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Research Paper

Application of a novel combination of near-infrared spectroscopy and a humidity-controlled 96-well plate to the characterization of the polymorphism of imidafenacin

Hiroshi Uchida[a,b], Tokuji Yoshinaga[a], Hirotoshi Mori[a] and Makoto Otsuka[b]

[a]Kyorin Pharmaceutical Co. Ltd, Tochigi, Japan, and [b]Research Institute of Pharmaceutical Science, Musashino University, Nishi-Tokyo Japan

Abstract

Objectives This study aimed to apply a currently available chemometric near-infrared spectroscopy technique to the characterization of the polymorphic properties of drug candidates. The technique requires only small quantities of samples and is therefore applicable to drugs in the early stages of development.

Methods The combination of near-infrared spectroscopy and a patented 96-well plate divided into 32 individual, humidity-controlled, three-well compartments was used in the characterization of a hygroscopic drug, imidafenacin, which has two polymorphs and one pseudo-polymorph. Characterization was also conducted with powder X-ray diffraction and thermal analysis. The results were compared with those from routinely used conventional analyses.

Key findings Both the microanalysis and conventional analysis successfully characterised the substance (transformation and relative stability among the two polymorphs and a pseudo-polymorph) depending on the storage conditions. Near-infrared spectroscopic analyses utilizing a humidity-controlled 96-well plate required only small amounts of the sample for characterization under the various conditions of relative humidity.

Conclusions Near-infrared microanalysis can be applied to polymorphic studies of small quantities of a drug candidate. The results also suggest that the method will predict the behaviors of a hygroscopic candidate in solid pharmaceutical preparations at the early stages of drug development.

Keywords imidafenacin; microanalysis; near-infrared spectroscopy; polymorphic transformation; 96-well plate

Introduction

It is well known that many organic pharmaceutical compounds can exist in several different crystalline forms or solvates.[1] These polymorphs or pseudo-polymorphs exhibit individually distinct physicochemical properties (solubility, dissolution rate, stability, hygroscopicity, etc.) and distinct pharmaceutical properties (bioavailability, efficacy, degradation, toxicity). In particular, hygroscopicity can have a significant impact on the physical and chemical stability of a compound *per se*[2] and also on the manufacturing processes for its solid pharmaceutical products (wet-granulation, aqueous film-coating, spray-drying).

Hygroscopic compounds in a solid dosage form often come into contact with water stemming from manufacturing processes and, in some cases, may uptake moisture from the air during storage. Thus, such compounds may change their crystal form, resulting in a different polymorph or pseudo-polymorph. Such transformation can induce problems in the manufacturing process and/or in the properties of the pharmaceutical product. It is therefore important to compare and evaluate the physicochemical stabilities of the distinct crystal forms at various conditions of relative humidity. In particular, it is useful and desirable that the physicochemical stability of drug substances should be evaluated at an early stage of drug development, i.e. before planning of the solid dosage form, formulation and packaging.

At early development stages, however, the bulk amount of any drug candidate is generally limited because of the high costs of the synthesis of materials that may not be commercially

Correspondence: Makoto Otsuka, Research Institute of Pharmaceutical Science, Faculty of Pharmacy, Musashino University, 1–1-20, Shinmachi, Nishi-Tokyo 202–8585, Japan. E-mail: motsuka@musashino-u.ac.jp

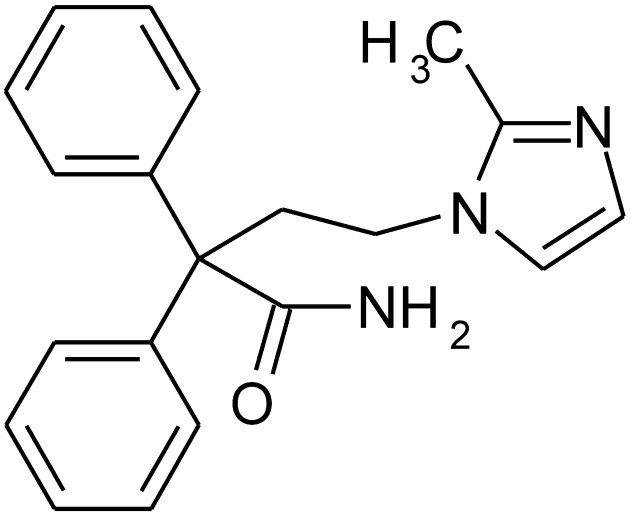

Figure 1 Chemical structure of imidafenacin.

available. It would be therefore valuable to develop a microanalytical method for the stability testing of the potential crystal forms of these substances that only required a very limited quantity to be available.

Traditionally, the stability of the crystal forms under various conditions has been monitored by polymorphic analysis with powder X-ray diffraction (XRD),[3] differential scanning calorimetry,[4] thermal gravimetric analysis (TG), microcalorimetry,[5] infrared spectroscopy[6] and dissolution kinetics.[7] However, these analyses are all time-consuming in terms of sample preparation and/or measurement and require relatively large amounts of the samples. In comparison with these conventional analyses, near-infrared (NIR) spectroscopy is simpler and more useful because it requires neither direct contact between a sample compound and a NIR probe[8] nor destruction of the sample. NIR spectroscopy is rapidly becoming an important technique for pharmaceutical analyses. For example, the chemometric NIR spectroscopic method [9] has been used to determine drug content,[10] drug stability,[11] the polymorphic content of pharmaceuticals,[12–16] the particle size of powders[17–22] and tablet mechanical strength.[23–27] In addition, NIR spectroscopy has proven to be one of the most suitable methods for the qualitative and quantitative evaluation of pseudo-polymorphs, especially water solvates (hydrates and anhydrates).[28–31]

We recently encountered polymorphic transformation (different crystalline forms) of imidafenacin (Figure 1), an orally active anticholinergic drug for the treatment of overactive bladder, which was under development in Japan.[32] In the present study, therefore, the stability and polymorphic transformation of this substance were evaluated by applying a novel microanalysis, that is, the combination of chemometric NIR spectroscopy and a humidity-controlled 96-well plate under various levels of relative humidity (RH). For comparison, the crystalline forms were also characterised by XRD, thermogravimetry and differential thermal analyses (TG/DTA), as well as hygroscopicity measurements. This microanalysis was found to be useful to characterise the physicochemical properties, including polymorphic transformation, of a drug substance in limited amounts stored under various RH levels.

Materials and Methods

Materials

Bulk powder of imidafenacin, 4-(2-methyl-1H-imidazol-1-yl)-2,2-diphenylbutanamide, was provided by Kyorin Pharmaceutical Co., Ltd (Tokyo, Japan) as Form I. All other chemicals were of analytical grade, and the water used was filtered through a Mill-Q Water Purification System (Millipore, Bedford, MA) prior to use.

Preparation of three crystalline forms of imidafenacin

Imidafenacin was dissolved in hydrochloric acid solution, and then the solution was neutralised with sodium hydroxide solution to obtain precipitated crystals. This crystal form was dried at 25°C and 22% RH for 48 h, yielding the sample powder B (form III) in a crystalline form. The sample powder A (form II) was prepared by drying the sample powder B (form III) at 60°C for 5 h.

X-ray powder diffraction

The XRD patterns were recorded using a θ/θ diffractometer (RINT-ULTIMA III, Rigaku Co., Tokyo, Japan) at 40 kV, 40 mA with a Cu-Kα source between 5 and 40° 2θ with a scan speed of 2.0°/min in the step scan mode.

Thermal gravimetric and differential thermal analysis

Thermal gravimetric and differential thermal analysis (TG/DTA) was performed using a thermogravimeter and differential thermal analyser (Thermo plus TG 8120, Rigaku Co., Tokyo, Japan). The samples (about 10 mg) were analysed in an open aluminum pan under nitrogen purge (100 ml/min) from 30 to 250°C at a heating rate of 5°C/min.

High-performance liquid chromatography analysis

The high-performance liquid chromatography (HPLC) analyses were performed using a Waters Alliance system (Waters, Milford, MA) equipped with an Inertsil ODS 3 V column (150 × 4.6 mm, 5 μm, GL Sciences Inc., Japan), where the column-oven temperature was maintained at 30°C. The separation flow was controlled by gradient using 0.1% (v/v) phosphoric acid solution (A, containing sodium 1-octanesulfonate), acetonitrile (B) and methanol (C) as the mobile phase at a flow rate of 1.0 ml/min. The gradient conditions of the mobile phase were as follows: phase A was decreased linearly from an initial 60 to 25%, phase B was increased linearly from an initial 25 to 60% during the first 40 min, and then phases A and B were flowed at the final combination (25 and 60%) for 10 min. Phase C was flowed at a constant rate of 15% throughout the analysis. The detection wavelength was set at 227 nm.

Hygroscopicity measurements

Conventional method

About 500 mg of sample powder A (form II) was placed in a 50-ml glass bottle and exposed to the atmosphere at 35°C and 69% RH. The RH value was controlled with a dessicator containing a saturated salt solution with a known corresponding RH value.[33]

Humidity-controlled 96-well plate method

A schematic diagram of the humidity-controlled 96-well plate is shown in Figure 2. The method for attaining and controlling target RH values in a humidity-controlled 96-well plate is described in Japanese Patent Kokai 2007–024562 (February 1, 2007). In brief, the quartz glass 96-well plate can be divided into 32 small compartments, each consisting of three wells. The sample powder and a saturated salt solution are placed in two different wells (Figure 2). The 96-well plate is then sealed up with a quartz glass plate and stored at a controlled temperature. The equilibrated RH values in the respective small compartments can be monitored using a capacitance changing-type humidity-sensor element TI-A (7×5 mm, 2 mm in thickness) through its transducer TA502 (Toplas Engineering Co., Tokyo, Japan). In our case, about 3 mg of each of the sample powders A (form II) was placed in one well of each compartment, with the saturated salt solution in a second. The sealed, humidity-controlled, 96-well quartz plate was then stored at 35°C for 300 min.

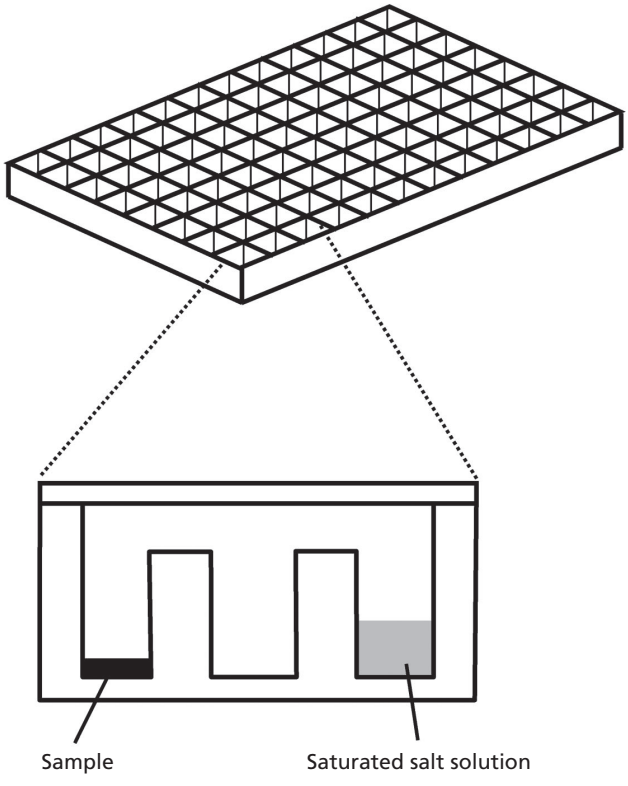

Figure 2 Schematic representation of a humidity-controlled quarts 96-well plate. The quartz glass 96-well plate is divided in 32 small compartments, each consisting of three wells. The sample powder and saturated salt solutions are placed in two different wells for storage under controlled levels of relative humidity, and the 96-well plate is sealed up with a quartz glass plate (Japan Patent Kokai No. 2007–024562).

Table 1 Recrystallization solvents and crystal forms

Solvent	Imidafenacin concentration	Crystal form
Ethanol (99.5%)	5000 mg/22 ml	I
Methanol	5000 mg/22 ml	I
Acetonitrile	5000 mg/200 ml	I
2-Propanol	5000 mg/50 ml	I
Acetone	5000 mg/400 ml	I
Ethyl acetate	5000 mg/450 ml	I
Chloroform	5000 mg/32 ml	I

Fourier transform near-infrared spectroscopy

Fourier transform reflectance NIR (FT-NIR) spectra were obtained using an NIR spectrometer (MPA; Bruker Optics, K.K., Tokyo, Japan). Each sample was scanned 16 times through a fibre-optic probe, and was averaged into one scan with 8 cm^{-1} resolution at reflectance mode in the 12500 to 4000 cm^{-1} frequency range. The fibre-optic probe was applied to the bottom of the sample-containing well of each compartment for recordings of scans in the 96-well quartz plate. Five spectra per sample were recorded at 0, 15, 30, 45, 60, 90, 120, 180, 240 and 300 min. The reference spectrum was obtained from an empty compartment (well) of the 96-well quartz plate. All spectra recorded were analysed using Pirouette software (Ver. 3.11, InfoMetrix Co., USA).

Results

Crystallisation solvents and subsequent crystal forms

Imidafenacin was crystallised from seven different solvents (Table 1). After excess imidafenacin was completely dissolved at around the boiling point of each solvent, the solution was cooled to room temperature overnight. No significant differences were observed in the HPLC impurities (<0.05%) of the seven crystallised materials. All of the crystals showed an identical XRD profile, which is also identical with that of form I (Table 1). When imidafenacin was dissolved in hydrochloric acid solution then recrystallised by neutralising with sodium hydroxide solution and dried at 25°C and 22% RH for 48 h, a new crystal was produced with an XRD profile different from that of form I (Figure 3a). This crystal was therefore defined as form III (Figure 3c). In addition, when form III was dried at 60°C for 5 h, another crystal was produced, again showing an XRD profile different from forms I and III. This crystal was defined as form II (Figure 3b).

Characterisation of three crystal forms of imidafenacin

The three crystalline forms (two polymorphs and one hydrate) of imidafenacin have different XRD patterns (Figure 3a, b and c). One polymorph, labelled form I, showed specific peaks at $2\theta = 6.6$, 9.4 and 19.0°. Another crystal prepared through recrystallisation of form I showed specific peaks at $2\theta = 16.7$,

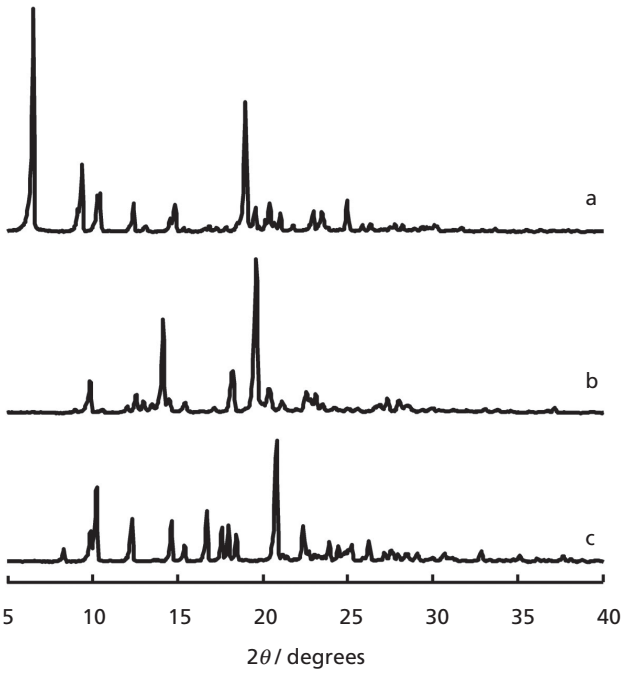

Figure 3 X-ray diffraction patterns of the different forms of imidafenacin. a, Form I; b, form II; c, form III.

20.8 and 22.4° and was defined as form III. A third crystal prepared by drying the form III showed another XRD pattern (b) different from those of forms I and III. The XRD pattern (b) had characteristic peaks at $2\theta = 14.1$ and 19.6°. Thus, the crystal was defined as form II.

Forms I, II and III of imidafenacin were differently characterised by TG/DTA as shown in Figure 4. While TG analysis from 30 to 250°C revealed no mass loss in either of forms I and II, analysis of form III yielded about 5.3% mass loss between 30 and 100°C. This loss corresponds to a stoichiometric drug/water ratio of 1 : 1 in form III. Form I showed only an endothermic peak at 191°C due to melting, while form II showed, besides an endothermic peak at 191°C due to melting, a small exothermic signal with its peak at 129°C. On the other hand, form III exhibited an endothermic signal with its peak at 65°C, a small exothermic peak with its peak at 127°C and then an endothermic peak due to melting at 191°C.

Novel hygroscopic characterisation by the combination of chemometric NIR spectroscopy and a humidity-controlled 96-well plate

Figure 5 shows the time-related changes in RH values controlled by known saturated salt solutions in the humidity-controlled 96-well plate. The 96-well plate was divided into separate compartments, each containing a saturated salt solution (Figure 2), which were then sealed up with quartz glass plates and stored at a controlled temperature. Each compartment reached an equilibrated RH state in about 20 min, a shorter time than under conventional methods, for example in a humidity-controlled desiccator.

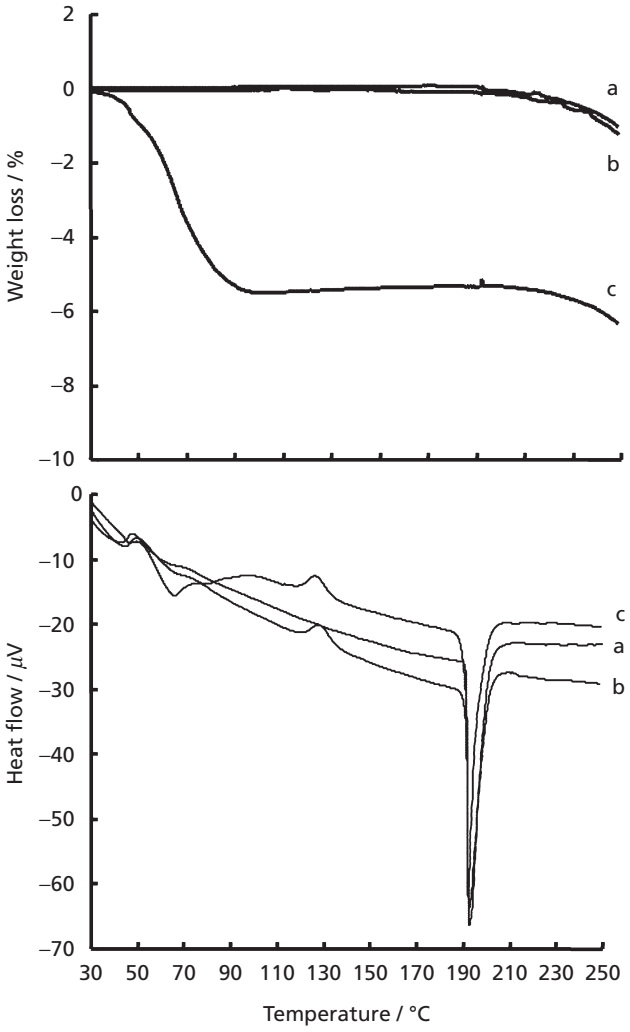

Figure 4 Thermal gravimetric and differential thermal analyses curves of the different forms of imidafenacin. a, Form I; b, form II; c, form III.

Figure 6 shows the NIR spectra of the three forms of imidafenacin. In particular, the NIR spectrum of form III shows the distinct absorbance characteristic of water (due to the combination of O–H stretching and bending vibrations) at around 5280 cm^{-1}, while form II demonstrated no absorption bands in this spectral region. Three other characteristic peaks of all these three NIR spectra were assigned to the second overtone C–H stretching of the benzene ring at 8750 cm^{-1}, the first overtone N–H stretching of $CONH_2$ at 7150 cm^{-1} and the first overtone C–H stretching of the benzene ring at 5940 cm^{-1}.[34] The NIR spectral patterns of the two crystal forms (forms I and II) of imidafenacin were different.

Figure 7 shows chronological changes in the raw NIR spectral patterns of form II stored at 69% RH in the 96-well plate. The baselines of the NIR spectra were shifted, and it was therefore difficult to identify time-dependent changes in any of the NIR spectral peaks.

To clarify the presence or absence of any time-dependent changes in any of the NIR spectral peaks, the raw NIR spectra

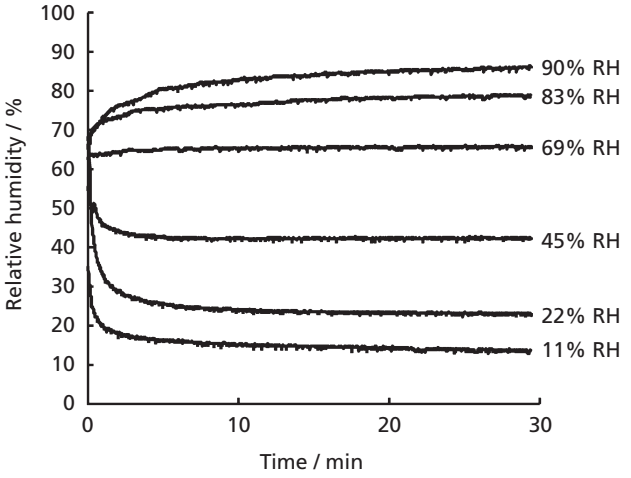

Figure 5 Changes in relative humidity values in the 96-well plate.

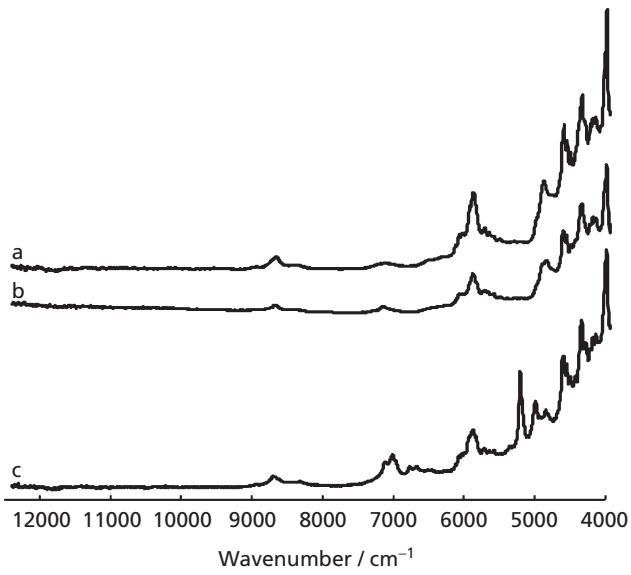

Figure 6 Near-infrared spectra of the different forms of imidafenacin. a, Form I; b, form II; c, form III.

were converted to the second-derivative NIR spectra. In the converted NIR spectra (Figure 8), the peak intensity at 5280 cm^{-1} associated with the O–H from water molecules decreased significantly with storage-time prolongation. Likewise, the peak intensities at 5060 and 4960 cm^{-1}, both associated with N–H asymmetric stretching of the amide II group (ν_{N-H} in amide II), changed time-dependently, with a central focus on a common isosbestic point.

To clarify time-related changes in some selected peaks, inverse logarithmic transformation was applied to the peak intensities from the second-derivative NIR spectra. Figure 9 shows individual changes in the peak intensities at 5280 cm^{-1} (O–H) (a) and 5060 cm^{-1} (ν_{N-H} in amide II) (b) from form II stored at 69% RH for 300 min in the 96-well plate. The peak intensity at 5280 cm^{-1} decreased rapidly at the earlier stage of storage, but remained almost constant from 180 min onwards.

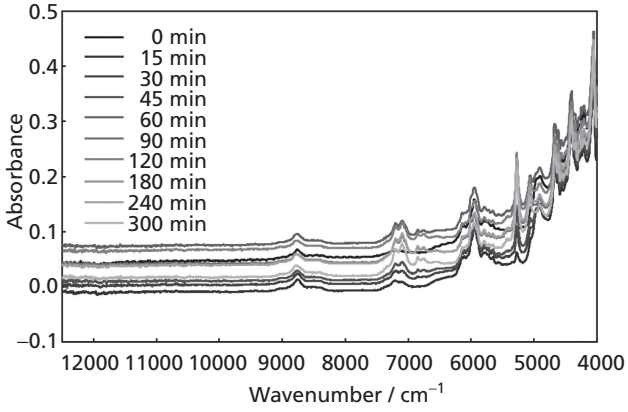

Figure 7 Near-infrared spectra of form II stored at 69% relative humidity for 300 min in a 96-well plate.

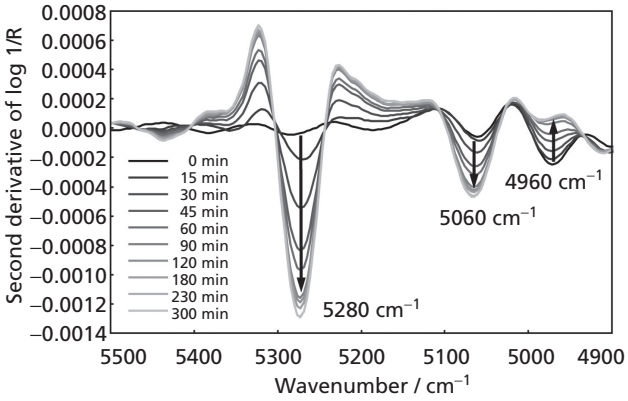

Figure 8 Second-derivative near-infrared spectra of form II stored at 69% relative humidity for 300 min in a 96-well plate.

Similarly, the peak intensity at 5060 cm^{-1} decreased rapidly at the earlier stage of storage, almost as quickly as that at 5280 cm^{-1}. The relationship between the peak intensities and the amide II group (Figure 9c) was obviously linear, and the peak intensity at 5280 cm^{-1} (O–H) increased in proportion to increases in the peak at 5060 cm^{-1} (N–H in the amide II group).

Discussion

All seven solvents for crystallization of imidafenacin yielded an identical crystalline form (form I). Recrystallization of imidafenacin from aqueous solution and subsequent drying at 25°C and 22% RH for 48 h yielded another crystal (form III). Further drying of form III at 60°C for 5 h resulted in formation of a third crystal (form II).

Results from the characterisation of the crystalline forms of imidafenacin by XRD indicate that imidafenacin can take at least three crystal forms (forms I, II and III) (Figure 3). These three crystal forms have different TG/DTA curves (Figure 4). The small exothermic signal on the DTA curve of form II is probably associated with transformation from form II to form I. The endothermic signal on the DTA curve of form

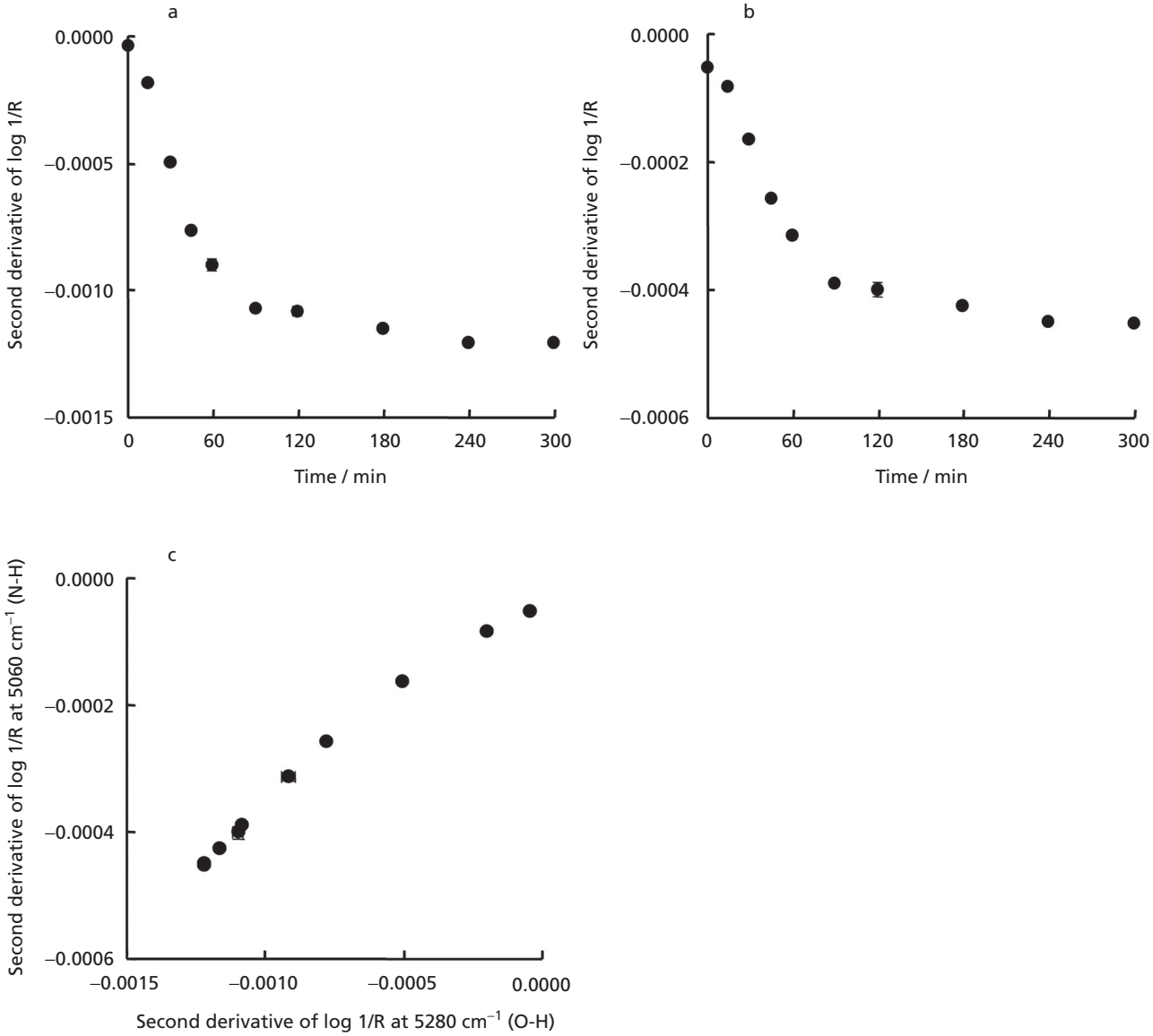

Figure 9 Changes in the water O-H and amide N-H peaks of the second-derivative near-infrared spectra of form II stored at 69% relative humidity for 300 min in a 96-well plate. (a) Peak at 5280 cm^{-1} from O-H of water; (b) peak at 5060 cm^{-1} from N-H of amide group; (c) relationship between the two peaks. Results represent mean ± SD, n = 5.

III is probably due to dehydration, resulting in form II. The subsequent small exothermic peak is probably attributable to thermal phase transformation from form II to form I; XRD analysis under heating conditions also confirmed this phase transformation (data not shown). In fact, when form II was stored under humid conditions created by the conventional method, it took up moisture rapidly and transformed into form III – this transformation was again confirmed by XRD analysis (data not shown). The different thermal behaviour of the three crystal forms suggests that form I is most stable, form II is metastable (forms I and II are considered true polymorphs) and form III is a monohydrate of free imidafenacin. These conventional analyses, however, were time-consuming in sample preparation and measurement and required relatively large amounts of the samples.

Chemometric NIR spectroscopy[9] is rapidly becoming an important technique and has many uses in the pharmaceutical industry, for example quantitative drug analysis,[10] drug stability,[11] polymorphic quantitification of pharmaceuticals,[12–16] particle size distribution[17–22] and tablet mechanical strength.[23–27].

In our present study, the combination of NIR spectroscopy and a humidity-controlled 96-well plate ('microanalysis') provided useful information about transformation between the different forms of imidafenacin. The NIR spectral patterns of the three crystal forms of imidafenacin were different (Figure 6), and this difference is considered to be due to the presence of polymorphs (including a pseudo-polymorph). The intensities of the NIR spectral peak associated with the O–H of water molecules and ν_{N-H} in amide II changed

with storage time (Figures 8 and 9). These results indicate that the more moisture (water) is absorbed, the more hydrogen-bond interaction develops between the O–H of the absorbed water and the N–H in amide II of the imidafenacin molecules. In other words, the findings suggest transformation of form II to form III. The relationship between the changes in the intensity of the peaks at 5280 cm^{-1} (O–H) and 5060 cm^{-1} (N–H in the amide II group) and time was obviously linear (Figure 9c). This result clearly suggests that the crystalline transformation of form II to form III corresponds to a hydration-based molecular interaction between the O–H of water and the N–H in amide II.

Hydrate formation of theophylline and caffeine, as model compounds, for example, has been well characterised using an NIR spectroscope as a single analytical tool.[16] The NIR spectrum detects a maximum associated with the O–H from water molecules absorbed and forming hydrates. Similarly, in the hydration of imidafenacin, the present microanalysis detected changes in the intensities of NIR spectral peak associated with the O–H from water molecules, as discussed above. This indicates that NIR spectroscopy, including the present microanalysis, can be applied to detect the hydration behaviour of active pharmaceutical ingredients. Thus, NIR spectroscopy offers a useful way of determining extent of hydration. The high sensitivity of NIR to water absorbance, however, may be one disadvantage of NIR because the strong O–H band may overlap with or hide other useful bands of interest. In such cases, NIR spectroscopy can be combined with Raman spectroscopy, which is less sensitive to water but more sensitive to the conformational and configurational rearrangement of the drug molecule, especially symmetric vibrations, such as C–C.

The present microanalysis confirms the transformation of imidafenacin that has been shown by conventional analyses and suggests the possibility of its application to the detection of hydration processes. Its use of only small quantities (of the order of milligrams) of samples makes this technique particularly relevant to drugs at the early phase of development.

NIR microanalysis is non-destructive of the samples, which can therefore be recovered, and can suggest detailed information about the transformation of polymorphs or pseudo-polymorphs. Once conventional instrumental analysis has indicated the presence of polymorphs or pseudo-polymorphs in a drug at an early stage of development, NIR can be applied to transformation analysis using a much smaller amount of a substance than a conventional analysis, for example using a humidity-controlled desiccator for the same purpose. Furthermore, NIR microanalysis has potential to evaluate quantitatively the crystal transformation of a polymorph, as NIR spectroscopy has been previously applied for such a purpose.

Conclusions

Imidafenacin was found to exist in at least three polymorphs (containing a pseudo-polymorph): forms I, II and III, as defined in the text. Analyses by XRD and TG/DTA suggest that form I is most stable, form II is metastable and form III is a monohydrate.

NIR spectroscopic analysis utilising a humidity-controlled 96-well plate requires only small amounts of the imidafenacin sample to evaluate the stability of the compound under the various conditions of relative humidity. The combination of reflectance NIR-chemometric analysis and this humidity-controlled 96-well plate was found to be useful to predict the polymorphic transformation of a drug substance in the solid pharmaceutical product. Thus, the present study indicates that NIR microanalysis can be applied effectively to polymorphism studies of drug candidates. It requires only small quantities of samples compared with conventional methods using glass bottles, suggesting that it is a particularly useful technique for drugs at the earlier stages of drug development.

Declarations

Conflict of interest

The authors declare that they have no conflicts of interest to disclose.

Funding

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