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Method Development and Validation for the Analysis of Perindopril Erbumine and Amlodipine Besilate by Rp-Hplc in Pure and Pharmaceutical Dosage Form

SOMA. PRASANTI

Under the Guidance of

Dr. P. SELVAM, M. Pharm., Ph.D., FNABS,
Professor & Principal

NOVA COLLEGE OF PHARMACEUTICAL EDUCATION & RESEARCH, JUPUDI, VIJAYAWADA

Abstract: *The objective of this present work was to develop and validate analytical method for quantitative determination of Perindopril Erbumine and Amlodipine Besylate in a tablet formulation. Chromatographic separation of the two drugs was achieved on an Eclipse XDB C-8 (150 mm X 4.6 mm), 5mm. The mobile phase constituted of Buffer: Acetonitrile (65:35) and pH adjusted to 2.6 with dilute Ortho-Phosphoric Acid was delivered at the flow rate 1.0 mL/min. Detection was performed at 210 nm. Separation was completed within 8 min. Calibration curves were linear with correlation coefficient between 0.99 to 1.0 over a concentration range of 8 to 60 mg/mL of Perindopril Erbumine and 10 to 75 mg/mL of Amlodipine Besylate. The relative standard deviation (R.S.D) was found<2.0%.*

I.INTRODUCTION

This thesis deals with the studies carried out on the stability indicating RP-HPLC method for the estimation of "Perindopril Erbumine And Amlodipine Besilate" in pharmaceutical dosage form determination of Perindopril Erbumine And Amlodipine Besilate in bulk and pharmaceutical dosage form.

Drug:

Various authors defined the term drug in various stages. The most widely accepted definition of a drug is as any chemical agent that affects the living process. Further the term drug includes all chemicals and medicines meant for treatment, mitigation or prevention of diseases in human beings or animals for internal or external use. Pharmaceutical chemistry is a science that makes use of the general laws of chemistry to study drugs i.e., their preparation, chemical nature, composition, structure, influence on an organism and studies the physical and chemical properties, the methods of quality control and conditions of their storage. According to their chemical structure or therapeutic action, the drugs may be classified as Antibacterial agents, Anti hypertensive drugs, Antidiabetic drugs, Pharmacodynamic agents, gastrointestinal agents and Prokinetic drugs etc.

Active pharmaceutical ingredient is defined as any component that provides pharmacological activity or other effect in the diagnosis, cure, mitigation, treatment or prevention of disease or to affect the structure or any function of the body.

Pharmaceutical Analysis is defined as the application of analytical procedures used to determine the purity , safety and quality of the drugs and chemicals.

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Pharmaceutical Analysis plays a vital role in the quality assurance and quality control of bulk drugs and their formulations. It is a branch of chemistry that deals with identification of compounds and mixtures (qualitative analysis) or the determination of the proportions of the constituents (quantitative analysis).

Analytical chemistry is the science that seeks ever improved means of measuring the chemical composition of natural and artificial materials. Pharmaceutical analysis is a specialized branch of analytical chemistry, which involves separating, identifying and determining the relative amounts of components in a sample of matter. It is concerned with the chemical characterization of matter both quantitative and qualitative.[18]

The complete analysis of a substance consists of four main steps.

1. Sample preparation / Sampling
2. Dissolution of the sample, conversion of the analyte into a form suitable for measurement.
3. Qualitative or quantitative analysis of sample
4. Calculation and interpretation of the measurement of sample.

One of the major decisions to be made by an analyst is the choice of the most effective procedure for a given analysis, for this he must be familiar with the practical details, the theoretical principles and also that he must be conversant with the conditions under which each method is reliable, aware of possible interferences which may arise and capable of minimizing or circumventing such problems. He must also be concerned with question regarding accuracy and precision. In addition he must not over look factors such as time and costing.

Factors affecting the choice of analytical methods:

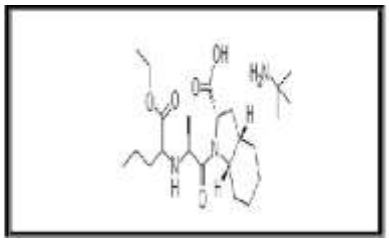
a) The type of analysis required.
b) Problem arising from the nature of the material.
c) Possible interference from the components of the material other than those of interest.
d) The concentration range, which needs to be investigated.
e) The accuracy required.
f) The facilities available.
g) The time required for complete analysis.

II.DRUG PROFILE

PERINDOPRIL

Perindopril is an ACE inhibitor used in treatment of hypertension &heart failure. It is converted into active metabolite Perindoprilat in the body . 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. Reduces B.P without increasing heartrate. Mostly used in patients having diabetes, naphropathy,CHF left ventricular hypertrophy & post-myocardial infraction .

Fig 1: Chemical structure of Perindopril



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- ***Drug category:*** Angiotensin-Converting Enzyme (ACE) Inhibitors
- ***IUPAC name:*** (2S,3aS,7aS)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxopentan-yl]amino}propanoyl]-octahydro-1H-indole-2-carboxylic acid.
- ***Molecular Formula:*** $C9H_{32}N_2O_5$.
- ***Molecular Weight:*** 368.46 g/mol.
- ***Half life:*** 1.2 hrs
- Melting point: 159.2-160.7 °C.

MECHANISM OF ACTION

Perindopril belongs to a class of medicines called angiotensin-converting enzyme inhibitors, or ACE inhibitors. It is prescribed for a number of different reasons. You may have been prescribed it to reduce high blood pressure (hypertension), or to treat heart failure, or to protect your heart and blood vessels from damage following a heart attack or following certain procedures. Your doctor will tell you why it has been prescribed for you.

ACE inhibitors like perindopril prevent your body from creating a hormone known as angiotensin II. They do this by blocking (inhibiting) a chemical called angiotensin-converting enzyme. This widens your blood vessels and helps to reduce the amount of water put back into your blood by your kidneys.

These actions help to decrease blood pressure in people who have blood pressure which is higher than normal. Although people with high blood pressure often do not feel unwell, if left untreated, high blood pressure can harm the heart and damage blood vessels, leading to a heart attack or stroke.

Heart failure is a condition where your heart does not work as well as it should. Because of this, there may be too much circulating fluid in your blood vessels. Perindopril helps to reduce this. It also appears to have a protective effect on the heart.

You may be prescribed perindopril on its own, or alongside other medicines to help your condition. The brand of perindopril called Coversyl® Arginine Plus contains a medicine called indapamide. This brand is prescribed for people with high blood pressure. Combination brands like this help to reduce the total number of tablets you need to take each day.

CLINICAL PHARMACOLOGY

PHARMACOKINETICS

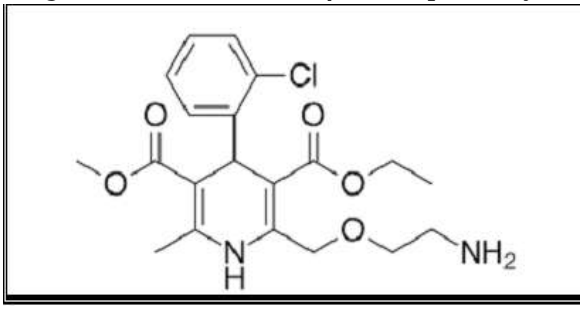
Absorption

After oral administration, perindopril undergoes extensive metabolic changes that include the formation of perindoprilat by hydrolysis. The bioavailability of perindopril ranges between 65.6 and 95.1%; 16.8% of an oral dose of perindopril is present in plasma as perindoprilat. Perindopril is 74% plasma protein-bound at steady-state concentrations and perindoprilat is 15% bound to plasma proteins.

AMLODIPINE BESYLATE

Amlodipine is in a group of drugs called calcium channel blockers. Amlodipine relaxes (widens) blood vessels and improves blood flow. Amlodipine is used to treat high blood pressure (hypertension) or chest pain (angina) and other conditions caused by coronary artery disease.

Fig 2: Chemical structure of **Amlodipine Besylate**



- ***Drug category:*** High Blood Pressure (Hypertension) Or Chest Pain (Angina) And Other Conditions Caused By Coronary Artery Disease.
- ***IUPAC name:*** Benzenesulfonic Acid;3-O-Ethyl 5-O-Methyl 2-(2-Aminoethoxymethyl)-4-(2-Chlorophenyl)-6-Methyl-1,4-Dihydropyridine-3,5-Dicarboxylate.
- ***Molecular Formula:*** $C_{26}H_{31}ClN_2O_8S$.
- ***Molecular Weight:*** 567.05094 g/mol.
- ***Half life:*** 30 to 50 Hours
- Melting point: 178-179 °C.

MECHANISM OF ACTION

Amlodipine is a dihydropyridine calcium antagonist (calcium ion antagonist or slow-channel blocker) that inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. Experimental data suggest that amlodipine binds to both

dihydropyridine and nondihydropyridine binding sites. The contractile processes of cardiac muscle and vascular smooth muscle are dependent upon the movement of extracellular calcium ions into these cells through specific ion channels. Amlodipine inhibits calcium ion influx across cell membranes selectively, with a greater effect on vascular smooth muscle cells than on cardiac muscle cells. Negative inotropic effects can be detected *in vitro* but such effects have not been seen in intact animals at therapeutic doses. Serum calcium concentration is not affected by amlodipine. Within the physiologic pH range, amlodipine is an ionized compound (pKa=8.6), and its kinetic interaction with the calcium channel receptor is characterized by a gradual rate of association and dissociationwith the receptor binding site, resulting in a gradual onset of effect.

Amlodipine is a periph eral arterial vasodilator that acts directly on vascular smooth muscle to cause a reduction in peripheral vascular resistance and reduction in blood pressure.

CLINICAL PHARMACOLOGY

PHARMACOKINETICS

After oral administration of therapeutic doses of NORVASC, absorption produces peak plasma concentrations between 6 and 12 hours. Absolute bioavailability has been estimated to be between 64 and 90%. The bioavailability of NORVASC is not altered by the presence of food.

Amlodipine is extensively (about 90%) converted to inactive metabolites via hepatic metabolism with 10% of the parent compound and 60% of the metabolites excreted in the urine. Ex vivo studies have shown that approximately 93% of the circulating drug is bound to plasma proteins in hypertensive patients. Elimination from the plasma is biphasic with a terminal elimination half-life of about 30–50 hours. Steady-state plasma levels of amlodipine are reached after 7 to 8 days of consecutive daily dosing.

III.OBJECTIVE:

To develop and validate RP-HPLC method for the determination of perindopril erbumine and amlodipine besilate dosage form and to develop and validate calibration curve method and derivative method for the determination of perindopril erbumine and amlodipine besilate in bulk and tablet dosage forms.

3.1 SPECIFIC AIMS:

To develop a simple, rapid and specific RP-HPLC method for the estimation of perindopril erbumine and amlodipine besilate in bulk and combined pharmaceutical dosage forms. To develop a simple, rapid, accurate eco friendly less cost methods for the determination of perindopril erbumine and amlodipine besilate in bulk and tablet dosage forms. To validate the proposed methods in accordance with the analytical parameters mentioned in the ICH guidelines, such as system suitability, accuracy, precision, specificity, linearity, robustness.

IV. MATERIALS AND INSTRUMENTS

REAGENTS AMD CHEMICALS:

Table 5: List of chemicals used in HPLC Method

S.No	Name	Grade	Manufacturer
1.	Water	HPLC	In house production
2.	Ortho Phosphoric Acid	HPLC	Merck
3.	Acetonitrile	HPLC	Merck
4.	Methanol	HPLC	Merck

INSTRUMENTS:

Table 6: List of Apparatus used in HPLC

S.No	Name	Model	Manufacturer
1.	pH meter	-	Eutech
2.	Weighing balance	-	Denver
3.	Ultrasonicator	UCA 701	Unichrome
4.	HPLC	Alliance	Waters 2695 - Empower software
5.	Flowrate	1mL/min	-
6.	Pump	Isocratic model	-

V. EXPERIMENTAL WORK

5.1 DETERMENATION OF WORKING WAVELENGTH (λ_{max})

The wavelength of maximum absorption of the solution of the drug in acetonitrile were scanned using Photodiode spectrophotometer within the wavelength region of 200–400 nm against acetonitrile as blank. The spectra of drug shows at 210 nm **(Fig.3)**, Thus 220 nm was selected as detector wavelength for the HPLC chromatographic method.

CHROMATOGRAPHIC CONDITIONS

During the selection of chromatographic conditions number of trails were carried out and the best trail was selected for optimized method.

Trails in optimization of chromatographic conditions

TRAIL-1

Chromatographic conditions

Column: Luna C18 250mmx4.6mm, 5µ particle size
Elution mode: Isocratic
Mobile phase: Water : Buffer (15 : 85) (v/v)
Flow rate: 1.0 mL /min
Detection wavelength: 190-400 nm
Injection volume: 20 µL
Run time: 10 min

Preparation of standard stock solution:

Accurately each 50 mg of perindopril erbumine and amlodipine besilate was weighed and transferred into 100 mL volumetric flasks and made up the volume with mobile phase. From above stock solution 100 µg/mL of perindopril erbumine and amlodipine besilate was prepared by transferring 5mL of stock into a 50 mL volumetric flask and diluted with mobile phase. This solution was used for recording chromatogram.

Observation

Peak retention time is very low (**fig : 4**) and it was not clear, because of that this trail was not considered

TRAIL-2

Chromatographic conditions

Column: Luna C18 250mmx4.6mm, 5µ particle size
Elution mode: Isocratic
Mobile phase: Buffer : ACN (20 : 80) (v/v)
Flow rate: 1.0 mL /min
Detection wavelength: 210 nm
Injection volume: 20 µL
Run time: 12 min

Observation

Peak was broad (**fig :5**). So this trail was not considered.

TRAIL-3

Chromatographic conditions

Buffer: 1ml OPA is dissolved in 1lt water.
Column: Luna C18 250mmx4.6mm, 5µ particle size
Elution mode: Isocratic
Mobile phase: Buffer : ACN(22 : 78) (v/v)
Flow rate: 1.0 mL /min
Detection wavelength: 210 nm
Injection volume: 10 µL
Run time: 12 min

Observation

Peak is eluted fastly (**fig :6**) . So, this trail was not considered.

TRAIL-4

Chromatographic conditions

Buffer: 1ml OPA is dissolved in 1lt water.
Column: Luna C18 250mmx4.6mm, 5µ particle size
Elution mode: Isocratic
Mobile phase: Buffer : ACN (25 : 75) (v/v)
Flow rate: 1.0 mL /min
Detection wavelength: 210 nm
Injection volume: 10 µL
Run time: 12 min

Observation

Peak is not clear (**fig : 7**) because of that this trail was not considered.

TRAIL-5

Chromatographic conditions

Buffer: 1ml OPA is dissolved in 1lt water.
Column: Luna C18 250mmx4.6mm, 5µ particle size
Elution mode: Isocratic
Mobile phase: Buffer : ACN (30 : 70) (v/v)
Flow rate: 1.0 mL /min
Detection wavelength: 210 nm
Injection volume: 10 µL
Run time: 12 min

Observation

Peak is eluted fastly (**fig: 8**), because of that this trail was not considered.

TRAIL-8 (OPTIMIZED METHOD)

Chromatographic conditions

Buffer: 1ml OPA is dissolved in 1lt water.
Column: Luna C18 250mmx4.6mm, 5µ particle size
Elution mode: Isocratic
Mobile phase: Buffer : ACN (50 : 50) (v/v)
Flow rate: 1.0 mL /min
Detection wavelength: 210 nm
Injection volume: 20 µL
Run time: 10 min

Observation:

A sharp pin pointed peak 5.649 (**fig : 11**) was observed, so this trail was considered.at ***Conclusion:***The perindopril erbumin was observed at 3.753 min with peak area 1200084, theoretical plates 3199 and tailing factor 1.59 and Amlodipine Besilate was observed at 5.042 min with peak area 850702, theoretical plates 3768 and tailing

factor 1.48 . Because of the satisfactory results, less retention time, this trial was optimized.

%ASSAY OF FORMULATION:

% Assay of Perindopril erbumine and Amlodipine besilate was carried out in tablet formulation with 100 µg/mL and 150 µg/mL results were calculated by using the formula given below and reported in

Test area x STD weight x Test dilution x Avg. Weight x Potency x 100

= STD area x test weight x STD dilution x label claim x 100

5.4 GENERAL PREPARATIONS

Preparation of Buffer

1ml Ortho Phosphoric acid was dissolved in 1lt Water was Filtered through 0.45µ membrane filter.

Preparation of Standard Solution and sample solution:

Weigh accurately about 5 mg of Perindopril Erbumine working standard and 6.25 mg of Amlodipine Besilate working standard into a 100 mL volumetric flask. Add 80 mL of diluent, sonicate to dissolve and dilute to volume with diluent. Further dilute 1mL of the above solution to 10 mL with the diluent. Further dilute 1mL of the above solution to 10 mL with the diluent.

Preparation of Sample solution:

Weigh 10 tablets and take average weight, then crush the tablets to powder form after take one tablet equivalent weight to weigh accurately about 11.5 mg of sample taken into a 100 mL volumetric flask. Add 70 mL of diluent, sonicate to dissolve and dilute to volume diluent. Further dilute 1 mL to 10 mL with the diluent. Further dilute 1 mL to 10 mL with the diluent. Filter through 0.45µ Nylon syringe filter.

5.5 METHOD VALIDATION

The validation of HPLC method for the determination of Perindopril Erbumine and Amlodipine Besilate as per the protocol and to demonstrate that the method is appropriate for its intended use was studied for the following parameters. All the validation parameters were carried out according to ICH.

System suitability:

The system suitability of developed method was conducted through the validation studies by using 5+6.25 µg/mL Perindopril Erbumine and Amlodipine Besilate. System suitability prior to analysis was investigated by checking parameters like tailing factor, retention times and number of theoretical plates. The results were found to be within the limits.

Acceptance Criteria

1. The no. of Theoretical plates should not be less than 3000.
2. The Tailing factor should not be more than 2.0
3. The %RSD should not more than 2.0

Linearity and Range

Linearity of an analytical method is its ability to elicit the test results that are directly, or by well-defined mathematical transformation, proportional to the concentration of analyte in sample within a given range. Linear correlation was obtained between peak area Vs concentration of Perindopril Erbumine and Amlodipine Besilate were in the range of 0.5-7.5µg/mL and 0.625-9.375. The linearity of the calibration curve was validated by the high value of correlation co-efficient of regression equation.

The Range of an analytical method is the interval between the upper and lower levels of analyte (including these levels) that have been demonstrated with precision, accuracy and linearity.

Acceptance Criteria:

Correlation coefficient should be not less than 0.999.

VI. RESULTS AND DISCUSSION

HPLC METHOD

Determination of Working Wavelength (λ_{max})

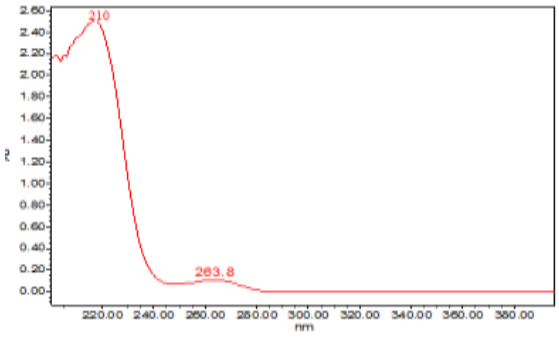


Figure 3: PDA-Spectrum of Perindopril Erbumine and Amlodipine Besilate

Optimization of chromatographic conditions

Method Development Trails

Trail-1

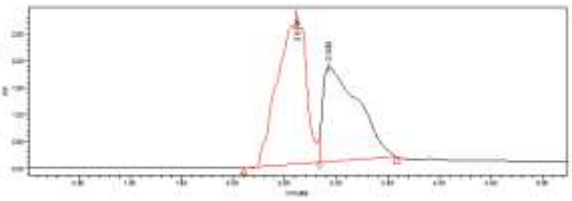


Figure 4 : Chromatogram of Trial -1

Trial -2

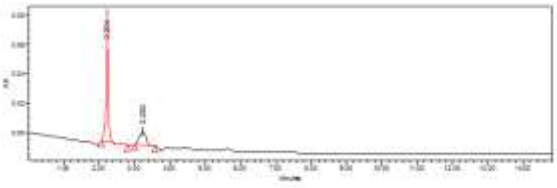


Figure 5 : Chromatogram of Trial -2

Trial -3

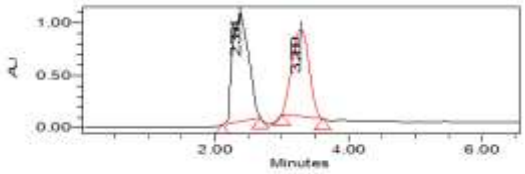


Figure 6: Chromatogram of Trial -3

Trial -4

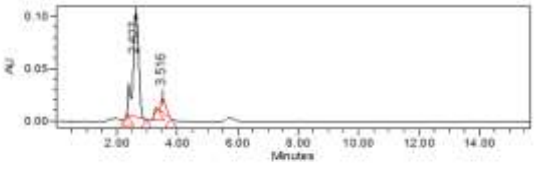


Figure 7: Chromatogram of Trial -4

Trial -5

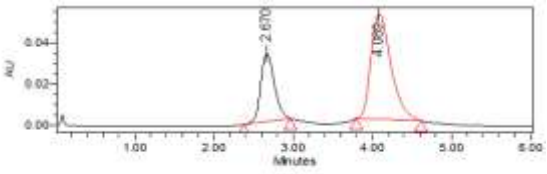


Figure 8: Chromatogram of Trial -5

Trial -6

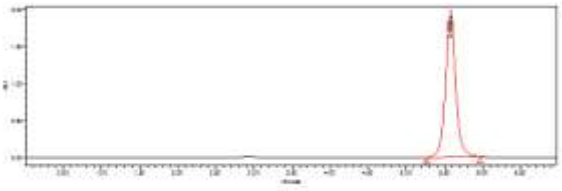


Figure 9: Chromatogram of Trial -6

Trial -7

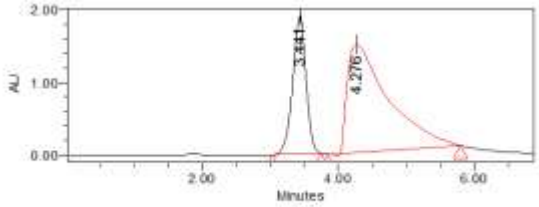


Figure 10: Chromatogram of Trial -7

Trail 8: (optimized trail)

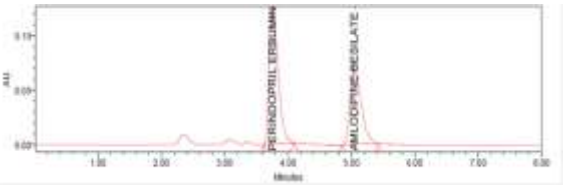


Figure 11: Chromatogram of Trial -8

Table 7: Optimized chromatography

	Name	Retention Time	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
1	Perndopril Erumine	3.753	1200084	68.25		0.87	5388
2	Amlodipine Besilate	5.042	850702	32.38	2.16	1.58	4058

Table 8: Optimized chromatographic conditions

PARAMETERS	OBSERVATION
ELUTION	Isocratic
TEMPERATURE	Ambient
MOBILE PHASE	Buffer : Acetonitile (50:50)(v/v).
COLUMN	Luna C18, 250mm x 4.6mm, 5µm.
DETECTION WAVE LENGTH	210 nm
FLOW RATE	1 mL/min
RUNTIME	8 min

Assay:

Table 9:% Assay of Perndopril Erumine and Amlodipine Besilate

Drug	Avg std area (n=6)	Avg sample area(n=6)	Avg wt of tab (mg)	Stdwt (mg)	Sample wt(mg)	Lable amount (mg)	Std purity	Amount found (mg)	% assay
Perndopril Erumine	1200084	12432132	542.3	100.8	782.3	590	100.1	100.1	100.3
Amlodipine Besilate	850702	2783328	542.3	100.4	782.3	425	100.1	100.1	100.3

6.2. ANALYTICAL METHOD VALIDATION (HPLC)

The method was validated for its linearity range, accuracy, precision. Method validation is carried out as per ICH guidelines

System Suitability Studies:

Table 10: System suitability parameters

S.No	Drug	Retention time(min)	Plate count	Tailing factor
1	Perndopril Erumine	3.753	3199	1.18
2	Amlodipine Besilate	5.042	3768	1.04

Linearity:

Linearity chromatograms of Perndopril Erumine and Amlodipine Besilate:

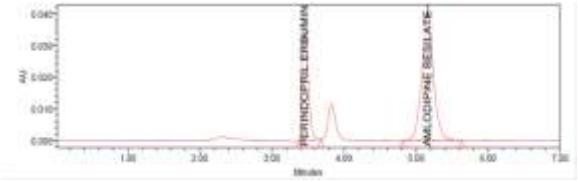


Figure 12: Chromatogram of Linearity-1

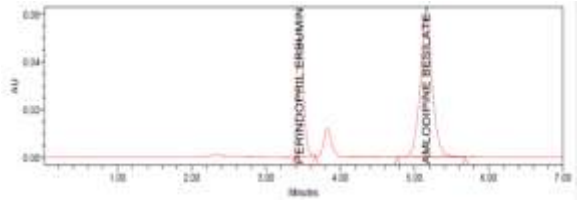


Figure 13 : Chromatogram of Linearity-2

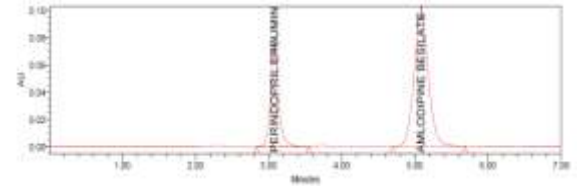


Figure 14 : Chromatogram of Linearity-3

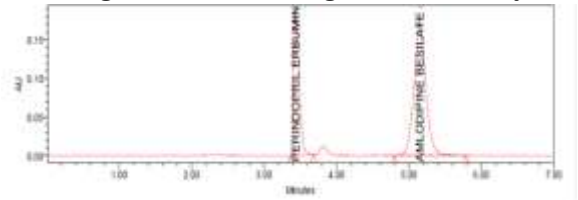


Figure 15 : Chromatogram of Linearity-4

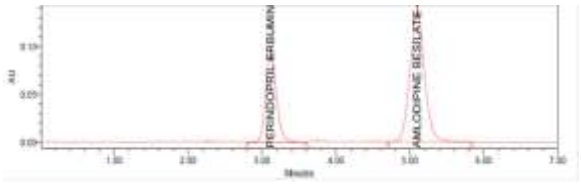


Figure 16 : Chromatogram of Linearity-5

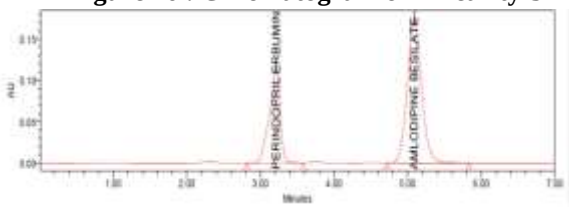


Figure 17 : Chromatogram of Linearity-6

Table 11: Results of linearity for Perindopril Erbumine

S.No	Perindopril Erbumine	
	Conc (µg/mL)	Peak area
1	0.50	101976
2	1.25	274826
3	2.50	525571
4	5.00	1044111
5	6.25	1274816
6	7.50	1528456
Regression equation	y = 203654x-9386.3	
Slope	203654.44	
Intercept	9386.26	
R^2	0.9997	

Linearity of Perindopril Erbumine:

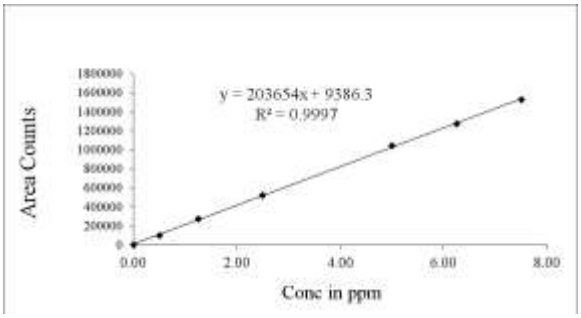


Figure 20 : calibration curve for Perindopril Erbumine at 210 nm

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Volume 04 Issue 01
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Table 11: Results of linearity for Amlodipine Besilate

S.No	Amlodipine Besilate	
	Conc.(µg/mL)	Peak area
1	0.78	197456
2	1.56	487403
3	3.12	988463
4	6.25	1944416
5	7.81	2387463
6	9.37	2847856
Regression equation	y = 304419x+14437	
Slope	304419.48	
Intercept	14438.69	
R^2	0.999	

Linearity of Amlodipine Besilate:

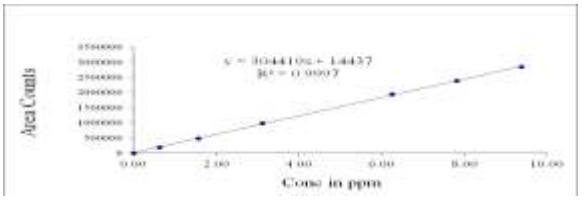


Figure 20 : calibration curve for Amlodipine Besilate at 210 nm

Accuracy:

Table 12: Accuracy results of Perindopril Erbumine by RP-HPLC method

S.NO	%Level of Std	Conc. Of working std. Added (µg/mL)	Peak area	Amount recovered	% recovery	Mean recovery	% R.S.D
1	2.5	2.5+250	504856	100.51	100.01	100.32	0.0584
			504863				
			502746				
2	5	5+500	1044111	100.04	100.58		
			1048521				
			1047563				
3	7.5	7.5+750	1587465	100.53	100.07		
			1588632				
			1587632				

Table 13: Accuracy results of Amlodipine Besilate by RP-HPLC method

S.NO	%Level of Std	Conc. Of working std. Added (µg/mL)	Peak area	Amount recovered	% recovery	Mean recovery	% R.S.D
1	2.5	3.125+250	984856	100.44	100.01	100.88	0.0586
			984863				
			982746				
2	5	6.25+500	1944111	100.84	100.42		
			1948521				
			1947563				
3	7.5	7.5+750	2928465	100.52	100.18		
			2928632				
			2927632				

Precision:

Table 13 : Precision studies by RP-HPLC method

S.NO	TYPE	Amlodipine Besilate		
		Mean area(n=6)	Std. deviation	%RSD
1	System precision	1956779	9700.89	1.269
2	Method precision	1955752	9462.25	0.383
3	Intermediate precision	1985472	7954.89	1.673

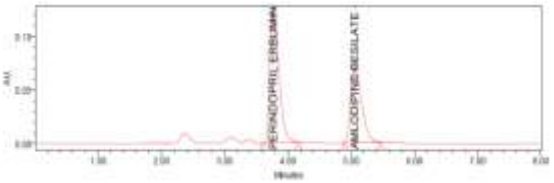
Ruggedness and Robustness:

Table 14 (i): Results of Ruggedness study by RP-HPLC

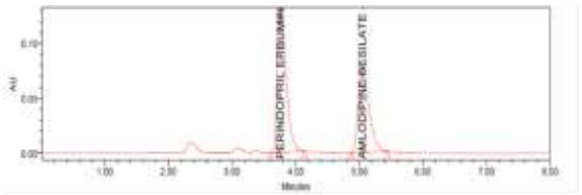
S.No	Parameter	Perindopril Erbumine	Limit
1	%RSD	1.450	NMT 2.0%

S.No	Parameter	Amlodipine Besilate	Limit
1	%RSD	1.124	NMT 2.0%

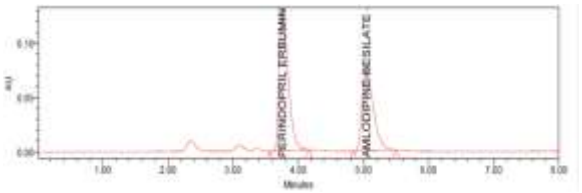
STABILITY:



(a) Chromatogram of stability at Initial



(b) Chromatogram of stability at 6 hrs



(c)Chromatogram of stability at 12 hrs

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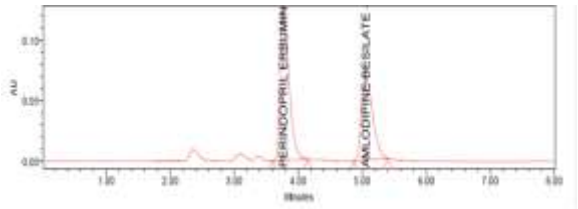
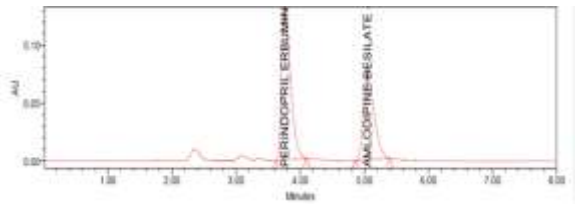


(d)Chromatogram of at 18 hrs



(e) Chromatogram of at 24 hrs

Table 15:Results of stability study

Time period (hours)	Perindopril Erbumine			
	Retention time	Peak area	Tailing factor	Plate count
Initial	3.767	1212375	1.63	3305
6 Hrs	3.760	1251718	1.63	3301
12 Hrs	3.754	1252040	1.62	3291
18Hrs	3.767	1209441	1.63	3789
24 Hrs	3.760	1243155	1.61	3313

Table 16:Results of stability study

Time period (hours)	Amlodipine Besilate			
	Retention time	Peak area	Tailing factor	Plate count
Initial	5.054	844774	1.34	3780
6 Hrs	5.046	888058	1.52	3833
12 Hrs	5.046	880327	1.53	3849
18Hrs	5.054	842147	1.46	3789
24 Hrs	5.046	876407	1.48	3868

Forced Degradation study:

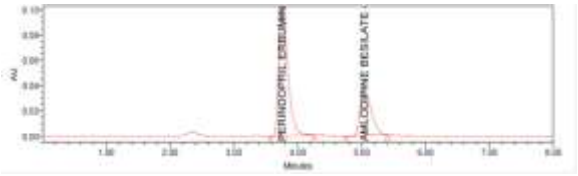


Figure 22(a): Chromatogram of acid degradation

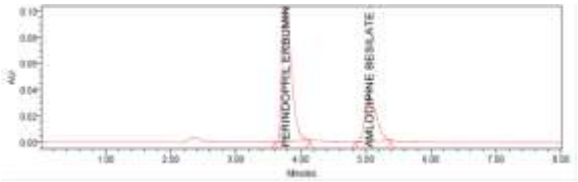


Figure 22(b): Chromatogram of alkali degradation

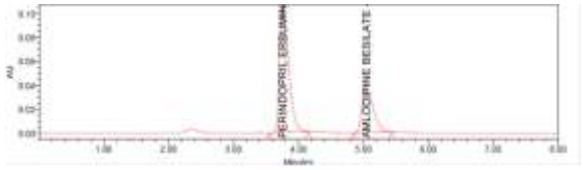


Figure 22(c): Chromatogram of peroxide degradation

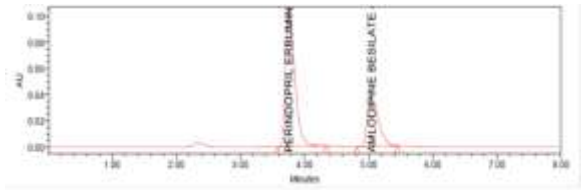


Figure 22(d): Chromatogram of reduction degradation

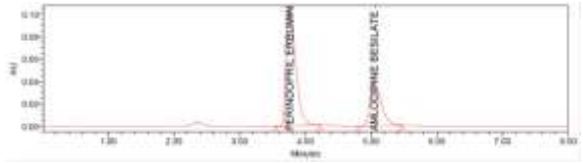


Figure 22(e): Chromatogram of thermal degradation

8. SUMMARY AND CONCLUSION

SUMMARY

An attempt has been made to develop a new stability indicating validated RP-HPLC method for the estimation of perindopril erbumine and amlodipine besilate in bulk and in dosage form. As the literature survey revealed that only two methods are available for estimation of perindopril erbumine and amlodipine besilate in bulk and in dosage forms so there is a need for a simple, economical and proper method of estimation of perindopril erbumine and amlodipine besilate bulk and in dosage form.

Luna C18, 250mm x 4.6mm, 5µm column with UV detector with an injection volume of 10 µL was injected and eluted with the mobile phase containing buffer : acetonitrile (50: 50v/v). This is pumped at a flow rate of 1mL/min and detected by UV (210 nm) detector. The peaks of perindopril erbumine and amlodipine besilate were eluted at retention times of 3.75 and 5.042 min respectively.

After method was developed, it was validated according to ICH guidelines for system suitability,

specificity and linearity, sensitivity parameters, precision, accuracy and robustness studies. the validation results were found well within the limits(%RSD of areas were<2 for assay and recoveries in the range of 98%-102% for assay,r^2>0.999) indicating that the developed method is simple, rapid, accurate, precise, specific, robust and economical and less time consuming.

CONCLUSION:

The proposed RP-HPLC, method were suitable methods for the determination of perindopril erbumine and amlodipine besilate in dosage forms. All the parameters of developed methods met the criteria of ICH guidelines for method validation.

The developed HPLC method has the following advantages: No tedious extraction procedures were involved. These methods are also having an advantage than reported method of good resolution and with retention time.The developed method has good recovery and sensitivity. The run time required for recording chromatogram was below 10.0mins. Suitable for the analysis of raw materials and formulations. Hence, the developed chromatographic method for perindopril erbumine and amlodipine besilate is said to be rapid, simple, precise, accurate, specific and cost effective that can be effectively applied for the routine analysis.

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