

**Near Infrared Spectroscopy****Authors:**

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## DairyGuard: Augmenting Nutritional Testing of Milk Powder with Adulterant Screening

**Introduction**

Milk powder is one of the most widely traded food commodities, with over 2.5 million metric tons exported annually<sup>1</sup>, and is used in a huge

array of food products, from infant formula to baked goods and confectionary. Unfortunately, dairy products are also a frequent target of food fraud, with 137 cases of economically motivated adulteration worldwide recorded by the United States Pharmacopoeia in 2011-2012<sup>2</sup>. The value of milk powder is linked to its protein content, and standard methods for protein analysis rely on a simple nitrogen assay, with the protein concentration inferred from the nitrogen content. Consequently, the addition of chemicals rich in nitrogen can artificially increase the apparent protein and thus the price demanded.

These known incidences of economically motivated adulteration have led to strict limits on melamine content. For example, the U.S. FDA states that melamine or cyanuric acid should not be present in foods at levels greater than 2.5 ppm, or 1 ppm for infant formula<sup>3</sup>. Establishing the absence of these materials above such levels requires highly sensitive techniques such as LC/MS/MS<sup>4</sup>. While it is important to have laboratory methods with the highest possible sensitivity, often such methods are comparatively expensive and time-consuming to run and this may limit their ability to obtain representative samples. Moreover, there are two additional concerns that are specific to economically motivated adulteration. The first is that ppm-level adulteration is not economically worthwhile, so genuinely adulterated samples are likely to have higher concentrations. For example, to increase the total nitrogen in skim milk powder by 0.16% (corresponding to an apparent protein increase of 1% total mass), it is necessary to add 2400 ppm of melamine. Second, and more troubling, is that while there are published cases of adulteration with melamine, “chemical space” is vast and there are many more high-nitrogen compounds that could potentially be used in the same way<sup>5</sup>. To stay ahead of the criminals, it’s important to look beyond currently known adulterants and consider other possibilities.

For these reasons, “fingerprinting” tools that measure the response of the entire sample without separation have a very important role to play in the fight against economically motivated adulteration. Near infrared (NIR) spectroscopy, in particular, is already very widely used in food, feed and agricultural industries for quantitative analysis of nutritional and quality parameters such as protein, moisture and fat. What is less well known is that it can be a superb tool for verifying the integrity of ingredient samples in the face of potential adulteration. NIR spectra can be measured in seconds, and contain information about the whole sample –

including any adulterants present. There is no physical separation process at work, so the spectra must be processed with appropriate chemometric tools to differentiate the contributions of the milk powder matrix and any adulterants. In this note, we describe the use of the DairyGuard™ Milk Powder Analyzer and the novel Adulterant Screen™ algorithm to detect seven potential adulterants in milk powder at levels well below 1%, without any time-consuming PLS or other chemometric calibrations.

### What is Adulterant Screen?

Previous applications of NIR to adulterant detection have utilized standard chemometrics tools (Figure 1). Quantitative methods using PLS regression have been developed for melamine and shown good performance. However, such targeted methods are only applicable to the adulterants they are calibrated for, and the calibration can be a very time-consuming process, involving the preparation of dozens to hundreds of samples with precisely known concentrations of the adulterant.

An alternative approach is to use a principal components analysis (PCA)-based method such as SIMCA, in which a model is built for the unadulterated material, and the quality of match of the sample spectrum to this model is used to determine whether the result is a pass or a fail. While this approach is truly non-targeted and potentially sensitive to any adulterant, there is no indication of why a failing sample has failed (no identification of the adulterant) and, because the method makes no use of the adulterant spectrum, the sensitivity cannot be expected to be as high as for a quantitative method.

Finally, methods that rely on conventional library searching – even with multivariate algorithms – suffer from an inability to model accurately the variation in the matrix, and are often used with commercial libraries that may be of limited applicability.

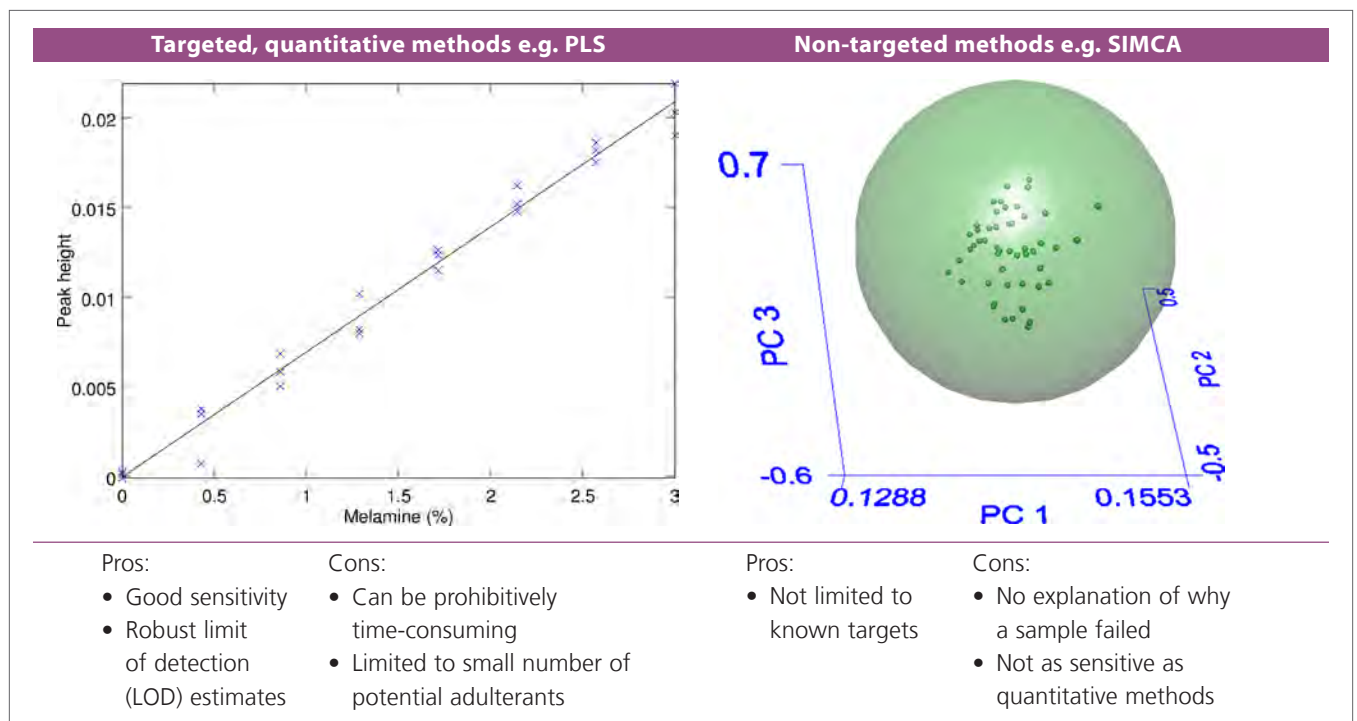


Figure 1. Schematic illustrations of existing chemometrics tools for adulterant analysis. Left: quantitative methods e.g. single peak height, or PLS regression. Right: non-targeted, factor-based methods e.g. SIMCA.

Adulterant Screen is a new algorithm designed specifically to address the problem of screening for potentially numerous adulterants in a complex matrix. It combines the generality and simplicity of non-targeted screening with some of the sensitivity benefits of a targeted approach.

### How Does it Work?

The first step is to generate a library of spectra of samples of the unadulterated material, exactly as for SIMCA. This library should span as much as possible of the natural variation of the material – due to differences between batches, suppliers or processing parameters, for example. However, these are all “normal” samples: there is no need to obtain “high-leverage” samples (e.g. those with unusual nutritional parameters), as is often the case for quantitative methods for nutritional properties. The number of spectra required depends on the complexity of the matrix and the desired sensitivity: a typical library for milk powder might contain a few dozen samples.

The second step is to prepare a library of adulterant spectra. These are spectra of the pure adulterants: there is no need to create mixtures. With DairyGuard, this has already been done: a spectral library of 19 high-nitrogen agricultural and industrial chemicals is included with the system. Adding a new adulterant to the library is as simple as measuring the pure adulterant, and then copying the spectrum to the library folder.

These two sets of spectra are registered in the software, and the method is ready to use.

### Performance Compared with SIMCA: physically Spiked Samples

Sixty-six samples of whole milk powder were prepared by spray-drying: forty-eight were used for the material library; twelve were used to prepare contaminated samples; and six used as blank controls. The following potential adulterants were used: melamine, urea, biuret, dicyandiamide, cyromazine and cyanuric acid. Each compound was ground finely then mixed thoroughly with the milk powder at concentrations of 0.2% and 2% mass. Samples prepared in a different manner (e.g. “wet blending”) may yield different results and require modified library spectra. Spectra were measured on a PerkinElmer DairyGuard Milk Powder Analyzer, which consists of a Frontier™ near-infrared (NIR) spectrometer using a NIRA II diffuse reflectance accessory. An accumulation time of 20s per sample at a resolution of 16 cm<sup>-1</sup> was used.

A SIMCA method was built using PerkinElmer AssureID™ software at the default confidence level (99%). All 48 spectra from the material library were used.

Adulterant Screen was configured with 24 of the spectra for calibration and 24 for validation (threshold setting).

The results are summarized in Table 1 (Page 4), showing that Adulterant Screen provides significantly greater sensitivity than SIMCA.

## The Adulterant Screen Algorithm

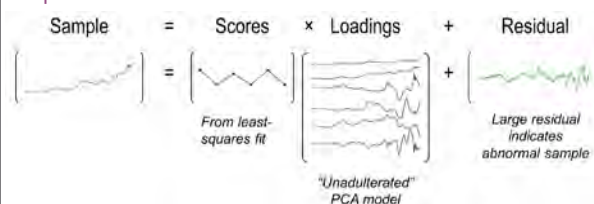
When a sample spectrum is scanned, the algorithm first compares it to a PCA model generated from the reference materials. This model is then augmented with each of the adulterant spectra in turn. If including a given adulterant in the model greatly increases the fit of the sample spectrum, it is likely that the adulterant is actually present in the sample. The algorithm also accounts for contamination with multiple adulterants, searching for every combination of up to three potential adulterants. The output of the algorithm is an estimated concentration, detection limit and confidence indicator for each adulterant in the library.

The concentration estimate is based on the relative intensity of the library spectrum of the adulterant and the amount found in the sample spectrum, without considering effective pathlength differences. As such, it is a semi-quantitative estimate. The detection limit estimate is expressed in the same terms. Finally, the reported confidence indicates the likelihood that the adulterant is actually present.

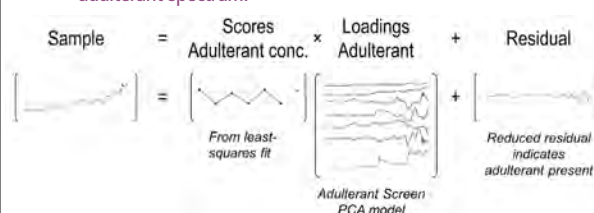
The confidence indicator and detection limit estimate are important because the sensitivity of the method is dependent on the similarity between the adulterant spectrum and the material spectrum. To validate detection limits, it is recommended to prepare a small number of samples with concentrations slightly above the estimated detection limits and verify that the adulterants are detected with high confidence.

If the sample is contaminated with some species that is not present in the library, or if the milk powder itself is a poor match to the original calibration data, the software will warn the user that unidentified components may be present.

#### Step 1. Residual calculated from standard PCA model.



#### Step 2. Residual calculated from augmented PCA model including adulterant spectrum.



Step 3. The residuals are compared against thresholds derived automatically from the validation samples. A detection limit is estimated and concentration and confidence scores assigned to the sample.

Table 1. Performance of SIMCA (with 99% confidence level) and Adulterant Screen for physically spiked whole milk powder samples. DCD = dicyandiamide; AS = Adulterant Screen. Incorrect results (false negatives) are highlighted. See the sidebar on prior page for an explanation of the detection limit and confidence estimates.

Sample	SIMCA pass?	AS pass?	AS level (%)	AS det. limit (%)	AS confidence
Blanks 1-6	Yes	Yes	Below LOD	-	-
Adulterants at 2%	No	No	All adulterants correctly identified		
Biuret 0.2%	Yes	No	0.19	0.15	Possible
Cyanuric acid 0.6%	Yes	No	0.37	0.25	Likely
Cyromazine 0.2%	Yes	Yes	0.017	0.11	Unlikely
DCD 0.2%	Yes	Yes	0.019	0.095	Very unlikely
Melamine 0.2%	No	No	0.21	0.12	Likely
Urea 0.2%	No	No	0.14	0.07	Likely

Both methods correctly recognized the blank samples as uncontaminated. SIMCA had no difficulty detecting adulteration at the percent level, and in some cases, 0.2% was sufficient to trigger a failure (melamine and urea). In terms of sensitivity, Adulterant Screen fared better, detecting cyanuric acid at 0.6% and biuret at 0.2% (but not cyromazine or DCD at 0.2%).

In addition to the improved sensitivity, Adulterant Screen also provided correct identifications for the adulterants: SIMCA merely indicated a pass or fail, without any indication as to the reason.

### Enhanced Diagnostics

Spectrum™ software provides a suite of diagnostic tools for advanced users. An Adulterant Screen results screen is shown in Figure 2 below. Note that, while adulterants are listed for the blanks, the levels and confidence values are extremely low. The residual spectrum for one of the blank samples is shown: there is no evidence of any structure that may be associated with an unmodeled component.

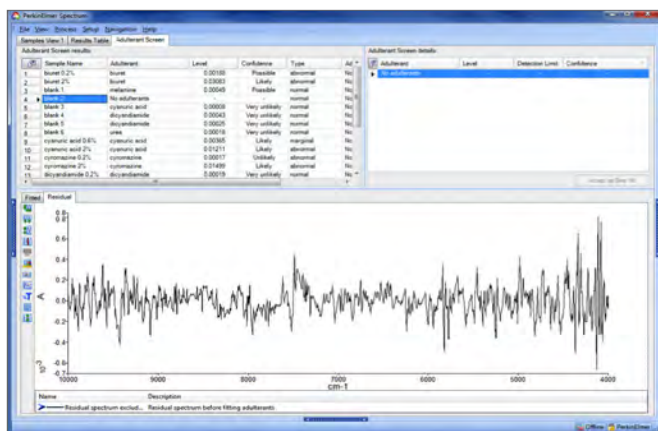


Figure 2. Typical results screen for Adulterant Screen in Spectrum Software version 10, showing the residual spectrum for an uncontaminated sample.

In comparison, the residuals from a contaminated sample (2% urea) show considerable structure (red trace in Figure 3). When the adulterant spectrum is included in the fit, most of this structure is eliminated (green trace).

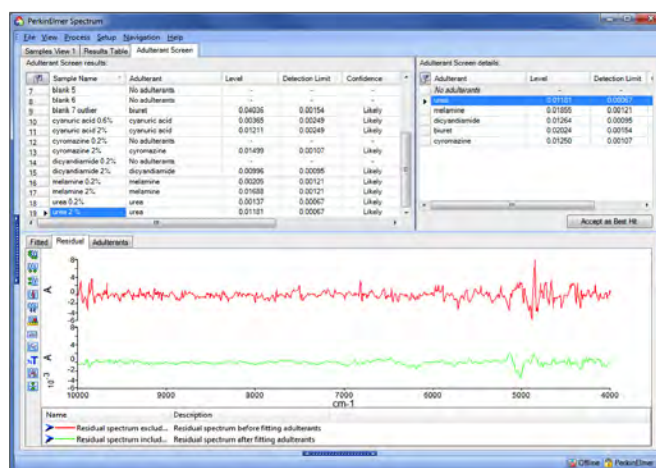


Figure 3. Residual spectra for a contaminated sample. Red trace: PCA residual, showing evidence of unmodeled components. Green trace: Adulterant Screen residual, showing a much improved fit.

Even if the structure in the residual is caused by the presence of an adulterant, it is not generally true that the structure will be recognizable as the spectrum of the adulterant. There will usually be both positive and negative spectral features in positions corresponding to absorption bands of both the uncontaminated material (milk powder) and the adulterant.

To provide a more interpretable spectrum, Adulterant Screen estimates the spectrum of the adulterant from the sample spectrum, using a least-squares fit to both the uncontaminated material and the adulterant reference spectrum. Particularly for chemical adulterants with distinctive spectra, good agreement between the extracted adulterant spectrum and the library spectrum is a strong indicator that the adulterant is really present. Conversely, if bands from the library spectrum are missing in the extracted spectrum, this may indicate that the sample is adulterated with something that is not present in the library. Figure 4 shows the extracted (black) and library (red) spectra for the 2% urea sample. Every peak in the library spectrum is matched by a peak in the estimated spectrum, so we can be confident that urea really is present in the sample.

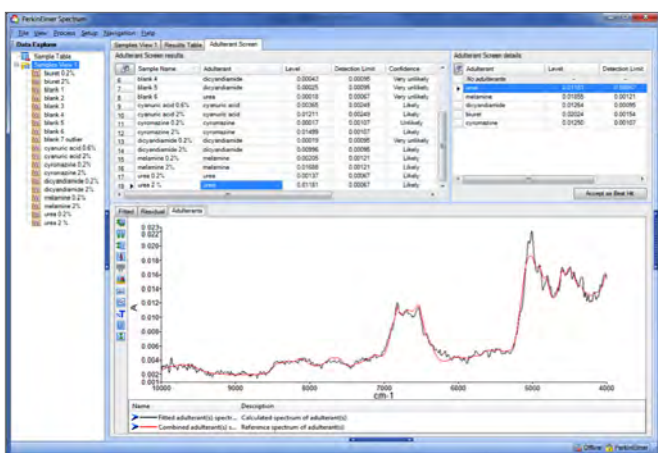


Figure 4. Estimated (black trace) and library (red trace) adulterant spectra for a sample contaminated with 2% urea.

This mode of results visualization is especially helpful when investigating results at or near the detection limit, as in the case of a sample contaminated with 0.2% melamine (Figure 5). While this sample triggered a “fail” result for both SIMCA and Adulterant Screen, indicating a need for further analysis, the Adulterant Screen result strongly indicates that melamine may be present, giving significantly more information to guide the next step to take.

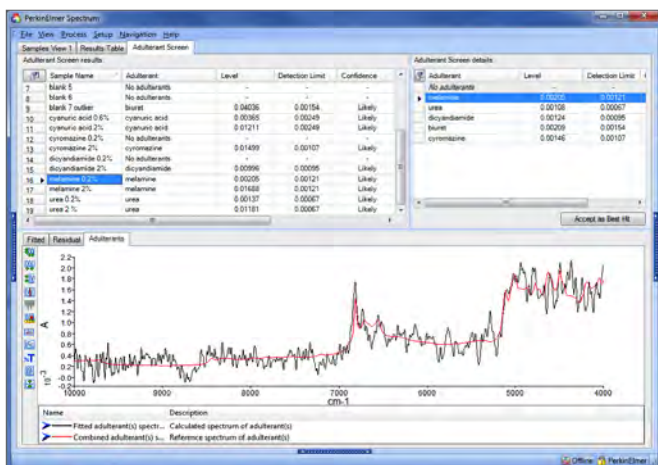


Figure 5. Estimated (black trace) and library (red trace) adulterant spectra for a sample contaminated with 0.2% melamine.

### Comparison with SIMCA: Synthetically Spiked Samples

The sensitivity of SIMCA and Adulterant Screen was further investigated using synthetically spiked spectra created by adding between 0.1% and 2.0% of each adulterant spectrum to one of the blank spectra. The spectra so produced are not equivalent to spectra of physically spiked samples, since the effective pathlength for the adulterant is dependent on the measurement matrix (i.e. either the adulterant itself or the milk powder), but do allow a comparison of the relative sensitivities of the two methods.

Table 2. Performance of Adulterant Screen compared with SIMCA (at the indicated confidence level) for synthetically spiked samples.

Adulterant	Lowest Detected Concentration (Synthetic Spiking)		
	SIMCA (99%)	SIMCA (95%)	Adulterant Screen
Biuret	>2%	>2%	0.2%
Cyanuric acid	>2%	>2%	0.3%
Cyromazine	0.6%	0.5%	0.3%
Dicyandiamide	1.4%	1.2%	0.3%
Melamine	0.7%	0.6%	0.3%
Urea	1.7%	1.4%	0.3%

Clearly, Adulterant Screen offers significantly better performance. This is because while SIMCA considers only the magnitude of the residuals, Adulterant Screen is actively searching for structure corresponding to the spectra of identified adulterant threats.

While the difference in performance on the physically spiked samples was not as dramatic, Adulterant Screen consistently outperformed SIMCA for the detection of contaminated samples.

### Integration Within a Complete Workflow

Pass/fail criteria, considering both level (concentration) and confidence, can be set, enabling Adulterant Screen to be used as part of a routine incoming-material test. The process can be incorporated as part of a Spectrum Touch™ App, enabling simple, reliable operation by non-specialist users. The DairyGuard Milk Powder Analyzer system includes the Spectrum Touch App (Figure 6) containing SIMCA and Adulterant Screen analyses as well as example quantitative methods, which can be used as a starting point for developing your own apps.

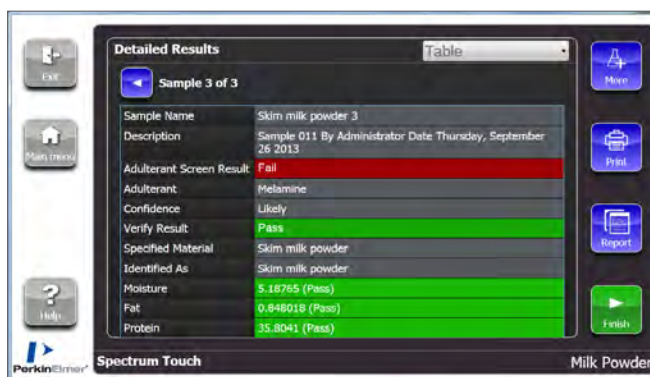


Figure 6. Results screen for the DairyGuard Touch App, showing a sample that has passed the Certificate of Analysis (COA confirmation tests and the SIMCA non-targeted screen, but failed on the more sensitive Adulterant Screen due to a low concentration of melamine.

## Summary

Adulteration of food and food ingredients for economic gain is an old practice and, sadly, one that is unlikely to be eliminated in the near future. This problem needs to be tackled with all the analytical techniques at our disposal: NIR spectroscopy clearly has a role to play, given its ubiquity in raw materials testing. The unique Adulterant Screen algorithm from PerkinElmer retains the strengths of non-targeted chemometric methods like SIMCA, but obtains greater sensitivity by utilizing a library of spectra of potential adulterants. The method can be easily adapted to screen new products or for new adulterants, without having to prepare mixture samples for calibration. The use of an adulterant library also allows much richer diagnostic information to be produced, giving much greater confidence in the results and informing the next analytical steps to take for a suspect sample. The PerkinElmer DairyGuard Milk Powder Analyzer is a complete solution pre-configured for Adulterant Screen analysis of milk powders (along with the standard quantitative analyses), with all the features integrated into a simple, touchscreen-based interface.

## References

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