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Investigation on the photochemical stability of lercanidipine and its determination in tablets by HPLC–UV and LC–ESI–MS/MS☆

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Abstract

The photostability of lercanidipine, a dihydropyridine calcium-channel blocker used in the treatment of the hypertension, was studied. Drug substance and its solutions and formulations were exposed to UV-A radiations (solar simulator) and the photodegradation process was monitored by UV spectrophotometry, HPLC and HPLC–mass spectrometry. The effect of the solvent (ethanol and ethanol/PBS 1:1 v/v) on the photodegradation pathway and kinetic was evaluated.

Lercanidipine and its photodegradation products were separated by a selective reversed-phase HPLC method and the main photoproducts were characterized by HPLC–MS/MS analysis, using an electrospray ionization source (ESI) and an ion trap analyzer. Photochemical reactions, involved in the photodegradation of lercanidipine, include aromatisation of the dihydropyridine moiety, formation of nitrosoderivatives and *N*-dealkylation in the side chain. The developed stability-indicating HPLC method was then applied to the quality control of commercially available lercanidipine formulations (tablets).

Keywords: Lercanidipine; Photostability; HPLC; LC–MS/MS; Quality control; Tablets

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1. Introduction

Lercanidipine, (±)2-[(3,3-diphenylpropyl)methylamino]-1, 1dimethylethyl methyl (4*RS*)-2,6-dimethyl-4-(3-nitrophenyl)-1-4-dihydropyridine-3,5-dicarboxylate monohydrochloride (Table 1), is a dihydropyridine calcium-channel blocker developed for an oral administration and used in the treatment of mild to moderate hypertension [1,2].

The chemical structure of lercanidipine is characterized by the presence of a side chain containing a 3,3-diphenylpropyl-methylamine-2-methyl-2-propyl group that was introduced to improve the lipophilic properties and the activity duration of the drug. From a physico-chemical point of view, lercanidipine is slightly soluble in water, but it is more soluble in some widely used solvents as well as ethanol and methanol, or mixture water–organic solvents. Lercanidipine is orally administered as racemate lercanidipine hydrochloride tablets. The drug is sensitive to light, according to the well-known photosensitivity of diihydropyridine class [3,4]. The photoreactivity of lercanidipine has been only in part investigated [4] and few methods, based on HPLC [4,5], spectrophotometry [6] and differential pulse voltammetry [7] have been developed for its determination in pharmaceutical dosage forms.

The aim of the present study was to investigate the photostability of lercanidipine in solutions and in pharmaceutical products, exposed to UV-A radiations, using a xenon arc lamp (solar simulator). The effect of the solvent (ethanol and ethanol/PBS 50:50) on the drug photostability and on the degradation kinetic was evaluated. Lercanidipine and its photodegradation products were separated by a selective liquid chromatographic (HPLC) method, under reverse phase conditions. The main photoproducts were characterized by LC–MS/MS analysis, using an electrospray ionization source (ESI) and an ion trap analyzer.

Therefore, a stability-indicating HPLC method was then developed and applied to the quality control of a commercial lercanidipine pharmaceutical formulation (tablets).

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2. Experimental

2.1. Materials

Lercanidipine standard was kindly supplied by Recordati Industria Chimica e Farmaceutica S.p.A (Milan, Italy). Zanedip® filmed tablets for oral administration, containing lercanidipine (9.4 mg/tab), were commercially available. Triethylamine (TEA) and acetic acid were from Sigma–Aldrich (Milan, Italy), solvents for chromatography were of HPLC grade from Romil Pure Chemistry (Cambridge, UK) and all other chemicals were of reagent grade from Carlo Erba Reagents (Milan, Italy). A Milli-Q® (Millipore, France) water purification system was used to obtain the purified water for photostability studies and HPLC analysis. Photodegradation studies were performed on drug solutions in ethanol and ethanol/phosphate buffer saline (PBS) 50:50 (v/v). PBS consisted of phosphate buffer (0.02 M; pH 7.4) containing 0.135 M sodium chloride.

2.2. Apparatus and experimental conditions

Tests on the photochemical stability were carried out using a xenon arc source to simulate the natural sunlight exposure. A 150 W xenon arc lamp (solar simulator, model 68805, Oriel Corporation, Stratford, CT, USA) was used, with a dichroic mirror (Oriel, model 81405) to block visible and IR radiation in order to minimize sample heating. An air-mass filter 1.5 (Oriel, model 81090) was used to simulate solar conditions and a UV-B-C blocking filter was employed to attenuate the UV-B component. The output beam was directed downward by a “beam-turning assembly”, containing the dichroic mirror. The UV dose (μW/cm^2) from the Xe-arc lamp was measured using a Oriel Goldilux radiometer (model 70127), fitted with external interchangeable probes for UV-A (dose: 82.2 μW/cm^2 min) and UV-B (dose: 9.1 μW/cm^2 min).

Spectrophotometric analyses were performed on a HP 8453 UV–vis photodiode spectrophotometer (Agilent Technologies, Palo Alto, CA), using 1 cm quartz cells.

LC–MS analysis was carried out on a Jasco PU-1585 Liquid Chromatograph (Jasco Corporation, Tokyo, Japan) interfaced with a Jasco 1575 UV–vis detector ($\lambda = 265$ nm) and with a LCQ-Duo Mass Spectrometer (Thermo Finnigan, San Jose, CA, USA), by a splitting flow T-valve. The mass spectrometer was equipped with heated capillary interface and electrospray ionization (ESI) source, and operated with an Ion Trap analyser. ESI system employed a 4.5 kV (positive polarity) spray voltage and a heated capillary temperature of 220 °C. The sheath gas and the auxiliary gas (nitrogen) flow rates were set to 0.75 and 1.2 l/min, respectively. Electrospray ionization was optimised using lercanidipine as reference compounds. The mass chromatograms were acquired in total ion current (TIC) modality from 100 to 900 *m/z*, in MS/MS mode (relative collision energy 24–27%) on the ESI generated ions of lercanidipine and its photoproducts. Reverse phase chromatographic analysis were performed on a Phenomenex Luna C_{18}, 3.5 μm (2.0 × 150 mm i.d.) column, using a mobile phase consisting of methanol/TEA buffer (0.01 M) 60:40 (v/v), adjusted to pH 4 with acetic acid and a flow rate of 250 μl/min. The injector was a Rheodyne valve (model 7725i) with a 20 μl loop.

2.3. Photostability testing

Solutions of lercanidipine in ethanol and in the mixture ethanol/PBS 50:50 (v/v) at a concentration of 0.2 mg/ml were placed into quartz cells (1 cm path length) closed with screw caps. Quartz cells were placed horizontally and exposed to UV-A and -B radiations (Xe-arc lamp) for increasing time, corresponding to increasing UV doses. The photoexposed solutions were then analysed by both spectrophotometry, to detect any absorption spectral changes, and LC–MS analysis (after dilution 1:5 with mobile phase to the final concentration of 40 μg/ml), to follow the lercanidipine photodegradation rate and pathway. These analyses were also carried out on samples stored in darkness: sample solutions in quartz cells wrapped in aluminium foils were exposed to radiation exposure.

To gain information on the kinetics of the process, solutions of lercanidipine (0.1 mg/ml) in ethanol and in the mixture ethanol/PBS 50:50 (v/v) were exposed to UV radiations and the disappearance of the drug was evaluated by HPLC–UV. Aliquots of 100 μl of the exposed solutions were withdrawn every 10 min (total time 45 min) for ethanolic solutions and every 30 min (total time 360 min) for solutions in ethanol/PBS. The photoexposed solutions were previous diluted with the mobile phase to the final concentration of 20 μg/ml before injection in HPLC apparatus.

Photostability at the solid state was also evaluated by exposing lercanidipine drug substance and lercanidipine commercial preparation Zanedip®, as entire and powdered tablets, to UV radiations for increasing time. Each tablet was turned upside down every hour to have the tablets surface exposed homogeneously. The solid forms were then analysed by LC–MS. Photoexposed tablets were treated as described in Section 2.5; photoexposed lercanidipine substance was dissolved in ethanol (0.2 mg/ml) and the resulting solution was diluted with the mobile phase to the final concentration of 40 μg/ml.

2.4. Photoproducts characterization: LC–ESI–MS/MS analysis

The photoexposed solutions in ethanol and in ethanol/PBS, diluted 1:5 with mobile phase, and the irradiated solid forms, treated as described in Section 2.5, were subjected to LC–MS analysis using the following conditions. The mass spectra were recorded within 100–900 *m/z* full scan (positive polarity), providing the total ion current (TIC) chromatograms (Fig. 2) and the pseudomolecular mass of the analytes. The MS/MS spectra of lercanidipine and its photoproducts, named PP (in the mixture ethanol/PBS) and PE (in ethanol) were also obtained by on-line LC–ESI–MS analyses. An explicative MS/MS spectrum of lercanidipine and a representative scheme of the fragmentation pattern, in agreement with literature data [8] is presented in Fig. 4. This fragmentation was found to be valid also for the photoproducts (Table 1), as below reported.