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A Validated RP-HPLC Method for Simultaneous Estimation of Ramipril and Valsartan in Pharmaceutical Dosage Form.

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ABSTRACT

A rapid, specific, sensitive and simple high performance liquid chromatography was developed for simultaneous estimation of Ramipril and Valsartan in capsule formulation. The separation was achieved by Thermo scientific C_{18} column (4.6 × 250 mm, particle size 5µm) with a mobile phase consisting of Acetonitrile: water (60:40 v/v, P^H 3.6 adjusted with anhydrous disodium hydrogen phosphate), at a flow rate of 1.2ml/min. Detection was carried out at 235nm. Retention time of Ramipril and Valsartan were found to be 2.46 and 6.37 min, respectively. The linear dynamic range was 1-20µg/ml and 20-100µg/ml Ramipril and Valsartan respectively. The method is validated for Accuracy, Precision, Ruggedness and Robustness. The proposed method is successfully applied for the simultaneous determination of both drugs in commercial capsule preparation. The results of the analysis have been validated statistically and by recovery studies.

Keywords: Ramipril, Valsartan, High performance liquid chromatography.

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INTRODUCTION

Ramipril is chemically (2*S*,3*aS*,6*aS*)-1-[(2*S*)-2-{[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino}propanoyl]-octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid and it belongs to a class of drugs called angiotensin converting enzyme (ACE) inhibitors which are used for treating high blood pressure, heart failure and for preventing kidney failure due to high blood pressure and diabetes. Valsartan is chemically (*S*)-3-methyl-2-(*N*-{[2'-(2*H*-1,2,3,4-tetrazol-5-yl)biphenyl-4-yl]methyl}pentanamido) butanoic acid and is an angiotensin II receptor antagonist (more commonly called an "ARB", or angiotensin receptor blocker), with particularly high affinity for the type I (AT_1) angiotensin receptor. By blocking the action of angiotensin, valsartan dilates blood vessels and reduces blood pressure [1-3]. Kurade V. P et al., estimated Ramipril and Telmisartan in Tablet dosage form [4]. Santosh R Karajgi estimated Atorvastatin and Ramipril by First Derivative Spectrophotometric method [5]. Popat B Mohite et al., performed Simultaneous estimation of ramipril and Telmisartan in Tablet Dosage Form by Spectrophotometry [6].
Atana.E.S et al., determined valsartan and hydrochlorothiazide in tablet dosage form by first derivative UV spectrophotometry and LC [7]. The literature review reveals that there is no method has been so far reported for the simultaneous determination of these drugs in pharmaceutical preparations [4-8]. Hence, it is necessary to develop a rapid, accurate and validated RP-HPLC method for the simultaneous estimation of Ramipril and Valsartan in capsule dosage forms. The developed method validated according to ICH guidelines The present manuscript describes a novel LC method which is simple, rapid, precise and accurate isocratic reverse phase HPLC for simultaneous determination of Ramipril and Valsartan.

MATERIALS AND METHODS

Equipment:

Chromatographic separation was performed on Waters HPLC system consist of model 2695 having PDA detector and Rheodyne injector with 20µl loop volume. Waters Empower software was applied for data collecting and processing.

Reagents and chemicals:

Acetonitrile and water of HPLC grade were procured from Rankem lab ltd. Ramipril and Valsartan standards were received as gift samples from Ranbaxy Laboratory Hyderabad, India, respectively. Ortho phosphoric acid A.R grade and citric acid monohydrate were purchased from E. Merck chemicals Mumbai, India. Capsule (Valent-R-5) having combination Ramipril (5mg) and Valsartan (80mg) was used.

HPLC Conditions:

A Thermo scientific C_{18} (25cm×4.6mm, 5µ) column was used as the stationary phase. A mixture of Acetonitrile and water in the ratio of (60:40v/v) was used as a mobile phase and P^H

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3.6 adjusted with anhydrous disodium hydrogen phosphate. It was filtered through 0.45µ membrane filter and degassed. The mobile phase was pumped at 1.2 ml/min. The eluents were monitored at 235nm.The injection volumes of sample and standard were 20µl.

Standard solutions:

A stock solution containing 1000µg/ml of Ramipril and Valsartan were prepared separately by dissolving in Acetonitrile. A working standard solution containing 1-20µg/ml and 20-100µg/ml of Ramipril and Valsartan were prepared from the above stock solution. All the stock solutions were covered with aluminum foil to prevent photolytic degradation until the time of analysis.

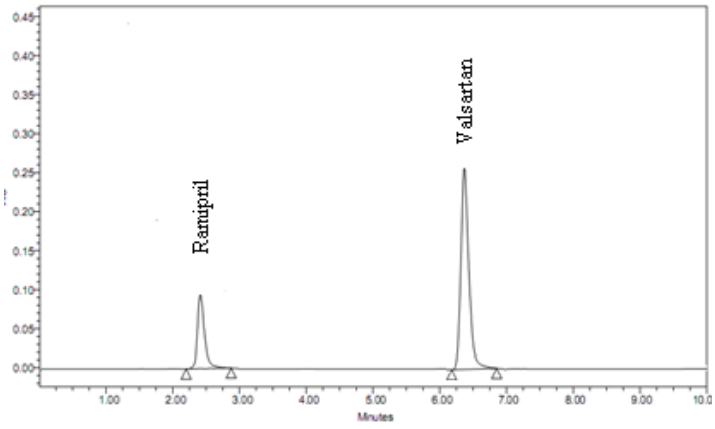
ASSAY OF TABLET FORMULATION:

Twenty capsules were weighed, each containing 5 mg of Ramipril and 80 mg of Valsartan were weighed and finely powdered. A quantity of powder equivalent to 5mg of Ramipril and 80mg of Valsartan was weighed and transferred to a 50ml standard flask. The drug was initially dissolved in Acetonitrile and sonicated for 10 minutes. The volume was made up to 50ml with mobile phase. The solution was filtered using 0.2µm membrane filter. The aliquot was then suitably diluted to get final concentrations of 5µg/ml of Ramipril and 80 µg/ml of Valsartan. Then 20µl of these solutions was injected in to the column, recorded and chromatogram was shown in Fig.1. Concentrations of Ramipril and Valsartan in the tablet formulation were calculated by comparing area of the sample with that of standard. The percentage assay of individual drug was calculated and presented in Table 1.

Table 1: Report for Assay

S.No	Drug	Amount present (mg/tab)	Amount found* (mg/tab)	% label claim*
1	Ramipril	5	4.96	99.20%
2	Valsartan	80	80.34	100.42%

Fig 1. Chromatogram of the sample.



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VALIDATION OF THE METHOD:

Linearity and range:

The developed method has been validated as per ICH guidelines. Every 20µl of the working standard solution of Ramipril in the concentration range of 1-20 µg/ml (Fig 2) and for Valsartan in the concentration range of 20- 100 µg/ml (Fig 3) were injected into the chromatographic system. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curves of Ramipril and Valsartan were obtained by plotting the peak area ratio versus the applied concentrations of Ramipril and Valsartan. The linearity curves of Ramipril and Valsartan were shown in Figure 3 and 4 and Linearity data's were shown in Table. 2.

Table 2: Statistical data of calibration curve of Ramipril and Valsartan

S.No	Parameters	Ramipril	Valsartan
1	Linearity range µg/ml	1-20 µg/ml	20- 100 µg/ml
2	Correlation Coefficient	0.999	0.999
3	LOD µg/ml	0.17µg/ml	2µg/ml
4	LOQ µg/ml	0.52µg/ml	5µg/ml

Fig 2: Linearity of detector response in HPLC method for Ramipril Concentration level Verses Analyte response

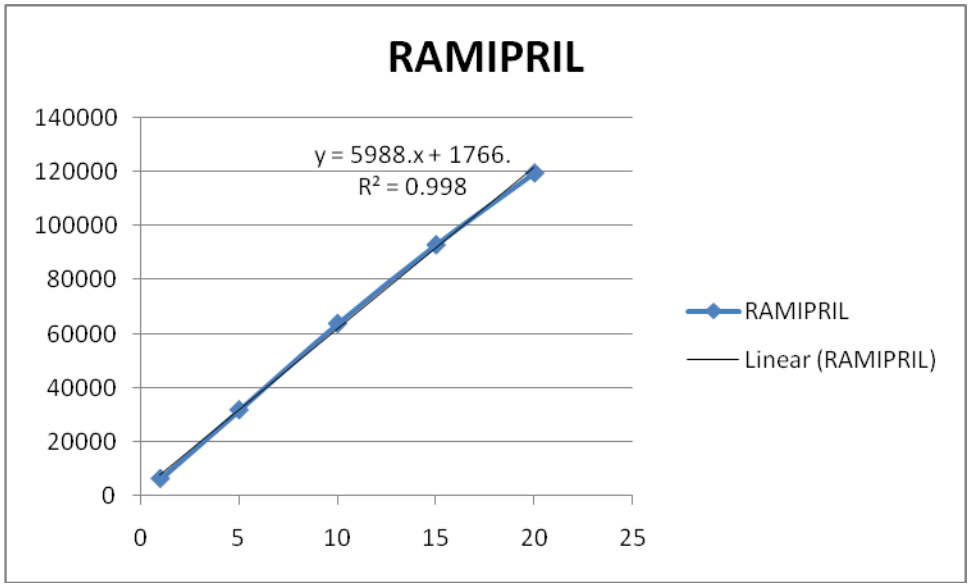

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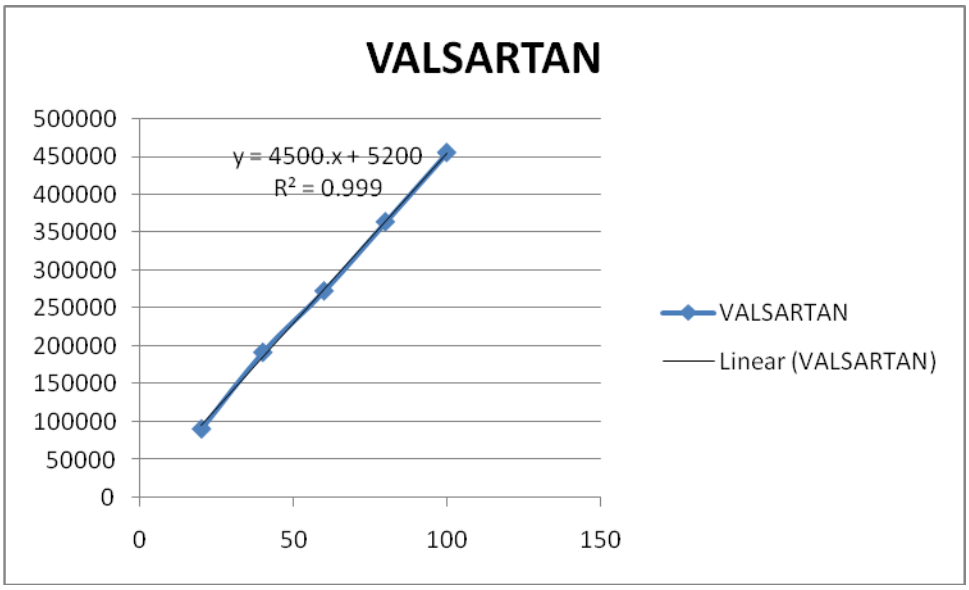
Fig 3: Linearity of detector response in HPLC method for Valsartan Concentration level Verses Analyte response



LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 3.3∂/*S* and 10∂/*S*, respectively as per ICH guidelines, where ∂ is the standard deviation of the response (*y*-intercept) and *S* is the slope of the calibration plot. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOQ is the smallest concentration of the analyte which gives response that can be accurately quantified (signal to noise ratio of 10). (Table. 2)

System suitability:

The resolution and peak symmetry were calculated for the standard solutions. (Table. 3).The values obtained demonstrated the suitability of the system for the analysis of this drug combination and the system suitability parameters fall within ±3% standard deviation range during performance of the method. Here tailing factor for peaks of Ramipril and Valsartan was less than 2 and resolution was satisfactory. The peaks obtained for Ramipril and Valsartan were sharp and have clear base line separation.

Table 3: System Suitability Report

S.No	Parameters	Ramipril	Valsartan
1	Theoretical plate	12462	32465
2	Asymmetry of the peak	0.74	0.63
3	Retention time	2.46 min.	6.37 min.
4	Resolution	7.52	

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Accuracy:

Recovery studies were carried out by applying the standard addition method. A known amount of standard Ramipril and Valsartan corresponding to 50%, 100%, and 150% of the label claim was added to pre analyzed sample of tablet dosage form separately. The recovery studies were carried out three times, at each level of recovery. The data's of accuracy were shown in (Table. 4)

Table 4: Recovery studies of Ramipril and Valsartan

Drug	%Concentration	Amount added(µg/ml)	Total amount found (µg/ml)	Recovery (%)	Mean Recovery (%)
Ramipril	50%	2.5	2.52	100.8	
	100%	5	5.14	102.8	101.11
	150%	7.5	7.48	99.73	
Valsartan	50%	40	39.87	99.67	
	100%	80	80.04	100.05	99.99
	150%	120	120.32	100.26	

System precision and Method Precision:

The Precision of the method was demonstrated by system precision and method precision studies. In the system precision studies, six replicate injections of the working standard solution prepared as per the proposed method and chromatograms were recorded. Standard deviation and relative standard deviation for the area was calculated and presented in (Table .5). In the method precision studies, six replicate injections of the analyte solution prepared as per the proposed method and chromatograms were recorded. Standard deviation and relative standard deviation for the area was calculated and presented in (Table.6).

Table 5: System Precision Report

Parameters	Area of Ramipril	Area of Valsartan
Trail 1	32163	2276534
Trail 2	31882	2281274
Trail 3	32785	2278346
Trail 4	31926	2275348
Trail 5	32439	2273427
Trail 6	32612	2277823
Average	32301.17	2277125
Standard deviation	370.2029	2697.201
%RSD	1.146098	0.118448

Table 6: Method Precision Report

Parameters	Area of Ramipril	Area of Valsartan
Trail 1	31954	2275126
Trail 2	31765	2274238
Trail 3	31654	2269374
Trail 4	31427	2272849

Trail 5	31782	2271254
Trail 6	31254	2272654
Average	31639.33	2272583
S.D	256.493	2066.184
%RSD	0.810678	0.090918

Solution Stability:

Solution stability was evaluated at room temperature for 48hrs. The percentage difference of relative standard deviation was not more than 2% from initial assay value result, thus indicated that both sample and standard solutions were stable for 24hrs, which was sufficient to complete the whole analytical process.

Ruggedness and Robustness:

The ruggedness of the method was determined by carrying out the experiment on different instrument like Waters HPLC and Shimadzu HPLC by different operators using different columns of similar type like Phenomenex C_{18},Hypersil C_{18}.Robustness of the method was determined by making slight changes in the experimental conditions such as the composition of the mobile phase, pH of the mobile phase, and flow rate of the mobile phase and the chromatographic characteristics were evaluated.

RESULTS AND DISCUSSION

The proposed method was found to be simple and sensitive with linearity in the concentration range of 1-20µg/ml and 20-100µg/ml of Ramipril and Valsartan. System suitability parameter indicates good resolution of both the peaks >2. In addition high column efficiency was indicated from the large number of theoretical plates (>1000). The degree of asymmetry was also evaluated using the tailing factor which did not exceed the critical value (1.5) indicating acceptable degree of peak asymmetry. The method was found to be accurate and precise as indicated by results of recovery studies and precision studies %RSD not more than 2%. There were no marked changes in the chromatograms which confirmed the ruggedness of the method. The standard deviation of % assay for sample was calculated for each parameter in robustness studies and relative standard deviation was found less than 2%. The low RSD value confirms the robustness of the method.

CONCLUSION

The developed RP-HPLC method for simultaneous determination of Ramipril and Valsartan can be used for routine analysis of both these components in combined dosage form.

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REFERENCES

[1] Indian Pharmacopoeia - Addendum, The Indian Pharmacopoeia Commission, 6th edition, Ghaziabad: 2010; 2038-2042, 2286-2290.

[2] Martindale. The Complete Drug Reference. 34th edition, Pharmaceutical Press; 2005; 994, 1018.

[3] The Merck Index. Merck & Co., Inc; 14th edition, Whitehouse Station. NJ, 2006; 8103, 9916.

[4] Kurade VP, Pai MG and R, Gude R. Indian J Pharm Sci 2009; 71(2): 148–151.

[5] Santosh R Karajgi. J Pharm Res 2009; 2:5.

[6] Popat B Mohite, Ramdas B Pandhare, Vaidhun H, Bhaskar. Eurasian J Anal Chem 2010; 5:1.

[7] Atana ES, Altmay SA, Goger NG, Ozkanab SA, Senturk Z. Simultaneous determination of valsartan and hydrochlorothiazide in tablets by first derivative UV spectrophotometry and LC. J Pharm Biomed Anal 2001; 25:009-13.

[8] Tian DF, Tian XL, Tian T, Wang ZY and Mo FK. I J Pharm Sci 2008; 70(3): 372–374.