Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 5, 2015

Original Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTENEOUS ESTIMATION OF OLMESARTAN MEDOXOMIL AND CHLORTHALIDONE IN TABLET DOSAGE FORM

VILAS SAWALE*, PANKAJ DANGRE, DISHA DHABARDE

Department of Pharmaceutical Chemistry, Kamla Nehru College of Pharmacy, Butibori, Nagpur, Maharashtra, India
Email: sawalevilas@gmail.com

Received: 20 Feb 2015 Revised and Accepted: 18 Mar 2015

ABTRACT

Objective: To develop and validate a simple, rapid, accurate and precise RP-HPLC method for the simultaneous determination of olmesartan medoxomil (OLM) and chlorthalidone (CHL) in pharmaceutical formulation.

Methods: Chromatographic separation was performed on a Phenomenox, Gemini C18 (250×4.6 mm, 5 µm) column from thermo isocratic mode with mobile phase 55:45 water: acetonitrile with pH adjusted to 3.0 with ortho phosphoric acid at flow rate 1 ml/min. Peak intensity of both the drugs was monitored at 250 nm with UV detection.

Results: The retention time (RT) of OLM and CHL was found to be 2.95 and 3.91 min, respectively. The linearity of OLM and CHL were found in the range of 10-60 µg/ml for OLM and 5-30 µg/ml for CHL. The limit of detection and limit of quantitation was 15ng/ml and 70 ng/ml for OLM and 20 ng/ml and 60 ng/ml for CHL.

Conclusion: The proposed method was validated in terms of accuracy, precision, linearity, limit of detection and limit of quantification. Furthermore, no interference was observed with extra pharmacopoeial excipients in tablet suggesting its utility for routine quality control analysis of OLM and CHL in pharmaceutical formulations.

Keywords: Olmesartan medoxomil, Chlorthalidone, RP-HPLC.

INTRODUCTION

Olmesartan medoxamil (OLM) is a (5-methyl-2 oxo-1,3-dioxol-4-yl) methyl ester of 4-(1-hydroxy-1-methylethyl)-2-propyl-1{[2-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-1H-imidazole-5-carboxylate [1,2] (fig. 1A). It is an AT_1 subtype angiotensin II receptor antagonist used in the treatment of hypertension. Chlorthalidone (CHL) is an oral diuretic used along with oral antihypertensive agent. Chemically it is (RS) 2-chloro-5-(1-hydroxy-3-oxo-2, 3-dihydro-1H-isoindol-1-yl) benzene-1-sulfonamide [3] (fig. 1B). Many analytical methods are reported in the literature for the determination of OLM by UV-Spectrophotometry [4], RP-HPLC [5-7] and OLM with hydrochlorothiazide by HPTLC [8]. Several methods have been described for determination of CHL by UV-Spectrophotometry [9], RP-HPLC [10-11] and by HPTLC [12]. Although there are several chromatographic methods reported for determination of both these drugs. However, to the best of our knowledge, there is no LC analytical method reported for simultaneous determination of OLM and CHL in combined dosage form. The objective of the present work was to develop accurate and precise RP-HPLC method with UV-detection for the quantification of these drugs in pharmaceutical formulation.

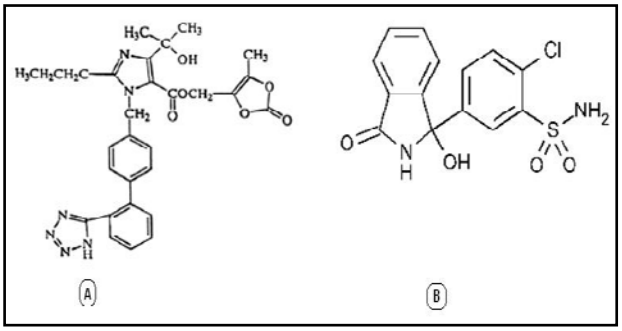

Fig. 1: Chemical structures of analytes. A. Olmesartan medoxomil, B. Chlorthalidone

MATERIALS AND METHODS

Material and reagent

Olmesartan medoxomil was kind gift of Ajanta pharmaceutical Ltd. (Mumbai, India), Chlorthalidone was provided by Ipca laboratories (Mumbai, India). Pharmaceutical formulation of tablets containing olmesartan medoxomil 20 mg and chlorthalidone 12.5 mg was purchased from local market of Nagpur. HPLC grade acetonitrile and phosphoric acid was procured from Merck, Mumbai. Double distilled de-ionized water was used throughout the study. All solutions were filtered through a Millipore vacuum filter system (0.22 µm) and degassed by sonicator.

Instrument

A HPLC system consisted of LC solution data handling system (Shimadzu LC-20AD), a SPD-20A Shimadzu UV visible detector, Rheodyne injector with 20 µl sample loop. A 25 µl Hamilton syringe was used for injecting the samples. Data acquisition were performed by using LC 2010 solution software. Ultrasonic bath sonicator was used for degassing of the mobile phase. Weighing of the materials was carried on a Shimadzu balance with high accuracy.

Chromatographic condition

The mobile phase consisted of water and acetonitrile (55:45 v/v), pH of which is maintained at 3.0 using orthophosphoric acid (85%). The mobile phase was always freshly prepared and filtered through a Millipore vacuum filter system equipped with 0.22 µm filter and degassed by sonicator. Chromatography was performed at ambient temperature by pumping the mobile phase at a flow rate of 1.0 ml/min. The column effluent was monitored at 250 nm.

Standard and working standard

Accurately 10 mg of standard OLM and CHL were weighed and dissolved into 100 ml mobile phase to obtain the final concentration of 100 µg/ml. Appropriate dilutions were made into 10 ml volumetric flasks with the mobile phase to produce working solutions in the concentrations range 10-60 µg/ml and 5-30 µg/ml

for OLM and CHL, respectively. 20 µl of samples were injected into the chromatographic system and peak areas were measured.

Pharmaceutical formulation

Twenty tablets were crushed in the mortar and the powdered equivalent to 20 mg OLM and 12.5 mg of CHL were transferred into 100 ml volumetric flask and dissolved in the mobile phase. The solution was sonicated to dissolve solid powder completely. The undissolved material is removed by filtration through Whatman filter paper no-41. The volume was made up with the mobile phase and further dilutions were made with the mobile phase to get final concentrations of 20 µg/ml of OLM and 12.5 µg/ml of CHL. The solutions were injected and respective Chromatogram was recorded.

RESULTS AND DISCUSSION

Method development

Different trials were carried out by varying the ratio of water: acetonitrile (v/v) and optimizing the chromatographic conditions. The mobile phase consisting of water: acetonitrile (55: 45 v/v), pH 3.0 was found to have good resolution at wavelength 250 nm with 1.0 ml/min flow rate. The optimized conditions give well resolved and sharp peak for both the drugs (fig. 2). Retention times for OLM and CHL were determined as 2.95 and 3.91 min, respectively (fig. 3).

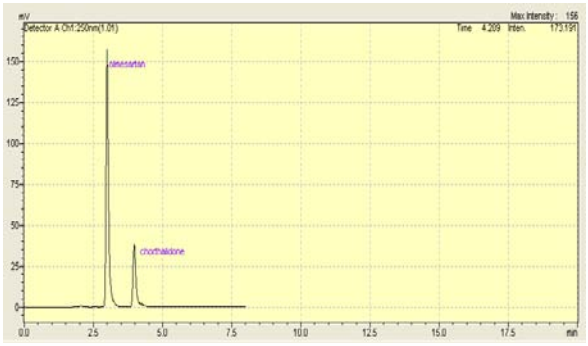

Fig. 2: A typical chromatogram showing well resolved and sharp peaks of OLM and CHL

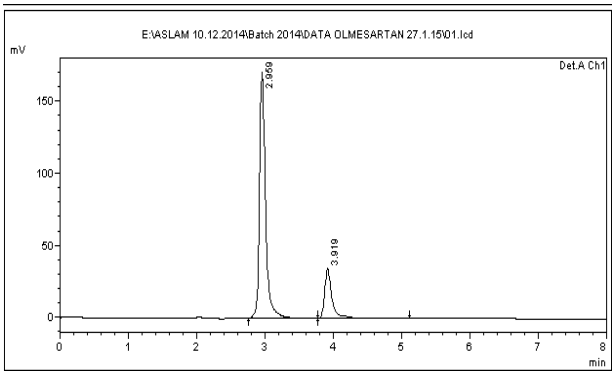

Fig. 3: Representative chromatogram of OLM and CHL having RT 2.95 and 3.91 min respectively

Method validation

Method validation was carried out for different parameters including linearity, accuracy, inter-day and intra-day precision, reproducibility. The limit of detection (LOD) and limit of quantification (LOQ), was also determined. All the parameters were studied considering the ICH guidelines [13].

System suitability

The efficiency of the column is expressed in terms of system suitability parameters, such as number of theoretical plates, resolution, tailing factor. The data are summarized in table 1.

Linearity

The linearity of the method was determined at six concentration levels ranging from 10-60 µg/ml for OLM and 5-30 µg/ml for CHL. The calibration curve was constructed by plotting peak area against concentration of drugs (fig. 4 and 5).

The results show that an excellent correlation exists between the peak area and concentration of drugs. Table 2, lists the linearity parameters of the calibration curves for OLM and CHL.

Table 1: System suitability parameter

Parameters	Retention time (t_r)	Theoretical plates (*N*)	Tailing factor (T_f)	Resolution (R_s)
OLM				
pH				
2.9	2.97	4312	1.67	0.864
3.0	2.96	4851	1.49	0.504
3.1	2.95	4765	1.64	0.626
Flow rate				
0.9	3.29	4995	1.48	0.652
1.0	2.94	4895	1.47	0.566
1.1	2.52	4754	1.67	0.588
Mobile phase				
43:57	2.85	4696	1.52	0.675
45:55	2.94	4725	1.49	0.525
47:53	3.12	4915	1.50	0.618
CHL				
pH				
2.9	3.89	6958	1.74	5.989
3.0	3.91	7551	1.69	5.718
3.1	3.88	7842	1.66	5.700
Flow rate				
0.9	4.34	7154	1.65	5.738
1.0	3.94	7581	1.66	5.506
1.1	3.47	7652	1.64	5.593
Mobile phase				
43:57	3.90	7287	1.66	5.824
45:55	3.91	7425	1.65	5.542
47:53	3.92	7894	1.79	5.620

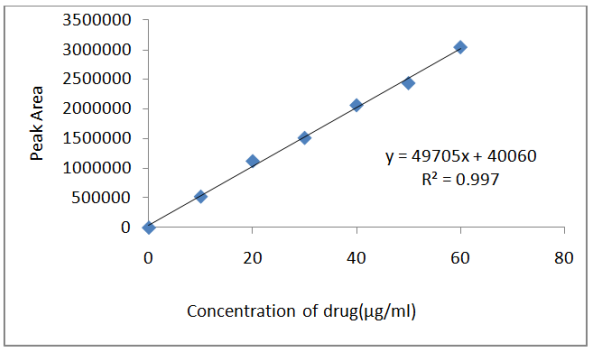

Fig. 4: Standard calibration curve of olmesartan medoxomil

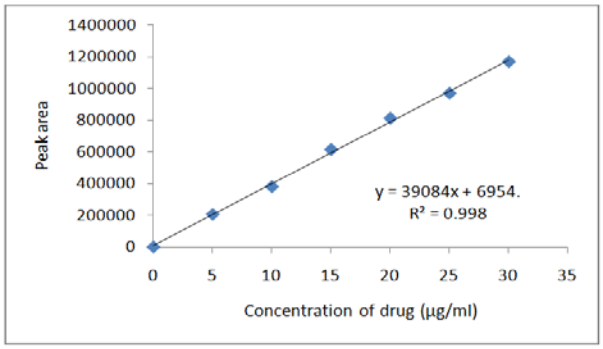

Fig. 5: Standard calibration curve of chlorthalidone

Accuracy

The accuracy of analytical procedure measures the closeness of measured values to the true value. Standard solution of accuracy of 80%, 100% and 120% solutions were injected into the chromatographic system. Table 3, represents the high percent recovery values indicating that the proposed method is accurate and reproducible.

Table 2: Regression characteristics of pure drug

Parameters	Olmesartan medoxomil	Chlorthalidone
Conc. Range (µg/ml)	10 to 60	05 to 30
Correlation coefficient (r^2)	0.997	0.998
Slope (m)	49679	39084
Intercept (b)	41346	6954
LOD (ng/ml)	15	20
LOQ (ng/ml)	70	60

Precision

The precision of the method was evaluated by inter day and Intraday variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage of RSD were calculated. In the inter day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and the response factor of drug peaks and percentage of RSD were calculated. From the result obtained table 4, the proposed HPLC method was found to be precise.

Table 3: Result for recovery studies

Analytes	Label claim	Amount added (%)	Total amount (mg)	Amount recovered* (mg)	Mean recovery (%)	RSD(%)
Olmesartan medoxomil	20 mg/tab	80	16	15.94	99.69±0.49	0.49
		100	20	19.96	99.80±1.20	1.20
		120	24	23.86	99.43±0.21	0.21
Chlorthalidone	12.50 mg/tab	80	10	9.92	99.30±0.18	0.18
		100	12.5	12.41	99.32±0.86	0.86
		120	15	14.91	99.45±0.31	0.31

* Mean of three determinations

Table 4: Result of precision study

	%Mean*		SD		% RSD	
	OLM	CHL	OLM	CHL	OLM	CHL
Intraday precision	99.55	99.14	0.42	1.026	0.42	1.03
Inter-day precision	99.41	99.47	0.68	1.048	0.68	1.05

* Mean of six determinations

Detection and quantitation limits

The limit of detection (LOD) and limit of quantitation (LOQ) of the developed method was determined by injecting progressively low concentrations of the standard solution using the developed RP-HPLC method. The LOD is the smallest concentration of analyte that gives measurable response. The LOD of OLM and CHL was found to be 20 ng/ml and 15 ng/ml, respectively. LOQ was 70 ng/ml and 60 ng/ml for OLM and CHL, respectively.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions. It has been observed that the proposed method is able to withstand relatively minor alterations in pH, compositions of mobile phase and flow rate (table 1), but retention times for OLM and CHL were found to be altered.

CONCLUSION

The RP-HPLC method was developed and validated successfully in terms of accuracy, precision, linearity etc. The proposed method was found to be simple, rapid and accurate. Hence, this method can be used for easy and efficient routine analysis of OLM and CHL in the quality control laboratory.

ACKNOWLEDGEMENT

We thank to IPCA Laboratories, and Ajanta Pharma Ltd. Mumbai, India, for providing the chlorthalidone and olmesartan medoxomil.

We also thankful to the secretary of the Kamla Nehru College of Pharmacy, Butibori, Nagpur, India for providing instrumental facility for carrying out the research work.

REFERENCES

1. Sweetman SC. Martindale, The complete drug reference. 36 th ed. Pharmaceutical press: London; 2009;975:3.
2. Budavari S. The Merck Index. 14thed. Merck and Co. Inc Whitehouse station NJ: Merck; 2006. p. 22.
3. Oneil MJ, Smith A, Heckelman PE. The Merck Index. 14thed. Merck and Co. Inc Whitehouse station NJ: Merck; 2010. p. 884.
4. Celebier M, Altinoz S. Determination of olmesartan medoxomil in tablets by UV-Vis Spectrophotometry. Pharm 2007; 62(2):419-22.
5. Ganduri RB, Lanka RA, Pamidi S, Peddareddigari RJ, Mohamod M. New RP-HPLC method for the determination of olmesartan medoxomil in tablet dosage form. Eurasian J Anal Chem 2010;5(2):145-51.
6. Sharma RN, Pancholi SS. RP-HPLC-DAD method for determination of olmesartan medoxomil in bulk and tablets exposed to forced conditions. Acta Pharm 2010;60:13-24.
7. Pai NR, Sawant SS. Development and validation of new RP-HPLC method for determining impurity profiling in olmesartan medoxomil drug as well as in tablet dosage form. Pharma Chem 2013;5(4):274-81.
8. Shah NJ, Suhagia BN, Shah RR, Patel NM. Development and validation of a simultaneous HPTLC method for the estimation of olmesartan medoxamil and hydrochlorthiazide in tablet dosage form. Indian J Pharm Sci 2007;69(6):834-6.
9. Akifulhaque M, Nivedita G, Prashanthkumar K, Pradeepkumar T. Simultaneous estimation of atenolol and chlorthalidone as bulk and in tablet dosage form using UV-Spectrophotometry. J Pharm Bio Sci 2012;1(4):20-3.
10. Singh B, Patel DK, Ghosh SK. A Reversed phase high performance liquid chromatographic method for determination of chlorthalidone in pharmaceutical formulation. Int J Pharm Pharm Sci 2009;1(2):24-9.
11. Kumar GS, Ramya V. Mondal S, Saipavankumar. Development and validation of RP-HPLC for simultaneous estimation of atenolol and chlorthalidone from pharmaceutical formulation. Int Res J Pharm 2012;3(10):215-9.
12. Mohamed S, Elgawishsamiam M, Mustafa, Abdalla E. Simple and rapid HPLC method for simultaneous of atenolol and chlorthalidone in spiked human plasma. Saudi Pharm J 2011;19:43-9.
13. Validation of analytical procedures, ICH Hormonised Tripartite Guidelines, Q2B; 1997.