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Developement of Difference Spectrophotometry Method for Estimation of Lercanidipine in Tablet Dosage Form

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ABSTRACT

A new, simple, rapid, sensitive and economical spectrophotometric method has been developed and validated for estimation of lercanidipine in pure and its pharmaceutical formulation like tablet. During the development of formulations containing lercanidipine in its solid dosage form, analytical methods will serve as assay method for quantitation of the lercanidipine during product developmental stages. The present work consist of estimation of lercanidipine by difference spectrophotometry which is based on shifting of λ_{max} by changing the pH of the solution by adding 0.1M HCl and 0.1M NaOH the absorption maximum was obtained. Linearity of the response was demonstrated for the drug for a range fulfilling Beer's law, which is 5-25 µg/ml. The absorption maxima of lercanidipine were obtained at 260 nm in 0.1M NaOH, and 240 nm in 0.1M HCl. The results of analysis have been validated statistically and by recovery studies. The method was extended to pharmaceutical formulation and there were no interferences from any excipients and diluents. The full analytical validation was performed according to International Conference on Harmonization Q2R1 guidelines for validation of analytical procedures.

Keywords: Lercanidipine, Beer's law, Difference spectrophotometry, International Conference on Harmonization.

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INTRODUCTION

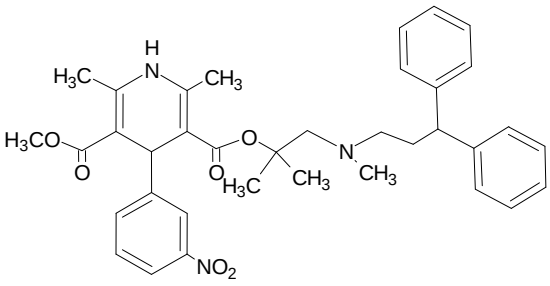

Figure 1: Structure of Lercandipine

Lercanidipine(2-[(3,3-diphenylpropyl)methylamine]-1,1-dimethylethylmethyl1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5pyridinedicarboxylic ester (LER), is a calcium channel blocker (figure 1). Lercanidipine is used for treating angina pectoris and hypertension [1]. A literature survey revealed different analytical methods for estimation from its pharmaceutical dosage forms including pharmacokinetic, bioanalytical and stability studies [2-5]. The development and validation of high performance liquid chromatographic method for estimation of lercanidipine in rabbit serum [6] and determination of lercanidipine and its impurities using DryLab software also reported [7] . The combination therapy of β-blocker and calcium channel blocker becomes often necessary and such dosage forms have been formulated. No difference spectrophotometric method has been reported. Hence the present study describes simple, sensitive, accurate, rapid and economical spectrophotometric method for the estimation of Lercanidipine (LER) in bulk & its tablet dosage form.

MATERIALS AND METHODS

Instrument:

Spectrophotometric analysis was carried out on a JASCO UV-spectrophotometer 530 using a 1 cm quartz cell. and band width of 2.0 nm in the range of 200–350 nm.

Reagents and Chemicals:

Lercanidipine was gifted from Cipla Ltd, Kurkumbh, India. All solvents were spectrophotometric grade obtained from SD fine chemicals. Water purified by glass distillation apparatus. The commercially available tablets 10 mg were obtained from local market.

Preparation of standard drug solution:

Standard Stock solutions containing lercanidipine (LER) was prepared by dissolving10 mg of drug separately in 50 ml of 0.1M NaOH and 50 ml of 0.1M HCl, sonicated for 5 minutes and

final volume of both the solution was made up to 100 ml with the solvents to get stock solutions containing 100µg/ml of lercanidipine in of 0.1M NaOH and 50 ml of 0.1M HCl in two different volumetric flask.

Procedure for determining the sampling wavelength analysis:

By appropriate dilution of the two standard drug solutions with 0.1M NaOH and 0.1M HCl, solution containing 10 µg/ml of LER was scanned separately in the range of 200-400 nm. In the difference spectrophotometric method developed for analysis of LER, one wavelength was selected for the estimation of LER from the overlain spectra. The wavelength selected for the estimation of drug in 0.1M NaOH and 0.1M HCl were found to be 260 and 240 nm respectively.

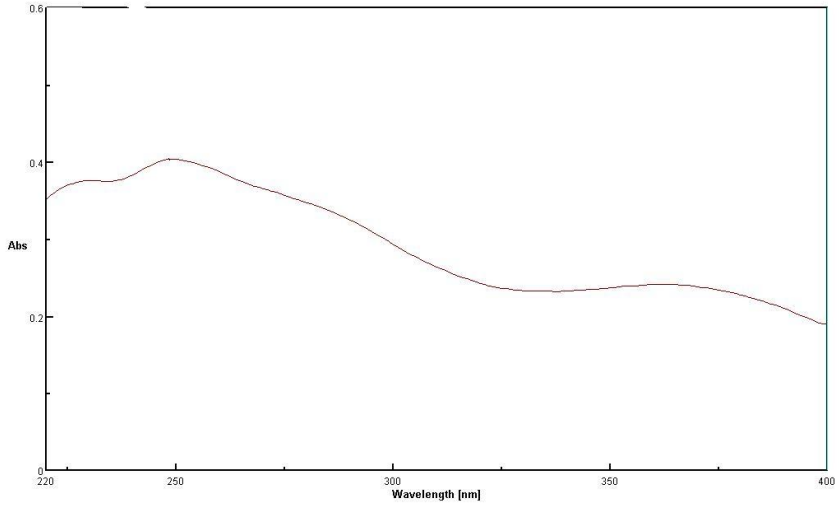

Figure 2: Spectra of Lercandipine in 0.1 N NaOH at 260 nm

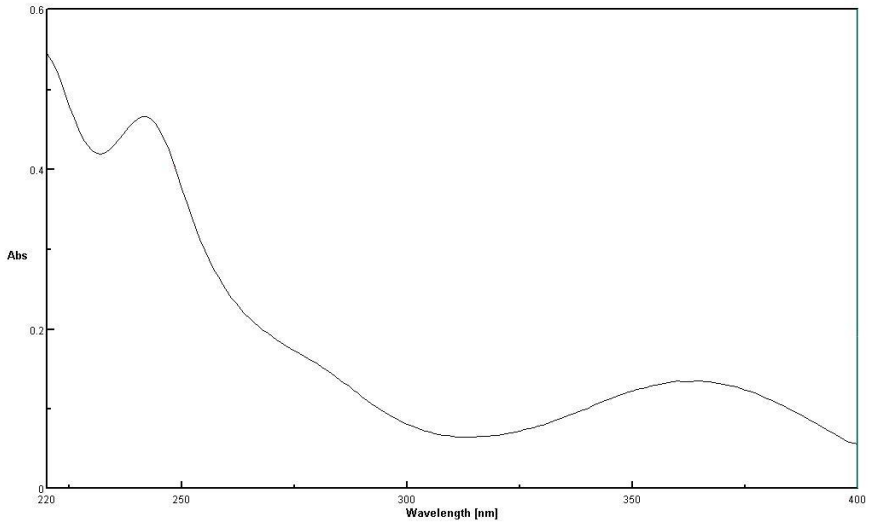

Figure 3: Spectra of Lercandipine in 0.1 N HCl at 240 nm

Method for calibration curve:

From standard stock solution of both drugs, six working standard were prepared. The appropriate aliquots of drug were pipette out from standard stock solution of the drug in 0.1M NaOH and 0.1M HCl in to series of 10 ml volumetric flask. The volume was made up to mark to get solutions of concentrations 5, 10, 15, 20, 25 µg/ml of LER in both 0.1M NaOH and 0.1M HCl separately. Calibration curve were constructed at wavelength 260 nm and 240nm by recording absorbance difference between 0.1 N NaOH and 0.1 N HCl respectively. By using quantitative modes of instrument intercept, slope and coefficient of correlation values for calibration curve was obtained.

Difference spectroscopy:

The selectivity and accuracy of spectrophotometric analysis of sample containing absorbing interference may be markedly improved by the technique of difference spectrophotometry. The essential feature of difference spectrophotometric assay is that the measured value is the difference in absorbance between two equimolar solutions of the analyte in different chemical forms which exhibit different spectral characteristics.

For the preparation and analysis of sample solution, each tablet containing 10 mg of LER, 20 tablets were accurately weighed and average weight per tablet was determined. The tablets were powdered and powders equivalent to 10 mg of drug was taken and treated in similar manner as that of standard. Marketed tablet formulation containing 10 mg of LER was analysed using this method. An amount equivalent to 10mg of LER was taken, triturate of 20 tablets and weighed. The contents of the flasks were dissolved in the 50 ml the 0.1 M NaOH and 0.1M HCl separately with aid of ultrasonication for 10 minutes. And then final volume of the solution was made up to 100 ml with same solvents. Then the solutions were filtered through Whatmann filter paper no.41 to get stock solution containing 100 µg/ml of LER in 0.1 M NaOH and 0.1M HCl. Then the absorbance was measured by making the appropriate dilutions and the concentration of analyte was determined with the equation obtained from calibration curve. The statistical data obtained after replicate determination.

The absorbance was measured at 260 nm and 240 nm in basic and acidic solution respectively against reagent blank. For both the solutions, calibration curve was prepared by plotting concentration versus difference in absorbance and found to be linear in the concentration range of 5 - 25 µg/ml (Table 1).

Method validation [8]

Linearity:

The linearity of an analytical method is its ability to elicit test results that are directly or by a well- defined mathematical transformation proportional to the concentration of analyte in samples within a given range. The range of analytical method is the interval between upper and

lower level of analyte including levels that have been demonstrated to be determining with precision and accuracy using the method. The linear response of LER was determined by analyzing five independent levels of the calibration curve in the range of 5-25 µg/ml.

Precision:

The precision is measure of either the degree of reproducibility or repeatability of analytical method. It provides an indication of random error. The precision of an analytical method is usually expressed as the standard deviation, Relative standard deviation or coefficient of variance of a series of measurements.

Recovery Studies:

Accuracy and sensitivity of analysis was determined by performing recovery studies by spiking different concentrations of pure drug in the preanalyzed tablet sample. Results of recovery studies indicated that the method is rapid, accurate and reproducible.

Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the proposed procedures was examined by evaluating the influence of small variation in the solvent on the analytical performance of the proposed methods.

Ruggedness:

Ruggedness was ascertained by getting the sample analysed from different analysis and carrying out analysis for interday and variation, intraday variation and for different instrument ascertained ruggedness by proposed method.

Limit of Detection:

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantities under the stated experimental conditions. Limit of detection can be calculated using following equation as per ICH guidelines.LOD = 3.3 × N/S. Where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

Limit of Quantification:

It is the lowest concentration of analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions. Limit of quantification can be calculated using following equation as per ICH guidelines. LOQ = 10 × N/S. Where, N is

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the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

RESULTS AND DISCUSSION

Calibration curve data was constructed in the range of the expected concentrations of 5 to 25 µg/ml. Beer's law was obeyed over this concentration range shown in Table 1. The regression equation was found to be Y=0.020 x+0.01. The correlation coefficient (r) of the standard curve was found to be 0.9987. The characteristic of the calibration plot is presented in (Table 2). The optical characteristics such as Beer's law limits, percent relative standard deviation and % range of error were found to be within the limits and satisfactory. All of the analytical validation parameters for the proposed method were determined according to ICH guidelines.

Table 1: Linearity of LER by difference spectrophotometry

Sr. No.	Conc. (µg/ml)	Absorbance at 260 nm (0.1 N NaOH)	Absorbance at 240 nm (0.1 N HCl)	Difference in Absorbance
1	5	0.132	0.098	0.034
2	10	0.245	0.187	0.058
3	15	0.377	0.299	0.078
4	20	0.520	0.392	0.128
5	25	0.641	0.490	0.151

Table 2: Optical characteristics of the proposed method

Parameters	Result
Measured wavelength (λmax)	260 nm (0.1 N NaOH), 240 nm (0.1 N HCl)
Beers law limit (µg/ml)	5-25
Regression equation (y = m x + c)	Y=0.020x+0.01
Slope	0.020
Intercept	0.01
Correlation coefficient (r)	0.9987
LOD µg/ml	0.26
LOQ µg/ml	0.53

***Y=mx+c, where x is the concentration in (µg/ml) and Y is difference in absorbance**

The recovery experiments were performed by adding known amounts of the drug to the pre-analysed formulation and reanalysing the mixture by proposed methods for both the solutions (Table 3).

Table: 3 Analysis of marketed formulation

Method	Label Claim (mg)	Amount estimated	% RSD	Standard Deviation	% Recovered
LER	10	9.8	0.12	0.032	99.78

R.S.D., Relative Standard Deviation.

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Table 4: Results of intermediate precision

Intermediate Precision		
		% R.S.D.
Day 1	Morning	99.98±0.38
	Evening	100.12±1.03
Day 2	Morning	100.11±0.67
	Evening	100.02±0.96

R.S.D., Relative Standard Deviation.

Table 5: Results of robustness and ruggedness

Results of robustness Using MeOH : Water (60:40)				
Label claim LER (mg/tab)	% Label claim estimated* (Mean ± %R.S.D.)		% Recovery estimated* (Mean ± %R.S.D.)	
10	98.34±0.97	99.67±1.27	99.54±1.11	100.15±0.97
Results of ruggedness by changing instrument				
Label claim LER (mg/tab)	% Label claim estimated* (Mean ± %R.S.D.)	% Recovery estimated* (Mean ± %R.S.D.)	Label claim LER (mg/tab)	% Label claim estimated* (Mean ± %R.S.D.)
10	101.07 ± 0.71	101.36 ± 0.20	102.74 ± 0.35	103.18 ± 0.31

*** Average of nine determinations; R.S.D., Relative Standard Deviation**

Performing replicate analyses of the standard solutions was used to assess the precision of the proposed method (Table 4). Results of robustness and ruggedness were shown in (Table 5). The LOD and LOQ were found to be 0.26µg/ml and 0.53 µg/ml respectively. The method was found to provide high degree of precision and reproducibility. The recovery studies showed that the results were within the limit indicating no interference.

CONCLUSION

The proposed method is simple, sensitive, accurate and precise and can be successfully employed for the routine analysis of the LER in Pharmaceutical formulations. The method was extended to pharmaceutical formulation and there were no interferences from any excipients and diluents.

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