



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 x 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 $\mu\text{g/ml}$ and 6.25 $\mu\text{g/ml}$ 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-biphenyl]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 AT1 receptors 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 25thFeb 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.

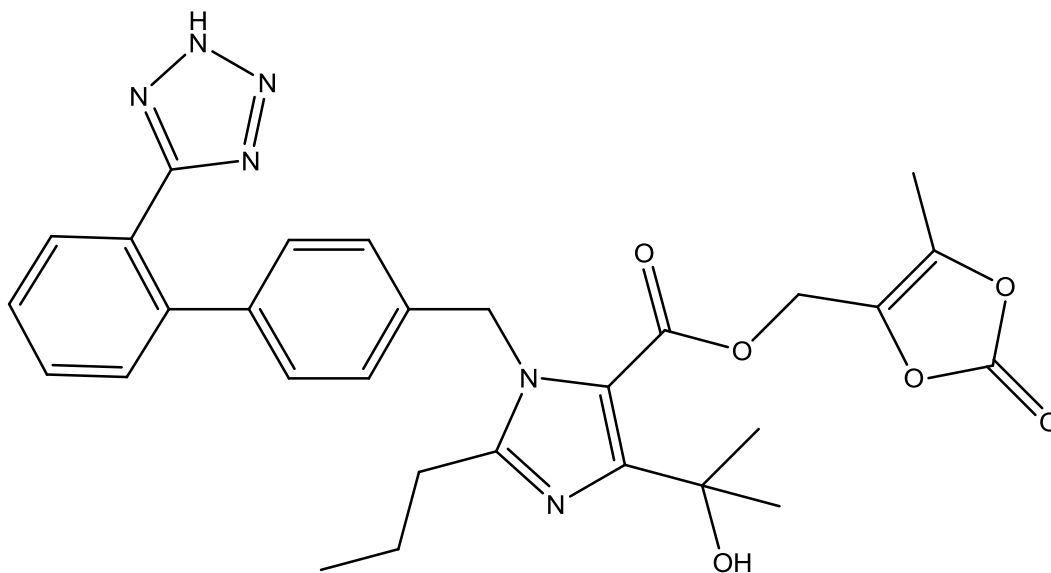


Fig. 1. Structure of olmesartan medoxomil.

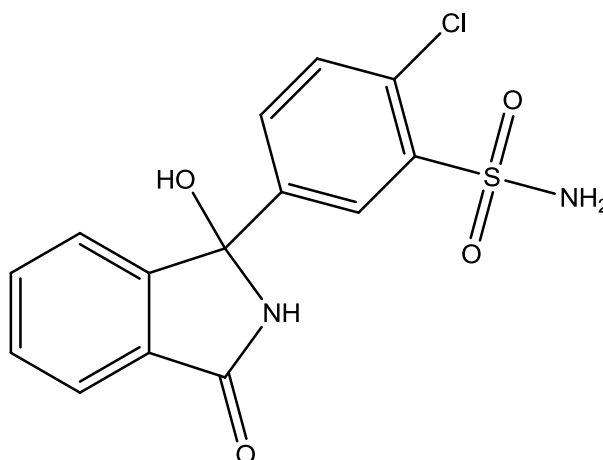


Fig. 2. Structure of chlorthalidone.

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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 $^{\circ}$ C |
| Sample cooler temperature | : | 5 $^{\circ}$ C, |
| Flow Rate | : | 1.5 ml/min, |
| Wave Length | : | 220 nm. |
| Injection volume | : | 20 μ L |

2. 3. Preparation of buffer (pH 3.0)

Accurately weighed and transferred 2.0 gm of ammonium dihydrogen orthophosphate 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 μ L, 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.

| Validation Parameter | Results | | Acceptance Criteria |
|-------------------------------------|---|------------|--|
| Specificity and system suitability: | | | |
| RT of Olmesartan peak | Standard | Sample | Standard and sample were comparable with respect to RT. |
| | 10.74min | 10.74 min. | |
| RT of Chlorthalidone peak | Standard | Sample | |
| | 4.87 min. | 4.88 min | |
| Blank Interference: | | | |
| At RT of Olmesartan peak | No peak observed at the RT of olmesartan | | Blank not showed no interference at the RT of Olmesartan peak. |
| At RT of Chlorthalidone peak | No peak observed at the RT of ohlorthalidone | | Blank not showed no interference at the RT of Chlorthalidone peak. |
| System Suitability: | | | |
| Olmesartan | Theoretical Plates | 25129 | NLT 10000 |
| | Tailing factor | 0.9 | Between 0.8 and 1.5 |
| | 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 | Between 0.8 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 °C | 27.5 °C | --- |
| | 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 ₂ O 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

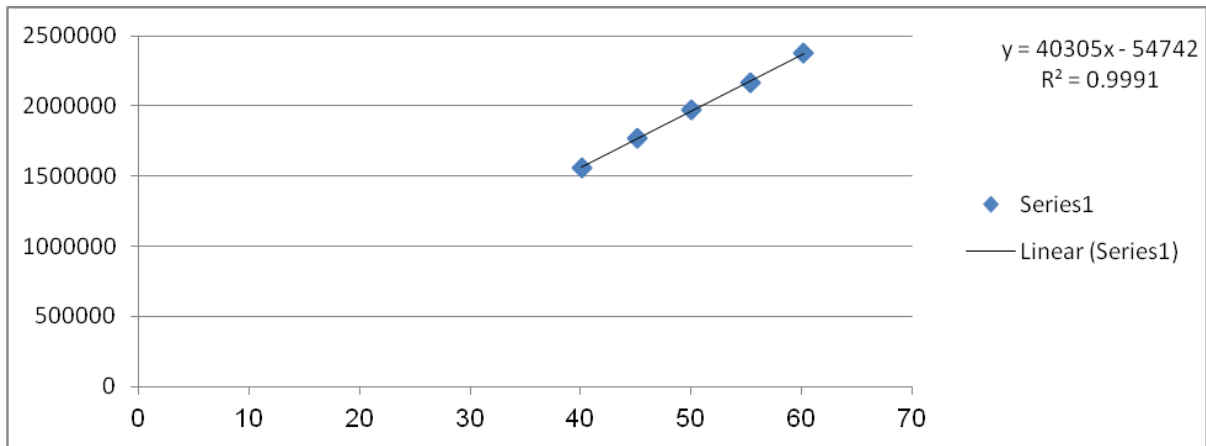


Fig. 3. Olmesartan linearity plot.

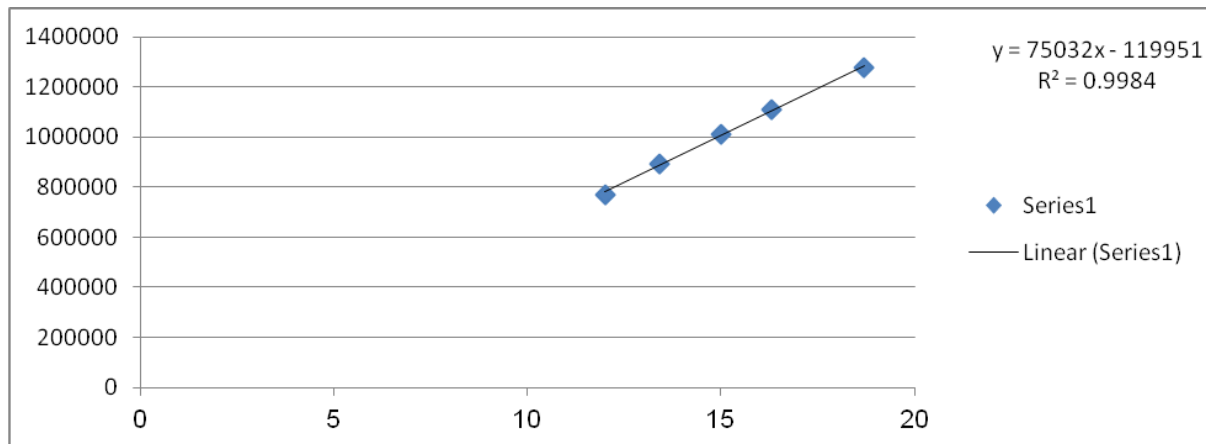


Fig. 4. Chlorthalidone linearity plot.

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 |
|---|
| <p>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</p> |

| 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 | 12 th Hour | 24 th 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 | 12 th Hour | 24 th 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 °C to 27.5 °C.

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 | 22.5°C | Difference | Average Precision Result | 22.5 °C | Difference |
| 1 | 99.32 | 100.12 | 0.8 | 100.96 | 100.4 | 0.56 |
| S.No. | % Assay Olmesartan | | | % Assay Chlorthalidone | | |
| | Average | 27.5°C | Difference | Average | 27.5 °C | Difference |

| | | | | | | |
|---|------------------|-------|------|------------------|-------|------|
| | Precision Result | | | Precision Result | | |
| 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 ₂ O ₂ stay for 30 min.) UV Stressed (12 Hours at 254 μm) Thermal Stressed (60 °C 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 ₂ O 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 °C 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.

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