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Short Communication

NIR spectroscopy as a process analytical technology (PAT) tool for monitoring and understanding of a hydrolysis process

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HIGHLIGHTS

- Amino acids concentration profile during hydrolysis process was rapid monitored.
- Desirability index and multivariate quantification limits were used to assess models.
- NIR band of each amino acid compound was investigated.

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ABSTRACT

The use of near infrared spectroscopy was investigated as a process analytical technology to monitor the amino acids concentration profile during hydrolysis process of Cornu Bubali. A protocol was followed, including outlier selection using relationship plot of residuals versus the leverage level, calibration models using interval partial least squares and synergy interval partial least squares (SiPLS). A strategy of four robust root mean square error of predictions (RMSEP) values have been developed to assess calibration models by means of the desirability index. Furthermore, multivariate quantification limits (MQL) values of the optimum model were determined using two types of error. The $\text{SiPLS}_{(3)}$ models for L-proline, L-tyrosine, L-valine, L-phenylalanine and L-lysine provided excellent accuracies with RMSEP values of 0.0915 mg/mL, 0.1605 mg/mL, 0.0515 mg/mL, 0.0586 mg/mL and 0.0613 mg/mL, respectively. The MQL ranged from 90 ppm to 810 ppm, which confirmed that these models can be suitable for most applications.

1. Introduction

Cornu Bubali (CB), one of the major sources of biological agents and ethnodrugs, have been used in Traditional Chinese Medicine for several thousand years (Liu et al., 2010a). And appeared in lots of famous and effective prescriptions, such as Qingkailing injection. In the manufacturing process of Qingkailing injection, hydrolysis process plays a major role in the pretreatment of CB, and is thus taken as our research object. Hydrolysis process of CB is a complex chemical reaction process. This is mainly due to the decomposition of the peptides into desirable amino acids during hydrolysis process. Besides, many process parameters can influence the hydrolysis, such as the temperature, alkaline conditions, speed of agitation, etc. (Liu et al., 2010b). Although these parameters can be well operated, the type and content of amino acids could not be controlled so far, indicating that there are some uncertainties in the hydrolysis process. Therefore, process monitoring will not only help improving the hydrolysis efficiency, but also might reduce hydrolysis time which directly affects process productivity.

In recent years, near infrared (NIR) spectroscopy has been broadly applied as a quick assay for biological component and property analysis (He et al., 2012; Jacobi et al., 2012; Richard et al., 2011). There is an increasing trend in the use of NIR for process monitoring because its effectiveness for both qualitative and quantitative analysis in different fields (McClure, 2003). In this paper, the possibility of NIR to quantify the content of amino acids in hydrolysis process of CB was investigated. The NIR linear model is mainly established based on partial least squares (PLS). Recently, PLS model has been developed including interval partial least squares (iPLS) and synergy interval partial least-squares (SiPLS). These PLS algorithms were previously assessed according to usual chemometric indicators: root mean square error of calibration (RMSEC), root mean square error of prediction (RMSEP), residual predictive deviation (RPD), determination coefficient (R^2) and bias (Zou et al., 2007).

However, all of these previous works about indicators cannot be fully used to assess a robust PLS algorithm (Wu et al., 2012a,b, 2013). This paper opens a new window for combinational

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assessment of NIR model using desirability index (DI) and multivariate quantification limits (MQL).

Several root RMSEP values were used to obtain DI, a concept from industrial quality control to produce a one-number summary of a range of scores on different dimensions (Verboven et al., 2012). This DI allows us to make the model selection procedure fully automatic, which will save time as it does not require any user intervention. In addition, this has become a point of interest and a review on the MQL of multivariate calibration methods has recently appeared (Alcala et al., 2008; Lorber and Kowalski, 1988; Ostra et al., 2008). In this work, we performed a NIR assay for hydrolysis process of CB upon various times, and presented several equations that can provide a quick and consistent prediction of amino acids from large populations of samples using MQL (MQL theory was introduced into the supporting information) and DI assessment parameters.

2. Methods

2.1. Materials

Cornu Bubali was purchased from Yabao Beizhongda Pharmaceutical Co., Ltd. (Beijing, China), and deposited in the Key Laboratory of TCM-information Engineering of State Administration of Traditional Chinese Medicine (Nos. 1211081, 1211082 and 1211083). Other reagents and materials were shown in supporting information.

2.2. Hydrolysis process

$Ba(OH)_2$ (630 g) was put in a 2 L general single layer chemical reactor and dissolved with 1000 mL boiling water. Then CB (125 g) was added into the reactor for hydrolysis. Stirring paddle kept running at a speed of 30 rpm during the whole hydrolysis process. Along with the hydrolysis process with a period of 11.5 h, sample of 1 mL was drawn by a pipette gun from the reactor at the fixed position every 10 min. A total of 114 samples of three batches were prepared.

2.3. NIR equipment and software

The NIR spectra were collected in transmission mode with an Antaris Nicolet FT-NIR system (Thermo Fisher Scientific Inc., USA). Each spectrum was the result of 32 scans in the range between 10,000 cm^{-1} and 4000 cm^{-1} at ambient temperature using 8 cm^{-1} resolution, and was recorded as absorbance with air as reference. Every sample was scanned three times, and the final spectrum used for each sample was an average of three results. All NIR spectra were collected and archived using the Thermo Scientific Result software.

2.4. Reference method

2.4.1. Derivatization of amino acids

Prior to the derivatization reaction, standard solution (50 μL) or hydrolysate of CB (50 μL) was mixed with 100 μL of 0.1 mol/L phenylisothiocyanate (PTIC) solution and 100 μL of 0.1 mol/L triethylamine (TEA) solution in an Eppendorf tube. The mixture solution was agitated and left to stand at room temperature for 60 min. Furthermore, hexane (400 μL) was added to the mixture solution and swirled for 60 s. The underlying solution was filtered through a Millipore membrane filter with an average pore diameter of 0.45 μm, and 10 μL filtrate was directly injected into the HPLC system for analysis. All HPLC determinations were conducted in duplicate.

2.4.2. HPLC equipment and chromatography conditions

An Agilent 1100 HPLC system (Agilent Technologies, USA) with a vacuum degasser and an auto-sampler, a diode array detector (DAD) were used. Separation was performed on Dikma Diamonsil C18 column (250 mm × 4.6 mm with 5 μm particle size) at 45 °C. The mobile phase was acetonitrile (A) and sodium acetate buffer solution (B), and the signal was monitored at 254 nm. The flow rate was maintained at 1 mL/min. The eluted gradient was shown in Table S1 (supporting information).

2.5. Data preprocessing and calibration development

The Kennard–Stone (KS) algorithm was used to split the data set into calibration and prediction (76:28). Orthogonal signal correction (OSC) is a suitable preprocessing method for PLS model of mixtures without loss of prediction capacity using spectrophotometric method (Chalus et al., 2005). In this study, pretreatment of raw data were performed using OSC (SIMCA P + 11.5, Umetrics, Sweden). In addition, to identify outlier samples, standardized residual based on the training estimates is computed and compared to a cutoff value (Verboven et al., 2012). Finally, a new index by quartiles was added, which could better represent the spread of the population. The quartiles are milestones in the population range ($RMSEP_{0.25}$, $RMSEP_{0.5}$, $RMSEP_{0.75}$ and $RMSEP_{1.0}$). We propose to combine four RMSEP values to completely assess models using the largest DI.

Data analysis was performed by Unscrambler 9.7 software package (Camo Software AS, Norway) and Matlab version 7.0 (MathWorks Inc., USA). Some of the algorithms were developed by Norgaard et al. which we downloaded from http://www.models.kvl.dk/. Others were developed by ourselves, which can be recognized as modifications of the algorithms described by Norgaard et al.

3. Results and discussion

3.1. Quantitative analysis of 12 amino acids with HPLC–DAD

Representative chromatograms are shown in Fig. S1 (supporting information). All the target amino acids are baseline separated and can be determined accurately. Because the main purpose of this article is to establish an NIR method, more detailed description about the HPLC method validation is shown in the supporting information (Table S2–S4). The response linearity, precision (repeatability and intermediate precision), stability and accuracy (recovery studies) satisfied the demand of quantitative analysis.

3.2. NIR spectral features and outliers selection

The raw spectra are shown in Fig. S2 (supporting information). It can be seen that two outlying observations are outliers. In addition, taking the L-lysine (LYS) calibration model as representative, when we perform PLS model with latent variables (LV) = 9 on the quantitative determination of LYS, we obtain the outlier maps in Fig. S3 (supporting information). We notice on the right of the figure that observation 28 has a huge leverage level but a small absolute regression residual. This is an illustration of a good regression leverage point. However, sample 57 and sample 20 on the other hand have both a large residual and a relative small leverage level, which indicates that they are outlying observations. Similar results were founded in the calibration models of other amino acids. Therefore, sample 57 and sample 20 should be discarded in the calibration set.