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Overall uncertainty measurement for near infrared analysis of cryptotanshinone in tanshinone extract

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ABSTRACT

This study presented a new strategy of overall uncertainty measurement for near infrared (NIR) quantitative analysis of cryptotanshinone in tanshinone extract powders. The overall uncertainty of NIR analysis from validation data of precision, trueness and robustness study was fully investigated and discussed. Quality by design (QbD) elements, such as risk assessment and design of experiment (DOE) were utilized to organize the validation data. An "$I \times J \times K$" (series I, the number of repetitions J and level of concentrations K) full factorial design was used to calculate uncertainty from the precision and trueness data. And a 2^{7-4} Plackett–Burmann matrix with four different influence factors resulted from the failure mode and effect analysis (FMEA) analysis was adapted for the robustness study. The overall uncertainty profile was introduced as a graphical decision making tool to evaluate the validity of NIR method over the predefined concentration range. In comparison with the T. Saffaj's method (*Analyst*, 2013, 138, 4677.) for overall uncertainty assessment, the proposed approach gave almost the same results, demonstrating that the proposed method was reasonable and valid. Moreover, the proposed method can help identify critical factors that influence the NIR prediction performance, which could be used for further optimization of the NIR analytical procedures in routine use.

1. Introduction

Near infrared (NIR) spectroscopy has been more and more considered as an attractive and promising process analytical tool for pharmaceutical industry [1–3]. In comparison with classical analysis methods, NIR analysis is rapid, non-destructive and requires little or no sample preparation [4], and can provide both chemical and physical information about samples [5]. Besides these advantages, NIR quantitative analysis belongs to the indirect analysis methods in nature, which are based on multivariate calibration models {e.g. partial least squares (PLS)} and require chemometric approaches to interpret the complex spectra signal of analyte. In other words, the speed and efficiency of analysis is guaranteed by using NIR, while the risk of obtaining accurate analytical results is increased. Therefore, in order to release the batch products with certain confidence, it is critical to evaluate the quality and reliability of the NIR-based method for its rational implementation.

Conventionally, the performance of NIR analysis was evaluated by chemometric indicators, such as square error of calibration (SEC), square error of prediction (SEP), determination coefficient (R^2), the ratio of performance to deviation (RPD), etc. These indicators were generally found in the literature to qualify the accuracy of NIR calibration models [6–9]. However, these chemometric measurements only gave the average level of information about errors and bias of the method, and they did not provide the uncertainty of each individual prediction over the range of measurand [10]. Moreover, they are not sufficient towards the pharmaceutical regulatory requirements.

Concerning the limitation of conventional chemometric indicators, more and more researchers have adopted the validation criteria in agreement with the ICH Q2 guideline to assess the validity of NIR method. Measure of linearity, trueness, repeatability, intermediate precision, accuracy and limits of quantification could be found [11–13]. If all validation results fall into the well predefined limits, the built NIR analytical method is proved to be reliable or fit for the intended purpose. An alternative approach is to use the accuracy profile (AP) validation strategy brought forward by the SFSTP (Société Française des Sciences et Techniques Pharmaceutiques) commission [14–16]. The AP methodology fully complies with the ICH Q2 regulatory documents since it integrates all the useful required validation criteria such as accuracy, trueness, precision, limits of quantification, range and linearity. By using the β-expectation tolerance interval (β-ETI), the accuracy profile makes possible a visual and reliable representation of the actual and future performances of the analytical method. Many studies reported using AP strategy for the validation of NIR method [17–21].

Indeed, the purposes of validation are not only to obtain estimates of validation criteria but also to evaluate the risk that can be expressed by the measurement uncertainty associated with the result. The expression

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of uncertainty is also an important parameter to assess the performance of an analytical method. The International Vocabulary of Basic and General Terms in Metrology (VIM) proposed the following definition for the measurement uncertainty: "A parameter associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measurand" [22]. Without information of the measurement uncertainty, there is a risk of either under- or over- interpretation of results. Until now, several standards and guidelines have been published and dedicated to assist analysts for assessing measurement uncertainty [23–27]. Nevertheless, few of these methods are reported to be used in the NIR analysis, because the suitability of an NIR procedure is related to many factors, including the instrumentation, the applied chemometrics, etc. [28].

In 2004, Feinberg et al. [29] proposed a relationship between the measurement uncertainty and the β-expectation tolerance interval. That's to say, measurement uncertainty and accuracy profile are related topics, so either can be evaluated using the other. For instance, Mantanus et al. used β-ETI to assess uncertainty of NIR determination of the moisture content in pharmaceutical pellets [30]. Similar reports could be found in other literatures [31,32]. Recently, T. Saffaj et al. [33, 34] have reported that the β-ETI may underestimate the measurement uncertainty. So he recommended using the β-content tolerance interval (β-CTI) to estimate measurement uncertainty from validation data and proposed the uncertainty profile (UP) [35]. In our previous work, the UP method was used to estimate the uncertainty of NIR quantification of *Licorice* acid content in the mixture of *Licorice* and talcum powders [36]. In essence, the models used to estimate measurement uncertainty in AP and UP methods were expressed as tolerance intervals, which extracted information from the trueness and precision validation data. However, some method parameters may not have been varied sufficiently during the precision and trueness study [37].

In this paper, we propose the concept of overall measurement uncertainty of NIR analysis that allows taking into account all sources of uncertainty. Models for overall uncertainty assessment were adapted from the LGC/VAM protocol [27] which combined different sources of uncertainty from validation data according to the precision, trueness and robustness experiments. The results from robustness studies can be used in the evaluation of uncertainties associated with method parameters not fully covered by the precision and trueness studies. The NIR determination of cryptotanshinone content in tanshinone extract powders was taken as the research object. Considering the complexity of NIR analytical procedures, the quality by design (QbD) elements, such as risk assessment and design of experiment (DOE) were utilized to organize the validation data. Failure mode and effect analysis (FMEA) was used to identify the critical factors. The effects of deliberate variations in the method parameters on the result were investigated. The overall uncertainty profile was constructed as a decision tool to assess the fitness of the purpose of NIR analytical methods. At last, the proposed overall uncertainty measurement method was compared with the T. Saffaj's method [38], and the results clearly confirmed the rationality and advantage of the proposed method.

2. Theory

In accordance with the LGC/VAM protocol [27] and the ISO/DTS 21748 guide [23], a basic model for estimating the overall uncertainty of the measured Y, is given as follows:

$$u^2(Y) = S_R^2 + u^2(\delta) + u_{rob}^2(Y) \tag{1}$$

where,S_R^2 is the intermediate precision derived from the precision study; $u^2(\delta)$is the uncertainty associated with the bias of the method which is obtained by the trueness study; $u_{rob}^2(Y)$ is the uncertainty coming from the robustness test. In order to estimate the overall uncertainty $u(Y)$ effectively, the three terms in the right part of Eq. (1) are divided into two parts, i.e. trueness and precision study and robustness study. The validation data from the trueness and precision study are generated by the "$I \times J \times K$" full factorial experimental design, and the validation data from the robustness study are generated by the Plackett–Burmann (PB) experimental design.

2.1. The trueness and precision study

The "$I \times J \times K$" full factorial validation protocol was utilized to design the trueness and precision experiments, where the effects of three aspects, i.e. series (I), the number of repetitions (J) and the level of concentrations (K) are taken into account. The one way ANOVA was performed on the validation data set. From the ANOVA analysis, the estimation of the within day variance σ_E^2 and the between day variance σ_B^2 can be easily obtained. The intermediate precision can then be expressed by:

$$S_R^2 = \sigma_M^2 = \sigma_B^2 + \sigma_E^2 \tag{2}$$

The uncertainty from the method bias can be estimated according to the ISO/DTS 21748 guide:

$$u^2(\delta) = \frac{S_R^2(1-\gamma+\gamma/n)}{m} \tag{3}$$

where m denotes the number of series (or conditions), and n is the number of independent replicates per each condition. γ equals the ratio of σ_E^2 to σ_M^2, i.e. $\gamma = \sigma_E^2/\sigma_M^2$.

2.2. The robustness study

Robustness, considered in the sense of internal validation, deals with the effect of experimental variables, called factors, inherent in the analytical procedure (e.g., temperature, humidity, detection wavelength, and pH), on the analytical result [39,40]. If control limits have been set in the method for a factor (e.g., temperature at 100 ± 5 °C), the factor should be investigated at the extremes of the permitted range (i.e., 95 °C and 105 °C in the example given). If no control limits have been specified, it is up to the analyst to choose suitable values for the robustness test. This can be based on knowledge gained from similar methods or during the development of the method being studied, or from knowledge of the normal variation of the factor. The robustness study was performed under the guidance of the LGC/VAM protocol and the procedures were suggested by Youden [41] as follows:

(1) Identify the influential factors named x_1,x_2, … and x_z.
(2) For each factor, define the nominal and extreme values expected in routine work and encode them as follows: high value = +1, low value = −1, nominal value = 0.
(3) Arrange the experiments according to the Plackett–Burmann experimental design, in which the experimental runs are split into two groups on the basis of the levels +1 or −1
(4) Perform the experiments randomly on control samples whose concentration lie in the middle of the concentration range in the trueness and precision study.

The effect of each factor $D(x)$ is estimated as the difference between the mean result obtained at the level +1 and the mean result obtained at the level −1:

$$D(x) = \frac{1}{N}\left(\left(\sum_{i=1}^{N} Y_i\right)_{(x=+1)} - \left(\sum_{i=1}^{N} Y_i\right)_{x=-1}\right) \tag{4}$$

Here, N is the number of experiments carried out at each level for each factor.