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Research Article

STABILITY INDICATING RP-HPLC METHOD FOR DETERMINATION OF RAMIPRIL IN PURE AND PHARMACEUTICAL FORMULATION

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ABSTRACT

A simple, rapid and accurate and stability indicating RP-HPLC method was developed for the determination of ramipril in pure and tablet forms. The method showed a linear response for concentrations in the range of 100-500 µg/mL using Acetonitrile: Buffer solution in the ratio (70:30) as the mobile phase with detection at 225 nm and a flow rate of 0.8 mL/min and retention time 2.287min. The value of correlation coefficient, slope and intercept were, 0.999, 9318.72and179702, respectively. The method was validated for precision,recovery, ruggedness and robustness. The drug undergoes degradation under acidic, basic, peroxide and thermal degradation conditions. All the peaks of degraded product were resolved from the active pharmaceutical ingredient with significantly different retention time. As the method could effectively separate the drug from its degradation product, it can be employed as a stabilityindicatingone.

Keywords: Eprosartan, RP-HPLC, Degradation studies.

INTRODUCTION

Stability indicating methods have become an important aspect of any analytical method validationandapartofUSFDArequirements[1].Chemically,Ramipril,2-[N-[(S)-1-(ethoxycarbonyl)-3-phenylpropyl)]-L-alanyl]-(1S,3S,5S)-2-azabicyclo[3-3-0]octane carboxylic acid (Fig 1),

is an angiotensin-converting enzyme (ACE) inhibitor. It acts on the renin–angiotensin aldosterone system. It inhibits the conversion of the inactive angiotensin-I to the highly potent vasoconstrictor, angiotensin-II, and also reduce the degradation of bradykinin1.

Hydrochlorothiazide, 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-Sulphonamide 1, 1-dioxide (Fig.2), is a diuretic, which inhibits active chloride reabsorption at the early distal tubule via the Na-Cl co-transporter, resulting in an increase in the excretion of sodium, chloride, and water1.Literature survey reveals few analytical methods for the determination of ramipril in pharmaceutical preparations and biological fluids, viz.radioimmunoassay[2], spectrophotometry[3,4],potentiometry [5,6] GC, [7,8] and HPLC [9,10]. and LCMS[11].UV Spectroscopy [12-15], Ratio Spectra Derivative Spectrophotometry[16]. HPLC [16, 17, 18] and HPTLC [19] methods are reported for simultaneous estimation of Hydrochlothiazide in combined dosage form. There are no reports yet for determination of this combination by proposed methods. Present work emphasizes on the quantitative estimation of Ramipril and Hydrochlorothiazide in their combined dosage form by UV Spectroscopic methods.

Figure 1: chemical structure of ramipril

MATERIALS AND METHODS

Chromatographic conditions: The analysis of the drug was carried out on a Waters HPLC system equipped with a reverse phase Xterra C18 column (100 mmx4.6mm; 3.5µm), a 2695 binary pump, a 20 µl injection loop and a 2487 dual absorbance detector and running on Waters Empower software.

Chemicals and Solvents: The reference sample of ramipril was supplied by Sun Pharmaceutical Industries Ltd., Baroda.

HPLC grade water and acetonitrile were purchased from E. Merck (India) Ltd., Mumbai.

Potassium dihydrogen phosphate and orthophosphoric acid of AR Grade were obtained from S.D. Fine Chemicals Ltd., Mumbai.

Preparation of phosphate buffer: Seven grams of KH2PO4 was weighed into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC water. 2 ml of Triethylamine was added and pH adjusted to 3.0 with orthophosporic acid.

Preparation of mobile phase and diluents 300 ml of the phosphate buffer was mixed with 700 ml of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45µ filter under vacuum.

Chromatographic conditions:

Mobile phase consist Conditions of ACN-Phosphate buffer in the ratio of70:30 at a flow rate of 08 ml/min and pH of buffer was adjusted to 3.0 UV detection was performed at 225nm. The mobile phase was degassed by an ultrasonic water bath for 5 min. Filter through 0.45µ filter under vacuum filtration. The column was equilibrated for at least 30 min with the mobile phase flowing through the system.

Standard Solution Preparation:

Accurately weigh and transfer 10mg of ramipril working standard into a 10 ml volumetric flask add about 7 ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 3.0ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Sample Solution Preparation

Weigh 5 ramipril Tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10 mg of ramipril into a 10 ml volumetric flask. Add about 7mL of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45µm filter.

Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Preparation of calibration graph

The linearity of response for ramipril assay method was determined by preparing and injecting solutions with concentrations of about 100,200, 300,400,500 µg/ml of ramipril.

Validation of the Proposed Method

After chromatographic method development and optimization it was 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 according to ICH guidelines for linearity, precision, sensitivity, and recovery. For linearity studies, working standard solutions equivalent to100 to500 µg/ml of ramipril were prepared with the mobile phase.

Precision & Accuracy

According to ICH, precision is the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions and may be considered at three levels: repeatability, intermediate precision and reproducibility. The intra-day and inter-day variations of the method were determined using five replicate injections and analyzed on the same day and different days.

The accuracy of an analytical method expresses the closeness between the theoretical value and experimental value. To ensure the reliability and accuracy of the method, the recovery studies were carried out to ensure the reliability and accuracy of the method. Accuracy was evaluated by injecting the ramipril about five times, at three different concentrations equivalent to 50, 100, and 150% of the active ingredient, by adding a known amount of ramipril standard to a sample of known concentration and calculating the recovery of ramipril for each concentration.

Detection and Quantification Limits

The limits of detection and quantification were calculated by the method based on standard deviation (σ) and slope (*S*) of the calibration plot using the formula LOD = 3.2 σ/*S* and LOQ =9.9 σ/*S*.

Specificity

The specificity test of proposed method demonstrated that excipients from tablets do not interfere in the drug peak. Furthermore, well shaped peaks indicate the specificity of the method.

Assay

Twenty tablets were weighed and powdered equivalent to 10 mg of ramipril was accurately weighed and diluted up to 10 ml. Working dilution was prepared using same diluents and used for analysis.

Forced degradation studies

Acid Degradation

Accurately weigh and transfer 10mg of ramipril working standard into a 10mL volumetric flask add about 3 ml of 0.5N HCl and sonicated for 5minutes. Refluxed under heat at 60 degrees in a heating mantle for 2 hours. Neutralized the sample solution using 0.5N NaOH and diluted up to the mark with diluents. (Stock solution).

Further pipette 0.3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Base Degradation

Accurately weigh and transfer 10mg of ramipril working standard into a 10mL volumetric flask add about 3mL of 0.5N NaOH and sonicated for 5 minutes. Refluxed under heat at 60 degrees in a heating mantle for 2 hours. Neutralized the sample solution using 0.5N HCl and diluted up to the mark with diluents. (Stock solution).

Further pipette 0.3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Thermo Degradation

Accurately weigh and transfer 10mg of ramipril working standard into a 10mL volumetric flask and oven under heat at 105 degrees for 12 hours. (Stock solution).

Further pipette 0.3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Peroxide Degradation

Accurately weigh and transfer 10mg of ramipril Working standard into a 10mL volumetric flask add about 1 ml of 30% Hydrogen Peroxide (H_2O_2)and sonicated for 5minutes and Refluxed under heat at 60 degrees in a heating mantle for 2 hours

Further pipette 0.3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

RESULTS AND DISCUSSION

In the proposed method, the retention time of ramipril was found to be 2.287 min. Quantification was linear in the concentration range of 100-500µg/ml. The regression equation of the linearity plot of concentration of ramipril over its peak area was found to be Y=179720+9316.7X (r2=0.999), where X is the concentration of ramipril (µg/ml) and Y is the corresponding peak area. The number of theoretical plates calculated was 2533.7, which indicates efficient performance of the column. The limit of detection and limit of quantification were found to be 0.12µg/ml and 0.42 µg/ml respectively, which indicate the sensitivity of the method. The use of phosphate buffer and acetonitrile in the ratio of 30:70v/v resulted in peak with good shape and resolution.

Calibration curve

These results indicate that the response is linear over the range of 100,200,300,400and 500µg/ml of ramipril with coefficient of regression, R2, value as 0.999 as shown in Table: 1. the value of correlation coefficient, slope and intercept were 0.999, 9318.72, and179702 respectively

Table: 1: Regression characteristics of the ramipril for proposed HPLC method

S.No.	parameter	Result
1.	Range (µg/ml)	100-500
2.	Detection wavelength	225
3.	. Mean 'R2' value	0.999
4.	Slope (m).	9318.72
5.	Intercept (c)	179702
6.	Run time(MIN)	6
7.	Retention Time (min)	2.287
8.	Theoretical Plates (N)	2533.7
9.	Tailing Factor	1.2
10.	LOD	0.12
11.	LOQ	0.42

Validation of the proposed method

The specificity, linearity, precision, accuracy, limit of detection, limit of quantification, robustness and system suitability parameters were studied systematically to validate the proposed HPLC method for the determination of the diluent and the solution was filtered through a 0.45 µ membrane filter. This solution containing 15 µg/ml of

ramipril was injected into the column six times. The average peak area of the drug was computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug. The relevant results are furnish Solution containing 40 µg/ml ramipril of was subjected to the proposed HPLC analysis to check intra-day and inter-day variation of the method and the results are furnished in Table-2.

The accuracy of the HPLC method was assessed by concentrated levels by the proposed method. The results are furnished in Table-3.

Table2: Precision of the proposed HPLC method

Concentration of ramipril(µg|ml)	Intra day	Inter day
Injection-1	2977138	3045134
Injection-2	2917756	3042317
Injection-3	2861139	3041284
Injection-4	2922541	3047977
Injection-5	2908272	3056220
Average	2917369	3046586
Standard Deviation	41363.5	5983.0
%RSD	1.42	0.20

Table3: Accuracy studies

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	4057403	5.0	5.01	100.20%	
100%	5560714	10.0	9.87	98.76%	99.2%
150%	6796343	14.8	14.60	98.66%	

Estimation of ramipril in tablet dosage forms

Two commercial brands of tablets were chosen for testing the suitability of the proposed method to estimate ramipril in tablet formulations Twenty tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 25 mg of ramipril was transferred into a 100 ml volumetric flask and dissolved in 25 ml of a 35:65 v/v mixture of phosphate buffer and acetonitrile. The contents of the flask were sonicated for 15 min and a further 25 ml of the diluent was added, the flask was shaken continuously for 15 min to ensure complete solubility of the drug. The volume was made up with the diluent and the solution was filtered through a 0.45 µ membrane filter. This solution containing 15 µg/ml of ramipril as injected into the column six times. The average peak area of the drug was computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug. The relevant results are furnished in table-5

Formulation	Label claim (mg)	Amount found (mg)	% Amount found
1	5	5.01	100.20
2	5	4.9	99.99

Stability- Indicating property

The stress studies were conducted and the data were depicted in Table: 5. the chromatogram of no stress treatment of control and sample showed no additional peaks (Figure: 2 & 3)

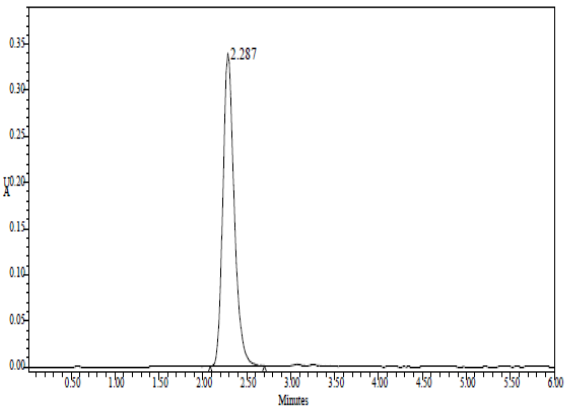

Figure 2: The simple chromatogram of standard ramipril.

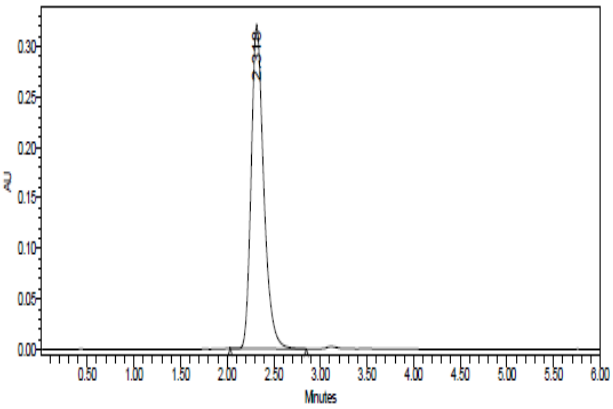

Figure 3: The simple chromatogram of test ramipril

The retention time (RT) of standard and sample were 2.287min&2.318min .The chromatogram of acid degraded sample showed no additional peaks (fig: 4). The chromatogram of alkali degraded sample showed no additional peaks (fig: 5). The chromatogram of thermal degraded sample showed no additional peaks (fig: 6**).** and the values were shown in Table 4.

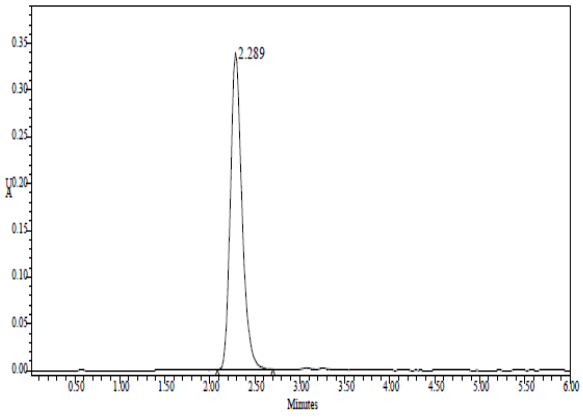

Figure4: The chromatogram of no stress treatment sample

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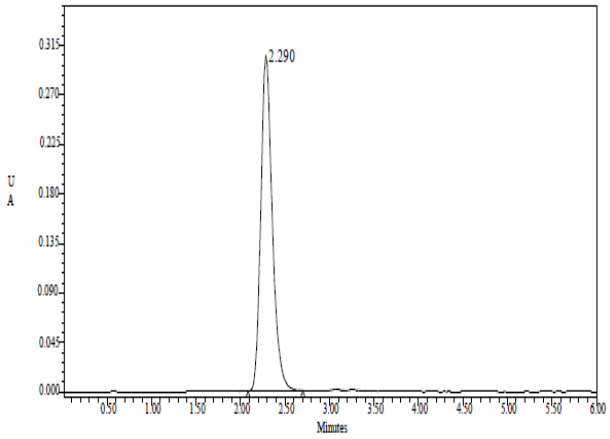

Figure 5: The chromatogram of acid degraded sample

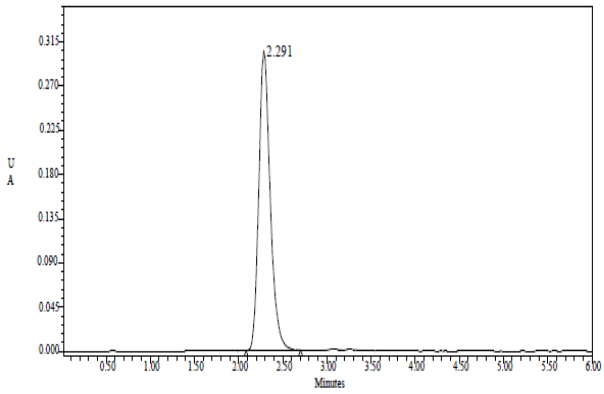

Figure 6: The chromatogram of alkali degraded sample

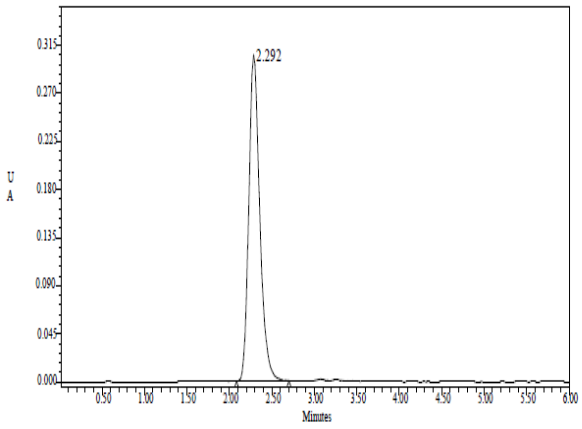

Figure 7: The chromatogram of thermal degraded sample

Table 4: Stressed study data of ramipril

S. No	Condition	Time(hrs)	Assayof ramipril	Retention time of ramipril	%degradation
1	No stress treatment	-	98.0	2.289	Nil
2	Acid	2	-	-	Nil
3	Alkali	2	-	-	Nil
4	Thermal	12	-	-	Nil

CONCLUSION

The developed HPLC technique is precise, specific, accurate and stability-indicating. Statistical analysis proves that the method is suitable for the analysis ramipril of as bulk drug and in Pharmaceutical formulation without any interference from the excipients. This study is a typical example of a stability-indicating assay, established following the recommendations of ICH guidelines. The method can be used to determine the purity of drug available from various sources by detecting any related impurities. The method has been found to be better than previously reported methods, because of use of a less economical and readily available mobile phase, lack of extraction procedures, no internal standard, and use of the same mobile phase for washing of the column. All these factors make this method suitable for quantification of in bulk drugs and in pharmaceutical dosage forms. It can therefore be concluded that use of the method can save much time and money and it can be used in small laboratories with very high accuracy and a wide linear range.

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REFERENCES

1. Goodman and Gilman's, in: Gilman A G, Rall T W, Nies A S,Taylor P. The Pharmacological Basis of Therapeutics. 11th Ed.Pergamon Press: Oxford; 1996, p. 743.
2. Eckert H G, Muenscher G, Oekonomopulos R, et al. Aradioimmunoassay for the angiotensin converting enzymeinhibitor ramipril and its active metabolite. Arzeneim, Forsch/Drug research. 1985; 35 (8): 1251–1256.
3. Bonazzi D, Gotti V, Andrisano V, Cavrini V. Analysis of ACEinhibitors in pharmaceutical dosage forms by derivative UVspectroscopy and liquid chromatography (HPLC). J. Pharm. Biomed. Anal. 1997; 16: 431–438.
4. Rahman N. and Azmi S.N.H. Kinetic SpectrophotometricMethod for the Determination of Ramipril inPharmaceutical Formulations, AAPS Pharm. Sci. Tech., 6(3),2005, E543-E551.
5. Aboul-Enein H. Y., Raluca I. S, Jacobus F V. Analysis of severalangiotensin-converting enzyme inhibitors usingpotentiometric, enantioselective membrane electrodes. Anal.Lett. 1999; 32: 623–632.
6. Aboul-Enein H Y, Bunaciu A, Bala C, Fleischin S. Enalapril andramipril selective membranes, Anal. Lett.1997; 30: 1999–2008.
7. Hans H M, Thomas K, Joachim W A. Screening for the Detectionof Angiotensin-Converting Enzyme Inhibitors, TheirMetabolites, and AT II Receptor Antagonists. Therap. Drug Monitor. 1998; 20: 706–713.
8. Sereda K M, Hardman T C, Dilloway M R, Lant A F. Developmentof a Method for the Detection of Angiotensin ConvertingEnzyme Inhibitors Using Electron Capture-Gas Chromatography Detection. Anal. Proc. 1993; 30: 371–372.
9. Aboul-Enein H Y, Thiffault C. Determination of ramipril and itsprecursors by reversed phase high performance liquidchromatography, Anal. Lett. 1991; 24: 2217–2224.
10. Motofumi I, Takeo K, Junichi G, Toshio N, Separation ofRamipril Optical Isomers by High-Performance LiquidChromatography. J. Liq. Chromatogr. 1990; 13: 991–1000.
11. Zhu. Z. and Vachareau. A. Liquid chromatography massspectrometry method for determination of ramipril and itsactive metabolite ramipril in human plasma, J. Chromatogra. B, 779(2), 2002, 297-306.