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DETERMINATION OF OLMESARTAN AND HYDROCHLORTHIAZIDE IN PHARMACEUTICAL FORMULATIONS BY RP-HPLC

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ABSTRACT

A simple and sensitive reverse phase high performance liquid chromatographic method for the determination of Olmesartan (OLM) and Hydrochlorthiazide (HCZ) was developed on a Shimadzu Class vp series HPLC system with a phenomenex C_{18} column (150 x 4.6 mm, 5 µ) using a mobile phase mixture containing acetonitrile and ammonium acetate buffer (pH-3.5) in the ratio of 55 : 45. The flow rate was 1.0 ml/min and effluents were monitored at 252 nm and eluted at 2.14 min (OLM) and 4.62 min (HCZ). Calibration curve was plotted with a range from 2.0-20 µg/mL for OLM and 1.0 -10 µg/mL for HCZ. The assay was validated for the parameters like accuracy, precision, robustness and system suitability parameters. The results were found to be satisfactory and the method can be adapted for the routine quality control of the drugs in formulations.

Key words: Olmesartan, Hydrochlorthiazide, Reverse phase HPLC, Validation.

INTRODUCTION

Olmesartan (shown in Fig. 1a) is 4-(1-Hydroxy-1-methylethyl)-2-propyl-1- [2′-(1H-tetrazoyl)[1,1′biphenyl]-4-yl]methyl]-1H-imidazole-5-carboxylic acid having anti hypertensive activity where as Hydrochlorthiazide (shown in Fig. 1b) is 6-Chloro-3,4-dihydro-2H-1,2, 4-benzothiadiazine-7-sulfonamide 1, 1 dioxide, used in the treatment of diuretics. Olmesartan is anti-hypertensive drug with prolonged effect. It selectively inhibits the binding of angiotensin II to angiotensin I receptors in vascular smooth muscles and thus blocks the vasoconstrictive effect, suggesting that there may be less risk of hypertension. Hydrochlorthiazide is the benzthiazide class of sulfonamide derivative, its action is to

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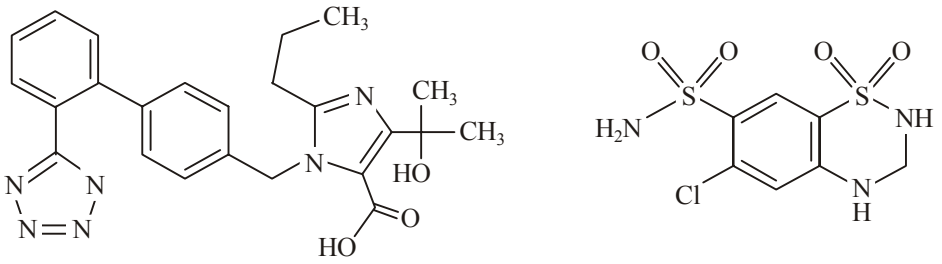
deplete the body's sodium stores. The drug reduces the loss of calcium, which inhibits the cells reabsorption of NaCl. Thiazides bind to the site of the Cl^- on the sodium chloride transporter molecule preventing it from picking up NaCl. The depletion of NaCl stores in the body reduces blood pressure and calcium output. The addition of HCZ to OLM was more effective than each agent at lowering blood pressure in patient with blood pressure.



(a) **(b)**

Fig. 1: (a) Olmesartan, (b) Hydrochlorthiazide

The literature reveals that there are some of the methods have been reported for olmesartan and hydrochlorthiazide in single dosage forms and only few reports were found in combined dosage forms by UV[1], HPLC[2-4] LCMS[5,6] and HPTLC[7,8].

The present paper describes a simple, sensitive, validated and economic method for the determination of olmesartan and hydrochlorthiazide

EXPERIMENTAL

Materials and reagents

Olmesartan and Hydrochlorthiazide were obtained from Burgeon Pharmaceuticals Ltd., Chennai, India. Acetonitrile (HPLC grade, Qualigens, Mumbai), MilliQ water was used through out the analysis. All the other reagents were of AR grade.

Instrumentation

The HPLC system consisted of a Shimadzu Class LC-10AT vp and LC-20AD pumps connected with SPD-10A vp UV-Visible detector. The data acquisition was performed by Spinco tech 1.7 software. The method was developed on a Gemini C_{18} (150 x 4.6 mm i.d, 5 µm) {Phenomenex,Torrance, USA}. Column maintained at ambient temperature. The mobile consisted of a mixture of acetonitrile and ammonium acetate buffer (pH-3.5) fixed in the ratio of 55 : 45 at a flow rate of 1.0 mL/min.

Preparation of stock and sample solutions

The standard stock solutions were prepared with methanol to give the final concentration of 1000 µg/mL. The working standard solutions of OLM and HCZ were prepared by taking suitable aliquots of drug solution from the standard solutions and the volume was made up to 10 mL with mobile phase to get concentrations of 2.0-20 µg/mL for OLM and 1.0-10 µg/mL for HCZ. For the analysis of pharmaceutical formulations, ten tablets were weighed and powdered. A quantity equivalent to labeled amount was weighed and transferred into extraction flask, to this suitable amount of acetonitrile was added and the mixture was subjected to vigorous shaking for 30 min. for complete extraction of drugs, and then centrifuged at 5000 rpm for 20 min. (Remi R8C laboratory centrifuge). Supernatant was collected from each set and diluted with mobile phase and injected to HPLC system for the analysis.

RESULTS AND DISCUSSION

Optimization of the method

A reversed-phase column procedure was proposed as a suitable method for the simultaneous determination of olmesartan and hydrochlorthiazide in combined dosage form. The chromatographic conditions were optimized by changing the mobile phase composition, pH, and buffers used in the mobile phase. Different ratios were tried to optimize the mobile phase. Finally a mixture of acetonitrile and ammonium acetate buffer (pH-3.5) in the ratio of 55 : 45 was used fixed at flow rate of 1.0 mL/min was used for the elution of the drugs.

A typical chromatogram obtained by using the afore mentioned mobile phase from 20 µL of the assay preparation is illustrated in Fig. 2. The retention factors of OLM and HCZ were 2.14 and 4.62 min, respectively.

Validation of the method

The linearity of the method was tested from 2.0-20 µg/mL for OLM and 1.0-10 µg/mL for HCZ. Linearity solutions were injected in triplicate and the calibration graphs were plotted as peak area of the analyte against the concentration of the drug in µg/mL. In the simultaneous determination, the calibration graphs were found to be linear for both the analytes in the mentioned concentrations and the correlation coefficients for the regression line were 0.9991 and 0.9996 for OLM and HCZ respectively. The accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drug to the placebo. The recovery was determined at three levels, viz. 100%, 110%, and 120% of the selected concentrations. Three samples were prepared for

each recovery level. The recovery values for OLM and HCZ ranged from 99.7-101.3% and 98.4-100.3%, respectively (Table 1). The precision (repeatability and intermediate precision) of the method was determined from one lot of combined dosage form. Intra and Inter day studies were performed by taking six replicates of three concentrations. The results are shown in (Table 2). The limit of detection (LOD) and limit of quantitation (LOQ) for OLM and HCZ were found to be 0.06 µg/mL, 0.20 µg/mL and 0.03 µg/mL, 0.1 µg/mL, respectively indicates the sensitivity of the method. To determine the robustness of the developed method experimental conditions were purposely altered and RSD of the peak areas of OLM and HCZ were found not greater than 2.0 illustrate the robustness of the method (Table 3).

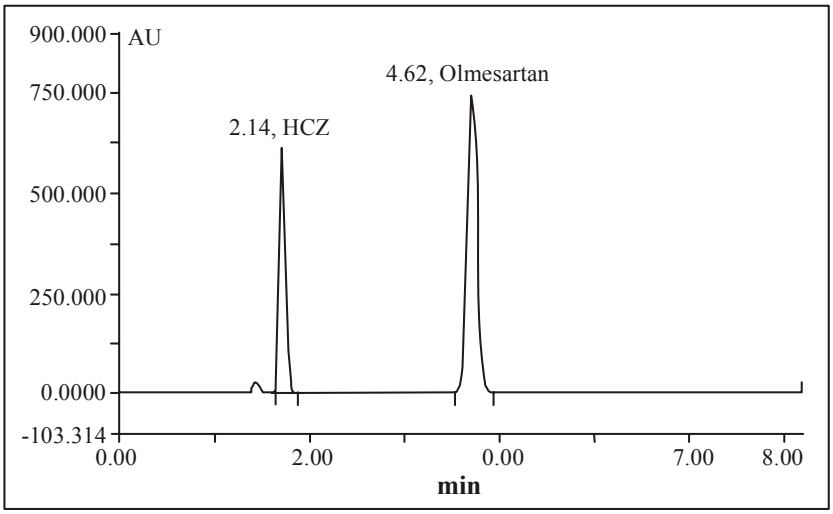


Fig. 2: Typical chromatogram showing the peaks of olmesartan (2.14 min) and hydrochlorthiazide (4.62 min) in pure forms

Table 1: Recovery of OLM and HCZ

Sample ID	Concentration of drug (µg/mL)				% Recovery	
	Pure drug		Formulation			
	OLM	HCZ	OLM	HCZ	OLM	HCZ
100%	0	0	100	100	99.75	100.37
110%	9.97	10.27	100	100	100.47	98.58
120%	19.94	20.54	100	100	101.37	98.48
N = 3						

Table 2: Precision data for OLM and HCZ

Nominal concentrations (µg/mL)		Mean ± S.D, % RSD	
OLM	**HCZ**	**OLM**	**HCZ**
2.0	1.0	1.97 ± 0.28, 0.56	1.06 ± 0.021, 1.27
3.0	2.0	2.89 ± 0.65, 0.93	2.03 ± 0.045, 1.15
4.0	3.0	3.96.36 ± 0.91, 2.05	3.06 ± 0.113, 1.43

Each mean value is the result of triplicate analysis for three times a day for three days
% R.S.D = (S.D/mean) x 100

Table 3: Robustness data (n = 3)

Condition	OLM[a]	HCZ[a]
Standard condition	0.81	0.87
Acetonitrile 54%	1.19	1.09
Acetonitrile 56%	1.23	1.39
Flow rate 1.4 mL/min	1.39	1.31
Flow rate 1.6 mL/min	1.42	1.38
pH 3.3	1.24	1.57
pH 3.7	1.79	1.81

Each value obtained from 8 µg/mL and 5 µg/mL of OLM and HCZ respectively
[a] % RSD value

Table 4: Results of the determination of olmesartan and hydrochlorthiazide tablets (n = 6)

Brand	Amount taken (mg)		Amount found (mg)		% RSD	Assay (%)	
	OLM	**HCZ**	**OLM**	**HCZ**		**OLM**	**HCZ**
Olmeszest	20	12.5	19.79	12.62	1.33	100.3	99.6
Olmat	20	12.5	19.81	12.44	1.63	99.7	99.9

Application of the method to pharmaceutical formulations

The method is sensitive and specific for the quantitative determination of OLM and HCZ and also subjected to validation for different parameters, hence has been applied for the estimation of drug in pharmaceutical dosage forms. Each sample was analyzed in triplicate after extracting the drug as mentioned above in experimental section. The amount of recombant was found be within the range of 99% – 100.6%. None of the tablet excipients were found to interfere with the analyte peak and the results were shown in Table 4.

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for simultaneous determination of olmesartan and hydrochlorthizide from pure and pharmaceutical formulations. The mobile phase is simple to prepare and the run time was less than 10 min., which consumes only 5 mL of mobile phase shows that the method was economical. The sample recoveries in all formulations were in good agreement with their respective label claims and suggested non-interference in the estimation. Hence, the method can be easily and conveniently adopted for routine analysis of olmesartan and hydrochlorthiazide in combined dosage forms.

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