International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 3, 2016

Original Article

METHOD DEVELOPMENT AND VALIDATION OF FAST DISSOLVING TABLET OF RAMIPRIL BY HPLC METHOD

VAIBHAV RAJORIYA, AMRITA SONI, VARSHA KASHAW*

Sagar Institute of Pharmaceutical Sciences (SIPS), NH-26, Narsinghpur Road, Sironja, Sagar, 470228 (M. P.) India
Email: varshakashaw@gmail.com

Received: 23 Oct 2015 Revised and Accepted: 25 Jan 2016

ABSTRACT

Objective: The Objective of present work is method development and validation of HPLC method for the quantitation of Ramipril in Fast dissolving tablet (FDT).

Methods: A stable, linear, rapid, accurate and selective HPLC method has been developed for the quantification of Ramipril in FDT using buffer and acetonitrile: methanol (60:40 v/v) ratio in combination as mobile phase and at the flow rate of 1 ml/minute at λ_{max} 210 nm. Chromatographic separation was performed on Shimadzu SPD-20A, SD-M10 AVP-Shimadzu, an ODS C-18 Kromacil (250 mm × 4.60 mm) column used as stationary phase. The quantitation of Ramipril done by HPLC, parameters studied were retention time, linearity, accuracy, precision, detection limit, quantitation limit and stability.

Results: Linear regression analysis data show a good linear relationship between response and concentration in the range of 5-30 µg/ml; detection carried out at λ_{max}210 nm; the linear regression equation for Ramipril was Y=10327x+72877; R^2=0.998. The retention time of the Ramipril was 2.910 min. Percent recoveries obtained for Ramipril was 99.58-100.15%. LOD and LOQ value was 0.802µg/ml and 1.4µg/ml for Ramipril respectively.

Conclusion: The result suggested that proposed method gives good peak resolution of Ramipril within short analysis time (<10 min) and high percentages of the recovery shown that method is free from interference of excipient present in the formulation. The % RSD of each parameter lies below the limit of 2%, proven the suitability. The statistical analysis proved that the proposed method is precise, accurate, selective and rapid for the HPLC estimation of Ramipril.

Keywords: Fast dissolving tablet, Ramipril, Accuracy, HPLC, Linearity

INTRODUCTION

Fast dissolving tablets (FDT) have more advantage for pediatric, geriatric [1-2], bedridden, disabled patients and also for those have difficulty in swallowing conventional tablets, capsules and liquid orals. FDT are the tablets which will rapidly disintegrate in the mouth without the need of water [3]. Pre gastric absorption of FDT can result in improved bioavailability and as a result of reduced dose [4]. Ramipril (fig. 1) is (2S, 3aS, 6aS)-1-[(S)-2-[[(S)-1-(ethoxy-carbonyl)-3-phenylpropyl]-amino]-propanoyl]-octahydrocyclopenta-pyrrole-2-carboxylic acid. Ramipril is indicated for the treatment of mild to moderate hypertension [5], congestive heart failure, myocardial infarction in patients with clinical evidence of heart failure [6]. Ramipril, a prodrug, is converted to the active metabolite ramiprilat by liver esterase enzymes [7]. However, it may cause hypotension, cough and other side effect [8]. The extensive literature survey has revealed that various methods was used for estimation of Ramipril by HPLC [9-12], HPTLC [13], Spectrophotometer [14], LC [15], LC-MS (Liquid chromatography-mass spectrophotometry) [16], Atomic-absorption spectrometry [17-18], and Capillary electrophoresis [19] has been reported earlier.

FDT of Ramipril was previously prepared [20]. The aim of the present work is method development and validation of prepared FDT of Ramipril by HPLC method.

MATERIALS AND METHODS

Chemicals and reagent

HPLC grade methanol and acetonitrile were procured from Sigma Aldrich, India. Sodium acetate (CAS No. 6131-90-4) and ammonium acetate (CAS No. 631-61-8) was procured from Chemical Drug House, New Delhi, India. HPLC grade water was obtained from Milli-Q system. Gift sample of Ramipril procured from Alkam Pharmaceutical Ltd, Baddi, India.

Apparatus and chromatographic condition

Chromatographic separation was performed on Shimadzu SPD-20A, SD-M10 AVP-Shimadzu, UV/Vis Diode Array Detector with ODS C_{18} Kromacil (250 mm × 4.60 mm). The elution was carried out isocratically at flow rate 1 ml/min.

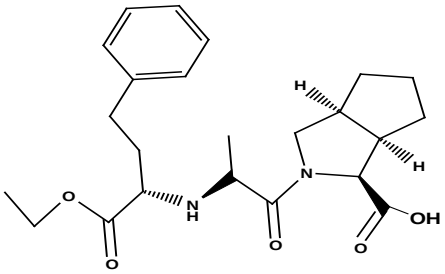

Fig. 1: Chemical structure of ramipril

Mobile phase selection

An optimized chromatogram is the one in which all the peaks are symmetrical and are well separated in less run time. The mobile phase was selected on the basis of best separation, peak purity index, peak symmetry, theoretical plate etc. The standard solution of Ramipril was run and combination of solvent have been tried to get a symmetry and stable peak (each mobile phase was filtered & degassed by solution through 0.45µ membrane filter to remove particulate matter) mobile phase selection for HPLC analysis are shown in table 1. Flow rate employed for analysis was 1.0 ml/min.

Table 1: Mobile phase selection for HPLC analysis

S. No.	Mobile phase	Flow rate	Ratio	Retention time	Suitable
1	methanol: acetonitrile	1.0 ml/min	80:20	-	not
2	methanol: acetonitrile	1.0 ml/min	70:30	-	not
3	(methanol: acetonitrile 70:30): acetate buffer	1.0 ml/min	50:50	8.910	Less suitable
4	(methanol: acetonitrile; 70:30): acetate buffer	1.0 ml/min	60:40	2.910	Suitable

Preparation of mobile phase

The mobile phase was prepared by mixing of methanol and acetonitrile in the ratio 70:30 (%v/v), the solution was filtered, this solution called mobile phase-A and dissolved 136 gm of sodium acetate and 77 gm of ammonium acetate in distilled water and dilute with distilled water to 1000 ml. Then add 250 ml of glacial acetic acid and mix, this solution called mobile phase-B. The final mobile phase was prepared by mixing both mobile phase A and B in the ratio of 60:40 (%v/v). 100 mg of Ramipril was weighed accurately and transferred to a 100 ml volumetric flask and volume was adjusted to the mark with the mobile phase in the ratio of 60:40 (%v/v), (1000 µg/ml). From the above solution 1 ml was transferred to 10 ml volumetric flask, and make up the volume up to mark with mobile phase to get the concentration of 100µg/ml. Then take 2 ml form the above solution and volume make up to the mark with mobile phase to get the concentration of 20µg/ml. Resulting solution was scanned over UV range (200-400 nm), maximum absorbance was found at λ_{max} 210 nm.

Preparation of standard stock solution

10 mg of Ramipril was weighed accurately and transferred to 10 ml volumetric flask and then volume was adjusted to the mark with the mobile phase, to give a stock solution of 1000 µg/ml. From stock solution of Ramipril 1 ml was taken and diluted up to 10 ml with the mobile phase, to give a stock solution 100 µg/ml. From this solution 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 ml solution was transferred to 10 ml volumetric flask and make up the volume up to 10 ml with mobile phase gives standard drug solution concentration ranges of 5, 10, 15, 20, 25, 30 µg/ml. Each of the standard drug solution was injected 3 times and the mean peak area of drug was calculated and plotted against the concentration of the drug. The regression equation was found out by using this curve. Mean AUC recorded for all the concentration range and typical calibration curve was shown in fig. 2.

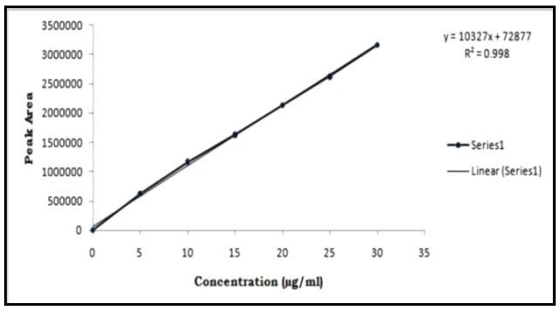

Fig. 2: Calibration curve of ramipril by HPLC (λ_{max}210 nm)

Analysis of FDT of ramipril (F4)

Twenty tablets were weighted individually from optimized formulation F4 (FDT of Ramipril) and powdered. Weight equivalent to 100 mg of Ramipril was transferred to 100 ml volumetric flask and dissolve in mobile phase. The solution was shaking vigorously for 10 min and filtered through 0.45 µ membrane filter paper and then volume make up to the mark with mobile phase to get a solution containing concentration 1000 µg/ml.

From the above solution 1 ml of solution was taken and diluted to 10 ml with mobile phase to get a solution containing 100µg/ml. From the above solution 1 ml of solution was taken and diluted to 10 ml with mobile phase to get a solution containing 10 µg/ml concentration of Ramipril. The amount of Ramipril per tablets was calculated by extrapolating the value of peak area from the calibration curve. Analysis procedure was repeated six times with tablet formulation shown in table 2.

Table 2: Analysis of FDT of ramipril (F4)

S. No.	Actual amount (mg/tab)	Peak area	Amount found (mg/tab)	% Amount found
1	20	1366565	20.05	100.4
2	20	1366555	19.97	99.4
3	20	1366565	19.90	99.60
4	20	1366656	19.91	99.70
5	20	1366541	19.95	99.75
6	20	1366532	20.01	100.01
	Mean	1366569	19.99	99.81
	SD	44.609	0.0578	0.350
	%RSD	0.0032	0.2891	0.3506

n=6; F4 optimized formulation for analysis; RS. Denotes for relative standard deviation; SD. denotes for standard deviation.

Table 3: Linearity

Parameter	Ramipril
λ_{max} (nm)	210
Beer's law limit (µg/ml)	5-30
Regression equation (y =mx+c)	Y=10327x+72877
Slope (m)	10317
Intercept (c)	72877
Correlation coefficient (R^2)	0.998
LOD	0.802 µg/ml
LOQ	1.4 µg/ml

n=3; LOD denoted for limit of detection; LOQ denote for limit of quantization; maximum absorbance 210 nm. Concentration range 5-30 µg/ml

Linearity

The linearity of the method was determined by analyze several aliquots of Ramipril in concentrations range of 5-30 µg/ml, repeated three times. Linearity was observed in the final concentration range of 5-30 µg/ml; with the correlation coefficient of 0.998 for Ramipril. Calibration curve was plotted using AUC versus concentration of standard solution. Peak area recorded for all the peaks was given in table 3. The slope and intercept value in calibration curve of Ramipril was Y=10327x+72877; R^2=0.998. The result shows an excellent correlation exists between peak area and concentration of the drugs within the concentration range 5-30 µg/ml.

Assay

20 µl of standard and sample solution were injected into injector of the liquid chromatogram, form the peak area of Ramipril, amount of drug in sample were computed.

Method validation

Validation of the method was done according to ICH guidelines [21] by Simultaneous equation method**.**

Accuracy

The accuracy of the method was determined by recovery study using the standard addition method. Pre analyzed samples were spiked with standard drug (Ramipril) at three different concentration levels, i.e., 80, 100 and 120%, and the mixtures were reanalyzed by the proposed method. Each level was repeated for three times. Data obtained was analyzed for percent recovery. Standard dilutions were prepared and three replicates of each dilution were analyzed in same day for repeatability and result was subjected to statistical analysis.

Precision

The precision of the method was carried out by intermediate precision. In intermediate precision, day to day precisions and analyst to analyst precision were performed. Day to day precisions and analyst to analyst precision were performed by preparing and applying concentrations; 25 µg/ml of Ramipril in day 1 & day 2 and Analyst 1 & Analyst 2 respectively. Assay for each analysis was calculated and mean, S. D., % RSD was determined.

Limit of detection and limit of quantification

Limit of Detection (LOD) and Limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentration of the standard solution using the developed HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio 3). The LOQ is the smallest concentration of the analyte which gives response that can be accurately quantified (signal to noise ratio 10). LOD and LOQ value measured by the following formula;

The LOD = 3.3σ/S and LOQ = 10σ/S;

Where σ = Standard deviation of the response; S = Slope of the deviation curve. In order to demonstrate the stability of both standard and sample solution during analysis, both solutions were analyzed over a period of 5 h at room temperature.

Ruggedness and robustness

The Ruggedness of the method was determined by carrying out the experiment on different instrument like Shimadzu SPD-20A and Agilent HPLC by different operator using different columns of similar type like Hypersil C18, Intersile C18. The Robustness of the method was determined by making slight changes in the chromatographic condition. As per ICH guidelines, small but deliberate variations in concentration of the mobile phase were made to check the method. Mobile phase varies methanol: acetonitrile (80:20)), (methanol: acetonitrile (70:30)), (methanol: acetonitrile, (70:30): acetate buffer {50:50}; (methanol: acetonitrile, (70:30): acetate buffer {60:40} ratio and flow rate varies 0.5-1.5 ml/min.

Solution stability

In order to demonstrate the stability of both standard and sample solution during analysis, both solutions were analyzed over a period of 5 h at room temperature.

RESULT AND DISCUSSION

Accuracy

The accuracy of the method was determined by recovery studies by standard addition method according to ICH guidelines. The pre-analyzed samples were spiked with standard drug Ramipril at three different concentration levels, i.e., 80%, 100% and 120%. The mixtures were reanalyzed by the proposed method and found to be within the limit of 99.856-100.008% for Ramipril. The values of percent recovery are listed in table 4. The % RSD below than 2, states that method is accurate. So the method can be used for the estimation of Ramipril from its tablet dosage form, were found more accurate without any interference.

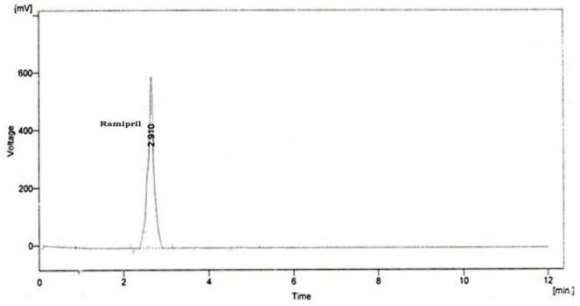

Fig. 3: Retention time of ramipril by HPLC method

Table 4: Drug recovery study of ramipril

Description	80	100	120
Amount present (µg/ml)	10	10	10
	10	10	10
	10	10	10
Amount of Std. added (µg/ml)	8	10	12
	8	10	12
	8	10	12
Amount recovery (µg/ml)	18.01	19.98	21.99
	17.99	19.99	21.95
	18.00	20.00	22.01
%Recovery	100.15	99.80	99.91
	99.875	99.90	99.58
	100.00	100.00	100.08
% Mean Recovery	100.008	99.900	99.856
SD* (n=3)	0.137689	0.100	0.2542
%RSD	0.133	0.100	0.254

n=3, RSD denotes for relative standard deviation; SD denotes for standard deviation.

The HPLC procedure was optimized with a view to develop accurate and stable assay method. The drugs Ramipril was run in mobile phase composition with different ODS C_{18} columns (Kromacil; 250 mm ×4.60 mm). The flow rate was maintained at 1 ml/min with temperature at 44ºC; detection at 210 nm gave sharp and symmetrical peak with retention time 2.910 min of Ramipril. The typical chromatogram of the Ramipril was shown in fig. 3.

Precision

The precision of the method was carried out by intermediate precision. In intermediate precision, day to day precisions and analyst to analyst precision were performed. 25µg/ml concentration was used for Ramipril. In day to day precision, for Ramipril % R. S. D. were found to be 0.583 and 0.567 at day 1 and day 2 respectively. In analyst to analyst precision for Ramipril % R. S. D. were found to be 0.854 and 0.654 at analyst 1 and analyst 2 respectively. Assay for each analysis was calculated and mean, S. D., % RSD was determined and data presented in table 5. The % R. S. D value for day to day precision and analyst to analyst was below than 2, as according to the ICH guidelines. These results were states that validation method was precise.

Limit of detection and limit of quantification

The LOD and LOQ for Ramipril were found to be 0.802µg/ml and 1.4µg/ml respectively. The LOD and LOQ value for Ramipril was below than 2, conclude that developed method is detected and quantify the drug in small quantity in dosages form (table 3). The retention time of Ramipril was 2.910 min. shown in fig. 3 and peak area of Ramipril remained almost unchanged (% RSD was less than 2.0) and there was no significant degradation found in indicated period, for at least 5 h which was sufficient to complete the whole analytical process.

Ruggedness

The Ruggedness of the method was determined by carrying out the experiment on different instrument like Shimadzu SPD-20A and Agilent HPLC by different operator using different columns of similar type like Hypersil C18, Intersile C18. The robustness of the method was determined by making slight changes in the chromatographic condition. These variations did not cause any significant difference in resolution of HPLC method, represent its robustness. The flow rate varies from 0.5-1.5 ml/min does not cause any variation in the chromatogram.

Table 5: Precision

(A) Intermediate precision: day-to-day

Ramipril	Day-1		Day-2	
Concentration (µg/ml)	Peak area	Retention time (min.)	Peak area	Retention time (min.)
25	2561347	2.35	2585958	2.32
25	2582789	2.32	2572418	2.36
25	2525958	2.36	2539921	2.34
25	2569921	2.34	2561347	2.35
25	2572418	2.35	2521347	2.34
25	2535958	2.34	2582789	2.34
Mean	2558065	2.343	2560630	2.341
SD	22307.85	0.0136	25471.62	0.0132
%RSD	0.87206	0.58304	0.99474	0.56763

(B) Intermediate precision: analyst-to analyst

Ramipril	Analyst-1		Analyst-2	
Concentration (µg/ml)	Peak area	Retention time (min.)	Peak area	Retention time (min.)
25	2595958	2.32	2561347	2.35
25	2572418	2.36	2582789	2.32
25	2569921	2.34	2542431	2.33
Mean	2579432	2.34	2562189	2.333
SD	14366	0.02	20192.17	0.015
%RSD	0.55694	0.8547	0.78808	0.6547

n=5; for day to day precision; n=3; for analyst to analyst precision; RSD denotes for relative standard deviation; SD. Denotes for standard deviation.

Solution stability

The retention time of ramipril was 2.910 min. shown in fig. 3 and peak area of ramipril was remained almost unchanged (% R. S. D. was less than 2.0) and there was no significant degradation found in indicated period, for at least 5 h which was sufficient to complete the whole analytical process.

Recovery studies

To study the accuracy and reproducibility of the proposed method recovery experiment study were carried out. A fixed amount of pre analyzed sample taken and standard drug was added at 80%, 100%, and 120% level. Each level was repeated for three times. The mean recovery of Ramipril was in the range of 99.856%-100.008% (table 4).

DISCUSSION

The LOD and LOQ value for the Ramipril obtained, demonstrate the suitability of the system for the analysis of the drug; system suitability parameter may fall within±2% range during routine performance of the method. To study the accuracy and reproducibility of the proposed method recovery experiment study were carried out, in this a fixed amount of pre analyzed sample taken and standard drug was added at 80%, 100%, and 120% level. Each level was repeated for three times. The mean recovery of Ramipril was in the range of 99.856%-100.008%. Recovery data states that the proposed method is accurate and reproducible. The lower value of the % RSD of assay indicates that the method is accurate. These results concluded that the proposed method is better in comparison to previously reported method.

In present method retention time of the Ramipril is 2.910 min is least as compare to other method reported by Kurade *et al.*, 2009; Rao *et al.*, 2010; Sharma *et al.*, 2012;Kumari *et al.*, 2014, that state the retention time (run time) of Ramipril is 3.68 min, 3.30 min, 3.620 min & 4.003 min respectively [22-24]. This shown that the run time of Ramipril is less than previous reported method. The LOD & LOQ value of Ramipril is 0.802µg/ml and 1.4µg/ml respectively, is

better than previously reported by Kumari *et al*., 2014 was 1.2µg/ml and 4.9µg/ml respectively; but Yadav *et al*., 2012 was states that the LOD and LOQ value of Ramipril is 0.5µg/ml and 1.0µg/ml respectively [25]. This shown that the less sample is required for the quantitation and quantification of the drug. Accuracy and percent recovery of Ramipril is 99-100% better than reported by Yadav *et al*., 2012, that state the percent recovery 98.00%. These data represent that this method is better than existing method and have less runtime compared to the previously reported method.

CONCLUSION

The proposed method gives good peak resolution of Ramipril within short analysis time (<5 min). The method is very simple, rapid. High percentages of the recovery show that this method is free from interference of excipient present in the formulation. The % RSD of each parameter lies below the limit of 2%, as per the ICH guidelines. The methods developed were found to be simple, rapid, selective, accurate, precise and economical for the estimation of Ramipril in solid dosage form.

ACKNOWLEDGEMENT

We are thankful to the Dr. Ashok Kumar Jain, Principal, SIPS, Sagar, for providing us necessary facilities to carry out the research work. We are also thankful to Alkam Pharmaceutical Ltd, Baddi, (India), for providing gift samples of Ramipril.

CONFLICT OF INTERESTS

Declare none

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