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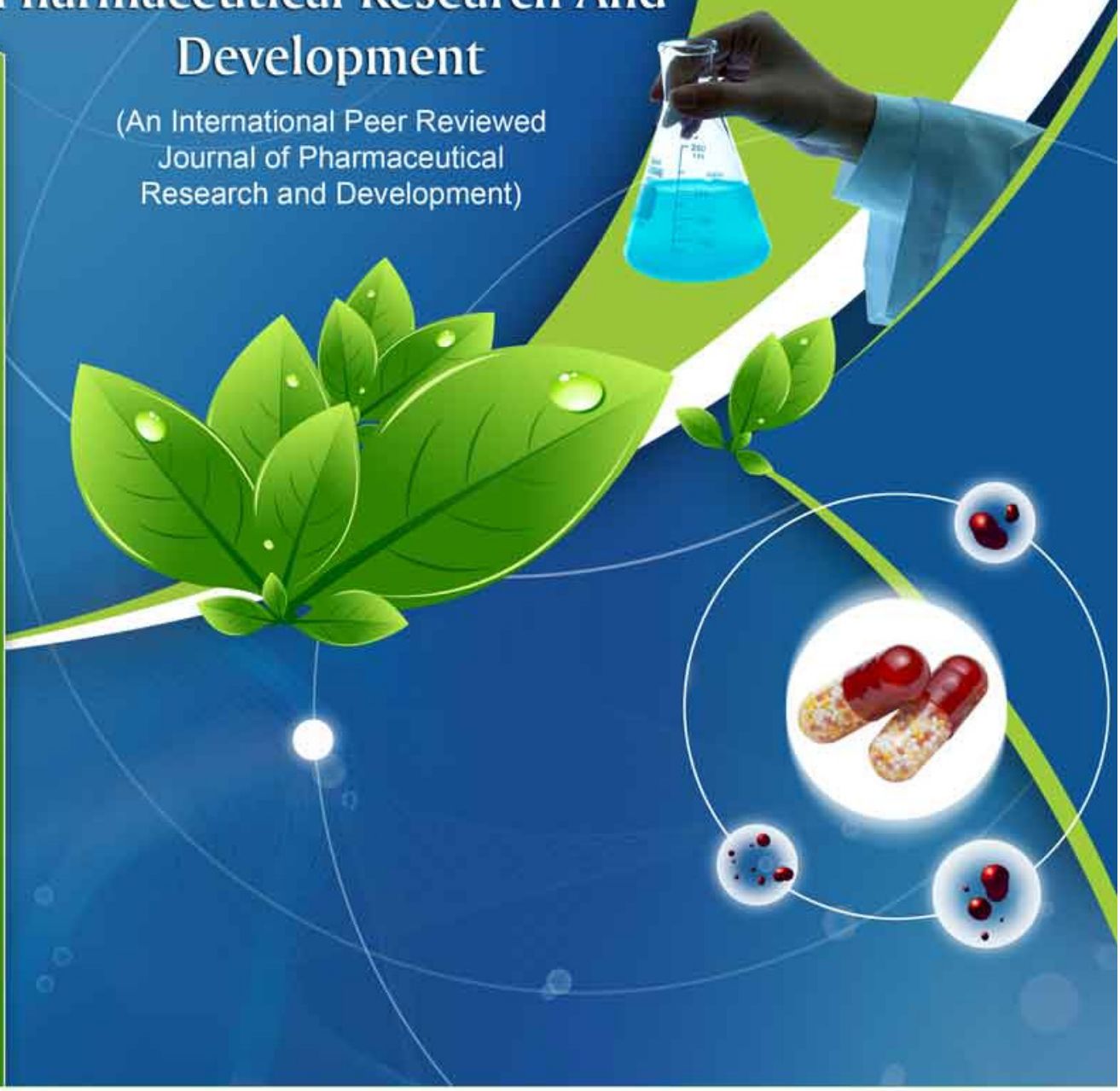

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Research Article

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF RAMIPRIL AND S (-) AMLODIPINE IN TABLET DOSAGE FORM

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ABSTRACT

*The main objective of this study is to develop and validate a simple and rapid isocratic reversed-phase high-performance liquid chromatographic method (RP-HPLC) for the simultaneous estimation of S (-) Amlodipine besylate and Ramipril in combined dosage form. The HPLC system was operated isocratically at flow rate of 1ml/min at 40°C ± 0.5° C for 15 min. The mobile phase found to be most suitable for analysis was Acetonitrile: 0.02M Potassium dihydrogen ortho phosphate buffer (0.1 % of triethylamine, 0.1 % of 6-heptane sulphonic acid salt): 35:65% v/v, pH adjusted to 2.5 with O-phosphoric acid, detection was carried out at 210nm using Hypersil BDS C-18 (150*4.6mm) 5µ column with injection volume 20µl. The retention time of Ramipril and S (-) Amlodipine Besylate were 5.61±0.3and 7.41±0.3 respectively. The proposed method was validated according to International Conference on Harmonization [ICH Q_2 (R_1)] and was found to be precise, accurate, selective and rapid for the simultaneous determination of Ramipril and S (-) Amlodipine Besylate in bulk and tablet dosage forms. The linearity for Ramipril (r^2=0.9914) and S (-) Amlodipine besylate (r^2=0.9930) was established in the range of 25.14-75.41 and 25.46-76.38µg/mL respectively. This new developed method was found to be precise with satisfactory %RSD values for inter and intraday precision.*

KEYWORDS: *S (-) Amlodipine besylate, Antihypertensive, High Performance Liquid Chromatography, Ramipril, Validation*

INTRODUCTION

S (-) Amlodipine besylate (Figure1a) belongs to the dihydropyridine group of calcium channel blocker used as an antihypertensive and antianginal agent [1]. S (-) Amlodipine besylate avoids the adverse effect of Amlodipine in racemic mixtures. S (-) Amlodipine besylate is an active and potent enantiomers of Amlodipine having IUPAC name 3-ethyl 5-methyl2-[(2-aminoethoxymethyl]-4-(2-chlorophenyl)-l, 4-dihydro6-methy1-3, 5-pyridinedicarboxylate [2, 3]. Ramipril (Figure 1b) is an angiotensin-converting enzyme (ACE) inhibitor, used in treatment of high blood pressure and congestive heart failure. 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 [4]. Ramipril having IUPAC name 2-[N-[(S)-1-(ethoxycarbonyl)-3-phenylpropyl)]-L-alanyl]-(1S,3S,5S)-2-azabicyclo [3-3-0] octane carboxylic acid [5].

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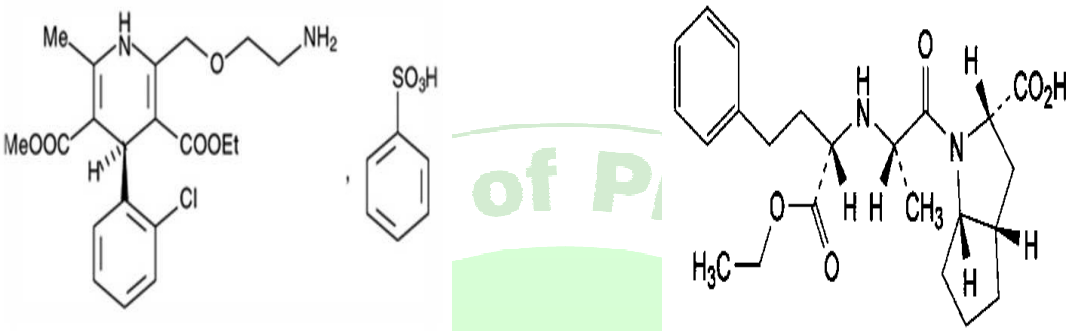
Various analytical methods were found for the determination of Amlodipine and Ramipril as single or in combination with other drugs [6-13]. Very few methods have been reported for quantitative estimation of Ramipril and Amlodipine besylate in combination [14-16]. Hence, the aim of the present work is to develop a simple and rapid method for simultaneous estimation of S (-) Amlodipine besylate and Ramipril in bulk and tablet dosage form.



(a) (b)

Figure 1; Structure of S (-) Amlodipine besylate (a) and Ramipril (b)

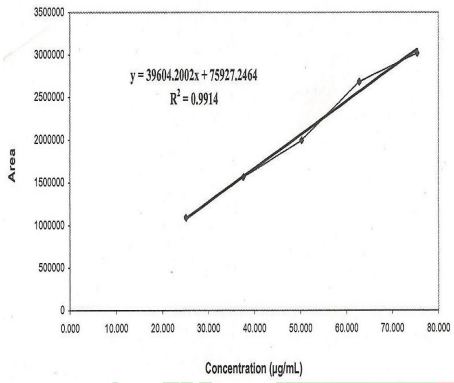


Figure 2 Calibration curve of Ramipril

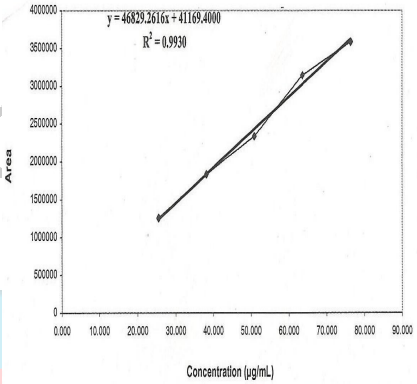


Figure 3 Calibration curve of S(-) Amlodipine Besylate

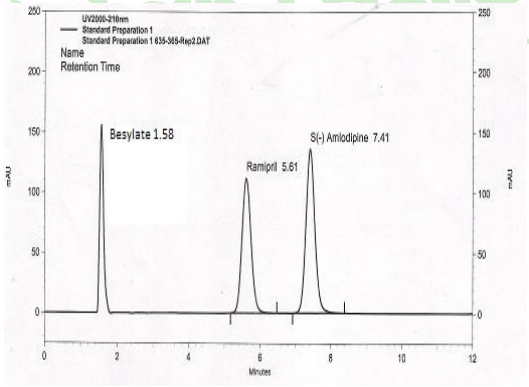


Figure 4 Chromatogram of API

MATERIAL AND METHODS

Chemicals and reagents:

Working standard of S (-) Amlodipine besylate and Ramipril were obtained from Emcure House, Bhosari, Pune. Acetonitrile and Methanol were obtained from RFCL Limited, Mumbai, India. Potassium dihydrogen orthophosphate, Tri-ethylamine and 6-heptane sulphonic acid salt were procured from Merck Chemicals, Mumbai, India. Purified water used was obtained from Milli Q.

Preparation of Phosphate buffer:

2.72 gram of Potassium dihydrogen orthophosphate was accurately weighed and transferred into a 1000ml beaker, and dissolved with water. 1ml of triethylamine and 1gm of 6-heptane sulphonic acid salt were added and the contents were diluted up to 1000ml with purified water.

Preparation of mobile phase:

The mobile phase was prepared by addition of Phosphate buffer 650 ml (65%) and 350 ml of Acetonitrile (35%). The pH of the solution was adjusted to 2.5 with Orthophosporic acid, dihydrogen ortho phosphate buffer (0.1 % triethylamine, 0.1 % 6-heptane sulphonic acid salt): 35:65% v/v, pH adjusted to 2.5 with O-phosphoric acid, detection was carried out at 210nm using Hypersil BDS C-18 (150*4.6mm) 5µ column with injection volume 20µl.

Validation procedure:

Accuracy (% recovery):

Linearity:

Stock solution was prepared by dissolving 47.5mg S (-) Amlodipine besylate and 31.5mg Ramipril in 100ml diluent in volumetric flask. Aliquots were prepared in the range of 50-150 % by taking 2, 3, 4, 5, 6ml from standard stock solution to 5 different 25ml volumetric flasks, diluted to 25ml with diluent. Linearity was established by calibration curve (Fig-2, 3) and the date for regration analysis of the calibration was shown in Table II.

degassed in ultrasonic water bath for 5 minutes. The solution was further filtered using 0.45 µ nylon filter.

Diluent Preparation:

Diluent was prepared by addition of 0.02M Potassium dihydrogen orthophosphate buffer 50% and Acetonitrile 50% then finally the resultant mixture was filtered using 0.45 µ nylon filter.

Standard preparation:

38mg S(-) Amlodipine besylate and 25mg Ramipril were accurately weighed and dissolved with 70 ml diluent into a 100ml volumetric flask. The contents were sonicated for about 10 minutes and then 5ml of above solution was transferred to 25ml volumetric flask, diluted with diluent to give a solution of 50µg/ml of both the drugs [S(-) Amlodipine and Ramipril].

Experimental Conditions:

The HPLC system was operated isocratically at the flow rate of 1ml/min at 40°C ± 0.5° C for 15 minutes. The mobile phase selected for analysis was Acetonitrile: 0.02M Potassium The accuracy of the method was determined by calculating the recovery of S (-) Amlodipine besylate and Ramipril by the standard addition method. Known amounts of standard solution of S (-) Amlodipine besylate and Ramipril (25, 50, 75 µg/ml) were added to prequantified sample solutions of tablet dosage form. The amount of S (-) Amlodipine besylate and Ramipril was estimated by applying values of peak area to the regression equations of the calibration curve (Table-I).

Precision:

The method precision studies (Intraday and Interday) were carried out by estimating the response from six sets of S(-) Amlodipine besylate and Ramipril of concentration 50 µg/ml, two times each. The results are reported in terms of relative standard deviation and were found to be satisfactory (Table-III).

Robustness:

Robustness of the method was studied by changing the flow rate (±0.1ml/min), column temperature (±5^{0}C), mobile phase composition (±5), wavelength (±2nm), and pH (±0.2) (Table-IV).

Solution stability:

Stability of sample solutions (50µg/ml) was studied at ambient temperature for 24 h. Both the drugs solutions were found stable up to 20 hours (Table-V).

System suitability test:

The system suitability test was carried out to evaluate the resolution and reproducibility of the system for the analysis to be performed by using five replicate injections of a reference solution containing S (-) Amlodipine besylate and Ramipril. Peak area, retention time, theoretical plates, and tailing factor, were measured (Table-VI)

Specificity:

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities and degradation products. The pharmaceutical formulation was subjected to forced degradation studies and no interferences of placebo as well as degradant was observed. Hence, the method was found to be specific.

Assay of Tablet formulation:

20 tablets were accurately weighed and crushed into a fine powder. Powder equivalent to 38 mg of S (-) Amlodipine besylate (or 25 mg of Ramipril) was weighed and transferred into 100ml volumetric flask and dissolved with 70ml diluent. The contents were sonicated for 10 minutes and volume was made up to 100ml with diluent. The solution was filtered through Whatman filter paper No-41. The final dilution was made by transferring 5ml of filtered solution to 25ml volumetric flask and volume was made up to 25ml with diluent, to give concentration 50µg/ml. Assay was performed by injecting 20µl of sample solution twice and the area were compared with that of mean area of five injections (20µl) of standard solution and % area was calculated (Table-VII).

Table I Accuracy (n=3) S (-) Amlodipine besylate

Level	*Mean % Recovery*	*% RSD*
50	99.66	0.39
100	101.06	0.28
150	101.64	0.33

Ramipril

Level	*Mean % Recovery*	*% RSD*
50	101.33	0.17
100	99.94	0.22
150	100.54	0.45

Table II Linearity (n=3)

Parameters	*S(-)Amlodipine Besylate*	*Ramipril*
Linearity range (µg/ml)	(25.46-76.38)	(25.14-75.41)
r^2	0.9930	0.9914
Slope	46829.2616	39604.2002
Intercept	41169.400	75927.246
Y= mx + c	46829.2616x +41169.400	39604.2002x +75927.246

Table III Precision (n=2)

Parameter	S(-) Amlodipine besylate	Ramipril
Interday(%RSD)	0.34	0.38
Intraday(%RSD)	1.92	0.68

Table IV Robustness

Parameter	Change	Area	
		%RSD of S(-) Amlodipine besylate	%RSD of Ramipril
Wavelength(±2)	208nm	0.27	0.84
	212nm	0.56	0.45
Temperature(±5℃)	35˚C	0.16	0.24
	45˚C	0.71	0.60
Flow rate(±0.1)	0.9ml/min	0.20	0.34
	1.1ml/min	0.50	0.49
pH(±0.2)	2.3	0.47	0.40
	2.7	1.32	1.02
Mobile phase composition (±5%)	ACN:Buffer(30:70)	0.61	1.17
	ACN:Buffer(40:60)	0.85	0.33

Table V Solution stability (at 50µg/ml)

Parameter	Mean area		SD		%RSD	
	S(-)Amlo-Dipine besylate	Ramipril	S(-) Amlo-Dipine besylate	Ramipril	S(-) Amlo-Dipine besylate	Ramipril
Initial	2349521	2006433	7212.82	8621.7	0.31	0.36
4^{th} hr	2348675	2005325	23581.557	7292.478	1.01	0.46
8^{th} hr	2345424	2002465	21089.535	9145.09	0.9	0.42
12^{th} hr	2347685	2002818	27908.299	8486.975	1.19	0.4
16^{th} hr	2349693	2003218	33909.889	7963.817	1.44	0.52
20^{th} hr	2351751	2005391	40456.139	10477.705	1.71	0.79
24^{th} hr	2385014	2019702	49788.224	48696.054	2.09	2.41

Table VI System Suitability

Parameter	Ramipril	S(-)Amlodipine besylate
Area	1974987	2397606
SD	6422.977	20433.552
%RSD	0.33	0.85
RT(min)	5.61±0.3	7.41±0.3
Theoretical Plate	2240	3939
Tailing Factor	1.13	1.12

Table VII Assay of Marketed Formulation

Parameters	*S(-)Amlodipine besylate*	*Ramipril*
Label Claim (mg)	2.5	2.5
Actual content found (mg)	2.512	2.49
% RSD	0.86	0.16
%Assay	100.48	99.6

RESULTS AND DISCUSSION

Selection of analytical wavelength was done with stock solutions of 50µg/ml in various organic solvents and scanned in UV range. Maximum absorbance of S (-) Amlodipine besylate and Ramipril was found at 232 nm and 210nm respectively so these wavelengths were taken for analysis but further study was done with 210nm because both the drugs chromatogram were seen at this wavelength. The initial method development was started with mobile phase of 0.05M Sodium phosphate buffer: Acetonitrile (50:50) with different pH values and columns. Further trials were done with 0.02M Phosphate dihydrogen orthophosphate buffer: Acetonitrile combinations (40:60, 50:50, 60:40, and 70:30v/v) and the column used was C8. The satisfactory peak area, asymmetry, theoretical plate, resolution factor were seen with final chromatographic conditions set for the method were mobile phase of Acetonitrile:0.02MPotassium dihydrogen ortho phosphate buffer (0.1 % triethylamine, 0.1 % 6-heptane sulphonic acid salt) 35:65% v/v, pH adjusted to 2.5 with O-phosphoric acid with flow rate of 1 ml/min and temperature of 40 ^{0}C. The column used was Hypersil BDS C-18 (150*4.6mm) 5µ with 20µl injection volume and detection was carried out at 210 nm (Fig-4). The calibration curve was obtained by plotting area verses the concentration of the solution and it was found linear for S (-) Amlodipine Besylate (r^2= 0.9930) and Ramipril (r^2= 0.9914) over the concentration range of 25.46-76.38 and 25.14-75.41µg/ml respectively. The %RSD values for accuracy, precision and robustness obtained were less than 2 which conclude that developed method was accurate, precise and robust. Both the drugs show stability in solution form up to 20th h. Assay of marketed formulation was found to be within 98-102 %.

CONCLUSION

A new, simple and rapid RP-HPLC method has been developed for the estimation of S (-) Amlodipine Besylate and Ramipril in bulk and tablet dosage forms. This new method was validated and found to be simple, precise and accurate. It can be used for quantification of S (-) Amlodipine Besylate and Ramipril simultaneously in bulk and tablet dosage forms as well as for routine analysis in quality control.

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