Journal of Pharmaceutical and Biomedical Analysis 50 (2009) 597–601

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Photostability of pitavastatin—A novel HMG-CoA reductase inhibitor

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ARTICLE INFO

Article history:
Received 15 July 2008
Received in revised form 3 October 2008
Accepted 6 October 2008
Available online 17 October 2008

Keywords:
Statins
HMG-CoA reductase inhibitors
Pitavastatin
Photostability
Photodegradation

ABSTRACT

The photostability of pitavastatin, an HMG-CoA reductase inhibitor used in the treatment of hypercholesterolemia, was investigated. The sample solution was exposed to UV-A radiation and the photodegradation process was monitored by means of spectrophotometric method and HPLC–DAD. Pitavastatin was shown to be photolabile and its photodegradation reaction followed the first-order kinetics with the rate constant $k = 3.54 \times 10^{-4} \pm 9.43 \times 10^{-6}\ s^{-1}$.

The chromatograms revealed the presence of four major photoproducts (PP-1–PP-4). The separated and isolated photolytic products were identified using a mass spectrometer coupled with a time of flight (TOF) analyzer. The main reaction observed during exposure to radiation of pitavastatin was photocyclisation leading to formation of four-ring photoproducts.

1. Introduction

Pitavastatin, NK-104, monocalcium bis(3*R*,5*S*,6*E*)-7-(2-cyclopropyl-4-[4-fluorophenyl]-3-quinolyl)-3,5-dihydroxy-6-heptenoate (PIT), is a totally synthetic HMG-CoA reductase inhibitor that significantly reduces serum total cholesterol, LDL cholesterol, and triglyceride levels while modestly raising HDL cholesterol [1,2]. The cellular mechanism of action is attributed to the inhibition of cholesterol biosynthesis in the liver and the drug is the first-line agent for lipid lowering in patients with atherosclerosis and cardiovascular disease [3–6]. Blocking the mevalonate pathway depletes cells not only of cholesterol but also of numerous metabolites involved in different cell functions [7–10]. Additionally, recent reports have suggested that pitavastatin has many pleiotropic effects and is now being tried for treatment of other diseases, including Alzheimer's disease and osteoporosis [11–15]. Recent research has shown that statins have potential anti-cancer effects [16]. They may be a result of the reduction of GTP-biding proteins, which are produced during cholesterol biosynthesis [17,18].

Metabolism of pitavastatin by the cytochrome P450 (CYP) system is minimal, principally through CYP 2C9, with little involvement of the CYP 3A4 isoenzyme, potentially reducing the risk of drug–drug interactions [19–21].

Nowadays, photostability studies are an integral part of the drug development process and are widely recognized as one of the most important procedures in registration of pharmaceutical products [22]. Knowledge of the photochemical and photophysical properties of the compound is necessary for appropriate handling, packaging and labelling the drug substance and drug product [23]. Radiation has two main effects on drugs. The first is the influence of light on the stability of the drug substances and drug formulations [24]. The second aspect of drug–light interactions is that of the biological effects caused by the reaction of drugs, photoproducts or metabolites of drugs with light and biomolecules, resulting in drug induced photosensitivity [25,26].

As follows from a literature survey, certain statins including cerivastatin, atorvastatin, fluvastatin and rosuvastatin are highly photochemically reactive [27–34]. The post-column photolytic degradation of cerivastatin led to the formation of a compound characterised by 75-times more intensive fluorescence than the parent compound [28]. Most attention in the literature has been devoted to the atorvastatin photodegradation. The changes appearing upon its irradiation were studied with the use of different irradiation sources and in the presence of methylene blue as a photosensitizer. The degradation products were isolated and characterised [29]. The photooxygenation of atorvas-

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doi:10.1016/j.jpba.2008.10.004

tatin in water as well as photochemistry of fluvastatin has been the subject of interest of Cermola et al. [30,31]. The photochemical sensitivity of rosuvastatin has been described by Mehta et al. [32]. Experiments focused on the development and validation of chromatographic method which proved the formation of many photoproducts—however the structural characterization of them was not performed [29,32]. The detailed studies on rosuvastatin photodegradation including structure elucidation of photoproducts were performed by Astarita et al. [33].

In this work, we have investigated the photochemical transformation processes of pitavastatin in solution and reported the structure elucidation of the main photoproducts.

2. Experimental

2.1. Materials

Pitavastatin calcium was a generous gift from Zydus Cadila, Ahmedabad, India. Acetonitrile (Baker, HPLC-reagent) was used as the mobile phase in chromatographic runs. Potassium dihydrogen phosphate and orthophosphoric acid (85%) were purchased from POCH, Gliwice, Poland.

Ultra-pure water was obtained from the Millipore Simplicity system.

Pitavastatin calcium solution (1×10^{-4} M) was prepared in acetonitrile:deionized water (50:50, v/v).

2.2. Photodegradation conditions

Pitavastatin calcium solution was placed in quartz, cylindrical cells (V = 2.5 mL, l = 1 cm) and exposed to UV-A radiation. A sample cell protected against light by aluminium foil was used as a dark control [35]. Photochemical experiments were carried out using a high-pressure mercury lamp equipped (HBO-50) with Wood's filter to isolate the 365 nm wavelength region. The UV-A dose was determined by means of a radiometer type VLX-3W, Vilber Lourmat, with a sensor CX-365, to be each time of 0.25 J cm^{-2} min^{-1}.

All procedures related to the experiment testing the photostability of pitavastatin were performed in a special room with no access of daylight.

2.3. Apparatus

The UV–vis absorbance spectra were recorded by a Shimadzu 1601 PC double-beam spectrophotometer, interfaced to a PC for data processing (PC 160 Plus software).

Chromatographic separations and analyses were performed with an Agilent 1200 LC system (Agilent Technologies, Waldbronn, Germany), equipped with a vacuum degasser, a quaternary pump, an autosampler and a diode array detector. Chromatographic separations were conducted on a LiChrospher RP-18 analytical column, 5 μm particle size, 250 mm × 4 mm (Merck, Darmstadt, Germany) maintained at 25 °C. The pump flow rate was 1 mL min^{-1} and injection volume was 20 μL. The mobile phase was acetonitrile (solvent A) and 10 mM phosphate buffer solution (potassium dihydrogen phosphate, KH_2PO_4), adjusted to pH 4,0 with phosphoric acid 85% (solvent B); gradient elution was employed starting at 40% A, increasing linearly to 100% over 30 min, and then maintained for 10 min.

2.4. Calibration curve

Calibration curve was obtained by plotting the peak area of pitavastatin versus the theoretical concentration over a range 1×10^{-5} to 1×10^{-3} M. The data were subjected to the least squares regression analysis. Inspection of the plotted calibration curve described by equation: $y = 1.61 \times 10^7 + 76.08$ and correlation coefficient (r = 0.999) confirmed that the calibration curve was linear over the concentration range.

2.5. Photodegradation kinetic

HPLC was adopted to follow the kinetics of pitavastatin photodegradation. At appropriate time intervals the samples were assayed by HPLC. The results were subjected to kinetic analysis based on the pitavastatin concentration at zero time (c_0) proportional to the initial concentration, and the concentration (c_t) after irradiation.

2.6. Photoproducts identification—mass spectrometry

The spectra were obtained by means of a Mariner API–TOF spectrometer (PerSeptive Biosystems, Stafford, TX), by direct injection of the samples dissolved in methanol–formic acid (99:1). To obtain the exact mass values, the instrument was calibrated with the use of an internal standard composed of 4,8-dimethyl-7-hydroxycoumarin, desipramine, and dansylglycyltryptophan.

3. Results and discussion

3.1. Photodegradation studies

The process of photodegradation was followed by analysis of UV–vis spectra recorded after irradiation of pitavastatin with increasing doses of irradiation. As shown in Fig. 1, the absorption spectra of pitavastatin in acetonitrile:water (50:50, v/v) showed three main bands centred at 233, 240, 317 nm, respectively. No changes in absorbance were detected in the reference samples (spectra not shown). In contrast, after exposure to UV-A the changes in spectrum occurred—the appearance of a new band at 223 and 317 nm and the disappearance of the broad band at 244 nm. The four isosbestic points at 207, 212, 228.5 and 292 nm appeared.

In addition, HPLC analyses were performed to monitor the changes of pitavastatin solution as a function of the exposure time (Figs. 2 and 3). Optimization of the HPLC method permitted detection of four photoproducts, namely PP-1 (t_R = 6.39 min), PP-2 (t_R = 8.21 min), PP-3 (t_R = 29.22 min) and PP-4 (t_R = 31.10 min).

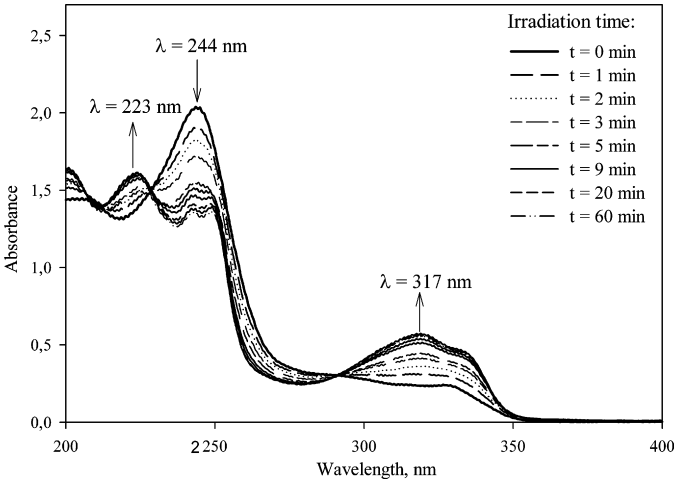

Fig. 1. Absorption spectra of pitavastatin solution exposed to UV-A radiation. The arrows indicate the changes in the absorption spectrum after UV-A exposure (0 → 60 min).