International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 1(2): September-October 2011

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Pharmaceutical Sciences **Research Article……!!!**

Received: 10-10-2011; Accepted: 14-10-2011

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

S.Arunkumar[1]*, D.Sridharan[1], A.Thenmozhi[1], M.Mahes[2]

1. Arulmigu Kalasalingam College of Pharmacy, Anand nagar, Krishnankoil, Tamilnadu, INDIA

2. Surya College of Pharmacy, Vilupuram, Tamilnadu, INDIA

Keywords:

Ramipril, Amlodipine besylate, RP-HPLC, simultaneous

For Correspondence:

S. Arunkumar

Arulmigu Kalasalingam College of Pharmacy, Anand nagar, Krishnankoil, Tamilnadu, INDIA

E-mail:

arunkumarpaper@gmail.com

ABSTRACT

The objective of this present work was to develop and validate an analytical method for the quantitative determination of Ramipril and Amlodipine besylate in a tablet formulation. Chromatographic separation of the two drugs were analyzed on a Phenomenex Hypersil C_{18} column (250mm X 4.6mm, 5µm) using 25mM Phosphate buffer, acetonitrile and methanol (55:30:15) as a mobile phase. The flow rate was 1mL/minute and the effluent was monitored at 210nm. The validation of the method was performed as per ICH guidelines.

INTRODUCTION

Ramipril is chemically 1S(1S,5S,7S)-8-((2S)-2-(((1S)-1-ethoxy carbonyl-3-phenly-propyl)amino) propanoyl)-8-azabicyclo [3-3-0] octane -7- carboxylic acid[1] (fig-1). It is a highly lipophilic, long acting ACE inhibitor. It inhibits angiotension-converting enzyme which has two fold effect in reduction of angiotension II levels (AT II) and increase in bradykinin levels. Both are contributes to fall in blood pressure[2]. Amlodipine is chemically 2[(2-aminoethoxy)methyl]-4-(2-chloro-phenyl)-1,4-dihydro-6-methyl-3,5-pyridine carboxylic acid, 3-ethyl, 5-methyl ester[3] (fig-2). It is a dihydropyridine derivative with calcium antagonist activity. It is used in the management of hypertension, chronic stable angina pectoris and prinzmetal variant angina[4]. From the literature survey, it was found that there are few analytical methods were reported for Ramipril and Amlodipine either individually by HPLC[5,6], UV spectrophotometric[7], kinetic spectrophotometry[8] and LC-MS[9]; or combination with other drugs by HPLC[10], HPTLC[11], UV spectrophotometry[12]. Only one HPTLC method[13] is available for this combination but there is no HPLC method has been reported for the simultaneous estimation of the Ramipril and Amlodipine in tablet dosage form. So it was felt that there is a need to develop a analytical method for the estimation of Ramipril and Amlodipine simultaneously in a single step process. This paper presents a rapid, sensitive and accurate HPLC method for the simultaneous estimation of Ramipril and Amlodipine.

EXPERIMENTAL

Standards and Reagents

Ramipril and Amlodipine besylate were provided by Rakshit drugs Ltd (Chennai, India.) was used as a working standard. The commercially available tablets RAMISTAR-A was purchased from local market. Potassium dihydrogen ortho phosphate was of analytical grade. HPLC grade Acetonitrile and methanol were purchased from Merck Chemical Ltd., Mumbai. Millipore water (Milli-Q, USA) was used in the preparation of buffer solution.

Apparatus:

Chromatographic separation was performed on SHIMADZU liquid chromatographic system LC 2010 AT equipped with quaternary pump, Shimadzu variable UV/Vis detector SPD-20A and auto Injector. LC solution software was employed for data collection and processing. Weighing was done on SHIMADZU balance (AY-120).

Chromatographic conditions:

Chromatographic Separation was achieved on Phenomenex C_{18} (250mm × 4. 6 mm, 5µ.,) column. The mobile phase consisting 25mM Phosphate buffer, acetonitrile and methanol (55:30:15) adjusted to pH5.0 with Potassium hydroxide, was delivered at rate of 1.0mL/ minute. The mobile phase was filtered through 0.45 µm membrane filter (Millipore, USA.) and degassed prior to use. Separation was performed at ambient temperature i. e. 25ºC and detection was made at 210 nm**.** The injection volume was 20 µL with a run time of 10 min.

Preparation of mixed standard stock solution

An accurately weighed quantity of 50mg of Ramipril and 138mg of Amlodipine besylate were taken in a 100mL volumetric flask and dissolved in 25mL mobile phase by sonicating for 15 minutes allowed to attain room temperature and made to volume with mobile phase. The solution was filtered through whatman filter paper no.1.

Preparation of working standard solution

Working standard solution was prepared by diluting 10mL of the above stock solution and transfers into a 250mL volumetric flask, and diluted up to the mark with mobile phase. This will give the solution of Ramipril and Amlodipine with concentration of 20 and 40µg/mL respectively.

Estimation of drug in commercial tablet formulation

For the estimation of the drugs in tablet formulation twenty tablets were weighed and their average weight was determined. The tablets were then finely powdered. Accurately weighed tablet powder equivalent to 50mg of Ramipril and 100mg of Amlodipine was dissolved in

mobile phase and make the volume to 100mL. Then it was sonicated for 15minutes and filtered through a whatman filter paper. Further dilutions were made similar to preparation of working standard solution. All determinations were conducted in triplicate. Both the standard and sample preparation was injected separately, and the peak area responses were recorded. The percentage label claim was calculated and given in table-1.

Table- 1: Summary of system suitability and other validation parameters

S.No	Parameters	Observed values	
		Ramipril	Amlodipine
1	Resolution	4.01	
2	Retention time	7.158	8.932
3	Tailing factor	0.965	0.99
4	Plate count	5997.55	5677.75
5.	Capacity factor	7.11	8.93

Table-2:- Assay of tablets.

BRAND NAME	Drug name	Label Claim mg/tab	Mean Peak Area		Amount found* ± SD (mg/tab)	%Label claim ± SD
			Standard	Sample		
RAMISTAR-A	RAMIPRIL	2.5mg	509715	504835	2.485±0.11	99.40%±0.12
	AMLODIPINE	5.0mg	11305270	11295328	4.995±0.601	99.90%±0.62%

***Mean of three values**

Table.3. Summary of validation parameters

Parameters	Data	
	RAMIPRIL	**AMLODIPINE**
Specificity	No interference at retention time of the analyte	
Linearity range	12 to 34 µg/mL	21 to 68 µg/mL
Correlation coefficient	0.9999	0.9998
Limit of Detection	1.39 µg/mL	4.20 µg/mL
Limit of Quantitation	12.99 µg/mL	4.22 µg/mL
%Recovery (n=6)	99.24 to 101.20%	97.60 to 99.58%
Precision (%RSD)		
System Precision	1.264%	0.659%
Method precision	0.322%	0.184%
Robustness (%RSD)	0.325	0.517
Ruggedness (%RSD)	0.473	0.424

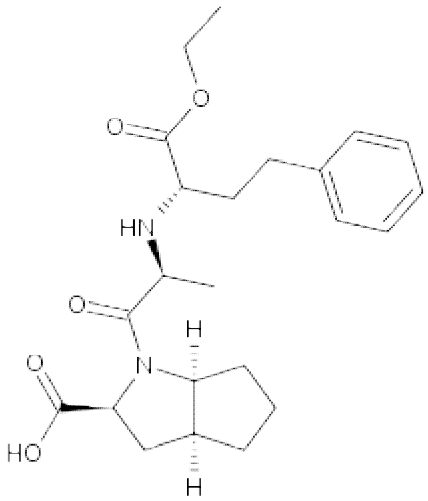

Fig-1. Showing the chemical structure of Ramipril

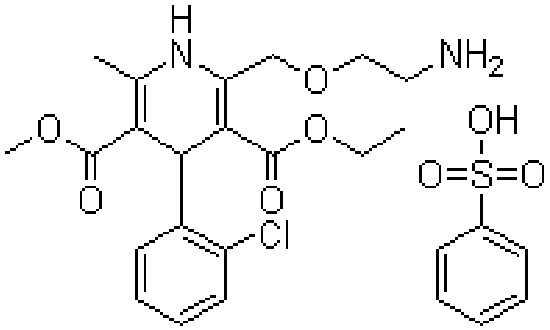

Fig-2. Showing the chemical structure of Amlodipine besylate

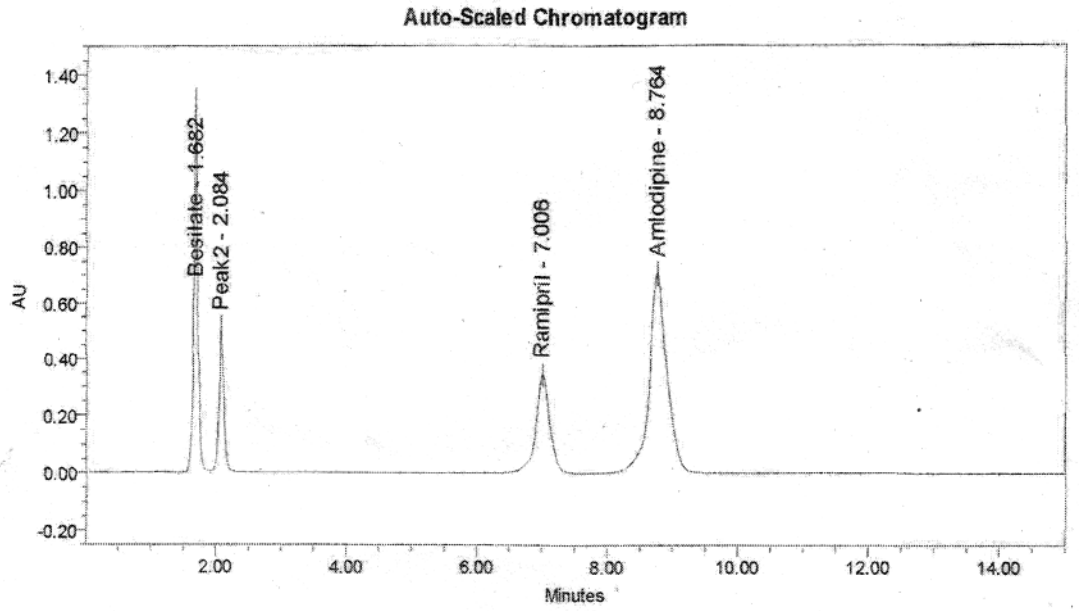

Fig-3: Typical chromatogram of Ramipril and Amlodipine besylate

RESULTS AND DISCUSSION

Estimation of Ramipril and Amlodipine in dosage form by RP-HPLC method was carried out using optimized chromatographic conditions. The typical chromatogram of Ramipril and Amlodipine for standard drug is shown in figure-1. The developed method was validated as per ICH guidelines[14]. System suitability tests were carried out using freshly prepared standard stock solution of Ramipril & Amlodipine and the parameters obtained are summarized in table-1. The percentage of individual drug found in the formulations, along with SD value is shown in table - 2. The results of analysis show that the amount of drugs was in good agreement with the label claims of the formulation. The recoveries of drug were determined at 80,100 and 120% level. The recovery of Ramipril from 99.24 to 101.20% and recovery of Amlodipine ranges from 97.60 to 99.58%, which shows the accuracy of proposed method. Ramipril was found to be linear in the concentration range of 12 to 34µg/mL, while Amlodipine was found to be linear in the concentration range of 21 to 68µg/mL. Method precision and system precision were determined by analyzing the drug sample at three different concentration levels.

CONCULSION

On the basis of result of assay and validation parameters it was concluded that proposed method was simple, accurate, and precise for the simultaneous estimation of Ramipril and Amlodipine in combined tablet dosage form and can be applied for the routine estimation of Ramipril and Amlodipine tablet dosage form.

REFERENCES

1. The Merck index - An encyclopedia of chemicals drugs and biological published by Merck research lab Division of Merck and congress, NJ,13th edition, 2001, pp 8194.
2. T.G.Warner and M.C.Perry, "Ramipril: a review of its use in the prevention of cardiovascular outcomes", Drugs, 2002; vol.62, pp.1381-1405.
3. M.J. O´ Neil, A. Smith, P.E. Heckelman, S. Budavari, The Merck Index, an Encyclopedia of Chemicals, Drugs and Biologicals, 13th ed., Merck & Co. Inc., White House Station, New Jersey, 2001, pp. 488, 865.
4. A.G. Goodman, L.S. Gilman, in: A.G. Gilman, T.W. Rall, A.S. Nies, P. Taylor (Eds.), The Pharmacological Basis of Therapeutics, 8th ed., Pergamon Press, Oxford, 1990, p. 774.
5. Bilal YILMAZ, "Determination of Ramipril in pharmaceutical preparations by High-Performance Liquid Chromatography", Int. J Pharm. Sciences Review and Research 2010; vol-1(1), pp 39-42.
6. Kanakapura Basavaiah et al, "Spectrophotometric and High Performance Liquid Chromatographic determination of Amlodipine Besylate in pharmaceuticals", Science Asia, 2005; vol-31, pp 13-21.
7. A.A.Al-Majed et al " Spectrophotometric Determination of Ramipril (A novel ACE inhibitor) in dosage forms", Spectroscopy Letters, 2001; vol-34 (2), pp211-220.N.R
8. N.Rahman et al, "Kinetic spectrophotometric method for the determination of Ramipril in pharmaceutical formulations", AAPS Pharma Sci. Tech., 2005; vol-6, pp 543-551.
9. Zhimeng Zhu et al, "Liquid chromatography –mass spectrometry method for determination of Ramipril and this active metabolic Ramipril in rat plasma", Journal of chromatography, 2002, Vol- 779(2), pp 297-306.

10. V.P.Kurade et al, "RP-HPLC estimation of Ramipril and Telmisartan in tablets", Ind. J.Pharm Sci; 2009, Mar-Apr, 71(2), pp 148-151.
11. K.Srinivasarao et al, "RP HPLC method for the determination of Iosartan potassium and Ramipril in combined dosage form", Ind J Pharm Sci,; 2010, Vol 72(1) pp 108-111.
12. Lincy Joseph et al, "Simultaneous estimation of Atorvastatin and Ramipril by RP-HPLC and spectroscopy", Pakistan J Pharm. Sci., 2008; vol 21(3), July, pp 282-284.
13. K.R.Gupta et al, "Simultaneous determination of Amlodipine and Ramipril by HPTLC, Asian J of Chemistry, 2007; Vol 19 (6), 4117-4182.
14. ICH of Technical Requirements for the registration of Pharmaceutical for human use, "Validation of analytical procedure methodology, ICH-Q2B, Geneva, 1996.