Method Validation for the Analysis of Licorice Acid in the Blending Process by Near Infrared Diffuse Reflectance Spectroscopy

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Abstract:

The present work described validation of near infrared (NIR) method for the quantification of the concentration of Licorice acid in the blending process of Licorice and talcum powder mixtures. The NIR diffuse reflectance spectra of samples were collected during the mixing process and the partial least square (PLS) model was developed. The accuracy profile (AP) approach that was fully compliant with the ICH Q2 (R1) guideline was used in order to assess the validity of the NIR chemometric method. Particularly, the $\beta$-content, $\gamma$-confidence tolerance interval, instead of $\beta$-expectation tolerance interval in the AP methodology, was introduced to provide a better estimate of measurement risk. The quantitative validation criteria such as trueness, precision (both repeatability and intermediate precision), results accuracy and valid range were obtained. The lower limit of quantification (LLOQ) was 1.26 mg·g\textsuperscript{-1}. Results demonstrated that NIR spectroscopy is suitable for the analysis of the concentration of Licorice acid. And the risk of using the established analytical method

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in routine phase could be well visualized and controlled.

**Key words:** NIR diffuse reflectance spectroscopy; Method validation; *Licorice* acid; Accuracy profile; $\beta$-content, $\gamma$-confidence tolerance interval

1. Introduction

Near infrared (NIR) spectroscopy has become a widely used analytical technique in pharmaceutical industry due to its high speed acquisition, non-destructive nature, capacity to measure both physical and chemical properties, and the fact that it needs little or no sample preparation[1]. Therefore, near infrared spectroscopy (NIRS) is more and more considered as an attractive and promising analytical tool for process analytical technology (PAT).

Once a calibration model for NIR analysis is developed and favorable predictions are expected, the method must be validated to comply with regulatory requirements. Like any analytical methods, the validation of NIRS method is a mandatory step at the end of the method development in order to give enough guarantees that each of future results during routine use will be close enough to the true value [2].

Generally, validation strategies can be classified into two possible approaches: the traditional approach and the accuracy profile approach. The traditional approach relies on the validation of specific aspects of the method performance step by step, such as accuracy, precision (repeatability and intermediate precision), linearity, range of application, etc. These validation parameters are consistent with recommendations...
of international conference of harmonization (ICH) and other regulatory guidelines from EMA or FDA regulations. Examples of NIRS method validated following this strategy can be found in the determination of water content or API (active pharmaceutical ingredient) content of drug products[3-5], and in the quantification of excipients of antifungal and antibacterial agents[6, 7]. But this type of validation is time consuming and laborious. Furthermore, this strategy can be concluded wrongly that a method giving imprecise results can be more easily validated than a precise one [8].

The accuracy profile (AP) validation protocol was brought forward by the SFSTP (La Société Francaise des Sciences et Techniques Pharmaceutiques) [9–11]. Accuracy profile is based on the combination in the same graph of the tolerance interval and the acceptable limits, and circumvents some drawbacks of the traditional validation procedures. Compared with the traditional approach, the accuracy profile approach not only simplifies the validation process of an analytical procedure, but also allows monitoring the risk of utilization. Moreover, it can declared an analytical method is valid or not, and the analytical result is guaranteed to be fit for the intended purpose of the analytical method [12, 13]. Several applications of NIR spectroscopy used this approach can be found as follows. Schaefer used the accuracy profile approach to validate the on-line NIR method to control an API crystallization step [14]. Tomuta used this approach to demonstrate that the NIR chemometric methods meet the requirements of a high throughput method for the determination of drug content and pharmaceutical properties of indapamide tablets [15]. Wu successfully
used PLS model and accuracy profile method for accurate determination of chlorogenic acid content in *L. japonica* solution in the ethanol precipitation process [16]. Ziémons studied to develop a robust NIR calibration model to determine the acetaminophen content of a low-dose syrup formulation, where the accuracy profile confirmed the adequate accuracy of results generated by the method all over the investigated API concentration range [17]. Fonteyne assessed the in-line moisture content during the drying process in a six-segmented fluid bed dryer of a continuous tablet production line by the accuracy profile, and it was statistically demonstrated that the new NIR method performed at least as good as the Karl Fischer reference method [18]. The AP validation strategy is also fully compliant with the ICH Q2 (R1) guideline [12], and the prediction interval is built by the $\beta$-expectation tolerance interval.

However, Saffaj recently reported that the $\beta$-expectation tolerance interval cannot accurately predicted future measurements of the method in routine phase since it was incapable to assess the routine uncertainty rightly and was unfortunately not able to protect the laboratory and the client interests at the same time [20-22]. Since the $\beta$-expectation tolerance interval only contains the information of trueness and precision about the analysis method, it may underestimate the measurement risk. While, the $\beta$-content, $\gamma$-confidence tolerance interval provides a good estimate of measurement risk, and gives the best guarantees concerning the decision of declaring a method as valid.

Therefore, in the present work, the $\beta$-content, $\gamma$-confidence tolerance interval is
recommended to build the accuracy profile for the validation of NIR analytical method. And the NIR quantitative analysis of the concentration of Licorice acid in the mixture of Licorice and talcum powder collected during the blending process was taken as the research object. The aim of this study is to apply the new validation strategy to study whether the NIR spectroscopy is suitable for the analysis of the concentration of Licorice acid.

2. Theory

Accuracy profile is a graphical decision making tool aiming to help the analyst in deciding whether an analytical procedure is valid. It is 2D-graphical representation results for trueness, tolerance intervals and acceptance limits [8]. Whereas, validation must cover up the whole application domain of the method. The trueness and precision are needed to be calculated at each concentration levels. This ideal acceptance criteria would ensure that a high proportion (say $\beta$) of future observations lie within acceptance limits (say $\lambda$), with a high degree of confidence (say $\gamma$), where the $\beta$-content, $\gamma$-confidence tolerance interval could help fulfill this task [29].

2.1 Estimation of trueness and precision

The $I \times J \times K$ full factorial validation protocol was utilized to design the validation data set, where the effect of three aspects, i.e. conditions ($I$), the number of repetitions ($J$) and level of concentrations ($K$) were taken into account [23]. The estimate of the trueness and precision of the method was carried out at each of the considered $k$ concentration levels, using the following statistical model [11]:
\[ Y_{ij} = \mu + \alpha_i + \varepsilon_{ij} \quad (i = 1, 2, \ldots, m; j = 1, 2, \ldots, n) \]  

(1)

Where, \( Y_{ij} \) is the \( j \)-th measured value of the \( i \)-th condition at the concentration level \( k \). \( \mu \) is the mean of the measured values at each concentration level. \( m \) is the number of series, \( n \) is the number of independent replicates per series. \( \alpha_i \) is the difference between the \( i \)-th series average and the \( \mu \) at level \( k \). \( \alpha_i \) is considered as a normal random variable with 0 as the average and \( \hat{\sigma}_B^2 \) as the variance. \( \varepsilon_{ij} \) is the experimental error considered as a normal random variable with an average of 0 and a variance of \( \hat{\sigma}_E^2 \).

The experimental error is supposed to be independent of the series. The \( \hat{\sigma}_B^2 \) and \( \hat{\sigma}_E^2 \) variances represent the inter-series and intra-series variances, respectively. The restricted maximum likelihood method is used to estimate, at every concentration level, the parameters \( u_k \), \( \hat{\sigma}_B^2 \) and \( \hat{\sigma}_E^2 \) of the model. Define \( MS_B \) and \( MS_E \), the mean square of inter-series and intra-series, respectively.

\[ MS_B = \frac{n}{m-1} \sum_{i=1}^{m} (\bar{Y}_i - \bar{Y})^2 \]  

(2)

Where \( \bar{Y}_i = \frac{1}{n} \sum_{j=1}^{n} Y_{ij} \), \( \bar{Y} = \frac{1}{mn} \sum_{i=1}^{m} \sum_{j=1}^{n} Y_{ij} \)

\[ MS_E = \frac{1}{m(n-1)} \sum_{i=1}^{m} \sum_{j=1}^{n} (Y_{ij} - \bar{Y})^2 \]  

(3)

If \( MS_E < MS_B \), then

\[ \hat{\sigma}_B^2 = \frac{MS_B - MS_E}{n} \]  

(4)

\[ \hat{\sigma}_E^2 = MS_E \]  

(5)

Otherwise

\[ \hat{\sigma}_B^2 = 0 \]  

(6)

\[ \hat{\sigma}_E^2 = \frac{1}{mn-1} \sum_{i=1}^{m} \sum_{j=1}^{n} (Y_{ij} - \bar{Y})^2 \]  

(7)
Precision:
The ICH defines precision as the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed condition. Precision is evaluated at two levels: repeatability and intermediate precision [19].

Repeatability expresses the precision under the same operating conditions over a short interval of time, and should be assessed using a minimum of 9 determinations covering the specified range for the procedure [12]. Therefore, the intra-series variance in Eq. (5) or Eq. (7) provides the repeatability variance estimate:

\[ \hat{\sigma}_{R}^{2} = \hat{\sigma}_{E}^{2} \]  

(8)

Intermediate precision expresses within laboratories variations generated from different equipment, different days or different analysts. The sum of intra- and inter-series variance provides an estimation of the intermediate precision:

\[ \hat{\sigma}_{M}^{2} = \hat{\sigma}_{B}^{2} + \hat{\sigma}_{E}^{2} \]  

(9)

Trueness:
The trueness of an analytical procedure, also called theoretical true value, express the closeness of agreement between the average of the results calculated by the method and the accepted reference value [19]. The trueness is expressed as bias and recovery in relative form.

\[ \text{bias}(\%) = \frac{\bar{Y} - Xr}{Xr} \times 100 \]  

(10)

\[ \text{recovery}(\%) = \frac{\bar{Y}}{Xr} \times 100 \]  

(11)
2.2 Estimation of the $\beta$-content, $\gamma$-confidence tolerance interval

The $\beta$-content, $\gamma$-confidence tolerance interval is defined as follows [24]:

$$P(P[L \leq x_i \leq U] \geq \beta) = \gamma$$  \hspace{1cm} (12)

The $\beta$-content, $\gamma$-confidence tolerance interval provides lower ($L$) and upper ($U$) limits that claim a specified proportion $\beta$ of assayed values will lie within the interval $[L, U]$, with specified confidence level $\gamma$. For example, for $\beta = 0.70$ and $\gamma = 0.95$, the $\beta$-content, $\gamma$-confidence tolerance interval expresses that there is a probability ($P$) of 0.95 that 70% of the individual observations of the population are included in the interval $[L, U]$.

According to the Mee’s approach [25], the $\beta$-content, $\gamma$-confidence tolerance interval under this method takes the following form:

$$[L, U] = \left[ \bar{Y} - k_c \hat{\sigma}_M, \bar{Y} + k_c \hat{\sigma}_M \right]$$  \hspace{1cm} (13)

With

$$k_c = \sqrt{\frac{\nu' \chi^2_{1, \beta}(\tau)}{\chi^2_{\nu', \gamma}}}$$  \hspace{1cm} (14)

In Eq. (13), $k_c$ represents a Chi-square distribution associated with $\beta$ and $\gamma$ for interval estimation [25]. $\chi^2_{1, \beta}(\tau)$ is the $\beta$ quantile of a noncentral Chi-square distribution with the degree of freedom 1. $\tau$ is noncentrality parameter. $\chi^2_{\nu', \gamma}$ denotes the $1-\gamma$ quantile of a non-central Chi-square distribution with degrees of freedom $\nu'$.
\[ v' = \frac{(R' + 1)^2}{(R' + (1/n))^2 / (m-1) + (1 - (1/n))/mn} \]  
(15)

\[ \tau = \frac{1}{mnB'} \]  
(16)

\[ R' = \max \left[ 0, \frac{1}{n} \left( \frac{F}{F_n} - 1 \right) \right] \]  
(17)

And \[ B' = \frac{R' + 1}{nR' + 1} \]  
(18)

Where \( F \) is the mean square ratio MSb/MSe, and \( F_n \) is the 100\( \eta \) percentile of an \( F \) distribution with \( v_1 = m(n-1) \) and \( v_2 = (m-1) \). However, based on numerical results, the recommended values of \( \eta \) are 0.85, 0.905 and 0.975, corresponding to \( \gamma = 0.90 \), 0.95 and 0.99, respectively [26].

Thus, the \( \beta \)-content, \( \gamma \)-confidence tolerance interval can be rewritten in relative form as follows:

\[ [L(\%), U(\%)] = [\text{bias}(\%) - k_cRSD(\%), \text{bias}(\%) + k_cRSD(\%)] \]  
(19)

Where:

\[ RSD(\%) = \frac{\sigma_m}{Xr} \times 100 \]  
(20)

### 2.3 Establishment of the accuracy profile

The proposed building procedures of accuracy profile are as follows:

1) Set acceptance limits \((-\lambda, +\lambda)\).

2) Construct \( \beta \)-content, \( \gamma \)-confidence tolerance intervals \([L, U] \) or \([L(\%), U(\%)]\) for each level according to Eq. (13) or Eq. (19) with desired confidence level \( \gamma \).

3) Make a 2D-graphical representation of results with the horizontal axis for the concentration levels and vertical axis for the tolerance interval limits \( (L, U) \) and accuracy.
4) Compare the tolerance interval limits \((L, U)\) to the acceptance limits \((-\lambda, +\lambda)\).

5) If \((L, U)\) falls completely within \((-\lambda, +\lambda)\), the method is accepted; otherwise, the method is not accepted.

3. Experimental

3.1 Materials

Licorice powder (lot number: 20120926) and medicinal talc (lot number: 20120514) were purchased from Ben Cao Fang Yuan Medicine Co., Ltd. (Beijing, China). Licorice acid monoammonium salt (lot number: 111229) was supplied by National Institutes for Food and Drug Control (Beijing, China). HPLC grade methanol and phosphoric acid were purchased from Fisher Scientific (USA). HPLC grade ammonium dihydrogen phosphate was purchased from Acros Organics (USA), and the pure water was purchased from Wahaha Co., Ltd. (Hangzhou, China).

3.2 Acquisition of spectroscopic data

An Antaris near infrared spectrometer (Thermo Fisher Scientific Inc., USA) was used to collect the spectroscopic data. Each spectrum was an average of 64 scans with the resolution 8 cm\(^{-1}\) over the range 10000 ~ 4000 cm\(^{-1}\). A background spectrum was taken daily in air. And the integrating sphere diffuse mode with rotating sample cup was applied.

3.3 Reference method

The reference method used for the Licorice acid determination was HPLC assay recommended by the Chinese Pharmacopoeia (Ch. P., 2010 Edition). An Agilent 1100 HPLC apparatus, equipped with a quaternary solvent delivery system, an auto sampler,
a DAD detector and HP workstation for data processing were used. The concentration of Licorice acid was analyzed by the reverse phase chromatography on an Agilent C18 column (4.6×250 mm, 5µm) with isocratic elution of the mobile phase consisted of methanol, ammonium dihydrogen phosphate buffer (65:35, v/v) at the flow rate of 1.0 mL·min$^{-1}$. A column temperature of 30°C, injection volume of 20 µL and detection wavelength at 250 nm were used.

3.4 Calibration and validation protocols

The licorice and talcum powders with the mass ratio of 1:6 were mixed by the 10L three dimensional blender (ZNW-10, Beijing Xing Shi Li He Co., Ltd., China). The filling coefficient was set at 70%, and the spindle speed was 13 rpm. During the mixing process, the blender stopped at 5, 7, 9, 11, 12, 13, 14, 15, 17, 19 mins, and the time required to reach a homogeneous blend is 17mins as assured by HPLC analysis. And then, 5 g powders are respectively sampled at 5 positions preset, as shown in Fig.1. These samples were directly analyzed by NIR under the conditions specified in Section 3.2. Two batches of mixing experiments were carried out, and 100 (10×5×2) samples were finally got.

The validation protocol used the 3×5×3 full factorial experiment design. Five different Licorice acid concentrations levels (0.78 mg·g$^{-1}$, 1.56 mg·g$^{-1}$, 2.34 mg·g$^{-1}$, 3.12 mg·g$^{-1}$ and 3.89 mg·g$^{-1}$) were investigated, and each level was performed in 3 replicates on 3 different days, resulting in 45 samples in the validation set.

3.5 Data Processing
SIMCA-P 11.5 (Umetrics, US) and Unscrambler 7.0 (CAMO, Norway) were used to perform spectral pretreatments. The PLS regression was performed on Matlab version 7.0 (Math Works Inc., USA) with PLS Toolbox 2.1 (Eigenvector Research Inc., USA). The calculation of the β-content, γ-confidence tolerance interval and the construction of the accuracy profile were realized using homemade programs.

4. Results and discussion

4.1 NIR method development

Before model building, the first step of NIR method development is outlier detection to improve the performance of the model. First, the raw NIR spectra of 100 samples were analyzed by the principal component analysis (PCA) with the first principal components explaining 87.21% variation and the first two principal components explaining the 99.8% variation of samples. Then, score plots with two principal components are used to identify spectra clusters and to reveal the spatial distribution of samples as shown in Fig. 2A. The Hotelling T² ellipse with 95% confidence is calculated to identify the potential outliers as shown in Fig. 2B. As a result, 4 abnormal samples were removed. And the rest 96 samples were divided into the calibration set (56 samples) and the validation set (40 samples) by the Kennard-Stone (K-S) method.

The second step for NIR method development is spectral pretreatment. In order to improve the prediction ability of model, different spectral pretreatment methods...
were investigated. Multiplicative signal correction (MSC) and standard normal variate (SNV) were used to eliminate the impact of light scattering generated by the uneven distribution of the particles size. The first derivative (1std) and second derivative (2ndd) treatments for spectral data were used to eliminate the spectral baseline drift, strengthen band characteristics and overcome overlapping bands. The Savitzky-Golay (S-G) smoothing and wavelet de-nosing of spectra (WDS) were used to effectively smooth the high frequency noise, improve the signal to noise ratio and reduce the noise impact. For S-G smoothing, the filter width was set at 9 wavenumbers and the polynomial order was 2. And then, the optimal number of latent variable was optimized using the leave-one-out (LOO) cross validation method. Conventional correlation coefficient $r$ for both calibration and validation sets, the root mean squared error of calibration (RMSEC), the root mean squared error of cross-validation (RMSECV), the root mean squared error of prediction (RMSEP) and ratio of performance deviation (RPD) were used to select several model candidates.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>LVs</th>
<th>Calibration set</th>
<th>Validation set</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$r_{cal}$</td>
<td>RMSEC</td>
</tr>
<tr>
<td>Origin</td>
<td>9</td>
<td>0.9932</td>
<td>0.085</td>
</tr>
<tr>
<td>S-G</td>
<td>9</td>
<td>0.9921</td>
<td>0.092</td>
</tr>
<tr>
<td>1std</td>
<td>5</td>
<td>0.9956</td>
<td>0.068</td>
</tr>
<tr>
<td>2ndd</td>
<td>3</td>
<td>0.9757</td>
<td>0.160</td>
</tr>
<tr>
<td>S-G +1std</td>
<td>8</td>
<td>0.9986</td>
<td>0.039</td>
</tr>
<tr>
<td>1std+S-G</td>
<td>5</td>
<td>0.9959</td>
<td>0.066</td>
</tr>
<tr>
<td>MSC</td>
<td>9</td>
<td>0.9925</td>
<td>0.089</td>
</tr>
<tr>
<td>SNV</td>
<td>9</td>
<td>0.9921</td>
<td>0.092</td>
</tr>
<tr>
<td>WDS</td>
<td>10</td>
<td>0.9871</td>
<td>0.117</td>
</tr>
</tbody>
</table>

Note: Origin means using the original spectra
As presented in Table 1, after spectral preprocessing, the prediction accuracy of the model is not significantly improved. Compared to other preprocessing methods, the 1std+S-G preprocessing method used relatively fewer PLS factors with values of $r_{cal}$ (0.9959) close to 1, and the smaller values of RMSEC (0.066 mg·g$^{-1}$), RMSECV (0.160 mg·g$^{-1}$) were also indications of the good quantitative performances of the NIRS method developed. The RPD value 3.10 was greater than 3, demonstrating that the predictive performance of the developed NIR calibration model was good.

Additionally, variables selection was done after preprocessing of spectra, but the model performance did not improve (the data were shown in Table S1 and the results were shown in Table S2). Therefore, the 1std+S-G preprocessing method was chosen to build the PLS regression model. Fig.3A showed that the model performance changes with the latent variable (LV) factors. And it could be seen that the RMSEC and RMSEP values did not change at 5 LVs, based on which the PLS model was established. The relationship between the calibration set and the prediction set of the regression model was shown in Fig. 3B.

Fig. 3

4.2 NIR method validation

In agreement with the guideline of ICH Q2, the typical validation characteristics for assay procedures like accuracy, precision, range, and linearity were determined, and the accuracy profile was established. Due to the quality control of traditional Chinese medicine is similar to that of biological products, the acceptance limits ($-\lambda$, $+\lambda$) were set at ± 20% for the validation of the NIR method [11, 19, 20, 27]. To
compute the $\beta$-content, $\gamma$-confidence tolerance intervals and build the accuracy profile, the present work had opted for the 4-6-\(\lambda\) rule adopted by the FDA for the validation of bioanalytical procedures. And this rule was translated into $\beta = 66.7\%$ and $\gamma = 90\%$ by Hoffman and Kringle [28-30].

4.2.1 Accuracy

Accuracy takes into account the total error which is the sum of systematic and random errors, related to the validation result. As presented in Table 2 and Fig. 4, the lower and upper $\beta$-content, $\gamma$-confidence tolerances for 1.56 mg·g\(^{-1}\) (-1.82, 8.37), 2.34 mg·g\(^{-1}\) (-12.2, 17.1), 3.12 mg·g\(^{-1}\) (-6.20, 2.29), 3.89 mg·g\(^{-1}\) (-19.4, 4.00) concentration levels were all within the acceptance limits of ±20%. Consequently, the method can be considered as valid over the concentration range from 1.56 mg·g\(^{-1}\) to 3.89 mg·g\(^{-1}\).

Nevertheless, the accuracy was outside the acceptance limits for the level 0.78 mg·g\(^{-1}\). This result can be explained by: with a reduced concentration levels, system and random errors would increase.

4.2.2 Precision

Precision is evaluated at two levels: repeatability and intermediate precision at five concentration levels. The variance of repeatability and time dependent intermediate precision as well as the relative standard deviation (RSD) were calculated from estimated concentrations. From the precision results in Table 2, it is obvious that the intermediate precision is worse than the repeatability, which means that there is an important operator and/or day effect at these concentration levels. As can be seen from Fig. 4, the dispersion of the results is good for 1.56 mg·g\(^{-1}\), 2.34
mg·g⁻¹, 3.12 mg·g⁻¹ and 3.89 mg·g⁻¹ concentration levels, leading to good repeatability and intermediate precision values. However, with the decrease of concentration, the repeatability and intermediate precision values significantly increased. The values at the 0.78 mg·g⁻¹ concentration level were too large to satisfy the analytical requirements.

4.2.3 Range

The intersection between the accuracy profile and the acceptance limits defines the lower limit of quantification (LLOQ) as well as the upper limit of quantification (ULOQ) of the procedure. The lower and upper limits of quantification (LLOQ and ULOQ) define the range where an analytical method is able to quantify accurately. They are respectively the smallest and highest concentration levels where the β-content, γ-confidence tolerance intervals are included within the acceptance limits.

If the β-content, γ-confidence tolerance intervals never cross the acceptance limits, then the LLOQ and ULOQ are located at the beginning and at the end of the active content range investigated.

In our case, the LLOQ value was 1.26 mg·g⁻¹ via interpolation from the accuracy profile (Fig. 4), and ULOQ value was 3.89 mg·g⁻¹. So the quantitative range was defined from 1.26 mg·g⁻¹ to 3.89 mg·g⁻¹.

**Table 2** ICH Q2 (R1) validation criteria for the NIR method

<table>
<thead>
<tr>
<th>Level (mg·g⁻¹)</th>
<th>Mean calculated concentration</th>
<th>Trueness</th>
<th>Precision</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Relative bias (%)</td>
<td>Recovery (%)</td>
<td>Repeatability (%)</td>
</tr>
<tr>
<td>0.78</td>
<td>0.65</td>
<td>-16.8</td>
<td>83.2</td>
<td>9.82</td>
</tr>
<tr>
<td>1.56</td>
<td>1.61</td>
<td>3.28</td>
<td>103.3</td>
<td>2.87</td>
</tr>
</tbody>
</table>
2.34 2.40 2.47 102.5 2.45 4.53 [-12.2,17.1] [2.05,2.74]
3.12 3.06 -1.96 98.0 1.40 1.60 [-6.20,2.29] [2.93,3.19]
3.89 3.59 -7.68 92.3 1.51 3.50 [-19.4,4.00] [3.14,4.05]

Note: The $\beta$-CTI (%) is relative $\beta$-content, $\gamma$-confidence tolerance interval; Abs $\beta$-CTI is absolute $\beta$-content, $\gamma$-confidence tolerance interval.

Fig.4

4.2.4 Linearity

The linearity of an analytical method is its ability within a definite range to obtain results directly proportional to the concentrations (quantities) of the analyte in the sample. Therefore, a linear model was fitted on the calculated concentrations of the validation standards for all series as a function of the introduced concentrations.

The relationship between the NIR predictions and the theoretical values was evaluated by the linear equation: $y = 0.9426\times x + 0.0572$ with $R^2$ of 0.9820. The intercept, the slope and the $R^2$ values demonstrated good agreement between the NIR predictions and the theoretical values. In order to prove the method linearity, the absolute $\beta$-content, $\gamma$-confidence tolerance intervals were applied. The linearity over the Licorice acid content range 1.56~3.89 mg·g$^{-1}$ was demonstrated since the $\beta$-content, $\gamma$-confidence tolerance interval ($\gamma = 90\%$) limits were within the absolute acceptance limits as shown in Fig. 5.

Fig.5

5. Conclusion

In this paper, a new strategy based on the accuracy profile methodology which incorporates the $\beta$-content, $\gamma$-confidence tolerance interval has been successfully managed to validate the NIR quantitative analytical procedures in a traditional...
Chinese medicine blending process. Results demonstrated the developed NIR method was suitable for the analysis of the concentration of Licorice acid. The proposed approach offered a formal statistical framework by which the performance of the method was assessed. The method validation characteristics such as accuracy, precision, range, linearity and limit of quantification could be obtained for customers. In addition, the improved accuracy profile approach gave a good estimate of measurement risk, and provided visual and reliable method decision tool in the validation stage and controlled the risk of using the analytical method in routine phase.

Moreover, it is believed that the improved accuracy profile approach is not only suitable for NIR method, but can also be used for other analytic procedures.

Acknowledgement

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References


[19] International Conference on Harmonization (ICH), Validation of analytical procedures: text and methodology, Q2 (R1), 2005.


Graphical Abstract

Blending → NIR → Calibration → β-CTI → Accuracy profile
Figure captions

**Figure 1** Blend equipment and sampling points.

**Figure 2** A Scores plot; B Outliers detection by the Hotelling $T^2$ ellipse.

**Figure 3** A Calibration characteristics vs. number of latent factors; B Correlation graph of NIR predictive values with reference values.

**Figure 4** Accuracy profile for the *Licorice* acid content. The red line is the relative bias; the medium dashed lines are the $\beta$-content; $\gamma$-confidence tolerance intervals ($\gamma = 90\%$) and the red short dashed lines are the acceptance limits ($\pm 20\%$), the 9 black points at each concentration level are relative bias for each predictive value.

**Figure 5** Linear profile for NIR analysis of the *Licorice* acid content. The blue medium dashed lines are absolute $\beta$-content, $\gamma$-confidence tolerance intervals ($\gamma = 90\%$), and red short dashed lines represent the accepted limits at $\pm 20\%$. The continuous line is the identity line $y = x$. 
Fig. 1
Fig. 2

A

Scores on PC 1 (87%)

Scores on PC 2 (13%)

B

Value of \( T^2 \) vs. Number of Samples

- 95% Hotelling limit
Fig. 3

A

![Graph of RMSE against NO. of factors showing RMSEC, RMSECV, Cumulative PRESS, and RMSEP](image)

B

![Graph of Predicted by NIR vs. HPLC reference value showing val and cal points](image)
Fig. 4

Theoretical Concentration (mg.g\(^{-1}\))

Relative error(\%)

LLOD

Range

ULOD

Theoretical Concentration (mg.g\(^{-1}\))

-40

-20

0

20

20

-20

-40
Fig. 5

\[ y = 0.9426x + 0.0572 \]

\[ R^2 = 0.9820 \]