Thermal Stability of Simvastatin under Different Atmospheres

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ABSTRACT: Simvastatin (SV) is a widely used drug for the treatment of hypercholesterolemia in humans. Nevertheless, serious efforts are still being made to develop new SV formulations with, for example, improved tabletability or bioavailability properties. These efforts frequently involve heating the compound well above ambient temperature or even fusion. In this work, the thermal stability of solid SV under different atmospheres was investigated by using isothermal tests in glass ampules, differential scanning calorimetry, and Calvet-drop microcalorimetry experiments. These tests were combined with analytical data from diffuse reflectance infrared Fourier-transform spectroscopy and liquid chromatography coupled with tandem mass spectrometry or Fourier transform ion cyclotron resonance mass spectrometry (LC–FT–ICR–MS). No decomposition was observed when the sample was kept at a temperature $\leq$373 K under N_2 or reduced pressure (13.3 Pa) atmospheres. Thermal degradation was, however, observed for temperatures $\geq$353 K in the presence of pure or atmospheric oxygen. The nature of the two main oxidative degradation products was determined through MS/MS experiments and accurate mass measurements of the precursor ions using FT–ICR–MS. The obtained results indicated that the decomposition process involves the oxidation of the hexahydronaphthalene fragment of SV. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:241–248, 2014

Keywords: calorimetry; mass spectrometry; thermal analysis; degradation products; diffuse reflectance; solid-state stability

INTRODUCTION

Statins are the main class of active pharmaceutical ingredients currently in use to control hypercholesterolemia in humans. They reduce plasma levels of low-density lipoprotein cholesterol (LDL-c) particles by inhibiting hydroxymethylglutaryl-CoA reductase (EC 1.1.1.34).[1–3] Several studies have shown that high plasma concentration of LDL-c lead to atherosclerosis, a predisposing factor for the development of cardiovascular diseases. Therefore, statin therapy has been playing a major role in the pharmaceutical armamentarium for the prevention and treatment of such diseases.[3–5]

An important member of the statin family is simvastatin (SV) (Fig. 1; $C_{25}H_{38}O_5$, CAS number [79902–63–9], 1*S*,3*R*,7*S*,8*S*,8a*R*)-8-{2-[(2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate), which was approved for marketing by the United States Food and Drug Administration in 1991 and is still widely prescribed to treat hypercholesterolemia.[1–3,6] It is normally administered in the lactone form (Fig. 1), which is traditionally prepared by direct alkylation of lovastatin, a similar statin obtained as a secondary metabolite from the filamentous fungus *Aspergillus terreus*.[7–9]

Thermal stability studies of crystalline[10,11] and amorphous[11–14] SV from ambient temperature (~298 K) to the melting point (~412 K)[10,11,15–19] are scarce, and, to the best of our knowledge, a detailed characterization of degradation products has not yet been reported.[20] This information is, however, very important for various aspects of SV use, such as the reliable definition of storage conditions or the development of new formulations that imply heating.

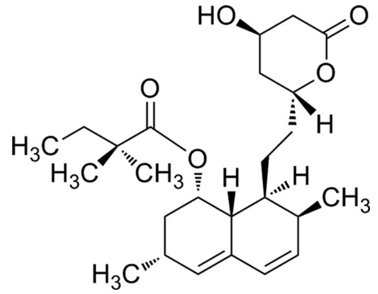

Figure 1. Molecular structure of simvastatin (SV) lactone form.

There have been, for example, serious efforts to develop efficient methodologies for solubility enhancement of SV because its bioavailability is essentially limited by a poor solubility in aqueous media.[2,21] Some promising strategies involve the production of SV glasses or solid dispersions in hydrophilic carriers (e.g., polyvinylpyrrolidone).[2,21] Such methods require fusion of SV or at least heating the sample well above ambient temperature.

Organic pharmaceuticals often degrade through oxidation reactions[22] and the picture emerging from reports on the thermal behavior of solid SV indicate that the compound may undergo an exothermic oxidative degradation in air, which is very slow at ambient temperature and has an onset at approximately 401 K when the sample is heated in a differential

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scanning calorimeter at a rate of 2 K/min.[20] Under inert atmosphere (e.g., N_2), no thermal events are observed between 298 K and the fusion temperature (412 K), except for a glass transition at approximately 306 K if an amorphous phase is present.[10,11,13,14] Thermogravimetry experiments carried out at 10 K/min indicate that after fusion SV undergoes pyrolysis with onset at approximately 443 or 476 K in dynamic air or dynamic N_2 atmospheres, respectively.[10,23]

In this work, a comprehensive experimental study of the thermal stability of SV under different atmospheres with identification of the major degradation products is reported. In addition to isothermal tests carried out in glass ampules, dynamic and isothermal experiments were also carried out by differential scanning calorimetry (DSC) and Calvet-drop microcalorimetry, respectively. These two types of technique are widely used in accelerated stability tests of solid-state APIs, and provide important information such as the temperature onsets and exothermic or endothermic nature of the degradation processes.[24]

MATERIALS AND METHODS

Materials

The SV sample (Jubilant Organosys, Mysore, Karnataka, India) was previously characterized in terms of chemical purity (98.88±0.12%), phase purity (form I, orthorhombic, $P2_12_12_1$, $Z'/Z = 1/4$), and morphology by elemental analysis, diffuse reflectance infrared Fourier-transform (DRIFT) spectroscopy, ^{1}H and ^{13}C NMR, X-ray powder diffraction, scanning electron microscopy, DSC, thermogravimetry, and LC–ESI/MS.[18] Acetonitrile (LC-MS grade; Fisher Scientific, Loughborough, Leicestershire, U.K.), acetic acid (p.a.; Sigma–Aldrich, Sintra, Portugal), and deionized water (Millipore Simplicity® Simpak 2, $R = 18.2$ MΩ cm, U.S.A.) were used in the LC–MS analysis. Nitrogen (Alphagaz N_2-1; , Ar Líquido, Lisbon, Portugal), oxygen (Alphagaz O_2-1; Ar Líquido, Lisbon, Portugal), and dry air obtained from a compressed air apparatus equipped with a desiccant dryer element were used. Water saturated air (air+H_2O), nitrogen (N_2+H_2O), or oxygen (O_2+H_2O) atmospheres were produced by passing the gases through a water bubbler at a flow of 26±1 cm^3/s. The relative humidity of atmospheric air (57%–60%) was monitored with a TFA 30502 sensor.

DRIFT Spectroscopy

DRIFT measurements were performed in the range 400–4000 cm^{-1}, with a resolution of 2 cm^{-1}, on a Nicolet 6700 spectrometer (Thermo Fisher Scientific, Waltham, MA, U.S.A.). The samples were dispersed in KBr.

Liquid Chromatography–Tandem Mass Spectrometry

Sample solutions (about 0.4 mg/cm^3) prepared in acetonitrile were analyzed with a ProStar 410 autosampler, two 210-LC chromatography pumps, a ProStar 335 diode array detector, and a 500-MS ion trap mass spectrometer equipped with an electrospray ionization (ESI) source (Varian, Palo Alto, CA, U.S.A.). Data acquisition and processing were performed using the Varian MS Control 6.9.3 software. The samples were injected onto the column *via* a Rheodyne injector with a 100 μL loop in the pick-up injection mode. Separations were carried out with a Phenomenex Luna C18 (2) column (150 × 2 mm^2, 3 μm), at a flow rate of 200 μL/min, using a 5 min linear gradient from 50% to 70% (v/v) acetonitrile in $2{\times}10^{-3}$ mol/dm^3 ammonium acetate, pH 4.0, followed by a 10 min linear gradient to 100% acetonitrile, and an 8 min isocratic elution with acetonitrile. The UV absorbance was monitored at 238 nm.

The mass spectrometer was operated in the positive and negative ESI mode, with the following optimized parameters: ion spray voltage, ±4.5 kV; capillary voltage, 20 V, and RF loading, 80%. Nitrogen was used as nebulizing and drying gas, at pressures of 35 and 10 psi, respectively; the drying gas temperature was set at 623 K. The positive tandem mass spectra (MS/MS) were obtained with an isolation window of 2.0 Da, excitation energy values between 0.9 and 1.2 V, and an excitation time of 10 ms.

Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

High-resolution mass measurements were performed on an Apex Qe FTICR 7 Tesla mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with an Apollo II dual ESI/MALDI source (Bruker Daltonics, Bremen, Germany). Spectra were acquired in positive ESI mode with external calibration. For LC–MS analysis, an Agilent 1200 capLC system (Agilent Technologies, Santa Clara, CA, U.S.A.) was used, at a flow rate of 10 cm^3/min. The gradient program and the solvents were identical to those used in the LC–MS/MS analysis, the column was a Zorbax C18 150 × 0.5 mm^2. Spectra were processed with the data Analysis 4.0 software package from Bruker Daltonics.

Thermal Stability Studies in Glass Ampules

Glass ampules (ca. 90 mm length and 7 mm internal diameter) were loaded with 51–380 mg of SV and closed under the following atmospheres: reduced pressure of 13.3 Pa, atmospheric air (57%–60% relative humidity), air+H_2O, N_2, N_2+H_2O, O_2, and O_2+H_2O. The ampules were sealed by means of a blow-torch, except in the case of the oxygen atmospheres where a rubber stopper reinforced with an Apiezon Q seal was employed. All weightings were performed with a precision of ±0.01 mg in a Mettler AT201 balance (Mettler-Toledo, Columbus, OH, U.S.A.).

The reduced pressure atmosphere was attained by pumping the ampule containing the SV sample with a rotary pump for 30 min before sealing. The ampule charged with atmospheric air was sealed under normal laboratory conditions (295±1 K, 57%–60% relative humidity). The dry oxygen and all water saturated atmospheres were achieved by purging the ampule for 30 min with the appropriate gas, before sealing. The N_2 atmosphere was obtained by connecting the ampule to a vacuum/N_2 line and performing three cycles consisting of pumping to 13.3 Pa and then filling with N_2 at approximately 1 bar.

The thermal stability of SV under the various atmospheres mentioned above was first tested by immersing the ampules containing the sample in a silicon oil bath kept at 373±1 K for 14 h. The effect of increasing the duration of the experiment at 373±1 K to 26 h was investigated in air and N_2 atmospheres. Experiments with fixed 18 h duration were performed at different temperatures (343±1 K, 353±1 K, 363±1 K, and 373±1 K) for atmospheric air. Finally, a test was made where a sample under reduced pressure was kept at 423 K (above the fusion temperature) for 5 min, and left to cool to ambient temperature after removal from the bath.