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Research paper

Prospects for multivariate classification of a pharmaceutical intermediate with near-infrared spectroscopy as a process analytical technology (PAT) production control supplement

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ABSTRACT

NIR spectroscopy was applied to develop a fast and reliable quality control system for a pharmaceutical substance to support information obtained through PAT surveillance of its manufacturing process. After calculating different quantitative calibrations of the substance's key quality parameters, a general classification model has been derived to capture the over-all product grade. The final spectral quality conformity model consisting of 96 representative batches – covering high process variability – was sensibilized toward five important quality parameters by their incorporation as PLS responses. The model characteristics were extensively investigated and interpreted to derive a reasonable limit for the reduced chemometric summary quality measure (Hotteling's T^2). Through this parameter new batches can be assessed easily by their NIR spectra, using versatile test batches for confirmation. Different sets of good quality batches, bad production batches beyond the respective chemical quality limit and synthetic batches exactly at the limit could be accurately assigned through their multivariate evaluation to a large extend. However, high model sensitivity to non-relevant product properties can lead to limited applicability of the model. This may be caused by restricted bandwidth of quality parameters in production environment for calibration, repack effects and high process instability.

1. Introduction

In pharmaceutical industry, various conditions for quality assurance and control of active ingredients, intermediates and products are imposed in a rigid regulatory system. For every substance, a certain set of quality parameters is defined, which has to be monitored and documented extensively according to quality acceptance standards, before further processing or product release is approved. In a conventional way, testing of product quality at batch processes is performed by sample drawing and subsequent laboratory analysis as a statistical control method of critical parameters. Restricted sample representativeness and inherent, undetectable variability in raw and in-process materials might lead to considerable end product quality fluctuations.

The PAT initiative, which is actively supported by the United States Food and Drug Administration (FDA) in terms of research and development, should enable an efficient quality improvement by adding a more dynamic dimension [1]. By definition, PAT is a mechanism to design, analyze and control pharmaceutical manufacturing processes through the measurement of critical process parameters which affect critical quality attributes to ensure the stated end product quality [2]. This systematic strategy provides a basis for the recognition of multifactorial interrelations of materials and process conditions [3] and often involves multivariate data analysis for interpretation. The major advantages of this new approach lie within the high degree of process understanding and the facilitated interpretation of the impact of production parameters on the final product quality. Therefore, production efficiency can be increased due to reduced production and release times and the avoidance of rework processes.

Various optical techniques including Infrared (IR), Near-Infrared (NIR), Raman and UV–VIS spectroscopy as well as NIR chemical imaging (CI) proved to be capable PAT control tools in pharmaceutical industry [4–8]. Radiation in the near infrared energetic range excites overtone and combination vibrations in the sample material, revealing a high content of chemical as well as physical information at the same time dispersed in the whole spectral area [9–11]. Together with multivariate data evaluation methods [12,13] required for information extraction, NIRS has become a popular process control tool with a broad range of applications for precise material characterization [14–17]. Even if the NIR

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method development and validation requires a comparatively high timely expense and background knowledge, the profitability of such investments is guaranteed by various advantages [18–22]. The possibility of direct, fast measurements without sample preparation due to weak absorption intensities, the robust, flexible equipment – capable in explosion proof zones and for long measuring distances achieved with fiber optics, no necessitated chemicals, sophisticated software and a comparable low financial expense turned this technique into a preferable monitoring tool. Its noninvasive application range covers off – in and online configurations [23–30]. Due to a high penetration depth compared to other spectroscopic methods [31], larger sample areas can be measured as surface contamination and inhomogeneity effects are compensated to a certain extend. In pharmaceutical industry, NIRS is meanwhile mainly established for identification and assay determination of liquid and solid samples in all different phases of production [32–38]. Adjacent to qualitative and quantitative methods, semiquantitative calibrations can be applied for quality-related classification of materials [39–41].

7-Aminocephalosporanic acid (7-ACA) [957-68-6] is an antibiotic intermediate, whose production process has recently been put under the control of a PAT control system. The objective of this study was the application of NIR spectroscopic methods in combination with multivariate data analysis to reliably determine the quality of the end product at minimum measuring effort and to at least partially replace laborious, conventional analysis methods in a long term. The quality of the substance is mainly varying regarding the content of active ingredient, the proportion of byproducts, water and residual solvent content as well as its color value, describing the slight yellowy staining of the substance. Different calibration methods and modeling techniques were tested and served for the development of a single summary quality conformity test as a supplement for the PAT control.

2. Experimental

2.1. Instrumentation

The spectra for the quantification models were recorded with a BÜCHI NIRFlex N-400 spectrometer (BÜCHI Labortechnik AG, Flawil, Switzerland), a polarization interferometer device equipped with a 2-m laboratory probe for measurements in diffuse reflection. The available wavenumber range is 4000–10,000 cm^{-1} at a fixed 12 cm^{-1} resolution (500 data points). Spectra were recorded using a probe fixture and a sample hoist with a 3 scans/3 measurement option, and 3 spectra were averaged for each sample.

A BRUKER Vector 22 N (BRUKER Optik GmbH, Ettlingen, Germany) was employed to record the spectra for the continuative conformity model. With this moving mirror interferometer system exclusively measurements in diffuse reflection using an integrating sphere with sample desk can be conducted. The accessible wavenumber region is 4000–9090 cm^{-1} with variable resolution. Samples were prepared in a glass cup with special optical bottom (injection flasks FIOLAX, clear HGB 1/ISO 719 52, 0x22, 00/1.20 mm. 411874) to minimize scattering and reflection effects with a standardized compaction procedure. Every spectrum was recorded at the highest feasible resolution of approximately 1 cm^{-1} with 128 scans (5280 data points).

2.2. Reference analysis

2.2.1. HPLC

ACA content as well as byproducts – Desacetyl-7-ACA, Desacetyl-7-ACA-Lacton, Desacetoxy-7-ACA, Cefalosporin C-Natrium – and the residual solvent Dimethylanilin were analyzed by reversed-phase high-performance liquid chromatography (HPLC). The separation was conducted with an Agilent 1090 series (Agilent Technologies, Palo Alto, CA, USA) system combined with a silica column C 18 (5 μm, 125 × 4 mm). A concentration of 36–44 mg of sample powder was weighted and dissolved in an ultrasonic bath. An autosampler cooled down to 4 °C was used for sample injection. For the 7-ACA content determination, an isocratic method was applied, while byproducts and DMA were quantified using gradient elution. UV detection was accomplished at 254 nm.

2.2.2. Karl-Fischer

Water content was analyzed according to the principle of dry coulometric Karl-Fischer back titration using a Mettler Toledo DL 39 Coulometer device with a Stromboli sample changer oven (Mettler-Toledo GmbH, Greifensee, Switzerland). Temperature was set to 130 °C. The sample was weighted to 0.5–1.0 g and swept with a molecular-sieve dried air stream for approximately 8 min.

2.2.3. Color value

The color value – defined as the extinction at the wavelength of 425 nm – corresponding to absorption maximum at this wavelength – is measured with a UV–VIS photospectrometer (Beckman Coulter TM DU520, California, USA). A concentration of 0.5 g of the powder are tentatively dissolved, filtrated and measured inside a 1-cm quartz cuvette.

2.3. Software

HPLC programming and data evaluation were performed with Chromeleon V67 (Dionex Corporation, Sunnyvale, USA). To control spectra recording BÜCHI NIRCal 4.21 and BRUKER OPUS 6.5 were used for the quantitative calibrations and the conformity model, respectively. SIMCA P + 11.5 (Umetrics, Umeå, Sweden) served as extensive chemometric analysis tool for multivariate modeling, visualization and interpretation.

3. Results and discussion

The production of β-Lactam antibiotics proceeds through fermentation in sterile, cooled bioreactors [42]. The fermentation broth is harvested, purified and further processed by chemical cleavage. The respective intermediate 7-ACA serves as a basic substance for almost all semisynthetic Cephalosporin antibiotics. Every 7-ACA batch is inspected by the quality control department regarding all norms as documented in the products control regulations by default. Some of these conditions are cited in Table 1.

3.1. Product release regulations

This extensive laborious test procedure should be reduced and partly replaced by the implementation of a new quality control

Table 1
Some quality parameters of 7-ACA and their specified acceptance criteria.

Quality parameters	Allowed range/limit
7-ACA assay (based on the anhydrous substance)	95–102%
Water content	<0.4%
Color value[a]	<50
Byproducts	
Cefalosporin C = CefC	⩽1.0%
Desacetyl-7-ACA-Lacton = HACA-Lacton	⩽0.5%
Desacetyl-7-ACA = HACA	⩽0.5%
Desacetoxy-7-ACA = ADCA	⩽0.5%
Residual solvent	
N,N-dimethylaniline = DMA	⩽1500 ppm

[a] CV = 1000 × extinction at 425 nm.

system. Regarding the PAT surveillance of the cleavage to ACA by a series of complex chemical reactions, the product quality is stated to be already compliant at an ordinary, systematic process progression. To supplement and confirm the PAT information, an additional fast and reliable testing procedure of the final product composition should be developed by NIRS. The high degree of interrelation between chemical quality data and spectral information of 7-ACA has already been demonstrated and described [43]. In a continuative study, various quantification and classification models with optimized spectral recording conditions were investigated and tested thoroughly to aim a multivariate evaluation model, which is preferably simple and robust toward different sources of variation. The final release of a product batch should be accomplished by the combined judgment of the process conformity and the NIR quality conformity. Process conformity is defined by PAT compliance of every defined unit operation of the whole process. Therefore, a set of critical single technical and chemical process parameters as well as the so called batch level plot – a multivariate measure derived from the time resolved process progress – have to be checked in respect of well-established limits. As an additional control mechanism for deviation detection, the precursor product is tested for its assay, water and residual solvent content by quantitative NIRS directly in the production area before its admission to the production. This course of action is finalized with the quality conformity test based on the intermediates NIR spectra. A comparison between the classical process control system and the PAT approach as displayed in Scheme 1 points out the efficiency of the new method.

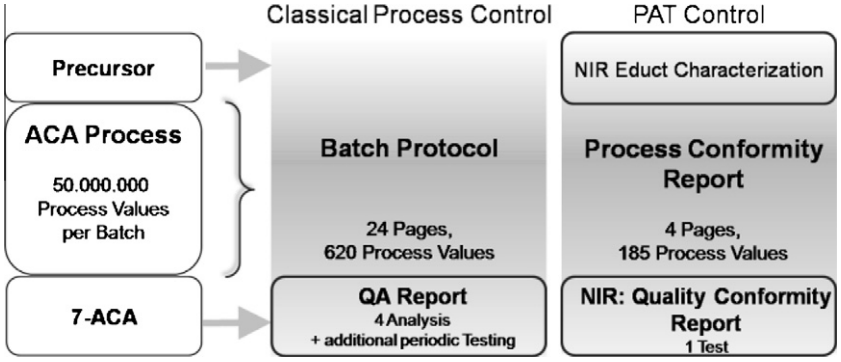
3.2. Quantitative approach

To estimate the NIR activity of several quality parameters and therefore the intermediates principal potential for spectral calibration, quantitative models have been calculated in a first step for a large set of available production samples. All batches starting from 2003, with the whole range of quality parameters investigated by default were taken into consideration. An elected subset offering the highest possible variance in all quality characteristics was complemented with recent production samples. To expand the respective calibration range, a set of samples exposed to physical stress such as temperature and humidity for different time periods at various conditions were integrated in the calibration set. Four major quality parameters – product assay, color value, water and CefC content – showed reasonable good capability for a quantitative NIRS calibration using PLS methods. The calibration details are summarized in Table 2. Calibrations of additional parameters mainly suffered from lack of sufficient variance of reference values in the present production plant environment. The parameter of remanent solvent DMA was already proved to be highly detectable with NIR spectroscopy in the former study. Therefore, the general conclusion could be drawn that the product has a high potential to be characterized by this technique.

3.3. Quality conformity model

Without the need of gathering specific quantitative information about the sample, an over-all quality conformity test was developed based on spectral information in the NIR region. The spectral model had to be constructed systematically, to reliably assess deviations in quality with multivariate methods. In current production, the values for several parameters (HACA, ADCA, HACA-Lacton and water) are very close to the limit, further challenging the required significance of the model.

To ensure sufficient accuracy of the model and to comply for the demands on highest sensitivity, the most suitable spectral recording condition proved to be the application of an integrating sphere. Collecting the radiation from all dihedral angles, this setup offers much higher reproducibility, minor spectral offset variability and less noise possibly caused by fiber optics. With the large beam spot the spectral information can be gathered for a bigger sample surface. Therefore, fewer intricacies arise due to inhomogeneities and differences in powder granularity. At very high resolution, subtle spectral features – which could retain relevant information – are visualized while noise can be compensated with an elevated number of scans and multivariate evaluation methods. Prior to spectral recording, the sample inside the vial was slightly fluffed



Scheme 1. Schematic illustration of the PAT system in comparison with classical process control.

Table 2
Summary of calibration data for four main quality parameters.

Parameter	PCs	Pretreatments	Cal./val.	Wavenumber range (cm^{-1})	R^2	SEE (%)	SEP (%)
Assay	4	1st Derivative	108/51	4596–9996	0.91	0.57	0.57
Color value	5	1st Derivative	125/69	4596–9996	0.98	5.70	6.23
Water	4	Norm. by closure	147/60	4596–9996	0.98	0.05	0.05
CefC	4	1st Derivative	98/37	4440–9000	0.92	0.07	0.09

PCs: principal components, Cal./val.: number of spectra in the calibration and validation set, R^2: coefficient of determination of the calibration straight line, SEE: standard error of estimation, SEP: standard error of prediction (cross validation).

up and subsequently compacted by tapping it on a soft surface 2–3 times. This method should assure a roughly unified packing density. Variations in powder compaction were integrated into the model by a high number of calibration samples.

The basic calibration set consisted of good quality batches offering each disposable quality parameter better than average (assay >97%, DMA ⩽450 ppm, CefC ⩽0.8%, water ⩽0.2% , color value <20, ADCA ⩽0.5%, sum unknown byproducts <0.7%). It was supplemented with few production batches revealing quality characteristics up to the stated limits to assure a representative model. The data set should combine as many approvable sources of variations as possible, covering a broad production time range and recent batches to assure model topicality. The final set of calibration spectra is displayed in Fig. 1.

The spectra are mainly dominated by CH, CH_2 and CH_3 vibrations, which can be seen in the range from 4080 to 4400 cm^{-1} as combination vibrations, 5650 to 5960 cm^{-1} as CH 1st overtone vibrations, around 7140 cm^{-1} as weak signal of the 1st overtone of CH combination vibrations and as the low intensity 2nd overtone of CH vibrations in the spectral range at 8330 cm^{-1}. Small water peaks can be identified around 5200 cm^{-1} and 7000 cm^{-1}. Further weak spectral characteristics include signals at 4830 cm^{-1} and 4659 cm^{-1} corresponding to the ROH and the amino group vibration, respectively. The different spectra show a significant shift originating in the recording conditions mainly depending on the powder compaction state. After centering and Standard Normal Variate (SNV) transformation to remove most of the non chemical spectral variance, only few spectral differences remain, corresponding to the general uniform quality of the whole set.

Ninety-six batches with their whole spectral range (9090–4000 cm^{-1}) corresponding to 5280 *X* variables were used for calculation. Using spectral unit-variance scaling, the sufficient incorporation of minor bands into the model can be guaranteed to gain the required sensibility. A PLS2 model on five important product parameters (DMA, HACA, ADCA, CefC and assay) as responses was computed to sensitize the qualitative model to these quantitative measures. Those chosen parameters have to be correlated to form a reasonable score hyper-plane. Theoretically one factor has to be dedicated for each interfering absorber to entirely subtract its contribution from the calibration model [44]. The extraction of six principal components (PCs) provides a sufficient degree of cumulative explanation of 96% of the calibration set spectra's *X* variables (wavenumbers) and 42% for the spectra's *Y* variables (chosen quality parameters) accordingly. Seventeen percentage of the *Y* variable variation can be predicted by cross validation. The total explained and predicted variation of the model itemized for the different responses is displayed in the upper part of Fig. 2. The degree of Y data explanation can be referred as satisfactory above 30% for all parameters except HACA. DMA and HACA both are not sufficiently quantitatively captured at all due to non

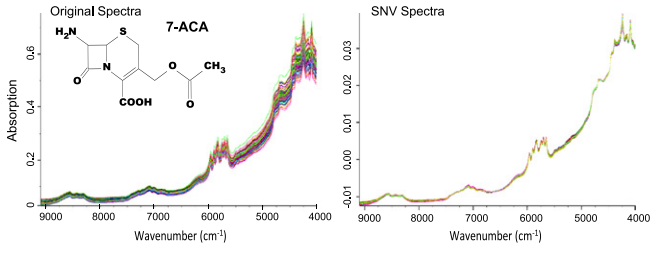


Fig. 1. Original calibration spectra (left) and Standard Normal Variate preprocessed spectra (right) with the chemical structure of the 7-ACA molecule. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

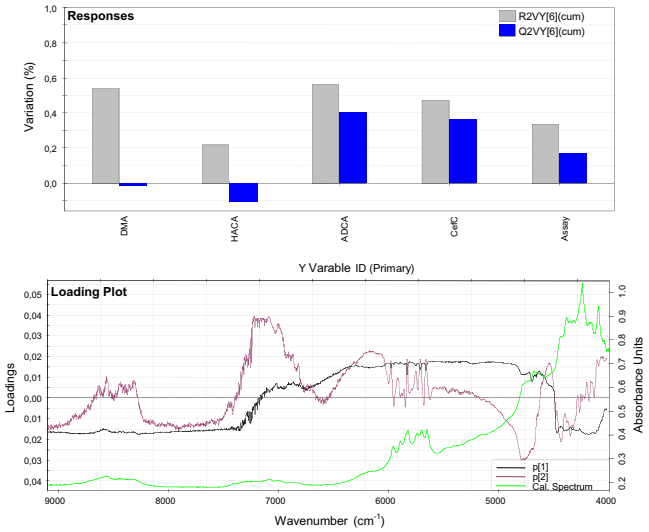


Fig. 2. Cumulative explained *Y* variables R2VY (grey) and cumulative predicted *Y* variables using cross validation Q2VY (blue) in % and the model's loading plot comparing the first two loading vectors p1 (black) and p2 (purple) to a typical absorption spectrum of 7-ACA (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

effectual variation in the reference set, while ADCA, CefC and assay show reasonable good prediction values.

In the bottom part of Fig. 2, the loading values for the first 2 PCs are presented together with a typical sample spectrum. Their characteristics are synchronous to the weight values, which establish the connection to the responses. Those spectral areas with highly positive or negative loadings have a stronger impact on the model. The systematic course of the first loading vector indicates that the corresponding PC is mainly describing physical effects, which affect large wave number regions that have not been sufficiently compensated by the normalization procedure. The structure of the 2nd loading vector clearly denotes a complex weighting of the spectral regions in the area of CH, CH_2 and CH_3 combination vibrations (4100–4500 cm^{-1}) and in the 1st overtone region (5500–6100 cm^{-1}). The further loading vectors have increasingly complicated structures with rising noise components (data not shown).

For visualization and interpretation, a certain % confidence interval is calculated for each PC dimension assuming a normal distribution of score value positions. Within that limit, the stated percentage of score points can be found statistically. As the variation of the data is decreasing steadily with each PC, the limit is declining in each dimension corresponding to a semi axis of an ellipsoid. This approach is implemented using a 95% significance interval in Fig. 3. The model is displayed as an ellipse in the first two dimensions to gain a good overview of the data. The homogenous arrangement of score positions confirms a representative set of calibration spectra with their comparatively decentralized distribution inducing a large confidence area.

As an efficient summary quality measure, the Hotteling's T^2 value is used to determine the quality integrity for unknown batch samples. This parameter can be mathematically derived depending on the score value variance of the reference set. It refers to the total quadratic distance from the center point of the 6-dimensional ellipsoid weighted with each PC's variance respectively [45].

$$T_i^2 = \sum_{a=1}^{A} \frac{t_{ia}^2}{s_{ta}^2} \quad (1)$$

A describes the number of PCs, t_a the score value of each PC of the sample spectra *i* and s_{ta}^2 refers to the variance of t_a according to the

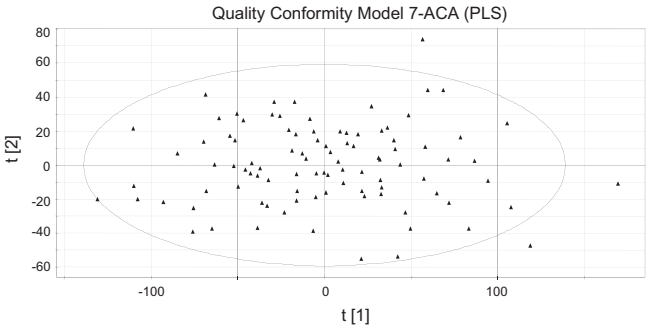


Fig. 3. Ellipse plot of the quality conformity model showing the score value distribution in the two first PCs with *t* referring to the respective score vector.

class model [12]. Through weighting by the variance, the decrease of variation of the score values with each component it taken into account so that the summary value is not only determined by the first few PCs. Every component is therefore tested separately and outliers in any dimension can be detected to highlight batches deviating from the systematic part of the model data. The Hotteling's T^2 parameter allows for the efficient assessment of the similarity of the sample batch spectra and the reference set of model spectra in respect of spectral features containing the most important quality parameters.

The confidence limit for quality control – formally depending on the definition of the number of degrees of freedom of the model – has to be set empirically based on an extensive testing effort. 95% (corresponding to a T^2 Limit of 13.9) proved to be a reasonable value in the following discussion of the model testing procedure with different batch sets. The limit could be shifted if this can be sufficiently justified and demonstrated. As T^2 values capture only the deviations included in the models reference set by spectral variability, the DMODX (Distance to the Model in X) value can be used as a complementary measure to assure model conformity of a new tested batch. This parameter detects abnormalities orthogonal to the model hyper-plane describing unmodeled variation by the spectral residuals.

3.4. Testing and validation

A first series of validation batches extensively tested for all chemical quality parameters, also used for the PAT control validation, could be accurately approved by their NIR spectra based on the derived T^2 value. Those 10 subsequent on-going production batches reveal T^2 values far below the critical limit between 2 and 7 and do not show any deviation regarding their DMODX values. For further testing, a set of conspicuous former production batches within the observation period of the last year was investigated. These batches originate from problematic production runs and rework processes that led to special 7-ACA compositions with certain parameters at (Out-Of-Expectation: OOE) and above (Out-Of-Specification: OOS) the limit. As a consequence of troubles with the solvent regeneration, eight batches showed elevated CefC levels with two of them exceeding the limit of 1%. On average, the ADCA content lies between 0.3% and 0.4%, rarely rising up to 0.5%. Four of the illustrated batches show ADACA levels of 0.5% and two more contain 0.6% ADCA, which is already beyond the demands of critical customers. Six further batches within the observation period appeared to have elevated levels of DMA – two of them crossing the limit of 1500 ppm – due to abnormalities of the solvent employed during the production. All those bad production samples could be detected by elevated T^2 levels, with OOS batches exceeding the confidence limit as displayed in Fig. 4. For a clear demonstration, the respective batch sample spectra were projected on the appropriate score plane in which they show the highest deviations from the model set. On the basis of these plots, the spectral differences of the bad production samples weighted with the selected model features can be clearly pointed out. CefC content can already be differentiated along the 1st PC. Therefore, the model is heavily dominated by the varying CefC content or by chemical or physical variations of the calibration spectra, which are related to this parameter. Elevated ADCA levels can be illustrated best in the PC 4 versus PC 3 plot. As a consequence, these two PCs are related to differences in ADCA content. Batches deviating due to their high DMA content can as well be determined with the 1st PC, with PC 6 additionally describing variations in the NIR active spectral regions of DMA.

Another approach to determine the applicability and performance of the model was the use of synthetic samples [32], to which byproducts or water have been added to obtain samples just at the quality limit. In some cases, artificial samples are not useful for the development of a calibration model, as many additional physical changes may occur during fabrication [46]. But for testing purpose only, such samples with special compositions can be of great value to review the empirical limit and confirm the critical distance. Minor inhomogeneities are expected to be sufficiently averaged regarding the whole illuminated sample volume. A good quality batch with a score position close to the center of the ellipsoid was weighted inside a glass vial and complemented to 1.4 g with the respective byproduct standard substance or purified water, respectively. Cautiously following a defined stepwise mixing procedure, sample spectra were recorded continuously until there were no further spectral changes – conferring to a stable score position and T^2 value at a sufficient degree of homogeneity. As shown in Fig. 5, the emerging accretive mixing, segregation and remaining inhomogeneity effects can be observed by the T^2 values. For ADCA and CefC, an increasing stirring level leads to a constant rising of the T^2 values, finally reaching the confidence limit. HACA is not well represented by the model, therefore such low concentrations cannot be detected by the spectra and do not comply with the multivariate limit. There were no reproducible T^2 values for HACA-Lacton as this coarse-grained substance cannot be mixed well with ACA. The detection of elevated humidity within the sample is simple, even before mixing the highly NIR active water leads to excessive T^2 values.

3.5. Compaction and particle size

Particle size and packing density both have a high impact on the scattering properties of the powder, as NIR spectroscopy is frequently used for particle size determination. With large particles the radiation penetrates deeper into the sample material leading to less reflection, weak band characteristics and a higher over-all absorption signal. Strong differences in particle size and compaction are limiting the spectral reproducibility [47] and may lead to nonlinearities in PLS calibration models [48]. As different spectral pretreatments may not entirely compensate for variably compressed sample material, compaction often constitutes a high fraction of the variation described by the model [49]. For 7-ACA, a bimodal particle distribution with a basic particle size of around 100 μm and a second particle size distribution maximum occurring at around 10 times this size was assessed during the investigations. These special particle size particularities – either caused by agglomeration effects or due to production techniques – are influencing the material's flow and repack characteristics. Therefore, quality-related non-relevant physical deviations could confine spectral reproducibility, lead to prediction value variability and may limit the model's applicability. Continuative experimental investigations have been done to achieve well-defined conditions using a more uniform compacting mechanism. A Teflon plunger

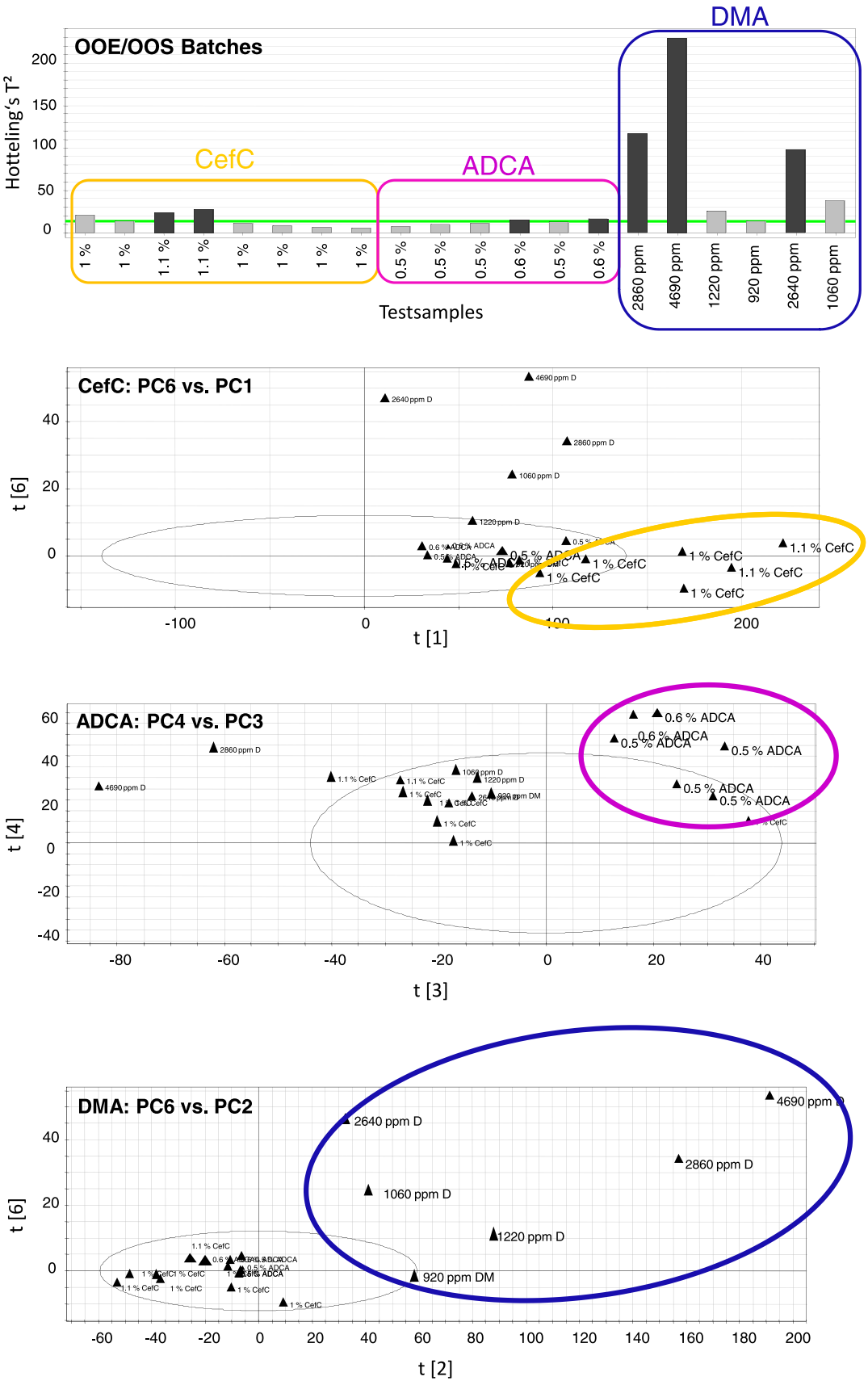


Fig. 4. Examples for quality predictability with Hotteling's T^2 values and the investigation of detected outliers by their score positions in different PC dimensions for CefC, ADCA and DMA. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

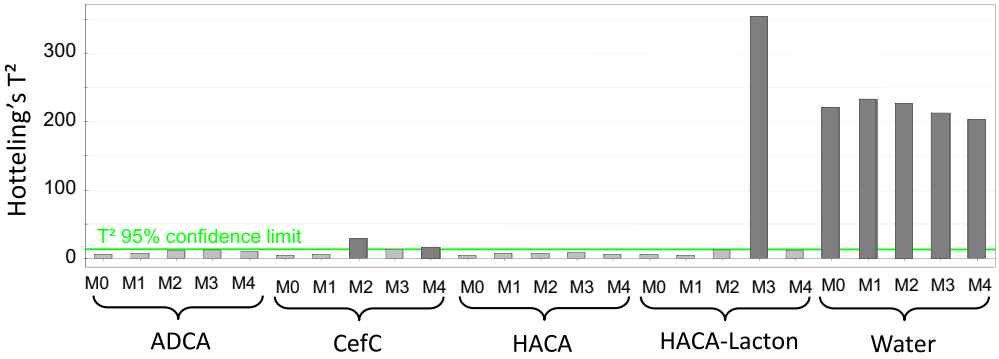


Fig. 5. Hotteling's T^2 values for a set of laboratory samples stepwisely mixed with byproduct and water up the respected quality limit. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

combined with a quartz crystal windowed sampling cup and a special compaction cup densely pressing a defined amount of material helped to raise spectral reproducibility at the cost of higher measuring expenditure.

4. Conclusion

The presented NIRS classification technique is a very suitable method to verify compliant batch quality. Especially in combination with the PAT control it allows for the easy and reliable product assessment at almost no effort. Through chemometric analysis, the potential of NIR spectroscopic data to gain material know how could be demonstrated, although certain premises remain to assure full applicability. The substance to be classified has to be available in a broad quality range for calibration and the production process must not reveal high variability or drifts to ensure reliable spectral evaluation. Routine application recording conditions have to be adjusted and optimized for the substance properties.

In case of ACA, several substance characteristics confined the implementation of the spectral model surveillance at the time being. During the testing phase, some batches without chemical peculiarities showed T^2 value exceeding the limit due to the high sensitivity of the model. A combination of several elevated quality parameters led to an over-all T^2 signal above the confidence limit in some cases, as the whole allowed quality range cannot be sufficiently systematically covered with independent variations of the reference samples. This restriction is also responsible for the insufficient incorporation of certain parameters into the model, which as a consequence cannot be determined by the present model. Variability in the on-going PAT controlled and non-effectually stable production process can induce chemical drifts in product composition and powder matrix modifications that may lead to unjustified negative quality assessment by NIRS. Therefore, this study constitutes a basis for the replanning of the release procedure and is meanwhile perceived as an additional control tool. Currently, a highly promising approach for PAT applications is the combination of spectroscopic "fingerprint" information like IR, NIR or Raman together with " high speed" HPLC gaining the most comprehensive quality information of bulk products. Both methods can be performed within minutes, a requirement for most PAT applications and Realtime-Release systems.

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