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P/N 1026513, Issue 2, May 2014

Dedicated Analytical Solutions

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Abnormal spectrum screening is a technique employing infrared analysis technology to rapidly identify milk samples that are abnormal and are therefore relevant for further investigation.

Samples can be abnormal due to different causes. Potential adulterants might be starch, whey, water, soap, plant oil or hydrolyzed protein; substances which are added to preserve milk or to increase quantity, or accidental cleaning agents or other fluid in the line that are not normally screened. These substances are added in concentrations which are similar to milk, e.g. matching the protein content of normal milk. These substances can also be mixed to produce artificial milk with the exact same apparent protein, fat and lactose concentrations. Generally, detection of fraud and adulteration of milk is time-, cost- and labor intensive.

Why multivariate?

Differentiation between cow's milk and milk from sheep, goat or buffalo milk cannot be based on a single component such as fat or protein. This means that samples that have protein, fat and total solids concentrations within the normal limits may not be detected by the regular software. Consequently, monitoring only one constituent at a time, some abnormal samples may not be caught. However, using the Abnormal Spectrum Screening Module (ASM), a rapid screening method, milk samples can be tested for adulteration. ASM utilize the whole FT-IR spectrum to screen samples for abnormalities. This analysis demands multivariate pattern-recognition techniques due to the complexity and variability of milk samples and the potential adulterants.

Multivariate data analysis, PCA

To reduce dimensionality of the spectra, Principal Component Analysis (PCA) is applied to extract the most important information from the spectrum. The PCA model consists of a number of significant factors which describe the variability of normal samples. This model can be 'trained' to recognize normal samples within e.g. a specific region, breed or country and the model can hereafter be applied to new samples. New samples which resemble the samples included in the model are assigned as normal and samples which have a deviating spectral pattern can be assigned as abnormal (outlier).

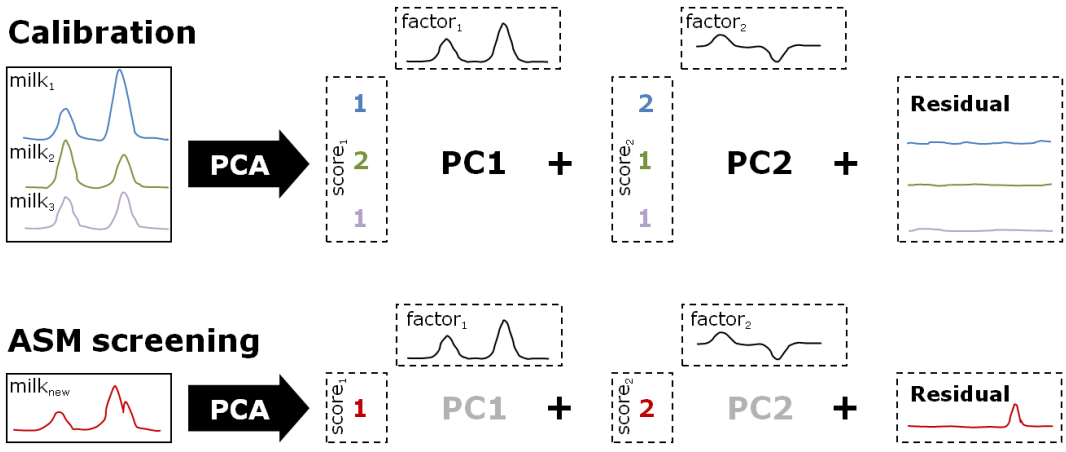

Figure 1 (1): The PCA model is based on normal samples describing the normal variation. (2): A new sample is tested how well the model describes the sample. The residual is the residue from what is not explained by the model

Abnormal samples (outliers)

When modeling normal samples with PCA, it becomes possible to recognize abnormal samples, so-called outliers. In PCA, a sample may be rejected as an outlier on the basis of its residual and Mahalanobis distance – both diagnostics are distance measures. In Figure 1, in the calibration, normal samples are modelled using a 2 factor PCA model. The residual, which is the spectral variation not described by the model, is insignificant. In ASM screening, a new sample is tested by the ASM model. The sample is poorly described by the model due to a deviating spectral pattern compared to the normal samples. The spectral residual is large. The second type of outlier includes samples which have a similar spectral pattern but have abnormal (extreme) spectral intensities. This is illustrated in Figure 2.These samples are well described by the model (low residuals) but are far from the model center, as illustrated by the outlying samples 'a' and 'b' in Figure 2. The distance to the model centre is normalized by the standard deviation from each factor giving the Mahalanobis distance. In ASM these two methods for outlier detection are combined.

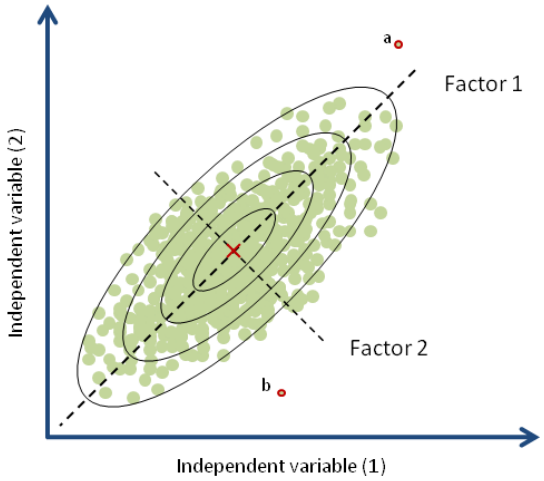

Figure 2 plot. Illustration of the first and second factor, representing maximum variation in data cloud together with Mahalanobis distances (ellipsoids). Sample 'a' and 'b' are examples of outliers

Procedure in the ASM

A spectral 'distance' between a new sample and the model based on normal samples is calculated. The residual and Mahalanobis distance of the new sample are calculated and a squared sum of the two is used as a new distance measure called score. The score will be low for samples that are very similar to the samples included in the model, and will be high for samples that differ considerably from the model samples. This score value will be compared to the selected threshold during the prediction stage.

Calibration model

To build an AMS model for screening of milk abnormality, two sets of samples must be analyzed when setting up the module; a calibration data set (unadulterated milk samples) for modeling and a validation data set for evaluating and adjusting the performance of the calibration. The calibration samples can of course not contain contaminants such as cleaning agents or adulterant since the model will be unable to discriminate between the pure and the abnormal milk samples. The selection of the calibration samples which should be included in the model has to be done with great care. These normal milk samples should include the entire range of normal incoming milk to get the most descriptive normal milk profile. From a spectroscopic point of view the definition of a

normal milk sample is influenced by many factors, incl. regional differences, seasonal variation, feeding scheme, lactation stage and herd individuality. However, it is a good idea to exclude some extreme samples (e.g. 1%) to avoid adulterated samples in the calibration set.

Number of factors

In order to optimize the sensitivity and selectivity it is crucial to select the correct number of factors in the calibration model which is set during calibration (Figure 3). The number of false positive results decreases when increasing the number of factors in the model. However, using too many factors the number of false positive results will start to increase without necessarily detecting true adulterated samples only. This occurs when the model starts to describe random noise and not systematic variation. The optimal number of factors is reached when the number of false positives reaches a minimum. Normally there is a quite wide buffer zone, before the number of false positives starts to increase. In general the number of factors should normally be between 5 and 15 to have the best model, but it heavily depends on the number of included samples, the variation between samples and the number of acceptable false positive/false negatives results.

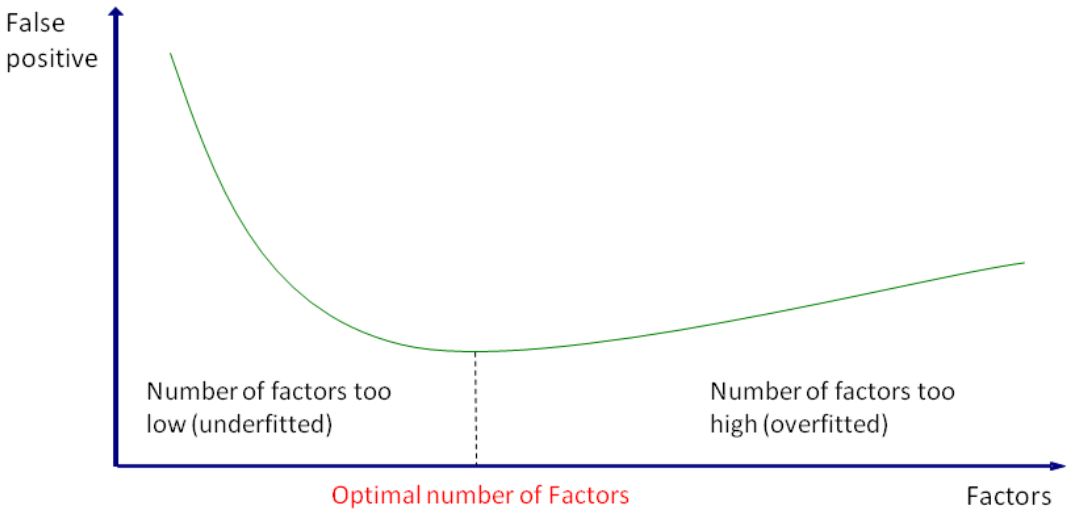

Figure 3 Increasing number of factors will decrease the number of false positive (risk of overfit). When the optimal number of factors has been reached will the number of false positives starts to increase.

Threshold

A threshold on the maximum acceptable distance between normal and abnormal samples can be defined. This threshold differentiates between the normal and the abnormal spectra. If the score is larger than the threshold, the samples are marked as 'failed'. A too narrow threshold will give more false positive results and a too wide threshold will give more false negative (adulterated samples which are identified as normal) results. The threshold should be set using a large set of normal samples ensuring that none of these come out as false positives. Results from several tests using al large set of validation samples indicate that a score threshold of 3 is optimal. However, it is possible to increase or decrease sensitivity of the model depending on the wanted specificity of the model. A threshold of 3 will reflect that approximate 99% of all samples will be graded as normal or 1% of samples will be graded abnormal Figure 4.

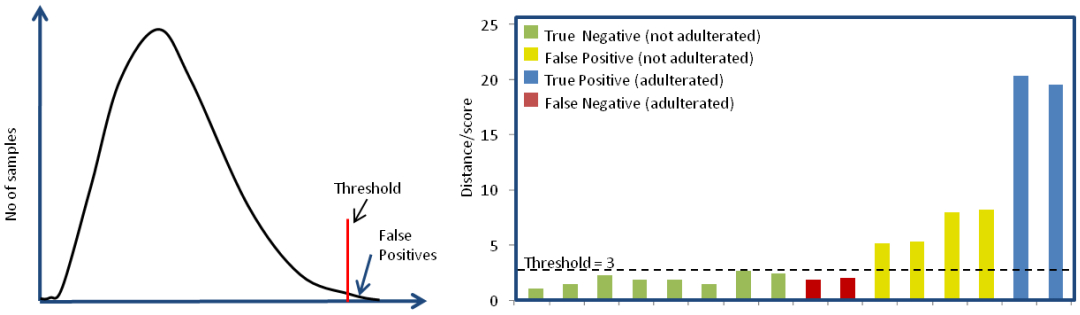

Figure 4 Left: 99% of all samples will be graded as normal (left). Right: samples classified as unadulterated and tested to be unadulterated/below threshold (green), samples classified as unadulterated but tested to be adulterated /exceeding the threshold (yellow), Samples classified as adulterated and tested to be adulterated/exceeding threshold (blue) and samples classified as adulterated and tested to be unadulterated/ below threshold (red)

Figure 4 (right) it is shown that normal samples with a score value less than 3 will be classified as true negative (not adulterated, green) and normal samples which have a score value of more than 3 are false positive (yellow) and assigned as abnormal. Adulterated samples with a score value of more than 3 are true positive (blue) and adulterated samples which have a score value of less than 3 are false negative (red).

Limit of detection (LOD)

There are different detection limits for each adulterant added to milk. The LOD is low for adulterants with large absorption in the spectrum (e.g. ammonium sulphate) and the LOD is high for adulterants with small impact on the spectrum (e.g. melamine). The LOD is even higher for adulterants which are naturally present in milk (e.g. urea). In Figure 5 (left) adulterant x and y has different detection limit. For a given detection limit, the number of detected % false positive will be less for adulterant y compared to x. Using this graph, it is possible to choose the acceptable number of false positive for each adulterant.

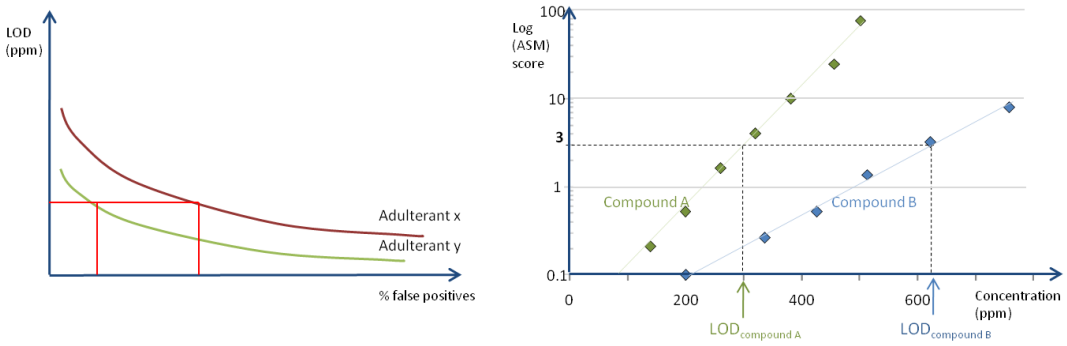

Figure 5 Log ASM score versus concentration of adulterant. The linearity makes it possible to read the LoD concentration for each compound.

Investigation shows that there are a linear correlation between the concentration of adulterant and the logarithm to the distance score. Figure 5 (right) shows two dilution series of compound A and B plotted against the logarithm of the score value for each sample. The linear correlation makes it is possible to read the detection limits of each adulterant on the x-axis. In the example in Figure 5, a threshold of 3 will give a LOD of 300 ppm for compound A and 620 ppm for compound B. Thereby the LOD of

different adulterants can be determined for different thresholds by plotting a dilution series against the score values. However, it is also possible to select a minimum LOD for known adulterants by the acceptable detection limit for lab conditions and read the threshold on y-axis. Apparent from Figure 5, in order to detect 500 ppm of compound B, the ASM threshold has to be set to 1.

Conclusion

Abnormal spectrum screening is a rapid screening method that, through the use of particle component analysis (PCA), extracts key information about potential adulterants in milk. Limit and detection settings need be set up according to requirements no matter whether you use 'out-of-the-box' calibrations or choose to make you own calibrations derived from local milk samples, as outlined in this paper.

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