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# Development and Validation of RP-HPLC Method For the Simultaneous Estimation of Ramipril and Olmesartan Medoxomil

**K. Pravalika[1], Sashmitha Samuel.B*[2], M. Bindu[1] and Sravanthi Vanama[1]**

[1]Assistant Professor, Department of Pharmaceutical Analysis, MLRIP, Hyderabad500043

[2]Associate Professor, Department of Pharmaceutical Chemistry, MLRIP, Hyderabad500043

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***Corresponding Author Email:** sashmitha.mlrip@mlrinstitutions.ac.in

## Abstract

An RP-HPLC method was developed for the simultaneous estimation of Ramipril & Olmesartan medoxomil in bulk and tablet dosage forms using Hypersil $C_{18}$ (4.6x250mm,5µm) column. 0.01M Potassium dihydrogen Phosphate pH 3(adjusted with orthophosphoric acid): acetonitrile (40:60) is used as the mobile phase. 228nm is used as the wavelength of detection. Linearity range is 5µg/ml to 25µg/ml for Ramipril & 20µg/ml to 100µg/ml for Olmesartan medoxomil. Retention times for Ramipril & Olmesartan medoxomil were 2.837 & 4.066 respectively. The developed method was validated as per ICH guidelines and the developed method was found to be precise, accurate, sensitive, and economical.

**Keywords**

Acetonitrile, ICH guidelines, Olmesartan medoxomil, Potassium dihydrogen phosphate, Ramipril, RP-HPLC

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## INTRODUCTION

Ramipril is a prodrug belonging to the angiotensin converting enzyme (ACE). It is metabolized to Ramiprilate in the liver and to a lesser extent in the kidneys. Ramiprilate is a potent competitive inhibitor of ACE, the enzyme responsible for the conversion of angiotensin I (ATI) to angiotensin II (ATII). ATII regulates blood pressure and is a key component of the renin-angiotensin-aldosterone system (RAAS).

Olmesartan is an angiotensin receptor blocker (ARB) that selectively inhibits the binding of angiotensin II to AT1, which is found in many tissues such as vascular smooth muscles and adrenal glands. This inhibits the AT1-mediated vasoconstrictive and aldosterone secreting effects of angiotensin II and results in decrease in vascular resistance and blood pressure.

Ramipril and Olmesartan is commonly used to treat high blood pressure and it is proved to be safe when used in combination. Olmy R is the brand name for the combination drug of Ramipril & Olmesartan medoxomil. The dosage form contains 5mg of Ramipril & 20mg of Olmesartan medoxomil. [1,2]

As per literature survey there are many methods [6-16] available for the determination of ramipril and Olmesartan individually and in combination with some other drugs. Only one method is found for this combination. Therefore, the present study has been undertaken in order to develop a new, simple, rapid, efficient and reproducible method for the

simultaneous estimation of Ramipril and Olmesartan.

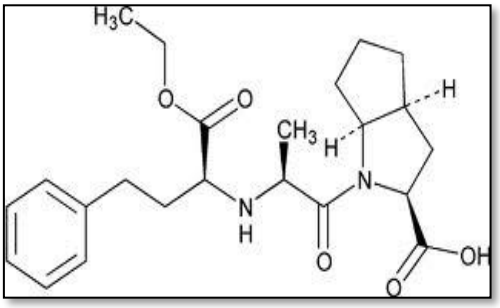

**Fig.1: Structure of Ramipril**

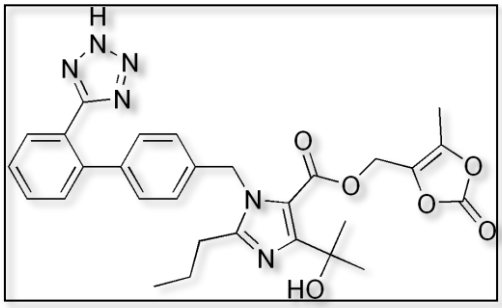

**Fig.2: Structure of Olmesartan**

**MATERIALS AND METHODS**

All the chemicals used in this study are of HPLC grade and the reference standards of Ramipril and Olmesartan medoxomil were obtained as gift samples. Purified water was prepared by using 0.22µ Millipore Milli-Q water purification systems. HPLC grade acetonitrile and methanol (Merck, Mumbai) were used for preparing the mobile phase and the diluent.

**Method Development**

After various trails, the combination of 0.01M potassium dihydrogen phosphate buffer (adjusted to pH 3.00 with orthophosphoric acid): acetonitrile in the ratio 40: 60 was selected as a suitable mobile phase for the simultaneous estimation of Ramipril & Olmesartan.

**Optimized HPLC Chromatographic Conditions**

Equipment: HPLC (Waters)
Column : Hypersil C18 (4.6x250mm,5µm)
Mobile phase: Phosphate buffer (pH 3):
ACN [40:60]
Flow rate: 1ml per min
Wavelength: 228 nm
Injection volume: 20 µl
Column Temperature: Ambient
Run time: 8min.

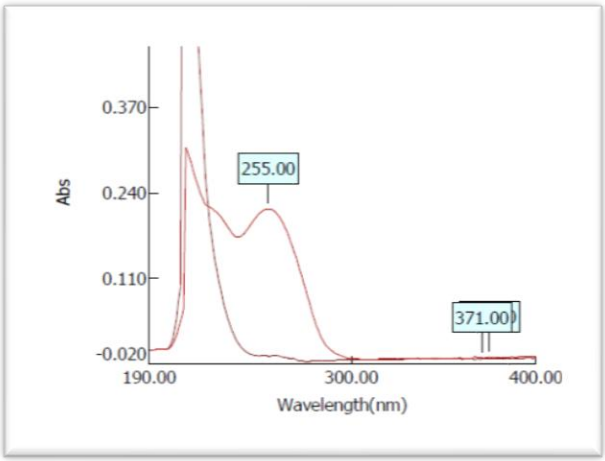


**Fig.3: Overlapped Spectrum of Ramipril & Olmesartan medoxomil**

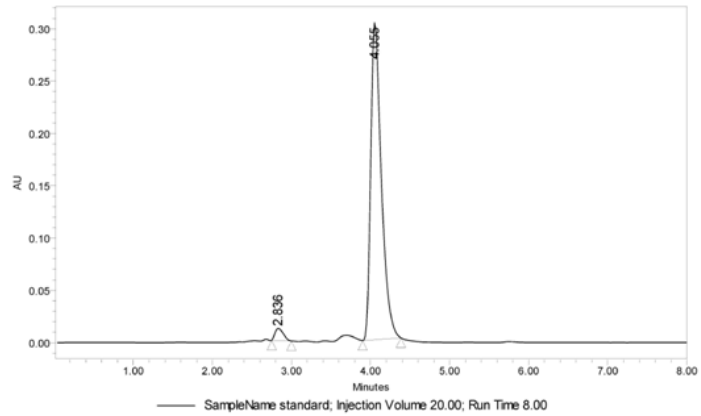


**Fig.4: Optimized method**

| | Peak Name | RT | Area | Height | USP Rate Count | USP Tailing | USP Resolution |
|---|---|---|---|---|---|---|---|
| 1 | Ramipril | 2.836 | 105962 | 11674 | 3041 | 1.34 | |
| 2 | Olmesartan | 4.055 | 3101062 | 303798 | 4361 | 1.50 | 4.05 |

**Table 1: Data of optimized method**

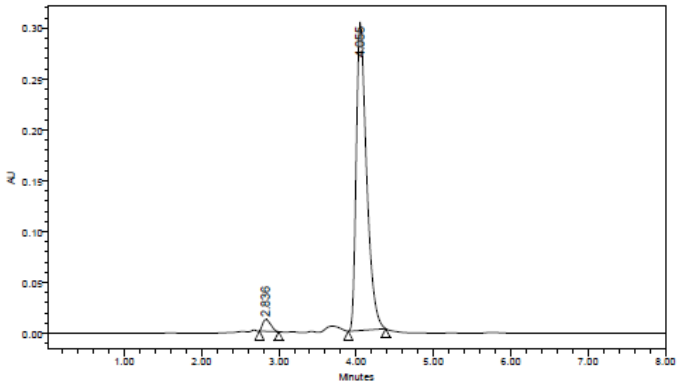


**Fig.5: Chromatogram for System Suitability**

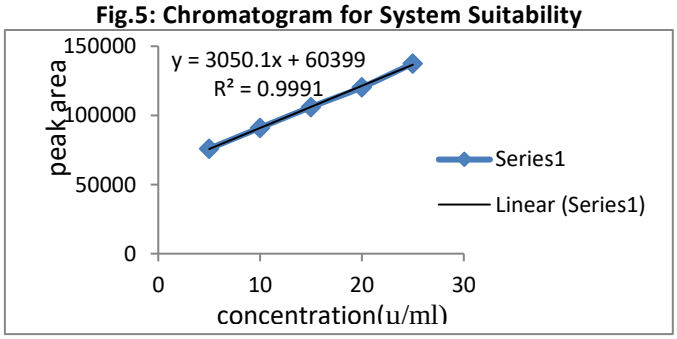


**Fig.6: Linearity graph of Ramipril**

| S.No | Linearity Level | Concentration | Area |
|---|---|---|---|
| 1 | I | 5ppm | 75890 |
| 2 | II | 10ppm | 91024 |
| 3 | III | 15ppm | 106032 |
| 4 | IV | 20ppm | 120298 |
| 5 | V | 25ppm | 137505 |
| Correlation Coefficient | | | 0.999 |

**Table 3: Results of linearity of Ramipril**

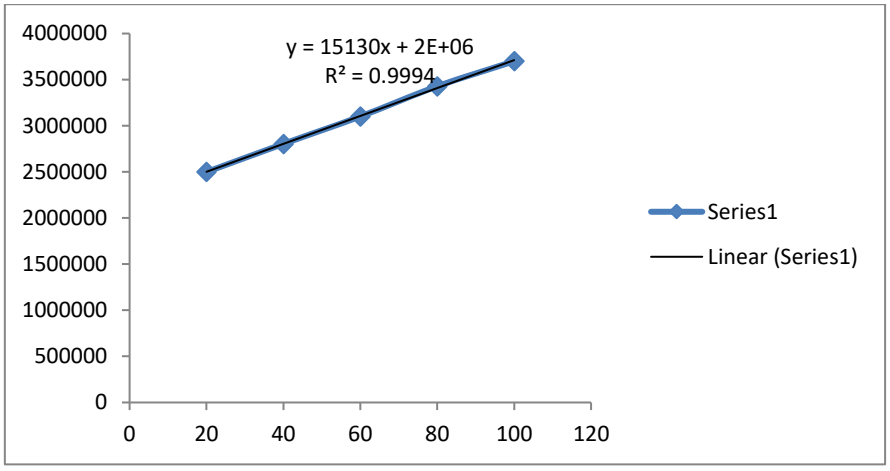


**Fig.7: Linearity graph of Olmesartan**

| S.No | Linearity Level | Concentration | Area |
|---|---|---|---|
| 1 | I | 20ppm | 2498860 |
| 2 | II | 40ppm | 2803763 |
| 3 | III | 60ppm | 3101056 |
| 4 | IV | 80ppm | 3427879 |
| 5 | V | 100ppm | 3699787 |
| Correlation Coefficient | | | 0.999 |

**Table 4: Results of linearity of Olmesartan**

| Injection | Area |
|---|---|
| Injection-1 | 100867 |
| Injection-2 | 102068 |
| Injection-3 | 102123 |
| Injection-4 | 103423 |
| Injection-5 | 101889 |
| Injection-6 | 101942 |
| Average | 102074 |
| Standard Deviation | 910.1253 |
| %RSD | 0.89 |

**Table 5: Results of precision of Ramipril**

| Injection | Area |
|---|---|
| Injection-1 | 3078309 |
| Injection-2 | 3090904 |
| Injection-3 | 3100266 |
| Injection-4 | 3102840 |
| Injection-5 | 3087412 |
| Injection-6 | 3088561 |
| Average | 3091946 |
| Standard Deviation | 9943.746 |
| %RSD | 0.32 |

**Table 6: Results of precision of Olmesartan**

| Injection | Area |
|---|---|
| Injection-1 | 99930 |
| Injection-2 | 101377 |
| Injection-3 | 103567 |
| Injection-4 | 102905 |
| Injection-5 | 101033 |
| Injection-6 | 101324 |
| Average | 101762.4 |
| Standard Deviation | 1466.319 |
| %RSD | 1.44 |

**Table 7: Results of intermediate precision of Ramipril**

| Injection | Area |
|---|---|
| Injection-1 | 3076573 |
| Injection-2 | 3108802 |
| Injection-3 | 3104529 |
| Injection-4 | 3092276 |
| Injection-5 | 3084879 |
| Injection-6 | 3092423 |
| Average | 3093412 |
| Standard Deviation | 13398.64 |
| %RSD | 0.43 |

**Table 8: Results of intermediate precision of Olmesartan**

| %Concentration | Area | Amount Added (mg) | Amount Found (mg) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 55127 | 2.5 | 2.44 | 98.94% | |
| 100% | 109501 | 5 | 5.1 | 101.30% | 99.84% |
| 150% | 171587 | 10.1 | 9.98 | 99.30% | |

**Table 9: Results of recovery studies of Ramipril**

| % Concentration | Area | Amount Added (mg) | Amount Found (mg) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 1550065 | 10 | 10.5 | 101.8% | |
| 100% | 2995918 | 20 | 20.6 | 101.8% | 101.7% |
| 150% | 4505441 | 30 | 30.4 | 101.5% | |

**Table 10: Results of recovery studies of Olmesartan**

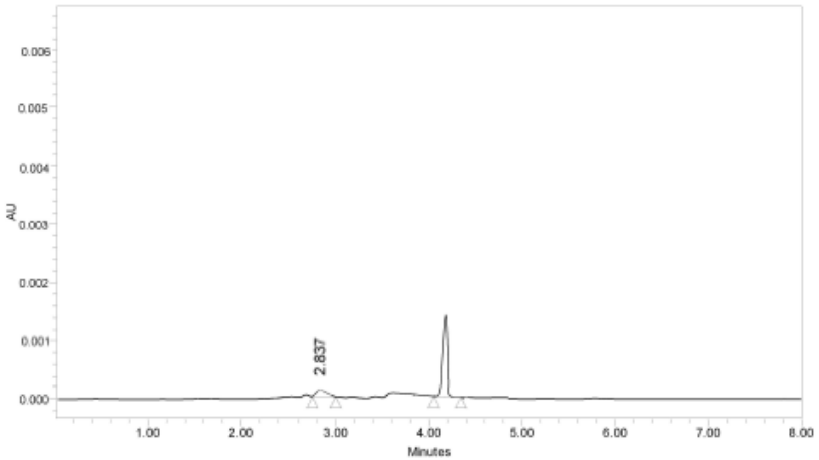


**Fig.8: LOD of Ramipril & Olmesartan**

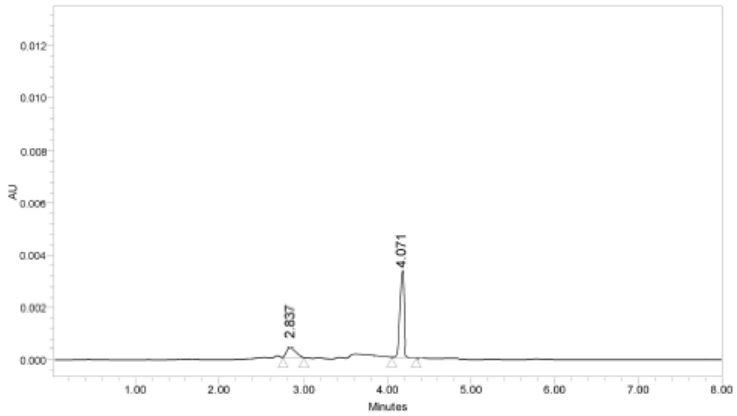


**Fig.9: LOQ of Ramipril & Olmesartan**

| S.No | Tablet Sample | Label claim mg/tablet | %Avg Assay (n=3) |
|---|---|---|---|
| 1 | Ramipril | 5 | 99.2% |
| 2 | Olmesartan medoxomil | 20 | 100.6% |

**Table 11: Results of assay of Ramipril & Olmesartan medoxomil**

**Analytical Method Validation**

Validation of analytical method is a process of establishing documental evidence which provides a high of assurance that a specific process will consistently produce a product of predetermined specifications and quantity attributes.

**Specificity**

The blank solution (Mobile Phase), 15µg/ml Ramipril standard solution, 60µg/ml of Olmesartan standard solution and mixed standard solution of Ramipril and Olmesartan were injected into the chromatographic system and the chromatogram was recorded.

**System Suitability Studies**

15µg/ml Ramipril and 60µg/ml Olmesartan standard solution as per test method were prepared. Six replicate injections were given. Then the system suitability parameters like Resolution, USP tailing and USP plate count were studied with the help of standard chromatogram.

**Linearity:**

**Preparation of stock solution:**

Accurately weigh and transfer 5 mg of Ramipril and 20 mg of Olmesartan working standard into a 100mL clean dry volumetric flask add about 70mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

**Preparation of Level – I (5ppm Ramipril & 20ppm Olmesartan):**

1ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

**Preparation of Level – II (10ppm Ramipril & 40ppm Olmesartan):**

2ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

**Preparation of Level – III (15ppm Ramipril & 60ppm Olmesartan):**

3ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

**Preparation of Level – IV (20ppm Ramipril & 80ppm Olmesartan):**

4ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

**Preparation of Level – V (25ppm Ramipril & 100ppm Olmesartan):**

5ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

**Precision:**

**Preparation of stock solution:**

Accurately weigh and transfer 5mg of Ramipril and 20mg of Olmesartan working standards into a 100ml clean dry volumetric flask add about 70ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette out 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Procedure:**

The standard solution was injected six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

**Intermediate Precision/Ruggedness:**

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by different analyst.

**Preparation of stock solution**:

Accurately weigh and transfer 5mg of Ramipril and 20mg of Olmesartan working standard into a 100ml clean dry volumetric flask add about 70ml of diluent and sonicate to dissolve it completely and make

volume up to the mark with the same solvent. (Stock solution). Further pipette 3ml of Ramipril & Olmesartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Procedure:**

The standard solution was injected six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

**Accuracy:**

**Preparation of Standard stock solution:**

Accurately weigh and transfer 5 mg of Ramipril and 10 mg of Olmesartan working standard into a 100ml clean dry volumetric flask add about 70ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 3ml of Ramipril & Olmesartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Preparation Sample solutions:**

**For preparation of 50% solution:**

Accurately weigh and transfer 2.5mg of Ramipril and 10mg of Olmesartan working standard into a 100ml clean dry volumetric flask add about 70ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock Solution). Further pipette 3ml of Ramipril & Olmesartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**For preparation of 100% solution:**

Accurately weigh and transfer 5mg of Ramipril and 20mg of Olmesartan working standards into a 100ml clean dry volumetric flask add about 70ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 3ml of Ramipril & Olmesartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**For preparation of 150% solution:**

Accurately weigh and transfer 7.5mg of Ramipril and 30mg of Olmesartan working standards into a 100ml clean dry volumetric flask add about 70ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 3ml of Ramipril & Olmesartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Procedure:**

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the amount found and amount added for Ramipril & Olmesartan and calculate the individual recovery and mean recovery values.

**Limit of Detection & Limit of Quantification:**

**Limit of detection**

The minimum concentration at which the analyte can be detected is determined from the linearity curve and standard deviation of response from precision by applying the formula.

Limit of detection = 3.3 σ / S

Where

σ is standard deviation from response

S is slope from calibration curve

The lowest concentration of Ramipril that can be detected, was determined from standard curve was 0.89µg/ml. The lowest concentration of Olmesartan that can be detected, was determined from standard curve was 1.97µg/ml.

**Limit of detection**

Limit of quantification can be obtained from the linearity curve and standard deviation of response from precision by applying the formula.

Limit of quantification=10 σ / S

Where

σ is standard deviation from response

S is slope from calibration curve

The lowest concentration at which peak can be quantified is called LOQ, was found to be 2.98µg/ml for Ramipril and 6.57µg/ml for Olmesartan.

**Robustness:**

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

**Assay**

**Preparation of Ramipril and Olmesartan Standard & Sample Solution:**

**Standard Solution Preparation:** Accurately weigh and transfer 5 mg of Ramipril and 20mg of Olmesartan working standard into a 100ml clean dry volumetric flask add about 70ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 3ml of Ramipril and Olmesartan from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Sample Solution Preparation:**

The average weight of 20 tablets was taken & accurately weigh and transfer equivalent to 5 mg of Ramipril and 20mg of Olmesartan sample [1tab] into a clean dry volumetric flask add about 70ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution). Further pipette 3ml of Ramipril & Olmesartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Procedure:**

Inject 20 µL of the standard & sample for 3 times into the chromatographic system and the amount of Ramipril and Olmesartan present in each tablet marketed sample is calculated by comparing the peak area of standard.

**Calculation:**

Calculate the amount of drug by using the following formula.

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{\text{Avg. Wt.}}{\text{Label Claim}} \times P$$

Where:

AT = Average area counts of sample preparation.
AS = Average area counts of standard preparation.
WS = Weight of working standard taken in mg.
WT = Weight of sample taken in mg.
DT = Dilution of sample solution
DS = Dilution of standard solution
P = Percentage purity of working standard

## RESULTS AND DISCUSSION

The RP-HPLC method has been validated as per ICH guidelines [3-5] for linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ. The system suitability parameters are reported, and all the parameters are found to be within the acceptance criteria.

## CONCLUSION

This work describes RP-HPLC method which has been developed and validated for simultaneous estimation of Ramipril and Olmesartan medoxomil in bulk drug and in combined dosage forms. RP-HPLC separation was achieved on a Hypersil $C_{18}$ column (4.6x150mm,5µm) with the phosphate buffer (0.01M potassium dihydrogen phosphate) pH 3 (adjusted with orthophosphoric acid): acetonitrile (40:60) and detection at 228nm.The flow rate was kept at 1ml/min and injection volume 20µl. The separation was performed at ambient temperature. Retention time of Ramipril and Olmesartan medoxomil was found to be 2.837 & 4.066 minutes respectively. Linearity of the method was found to be 5 to 25ppm (Ramipril) and 20 to 100ppm (Olmesartan medoxomil) respectively. The correlation coefficient of Ramipril was found to be 0.999 & Olmesartan medoxomil is 0.999. Accuracy of the method was determined and was found to be 99.84% to 101.3% for Ramipril and 101.7-101.8% for Olmesartan medoxomil respectively and precision of the method was demonstrated which is less than 2% in all instances. The systemic suitability parameters such as theoretical plates and tailing factor were found to be 3287 &1.24 and 4275 &1.50 respectively for Ramipril and Olmesartan medoxomil. This method was validated according to ICH guidelines and can be used for routine analysis.

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