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Evaluation of thermal stability of indinavir sulphate using diffuse reflectance infrared spectroscopy

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Abstract

Indinavir sulphate is a potent and specific protease inhibitor of human immunodeficiency virus (HIV). It is used for the treatment of acquired immune deficiency syndrome (AIDS). At elevated temperature the drug which otherwise remains crystalline undergoes a phase transition to an amorphous phase to form degradation products. In the present study, thermal stability of indinavir sulphate is evaluated using diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy. Infrared spectra of the drug before and after the exposure to thermal radiation at different temperatures were acquired in the diffuse reflectance mode using a Fourier transform infrared (FTIR) spectrophotometer. The differential scanning calorimetry (DSC) and the X-ray diffraction (XRD) studies were used as complimentary techniques to adequately implement and assist the interpretation of the infrared spectroscopy results. The DRIFT spectra reveal that the drug remains stable up to 100 °C, degrades slightly at 125 °C and undergoes complete degradation at about 150 °C to produce degradation products. The degradation products can easily be characterized using the infrared spectra.

Keywords: Indinavir sulphate; HIV protease inhibitors; Diffuse reflectance; Infrared spectroscopy; Thermal stability; ICH

1. Introduction

Indinavir sulphate [*N*-[2(R)-hydroxy-1(S)-indanyl]-5-{[2(S)-tertiary-butylaminocarbonyl]-4-(3-pyridylmethyl)piperazino}-4(S)-hydroxyl-2(R)-phenyl-methyl-pentanamide] (Fig. 1) is among the selective, potent and specific reversible inhibitors of the human immunodeficiency syndrome (HIV) protease. HIV protease is an enzyme that plays an essential role in HIV replication and the formation of an infectious virus. Indinavir is a structural analogue of the HIV phe-Pro protease cleavage site. The drug's structure inhibits the function of HIV protease, blocking virus replication and causing the formation of immature, noninfectious virions [1,2]. Indinavir is active in both acutely and chronically infected cells. At elevated temperatures the drug which otherwise is crystalline undergoes a phase transition to an amorphous substance leading to the formation of degradation products [3]. The primary degradation pathway for indinavir sulphate is amide bond hydrolysis to form lactone and aminoindanol [4,5] (Fig. 1).

Several high-performance liquid chromatography (HPLC) methods [6–9], liquid chromatography/mass spectroscopy (LC/MS) methods [10,11], capillary electrophoresis [12–14] and thin-layer chromatography (TLC) methods [15,16] were developed for the determination of indinavir sulphate in blood and plasma. A capillary electrophoresis (CE) method was developed for the separation of indinavir from its degradation impurities in capsules [17]. A few liquid chromatography (LC) methods [18,19] were also developed for the assay and purity control of indinavir formulations.

According to the International Conference on Harmonization (ICH) parent drug stability test guidelines "Q1A (R2)" [20], the stress testing is mandatory to determine inherent stability characteristics of the drug substances. Heat treatment is one of the important stress conditions defined by ICH guidelines. To the

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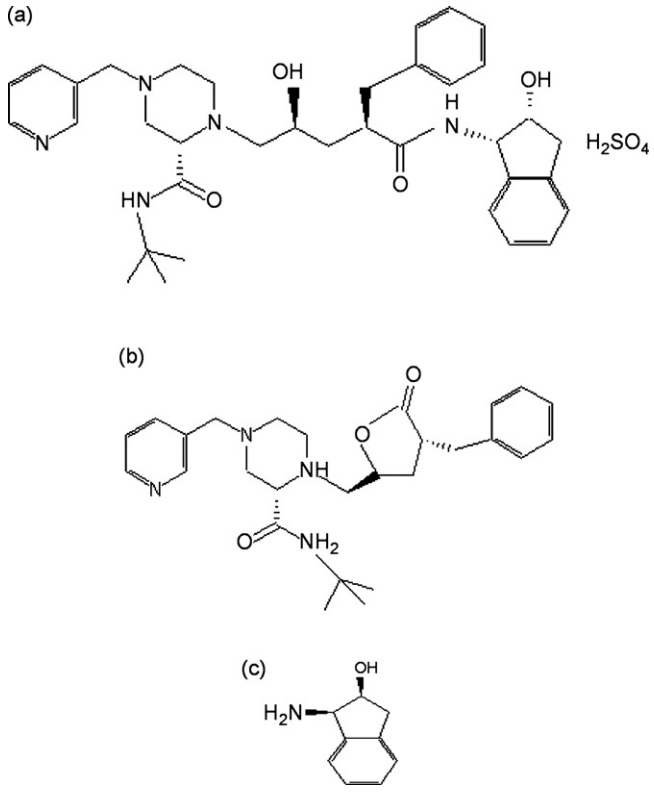

Fig. 1. Chemical structure of indinavir sulphate (a), indinavir lactone (b), and aminoindanol (c).

best of our knowledge, a very few articles related to the stability studies of indinavir sulphate in the pharmaceutical dosage form have been reported in literature. Kaul et al. [21] developed a stability indicating high-performance thin-layer chromatographic (HPTLC) method for the analysis of indinavir sulphate. As the degradation of indinavir sulphate is temperature dependent, so there is an urging need to develop a simple, fast and accurate stability indicating method to evaluate the thermal stability of indinavir sulphate.

Infrared spectroscopy is gaining importance as a tool to evaluate the stability of the pharmaceuticals. This technique has an edge over other techniques by being rapid, nondestructive, noninvasive nor does it require hazardous organic solvents. Several workers have successfully used infrared spectroscopy to evaluate the photostability of drugs [22–24]. Teraoka et al. [22] evaluated the photostability of nicardipine hydrochloride using Fourier transformed infrared spectroscopy. Photostability of cabamazepine polymorphs [23] and nifedipine [24] was investigated by using Fourier transformed reflection–absorption spectroscopy. The physical and thermal characterization of chiral omeprazole sodium salts was reported using DRIFT, scanning electron microscopy (SEM), DSC and powder X-ray diffraction [25]. To the best of our knowledge evaluation of thermal stability of any drug by infrared spectroscopy has not yet been reported in literature.

The aim of the present work is to evaluate the stability of indinavir sulphate under stress condition of heat as defined by ICH using diffuse reflectance infrared spectroscopy. X-ray diffraction (XRD) Differential scanning calorimetry (DSC) and Thermo gravimetric analysis (TGA) were carried out simultaneously to confirm and support the results of infrared spectroscopy. DSC is a thermoanalytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference are measured as a function of temperature and time. DSC is used extensively in the pharmaceutical industries to determine the melting point, purity, glass transition temperature and thermal decomposition of drug substances [26].

2. Experimental

2.1. Materials

Indinavir sulphate used in this study was procured from Cipla Pharmaceuticals Ltd., India. Potassium bromide (KBr) used in this study was of spectroscopy grade and was procured from BDH Laboratory Suppliers, England. All chemicals and reagents were of analytical reagent grade and were used without further purification.

2.2. Thermal degradation studies

Linkam TP 92, HFS 91/hot stage plate with platinum resistor was used for thermal treatment of the drug. The drug powder in a small aluminum dish was kept on the silver block on a hot stage. During the thermal treatment the samples were held on the silver block of the hot stage and the temperature was varied from 25 to 150 °C in 10 °C steps. A fresh sample is used at each temperature. For optimization of the time period for heating, the sample was treated at fixed temperature for different time period between 15 min and 1 h. Finally time period of 1 h is selected for subsequent studies to allow the samples to degrade completely and to minimize the error. After heating, the samples were allowed to cool down to room temperature before further experiments. The heating and cooling rate was maintained to be 10 °C min^{-1}.

2.3. Diffuse reflectance infrared Fourier transformed spectroscopic (DRIFT) measurements

DRIFT spectra of the drug powder were recorded at room temperature in the range of 400–4000 cm^{-1} using a Bio Rad 175 C Fourier transformed spectrophotometer operating with a Globar source, in combination with a KBr beam splitter and deuterated triglycine sulphate (DTGS) detector. The instrument was equipped of Pike Technologies, diffuse reflectance accessory. In diffuse reflectance spectroscopy, the sample to be analyzed must be diluted with an infrared transmitting matrix. Therefore After exposing the drug to thermal radiation at different temperatures, the sample mixtures was prepared by dispersing 5% (w/w) of the treated drug powder in spectroscopy grade potassium bromide (KBr). The variation in particle size can have a significant influence on the DRIFT measurement, which can cause differences in reflection pattern and consequently a high noise level. Therefore the sample mixtures were well ground before measurement in order to make samples more homogenous and increase relative reflectance coming out of the

samples. Sample mixtures were then placed in a small sample cup and kept in the sample holder. The spectra with a resolution of 4 cm^{-1} were recorded. 128 scans were collected for each spectrum. Background spectra were obtained with ground KBr powder for each experimental condition.

Spectroscopic manipulation such as smoothing, normalization and derivatization were performed using Unscrambler 9.1 software. Savitzky-Golay second-order polynomial was used with 21 data points to obtain the second-order derivative spectra

2.4. Differential scanning calorimetric (DSC) and thermogravimetric analysis (TGA)

A PerkinElmer Pyris 6 DSC was used for recording DSC thermogram of original indinavir sulphate. About 5 mg sample of pure indinavir sulphate was weighed accurately using PerkinElmer Diamond TG/DTA balance. Weighed sample was heated in a closed aluminum pan at a programmed rate of 10 °C min^{-1} in the temperature range from 30 to 400 °C under nitrogen flow of 40 mL min^{-1}. Empty aluminum pan was used as a reference. The experiment was performed in triplicate to check the reproducibility.

TGA measurements of original indinavir sulphate was carried out by Shimadzu TA 60 thermal analyzer with 20–25 mg of sample under a nitrogen flow of 40 ml min^{-1} at a heating rate of 10 °C min^{-1} from 35 to 400 °C. It is important that the heating rate and purge flow rate be the same for direct comparison of thermal events in both DSC and TGA.

2.5. X-ray powder diffraction (XRD) analysis

X-ray powder diffraction patterns were measured at room temperature by Bruker D8 advance X-ray diffractometer. The scanning rate employed was 1° min^{-1} over the 10–70° 2θ range in step of 0.025° 2θ for 1 s per step. The XRD patterns of original indinavir sulphate before thermal treatment and after exposing the drug to thermal radiation at 150 °C were recorded.

3. Results and discussion

3.1. Differential scanning calorimetry and thermogravimetric analysis

As the temperature increases the sample eventually reaches its melting temperature (T_m). The melting process results in an endothermic peak in the DSC curve. The melting point of indinavir sulphate is in the range 150–153 °C [27]. The DSC thermogram of indinavir sulphate (Fig. 2a) shows a sharp endotherm with T_m at around 153 °C that corresponds to the melting point of the drug. The small broad endotherms at low temperature between 40 and 50 °C may be due to the presence of some volatile material in the sample. The endotherm at about 160 °C may be due to the degradation products of indinavir. There are several endotherms from 180 to 210 °C. It may be due to melting temperature (T_m) of indinavir lactone (one of the degradation products of indinavir sulphate) and glass transition temperature (T_g) of degradation products of indinavir sulphate.

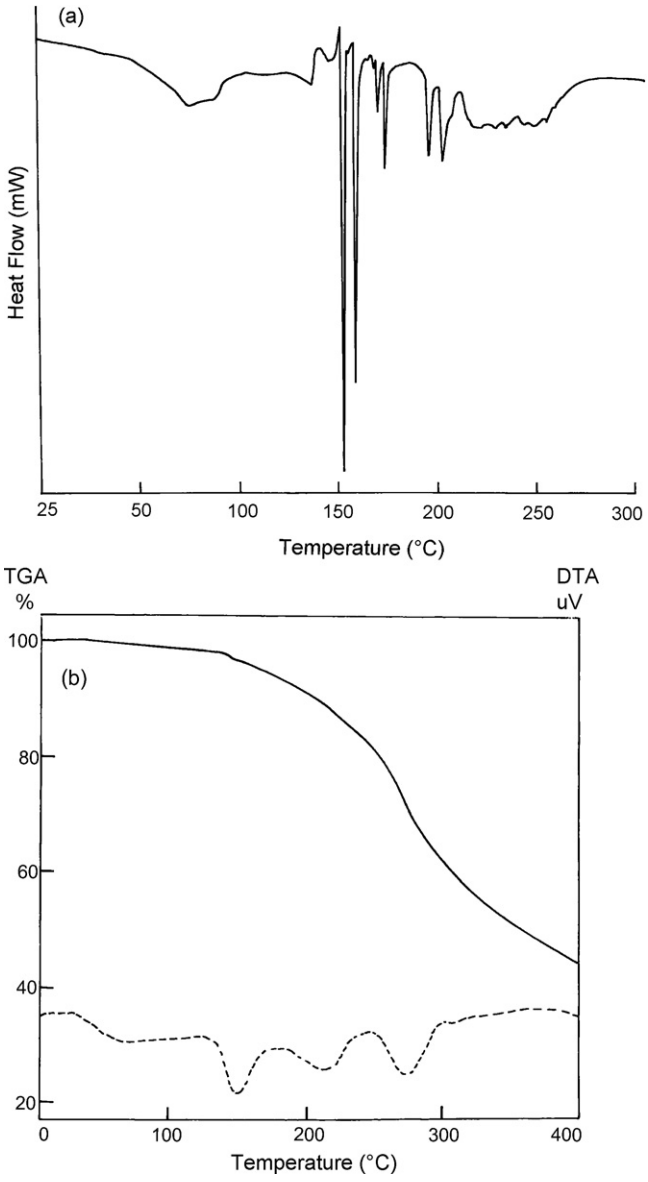

Fig. 2. (a) DSC thermogram and (b) TGA/DTA curve of indinavir sulphate.

This indicates that indinavir is no longer present as a crystalline material at elevated temperature but is converted in to amorphous state. The results of DSC suggest that indinavir sulphate undergoes degradation when exposed to elevated temperature.

DSC results are further confirmed by TGA/DTA curve. In general these techniques are complementary with one confirming the results of another. The TGA/DTA curves of indinavir sulphate are shown in Fig. 2b. In the lower temperature range there is a small weight loss that may be due to removal of moisture or low-boiling solvents from the sample. The thermal curve reveals that the onset of thermal degradation of indinavir sulphate occurs above 150 °C. The major weight loss transition occurs between 150 and 280 °C. DTA curve also shows that major decomposition of indinavir sulphate occurs at around 150 °C (melting point of the drug). The thermal degradation continued to the region 280 °C and appears to occur in three steps. These several transition in the drug may be correlated to

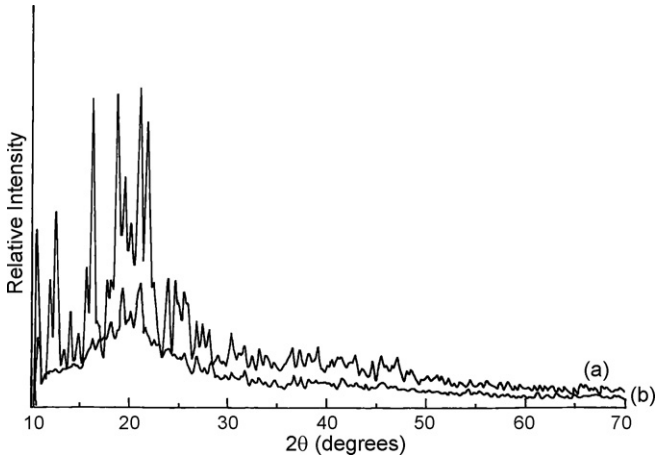

Fig. 3. XRD spectra of intact indinavir sulphate (a) and degraded indinavir sulphate (b).

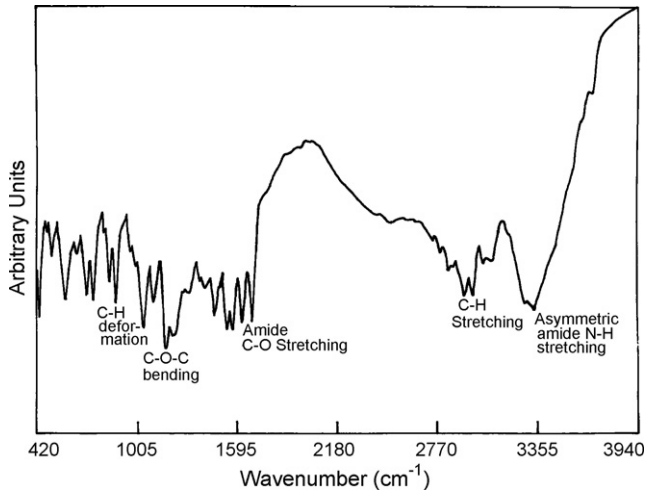

Fig. 4. DRIFT spectra of intact indinavir sulphate.

the degradation products of drugs. These results of thermal analysis suggest that indinavir sulphate undergoes degradation when exposed to elevated temperature.

3.2. X-ray powder diffraction

Fig. 3 shows the X-ray diffraction patterns of intact and degraded drug. In the X-ray diffraction spectrum of intact indinavir sulphate several sharp peaks at diffraction angle of 2θ 10.85, 12.91, 18.59, 19.40, 20.91, 21.69, 23.80 and 24.54° are present. The presence of these distinct peaks indicates that the drug is present as a crystalline material. It is clear from X-ray diffractogram of degraded drug that the crystallinity peaks of the drug diminish significantly after exposure of the drug to thermal radiation at 150 °C. These results confirm that the drug that was present as a crystalline material degraded in to an amorphous substance at a temperature of 150 °C.

3.3. Diffuse reflectance infrared spectroscopy

DRIFT spectrum of intact indinavir sulphate in the wavelength region 420–3940 cm^{-1} is shown in Fig. 4. The band at about 3350 cm^{-1} may be attributable to the asymmetric amide N–H stretching vibration. The band at 1680 cm^{-1} corresponds to amide C=O stretching vibration with N–H bending of amide group at about 1620 cm^{-1} [28]. The absorption band at 2970 cm^{-1} may be attributed to the C–H stretching vibration. The band at 3030 cm^{-1} may be attributed to C–H stretching vibration of nitrogen containing heterocyclic aromatic compounds. Interaction between ring C=C and C=N stretching vibration in pyridine and pyrimidine result in strong to medium intensity absorptions band at about 1457 cm^{-1} [28]. The band at about 1170 cm^{-1} may be attributed to C–O–C bending vibration. The bands in the region of 800–900 cm^{-1} may be attributed to –CH deformation in substituted aromatics. The band at about 580 cm^{-1} may be due to aromatic amide N–C=O bending vibration.

Fig. 5a–c shows the overlaid DRIFT spectra of indinavir sulphate in different wavelength region collected after exposing the drug to thermal radiation at different temperatures. It is evident from the overlaid spectra that at 125 °C there is a slight change in the spectra, which reveals that the drug starts degrading. There are remarkable changes in the spectra after exposing the drug to thermal radiation at 150 °C (Table 1). The absorption bands in the region 800–900 cm^{-1} (Fig. 5a) attributable to –CH deformation in substituted aromatics diminishes significantly. The band at about 580 cm^{-1} attributed to N–C=O bending in aromatic amides shifts towards higher wave number. This shifting may be an evidence of degradation of the drug. It may be due to amide bond hydrolysis of indinavir sulphate to form lactone and aminoindanol derivatives. The most significant region of the spectra that shows the appearance of new bands due to degradation of indinavir sulphate and formation of degradation products is shown in Fig. 5b. The major changes are in the regions 1200–1400 cm^{-1} and 1600–1800 cm^{-1}. A new band that appears at about 1790 cm^{-1} after exposing the drug to elevated temperature may be due to the formation of lactone derivative. Lactones generally have bands due to the stretching of the C=O and C–O groups. After degradation the new band at about 1790 cm^{-1} may be attributable to the lactone C=O stretching [28]. Two new bands in the region 1160–1370 cm^{-1} may be due to the lactone C–O stretching and alcohol C–O stretching of aminoindanol [28]. These new bands at 1790 cm^{-1} and 1160–1370 cm^{-1} may be used as band marker for the degradation of indinavir sulphate. The band at about 1680 cm^{-1} which is due to the amide C=O stretching has shifted slightly towards lower wave number after exposing the drug to elevated temperatures. The absorption band at about 3330 cm^{-1} (Fig. 5c) that is attributable to amide N–H stretching in intact drug has shifted towards lower wavenumber after exposing the drug to thermal radiation at 150 °C. This may be due to the –OH stretching vibration in amino indanol that is one of the degradation products of indinavir sulphate.

The analysis of thermal stability of indinavir sulphate by infrared spectroscopy may be enhanced by computing the second derivative of the spectra. Second derivative of $\log(1/R)$ data with respect to wavelength enhances spectral features and

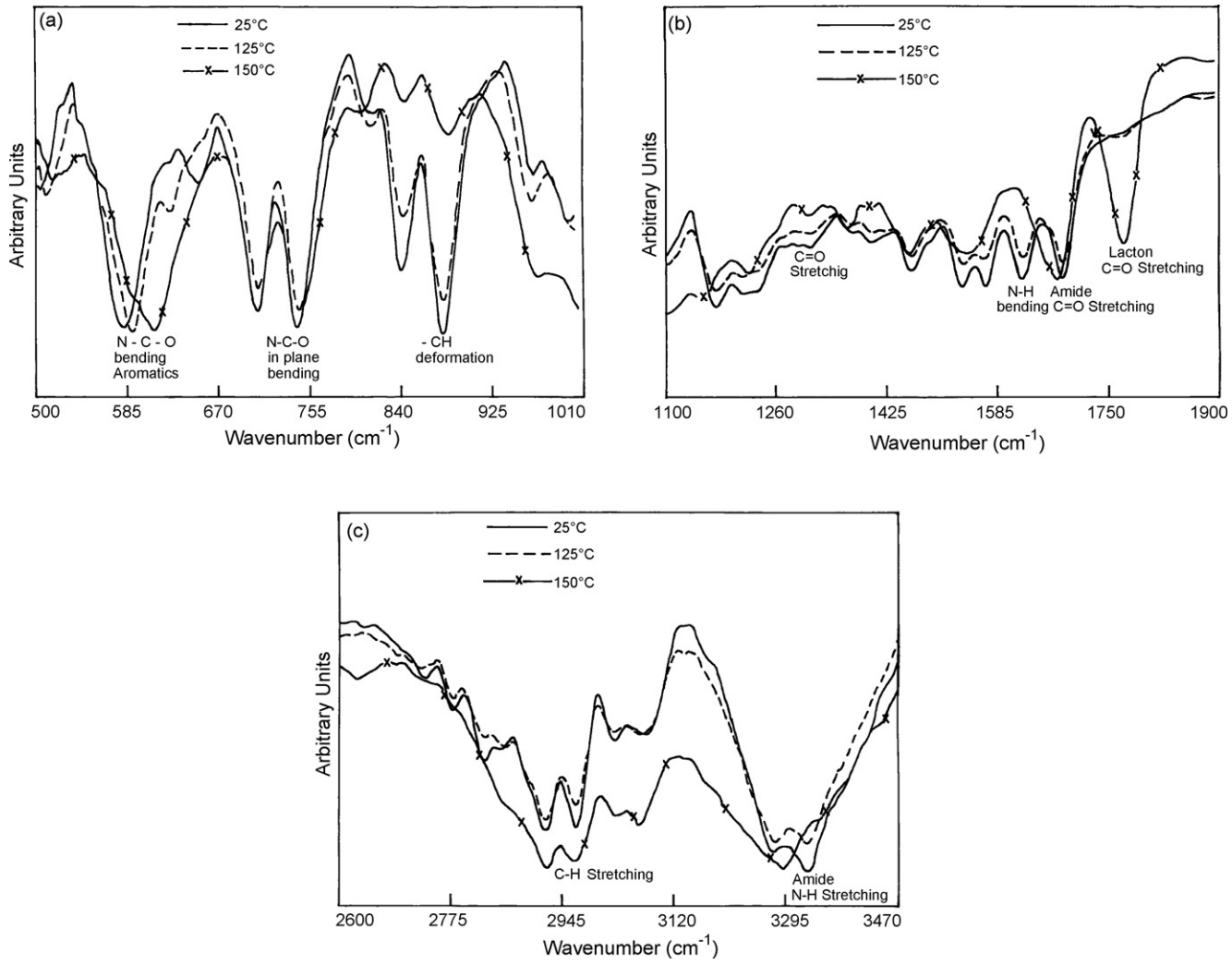

Fig. 5. Overlaid DRIFT spectra of indinavir sulphate at different temperatures in the region of (a) 500–1010 cm^{-1}, (b) 1100–1900 cm^{-1}, and (c) 2600–3470 cm^{-1}.

Table 1
Changes in the DRIFT spectra of indinavir sulphate after exposure to thermal radiation at 150 °C

Wavenumber (cm^{-1})	Assignments	Remarks
580	N—C═O bending in aromatics	The band shifted towards higher wavenumber in the spectra of degraded drug. This may be due to amide bond hydrolysis of indinavir sulphate
1160–1370	Region of C—O stretching	Two new bands appear in this region after exposing the drug to thermal radiations. These bands may be due to lactone and aminoindanol C—O stretching
1680	Amide C═O stretching	The band shifted towards lower wavenumber after degradation of drug
1790	Lactone C═O stretching	The band appearing in the spectra of degraded drug may be due to the formation of lactone derivative
3330	Amide N—H stretching	The band shifted towards lower wavenumber in the spectra of degraded drug. It may be due to —OH stretching vibration of aminoindanol

also compensates for the baseline shifts. The Savitzky–Golay second-order polynomial was used with 21 data points to obtain second derivative spectra. As the results illustrate that the major changes in the spectra after degradation occur in the region 1200–1400 cm^{-1} and 1600–1800 cm^{-1}, so we focused our attention on these ranges. These regions may prove useful for assessing the thermal stability of indinavir sulphate. The overlaid second derivative spectra of indinavir sulphate in the region 1165–1377 cm^{-1} is shown in Fig. 6a. After degradation of the drug two new bands in the region 1165–1370 cm^{-1} appear which

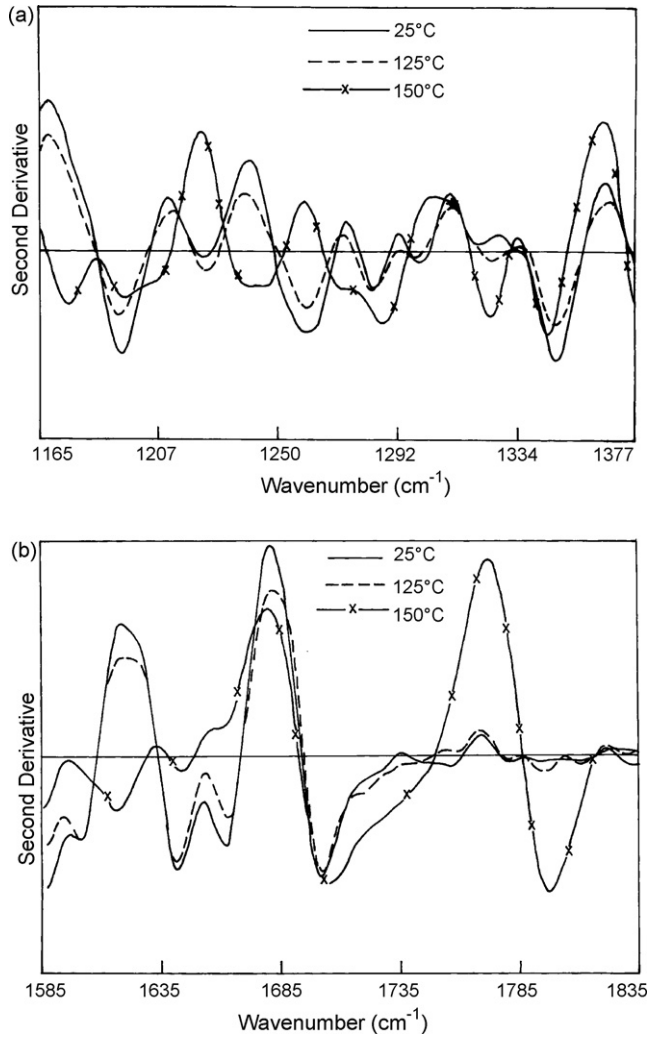

Fig. 6. Overlaid second derivative spectra of indinavir sulphate at different temperatures in the region of (a) 1165–1377 cm^{-1} and (b) 1585–1835 cm^{-1}.

becomes rather clear in the second derivative spectra than the original spectra. These bands may be due to the formation of lactone derivative and aminoindanol as described earlier. Fig. 6b shows the overlaid second derivative spectra of indinavir in the range 1585–1835 cm^{-1} at different temperatures. In this region second derivative spectra confirm the appearance of a new band at about 1790 cm^{-1} after exposing the drug to thermal radiation at elevated temperature as in original spectra (Fig. 5b). The band appears with less intensity at 125 °C and as the temperature increases to 150 °C it becomes more intense. This band at about 1790 cm^{-1} may be due to the formation of lactone C=O stretching and might be used as a band marker for the degradation of indinavir to form lactone derivative.

The results suggest that indinavir sulphate starts degrading slightly at about 125 °C and undergoes amide bond hydrolysis to give lactone derivative and aminoindanol at 150 °C. The results of DSC, TGA and XRD measurements also support the results of infrared spectroscopy. The degradation products can also be easily identified from infrared spectra of the drug.

4. Conclusions

The present study evaluates the thermal stability of indinavir sulphate under stress condition of heat by diffuse reflectance infrared Fourier transformed spectroscopy (DRIFT). The DRIFT spectra of intact indinavir sulphate was analyzed and compared with the spectra of the drug after exposure to thermal radiation at different temperatures. The spectra reveal that there is no change in the spectra up to 100 °C. The changes in the spectra start after exposing the drug to 125 °C and it change significantly at 150 °C. The DRIFT spectra show that indinavir sulphate undergoes degradation at 150 °C to give lactone derivative and amino indanol. The strong absorption bands at about 1790 cm^{-1} due to lactone C=O stretching and two bands in the region 1160–1370 cm^{-1} due to lactone and alcohol C–O stretching could clearly distinguish the intact indinavir and its degradation products. The results of DSC, TGA and XRD confirm the results of infrared spectroscopy. This study would be carried out further for evaluating the stability of indinavir sulphate under other stress conditions defined by ICH such as oxidation, acid and alkali hydrolysis and photodegradation.

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