Talanta 120 (2014) 114–125

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

A Process Analytical Technology (PAT) approach to control a new API manufacturing process: Development, validation and implementation

Cédric Schaefer [a,d,*], David Clicq [a], Clémence Lecomte [b], Alain Merschaert [b], Edith Norrant [c], Frédéric Fotiadu [d]

[a] UCB Pharma, Analytical Development Chemicals, Avenue de l'Industrie, 1420 Braine-l'Alleud, Belgium
[b] UCB Pharma, Chemical Process Development, Avenue de l'Industrie, 1420 Braine-l'Alleud, Belgium
[c] UCB Pharma, Innovation & Technology Development, Avenue de l'Industrie, 1420 Braine-l'Alleud, Belgium
[d] Institut des Sciences Moléculaires de Marseille, CNRS, UMR 7313, École Centrale Marseille, Aix Marseille Université, Avenue Escadrille Normandie-Niemen, Case A62, 13397 Marseille cedex 20, France

ARTICLE INFO

Article history:
Received 27 May 2013
Received in revised form
19 November 2013
Accepted 26 November 2013
Available online 2 December 2013

Keywords:
Process Analytical Technology (PAT)
Quality-by-Design (QbD)
Near infrared spectroscopy
API crystallization
In-process control (IPC)
Method validation

ABSTRACT

Pharmaceutical companies are progressively adopting and introducing Process Analytical Technology (PAT) and Quality-by-Design (QbD) concepts promoted by the regulatory agencies, aiming the building of the quality directly into the product by combining thorough scientific understanding and quality risk management. An analytical method based on near infrared (NIR) spectroscopy was developed as a PAT tool to control on-line an API (active pharmaceutical ingredient) manufacturing crystallization step during which the API and residual solvent contents need to be precisely determined to reach the predefined seeding point. An original methodology based on the QbD principles was designed to conduct the development and validation of the NIR method and to ensure that it is fitted for its intended use. On this basis, Partial least squares (PLS) models were developed and optimized using chemometrics methods. The method was fully validated according to the ICH Q2(R1) guideline and using the accuracy profile approach. The dosing ranges were evaluated to 9.0–12.0% w/w for the API and 0.18–1.50% w/w for the residual methanol. As by nature the variability of the sampling method and the reference method are included in the variability obtained for the NIR method during the validation phase, a real-time process monitoring exercise was performed to prove its fit for purpose. The implementation of this in-process control (IPC) method on the industrial plant from the launch of the new API synthesis process will enable automatic control of the final crystallization step in order to ensure a predefined quality level of the API. In addition, several valuable benefits are expected including reduction of the process time, suppression of a rather difficult sampling and tedious off-line analyses.

1. Introduction

The Process Analytical Technology (PAT) initiative promoted by the Food and Drug Administration (FDA) has encouraged the pharmaceutical companies to increase research and use of new analytical technologies to perform timely measurements of the critical quality attributes of raw materials and intermediates allowing process understanding and control [1].

The PAT concept is embraced in the Quality-by-Design (QbD) framework, introduced and developed by the ICH Q8(R2), Q9 and Q10 guidelines [2–4] that aims product and process understanding and process control, based on the scientific background and quality risk management, with the goal of ensuring a predefined final product quality. Although the ICH Q8(R2) guideline does not explicitly mention analytical method development, QbD principles can be extended to the development of analytical methods [5,6], and consequently to these used within PAT concept.

Near infrared (NIR) spectroscopy has become a widely used analytical technique in pharmaceutical industry due to its high speed acquisition and non-destructive nature, its capacity to measure both physical and chemical properties, and the fact it needs little or no sample preparation [7,8]. Moreover the possibility to use high dimension optical fibers connected to process probes allows remote measurements and consequently in- or on-line implementation at manufacturing plant. As NIR spectra are characterized by broad and overlapping absorption bands and may have thousands of wavelength variables, assignment to specific chemical group vibrations may be rather difficult. To overcome these drawbacks, chemometrical tools such as multivariate data

* Corresponding author at: UCB Pharma, Analytical Development Chemicals, Avenue de l'Industrie, Bâtiment T1, B1420 Braine-l'Alleud, Belgium. Tel.: +32 2 386 64 52; fax: +32 2 386 32 97.
E-mail address: cedric.schaefer@ucb.com (C. Schaefer).

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.11.072

analysis are used to extract the useful information from NIR spectra and to correlate them with reference values [9].

Several NIR spectroscopy applications in the pharmaceutical field concerning the monitoring of analyte solute concentration were reported. Most of them were developed and tested at laboratory scale [10–14], whereas the ones monitoring industrial processes were not used as primary analysis but in substitution of classical reference methods [15–20]. Peinado et al. [21] described the validation of an in-line drying monitoring by NIR spectroscopy for a commercial oral solid dose formulation, fully compliant with the ICH Q2(R1) guideline [22]. De Bleye et al. [23] exposed that this approach did not fit entirely with the revised version of the European Pharmacopeia (2.2.40) [24], stipulating that NIR method like any analytical method, must be validated consistently with its intended use.

Crystallization is a purification operation resulting in a solid intermediate or API (Active Pharmaceutical Ingredient), commonly used in pharmaceutical processes. In order to obtain API products with desired and highly reproducible solid state properties (crystal purity, polymorphism, crystal size distribution, density and flowability), understanding and controlling the crystallization process are critical [25].

The API physicochemical properties can have a large impact on the downstream unit operations such as filtration and drying, as well as on the bioavailability of the final drug product. Consequently, real-time in-process monitoring of solute concentration can be beneficial to control the level of supersaturation that drives nucleation and crystals growth.

This study focuses on a seeded crystallization forming the last step of a new API organic synthesis process (Supplementary material, Fig. S1). After a purification step the solution is concentrated in API while the methanol is eliminated by distillation. During this distillation process the API and methanol contents must be accurately controlled in order to reach a predefined seeding point. The normal operating ranges (NOR) of the critical parameters for the seeding were set as $10.5 \pm 0.4\%$ w/w for the API content, not more than 0.50% w/w for the methanol content and $65 \pm 2\ ^{\circ}C$ for the temperature. It was found during previous development studies (internal communications) that under these precise experimental conditions the crystal growth is predominant over the nucleation leading to a controlled crystallization and the formation of API in a predefined polymorphic form with no risk of seeds dissolution. Previous results proved the feasibility to use on-line NIR spectroscopy as a PAT tool during the crystallization step of the new industrial API process [26].

The present study describes the development and validation of a NIR method according to the QbD and PAT principles for the on-line monitoring of both the API and methanol contents during the crystallization step of an API manufacturing process. This primary method enables an automated unequivocal decision once the predefined seeding point is reached. The method will be implemented in the manufacturing plant to control the final API crystallization process without sampling and off-line analyses.

2. Materials and methods

2.1. Materials

API batches used for this study were manufactured in-house with a purity not less than 99.7% (assessed by high performance liquid chromatography). Ethyl acetate used as the crystallization solvent and methanol used during previous process steps were purchased from commercial suppliers with a purity not less than 100.0%. The impurities generated during previous process steps were synthesized and purchased from commercial suppliers compliant with internal analytical specifications.

2.2. Spectroscopic data

An ABB Fourier Transform Process Analyser (ABB-FTPA2000-260) Near Infrared spectrometer with InGaAs (indium gallium arsenide) detectors and equipped with an immersion transmission probe, optical path light of 5 mm, Zafiro-X (Solvias); was used to record in and on-line data respectively at laboratory and pilot scale. The probe was connected to the spectrometer by a 65 m optical fiber, core diameter 600 μm. A dedicated computer system was used to collect the spectra with the GRAMS/AI software version 7.0 (Thermo Galactics) during the development phase and with the real-time monitoring software FTSW100 Process Software version 2.71 (ABB) during the GMP (Good Manufacturing Practices) validation phase. AIRS software version 3.21 (Thermo Galactics) was run to verify the spectrometer spectral qualities. Each spectrum was the average of 256, 128 or 64 scans (2 min 42 s, 1 min 24 s and 42 s) with a resolution of 8 cm^{-1} over the range from 3800 cm^{-1} to 14,000 cm^{-1}. A background spectrum was daily taken in air at laboratory scale and at the beginning of each batch in N_2 at pilot scale. The spectrometer was installed in a thermostatic box to maintain the temperature in the range 20–25 °C.

2.3. Experimental procedure and setup

2.3.1. Laboratory scale

The NIR immersion probe was directly inserted in a Büchi Autoclave system equipped with a 250 mL jacketed glass reactor

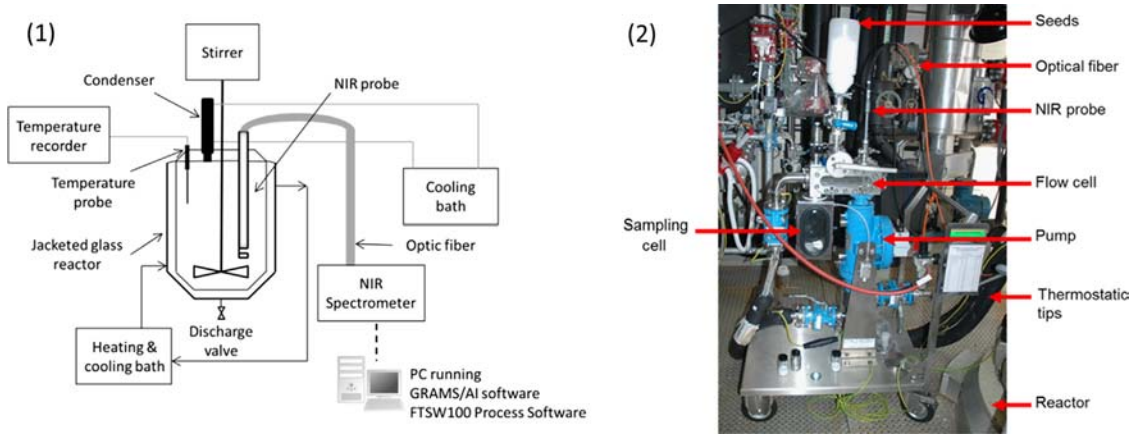

Fig. 1. (1) Schematic of the experimental setup at laboratory scale and (2) photograph of the experimental setup at pilot scale.

with temperature controlled through a Hüber CC 240 wl thermostated bath. The solution was stirred between 200 and 300 rpm using a blade impeller. A platinum resistance thermometer with a temperature recorder (Fuji Electric) was used to measure the solution temperature. To prevent loss of solvent, the condensate was refluxed back into the reactor using a mixture of glycol and water cooled at 5 °C and the condensate refluxed back into the reactor. The laboratory scale experimental setup is shown schematically in Fig. 1(1).

2.3.2. Pilot scale

NIR measurements were performed on-line by inserting the immersion probe in a Prosys recirculation loop equipped with a 0.450 L flow cell and a valve sample cell. The loop pump speed was set to 1 pulse every 3 s with a compressed air pressure of 2.5 bar to minimize the generation of bubbles in the flow cell. The loop was connected to a 200 L jacketed Hastelloy reactor with thermostatic tips. The solution was stirred using a two-story pitched blade impeller at the speed of 100 rpm. The solution temperature was monitored with an *in situ* probe. A mixture of glycol and water was used for heating and cooling the solution. Reflux condensers were used to prevent loss of solvent due to evaporation. The pilot plant experimental setup is shown by pictorial representation in Fig. 1(2).

2.4. Sampling and reference methods

Thieved samples were collected in duplicate for API and methanol reference content determination just after the NIR spectra recordings. At laboratory scale, heated syringes with metal needles were used to take samples into the reactor through a septum. At pilot scale, samples were collected through the sampling cell on the recirculation loop (Fig. 1(2)). API content was quantified by a validated reversed-phase ultra high performance liquid chromatography (UHPLC) performed on a Waters Acquity UPLC system. Methanol content was quantified by a validated head-space gas chromatography (HS-GC) method performed on an Agilent Technologies G1888 Network GC System coupled to a G1888 Network Headspace Sampler. The performances of these two methods are described in Supplementary material, Tables S1 and S2.

2.5. Chemometrics

Design of experiments (DOE) were executed in JMP 8 (SAS Institute). Spectral pretreatments, PCA and PLS models were computed during the development phase using Unscrambler software version 9.8 (Camo). NIR predictions during the GMP validation phase were established by the software FTSW100 Process Software version 2.71 (ABB). The validation results were treated by the software e.noval version 3.0 (Arlenda).

2.6. NIR method development and validation approach

Recently, several guidelines were published regarding the use of NIR spectroscopy in the pharmaceutical industry [27,28].

Quantitative NIR methods are generally applied to detect and determine the analyte as it exists in the sample matrix (*i.e.* without any sample preparation) and require multivariate calibration to link the NIR spectrum with the analytical reference method result. This implies that NIR method is conceptually different from conventional analytical techniques; the design, the development and the validation of such a method are inextricably linked and must be considered holistically. Fig. 2 lists all the steps performed during the calibration and validation phases. To ensure the fit for purpose use of the presented NIR method, calibration and validation designs were built following the QbD framework; including analytical target profile (ATP) risk assessment [29], design of experiments (DOE) and robustness/ruggedeness evaluation.

The ATP requested an in-process control (IPC) analysis able to determine the API and methanol contents in their seeding NOR with sufficient accuracy to allow an automated unequivocal decision when the seeding should be initiated. Accuracy acceptance limits of 5% and 25% respectively for API and methanol were set with a risk of 5%. Out of practical and safety reasons, it was preferred to have a rapid analysis without sampling issues.

Robust calibration design is critical in multivariate quantification analysis and should be selected to cover the entire all sources

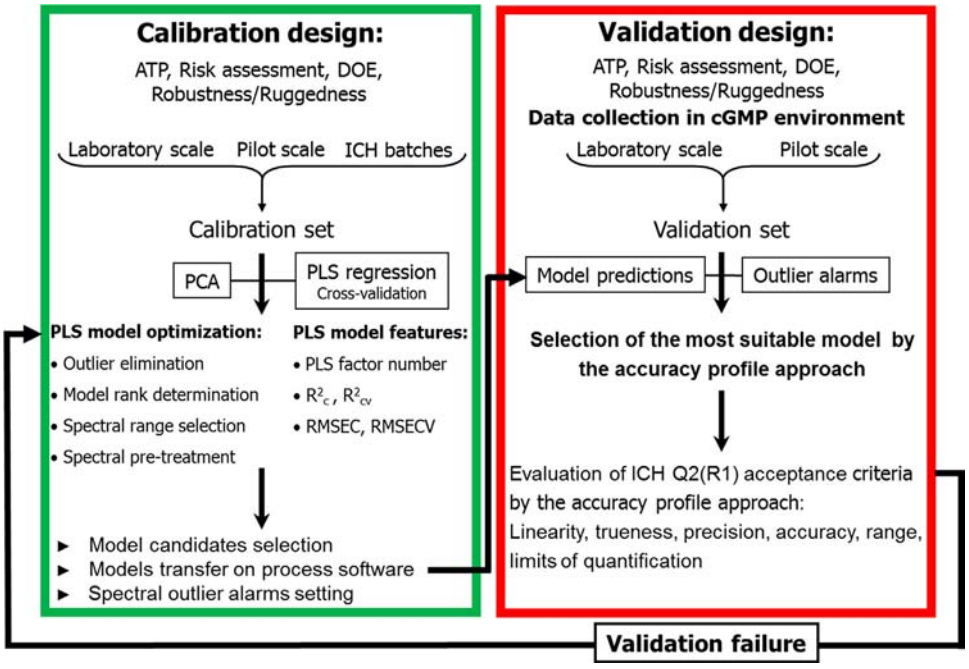

Fig. 2. Method development and validation methodology.

of variability expected in the manufacturing process [30]. Therefore, a mixed calibration set including both synthetic samples and samples from different ICH production batches was used. As the feasibility study has proved that the use of laboratory scale samples was effective to ensure the method variability in the industrial environment [26], and considering the balance between experimental cost and data representativeness, an intertwined design of synthetic samples at laboratory and pilot scale was used.

Calibration data were first checked using PCA and subsequently PLS models using cross-validation were developed and optimized. Conventional calibration criteria such as the R^2 of calibration (R^2_c) and cross-validation (R^2_{cv}), the root mean square error of calibration (RMSEC), the root mean square error of cross-validation (RMSECV) and the number of PLS factors were used to select several model candidates.

After the definition, characterization and robustness verification of the calibration models, the efforts were shift away from calibration and optimization towards the validation of the method.

A validation set was made up of independent data collected in a full GMP environment, allowing the evaluation of the predictive performance of the model candidates. The validation set (in line with the calibration set) was composed of synthetic samples both at laboratory and pilot scale covering the same variability as applied during the model calibration.

The accuracy profile approach was used to select the best model for each analyte [31]. The accuracy profile based on β-expectation tolerance intervals enables a visual and reliable representation of the future routine performances of the method [32], matching entirely with the risk assessment trend described in the ICH Q9 guideline [3].

Hubert et al. exposed the accuracy profile approach based on the total error theory is also fully compliant with the ICH Q2(R1) requirements for analytical method validation [33–35].

As by nature the variability of the reference methods is included in the variability obtained for the NIR method during the validation, a real-time process monitoring was performed as an addition to the validation in order to prove and document that the NIR method is appropriate for its intended use.

2.7. Calibration protocol

As described in Section 2.6, the calibration set encompasses on the one hand, data from three ICH production batches at pilot scale, and on the other hand, data from synthetic samples obtained by mixing known quantities of raw materials both at laboratory and pilot scales. The synthetic samples were collected according to a calibration protocol (Supplementary material, Fig. S2 (1)) involving the parameters identified as high risk priority during the risk assessment (3.1).

The calibration protocol covered both 3 different API levels (9.0%, 10.5% and 12.0% w/w) and 5 different methanol levels (0.00%, 0.10%, 0.55%, 1.00% and 1.50% w/w). Synthetic samples were executed according to DOEs in order to improve the model robustness and to reduce the number of experiments [36].

At laboratory scale, a custom DOE with 28 experiments was repeated 2 times (DOE 1 and 2). Beside the 2 analytes of interest, the DOE also covers the variation in composition of the matrix expected during the process by means of a randomized 2^{5-1} factorial fractional design (see Supplementary material, Tables S3 and S4).

At pilot scale, a custom DOE with 13 experiments encompassing only combinations between the levels of API and methanol was executed 2 times (DOE 3 and 4), as described in Supplementary material, Tables S3 and S5. Each DOE was performed with different batches of API and solvents with the aim to increase the method ruggedness.

Each spectrum was an average of 256 scans, in order to reduce the effect of some interferences due to harsh experimental conditions expected at manufacturing plant [17]. As the NIR measurement is planned to be perform around the reflux temperature during the process, all synthetic samples were scanned under two different temperatures, reflux (T1) and reflux – 5 °C (T2), to take into account variations in the spectral response due to temperature fluctuation [37]. Sampling for reference analysis was executed at the two temperature levels according to the procedure described in Section 2.4. Spectra were recorded in duplicate (R1 and R2) just before the sampling.

Finally 356 spectra and 176 reference measurements for each analyte were recorded during the calibration.

2.8. Validation protocol

As described in Section 2.6, the validation set encompasses data collected from synthetic samples collected both at laboratory and pilot scale. The experimental validation protocol (Supplementary material, Fig. S2 (2)) includes the parameters identified as high risk priority during the risk assessment (see Section 3.1).

The validation protocol covered 5 different API levels (9.0%, 10.0%, 10.5%, 11.0% and 12.0% w/w) and 6 different methanol levels (0.00%, 0.10%, 0.30%, 0.50%, 1.00% and 1.50% w/w). In total 4 series, 2 series at laboratory scale and 2 series at pilot scale, were performed. Each series was based on a custom randomized DOE containing at least 2 repetitions for each level of API and methanol. At pilot scale the DOE included only the variations of analytes content, whereas at laboratory scale it also covered the potential variation in matrix impurities. Each series was performed with different batches of API and solvents with the aim to increase the method ruggedness.

Furthermore, identically to the NIR calibration, all samples were recorded under two different temperatures, reflux (T1) and reflux −5 °C (T2) and sampling to reference analysis was executed at these two temperature levels according to the procedure described in Section 2.4. Each NIR measurement was recorded under 3 different number of scans (256 (2 min 42 s), 128 (1 min 24 s) and 64 scans(42 s)) in duplicate (R1 and R2).

Finally 184 spectra for each scan number and 92 reference measurements for each analyte were recorded during the validation.

3. Results and discussion

3.1. Risk assessment

The ICH Q9 guideline [3] describes the principles and tools of quality risk management to guide development efforts. A risk assessment was conducted by involving internal experts of the process development, analytical development, API pilot plant and manufacturing.

A fishbone diagram, as shown in Fig. 3, was created to map potential variables that can have an impact on the desired NIR method quality attributes. In total 6 categories were identified: equipment, measurement, environment, materials, man and process.

Subsequently a Risk assessment was performed according to the Failure Mode Effects Analysis (FMEA) method. FMEA risk management strategy allowed risk mitigation and brought into focus the main process variables or high risk variables of the NIR measurement dynamics [38].

Table 1 summarizes the risks, either identified as having a key-influence on the models development, on the method validation or on the future routine application. Risks were first categorized as controlled, noise or experimental. For each risk, severity, probability

and detectability were evaluated, resulting in a risk priority level categorized as low, medium or high. Factors with high risk priority were included in the experimental design both in development and validation (*e.g.* the analytes contents as DOE factors, the temperature as experimental protocol parameter and the optical fiber temperature as ruggedness parameter).

3.2. NIR method development

3.2.1. NIR spectra

Fig. 4 plots an overlay of the raw NIR spectra recorded forming the calibration set in the range 4660–10,020 cm^{-1}. Band assignment to specific chemicals groups is difficult. However the bands of interest of API and methanol were identified using NIR bands correlation table [39]. The range 4860–5370 cm^{-1} encompasses N–H and O–H combination bands. The peak at 6730 cm^{-1} is assigned to the N–H 1st overtone, the range 6800–7400 cm^{-1} contains the O–H 1st overtone band and combinations of this band. Moreover, the ranges 5640–6030 and 8380–8900 cm^{-1} corresponding respectively to the 1st overtone and 2nd overtone of –CH, $–CH_2$, $–CH_3$ bonds, can be attributed predominantly to the organic solvents.

3.2.2. Qualitative analysis

A PCA [40] was computed based on the raw spectra used for the calibration set in the range 4660–10,020 cm^{-1}. A random

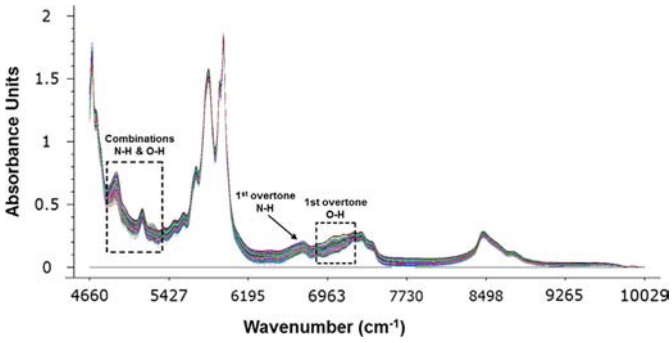

Fig. 4. Overlay of the NIR spectra included in the calibration set.

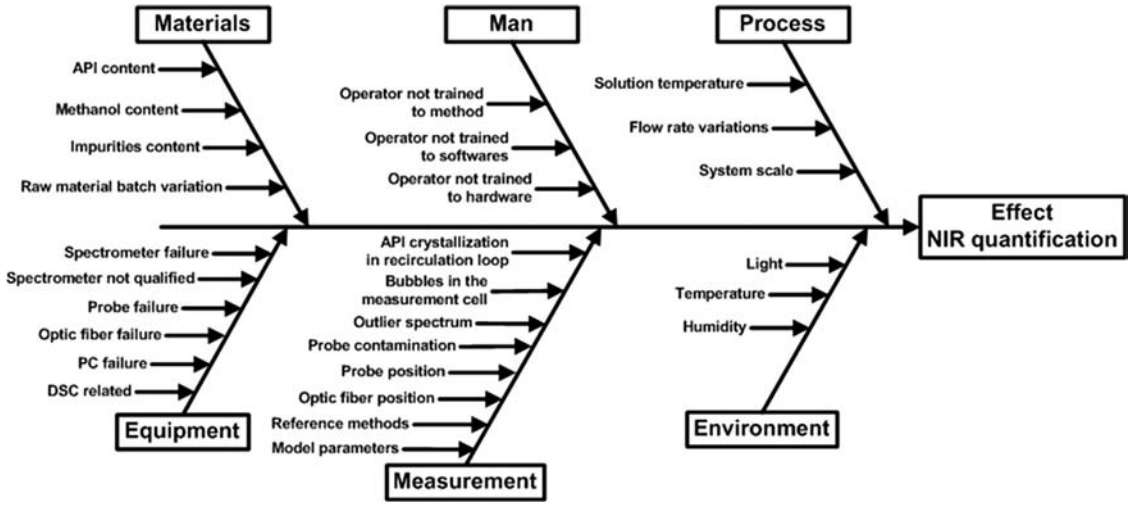

Fig. 3. Fishbone diagram for the development of the NIR method and its routine use.

Table 1
Risk assessment using FMEA methodology for the development, the validation and the routine use of the NIR method. SQC stands for spectral quality check.

Risk	Risk nature	Risk priority	Evaluation
Spectrometer failure	Controlled	Low	Spectral Quality Check test/maintenance
Probe position	Noise	Low	Ruggedness parameter Probe position will be fixed in routing use
Probe contamination	Controlled	Low	Spectral Quality Check test/cleaning
Optical fiber position	Noise	Low	Ruggedness parameter Fiber position will be fixed in routing use
Flow rate	Experimental	High	Robustness parameter Flow rate will be fixed in routine application
System scale	Experimental	High	Experimental protocol parameter
API content	Experimental	High	DOE factor
Methanol content	Experimental	High	DOE factor
Impurities content	Experimental	High	DOE factor
Solution temperature	Experimental	High	Experimental protocol parameter
Raw material batch variation	Noise	Low	Ruggedness parameter
API crystallization in the recirculation loop	NA	NA	Out of scope regarding the method development and validation
Presence of bubbles	Noise	High	Ruggedness parameter
Spectrometer temperature	Controlled	Low	The spectrometer was placed in a thermostatic box
Optical fiber temperature	Noise	High	Ruggedness parameter
Reference methods and sampling	Controlled	Low	Reference methods are validated. Sampling accuracy was assessed
Model parameters	Experimental	High	Evaluated during the method development
Outlier spectrum	Controlled	Low	Outlier detection mechanisms

subsets cross-validation with 10 segments and 10 iterations was carried out.

Score plots were used to identify spectra clusters and to explain their differences and similarities in reference to the high risk priority parameters (Fig. 5). A 95% confidence Hotelling T^2 ellipse allowing identification of potential outliers was calculated [41].

Fig. 5 displays that combinations of PC 2/PC 3 and PC 2/PC 4 discriminate spectra according to API and methanol content respectively. PC 7 explains almost all the variance due to the spectra temperature, however this variable explains only 0.05% of the model variance. Combination of PC1 and PC5 is able to separate spectra according to their origin. The 10 first PCs were not able to differentiate spectra according to the to the raw material batch and the impurity content factors.

These results demonstrated an important effect, highly modeled by the PC1, of the scale factor on the spectra collected. This strong influence was taken into account both in the calibration and the validation design (see Fig. 2). These results also confirmed the ability of the NIR method to quantitatively monitor the 2 analytes. Finally, a weak influence of the temperature as well as a negligible effect of the impurity content and the raw material batch variation was observed.

3.2.3. *Quantitative models building*

Predictive NIR models based on PLS regression [42] were computed to quantify the 2 analytes using the data from the calibration set.

The calibration model optimization encompassed the following steps: number of PLS factors selection, outliers detection and elimination, spectral range and spectral pre-treatment selection. Internal validation using a random subsets cross-validation with 10 segments and 10 iterations was carried out to estimate models performance.

The number of PLS factors was chosen such as the prime factor for which no significant variation of the RMSECV value was observed.

Outliers detection and elimination were performed according to an in-house methodology including: leverage, spectral residual variance and reference residual variance statistics, as well as the previous PCA. The outlier spectra eliminated represent respectively 4.4% and 3.4% of the calibration set for the API and methanol.

A manual approach was used to select the useful spectral variables. The aim was to maximize the contribution of the analytes of interest and, at the same time, minimize the contribution of irrelevant information [39]. The regions 4770–5050 cm^{-1} and 6560–7350 cm^{-1} for the API, 4780–5350 cm^{-1} and 6450–7200 cm^{-1} for methanol, marked in the wavenumber loading weight plots (see Supplementary material, Fig. S3), have the strongest influence on the PLS models. Moreover these regions encompass the functional absorbance bands identified in Fig. 4. However the highly informative region 4660–4690 cm^{-1} for the 2 analytes, characterized by an absorbance value high than 1.5 was not selected due to signal saturation [43].

Different spectral pretreatments were investigated in order to improve the models prediction ability. Three pre-treatments were selected: linear baseline correction, 1st and 2nd derivative. The linear baseline correction was performed between 2 points at 7580 and 10,020 cm^{-1}, with the aim to reduce spectral drift. The 1st and 2nd derivatives were computed according to the Savitsky-Golay algorithm based on 5 smoothing points and a second order polynomial, in order to improve the resolution of overlapped bands and attenuate baseline offsets [44].

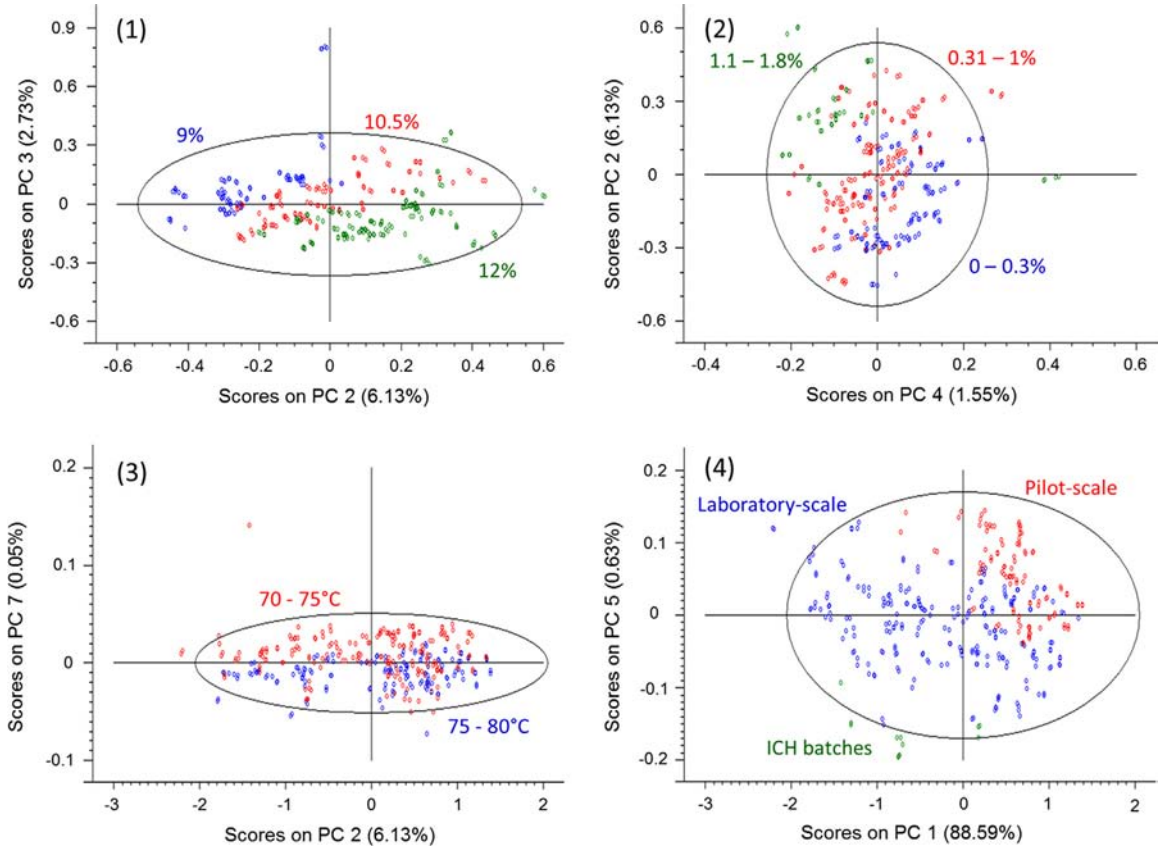

Fig. 5. PCA plots used to discriminate the spectra according to (1) the API concentration, (2) the residual solvent concentration, (3) the temperature and (4) the spectra origin. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

Conventional statistical parameters *e.g.* R^2 of calibration (R^2_c), R^2 of cross-validation (R^2_{cv}), root mean square error of calibration (RMSEC), root mean square error of cross-validation (RMSECV) and model number of PLS factors were used to select 3 model candidates with the most predictive ability for each analyte.

As shown in Fig. S4, a good agreement between the NIR predictions and the chromatographic reference values both during calibration and internal validation for the 2 analytes. Tables 2 and 3 show the parameters of the 3 model candidates for the API and methanol respectively. All model candidates have a small number of PLS factors, *i.e.* 4 or 2 factors for the API and 3 or 2 for the methanol. Consequently the risk of overfitting is limited. The R^2_c and R^2_{cv} values are equal to 0.98 for the API models and equal to 0.99 for the methanol models. For each analyte model, the RMSEC and RMSECV values as well as their difference values are low. These results demonstrate a good global predictive performance and robustness of the candidate models for both analytes.

Finally, the 3 models candidates for each analytes were tested during the method validation (see Section 3.3).

3.2.4. Spectral outlier detection

Once the calibration models were developed, a strategy for detecting spectral outliers was put in place.

The mechanism of detection of the spectral outliers was based on the leverage and the residual variance statistics. These statistics were evaluated for each spectrum of each model candidate. A "warning" and an "alarm" threshold were set in the real-time monitoring software for each model candidate (see Supplementary material, Fig. S5). The "warning" threshold is defined as 2 times the maximum values of the statistics parameter whereas the "alarm" thresholds is defined as 3 times the maximum values of the statistics parameter. Future predictions identified as "warning" will be highlighted whereas predictions identified as "alarm" will be highlighted and rejected.

Table 2
PLS model characteristics for the API content.

	Model		
Model feature	(a)	(b)	(c)
Pre-treatment	Linear baseline correction	1st derivative	2nd derivative
Range (cm^{-1})	4810–5042 6585–7356	4810–5042 6585–7356	4810–5042 6585–7356
PLS factor number	4	2	4
Development			
R^2_c	0.9851	0.9771	0.9853
R^2_{cv}	0.9847	0.9767	0.9837
RMSEC (% w/w)	0.153	0.189	0.152
RMSECV (% w/w)	0.155	0.191	0.160
Validation			
R^2_v	0.9620	0.9829	0.9797
RMSEP (% w/w)	0.201	0.138	0.150

Table 3
PLS model characteristics for the methanol content.

	Model		
Model feature	(d)	(e)	(f)
Pre-treatment	Linear baseline correction	1st derivative	2nd derivative
Range (cm^{-1})	4810–5350 6488–7240	4810–5350 6488–7240	4810–5350 6488–7240
PLS factor number	2	2	3
Development			
R^2_c	0.9895	0.9910	0.9939
R^2_{cv}	0.9894	0.9909	0.9938
RMSEC (% w/w)	0.046	0.042	0.035
RMSECV (% w/w)	0.047	0.043	0.035
Validation			
R^2_v	0.9905	0.9925	0.9912
RMSEP (% w/w)	0.047	0.041	0.045

3.3. Method validation

3.3.1. Models selection

PLS model candidates selected in Section 3.2.3 were validated using an independent external validation set. All spectra recorded during the quantitative validation exercise passed the spectral outlier rejection mechanism described in Section 3.2.4. Conventional statistical parameters such as R^2 of validation (R^2_v) and root mean square errors of prediction (RMSEP) were estimated for each analyte (Tables 2 and 3). As no significant difference of these parameters was observed between models computed with 256, 128 or 64 scans (data not shown), only the model candidates computed with 64 scans, enabling higher frequency measurement, were studied.

Model (b) for API and model (e) for methanol display the highest predictive performance, with a R^2_v respectively equal to 0.9829 and 0.9925, and a RMSEP value of respectively equal to 0.138 and 0.041% w/w. Nevertheless these results do not guarantee the good predictive ability of the models to quantify the analytes at each content levels of interest during the future routine application [23]. Therefore, the predictive performance of the model candidates was assessed with accuracy profiles.

Fig. 6 displays, respectively for API and methanol, the accuracy profiles computed with the validation set results for each model candidates. The β-expectation tolerance limits were built with a risk $(1-\beta)$ of 5%. The acceptance limits were fixed at 5% for the API quantification and at 25% for the methanol quantification.

As seen in Fig. 6, only model (b) has the tolerance limits (blue dotted lines) entirely included within the acceptance limits over the complete API content range. This means that 95% of the future predictions based on model (b) will be computed with an error not more than 5% over the API content range 9.0–12.0% w/w. Moreover, model (b) uses the lowest number of PLS factors (only 2) characterizing its good robustness.

Out of Fig. 6, model (e) is characterized by both the largest validated range and the smallest lower limit of quantification (LLOQ). Indeed the tolerance limits are located outside the acceptance limits for methanol contents lower than 0.18% w/w. Thus, these results guarantee that 95% of the future predictions based on model (e) will be computed with an error not more than 25% over the methanol content range 0.18–1.50% w/w. Furthermore model (e) uses the lowest number of PLS factors (only 2), which indicates its robustness.

In conclusion, model (b) for API quantification and model (e) for methanol quantification were selected to assess the method validation.

3.3.2. Method validation results

ICH Q2(R1) validation criteria results of the models (b) and (e) are shown respectively in Table 4. Facing to the confusion between accuracy and trueness observed in the ICH guidelines, it seems important to note that the accuracy represents the total error which is the sum of the trueness (systematic error) and precision (random error) [33–35].

3.3.2.1. Trueness. Fig. 6(b) and Fig. 6(e) show that the bias is low and stable over the entire range of the API and over the range

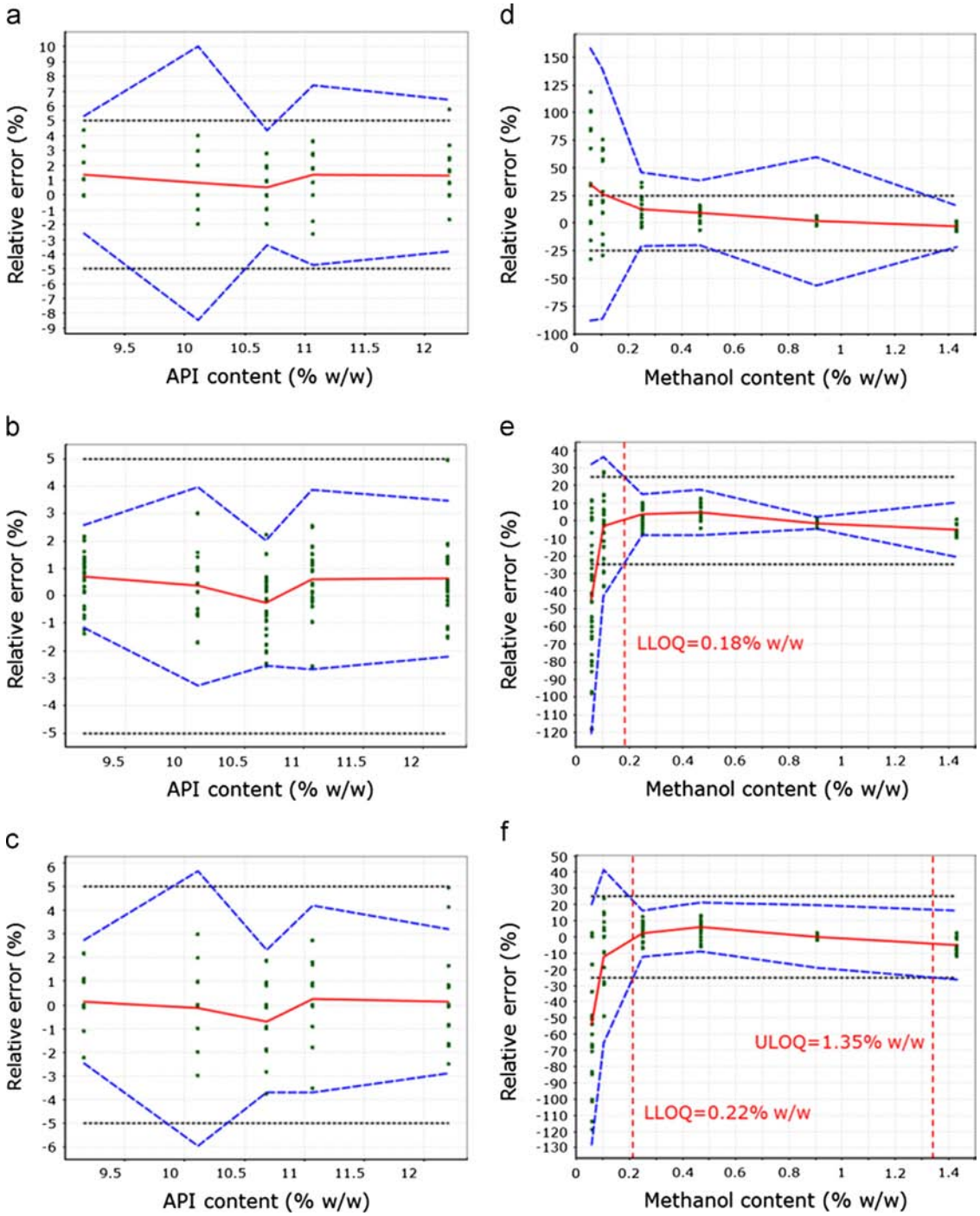

Fig. 6. Accuracy profiles of models (a)–(c) for the API content determination, models (d)–(f) for the methanol content determination The plain line represents the model relative bias, the dashed lines represent the β-expectations tolerance limits (β=95%), the dotted lines are the acceptance limits set respectively to $\pm$5% for the models (a)–(c) and $\pm$25% for the models (d)–(f). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

0.10–1.50% w/w of the methanol. Nevertheless, it becomes important for methanol content lower than 0.10% w/w.

3.3.2.2. Precision. Precision is evaluated at two levels: repeatability and intermediate precision. For the API, the intermediate precision as well as the repeatability, calculated through the relative standard deviation (RSD%), are not larger than 1%. Over the range 0.30–1.50% w/w of methanol, the repeatability and the intermediate precision are not larger than respectively 5% and 6%. These values significantly increase for the levels 0.10% and 0.05% w/w.

3.3.2.3. Accuracy. For the API, the accuracy calculated through the relative β-expectation tolerance limits is entirely included within the acceptance limits of $\pm$5%. For the methanol, the accuracy over the range 0.30–1.50% w/w is located within the acceptance limits of $\pm$25%. Nevertheless the accuracy is outside the acceptance limits for the levels 0.10% and 0.05% w/w. This result can be

Table 4
ICH Q2(R1) validation criteria of the model (b) for the API content and the model (e) for the methanol content.

	Trueness		Precision		Accuracy
Analyte content level (% w/w)	Relative bias (%)	Recovery (%)	Repeatability (RSD%)	Intermediate precision (RSD%)	Relative β-expectation tolerance limits (%)
API					
9.0	0.7	100.7	0.9	0.9	[−1.1; 2.6]
10.0	0.4	99.9	0.9	1.3	[−3.2; 4.0]
10.5	−0.3	99.7	1.0	1.1	[−2.5; 2.0]
11.0	0.6	100.6	1.1	1.4	[−2.7; 3.9]
12.0	0.6	100.6	1.1	1.3	[−2.2; 3.5]
Methanol					
0.05	−44.3	55.7	34.3	36.4	[−120.4; 31.8]
0.10	−3.4	96.6	18.1	18.9	[−43.2; 36.3]
0.30	3.6	103.5	5.1	5.5	[−8.1; 15.2]
0.50	4.5	104.5	2.9	4.6	[−8.5; 17.5]
1.00	−1.6	98.4	1.3	1.4	[−4.9; 1.8]
1.50	−5.3	94.7	1.3	3.8	[−20.7; 10.1]

explained by: first, these content levels are close to the sensitivity limit of NIR spectroscopy [45], second the HS-GC reference method also shows a lack of accuracy for these levels (see Supplementary material, Fig. S6).

3.3.2.4. Dosing range. The dosing range is defined to 9.0–12.0% w/w for the API and to 0.18–1.50% w/w for the methanol. In a first instance the LLOQ value of 0.18% w/w for the methanol was obtained via interpolation from the accuracy profile (Fig. 6). This value was confirmed by additional experiments performed at laboratory scale in the range: 0.16–0.21% w/w. 12 NIR measurements and 6 reference analyses were collected. The recoveries between the NIR measurements and the reference method (see Section 2.4) were in the range 85.7–118.8% with a relative standard deviation (RSD) of 9.2% attesting the decision of setting the LOQ at 0.18% w/w (Supplementary material, Table S6).

3.3.2.5. Linearity. The relationship between the NIR predictions and the reference methods from the external validation phase was evaluated by the linear equations: $y=1.006\ x - 0.027$ with R^2 of 0.9843 for the API and $y=0.960\ x+0.006$ with R^2 of 0.9942 for the methanol. The intercept, the slope and the R^2 values demonstrate good agreement between the NIR predictions and the reference methods in the two models (Supplementary material, Fig. S7). The linearity over the API content range 9.0–12.0% w/w and over the methanol content range 0.18–1.50% w/w is demonstrated since the β-expectation tolerance limits are within the absolute acceptance limits (see Fig. 6).

3.3.2.6. Specificity. The specificity of the models (b) and (e) to quantify respectively the API and the methanol contents was demonstrated by three different parameters, as exposed by Peinado et al. [21]. First, the spectral ranges used to build the models contain the specific absorbance bands of the two analytes (Fig. 4), the selected spectral ranges having a high influence on the predictive models (Fig. S3) and including maximum spectral difference between high and low content for each analyte (see Supplementary material, Fig. S8).

3.3.2.7. Robustness and ruggedness. The experimental design used during the calibration and the validation guarantees the robustness and the ruggedness of the method. The methodology of the method development and validation presented in Fig. 2 guarantees the robustness and the ruggedness of the method.

Out of Table 4 it is clear that the intermediate precision values are close to the repeatability ones. This means that variances between the series is low and thus the ruggedness parameters, defined in the risk assessment (Section 3.1) and varied between the series (batches variation, probe and optical fiber position, …), have a weak impact on the method performance.

Table 5
NIR method uncertainty for the API and the methanol content determination.

Analyte	Analyte content level (% w/w)	NIR method uncertainty (%)
API	9.0	1.8
	10.0	2.9
	10.5	2.3
	11.0	2.9
	12.0	2.7
Methanol	0.05	74.7
	0.10	38.8
	0.30	11.3
	0.50	10.1
	1.00	3.0
	1.50	8.7

3.4. NIR method uncertainty assessment

The International Organization for Standardization (ISO) describes the uncertainty as parameter characterizing the dispersion of the values that could reasonably be attributed to the measurand [46,47].

Table 5 displays the uncertainty of the NIR method for each analyte content level of validation. The uncertainty values were calculated according to the expanded uncertainty approach [48,49].

Over the entire API validated range (9.0–12.0% w/w) the NIR method uncertainty is not more than 2.9%, *i.e.* the unknown true value is located at a maximum of $\pm$ 2.9% around the NIR method result with a confidence level of 95%.

As the LLOQ extracted from the accuracy profile for the methanol (0.18% w/w) is located between the level of validation 0.10% and 0.30% w/w, NIR predictions values in the range 0.18–0.30% w/w present an uncertainty of 38.8% whereas NIR predictions values in the range 0.31–1.50% w/w have an uncertainty of 10.1%. Therefore, with a confidence level of 95%, the unknown true value is located at a maximum of $\pm$ 38.8% and 10.1% respectively around the NIR method result included in the range of 0.18–0.30% w/w and 0.31–1.50% w/w.

3.5. Method performance vs. process requirements

During the future API crystallization process, the seeding should be initiated once the NOR for each critical parameter (temperature, API and methanol contents) is reached.

As already mentioned, the scope of the validated NIR method is to monitor in real-time the 2 analytes content in solution during a distillation process prior the seeding. It was assessed that at production plant the cooling of the solution from reflux to the target temperature (65 °C) has no significant impact on the analytes content.

After the validation we had to link the validation results with the intended purpose of the method, *i.e.* reaching the target seeding parameters based on the real-time monitoring. It is important to note that the error on the NIR measurement assessed during the method validation through the accuracy profiles also includes the error of the chromatographic reference methods and the error of the sampling.

The NIR method was validated over the range 9.0–12.0% w/w for the API content with acceptance limits fixed at ± 5%. In routine the challenge is to avoid both overestimation of the API content values less than the NOR (< 10.1% w/w) and underestimation of content values more than the NOR (> 10.9% w/w). The method uncertainty assessment described in Table 5 shows a maximum uncertainty of the NIR method of 2.9% over the entire range of API. The key difficulty is that the uncertainty region also covers almost the entire NOR of the API content, involving very limited flexibility during the process. From Table 4 it was noted that the relative bias is low and stable over the entire range of validation. This means that repetition of the NIR measurements is able to decrease the final result error. As during routine applications one NIR measurement with 64 scans takes about 40 s, it was calculated that in production environment a variation of 0.03% w/w of API content between two successive NIR measurements is expected. Therefore, in order to ensure that the final decision result to initiate the seeding is included in the NOR, an API content decision criterion for the API content was set as the result of moving average on three successive NIR predictions included in the range 10.3–10.7% w/w.

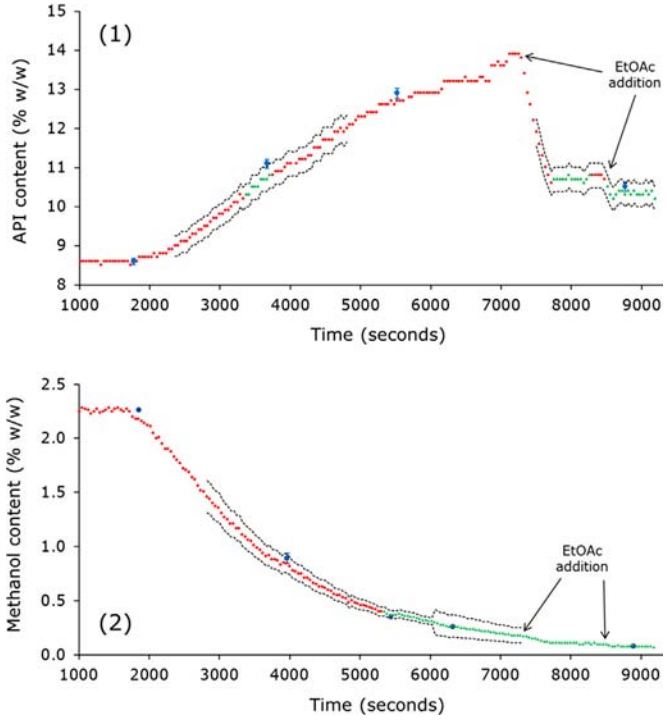

Fig. 7. Real-time monitoring of the API content (1) and the methanol content (2) performed by the NIR method during the distillation process. The green triangles represent the predictions in the range of the seeding acceptance criterion whereas the red points represent the predictions out of this range. The dashed lines represent the uncertainty of the method over the range of validation (± 2.9% over the range 9–12% w/w for the API, ± 38.8% and 10.1% over the respective ranges 0.18–0.30 and 0.31–1.50% w/w for the methanol). On graphic (1) the blue points are the UHPLC reference results and the error bars correspond to the UHPLC method acceptance limit. On graphic (2) the blue points represent the HS-GC reference results and the error bars stand for the HS-GC method uncertainty. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

For methanol, the method uncertainty results reported in Table 5 display an uncertainty value of 10.1% at 0.50% w/w content level. In this case the risk is the underestimation of methanol content values higher than the NOR (> 0.50% w/w). Hence, to ensure that the final decision to initiate the seeding is included in the NOR, a decision criterion for the methanol content was established as three successive NIR predictions below 0.40% w/w.

3.6. *Real-time monitoring of the API and methanol contents and detection of the seeding point*

The ability of the NIR method to monitor in real-time and simultaneously the 2 analytes contents, and consequently to detect the seeding point, was assessed by mimicking the distillation process at laboratory scale. The experimental setup described in Fig. 1(1) was used in distillation mode, *i.e.* solvent vapors were not refluxing back into the reactor. Sampling to reference analysis was executed according to the procedure described in Section 2.4.

Out of Fig. 7, it can be noticed that the NIR predictions of the two analytes present good agreement with the reference values taking into account the uncertainty of the NIR method. Out of the obtained results, it seems clear that first, the NIR method is more accurate than evaluated in the accuracy profiles (see Section 3.3.2)

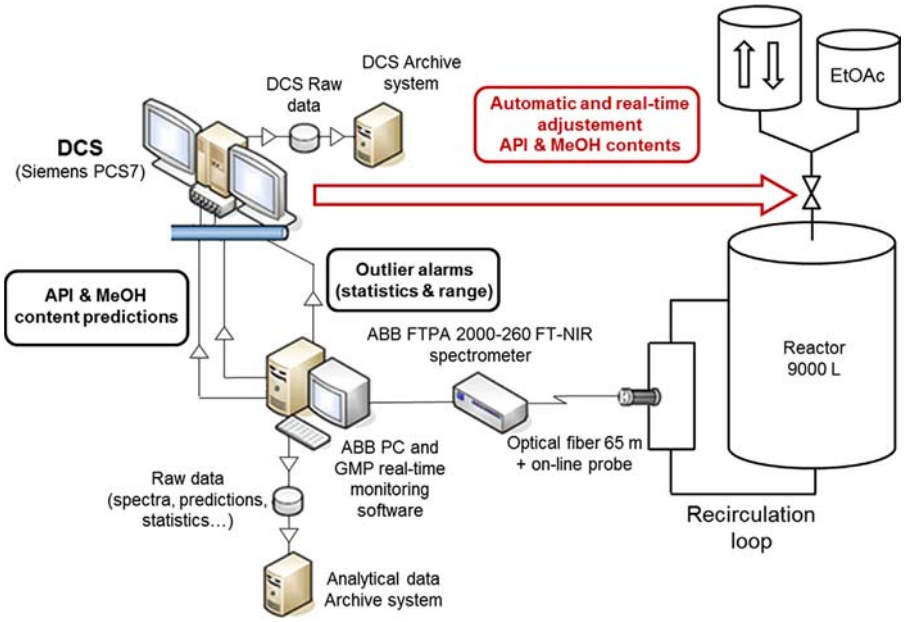

Fig. 8. Process scheme of the real-time monitoring and control system at manufacturing plant.

even for methanol content lower than the LLOQ (0.18% w/w). Second, the decision criteria defined in Section 3.5 can be applied in routine process to determine the seeding point. In addition, the NIR method presents a good repeatability of the API content predictions with a RSD of 0.38% evaluated on 13 measurements at decision criterion level (Fig. 7(1)), which is better than the validation results, assessed to 1.04% result at the level of 10.5% w/w (Table 4).

3.7. Implementation at manufacturing plant

Fig. 8 shows the future implementation of the NIR method at manufacturing plant to control the crystallization step of a new API synthesis process. A distributed control system (DCS), based on the NIR predictions, will be able to adjust automatically and in real-time the composition of the solution by regulating the distillation and by adding ethyl acetate if needed. Once the decision criteria defined in Section 3.5 are reached, the DCS will set the solution to the right temperature and will initiate the seeding.

3.8. Control strategy and lifecycle management

As mentioned by the EMA, a NIR method is known to evolve during time [27]. Consequently, a control strategy and a lifecycle management are required to maintain the method performances at a high level.

The goal of a control strategy is to determine for each measurement in real-time the correct functioning of the instrument and the predictive ability of the model [50]. An instrument performance check also called SQC (spectral quality check) will be performed before each experiment to test the equipment's parameters of frequency, linearity, modulation, baseline and noise. The models prediction performances will be evaluated via 3 parameters: spectral leverage, spectral residuals and models lack of fit (recovery of results obtained via NIR towards the reference analysis). The control strategy for the models predictive performances will be based on the use of control charts with the aim of monitoring the evolution of the 3 selected parameters [51]. The spectral leverage and spectral residuals statistics will be evaluated for each measurement whereas periodic controls are planned for the models lack of fit. Any out of trend observation in the control charts will result in an investigation on the models performance (which can lead to a models up-date and an associated method re-validation if appropriate).

4. Conclusion

An analytical method based on NIR spectroscopy was developed as a PAT tool in order to determine on-line both the API and residual solvent contents during the final crystallization step of an API manufacturing process.

A methodology based on the QbD principles was designed to conduct the development and validation of the NIR method and to ensure that it is fitted for its intended use. An intertwined experimental design was established with a large part of synthetic samples prepared both at laboratory and pilot scales.

Robust PLS models based on chemometrics methods were built to determine the API and residual solvent contents in solution prior the seeding. The NIR predictions of the 2 analytes presented a good agreement with the references methods.

After development, the method was fully validated according to the ICH Q2(R1) guideline, using the accuracy profile approach. The validation results matched with the requirements defined in the ATP.

A procedure was set with the aim of providing an automated unequivocal decision once the target solution composition to initiate the seeding was reached.

A real-time process monitoring was performed following the validation phase to prove and document that the method is fitted for purpose.

The method is ready for implementation in the manufacturing plant from the launch of the new API process. Its use as a primary method to control the final API crystallization step will enable several valuable benefits including reduction of the process time, suppression of a particular difficult sampling and tedious off-line analyses. Hence, return of investment within 2 years is expected.

The method development and validation approach were presented and approved by the European Medicines Agency (EMA). Nevertheless the 10 first batches should be controlled by both the NIR and the chromatographic reference methods.

Finally, it is important to highlight that this entire work has required a strong cross functional team work, encompassing a lot of different departments present in any classical pharmaceutical company such as process development, analytical development, API pilot plant, manufacturing; qualification, quality assurance (QA) and information technologies (IT).

Acknowledgments

The authors would like to thank Alain Clerbaux, Ineke Nackaerts and André Bernard (UCB Pharma, API Pilot Plant, Braine-l'Alleud, Belgium) for the help during experiments performed in pilot plant, as well as Filip De Boever, Mustapha Msellek, Christelle Bapsalle, Laurent Simon, Rodrigo Di Nicolantonio and Vanessa Busuito (UCB Pharma, Analytical Development Chemicals, Braine-l'Alleud, Belgium) for the analytical support. The authors also thank Nicolas Otis, Mathieu Côté and Stéphane Breton (ABB Analytical Measurements, Quebec, Canada) for their support during the setting of the real-time monitoring software. Finally, a special thank is addressed to Jérôme Mantanus (UCB Pharma, Chemical Pharmaceutical Development, Braine-l'Alleud, Belgium) for his valuable expertise in NIR method development and validation.

Cédric Schaefer is supported by a CIFRE Convention granted by UCB Pharma (France) and the French Ministry of Higher Education and Research through the ANRT.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.11.072.

References

[1] United States Food and Drug Administration (FDA), Guidance for industry PAT – A Framework for Innovative Pharmaceutical Manufacturing and Quality Assurance, 2004.
[2] International Conference on Harmonization (ICH), Pharmaceutical Development, Q8(R2), 2009.
[3] International Conference on Harmonization (ICH), Quality Risk Management, Q9, 2005.
[4] International Conference on Harmonization (ICH), Pharmaceutical Quality System, Q10, 2008.
[5] P.J. Borman, P. Nethercote, M.J. Chatfield, D. Thompson, K. Truman, Pharm. Technol. 31 (2007) 142–152.
[6] F.G. Vogt, A.S. Kord, J. Pharm. Sci. 100 (2011) 797–812.
[7] M. Blanco, J. Coello, H. Iturriaga, S. Maspoch, C. de la Pezuela, Analyst 123 (1998) 135R–150R.
[8] G. Reich, Adv. Drug Deliv. Rev. 57 (2005) 1109–1143.
[9] Y. Roggo, P. Chalus, L. Maurer, C. Lema-Martinez, A. Edmond, N. Jent, J. Pharm. Biomed. Anal. 44 (2007) 683–700.
[10] E. Ziemons, J. Mantanus, P. Lebrun, E. Rozet, B. Evrard, Ph. Hubert, J. Pharm. Biomed. Anal. 53 (2010) 510–516.

[11] X. Liu, D. Sun, F. Wang, Y. Wu, Y. Chen, L. Wang, J. Pharm. Sci. 6 (2011) 2452–2459.
[12] R.F. Li, X.Z. Wang, S.B. Abebe, Part. Syst. Charact. 25 (2008) 314–327.
[13] C.Y. Ma, X.Z. Wang, Particuology 9 (2011) 589–597.
[14] Y. Wu, Y. Jin, H. Ding, L. Luan, Y. Chen, X. Liu, Spectrochim. Acta A 5 (2011) 934–939.
[15] H.W. Ward, S.S. Sekulic, M.J. Wheeler, G. Taber, F.J. Urbanski, F.E. Sistare, T. Norris, P.K. Aldridge, Appl. Spectrosc. 52 (1998) 17–21.
[16] H.W. Ward, F.E. Sistare, Anal. Chim. Acta 595 (2007) 319–322.
[17] S.A. Arnold, R. Gaensakoo, L.M. Harvey, B. McNeil, Biotechnol. Bioeng. 80 (2002) 405–413.
[18] S. Vaidyanathan, S.A. Arnold, L. Matheson, P. Mohan, G. Macaloney, B. McNeil, L.M. Harvey, Biotechnol. Prog. 16 (2000) 1098–1105.
[19] C. Hakemeyer, U. Strauss, S. Werz, G.E. Jose, F. Folque, J.C. Menezes, Talanta 90 (2012) 12–21.
[20] L.O. Rodrigues, J.P. Cardoso, J.C. Menezes, Talanta 75 (2008) 1203–1207.
[21] A. Peinado, J. Hammond, Andrew Scott, J. Pharm. Biomed. Anal. 54 (2011) 13–20.
[22] International Conference on Harmonization (ICH), Validation of analytical procedures: text and methodology, Q2 (R1), 2005.
[23] C. De Bleye, P.-F. Chavez, J. Mantanus, R. Marini, Ph. Hubert, E. Rozet, E. Ziemons, J. Pharm. Biomed. Anal. 69 (2012) 125–132.
[24] European Pharmacopoeia Commission, Group PAT, Near-infrared spectrophotometry (2.2.40), Pharmeuropa 23 (2011) 3.
[25] Z.-P. Chen, J. Morris, A. Borissova, S. Khan, T. Mahmud, R. Penchev, K.J. Roberts, Chemometr. Intell. Lab. 96 (2009) 49–58.
[26] C. Schaefer, C. Lecomte, D. Clicq, A. Merschaert, E. Norrant, F. Fotiadu, J. Pharm. Biomed. Anal. 83 (2013) 194–201.
[27] European Medicines Agency (EMA), Guideline on the Use of near Infrared Spectroscopy (NIRS) by the Pharmaceutical Industry and the Data Requirements for New Submissions and Variations, Draft, 2012.
[28] Pharmaceutical Analytical Sciences Group (PASG), NIR Sub-group Guidelines for the Development and Validation of Near-Infrared Spectroscopic Methods in the Pharmaceutical Industry, PASG, 2002.
[29] M. Schweitzer, M. Pohl, M. Hanna-Brown, P. Nethercote, P. Borman, G. Hansen, K. Smith, Pharm. Tech. Eur. 22 (2010) 29.
[30] M. Blanco, J. Coello, A. Eustaquio, H. Iturriaga, S. Maspoch, Anal. Chim. Acta 392 (1999) 237–246.
[31] J. Mantanus, E. Ziemons, P. Lebrun, E. Rozet, R. Klinkenberg, B. Streel, B. Evrard, Ph. Hubert, Anal. Chim. Acta 642 (2009) 186–192.
[32] Ph. Hubert, P. Chiap, J. Crommen, B. Boulanger, E. Chapuzet, N. Mercier, S. Bervoas-Martin, P. Chevalier, D. Grandjean, P. Lagorce, M. Lallier, M.C. Laparra, M. Laurentie, J.C. Nivet, Anal. Chim. Acta 391 (1999) 135–148.
[33] 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, J. Pharm. Biomed. Anal. 36 (2004) 579–586.
[34] Ph. Hubert, J.J. Nguyen-Huu, B. Boulanger, E. Chapuzet, P. Chiap, N. Cohen, P.A. Compagnon, W. Dewe, M. Feinberg, M. Lallier, M. Laurentie, N. Mercier, G. Muzard, C. Nivet, L. Valat, E. Rozet, J. Pharm. Biomed. Anal. 45 (2007) 70–81.
[35] Ph. Hubert, J.J. Nguyen-Huu, B. Boulanger, E. Chapuzet, N. Cohen, P.-A. Compagnon, W. Dewé, M. Feinberg, M. Laurentie, N. Mercier, G. Muzard, L. Valat, E. Rozet, J. Pharm. Biomed. Anal. 45 (2007) 82–96.
[36] H. Wu, M. Tawakkul, M. White, M.A. Khan, Int. J. Pharm. 372 (2009) 39–48.
[37] F. Wulfert, W.Th. Kok, A.K. Smilde, Anal. Chem. 70 (1998) 1761–1767.
[38] S. Adam, D. Suzzi, C. Radeke, J.G. Khinast, Eur. J. Pharm. Sci. 42 (2011) 106–115.
[39] Z. Xiaobo, Z. Jiewen, M.J.W. Povey, M. Holmes, M. Hanpin, Anal. Chim. Acta 667 (2010) 14–32.
[40] S. Wold, K. Esbensen, P. Geladi, Chemometr. Intell. Lab. Syst. 2 (1987) 37–52.
[41] The Unscrambler Appendices: Method References, Camo ⟨http:/www.camo.com/downloads/U9.6%20pdf%20manual/The%20Unscrambler%20Method%20References.pdf⟩ (accessed October 20, 2013).
[42] S. Wold, M. Sjostrom, L. Eriksson, Chem. Int. Lab. Syst. 58 (2001) 109–130.
[43] X.Y. Niu, F. Shen, Y.F. Yu, Z. Yan, K. Xu, H.Y. Yu, Y.B. Ying, J. Agric. Food Chem. 56 (2008) 7271–7278.
[44] A. Savitzky, M.J. Golay, Anal. Chem. 36 (1964) 1627–1639.
[45] M. Jamrogiewicz, J. Pharm. Biomed. Anal. 66 (2012) 1–10.
[46] International Vocabulary of Basic and General Terms in Metrology, 2nd edition, ISO, Geneva, 1993.
[47] Guide to the Expression of Uncertainty in Measurement, ISO, Geneva, 1993.
[48] Analytical Methods Committee, Analyst 120 (1995) 2303–2308.
[49] R.D. Marini, P. Chiap, B. Boulanger, S. Rudaz, E. Rozet, J. Crommen, Ph. Hubert, Talanta 68 (2006) 1166–1175.
[50] R.P. Cogdill, C.A. Anderson, J.K. Drennen III, AAPS PharmSciTech 6 (2005) E284–E297.
[51] D.C. Montgomery, Statistical Quality Control: A Modern Introduction, 7th edition., Wiley, New York, 2013.