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Research Article

Simultaneous Estimation of Olmesartan Medoxomil and Hydrochlorothiazide by RP-HPLC Method from Combined Dosage Forms

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ABSTRACT

A simple, reproducible and efficient reverse phase high performance liquid chromatographic method was developed for simultaneous estimation of olmesartan medoxomil (OLM) and hydrochlorothiazide (HCTZ) in combined tablet dosage form. Formulation containing OLM with HCTZ are used as antihypertensive angiotensin II receptor blocker. Chromatography was performed on a 250 mm x 4.6 mm, 5-µm particle size, C_8 Qualisil BDS column with a 50:50 (*v/v*) mixture of buffer and acetonitrile as a mobile phase and the pH was adjusted to 4.7 by adding dilute phosphoric acid. The detection of the combined dosage form was carried out at 225 nm and a flow rate employed was 1 ml min^{-1}. The retention times were 5.074 & 7.242 min for olmesartan medoxomil and hydrochlorothiazide, respectively. Linearity was obtained in the concentration range 20 to 100 µg mL^{-1} for olmesartan medoxomil and in the range 12.5 to 62.5 µg mL^{-1} for hydrochlorothiazide, with a correlation coefficient of 0.9956 and 0.989. The result of the analysis were validated statistically and recovery studies confirmed the accuracy and precision of the proposed method.

Keywords: Olmesartan Medoxomil, Hydrochlorothiazide, RP-HPLC, Simultaneous Estimation.

INTRODUCTION

Olmesartan medoxomil belongs to angiotensin II receptor blocker effective in lowering blood pressure in hypertensive patients. Chemically it is known as 2,3-dihydroxy-2-butenyl 4-[1-hydroxy-1-methylethy]-2-propyl-1-[p(o-1H-tetrazol-5-ylphenyl) benzyl] imidazole-5-carboxylate, cyclic 2,3-carbonate.It's molecular weight is 558.59 [Andrew Whittaker 2005, Black HR *et al.,* 2003*,* The Merck Index 3., 2006].

Hydrochlorothiazide is a diuretic of the class of benzothiadiazine widely used in antihypertensive pharmaceutical formulations, alone or combination with other drugs, which decreases active sodium reabsorption and reduced pheripheral vascular resistance. It is chemically 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide, and was successfully used as one content in association with other drugs in the treatment of hypertension. Its molecular weight is 297.7[The Merck Index 3, 2006; Markhan A *et al.,* 1997]

There are very few methods appearing in the literature for the simultaneous estimation of angiotensin II receptor antagonist and hydrochlorothiazide in tablets [Carlucci G *et al.,* 200; Schoenberg JA *et al.,* 1995; Del Castillo D *et al.,* 1998].

Since these methods were based on HPLC, Capillary electrophoresis and UV derivative spectrophotometry [Daneshtalab N *et al.,* 2002; Hillaert S *et al.,* 2003, Satana E *et al.,* 2001], the procedure was inconvenient for determination and run time were rather long.

Thus, an attempt was made to develop a simple, precise, accurate and economical RP-HPLC method for the simultaneous estimation of olmesartan medoxomil and hydrochlorothiazide in tablets.

The chemical structures of olmesartan medoxomil and hydrochlorothiazide are shown in Fig. 1.

EXPERIMENTAL

Chemicals and Reagents

HPLC-grade acetonitrile and sodium dihydrogen ortho phosphate analytical grade were procured from Rankem (Mumbai, India) and pure standards of olmesartan medoxomil (99.26%) and hydrochlorothiazide (99.89%) were obtained as gift samples from Caplin point laboratories (Pondicherry, India). Hydrochloric acid was procured from E. Merck (Mumbai, India). HPLC grade water was procured from Ranbaxy (Mumbai, India).

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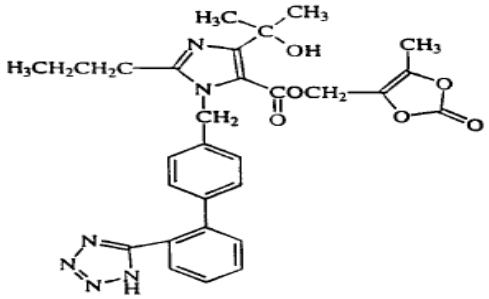
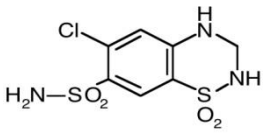
Instrumentation and Chromatographic Conditions

Chromatography was performed with a Agilent technologies 1120 compact LC (Germany) gradient pump,



Olmesartan medoxomil



Hydrochlorothiazide

Fig. 1 The chemical structure of olmesartan medoxomil and hydrochlorothiazide

an variable wavelength detector and a rheodyne 9013 injector with 20-µL loop, C8 Qualisil BDS column (250 x 4.6nm., 5-µm particles) was used for chromatographic separation under suitable condition. Detection was carried out at 225 nm and the software used was EZ chrom elite version 3.3.

The mobile phase was a 50:50 (*v/v*) mixture of freshly prepared buffer (sodium dihydrogen ortho phosphate and triethyl amine) and acetonitrile. The mobile phase was filtered through 0.45 µ membrane filter and sonicated before use. The flow rate of mobile phase was maintained at 1 mL min^{-1}.The column temperature was maintained at ambient temperature. The detection wavelength was 225nm for both drugs. The injection volume was 20 µL and total run time was 10 min. The peaks were identified by retention time; a typical chromatogram is shown in Fig. 2.

Preparation of standard solution for calibration Plots

Weigh accurately about 0.100g of olmesartan medoxomil working standard and 0.0625g of hydrochlorothiazide transferred to a 100 ml volumetric flask. Then, add 30 ml of 0.1N HCL, sonicated to dissolve the content and make up the volume with diluents (acetonitrile & water (80:20)). Filter through 0.45µ membrane filter paper. Dilute 5ml of resulting solution to 50 ml with diluent to give a concentration of 100 µg mL^{-1} of olmesartan medoxomil & 62.5 µg mL^{-1} of hydrochlorothiazide. Stock solution was diluted with mobile phase to give working standard solution containing 20 to 100 µg mL^{-1} of olmesartan medoxomil and 12.5 to 62.5 µg mL^{-1} hydrochlorothiazide. These standard solutions were injected for construction of calibration plots by plotting drug peak-area ratio (*y*) for each of the drug against concentration (*x*). Analysis was performed at ambient temperature. The retention times of olmesartan medoxomil and hydrochlorothiazide under these conditions were 5.07 and 7.24 min respectively.

Assay procedure

Twenty tablets, each containing OLM (20 mg) and HCTZ (12.5 mg) were weighed, finely powdered, and an amount of powder sample equivalent to 100 mg of olmesartan medoxomil and 62.5mg hydrochlorothiazide was taken in 100 mL volumetric flask and dissolved in 30 ml of 0.1N HCL and extracted by sonication to ensure complete solubility of the drug.

The excipients were separated by filtration. The mixture was then made up to 100 mL with diluents, thoroughly mixed and filtered through a 0.45 µ membrane filter. The filtrate concentration of olmesartan medoxomil was 100 µg mL^{-1} and for hydrochlorothiazide was 62.5 µg mL^{-1} from tablets. Then the solutions were injected five times into the column. Each injection was replicated five times. From the peak area, the drug content in the tablets was qualified using the regression equation obtained from the pure samples.

A typical chromatogram obtained from a sample solution is shown in Fig. 2.

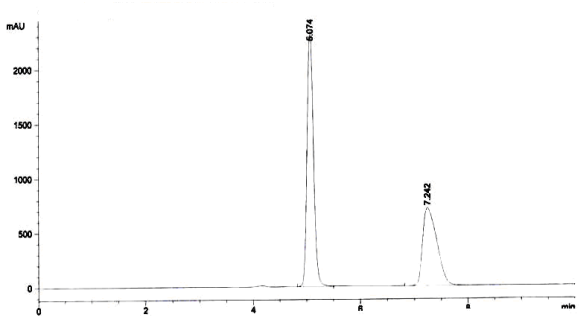


Fig. 2 Typical chromatogram obtained from olmesartan medoxomil and hydrochlorothiazide

RESULTS AND DISCUSSION

Method development

The objective of this study was to develop simultaneous estimation of two components under isocratic conditions. The mobile phase used was the mixture of acetonitrile with buffer in different ratios. Finally a mixture of acetonitrile- buffer (pH 4.7) in the ratio of 50:50 (*v/v*), proved to be effective mixture than the other mixture used for the separation. Then the flow rate tested includes 0.4, 0.8, 1.0, 1.2 and 1.5 ml. Among the flow rates 1.0 ml was selected for the assay because of better resolution of the peaks.

The mentioned chromatographic conditions revealed to provide better resolution between olmesartan medoxomil and hydrochlorothiazide in a reasonable time of 5.074 and 7.242 min, respectively. The optimum wave length for detection was 225 nm, no indigenous

interfering compounds eluted at the retention times of the drugs.

and 62.5 µg mL^{-1} HCTZ for tablets was prepared from the stock solutions and was spiked with amounts of the standard drugs equivalent to 50,100 and 150% of the amounts present in the original solution. These

Table. 1 Results from validation and system-suitability studies

Method characteristic	Olmesartan medoxomil	Hydrochlorothiazide
Theoretical plates	8956	3427
Resolution	-	6.11
Linearity range (µg mL^{-1})	20-100	12.5-62.5
Percentage Recovery (%) *	96.7	97.5
Symmetry factor	0.88	0.49
Correlation coefficient	-	2.63
Capacity factor	0.36	0.94
Accuracy (RSD (%))**	0.68	0.74
Intra-day precision (RSD (%))*	0.39	0.31
Inter-day precision (RSD (%))*	0.50	0.54

* Results are mean five replications

* * Results are mean three replications with three different drug concentrations

Validation of the Method

The method was validated, in accordance with ICH guidelines, for linearity, accuracy, precision, specificity, sensitivity, ruggedness, and robustness.

Linearity

Linearity was assessed with the aid of serially diluted calibration solutions as mentioned above. The standards were injected separately. Calibration graphs were plotted on the basis of triplicate analysis of each calibration solutions. Linear correlations were obtained over the range studied, with correlation coefficients ≥ 0. 99 for the drugs. In case of tablets, the regression equation was y = 0.0198x (R2=0.9956) for olmesartan medoxomil and y = 0.02172x (R2=0.989) for hydrochlorothiazide.

Precision

The precision of the method was done by replicate (n=5) analysis of tablet preparations. The precision was also studied in terms of intra-day changes in peak area of drug solution on the same day and on three different days over a period of one week. The intra-day and inter-day variation was calculated in terms of percentage relative standard deviation and the results are given in Table. 1.

solutions were then analyzed for recovery studies and consistent values by replicated injections cum analysis. Results for determination of precision and accuracy are presented in Table. 1.

Specificity

Specificity was tested against standard compounds and against potential interferences in the presence of placebo. No interference was detected at the retention time of OLM and HCTZ in sample solution. Peak purity for OLM and HCTZ was tested by comparing spectra acquired at the start (S), apex (A), and end (E) of the peaks.

Recovery

Recovery was determined by spiking the formulation with standards of each drug equivalent to 50,100, and 150 % of the amount originally present. Average recoveries ranged from 97 to 100, as reported in Table. 1.

Stability

To demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 h at room temperature. The results showed that for both solutions, the retention times and peak areas of OLM and HCTZ remained almost unchanged (RSD<2.0%) indicating that no signif-

Table. 2 Results from validation and system-suitability studies

Formulation	Labeled amount (mg per dose)	Amount found (mg per dose)	Recovery (%, n = 5)
Olmesartan medoxomil	20	19.70	98.52
Hydrochlorothiazide	12.5	12.38	99.06

Accuracy

Accuracy was determined by the method of standard additions at three different levels, by multiple level recovery studies. Solution containing 100 µg mL^{-1}OLM

icant degradation occurred within this period, i.e. both solutions were stable for at least 24 h, which was sufficient to complete the whole analytical process. Sample solution were then stored at 4 and 25° C and checked

after three days of storage. When results were compared with those from freshly prepared sample in each case no significant degradation occurred within the indicated period.

Ruggedness and Robustness

The ruggedness of the method was determined by using different instrument (Waters 2489) and different column (Symmetry C_8) of similar type. The robustness of the method was determined by making slight changes in the chromatographic conditions (buffer pH±0.5, flow rate±0.2 min). Again there was no marked change in the chromatograms. These results indicated that the method was rugged and robust with regard to these conditions. When mobile phase composition was changed by ± 10%, however, proper resolution could not be achieved; separation of the drugs was very sensitive to mobile phase ratio.

System suitability tests are an integral part of chromatographic method. They were used to verify that the reproducibility of the chromatographic system is adequate for the analysis. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared standard stock solution of olmesartan medoxomil and hydrochlorothiazide. In addition, standard deviation of olmesartan medoxomil and hydrochlorothiazide standards were evaluated by injecting mixed standard of both olmesartan medoxomil and hydrochlorothiazide (100 and 62.5 µg/mL). All the parameters are shown in Table. 2

CONCLUSION

The proposed RP-HPLC method for simultaneous assay of olmesartan medoxomil and hydrochlorothiazide in combined tablets dosage forms is simple, precise, specific and highly accurate and less time consumption for analysis could be recorded. So, it can definitely be employed for the routine analysis. Hence this RP-HPLC method is suitable for quality control of raw materials and formulations, and also for dissolution studies. It can be used for bioequivalence studies in plasma.

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