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A Review Study of Stability Indicating Based On RP-HPLC for Perindopril–Amlodipine Besylate

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***Abstract*— An accurate and precise HPLC method was developed for the simultaneous determination of perindopril and amlodipine. Separation of the drugs was achieved on a reverse phase C 18 column using mobile phase consisting of phosphate buffer 0.04M Borate cradle pH 3.0 the detection wavelength was 293nm & 339nm. The linearity was observed in the range of 10-90 µg/mL for amlodipine and 5-30 µg/mL for perindopril. The proposed method was validated for its linearity, accuracy, precision and robustness. The proposed method can be employed to estimate the drug contents in marketed formulations.**

Moreover the % RSD estimation of Intraday as well as Inter day investigation are 0.7026 as well as 0.1175 for Amlodipine besylate, 1.1703 as well as 1.1783 for Perindopril separately.

Methods: This review paper will focus on Amlodipine A simple, precise and accurate RP-HPLC method has been developed and subsequently validated for simultaneous estimation of Perindopril–Amlodipine Besylate from their combination dosage form.

***Index Terms*— Perindopril–Amlodipine Besylate, RP-HPLC method; tablet dosage forms.**

I. INTRODUCTION

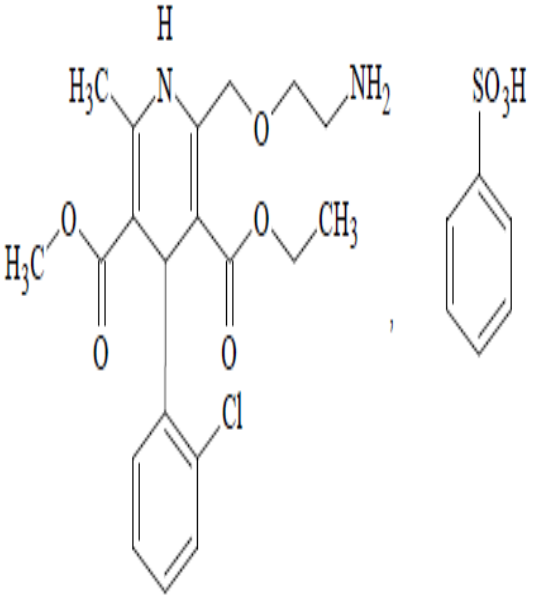
Methods: This review paper will focus on Amlodipine A simple, precise and accurate RP-HPLC method has been developed and subsequently validated for simultaneous estimation of Perindopril–Amlodipine Besylate from their combination dosage form.

Hypertension is well established as a major risk factor for cardiovascular disease. Although there is undeniable evidence to support the beneficial effects of antihypertensive therapy on morbidity and mortality, adequate blood pressure management still remains suboptimal. Perindopril and amlodipine is a combination of medicines that are used to treat high blood pressure (hypertension). High blood pressure adds to the workload of the heart and arteries. If it continues for a long time, the heart and arteries may not function properly. This can damage the blood vessels of the brain, heart, and kidneys, resulting in a stroke, heart failure, or kidney failure. Lowering blood pressure can reduce the risk of strokes and heart attacks.

Perindopril is an angiotensin-converting enzyme (ACE) inhibitor. It works by blocking a substance in the body that causes the blood vessels to tighten. This relaxes the blood vessels and lowers blood pressure.

Amlodipine is a calcium channel blocker (CCB). It affects the movement of calcium into the cells of the heart and blood vessels. This relaxes the blood vessels and lowers blood pressure. A lower blood pressure will increase the supply of blood and oxygen to the heart.

Amlodipine Besylate acts as a calcium channel blocker, vasodilator, and anti-hypertensive agent Amlodipine Besylate is capable of controlling the movement of calcium ions in the body. **Chemical structure and name**



3-ethyl5-methyl2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3, 5-dicarboxylate benzene sulphonate.

Perindopril It is used as an antihypertensive agent and diuretics at lower dosage shows antihypertensive effects. At higher doses shows diuretic actions also. It relaxes the vascular muscles by inhibiting net Ca2+ inflow. Perindopril obstructs the sluggish section of the delayed rectifier potassium current (IKs) without varying the speedy section (IKr) or the inward rectifier current. It specifically blocks or antagonizes the actions of two proteins: KCNQ1 and KCNE1. Perindopril is also thought to stimulate the separation of the vasodilator hypertensive prostaglandin PGE2

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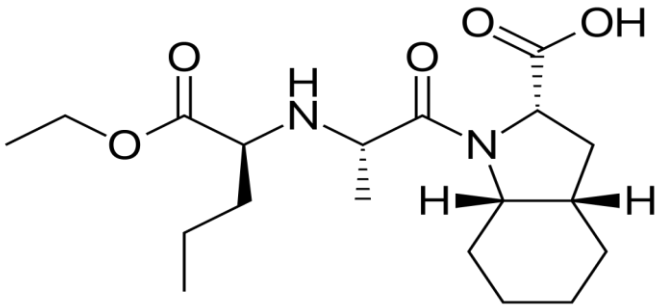
Chemical structure and name



(2S)-2-[(1S)-1-Carbethoxybutylamino]-1-oxopropyl-(2S,3aS,7aS)-perhydroindole-2-carboxylic Acid

Through literature survey reveals that there square measure few analytical ways such as RP-HPLC and UVlight ways are rumored for synchronous estimate

Perindopril–Amlodipine Besylate in pharmaceutical dose kind. Present work emphasizes on the quantitative estimation of Perindopril–Amlodipine Besylate in their combined dosage form (Tablets) by RP-HPLC. The proposed technique was also successfully used to separate the degraded product from the samples.

II. MATERIALS AND METHODS

Drugs

Amlodipine Besylate was openhandedly gifted by Aurobindo Pharmaceuticals, Hyderabad. And, Perindopril was gifted by Micro Labs, Bangalore. commercial pill dose kind Coversyl-AM was purchased from native market.

Formulation used

Nitrile was procured from a local pharmacy. It is composed of Amlodipine besylate that is equivalent to 5mg Amlodipine and 4 mg of Prindopril.

Chemicals and Solvents Used

Acetonitrile (HPLC grade), Methanol (HPLC grade), Water (HPLC grade), Methanol (Analytical grade), Glacial Acetic acid (Analytical grade), Boric acid (Analytical grade), and Sodium hydroxide (Analytical grade).

Instruments Used

Following instruments are used to carry out this research work:

- Shimadzu AUX-220 Digital balance
- Shimadzu- 1700 Double Beam- UV- Visible spectrophotometer
- Sonorex Bendelin Digital 10p apparatus
- Micropipette
- Elico LI 127 pH meter
- Milli-Q-Millipore Integral 3
- Mettler Toledo AB204-S/FACT
- Alliance Waters E2695 modular HPLC System
- Waters 2489 UV/Visible Detector
- Waters 2489 PDA Detector

Selection of solvent

Solubility of both Amlodipine besylate, Perindopril has been found in varied solvents according to the standards of Indian Pharmacopoeia. The Solubility of both has been carried in non-polar and polar solvents. Further, the solvent common in both is methanol along with 0.2M Borate buffer and has a pH of 8.0. This is ought to be the most economic solvent used for analyzing the Amlodipine besylate or Perindopril being the methods proposed. As evident from the study of solubility, Methanol on dilution with Borate Buffer having concentration 0.2M (pH-8.0) has been used as the solvent for the method Spectrophotometry. Also, it has been selected based on its cost factor, availability and the wavelength cutoff.

Preparation of 0.2M Borate buffer – pH 8.0

Boric acid with 3.09 gm and Potassium Chloride 3.72 gm with a water of 500ml is used for dissolving and has the pH 8.0 for the Sodium Hydroxide with concentration 0.2M and further diluted in 1000 ml water.

Preparation of Amlodipine besylate standard stock solution

With an accurate weight as 25mg of Amlodipine besylate which is then transferred to a volumetric flask of 100 mL separately, and then dissolving the same into methanol to abide by the methanol volume. The above solution has been found to have Amlodipine having concentration 250 mcg/ml.

Preparation of Prindopril standard stock solution

Perindopril as weighed in to have 7.5 mg is then transferred to a volumetric flask of 100 mL and the dissolved in methanol to stand by methanol volume. The above solution has been found to have Perindopril having concentration 75 mcg/ml.

Preparation of calibration graph for Amlodipine besylate

Weighing the Amlodipine besylate with 132.10 Equivalent is transferred to a volumetric flask 25 ml dissolving in Methanol to make it till the methanol volume (3750 µg/ml). Further, the solution with 1ml concentration has been transferred to a volumetric flask with 25 ml concentration to map the needed volume with Borate Buffer having 0.2M and pH 8.0 to attain the desired concentration of 150 µg/ml. Next, transferring the 1–6 ml to series of volumetric flask of 10 ml to map with the Borate buffer. Further, absorbance of varied concentration solutions has been measured to be 339.0 nm against the blank. Plot the calibration curve of concentration against the absorbance. It has been found that the solutions are linear with concentration range between 15 – 90 µg/ml. Repeat the procedure thrice.

For Perindopril

31.25 mg of Perindopril raw material was weighed and transferred into 25 ml volumetric flask. Dissolved in methanol and made up to the volume with methanol (1250 µg/ml). 1ml of the solution was transferred into a 25 ml volumetric flask and made up to the required volume with 0.2M Borate buffer–pH 8.0 to get the concentration 50 µg/ml. From the aliquots of stock solution of Prindopril 1–6 ml were transferred into a series of 10 ml volumetric flasks and made up to the mark with Borate buffer. The absorbance of different concentration solutions was measured at 293.0 against blank. The calibration curve was plotted using concentration against absorbance. The solutions were found to be linear with the concentration range of 5 – 30 µg/ml. The procedure was repeated for three times.

RP-HPLC method development and optimization of chromatographic conditions Selection of chromatographic method

Method of Proper selection relies on sample nature, polarity, the molecular weight, value of Pka and solubility. Drugs Prindopril and Amlodipine Besylate for the current study are polar. Hence, the technique of Reverse Phase

Chromatographic has been selected using the C18 column owing the stationary phase having varied mobile phase.

Selection of mobile phase and wavelength

Mobile phase variant having different ratios has been selected along with the recording of chromatograms. As an outcome, the Acetonitrile: Methanol: with concentration of 0.04M Phosphate buffer-pH 3.0 (25:30:45 v/v/v) has been selected for the mobile phase. Both the drugs have been eluted having sharp peak along with efficient resolution as using the above given ratio. So the phase has been used for optimization of chromatographic conditions.

Further, they are scanned in a UV region having wavelength 200 – 400 nm with being recorded at spectrums. It is seen that the two drugs mark absorbance at wavelength of 240.0 nm and could be efficiently used to estimate multiple drugs without being interfered. Further, wavelength 240.0 nm has been selected as the detection wavelength to estimate the two drugs via RP method of HPLC with techniques of Isocratic elution.

III. RESULTS AND DISCUSSION

Evaluation of numerous medications in plans has an edge over techniques which are tedious and furthermore utilization of dissolvable is limited. Two straightforward, quick, exact and precise Spectrophotometric techniques as well as isocratic RP – HPLC strategy were created and approved for evaluation of Amlodipine besylate as well as Perindopril in unadulterated structure and in consolidated tablet measurement structure. The techniques utilized that are

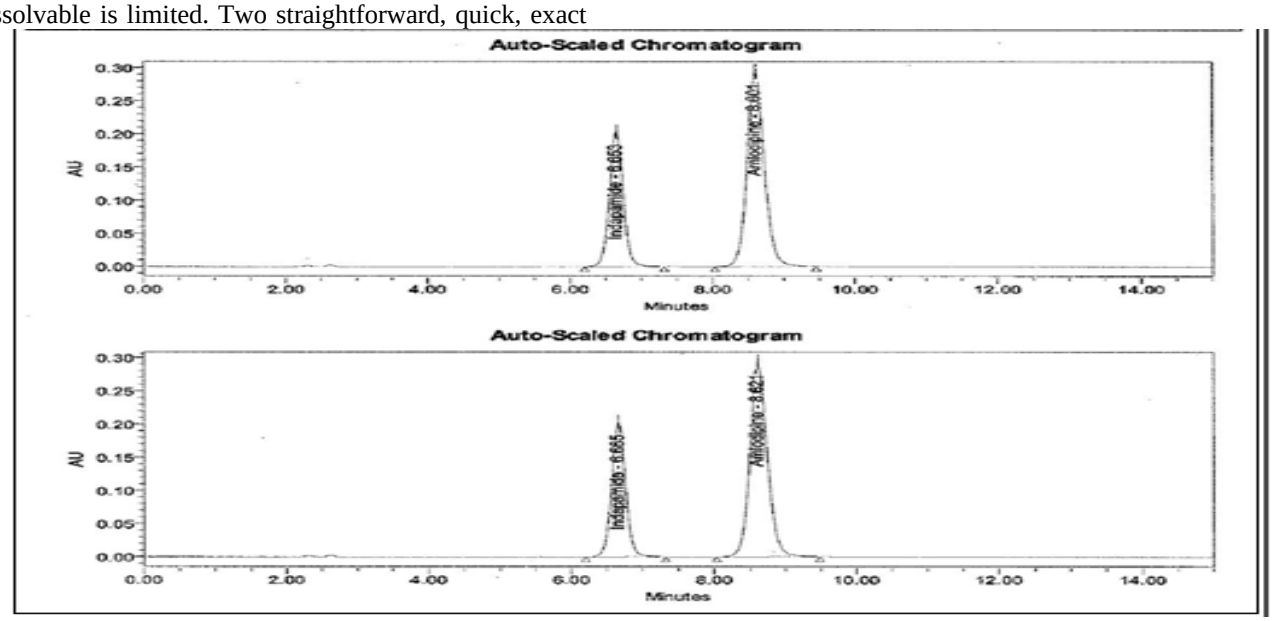
- UV Spectrophotometric techniques
- Subsidiary Spectrophotometric strategy
- RP – HPLC technique

Method validation

The validation was performed with above developed RP HPLC technique for synchronous estimation of Prindopril and Amlodipine Besylate according to ICH tips. Various parameters were evaluated such as system quality, precision, accuracy, linearity, robustness, LOD, and LOQ

System suitability parameters

System suitability was performed to verify the acceptableness of the resolution and repeatability of the system. System suitability was performed by injecting six replicate injections of the standard resolution (100%) and parameters such as peak space, USP tailing, theoretical plates, retention time, and peak asymmetry were evaluated. The % RSD determined and rumoured inside the bounds.



	Sample Name	Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	LINEARITY 90%	Amlodipine	8.566	3105412	6356.69	1.06	4.92
2	LINEARITY 90%	Amlodipine	8.556	3105741	6325.78	1.06	4.90
Mean			8.561	3105576.501	6341.20	1.1	4.9
Std. Dev.			0.007	1355.019			
% RSD			0.1	0.0			

	Sample Name	Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	LINEARITY 90%	Perindopril	6.625	1894574	6015.56	0.99	
2	LINEARITY 90%	Perindopril	6.619	1890654	5935.73	1.00	
Mean			6.622	1896114.001	5975.0	1.00	
Std. Dev.			0.004	124.135			
% RSD			0.1	0.0			

	Sample Name	Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	LINEARITY 100%	Amlodipine	8.564	3453627	6290.54	1.06	4.89

2	**LINEARITY 100%**	**Amlodipine**	8.583	3456961	6260.71	1.06	4.89
Mean			8.574	3455254.011	6275.6	1.1	4.9
Std. Dev.			0.013	703.571			
% RSD			0.2	0.1			

	Sample Name	**Name**	**RT**	**Area**	**USP Plate Count**	**USP Tailing**	**USP Resolution**
1	**LINEARITY 100%**	**Perindopril**	6.625	2125415	5974.65	0.99	
2	**LINEARITY 100%**	**Perindopril**	6.637	2123954	5876.73	1.00	
Mean			6.531	2124684.012	5925.70	1.00	
Std. Dev.			0.008	1131.371			
% RSD			0.1	0.1			

	Sample Name	**Name**	**RT**	**Area**	**USP Plate Count**	**USP Tailing**	**USP Resolution**
1	**LINEARITY 110%**	**Amlodipine**	8.601	3792687	6201.27	1.06	4.86
2	**LINEARITY 110%**	**Amlodipine**	8.621	3792546	6188.90	1.06	4.87
Mean			8.611	3793616.501	6195.1	1.1	4.9
Std. Dev.			0.014	1131.372			
% RSD			0.2	0.0			

	Sample Name	**Name**	**RT**	**Area**	**USP Plate Count**	**USP Tailing**	**USP Resolution**
1	**LINEARITY 110%**	**Perindopril**	6.653	2334152	5889.43	0.90	
2	**LINEARITY 110%**	**Perindopril**	6.665	2325745	5856.47	1.00	
Mean			6.659	2329948.501	5873.0	1.00	
Std. Dev.			0.008	2147.219			
% RSD			0.1	0.1			

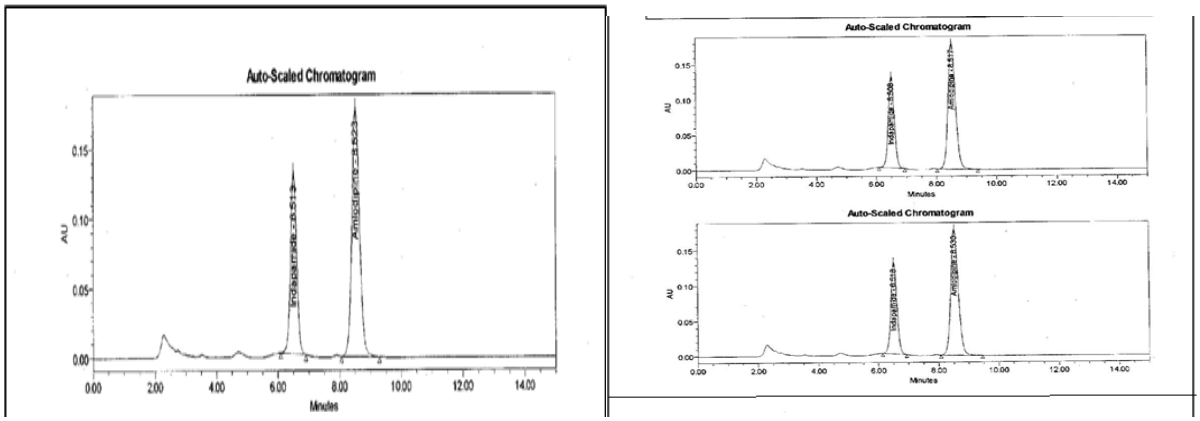


FIGURE CHROMATOGRAM FOR RECOVERY STUDIES (80%)

	Sample Name	**Name**	**RT**	**Area**	**USP Plate Count**	**USP Tailing**	**USP Resolution**
1	**Recovery 80%**	**Amlodipine**	8.517	3072827	5810.14	1.07	4.90
2	**Recovery 80%**	**Amlodipine**	8.530	3083012	5806.61	1.07	4.89
3	**Recovery 80%**	**Amlodipine**	8.523	3079904	5803.77	1.06	4.90
Mean			8.523	3078580	5805.90	1.1	4.9
Std. Dev.			0.006	5219.469			
% RSD			0.1	0.2			

	Sample Name	Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	**Recovery 80%**	**Perindopril**	6.508	1790460	5277.99	1.02	
2	**Recovery 80%**	**Perindopril**	6.518	1786129	5260.70	1.02	
3	**Recovery 80%**	**Perindopril**	6.513	1788616	5317.22	1.01	
Mean			6.513	1788401.647	5285.30	1.0	
Std. Dev.			0.005	2173.208			
% RSD			0.1	0.1			

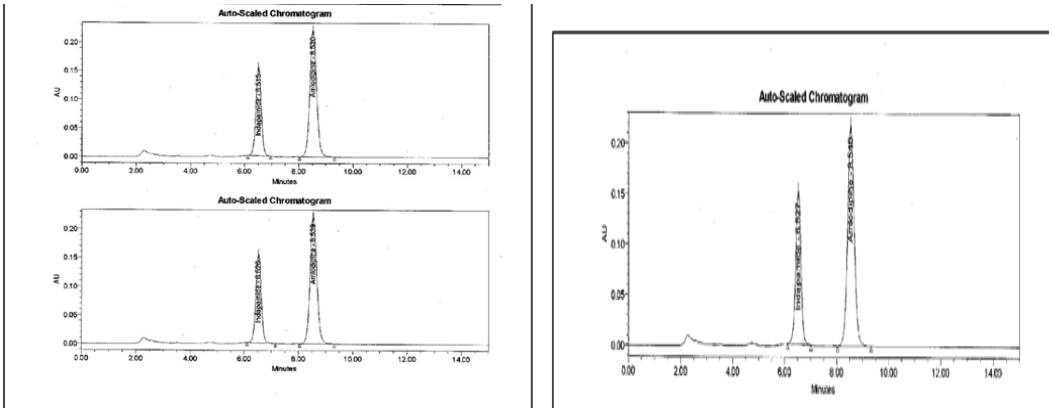


FIGURE CHROMATOGRAM FOR RECOVERY STUDIES (100%)

	Sample Name	Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	**Recovery 100%**	**Amlodipine**	8.520	3835054	5742.65	1.07	4.87
2	**Recovery 100%**	**Amlodipine**	8.539	3843226	5716.82	1.07	4.85
3	**Recovery 100%**	**Amlodipine**	8.540	3849522	5558.34	1.06	4.77
Mean			8.533	3842604.016	5672.60	1.1	4.8
Std. Dev.			0.011	7249.090			
% RSD			0.1	0.2			

	Sample Name	Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	**Recovery 100%**	**Perindopril**	6.515	2133181	5202.58	1.02	
2	**Recovery 100%**	**Perindopril**	6.526	2160726	5223.11	1.02	
3	**Recovery 100%**	**Perindopril**	6.527	2150648	5014.07	1.01	
Mean			6.523	2140185.066	5166.80	1.0	
Std. Dev.			0.007	13936.613			
% RSD			0.1	0.0			

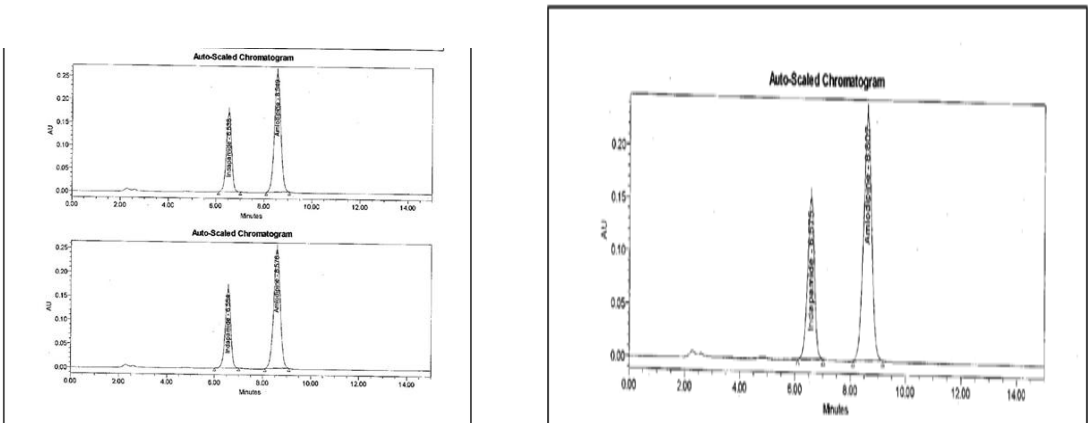


FIGURE CHROMATOGRAM FOR RECOVERY STUDIES (120%)

	Sample Name	Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	**Recovery 120%**	**Amlodipine**	8.549	4709741	5133.21	1.05	4.56
2	**Recovery 120%**	**Amlodipine**	8.576	4720646	4799.57	1.02	4.41
3	**Recovery 120%**	**Amlodipine**	8.607	4759012	4739.50	1.00	4.06
Mean			8.577	4729799.443	4690.9	1.0	4.3
Std. Dev.			0.029	25579.715			
% RSD			0.3	0.5			

	Sample Name	Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	**Recovery 120%**	**Perindopril**	6.538	2576781	4550.59	0.99	
2	**Recovery 120%**	**Perindopril**	6.554	2582619	4225.93	0.96	
3	**Recovery 120%**	**Perindopril**	6.575	2581354	3468.25	0.94	
Mean			6.556	2580251.098	4066.30	1.0	
Std. Dev.			0.019	3071.367			
% RSD			0.3	0.1			

TABLE

OPTICAL CHARACTERISTICS OF AMLODIPINE BESYLATE AND PERINDOPRIL BY RP-HPLC

PARAMETER	AMLODIPINE	PERINDOPRIL
$\lambda_{}$ Max	240	285
Beers law	80-120	5-40
Correlation coefficient (r)	0.99993	0.99956
Regression equation	Y= (34513.19)x+717.1428	Y= (70554.93) x (-1552.982143)
Slope (m)	34513.19	70554.93929
Intercept (c)	717.1428	-1552.982143
LOD	0.07491703	0.066817129
LOQ	0.227021308	0.202476148
Standard Error	3191.47	8481.610289

TABLE OPTICAL CHARACTERSTICS OF AMLODIPINE BESYLATE AND PERINDOPRIL BY FIRST ORDER DERIVATIVE SPECTROSCOPIC METHOD

PARAMETER	AMLODIPINE BESYLATE	PERINDOPRIL
Beers law limit ug/mL/1	15-90 ug/mL/1	5-35 ug/mL/1
Molar Specificity	176.6787	236.2271
Sandells sensitivity	3.032491	1.590899
Correlation coefficient (r)	0.999842	0.999876
Regression equation	Y=0.0033x - (-) 0.00018	Y= 0.0006X0.000171
Slop (m)	0.00033	0.000629
Intercept (c)	-0.00018	0.000171
LOD	0.3973	0.2177
LOQ	1.2041	0.6598

*****Mean of six observations

TABLE RECOVERY STUDY OF AMLODIPINE BESYLATE AND PERINDOPRIL BY RP-HPLC METHOD

Drug	%	Amount Added	Amount Recovered	% Recovery	S.D.	%RSD	S.E.
AML	80	80	79.70	99.53	0.34219	0.34389	0.03902
	100	100	99.47	99.47	0.15821	0.15905	0.01755
	120	120	122.46	102.04	0.55018	0.39182	0.06113
PER	80	24	24.68	102.55	0.41061	0.39995	0.04555
	100	30	29.63	98.83	0.62601	0.63343	0.06955
	120	36	53.61	99.26	0.61808	0.62226	0.06867

*Mean of three observations

IV. SUMMARY & CONCLUSION

Basic, quick, exact and precise RP-HPLC technique were produced and approved for evaluation of Amlodipine besylate as well as Perindopril in the tablet measurement structure.

V. ACKNOWLEDGEMENTS

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VI. CONFLICT OF INTEREST

Authors declares that there is no conflict of interest.

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