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Development and validation of reversed phase gradient HPLC method for the simultaneous estimation of olmesartan medoxomil and chlorthalidone in dosage forms

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ABSTRACT

The main objective of the proposed study is to develop and validate a new stability indicating reverse phase HPLC gradient method for the simultaneous estimation of olmesartan medoxomil and chlorthalidone in combined dosage form. The method is optimized by using zorbax phenyl column (250 × 4.6 mm, 5 µ). For pump-A, ammonium dihydrogen orthophosphate and pump-B, acetonitrile: are used. Phosphate buffer pH was adjusted with orthophosphoric acid to 3.0. Water and acetonitrile (45:50) are used as diluent. The flow rate is 1.5 ml/min and the elutant is monitored at 220 nm with uv detector. The retention time of olmesatran medoxomil and chlorthalidone are 10.70 ±0.1 mins and 4.8 ± 0.1 mins respectively. Precision shows that % Relative standard deviation of olmesartan medoxomil and chlorthalidone is about 0.23 and 0.56 respectively. The percentage recoveries of both the drugs olmesartan medoxomil and chlorthalidone from the dosage formulation are 100.3% and 99.20% respectively. Linearity of olmesartan medoxomil and chlorthalidone is in the range of 10.0 to 60.0 µg/ml and 6.25 µg/ml respectively. Calibration curve shows good linearity and range. The correlation coefficient of olmesartan medoxomil and chlorthalidone is 0.999. And the results obtained for Robustness and Ruggedness are well within the acceptance criteria. The proposed method is found to be simple, rapid, accurate and precise. It is found to be economical and suitable for simultaneous determination of olmesartan medoxomil and chlorthalidone in pharmaceutical dosage form.

***Keywords*:** Olmesartan medoxomil, Chlorthalidone, RP-gradient HPLC, Simultaneous estimation, Buffer pH 3.0, Acetonitrile, Linearity, Precision, Accuracy, Robustness, Forced degradation

1. INTRODUCTION

Olmesartan medoxomil and chlorthalidone dose combination is found to show superior antihypertensive efficacy in blood pressure reduction in patients with stage 2 hypertension when compared with the maximum approved dose of olmesartan [1]. Medoxomil is an angiotensin II receptor antagonist which has the chemical name [(5-Methyl-2-oxo-1-3-dioxol-4-yl) methyl 4-(2-hydroxy-2-propanyl)-2-propyl-1-{[2'-(2H-tetrazol-5-yl)-4-biphenylyl]methyl}-1H-imidazole-5-carboxylate (Fig. 1) it may be used alone or in combination olmesartan/chlorthalidone. Olmesartan medoxomil is an angiotensin II receptor antagonist which has the chemical name (5-Methyl-2-oxo-1,3-dioxol-4-yl) methyl 2-ethoxy-1-{[2'-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]methyl}-1H-benzimidazole-7-carboxylate monopotassium salt. It is a white crystalline powder which is practically insoluble in water, freely soluble in methanol, dimethyl sulfoxide and dimethyl formamide, soluble in acetic acid, slightly soluble in acetone and acetonitrile and very slightly soluble in tetrahydrofuran and 1-octanol.

Chlorthalidone [2-chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1H-isoindol-1-yl)benzene-1-sulfonamide] (Fig. 2), is a diuretic drug used to treat hypertension [2]. CHT increases the excretion of sodium, chloride and water into the renal lumen by inhibiting sodium ion transport across the renal tubular epithelium. Olmesartan Medoxomil can be used alone or with other antihypertensive agents. OLM is a prodrug that works by blocking the binding of angiotensin II to the AT1receptors in vascular muscle. The review of literature revealed that various analytical methods involving UV spectrophotometry, HPLC, potentiometry and Electrophoresis have been reported for CHT in single form and in combination with other drugs [3-5]. Several analytical methods have been reported for OLM in single form and in combination with other drugs for combination of olmesartan and chlorthalidone [6,7]. Therefore the present research work aims to develop a simple, sensitive, accurate and reproducible method for simultaneous estimation of chlorthalidone and olmesartan medoxomil combined dosage form by HPLC method.

It is US FDA approved as edarby tablets on 25th Feb 2011, to treat hypertension in adults. It is available in 40mg and 80 mg dosages, with the recommended dosage at 80mg once in a day. The active moiety of olmesartan medoxomil is released by hydrolysis of medoxomil ester. It is an active ARB (AT1) type and is more effective in lowering blood pressure within 24 hours as compared to other ARBs. Olmesartan Medoxomil an ARB is combined with chlorthalidone, a thiazide type diuretic in treating hypertension significantly when compared to other fixed dose antihypertensive combination without the difference in safety measurements. Chlorthalidone is practically insoluble in water, ether and chloroform, soluble in methanol and slightly soluble in alcohol. It is a thiazide type diuretic used to treat hypertension. It acts similarly to the thiazides in causing diuresis but does not have benzothiadiazine moiety in it. It acts at the proximal portion of the distal convoluted tubule of the nephron and shows longest duration of action when compared to other thiazide diuretics. The literature survey shows that spectroscopic and chromatographic methods for individual

drugs but there is only a single method available for quantitation of olmesartan medoxomil and chlorthalidone in solid dosage forms simultaneously. Thus it is inevitable to develop such a sensitive, accurate, precise, rapid and economical method for routine analysis of this combination in pharmaceutical dosage.

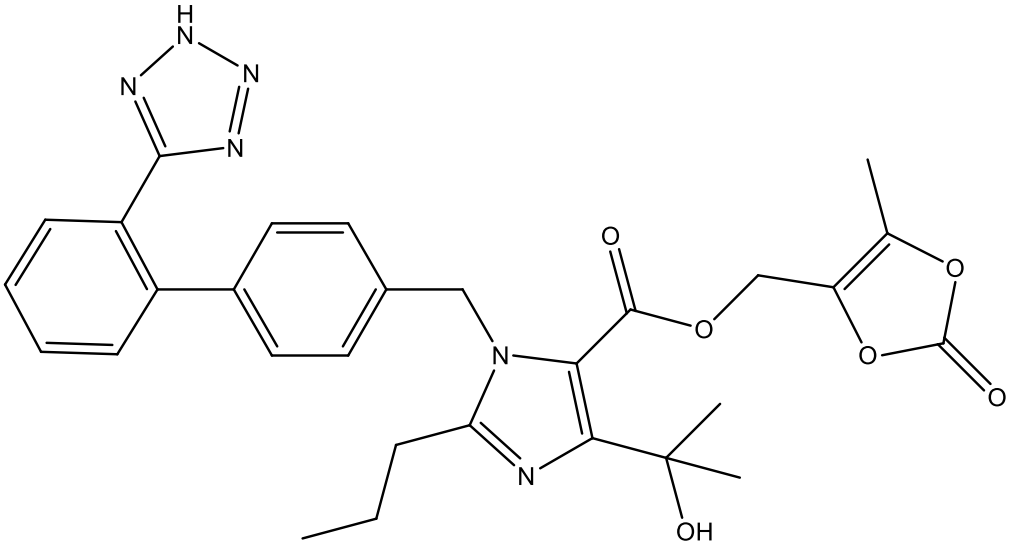

Fig. 1. Structure of olmesartan medoxomil.

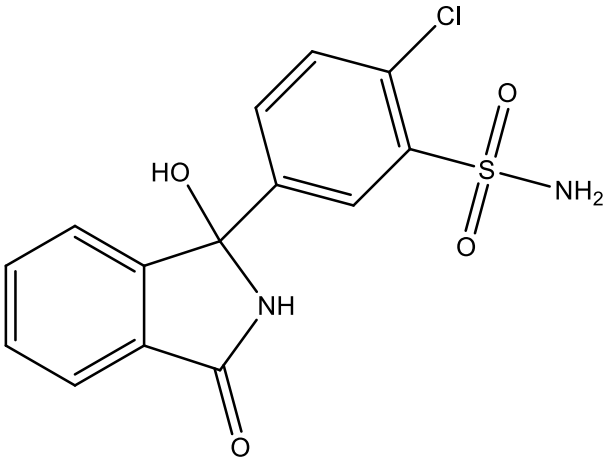

Fig. 2. Structure of chlorthalidone.

It is US FDA approved as edarby tablets on 25th Feb 2011, to treat hypertension in adults. It is available in 40 mg and 80 mg dosages, with the recommended dosage at 80 mg once in a day. The active moiety of olmesartan medoxomil is released by hydrolysis of

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2. MATERIALS AND METHODS

2. 1. Instrumentation

A high performance liquid chromatography system consisting of Waters 2695 Separation (Alliance) Module with UV detector was used with data handling system Empower. Chemicals were weighed using Analytical balance, Sartorius and LC GC, all pH measurements were done on pH meter. Reagents, Chemicals, HPLC grade solvents and orthophosphoric acid and acetonitrile were obtained from Merck Specialities Pvt Ltd, India. Water was deionized and further purified by means of Milli-Q water purification system, Millipore Ltd (U.S.A). AR grade ammonium dihydrogen orthophosphate was obtained from Rankem Pharmaceuticals India Ltd. Olmesartan Medoxomil and Chlorthalidone were obtained as pure standards and samples [tablets of Olmesartan Medoxomil (40 mg) and Chlorthalidone (6.25 mg)] from Swiss garnier, Himachel pradesh, India.

2. 2. Chromatographic conditions and measurement procedure

Column	:	Zorbax Phenyl (250 x 4.6 mm), 5 µ ,
Column oven temperature	:	25 °C
Sample cooler temperature	:	5 °C,
Flow Rate	:	1.5 ml/min,
Wave Length	:	220 nm.
Injection volume	:	20 µL

2. 3. Preparation of buffer (pH 3.0)

Accurately weighed and transferred 2.0 gm of ammonium dihydrogen arthophosphate in a 1000 ml of volumetric flask, with 900 ml of milli-Q water, sonicated to degas and finally

made up the volume with water. Then pH was adjusted to 3.0 with dil. ortho phosphoric acid solution. The solution was filtered through 0.45 μm membrane filter.
Preparation of mobile phase A: Buffer (pH 3.0) was used as mobile phase A. Preparation of mobile phase B: Acetonitrile was used as mobile phase B.
Preparation of diluent: Degassed mixture of water and acetonitrile in the ratio of 50:50 was used as diluent.

2. 4. Standard stock preparation (a)

Weighed accurately about 50 mg of olmesartan medoxomil working standard to a 50 ml volumetric flask. Added about 30 ml of diluent, sonicated to dissolve and made up to volume with diluent.

2. 5. Standard stock preparation (b)

Weighed accurately about 31 mg of chlorthalidone working standard to a 100 ml volumetric flask. Added about 70 ml of diluent, sonicated to dissolve and made up to volume with diluent.

2. 6. Standard preparation

About 5 ml of standard stock preparation (a) and standard stock preparation (b) were made up to volume with 100 ml volumetric flask.

2. 7. Sample preparation

Accurately weighed and transferred 5 tablets to a 100 ml volumetric flask. Added 70 ml of diluent, sonicated for 20 minutes (maintaining the temp NMT 10 °C) to dissolve and made up to volume with diluent. Centrifuged at 4000 rpm for 10 min. Diluted 5 mL of the supernatant solution to 100 mL with diluent.

2. 8. Gradient programme

The Gradient parameter programme was tabulated in Table 1. In this table each time period the percentage of mobile phase A and B given.

Table 1. Gradient parameter of mobile phase A and B.

Time (min)	Mobile phase - A%	Mobile phase - B%
0.01	76	24
7.00	76	24
7.01	55	45
13.00	55	45
13.01	76	24
18.00	76	24

2. 9. Evaluation of system suitability

The prepared standards were injected into the chromatograph and recorded the chromatograms

The system is suitable for analysis, if;

1. The relative standard deviation for five replicate injections is not more than 2.0 %.
2. The tailing factor is between 0.8 and 1.5
3. The column efficiency determined is not less than 1900 theoretical plates.

2. 9. 1. Procedure

The prepared sample was injected into chromatograph and chromatograms were recorded

2. 9. 2. Calculation

The percentage content of Olmesartan medoxomil in tablet was calculated by using following formula

$$\frac{A_1 \times WS \times 5 \times 100 \times 100 \times P \times 100}{A_2 \times 25 \times 100 \times SW \times 5 \times 100 \times LC} \times WA$$

Where

A_1 = Average peak area of sample preparation; A_2 = Average peak area of standard preparation; WS = Weight of standard taken; SW = Weight of sample taken; P = % purity of standard (on as is basis); WA = Average weight of tablets in mg; LC = Label claim.

The percentage content of chlorthalidone in tablet was calculated by using following formula

$$\frac{A_1 \times WS \times 5 \times 100 \times 100 \times P \times 100}{A_2 \times 100 \times 100 \times SW \times 5 \times 100 \times LC} \times WA$$

where

A_1 = Average peak area of sample preparation; A_2 = Average peak area of standard preparation; WS = Weight of standard taken; SW = Weight of sample taken; P = % purity of standard (as such); WA= Average weight of tablets in mg; LC = Label claim.

Using the chromatographic conditions, various trials were made for the determination of assay. Each trial mixture of known components were injected and observed for resolution and tailing factor of the peaks. Different flow rates of the mobile phase were tried for good resolution. Both the drugs olmesartan medoxomil and chlorthalidone were found to be soluble and stable in a mixture of buffer pH 3.0 and acetonitrile. Finally the chromatographic conditions were optimized at flow rate 1.50 ml/min, injection volume of 20 µL, run time of

18 minutes, at column oven temp 25 °C with acetonitrile and water in the ratio(50:50) (sonicated and degassed) as diluents, in a zorbax phenyl (250 x 4.6 mm), 5 µ column. The %RSD for both the drugs olmesartan medoxomil and chlorthalidone were found to be 0.2 and 0.5 respectively and tailing factor was less than 1.5. The retention time for olmesartan medoxomil and chlorthalidone was found to be 10.7 minutes and 4.8 minutes respectively. Absorption maximum was found to be 220 nm. Shapes of peaks were good. The method was further validated under the chromatographic conditions.

3. RESULTS AND DISCUSSION

3. 1. Validation parameters

3. 1. 1. Specificity and system suitability

The validation and specificity and system suitability parameters of Olmesartan peak and Chlorthalidone peak [8-11] were tabulated in Table 2. From the table, the various parameter related to the specificity and suitability of HPLC analysis of Olmesartan peak and Chlorthalidone peak tablets given respected to acceptance criteria.

Table 2. Validation parameters.

<table>
<tr><th>Validation Parameter</th><th colspan="2">Results</th><th colspan="2">Acceptance Criteria</th></tr>
<tr><td colspan="5">Specificity and system suitability:</td></tr>
<tr><td rowspan="2">RT of Olmesartan peak</td><td>Standard</td><td>Sample</td><td colspan="2" rowspan="4">Standard and sample were comparable with respect to RT.</td></tr>
<tr><td>10.74min</td><td>10.74 min.</td></tr>
<tr><td rowspan="2">RT of Chlorthalidone peak</td><td>Standard</td><td>Sample</td></tr>
<tr><td>4.87 min.</td><td>4.88 min</td></tr>
<tr><td colspan="5">Blank Interference:</td></tr>
<tr><td>At RT of Olmesartan peak</td><td colspan="2">No peak observed at the RT of olmesartan</td><td colspan="2">Blank not showed no interference at the RT of Olmesartan peak.</td></tr>
<tr><td>At RT of Chlorthalidone peak</td><td colspan="2">No peak observed at the RT of ohlorthalidone</td><td colspan="2">Blank not showed no interference at the RT of Chlorthalidone peak.</td></tr>
<tr><td colspan="5">System Suitability:</td></tr>
<tr><td rowspan="2">Olmesartan</td><td>Theoretical Plates</td><td>25129</td><td colspan="2">NLT 10000</td></tr>
<tr><td>Tailing factor</td><td>0.9</td><td colspan="2">Between 0.8 and 1.5</td></tr>
</table>

	The relative standard deviation for five replicate injections	0.12	The relative standard deviation for five replicate injections is not more than 2.0 %.
Chlorthalidone	Theoretical Plates	2619	NLT 2000
	Tailing factor	0.9	Between 0.8 and 1.5
	The relative standard deviation for five replicate injections	0.25	The relative standard deviation for five replicate injections is not more than 2.0 %.

3. 1. 2. Linearity

The linearity of Olmesartan peak and Chlorthalidone peak tablets given respected to acceptance criteria. Here the observed correlation coefficient was 0.999 and is shown in Table 3.

Table 3. Linear analysis of Olmesartan peak and Chlorthalidone peak tablets.

Name	Results		Acceptance Criteria
Olmesartan	Correlation coefficient	0.999	Correlation coefficient should be NLT 0.99
	% y intercept	-0.547	%y intercept should be in between ±2.0
Chlorthalidone	Correlation coefficient	0.999	Correlation coefficient should be NLT 0.99
	% y intercept	-1.199	%y intercept should be in between ±2.0

3. 1. 3. Precision

The precision analysis of Olmesartan peak and Chlorthalidone peak tablets given respected to acceptance criteria. Here the observed precision was 0.64 and 1.999 and is shown in Table 4.

Table 4. Precision analysis of Olmesartan peak and Chlorthalidone peak tablets.

Parmeter	Results		Acceptance Criteria
System Precision:			
Olmesartan	Theoretical Plates	26995	NLT 10000
	Tailing factor	0.91	Between0.8 and 1.2

	The relative standard deviation for five replicate injections	0.26	The relative standard deviation for five replicate injections is not more than 2.0 %.
Chlorthalidone	Theoretical Plates	2817	NLT 2000
	Tailing factor	0.67	NMT 2.0
	The relative standard deviation for five replicate injections	0.43	The relative standard deviation for five replicate injections is not more than 2.0 %
Method Precision:			
Olmesartan	The %RSD of Assay from six sample preparation	0.64	The %RSD of Assay from six sample preparation should be NMT 2.0
Chlorthalidone	The %RSD of Assay from six sample preparation	1.00	

3. 1. 4. Intermediate precision

The intermediate precision analysis of Olmesartan peak and Chlorthalidone peak tablets given respected to acceptance criteria. Here the observed precision was shown in Table 5.

Table 5. Precision analysis of Olmesartan peak and Chlorthalidone peak tablets.

Validation Parameter	**Results**		**Acceptance Criteria**
System Precision:			
Olmesartan	Theoretical Plates	32012	NLT 10000
	Tailing factor	1.1	Between 0.8 and 1.2
	The relative standard deviation for five replicate injections	0.57	The relative standard deviation for five replicate injections is not more than 2.0 %.
Chlorthalidone	Theoretical Plates	3517	NLT 2000
	Tailing factor	1.05	Between 0.8 and 1.2
	The relative standard deviation for five replicate injections	0.61	The relative standard deviation for five replicate injections are not more than 2.0 %.

3. 1. 5. Accuracy and range

The accuracy and range analysis of Olmesartan peak and Chlorthalidone peak tablets given respected to acceptance criteria. Here the observed accuracy and range was shown in Table 6.

Table 6. The accuracy and range analysis of Olmesartan peak and Chlorthalidone peak tablets.

Validation Parameter	**Results**	**Acceptance Criteria**
% Mean Accuracy (from 80% to 120% of the target concentration)		
Olmesartan	100.23	The % recovery should be in between 98.0 to 102.0.
Chlorthalidone	98.79	
Validation Parameter	Results	Acceptance Criteria
Range	80% to 120% of the target concentration	---

3. 1. 6. Solution stability

The solution stability of Olmesartan peak and Chlorthalidone peak tablets given respected to acceptance criteria. Here the observed data was shown in Table 7.

Table 7. The solution stability analysis of Olmesartan peak and Chlorthalidone peak tablets.

Validation Parameter					**Acceptance Criteria**
Olmesartan	Parameter	Initial	12^{th} Hour	24^{th} Hour	
	Theoretical Plates	21269	21756	21221	NLT 10000
	Tailing factor	0.95	0.91	0.97	Between 0.8 and 1.2
	The relative standard replicate injections for five replicate injections	0.35	0.29	0.46	The relative standard deviation for five replicate injections and bracketing together was not more than 2.0 %

Chlorthalidone	Theoretical Plates	2517	2439	2388	NLT 2000
	Tailing factor	0.97	1.01	1.01	Between 0.8 and 1.2
	The relative standard deviation for five replicate injections	0.32	0.43	0.67	The relative standard deviation for five replicate injections and bracketing together was not more than 2.0 %.

3. 1. 7. Robustness

The robustness analysis of Olmesartan peak and Chlorthalidone peak tablets given respected to acceptance criteria. Here the observed data was shown in Table 7.

Table 7. The robustness analysis of Olmesartan peak and Chlorthalidone peak tablets.

Validation Parameter	**Results**		**Acceptance Criteria**
Robustness:			
Filter variability:			
Olmesartan	Centrifuged Vs Nylon	Centrifuged Vs PVDF	---
	0.25%	0.63	The % Assay difference should be NMT 2.0.
Chlorthalidone	0.93%	0.05	
Flow variation:			
Olmesartan	1.35mL/min.	1.65mL/min.	---
	1.23%	1.47%	The % Assay difference should be NMT 2.0.
Chlorthalidone	0.49	1.32%	
Temperature variation:			
Olmesartan	22.5 ºC	27.5 ºC	---
	0.80%	0.86%	The % Assay difference should be NMT 2.0.
Chlorthalidone	0.24%	1.24%	

3. 1. 8. Forced degradation

The forced degradation analysis of Olmesartan peak and Chlorthalidone peak tablets given respected to acceptance criteria. Here the observed data was shown in Table 8.

Table 8. The forced degradation analysis of Olmesartan peak and Chlorthalidone peak tablets.

Validation Parameter	**Results**		**Acceptance Criteria**
Forced Degradation			
Type of Stress	---		---
Acid Stressed (5ml of 2N HCL stay for 30 min.)	Drug Substance	Complies	Drug Substance: Peak should be uniform and there should not be co eluting peaks. Sample: % Assay degradation Should be NMT 10.0%
	Sample	Complies	
Base Stressed (5 ml of 2N NaOH stay for 30 min.)	Drug Substance	Complies	
	Sample	Complies	
Peroxide Stressed (5ml of H_2O_2 stay for 30 min.)	Drug Substance	Complies	
	Sample	Complies	

3. 2. Validation data

3. 2. 1. Specificity

In this section the specificity of the two tablets are analyzed and is presented in Table 9. From the table, there was no interference observed due to blank at the retention time of Olmesartan and Chlorthalidone peak. The theoretical plate for Olmesartan is 27123 and for Chlorthalidone is 2617. The tailing factor for Olmesartan is 0.71 and for Chlorthalidone is 0.41. The relative standard deviation for five replicate injections for Olmesartan is 0.12 and for Chlorthalidone is 0.25. From the chromatogram of the standard and sample, it was concluded that the standard and sample were comparable with respect to retention time. From blank and placebo chromatograms, it was concluded that no interference at the retention time of Olmesartan and Chlorthalidone peak. Hence it could be concluded that no interference due to blank and placebo for the determination of assay of Olmesartan and Chlorthalidone Tablets. Hence the method was specific.

Table 9. Specificity analysis of the two tablets.

Procedure
Blank and interference: Prepared blank solutions as per the test method and injected into the chromatographic system.
System suitability: Prepared system suitability solution and standard solution as per the test method and injected into the chromatographic system.
Result

Identification:
Acceptance Criteria:
The standard and sample solution should be comparable with respect to retention time.

Type of Chromatogram	RT of Olmesartan Peak	RT of Chlorthalidone Peak
Standard	10.74	4.87
Sample	10.74	4.88

Result: The standard and sample were comparable with respect to retention time.

Blank interference:
Acceptance criteria: Blank should not show any interference at the retention time of Olmesartan and Chlorthalidone peak.

Type of Chromatogram	Area of peak observed at the retention time of Olmesartan	Area of peak observed at the retention time of Chlorthalidone
Blank	0.000	0.000
Placebo	0.000	0.000

Observation

Injection No.	Area		Tailing Factor		Theoretical Plate	
	Olmesartan	Chlorthalidone	Olmesartan	Chlorthalidone	Olmesartan	Chlorthalidone
01	1983193	1012121	0.71	0.41	27123	2617
02	1979123	1013171				
03	1977129	1012179				
04	1979301	1012178				
05	1977923	1017911				
Average	1979333.8	1013512				
SD	2334	2498				
%RSD	0.12	0.25				

3. 2. 2. Linearity

The linear regression analysis [12-15] of the two tablets is presented in Table 10. From the table the observed correlation coefficients were 0.999 and 0.998 and are good. The single plots are shown in **Figs 3** and **4**.

Table 10. The linear regression analysis results of two tablets.

Method/Procedure
Series of solutions were prepared by using Olmesartan medoxomil and Chlorthalidone working standard at concentration level from 80% to 120% of the target concentration and injected (three replicate) into the chromatographic system. Acceptances criteria: Correlation coefficient should be NLT 0.999 %y intercept should be between ±2.0

Level	Concentration (ppm)		Average area		Statistical Analysis		
---	Olmesartan	Chlorthalidone	Olmesartan	Chlorthalidone	Parameter	Olmesartan	Chlorthalidone
80% conc. Level	40.1	12.0	1559123	771211	Correlation Coefficient	0.999	0.998
90% conc. Level	45.1	13.4	1766129	921219	%y intercept	0.5471	-1.199
100% conc. Level	50.1	15.01	1972129	1010171			
110% conc. Level	55.4	16.3	2162323	1110121	---	---	---
120% conc. Level	60.2	18.7	2379129	1275670			

Result:
For Olmesartan: Correlation Coefficient: 0.999; %y intercept : -0.547
For Chlorthalidone: Correlation Coefficient : 0.998; %y intercept: -1.199

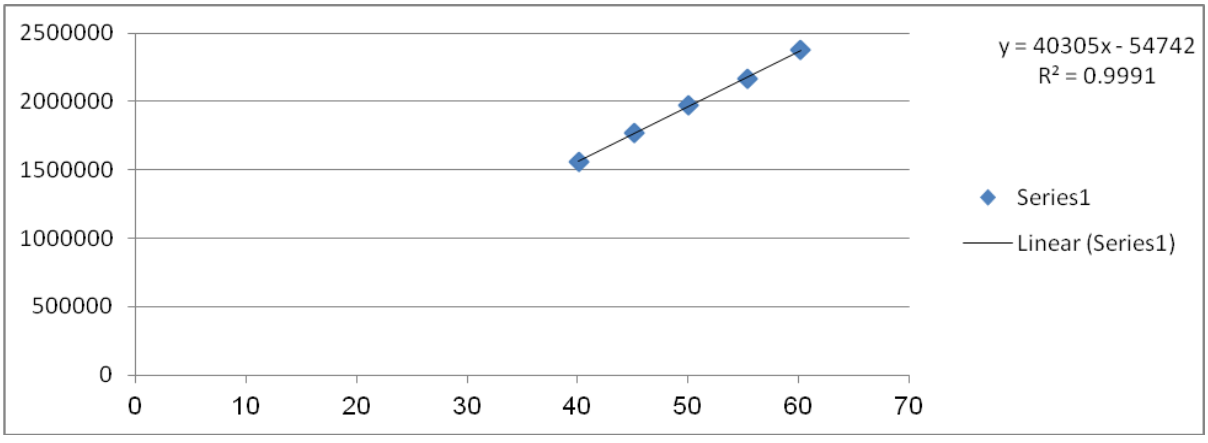

Fig. 3. Olmesartan linearity plot.

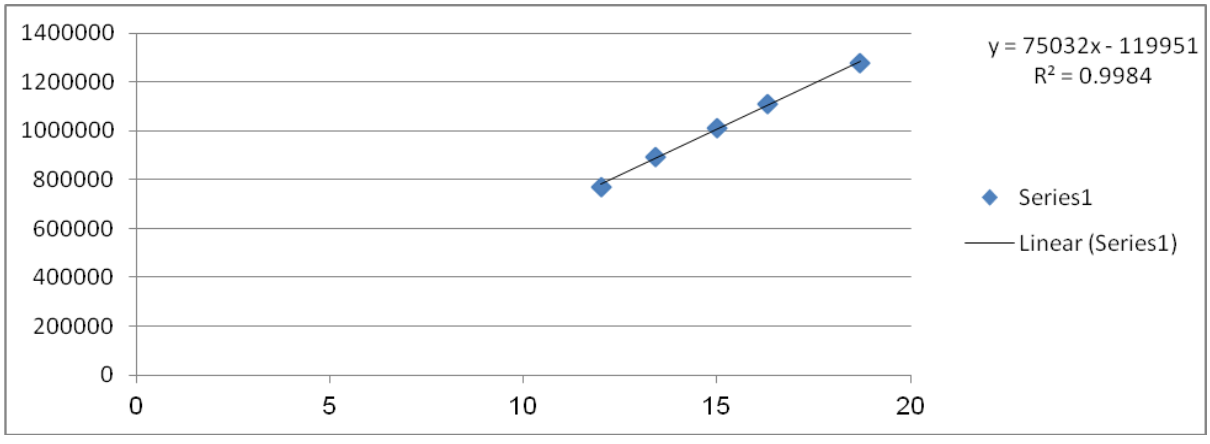

Fig. 4. Chlorthalidone linearity plot.

3. 3. Precision

The precision and the intermediate precision analysis data of the two tablets were presented in Tables 11 and 12. From the Tables 11 and 12, The theoretical plate for Olmesartan is 27129 and for Chlorthalidone is 2719. The tailing factor for Olmesartan is 0.91 and for Chlorthalidone is 0.89. The relative standard deviation for five replicate injections for Olmesartan is 0.26 and for Chlorthalidone is 0.43. The above results showed that the system is precise. The %RSD of Olmesartan assay from six samples is 0.64. The %RSD of Chlorthalidone assay from six sample is 1.00. Conclusion: The above results showed that the method is precise.

Table 11. The precision analysis data of the two tablets.

System Precision: Prepared standard solution as per the test method injected into the chromatographic system.						
Observation/Results						
Inj.No.	Area		Tailing Factor		Theoretical Plate	
	Olmesartan	Chlorthalidone	Olmesartan	Chlorthalidone	Olmesartan	Chlorthalidone
01	1972179	1071033	0.91	0.67	26995	2817
02	1974147	1073005				---
03	1969121	1066049				---
04	1966231	1073029				---
05	1979912	1078759				---
Average	1972318	1072375				---
SD	5203	4566				---
%RSD	0.26	0.43				---

Sample ID	%Assay Olmesartan	% Assay Chlorthalidone
Sample-1	100.06	100.21
Sample-2	100.21	102.23
Sample-3	98.95	100.27
Sample-4	98.75	101.08
Sample-5	98.99	102.10
Sample-6	98.98	99.89
Average	99.323	100.963
STDEV	0.637	1.011
%RSD	0.64	1.00

Table 12. Summary of Precision and Intermediate precision results

Observation/Results				
Sample ID	% Assay Olmesartan		% Assay Chlorthalidone	
	Set-I	Set-II	Set-I	Set-II
1	100.06	100.12	100.21	100.71
2	100.21	100.01	102.23	101.73
3	98.95	98.99	100.27	100.67
4	98.75	98.98	101.08	101.08
5	98.99	99.69	102.10	101.1
6	98.98	99.92	99.89	99.79
Average	99.323	99.618	100.963	100.847
SD	0.637	0.511	1.011	0.643
%RSD	0.64	0.51	1.00	0.64
Overall average	99.471		100.847	
Overall SD	0.571		0.613	
Overall %RSD	0.57		0.61	

3. 4. Accuracy

The accuracy analysis method of precision results were tabulated in Table 13. For Olmesartan the average % recovery was 100.23. For Chlorthalidone the average % recovery was 98.79. It was concluded that the test method was accurate from 80% to 120% of the target concentration.

Table 13. The accuracy analysis method of precision results of two tablets.

Method/Procedure
Prepared five concentration solutions in triplicate by spiking Olmesartan and Chlorthalidone drug substances to placebo from 80% to 120% of the target concentration of the target concentration and analyzed as per test method Acceptance criteria:

The % recovery should be in between 98.0 to 102.0						
Observation/Results						
Olmesartan medoxomil:						
S.No.		Amount added (mg/mL)	Amount found (mg/mL)	% Recovery	Statistical Analysis	
1	Sample-1	0.040320	0.040209	99.72	Average	100.74
	Sample-2	0.040280	0.040126	99.62		
	Sample-3	0.040250	0.040196	99.87		
2	Sample-1	0.045420	0.045315	99.77	Average	99.98
	Sample-2	0.045260	0.045345	100.19		
	Sample-3	0.045200	0.045186	99.97		
3	Sample-1	0.050130	0.050102	99.94	Average	100.17
	Sample-2	0.050180	0.050227	100.09		
	Sample-3	0.050100	0.050339	100.48		
4	Sample-1	0.055220	0.055523	100.55	Average	100.60
	Sample-2	0.055260	0.055548	100.52		
	Sample-3	0.055200	0.055604	100.54		
5	Sample-1	0.060110	0.060452	100.57	Average	100.68
	Sample-2	0.060160	0.060587	100.71		
	Sample-3	0.060100	0.060551	100.75		
Average Overall Statistical Analysis						100.23
Chlorthalidone:						
S.No.		Amount added (mg/mL)	Amount found (mg/mL)	% Recovery	Statistical Analysis	
1	Sample-1	0.012550	0.012414	98.92	Average	99.30

	Sample-2	0.012550	0.012464	99.31		
	Sample-3	0.012500	0.012414	99.93		
2	Sample-1	0.014050	0.014106	100.40	Average	99.44
	Sample-2	0.014200	0.013962	98.32		
	Sample-3	0.014150	0.014092	99.59		
3	Sample-1	0.015650	0.015363	98.17	Average	98.06
	Sample-2	0.015700	0.015392	98.04		
	Sample-3	0.015800	0.015478	97.96		
4	Sample-1	0.017020	0.016025	94.15	Average	97.54
	Sample-2	0.017159	0.017049	99.36		
	Sample-3	0.017158	0.017005	99.11		
5	Sample-1	0.018750	0.018554	99.95	Average	99.61
	Sample-2	0.018650	0.018586	99.66		
	Sample-3	0.018700	0.018739	100.21		
Average Overall Statistical Analysis						98.79

3. 5. Range

The range of the analytical method was obtained from linearity, precision and accuracy data. It is concluded that data from linearity, precision and accuracy, that the range of the analytical method for the determination of % Assay of Olmesartan - 40 mg with Chlorthalidone - 6.25 mg Tablets was from 80% to 120% of the target concentration

3. 6. Solution stability

The solution stability test results were tabulated in Table 14. From the above data it was concluded that the standard and sample solution were stable up to 24 hours on bench top.

Table 14. The solution stability test results of two tablets.

Method/Procedure
Sample solution: Prepared standard and sample solution as per the test method and injected into the

chromatographic system. At each interval injected the resolution solution, standard solution and sample solution into the chromatographic system. Calculate the %assay of initial standard solution and sample solution for each interval was calculated
Acceptance Criteria:
The %Assay difference should be NMT 2.0 from initial.

Validation Parameter		Results			Acceptance Criteria
Standard Solution Stability:					
Olmesartan	Parameter	Initial	12th Hour	24th H	---
	Theoretical Plates	24129	23759	23541	NLT 10000
	Tailing factor	0.91	0.89	0.90	The tailing factor is not more than 2.0.
	The relative standard deviation for five replicate injections	0.21	0.52	0.76	The relative standard deviation for five replicate injections is not more than 2.0 %.
Chlorthalidone	Theoretical Plates	2417	2513	2412	NLT 2000
	Tailing factor	0.91	0.93	0.91	The tailing factor is not more than 2.0.
	The relative standard deviation for five replicate injections	0.21	0.42	0.887	The relative standard deviation for five replicate injections is not more than 2.0 %.
Sample Solution Stability:					
Olmesartan	Parameter	Initial	12th Hour	24th H	----
	%Assay	100.03	100.44	100.18	---
	% Assay difference from initial	---	0.41	0.15	% Assay difference from initial should be NMT 2.0
Chlorthalidone	% Assay	99.54	99.74	99.55	% Assay difference from initial should be NMT 2.0
	% Assay difference from initial	---	0.20	0.01	

3. 7. Robustness

The robustness results were tabulated in Table-15. The results showed that the method was robust and results were not affecting when the test sample was filtered through nylon and PVDF filters. The observed results showed that the method was robust and results were not affecting when the flow of the mobile phase was changed from 1.35 ml/min. to 1.65 ml/minute. The above results showed that the method was robust and results were not affecting when the temperature was changed from 22.5 ºC to 27.5 ºC.

Table 15. Observation/Results of robustness.

Method/Procedure

Filter variability:
Prepared six sample solutions as per the test method, centrifuged one portion of the sample was filtered other portion of samples through at least two types of filters.
Eg. Nylon and PVDF.
Acceptance criteria: The % difference between the % assay values for centrifuged Vs filtered samples should be NMT 2.0.

Method/Procedure

Flow variation: The flow rate variation results were tabulated in table-19
Prepared standard and sample solutions as per the test method and injected into the chromatographic condition.
Acceptance criteria:
The % assay difference should be NMT 2.0 from average precision result.

Observation/Results

S.No.	% Assay Olmesartan			% Assay Chlorthalidone		
	Average Precision Result	1.35ml/min.	Difference	Average Precision Result	1.35 ml/min.	Difference
1	99.32	100.55	1.23	100.96	99.49	1.47

S.No.	% Assay Olmesartan			% Assay Chlorthalidone		
	Average Precision Result	1.65ml/min	Difference	Average Precision Result	1.65ml/min.	Difference
1	99.32	99.81	0.49	100.96	99.64	1.32

Method/Procedure

Temperature variation: The temperature variation results were tabulated in table-20
Prepared standard and sample solutions as per the test method and injected into the chromatographic condition.
Acceptance criteria:
The % assay difference should be NMT 2.0 from average precision result.

Table-20 Observation/Results

S.No.	% Assay Olmesartan			% Assay Chlorthalidone		
	Average Precision Result	22.5ºC	Difference	Average Precision Result	22.5 ºC	Difference
1	99.32	100.12	0.8	100.96	100.4	0.56
S.No.	% Assay Olmesartan			% Assay Chlorthalidone		

	Average Precision Result	27.5ºC	Difference	Average Precision Result	27.5 ºC	Difference
1	99.32	99.56	0.24	100.96	99.72	1.24

3. 8. Forced degradation

The forced degradation results were tabulated in Table 16. From the table, the percentage of assay of forced degradation analysis of Olmesartan – 20 mg with Chlorthalidone - 6.25 mg measured with acid, base, peroxide, UV and thermal stressed methods. All analyzed gave passed results.

Table 16. The forced degradation results.

Method/Procedure					
Olmesartan-20mg with Chlorthalidone - 6.25 mg placebo, drug substances and sample was stressed with following conditions and solutions were prepared as per the test method and were injected into the chromatographic system. Acid Stressed (5ml of 2N HCl stay for 30 min.) Base Stressed (5ml of 2N NaOH stay for 30 min.) Peroxide Stressed (5ml of H_2O_2 stay for 30 min.) UV Stressed (12 Hours at 254 µm) Thermal Stressed (60 ºC for 2 Hours)					
Forced degradation: Sample Acceptance criteria: % Assay degradation should be NMT 10%.					
Degradation mechanism/ condition	Observation				Remarks
	Olmesartan		Chlorthalidone		
	% Assay	% Assay difference from undegraded	% Assay	% Assay difference from undegraded	
Un degraded	99.32	---	100.96	---	Passed
Acid Stressed (5 ml of 2N HCL stay for 30 min.)	99.81	0.41	99.74	1.22	Passed
Base Stressed (5 ml of 2N NaOH stay for 30 min.)	99.22	0.1	99.72	1.24	Passed
Peroxide Stressed (5 ml of H_2O_2 stay for 30 min.)	98.21	1.11	99.41	1.55	Passed
UV Stressed (12 Hours at 254 µm)	99.00	0.32	99.63	133	Passed
Thermal Stressed (60 ºC for 2 Hours)	99.13	0.68	99.23	1.73	Passed

4. CONCLUSIONS

The all test method is validated for specificity, precision, intermediate precision, accuracy, linearity, range, stability of solution and robustness and found to meets the pre-determined acceptance criteria. The validated method is specific, linear, accurate, robust and rugged for assay of the Olmesartan-20mg with Chlorthalidone-6.25mg Tablets.

References

[1] G.K. Aulakh, R.K. Sodhi, M. Singh, *Life Sciences*, 81(2007) 615-639.

[2] Kusum lata et. al., *International Journal of Pharmachutical and Quality Assurance,* 1 (2010) 60-66.

[3] Parikh et. Al., *Pharma Science Monitor,* 4, Supp-1, (2013)

[4] M. Yunoos and D. Gowri Sankar, *Journal of Chemical and Pharmaceutical Research*, 7(3) (2015) 2442-2448.

[5] S. Hillaert, W. Van den Bossche, *Journal of Pharmaceutical and biomedical analysis* 31 (2003) 329-339.

[6] (a) D. Suneetha and A. Lakshmana Rao, *International Journal Of Research In Pharmacy And Chemistry* 1 (2011) 1-7.

(b) V. Bhaskara Raju and A. Lakshmana Rao, *International Journal Of Research In Pharmacy And Chemistry* 1 (2011) 25-28.

(c) I. Venkata Rayanam, A. Lakshmana Rao and M.V. Ramana, *International Journal Of Research In Pharmacy And Chemistry* 1 (2011) 50-54.

[7] S. Naazneen and A. Sridevi, *International Journal of Pharmacy and Pharmaceutical Sciences*, 6 (2014) 236-243.

[8] M. Bakshi and S. Singh, *Journal of Pharmaceutical and Biomedical Analysis* 28 (2002) 1011-1040

[9] The Formulary US, Rockyvilley USP. The United State pharmacopoeial Convention Inde. NF 32, 2014;36

[10] ICH (International Conference on Harmonization) –Guidelines Q2A, Validation of Analytical Procedures: Definition and terminology (CPMP III/5626/94) March Geneva, Switzerland. Journal of AOAC International 1995.

[11] ICH (International Conference on Harmonization) – Guidelines Q2B, Validation of Analytical Procedures: Methodology (CPMP/ICH/281/95) November Geneva, Switzerland. Journal of AOAC International 1996.

[12] G. Thirunarayanan, *J. Korean Chem. Soc*., 52(4) (2008) 369-379.

[13] R. Arulkumaran, R. Sundararajan, G. Vanangamudi, M. Subramanian, K. Ravi, V. Sathiyendiran, S. Srinivasan, and G. Thirunarayanan, *IUP. J. Chem*., 3(1), (2010) 82-98.

[14] G. Thirunarayanan, P. Mayavel, K. Thirumurthy, G. Vanangamudi, K. Lakshmanan and K. G. Sekar, *Int. J. Chem*. 1(2) (2012) 166-172.

[15] G. Thirunarayanan, G. Vanangamudi and M. Subramanian, *Org. Chem.: An Indian Journal*, 9(1) (2013) 1-16. UPDI: http://www.updi.info/09747516.9/165555

[16] K. G. Sekar and G. Thirunarayanan, *Int. Lett. Chem. Phys. Astro*. 8(2) (2013) 160-174.

[17] M. Subramanian, G. Vanangamudi and G. Thirunarayanan, *Spectrochim Acta*, 110A, (2013) 116-123. DOI: http://dx.doi.org/10.1016/j.saa.2013.03.023

[18] G. Vanangamudi, M. Subramanian and G. Thirunarayanan, *Arabian J. Chem*. 2013. DOI: 10.1016/j.arabjc.2013.03.006.

[19] R. Arulkumaran, S. Vijayakumar, R. Sundararajan, D. Kamalakkannan, K. Ranganathan, R. Suresh, S.P. Sakthinathan, G. Vanangamudi, G. Thirunarayanan, *Indian J. Adv. Chem. Sci*. 2(1) (2013) 6-15.

[20] R. Vijayakumar, M. Rajarajan, S. Balaji, V. Manikandan, R. Senbagam G. Vanangamudi, G. Thirunarayanan, *World Scientific News* 9 (2015) 70-87

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