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CHEMOMETRIC CORNER

Qualification: Adulteration screening with NIR – a case on skim milk powder

Qualitative methods for adulteration screening are introduced and a case is presented: the detection of melamine in skim milk powder by NIR and chemometrics.

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P/N 1026716, Issue 1, May 2014

Dedicated Analytical Solutions

Introduction

High quality in food and feed production is based on reliable determination of the composition of the raw materials as well as intermediate and end products. However, it is equally important to be able to detect deviating sample spectra that might be caused by adulteration, process mishaps or quality deviations. The detection of samples deviating from a good product is called *qualification* – we refer to the InFocus whitepaper on terminology for a description of *identification* and *qualification* [1].

The need for efficient methods for detection of adulteration and other deviations is obvious – here we describe the method background and provide an example of how to apply the methods for adulteration detection in dairy powder analysis with FOSS NIRS DS2500 solution combined with the latest version of the WinISI chemometric software.

The mathematics

As the basis for the qualitative model development, we have a training data set with good product spectra of a number of samples spanning the expected variation of the relevant product. The methods applied are based on Principal Component Analysis (PCA) and derived distances in a PCA model of the data. As seen in Figure 1, PCA can be described as a decomposition of the pre-processed spectral data, **X**, into a matrix **T**, **P** and **E** for *A* components. The **P** matrix contains the loadings, or the hidden spectra/ common structures, the **T** matrix the concentration of each of the loadings for each sample and the **E** matrix contains the residuals – the part not described by the model (see [2] for more detailed description of PCA applied to NIR spectra).

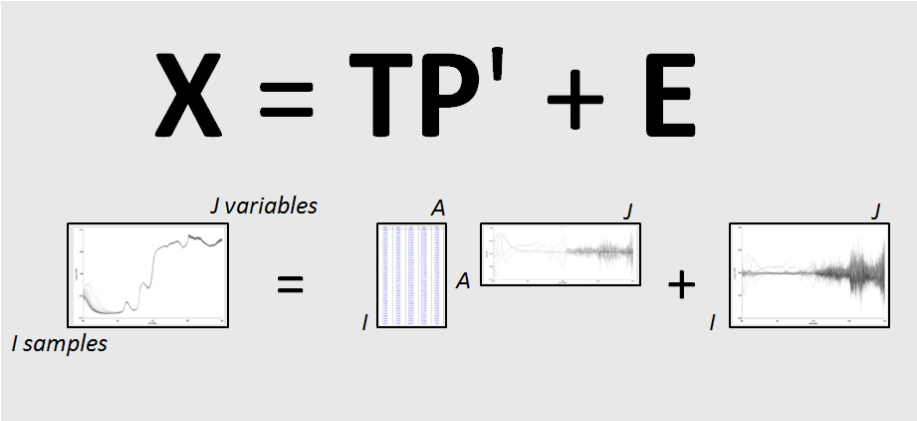

Figure 1. The PCA model.

There are two ways to analyse a given sample's distance to another sample: it can be measured as the distance to the center *within* the model – this is calculated from the scores, or the distances *to* the model – calculated from the residuals:

- A sample spectrum for a new sample that deviates only with respect to score space can be called a *concentration outlier* – the spectral pattern is comparable to the good product spectra but the sample either contains very high or very low concentrations compared to the good product samples.

- A sample spectrum for a new sample that deviates only with respect to the residuals can be called a *spectral pattern outlier* – the spectral pattern deviates compared to the good product spectra but the sample concentration levels are comparable to the good product samples.

- A spectrum that deviates on both distances – scores and residuals – is both a concentration and spectral pattern outlier.

WinISI qualification methods are designed to characterize a set of spectra representing product samples that meet quality specifications and to detect departures from the data set. The following distances are available:

- **Mahalanobis distance (squared)**: measures the distance between a spectrum and the average spectrum of the good product dataset using PCA scores (T). The values are scaled so that the average distance for all samples in the PCA is 1.0. This measure is useful to detect if a new sample spectrum close to the average spectrum in a way that resembles the good product samples.

- **Mahalanobis neighbor distance (squared)**: measures the distance between a spectrum and the *closest* spectrum in the good product dataset. The values are scaled with the same factor used in the Mahalanobis distance measure. This measure is useful to detect the distance to the nearest neighbor spectrum and compare this with the average neighbor distance for the whole data set.

- **RMS X residuals**: the portion of a spectrum that is not explained by the PCA model. A square root of the average squared (RMS) residual is computed to provide the RMS X residuals. This measure is useful when we want to detect spectral variation not similar to the spectral variation in the good product data set.

- **Maximum X Residual**: Instead of computing the RMS, this method finds the largest absolute X residual for the new sample spectral residual. It is more sensitive to unmodelled variation at just a few wavelengths than the RMS X residuals method. If for instance an adulterant has a specific peak at 2100 nm and 2300 nm it might be easier to detect by focusing on the specific part of the spectrum where the deviation is largest.

- **Maximum X Residual T**: is a modification of the Maximum X Residual method. Each X residual wavelength is turned into a 't' statistic by dividing by the standard deviation of all corresponding residuals at that wavelength in the good product set. The method reports the largest absolute 't' value. This method is more sensitive to small departures of the residuals compared to the residuals in the good product dataset.

- **Maximum Peak T**: this method is also based on the X residuals in a PCA model as the previous three methods. The method looks for a peak shape in the X residuals. This method computes a 't' statistic by dividing the intensity of each peak by the standard deviation of all corresponding peak intensities. The method reports the largest positive 't' value over all wavelengths. This method is sensitive to the addition of new ingredients not present in the good product dataset and will react on spectral deviations.

- **ASM method**: this method is a combination of the Mahalanobis distance and the RMS X residuals. It is the square root of the sum of Mahalanobis distance squared and a suitably scaled RMS X residuals squared. This measure will indicate that a sample is good only if its spectrum is similar to the average good product spectrum **and** the X residuals are small. In this way, the ASM methods combine information from both the model and outside the model.

The Mahalanobis based methods are suitable for concentration outlier detection while the residual based methods are relevant for detection of a variety of spectral patterns; the ASM merges the concentration and spectral pattern outlier detection.

Case study - the data

NIR spectra of 214 skim milk powder samples were recorded by a NIRS DS2500 instrument to provide spectra from 400 to 2500 nm with 0.5 nm intervals – in total 4200 variables per spectrum (Figure 2). These were split into an 81 sample training set, a 40 sample tuning (or test) set and a 93 sample validation set. The splits were made from the 214 samples ordered by analysis time with the training set being the oldest, the tuning set being in the middle and the validation set being the most recent scans. The tuning and validation spectra were also spiked with 0.5% melamine (Figure 3). Since neither the skim milk powder nor melamine had distinctive peaks in the 400 – 1100 nm range, only the NIR range was used in the study.

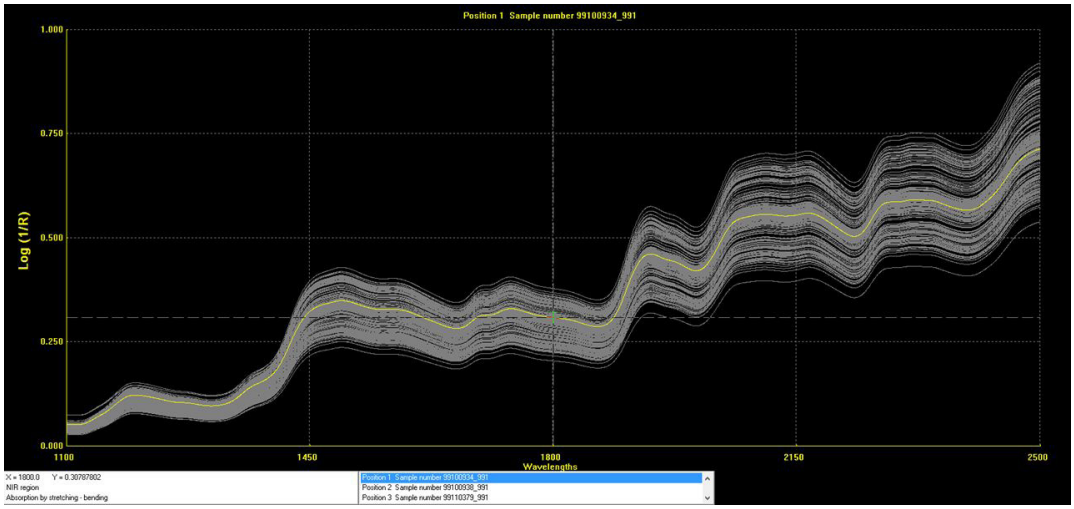

Figure 2. NIR spectra from 1100-2500 nm of 214 skim milk powder samples.

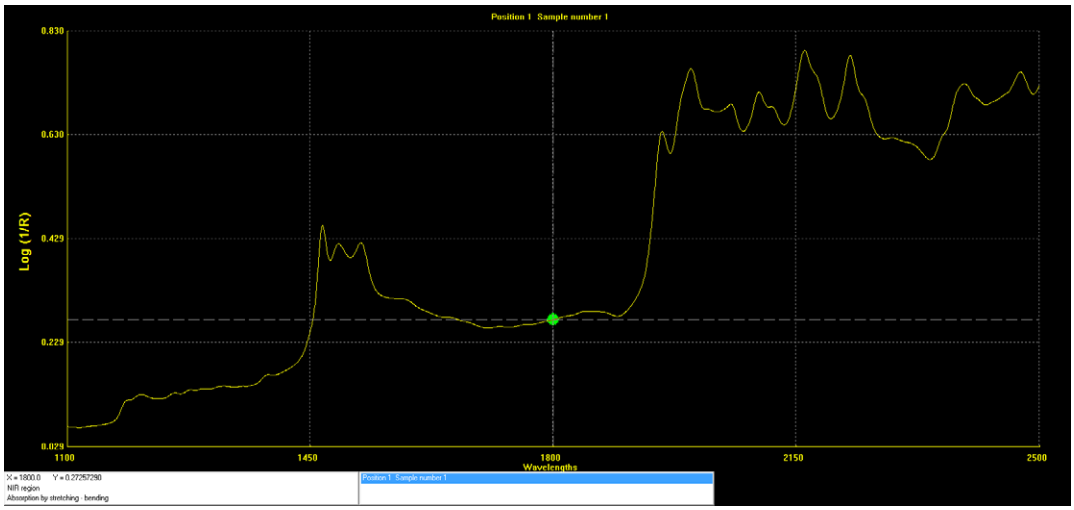

Figure 3. NIR spectrum of melamine from 1100-2500 nm.

The same conditions as for sample selection for quantitative analysis holds here – it is important to span the expected variation to be seen in future 'good' samples. This might be chosen to be a narrow variation, e.g. in a process context the last month of production or for laboratory solutions a broader variation.

The qualification model

We are interested in the detection of melamine which has distinct peaks around 1467 nm and several between 1955 and 2227 nm, and preprocessing with a second derivative (2,20,1,1) was used to emphasize these peaks.

A qualification model (Good Product Definition, GPD, in WinISI) model was developed using the 81 training samples. The output is shown in Figure 4. The tricky part is to determine the number of components to use in the model. For this we use the 40 sample tuning set and some spiked samples.

A comparison of the seven methods available for qualification clearly showed that the Maximum Peak T method was very efficient in detecting the melamine, so we will base the analysis on this method.

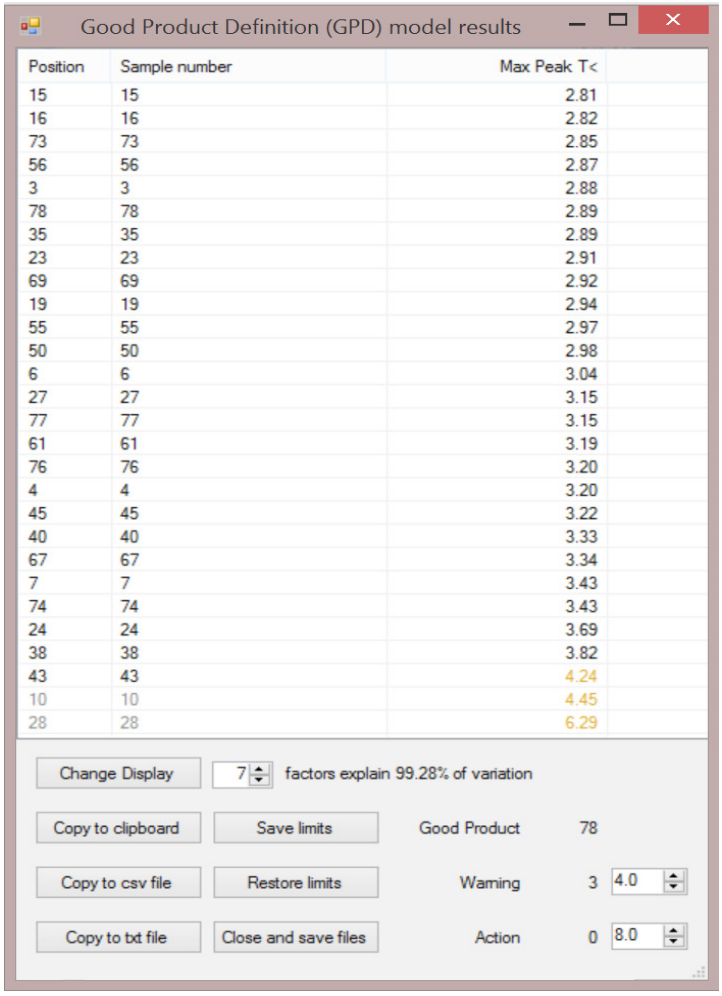

Figure 4. Output from the qualification model application.

If the number of factors in the qualification model is too low, the modelling of product will be incomplete (under-fitting) and natural sample constituents might contribute to a high Maximum Peak T value. If the number of factors is too high (over-fitting), random noise might contribute to a high Maximum Peak T value. Table 1 shows the lowest and highest Maximum Peak T values for the tuning set and the spiked tuning set. It is desirable to have the minimum spiked value larger than the maximum un-spiked value. Using the ratio of minimum spiked value divided by maximum un-spiked value, seven factors do a very good job of identifying melamine.

Factors	Tuning Set		Spiked Tuning Set	
	Min	Max	Min	Max
3	1.97	7.44	12.57	17.43
4	2.09	7.87	12.40	17.92
5	2.36	7.87	12.28	20.76
6	3.08	7.69	51.13	58.27
7	3.09	7.43	51.69	58.75
8	3.45	7.88	48.56	56.91
9	3.59	7.90	48.91	56.93
10	3.27	7.58	47.26	55.54

Table 1. Range of Maximum Peak T values for the tuning set and the spiked tuning set.

A threshold value should be chosen slightly higher than the observed un-spiked maximum. In this case, 8.0 would be an appropriate threshold – for this value the number of false positives should also be recorded for a sufficient large number of blank samples. When the seven factor qualification model is applied to the validation set, we get an un-spiked range of 2.27 – 7.79 and a spiked range of 50.74 – 59.44, clearly identifying the melamine in the spectra.

If a general screening for adulteration with NIR is to be setup it is possible in the Mosaic-Nova software to combine e.g. a) Mahalanobis distance to detect concentration outliers, b) RMS X residuals to catch broad spectral pattern outliers and c) Maximum Peak T to detect narrow and specific spectral pattern outliers.

Conclusion

With the new WinISI features, it is easy to develop qualification models for the NIRS DS2500 platform; the models are seamlessly integrated into an IQ^2 flow with *Identification* (select the right product group; i.e. skim milk powder), *Qualification* (is it 'good' product and not adulterated) and *Quantification* (prediction of protein and moisture) providing a strong tool to secure good product.

References

[1] Chemometric terminology for qualitative and quantitative analysis – IQ^2, InFocus Vol. 38, No. 1, 2014.

[2] Principal Component Analysis and Near Infrared Spectroscopy, In Focus Vol. 36, No. 1, 2012 (http://viewer.zmags.com/publication/b452b1b8#/b452b1b8/33)

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