# Simultaneous estimation of Ramipril and Olmesartan Medoximil by RP-HPLC method

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## Abstract
After thoroughly surveying literature, it was evident that there are few analytical methods reporting for the estimation of ramipril and olmesartan medoximil by reverse phase HPLC method. At present the new formulation ramipril and olmesartan medoximil is obtained in the market. At present, there is no specific and economical method determination of ramipril and olmesartan medoximil in a combined pharmaceutical dosage form. So there arises a need for developing a sensitised analytical method for the simultaneous estimation of ramipril and olmesartan medoximil in many pharmaceutical dosage forms which are commonly obtained in the market.

**Keywords:** Ramipril, Olmesartan Medoximil, RP-HPLC, Tablets.

## Introduction
Ramipril: Binding and inhibiting activity is done by ACE inhibitors, which have a greater inhibitory activity towards the C-domain. Active metabolite of Ramipril is Ramipril at, which combats with AT I to bind for ACE by inhibiting and conversion of ATI to ATII which is an enzymatic proteolysis. There is a proportionate reduction in blood pressure when there is decrease in the AT II levels and there by inhibition of the pressor effects of ATII. There is also a proportionate increase in plasma renin activity because of the loss of feedback inhibition mediated by AT II on the stimulation of the reflex mechanisms via baroreceptors and release of renin.

Olmesartan: This is a type of an ARB where we observe the selective inhibition of binding of the AT II to AT I, which can be observed in many tissues such as vascular smooth muscle and the adrenal glands. This preferentially blocks the AT I mediated vasoconstriction and aldosterone- secreting effects of angiotensin II and as a result we observe the decrease in the vascular resistance as well as the blood pressure. Olmesartan is selective for AT I and has an affinity 12,500 times greater for AT I than the AT II receptor.

## Materials and Methods
Chromatographic Conditions: 2489 UV-Visible detector and Waters e2695 separation module with the HPLC instrument provided with a Inertsil C8 column (100 mm x 4.6 mm; 5m), auto-injector, the auto sampler with Empower software from Waters Corporation, Milford USA was employed in the study. HPLC grade acetonitrile, was procured from Ranbaxy, India, and Potassium dihydrogen phosphate, Di potassium hydrogen orthophosphate, potassium hydroxide, ortho phosphoric acid AR grade was procured from SD Fine Chem Mumbai, India was employed in the study.

Reverse Phase Chromatography technique: Reversed-phase liquid chromatography (RPLC) is beneficial method for the determination of the pharmaceutical compounds for multiple reasons because of its compatibility with aqueous and organic solutions along with different detection systems and its repeatability and high consistency. RPLC analysis is sensitised and accurate, whether it can be a pharmaceutical or bioanalytical field, preferentially has the use of stationary phases which give symmetrical and efficient peaks being obtained.

### Drug Samples
Formulation samples were procured from the local market and Reference samples were procured from Bio-Leo Analytical Labs India Pvt Ltd, Hyderabad, India, and the mobile phase.

Accurately mixed and weight 0.4g of dipotassium hydrogen phosphate and 1.6g of potassium dihydrogen phosphate in 1000ml of water and adjust the pH 6.8 with dilute potassium hydroxide and acetonitrile in the ratio of 65:35 v/v was to be degassed after filtering through a 0.22mm membrane filter. The mobile phase is a diluent for preparing the working solution of the drug. Flow rate of the mobile phase was maintained constantly at 1ml/min. The column temperature was constantly maintained at 30°C and the determining of the drug was carried out at the wavelength 219nm.

Preparation of working stock and working standard solution of Ramipril and Olmesartan Cubis precision balance MSE524P-1CE-DA is used and weight about 5 mg of Ramipril and 20 mg of Olmesartan and to be transferred into a 50ml volumetric flask and the solution was to be sonicated, which was diluted with the mobile phase to get a working standard solution of 100 g/ml Ramipril and 400 g/ml Olmesartan. Furtherly

10 ml diluted to 100 ml with mobile phase gives 10 g/ml Ramipril and 40 g/ ml.

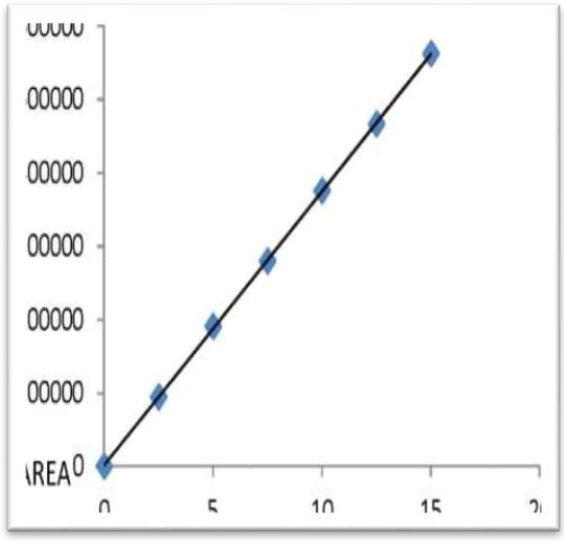
## Results and Discussions

- **Method of development:** development and optimization were done using different mobile phase compositions and columns. Some of the trails show less resolution, peak asymmetry and less theoretical plates show less than 2000. Out of all trails, one trial shows good peak shape, good efficiency, and resolution. This trail conducted by using acetonitrile: phosphate buffer atpH 6.8 in the ratio of 35:65 v/v with inertcil C8 (100x 4.6 mm x 5 m), column at a flow rate of 1 mL/min and determined wavelength was 219 nm. The retention times of Ramipril was found to be 2.28 min and Olmesartan was found to be 3.76 min.
- **Chromatographic conditions:** The mobile phase is isocratic and has a buffer (pH -2.0), methanol and acetonitrile (30:20:50% v/v/v), flowing through the Inertsil ODS C18 column (make: 150 mm x 4.8 mm i.d; particle size 5µm) at a constant flow rate of 1.0 ml/min at column temperature which is ambient. The mobile phase was pumped through the column at a flow rate of 1.0ml/min with a sample injection volume of 10µl. Detection of the analytes was carried out at a wavelength of 267 nm.

**Optimized chromatographic conditions for assay**

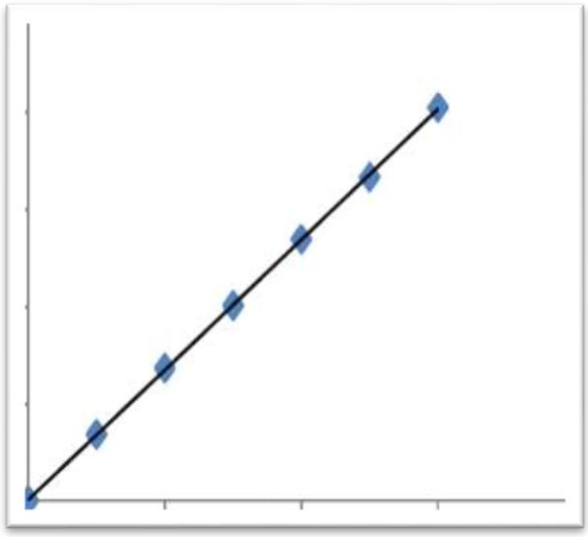
Waters e2695 separation module with the high pressure liquid chromatographic instrument provided with a Inertsil ODS 3 column (100 mm x 4.6 mm; 5m and 2489 UV-Visible detector, auto-injector, autosampler with Empower 3 software from Waters Corporation, Milford USA was employed in the study. HPLC grade acetonitrile, was purchased from Merck, Mumbai, India and Potassium dihydrogen phosphate, Di potassium hydrogen orthophosphate, potassium hydroxide, orthophosphoric acid ACS,ISO,Reag.Ph Eur grade were purchased from Merck Mumbai, India was used in the study.

**Linearity of Ramipril** y = 37345x + 1546.2
**$R^2$=0.99999**




**Concentration (^g/ml)**

**Linearity of Olmesartan** y = 33551x + 5231.2
**R2 = 0.9999**



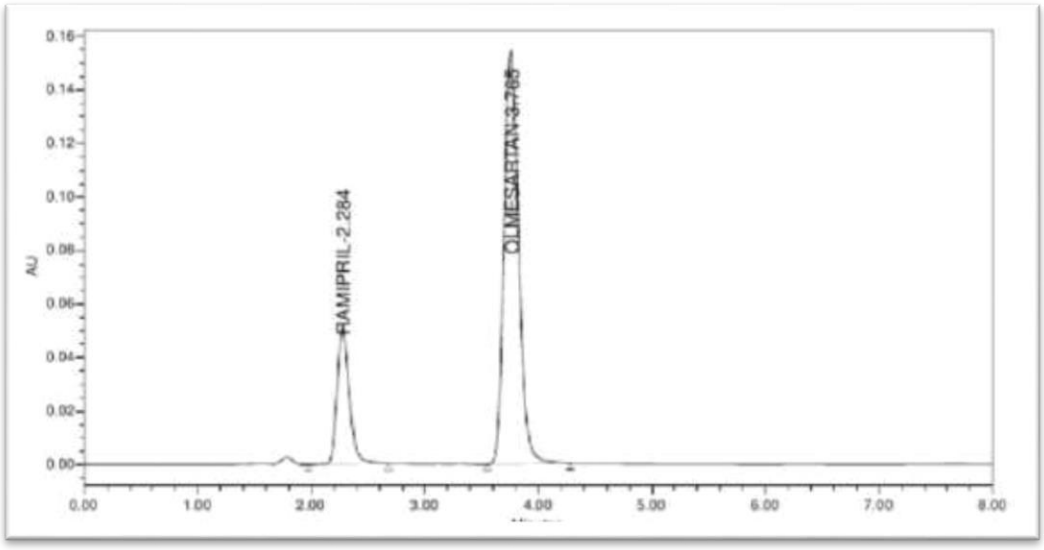
**Concentration (^g/ml)**

| | Peak | RT | Area | Height | % | Resolution | Symmetr | USP Plate |
|---|---|---|---|---|---|---|---|---|
| 1 | Ramipril | 2.284 | 381237 | 50656 | 21.78 | | 1.22 | 2434 |
| 2 | Olmesartan | 3.765 | 1364023 | 154786 | 78.22 | 7.06 | 1.16 | 4243 |

**Acceptance criteria**

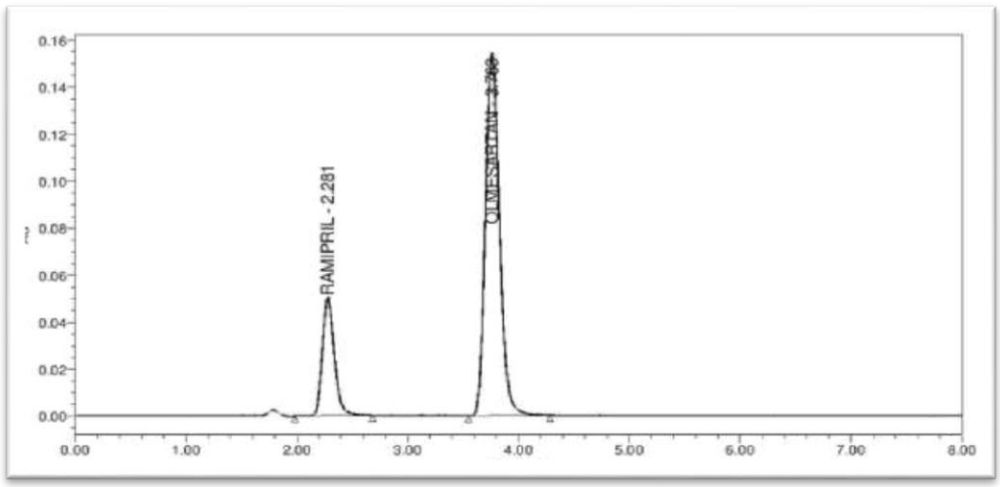
This is the relationship between the concentration (%) of RAMI and OLMI and area of RAMI and OLMI should be linear in the specified range and the correlation should not be less than 0.99.

**Prerision (Repeatability):**




**System Precision:**

| | Peak Name | RT | Area | Height | % Area | Resolution | Symmetry Factor | USP Plate Count |
|---|---|---|---|---|---|---|---|---|
| 1 | Ramipril | 2.281 | 379787 | 50571 | 21.78 | | 1.23 | 2191 |
| 2 | Olmesartan | 3.762 | 1363665 | 154754 | 78.22 | 7.03 | 1.16 | 4275 |



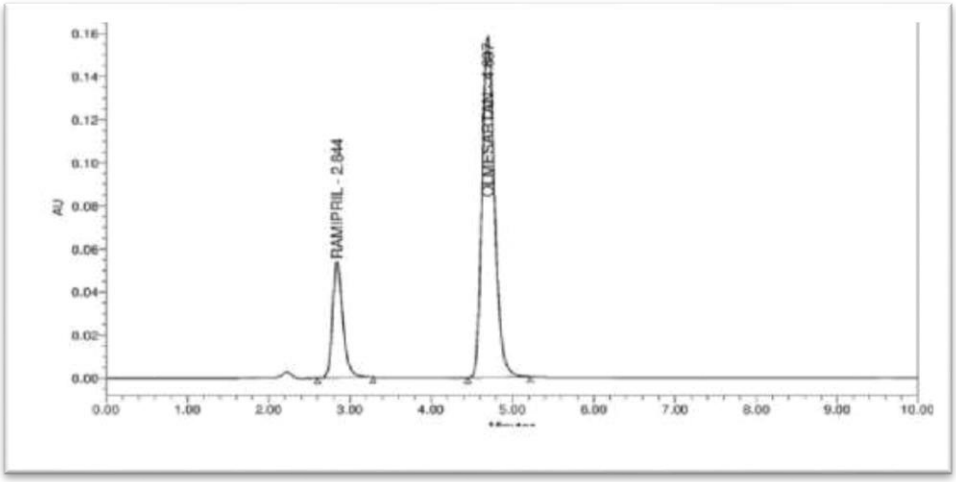

**Acceptance criteria:**

The results of the plate count and tailing factor are found to be within the limit and the % RSD was found to be less than 2%.

**150% Spiked**

| S. No | Name | Vial | Inj | Retention Time | Area |
|---|---|---|---|---|---|
| 1 | RAMI Accuracy150% | 30 | 1 | 2.283 | 565466 |
| 2 | RAMI Accuracy150% | 30 | 2 | 2.285 | 565977 |
| 3 | RAMI Accuracy150% | 30 | 3 | 2.286 | 565032 |
| Mean | | | | 2.28 | 565491.7 |
| Std. Dev | | | | 0.002 | 473 |
| %RSD | | | | 0.1 | 0.1 |

**Robustness:** The robustness of the method developed was evaluated by altering few experimental conditions and evaluating the resolution between two adjacent peaks of ramipril and olmesartan.
Robustness Flow -1(std) (0.8ml/min)




| Peak Name | RT | Area | Height | % Area | Resolution | Symmetry Factor | USP Plate Count |
|---|---|---|---|---|---|---|---|
| Ramipril | 2.844 | 472613 | 53963 | 21.72 | | 1.29 | 2530 |
| Olmesartan | 4.697 | 1702930 | 158438 | 78.28 | 7.36 | 1.21 | 4462 |

**Acceptance criteria:** RSD value is within the limit.

**%Assay and Recovery Data:**

| Amount claim(mg/tablet) | Sample | Amt. found by proposed method | | | %Recovery | | |
|---|---|---|---|---|---|---|---|
| | | 50%Lev | 100LEV | 150%LEV | 50%LEV | 100LEV | 150%LEV |
| Ramipril-5 mg | 1. | 4.991 | 9.986 | 15.008 | 99.82 | 99.86 | 100.05 |
| Olmesartan-20mg | 2. | 20 | 39.94 | 59.376 | 100 | 99.85 | 99.96 |

**Validation Parameters:**

| S.NO | Parameters | Acceptance criteria | Observed Values | | | |
|---|---|---|---|---|---|---|
| | | | Ramipril | | Olmesartan | |
| 1. | SYSTEM Suitability Theoretical plates | NLT-2000 | 2186 | | 4268 | |
| | > Tailing factor | T >0.5 and < 2 | **1.2** | | **1.1** | |
| | ^ Retention time | | 2.28 | | 3.76 | |
| | > Resolution | R of > 2 | | | 7.03 | |
| | > %Area | | 21.26 | | 78.24 | |
| 2. | Assay | NLT-98% | 99.88% | | 99.91 | |
| 3. | Specificity | | 2.281 | | 3.76 | |
| 3. | Linearity Correlation coefficient | NLT 0.99 | 0.9999 | | 0.9999 | |
| 4. | Method precision | %RSD NMT 2 | RT 1 (RA) | RT2(OL) | AREA1(RA) | AREA2(OL) |
| | | | 0.04 | **0.1** | **0.6** | **0.1** |
| 4. | System precision | %RSD NMT 2 | **0.1** | **0.1** | 0.4 | 0.5 |
| 5. | Accuracy 50% | NLT 98% | 99.82% | | 99.85% | |
| 6. | Accuracy 100% | NLT 98% | 99.86% | | 99.85% | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 7. | Accuracy 150% | NLT 98% | **100**% | | 98.96% | |
| 8. | Ruggedness | % RSD NMT 2 | **0.1** | **0.1** | **0.1** | 0.3 |
| 9. | Robustness-Flow-**0**.8ml/min | % RSD NMT 2 | 0.7 | **0.2** | 0.7 | **0.2** |
| 10. | Robustness-Flow-**1**.2ml/min | % RSD NMT **2** | 0.5 | 0.3 | **0.8** | **0.1** |
| 11. | Robustness buffer-63%v/v | %RSD NMT 2 | 0.4 | **0.2** | **0.1** | **0.1** |
| 12. | Robustness buffer-67%v/v | %RSD NMT 2 | 0.4 | **0.2** | **0.2** | **0.1** |
| 14. | LOD( mcg/ml) | | 0.145 | | 0.6032 | |
| 15. | LOQ(mcg/ml) | | 0.4319 | | 1.8285 | |

## Summary and Conclusion

We have developed a consistent, simple, accurately done RP-HPLC method for the determination of Ramipril and Olmesartan in a tablet dosage form. The separation was eluted on Inertsil ODS C18 column (100x4.6 mm;5) using a mobile phase mixture of acetonitrile and mixed phosphate buffer 6.8 in a ratio of 35:65v/v at a flow rate of 1.0 ml/min .The determination was made at the wavelength 219 nm. The retention times was 2.28 min for Ramipril and 3.76 min for Olmesartan. Caliberation curve was linear over the concentration range of 2.5-15g/ml for Olmesartan. The present method being validated as per ICH guidelines parameters like Linearity, Specificity, Precision, Accuracy, Robustness, Ruggedness and Lod & Loq. This is the method where we can be precise, specific and rapid proved to be preferential for the quantitative analysis of the dosage form and drug.

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