Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(6):725-733

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

RP-HPLC method development and validation for the simultaneous estimation of ramipril and amlodipine besylate in capsule dosage form

Jignesh Patel[1]* and Mandev Patel[2]

[1]Department of Quality Assurance, Bhagwant University, Ajmer, Rajasthan, India
[2]A-One Pharmacy College, Enasan, Ahmedabad, Gujarat, India

ABSTRACT

A simple, rapid, and accurate reversed phase High-performance liquid chromatographic (RP-HPLC) method has been developed and subsequently validated for the simultaneous determination of Ramipril (RAM) and Amlodipine Besylate(AML) in Capsule dosage form. The separation is carried out using a mobile phase consisting of Buffer: Acetonitrile (70:30). The column used is Hypersil BDS C8 (150 × 4.6 mm, 5 µm) with flow rate of 1.2 mL/min using UV detection at 210nm. The total run time is 25 min and the retention time of RAM and AML is 5.9 min and 7.5 min respectively. The described method is linear for the assay of RAM and AML over a concentration range of 15-35µg/ml and 30-70 µg/mL respectively. Results of the analysis have been validated statistically and by recovery studies. The results of the studies showed that the proposed RP-HPLC method is simple, rapid, precise, and accurate, which is useful for the routine determination of RAM and AML in capsule dosage form.

Key words: Ramipril, Amlodipine besylate, HPLC

INTRODUCTION

Ramipril (2S, 3aS, 6aS)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl] amino} propanoyl]-octahydrocyclopenta[b]pyrrole-2-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]. Various analytical methods have been reported for estimation of Ramipril in pure form and in combination with other compounds by Spectroscopically[2-3] and HPLC[4-5].

Amlodipine chemically (RS)-3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate. Long- acting calcium channel blocker used as an anti-hypertensive and in the treatment of angina[6]. Like other calcium channel blockers, amlodipine acts by relaxing the smooth muscle in the arterial wall, decreasing total peripheral resistance and hence reducing blood pressure, in angina it increases blood flow to the heart muscle. Literature survey reveals that few analytical methods were reported on Ramipril and in combination with other compounds by Spectroscopically[7-9] HPTLC[10] and HPLC[11-12].

Literature survey reveals that a variety of spectrophotometric and chromatographic methods including UV, colorimetric determination, ratio derivative, and a HPLC methods have been reported for determination of RAM and AML either single or in combination with other drugs. Whereas no liquid chromatography method has been reported for simultaneous quantitative determination of RAM and AML in capsule dosage form.

Hence a rapid simple reproducible Ultra performance liquid chromatography method was developed for simultaneous quantitative determination of RAM and AML in capsule dosage form in presence of degradation product.

EXPERIMENTAL SECTION

2.1 Reagents and chemicals

HPLC grade Acetonitrile, methanol, NaOH and HCl were procured from Merck, Mumbai (India). Concentrated HCl was of analytical grade delivered by Renkem, India. Ramipril and Amlodipine besylate working standard were gifted from Drakt Pharmaceutical Ltd, Vadodara.

2.2 Instrumentation:

Chromatographic separation was performed using Shimadzu LC 2010CHT high performance liquid chromatography system in isocratic mode, equipped with auto sampler and a photo-diode array detector. Chromatograms and data were recorded by means of LC solution software. waterbaths equipped with MV controller (Julabo, Seelbach, Germany) was used for hydrolysis studies. Photo stability studies were carried out in a photo stability chamber (SVI equipments, Germany.). Thermal stability studies were performed in a dry air oven (Labline, India).

2.3 Chromatographic Conditions:

The chromatographic column was a hypersil BDS C8, 150 mm X 4.6 mm i.d with 5µm particles. Buffer for Mobile phase was prepared by dissolving 3.42 g of potassium dihydrogen phosphate in 1000ml of water, add 5 ml triethylamine and adjust pH 2.5 with 10% Ortho-phosphoric acid and mix. Mobile phase was prepared by mixing buffer and acetonitrile in the ration of 70:30 v/v. The flow rate of mobile phase was 1.2 mL min^{-1} and the detection was monitored at a wavelength of 210 nm. The column temperature was maintained at $55^{O}C$ and injection volume was 10 µL. Diluent was prepared by mixing water and methanol in the ratio of (50:50).

2.4 Preparation of standard solution

Accurately weigh about 25 mg of Ramipril working standard and 70 mg of Amlodipine Besylate (equivalent to 50 mg of Amlodipine) and transfer into 100 ml volumetric flask. Dissolve in dilute and make up to mark with it. Several aliquots of these standard stock solutions were taken in different 50 mL volumetric flask and diluted up to the mark with diluents to achieve final linearity concentrations of RAM and AML were 15-35µg/mL and 30-70µg/mL, respectively.

2.5 Preparation of sample solution

Weigh the content not less than 20 capsules and mix. Weigh accurately about granules equivalent to 12.5mg of Ramipril and 25mg of Amlodipine and transfer into 500ml volumetric flask. Add about 30ml of water and shake mechanically to disperse the granules till granules dissolve completely and then add about 300ml of diluent and sonicate for 45 minutes with intermittent shaking. Allow to attain room temperature, make up the volume with diluent and centrifuge the solution at 3000rpm for 15minutes. Filter the solution through 0.45µ membrane filter (Nylon).

RESULTS AND DISCUSSION

3.1 METHOD DEVELOPMENT AND OPTIMIZAION

Some important parameters like pH of the mobile phase, percentage and type of the organic modifier and buffer, etc., were tested for a good chromatographic separation. Trials showed that an acidic mobile phase with reverse phase hypersil BDS C8 column gives symmetric and sharp peaks. For this reason, phosphate buffer containing triethyalamine and adjust pH 2.5 with O-Phosphoric acid was preferred as a buffer. Acetonitrile was chosen as the organic modifier because it dissolves drugs very well. The simple isocratic program was applied for the analysis at a flow rate of 1.2 mL/min showed good resolution. The resolution between RAM and AML was much greater than 3.0 with a decrease in peak tailing. Retention times of the drugs obtained under these conditions were 5.9 and 7.5 min for RAM and AML, respectively. To determine the wave length of simultaneous determination of RAM and AML were injected into the HPLC system and obtained the UV spectra in the range 200-400nm by DAD. The UV spectra of the solutions obtained shown maximum absorbance at 210nm. For the quantitative analytical purposes the wavelength was set at 210 nm. The typical chromatogram of blank and sample is shown in Figure 1and 2.

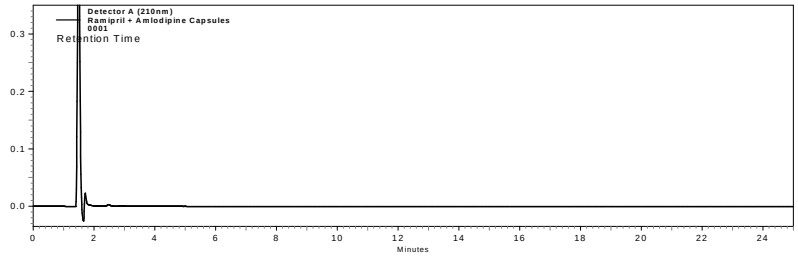

Figure1: - Chromatogram of Blank-Diluent

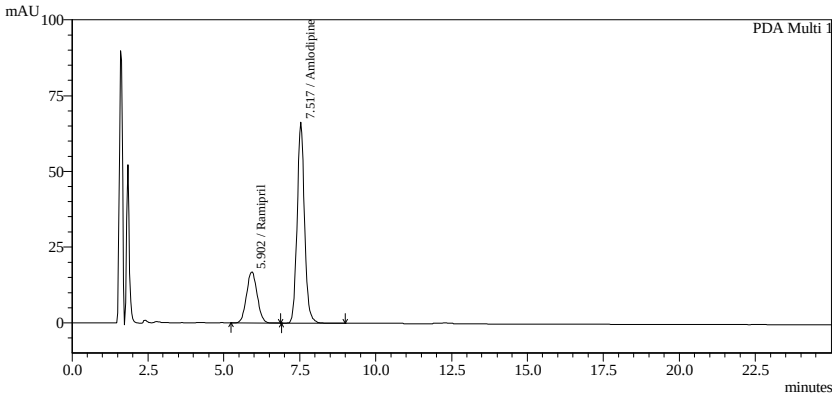

Figure 2: Typical chromatogram of Ramipril and Amlodipine

3.2 System suitability studies

The column efficiency, resolution, and peak asymmetry were calculated for the standard solutions. The values obtained (Table 1) demonstrated the suitability of the system for the analysis of this drug combination.

Table .1 System Suitability Parameters

Parameter	RAM	AML
Retention time	5.9	7.5
% RSD(n=6)	0.9	0.7
Asymmetric factor	1.0	1.0
Theoretical plates	2800	9927
Resolution factor	-	3.12

3.3 Linearity

The linearity was determined for RAM and AML. The Calibration curve was plotted using 60%, 80%, 100%, 120% and 140% standard solution of RAM and AML with respect to test concentration of 25 µg/mL and 50 µg/mL, respectively.

The linear regression equation for two drugs:

RAM:$Y = 8228.03X - 10992.00$ ($r = 0.9990$)(n=5)
AML:$Y = 22522.71\ X - 22525.93$ ($r = 0.999$) (n=5)

The results showed that an excellent correlation exists between peak area and concentration of the drugs within the concentration range indicated previously. The data was analyzed by "linear regression least squares fit," and the parameters are listed in Table 2

Table .2 Linearity Study

Analyte	Concentration range	R^2	Slope	Intercept
RAM	15-35 µg/mL	0.999	8228.03	– 10992.00
AML	30-70 µg/mL	0.999	22522.71	– 22525.93

3.4 Accuracy

To check the accuracy of the developed method, analytical recovery study experiments were carried out by the standard addition method. From that total amount of the drug found, the percentage recovery was calculated. The recovery values for the two drug compounds are in the range 99.5 - 100.8 for RAM and 98.3 - 99.3 for AML. The Accuracy data are reported in Table 3and 4.

Table 3:- Accuracy data for Ramipril

Level	Replicate	Amount		Recovery		
		Added (µg/ml)	Found (µg/ml)	% Recovery	Mean	% RSD
Level-1 (60%)	1	14.75	14.87	100.8	100.8	0.0
	2	14.75	14.83	100.6		
	3	14.75	14.88	100.9		
Level-2 (100%)	1	24.59	24.53	99.8	99.5	0.1
	2	24.59	24.44	99.4		
	3	24.59	24.43	99.4		
Level-3 (140%)	1	34.42	34.27	99.6	99.5	0.1
	2	34.42	34.27	99.6		
	3	34.42	34.17	99.3		

Table 4:- Accuracy data for Amlodipine

Level	Replicate	Amount		Recovery		
		Added (µg/ml)	Found (µg/ml)	% Recovery	Mean	% RSD
Level-1 (60%)	1	30.28	29.95	98.9	99.3	0.12
	2	30.28	30.10	99.4		
	3	30.28	30.18	99.7		
Level-2 (100%)	1	50.48	49.41	97.9	98.3	0.2
	2	50.48	49.70	98.5		
	3	50.48	49.77	98.6		
Level-3 (140%)	1	70.67	69.63	98.5	98.6	0.1
	2	70.67	69.85	98.8		
	3	70.67	69.67	98.6		

3.5 Precision

Precision was determined by studying the repeatability and intermediate precision. Repeatability results indicate the precision under the same operating conditions over a short interval time inter assay precision. The relative standard deviation, were calculated for two drugs. The results are mentioned in Table 5. Intermediate precision was carried out by doing intra and inter day precision studies. In the intraday study, the concentrations of two drugs were calculated on the same day at interval of 1hr. In the inter day study, the concentrations of drug contents were calculated on three different days, and the study express with in laboratory variation in different days. In both intra and inter day precision studies for the methods, % RSD values were not more than 2%, which indicates good intermediate precision (Table 5).

Table 5:- Precision data for Ramipril and Amlodipine

	Ramipril		Amlodipine	
Replicate	Repeatability	Intermediate Precision	Repeatability	Intermediate Precision
1	104.3	104.4	101.5	100.3
2	104.5	104.3	102.0	100.9
3	102.5	105.2	102.0	104.5
4	102.4	104.5	102.4	104.3
5	102.2	104.0	101.1	104.1
6	103.6	103.2	103.4	104.7
Statistics-Set level				
Mean	103.3	104.3	102.1	103.1
% RSD	0.9	0.7	0.5	1.8

3.6 Specificity:
Specificity of the method was assessed by comparing the chromatograms obtained from standard and sample solution. Retention time of RAM and AML in standard and sample solution was same, so the method was specific. The method was selective because no interference of excipient was found.

3.7 Robustness
The robustness of the method was established by making deliberate minor variations in the flow rate was changed by 10%, change mobile composition ± 2% absolute, column oven temperature changed by ± 5°C and pH change by 0.2 unit absolute. The result shows that %RSD, % difference in assay were not more than 2%.

Table 6:- Results of Robustness parameter

Condition	% RSD		%Recovery		%Difference in Assay	
LIMIT	NMT 2.0		98 to 102%		NMT 2%	
1) Change in Flow rate	**Ramipril**	**Amlodipine**	**Ramipril**	**Amlodipine**	**Ramipril**	**Amlodipine**
Normal Condition(1.2 ml per minute)	0.1	0.1	104.3	101.5	-	-
Change in flow rate by - 0.12 ml per minute (1.08 ml per minute)	0.1	0.1	103.4	102.7	0.9	1.2
Change in flow rate by + 0.12 ml per minute (1.32 ml per minute)	0.1	0.2	104.9	102.5	0.6	1.0
2) Change in Mobile Phase composition						
Normal Condition Buffer: Acetonitrile(70:30)	0.1	0.1	104.3	101.5	-	-
Change in organic phase Ratio by -2.0 Buffer: Acetonitrile(68:32)	0.8	1.7	102.9	102.7	1.4	1.6
Change in organic phase Ratio by +2.0 Buffer: Acetonitrile (72:28)	0.2	0.2	101.3	101.8	0.2	0.3
3) Change in column oven temperature						
Normal Condition (55°C)	0.1	0.1	104.3	101.5	-	-
Change in oven temperature by -5°C (50°C)	0.4	0.2	104.7	103.3	0.4	1.8
Change in oven temperature by +5°C (60°C)	0.2	0.1	105.1	103.2	0.8	1.7
4) Change in pH						
Normal Condition(pH 2.5)	0.1	0.1	104.3	101.5	-	-
Change in -0.2 unit (2.3)	0.8	0.3	102.6	99.8	1.7	1.7
Change in +0.2 unit (2.7)	0.9	0.6	103.0	99.8	1.3	1.7

3.8 Forced Degradation Studies:
Stress testing of a drug substance can help to identify the likely degradation products, which can help to establish the degradation path ways and the intrinsic stability of the molecule. All stress decomposition studies were performed at initial drug concentration 25µg/ml of RAM and 50 µg/ml of AML. The degradation conditions were selected on the basis of literature survey.

3.8.1 Hydrogen Peroxide Induced Degradation
For hydrogen peroxide-Induced degradation, the studies were carried out at 100^0C temperature in 5% Hydrogen Peroxide for 15 min and Showed the minor degradation found at RT 4.9min. All the major and minor degradation products were well separated from RAM and AML Peaks (figure no 3, 4). The peak Purity is checked for RAM and AML. The results are summarized in Table 7.

3.8.2 Acid Induced Degradation
Acid hydrolysis was performed in 0.1N HCl at 100^0C for 30min under reflux condition showed the minor degradation found at RT 2.6, 2.9 and 4.8min and all the major and minor degradation products were well separated from RAM and AML peaks (figure no. 5, 6). The Peak purity is checked for RAM and AML and the results are summarized in Table 7.

3.8.3 Base Induced Degradation
Base hydrolysis was performed in 0.1N NaOH at room temperature for 5 min and showed the major degradation found at RT 2.6 min and all the major and minor degradation products were well separated from RAM and AML peaks (figure no.7, 8). The Peak purity is checked for RAM and AML and the results are summarized in Table 7.

3.8.4 Photo degradation

Photo degradation studies were carried out at exposed to light for an overall illumination of 1.2 million lux hours. Samples were withdrawn at appropriate time and subjected to HPLC analysis after suitable dilution (25µg/ml of AML and 50 µg/ml of AML). The drugs RAM and AML were stable under Photolytic condition (figure no. 9). The peak purity is checked for RAM and AML and the results are summarized in Table 7.

3.8.5 Thermal Degradation

Thermal degradation studies were carried out at 100°C for 72 hours Samples were withdrawn at appropriate time and subjected to HPLC analysis after suitable dilution (25µg/ml of AML and 50 µg/ml of AML). The drugs RAM and AML were stable under thermal degradation condition (figure no. 10, 11). The peak purity is checked for RAM and AML and the results are summarized in Table 7.

Table 7:- Degradation condition for Ramipril API and Amlodipine API and Formulation

Sample Name	Peak purity index
Ramipril API, Amlodipine API and Formulation–Acid Degradation (0.1N HCl at 100 °C for 30 minutes)	1.000
Ramipril API, Amlodipine API and Formulation –Base Degradation (0.1 N NaOH at room temperature for 5 minutes)	1.000
Ramipril API, Amlodipine API and Formulation –Oxidative degradation (5% H_2O_2 at 100°C for 15minutes)	1.000
Ramipril API, Amlodipine API and Formulation –Thermal Degradation (100°C for 72 hours)	1.000
Ramipril API, Amlodipine API and Formulation –Photo Degradation (1.2 million lux hours)	1.000

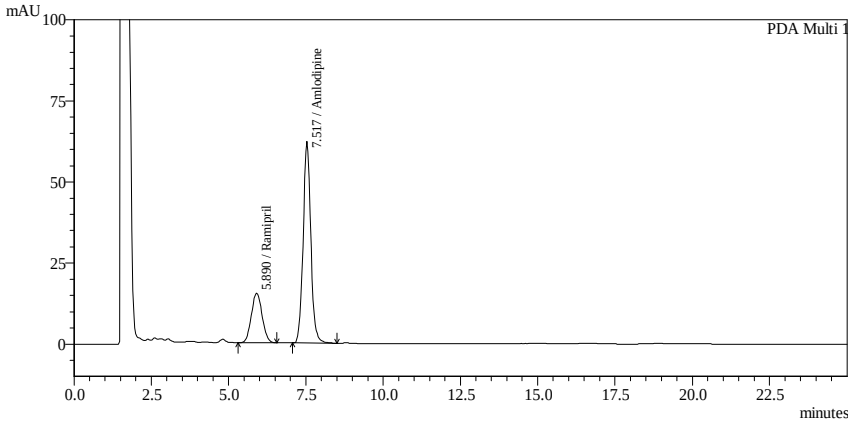

Figure 3: - Chromatogram of Sample treated with 5% H_2O_2 at 100°C for 15minutes

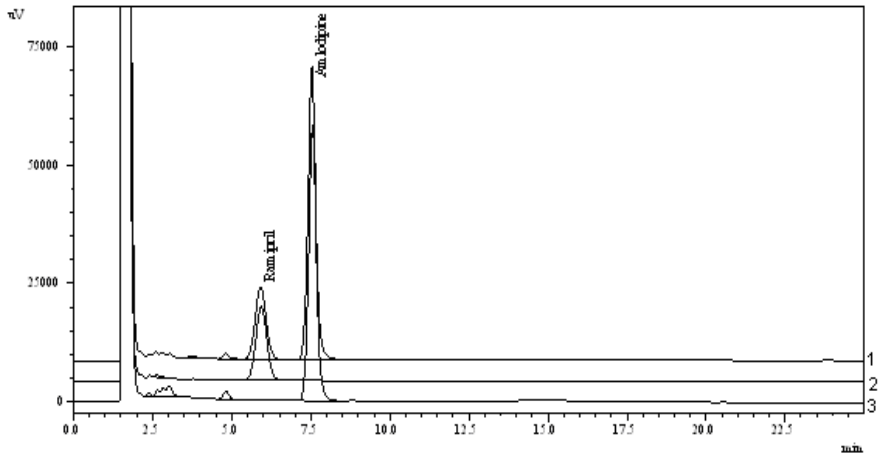

Figure: 4 Typical HPLC chromatogram of Oxidative hydrolysis - degraded sample preparation of Formulation (1), Ramipril API (2) and Amlodipine API (3)

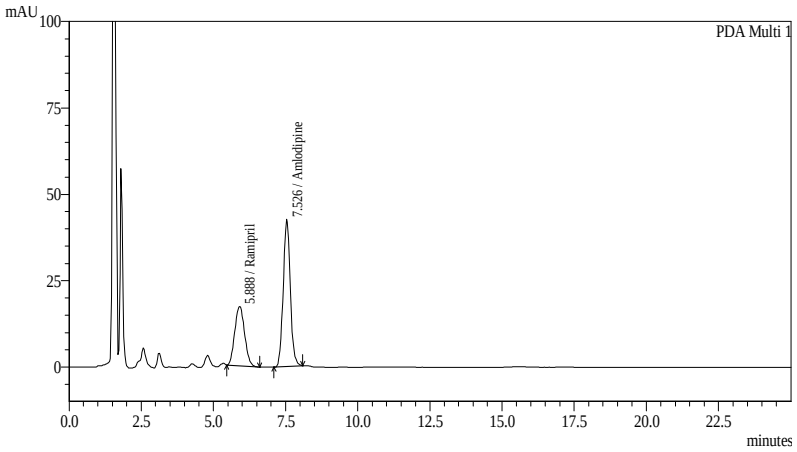

Figure 5: - Chromatogram of Sample treated with 0.1N HCl reflux for 30 minute at 100°C

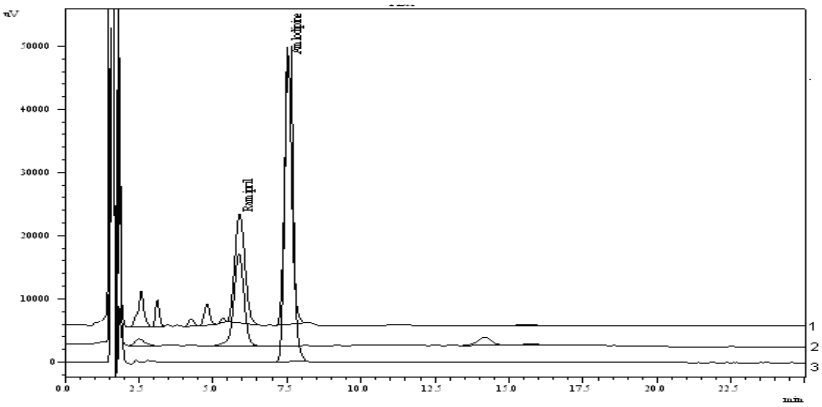

Figure: 6 Typical HPLC chromatogram of Acid hydrolysis – degraded sample preparation of Formulation (1), Ramipril API (2) and Amlodipine API (3)

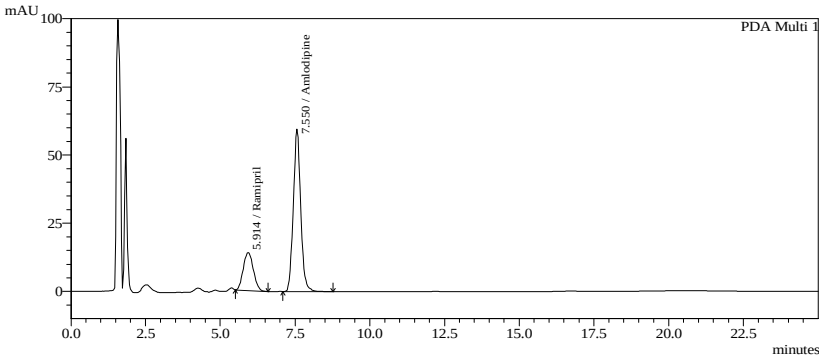

Figure 7: - Chromatogram of Sample treated with 0.1N NaOH for 5 minutes at room temperature

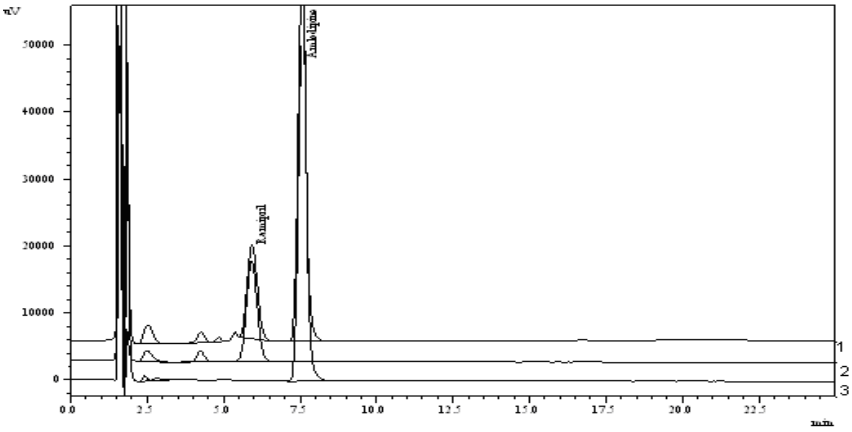

Figure: 8 Typical HPLC chromatogram of Alkali hydrolysis - degraded sample preparation of Formulation (1), Ramipril API (2) and Amlodipine API (3)

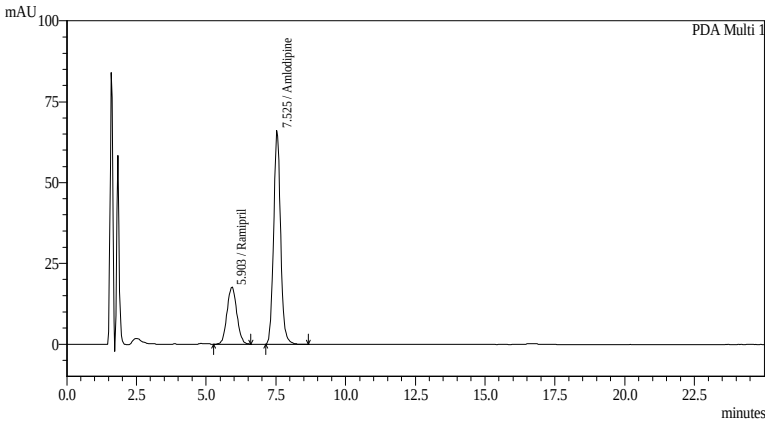

Figure 9: - Chromatogram of Sample placed in photo light chamber for 1.2 million lux hours

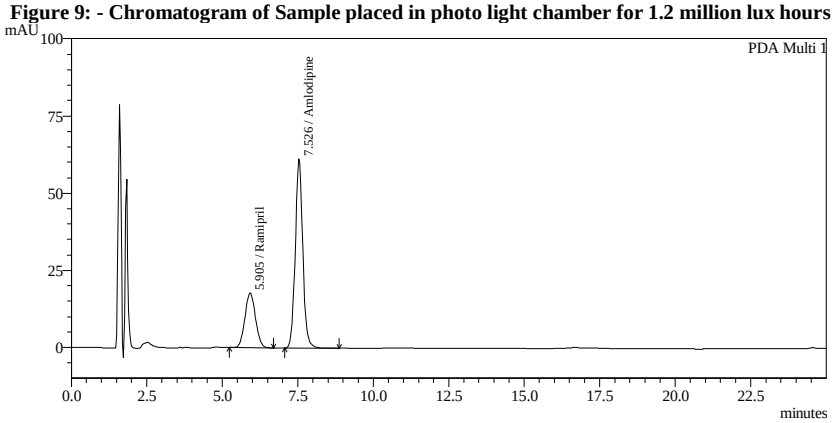

Figure 10: - Chromatogram of Sample treated with 100°C for 72 hours (Thermal Degradation)

3.9 Solution stability and Mobile Phase stability

The solution stability of RAM and AML was carried out by leaving the test solution in tightly capped volumetric flask at room temperature for 24hrs. The same sample solution was assayed after 12 hrs interval up to the study period against freshly prepared standard solution of RAM and AML. The percentage RSD of assay of RAM and AML was calculated for the study period during solution stability experiments. The % RSD of the assay of RAM and AML during solution stability experiment was within 1% and it indicated that both standard and test preparation were stable for 1 days on bench top at room temperature.

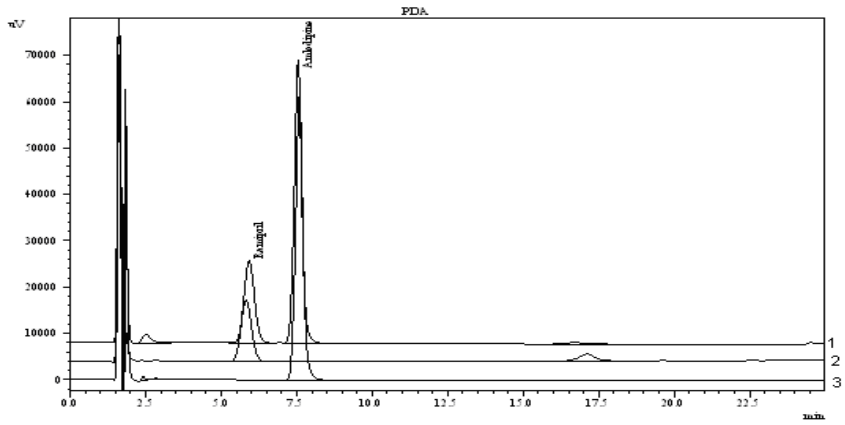

Figure: 11 Typical HPLC chromatogram of Thermal - degraded sample preparation of Formulation (1), Ramipril API (2) and Amlodipine API (3)

CONCLUSION

The proposed method gave good resolution between RAM and AML. Solution stability studies showed that the active pharmaceutical ingredients remained stable for 24 hr at room temperature. The changes in flow rate, composition of mobile phase, and temperature of column did not affect the percentage assay of drug, confirming the robustness of the method. High percentage recovery of drug shows the method is free from interference of excipients present in the formulation. Thus the proposed method is simple, rapid, sensitive, specific, accurate, and precise, and does not involve complicated sample preparation procedures.

Acknowledgments
The authors would like to thank the principal of A-one college of pharmacy and director of Drakt Pharmaceutical Ltd for their support to carry this investigation.

REFERENCES

[1] AG Gilman, TW Rall, S Niesa, P Taylor. The Pharmacological Basis of Therapeutics.1st edition, Oxford, **1996**, 743.
[2] S Bankey; GG Tapadiya; SS Saboo; et al. *International J. of chem. tech Res.*, **2009**, 1(2), 183-188.
[3] PR Patil; SU Rakesh; PN Dhabale; KB Burade. *Research J. Pharm. and Tech.* **2009**, 2(2), 304-307.
[4] B Yilmaz; *International J. of Pharm. Sciences Review and Research*, **2010**, 1(1), 39-42.
[5] F Belal; EA Gadkariem; MA Abounassif. *J. Pharm. Biomed Anal*. **2001**, 24, 335 - 342.
[6] The Merck Index, 14th ed., **2006**, 493.
[7] BG Chaudhari; AB Patel. *International J. of chem. tech Res.*, **2010**, 2(1), 633-639.
[8] KR Gupta; AD Mahapatra; AR Wadodkar; SG Wadodkar. *International J. of chem. tech Res.*, **2010**, 2(1), 551-556.
[9] PR Patil; SU Rakesh; PN Dhabale; KB Burade. *Research J. Pharm. and Tech.* **2009**, 2(2), 304-307.
[10] AP Argekar; SG Powar. *J. Pharm. Biomed analysis*, **2000**, 21(6), 1137 - 1142.
[11] KR Naidu; UN Kale; MS Shingare. *J. Pharm. Biomed analysis*, **2005**, 39, 147-155.
[12] MM Maste; MC Kalekar; N Kadian; AR Bhat. *Asian J. Research Chem.***2011**, 4(8), 15-18.