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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR DETERMINATION OF RAMIPRIL

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ABSTRACT

A Simple and precise HPLC method was developed for the determination of Ramipril of pure drug pure drug. It was carried out using C18 Column (250 × 4.6 mm i.d. 5 µm particle size), with mobile phase compressing of Sodium phosphate buffer and Methanol in the ratio of 90:10 v/v/v, pH= 2.8 (pH adjusted with OPA). The flow rate was 1.0 ml/min and the detection was carried out using PDA detector at 208 nm. The retention times were 2.633 minute for Ramipril and Calibration curves were linear with correlation coefficient 0.9996 over concentration range of 100 - 300µg/ml for Ramipril µg/ml. Recovery was found in between 100.6% for Ramipril. Method was found to be reproducible with relative standard deviation (R.S.D) for intra and inter day precision less than 2%. The method was validated by evaluation of different parameters such as accuracy, linearity, precision, LOD and LOQ.

INTRODUCTION

Ramipril (RM) chemically as (2*S*, 3a*S*, 6a*S*)-1-[(*S*)-2-[[(*S*)-1-(ethoxycarbonyl)-3henylpropyl] amino] propanoyl] octahydrocyclo-penta[*b*] pyrrole-2-carboxylic acid (Fig.1). Themolecular weight of RM is 416.5 and melting point is 109°C. Ramipril is soluble in methanol (Clarke's, 2005). It is a highly lipophilic, long acting ACE inhibitor. It inhibits angiotensinconverting enzyme which has two fold effect in reduction of angiotensin-II levels (AT-II) and increase in bradykinin levels. Both are contributes to fall in blood pressure. It acts on the renin–angiotensin aldosterone system (Tripathi, 2003). It inhibits the conversion of the inactive angiotensin-I to the highly potent vasoconstrictor, angiotensin II, and also reduces the degradation of bradykinin.

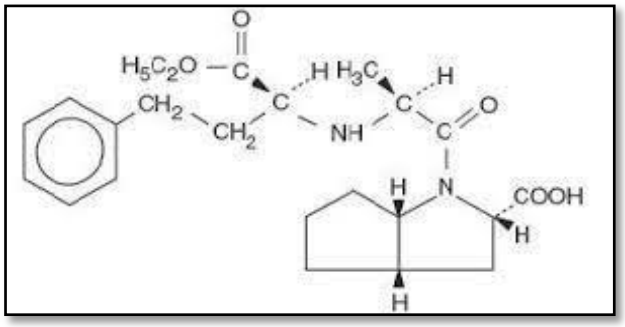

Fig. 1: Structure of Ramipril.

Ramipril is indicated for the treatment of Mild to moderate hypertension, Congestive heart failure, following myocardial infarction in patients with clinical evidence of heart failure.

EXPERIMENTAL

RM of Standard drug were obtained as a gift sample from AratipharmaceuticalsLtd. (H.P., India).All analytical grade chemicals and solvents were supplied by S.D. Fine Chemicals, Mumbai, India. Distilled water was used to prepare all solution. Freshly prepared solutions were always employed.

INSTRUMENTAL

Determination was carried out using gradient binary pumps system combined with a PDA 2998 series photo diode array detector. The column used was C18 Column (250×4.6mm i.e.;5µm particle size). UV detection was performed at 208 nm. Analyte weighing, for preparation of calibration standards and quality controls, was done on a microbalance, Mettler Toledo AB 204-S.All mobile phase solutions were degassed ultrasonically by Steryl 40050 bath Sonicator before use. The HPLC system was controlled by a PC workstation using Empower software 2.

CHROMATOGRAPHIC CONDITIONS

C_{18} column (250mm× 4.6mm) was used for the separation; mobile phase consisted of mixture of methanol and phosphate buffer [pH 2.8 adjusted with ortho-phosphoric acid (dil.)]in ratio (90:10 v/v) was delivered at a flow rate 1.0 ml/min with detection wavelength 208 nm for Ramipril. The mobile phase was filtered through a 0.45µm membrane filter and sonicated for 10 min. the injection volume was 20µl. Analysis was performed at a temperature of 40^0C.

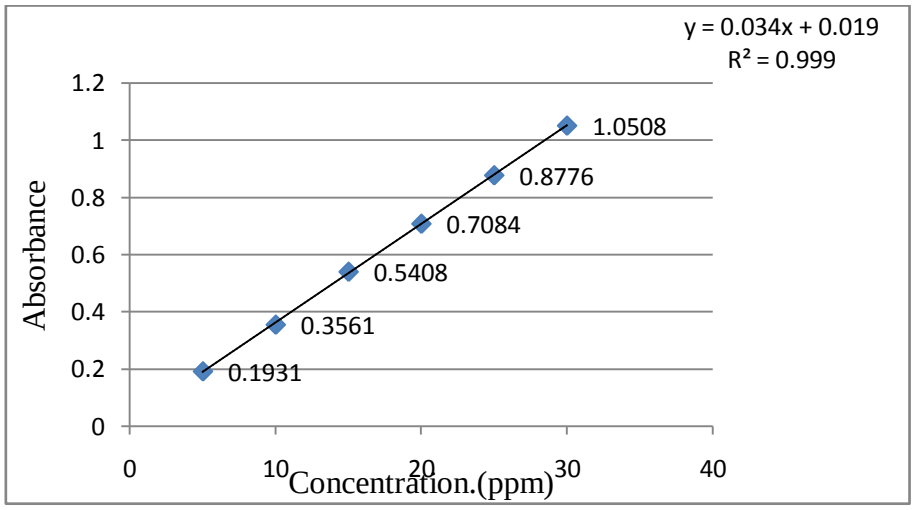

Fig. 2: Calibration curve for Ramipril.

Preparation of standard stock solution:

A stock solution of drug Ramipril (100µg/mL) was prepared by dissolving 10 mg of drug in 100mL of selected solvent (methanol + phosphate buffer pH 2.8).

Optimization of chromatographic conditions

Sometimes, the effects of different chromatographic conditions on the instrumental responses create a situation where one has to compromise between different experimental variables in order to achieve the best chromatographic separation.Chromatographic separations are significantly affected by themobile phase conditions, such as the type and composition of the organic modifiers. The optimized chromatographic peaks with best resolutions show in **Figure 3**.

Sample Name: Ramipril 100ppm

Wavelength: 208nm

Mobile Phase: Methanol: Buffer (KH_2PO_4) pH: 2.8 (90:10)

Sample volume: 20µl

Flow rate: 1.2 ml/min

Pressure:9-10MPa

Run Time: 5.62min

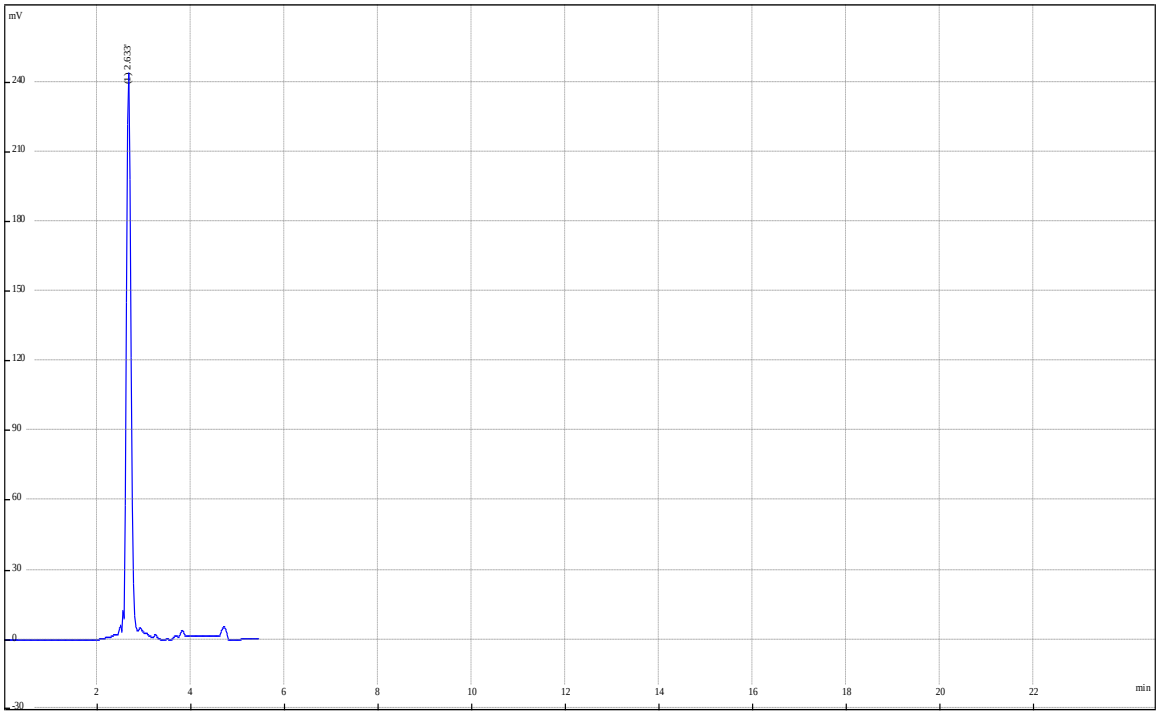

Fig. 3: Typical chromatogram of Ramipril

Validation

Once the chromatographic method had been developed and optimized, it must be validated. The validation of an analytical method verifies that the characteristics of the method satisfy the requirements of the application domain. The proposed method was validated in the light of ICH Guidelines for linearity, precision, recovery. Consequently, the following were performed (ICH Guidelines, 1996).

Linearity:

Series of aliquots of the standard stock solution[100µg/ml-300µg/ml] were transferred into a series of 10 mL volumetric flasks and volume was made up to the mark using selected solvent. Each solution was injected and chromatogram was recorded. Linearity was evaluated by determining by five standard working solutions each in triplicate for HPLC. Correlation coefficient and regression equation were obtained from the calibration curve. The range for which minimum value of sum of squares of error was obtained was chosen for the study. An acceptance criterion for Linearity is< 0.999.

Precision:

Prepare six different test solutions of 100% test concentration from same sample matrix. Inject duplicate injection of each test solutions. For standard solution, 100 mg of Ramipril was dissolved in 100 ml solvent mixture to make 1000µg/ml. sonicted it for 15 min. then 1

ml withdrawn from stock solution and diluted in 10 ml of solvent to make 100 µg/ml. six replicates are taken.

Accuracy: To check the accuracy of method, recovery studies were carried out by addition of standard drug solution to pre-analysed sample solution at three different levels 50%, 100%, 150% recovery study.

1. **50%Accuracy:**Prepare 100 µg/ml of standard solution from 1000µg/ml. take 1 ml from it and 0.5 ml of ramipril solution of 100µg/ml about 9.5 ml of diluent was added. Shake well and sonicated to dissolve the content then filter through sintered glass membrane filter.
2. **100% Accuracy:** Prepare 100 µg/ml of standard solution from 1000µg/ml. take 1 ml withdrawn from solution and 1 ml of ramipril solution of 100µg/ml about 9 ml of diluent was added. Shake well and sonicated to dissolve the content then filter through sintered glass membrane filter.
3. **150%Accuracy:** Prepare 100 µg/ml of standard solution from 1000µg/ml. take 1 ml withdrawn from solution and 1.5 ml of ramipril solution of 100µg/ml about 8.5 ml of diluent was added. Shake well and sonicated to dissolve the content then filter through sintered glass membrane filter. For Accuracy, the acceptance criteria are 98-102% and R.S.D. is 2.0%.

Robustness: the robustness of method is its ability to remain unaffected by small deliberate changes in parameters. For e.g. Change in flow rate of mobile phase ± 0.20 ml/min or change in composition of mobile phase ± 2.0%, change in column oven temperature ± 5^0C. Acceptance criteria for Robustness is<2.0%.

Sensitivity

Limit of detection (LOD) and limit of quantitation (LOQ) were calculated by

$$S/N = 2/1 \text{ or } 3/1$$

Where, S= signal

N= Noise.

LOD is calculated from the formula:

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

LOQ is calculated from the formula:

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

σ = Standard deviation of the response

S = Slope of the calibration curve

RESULTS AND DISCUSSION

Optimization of Chromatographic Condition

The chromatographic parameters were initially evaluated using a Symmetry C18 column and a mobile phase composed of Sodium phosphate buffer: Methanol (9.:10).Under these conditions, the retention time obtained for RM was 2.633 min, Optimized chromatographic peak shows in Figure 3.

Validation

Linearity/Calibration Curve

A linear correlation was found between the peak areas and the concentrations of RM in the range 100-300 of µg/ml for RM The regression analysis data are presented in Table 2 the regression coefficients (r2) obtained for RM is 0.996 Both compounds show the linearity of the method. Calibration curve for RM is given in Figure 4.

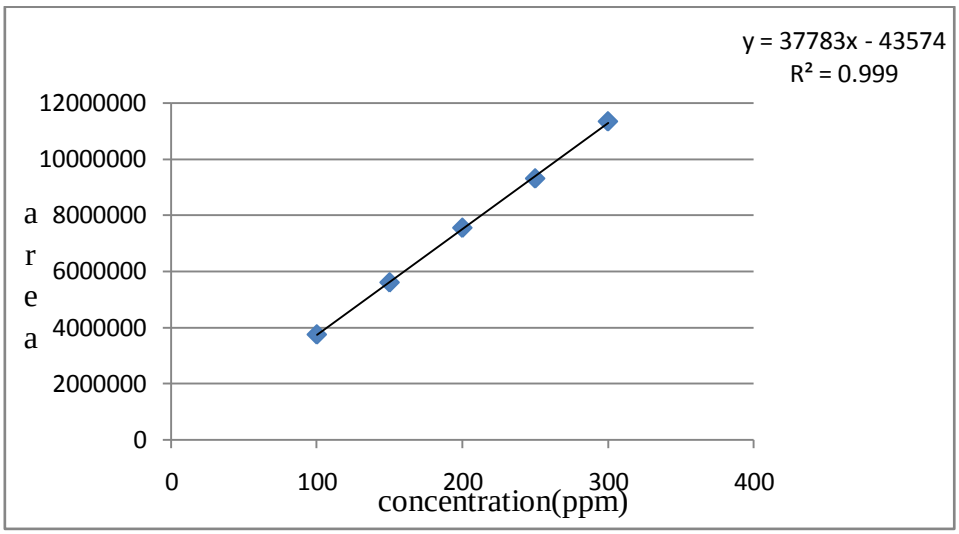

Fig No: 4.Linearity curve ofRamipril

Table No. 1 . Linearity of Ramipril

Sr. No.	Concentration of sample in ppm	Area	Theoretical plate
1	100	3750671	5291
2	150	5606598	5347
3	200	7552733	5288
4	250	9311282	5175
5	300	11344157	5269

Precision

The reproducibility of the method was estimated by analyzing samples. Six injections of the standard mixture were analyzed for the determination of system precision. Similarly six solutions of the individual standards were prepared and assayed for the determination of method intra-precision and inter-precision results are shown in the table 3 & 4.

Table no. 2. Intra-day Precision of Ramipril

Sr.no.	Injection of sample	Ramipril Area
1	Injection 1	673842
2	Injection 2	674564
3	Injection 3	676347
4	Injection 4	676347
5	Injection 5	669531
6	Injection 6	672462
	Average area	672583
	S.D.	38574
	% RSD	0.96323
	Acceptance criteria	≤ 2%

Table no. 3. Inter-day Precision of Ramipril

Sr.no.	Injection of sample	Ramipril Area
1	Injection 1	648573
2	Injection 2	644731
3	Injection 3	632317
4	Injection 4	635203
5	Injection 5	635345
6	Injection 6	645925
	Average area	640349
	S.D.	34073.15
	% RSD	0.7539
	Acceptance criteria	≤ 2%

Accuracy of Ramipril

Accuracy of method was demonstrated at three different concentration level (50-150%) by spiking a known quantity of standard drugs into analyzed sample in triplicate. The result of Accuracy revealed than the method was more accurate.i.e. % Recovery of RM was found to be 100.6% & % RSD was 1.1

Table no. 4. Accuracy of Ramipril

Sr. no.	Conc.level (%)	Vol. of Stock solution	Amount Added (µg)	Average Area	Amount Recovered (µg)	% Recovery	Average Recovery %	% RSD
1	50	1.0	10	6592453	9.90	99.0	99.3	1.111
2	50	1.0	10	6745425	9.94	99.4		
3	50	1.0	10	6792341	9.97	99.7		
4	100	2.0	10	7583523	10.05	100.5	100.4	0.79
5	100	2.0	10	7602433	10.03	100.3		
6	100	2.0	10	7579421	10.06	100.6		
7	150	3.0	10	8347392	10.08	100.8	100.6	0.79
8	150	3.0	10	8284367	10.05	100.5		
9	150	3.0	10	8298640	10.07	100.7		
					Overall recovery		100.6	1.1

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.

Table no. 5. Robustness of Ramipril

Sr. No.	Concentration	Area (↓ flow rate ± 0.2 ml)	Area (↑ flow rate ± 0.2ml)
1	100	675489	66895
2	100	668753	66947
3	100	666132	66486
	Average	670125	667763
	S.D	54297.6376	589643.27
	%RSD (≤ 2%)	0.9818506	0.9114325

Detection and quantitation limits

Limit of detection and Limit of Quantitation of developed method of Ramipril was found to be 1.8µg/ml 0.601µg/ml respectively. It is expressed as the concentration of analyses in sample. S/N ratio should not be less than 10 & RSD ≤ 3%

CONCLUSION

The proposed method was found to be simple, fast, robust, more precise and accurate under the present experimental conditions. Therefore the developed method can be used for routine analysis for simultaneous estimation of Ramipril in pharmaceutical dosage form.

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