

Isocratic Reverse Phase High Performance Liquid Chromatographic Estimation of Ramipril and Amlodipine in Pharmaceutical Dosage Form

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ABSTRACT

A new simple, rapid, specific, accurate, precise and novel reverse phase High Performance Liquid Chromatography (RP-HPLC) method has been developed for the simultaneous estimation of Ramipril and amlodipine besylate in the combined pharmaceutical dosage form. The chromatographic separation for ramipril and amlodipine besylate were achieved with mobile phase containing mixed phosphate buffer (0.02 M potassium di-hydrogen orthophosphate and 0.002 M di-potassium hydrogen phosphate anhydrous, pH 6.8) and acetonitrile (60:40 % v/v), phenomenex C18 column (150 × 4.6mm i.d, particle size of 5 μ) at 5^o C and UV detection at 237 nm. The compounds were eluted in the isocratic mode at a flow rate of 1 ml min⁻¹. The retention times of Ramipril 2.13 ± 0.09 min and amlodipine besylate at 5.18 ± 0.12 min. The above method was validated in terms of linearity, accuracy, precision, LOD, LOQ etc. in accordance with ICH guidelines.

Keyword: Ramipril, Amlodipine Besylate, RP-HPLC, Validation.

INTRODUCTION

Ramipril, chemically (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 [1]. It acts on the renin-angiotensin aldosterone system by inhibiting the conversion of the inactive angiotensin I to the highly potent vasoconstrictor, angiotensin II, and also reduces the degradation of bradykinin. Amlodipine besylate (AMLO), chemically,

(2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1, 4-dihydro- 6-methyl-3, 5-pyridinedicarboxylic acid 3-ethyl, 5-methyl ester) [2] (Fig.2). It is an anti-hypertensive and an antianginal agent in the form of the besylate salt, Amlodipine besylate. It is not official in any Pharmacopoeia.

Literature survey reveals that there are several analytical methods for the estimation of amlodipine besylate and ramipril individually or in combination with other drugs [3-10]. Although the combination use of amlodipine besylate and ramipril is continuously increasing, there is no simple and economical RP-HPLC method for the determination of these drugs in the combined pharmaceutical dosage form. The purpose of present study is to investigate a simple, precise, accurate and economic RP-HPLC method in simultaneous determination of amlodipine besylate and ramipril in the combined pharmaceutical dosage form.

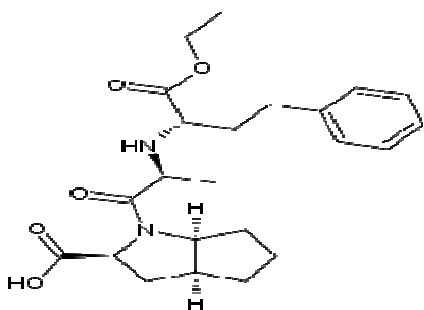


Fig. 1. Structure of Ramipril

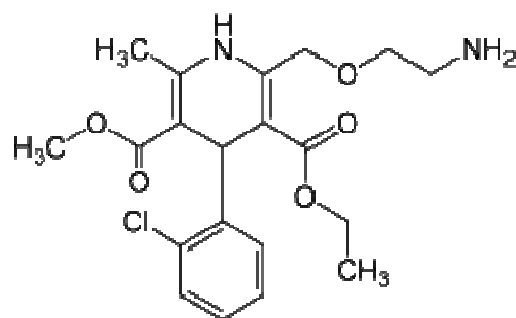


Fig. 2. Structure of Amlodipine

MATERIALS AND METHODS

Ramipril and Amlodipine besylate gift samples were obtained from Taj Pharmaceuticals Ltd. India. Acetonitrile (HPLC grade) and dipotassium hydrogen phosphate (AR grade) were purchased from Merck Ltd, India. Water for HPLC was obtained from Qualigen fine chemicals, Mumbai, India. Analytical reagent potassium dihydrogen orthophosphate (AR grade) was obtained from Rankem Pvt. Ltd, Mumbai. Chromatographic separation was performed on Waters® HPLC system equipped with Waters 2489 UV/Visible detector and Empower software. Phenomenex C18 Column (150 × 4.6 mm i.d, particle size of 5 μ) and constant flow pump and Auto injector with 20 μ L loop were used. The composition of the mobile phase was in the ratio of mixed phosphate buffer (0.02 M potassium di-hydrogen orthophosphate and 0.002 M di-potassium hydrogen phosphate anhydrous, pH 6.8) and acetonitrile (60:40 % v/v) and was delivered at a flow rate of 1.0 ml min⁻¹. The mobile

phase was filtered through a 0.45 μ membrane filter and sonicated for 15 min. Analysis was performed at 5^o C temperature.

Preparation of standard solutions

5 mg of Ramipril and 5 mg of amlodipine besylate were accurately weighed and dissolved in 50 ml of mobile phase to obtain standard stock solution of 50 μ g mL⁻¹ and 100 μ g mL⁻¹ respectively. Further working standard solutions of Ramipril and amlodipine besylate, 1.25-7.5 μ g mL⁻¹ and 2.5-15 μ g mL⁻¹ respectively, were prepared by suitable dilution of the stock solution with mobile phase. A typical chromatogram obtained from the analysis of drugs using the developed method was shown in Fig. 3.

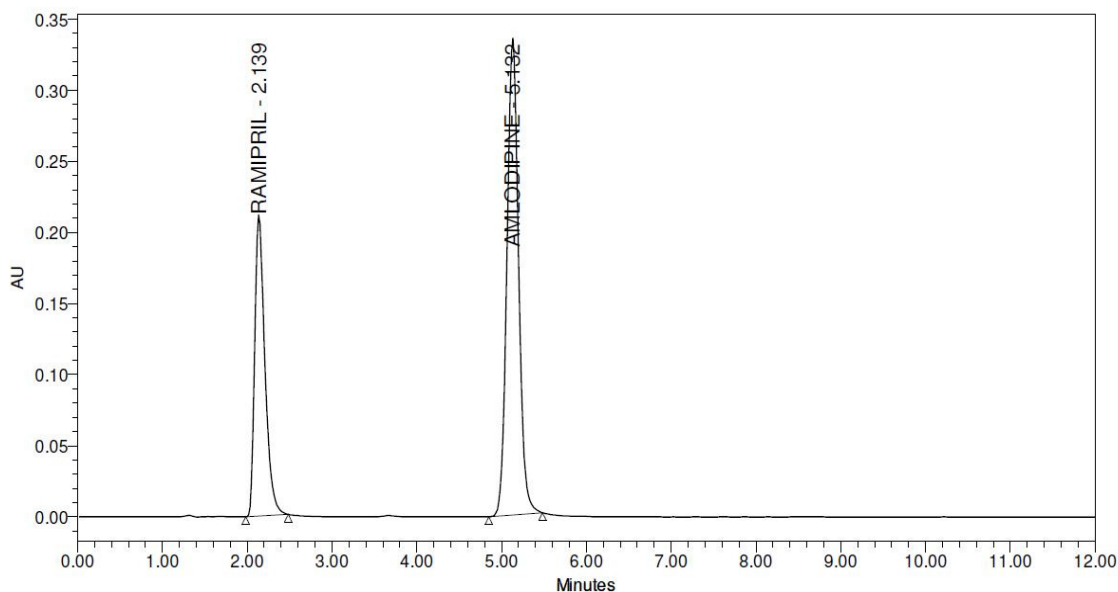


Fig.3: A typical chromatogram of standard Ramipril (2.5 μ g ml⁻¹) and amlodipine besylate (5 μ g ml⁻¹) measured at 237 nm

Preparation of capsules for assay

For analysis of commercial formulations, 20 capsules were weighed and average weight was calculated. Weight equivalent to 2.5 mg and 5 mg of Ramipril and amlodipine besylate was taken and transferred into 100 ml volumetric flask and dissolved with mobile phase, filtered through a whatman filter paper. This solution was further diluted stepwise with mobile phase to get the concentration within the linearity range.

RESULTS AND DISCUSSION

Phenomenex c18 (150 × 4.6 mm i.d, particle size of 5 μ), column maintained at ambient temperature 5^o C was used for the separation and the method was validated for the estimation of Ramipril and amlodipine besylate in capsules. The composition, pH and the flow rate of the mobile phase were optimized. A mobile phase consisting of mixture of 0.02 M potassium dihydrogen phosphate and 0.002 M dipotassium hydrogen phosphate (pH 6.8): acetonitrile (60:40 % v/v) set at a flow rate of 1 ml min⁻¹ was selected for use of further studies after several preliminary investigatory chromatographic runs. Under the described experimental conditions, all peaks were well defined and free from tailing. The effects of small deliberate changes in the mobile phase composition, and flow rate were evaluated as a part of testing for method robustness.

Method validation:

The proposed method was validated as per International Conference on Harmonization (ICH) guidelines.

Linearity and Range

Linearity was established by least squares linear regression analysis of the calibration curve. The calibration curves were linear over the concentration range of 1.5-7.5 μ g mL⁻¹ for Ramipril, 2.5-15 μ g mL⁻¹ for amlodipine besylate. Peak areas were plotted versus respective concentrations and linear regression analysis was performed on the resultant curves. Correlation coefficient values were found to be 0.999 and 0.999 for Ramipril and Amlodipine besylate respectively, (Fig. 4 and Fig. 5). The results are given in Table 1.

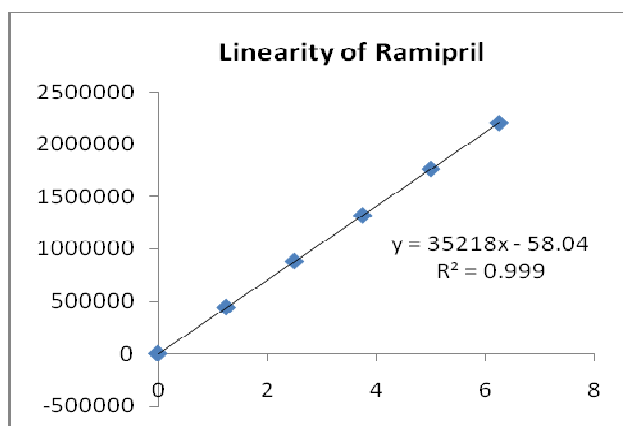


Fig. 4: Calibration curve of Ramipril

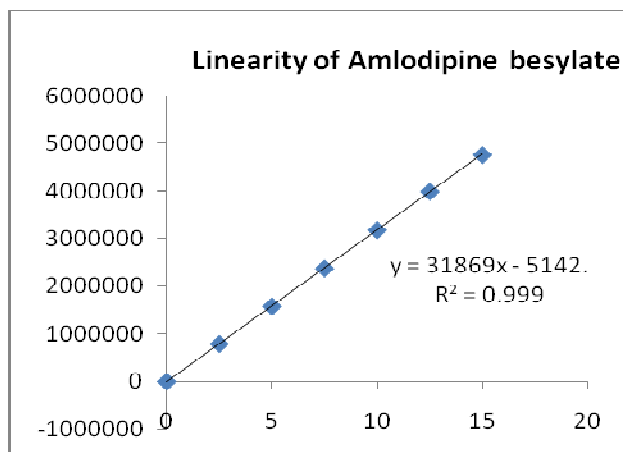


Fig. 5: Calibration curve of Amlodipine besylate

Precision

Intra-day precision was investigated by injecting six replicate samples of each of the samples on the same day. The % RSD obtained for ramipril and amlodipine besylate were found to be 0.32 and 0.31 respectively. Inter-day precision was assessed by injecting the same two samples over six consecutive days. The % RSD obtained for ramipril and amlodipine besylate were found to be 0.28 and 0.32 respectively. The results are given in Table 1.

Accuracy

Recovery studies were carried out by applying the method to drug sample to which known amount of standard corresponding ramipril and amlodipine besylate to 50, 100 and 150% of label claim had been added. At each level of the amount six determinations were performed. The mean recoveries obtained for ramipril and amlodipine besylate were 100.01 and 100.5%, respectively. The results are given in Table 1.

Specificity

The method specificity was assessed by comparing the chromatograms obtained from the drug and the most commonly used excipients mixture with those obtained from blank

(excipients solution in water without drug). The method was specific as none of the excipients interfered with the analytes of interest.

LOD and LOQ

LOD and LOQ of ramipril and amlodipine besylate were determined by calibration curve method. LOD and LOQ for ramipril were 0.46 and 1.42 $\mu\text{g mL}^{-1}$, for amlodipine besylate were 1.05 and 3.18 $\mu\text{g mL}^{-1}$. The results are given in Table 1.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the method was investigated under a variety of conditions including changes of composition of buffer in the mobile phase and flow rate. % RSD of assay was calculated for each condition. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust.

Ruggedness

The ruggedness of the method was assessed by comparison of the intra-day and inter-day assay results for ramipril and amlodipine besylate that has been performed by two analysts. The % RSD values for assays performed in the same laboratory by two analysts did not exceed 2, indicating the ruggedness of the method

Analysis of marketed formulation

The proposed RP-HPLC method was applied to the simultaneous estimation of ramipril and amlodipine besylate in Stamace plus capsules. Satisfactory results were obtained which is given in Table 2, for compound in good agreement with label claims. No interference due to excipients was detected in the chromatograms produced (Fig.6).

Table 1: Summary of validation parameters

Parameters (Units)	Ramipril	Amlodipine besylate
Linearity range ($\mu\text{g/ml}$)	12.5-7.5	2.5-15
Correlation coefficient	0.999	0.999
Slope	35219	31869
Intercept	58.04	5142
LOD ($\mu\text{g/ml}$)	0.46	1.05
LOQ ($\mu\text{g/ml}$)	1.42	3.18
Recovery (%)		
50	100.06	100.43
100	100.55	100.95
150	99.37	100.56
Precision (% RSD)		
Intraday (n=3)	0.32	0.28
Inter day(n=3)	0.31	0.32

Table 2: Analysis of marketed formulation

Drugs	Labelled amount, mg capsule ⁻¹	Amount found, mg capsule ⁻¹	% *RSD
Ramipril	2.5	2.49	0.521
Amlodipine besylate	5	4.975	0.362

* Mean of six observations

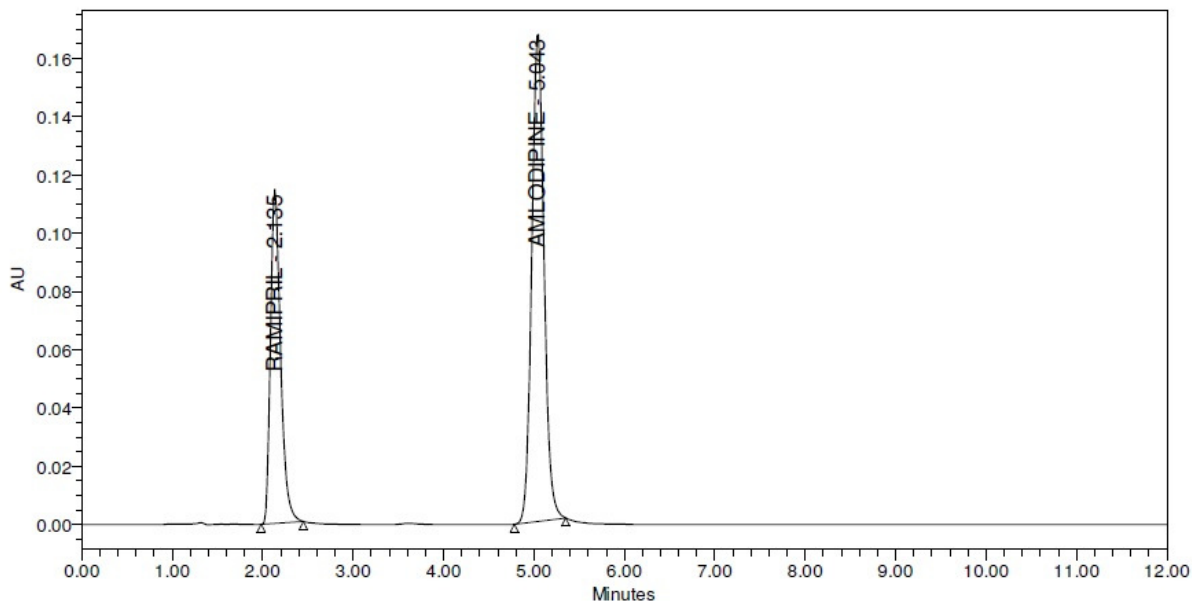


Fig. 6: A typical chromatogram of formulation Ramipril ($2.5 \mu\text{g ml}^{-1}$) and amlodipine besylate ($5 \mu\text{g ml}^{-1}$) measured at 237 nm

CONCLUSION

The proposed RP-HPLC method is simple, reliable and selective providing satisfactory accuracy and precision with lower limits of detection and quantification. Moreover the shorter duration of analysis for ramipril and amlodipine besylate make the reported method suitable for routine quantitative analysis in pharmaceutical dosage forms.

ACKNOWLEDGEMENT

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