International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Analytical Method Development and Validation for Larcenidepin and its Stages by High Performance Liquid Chromatography

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Abstract: ***Objective: To develop and validate a rapid, precise, robust, cost-effective HPLC method for separating Lercanidipine and its impurities as per the latest regulatory requirement and regulatory expectations. Methods: The Lercanidipine and Impurities resolved on Inertsil ODS 3V 150 X 4.6 mm, C18 column using a mobile phase system containing 0.1% TFA: Acetonitrile (50:50 v/v.) at detector wavelength 225 nm, with flow rate 1.0 ml/min and column temperature 30^{0}C. Results: Good linearity was observed for Lercanidipine impurity over the concentration range of 50 – 250µg/ml, with the linear regression (Correlation coefficient R = 0.999) and proved to be robust. The LOD and LOQ of Lercanidipine were 50 ng/ml and 500ng/ml, respectively, for a 10 µL injection volume. Lercanidipine sample solution and mobile phase were stable for at least 48 hours. The % RSD for the assay content in six sample solutions is 0.04. Hence the method for the determination of Lercanidipine assay is precise. Conclusion: The developed HPLC method was simple, reliable, and cost-effective for Lercanidipine using the reverse mobile phase and validated as per ICH guidelines. The developed method can be used to quantitatively determine Lercanidipine in bulk drug materials in the pharmaceutical industry.***

Keywords: Lercanidipine, Reverse phase, HPLC, Validation.

1. Introduction

Lercanidipine, 1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylic acid 2-[(3,3-diphenylpropyl)methylamino]-1,1-dimethyl ethyl methyl ester (Fig. 1), is a new drug which belongs pharmacological active compound series classified as 1,4-dihydropyridine calcium channel blockers. This drug is used for treating hypertension [1,2]. It was seen that the lercanidipine was estimated alone or in a combination of other antihypertensive agents[3,4]. The available reported work for estimating lercanidipine using HPLC was very few, and each of the methods has its own disadvantages. In another reported method[5], the pH of the mobile phase is maintained at 3pH, and This acidic pH may be harmful to the shelf life of the analytical column; the utilization of a high amount of Phosphate buffer is unjustifiable because of its cost in reported method [6], In another method reported[7] use of the 90 % amount of acetonitrile.

In the literature, there is no method for the separation and Validation of Lercanidipine and its impurities in bulk drugs using reverse-phase containing buffer 0.1% TFA: Acetonitrile by high-performance liquid chromatography. This paper describes a reverse-phase LC method for the rapid separation and Assay of Lercanidipine and its impurities to overcome all the possible unfavorable conditions and develop a reliable, economical, and cost-effective method for estimating lercanidipine and to validated[8] the method as per ICH guidelines.

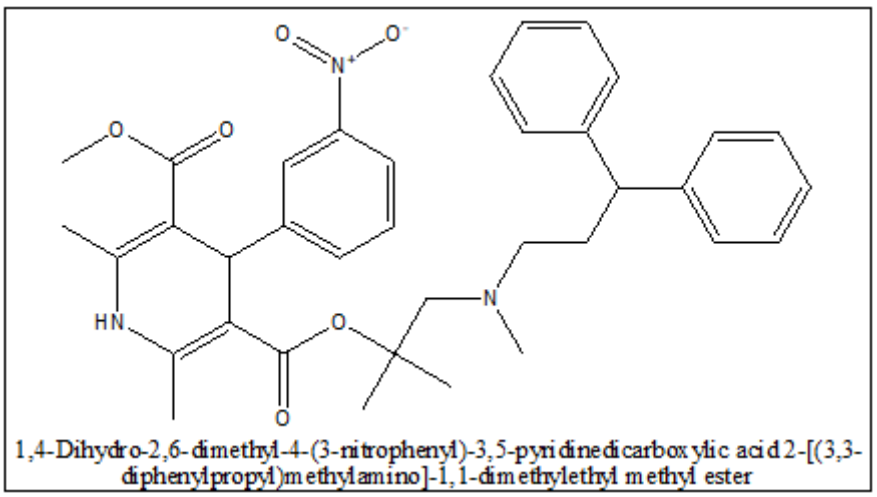

Figure 1: Structure of Lercanidipine

2. Material and Methods

Chemicals
Lercanidipine kindly supplied by Lavender lab Pune, Maharashtra, India. HPLC grade Acetonitrile, TFA was purchased from Merck.

Equipment
Agilent 1260 series LC system with UV detector and inbuilt auto-injector utilized for method development and validation.

Mobile Phase Preparation
Take 1 ml TFA and carefully transfer into 1000 mL measuring cylinder and add 500 mL of Milli-Q water and dissolve. Makeup to the mark with acetonitrile to obtain the below ratio.
Mobile phase: 0.1% TFA: Acetonitrile (50:50)

Sample Preparation
Weighed accurately about 25 mg of Lercanidipine sample, transferred it into a 50 mL volumetric flask, added about 30 mL of diluent dissolved, and mixed. Makeup to the mark with diluent and mixed. Further diluted 5 mL of this solution to 50 ml with diluent.to obtain 0.01mg/mL

Diluent: Mobile Phase
The chromatographic conditions were optimized using Inertsil ODS 3V 150 X 4.6 mm, C18column using a mobile phase system containing 0.1% TFA: Acetonitrile (50:50 v/v.) at detector wavelength 225 nm with a flow rate of 1.0 ml/min and column temperature 30^0C. Diluents (50:50 v/v) acetonitrile:water. The injection volume was 10µl.

Validation of the method

Accuracy
A series of sample preparations of Lercanidipine was prepared at 50%, 100%, and 150% in three triplicates concerning the target test concentration. The Recovery percentage was calculated from the slope and Y-intercept of the calibration curve obtained in the linearity section.

Precision
The repeatability of the method was checked by injecting replicate injections of 500 µg/mL of the solution six times on the same day as the intraday precision study of Lercanidipine, and the % RSD was found.

LOD and LOQ of Lercanidipine
The limit of detection, defined as the lowest concentration of analyte that can be clearly detected above the baseline signal, is estimated as three times the signal-to-noise ratio [9]. The limit of quantitation, defined as the lowest concentration of analyte that can be quantified with suitable precision and accuracy, is estimated as ten times the signal-to-noise ratio [9]. LOD and LOQ were achieved by injecting a series of dilute solutions of Lercanidipine.

The precision of the developed method for Lercanidipine at the limit of quantification was checked by analyzing six test solutions of Lercanidipine prepared at LOQ level and calculating the percentage relative standard deviation of area.

Linearity of Lercanidipine
Detector response linearity was assessed by preparing five calibration sample solutions of Lercanidipine covering from 500 ng/ml (LOQ) to 2500 ng/ml (500 ng/ml, 1000 ng/ml, 1500 ng/ml, 2000 ng/ml, and 2500 ng/ml), prepared in mobile phase from a stock solution of Lercanidipine.

The linearity regression curve was obtained by plotting peak area versus concentration. Linearity was checked for 3 consecutive days in the same concentration range from the same stock solution. Percentage deviation of the slope and Y-intercept of the calibration curve were calculated.

Robustness
To determine the robustness of the method, experimental conditions such as mobile phase composition, flow rate, and column temperature were purposely altered of Lercanidipine.

Solution stability and mobile phase stability

Prepared standard and Sample Solutions of Lercanidipine 0.05 mg/ml. The stability of Lercanidipine in solution at analyte concentration was Injected Blank, Standard Solution in five replicates, and Sample Solution at 0hrs, 6hrs, 24hrs, and 48hrs into the Chromatographic system**.** Content of Lercanidipine has been checked for six hours intervals up to the study period. The stability of mobile phases was carried out by evaluating the content of Lercanidipine sample solutions prepared freshly at six hours intervals for two days. The same mobile phase was used during the study period.

3. Results and Discussion

Method development
The main aim of the chromatographic method is to obtain separation of Lercanidipine from Impurity-A, Impurity-B, and Impurity-C in a very short time and to reduce the cost of analysis. Impurities were separated using different stationary phases such as C18, C8, Phenyl, and cyano columns, as well as different mobile phases, were employed. The Accurate separation was obtained on Inertsil ODS 3V 150 X 4.6 mm, C18 column using a mobile phase system containing 0.1% TFA: Acetonitrile (50:50 v/v.) at detector wavelength 225 nm with a flow rate of 1.0 ml/min,15 minutes of run time with column temperature 30^0C and Diluents (50:50 v/v) acetonitrile: water was used. Numerous experiments were conducted to select the best mobile phases and stationery that would give optimum resolution and selectivity for separation within less time.

In the Presized method, the typical retention times of Lercanidipine and Impurity-A, Impurity-B, and Impurity-C were about 5.6 and 4.0, 12.16.5 minutes, respectively. The system suitability chromatogram shows the identical separation of Lercanidipine and its impurities (Figure.2). A typical HPLC chromatogram of a Lercanidipine bulk sample (500 µg/ml) spiked with Impurities (1 %) is shown in(Figure.3).

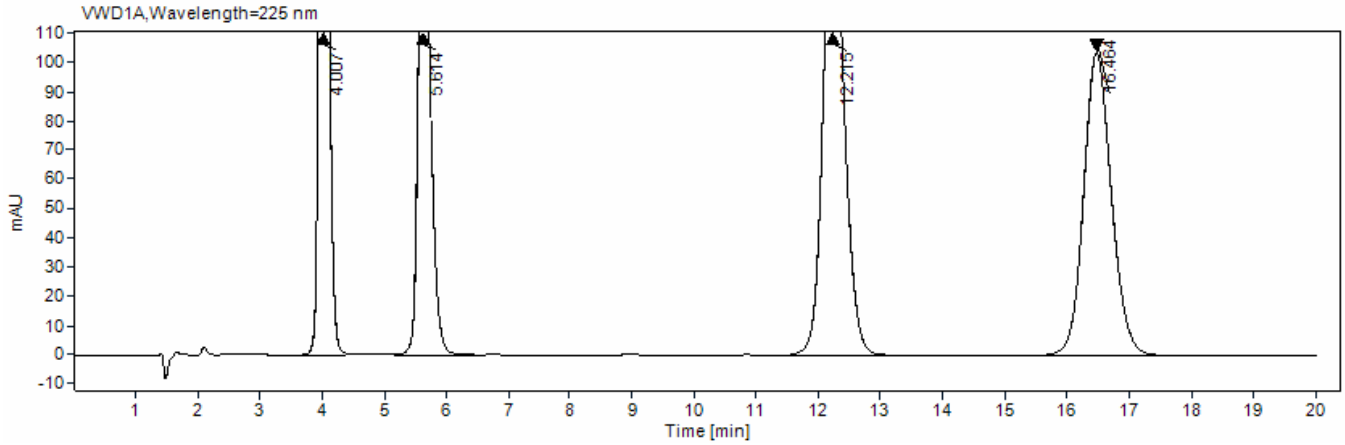

Figure 2: Typical HPLC chromatogram of System suitability

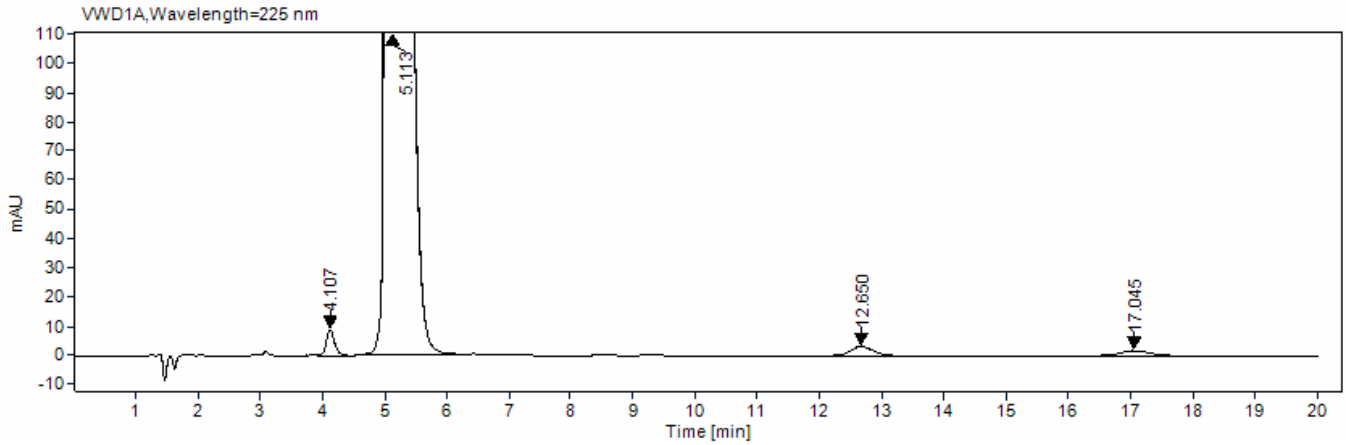

Figure 3: Typical HPLC chromatogram of Lercanidipine bulk sample (100 µg/ml) spiked with Impurities (1 %)

Validation results of the method

The system suitability test results are presented in (Table. 1).In the repeatability study, the relative standard deviation (RSD) was 0.04% for the Lercanidipine peak area (Table. 2). In the intermediate precision study, results show that RSD values were in the same order of magnitude as those obtained for repeatability (Table 2).

The limit of detection (LOD) and limit of quantification (LOQ) concentrations were estimated to be 150 and 500 ng/ml for Lercanidipine when a signal-to-noise ratio of 3 and 10 was used as the criteria. The method precision for Lercanidipine at the limit of quantification was less than 3 % RSD (Table. 2).

Good linearity was observed for Lercanidipine over the 500 – 2500 ng/ml concentration range, with the linear regression equation y = 10922X+542 (Correlation coefficient R = 0.999). Linearity was checked for Lercanidipine over the same concentration range for three consecutive days. The percentage deviation of the slope and Y-intercept of the calibration curve were 10922 and 542, respectively (Fig.6 and Table. 2).

The standard addition and recovery experiments were conducted for Lercanidipine in bulk samples in triplicate at 0.125, 0.250, and 0.375 percent of analyte concentration. Recovery was calculated from the slope and Y-intercept of the calibration curve obtained in the linearity study, and percentage recovery ranged from 98.0 to 102.0 (Table. 3).

The chromatographic resolution of Lercanidipine peaks evaluated the method's robustness under modified conditions. (Table. 4), demonstrating sufficient robustness.

No significant change in the Lercanidipine content was observed in the sample during solution stability and mobile phase stability experiments. Hence Lercanidipine sample solution and mobile phase are stable for at least 48 hours.

Table 1: System- suitability report

Compound (n=3)	Rt	R_S	N	T
Impurity-A	4.0	-	4998	1.2
Lercanidipine	5.6	5.6	4393	1.3
Impurity-B	12.1	13.92	6418	1.1
Impurity-C	16.5	5.95	6506	1.1

n =3 determinations

R_S - USP resolution, N- number of theoretical plates (USP tangent method), T- USP tailing factor

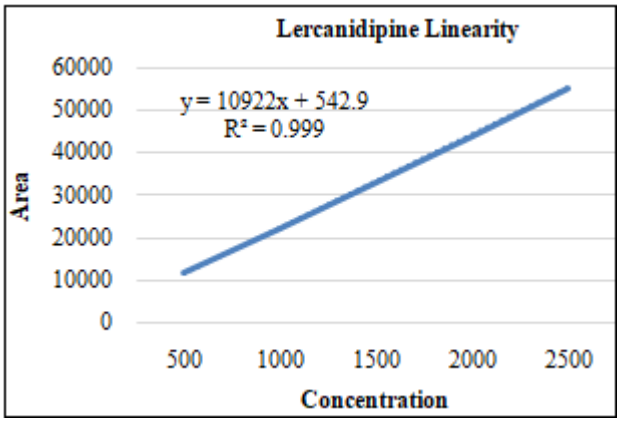

Figure 6: Linearity

Table 2: Validation results of the developed reverse phase method

Validation parameter	Results
Repeatability (n=6, % RSD)	
Retention time (Lercanidipine)	0.9
Area (Lercanidipine)	0.04
Intermediate precision (n=18, % RSD)	
Retention time (Lercanidipine)	1
Area (Lercanidipine)	0.9
LOD-LOQ (Lercanidipine)	
Limit of detection (ng/ml)	150
Limit of quantification (ng/ml)	500
Precision at LOQ (% RSD)	2.4
Linearity (Lercanidipine)	
Calibration range (ng/ml)	500-1500
Calibration points	5
Correlation coefficient	0.999
Slope (% RSD)	0.9
Intercept (% RSD)	1.2

Table 3: Recovery results of Lercanidipine in bulk drugs

Added (ng) (n=3)	Recovered (ng)	% Recovery	%RSD
1215	1195	98	2.2
2517	2547	101.4	2.3
3728	3710	99.6	2.2

Table 4: Robustness of the method

Parameter	USP resolution between Lercanidipine and Impurity-A
Flow rate (ml/min)	
0.8	5.4
1.0	5.6
1.2	5.7
Column temperature (°C)	
25	5.5
30	5.6
35	5.7
Acetonitrile percentage in the mobile phase (%)	
45	5.3
50	5.6
55	5.7

4. Conclusion

The developed HPLC method was simple, reliable, cost-effective, and rapid separation of Lercanidipine using a reverse mobile phase system containing 0.1% TFA: Acetonitrile (50:50 v/v.) at detector wavelength 225 nm with a flow rate of 1.0 ml/min and column temperature 30^0C and validated as per ICH guidelines. The validation method was carried out using Inertsil ODS 3V 150 X 4.6 mm, C18 column. The developed method could be successfully employed for the determination of Lercanidipine and its impurities in bulk drug materials in the pharmaceutical industry.

Acknowledgments

We would like to thank the management of Lavender Lab Pune, India, for donating drugs and for providing the necessary facilities to develop and complete this work.

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