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A Comparative Study on Quantification of Binary Polymorphic Mixtures of Nateglinide by Using Powder X-Ray Diffraction, Differential Scanning Calorimetry and Near Infrared Spectroscopy

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Abstract: The aim of this study is to evaluate the ability of powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC) and near infrared (NIR) spectroscopy to quantify two polymorphic forms (B, H) of nateglinide in binary powder mixtures. For this purpose, univariate and multivariate method are developed. The results show that PXRD (with PLS regression) provides a reliable determination of nateglinide samples (predicted accuracy of 89.1%), which is the best choice for quantification of nateglinide polymorphic mixtures in practice, while NIR spectroscopy is the least accurate (predicted accuracy of 79.6%) mainly due to the effect of sample inhomogeneity. Though a good linear relation and a high predicted accuracy (93.4%) for DSC are successfully obtained, it could not be a practical way for low-content quantification in nateglinide polymorphic mixtures.

Key words: nateglinide polymorphs, quantification, powder X-ray diffraction, differential scanning calorimetry, near infrared spectroscopy

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基于 PXRD、DSC 和 NIR 技术的那格列奈多晶型的定量分析方法研究

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[**摘要**] 从晶体学、热力学和光谱学三个角度展开对那格列奈多晶型的定量分析研究,通过 X-射线粉末衍射法、差示扫描量热法及近红外光谱分析技术,并结合化学计量学的方法,建立了那格列奈 B 晶型和 H 晶型二元混合体系的定量分析模型. 结果表明,近红外光谱法虽然操作简单快速,但模型的预测准确率和方法的灵敏度较低;差示扫描量热法虽然准确度较高,但实用性较差;利用 PXRD 结合 PLS 法得到的结果最好,模型比较稳定,不易受样品的影响,可用于实际工作中. 为那格列奈制剂产品及其他多晶型药物的定量分析奠定了理论基础.

[**关键词**] 那格列奈多晶型,定量分析,X-射线粉末衍射法,差示扫描量热法,近红外光谱法

Polymorphism is a well recognized phenomenon, i.e. a solid material existing in two or more crystalline phases with different arrangements or conformations in the crystal lattice[1-2]. Drug polymorphs are different crystalline forms of the same drug substance. It has been estimated that more than half of active pharmaceutical ingredients (APIs) exist in more than one polymorphic form. Different API polymorphs have different physical and chemical properties, thus causing changes in the solubility, stability, dissolution, bioavailability, and final efficacy of drugs[3-4]. The stringent regulatory constraints forces pharmaceutical companies to deal with polymorphism of

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APIs and the pharmaceutical polymorph analysis improvments[5-8]. The quantitative solid state analysis in polymorph has also been increased. Detection of low level impurity is a must for quality assurance[1,9].

Many analytical techniques have proven suitable for quantification of polymorphs in polymorphic mixtures, including powder X-ray diffraction(PXRD)[9-19], thermal analysis[16,20-24], solid-state nuclear magnetic resonance spectroscopy(ssNMR)[9,25-26], optical microscopy and vibrational spectroscopy (including infrared (IR), Raman)[9-10,13,27-31]. Among these techniques, Powder X-ray diffraction(PXRD) has been the gold standard for polymorph analysis as polymorphs have unique crystal structure[31-32]. There are two primary quantification methods, i.e. single peak method and whole pattern method[9]. The single peak quantification is based on the intensity of individual diffraction peaks which is related to the concentration of the component in the mixture[33]. However, this method should be used when the preferred orientation is minimized[18]. Therefore, it is important to choose right instrument settings and sample preparation parameters to reduce preferred orientation and attain good homogeneity in the calibration samples. The whole pattern method such as the factor-based Partial Least Square (PLS)[11,13,34-35], the Reference Intensity Ratio (RIR) method[34], the whole powder-pattern decomposition method(WPPD)[36-37] and the Rietveld method[38] uses the whole pattern to establish the relationship between phase composition and the intensity of patterns of the phases being quantified. The Rietveld method permits more accurate and precise quantification, since it is less sensitive for experimental errors concerning the sample properties and preparation, especially preferred orientation effects. However, its use in practical aspects of quantitative phase analysis is still limited by its requiring prior information about the sample's structure. Although there are adaptions for the quantitative analysis of materials with partially or unknown structures[39], reports on polymorphic quantification of drug subtances with unknown structures using the Rietveld method are scarce perhaps because organic drugs have worse stability in contrast to inorganic materials.

Vibrational spectroscopic methods can also be used. NIR is concerned with the overtones and combination modes of fundamental molecular vibrations[13]. Univariate calibration is sometimes enough to provide accurate and precise quantification of polymorph composition. Multivariate analysis such as partial least square (PLS) regression and principal component regression(PCR) should be used to improve the accuracy and robustness of the calibration model due to overlapping peaks or peak shifting[10-11,13,40-44].

Differential scanning calorimetry(DSC) has also been recently used as a method for quantifying polymorphs with well-resolved endothermic or exothermic transitions[45]. DSC does not require a large amount of sample, nor chemical treatment with organic solvents or time-consuming manipulation practices prior to measurement[23]. Bruni et al[20] suggested a method for the assessment of the polymorphic purity of nateglinide in binary mixtures by DSC with the B one from the melting peak of B. Although a good linear relationship($R^2 = 0.997$) was obtained between the melting enthalpy of the B form against the H nominal content in mixtures of known composition, there is no further study on the validation of the proposed method. It is very difficult to ensure accurate and reliable quantification by using only a technique. Nevertheless, to the best of our knowledge, there have been no reports dealing with the quantification of nateglinide polymorphs with a comparative method using different techniques. There are distinct X-ray diffraction reflections and also unique NIR bands for both nateglinide polymorphs, indicating that these two analytical techniques are suitable for quantitative purpose. Considering these aspects, the development and comparision of quantification models for binary polymorphic mixtures of nateglinide using PXRD, DSC and NIR spectroscopy are described in this paper.

Nateglinide, (Fig. 1) (−)-N-[(trans-4-isopropylcyclohexane carbonyl)-D-phenylanine], is a drug used to lower sugar levers in patients with type 2 diabetes by stimulating release of insulin[46] that can exist in four polymorphs(H, B, S and X2)[47-49]. In the

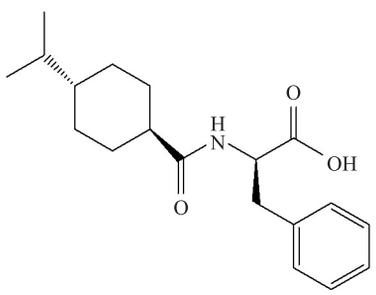

Fig. 1 Chemical structure of nateglinide

case of nateglinide, Sumikawa and co-workers[49] first reported the crystals of form B and form H. According to the report, the form B suffers from problems of instability, especially when subjected to the mechanical grinding. So it is not ideal for use in medicine. Form H is pharmaceutically preferred because they have better stability. In our early studies, we have found new crystal forms of nateglinide named form S and form X2[47-48]. We have studied the solubility and stability of the three crystal forms (H, B, and S) and determined the crystal structure and drug effect of form H and form S[47-48,50]. Although the result indicates that S-form nateglinide has potential usage as a new oral antidiabetic agent, the form H is still in great demand on the market. Occasionally in our work we detect unquantified amounts of form B in the tested nateglinide form H. Thus, it is necessary to develop a simple, sensitive and accurate method to quantify the amount of form B in polymorphic (forms B and H) mixtures of nateglinide. In this study we develop and validate three methods to quantify the nateglinide polymorphs and evaluate their application by studying an unquantified commercial nateglinide sample. The results provide valuable information with regard to the evaluation of the quality of commercially available nateglinide raw material products.

1 Materials and Methods

1.1 Materials and polymorph preparation

Nateglinide polymorph H was courtesy of Deyuan Pharmaceutical Co., Ltd. (Jiangsu, China) and used as received. Pure polymorph B was obtained by recrystallization method[50]. For this, 500 mg of polymorph H were dissolved in ethanol-water (60:40, v/v) at 30 ℃, and the solution was cooled to 5 ℃, with stirring. The precipitated crystals were filtered, dried at 90 ℃, under reduced pressure overnight, and then stored under ambient conditions for 3 ds. All solvents used were reagent grade. Solvent mixture was prepared by mixing two solvents of predetermined volume. A confirmation of the polymorph identity was performed through SEM, PXRD, DSC and FTIR, comparing with previous results described in the literature[48-49].

All binary mixtures of polymorphs were obtained by mixing the pure dry samples. Because the grinding of nateglinide polymorph B will induce the phase transformation[20], the samples were mixed in an ultrasonic method instead of a ground method. The possibility of any phase change occurring during ultrasonic stirring was ruled out by testing pure nateglinide form B and H before and after ultrasonic stirring. For PXRD and NIR analysis, 100 mg binary calibration mixtures containing 0, 1%, 2%, 4%, 6%, 8%, 10%, 20%, 30%, 40%, 60%, 80% and 100% of form B, separately, with the remaining mass balance provided by form H, were dispersed for 15 min with ultrasonic stirring (40 kHz). The mixtures were filtered and dried for 3 ds at room temperature. Validation mixtures containing 5%, 15%, 25%, 50% and 75% of form B were prepared in the same way. For DSC analysis, the two forms were individually weighted in the DSC pans.

1.2 Instrumentation, data collection, and analysis

Scanning electron microscopy (SEM) (JEOL, JSM 5610LV) on gold-coated specimens was used to examine the morphological features of the particles of nateglinide form B and H, using an accelerating voltage of 15 kV.

PXRD patterns were obtained at room temperature on Rigaku D/max-2500VL/PC diffractometer (Rigaku Co., Japan) using Cu-Kα X-radiation ($\lambda = 1.541\ 8$ Å) at 40 kV, 200 mA passing through a curved graphite single crystal monochromator with divergence slit of 1°, antiscattering slit of 1°, and receiving slit of 0.15 mm. Data were recorded over the range 3°-40° (2θ), using a step size of 0.02° (2θ), a count time 1s per step. Each studied sample was manually loaded in a glass holder (20 mm×20 mm×0.2 mm) made of boron and pressed by a clean glass slide to ensure coplanarity of the powder surface with the surface of the holder. All the X-ray diffraction experiments were performed in triplicate and a rotating sample holder was used to reduce preferred orientation effect. Obtained diffractograms were analyzed with MDI Jade6.5 software furnished with the PXRD.

DSC measurements were performed in triplicate using an Analysis Diamond DSC (Perkin Elmer Co., USA). The instrument was calibrated for temperature and heat flow using indium as the standard. The samples

(~2.0 mg) were placed in sealed aluminum pans under nitrogen purge at flow rate of 25 mL min^{-1}. The temperature range was 25 ℃-160 ℃ at a ramp of 10 ℃/min. Data acquisition and analysis were performed using the Pyris software. For enthalpy calculation, the start and end points for the integration of the thermal peak were identified by visual inspection.

The FTIR spectra were obtained on a NEXUS-670 FTIR spectrophotometer (Thermo Nicolet Co., USA), in transmission mode by KBr pellets. The spectrum for each sample was recorded using Omnic 5.1 software over the 4 000 cm^{-1}-400 cm^{-1} spectral region with 32 accumulations at a resolution of 4 cm^{-1}.

NIR data were collected using a Cray 5000 UV-Vis-NIR spectrophotometer (Varian Co., USA) fitted with a specular reflectance accessory (SRA), in the spectral region of 10 000 cm^{-1}-4 000 cm^{-1} (1 000 nm-2 500 nm), at a spectral resolution of 0.2 nm. NIR spectra were acquired using the Cray WinUV software. Before each analysis, a background spectrum of calcium sulfate was recorded. Sample vials were repositioned between triplicate measurements of each sample.

Multivariate data analysis was carried out using the Unscrambler v9.7 software (Camo, Norway). The data was divided into calibration set to build a quantification model and a prediction set to validate the model. Partial least square (PLS) regression was used to build calibration models from PXRD and NIR spectra collected for nateglinide polymorph mixtures at different polymorphic contents. To remove unimportant baseline (offset) interferences from samples or correct scatter effects and accentuate spectral signals of interest, different pre-processing methods, including multiplicative scatter correction (MSC), standard normal variate (SNV), the first and second derivative and their combinations were applied for 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. A full cross validation method (Leave-One-Out validation) was carried out to select the optimal number of PLS factors. PLS model quality was assessed on the basis of: root-mean-square error (RMSE) of calibration (RMSEC), root-mean-square error from cross-validation (RMSECV), root-mean-square error of prediction (RMSEP), and the coefficient of determination (R^2) between predicted and measured values. RMSE was calculated as follows,

$$\mathrm{RMSE} = \sqrt{\frac{\sum_{i=1}^{n} (y_i - Y_i)^2}{n}}. \quad (1)$$

where y_i, Y_i and n are calculated value, the theoretical value (neglecting the possiblity of inaccurate mixture preparation, this is considered to be equal to the nominal value) and the number of measurements, for calibration, cross-validation and prediction. The predicted accuracy was used to evaluate the accuarcy of the developed models. The predicted accuracy was calculated as follows,

$$\text{Predicted accuracy}(\%) = 1 - |y_i - y| / y. \quad (2)$$

where y is the measured value of form B in nateglinide binary mixtures, y_i is the mean value of predicted form B content in nateglinide binary mixtures.

The limit of detection (LOD) and quantitation (LOQ) were estimated as 3.3 and 10 times the standard deviation of blank divided by the slope of the calibration curve, respectively[51], with the modification that instead of blank (corresponding pure form) the 5% mixtures were used, which were measured three times.

The practical application value of the methods developed was evaluated by an unquantified sample from a pharmaceutical manufacturer.

2 Results and Discussion

2.1 Characterization of nateglinide polymorphs

Pictures of nateglinide form B and form H crystals are shown in Fig. 2. Form B crystals are rod-like while form H are acicular. Fig. 3 shows the PXRD patterns of nateglinide polymorphs before and after ultrasonic

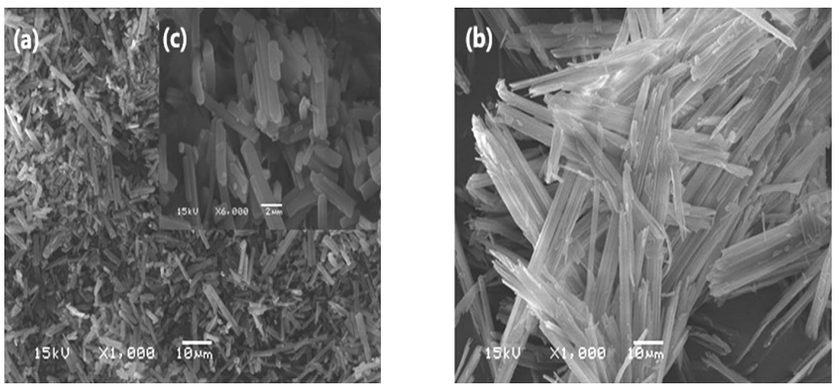

Fig. 2 SEM images of form B(a), form H(b) of nateglinide and partial enlarged image(c)

stirring. The results show that the form B and form H do not induce polymorph transition after ultrasonic stirring. The diffraction pattern of form H shows maxima at 2θ values of 8.1°, 13.1°, 19.6° and 19.9° while the form B gives strong reflections at 2θ values of 4.8° and 14.0°. The experimental patterns of form B and form H correspond well with the patterns reported in US patent 5,488,150[49], and show no evidence of contamination by other polymorphic forms. Both forms show intense peaks indicating their crystalline nature. The DSC curves for nateglinide form H and form B at a ramp of 10 ℃/min are shown in Fig. 4(a). For form H, only a single endothermic melting peak with an onset temperature of 139.0 ℃ and an enthalpy of 95.1 J/g is observed at the heating rate. Form B on the other hand, also shows only a single endothermic melting peak with an onset temperature of 129.7 ℃ and an enthalpy of 90.1 J/g. These results are concordant to a previously reported study[20]. The single melting peak for either polymorph indicates the presence of only one polymorph and there are no polymorphic impurities within the sample and no phase transformation during heating. In FTIR spectra (Fig. 4(b)), the main region of 1 750 cm^{-1}–1 650 cm^{-1} (C=O stretching) identifies and distinguishes the form B and from H of nateglinide. Form B absorptions occure at 1 746 cm^{-1}, while peak 1 714 cm^{-1} is shown in form H.

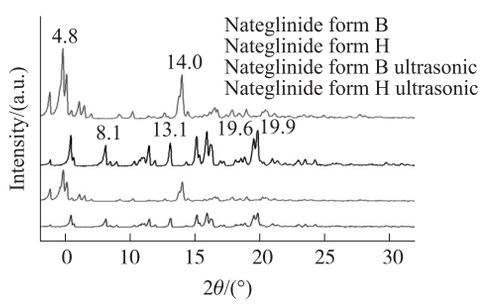

Fig. 3 PXRD patterns of form B and H of nateglinide, as well as ultrasonic form B and ultrasonic form H, from top to bottom, respectively

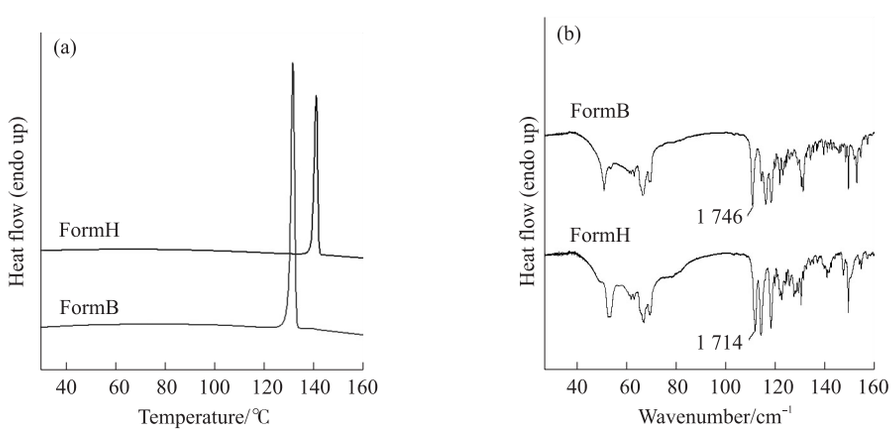

Fig. 4 (a) DSC curves of nateglinide form H and form B at 10 ℃/min heating rate. (b) FTIR spectra of nateglinide form B and H

2.2 Calibration models built using PXRD, NIR and DSC

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 is desired. For nateglinide form B, the peak at 4.8° 2θ is selected for quantification. Out of the four characteristic peaks of form H at 2θ values of 8.1°, 13.1°, 19.6° and 19.9°, peaks at 19.6° and 19.9° 2θ are selected for quantification of form H in binary mixtures. The differences are radically interpretable at 2θ with the change in concentration(Fig. 5). The other two intensity peaks exhibit somewhat lower R^2 values(less than 0.946) and are thus not used for quantification. Also, the peaks selected are used for identifying the crystalline nature of nateglinide form H. Two peaks at 2θ values of 19.6° and 19.9°(2θ) must be separated and the peak at 4.8° 2θ can not be existed. Thus a simple univariate quantification method is tried using PXRD data. The data are calculated with MDI Jade6.5 software.The peak at 4.8° 2θ is integrated from 3.90° to 4.88°(2θ) and this area monitors the level of form B in the samples. The separate peaks at 19.6° and 19.9°(2θ) are integrated from 19.20° 2θ to 19.94°(2θ). The calibration curve is constructed by calculating the percentage of form B using peak height intensity and area(Eq.(3)) and relating this to the measured percentage weight composition. The univariate calibration correlations for form B calculated using peak height and peak area are presented in Fig. 6. From Fig. 6, good linear correlations(R^2 = 0.991 and 0.988) are both achieved with peak height intensity data and peak area within the range of 0%−100% of form B. The calibration models are tested by using a set of validation standards, and the RMSEP values are presented in Table 1.

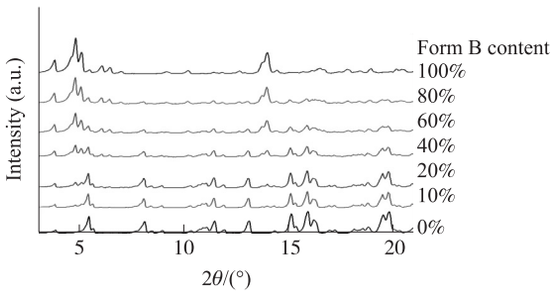

Fig. 5　PXRD patterns of different nateglinide form B and form H binary mixtures

$$X_A = \frac{I_A}{I_A + I_B} . \tag{3}$$

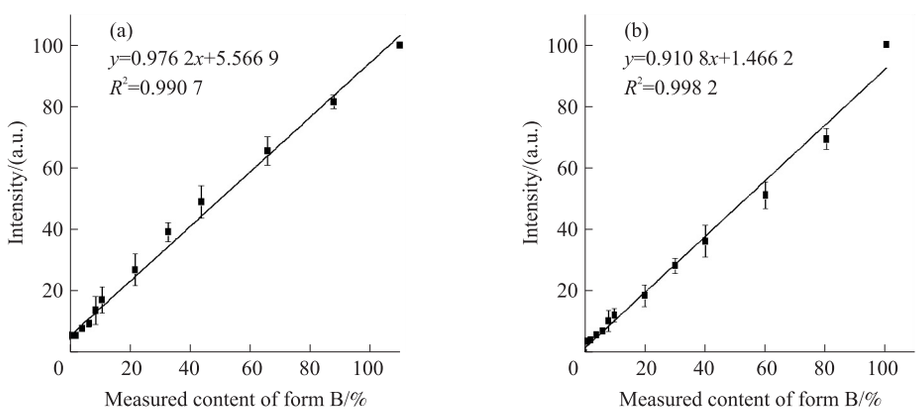

Fig. 6　Calibration curve for determination of the%(by mass) in mixtures of form B and form H using (a) peak height and (b) peak area as per Eqn.(3)(n=3)

Table 1　Performance of regression models for quantifying form B in binary mixtures using different analytical methods

Methods	Pretreatments	R^2	PLS factors	RMSEC/%	RMSECV/%	RMSECP/%
PXRD(peak height)		0.991		3.13		2.68
PXRD(peak area)		0.988		3.29		3.42
DSC		0.998		1.44		1.64
PXRD(PLS)	Mean normalization	0.997	3	1.50	1.53	1.75
NIR(PLS)	SNV	0.974	5	1.33	2.95	4.53

Multivariate calibrations were performed on the 3.9°−20.1°(2θ) range, as shown in Fig. 5. The PLS regression analysis uses 39 PXRD scans from 13 calibration samples. All the diffractograms are subjected to baseline correction in the selected range prior to PLS regression. During regression, 4 inaccurate diffractograms are rejected and the remaining 35 diffractograms are selecteded for calibration. 12 diffractograms are used for valida-

tion. The best calibration model (requiring 3 PLS factors) is achieved for the quantification of form B in the binary mixtures when PXRD patterns of calibration samples are subjected to mean normalization. The predicted form B contens from the PLS model are plotted against the actual form B contents in Fig. 9 (a). The entire validation data set is within the 95% confidence intervals indicating reasonable reliability of our model. Table 1 summarizes the quality indicators of our calibration model and the results show a significant improvement over the univariate analysis using peak height or peak area. The results of LOD and LOQ for PXRD method are listed in Table 2.

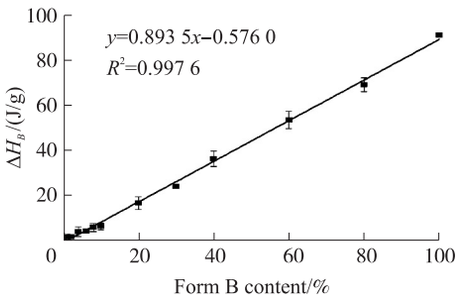

Fig. 7 Calibration curve of nateglinide form B from DSC (n=3)

The melting enthalpies of form B are used for calibration and the calibration curve is plotted in Fig. 7. The enthalpy of form B shows good linear relationship (R^2=0.998) to form B content. The result is similar to the calibration curve reported by Bruni G. et al[20].

Table 2 Comparison of the three techniques for the quantification of form B in an unknown mixture and calculated limts of detection (LOD) and quantification (LOQ)

	Form B content (%) determined by				
	PXRD (peak height)	PXRD (peak area)	DSC	PXRD (PLS)	NIR (PLS)
Sample	2.46±0.59	4.07±0.80	0.65±0.00	5.56±0.70	28.21±1.57
LOD	4.16	2.50	3.86	1.79	11.58
LOQ	12.60	7.58	12.87	5.42	35.07

Fig. 8 shows NIR spectra for pure and mixtures of nateglinide polymorphs in the spectral region from 10 000 cm^{-1} to 4 000 cm^{-1}. From Fig. 8(a), the bands between 5 000 cm^{-1}−4 000 cm^{-1} are assigned to combinations of C−H and N−H stretching and bending vibrations. The first overtone of O−H and C−H stretching vibrations are observed around 6 600 cm^{-1} and 5800 cm^{-1}, respectively. In addition, the second overtone of C−H stretching arises around 8 700 cm^{-1} and 8 400 cm^{-1}. Comparing the NIR spectra for each polymorph, it can be seen that form B has the almost same absorbance as form H in the range of 10 000 cm^{-1}−7 000 cm^{-1}, and the main differences between them are related to intensity and width of bands for the first overtone of O−H stretching (6 600 cm^{-1}). As such, PLS regression anlysis is carried out using the range of 7 000 cm^{-1}−4 000 cm^{-1}. Due to scattering and other effects, a set of NIR spectra on similar samples often exhibit constant baseline offsets from one to the next. To eliminate baseline offset differences, reduce scattering effects, and increase the resolution of neighboring peaks, first- or second-derivatization is often applied to NIR spectra prior to their use in calculations. Other preprocessing techniques, such as SNV or MSC, may be applied to more effectively reduce scattering effects that arise from partical size differences among samples[9]. Therefore, the optimal PLS model is generated after assessing the effects of several different pretreatments including SNV, MSC, derivative and their combinations. 54 NIR spectra from 13 calibration samples and 5 validation samples are used for the PLS regression analysis. Initially, the optimal PLS model was obtained when NIR data are subjected to SNV pretreatment. During regression, 4 inaccurate spectra are rejected and the remaining 35 spectra are selecteded for calibration. 12 spectra are used for validation (independent test). The RMSECV is plotted as a function of the number of PLS factors in the model. The best PLS model (requiring 5 PLS factors) is achieved for the quantification of form B in these two binary mixtures. A good linear correlation (R^2 = 0.998) is obtained, but with higher RMSECV and RMSEP values (relative to PXRD). And the performance of the NIR correlation curve between the predicted and measured content of form B in nateglinide binary mixtures is obviously inferior to the PXRD model. As seen in Table 1 and Fig. 9(b), there are data points outside the 95% confidence intervals and the 95% confidence intervals are much wider than that of PXRD. The higher RMSECV and RMSEP values by NIR may be due to sample inhomogeneity and sample

packing. The polymorphs may be unevenly distributed in the mixture and the sampling area may not be representative for the overall content in the sample. The results of LOD and LOQ for the multivariate NIR based models are listed in Table 2.

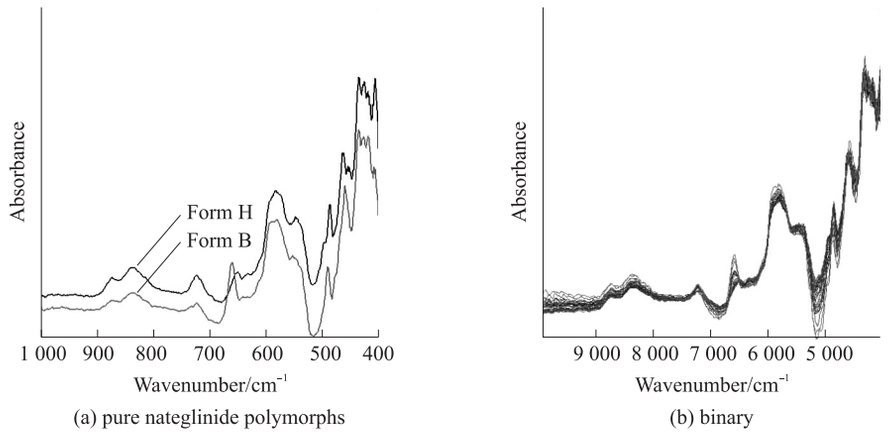

Fig. 8 NIR spectra of nateglinide polymorphs: (a) pure nateglinide polymorphs (b) binary mixtures

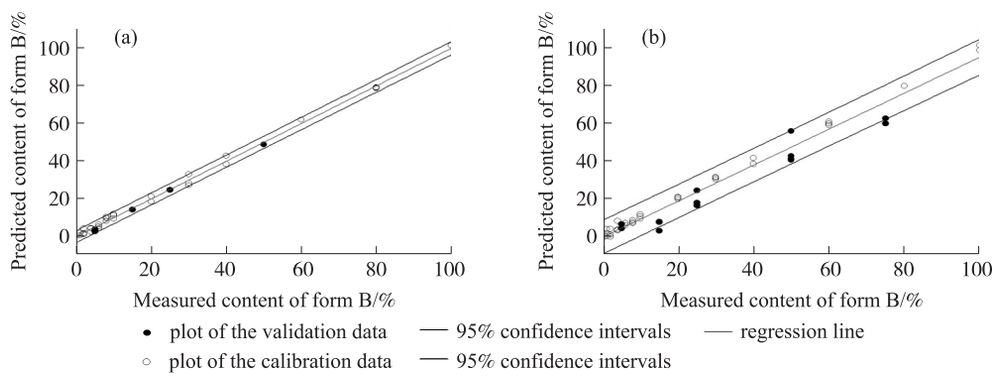

Fig. 9 (a) Correlation curve of predicted vs. measured content of form B in nateglinide binary mixtures using PXRD by PLS analysis. (b) Correlation curve of predicted vs. measured content of form B in nateglinide binary mixtures using NIR data by PLS analysis

2.3 Comparision of the three techniques

The above results clearly show quantification of nateglinide form B in binary mixtures with form H by PXRD, DSC and NIR. Among them, NIR and PXRD require careful sample preparation. In contract, DSC appears to be a more convenient method since no special sample preparation is required and calibration is simple and straightforward. Besides, the predicted accuracy of regression models for quantifying form B in binary mixtures using different analytical methods is calculated (Table 3).

Table 3 Results of the predicted accuracy of regression models for quantifying form B in binary mixtures using different analytical methods

Form B content/%	Predicted accuracy/%				
	PXRD(peak height)	PXRD(peak area)	DSC	PXRD(PLS)	NIR(PLS)
5	59.6	97.4	96.0	70.2	99.8
15	96.3	84.1	83.3	93.4	48.2
25	98.4	81.4	99.1	98.1	80.8
50	95.7	89.5	95.1	97.1	89.7
Mean value(%)	87.5	88.1	93.4	89.7	79.6

The results show that DSC proves to be the most accurate (predicted accuracy of 93.4%), while NIR is the least accurate (predicted accuracy of 79.6%). PXRD with peak height, peak area and PLS analysis shows similar results. Calculated values are 87.5%, 88.1% and 89.1%, respectively. PXRD with peak height and PLS analysis give a lower predicted accuracy for low content quantification in polymorphic mixtures. As can be seen from Table

2, PXRD with peak area and PLS analysis give lower LOD and LOQ values. Similar results are observed for DSC and PXRD with peak height, while higher values (LOD = 11.58%, LOQ = 35.07%) are observed for NIR. To further compare the practical application values of these three techniques, an unqualified sample from a pharmaceutical manufacturer are analyzed (Table 2). It is obvious that PXRD provides similar determination of the polymorphic content (between 2% and 6%) in our system, while NIR gives a high determination of form B content (~30%). Surprisingly, DSC gives a very low determination of the polymorphic content (~0%) in our system.

The poor performance by NIR and DSC is mainly due to sample inhomogeneity and sample packing. The polymorphs may be unevenly distributed in the mixture and the sampling area may not be representative for the overall content in the sample. Despite DSC appears to be a more convenient method and the calibration is simple, accurate and straightforward, there are potential drawbacks concerned with our current approach. An obvious problem is that DSC method requires only a small amount of sample (2 mg-10 mg), whereas PXRD method requires at least 100 mg, low-content quantification in powder mixtures using DSC may not be easily detected with sampling inhomogeneity. This means that the DSC method will not be suitable for quantifying low content in polymorphic mixtures. In other words, the DSC method is not applicable in practice. Therefore, PXRD technique is the best choice for quantification in nateglinide polymorphic mixtures.

3 Conclusions

Binary polymorphic mixtures of nateglinide have been prepared and analyzed quantitatively by PXRD, DSC and NIR spectroscopy, coupled with univariate and multivariate analysis. Practically comparing the three techniques, PXRD (with PLS regression) provides a reliable determination of nateglinide mixtures. It is the best choice for quantification in nateglinide polymorphic mixtures in practice. NIR spectroscopy is found to be the least accurate method for the model compound studied. Though a good linear calibration curve for DSC is successfully obtained, it could not be a practical way for low-content quantification in nateglinide polymorphic mixtures.

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