Development and validation of reversed phase high-performance liquid chromatography method for estimation of lercanidipine HCl in pure form and from nanosuspension formulation

Abstract

**Aim:** Quantitative estimation of lercanidipine HCl in bulk material as well as from nanosuspension formulations via a developed reverse phase HPLC method.

**Materials and Methods:** Optimized chromatographic condition was used to achieve separation on a Kromasil (100-5c18 250 x 4.6 mm) column using Shimadzu HPLC system. The mobile phase consisted of a mixture of acetate buffer (20 mM pH 4.5) and acetonitrile in the ratio of 10:90, v/v. It is pumped through the chromatographic system at a flow rate of 1 ml/min. The detection was carried out at 240 nm using ultraviolet-visible spectrophotometry detector. The method was validated as per Q2 (R1) guidelines, and suitability of the developed method was established by optimized nanosuspension formulation.

**Results:** The method is specific to lercanidipine (RT: 7.7 min), and has ability to resolve the analyte peak from excipient interferences. It is linear (regression coefficient: 0.9993), accurate (average recovery: 100%), and passed all the system suitability requirements.

**Conclusion:** Developed method was found applicable for evaluation of drug content, content uniformity, and analyzing samples of dissolution studies of nanosuspension.

**Key words:** Lercanidipine HCl, liquid chromatography, nanosuspension, validation

Introduction

Lercanidipine, 5-O-(1-[3,3-diphenylpropyl(methyl)amino]-2-methylpropan-2-yl) 3-O-methyl 2,6-dimethyl-4-(3-nitropheryl)-4-dihydropyridine-3,5-dicarboxylate is a dihydropyridine calcium antagonist [Figure 1]. It is used for the management of Stage I and Stage II hypertension and is also perhaps useful in relieving angina pectoris.[1,2]

Lercanidipine HCl is soluble in dimethylformamide, dichloromethane, and methanol.[3] It has a pKa value of about 6.83.[4] It is extremely lipophilic, possess octanol: water partition coefficient (Log P) value of about 6.4.[1]

Preclinical and clinical findings propose that lercanidipine may have protective effects on the kidneys, cardiovascular system, and target organs. Lercanidipine (10 mg/day)

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produces a smooth antihypertensive effect without unfavorable hemodynamic or sympathetic effects due to its vascular selectivity. Lercanidipine emerged as a flexible choice for antihypertensive treatment across a wide range of patients due to its favorable efficacy and safety profile. Lercanidipine attains maximum plasma concentration within 2–3 h after oral administration and exhibits a slow onset of action. However, it is extensively metabolized by cytochrome P450 3A4. Its low water solubility (5 µg/ml), poor permeability, extensive first pass metabolism, and food dependent absorption result in its low bioavailability of 10%. There are few HPLC methods described in literature for the analysis of lercanidipine hydrochloride, its impurities and degradation products in bulk as well as in commercial tablets. In the present research work, nanosuspensions of lercanidipine hydrochloride were formulated using a wide range of surfactants and polymers as nanosuspension stabilizers and analyzed by proposed method. As lercanidipine is a poorly bioavailable drug, we may expect continuing the research work, in the context of its solubility and permeation enhancement using a broad range of excipients. Therefore, the proposed method has been developed and validated with a definite aim of having a method, which is simple in operation, cost-effective, and able to analyze bulk material, uniformity of dosage, in vitro release samples, and to detect small changes that may occur when drug is processed with various excipients. The method is validated as per ICH Q2 (R1) (validation of analytical procedures: Text and Methodology) guideline. "Typical validation characteristics such as accuracy, precision, repeatability, intermediate precision, specificity, limit of detection, limit of quantification (LOQ), linearity, range, and robustness were evaluated.

Materials and Methods

Instrumentation

A Shimadzu HPLC system (LC 2010 HT) equipped with degassing unit, low-pressure gradient unit, pump unit, ultra-fast autosampler, column oven, and an ultraviolet-visible spectrophotometry detector with a thermostatted flow cell was utilized. LC solution software (version 1.24 SP 1, It is the software by SHIMADZU for processing of chromatographic data. Make and model of HPLC system used mentioned under Instrumentation section) was used for data acquisition and system suitability calculations. In addition, Equitron bath sonicator, Millipore Direct Q-3 water purification system, and electronic balances (Denver Instruments, USA and Sartorius, India) were used in the study. Chromatographic parameters used for the determination of lercanidipine HCl are given in Table 1.

Materials

Lercanidipine HCl hemihydrate obtained as gift sample from Lupin Limited, Research Park, Pune. HPLC grade methanol and acetonitrile were obtained from Finar Chemicals, Gujarat (India). All the surfactants and/or polymers were procured from Loba Chemie Ltd. Mumbai and Sigma-Aldrich Bengaluru, India.

Preparation of nanosuspension of lercanidipine

Lercanidipine (LR) nanosuspensions were prepared by precipitation technique. 50 mg of Lercanidipine was dissolved in sufficient volume (500 µl) of methanol. Several polymers and/or surfactants such as PEG (polyethylene glycol) 400, HPMC (hydroxypropyl methyl cellulose) E15, PVA (poly vinyl alcohol), sodium alginate, methyl cellulose, HPMC E5, and SLS (sodium lauryl sulphate) were used singly or in a combination to act as a stabilizer. The polymers/surfactants were dissolved in water (10 ml) separately. The resulting mixture was kept under high-speed homogenization (Polytron PT 3100) for 15 min at 10000 rpm. The temperature of the stabilizer solution was maintained at 10°C. The drug solution was added all at once in stabilizer solution kept under high-speed homogenization. After complete addition of the drug solution, homogenization was continued for 15 min to get nanosuspension.

Method validation

The developed method was validated for the parameters such as accuracy, precision, repeatability, intermediate precision, specificity, LOD, LOQ, linearity, range, system suitability, and robustness as described below.

Linearity

The linearity of an analytical method is its capability to obtain a test result, which has a certain mathematical relationship to the concentration of analytes. A standard solution of lercanidipine HCl (500 µg/mL) was prepared by dissolving

| Table 1: Chromatographic parameters for determination of lercanidipine HCl |
|----------------|------------------|
| Parameter      | Condition        |
| Stationary phase | Kromasil 100-5c18 250 × 4.6 mm |
| Mobile phase | Acetonitrile: 20 mM acetate buffer pH 4.5 (90:10) |
| pH | Adjusted to 4.5 with glacial acetic acid |
| Detection wavelength | 240 nm |
| Run time | 12 min |
| Column oven temperature | 25°C |
| Flow rate | 1 ml/min |
| Injection volume | 20 µl |
exactly weighed 5 mg of lercanidipine HCl in 10 ml methanol. Different volumes of stock solution were transferred into 10 ml volumetric flasks separately and diluted with mobile phase to yield 5.0–25.0 µg/ml concentration range. Each dilution was prepared in duplicate. Areas for five injections were determined and graph was prepared. Slope and intercept were estimated.

**Precision**

**Method precision (repeatability)**
Method precision (repeatability) is the result of the method working over a short time interval under the identical conditions (inter-assay precision). Six replicates of standard solution of lercanidipine HCl (10.0 µg/mL) were analyzed, and chromatograms were recorded. The mean area and % relative standard deviation (RSD) for those injections were calculated.

**Intermediate precision**
Intermediate precision of the method was checked by repeating the entire procedure on the next day for 3 replicates of standard solution of lercanidipine HCl (10.0 µg/mL). The % RSD for response area and retention time was calculated.

**Formulation precision**
Prepared nanosuspensions were assessed using the present analytical method. Theoretical drug content of nanosuspension is 5 mg/ml. Different aliquots of 1 ml, 2 ml, and 3 ml were taken from nanosuspensions and transferred to 10 ml volumetric flask separately. Volume was made up with methanol and mixed thoroughly using vortex mixer to extract the drug completely. These solutions were filtered through 0.45 µm cellulose membrane filter paper and diluted appropriately to get the concentrations of 5 µg/ml, 10 µg/ml, and 15 µg/ml (50, 100, and 150% of assay concentration).

**Filter validation**
Filter validation was performed by analyzing solutions at 5 and 40 µg/ml (the lowest and highest concentrations of the working solutions). The solutions were analyzed in duplicate after filtration through 0.45 µm cellulose membrane filter. The results were compared with the unfiltered sample injected at the same concentration levels.

**System suitability**
Data from six injections (at 100% assay concentration) were used for computing system suitability parameters such as tailing factor and number of theoretical plates using software.

**Limit of detection and limit of quantification**
The LOD and LOQ were calculated on the basis of response and slope of regression equation.

LOD = 3.3 × σ/S and LOD = 10 × σ/S, where σ = the standard deviation of the response, S = the slope of the calibration curve of analyte.

**Robustness**
The robustness of an analytical procedure is an extent of its ability to remain unaffected by small, but deliberate changes in method parameters and provides a clue of its reliability during normal usage. The factors chosen for this study were detection wavelength (nm), temperature (°C), flow rate (ml/min), mobile phase (percentage acetonitrile), and pH of the acetate buffer. These factors were changed as per manner shown in Table 2.

Influence of change in the above factors on analysis parameters such as response area, retention time, tailing factor, and theoretical plates were determined by calculating correlation coefficient using “one-factor response surface method” (StatEase Design Expert® Software Version 9).
Results and Discussion

The chromatogram of lercanidipine acquired by developed method is indicated in Figure 2. Lercanidipine elutes at retention time 7.73 min with an average tailing factor of 0.8.

Linearity

Table 3 indicates the regression statistics obtained for linearity test. The linearity of an analytical method is its capability to produce test results that are directly proportional to the concentration of analyte within a given range [Figure 3]. The method was found linear in the expected concentration range. The regression coefficient was found to be 0.9993, demonstrating its suitability for analysis.

Repeatability

The precision of an analytical method is the extent of agreement between individual test results when the method is applied repetitively to multiple sampling of homologous sample. The method passed the test for repeatability, as percent RSD (0.7%) of the area of the peaks of six replicates injection at 100% assay concentration was within the limits of 2%. % RSD of response area and retention time was calculated and presented in Table 4.

Intermediate precision

The procedure followed for assay method in method precision was repeated on the next day. As the percent RSD [Table 4] of the response areas obtained was within the limits of 2%, method passed the test for intermediate precision. Therefore, proposed analytical technique has a good intermediate precision.

Specificity of the method

Specificity is the capability of an analytical method to assess unambiguously the analyte in the presence of other components that are present in the sample matrix. The representative chromatogram [Figure 4] of excipient blend indicates that excipients do not interfere with the drug peak [Figure 2], which shows the specificity of the method for lercanidipine.

When the developed method was used for the estimation of lercanidipine in dosage form, it was evaluated for the spectral purity in the diode array detector. The purity of the peak constituting for lercanidipine passes the test. The total peak purity index (1.000000) was found to be greater than the
single point threshold (0.999714) [Figure 5]. It indicates that the method is highly specific and no other components were co-eluting with the analytes.

Summary of system suitability studies
System suitability test is an essential part of chromatographic methods and performed to confirm that the resolution and reproducibility of the system are suitable for the analysis to be done. The results of system suitability are given in Table 5. All the values for the system suitability parameters are well within the acceptance criteria.[9]

Accuracy
The accuracy of an analytical method determines the closeness of test results obtained by the method to the true value. It can be determined by applying analytical procedure to an analyte of known purity (for a drug substance) or by recovery studies, where known amount of standard is spiked in the placebo (for the drug product). The results of accuracy studies from drug solution, excipient matrix, and formulation are shown in Tables 6 and 7, and it is observed that the method is accurate within desired range. Low % RSD values of drug recovery from formulation at each concentration level indicate uniformity of drug content in dosage form.[9]

Figure 5: Peak purity curve of lercanidipine HCl

Table 5: System suitability parameters

| System suitability parameter | Observation | Acceptance criteria |
|-----------------------------|-------------|---------------------|
| Tailing factor              | 0.812±0.00  | Should NMT 2.0      |
| Theoretical plates (n)      | 8835.71±81.60 | Should NLT 2000    |

Table 6: Accuracy/recovery data for lercanidipine HCl from solution

| Level of percentage | Added amount | Amount recovered | Percentage of recovery | Percentage of RSD |
|---------------------|--------------|------------------|------------------------|-------------------|
| 50                  | 5            | 4.9853           | 99.70                  | 0.783             |
|                     | 5            | 5.063            | 101.26                 |                   |
|                     | 5            | 5.074            | 101.48                 |                   |
| 100                 | 10           | 10.2113          | 102.11                 | 0.284             |
|                     | 10           | 10.2549          | 102.54                 |                   |
|                     | 10           | 10.282           | 102.82                 |                   |
| 150                 | 15           | 15.2506          | 101.67                 | 0.137             |
|                     | 15           | 15.3033          | 102.02                 |                   |
|                     | 15           | 15.2891          | 101.92                 |                   |

RSD: Relative standard deviation

Table 7: Accuracy/recovery data for lercanidipine HCl from excipient matrix and formulation

| Parameter                          | Concentration level | Percentage of recovery | Percentage of RSD |
|------------------------------------|---------------------|------------------------|-------------------|
| Assay (spiking)                    | 50                  | 96.77                  | 1.93              |
|                                   | 100                 | 99.21                  | 1.55              |
|                                   | 150                 | 100.58                 | 1.50              |
| Recovery from formulation          | 50                  | 81.49                  | 1.45              |
|                                   | 100                 | 82.91                  | 1.52              |
|                                   | 150                 | 83.96                  | 1.92              |

RSD: Relative standard deviation

Table 8: Effect of changes in independent factors on chromatographic profile

| Parameter                          | Mean peak area | Retention time (min) | Tailing factor |
|------------------------------------|----------------|----------------------|---------------|
| Column temperature                 |                |                      |               |
| 20°C                               | 488,239        | 8.0                  | 0.93          |
| 25°C                               | 438,407        | 7.7                  | 0.80          |
| 30°C                               | 494,136        | 7.3                  | 0.86          |
| Detection wavelength               |                |                      |               |
| 238                                | 476,452        | 7.7                  | 0.8           |
| 240                                | 438,407        | 7.7                  | 0.80          |
| 242                                | 405,298        | 7.7                  | 0.8           |
| Buffer pH                          |                |                      |               |
| 4.3                                | 436,114        | 7.9                  | 0.80          |
| 4.5                                | 438,407        | 7.7                  | 0.80          |
| 4.7                                | 438,429        | 7.3                  | 0.81          |
| Composition                        |                |                      |               |
| 10:88                              | 483,465        | 10.2                 | 0.75          |
| 10:90                              | 438,407        | 7.7                  | 0.8           |
| 10:92                              | 476,492        | 7.3                  | 0.8           |
| Flow rate (ml/min)                 |                |                      |               |
| 0.9                                | 477,716        | 8.5                  | 0.8           |
| 1                                  | 438,407        | 7.7                  | 0.8           |
| 1.1                                | 389,578        | 6.9                  | 0.81          |

RSD: Relative standard deviation

Limit of detection and limit of quantification
According to the study carried out, the LOD and LOQ of lercanidipine were found 0.219 µg/ml and 0.663 µg/ml, respectively.

Filter validation
The RSD obtained at lower concentration (0.68%) and higher concentration (0.59%) shows suitability of the cellulose membrane filter for the dissolution sample filtration, as the RSD is <2%.[10]

Robustness
Effects of change in factors such as detection wavelength (nm), temperature (°C), flow rate (ml/min), mobile phase (percentage acetonitrile), and pH of the acetate buffer on analysis parameters such as response area, retention time, tailing factor, and theoretical plates were observed in robustness studies and results obtained were indicated in Table 8.
Table 9: Correction coefficients to show the effect of the different variables studied on chromatographic profile of lercanidipine HCl

|                          | Column temperature | Detection wavelength | Flow rate | Percentage of acetonitrile | pH of acetate buffer |
|--------------------------|--------------------|----------------------|-----------|---------------------------|----------------------|
| Mean peak area           | 0.123              | −0.998               | −0.994    | −0.183                    | 0.550                |
| Retention time           | −0.995             | 0.525                | −0.998    | −0.885                    | −0.941               |
| Tailing factor           | −0.659             | 0.000                | 0.953     | 0.828                     | 0.290                |
| Theoretical plates       | −0.973             | 0.170                | −0.988    | −0.998                    | −0.941               |

Effects of changes in independent factors on chromatographic profile were calculated by determining correlation coefficient using “One-Factor Response Surface Method” (Stat-ease Design Expert 9) [Table 9]. All the independent factors are listed in different columns and all the analysis parameters are listed in different rows. Correlation coefficient of particular factor and analysis parameter is indicated by the intersection of respective column and row. The value of correlation coefficient always lies between −1 and +1.

Positive value of correlation coefficient indicates a positive association. Negation value of correction coefficient indicates a negative association. The value of correlation coefficient in between ± 0.70 and ± 1 indicates a strong association. Therefore, it can be observed from the above table that change in column temperature has a strong influence on the parameters such as retention time and number of theoretical plates. Change in detection wavelength does not influence tailing factor at all. Flow rate has a strong influence on all the analysis parameters. The value of correlation coefficient in between ± 0.50 and ± 0.70 indicates a moderate association. pH of acetic buffer and peak area have a moderate positive association. The value of correlation coefficient in between ± 0.30 to ± 0.50 indicates a low association. The value of correlation coefficient in between -0.30 to +0.30 indicates no association. This indicated that there is a no correlation between column temperature and mean peak area.

Conflicts of interest
There are no conflicts of interest.

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