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RP-HPLC Method Development for Simultaneous Estimation of Azilsartan Medoxomil and Chlorthalidone In Pharmaceutical Formulations

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A B S T R A C T

Simple, accurate, economical and reproducible RP-HPLC method for simultaneous estimation of two component drug mixture AzilsartanMedoxomil and Chlorthalidone in combined tablet dosage form has been developed. Developed HPLC method is reversed phase chromatographic method using Inertsil C_{18} column and gradient mobile phase, detection of eluent was carried out using PDA detector at a flow rate of 1.2ml/ min. Forced degradation studies under various stress conditions, including hydrolysis, oxidation, thermal, humidity, and photolytic degradation, were performed. The developed method was validated in terms of specificity, linearity, precision, intermediate precision, accuracy, robustness and solution stability. The linearity was observed in concentration range of 12.5 ppm to 37.5 ppm for AzilsartanMedoxomil and from 3.5 ppm to 22.7ppm for Chlorthalidone. Results of analysis were validated statistically and by recovery studies. The proposed RP-HPLC method achieved satisfactory resolution between AzilsartanMedoxomil and Chlorthalidone. It can be used for determination of AzilsartanMedoxomil in drug substance and pharmaceutical preparation. Thismethod is suitable for routine quality control and stability testing of AzilsartanMedoxomil and Chlorthalidone in pharmaceutical formulations.

Key words: HPLC, Simultaneous estimation, AzilsartanMedoxomil, Chlorthalidone.

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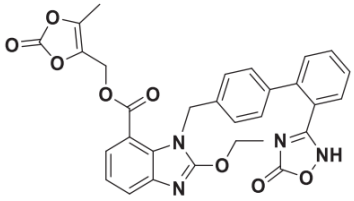
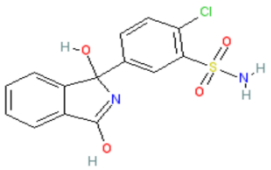
INTRODUCTION:

AzilsartanMedoxomil and Chlorthalidone is an antihypertensive drug used to treat major congestive heart failure [1]. This work, is a novel, simple, rapid, accurate, precise, selective, stability-indicating, and fully validated high-performance liquid chromatography method. Stability is an essential factor for a drug product's quality, safety, and efficacy. A literature search reveals that there are very few methods reported for the determination of Azilsartan Medoxomil and Chlorthalidone HPLC [2,3,4,5,6,7].The present study aimed to develop and validate stability indicating the HPLC method for the simultaneous estimation of AzilsartanMedoxomil and Chlorthalidone. As compared to other analytical techniques, the HPLC method is a highly powerful, quick, automated, accurate, efficient, reproducible, sensitive, and extremely precise analytical technique [8,9,10]. Solubility studies of Azilsartan and Chlorthalidone is evaluated for selection of mobile phase. Validation of the method for determination of assay will be performed according to the ICH requirements after stress testing on the drug tablets under a variety of conditions, including hydrolysis, oxidation, photolysis, and thermal degradation [11,12]. In the current article, we are reporting the development and validation of a fast, precise, accurate, linear, robustness, and stability-indicatinghigh-performance liquid chromatography method [11].

Equipment & chemicals: HPLC (Agilent); Analytical Balance (Sartorius / MSU6.6S-000-DM); Orbital Shaker (Sartorius); Ultrasonic bath (Mettler Toledo); pH Meter (Mettler Toledo); Centrifuge Machine (Sartorius); AzilsartanMedoxomil (Glenmark Pharm. Ltd.) and Chlorthalidone (Glenmark Pharm. Ltd.)



AzilsartanMedomoxil



Chlorthalidone

MATERIAL AND METHODS:

Preparation of Buffer (Mobile Phase-A): Dissolve about 2.99 g of potassium dihydrogen phosphate in 1000 mL of water. Sonicate to dissolve and mix. Adjust pH 4.0 ± 0.1 withdilute Ammonia solution. Filter through a 0.45µ membrane filter.

Mobile Phase-B: Acetonitrile

Preparation of Diluent 1: Mix Buffer pH 6.8 and Acetonitrile in the ratio of 50:50 v/v

Preparation of Diluent 2: Mix Water and Acetonitrile in the ratio of 40:60 v/v

Preparation of Standard Stock Solution:

Standard Stock Solution (Chlorthalidone): Weigh accurately about 50 mg of Chlorthalidone standard into 100 mL volumetric flask, add about 60 mL of diluent-1 and sonicate the solution to dissolve. Dilute to the volume with diluent-1 and mix well.

Standard Stock Solution (AzilsartanMedoxomil): Weigh accurately about 53.4 mg of AzilsartanMedoxomil standard into 100 mL volumetric flask, add about 60 mL of diluent-1 and sonicate the solution to dissolve. Dilute to the volume with diluent-1 and mix well.Pipette out 5.0 mL of AzilsartanMedoxomil Standard above stock solution & 3.0 mL Chlorthalidone Standard stock solution into 100 mL volumetric flask, dilute to the volume with diluent-2 and mix well.

Preparation of Sample Solution: Weigh and triturate not less than 6 tablets. Transfer equivalent 5 tablets powder into a 500 mL volumetric flask add 150 mL of phosphate buffer pH-6.8. Shake the sample in orbital shaker for about 30 minutes at room temperature followed by addition of 250 mL of acetonitrile. Dilute upto mark with acetonitrile. Filter the resulting solution through 0.45 µm Whatman PVDF filter. Further dilute 3 mL of above filtrate solution to 50 mL with diluent-2, mix well [12].

METHOD VALIDATION

Specificity: The standard solution is prepared as given in the procedure and injected in six replicates and the suitability parameters are calculated. The column efficiency, resolution and tailing factor were calculated for the standard solutions. System suitability parameters may fall within 2% relative standard deviation range during routine performance of the method.The system suitability parameters are calculated (Table 3). There is no interference from the blank at the retention time of analytes.

Precision:

Repeatability (method precision): Six independent samples are prepared and analyzed as per the method. % Assay of AzilsartanMedoxomil and Chlorthalidone Tablets in each determination is calculated and the % RSD of the same is determined.

Repeatability of standard *(System Precision):* System precision was evaluated by determining the %RSD of retention time and peak area response of AzilsartanMedoxomil and Chlorthalidone peak obtained from five replicate injections of standard solution.

Intermediate Precision (*Ruggedness):* The intermediate precision was determined by three replicates of the prepared sample solutions. The intermediate precision of the sample application and measurement of peak area was obtained by the assay of three sample sets on different days and instrument at concentration levels of 25µg/mL for AzilsartanMedoxomil and 15µg/mL for Chlorthalidone in different day time interval for inter-day precision. Peak areas were determined and RSD was calculated [13].

Accuracy: The accuracy of the methods was assured by the use of the standard addition technique, involving analysis of formulation samples to which certain amounts of authentic drugs were added. The resulting mixtures were assayed, and the results obtained for both drugs were compared to those expected. The recovery experiments were carried out in triplicate by spiking previously analysed sample. Accuracy (recovery in %) evaluated in the range to cover 50% to 150% of stock concentration for Assay of AzilsartanMedoxomil (i.e. from 200.0 µg per mL to 600.0 µg per mL) and 25% to 150% of stock concentration for Assay of Chlorthalidone (i.e. from 75.0 µg per mL to 375.0 µg per mL). Chromatographic conditions are followed as per the method and the criteria for conclusion are analysed.

Linearity and range:

Preparation of Linearity solutions:7 different solutions of AzilsartanMedoxomil and Chlorthalidone ranging from 12.5 ppm to 37.5 ppm and 3.5 ppm to 22.7 ppm respectively are prepared as follows:First level and last level were injected in six replicates and % RSD of the area responses of six replicates of first level and last level was determined. The other levels were injected in duplicate. A graph of mean peak area response vs. concentration was plotted [14].

Solution Stability: The stability of the sample solution is checked by injecting this solution stored at room temperature at 0 hour and up to 50 hours. The sample is compared against initially injected standard. The change in the % Assay is calculated for time interval with respect to the initial value [15].

Filter Suitability: Filter Suitability performed by analysing test solution with and without filtration (centrifuged sample) using six preparations of sample solutions. Calculated assay of 12 test solutions (six filtered samples and six centrifuged samples) and determine % recovery. A portion from each aliquot was filtered through 25mm, 0.45 µm Whatman PVDF syringe filter, discarding about first 10 mL of filtrate. Remaining portion of same test solution is subjected to centrifugation at 2000 rpm for 10 minutes and upper clear supernatant solution is used for the analysis [16].

RESULTS AND DISCUSSION

Specificity and Forced Degradation: Peak purity analysis is conducted for as such test sample and spiked test sample, which exhibits that there is no interference due to blank. No inference from tablet excipients and mobile phase. No peak was found at retention time of Chlorthalidone and Azilsartan Medoxomil. This was very favourable conditions for routine analysis.Peak purity analysis is also conducted for the samples under stressed conditions of acid, alkali, peroxide, thermal, humidity, and photolytic degradations. The known related substances and Azilsartan Medoxomil and Chlorthalidone peaks are pure and there are no co-eluting peaks.In assay and related substances method, expected degradation between 5% to 20% was achieved for Azilsartan Medoxomil and Chlorthalidone Tablets after exposure to stressed conditions, viz., elevated temperature, acid hydrolysis, base hydrolysis, oxidation and water hydrolysis degradation conditions. Hence, Azilsartan Medoxomil and Chlorthalidone Tablets are found sensitive towards these degradation conditions.

The resolution between the Azilsartan Medoxomil and Chlorthalidone and any impurity or degradant is adequate and the analytical method for related substances test is capable of detecting the degradant. Azilsartan Medoxomil peak, Chlorthalidone peak and its known impurities are found to be spectrally pure. Hence, proposed method is specific and stability indicating.

Precision and Ruggedness:

System precision: The system precision is evaluated by RSD of peak area of the analyte. The obtained %RSD value 0.10 and 0.08 for Azilsartan Medoxomil and Chlorthalidone respectively which is less than 5.0 % meets the acceptance criteria. This indicates good system and method precision.

Table 1: Chromatographic parameters

Instrument	High-performance liquid Chromatography
Column	Inertsil C18, (150 x 4.6) mm, 5µ
Detector	UV
Wavelength	235nm
Column oven Temp	40°C
Flow rate	1.2 mL/minute
Run Time	15 minutes

Table 2: Gradient details

Time (Minutes)	Mobile phase-A (%)	Mobile phase-B (%)
0	72	28
5	72	28
6	45	55
14	50	50
14.5	25	75
16.5	25	75
17	25	75
20	75	25

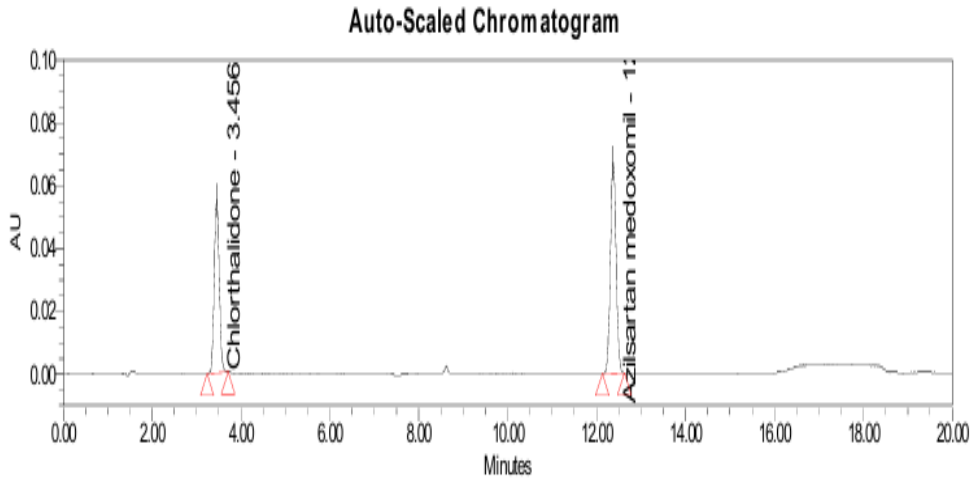


Figure 1: Chromatogram of developed method

Method precision: In method precision the % RSD of six individual test results is less than 10.0 %, which meets the acceptance criteria.The obtained %RSD value was 0.52 and 0.81 for Azilsartan Medoxomil and Chlorthalidone respectively

Intermediate precision:

In the intermediate precision/ruggedness study, six different sample preparations were analysed by different analysts, using different systems, and on different days, the % RS.D. Result was found to be less than 10.0 %, and overall % RSD of the test result on comparison with Method precision also found to be less than 10.0 %, thus it is concluded that the method is precise and rugged, meets the acceptance criteria.

Accuracy: The accuracy of the method is checked at 50 %, 100 %, and 150 % of the specification limit. The % recovery for Azilsartan Medoxomil and Chlorthalidone obtained is between 80 % to 120 %; meets the acceptance criteria.

Linearity and range:

The linearity of the method was determined seven concentrations; the equation for calibration curve was linear. The results shows excellent correlation exists between peak area and concentration of drug. The correlation coefficient values are found to be more than 0.98. The proposed analytical method is linear in the range from concentration 12.5 ppm to 37.5 ppm for Azilsartan Medoxomil and from 3.5 ppm to 22.7ppm for Chlorthalidone.

Range was evaluated % RSD of area, the levels given above data determined the % RSD 0.12 and 0.25 (level 1 and 7) of AzilsartanMedoxomil and Chlorthalidone respectively and the % RSD 0.15 and 0.30 (level 1 and 7) of AzilsartanMedoxomil and Chlorthalidone respectively. The results show excellent (% RSD less than 2) range.

Table 3: Linearity and range of Azilsartan lower strength

Linearity Levels	Concentration in		Mean peak area	% RSD of peak area
	% w.r.t. working level conc.	µg/mL		
1	50	12.5486	305883.2	**0.12**
2	60	15.0583	366325.0	NA
3	80	20.0778	489313.5	
4	100	25.0972	614120.5	
5	120	30.1167	735952.5	
6	140	35.1361	859341.5	
7	150	37.6458	916917.8	**0.25**
Correlation coefficient			**1.000**	
Slope			**24440.586**	
% y-intercept			**-0.137**	

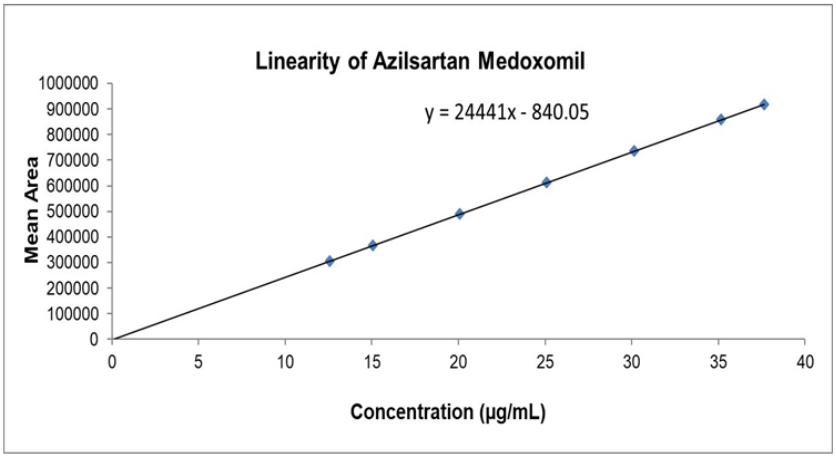


Figure 2: Calibration curve between area and concentration

Stability in analytical solution: The results show that for both solutions, the retention time and peak area for both drugs remain almost unchanged (%RSD less than 2) and no significant degradation was observed within the indicated period, indicating that both solutions were stable for at least 50 hr, which was sufficient for the whole analytical process.

Robustness: The validation studies show that the method is robust under deliberately varied conditions of column oven temperature (± 5^{O}C) and pH of mobile phase (± 0.1). RRT obtained from each experiment compared with RRT obtained from specificity experiment; meets the acceptance criteria. The robustness, variation in pH of mobile phase composition was evaluated by % RSD, % assay and comparative study with the precision. In the comparison study % RSD and % assay was found to be in acceptable criteria.All the conditions used to evaluate were found to be less than 2(%RSD) so this shows method is robust.

Table 4: Linearity of Chlorthalidone lower strength

Linearity Levels	Concentration in		Mean peak area	% RSD of peak area
	% w.r.t. working level conc	µg/mL		
1	23	3.5441	105212.3	0.15
2	33	5.0630	147638.5	
3	50	7.5945	221469.5	
4	67	10.1260	298088.5	
5	100	15.1890	443090.0	
6	123	18.7331	550698.0	
7	150	22.7835	668693.3	0.30
Correlation coefficient			**1.000**	
Slope			**29344.221**	
% y-intercept			**-0.053**	

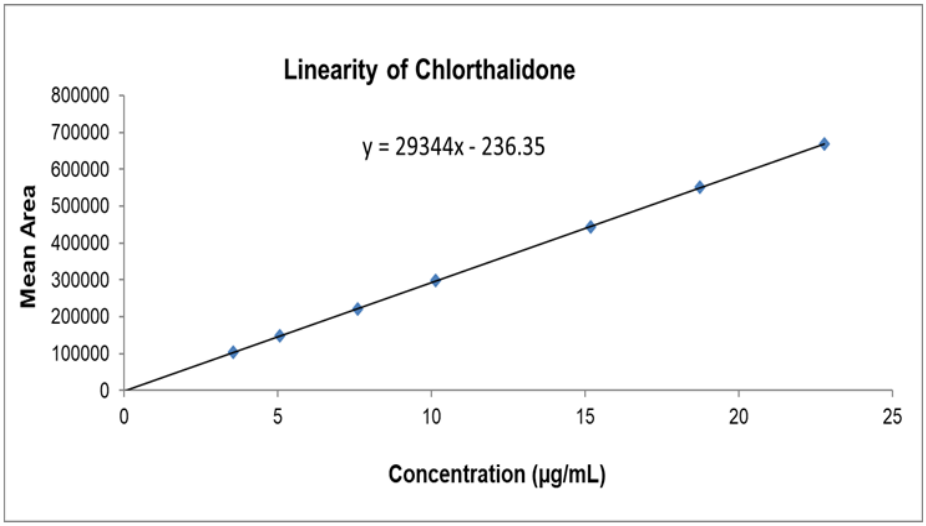


Figure 3: Calibration curve between area and concentration

CONCLUSION:

The developed RP-HPLC method is simple and selective for simultaneous determination of Azilsartan Medoxomil and Chlorthalidone in combined tablet dosage form was found to be accurate, rapid and economical. The values of coefficient of variance were satisfactorily low and recovery was close to 100% indicating reproducibility of the method. The linearity was observed within limit hence method is linear.Developed method for simultaneous estimation of two drugs from combined dosage form is reverse phase chromatographic method utilizing C18 column and gradient mobile phase, detection of eluent was carried out using UV detector. The method was developed having 15 min. run time only. The excipients in the formulation did not interfere in the accurate estimation of Azilsartan Medoxomil and Chlorthalidone. The method exhibited high specificity, with clear separation of peaks and no interference from other substances. Stressed conditions, viz., elevated temperature, acid hydrolysis, base hydrolysis, oxidation and water hydrolysis degradation led to significant impurity formation but the method remained precise and reliable in quantifying this degradation Products.The system precision and ruggedness studies confirmed the method's reproducibility, with relative standard deviation (RSD) values well within acceptable limits. The method precision results had RSDs of less than 10%, confirming its consistency across different conditions, analysts, and instruments. In terms of accuracy, the recovery of AzilsartanMedoxomil and Chlorthalidone samples ranged between 98.1% and 101.9%, while the method showed excellent linearity ($R^2 > 0.99$) across a wide concentration range. Finally, the robustness tests demonstrated that the method could withstand variations in experimental conditions without compromising performance. Overall, this validated method is precise, robust, and reliable for the routine analysis and quality control of AzilsartanMedoxomil and Chlorthalidone.The HPLC method is simple, selective and economical for simultaneous determination of Azilsartan Medoxomil and Chlorthalidone in the available marketed product “Zilokem CT” of Alkempharmaceuticals limited and other pharmaceutical preparations

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