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# STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF OLMESARTAN, CHLORTHALIDONE AND CILNIDIPINE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC

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**ABSTRACT**

The purpose of the investigation was to develop a simple, rapid and accurate RP-HPLC method to determine assay of Olmesartan, chlorthalidone And Cilnidipine in Bulk and Pharmaceutical Dosage Form. The chromatographic separation was performed on Kromosil 250 x 4.6 mm, 5µm. Eluents were monitored on PDA detector at a wavelength of 240 nm using a mixture Buffer: Acetonitrile: methanol (45:50:5v/v). The column temperature was maintained at 30°C. Validation parameters such as system suitability, linearity, precision, accuracy, specificity, limit of detection (LOD), limit of quantification (LOQ), Stability of sample and standard stock solutions and robustness were studied as reported in the ICH guidelines. The retention time for Olmesartan, chlorthalidone And Cilnidipine was 3.357 min, 2.838 min and 3.722min respectively. Assay method further evaluated for Olmesartan, chlorthalidone And Cilnidipine analysis at low concentration of analyte and found limit of detection is 0.03, 0.05 and 0.02 ppm respectively and limit of Quantitation is 0.08, 0.16 and 0.07 ppm respectively. The percentage recovery of Olmesartan, chlorthalidone And Cilnidipine was 100.70%, 100.34% and 100.47% respectively. The %RSD for Olmesartan, chlorthalidone And Cilnidipine was found to be less than 1.08%, 0.9% and 1.3% respectively. Linearity of Olmesartan chlorthalidone, and Cilnidipine performed from 25% to 150% and the $R^2$ is 0.999, intercept and slope found to be y = 43191x + 562.0, y = 65434x + 1255 and y = 76821x + 1619 respectively. The method was fast, accurate, precise and sensitive hence it can be employed for routine quality control of Olmesartan, chlorthalidone And Cilnidipine containing drug in quality control laboratories and pharmaceutical industries.

**Key words:** RP-HPLC, Olmesartan, chlorthalidone, Cilnidipine

## INTRODUCTION

Olmesartan is an angiotensin II receptor antagonist (angiotensin receptor blocker, ARB) used in the management of hypertension. Chemically it is known as 4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(1H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)-1H-imidazole-5-carboxylic acid. It is marketed under the trade name BENICAR®. Cilnidipine is a calcium channel blocker[1-6]. Cilnidipine is the novel calcium antagonist accompanied with L-type and N-type calcium channel blocking function. Chemically it is known as O3-(2-methoxyethyl) O5-[(E)-3-phenylprop-2-enyl] 2,6-dimethyl-4-(3-nitrophenyl)-1,4- dihydropyridine- 3,5-dicarboxylate. It was jointly developed by Fuji Viscera Pharmaceutical

Company, Japan and Ajinomoto, Japan and approved to come into market for the first time and used for high blood pressure treatment in 1995[7-11]. Chlorthalidone or chlorthalidone is a diuretic drug used to treat hypertension. Chemically it is known as (RS)-2-Chloro-5-(1-hydroxy-3-oxo-2,3- dihydro-1H-isoindol-1-yl)benzene-1-sulfonamide. It is originally marketed as Hygroton in the USA[12-14].

The literature survey reveals that there is no analytical method available for estimation of Olmesartan , Cilnidipine and Chlorthalidone The reported methods available for the estimation of Olmesartan, Cilnidipine and Chlorthalidone individually are spectro photometric method[15-26] Since the lack of official high performance liquid chromatographic methods for the simultaneous estimation of Olmesartan, Cilnidipine and Chlorthalidone, we have planned to develop a simple, precise, economic and accurate Stability indicating RP-HPLC method development and validation for the estimation of Olmesartan, Cilnidipine and Chlorthalidone in pharmaceutical dosage form.

## EXPERIMENTAL

### Materials and methods

Active pharmaceutical ingredients Olmesartan, Cilnidipine and Chlorthalidone were obtained as a gift sample from Spectrum pharma research solutions, Hyderabad. The pharmaceutical dosage form (Trinexovas (10mg/40mg/12.5mg) Macleods(Procare CV) was purchased from local pharmacy. The solvents used in this work were of HPLC grade and obtained from Merck Specialties Private Limited, Mumbai.

### Instrumentation and chromatographic conditions

The analysis was performed on a high performance liquid chromatography system consists of waters 2695 with 2996 module Photo Diode Array detector equipped with a quaternary solvent delivery pump, automatic sample injector and column thermostat. The data acquisition and analysis was performed by using Empower2 software. The chromatographic separation was performed on Kromosil 250 x 4.6 mm, 5µm. The flow rate was kept at 1ml/min. The column temperature was maintained at 30°C.The mobile phase was made of 0.1% Perchloric acid Buffer, Acetonitrile and Methanol taken in the ratio 45:50:5 ratio had gave acceptable retention time and good resolution between Olmesartan, Cilnidipine and Chlorthalidone. The method was optimized at 240nm. Data acquisition and processing was performed by using empower2 system software. The run time was taken as 7min. All the determinations are carried out at an ambient temperature.

**Preparation of Standard stock solutions:** Accurately Weighed and transferred 40mg of Olmesartan and 12.5mg of chlorthalidone and 10 mg of Cilnidipine working Standards into a 50ml clean dry volumetric flask, add 3/4$^{th}$ volume of diluents, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

**Preparation of Sample stock solutions:** 1tablet was weighed, powdered and then the weight 380mg (equivalent to 40mg of Olmesartan and 12.5mg of chlorthalidone and 10 mg of Cilnidipine) was transferred into a 50mL volumetric flask, 30mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

### Preparation of buffer

**Buffer: (0.1%Perchloric acid)**

1ML of Perchloric acid solution in a 1000ml of Volumetric flask add about 100ml of milli-Q water and final volume make up to 1000 ml with milli-Q water

### Method validation

The method was validated according to ICH guidelines. The different validation characteristics which were performed are following: Linearity, accuracy, Precision, limit of detection, limit of quantification, robustness and the stability indicating capability.

### System suitability parameters

The system suitability parameters were determined by preparing standard solutions of Olmesartan, Cilnidipine and Chlorthalidone and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

### Linearity

The linearity of the method is determined by preparing three individual series of solutions in the range of Olmesartan (20-120µg/ml), Cilnidipine (5-30µg/ml) and Chlorthalidone (6.25-37.5µg/ml). The obtained peak areas are plotted against concentration.

### Preparation of linearity solutions

**Preparation of Standard stock solutions:** Accurately weighed 40mg of Olmesartan and 12.5mg

of chlorthalidone and 10 mg of Cilnidipine working Standards into a 50ml clean dry volumetric flask, add 3/4$^{th}$ volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents.
From three stock solutions pipette out 0.25ml, 0.5ml, 0.75ml, 1.0ml, 1.25ml, 1.50ml into 10ml volumetric flak to get 25%,50%, 75%, 100%, 125%, 150% of standard solutions.

**Precision**

***a) Method precision (repeatability)***

The method precision/ repeatability can be determined by injecting six working standard solutions and six sample injections. The areas of all the injections were taken and standard deviation, %Relative standard deviation, %assay were calculated.

***b) Intermediate precision***

The intermediate precision can be determined by injecting six working standard solutions and six sample injections on different days by different operators or by different instruments. The areas of all the injections were taken and standard deviation, %Relative standard deviation, %assay were calculated. The results obtained were within the acceptance criteria.

**Accuracy**

Accuracy is tested by the standard addition method at three different levels 50, 100 and 150%. The percentage recoveries of Olmesartan, Cilnidipine and Chlorthalidone present in the pharmaceutical dosage form were calculated.

**Preparation of 50% Spiked Solution:** 1tablet was weighed, powdered and then the weight 380mg (equivalent to 40mg of Olmesartan and 12.5mg of chlorthalidone and 10 mg of Cilnidipine) was transferred into a 50mL volumetric flask, 30mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.5ml was pipeted out into a 10 ml volumetric flask and made up to 10ml with diluent.

**Preparation of 100% Spiked Solution:** 1tablet was weighed, powdered and then the weight 380mg (equivalent to 40mg of Olmesartan and 12.5mg of chlorthalidone and 10 mg of Cilnidipine) was transferred into a 50mL volumetric flask, 30mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

**Preparation of 150% Spiked Solution:** 1tablet was weighed, powdered and then the weight 380mg (equivalent to 40mg of Olmesartan and 12.5mg of chlorthalidone and 10 mg of Cilnidipine) was transferred into a 50mL volumetric flask, 30mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1.5ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

**Limit of detection and limit of quantification**

Limit of detection (LOD) and limit of quantification (LOQ) of Olmesartan, Cilnidipine and Chlorthalidone were determined by calibration curve method. Solutions of Olmesartan, Cilnidipine and Chlorthalidone were prepared in linearity range and injected in triplicate. Average peak area of three analyses was plotted against concentration

**Method robustness**

The robustness can be determined by varying the following parameters:
Robustness of the developed method was determined by making small deliberate changes in flow rate (±1ml/min), column temperature (±5%), organic mobile phase ratio (±10%), along with the optimized method.

**Forced degradation studies**

**Oxidation:**

To 1 ml of stock solution of Olmesartan, Cilnidipine and Chlorthalidone 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 600 c. For HPLC study, the resultant solution was diluted to obtain 80µg/ml, 20µg/ml and 13µg/ml of all components and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Acid Degradation Studies:**

To 1 ml of stock s solution Olmesartan, Cilnidipine and Chlorthalidone, 1 ml of 2N Hydrochloric acid was added and refl uxed for 30mins at 600 c .The resultant solution was diluted to obtain 80µg/ml, 20µg/ml and 25µg/ml of all components and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Alkali Degradation Studies:**

To 1 ml of stock solution Olmesartan, Cilnidipine and Chlorthalidone, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 600 c. The resultant solution was diluted to obtain 80µg/ml, 20µg/ml and 25µg/ml of all components and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Dry Heat Degradation Studies:**
The standard drug solution w a s placed in oven at 1050 c for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted obtain 80µg/ml, 20µg/ml and 25µg/ml of all components and10µl were inject d into the system and the chromatograms were recorded to assess the stability of the sample**.**

**Photo Stability studies:**
The photochemical stability of the drug was also studied by exposing the 800µg/ml, 200µg/ml and 250µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 80µg/ml, 20µg/ml and 25µg/ml of all components and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Neutral Degradation Studies:**
Stress testing under neutral conditions was studied by refluxing the drug in water for 6h r s at a temperature of 60º. For HPLC study, the resultant solution was diluted to obtain 80µg/ml, 20µg/ml and 25µg/ml of all components and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

## RESULTS AND DISCUSSIONS

**Development and optimization of HPLC method**
The present work was focused to develop a stability indicating RP-HPLC method for the simultaneous estimation of Olmesartan, Cilnidipine and Chlorthalidone in pharmaceutical dosage form. The solubility of the active pharmaceutical ingredient was checked in different solvents like methanol, water, Acetonitrile and in different ratios but finally the standard is soluble in water: methanol (50:50) so it was chosen as a diluent. The different mobile phases like Acetonitrile and potassium dihydrogen phosphate buffer and Acetonitrile and sodium dihydrogen phosphate buffer were used in compositions with a flow rate of 1ml/min but the peak resolution, retention time and tailing factor were not satisfactory, so at last 0.1% perchloric acid and Acetonitrile was selected as a buffer at flow rate of 1ml/min. Initially and "BDS®" (150mm x 4.6mm x 5µ) columns with different temperatures like 30, 35, 40, 45°C were used but the retention time, run time and peak resolution were not exact and the problem was get rid by using kromosil®"(250mm x 4.6mm x 5µ) kept at 30°c with a run time of 8 minutes. Finally the method was optimized by altering the mobile phase composition / ratio and the optimized wavelength of three drugs Olmesartan, Cilnidipine and Chlorthalidone was found to be at 240nm.

***Forced degradation studies:***
The stability studies were conducted by exposing the dosage form to different stress conditions like acid, base, peroxide, thermal, light and water. It was found that the dosage form was slightly degraded in acid, base peroxide and thermal conditions but stable in photolytic and hydrolytic conditions

**System suitability parameters**
The system suitability tests were conducted before performing the validation and the parameters were within the acceptance criteria like retention times were 3.357 min, 2.838 min and 3.722min for Olmesartan, chlorthalidone And Cilnidipine, plate count was >2000, peak tailing was <2 and the %RSD of peak areas of six injections were ≤ 2% (Table 1). Hence the proposed method was successfully applied to routine analysis without any problems.

**Linearity range**
The linearity range was in the interval of Olmesartan (20-120µg/ml), Cilnidipine (5-30µg/ml) and Chlorthalidone (6.25-37.5µg/ml), respectively. These were represented by a linear regression equation as follows: y (Olmesartan) = 43191x + 562.0. (r2=0.999), y (Cilnidipine) = 76821x + 1619 (r2 =0.999) and y (Chlorthalidone) = 65434x + 1255. Regression line was established by least squares method and correlation coefficient (r2) for Olmesartan, Cilnidipine and Chlorthalidone were found to be greater than 0.999. Hence the curves established were linear. (Table 2).

**Precision**
Six replicates injections at the same concentration were analyzed on same day and two different days for verifying the variation in the precision and the % RSD for Olmesartan, Cilnidipine and Chlorthalidone were within acceptable limit of ≤2. Hence the method is reproducible on different days with different analyst and column. This indicates that the method is precise (Table 3).

**Accuracy**
The percentage recoveries for Olmesartan, Cilnidipine and Chlorthalidone were found to be 100.70%, 100.47% and 100.34% respectively (Table 4, 5, 6). The results of the recovery studies undoubtedly demonstrate accuracy of the proposed method.

**Limit of detection (LOD) and limit of quantification (LOQ)**

The determined values of LOD and LOQ were calculated by using slope and Y-intercept. The LOD and LOQ values for Olmesartan were found to be 0.03 and 0.08 µg/ml and Cilnidipine were found to be 0.02 and 0.07µg/ml, Chlorthalidone were found to be 0.05 and 0.16µg/ml respectively (Table 7).

**Robustness**

Robustness of the proposed method demonstrated a non-significant alteration through analysis of the sample and standard Olmesartan, Cilnidipine and Chlorthalidone solution (Table 6). After this the results obtained were compared with that of optimized method. It was confirmed that by the deliberate changes in the parameters there was no any significant changes in standard deviation, relative standard deviation, theoretical plates, retention time and USP tailing factor.

**Assay**

The Content of Olmesartan, Cilnidipine and Chlorthalidone in the pharmaceutical dosage form was found by using the developed method. The percentage purity of Olmesartan, Cilnidipine and Chlorthalidone were found to be 100.68%, 100.88% and 100.71% and %RSD values for Olmesartan, Cilnidipine and Chlorthalidone were within limit of ≤2.

**Forced degradation studies**

The forced degradation studies were conducted and all the parameters for Olmesartan, Cilnidipine and Chlorthalidone were within the limits. Olmesartan, Cilnidipine and Chlorthalidone have shown significant sensitivity towards the treatment of HCl, NaOH and peroxide solutions. The drugs gradually undergone degradation with time and prominent degradation was observed. Olmesartan, Cilnidipine and Chlorthalidone were stable under forced thermal degradation, photolytic and neutral degradations. From the degradation studies, Peak purity test results derived from PDA detector, confirmed that the Olmesartan, Cilnidipine and Chlorthalidone peaks were homogeneous and pure in all the analyzed stress samples. The mass balance of stressed samples was close to 97.61%.

## CONCLUSION

A new, simple, rapid and precise stability indicating high performance liquid chromatographic method was developed for the simultaneous estimation of Olmesartan, Cilnidipine and Chlorthalidone in pharmaceutical dosage form. Hence this method can be applied for the estimation of Olmesartan, Cilnidipine and Chlorthalidone in drug testing laboratories and pharmaceutical industries.

## ACKNOWLEDGEMENTS

The authors were thankful for Spectra pharma research solutions, Hyderabad for providing Olmesartan, Cilnidipine and Chlorthalidone reference standards as a gift sample to carry out the research work.

## DISCLOSURE OF INTEREST

The authors declare that they have no conflicts of interest concerning this article.

**Table 1: System suitability parameters for Olmesartan, Cilnidipine, Chlorthalidone.**

| S no | Olmesartan | | | Cilnidipine | | | Chlorthalidone | | |
|---|---|---|---|---|---|---|---|---|---|
| Inj | RT(min) | TP | Tailing | RT(min) | TP | Tailing | RT(min) | TP | Tailing |
| 1 | 3.352 | 8887 | 1.36 | 3.705 | 8764 | 1.31 | 2.814 | 8064 | 1.29 |
| 2 | 3.353 | 9587 | 1.33 | 3.706 | 7616 | 1.33 | 2.837 | 9327 | 1.28 |
| 3 | 3.356 | 9651 | 1.33 | 3.708 | 8131 | 1.35 | 2.839 | 9087 | 1.31 |
| 4 | 3.369 | 9664 | 1.31 | 3.725 | 7116 | 1.31 | 2.851 | 9964 | 1.27 |
| 5 | 3.371 | 9304 | 1.34 | 3.732 | 8656 | 1.30 | 2.852 | 9734 | 1.27 |
| 6 | 3.374 | 10416 | 1.36 | 3.747 | 8029 | 1.27 | 2.852 | 9697 | 1.26 |

**Table 2: Linearity table for Olmesartan, Cilnidipine, Chlorthalidone.**

| Olmesartan | | Cilnidipine | | Chlorthalidone | |
|---|---|---|---|---|---|
| Conc (µg/mL) | Peak area | Conc (µg/mL) | Peak area | Conc (µg/mL) | Peak area |
| 20 | 850903 | 5 | 375555 | 6.25 | 404876 |
| 40 | 1717834 | 10 | 751655 | 12.5 | 825891 |
| 60 | 2593625 | 15 | 1186445 | 18.75 | 1244615 |
| 80 | 3503282 | 20 | 1555518 | 25 | 1621826 |
| 100 | 4332105 | 25 | 1922876 | 32.5 | 2113599 |
| 120 | 5146342 | 30 | 2285465 | 37.5 | 2468016 |

**Table 3 Determination of repeatability and intermediate precision**

| Drug Name | Repeatability | | | Intermediate | | |
|---|---|---|---|---|---|---|
| | Peak Area | Std Dev | %RSD | Peak Area | Std Dev | %RSD |
| Olmesartan | 3625642 | 34880.82 | 0.96 | 3635755 | 27455.3 | 0.8 |
| Cilnidipine | 1581115 | 16732.18 | 1.06 | 1586206 | 12474.4 | 0.8 |
| Chlorthalidone | 1614585 | 16276.35 | 1.01 | 1619363 | 12645.5 | 0.8 |

**Table 4 Determination of Accuracy of Olmesartan**

| % Level | Amount Spiked (µg/mL) | Total amount found(µg/mL) | Amount recovered (µg/mL) | % Recovery | Mean %Recovery |
|---|---|---|---|---|---|
| 50% | 40 | 121.4 | 40.5 | 101.2 | 100.70% |
| | 40 | 120.0 | 40.0 | 100.0 | |
| | 40 | 120.8 | 40.3 | 100.7 | |
| 100% | 80 | 160.7 | 80.3 | 100.4 | |
| | 80 | 162.4 | 81.2 | 101.5 | |
| | 80 | 159.1 | 79.5 | 99.4 | |
| 150% | 120 | 241.4 | 120.7 | 100.6 | |
| | 120 | 243.3 | 121.6 | 101.4 | |
| | 120 | 242.8 | 121.4 | 101.1 | |

**Table 5 Determination of Accuracy of Cilnidipine**

| % Level | Amount Spiked (µg/mL) | Total amount found(µg/mL) | Amount recovered (µg/mL) | % Recovery | Mean %Recovery |
|---|---|---|---|---|---|
| 50% | 10 | 29.92 | 9.97 | 99.74 | 100.47% |
| | 10 | 30.22 | 10.07 | 100.73 | |
| | 10 | 30.33 | 10.11 | 101.10 | |
| | 20 | 39.83 | 19.92 | 99.58 | |

| | | | | |
|---|---|---|---|---|
| 100% | 20 | 41.10 | 20.55 | 102.76 |
| | 20 | 40.21 | 20.11 | 100.53 |
| 150% | 30 | 49.15 | 29.49 | 98.30 |
| | 30 | 50.36 | 30.22 | 100.72 |
| | 30 | 50.37 | 30.22 | 100.74 |

**Table 6 Determination of Accuracy of Chlorthalidone**

| % Level | Amount Spiked (µg/mL) | Total amount found(µg/mL) | Amount recovered (µg/mL) | % Recovery | Mean %Recovery |
|---|---|---|---|---|---|
| 50% | 12.5 | 37.89 | 12.63 | 101.03 | 100.34% |
| | 12.5 | 37.27 | 12.42 | 99.39 | |
| | 12.5 | 37.55 | 12.52 | 100.15 | |
| 100% | 25 | 49.01 | 24.50 | 98.02 | |
| | 25 | 51.10 | 25.55 | 102.21 | |
| | 25 | 49.85 | 24.92 | 99.70 | |
| 150% | 37.5 | 63.42 | 38.05 | 101.48 | |
| | 37.5 | 63.15 | 37.89 | 101.04 | |
| | 37.5 | 62.50 | 37.50 | 100.01 | |

**Table 7 Sensitivity table of Olmesartan, Cilnidipine and Chlorthalidone**

| Molecule | LOD(µg/ml) | LOQ(µg/ml) |
|---|---|---|
| Olmesartan | 0.03 µg/ml | 0.08 µg/ml |
| Cilnidipine | 0.02 µg/ml | 0.07 µg/ml |
| Chlorthalidone | 0.05 µg/ml | 0.16 µg/ml |

**Table 8 Robustness data for Olmesartan, Cilnidipine and Chlorthalidone.**

| S.no | Condition | %RSD of Olmesartan | %RSD of Cilnidipine | %RSD of Chlorthalidone |
|---|---|---|---|---|
| 1 | Flow rate (-) 0.9ml/min | 1.1 | 1.1 | 1.1 |
| 2 | Flow rate (+) 1.1ml/min | 0.6 | 0.4 | 0.6 |
| 3 | Mobile phase (-) 38B:62A | 0.9 | 0.8 | 0.6 |
| 4 | Mobile phase (+) 26B:74A | 1.9 | 1.2 | 1.3 |
| 5 | Temperature (-) 25°C | 1.1 | 1.1 | 1.1 |
| 6 | Temperature (+) 35°C | 0.5 | 0.5 | 0.5 |

**Table 9 Degradation Data of Olmesartan**

| S.NO | Degradation Condition | % Drug Degraded | Purity Angle | Purity Threshold |
|---|---|---|---|---|
| 1 | Acid | 4.66 | 0.13 | 0.291 |
| 2 | Alkali | 1.50 | 0.137 | 0.293 |
| 3 | Oxidation | 7.27 | 0.125 | 0.302 |
| 4 | Thermal | 0.92 | 0.28 | 0.298 |
| 5 | UV | 1.06 | 0.179 | 0.296 |
| 6 | Water | 0.55 | 0.15 | 0.294 |

**Table 10 Degradation Data of Cilnidipine**

| S.NO | Degradation Condition | % Drug Degraded | Purity Angle | Purity Threshold |
|---|---|---|---|---|
| 1 | Acid | 3.39 | 0.188 | 0.33 |
| 2 | Alkali | 2.51 | 0.139 | 0.342 |
| 3 | Oxidation | 4.58 | 0.188 | 0.362 |
| 4 | Thermal | 0.66 | 0.189 | 0.341 |
| 5 | UV | 0.72 | 0.191 | 0.335 |
| 6 | Water | 0.92 | 0.103 | 0.337 |

**Table Degradation Data of Chlorthalidone**

| S.NO | Degradation Condition | % Drug Degraded | Purity Angle | Purity Threshold |
|---|---|---|---|---|
| 1 | Acid | 4.27 | 0.389 | 0.323 |
| 2 | Alkali | 2.88 | 0.259 | 0.332 |
| 3 | Oxidation | 5.60 | 0.277 | 0.369 |
| 4 | Thermal | 1.19 | 0.192 | 0.324 |
| 5 | UV | 0.16 | 0.325 | 0.327 |
| 6 | Water | 0.13 | 0.289 | 0.328 |

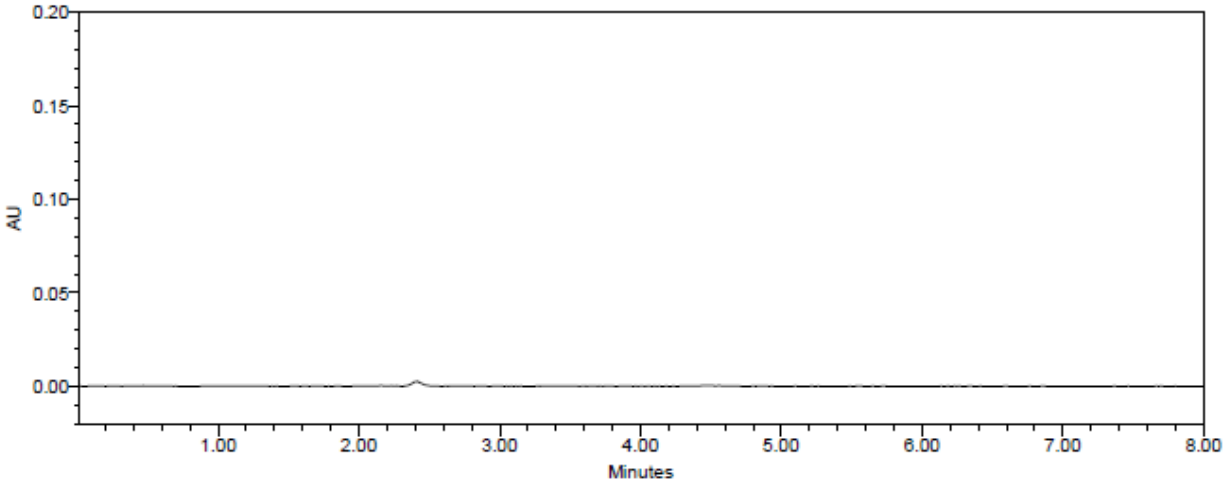


**Fig.1 Blank chromatogram of Olmesartan, Cilnidipine and Chlorthalidone**

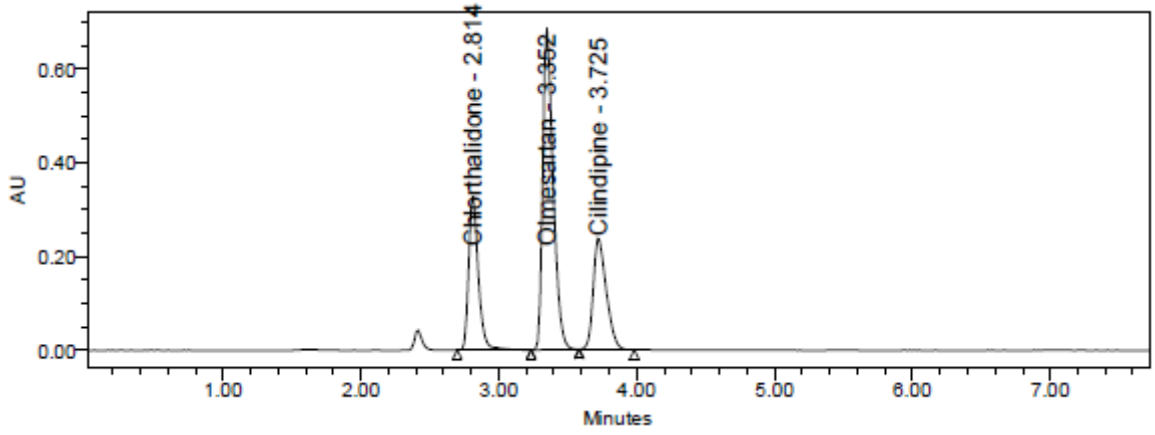

**Fig.2 Standard chromatogram of Olmesartan, Cilnidipine and Chlorthalidone**

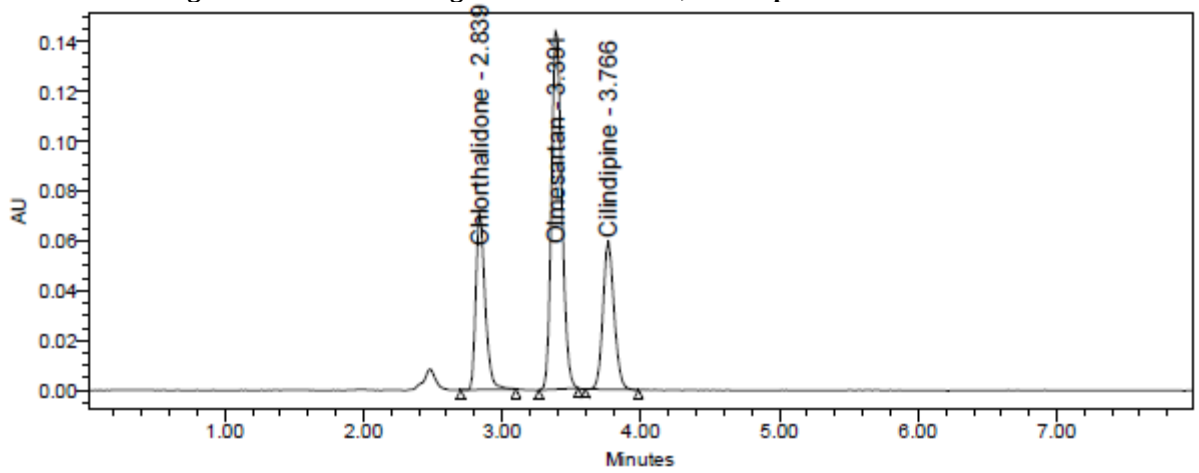


**Fig.3 Linearity chromatogram of Olmesartan, Cilnidipine and Chlorthalidone**

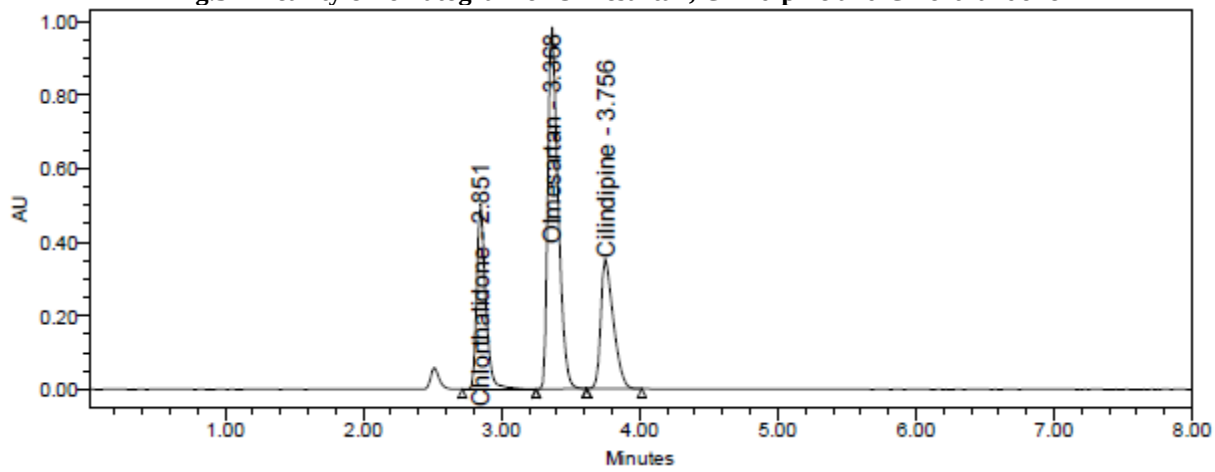


**Fig.4 Accuracy chromatogram of Olmesartan, Cilnidipine and Chlorthalidone**

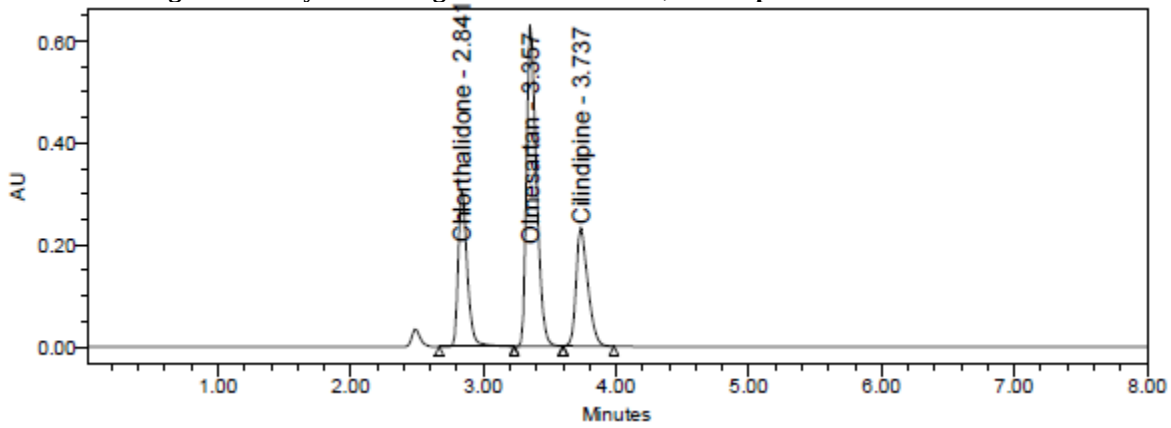


**Fig.5 sample chromatogram of Olmesartan, Cilnidipine and Chlorthalidone**

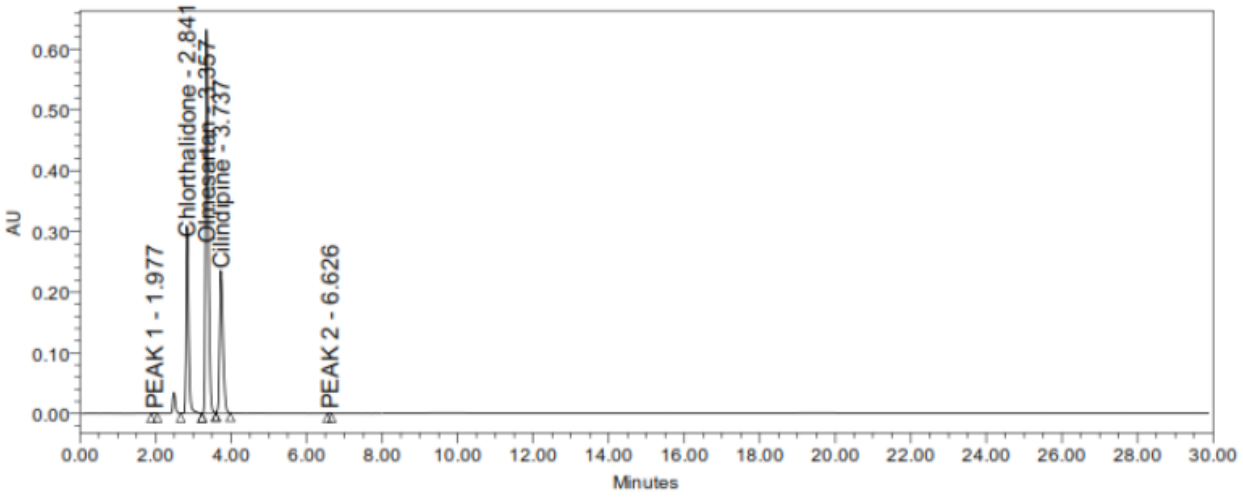


**Fig.6.1 Acid degradation chromatogram of Olmesartan, Cilnidipine and Chlorthalidone**

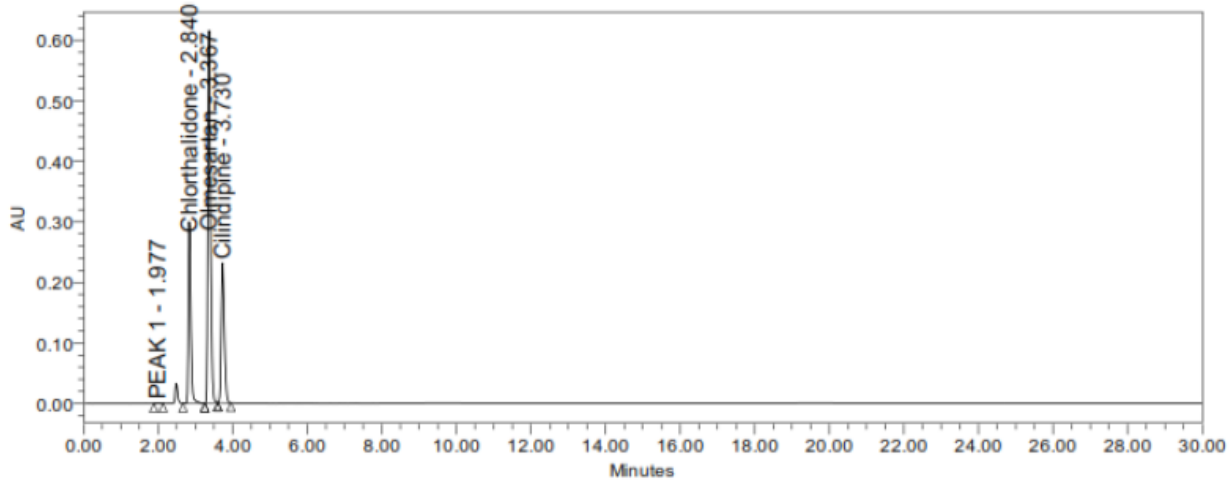


Fig.**6.2 Alkali degradation chromatogram of Olmesartan, Cilnidipine and Chlorthalidone**

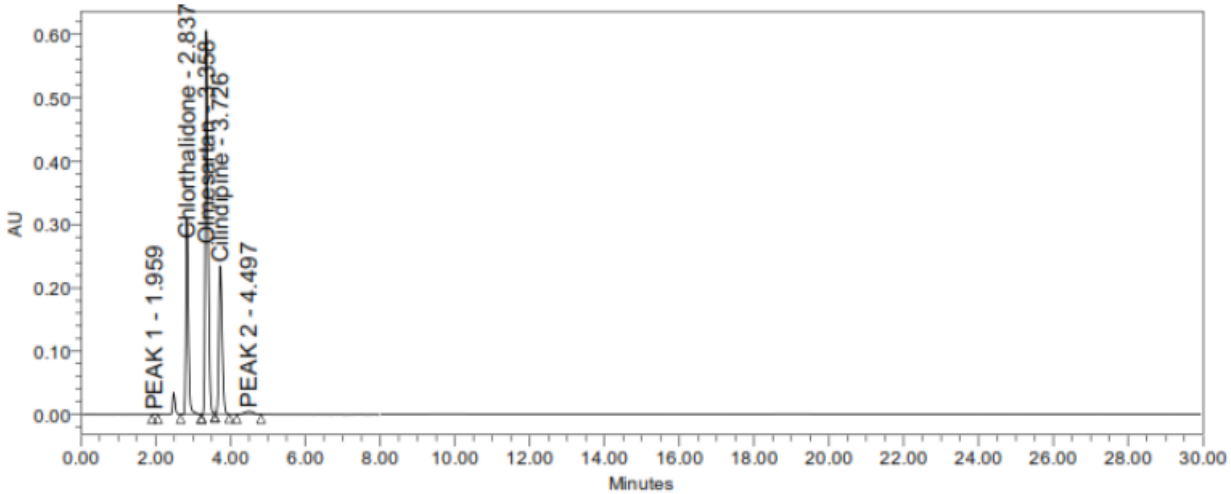


**Fig.6.3 Peroxide degradation chromatogram of Olmesartan, Cilnidipine and Chlorthalidone**

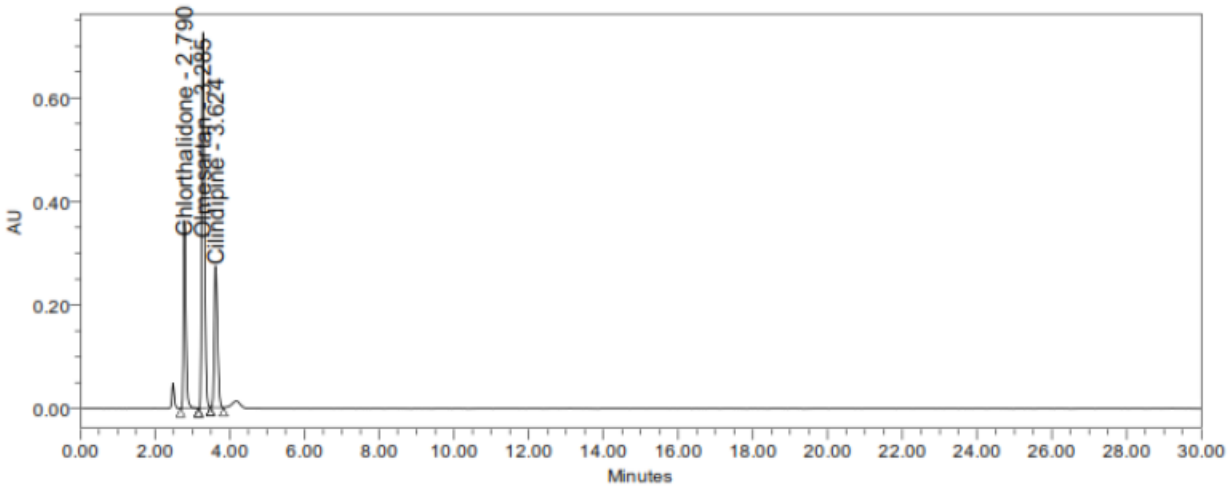


**Fig.6.4 Thermal degradation chromatogram of Olmesartan, Cilnidipine and Chlorthalidone**

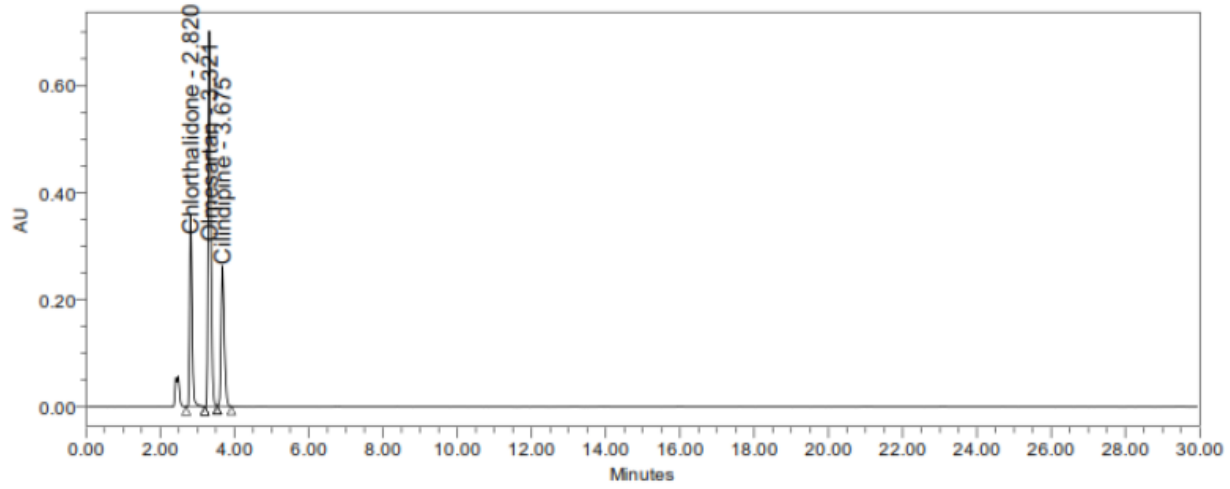


**Fig.6.5 UV degradation chromatogram of Olmesartan, Cilnidipine and Chlorthalidone**

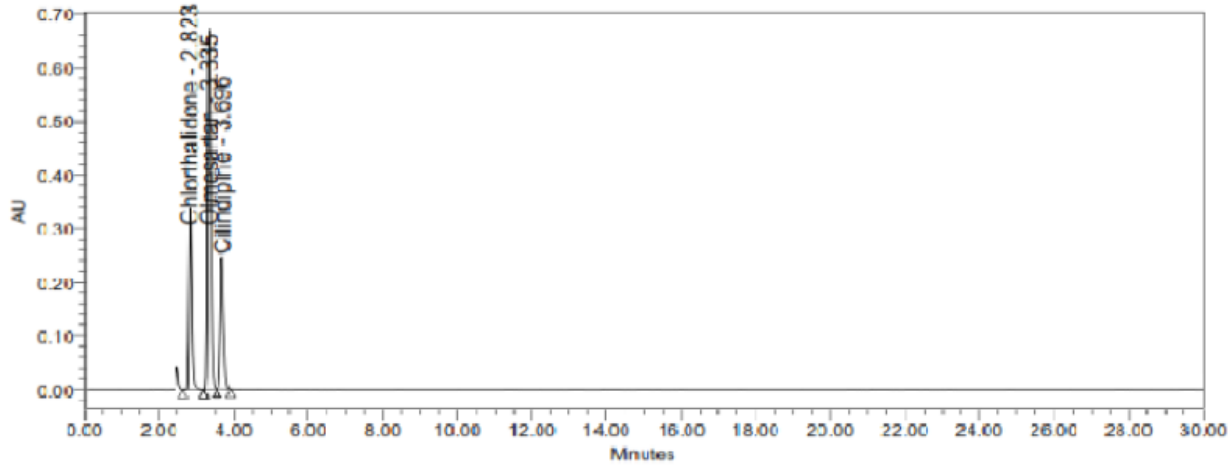


**Fig.6.6 Water degradation chromatogram of Olmesartan, Cilnidipine and Chlorthalidone**

**REFERENCES**


1. Chen X, Ji ZL, Chen YZ: TTD: Therapeutic Target Database. Nucleic Acids Res. 2002 Jan 1;30(1):412-5.
2. Koike H, Sada T, Mizuno M: In vitro and in vivo pharmacology of Olmesartan medoxomil, an angiotensin II type AT1 receptor antagonist. J Hypertens Suppl. 2001 Jun;19(1):S3-14.
3. Ochiai K, Hu Q, Lee J, Mansoor A, Liu J, Wang X, Gong G, Murakami Y, Ishibashi Y, Shimada T, Zhang J: Functional and bioenergetic consequences of AT1 antagonist Olmesartan medoxomil in hearts with postinfarction LV remodeling. J Cardiovasc Pharmacol. 2006 May;47(5):686-94.
4. Warner GT, Jarvis B: Olmesartan medoxomil. Drugs. 2002;62(9):1345-53; discussion 1354-6.
5. Mire DE, Silfani TN, Pugsley MK: A review of the structural and functional features of olmesartan medoxomil, an angiotensin receptor blocker. J Cardiovasc Pharmacol. 2005 Nov;46(5):585-93.
6. Kreutz R, Bolbrinker J, Huber M: Pharmacokinetics of olmesartan medoxomil plus hydrochlorothiazide combination in healthy subjects. Clin Drug Investig. 2006;26(1):29-34.
7. Löhn M, Muzzulini U, Essin K, et al. (May 2002). "Cilnidipine is a novel slow-acting blocker of vascular L-type calcium channels that does not target protein kinase C". *J. Hypertens*. 20 (5): 885–93
8. Takahara, A., et al. 2004. Biol. Pharm. Bull. 27: 1388-1391.
9. Hayashi, K., et al. 2007. Circ. Res. 100: 342-353.
10. Morimoto, S., et al. 2007. J. Hypertens. 25: 2178-2183.
11. Lee, Y.J., et al. 2009. J. Neurochem. 111: 90-100.
12. Beisenherz, et al.: Arch. Int. Pharmacodyn. Ther., 161, 76 (1966).
13. Zsoter, et al.: J. Pharmacol. Exp. Ther., 180, 723 (1972).
14. Singer, J.M., et al.: Anal. Profiles Drug Subs., 14, 1 (1985),
15. Virali thakkar, r.b.patel, h.p.parmar, g.h.patel , p.k.pradhan , u.m.upadhyay, rp-hplc method for simultaneous estimation of olmesartan medoxomil and chlorthalidone in tablet dosage form, *ijpr*, volume 6, issue 4, oct - dec, 2014 page no 63-67.
16. Raveendra babu ganduri, jayachandra reddy peddapapireddigari,new rp-hplc method for the determination of olmesartan medoxomil in tablet dosage form, *international journal of pharma sciences and research* vol 5 no 06 jun 2014 page no 275-282.
17. P. Murali, p. Sunil kumar chaitanya, c. Pavani, g. Rohini reddy and r. Ramalingamder development and validation of a stability-indicating rp-hplc method for the simultaneous quantification of olmesartan medoxomil and chlorthalidone in solid dosage form *pharmacia lettre*, 2014, 6 (6):148-157.
18. Pradip parikh, ujjwal sahoo, arti zanvar, a.k.seth pharma science monitor an international journal of pharmaceutical sciences derivative spectrophotometric method for simultaneous estimation of chlorthalidone and olmesartan medoxomil in their tablet dosage form, sept2013 issn: 0976-7908 parikh et al www.pharmasm. vol - 4, issue - 4, supl – 1.
19. D. Barot, p. K. Pradhan, g. Patel, s. Shah, h. P. Parmar, s. Dey, U. M. Upadhyay "s the simultaneous uv spectrophotometric estimation of olmesartan emedoxomil and chlorthalidone in tablet dosage Form pharma innovation journal 2014; 3(10): 76-806
20. Chaitanya prasad mk, vidyasagar g, Development of rp-hplc method for estimation of olmesartanmedoxomil in tablet dosage forms der pharma chemica, 2011, 3 (6):208-212.
21. Kumanan raghunathan, jitendra m reddy, manasa r. Stability indicating rp-hplc method development and validation of olmesartan medoxomil *asian j phar biol res*. 2011; 1(2):79-86.
22. Syed shanaz qutaba, syed naeem razzaqa,b, muhammad ashfaqb. Simultaneous quantitation of olmesartan medoxomil and amlodipine besylate in Combined tablets using hplc *j chil. Chem. Soc*., 54, no 3 (2009) 234.
23. Brijesh singh, d.k.patel and s.k.ghosh, Department of pharmaceutical sciences a reversed phase high performance liquid chromatographic Method for determination of chlorthalidone ipharmaceutical formulation ijppsjournal.com/vol1issue2/145.
24. P. Hari prasad, P. M. Patel, D. Vijaysree, simultaneous estimation of metoprolol tartrate and chlorthalidone by using rp-hplc and method development as per ich guidelines, *der pharma chemica*, 2013, 5(5):139-14.
25. Neelima Kudumula Development And Validation Of Rp-Hplc Method For The Simultaneous Estimation Of Chlorthalidone And Cilnidipine In Bulk And Combined Tablet Dosage Form Pharmacophore 2014, Vol. 5 (4), 442-450.
26. Sidhdhapara Mital J Development And Validation Of Rp-Hplc Method For Simultaneous Estimation Of Cilnidipine And Olmesartan Medoxomil In Their Combined Tablet Dosage IJPBS ;Volume 4:Issue 1 2014:157-160.