Academic Sciences Asian Journal of Pharmaceutical and Clinical Research
Vol 6, Issue 1, 2013 ISSN - 0974-2441

Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF RAMIPRIL AND VALSARTAN IN BULK AND PHARMACEUTICAL DOSAGE FORMS.

BRAHMAIAH.B*, K. SUJANA, A.PRAMEELA RANI

University College of Pharmaceutical Sciences, Acharya Nagarjuna University,Guntur, A.P, India. 522510, Email: bbinginapalli@gmail.com

Received: 9 September 2012, Revised and Accepted: 10 November 2012

ABSTRACT

A Simple, fast, accurate and precise method has been developed for the simultaneous estimation of Ramipril and valsartan in pharmaceutical dosage forms by RP- HPLC. The separation is carried out on C-18 Hypersil column using buffer: Acetonitrile (35:65) using pH 3.9 with phosphate buffer. The R_t times of valsartan 2.8 and Ramipril 2.1min. The developed method was validated as per ICH guidelines.

Keywords: HPLC, Ramipril, Valsartan.

INTRODUCTION

Ramipril is chemically (IUPAC NAME) [(2S,3aS, 6aS)-1[(S)-N-[S-1-carboxy-3-phenyl propyl] alanyl] and it belongs to antihypertensive activity. It is the ACE inhibitor, major control of blood pressure. Valsartan is Chemically (IUPAC NAME) [3-methyl-2-[pentanoyl-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl]methyl]amino]-butanoic acid]. It is angiotensin-II receptor antagonist particularly (AT1 receptor) blocker. Combined dosage form of these two drugs is used for management and treatment of hypertension.[1-3] Popat B Mohite et al., performed Simultaneous estimation of Ramipril and Telmisartan in Tablet Dosage Form by Spectrophotometry.[4] Literature survey revealed only few spectroscopic methods[5-6] and HPLC methods[7-8] have been developed on this combination. So it is necessary to develop the new analytical method better than those two existing methods. The developed method should be accurate, precise, sensitive, rapid and economic HPLC method in bulk and pharmaceutical dosage forms.[9-10]

The chemical structures of Ramipril and Valsartan are shown in Fig 1 and Fig 2.

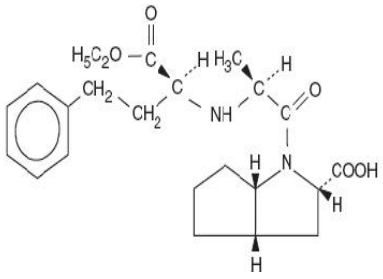

Fig 1: Structure of Ramipril.

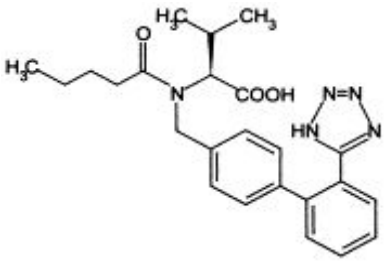

Fig 2: Structure of Valsartan.

MATERIALS AND METHODS

Reagents and Chemicals

Acetonitrile (HPLC grade) and potassium Dihydrogen orthophosphate (analytical grade) were procured from Merck Company. Ramipril & Valsartan gift samples were received from Hetero Laboratories Hyderabad, India. Formulation Valent-R5 (Valsartan 80mg & Ramipril 5mg) was purchased from local pharmacy.

Instrument

A waters 2695 model having empower 2 software and injection volume 20µl loop volume. C18 Hypersil (150 × 4.6 mm, 5µm) analytical column was used.

Chromatographic conditions

Mobile phase was prepared by mixing the 20mMolar phosphate buffer: Acetonitrile (35:65) and it was filtered using Whattman filter paper (0.22µm). The mobile phase pumped at the 0.8ml/minute. Inject each 20µl volume of sample & standard into the HPLC system individually. The eluents were detected at 220 nm. The typical chromatogram of optimized method was shown in Fig 3.

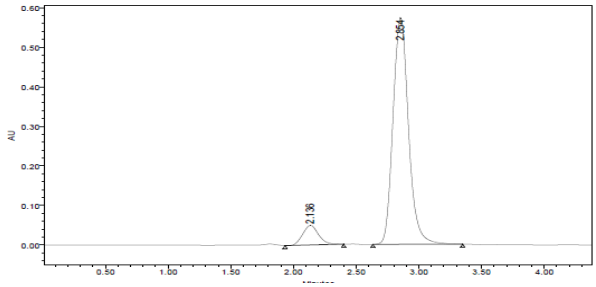

Fig 3: Typical chromatogram of Ramipril and Valsartan.

Standard stock solution preparation

Accurately weigh and transfer 5 mg of Ramipril and 80mg of Valsartan working standard into a 100ml clean and dry volumetric flask, then add about 70ml of diluent and sonicate to dissolve it completely and made up volume to the mark with the same solvent. From this Stock solution further pipette out 0.4ml into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample stock solution preparation

The capsule powder equivalent to label claim of the formulation was taken and transferred in to 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. From this Stock solution further pipette 0.4ml into a 10ml volumetric flask and dilute up to the mark with diluent.

Assay of formulation

Inject 20 µL of the standard and sample into the chromatographic system. Record the chromatogram then calculate the peak areas for

the Ramipril and Valsartan**.** The % Assay was calculated and the results were shown in table 1.

Table 1: Report for Assay

S. No	Drug	Amount present(mg/cap)	Amount found(mg/cap)	%label claim
1	Ramipril	5	4.98	99.7
2	Valsartan	80	79.2	99.0

System suitability parameters

The resolution and peak symmetry was calculated for standard solution. The tailing factor of both drugs was less than 2 and the plate count for both drugs was more than 2000. The peaks obtained for Ramipril and Valsartan were sharp and have clear baseline separation. The results were shown in Table 4 and Table 5.

VALIDATION OF THE METHOD

Linearity

The developed method has been validated as per ICH guidelines. Each 20µl of the standard solution of Ramipril in the concentration range of 1-5µg/ml and for valsartan in the concentration range of 10-50µg/ml 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 plotted by using the peak area versus the applied concentration of Ramipril and Valsartan. The linearity curves of Ramipril and Valsartan were shown in figure 4 and figure 5 and linearity data was shown in Table 2 and Table 3.

Accuracy

Accuracy studies were carried out by comparison method. 80%, 100%, and 120% were prepared from dosage form and compared to the standarad peak areas. The recovery studies carried out six times at each level of recovery. The data of accuracy was shown in Table 6 and Table 7.

System precision

In the system precision mode, six replicate injections of working standard solution were prepared and injected and measure the peak area of chromatogram. Standard deviation and relative standard deviation were calculated.

Method precision

In the method precision studies, five/six replicates injections of the analyte solution prepared as per the proposed method and chromatograms were recorded. The standard deviation and relative standard deviation were calculated. The results were shown in Table 8 and Table 9.

Ruggedness

The ruggedness of method was determined by different instruments like Waters HPLC, Shimadzu HPLC by different operators using different columns.

Robustness

The robustness of the method was determined by slightly changing the parameters like temp, flow rate, mobile phase ratio, pH of the mobile phase etc and the chromatogram characteristics were evaluated.

LOD & LOQ

Limit of detection and limit of quantification were calculated as 3.3(s.d/s) and 10(s.d/s). Where s.d is the standard deviation of the response (y-intercept) and s is the slope of calibration plot. The LOD is the smallest concentration of the analyte that gives the measurable response (S/N ratio3). The LOQ is the smallest concentration of the analyte which gives response that can be accurately quantified(S/N ratio).

RESULTS AND DISCUSSION

The proposed method was found to be simple and sensitive with linearity in the concentration range of 1-5µg/ml and 10-50µg/ml of Ramipril and Valsartan. System suitability parameters were found to be within the limit. The method is accurate and precise as indicated by results of recovery studies and precision. %RSD was not more than 2%. There were no marked changes in the chromatogram which confirmed the ruggedness of the method. The low RSD value confirms the robustness of the method.

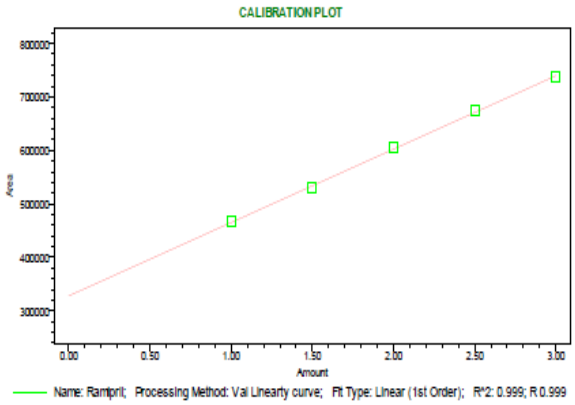

Fig 4: Linearity curve of Ramipril.

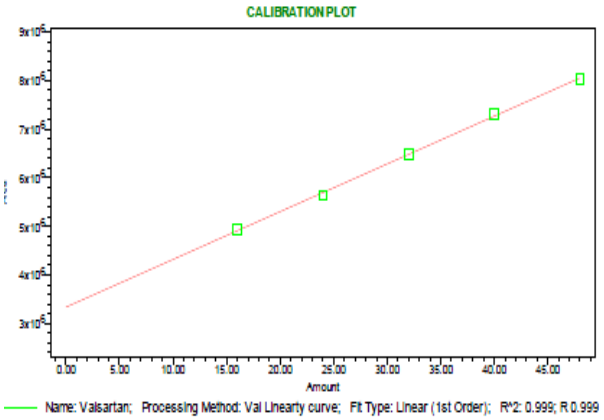

Fig 5: Linearity curve of Valsartan.

Table 2: Linearity data of Ramipril.

S. No	Concentration(µg/ml)	Peak area
1	1	976285
2	1.5	1463515
3	2	1932890
4	2.5	2447921
5	3	2911423
Slope	19459	
Intercept	0301.9	
Correlation coefficient	0.999	

Table 3: Linearity data of Valsartan.

S.no	Concentration(µg/ml)	Peak area
1	16	4934705
2	24	5634434
3	32	6494192
4	40	7314905
5	48	8023079
Slope	98215	
Intercept	32772	
Correlation coefficient	0.999	

Table 4: System suitability results for Ramipril.

S.no	Change organic in the mobile phase	Usp plate count	Usp tailing
1	10% organic less	2195.0	1.1
2	Actual	2164.0	1.2
3	10%organic more	2170.0	1.0

S.no	Change in flow rate	Usp plate count	Usp tailing
1	0.6	2430.0	1.1
2	0.8	2164.0	1.2
3	1.0	2369.0	1.1

Table 5: System suitability results for Valsartan.

S.no	Change organic in the mobile phase	Usp plate count	Usp tailing
1	10% organic less	3187.0	1.1
2	Actual	2789.0	1.2
3	10%organic more	2569.0	1.2

S.no	Change in flow rate	Usp plate count	Usp tailing
1	0.6	3543.0	1.1
2	0.8	2789.0	1.2
3	1.0	3226.0	1.1

Table 6: Accuracy results for Ramipril.

%Concentration (at specification level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
80%	546834	4.0	3.99	99.9%	
100%	593263	5.0	4.92	98.5%	99.3%
120%	654584	6.0	5.97	99.6%	

Table 7: Accuracy results for Valsartan.

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
80%	5474132	64.0	63.7	99.6%	
100%	6484152	80.0	79.6	99.8%	99.2%
120%	7302305	96.0	94.6	98.4%	

Table 8: Intraday precision.

Injection	Ramipril Area	Valsartan Area	Ramipril Area
Injection-1	596886	6423669	596886
Injection-2	597766	6418299	597766
Injection-3	600318	6435957	600318
Injection-4	599337	6426016	600832
Injection-5	598787	6425928	600884
Average	599337	6425974	599337
Standard Deviation	1875.2	6400.9	1875.2
%RSD	0.10	0.34	0.31

Table 9: Inter day precision.

Injection	Ramipril Area	Valsartan Area
Injection-1	628573	6609039
Injection-2	624731	6625558
Injection-3	619076	6633630
Injection-4	622317	6643244
Injection-5	625203	6628255
Average	623980	6627945
Standard Deviation	3534.5	12545.9
%RSD	0.57	0.19

CONCLUSION

The results of study indicate that the proposed RP-HPLC method was simple, precise, highly accurate and rapid for the simultaneous estimation of Ramipril and Valsartan in bulk and pharmaceutical dosage forms.

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