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Research Article

Validated UPLC method for simultaneous estimation of Olmesartan medoxomil and Hydrochlorothiazide in combined dosage forms

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ABSTRACT

A simple, accurate, sensitive and validated UPLC method for simultaneous determination of Olmesartan and hydrochlorothiazide in combined tablet dosage form has been developed. Separation carried out on UPLC system equipped with Waters Acquity UPLC BEH C18 Column (100 × 2.1 mm i.d., 1.7µm particle size)using Mobile phase-A of Acetonitrile and phosphate buffer adjusted to the pH to 2.5 in the ratio of 95:5 v/v and Mobile phase-B of Acetonitrile and Methanol in the ratio of 70:30 v/vat a flow rate of 0.04 mL/min in the Gradient program with run time of 6.5 minutes and detection using PDA detector was carried out at 260 nm. Results were linear in the range of 7 – 112µg/ml and 11-177µg/ml for both Hydrochlorothiazide and Olmesartan Medoximil respectively. The method has been successfully applied for the analysis of drugs in pharmaceutical formulation. Results of analysis were validated statistically and by recovery studies.

Key words: UPLC, Olmesartan medoximil, Hydrochlorothiazide, Tablet dosage form.

INTRODUCTION

Olmesartan medoxomil is described chemically as 2,3-dihydroxy-2-butenyl 4(1-hydroxy-1-methylethyl)-2-propyl-1-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]imidazole5-carboxylate, cyclic 2,3-carbonate and is a selective AT1 subtype angiotensin II receptor antagonist[1,2]. Hydrochlorothiazide, a thiazide diuretic, inhibits water reabsorption in the nephron by inhibiting the sodium-chloride symporter (SLC12A3) in the distal convoluted tubule and chemically 6-chloro-1, 1-dioxo-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide[3]. Literature survey reveals High Performance Liquid Chromatographic (HPLC) for determination of Olmesartan Medoximil and Hydrochlorothiazide combination are not official in Pharmacopeias of USP and BP. And their determination is official as single compound in Pharmacopeias. Various analytical methods have been reported for the assay of Olmesartan Medoximil, Hydrochlorothiazide alone or in combination with other antihypertensive agents in pharmaceutical formulations. They include UV spectroscopy[4-16], high performance liquid chromatography[4, 17-31].

A **B**

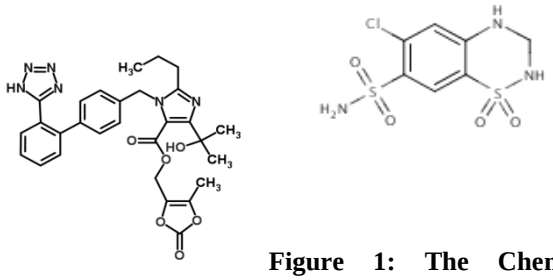

Figure 1: The Chemical Structures of (A) Olmesartan medoximil and (B) Hydrochlorothiazide.

As on only few methods is available for their simultaneous determination, however, it is essential to develop a suitable analytical method for simultaneous estimation of Olmesartan Medoximil and Hydrochlorothiazide in bulk

and in pharmaceutical preparations, because HPLC methods have been widely used for routine quality control assessment of drugs, because of their accuracy, repeatability, selectivity, sensitivity and specificity. We have developed a simple, precise, Olmesartan Medoximil and Hydrochlorothiazide in bulk and in pharmaceutical dosage forms. Because analytical methods must be validated before use by the pharmaceutical industry, the proposed HPLC- UV detection method was validated in accordance with International conference on Harmonization (ICH).

MATERIALS AND METHODS

Chemical and Reagents

Pharmaceutically pure samples of Olmesartan Medoximil and Hydrochlorothiazide were obtained as a gift samples from Dr.Reddy's, Hyderabad used as such without further purification. A combination of Olmesartan Medoximil (20 mg) and Hydrochlorothiazide (12.5 mg) in tablet formulation (Benicar HCT) was procured from Indian market (Indoco Remedies Limited, Mumbai), HPLC grade methanol, Acetonitrile, methanol, water and potassium dihydrogen phosphate (AR grade) purchased from Merck Chemicals India Pvt. Limited, Mumbai, India.

Instrumentation and Chromatographic Condition

Analysis was performed with a Waters Acquity UPLC system with DAD detector set at 260 nm. Compounds were separated on a Waters Acquity UPLC® with PDA Detector BHE C18 Column (100 × 2.1 mm i.d., 1.7µm particle size) under reversed phase partition conditions. The mobile phase-A of Acetonitrile and pH -2.5 phosphate buffer (pH 2.5 ±0.05, adjusted with diluted Orthophosphoric acid) and mobile phase-B of Acetonitrile and Methanol. The flow rate was 0.04ml/min and the run time was 6.5 minutes with gradient elution. Samples were injected using Rheodyne injector with 10 µL loop and detection was carried out at 260 nm. Before analysis mobile phase were degassed by the use of a sonicator (Ultrasonic Cleaner, Power Sonic 420) and filtered through a 0.22µm PVDF syringe filter. The identity of the compounds was established by comparing the retention times of compounds in the sample solution with those in standard solutions. Chromatography was performed in column temperature maintained at 30±5°C. The UV spectrum of Olmesartan Medoximil and Hydrochlorothiazide for selecting the working wavelength of detection was taken using a shimadzu UV-1800, With UV Probe software UV-Visible spectrophotometer (shimadzu, Kyoto, Japan). All Weighing were done on Shimadzu balance (Model AY-120).

Preparation of Standard Stock Solution

(About 88 ppm for Olmesartan and about 56 ppm for Hydrochlorothiazide)**:** Precisely weight and transfer about 45 mg of Olmesartan Medoxomil and 28 mg of Hydrochlorothiazide into a 50mL volumetric flask, add 5 mL of Diluent-1(0.1N HCL), sonicated for 2 minutes, further add 25 mL of Diluent-2{Water and Acetonitrile in 20:80 (%v/v)} and sonicate for 3 minutes and make up the volume with Dilute-2 and mix well. Pipette out 5mL of the above solution into 50mL volumetric flask and make up to volume with Dilute-3 (pH 2.5 phosphate buffer). And mix well.

Procedure for Analysis of Tablet Formulation

Seven tablets were weighed accurately and transferred in to a 250ml volumetric flask and add about 20 ml of diluent-1. The contents were sonicated for 5min with intermediate shaking, to ensure the complete solubility of drugs and further add 100mL of Diluent-2 and sonicate for 15 min and volume was made up to the mark with the diluent-2 and mix well. The solution was then centrifuged at 4000rpm for 10min and the clear supernatant was collected. From that, further dilutions were made by diluting 4 ml into 50ml with diluent-3; filtered through Filter through 0.22µm PVDF syringe filter. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the tablet sample solutions were injected, chromatogram was obtained and the peak areas were recorded. The injections were repeated six times and the amount of each drug present per tablet was estimated from the respective calibration curves.

Method Validation

The method was validated for specificity, linearity, accuracy, intra-day and inter-day precision and robustness, in accordance with ICH guidelines.

RESULTS AND DISSCUSSION

Method development

Several tests were performed in order to get satisfactory separation-resolution Olmesartan Medoximil, Hydrochlorothiazide in different mobile phases with various ratios of buffers and organic phases by using different columns. The ideal mobile phase was found to be composition of mobile phase-A is Acetonitrile and phosphate buffer (pH 2.5 ±0.05, adjusted with dil. orthophosphoric acid) in the ratio of 95:5 v/v and Mobile phase-B is Acetonitrile and Methanol in the ratio of 70:30 v/v. This Mobile phase used under gradient elution gave a very satisfactory and good resolution of Olmesartan Medoximil, Hydrochlorothiazide. Increasing or decreasing pH of mobile phase by ± 0.2 did not show significant change in retention time of each analyte. The retention time of Olmesartan Medoximil, Hydrochlorothiazide on the analytical column was evaluated at a flow rate of 0.04 ml/min. The injection volume was 10 µL. The retention time of standard and sample for Olmesartan Medoximil, Hydrochlorothiazide was satisfactory with good resolution. This work was focused on optimization of the conditions for the simple and rapid as well as low cost effective analysis including a selection of the proper column or mobile phase to obtain satisfactory results. Solvent type, solvent strength (volume fraction of organic solvent(s) in the mobile phase and pH of the buffer solution), detection wavelength, and flow rate were varied to determine the chromatographic conditions giving the best separation. The

mobile phase conditions were optimized so there was no interference from solvent and excipients. Finalized chromatographic conditions were mentioned on below Table-1.

Table 1: Finalized chromatographic condition

Flow rate:0.04 ml/min	**Column temperature**: 30±5˚C	**Injection Volume**:10µL
Wave length:260 nm	**Sample temperature**: Ambient	**Run time**:6.5 minutes
Gradient programme		
Time (in mins)	**Mobile phase-A (%v/v)** (pH 2.50 phosphate buffer and Acetonitrile)	**Mobile phase-B (%v/v)** (Acetonitrile and methanol)
0.0	90	10
1	90	10
1.5	65	35
3	60	40
4	50	50
5	60	40
5.5	90	10
6.5	90	10

To inject the standards on above finalized chromatographic conditions and their results was mentioned on below Table-2.

Table2: Results from system suitability study of Olmesartan & Hydrochlorothiazide

System Suitability Parameters	**Results**		**Acceptance Criteria**
	Olmesartan	**Hydrochloro-thiazide**	
Retention time	1.3	3.9	
%RSD for area of Olmesartan and Hydrochlorothiazide for five replicate injections of standard solution	0.05	0.15	NMT 2.0
Tailing factor for Olmesartan and Hydrochlorothiazide peak	1.2	1.2	NMT 2.0
Theoretical plates for Olmesartan and Hydrochlorothiazide	64100	2698	NLT 2000

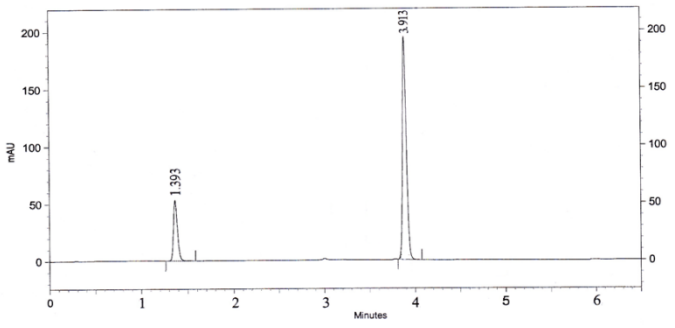

Figure 2: Optimized chromatogram for Olmesartan Medoximil (88 ppm) and Hydrochlorothiazide (56 ppm)

Linearity

Aliquots 2.5,2.5,5 mL of working standard solution in to 20,10,10 mL volumetric flask respectively for 12.5, 25, 50 % levels, 2.5, 3.8, 5.0 mL of stock solution of Olmesartan Medoximil and Hydrochlorothiazide were transferred in a series of 25 mL volumetric flasks for 100, 150, 200 % levels. Finally the volume was made up to the mark with the diluent. Two replicates per concentration were injected and chromatograms were recorded. The peak area ratios of olmesartan and hydrochlorothiazide were calculated and respective calibration curves were plotted of response against concentration of each drug. Calibration curves for olmesartan and hydrochlorothiazide were plotted separately of response against respective concentration of olmesartan and hydrochlorothiazide. The slope and intercept value for calibration curve were y = 24560 x + 11151 (R^2 = 0.9995) for olmesartan and y = 60517x + 22126 (R^2 = 0.9996) for hydrochlorothiazide, where Y represents the peak area of analyte and X represents analyte concentration. The results are satisfactory, because there is a significant correlation between response factor and concentration of drugs within the concentration range. The calibration curves of olmesartan and hydrochlorothiazide are given in Figures 3 and 4 respectively.

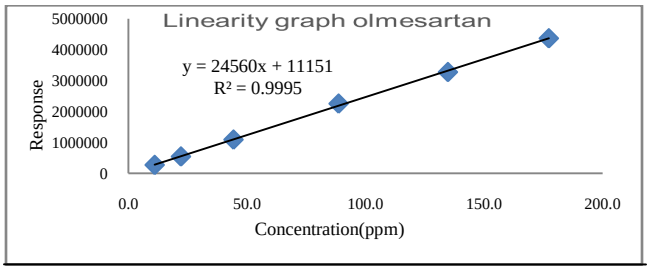

Figure 3: Linearity curve for Olmesartan

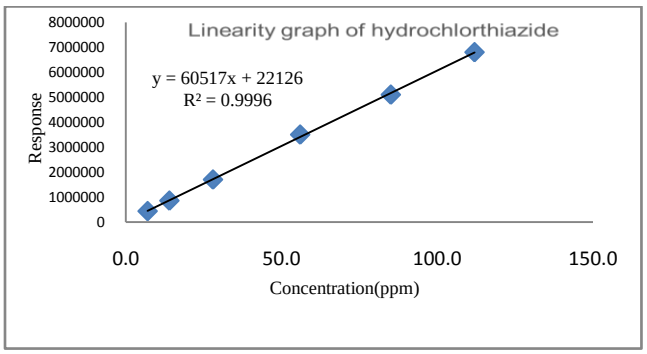

Figure 4: Linearity curve for Hydrochlorothiazide

Precision

Precision of the method was confirmed by the repeated analysis of formulation for six times. The% RSD values were found to be satisfactory. The low % RSD values indicated that drugs showed good agreement with the label claim ensures the precision of the method. Intraday and Interday precision was determined by preparing six (n=6)

replicate samples and analyzed on same day for intraday The peak areas were recorded and Relative standard deviation (RSD) was calculated for both series of analyses. For strength 40/25 mg of intraday precision %RSD of olmesartan and hydrochlorothiazide are 1.7, 1.1 and interday precision %RSD of olmesartan and hydrochlorothiazide are 0.8, 0.9 respectively. For strength 20/12.5 mg of intraday precision %RSD of olmesartan and hydrochlorothiazide are 0.7,1.8 and interday precision %RSD of olmesartan and hydrochlorothiazide are 0.9, 1.2 respectively and overall %RSD for Olmesartan are 1.3, 0.8 and Hydrochlorothiazide are 1.0, 1.4 for 40/25 mg and 20/12.5 mg strength respectively (Table3)

Accuracy

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100% and 150%. The percentages of recoveries were calculated, results of which are represented in Table 4.

Table 4: Recovery studies of olmesartan and hydrochlorothiazide

Level of % Recovery	% Mean Recovery*		% R.S.D.*	
	Olmesartan	Hydrochlor-thiazide	Olmesartan	Hydrochlor-thiazide
50	99.4	99.2	0.59	0.27
100	99.6	99.9	0.32	0.32
150	99.6	99.6	0.78	0.65

**Avg. of six determinations for 50 & 150, three determinations for 100%, R.S.D. is relative standard deviation*

LOD and LOQ

LOD and LOQ were calculated as 3.3 σ /S and 10 σ /S respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

and on different days for Interday precision. (Table3). The

Robustness:

As defined by ICH, The robustness of an analytical procedure describes to its capability to remain unaffected by small and deliberate variations in method parameters. Robustness was performed to injected the standard and samples by small variation in the chromatographic conditions and found to be unaffected by small variations like ± 2% variation in volume of mobile phase composition with respect to acetonitrile, ± 0.2 mL/min in flow rate of mobile phase ,± 0.2 variation in pH, different type of filters and ± 5 column temperature variation. It was observed that there were no marked changes in the chromatograms, which demonstrated that the UPLC method developed is robust.

Specificity

Specificity was tested against standard compounds and against potential interferences. Specificity was determined by comparing the responses of standard and sample solution. No interference was detected at the retention times of both olmesartan and hydrochlorothiazide in sample solution.

Table 5: Summary of validation parameters of proposed UPLC method

Parameters	Olmesartan		Hydrochlorothiazide	
Linearity range (µg/mL)	11 – 177		7 – 112	
Correlation co-efficient	0.9995		0.9996	
LOD [a] (µg/mL)	3.4		2.15	
LOQ [b] (µg/mL)	10.3		6.5	
Accuracy (% Recovery)	99.4 - 99.6		99.2 - 99.9	
Precision (% RSD)[c]	Tab 1	Tab 2	Tab 1	Tab 2
Intraday (n^d = 6)	1.7	0.7	1.1	1.8
Interday (n^d = 6)	0.8	0.9	0.9	1.2

[a] LOD = Limit of detection, [b] LOQ =Limit of quantitation.
[c] RSD = Relative standard deviation, [d] n = Number of determination

Table3: Precision studies

S.No	% Assay							
	Olmesartan				Hydrochlorothiazide			
	Tab-1(n=6)		Tab-2(n=6)		Tab-1(n=6)		Tab-2(n=6)	
	Intraday precision	Interday precision	Intraday precision	Interday precision	Intraday precision	Interday precision	Intraday precision	Interday precision
1	98.8	98.8	100.8	99.2	100.8	101.0	99.3	99.5
2	96.8	100.4	98.7	100.3	99.6	100.4	98.6	101.0
3	98.1	99.6	99.5	98.7	100.4	100.2	98.4	99.5
4	101.0	100.8	99.2	100.0	102.3	102.1	102.2	101.6
5	100.6	100.4	99.3	98.6	102.0	102.0	101.7	99.7
6	100.8	100.6	99.3	100.8	102.2	102.4	102.0	102.2
Mean	99.4	100.1	99.5	99.6	101.2	101.4	100.4	100.6
%RSD	1.7	0.8	0.7	0.9	1.1	0.9	1.8	1.2
Over all % RSD (n=12)	1.3		0.8		1.0		1.4	

Tab-1 is 40/25 mg and Tab – 2 is 20/12.5 mg of Olmesartan and Hydrochlorothiazide respectively

CONCLUSION

The validated UPLC method employed here provided to be simple, fast, accurate, precise and robust, thus can be used for routine analysis of Olmesartan and hydrochlorothiazide in combined tablet dosage form.

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