International Journal of Multidisciplinary Research and Growth Evaluation.

Analytical method development and validation for the estimation of Lisinopril by reverse-phase high-performance liquid chromatography

Y Ganesh Kumar [1*], G Sruthi [2], Ejajahmed [3], Md. Sahin Ali [4], Malipatel Mani Vardhan Reddy [5]
[1-5] Department of Pharmaceutical Analysis, Sree Dattha Institute of Pharmacy, Sagar Road, Sheriguda, Ibrahimpatnam, Telangana, India

* Corresponding Author: **Y Ganesh Kumar**

Article Info

ISSN (online): 2582-7138
Volume: 03
Issue: 06
November-December 2022
Received: 28-10-2022;
Accepted: 19-11-2022
Page No: 469-475

Abstract
A simple isocratic RP-HPLC method has been developed and subsequently validated for the determination of Lamivudine in API form and pharmaceutical dosage forms as per ICH guidelines. The separation achieved on a reversed phase Symmetry ODS (C18) RP Column, 250 mm x 4.6 mm, 5µm as a stationary phase and Phosphate Buffer and Methanol in the ratio of 46:54 (pH-3.2) as mobile phase at a flow rate of 1.0 ml/min. The UV detection was performed at 206 nm. The retention time for Lamivudine was found to be 3.622min. The detector response was linear in the concentration range of 60-140 µg/ml. The respective linear regression equation being Y= 48313.x + 71968 with R2 = 0.9993. The percentage of Lamivudine in pharmaceutical dosage form was found to be within in the limits. The limit of detection and the limit of quantification were found to be 0.08µg/ml and 0.24µg/ml respectively. The results of the study showed that, the proposed RP-HPLC method was simple, rapid, precise, accurate and specific, which can be used for the routine determination of Lamivudine in API form and pharmaceutical dosage form.

Keywords: in API form and, RP-HPLC, Method Development, Validation, ICH Guidelines

Introduction

Lisinopril is an angiotensin-converting enzyme (ACE) inhibitor widely used in the therapy of hypertension and heart failure. Lisinopril [1] is associated with a low rate of transient serum aminotransferase elevations and has been linked to rare instances of acute liver injury that can be severe and even fatal. Lisinopril is indicated for the treatment of acute myocardial infarction, hypertension in patient's ≥6 years, and as an adjunct therapy for heart failure. A combination product with hydrochlorothiazide is indicated for the treatment of hypertension. Lisinopril [2] is an angiotensin converting enzyme inhibitor used to treat hypertension, heart failure, and myocardial infarction. Lisinopril is not a prodrug, and functions by inhibition of angiotensin converting enzyme as well as the renin angiotensin aldosterone system. It has a wide therapeutic index and a long duration of action as patients are generally given 10-80mg daily. Angiotensin II constricts coronary blood vessels and is positively inotropic, which under normal circumstances, would increase vascular resistance and oxygen consumption. This action can eventually lead to myocyte hypertrophy and vascular smooth muscle cell proliferation. Lisinopril [3] is an angiotensin converting enzyme inhibitor (ACEI), preventing the conversion of angiotensin I to angiotensin II. This action prevents myocyte hypertrophy and vascular smooth muscle cell proliferation seen in untreated patients. Increased levels of bradykinin also exhibit vasodilating effects for patients taking ACEIs. Lisinopril also inhibits renin's conversion of angiotensin to angiotensin I. Lisinopril is used alone or in combination with other medications to treat high blood pressure in adults and children 6 years of age and older. It is used in combination with other medications to treat heart failure. Lisinopril is also used to improve survival after a heart attack. The IUPAC Name of Lisinopril is (2S)-1-[(2S)-6-amino-2-[[(1S)-1-carboxy-3-phenyl propyl] amino] hexanoyl] pyrrolidine-2-carboxylic acid. The Chemical Structure of Lisinopril is as following.

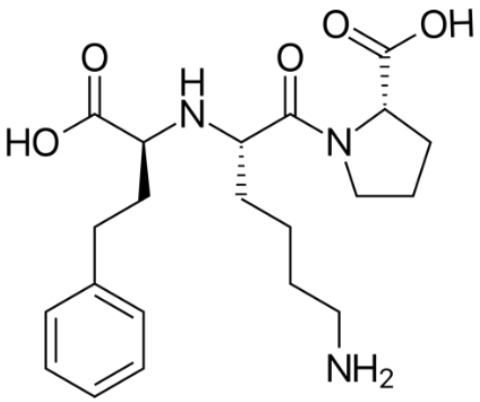

Fig 1: Chemical Structure of Lisinopril

Experimental

Table 1: List of Instrument used

S. No.	Instruments/Equipments/Apparatus
1.	**HPLC** with Empower2 Software with Isocratic with UV-Visible Detector (Waters).
2.	**T60-LAB INDIA** UV – Vis spectrophotometer
3.	Electronic Balance **(SHIMADZU ATY224)**
4.	Ultra Sonicator **(Wensar wuc-2L)**
5.	Thermal Oven
6.	Symmetry ODS RP C_{18},5µm, 15mm x 4.6mm i.d.
7.	P^H Analyzer **(ELICO)**
8.	Vacuum filtration kit **(BOROSIL)**

Table 2: List of Chemicals used

S. No.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
2.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
3.	Dipotassium hydrogen orthophosphate	96%	A.R.	Sd fine-Chem ltd; Mumbai
4.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
5.	Potassium dihydrogen orthophosphate	99.9%	A.R.	Sd fine-Chem ltd; Mumbai
6.	Sodium hydroxide	99.9%	A.R.	Sd fine-Chem ltd; Mumbai
7.	Hydrochloric acid	99.9%	A.R.	Loba Chem; Mumbai.
8.	Hydrogen Peroxide	99.9%	A.R.	Loba Chem; Mumbai.

Selection of Wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase [4] diluting with the same solvent.(After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Lisinopril, so that the same wave number can be utilized in HPLC UV detector for estimating the Lisinopril. The scanned UV spectrum is attached in the following page,

Sample & Standard Preparation for the UV-Spectrophotometer Analysis

25 mg of Lisinopril standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase.

Further dilution was done by transferring 0.5 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

Optimization of Chromatographic Conditions

The chromatographic conditions were optimized by different means. (Using different column, different mobile phase, different flow rate, different detection wavelength & different diluents for sample preparation etc.

Table 3: Summary of Process Optimization

Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Symmetry C_{18}, ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	Methanol: Acetonitrile = 40: 60	1.0ml/min	235nm	Very Low response	Method rejected
Symmetry C_{18}, ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	Methanol: Acetonitrile = 55: 45	1.0ml/min	235nm	Low response	Method rejected
Symmetry C_{18}, ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	Acetonitrile: Water = 50:50	1.0ml/min	235nm	Tailing peaks	Method rejected
Symmetry C_{18}, ODS, Reverse Phase,	Methanol: Water = 70:30	1.0ml/min	235nm	Resolution was not good	Method rejected

250 mm x 4.6 mm, 5µm, Column.					
Symmetry C_{18}, ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	ACN : Methanol: 0.1% OPA = 70:25:5	1.0ml/min	235nm	Tailing peak	Method rejected
Symmetry C_{18}, ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	ACN : Methanol: 0.1% OPA = 60:30:10	1.0ml/min	235nm	Nice peak	Method accepted

Preparation of Mobile Phase

600ml of HPLC Grade Acetonitrile, 300ml of HPLC Grade Methanol and 100ml 0.1% OPA were mixed well and degassed [5] in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration.

Results and Discussion

Method Development

Selection of Wavelength

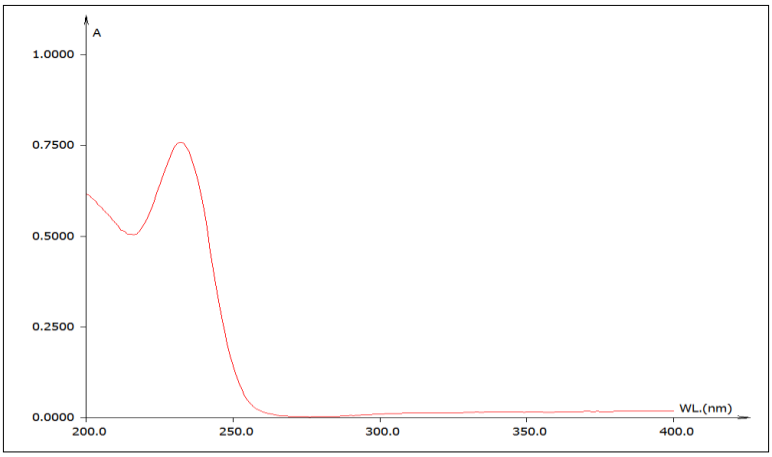

Fig 2: UV Spectrum for Lisinopril

Observation: While scanning the Lisinopril solution we observed the maxima at 235nm. The UV spectrum has been recorded on T60-LAB INDIA make UV-Vis spectrophotometer model UV-2450.

Summary of Optimized Chromatographic Conditions

The Optimum Chromatographic conditions[6] obtained from experiments can be summarized as below:

Table 4: Summary of Optimised Chromatographic Conditions

Mobile phase	**ACN : Methanol: 0.1% OPA = 60:30:10**
Column	Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm
Column Temperature	Ambient
Detection Wavelength	235 nm
Flow rate	1.0 ml/ min.
Run time	06 min.
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase
Injection Volume	10µl
Type of Elution	Isocratic
Retention time	2.570 minutes

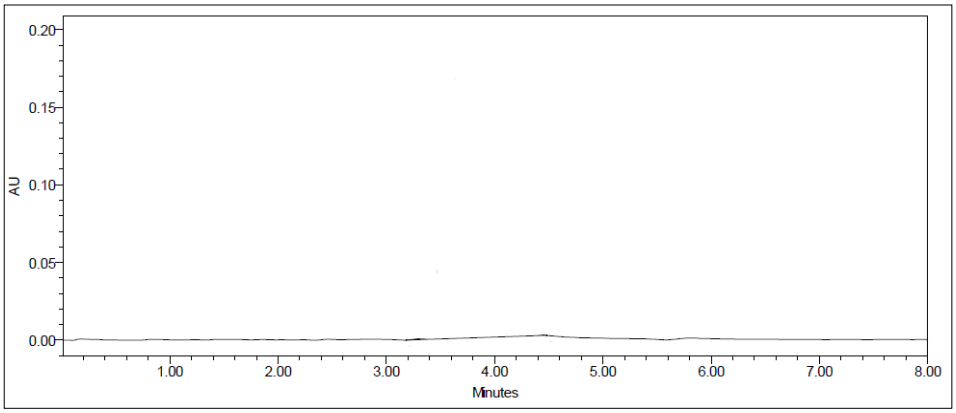

Fig 3: Chromatogram for Blank Solution

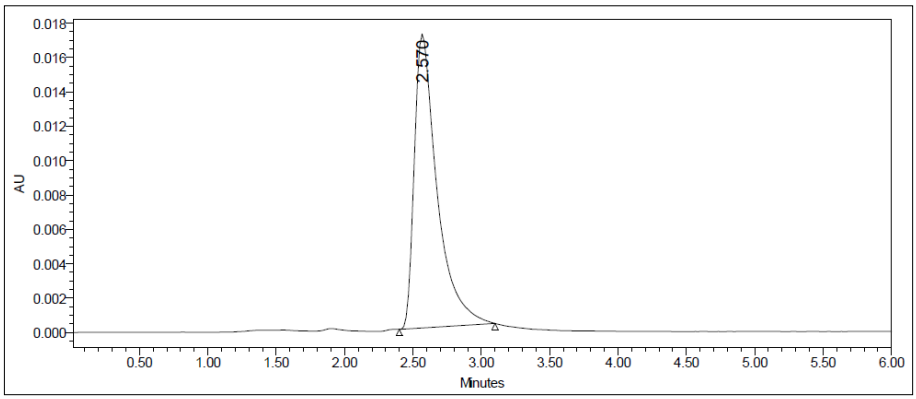

Fig 4: Chromatogram of Lisinopril in Optimized Condition

Method Validation

1. Accuracy

Recovery study

To decide the exactness [7] of the proposed strategy, recuperation thinks about were done by including diverse sums (80%, 100%, and 120%) of unadulterated medication [8] of LISINOPRIL were taken and added to the pre-broke down plan of fixation 10µg/ml. From that rate recuperation [9] esteems were computed. The outcomes were appeared in table-5.

Table 5: Readings of Accuracy

Conc. In ppm	Conc. Found	Peak Area		% Recovery
8	8.035	161523		100.437
8	8.153	163815		101.912
8	8.061	162023		100.762
			Avg.	101.037
			S.D	0.775
			%RSD	0.767046
Conc. In ppm	**Conc. Found**	**Peak Area**		% Recovery
10	9.930	198315		99.30
10	10.033	200320		100.33
10	10.044	200540		100.44
			Avg.	100.0233
			S.D	0.628835
			%RSD	0.628688
Conc. In ppm	**Conc. Found**	**Peak Area**		% Recovery
12	11.981	238151		99.841
12	12.066	239819		100.55
12	12.215	242712		101.791
			Avg.	100.7273
			S.D	0.987021
			%RSD	0.979894

2. Precision

2.1. Repeatability

The precision [10] of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Lisinopril (API). The percent relative standard deviation [11-12] was calculated for Lisinopril are presented in the table-6.

Table 6: Readings of Repeatability

HPLC Injection Replicates of Lisinopril	Retention Time (Minutes)	Peak Area (AUC)
Replicate – 1	2.572	197236
Replicate – 2	2.570	197762
Replicate – 3	2.573	195969
Replicate – 4	2.570	194724
Replicate – 5	2.574	198327
Replicate – 6	2.573	198711
Average		**197121.5**
Standard Deviation		**1515.213**
% RSD		**0.768667**

2.2. Intermediate Precision

2.2.1. Intra-assay & inter-assay

The intra & inter day variation [13-16] of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Lisinopril revealed that the proposed method is precise.

Table-7: Results of Intra-Assay & Inter-Assay

Conc. Of Lisinopril (API) (µg/ml)	Observed Conc. of Lisinopril (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
8	7.46	0.62	8.05	0.96
10	10.87	0.85	9.43	0.71
12	11.81	0.92	12.04	0.65

3. Linearity & Range

The calibration curve showed good linearity[17] in the range of

6-14 µg/ml, for Lisinopril (API) with correlation coefficient [18-19] (r^2) of 0.999 (Fig-5). A typical calibration curve [20] has the regression equation of y = 19423x + 5444 for Lisinopril.

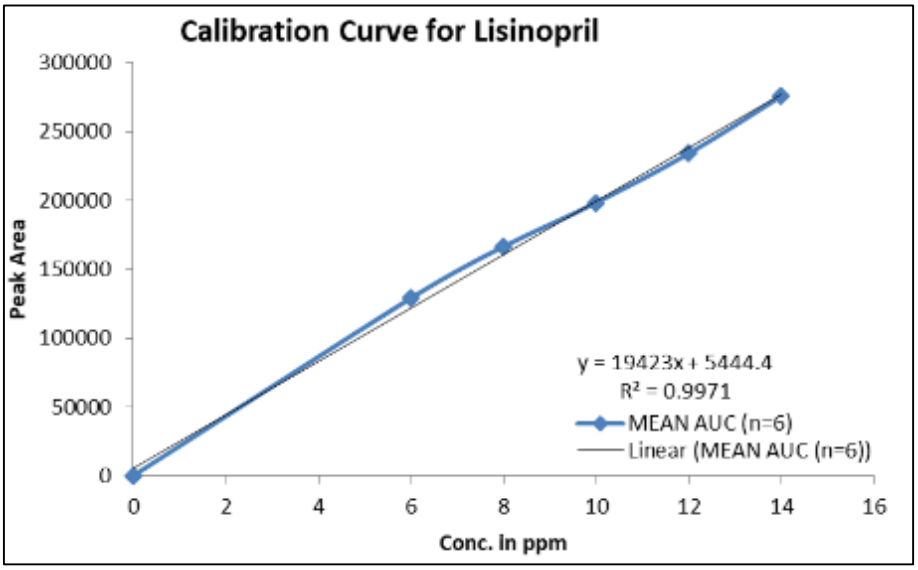

Fig 5: Calibration Curve of Lisinopril (API)

Table 8: Linearity Results

CONC.(µg/ml)	MEAN AUC (n=6)
0ppm	0
6ppm	129013
8ppm	166523
10ppm	198315
12ppm	234151
14ppm	275819

4. Method Robustness

Influence of small changes in chromatographic conditions [21] such as change in flow rate [22] (± 0.1ml/min), Wavelength of detection (±2nm) & organic phase in mobile phase (±5%) studied to determine the robustness [23] of the method are also in favour of (Table-9, % RSD < 2%) the developed RP-HPLC method for the analysis [24] of Lisinopril (API).

Table 9: Result of Method Robustness Test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.68
Flow (0.9 ml/min)	0.39
More Organic	0.54
Less Organic	0.63
Wavelength of Detection (237 nm)	0.91
Wavelength of detection (233 nm)	0.93

5. LOD & LOQ

The Minimum concentration level at which the analyte can be reliable detected (LOD [25]) & quantified (LOQ [26]) were found to be 0.08 & 0.24µg/ml respectively.

6. System Suitability Parameter

Framework appropriateness testing [27] is an essential piece of numerous scientific techniques. The tests depend on the idea that the gear, hardware, explanatory activities and tests to be broke down establish a vital framework that can be assessed all things considered. Following framework appropriateness test [28] parameters were built up. The information is appeared in Table-10.

Table 10: Data of System Suitability Parameter

S. No.	Parameter	Limit	Result
1	Resolution	Rs > 2	8.47
2	Asymmetry	T ≤ 2	Lisinopril=0.23
3	Theoretical plate	N > 2000	Lisinopril=2987
4	Tailing Factor	T<2	Lisinopril=1.17

7. Estimation of Lisinopril in Pharmaceutical Dosage Form

Twenty pharmaceutical dosage forms were taken and the I.P. strategy was taken after to decide the normal weight [29]. Above measured tablets were at last powdered and triturated well. An amount of powder proportionate to 25 mg of medications were exchanged to 25 ml volumetric flagon, make and arrangement was sonicated for 15 minutes, there after volume was made up to 25 ml with same dissolvable. At that point 10 ml of the above arrangement was weakened to 100 ml with versatile stage. The arrangement was separated through a layer channel (0.45 µm) and sonicated to degas [30]. The arrangement arranged was infused in five reproduces into the HPLC framework and the perceptions were recorded. A copy infusion of the standard arrangement was additionally infused into the HPLC framework and the peak regions were recorded. The information is appeared in Table-11.

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt} = \text{mg/tab}$$

Where:
AT = Peak Area of medication acquired with test arrangement
AS = Peak Area of medication acquired with standard arrangement
WS = Weight of working standard taken in mg
WT = Weight of test taken in mg
DS = Dilution of Standard arrangement
DT = Dilution of test arrangement
P = Percentage virtue of working standard

Table 11: Recovery Data for estimation Lisinopril in Zestril 10

Brand Name of Lisinopril	Labelled amount of Drug (mg)	Mean (± SD) amount (mg) found by the proposed method (n=6)	Assay % (± SD)
Zestril 10 Tablets (Astra Zeneca)	10mg	9.896 (±0.627)	99.79 (±0.277)

Result & Discussion

The amount of drug in Zestril Tablets was found to be 9.896 (±0.627) mg/tab for Lisinopril& % assay [31] was 99.79 %.

Summary and Conclusion

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Lisinopril, different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here Symmetry ODS RP C_{18}, 5µm, 15mmx4.6mm i.d. Column was preferred because using this column peak shape, resolution and absorbance were good. Discovery wavelength was chosen in the wake of examining the standard arrangement of medication more than 200 to 400nm. From the U.V range of Lisinopril it is apparent that a large portion of the HPLC works can be proficient in the wavelength scope of 210-300 nm helpfully. Further, a stream rate of 1 ml/min and an infusion volume of 10µl were observed to be the best investigation. The outcome demonstrates the created technique is amazingly, one more reasonable strategy for measure and dependability related debasement examines which can help in the investigation of Lisinopril in various details.

References

1. https://go.drugbank.com/drugs/DB00722
2. https://pubchem.ncbi.nlm.nih.gov/compound/Lisinopril
3. https://en.wikipedia.org/wiki/Lisinopril
4. Morgan David J. Fraction collector (post on Flickr)". Flickr. Retrieved, 2015.
5. Karger Barry L. HPLC: Early and Recent Perspectives. Journal of Chemical Education. 74: 45. Bibcode: 1997JChEd. 1997; 74:45K.
6. Henry Richard A. The Early Days of HPLC at Dupont. Chromatography Online. Avanstar Communications Inc, 2009.
7. Iler RK. The Chemistry of Silica. John Wiley & Sons. New York, 1979.
8. Karger BL, Berry LV. Rapid liquid-chromatographic separation of steroids on columns heavily loaded with stationary phase. Clin. Chem. 1971; 17(8):757-64.
9. Giddings J. Calvin, Dynamics of Chromatography, Part I. Principles and Theory. Marcel Dekker, Inc., New York, 281, 1965.
10. Ettre C. Milestones in Chromatography: The Birth of Partition Chromatography (PDF). LCGC. 2001-2016; 19(5):506-512.
11. Martin AJP, Synge RLM. Separation of the higher monoamino-acids by counter-current liquid-liquid extraction: the amino-acid composition of wool. Biochemical Journal. 1941; 35(1-2):91-121.
12. Lindsay S, Kealey D. High performance liquid chromatography. Wiley. from review Hung, L. B.; Parcher, J. F.; Shores, J. C.; Ward, E. H. (1988). "Theoretical and experimental foundation for surface-coverage programming in gas–solid chromatography with an adsorbable carrier gas. J Am. Chem. Soc. 1987; 110(11):1090-1096.
13. Journal of Pharmaceutical and Biomedical Analysis. 1999; 21(2):371-382.
14. Tropical Journal of Pharmaceutical Research, © Pharmacotherapy Group. 2009: 8(5):449-454.
15. Rabi Sankar, Instrumental Method of Analysis, P-18-6, P-18-3.
16. Lloyd R Snyder. Practical HPLC Method Development, 2nd edition, P-503.
17. Guidance for industry, Analytical Procedure and Method Validation, U.S. Department of Health and Human Services FDA, 2000.
18. Validation of analytical procedures, methodology, ICH harmonized tripartite guideline, 1996, 108.
19. BK. Sharma, Instrumental Methods of Chemical Analysis, 75-78, 113-115.
20. Galen W. Ewing, Instrumental Methods of Chemical Analysis, Vth Ed., 1.
21. Takeru Higuchi, Einar Brochmann, Hanffen Hanssen, Pharmaceutical Analysis, Ist edition, 1-10.
22. A.H. Beckett, J.B. Stenlake, Practical Pharmaceutical Chemistry, IV edition, 2:275-298.
23. Quality Assurance, worth the effort, Inforum, volume 7; number, 2003, 4.
24. PD Sethi. Quantitative Analysis of drugs in Pharmaceutical formulation, IIIrd Ed., pp.1-21, 51-56.
25. Text on Validation of Analytical Procedures, ICH Harmonized Tripartite Guidelines, 1994.
26. Validation of Analytical Procedures: Methodology. ICH-Guidelines Q2B, Geneva. 1996, 11. (CPMP/ICH/281/95).
27. Development and validation of HPLC method - A Review, Vibha Gupta *et al*, International Research Journal of Pharmaceutical and Applied Sciences. 2012; 2(4):17-25.
28. A Review: HPLC Method Development and Validation, Santosh Kumar Bhardwaj **et al*. International Journal of Analytical and Bioanalytical Chemistry, 2015.
29. Method Development: A Guide to Basics Quantitative & Qualitative HPLC, LC, GC chromacademy.
30. Lalit V Sonawane, Bioanalytical Method Validation and Its Pharmaceutical Application- A Review Pharmaceutica Analytical Acta Center for Drug Evaluation and Research (CDER) Reviewer Guidance, 2014; 5:3.
31. ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology.
32. Vikas Chander, Manindra Mohan, Richa Seth, Prashant Singh, Rakesh Singh, Sanjay Gupta. Development and Validation of RP-HPLC Method for the Estimation of Lisinopril in Tablet Dosage Form, Analytical Chemistry Letters, ACL. 2012; 2(5):309-313.
33. Vijaykumar T Pawar, Harinath N, More, Manish S Bhatia. Development and Validation of RP-HPLC Method for the Determination of Lisinopril and Amlodipine in Bulk and Multicomponent Pharmaceutical Cardiovascular Dosage Form, Natural Volatiles &

Essential Oils, 2021; 8(4):9441-9451.
34. Khairi MS Fahelelbom, Moawia MM Al-Tabakha, Nermin AM Eissa, Dana Emad Eddin Obaid, Sadik Sayed. Development and Validation of an RP-HPLC Analytical Method for Determination of Lisinopril in Full and Split Tablets, Research J. Pharm. and Tech 2020; 13(6):2647-2652. Doi: 10.5958/0974-360X.2020.00470.9.
35. K Raghu Babu, ESRS Sarma, N Aruna kumari, GMJ Raju, V Mallikarjuna Sarma. Simple and stability indicating RP-HPLC assay method development and validation Lisinopril dihydrate by RP-HPLC in bulk and dosage form, International Journal of Current research and Academic Review. 2015; 3(4):226-233.