“VALIDATED RP-HPLC METHOD DEVELOPMENT FOR THE SIMULTANEOUS ESTIMATION OF LISINOPRIL AND HYDROCHLOROTHIAZIDE IN BULK PHARMACEUTICAL DOSAGE FORM”

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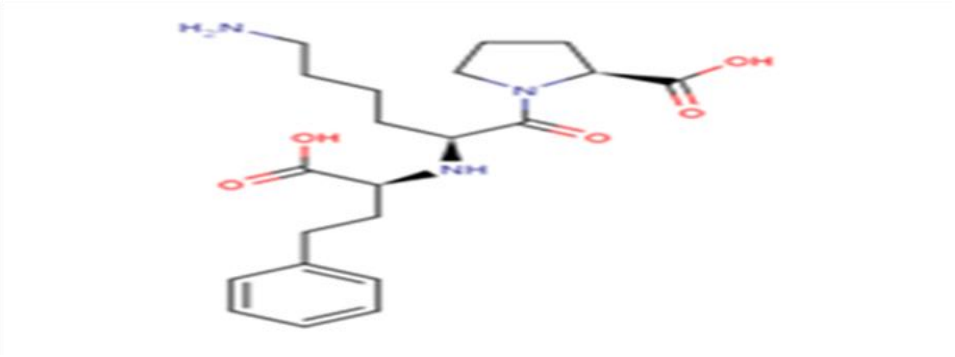
Drug profile

Lisinopril:

Generic Name	: Lisinopril
Molecular Formula	: C21H31N3O5
Molecular Weight	: 408.48
Melting Point	: 152-156ºC
Category	: Anti Hypertensive



Chemical Name :.(2S)-1-[(2S)-6-amino-2-[[(2S)-1-hydroxy- 1-oxo- 4-phenylbutan-2-yl]amino]hexanoyl] pyrrolidine-2-carboxylic acid

Description :A white powder.

- **Solubility:**
- Freely soluble in methanol, acetonitrile and n-butyl acetate, dichloromethane increases with temperature. Slightly soluble in water

Mechanism of action:

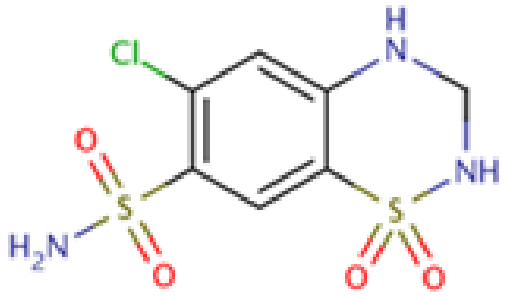
- Lisinopril competes with angiotensin I for its binding site on the angiotensin-converting enzyme (ACE), an enzyme which converts angiotensin I to angiotensin II.

Therapeutic Dosage:

Adult: 2.5 to 20 mg daily.

Hydrochlorothaizide

Generic Name	: Hydrochlorothiazide
Molecular Formula	: C7H8ClN3O4S2
Molecular Weight	: 297.73
Melting Point	: 158-160º C
Category	: Anti Hypertensive,Diuretic.



Chemical Name: Ethyl methyl (4RS)-2,6-dimethyl-4-(3-nitrophenyl)-1,4 dihydropyridine-3,5-dicarboxylate.

Description: Olmesartan medoxomil is a Yellow, crystalline powder.

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- **Solubility:**

  Freely soluble in ethyl acetate, soluble in ethanol and methanol, practically insoluble in water.

- **Mechanism of action**:

  As a diuretic, hydrochlorthiazide inhibits active chloride reabsorption at the early distal tubule via the Na-Cl cotransporter, resulting in an increase in the excretion of sodium, chloride, and water. Hydrochlorothiazide also inhibit sodium ion transport across the renal tubular epithelium through binding to the thiazide sensitive sodium-chloride transporter. This results in an increase in potassium excretion via the sodium-potassium exchange mechanism.

- **Therapeutic Dosage:**

  Adult: 25 to 200 mg daily

Literature review

- **Lisinopril**
- Linisopril
- Randa Hilal-Dandan "Renin and Angiotensin". Chapter 26 in Goodman & Gilman's The Pharmacological Basis of Therapeutics, 12e, eds. Laurence L. Brunton, Bruce A. Chabner, Björn C. Knollmann. The McGraw-Hill Companies, Inc. 2011. ISBN 978-0-07-162442-8

- "Diovan prescribing information". Novartis.
- Lexi-Drugs Online. "Valsartan". Lexi-Comp.
- Haberfeld, H, ed. (2009). Austria-Codex (in German) (2009/2010 ed.). Vienna: Österreichischer Apothekerverlag. ISBN 3-85200-196-X.

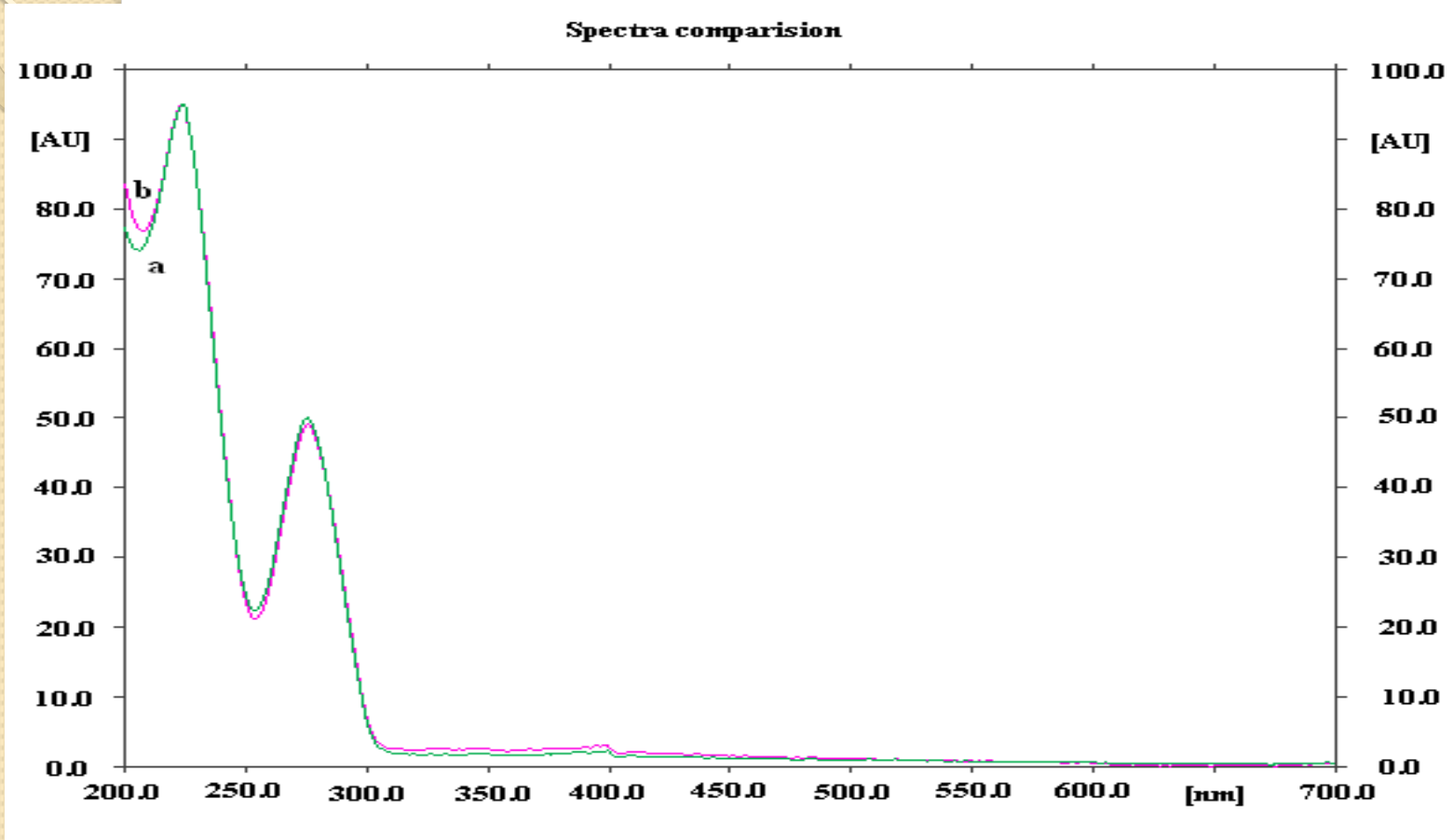
- Hydrochlrothiazide:
- Beermann B, Groschinsky-Grind M, Rosén A. (1976). "Absorption, metabolism, and excretion of hydrochlorothiazide". Clin Pharmacol Ther 19 (5 (Pt 1)): 531–7.
- "Hydrochlorothiazide". The American Society of Health-System Pharmacists. Retrieved Jan 2015.
- Wright, JM; Musini, VM (8 July 2009). "First-line drugs for hypertension.". The Cochrane database of systematic reviews (3): CD001841. PMID 19588327.

Aim of work

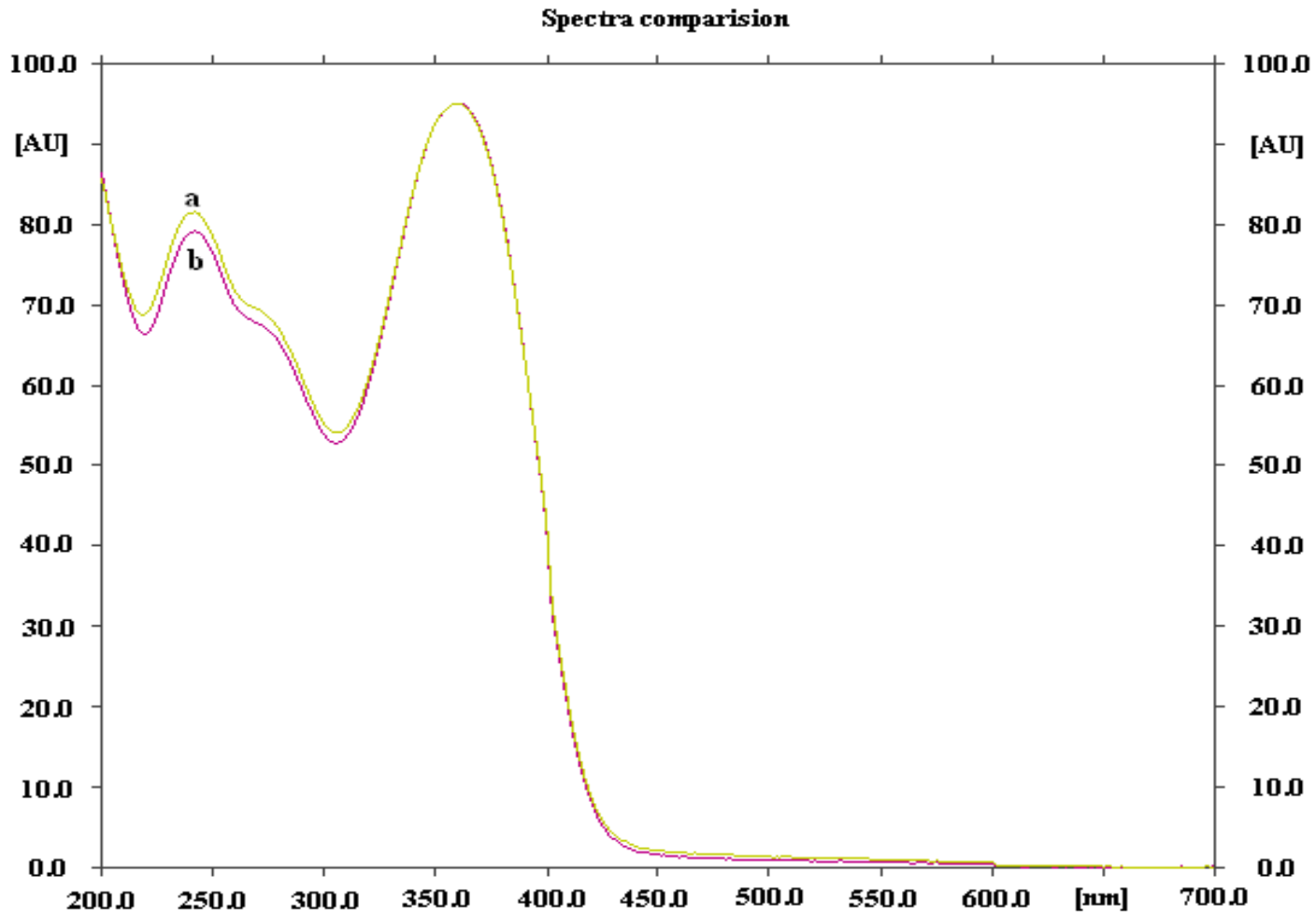
There is no specific HPLC method for simultaneous estimation of Lisinopril and Hydrochlorothiazide in combined dosage form. So the aim of work is develop a Rp-Hplc method and its validation for simultaneous estimation Lisinopril and Hydrochlorothiazide in tablet dosage form

Method Development

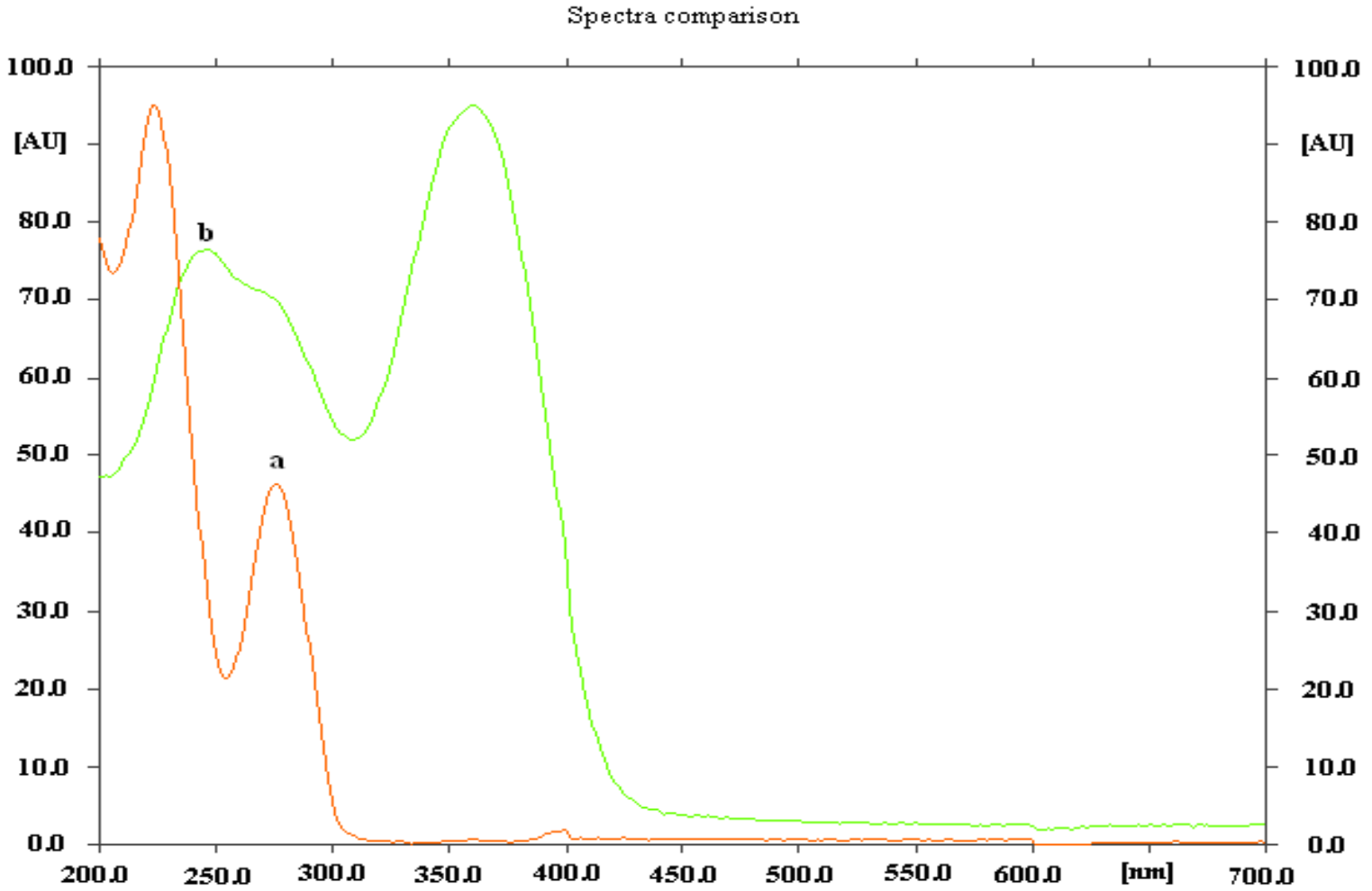
Wavelength Detection of Lisinopril in methonol: 224nm



Wavelength Detection of Hydrochlorothiazide in methonol: 238nm



UV overlay spectra of Lisinopril (a) and Hydrochlorothaizide (b) in methanol (235 nm)



- **Initialization of The Instrument**

  First the column was placed on the instrument and switch on the instrument and washed with Double Distilled water for 30 min. Then run the mobile phase for 30 min for column saturation

- **Preparation of buffer solution:**

  1.54gm of ammonium acetate was dissolved in 1000ml of double distilled water.

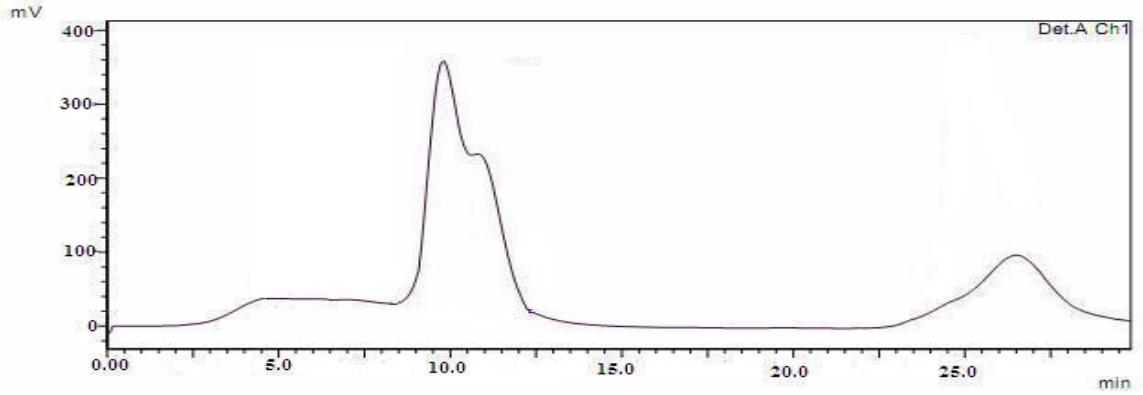
- **Preparation of Standard Solution**

  About 50mg of Lisinopril and 10mg of Hydrochlorothiazide working standard was weighed and transferred into a 100ml volumetric flask, and dissolved separately in small quantity of methanol and was made up to volume with mobile phase. 5ml of stock solution was pipetted out and transferred into a 50ml volumetric flask and made up to the volume with mobile phase.

Method Development Trials

Trial 1: mobile phase

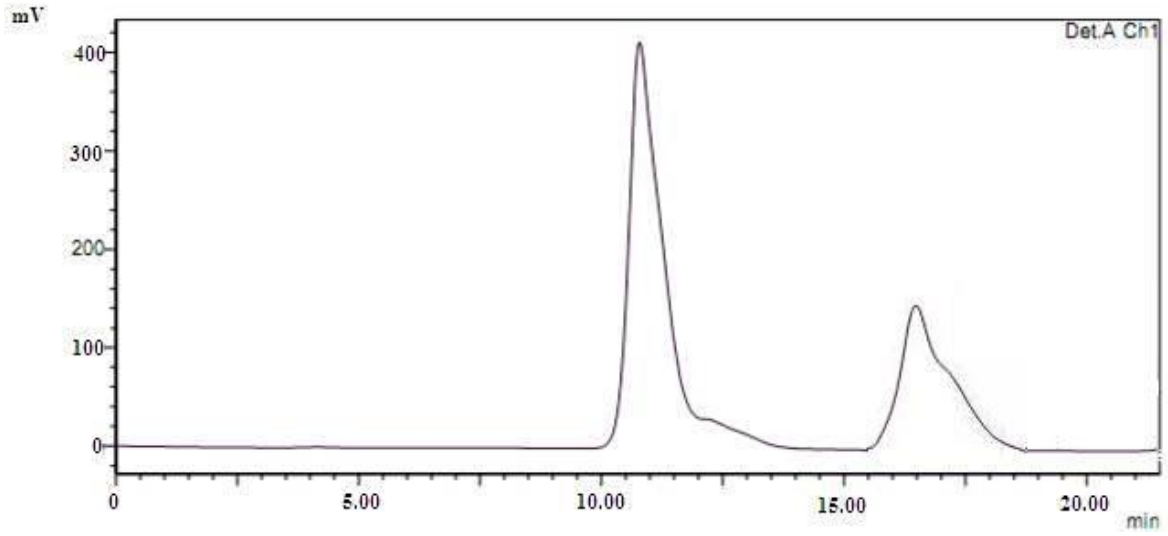
The mobile phase was prepared by mixing buffer: acetonitrile: methanol in the ratio of 35:30:35. pH was adjusted to 3.0 with ortho phosphoric acid. Filtered through 0.45µ membrane filter paper, then sonicated for 2-3 min for degassing the air from mobile phase.



Result: Peak shape was not good, retention time was more and tailing was more than limit

Trial 2:Mobile phase:

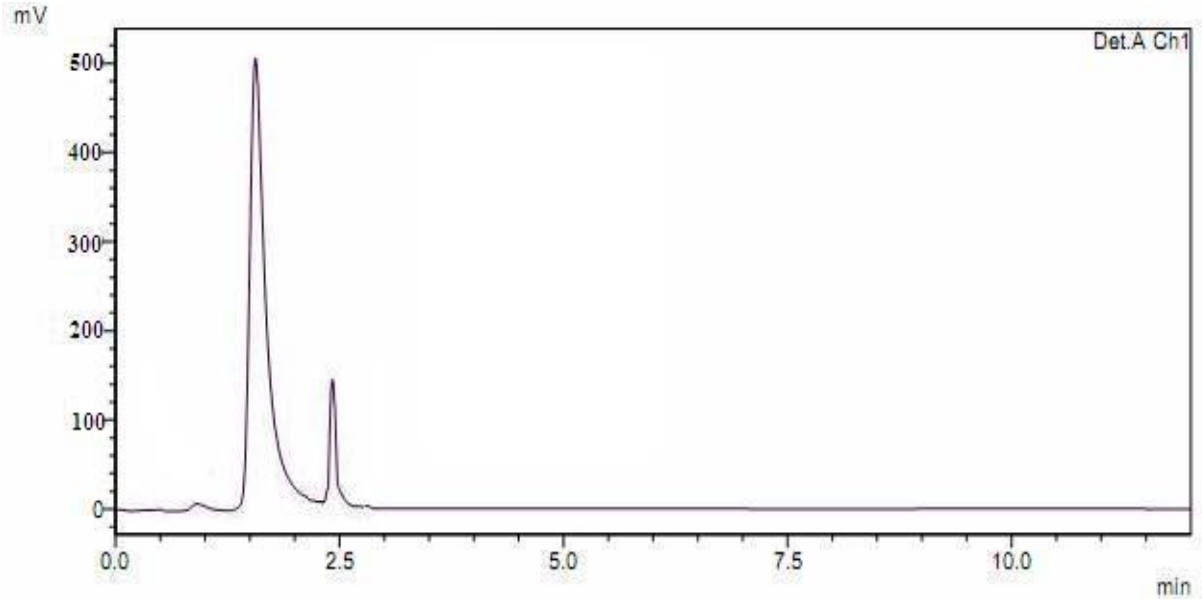
The mobile phase was prepared by mixing methanol : water in the ratio of 80:20. and pH was adjusted to 3.0 with Orthophosphoric acid, Above solvent was filtered and degassed



Result: Peak shape was not good, retention time was more and tailing was more than limit.

Trial 3:Mobile phase:

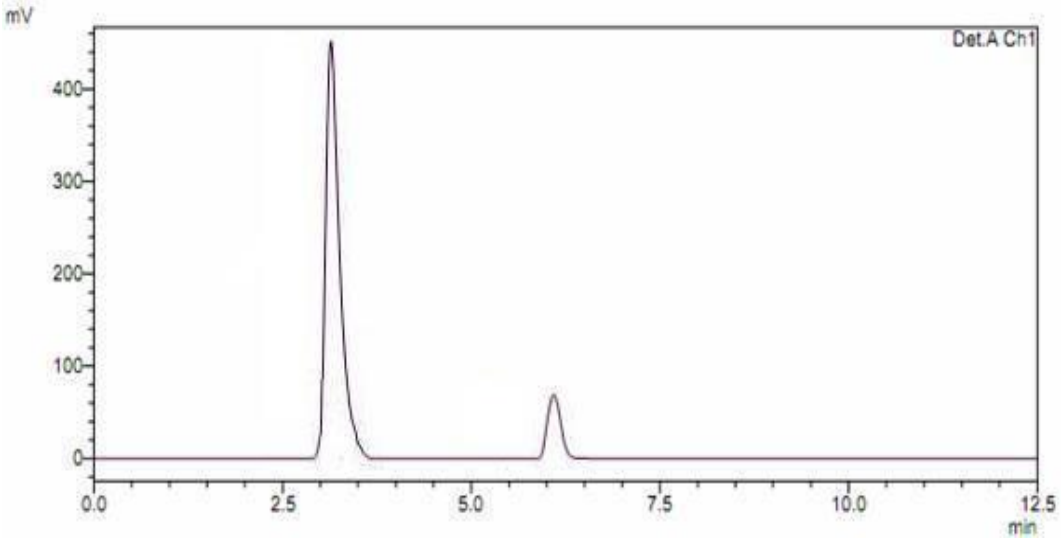
The mobile phase was prepared by mixing of Acetonitrile : Water in ratio of 80:20 and pH was adjusted to 3.0 with Orthophosphoric acid filtered and degassed it.



Result: Theoretical plates were less and tailing was more than limit

Trial 4:mobile phase

The mobile phase was prepared by mixing Methanol : Acetonitrile : Water in the ratio of 50 : 30 : 20 and pH was adjusted to 3.0 with Orthophosphoric acid and was filtered and degassed.

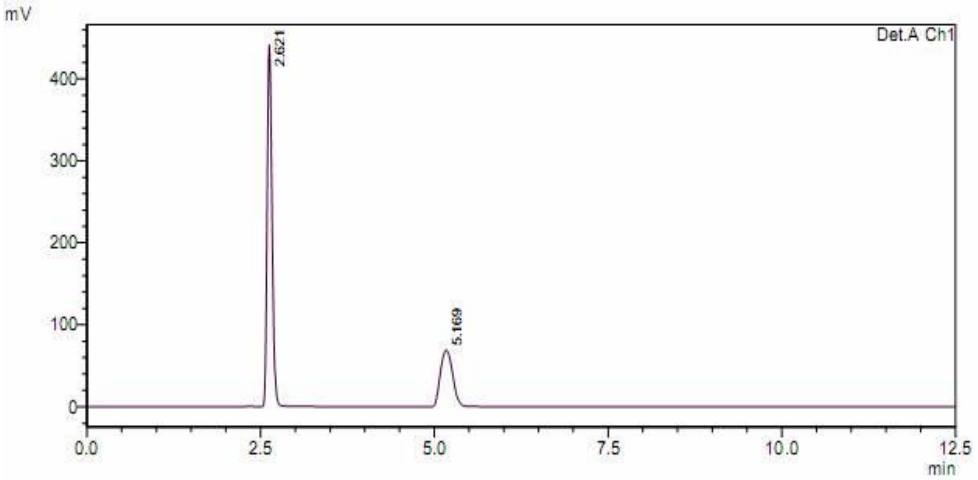


Result: Theoretical plates were less and tailing was more than limit

Trial 5:OPTIMIZED METHOD

mobile phase

The mobile phase was prepared by mixing Methanol: Acetonitrile: water in the ratio of (40:40:20) and was filtered and degassed



Result: The retention time of Atenolol was 2.621 min and for Nitrendipine was 5.169 min. The peaks are well separated with a resolution of 11.079.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Stationary Phase	: Phenomenex Luna C18 5µ (250 × 4.6mm)
Instrument	: Shimadzu HPLC LC-2010
Injection Volume	: 20µl
Flow rate	: 1.3 ml/min
Operating temperature	: Room temperature
detector wave length	: 235 nm
Mobile phase ratio	: methonol: Acetonitrile:water (40:40:20 V/V)
Diluent	: Mobile Phase
run time	: 12.5 min

VALIDATION

System suitability studies

lisinopril

System suitability parameters	RT (min)	AUC	No. of Theoretical plates (n)	Tailing factor
Rep-1	2.621	2158951	5472.439	1.221
Rep-2	2.622	2150164	5508.321	1.217
Rep-3	2.624	2167349	5488.255	1.229
Mean	2.622	2158821.333	5489.672	1.222
S.D	0.0015	8593.234	17.982	0.0061
R.S.D	0.0582%	0.398%	0.327%	0.499%

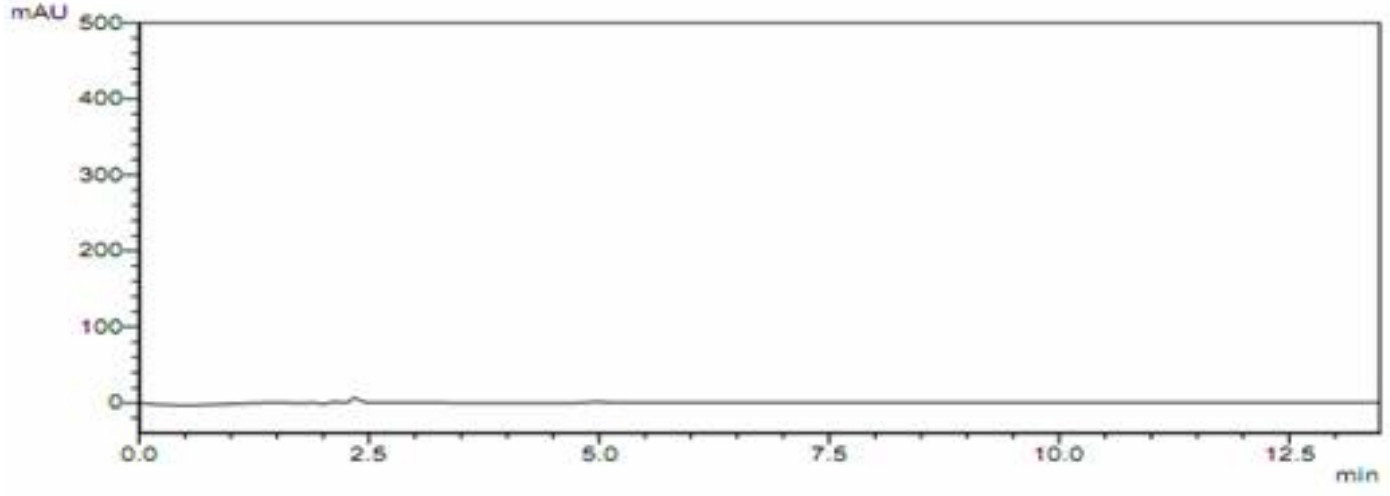
Hydrochlorothiazide

System suitability parameters	RT (min)	AUC	No. of Theoretical plates (n)	Tailing factor
Rep-1	5.169	852497	4078.964	1.158
Rep-2	5.161	841482	4067.803	1.159
Rep-3	5.156	845166	4061.306	1.164
Mean	5.162	846381.666	4069.358	1.160
S.D	0.0053	4578.278	7.292	0.0026
R.S.D	0.103%	0.540%	0.179%	0.226%

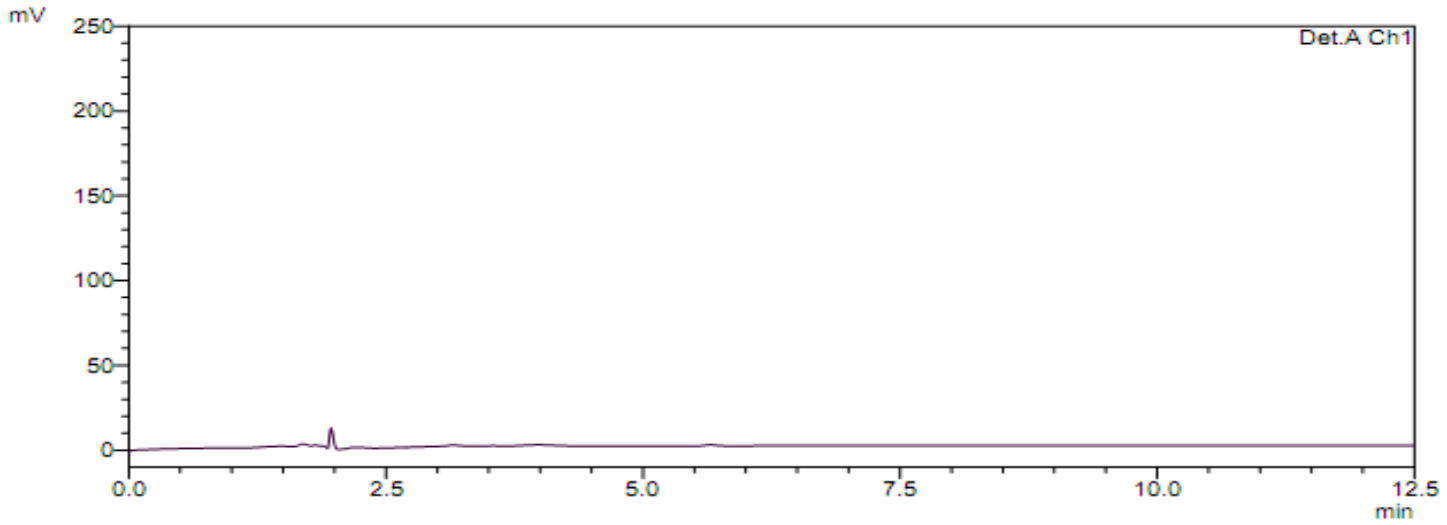
Result

- In System Suitability R.S.D. for Lisinopril was 0.398% for Hydrocholorothiazide it was 0.540%. For Lisinopril Mean RT, Theoretical plate and Tailing factor was found to be 2.6 min, 5489 and 1.222 respectively. For Hydrocholorothiazide Mean RT, Theoretical plate and Tailing factor was found to be 5.1 min, 4069 and 1.160 respectively.

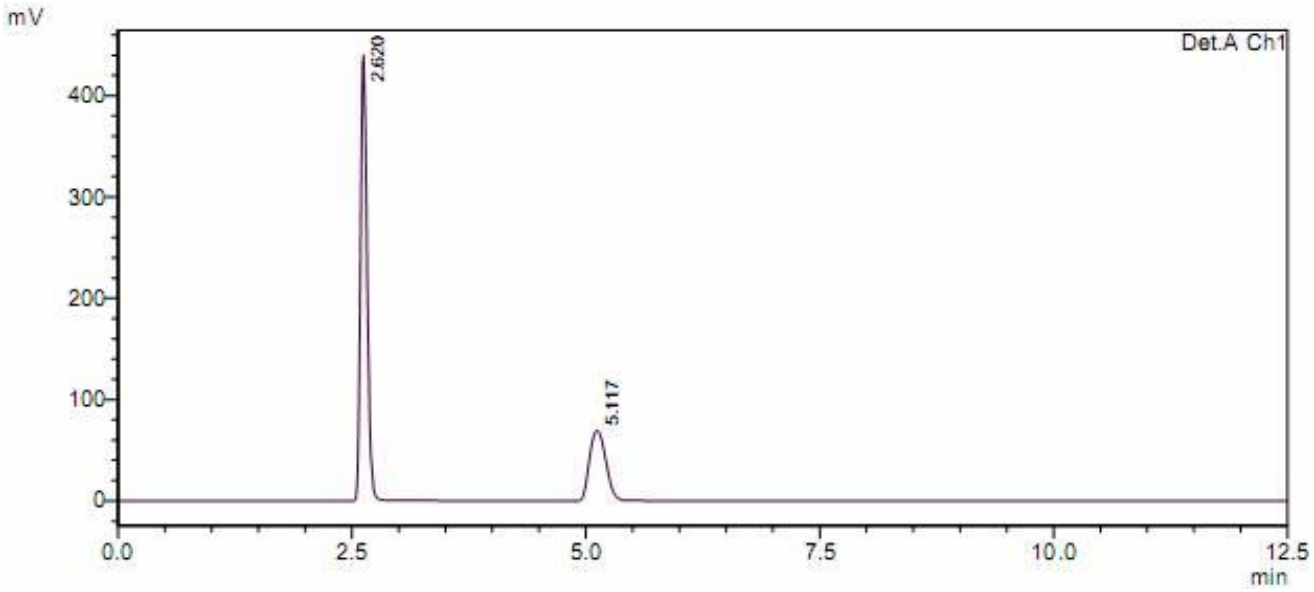
Specificity: Chromatograms for Specificity



Chromatogram for Mobile phase



Chromatogram for Placebo



Chromatogram for Lisinopril and Hydrochlorothiazide

Result for Specificity

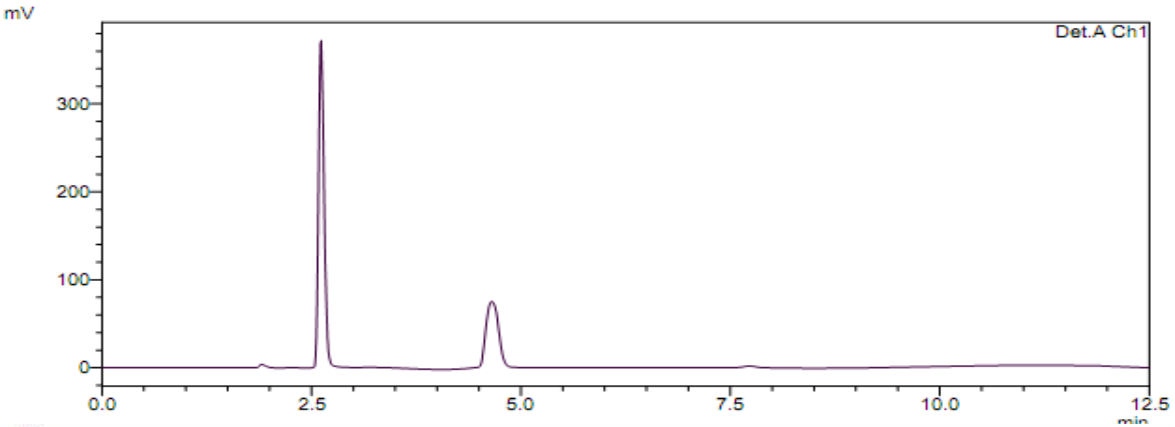
- According to above 3 graphs it was found that there was no interference by the placebo. That means no impurity was interfered.

Accuracy

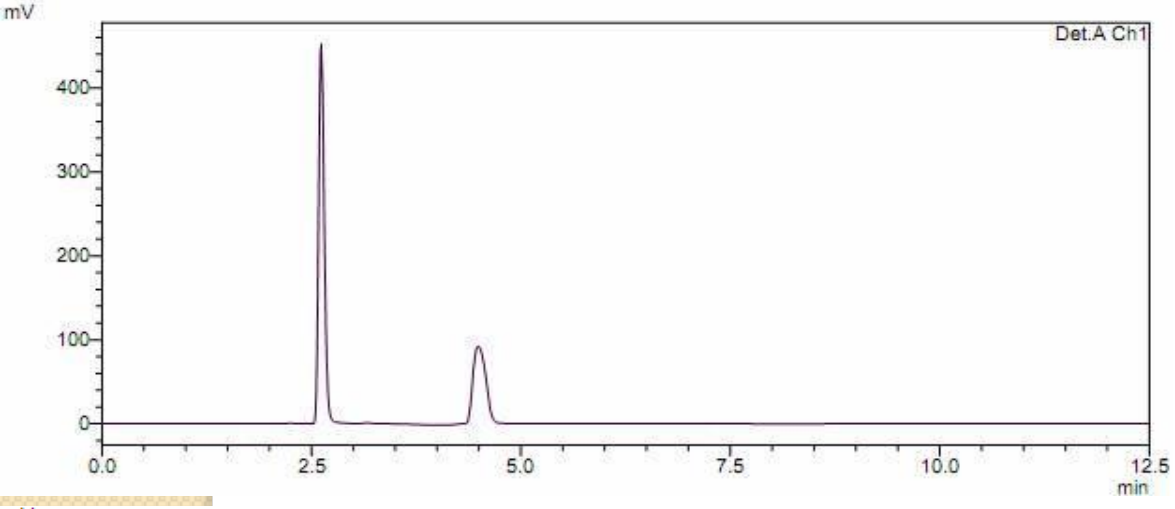
S.No	Inj. Sample	Spike level	s.d	r.s.d	% Recovered
1	lisinopril	80 %	0.691	0.692	99.865
2		100 %	0.691	0.573	99.525
3		120 %	0.665	0.675	100.014
4.	hydrochlorothaizide	80 %	0.995	0.990	100.460
5		100 %	0.770	0.764	100.71
6		120 %	0.807	0.809	99.750

Result for Accuracy:

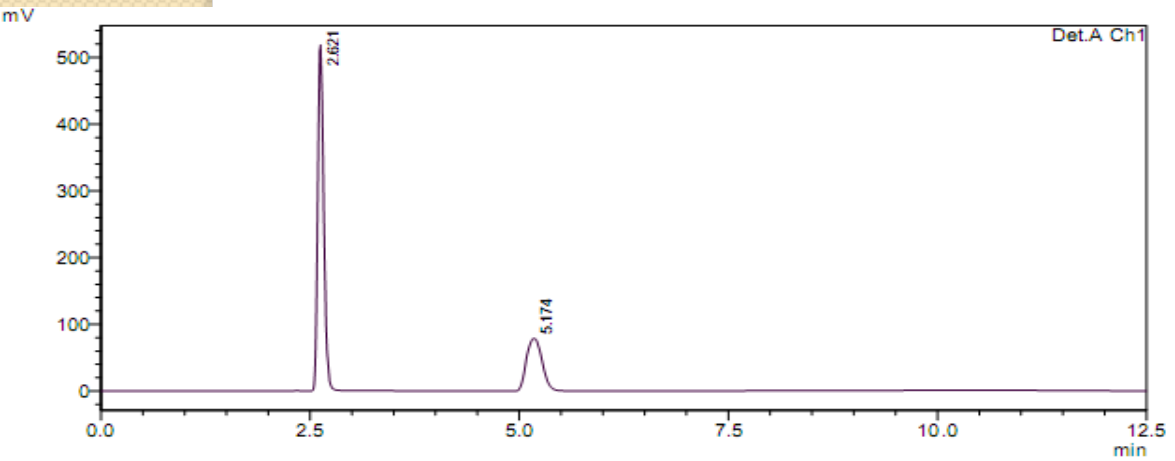
The % Recovery of lisinopril was **99.865, 99.525** & **100.014** at 80%, 100% & 120% level where as for Hydrocholo it was **100.460, 100.714 & 99.750** at 80%, 100% & 120% level



Recovery for Lisinopril and Hydrochlorothiazide at 80%



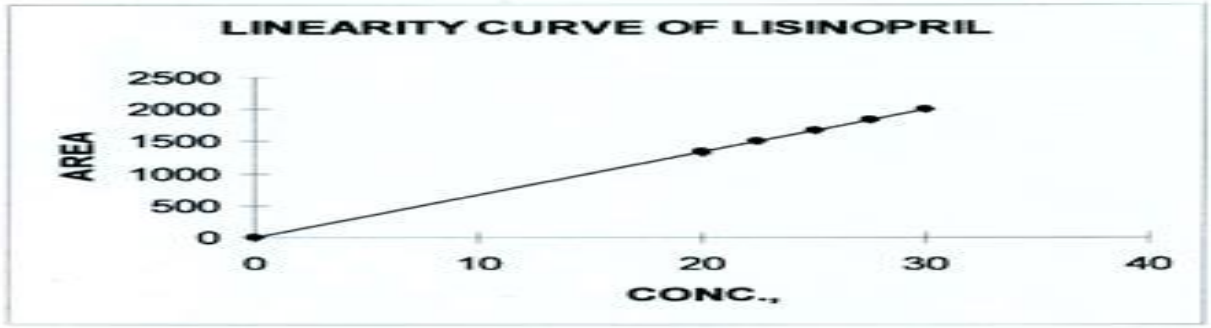
Recovery for Lisinopril and Hydrochlorothiazide at 100%



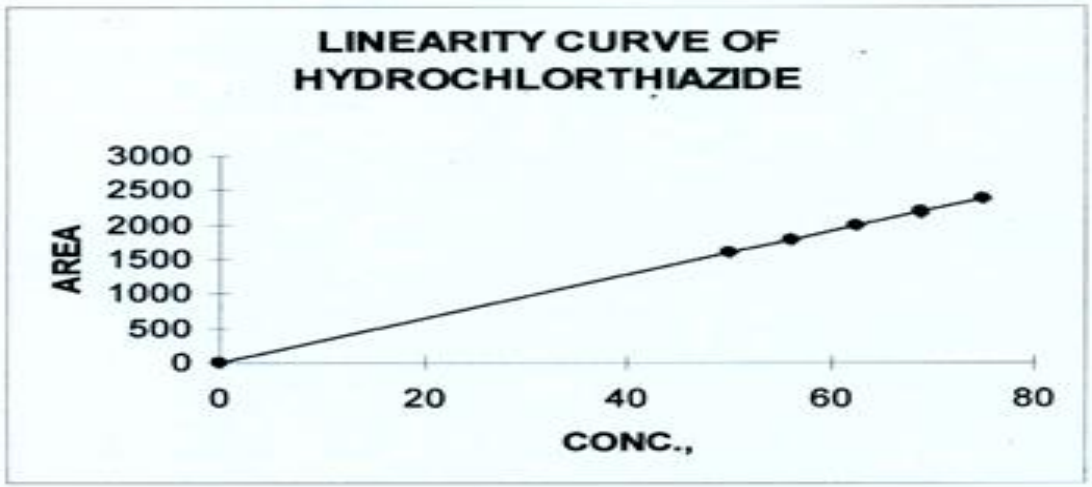
Recovery for Lisinopril and Hydrochlorothiazide at 120%

Linearity and Range

Lisinopril		**Hydrochlorothiazide**	
Concentration	AUC	Concentration	AUC
30 µg/ml	1218292	6 µg/ml	519916
40 µg/ml	1723148	8 µg/ml	667884
50 µg/ml	2157782	10 µg/ml	847280
60 µg/ml	2589426	12 µg/ml	1017826
70 µg/ml	3021724	14 µg/ml	1188728
Correlation Coefficient (R2)	0.9989	Correlation Coefficient (R2)	0.9992
Slope (m)	44731	Slope (m)	84378
Intercept (y)	94497	Intercept (y)	4543.8



LINEARITY CO-EFFICIENT :0.9999



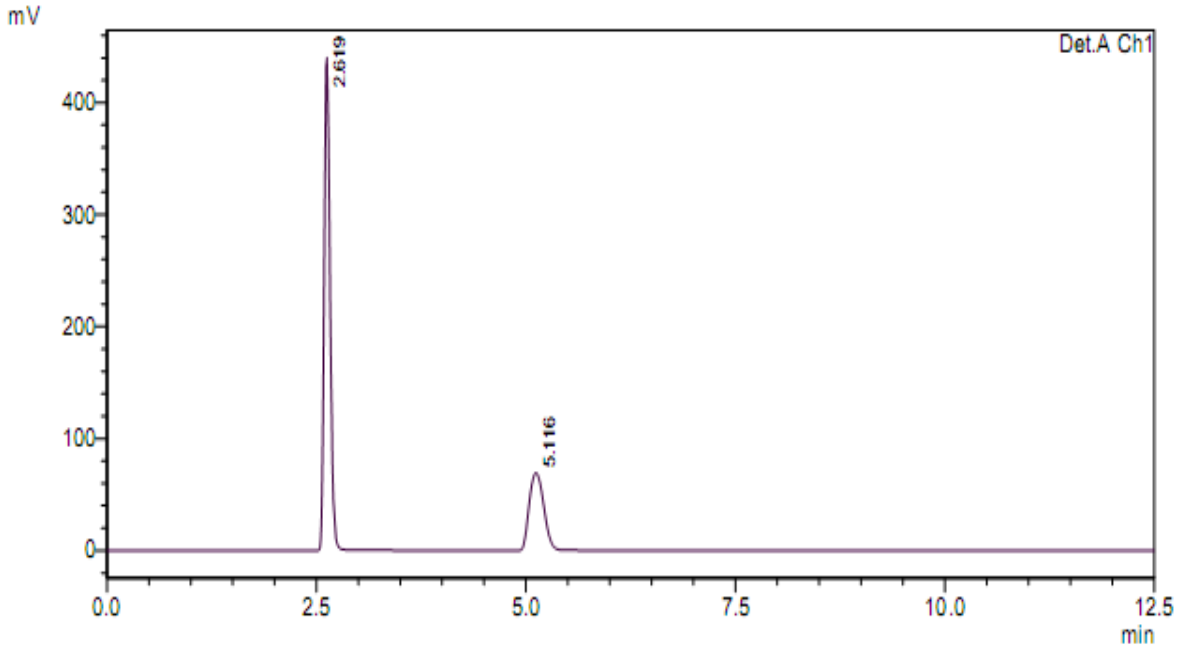
LINEARITY CO-EFFICIENT 0.9999

- LOD and LOQ determination is based on the standard deviation of the response and slope

- Limit of Detection for Lisinopril =0.10µg/ml
- Limit of Quantitation for Lisinopril = 0.10 µg/ml
- Limit of Detection for Hydrochlorothiazide = 0.30 µg/ml
- Limit of Quantitation for Hydrochlorothiazide = 0.32 µg/ml

Precision

i) Repeatability:



S. No	Lisinopril		Hydrochlorothiazide	
	Area of Lisinopril	Percentage amount	Area of Hydrochlorothaizide	Percentage amount
1	2156778	100.42	844122	100.97
2	2154088	100.29	839816	100.45
3	2157194	101.44	832488	99.57
4	158254	101.49	831982	99.51
5	2154682	100.32	838819	100.33
6	2118996	98.65	824978	98.67
Avg.	**2149998.66**	**100.10**	**835367.5**	**99.92**
S.D	**15268.95**	**0.710**	**6870.780**	**0.821**
R.S.D	**0.710**	**0.710**	**0.822**	**0.822**

Result for Precision:

The S.D of % amount of Lisinopril was 0.710 and Hydrochlorothaizide was 0.821. The R.S.D of Lisinopril was 0.710% and Hydrocholorothiazide was 0.822%.

The R.S.D of both was found less than **2.** Therefore this method has good reproducibility.

Robustness

Change in Flow Rate Parameter

In this parameter the Flow rate was changed to 1.3 ml/min.

S.NO	lisinopril			Hydrochlorothiazide		
	AUC	USP Tailing	R.T.	AUC	USP Tailing	R.T.
1	2462076	1.214	3.569	952120	1.157	5.971
2	2448472	1.217	3.564	954208	1.151	5.973
3	2458202	1.214	3.561	956694	1.156	5.982
Mean Area	2456250	1.215	3.564	954340	1.154	5.975
S.D.	7008.918			1869.682		
R.S.D.	0.285			0.195		

In this parameter the Flow rate was changed to 1.7 ml/min.

S.NO	lisinopril			Hydrochlorothiazide		
	AUC	USP Tailing	R.T.	AUC	USP Tailing	R.T.
1	1891056	1.216	1.841	748820	1.159	3.918
2	1893904	1.215	1.843	752209	1.161	3.915
3	1899500	1.224	1.846	745350	1.151	3.919
Mean Area	1894820	1.218	1.843	748793	1.157	3.91733
S.D.	4295.879			2800.240		
R.S.D.	0.226			0.373		

RESULT:

S.D.and RSD with decrease flow rate of mobile phase for lisinopril is 7008.918 and 0.285% for Hydrocholorothiazide is 1869.682 and 0.195 %.

S.D.and RSD with increase flow rate of mobile phase for lisinopril is 4295.879 and 0.226% for Hydrochlorothiazide is 2800.240 and 0.373 %.

RESULT:

S.D. and RSD of Lisinopril and Hydrochlorothiazide sample solution stability was 13386.29 and 0.6274%. and for Hydrochlorothaizide sample solution 9044.92 and 1.112%. S.D. and RSD of Lisinopril standard solution stability was 6716.582 and 0.312%. and for Hydrochlorothaizide standard solution it was 12130.314 and 1.432%.

Results with acceptance criteria

S. No	Parameter	Acceptance Criteria		Results Obtained	
				lisinopril	hydrocholorothiazide
1	System Suitability	R. S. D. NMT 2 %		0.398	0.540
2	Specificity	No Interference		No Impurity	No Impurity
3	Linearity	Correlation coefficient not less than 0.998		0.9989	0.9992
4	Precision	R. S. D. NMT 2 %	Repeatability	0.710	0.822
			Intermediate precision	0.445	0.668
5	Accuracy	R. S. D. NMT 2 %	80%	0.692	0.990
			100%	0.573	0.764
			120%	0.665	0.809
		Recovery of the spiked drug (98-102 %)	80%	99.865	100.460
			100%	99.525	100.714
			120%	100.014	99.750
6	Robustness	R.S.D.NMT 2 %	Flow Rate 1.3 ml/min	0.285	0.195
			Flow Rate 1.7 ml/min	0.226	0.373
			Wave length 230nm	0.263	0.874
			Wave length 240nm	0.449	0.191
8.	Solution Stability	R. S. D. NMT 2 %	Standard	0.312	1.432
			Sample	0.6274	1.112

SUMMARY AND CONCLUSION

A simple reverse phase HPLC method was developed for the simultaneous determination of Lisinopril and Hydrochlorothaizide in pharmaceutical dosage form. A Phenomenex Luna C18 5µ (250 × 4.6mm)5µ column from Thermo in isocratic mode, with mobile phases : methonol: Acetonitrile:water (40:40:20V/V)was used. The flow rate was 1.0-ml/ min and effluent was monitored at 235 nm.

The retention times The retention time of Lisinopril was 2.621 min and for Hydrochlorothiazide was 5.169 min. The peaks are well separated with a resolution of 11.079. As per ICH guidelines the method was validated.

BIBLIOGRAPHY

- Skoog W. Fundamental of Analytical Chemistry, Saunders College, Publishing, 7th Edn, 1992, pp.1-3
- Corners' K. A., Textbook of Pharmaceutical Analysis, A Wiley Interscience Publication, 1st Edn 1967, pp.475-78
- Kasture A.V., Wadodkar S.G., Mahadik K.R., and More H.N., Textbook of Pharmaceutical Analysis – II, Published by Nirali Prakashan, 13th Edn, 2005, pp.47-56
- Beckett A.H. and Stanlake J.B., Practical Pharmaceutical Chemistry, Part 2, CBS Publishers and Distributors, 4th Edn, 2002, pp.157-174
- Remington, The Science & Practice of a Pharmacy Vol. I, 20th Edn. pp 587-613
- Snyder L.R., Kirkland J.J. and Glajch L.J., Practical HPLC Method Development, John Wiley and Sons, INC., 2nd Edn. 1997, pp. 98-102
- ICH-Guidelines Q2A, Validation of Analytical Procedures: Definition and terminology Geneva, Switzerland 1995. 68-76.
- The United States Pharmacopoeia-32(2009),National Formulary 27,U.S.Pharmacopeial convention Inc., Rockville, MD, p. 1956-57.
- Willard H. H., Merritt, L. L., Dean, J. A. and Settle, F. A., Instrumental Methods of Analysis, CBS Publishers And Distributors, New Delhi, 7th Edn, pp.580-608

THANK YOU