International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 5, 2016

Original Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF IMPURITIES FROM OLMESARTAN MEDOXIMIL AND HYDROCHLOROTHIAZIDE TABLET

A. D. MALI*, U. B. MORE

Department of Chemistry, Shri Jagdish Prasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan, 333001, India
Email: mali.ad@rediffmail.com

Received: 26 Jan 2016 Revised and Accepted: 15 Mar 2016

ABSTRACT

Objective: To develop and validate stability indicating RP-HPLC gradient method for simultaneous estimation of impurities and degradation products from Olmesartan Medoximil and Hydrochlorothiazide tablet.

Methods: The chromatographic separation was achieved by using Inertsil ODS (250 mm x 4.6 mm, 5µ) column. The mobile phase-A consists of 0.01M potassium dihydrogen phosphate buffer pH 3.2 adjusted using orthophosphoric acids and acetonitrile as mobile phase-B. The flow rate was 1 ml/min, and chromatograms extracted at wavelength 225 nm.

Results: The method was found linear from LOQ to 0.4% level with respect to target concentrations of Olmesartan Medoximil (1.6 mg/ml) and Hydrochlorothiazide (0.5 m/ml) for all impurities, with correlation coefficient found greater than 0.99. The method found robust in all deliberate variations of method parameters as a resolution between adjacent peaks found greater than 2.0. The % RSD results for precision and intermediate precision found less than 5.0%.

Conclusion: The proposed analytical method was found to be robust, stability indicating and can be used for estimation of impurities and degradation products of Olmesartan Medoximil and Hydrochlorothiazide from tablet dosage form.

Keywords: Stability indicating, RP-HPLC, ICH, Olmesartan Medoximil and Hydrochlorothiazide

INTRODUCTION

Olmesartan Medoximil chemically, it is 4-(1-Hydroxy-1-methylethyl) -2-propyl-1-((2'-(1H-tetazol-5-yl) (1, 1'-biphenyl)-4-yl) methyl)-1H-imidazole-5-carboxylic acid (5-Methyl-2-oxo-1, 3-dioxol-4-yl) methyl ester. It works by blocking a substance in the body that causes blood vessels to tighten. As a result, Olmesartan relaxes blood vessels. This lowers blood pressure and increases the supply of blood and oxygen to the heart. Olmesartan prevents the constriction of blood vessels (1). Olmesartan is a non-peptide molecule. Hydrochlorothiazide chemically, it is 6-Chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide, reduces the amount of water in the body by increasing the flow of urine, which helps lower the blood pressure (2).

The levels of impurity changes due to route of synthesis, reaction condition, sources and quality of starting material, reagents and solvents used during synthesis, the purification steps, and conditions of crystallization, drying, distillation and storage of bulk drug materials. The same things were applied to formulation too thereof have to check repeatedly on impurity profiling during the research and development as drug interact with various excipients. In order to ensure the quality and efficacy of drug product regulatory authorities such as US FDA, CGMP, TGA and MCA insist on the impurity profiling of drugs.

Literature review reveals that the methods for Olmesartan Medoximil alone are developed and validated using Ultra violet-visible Spectrophotometry and Reverse-Phase High-Performance Liquid Chromatography from Tablet dosage form (3, 4). Also, there are analytical methods available for determination of Olmesartan Medoximil and Hydrochlorothiazide from the biological matrix (5, 8). Some of the methods have been reported for Olmesartan and Hydrochlorothiazide by HPLC (9, 13), LCMS (14, 15) and HPTLC (16). Most of available RP-LC methods were for estimation of Olmesartan Medoximil and Hydrochlorothiazide from a combination of the dosage form. There was no any official pharmacopoeial method for Simultaneous estimation of impurities and degradation products from Olmesartan Medoximil and Hydrochlorothiazide reported till date. The present analytical method was robust, economical and stability-indicating for estimation of impurities and degradation products from the combination of tablet dosage form.

MATERIALS AND METHODS

Instrumentation

Waters HPLC system with photodiode array detector was used for method development and forced degradation studies. The HPLC system consists of 2695 separation module and 2998 photodiode array detector. The output signal was monitored and processed using Empower 2 software. Hydrolysis studies were performed in water bath (Make-Bio-analytical ltd) and thermal stability studies were performed in oven (Make-Bio-analytical ltd)

Chemicals and reagents

Active pharmaceutical ingredients are Olmesartan Medoximil and Hydrochlorothiazide and its related impurities obtained from veeprho laboratories pvt. Ltd. Marketed formulation of Olmesartan Medoximil and Hydrochlorothiazide obtained from Ajanta pharmaceuticals Ltd. Analytical grade potassium dihydrogen phosphate obtained from Merck (Mumbai India), HPLC grade Acetonitrile obtained from Merck (Darmstadt, Germany), water from milli-Q purification system (Millipore, Bedford, USA) and GR grade orthophosphoric acid obtained from (Merck, Mumbai).

Chromatographic conditions

The chromatographic separation was achieved by Inertsil C18, 250 mm x 4.6 mm column. The mobile phase-A consists of 0.01M KH_2PO_4 Buffer (pH 3.2) adjusted using diluted orthophosphoric acid and Acetonitrile as mobile phase-B. The flow rate was 1.0 ml/min throughout the gradient program. The eluents were monitored at 225 nm. The column temperature was maintained at 30 °C. The injection volume was 10 µl. The diluent was prepared by mixing of buffer pH 3.2 and acetonitrile in the ratio of 1:1 (v/v). The gradient program was set as: time (min)/% mobile phase B: 0/10, 10/20, 20/35, 35/50, 45/85, 55/85, 60/10 and 70/10.

Preparation of stock solutions

A stock solution of Olmesartan Medoximil (1.60 mg/ml) and Hydrochlorothiazide (0.5 mg/ml) was prepared by dissolving an appropriate amount in the diluent. A stock solution of impurities (0.1 mg/ml) was prepared individually in the diluent. Working solutions were prepared from stock solutions respectively.

Preparation of sample solutions

Weigh and transferred powder equivalent to 25 mg of Hydrochlorothiazide into 50 ml volumetric flask added about 35 ml diluent and sonicated for 20 min with intermittent shaking. The solution was diluted to 50 ml with diluent and mix well keep the solution on the bench for 2 min and filtered above solution through 0.45 µm Nylon syringe filter.

RESULTS AND DISCUSSION

Method development and optimization

The main objective of the chromatographic method was to separate all impurities from each other and from Olmesartan Medoximil and Hydrochlorothiazide peaks. As isocratic method was not able to give adequate selectivity and rapid separation of impurities hence, the gradient method was developed. The stress conditions used for forced degradation includes acid hydrolysis (0.1N HCl at bench top for 1Hr), Base hydrolysis (0.01M NaoH at bench top for 30 min), Peroxide stress (10% H2O2 at bench top for 5 min), Humidity stress (40 °C/75% RH), Heat stress (80 °C for 24 Hr) and Photolytic stress. In stress conditions, Olmesartan medoximil acid impurity was major degradent.

System suitability

System suitability evaluated with the parameters such as tailing factor (should be<2.0), theoretical plate (should be>10,000) and %RSD for replicate injections of standard solution (should<5.0). The results for proposed method depicted in (table 1). In system suitability found the resolution for all adjacent peaks more than2.0, tailing factor less than 2.0, theoretical plates more than 10,000 and % RSD for six replicate injections of a diluted standard below 5.0%.

Specificity and forced degradation

All the degradation study samples were analyzed using a PDA detector with respective concentration of Olmesartan medoximil (1.6 mg/ml) and Hydrochlorothiazide (0.5 mg/ml). During stress study found that Olmesartan Medoximil labile to degradation in acid, base, and peroxide stress conditions, Hydrochlorothiazide labile to Heat and Humidity degradation. The results of forced degradation study were depicted in (table 2) and chromatograms in (fig. 1). The forced degradation study found that no interference found from blank and placebo at retention times of the main peak and impurities. All peaks found homogeneous and the peak purity data found within the acceptance limit.

Precision

The result of precision and intermediate precision was depicted in (table 3). The % relative standard deviation for each impurity in six determinations for precision and intermediate precision found below 2.0% confirms the preciseness of the method.

Limit of detection and quantification

The results for LOD and LOQ values were depicted in (table 3). The precision at LOQ level with six preparations found less than 5.0%.

Linearity

The linearity was evaluated from LOQ to 0.4% with respect to a target concentration of Olmesartan Medoximil 1.6 mg/ml and Hydrochlorothiazide 0.5 mg/ml. The results for linearity like slope, intercept and correlation coefficient depicted in (table 3). The correlation coefficient for impurities found more than 0.99. There was an excellent correlation between the peak area response and the concentration.

Table 1: Chromatographic performance data

Compound	Retention time (min)	Tailing factor	Theoretical plates	%RSD*
Hydrochlorothiazide	10.8	1.04	27643	1.43
Olmesartan Medoximil	30.7	1.03	161320	0.83

*Six determinations of standard solution

Table 2: Forced degradation data

Conditions	%Degradation	Purity angle for Olme	Purity threshold for olme	Purity angle for hctz	Purity threshold for hctz	Purity flag
As such condition	0.4	0.519	1.062	0.372	1.029	No
Acid Stress	14.9	0.527	1.059	0.362	1.032	No
Base Stress	12.4	0.528	1.051	0.359	1.027	No
Peroxide Stress	9.9	0.531	1.060	0.364	1.033	No
Heat Stress	3.6	0.536	1.058	0.363	1.030	No
Humidity Stress	4.6	0.545	1.066	0.384	1.012	No

Olme: Olmesartan Medoximil, Hctz: Hydrochlorothiazide

Table 3: Linearity, LOD, LOQ and precision data

	Hydrochlorothiazide impurities			Olmesartan medoximil impurities				
Parameter	Imp-B	Chlorothiazide	Imp-C	Acid	Ester	Dehydro	Imp-IV	Imp-III
LOD (%)	0.005	0.011	0.005	0.006	0.014	0.007	0.007	0.010
LOQ (%)	0.016	0.032	0.014	0.019	0.042	0.020	0.020	0.029
Slope (*b*)	81680.9	82523.8	35115.5	39650.6	30458.5	27942.9	17889.5	17047.0
Intercept(a)	-3043.9	-1220.8	1073.5	267.4	1172.6	-194.4	-829.9	-2280.3
Corr. Coeff.	0.9988	0.9999	0.9993	0.9993	0.9996	0.9991	0.9997	0.9996
% Y Intercept @100% Level	-3.61	-1.41	2.75	0.21	1.13	-0.24	-1.49	-4.36
Precision (% RSD)#	0.99	1.36	1.85	1.28	0.98	0.40	0.85	1.43
Intermediate precision (% RSD)#	0.50	1.47	1.49	0.82	1.05	1.10	1.15	1.17
Precision at LOQ (% RSD)#	0.71	3.65	1.30	2.65	2.47	1.08	1.53	1.82

Average %RSD for six determinations.

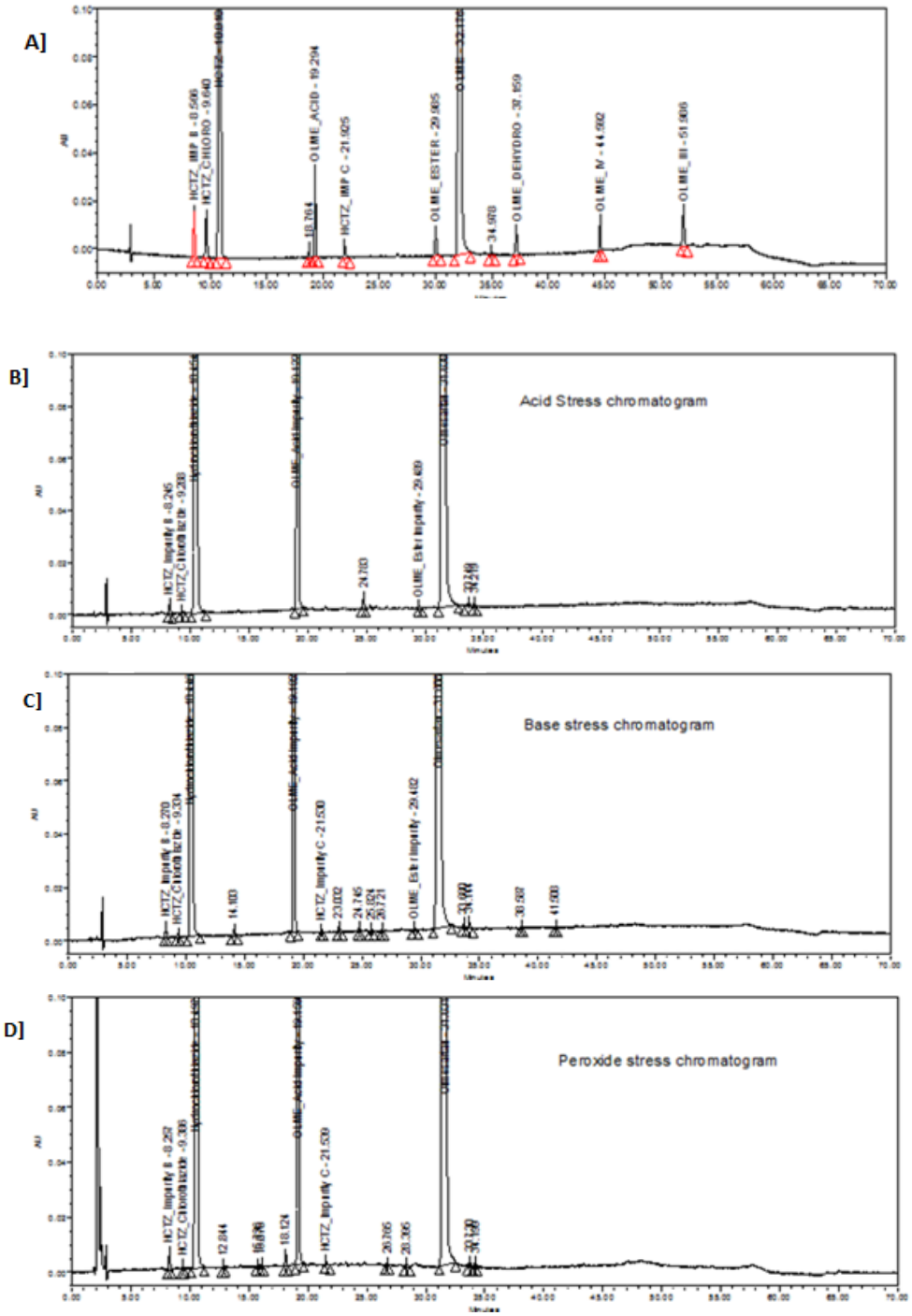

Fig. 1: Typical chromatograms of impurity spik sample (A), Acid stress sample (B), Base stress sample (C) and peroxide stress sample (D)

Table 4: Accuracy data

	Hydrochlorothiazide impurities		Olmesartan medoximil impurities					
Amount spiked€	**Imp-B$**	**Chlorothiazide$**	**Imp-C$**	**Acid$**	**Ester$**	**Dehydro$**	**Imp-IV$**	**Imp-III$**
50%	103.4	102.3	102.1	104.3	103.4	100.6	98.5	98.4
	±2.36	±0.92	±0.33	±1.78	±0.77	±0.73	±2.63	±2.92
100%	102.6	103.8	101.3	101.9	104.0	99.6	98.5	99.3
	±1.52	±0.84	±1.48	±1.85	±1.28	±0.56	±1.15	±1.33
200%	101.1	100.1	99.9	100.3	99.7	98.5	99.5	99.2
	±0.35	±0.53	±1.09	±1.62	±1.63	±1.21	±1.71	±0.25

€ Amount spiked with respect to test the concentration of Olmesartan Medoximil (1.6 mg/ml) and Hydrochlorothiazide (0.5 mg/ml)., $ mean±%RSD for three determinations.

Table 5: Robustness data

Compound↓/Variations→	As such	Flow 0.8R	Flow 1.2R	Temp 25R	Temp 35R	pH 3.0R	pH 3.4R
HCTZ_Imp-B	NA	NA	NA	NA	NA	NA	NA
HCTZ_Chlorothiazide	3.9	3.9	4.0	4.1	3.	3.9	4.0
HCTZ	4.3	4.5	4.2	4.4	4.2	4.4	4.4
OLME_Acid	33.4	30.4	35.6	29.9	37.2	33.2	32.6
HCTZ_Imp-C	11.6	11.8	10.2	12.8	8.2	11.3	11.6
OLME_Ester	26.5	25.6	26.8	19.8	32.9	23.1	22.9
OLME	6.7	6.5	6.7	7.2	6.0	7.1	4.8
OLME_Dehdro	14.7	15.0	14.7	14.9	14.3	15.1	15.1
OLME_Imp-IV	28.0	27.2	29.9	28.5	26.5	30.7	25.2
OLME_Imp-III	26.88	27.16	27.4	26.43	27.34	22.19	20.2

HCTZ-Hydrochlorothiazide, OLME-Olmesartan Medoximil, R-Resolution between two adjacent peaks.

Accuracy

The accuracy was evaluated in triplicate by spiking respective impurities in the sample at 50%, 100% and 200% with respect to an analyte concentration of Olmesartan Medoximil 1.6 mg/ml and Hydrochloro-thiazide 0.5 mg/ml. The % RSD result for all impurities was depicted in (table 4). The percent recovery found varied from 98.0% to 105.0% and the % RSD for three determinations found below 3.0%.

Robustness

With all deliberate variations of method parameters (flow, pH and temperature), the resolution between all the adjacent peaks found more than 2.0. The results for robustness study depicted in (table 5).

Solution stability

The similarity factor for freshly prepared standard and solution after 24 Hr found between 0.95 to 1.05 was as for Olmesartan Medoximil acid impurity up to 3Hr solution less than 0.95. Hence, the standard solution found a stable for 24 Hr and sample solution found stable for less than 3 Hr.

CONCLUSION

A specific, precise, accurate, linear and robust RP-HPLC method developed for simultaneous estimation of related substances from a combination of Olmesartan Medoximil and Hydrochlorothiazide in pharmaceutical tablet dosage form. The method was stability-indicating and can be used for routine analysis of production samples.

ACKNOWLEDGEMENT

The author wishes to thanks to Mr. Venkat Shinde (MD and CEO) Veeprho Laboratories Pvt. Ltd. for providing resources, guiding during the work and co-operation from colleagues appreciated.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Raj ND, Anbazhagan S, Kunapareddy A, Sunkara N, Chusena N. Validated stability indicating gradient RP-HPLC method for the estimation of antihypertensive drugs in bulk and pharmaceutical dosage. Int Curr Pharm J 2012;1:336-40.
2. Rai M, Kawde PB. Simultaneous determination of olmesartan amlodipine besylate and hydrochlorothiazide in tablet dosage form by using stability-indicating HPLC method. Res J Pharm Biol Chem Sci 2013;4:560-7.
3. Celebier M, Altinoz S. Determination of olmesartan medoximil in tablets by UV-VIS spectrophotometry. Pharmazie 2007;62:419.
4. Jain P, Jain A, Maliwal D, Jain V. Development and validation of a spectrophotometric and RP-HPLC method for estimation of Olmesartan Medoximil in tablet dosage form. Int J Pharma Bio Sci 2010;1:1-7.
5. Chrysant SG, Chrysant GS. Antihypertensive efficacy of olmesartan medoximil alone and in combination with hydrochlorothiazide. Expert Opinion Pharmacother 2004;37:657.
6. Chrysant SG, Wang MA, Hinman DJ. Evaluation of antihypertensive therapy with the combination of olmesartan medoximil and hydrochlorothiazide. Am J Hypertens 2004;17:252.
7. Haung T, Zhong H, Bei Yang, Bei Luping Shao, Xiaowei Zheng, Gengli Duan. Simultaneous determination of captopril and hydrochlorothiazide in human plasma by RP-HPLC from linear gradient elution. J Pharm Biomed 2006;41:644.
8. Laeis P, Puchler K, Kirch W. The pharmacokinetic and metabolic profile of olmesartan medoximil limits the risk of clinically relevant drug interaction. J Hypertension 2001;19:21-32.
9. Shah NJ, Suhagia BN, Shah PB, Shah RR. Development and validation of a simultaneous RP-HPLC method for the estimation of Olmesartan Medoximil and Hydrochlorothiazide in tablet dosage form. Indian J Pharm Sci 2007;69:202.
10. Patel LJ, Suhagia BN, Shah PB, Shah RR. Simultaneous estimation of bisoprolo fumarate and hydrochlorothiazide in tablet dosage form by using RP-HPLC method. Indian J Pharm Sci 2006;68:635.
11. Wankhede SB, Tajne MR, Gupta KR, Wadodkar SJ. RP-HPLC method for simultaneous estimation of telmisartan and hydrochlorothiazide in tablet dosage form. Indian J Pharm Sci 2007;69:202.
12. Suryadevara V, Reddyvalam L, Ballipalli V, Koduri T, Marupudi R. Method development and validation for simultaneous estimation of Olmesartan Medoxomil and Hydrochlorothiazide by Rp-Hplc. Oriental J Chem 2004;30:195-201.
13. Patel U, Chokshi A, Desai P. Development and validation of rp-hplc method for determination of hydrochlorothiazide, Olmesartan medoxomil and their related substances in combined tablet dosage form. Int J Pharm Pharm Sci 2014;6:9.
14. Parekh SA, Pudage A, Joshi SS, Vaidya VV, James NA, Kamat SS. Simultaneous estimation of hydrochlorothiazide, Quinapril and quinaprilat in human plasma by liquid chromatography coupled to tandem mass spectrometry. Indian J Chromatography B 2008;59:873.
15. Dongyang L, Hua Pei B, Nobuka A, Xiaoming Ji. Quantitative determination of Olmesartan in human plasma and urine by liquid chromatography coupled to tandem mass spectrometry. J Chromatogr B: Biomed Sci Appl 2007;856:190-7.
16. Shah NJ, Suhagia BN, Shah RR, Patel NM. Development and validation of HPTLC method for simultaneous estimation telmisartan and Hydrochlorothiazide in tablet dosage form. Indian J Pharm Sci 2007;69:834.