CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

Available online at: http://www.iajps.com Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF AMLODIPINE AND PERINDOPRIL IN PURE AND PHARMACEUTICAL TABLET DOSAGE FORM BY USING RP-HPLC

Kantlam Chamakuri*[1], Suchitra Cheekuri[2], Manasa Sikulammetla[3], Kalpana Bommagoni[4]

[1]Associate Professor, Dept. of Pharmaceutical Analysis & Q.A., Netaji Institute of Pharmaceutical Sciences, Toopranpet, Choutuppal, Nalgonda-508252, Telangana state, India

[3]Assistant Professor, Dept. of Pharmaceutical Analysis & Q.A., Netaji Institute of Pharmaceutical Sciences, Toopranpet, Choutuppal, Nalgonda-508252, Telangana state, India

[2,4]M.Pharmacy, Dept. of Pharmaceutical Analysis & Q.A., Netaji Institute of Pharmaceutical Sciences, Toopranpet, Choutuppal, Nalgonda-508252, Telangana state, India.

Article Received: December 2019 **Accepted:** December 2019 **Published:** December 2019

Abstract:

The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Amlodipine and Perindopril in tablet dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statistically according to ICH guidelines. The Sample recoveries in all formulations were in good agreement with their respective label claims. From literature review and solubility analysis initial chromatographic conditions Mobile phase ortho phosphoric acid buffer: Methanol 65:35 were set (Buffer PH 2.45 adjusted with Triethylamine), Kromosil C 18 (250×4.6mm, 5µ) Column, Flow rate 1.0 ml/min and temperature was ambient, eluent was scanned with PDA detector in system and it showed maximum absorbance at 254 nm. As the methanol content was increased Amlodipine and Perindopril got eluted with good peak symmetric properties. The retention times for Amlodipine and Perindopril was found to be 2.589 min and 3.711 min respectively. System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria. Linearity study was carried out between 50% to150 % levels, R2 value was found to be as 0.999. By using above method assay of marketed formulation was carried out, 100.7% was present. Full length method was not performed; if it is done this method can be used for routine analysis of Amlodipine and Perindopril.

KEYWORDS: Kromosil C 18, Amlodipine and Perindopril, HPLC.

Corresponding author:

Dr. Kantlam Chamakuri,

Associate Professor, Dept. of Pharmaceutical Analysis & Q.A.,
Netaji Institute of Pharmaceutical Sciences,
Toopranpet, Choutuppal, Nalgonda-508252.
Telangana state, India.
E Mail: kantlam3@gmail.com.

QR code

Please cite this article in press Kantlam Chamakuri et al., ***Analytical Method Development And Validation For The Simultaneous Estimation Of Amlodipine And Perindopril In Pure And Pharmaceutical Tablet Dosage Form By Using RP-HPLC.,*** *Indo Am. J. P. Sci, 2019; 06[12].*

1.INTRODUCTION:

Pharmaceutical analysis shows a significant role within the quality assurance as well as control of bulk medication. Analytical chemistry involves separating, unique identifying, and decisive the relative amounts of parts in the sample matrix. Pharmaceutical analysis is a specialized branch of analytical chemistry. Pharmaceutical analysis derives its principles as of varied branches of sciences like physics, microbiology, nuclear science, and electronics etc. Qualitative examination reveals the chemical uniqueness of the sample. Quantitative analyses establish the relative amount of 1 or a lot of those species or analytes in numerical terms. Qualitative analysis is required before a quantitative analysis can be undertaken. A separation step is sometimes a essential a part of each a qualitative and measurement. The results of typical quantitative analysis can computed from two measurements. One is the mass or volume of sample to be analyzed and second is the measurement of some quantity that is proportional to the amount of analyte in that sample and normally completes the analysis.

The methods of estimation of drugs are divided into physical, chemical, physicochemical and biological ones. Of them, physical and physicochemical methods are used mostly. Physical methods of analysis involve the studying of the physical properties of a substance. They include determination of the solubility, transparency or degree of turbidity, colour density or specific gravity (for liquids), moisture content, melting, freezing and boiling points.

Physicochemical methods are used to study the physical phenomenon's that occur as a result of chemical reactions. Among the physicochemical methods are optical refractometry, polarimetry, emission and fluorescent methods of analysis, photometry including photo colorimetry, spectrophotometry, nephelometry and turbidimetry electrochemical (potentiometry, amperometry, coulometry, voltametry, polarography) and chromatography (column, paper, thin layer, gas-liquid, high performance liquid chromatography) methods are generally preferable. Methods involving nuclear reactions such as nuclear magnetic resonance (NMR) and paramagnetic resonance (PMR) are becoming more and more popular. The chemical methods include the gravimetric and volumetric procedures, which are based on complex formation, acid-base, precipitation and redox reactions. Titrations in non-aqueous media and complexometry have been widely used in pharmaceutical analysis whenever the existing amounts are in milligram level and the interferences are negligible. The methods (HPLC, GLC, NMR and Mass spectroscopy) of choice for assay involve sophisticated equipment that are very costly and pose problems of maintenance. Hence they are not in the reach of most laboratories and small-scale industries, which produce bulk drugs and pharmaceutical formulations. However this sophisticated equipment usage eliminate the difficulties encountered in the determination of minute amounts of degradation products or the analysis of the metabolites of drugs in body fluids.

Advances in both chemistry and technology are making new techniques available and expanding the use of existing ones. Photo acoustic spectroscopy is an example of an emerging analytical technique. A number of existing techniques have been combined to expand the utility of the component methods. Gas chromatography-mass spectrometry (GC-MS), (ICP-MS) and Gas chromatography-infrared spectroscopy (GC-IR) are examples of successful hyphenated methods.

2. METERIALS AND METHODS:

2.1. METERIALS

The marketed formulation is Midazole Tablet (Chemida lab Pvt Ltd) containing, Amlodipine – 400 mg and Perindopril - 150 mg , which were purchased From Local Pharmacy shop. HPLC Grade chemicals and solvents, Methanol, Ortho phosphoric acid, Potassium dihydrogen ortho phosphate and Tri ethyl amine, were purchased from Merck laboratories. The instruments and Equipments are Shimadzu UFLC-20 AD Chromatographic system (japan), Analytical HPLC isocratic pump, SPD-M20A diode array detector, LC 20 software, Kromosil (250×4.6mm, 5µ) ODS C-18 RP-column and Rheodyne injector with 20µ capacity, which were use for performing the experiments.

2.2. METHOD DEVELOPMENT AND OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS

Solubility

Amlodipine and Perindopril are sparingly soluble in Methanol and Acetonitrile.

Table.1. Solubility of Amlodipine and Perindopril

Drugs	Solvents				
	Water	Methanol	Acetonitrile	0.1N NaOH	Buffer:Methanol:65:35
AMLODIPINE	-	+	+	+	+
PERINDOPRIL	-	+	+	-	+

[Whereas (+) indicates solubility, (-) indicates insolubility

Selection of chromatographic condition

Proper selection of the method depends upon the nature of the sample, its molecular weight and solubility. The drugs selected in the present study are polar in nature and hence reversed phase or ion-pair or ion exchange chromatography method may be used. The reversed phase HPLC was selected for the separation because of its simplicity and suitability.

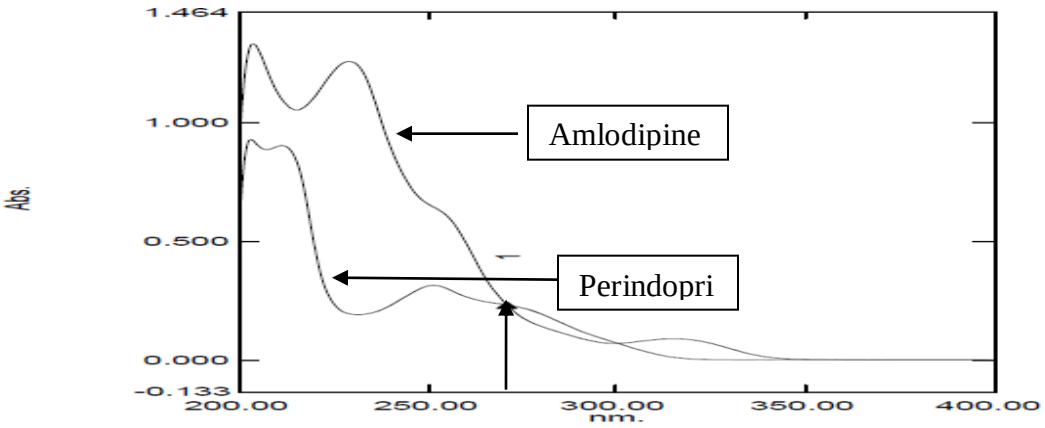
of detection wavelength:

.

The sensitivity of method that uses UV- Vis detector depends upon the proper selection of wavelength. An ideal wavelength is that gives maximum absorbance and good response for both the drugs to be detected.

Standard solutions of Amlodipine and Perindopril were scanned in the UV range (200-400nm) and the spectrums obtained were overlaid and the overlain spectrum was recorded. From the overlain spectrum, 254 nm was selected as the detection wavelength for the present study

Figure 1. Over line Spectrum of Amlodipine and Perindopril



Selection of mobile phase:

Initially the mobile phase tried was methanol and water, methanol and Methanol, buffer and water in various proportions. Finally, the mobile phase was optimized to Buffer: Methanol in proportion 65:35 v/v respectively.

Optimization of flowrate

The method was performed with flow rates 0.8ml, 1.5ml and 1ml/min. Flowrate of 1ml/min was found to be ideal as it gave sharp peak.

Based on the above study, the following chromatographic conditions were selected for the simultaneous estimation of drugs in multi component dosage forms

3. RESULTS AND DISCUSSION:

Trial-1

Table 2. Chromatographic condition

Parameters	Description
Flow rate	1ml min^{-1}
Column	Chromosil C_{18} Column(250mm x 4.6mm)5µ
Mobile Phase	Buffer: Methanol P^H 2.5 (30:70 v/v)
Buffer	Potassium dihydrogen orthophosphate PH 2.5 adjust with Orthophosphoric acid

Detector	PDA
Column temperature	Ambient
Wavelength	254 nm
Type of elution	Isocratic
Injection volume	20µl
Run time	10min

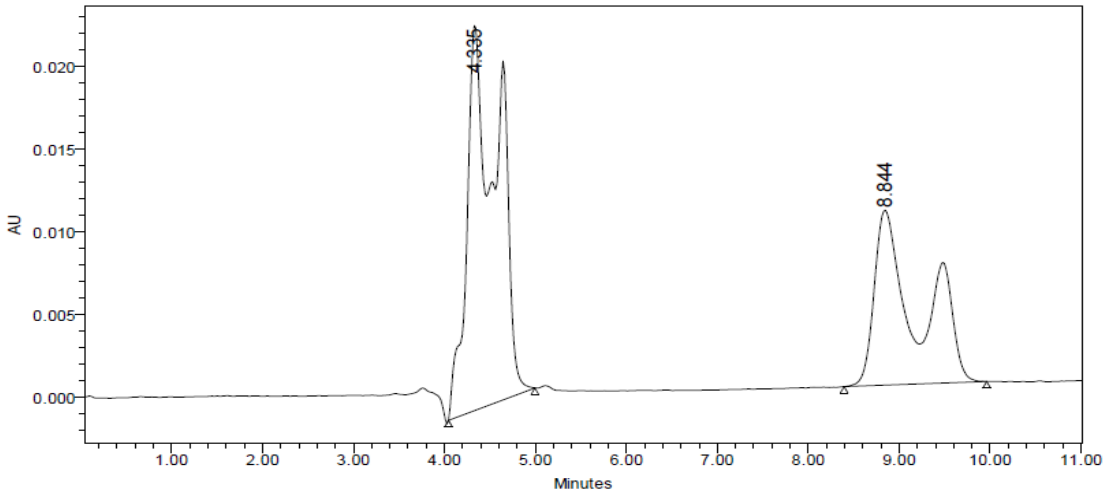


Fig.2 Chromatogram of Trial-1

Observation: The separation of two analytical peaks was not proper, so the mobile phase ratio has been changed for next trial.

Trial-2

Table 3. Chromatographic condition

Parameters	**Description**
Flow rate	1ml min^{-1}
Column	Chromosil C_{18} Column (250mm x 4.6mm)5µg.
Mobile Phase	Buffer: Methanol P^H 2.5 (30:70 v/v)
Buffer	Potassium dihydrogen orthophosphate ph2.5 adjusted with Orthophosphoric acid
Detector	PDA
Column temperature	Ambient
Type of elution	Isocratic
Wavelength	254nm
Injection volume	20µl

Run time	10min

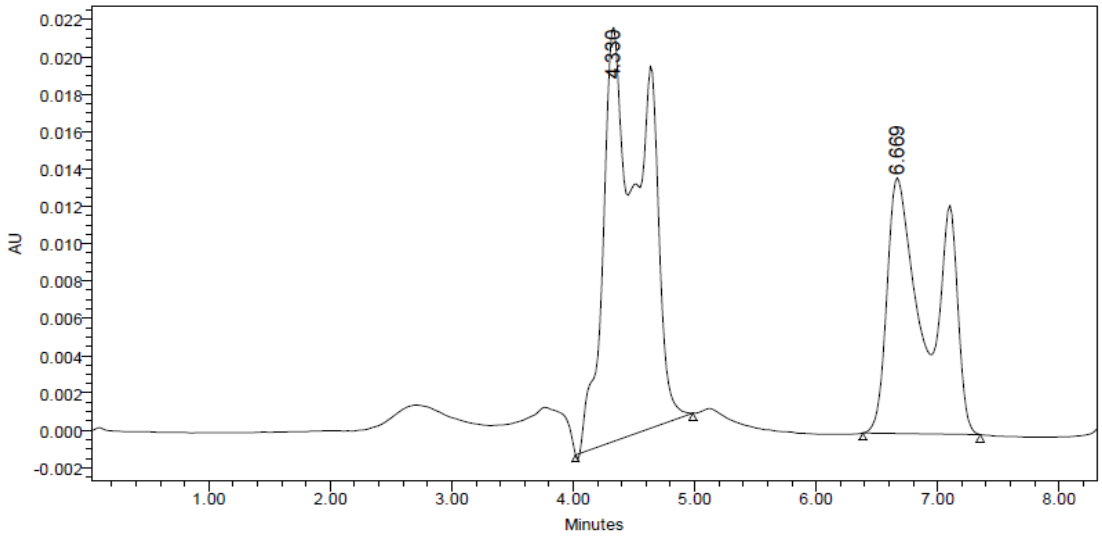


Fig.3 Chromatogram of Trial-2

Observation: The separation of two analytical peaks was not proper, so the mobile phase ratio has been changed for next trial.

Trial-3

Table 4. Chromatographic condition

Parameters	**Description**
Flow rate	1ml min^{-1}
Column	chromosil C_{18} Column (250mm x 4.6mm)5µg.
Mobile Phase	Buffer: Methanol P^H 2.5 (60:40 v/v)
Buffer	Potassium dihydrogen orthophosphate PH 2.5 adjusted with OPA
Detector	PDA
Column temperature	Ambient
Type of elution	Isocratic
Wavelength	254 nm
Injection volume	20µl
Run time	10min

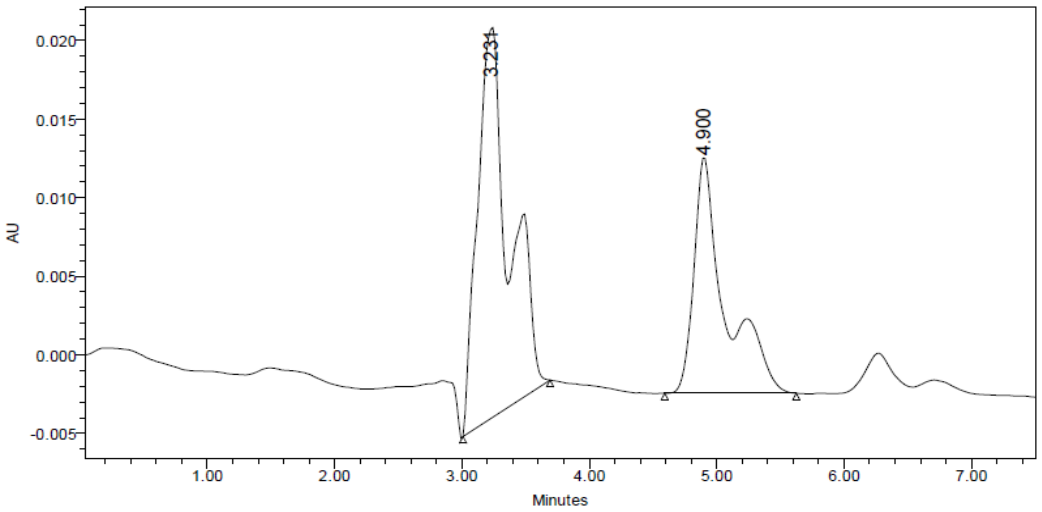


Fig 4. Chromatogram of Trial-3

Observation: The separation of two analytical peaks was not proper, so the mobile phase ratio has been changed for next trial.

Trial-4

Table 5.Chromatographic condition

Parameters	**Description**
Flow rate	1ml min^{-1}
Column	Chromosil C_{18} Column (250mm x 4.6mm)5µg.
Mobile Phase	Phosphate buffer: Methanol P^H 2.5 (20:80 v/v)
Buffer	Potassium dihydrogen orthophosphate PH 2.5 adjust with orthophosphoric acid
Detector	PDA
Column temperature	Ambient
Type of elution	Isocratic
Wavelength	254 nm
Injection volume	20µl
Run time	10min

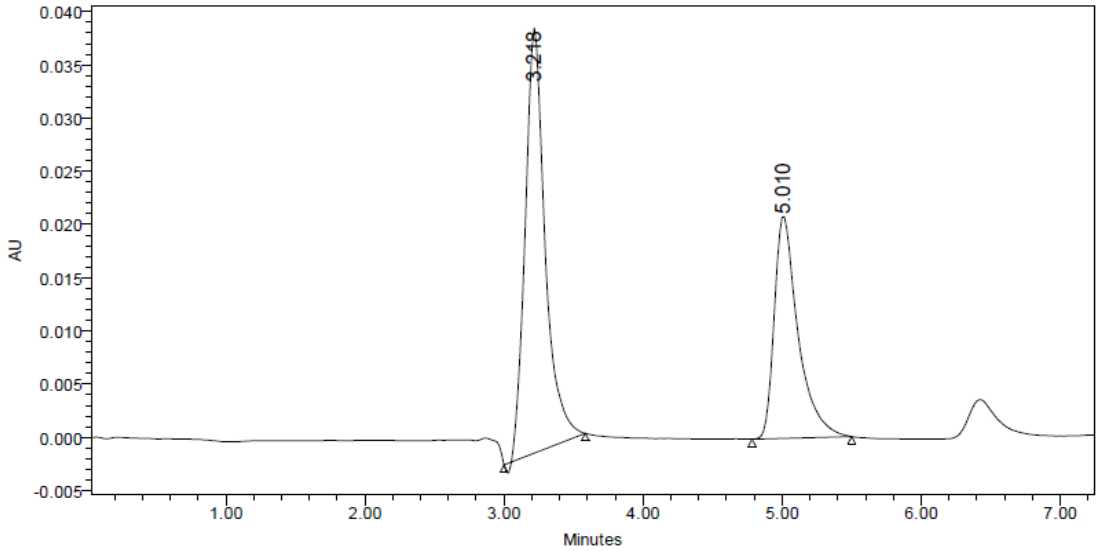


Fig 5. Chromatogram of Trial-4

Observation: The separation of two analytical peaks is occurred but fronting occurs in Amlodipine peak.

Trial-5

Table 6. Chromatographic condition

Parameters	**Description**
Flow rate	1ml min^{-1}
Column	Chromosil C_{18} Column (250mm x 4.6mm)5µg.
Mobile Phase	Phosphate buffer: Methanol P^H 2.5 (55:45 v/v)
Buffer	Potassium dihydrogen orthophosphate PH 2.5 adjust with Orthophosphoric acid
Detector	PDA
Column temperature	Ambient
Type of elution	Isocratic
Wavelength	254 nm
Injection volume	20µl
Run time	10min

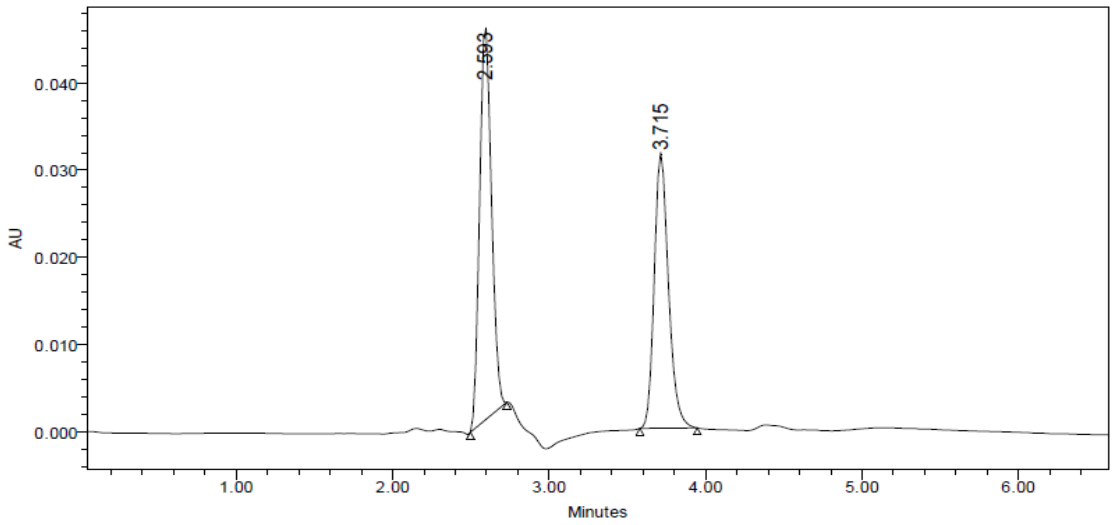


Fig 6. Chromatogram of Trial-5

Observation: The separation of two analytical peaks was good but base line noise is occurred. So the mobile phase ratio has been changed for next trial.

Trial-6

Table 7: Chromatographic condition

Parameters	**Description**
Flow rate	1ml min^{-1}
Column	kromosil C_{18} Column (250mm x 4.6mm)5µg.
Mobile Phase	Phosphate buffer:Methanol P^H 2.5 (65:35 v/v)
Buffer	Potassium dihydrogen orthophosphate PH 2.5 adjusted with Orthophosphoric acid
Detector	PDA
Column temperature	Ambient
Type of elution	Isocratic
Wavelength	254 nm
Injection volume	20µl
Run time	10min

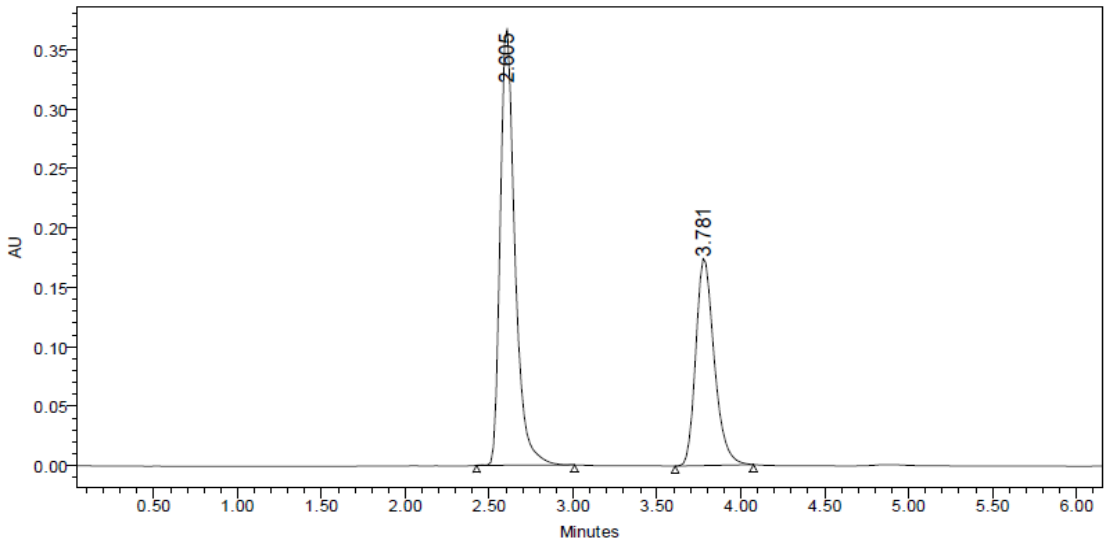


Fig 7. Chromatogram of Trail-6

Observation: The separation of two analytical peaks was good. The plate count also above 2000, tailing factor below 2, and the resolution is above 2. The condition is taken as optimized method.

OPTIMIZED METHOD

Preparation of Buffer:

About 7.0g of potassium dihydrogen orthophosphate was dissolved in 1000ml of HPLC grade water and pH 2.5 was adjusted with orthophosphoric acid. It was filtered through 0.45µm nylon membrane filter and degassed with sonicator. It was used as a diluent for the preparation of sample and standard solution.

Preparation of mobile phase:

Mobile phase consist of buffer: Methanol of P^H 2.5 (35:65) was taken sonicated and degassed for 10min and filtered through 0.45 µm nylon membrane filter

Standard Preparation:

Weigh accurately 10 mg Amlodipine Working Reference Standard and 15mg of Perindopril Working Reference Standard is taken in to 100ml volumetric flask and then it was dissolved and diluted to volume with mobile phase up to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase.(Stock solution) Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent. The chromatogram was shown in Table -8.

Parameters	**Description**
Flow rate	1ml min^{-1}
Column	Kromosil C_{18} Column (100mm x 4.6mm)5µg.
Mobile Phase	Methanol: Phosphate buffer P^H 2.5 (35:65 v/v)
Buffer	Potassium dihydrogen orthophosphate PH 2.5 adjusted with Orthophosphoric acid
Detector	PDA
Column temperature	Ambient
Type of elution	Isocratic
Wavelength	254 nm
Injection volume	20µl
Run time	10min

6.5. Assay

Preparation of samples for Assay

Standard preparation:

Weigh accurately 10mg Amlodipine Working Reference Standard and 15mg of Perindopril Working Reference Standard is taken in to 100ml volumetric flask and then it was dissolved and diluted to volume with mobile phase up to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase. (Stock solution) Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

Sample preparation:

10 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 10 tablets was transferred into a 100ml standard flask. A volume of 70ml of mobile phase was added and sonicate for 30min.Then the solution was cooled and diluted to volume with mobile phase and filtered through 0.45µm membrane filter. (Stock solution) Further pipette 0.25ml of Amlodipine and Perindopril of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

Assay procedure

20µL of the standard and sample solutions of Amlodipine and Perindopril were injected into the HPLC system and the chromatograms were recorded. Amount of drug present in the capsules were calculated using the peak areas.

Amount of drug in tablet was calculated using following formula :

$$\% \text{ Label claim} = \frac{Asp}{Ast} \times \frac{Dst}{Dsp} \times \frac{A}{Lc} \times P$$

Where,

Asp = Area for sample solution.

Ast = Area for standard solution.
Dst = Dilution factor for standard.
Dsp = Dilution factor for sample.
Lc = Label claim.
A = Average weight.
P = Potency

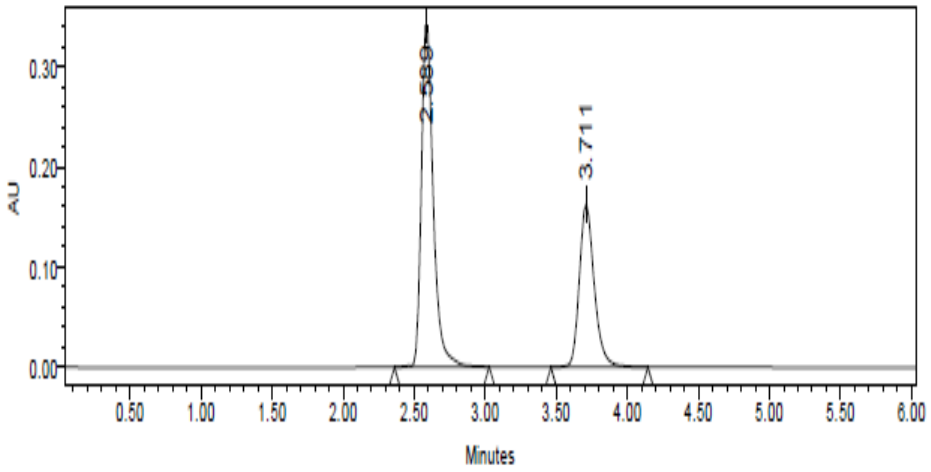


Fig 8. Chromatogram of standard

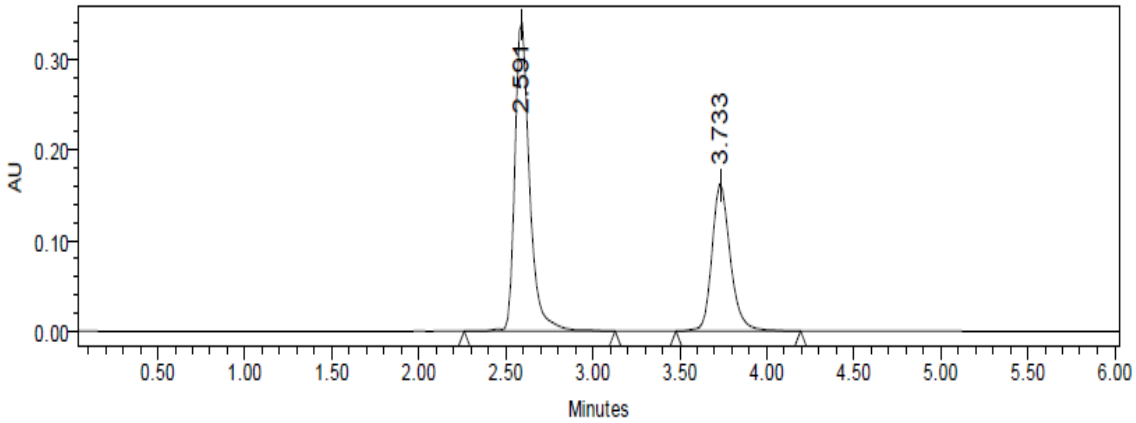


Fig 9. Chromatogram of Test

Table.9. Peak results of Standard & Test Chromatograms for Assay

Parameter	Standard		Test	
	Amlodipine	Perindopril	Amlodipine	Perindopril
Retention time	2.589	3.711	2.591	3.733
Peak Area	2008408	1185786	2005829	1189695
USP Plate Count	6167	6389	5752	7187
Tailing Factor	1.3	1.3	1.4	1.2
USP Resolution	-	6.6	-	9.3

Table 10: Results of Assay

Parameters	Amlodipine	Perindopril
Standard peak area	2008408	1185786
Test peak area (mean)	2005829	1189695
Average Weight	694.2mg	694.2mg
Label claim	400 mg	150 mg
% Purity of Standard	99.50	99.58
Amt obtained	399.88 mg	150.10 mg
% Assay	99.77%	100.12%

The % assays of Amlodipine and Perindopril were found to be 99.77% and 100.12% respectively. Thus, % Assay results were found to be within the limits i.e., 98-102% for both the drugs. Hence the developed method can be routinely used for the simultaneous estimation of Amlodipine and Perindopril in the marketed formulations.

VALIDATION:

Validation of an analytical method is the process to establish by laboratory studies that the performance characteristic of the method meets the requirements for the intended analytical application. Performance characteristics were expressed in terms of analytical parameters. After development of RP-HPLC method for estimation of Amlodipine and Perindopril, validation of the method was carried out according to ICH guidelines

The developed method was validated for the following parameters.

A. System suitability, B. Linearity, C. Specificity, D. Precision, E. Accuracy, F. LOD & LOQ, G. Robustness

SYSTEM SUITABILITY:

A Standard solution of Amlodipine and Perindopril working standard was prepared as per procedure and was injected five times into the HPLC system. The system suitability parameters were evaluated from standard Chromatograms obtained by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from five replicate injections.

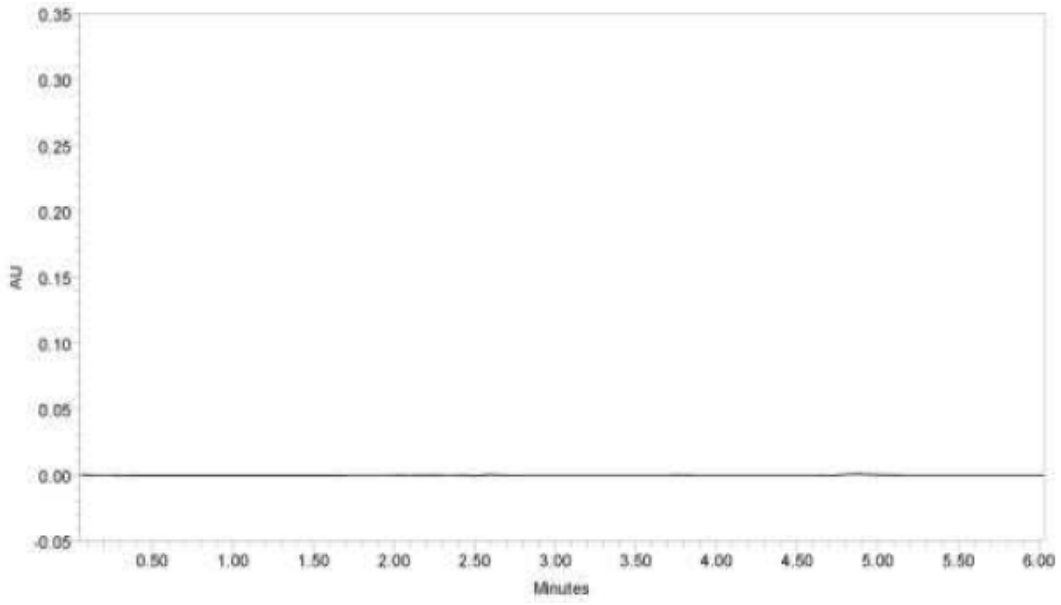


Figure 10. Chromatogram of Blank

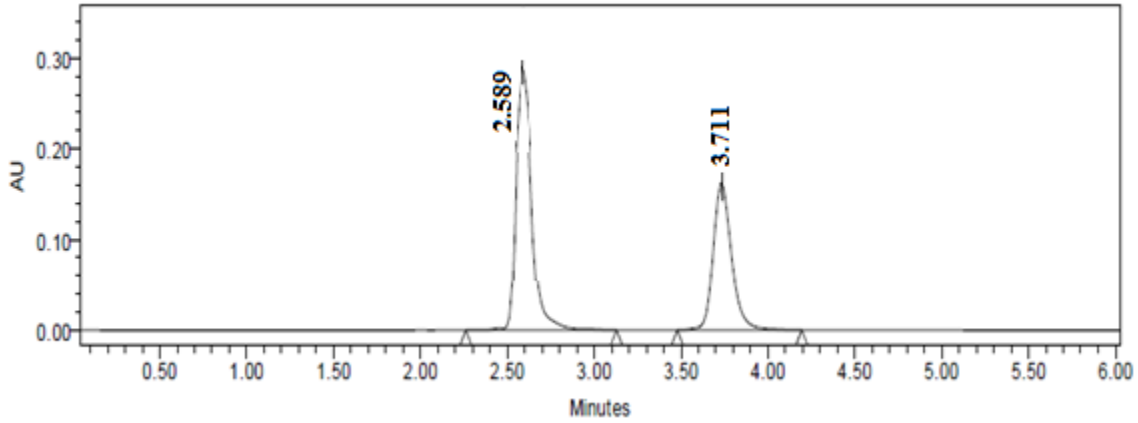
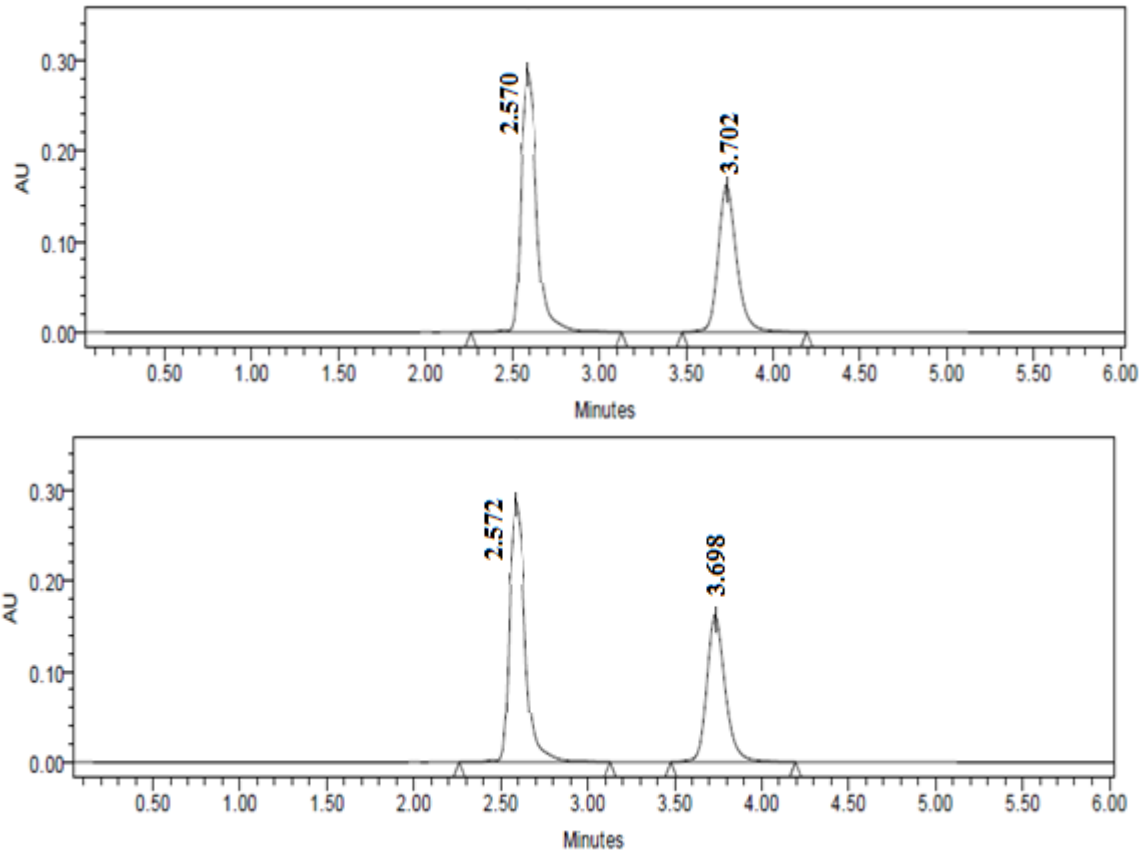


Figure 11. Chromatogram of system suitability 1 to 5



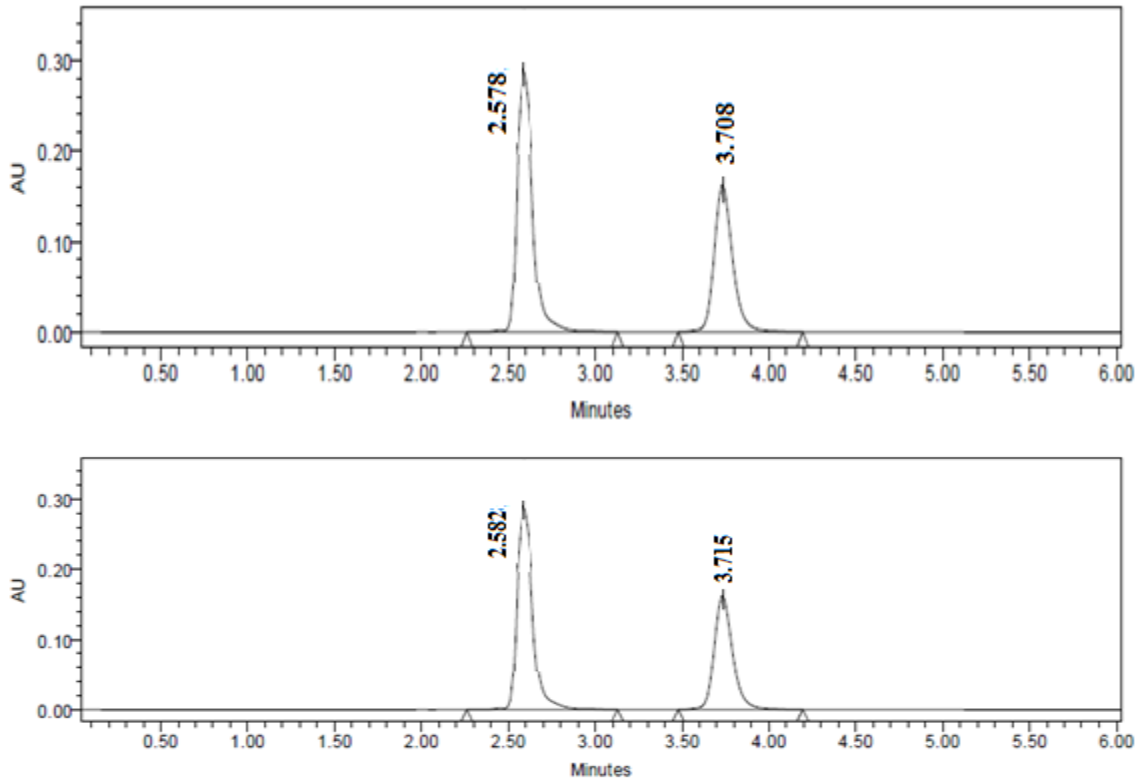


Table 11. Results of System suitability Test for Perindopril

Injection	Retention time (t_R)	Peak Area	Plate count	Tailing factor
1	3.711	1185786	6389	1.3
2	3.702	1184759	6455	1.3
3	3.698	1187496	6234	1.6
4	3.708	1190478	6478	1.3
5	3.715	1183897	6502	1.30
Mean	-	1186196	-	-
SD	-	2433.47	-	-
% RSD	-	0.20	-	-

Table 12 Results of System suitability Test for Amlodipine

Injection	Retention time (t_R)	Peak Area	Plate count	Tailing Factor
1	2.589	2008408	5752	1.4
2	2.570	2008412	5758	1.3
3	2.572	2008357	5672	1.2
4	2.578	2007478	5674	1.4
5	2.582	2008475	5749	1.3
Mean	-	2008249	-	-
SD	-	380.0	-	-
% RSD	-	0.01	-	-

Report:

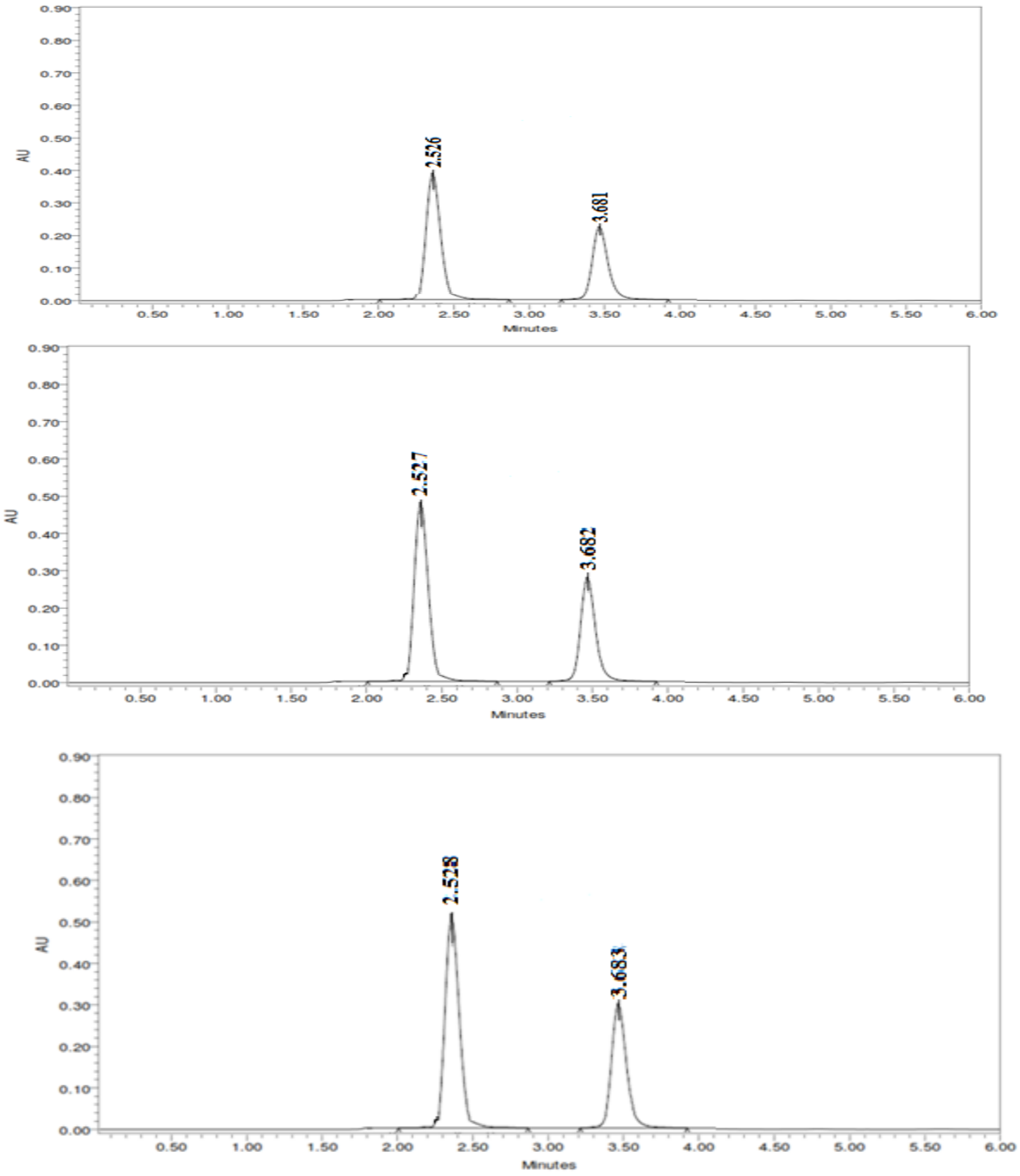
All the System suitability parameters were satisfied, thus the method passed the System suitability test.

LINEARITY:

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.

Serial dilutions of Amlodipine and Perindopril (20-60µg/ml and 10-30 µg/ml) were injected into the column and detected at a wavelength set at 254 nm. The calibration curve was obtained by plotting the concentration vs. peak area.

Fig 12. Linearity showing chromatograms 1 to 5



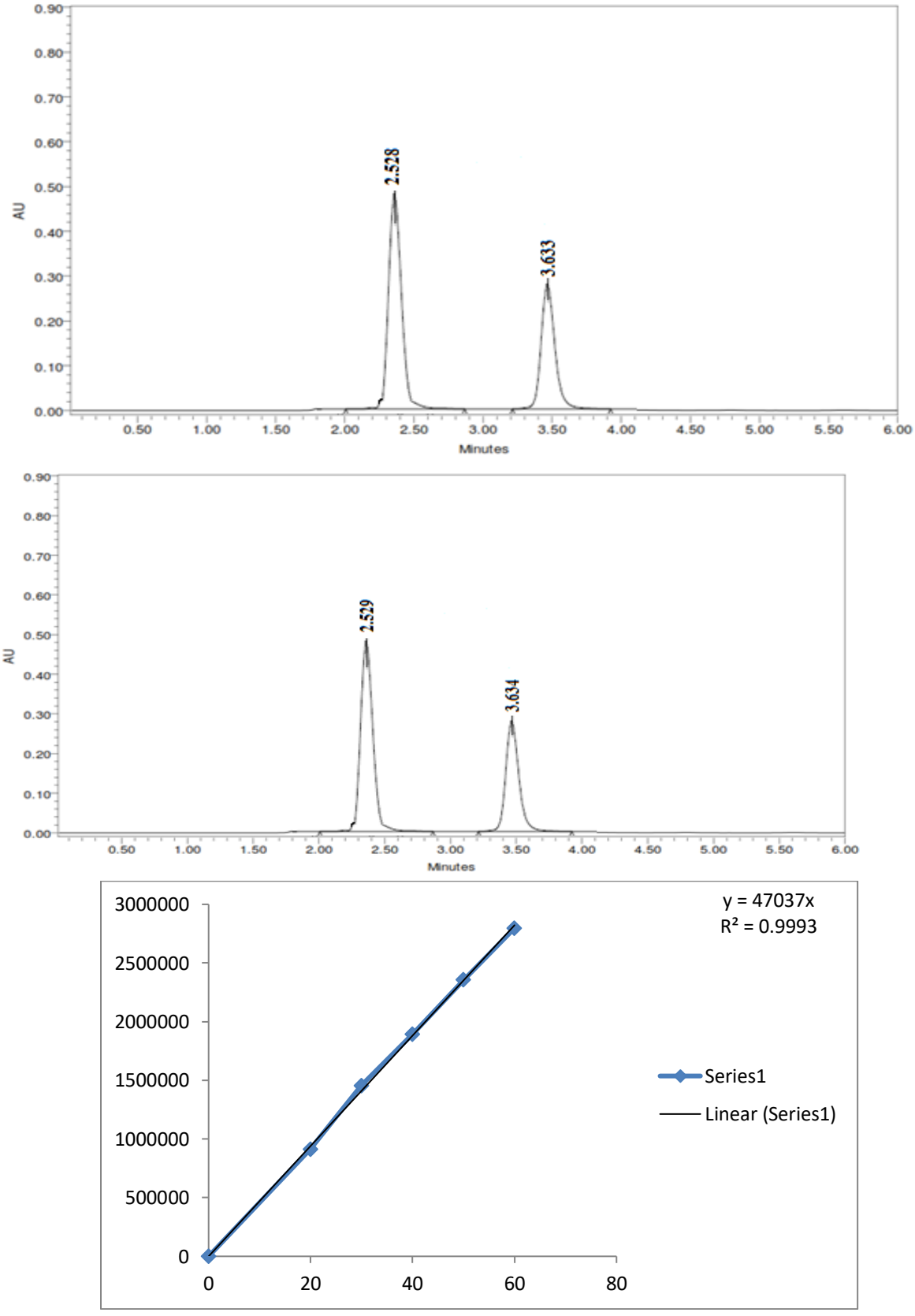


Figure 13. Linearity Graph of Perindopril

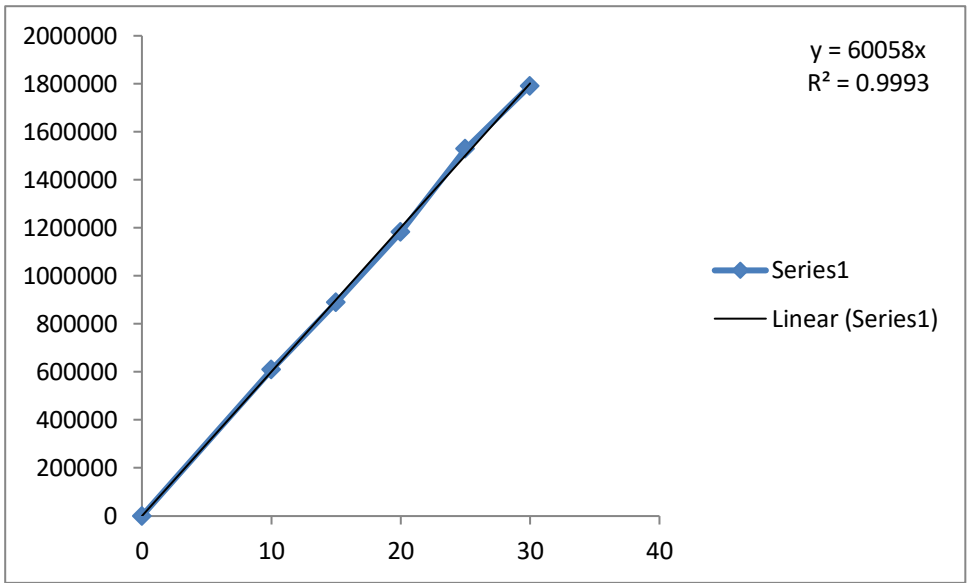


Figure 14. Linearity Graph of Amlodipine

Table 13. Preparation of Working standard solutions for Linearity

Sample ID	Perindopril		Amlodipine	
	Concentration (mcg/ml)	**Area**	**Concentration (mcg/ml)**	**Area**
20% of operating concentration	20	914140	10	610046
40% of operating concentration	30	1455681	15	890204
60% of operating concentration	**40***	**1892966**	**20***	**1183023**
80% of operating concentration	50	2356546	25	1529886
100% of operating concentration	60	2797214	30	1792302
Correlation Coefficient			0.999	

SPECIFICITY:

ICH defines specificity as "the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically this might include impurities, degradants, matrix, etc.

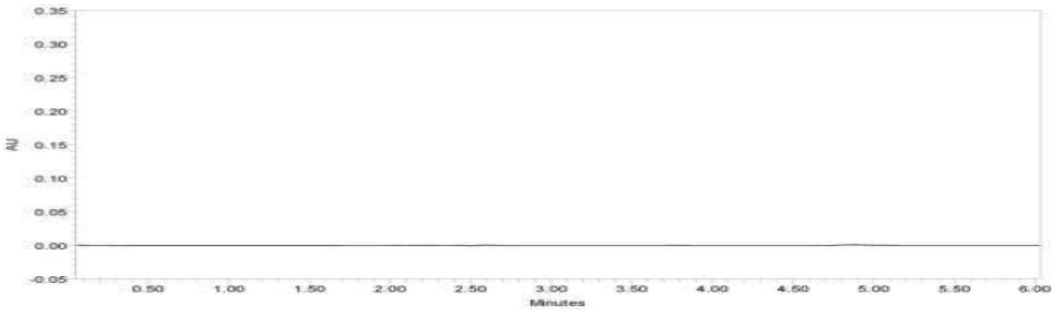


Figure 15. Chromatogram of Blank

PRECISION:

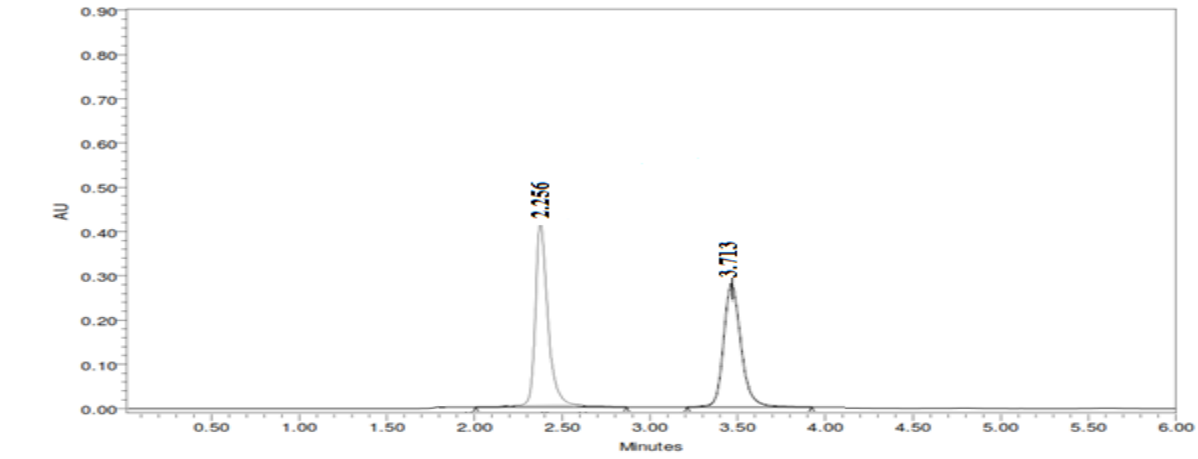
The precision of the method was demonstrated by intra-day and inter-day precision studies. Intra-day studies were performed by injecting three (3) repeated injections within a day. Peak area and %RSD were calculated and reported.

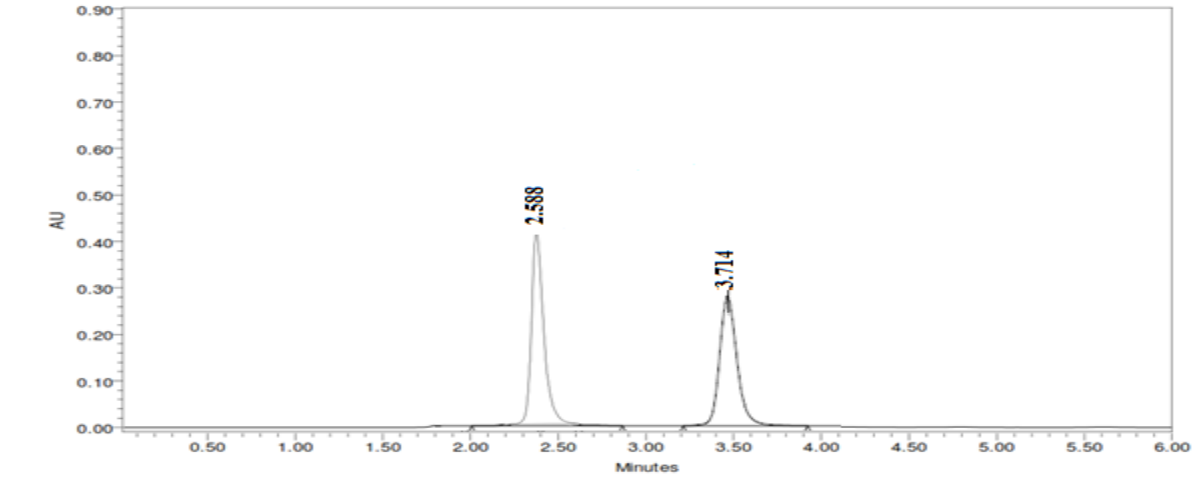
The chromatograms of intra-day precision studies were shown. Inter-day precision studies, was done by injecting three (3) repeated injections for three consecutive days. Peak area and %RSD were calculated and reported.

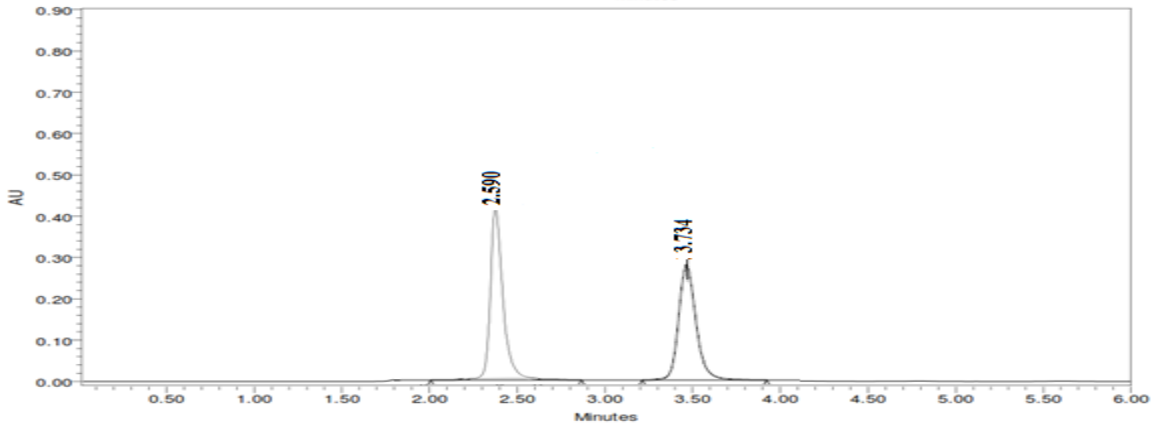
METHOD PRECISION:

Method precision also called as repeatability/Intra-day precision indicates whether a method gives consistent results for a single batch. Method precision was demonstrated by preparing six test solutions at 100% concentration as per the test procedure & recording the chromatograms of six test solutions.

The % RSD of peak areas of six samples was calculated. The method precision was performed on Amlodipine and Perindopril formulation. The % RSD of the assay value for six determinations should not be more than 2.0%.







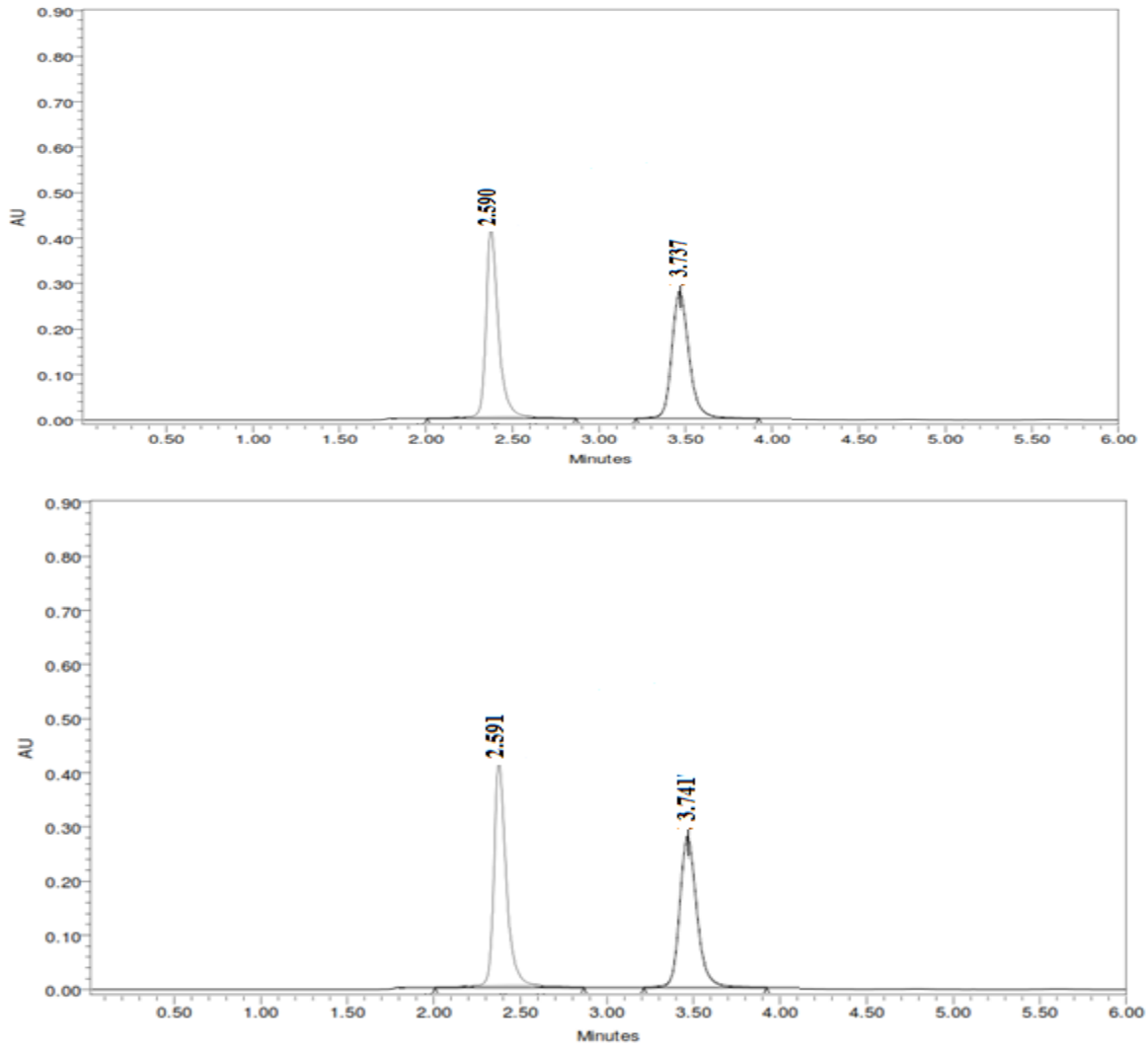


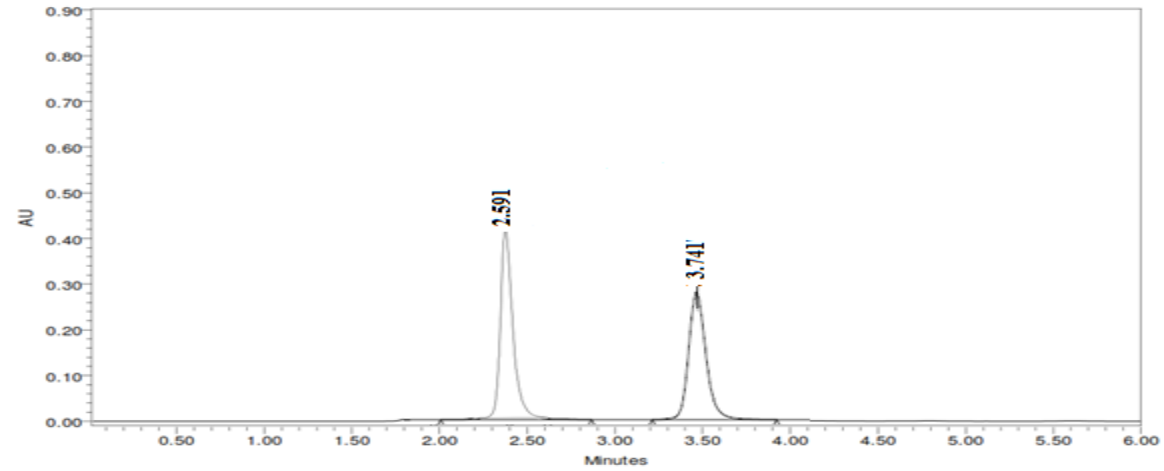
Fig 16. Chromatograms showing precision 1to 5

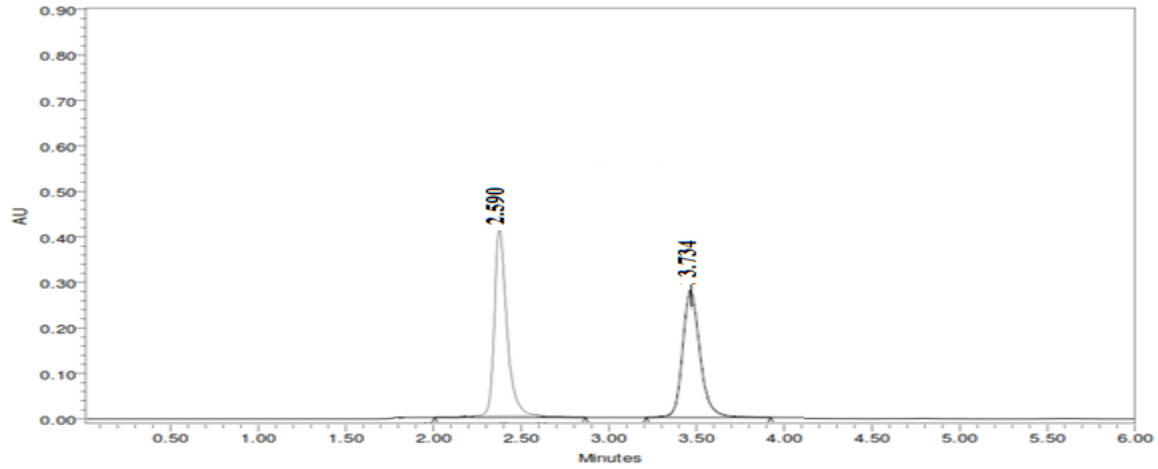
Table 14. Method Precision data for Amlodipine & Perindopril

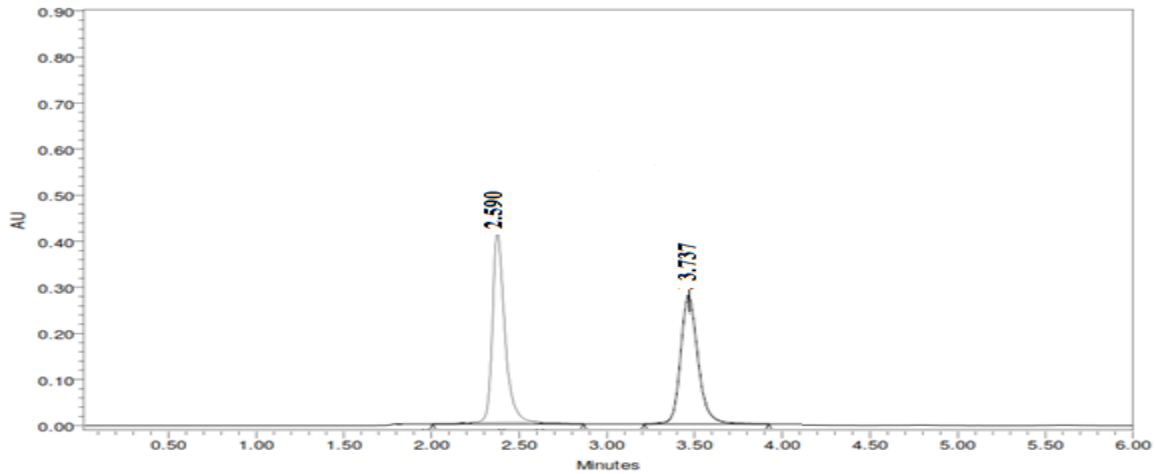
S.No.	Concentration (µg/ml)	Amlodipine		Perindopril	
		Retention time(Rt)	Peak Area	Retention time(Rt)	Peak Area
1	40 & 20	2.586	2010800	3.713	1184689
2	40 & 20	2.588	2002956	3.714	1188199
3	40 & 20	2.590	2012800	3.734	1195842
4	40 & 20	2.590	2005243	3.737	1184210
5	40 & 20	2.591	2011092	3.741	1198327
Avg			2008998		1191598
SD			3920.9		6668.5
%RSD			**0.19**		**0.55**

INTERMEDIATE PRECISION:

Intermediate precision of the analytical method was determined by performing method precision on another day by different analysts under same experimental condition. Assay of all six replicate sample preparations was determined and mean %assay value, standard deviation & %RSD was calculated.


AU
Minutes
2.591
3.741


AU
Minutes
2.590
3.734


AU
Minutes
2.590
3.737

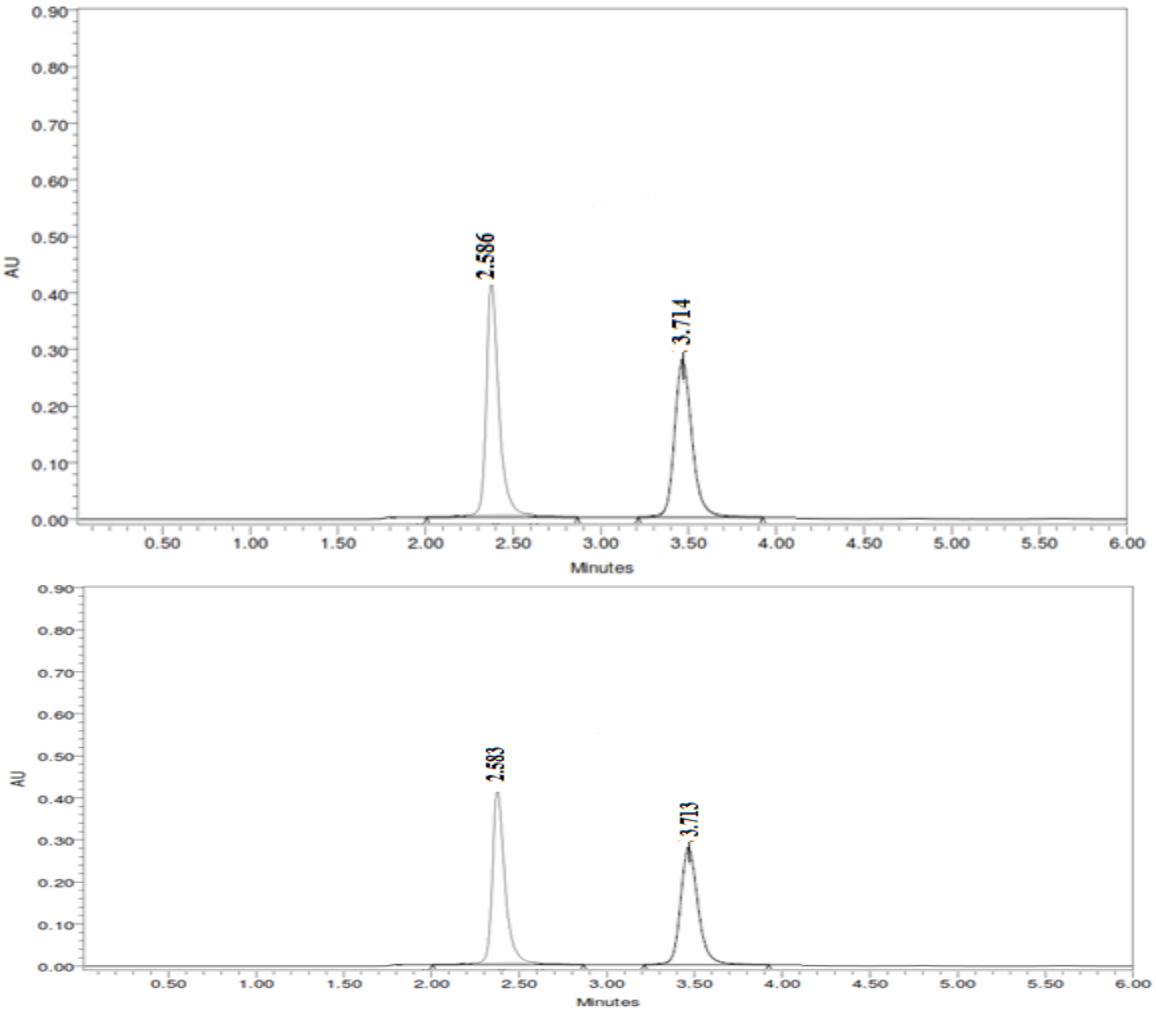


Fig 17.Chromatogram showing ID Precision 1 to 5

Table 15 Intermediate Precision data for Amlodipine and Perindopril

S.No.	Concentration (µg/ml)	Intermediate Precision			
		Day 1 Amlodipine		Day 1 Perindopril	
		Retention time	Peak Area	Retention time	Peak Area
1	40&20	2.591	2005053	3.741	1183951
2	40&20	2.590	2007362	3.734	1184689
3	40&20	2.590	2007473	3.737	1186232
4	40&20	2.586	2009153	3.714	1186406
5	40&20	2.583	2012800	3.713	1188564
Avg			2009104		1186244
SD			3140.6		1730.9
%RSD			**0.15**		**0.14**

Table 16 Intermediate Precision data for Amlodipine and Perindopril

S.No.	Concentration (µg/ml)	Day 2 Amlodipine		Day 2 Perindopril	
		Retention time(Rt)	Peak Area	Retention time(Rt)	Peak Area
1	40 & 20	2.586	2010800	3.713	1184689
2	40 & 20	2.588	2002956	3.714	1188199
3	40 & 20	2.590	2012800	3.734	1195842
4	40 & 20	2.590	2005243	3.737	1184210
5	40 & 20	2.591	2011092	3.741	1198327
6	40 & 20	2.589	2011098	3.740	1198320
Avg			2008998		1191598
SD			3920.9		6668.5
%RSD			**0.19**		**0.55**

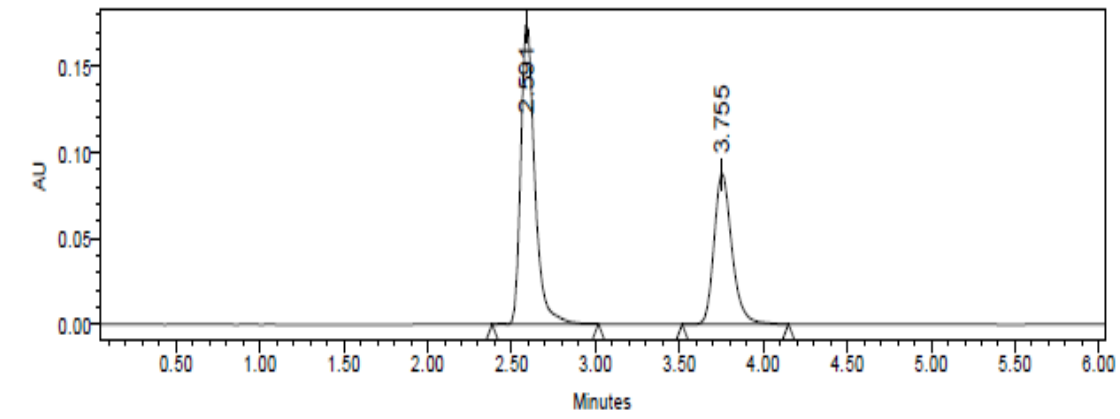
ACCURACY:

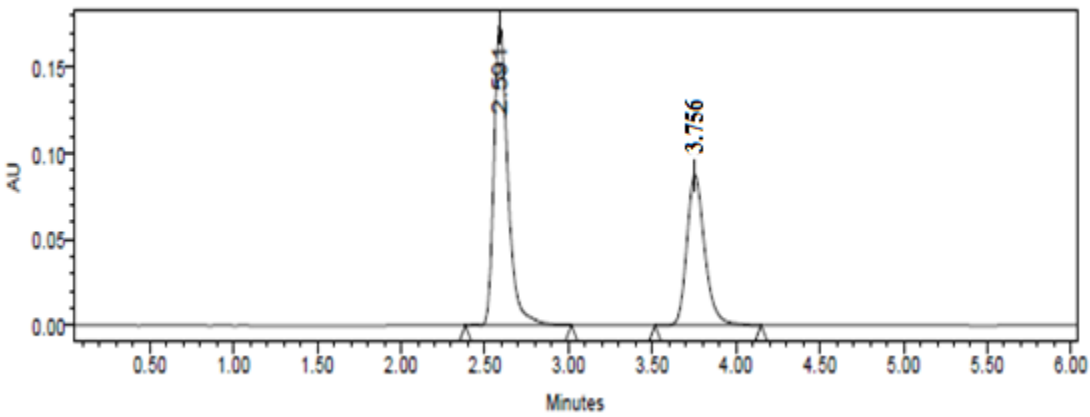
Accuracy of the method was determined by recovery experiments. There are mainly 2types of recovery studies are there.

a) Standard addition method: To the formulation, the reference standard of the respective drug of known concentration was added, analyzed by HPLC and compared with the standard drug concentration.

b) Percentage method: For these assay method samples are prepared in three concentrations of 50%, 100%, and 150% respectively.

Acceptance criteria: The mean % recovery of the Amlodipine and Perindopril at each level should be not less than 95.0% and not more than 105.0%.





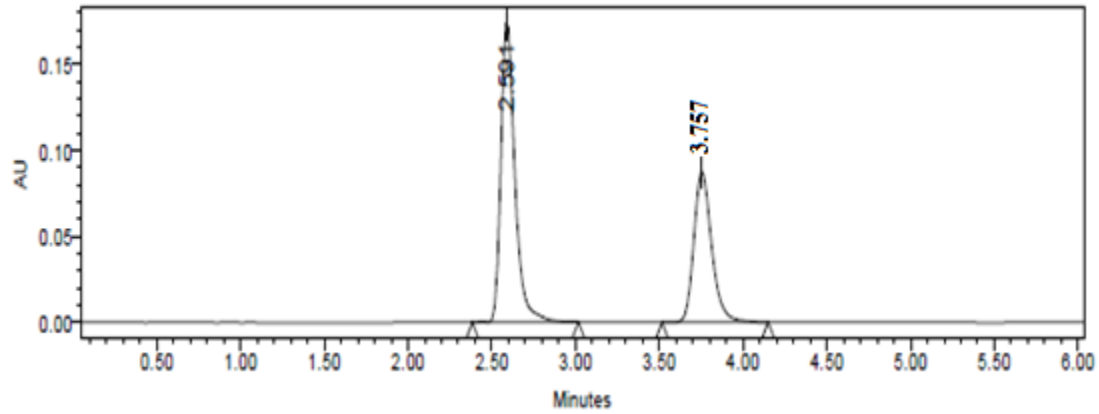


Figure 18. Chromatogram of Accuracy 50%

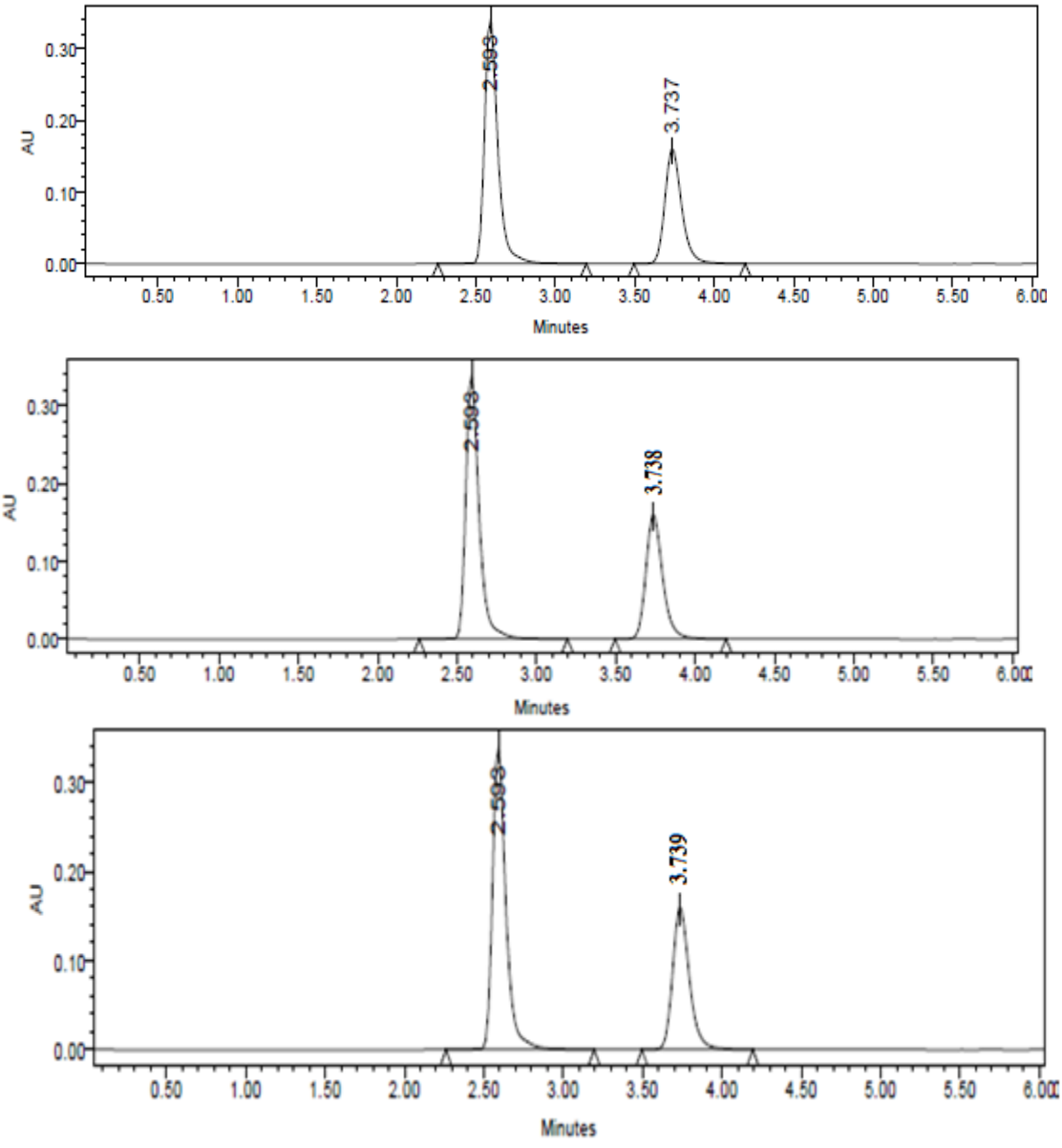


Figure 19. Chromatogram of Accuracy 100%

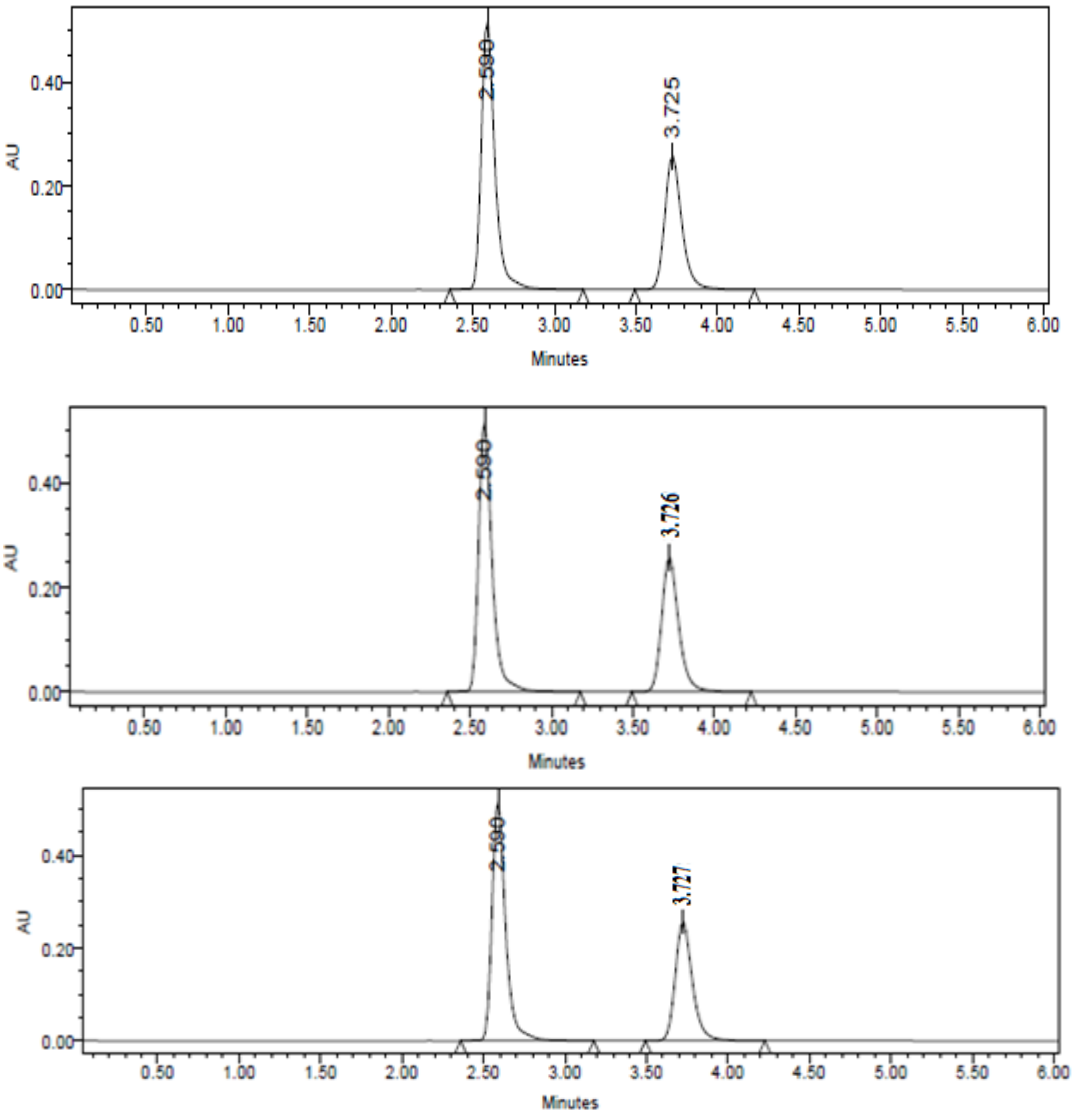


Figure 20. Chromatogram of Accuracy 150%

Table 17. Accuracy Study of Amlodipine

Sample Id	Conc found (µg/ml)	Concn Obtained (µg/ml)	%Recovery	Mean recovery	Statistical Analysis
50%	5	5.01	100.2		
50%	5	4.96	99.2	99.73	
50%	5	4.99	99.8		**%RSD= 0.505**
100%	10	9.95	99.5		
100%	10	9.87	98.7	98.8	
100%	10	9.82	98.2		**%RSD=0.66**
150%	15	14.64	97.6		
150%	15	14.76	98.4	98.8	
150%	15	15.06	100.4		**%RSD=1.45**

Table 18. Accuracy Study of Perindopril

Conc (µg/ml)	Concn Obtained(µg/ml)	%Recovery of drug	Mean accuracy	%RSD
5	4.92	98.0		
5	4.96	99.2		
5	5.02	100.4	**99.2**	**1.2**
10	9.95	99.5		
10	9.94	99.4		
10	9.98	99.8	**99.5**	**0.2**
15	14.78	98.6		
15	14.94	99.6	**99.0**	**0.530**
15	14.83	98.8		

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION:

The Sensitivity of measurement of Amlodipine and Perindopril by use of the proposed method was estimated in terms of the Limit of Detection (LOD) and the Limit of Quantitation (LOQ). The LOD and LOQ were calculated by the use of the equations:

$$LOD = 3.3 \times \frac{\sigma}{S}$$

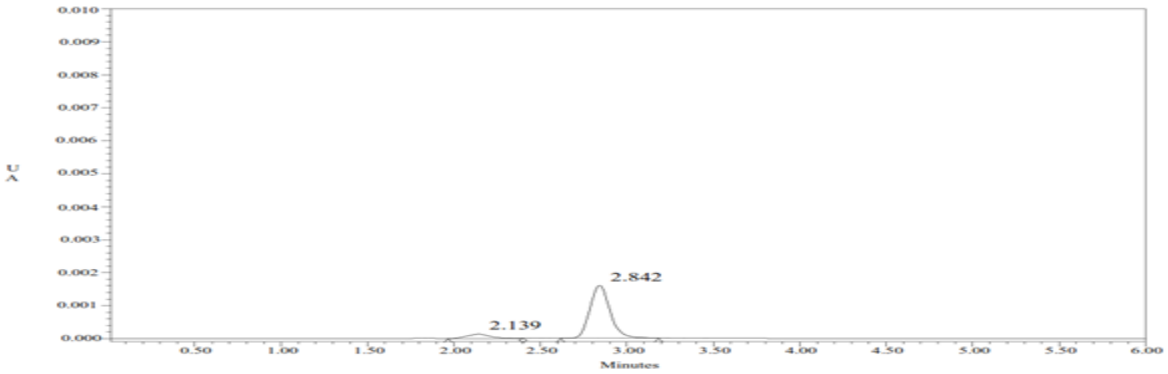


Fig.21. Results for LOD

$$LOQ = 10 \times \frac{\sigma}{S}$$

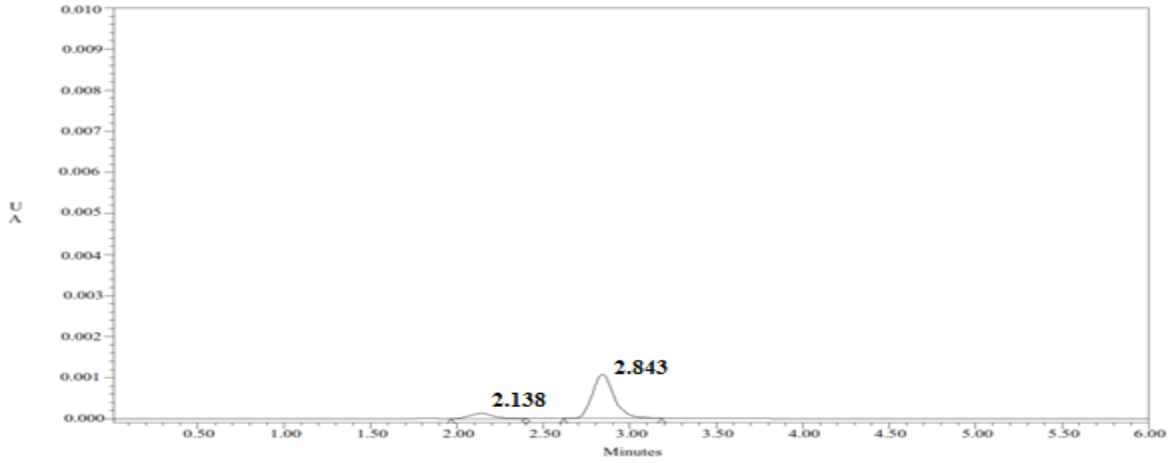


Fig.22. Results for LOQ

Where, σ is the standard deviation of intercept of calibration plot and S is the average of the slope of the corresponding calibration plot. The LOD and LOQ values for Amlodipine and Perindopril were reported in the Table.

Table 19. LOD and LOQ Data of Amlodipine and Perindopril

Amlodipine			**Perindopril**		
Conc.(x) (µg/ml)	**Peak Areas (y)**	**Statistical Analysis**	**Conc.(x) (µg/ml)**	**Peak Areas (y)**	**Statistical Analysis**
40	2004682	S = 39092 c = 618048 LOD: 3.2µg/ml LOQ: 10.2µg/ml	20	1184227	S = 39092 c =369381 LOD:3.3µg/ml LOQ: 10.3µg/ml
40	2004587		20	1186425	

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. For the determination of a method's robustness, deliberate change in the Flow rate was made to evaluate the impact on the method.

Effect of variation in flow rate:

A study was conducted to determine the effect of variation in flow rate. Standard and Test solutions of 100% concentration was prepared & injected into the HPLC system by keeping flow rates 0.8 ml/min& 1.2 ml/min. The effect of variation of flow rate was evaluated.

Effect of variation in mobile phase composition: A study was conducted to determine the effect of variation in mobile phase ratio by changing the ratio of organic solvent i.e., Buffer: Methanol by ±2ml. Standard & test solutions of 100% concentration were prepared and injected into the HPLC system and the chromatograms were recorded. The retention times, tailing factors & %RSD values were calculated.

Results:

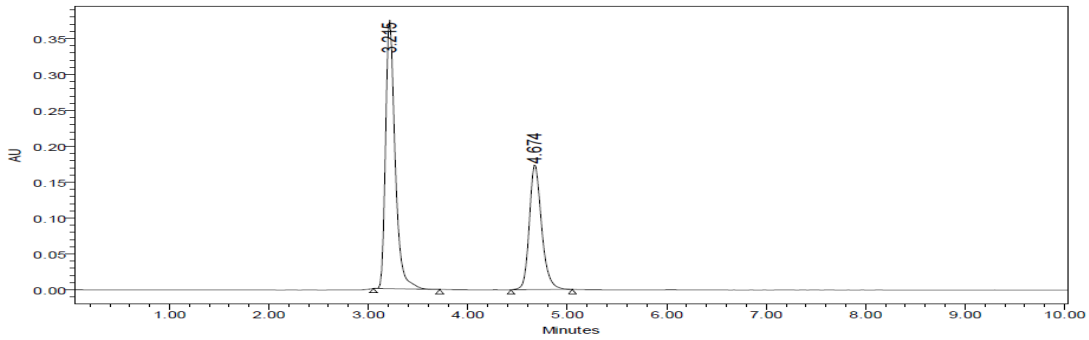


Figure. 23. Representative Chromatogram at Flow rate of 0.8 ml/min

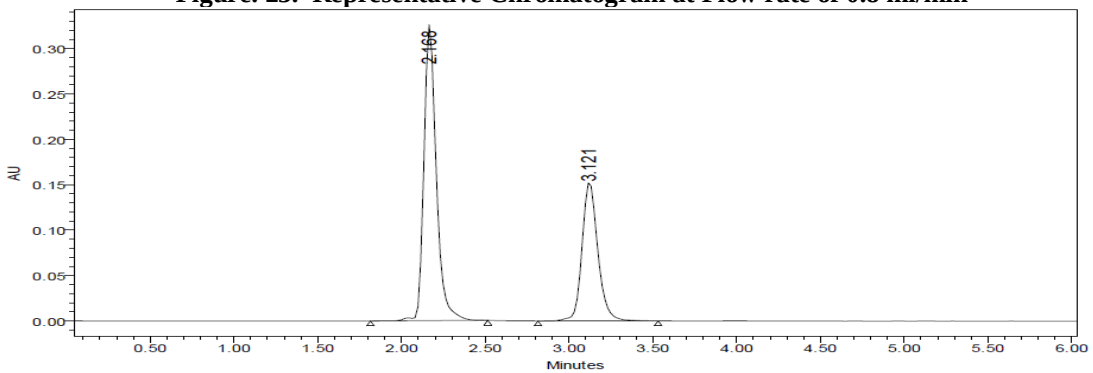


Figure. 24. Representative Chromatogram at Flow rate of 1.2 ml/min

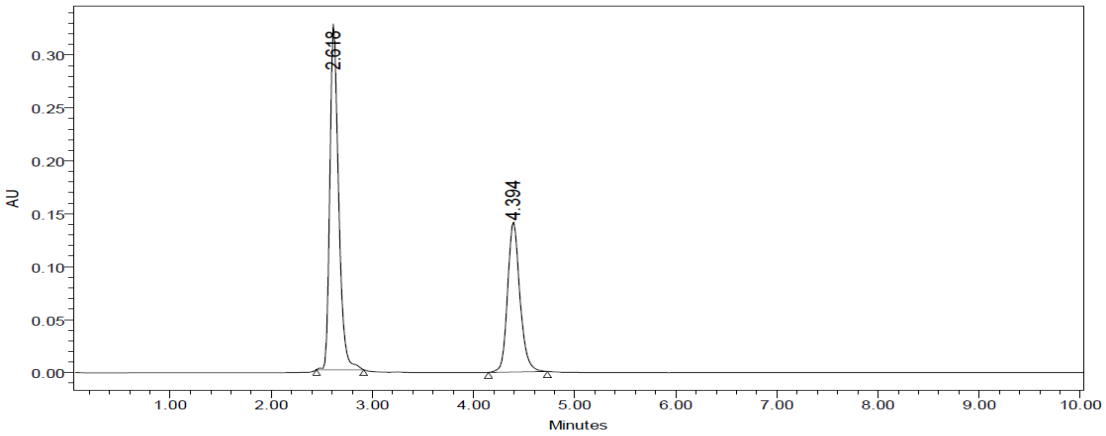


Figure. 25. Representative Chromatogram for Mobile phase composition (Buffer: Methanol: 40:60)

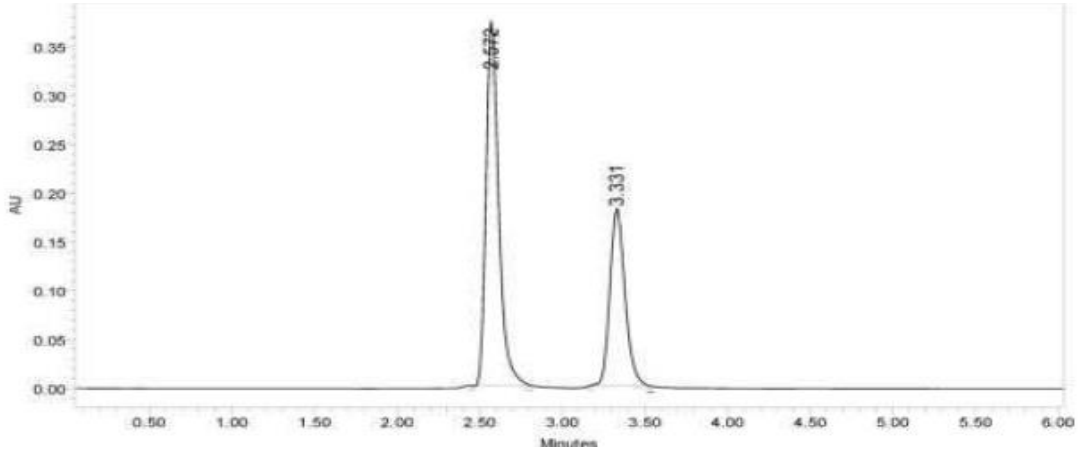


Figure. 26. Representative Chromatogram for Mobile phase composition (Buffer: Methanol:30:70)

Table.20. Robustness data for Amlodipine

Std. Replicate	Variation in flow rate		Variation in Mobile phase composition	
	Flow Rate 0.8ml/min	Flow Rate 1.2ml/min	Buffer: Methanol (40:60)	Buffer: Methanol (30:70)
1	2492492	1676589	1951632	1979168
2	2495874	1675428	1954783	1967452
Mean	2494183	1676009	1953208.0	1973310
SD	2391.4	820.9	2228.0	8284.46
%RSD	0.09	0.04	0.11	0.4
Retention time	3.150	2.168	2.618	2.572
Tailing factor	1.4	1.3	1.3	1.3
Theoretical plates	5752	4207	4577	4476

Table. 21. Robustness data for Perindopril

Parameter	Variation in flow rate		Variation in Mobile phase composition	
Standard	**Flow Rate 0.8ml/min**	**Flow Rate 1.2ml/min**	**Buffer: Methanol (40:60)**	**Buffer: Methanol (30:70)**
1	1500192	100524	1196996	1153397
2	1500426	100468	1198547	1154782
Mean	1500309	100496	1197772	1154090
SD	165.5	39.59	1096.2	979.34
%RSD	0.01	0.03	0.09	0.08
Retention time	4.674	3.121	4.394	3.331
Tailing factor	1.2	1.2	1.2	1.2
Theoretical plates	7187	5412	6498	6471

Report:
Amlodipine & Perindopril peaks in the chromatogram passed the system suitability criteria. %RSD of peak areas of Amlodipine & Perindoprwas not more than 2.0% for variation in mobile phase composition. From the above data, it was concluded that the method was robust

4.DISCUSSION:

In RP-HPLC method, the conditions were optimized to obtain an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to separate title ingredients. Mobile phase and flow rate selection was based on eak parameters (height, tailing, theoretical plates, capacity or symmetry factor), run time and resolution. The mobile phase containing mixture of orthophosphoric acid buffer solution: Methanol (65:35v/v, pH 2.45) with a flow rate of 1.0 ml/min is quite robust.

The optimum wavelength for detection was 254 nm at which better detector response for both the drugs was obtained. The retention times for Amlodipine and Perindopril was found to be 2.589 ± 0.004 min and 3.711 ± 0.005 min, respectively. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The calibration was linear in concentration range of 20 to 60 µg/ ml and 10 to 30 µg/ml, with regression 0.9979 and 0.9999, Amlodipine and Perindopril respectively. The low values of % R.S.D indicate the method is precise and accurate. The mean recoveries were found above 99.3 % for both the drugs.

Robustness of the proposed method was determined by varying various parameters, the %RSD reported was found to be less than 2 %. The proposed method was validated in accordance with ICH parameters and the applied for analysis of the same in marketed formulations.

5. CONCLUSION:

The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Amlodipine and Perindopril in tablet dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statistically according to ICH guidelines. The Sample recoveries in all formulations were in good agreement with their respective label claims.

From literature review and solubility analysis initial chromatographic conditions Mobile phase ortho phosphoric acid buffer:Methanol 65:35 were set (Buffer PH 2.45 adjusted with Triethylamine), Kromosil C 18 (250×4.6mm, 5µ) Column, Flow rate 1.0 ml/min and temperature was ambient, eluent was scanned with PDA detector in system and it showed maximum absorbance at 254 nm. As the methanol content was increased Amlodipine and Perindopril got eluted with good peak symmetric properties. The retention times for Amlodipine and Perindopril was found to be 2.589 min and 3.711 min respectively.

System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria.

Linearity study was carried out between 50% to150 % levels, R2 value was found to be as 0.999.

By using above method assay of marketed formulation was carried out, 100.7% was present.

Full length method was not performed; if it is done this method can be used for routine analysis of Amlodipine and Perindopril.

6. REFERENCES:

1. Samya M. El-Gizawy. James Holler & Stanley R. Crouch. Instrumental analysis, perindopril India edition, 2007, pg: 13-14.
2. Gurdeep R. Chatwal & Sham K. Anand. Instrumental Methods Of Chemical Analysis (Analytical Chemistry) , pg: 2.566-2.567.
3. Satinder Ahuja & Neil Jespersen. Modern Instrumental Analysis 47 (Comprehensive Analytical Chemistry) volume-47, pg-7-8.
4. Kumar GVS, Mrunali R. Patel, An Introduction to Analytical method development for pharmaceutical formulations, Pharmainfo.net 2008; 17:19.
5. Dipti B. Patel, N. J. Patel, S. K. Patel, A. M. Prajapati, and S. A. Patel, Rp hplc method for the estimation of Dutasteride in tablet dosage form. Indian Journal of Pharmaceutical Sciences, 2010 Jan-Feb; 72(1): 113–116.
6. Kamepalli Sujana, D. Gowri Sankar, Konda Abbulu and O.Bala Souri, Simultaneous estimation of finasteride and tamsulosin hydrochloride by reverse phase HPLC in bulk and pharmaceutical dosage form. International Journal of Pharmacy & Life sciences,Vol. 3, Issue 8: August: 2012, Pg.no:1905-1908.
7. Chandan, M. Vasudevan World Academy of Science, Engineering and Technology International Journal of Medical, Health, Pharmaceutical and Biomedical Engineering Vol:7 No:12, 2013.
8. P. Ravisankar1, G. Devala Rao et al Asian journal of pharmaceutical and clinical research Vol 6, Supply 3, 2013 ISSN - 0974-244.
9. Kullai Reddy Ulavapalli1 et al Indian Journal of Novel Drug delivery 3(2), Apr-Jun, 2011, 134-142.
10. Anil Waldiaa, Shubash Guptab et al. Journal of Chemical Technology Vol. 15, November 2008, pp. 617-620.
11. Shreya R. Shah, , , S. Dey, et alJournal of Taibah University for Science, Volume 8, Issue 1, January 2014, Pages 54–63.
12. Peyami Sari, Majid Razzak journal of Liquid Chromatography & Related Technologies Volume 27, Issue 2, 2005.
13. Celebier M, Altinoz S. Determination of Olmesartan medoxomil in tablets by UV-Vis spectrophotometry, Pharmazie, 2007; 64: 419-422.
14. Shah NJ, Suhagia BN. Development and Validation of a Simultaneous HPTLC Method for the Estimation of Olmesartan medoxomil and Hydrochlorothiazide in Tablet Dosage Form. Indian J. Pharm. Sci. 2007; 69: 834-836.
15. Dongyang Liu, Pei Hu, Matsushima N. Quantitative determination of olmesartan in human plasma and urine by liquid chromatography coupled to tandem mass spectrometry. J. Chromatography B. 2007; 856: 190-197.
16. Vaidya VV, Shikha MN. LC-MS-MS Determination of Olmesartan in Human Plasma. Chromatographia. 2008; 67: 147-150.
17. Celebier M, Sacide A, Development of a CZE Method for the Determination of Olmesartan medoxomil in Tablets, Chromatographia, 2007; 66: 929-933.
18. Patel CV, Khandhar AP. Validated Absorption Factor Spectorphtometric and Reversed-Phase High-Peroformance Liqid Chromatographic Methods for the Determination of Ramipril and Olmesartan Medoxomil in Pharmaceutical Formulations. Eurasian J. Anal. Chem. 2007; 2(3): 159-171.
19. Murakami T, Konno H, Fukutsu N. Identification of a degradation product in stressed tablets of Olmesartan medoxomil by the complementary use of HPLC hyphenated techniques, J. Pharm. Biomed. Anal. 2008; 1-7.
20. Naidu KR, Kale UN, Shingare MS. Stability indicating RP-HPLC method for simultaneous determination of amlodipine and benazepril hydrochloride from their combination drug product. J Pharm Biomed Anal 2005; 39: 147-155.
21. Chaudhari BG, Patel NM,Shah PB. Stability indicating RP-HPLC method for simultaneous determination of atorvastatin and amlodipine from their combination drug products. Chem Pharm Bull 2007; 55: 241-246.
22. Shah DA, Bhatt KK, Shankar MB, Mehta RS, Gandhi TR, Baldania SL. RP-HPLC determination of atorvastatin calcium and amlodipine besylate combination in tablets. Indian J Pharm Sci 2006; 68: 796-799.
23. Valiyare GR, Chandra A, Apte SK, Mahadik AA. HPLC determination of amlodipine,losartan and ramipril in pharmaceutical formulations. Indian Drugs 2005; 42: 309-312.
24. Bahrami Gh, Mirzaeei Sh. Simple and rapid HPLC method for determination of Amlodipine in human serum with fluorescence detection and its use in pharmacokinetic studies. J Pharm Biomed Anal 2004; 36: 163-168.

25. Kanakapura B, Umakanthappa C, Paregowda N. Spectrophotometric and high performance liquid chromatographic determination of amlodipine besylate in pharmaceuticals. Science Asia 2005; 31: 13-21.
26. Klinkeberg R, Streel B, Ceccato A. Development and validation of a liquid chromatographic method for the determination of Amlodipine residues on manufacturing equipment surfaces. J Pharm Biomed Anal 2003; 32: 345-352.
27. Kulkarni AP, Gat GV, Pimple SV, Joshi MA. HPLC method for determination of losartan potassium and amlodipine besylate in tablets. Indian Drugs 2003; 40: 298- 299.
28. Rao JR, Kadam SS, Mahadik KR. Reverse phase high performance liquid chromatographic determination of amlodipine and benazepril hydrochloride in tablets. Indian Drugs 2002; 39: 378-381.
29. Zarapkar S, Kanyawar N. Simultaneous estimation of amlodipine and losartan potassium in pharmaceutical dosage by RP-HPLC. Indian Drugs 2002; 39: 338-341.
30. Gowri N, Vaidhyalingam V, Shantha A. Simultaneous estimation of amlodipine and benazepril tablets by RP-HPLC. Indian Drugs 2002; 39: 532-535.
31. Ilango K, Pabbisetty BSK, Karunanidhi SL. Simple and rapid high performance thin layer chromatographic estimation of amlodipine and atenolol from pharmaceutical dosages. Indian Drugs 2000; 37: 497-499.
32. Patki RV, Tamhankar CP, Tipnis HP. Simple and rapid high performance liquid chromatographic estimation of amlodipine from pharmaceutical dosages. Indian Drugs 1994; 31: 560-561.
33. Sane RT, Desai AJ, Valiyare GR, Ghadge AJ. High performance liquid chromatographic determination of amlodipine besylate from its pharmaceutical preparation. Indian Drugs 1993; 30: 501-505.
34. Chitlange SS, Bagri K. Stability Indicating RP- HPLC Method for Simultaneous Estimation of Valsartan and Amlodipine in Capsule Formulation, Asian J. Research Chem., 2008; 1:15- 18.
35. Mohammadia A, Rezanour N. A stability-indicating high performance liquid chromatographic (HPLC) assay for the simultaneous determination of atorvastatin and amlodipine in commercial tablets, J. of Chromatography B, 2007; 846: 215– 221.
36. Chaudhari BG, Patel NM, Shah PB. Simultaneous estimation of atorvastatin and amlodipine from formulation by HPTLC. Indian Drugs 2006; 43: 649-655.
37. Kasture AV, Ramteke M. Simultaneous UV-Spectrophotometric method for the estimation of atenolol and amlodipine besylate in combined dosage form. Ind J Pharm Sci 2006; 68: 394-396.
38. Sahu R, Patel VB. Simultaneous spectrophotometric determination of amlodipine esylate and atorvastatin calcium from binary mixture by dual wavelength and zero absorbance measurement. Indian Drugs 2005; 43: 160-161.
39. Gohil K, Trivedi P, Molvi KI. Spectrophotometric analysis of amlodipine besylate in bulk and tablet dosage form. Ind J Pharm Sci 2005; 67: 376-378.
40. Mashru RC, Parikh PP. Development of a method for simultaneous estimationof amlodipine besylate and lisinopril in their combined dosage form. The Eastern Pharmacist 2000; XLIII, No. 513: 111-112.
41. Lokesh BVS, Reddy MN, Sankar DG, Sreedhar K. Extractive spectrophotometric determination of amlodipine. The Eastern Pharmacist 1996; XXXIX, No. 458: 125-126.
42. Bhagirath K. Patel, Subhaschandra K. Patel, Swati R. Patel*. Analytical method development and validation forsimultaneous estimation of amlodipine besylate andperindopril arginine in combined pharmaceutical dosage form. Pharmaceutical and Biological Evaluations; June 2016; vol. 3 (Issue 3): 343-350: ISSN 2394-0859.
43. Naser F. Al-Tannak. UHPLC-UV Method for Simultaneous Determination of Perindopril Arginine and Indapamide Hemihydrate in Combined Dosage Form: A Stability-Indicating Assay Method. Sci. Pharm. 2018, 86(1), 7; https://doi.org/10.3390/scipharm86010007; 22 February 2018.