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

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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 (4.6D 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 r²=0.995) and (y =13444+27405 r² = 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].

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 (4.6D 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 Specialties 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.
Preparation of stock, working standard

Standard stock solutions containing Azilsartan Medoxomil and Chlorthalidone prepared individually by dissolving 10 mg of Azilsartan Medoxomil 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.

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 0.996 and 0.995 respectively.

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    |
| Corr. 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     |
| Corr. Coeff.| 0.995       |
| Intercept  | 27405       |
Accuracy

Wavelength was 244 nm [8]. The retention time for Azilsartan 2.8 as mobile phase at a flow rate of 0.9 ml/min and the detection particle size: 5 micron) using Acetonitrile and Water (70:30 v/v, pH dosage form on RP C-18 Column, Cosmosil C18 (4.6ID x 250 mm, estimation of Azilsartan Medoxomil and Chlorthalidone in tablet.

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 (4.6 ID x 250 mm, Particle size: 5 micron) using Acetonitrile and Water (70:30 v/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 in concentration range of 80-400 μg/ml and 25-125 μg/ml respectively, in the linearity study, regression equation and coefficient of correlation for Azilsartan Medoxomil and Chlorthalidone were found to be and % RSD for Intra-day precision is expressed in % RSD. Percent RSD for Intra-day precision was found to be 1.64 Chlorthalidone, and 0.98 Azilsartan medoxomil. Inter-day precision was found to be 1.64 Chlorthalidone, and 0.98 Azilsartan medoxomil.

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 Intra-day assay precision was found to be 1.64 Chlorthalidone, and 0.98 Azilsartan medoxomil. Inter-day assay precision was found to be 1.08 Chlorthalidone and 0.50 Azilsartan medoxomil.

Robustness

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 Azilsartan Medoximil and Chlorthalidone 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 Azilsartan 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 Azilsartan Medoxomil and Chlorthalidone in tablet dosage formulation.

CONCLUSION

A simple rapid, precise and reliable method was developed for the estimation of Azilsartan Medoxomil and Chlorthalidone tablet dosage formulation. The results obtained are within the specified limits of accuracy, precision, and reliability. This method can be employed for the routine analysis of Azilsartan Medoxomil and Chlorthalidone in pharmaceutical dosage form.
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 authors have contributed equally

CONFLICT OF INTERESTS
Declared none

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