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Stress degradation studies of nelfinavir mesylate by Fourier transform infrared spectroscopy

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ABSTRACT

Nelfinavir mesylate is the first nonpeptidic protease inhibitor available in pediatric formulation. In the present paper the stability of nelfinavir mesylate under different stress conditions is evaluated using Fourier transform infrared spectroscopy. The drug is subjected to thermal degradation, photodegradation, acid hydrolysis, base hydrolysis and oxidation as per ICH guidelines. Differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), X-ray diffraction (XRD) and high performance liquid chromatography (HPLC) are carried out to support the implementation of infrared spectroscopy for the stability studies of nelfinavir mesylate. Significant changes are observed in the IR spectra collected after exposing the drug to thermal radiations, acid and base hydrolysis and oxidative degradation. No change is observed in the spectra of the drug after exposing it to sunlight indicating the good photostability of nelfinavir mesylate. The results of infrared spectroscopy agree well with that of other complementary techniques as DSC, TGA, XRD and HPLC.

1. Introduction

Nelfinavir mesylate (mol. formula $C_{32}H_{45}N_3O_4S{\cdot}CH_4O_3S$) is the first nonpeptidic protease inhibitor available in pediatric formulation. The molecule contains 5-chiral carbon (Fig. 1) and the drug substance is presented as a single isomer. The chemical name of the nelfinavir mesylate is [3S-[2(2S*,3S*),3α,4aβ,8aβ]]-N-(1,1-dimethylethyl)decahydro-2-[2-hydroxy-3-[(3-hydroxy-2-methylbenzoyl)amino]-4-(phenylthio)butyl]-3-isoquinoline carboxamide monomethanesulphonate (salt). The HIV protease is an enzyme required for the proteolytic cleavage of the viral polyprotien precursors found in infectious HIV. Nelfinavir reversibly binds to the active site of the HIV protease and prevents it from cleaving the *gag-pol* polyprotien resulting in the formation of immature noninfectious viral particles [1].

Several reports are available on the simultaneous determination of nelfinavir mesylate with other protease inhibitors and nonnucleoside reverse transcriptase inhibitors using high performance liquid chromatography (HPLC) [2–8], ion pair HPLC [9], liquid chromatography–mass spectroscopy (LC–MS) [10] and HPLC–MS [11]. The determination of nelfinavir in bulk drugs and dosage form has been reported by some researchers [12–14]. Nelfinavir mesylate was determined in the presence of its degradation products using high performance thin layer chromatography (HPTLC) [12]. Another stability indicating HPLC method was reported for the determination of nelfinavir mesylate as bulk drug and in pharmaceutical dosage form [13]. Spectrophotometry [14,15], ultraviolet (UV) spectrophotometry [16], and potentiometry [17] methods have been described for the determination of nelfinavir mesylate. An evaluation of the liquid chromatography based international pharmacopeia's method for the nelfinavir mesylate purity control was published by Yekkala et al. [18]. A reverse phase (RP) HPLC method has been described for the determination of nelfinavir mesylate in tablet dosage form [19]. More recently Seshachalam et al. [20] developed a stability indicating RP-LC method for the determination of nelfinavir mesylate and its related impurities in drug substances and pharmaceutical formulations.

Stress testing provides information about degradation mechanisms and potential degradation products. This information can be used to develop manufacturing processes or to select proper packaging. It may also help in preparing reference material for identified degradation products. There is a need to develop a simple, fast and accurate method to determine the stability of drug substances under different stress conditions. Susceptibility to oxidation, acidic hydrolysis, base hydrolysis, thermal stability and photostability are some of the important stress conditions defined by ICH.

In the present scenario infrared spectroscopic techniques are becoming increasingly important and popular in pharmaceutical industries because they are nondestructive in nature, can be applied

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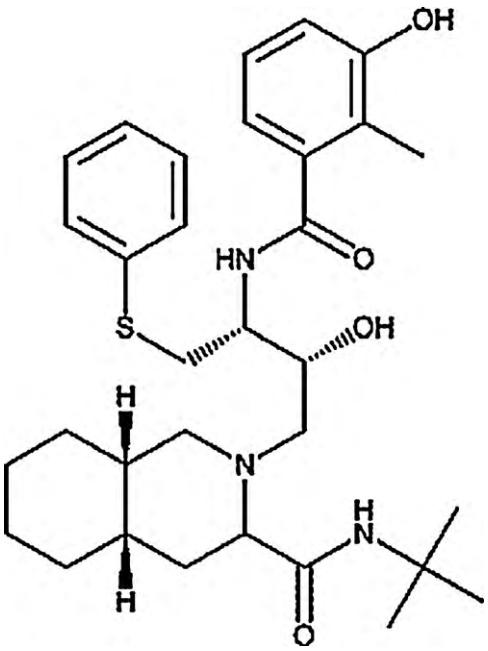

Fig. 1. Chemical structure of nelfinavir mesylate.

online and have the potential to provide rapid and convenient solutions to routine analytical problems. Moreover, it needs very little sample preparation and does not require hazardous organic solvents as is necessary in the most widely used HPLC method. Infrared spectroscopy has been used successfully by several researchers to evaluate the photostability of drugs. Photostability of nicardipine hydrochloride was determined by Fourier transform infrared spectroscopy [21]. Fourier transform-reflection–absorption spectroscopy was used to evaluate the photostability of carbamazepine polymorphs [22] and nifedipine [23]. Physical and thermal characterization of chiral omeprazole sodium salts was reported using diffuse reflectance infrared spectroscopy [24]. In previous publications we have reported the thermal stability studies of indinavir sulphate [25] and 5-fluorouracil [26] using diffuse reflectance infrared spectroscopy. Masmoudi et al. [27] reported the utilization of FTIR spectroscopy in stability study of cosmetic or pharmaceutical oil in water emulsions. The photolysis and thermolysis of four 1,5-diaryl-3-methyl-pentazadiene compounds in KBr matrix have been investigated by infrared spectroscopy [28].

In the present study the stability of nelfinavir mesylate under different stress conditions is evaluated using Fourier transform infrared spectroscopy. The drug is subjected to acid hydrolysis, base hydrolysis, oxidation and thermal degradation. Differential scanning calorimetry (DSC), thermo gravimetric analysis (TGA), X-ray diffraction (XRD) and high performance liquid chromatography (HPLC) are carried out to support the implementation of infrared spectroscopy for the stability studies of nelfinavir under different stress conditions.

2. Experimental

2.1. Materials

Nelfinavir mesylate used in the study was procured from Cipla Pharmaceuticals Ltd., India. Spectroscopy grade potassium bromide (KBr) was obtained from BDH Laboratory Suppliers, England. Methanol and acetonitrile used in the study were of HPLC grade and procured from Qualigens Fine Chemicals, India. HPLC grade potassium phosphate was obtained from Qualigens Fine Chemicals, India. Water used for HPLC analysis was filtered through Milli Pore water purification system. All other reagents were of analytical grade and used without further purification.

2.2. Stress degradation of nelfinavir mesylate

For stress degradation studies the drug substance, nelfinavir mesylate was subjected to thermal degradation, photodegradation, acid degradation, base degradation and oxidative degradation. Thermal and photodegradation studies were performed in solid state. For acid, base and oxidative degradation the methanolic solution of the drug was prepared at the concentration level of 1 mg/ml. The details of the degradation studies performed are given below.

2.2.1. Thermal degradation studies

Linkam TP 92, HFS 91/hot stage plate with platinum resistor was used for thermal degradation of the drug. The drug powder in a small aluminum dish was kept on the silver block on the hot stage. During the thermal treatment the drug powder was held on the silver block of the hot stage in a small aluminum dish. The temperature of the hot stage was varied from 25 to 300 °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 30 min 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}.

2.2.2. Photodegradation studies

The photochemical stability of the drugs was studied by exposing the drug substance to direct sunlight for 7 days (from 11:00 to 17:00 h at about 42 °C, total 42 h) by keeping the drug substance at terrace.

2.2.3. Acid and base degradation studies

For preparing acid and base induced degradation products, 5 ml of HCl and 5 ml of NaOH were added separately to 15 ml of methanolic solution of the drug. The solution was then refluxed at 70 °C for 1.0 h. The degradation conditions were controlled and optimized by varying the concentration of acid and base from 0.1 to 1N. Finally the concentration of acid and base was selected as 1N for this study. The solution was cooled to room temperature before further experiments.

2.2.4. Oxidative degradation studies

For optimizing the oxidative degradation conditions, to 15 ml of methanolic solution of the drug 5 ml of 5–30.0% (v/v) hydrogen peroxide was added. The solution was heated on a boiling water bath for 10 min to remove the excess of hydrogen peroxide. The solution was then refluxed for 30 min at 70 °C and cooled to room temperature before further experiments. 30% (v/v) hydrogen peroxide solution was finally chosen for the oxidative degradation of the drug.

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

A Perkin-Elmer Pyris 6 DSC was used for recording DSC thermogram of nelfinavir mesylate. About 5 mg sample of nelfinavir was weighed accurately using Perkin-Elmer 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 nelfinavir mesylate were 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.

2.4. Diffuse reflectance infrared Fourier transform spectroscopic (DRIFT) measurements

Thermal and photodegradation studies were carried out by diffuse reflectance Fourier transform infrared spectroscopy. DRIFT spectra of the drug powder before and after exposure to thermal and photo radiations were recorded in the range of 400–4000 cm^{-1}, using a Varian 660 Fourier transform infrared spectrophotometer operating with a Globar source, in combination with a KBr beam splitter and deuterated triglycine sulphate (DTGS) detector. The instrument was equipped with 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 were 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. The grounding of sample does not have any influence on the degradation of drug. Sample mixtures were then placed in a small sample cup and kept in the sample holder. The spectra were recorded with a resolution of 4 cm^{-1}. 128 scans were collected for each spectrum. Background spectra were obtained with ground KBr powder for each experimental condition.

2.5. Attenuated total reflectance (ATR) Fourier transform infrared spectroscopic (FTIR) measurements

Acid degradation, base degradation and oxidative degradation studies of nelfinavir mesylate were carried out by ATR-FTIR spectroscopy. ATR-FTIR spectroscopy has proven its applicability in various disciplines of chemical research. The advent and ease of use of ATR accessory have made it possible to analyze sample without extensive sample preparation. Attenuated total reflectance Fourier transform infrared spectroscopy was performed using the Varian 660 IR spectrometer equipped with KBr beam splitter and DTGS detector. The experimental setup utilized a PIKE MIRacle™ horizontal ATR accessory with a covered sample trough. The reflection element was a $\theta = 45°$ ZnSe crystal with multiple internal reflection. ATR spectra of methanolic solution of drug, acid degradation products, base degradation products and oxidative degradation products were acquired simply by pouring the solution on the crystal. Crystal was cleaned after analysis of each sample and a new background was taken before recommencing spectral analysis. All spectra were collected by averaging 128 scans with a resolution of 4 cm^{-1}.

2.6. 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/step. The XRD patterns of nelfinavir mesylate before thermal treatment and after exposing the drug to thermal radiation at 200 °C were recorded.

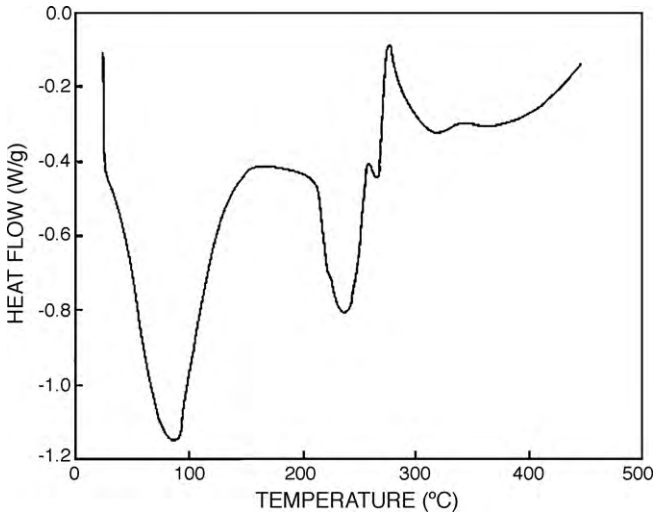

Fig. 2. DSC thermogram of nelfinavir mesylate.

2.7. High performance liquid chromatographic (HPLC) analysis

HPLC analysis was performed on a Shimadzu HPLC (UFLC, Prominence) equipped with a LC-20AD binary pump, a 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 controller by LC software. A Phenomenex C18 column (250 mm × 4.6 mm, 5 μm) was used for analysis. The mobile phase consisted of a mixture of 25 mM potassium phosphate buffer (pH 3.4 adjusted with phosphoric acid) and acetonitrile (60:40, v/v). The flow rate was maintained at 1.0 ml/min. The column temperature was maintained at 40 °C and the detector wavelength was set at 220 nm. The injection volume was 20 μl.

3. Results and discussion

3.1. Thermal degradation studies

3.1.1. Differential scanning calorimetry and thermogravimetric analysis

Fig. 2 shows the DSC thermogram of nelfinavir mesylate. The thermogram shows an endotherm at about 90 °C that may be due to the presence of some volatile materials/moisture in the sample. The endotherm with T_m at around 250 °C corresponds to the melting point of nelfinavir mesylate. There is an exothermic peak at about 280 °C in the thermogram of nelfinavir mesylate that may be due to the glass transition temperature (T_g) of the drug. DSC results show that nelfinavir mesylate gets degraded at about 250 °C.

DSC and TGA/DTA techniques are complementary to each other. In TGA the change in sample mass is measured by a thermobalance as a function of temperature. Fig. 3 shows the TGA/DTA curves of nelfinavir mesylate. It is evident from the thermal curve that the onset of thermal degradation starts at about 250 °C. The major weight loss transition occurs between 250 and 300 °C. The main weight loss step in TGA coincides with the exothermic decomposition peak in the DSC curve. DTA curve also confirm the similar results.

3.1.2. Diffuse reflectance infrared spectroscopy

Fig. 4 shows the DRIFT spectra of nelfinavir mesylate in the region 450–3400 cm^{-1}. The band at about 3083 cm^{-1} may be due to

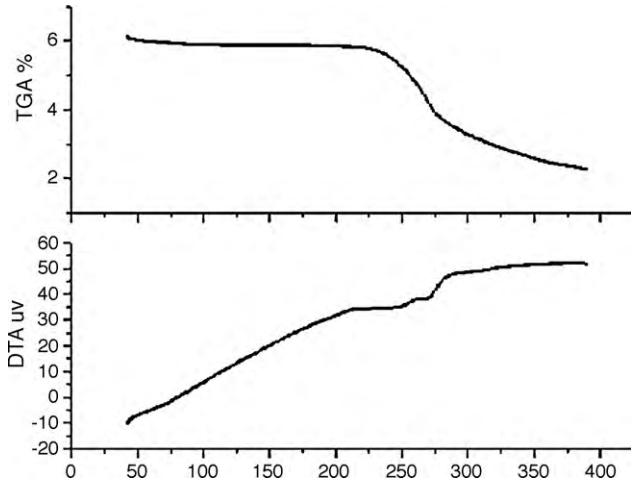

Fig. 3. TGA/DTA curve of nelfinavir mesylate.

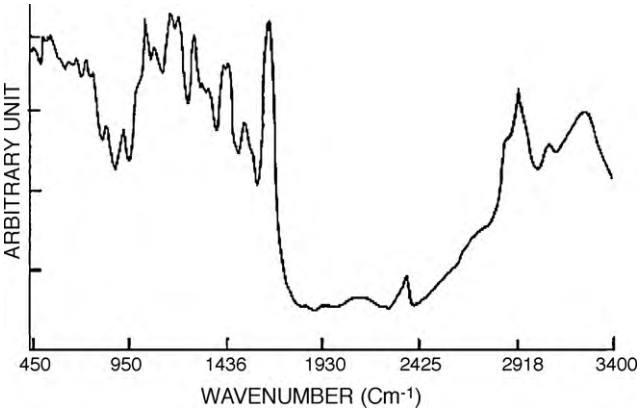

Fig. 4. DRIFT spectra of intact nelfinavir mesylate.

aromatic –CH stretching. The absorption band at about 2931 cm^{-1} may be attributed to –CH stretching. C=O stretching vibration frequencies appears at about 1720 cm^{-1} [29]. There is an absorption band at about 1437 cm^{-1} that may be assigned to N–H bending vibrations [29]. The absorption band at about 1165 cm^{-1} may be corresponds to –COCN stretching. C–O stretching vibration appears at about 1043 cm^{-1}. There is an absorption band at about 930 cm^{-1} which may be attributed to =CH out of plane deformations. An absorption band appears at about 692 cm^{-1} which may be assigned to C–S stretching [29]. The band at about 553 cm^{-1} may be due to aromatic ring deformations. The characteristic absorption bands of nelfinavir mesylate are listed in Table 1.

The overlaid DRIFT spectra of nelfinavir mesylate in different wavelength regions collected after exposing the drug to thermal radiations at different temperature are presented in Fig. 5a and b. It

Table 1
Characteristics infrared absorption bands of nelfinavir mesylate.

Frequency (cm^{-1})	Vibrational assignments
3083	Aromatic –CH stretching
2931	Antisymmetric –CH stretching
1720	C=O stretching
1437	–NH bending
1165	–COCN stretching
1043	C–O stretching
930	=CH out of plane deformations
692	C–S stretching
553	Aromatic ring deformations

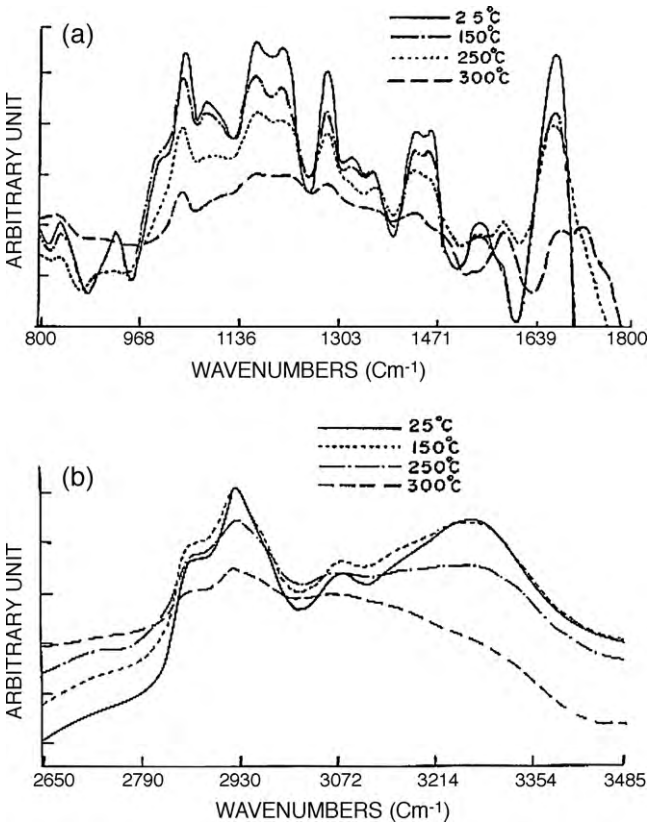

Fig. 5. Overlaid DRIFT spectra of nelfinavir mesylate at different temperatures in the region of (a) 800–1800 cm^{-1} and (b) 2650–3485 cm^{-1}.

is clear from the spectra that up to 150 °C the drug remains stable as no characteristic difference is observed in the DRIFT spectra up to 150 °C. The spectra reveal that the drug starts degrading at 250 °C and degrade completely at 300 °C. All the characteristic absorption bands diminish significantly at 250 °C and almost disappear at 300 °C. The absorption band at about 3083 cm^{-1} which is due to aromatic –CH stretching becomes less intense at 250 °C and disappears at 300 °C. The band at about 2930 cm^{-1} also becomes less intense at 250 °C and intensity is further decreased at 300 °C. It is an evidence of degradation of drug at elevated temperature. The absorption band at about 930 cm^{-1}, which is due to =C–H out of plane deformations, disappears in the spectra of the nelfinavir mesylate collected after exposing the drug to thermal radiations at 250 and 300 °C. The band at about 1043 cm^{-1} that is attributed to C–O stretching vibration also diminishes significantly at 250 and 300 °C. The absorption band at 1286 cm^{-1} becomes less intense at 250 °C and disappears in the spectra of the drug collected after thermal degradation at 300 °C. The C=O stretching vibration band which appears at about 1720 cm^{-1} in the spectra of intact drug get disappears in the spectra of drug at 300 °C. This band remains unchanged after exposing the drug to 250 °C. All other characteristic absorption bands also weaken significantly at discussed temperatures. The results confirm that the thermal break down of nelfinavir mesylate starts at 250 °C and degrades completely at 300 °C.

3.1.3. X-ray powder diffraction

The X-ray diffraction patterns of untreated and thermally degraded nelfinavir mesylate are shown in Fig. 6a and b respectively. In the X-ray diffraction pattern of untreated drug several peaks are observed. The crystallinity peaks decline in the XRD pattern of thermally treated drug at 250 °C (Fig. 6b). These results confirm that the drug starts degrading at 250 °C.

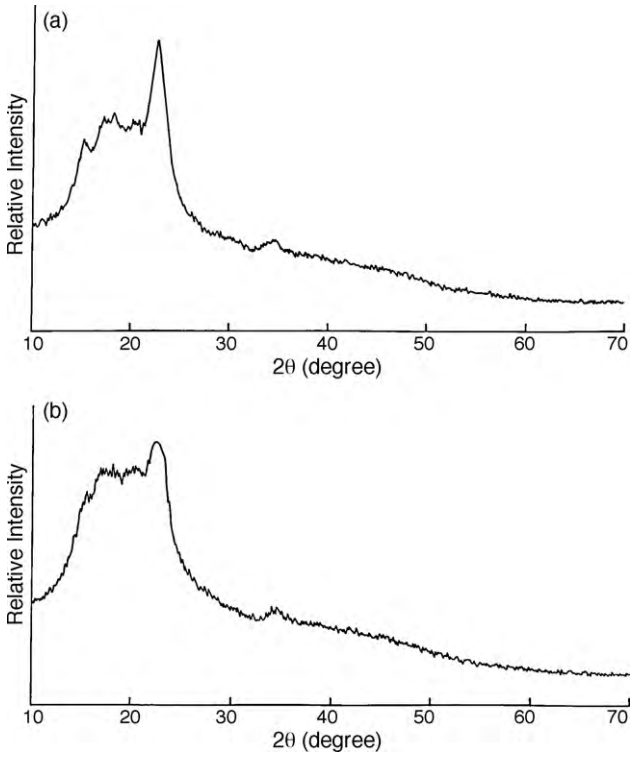

Fig. 6. XRD spectra of (a) intact nelfinavir mesylate and (b) thermal degraded nelfinavir mesylate

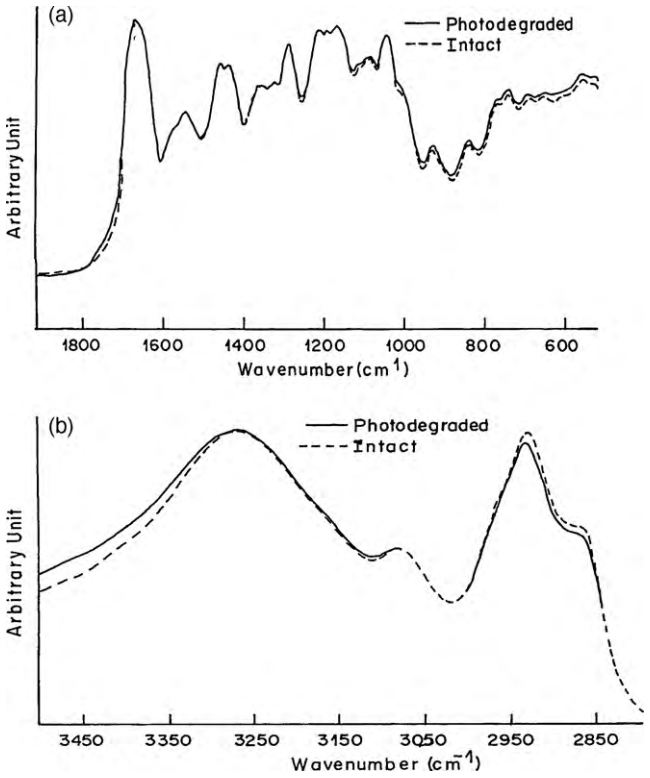

Fig. 8. Overlaid infrared spectra of nelfinavir mesylate after and before photodegradation in different wavelength regions

3.1.4. High performance liquid chromatographic (HPLC) analysis

HPLC chromatogram of intact nelfinavir mesylate is presented in Fig. 7a. It is depicted in the chromatogram that nelfinavir mesylate separates at a retention time of 12.2 min. The chromatogram of thermally degraded drug at 250 °C is shown in Fig. 7b. It shows several peaks in addition to that of nelfinavir mesylate. The additional peaks may be due to the formation of degradation products and impurities. It is clear from the HPLC results that nelfinavir mesylate undergoes degradation when exposed to thermal radiations at elevated temperatures.

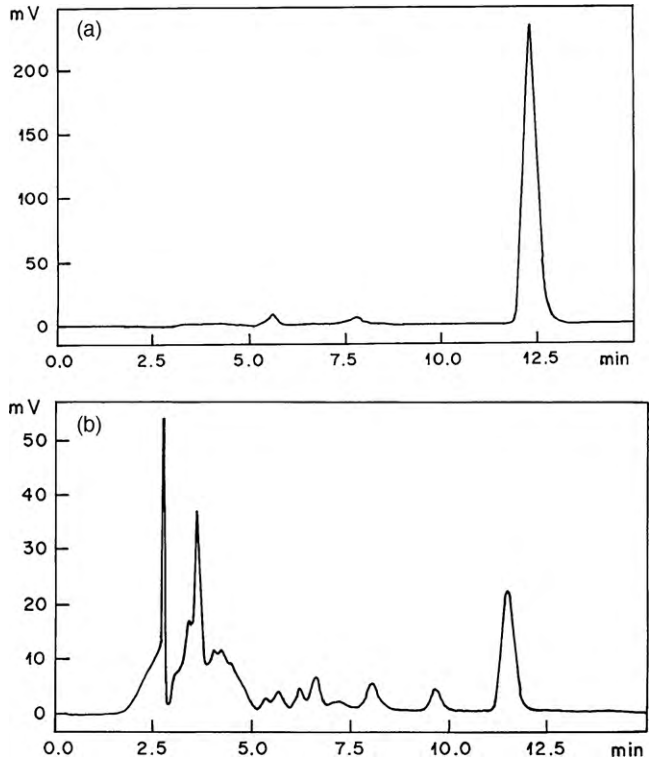

Fig. 7. HPLC chromatogram of (a) intact nelfinavir mesylate and (b) thermal degraded nelfinavir mesylate

3.2. Photodegradation studies

Fig. 8 shows the overlaid diffuse reflectance infrared spectra of nelfinavir mesylate before and after exposure to UV radiations. The spectra remain unchanged suggesting that the drug remains stable under stress condition of UV radiations. HPLC results also show that the retention time and peak area of nelfinavir mesylate remains unchanged and no significant degradation was observed upon photo exposure within the indicated period, suggesting that nelfinavir mesylate is photostable for 42 h. Further exposure is required to determine the photodegradation of nelfinavir mesylate.

3.3. Chemical degradation studies

3.3.1. ATR-FTIR spectroscopy

Fig. 9 shows the overlaid ATR-FTIR spectra of nelfinavir mesylate in different wavelength regions collected before and after exposing the drug to acidic condition. The spectra reveal the degradation of nelfinavir mesylate under acidic condition. Significant differences are observed in the IR spectra of acid degraded nelfinavir mesylate. The absorption band observed at about 3320 cm^{-1} in the IR spectra of intact nelfinavir mesylate weakens remarkably after acid degradation of the drug. The absorption bands at about 2930 and 2850 cm^{-1} become less intense in the spectra of acid degraded nelfinavir mesylate. The absorption band observed at about 2050 cm^{-1} before acid degradation gets diminish significantly after acid degradation of the drug. The absorption band at

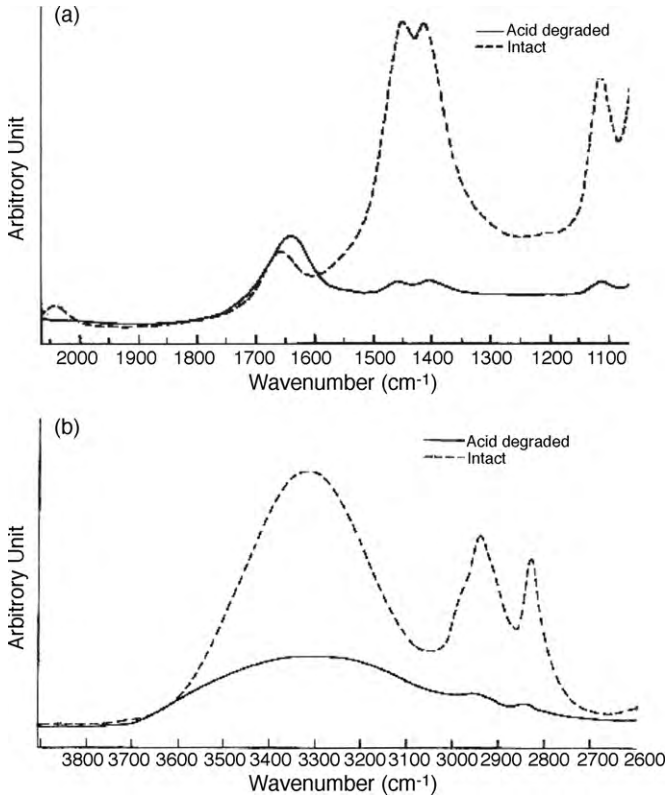

Fig. 9. Overlaid infrared spectra of nelfinavir mesylate before and after acid degradation in the region of (a) 2100–1100 cm^{-1} and (b) 3900–2600 cm^{-1}.

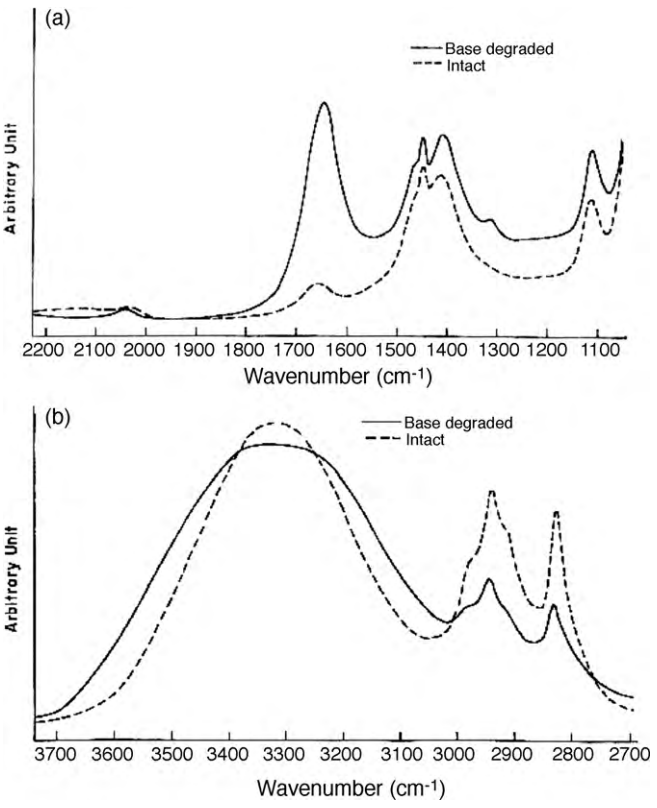

Fig. 10. Overlaid infrared spectra of nelfinavir mesylate before and after base degradation in the region of (a) 2200–1100 cm^{-1} and (b) 3700–2700 cm^{-1}.

about 1639 cm^{-1} becomes more intense in the IR spectra of acid degraded drug. It may be due to the formation of degradation products. The differences are also observed at 1458 and 1110 cm^{-1}, both the absorption bands become less intense in the IR spectra of the drug collected after exposing the drug to stress condition of acidic hydrolysis.

Similar changes are observed in the IR spectra of the drug collected after exposure to basic condition as is evident from the overlaid ATR-FTIR spectra of nelfinavir mesylate before and after basic hydrolysis (Fig. 10). The results suggest that nelfinavir mesylate undergoes degradation when exposed to stress conditions of acidic and basic hydrolyses.

The overlaid IR spectra of nelfinavir mesylate before and after oxidation are presented in Fig. 11. Noticeable changes are observed in the spectra after oxidative degradation. The absorption band at about 3320 cm^{-1}, which is due to aromatic –CH stretching, shifts towards lower wavenumber. The bands at about 2930 and 2830 cm^{-1} attributed to –CH stretching frequency almost disappears in the IR spectra of drug after exposure to oxidative condition. It may be due to the degradation of drug on oxidation. The absorption band observed at about 2050 cm^{-1} in the IR spectra of intact nelfinavir mesylate disappears after oxidation. The absorption band at about 1639 cm^{-1} becomes more intense in the spectra of degraded drug. The band observed at about 1458 cm^{-1} due to –NH bending vibrations shifts towards lower wavenumber after oxidative degradation of the drug. The C–O stretching vibration observed at about 1110 cm^{-1} in the spectra of intact nelfinavir mesylate get disappears in the spectra of degraded drug.

It is clear from the results that changes in the IR spectra of nelfinavir mesylate after exposure to stress conditions of acid, base and oxidative degradation are quite similar. It suggests that the drug undergoes degradation through similar pathway under acidic hydrolysis, basic hydrolysis and oxidation. The changes in absorp-

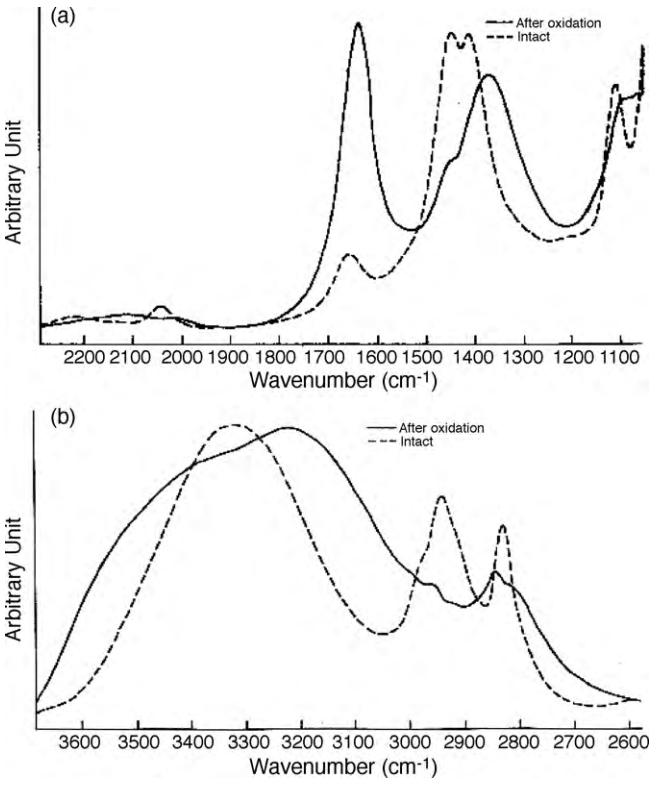

Fig. 11. Overlaid infrared spectra of nelfinavir mesylate before and after oxidation in the region of (a) 2100–1100 cm^{-1} and (b) 3900–2600 cm^{-1}.

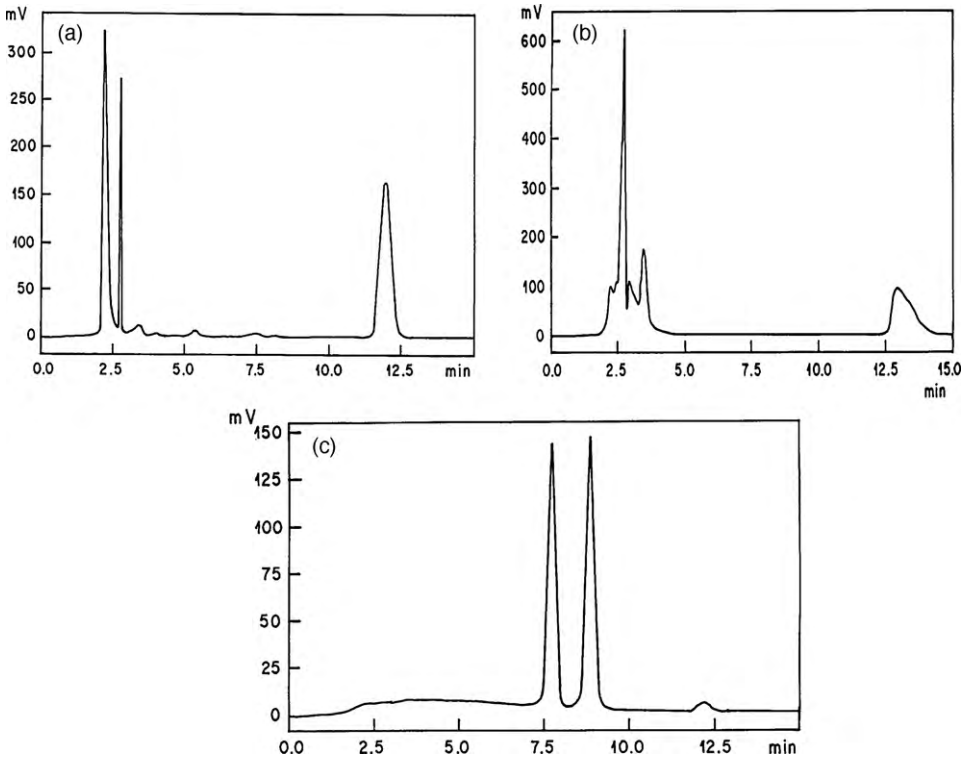

Fig. 12. HPLC chromatogram of (a) acid degraded nelfinavir mesylate, (b) base degraded nelfinavir mesylate and (c) oxidative degraded nelfinavir mesylate.

tion bands may be used as marker bands for the degradation of nelfinavir mesylate under different stress conditions.

3.3.2. High performance liquid chromatographic (HPLC) analysis

The representative chromatograms obtained after acidic degradation, basic degradation and oxidative degradation of nelfinavir mesylate are shown in Fig. 12a–c, respectively. The chromatograms show some additional peaks other than nelfinavir mesylate. The additional peaks may be due to the degradation products of nelfinavir mesylate. This confirms the degradation of nelfinavir mesylate under stress conditions of acid, base and oxidation that is also clear from the FTIR analysis.

4. Conclusions

The present study evaluates the stability of nelfinavir mesylate under different stress conditions using Fourier transform infrared spectroscopy. The infrared spectra of intact nelfinavir mesylate were analyzed and compared with the spectra of the drug after exposure to different stress conditions. The spectra suggest the degradation of nelfinavir mesylate under stress condition of heat, acid hydrolysis, base hydrolysis and oxidation. Spectra reveal that the drug remains photostable when exposed to sunlight for 42 h as no change is observed in the spectra. The results of infrared spectroscopy agree well with that of other complementary techniques as DSC, TGA, XRD and HPLC. This study supports the feasibility of infrared spectroscopic technique to evaluate the stability of nelfinavir mesylate under different stress conditions.

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