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Research Article

SIMULTANEOUS ESTIMATION OF RAMIPRIL AND AMLODIPINE IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT

A simple, specific, accurate and precise reverse phase high pressure liquid chromatographic method has been developed for the simultaneous determination of Ramipril and Amlodipine besylate from combined dosage form by reverse phase C18 column (BDS, C18, 5µ, 250mm x 4.6mm). The sample was analysed using (1.36gm of potassium dihydrogen ortho phosphate and 1ml of triethy amine in 1000ml of water) Buffer:Acetonitrile in the ratio of 45:55(pH adjusted to 6.5 with Orthrophosphric acid) as a mobile phase at a flow rate of 1ml/min and detection at 230nm. The retention time for Ramipril and Amlodipine besylate was found to be 2.6 and 4.3 min respectively, and recoveries from combined dosage form were between 98 and 102%. The method can be used for estimation of combination of these drugs in combined dosage form.

Keywords: Ramipril, Amlodipine besylate, RP-HPLC.

INTRODUCTION

Ramipril is a 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 reduce 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, 9]

Amlodipine besylate is a 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine, is a benzene sulfonate(besylate) salt of amlodipine, which is a dihydropyridine calcium channel blocker. Various analytical methods have been reported for the assay of Amlodipine besylate[10] in pure form as well as in pharmaceutical formulations.They include high performance liquid chromatography[11-16], reversed phase high performance liquid chromatography[17-20], high performance thin layer chromatography[21-24], gas chromatography[25],liquid chromatography with tandem mass spectrometry[26] and derivative spectroscopy[27,28.] No RP-HPLC study on Ramipril and Amlodipine in combined dosage form in pharmaceutical preparations has been found to in recent literature survey.

MATERIAL AND METHODS

Instrumentation

A High Performance Liquid Chromatography system, with LC solutions data handling system (WATERS ALLIANCE 2695 Separation module) with an auto sampler was used for the analysis. The data was recorded using WATERS EMPOWER software. The purity determination performed on a stainless steel column 250mm long, 4.6mm internal diameter filled with Octadecyl silane chemically bonded to porous silica particles of 5µm diameter (BDS C18, 5µ, 250mm x 4.6mm).

Reagent and chemicals

Pure samples of Ramipril and Amlodipine besylate were obtained from Medopharm and Global pharma health care Pvt.Ltd., respectively for the estimation of Ramipril and Amlodipine besylate in commercial formulations. HPLC grade Orthrophosphric acid, Acetonitrile and Triethyl amine were procured from Qualigens fine chemicals. High pure water prepared by using Millipore Milli Q pluspurification system.

Experimental Conditions

The HPLC system was operated isocratically at flow rate of 1ml/min at 25°C ± 0.5° C for 15 min. The mobile phase found to be most suitable for analysis was Acetonitrile:Potassium dihydrogen ortho phosphate buffer 55:45% v/v, 0.5% triethylamine, pH adjusted to 6.5 with O-phosphoric acid, detection was carried out at 230nm.

Preparation of phosphate buffer

1.36 grams of KH2PO4 was weighed into a 1000ml beaker, dissolved and 1ml of triethyl amine into it and it is diluted to1000ml with HPLC grade water. Adjusted the pH to 6.5 with Orthophosporic acid.

Preparation of mobile phase

Mix a mixture of above buffer 450 ml (45%) and 550 ml of Acetonitrile HPLC grade (55%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Preparation of Standard solution

Standard stock solution of 1000µg/ml of each Ramipril and Amlodipine besylate were prepared by dissolving 10 mg of each drug in mobile phase. Sub stock solution was prepared from stock solution by diluting each standard stock solution (1ml) up to 10ml to get 100µg/ml of each drug. The normal concentrations in range 20-100µg/ml were prepared for calibration. All solutions were stored at room temperature. Each standard solution (20 µl) was injected into the column after filtration using 0.2 micron membrane filter.

Sample preparation

Twenty tablets were weighed and crushed to fine powder. Powder equivalent to 5 mg of Ramipril and 5 mg of Amlodipine besylate was accurately weighed and dissolved in mobile phase, sonicated for 10 min and filtered through Whatman filter paper no. 42, finally different concentrations of tablet sample were prepared by serial dilution technique.

Procedure

Inject 20 mL of the standard, sample into the chromatographic system and measure the areas for the Ramipril and Amlodipine besylate.

RESULTS AND DISCUSSION

The purpose of the present study was to develop a rapid and sensitive RP-HPLC method for the simultaneous estimation of Ramipril and Amlodipine besylate in combined dosage form using BDS C18 analytical column with UV detection

Validation

The described method has been validated for the simultaneous estimation of Ramipril and Amlodipine besylate using following parameters.

Accuracy

Accuracy of the method was demonstrated at three different concentration levels (80,100,120%) by spiking a known quantity of standard drugs into a analyzed sample in triplicate. The results of accuracy (Table3) revealed that the method was more accurate.

Precision

For the precision of the method, three replicate were injected into the system on two different non consecutive days, in each case %RSD was calculated. Results of precision are given in Table 1, which indicated that the method is precise.

Linearity

Linearity was demonstrated by analysing six different concentrations of active compound. Peak areas were recorded for all the peaks and calibration plot was constructed by plotting peak area vs. concentrations of Ramipril and Amlodipine besylate which were found to be linear in the range of 20µg/ml-100 µg/ml. Coefficient of correlation of Ramipril and Amlodipine besylate were determined and mentioned in Table no.1

Limit of detection (LOD) and limit of quantization (LOQ)

The limit of detection and limit of quantification for Ramipril and Amlodipine besylate were calculated from the linearity data using relative standard deviation of the response and slope of the calibration curve. The limit of detection of a compound is defined as the lowest concentration of analyte that can be detected. LOD value of Ramipril and Amlodipine besylate was found to be 1.61 and 0.95 µg/ml, respectively. The limit of quantification is the lowest concentration of a compound that can be quantified with acceptable precision and accuracy. LOQ value of Ramipril and Amlodipine besylate was found to be 4.89 and 2.88 µg/ml, respectively.

Specificity

No interference of peaks were found in the chromatogram indicating that excipients used in the tablet formulation did not interfere with the estimation of the drugs by the proposed method for the simultaneous determination of Ramipril and Amlodipine besylate in the combined dosage form, hence the method is specific.

Robustness

In order to demonstrate the robustness of the method, system suitability parameters were verified by making deliberate changes in the chromatographic conditions, viz. change in flow rate by ±0.05 mL min-1, change in pH of the buffer by ±0.1 unit and change in the ratio of mobile phase (±2% absolute). The method was demonstrated to be robust over an acceptable working range of its HPLC operational parameters. To ascertain the system suitability for the proposed method a number of statistical values such as theoretical plates, HETP, resolution have been calculated with the observed readings and the results are tabulated in Table 1. The HPLC method developed in the present study has been used to quantify Ramipril and Amlodipine besylate in the combined dosage form (Fig.1). The average drug content of Ramipril and Amlodipine besylate was found to be 4.96 and 4.99 mg of the labeled amount and mentioned in Table 3.

Table 1: Validation and system suitability parameters

Parameters	Ramipril	Amlodipine besylate
Linearity range (µg/ml)	20 – 100 µg/ml	20 – 100 µg/ml
Correlation coefficient	0.9997	0.9998
Retention time (min)	2.6	4.3
Resolution	6.27	
Tailing factor	1.24	1.94
Limit of detection (µg/ml)	1.61µg/ml	0.95µg/ml
Limit of quantification(µg/ml)	4.89µg/ml	2.88µg/ml
Precision (RSD %)	0.13 %	0.21 %

Table 2: Mean (±S.D.) amount of Ramipril and Amlodipine besylate in tablet dosage forms by proposed HPLC method

S. No	Drug Name	Label claim	Amount found	% Purity
1	. Ramipril	5mg	4.96	99.20
2	Amlodipine besylate	5mg	4.99	99.80

Table 3: Recovery study of Ramipril and Amlodipine besylate using the proposed HPLC method

S. No	Spiked level	% Recovery of Ramipril	% Recovery of Amlodipine besylate	% RSD of Ramipril	% RSD of Amlodipine besylate
1	80%	99.13	101.08	0.03	0.17
2	100%	100.43	99.28	0.23	0.05
3	120%	100.02	100.37	0.53	0.28

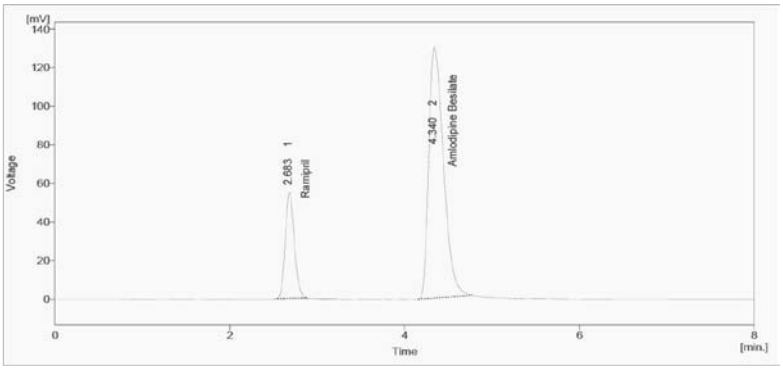

Fig. 1: Chromatogram of Ramipril and Amlodipine besylate

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 and Amlodipine besylate in bulk and pharmaceutical dosage form.

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