An Accurate Quantitative Analysis of Polymorphic Content by Chemometric X-ray Powder Diffraction

Masafumi SUDA,* Kozo TAKAYAMA,* and Makoto OTSUKA**†

*Department of Pharmaceutics, Hoshi University, 2-4-41 Ebara, Shinagawa, Tokyo 142-8501, Japan

**Research Institute of Pharmaceutical Sciences, Faculty of Pharmacy, Musashino University,
1-1-20 Shinmachi, Nishi-Tokyo 202-8585, Japan

Data from X-ray powder diffraction (XRD) were subjected to a partial least-squares regression analysis (PLS) to build a calibration model for predicting the polymorphic content of carbamazepine (CBZ). The effectiveness of the PLS method in the construction of calibration models was analyzed by a scientific approach based on a regulation vector. CBZ forms I and III were characterized by differential scanning calorimetry (DSC) and XRD. Powder mixtures of forms I and III at various ratios (0 – 100% w/w; form III) were subjected to XRD. Five diffraction peaks were used for the peak-area method to compare with PLS. The results obtained by PLS had a better predictive accuracy compared to those of the peak-area method. The XRD-PLS method was established as a non-destructive, non-contact way to avoid the particle orientation effect based on statistical theory.

(Received October 19, 2007; Accepted February 6, 2008; Published April 10, 2008)

Introduction

Many organic pharmaceutical compounds can exist in different crystalline forms or solvates.¹ These polymorphs exhibit differing physicochemical properties, such as solubility, dissolution rate, stability, and hygroscopicity, and differing pharmaceutical properties, such as bioavailability, efficacy, degradation, and toxicity. Therefore, for drug substances which have polymorphism, pharmaceutical regulations require that an appropriate solid-state of bulk materials is provided in which only one polymorphic form is included, or specified polymorphic contents are controlled.² It is important to evaluate the polymorphic transition mechanism, and then to control the crystalline forms under stable manufacturing processes and storage conditions.³ Chemical and/or physicochemical properties concerning the polymorphic problem with pharmaceuticals have been obtained by using conventional scientific approaches, such as DTA, TG, differential scanning calorimetry (DSC), and X-ray powder diffraction (XRD) analysis. The food and drug administration discussed the adoption of process analytical technology (PAT) for regulating the manufacturing of medicines. Noncontact methods, such as near infrared spectroscopy (NIR) and Raman spectroscopy have been a focus of attention as important tools for PAT.

Chemoinformatics are employed in NIR quantitative and qualitative analyses, and have received the attention by preformulation scientists,⁴⁻⁷ since chemoinformatics, such as the classical least-squares (CLS) method, principal component analysis (PCA) and partial least squares (PLS) method provide an ideal means for extracting quantitative information from complex spectra containing multi-component samples. This analytical method combines both nondestructive inspection and

rapidly measured velocity values, since it is spectroscopic and statistical.

In a previous paper,⁸ the crystallinity and polymorphic content of crystalline medicines were evaluated by using a combination of chemoinformatics and NIR spectroscopy. The combination of chemometrics and temperature-induced Fourier-transform infrared spectroscopy (FT-IR) with attenuated total reflection (IR-ATR) spectroscopy has resulted in a greatly increased ability of achieving the precise quantification of various pharmaceutical systems.

XRD is also effective for the on-line monitoring of the manufacturing of medicines as a non-contact, non-destructive inspection technique to investigate the amount of medical substance and polymorph in medicine.⁹ However, generally, the relative amounts of two crystalline polymorphs are determined based on calibration curves constructed from a quantitative analysis of standard powders at various weight ratios physically mixed using an agate pestle in a mortar, *etc.*^{10,11} A limitation of the accuracy of the quantitative analysis is caused by insufficient homogeneity in the crystalline standard samples and the crystal orientation in the sample holder. For example, since the peak area of an XRD profile is affected by the crystal orientation and homogeneity of standard samples, the calibration curve calculated by the peak-area method is not reliable. Therefore, it is important to obtain an accurate calibration curve, but it is difficult to obtain an XRD profile without changing the crystal orientation in the packing on the glass plate, since most polymorphic crystals are very sensitive to mechanical and environmental stress. Okumura *et al.* developed a jet mill micronization and rubber ball mixing method (JMRM) to avoid a change to the crystallinity or polymorphic content during mixing of the samples.¹² However, the obtained samples were well mixed, though still heterogeneous. The results indicated the importance of the standard sample quality and the usefulness of an artificial neural network¹¹ for the accuracy of a quantitative XRD analysis.

† To whom correspondence should be addressed.
E-mail: motsuka@musashino-u.ac.jp

Carbamazepine (CBZ), an anticonvulsant used to treat epilepsy and trigeminal neuralgia, has four polymorphs and a dihydrate form.^{13–20} The three principal polymorphs (*e.g.* the primitive monoclinic polymorph (form I), trigonal polymorph (form II) and triclinic polymorph (form III)) and dihydrate (form IV) have been well characterized by XRD, DSC, ¹³C-NMR, FT-IR, and Raman spectroscopy.^{21–25}

The polymorphic content of pharmaceuticals is determined and predicted by the XRD method, but this method has relatively large errors, of around $\pm 5\%$, due to the crystal orientation when the sample powder is loaded on the glass plate. If these problems are solved, the X-ray diffraction method might be applicable to on-line pharmaceutical manufacturing processes as a non-destructive measurement method. For this reason, the chemometric XRD method was investigated as a quantitative method for the prediction of polymorphic content.

In this study, we tried to construct a calibration model not influenced by the crystal orientation of CBZ forms I and III due to pressure in order to predict the polymorphic content. PLS was employed to quantitate the XRD pattern of the polymorphic mixture, and was used to calculate the optimum statistical model. The obtained calibration curves were evaluated for the accuracy of predicting the polymorphic content by cross validation. The effectiveness of the PLS method in constructing calibration models was discussed by a scientific approach based on a regulation vector.

Materials and Methods

Materials

CBZ form I was obtained from Shizuoka Coffein Co., Ltd. (Shizuoka, Japan) and used as received. CBZ form III was prepared as reported.⁷ The samples were identified by both XRD and DSC.⁷

In accordance with the JMRM method,^{11,12} mixtures of form I and form III were obtained as follows: a total of 500 mg of powders was mixed at various ratios (0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% w/w form III) in a MM-200 vibrating mill (Retsch GmbH & Co. KG, Haan, Germany) containing three teflon balls (diameter, 20 mm; weight, 4 – 5 g; Retsch GmbH & Co. KG) for 3 min.

Scanning electron microscopy

CBZ crystals and polymorphic mixtures were observed by a scanning electron microscope (SEM). The samples were subjected to both low pressure and high pressure using a glass plate, and were coated with gold in an ion sputter JFC-1100 (Jeol Datum Co., Japan); photomicrographs of samples were taken with a SEM (Model JSM-5200LV, Jeol Datum Co., Japan).

Differential scanning calorimetry

DSC measurements were carried out at a heating rate of $1.0^{\circ}\text{C min}^{-1}$ under a nitrogen-gas purge at 60 ml min^{-1} with a DSC 8230 (Rigaku Ltd. Co., Tokyo, Japan). The measurements were conducted at least three times ($n = 3$). The polymorphic transformation temperature was defined as the endothermic peak measured by the three-point method in the DSC curve. Each sample (5 mg) was sealed with an aluminum pan.

X-ray powder diffraction

XRD profiles were recorded on a RINT-ULTIMA III (Rigaku Ltd. Co.). A 50 mg loosely packed sample was prepared to put on a glass holder. A 50 mg tightly packed sample was prepared

for pressing by a flat glass plate on the glass holder. XRD measurements were carried out at 40 kV, 40 mA with a Cu-K α source between 5 and $40^{\circ} 2\theta$ with a scan speed of $0.4^{\circ} \text{ min}^{-1}$ in the step scan mode. An optical monochromator was used parallel to a beam splitter. XRD measurements were conducted four times each for 11 standard samples. The polymorphic content of the sample was evaluated based on 44 diffraction data sets obtained by the PLS and diffraction peak-area methods. Thirty-three XRD profiles of 11 standard sample sets were used to establish a calibration model with which to predict the polymorphic content by PLS. Eleven XRD profiles of other sample sets were used to validate the model. A chemometric analysis was performed using the PLS program associated with the Pirouette software (InfoMetrix Co., USA). The conditions were optimized to minimize the standard error of cross-validation (SEV) by using PLS with the leave-one-out cross-validation (LOO) method.

With the diffraction peak-area method, the peak area was measured by the three-point method using JADE (Materials Data Inc., CA). A calibration curve was obtained based on the total area of five diffraction peaks at $2\theta = 7.9, 8.6, 9.4, 12.2$, and 13.5° , respectively. The predictability of the peak area method was evaluated to be the same as that of PLS.

Partial least squares regression

PLS regression is one of the most common regression algorithms in the fields of chemometrics and spectroscopy. PLS regression is mainly used for modeling the linear regression between multi-dependent variables and multi-independent variables. It has many advantages over ordinary multiple linear regression. For example, it can avoid harmful effects of multicollinearity, and is particularly suitable for regressing when the number of observations is less than the number of variables. In addition, PLS regression combines the basic functions of a regressing model, PCA, and a canonical correlation analysis.^{7,26–29} PLS also has the advantage that the precision of the model parameters is improved by increasing the number of relevant variables and observations. For our purposes, PLS regression is a useful algorithm because the scores of the spectral space (*X*-variable) are always correlated to those of the concentration space (*Y*-variable). This correlation between the *X*-variable and *Y*-variable corresponds to a comparison of the responses. PLS regression modeling for concentration calibration is generally formulated with the following two equations:³⁰

$$\mathbf{A} = \sum_h \mathbf{t}_h \mathbf{p}_h + \mathbf{R}_A = \mathbf{TP} + \mathbf{R}_A, \quad (1)$$

$$\mathbf{c} = \sum_h \mathbf{u}_h \mathbf{q}_h + \mathbf{R}_C = \mathbf{UQ} + \mathbf{R}_C, \quad (2)$$

where $\mathbf{A}$ and $\mathbf{R}$ are spectra and residual (or error) matrices, respectively, and $\mathbf{c}$ is a concentration vector. $\mathbf{T}$ and $\mathbf{U}$ are the scores in each equation, while $\mathbf{P}$ and $\mathbf{Q}$ correspond to the loadings, respectively. Since $\mathbf{c}$ is a vector, the concentration score, $\mathbf{Q}$, becomes a vector as well. The components of the matrices ($\mathbf{T}$, $\mathbf{U}$, and $\mathbf{P}$) and $\mathbf{Q}$ are respectively represented by $\mathbf{t}_h$, $\mathbf{u}_h$, $\mathbf{p}_h$, and $\mathbf{q}_h$.

Results and Discussion

Physicochemical characterization of CBZ forms I and III

It is reported that a transformation from form I to III occurred at 161.5°C .^{6,14} To confirm the polymorphic type, these polymorphs were identified based on DSC and XRD measurements. Figure 1 presents the DSC curve of forms I and III upon heating.

The DSC curve of form I had an exothermic peak at around 155 – 165°C due to a polymorphic transformation into form III and an endothermic peak at 191°C due to melting. The curve of form III had an endothermic peak at 191°C due to melting.

Figure 2 presents XRD profiles of form III (a) and form I (b) at room temperature. The XRD pattern (a) was identified by specific peaks of form I at $2\theta = 7.9, 9.4, 12.3, 15.1$ and 19.8° , while the diffraction pattern (b) showed specific peaks attributable to form III at $2\theta = 15.1, 20.0, 24.5$, and 27.5° . These results reflect that the diffraction patterns were significantly different. The XRD data for forms I and III identified them as monoclinic and triclinic crystalline forms, respectively.^{14,16} Forms I and III were observed as cube- and rod-shaped particles by SEM, respectively.

SEM of the pressurized polymorphic powder mixture

The diffraction peak intensity of an XRD profile is affected by

Fig. 1 DSC curves of forms I and III upon heating.

Fig. 2 XRD patterns of forms I (a) and III (b) at room temperature.

the crystal orientation, since a quantitative relationship exists between the amount of crystalline solid and diffraction intensity, based on randomized particle loading in the sample holder. If there is a crystal orientation in the sample holder under the pressurized condition, the intensity of specific diffraction peaks related to the crystal orientation might be significantly increased or decreased. In order to observe the crystal orientation of polymorphs of forms I and III under the pressurized condition, a tightly packed powder sample was subjected to SEM measurements.

Figure 3 presents SEM photographs of a polymorphic mixture, 50% (w/w), in a loosely packed sample (a) and a tightly packed sample by high pressure (b, c). In the loosely packed sample rod-shaped particles of form III were randomly placed in the holder, as shown in photograph (a). In contrast, the particles were orderly packed in the tightly packed sample by pressurization (b, c). These results showed that the polymorphic powder mixture was oriented with pressurization. Since crystal orientation induced reduction of the quantity of the XRD profile, it directly affected the peak-area intensity of the solid. For these reasons, technology to control the crystal orientation, such as a rotating sample holder and the addition of a matrix component to the sample, is needed.

Influence of packing on the XRD profile of a polymorphic mixture

The XRD diffraction peak-area method is in general predictive of the polymorphic content. The method involves drawing up a calibration curve from fluctuation values of the large peak area in specific peaks. A high-accuracy calibration curve for a multi-component system is obtained based on reproducible XRD profiles. As discussed above, the XRD profile often fluctuates with pressure when crystalline samples are oriented.

Figure 4 presents the XRD profiles of polymorphic form III mixtures in the range 0 – 100% (w/w); (a) for loosely packed and (b) for tightly packed samples. The peaks indicated by closed circles in Fig. 4 were used for the diffraction peak-area method. The arrow in the figure shows the direction the peak intensity increasing, which fluctuates with an increase of the form III ratio.

The intensity of specific peaks increased and/or decreased according to the polymorphic ratio. In general, the intensity of peaks at a low angle of diffraction fluctuated significantly, which was related to the crystal orientation. In contrast, the peaks at a high diffraction angle showed the tendency of smaller fluctuation.

In Fig. 4 (a, loosely packed sample), the intensity of the peaks increase with an increase in form III content, but fluctuate due to an increase and/or decrease in the content. As shown in Fig. 4 (b, tightly packed sample), the intensity of the peak at $2\theta = 20.0^\circ$ is due to form III increasing with an increase in form III, but the

Fig. 3 SEM photographs of CBZ: (a) physical mixture of forms I and III under the loosely packed condition, (b, c) physical mixture of forms I and III under the tightly packed condition.

Fig. 4 XRD profiles of a CBZ polymorphic powder mixture: (a) loosely packed, (b) tightly packed. The arrow shows the direction of the increase in the peak intensity, which fluctuates with an increase of form III ratio. The plot shows the angle of diffractions used for the peak-area method.

Fig. 5 Changes in the intensity of XRD profiles with the polymorphic content of form III: (a) at $2\theta = 15.1^\circ$ and (b) at $2\theta = 20.0^\circ$ (open circle, loosely packed condition; closed circle, tightly packed condition).

peaks fluctuate more than those for loosely packed samples.

Figure 5 presents the effect of the packing of the powder on the intensity of XRD profiles of CBZ standard samples: (a) specific peak of form I at $2\theta = 15.1^\circ$ and (b) specific peak of form III at $2\theta = 20.0^\circ$. The open circle shows the intensity for a loosely packed sample, and the close circle, that for a tightly packed sample.

As shown by the observation with SEM (Fig. 3), form I is a cube-shape crystal that forms independently of the particle orientation characteristics. Accordingly, it would appear that the intensity of this peak has reproducibility as shown in Fig. 5(a). In contrast, the intensity of the diffraction peak due to form III fluctuates in the plot in Fig. 5(b). In particular, the intensity of the tightly packed powder (Fig. 5(b)) fluctuated significantly. As the observation with SEM shows, form III is a rod-shape polymorph dependent on the particle orientation characteristics. Their polymorphic shape induced a crystal orientation in the sample holder when tightly packed on a glass plate. It is suggested that this phenomenon decreased the quantitative characteristics of the XRD profile. When the calibration curve is prepared based on XRD by the peak-area method, the peak at $2\theta = 20.0^\circ$ should be chosen because it is larger. However, it is necessary to care about the reproducibility of the measuring diffraction peaks due to the sample preparation conditions. The

fluctuation will affect the quantitative results.

Predictability of the polymorphic content by the XRD-peak area method

The crystal orientation and the heterogeneous mixing of standard samples induced fluctuating diffraction peaks at low-angle in the XRD profiles. The effect of the fluctuation of specific diffraction peaks on the accuracy of the determination method is investigated.

The relationship between the actual and predicted polymorph contents of form III by the peak-area method was evaluated, and the results of validation for several relationships are summarized in Tables 1 and 2. The accuracy of the calibration curve was evaluated based on the validation data set using the mean bias and mean accuracy, Eqs. (3) and (4), respectively:

$$B_m = \frac{\sum_{i=1}^n (X_p - X_t)/X_t}{n} \times 100, \quad (3)$$

$$A_m = \frac{\sum_{i=1}^n |X_p - X_t|/X_t}{n} \times 100, \quad (4)$$

where B_m is the percentage of the mean bias, A_m the percentage of the mean accuracy, X_p the predicted value of the form I content, X_t the actual value of the form I content and n the

Table 1 Combination of data sets using the calibration model and LOO cross validation

No.	Calibration data set	Validation data set
1	Loosely packed	Loosely packed
2	Loosely packed	Tightly packed
3	Loosely packed	Loosely packed + Tightly packed
4	Tightly packed	Loosely packed
5	Tightly packed	Tightly packed
6	Tightly packed	Loosely packed + Tightly packed
7	Loosely packed + Tightly packed	Loosely packed
8	Loosely packed + Tightly packed	Tightly packed
9	Loosely packed + Tightly packed	Loosely packed + Tightly packed

Table 2 B_m and A_m for the peak-area method

No.	Correlation coefficient, r	Slope	Intercept	B_m , %	A_m , %
1	0.829	0.865	18.198	38.0	38.6
2	0.918	0.851	21.999	62.5	63.0
3	0.720	0.812	33.965	150.2	150.8
4	0.827	0.760	49.732	112.3	112.3
5	0.772	0.782	43.367	138.4	49.2
6	0.831	0.815	36.799	82.9	82.9
7	0.924	0.776	26.012	50.9	51.5
8	0.849	0.812	33.965	75.1	75.4
9	0.918	0.851	22.010	52.4	53.0

number of experiments. The results for these calibration models are summarized in Table 2.

The plot for conditions Nos. 2–4 and 6 for the calibration and validation had a more linear relationship and larger correlation coefficient constant than for different conditions. B_m was calculated to be in the range of 38.0–150.2%, and A_m was in the range of 38.6–150.8%. The B_m and A_m for conditions Nos. 2–4 and 6 were larger than those for the other conditions.

Figure 6 presents the relationship between the actual and predicted polymorph content of form III by the peak-area method under condition No. 9 in Table 2. The plot gives a straight line with a correlation coefficient constant, r , of 0.918, a slope of 0.851, and a Y -intercept of 22.01. These predicted values were all within the 95% predictive intervals, but were widely dispersed, indicating that the peak-area method has little predictive potential for polymorphic content.

Predictability of the polymorphic content by the XRD-PLS method

Chemometrics can be used to decompose raw data profiles and help us to understand the significant contributions of specific data groups. In the present study, in order to predict polymorphic content, calibration models were established based on XRD profiles of a powder mixture consisting of forms I and III and their actual content by PLS.

Table 3 presents correlation parameters of the relationship with several validation approaches. All of the calibration curves have good linearity. Then, the B_m for the validation of the PLS was calculated to be in the range of –13.3–73.4%, and the A_m

Fig. 6 Relationship between the actual and predicted polymorph content of form III by the peak-area method under condition No. 9 in Table 2. The open circle shows the data set used for calibration, and the closed circle, that used for validation. The straight line is the regression line, the broken line shows 95% confidence intervals, and the dotted line shows 95% prediction intervals.

Table 3 B_m and A_m for PLS

No.	Correlation coefficient, r	Slope	Intercept	B_m , %	A_m , %
1	0.984	0.959	1.258	–13.3	44.9
2	0.966	0.740	11.250	27.1	65.2
3	0.969	0.850	6.239	–13.3	44.9
4	0.994	0.248	–0.963	73.4	86.7
5	0.996	0.975	1.155	4.3	21.5
6	0.974	1.112	0.096	73.4	86.7
7	0.996	0.996	–0.912	–9.2	16.4
8	0.989	0.985	4.761	10.6	29.9
9	0.991	0.946	1.924	–9.2	16.4

was in the range of 16.4–86.7%. In particular, the B_m and A_m values of validations Nos. 7–9 were smaller than those of the others. Since these calibration models based on multi data from loosely and tightly packed samples gave information about the crystal orientation and random packing samples, the calibration models had robust predictability for the spectral data under any conditions. On the other hand, the B_m and A_m values were larger for validations Nos. 2–4 and 6. It was conceivable that calibration models Nos. 7–9 have tolerance for the fluctuated data with the crystal orientation, since the model was constructed based on the spectra for both loosely and tightly packed conditions. The values of No. 5 were relatively small as well as those of Nos. 7–9. This is because both the calibration and validation data set samples of No. 5 were packed under the same pressure condition, and happened to result in orientation at the same degree. In contrast, it was conceivable that the errors of Nos. 2 and 3 (loosely packed conditions) and of No. 6 (tightly packed condition) were large, since the model did not have any tolerance for spectral fluctuation.

Figure 7 presents the relationship between the actual and predicted polymorph content of form III by PLS under condition No. 4 in Table 3. The plot gives a straight line, with a slope of 1.248, a Y -intercept of –0.963, and a correlation coefficient constant, r , of 0.994. The result suggests that the linear calibration model was obtained based on the data from condition

Fig. 7 Relationship between the actual and predicted polymorph content of form III by PLS under condition No. 4 in Table 3. The open circle shows the data set used for calibration, and the closed circle, that used for validation. The straight line is the regression line, the broken line shows 95% confidence intervals, and the dotted line shows 95% prediction intervals.

Fig. 8 Relationship between the actual and predicted polymorph content of form III by PLS under condition No. 9 in Table 3. The open circle shows the data set used for calibration, and the closed circle, that used for validation. The straight line is the regression line, the broken line shows 95% confidence intervals, and the dotted line shows 95% prediction intervals.

No. 4. However, the validation result of the model was not good, and some of the data was out of the 95% predictive intervals, as shown in Fig. 7. These results indicate that this calibration model was not adequate for the quantitation of non-orientated samples.

Figure 8 presents the relationship between the actual and predicted polymorph content of form III by PLS under condition No. 9 in Table 3. The plot gives a straight line, with a slope of 0.946, a Y-intercept of 0.924, and a correlation coefficient, r , of 0.911. Since all data points were within the 95% predictive intervals, the model based on the spectral data with larger variability had robust and stable predictability for polymorphic content. In addition, the B_m and A_m values of the PLS-XRD method were smaller than those of the peak-area method. It is clear from the B_m and A_m values that the XRD-PLS method is more effective and robust than the peak-area method for predicting the polymorph content. These results suggested

Fig. 9 XRD profiles of the 50% (w/w) mixture prepared under loosely packed and tightly packed conditions ($n = 3$).

Fig. 10 The RV for the calibration model obtained by PLS.

that the calibration curve for the PLS-XRD method accounted for less weight for the intensity of the crystal-orientated diffraction peaks.

Effectiveness of the PLS method in constructing calibration models

As discussed previously, PLS was effective for constructing a calibration model having high predictability. The characteristics of this method are the extraction of only target information, and the cancellation of noise. However, its effectiveness has not been verified experimentally.

Figure 9 presents XRD profiles of a 50% (w/w) CBZ mixture prepared under loosely packed and tightly packed conditions ($n = 3$). The numbers in Fig. 9 indicate 12 randomly selected diffraction peaks as an index. As shown, there are 2 peaks depending on the packing (e.g. the peaks at $2\theta = 7.9, 8.6^\circ$), and the other does not (e.g. the peaks at $2\theta = 12.2, 20.0^\circ$).

In the present study, the coefficient of variation (CV) of the peak intensity and the regression vector (RV) as a weighting function of the calibration model were calculated on a scientific basis in order to clarify the effectiveness of the PLS. CV and RV were therefore analyzed quantitatively.

Figure 10 presents the RV for the calibration model obtained by PLS. This result indicates that the peak at $2\theta = 12.2^\circ$ was most responsible for the positive correlation, and the peak at $2\theta = 15.1^\circ$ was most responsible for the negative correlation

Fig. 11 The effect of the CV of peak intensity on the RV for the calibration model obtained by PLS.

against increasing form III.

Figure 11 presents the effect of the CV of the peak intensity on the absolute value of RV ($|RV|$) for the calibration model obtained by PLS. This relationship showed that $|RV|$ is inversely proportional to CV on a semi-logarithmic plot. For example, the 7th angle of diffraction is important in the present calibration model. In contrast, the 1st angle of diffraction was almost negligible. Since the CVs at the 7th and 4th angles of diffraction were smallest, and those at the 1st and 2nd angles of diffraction were largest, the relationship between CV and RV had a scientific basis, because the PLS regression can avoid any harmful effects in modeling due to multicollinearity. Accordingly, PLS was calculated quantitatively using the change in the diffraction angle, which has non- or less-fluctuation selectively. It was suggested that this selectivity contributed to an improvement in the accuracy of the calibration model; it was also demonstrated experimentally that the PLS efficiently extracted information from the diffraction profiles, and cancelled noise.

Conclusion

The XRD method is described in the pharmacopoeia as an important way to evaluate the polymorphism and crystallinity of medicines. Since there are some problems with XRD, such as particle orientation in preparing crystalline samples, the degree of precision is relatively low. However, if these problems are solved, the XRD method can be used for the high-quality control of medicine in a non-destructive, non-contact analysis. In the present study, the XRD-PLS method was established for a non-destructive, non-contact analysis to avoid the effect of particle orientation based on statistical theory. The XRD-PLS method was therefore demonstrated as being potentially effective for the non-destructive, non-contact analysis for PAT.

Acknowledgements

This work was supported in part by a Grant-in-Aid (Scientific Research, C, No. 17500322) for Scientific Research and HAITEKU (2004 - 2008) from the Ministry of Education, Culture, Sports, Sciences and Technology, Japan.

References

1. J. K. Halebian and W. McCrone, *J. Pharm. Sci.*, **1969**, 58, 911.
2. ICH Harmonized Guideline. Q6A Specifications, Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products, Chemical Substance, **1999**.
3. S. Byrn, R. Pfeiffer, M. Ganey, C. Hoiberg, and G. Poochikian, *Pharm. Res.*, **1995**, 12, 945.
4. A. J. Florence, A. Johnston, S. L. Price, H. Nowell, A. R. Kennedy, and N. Shankland, *J. Pharm. Sci.*, **2006**, 95, 1918.
5. I. M. Palabiyik, E. Dinç, and F. Onur, *J. Pharm. Biomed. Anal.*, **2004**, 34, 473.
6. R. Kramer, *“Chemometric Techniques for Quantitative Analysis”*, **1998**, Marcel Dekker, New York.
7. T. Hasegawa, *“Quantitative Analytical Techniques of Spectra”*, **2005**, Khodansya Scientific, Japan.
8. M. Otsuka, Y. Fukui, K. Otsuka, H. J. Kim, and Y. Ozaki, *J. Near Infrared Spectrosc.*, **2006**, 14, 9.
9. H. Yoshino, Y. Hagiwara, S. Kobayashi, and M. Samejima, *Chem. Pharm. Bull.*, **1984**, 32, 1523.
10. L. S. Zevin and G. Kimmel, *“Quantitative X-ray Diffractometry”*, **1995**, Springer, New York.
11. T. Okumura, M. Nakazono, M. Otsuka, and K. Takayama, *Colloids Surf., B*, **2006**, 49, 153.
12. T. Okumura and M. Otsuka, *J. Pharm. Sci.*, **2005**, 94, 1013.
13. T. Umeda, N. Ohnishi, T. Yokoyama, K. Kuroda, T. Kuroda, E. Tatsumi, and Y. Matsuda, *Yakuzaigaku Zasshi*, **1984**, 104, 786.
14. L. E. McMahon, P. Timmins, A. C. Williams, and P. York, *J. Pharm. Sci.*, **1996**, 85, 1064.
15. F. U. Krahn and J. B. Mielck, *Pharm. Acta Helv.*, **1987**, 62, 247.
16. J. Dugu'e, J. Ceolin, J. C. Rouland, and F. Lepage, *Pharm. Acta Helv.*, **1991**, 66, 307.
17. R. Ceolin, S. Toscani, M. F. Gardette, V. N. Agafonov, A. V. Dzyabchenko, and B. Bachet, *J. Pharm. Sci.*, **1997**, 86, 1062.
18. Y. Matsuda, R. Akasawa, R. Teraoka, and M. Otsuka, *J. Pharm. Pharmacol.*, **1994**, 46, 162.
19. C. Rustichelli, G. Gamberini, V. Ferioli, M. C. Gamberini, R. Ficarra, and S. Tommasini, *J. Pharm. Biomed. Anal.*, **2000**, 23, 41.
20. N. Kaneniwa, J. Ichikawa, T. Yamaguchi, K. Hayashi, N. Watari, and M. Sumi, *Yakuzaigaku Zasshi*, **1987**, 107, 808.
21. A. L. Grzesiak, M. Lang, K. Kim, and A. J. Matzger, *J. Pharm. Sci.*, **2003**, 92, 2260.
22. F. Tian, J. A. Zeitler, C. J. Strachan, D. J. Saville, K. C. Gordon, and T. Rades, *J. Pharm. Biomed. Anal.*, **2006**, 40, 271.
23. K. Park, J. M. B. Evans, A. S. Myerson, *Cryst. Growth Des.*, **2003**, 3, 991.
24. L. E. McMahon, P. Timmins, A. C. Williams, and P. York, *J. Pharm. Sci.*, **1996**, 85, 1064.
25. M. R. J. Hachey, A. Bogomolov, K. C. Gordon, and T. Rades, *Raman Tech. for Today's Spectroscopists*, **2004**, 16.
26. S. Wold, J. Trygg, A. Berglund, and H. Antti, *Chemom. Intell. Lab. Syst.*, **2001**, 58, 131.
27. I. S. Helland, *Chemom. Intell. Lab. Syst.*, **2001**, 58, 97.
28. P. Gramatica, P. Pilutti, and E. Papa, *J. Chem. Inf. Comput. Sci.*, **2004**, 44, 1794.
29. B. Nadler and R. R. Coifman, *J. Chemom.*, **2005**, 19, 45.
30. T. Hasegawa, J. Nishino, M. Sonoyama, and T. Nakano, *Appl. Spectrosc.*, **2002**, 56, 288.