Original Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF AZILSARTAN MEDOXOMIL AND CHLORTHALIDONE IN BULK AND TABLET DOSAGE FORM

S. S. AHER[1]*, R. B. SAUDAGAR, HEMANT KOTHARI

[1]R. G. Sapkal College of Pharmacy, Anjaneri, Nashik, [2]Pacific Academy of Higher Education and Research University, Udaipur
Email: smitarohokale@yahoo.com

ABSTRACT

Objective: A simple, precise, accurate method was developed for the simultaneous estimation of azilsartan and chlorthalidone in bulk and tablet dosage form by RP-HPLC technique.

Methods: Acetonitrile and water in the ratio of (70:30) pH 2.8 used as mobile phase run through (Cosmosil C18 (4.6ID x 250 mm, Particle size: 5 micron) column with a flow rate of 0.9 ml/min. The temperature of the column oven was maintained at 30 °C. Wavelength was selected 244 nm. Stock and working solutions were prepared by using the diluents water and acetonitrile in the ratio of 50:50. Runtime was fixed to 9 min.

Results: Chlorthalidone and azilsartan were eluted at 2.02 and 3.92 with good resolution the plate count, tailing factor and all system suitability parameters are within ICH range. Azilsartan Medoxomil and Chlorthalidone were found to be linear low in concentration range of 80-400µg/ ml and 25-125µg/ ml respectively in the linearity study, regression equation and coefficient of correlation for Azilsartan Medoxomil and Chlorthalidone were found to be (y = 28695x+15397 r^2=0.995) and (y=13444+27405 r^2 = 0.996) Percentage recovery for both Azilsartan Medoxomil and Chlorthalidone was found in range of 99.89%-99.96% indicating accuracy of the proposed work. Assay of the tablet was performed and found as 100.15%.

Conclusion: All the parameters were within the ICH guidelines, and the method was economical and simple as retention times were less than in literature and decreased run time.

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

INTRODUCTION

This Azilsartan Medoxomil and Chlorthalidone fixed-dose combination is found to show superior antihypertensive efficacy in blood pressure reduction in patients with stage 2 hypertension Azilsartan Medoxomil is an Angiotensin II receptor antagonist [1, 2] 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 mono-potassium salt [3, 4]. It is a white crystalline powder which is practically insoluble in water, freely soluble in methanol, dimethyl sulfoxide and dimethylformamide, soluble in acetic acid, slightly soluble in acetone and Acetonitrile and very slightly soluble in Tetra Hydro furan and 1-octanol [5, 6].
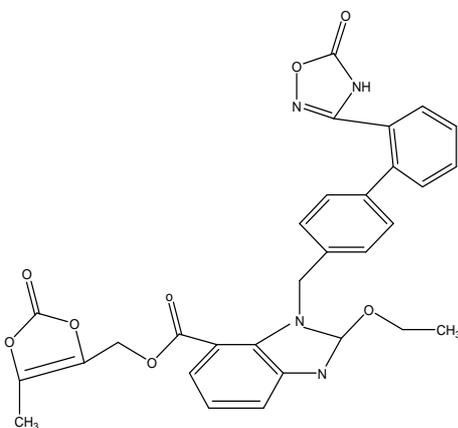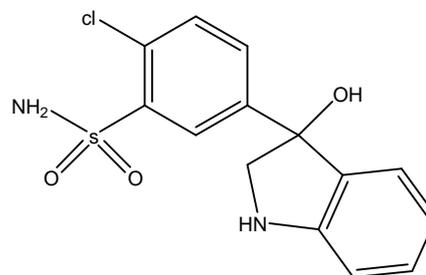

Fig. 2: Structure of chlorthalidone

MATERIALS AND METHODS

Instrumentation

A high-performance liquid chromatography system consisting of HPLC Binary Gradient System Model no HPLC 3000 Series Company: Analytical Technologies Ltd. UV detector-300 Reciprocating (40MPa) pump with Cosmosil C18 (4.6ID x 250 mm, Particle size: 5 micron) column Software HPLC Workstation High Precision Balance Model: max 100 gm Min: 0.001 gm.

Reagents and chemicals

HPLC grade solvents methanol, Acetonitrile and water were obtained from Merck Specialities Pvt Ltd, India. Water was deionized and further purified by means of Milli-Q plus water purification system; AR grade Potassium dihydrogen Orthophosphate was obtained from Ranchem Pharmaceuticals India Ltd.

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

Fig. 1: Structure of azilsartan medoxomil

Preparation of stock, working standard

Standard stock solutions containing Azilsartan Medoxomil and Chlorthalidone prepared individually by dissolving 10 mg of Azilsartan Medoximil and 10 mg of Chlorthalidone separately in 100 ml of mobile phase. It was then sonicated for 15 min, and the final volume of both the solutions was made up to 100 ml with methanol to get stock solutions containing 1000 µg/ml.
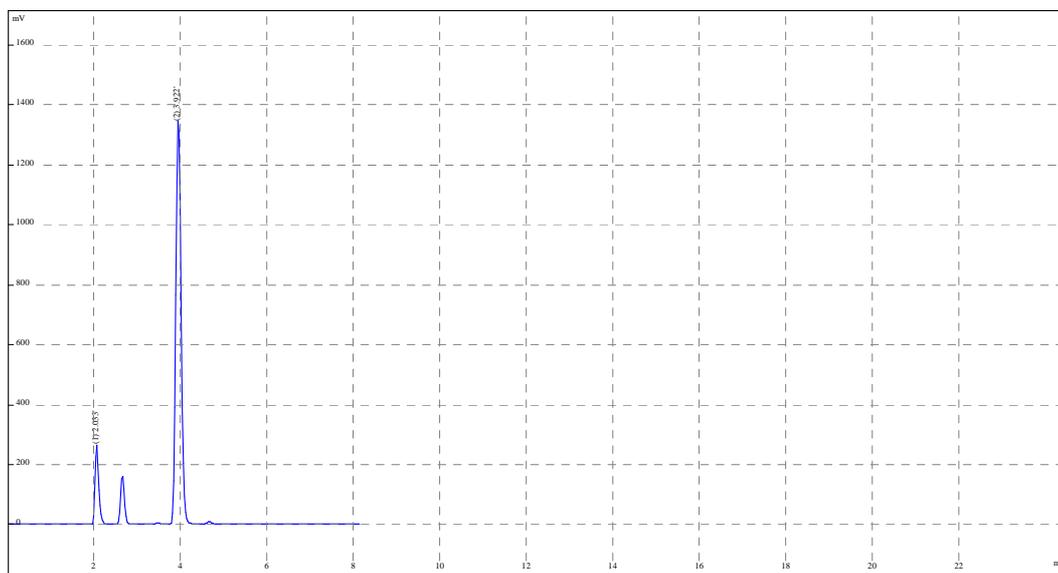

Fig. 3: Standard chromatogram of azilsartan medoxomil and chlorthalidone

Method validation

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, robustness and system suitability.

Linearity

The developed method has been validated as per ICH guidelines. Working standard solutions of Chlorthalidone and Azilsartan Medoxomil in the mass concentration range of 80-400 ppm and 25-125 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 Azilsartan Medoxomil and Chlorthalidone was obtained by plotting the peak area ratio versus the applied concentrations. The linear correlation coefficient was found to be 0.996and0.995 resp.
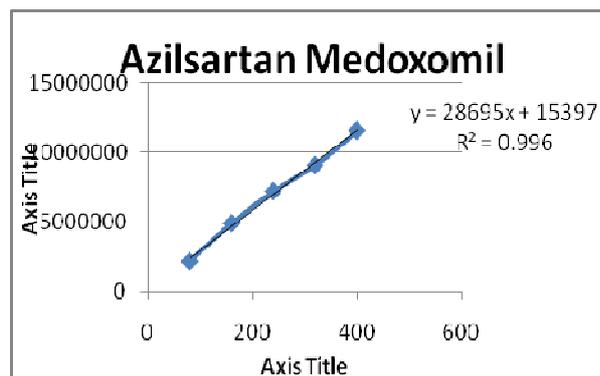

Fig. 4: Linearity graph of azilsartan medoxomil

Table 1: Linearity results showing correlation coefficient for azilsartan medoxomil

Conc(µg/ml)	Conc(µg/ml)
80	2224150
160	4961110
240	7269323
320	9132435
400	11616294
Corre. Coeff	0.996
Intercept	15397

Table 2: Linearity results showing correlation coefficient for chlorthalidone

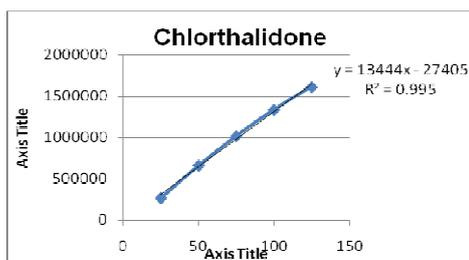| Conc(µg/ml)	Conc(µg/ml)
25	270195
50	663033
75	1019758
100	1338698
125	1612897
Corre. Coeff.	0.995
Intercept	27405

Fig. 5: Linearity graph of chlorthalidone

Precision and robustness

Precision

The reproducibility of the proposed method was determined by performing tablet assay at different time intervals (morning, afternoon and evening) on the same day (Intra-day assay precision) and on different days (Inter-day precision). Result of intra-day and inter-day precision is expressed in % RSD. Percent RSD for Intraday assay precision was found to be 1.64 Chlorthalidone, and 0.98Azilsartan medoxomil Inter-day assay precision was found to be 1.08 Chlorthalidone and 0.50Azilsartan medoxomil.

Table 3: Result of precision

Conc. of azilsartan medoxomil and chlorthalidone	Interday precision	Day1		Day 2			Mean	%RSD
75: 240 PPM	1019758	1006574	1011017	1004354	986824	1006748	1005878	1.08%
75:240 PPM	7269353	7309656	7279342	7314070	7293703	7371677	7306295	0.50%
	Intraday	Morning		Evening				
75:240 PPM	1019758	1006874	1011017	1020586	995025	1011221	1016197	1.64%
75:240 PPM	7269323	7309656	7279342	7387730	7247470	7427650	7320195	0.98%

Average of three readings

Table 4: Results of robustness

Parameter	% mean±SD		% RSD*	
Robustness	Azilsartan medoxomil and	Chlorthalidone	Azilsartan medoxomil	Chlorthalidone
75:240 PPM	98.62±0.10	99.03±0.21	0.95	1.20

Accuracy

The validity and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100%, and 120%) was found at three replicate and three concentrations level. The values of % mean just close to 100, SD and % RSD are less than 2 indicate the accuracy of the method. The result of recovery study showed in table 5.

Table 5: Results of recovery

Drug	Level	Conc. of std. (µg/ml)	Conc. of solution (µg/ml)	% recovery	%RSD
Azilisartan Medoximil	80%	25	20	99.36	0.324
		25	25		
		25	30	98.89	0.524
Chlorthalidone	100%	80	64		
	120%	80	80	99.26	0.216
		80	96		

Average of three readings

RESULTS AND DISCUSSION

The present work describes RP-HPLC method for simultaneous estimation of Azilsartan Medoxomil and Chlorthalidone in tablet dosage form on RP C-18 Column, Cosmosil C18 (4.6ID x 250 mm, Particle size: 5 micron) using Acetonitrile and Water (70:30v/v, pH 2.8) as mobile phase at a flow rate of 0.9 ml/min and the detection wavelength was 244 nm [8]. The retention time for Azilsartan Medoxomil and Chlorthalidone was found to be 2.0 and 3.92 min respectively detection response for both Azilsartan Medoxomil and Chlorthalidone were found to be linear low in concentration range of 80-400µg/ml and 25-125µg/ml respectively in the linearity study, regression equation and coefficient of correlation for Azilsartan Medoxomil and Chlorthalidone were found to be and (y=13444+27405 r=0.995 and (y=13444+27405 r^2 = 0.996) Percentage recovery for both Azilsartan Medoxomil and Chlorthalidone was found in range of 99.89%-99.96% indicating accuracy of the proposed work.

The percentage RSD for both the tablet analysis and recovery studies is less than 2% indicating high degree of precision. The results of recovery studies were found to be linear in 80-120% of final assay concentration range indicating linearity and range of the proposed method [9].

The robustness of the proposed method determined by analyzing the same batch of Azilsartan Medoximil and Chlorthalidone tablets by changing the wavelength the overall mean, standard deviation, and % RSD of the assay values were found to be less than 2% which shows the ruggedness of our method [10].

A sample solution of Azilisartan Medoximil and Chlorthalidone tablets was prepared as per the proposed method and analyzed initially and also analyzed at different time intervals by keeping the solution at room temperature. The cumulative %RSD for the area counts of Azilisartan Medoximil and Chlorthalidone was found to be less than 2% The results of the robustness study also indicated that the method is robust and is unaffected by deliberate variation in the chromatographic conditions. Hence, it can be concluded that the developed RP-HPLC method is accurate, precise, and selective and can be employed successfully for the estimation of Azilisartan Medoximil and Chlorthalidone in tablet dosage formulation.

CONCLUSION

A simple rapid, precise and reliable method was developed for the estimation of the Azilisartan Medoximil and Chlorthalidone tablet dosage formulation. The results obtained are within the specified

limit by the ICH guidelines. Analytical column used and the mobile phase provides good separation and gives the sharp results. The retention time observed for both the drugs was good hence the method can be used for routine analysis in quality control laboratories.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declared none

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