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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF AZILSARTAN MEDOXOMIL AND CHLORTHALIDONE BY RP-HPLC IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

The present study aims to develop a simple economical method for estimation of Azilsartan and chlorthalidone combined dosage form by RP-HPLC technique. Buffer used in this method was 0.1% OPA buffer of pH 2.5 used in the ratio of 40B:60A run through C18 BDS 250mm column with a flow rate of 1ml/min for 7min run time. The column was maintained at 30°C temperature and optimized wavelength was 220nm. Azilsartan eluted at 2.5min and Chlorthalidone at 3.5min retention time with 5.2 resolution. System suitability parameters like plate count and tailing factors were passed according to ICH guidelines. Repeatability of Azilsartan and chlorthalidone was found to be 0.4 and 0.34 respectively. Percentage recovery of azilsartan was 99.81% and of chlorthalidone was 99.71%. Linearity performed with range of 40-240µg/ml for azilsartan and 25-150µg/ml for chlorthalidone and correlation coefficient obtained was 0.999. Robustness was found to be within the limits i.e., less than 2. Stability studies were done and %degraded was with the limits. A simple method was developed for estimation of Azilsartan and chlorthalidone. All the validation parameters were succeeded and were within the range. This method can be used in the regular analysis of Azilsartan and chlorthalidone combination dosage form.

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Keywords: Azilsartan, Chlorthalidone, ICH guidelines, RP-HPLC.

INTRODUCTION

Azilsartan is used in the treatment of hypertention. It is a angiotensin II receptor antagonist. Its mechanism of action is blocking the angiotensin receptor by vasopressor harmone that stops vasoconstriction and thus decreases the blood pressure. Its IUPAC name was (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 and molecular formula $C_{30}H_{23}KN_4O_8$. Azilsartan was practicaly insoluble in water but soluble in DMSO and methanol. Pka of the drug was 9.21. Chlorthalidone is used in the treatment of hypertension, it is a thiazide diuretic drug which inhibits Na^+ and Cl^- ions re-absorption 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-1*H*-isoindol-1-yl)benzene-1-sulfonamide with molecular formula $C_{14}H_{11}ClN_2O_4S$. Chlorthalidone was soluble in Methanol, water and DMSO. Pka found was 9.57. According to literature two methods were available in which madhu et al., the retention time for Chlortalidone and Azilsartan Medoxomil were 3.923min and 7.208 min respectively. Naazneen et al., the retention time for Chlortalidone and Azilsartan Medoxomil were 2.36±0.1 mins and 5.54±0.5 mins respectively.

EXPERIMENTAL WORK

Materials and reagents: Edarbyclor combination tablet formulation with lable claim 40mg and 25mg of Azilsartan and Chlorthalidone from Takeda. Azilsartan and chlorthalidone bulk drugs were gifted samples by spectrum pharma research solutions, HPLC grade Acetonitrile and water from Merk.

Instruments: Instrument used in this work was Waters HPLC system 2695with quartarnary pump delivery system, automatic sampler, column oven and PDA detector 2996. Whole the system was integrated with empower 2 software. Labman Ultrasonic cleaner, Digisun Electronic pH meter 7007.

Preparation of Buffer: (0.1%OPA): In the preparation of 0.1%OPA buffer of pH 4.0, 1ml of Ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

Preparation of standard working solution: Accurately Weighed and transferred 40mg&25mg of Azilsartan and Chlorthalidone working Standards into a 50 ml clean dry volumetric flask, add 30ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solution, 1 ml was pipetted out in to a 10ml Volumetric flask and then make up to the final volume with diluent

Preparation of sample working solution: 5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 5 tablets was transferred into a 25ml volumetric flask, 30ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 0.5ml was pipetted out into a 10 ml volumetric flask and made upto 10ml with diluent

Chromatographic conditions: Column used in this method was BDS C18 of 250mm column length, through which mobile phase of composition 40:60A was pumped at a flow rate of 1ml/min. sample injected was 10µl, column was maintained at 30°C and the optimized wavelength selected was 220nm at with two drugs have optimum absorbance.

Method Validation: The optimized method was validated as per ICH guidelines to check whether the developed method could be used for the estimation of azilsartan and chlorthalidone in the regular analysis. ICH guidelines includes following parameters.

Specificity: specificity indicates whether the developed method detects and quantifies our molecule of interest without any interference like placebo and other dissolved contents. Placebo and blank was injected and compared to the optimized chromatogram to detect the interference at our molecule retention time.

Linearity: Linearity determines the increase in the response with linear to the concentration. Six different concentration solutions were prepared such as 40µg/ml, 80µg/ml, 120µg/ml, 160µg/ml, 200µg/ml and 240µg/ml of azilsartan and 25µg/ml, 50µg/ml, 75µg/ml, 100µg/ml, 125µg/ml, and 150µg/ml of chlorthalidone and injected to HPLC system.

Precision:

Repeatability: It is also known as repeatability, Six working sample solutions were prepared from the sample stock solution and injected on the same day, in to the same instrument, by the same analyst and %RSD was calculated.

Intermediate Precision: It is also known as Inter day precision, analyst to analyst precision, system to system precison. Six working sample solutions were prepared from the sample stock solution and injected on the next day or by another analyst or injected to another instrument and %RSD was calculated.

Accuracy: Three levels of standard solutions were prepared as level 50% (80µg/ml of azilsartan and 50µg/ml of chlorthalidone), level 100% (160µg/ml of azilsartan and 100µg/ml of chlorthalidone) and level 150% (240µg/ml of azilsartan and 150µg/ml of chlorthalidone) by placebo method in which standard was added to placebo and injected to HPLC system.

LOD: Limit of detection is the lowest concentration of the drug that can be detected at the detector level without necessary quantification and the concentration where the S/n ratio is 3:1

LOQ: Limit of quantification is the lowest concentration of the drug that can be quantified with accuracy and precision and the concentration where the S/n ratio is 10:1

Robustness: Small changes in the optimized method was done such as change in flow rate of 10% (±), Change in Mobile phase composition in 10% (±) of Acetonitrile and change in temperature 5°C (±). Maintaining these conditions samples were injected and %RSD was reported.

System suitability: System suitability for that method was tested by five replicate injections of standard preparation. Plate count, tailing factor, resolution and %RSD were reported.

Assay: Percentage labeled amount was found by performing assay. Sample and standard solutions of same concentrations were prepared and sample peak area was compared to the standard peak area.

Stability studies:

Oxidation: To 1 ml of stock solutionof Azilsartan and Chlorthalidone, 1 ml of 20% hydrogen peroxide

(H2O2) was added separately. The solution was kept for 30 min at 60°C. For HPLC study, the resultant solution was diluted to obtain 160µg/ml&100µg/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 1 ml of stock s solution Azilsartan and Chlorthalidone, 1ml of 2N Hydrochloric acid solution was added and kept a side for 30mins at 60°C after 30min the solution was neutralized with 1ml of 2N NaOH solution. The resultant solution was diluted to obtain 160µg/ml&100µg/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 and Chlorthalidone, 1 ml of 2N sodium hydroxide was added and kept a side for 30mins at 60°C after 30min 1ml of 2N hydrochloric acid was added and neutralized. The resultant solution was diluted to obtain 160µg/ml&100µg/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°C for 6hrs to study dry heat degradation. For HPLC study, the resultant solution was diluted to 160µg/ml&100µg/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 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 160µg/ml&100µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies: Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to 160µg/ml&100µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

RESULTS AND DISCUSSIONS

In the method development process many trials were done with different columns, mobile phase compositions, by changing the buffer and its pH. But the results were optimum with BDS 250mm length column of particle size 5µ. The mobile phase composition was pumped with the flow rate of 1ml/min through the column which was maintained at 30°C. Wavelength optimized in this method was 220nm. By injecting 10µl of the working standard solution in to hplc system azilsartan and chlorthalidone were eluted at 2.5min and 3.5min with a resolution of 5.2. All the system suitable parameters like Plate count and tailing factors were within the limits. The developed method was to be validated as per the guidelines of ICH. The method was found to be specific without any interference of blank and placebo constituents. Calibration curve was plotted, correlation coefficient obtained was 0.999. Regression equation found was y = 1109.x + 983.7 for azilsartan and y = 1842.x + 879.2 for chlorthalidone. %RSD obtained was 0.4 and 0.34 for azilsartan and chlorthalidone respectively. Recovery studies were done and azilsartan recovered was 99.81% and chlorthalidone was of 99.71%. LOD, LOQ concentrations of Azilsartan and chlorthalidone were 2.93µg/ml, 8.87µg/ml and 1.58µg/ml, 4.77µg/ml respectively. Robustness was passed and %RSD was within the limits.

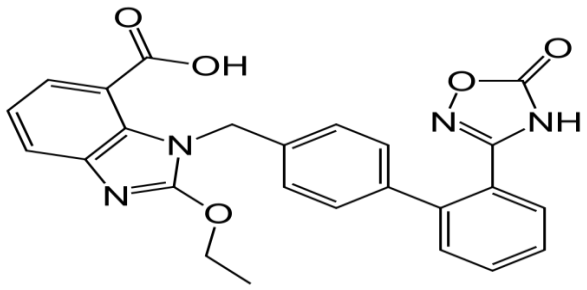

Fig.1: Azilsartan Medoxomil

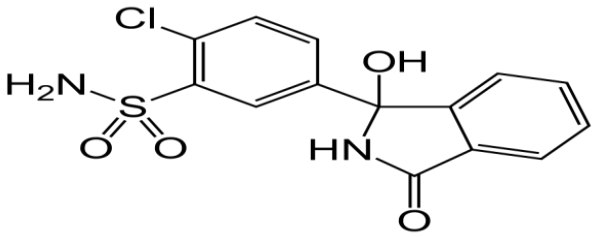

Fig.2: Chlorthalidone

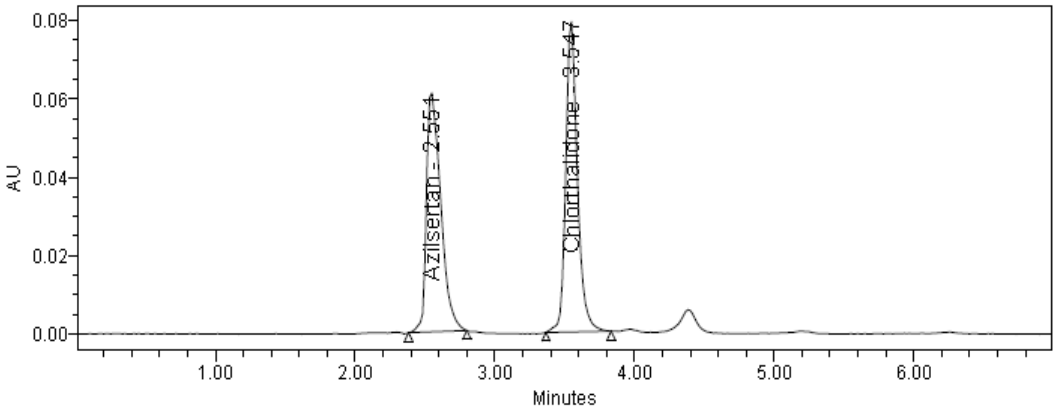

Fig.3: Chromatogram of sample solution

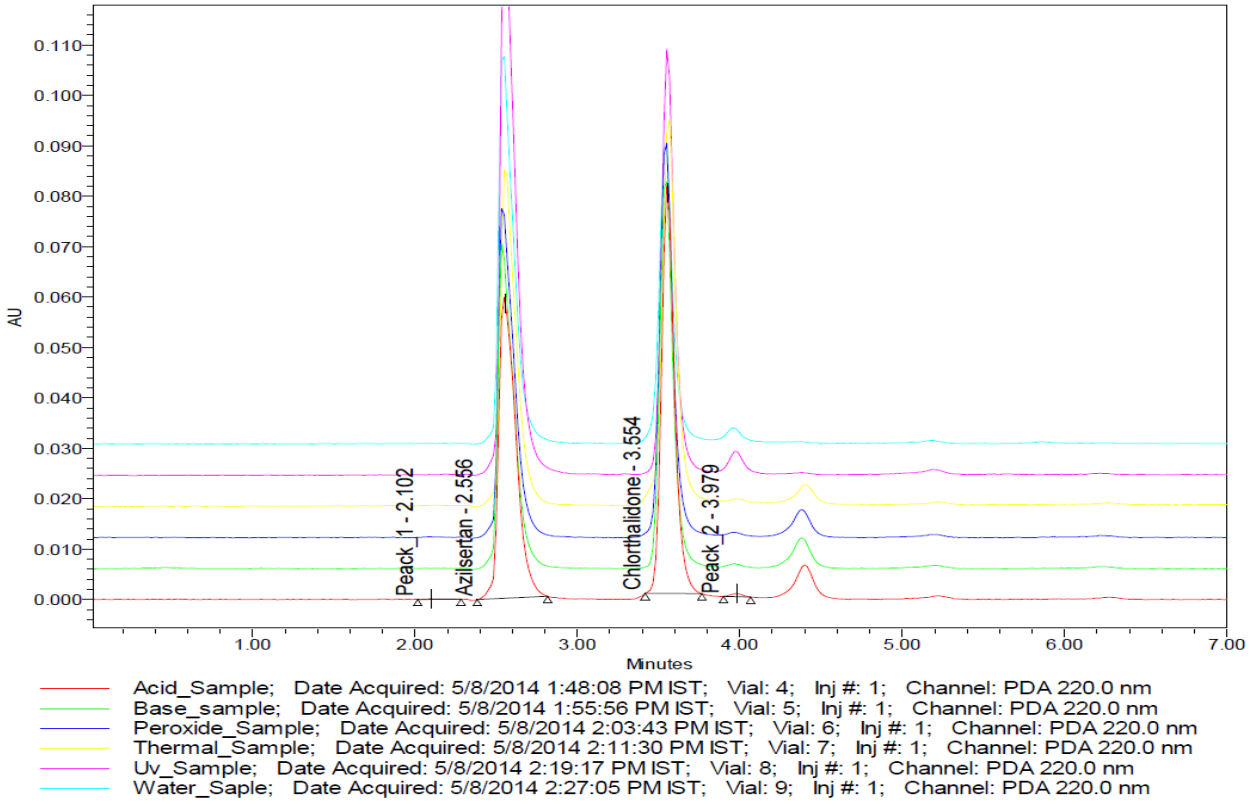

Fig.4: Overlay Chromatogram of degradation samples

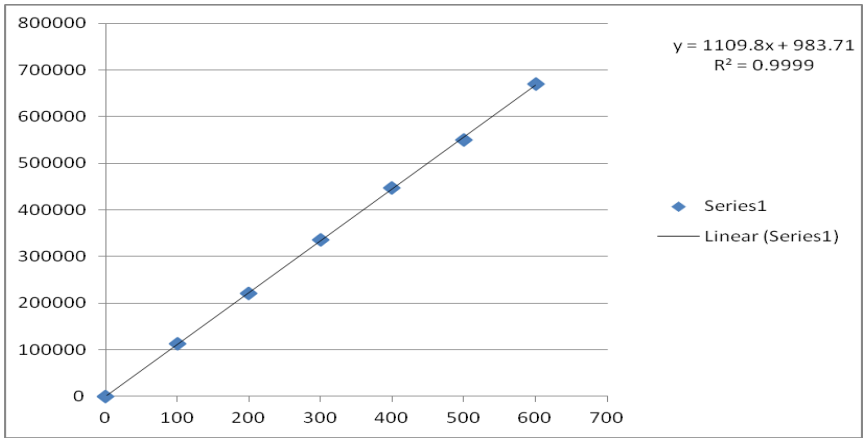

Fig.5: Calibration curve of azilsartan medoxomil

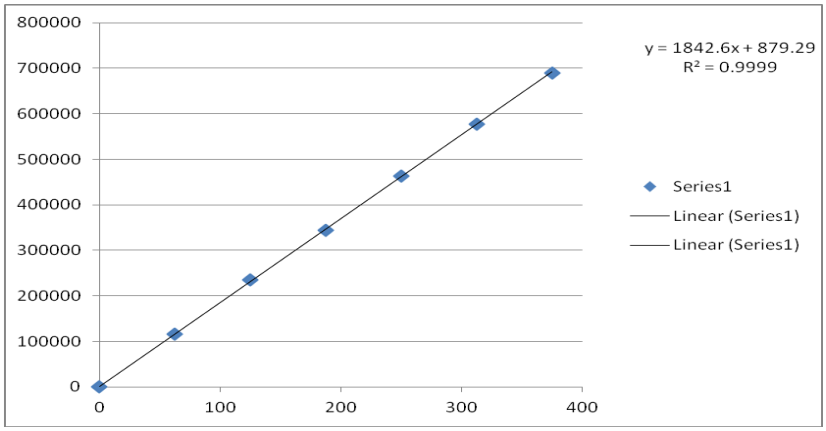

Fig.6: Calibration curve of chlorthalidone

Table.1: Validation parameters

Parameters	Azilsartan	Chlorthalidone
Recovery	99.81%	99.71%
Intraday precision	0.40	0.34
Inter day precision	0.70	1.31
LOD	2.93µg/ml	1.58µg/ml
LOQ	8.87µg/ml	4.77µg/ml
Specificity	Specific	Specific
Robustness	1.53	1.27
Solvent stability	Stable for 24 hrs	Stable for 24 hrs

Table.2: Calibration Data

Parameters	Azilsartan	Chlorthalidone
Optimized Wavelength	220nm	220nm
Linearity range	40ppm-240ppm	25ppm-150ppm
Intercept	983.7	879.2
Slope	1109	1842
Correlation Coefficient	0.999	0.999
Linearity Equation	y = 1109.x + 983.7	y = 1842.x + 879.2

Table.3: Robustness Data

Parameters	Azilsartan	Chlorthalidone
Flow minus	0.57	0.54
Flow Plus	0.35	1.75
Mobile phase minus	1.56	1.44
Mobile phase plus	1.29	1.10
Temperature minus	0.53	0.75
Temperature Plus	0.65	0.38

Table.4: Recovery Data

Parameters	Azilsartan			Chlorthalidone		
Level of Recovery	50%	100%	150%	50%	100%	150%
%Recovery	99.69	99.93	99.81	99.80	99.74	99.61
STDEV	0.962	0.170	0.152	0.403	0.297	0.393
%RSD	0.96	0.17	0.15	0.40	0.30	0.39

Table.5: Assay table

	Lable claim		Amount recovered		% Assay	
Formulation	AZIL	CHLO	AZIL	CHLO	AZIL	CHLO
Edarbyclor	40	25	39.77	24.89	99.43	99.55

Table.6: System suitability table

Parameters	Azilsartan	Chlorthalidone
Retention time	2.5±0.3min	3.5±0.3min
Plate count	2814	9441
Tailing Factor	1.45	1.25
Resolution		5.2
%RSD	0.4	0.34

Table.7 Degradation data of Azilsartan

Degradation	% Degraded	Purity Angle	Purity Threshold
Acid	92.21	0.399	0.429
Alkali	93.14	0.372	0.508
Peroxide	94.23	1.522	2.413
Thermal	95.05	1.456	2.431
Photolytic	98.07	0.210	0.349
Water	99.13	0.213	0.393

Table.8 Degradation data of chlorthalidone

Degradation	% Degraded	Purity Angle	Purity Threshold
Acid	92.28	0.283	0.384
Alkali	93.88	0.212	0.311
Peroxide	94.41	0.235	0.394
Thermal	95.10	1.610	2.434
Photolytic	98.55	0.265	0.392
Water	99.17	0.187	0.473

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