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Development and Validation of Stability Indicating RP-HPLC Method for Amlodipine Besylate and Perindopril Arginine in Synthetic Formulation

Priya R. Rajput¹, Atul Bendale², Shailesh V. Luhar³, Dr. Sachin B. Narkhede⁴

1. M. pharm Student, Smt. B.N.B Swaminarayan Pharmacy College, Salvav, Vapi-396191, Gujarat, India

2. Assistant Professor of Department of Quality Assurance, Smt. B.N.B Swaminarayan Pharmacy College, Salvav, Vapi-396191, Gujarat, India

3. Head of the department of quality assurance department, Smt. B.N.B Swaminarayan Pharmacy College, Salvav, Vapi-396191, Gujarat, India

4. Principal of Smt. B.N.B Swaminarayan Pharmacy College, Salvav, Vapi-396191, Gujarat, India

ABSTRACT:

A simple, rapid, precise and accurate stability-indicating RP-HPLC method was developed and validated for the simultaneous determination of Amlodipine Besylate (AMD) and Perindopril Arginine (PRD) pharmaceutical dosage form. Method was carried out by using Sheisedo C18 (250 * 4.6 mm, 5µm) column and Acetonitrile : Methanol : water (30:40:30 % v/v/v) as mobile phase at 1.0 ml/min flow rate. Detection was carried out at 227nm. Rt was found to be 3.90 min for AMD and 4.90 min for PRD. For stability study drugs were subjected to acid hydrolysis, alkaline hydrolysis, oxidative degradation and thermal degradation. AMD was highly susceptible to acidic and thermal condition. Pharmaceutical dosage form was more stable than active pharmaceutical ingredient. The linearity of the proposed method was investigated in the range of 50-250 µg/ml (r²= 0.997) for AMD and 50-250 µg/ml (r²= 0.998) for PRD. The limit of detection were 0.115µg/ml and 0.146µg/ml and the limit of quantification were 0.349µg/ml and 0.442µg/ml for AMD and PRD respectively.

KEY WORDS: Amlodipine Besylate, Perindopril Arginine, Stability indicating RP-HPLC Method, Validation

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*For Correspondence:

Priya R. Rajput

M. pharm Student, Smt. B.N.B Swaminarayan Pharmacy College, Salvav, Vapi-396191, Gujarat, India.

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1. INTRODUCTION^[1-6]

Amlodipine Besylate 3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl) 6-methyl-1,4-dihydropyridin dicarboxylate. It is dihydropyridine calcium channel blocker and used for treatment of antihypertensive. It is official in Indian pharmacopeia 2010. It is freely soluble in methanol. Molecular weight of Amlodipine Besylate is 567.050 gm/mol and formula is C₂₆H₃₁ClN₂O₈S.

Perindopril Arginine is chemically L-arginine (2S,3aS,7aS)-1-[(2S)-2-[[[(1S)-(ethoxycarbonyl) butyl] amino]propanoyl] octahydro-1H-indole-2-carboxylate. It is the Competitive inhibitor of ACE. It is not official in any Pharmacopeia. It is slightly soluble in Readily soluble in purified water. Molecular weight of Perindopril Arginine is 537.4 gm/mol and formula is C₁₉H₃₂N₂O₅•C₆H₁₄N₄O₂.

Literature Review reveals that there was no reported Stability Indicating RP-HPLC method for Simultaneous Estimation of Amlodipine Besylate and Perindopril Arginine in combined dosage form. So the present work is aimed for To develop an accurate, specific, repeatable Stability Indicating RP-HPLC

method for Simultaneous estimation of Amlodipine Besylate and Perindopril Arginine in synthetic formulation.

2. MATERIALS AND METHODS

2.1. Method development:

2.1.1 Materials:

Amlodipine Besylate was obtained as gift sample from Alkem laboratories, Ltd., Vapi. Perindopril Arginine was obtained as gift sample from Arti Pharmaceuticals, Mumbai. Methanol, Acetonitrile, Water and Ortho phosphoric acid – HPLC grade were purchased from Ran Kem Lab.

2.1.2 Instrumentation and Chromatographic method:

The analysis of the drug was carried out on a Peak HPLC system equipped with a reverse phase Shisedo C₁₈ column, peak pump with auto sampler and a detector running on Peak LC Solution Software. The mobile phase consists of Acetonitrile:Methanol: Water (30:40: 30% v/v/v) (pH 5.4) and the flow rate were maintained at 1.0 ml/min. The mobile phase was freshly prepared and passed through nylon membrane filter of pore size of 0.45µm and it was degassed by sonicating for 10min. before it was used. The elution was monitored at wavelength of 227 nm with UV detector, and the injection volume was 10µl.

2.1.3 Determination of maximum absorbance:

The standard solutions of Amlodipine Besylate and Perindopril Arginine were scanned in the range of 200-400 nm against mobile phase as blank. Isobestic point of Amlodipine Besylate and Perindopril Arginine at 227nm. Thus the wavelength selected for the determination of Amlodipine Besylate and Perindopril Arginine was 227nm.

2.1.4 Preparation of stock and standard solutions:

Accurately weighed 100mg of Amlodipine Besylate and 50mg of Perindopril Arginine were dissolved in 100 ml volumetric flask containing 100 ml of Methanol which is considered as stock solution. Working standard solution of Amlodipine Besylate and Desloratidine were prepared by making various dilutions of the drug solution from the stock solution. Five sets of the drug solution were prepared in the mobile phase containing 50-250µg/ml of

Amlodipine Besylate and 50-250 µg/ml of Perindopril Arginine. Each of this drug solution was injected into the column and the peak area and retention time was recorded.

2.1.5 Assay of marketed formulation (Brand name of tablet – Ventidox - DL):

Twenty tablets were weighed and average weight of a single tablet was calculated. Tablets were crushed and mixed using a mortar and pestle. Then drug sample equivalent to 10mg of Amlodipine Besylate and 15mg of Perindopril Arginine were accurately weighed and transferred into a 100ml volumetric flask and mixed with known amount of methanol and the active pharmaceutical ingredients were extracted into the methanol followed by ultra-sonication and then filtered through a nylon membrane of pore size 0.45µm. The drug sample was diluted by adding methanol to obtain a stock solution of 100µg/ml of Amlodipine Besylate and 150 µg/ml of Perindopril Arginine.

2.2 Method validation^[6]

The Proposed method was validated according to ICH guidelines. The parameters assessed were linearity, precision, accuracy, LOD and LOQ.

2.2.1 System Suitability

System suitability tests are an integral part of liquid chromatography. They are used to verify that resolution and reproducibility of chromatography system are adequate for the analysis to be done. System Suitability was performed on standard solution and system suitability parameters were calculated at the start of study for each parameter.

2.2.2 Linearity and Range.

The linearity was determined at Three levels over the range of 50-250 µg/ml Amlodipine Besylate and 50 -250 µg/ml Perindopril Arginine. Peak area of above linearity solution preparations were taken at each concentration three times.

2.2.3 Accuracy

Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (80%, 100% and 120%) taking into consideration percentage purity of added bulk drug samples. These

solutions were subjected to re-analysis by the proposed method and Results are Calculated.

2.2.4 Precision

A. Repeatability Study:

Standard solutions of 100,150,200 µg/ml Amlodipine Besylate and 100, 150, 200 µg/ml Perindopril Arginine were prepared and chromatograms were recorded. Area was measured of the same concentration solution three times and %RSD was calculated.

B. Intra-day precision

Mixed solutions containing 100,150,250 µg/ml Amlodipine Besylate and 100, 150, 200 µg/ml Perindopril Arginine were analyzed three times on the same day, % R.S.D was calculated.

C. Inter-day precision

Mixed solutions containing 100,150,250 µg/ml Amlodipine Besylate and 100,150,200µg/ml Perindopril Arginine were analyzed on three different days and % R.S.D was calculated.

2.2.5 Limit of Detection and Limits of Quantitation

Limit of Detection (LOD)

From the linearity curve equation, the standard deviation (SD) of the intercepts (response) was calculated. The limit of detection (LOD) of the drug was calculated by using the following equation designated by International Conference on Harmonization (ICH) guideline:

$$\text{LOD} = 3.3 \times \text{Intercept} / \text{Slope}$$

Limit of Quantitation (LOQ)

The limit of quantitation (LOQ) of the drug was calculated by using the following equation designated by International Conference on Harmonization (ICH) guideline:

$$\text{LOQ} = 10 \times \text{Intercept} / \text{Slope}$$

2.2.6 Robustness

The robustness of the method was established by making deliberate minor variations in the following method parameters

- a) pH of mobile phase: ± 0.2
- b) Flow rate : ± 0.2 ml/min
- c) Change in the ratio of component in the mobile phase: $\pm 2\%$.

2.3 Stability studies^[7]

Stability Studies was carried out on the drug in order to check the stability of the drug by providing various stress conditions like acid, base, oxidation and thermal degradation compared with normal conditions. The purpose of force degradation method is to provide evidence that the analytical method is efficient in determination of drug substances in commercial drug product in the presence of its degradation products.

2.3.1 Acidic hydrolysis

Take 1ml solution of AMD 1000 µg/ml and 1.5 ML of PRD 1000 µg/ml, 2 ml of 0.1M HCl was added. The solution was heated for 1 hr at 60°C and transferred to a 10ml volumetric flask, cooled, neutralized by 0.1M NaOH and diluted up to mark with methanol to get final concentration 100 µg/ml of AMD and 150 µg/ml of PRD.

2.3.2 Alkaline hydrolysis

Take 1 ml solution of AMD 1000 µg/ml and 1.5ml of PRD Arginine 1000 µg/ml, 2 ml of 0.1M NaOH was added. The solution was heated for 1 hr at 60°C and transferred to a 10ml volumetric flask, cooled, neutralized by 0.1M HCl and diluted up to mark with methanol to get final concentration 100 µg/ml of AMD and 150 µg/ml of PRD.

2.3.3 Oxidative degradation

Take 1 ml solution of AMD 1000 µg/ml and 1.5 ml of PRD 1000 µg/ml, 2 ml 6% H₂O₂ was added at room temperature for 4 hours at 60°C and transferred to a 10ml volumetric flask, cooled diluted up to mark with methanol to get final concentration 100 µg/ml of AMD and 150 µg/ml of PRD.

2.3.4 Thermal degradation

Take 1 ml solution of AMD 1000 µg/ml and 1.5ml of PRD 1000 µg/ml, heat the solution for 2 hr at 80°C and transferred to a 10ml volumetric flask, cooled diluted up to mark with methanol to get final concentration 100 µg/ml of AMD and 150 µg/ml of PRD.

3. RESULTS AND DISCUSSION

3.1 Linearity: The calibration curve showed (Fig.7) good linearity in the range of 50--250µg/ml for Amlodipine Besylate and 50-250µg/ml for Perindopril Arginine with correlation coefficient (r^2) of 0.997 and 0.998 for Amlodipine Besylate and Perindopril Arginine respectively. Results are given in Table 6.

3.2 Recovery: Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (80%, 100% and 120%) taking into consideration percentage purity of added bulk drug samples. And % recovery values found to be in the range of 99.27% - 101.40% for Amlodipine Besylate and 99.29 - 101.13% for. The result are given in table 7.

3.3 Precision: Intraday precision was carried out using test samples analyzed on the same day. Interday precision was assessed by analysis of the same solutions on consecutive days. The % RSD value were below 2 indicate that the method is precise. The results are given in table 8, 9 and 10.

3.4 Robustness: Small deliberate changes in chromatographic conditions such as change in mobile phase ratio ($\pm 2\%$), change in pH (± 2 units) and flow rate (± 2 units) were studied to determine the robustness of the method. The results were in favor of (% RSD < 2%) the developed RP-HPLC method for the analysis of Amlodipine Besylate and Perindopril Arginine. The results are given in table 12 and 13.

3.5 Limit of Detection (LOD) and Limit of Quantification (LOQ):

The LOD was found to be 0.115µg/ml and the LOQ 0.349 µg/ml for Amlodipine Besylate and the LOD was found to be 0.146µg/ml and the LOQ 0.442µg/ml for Perindopril Arginine estimated by using the standard formulas.

3.6 Stability studies: Stability indicating RP - HPLC method was performed in different stress condition using the Acetonitrile : Methanol : water (30:40:30 % v/v/v) as mobile phase suggested the following degradation behavior.

The chromatograms obtained on stress degradation, like photolytic degradation and similarly other conditions were shown in figure 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 and 19.

3.7 DISCUSSION

RP-HPLC method was found to be linear over the range of 50-250 µg/ml for Amlodipine Besylate and 50 - 250 µg/ml for Perindopril Arginine. The method has been validated by studying accuracy and precision, LOD, LOQ and system suitability according to ICH guideline. The Stability study of Amlodipine Besylate and Perindopril Arginine indicates that the drug significantly degrade under acidic and thermal conditions.

4. CONCLUSION:

A simple, economic, accurate and robust RP-HPLC method has been developed and validated for the estimation of Amlodipine Besylate and Perindopril Arginine bulk and pharmaceutical dosage form. The reverse phase liquid chromatography was performed using Shiseido-C₁₈ (250mm x 4.6mm, 5 µm) column. The mobile phase used was Acetonitrile: Methanol: Water (pH-5.4) (30:40:30 %v/v/v) with flow rate 1 ml/min. The detection was carried out at 227nm. The retention time were found be 3.90 ± 0.01 min and 4.90 ± 0.01 min for Amlodipine Besylate and Perindopril Arginine respectively. There was no interference from any excipients in the determination of drugs in dosage form which indicates the method is specific. All method validation parameters lie within its acceptance criteria as per ICH Q2 (R1) guideline so we can conclude that method is simple, linear, accurate and precise. Hence, it can be successfully used for the routine analysis of Amlodipine Besylate and Perindopril Arginine pharmaceutical dosage forms.

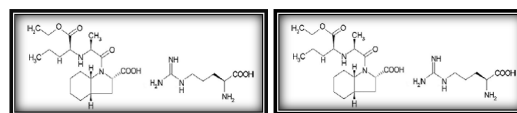
Stability indicating RP - HPLC method has been developed for Amlodipine Besylate and Perindopril Arginine pharmaceutical dosage form. Amlodipine Besylate is easily degrade in acidic and thermal condition while Perindopril Arginine was slightly degrade. Perindopril Arginine is more stable than Amlodipine Besylate in various stress condition. From the result, We can conclude that Pharmaceutical dosage form was more stable than Active pharmaceutical ingredient.

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TABLES AND FIGURES



Amlodipine Besylate Perindopril Arginine

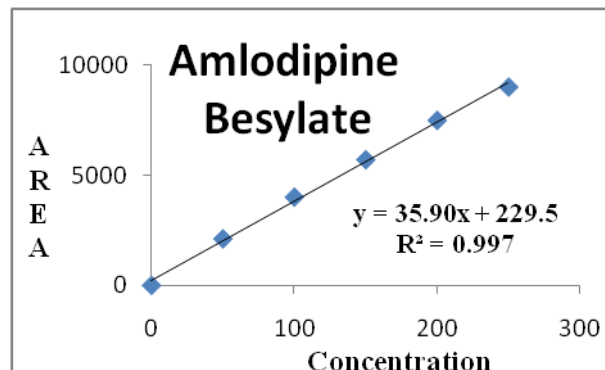


Figure 4 Calibration curve of Amlodipine Besylate

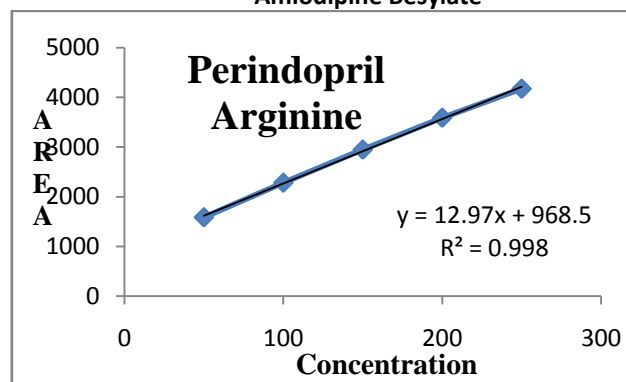


Figure 5 Calibration curve of Perindopril Arginine

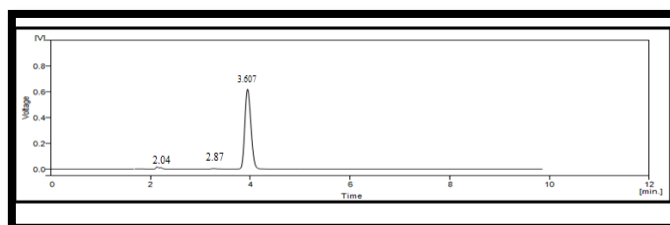
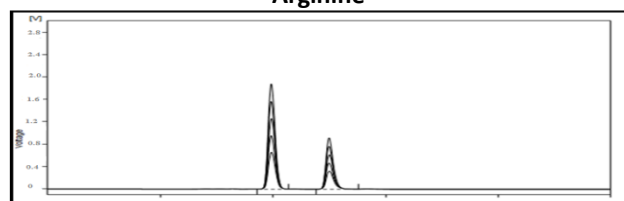


Figure 8 Acid hydrolysis peak of Amlodipine Besylate

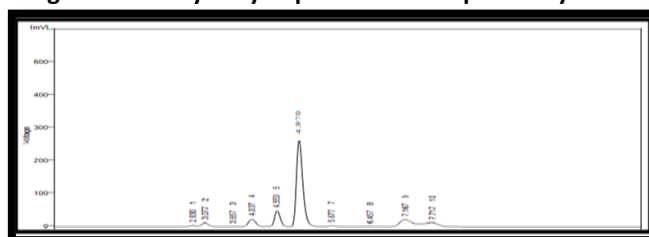


Figure 9 Acid hydrolysis peak of Perindopril Arginine

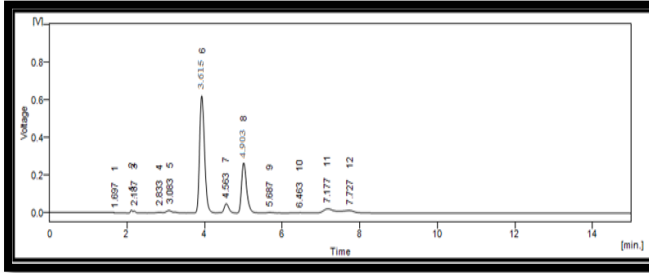


Figure 10 Acid hydrolysis peak of Pharmaceutical dosage form

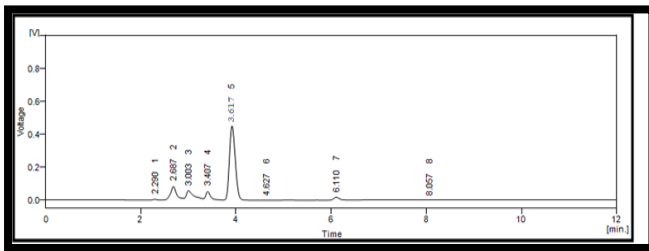


Figure 11 Alkali hydrolysis peak of Amlodipine Besylate

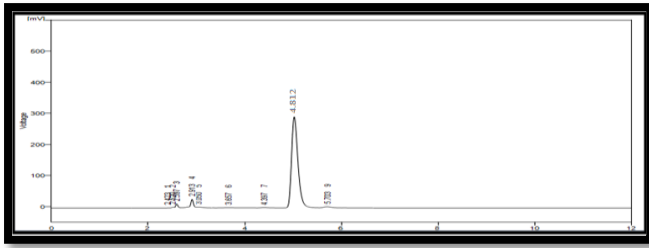


Figure 12 Alkali hydrolysis peak of Perindopril Arginine

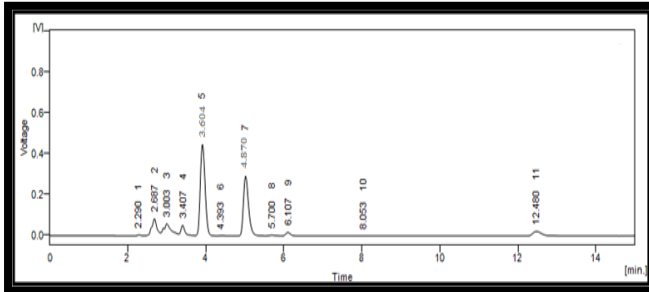


Figure 13 Alkali hydrolysis peak of Pharmaceutical dosage form

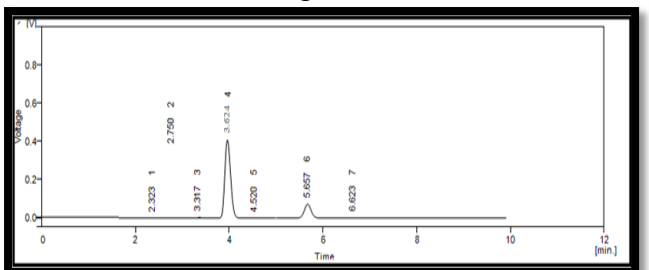


Figure 14 Oxidative degradation peak of Amlodipine Besylate

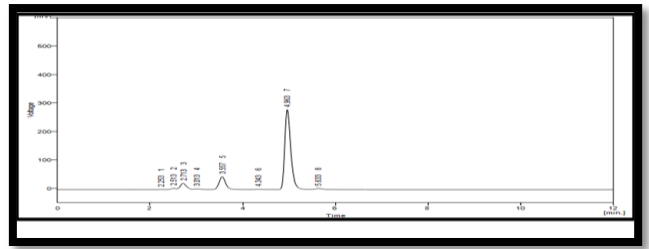


Figure 15 Oxidative degradation peak of Perindopril Arginine

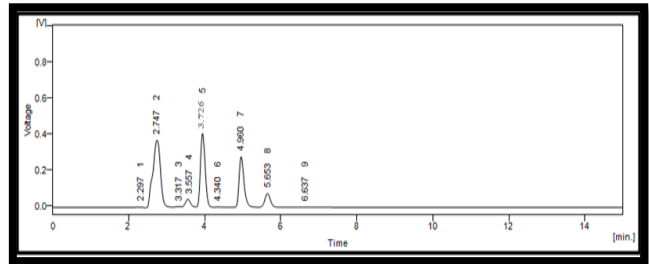


Figure 16 Oxidative degradation peak of Pharmaceutical dosage form

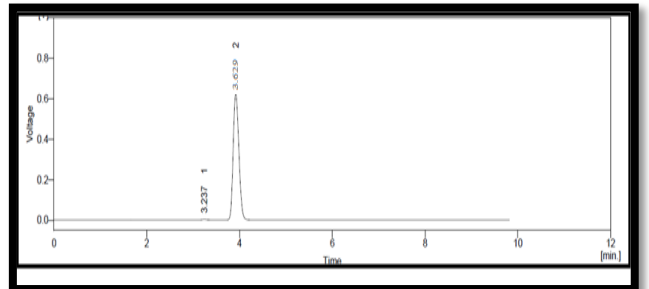


Figure 17 Thermal degradation peak of Amlodipine Besylate

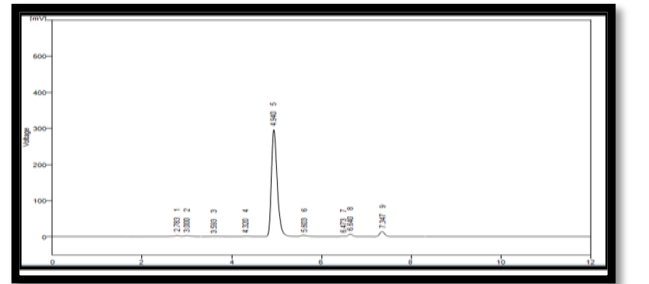


Figure 18 Thermal degradation peak of Perindopril Arginine

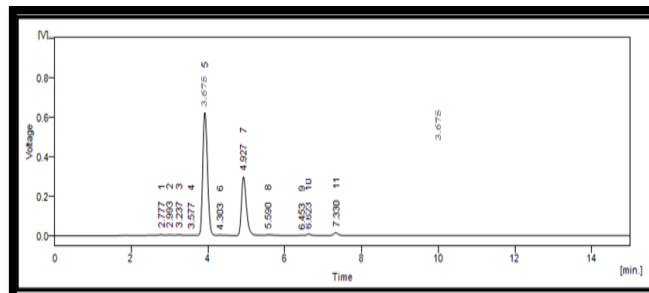


Figure 19 Thermal degradation peak of Pharmaceutical dosage form

Parameters	Specifications
Column	Sheisedo C ₁₈ (250mm*4.6mm, 5µm)
Mobile phase	Acetonitrile : Methanol :Water (35 : 40 : 25 % V/V/V) pH 3.2
Flow rate	1 ml/min
Run time	10 min
Detection wavelength	227 nm
Retention time	3.90 min for AMD and 4.90 min for PRD

Table 1 Finalization of Chromatographic condition

Amlodipine Besylate		Perindopril Arginine	
Conc. (µg/ml)	Mean. Area±S.D	Conc. (µg/ml)	Mean.Area±S.D
50	2003.78±1.047	50	1584.23 ± 0.756
100	3432.51±1.699	100	228014 ±0.608
150	4998.21±0.860	150	2947.38 ±0.434
200	6488.52±1.038	200	3589.14 ±0.437
250	7987.44±1.629	250	4173.67 ±0.637

Table 2 Calibration curve for Amlodipine Besylate and Perindopril Arginine

Parameters	Result	
	Amlodipine Besylate	Perindopril Arginine
Linearity Range (µg/ml)	50-250	50-250
Slope	35.90	12.97
Intercept	229.5	968.5
Retention Time (min)	3.90	4.90
Correlation Coefficient	0.997	0.998

Table 3 Amlodipine Besylate and Perindopril Arginine

Drug	Actual Conc. of Drug	Conc. Obtained	%Drug Found	Mean	SD	%RSD
AMD	10	9.84	98.4	98.53	0.51	0.52
	10	9.91	99.1	3	3	0
	10	9.81	98.1			
PRD	15	14.79	98.6	99.11	0.57	0.57
	15	14.85	99	1	4	9
	15	14.96	99.73			

Table 4 Assay Result of Synthetic Formulation

Factor	AMD	PRD
RT	3.90	4.90
Tailing Factor	1.515	1.477
Theoretical Plates	6608.475	4094.916
Resolution	5.202	1.784

Table suitability parameters for Amlodipine Besylate and Perindopril Arginine

Amlodipine Besylate			Perindopril Arginine		
Conc. (µg/ml)	Area Mean ± S.D.	%RSD	Conc. (µg/ml)	Area Mean ± S.D.	%RSD
50	2003.78±1.047	0.052	50	1584.2 ± 0.756	0.047
100	3432.51±1.699	0.049	100	228014 ±0.608	0.026
150	4998.21±0.860	0.017	150	2947.3 ± 0.434	0.014
200	6488.52±1.038	0.016	200	3589.1 ± 0.437	0.012
250	7987.44±1.629	0.020	250	4173.6 ± 0.637	0.015

Table 6 Linearity for Amlodipine Besylate and Perindopril Arginine

% Recovery	Target Conc. (µg/ml)	Spike conc. (µg/ml)	Final Conc. (µg/ml)	Conc., Obtained		% Assay	
				MT KT	DES	MTK T	DES
80%	10 + 15	8 + 12	18 + 27	18.0	26.81	100.44	99.2963
	10 + 15	8 + 12	18 + 27	17.9	27.81	99.544	100.463

	15	12	27	2	13	5	81
	10 +	8 +	18 +	17.8	27.	99.2	100.1
	15	12	27	7	05	7	85
100%	10 +	10 +	20 +	19.9	29.	99.7	99.73
	15	15	30	4	92	0	33
	10 +	10 +	20	20.0	30.	100.	100.2
	15	15	+30	5	07	25	33
	10 +	10 +	20 +	20.1	30.	100.	100.5
	15	15	30	4	15	70	
120%	10 +	12 +	22 +	21.8	32.	99.4	99.03
	15	18	33	7	68	0	03
	10 +	12 +	22 +	22.0	33.	100.	100.4
	15	18	33	9	14	40	24
	10 +	12 +	22 +	22.1	33.	100.	100.4
	15	18	33	2	14	54	24

Table 7 Accuracy for Amlodipine Besylate and Perindopril Arginine

Drug	Conc.(µg/ml)	Mean Abs. ± S.D.	%RSD
Amlodipine	100	4013.0±8.688	±0.926 0.216
	150	5712.57±9.758	±0.612 0.170
	200	7512.66±6.250	±0.848 0.583
	RT	3.956 ± 0.020	
Perindopril Arginine	100	2280.50 ± 0.860	±0.037
	150	2949.12 ± 2.230	±0.075
	200	3585.05 ± 6.087	±0.169
	RT	4.92 ± 0.047	

Table 8 Repeatability for Amlodipine Besylate and Perindopril Arginine

Amlodipine Besylate			Perindopril Arginine		
Conc. (µg/ml)	Area Mean ± S.D. (n=3)	%RSD	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	%RSD
100	4013.0 ± 8.688	0.216	100	2280.50 ± 0.860	0.037
150	5712.57 ± 9.758	0.170	150	2949.12 ± 2.230	0.075
200	7512.66 ± 6.250	0.583	200	3585.05 ± 6.087	0.169
RT	3.956 ± 0.020		RT	4.92 ± 0.047	

100	4033.3	0.82	100	2280.713±0.5	0.03
	8			55	6
	±13.45				
150	5752.1	0.79	150	2947.293±0.6	0.02
	2			64	2
	±15.55				
200	7548.9	0.73	200	3589.177±0.5	0.00
	1			25	7
	±19.78				

Table 9 Intra-day precision for Amlodipine Besylate and Perindopril Arginine

Amlodipine Besylate			Perindopril Arginine		
Conc. (µg/ml)	Area Mean ± S.D. (n=3)	%RSD	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	%RSD
100	4004.69	0.023	100	2280.75±0.356	0.019
150	5702.26	0.010	150	2947.65±0.525	0.023
200	7488.56	0.011	200	3589.10±0.451	0.007
RT	3.956 ± 0.020				

Table 10 Inter-day precision for Amlodipine Besylate and Perindopril Arginine

Sr. no.	Amlodipine Besylate (150 µg/ml)					
	pH		Flow rate		Mobile phase	
	+0.2 units	-0.2 units	+0.2 units	-0.2 units	+2.0 %	-2.0 %
1	5013.58	4856.27	5103.65	4836.50	5153.18	4906.57
2	5023.17	4862.28	5157.30	449.26	5172.62	4915.67
3	5036.69	4871.67	5139.42	4857.62	5185.28	4895.40
Me	5024.	4871.	5133.	4847.	5170.	490.8

an	48	67	45	79	36	8
S.D	11.61	7.763	21.31	10.63	16.17	10.15
	17	34	7	71	27	40
%	0.231	0.159	0.532	0.219	0.312	0.206
RSD	1	6	1	4	8	9

Table 12 Robustness for Amlodipine Besylate

Sr. no.	Perindopril Arginine(150 µg/ml)					
	Ph		Flow rate		Mobile phase	
	+ 0.2 units	-0.2 units	+0.2 units	-0.2 units	+ 2.0 %	- 2.0 %
1	3075.9	2864.3	3164.2	2756.1	3047.9	2745.9
	8	65	65	69	4	87
2	3065.2	2876.7	3174.6	2769.6	3057.5	2768.1
	6	8	9	4	48	2
3	3094.3	2882.3	3167.1	2764.3	3073.6	2768.6
	69	6	5	6	9	4
Me an	3078.5	2874.5	3168.7	2763.3	3059.7	2760.9
	36	02	02	9	26	16
S.D	14.721	9.2113	5.3829	6.7877	13.012	12.931
	91	03	27	18	43	22
%	0.4782	0.3204	0.1698	0.2456	0.4252	0.4683
R.S.D	11	49	78	3	81	67

Table 13 Robustness for Perindopril Arginine

Parameters	AMD	PRD
Detection wavelength (nm)	221 nm	
Concentration range (mcg/ml)	50-250	50-250
Slope	35.90	31.40
Intercept	229.5	968.5
Correlation coefficient	0.997	0.998
Regression Coefficient	y = -35.90x + 229.5	y = 12.97x + 968.5
LOD	0.115	0.146

LOQ	0.349	0.442
%Recovery	99.27-101.40	99.29-101.13
(Accuracy, n = 3)		
Repeatability	0.170-0.832	0.037-0.169
(RSD, n = 3) %		
Precision Data		
Interday	0.10-0.14	0.012-0.017
Intraday	0.09-0.54	0.014-0.024
Robustness (%RSD)		
pH(+0.2 units)	0.2311	0.478
pH(-0.2 units)	0.1596	0.320
Flow Rate(+0.2 units)	0.5321	0.169
Flow Rates(-0.2 units)	0.2194	0.245
Mobile Phase(+2%)	0.3128	0.425
Mobile Phase(-2%)	0.2096	0.468

Table 14 Summary of RP – HPLC Method

SR. NO.	STRESS TYPE	% DEGRADATION of SAMPLE		% DEGRADATION of SYNTHETIC MIXTURE	
		AMD	PRD	AMD	PRD
1	Acidic	8.02	6.99	7.25	6.02
2	Alkaline	7.42	7.32	6.06	6.13
3	Oxidation	7.92	8.61	7.01	7.81
4	Thermal	8.54	9.02	7.54	8.26

Table 14 Summary of Stability indicating RP – HPLC Method

