Research Article

Comparative Analytical Methods for the Simultaneous Estimation of Olmesartan and Chlorthalidone in Formulation Dosage Form Using UV Spectroscopy and RP-HPLC

Dr. Syed Rizwan Hussain*, Mudiganti Rakshitha Goud, Shaik Rahmath, Afreen Sultana, Saba Fatima
Deccan School of Pharmacy, Department of Pharmaceutical Analysis, Hyderabad, Telangana, 500001, India.
***Corresponding author's E-mail:** nawzirspectra@gmail.com

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ABSTRACT

Hypertension (HTN) affects millions globally, necessitating effective treatment strategies often involving combination drug therapy. This study focuses on developing and validating analytical methods for the simultaneous estimation of Olmesartan (OLM) and Chlorthalidone (CHL), two drugs frequently combined for hypertension management. Two methods were explored: UV spectrophotometry and High-Performance Liquid Chromatography (HPLC). The main aim is to compare the assay results for the above said combination using two a For the UV method, methanol and distilled water served as solvents, with OLM and CHL showing absorbance maxima at 258 nm and 214 nm, respectively. The method demonstrated linearity within 20-60 µg/ml for OLM and 2-10 µg/ml for CHL, with excellent precision, accuracy, and sensitivity. The HPLC method utilized a mobile phase of methanol, acetonitrile, and orthophosphoric acid, achieving separation on a C18 column with UV detection. Calibration curves for OLM and CHL displayed linearity over the same concentration ranges. Both methods were rigorously validated, showing robustness across varied parameters, including flow rates and mobile phase composition. This study provides reliable, efficient methodologies for the simultaneous analysis of OLM and CHL in pharmaceutical formulations, supporting enhanced quality control and patient adherence in antihypertensive therapy.

Keywords: Hypertension, Olmesartan, Chlorthalidone, UV spectrophotometry, HPLC, Analytical validation, Calibration curve, pharmaceutical analysis, Precision, Accuracy, Robustness, Recovery studies.

INTRODUCTION

In the field of pharmaceutical analysis, the need for precise, accurate, and efficient analytical methods for multi-component drug formulations is increasingly crucial, especially for medications with complementary therapeutic effects. Olmesartan and Chlorthalidone, often prescribed together for the treatment of hypertension, require reliable analytical techniques to ensure their simultaneous estimation in formulation dosage forms. This study explores and compares two widely used methods—UV spectroscopy and Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC)—for the simultaneous quantification of Olmesartan and Chlorthalidone. Each method is evaluated for parameters such as precision, accuracy, linearity, and sensitivity to determine the most effective approach for routine quality control. By investigating these analytical techniques, this study aims to offer valuable insights into their practical applications and suitability in pharmaceutical quality assurance, highlighting the advantages and limitations of each method for the analysis of combined antihypertensive agents.[1-2]

MATERIALS AND METHODS

EXPERIMENTAL WORK OF UV

Apparatus:

Digital Weighing Balance: Denver SI-234, Germany,

UV-Visible Spectrophotometer: Shimadzu UV-1800, Japan,

Filters: Whatman Filter Paper,

Glassware: Pipettes (1-10 ml): Borosil, Volumetric Flasks (10-100 ml): Borosil

Materials: Working standards of Olmesartan medoxomil were obtained from Merck Ltd., Village Mandoli, Delhi, India, and chlorthalidone was provided by Systopic Laboratories Pvt Ltd, Ranjit Nagar, New Delhi, India. They were used without further purification. Marketed formulations included Olmighty 20 Tablet (Merck Ltd., Delhi, India), Olmezest 20 (Sun Pharmaceutical Industries Ltd., East Sikkim, India), New CH 12.5 mg Tablet (Systopic Laboratories Pvt Ltd, New Delhi, India), and CTD-T 12.5/20 (Ipca Laboratories Ltd, Mumbai, Maharashtra, India). Additionally, Omen CT, a fixed-dose combination tablet containing 40 mg olmesartan medoxomil and 12.5 mg chlorthalidone, was obtained from Medley Pharmaceuticals, Andheri, Mumbai, India. All the chemicals were of analytical grade. Ethanol was purchased from CSS, while NaOH and HCl were sourced from SDFCL. Distilled water was used as the solvent and prepared in-house.[3-5]

Solubility studies: Olmesartan & Chlorthalidone are soluble in 0.1N HCl, Ethanol.

Preparation of working standard stock solution: Individual solutions of olmesartan and chlorthalidone were prepared by taking 0.09g powdered drug in 100 ml volumetric flask and the drug was dissolved using ethanol and makeup to mark using ethanol.

Working standard solution: From the stock solution, the solution was further used to prepare various dilution of concentration range of 20-60 mcg/mL of olmesartan and for

chlorthalidone having concentration range of 2-10mcg/ml using ethanol as blank. [6]

Selection of wavelength: For selection of the solvent for the UV-Visible spectroscopic method, both the selected drugs are freely soluble in ethanol. For selection of λ max for estimation, standard solutions of olmesartan having concentration range of 20-60 mcg/mL and chlorthalidone having concentration range of 2-10mcg/ml in ethanol were prepared and scanned in the range of 200 to 400 nm in double beam UV Visible spectrophotometer using ethanol as blank.[7-8]

METHODOLOGY FOR ASSAY USING UV SPECTROSCOPY

Simultaneous equation method: Also known as Vierodt's method.

If a mixture contains two absorbing drugs, each of which absorbs at λmax of each other, then the concentration of both the drugs can be calculated by using the simultaneous equation method. A mixture of two components X and Y are present in concentration Cx and Cy respectively, by measuring the absorbance of mixture at two wavelengths λ_1 and λ_2 concentration of each component can be determined. Let ax_1, and ax_2 and ay_1 and ay_2, are the absorptivity values determined at λ_1 and λ_2 for component X and Y respectively. The absorbance of mixture at λ_1 and λ_2 is A_1 and A_2 which is due to the absorbance of each component (X and Y) at λ_1 and λ_2 respectively.

At λ_1, $A_1 = ax_1bc_x + ay_1bc_y$

At λ_2, $A_2 = ax_2bc_x + ay_2bc_y$ After mathematical calculation,

$C_x = (A_2ay_1-A_1ay_2)/(ax_2ay_1-ax_1ay_2)$ $C_y = (A_1ax_2-A_2ax_1)/(ax_2ay_1-ax_1ay_2)$

Maximum precision is obtained if the ratio of $(A_2/A_1)/(ax_2/ax_1)$ and $(ay_2/ay_1)/(A_2/A_1)$ is outside the 0.1-2.0 range.[8-10]

FIRST ORDER DERIVATIVE SPECTRUM METHOD

Spectra obtained by derivatizing zero order spectrum once. It is a plot of change of absorbance with wavelength against wavelength i.e. rate of change of the absorbance with wavelength, $dA/d\lambda = f(\lambda)$.

First order spectra passes through zero as λ max of the absorbance band. Absorbance band of first order derivative shows certain positive and negative band with maxima and minima. By scanning the spectrum with a minimum and constant difference between two wavelengths, dual-wavelength spectrophotometer obtains first-derivative spectra. It is characterized by a maximum, a minimum and cross over point at the Amax of the absorption band.

VALIDATION PARAMETERS

Validation of analytical methods, in general, has been extensively covered in the ICH guidelines Q2A and Q2B, in the FDA guidance and by USP. Here validation has been carried out as per ICH guidelines Q2A and Q2B.[11-12]

RESULTS AND DISCUSSION

Linearity

Linearity studies were carried out by preparing a series of dilutions from working stock solution of both the drugs. For olmesartan, the linearity was studied from 20-60mcg/mL concentration with absorbance of 0.115, 0.153, 0.187, 0.256, 0.312 respectively. For chlorthalidone it was from 2-10mcg/mL concentration with absorbance of 0.117, 0.297, 0.396, 0. 561, 0.715 respectively.

Table 1: Intra-day Precision data of OLM & CHL at 258nm

Determination of Accuracy data for OLM and CHL					
Drug	**Level%**	**Added(ug/ml)**	**Found(ug/ml)**	**%Recovery**	**%RSD**
Olmesartan	80	8.1	7.8	98	1.37
	100	10	9.94	99.5	
	120	12.53	12.73	101.6	
Chlorthalidone	80	7.9	7.8	99.2	0.98
	100	10.2	10	98.7	
	120	12.46	12.52	99.9	

Intra-day Precision data of OLM & CHL at 258nm									
For olmesartan and chlorthalidone at 258nm and 214nm at 10-11am, 12- 1pm, and 2-3pm									
Absorbance						Mean ± SD		%RSD	
10-11 am		12-1 pm		2-3 pm					
CHL	OLM	OLM	CHL	OLM	CHL	OLM	CHL	OLM	CHL
0.171	0.667	0.669	0.170	0.668	0.173	0.00808	0.0344	1.2%	1.7%
0.195	0.667	0.674	0.180	0.670	0.189	0.678176	0.216458	0.6%	0.2%
0.239	0.681	0.678	0.220	0.674	0.222	0.673722	0.219654	0.4%	0.2%
0.465	2.626	2.623	0.452	2.620	0.450	0.05963	0.04250	0.5%	1.87%
0.06	2.660	2.623	0.505	2.659	0.500	1.734352	0.520484	1.7%	0.52%
0.550	2.742	2.740	0.509	2.739	0.504	0.83333	0.514104	1.8%	0.51%

Accuracy

The accuracy of the method was determined by calculating recovery of OLM and CHL by the standard addition method.

% Recovery = amount found / amount added x 100

Intraday precision studies: results shown in below (Table 1)

For olmesartan and chlorthalidone at 258nm and 214nm at 10-11am, 12- 1pm, and 2-3pm

Inter day precision

The inter-day precisions of the proposed method was determined by analyzing corresponding responses on 2 different days over a period of 1 week for 2 different concentrations of standard solutions of OLM (20 and 50 µg/ml) with absorbance of 0.110, 0.23 and CHL (2 and 8 µg/ml) with absorbance of 1.60 and 0.5.

Sensitivity

The sensitivity of measurement of OLM and CHL by the use of proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ).

Results of sensitivity data for OLM at 258 and CHL at 214nm: LOD (µg/ml) was 0.474 µg/ml, 0.472 µg/ml respectively & LOQ (µg/ml) was 1.422 µg/ml & 1.461 µg/ml respectively.[13-14]

SIMULTANEOUS EQUATION METHOD AND FIRST ORDER DERIVATIVE METHOD

Results are shown in below (Table 2).

Table 2: Statistical data of OLM and CHL.

Parameters	Simultaneous equation method and first order derivative method			
	OLM at 258nm	OLM at 214nm	CHL at 258nm	CHL at 214nm
Linearity (ug/ml)	20-60	20-60	2-10	2-10
Regression equation	Y=0.0051X+0.0021	Y=0.0235X+0.0329	Y=0.0132X+0.0013	Y=0.0689X+0.013
Slope (SD)	0.01975	0.01295	0.0135	0.059
Intercept (SD)	0.01876	0.0217	0.0514	0.0123
Correlation coefficient (r)	0.9919	0.9951	0.9994	0.9939

EXPERIMENTAL WORK OF HPLC

Chemicals and Standards Used: The study used high-grade chemicals and standards for accurate results, including HPLC- grade water, methanol, acetonitrile, and reagent-grade ortho-phosphoric acid, KH_2PO_4, and K_2HPO_4 from Merck. Filtration materials included a 0.22 µm nylon filter (Advanced Lab) and a 0.45 µm filter paper (Millipore). In-house standards of chlorthalidone and olmesartan were prepared.

Instruments Used: The analysis was conducted using an HPLC-auto sampler with a UV detector (Waters, 2695 model) and Empower software, along with a UV double beam spectrometer (Lab India, UV 3000+ model) and UV Win software. Additional equipment included a digital balance (Ascocet ER 200A), pH meter (ADWA AD 102U), and sonicator (Enertech SE60US).[15]

METHOD DEVELOPMENT FOR THE SIMULTANEOUS ESTIMATION OF CHLORTHALIDONE AND OLMESARTAN BY USING RP-HPLC

1. Selection of mobile phase: pH 3 phosphate buffer: Methanol (70: 30% v/v), Buffer pH should be between 2 to 8., Below 2: siloxane linkages are cleaved, Above 8: dissolution of silica, pH selected: 3 ±0.05

2. Selection of wavelength: 5 mg each of Chlorthalidone and Olmesartan was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Chlorthalidone and Olmesartan. The isosbestic point was taken as detection wavelength.

3. Selection of mobile phase: pH 3 phosphate buffer: Methanol (70 : 30% v/v), Buffer pH should be between 2 to 8., Below 2: siloxane linkages are cleaved, Above 8: dissolution of silica, pH selected: 3 ±0.05

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5. Selection of column: Column is selected based on solubility, polarity and chemical differences among analysts and Column selected i.e. Symmetry C18 column (4.6 x150 mm) 5 µ.

6. Selection of flow rate: Acceptable limit: - Not more than 2.5 ml/min, Flow rate selected was 1ml/min

7. Selection of diluent: Selection of diluent is based on the solubility of the analyte, Diluent selected: Methanol: phosphate buffer pH 3 (70 : 30v/v)

8. Selection of column temperature: Preferable temperature is ambient or room temperature.

9. Selection of test concentration and injection volume: The test concentration selected is 15 ppm and Injection volume selected was 10µL.

PROCEDURE

Preparation of phosphate buffer: 2.95 grams of KH2PO4 and 5.45 grams of K2HPO4 were weighed and taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water and pH was adjusted to 3 with orthophosphoric acid. The resulting solution was sonicated and filtered.

Preparation of mobile phase: Mix a mixture of above buffer 300 ml (30%) and 700 ml of methanol (HPLC grade-70%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration. Mobile phase Buffer: Methanol (30:70) V/V.

Diluents preparation: Mobile phase was used as the diluent.

Preparation of the Chlorthalidone and Olmesartan standard and sample solution: Accurately weigh and transfer 10 mg equivalent weight of Olmesartan and Chlorthalidone Tablet powder into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.6ml of Olmesartan & Chlorthalidone the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Standard solution preparation: Accurately weigh and transfer 12.5 mg & 8 mg of Olmesartan and Chlorthalidone working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution: Further pipette 0.6ml of Olmesartan & Chlorthalidone the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure: 10 µl of the blank, standard and sample were injected into the chromatographic system and areas for the Chlorthalidone and Olmesartan the peaks were used for calculating the % assay by using the formulae.[16-17]

RESULTS AND DISCUSSIONS

System suitability: Accurately weigh and transfer 12.5 mg &8 mg of Olmesartan and Chlorthalidone working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.6ml of Olmesartan & Chlorthalidone the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent as seen in (table 3).[18]

Table 3: Results for System Suitability of OLM & CHL

Injection	RT (min)		Peak area		TP		TF	
1	3.901	2.5263	434308	124652	4315.31	2554.31	1.17	1.28
2	4.016	2.767	436736	127376	4232.73	2634.55	1.17	1.31
3	4.012	2.764	436821	122803	4372.54	2623.37	1.17	1.31
4	4.140	2.808	435350	125382	4354.17	2622.73	1.17	1.23
5	4.077	2.789	425462	122153	4322.22	2460.39	1.17	1.32
6	4.056	2.799	438085	126345	4328.19	2634.88	1.18	1.27
Mean			44531.3	123634	-	-	-	-
SD			1257.3	631.0	-	-	-	-
%RSD			0.3	0.6	-	-	-	-

VALIDATION PARAMETERS

Specificity:

The specificity test was performed for Chlorthalidone and Olmesartan. It was found that there was no interference of impurities in retention time of analytical peak.

Linearity

Stock Solution Preparation: Dissolve 5 mg each of Olmesartan and Chlorthalidone in 7 mL diluent, sonicate, and dilute to 10 ml.

Levels:

Level I: 25 ppm Olmesartan, 16 ppm Chlorthalidone (0.2 mL stock in 10 mL diluent)

Level II: 50 ppm Olmesartan, 32 ppm Chlorthalidone (0.4 mL stock in 10 mL diluent)

Level III: 75 ppm Olmesartan, 48 ppm Chlorthalidone (0.6 mL stock in 10 mL diluent)

Level IV: 100 ppm Olmesartan, 64 ppm Chlorthalidone (0.8 mL stock in 10 mL diluent)

Level V: 125 ppm Olmesartan, 80 ppm Chlorthalidone (1.0 mL stock in 10 mL diluent)

Procedure: Inject each level, measure peak area, and plot concentration vs. peak area for correlation coefficient.

Criteria: Correlation coefficient ≥ 0.999. as seen in (figure 1).

Accuracy

The accuracy study was performed for 50%, 100% and 150 % for Chlorthalidone and

Olmesartan. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery, shown in (table 4).

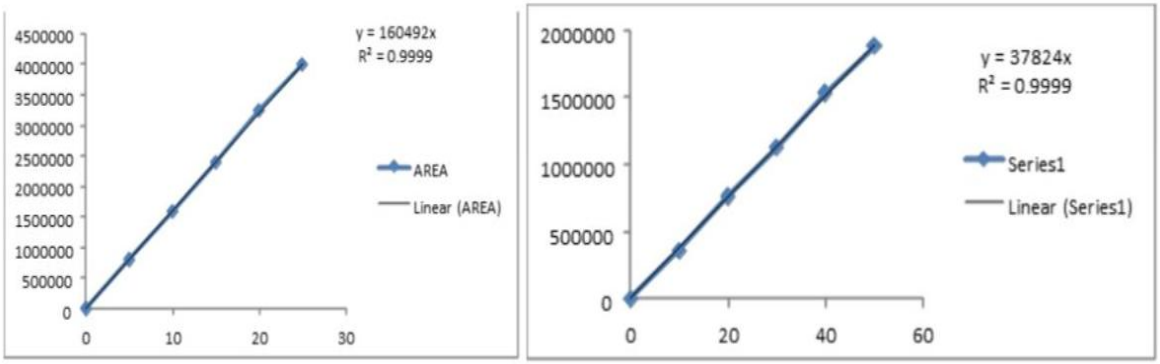

Figure 1: Showing calibration graph for Chlorthalidone and Olmesartan

Table 4: Accuracy results for Olmesartan & Chlorthalidone

%Conc	Area		Amount Added (mg)		Amount Found (mg)		% Recovery		Mean Recovery	
50%	1726242	765624	7.05	4.25	7.1	4.30	101.9%	101.2%	101.7%	101.4%
100%	3187170	1508055	13.1	8.25	13.2	8.48	101.3%	101.5%		
150%	4521881	2204983	18.5	12.2	18.8	12.39	101.8%	101.6%		

Repeatability

Preparation of stock solution

Accurately weigh and transfer 12.5 mg & 8 mg of Olmesartan and Chlorthalidone working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.6ml of Olmesartan & Chlorthalidone the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent

Procedure: The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits and the intermediate precision was performed for %RSD of Chlorthalidone and Olmesartan was found to be 1.99and 1.46 respectively (NMT 2) results are shown in (table 5).

Acceptance criteria

The % RSD for the area of five standard injections results should not be more than 2.

Table 5: %RSD results for Olmesartan & Chlorthalidone

Injection	Area	
	OLM	CHL
Injection-1	3045768	1475698
Injection-2	3030853	1461561
Injection-3	3063519	1481379
Injection-4	3065127	1467049
Injection-5	3099001	1472628
Average	3060854	1471663
Standard Deviation	25535.28	7664.08
%RSD	0.83	0.52

Results for Intermediate Precision of Chlorthalidone		
Injection	**Area**	**Injection**
	Concentration 15ppm	
	CHL	**OLM**
Injection-1	1419430	3098177
Injection-2	1437396	3075703
Injection-3	1461998	3135114
Injection-4	1484335	3173644
Injection-5	1486671	3179888
Injection-6	1488969	3184696
Average	1463133.2	3141203.7
Standard Deviation	29136.557	46085.033
%RSD	1.99%	1.46

The method precision study was performed for chlorthalidone and olmesartan precision of the analytical method was found to be 0.8 and 0.5 percent respectively (NMT 2).

Intermediate Precision/Ruggedness

To evaluate the intermediate precision (also known as ruggedness) of the method, precision was performed on different days by using different make column of same dimensions.[19]

Limit of detection (LOD): Limit of Detection was performed for Chlorthalidone and Olmesartan was found to be 2.85 µg/ml and 0.0372 µg/ml respectively. The result obtained is within the limit.

Limit of quantification (LOQ): The LOQ was performed for Chlorthalidone and Olmesartan was found to be 5ug/ml for both respectively. results for both LOD, LOQ are shown in (table 6).[20]

Table 6: Results of LOD & LOQ

S.NO	Name	Retention Time (min)	Area (µV*sec)	Peak Height
1	Chlorthalidone	2.184	1236	142
2	Olmesartan	7.50	1087	162

Drug name	Baseline noise (µV)	Signal obtained (µV)	Concentration
Chlorthalidone	31	121	2.85
Olmesartan	31	121	4.03

Results of LOQ			
Drug name	**Baseline noise (µV)**	**Signal obtained (µV)**	**Concentration**
Chlorthalidone	31	404	5.0
Olmesartan	31	413	5.0

COMPARATIVE STUDY BETWEEN UV ANALYTICAL METHODS & RP-HPLC

Results are shown in below (Table 7).

Table 7: Comparative study between UV analytical methods & RP-HPLC

UV Spectroscopy Simultaneous Equation Method			
	Linearity	Accuracy % Recovery	Assay
Chlorthalidone	0.991	99.9%	99.20%
Olmesartan	0.993	101.6%	99.02%
UV Spectroscopy First Order Derivative Spectroscopy			
	Linearity	Accuracy % Recovery	Assay
Chlorthalidone	**0.991**	**99.9%**	**99.20%**
Olmesartan	**0.993**	**101.6%**	**99.02%**
RP-HPLC Simultaneous Equation Method			
	Linearity	Accuracy % Recovery	Assay
Chlorthalidone	**0.999**	**101.7%**	**99.1%**
Olmesartan	**0.999**	**101.4%**	**98.2%**

CONCLUSION

This study successfully developed and validated both UV Spectroscopy and RP-HPLC methods for simultaneous estimation of Olmesartan and Chlorthalidone in pharmaceutical formulations. UV Spectroscopy was found to be a quick, cost-effective option suitable for routine quality control with good linearity and precision, while RP-HPLC offered greater sensitivity, specificity, and stability insights, making it ideal for in-depth R&D. The research underscores the importance of selecting appropriate analytical techniques based on specific needs and resources, supporting optimized quality control and contributing to improved patient outcomes in combination therapies.

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REFERENCES

1. Sawale V, Dhabarde DM, Kar Mahapatra D. Development and validation of UV spectrophotometric method for simultaneous estimation of olmesartan medoxomil and chlorthalidone in bulk and tablet. Eurasian J Anal Chem 2017; 12(1):55-66. DOI: 10.12973/ejac.2017.00144a.
2. Haque, A. M., Nivedita, G., Kumar, P. K., Amrohi, H. S., & Diwan, P. V. Simultaneous estimation of atenolol and chlorthalidone as bulk and in tablet dosage form using UV-spectrophotometry. IOSR Journal of Pharmacy and Biological Sciences, 2012;1:20-23.
3. Patel J, Garala K, Patel A, Raval M. Sheth N, Development of the UV spectrophotometric method of Olmesartan medoxomil in bulk drug and pharmaceutical formulation and stress degradation studies, Pharmaceutical Methods, 2011;2(1):36-41, ISSN No: 2229-4708.
4. Sharma T, Sudam Chandra S, Gowrishankar D, Rapid and selective UV Spectrophotometric method for the analysis of Olmesartan medoxomil in bulk and dosage form, International Journal of Drug Delivery, 2012;4(1):134-138, ISSN No:0975-0215.

5. Hemke AT, Bhure MV. Chouhan KS, Gupta KR and Wadodkar SG, UV Spectrophotometric Determination of Hydrochlorothiazide and Olmesartan Medoxomil in Pharmaceutical Formulation, E-Journal of Chemistry, 2010;7(4):1156-1161, 2090-9071.
6. Verma PK, Kamboj Vk, Ranjan S (2010). Spectrophotometric Estimation of Olmesartan Medoxomil in Tablet Dosage Form with Stability Studies, International Journal of Chem Tech Research, 2010;2(2):1129-1134, ISSN No:2455-9555.
7. Wankhede SB, Wadkar SB, Raka KC, Chitlange SS (2009), Simultaneous estimation of Amlodipine besilate and Olmesartan medoxomil in pharmaceutical dosage form, Indian Journal of Pharmaceutical Sciences, 2009;71(5):563-567.
8. Niraimathi V, Jerad SJ, Senthil, UV Spectrophotometric Methods for the Estimation of Chlorthalidone in Bulk and Oral Dosage Form, Indo American Journal of Pharmaceutical Research, 2013;3(9):7160-7167, ISSN No: 2231-6876.
9. Padmane SP, Jain ND, Ittadwar AM and Walde S, A Derivative UV- Spectrophotometric Method for the Simultaneous Determination of Metoprolol Succinate and Chlorthalidone in Combined Dose Tablet, International Journal of Analytical and Bio analytical Chemistry, 2014;4(1):33-41.
10. Barot D, Pradhan PK, Patel G, Shah S. Parmar HP, Dey S. Upadhyay UM, Simultaneous UV spectrophotometric estimation of Olmesartan medoxomil and Chlorthalidone in tablet dosage Form, The Pharma Innovation Journal, 2014;3(10):50-56.
11. Patel S, Patel D, Simultaneous Determination Of Metoprolol Succinate and Chlorthalidone by UV Spectrophotometric Method, Pharmagene, 2013;1(3):39- 43. ISSN No: 2231-6876.
12. Raval HR, Patel DM and Patel CN, Estimation of Metoprolol Tartrate and Chlorthalidone in Combined Dosage Form by UV-Spectrophotometric Methods, Research Journal of pharmacy and Technology, 2011;4(6):1132-1134, ISSN No: 0974- 3618.
13. Ganduri MR. Lanka RA. New RP-HPLC method for the determination of Olmesartan medoxomil in tablet dosage form, Eurasian Journal of Analytical Chemistry, 2010;5(2):145-151, ISSN No: 1306-3057.
14. Patil PS, More HN, Pishwikar SA, RP-HPLC Method For Simultaneous Estimation of Amlodipine Besylate And Olmesartan Medoxomil from Tablet, International Journal of Pharmacy and Pharmaceutical Sciences, 2011;3(3):146-149, ISSN No: 0975-1491.
15. Birajdar, Arunadevi S. Meyyanathan SN, Suresh B, Validated reversed phase HPLC method for the determination of Olmesartan medoxomil in combination with Hydrochlorothiazide, Saudi Pharmaceutical Journal, 2009;17(2):189-194.
16. Singh B. Patel DK. Gosh SK, A reversed phase high performance liquid chromatography for determination of Chlorthalidone in pharmaceutical formulation, International Journal of Pharmacy and Pharmaceutical Sciences, 2009;1(2):24-29, ISSN No: 0975-1491.
17. Santosh Kumar Bhardwaj et al, A Review: HPLC Method Development and Validation, International Journal of Analytical and Bioanalytical Chemistry, 2015;2(1) pg 1-25.
18. Vibha G et al., Development and validation of HPLC method a review. International Research Journal of Pharmaceutical and Applied Sciences. 2012;2(4) pg 22-23.
19. Bliesner DM. In: Validating Chromatographic Methods. John Wiley & sons Inc. 2006, pg 88- 92.
20. Mohammad Tet al., HPLC Method Development and Validation for Pharmaceutical Analysis- A Review. International Pharmaceutical Sciences, 2012;2(3) pg 14-21.