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An Improved RP – HPLC Method For Simultaneous Estimation of Ramipril and Olmesartan In Tablet Dosage Form

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ABSTRACT

A new simple fast accurate and economical reverse phase high performance liquid chromatographic method was developed for the determination of Ramipril and Olmesartan in bulk and tablet dosage form. The separation was eluted on a Inertsil C_8 column (100 mm x 4.6 mm; 5µ) using a mobile phase mixture of mixed phosphate buffer 6.8 and acetonitrile in a ratio of 65:35 v/v at a flow rate of 1.0ml/min. The detection was made at 219 nm. The retention times were 2.28min for Ramipril and 3.76min for Olmesartan. Calibration curve was linear over the concentration range of 2.5-15 µg/ml for Ramipril and 10 to 60 µg/ml for Olmesartan. The propose method was validated as per the ICH guidelines parameters like Linearity, specificity, precision, accuracy, robustness and ruggedness. The method was accurate, precise, specific and rapid found to be suitable for the quantitative analysis of the drug and dosage form.

Keywords: Method development and validation, Olmesartan, Tablets, C_8 column, RP-HPLC.

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INTRODUCTION

Ramipril(Figure1) (2S, 3aS, 6aS) -1[(S)-N-[(S)-1-Carboxy-3-phenylpropyl]alanyl] octahydrocyclopenta [b] pyrrole-2-carboxylic acid, 1-ethyl ester[1,2]. Olmesartan medoxomil(Figure2) is 2, 3-dihydroxy-2-butenyl4-(1-hydroxy-1-methylethyl)-2-propyl-1-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]imidazole-5-carboxylate,Ramipril is official in Indian Pharmacopoeia, European Pharmacopoeia and U.S. Pharmacopoeia. Olmesartan is non pharmacopeial drug. but there is no official method for the combination. Both drugs in combination of tablet dosage form in the ratio of 5:20 mg Ramipril and Olmesartan respectively. As per literature survey many methods have been reported the estimation of Ramipril and Olmesartan individually or in combination with some other drugs[3-6]. With this present proposed method both Ramipril and Olmesartan estimates simple and economical in tablet formulation.

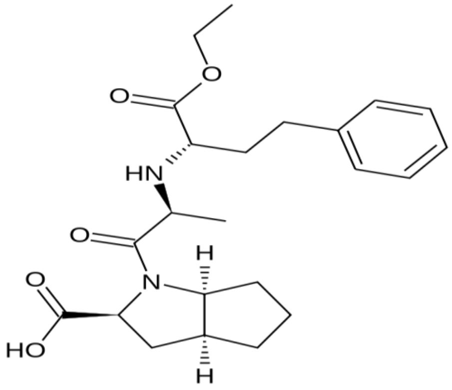

Figure 1: Structure of Ramipril

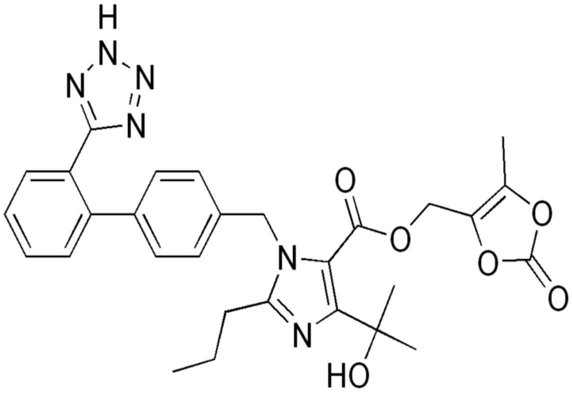

Figure 2: Structure of Olmesartan

MATERIAL AND METHODS

Chromatographic Conditions

Waters e 2695 separation module with high pressure liquid chromatographic instrument provided with a Inertsil C_8 column (100 mm x 4.6 mm ; 5 μ) and 2489 UV-Visible detector, auto injector, auto sampler with Empower 2 software from Waters corporation, Milford USA was employed in the study. HPLC grade acetonitrile, was purchased from Ranbaxy, India, and Potassium dihydrogen phosphate, Di potassium hydrogen ortho phosphate, potassium hydroxide, ortho phosphoric acid AR grade were purchased from SD Fine Chem Mumbai, India were used in the study.

Drug Samples

The reference samples were obtained from M/s. Bio-Leo Analytical Labs India Pvt Ltd, Hyderabad, India, and the formulation samples were purchased from local market.

Mobile phase

Mix an accurately weighed 1.5g of potassium dihydrogen phosphate and 0.3 g of dipotasssium hydrogen phosphate in 1000ml of water adjust pH 6.8 with dilute potassium hydroxide and acetonitrile in the ratio 65:35 v/v was filtered through 0.22µ membrane filter and was degassed. Mobile phase was used as diluent for preparing the working solution of the drug. The mobile phase was filtered and sonicated by using Bio-Technics India, Mumbai before use. The flow rate of the mobile phase was maintained at 1ml/min. The column temperature was maintained at 30ºC and the detection of the drug was carried out at 219nm.

Preparation of stock and working standard solution of Ramipril and Olmesartan

About 5.0 mg of Ramipril and 20 mg of Olmesartan was weighed accurately on Sartorius semi micro balance model-CPA225D and transfers in to 50ml volumetric flask the solution was sonicated and the resulting solution was diluted with the mobile phase to get a working standard solution of 100 µg/ml Ramipril and 400 µg/ml Olmesartan. Further dilute 10 ml to 100 ml with mobile phase gives 10 µg/ml Ramipril and 40 µg/ml Olmesartan.

Sample Preparation

Weighed accurately previously weighed and crushed 20 tablets powder equivalent 1 tablet weight which contains 5mg of Ramipril and 20mg of Olmesartan transferred to 50ml volumetric flask make up to the mark with mobile phase sonicated and filtered through whatman filter paper. Further dilute 10 ml to 100 ml with mobile phase.

Linearity and Construction of Calibration Curve

Linearity of the peak area response was determined by taking measurement at Six concentration prints (6 replicates at each point) working standard dilution of Ramipril and Olmesartan in the range of 2.5-15.0µg/ml and 10 to 60 µg/ml respectively. 20µl quantity of the dilution was injected each time in to the column. The drug elutes was monitored at 219 nm at a column temperature of 30ºC and the corresponding chromatograms were obtained. Form these chromatograms the mean peak areas were calculated and a plot of concentration over the peak area was constructed.

RESULTS AND DISCUSSION

The present study was aimed at developing a simple economical precise and accurate HPLC method for the analysis of Ramipril and Olmesartan in bulk drug and in pharmaceutical dosage form. In order to achieve optimum separation of the component peaks, mixture of acetonitrile with water in different combinations were tested as mobile phase on a C_8 stationary phase. A mixture of Phosphate buffer pH 6.8: acetonitrile in a proportion of 65:35 v/v was selected as the chromatographic peaks were well defined and resolved with no tailing, the optical and system suitability parameters are tabulated in Table 1. The retention time obtained for Ramipril was 2.28±0.1 min and for Olmesartan was 3.76±0.1min. Each of the samples was injected Six times and the Sample retention times were observed in all cases. The peak areas of Ramipril and Olmesartan were reproducible as indicated by low coefficient of variation. A good linear relationship (r^2 = 0.999) was observed for Ramipril and (r^2=0.999) was observed for Olmesartan, The linearity of six different concentrations of overlaid chromatogram of Ramipril and Olmesartan shown in figure 3. The regression concentration and areas are given in table 2. And the regression characters are given in figure 6&7. When test solutions were analyzed by the proposed method for finding out intra and inter-day variation, low co-efficient of variation was observed. The absence of additional peaks indicated non-interference of common excipients used in the tablets.

Table 1: Optical and System suitability parameters

Parameter	Ramipril	Olmesartan
Concentration(µg/ml)	2.5-15	10-60
Slope(m)	37345	33551
Correlation Coefficient(r^2)	0.999	0.999
Intercept(b)	1546	5231
Asymmetry Factor	1.22	1.15
Theoretical Plates	2186	4268
Resolution		7.03
LOD(µg/ml)	0.1425	0.6032
LOQ(µg/ml)	0.4319	1.828

Table 2: Calibration data of the proposed method

Ramipril Conc(mcg/ml)	Mean Area	Olmesartan Conc(mcg/ml)	Mean Area
0	0	0	0
2.5	95092	10	343271
5	191203	20	686697
7.5	280450	30	1007943
10	375975	40	1348299
12.5	466596	50	1669121
15	562126	60	2026989

High recovery values obtained from the different dosage form by the proposed method indicates the method is accurate. The drug content in tablets was quantified using the proposed analytical method are given in table 3.

The deliberate changes in the method have not much affected the peak tailing, Theoretical plates and the percent assay. This indicated the robustness of the method. The robustness study results are presented in table 4. The lowest value of LOD and LOQ as obtained by the proposed method by calculated using 3.3xstdev/slope for LOD and 10xstdev/slope for LOQ . The standard solution of the drug was stable up to 24 hrs as the difference in percent assay during the above period is within limit system suitability parameters were studied with six replicates standard solution of the drug and the calculated parameters are within the acceptance criteria. The tailing factor and the number theoretical plate are in the acceptable limits.

Table 3 : Accuracy data (Triplicate values at 50,100 &150 percent levels)

Parameter	Initial Amount(µg)	Amount added(µg)	Total Amount(µg)	Amount Recovered(µg)	%of mean recovery
Ramipril					
50% level	5	2.5	7.5	7.461	99.48
100%level	5	5	10	9.88	98.80
150%level	5	7.5	12.5	12.431	99.45
Olmesartan					
50% level	20	10	30	29.763	99.21
100%level	20	20	40	39.572	98.93
150%level	20	30	50	49.85	99.70

Table 4: Robustness Study

Drug	Parameter	Chromatographic Conditions			
		Retention time	Area	Theoretical Plates	Asymmetry
Ramipril	Buffer Change ±2%				
	63%v/v	2.182	348087	2201	1.22
	65%v/v	2.281	379787	2191	1.23
	67%v/v	2.343	345852	2239	1.22
	Flow Change±0.2				
	0.8ml/min	2.844	472613	2530	1.29
	1.0ml/min	2.281	379787	2191	1.23
	1.2ml/min	1.919	314473	2034	1.17
Olmesartan	Buffer Change ±2%				
	63%v/v	3.388	1243660	4499	1.21
	65%v/v	3.762	1363665	4275	1.16
	67%v/v	4.087	1239419	4462	1.22
	Flow Change±0.2				
	0.8ml/min	4.697	1702930	4462	1.29
	1.0ml/min	3.762	1363665	4275	1.16
	1.2ml/min	3.153	1131136	3881	1.12

The system precision was established by six replicate injections of the standard solution containing analytes of interest. The values of relative standard deviation were found within the limit, indicating the injection repeatability of the method. The method precision was established by carrying out the analyte six times using the proposed method. The relative standard deviation was found within the limit, indicating the injection repeatability of the method. The overlaid precision spectrum shown in figure 4 and the results were presented in Table 5&6.

Table 5: Precision Study

Ramipril			**Olmesartan**	
S.No.	**RT**	**Area**	**RT**	**Area**
1	2.281	379787	3.762	1363665
2	2.281	380108	3.763	1363958
3	2.281	377369	3.761	1356776
4	2.283	375783	3.763	1347133
5	2.284	378786	3.766	1357368
6	2.286	378740	3.769	1358723
Avg	2.283	378428.833	3.764	1357937
Stdev	0.00206	1613.21361	0.002966	6133.161
%RSD	**0.09**	**0.43**	**0.08**	**0.45**

Table 6: Method Precision study

Ramipril			**Olmesartan**	
S.No.	**RT**	**Area**	**RT**	**Area**
1	2.284	381237	3.765	1364023
2	2.282	380045	3.763	1361543
3	2.285	380343	3.766	1362655
4	2.284	385409	3.766	1366567
5	2.281	382680	3.762	1364360
6	2.283	384890	3.766	1365680
avg	2.283	382434	3.765	1364138
stdev	0.00147	2300.24	0.001751	1858.689
%RSD	**0.06**	**0.60**	**0.05**	**0.14**

The diluted preparations of marketed tablets were injected in duplicate and the results were calculated and presented in Table 7. The chromatogram of sample preparation shown in figure 5.

The specificity of the HPLC method was determined by the complete separation of Ramipril and Olmesartan. When it was subjected to forced degradation as per ICH guidelines which was carried out with 0.1N HCL, 0.1N NaOH and Heat degradation. The method does not permit detection of degradation product for Ramipril and Olmesartan.

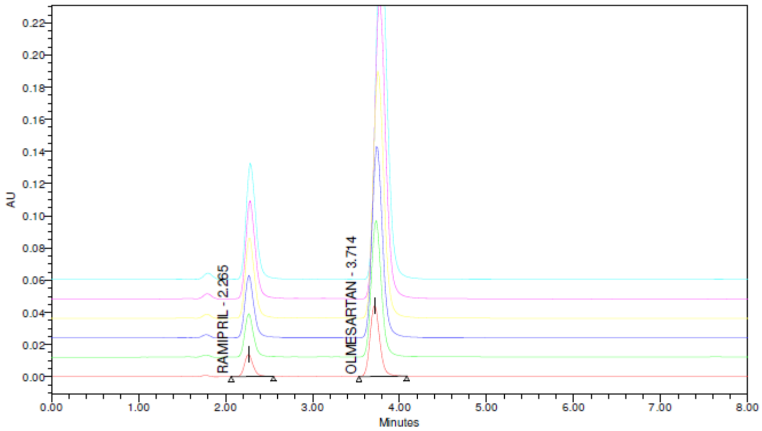

Figure 3: Ramipril and Olmesartan Overlaid linearity chromatogram

Table 7: Assay Results

Drug	Amount present/tablet	% of Assay
Ramipril	4.973 mg	99.46
Olmesartan	19.945 mg	99.72

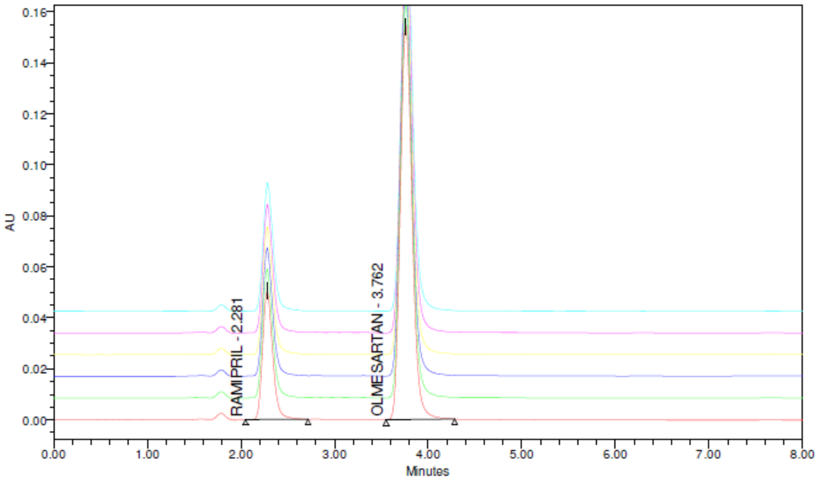

Figure 4: Ramipril and Olmesartan Overlaid precision chromatogram

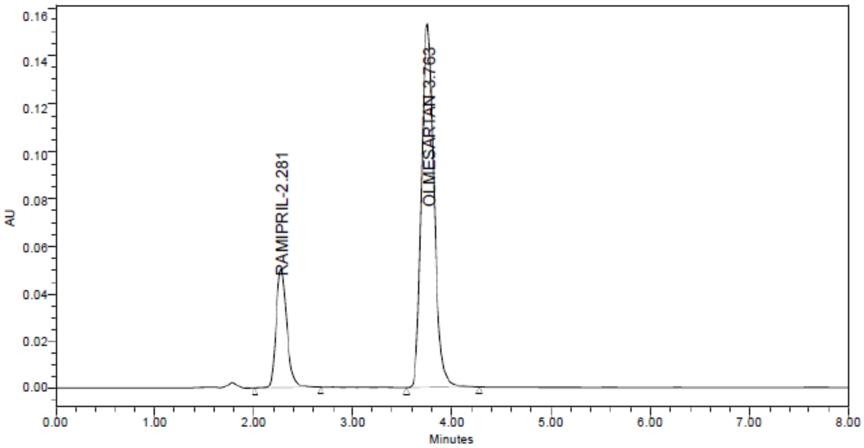

Figure 5: Ramipril and Olmesartan sample chromatogram

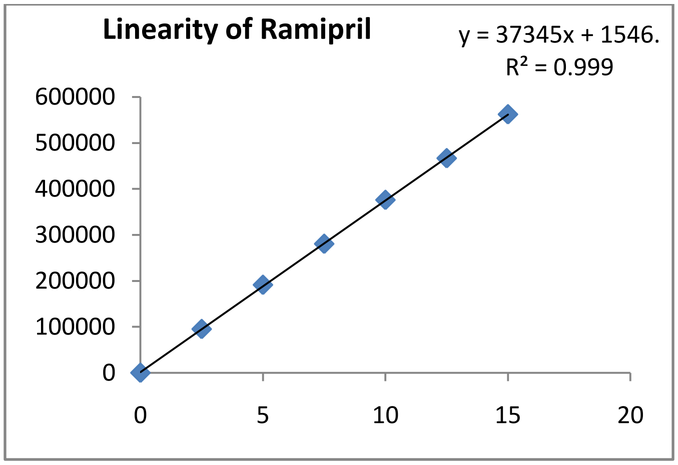

Figure 6: Linearity of Ramipril

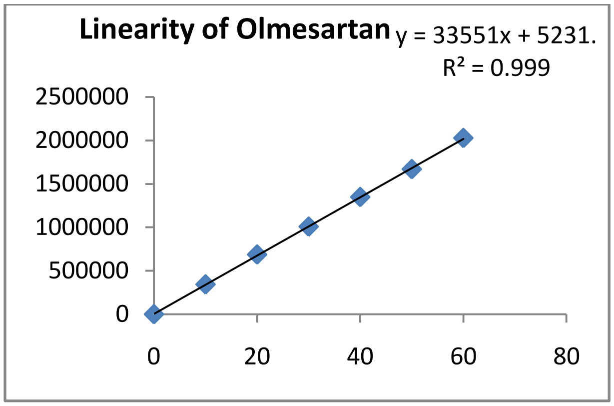

Figure 7: Linearity of Olmesartan

CONCLUSION

Hence it can be concluded that the proposed HPLC method is evident very fast and economical compared to the literature available.

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