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Original Article

Method Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimation of Perindopril Erbumine and Amlodipine Besylate in Bulk and its Pharmaceutical Formulations

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ABSTRACT

A new rapid, precise and sensitive reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed and validated for the estimation of Perindopril and Amlodipine simultaneously in combined dosage form. The two components Perindopril and Amlodipine were well resolved on an isocratic method, C18 column, utilizing a mobile phase composition of acetonitrile: methanol: a mixed buffer of 0.02M Potassium dihydrogen phosphate buffer and 0.02M Sodium dihydrogen Phosphate buffer with 1mL Tri ethyl amine (40:20:40), v/v, pH 5.0) at a flow rate of 1.0 mL/min with UV detection at 226 nm. The retention time of Perindopril and Amlodipine were 2.9 min and 4.9 min respectively. The developed method was validated for specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and robustness as per ICH guidelines. Linearity for Perindopril and Amlodipine were found in the range of 48-112 µg/ml and 60.0-140.0 µg/ml, respectively. The percentage recoveries for Perindopril and Amlodipine ranged from 98.9-100.4 % and 98.9-100.7 %, respectively. The proposed method could be used for routine analysis of Perindopril and Amlodipine in their combined dosage forms.

Keywords: Liquid chromatography, Perindopril, Amlodipine, Combined dosage forms, Simultaneous estimation, Validation.

INTRODUCTION

Perindopril hydrochloride (NEB) is (±) [2R* R* R* (S *)] œ, œ [imino bis (methylene)] bis- [6- fluoro- 3, 4 - dihydro- 2H- 1- benzopyran- 2-methanol] hydrochloride is an antihypertensive drug, It is a racemate of two enantiomers with four chiral centers. The SRRR-enantiomer (d-Perindopril) is a potent and cardio selective □1-adrenergicblocker. The RSSS-enantiomer (Perindopril) has a favourable hemodynamic profile 1, 2. Amlodipine (VAS) (N-valeryl-N[[2-(1H-tetrazol-5-yl) biphenyl-4-yl] methyl] valine, is an orally active, potent and specific competitive angiotensin II antagonist acting at the ATI receptor, which mediates all known effects of angiotensin II on the cardiovascular system. Amlodipine is widely used in the treatment of hypertension 1, 3. Combination of NEB and VAS is used as cardiovascular and 1-adrenergic blocker. The chemical structures of Perindopril and Amlodipine are shown in (Fig. 1).

To the best of our knowledge, no study has been reported for the simultaneous determination of Perindopril and Amlodipine in pharmaceutical formulations by UV-spectrophotometer and RP-HPLC method. The significance of the developed methods is to determine the content of both drugs simultaneously in commercially available capsule dosage form and can be used in future for bioequivalence study for the same formulations. The capsule solid dosage form in combination containing Perindopril HCl (5 mg) and Amlodipine (4mg) is available in the market. In this paper, we reported two spectrophotometric methods and one reverse-phase HPLC method for the quantification of Perindopril and Amlodipine simultaneously. The present RP-HPLC method was validated as per ICH guidelines[9,10].

However there is no analytical method reported for simultaneous estimation of both drugs in their combined tablet dosage form by reporting forced degradation studies to demonstrate stability indicating nature of the method. Present work describes rapid, simple, sensitive, accurate and reproducible stability indicating method.The present developed method was used determine the Perindopril and Amlodipine present in the formulation and method validated according to the ICH guidelines[18-19].

MATERIALS AND METHODS

Materials

HPLC grade Potassium dihydrogen phosphate, acetonitrile, methanol and water were procured from Merck India. All dilutions were performed in standard class-A, volumetric glassware. For the estimation of commercial formulation, Coversyl-AM having (Perindopril Erbumine-4mg and Amlodipine Besylate-5mg) manufactured by Serdia pharmaceuticals (India) ltd were procured from the local market.

Instrumentation

Agilent 1120 compact LC chromatographic system, with DAD detector and a fixed injector equipped with 20µL loop was used for the chromatographic separation. The chromatogram was recorded at and peaks quantified by means of Ez Chrome software. Chromatographic separation was carried out on a C18 column [Inertsil ODS 3V, 150mm x4.6mm 5µ]. Sartorius electronic balance was used for weighing the samples. Ultra-sonic bath sonicator was used for degassing and mixing of the mobile phase.

Chromatographic conditions

Chromatographic separation of Perindopril and Amlodipine was carried on a C18 column. The mobile phase was composed of acetonitrile, methanol and a mixed buffer of 0.02M potassium dihydrogen phosphate and Sodium Dihydrogen Phosphate

buffer with 1mL of Triethylamine (pH 5.0) in the ratio of 40:20:40 v/v. It was filtered through a 0.45 µ membrane filter and degassed for 15 minutes. The flow rate of the mobile phase was maintained at 1 ml/min. Detection was carried out at 226 nm at ambient temperature.

Method development

Preparation of standard stock solutions

Standard stock solutions were prepared by dissolving 50 mg of Amlodipine and 40 mg Perindopril working standard in two separate each 50 mL volumetric flasks using 30mL of mobile phase and made up to the mark with mobile phase to obtain a final concentration of 1000µg/mL and 800 µg/mL of each Perindopril and Amlodipine . From the above stock solutions, each 5ml of aliquots of Perindopril and Amlodipine were pipette in to a 50mLvolumetric flask and dissolved in 25mL of the mobile phase and made up to the mark with the solvent to obtain a final concentration of 80 µg/mL and 100 µg/mL for Perindopril and Amlodipine respectively.

Preparation of sample solutions

Weighed and finely powdered 20 Tablets. Accurately weighed and transferred equivalent to 40mg Perindopril and 50mg of Amlodipine into a 100 mL volumetric flask, added 70 mL of diluent, and sonicated for 30minutes with intermittent shaking at controlled temperature and diluted to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transferred 5.0 mL of the above solution into a 25 mL volumetric flask and diluted to volume with diluent to obtain a concentration of 80 and 100 µg/mL of Perindopril and Amlodipine respectively.

Method validation

The developed HPLC method for the simultaneous determination of Perindopril and Amlodipine was validated as per the ICHguidelines[13,14].

As part of method validation as per ICH guidelines, the following parameters are studied.

1. System Suitability and System Precision
2. Specificity Studies
   - Blank Interference
   - Placebo Interference
   - Forced degradation studies in different stress conditions to establishing stability indication of the developed method.
3. Method Precision
4. Accuracy studies
5. Linearity Studies including LOD/LOQ determination
6. Ruggedness
7. Robustness
8. Analysis of Marketed samples by applying the developed method.

Each parameter was explained separately in different sections under results and discussions.

RESULTS AND DISCUSSION

System suitability and system precision

System suitability for chromatographic separation was checked on each day of validation to evaluate the components of the analytical system in order to show that the performance of the system meet the standards required by the method. System suitability parameters established for the developed method include number of theoretical plates (efficiency), Resolution, Tailing factor. The HPLC system was equilibrated using the initial mobile phase composition, followed by 5 injections of the standard solution of 100% concentration containing 80 µg/mL Perindopril and 100 µg/ml Amlodipine. These 5 consecutive injections were used to evaluate the system suitability on each day of method validation. The result was given in the Table 1.

Specificity

Blank interference

A study to establish the interference of blank was conducted. Diluent was injected into the chromatograph in the defined above chromatographic conditions and the blank chromatograms were recorded. Chromatogram of Blank solution (Fig. no.-2) showed no peaks at the retention time of Perindopril and Amlodipine peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of Perindopril and Amlodipine in Perindopril and Amlodipine tablets. Similarly typical representative chromatogram of standard is also shown (Fig. No. -3)

Forced degradation

Control sample

Weighed and finely powdered 20 Tablets. Accurately weighed and transferred equivalent to 40mg Perindopril and 50mg of Amlodipine into a 100 mL volumetric flask, added 70 mL of diluent, and sonicated for 30minutes with intermittent shaking at controlled temperature and diluted to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transferred 5.0 mL of the above solution into a 25 mL volumetric flask and diluted to volume with diluent. Refer (Fig. no.-4A)

Acid degradation sample

Weighed and finely powdered 20 Tablets. Accurately weighed and transferred equivalent to 40mg Perindopril and 50mg of Amlodipine into a 100 mL volumetric flask, added 70 mL of diluent, and sonicated for 30minutes with intermittent shaking at controlled temperature. Then add 5mL of 1N acid, refluxed for 30min at 60°C, then cooled to room temperature, neutralize with 1N NaOH and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 5.0 mL of the above solution into a 25 mL volumetric flask and dilute to volume with diluent. Refer (Fig. no.-4B)

Base degradation sample

Weighed and finely powdered 20 Tablets. Accurately weighed and transferred equivalent to 40mg Perindopril and 50mg of Amlodipine into a 100 mL volumetric flask, added 70 mL of diluent, and sonicated for 30minutes with intermittent shaking at controlled temperature. Then add 5mL of 1N NaOH, refluxed for 30min at 60°C, then cooled to room temperature, neutralize with 1N NaOH and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 5.0 mL of the above solution into a 25 mL volumetric flask and dilute to volume with diluent. Refer (Fig. no.-4C)

Peroxide degradation sample

Weighed and finely powdered 20 Tablets. Accurately weighed and transferred equivalent to 40mg Perindopril and 50mg of Amlodipine into a 100 mL volumetric flask, added 70 mL of diluent, and sonicated for 30minutes with intermittent shaking at controlled temperature. Then add 5mL of Hydrogen Peroxide, refluxed for 30min at 60°C, then cooled to room temperature, and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 5.0 mL of the above solution into a 25 mL volumetric flask and dilute to volume with diluent. Refer (Fig. no.-4D)

Thermal degradation sample

Tablets are exposed to 105°c for five days. Weighed and finely powdered 20 Tablets. Accurately weighed and transferred equivalent to 40mg Perindopril and 50mg of Amlodipine into a 100 mL volumetric flask, added 70 mL of diluent, and sonicated for 30minutes with intermittent shaking at controlled temperature and dilute to volume

with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 5.0 mL of the above solution into a 100 mL volumetric flask and dilute to volume with diluent. Refer (Fig. no.-4E)

Similarly Humidity, UV-Light exposure, Sunlight exposure and Water hydrolysis stress samples are prepared and checked for their purity by proposed method.

Linearity and range

The standard curve was obtained in the concentration range of 48-112 µg/ml for Perindopril and 3-7 µg/mL for Amlodipine. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r2] of standard curve were calculated and given in Figure-5A(For Escitalopram) and Figure-5B(For Etizolam) to demonstrate the linearity of the proposed method. The result of regression analysis was given in the Table 2.

From the data obtained which given in Table-2 (For Perindopril and Amlodipine) the method was found to be linear within the proposed range.

Accuracy

The accuracy of an analytical method is the closeness of results obtained by that method to the true value for the sample. It is expressed as recovery (%), which is determined by the standard addition method. In the current study recovery at three spike levels 80%, 100% and 120% were carried out. The % recovery at each spike level was calculated and was given in Table 3.

Precision

The precision of an analytical method is the closeness of replicate results obtained from analysis of the same homogeneous sample. Precision was considered at different levels, i.e. method, system.

Inter day and intraday. Precision of the developed method was assessed by measuring the response on the same day (intraday precision) and next two consecutive days (inter day precision). The precision of the method was assessed by six replicate injections of 100% test concentration. Intra and inter-day precision of the method was assessed by determination of standard deviation and % RSD for the analyte response. The result was given in Table 4.

LOD and LOQ

LOD and LOQ values were determined by the formulae LOD = 3.3 σ/S and LOQ = 10 σ/S(Where, σ is the standard deviation of the responses and S is the slope of the calibration curves). In the present method σ is the mean of standard deviation of y intercepts of the three calibration curves and S is the mean of slopes of the calibration curves. The result was given in Table5.

Robustness

The robustness of the method was determined by assessing the ability of the developed method to remain unaffected by the small changes in the parameters such as percent organic content, pH of the mobile phase, buffer concentration, temperature, injection volume and flow rate. A deviation of ± 2nm in the detection wavelength, ± 0.1 mL/min in the flow rate, ± 5%change in the organic phase were tried individually. The result was given in the Table 5.

OVER ALL SUMMARY OF THE METHOD

Column chemistry, solvent selectivity, solvent strength (volume fraction of organic solvent(s) in the mobile phase), detection wavelength and flow rate were varied to determine the chromatographic conditions for giving the best separation. Several mobile phase compositions were tried to resolve the peaks of Perindopril and Amlodipine. The optimum results were attained with acetonitrile, methanol and potassium

dihydrogen phosphate buffer (pH 4.0) in the ratio of 50:20:30 (v/v) because it could resolve the peaks of Perindopril with retention time at 2.5 min and Amlodipine retention time at 5.3 min. The two peaks were symmetric and sufficiently resolved. System suitability was carried out by injecting 5 replicate injections of 100% concentration of Perindopril and Amlodipine. The resolution was found to be greater than 2 and the other parameters are presented in Table 1.

Specificity of the chromatographic method was tested by injecting mobile phase as blank and sample concentration prepared from marketed formulation. The response was compared with that obtained from the standard drug. The chromatogram confirms the presence of Perindopril and Amlodipine at 2.5 min and 5.3 min respectively without any interference. Thus the developed method was specific for analyzing the commercial formulations for Perindopril and Amlodipine. An optimized chromatogram with the retention times of Perindopril and Amlodipine was shown in the Figure 2.

The peak areas corresponding to the concentration range of Perindopril 48-112 µg/mL and Amlodipine 3-7 µg/ml prepared in triplicate were plotted against the respective concentrations. The calibration curves were linear in the range studied for Perindopril and Amlodipine, respectively, with mean correlation coefficients (n=3) of 0.999 and higher, the representative calibration curve is shown in Figure3. The regression analysis was given in Table 2.

Accuracy of the proposed method was assessed by standard addition method at 80%, 100% and 120% levels of recovery to the pre analyzed sample in triplicate. The recovery of the added standard to the sample was calculated and it was found to be 98.9-100.4 %w/w for Perindopril and 98.9-100.7 %w/w for Amlodipine respectively and the % RSD was less than 2 for both the drugs which indicates good accuracy of the method. The result of recovery was given in table 3.

LOD and LOQ were calculated from the average slope and standard deviation of y intercepts of the calibration curve. Limit of detection for Perindopril and Amlodipine were 3.03 µg/mL and 11.17 µg/mL respectively where as limit of quantitation of Perindopril and Amlodipine were 9.17 µg/mL and 33.85 µg/mL respectively indicating high sensitivity of the method. LOD and LOQ value was given in table 2. The method is precise with a %RSD of less than 2 for both Perindopril and Amlodipine respectively. The results of intraday and inter day precision was given in table 4. Robustness was carried out by change in the flow rate (±1mL/min), mobile phase variation (±5%) and variation in wavelength (± 2 nm).Solution of 100% concentration is prepared and injected in triplicate for each varied operational condition and % R.S.D was found to be less than 2. The result was given in table 5. The proposed method was applied for the assay of commercial formulation containing Perindopril and Amlodipine. Each sample was analyzed in triplicate. The mean recovery values were 101.5 and 98.4 for Perindopril and Amlodipine. The result of estimation was given in table 6.

CONCLUSION

The proposed RP-HPLC method for simultaneous assay Perindopril and Amlodipine in combined dosage forms was validated, and found to be applicable for routine quantitative analysis of Perindopril and Amlodipine. The results of linearity, precision, accuracy and specificity, were proved to be within the limits. The method provides selective quantification of Perindopril and Amlodipine with no interference from other formulation excipients. Therefore, this method can be employed for the routine analysis for simultaneous estimation Perindopril and

Amlodipine in quality control of formulations and also in the dissolution studies.

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Table 1. System suitability parameters for perindopril and amlodipine by proposed method

Name of the compound	Retention time	Tailing factor	Theoretical plate	USP resolution
Perindopril	2.840	1.792	3693	-
Amlodipine	4.970	1.639	5575	9.400

Table 2. Linearity studies for perindopril and amlodipine by proposed method

% Level (Approx.)	For amlodipine		For perindopril	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
60	60	1921.349	48	827.584
80	80	2603.317	64	1096.62
100	100	3309.843	80	1385.935
120	120	3888.959	96	1658.886
140	140	4512.575	112	1910.699
	Slope	32	Slope	17
	Intercept	13	Intercept	12
	% Y-Intercept	40.6	% Y-Intercept	0.7
	STYEX	44	STYEX	11
	CC	0.9993	CC	0.9998
	RSQ	0.9986	RSQ	0.9995
	Residual sum of squares	44	Residual sum of squares	11
	LLD	11.17	LLD	3.03
	LLQ	33.85	LLQ	9.17

Table 3A. Recovery studies for perindopril by proposed method

% Level	Recovery range	% RSD at each level	Over all % RSD
80	98.9-99.2	0.30	0.48
100	99.3-99.7	0.21	
120	99.6-100.4	0.41	

Table 3B. Recovery studies for amlodipine by proposed method

% Level	Recovery range	% RSD at each level	Over all % RSD
80	98.9-99.7	0.42	0.59
100	100.2-100.7	0.25	
120	99.1-99.9	0.41	

Table 4. Method precision (inter and intraday) studies for perindopril and amlodipine by proposed method

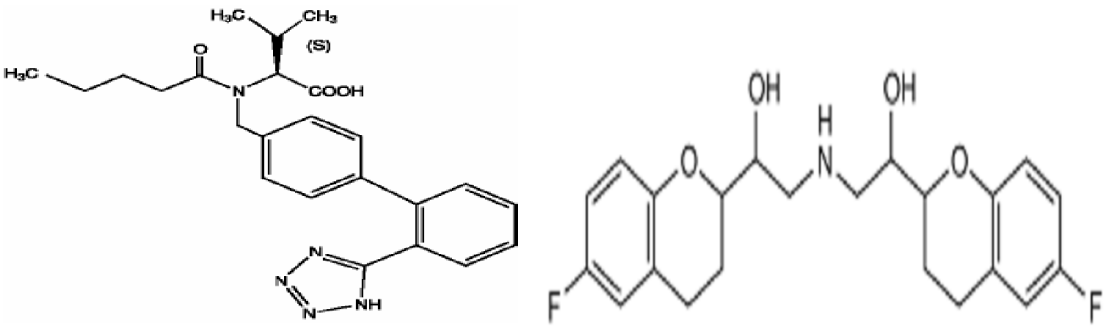
Summary showing method Precision by proposed method			
For perindopril		For amlodipine	
Method precision (Inter &Intra Day)		Method precision (Inter &Intra DAY)	
99.6	100.1	99.4	98.6
99.7	99.9	99.1	99.4
98.9	99.6	99.8	99.1
99.6	99.4	99.2	100.5
99.9	99.7	98.4	100.1
100.2	98.7	98.6	98.7
Overall avg.	99.61		99.24
Overage Std dev.	0.44		0.64
Over all % RSD	0.44		0.65

Table 5. Robustness studies for perindopril and amlodipine by proposed method

Parameter		% RSD	
		Perindopril	Amlodipine
Wavelength ±2	224 nm	0.32	0.34
	228 nm	0.36	0.78
Flow Rate mL /min	0.8 mL/min	0.52	0.39
	1.2mL.min	0.48	0.81

Table 6. Assay of marketed samples for perindopril and amlodipine by proposed method

Drug	Amount claimed in mg per tablet	Estimated amount in mg/tablet	% Assay
Perindopril	4	4.06	101.5
Amlodipine	5	4.92	98.4



(A) **(B)**

Figure 1. (A) Amlodipine, (B) Perindopril HCl

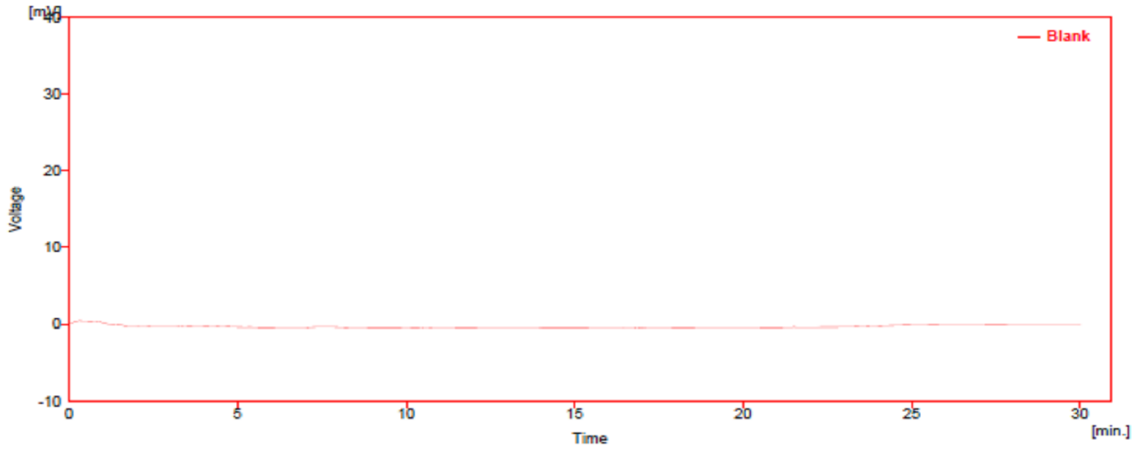


Figure 2. A typical HPLC chromatogram showing the no interference of diluent for perindopril and amlodipine

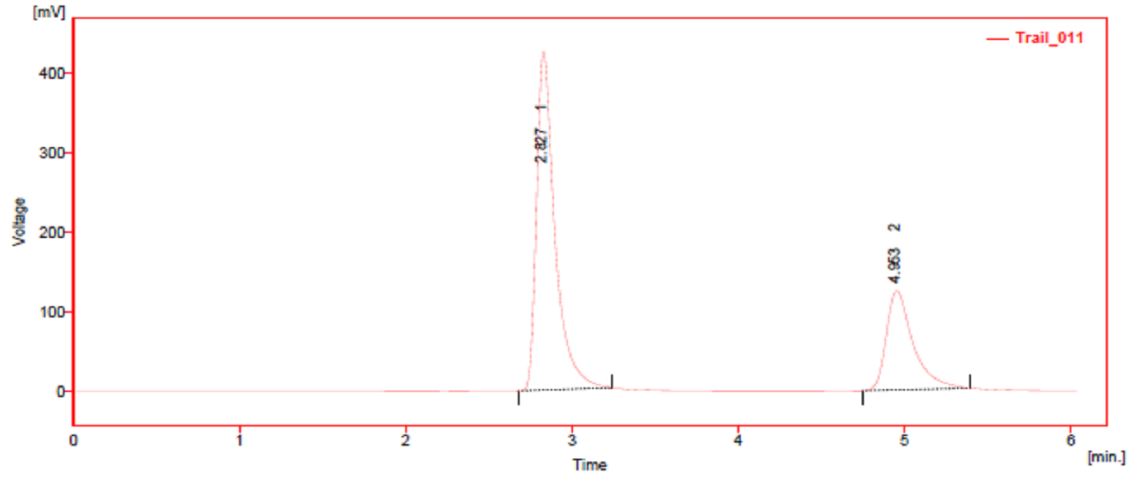


Figure 3. A typical HPLC chromatogram showing the peak of perindopril and amlodipine

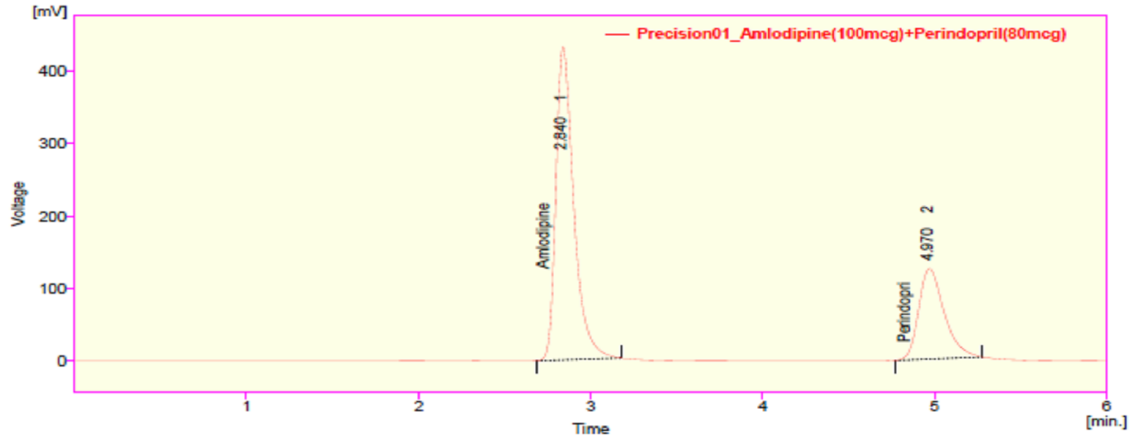


Figure 4A. A typical HPLC chromatogram showing the control sample profile of perindopril and amlodipine by proposed method

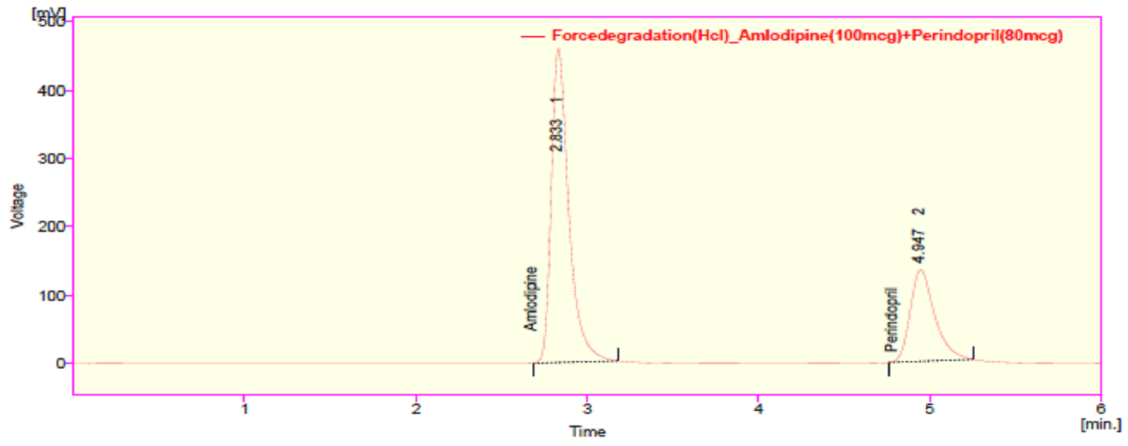


Figure 4B. A typical HPLC chromatogram showing the profile of perindopril and amlodipine in acidic hydrolysis by proposed method

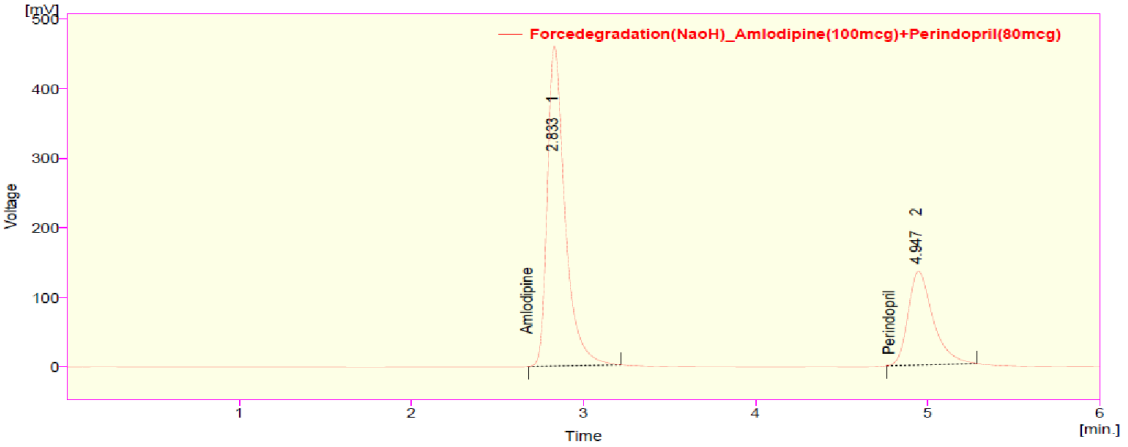


Figure 4C. A typical HPLC chromatogram showing the profile of perindopril and amlodipine in base hydrolysis by proposed method

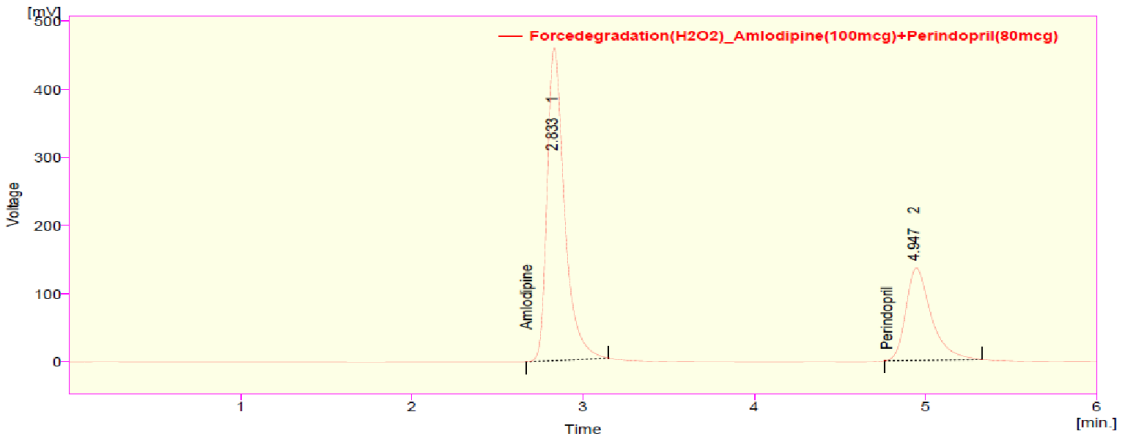


Figure 4D. A typical HPLC chromatogram showing the profile of perindopril and amlodipine in peroxide hydrolysis by proposed method

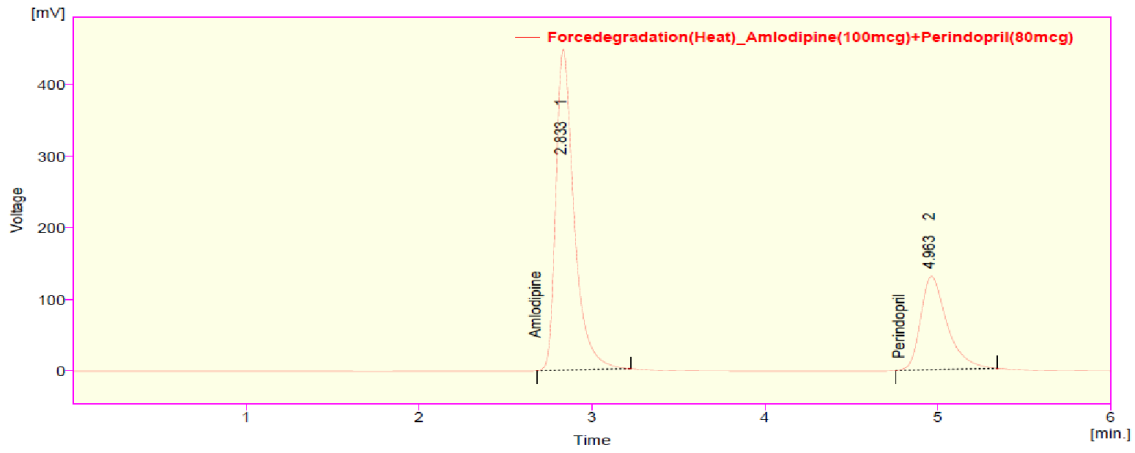


Figure 4E. A typical HPLC chromatogram showing the profile of perindopril and amlodipine in thermal hydrolysis by proposed method

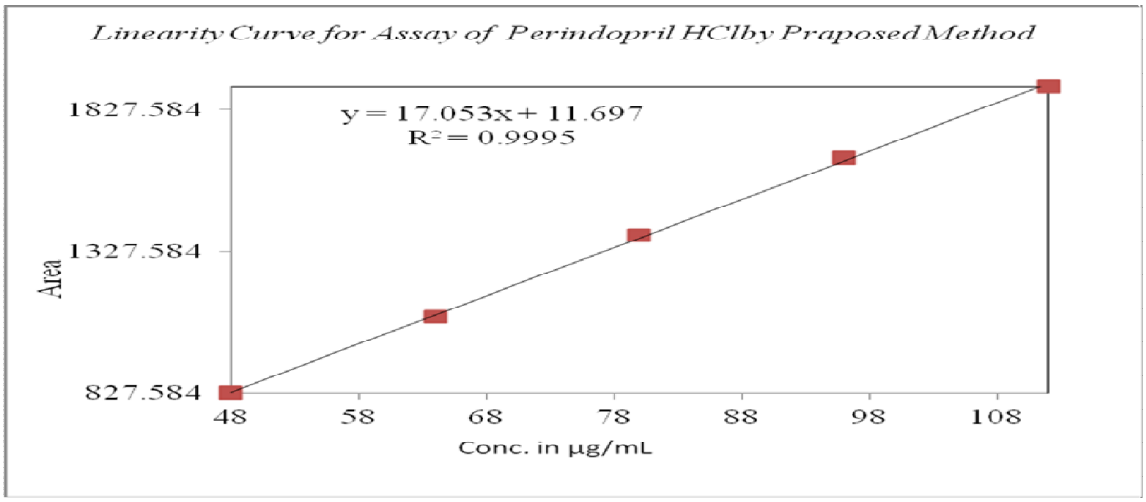


Figure 5A. Calibration curve for perindopril HCl

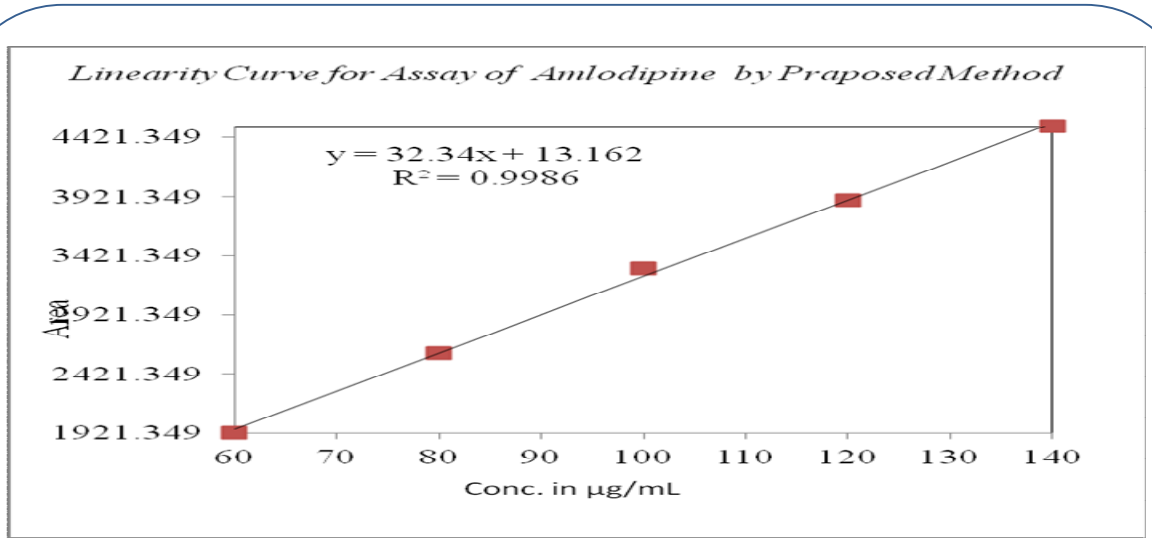


Figure 5B. Calibration curve for amlodipine