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Acetaminophen determination in low-dose pharmaceutical syrup by NIR spectroscopy

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ABSTRACT

The aim of the present study was first to develop a robust near infrared (NIR) calibration model able to determine the acetaminophen content of a low-dose syrup formulation (2%, w/v). Therefore, variability sources such as production campaigns, batches, API concentration, syrup basis, operators and sample temperatures were introduced in the calibration set. A prediction model was then built using partial least square (PLS) regression. First derivative followed by standard normal variate (SNV) were chosen as signal pre-processing. Based on the random subsets cross-validation, 4 PLS factors were selected for the prediction model. The method was then validated for an API concentration ranging from 16 to 24 mg/mL (1.6–2.4%, w/v) using an external validation set. The 0.26 mg/mL RMSEP suggested the global accuracy of the model. The accuracy profile obtained from the validation results, based on tolerance intervals, confirmed the adequate accuracy of the results generated by the method all over the investigated API concentration range. Finally, the NIR model was used to monitor in real time the API concentration while mixing syrups containing various amounts of API, a good agreement was found between the NIR method and the theoretical concentrations.

1. Introduction

FDA's process analytical technology (PAT) focuses on the real-time monitoring of pharmaceutical manufacturing processes [1]. While time consuming laboratory testings still nowadays slow down the batch release step, the data collected during the manufacturing could reduce the batch release time and eventually enable real-time batch release. A real-time monitoring system such as PAT requires a high data acquisition speed and has to be compatible with probes for on-line and in-line analysis.

Near infrared (NIR) spectroscopy is a well-established vibrational spectroscopic technique [2–4]. In the covered wavelength region (between 14,000 and 4000 cm^{-1}), relatively wide bands related to overtones and combination of fundamental vibration of chemical groups with hydrogen, such as C–H, N–H, O–H and S–H are observed. Such vibrations lead to overlapping bands which contain both physical and chemical information. Consequently, chemometric tools such as spectral pre-treatments and regression methods are needed to extract the significant information.

NIR spectroscopy has many advantages: fast spectral acquisition, minimization of sample preparation and/or destruction and the use of probes allowing at-line, on-line and in-line measurements. Based on those advantages, NIR spectroscopy matches the PAT requirements. It has been part of PAT applications to monitor critical process attributes such as blend homogeneity, the coating level, the moisture content and the active content [3–13].

The acetaminophen determination in solid dosage forms by near infrared spectroscopy has already been investigated in previous studies [14–16].

As one of the main drawbacks of NIR spectroscopy is its relatively low sensibility, few NIR quantitative applications have been performed on low dosage forms [17,18]. Therefore the challenge still remains to quantify low dosage pharmaceutical forms.

In full accordance with the PAT concept, the acetaminophen content of low-dose syrups could be determined by NIR spectroscopy. The NIR measurements could be performed off-line, at-line, on-line or in-line to check the conformity of the pharmaceutical syrups during the manufacturing and/or before the final packaging stage. Such a NIR method could then be the first step towards a real-time release quality-based system.

Validation is a crucial and mandatory step in the lifecycle of an analytical method [19]. Based on β-expectation tolerance intervals, the accuracy profile makes possible a visual and reliable representation of the actual and future performances of the analytical

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method. It enables a better risk management [20]. Besides, it fully complies with the ICH Q2(R1) regulatory documents as it integrates all the useful required validation criteria such as accuracy, trueness, precision, limits of quantification, range and linearity [21–23].

The aim of the present study was first to develop a reliable near infrared method able to determine the active content of a low-dose acetaminophen syrup. The second aim was to fully validate the method for an active content ranging from 16 to 24 mg/mL. Finally, the ability of the validated NIR method to monitor in real-time changes in API concentration was tested.

2. Materials and methods

2.1. Syrups preparation

Laboratory scale syrups containing an acetaminophen concentration between 16 and 24 mg/mL (1.6–2.4%, w/v) were manufactured. An accurately weight amount of acetaminophen (between 400 and 600 mg) was dissolved into 20 mL of syrup basis in a 25 mL volumetric flask using a sonic bath Branson 2510 (Branson Ultrasonics Corporation, Danbury, USA). After complete acetaminophen dissolution, syrup basis was added to 25 mL. The syrup basis is prepared using 60 mL of glycerol (Fagron, Certa), 10 mL of ethanol (Merck), 5 mL of water and syrup conservans (Fagron, Certa) was added to 100 mL.

2.2. Calibration samples

3 syrups production campaigns were included in the calibration of the NIR method. 16, 20 and 24 mg/mL (1.6, 2.0 and 2.4%, w/v) API syrups were manufactured for each production campaign, 3 syrups per concentration level.

2.3. External validation samples

3 new syrups production campaigns were used for the validation set. The validation set was built in the same way as the calibration set except that 18 and 22 mg/mL (1.8 and 2.2%, w/v) API syrups were also manufactured.

2.4. FT-NIR equipment and software

The syrups samples were analyzed with a multipurpose analyzer Fourier transform near infrared spectrometer (MPA, Bruker Optics, Ettlingen, Germany) equipped with a semiconductor room temperature sulfide lead (RT-PbS) detector. A transmittance probe for liquids with a fixed optical pathlength of 2 mm was used to collect the NIR spectra. The probe was directly in contact with the syrups. A background spectrum with the empty probe was acquired before each series of measurements. Between each measurement, the probe was cleaned with water and dryed with a nitrogen flow. The spectra were collected with the Opus Software 6.5 (Bruker Optics). Each spectrum was the average of 32 scans and the resolution was 8 cm^{-1} over the range from 12,500 to 3600 cm^{-1}. The time necessary for a NIR measurement was 15 s. The NIR spectrum of acetaminophen was collected using the integrating sphere module of the spectrometer allowing a reflectance measurement.

2.5. Reference method

The reference method used for the API determination was the HPLC assay recommended by the USP 32 for acetaminophen capsules [24]. The HPLC method was performed in a LaChrom HPLC system (Hitachi High-Tech, Tokyo, Japan). One determination was made for each sample and was used as the reference value.

2.6. Near infrared method: calibration and validation protocols

Variability sources such as production campaigns, batches, API levels, syrup basis, operators and temperatures were introduced in the calibration and validation protocols. The NIR spectra of the samples were first recorded before performing the reference measurements.

2.7. Multivariate data analysis

Partial least square (PLS) regression, first derivative (order: 2, window: 15 points, corresponding to 57.75 cm^{-1}) and standard normal variate (SNV) were carried out with PLS Toolbox 5.0 for Matlab version 7.6. PLS regression using cross-validation random subsets was performed on the calibration set to build the prediction model [25]. The random subsets cross-validation is performed as follows: if s is the number of data splits, n the total number of samples and r the number of iterations, s different test sets are determined through random selection of n/s objects in the data set, such that no single object is in more than one test set. This procedure is repeated r times [26].

The model ability to predict the API content was further tested with the external validation set.

The calculation of the accuracy profile based on the external validation set results was performed with e.noval version 3.0 (Arlenda, Liège, Belgium).

3. Results and discussion

3.1. Calibration

To build a robust NIR model, the calibration set has to contain the future expected variability that the model will meet in the routine environment. Therefore, 6 sources of variability were integrated in the calibration set, these are cited in Table 1. First of all, 3 different syrup production campaigns were manufactured. Indeed, during the lifecycle of an analytical method, many production campaigns will be analyzed. It is then necessary to include different production campaigns in the calibration set. In full accordance with the previous consideration, 9 independent batches were manufac-

Table 1
Variability sources included in the calibration and validation sets.

	Calibration set	Validation set
Variability sources	Amount of variability	
Production campaigns	3	
Batches per production campaign	9	15
API levels	3 (16, 20 and 24 mg/mL)	5 (16, 18, 20, 22 and 24 mg/mL)
Basis of excipients	2 brands of glycerol and syrup conservans, 1 batch per brand	2 brands of glycerol and syrup conservans, 1 new batch per brand
Operators	2	1
Sample temperature during the NIR acquisition	2 (25 and 30 °C)	

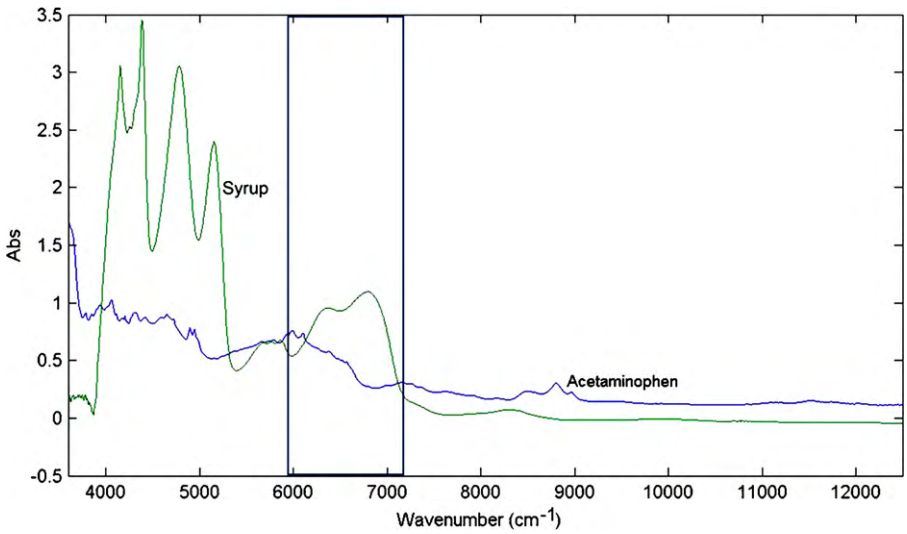

Fig. 1. Syrup transmittance and acetaminophen reflectance spectra. The highlighted area defines the selected spectral range for the NIR model.

tured per production campaign, 3 batches per API level. In order to cover a large part of the acetaminophen concentration range, each syrup was manufactured around the targeted concentration levels (between 16 and 24 mg/mL ±5%) by weighing various amounts of API. Besides, from one production campaign to another, variability was also introduced in the syrup basis: 2 brands of glycerol and syrup conservans were used, 1 batch per brand. They were combined randomly to manufacture 3 syrup bases per production campaign. Also, as an analytical method is not dedicated to only 1 operator, the calibration set was designed with 2 operators. Operator (a) manufactured the first syrup production campaign and performed the corresponding HPLC and NIR analyses. Operator (b) worked on the remaining production campaigns of the calibration set. Finally, as liquid samples are analyzed in the present study, temperature changes can induce spectral variations leading to increased Mahalanobis distances. With increasing temperatures, the water absorption bands are moving to higher wavenumbers and there is an increase in signal height [2]. Therefore samples were scanned while thermostated at 25 and 30 °C. The samples were then analyzed once by the HPLC reference method. Per production campaign, 18 NIR spectra were recorded and 9 reference measurements were carried out. As 3 different production campaigns were manufactured, the entire calibration set contains a total of 54 NIR spectra and 27 reference measurements.

3.2. External Validation

New batches were manufactured for the external validation set. As can be seen from Table 1, the introduced variability sources were the same as for the calibration set except that only operator (b) designed the external validation set. In order to demonstrate that 3 API concentration levels were sufficient to build a robust calibration set and to fully validate the model, 5 API concentration levels were integrated in the external validation set.

Per production campaign, 30 NIR spectra were recorded and 15 reference measurements were carried out. As 3 different production campaigns were manufactured, the entire validation set contains a total of 90 NIR spectra and 45 reference measurements.

3.3. Validation results of the NIR method

Fig. 1 displays the 20 mg/mL acetaminophen syrup formulation transmittance spectrum and the acetaminophen reflectance spectrum. From this figure, absorption bands coming from the syrup basis can be observed in the 3900–5300 and 6000–7200 cm^{-1} spectral areas. Besides, the reflectance spectrum of acetaminophen shows absorption bands from 4000 to 9000 cm^{-1}. Table 2 displays the conventional parameters of the NIR model. It can be seen that the 5955–7212 cm^{-1} spectral region was selected to build the quantitative model as it contains information about the API. PLS regression was performed with the calibration set. Cross-validation based on random subsets was carried out to select the model number of PLS factors. For the random subsets cross-validation, 9 splits and 10 iterations were selected.

About the number of PLS factors, Fig. 2 shows the evolution of the RMSECV according to the number of PLS factors. Based on this figure, 4 PLS factors were selected as the RMSECV value is minimal from 4 PLS factors.

Fig. 3 shows a good agreement between the NIR predictions and the HPLC reference method results for both the calibration and validation sets.

The RMSEC, RMSECV and RMSEP values are low. However, those criteria do not allow the assessment of the model ability to quantify accurately over the entire API range.

Therefore, the model predictive performance was evaluated with accuracy profiles computed on the external validation results. This innovative approach uses tolerance intervals as statistical methodology that allows predicting a region of concentration where each future result has a defined probability to fall. This probability is defined by the analyst.

Facing the confusion found between accuracy and trueness in the ICH document, it seems important to remind that accuracy represents the total error concept which is the sum of the trueness (systematic error) and precision (random error). In this aspect, the accuracy profile takes into account the total error [21,22].

Table 2
Spectral range, spectral pre-treatment, number of PLS factors, R^2 of validation, RMSEC, RMSECV and RMSEP of the NIR model.

NIR model	Selected parameter
Spectral range selected (cm^{-1})	5955–7212
Spectral pre-treatment	First derivative + SNV
Number of PLS factors	4
R^2 val	0.993
RMSEC (mg/mL)	0.34
RMSECV (mg/mL)	0.39
RMSEP (mg/mL)	0.26

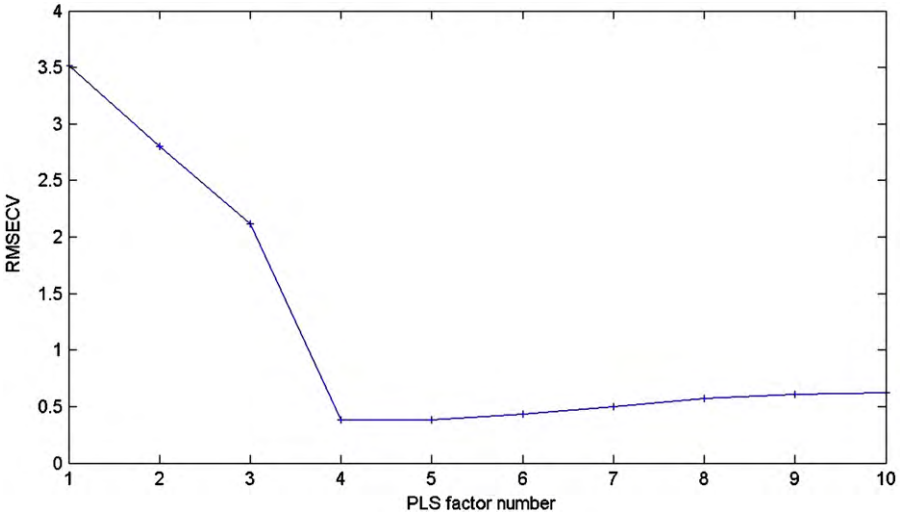

Fig. 2. RMSECV according to the PLS factor number.

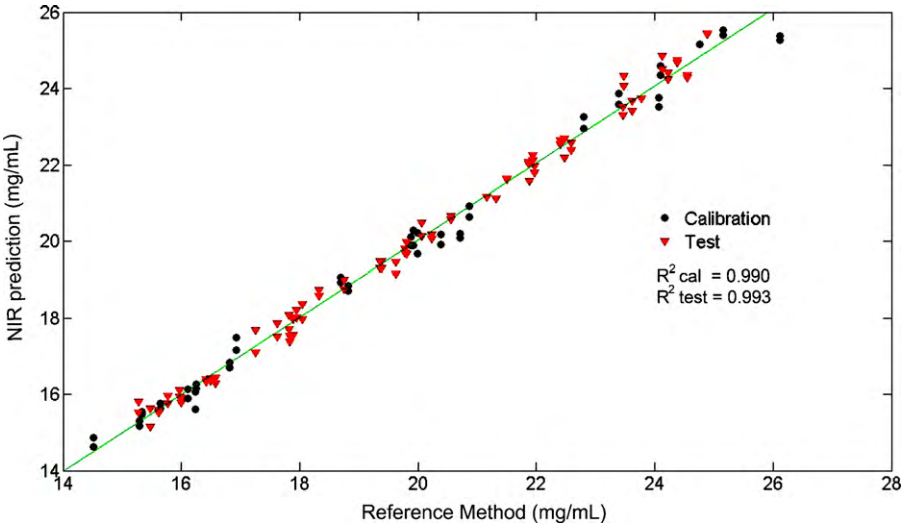

Fig. 3. API NIR predictions versus the reference method results. The black dots and the red triangles represent the results of the calibration and the external validation sets respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

As the focus of the present study is the determination of an API in a pharmaceutical formulation, the acceptance limits were set at ±5% for the validation of the NIR method while the probability to obtain results within the tolerance interval was set at 95%.

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-expectation tolerance intervals are included within the acceptance limits. If the β-expectation tolerance interval never crosses the acceptance limits, then the LLOQ and ULOQ are located at the beginning and at the end of the active content range investigated.

Fig. 4 displays the accuracy profile computed with the external validation set results. It can be seen from this figure that the validation results concentrations are different from the ones displayed in Fig. 3. For the accuracy profile calculation, it was necessary to perform an alignment on the mean concentration obtained by the reference method per API concentration level to compute repeatability and intermediate precision variance estimates [22]. It can be observed that the β-expectations tolerance limits are fully included within the ±5% acceptance limits. Therefore, each future result has at least 95% probability to fall within the ±5% acceptance limits.

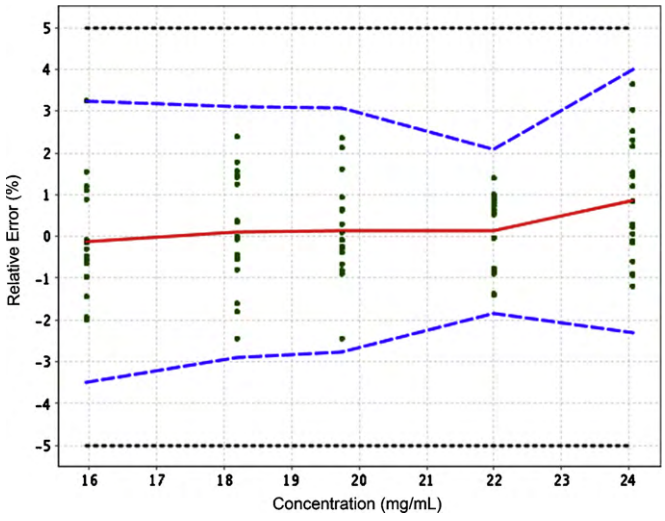

Fig. 4. Accuracy profile based on the validation results of the NIR model. The plain line is the relative bias, the dashed lines are the β-expectations tolerance limits (β = 95%) and the dotted lines represent the acceptance limits (±5%).

Table 3
ICH Q2(R1) validation criteria of the NIR method.

	Concentration level (mg/mL)	Mean introduced concentration (mg/mL)	Relative bias (%)
Trueness	16	15.9	−0.1
	18	18.2	0.1
	20	19.7	0.1
	22	22.0	0.1
	24	24.1	0.9

	Concentration level (mg/mL)	Mean introduced concentration (mg/mL)	Repeatability (RSD %)	Intermediate precision (RSD %)
Precision	16	15.9	1.1	1.4
	18	18.2	1.3	1.3
	20	19.7	1.0	1.2
	22	22.0	0.9	0.9
	24	24.1	1.4	1.4

	Concentration level (mg/mL)	Mean introduced concentration (mg/mL)	Relative β-expectation tolerance limits (%)
Accuracy	16	15.9	[−3.5, 3.2]
	18	18.2	[−2.9, 3.1]
	20	19.7	[−2.8, 3.1]
	22	22.0	[−1.8, 2.1]
	24	24.1	[−2.3, 4.0]

	Lower LOQ (mg/mL)	Upper LOQ (mg/mL)
Limits of quantification (LOQ)	15.9	24.1

Besides, it can be seen that the developed NIR model quantifies with the same level of accuracy API levels not included in the calibration set: 18 and 22 mg/mL API syrups.

Table 3 shows the ICH Q2(R1) validation criteria of the developed method. As seen in the accuracy profile, the bias is close to zero from 16 to 22 mg/mL. It is equal to 0.9% for the 24 mg/mL concentration level.

The precision of the method was estimated by measuring repeatability and intermediate precision at the 5 concentration levels investigated. As can be seen from Fig. 4, the dispersion of the results is the lowest for the 22 mg/mL concentration level, leading to the best repeatability and intermediate precision values. However, the other concentration levels repeatability and intermediate precision values are still very satisfactory and never exceed 1.4% as shown in Table 3.

The linear profile of the prediction model is shown in Fig. 5. A linear model was fitted on the calculated concentrations of the validation standards for all series as a function of the introduced concentration. The intercept, the slope and the R^2 values are also presented in Fig. 5. The slope and intercept are close to 1 and 0 respectively confirming the absence of proportional and constant systematic error of the model. The linearity of the results obtained by the NIR method for the 16–24 mg/mL concentration levels is demonstrated since the β-expectation tolerance limits were included in the absolute acceptance limits.

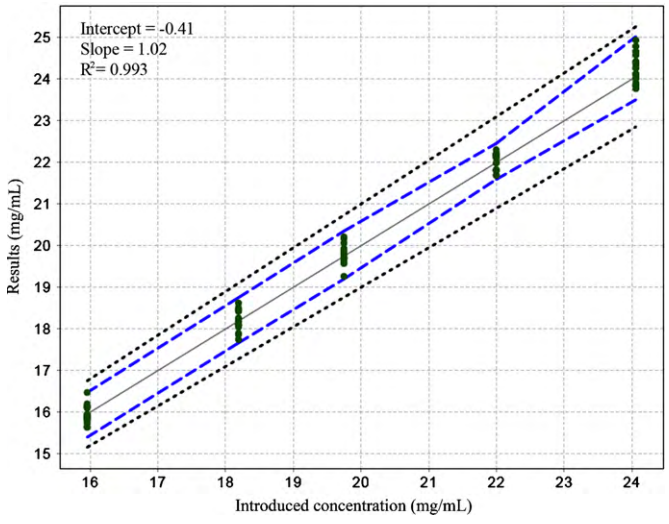

Fig. 5. Linear profile of the NIR model. The dashed limits on this graph correspond to the accuracy profile, i.e. the β-expectation tolerance limits expressed in absolute values. The dotted curves represent the acceptance limits at ±5% expressed in the concentration unit. The continuous line is the identity line $y = x$.

3.4. Uncertainty assessment of the NIR method

The uncertainty characterizes the dispersion of the values that could reasonably be attributed to the measurand [27,28]. Several uncertainty results were generated and are present in Table 4: the uncertainty of bias of the method at each concentration level of the validation standard, the uncertainty which combines the uncertainty of the bias with the uncertainty of the method obtained during the validation step, i.e. the intermediate precision standard deviation, and the expanded uncertainty which equals to the uncertainty multiplied by a coverage factor $k = 2$ representing an interval around the results where the unknown true value can be observed with a confidence level of 95% [29,30]. In addition, the relative expanded uncertainties with the corresponding introduced concentrations are not higher than 3%, which means that with a confidence level of 95%, the unknown true value is located at a maximum of ±3% around the measured results.

3.5. NIR monitoring of API concentration changes in syrups

The ability of the validated NIR method to monitor changes in API concentration was tested with 2 mixing experiments. The syrups used all belonged to the external validation set. First, 5 mL fractions of a 24 mg/mL syrup were added to a 15 mg/mL syrup to manufacture a 20 mg/mL syrup. The mixture was all the time under magnetic agitation. A NIR spectrum was acquired after each fraction addition. Secondly, the same type of experiment was performed except that 2 mL fractions of the syrup basis were added to a 24 mg/mL syrup to manufacture a 15 mg/mL syrup. Theoretical syrup concentrations were calculated to compare them with the NIR predictions.

Table 4
NIR method: estimates of measurements uncertainties related to the acetaminophen concentration in the syrups at each concentration level investigated.

Concentration level (mg/mL)	Mean introduced concentration (mg/mL)	Uncertainty of the bias (mg/mL)	Uncertainty (mg/mL)	Expanded uncertainty (mg/mL)	Relative expanded uncertainty (%)
16	15.9	0.08	0.23	0.47	2.9
18	18.2	0.07	0.26	0.51	2.8
20	19.7	0.09	0.25	0.51	2.6
22	22.0	0.05	0.20	0.41	1.9
24	24.1	0.08	0.36	0.72	3.0

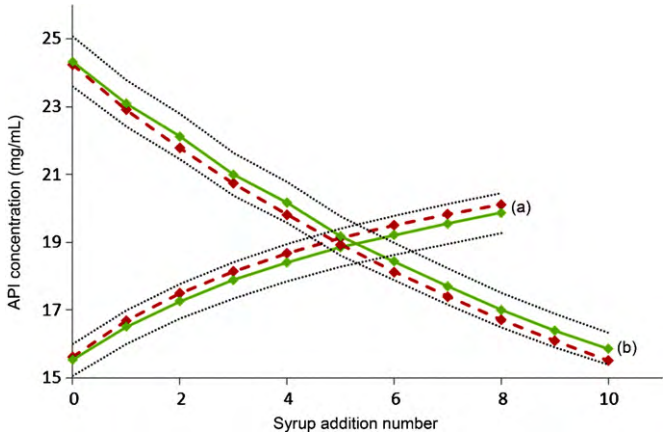

Fig. 6. Theoretical and NIR monitoring of the API content of (a) a 15 mg/mL API syrup, (b) a 24 mg/mL API syrup while addition of (a) a 24 mg/mL syrup and (b) the syrup basis. The green continuous lines and the red discontinuous lines represent respectively the NIR and the theoretical results. The dotted lines represent the ±3% uncertainty of the NIR method. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

From Fig. 6, it can be observed that the NIR predictions agree quite well with the theoretical concentration calculations, taking into account the uncertainty of the NIR method.

The above experiments clearly show that the monitoring ability of such NIR method could be used to prevent the risk to obtain out of specification products. Even though the present study was performed in a laboratory scale, the authors believe that the present methodology could be transposed in a real manufacturing environment while taking care of additional challenges such as the interfacing and advanced probe cleaning procedures. Applied before the final packaging steps, such method could be the first step towards a real-time release quality-based system.

4. Conclusions

A robust NIR model able to quantify acetaminophen in a low-dose pharmaceutical syrup formulation (2%, w/v) was developed.

The model was successfully validated for an active content ranging from 16 to 24 mg/mL using an external validation set. The RMSEP value of the model suggested the overall model accuracy. The accuracy profile on the validation results demonstrated the good accuracy of the results generated by the model all over the investigated API range. Indeed, based on the calculated tolerance interval, each future result will be included within the ±5% acceptance limits with a probability of at least 95%.

Finally, it was shown that the developed method is able to monitor in real time the API content of laboratory scale pharmaceutical syrups. Consequently, the present method transposed in the manufacturing line could be used to monitor the syrups API concentrations. The information provided by this monitoring system may eventually reduce the risk to obtain out of specification products. Applied to the manufacturing line, such method could be one key element towards a real-time release quality-based system.

References

[1] United States Food and Drug Administration (FDA), Guidance for industry PAT-A framework for innovative pharmaceutical manufacturing and quality assurance, FDA, 2004.
[2] D.A. Burns, E.W. Ciurczak, Handbook of Near-Infrared Analysis, vol. 35, 3rd ed., CRC Press, New York, 2008.
[3] J. Luypaert, D.L. Massart, Y. Vander Heyden, Near-infrared spectroscopy applications in pharmaceutical analysis, Talanta 72 (2007) 865–883.
[4] Y. Roggo, P. Chalus, L. Maurer, C. Lema-Martinez, A. Edmond, N. Jent, A review of near infrared spectroscopy and chemometrics in pharmaceutical technologies, J. Pharm. Biomed. Anal. 44 (2007) 683–700.
[5] C. Bodson, W. Dewé, Ph. Hubert, L. Delattre, Comparison of FT-NIR Transmission and UV–vis spectrophotometry to follow the mixing kinetics and to assay low-dose tablets containing riboflavin, J. Pharm. Biomed. Anal. 41 (2006) 783–790.
[6] J.J. Moes, M.M. Ruijken, E. Gout, H.W. Frijlink, M.I. Ugwoke, Application of process analytical technology in tablet process development using NIR spectroscopy: blend uniformity, content uniformity and coating thickness measurements, Int. J. Pharm. 357 (2008) 108–118.
[7] S.H. Tabasi, V. Moolchandani, R. Fahmy, S.W. Hoag, Sustained release dosage forms dissolution behavior prediction: a study of matrix tablets using NIR spectroscopy, Int. J. Pharm. 382 (2009) 1–6.
[8] W. Li, L. Bagnol, M. Berman, R.A. Chiarella, M. Gerber, Applications of NIR in early stage formulation development. Part II. Content uniformity evaluation of low dose tablets by principal component analysis, Int. J. Pharm. 380 (2009) 49–54.
[9] S.S. Rosa, P.A. Barata, J.M. Martins, J.C. Menezes, Development and validation of a method for active drug identification and content determination of ranitidine in pharmaceutical products using near-infrared reflectance spectroscopy: a parametric release approach, Talanta 75 (2008) 725–733.
[10] J. Mantanus, E. Ziémons, P. Lebrun, E. Rozet, R. Klinkenberg, B. Streel, B. Evrard, Ph. Hubert, Active content determination of non-coated pharmaceutical pellets by near infrared spectroscopy: method development, validation and reliability evaluation, Talanta 80 (2010) 1750–1757.
[11] J Mantanus, E. Ziémons, P. Lebrun, E. Rozet, R. Klinkenberg, B. Streel, B. Evrard, Ph. Hubert, Moisture content determination of pharmaceutical pellets by near infrared spectroscopy: method development, validation and reliability evaluation, Anal. Chim. Acta 642 (2009) 186–192.
[12] J. Rantanen, E. Räsänen, O. Antikainen, J.-P. Mannermaa, J. Yliruusi, In-line moisture measurement during granulation with a four-wavelength near-infrared sensor: an evaluation of process-related variables and a development of non-linear calibration model, Chemom. Intell. Lab. Syst. 56 (2001) 51–58.
[13] N. Sandler, J. Rantanen, J. Heinämäki, M. Römer, M. Marvola, J. Yliruusi, Pellet manufacturing by extrusion-spheronization using process analytical technology, AAPS Pharm. Sci. Tech. 6 (2005), Article 26.
[14] M. Blanco, R. Cueva-Mestanza, A. Peguero, Controlling individual steps in the production process of paracetamol tablets by use of NIR spectroscopy, J. Pharm. Biomed. Anal. 51 (2010) 797–804.
[15] A. Eustaquio, M. Blanco, R.D. Jee, A.C. Moffat, Determination of paracetamol in intact tablets by use of near infrared transmittance spectroscopy, Anal. Chim. Acta 383 (1999) 283–290.
[16] M. Ito, T. Suzuki, S. Yada, H. Nakagami, H. Teramoto, E. Yonemochi, K. Terada, Development of a method for nondestructive NIR transmittance spectroscopic analysis of acetaminophen and caffeine anhydrate in intact bilayer tablets, J. Pharm. Biomed. Anal. 53 (2010) 396–402.
[17] M. Blanco, M. Castillo, A. Peinado, R. Beneyto, Determination of low analyte concentrations by near-infrared spectroscopy: effect of spectral pretreatments and estimation of multivariate detection limits, Anal. Chim. Acta 581 (2007) 318–323.
[18] P. Chalus, Y. Roggo, S. Walter, M. Ulmschneider, Near-infrared determination of active substance content in intact low-dosage tablets, Talanta 66 (2005) 1294–1302.
[19] International Conference on Harmonization (ICH) of Technical Requirements for registration of Pharmaceuticals for human Use, Topic Q2 (R1): validation of analytical procedures: Text and methodology, Geneva, 2005.

[20] International Conference on Harmonisation (ICH) of technical requirements for registration of pharmaceuticals for human use, Topic Q9: Quality Risk Management, Geneva, 2005.
[21] Ph. Hubert, J.J. Nguyen-Huu, B. Boulanger, E. Chapuzet, P. Chiap, N. Cohen, P.A. Compagnon, W. Dewé, M. Feinberg, M. Lallier, M. Laurentie, N. Mercier, G. Muzard, C. Nivet, L. Valat, Harmonization of strategies for the validation of quantitative analytical procedures: a SFSTP proposal—part I, J. Pharm. Biomed. Anal. 36 (2004) 579–586.
[22] Ph. Hubert, J.J. Nguyen-Huu, B. Boulanger, E. Chapuzet, P. Chiap, N. Cohen, P.A. Compagnon, W. Dewé, M. Feinberg, M. Lallier, M. Laurentie, N. Mercier, G. Muzard, C. Nivet, L. Valat, E. Rozet, Harmonization of strategies for the validation of quantitative analytical procedures: a SFSTP proposal—part II, J. Pharm. Biomed. Anal. 45 (2007) 70–81.
[23] A. Bouabidi, E. Rozet, M. Fillet, E. Ziemons, E. Chapuzet, B. Mertens, R. Klinkenberg, A. Ceccato, M. Talbi, B. Streel, A. Bouklouze, B. Boulanger, Ph. Hubert, Critical analysis of several analytical method validation strategies in the framework of the fit for purpose concept, J. Chromatogr. A 1217 (2010) 3180–3192.
[24] Acetaminophen Capsules, United States Pharmacopoeia USP32NF27, The United States Pharmacopeial Convention, Rockville, 2009, p. 1338–1390.
[25] European Medicines Agency, Draft: guideline on the use of near infrared spectroscopy by the pharmaceutical industry and the data requirements for new submissions and variations, EMEA/CHMP/CVMP/QWP/17760/2009 Rev 1.
[26] http://wiki.eigenvector.com/index.php?title=Using_Cross-Validation (accessed 26.02.10).
[27] Analytical Methods Committee, Uncertainty of measurement: implication of its use in the analytical science, Analyst 120 (1995) 2303–2308.
[28] Eurachem/Citac Guide, Quantifying the Uncertainty in Analytical Measurement, 2nd ed., 2000.
[29] CB EA-4/16, EA guidelines on the expression of uncertainty in quantitative testing. <http://www.european-accreditation.org>, 2004.
[30] R.D. Marini, P. Chiap, B. Boulanger, S. Rudaz, E. Rozet, J. Crommen, Ph. Hubert, LC method for the determination of R-timolol in a S-timolol maleate: validation of its ability to quantify and uncertainty assessment, Talanta 68 (2006) 1166–1175.