A Comparative Study of the use of Powder X-ray Diffraction, Raman and Near Infrared spectroscopy for quantification of binary polymorphic mixtures of Piracetam

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ABSTRACT

Diffraction and spectroscopic methods were evaluated for quantitative analysis of binary powder mixtures of FII(6.403) and FIII(6.525) Piracetam. The two polymorphs of Piracetam could be distinguished using powder X-ray diffraction (PXRD), Raman and near-infrared (NIR) spectroscopy. The results demonstrated that Raman and NIR spectroscopy are most suitable for quantitative analysis of this polymorphic mixture. When the spectra are treated with the combination of multiplicative scatter correction (MSC) and second derivative data pretreatments, the partial least squared (PLS) regression model gave a root mean square error of calibration (RMSEC) of 0.94 and 0.99 % respectively. FIII(6.525) demonstrated some preferred orientation in PXRD analysis, making PXRD the least preferred method of quantification.

KEYWORDS: Piracetam polymorphs; Quantitative analysis; Powder X-ray diffraction; Raman spectroscopy; Near-infrared spectroscopy.

1. Introduction

Polymorphism is a well recognised phenomenon whereby a pure chemical compound may exist in two or more structural orientations, each displaying different physical characteristics. Pseudo-polymorph - solvates and hydrates which have a molecule of solvent included in the crystal structure, are also possible. As different polymorphs display individual physical properties, such as density, melting point and solubility, polymorph purity is vitally important to the manufacture of chemicals, in particular, pharmaceuticals. Production of an unwanted, or impure, polymorph will give a product that most likely will not satisfy the intended purpose, or processing characteristics, of the required polymorph. Identification and quantification of polymorphic forms has become a necessary requirement in the production of modern day pharmaceuticals[1, 2].

A range of analytical techniques have proven suitable for the analysis and quantification of polymorphic mixtures. Powder X-ray diffraction (PXRD) [3-11], near-infrared spectroscopy (NIR)[12-16], attenuated total reflectance infrared (ATR-IR) spectroscopy [14], diffuse reflectance infrared spectroscopy (DRIFTS) [4, 17], Raman spectroscopy [7, 9, 14, 18-21] and, more recently, solid state 13C CPMAS NMR spectroscopy [22] have been used to successfully quantify polymorphic mixtures with methods ranging from simple univariate correlations to more complicated multivariate chemometric approaches.

Piracetam (2-oxo-1-pyrrolidine acetamide) is a polymorphic drug compound (Figure 1) with five reported polymorphs, of which two (FIV and FV) are obtained only under high pressure (> 0.5 GPa) conditions [23]. The remaining polymorphs FI, FII and FIII, have been identified and structurally characterized under ambient conditions [24, 25]. FI is highly unstable at ambient conditions and can be isolated only by heating FII or FIII to 400 K and then quenching to room temperature. It transforms back to FII in the solid state within a few hours, and, as such, is not of much practical relevance. FII is metastable, and FIII is the stable polymorph at ambient conditions, as

determined by melting data [25]. A quantification model for polymorphic mixtures of FII and FIII Piracetam is desired to investigate the solution mediated polymorphic transformation of FII to FIII. To our knowledge, no efforts have been made in published literature to quantify mixtures of FII and FIII Piracetam. Throughout the literature there is some confusion over the nomenclature of the different polymorphs. In this work the system used for identifying the polymorphs is simply the form number followed by the *a* lattice parameter reported for the particular polymorph in the Cambridge Crystallographic Data Centre (CCDC) in brackets, so that Form II and Form III will subsequently be referred to as FII(6.403) and FIII(6.525) respectively. The reference codes for piracetam FII(6.403) and FIII(6.525) in the CCDC are BISMEV and BISMEV01 respectively.

As polymorphs differ fundamentally in their crystal structure, powder X-ray diffraction (PXRD) has become the gold standard for polymorph analysis [2, 26]. Quantification using PXRD is based on the principle that the intensity of diffraction peak for a component in a mixture is related to the concentration of that component in the mixture [27]. A number of different peak parameters on PXRD patterns can be used for this analysis: peak height intensity [4], a ratio of peak height intensity [6], and peak area [7, 8, 9] being the most common in univariate analysis. Pharmaceutical compounds present an issue for X-ray diffraction analysis in that these materials can tend to display a high degree of preferred orientation. This can lead to difficulty in obtaining good quality, representative, reproducible diffractograms. Campbell Roberts et al. completed a comprehensive quantitative study for binary mixtures of mannitol polymorphs, and investigated the effect of preferred orientation on the quantification [5]. For particle sizes below 125 μm, this effect was deemed negligible. However, grinding pharmaceutical products to achieve this small particle size is not always feasible, as grinding may induce some phase transformation.

In addition to PXRD methods, vibrational spectroscopy, such as NIR, MIR, and Raman spectroscopies, can be used for rapid characterisation and quantification of polymorphs of pharmaceutical materials. NIR is associated with the overtones and combination modes of fundamental molecular vibrations that occur in the NIR to IR spectral region [28]. The coupling of NIR with chemometrics allows for interpretation of the resulting broad spectra and it is a technique widely used in pharmaceutical environments, in reaction monitoring, quality control and quantification of pharmaceutical materials [29, 30]. For Raman spectroscopy the gross selection rule is that for a Raman active vibration to occur there must be a change in polarizability of the molecule during its molecular vibration[31]. As many active pharmaceutical ingredient (APIs) contain aromatic functional groups with symmetric vibrational modes, they are considered to be strong Raman scatterers. Raman spectroscopy requires little or no sample preparation, and allows for in-situ analysis and high chemical specificity. However fluorescence of samples can obscure useful spectral information, although this may be overcome by use of NIR excitation and/or the application of various data pre-processing methods. Raman Spectroscopy has been utilised successfully for the quantification of polymorphic mixtures, tablets, capsules and for inline analysis of fluid bed drying processes [32-36].

The objective of this work is to develop and compare quantification models for binary mixtures of FII(6.403) and FIII(6.525) Piracetam using PXRD, Raman, and NIR spectroscopy.

2. Materials & Methods

2.1 Preparation of polymorphs

Piracetam was supplied by AXO Industry Ltd, Belgium, and complies with European Pharmacopoeia standards (CAS Number: 7491-74-9). Methanol was reagent grade. 10 g of

the FIII(6.525) polymorph of piracetam was prepared by recrystallization from methanol [1]. The FII(6.403) polymorph was prepared by heating 5 g of FIII(6.403) crystals to 140 ºC for 72 hr, and storing under ambient conditions for 5 days[2]. Isolation of pure polymorphic forms was confirmed using PXRD, DSC, and ATR-FTIR spectroscopy (Supplementary Information).

2.2 Preparation of polymorphic standards

The pure forms were ground individually in an agate mortar with a pestle for 30 seconds each, and a 90 – 125 µm sieve fraction collected for standard preparation. The possibility of any phase change occurring during grinding was discounted by testing a control sample before and after grinding. 100 mg binary calibration mixtures containing 0, 1, 5, 10, 20, 30, 40, 50 60, 70, 80, 90, 95, 99 and 100 % of FII(6.403), with the remaining mass balance provided by FIII(6.525), were prepared by gentle mixing of weighed quantities of both polymorphs in an agate mortar with pestle. Validation mixtures containing 15, 50, 75 and 85% of FII(6.403) were prepared in the same way.

2.3 X-ray powder diffractometry

Diffraction patterns of the samples were obtained in reflectance mode using a Phillips PANalytical X'Pert MPD Pro instrument with a Cu Kα source (λ = 1.5418 Å), nickel filter, fixed divergence slit of ½°, and accelerating voltage and anode current set as 40 kV and 35 mA respectively. Data was recorded over the range 8 to 35 °2θ, using a step size of 0.017 °2θ, a count time of 33 sec per step, a scan speed of 0.064 °2θ/ sec, and a sample rotation of 4 rpm using PANanalytical Data Collector, version 2.0.

The polymorph mixture was placed on a silicon crystal zero-background disc, which was mounted into a sample holder using a clip. The sample surface was smoothed with a

glass slide. The samples were measured consecutively in triplicate. Sample preparation using pressed cellulose discs was also attempted, but this was found to result in amplified preferred orientation effects in the recorded diffraction patterns.

Reference PXRD patterns for the FII and FIII polymorph were generated with the CIF files BISMEV and BISMEV01 respectively, from the Cambridge Crystallographic Data Centre (CCDC), using Mercury 2.4.

2.4 Raman Spectroscopy

Raman spectra were collected at room temperature using a RamanStation spectrometer (AVALON Instruments Ltd., Belfast, Northern Ireland; now PerkinElmer) equipped with 785 nm laser diode excitation, cooled (-77 ºC) CCD detector, and a motorised XYZ sample stage. The samples were placed in aluminium crucibles (Thorn Scientific Services Ltd, UK) of 2 mm depth and 5 mm diameter and measured with a laser power of 79.9 mW at the sample (spot size ~200 µm). An exposure time of 2 s × 10 acquisitions was used for each measurement and spectra were collected over a range of 250 to 3310 cm^{-1} with 4 cm^{-1} resolution. Each sample was analyzed on a 3 x 3 grid with 0.5 mm spacing to reduce sub-sampling effects. An average spectrum was calculated from the 9 individual spectra.

2.5 NIR spectroscopy

NIR data were collected using a Perkin Elmer Spectrum One spectrometer fitted with an NIR reflectance attachment. NIR spectra were collected with interleaved scans in the 10000–4000cm^{-1} range with a resolution of 8 cm^{-1}, using 32 co-added scans. Sample vials (SUN-Sri Ltd.) were shaken and repositioned between triplicate measurements of each sample.

2.6 Data analysis

X'Pert HighScore Plus software (PANalytical) was used to calculate peak height intensity and area, and correct the shifts along the 2θ axis for PXRD scans. The cubic spline data interpolation technique was then used to reconstruct the PXRD scans. Multivariate data analysis was carried out using The Unscrambler v9.8 software (Camo, Norway). To remove unimportant baseline (offset) interferences from samples or correct scatter effects and accentuate spectral signals of interest, various different pre-processing methods, including multiplicative scatter correction (MSC), standard normal variate (SNV), first and second derivative and their combinations were applied for Raman and NIR data. Savitzki-Golay first and second derivative calculations were performed with a window size of 15 points and a second order polynomial. Mean normalization was used for PXRD data. The PXRD and spectroscopic data were subjected to mean centring prior to partial least squares (PLS) analysis. The optimal number of PLS factors was determined by using a leave-one-out cross validation procedure. The performance of the model was evaluated by using the correlation coefficient (R^2) and root mean square error (RMSE) of the calibration (RMSEC), cross-validation (RMSECV) and prediction (RMSEP). RMSE was calculated using the following equation,

$$\mathrm{RMSE} = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n}} \qquad (1)$$

where $\hat{y}_i$, y_i and n represent the calculated value, the actual value and the number of samples.

3. Results and Discussion

3.1 Characterisation of Piracetam polymorphs

Production of the pure FII(6.403) and FIII(6.525) polymorphs was confirmed with PXRD, DSC and FTIR analysis, as seen in Figure 2 and Supplementary Information. The

experimental PXRD patterns were compared to the theoretical patterns (Figure 2) and no polymorph impurity was detected. Some preferred orientation was evident in the FIII(6.525) pattern. Transmission mode PXRD could have provided for superior diffraction results, but this capability was not available in this study. The FTIR spectra and DSC thermograms (Supplementary Information) were compared to those published by Pavlova et al. [24] and Kuhnert-Brandstaetter et al. [25]. The endothermic peaks in the DSC thermograms indicted that the transformation to FI(6.747) at 114 and 120 ºC in FII(6.403) and FIII(6.525) respectively and melting of FI(6.747) at 152 ºC.

3.2 Powder X-Ray Diffraction Analysis

Diffraction patterns for the pure polymorphs were examined to identify regions of sufficient selectivity for either polymorph to be used for quantification studies. A region with a well resolved diffraction peak, showing no overlap with the corresponding region for the alternative polymorph was desired. The (101) peak of FII(6.403) at 15.8 º 2θ and the (014) peak of FIII(6.525) at 25.7 º 2θ were selected, and the change in intensity of these peaks as a function of FII(6.403) content is shown in Figure 3. Accordingly, a simple univariate quantification method was attempted using the PXRD data[37]. The PXRD patterns for calibration samples were analysed quantitatively by calculating the percentage of FII(6.403) using peak height intensities and area (equation 2, K=1), and relating this to the measured percentage weight composition. The use of a response factor, K, to account for the difference in peak intensity, or area, observed for the (101) peak of FII and the (014) peak of FIII(6.525) in the diffraction pattern of pure FII(6.403) and FIII(6.525) respectively, was also assessed.

$$X_A = \frac{I_A}{I_A + (I_B * K)} \qquad (2)$$

$$K = \frac{I_{Ao}}{I_{Bo}} \qquad (3)$$

The peak intensity and area for PXRD data were calculated with the X'Pert HighScore Plus software, which uses a mathematical function designed to identify the peaks present in the experimental pattern and their exact position and area. The settings used for this procedure were a minimum peak significance of 1.00, minimum tip width of 0.01 º 2θ, maximum tip width of 1.00 º 2θ, and a peak base width of 2.00 º 2θ. The univariate calibration correlations for FII calculated using peak intensity and peak area, without and with the response factor K, are presented in Figure 4. While linear correlations were achieved, there was significant scatter around the lines resulting in poor correlation coefficients (Table 1). A slightly higher linear correlation coefficient was achieved when a response factor K was used with the peak intensity data. The calibration model(s) was tested by using a set of validation standards, and the results are presented in Table 2.

The limit of detection (LOD) and limit of quantification (LOQ) for each correlation were calculated using equations 3 and 4, the standard deviation (STD) of three measurements of a sample with 95% FIII and the slope (m) of the calibration plot for the peak intensity and peak area:

$$LOD = (3 * STD)/ m \quad (4)$$

$$LOQ = (10 * STD)/ m \quad (5)$$

Multivariate calibrations were performed on the 15.1–21.0 and 22.8–26.1 º 2θ range excluding the reflection around 21.3 and 21.7 º which exhibited extraordinary intensity variation with the concentration change of FII(6.403), as shown in Figure 3. The PLS regression analysis used 45 PXRD scans from 15 calibration samples. The best calibration model (requiring only 1 PLS factor) was achieved for the quantification of FII(6.403) in the binary mixtures when PXRD patterns of calibration samples were subjected to mean normalization. The RMSEC, RMSECV and RMSEP values are presented in Table 1 and the

results show a significant improvement over the univariate analysis using peak intensity or peak area. A good linear relationship was observed between the PLS predicted content of FII(6.403) against the measured content (Figure 5) with R^2 of 0.997. LOD and LOQ for the multivariate method were estimated using the same sample, and similar results were obtained (Table 2).

3.3 Raman and NIR spectroscopic analysis

Raman spectroscopy has distinct advantages in the analysis of solid materials because of the minimal sample preparation required and the non-contact, non-destructive nature of the measurement. Different polymorphs of the same compound have different packing of molecules, and so the Raman spectra of the various polymorphs will be different due to subtle differences in molecular vibrations and rotations. Recently, the polymorphic form (FII(6.403) or FIII(6.525)) of Piracetam produced from a cooling crystallization in ethanol was successfully monitored *in situ* by Raman spectroscopy [38]. Figure 6a shows the Raman spectra (from 250 cm^{-1} to 3310 cm^{-1}) for the polymorphs FII and FIII of piracetam. The band at 3140 cm^{-1} is due to symmetric stretching vibrations of NH_2 while the 2750–2990 cm^{-1} bands can be assigned to the symmetric and anti symmetric stretching vibrations of the CH_2 groups. The 1680 and 1650 cm^{-1} bands are ascribed to the C=O stretching vibrations in the ring and acetamide respectively. The remaining Raman band assignments can be found in the literature. [39] It is noted that there are some differences in the Raman spectra of these two polymorphs. For example, FII(6.403) has one characteristic amide peak at 1654 cm^{-1}, while in FIII(6.525) the band is split into two at 1658 and 1648 cm^{-1}. There is also a peak specific to FIII(6.525) at 1410 cm^{-1} and band shifts in the region 1530–1370 cm^{-1}. Furthermore, band shifts are also observed between of 890–750 cm^{-1} which are related to changes in the bond lengths and angles made by atoms adjacent to the carbonyl group.

Partial least squares (PLS) regression analysis was carried out using the whole and several selected spectral regions of interest (RoI). In addition, various pretreatment methods, including SNV, MSC, derivative and their combinations were used to reduce the effect of systematic variations, which are not related to the measured parameters. The results suggested the spectral region from 1730–1370 cm^{-1} was the most suitable for quantification and therefore selected for the model. The best PLS model was achieved for the quantification of FII(6.403) in these two binary mixtures when Raman data subjected to MSC and 2nd derivative treatment. MSC has been proved useful as it eliminated any light scattering from the powders and then subsequent use of the 2nd derivative on these pre-processesd spectra gave more meaningful quantitative models with low RMSEC and RMSEP values of 0.94 % and 1.12 %, respectively. The plot of predicted vs. measured content of FII(6.403) is presented in Figure 7a and shows an R^2 value of 0.999 with high linearity.

NIR spectroscopy is also suitable for quantitative polymorph analysis and the method has been successfully applied to a wide variety of solid state characterizations [12-15, 18, 21]. The NIR spectra (Figure 6b and supplementary information) of these two polymorphs show some differences in the 5870–5600 and 4314–4080 cm^{-1} spectral regions. For example, FII(6.403) has one peak at 5724 cm^{-1}, while FIII(6.525) has two peaks at 5748 and 5708 cm^{-1}. In addition, FII(6.403) has one peak at 4364 cm^{-1}, while FIII(6.525) has two peaks at 4380 and 4358 cm^{-1}. These two spectral RoIs were combined and used to construct PLS model. The optimal PLS model was generated after assessing the effects of several different pretreatments and combinations thereof. This optimal model was obtained by using a combination of MSC and 2nd derivative pretreatments, which gave a good linear relationship (R^2 = 0.999) between the predicted and measured FII(6.403) content (Figure 7b). LOD and LOQ for the multivariate Raman and NIR based models were estimated from the mixtures containing 95% of FIII(6.525), and the results are listed in Table 2.

3.4 Comparison of the three techniques

The above results clearly show that accurate quantification of FII(6.403) in binary mixtures with FIII is possible by either PXRD or spectroscopic analysis, but the spectroscopic methods outperform PXRD. This is evident from the 95% confidence internals (Figure 5 and 7) where those of PXRD are much wider than for the Raman or NIR techniques. The RMSEC and RMSEP values for the Raman and NIR models are also significantly smaller than the PXRD model. To further compare the accuracy of these three techniques, a set of validation samples containing different amounts of FII(6.403) were analyzed (Table 2). For example, determination of the sample 15% FII(6.403), Raman and NIR proved to be the most accurate, followed by PXRD with PLS analysis, while PXRD with peak intensity was the least accurate. However as the FII(6.403) content increases the differences decrease, and, overall, NIR gives the most accurate predictions.

It is well known that sample homogeneity is very important in the quantification of solid powder mixtures. In this study, all the samples were sieved using a 90 – 125 μm sieve fraction and samples were rotated during the PXRD acquisition. Nine measurements (from a 3 x3 grid with 0.5 mm spacing) were performed on each sample for the Raman measurements to reduce potential errors which could arise from the small sampling size. To clarify this, the relative standard deviation (RSD) values of quantitation of the 95% FIII sample obtained from the average spectrum and from individual spectra were 6.22% and 58.81% respectively. The results clearly show that the increase in sampled volume using the 3 x 3 mapping reduces the errors associated with sub-sampling. NIR spectra were collected from glass sample vials and the spectra were recorded from a sample area which was 15 mm in diameter. Each sample was measured in triplicate to reduce sampling errors arising from local inhomogeneity [14].

4 Conclusion

Binary polymorphic mixtures of the nootropic drug Piracetam have been prepared and analyzed quantitatively by PXRD, Raman and NIR spectroscopy, coupled with univariate and multivariate analysis. The spectroscopic techniques gave superior correlation and proved accurate in quantifying a validation data set. The FIII(6.525) polymorph of piracetam exhibits a degree of preferred orientation in XRD analysis, making quantification with this technique less accurate.

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References

(1) Byrn, S.; Pfeiffer, R.; Ganey, M.; Hoiberg, C.; Poochikian, G., Pharmaceutical Solids: A Strategic Approach to Regulatory Considerations. *Pharmaceutical Research* **1995,** 12, (7), 945-954.

(2) Chieng, N.; Rades, T.; Aaltonen, J., An overview of recent studies on the analysis of pharmaceutical polymorphs. *Journal of Pharmaceutical and Biomedical Analysis* 2011, 55, (4), 618-644..

(3) Agatonovic-Kustrin, S.; Wu, V.; Rades, T.; Saville, D.; Tucker, I. G., Powder diffractometric assay of two polymorphic forms of ranitidine hydrochloride. *International Journal of Pharmaceutics* **1999,** 184, (1), 107-114.

(4) Bugay, D. E.; Newman, A. W.; Findlay, W. P., Quantitation of cefepime · 2HCl dihydrate in cefepime · 2HCl monohydrate by diffuse reflectance IR and powder X-ray

diffraction techniques. *Journal of Pharmaceutical and Biomedical Analysis* **1996,** 15, (1), 49-61.

(5) Campbell Roberts, S. N.; Williams, A. C.; Grimsey, I. M.; Booth, S. W., Quantitative analysis of mannitol polymorphs. X-ray powder diffractometry - exploring preferred orientation effects. *Journal of Pharmaceutical & Biomedical Analysis* **2002,** 28, 1149-1159.

(6) Dong, W.; Gilmore, C.; Barr, G.; Dallman, C.; Feeder, N.; Terry, S., A quick method for the quantitative analysis of mixtures.1.Powder X-ray diffraction. *Pharmaceutical Technology* **2008,** 97, (6), 2260-2276.

(7) Nemet, Z.; Kis, G. C.; Pokol, G.; Demeter, A., Quantitative determination of famotidine polymorphs: X-ray powder diffractometric and Raman spectrometric study. *Journal of Pharmaceutical & Biomedical Analysis* **2009,** 49, (3), 338-346.

(8) Suryanarayanan, R., Determination of the Relative Amounts of Anhydrous Carbamazepine and Carbamazepine Dihydrate in a Mixture by Powder X-ray Diffractometry. *Pharmaceutical Research* **1989,** 6, (12), 1017-1024.

(9) Tian, F.; Zhang, F.; Sandler, N.; Gordon, K. C.; McGoverin, C. M.; Strachan, C. J.; Saville, D. J.; Rades, T., Influence of sample characteristics on quantification of carbamazepine hydrate formation by X-ray powder diffraction and Raman spectroscopy. *European Journal of Pharmaceutics and Biopharmaceutics* **2007,** 66, (3), 466-474.

(10) Tiwari, M.; Chawla, G.; Bansal, A. K., Quantification of olanzapine polymorphs using powder X0-ray diffraction technique. *Journal of Pharmaceutical & Biomedical Analysis* **2007,** 43, 865-872.

(11) Li, Y.; Shan Cow, P.; Tan, R. B. H., Quantification of polymorphic impurity in an enantiotropic polymorph system using differential scanning calorimetry, X-ray powder diffraction and Raman spectroscopy. *International Journal of Pharmaceutics* **2011,** 415, (1-2), 110-118.

(12) McGoverin, C. M.; Ho, L. C. H.; Zeitler, J. A.; Strachan, C. J.; Gordon, K. C.; Rades, T., Quantification of binary polymorphic mixtures of ranitidine hydrochloride using NIR spectroscopy. *Vibrational Spectroscopy* **2006,** 41, (2), 225-231.

(13) Ziémons, E.; Bourichi, H.; Mantanus, J.; Rozet, E.; Lebrun, P.; Essassi, E.; Cherrah, Y.; Bouklouze, A.; Hubert, P., Determination of binary polymorphic mixtures of fluconazole using near infrared spectroscopy and X-ray powder diffraction: A comparative study based on the pre-validation stage results. *Journal of Pharmaceutical and Biomedical Analysis* **2011,** 55 (5), 1208-1212.

(14) Hu, Y.; Erxleben, A.; Ryder, A. G.; McArdle, P., Quantitative analysis of sulfathiazole polymorphs in ternary mixtures by attenuated total reflectance infrared, near-infrared and Raman spectroscopy. *Journal of Pharmaceutical and Biomedical Analysis* **2010,** 53, (3), 412-420.

(15) Patel, A. D.; Luner, P. E.; Kemper, M. S., Quantitative analysis of polymorphs in binary and multi-component powder mixtures by near-infrared reflectance spectroscopy. *International Journal of Pharmaceutics* **2000,** 206, (1-2), 63-74.

(16) Xie, Y.; Tao, W.; Morrison, H.; Chiu, R.; Jona, J.; Fang, J.; Cauchon, N., Quantitative determination of solid-state forms of a pharmaceutical development compound in drug substance and tablets. *International Journal of Pharmaceutics* **2008,** 362, (1-2), 29-36.

(17) Pöllänen, K.; Häkkinen, A.; Huhtanen, M.; Reinikainen, S.-P.; Karjalainen, M.; Rantanen, J.; Louhi-Kultanen, M.; Nyström, L., DRIFT-IR for quantitative characterization of polymorphic composition of sulfathiazole. *Analytica Chimica Acta* **2005,** 544, (1-2), 108-117.

(18) Deeley, C. M.; Spragg, R. A.; Threlfall, T. L., A comparison of Fourier transform infrared and near-infrared Fourier transform Raman spectroscopy for quantitative

measurements: An application in polymorphism. *Spectrochimica Acta Part A: Molecular Spectroscopy* **1991,** 47, (9-10), 1217-1223.

(19) Pelletier, M. J., Quantitative analysis using Raman spectrometry. *Applied Spectroscopy* **2003,** 57, (1), 20A-42A.

(20) Chieng, N.; Rehder, S.; Saville, D.; Rades, T.; Aaltonen, J., Quantitative solid-state analysis of three solid forms of ranitidine hydrochloride in ternary mixtures using Raman spectroscopy and X-ray powder diffraction. *Journal of Pharmaceutical and Biomedical Analysis* **2009,** 49, (1), 18-25.

(21) Heinz, A.; Savolainen, M.; Rades, T.; Strachan, C. J., Quantifying ternary mixtures of different solid-state forms of indomethacin by Raman and near-infrared spectroscopy. *European Journal of Pharmaceutical Sciences* **2007,** 32, (3), 182-192.

(22) Virtanen, T.; Maunu, S. L., Quantitation of a polymorphic mixture of an active pharmaceutical ingredient with solid state 13C CPMAS NMR spectroscopy. *International Journal of Pharmaceutics* **2010,** 394, (1-2), 18-25.

(23) Fabbiani, F. P. A.; Allan, D. R.; Parsons, S.; Pulham, C. R., An exploration of the polymorphism of piracetam using high pressure, *CrystEngComm* **2005,** 7, 179-186.

(24) Pavlova, A.,Polymorphisms of Piracetam (*translation*) *Pharmazie* **1979,** 34, (449-450).

(25) Kuhnert-Brandstaetter, M.; Burger, A.; Voellenkelee, R., Stability behaviour of Piracetam polymorphs. *Scientifica Pharmaceutica* **1994,** 62, (3), 307-316.

(26) Stephenson, G. A.; Forbes, R. A.; Reutzel-Edens, S. M., Characterization of the solid state: quantitative issues. *Advanced Drug Delivery Reviews* **2001,** 48, (1), 67-90.

(27) Alexander, L.; Klug, H. P., Basic Aspects of X-Ray Absorption in Quantitative Diffraction Analysis of Powder Mixtures. *Analytical Chemistry* **1948,** 20, (10), 886-889.

(28) Burns, D. A.; Ciurczak, E. W., *Handbook of Near-Infrared Analysis*. Second ed.; 2001; Vol. 27.

(29) Roggo, Y.; Chalus, P.; Maurer, L.; Lema-Martinez, C.; Edmond, A.; Jent, N., A review of near infrared spectroscopy and chemometrics in pharmaceutical technologies. *Journal of Pharmaceutical and Biomedical Analysis* **2007,** 44, (3), 683-700.

(30) Reich, G., Near-infrared spectroscopy and imaging: Basic principles and pharmaceutical applications. *Advanced Drug Delivery Reviews* **2005,** 57, (8), 1109-1143.

(31) Raman, C. V.; Krishnan, K. S., A New Type of Secondary Radiation. *Nature* **1928**, (121), 501-502.

(32) Strachan, C. J.; Rades, T.; Gordon, K. C.; Rantanen, J., Raman Spectroscopy for Quantitative Analysis of Pharmaceutical Solids. *Journal of Pharmacy and Pharmacology* **2007,** 59, 179-192.

(33) Kachrimanis, K.; Braun, D. E.; Griesser, U. J., Quantitative analysis of paracetamol polymorphs in powder mixtures by FT-Raman spectroscopy and PLS regression. *Journal of Pharmaceutical and Biomedical Analysis* **2007,** 43, (2), 407-412.

(34) Mazurek, S.; Szostak, R., Quantification of atorvastatin calcium in tablets by FT-Raman spectroscopy. *Journal of Pharmaceutical and Biomedical Analysis* **2009,** 49, (1), 168-172.

(35) Johansson, J.; Sparén, A.; Svensson, O.; Folestad, S.; Claybourn, M., Quantitative Transmission Raman Spectroscopy of Pharmaceutical Tablets and Capsules. *Appl. Spectrosc.* **2007,** 61, (11), 1211-1218.

(36) Févotte, G., In Situ Raman Spectroscopy for In-Line Control of Pharmaceutical Crystallization and Solids Elaboration Processes: A Review. *Chemical Engineering Research and Design* **2007,** 85, (7), 906-920.

(37) Cullity, B. D.; Stock, S. R., *Elements of X-Ray Diffraction*. 3rd ed.; Prentice Hall Inc.: 2001.

(38) Barrett, M.; Hao, H.; Maher, A.; Hodnett, K.; Glennon, B.; Croker, D., In Situ Monitoring of Supersaturation and Polymorphic Form of Piracetam during Batch Cooling Crystallization. *Organic Process Research & Development* **2011,** 15, (3), 681-687.

(39) Khamchukov, Y. D.; Shashkov, S. N.; Lukomskii, I. V., Vibrational Spectra of 2-oxo-pyrrolidineacetamide. *Journal of Applied Spectroscopy* **1990,** 53, (6), 1281-1286.