Research Article

Drug Testing and Analysis

Received: 4 April 2009 Revised: 21 May 2009 Accepted: 21 May 2009 Published online in Wiley Interscience: 15 July 2009

(www.drugtestinganalysis.com) DOI 10.1002/dta.41

Thermal stability studies of 5-fluorouracil using diffuse reflectance infrared spectroscopy

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5-Fluorouracil is one of the oldest chemotherapy drugs and it has been in use for decades. It is an active medicine against several types of cancer and effectively blocks the replication of DNA viruses. The present study assessed the potential of diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy to determine the thermal stability of 5-fluorouracil. Infrared spectra of the drug before and after exposure to thermal radiation at different temperatures were collected in diffuse reflectance mode using a Fourier transform infrared (FTIR) spectrophotometer. Differential scanning calorimetry (DSC) and X-ray diffraction (XRD) analysis were carried out simultaneously to confirm and support the results of infrared spectroscopy. The DRIFT spectra reveal that the drug shows good thermal stability up to 275 °C and undergoes complete thermal breakdown at about 285 °C. The results of DSC and XRD analysis also give the same information, which support the implementation of diffuse reflectance infrared spectroscopy for the determination of thermal stability of 5-fluorouracil. **Copyright © 2009 John Wiley & Sons, Ltd.**

Keywords: 5-fluorouracil; antimetabolites; diffuse reflectance; ICH; infrared spectroscopy; thermal stability

Introduction

5-Fluorouracil (Figure 1) is one of the oldest and most commonly used chemotherapy drugs. It is a pyrimidine analogue, belonging to the family of drugs called antimetabolites. It is used as an active medicine against several types of cancers,[1,2] particularly of the colon, liver and stomach.[3–6] The drug effectively blocks the replication of DNA viruses.[7,8] 5-Fluorouracil is estimated in pharmaceutical dosage and biological fluids by several methods such as high-performance liquid chromatography (HPLC),[9–12] gas chromatography–mass spectrometry (GC-MS),[13] solid phase extraction,[14] liquid-liquid extraction,[15] liquid chromatography with tandem mass spectral detection (LC-MS/MS)[16] and capillary electrophoresis.[17]

Stability testing is mandatory to determine the inherent stability characteristics of drug substances in accordance with International Conference on Harmonization (ICH) parent drug stability test guidelines.[18] Such testing is an important part of the drug development process. The purpose of stability testing is to provide evidence on how the quality of drug substances varies with different environmental conditions such as temperature, humidity and light. It determines storage conditions and shelf life of the drugs. Temperature is an important factor that influences the stability of the drugs and heat treatment is one of the important conditions defined by ICH guidelines. There is an urgent need to develop a simple, fast and accurate method to evaluate the thermal stability of drugs. Various HPLC-based methods to indicate stability have been developed to evaluate the stability of 5-fluorouracil.[19,20] Although HPLC is considered as an established technology in pharmaceutical industries, it has some disadvantages like complicated system operation, high cost of consumables and generation of hazardous organic solvents.

Infrared spectroscopy is emerging as an indispensable analytical technique to evaluate the stability of pharmaceuticals. This technique overcomes many of the disadvantages related to HPLC, as it is easy to operate, needs a very little sample preparation and does not require hazardous organic solvents. Infrared spectroscopy has been used successfully to evaluate the stability of drugs by several researchers. Most of the reported methods have focused on photostability studies of different drugs using Fourier-transformed infrared spectroscopy.[21–23] To the best of our knowledge, very little attention has been paid to thermal stability studies of pharmaceuticals using infrared spectroscopy. There are only a few reports concerning the use of infrared spectroscopy for thermal characterization[24,25] and thermal stability studies of pharmaceuticals.[26]

The aim of the present study was to assess the feasibility of diffuse reflectance infrared spectroscopy to evaluate the thermal stability of 5-fluorouracil. Differential scanning calorimetry (DSC) and X-ray diffraction (XRD) were used as complementary techniques to implement and assist in the interpretation of infrared spectroscopy results. Infrared spectroscopy has advantages over DSC and XRD by being nondestructive and noninvasive. The products after degradation can also be characterized using infrared spectroscopy.

Materials and Methods

Materials

5-Fluorouracil was generously supplied by Biochem Pharmaceutical Industries Limited, Daman, India as a gift sample. The potassium bromide (KBr) used in this study was of spectroscopy grade and was procured from BDH Laboratory Suppliers, England. All other

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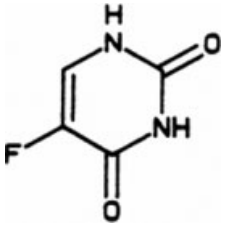

Figure 1. Chemical structure of 5-fluorouracil.

chemicals were of analytical reagent grade and used without further purification.

Thermal degradation

Linkam TP 92, HFS 91/hot stage plate with platinum resistor was used for thermal exposure of the drug sample. The drug powder was kept on the silver block of the hot stage in a small aluminium dish. During the thermal treatment the temperature of the hot stage was varied from 25 °C to 300 °C in 10 °C increments. A fresh sample was used at each temperature. A time period of one hour was selected for thermal exposure of the drug to allow the samples to degrade completely. After heating, the samples were allowed to cool down to room temperature before further experiments. The heating and cooling rate was maintained at 10 °C min^{-1}.

Diffuse reflectance infrared Fourier transform spectroscopic (DRIFT) measurements

Diffuse reflectance infrared Fourier transform (DRIFT) measurements were performed using a Bio Rad 175 C Fourier transform spectrophotometer accompanied with Globar source, KBr beam splitter and deuterated triglycine sulphate (DTGS) detector. The instrument was also equipped with Pike Technologies diffuse reflectance accessory. In diffuse reflectance spectroscopy, the sample to be analysed must be diluted with an infrared transmitting matrix so, after exposing the drug to thermal radiation at different temperatures, the samples 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 a high noise level. The samples were therefore ground well to a fine powder before measurements were made to make samples more homogenous and to increase relative reflectance coming out of the samples. Ground samples were then placed in a small sample cup and kept in the sample holder. The spectra were scanned between 4000 cm^{-1} and 400 cm^{-1} by averaging 128 scans for each spectrum with a resolution of 4 cm^{-1}. Each sample was scanned in triplicate to check the reproducibility. Ground spectroscopy grade KBr powder was used as background for each experimental condition. Unscrambler 9.1 software was used for spectroscopic data treatments. Smoothing of the spectra was done using the Savitzky-Golay method with 21 data points. After smoothing spectra were normalized and second order derivatization to compensate baseline shifting.

Differential scanning calorimetric (DSC) analysis

The thermogram of the original 5-fluorouracil was recorded on a Perkin Elmer Pyris 6 DSC. A sample of approximately 5 mg of the drug was accurately weighed using a Perkin Elmer Diamond TG/DTA microbalance. The weighed sample was heated from 30 °C to 350 °C at a heating rate of 10 °C min^{-1} in a closed aluminium pan under nitrogen purge gas flow of 40 ml min^{-1}. An empty aluminium pan was used as a reference. Reproducibility was checked by running the sample in triplicate.

Powder X-ray diffraction (XRD)

Powder X-ray diffraction patterns were collected at room temperature using a Bruker D8 advance diffractometer operating at the Cu Kα (1.54A°). The patterns were recorded in the range of 10°–70° 2θ at a step size of 0.025° 2θ for 1 s per step. The XRD pattern of original 5-fluorouracil before thermal exposure and after exposing the drug to thermal radiation at 275 °C and 285 °C was recorded.

Results and Discussion

Differential scanning calorimetry

Differential scanning calorimetry is a thermoanalytical technique in which the difference between the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature and time. It is used extensively in the pharmaceutical industries to determine the melting point, purity, glass transition temperature and thermal decomposition of drug substances.[27] The DSC thermogram of 5-fluorouracil is shown in Figure 2. It is evident from the thermogram that the drug shows good thermal stability up to its melting point. The onset melting peak of 5-fluorouracil is observed at about 283 °C. No other characteristic decomposition peak is observed in the DSC thermogram of 5-fluorouracil. This indicates that 5-fluorouracil remains stable up to 283 °C and undergoes degradation after that.

X-ray powder diffraction

The X-ray diffraction patterns of intact and after exposing the drug to thermal radiation at 285 °C are shown in Figures 3a and 3b respectively. In the X-ray diffraction spectrum of intact 5-fluorouracil, characteristic intense peaks are observed at diffraction angle of 2θ 16°, 19°, 22° and 29°. These peaks are due to its crystalline nature. Significant changes were not observed in the crystallinity peaks of the drug after exposing the drug to thermal radiation at 275 °C, which indicates that the drug is thermally stable up to this temperature. The crystallinity peaks

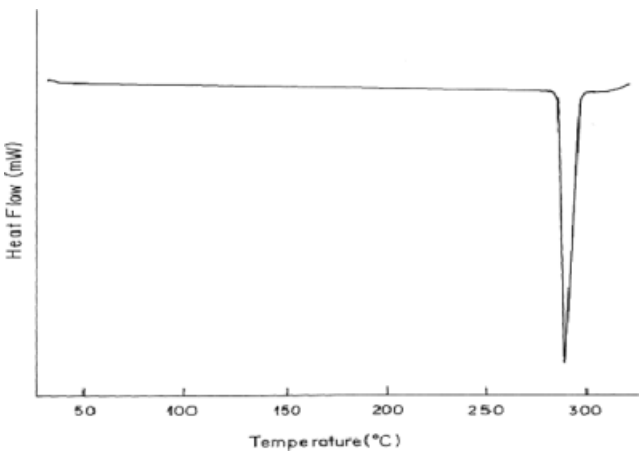

Figure 2. DSC thermogram of 5-fluorouracil.

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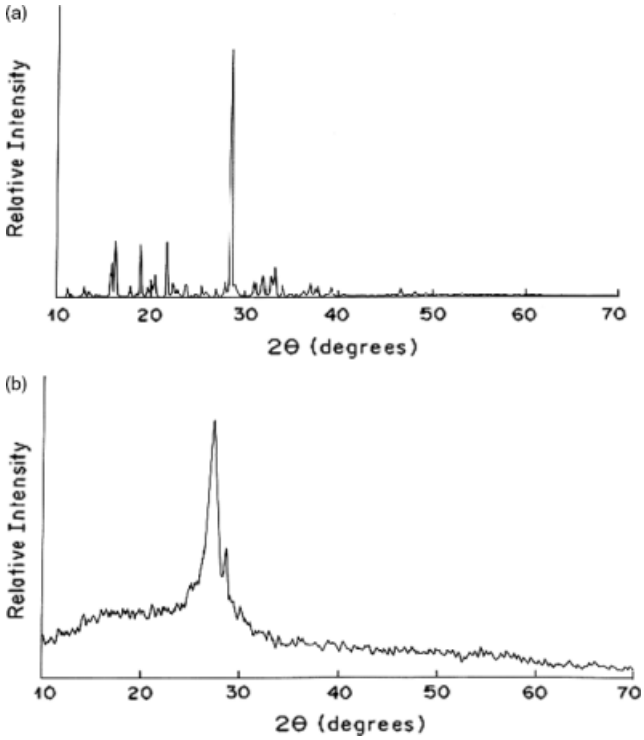

Figure 3. XRD spectra of (a) intact 5-fluorouracil; (b) degraded 5-fluorouracil.

diminished significantly after exposing the drug to thermal radiation at 285 °C as is clear from the XRD diffractogram of degraded drug. These results confirm that 5-fluorouracil remained thermally stable up to 275 °C and underwent decomposition at higher temperature.

Diffuse reflectance infrared spectroscopy

Figure 4 shows the overlaid DRIFT spectra of 5-fluorouracil in the region of 420–4000 cm^{-1}, collected after exposing the drug to thermal radiation at different temperatures. The characteristic absorption bands of intact 5-fluorouracil are summarized in Table 1. The medium intensity absorption band in the region 3100–3000 cm^{-1} represents = C–H stretching. The absorption bands at about 2938 cm^{-1} and 2831 cm^{-1} may be attributed to $-CH_2$. The drug shows bands in the region 1580–1650 cm^{-1} corresponding to the C=N and C=C ring stretching vibrations.[28] The absorption band at about 1724 cm^{-1} may be due to the stretching frequency of C=O group. The bands at about 1450 cm^{-1} and 1350 cm^{-1} are vibrations of the substituted pyrimidine compounds.[28] The absorption bands at 1180 cm^{-1} and 1251 cm^{-1} are assigned to the C–O and C–N vibrations respectively. The absorption band at about 1230 cm^{-1} is due to the fluorine atom on the ring.[29] The absorption bands are also observed in the region 820–550 cm^{-1}, which may be attributed to the C–F deformations.[28] Due to the bending of the ring halogen bond, aromatic fluoro compounds have a medium intensity band at about 420 cm^{-1},[28,29] which is observed in the spectrum of 5-fluorouracil (Figure 4).

It is evident from the overlaid spectra that up to 275 °C no remarkable changes are observed in the spectra, which confirms that the drug remains stable up to this temperature. Significant changes are observed in the spectra after exposing the drug at 285 °C as

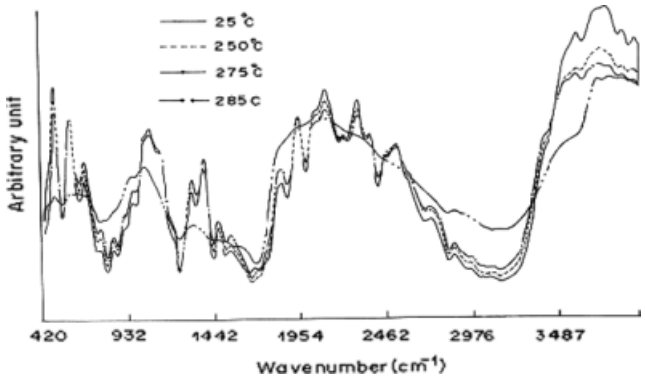

Figure 4. Overlaid DRIFT spectra of 5-fluorouracil at different temperatures in the region of 420–4000 cm^{-1}.

Table 1. Characteristics absorption bands of 5-fluorouracil at room temperature

Absorption bands (cm^{-1})	Assignments
3100–3000	=C–H stretching
2938–2831	$-CH_2$
1724	C=O stretching
1580–1650	C=N and C=C ring stretching vibrations in pyrimidines
1180 and 1251	Vibrations of C–O and C–N respectively
820–550	C–H deformations
420–375	Bending of ring halogen bands in aromatic fluoro compounds

is evident from Figure 4. All the characteristic absorption bands of intact 5-fluorouracil diminish significantly at this temperature, which reveals that the drug is no longer stable but is completely degraded. These changes can be related to those observed by DSC thermogram and X-ray diffraction pattern of the drug.

The analysis of thermal stability of 5-fluorouracil by infrared spectroscopy may be enhanced by computing the second derivative of the spectra. The second derivative of log (1/R) data with respect to wavelength enhances spectral features and also compensates for baseline shift. The Savitzky-Golay second order polynomial was used with 21 data points to obtain second derivative spectra. Figure 5 a-c shows the overlaid second derivative spectra of 5-fluorouracil in different wavelength region at different temperatures. The second derivative spectra further confirm that the drug shows good thermal stability up to 275 °C as no changes in the spectra are observed up to this temperature. Remarkable changes are observed in the second derivative spectra of the drug collected after exposing the drug to thermal radiation at 285 °C as in the original spectra. The shifting of the absorption band at about 500 cm^{-1} (C–F deformation) towards the lower wavenumber becomes rather clear in the second derivative spectra. The deterioration of all other characteristic bands becomes clear in the second derivative spectra, which again indicate the complete thermal breakdown of the drug at 285 °C. The results of infrared spectroscopy agree well with the DSC and XRD measurements, which support the use of diffuse reflectance infrared spectroscopy to assess the thermal stability of 5-fluorouracil.

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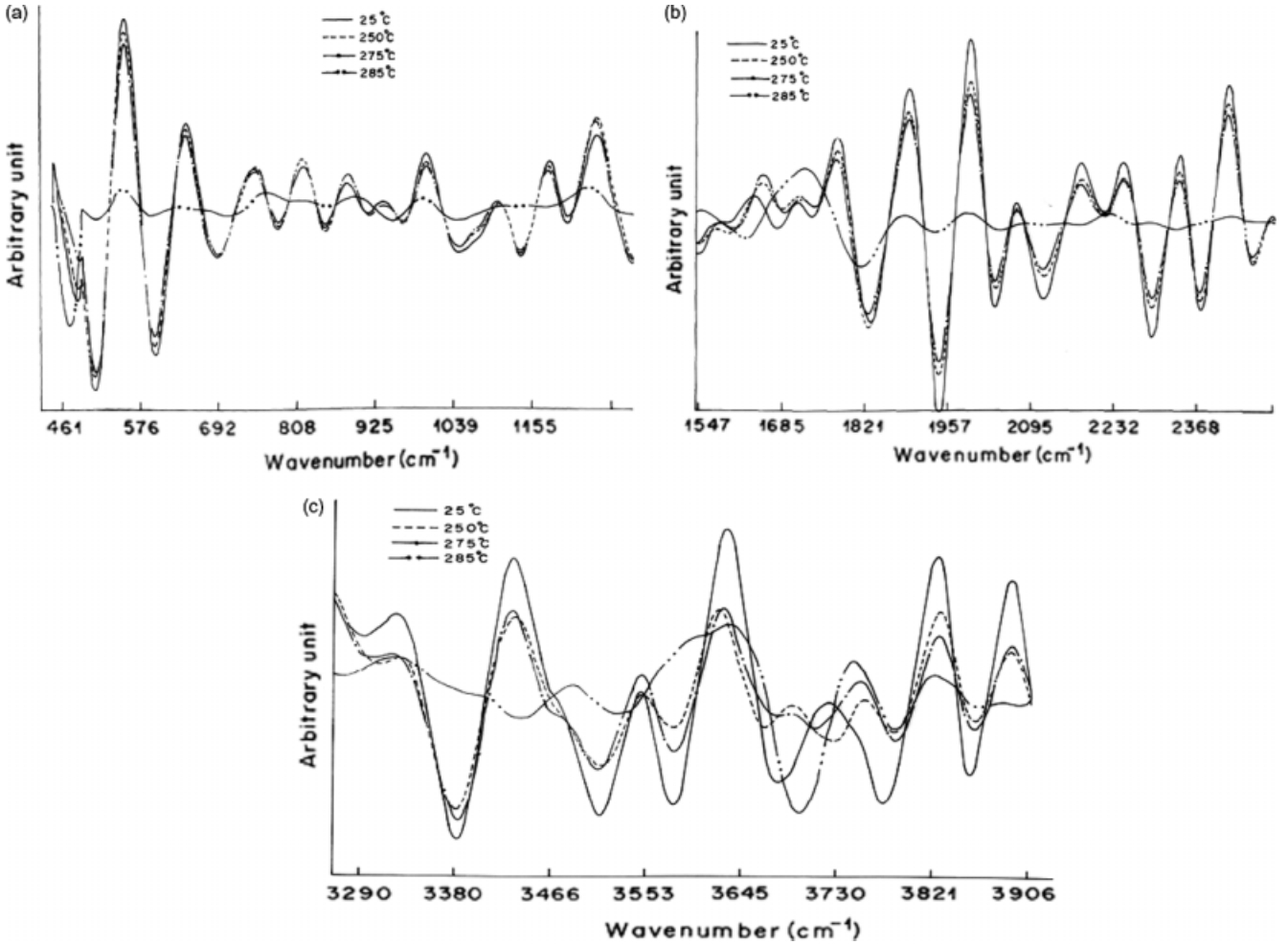

Figure 5. Overlaid second derivative spectra of 5-fluorouracil at different temperatures in the region of (a) 450–1250 cm^{-1}; (b) 1547–2450 cm^{-1}; (c) 3290–3906 cm^{-1}.

Conclusions

The present study assesses the feasibility of diffuse reflectance infrared spectroscopy to determine the thermal stability of 5-fluorouracil. The DRIFT spectra of intact 5-fluorouracil were analysed and compared with the spectra of the drug collected after exposing the drug to thermal radiation at different temperatures. It is evident from the spectra that the drug showed good thermal stability up to 275 °C and decomposed at about 285 °C. The results of DSC and XRD also confirm the results of infrared spectroscopy. The present study suggests that diffuse reflectance infrared spectroscopy might be used as a simple, rapid and reliable stability-indicating technique to assess the thermal stability of 5-fluorouracil. This study would be carried out further for evaluating the stability of 5-fluorouracil under other stress conditions defined by ICH.

Acknowledgements

The authors thank the Director, National Physical Laboratory, New Delhi, India for granting permission to publish the work. One of the authors, Parul Singh, is thankful to Indian Council of Medical Research (ICMR) for financial support as Senior Research Fellowship.

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