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ORIGINAL ARTICLE

RP-HPLC Method Development and Validation for Determination of Lercanidipine HCl in Bulk and Dosage Form

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ABSTRACT
This paper presents the development and validation of an RP-HPLC technique for lercanidipine quantification in dose and bulk forms. The technique optimizes chromatographic parameters like stationary phase, mobile phase composition, pH, and flow rate for effective separation and precise quantification. Validation was carried out according to ICH criteria, assessing robustness, specificity, linearity, accuracy, and precision. Tests for specificity confirmed the procedure's reliability in identifying lercanidipine in the presence of interferences. The approach demonstrated excellent linearity over the concentration range, with correlation values above 0.99. Precision studies showed repeatability and moderate precision. The RP-HPLC method is suitable for routine quality control examination in pharmaceutical formulations, offering a reliable and precise way to measure lercanidipine.
Keywords: *Lercanidipine HCl, RP-HPLC, Development, Validation, Stability, Lotensyl, Q2(R1).*

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INTRODUCTION

The study of the chemical composition of substances and the creation of evaluation techniques for these compositions is known as analytical chemistry. This field encompasses traditional wet laboratory techniques as well as advanced instrumental methods [1]. Analytical chemistry plays a critical role across various domains, including science, engineering, medicine, and industry. A variety of methods, including chemical tests, spectroscopy, spectrometry, microscopy, flame tests, and bead tests, are used in qualitative analysis to identify a material [2]. Quantitative analysis, on the other hand, focuses on measuring the mass or concentration of a sample [3]. Samples are purified for characterisation using analytical balances, gravimetric analysis, volumetric analysis, and separation methods such as filtration, centrifugation, and chromatography [4]. The pharmacological action of the drug lercanidipine HCl involves the inhibition of ion-regulating gating mechanisms, leading to the disruption of channels and suppression of calcium release from the sarcoplasmic reticulum. This results in decreased intracellular calcium levels, which in turn reduces the contractile activity of cardiac smooth muscle cells [5]. Consequently, this enhances oxygen delivery, diminishes peripheral resistance, lowers blood pressure, and alleviates cardiac afterload Lercanidipine HCl exerts its pharmacological effects as a calcium channel blocker due to the presence of a dihydropyridine ring in its molecular structure (as shown in Figure 1). Due to a structural property of the medication, calcium ions cannot enter cardiac and vascular smooth muscle cells. Instead, they are selectively targeted against l-type calcium channels [6]. By reducing intracellular calcium levels, lercanidipine HCl induces smooth muscle relaxation, resulting in vasodilation, lowered blood pressure, and decreased cardiac workload [7].

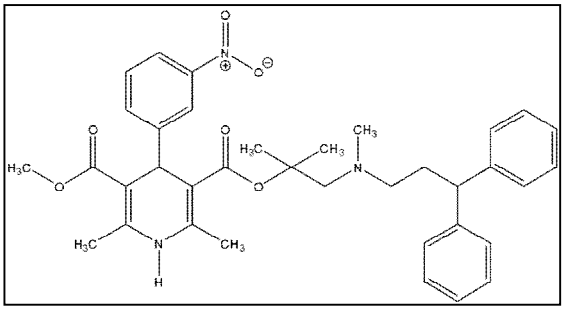

Figure1: Structure of lercanidipine

MATERIAL AND METHODS

Materials

The VIDISHA ANALYTICAL LAB and Training Center in Nashik kindly supplied a gift sample of the medication lercanidipine hydrochloride. Apart from the medication, VIDISHA ANALYTICAL LAB also provided the water and methanol needed for the analysis, guaranteeing the calibre and uniformity of the components utilized in the procedure

Chromatographic Conditions (RP-HPLC)

LERCANIDIPINE HCl in bulk and lotensyl tablets were quantitatively analyzed using an HPLC system that has an ultraviolet/visible detector. To analyze the lotensyl tablet, a Phenomenex C18 (250 mm X 4.6 mm i.d.) 5µm was utilized. The flow rate and injection volume have been adjusted to 20µL and 1.0 ml/min, respectively. A wavelength of 238 nm was chosen. We utilized a ratio of 55:45 for the addition of 0.05% orthophosphoric acid to water, which was then filtered through a 0.45µm PVDF filter, degassed using sonication, and employed as a phase of mobile.

Preparation of the Standard Solutions and Quality Control Samples

Preparation of the Standard Solutions

A 10-milligram solution of lercanidipine hydrochloride was made in methanol and then sonicated to achieve total dissolution to prepare the necessary solution. Next, using the mobile phase, A working concentration of 10 µg/ml was achieved by aliquoting and diluting 0.4 mL of the standard stock solution. Chromatograms were produced in these circumstances.

The Pharmacopoeia's system suitability criteria were applied to guarantee the chromatographic system's appropriateness for the planned analysis. Five replicate injections of the standard medication solution were used for data collection and documentation, resulting in consistent and dependable data for analysis.

Preparation of the Quality Control Samples

Twenty tablets were weighed, transferred to a pestle and mortar, and then finely powdered. Mix the ingredients with the butter paper equally. After weighing the 20-milligram powdered lercanidipine HCl, we added 35 millilitres of methanol, cleaned and dried the 50-millilitre flask, and sonicated it for fifteen minutes while periodically shaking it. After 15 minutes, let the solution come to room temperature before adding methanol to adjust the volume to the required amount. After filtering the mixture using a suitable 0.45 µ syringe filter, 3–5 mL of the initial filtrate was discarded. The filtered stock solution was further diluted to 20 ml from 0.5 ml using the mobile phase. After injecting 10 mg of lercanidipine HCl into the resultant solution, the chromatograms and outcomes were noted.

Method validation

The International Conference on Harmonization (ICH) requirements were followed in the development and validation of lercanidipine HCl tablets. During the validation procedure, a number of important factors were evaluated, such as accuracy and precision as well as system appropriateness, specificity, range, and linearity. To guarantee accuracy and consistency in the analysis, detection and quantitation limits were also established, and the solution's stability was assessed.

System suitability

The appropriateness of the quantitative analysis approach was evaluated by injecting the same lercanidipine HCl reference solution five times. The standard deviation of the peak regions of the active pharmaceutical component was used to assess the system's appropriateness. Reliability and precision of the analytical method were guaranteed by acceptance requirements for system suitability, which included a tailing factor of less than 2.0 % and an RSD NMT of 2.0 %.

Specificity

The capacity to measure APIs in impure form, such as contaminants, degradation products, and pharmaceutical excipients, with accuracy is known as specificity. A sample solution comprising pharmaceutical excipients and main degradation products was used to analyse the lercanidipine HCl tablet. Every chromatogram was examined using a sample, standard, placebo, and blank. Excipients included lactose, flour, magnesium stearate, talc, and cross povidone.

Linearity & range

The measured value was directly measured at each concentration, and the standard stock solution was diluted to test for linearity. Regarding lercanidipine HCl, five concentrations ranging from 10 to 150% of the typical stock solution's concentration (lercanidipine HCl 1.00 µg/mL to 15.00 µg/mL) were generated. Every solution underwent three evaluations. A linear regression equation was used to analyse the concentrations of the APIs in relation to the peak area of the examined chromatogram, and an evaluation of the calibration curves' linearity was done. The correlation coefficient (R^2) of 0.99 was used in this instance to assess the acceptance requirements.

Accuracy & precision

Nine consecutive measurements of three concentrations, including the prescribed range, were used to assess the accuracy using quantitative analysis. By comparing the measured QC test solution's API quantity to the recovery rate (%) numerical value, the amount of API was ascertained. Additionally, the RSD of the obtained value was used to assess precision. To verify accuracy and precision, the lercanidipine HCl pill was used to obtain the QC test solution. It was determined that the acceptance standards were lower than the 100 ± 5 % average recovery rate.

Intermediate Precision

Using the QC sample solution made from lercanidipine HCl tablets at 100 % concentration on several test days, using different testers and equipment in the same laboratory for two days in a row, intermediate precision was achieved. With a 2% RSD and the recovery rate (%) as the basis, the acceptance requirements were assessed.

Limit of Detection and Limit of Quantitation

The lowest detectable quantity of analyte in the sample that cannot be quantified is indicated by the limit of detection or LOD. The lowest quantity of API in the sample that may be stated is indicated by the limit of quantitation (LOQ). Here, S is the slope of the calibration curve and σ is the standard deviation of the y-intercept on the regression line. The following equations can be used to determine LOD & LOQ:

$$LOD = 3.3\frac{\sigma}{S}$$

$$LOQ = 10\frac{\sigma}{S}$$

Solution Stability

Robustness was employed to assess the test method's dependability when the analysis conditions were purposefully altered. The stability of the test solution served as a representative variation factor, and lercanidipine HCl's stabilities at 100% concentration and room temperature (25 ± 2°C) were compared. The assay of the APIs between the QC sample solution and the standard solution made with lercanidipine HCl was estimated using quantitative analysis. After 24 and 48 hours, the results of the QC test solution and the standard solution were compared to the baseline values, the recovery rate was determined to be less than 100 ± 5%.

RESULTS AND DISCUSSION

The lercanidipine HCl tablets were subjected to an analysis process called RP-HPLC. Additionally, method validation was used to verify the devised analytical method. The RSD of system appropriateness was 0.1%, and the API had a retention time of 7 minutes. Furthermore, the APIs showed linearity at concentrations between 10 and 100 %. For 48 hours, accuracy and precision remained steady and within allowable bounds. We could not find any peak interference with the excipients, and the lercanidipine HCl tablet quantitative analysis was accurate and selective. Using an HPLC system, the maximum absorption wavelength was found to be 238 nm, which is the wavelength that fulfils lercanidipine HCl. The API passed tests including accuracy and precision at 238 nm, as well as tests for specificity.

System Suitability

The RSDs of the standard solutions of lercanidipine HCl was 0.20%. Therefore, the RSD acceptance criteria for the two APIs were within 2.0%. In addition, the theoretical plate for the API was 6160 which is within acceptable limits (Table 1).

Specificity

A blank, placebo, standard, and sample were used to analyze each chromatogram to show the specificity of the quantitative analysis of the lercanidipine HCl tablet. There was no interference between the peaks of the API and the peaks of the API and excipients in the chromatogram, as seen in Fig. (2),(3),(4) & (5). The new RP-HPLC quantitative analysis method's specificity was verified.

Linearity and Range

Five concentrations of lercanidipine HCl (1.00 µg/mL to 15.00 µg/mL), which represent 10 to 150% of the normal stock solution concentration, were used to assess the tablet's linearity. For lercanidipine HCl, the linear regression equation was Y = 948961.1149 X + -1961.299689. For the two APIs, the correlation coefficient (R^2) was 0.9999 (Figure 6, Table 2).

Accuracy and Precision

The lercanidipine HCl pill was used to prepare the QC sample solution at 3 different concentrations: 50%; 100%, and 150%. Table 3 displays the accuracy and precision values. Based on the recovery rate and RSD of the QC sample solution for the lercanidipine HCl tablet, accuracy and precision were assessed. With RSDs of 1.025%, the precision of the API met the required standards, and the average recovery rate was 99.36%.

Table 1: System suitability for lercanidipine HCl

Sr No.	Standard solution	Area	Asymmetry (Tailing factor)	Theoretical plates
1	Std 1	9476937	1.41	6156
2	Std 2	9450374	1.42	6147
3	Std 3	9436204	1.41	6174
4	Std 4	9462002	1.41	6165
5	Std 5	9483193	1.40	6159
Mean		9461742	1.41	6160
STD Dev		19186.72649		
% RSD		0.20		

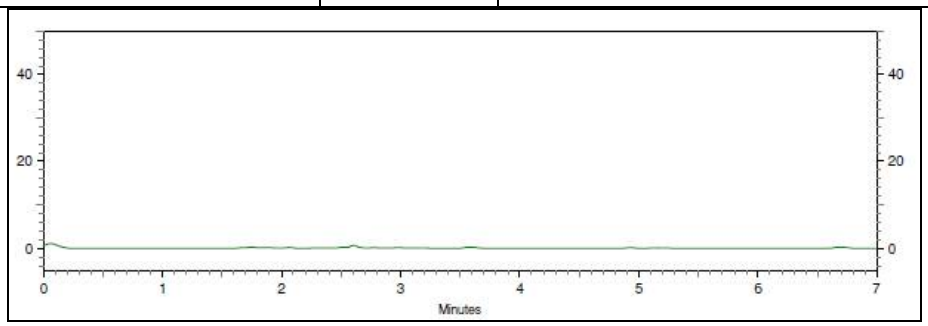

Figure 2: Chromatogram of Peak purity of Blank solution

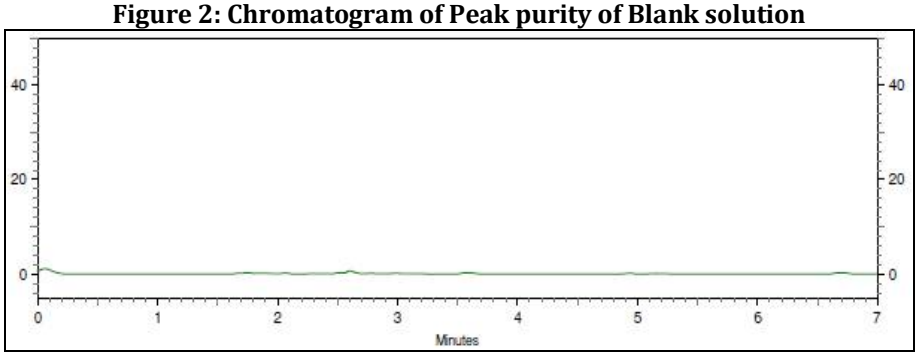

Figure 3: Chromatogram of Peak purity of Placebo solution

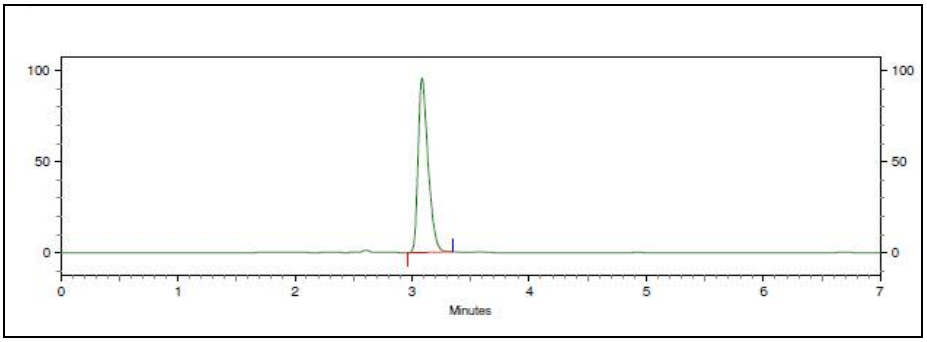

Figure 4: Chromatogram of Peak purity of Standard solution

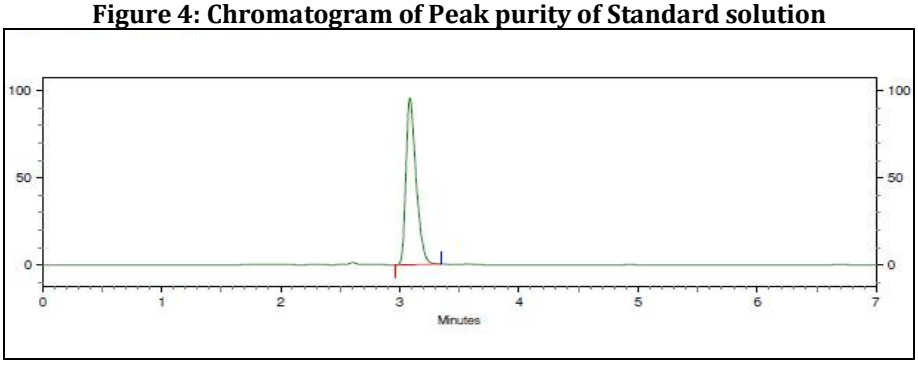

Figure 5: Chromatogram of Peak purity of Sample solution

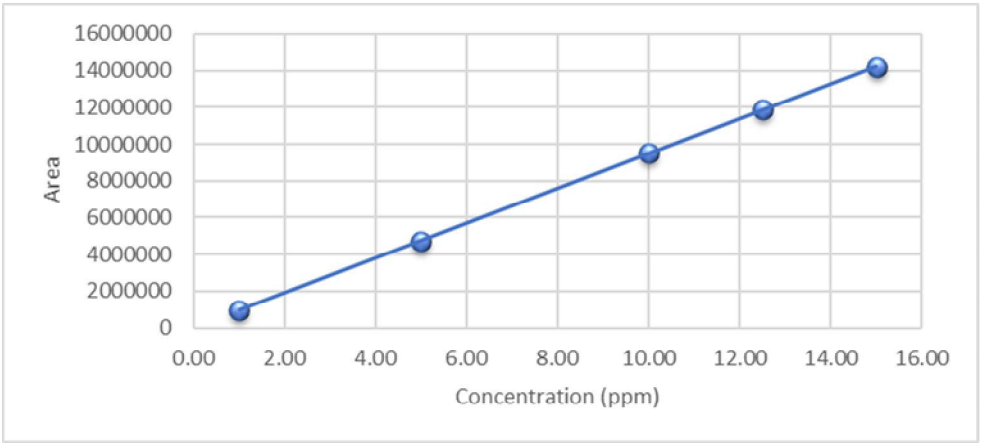

Figure 6: Calibration curve of Lercanidipine HCl

Table 2: Linearity Data for Lercanidipine HCl

Level	Conc. (µg/mL)	Area	Mean	% RSD
10%	1.00	948710	947859	0.194
		949121		
		945747		
50%	5.00	4722854	4733284	0.205
		4742047		
		4734951		
100%	10.00	9482395	9482017	0.146
		9468014		
		9495643		
125%	12.50	11899993	11904748	0.181
		11885950		
		11928302		
150%	15.00	14174183	14202094	0.188
		14227492		
		14204607		

Table 3: Result of Accuracy of Lercanidipine HCl

Level (%)	Recovered conc (µg/mL)	Added conc (µg/mL)	% Recovery	Mean Recovery	% RSD
50	5.03	5.05	99.60	**98.95**	**0.6021**
	5.04	5.10	98.82		
	5.02	5.10	98.43		
100	10.02	10.15	98.72	**99.38**	**0.8282**
	10.08	10.05	100.30		
	10.01	10.10	99.11		
150	14.97	15.05	99.47	**99.75**	**1.3213**
	14.79	15.00	98.60		
	15.33	15.15	101.19		

Table 4: Result Precision for Lercanidipine HCl test sample assay

Repeatability	Mean	99.01
	STD DEV	0.949177
	% RSD	0.959
Intermediate precision (Inter-Day)	Mean	99.39
	STD DEV	1.133043
	% RSD	1.140
Repeatability Plus Inter-day	Mean	99.201
	STD DEV	1.01660
	% RSD	1.025

Table 5: Results of Solution stability

Sample test solution			**Standard solution**		
Time point	**Area**	**% Absolute difference**	**Time point**	**Area**	**% Absolute difference**
Initial	9467382	NA	**Initial**	9470478	NA
12 Hours	9429365	0.40	**12 Hours**	9444893	0.27
24 Hours	9409460	0.61	**24 Hours**	9433595	0.39

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The detection and quantification limits were calculated using the slopes of the calibration curve. The quantification limit is 0.291µg/mL and the detection limit of 0.096 µg/mL for lercanidipine HCl, respectively.

Solution stability

The assay of the API was found to be stable within 2.0% for 48 hours at room temperature (25 ± 2°C) when compared between the standard solution and the QC sample solution (Table 5). It is vital to exhibit that slight modification to the experimental setup did not impact the analysis when employing the RP-HPLC technique for concurrent quantification. None of the experiments showed a significant change in the APIs' peak area, RSD, tailing factor, or theoretical plates. These findings corroborated the trustworthiness of the test results and methodology and established the stability of the APIs. According to the synthetic judgment, lercanidipine HCl showed a high absorption in the 238 nm wavelength range. The produced tablet did not interact with the mobile phase or the diluent, according to the solution stability results among the technique validation parameters. It was also confirmed that the tablet was highly efficient and separated quickly. Furthermore, lercanidipine HCl and the excipients in the tablet and mobile phase were shown to have no effect on the recovery rate or interfere with the analyte's ability to be detected, according to the specificity results. Differences in experience and competence did not affect intermediate precision evaluation, suggesting that an effective RP-HPLC quantitative analysis procedure had been devised.

CONCLUSION

A brand-new RP-HPLC technique has been created to measure lercanidipine HCl in bulk and dosage forms. This method is crucial for accurate measurement in medication formulations, as it ensures patient safety and therapeutic efficacy. The method uses chromatographic parameters to provide separation, sensitivity, and accuracy. The accuracy, repeatability, and dependability of the approach were confirmed upon validation in accordance with the requirements of the International Conference on Harmonization

(ICH). The method's high linearity over the concentration range and low relative standard deviations indicate its intermediate precision and repeatability. The method's suitability for routine quality control analysis was confirmed by examining commercially accessible dosage forms, showing outstanding agreement with the label claim. This advancement in analytical chemistry and pharmaceutical quality assurance is a significant advancement in the field.

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