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A NEW VALIDATED RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF RAMIPRIL AND OLMESARTAN MEDOXOMIL

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ABSTRACT

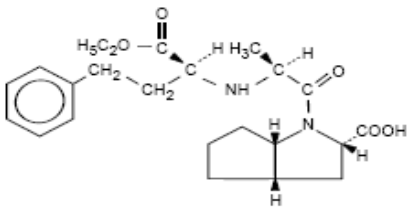
The present work describes a validated reverse phase high performance liquid chromatographic method for simultaneous estimation of Ramipril and and Olmesartan medoxomil in tablet formulation. Chromatography was performed on a Hypersil C18 (4.6mmx250mm, 5µm) column from inisocratic mode with mobile phase containing acetonitrile: 0.05 M KH_2PO_4 pH 3.0 (60:40). The flow rate was 1.0 ml/min and the eluent was monitored at 228 nm. The selected chromatographic conditions were found to effectively separate Ramipril (RT- 2.836 min) and Olmesartan (RT- 4.055 min). Linearity for Ramipril and Olmesartan medoxomil were found in the range of 5ppm-25ppm and 20ppm -100ppm respectively. The proposed method was found to be accurate, precise, reproducible and specific. The mean recovery was 99.84 ± 0.20% and 101.7 ± 0.20% for ramipril and olmesartan medoxomil respectively. The methods were validated according to the ICH guidelines.

Keywords: Ramipril, Olmesartan medoxomil and RP-HPLC

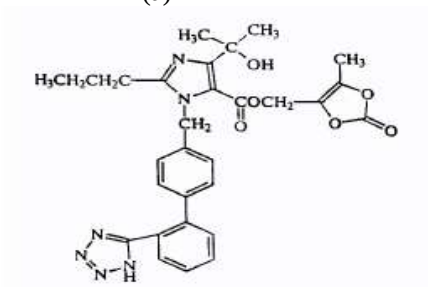
INTRODUCTION

Ramipril's chemical name is (2S, 3aS, 6aS) -1[(S)-N-[(S) -1-Carboxy-3-phenylpropyl] alanyl] octahydrocyclopenta[b] pyrrole-2-carboxylic acid, 1-ethyl ester. Ramipril is an angiotensin-converting enzyme (ACE) inhibitor.It is an inactive prodrug and is converted to ramiprilat in the liver and is used to treat hypertension and heart failure, reduces proteinuria renal disease in patients with nephropathies, prevents stroke, myocardial infarction, and cardiac death in high-risk patients.

Ramiprilat is an active metabolite which competes with angiotensin I for binding at the angiotensin-converting enzyme, blocking the conversion of angiotensin I to angiotensin II [1]. As angiotensin II is a vasoconstrictor and a negative-feedback mediator for renin activity, lower concentrations result in a decrease in blood pressure and an increase in plasma rennin. Ramiprilat may also act on kininase II, an enzyme identical to angiotensin-converting enzyme that degrades the vasodilator bradykinin [2].



(a)



(b)

Fig.1: a. Structure of Ramipril b. Structure of Olmesartan medoxomil

Olmesartan medoxomil is described chemically as 2, 3-dihydroxy-2-butenyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-[p-(o-1H-tetrazol-5-ylphenyl) benzyl] imidazole-5-carboxylate, cyclic 2, 3-carbonate.Olmesartan medoxomil, a specific angiotensin II type 1 antagonist, used alone or in combination with other antihypertensive agents to treat hypertension. Blockade of the angiotensin II receptor inhibits the negative regulatory feedback of angiotensin II on renin secretion, but the resulting increased plasma renin activity and circulating angiotensin II levels do not overcome the effect of olmesartan on blood pressure. Angiotensin II is formed from angiotensin I in a reaction catalyzed by angiotensin converting enzyme (ACE, kininase II) [3]. Angiotensin II is the principal presser agent of the renin-angiotensin system, with effects that include vasoconstriction, stimulation of synthesis and release of aldosterone, cardiac stimulation and renal reabsorption of sodium [4]. Olmesartan blocks the vasoconstrictor effects of angiotensin II by selectively blocking the binding of angiotensin II to the angiotensin 1 receptor in vascular smooth muscle [5, 6].

OBJECTIVES AND PLAN OF THE WORK

Objectives of Work

Ramipril and Olmesartan is commonly used to treat high blood pressure and it is proved to be safe when used in combination. As per literature survey there are many methods 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.

Plan of Work

i) To develop a method for the combination of Ramipril and Olmesartan in tablet dosage form by RP – HPLC.
ii) To validate the developed RP – HPLC method

EXPERIMENTAL

Analysis was performed by using HPLC, column used is Xterra C8 (4.6 x 250mm,5µm), with the flow rate of 1ml per min and trails were runned by taking five different mobile phases and remaining parameters (trail 1 – trail 5) are to be constant in to consideration. Finally, the optimized conditions were to be

Equipment: High performance liquid chromatography UV detector
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 oven : Ambient
Run time : 8min

METHOD DEVELOPMENT

Preparation of phosphate buffer:

Weighed 7.0 grams of KH_2PO_4 into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjust the pH to 3 with ortho phosphoric acid.

Preparation of mobile phase:

Mix a mixture of above buffer 400 ml (40%) and 600 ml of Acetonitrile HPLC (60%) degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

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.

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.
As mentioned in Table no- 1 and 2

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. As mentioned in Table no- 3 and 4.

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.
As mentioned in Table no-3 and 4.

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. As mentioned in Table no-3 and 4.

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.

PRECISION:
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 for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. As mentioned in Table no-5 and 6.

INTERMEDIATE PRECISION/RUGGEDNESS:
To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions.

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 for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. As mentioned in Table no-7 and 8.

LIMIT OF DETECTION & LIMIT OF QUANTIFICATION:
Limit of detection
Limit of detection is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental

conditions.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 Quantification
Limit of Quantification is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. Several approaches for determining the quantification limit are possible, depending on whether the procedure is a non-instrumental or instrumental.

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 1.97µg/ml for Ramipril and 6.57µg/ml for Olmesartan.

The data of LOD and LOQ is given in table no. 9

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.

a). The flow rate was varied at 0.9 ml/min to 1.1ml/min.
Standard solution 15ppm of Ramipril & 60ppm of Olmesartan was prepared and analysed using the varied flow rates along with method flow rate. The results are summarized. On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is not robust even by change in the flow rate ±10%. The method is robust only in less flow condition. As mentioned in Table no-10 and 11.

b). The Organic composition in the Mobile phase was varied from 50% to 70%.
Standard solution 15µg/ml of Ramipril & 60µg/ml Olmesartan was prepared and analysed using the varied mobile phase composition along with the actual mobile phase composition in the method. The results are summarized. On evaluation of the above results, it can be concluded that the variation in 10% Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is not robust even by change in the Mobile phase ±10%. As mentioned in Table no-12 and 13.
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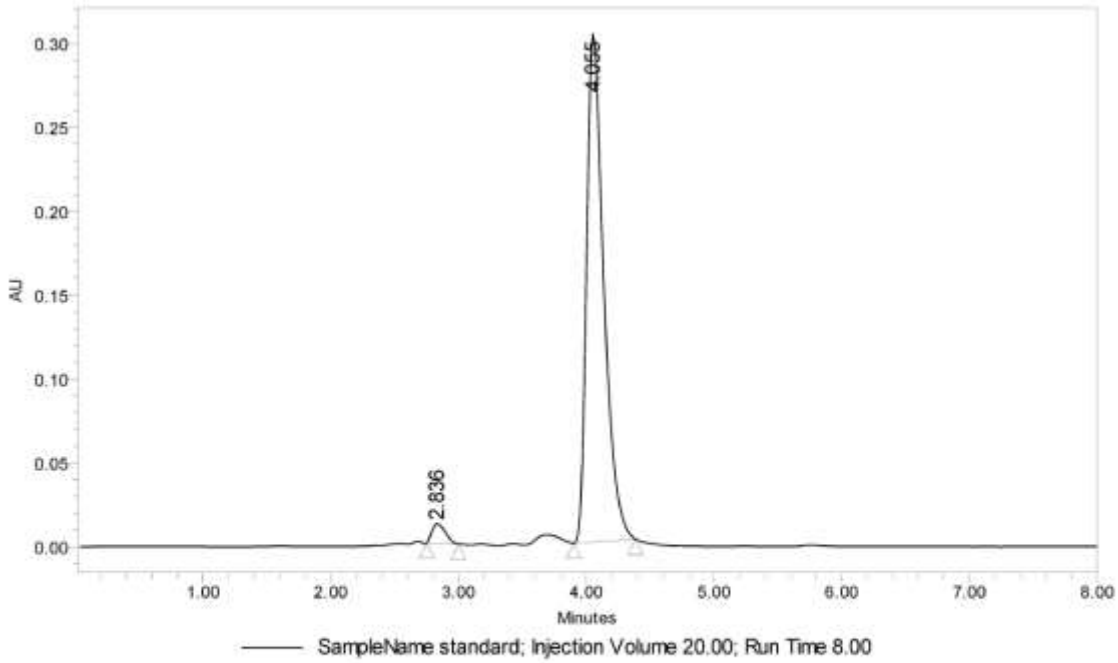
RESULTS AND DISCUSSION:

CHROMATOGRAPHIC PARAMETERS: TRAIL 6 (optimised trail)

Equipment	: High performance liquid chromatography UV detector
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 oven	: Ambient
Run time	:8min

OBSERVATION: Peaks obeying all system suitability parameters as shown in the figure below



	Peak Name	RT	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Ramipril	2.836	84443	11674	3041	1.34	
2	Olmisartan	4.055	2806834	303798	4361	1.53	4.05

Fig no. 2: chromatogram of Ramipril and Olmesartan (Trail 6)

LINEARITY:

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 no-1: Summary of Linearity: (Ramipril)

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 no-2: Summary of linearity: (Olmesartan medoxomil)

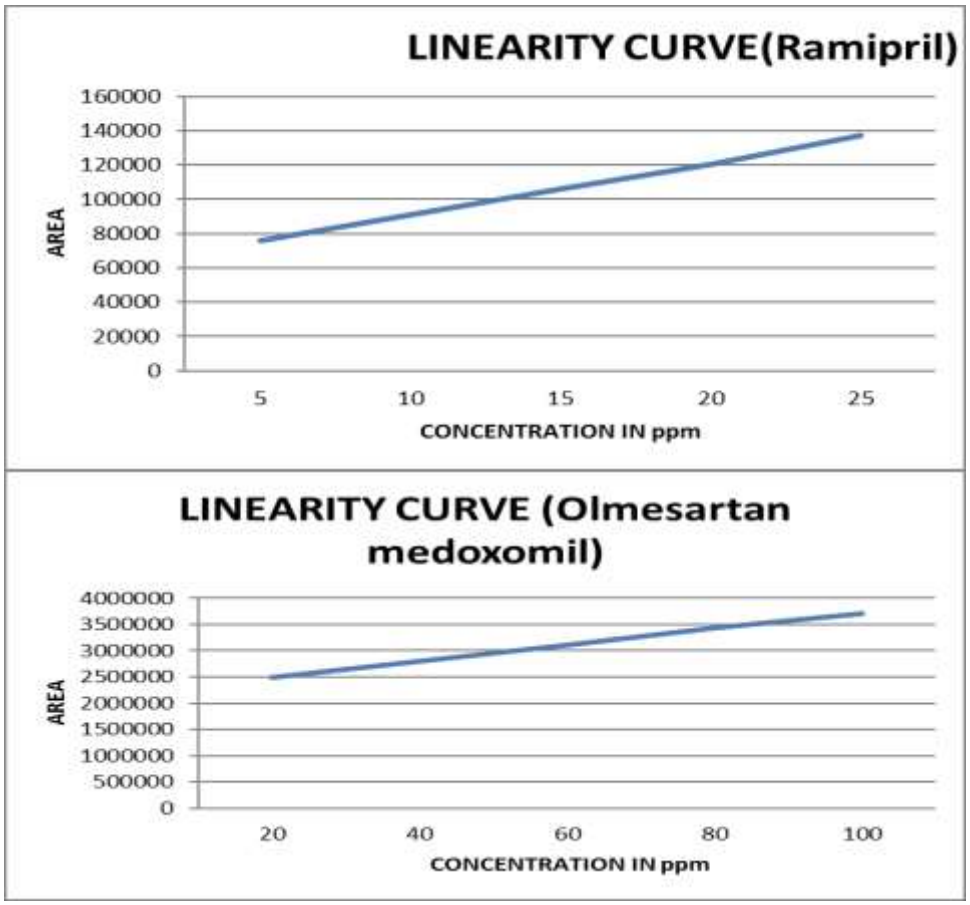


Fig 3: Linearity graphs

Acceptance Criteria:

Correlation coefficient should be not less than 0.999.

ACCURACY:

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

Table no-3: Summary of Recovery studies: (Ramipril)

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

Table no-4: Summary of Recovery studies: (Olmesartan medoxomil)

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102.0%

PRECISION:

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

Table no-5: Summary of precision: (Ramipril)

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

Table no-6: Summary of precision: (Olmesartan medoxomil)

Acceptance Criteria:
The % RSD for the area of five standard injections results should not be more than 2%

INTERMEDIATE PRECISION:

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

Table no-7: Summary of Intermediate precision: Ramipril)

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

Table no-8: Summary of Intermediate precision :(Olmesartan medoxomil)

Acceptance Criteria: The % RSD for the area of five standard injections results should not be more than 2%

LIMIT OF DETECTION & LIMIT OF QUANTIFICATION

S.No	Sample	LOD(µg/ml)	LOQ(µg/ml)
1	**Ramipril**	**0.89**	**2.98**
2	**Olmesartan**	**1.97**	**6.57**

Table no. 9 : LOD & LOQ data of Ramipril & Olmesartan medoxomil

ROBUSTNESS:

The flow rate was varied at 0.9 ml/min to 1.1ml/min.

On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is not robust even by change in the flow rate ±10%.

The method is robust only in less flow condition.

	Flow rate(ml/min)	Retention Time	USP Plate Count	USP Tailing
1	0.9	3.140	2774	1.24
2	1.0	2.837	3254	1.33
3	1.1	2.585	3287	1.36

Table no-10: Summary of Robustness: (Ramipril)

	Flow rate(ml/min)	Retention time	USP Plate Count	USP Tailing
1	0.9	4.506	4162	1.52
2	1.0	4.066	4275	1.53
3	1.1	3.709	4324	1.54

Table no-11: Summary of Robustness: (Olmesartan medoxomil)

The Organic composition in the Mobile phase was varied from 50% to 70%.

The results are summarized: On evaluation of the results, it can be concluded that the variation in 10% Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is not robust even by change in the Mobile phase ±10

S.No	Change in organic composition in the mobile phase	Retension time	USP Plate Count	USP Tailing
1	10% less	2.875	2866	1.24
2	Actual	2.863	3287	1.24
3	10% more	2.821	3306	1.45

Table no-12: Summary of Robustness: (Ramipril)

S.No	Change in organic composition in the mobile phase	Retention Time	USP Plate Count	USP Tailing
1	10% less	4.614	4244	1.55
2	Actual	4.072	4275	1.53
3	10% more	3.709	4261	1.52

Table no-13: Summary of Robustness: (Olmesartan medoxomil)

CONCLUSION

This project describes Reversed-phase high-performance liquid chromatographic 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(4.6x150, 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 & 4.055 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 &1.53 respectively for Ramipril and Olmesartan medoxomil. This method was validated according to ICH guidelines and can be used for routine analysis.

REFERENCES

1. Kukushkin S K, Lebedev A V, Manoshkina E M, Shamarin V M Ramipril effects on 24-hour profile of blood pressure in patients with mild and moderate hypertension. J Pharm Biomed Anal, 1998;70(9): 69-71.
2. Reinhard H A., Becker M D, Bernard M D, Sch.lkens M D Ramipril: Review of pharmacology. J Clin Pharmacol, 1987;59(10): 3-11.
3. Strawn W B, Chappell M C, Dean R H Inhibition of early atherogenesis by losartan in monkeys with diet-induced hypercholesterolemia, J.Pharm.Biomed Anal.,2000;101(7):1586.
4. P.chler K, Laeis P, Stumpe K O A comparison of the efficacy and safety of the oral angiotensin II antagonist Olmesartan medoxomil with those of atenolol inpatients with moderate to severe hypertension under continuous treatment with hydrochlorothiazide. J Pharm Biomed Anal , 2001;19 (2): 153.
5. http://www.fda.gov/medwatch/SAFETY/2004/nov_PI/Benicar_Tab_PI.pdf
6. Ball K, A Multicentre, double-blind, efficacy, tolerability and safety study of the oral angiotensin II antagonist Olmesartan medoxomil versus losartan in patients with mild to moderate essential hypertension. J Pharm Biomed Anal, 2001;19 (2): 153.
7. Zhimeng Z, Andre V, Len N,Liquid chromatography–mass spectrometry method for determination of Ramipril and its active metabolite ramiprilat in human plasma. J Chromatogr B,2002;779(2): 297-306.
8. Magda M, Ayad A, Shalaby E, Abdellate F ,Spectrophotometric and AAS determination of Ramipril and Enalapril through ternary complex formation J PharmBiomed Anal, 2002;28(2): 311. Eurasian J. Anal. Chem., 2007 ,2(3), **171**
9. Hillaert S, Grauwe K, Bossche W Simultaneous determination of hydrochlorothiazide and several inhibitors of angiotensin-converting enzyme by capillary electrophoresis. J Chromatogr A, 2001;924 (1):439.
10. Belal F, Al-Zaagi A, Gadkariem E, Abounassif M ,A stability-indicating LC method for the simultaneous determination of ramipril and hydrochlorothiazide in dosage forms J Pharm Biomed Anal, 2001;24(3):335.
11. Barry L, Mark Williams, H, Anna I, Veysoglu T, Parente E , Development and validation of a liquid chromatographic method for the determination of the related substances of ramipril in Altace capsules. J Pharm Biomed Anal, 2000;23(4):637-651.
12. Hisham E, Magda M, Elham A Spectrophotometric and atomic absorption spectrometric determination of ramipril and perindopril through ternary complex formation with eosin and Cu (II). J Pharm Biomed Anal, 1999;18(6):1027.
13. Bonazzi D, Gotti R, Andrisano V, Cavrini V, Analysis of ACE inhibitors in pharmaceutical dosage forms by derivative UV spectroscopy and liquid chromatography(HPLC). J Pharm Biomed Anal,1997;16(3):431-438.
14. Zarapkar S S, Rane S H , RP- HPLC determination of ramipril and hydrochlorothiazide in tablet Indian. J Pharma Sci, 2000;37(12):589-593.