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Original Article

Application of planar chromatography for estimation of lercanidipine hydrochloride in dosage form

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ABSTRACT

A new simple, precise, accurate, and selective high performance thin-layer chromatographic (HPTLC) method has been developed for analysis of lercanidipine hydrochloride (LER) in a tablet, using chloroform:toluene:methanol:acetic acid (8:1:1:1; *v/v/v/v*) as mobile phase. Chromatographic separation was achieved on precoated silica gel $60F_{254}$ plates with 0.2 mm thickness. Detection was performed densitometrically using a UV detector at 365 nm. The retention factor of lercanidipine hydrochloride was R_F 0.55 (±0.02). The reliability of the method was assessed by evaluation of linearity 30–210 ng per band. The suitability of this HPTLC method for quantitative determination of LER was proved by validation in accordance with the requirement of pharmaceutical regulatory standards. The method was found to be reproducible and convenient for determination of compound in commercial pharmaceutical formulations. The advantage of the method is simplicity, reasonable sensitivity, rapidity, excellent resolving power, low cost and is a more effective option than other chromatographic techniques in routine quality control.

1. Introduction

Lercanidipine hydrochloride (Fig. 1), 2-[(3,3-diphenylpropyl) methylamino]-1,1-dimethylethylmethyl-1,4-dihydro-2,6-dimethyl-4-(*m*-nitrophenyl)-3,5-pyridinedicarboxylate hydrochloride is a 1,4 dihydropyridine calcium-channel blocker used in the treatment of hypertension as it has good specificity on smooth vascular cells.[1] It is not official in any pharmacopoeia. The molecular weight of LER is 648.19 and melting point is 170–180 °C.[2] Spectrophotometric,[3] HPLC, and LC–MS,[4,5] HPTLC[6] methods have been reported for its determination in pharmaceutical formulations and biological fluids. This paper describes a reliable, rapid and accurate HPTLC method for determination of lercanidipine hydrochloride in tablets. The proposed HPTLC assays were validated in accordance with criteria stipulated by regulatory standards for pharmaceuticals.

2. Experimental

2.1. Chemicals and materials

Analytically pure sample of lercanidipine hydrochloride was supplied, as a gift sample by M/s Glenmark Pharmaceutical Ltd (Mumbai, India). All chemicals including chloroform,

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methanol, toluene, acetic acid were of analytical grade and were used without further purification. T1 = Lotensyl® 10 (Sun Pharmaceuticals Ltd., India) and T2 = Lervasc (Lupin Pharmaceuticals Ltd., India) containing labeled amount of 10 mg lercanidipine hydrochloride were procured from local market.

2.2. Chromatographic conditions

Chromatography was performed on 10 × 10 cm^2 aluminum HPTLC plates precoated with 0.2 mm layers of silica gel (E. Merck, Darmstadt, Germany; supplied by Merck India, Mumbai, India). Before chromatography, the plates were prewashed with methanol and dried in an oven at 50 °C for 5 min. Samples were applied as 6-mm wide bands, under a continuous flow of nitrogen, by means of a CAMAG (Muttenz, Switzerland) Linomat V sample applicator equipped with an applicator microsyringe (Hamilton, Bonaduz, Switzerland). A constant application rate of 0.1 μL s^{-1} was used. The plates were then conditioned for 20 min in a presaturated twin-trough glass chamber (10 × 10 cm^2) with the mobile phase of chloroform–toluene–methanol–acetic acid (8:1:1:1, v/v/v/v). The plates were then placed in the mobile phase and ascending development was performed to a distance of 70 mm from the point of application at ambient temperature, and the development time was 12 min. Subsequent to the development, the plates were dried in a current of air with the help of an air dryer and spots were visualized in CAMAG UV cabinet with dual wavelength UV lamp (254 and 366 nm); densitometric scanning was performed at 365 nm with CAMAG TLC scanner III operated in reflectance–absorbance mode and controlled by Wincats V software. The concentrations of compound were studied from the intensity of diffusely reflected light. Evaluation was based on linear regression of peak areas.

2.3. Preparation of standard solution

A stock solution containing 1 mg mL^{-1} LER was prepared in methanol. Calibration solutions were prepared by diluting the stock solution so that application of 1 μL volumes gave a series of spots covering the range 30–210 ng LER.

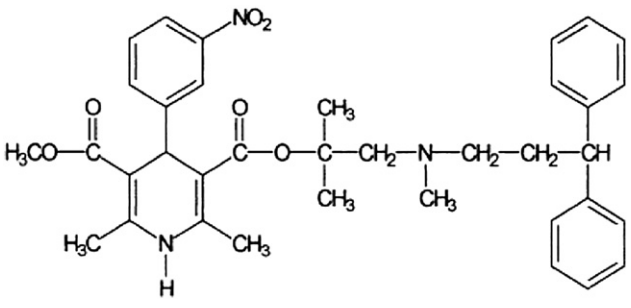

Fig. 1 – Structure of lercanidipine hydrochloride.

2.4. Method validation

The developed method was validated for linearity and range, specificity, precision, accuracy and robustness as per ICH guidelines.[7,8]

2.4.1. Linearity and range

Each concentration in the range of 30–210 ng per spot was spotted five times on individual plates and response was measured after scanning. For evaluation of linearity, peak area and concentrations were subjected to least square regression analysis to calculate calibration equation and correlation coefficient.

2.4.2. Specificity (interference from excipients)

The specificity of the method was ascertained by analyzing LER in presence of excipients of LER tablet formulations. The bands of LER in the sample were confirmed by comparing R_F values and respective spectra of the sample with those of the standard. The peak purity of LER was assured by comparing the spectra at three different levels, that is, peak-start (s), peak-apex (m) and peak-end (e) positions.

2.4.3. Precision

Precision was measured by using standard solutions containing LER at concentrations covering the entire calibration range. The precision of the method was evaluated by calculating the percent relative standard deviation (%RSD) of mean peak areas obtained from each spot of sample. Same procedure was performed at different time intervals on the same day, on different days and by different analysts.

2.4.4. Accuracy

The accuracy of the method was determined by recovery studies using standard additions at three different levels (approximately 80, 100 and 120% of label claim), i.e. multiple-level recovery studies. This was done to check for the recovery of the drug at different levels in the formulations.

2.4.5. Robustness

Robustness was assessed by deliberately changing the chromatographic conditions and studying the effects on the results obtained.

2.4.6. Limits of detection and limit of quantitation

Limits of detection and limit of quantitation were determined on the basis of the mathematical terms mentioned in ICH guidelines[7,8] for method validation from triplicate results of linearity.

Limit of detection was determined using equation 3.3 σ/s and limit of quantification was determined using equation 10 σ/s, where s is the slope of calibration curve and σ is standard deviation of responses.

2.4.7. Solution stability

The solutions at analytical concentration (1 mg mL^{-1}) were prepared and stored at room temperature protected from light for 48 h and analyzed at interval of 0, 6, 24 and 48 h for the presence of any band other than that of LER and the results