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A Stability-Indicating High Performance Liquid Chromatographic Assay for the Simultaneous Determination of Atenolol and Lercanidipine

Suresh Kumar Kankanala

Research Scholar, CMJ University, Shillong, Meghalaya

Abstract – A simple, rapid, precise and accurate isocratic reversed phase stability indicating HPLC method was developed and validated for the simultaneous determination of atenolol and Lercanidipine hydrochloride in commercial tablets. The chromatographic separation was achieved on phenomenex Gemini C18 (250×4.6 mm, 5 µm) column using a mobile phase consisting of acetonitrile and buffer (20 mM potassium dihydrogen phosphate pH 3.5) in the ratio of (55:45, v/v) at a flow rate of 1.0 ml/min and UV detection at 235 nm. The linearity of the proposed method was investigated in the range of 40-160 µg/ml (r^2 =0.9995) for atenolol and 8-32 µg/ml (r^2 =0.9993) for Lercanidipine. Degradation products produced as a result of stress studies did not interfere with the detection of atenolol and Lercanidipine and the assay can thus be considered stability-indicating.

Keywords: Atenolol, Antihypertensive, Lercanidipine Hydrochloride, Stability-Indicating Assay, Simultaneous HPLCUV, Validation.

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INTRODUCTION

Atenolol (ATE), 4-[2-hydroxy-3-[(1-methylethyl) amino] propoxy] benzeneacetamide (fig. 1) is a selective ß1 - adrenoceptor antagonist. Lercanidipine hydrochloride (LER), 1,4-dihydro-2,6-dimethyl- 4 - (3 - n i t r o p h e n y l) - 3 , 5 - p y r i d i n e d i c a b o x y l i c acid 2-[(3,3-diphenylpropyl)methyl amino]-1,1- dimethylethyl methyl ester hydrochloride (fig. 1). Lercanidipine is a third generation dihydropyridine calcium antagonist with a bulky bis-phenylalkylamine side chain, which makes it more lipophilic than most other in its class. There are many reported methods for analysis of ATE [1-4] or LER [5-7] either alone or in combination with other drugs in pharmaceutical dosage forms or individually in biological fluids. Simultaneous spectrometric estimation of atenolol and Lercanidipine hydrochloride in tablet dosage form has been reported [8]. None of the reported analytical methods describe a stability indicating method for the simultaneous determination of ATE and LER in presence of their degradation products. The objective of this work was to develop a stability–indicating HPLC method for combination drug products of ATE and LER. This method is also applicable for other commercially available drugs.

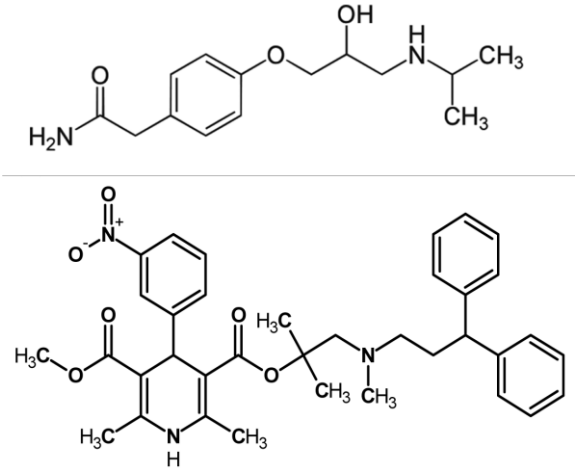

Fig. 1: Molecular structure of (a) atenolol and (b) Lercanidipine hydrochloride

MATERIALS AND METHODS

HPLC system (Waters 2489, Milford, USA) consisting of quaternary gradient pump (TM 600), rheodyne manual injector with 20 µl loop, column oven and UV detector was employed for analysis. Chromatographic data was acquired using Empower software. Working standards of atenolol and Lercanidipine were provided by Emcure pharmaceuticals, Pune, India. A tablet containing 50 mg ATE and 10 mg LER (Lotensyl AT; Sun Pharmaceuticals, Vadodara, India) was obtained

commercially. HPLC grade acetonitrile and orthophosphoric acid were obtained from Merck India Limited, Mumbai. Analytical grade hydrochloric acid, sodium hydroxide pellets and hydrogen peroxide solution 30% (v/v) were obtained from Ranbaxy Fine Chemical (New Delhi, India) and 0.45 µm membrane filter was obtained from Pall life sciences (Mumbai, India). High purity deionised water was obtained from Millipore, Milli-Q (Milford, USA) purification system.

Chromatographic conditions:

Phenomenex Gemini C18 (250×4.6 mm, 5 µm) column used as a stationary phase. The isocratic mobile phase consisting of acetonitrile and buffer (20 mM potassium dihydrogen phosphate, pH 3.5 adjusted with orthophosphoric acid) in the ratio of (55:45, v/v) was used throughout the analysis. The flow rate of the mobile phase was 1.0 ml/min. Detector signal was monitored at wavelength of 235 nm. The column temperature was kept ambient and injection volume was 20 µl.

Preparation of standard solution:

Standard stock solution containing ATE (500 µg/ml) and LER (100 µg/ml) was prepared by transferring 50 mg ATE and 10 mg LER working standard into a 100 ml volumetric flask. A 40 ml portion of diluents (acetonitrile-water 50:50, v/v) was added, sonicated and cooled to room temperature. The solution was diluted to the mark with diluents. Standard solution containing ATE (100 µg/ml) and LER (20 µg/ml) was prepared by pipetting 10 ml stock solution into a 50 ml volumetric flask and diluting to volume with diluents.

Preparation of sample solution:

Twenty tablets were weighed and the average weight was calculated. The tablets were crushed with a mortar and pestle for 10 min. A portion of powder equivalent to the weight of one tablet was accurately weighed and transferred to a 100 ml volumetric flask. Approximately 50 ml diluent was added and the mixture was sonicated for 15 min with intermittent shaking. The contents were restored to room temperature and diluted to volume with diluents to furnish stock test solution. The stock solution was filtered through 0.45 µm membrane filters and 10 ml of the filtered solution was transferred to a 50 ml volumetric flask and diluted to volume with diluents to give test solution containing 100 µg/ml ATE and 20 µg/ml LER.

Forced degradation studies:

Tablet powder equivalent to the weight of one tablet was transferred to 250 ml round bottomed flask and treated under acidic, alkaline, oxidizing, thermal and photolytic stress conditions. When degradation was complete, the solution were left to equilibrate to room temperature and diluted with diluents to furnish solutions of concentration equivalent to 100 µg/ml ATE and 20 µg/ml LER. The specific conditions are described below. In acidic degradation drug was heated under reflux with 1M hydrochloric acid for 30 min at 80° on oil bath and the drug was treated with 0.1N NaOH at room temperature for 2 h in alkaline degradation. Then resulting solution was neutralized. The drug was treated with 2% (v/v) H2 O2 at room temperature for 2 h in oxidative degradation. Thermal degradation was performed by exposing the solid drug to dry heat in a convection oven at 70° for 72 h and photolytic degradation was performed by exposing the drug to sunlight for 72 h.

Validation procedure:

In the system suitability standard solution of 100 µg/ml ATE and 20 µg/ml LER (n=5) was prepared and injected. Then system suitability parameters like retention time, theoretical plates, peak asymmetry and resolution were calculated from the chromatogram. The specificity of the method was evaluated by assessing interference from excipients in the pharmaceutical dosage form prepared as a placebo solution. The specificity of the method for the drug was also established by checking for interference with drug quantification from degradation products formed during the forced degradation study. Test solutions for assessment of the linearity of the method where prepared at seven concentrations from 40 to 160% of the analyte concentration in the assay (i.e. 40, 60, 80, 100, 120, 140 and 160 µg/ml for ATE and 8, 12, 16, 20, 24, 28 and 32 µg/ml for LER). Peak area and concentration data were then evaluated by linear regression analysis. The precision of the method, as intra-day repeatability was evaluated by performing six independent assays of the test sample preparation and calculating the RSD %. The intermediate (intraday) precision of the method was checked by performing same procedure on different days by another person under the same experimental conditions. Accuracy was studied by adding three different amounts (corresponding to 50, 100 and 150% of the test preparation concentrations) of ATE and LER to the placebo preparation and comparing the actual and measured concentrations. For each level, three solutions were prepared and each was injected in duplicate. The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. The conditions studied were flow rate (altered by ±0.1/min), mobile phase composition (by using 57:43 and 53:47 v/v acetonitrile: buffer pH 3.5), buffer pH (altered by ±0.2) and use of HPLC columns from different batches. Sample solution stability was evaluated by shorting the solution at ambient temperature and at 2-5° and analysis after 6, 12, 24, 36 and 48 h. The responses from the aged solutions were compared with those from freshly prepared standard solution.

RESULTS AND DISCUSSION

In this work HPLC method with UV detection for analysis of ATE and LER in a tablet formulation was

developed and validated. The analytical conditions were selected after testing the different condition effecting HPLC analysis, for example diluents and buffer composition, buffer concentration, organic solvent in the mobile phase, buffer to organic solvent ratio and other chromatographic conditions. Preliminary trials with mobile phases comprising mixtures of water with methanol or acetonitrile did not give good peak shape. The best peak shape was obtained by use of 0.02 M potassium dihydrogen phosphate buffer, adjusted to pH 3.5 with phosphoric acid and use of mobile phase of composition 55:45 (v/v) acetonitrile-0.02 M potassium dihydrogen phosphate buffer. Acetonitrile chosen as a organic constituent of the mobile phase to reduce retention times and buffer was chosen to reduce peak asymmetry and achieve good peak shape. The optimized mobile phase enabled good resolution of ATE and LER and of compounds generated during forced degradation. As shown in fig. 2, ATE and LER were eluted after 2.12 and 6.05 min, respectively. The newly developed analytical method was validated according to the ICH guidelines [9, 10] and its updated international convention [11], USP [12] and AOAC International. System suitability was verified by measurement of peak asymmetry (A3.0) and number of theoretical plates (N>2000) after chromatography of standard solution. The values of these properties were in accordance with in-house limits. The specificity of the method was determined by checking for interference with the analytes from placebo components by measuring peak purity for ATE and LER during the forced degradation study. Peak purity was satisfactory under the different stress conditions. There was no interference from any degradation product peak with the drug peaks. Fig. 2 shows the chromatograms of untreated drugs in tablet solution. ATE showed extensive degradation in oxidative conditions while extensive degradation of LER occurred in acidic conditions as shown in (fig. 3) and (fig. 4), respectively. Both drugs show normal degradation in alkaline conditions as shown in (fig. 5). the extent of degradation results of ATE and LER under various stress conditions.

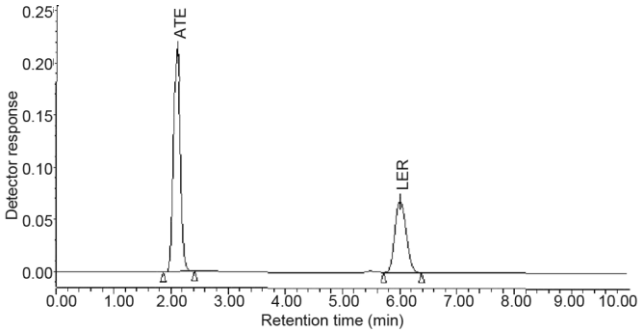

Fig. 2: Typical chromatogram of tablet extract, showing peaks of ATE and LER

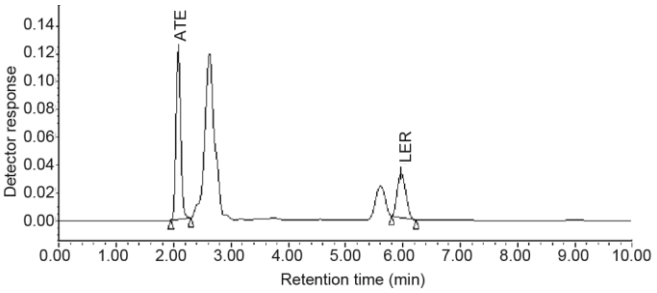

Fig. 3: Chromatographic separation of ATE and LER from their oxidative degradation products

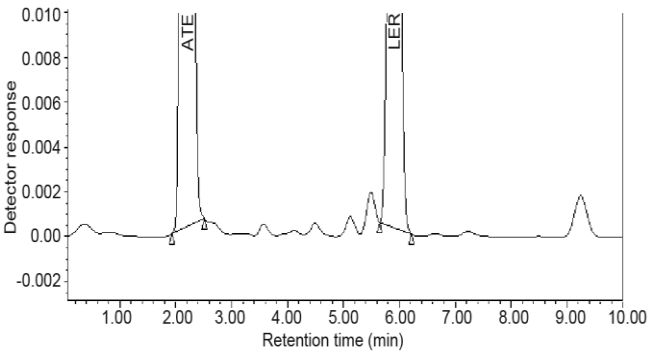

Fig. 4: Chromatographic separation of ATE and LER from their acidic degradation products

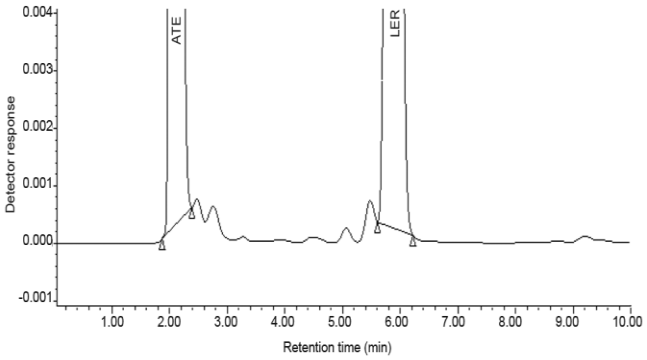

Fig. 5: Chromatographic separation of ATE and LER from their alkaline degradation products

ATE and LER showed linearity in the range of 40-160 µg/ml and 8-32 µg/ml, respectively. The linear regression equations were y=16456x+11776, correlation coefficient 0.9995 for ATE and y=47672x+7888, correlation coefficient 0.9993 for LER, where x is the concentration in µg/ml and y is the peak area in absorbance units. The limits of detection and quantification were evaluated by serial dilution of ATE and LER stock solution until the signal-to-noise ratios were 3:1 for LOD and 10:1 for LOQ. The LOD for ATE and LER were 0.02 and 0.05 µg/ml, respectively; the LOQ were 0.05 and 0.1 µg/ml, respectively. The relative standard deviations were 0.47% for ATE and 0.27% for LER, which are well within the acceptable limit of 2.0%. The RSDs for

intermediate precision were found to be 0.21% for ATE and 0.30% for LER. The recovery of ATE and LER from placebo was determined at three different concentrations. Mean recovery was 98.54-101.54% for ATE and 98.81–100.86% for LER. In all deliberately varied conditions, the RSD of peak areas of ATE and LER were found to be well within the acceptable limit of 2.0%. The tailing factor and asymmetry for both the peaks were found to be <2.0%.

Proposed HPLC method is specific, accurate and precise for the simultaneous determination of atenolol and Lercanidipine hydrochloride from pharmaceutical dosage form. The described method is suitable for routine analysis and quality control of pharmaceutical preparations containing atenolol, Lercanidipine, nifedipine, indapamide, hydrochlorothiazide and amlodipine either as such or in combination with atenolol. The method can be used to separate these drugs from their degradation products and excipients found in the tablet dosage form.

CONCLUSION

A stability-indicating HPLC method was developed, validated and applied for the determination of Letrozole in pharmaceutical dosage forms. The developed method was validated as per ICH guidelines and was found to be accurate, precise, robust and specific. The chromatographic separation was achieved on phenomenex Gemini C18 (250×4.6 mm, 5 µm) column using a mobile phase consisting of acetonitrile and buffer (20 mM potassium dihydrogen phosphate pH 3.5) in the ratio of (55:45, v/v) at a flow rate of 1.0 ml/min and UV detection at 235 nm. The linearity of the proposed method was investigated in the range of 40-160 µg/ml (r2 =0.9995) for atenolol and 8-32 µg/ml (r2 =0.9993) for Lercanidipine. Degradation products produced as a result of stress studies did not interfere with the detection of atenolol and Lercanidipine and the assay can thus be considered stability-indicating. The chromatographic elution step is undertaken in a short time (o4 min). No interference from any components of pharmaceutical dosage form or degradation products was observed and the method has been successfully used to perform long-term and accelerate stability studies of Letrozole formulations.

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