RESEARCH ARTICLE

HPLC Method Validation for Quantification of Lisinopril

Vijaykumar Pawar*, Harinath More

Department of Pharmaceutical Chemistry, Bharati Vidyapeeth College of Pharmacy, Kolhapur, Maharashtra, India

Received: 26th December, 2022; Revised: 10th March, 2023; Accepted: 26th May, 2023; Available Online: 25th June, 2023

ABSTRACT

This study aimed to develop a straightforward, sensitive, exact, quick, and accurate reverse phase high performance liquid chromatography (RP-HPLC) method for figuring out how much lisinopril is in pharmaceutical gels and other large amounts of medication. Agilent Zorbax Bonus-RP column (250 x 4.6 mm, 5µ) was used for the chromatographic separation. "A mobile phase composed of methanol and trifluoroacetic acid (50:50 v/v) was used to develop the analytical procedure. The flow was found to be occurring at a rate of 1-mL/min and with a wavelength of 215 nm. The retention time was 2.28 min. In a concentration range from 3–7 µg/mL (r2=0.998), the drug's response was determined to be linear. The LoQ was 1.11 µg/mL, while the LoD was 0.36 µg/mL. Lisinopril's %assay was determined to be 98.22%, while assays for the other medicines in the commercial formulation showed no interference from the excipients. This method functions well and can be applied to routine analysis.

Keywords: Lisinopril, Gel formulation, Validation and RP-HPLC.

International Journal of Pharmaceutical Quality Assurance (2023); DOI: 10.25258/ijpqa.14.2.10

How to cite this article: Pawar V, More H. HPLC Method Validation for Quantification of Lisinopril. International Journal of Pharmaceutical Quality Assurance. 2023;14(2):298-302.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The ACE inhibitor lisinopril, often known as LIS, has the chemical formula N2-[(1S)- 1-Carboxy-3-phenylpropyl]. -L-lysyl-L-proline.[1-5] Angiotensin-I, a vasodilator, is converted to angiotensin-II, a more powerful vasoconstrictor, by a peptidyldipeptidase termed ACE (Figure 1). A decrease in plasma angiotensin-I, which triggers a shift in blood pressure, and a decrease in aldosterone production, with the latter decrease possibly resulting in a modest increase in serum potassium are both effects of angiotensin-II.[6] The Expert inhibitor is also used to treat hypertension and cardiovascular collapse.[7] Using particular spectro-scopic, LC, and septrofluormetric approaches, the validation of LIS in mass and drug dose structures has been covered in previous publications. These studies have demonstrated that HPLC is the most feasible and accurate technique for ensuring LIS quality in terms of mass and identifying structures.[8-13]

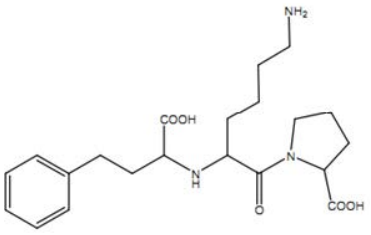

Figure 1: Structure of LIS

A few techniques recorded for LIS in the published literature are spectrophotometry,[14-16] gas-liquid chromatography,[17] capillary electrophoresis, and polarography.[18,19] By creating and validating an HPLC method for the analysis of LIS in both bulk and gel formulation, our research intends to close this gap. The method was validated in compliance with ICH guidelines.

MATERIALS AND METHODS

Materials

Instrument used

A quaternary pump (G7111A), an autosampler injector (G7129A), and a DAD detector (G7115A), all of which are controlled by the OpenlabEzchrom software, were installed on an Agilant1260 HPLC System for the experiment. The column is made by Phenomenex, USA.

Chemicals

Micro Labs Limited, Mumbai, India, 400072 generously provided a free sample of LIS's active medicinal component. The following supplies were acquired from local supermarkets: water, trifluoroacetic acid, and methanol (HPLC grade).

Methods

Chromatographic conditions

- Column oven temp: 32°C
- Flow rate: 1-mL/min.
- Mobile Phase: Buffer : Methanol (50 : 50)

*Author for Correspondence: vijaydash1982@rediffmail.com

Buffer: 0.1% TFA water
Preparation of Buffer:
Add 1-mL of Trifluoroacetic Acid in 1000 mL of Water,
Mix filter twice through
0.45p membrane filter and degas for 15 minute.

- Runtime: 10 minutes
- Injection Volume: 10 µL
- Wavelength: 215 nm
- Diluent: 0.1% TFA Water
- Column: Agilent Zorbax Bonus-RP (250 x 4.6 mm, 5 µ)

Preparation of standard solution

Mix 5 mg of lisinopril with 5 mL of diluent in a 10-mL volumetric flask, then sonicate the mixture for 5 minutes. Add enough diluent to get the volume up to 10 mL. (Dilution: 500 g/mL)

Pipette 1-mL of SSS-I into a volumetric flask with a 10 mL capacity. Add additional diluent until the required volume is reached after adding 5 mL of the diluent, stirring until a vortex forms. Add the SSS-II label.(Dilution: 50 g/mL)

Working Standard (WS): SSS-II Pipette 1-mL into a 10 mL volumetric flask. The method is as follows: add 5 mL of the diluent, mix, then add additional diluent until the volume is filled. Lastly, label the mixture as WS.(Conc. = 5 µg/mL)

Determination of absorption maxima

A total of 3 mL of the standard stock solution were further diluted with methanol to achieve the concentration of 5 µg/mL concentration. In the UV spectrum between 200 and 400 nm, this solution was compared with a blank made of distilled water. The medication is very absorbent at the 215 nm wavelength used for detection. Figure 2 displays the outcomes that were obtained.

Selection of mobile phase and chromatographic conditions

Methanol, water, and 0.1% t-butyl alcohol were used in the experiments. Different concentrations of water. During a 10 minute run, the drug was filtered using a millipore vacuum filter system and a 0.45 m high vacuum filter, producing excellent resolution and a strong peak. TFA dissolved in methanol at a ratio of 0.1 was determined to be the ideal mobile phase. The drug's retention time (Rt) and symmetrical peak shape were also optimized by adjusting chromatographic parameters such run duration, sample injection volume, flow rate, and detection wavelength. Figure 3 depicts a chromatogram of the sample.

RESULTS AND DISCUSSION

System Suitability Test

Standard solutions with 5 g/mL concentrations were injected into the chromatographic system to test their suitability. Table 1 displays the results of the parameters.

Preparation of Calibration Curve

For the purpose of making a linear concentration of LIS, we employed a typical stock solution. Methanol was used to dilute the LIS stock solution to the proper concentration, and aliquots

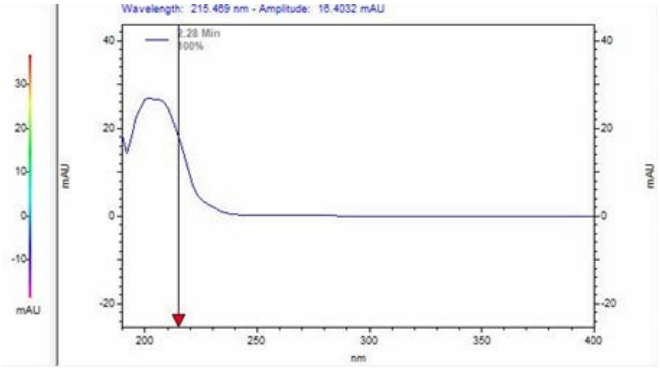

Figure 2: UV spectra of the standard LIS (λmax= 215 nm)

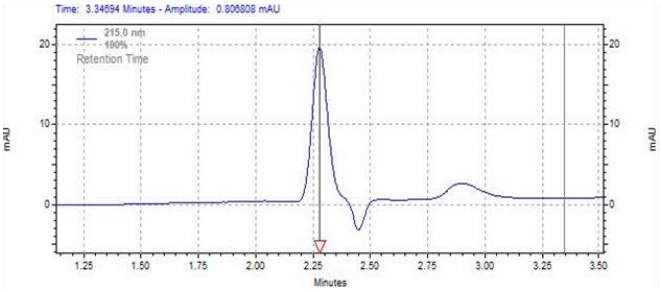

Figure 3: Chromatogram of the standard LIS (Rt = 2.28)

with sizes ranging from 0.6 to 1.4 mL were then transferred to a series of 10 mL containers. To create chromatograms, injections of five replicates of each concentration were made. To demonstrate how closely the two variables are related to one another, we evaluated the drug's peak area and created a calibration curve. The reaction was linear for concentrations between 3 and 7 µg/mL. The LIS correlation coefficient was found at 0.998.

Validation of Method[20]

The method was validated in compliance with ICH guidelines.

Linearity

In a series of 10 mL volumetric flasks, aliquots were transferred from a stock solution of LIS (50 µg/mL), and methanol was then added to the suitable concentration. After injecting five identical samples of each concentration, chromatograms were obtained. The drug's peak area was noted, and the data was compared against the drug's concentration to create a calibration curve. The concentration range from 3–7 µg/mL showed a linear response. Table 2 displays the acquired findings. The concentration range shown above shows a very good connection between peak area and drug concentration. Figure 4 depicts the LIS calibration curve, while Figure 5 demonstrates the LIS linearity overlay curve.

Accuracy

The accuracy of a measurement is defined as the degree to which it corresponds to a value that has been accepted as being either a conventionally true value or a recognized reference value found within acceptable ranges. Three different doses were used in the studies: 80, 100, and 120% of the mean

Table 1: System suitability test's result

Parameters	*LIS*
Detection Wavelength (nm)	215
Range for Linearity (µg/mL)	3-7
Correlation Coefficient	0.9986
Equation for linear regression (y=mx+c)	
Intercept (c)	-0.4095.6
Slope (m)	35879
Retention time	2.28
Peak area	172945
Number of Theoretical Plates	5700.7
Asymmetry	1.091

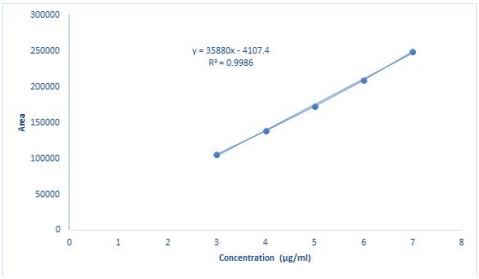

Figure 4: Calibration curve of LIS

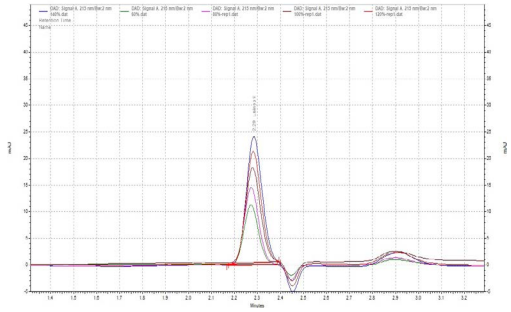

Figure 5: Linearity overlay curve of LIS

recovery of a standard known sample. The LIS recovery percentage was computed. Table 3 displays the findings.

Precision

Standard and relative standard deviations were commonly used to assess accuracy (coefficient of variance). Three sets of each sample were analyzed to determine accuracy. The same concentration sample is scanned many times in a repeatability investigation. The sampling was done by time interval for intermediate accuracy. Precision work was mostly done between days and within days. The results are shown in Table 4.

Table 2: Observations for Calibration Curve

Sr. No.	*%Level*	*Concentration (µg/mL)*	*Mean of peak area*
1	60	3	105465
2	80	4	138897
3	100	5	172935
4	120	6	209713
5	140	7	249458

Limit of detection (LoD) and Limit of quantitation (LoQ)

The limits of quantitation (LoQ) refer to the lowest concentration of analyte in a sample that can be quantitatively evaluated, as opposed to the limits of detection (LoD), which concern the smallest amount of analyte in a sample that can be roughly estimated but not quantified. A formula exists that may be used to determine this.

$LOD = 3.3\sigma/S$ $LOQ = 10\sigma/S$

Where,

σ = the standard deviation of the response

S = slope of calibration curve

The results are shown in Table 5.

Robustness

The stability of the method was evaluated by gradually altering chromatographic parameters such flow rate (1-mL/min) and wavelength (1-nm). The devised approach was shown to be very stable due to the lack of noticeable variation in the chromatogram and drug peak regions. Table 6 displays the results of the robustness test.

Assay of Formulation

Due to the lack of LIS-containing gel formulations on the Indian market, a gel containing 500 g of LIS has developed in-house by combining routinely used excipients. In a 10 mL volumetric flask, add 2 gm of gel, or 500 mcg of LIS, sonicate for 10 minutes, then add 5 mL of diluent and the remaining diluent. (Conc. = 50 µg/mL). Transfer 1-mL of the aforementioned solution to a 10-mL volumetric flask using a micropipette. Next, add 5 mL of the diluent and mix for 10 minutes. Finally, fill the flask to the top with the remaining diluent.(Conc. = 5 µg/mL). To make up the correct volume, water was added to the mixture before even being filtered through Whatman filter paper No. 41. Five mL of diluent were added to 1-mL of this solution, which was then put to a 10 mL volumetric flask and shaken vigorously for 10 minutes. A chromatogram was injected after the final volume had been

Table 3: Results of accuracy

Sample id	*Reps*	*Spiked conc. (Mg/ml)*	*Area*	*Amt recovered (µg/ml)*	*%Recovery*	*Average*	*Stdev*	*Rsd*
80%	Rep 1	3.9988	138907	3.94	98.32	98.38	0.060000	0.086
	Rep 2	3.9988	139071	3.95	98.44			
100%	Rep 1	4.9985	172945	4.90	97.93	99.03	1.095000	0.774
	Rep 2	4.9985	176805	5.01	100.12			
120%	Rep 1	5.9982	209723	5.96	98.97	98.92	0.049999	0.071
	Rep 2	5.9982	209513	5.94	98.87			

Table 4: Analysis of precision

Intra Day Precision			
Concentration (µg/mL)	*Average Area*	*S.D.*	*%R.S.D.**
3	105389	0.77	0.78
4	138976	0.29	0.28
5	172990	0.56	0.56
Inter-day precision			
3	105655	1.11	1.11
4	138988	0.42	0.42
5	172955	0.52	0.52

*n=3

Table 5: Result of LoD and LoQ

Name of Drug	*LoD*	*LoQ*
LIS	0.35	1.14

Table 6: Result of robustness studies

Drug	*%RSD Found for robustness study*			
	Flow Rate (1-mL/min)		*Wavelength (215 nm)*	
LIS	+1	-1	+1	-1
	2.28	2.28	2.28	2.28

*Average of three determinations

Table 7: Analysis of gel formulation

Drug	*Label Claim*	*Concentration injected (µg/mL)*	*Conc. Recovered (µg/mL)*	*%Drug content*	*%R.S.D.*
LIS	250	5	4.922	98.82	1.35

adjusted with diluent. After five different injections, peak areas were measured". An approximation of the medicine concentration in the sample was calculated using the calibration curve. There was 98.22% drug content, as measured by weight. Table 7 displays the acquired results.

CONCLUSION

The LIS in both bulk and gel formulations can be measured simultaneously using the RP-HPLC method, which was created and verified. The process's simplicity, accuracy, effectiveness, and cost-efficiency were all confirmed, and it was established that it complied with ICH criteria. The procedure was devised utilizing a mobile phase made up of methanol and TFA mixed 50:50 by volume. At a flow rate of 1-mL/min, the LIS retention time was found to be 2.28 minutes. Figure 3 depicts a chromatogram of the sample. The parameter for the system's appropriateness has been calculated, and the results are within acceptable limits. By comparing the peak area to the concentrations of interest, calibration curves were created, and it was discovered that the linearity range for LIS was 3–7 g/mL. The correlation coefficient (r2) value for LIS was 0.998. Figure 4 displays the calibration curve for the LIS. Three levels of accuracy (80, 100, and 120%) were examined. The average percentages of LIS recovery were determined to be 98.37, 99.02, and 98.91%, all of which were within the margin of error of the approach. Finding a mean %RSD of less than 2 in the precision study was indicative of the method's accuracy. The LoD was determined to be 0.36 µg/mL. 1.11 µg/mL was found to be the LIS LoQ. The LIS test's percentage result was 98.22%.

The proposed and validated RP-HPLC method for LIS measurement in gel formulation was shown to be feasible, sensitive, specific, and accurate. A strong correlation and a low relative standard deviation proved that each validation parameter had enough data. As a result of this innovation, the pharmaceutical sector may use the created approach for bulk and gel drug identification.

REFERENCES

1. Indian Pharmacopoeia. Ministry of Health and Family Welfare, New Delhi, India. 2007.
2. British Pharmacopoeia. Stationery Office Books (TSO) London, United Kingdom. 2005.
3. United States Pharmacopoiea-USP-24, NF-19. Asian Edition, United States Pharmacopoeial Convention, INC. Twin brook Parkway, Rockville, MD, USA. 2000.
4. European Pharmacopoeia. European Directorate for Quality Medicine and Health Care. 2007.
5. British National Formulary. British Medical Associations, Royal Pharmaceutical Society of Great Britain, United Kingdom. 2005.
6. Patel D, Kumar S, Yadav YC, Seth AK, Deshmukh GJ, Ghelani TK. RPHPLC method for development and validation of Lisinopril tablets. Pharma Science Monitor. 2011;2(3):Suppl-1:16-23.
7. Parfitt K. Martindale- The Complete Drug Reference. Pharmaceutical Press, 32nd ed., London. 1999.
8. Jamakhandi CM, Javali C, Disouza JI, Chougule US, Mullani AK. Spectrophotometric determination of lisinopril dosage form by condensation reaction. International Journal of Pharmacy and Pharmaceutical Science. 2011;3(2):185-187.
9. EI-Emam AA, Hansen SH, Moustafa MA, El-Ashry SM, El-Sherbiny DT. Determination of lisinopril in dosage forms and spiked human plasma through derivatization with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-C1) followed by spectrophotometry or HPLC with fluorimetric detection. Journal of Pharmaceutical and Biomedical Analysis. 2004;34(1):35-44.
10. Bakshi M, Singh S. Development of validated stability-indicating assay methods-Critical review. Journal of Pharmaceutical and Biomedical Analysis. 2008;28:1011-1040.
11. Chauhan V, Prajapati ST, Patel CN. A validated RP-HPLC method for simultaneous estimation of Amlodipine and Lisinopril in pharmaceutical dosage form. International Journal of Pharmaceutical Science and Research. 2011;2(7):1712-1715.
12. Raju VB, Rao AL. Development, estimation and validation of Lisinopril in bulk and its pharmaceutical formulation by HPLC method. E-Journal of Chemistry. 2012;9(1):340-344.
13. Adam E, Barakat I, Saeed A. Development and validation of a high performance liquid chromatography method for the determination of lisinopril dihydrate. Asian Journal of Pharmaceutical and Biological Research. 2012;2(2):94-99.
14. El-Gindy A, Ashour A, Abdel-Fattah L, Shabana MM. Spectrophotometric and LC determination of lisinopril. J Pharm Biomed Anal. 2001;25:913-922.
15. Paraskevas G, Atta-Politou J, Koupparis M. Spectrophotometric

determination of lisinopril in tablets using 1-fluoro-2,4-dinitrobenzene reagent. J Pharm Biomed Anal. 2002;29:865-872.
16. El-Yazbi FA, Abdine HH, Shaalan RA. Spectrophotometric and spectrofluorometric methods for the assay of lisinopril in single and multicomponent pharmaceutical dosage forms. J Pharm Biomed Anal. 1999;19:819-827.
17. Avadhamulu AB, Pantulu AR. Gas liquid chromatographic estimation of lisinopril in its pharmaceutical dosage forms. Indian Drugs. 1993;30:646-649.
18. Hillaert S, De Grauwe K, Van den Bossche W. Simultaneous determination of hydrochlorothiazide and several inhibitors of angiotensin converting enzyme by capillary electrophoresis. J Chromatogr A. 2001;924:439-449.
19. El-Enany N, Belal F, Al-Ghannam S. Polarographic determination of lisinopril in pharmaceuticals and biological fluids through treatment with nitrous acid. Microchimica Acta. 2003;141:55-61.
20. Wadher Sailesh J, Shivpuje Shivraj S, Shaikh Isak Liyakat. Development and validation of stability indicating rp-hplc method for the estimation of simvastatin in bulk and tablet dosage form. Research J. Pharm. and Tech. 2018;11(4):1553-1558.