

Development and Validation of A Reversed Phase-High Performance Liquid Chromatography Method for the Quantitative Estimation of Ramipril Drug Content from Formulated Dosage Form

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ABSTRACT

A reliable and reproducible reversed-phase high performance liquid chromatography (RP-HPLC) was developed for the quantitative determination of Ramipril drug content from marketed bulk tablets. The active ingredient of Ramipril separation achieved with C¹⁸ column using the methanol water mobile phase in the ratio of 40:60 (v/v). The active ingredient of the drug content quantify with UV detector at 215 nm. The retention time of Ramipril is 5.63 min. A good linearity relation (R²=0.999) was obtained between drug concentration and average peak areas. The limit of detection and limit of quantification of the instrument were calculated 0.03 and 0.09 µg/mL, respectively. The accuracy of the method validation was determined 102.72% by recoveries method.

Keywords: Ramipril, RP-HPLC, UV-detector.

INTRODUCTION

The chemical name of active ingredient Ramipril is (2S, 3aS, 6aS)-1 [(S)-N- [(S)-1-carboxy-3-phenylpropyl]alanyl] octahydro cyclopenta [b]pyrrole-2-carboxylic acid-1-ethyl ester. It is highly lipophilic in nature. It is inhibit to angiotensin converting enzyme (ACE). Ramipril is used against hypertension, congestive heart failure and diabetic nephropathy¹. It is white crystalline powder. It is freely soluble in Methanol, Slightly soluble in Water, Which is melts in the range of 109 -110 °C. The various methods have been reported of method validation of Ramipril with different separation techniques like UV-Visible Spectroscopy², RP-HPLC³⁻⁵ etc. The main purpose of the present study was to establish according ICH Guidelines for the determination and estimation of Ramipril drug content in pure form and in pharmaceutical dosage forms⁶⁻⁷.

EXPERIMENTAL

Chemicals and reagents

The 99.5% standard drug of Ramipril, HPLC grade methanol, distilled water and 0.45 nylon filter membrane were purchased from Merck India Ltd.

Instrumentation

A binary pump CYBERLABTM HPLC chromatograph was used for the analysis. The separation has done on reversed phase C₁₈-column. The analyte were monitored with UV detector at 215 nm. The reversed phase HPLC was operated

at isocratic elution mode with 40:60 (v/v) methanol-water mobile phases. The flow rate of elution was 1.0 mL/min. An ultrasonic sonicator was used for the degassing of mobile phase, standard solution and sample solution.

Preparation of mobile phase

The solvent mixture of methanol /water in the ratio of 40:60 was used as mobile phase. The mobile phase were filtered through a 0.45µm nylon membrane and degassed by sonication.

Preparation of Ramipril stock solution

A 200 ppm stock solution was prepared for preparation the serial dilutions. Ramipril transferred (20 mg) into a 200 mL volumetric flask and mixed with mobile phase and make up it to meniscus, and then it filtered through 0.45µm nylon filter membrane.

Preparation of sample solution

The 10 tablets of Ramipril were weight and crushed by mortar piston. The crushed tablets were mixed well, and then an equivalent amount of 20 mg was transferred into a small conical flask and extract with mobile phase. The extract was filtered into a 200 ml volumetric flask and the volume make up to 200 ml. Achieved aliquots was covered the working concentration range 200 ppm.

Preparation of calibration curve

A calibration curve was constructed to evolution the linearity. The calibration curve was plotted between average peak area and its drug concentration (µg/mL)

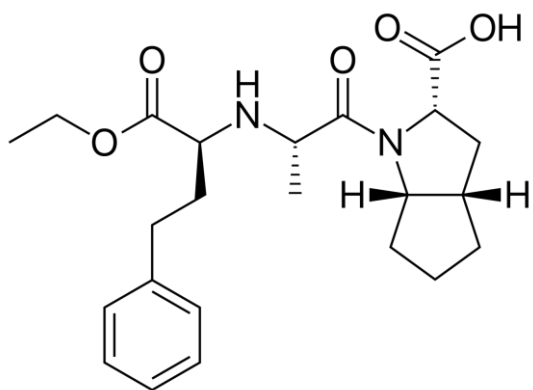


Figure 1: Structure of Ramipril

Table 1: The results for system suitability test

Sl. No.	Parameters	Mean	CV %
1	Retention Factor	5.89	2.28
2	Tailing Factor	0.95	2.00
3	Resolution Factor	1.14	1.49
4	Theoretical Plates	1272	2.46

CV=coefficient variance

Table 2: Summary for linearity study

Sl No.	Parameters	RP-HPLC Analysis
1	Correlation range	5-100 µg/mL
2	Regression equation	y=711.6x-183.1
3	Regression coefficient (R ²)	0.999

Table 3: Results for Limit of Detection and Limit of Quantification

Sl. No.	Parameters	Concentration in µg/mL
1	Limit of Detection	0.03
2	Limit of Quantification	0.09

Table 4: Results of recovery experiment by HPLC Method

Sl. No.	Added in µg	Recover in µg	Recovery in %
1	5	4.84	96.80
2	10	9.8	98
3	25	25.68	102.72

levels. The serial dilutions 5, 10, 20, 25 and 50 and 100 µg/mL were prepared from the stock solution of Ramipril. A total volume of 20 ml was maintained with mobile phase. These different serial dilutions were filtered through a 0.45µm nylon membrane. Each solution of 20 µL was injected into the column in thrice replication.

Method validation

The describe method was validated according to ICH guidelines with respect to linearity, specificity, accuracy, limit of detection (LOD) and limit of quantification (LOQ).

Linearity

According to ICH guidelines the linearity of an analytical procedure is its ability (within a given range) to obtain test

results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity can be calculated by using following equation as per ICH guidelines ^{6,7}.

$$y = mx + b$$

Where the constant 'm' determines the slope or gradient of that line and the constant term 'b' determines the point at which the line crosses the y-axis, otherwise known as the y-intercept.

Specificity

Specificity was determined with excipients of formulated tablets. An equivalent weight was taken and solution prepared similarly to the sample solution. The prepared solution was determined as per the ICH guidelines. The specificity can calculated from the following relation:

$$\text{Specificity} = \frac{\text{Number of true negatives}}{\text{Number of true negatives} + \text{number of false positives}}$$

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy of the proposed methods was checked by recovery studies, by addition of standard drug solution to reanalyzed sample solution at three different concentration levels 5, 10 and 25 µg/mL. The chromatograms were recorded and the percentage recovery was calculated.

Accuracy can be calculated by using following equation as per ICH guidelines.

$$\text{Percentage Recovery} = \frac{\text{Peak Area of the Drug in Standard}}{\text{Peak Area of the Drug in Sample Mix}} \times 100$$

System suitability test

The reproducibility of sample was checked of the system to measurement of peak area and was carried out using three replicates of same concentration of standard and sample, respectively.

Limit of detection (LOD)

According to ICH guidelines 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.

Limit of detection can be calculated using following equation as per ICH guidelines.

$$\text{Limit of Detection} = 3.3 \times \frac{\text{Standard deviation of the Peak Area of the Drug}}{\text{Slope of the Corresponding Calibration Curve}}$$

Limit of quantification (LOQ)

The quantization 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. The quantization limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

Limit of quantification can be calculated using following equation as per ICH guidelines.

$$\text{Limit of Quantisation} = 10 \times \frac{\text{Standard deviation of the Peak Area of the Drug}}{\text{Slope of the Corresponding Calibration Curve}}$$

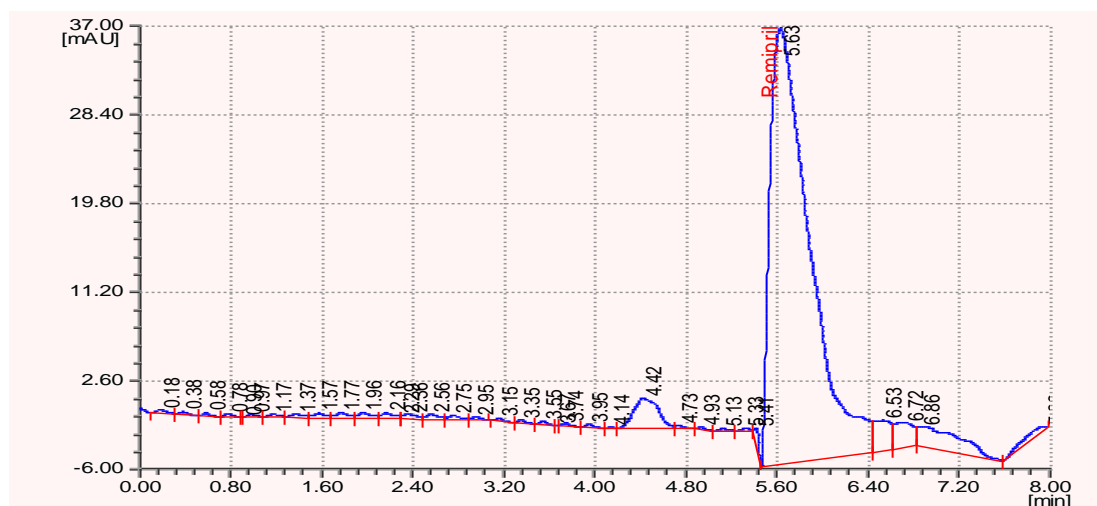


Figure 2: Standard Chromatogram of Ramipril

RESULTS

Selection of mobile phase

It is a basic need of high performance liquid chromatography (HPLC) technique. The mobile phase have been selected to check the mobile phase at various composition with the solvent of HPLC grade methanol and water 70:30, 60:40, 50:50, 40:60 and 30:70 (v/v) on reversed phase C₁₈ column with the wave length 215 nm. The mobile phase methanol and water in the ratio of 60:40 (v/v) selected.

Chromatographic Conditions

The separation and identification of the Ramipril drug content have been done with the mobile phase 40:60 (methanol:water) at the flow rate of 1.0 mL/min. The drug content optimized at 215 nm. These all parameters showed a well-defined chromatographic separation within a run time of about 5.63 min. The retention time of Ramipril was 5.63 min.

System stability test

The System suitability test was performed to stabilization the chromatographic conditions. The test was performed by injecting the standard mixture in thrice replication. The various parameters Retention Time (R_t), Tailing Factor (T_f), Resolution Factor (R_f), and Theoretical Plates (T_p) were computed. The all parameters were statistically calculated. The CV % to the retention, tailing factor, resolution factor and theoretical plates were reported 2.28, 2.0, 1.49 and 2.46. The calculated system suitability parameters were shown in the following table 1.

The calculated CV % value of all parameters are less than 10 (<10)^{9,10}, thus all CV% values are significant.

Specificity

The specificity of the method was determined by checking the interference with the components from placebo, No interference was observed for any of the components like excipients. For the repeatability of the peaks and retention time the required temperature was 20 °C⁸. The above Chromatogram is showing that the specificity of the method (fig No. 2).

Linearity

The detector response for the proposed method determined to be linear over the range. The six concentration levels 5, 10, 20, 25 and 50 and 100 µg/mL were prepared and injected. The calibration curve was plotted between drug concentrations level and peak area (average value) for HPLC. The linearity of the method was evaluated by linear regression analysis. The following results are obtained on the analysis of the data:

The correlation range was determined for HPLC 5-100 µg/mL, respectively. Regression coefficient was calculated 0.999 which significant at level of 0.05 % error. Hence the above calculated data are showing that the detector response is Linear

Limits of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) were determined by calculating signal to noise ratio for metformine hydrochloride 3 and 10 respectively. The limit of detection (LOD) and limit of quantification (LOQ) values for RP-HPLC were found 0.03 µg/mL and 0.09 µg/mL.

Accuracy

The accuracy of the method validation was determined by recovery method. The recovery was calculated in percentage for HPLC method 96.80, 98 and 102.72 % at three concentrations level 5, 10 and 25 µg.

The recovery results were show good co-relation with adding Ramipril drug content. Thus, laboratories experimental method was success for the quantitative determination and estimation of Ramipril drug content.

DISCUSSION

The analysis parameter such as system suitability study is indicate that the applied method was suitable for the analysis. Wave length selection is the primary need for the chromatographic analysis. To selection the wave length for Ramipril was investigated in order to determine a suitable wavelength for the assay evaluation. The suitable wave length was found 215 nm. The selection of mobile phase is an important secondary basic need for chromatographic analysis. The mobile phase was select under isocratic chromatographic mode.

CONCLUSION

The development of method and validation for Ramipril by reverse phase-high performance liquid chromatography (RP-HPLC) technique was successfully applied in the laboratory for the determination of Ramipril drug content from the marketed dosage form. Thus, the above method can be recommended for simultaneous determination of Ramipril drug content from formulated dosage forms estimation.

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