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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF OLMESARTAN MEDOXOMIL AND HYDROCHLOROTHIAZIDE

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ABSTRACT

A Simple, Fast, Precise reverse phase isocratic high performance liquid chromatographic (HPLC) technique has been developed for the simultaneous estimation of Olmesartan medoxomil (OLM) and Hydrochlorothiazide (HCTZ) in marketed formulations. Estimation of these combined formulation was performed using Waters Symmetry C_{18} column (150 x 4.6 mm, 5µm) using the mobile phase of composition Methanol , Acetonitrile and Ammonium acetate buffer (50:25:25 v/v/v, pH 3.5). The flow rate was 1.0ml/min and the seperation was observed at 240nm. The retention time of OLM and HCTZ was found to be 3.34 min and 1.69 min respectively. The method was found to be linear over a range of 30 µg/ml for OLM and 18.75 µg/ml for HCTZ. The method was validated according to the guidelines of International Conference on Harmonisation (ICH) and was successfully employed in the estimation of commercial formulations.

KEYWORDS: **Olmesartan medoxomil , Hydrochlorothiazide , RP-HPLC, Method validation**.

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INTRODUCTION

Olmesartan medoxomil, a prodrug, chemically Cyclic 2,3 carbonate 2,3 - dihydroxy - 2 - butenyl - 4 - (1 - hydroxy - 1 -methylethyl) - 1 - propyl - 1 - [p - (o - 1H - tetrazol - 5 - ylphenyl)benzyl]imidazole - 5 - carboxylate is hydrolyzed to olmesartan during absorption from the gastrointestinal tract. Olmesartan is a selective AT1 subtype angiotensin II receptor antagonist[1]. Hydrochlorothiazide , a diuretic chemically 6-chloro-1,1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7- sulfonamide is a a thiazide diuretic works by inhibiting water reabsorption in the nephron by inhibiting the sodium-chloride symporter (SLC12A3) in the distal convoluted tubule[2-3]. Chemical structures of both drugs are given in figure 1. Extensive literature survey revealed that very few methods were reported for the simultaneous estimation of Olmesartan medoxomil and Hydrochlorothiazide .So, an attempt has been made to develop an accurate, precise and economically viable RP-HPLC method for the simultaneous estimation of combination of interest in the current research.[4-10]

MATERIALS AND METHODS

Equipment Used

The chromatographic seperation was performed on Waters alliance 2695 seperation module integrated with Waters 996 PDA detector. A reverse phase Symmetry C_{18} (4.6 x 150mm, 5μm, Make: Waters) was used. LABINDIA UV spectrometer and Sartorius electronic balance were used for Ultravoilet spectroscopy and weighing purposes respectively.

Chemicals and Reagents

Pharmaceutical pure grade Olmesartan medoxomil and Hydrochlorothiazide were obtained from Sura Labs and Marketed formulation OLMAT-H ® with dosage of 20mg of olmesartan medoxomil and 12.5mg of Hydrochlorothiazide were procured from local market . HPLC grade Acetonitrile, Methanol and Water were procured from Merck specialities private limited, Mumbai.

Chromatographic conditions

Symmetry C_{18} (4.6 x 150mm, 5μm, Make: Waters) was used for the chromatographic separation at a detection wavelength of 240nm. Mobile phase of composition Methanol, Acetonitrile and Ammonium acetate buffer, pH 3.5 in a ratio of 50:25:25 was selected for elution and same mixture was used in the preparation of standard and sample solutions. Flow rate was adjusted to 1ml/min and the injection volume was 10μL.

Preparation of mobile phase

Ammonium acetate buffer - Accurately weighed 25 grams of ammonium acetate was taken in a 25ml distilled water, dissolved and adjusted to pH 3.3-3.5 with Orthophosphoric acid and final volume was made up with distilled water. Accurately measured 500 ml (50%) of Methanol, 250 ml of Acetonitrile HPLC (25%) and Ammonium acetate buffer 3.5 pH (25%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 μ filter under vacuum filtration.

Preparation of standard solution

Accurately weighed amounts 4 of 10 mg Olmesartan medoxomil and 10 mg hydrochlorothiazide were taken to a 10 ml cleaned and dried volumetric flask. This was then diluted with 7ml of diluent and was sonicated. The volume was made to10 ml with the same solvent. This was marked and labeled as Stock solution. Further, an amount of 0.3 ml Olmesartan medoxomil and 0.1875 mL Hydrochlorothiazide each were pipetted from the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluents to get 30 μg/ml of Olmesartan medoxomil and 18.75 μg/ml of Hydrochlorothiazide.

Preparation of sample solution

10 mg equivalent amount of Olmesartan medoxomil in combination tablet (Label claim of olmesartan- 20 mg, Hydrochlorothiazide - 12.5mg) is being taken to a 10 ml cleaned and dried volumetric flask. This was then diluted with 7ml of diluent and was sonicated. The volume was made with 10 ml with the same

solvent. This was marked and labeled as Stock solution. Further, an amount of 0.3 ml of the above stock solution was pipette into a 10ml volumetric flask and diluted up to the mark with diluents to get 30 µg/ml of Olmesartan medoxomil and 18.75 µg/ml of Hydrochlorothiazide.

Optimisation of HPLC method

The HPLC method was optimized with an aim to develop a simultaneous estimation procedure for the assay of Olmesartan medoxomil and Hydrochlorothiazide. For the method optimization, different mobile phases were tried, but acceptable retention times, theoretical plates and good resolution were observed with Methanol, Acetonitrile and Ammonium acetate buffer pH 3.5 (50:25:25 v/v/v) using Symmetry C_{18} (4.6 x 150mm, 5µm, Make: Waters)

Validation of the RP-HPLC method

Validation of the optimized method was performed according to the ICH Q2 (B) guidelines.

System suitability

System suitability was carried out with an injection of a solution of 100% concentration having 30µg/ml of Olmesartan medoxomil and 18.75 µg/ml of Hydrochlorothiazide the chromatographic system. Number of theoretical plates (USP plate Count) obtained and calculated tailing factor (USP tailing) were reported in table 1.

Linearity

For the determination of linearity, appropriate aliquots were pipetted out from working stock solution to a series of 10ml volumetric flasks and the volume was made up with the solvent to obtain concentrations ranging from 10-50 µg/ml of Olmesartan medoxomil and 6.25-31.25 µg/ml of Hydrochlorothiazide. Each solution was injected in triplicate. Calibration curves were plotted with observed peak areas against concentration followed by the determination of regression equations and calculation of the correlation coefficients. The calibration curves for Olmesartan medoxomil and Hydrochlorothiazide were shown in Figure 3 and their corresponding linearity parameters were given in table 2.

Accuracy

To ensure the reliability and accuracy of the method, recovery studies were carried out Three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same and the percent recovery was reported. The results were given in Table 4.

Precision

For intermediate precision 18.75 µg/ml of Hydrochlorothiazide and 30 µg/ml of Olmesartan medoxomil of the above sample solution was injected for five times in five different days and peak areas were recorded. The results were given in Table 3.

Specificity

Specificity of a method was determined by testing standard substances against potential interferences. The method was found to be specific when the test solution was injected and no interferences or disturbances were found because of the presence of excipients.

Limit of detection (LOD) and Limit of quantitation (LOQ)

The LOD and LOQ were calculated from the slope(s) of the calibration plot and the standard deviation (SD) of the peak areas using the formulae LOD = 3.3 σ/S and LOQ = 10 σ/S. the values were given in table 6.

Robustness

Robustness of the method were verified by altering the chromatographic conditions like mobile phase composition, flow rate, detection wavelength, etc.[11] and the % RSD should be reported. Small changes in the operational conditions were allowed and the extent to which the method was robust was determined. A deviation of retention times was observed with ±0.1 mL/min in the flow rate, and deviations in retention times were observed with increasing and decreasing organic content in the mobile phase. The results are shown in table 5.

Assay of marketed formulation

10µl of sample solution of concentration 30µg/ml of Olmesartan medoxomil and 18.75µg/ml of Hydrochlorothiazide was injected into chromatographic system and the peak responses were measured. The solution was injected three times into the column. The amount present in each tablet was calculated by comparing the areas of standards with the test samples.

RESULTS AND DISCUSSION

After a number of trials with mobile phases of different composition, acetonitrile and phosphate buffer, pH 6.8 50:50 v/v was selected as mobile phase because of better resolution and symmetrical peaks. OM and HCTZ were found to show appreciable absorbance at 260nm when determined spectrophotometrically and hence it was selected as the detection wavelength. An optimized chromatogram showing the separation of Olmesartan medoxomil and Hydrochlorothiazide at different RTs was shown in figure 2. System suitability was carried out by injecting 5 replicate injections of 100% test concentration, the number of theoretical plates, and resolution were satisfactory. The chromatogram confirms the presence of Olmesartan medoxomil and Hydrochlorothiazide at 3.34 min and 1.69 min respectively without any interferences. The parameters were given in table 1.

Table 1
System suitability parameters

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Hydrochlorothiazide	1.688	467354	984876	-	1.28	2842
2	Olmesartan medoxomil	3.338	1121479	161751	10.62	1.13	5525

Table 2
Results for Linearity

Concentrations (µg/ml)	Hydrochlorothiazide area	Concentrations (µg/ml)	Olmesartan Medoxomil area
6.25	473592	10	386642
12.5	474087	20	715547
18.75	474584	30	1079209
25	475118	40	1416945
31.25	475586	50	1769025

Table 3
Results for precision

Drug	STD.DEV	%RSD
Olmesartan medoxomil	7654.6	0.7
Hydrochlorothiazide	3732.3	0.8

Table 4
Results for Accuracy
4(a).Accuracy (recovery) data for Hydrochlorothiazide

%Concentration	Area	Amount present (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	681496	5	4.91	98.2%	99.26%
100%	900935	10	9.98	99.8%	
150%	1472830	15	14.97	99.8%	

4(b).Accuracy (recovery) data for Olmesartan Medoxomil

%Concentration	Area	Amount present (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1609146	5	4.95	99%	99.36%
100%	2187730	10	9.93	99.3%	
150%	1659574	15	14.98	99.8%	

Table 5
Results for Robustness
5(a).Results for variation in flow and mobile phase composition

Hydrochlorothiazide					
Condition	**RT**	**Area**	**Height**	**USP tailing**	**USP plate count**
Less Flow	1.867	518500	93362	1.25	2704
More flow	1.544	422704	81335	1.23	2264
Less organic	1.731	465830	75693	1.17	2020
More organic	1.674	473756	80966	1.27	1942

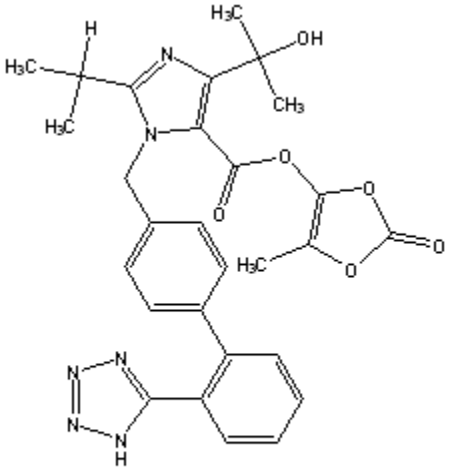
5(b).Results for variation in flow and mobile phase composition

Olmesartan medoxomil					
Condition	**RT**	**Area**	**Height**	**USP tailing**	**USP plate count**
Less Flow	3.621	1253302	148818	1.11	4405
More flow	2.997	1016303	132846	1.12	3515
Less organic	6.242	1158297	74259	1.17	3552
More organic	2.302	1093816	188630	1.15	3844

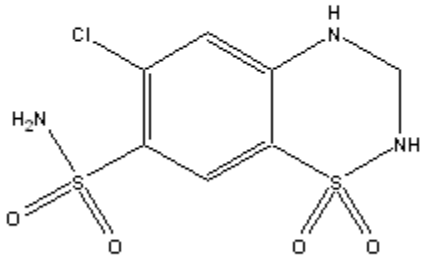
Table 6
Results for LOD and LOQ

	LOD	LOQ
Hydrochlorothiazide	3.05	10.42
Olmesartan medoxomil	2.92	9.96

Figure 1
Chemical structures



Olmesartan medoxomil (left)



Hydrochlorothiazide (right)

Concentration range of 10-50 µg/ml for Olmesartan medoxomil and 6.25-31.25µg/ml for Hydrochlorothiazide were found to be linear with correlation coefficients 0.999 and 0.999 for Olmesartan medoxomil and Hydrochlorothiazide respectively.

Figure 2
Linearity Graph

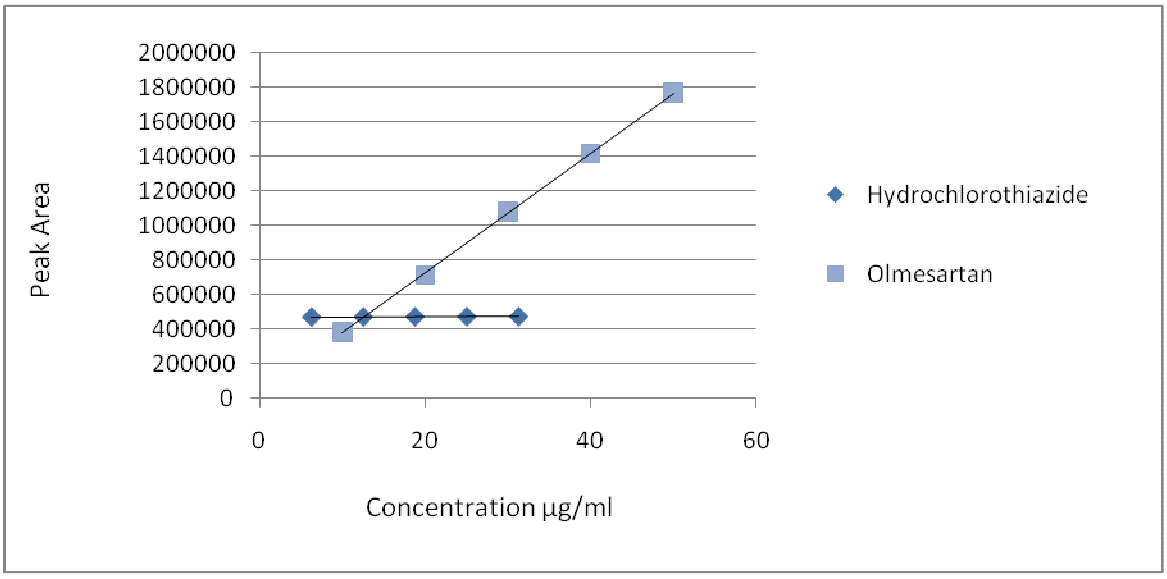
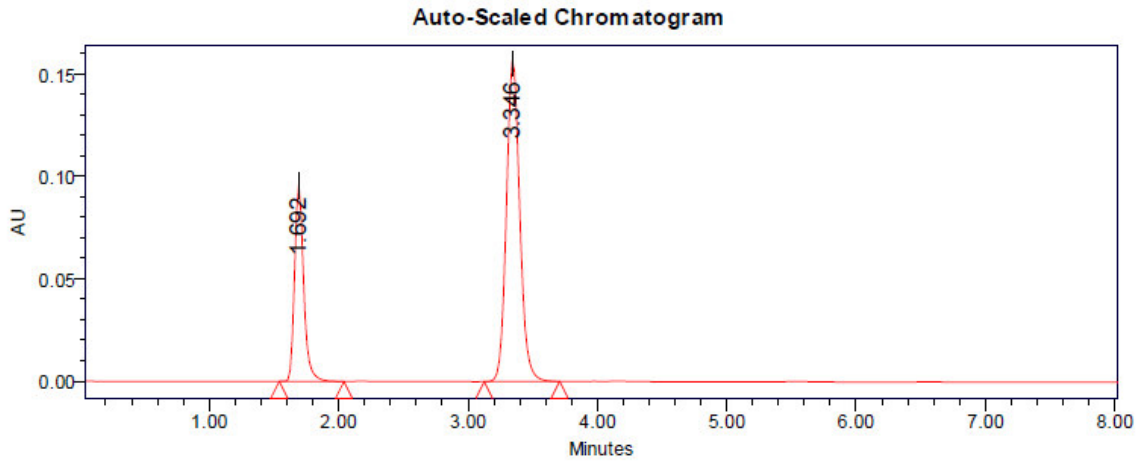
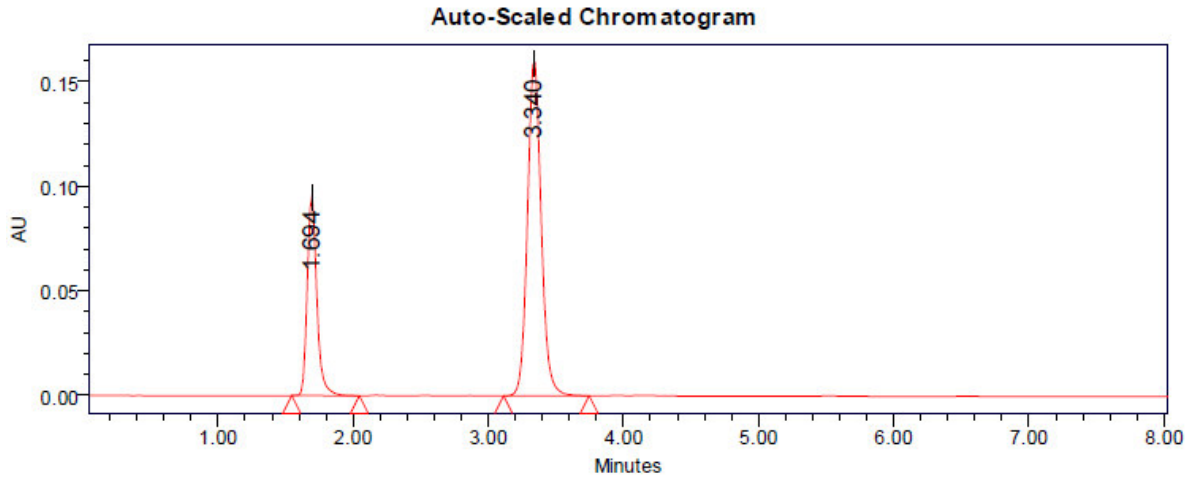


Figure 3
Optimised Chromatograph for Olmesartan medoxomil and Hydrochlorothiazide using RP-HPLC



A typical chromatogram for assay of marketed tablet formulation containing 18.75 µg/ml of Hydrochlorothiazide and 30 µg/ml of Olmesartan medoxomil.



CONCLUSION

The HPLC method developed and validated allows a simple and fast quantitative determination of Olmesartan medoxomil and Hydrochlorothiazide from its formulation. All the validation parameters were found to be within the limits according to the ICH guidelines. The proposed method was found to be specific for the drugs of interest irrespective of the excipients present and the method was found to be simple, accurate, precise, rugged, robust and can be involved in the routine analysis of the marketed formulations.

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