



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

Aswini GL¹, Dachinamoorthy D², Rao SJVLN³

¹School of Pharmaceutical Sciences, JNTUK, Kakinada, Andhra Pradesh- 533003, ²QIS College of Pharmacy, Ongole, Andhra Pradesh- 523272, ³Yalamarty Pharmacy College, Anandhapuram, Vishakhapatnam, Andhra Pradesh- 530052

Address for Correspondence
Ganipisetty Lakshmi Aswini
E-mail :
ganipisettyaswini@gmail.com

Received: 03-07-2014
Review completed: 02-08-2014
Accepted: 21-09-2014

Access this article online

QR Code



Website:
www.ijrpsonline.com

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/v at 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.

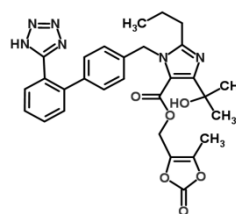
Key words: UPLC, Olmesartan medoximil, Hydrochlorothiazide, Tablet dosage form.

INTRODUCTION

Olmesartan medoximil is described chemically as 2,3-dihydroxy-2-butenyl 4(1-hydroxy-1-methylethyl)-2-propyl-1-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]imidazole-5-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-sulfonamide³. 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

spectroscopy⁴⁻¹⁶, high performance liquid chromatography^{4, 17-31}.

A



B

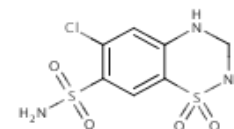


Figure 1: The Chemical Structures of (A) Olmesartan medoximil and (B) Hydrochlorothiazide.

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 Medoximil 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	Hydrochlorothiazide	
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

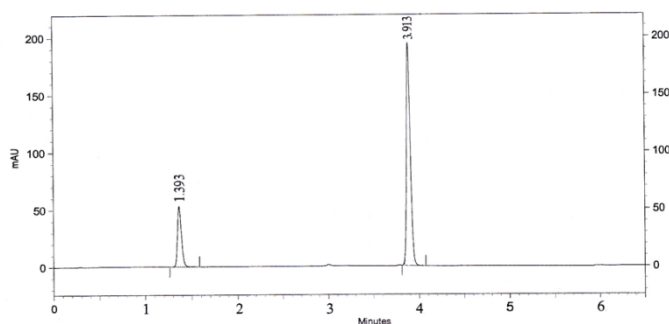


Figure 2: Optimized chromatogram for Olmesartan Medoximil (88 ppm) and Hydrochlorothiazide (56 ppm)

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 $y = 24560x + 11151$ ($R^2 = 0.9995$) 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.

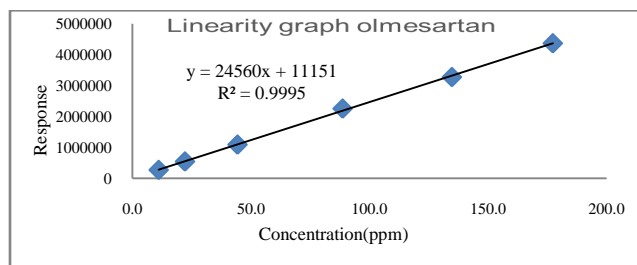


Figure 3: Linearity curve for Olmesartan

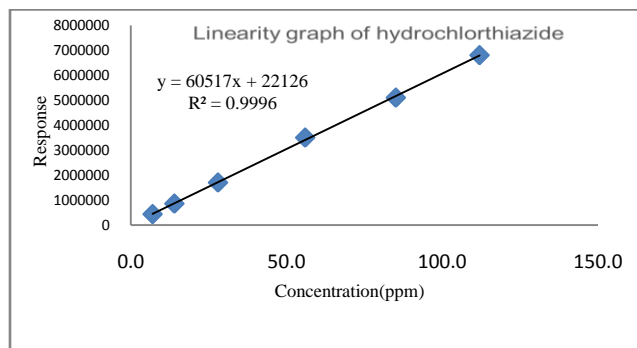


Figure 4: Linearity curve for Hydrochlorothiazide

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	Hydrochlorothiazide	Olmesartan	Hydrochlorothiazide
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 $3.3 \sigma / S$ and $10 \sigma / S$ 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 $\pm 2\%$ 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 ($\mu\text{g/mL}$)	11 – 177		7 – 112	
Correlation co-efficient	0.9995		0.9996	
LOD ^a ($\mu\text{g/mL}$)	3.4		2.15	
LOQ ^b ($\mu\text{g/mL}$)	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.

REFERENCE

- Martindale, The complete drug reference, Lambeta high street, London, 35th Edition, 2006.
- Julie AB and John MB. Clin Therap, 2004; 26(1): 28-32.
- British pharmacopoeia, The Stationery Office, London, 2007; 1:1036-1037.
- Wankhede S.B, Wadkar S.B and Raka K.C et al. Simultaneous Estimation of amlodipine Besilate and Olmesartan Medoxomil in Pharmaceutical Dosage Form. Indian J Pharm Sci. 2009; 71(5):563-567.
- Jothieswari. D, Anandakumar. K, Vijaya Santhi. et al. Validated UV Spectrophotometric Method for the Simultaneous Estimation of amlodipine Besylate, Valsartan and Hydrochlorothiazide in Bulk and in Combined Tablet Dosage Form. J Pharm Biomed Sci. 2010; 5(13): 1-5.
- Sumita singh, vikas Bali, kamla Pathak. Development and validation of a novel spectrophotometric analytical method for the determination of olmesartan medoxomil in pharmaceutical formulations. International. 2011; 3(5): 487-490.
- Hacioğlu F, Onal A. Determination of eprosartan mesylate and hydrochlorothiazide in tablets by derivative spectrophotometric and high-performance liquid chromatographic methods. J Chromatogr Sci. 2012; 50(8):688-93.
- Rote AR, Bari PD. Ratio spectra derivative and zero-crossing difference spectrophotometric determination of olmesartan medoxomil and hydrochlorothiazide in combined pharmaceutical dosage form. AAPS Pharm Sci Tech. 2009; 10(4):1200-5.
- Celebier M1, Altinoz S. Determination of olmesartan medoxomil in tablets by UV-Vis spectrophotometry. Pharmazie, 2007; 62(6): 419-22.
- Pandurang N. Dhabale and Sunita R Bhagade. Simultaneous UV Spectrophotometric Methods for Estimation of Amlodipine Besilate and Olmesartan Medoxomil in Tablet Dosage Form. J. Chem. Pharm. Res. 2011; 3(2):650-656.
- Kardile P.D, Kalyane V.N, Thakkar H.T. et al. Simultaneous estimation of amlodipine besylate and olmesartan medoxomil drug formulations by HPLC and UV-spectrophotometric methods. J. Pharm. Sci. & Res. 2010; 2(9): 599-614.
- Kereiakes DJ, Neutel JM, Punzi HA. Efficacy and safety of olmesartan medoxomil and hydrochlorothiazide compared with benazepril and amlodipine besylate. Am J Cardiovasc Drugs. 2007; 7(5): 361-72.
- Thomas AB, Chavan UB, Nanda RK, et al. Simultaneous spectrophotometric estimation of Hydrochlorothiazide, Atenolol and Losartan potassium in tablet dosage form. Hindustan Antibiot Bull. 2009; 51(1-4): 33-8.
- Bhusari.K.P, Khedekar.P.B and Banode V.S. Derivative and Q-analysis Spectrophotometric Methods for Estimation of Hydrochlorothiazide and Olmesartan Medoxomil in Tablets. Indian J Pharm Sci. 2009; 71(5): 501-08.
- Kartal M, Erk N. Simultaneous determination of hydrochlorothiazide and amiloride hydrochloride by ratio spectra derivative spectrophotometry and high-performance liquid chromatography. J Pharm Biomed Anal. 1999; 19(3-4):477-85.
- Ertürk S, Cetin SM, Atmaca S. Simultaneous determination of moexipril hydrochloride and hydrochlorothiazide in tablets by derivative spectrophotometric and high-performance liquid chromatographic methods. J Pharm Biomed Anal. 2003; 33(3):505-11.
- Shah S.K, Asnani A.J, Kawade D.P, et al. Simultaneous Quantitative Analysis of Olmesartan Medoxomil and amlodipine Besylate in Plasma by High-performance Liquid Chromatography Technique. Journal of Young Pharmacists. 2012; 4(2): 88-94.
- Jain PS, Patel MK and Gorle AP et al. Stability-indicating method for simultaneous estimation of olmesartan medoxomile, amlodipine besylate and hydrochlorothiazide by RP-HPLC in tablet dosage form. J Chromatogr Sci. 2012; 50(8): 680-7.
- Patil, Pournima S, More, Harinath N, Pishwikar, Sachin A. RP-HPLC method for simultaneous estimation of amlodipine besylate and olmesartan medoxomil from tablet. Int J Pharm Pharm Sci. 2011; 3(3): 146-149.
- 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(1):13-24.
- Sultana N, Arayne MS, Ali SS, et al. Simultaneous determination of olmesartan medoxomil and irbesartan and hydrochlorothiazide in pharmaceutical formulations and human serum using high performance liquid chromatography. Se Pu. 2008; 26(5):544-9.
- Carlucci G1, Di Federico L, Iuliani P. HPLC-DAD method for the simultaneous determination of zofenopril and hydrochlorothiazide in oral pharmaceutical formulations. J Sep Sci. 2010; 33(12):1717-22.
- Meyyanathan SN, Rajan S, Muralidharan S, Birajdar AS, Suresh B. A Validated RP-HPLC Method for Simultaneous Estimation of Nebivolol and Hydrochlorothiazide in Tablets. Indian J Pharm Sci. 2008; 70(5):687-9.

24. Thakker NM, Panchal HB, Rakholiya DR, et al. Development and validation of a stability indicating RP-HPLC method for simultaneous estimation of Olmesartan Medoxomil and Metoprolol Succinate in pharmaceutical dosage form. *Pharm Methods*.2012;3(2):84-89.
25. Patel HU, Suhagia BN, Patel CN. Simultaneous analysis of eprosartan and hydrochlorothiazide in tablets by high-performance liquid chromatography. *Pharm Methods*. 2011;2(2): 143-147.
26. Nitin Dubey, Ankit Jain, Ajay K. Raghuwanshi, Dinesh K. Jain. Simultaneous Determination and Validation of Olmesartan Medoxomil, amlodipine RP-HPLC Method. *Asian Journal of Chemistry*.2012; 24(10):4535-4537.
27. Ashok kumar.j, Sathya A, Senthil kumar .k, et al .Simultaneous estimation of olmesartan and Hydrochlorothiazide by RP-HPLC Method from Combined Dosage Forms. *Int. J. Res. Pharm. Sci*.2010; 1(1):24-27.
28. Jain, Prabhat, Anurekha, et al. Development and validation of spectrophotometric and RP-HPLC method for estimation of olmesartan medoxomil in tablet dosage form. *Int J Pharm. Bio. Sci*. 2010; 1(2):1-7.
29. Devanaboyina, Narendra, Satyanarayana, et al. Simultaneous determination of olmesartan and hydrochlorothiazide in combined pharmaceutical dosage form by RP-HPLC method. *Int J Pharm. Bio. Sci*. 2012; 3(2): 107-115.
30. Buchi Nalluri N, Venkateswara Naik D, Sunandana B, et al. Development and validation of RP-HPLC-PDA method for the simultaneous estimation of hydrochlorothiazide, amlodipine besylate and olmesartan medoxomil in bulk and pharmaceutical dosage forms. *J Chem Pharm Res*. 2013; 5(1): 329-335.