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Simple and Accurate Validation of Lercanidipine in Human Plasma by RP-HPLC

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ABSTRACT

A rapid and sensitive High Performance liquid chromatography (HPLC) method has been developed and validated. The analyte was extracted from human plasma by simple precipitation technique. Nifedipine was used as the internal standard. A Princeton C_{18} column provided chromatographic separation of the analyte. A simple, selective, rapid, precise and economical reverse phase High Pressure Liquid Chromatographic method has been developed for the estimation of Lercanidipine in human Plasma. The method was carried out on a Princeton C_{18} (250 mm x 4.6 mm i.d., 5µ) column with a mobile phase consisting of acetonitrile: Water (adjusted to pH 3.5 using orthophosphoric acid) (55:45 v/v) at a flow rate of 1.0 ml/min. Detection was carried out at 235 nm. The retention time of Lercanidipine Nifedipine, was 5.31, 10.00 min, respectively. The proposed method has been validated with linear range of 5.0-250.0 ng/ml for Lercanidipine. The precision and accuracy values are within 10%. The overall recovery of Lercanidipine was 96.4 %. The developed and validated method was applicable for the pharmacokinetics studies.

Keywords: Lercanidipine, HPLC, Validation.

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INTRODUCTION

Lercanidipine,2-[(3,3-diphenylpropyl)methylamine]1,1-dimethylethyl 1,4-dihydro-2,6 dimethyl 1,4(3-nitrophenyl)-3,5 pyridinedicarboxylic ester. This drug is used in hypertension treatments based on its selectivity and specificity on the smooth vascular cells. There are many works published in the last two decades regarding HPLC methods for determination of Lercanidipine in biological fluids of different species. For a laboratory, to develop a method is sometimes a compromise between cost, time consuming and purpose of study. Some of the reported methods about Lercanidipine quantification in human plasma supposed expensive sample extractions [1-4] Number of articles about determination of lercanidipine in human plasma after protein precipitation is great due to the relative simplicity of the sample treatment. In many works acetonitrile or methanol was used, as precipitating agent .The aim of this study was to propose a simple method for quantification of lercanidipine in human plasma after protein precipitation with 10% perchloric acid, with internal standard. The method was validated to provide enough selectivity, sensitivity and stability in pharmacokinetic and bioequivalence studies [5-6].

MATERIALS AND METHODS

Chemicals and reagents

The reference standards of lercanidipine (purity: 99.67%) and nifedipine (purity: 98.44%) were obtained from Glen mark Pharma, Mumbai. and Aurobindo Pharma (Hyderabad, India) respectively. High purity water was prepared in-house using a Milli-Q water purification system obtained from Millipore (India) Pvt. Ltd. (Bangalore, India). HPLC grade methanol and acetonitrile were purchased from E. Merck Ltd. (Mumbai, India). Drug free (blank) heparinized human plasma was obtained from a local Nursing home (Ootacamund, India) and was stored at −20 C prior to use.

Calibration curves

The Stock solutions of lercanidipine and nifedipine were prepared in acetonitrile at free base concentration of 1000 µg/ml. Secondary and working standard solutions were prepared from stock solutions by dilution with water: acetonitrile (50:50, v/v). These diluted working standard solutions were used to prepare the calibration curve and quality control samples. Blank human plasma was screened prior to spiking to ensure it was free of endogenous interference at retention times of Lercanidipine and the internal standard Nifedipine**.** The calibration curve ranged from 5.0-250.0 ng/ml. Quality control samples were prepared at three concentration levels of 20.0, 100.0 and 200.0 ng/ml for lercanidipine. The samples were vortexed and stored at

–70 ± 2 ^{0}C until processing.

Sample preparation

A 0.5 ml aliquot of human plasma sample was mixed with 0.1 ml of internal standard working solution (2000.0 ng mL^{-1} of Nifedipine) and 500 µL of 10 % Perchloric acid (precipitating agent). The resulting solution was vortexed for 5 minutes and centrifuged at 4000 rpm for 10 min. Supernatants from the above solutions were separated and used for the analysis. 20 µL of the eluent was injected into the HPLC system. The sample chromatogram is presented in figure-1.

Instrumentation

Chromatographic separation was performed on a Shimadzu® liquid chromatographic system equipped with a LC-10AT-vp solvent delivery system (pump), SPD M-10AVP photo diode array detector, Rheodyne 7725i injector with 20 µL loop volume. Class-VP 6.01 data station was applied for data collecting and processing (Shimadzu, Japan). A Princeton C_{18} column (250 mm x 4.6mm i.d., 5µ) was used for the separation, mobile phase of a mixture of acetonitrile and water (adjusted to pH 3.5 using orthophosphoric acid), (55:45 v/v) was delivered at a flow rate of 1.0 mL min^{-1} with detection at 230 nm. The mobile phase was filtered through a 0.2µ membrane filter and degassed. The injection volume was 25 µL; Analysis was performed at ambient temperature.

Validation

The method was validated for selectivity, sensitivity, linearity, precision, accuracy, and stability. The selectivity of the method was evaluated by comparing the chromatograms obtained from the samples containing lercanidipine and the internal standard with those obtained from blank samples. Sensitivity was determined in terms of LLOQ (Lower Limit of Quantification) where the response of LLOQ was at least five times greater than the response of interference in blank matrix at the retention time of the analyte. For linearity, different concentrations of standard solutions were prepared to contain 5.0 ng/ml to 250.0 ng/ml of lercanidipine containing 2000.0 ng/ml of nifedipine. These solutions were analysed and the peak areas and response factors were calculated. The calibration curve was plotted using response factor vs concentration of the standard solutions. Standard curve fitting was determined by applying the simplest model that adequately describes the concentration-response relationship using appropriate weighing and statistical tests for goodness of fit. The precision of the method was determined by intraday precision and interday precision. The intra-assay precision and accuracy was calculated for five replicates at each Lower Limit of Quantification (LLOQ), Low Quality Control (LQC), Middle

Quality Control (MQC) and High Quality Control (HQC) levels, each on the same analytical run, and inter-assay precision and accuracy was calculated after repeated analysis in three different analytical runs.

Accuracy of the developed method was determined by relative and absolute recovery experiments. The relative recovery of the drug was calculated by comparing the amount of the drug obtained from the drug supplemented plasma with the actually added amount. Recovery studies were carried out for three levels at six times and the percentage recovery, mean, standard deviation and coefficients of variation were calculated.

As part of the method validation, stability studying was carried out. Room temperature stock solution stability, refrigerated stock solution stability, freeze thaw stability, short term stability and long term stability were determined. Room temperature stock solution stability was carried out at 0, 3 and 8 hours by injecting four replicates of prepared stock dilutions of lercanidipine equivalent to middle quality control sample concentration and the stock dilution of internal standard equivalent to the working concentration.

Comparison of the mean area response of lercanidipine and internal standard at 3 and 8 hours was carried out against the zero hour value. Refrigerated stock solution stability was determined at 7, 14 and 27 days by injecting four replicates of prepared stock dilutions of the analyte equivalent to the middle quality control sample concentration and the stock dilution of internal standard equivalent to the working concentration.

The stability studies of plasma samples spiked with lercanidipine were subjected to three freeze - thaw cycles, short term stability at room temperature for 3 h and long term stability at – 70^{o} C over four weeks. In addition, stability of standard solutions was performed at room temperature for 6 h and freeze condition for four weeks.

The stability of triplicate spiked human plasma samples following three freeze thaw cycles was analysed. The mean concentrations of the stability samples were compared to the theoretical concentrations. The stability of triplicate short term samples spiked with lercanidipine was kept at room temperature for 1.00 to 3.00 h before extraction.

The plasma samples for long term stability were stored in the freezer at -70 ^{0}C until the time of analysis. FDA Guidance for industry.

RESULTS AND DISCUSSION

Validation

The validation parameters such as accuracy, precision (repeatability and reproducibility),

linearity and range, sensitivity (LOD and LOQ), robustness/ruggedness, stability, selectivity/specificity and system suitability were evaluated.

Accuracy

The accuracy of the optimised methods was determined by relative and absolute recovery experiments. The percentage recovery values for lercanidipine ranged from 93.01 to 99.14%, and their relative recovery values from 92.54 to 99.85% (Table 1). The coefficient of variation (%) of these values was less than 10.00%. It is indicative that the developed methods are accurate and reliable.

Table 1. Percentage recoveries of lercanidipine from different concentrations of human plasma

Concentration	% Recovery
20	93.01
100	94.15
200	97.54
300	98.24
400	99.14

Precision

The optimized method for the estimation of lercanidipine was found to be precise. This was evident from the coefficient of variation values, which were less than 10.00% at all concentrations. The validation results are given in Table 2.

Table 2. Interday and Intraday precision for plasma lercanidipine determination

Paramete	Concentration (ng/ml)	% Coefficient variation
Interday	20.0	4.47
	100.0	1.18
	200.0	3.17
Intraday	20.0	4.23
	100.0	1.48
	200.0	3.95

Selectivity

The selectivity of the method was evaluated by comparing the chromatograms obtained from the samples containing lercanidipine and the internal standard with those obtained from blank samples. These chromatograms were compared with the chromatograms obtained from standard solutions. Each chromatogram was tested for interference. The combination of the sample preparation procedure and chromatography provided an assay which is free from significant interfering endogenous plasma components at the retention times of the selected drugs and the internal standard.

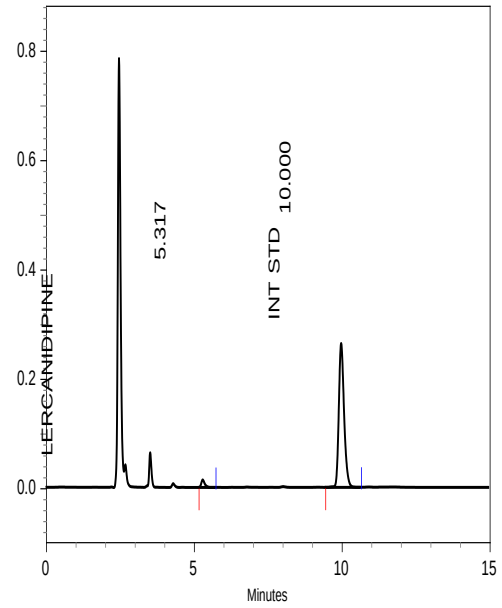

Figure 1. Sample chromatogram of lercanidipine

Linearity

It was observed that the optimised methods were linear within a specific concentration range for lercanidipine. The calibration curves were plotted between response factor and concentration of the standard solutions. The linearity ranges were found to be 5.0-250.0 ng/ml (figure-2). The calibration curves were constructed on 11 different days over a period of four weeks to determine the variability of the slopes and intercepts. The results indicated no significant interday variability of slopes and intercepts over the optimised concentration range.

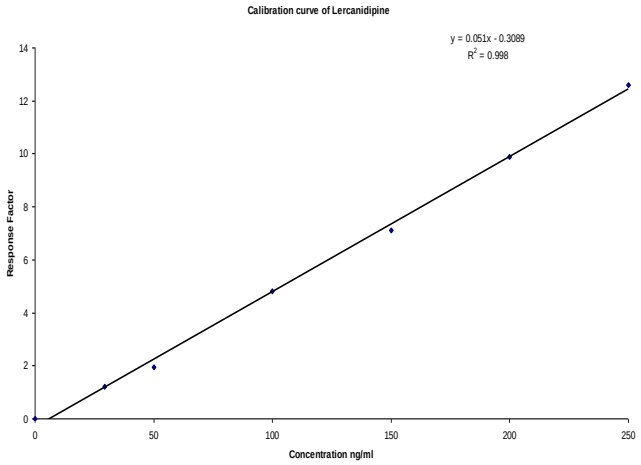

Figure 2. Calibration Curve of Lercanidipine

Ruggedness and Robustness

The ruggedness and robustness of the methods were studied by changing the experimental

conditions. No significant changes in the chromatographic parameters were observed when changing the experimental conditions (operators, instruments, source of reagents and column of similar type) and optimised conditions (pH, mobile phase ratio and flow rate) (Table 3).

Table 3 System sutability studies

Linearity Reange	5-250 ng/ml
Correlation coefficient	0.998
Theoretical plate/meter	2498
Asymmetric factor	1.2
LOD (ng/ml)	5 ng/ml
LOQ (ng/ml)	2.5 ng/ml

Stability studies

The stability studies of plasma samples spiked with selected drugs were subjected to three freeze-thaw cycles, short term stability at room temperature for 3 h and long term stability at – 70 ^{o}C over four weeks. In addition, stability of standard solutions was performed at room temperature for 6 h and freeze condition for four weeks. The mean concentrations of the stability samples were compared to the theoretical concentrations. The results indicate that selected drugs in plasma samples can be stored for a month without degradation in frozen state. The results of short term storage at room temperature stability and freeze-thaw cycles indicate no degradation of the selected drugs in plasma as well as in sample solution and hence plasma samples could be handled without special precautions.

CONCLUSION

In conclusion, the developed method for the estimation of lercanidipine in plasma is accurate, precise, selective and linear and it can be applicable for the Pharmacokinetics studies. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments such as LC–MS/MS or GC–MS/MS that are complicated, costly and time consuming rather than a simple HPLC–UV method. Considering the possible worldwide development of counterfeit lercanidipine, the proposed method could be useful for the national quality control laboratories in developing countries.

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