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Validation of Raman spectroscopic procedures in agreement with ICH guideline Q2 with considering the transfer to real time monitoring of an active coating process

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ABSTRACT

A multivariate model was constructed by correlating Raman spectral data with coated amount of the API diprophylline using Partial Least Squares. In agreement with ICH guideline Q2 the method was validated in order to achieve the requirement of demonstrating that Raman spectroscopy is suitable as rapid PAT tool for inline quantitative monitoring of active coating. The present work presents an appropriate approach to transfer the requirements of the guidelines to the Raman method used for inline measurements and demonstrates that the requirements of the validation characteristics were achieved.

1. Introduction

Process analytical technologies (PAT) are common for accompanying manufacturing processes like in the pulp and paper or chemical and petroleum industry [1–3]. The US Food and Drug Administration (FDA) has redefined the phrase and implemented it into an initiative focusing on improving several aspects of the pharmaceutical industry. Process understanding, optimization of manufacturing efficiency, and reproducibility of product quality are the primary objectives of the process analytical technology (PAT) guidance issued by the FDA [4]. The ultimate goal is the Real Time Release (RTR) whereby batch release is based on data collected throughout the process without offline testing of manufactured products.

An overview of the applications of PAT in the pharmaceutical industry is given in the literature [5–7]. The implementation of PAT reaches from monitoring of the synthesis of the active pharmaceutical ingredient and identifying the raw material up to determining the concentration of the API in the finished dosage form [7]. It has been shown that spectroscopic methods in combination with multivariate data analysis are adequate for PAT applications. Particularly, near-infrared spectroscopy (NIR) has been established as PAT tool for pharmaceutical applications and is now used for monitoring blending, process control of granulation, drying and coating operations and many other applications [8–15]. Recently Raman spectroscopy has expanded to an adequate alternative method and has been applied as PAT tool for pharmaceutical applications [16–26].

In the formulation of solid dosage forms film coating represents an important unit operation which can fulfill different functions like taste masking, product identification and protective layering. Furthermore, film coating is frequently used to improve the therapeutic effect, for example enteric or controlled release coatings, which influence location and period of drug release. Active coating is a specific application of film coating where the active ingredient is comprised in the coating layer.

Both functional and active coatings are challenging operations regarding the achievement of desired amount of coating and coating uniformity. In order to guarantee the quality of such dosage forms it is desirable to develop a tool that is able to monitor the coating operation and to determine the coating uniformity, respectively. NIR and Raman spectroscopy are suitable analytical methods for inline monitoring and have been frequently applied for tablet coating processes [12–15,22–26].

The work focused on demonstrating the suitability of Raman spectroscopy as PAT tool for inline quantitative monitoring of active coating by validating the Raman analytical method in agreement with ICH guideline Q2 [27].

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The guideline Validation of Analytical Procedures (Q2) arrogates that every analytical technique must be shown to be suitable for its intended purpose. In dependence on the analytical procedure typical validation characteristics are required. In the case of assay procedures the accuracy, specificity, precision, range, and linearity are required. In the present work typical validation characteristics were examined while focusing on the transfer of the method to real time monitoring. Detection limit and quantitation limit are normally not required for assay procedures. In this case the assay was included in the investigation, as it is useful to know the detection and the quantitation limit in order to assign the area in which inline quantitative monitoring of active coating is possible. Furthermore, the ICH guideline postulates that the repeatability should be assessed using samples, which cover the specified range for the procedure. Alternatively, a minimum of six determinations at 100% of the test concentration is applicable. In the literature [28–30] the precision is examined in the range from 80% to 120% of the target API concentration in the case of assay procedures. But as in this case the whole process was monitored a tablet set covering the specified range of the API amount during the coating procedure was investigated. Finally it must be noted that multivariate methods of analysis like Partial Least Squares (PLS) are not considered in the development of ICH guidelines Q2 A&B which are necessary in combination with spectroscopic methods for application as PAT tool in order to handle the mounds of data. This problem was already discussed in the literature [28–30] but the guidelines are general and flexible enough that they can conform to the requirements and limitations of the method.

The model drug diprophylline (dph) was coated on placebo tablets and a multivariate quantitative calibration was performed using tablets collected at different stages of coating. The spectral measurements were correlated with the amount of coated active ingredient at each time point by using PLS. Afterwards the developed model was validated in agreement with ICH guideline Q2 whereby the focus was the transfer to real time monitoring. The application of the method was described recently [26].

2. Material and methods

2.1. Materials

2.1.1. Drug

The water soluble caffeine derivative diprophylline (dph; BASF, Ludwigshafen, Germany) was used as model drug for the active coating.

2.1.2. Tablets

The tablet cores were biconvex (4 mm in height, 7 mm in diameter) and were composed of 49.75% w/w lactose monohydrate (Tablettose® 80, Meggle, Wasserburg, Germany), 49.75% w/w microcrystalline cellulose (Avicel® PH 102, FMC International, Little Island Cork, Ireland) and 0.5% w/w magnesium stearate (Welding, Hamburg, Germany).

2.1.3. Coating solution

The composition of the solid fraction of the aqueous coating solution was 30% w/w hydroxypropyl methylcellulose (HPMC, Walocel® HM5 PA2910, Wolff Cellulosics, Walsrode, Germany), 10% w/w polyethylene glycol 1500 (Clariant GmbH, Frankfurt am Main, Germany) and 60% w/w diprophylline, whereby the aqueous coating solution contained 20% solids.

For preparing the blank a coating solution without diprophylline was used, which contained 9% of solids and was composed of 75% (w/w) HPMC and 25% (w/w) polyethylene glycol 1500 related to the solid fraction.

2.2. Methods

2.2.1. Tablet coating

In each case a batch size of 3.5 kg was coated in a Laboratory Film Coater BFC 5 (L. B. Bohle, Ennigerloh, Germany) with a pan diameter of 316 mm and a length of 356 mm, as described in the previous work [26].

2.2.2. Raman equipment

Raman spectra of tablets were collected using a PhAT System (Kaiser Optical Systems, Ann Arbor, USA) equipped with a non-contact optic sampling device. The excitation laser (785 nm diode laser) was introduced and magnified to form a circular illumination area of 6 mm diameter (area: 28.3 mm^2) to cover a large sample area. This wide area illumination (WAI) scheme improves the reliability of sample representation and the reproducibility of sampling due to less sensitivity of sample placement with regard to the focal plane [31,32]. The scattered radiation was collected by an array of 50 optical fibers and delivered to an air cooled CCD detector. A holographic transmission grating dispersed the radiation from the optical fibers and integrated a combined signal over the total illuminated area. Data collection and data transfer were automated using the HoloGRAMS™ (Kaiser Optical Systems, Ann Arbor, USA) data collection software package, the HoloREACT™ (Kaiser Optical Systems, Ann Arbor, USA) reaction analysis and profiling package, the Matlab® software package (version 6.5, The MathWorks, Inc., Natick, MA, USA), and Excel®.

In either case of the experiment the spectral data were preprocessed by standard normal variate (SNV) and mean centering in order to facilitate calibration development.

2.2.3. Validation of instrument operation

The European Pharmacopoeia [33] and the USP 33 [34] have published procedural methods for calibrating Raman spectrometers. The test of accuracy and precision for wavelength and photometry are routine quality control tests of the instrument performance and as such they were performed to verify correct instrument performance without being considered part of the current study.

2.2.4. Reference analysis

A reference method is needed in order to provide the values for the calibration calculation. Furthermore, reference values are needed to compare with the values calculated by the Raman measurement, in order to determine the accuracy of the Raman analytical method. The amount of coated diprophylline was determined by the reference analytical method UV spectroscopy (Lambda-2, PerkinElmer, Ueberlingen, Germany). On basis of the ICH guideline Q2 the UV spectroscopy was validated by performing the typical validation characteristics for assay procedures, in order to provide an appropriate reference method for the development of the Raman analytical method. The performance and results of the validation of the reference method are not discussed in the current study. For the UV spectroscopy a calibration for the range of diprophylline concentrations 0.7 mg/500 ml–18 mg/500 ml was performed with 10 different concentrations and three repeated measurements in each case. After dissolving the coated tablet in 500 ml water the absorption was measured at 273 nm.

2.2.5. Sample sets

For the calibration a tablet set ($n = 52$) from 0 mg to 11.2 mg dph amount collected at different stages of coating was used. An extra set of samples ($n = 24$) from 0 mg to 10.3 mg dph amount was used for model validation, which arose from the same batch as the calibration set. Additionally, a second validation set ($n = 36$) from

1.5 mg to 9.6 mg dph amount of an independent batch was available in order to test the model.

2.2.6. Calibration development

For the offline quantitative calibration development the tablets of the calibration set were measured with the Raman probe with a working distance of 22 cm and a scanning time of 10 s followed by cosmic ray filtering and a dark subtraction. Each tablet was measured three times and the respective average spectrum was used for the correlation. The tablets of the validation set were measured in the same way. In either case of the experiment the spectral data were preprocessed by standard normal variate (SNV) and mean centering to facilitate calibration development. A multivariate model was constructed by correlating preprocessed Raman spectral data in the region 1200–1400 cm^{-1} with coated amount of diprophylline (mg) using Partial Least Squares (PLS). In each case PLS models and data preprocessing (mean centering and SNV) were carried out using the Simca-P+ 11.5 software (Umetrics AB, Umeå, Sweden).

2.3. Validation of the Raman analytical method

The developed model was validated in agreement with ICH guideline Q2 by performing the typical validation characteristics with considering the transfer to real time monitoring. In each case the Raman measurement was performed with a working distance of 22 cm and a scanning time of 10 s followed by cosmic ray filtering and a dark subtraction. It must be noted that the methodology presented here is an appropriate approach and not the only suitable one which must be followed.

2.3.1. Accuracy

For determining the accuracy the calibration set ($n = 52$) and the two validation sets ($n = 24$; $n = 36$) were investigated. The accuracy was evaluated by comparing the results of the Raman analytical method with those of the validated reference method UV spectroscopy by calculating the statistical quantities standard error of calibration and standard error of prediction (SEC and SEP). These statistics describe in quantitative terms the agreement between the Raman values and the values from the reference method from the same samples in accordance with the ICH guidelines. While the calculation of SEC based on data from samples which were used to develop the calibration model, the SEP is calculated by using samples which were not included in the calibration calculations. Another appropriate statistic for comparing the results from the Raman method and the results from the reference method is the bias of the readings from the independent set of validation samples. The bias, which represents the systematic error, is the arithmetic mean of the differences between the two methods and should be small. In the case of the calibration set the bias should be nearly zero for a good calibration.

2.3.2. Specificity

The ICH guideline [27] postulates specificity for assay procedures in order to provide an exact result which allows an accurate statement on the content of the analyte in a sample. Specificity is defined as the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. In our case the coated dph amount is the analyte to be determined and the uncoated placebo tablet represents the matrix. Furthermore, the other components of the coating solution must be noted, whose amount increases in dependence on the coating stage, too. In order to investigate the specificity of the method the changes of the Raman spectra in dependence on the coated amount of dph were examined by using multivariate data analysis. In addition, blanks were prepared by coating tablets in the same way like the tablets of the calibration set but with a coating solution without dph. Afterwards, the blanks were used to test the model. In order to demonstrate the specificity the method must be able to differentiate between the blanks and samples coated with dph.

2.3.3. Precision

The ICH defines precision as the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed condition. In the worked example, precision was investigated by assaying the same five samples with different dph amount (tablets collected at different coating level). The investigated tablet set covered the specified range of the dph amount during the coating procedure with 1 mg dph amount representing the early stage of coating to 9.9 mg dph amount typifying the process end. After finishing the precision analysis the dph amounts of the five samples were determined by the reference method UV spectroscopy and were compared to the results of the Raman method.

Furthermore, precision can be assessed at three levels: repeatability (intra-assay precision), intermediate precision (inter-assay precision) and reproducibility.

2.3.3.1. Repeatability. Repeatability expresses the precision under the same operating conditions over a short interval of time. The repeatability was determined by six-fold measurement of the five samples without moving the sample (static measurement) during the series of measurements.

2.3.3.2. Intermediate precision. Intermediate precision expresses within-laboratories variations like different equipment, different days, and different analysts. The sample presentation is a critical factor in the case of the Raman method and in the USP 33 [34,35] it is postulated that the method validation must also encompass sample position. Therefore, the intermediate precision was examined by measuring the five samples six times and removing them from the holder and replacing them after each measurement (replaced measurement). Additionally, the intermediate precision was established by six-fold measurement of the five samples on six different days without moving the sample during the series of measurements. The same procedure was followed by a different analyst. While the first analyst performed the measurement in the morning, the second analyst measured the sample in the afternoon. Additionally, the photometric precision was investigated at the according six days in agreement with the USP 33 [34] for the interesting spectral area 1200–1400 cm^{-1} by using cyclohexane as reference standard. Therefore, a normalization and baseline correction was necessary which is only applied to the cyclohexane spectra. The peak at 801.3 cm^{-1} was set as reference peak and the areas of the peaks at 1266.4 and 1444.4 cm^{-1} were calculated.

2.3.3.3. Reproducibility. The reproducibility, which expresses the precision between laboratories, was not investigated.

2.3.4. Homogeneity of variance

The German standard DIN 38402 [36] postulates that the standard deviation of the repeated measurements is independent of the concentration within the working range of the analytical method. By investigating the repeatability of the five samples the homogeneity of variance over the working range is simultaneously estimated. The German standard DIN 38402 part 51 requires 10 repeat measurements for the lowest and highest concentration. Afterwards, the variance of the two samples has to be tested on significance by using the *F*-test. Therefore, a sample containing 0 mg (placebo tablet) coated dph amount, representing the lowest concentration of the working range and the sample with 11 mg coated

dph amount, representing the highest concentration, were measured additionally 10-fold in order to fulfill the requirements of the German standard. Furthermore, a sample with 0.5 mg dph amount, which is approximately the detection limit, was included in the investigation.

2.3.5. Range

For developing a quantitative method it is necessary to determine the range of analyte concentration/amount over which the method may be applied. The range of an analytical procedure is the interval between the upper and lower concentration/amount of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity [27]. The range depends on the application intended for the analytical procedure. In the present work the Raman method is used to monitor the coating process. The required range included the whole calibration set which presents the different stages of coating from beginning (0 mg dph) to the process end (11 mg dph). Generally, the values of the limits of detection (LD) and/or quantitation (QL) are the limiting factors at the lower end of the concentration range. At the upper end of the concentration range limitations will be imposed by various effects depending on the instrument response system [37].

2.3.6. Linearity

The ICH guidelines require the evaluation of linearity across the range of the analytical procedure in order to provide its ability to obtain test results which are directly proportional to the concentration/amount of the analyte in the sample. Besides a visual evaluation of the analyte signal as a function of the concentration, appropriate statistical calculations are recommended, such as linear regression. The ICH guidelines instruct calculation of the correlation coefficient, *y*-intercept, slope of the regression line and residual sum of squares by using linear regression for evaluating linearity. Ideally the intercept a and the slope b should be 0 and 1, respectively, in order to exclude a systematic error. Linear regression was applied and the 95% confidence interval (CI) was calculated for the intercept a and the slope b (equations (1) and (2)) of the calibration data set [30].

$$\mathrm{CI}_a = a \pm tS_{y1}\sqrt{\frac{1}{n} + \frac{\bar{x}^2}{S_{xx}}} \tag{1}$$

$$\mathrm{CI}_b = b \pm t\frac{S_{y1}}{\sqrt{S_{xx}}}. \tag{2}$$

In equations (1) and (2) S_{y1} stands for the residual standard deviation of the linear regression function, S_{xx} for the sum of squares and t for the student's t-value for the probability of error α and the degree of freedom f ($f = n - 2$). For the establishment of linearity, a minimum of five concentrations are recommended. The linearity was evaluated for the calibration set with 52 different concentrations, which were used to develop the model. In addition the *F*-test after Mandel (linearity test) was performed, which is described in the German standard DIN 38402 Part 51 [36]**.** The test after Mandel compared the linear regression function with a polynomial regression of second degree. The reduction of the residual variance by taking the polynomial regression of second degree is tested on significance by using an *F*-test. Therefore it is necessary to calculate the linear regression function $y = a + bx$ and the residual standard deviation $S_{y1,}$ and also the quadratic regression function $y = a + bx + cx^2$ and the residual standard deviation $S_{y2.}$

By the residual standard deviations S_{y1} and S_{y2} the difference of the residual sum of squares (DS^2) is calculated, $DS^2 = (n-2){S_{y1}}^2 - (n-3){S_{y2}}^2$ whereby the residual standard deviations S_y are defined as described in equations (3) and (4):

$$S_{y1} = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n-2}} \tag{3}$$

$$S_{y2} = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n-3}}, \tag{4}$$

whereby y_i stands for the observed value by the reference method and $\hat{y}_i$ stands for the predicted value by the Raman method. Finally, the test value $\mathrm{PW} = DS^2/{S_{y2}}^2$ is calculated for the *F*-test and is compared to the reference value of the *F*-table for $f_1 = 1$, $f_2 = n - 3$ and for the probability of error α=0.01. Besides for the calibration set, the *F*-test after Mandel was performed additionally for the two validation sets with 24 (1st validation set) and 36 (2nd validation set) different concentrations.

2.3.7. Detection and quantitation limit

The detection limit (DL) is a qualitative value and is defined as the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Only on the quantitation limit (QL) a quantitative statement is possible. The QL is defined as the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The detection (DL) and quantitation limits (QL) were determined by using the German standard DIN 32645 [38]. Additionally, the determination of the DL and QL was adopted according to the ICH guideline [27], where the use of multivariate methods like PLS is not considered. The ICH guideline described several methods for determining the DL and QL. Besides, the visual evaluation and the signal-to-noise approach the DL and the QL can be calculated by means of the standard deviation of the response (σ) and the slope of the calibration curve, which conformed to the sensitivity of the measurement procedure. In the present work not a real function which related signal and concentration but a PLS calibration was used to establish a regression between predicted values and reference values. After data reduction by performing the PLS the principal components conformed to the main information of the Raman spectra which correlated to the dph amount. In this work three principal components were needed to construct the PLS model and consequently the predicted values represent the spectral information condensed by the three principal components, which are related to the dph amount. In order to calculate DL and QL the standard deviation of the predicted value (apparent response (σ)) of the blank and the slope (b) of the PLS calibration (apparent sensitivity) were used. The approach of the German standard DIN 32645 based on the variability of the concentration dependent experimental determination. The German standard DIN 32645 suggests two approaches to assess the detection and quantitation limit.

The first approach is called "direct method" whereby the uncertainty is determined by repeated measurements of the blank. Additionally, the slope of the calibration function is required. Therefore the calibration for the whole working range can be used. The second approach is called "indirect method". In this case the uncertainty is determined by extrapolation of the calibration curve. It must be noted that the calculation is performed in the range of zero until the 10-fold of the detection limit and not with the whole working range of the calibration. In the present work the first approach was performed and the results were compared to the calculation based on the method of the ICH guideline. Therefore, 10 prepared blanks were measured one time in each case with the Raman probe and the dph amount was determined by the developed model.

The average predicted dph amount of 10 different blanks ($n = 10$) was 0.2 mg dph with a standard deviation of 0.07 mg. It must be noted, that the German standard postulated homogeneity of variance between the measurement of the blank and an analyte with a concentration which is approximately the detection limit. Therefore, one blank and two coated tablets containing 0.5 mg and 1.3 mg dph amount were measured 10-fold and the variance of the three samples was tested on significance by using the *F*-test.

Afterwards the "critical value (y_k)" was determined in order to calculate the DL. With a defined probability of error ($a = 0.01$) taking as basis the critical value represents the measured value, which indicates that the analyte concentration in the sample is greater than in the blank.

$$y_k = \bar{y}_L + s_L t \sqrt{\frac{1}{m} + \frac{1}{n}}. \quad (5)$$

y_k is calculated by equation (5) with m is equal to number of measurements per blank, n is equal for the number of blanks and $\bar{y}_L$ stands for the mean of the measured blank. By means of the y_k and the slope (b) of the calibration function the DL can be calculated with equation (6).

$$\mathrm{DL} = \frac{(y_k - \bar{y}_L)}{b} \quad (6)$$

$$QL = k\frac{S_L}{b} t_{f;\frac{a}{2}} \sqrt{\frac{1}{m} + \frac{1}{n} + \frac{((kDL) - \bar{x})^2}{S_{xx}}}. \quad (7)$$

The QL according to the German standard is calculated by using the factor k, which corresponds to the reciprocal of relative uncertainty. Usually, a factor of 3 is applied, which corresponds to an uncertainty of 33.3% (equation (7)). According to the ICH the DL and QL were calculated additionally by means of the standard deviation of the predicted values of the blank (S_L) and the slope (b) of the PLS calibration (apparent sensitivity). The factors 3.3 ($DL = 3.3S_L/b$) and 10 ($QL = 10S_L/b$), respectively, are used to discriminate between distribution of blank and analyte and are based on the signal-to-noise approach.

2.4. Application of the method for real time monitoring

2.4.1. Inline measurements

As described in the previous work [26] the Raman probe was fixed outside the coating pan to collect spectra during the process with a working distance of about 22 cm. To protect the probe against dust compressed air was blown through an iron pipe (95 mm length, 33 mm diameter), which was attached in front of the probe. The scanning time for every spectrum was 30 s followed by cosmic ray filtering that was offset to one single spectrum per minute. The coated dph amount was predicted on the basis of the inline data by means of the developed model.

2.4.2. Transfer of the developed model to real time monitoring

The goal of the work was to transfer the developed method to real time monitoring. Compared to the offline calibration, in which static measurements of one tablet in each case were performed, the inline measurements acquired moving tablets and every measurement covered several tablets. Consequently, the prediction of the coated dph amount by the inline data represents the averaged value over multiple tablets. In order to investigate the influence of the movement of the tablets during the process compared to the static samples by the model development Raman spectra of the offline measurements ($n = 6$) and the inline measurements ($n = 6$) at the according coating stages were compared with each other, and the corresponding loadings and scores were examined.

2.4.3. Robustness

Furthermore, it must be pointed out that the measurement conditions are not equal during the coating process. As described in the previous work the pan rotation speed during the process is not the same and varied in dependence on the process step. In order to estimate the effect of the pan rotation speed on the Raman signal speed trials were performed [26]. Furthermore, it is not possible to ensure the same working distance during the process. Therefore, measurements were performed between 12.5 cm and 28 cm in order to investigate if little displacements of the sample related to the probe disturb the chemometric model.

3. Results and discussion

3.1. Validation of the Raman analytical method

In agreement with ICH guideline Q2 the typical validation characteristics for assay procedures like accuracy, specificity, precision, range, and linearity were determined. As discussed in the previous section the detection and quantitation limits were calculated additionally for the transfer to real time monitoring.

3.1.1. Accuracy

Determination of accuracy was performed by calculating the statistical quantities standard error of calibration and prediction (SEC and SEP). For the calibration set a SEC of 0.29 mg was calculated. The validation set ($n = 24$) resulted in a SEP of 0.29 mg and the second validation set ($n = 36$) of the independent batch gave a SEP of 0.26 mg. In the case of the calibration set the bias should be nearly zero which applied for the investigated calibration set with a bias of −0.000001 which indicates a good calibration. For the validation set a small bias with −0.07 (1st validation set) and 0.37 (2nd validation set) was calculated too indicating the accuracy of the method. The results of the calculated SEC, SEP, and the bias of the two validation sets illustrate the accuracy of the Raman method for determining the coated amount of dph. It must be noted that the independent batch, which was used as second validation set, was coated one month later than the batch which was used for the calibration and first validation set. By comparing the resulted bias of the second and of the first validation set it becomes clear that the bias of the second validation set is noticeably greater than the bias of the first validation set. This can be explained by the intensity variability of the excitation laser and confirms the need of intensity calibration at regular intervals. Furthermore, despite the stability of the instrument, changes in the instrumental throughput [39], especially over long time periods must be considered. Consequently the necessity of continuous verification and revalidation of the developed model is pointed out by using the model over a longer period in order to guarantee the suitability of the method. In order to eliminate variations caused by absolute intensity fluctuations an appropriate approach is to use an internal or external standard. Exemplary for the internal standard an excipient peak can be used if it is existing in an adequate amount compared to the analyte. In the case of the coating procedure it is not applicable because the signal of the core is attenuated by the coated film during the process. Furthermore, the other components of the coating solution (HPMC, PEG) give no usable Raman signal, as described below in the specificity section. Kim et al. [31] demonstrated an appropriate approach to correct the problematic variation of Raman intensity from the change of laser power by using isobutyric anhydride as external standard for the offline measurements of naproxen tablets. But in the case of inline measurements, where the instrument is installed in a process location, the positioning of an external standard will be difficult.

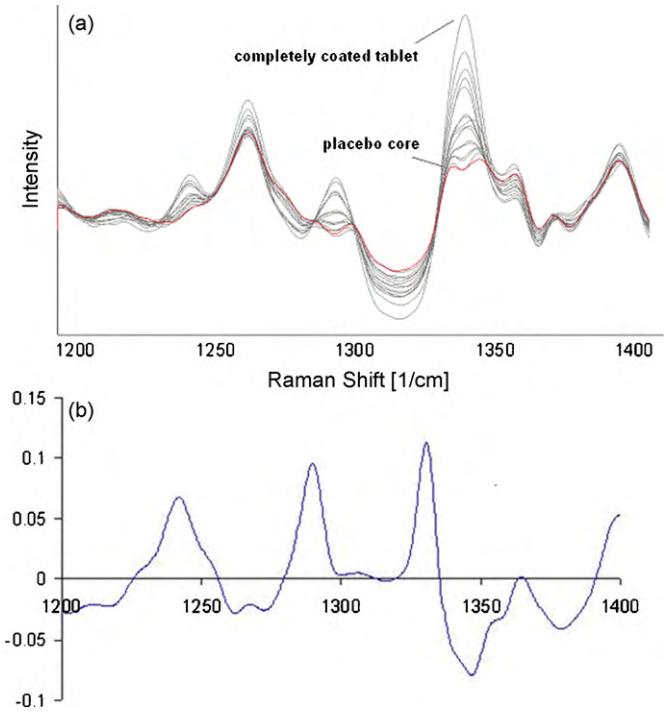

Fig. 1. Comparison of the (a) Raman spectra of tablets in dependence on the coating level with the (b) loading plot of the first principal component.

3.1.2. Specificity

The sensitivity of the Raman signal to changes in the coating level on tablets can be seen in Fig. 1(a), which demonstrates baseline-corrected Raman spectra in the region 1200–1400 cm^{-1} of the calibration samples, which were collected at different stages of coating. The intensity of the peaks 1290 cm^{-1} and 1330 cm^{-1} increases as function of coating time and can be assigned to the amount of dph. The methylated N3 atom (CN) stretch occurs at 1290 cm^{-1} and the imidazole ring stretch at 1330 cm^{-1}. The constructed PLS model needed three principal components whereby the first principal component explained 94% of the variance which grew up to 99% of the variance by using three principal components. The loading plot (Fig. 1 (b)) of the first principal component shows strong analogy with the Raman spectra of dph and confirms that the most part of the variance of the Raman spectra can be assigned to dph. Fig. 2 presents the score plot ($u_1 \times t_1$) of the first principal component. In general these plots display the observations in the projected X (T) and Y (U) space, and show how well the Y space (dph amount) correlates to the X space (Raman spectra) [40]. In evidence there exists a correlation between the variance of the *x*- and *y*-variables which verifies the sensitivity of the Raman signal to changes in the coating level. In addition the calibration set was analyzed by Multivariate Curve Resolution (MCR). MCR is a profitable tool for the operator of analytical spectroscopy because all spectra of the collective mixture are fragmented into chemically interpretable basis spectra that reflect the fundamental components of the whole data set. Increment or decrement of a defined component within the data matrix is pointed by the score values. Finally, it is possible to calculate the individual spectra of the single components from the mixture. The results of the MCR of the baseline-corrected spectra indicated that the resulting data could be described sufficiently with a two-factorial model. Fig. 3 shows the resultant basis spectra of the two components. The reproduced spectra were similar to the spectra of the dph and the placebo core (figure not shown). By examining the score values of the components (Fig. 4) it was obvious that the contribution of the dph to the signal increased with the coating time, which verifies additionally the specificity of the method. But it must be noted, that the contribution of the core to the analytical signal decreased with the coating time as consequence of the attenuation of the core signal by the coated film. By comparing the Raman spectra of the coating solution without dph (figure not shown) with the progress of the Raman spectra in dependence on the coating level, it becomes clear, that the other coating elements (HPMC, PEG 1500) have no great contribution to the changes of the Raman spectra in dependence on the coating amount. Additionally, the above mentioned loading plot of the first principal component and the MCR verified the small influence of the other components of the coating solution by resembling the spectra of dph. Furthermore, the Raman spectra of one prepared blank, a placebo tablet, a sample with a coated dph amount near the DL (0.3 mg) and a finished coated tablet (11 mg) were examined (Fig. 5). By means of a characteristic peak of the

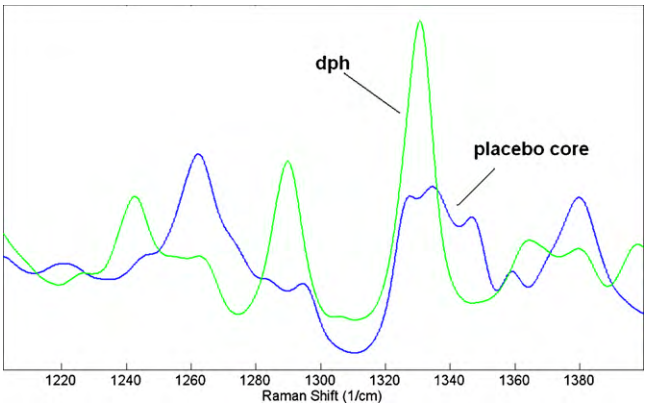

Fig. 3. Spectra of dph and placebo core reproduced by MCR.

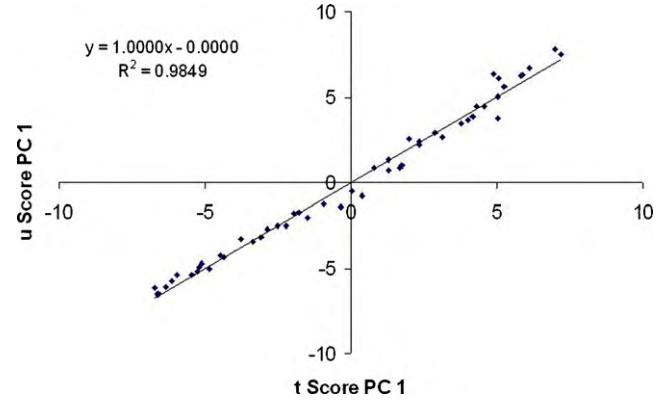

Fig. 2. Score plot ($u_1 \times t_1$) of the first principal component.

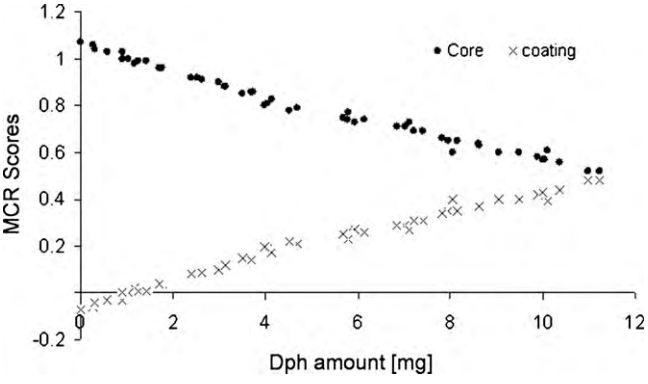

Fig. 4. MCR scores in dependence on the dph amount [mg].

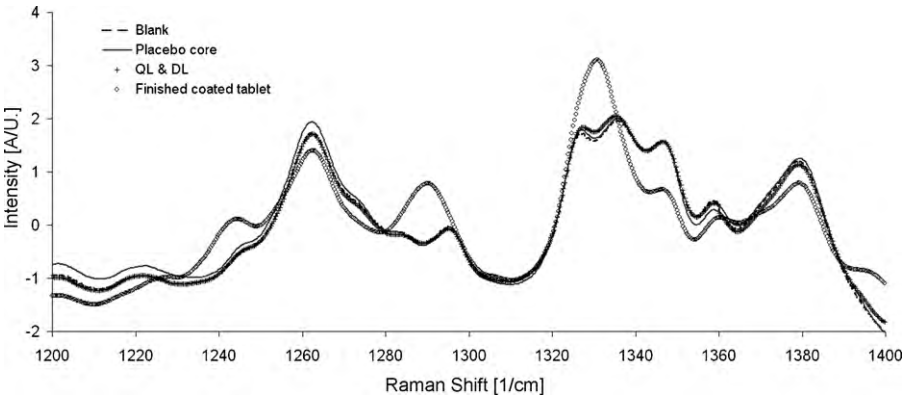

Fig. 5. Raman spectra of the blank, placebo cores, sample with a coated dph amount near DL and a finished coated tablet.

placebo tablet (1260 cm^{-1}) and a peak which represents the dph (1330 cm^{-1}) it was possible to distinguish between the tablets. In the case of peak 1260 cm^{-1} it was possible to differentiate clearly between the finished coated tablet and the placebo tablet. The sample with the coated dph amount near the DL and the blank differ from the placebo tablet and the finished coated tablet. But it was not possible to distinguish between the blank and the sample with low dph amount. In the case of peak 1330 cm^{-1} it was possible to distinguish between the blank and the sample with a coated dph amount near the DL.

This indicates that the blanks can be distinguished from the coated samples by the Raman method. Afterwards, the coated dph amounts of 10 blanks were determined by the Raman method requiring an amount of 0 mg. The average predicted dph amount of the blanks was 0.2 mg dph. The largest part of the variance of the Raman spectra during the process can be assigned to dph. But as mentioned above the amount of the other components of the coating solution increases during the coating process too and influences the prediction of the model to a small extent. The placebo cores were included in the calibration set and represented 0 mg dph amount. The Raman spectra of the blanks differ from the spectra of the placebo cores and resulted in the small uncertainty. The results of the multivariate data analysis and the changes of the Raman spectra in dependence on the coating level demonstrate the specificity of the Raman method for its intended purpose.

3.1.3. Repeatability

The results (Table 1) show by means of the standard deviation that the repeatability of the method is constant over the investigated working range 1.0–9.9 mg dph. The *F*-test with a probability of error $\alpha = 0.01$ verifies that the variance of the individual samples is not significantly different. But it must be noted that with a higher dph amount the calculated coefficient of variation (CV) and the resulting accuracy of the investigated samples were improved compared to the samples with low dph content. As mentioned in Section 2.3.7 the QL assigns the lowest dph amount, which can be determined quantitatively with suitable precision and accuracy. But by increasing the dph amount the accuracy and prediction are advanced as consequence of the detected stronger Raman signal. The results show, that the calculated CV in dependence on the dph amount fulfills the requirements of the intended approach. Starting from the 2.1 mg dph amount the calculated CV is below 5% and decreases to 1% towards the end of the coating process (9.9 mg dph), when the coating information is strongly needed for process monitoring. Furthermore, the mean (±95% confidence interval (CI)) of the Raman predicted dph amount shows no evidence for a difference in values compared with the reference method UV spectroscopy.

3.1.4. Intermediate precision

Table 2 illustrates the results of the measurements with replacing the tablets after each measurement and in analogy with the static measurements (Section 3.1.3) the CV decreased by increasing dph amount. As expected the standard deviation is higher compared to the static measurements, except in the case of sample 3. Thus, the sample position during the measurement must be considered in order to provide the precision of the method. But the results of the static and replaced measurement are comparable, which could be related to the Phat® probe. Additionally the *F*-test with a probability of error $\alpha = 0.01$ verifies that the variance

Table 1
Results of the investigated samples for the repeatability experiments ($n = 6$; static measurements).

	Predicted dph amount (mg)	Standard deviation (mg)	Coefficient of variation (%)	Confidence interval (mg)	Observed value by UV (mg)
1	0.95	0.07	7.48	0.06	0.96
2	2.11	0.10	4.69	0.08	2.07
3	3.66	0.18	5.00	0.15	3.60
4	7.44	0.08	1.11	0.07	7.40
5	9.86	0.10	0.96	0.08	9.90

Table 2
Results of the investigated samples for the experiments after removing and replacing the tablet after each measurement ($n = 6$; replaced measurement).

	Predicted dph amount (mg)	Standard deviation (mg)	Coefficient of variation (%)	Confidence interval (mg)	Observed value by UV (mg)
1	1.05	0.17	16.18	0.14	0.96
2	2.12	0.16	7.66	0.13	2.07
3	3.67	0.11	3.05	0.09	3.60
4	7.35	0.14	1.96	0.12	7.40
5	10.05	0.18	1.82	0.15	9.90

Table 3
Results of the investigated samples for the intermediate precision experiments by analyst 1 and 2 ($n = 36$).

	Predicted dph amount (mg)	Standard deviation (mg)	Coefficient of variation (%)	Confidence interval (mg)	Observed value by UV (mg)
Analyst 1					
1	0.90	0.21	23.13	0.07	0.96
2	2.06	0.13	6.27	0.04	2.07
3	3.55	0.17	4.69	0.05	3.60
4	7.36	0.18	2.46	0.06	7.40
5	9.85	0.16	1.65	0.05	9.90
Analyst 2					
1	0.91	0.25	27.63	0.08	0.96
2	2.21	0.14	6.27	0.05	2.07
3	3.62	0.19	5.15	0.06	3.60
4	7.44	0.26	3.52	0.09	7.40
5	9.90	0.14	1.41	0.05	9.90

of the according samples from the static and replaced measurement are not significantly different. Kim et al. [31] has already discussed that the Phat® probe use a wide area illumination (WAI) scheme for Raman collection to cover a large surface area (coverage area: 28.3 mm^2) of the sample. This improves dramatically the reliability in sample representation and the reproducibility of sampling due to less sensitivity of sample placement with regard to the focal plane. Additionally the mean (±95% confidence interval (CI)) of the results achieved by the replaced measurement shows no evidence for a difference in values compared to the static measurement. Table 3 shows the results of the intermediate precision observed on six different days and by two different analysts. The mean (±95% confidence interval (CI)) of the achieved results shows no evidence for a difference in values by comparing them with the reference method UV spectroscopy, except in the case of sample 2 of the second analyst. Noticeably the resulted standard deviation is higher compared to the repeatability measurements and the replaced measurements. Thereby it must be noted that the standard deviation of the according samples resulting from the measurement series of one day are comparable with the standard deviation of the repeatability measurements. But the mean (±95% confidence interval (CI)) of the achieved results of the according samples differ between the six days. The dph amount obtained from the validated reference method UV spectroscopy was the known value of the according sample and was used as "true value (100%)". By comparing the observed value in dependence on the days with the "true value" (Table 4) it becomes clear, that except in the case of sample 1 the aberration from the true value fluctuates up to 10%. The differences in the case of sample 1 are larger and were in dependence on the day not consistent with the reference value. Sample 1 contained 0.96 mg dph which is currently above the QL while the dph amount increased in the following samples up to 9.9 mg dph (Tables 1–3). In analogy with the repeatability experiments in Section 3.1.3 the CV and the aberration decreased by increasing dph amount, which can be related to the random noise. The random noise is composed of the random error and the shot (statistical) noise [39]. By collecting two spectra for the same sample, assuming that there are no changes in the sample or the analyzer, the difference between the two spectra will be the random error associated with the measurement. As described in Sections 2.2.6 and 2.3 every measurement is followed by cosmic ray filtering and a dark subtraction. The cosmic ray filtering eliminates the random error arising from cosmic rays which impact the detector during the exposure. Additionally the CCD detector is sensitive to dark current, which arises from thermal energy within the silicon lattice comprising the CCD. Thereby electrons are created over time that are independent of the light falling on the detector. These electrons are captured by the CCD's potential wells and counted as signal. In order to avoid the dark current the detector was cooled down to −40 °C and a dark subtraction was performed after every measurement. The shot noise arises from the random probability associated with actually observing a photon at a given wavelength, whereby the random variation for a given measurement is the square root of the number of counts measured [39]. Thus the shot noise associated with a measurement of n counts will be nearly $n^{1/2}$ which is often described in terms of a signal-to-noise ratio or relative error. Consequently the shot noise increases if the total number of counts increases but the relative error decreases because of the square root relationship. Transferred to the worked example the relative error decreases in the case of samples containing higher dph amount which leads to an increase of the acquired total number of counts. Table 3 shows clearly that starting from sample 2 (2.1 mg dph) the CV decreased significantly to 6% compared to sample 1 (0.96 mg dph) with a CV over 20%. Additionally as described in Section 3.1.1 the absolute intensity fluctuation is a critical factor for the method development based on Raman spectroscopy. In agreement with the USP 33 [34] the photometric precision was investigated for the interested spectral area 1200–1400 cm^{-1}.by using cyclohexane as reference standard. The variation of the calculated areas of the peaks 1266.4 cm^{-1} and 1444.4 cm^{-1} between the days were below 10% which fulfill the requirements. However the intensity fluctuations between the different days influence the precision of the method and should be considered. In addition the intermediate precision is affected by the placement of the sample, which varied between the different days and the analysts.

Table 4
Observed values (%) compared to the reference method in dependence on the day by analyst 1 and 2 ($n = 6$).

	1 day	2 day	3 day	4 day	5 day	6 day
Analyst 1						
1	77.27	63.37	102.33	98.94	126.36	95.47
2	91.63	91.87	104.78	101.97	102.63	105.13
3	91.84	94.43	98.70	101.79	104.20	100.13
4	94.80	101.17	99.30	100.42	101.52	99.24
5	97.09	99.16	102.13	99.60	100.20	99.06
Analyst 2						
1	70.21	66.62	105.26	85.94	107.63	136.24
2	94.93	110.59	110.67	110.80	102.26	110.88
3	92.66	105.77	105.56	97.63	98.67	103.36
4	94.45	103.27	103.51	98.40	100.86	102.69
5	100.17	100.17	100.51	102.08	97.92	99.05

3.1.5. Linearity

Fig. 6 presents the plot of the Raman (predicted) versus UV (observed) results of the calibration set, which shows no visible evidence of non-linearity. The same results are estimated for the two validation sets (figures not shown). Table 5 illustrates the various statistics required by the ICH guidelines for evaluating linearity through the use of a linear regression relating the Raman to the UV values. In the case of the calibration set the 95% confidence interval

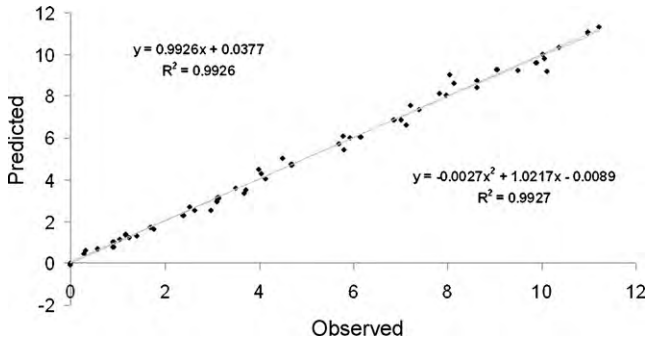

Fig. 6. Plot of Raman values (predicted) versus UV values (observed) for testing linearity.

for the intercept (−0.111 to 0.187) included zero and the 95% confidence interval for the slope (0.968–1.016) included one, indicating there is no systematic error in the calibration function. The linear and quadratic regression functions required for the *F*-test after Mandel are displayed in Fig. 6 for the calibration set. The residual standard deviations S_{y1}/S_{y2} resulted in 0.295/0.296 for the calibration, 0.237/0.239 for the first validation and 0.257/0.259 for the second validation set and there is obviously no significant difference in each case. The calculated test value PW for the calibration (0.41), the first validation (0.72), and second validation set (0.41) were smaller than Ff1, f2; 99% (7.2 for calibration, 8.02 for the 1st and 7.47 for the 2nd validation set). This indicates that there is no significant difference in the residual variance by taking the quadratic regression function and that the Raman analytical signal as function of the dph amount can be characterized as linear for the evaluated working range. The results of the visual evaluation and the statistical calculations verify the linear relationship across the working range of the developed analytical method.

3.1.6. Detection and quantitation limit

The homogeneity of variance was investigated for one blank and two coated tablets (samples 1 and 2) collected at the early stage of the process. In the case of the examined blank the predicted dph amount (mean ±SD) was 0.27 mg ± 0.13 mg and 0.52 mg ± 0.15 mg (sample 1) and 1.28 mg ± 0.15 mg (sample 2) for the coated tablets. The results of the *F*-test verified that the variance of the individual samples was not significantly different. The test values of SD^2 sample1/SD^2 blank (1.30) and SD^2 sample2/SD^2 blank (1.27) were smaller than $F_{9.9;99\%}$ (5.35). The Raman calibration is presented as a plot of Raman predicted values versus observed values of the reference method (UV spectroscopy). Thereby, the Raman predicted values represent the information of the *y*-variable (dph amount) in the *x*-variables (Raman spectra). The calculated critical value y_k ($\alpha = 0.01$) of 0.43 represents the Raman predicted value which indicates that the dph amount in the sample is higher than in the blank. The calculation according to the German standard with a probability of error 0.01 resulted in 0.22 mg for the DL and 0.79 mg for the QL. By using the transferred ICH methodology for the calculation the DL resulted in 0.24 mg and the QL in 0.74 mg. The resulting DL and QL of the two approaches gave similar results and give a remark for the limit of coated dph amount which is needed for the quantitative inline monitoring. But it must be considered that the calculated DL and QL based on static measurements compared to the inline measurements which acquired moving tablets, which lead to higher values for DL and QL as calculated. The previous work [26] demonstrated that Raman spectroscopy is an appropriate PAT tool to monitor the process of active coating. The predicted dph amount by the inline measurements was consistent with the offline measured samples, which were collected at the according sampling points. The samples were collected at 30 min intervals whereby after 30 min an average of 1.6 mg dph was coated on the placebo tablets. As consequence it can be concluded that after 30 min coating time or 1.6 mg coated dph amount the developed Raman method predicted reliable results by the inline measurements.

Table 5
Statistics for evaluating linearity; ICH requirements.

Statistics	Calibration set	1st Validation set	2nd Validation set
Correlation coefficient	0.9926	0.9954	0.9904
y-intercept	0.0377	0.0898	0.2082
Slope of PLS calibration	0.9926	0.9699	1.0306
Residual sum of squares	4.38	1.60	7.27

3.1.7. Range

An appropriate accuracy and linearity was shown for the whole calibration set. However the lower end of the concentration range is limited by the limit of quantitation (0.8 mg dph) and the precision of the method. A suitability level of precision was shown for a dph amount about 2 mg, where the CV was below 10% in the case of the repeatability and the intermediate precision. Consequently the requirements were fulfilled for a working range of 2–11.2 mg dph for the developed analytical procedure, which is suitable to monitor the coating process. The early stage of the coating process can be detected starting from the dph amount between the calculated DL and QL, but for the inline quantitative monitoring a sufficient coated dph amount (2 mg) is necessary. This would be above the calculated QL which is estimated to start at 30 min coating time as consequence of the results of the previous work [26] and of the precision experiments. But altogether the most part of the process could be monitored by the inline measurements.

3.2. Application of the method to real time monitoring

3.2.1. Transfer of the developed model for real time monitoring

As described above a critical factor for the transfer of the developed method to real time monitoring is that compared to the offline calibration, in which static measurements of individual tablets were performed, the inline measurements acquired moving tablets. As described above changes in observed intensity of the Raman spectra are not only due to changes in concentration of the analyte. In the accuracy and precision section the problem of laser intensity fluctuation was mentioned. But in addition changes of the interrogated volume [39] are a critical factor, which is affected exemplary by changes in a sample's refractive index, opacity, position and density. Such changes are mostly observed with inline process analysis applications. By examining the Raman spectra (Fig. 7) resulting from the offline and inline measurements a difference is noticeable for the peaks 1290 cm^{-1} and 1330 cm^{-1}, which are characteristic for dph (Section 3.1.2). Fig. 8 illustrates the Raman spectra reproduced with the loadings and the scores of the second principal component of a finished coated tablet in the case of the offline and inline measurements. Thereby the reproduced Raman spectrum of the inline measurement is mirror-inverted compared to the offline measurement. The second principal component discriminates between inline and offline measurement and indicates that the fact measuring moving tablets compared to the static offline measurement must be considered. The loadings of the second principal component are more difficult to interpret. But the peak at 1260 cm^{-1} (placebo tablet) and the peak at 1330 cm^{-1} are visible. The discrimination can be related to changes in the interrogated volume. As described in the previous work changes in the packing density in dependence on the pan rotation speed and the sample position can affect the observed Raman signal. Thus for the inline measurement a scanning time of 30 s was neces-

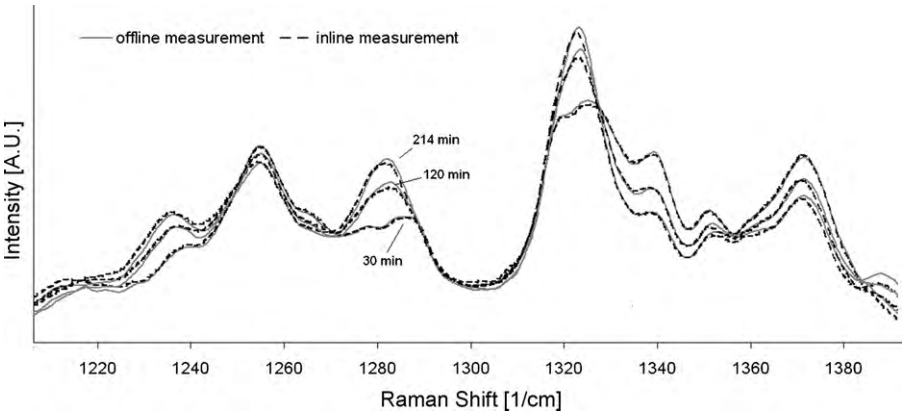

Fig. 7. Raman spectra of the inline and offline measurements in dependence on the coating time ($n = 6$).

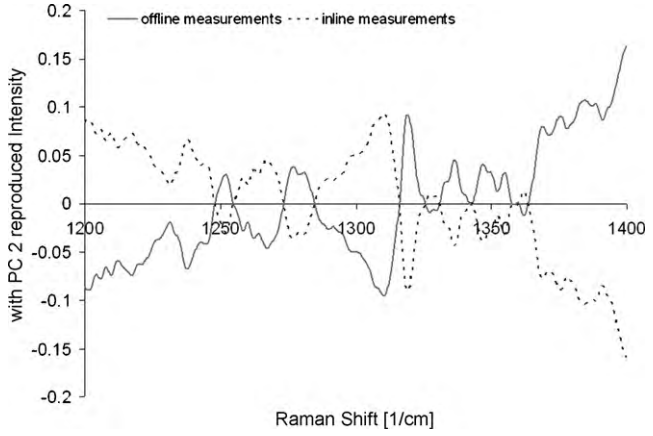

Fig. 8. Raman spectra reproduced with the second principal component of the offline and inline measurements of a finished coated tablet.

sary in order to get an adequate Raman signal compared to the offline measurement, where a scanning time of 10 s was sufficient. Furthermore, it must be noted that other conditions like the refractive index were changed during the process. As result of the coated film and changes of the moisture of the tablets during the process, the refractive index can be affected and influence the Raman signal. The previous work [26] demonstrated the feasibility of Raman spectroscopy as PAT tool for monitoring the progress of active coating with dph as model drug. The constructed model was applicable to determine the amount of coated active ingredient during the coating process by inline measurements. Furthermore, it was possible to detect the amount of coated active ingredient on cores with the API itself, which is beneficial and applicable for dosage forms with a delayed release core coated with an immediate dose.

3.2.2. Robustness

The results of the previous work [26] indicate that the developed method is not vigorously disturbed by variation of the process parameters or measurement conditions within a restricted range. In the case of the speed trials the prediction of the dph amount was not affected decisively by the pan speed. Furthermore in a range of 15–28 cm the predicted dph amount of the different working distances is not significantly different.

4. Conclusion

A Raman spectroscopic procedure was validated in agreement with the ICH guideline Q2. The typical validation characteristics for assay procedures were examined with considering the transfer to real time monitoring and fulfilled the requirement for the intended purpose of the analytical procedure. Critical factors of the method based on the technical feasibility and accuracy of the Raman equipment like fluctuation of the total laser intensity and changes in the instrumental throughput, which influence the precision of the method. Therefore a continuous verification and revalidation of the developed model is necessary by using the model over a longer period in order to guarantee the suitability of the method. In order to improve the precision of the method more investigations are necessary like the implementation of an external standard. As described in the previous work the Raman spectroscopy is an appropriate PAT tool in active coating. But for the application of the developed method to real time monitoring the factors like moving samples or changes of the interrogated volume during the process must be noted.

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