# At-Site Screening and Measurement of Adulterant Levels in Bovine Milk by Mid FTIR Spectroscopy

## Application note

Food, QA/QC


**Authors**

Alan Rein, Ph.D.
Agilent Technologies, Inc.

Professor Luis Rodriguez-Saona
Food Science and Technology
Department of Ohio State University


## Introduction

Milk is a common target for adulteration, which is of significant concern to both producers and consumers. Some common milk adulterants include water, whey, sodium hydroxide, urea, melamine and other potentially harmful substances. The purpose of adulterating milk is to artificially increase the volume and/or mask inferior quality product for economic gain.

For this reason, there is significant interest in rapid, easy to use analytical methods that can detect if milk is adulterated and then measure the levels of the adulterants in milk. In a recent publication [1], researchers measured adulterants in milk in the laboratory using the Agilent Cary 630 FTIR spectrometer and showed that the mid FTIR system is superior to NIR spectroscopy for these determinations.

With the recent availability of easy-to-use, dedicated FTIR analyzers, screening milk for adulteration and then measuring the specific contaminant levels is easier and faster than with traditional analytical methods. These FTIR analyzers are designed for use in at-site production locations by less experienced personnel and thus offer the dairy industry a means to improve productivity.

### Compact, at-site FTIR systems for methods development and methods deployment in the dairy industry

Agilent now offers a family of ultra-compact FTIR spectrometers and analyzers (Figure 1), which all utilize the same optics, software and sampling technology [2]. If multi-purpose methods development and QA/QC is of primary interest, the Cary 630 FTIR spectrometer is ideal. For deployment of specific FTIR based solutions, the Agilent 5500 FTIR analyzer is an excellent platform for routine at-site analysis. Both of these systems use diamond ATR sampling technology for the analysis of solids and films; Agilent's exclusive DialPath transmission sampling technology is used for the analysis of liquid milk samples.

**Figure 1.** Agilent Cary 630 FTIR spectrometer for routine QA/QC and methods development and the Agilent 5500 FTIR analyzer for deployment of methods in at-site production labs.

This application note will describe two distinct methods for measuring adulteration in milk:

- A screening method to detect that an adulteration has taken place, using an Agilent FTIR analyzer equipped with DialPath sampling technology
- Identification and measurement of specific adulterants, using an Agilent FTIR analyzer equipped with diamond ATR sampling technology

In the screening method, the milk is measured directly with no sample preparation, leading to an extremely fast analysis. Using this technique on the dedicated Agilent 5500 or 4500 FTIR spectrometer allows for efficient screening at the point of delivery.

To get accurate identification and measurement of the adulterant, a second method is employed that uses simple sample preparation steps. This latter technique provides a fast alternative to classical analytical methods of determining milk contamination levels.

## Experimental

### Instrumentation

**Table 1.** Spectrometer parameters used for both screening and specific identification methods

| Parameter | Settings |
|---|---|
| Screening | DialPath technology 30 micron pathlength |
| Measurement | ATR technology single reflection diamond ATR |
| FTIR spectra | 64 co-added interferograms |
| Resolution | 4 $cm^{-1}$ |
| Measurement time per sample | Approx. 30 seconds |

### Materials and reagents

#### Preparation of milk standards for analysis

Commercial bovine milk samples were mixed with varying amounts of tap water, whey, synthetic milk, synthetic urine, urea and hydrogen peroxide. These materials were chosen because they are reported as common adulterants in certain countries for either increasing volume, adding nitrogen (to provide better results from Kjeldahl protein assays) or to sanitize the milk (hydrogen peroxide).

The overall dilution of the milk by adulterants was from 3 to 50% v/v. The concentrations of specific adulterants spiked into milk samples was: 1.87 – 30 g/L for whey; 0.78 – 12.5 g/L of urea and synthetic urine; 0.05 – 0.8 g/L of urea for synthetic milk and 0.009 – 0.15 g/L for hydrogen peroxide. Six lots of milk were adulterated with five levels of contaminants resulting in 30 adulterated samples per lot.

For adulerant screening, two drops each of the spiked liquid samples were measured with the DialPath sampling technology (Table 1).

For measuring each adulterant individually, the spiked samples were mixed in equal volumes with chloroform to extract the fat matrix interference. The water soluble supernatant was then applied to the diamond ATR sensor and vacuum dried to form a film (Table 1).

## Results and discussion

### Screening

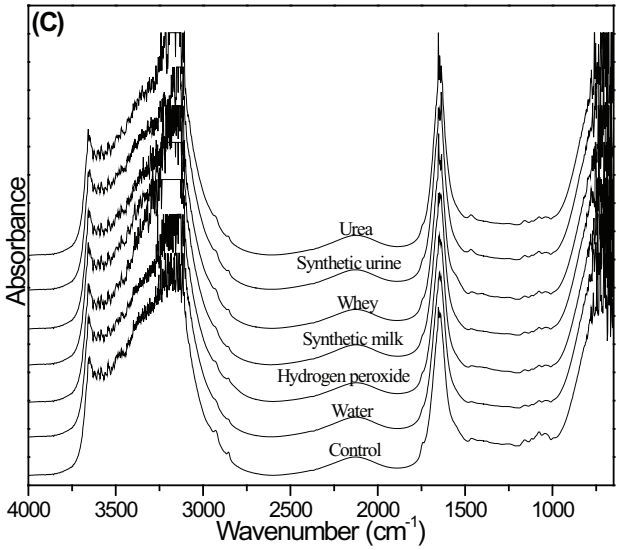


**Figure 2.** MIR spectra of diluted milk are dominated by contribution from water. Using a 30 micron pathlength allows MIR fingerprint region spectral information to be used for analysis.

The spectra of the samples are dominated by the strong absorbance from water. Whereas the O-H stretch centered at 3300 $cm^{-1}$ is fully absorbing of MIR light, the information rich fingerprint region from 2000 to 800 $cm^{-1}$ contains accessible information.

A partial least square model was developed based on overall dilution levels and good correlation was found between the infrared estimated concentrations and the level of dilution that resulted from the spiking process (Table 2).

Cleaning the DialPath sampling technology consists of simply wiping the windows in preparation for the next milk sample. This screening method takes less than two minutes from introduction of sample to clean-up, providing a highly efficient means of screening milk for dilution as a result of one or more contaminants. Agilent's Microlab software uses color coded alerts to advise the operator if the milk sample is diluted.

### Analysis of specific contaminants in milk

The dried milk film showed major differences between the control sample and the spiked samples. For example, milk spiked with whey showed two strong absorbances associated with the protein Amide I and Amide II bands at 1635 $cm^{-1}$ and 1530 $cm^{-1}$, respectively. Samples adulterated with urea, synthetic milk and urine exhibited strong bands at 1615 $cm^{-1}$ and 1454 $cm^{-1}$ arising from C=O and $NH_4^+$ absorbances.

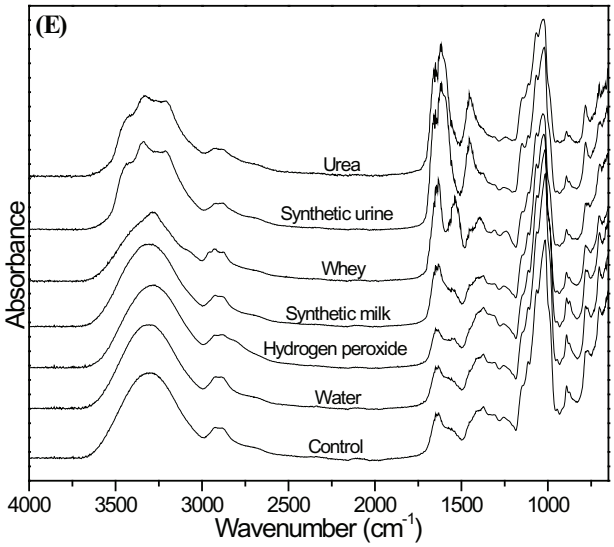


**Figure 3.** Spectra of dried milk film recorded with FTIR spectrometer using single reflection ATR

Partial least square regression analysis of the dried films (Table 3) demonstrates good correlation between the infrared estimated concentrations and the spike adulterant levels. Prediction ability of the models showed SEP values of 1.18 g/L (whey), 0.009 g/L (hydrogen peroxide), 0.028 g/L (synthetic milk), 0.412 g/L (synthetic urine) and 0.232 g/L (urea). These excellent results, as evidenced by low SEP, result from the ability to differentiate specific MIR absorbance bands for each of the adulterants used in the study.

**Table 2.** Results from PLS model correlating level of dilution with mid-infrared fingerprint region spectra

| | Number of milk lots | Number of samples | Factors | SEC | SECV | SEP | $R^2$cal | $R^2$val |
|---|---|---|---|---|---|---|---|---|
| Agilent FTIR (1300 – 950 $cm^{-1}$) | | | | | | | | |
| All adulterants | 4 | 372 | 4 | 0.74 | 0.76 | 0.83 | 0.98 | 0.98 |

**Table 3.** PLS results from spectra of dried milk measured by Agilent FTIR analyzer equipped with single reflection diamond ATR

| | Number of milk lots | Number of samples | Factors | SEC | SECV | SEP | $R^2$cal | $R^2$val |
|---|---|---|---|---|---|---|---|---|
| Whey | 5 | 90 | 5 | 1.03 | 1.16 | 1.18 | 0.98 | 0.98 |
| Hydrogen peroxide | 5 | 90 | 4 | 0.008 | 0.009 | 0.009 | 0.96 | 0.94 |
| Synthetic milk | 5 | 90 | 5 | 0.023 | 0.027 | 0.028 | 0.98 | 0.98 |
| Synthetic urine | 5 | 90 | 4 | 0.333 | 0.364 | 0.412 | 0.98 | 0.98 |
| Urea | 5 | 90 | 5 | 0.175 | 0.210 | 0.232 | 0.98 | 0.98 |

SEC and SECV values in g/L

## Conclusion

Agilent FTIR analyzers equipped with DialPath transmission sampling technology provide an easy-to-implement, rapid method for screening milk samples for tampering to as low as 3% v/v dilution. These systems equipped with single reflection diamond ATR sampling technology afford measurement of specific common adulterants in dry milk films. For laboratories located at dairy processing sites, this combination of technology and methodology offers a time and cost saving alternative to traditional methods of milk analysis.

## References


1. P. M. Santos, E. R. Pereira-Filho, L. E. Rodriguez-Saona, "Application of handheld and portable infrared spectrometers in bovine milk analysis", Journal of Agricultural and Food Chemistry, February 13, 2013, 61(6), 1205-1211.
2. "Agilent's FTIR family - lab results, anywhere you want" Agilent PN: 5991-1405EN



Agilent wishes to acknowledge Professor Luis Rodriguez-Saona's group at the Food Science and Technology Department of Ohio State University for methods, data and helpful discussion.


**www.agilent.com/chem**


Agilent shall not be liable for errors contained herein or for incidental or consequential damages in connection with the furnishing, performance or use of this material.

Information, descriptions, and specifications in this publication are subject to change without notice.

© Agilent Technologies, Inc. 2013



Published February 21, 2013
Publication number: 5991-1953EN


Agilent Technologies