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A Facile and Isocratic RP-HPLC Method and Spectroscopy for Simultaneous estimation of Ramipril in Formulation: dissolution method

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Abstract: A precise, accurate and reproducible Reverse phase High Performance Liquid Chromatographic (RP-HPLC) method for simultaneous estimation of Ramipril on RP C-18 Column (Kromasil, 250 x 4.6 mm) using Acetonitrile: 10 µM potassium dihydrogen phosphate buffer, Methanol, ammonia (40:30:30, v/v) as mobile phase as mobile phase at a flow rate of 0.8 ml/min and the detection wavelength was 253 nm. The retention time for Ramipril was found to be 7.40 min, respectively. Amoxicillin used as internal standard. The method was also applied for the determination of Ramipril in the presence of their degradation products formed under variety of stress conditions. Proposed method was validated for precision, accuracy, linearity, robustness and ruggedness. Significant degradation was found in alkali medium. Mild degradation of drug occurred in acidic medium, higher oxidative stress and thermal conditions. The drug was found almost stable to neutral and photolytic condition.
Key words: Ramipril, HPLC, Micellar method.

Introduction

Ramipril,2-[N-[(S)-1-(ethoxycarbonyl)-3-phenylpropyl)] - L-alanyl]-(1S, 3S, 5S)-2-azabicyclo [3-3-0] octane carboxylic acid, 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 reduces the degradation of bradykinin [1]. Literature survey reveals few analytical methods for the determination of ramipril in pharmaceutical preparations and biological fluids, viz. Radioimmunoassay [2], Spectrophotometry [3], potentiometry [4, 5] GC, [6, 7] and HPLC [8-10]. Miceller liquid chromatography (MLC) is a reversed phase liquid chromatographic (RPLC) mode with mobile phases containing a surfactant (Ionic or Non ionic) above its critical concentration (CMC) [11]. In these conditions the stationary phase is modified with an approximately constant amount of surfactants monomers, and solubilizing capability of mobile phase is altered by the presence of micelles, giving rise to diverse interactions (Hydrophobic, ionic and satiric) with major implications and selectivity. Author of the article and his research team has developed a HPLC method development in different pharmaceutical dosage form [12-24]. The objective of present work to study degradation of Ramipril under different ICH recommended stress conditions, and to established a validated stability-indicating HPLC method.

Material and methods

Chromatographic condition and Reagents

Tween-20, n-butanol and water were obtained from Merck. All reagents were of HPLC grade unless otherwise specified. The HPLC system equipped with an LC-10 AT *vp* solvent-delivery system with universal loop injector (Rheodyne 7725 i) of injection capacity of 20 µL. Detector consists of photodiode array detector SPD-10 AVP UV-Visible detector. Separation was carried out on C_8 (150 x 4.6 mm i.d.) column using and mobile phase considered 15% n-butanol in 8.0 $molL^{-1}$ Tween-20 pH adjusted to 3.1 $\pm$

0.3 with o-phosphoric acid. It was pumped at flow rate of 1ml /min. the mobile phase was passed through nylon 0.45 µm membrane filters and degassed before use. Samples were injected using Rheodyne injector with 20 µL loop and detection was carried out at 240 nm. All Weighing were done on Shimadzu balance (Model AY-120). The equipment was controlled by a PC workstation. The work was carried out in an air-conditioned room maintained at temperature 25±2^{0}C. Chromatograms were recorded using CLASS-VP software.

Dissolution Apparatus

For dosage forms[25] the most frequently used apparatus are Apparatus 1 (basket) and Apparatus 2 (paddle). Basket type is used for testing of capsules and hence it was used. Agitation is also an important part of the dissolution procedure. Apparatus 1 (baskets) at 60 rpm or Apparatus 2 (paddles) at 50 or 100 rpm are used most commonly. Higher or lower rates are usually inappropriate because of the inconsistency of hydrodynamics below 40 rpm and increased turbulence above 100 rpm. Hence 75 rpm speed is selected.

Preparation of Standard Stock Solutions

Standard stock solution of Ramipril was prepared separately by dissolving 2 mg of drug in 10 mL mobile phase to get concentration of 100 µg mL^{-1}. One mL of this stock solution was further diluted to 10 mL with mobile phase to get a working standard solution having concentration 10 µg mL^{-1}.

System Suitability

The system suitability was assessed by six replicate injections of the mixture containing 10 µg mL^{-1} of the drug. The resolution, peak asymmetry, number of theoretical plates and HETP were calculated as represented in *Table 1*. The values obtained demonstrated the suitability of the system for the analysis of these drugs in combination. Mean retention time and standard deviation was found to be 1.362 ± 0.0221 for Ramipril.

Degradation studies:

Drug at a concentration of 1 mg ml^{-1} was used in all degradation studies. The pH of the potassium dihydrogen phosphate buffer was checked before and after reaction and no change was observed. Conditions employed for stability studies were as follows.

Hydrolytic studies:

For acid hydrolysis studies, 1mg ml^{-1} solution of the drug was prepared by diluting required amount of drug in 0.1N HCl and the solution was refluxed for 12h and then for 14h. Same concentration of drug was subsequently prepared in 1 N HCl and refluxed for 12h. Studies in alkali conditions were done at a drug concentration of 1mg ml^{-1} in 0.1 N NaOH and the solution was refluxed for 8h. For neutral condition 1mg ml^{-1} solution of the drug was prepared in water and refluxed initially for 12h and subsequently for 24h.

Oxidative studies:

For oxidative degradation study, initially 1mg ml^{-1} strength of drug was prepared in 3% H_2O_2.The drug was kept under the conditions of room temperature for a period 12h and then for 24h. Subsequently the drug was exposed to 10% H_2O_2 at room temperature for a period of 24h.

Thermal (dry heat) studies:

Susceptibility of the drug to dry heat was studied by exposing the solid drug to 60^0 C for 15 days in a hot air oven. Sampling was carried out every day to study its degradation behavior. For all the stability study, the formation of degradable product was confirmed by comparing the chromatogram of the degradable mixture with the blank solvent stored under normal condition and the drug solution kept under normal condition.

Preparation of samples for HPLC analyses:

For hydrolysis study during 1.0 N HCl, 0.1 N NaOH and oxidative study during 10% H_2O_2 the samples were diluted 10 times with water where as the samples were diluted 100 times with water during higher acidic, higher alkali and 30 % H_2O_2 conditions. The solid samples for neutral, thermal and photolytic degradation study were suitably diluted with water.

Separation studies on stressed samples:

In all HPLC runs, the mobile phase was filtered through 0.2µm nylon membrane under vacuum and degassed before use. The injection volume was 20µl and the mobile phase flow rate was 0.8 ml min^{-1}, the analytical wavelength selected was 253nm.HPLC studies were carried out on all reaction solution individually. Initially analyses were performed C_{18} column and mobile phase composed of acetonitrile: potassium dihydrogen phosphate buffer (pH 3 adjusted with orthophosphoric acid). As the satisfactory resolution of the drug and the degradation products was not achieved, hence to get good resolution the method was further optimized by increasing the ratio of acetonitrile and it was found good resolution in the ratio of Acetonitrile: 10 mM potassium dihydrogen phosphate buffer (60:40, v/v) as mobile phase .

Table 1. System suitability parameters for RP-HPLC method

Sr. No.	Parameters	Ramipril
1	Theoretical plates	9832
2	HETP (cm)	0.3265
3	Resolution*	2.36
4	Asymmetry factor	1.38

* With respect to previous peak

Table 2. Summary of validation parameters of proposed RP-HPLC method

Parameters	Ramipril
Linearity range (µg mL^{-1})	2-10
Correlation co-efficient	0.9994
Slope (m)	65109
Intercept (c)	121776
LOD[a] (µg mL^{-1})	0.94
LOQ[b] (µg mL^{-1})	1.73
Accuracy (% Recovery)	99.93-101.08
Precision (% RSD)[c]	
Intra day (n[d] = 3)	0.287
Inter day (n = 3)	0.851

[a]LOD = Limit of detection,[b]LOQ =Limit of quantitation,
[c]RSD = Relative standard deviation,dn = Number of determination.

Table 3 Recovery studies (n=3)

Actual concentration (µg ml^{-1})	Calculated concentration (µg ml^{-1}) ±S.D.; % COV	Recovery (%)
100	100.16 ± 0.327; 0.22	100.08
300	300.09 ± 0.198; 0.49	100.31
600	600.18 ± 0.332; 0.28	100.16

Table 4 -Result from system-suitability study

Property (n=5)	Ramipril
R_t	7.40
T_f	1.19
k'	3.98
N	7626
Rs	3.66

Rt : Retention time, T_f : Tailing factor,
k' : Capacity factor,
N : Theoretical plates number, Rs: Resolution

Table 4- stability Result of assay of tablet formulation

Experiment	Percencentage degragradation	Purity threshold
Photolytic (5-Days in Sun Light)	12.85	2.44
Thermal (6-Days in 110°C)	22.54	1.16
Alkali Deg (0.1M NaOH 1ml)	31.85	1.98
Acid Deg (0.1-N HCl 1ml)	48.99	3.27
3% H_2O_2	60.38	4.82

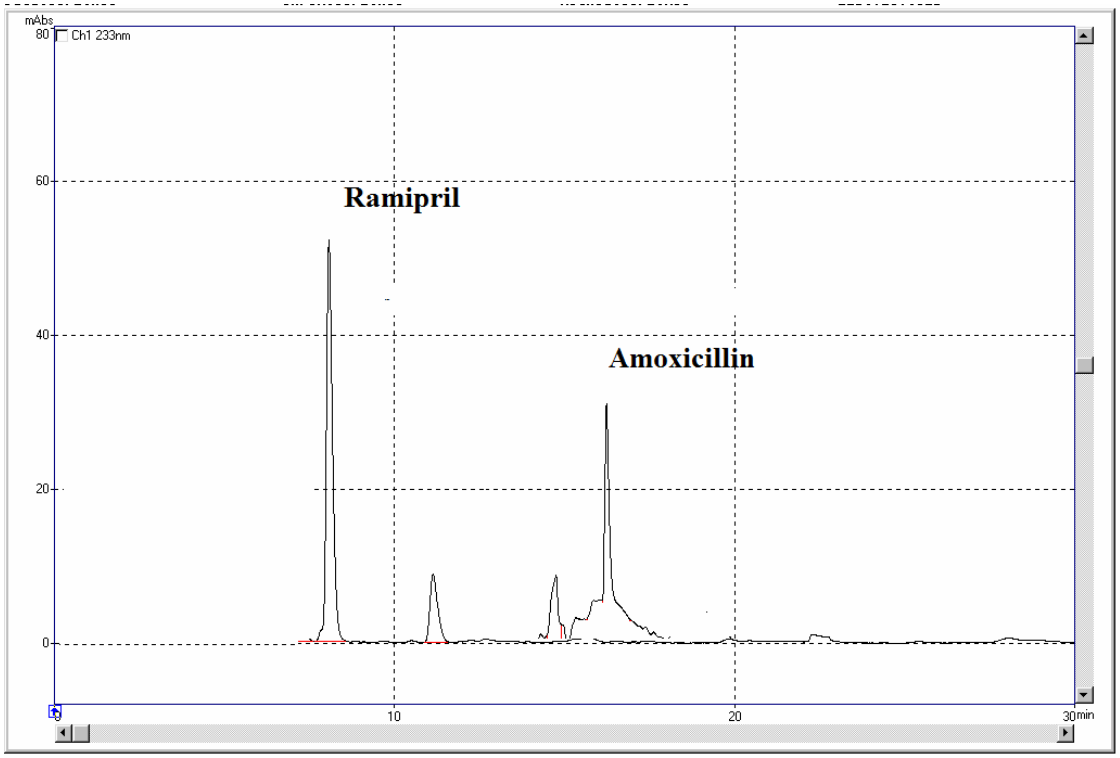

Fig. Chromatogram of Ramipril with standard Amoxicillin

Result and Discussion

A stock solution of the drug (1mg ml^{-1}) was prepared in water. From this stock solution five concentrations of the drug were prepared within the concentration range of 10-100 µg ml^{-1}. The solutions were injected in triplicate into the HPLC column, keeping all the conditions constant. Precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analyses of three different concentration of (100,300,600 µg ml^{-1}) drug in hexaplicate on the same day. Intermediate precision of the method was checked by repeating the studies on same day at an interval of one hour (intraday precision) for three hours and on three different days (interday precision).Accuracy of the method was tested by fortifying a mixture of decomposed reaction solutions with three concentration of the drug and determining the percentage of recovery of added drug. The specificity of the method towards the drug was established through study of resolution factor of the drug peak from the nearest resolving peak. Whereas selectivity was established through determination of purity for each degradation product peak using PDA detector. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50 %, 100 % and 150 %. The percentages of recoveries were calculated, results of which are represented in Table 3. LOD and LOQ were calculated as 3.3 σ /S and 10 σ /S respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. In the robustness study, the influence of small, deliberate variations of the analytical parameters on retention time of the drugs was examined. The following three factors were selected for change: flow rate of the mobile phase (0.8 ± 0.02 mL min^{-1}), a wavelength at which the drugs were recorded (253 nm) and mobile phase percentage with respect to acetonitrile (± 2 %). One factor at the time was changed to estimate the effect. The solutions containing 10 µg mL^{-1} of Ramipril were applied onto the column. It was observed that the drug gets slowly degraded in strongly acidic conditions over a period of time. On reflux in 0.1 N HCl (12h) and further for 14h there is no degradation. The degradation of the drug resulted in the rise of one extra peak at 2.661 in 1.0 N HCl (12h). This indicates that the drug is hydrolysed under acid conditions, to a chromatographic compound. In alkali, the drug was found to decompose almost 60-70% and then 70-80% after refluxing for 2h and then continuing 3h in 0.1 N NaOH respectively. As shown in chromatogram, degradation of the drug resulted in the rise of one extra peak at 2.532 minute results of the stress studies indicated the specificity of the method that has been developed. Ramipril was degraded only in 3% H_2O_2 and in temperature stress

conditions whereas Ramipril was degraded in all conditions. The degraded products appeared at retention time (R_{Ts}) 3.08 in 0.1 M HCl, 4.36 in 0.1 M NaOH, 3.87 in 3% H_2O_2 in temperature degradation studies. To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate, mobile phase ratio and column temperature on the retention time and tailing factor were studied. The method was found to be unaffected by small changes like ± 0.1 change in pH, ± 0.1 change in flow rate and ± 1 change in mobile phase.

Conclusion

The proposed method is simple, sensitive and reproducible and hence can be used in routine for simultaneous determination of Ramipril in bulk as well as in pharmaceutical preparations. Statistical analysis of the results has been carried out revealing high accuracy and good precision.

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