International Journal of Pharmaceutics 362 (2008) 29–36

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Quantitative determination of solid-state forms of a pharmaceutical development compound in drug substance and tablets

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ARTICLE INFO

Article history:
Received 14 February 2008
Received in revised form 20 May 2008
Accepted 29 May 2008
Available online 7 June 2008

Keywords:
Solid-state forms
Quantitation
Raman spectroscopy
Form stability
Tablet

ABSTRACT

Common analytical techniques including Raman, NIR, and XRD were evaluated for quantitative determination of three solid-state forms (amorphous, Form B and Form C) of a development compound. Raman spectroscopy was selected as the primary analytical technique with sufficient sensitivity to monitor and quantify the neat drug substance alone and in the drug product. A reliable multivariate curve resolution (MCR) method based on the second derivative Raman measurements of the three pure physical forms was developed and validated with 3.5% root mean square error of prediction (RMSEP) for Form B, which was selected as the preferred form for further development. A partial least squares (PLS) algorithm was also used for the multivariate calibration of both the NIR and Raman measurements. The long-term stability of Form B as a neat active pharmaceutical ingredient (API) and in a tablet formulation was quantitatively monitored under various stress conditions of temperature and moisture. Moisture, temperature, excipients and compression were found to have significant effects on the phase transition behavior of Form B.

1. Introduction

Physical forms of a drug substance may exhibit different physical and chemical properties. The differences caused by polymorphism may affect the pharmaceutical behavior including stability and bioavailability of the drug substance in the final drug product (FDA, 2007). When the polymorphism impacts drug product performance, it is required to establish method and criteria for the appropriate solid-state form in the final specifications for new drug substances and drug products (ICH, 2000).

Numerous methods have been used to characterize the solid-state forms of pharmaceuticals, including X-ray diffraction (XRD), thermal analysis (differential scanning calorimetry (DSC)), solid-state nuclear magnetic resonance (NMR) spectroscopy, vibrational spectroscopy (including infrared (IR), near infrared (NIR), and Raman), and optical microscopy. XRD is recognized as a powerful technique for the definitive identification of polymorphs. However, its use in quantitative determination of the amorphous and crystalline phases may be limited by the preferred orientation related peak intensity changes, as well as the difficulty of separating diffraction peaks of a single component (like the amorphous form) from a complex mixture (Shah et al., 2006). As alternatives, vibrational spectroscopic techniques such as Raman, IR and NIR, are very useful in obtaining both structural information and polymorphic quantitation (Stephenson et al., 2001).

Vibrational spectroscopic techniques are "fingerprinting" techniques for identification of given chemicals and materials (Smith and Carabatos-Nedelec, 2001). Infrared spectroscopy, including mid-IR and NIR technologies, has traditionally been heavily utilized in the chemical and pharmaceutical industries (Burns and Ciurczak, 2001; Kipouros et al., 2006; Roston et al., 1993). Recently, Raman spectroscopy has also gained popularity in different areas of the pharmaceutical industry (Kachrimanis et al., 2007; Strachan et al., 2007; Wartewig and Neubert, 2005). The main advantages of using Raman techniques in the analysis of pharmaceutical solids include: (1) flexible sampling approaches with minimum sample preparation; (2) minor interference of water compared with infrared techniques; and (3) large Raman scattering cross-sections for most APIs compared with aliphatic excipients (weak Raman scatterers) (Strachan et al., 2007).

Quantitative analysis of the solid-state form composition using vibrational spectroscopy is of much interest for APIs. Most work in the literature uses the gravimetric preparation of reference physical mixtures for the quantitative calibration. The multivariate techniques used mainly include principal component analysis (PCA) and PLS and were applied to the treatment of spectroscopic data collected from both reference physical mixtures and test samples. In recent years, vibrational spectroscopic technologies including Raman (Mazurek and Szostak, 2006), IR (Braga and Poppi, 2004), and NIR (Blanco and Villar, 2000) spectroscopy were

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doi:10.1016/j.ijpharm.2008.05.038

used for the quantitative determination of different APIs and their physical forms presented in the drug substance and product formulations. Poellaenen et al. used IR spectroscopy together with a PLS data processing technique to quantify different crystalline forms (Poellaenen et al., 2005). In this case, the quantification of polymorphic composition was based on the relative integrated XRPD peak intensity. However, this method cannot be directly applied to the quantitation of amorphous materials due to the lack of characteristic diffraction peaks. Kogermann et al. reported a qualitative in-situ analysis of solid-state form transition using Raman and NIR spectroscopy with PLS modeling, which was based on the spectroscopic data of pure physical forms (Kogermann et al., 2007). Their work provided useful information prior to quantitative analysis of those forms, and it could be more meaningful to generate a quantitative method based on the spectroscopic data of pure solid-state forms.

This paper describes the use of Raman spectroscopy to quantitatively analyze the solid-state forms of a development compound in neat drug substance and in tablets. XRD, NIR and DSC analyses were also performed on the neat drug substance as reference techniques. The development compound was found to exist as an amorphous form, and as two hydrate crystal forms (Form B and Form C). The two hydrate crystal forms can each undergo phase transition to the amorphous state upon dehydration under certain conditions, including elevated temperature, humidity and compression. The abundance of each polymorph under various conditions has been quantitatively determined based on MCR treatment of the Raman spectroscopic data of the three pure physical forms and test samples. The PLS-based analysis of the NIR and Raman spectroscopic data is also described as the secondary method for the purpose of evaluating the MCR-predicted results. In the present study, the results of the solid-state form quantitation help us gain a better understanding of the solid-state conversion behavior of the compound, as well as the stability of Form B during manufacturing, processing, and storage.

2. Materials and methods

2.1. Materials

The development compound was obtained from Amgen Inc. (Thousand Oaks, CA). The amorphous and Form B crystalline materials were used as received and each was confirmed by their XRD patterns. Form C crystalline material was prepared by a recrystallization step from an ethanol/water (50/50, v/v) slurry at 40 °C. Two sets of physical mixtures of all three forms were prepared gravimetrically with 16 different compositions covering a 0–100% range for all three forms. The weight of each mixture was about 30 mg. A wet mixing step was utilized to minimize the heterogeneity of the sample mixtures. Each mixture was first dispersed in hexane, in which the development compound has been shown to have very limited solubility, and then stirred for 5 min. The mixtures were then filtered and allowed to dry. All three phases were slurried separately in hexane and then characterized post-slurry by their Raman spectra to insure that phase conversion was minimal during the mixing process. A drug blend was prepared with 5.3% Form B crystalline material in a mixture of excipients. Placebo tablets were made to have the same proportions of excipients as within the active tablets which contain the same composition as the drug blend.

2.2. Raman measurements

Dispersive Raman spectra were collected using a FALCON II^{TM} molecular chemical imaging (MCI) system from ChemImage Corporation (Pittsburgh, PA). The excitation source is a Millennia IIi Laser Source (Spectra–Physics, Mountain View, CA) at 532 nm with a power of ca. 300 mW at the sample for the powder sample and ca. 100 mW at the sample for tablets. The effect of laser power on the stability of physical forms was excluded with no apparent spectral change after 2 h laser exposure. The Raman signal was collected using a 5× objective with a sample area of about 450 μm in diameter on a Model BX51 Olympus microscope platform (Center Valley, PA). A back illuminated CCD (Model: 1340× 100B XP, Princeton Instruments, Trenton, NJ) cooled at −70 °C was used to record the Raman signal after reflection from a 1200 g/mm grating in the spectrograph. Twenty measurements were performed for each sample, and a fresh sampling area was used for each replicate measurement.

2.3. NIR measurements

Reflectance NIR spectra were measured on a Nicolet Antaris NIR Analyzer (Thermo Electron. Corp., Waltham, MA). Each spectrum was an average of 10 scans at 4 cm^{-1} resolution over the range 10,000–4000 cm^{-1}. Twenty measurements were obtained for each sample, and sample vials were shaken and repositioned between replicate measurements.

2.4. XRD measurements

XRD analysis was carried out using a θ/θ diffractometer (X'pert MPD, Philips Analytical, Natick, MA) with Cu Kα radiation. All samples were analyzed with Bragg-Brentano geometry from 3° to 40° 2θ at a step size of 0.01° 2θ on zero-background silicon wafers.

2.5. DSC/TGA measurements

A DSC Q1000 instrument (TA instruments Inc., New Castle, DE) was used for differential scanning calorimetry (DSC) measurements with a scanning rate of 2 °C/min, and a TGA Q500 instrument (TA Instruments Inc., New Castle, DE) was used for thermogravimetric determinations with a scanning rate of 2 °C/min.

2.6. Multivariate data analysis

All multivariate data analysis was done using Matlab 7.01 (MathWorks Inc., Natick, MA) and PLS toolbox 3.5 (Eigenvector Research, Inc., Wenatchee, WA). PLS calibration models were built based on the mean centered second derivative spectra for both NIR and Raman spectral data. The normalized second derivative steps were reported previously (Madden, 1978; Savitzky and Golay, 1964; Zhang and Ben-Amotz, 2000). PLS calibration models were applied to NIR and Raman spectral data, as a standard approach, which used the spectral measurements (NIR and Raman) of one set of physical mixtures for calibration (calibration set), while the second set of spectral data was designated as the test set for the purpose of batch-to-batch validation. The MCR-Raman calibration method was also used for the quantitative determination of solid-state forms based on second derivatives of the Raman spectra that were taken from three individual pure forms of the development compound (amorphous, Form B, and Form C). An MCR algorithm was used to deconvolute the second-derivative Raman spectra of samples into the second derivative Raman spectra of three pure solid-state forms (amorphous, Form B and Form C) (Xie et al., 2008). Raman data measured from the two sets of physical mixtures were both used as test sets for the evaluation of this MCR-Raman method.

The root-mean square error of calibration (RMSEC) together with the root-mean square error of prediction (RMSEP) were evaluated to test the quality of the model by using the two following

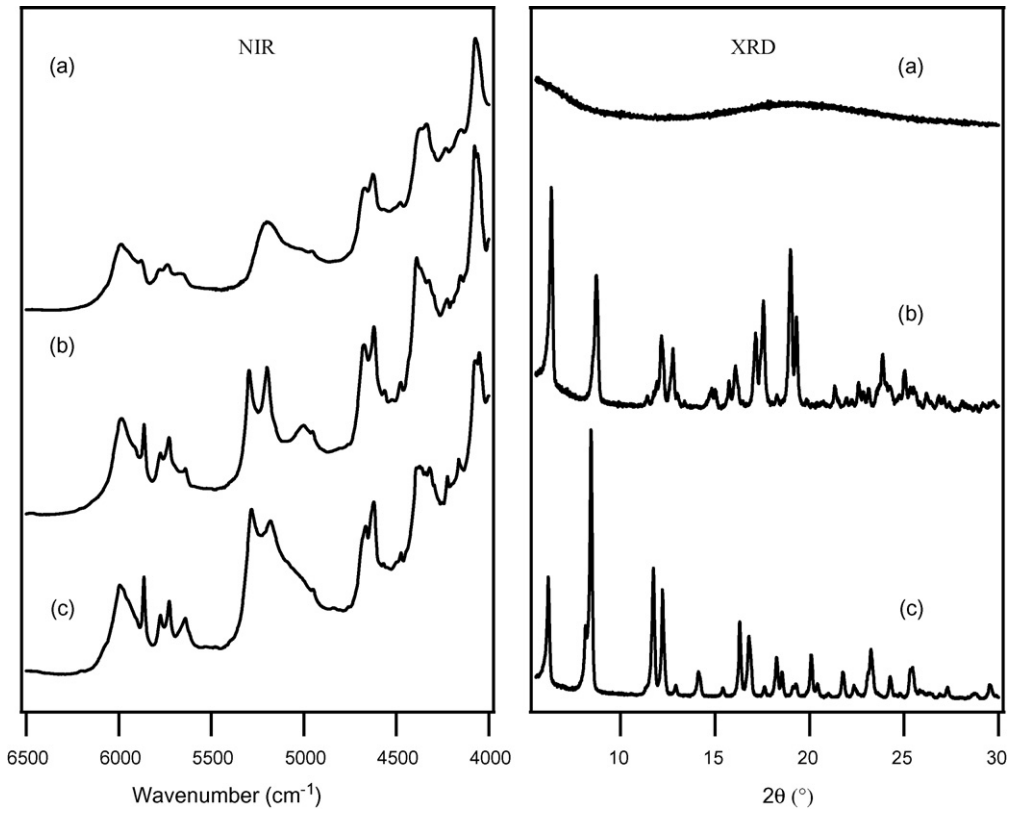

Fig. 1. The NIR spectra (left) and XRD diffraction patterns (right) of (a) amorphous, (b) Form B, and (c) Form C physical forms of the development compound.

equations (Nas et al., 2002):

$$\text{RMSEC} = \sqrt{\frac{\sum_{i=1}^{N}(\hat{y}_i - y_i)^2}{N - A - 1}}, \quad \text{and} \quad \text{RMSEP} = \sqrt{\frac{\sum_{i=1}^{N}(\hat{y}_i - y_i)^2}{N}}$$

where N is the total number of spectra collected, and A stands for the number of latent variables used in the PLS model. $\hat{y}_i$ is the form percentage (w/w) determined by weight, and y_i is the predicted percentage of the specific form of the development compound for the sample corresponding to the i-th spectrum. As the main source of error in a Raman quantitation method comes from sample heterogeneity and small sampling area (Johansson et al., 2005; Roberts et al., 2002; Strachan et al., 2007; Taylor and Zografi, 1998), the RMSEC and the RMSEP values here probably are indicative of the heterogeneity of the mixtures used for the measurements.

3. Results and discussion

3.1. Characterization of the three physical forms

The amorphous and two hydrate crystal forms (Form B and Form C) of the development compound were characterized by X-ray diffractometry (Fig. 1), NIR spectroscopy (Fig. 1), and Raman spectroscopy (Fig. 2).

All three methods show clear differences between the phases, which can be used for the qualitative determination of the three forms. Further evaluation of each method was necessary to determine which technique was best suited for quantitation of the three phases, especially the amorphous form.

3.1.1. XRD

The XRD pattern for the amorphous form of the development compound is displayed in Fig. 1a. Unlike the distinctive XRD patterns for the two crystal forms (Fig. 1b and c), the XRD pattern for the amorphous form consists of non-chemically specific features due to the random arrangement of the molecules. Therefore, it becomes problematic to quantify the amorphous form via XRD. Not only does the weak signal and lack of unique reflections from the amorphous phase make XRD a less sensitive technique for quantifying an amorphous phase, but also the presence of other excipients in a mixture can complicate the interpretation of the quantitation results.

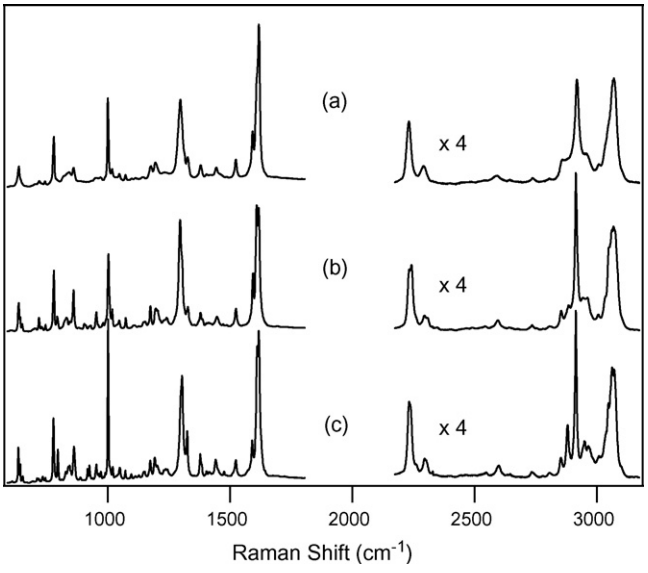

Fig. 2. Raman spectra of (a) amorphous, (b) Form B, and (c) Form C forms of the development compound.

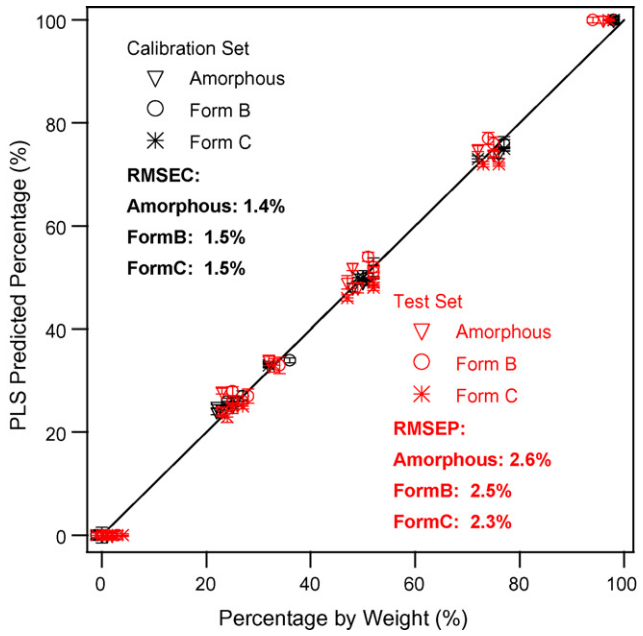

Fig. 3. The RMSECs for the calibration set and the RMSEPs for the test set of physical mixtures based on the PLS analysis of NIR measurements for three physical forms.

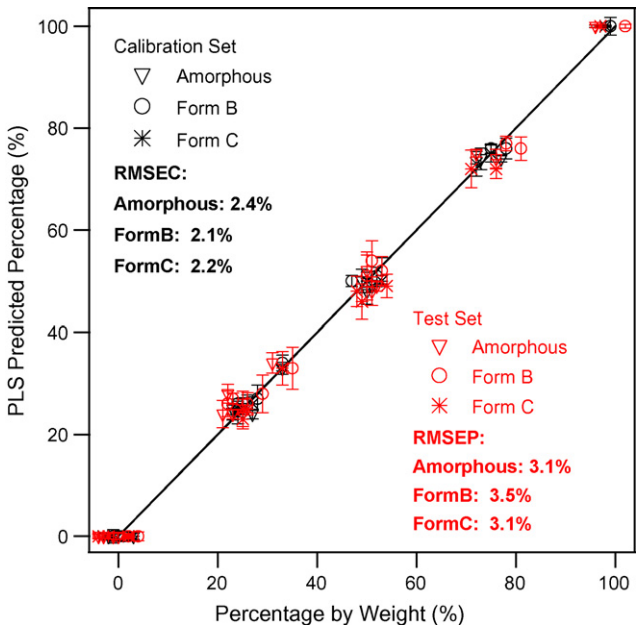

Fig. 4. The RMSECs for the calibration set and the RMSEPs for the test set of physical mixtures based on the PLS analysis of Raman measurements for three physical forms.

3.1.2. NIR

The unique NIR spectra for the amorphous form, Form B, and Form C are shown in Fig. 1. Unlike XRD, the amorphous form has unique NIR spectral features, as well as the two crystal forms. The spectral region at 5000–5500 cm^{-1} that represents the water in the hydrated crystal provides clear characteristics for the qualitative and quantitative determination of each form within mixtures (Rasanen et al., 2001). Fig. 3 demonstrates the multivariate calibration established by NIR measurements using a PLS algorithm with 10 latent variables. NIR spectra measured from one set of physical mixtures were used for the PLS-calibration with RMSECs being less than 2%. The PLS prediction that was applied to the NIR measurements of the second set of physical mixtures also shows small prediction errors with all RMSEPs being less than 3%.

However, the NIR technique is known to be sensitive to changes in moisture content and particle size distribution (Nieuwmeyer et al., 2007; Rasanen et al., 2001), and it could generate significant prediction errors when applied to different lots of the same material with different water content and particle sizes. NIR applications to polymorphic quantitation in drug products are also limited because of significant signal interference from excipients. Overlapped NIR peaks from both the drug substance and excipients can be a critical challenge for the development of a NIR method, especially when the drug load is small.

3.1.3. Raman

Raman spectroscopy is another vibrational spectroscopic technology, which gives different physical forms their unique Raman spectral features, as shown in Fig. 2. Comparing IR and NIR spectroscopy, Raman spectroscopy shows stronger spectral intensity for the aromatic molecule with less sensitivity to the presence of water. The spectral difference between the three forms mainly lies in the aromatic structural related vibrational modes such as peaks at approximately 1000 cm^{-1} (ring breathing mode of benzene ring) and approximately 1610 cm^{-1} (ring deformation of benzene ring).

Fig. 4 shows the PLS quantitation results using Raman measurements for the same two sets of physical mixtures with slightly larger RMSECs and RMSEPs, compared with those obtained using NIR spectroscopy. Again, the major source of quantitation error in Raman methods comes from the small sampling size and the sample heterogeneity. The larger prediction errors showed in Fig. 4 are probably associated with the smaller sampling size used in Raman spectroscopy (0.45 mm in diameter) when compared with NIR spectroscopy (8 mm in diameter).

In order to overcome the sample heterogeneity in calibration mixtures, an MCR-Raman prediction model was also utilized rather than the standard approach using the Raman measurement of reference physical mixtures. The detailed procedures for the MCR-Raman method have been recently described (Xie et al., 2008). Briefly, only the Raman spectra of the three pure forms (amorphous, Form B, and Form C) were used, and the normalization for achieving the mass related intensity was performed using the integrated peak intensity of the spectral region at 2800–3200 cm^{-1} (C–H stretching mode, V_{CH}), as shown in Fig. 2. Second derivatives of Raman spectroscopic data were used to minimize the changes of the Raman spectral baseline related to fluorescent impurities in test samples (Zhang and Ben-Amotz, 2000). The Raman spectral regions used for the quantitative analysis were purposely selected to satisfy both the maximal difference among the three physical forms and the minimal sensitivity to interactions between different compositions in a test sample. Generally, the low-frequency spectral region below 400 cm^{-1} was avoided, and this low-frequency region is commonly considered for the study of intermolecular interactions in condensed systems (Nielsen, 2001). Therefore, the MCR quantification method could be achieved based on the previous assumption that the second derivative Raman spectrum of a test sample is a linear combination of the second derivative spectra of its solid-state components (Xie et al., 2008).

The MCR-Raman calibration model was evaluated by testing Raman measurements of physical mixtures. Table 1 summarizes the RMSEC and RMSEP values obtained from the Raman spectral data of the two sets of physical mixtures using both PLS and MCR methods. Because the calibration model used for the MCR-Raman method only utilizes the Raman spectra of the three pure solid-state forms instead of their physical mixtures, there were no RMSEC values calculated for the MCR-Raman method. Instead, the RMSEP

Table 1
Comparison of the RMSEC and the RMSEP values obtained from the Raman spectral data

Multivariate methods		Amorphous (%)	Form B (%)	Form C (%)
Raman (PLS)[a]	RMSEC	2.4	2.1	2.2
	RMSEP	3.1	3.5	3.1
Raman (PLS)[b]	RMSEC	3.1	2.7	2.6
	RMSEP	3.2	3.9	3.4
Raman (MCR)[a]	RMSEP	3.6	3.4	2.6
Raman (MCR)[b]	RMSEP	3.6	3.5	3.6

[a] With 600–1800 cm^{-1} spectral region.
[b] With 1500–1650 cm^{-1} spectral region.

values obtained for the MCR-Raman method were based on the two sets of mixtures. The spectral region at 1550–1650 cm^{-1}, which has no Raman signal interference from excipients, was selected for the quantitative analysis of API physical forms in the presence of excipients. The similarities of the RMSEP values indicate that the main prediction errors may be derived from the sample heterogeneity, because the MCR-Raman method does not account for the sample uniformity-related factors in its calibration model. The MCR-Raman method was used for quantifying the solid-state forms in the following studies.

Commonly used excipients in solid dosage forms are aliphatic molecules, while many drug candidates have aromatic structures. The nature of Raman scattering makes Raman technology more attractive for studying API in formulations, compared with NIR spectroscopy. The development compound in this study not only shows isolated Raman peaks of aromatic vibrations (at 1500–1650 cm^{-1}), but also gives relatively stronger intensity due to its larger Raman scattering cross-section. Fig. 5 shows the Raman spectra of (a) the tablet with 5.3% drug load of the development compound; (b) the placebo blend with excipients; and (c) Form B API. The NIR spectra for the same sample set are also listed in Fig. 5. There is visually no difference between the placebo and the active tablet in the NIR spectra, which is consistent with the fact that there is only 5.3% (w/w) API load in the tablet. In NIR spectroscopy, major NIR spectral features consist of combination and overtone bands of the X–H bands (X=C, O, N, S) of the analytes. In this case, the development compound contains multiple aromatic rings with a few short alkyl-substituted side chains, making its NIR detection challenging in the presence of 94.7% (w/w) excipients with most alkyl groups. However, a compound with aromatic structures always gives much larger Raman scattering cross-section in comparison with alkyl molecules such as those present excipients (weak Raman scatterers). Therefore, Raman spectroscopy is more sensitive to the existence of this API in the presence of excipients. The API shows distinct peaks around 1610 cm^{-1} in the Raman spectrum of the tablet formulation with 5.3% (w/w) API load (Fig. 5a).

Because only the phase transition from Form B to the amorphous form was observed during the stability study of Form B API, the extent of conversion was monitored by tracking the percentage of the remaining Form B in the sample. The performance of the MCR-Raman quantitation based on the spectral region at 1550–1650 cm^{-1} for Form B is demonstrated by analyzing the mixture samples only containing Form B and the amorphous form, as shown in Fig. 6. Each data point represents the mean of 20 measurements for a mixture sample, with an overall RMSEP of 3.5% in a range from 0% to 100% Form B. As there is no excipient interference in the selected spectral region at 1550–1650 cm^{-1}, the MCR-Raman prediction method can be applied to quantitatively monitor the form stability for both the neat API and tablets.

3.2. Stability monitoring of Form B

Preliminary data suggested that the Form B crystal was more thermodynamically stable than the other two solid-state forms

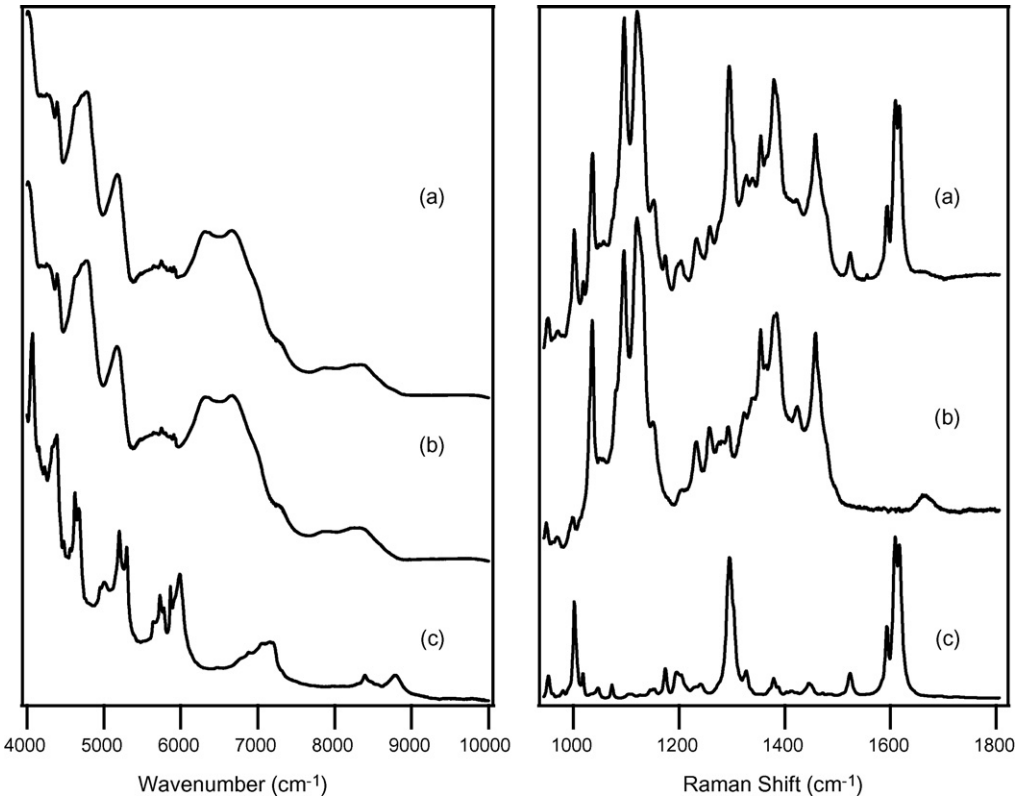

Fig. 5. The NIR spectra (left) and the Raman spectra (right) of (a) the tablet of the development compound with 5.3% drug load, (b) the excipient blend, and (c) the neat Form B crystal.

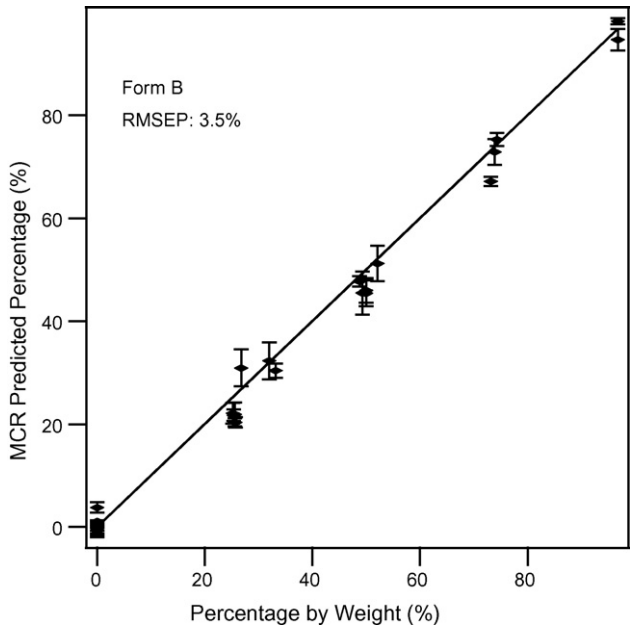

Fig. 6. MCR-predicted percentages of Form B crystal based on the MCR-Raman method for the two sets of physical mixtures. The overall RMSEP of Form B prediction is 3.5%.

based on the solubility measurements at 21 °C, and it was selected for further drug development since it had the lowest aqueous solubility. For this reason, a study was designed to investigate the physical behavior of Form B in greater detail. Form B can undergo a dehydration process at a temperature of 83.1 °C resulting in an amorphous form, based on the DSC/TGA curves, shown in Fig. 7. Therefore, monitoring this phase conversion for further formulation development became essential. In order to obtain a better understanding of the physical form stability of Form B as neat API and in the formulation, various temperature and humidity conditions (25 °C/60% RH, 40 °C/75% RH, and 50 °C/75% RH) were selected for testing the long-term stability of (1) Form B as API; (2) Form B at 5.3% (w/w) in an excipient blend (microcrystalline cellulose, mannitol, polyvinyl pyrrolidone, sodium starch glycolate, fused silica, and magnesium stearate); and (3) Form B in active tablets. All samples were tested for a minimum of 3 months in both sealed

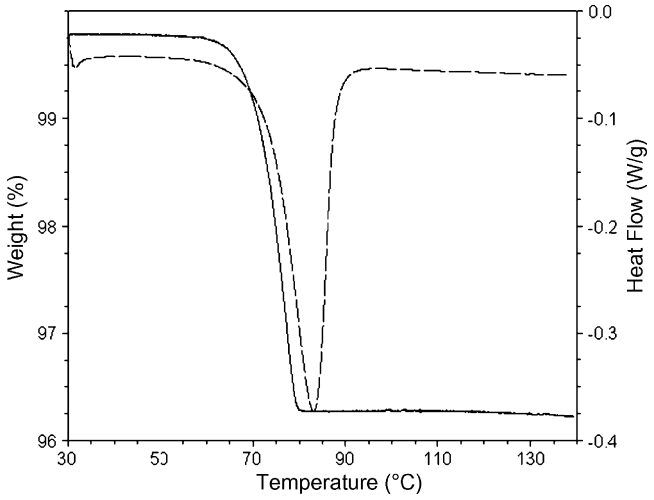

Fig. 7. The DSC (dashed line) and TGA (solid line) scans of the Form B crystal of the development compound.

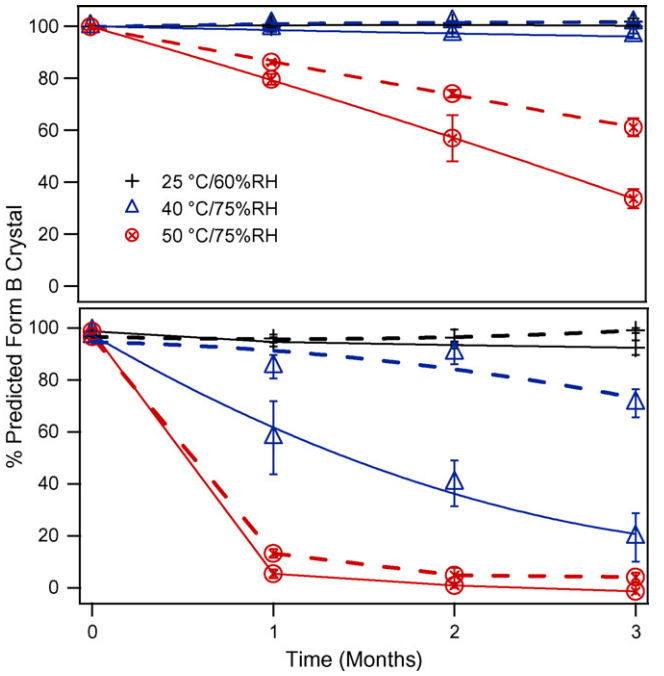

Fig. 8. The predicted % Form B in stability samples of API (top) and the drug blend with 5.3% drug load (bottom) by using the MCR-Raman method. Both results from the sealed condition (broken lines) and the open-dish condition (solid lines) are demonstrated with standard deviations from five measurements.

and open-dish conditions. As shown in Fig. 8 (top), Form B retains its crystalline structure when stored at 25 °C/60% RH even in the open-dish condition. For samples stored at 40 °C/75% RH, only a slight drop in crystallinity was observed in the open-dish condition, and the crystallinity remained intact when the container was sealed. However, a significant drop in crystallinity was observed at 50 °C/75% RH with a higher dehydration rate for samples stored in an open dish. Only 34% of Form B remained after 3-month storage in an open dish compared to 61% Form B in the sealed sample stored under the same conditions at 50 °C/75% RH.

The excipient effect may be an important factor that impacts the chemical and physical stability of a drug substance in a drug product. A drug blend containing 5.3% Form B API was tested at the same stress conditions as the neat Form B API. Fig. 8 (bottom) shows time dependent plots for the drug blend at all conditions. Higher conversion rates were detected with higher temperature/humidity conditions, similar to what was observed for the neat drug substance. However, faster conversion rates were observed at 40 °C/75% RH and 50 °C/75% RH for the blend as compared to the neat drug substance. At 40 °C/75% RH, the blend sample stored in the open dish showed a faster conversion rate, with only 20% crystallinity remaining after 3 months, compared with 70% crystallinity remaining for the blend sample stored under sealed conditions. There was also approximately a 90% decrease in crystallinity after 1 month for blend samples stored at 50 °C/75% RH.

The trend of the dehydration rate may be correlated with the combined effect of (1) the temperature applied and (2) the amount of water sorption by test samples containing excipients (especially microcrystalline cellulose and mannitol). With elevated temperature at 50 °C, Form B API exhibited much higher dehydration rates at all conditions. However, it is not straightforward to relate water sorption to the dehydration rate. One explanation can be due to a kinetically favorable process. Even with limited solubility in water (1 μg/mL at 20 °C), the Form B crystal can still partially dissolve in water especially at 40 and 50 °C. As the polarity of water does not

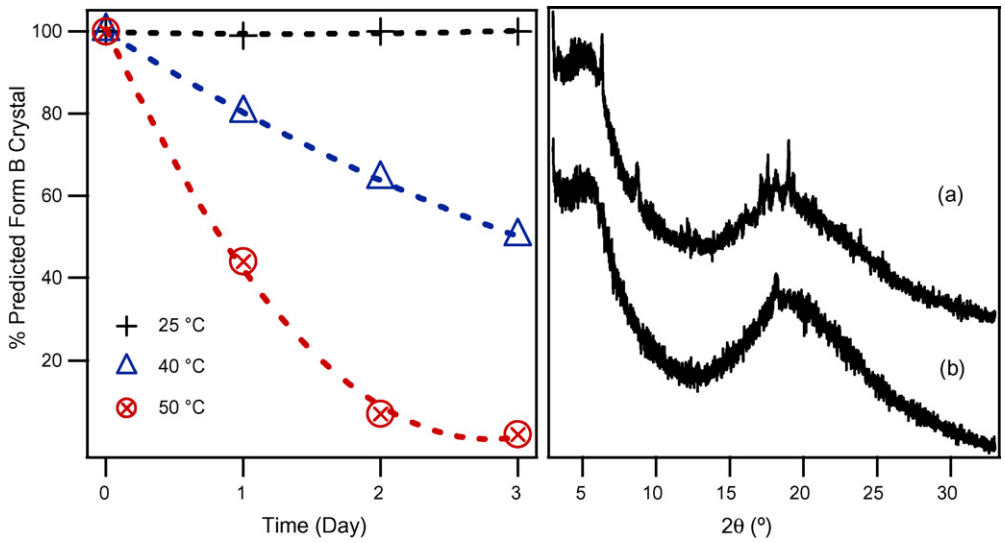

Fig. 9. (Left) The MCR-Raman predicted % Form B from samples slurried in water. (Right) The XRPD patterns of (a) the day 2 sample and (b) the day 3 sample at 50 °C.

favor the crystallization of the Form B crystal, the solute in water can only precipitate as the amorphous form. In order to prove this hypothesis, an extreme condition was tested by stirring Form B material as a water slurry at 25, 40, and 50 °C (200 mg in 50 mL water). The results are shown in Fig. 9 (left). A highly accelerated phase transition was observed over the 3-day period. At day 3, only 2% (50 °C) and 50% (40 °C) Form B crystal remained in the solid. This was confirmed by the X-ray diffraction pattern of the undissolved solids. In Fig. 9 (right), the diffraction pattern (a) corresponds to the remaining solid at day 2 (50 °C), where the MCR-Raman analysis showed that about 7% Form B crystal remained. The peaks appearing on top of the amorphous halo were consistent with the Bragg peaks of the Form B crystal. The diffraction pattern at day 3 (b) only indicates the existence of amorphous material, whereas the MCR-Raman analysis showed that 2% Form B remained. Therefore, this result confirms that the phase transition in water is actually from Form B hydrate to the amorphous form.

3.3. Phase transition under compression

The same drug blend as above (5.3% drug load) was compressed into active tablets under 280 MPa compression force. The resulting tablets were monitored under the same conditions as the drug blend, and the results are shown in Fig. 10. The trend of phase transition of the tablets at various temperature/moisture conditions is consistent with the observations for the drug blend (Fig. 8 (bottom)) under sealed conditions. It is clear that the compaction force has a significant effect on the phase transition of Form B into amorphous form, with an 18% decrease in crystallinity.

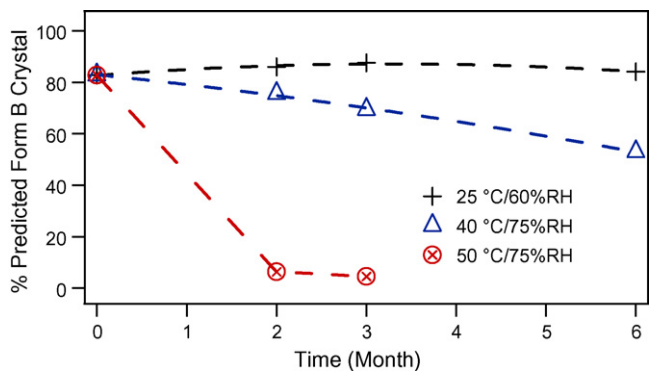

Fig. 10. The predicted % Form B in stability samples of tablets with 5.3% drug load by using the MCR-Raman method. Tablets were stored under sealed conditions.

In order to confirm the effect of compression on the tablet samples, neat API was compressed at different compaction forces and tested after gently grinding to a fine powder with mortar and pestle. As shown in Table 2, the 220 MPa compaction force was enough to convert approximately 20% form B to the amorphous form, which is consistent with the tablet dosage form data. Compaction forces higher than 220 MPa do not show any greater effects on the extent of form conversion. A similar compression-induced from conversion phenomenon was reported in the literature for chlorpropamide (Otsuka and Matsuda, 1993; Wildfong et al., 2005).

DSC was used to confirm the results measured by the MCR-Raman method. The same ground powder samples of neat API tablets that were measured by the Raman technique were tested again using DSC. As shown in Fig. 11, the DSC data of compressed Form B powder samples show lower integrated heat of dehydration compared with that of the non-compressed Form B. The dehydration temperature of compressed Form B samples is also consistently lower than that of the non-compressed Form B. The dehydration peak temperature is lowered to 77.6 °C for the sample compressed at 220 MPa, compared with 83.1 °C observed for the non-compressed Form B sample. One possible reason for this difference might be due to some level of distortion in the crystalline structure upon compaction (Tawashi, 1968). The DSC curve of the amorphous form is also shown in Fig. 11, which does not have heat events overlapped with the dehydration peak. The Tg' of the amorphous form is 55 °C. The quantitation method using DSC data was based on the calculation of percent remaining heat of dehydration after tablet compaction and grinding. The dehydration heat of the

Table 2
Compression-induced form transition measured by DSC and Raman spectroscopy

Compaction pressure (MPa)	By DSC		By Raman
	J/g	Form B (%)	Form B (%)
0	94.4	100	100
220	74.6	79	82
390	74.2	79	82
550	73.8	78	82
870	72.8	77	80

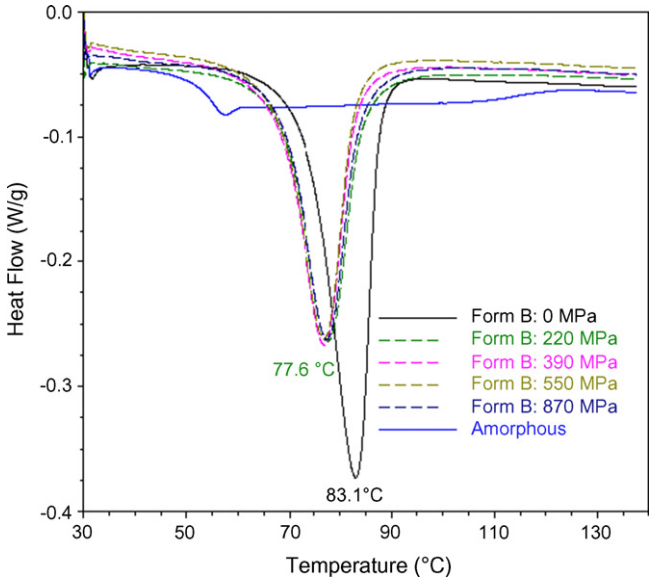

Fig. 11. The DSC scans of Form B before (solid line) and after (dashed lines) compression under 220, 390, 550, and 870 MPa.

uncompressed Form B sample is 94.4 ± 0.9 J/g ($N = 7$). Table 2 lists the quantitative results obtained by both Raman and DSC methods. Both techniques gave consistent results for the remaining Form B crystallinity in the ground powder samples of neat API tablets. However, only the Raman technique can be applied to a tablet formulation with a low drug load.

4. Conclusion

In conjunction with multivariate analysis, Raman spectroscopy has been successfully used to quantify different solid-state forms of a pharmaceutical development compound in the drug substance and tablets. Raman spectroscopy was demonstrated to be advantageous over NIR and XRD for quantifying the amorphous form in blend and tablets with a low drug load (5.3%).

It was also shown that an MCR-Raman method for the development compound could be generated without requiring actual measurements from physical mixtures for the purpose of calibration. For Form B, a RMSEP of 3.5% was obtained with various mixtures containing all three polymorphs and covering a range from 0% to 100%. Although physical mixtures of different polymorphic forms were used in this work to validate the PLS calibration model, the MCR-Raman method, with the advantages demonstrated above, may be directly applied to other pharmaceutical compounds under circumstances where reliable physical mixtures are not available (such as the existence of metastable solid forms).

Acknowledgement

The authors thank Melissa Mrozek-Morrison, Lan Li, Zinah Hong, Sekhar Kanapuram, Armen Pirjanian, Shawn Walker and Yuan-Hon Kiang for technical assistance and scientific discussion and are thankful for support from the small molecule pharmaceutics group at Amgen.

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