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**NIR SPECTROSCOPIC METHOD FOR THE IN-LINE MOISTURE ASSESSMENT DURING DRYING
IN A SIX-SEGMENTED FLUID BED DRYER OF A CONTINUOUS TABLET PRODUCTION LINE:
VALIDATION OF QUANTIFYING ABILITIES AND UNCERTAINTY ASSESSMENT**

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ABBREVIATIONS

FDA	Food and Drug Administration
ICH	international conference on harmonisation
KF	Karl Fischer
LHP	LightHouseProbe™
NIR	Near Infrared
PAT	Process Analytical Technology
PCA	principal component analysis
PLS	partial least squares
RMSEC	root mean square error of calibration
RMSECV	root mean square error of cross-validation
RMSEP	root mean square error of prediction
SEC	standard error of calibration
SEP	standard error of prediction
	Société Française des Sciences et Techniques
SFSTP	Pharmaceutiques
SNV	standard normale variate

ABSTRACT

This study focuses on the thorough validation of an in-line NIR based moisture quantification method in the six-segmented fluid bed dryer of a continuous from-powder-to-tablet manufacturing line (ConsiGma™ 25, GEA Pharma Systems nv, Wommelgem, Belgium). The moisture assessment ability of an FT-NIR spectrometer (Matrix™-F Duplex, Bruker Optics Ltd, UK) equipped with a fiber-optic Lighthouse Probe™ (LHP, GEA Pharma Systems nv, Wommelgem, Belgium) was investigated. Although NIR spectroscopy is a widely used technique for in-process moisture determination, a minority of NIR spectroscopy methods is thoroughly validated.

A moisture quantification PLS model was developed. Twenty calibration experiments were conducted, during which spectra were collected at-line and then regressed versus the corresponding residual moisture values obtained via Karl Fischer measurements. The developed NIR moisture quantification model was then validated by calculating the accuracy profiles on the basis of the analysis results of independent in-line validation experiments. Furthermore, as the aim of the NIR method is to replace the destructive, time-consuming Karl Fischer titration, it was statistically demonstrated that the new NIR method performs at least as good as the Karl Fischer reference method.

34 INTRODUCTION

35 In 2004, the Food and Drug Administration (FDA) launched their (Process Analytical
36 Technology) PAT-Guidance for Industry' [1]. One of the goals of this guidance is to encourage
37 the pharmaceutical industry to imply innovative methods for quality assurance, based on
38 measurements during processing and enhancing in-depth process understanding. It is stated
39 that the use of PAT tools and -principles will lead to an increased process knowledge which
40 should allow better process control. Real-time process information can be used to overcome
41 the limitations of time-defined process end points, which are commonly used in
42 pharmaceutical industry. In case of fluid bed drying for example, it is not opportune to dry
43 longer than necessary. Thanks to these industrial drivers, recent technological advances in
44 process analyzers make real-time measurement of critical process parameters and quality
45 attributes possible. To allow in-line monitoring, analyzers should be non-destructive, fast and
46 mountable in the process. Furthermore, the analyzer should be capable of collecting data
47 without sample-pretreatment. Therefore, Near Infrared (NIR) Spectroscopy has found already
48 for a long time its way to the pharmaceutical industry.

49 NIR has been reported to be a powerful tool for water determination. It has found multiple
50 applications for the quantitative determination of moisture content during the manufacturing
51 of pharmaceutical solid dosage forms [2]. Several authors reported the in-line, on-line and at-
52 line use of NIR spectroscopy to determine the moisture content of pharmaceutical products
53 during granulation and drying [3-18]. These authors developed multivariate calibration
54 models, e.g. Partial Least Squares (PLS)-models, for the prediction of moisture content, using

either Loss on Drying or Karl Fischer titration as reference method. They validated their models by means of validation parameters, such as the Standard Error of Calibration (SEC), the Standard Error of Prediction (SEP) as well as the Root Mean Square Error of Calibration (RMSEC), the RMSE of Cross Validation (RMSECV), the RMSE of Prediction (RMSEP) and the R^2 . Among these, the best measure for the predictive power of the model is the RMSEP, since this includes the evaluation of an external test-set.

However, all these validation parameters do not guarantee that future measures will be inside predefined acceptance limits [19]. Hubert et al. presented a validation strategy allowing to evaluate the proportion of expected future measures that will fall inside the acceptance limits. The acceptance limit is the accepted difference between the 'measured value' and the unknown true value [20-22]. This validation strategy is based on the use of accuracy profiles and is in full compliance with the regulatory quality guidelines. Mantanus et al. constructed for example a PLS model, which predicted the moisture content in pellets by means of NIR spectroscopy [23]. They concluded that the SEC and SEP were not sufficient to validate the method and therefore applied the accuracy profile approach. More case studies for the application of accuracy profiles to mainly off-line NIR spectroscopic methods have been reported by De Bleye et al. [19]. Raman spectroscopic methods for the determination of medroxyprogesterone in pharmaceutical suspensions [24] and metoprolol tartrate during hot-melt extrusion [25] have been validated using accuracy profiles as well.

The **aim** of this study was to validate a method for the in-line moisture determination of granules via NIR spectroscopy in the six-segmented fluid bed dryer of a fully continuous from powder-to-tablet production line. An NIR system equipped with a fiber-optic Lighthouse

77 Probe™ implemented in the dryer was used. The granulates were produced via high shear
78 twin screw wet granulation. The validation of the NIR based moisture assessment was based
79 on the use of accuracy profiles as presented by the SFSTP (La Société Française des Sciences
80 et Techniques Pharmaceutiques) [20-22]. Furthermore, from the validation measurements
81 performed to apply the accuracy profile validation strategy, the measurement uncertainty was
82 estimated as described by Feinberg et al. [26, 27]. As the final aim was to evaluate whether
83 the in-line NIR method is able to replace the off-line Karl Fischer method, the proposed
84 method must perform at least as good as the reference method. Therefore, a comparison of
85 the performance (precision and bias) of both methods was made via the approach proposed
86 by [28, 29].

87 MATERIALS AND METHODS

88 Materials

89 All continuous granulation and fluid bed drying experiments were performed with the same
90 powder premix. Anhydrous theophylline (Farma-Quimica sur SL, Malaga, Spain) (30%, w/w)
91 was granulated with lactose monohydrate 200 M (Caldic, Hemiksem, Belgium).
92 Polyvinylpyrrolidone (Kollidon 30®, BASF, Burgbernheim, Germany) was used as a binder and
93 added to the dry powder mixture at a concentration of 2.5% (w/w). Distilled water was used
94 as granulation liquid.

95 NIR equipment

96 An FT-NIR spectrometer (Matrix™-F Duplex, Bruker Optics Ltd, UK) equipped with a fiber optic
97 Lighthouse Probe™ (LHP, GEA Pharma Systems nv, Wommelgem, Belgium) was used for this
98 study. The Lighthouse Probe™ was mounted in cell 2 of the fluid bed dryer (see description of
99 the continuous system below). For all spectra a resolution of 8 cm⁻¹ was applied and each
100 spectrum was the average of 32 scans. This resulted in one spectrum collected every 20
101 seconds. The applied spectral range was 10,000 to 4,500cm⁻¹.

102 Karl Fischer moisture determination

103 The residual moisture content of the granules after drying was determined by volumetric Karl
104 Fischer titration using a V30 volumetric Karl Fischer titrator (Mettler Toledo, USA). Methanol
105 (Hydranal, Sigma Aldrich, Germany) was used as solvent. Before titration, granules were
106 stirred and dissolved during three minutes. Measurements were done in triplicate.

Continuous granulation and drying

For continuous granulation and drying, the ConsiGma™ 25 unit (GEA Pharma Systems, Collette™, Wommelgem, Belgium) was used [30]. It consists of three major parts: a continuous twin screw high shear granulator, a six-segmented fluid bed dryer and a discharge system. The six-segmented dryer is equipped with two probe interfacing places, namely in cell 2 and cell 5. PAT probes can be mounted in these cells, hence enabling in-line measurements. For both the calibration and validation experiments (see below), the granulator settings were kept constant. The raw material premix was fed at a rate of 17.5 kg/h, a screw speed of 950 rpm was applied and the barrel temperature was kept constant at 25°C. The distilled water was added with a rate of 32 g/min. The same airflow was applied in the fluid bed dryer for all experiments: 360 m³/h.

Development of the NIR moisture determination calibration model

For the development of the calibration model, granules with five different moisture levels were prepared (Figure 1). To obtain these granules, five granulation runs were performed in which the applied drying air temperature was varied between 35°C and 80°C. The cell filling time was kept constant at 180s and granules were dried during 600s. The granules for which the highest moisture content was aimed, were only dried for 300s. Each moisture level experiment was repeated four times, spread over two days (hence resulting in a total of 5 x 4 = 20 calibration experiments). After each calibration experiment, five NIR spectra were immediately collected at-line. Simultaneously, three samples were collected for Karl Fischer measurements. These measurements were performed immediately. Nevertheless, since only

one Karl Fischer titrator was available, the second and third Karl Fischer measurement had a certain 'lag time' before they were measured. Spectra were Standard Normal Variate (SNV) corrected prior to further analysis and the three Karl Fischer measurements were averaged in order to obtain one moisture content value for each calibration experiment that was then correlated to the five corresponding collected NIR spectra from that run. Taking the 20 calibration experiments into account, the obtained KF moisture contents varied between 3.4% and 7.21%, which is a relevant range for the residual moisture content of twin screw granulated theophylline-lactose granules REF DROOGPAPER.

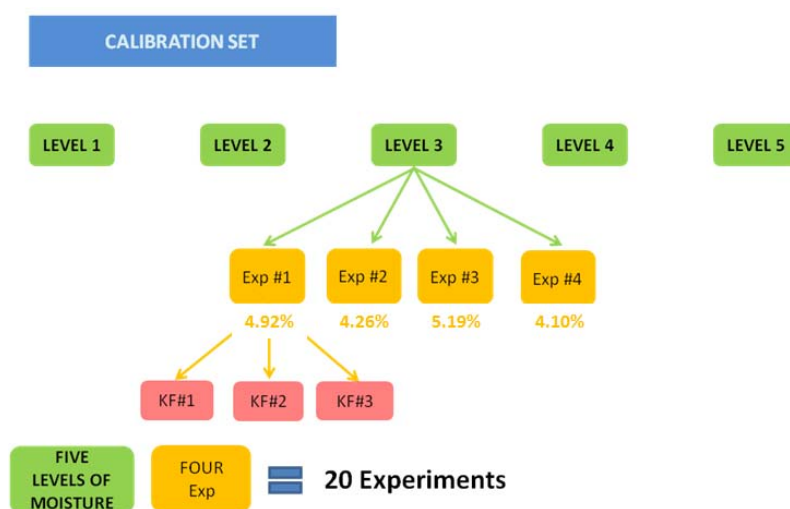


Figure 1. Overview of the performed calibration experiments.

Validation of the in-line NIR moisture assessment method

For the validation experiments the drying cells were filled during 300s and a total drying time of 900s was applied. The temperature of the drying air was changed between 35°C and 75°C in order to obtain three different moisture content (Figure 2). The low level had an average moisture content of 3.67%, the medium moisture content was 4.79% and the highest moisture

content was 6.13% (determined by KF). Each level was performed in triplicate and repeated on four different days. A total of four blocks of three experiments resulted in twelve experiments per moisture level. The validation experiments were repeated on four different days in order to evaluate the intermediate precision. Karl Fischer measurements were performed after each validation experiment, as was done for the calibration experiments.

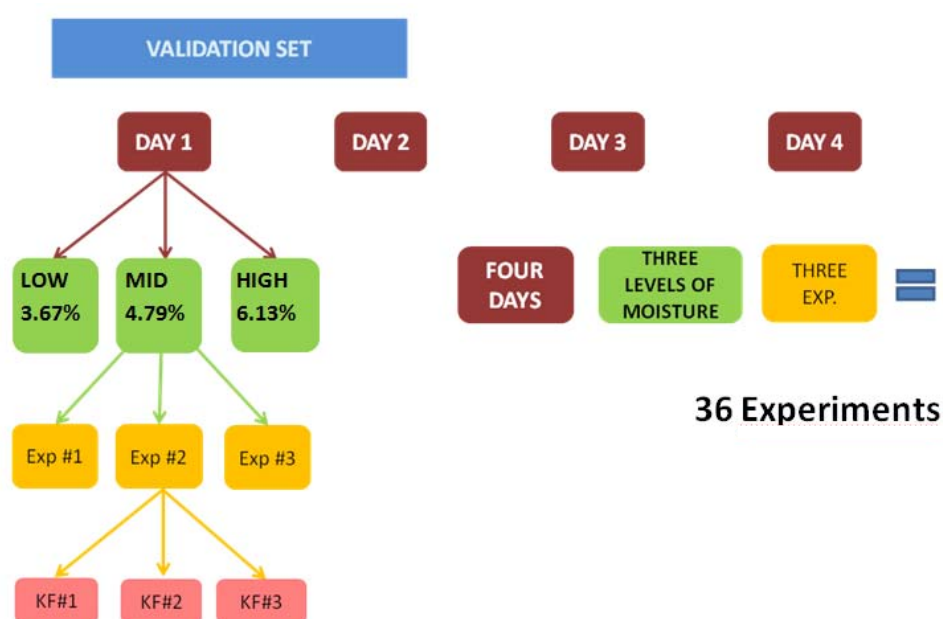


Figure 2. Overview of the performed validation experiments.

NIR spectra were collected in-line during drying. One spectrum was collected each 20 seconds, hence providing real-time spectral information. Each collected NIR spectrum was fed into the PLS model calibration model resulting in a predicted moisture content each time an NIR spectrum was collected during processing. After each validation experiment, three samples for Karl Fischer titration, as well as five at-line spectra were collected. The at-line spectra were collected for verification purposes only.

In order to evaluate the in-line collected spectra, Principal Component Analysis was performed on all in-line spectra per experiment. Scatter plots of the first and second component were used in order to detect and exclude outliers. With this information the last ‘valid’ spectrum of each run was selected. In this manner a set of 36 in-line spectra were constructed as validation test-set.

Multivariate data-analysis and modeling

Principal Component Analysis (PCA) and Partial least squares (PLS) calibration models were developed using a multivariate data analysis software package (Simca P+ 12.0, Umetrics AB, Umeå, Sweden).

Validation of the in-line moisture assessment method

The validation strategy introduced by Hubert et al. [20-22] proposed to review the objectives of the validation according to the intended use of the analytical procedure: to ensure that the difference between the “measured value” (y_i) and the unknown “true value” (μT), is at least lower than an acceptance limit (λ):

$$|y_i - \mu T| < \lambda$$

In this paper, λ is set at 20% for the in-line determination of the moisture content of granules during drying. Thus, as the aim of validation is to determine if an analytical procedure can be guaranteed as acceptable, it must be assured that the probability that a measurement will fall

175 outside the acceptance limits is less or equal to the maximum risk (5%) that the analyst is able
176 to take during routine use:

$$177 \Pr (|y_i - \mu_T| < \lambda) \geq \beta$$

178 If so, the analytical procedure can be considered as acceptable. In this study, the wished
179 proportion of measurements inside the acceptance limits (β) was set at 95%. It is clear that
180 the classical determinations of trueness, precision, linearity etc.. as they are described in the
181 ICH Q2 are not sufficient to decide whether the objectives of an analytical procedure are
182 guaranteed. The adapted decision tool therefore is the accuracy profile of the analytical
183 procedure, which is constructed from the total error (bias + standard deviation) of the
184 procedure. This is obtained by computing the β -expectation tolerance interval at each
185 concentration level and allows evaluating the proportion of expected future measurements
186 that will fall inside the acceptance limits. This enables to control the risk associated to the use
187 of the method. Using the data collected for the development of the accuracy profile, the
188 measurement uncertainty was estimated as described by Feinberg et al [26, 27]. During
189 validation, the within day and between day variability were taken into account (Figure 2).

190

191

192

193 **Comparison of the NIR method with the KF reference method**

194 The final aim of the present work is to replace the slow and destructive Karl Fischer reference
195 method for the quantitative moisture assessment during drying by a fast and non-destructive
196 alternative in-line analysis method. Therefore, it is necessary to demonstrate that the
197 envisaged method does not have worse performance characteristics than the reference
198 method. Therefore, a comparison of the two fundamental performance parameters, precision
199 and bias, was performed as described by [28, 29] The approach for method comparison
200 proposed in those papers, calculates the number of measurements needed to assure the
201 preset probability ($1-\beta$) of not adopting an alternative method with unacceptable
202 performance (α and β error are under control). This may require a higher number of
203 measurements than a laboratory can perform. This was the case in our study (15
204 measurement days required) due to practical, time and cost reasons. Therefore, a user-
205 defined number of measurements was used and only the α error (risk to reject the null
206 hypothesis, when it should be accepted) was under control. On the other hand, it was possible
207 to calculate the β error [31] The comparison of the precision parameters (repeatability and
208 intermediate precision) between both methods was done using F-tests. Bias was compared
209 using the interval hypothesis t-test instead of the point hypothesis t-test, as it is more relevant
210 to test whether bias between two methods is not too large (for this study, smaller than or
211 equal to 0.5%) instead of testing that the bias between methods is zero [32]. From a chemical
212 point of view, there is always a bias. Furthermore, the traditional null and alternative
213 hypotheses were exchanged (thus the α - and β -error are exchanged too) for the interval
214 hypothesis testing. This is, since it is more important to control the risk to wrongly accept
215 there is no bias while in fact here is, instead of wrongly accepting there is bias, while there is

216 none. For this study the 'new' α risk (= risk to wrongly accept that there is no bias) was chosen
217 at 0.05.

218 **RESULTS AND DISCUSSION**

219 **Development of a NIR based calibration model**

220 Hundred spectra were obtained during the calibration experiments and used to build the
221 calibration model (5 moisture levels * 4 repetitions * 5 spectra/experiment = 100 spectra, see
222 Figure 1). All spectra were mean centered and SNV-corrected after which a principal
223 component analysis (PCA) was performed, using the full spectral region from 10000 to 4500
224 cm^{-1} . The first principal component, capturing a variance of 90.58%, represented moisture
225 content. The loadings of the first principal component clearly indicated the spectral region
226 between 5350 cm^{-1} and 4775 cm^{-1} enclosed the most variance, which is indeed where the
227 water bands are expected. Hence, the 5350 to 4775 cm^{-1} spectral region was selected,
228 centered and SNV-corrected to build a PLS calibration model for the prediction of moisture
229 content. This PLS model consisted of two principal components, resulting in an R^2 of 99.7%, a
230 Q^2 of 96.4% and an RMSEE of 0.2342% and an RMSECV of 0.2331%.

231 **Validation of the in-line moisture assessment method**

232 Using the above developed PLS moisture calibration model, the moisture content at the end
233 of each drying validation experiment was predicted (Table 1, y_{ipred}) based on the last valid in-
234 line collected spectrum (Figure 3). Due to process variation it was not possible to obtain the
235 exactly equal moisture contents when repeating the validation runs. (e.g. the low level
236 moisture content validation experiment of day 1 resulted in a slightly different moisture
237 content compared to the low level moisture content experiment of day 2). Therefore, at each

validation moisture level all the NIR predicted moisture content values were normalized as follows (see Table 1):

Equation 1.

$$y_{inor} = \frac{y_{ipred} * mean}{y_{iref}}$$

With

y_{inor} = normalized NIR predicted moisture content of validation experiment i

y_{ipred} = moisture content as predicted by the NIR calibration model

$mean$ = mean Karl Fischer moisture content at each moisture level: 3.67%, 4.76% or 6.13%, respectively

y_{iref} = moisture content as obtained via the Karl Fischer titration for validation experiment i

Table 1. The moisture content obtained after each validation experiment by means of Karl Fischer (y_{iref}), the value predicted by the PLS-calibration model (y_{ipred}) and the normalized value (y_{inor}).

Experiment	y_{iref}	y_{ipred}	y_{inor}
Low Day 1	3.51	3.56	3.73
Low Day 1	3.53	3.43	3.57
Low Day 1	3.73	3.49	3.43
Low Day 2	3.63	3.50	3.54

Low Day 2	3.58	3.46	3.54
Low Day 2	3.57	3.45	3.55
Low Day 3	3.78	3.48	3.38
Low Day 3	3.54	3.38	3.50
Low Day 3	3.73	3.45	3.40
Low Day 4	3.72	3.83	3.78
Low Day 4	3.79	3.78	3.66
Low Day 4	3.95	3.81	3.55
Mid Day 1	4.74	4.80	4.85
Mid Day 1	4.81	4.74	4.71
Mid Day 1	4.77	4.77	4.78
Mid Day 2	5.07	4.94	4.66
Mid Day 2	5.14	4.87	4.53
Mid Day 2	4.87	4.62	4.54
Mid Day 3	4.28	4.03	4.50
Mid Day 3	4.89	4.33	4.24
Mid Day 3	4.75	4.29	4.32
Mid Day 4	4.15	3.85	4.44
Mid Day 4	5.19	4.94	4.56
Mid Day 4	4.77	4.69	4.71
High Day 1	5.71	5.69	6.11
High Day 1	5.85	5.81	6.08
High Day 1	5.69	5.38	5.80
High Day 2	6.7	6.44	5.89

High Day 2	6.5	6.23	5.87
High Day 2	6.44	6.07	5.77
High Day 3	5.7	5.91	6.36
High Day 3	6.28	5.98	5.83
High Day 3	6.09	6.15	6.19
High Day 4	6.09	6.10	6.14
High Day 4	6.16	6.12	6.09
High Day 4	6.33	6.14	5.94

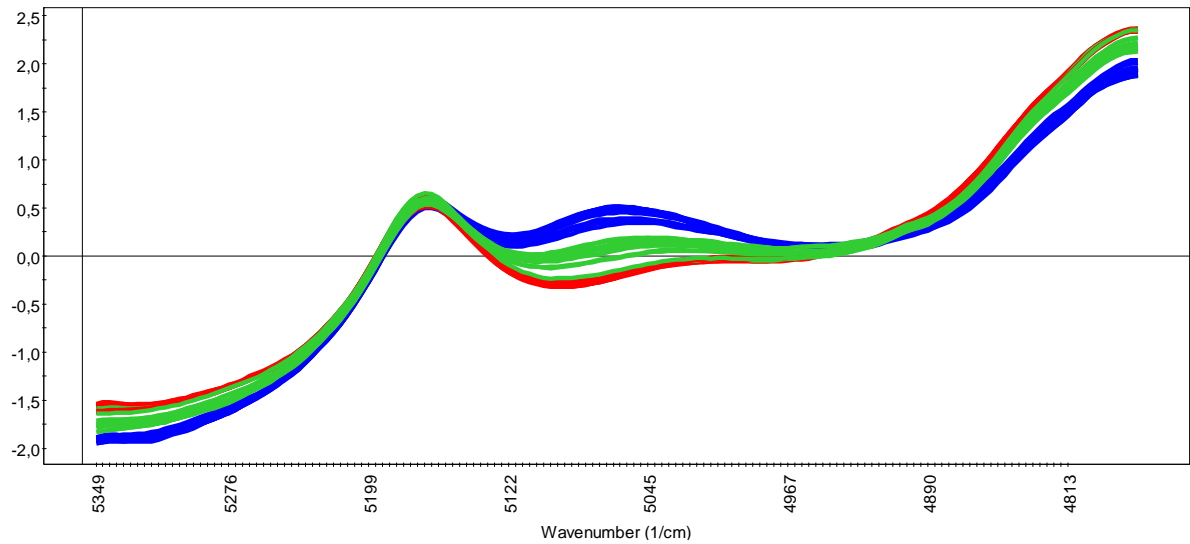


Figure 3. The last valid in-line collected spectrum for each validation run in the spectral range from 5350 cm^{-1} to 4775 cm^{-1} . Spectra are colored after moisture content: high-blue, mid-green and low-red.

The calculated RMSEP for the y_{iref} compared to the y_{ipred} is 0.2236. The normalized (Table 1, y_{inor}) values were used to calculate the trueness, precision, accuracy (Table 2) and accuracy profile (Figure 4) for each validated residual moisture level. It can be noticed from the accuracy

profile that the β -expectation tolerance intervals do not exceed the acceptance limits of 20% over the validated concentration range. Ninety-five times out of 100, the future in-line measurements will be included within the acceptance limits of 20%. Thus, using this validation strategy, the accuracy profile is used to *decide* whether the in-line NIR method can be declared valid or not. The validation data calculated in Table 2 help to make a *diagnosis*.

From the data used for the calculation of the accuracy profile, the measurement uncertainty was estimated [26]. The uncertainty results are presented in Table 3. The expanded uncertainty defines an interval around the mean value in which the unknown 'true value' is retrieved with a probability of 95%. From the relative expanded uncertainties, it can be seen that for each examined moisture level, the interval in which there is a probability of 95% that the true value, estimated by the measured value, occurs, is larger than 5%.

Table 2. Calculated in-line NIR method validation parameters.

Trueness		
Level	Relative bias	Recovery
Low	-3.25 %	96.75 %
Mid	-4.50 %	95.50 %
High	-1.99 %	98.01 %

Precision

Level	Repeatability	Intermediate precision
Low	2.78%	3.55 %
Mid	2.30%	4.24 %
High	2.85%	3.05 %

273

274

Accuracy

Level	Absolute total error	Relative total error	Relative β -expectation tolerance limits
Low	0.245 %	6.68 %	[-11.94; 5.43] %
Mid	0.409 %	8.54 %	[-16.81; 7.81] %
High	0.305 %	4.98 %	[-9.11; 5.12] %

275

276 **Table 3.** Point estimates of the different uncertainties related to moisture content at each
277 moisture content level of the accuracy profile

Level	Uncertainty of the bias (%)	Uncertainty (%)	Expanded uncertainty (%)	Relative expanded uncertainty (%)
Low	0.048	0.135	0.270	7.35

Mid	0.087	0.212	0.424	8.87
High	0.060	0.193	0.386	6.29

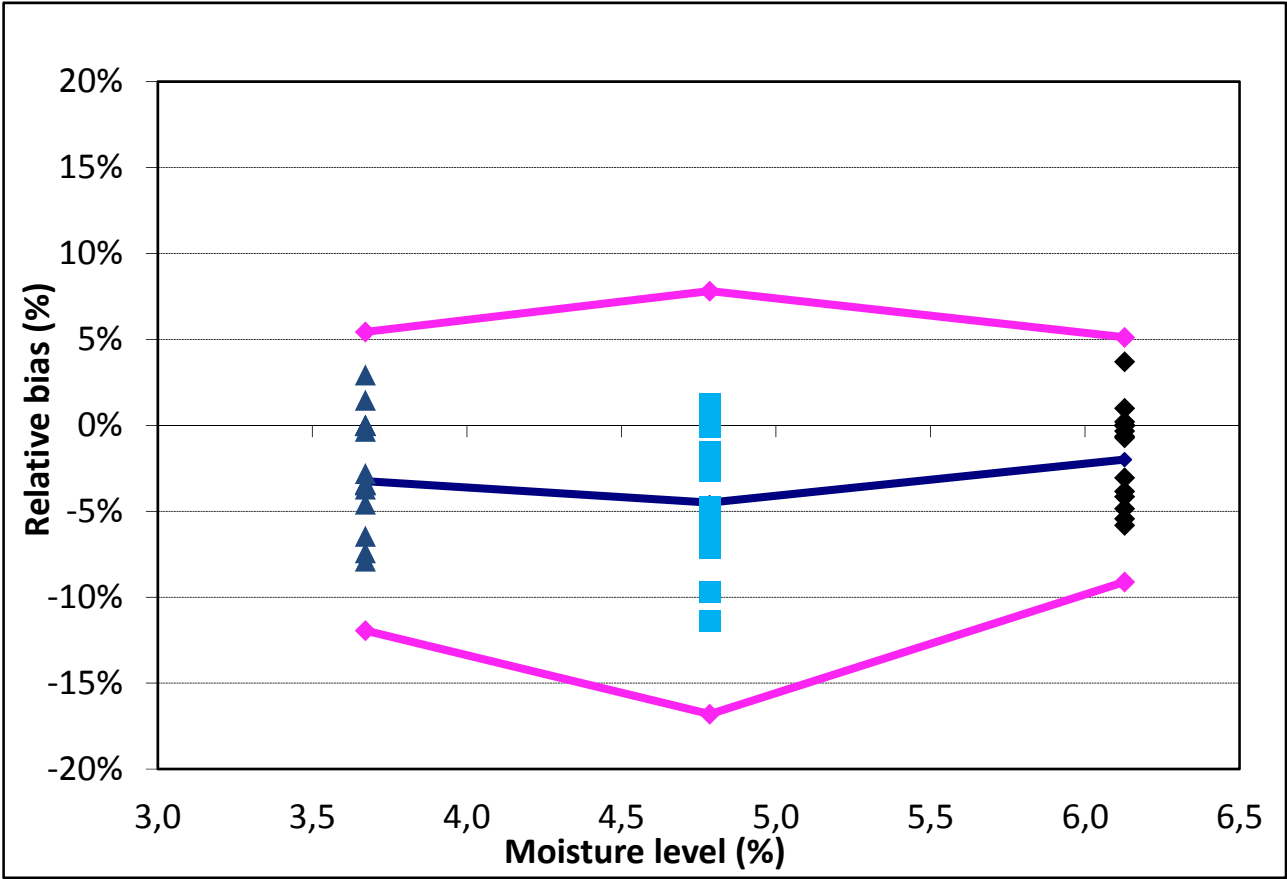


Figure 4. Accuracy profile.

Comparison of alternative in-line NIR method with Karl Fischer reference method

The NIR as well as the Karl Fischer validation data used for the comparison of both methods at the lowest examined residual moisture content level are mentioned in Table 4. In a first step, the different types of variances at this low level were calculated (Table 5). These variances were used for the calculation of the F-tests for the evaluation of the different precision parameters (Table 6). From these tests, it can be concluded that the repeatability as

well as the intermediate precision are at least as good as those of the KF reference method. The α error of these F-tests (risk to wrongly reject the conclusion that the precision parameters of the NIR based prediction method are better or equal to the precision parameters of the KF reference method) was set at 5%. For the evaluation of the bias, the estimated bias d (grand mean of alternative validation data – grand mean of reference validation data = 0.12%), was calculated together with its 95% upper confidence limit (UCL = 0.317%) and lower confidence limit (LCL = -0.077%). If UCL is smaller or equal or LCL is higher or equal to the acceptable bias (0.5%), the bias is acceptable since there is probability smaller than 5% that the absolute bias as estimated by d is larger than 0.5%. Using the interval hypothesis, the risk to wrongly accept that there is no bias ('new' α error) was kept at 5%. The probability to reject wrongly a non biased method was calculated as 2.40% ('new' β error).

Table 4. KF reference data and NIR validation data used for method comparison.

			KF	predicted by NIR calibration model
day 1	low (3,6%)	exp 1	3,51	3,56
day 1	low (3,6%)	exp 2	3,53	3,43
day 1	low (3,6%)	exp 3	3,73	3,49
day 1	mid (4,5%)	exp 1	4,74	4,80
day 1	mid (4,5%)	exp 2	4,81	4,74
day 1	mid (4,5%)	exp 3	4,77	4,77
day 1	high (6,0%)	exp 1	5,71	5,69

day 1	high (6,0%)	exp 2	5,85	5,81
day 1	high (6,0%)	exp 3	5,69	5,38
day 2	low (3,6%)	exp 1	3,63	3,50
day 2	low (3,6%)	exp 2	3,58	3,46
day 2	low (3,6%)	exp 3	3,57	3,45
day 2	mid (4,5%)	exp 1	5,07	4,94
day 2	mid (4,5%)	exp 2	5,14	4,87
day 2	mid (4,5%)	exp 3	4,87	4,62
day 2	high (6,0%)	exp 1	6,7	6,44
day 2	high (6,0%)	exp 2	6,5	6,23
day 2	high (6,0%)	exp 3	6,44	6,07
day 3	low (3,6%)	exp 1	3,78	3,48
day 3	low (3,6%)	exp 2	3,54	3,38
day 3	low (3,6%)	exp 3	3,73	3,45
day 3	mid (4,5%)	exp 1	4,28	4,03
day 3	mid (4,5%)	exp 2	4,89	4,33
day 3	mid (4,5%)	exp 3	4,75	4,29
day 3	high (6,0%)	exp 1	5,7	5,91
day 3	high (6,0%)	exp 2	6,28	5,98
day 3	high (6,0%)	exp 3	6,09	6,15
day 4	low (3,6%)	exp 1	3,72	3,83
day 4	low (3,6%)	exp 2	3,79	3,78

day 4	low (3,6%)	exp 3	3,95	3,81
day 4	mid (4,5%)	exp 1	5,19	4,94
day 4	mid (4,5%)	exp 2	4,77	4,69
day 4	mid (4,5%)	exp 3	4,15	3,85
day 4	high (6,0%)	exp 1	6,09	6,10
day 4	high (6,0%)	exp 2	6,16	6,12
day 4	high (6,0%)	exp 3	6,33	6,14

Table 5. Overview of the different types of variances of the in-line NIR and KF validation used for method comparison.

Variance estimates	KF method	NIR method
Repeatability variance (s_r^2)	0.0114	0.0021
(Operator + day) variance (s_{OD}^2)	0.0078	0.0289
(Operator + day) intermediate precision	0.0193	0.0310
Variance of the day means	0.0116	0.0296

300

Table 6. Overview of the F-test results for the comparison of the repeatability and intermediate precision between the in-line NIR and KF reference method.

302

Evaluated precision comparison	Calculated F-value	Tabulated F-value	Conclusion
Repeatability	0.186	3.44	Repeatability of the in-line NIR method is acceptable
Time + operator different intermediate precision	1.61	4.35	Operator + time-different intermediate precision of the in-line NIR method is acceptable

304 CONCLUSION

305 The results show that the developed fast and non-destructive in-line NIR method is a reliable
306 alternative for the traditionally used destructive and time-consuming KF method for the in-
307 line moisture determination of granules in the six-segmented fluid bed dryer of a continuous
308 from-powder-to-tablet production line. The NIR spectra were collected in-line by means of a
309 Lighthouse ProbeTM, connected to a Brüker-system. The accuracy profile, calculated during
310 the validation of the in-line NIR method, assures that at least 95% of future routine
311 experiments will be included within the preset acceptance limits (20%), which suits with the
312 aim of the developed analytical procedure. Moreover, the data used in this validation
313 approach were also used to estimate the uncertainty of the bias as well as the expanded
314 uncertainty at each concentration level. Finally, the new NIR method has at least as good
315 performance characteristics (precision and bias) as the KF method.

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