Volume 2 (6), 2014, Page-457-462
CODEN (USA)-IJPRUR, e-ISSN: 2348-6465

PHS Scientific House
PHARMAHEALTHSCIENCES

International Journal of Pharma Research and Health Sciences

Available online at www.pharmahealthsciences.net

Original Article

Method Development and Validation of RP-HPLC Method for Simultaneous Estimation of Olmesartan Medoxomil and Hydrochlorothiazide in Bulk and Pharmaceutical Dosage Form

S Gayathri *, D Sireesha, M Akiful Haque, S Harshini, Vasudha Bhakshi, Sowjanya Kumar Reddy
Department of Pharmaceutical Analysis and Quality Assurance, School of Pharmacy, Anurag Group Of Institutions, Hyderabad , India

ARTICLE INFO

Received: 25 Nov 2014
Accepted: 12 Dec 2014

ABSTRACT

Present work describes a simple, rapid, precise, accurate, economical and reproducible reverse phase high performance liquid chromatographic (RP-HPLC) method and its validation for the estimation of Olmesartan medoxomil and Hydrochlorothiazide in bulk and pharmaceutical dosage form on a Develosil ODS HG-5 RP C_{18}, (5µm, 15cmx4.6mm i.d.) using a mixture of Potassium dihydrogen phosphate buffer+Dipotassium hydrogen phosphate (0.02 M, pH 5.0): acetonitrile (40:60) %v/v as mobile phase in an isocratic elution mode, at a flow rate of 1.0 ml/min. The detection was monitored at 258 nm.The retention times of Olmesartan and Hydrochlorothiazide were found to be 9.93 min and 2.02 min respectively.Excellent linearity range was found between 0-60 µg/ml for Olmesartan and Hydrochlorothiazide. Proposed method was validated with respect to linearity, precision, accuracy, specificity and ruggedness. Method was successfully applied for the simultaneous determination of Olmesartan and Hydrochlorothiazide from the combined dosage form.

Keywords: Olmesartanmedoxomil (OLM), Hydrochlorothiazide (HCTZ), R HPLC,Validation

Corresponding author *
Ms Gayathri,
Department of Pharmaceutical Analysis and Quality Assurance, School Of Pharmacy,Anurag Group Of Institutions,Hyderabad
Email:gayathri4sv@gmail.com

1. INTRODUCTION

Chemically Olmesartanmedoxomil is 5-methyl-2-oxo-2H-1,3-dioxol-4-yl)methyl 4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(2H-1,2,3,4-tetrazol-5-

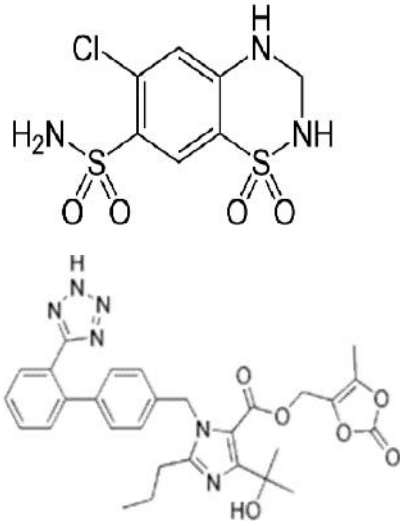
yl)phenyl]phenyl]methyl)-1H-imidazole-5-carboxylate. Molecularformula$C_{29}H_{30}N_6O_6$Molecular weight558.585.

Olmesartan is indicated for the treatment of Hypertension. It is a specific angiotensin II type 1 (AT1) receptor antagonist, which blocks the blood pressure increasing effects of angiotensin II via the renin-angiotensin-aldosterone system (RAAS). Olmesartan is an ARB that selectively inhibits the binding of angiotensin II to AT1, which is found in many tissues such as vascular smooth muscle and the adrenal glands. This effectively inhibits the AT1-mediated vasoconstrictive and aldosterone-secreting effects of angiotensin II and results in a decrease in vascular resistance and blood pressure. [1, 2]

Hydrochlorothiazide is 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4- benzothiadiazine -7-sulfonamide.Mol.formula $C_7H_8ClN_3O_4S_2$ Molecular weight297.74.

Hydrochlorothiazide is frequently used for the treatment of hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis, and the prevention of kidney stones.Hydrochlorothiazide, a thiazide diuretic, inhibits water reabsorption in the nephron by inhibiting the sodium-chloride symporter in the distal convoluted tubule, which is responsible for 5% of total sodium reabsorption. By blocking the sodium-chloride symporter, hydrochlorothiazide effectively reduces the osmotic gradient and water reabsorption throughout the nephron.

Several analytical methods have been reported for the estimation of HCTZ in marketed formulations and United States Pharmacopoeia (USP) describes a RP-HPLC method for its estimation .OM has not been described officially in any pharmacopoeia yet. Extensive literature survey revealed that very few methods were reported for the simultaneous estimation of OM and HCTZ. 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. [3-5]



2. MATERIALS AND METHOD

Apparatus and chromatographic condition:

The chromatographic separation was performed on a Waters 2690/5, integrated with Auto Sampler and PDA detector and Empower2 software. The analytical DevelosilODS HG-5 RP C_{18}, (5µm, 15cmx4.6mm i.d.)was used for the separation. The mobile phase consisted of Potassium dihydrogen phosphate buffer+Dipotassium hydrogen phosphate (0.02 M, pH 5.0): acetonitrile (40:60) %v/v.

The mobile phase was prepared freshly,filtered, sonicated before use and delivered at a flow rate of 1.0ml/min and the detector wavelength was set at 258 nm. The injection volume was 20 µl. [6, 7]

Chemicals and Reagents:

The pharmaceutical grade pure Olmesartan medoxomil and Hydrochlorothiazide were obtained as gift samples from Matrix Laboratories Ltd,Hyderabad andAurobindoPharma Ltd, respectively. HPLCgrade Acetonitrile and Analytical grade dipotassiumhydrogen phosphate,Methanol,Orthophosphoricacid and HPLC

grade water were obtained from S.D Fine Chemicals Ltd., Mumbai.

Preparation of mobile phase:

Mobile phase was prepared by taking Potassium dihydrogen phosphate buffer+Dipotassium hydrogen phosphate (0.02 M, pH 5.0): acetonitrile (40:60) Mobile phase was filtered through 0.45 µm membrane filter and degassed under ultrasonic bath prior to use. The mobile phase was pumped through the column at a flow rate of 1.0 ml/min.

Preparation of standard stock solutions:

Accurately weighed 100 mg of Hydrochlorothiazide and 100 mg of Olmesartan were transferred to 100 ml volumetric flask. About 40 ml of HPLC grade methanol was added and sonicated to dissolve. The volume was made up to mark with same solvent. Then 10 ml of the above solution was diluted to 100 ml with the solvent system. The resultant solution was filtered through a 0.45 µm membrane filter and degassed under ultrasonic bath prior to use. From the above standard solution several working standard solutions are prepared by serial dilution technique. [8-10]

Preparation of sample solutions:

Analysis of marketed Formulation:

Twenty Olmax-H tablets each containing 20 mg of Olmesartan medoxomil and 12.5 mg of Hydrochlorothiazide were weighed, average weight was calculated and powdered. A quantity equivalent to 20 mg of Olmesartan medoxomil and 12.5 mg of Hydrochlorothiazide was weighed and transferred into 100 ml volumetric flask. It is extracted with mobile phase. The volumetric flask was sonicated for 20 minutes to affect the complete dissolution of the drugs and the solution was made up to the volume with mobile phase and filtered.

Procedure:

Inject 20µl of the standard and sample solution into the chromatographic system and measure the areas for the Hydrochlorothiazide and Olmesartan peaks and calculate the % Assay by using the formulae.The assay values are shown in Table 1.

Method Development:

The Method for the estimation of Olmesartan and Hydrochlorothiazide is developed using different mobile phases at different pH.The mobile phase acetonitrile: potassium dihydrogen phosphate buffer (0.05 M, pH 4.0) (60:40 v/v) was found to be ideal mobile phase for determination of Olmesartan and Hydrochlorothiazide at ambient temperature. Fig 1 and Fig 2.

Method Validation:

The proposed HPLC method was validated as per ICHguidelines.

System suitability:

The system suitability of Olmesartan and Hydrochlorothiazide were assessed by comparing the Theoretical plates, Asymmetry,% RSD for Area, % RSD for Retention time of standard Olmesartan and Hydrochlorothiazide. Results are shown in Table 2.

Linearity:

Linearity was studied by preparing standard solutions at different concentration levels. The linearity range for Olmesartan and Hydrochlorothiazide was found to be 0-60 µg/ml and 10-90 µg/ml. The regression equation for Olmesartan and Hydrochlorothiazide were found to be y = 33748x - 20461and y = 41653x - 11677 with coefficient of correlation, (r) 0.999 and 0.999 respectively.Table3 and 4...Fig3 and Fig4. [11]

Accuracy(Recovery studies):

To check the degree of accuracy of the method, recovery studies were performed at 80%, 100% and 120% levels.Known amounts of standard Olmesartan and Hydrochlorothiazide were added to pre-analyzed samples and were subjected to the proposed HPLC method. Results of recovery studies are shown in Table 5.

Precision:

Repeatability

The precision of the method were assessed by repeatability in which five replicates of a fixed amount of drug were injected and analysed. The relative standard deviation for Olmesartan and Hydrochlorothiazide were calculated and are shown in the Table 6.The % R.S.D. values of the measurements ranged between 0.709 and 0.832%, confirming good precision of the proposed method. [12]

Intermediate precision

For intraday studies, drug solutions of three concentrations were injected six times each into the HPLC system and for inter day studies, the solutions of three concentrations were injected into the HPLC on different days. Data obtained shown in Table 7 were analysed. The % R.S.D. of results obtained in intermediate precision study was not greater than 2% confirming good precision. [13]

Specificity:

Specificity can be described as the capability of the method to accurately measure the response of the analysed compound with no interferences originating from sample matrix. For the specificity of the method the marketed formulation has been taken & the solution was injected into the HPLC system**.** The method was found to be specific when the test solution was injected and no interferences 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 weregiven in table 8.

Robustness:

For testing the robustness of method a few parameters like flow rate, percentage of composition of acetonitrile in the mobile phase were deliberately changed. One parameter was changed at one time to evaluate the effect in results. The % R.S.D. of results of samples obtained for robustness with respect to change in flow & change in composition were within 2% of method precision & thus ensures that the method is Robust.

3. RESULTS AND DISCUSSION

To develop a precise, accurate and suitable RP- HPLC method for the simultaneous estimation of Olmesartan and Hydrochlorothiazide, different mobile phases were tried and the proposed chromatographic conditions were found to be appropriate for the quantitative determination. The results obtained by the assay of marketed formulation are summarized in Table.1.

Table1: Assay of marketed formulation

Brand name of tablets (OLMAX-H)	Labelled amount of Drug (mg)	Mean amount (mg) (n=6)	Mean Assay (n = 6)
Hydrochlorothiazide	12.5mg	12.43(±0.06)	99.44(±0.48)
Olmesartan medoxomil	20mg	20.01(±0.04)	100.05(±0.12)

Table 2: Result of System suitability for standard Olmesartan and Hydrochlorothiazide

Parameter	Olmesartan Medoxomil	Hydrochlorothiazide
Theoretical Plate	4693	3246
Assymmetry	0.5	0.12
% RSD for Area	0.709605462	0.83258576
% RSD for Retention time	0.055181	0.221612

Table 3: Standard curve for Olmesartan

CONC.(µg/ml)	MEAN AUC
0	0
20	635359
30	981464
40	1314976
50	1681308
60	2013768

Table 4: Standard curve for Hydrochlorothiazide

CONC.	AUC
0	0
20	811367

30	1116983
40	1586487
50	2039569
60	2586059

Table 5: Result for Summary of Accuracy

Parameter	Olmesartan Medoxomil	Hydrochlorothiazide
Recovery 80%	99.92	99.67
Recovery 100%	100.72	99.19
Recovery 120%	100.40	99.49

Table 6: Repeatability of Olmesartan and Hydrochlorothiazide

Concentration 60ppm	5 Injections	Peak Areas of Olmesartan medoxomil	%Assay	Peak Areas of Hydrochlorothiazide	%Assay
Statistical Analysis	Mean	1976544.6	100.4	1538434.6	99.91
	SD	14025.66844	0.469361268	12808.78741	0.223681
	% RSD	0.709605462	0.469737058	0.83258576	0.224407

Table 7: Interday and Intraday precision

Drug	Intraday Precision (% RSD)	Interday Precision (% RSD
Olmesartan Medoxomil	0.86	0.87
Hydrochlorothiazide	1.50	0.24

Table8: Results of LOD and LOQ

Drug	LOD(µg/ml)	LOQ(µg/ml)
Olmesartan medoxomil	0.96	4.32
Hydrochlorothiazide	0.32	1.44

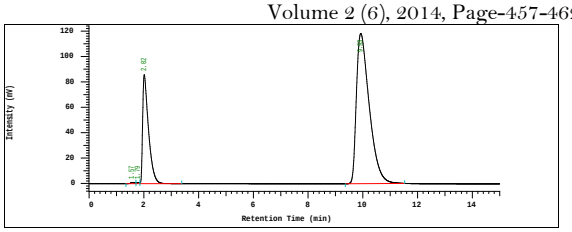


Fig1: Chromatogram of standard

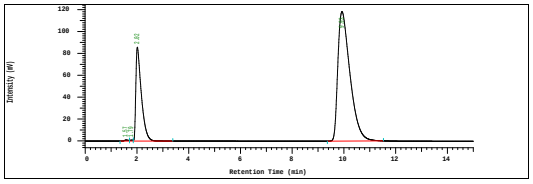


Fig2: Chromatogram of sample

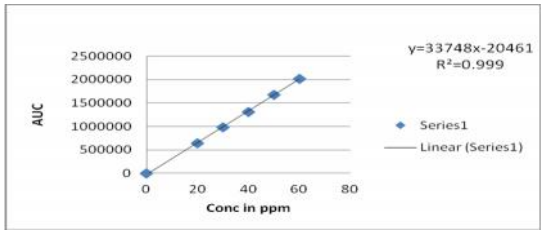


Fig3: Linearity curve for Olmesartanmedoxomil

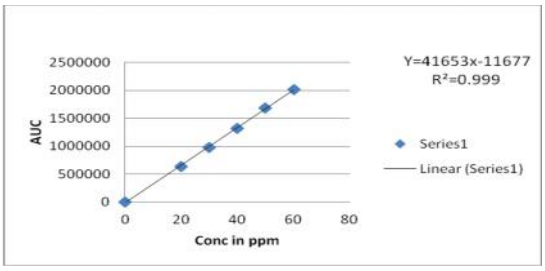


Fig 4: Linearity curve for Hydrochlorothiazide

4. CONCLUSION

The proposed method is simple, sensitive and reproducible and hence can be used in routine for simultaneous determination of Olmesartan medoxomil and Hydrochlorothiazide in bulk as well as in pharmaceutical preparations. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The RSD for all parameters was found to be less than two, which indicates the validity of method and assay results obtained by this method are in fair agreement.

5. REFERENCES

1. The Merck Index, an encyclopedia of chemicals, drugs and biological. Fourteenth Edn. USA; 2006.
2. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry, 4thEdn., C.B.S. Publications,
3. Mendham J, Denney RC, Barnes V, Thomas, MJK. Vogel's Text book of Qualitative Chemical Analysis, 6thEdn., 261-287.
4. Validation of Analytical Procedures, ICH Harmonized Tripartite Guidelines 1994.
5. ICH, Q2A Text on validation of analytical procedures, Oct, 1994www.ich.org.
6. ICH, Q3B Validation of analytical procedures: methodology, Nov, 1996.
7. MaitreyiZaveri et al. Simultaneous Estimation Of OlmesartanMedoxomil And Hydrochlorothiazide By Validated RP-HPLC. International Journal Of Pharmaceutical Research and Bio-Science._ 2012, 1(1).
8. Vijaya P Godseet al. Validated Stability-Indicating HPLC Method For Simultaneous Determination Of OlmesartanMedoximil And Hydrochlorothiazide In Combination Drug Products. Eurasian Journal of Analytical chemistry 2010; 5(2): 137-144.
9. GS Devika, M Sudhakar, J VenkateshwarRao, Orient J Chem 2012; 28(2): 887-893.
10. Sharma BK. Instrumental methods of Chemical analysis, 19th Edn., 2000.
11. Willard H.H.; Merrit L.L.; Dean. J.A., Instrumental methods of analysis, 7thEdn., CBS Publishers, New Delhi.
12. Khopkar, S.M. Basic concepts of analytical chemistry, 2nd edition, 2005.
13. Tips on Liquid Chromatography, Waters www.waters.com. http://www.drugbank.ca/drugs