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Research paper

A new PAT/QbD approach for the determination of blend homogeneity: Combination of on-line NIRS analysis with PC Scores Distance Analysis (*PC-SDA*)

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ABSTRACT

A novel and straightforward multivariate analytical tool for the qualitative determination of powder blend uniformity using on-line Near-Infrared Spectroscopy (NIRS) is presented. The approach combines current chemometric methods, e.g. spectral pre-processing and Principal Component Analysis (PCA), with (1) a new approach of data analysis to determine the end-point of the blending process, (2) building a design space (DS) for blend homogeneity and (3) developing a solid statistical rationale to stop blending according to Quality-by-Design (QbD) principles of FDA's Process Analytical Technology (PAT) initiative. The new approach comprises calculation of Euclidean distances between PCA scores in a multidimensional space and determination of Moving Block Standard Deviations (MBSDs) of successive Principal Component (PC) scores distances to estimate a time-window during blending where spectral variability decreases to a preset minimum. Hotelling's T^2 statistics is then used to monitor and report blend homogeneity. This technique is called "Principal Component Scores Distance Analysis" (*PC-SDA*).

A Central Composite Design resulting in 10 batches mixed in a bin-blender (same composition, different blender fill level, different number of revolutions) was executed.

NIR Chemical Imaging (NIR-CI) in combination with Symmetry Parameter Image Analysis (*SPIA*) was used to verify the NIRS analyzer response and assess homogeneity of all NIR-active components.

1. Introduction

Powder mixing is a pharmaceutical unit operation required for the production of all solid pharmaceutical products. In the blending process, blend uniformity analysis (BUA) is an important aspect that needs to be controlled during the manufacturing of solid dosage forms, since only a homogeneous mixture can be subdivided into individual doses, which provide the correct proportion of the active pharmaceutical ingredient (API) [1]. The current approach for ensuring homogeneity of a powder blend is based on collecting samples at different powder bed locations within the vessel at predefined time intervals. Collected samples are then analysed off-line by time-consuming and laborious procedures – e.g., UV–VIS spectroscopy [2] or high-performance liquid chromatography (HPLC) [3]. A blend, in classical pharmaceutical sense, is considered to be homogeneous when the API content of the blend samples is within specification, while assuming that all excipients are also evenly distributed [4].

FDA's Process Analytical Technology (PAT) initiative [5] has stimulated the pharmaceutical industry to increase research and use of new analytical technologies, which could support manufacturing through Quality-by-Design (QbD) principles [6]. QbD is aimed to design and develop formulations and manufacturing processes that ensure a predefined quality. Thus, QbD requires the understanding how formulation and process variables influence product quality. Relevant documents from the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) — ICH Q8 [7], Pharmaceutical Development, along with ICH Q9 [8], Quality Risk Management, and ICH Q10 [9], Pharmaceutical Quality Systems — indicate how Quality-by-Design ensures drug product quality [10]. Additionally, the annex to ICH Q8 [11] describes how to define a design space (DS) in a pharmaceutical dossier, as opposed to classical nominal operating ranges, i.e. proven acceptable ranges (PAR) by univariate studies.

Near-Infrared Spectroscopy (NIRS) is a powerful non-invasive analytical technique, since it is multiparametric, sensitive to both physical and chemical attributes, fast and easy to interface with the process and capable of providing whole matrix fingerprinting.

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It has been investigated for e.g. BUA and is supported by the PAT initiative [12]. The potential of this type of approach to homogeneity determinations is that the assumption of excipient homogeneity will be removed, since all components of the blend mixture will contribute to the resultant NIR spectrum and are thus measured implicitly [4]. In this context, a number of studies have been published over the last decades dealing with at-, on- and in-line NIRS measurements in pharmaceutical blending (Table 1).

One of the most common approaches for qualitative assessment of homogeneity is to calculate the Moving Block Standard Deviation (MBSD) between consecutive spectra. The data are arranged into a time by wavelength matrix. A new matrix is calculated over all wavelengths by determining the standard deviation (SD) of intensity values over a predefined time-window or block. Finally, a mean value is calculated from each of the resulting SD spectra over all wavelengths. The mean SD can be plotted as a function of time, and the blending end-point is determined to be the time interval at which the mean SD profile reaches a minimum value [4]. One advantage of this approach is that no sample quantification is required. However, it is arguable to calculate the mean SD of SD's. The definition of a "minimum SD value" has not been described yet. Additionally, MBSD does not ensure that a possible absence of the API or some excipients will be noticeable. On the other hand, quantitative approaches constructing univariate or multivariate calibration models have been used to express blending processes in terms of concentration variation, which is comparable to the standard criteria of current regulatory requirements [26]. Quantification using off-line samples to predict the concentration of the drug and/or excipients during blending is time-consuming, cost-intensive and laborious. Compared to quantitative approaches, qualitative approaches require less data and are therefore more suitable for early process development stages. There are many techniques and various approaches available. A detailed explanation is beyond the scope of this paper. The interested reader is referred to reviews by Reich [27], Roggo et al. [28] and a book chapter by Ciurczak and Drennen [29].

At-line NIR Chemical Imaging (NIR-CI) was used as reference method in this study to demonstrate an effective verification of the NIRS analyzer response. NIR-CI is a non-destructive method that allows a fast evaluation of homogeneity of all NIR-active components. Amigo et al. [30] used NIR-CI for the determination of powder blend homogeneity and studied the benefits and drawbacks of PCA, cluster analysis and correlation coefficients for qualitative purposes and Classical Least Squares (CLS) and Multivariate Curve Resolution – Alternating Least Squares (MCR-ALS) for quantitative purposes. The standard deviation of single-channel-image pixels as a function of time was calculated univariately by El-Hagrasy et al. [20], while Lewis et al. [31] have used in addition symmetry descriptors, such as SD, skewness and kurtosis, to determine API distribution. Based on Partial Least Squares (PLS) predicted images of ingredients, Li et al. [32] have generated binary images, which were used to calculate the number and size of the constituent particles/domains within images. Ma et al. [33] performed multivariate analysis including pure-component PCA and discriminant PLS to treat imaging data. Puchert et al. [34] utilized Symmetry Parameter Image Analysis (*SPIA*), an approach for the statistical evaluation of NIR-CI in terms of a multivariate treatment of univariate statistical descriptors (e.g. skewness and kurtosis) characterizing image pixels.

With respect to blend homogeneity using NIRS, none of the available methods *fully* describes how to detect blending endpoints based on a solid statistical rationale (Table 1).

Therefore, our aim was (1) to develop a simple multivariate analysis tool for on-line NIRS measurements, which combines current chemometric methods with a new approach of data analysis, (2) building a design space for blend end-point determination and (3) reporting the blend homogeneity. This technique was called by the authors "Principal Component Scores Distance Analysis" (*PC-SDA*) and will be first described and later on applied to several powder blend experiments at a pre-industrial scale. The process settings were previously defined by designed experiments with factors chosen from a risk analysis. Furthermore, it will be explained how a real-time application can be implemented to monitor powder blend processes. The procedure requires no sample quantification, while ensuring that a possible absence of the API or excipients will be noticeable. Additionally, the final mixtures were investigated off-line by HPLC to determine API blend uniformity and NIR-CI to verify the on-line NIRS sensor response, i.e. to decide whether the whole blend was homogeneous, meaning that the API and the excipients were well distributed. In addition, tablets of each powder blend were produced to be analysed by NIR-CI in terms of API distribution. Furthermore, tablet hardness, tablet thickness and tablet weight were determined.

Table 1
Applications and approaches for the determination of blend homogeneity using NIRS.

Method	At-line	On-line	In-line
Average standard deviation of spectra	[13]	[1]	
Bootstrap error-adjusted single-sample technique (BEST)	[14]	[15]	
Change in absorbance of one of the components		[16]	
Chi-square analysis	[14]		
Dissimilarity between spectra and ideal mixture		[1]	[4]
Euclidean distance between spectra		[1]	
Hidden Markov Model (HMM)		[17]	
Mean square differences between consecutive spectra			[18]
Moving Block Standard Deviation (MBSD) of PC scores			[19]
Moving Block Standard Deviation (MBSD) of spectra		[20]	[4,12,19]
Net Analyte Signal (NAS) of spectra			[21]
Non-linear iterative partial least square (NIPLS)		[22]	
Principal Component Analysis (PCA)	[23]	[1,20,24]	[25]
Quantitative analysis – multivariate	[13]	[15,24]	[19,21,25]
Quantitative analysis – univariate		[16]	[18]
Soft Independent Modeling of Class Analogy (SIMCA)		[15,20]	[4]
Simple Interactive Self-modeling Mixture Analysis (SIMPLISMA)	[23]		
End-point determination		**[15,20,22,24]**	**[4,25]**
Confidence limit (threshold) for blend homogeneity	**[14]**	**[1]**	**[4]**
Design space for blend homogeneity	**–**	**–**	**–**
Statistical report for blend homogeneity	**–**	**–**	**–**

2. Materials and methods

2.1. Materials and equipment for on-line NIRS

All powder blends had the same composition with a low dose API (~2.5% w/w API, Crospovidone, Microcrystalline Cellulose, Corn Starch and Magnesium Stearate). All ingredients were of the highest available pharmaceutical grade and complied with their corresponding European Pharmacopoeia monographs. After weighing, the components of each formulation were transferred to a 100L bin-blender (Servolift GmbH, Offenburg, Germany).

A LANCIR II® spectrometer together with the OPUS PROCESS® software (both BRUKER OPTIK GmbH, Ettlingen, Germany) was used for spectra acquisition in diffuse reflectance. This spectrometer is equipped with a 256 element diode array detector that covers the wavelength range from 1100 to 2200 nm. It was mounted directly onto the blender, and spectra were obtained through a sapphire window without direct sample contact. The 25 mm in diameter beam size of the on-line sensor corresponds to about 150–200 mg of sample mass, which in our case was adequate to effectively use the on-line NIR sensor for quantitative blend uniformity analysis, where the beam size has to be equivalent to one single dosage unit [35]. An acceleration sensor, which measures the total acceleration resulting from the gravitation and the centrifugational forces, ensures correct spectra acquisition. The LANCIR II is therefore able to determine its actual orientation and whenever it was located at the bottom position during rotation a trigger device signalled the start of the measurements. For all on-line blending acquisitions in this study, a trigger angle of −45° to +45° was found to be optimal. Measured NIR spectral data were then transferred via a wireless network from the spectrometer unit to a nearby notebook. Dark measurement was performed to correct both stray light influences and effects caused by the sampling technique. White measurement was performed using a Spectralon standard. Dark and white spectra were taken before starting each blending batch. Acquisition parameters were similar to those used in sample measurements. The instrumental setup is shown in Fig. 1.

2.2. Experimental design

QbD requires statistical methods to be used in pharmaceutical formulation and process development. For quality and productivity improvement, the most cost beneficial of these methods is statistical Design of Experiments (DoE). A trial-and-error search for the *vital few* factors that most affect quality is costly and time-consuming [36]. Based on a risk analysis, a Central Composite Design (CCD) [37] with two factors was designed using Design-Expert (Stat-Ease Inc., Minneapolis, USA). Two critical process parameters (CPP) [11] of the blending process (*blender fill level (bfl) and number of revolutions*) were identified (data not shown). The fill level of the blender covered a range from 30% (v/v) up to 94% (v/v), which is within the range of filling levels commonly used in the industry [38]. The rotational speed of the blender was set at 12 rpm, while the number of revolutions comprised a range from 10 (~1 min) up to 420 (~35 min). A total of 10 runs were carried out including two centre-point runs. All experimental points are symmetrical with reference to the experimental centre. The various combinations are shown in Table 2.

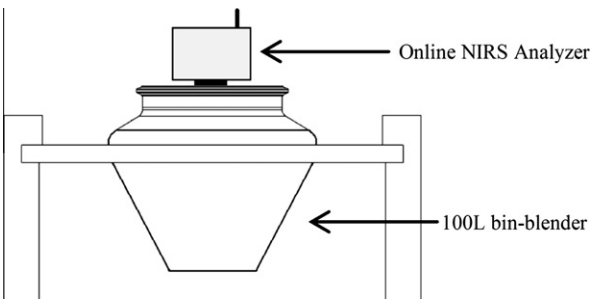

Fig. 1. Instrumental setup for on-line NIRS blend monitoring.

2.3. Spectral pre-processing (chemometric optimisation)

As the goal of the final method is to monitor blend uniformity, spectra were continuously obtained over the entire spectral region available (1100–2200 nm) at a fixed resolution of 4 nm to provide absorbance values (log (1/*R*)) in function of wavelength (nm). This enabled four spectra co-averaged (integration time: 50 μs) into one spectrum to be acquired in each revolution. The raw spectra were mathematically transformed and visualized using the software SIMCA-P+ 12.0 (Umetrics, Umeå, Sweden) with the aim to reduce the spectral variability associated with the physical characteristics of powders and the instrumental variability. For this purpose, a standard normal variate (SNV) algorithm [39] followed by second derivative Savitzky–Golay [40] using third-order polynomials across 21 data points was applied. This approach was used to eliminate non-chemical spectral variations emanating from the blending process such as the baseline shifts. SNV, applied spectrum by spectrum, results in centring and reduction of each intensity value for all the different variables included in the spectra [41] – this reduces the multiplicative interferences of scatter and particle size.

2.4. Data analysis – Principal Component Scores Distance Analysis (PC-SDA)

The approach presented here is a simple and straightforward multivariate analysis tool, which combines current chemometric methods with a new approach of data analysis to determine the end-point of the blending process, building a design space for blending process trajectories and then reporting relevant statistics near and at the end-point evaluated over the entire NIR spectral range. The approach is schematically illustrated in Fig. 2 and will be described by means of a powder blending process with a total validated blend time of 18 min.

As previously described, spectra were mathematically transformed. The pre-processed spectra are plotted in Fig. 2a. When the blending is incomplete, the NIR spectra show significant variations; however, the variability is much reduced after a certain time. It is almost impossible to tell when and if the spectral variability converges. The band intensities are related to the concentrations of different chemical components. Though, bands may not be sharply separated and overlap is common. Hence, it is necessary to use chemometric methods to extract the characteristic features of the spectrum.

For this reason, we performed Principal Component Analysis (PCA) of spectral data. Vickerman et al. [42] well describe PCA in general, while among other things Kirsch et al. [43] focus on PCA

Table 2
Experimental design (centre-point runs are printed in bold).

Run no.	Revolutions (n)	Fill level (%)	Blend time (min)
1	**215**	**62**	**18**
2	420	62	35
3	215	30	18
4	70	85	6
5	70	40	6
6	**215**	**62**	**18**
7	10	62	1
8	360	40	30
9	215	94	18
10	360	85	30

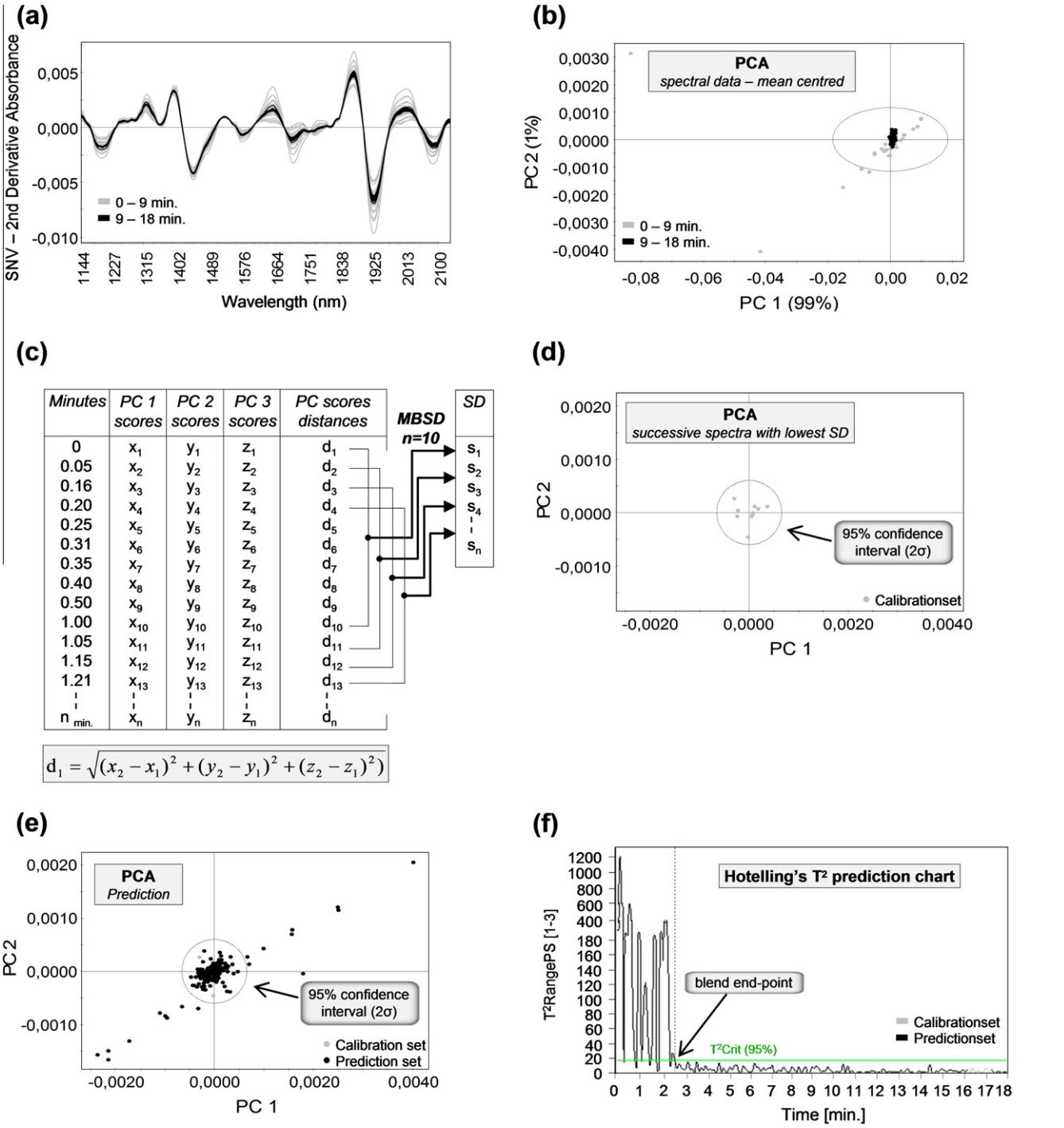

Fig. 2. Description of a new approach of data analysis (*PC-SDA*): (a) pre-processed spectra; (b) PCA of spectral data; (c) calculation of successive PC scores distances according to the illustrated equation, calculation of Moving Block Standard Deviations of successive PC scores distances; (d) PCA – calibration set; (e) PCA – prediction set; (f) predicted Hotelling's T^2 chart for the determination of the blend end-point (dashed line) and the proposed design space (T^2 Crit. 95%, solid line, green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in terms of spectral analysis. PCA is an unsupervised multivariate statistical technique commonly used to reduce the dimensionality of data sets (e.g., near-infrared spectra). PCA describes the major sources of variability in the spectra with a small number of orthogonal (uncorrelated) axes, or Principal Components (PCs). Each spectrum is plotted as a single point in the multidimensional space, resulting in a cluster of single-point spectra in *n*-dimensional space. The first PC describes the largest amount of spectral variation, the second axis the second greatest source, and so forth, until all sources of variability have been explained.

A two dimensional graphical representation of the PCA, applied after mean centring of the pre-processed spectra, is shown in Fig. 2b. In mean centring, each variable is centred by the subtraction of its mean value across all samples. In the case of spectral data, this is equivalent to subtracting the mean spectrum of the data set from each sample [42].

In this study, the major source of spectral variability was expected to be due to chemical variations. Pre-processing algorithms such as SNV followed by a second derivative were used to highlight chemical variability.

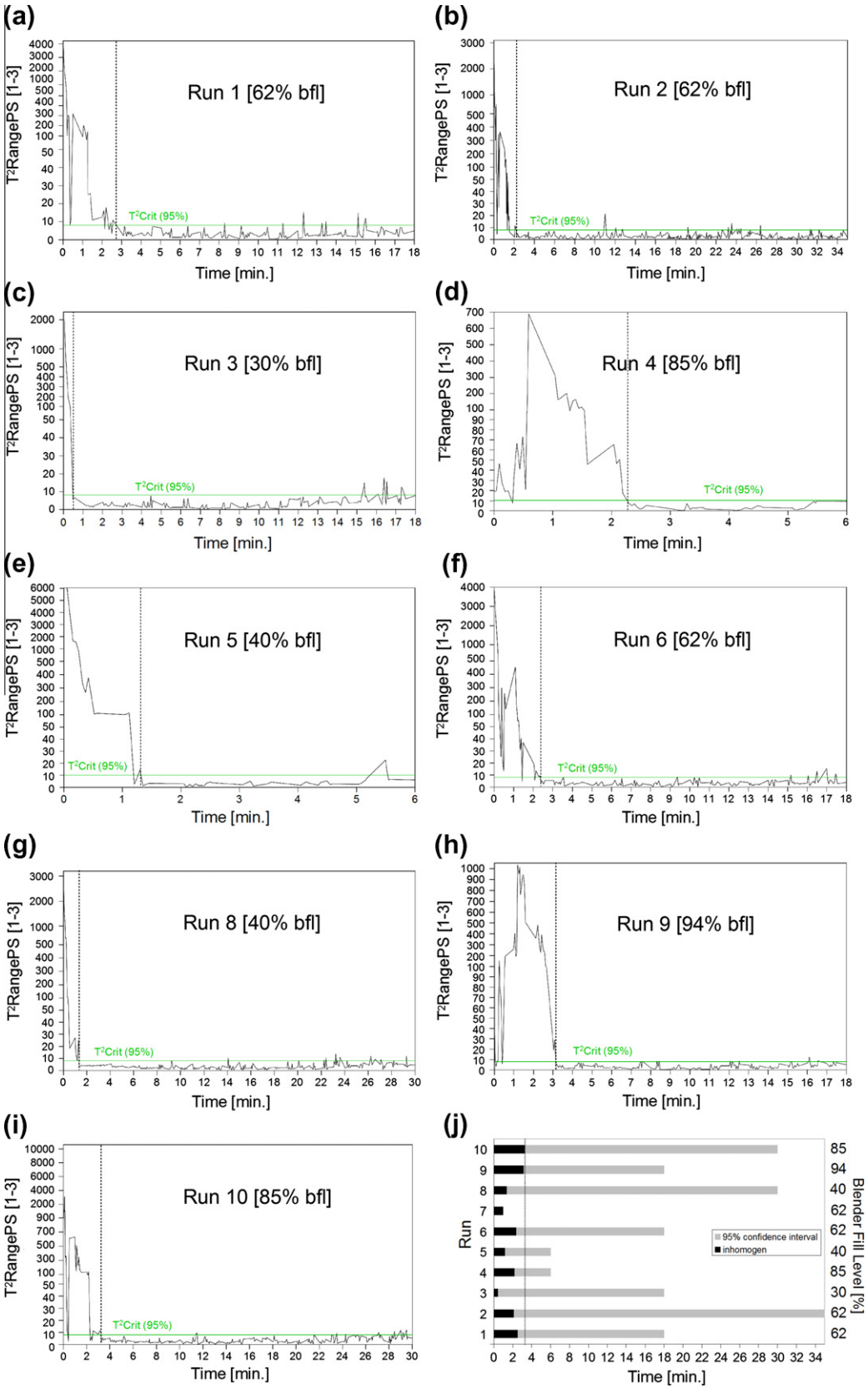

Fig. 3. (a–i) Predicted Hotelling's T^2 charts for the blend processes, blend end-points (dashed lines), design spaces (T^2 Crit. 95%, solid lines, green); (j) summary plot with overall blend end-point (dashed line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

As next step, Moving Block Standard Deviations (MBSDs) of successive PC scores distances (as a function of time) were calculated. This was carried out by a spreadsheet application (Microsoft EXCEL 2002). Storme-Paris et al. [19] described a quite similar approach with the difference of calculating Moving Block Standard Deviations between PC scores values for each PC separately. Instead,

we used the first three PC scores for calculating the Euclidean distances between successive PC scores. The usage of more than three PCs is possible, depending on the%-explained variance of *n*PCs. Euclidean distance examines the root of square differences between coordinates of a pair of objects. In Cartesian coordinates, if $p = (x_1, y_1, z_1)$ and $q = (x_2, y_2, z_2)$ are two points in three-dimensional Euclidean space, then the distance from p to q is given by [44]:

$$d(p,q) = \sqrt{\sum_{i=1}^{n}(p_i - q_i)^2} \quad (1)$$

In the PC space, the scores on PC 1, PC 2 and PC 3 are weighted according to the amount of the variance explained by PC 1, PC 2 and PC 3 [45].

Fig. 2c schematically describes the calculations using PCA results from the first minute of blending. PC values are referred as follows: PC 1 = *x*-axis, PC 2 = *y*-axis and PC 3 = *z*-axis. The calculation of distances between successive PC scores was carried out (see also equation for calculating e.g. the first distance value in Fig. 2c) and resulted in a column of PC scores distances ($d_1 - d_n$) – i.e., the distance in a three-dimensional space from one score to the next and so forth. Next, the standard deviations of successive distances were calculated. Since the rotational speed of the blender was set to 12 rpm for all experiments, ~10 spectra were collected per minute and a block-frame of 10 successive PC scores distances was used for calculating the standard deviation. This resulted in a column of standard deviations ($s_1 - s_n$).

Since low standard deviation indicates less spectral variability and therefore good homogeneity, the next step was to search for the lowest standard deviation. Although a specification of acceptable standard deviation will be problem-specific (e.g., depending on criticality of API content, potency or toxicity), one could set it as a general rule of thumb at the level of HPLC (i.e., the golden-standard) repeatability (<1%). Since one standard deviation resulted from 10 successive distances, and these in turn derived from 11 successive PC 1–3 scores, the allocation of the lowest standard deviation to its corresponding PC scores and thus corresponding spectra was carried out. This approach is both novel and innovative.

Again, PCA was performed, but only containing the previously determined 11 successive measured spectra with the lowest variability (Fig. 2d). The reason for calculating a PCA merely based on data with the lowest variability (=*calibration set*) is that a 95% confidence interval for good homogeneity can be computed more accurately and under much more challenging conditions than based on estimates made on the entire time trajectory. That 95% confidence ellipse in the PCA scores plot (Fig. 2d) describes the boundary of the targeted blend homogeneity design space. It should be noted that for calibration purposes the decision to simply select spectra close to the end-point of mixing for building the design space could lead to false interpretation. A prolonged blend time is not a guarantee of homogeneity, since segregation or lubrication phenomena can occur when blending is continued for an excessive period [19].

The remaining spectra were now part of the *prediction set* to be determined by the calculated PCA model. Fig. 2e shows the predicted scores plot. All PC scores respectively corresponding spectra within the 95% confidence interval meet the quality attribute of good homogeneity.

Blend homogeneity can be monitored through a multivariate Hotelling's T^2 [46] control chart. The Hotelling's T^2 for observation *i*, based on *A* components is:

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

where s_{ta}^2 = variance of t_a according to the class model [46].

Fig. 2f illustrates the predicted Hotelling's T^2 chart as a function of time in minutes. For a new spectrum taken, it can be determined whether it falls within or outside the confidence limit. In this context, the time at which the spectra scores fall into the 95% confidence interval determines the end-point for blending. Additionally, the determination of a maximum limit of acceptable standard deviation of PC scores distances is proposed by using the standard deviation value, which corresponded to the time at which the scores fall into the 95% confidence interval. Consequently, this approach could be used in a pharmaceutical dossier to statistically support a QbD filing on determination of the blending end-point and definition of a design space for blend homogeneity.

2.5. Reference analysis – powder blends

Reference data were measured for purposes of homogeneous distribution (API and excipients), i.e. to verify the on-line NIRS response with a SyNIRgi™ Chemical Imaging System (Malvern Instruments, Malvern, UK). According to the *Guidance for Industry: Powder Blends and Finished Dosage Units – Stratified In-Process Dosage Unit Sampling and Assessment* [47], aliquots of a predefined amount (3× the sample mass of one single dosage unit) were withdrawn at 10 different powder bed locations within the vessel after each blending process to be analysed by NIR-CI. The determination of batch powder blend homogeneity was carried out using the approach of Symmetry Parameter Image Analysis (*SPIA*). The results from image histogram statistics were processed and visualized using the software SIMCA-P+ 12.0 (Umetrics, Umeå, Sweden). For a more detailed description of *SPIA* and the Imaging System, we refer to our previous publication [34].

Furthermore, the same blend thief samples were corrected for weight and tested for API content utilizing high-performance liquid chromatography (HPLC) on a Chromolith® Performance RP-18 column (Merck KGaA, Darmstadt, Germany) with pH 2.5 ammonium dihydrogen phosphate buffer and acetonitrile as the mobile phase at a column temperature of 30 °C with UV detection (LaChrom Elite® L-2400, VWR-Hitachi International GmbH, Darmstadt, Germany).

2.6. Process capability – compaction of powder blends

Each blend was compressed into tablets on a Fette 2090 single rotary tablet press (Fette GmbH, Schwarzenbek, Germany) equipped with 43 punches. For reasons of comparability, compression force (17.5 kN) and rotational speed (180,000 tablets/h) were kept constant. During compaction, every 30 min 10 tablets were automatically withdrawn to be analysed for hardness, thickness and weight by a Checkmaster 4.1 (Fette GmbH, Schwarzenbek, Germany). The following specifications were set: hardness (69–90 N); thickness (2.55 mm ± 10%); and weight (170 mg ± 3%).

For each batch, tablet samples (n = 10, randomly gathered during compaction) were tested for API distribution utilizing NIR-CI and *SPIA*.

3. Results and discussion

3.1. Powder blend on-line NIRS analysis

According to the chosen experimental design, a total of 10 batches were mixed under predefined conditions (i.e. same composition, different blender fill level, different number of revolutions) and on-line monitored by NIRS. The spectral data were statistically analysed by the new approach described in Section 2.4, each batch separately. In all cases, three PCs were sufficient to explain ~99% of variance. These 10 independent PCA models could be combined into a single design space. We defined an overall limit for accept-

able standard deviation of PC scores distances with equal to or less than 5×10^{-4}. The standard deviations within the 95% confidence interval of all batches were below that value. Fig. 3 illustrates the predicted Hotelling's T^2 charts for the blend processes except run 7. This batch was blended for only one minute at a fill level of 62%, and the calculated standard deviation of PC scores distances was not yet within the defined specification. For interpretation purposes, Fig. 3a–i display the blending end-points as a function of time (dashed lines) and the corresponding multivariate process trajectories (T^2 Crit. 95%, solid lines, green). The influence of the blender fill level was very well observable. Batches with a low fill level (e.g. Fig. 3c) reached homogeneity much faster than batches with a high fill level (e.g. Fig. 3h). A statistical analysis including intermediate blender fill levels was not carried out, since a systematic investigation into the effect of blender fill levels on blend kinetics was beyond the scope of this study.

From Fig. 3, it is evident that blending for more than 4 min is not necessary, since for 9 of 10 runs the blend was apparently homogeneous before 3.5 min, regardless of blender fill level (except for run 7 as previously mentioned). The adjustment of the mixing time within a range of 1.5 min up to 4 min is proposed not based on any operational benefits, but based on potentially increased risks of demixing or segregation, if blend time is extended.

Since all batches contained the same composition, it was now even feasible to build an "overall design space" in one PCA model. The predefined spectra with low variability (11 spectra per batch = 110) were used as *calibration set* to build the design space for blend homogeneity. The remaining spectra were now part of the *prediction set* to be determined by the calculated PCA model. Fig. 4 illustrates the predicted scores plot. All PC scores respectively corresponding spectra within the 95% confidence interval (design space) meet the quality attribute of good homogeneity.

3.1.1. Real-time application for on-line NIRS monitoring of powder blend processes

Once a design space for blend homogeneity is developed, a real-time implementation can be suggested as follows. Each new spectrum will be projected onto the design space. If successively measured spectra fall into and remain inside the 95% confidence ellipse within a period of ~2 min (incl. safety margin), then the blending process can be stopped. The absence or false dosage of the API and excipients caused by e.g. dead spots within the blender will be made noticeable, since the measured spectra will not fall into the 95% confidence interval. This is due to the increased sensitivity to consecutive spectra variability and also to the use of the entire spectral range.

3.2. NIR Chemical Imaging of powder samples (verification of the sensor response)

To test and challenge the proposed approach, blend uniformity analysis was carried out. The first step was the development of a hyperspectral reference library [34] with powder samples of the pure API and four major NIR-active excipients (Crospovidone, Microcrystalline Cellulose, Corn Starch and Magnesium Stearate). Based on the reference library, a PLS calibration model for discriminant analysis was built. The next step was to apply the PLS model to all powder blend samples which were collected after blending. Fig. 5 shows the PLS predicted API images (10 samples per run, A–J). The score values over the images show that the maximum value at any pixel is only ~0.025, which would equate to an abundance of ~2.5%, i.e. 97.5% of the pixel contribution is from the other components.

Blends with a long blending time (runs 2, 8, 10) showed strong variability within the samples. Samples from run 7 – blended for less than one minute – also differed with respect to the relative abundance of the API. Generally, samples blended for 6 min (runs 4, 5) and 18 min (runs 1, 3, 6, 9) were evenly distributed, which

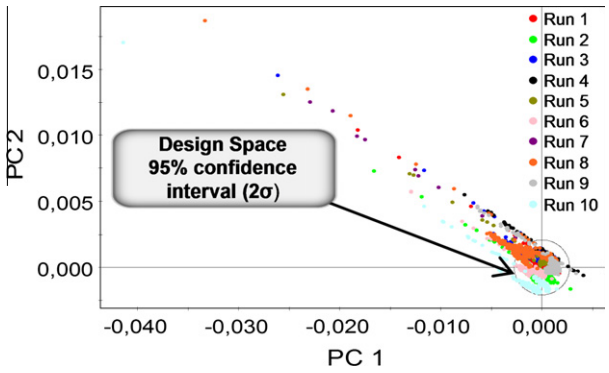

Fig. 4. Predicted scores plot of all batches; design space = Hotelling's T^2 ellipse. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

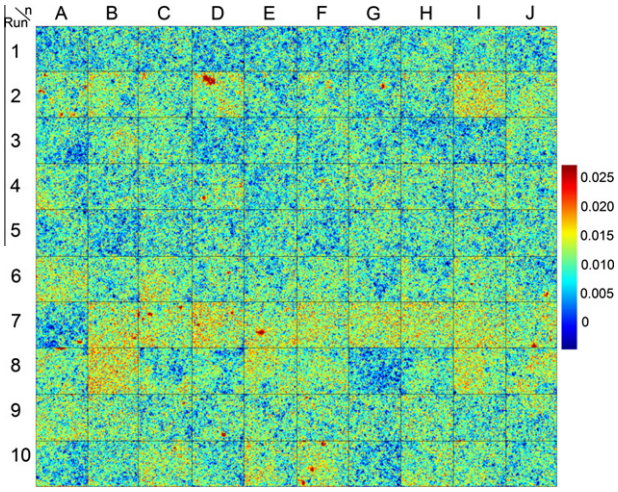

Fig. 5. PLS predicted API images. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

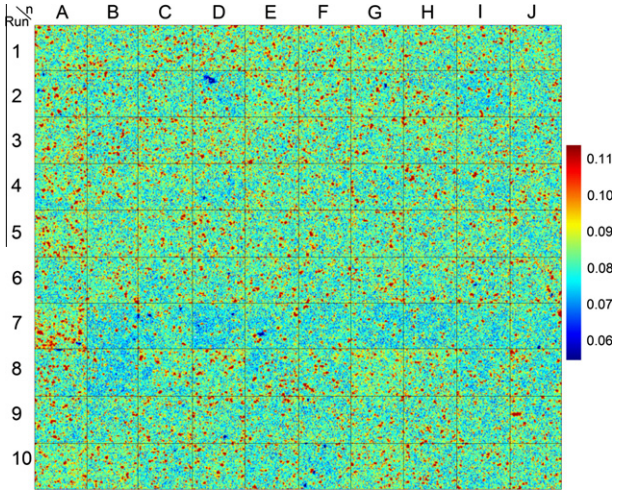

Fig. 6. PLS predicted Crospovidone images. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

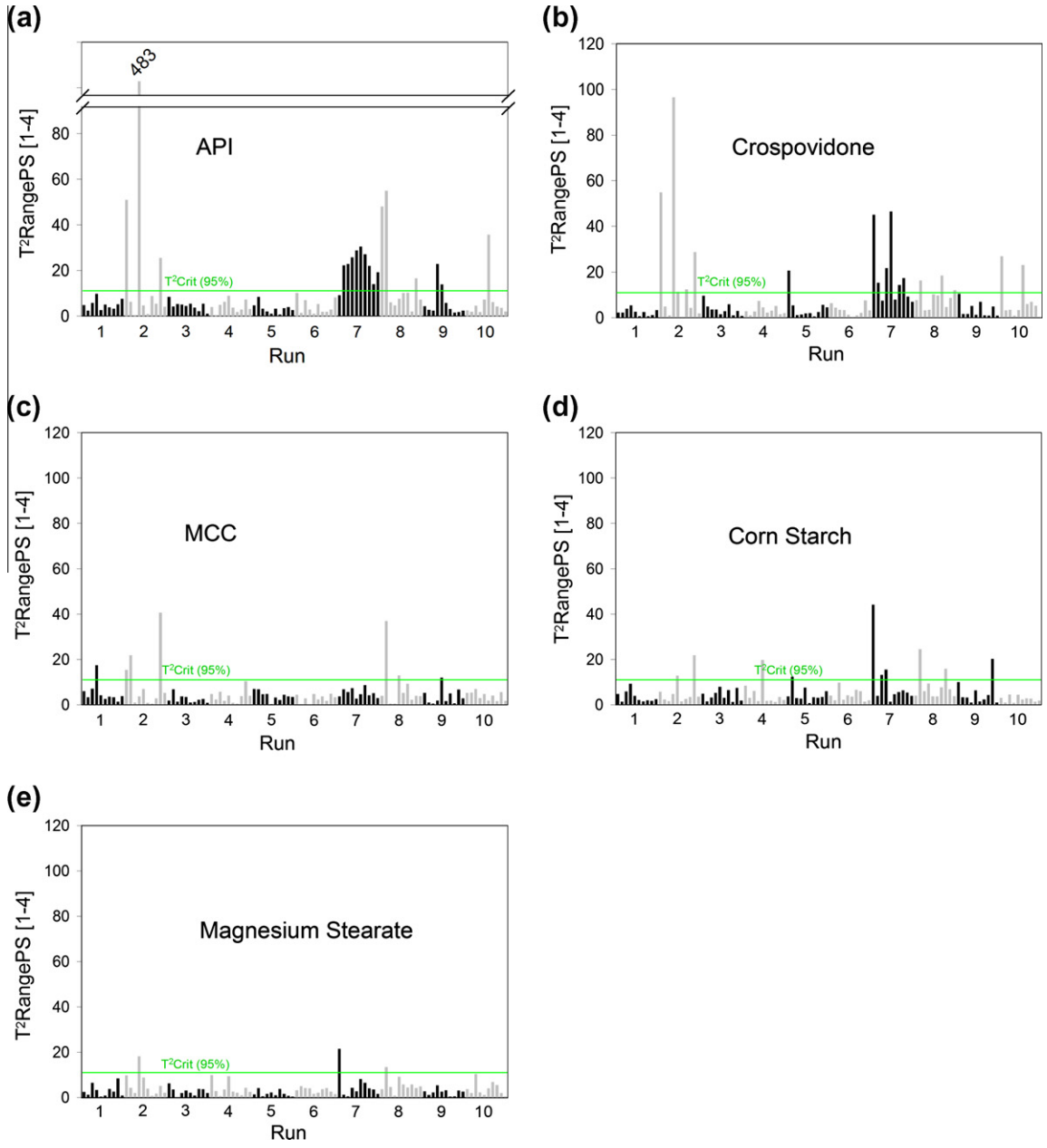

Fig. 7. Symmetry Parameter Image Analysis (*SPIA*) of powder blends – predicted Hotelling's T^2 chart for: (a) API; (b) Crospovidone; (c) Microcrystalline Cellulose; (d) Corn Starch; (e) Magnesium Stearate – confidence interval (T^2 Crit. 95%, solid lines, green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

indicated good homogeneity. In this case, it was obvious that unnecessarily long blending affected the quality.

The visual inspection of images of the excipients also indicated differences in the relative abundance and the spatial distribution of components. The PLS predicted images of Crospovidone are illustrated in Fig. 6.

Symmetry Parameter Image Analysis (*SPIA*) [34] was used to analyse sample variability of all components. Fig. 7 illustrates the predicted Hotelling's T^2 charts for each ingredient.

The Hotelling's T^2 chart results confirmed the findings of image visualization. Samples outside the 95% confidence interval in the T^2 charts also showed significant image characteristics related to inhomogeneity. The PLS predicted images of the API and Crospovidone showed the largest variance in terms of pixel intensity and spatial distribution, while Microcrystalline Cellulose, Corn Starch (except one sample of run 7) and Magnesium Stearate were more or less evenly distributed.

3.3. HPLC for the determination of blend uniformity (API)

The weighted powder samples were analysed by HPLC to determine the blend uniformity of the API. Fig. 8 shows the results given by HPLC.

The individual content was between 85% and 115% of the average content. This complied with the current European Pharmacopoeia method "*2.9.6. uniformity of content of single-dose preparations*" and "*2.9.40. uniformity of dosage units*" [48,49]. However, a trend is apparent that samples of run 7 contained more API. This confirmed the findings by NIR-CI.

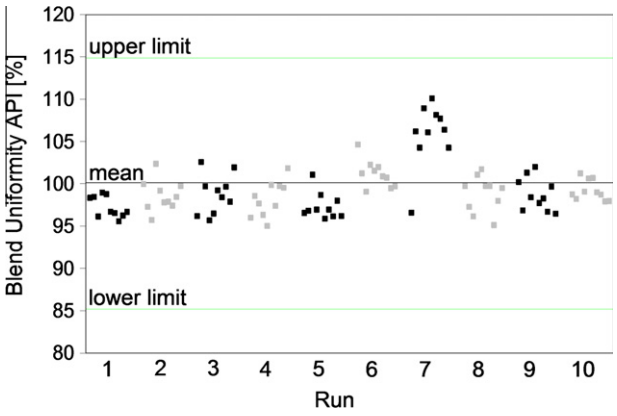

Fig. 8. Powder blend uniformity of the API (HPLC Analysis); limits according to European Pharmacopoeia 7th edition (solid lines, green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

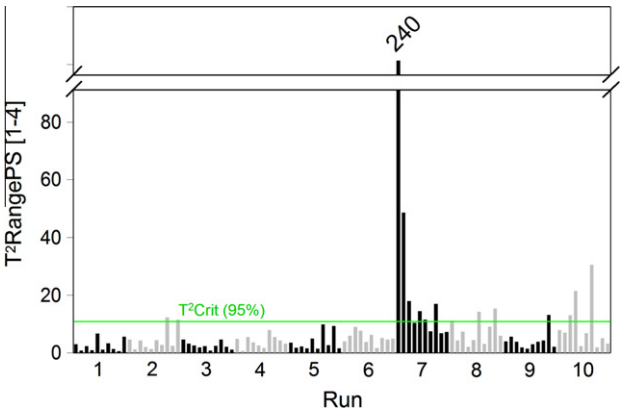

Fig. 10. Symmetry Parameter Image Analysis (*SPIA*) of tablets – predicted Hotelling's T^2 chart for the API – confidence interval (T^2 Crit. 95%, solid line, green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

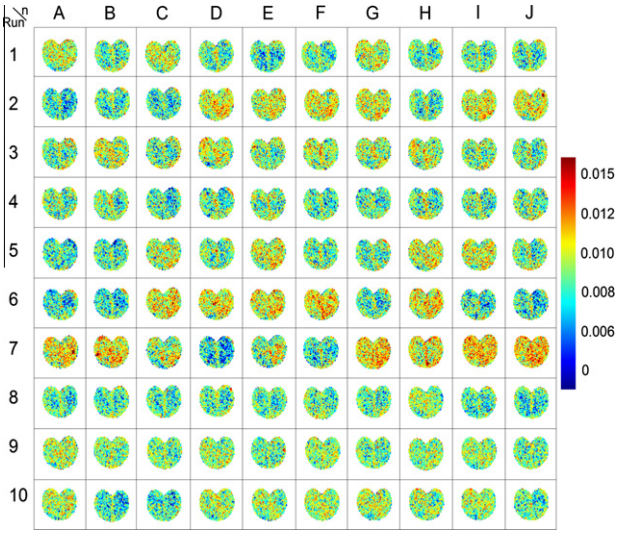

Fig. 9. PLS predicted API images from manufactured tablets (10 tablets/run). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.4. Compaction of powder blends (process capability)

The aim was to investigate whether the powder blends are compactable into tablets and additionally meet the desired quality attributes (distribution of the API, hardness, thickness and weight).

Again, NIR-CI was carried out. Image acquisition and pre-treatments were processed in the same manner as for the powder blend study. The previously developed hyperspectral reference library and the PLS calibration model were applied to the tablet samples with the difference that only the PLS predicted API images were used for further investigation. Fig. 9 shows the PLS predicted API images.

In terms of the API distribution, tablets which originated from long time blended mixtures (runs 2, 8, 10) showed higher variability than tablets from short time blended mixtures (e.g. runs 4, 5). However, run 7 remains a so-called "outlier" batch. This was statistically confirmed by *SPIA*. Fig. 10 illustrates the API predicted Hotelling's T^2 chart for the tablets.

The measurement of hardness, thickness and weight of tablets revealed non-significant variability. All values were within the required specifications – even for tablets from run 7 (data not shown).

4. Conclusions

A new PAT/QbD approach for the determination of powder blend homogeneity combining on-line NIRS and a new approach of multivariate data analysis – called by the authors "Principal Component Scores Distance Analysis, (*PC-SDA*)" was developed. Based on PCA of pre-treated spectra, the Euclidean distances between successive PC scores (as a function of blend time) followed by the determination of MBSDs of successive PC scores distances in a multidimensional space were calculated. This approach successfully enabled the *estimation* of a time-window during blending where the spectral variability was lower than a preset threshold. In that context, a maximum limit of acceptable standard deviation of consecutive spectra was set in terms of spectra scores. Spectra with low variability were used to successfully build the target multivariate end-point space for blend homogeneity by which real-time determination of blend homogeneity and the adjustment of blending time is feasible. The approach was demonstrated utilizing Hotelling's T^2 statistics which could be used as an innovative way of reporting blend homogeneity in pharmaceutical dossiers and QbD filings.

NIR-CI verified the findings of on-line NIRS analysis of powder blends in terms of the spatial distribution of ingredients. However, HPLC analysis of powder samples showed non-significant differences in content uniformity of the API. Furthermore, it was less sensitive to ensure blend homogeneity.

Compaction into tablets was performed for all powder blends and measurements of hardness, thickness and weight complied with the required specifications. Additionally, investigation of tablets utilizing NIR-CI analysis in combination with Symmetry Parameter Image Analysis (*SPIA*) was able to detect an "outlier" batch, as that batch had an extremely short blend time.

Both NIR-CI and on-line NIRS blend monitoring combined with multivariate data analysis are powerful PAT tools for process development and manufacturing according to Quality-by-Design principles.

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