IJBPAS, November, 2021, 10(11): 4089-4097

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF RAMIPRIL IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

D. HARITHA CHOWDARY*, PRACHET P AND RAMA RAO N

Department of Pharmaceutical Analysis, Chalapathi Institute of Pharmaceutical Sciences, Chalapathi Nagar, Lam, Guntur-522034

***Corresponding Author: D. Haritha Chowdary: E-Mail: darapaneniharitha28@gmail.com**

Received 21st Jan. 2021; Revised 20th Feb. 2021; Accepted 24th March 2021; Available online 1st Nov. 2021

https://doi.org/10.31032/IJBPAS/2021/10.11.5730

ABSTRACT

Ramipril is an Angiotensin Converting Enzyme (ACE) inhibitor, an anti-hypertensive drug. The aim of the present work was to develop simple, precise, accurate, specific, stability-indicating RP-HPLC method for the assay determination of Ramipril in bulk and pharmaceutical dosage form. The separation is achieved using Agilent TC-C18 (2), 5µm, 4.6×250mm column in isocratic mode with mobile phase, Acetonitrile:Methanol:Phosphate buffer (60:20:20v/v/v) at a flow rate of 1.0ml/min. Detection is carried out at 215nm. The retention time for ramipril is 1.635 minutes. The RP-HPLC is validated with respect to specificity, accuracy, precision, linearity range, limit of detection (LOD), limit of quantification (LOQ), system stability studies, robustness, ruggedness with intraday and interday variation studies. The percent recoveries ranged between 95-105 % and RSD < 2%. The developed method was validated as per International Conference on Harmonization (ICH) guidelines. Linearity was observed in the concentration range of 10 to 100% with correlation coefficient >0.995. The percent of relative standard deviation of six replicate measurements was found to be 1.76 which indicates that the proposed method was precise. The method could be successfully used for the analysis of Ramipril in bulk and pharmaceutical dosage form.

Keywords: Ramipril, ACE inhibitor, RP-HPLC

INTRODUCTION

Hypertension is a disease characterized by abnormally which the blood pressure in the arteries is elevated. It is classified as either primary or secondary. About 90-95% of cases are termed "primary (idiopathic) hypertension", which refers to high blood pressure for which no exact cause can be found the remaining 510% of secondary hypertension cases are caused by another condition that affect the renal, vascular lesions or endocrine system. Blood pressure is classified based on two types of measurements; the 140mmHg systolic and 90mmHg diastolic blood pressure is the blood pressure expressed as ratio such as '120 or 80 (120/80)mmHg. Systolic blood pressure is the pressure in vessels during a heartbeat. Diastolic pressure between heartbeats **[1]**.

Ramipril (RAM) is (2S,3aS,6aS)-1-[(2S)-2[[(1S)-1-(ethoxy carbonyl)-3-phenylpropyl]amino]propanoyl] octahydro cyclo penta[b]pyrrole-2-carboxylic acid, used as angiotensin converting enzyme inhibitor (ACE inhibitor) **Figure 1**. Angiotensin-converting enzyme (ACE) inhibitiors lower blood pressure by reducing peripheral vascular resistance without reflexively increasing cardiac output, heart rate or contractility. These drugs block the enzyme ACE which cleaves angiotensin I to form the potent vasoconstrictor angiotensin II **[2]**.

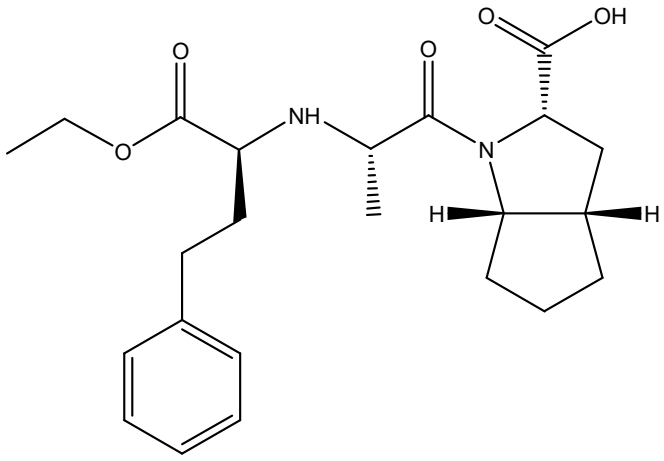

Figure 1: Structure of Ramipril

MATERIALS AND METHODS

Table 1: Chemicals and standards used

S. No	Chemicals	Manufacturer	Grade
1.	Ramipril	Mylan labs	-
2.	Potassium dihydrogen orthophosphate	Fischer scientific chemicals	Agilent
3.	Acetonitrile	Fischer scientific chemicals	HPLC
4.	Water	Fischer scientific chemicals	HPLC

Table 2: Instruments used

S. No	Instrument	Software	Manufacturer
1.	HPLC-PDA detector	Lab solutions	Schimadzu
2.	Weighing balance	-	Schimadzu
3.	pH meter	-	Lab india
4.	Sonicator	-	Lobalife
5.	Vacuum filter	-	-

Chromatographic conditions

A HPLC equipped with PDA detector was used. The chromatographic analysis was performed on column of Agilent TC-C18 (2), 5µm, 4.6×250mm. Mobile phase consisting of Acetonitrile: Methanol: Phosphate buffer (60:20:20v/v/v) was used in isocratic mode, with detection at 215nm. An injection volume of 20µl was used, keeping the flow rate of 1.0ml/min. The retention time of ramipril under the optimized conditions was found to be 1.635min and the typical chromatogram is shown in **Figure 2**.

Preparation of Phosphate buffer

About 0.136g of potassium dihydrogen ortho phosphate was dissolved in 100ml of HPLC water and adjusted to pH-3.0 with 0.1% ortho phosphoric acid and filtered through 0.45µ membrane filter.

Preparation of Mobile phase

Acetonitrile, HPLC water and phosphate buffer (adjusted to pH-3.0 with orthophosphoric acid) in the ratio 60:20:20v/v/v, the solution was filtered by vacuum filtration and degassed by ultra-sonication.

Preparation of Standard solution

About 10mg of Ramipril was accurately weighed and transferred into 10ml volumetric flask. Some amount of mobile phase was added and dissolved, and then the volume was made up to the mark with mobile phase.

Method development

Optimized chromatographic conditions

Mobile phase:

Acetonitrile:Methanol:Phosphate buffer (60:20:20v/v/v)

Column: Agilent TC-C18 (2), 5µm, 4.6×250mm

Flow rate: 1.0ml/min

Detector: PDA detector

Injection volume: 20µl

Column temperature: 25ºC

Run time: 6min

Retention time: 1.635min

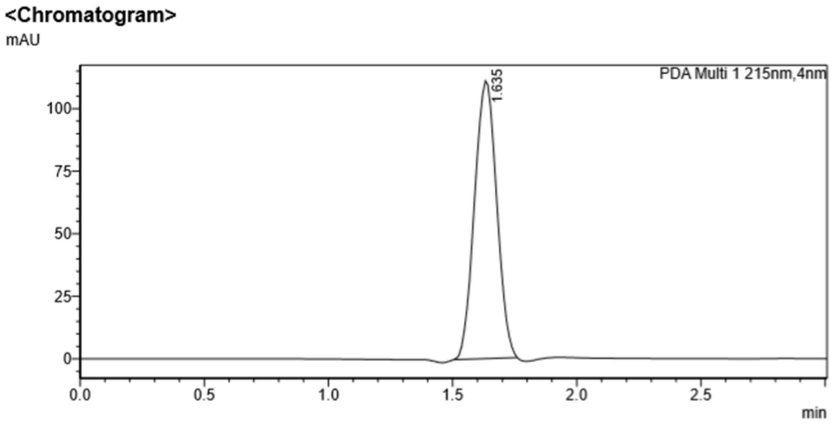

Figure 2: Chromatogram of HPLC

RESULTS

Linearity

Linearity of an analytical procedure is its ability to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample. It was performed by preparing ramipril standard solution in the range above 20-100% of target concentration and measured the peak responses **(Table 3)**.

Appropriate aliquots were pipette out from the standard stocks A and B solutions into a series of 10ml volumetric flasks. The volume was made up to the mark with Acetonitrile:Methanol:Phosphate buffer (60:20:20v/v/v) to get a set of solutions having the concentrations ranging from 20-100µg/ml of ramipril. Absorbance of the solutions was measured at 215nm.

Precision

The precision of an analytical procedure express the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision may be considered at three levels such as system precision and intermediate precision (ruggedness).

The precision of the test procedure was evaluated by injecting the six standard solutions. The relative standard deviation of the six injections was calculated **(Table 4)**.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value that is accepted either as a conventional true value or as an accepted reference value and the value found.

It was carried out by spiking known amounts of drug substance with placebo at 50%, 100% and 150% in triplicate for each level.

To study the reliability and suitability of the developed method, recovery experiments were carried out. Accuracy of the proposed method was determined using recovery studies by standard addition method. The recovery studies were carried

out by adding known amounts (50%, 100% and 150%) of the pure drug to the pre-analyzed formulation. The solutions were prepared in triplicates and the % recovery was calculated **(Table 5)**.

Robustness

Robustness of the method is performed by altering the chromatographic conditions such as flow rate, wavelength, mobile phase composition and observed the variation of the results which should be within the acceptance criteria **(Table 6)**.

Limit of Detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value **(Table 7)**.

1. Based on Signal-to-Noise for LOD (3:1), LOQ (10:1)
2. Based on the Standard Deviation of the response and the slope

$$LOD = \frac{3.3\ \sigma}{S}$$

Limit of Quantification

The quantification limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

From the linearity data, the limit of detection and quantification were calculated using the following formula.

$$LOQ = \frac{10\ \sigma}{S}$$

LOD and LOQ of Ramipril are performed by spiking of known concentrations of the sample into the placebo of formulation and inject the sample.

Table 3: Linearity of Ramipril

S. No	Standard Concentration (µg/ml)	Peak Area
1.	20	2705580
2.	40	5717935
3.	60	8650214
4.	80	11911656
5.	100	15093253

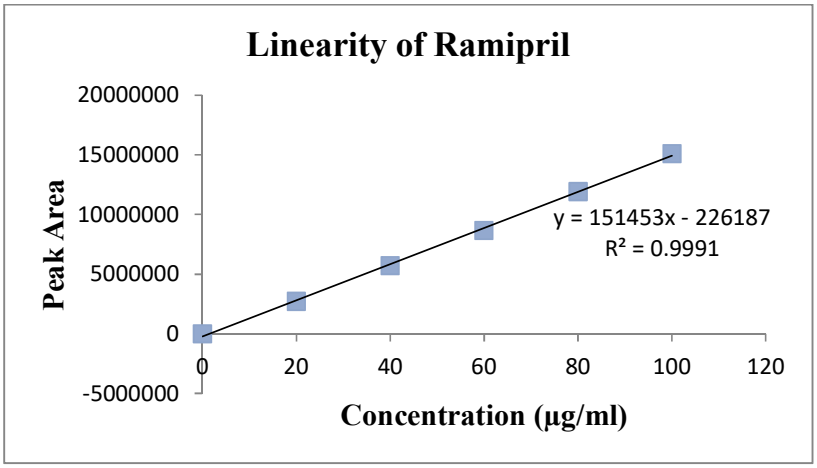

Figure 3: Calibration curve of Ramipril

Table 4: Precision of Ramipril

S. No	Injection Number	Peak Area
1.	1	8698940
2.	2	8514990
3.	3	8679991
4.	4	8682921
5.	5	8568689
6.	6	8567995
7.	Mean	8618921
8.	%RSD	0.96

Table 5: Accuracy of Ramipril

S. No	Accuracy level	% Recovery	Mean recovery	Overall mean recovery
1.	50%	99.2 98.6 99.1	98.96	98.99
2.	100%	99.8 99.0 98.9	99.23	
3.	150%	98.4 99.2 98.8	98.80	

Table 6: Robustness of Ramipril

S. No	Parameters	Condition	System suitability results		
			%RSD	USP tailing	USP Plate Count
1.	Flow rate by ± 0.2 ml/min	0.8	0.87	1.34	2830
		1.2	0.72	1.54	2783
2.	Wavelength of analysis ±5nm	210	1.01	1.71	2486
		220	0.86	1.02	2378
3.	Organic composition of mobile phase(Acetonitrile:Methanol:Phosphate buffer v/v/v)	65:20:15	0.90	1.16	2406
		55:20:25	1.47	1.88	2063

Table 7: Results of LOD and LOQ

Sample	LOD	LOQ
Ramipril	0.97µg/ml	4.69µg/ml

DISCUSSION

The developed RP-HPLC method was validated as per the ICH guidelines for linearity, precision, accuracy, LOD, LOQ and robustness. The method shows the recoveries ranged 95-105% and %RSD <2% which prove that the method is accurate and precise. Linearity was observed in the concentration range between 20 to 100% with correlation coefficient >0.995%.

CONCLUSION

In the present work, an attempt was made to provide a newer, sensitive, simple, accurate and low cost RP-HPLC method. It is successfully applied for the determination of Ramipril in pharmaceutical preparations without the interferences of other constituent in the formulations. The system with Acetonitrile, Methanol and Phosphate buffer in 60:20:20v/v/v ratio with 1.0ml/min flow rate is quite robust. The optimum

wavelength for detection was 215nm at which better detector response for drug was obtained. The average retention time for Ramipril was found to be 1.635minutes. The linearity was observed in the range of 20-100µg/ml. For the drug with a correlation coefficient of 0.999. The low values of %RSD indicate the method is precise and accurate. The mean recoveries were found in the range of 95.0%-105%. Hence, the chromatographic method developed for Ramipril said to be rapid, simple, specific, sensitive, precise, accurate and reliable that can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutics and bio-equivalence studies and in clinical pharmacokinetic studies.

ACKNOWLEDGEMENT

I am very grateful to Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur, for providing support, guidance and facilities.

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