An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

SIMULTANEOUS ESTIMATION OF ENALAPRIL MALEATE AND FELODIPINE BY USING RP-HPLC METHOD IN TABLET DOSAGE FORM

M. Prasanthi Evangelin *, Chennupalli Jhansi Rani and Manohar Babu S

SIMS College of Pharmacy, SIMS Group of Institutions, Mangaldas Nagar, Guntur,-522001, Andhra Pradesh, India.

ARTICLE INFO

Key Words

Enalapril Maleate, Felodipine,

RP-HPLC.

ABSTRACT

Estimation of Enalapril maleate and Felodipine simultaneously in tablet dosage forms by RP - HPLCmethod.The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 215nm Enalapril Maleate for and 248nm for Felodipine. Common wavelength will be 237nm and the peaks purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Inertsil C_{18}, ODS chosen good peak shape. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area, satisfactory retention time and good resolution. Different ratios of mobile phase were studied, mobile phase with ratio of 60:40 Methanol: Buffer was fixed due to good symmetrical peaks and for good resolution. So this mobile phase was used for the proposed study.The present recovery was found to be 98.0-101.50 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. Detection limit was found to be 0.25 Enalapril Maleate and 0.34 for Felodipine. Linearity study was, correlation coefficient and curve fitting was found to be. The analytical method was found linearity over the range of 20-80ppm of the target concentration for both the drugs. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

INTRODUCTION:

Pharmaceutical Analysis plays a very vital role in the quality assurance and quality control of bulk drugs and their formulations. 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.In recent years, several analytical techniques have been evolved[1-3.]

SPECTROPHOTOMETRICMETHODS[4-6]

Spectrophotometry is generally preferred especially by small-scale industries as the cost of the equipment is less and the maintenance problems are minimal. The method of analysis is based on measuring the absorption of a monochromatic light by colorless compounds in the near ultraviolet path of spectrum (200-380nm). The photometric methods of analysis are based on the Bouger-Lambert-Beer‟s law, which establishes the absorbance of a solution is directly proportional to the concentration of the analyte. The fundamental principle of operation of spectrophotometer covering UV region consists in that light of definite interval of wavelength passes through a cell with solvent and falls on to the photoelectric cell that transforms the radiant energy into electrical energy measured by agalvanometer.

The important applications are: Identification of many types of organic, inorganic molecules and ions. Quantitative determination of many biological, organic and inorganic species. Monitoring and identification of chromatographic of effluents.

HPLC METHODDEVELOPMENT[7-10]

The term "Chromatography" covers those processes aimed at the separation of the various species of a mixture on the basis of their distribution characteristics between a stationary and a mobile phase.

MODES OF CHROMATOGRAPHY [10-12]

Modes of chromatography are defined essentially according to the nature of the interactions between the solute and the stationary phase, which may arise from hydrogen bonding, Vander walls forces, electrostatic forces or hydrophobic forces or basing on the size of the particles (e.g. Size exclusion chromatography).

MATERIALS USED: Enalapril Maleate and Felodipine Working Standards. Methanol HPLCGrade, Buffer(KH2PO4) HPLC Grade.

Different modes of chromatography are as follows:

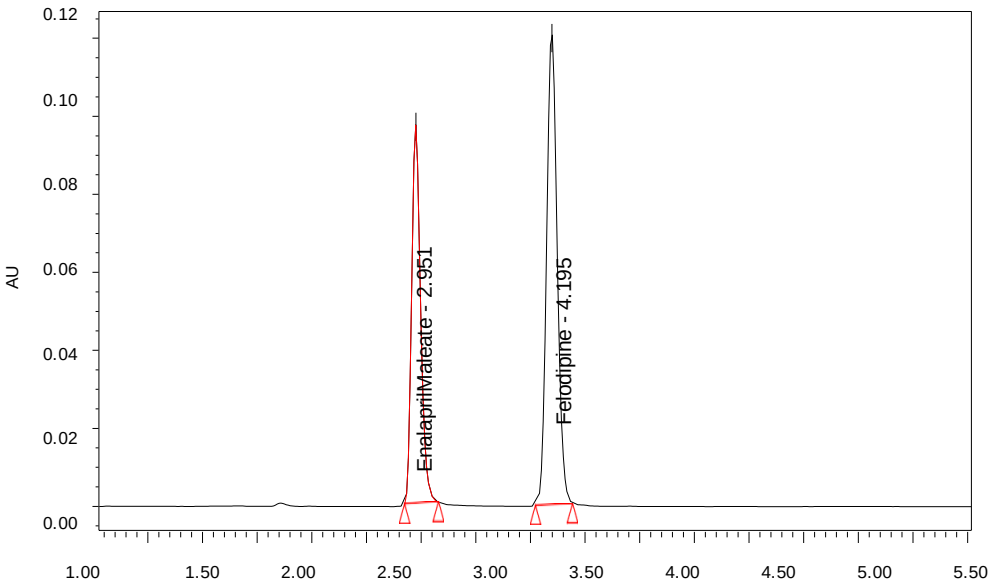
- Normal Phase Chromatography
- Reversed Phase Chromatography
- Reversed Phase – ion pairChromatography
- Ion-Exchange Chromatography
- Size Exclusion Chromatography

The modern form of column chromatography has been called high performance, high pressure, and high-resolution and high-speed liquid chromatography.

High-Performance-Liquid-Chromatography (HPLC)[12]

is a special branch of column chromatography in which the mobile phase is forced through the column at high speed. As a result the analysis time is reduced by 1-2 orders of magnitude relative to classical column chromatography and the use of much smaller particles of the adsorbent or support becomes possible increasing the column efficiency substantially.

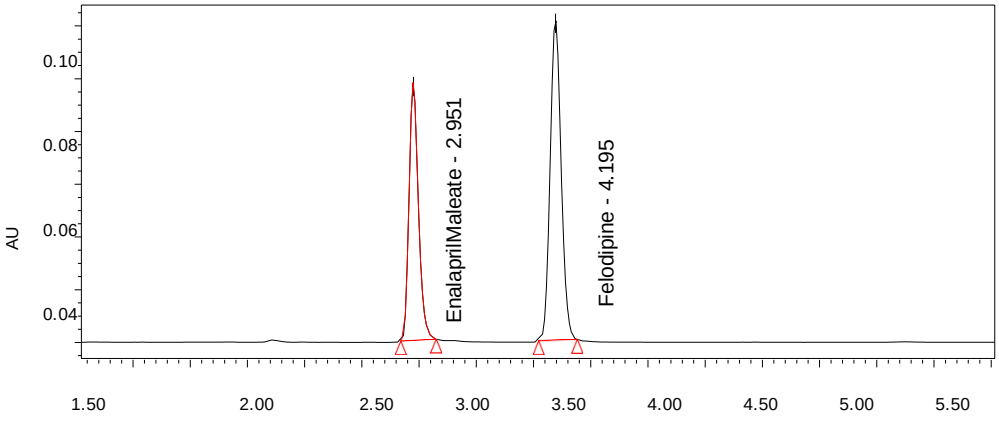
Fig 1: Chromatogram of standard



Inference: Got chromatogram at RT's of 2.951min to Enalapril Maleateand 4.195 min to Felodipine

S.NO	Name of the peak	Retention Time(min)
1	**Enalapril Maleate**	2.790
2	**Felodipine**	3.481

Fig 2:Chromatogram of sample



Inference: Got same chromatogram with same RT values as of standard

S.NO	Name of the peak	Retention

		time(min)
1	**Enalapril Maleate**	2.950
2	**Felodipine**	4.193

TABLE-2- Data of System Suitability forEnalapril Maleate

Injection	**RT**	**Peak Area**	**USP Plate count**	**USP Tailing**
1	2.951	674753	10953.609752	1.153539
2	2.950	674261	10951.014286	1.155271
3	2.948	675298	10003.278630	1.157740
4	2.948	679221	10986.906427	1.159499
5	2.949	688636	10946.878423	1.152820
Mean	2.94735	678433.8	10768.34	1.155774
SD	0.001817	6031.135	-------	-------
% RSD	0.05221	0.888979	-------	-------

TABLE-3- Data of System Suitability for Felodipine

Injection	**RT**	**Peak Area**	**USP Plate count**	**USP Tailing**
1	4.195	1218805	9478.317159	0.899633
2	4.193	1214014	9452.196217	0.893423
3	4.189	1215474	9569.928335	0.894443
4	4.189	1227655	9619.633847	0.882222
5	4.190	1267019	9749.907462	0.892316
Mean	4.19422	1228593	9573.997	0.892407
SD	0.00707	122124.07	-------	-------
% RSD	0.025353	1.800764	-------	-------

Fig: 3 Chromatograms of system suitability standards- 1

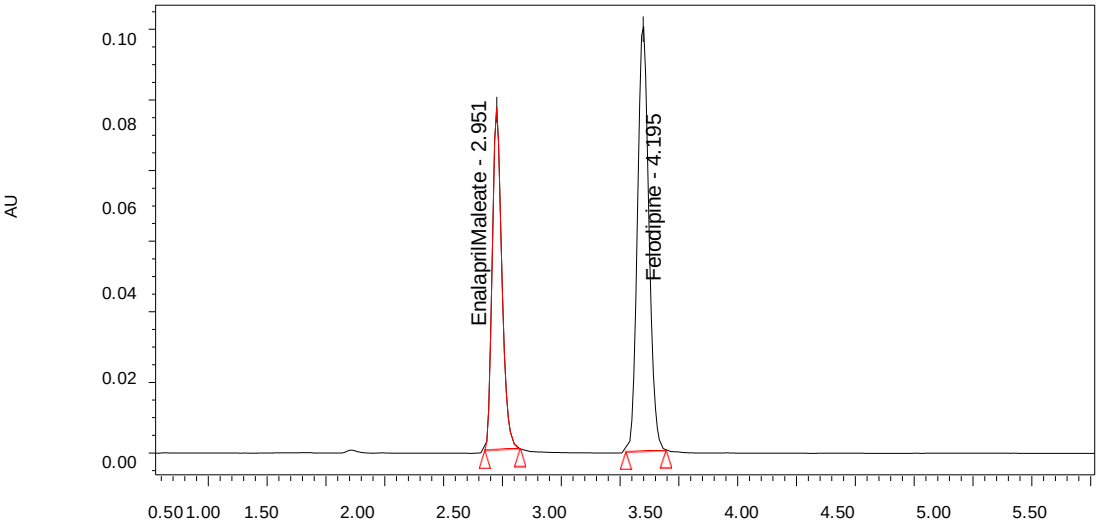


Fig: 4 System suitability Chromatogram for standard – 2

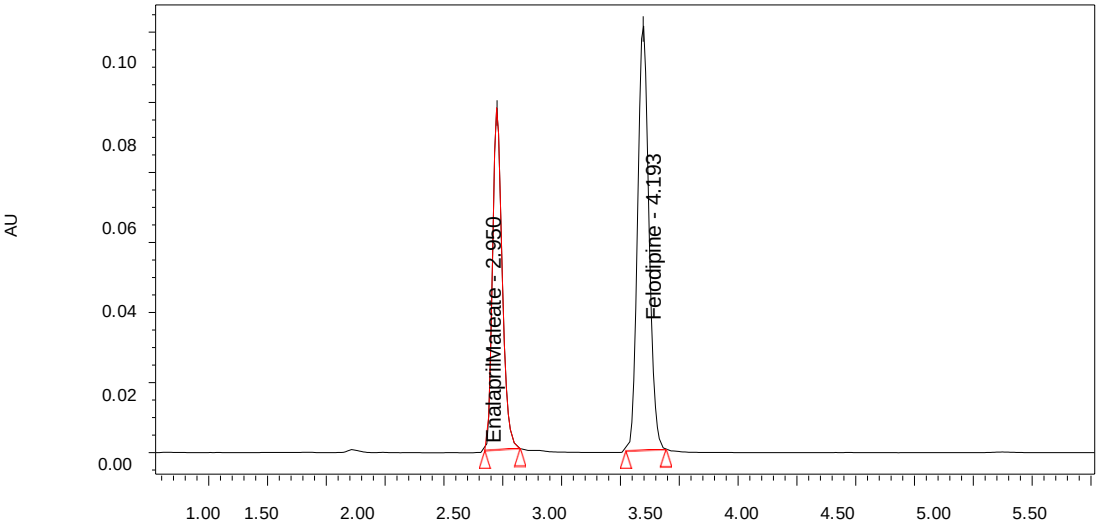


Fig: 5 System suitability Chromatogram for standard – 3

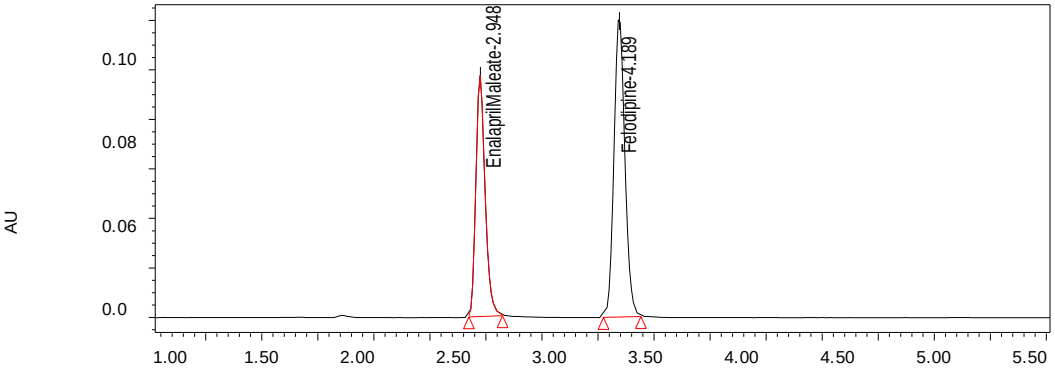


Table-4- Data of Repeatability (System precision) for Enalapril Maleate

	Injection	*Peak Areas of Enalapril Maleate*	*%Assay*
Concentration 40ppm	*1*	*674753*	*98.66*
	2	*674261*	*99.30*
	3	*675298*	*101.53*
	4	*679221*	*100.53*
	5	*688636*	*99.98*
Statistical Analysis	*Mean*	*678433.8*	*100.00*
	SD	*6031.135*	*1.107678*
	% RSD	*0.888979*	*1.10*

TABLE-5-Data of Repeatability (System precision) for Felodipine

	Injection	*Peak Areas of Felodipine*	*%Assay*
Concentration 40ppm	*1*	*1218805*	*99.95*
	2	*1214014*	*100.24*
	3	*1215474*	*100.06*
	4	*1227655*	*99.30*
	5	*1267019*	*100.00*
Statistical Analysis Analysis	*Mean*	*1228593*	*99.91*
	SD	*22124.07*	*0.35819*
	% RSD	*1.800764*	*0.35*

Fig: 6 Chromatogram for system precision (standard - 1)

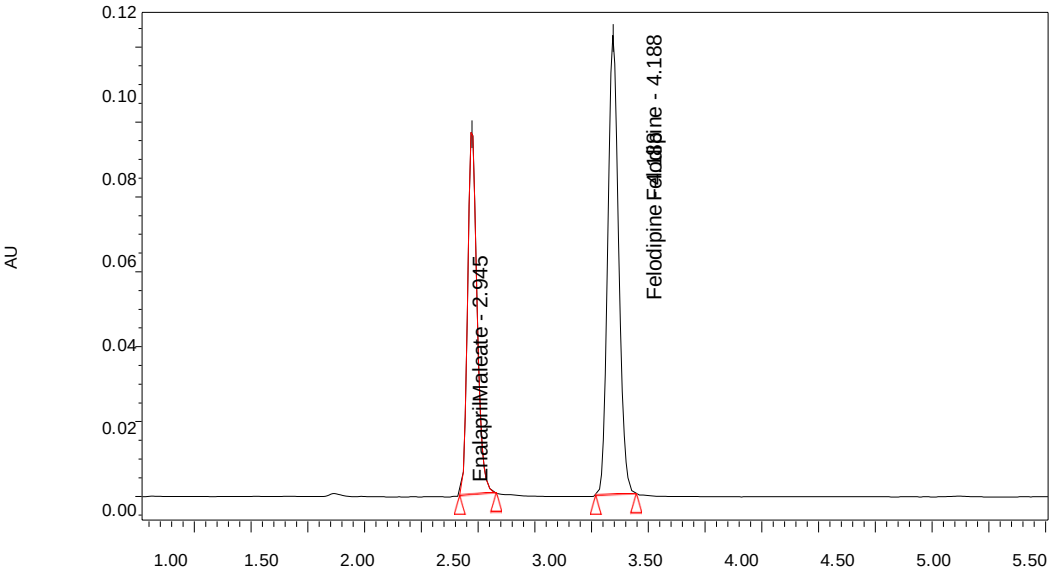


Fig: 7 Chromatogram for system precision (standard - 2)

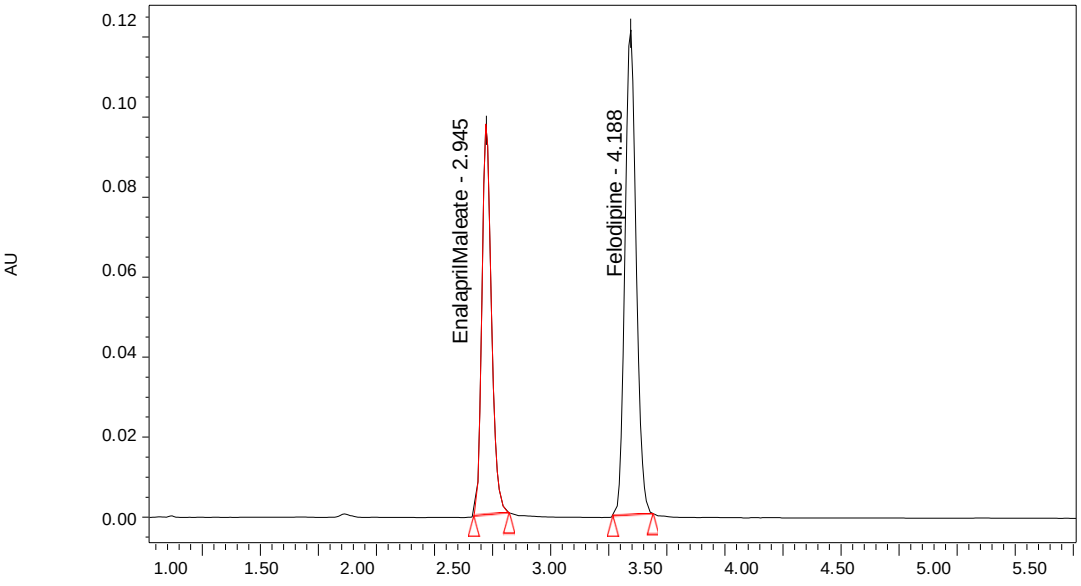


Fig: 8 Chromatogram for system precision (standard - 3)

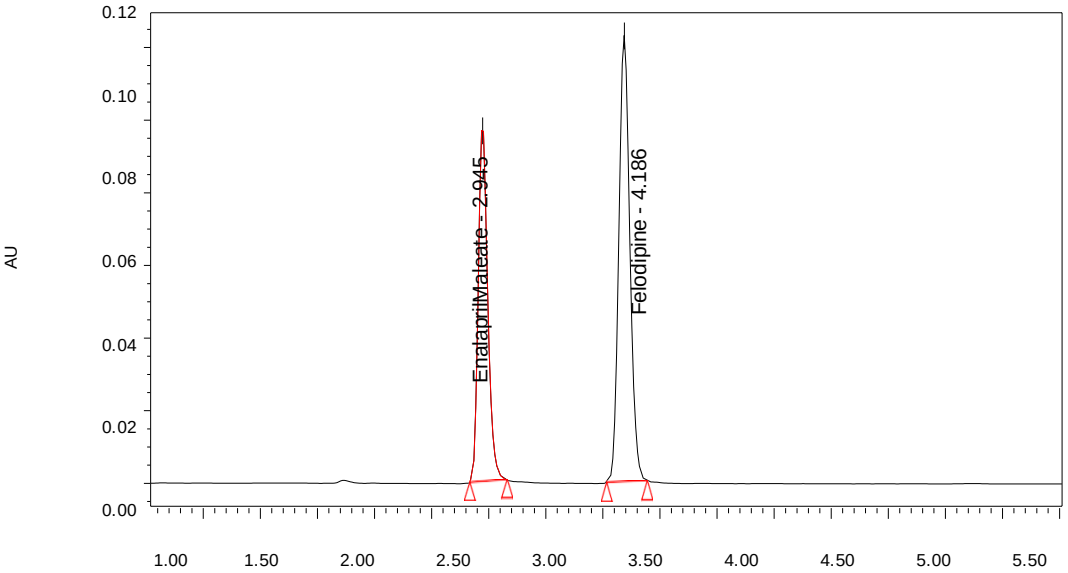


TABLE-6- Data of Repeatability (Method precision) for Enalapril Maleate

	Injection	*Peak Areas of Enalapril Maleate*	*%Assay*
Concentration 40ppm	***1***	***633495***	***98.55***
	2	***635992***	***98.88***
	3	***639828***	***99.40***
	4	***639098***	***99.30***
	5	***648289***	***100.53***
Statistical Analysis	***6***	***631322***	***98.28***
	Mean	***637312***	***99.278***
	SD	***5988.879***	***0.827236***
	% RSD	***0.0891***	***0.83***

TABLE-7-Data of Repeatability (Method precision) for Felodipine

	Injection	*Peak Areas of Felodipine*	*%Assay*
Concentration 40ppm	*1*	*1202110*	*98.6*
	2	*1203700*	*99.02*
	3	*1201851*	*98.12*
	4	*1202255*	*98.31*
	5	*1203283*	*98.81*
	6	*1202349*	*98.36*

Fig: 9 Chromatogram for Repeatability

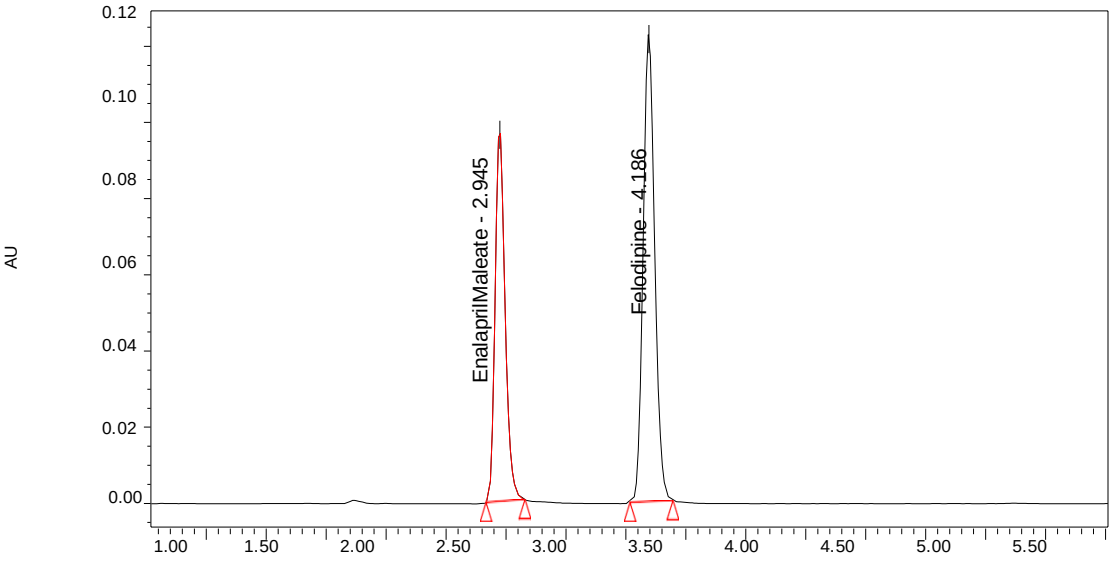


Table-8-Data of Intermediate precision (Analyst 2) for Enalapril Maleate

	Injection	Peak Areas of Enalapril Maleate	%Assay
Concentration on 40ppm	1	636792	99.99
	2	634360	99.66
	3	655696	101.53
	4	644147	99.98
	5	644127	99.97
	6	652525	101.10
Statistical Analysis	**Mean**	644607.8	100.37
	SD	6392.59	0.753536
	% RSD	1.183	0.75

Table-9-Data of Intermediate precision (Analyst 2) for Felodipine

	Injection	Peak Areas of Felodipine	%Assay
Concentration 40ppm	1	1205267	99.78
	2	1205625	99.95
	3	1205840	100.00
	4	1202735	98.55
	5	1208991	101.50
	6	1208543	101.37
***Statistical* Analysis**	**Mean**	1206333.5	100.19
	SD	12572.599	1.100898
	% RSD	1.24	1.09

Table-10-Data of Accuracy for EnalaprilMaleate (50%)

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Injection 1	20	20.04	100.22	MEAN	100.06
50% Injection 2	20	19.97	99.85		
50% Injection 3	20	20.02	100.11	%RSD	0.18

Table-11-Data of Linearity (Enalapril Maleate)

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	18600
20	632546	y-Intercept	276.2
30	658296	Correlation Coefficient	1
40	694400		
50	730308		
60	916282		
70	9402046		
80	9788277		

Table-12-Data of Linearity (Felodipine)

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	5140
20	1202965	y-Intercept	114.7
30	1254371	Correlation Coefficient	1
40	1295856		
50	1297167		
60	1308577		
70	1359903		

Fig: 11 Linearity graph of Felodipine

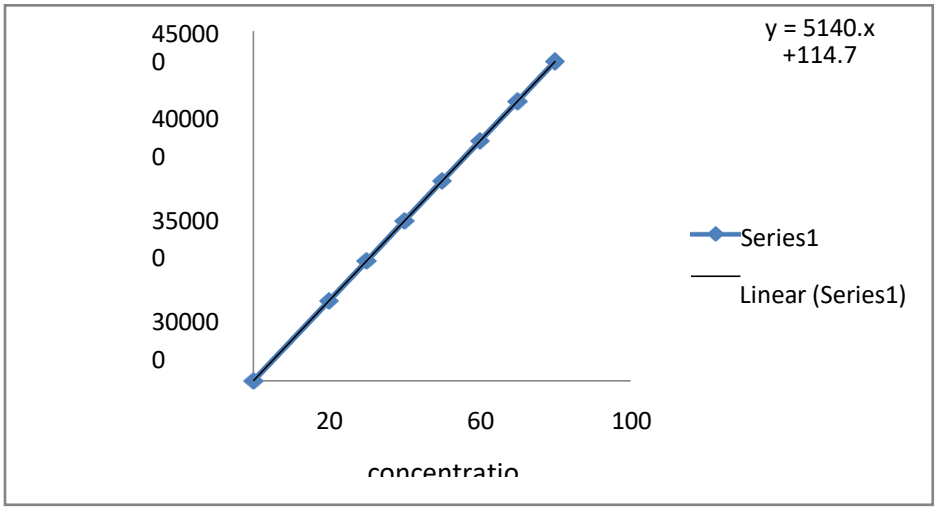


Fig: 12 Chromatogram for 30 ppm standard 1

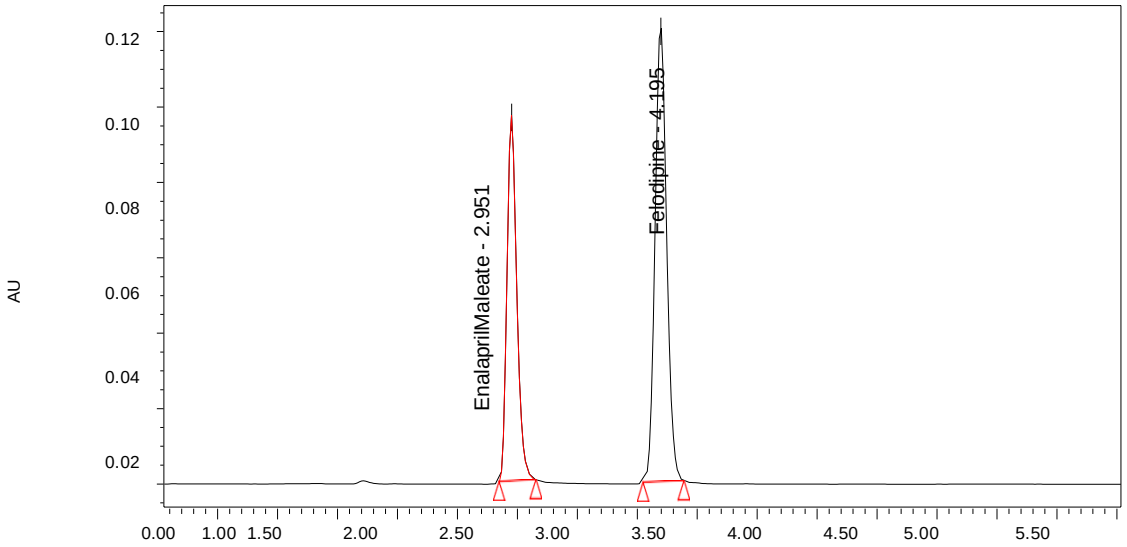


Fig: 13 Chromatograms for 50ppm

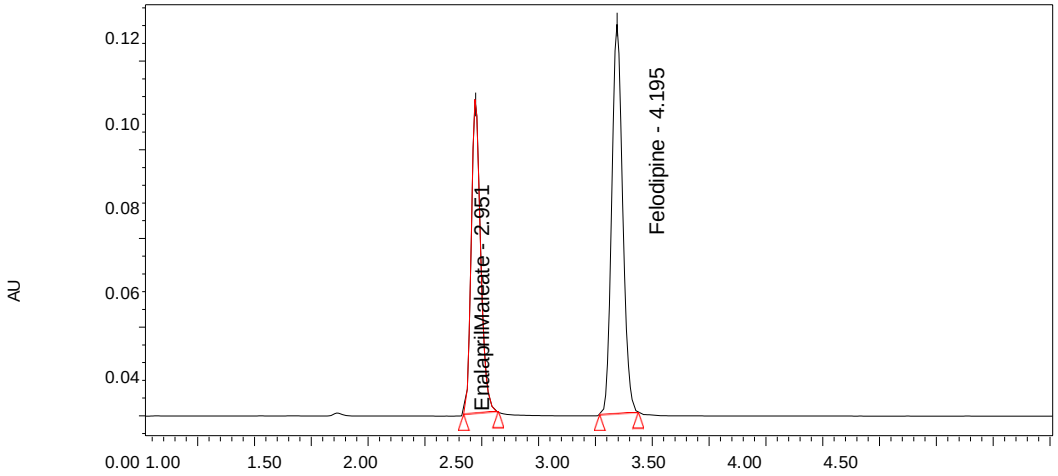


Fig: 14 Chromatogram for 70 ppm standard

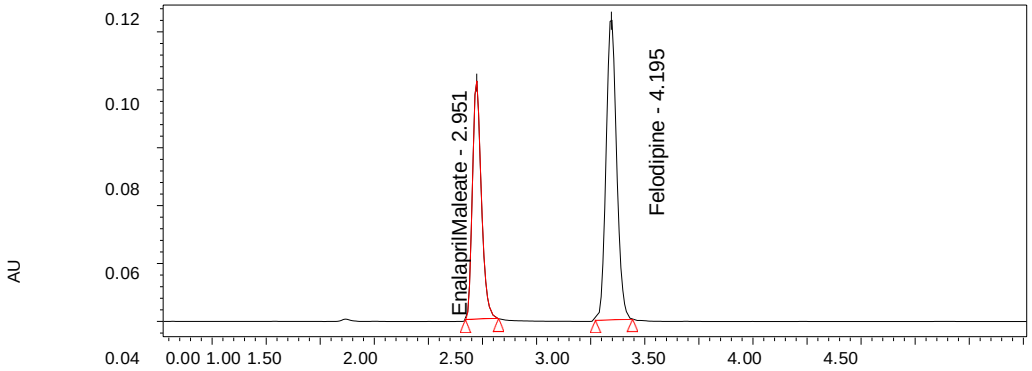


Fig: 15 Chromatograms of system to system variability

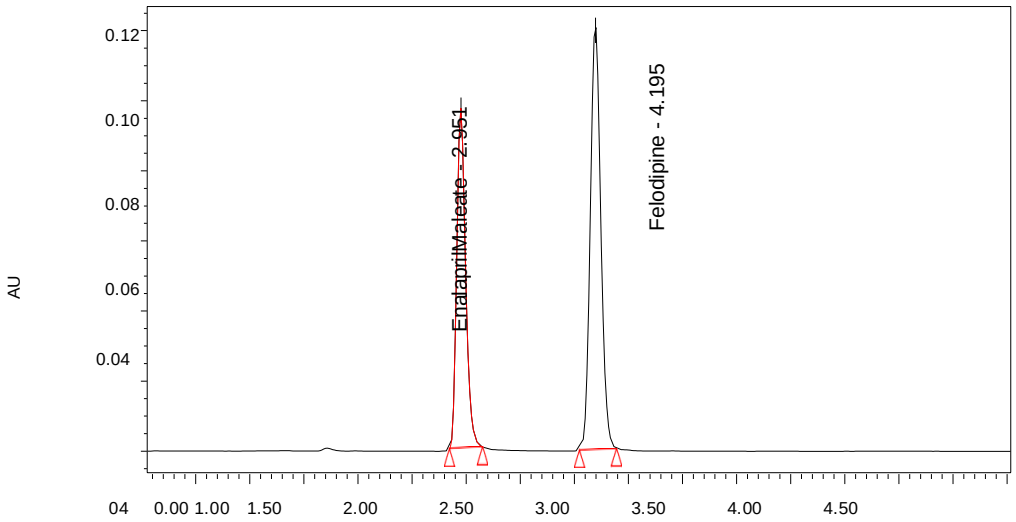


Fig: 16 Chromatogram of system to system variability std- 1

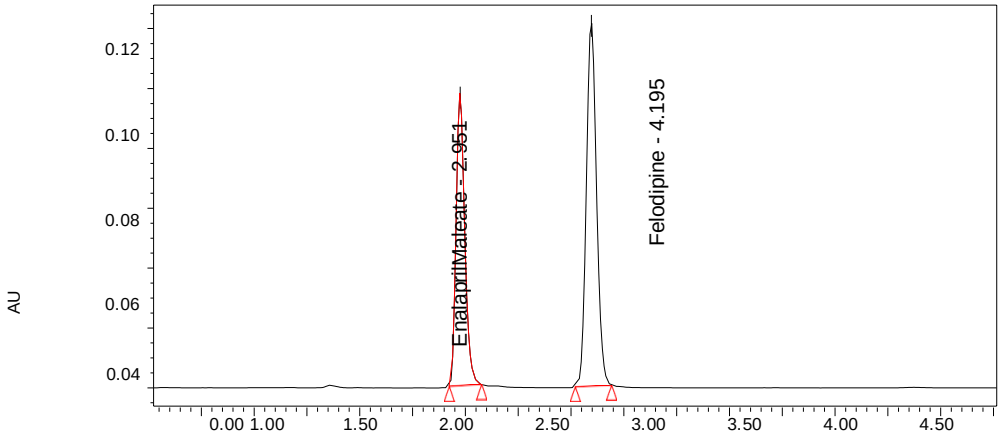


TABLE-14- Data for Effect of variation in flow rate (Felodipine)

	Std Area	Tailing factor		Std Area	Tailing factor		Std Area	Tailing Factor
	1273707	1.362089		1206349	1.280574		1266195	1.285372
Flow 0.8 ml	1273211	1.352617	**Flow 1.0 ml**	1205267	1.279932	**Flow 1.2 ml**	1265885	1.299385
	1273948	1.376926		1205625	1.261721		1266303	1.308063
	1273465	1.345752		1205840	1.276089		1267243	1.274662

SUMMARY AND CONCLUSION:

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 215nm Enalapril Maleate for and 248nm for Felodipine. Common wavelength will be 237nm and the peaks purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Inertsil C_{18}, ODS chosen good peak shape. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area, satisfactory retention time and good resolution. Different ratios of mobile phase were studied, mobile phase with ratio of 60:40 Methanol: Buffer was fixed due to good symmetrical peaks and for good resolution. So this mobile phase was used for the proposed study. The present recovery was found to be 98.0-101.50 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. Detection limit was found to be 0.25 Enalapril Maleate and 0.34 for Felodipine. Linearity study was, correlation coefficient and curve fitting was found to be. The analytical method was found linearity over the range of 20-80ppm of the target concentration for both the drugs. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

REFERENCES:

1. BeermannB, Groschinsky-Grind M, RosénA. (1976). "Absorption, metabolism, and excretion of hydrochlorothiazide". ClinPharmacolTher**19** (5 (Pt 1)):5317.
2. "Hydrochlorothiazide". The American Society of Health- System Pharmacists. Retrieved Jan 2015.
3. Wright, JM; Musini, VM (8 July 2009). "First-line drugs for hypertension.". The Cochrane database of systematic reviews

(3):CD001841.doi:10.1002/14651858.CD001841.pub2.PMID19588327.

4. Ravina, Enrique (2011). *The evolution of drug discovery: from traditional medicines to modern drugs* (1 Ed.). Weinheim: Wiley-VCH.p.74. ISBN9783527326693.

5. "WHO Model List of EssentialMedicines" (PDF). World Health Organization. October 2013. Retrieved 22April2014.

6. "Best drugs to treat high blood pressure The least expensive medications may be the best for many people". *http://www.consumerreports.org/*. November 2014. Retrieved 10 January2015.

7. Messerli, Franz; Makani, Harikrishna; Benjo, Alexandre; Romero, Jorge; Alviar, Carlos; Bangalore, Sripal (2011). "Antihypertensive Efficacy of Hydrochlorothiazide as Evaluated by Ambulatory Blood Pressure Monitoring: A Meta- Analysis of

8. Mitchell, Deborah. "Long-Term Follow-Up of Patients with Hypo para thyroidism". J Clin Endocrin Metab. Endocrine Society. Retrieved 19 June2013. Randomized Trials". J.Am. Coll. Cardiol. **57**(5):590–600. Doi:10.1016/j.jacc.2010.07.053.

9. Johnson, KK; Green, DL, Rife, JP, Limon, L (February 2005). "Sulfonamide cross-reactivity: fact or fiction?". The Annals of pharmacotherapy **39** (2): 290–301.doi:10.1345/aph.1E350. PMID15644481.

10. Uniformed Services University Pharmacology Note Set #3 2010,Lectures #39 & #40, Eric Marks

11. Duarte, JD; Cooper-Dehoff, RM (2010). "Mechanisms for blood pressure lowering and metabolic effects of thiazide and thiazide-like diuretics". Expert review of cardiovasculartherapy**8** (6):793–802.doi:10.1586/erc.10.27. PMC 2904515.PMID 20528637. NIHMSID: NIHMS215063

12. "Tour de France: Alexandr Kolobnev positive for banned diuretic". Velonation. 2011-07-11. Archived from the original on 2011-07-12. Retrieved 2011-07- 12.

13. "Kolobnev denies knowledge of doping product, says not fired by Katusha".Velonation. 2011-07-12. Archived fromthe original on 2011-07-12. Retrieved2011-07.