DEVELOPMENT AND VALIDATION OF A NOVEL STABILITY INDICATING RPHPLC METHOD FOR SIMULTANEOUS ESTIMATION OF PERINDOPRIL, INDAPAMIDE AND AMLODIPINE IN BULK AND COMBINED TABLET DOSAGE FORMS

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ABSTRACT

An accurate and precise linear stability indicating RP-HPLC method is proposed for simultaneous determination of Perindopril, Indapamide and Amlodipine in bulk sample and Combined Tablet dosage forms.The analysis was carried out on a zodiac Sil RP C_{18} Coloun using Mixture of potassium dihydrogen phosphate buffer (pH 3.0) and Acetonitrile in70:30 %v/v ratio at flow rate of 1.0 mL/min thedetection was carried out 260 nm. The retention times were found to be 1.76, 2.24 and3.176 min. for Perindopril, Indapamide and Amlodipine. Linearity was observed in the concentration ranges of 50-250μg/mL for Perindopril, Amlodipine 10-50 μg/ml for Indapamide. The percent recoveries were found to be 100.88 and 99.50% for Perindopril, Amlodipine. 100.62% for Indapamide.(average % recoveries).for the limit quantifications for found to be 9.8,9.05 and 8.05 μg/mL for

Perindopril, Indapamide and Amlodipine respectively Forced degradation studies on the drugs in combinations were also carried out and results present it.

KEYWORDS: RP-HPLC Perindopril, Indapamide and Amlodipine, Forced Degradation Studies; Tablet Dosage Form.

INTRODUCTION

Perindopril

Perindopril [Figure.1 a)], is an ACE inhibitor used in treatment of hypertension & heart failure. It is converted into active metabolite Perindoprilat in the body.[1] It blocks conversion of AT I to AT II & also inhibit degradation bradykinin which is a potent vasodialator, thus decreases peripheral resistance, lowers B.P & produces mild natriuresis.[2] Reduces B.P without increasing heartrate. Mostly used in patients having diabetes, naphropathy, CHF left ventricular hypertrophy & post-myocardial infraction.[3]
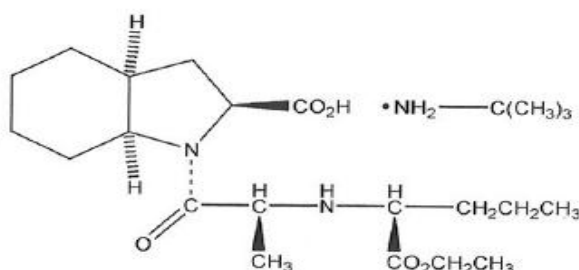
(A)
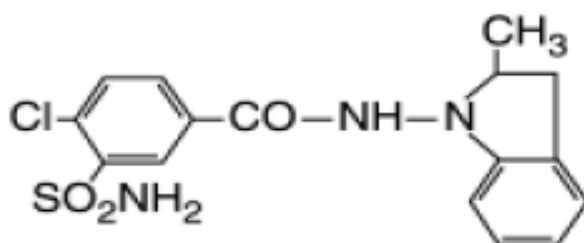
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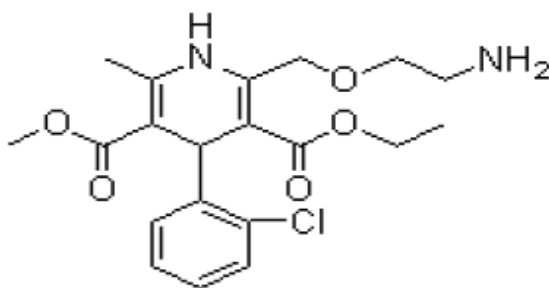
(C)

Fig.No.1. Chemical structures of (a) Perindopril erbumine b) Indapamide c) Amlodipine

Perindopril erbumine is chemically described as (2S,3∝S,7∝S)-1-[(S)-N-[(S)-1-Carboxy-butyl]alanyl]hexahydro-2-indolinecarboxylic acid, 1-ethyl ester, compound with tert-butylamine (1:1).

Indapamide [Figure 1 B] is an oral antihypertensive/diuretic. Its moleculecontains both a polar sulfamoylchlorobenzamide moiety and a lipid-soluble methylindoline moiety. It differs chemically from the thiazides in that it does not possess the thiazide ring system and contains only one sulfonamide group. The chemical name of Lozol (indapamide) is 1-(4-chloro-3sulfamoylbenzamido)-2-methylindoline, and its molecular weight is 365.84. The compound is a weak acid, pK_a=8.8, and is soluble in aqueous solutions of strong bases. It is a white to yellow-white crystalline (tetragonal) powder.

Amlodipine

Amlodipine [Figure 1 C] is a dihydropyridine calcium channel blocker used in management of hypertension & angina pectoris.[1] It has greater selectivity for vascular smooth muscle.[2] It has longer t½. It has lesser 1st pass metabolism; hence bioavailability is consistent.

EXPERIMENTAL

A Waters 2695 module equipped with Quaternary constant flow pump with Auto injector a Zodiac silica RP ODS C18 column (150 mm × 4.6 mm I.d.,5 μ size particle) and 2996 Photodiode Array Detector and running an Empoer-2 software are used to reduced all the weighings were doneon an Metler AE160 Electronic balance. Certified chemicals and Reagents.

The percentage recoveries was found to be 99.50, 100.62, 100.88% v/vPerindropil, Amlodipine and Indapamide (percentage of recoveries).for the limit of quantifications were found to be 9.8μg for respective PND, INDP and AMD. Forced degradation studies on the drops in combinations were also carried out and results present it.

OPTIMIZED METHOD

Chromatographic conditions

Column	:	Zodiac silica RP C_{18} 4.6×250mm 3.0μm
Mobile phase ratio	:	ACN: pH 3 buffer (70: 30 % v/v)
Detection wavelength	:	260 nm
Flow rate	:	1.0mL/min

Injection volume   :   20µl

Column temperature   :   35º C

Run time   :   10min.

Retention time   :   2.36.5 & 3.90 min.

PROCEDURE

Preparation of phosphate buffer

2.95 grams of potassiumdihydrogen phosphate was accurately weigh in about 900 mL of water along with 2.0 mLof Triethyl amine .The pH was adjusted to 3.0 with OPA. And the volume was made up to 1Litere.

Preparation of mobile phase

Mix a mixture of above buffer 300 mLand 700 mL of methanol and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration.

Preparation of Diluents

Mobile phase was used as the diluent.

Preparation of the individual Perindopril, Indapamide and Amlodipine standard preparation
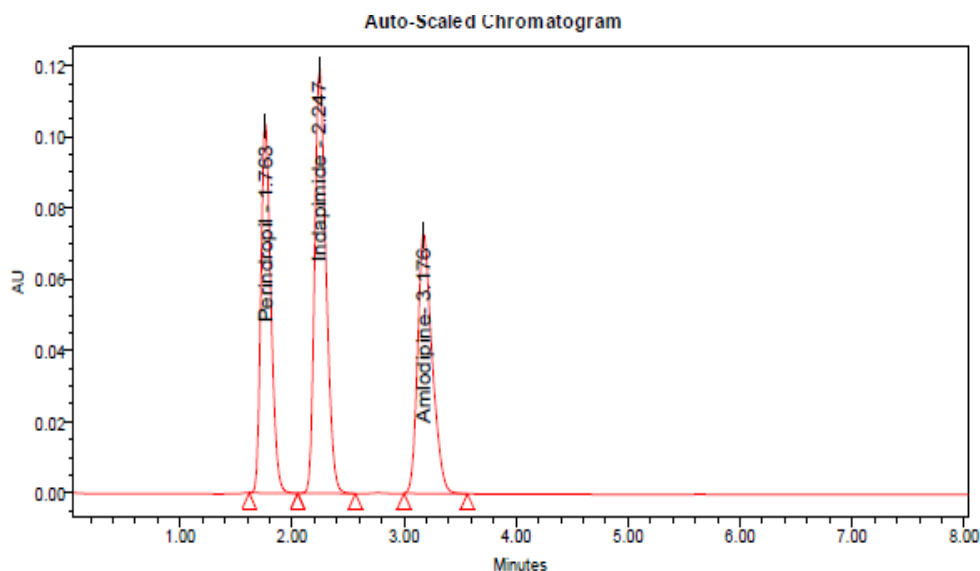
10 mg of Perindopril, Indapamide and Amlodipine working standard was accurately weighed and transferred into a 10 mL clean dry volumetric flask and add about 2 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1.5 mL from the above stock solution into a 10 mL volumetric flask and was diluted up to the mark with diluent. The chromatogram is shown in Fig.

Preparation of the Perindopril, Indapamide and Amlodipine standard and sample solution

Sample solution preparation: 10 mg Equivalent of Perindopril, Indapamide and Amlodipine tablet powder were accurately weighed and transferred into a 10 mL clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10 mL of the above stock solution into a 100 mL volumetric flask and was diluted up to the mark with diluent. The chromatograms are shown in Fig. and results are tabulated in Table.

Standard solution preparation

10 mg Perindopril, Indapamide and Amlodipine working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent. The chromatograms are shown in Fig.2.

**Fig.2 Typical Chromatogram of standard Perindopril, Indapamide and Amlodipine.**

Procedure

10μL of the blank, standard and sample were injected into the chromatographic system and areas for the Perindopril, Indapamide and Amlodipine the peaks were used for calculating the % assay by using the formulae.

System suitability

➤ Tailing factor for the peaks due to Perindopril, Indapamide and Amlodipinein standard solution should not be more than 1.5.

➤ Theoretical plates for the Perindopril, Indapamide and Amlodipine peaks in standard solution should not be less than 2000.

METHOD VALIDATION

1. Specificity: The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank. The chromatograms are shown in Fig.3.

	Baseline Noise (mV)
1	0.048
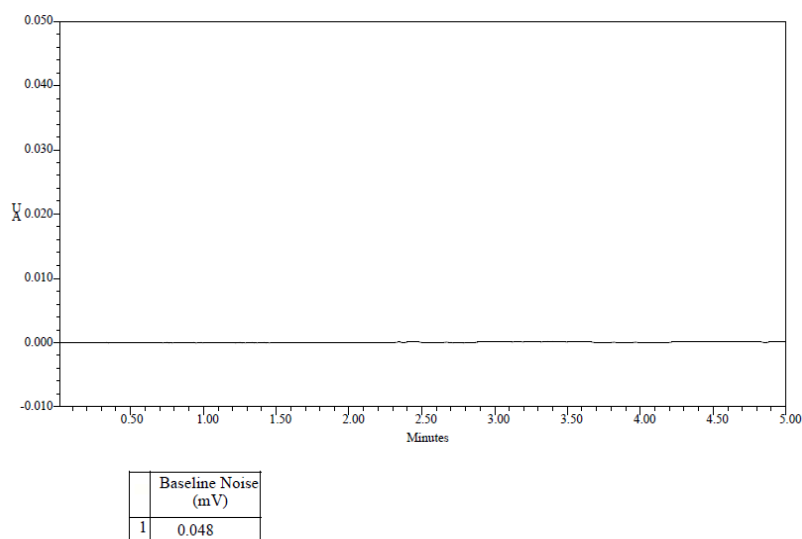
Fig.3.Chromatogram of placebo

2. Linearity

Preparation of stock solution

10 mg of Perindopril, Amlodipine and 1mg of Indapamide working standard were accurately weighed and were transferred into a 10ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Preparation of Level – I (50ppm of Perindopril, Amlodipine and 5 ppm of Indapamide)
0.5 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

Preparation of Level–II (100ppm of Perindopril, Amlodipine and 10ppm of Indapamide)
1 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

Preparation of Level–III (150ppm of Perindopril, Amlodipine and 15ppm of Indapamide): 1.5 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

Preparation of Level–IV (200 ppm of Perindopril, Amlodipine and 20ppm of Indapamide): 2 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

Preparation of Level–V (250 ppm of Perindopril, Amlodipine and 25ppm of Indapamide)

2.5ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

Procedure

Each level was injected into the chromatographic system and peak area was measured.Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and the correlation coefficient was calculated.The chromatograms are shown in Fig.4.and results are tabulated in Table 1.

Table.1. Linearity results

Linearity Level	Concentration µg/mL		Peak Area		
	PRD & AMDP	INDP	Perindropil	Amlodipine	Indapamide
I	50	10	209089	183117	292892
II	100	20	447565	355053	540359
III	150	30	644723	527075	785680
IV	200	40	858788	707244	1044819
V	250	50	981805	859557	1302210
Correlation Coefficient r^2			0.9966	0.9999	0.9997
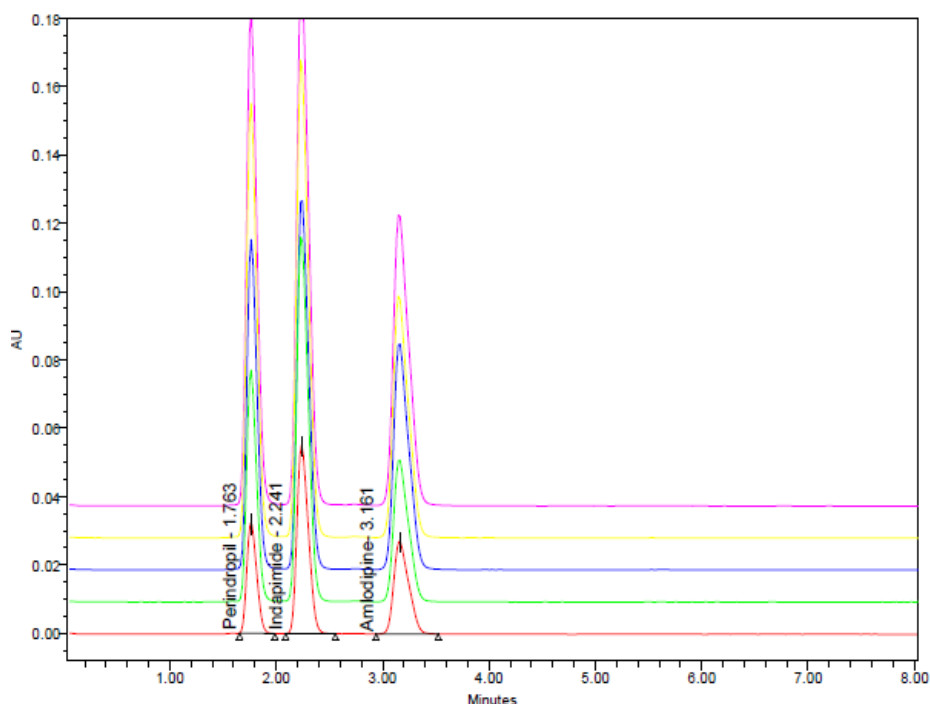
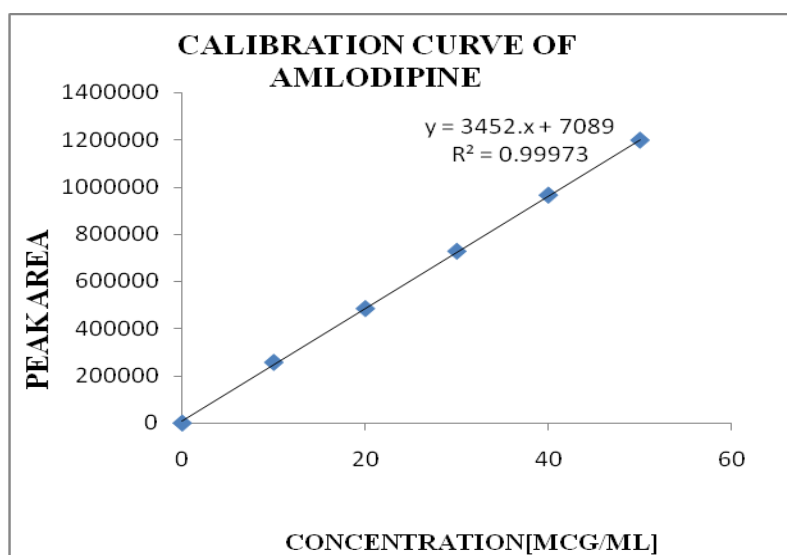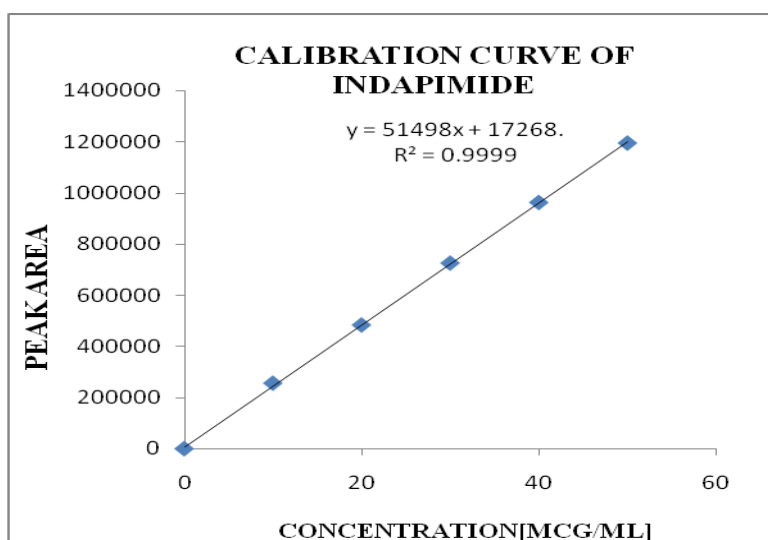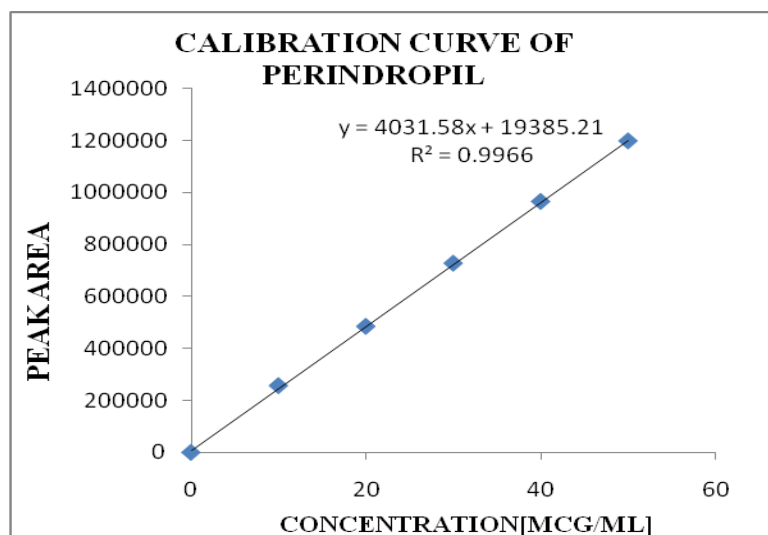**Fig.3: Overlain Chromatogram of Indapamide, Amlodipine and Perindropil.**

Fig.No.4: Linerty curves of Perindopril (a), Indapamide (b) and Amlodipine (c).

3. Range: Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 50μg/ml-250μg/ml and 10μg/ml-60 μg/ml of Perindopril, Amlodipine and Indapamide respectively.

4. Accuracy

Preparation of standard stock solution: 10mg of Perindopril, Amlodipine and 1mg of Indapamide working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1 ml of the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of sample solutions

For preparation of 50% solution (with respect to target assay concentration)
5 mg of Perindopril, Amlodipine and 0.5mg of Indapamide working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1 ml of the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

For preparation of 100% solution (with respect to target assay concentration)
10mg of Perindopril, Amlodipine and 1mg of Indapamide working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1 ml of the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

For preparation of 150% solution (with respect to target assay concentration)
15mg of Perindopril, Amlodipine and 1.5 mg of Indapamide working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1 ml of the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Procedure: The standard solutions of accuracy 50%, 100% and 150%were injected into chromatographic system. Calculate the amount found and amount added for Perindopril, Indapamideand and Amlodipineand calculate the individual % recovery and mean % recovery values present it Table 2.&3.

Table 2: Accuracy values for Perindopril &Amlodipine

%Concentration (at specification Level)	Amount Added (mg)		Amount Found (mg)		%Recovery		Mean Recovery	
	PRD	**AMD**	**PRD**	**AMD**	**PRD**	**AMD**	**PRD**	**AMD**
50%	5	5	5.12	4.86	98.9%	98.9%		
100%	10	10	10.02	9.96	99.6%	99.6%	100.88%	99.50%
150%	15	15	15.06	15.01	100.0%	100.0%		

PRD = Perindopril; AMD =Amlodipine.

Table 3: Accuracy studies Perindopril

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	991041	0.5	0.51	101.67	
100%	1556423	1.0	1.12	100.2%	100.62%
150%	2079793	1.5	1.56	100.0%	

5. Precision

5.1 Repeatability

Preparation of stock solution: 10mg of Perindopril, Amlodipine and 1mg of Indapamide working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1 ml of the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Procedure: The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

5.2 Intermediate Precision

To evaluate the intermediate precision (also known as ruggedness) of the method, precision was performed on different days by using different make column of same dimensions.

Preparation of stock solution

10mg of Perindopril, Amlodipine and 1mg of Indapamide working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1 ml of the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Procedure

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

6. Limit of detection (LOD)

LOD's can be calculated based on the standard deviation of the response (SD)and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

7. Limit of quantification

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Robustness

As part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method.

a) The flow rate was varied at 0.4ml/min to 0.6 ml/min. Standard solution 150 ppm of Perindopril, Amlodipine and 15 ppm of Indapamidewas prepared and analysed using the varied flow rates along with method flow rate.

b) The organic composition in the mobile phase was varied from 65% to75 %standard solution 150 µg/ml of Perindopril, Amlodipine and 15 µg/ml of Indapamide were prepared and analysed using the varied mobile phase composition along with the actual mobile phase composition in the method.

9. System suitability

10mg of Perindopril, Amlodipine and 1mg of Indapamide working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1 ml of the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Degradation study

In a order to the determine whether the analytical methods were stable Perindopril, Indapamide And Amlodipine dosage forms are stressed on the different conditions to applied degradation studies. The guidelines are expressed in ICH Q2A, Q3B, Q2B & FDA 21 CFR section of 211 all the required for development & for the validation of stability study.

The degradation of a sample was prepared by the transfer the individual tablet powder was equivalent to the weight of each tablet was transfer into 100 ml flask & it was treated under the acidic, alkaline, thermal, oxidizing and photolytic conditions. When degradation was complete the solution were left to equilibrate to the room temp & dil. with mobile phase to furnish the solutions of a concentration equivalent to a 30 µg/mL of Perindopril, Indapamide and Amlodipine. The specific degradative conditions are described below.
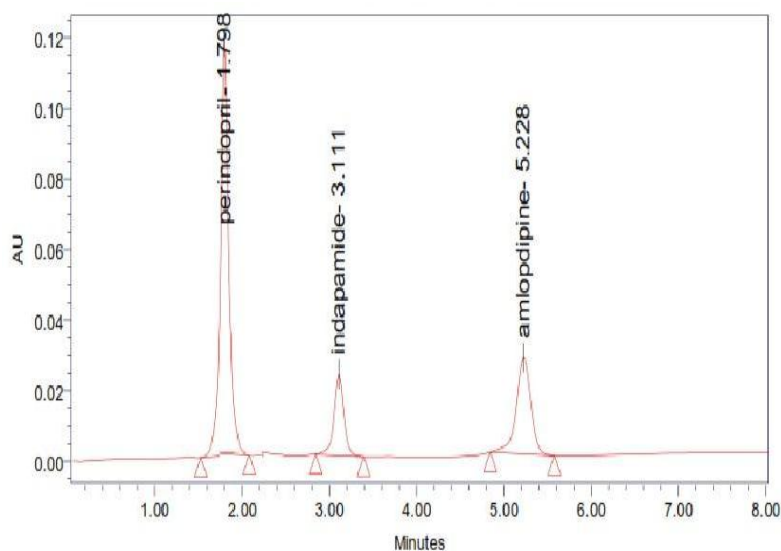
Figure 4: Chromatogram of acidic forced degradation of Perindopril, Indapamide and Amlodipine

1. Acid degradation study: TheAcid degradation was done by sample was treated with 3ml of 1N hydrochloric acid and kept for 10hrs at 60°C. After 10hrs the solution was neutralized with 3ml of 1N sodium hydroxide, made the volume up to the mark with mobile phase and analyzed using HPLC. The degrading drug content was found up to 6.8% in the acidic condition (Figure 4).

2. Alkaline degradation: TheAlkaline degradation was done by sample was treated with 3ml of 1N sodium hydroxide and kept the sample for 10hr. After 10hr solution was neutralized to add 3ml of 1N hydrochloric acid, made the volume up to the mark with irrelevant media and analyzed using HPLC. In alkali degradation study, it was found to be 9.7% of the degraded drug (Figure 5).
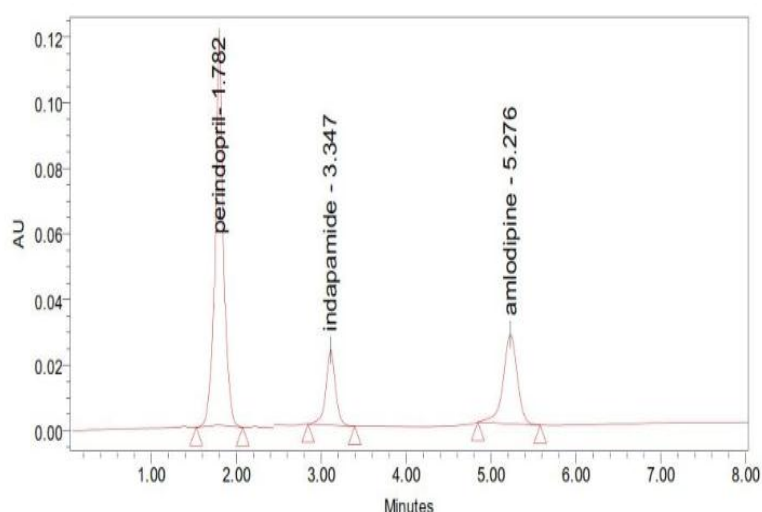

Figure 5: Chromatogram of alkali forced degradation of Perindopril, Indapamide and Amlodipine

3. Oxidative degradation: Theoxidative degradation was done by sample was mixed with 3mL of 30%v/v aqueous hydrogen peroxide solution and kept for 10hrs. After 10hrs made the volume upto the mark with mobile phase and analyzed using HPLC. In oxidative degradation, it was found to be 6.9% of the degraded drug (Figure 6).
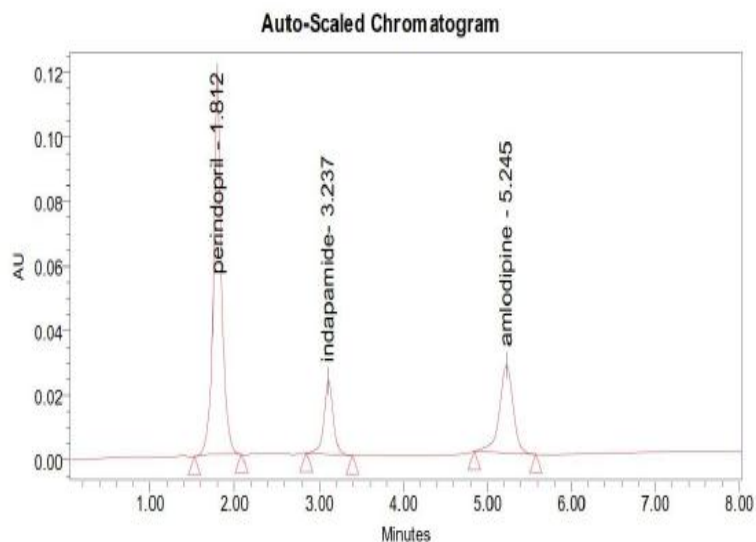
Figure 6: Chromatogram of oxidative forced degradation of Perindopril, Indapamide and Amlodipine.

4. Photolytic degradation: Thephotolytic degradation was done by exposingof drug content under the UV light for 15mins to 7days. There is 5.8% of the drug degradation observed in the above specific photolytic degradation condition (Figure 7).
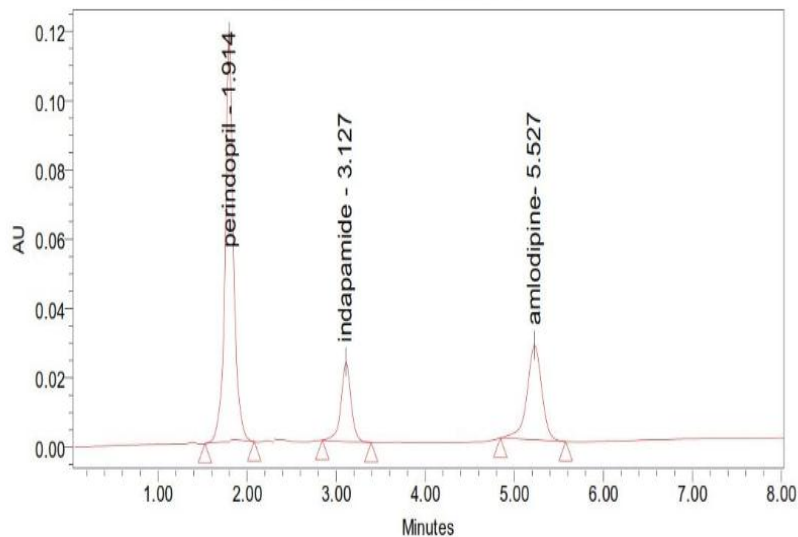
Figure 7: Chromatogram of UV-light degradation of Perindopril, Indapamide and Amlodipine

3.4.3.5. Thermal degradation: TheThermal degradation is to be performing by the exposing the solid drug at the80°C for 15mins to 60mins and at 220°C for 2-5mins. Resultant chromatogram of thermal degradation study (Figure 37, 38 & Table 10, 11) was indicates that

the drug was found to be slightly stable under thermal condition. It was only 7.7% of the drug content were degraded.(Figure-8.)
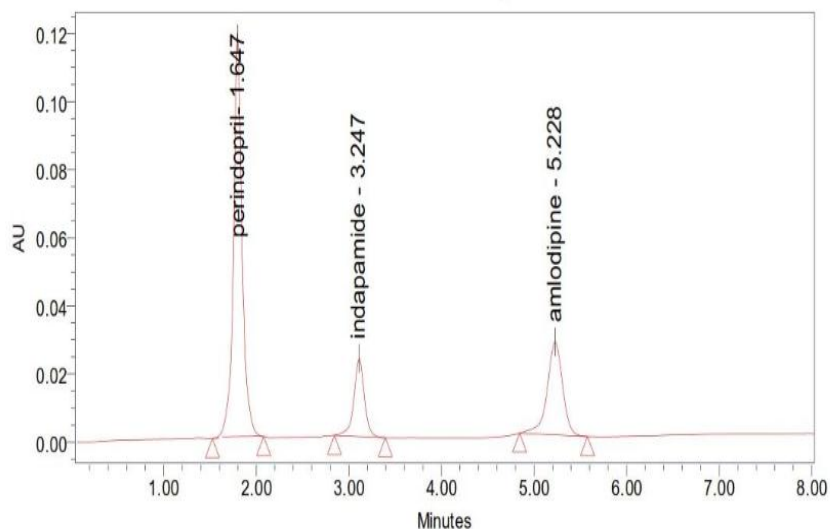
Figure 8: Chromatogram of thermal degradation of Perindopril, Indapamide and Amlodipine.

RESULTS AND DISCUSSION

Table 1: Peak purity results of Perindopril, Indapamide and Amlodipine

Stress Condition	Purity Angle			Purity Threshold		
	Perindopr	**Indapamid**		**Perindopr**	**Indapamid**	**Amlodipine**
Acid Degradation	0.589	0.428	0.067	1.619	0.574	0.354
Alkali Degradation	0.197	1.162	0.323	1.753	5.129	2.400
Oxidative Degradation	0.589	0.428	0.067	1.619	0.574	0.354
PhotolyticDegradation	0.289	0.428	0.267	1.619	0.574	1.154
Thermal Degradation	0.589	0.428	0.167	1.619	0.574	0.354

Table 2: Percentage of degradation of Perindopril, Indapamide and Amlodipine

Drug Name		*Acid*	*Alkali*	*Oxidative*	*Photolytic*	*Thermal*
Perindopril	*Std Area*	639198.6				
	Sample Area	589116	601257	592575	612547	586258
	% of Degradation	7.8%	5.9%	7.2%	4.1%	8.2%
Indapamide	*Std Area*	835566.6				
	Sample Area	779391	728274	760578	792587	785487
	% of Degradation	6.7%	12.8%	8.9%	5.1%	5.9%
Amlodipine	*Std Area*	655354.3				
	Sample Area	614934	585846	624635	601578	594892
	% of Degradation	6.1%	10.6%	4.6%	8.2%	9.2%
% Average of Degradation		6.8%	9.7%	6.9%	5.8%	7.7%

The present study was aimed at developing a simple, sensitive, precise andaccurate HPLC method for the simultaneous determination of Perindopril and Amlodipinefrom bulk samples and their tablet dosage forms. A non-polar C_{18} analyticalchromatographic column was chosen as the stationary phase for the separation andsimultaneous determination of Perindopril and Amlodipine. Mixtures of commonly usedsolvents like water, methanol and acetonitrile with or without buffers in differentcombinations were tested as mobile phases. The choice of the optimum composition isbased on the chromatographic response factor, a good peak shape with minimum tailing.A mixture of acetonitrile and potassium di hydrogen phosphate buffer (pH 3.0 adjusted with OPA)in the ratio of 70:30 v/v was proved to be the mostsuitable of all the combinations since the chromatographic peak obtained was welldefined, better resolved and almost free from tailing. The retention times of the Perindopril and Amlodipine were found to be 1.76, 2.24 and 3.176 min respectively. The linearity was found satisfactory for the drugs in the range 50 to 250μg/ml for Perindopril andIndapamide10-50 μg/mL for Amlodipine (Table 13.4 & 13.5). The regression equation of the linearity curve between concentrations of Perindopril and Amlodipine over its peak areas were found to be Y=4031X+19385.21 (where Y is the peak area and X is the concentration of Perindopril in μg/mL), Y=51498X+17268 and Y=3452X+7089 (where Y is the peak area and X is the concentration of Amlodipine in μg/mL) respectively. Precision of the method was studied by repeated injection of tablet solution and results showed lower %RSD values (Table 13.6-13.8). This reveals that the method is quite precise. The percent recoveries of the drug solutions were studied at three different concentrationlevels. The percent individual recovery and the %RSD at each level were within the acceptable limits. This indicates that the method is accurate. The absence of additional peaks in the chromatogram indicates non-interference of the commonly used excipients in the tablets and hence the method is specific.

The deliberate changes in the method have not much affected the peak tailing, theoretical plates and the percent assay. This indicates that the present method is robust (Table). The system suitability studies were carried out to check various parameters such as theoretical plates and tailing factor (Table). The lowest 45 .5values of LOD and LOQ as obtained by the proposed method indicate that the method issensitive (Table). The solution stability studies indicate that both the drugs were stable up to 24 hours. The forced degradation studies indicate that both the drugs were stable in stability studies (Table).

CONCLUSION

The proposed stability-indicating RP-HPLC method was simple, specific, sensitive, accurate and precise and can be used for simultaneous analysis of Perindopril and Amlodipine in bulk samples and its tablet dosage forms.

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