



## IN-DEPTH FOCUS

# Validation methodologies of near infrared spectroscopy methods in pharmaceutical applications

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**As in any analytical method, a mandatory step at the end of the development of a near infrared spectroscopy (NIRS) method is the validation. This step ensures that future results coming from the application of the method in routine will be close enough to the true value. However, from the literature, a minority of NIRS methods are thoroughly validated despite the guidelines published by different group and regulatory authorities to help an analyst adequately decide if his method can be considered as valid. In this context, the aim of this review is to offer a critical overview of the different validation methodologies applied to assess the validity of quantitative methods using near infrared spectroscopy used in the field of pharmacy.**

In the last decade, near-infrared spectroscopy (NIRS) has taken an important place in the industrial field, especially in the pharmaceutical industry. Indeed, this technique, which belongs to vibrational spectroscopy, shows numerous advantages including fast acquisition, non-invasive, non-destructive and minimisation of sample preparation and offers

the possibility to use probes. Furthermore, this solvent-free technique is a promising tool in Process Analytical Technology (PAT) and Green Chemistry frameworks.

As any analytical procedures, NIRS quantitative methods require a thorough validation before their applications in the pharmaceutical industry. Several notes and drafts for guidance

were published by different industrial or scientist groups and regulatory authorities in order to help analysts to decide if their quantitative methods are valid<sup>1-4</sup>. Despite these publications, a lack of thorough validation of NIRS methods remains in literature.

Facing this situation, the objective of this review is to provide a new tool which offers analysts the possibility of having a critical overview of methodologies applied to assess the validity of quantitative methods using near-infrared spectroscopy. This review is oriented in pharmaceutical applications.

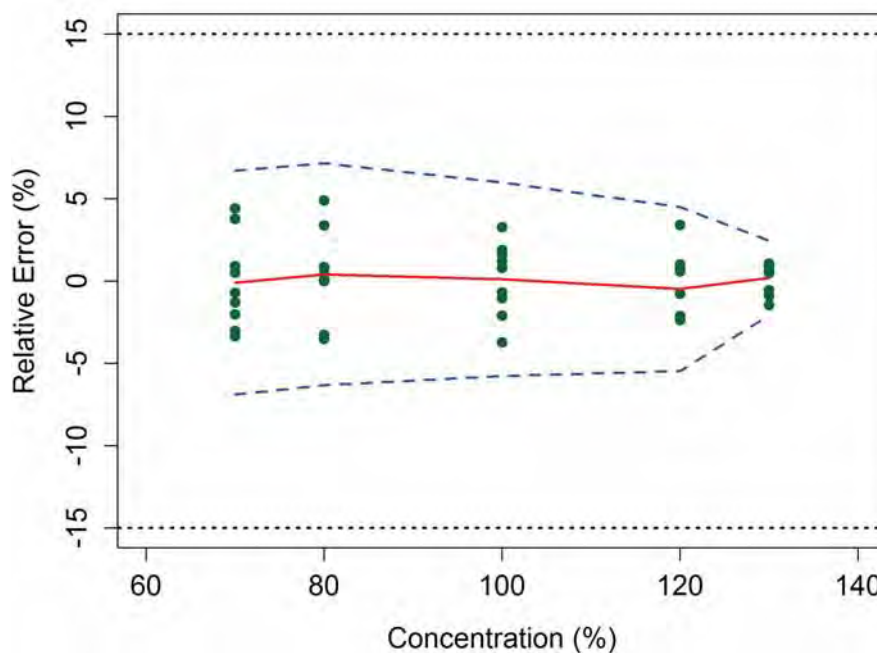
### **Decision methodologies used to validate NIRS methods**

There are four main decision methodologies used to assess the validity of analytical methods, namely the descriptive approach

using statistical descriptive parameters, the different approach using statistical hypothesis tests, the equivalence approach and the accuracy profile which is based on the statistical methodology of tolerance intervals<sup>5-8</sup>.

Most of the validation procedures performed for NIRS methods in pharmaceutical applications fall in the descriptive approach which includes two main methodologies: the use of traditional chemometric parameters and the criteria from the ICH Q2 guideline<sup>9</sup>.

Concerning traditional chemometric parameters, two steps are involved to validate a multivariate calibration model. The first step entails the use of a calibration set of data and using the cross-validation technique such as leave-one-out to assess the suitability of the  $R_{cal}^2$ , the root mean square error of calibration (RMSEC) and the root mean square error of the cross-validation (RMSECV) are computed. To optimise this calibration model, it is necessary to determine the optimal number of factors by plotting RMSEC and more especially RMSECV versus the number of factors. The RMSEC is decreased by increasing the number of factors and it also provides the most



**Figure 1** Accuracy profile obtained from an NIR method for the determination of an API in pharmaceutical tablets. The plain line is the relative bias, the dashed lines are the  $\beta$ -expectation tolerance limits ( $\beta=95$  per cent) and the dotted lines represent the acceptance limits ( $\pm 15$  per cent)

optimistic estimate of the partial least squares (PLS) calibration model performance showing the ability of this multivariate calibration model to fit the observed data of the calibration

set. The second step consists of the use of an external set of data, not involved in the calibration procedure, to validate the NIR predictive model on future data. The  $R_{pred}^2$

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and the root mean square error of prediction (RMSEP) are used to evaluate the quantitative performance of the NIR model. At the end of this process, it is advised to readjust the number of factors in order to improve the performance of the developed NIR model by observing the criteria values. In this context, the closer to one of the value of  $R_{cal}^2$  and  $R_{pred}^2$  are and the smaller the values of RMSEC, RMSECV and RMSEP are, better is the developed NIR method.

The problem of the traditional chemometric validation application in literature is that, most of the time, there is no evaluation of quantitative criteria which are trueness, precision, results accuracy, linearity and valid dosing range. Therefore, the pharmaceutical regulatory requirements concerning method validation found in the ICH Q2 document are not always respected<sup>9</sup>. In addition, there is no information about the suitability of the method for its intended use or evaluation of the future results and their reliability coming from the future application of the NIR method in routine.

The second main approach used to validate a NIR multivariate quantitative method is the use of validation criteria found in the ICH Q2 document<sup>9</sup>. The main limitation of this validation methodology concerns the evaluation of the linearity which is the relationship between the predicted results coming from the NIRS and the reference results obtained by a reference method (e.g. HPLC). Indeed, the linearity is evaluated by observing the value of the correlation coefficient: the closer to one its value is, the better the results are. However, it has been demonstrated that a value close to one is not sufficient to guarantee the comparability and the exchangeability of the analytical method investigated<sup>10</sup>.

What about the differential approach, where statistical hypothesis tests are used to assess if the observed effects are statistically significant. This approach is generally used to evaluate if the average difference of the results coming from the reference and the NIRS methods are statistically different in order to evaluate the average accuracy of the method. But it is also used to assess the linearity criterion by demonstrating that the slope is equal to one and the intercept equal to zero. However, there is an important risk with this approach, to accept a method as valid even though it is not. It is important to avoid flawed conclusions. The only conclusion that can be drawn with this approach is the ability to demonstrate a difference and not equivalence. The equivalence approach, which is not as well used, corrects these flaws.

The last approach is the accuracy profile based on tolerance intervals. The tolerance interval used is a ' $\beta$ -expectation tolerance interval' which defines an interval where an expected proportion of NIR results will fall with a defined probability  $\beta$ . An example of an accuracy profile is given in **Figure 1** opposite.

In this representation, the plain line represents the relative bias and the dashed lines are the  $\beta$ -expectation tolerance limits which links  $\beta$ -expectation tolerance intervals calculated from each concentration levels tested using their estimated bias and standard deviation of intermediate precision. If  $\beta$ -expectation tolerance limits are comprised within the acceptance limits, which are represented in dotted lines and fixed according to the regulatory requirements (five per cent on pharmaceutical specialties), the method can be considered as valid on the whole concentration range. This fully ICH compliant approach can declare the method as valid and guarantees



## Process Analytical Technology

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the quality, reliability and accuracy of the individual results for the intended use of the NIRS method. Accuracy profiles have been computed from results coming from some NIRS applications validated according to the descriptive approach. It has been shown that some of them which present low RMSEP could not be declared as valid based on their accuracy profiles<sup>11</sup>. This case demonstrates that small RMSEP is not sufficient to guarantee the validity of NIRS methods.

### Conclusion

The consideration of an analytical method as valid depends on the responsibility of the analyst and of the methodology which he chooses to make this conclusion. This review serves to highlight that it is important to avoid flawed conclusions. Some approaches are not sufficient to consider a NIRS method as valid. The accuracy profile approach which is based on tolerance interval is probably more adapted and is fully ICH compliant.

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### Biographies



Pierre-François Chavez is a PhD Student at the Laboratory of Analytical Chemistry of Department of Pharmacy of University of Liege. His thesis topic is implementation of vibrational spectroscopy including Near Infrared as Process Analytical Technology tool in pharmaceutical industry. He obtained his Master's degree in Pharmacy from University of Liege.



Charlotte De Bleye received her degree in pharmaceutical sciences from the University of Liege in June 2011. She is currently a PhD student in pharmaceutical and biological sciences working on the development of Surface Enhanced Raman Scattering methods to identify and quantify small pharmaceutical and biological molecules in complex matrices.



Dr. Pierre-Yves Sacré received his PhD in 2011 in Pharmaceutical and Biomedical Sciences from the University of Liege for his researches on the detection of counterfeit medicines. He is currently involved in the development of hyperspectral imaging analysis of pharmaceuticals in Professor Hubert's Analytical Chemistry Laboratory at the University of Liege.



Eric Rozet is Postdoctoral Researcher at the Analytical Chemistry Laboratory of the University of Liege. He has more than nine years of experience in non-clinical statistics: robust optimisation, validation, transfer, inter-laboratories studies and uncertainty assessment. Eric has a BSc degree in Bio-engineering and a PhD degree in Pharmaceutical Sciences.



Philippe Hubert is Professor of Analytical Chemistry and head of the Department of Pharmaceutical Sciences at the University of Liege. He has published more than 200 peer reviewed articles. His research focuses on separation techniques for the determination of active ingredients, vibrational spectroscopy in the framework of PAT and analytical methods validation.



Eric Ziemons received his PhD in 2006 in Pharmaceutical Sciences from the University of Liege. His researches are focused on vibrational spectroscopy (NIR and Raman spectroscopy) applied in the framework of Process Analytical Technology (PAT) and on Surface Enhanced Raman Scattering (SERS). He is the author of more than 40 peer reviewed articles.