentration of 40 ppm.

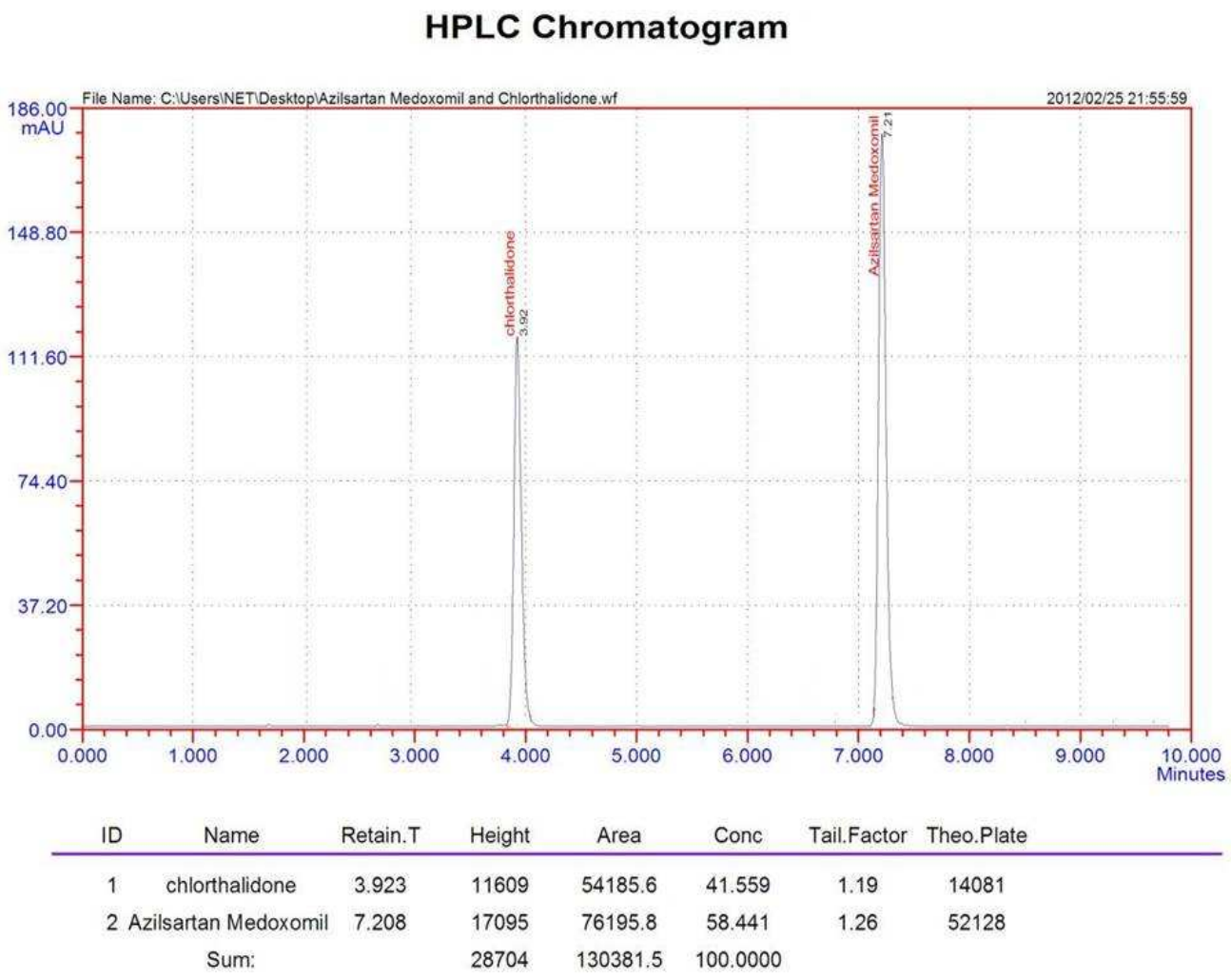
S. No	H.P.L.C Conditions	Results
1	Elution	Isocratic
2	API Concentration	6 ppm
3	Mobile Phase	MeOH:H_2O:ACN:0.1%OPA 30:35:15:5 (v/v/v/v)
4	P^H	4.6
5	Column	C_{18}
6	Wavelength	251nm
7	Flow	0.9ml / min
8	Runtime	10 min
9	Retention Time	Azilsartan Medoxomil–7.208 Chlortalidone – 3.923
10	Area	Azilsartan Medoxomil – 6195 Chlortalidone – 54185
11	Theoritical Plates	Azilsartan Medoxomil – 2128 Chlortalidone – 14081
12	Tailing Factor	Azilsartan Medoxomil –1.26 Chlortalidone – 1.19
13	Pump Pressure	6.81 Psi

Table 1 : Chromatographic conditions.

MeOH : Methanol,

ACN : Aceto Nitrile,

OPA : Ortho Phosphoric Acid.



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1	chlorthalidone	3.923	11609	54185.6	41.559	1.19	14081
2	Azilsartan Medoxomil	7.208	17095	76195.8	58.441	1.26	52128
	Sum:		28704	130381.5	100.0000		

Figure 3: Standard chromatogram of Azilsartan Medoxomil and Chlorthalidone

Method Validation procedure

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity precision, accuracy, specificity, and limit of quantification, robustness and system suitability.

Linearity

The developed method has been validated as per ICH guidelines (Zucman D, 2007). Working standard solutions of Chlorthalidone and Azilsartan Medoxomil in the mass concentration range of 10 ppm to 80 ppm was injected into the chromatographic system. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curve of Chlorthalidone and Azilsartan Medoxomil was obtained by plotting the peak area ratio versus the applied concentrations. The linear correlation coefficient was found to be 0.999.

S.No	Conc (PPM)	Area of Chlorthalidone	Area of Azilsartan Medoxomil
1	10	15697	20217
2	20	28974	39862
3	30	42682	57863
4	40	54185	76195
5	50	70936	94367
6	60	85642	113674
7	70	98751	136472
8	80	113257	157691
Correlation Coefficient		0.9998	0.9998
Slope		45166	55927
Intercept		1866	- 5140

Table 2: Linearity of Azilsartan Medoxomil and Chlorthalidone

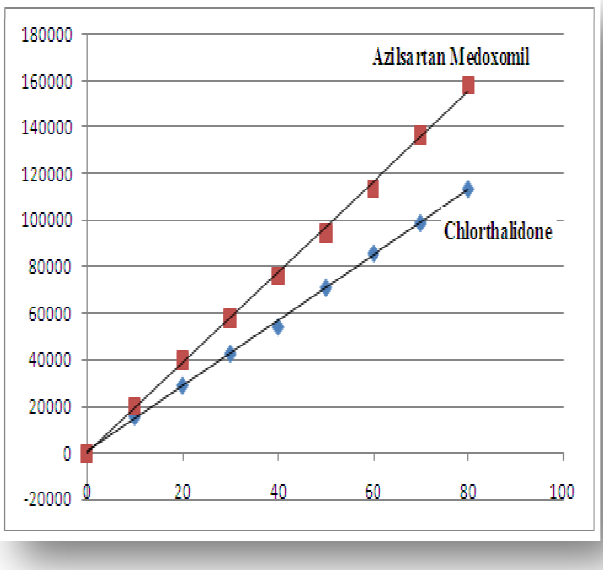


Figure 4: Calibration curve of Azilsartan Medoxomil and Chlorthalidone

Precision

Repeatability of the method was checked by injecting replicate injections of 40 ppm of the solution for six times on the same day as intraday precision study of Azilsartan Medoxomil and Chlorthalidone and the RSD was found to be for 0.596 Azilsartan Medoxomil and 0.73 for Chlorthalidone.

Injection	Conc. (ppm)	Azilsartan Medoxomil	Chlorthalidone
1	40	76195	54185
2	40	76847	54193
3	40	76512	54039
4	40	76951	54987
5	40	75746	54820
6	40	76169	54728
	RSD	0.596	0.73

Table 3: Precision parameters of Azilsartan Medoxomil and Chlorthalidone

Accuracy

The accuracy of the method was determined by calculating recovery of Azilsartan Medoxomil and Chlorthalidone by the method of standard addition. Known amount of Azilsartan Medoxomil and Chlorthalidone (20ppm, 40ppm and 60ppm) was added to a pre quantified sample solution and the amount of Azilsartan Medoxomil and Chlorthalidone were estimated by measuring the peak area

ratios and by fitting these values to the straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range and amount of Azilsartan Medoxomil and Chlorthalidone was estimated by measuring the peak area ratios by fitting these values to the straight line equation of calibration curve. From the above determination, percentage recovery was calculated and the average recovery was found to be 99.735 for Azilsartan Medoxomil and 99.80 for Chlorthalidone.

Recovery	Conc. of sample	Recovery of Azilsartan Medoxomil	Recovery of Chlorthalidone	% Recovery of Azilsartan Medoxomil	% of recovery of Chlorthalidone
50%	20 ppm	19.89	19.91	99.45	99.55
100%	40 ppm	39.79	40.08	99.475	100.2
150 %	60 ppm	60.17	59.79	100.28	99.65

Table 4 :Recovery results

LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as per ICH guide-lines. Results are shown in table 5.

Parameter	Measured for Azilsartan Medoxomil	Measured for Chlorthalidone
LOD	0.07ppm	0.009ppm
LOQ	0.02ppm	0.03ppm

Table 5: Results of LOD and LOQ.

Robustness

To determine the robustness of the method, two parameters from the optimized chromatographic conditions were varied. Results of Robustness are shown in table 6.

Parameter	Modifications	Azilsartan Medoxomil		Chlorthalidone	
		Peak Area	% of Change	Peak Area	% of Change
Standard	No change	76195	------	54185	----
Mobile Phase	Methonal : Water : Acetonitrile : 0.1% Ortho phosphoric acid 30:30:20:5(v/v/v/v)	75914	1.6	54092	0.17
P^H	5.3	76252	0.08	54284	0.183
Wavelength	256 nm	76024	0.22	54057	0.24

Table 6: Robustness results

Results and Discussion

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase. The drugs Azilsartan Medoxomil and Chlorthalidone preferably analyzed by reverse phase columns and accordingly C_{18} column was selected. So the elution of the compound from the column was influenced by polar mobile phase. The concentration of the Water, Methanol and Acetonitrile were optimized to give symmetric peak with short run time based on asymmetric factor and peak area obtained. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase Methonal : Water : Acetonitrile : 0.1% Ortho phosphoric acid 30:35:15:5(v/v/v/v). The retention time of Azilsartan Medoxomil was 7.208 and Chlorthalidone was found to be 3.923 min, which indicates a good base line. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The system suitability and validation parameters are given in Table 7. The average recovery was found to be 99.735 for Azilsartan Medoxomil and 99.80 for Chlorthalidone indicating that the proposed method is highly accurate. Proposed liquid chromatographic method was applied for the determination of Azilsartan Medoxomil and Chlorthalidone in tablet formulation. The result for Azilsartan Medoxomil and Chlorthalidone was comparable with a

corresponding labeled amount. The absence of additional peaks indicates no interference of the excipients used in the tablets.

S.NO	Formulation	Brand	Concentration	Amount found	% Estimation
1	Azilsartan Medoxomil	**EDARBYCLOR 800mg**	40ppm	39.757	99.4
2	Chlorthalidone	**EDARBYCLOR 25mg**	40ppm	39.82	99.55

Table 7: Formulation Results of Azilsartan Medoxomil and Chlorthalidone.

Conclusion

A validated RP-HPLC method has been developed for the determination of Azilsartan Medoxomil and Chlorthalidone in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 10 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of Azilsartan Medoxomil and Chlorthalidone in pharmaceutical dosage form.

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