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Original Article

Thermal stability and hydration behavior of ritonavir sulfate: A vibrational spectroscopic approach ☆

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ABSTRACT

Ritonavir sulfate is a protease inhibitor widely used in the treatment of acquired immunodeficiency syndrome. In order to elucidate the inherent stability and sensitivity characteristics of ritonavir sulfate, it was investigated under forced thermal and hydration stress conditions as recommended by the International Conference on Harmonization guidelines. In addition, competency of vibrational (infrared and Raman) spectroscopy was assessed to identify structural changes of the drug symbolizing its stress degradation. High performance liquid chromatography was used as a confirmatory technique for both thermal and hydration stress study, while thermogravimetric analysis/differential thermal analysis and atomic force microscopy substantiated the implementation of vibrational spectroscopy in this framework. The results exhibited high thermal stability of the drug as significant variations were observed in the diffuse reflectance infrared Fourier transform spectra only after the drug exposure to thermal radiations at 100 °C. Hydration behavior of ritonavir sulfate was evaluated using Raman spectroscopy and the value of critical relative humidity was found to be $>67\%$. An important aspect of this study was to utilize vibrational spectroscopic technique to address stability issues of pharmacological molecules, not only for their processing in pharmaceutical industry, but also for predicting their shelf lives and suitable storage conditions.

1. Introduction

Inhibition of human immunodeficiency virus (HIV) protease has been recognized as an important approach for therapeutic intervention of acquired immunodeficiency syndrome (AIDS) [1]. Protease inhibitors are the drugs which play an instrumental role in the reduction of morbidity and mortality among people with HIV infection [2,3]. Amongst the family of protease inhibitors, ritonavir sulfate has revolutionized HIV therapy due to its selective, competitive and reversible inhibitory effects on both HIV-1 and HIV-2 proteases [4]. Ritonavir, {[5S-(5R*,8R*,10R*,11R*)]-10-hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4-thiazolyl]-3,6-dioxo-8,11-bis (phenylmethyl)-2,4,7,12-tetraazatridecan-13-oic acid, 5-thiazolylmethyl ester} (Fig. 1), is a synthetic organic compound derived from N-carbamoyl-alpha amino acids and their derivatives.

The stability of pharmaceutical molecules is a matter of great concern as it affects the safety and efficacy of the drug product. Therefore, the International Conference on Harmonization (ICH) Q1A guideline entitled "Stability testing of new drug substances and products" requires stress testing to be carried out in order to elucidate the inherent stability and sensitivity characteristics of the active substance [5]. However, literature supports limited analytical methods established for the stability studies of the solid dosage form of ritonavir sulfate. While high performance thin layer chromatography (HPTLC) has been used for simultaneous determination of ritonavir and lopinavir in capsules [6]. A few liquid chromatography–mass spectroscopy (LC–MS) methods have been reported for analysis of ritonavir and its metabolites in biological fluids [7–10]. International Pharmacopoeia (Ph. Int.) describes a liquid chromatography method to separate ritonavir and its impurities [4]. Determination of ritonavir in bulk dosage form using spectrophotometric and potentiometric methods has also been described [11–15]. In recent years, several high performance liquid chromatography (HPLC) methods for simultaneous determination of antiretroviral drugs in plasma have been demonstrated [16,17]. Nevertheless, HPLC has been widely used for the stress degradation study of pharmaceuticals, it has some disadvantages in terms of cost performance, time consumption and necessary equipment, such as the use of expensive disposable cartridges at solid-phase drug extraction, gradient elution control by a gradient HPLC pump system and the ultraviolet detection at multiple wavelengths. Therefore, in the present scenario, a simplified technique is desirable for addressing these issues.

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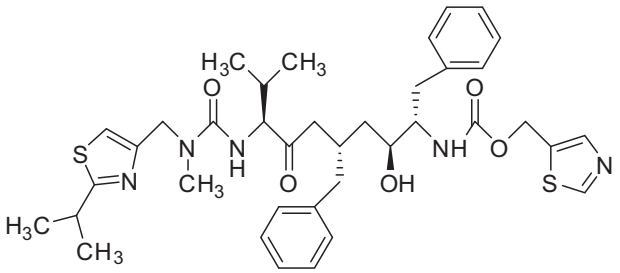

Fig. 1. Chemical structure of ritonavir sulfate.

Vibrational spectroscopy has an edge over the above mentioned techniques for studying pharmaceutical systems at molecular level as it is a nondestructive and noninvasive technique, and is sensitive to structural conformational aspects and the environment of the compound to be analyzed [18]. It also offers substantial advantages in terms of speed, lends them to in-process monitoring of the structure and does not require hazardous organic solvents. Vibrational spectroscopy has been employed by various researchers to evaluate the photo-stability of drugs. The photo-stability of nicardipine and corresponding transformations during the shelf-life of the solid dosage form of a drug has been determined using Fourier transform infrared (FTIR) spectroscopy [19]. Similarly, the photo-stability of carbamazepine polymorphs and nifedipine has been studied using Fourier transform reflection-absorption spectroscopy [20,21]. In our previous study, we applied diffuse reflectance infrared spectroscopy to evaluate the stability of some antiretroviral and anticancer drugs [22–24]. Raman spectroscopy has been successfully used for the quantitative analysis of polymorphic mixture of carbamazepine [25]. Sardo et al. [26] used Raman spectroscopy to monitor reversible-hydration kinetic processes of niclosamide. Moreover, hydration and dehydration characteristics of theophylline have been monitored using Raman spectroscopy [27].

The present study was conducted to assess the feasibility of vibrational spectroscopy to address thermal as well as hydration stability issues of ritonavir sulfate. The critical temperature and critical relative humidity (RH) of ritonavir sulfate estimated through this work are necessary not only for its processing in pharmaceutical industry but also for predicting its shelf life and suitable storage conditions. In this framework, diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy was used to evaluate the thermal stability of the drug. HPLC and thermogravimetric analysis (TGA)/differential thermal analysis (DTA) were used to substantiate the inferences drawn from the spectroscopic analysis of the thermally degraded drug. Furthermore, Raman spectroscopy, a water transparent technique, was used to study the hydration stress behavior of ritonavir, and HPLC was also used as a confirmatory technique for hydration stress behavior. Moreover, atomic force microscopy (AFM) analysis was carried out to depict changes in the topographical morphology of the hydrated form of ritonavir sulfate.

2. Experimental

2.1. Materials and methods

Tablets of ritonavir sulfate used in this investigation were procured from Cipla Pharmaceuticals Limited, India. Spectroscopic grade potassium bromide (KBr) was bought from BDH Laboratory Suppliers, England. Methanol (CH_4O), potassium phosphate (KH_2PO_4), acetonitrile (C_2H_3N) and 0.05 M phosphoric acid (H_3PO_4) used in the study were of HPLC grade and obtained from Qualigens Fine Chemicals. Millipore purified water (resistance ~18.2 MΩ) from Scholar-UV Nex UP 1000 system was used for HPLC analysis. All other reagents were of analytical grade and used without further purification.

2.2. Thermal degradation studies

2.2.1. Sample preparation

Ritonavir sulfate was thermally degraded using Linkam TP 92, HFS 91/Hot stage plate with platinum resistor. The setup included a small aluminum dish in which the drug powder was kept on the silver block in the hot stage. The drug samples for the study were heated at different temperatures ranging from 30 to 120 °C with an increment of 10 °C for a period of 1–6 h with an increment of 1 h at each temperature. Besides this, a fresh drug sample was used at each temperature and retention time. Then, the thermally treated samples were cooled down to room temperature before being subjected to DRIFT measurements. The heating and cooling rate of the hot stage was maintained at 10 °C/min.

2.2.2. DRIFT spectroscopic measurements

To characterize thermally treated ritonavir sulfate samples, homogenous sample mixtures were prepared by dispersing 5% (m/m) of thermally treated drug powder in spectroscopic grade potassium bromide. The sample mixtures were then kept in the sample holder of Varian 660 Fourier transform infrared spectrophotometer equipped with Pike Technologies, diffuse reflectance accessory operating with a Globar source, in combination with a KBr beam splitter and deuterated triglycine sulfate (DTGS) detector. The infrared spectra of the drug powder before and after exposure to thermal radiation were recorded in the scan range of 400–4000 cm^{-1} with a resolution of 4 cm^{-1}. A total of 256 scans were collected for each spectrum. Background spectrum was also recorded with ground potassium bromide powder under the same experimental conditions before collecting each sample scan.

2.2.3. HPLC measurements

HPLC analysis of thermally degraded ritonavir sulfate was performed on a Shimadzu HPLC (UFLC, Prominence) equipped with an LC-20AD binary pump, an SPD-20A variable wavelength UV–vis detector, a CTO-20A column oven, degasser and a manual injector fitted with 20 μL sample loop. The instrument was controlled by LC software. A Phenomenex C_{18} column (250 mm × 4.6 mm, 5 μm) was used for analysis. The mobile phase consisting of acetonitrile: 0.05 M phosphoric acid (55:45, v/v), was ultrasonicated and vacuum filtered by passing through a 0.44 μm pore size membrane filter prior to use. The standard stock solutions of fresh ritonavir sulfate at a concentration of 1.0 mg/mL, after exposure to thermal radiations and different RH treated samples, were prepared in the mobile phase. The prepared solution was ultrasonicated for 20 min, vacuum filtered through a 0.44 μm membrane filter and then a 0.22 μm membrane filter before being fed to the manual injector. The flow rate was adjusted to 1.0 mL/min and the injection volume of 20 μL was maintained. All the chromatograms were recorded at a wavelength of 210 nm with the column temperature maintained at 40 °C.

2.2.4. TGA/DTA studies

For comprehensive analysis of ritonavir sulfate's thermal behavior, TGA/DTA measurements of the drug were conducted on a Shimadzu TA 60 thermal analyzer with 10 mg of sample under a nitrogen flow of 40 mL/min with a heating rate of 10 °C/min from 35 to 350 °C. The experiment was performed in triplicate to check the reproducibility.