Real-Time NIR Monitoring of Blending Process using Inlier Diagnostics

Angela Schmidt: as@camo.no - **CAMO Software AS**,
Bhanu Kalvakota: bhanuk@amgen.com - **Amgen Inc.**

In this example a quantitative NIR model for real-time monitoring of a pharmaceutical blending process was developed. The difference between those blend batches is the active pharmaceutical ingredient (API) dosages: 17, 25, 34 and 62 % by weight. It is expected that these levels of the API are constant towards the end of the blending processes. So the objective was to determine a steady-state concentration and to find out how long it takes to reach this steady state behavior.

As a process analytical tool an in-line NIR spectrometer (Brimrose, AOTF technology) was used, mounted onto a pilot scale (5 L) tumble bin blender, whose speed (12 rotations per minute) and filling level (75%) were kept constant. The only difference between the batch process parameters was the level of API. During each batch blending process an NIR spectrum was acquired at every revolution of the blender, with 36 scans corresponding to a measurement time of 1.2 seconds. NIR spectra were acquired between 1300 – 2100 nm in 2 nm increments.

For quantitative method development, a calibration model with NIR spectra from 50 artificial samples prepared from the same ingredients were recorded in an off-line mode, with 256 scans (over 7-8 seconds) and replicated 5 times. The range of the API concentrations from 2 to 84 % covered the API levels of the in-line batches. The most obvious approach, i.e. using the quantitative off-line model from laboratory samples to predict the batch spectra, failed, not because the batch spectra were marked as outliers, but because they were labeled as Inliers. This tool according to the ASTM guidelines D6122-06 (2006) is also called "Nearest neighbor distance Inlier statistics". An Inlier detection test is conducted to determine if a spectrum resides within the region of the multivariate calibration space that is sparsely populated. If so, the prediction result might be subject to possible interpolation error. Therefore inlier statistics would be a potential tool to qualify appropriate validation samples in order to perform successful method validation procedures required for real-time monitoring of the pharmaceutical processes.

With this example it will be shown how the inlier test works, and that a quantitative model built from both off-line and in-line NIR spectra is applicable without any losses in prediction quality (RMSEP, RMSEC).

Keywords: Blending, NIRS, PLS1, validation, Inlier, Ydeviaton
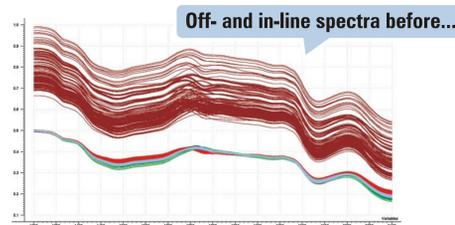
Materials: 2 types of spectral data

Figure 1: Original NIR Reflectance spectra from blending mixtures acquired in off-line mode (n=251, brown, 50 API levels) and in-line mode (n=1363, colored, 4 API levels). The offset between in-line and off-line spectra could be due to packing density and sample weights at the saphire window.
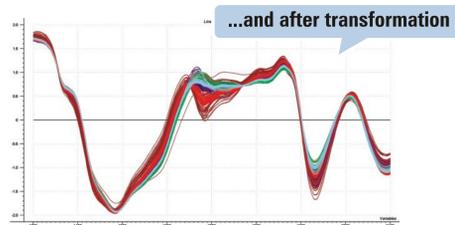
Figure 2: Transformed* NIR spectra from blending mixtures acquired in off-line mode (n=251, brown) and in-line mode (n=1363, colored). *DeTrend (2nd polynomial order) + Standard Normal Variate (SNV). Transformed off-line spectra cover all of the in-line spectra (colored).

Methods: Explorative Analysis
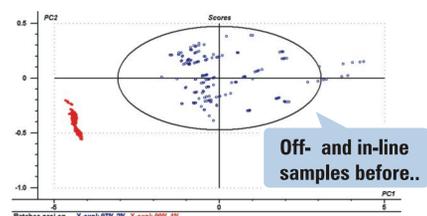### PCA Projection of 4 Batches onto PCA model from off-line samples

Figure 3: Original NIR Reflectance spectra from blending mixtures acquired in-line (n=1363, red) are projected onto PCA model from off-line spectra (n=251, blue). The 95 % confidence Hotelling T2 ellipse indicates in-line data are outside. Explained Variance of both groups is similar for only 2 PCs:99%, 100%.
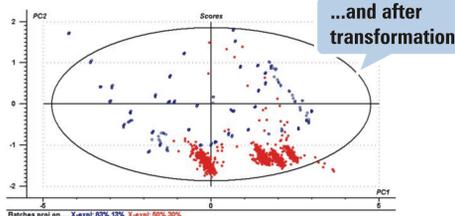
Figure 4: Transformed NIR Reflectance spectra from blending mixtures acquired in-line (n=1363, red) are projected onto PCA model from off-line spectra (n=251, blue). After DeTrend(2) + SNV the 95 % confidence Hotelling T2 ellipse covers the majority of all spectra. But Explained Variance of 96% (for blue) and 80% (for red) indicates that 2 PCs might not be sufficient.

Methods: Regression
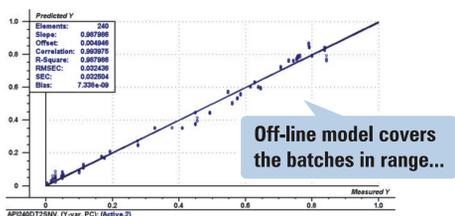### PLSR-1 from off-line spectra, Prediction of in-line spectra

Figure 5: Calibration statistics for PLS1-model from 240 transformed off-line spectra. At rank 2 the RMSEC is 3.24 % API, and the R2 = 0.988 for calibration; moreover RMSEP = 3.40%, R2 = 0.987 for a 50 segment, systematic Cross Validation.
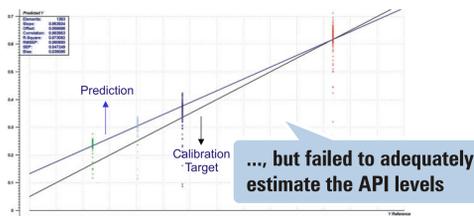
Figure 6: Prediction of 1363 in-line spectra with a calibration model built from 240 off-line spectra. The RMSEP is 6.07 % and R2 0.873. Obvious is a high prediction bias of 3.8%, expressed by a tilting regression line when compared to the target line.

Results: Prediction
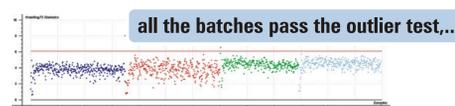### of in-line samples from 4 batches using off-line model

Figure 7: Hotelling T2 values at PC2 and 5 % significance level for 1363 in-line spectra predicted with PLS1 model built from 240 off-line spectra: there are hardly any outliers. The 4 batches with different levels of API are grouped by different colors.

Figure 8: Inlier distances for 1363 in-line spectra predicted with a calibration model built from 240 off-line spectra. Almost all in-line spectra are above the inlier distance limit (red line), i.e. they are having a distance to the nearest calibration sample that is higher than the maximum distance between the 2 calibration samples being most far off. So they are falling into sparsely populated areas of the region in the multivariate model space.
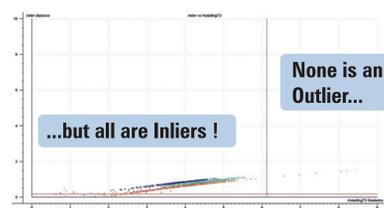

Figure 9: Prediction of 1363 in-line spectra with a calibration model built from 240 in-line spectra. The Inlier vs. Outlier plot shows, that almost all in-line spectra are inliers, but none is an Hotelling T2 outlier. The sample points can be grouped well according to the 4 batches with differing API levels.
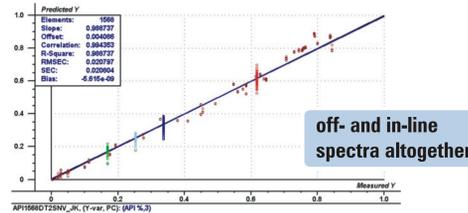
Methods: PLS-Regression

Figure 10: Calibration statistics for PLS1-model from 1568 transformed in- and off-line spectra altogether. Now 1 PC more is required to describe the additional variance. At rank 3 the RMSEC is 2.07 % API, and the R2 0.989 for calibration; moreover the RMSEP = 2.16 % and R2 = 0.987 for a 79 segment, systematic Cross Validation.
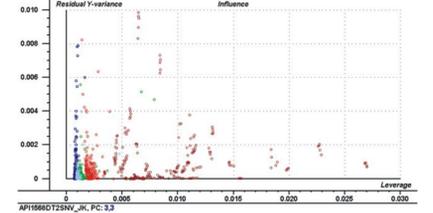
Figure 11: The influence plot shows that the calibration off-line samples (brown) are more influential to the model due to higher leverage values than colored batch spectra, acquired in in-line mode.

Results: Prediction using "entire" model
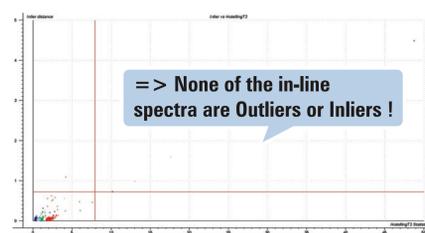### (from off- and in-line spectra)

Figure 12: Prediction of 681 in-line spectra with a calibration model built from 896 off- and in-line spectra. The Inlier vs. Outlier plot shows that the calibration model is more robust with almost all predicted spectra neither inliers nor Hotelling T2 outliers. Again the sample points are grouped according to the 4 batches with differing API levels.
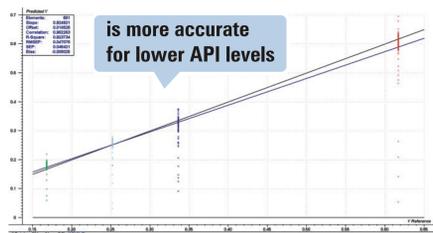
Figure 13: Prediction of 681 on-line (every 2nd) spectra with a calibration model built from 896 (remaining) in- plus off-line spectra. The RMSEP is 4.7 % and R2 0.924, whereas the bias is much lower at 0.8% now.
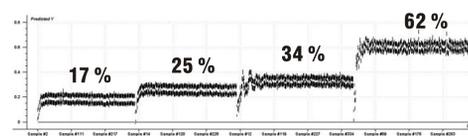

Figure 14: Predictions and YDeviations of 681 (every 2nd) in-line spectra from 4 batches over number of revolutions. After initial increase the predicted Y from all the batches are moving towards a steady state, corresponding to the supplied API concentrations given in % by weight (API b.w.)
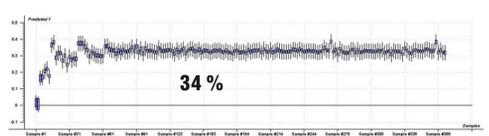
Figure 15: Predictions of 184 on-line spectra from 34% API b.w. level over number of revolutions. After initial increase the predicted Y values are moving towards a steady state, corresponding to the supplied API level of 34 % b.w. Here the yDeviations are the blue boxes around the predicted Y (white line inside).

Conclusion:

- PLS1 model for the pharmaceutical blending process was built.
- Quantitative model based only on off-line samples, resulted in real-time batch predictions pass the Hotelling T2 outlier test but fail the inlier diagnostic test.
- Thus the NIR spectra from both off-line and in-line acquisitions in spite of deviating dynamics were included in the quantitative model
- The prediction quality expressed by RMSEC / RMSEP of the quantitative model did not suffer from including in-line spectra.
- The entire model just requires 1 more principal component (PC) to explain the additional variation due to sample dynamics.
- The RMS errors for "entire" model are even lower compared to models with fewer number of spectra (n) — summary in table 1.
- A Calibration model from only in-line spectra with API 17 to 62 % gave the best (cross-validation) RMSEP.

Transformation	Data Type	n spc	Set	Rank	Xexpl PC1	Xexpl PC2	Yexpl PC1	Yexpl PC2	Validation RMSEP	R^2	Bias	Calibration RMSEC	R^2
ABS+DT(2)+SNV	off-line	240	CAL	2	77	18	86	12	4,26 CV	0.980		4.01	0.982
KM +DT(2)+SNV	off-line	240	CAL	2	71	23	68	29	5,48 CV	0.967		5.13	0.97
	off-line	240	CAL	2	84	13	91	8	3,40 CV	0.987		3.21	0.988
	all on-line	1363	VAL	2	-		-		6,07 TestSet	0.873	3.8		
	off-line	240	CAL	3	84	13	91	8	2,99 CV	0.990		2.75	0.992
	all on-line	1363	VAL	3	-		-		5,13 TestSet	0.909	0.9		
	"entire" off- /on-line	1568	CAL	3	78	19	88	10	2,16 CV	0.986		2.08	0.989
	all off-/on-line every 2nd	896	CAL	3	74	22	85	13	2,28 CV	0.988		2.14	0.99
	on-line remaining	681	VAL	3	-		-		4,71 TestSet	0.924	-0.8		
	all off-/on-line superblend	491	CAL	3	75	21	84	14	2,74 CV	0.987		2.58	0.988
	on-line starting	1118	CAL	3	-		-		5,14 TestSet	0.909	-0.9		
DT(2) + SNV		1326	CAL	2	97		98		1,60 CV	0.991		1.60	0.991
	all on-line	241	CAL	2	1	98	1	99	2,50 CV	0.987		2.00	0.986

Table 1: Summary of Statistics for Regression (Calibration, Cross-Validation CV) and Prediction (TestSet) models.
Transformations: ABS = Absorbance; DT (2) = DeTrending with 2nd order polynomial; SNV = Standard Normal Variate; KM = Kubelka Munk.

Predictions of Real-time Blending Process

- Real-time batch predictions using a model, that combined off- and in-line data, resulted in none of the batch samples identified as inlier.
- The inlier statistics tool is useful to finding interpolation errors of the quantitative model used, and thus testing the model's robustness for prediction of in-line samples. Inlier statistics could be suggested as a tool to qualify validation samples and assess model robustness.
- yDeviations from prediction samples (shown in figure 14) were found to be useful to describe a stable process, rather than simple differences between predicted Y ± measured Y (reference).
- yDeviation is an Unscrambler specific term, and will relate X-residuals from this prediction to those from the quantitative model and include validated Y-residuals from the calibration model as well. So it is like an individual RMS-error for each prediction sample, representing its position in the model space and thus ideal for trend analysis.
- yDeviations for higher API-level batches are relatively more stable than lower API level batches, and thus will require shorter blending times. This fact corresponds fully well to blending experiences even without NIR technology.
- Predictions from a pure off-line model were least accurate (high Y-deviations i.e. 10 fold higher), but with a good precision (low %RSD).
- Predictions from a pure in-line calibration model were most accurate (also low Ydev), but least precise due to doubled %RSD.
- Predictions from a "mixed" model that included off-line samples and selected super-blended in-line samples from the last 5 minutes of the batch process resulted in the best compromise with regard to accuracy and precision (Table 2).

% Active	End-point	Offline Calibration Pred%	Offline Calibration %RSD	Offline Calibration Y-devn	Offline Calibration Accuracy	Off-/ In-line (from last 5 min) Calibration Pred	Off-/ In-line (from last 5 min) Calibration %RSD	Off-/ In-line (from last 5 min) Calibration Y-devn	Off-/ In-line (from last 5 min) Calibration Accuracy	In-line (from last 5 min) Calibration Pred	In-line (from last 5 min) Calibration %RSD	In-line (from last 5 min) Calibration Y-devn	In-line (from last 5 min) Calibration Accuracy
16,8	125	21,8	3,1	0,124	1,30	19,1	3,4	0,019	1,17	18,2	7,4	0,011	1,08
25,5	90	28,9	2,6	0,123	1,13	26,6	2,6	0,015	1,04	24,8	5,2	0,014	0,97
33,6	80	34,2	2,8	0,103	1,02	32,8	2,7	0,010	0,98	32,9	4,3	0,017	0,98
61,7	70	59,4	2,3	0,099	0,96	59,4	2,5	0,017	0,96	61,4	4,8	0,019	1,00

Table 2: Comparison of traditional Statistics and Y-Deviations from Predictions of in-line Validation Samples beyond the end-point (based on MBSD),
Note: When in-line data is deployed in calibration models, only the remaining data beyond the end-point is used for prediction.

MBSD = Moving Block Standard Deviation

Pred = Predicted Y, mean
Y-devn = Y Deviation = Uncertainty

% RSD = Relative Standard Deviation
% Accuracy = Pred/ Active