SUMMARY OF LA LETTRE DE CECALAIT, N° 33 (2nd quarter 2000)

Evaluation of the Milkoscan 6000

Milkoscan 6000 is an automatic FTIR (Fourier transform infrared) analyser, developed and marketed by Foss, for analysis of fat, protein and lactose in milk. It can also measure other parameters such as urea or a freezing point equivalent (FPD). CECALAIT has recently evaluated its analytical characteristics (phase I assay, see above)

APPARATUS

Run by a Windows-based micro-computer for analyses and calibration, its analytical speed is 450 samples/h. Two different ways are available to develop the calibrations for the components.

- one is based upon the partial least squares regression (PLS) method, using the absorbances at the wavelengths usually taken in the multiple linear regression (MLR) method, ie 3 wavelengths for fat and 4 for protein (cf standard IDF 141 B: 1996). This calibration is called «traditional P.L.S. calibration» and predicts the concentration of fat, protein and lactose. The results are the same as the ones which would have been given by MLR.
- the other is based upon the partial least squares regression (PLS) method, using a set of absorbances from the spectrum of calibration samples. This calibration is called « P.L.S. Spectrum calibration» and predicts urea and FPD, but also fat, protein and lactose.

TESTS PERFORMED

Tests were performed from July to October 1999 for the following components : fat, protein and FPD.

The following characteristics were evaluated, according to IDF standard 141B :1996 and to the guidebook for infrared analysers issued in France by CNIEL (the milk payment body):

- stability
- carry-over effect
- linearity
- repeatability
- accuracy

❶ STABILITY

The stability was evaluated by the duplicate automatic analysis of a set of three milks, corresponding to the usual range of fat and protein, every 15 mn for half a day.

Whatever the calibration used, the results show that the standard relative deviation of reproducibility S_R is always lower than the value inferred by the IDF standard 141B, S_R < 0.27g/kg. For the FPD, the values are all lower than R=5 m°C, given by IDF standard 108.

❷ CARRY-OVER EFFECT

The carry-over effect was evaluated by analysing the same individual milk and distilled water, 20 times, in the following sequence : milk – milk – water* - water*.

The carry-over effect (Tc %) was estimated with following equation :

Tc % = [(S(water 1) - S(water 2))/ (S(milk 2) - S(water 2))] x 100

Tc values are in the interval of **0.39% to 0.97%.**

These values comply with the maximum limit of 1% usually allowed, for instance in routine methods of determination of milk composition, used for milk payment purposes.

NB : for conductivity reasons, water samples were spiked with 0.4 % KCl, according to the manufacturer's recommendations.

❸ LINEARITY

Linearity was evaluated for each channel by automatic analysis, in duplicate, of a set of 11 milks with :

- fat ranging from 0 to 120 g/l,
- protein ranging from 0 to 83 g/l.

The analysis followed first increasing, then decreasing fat levels. Linearity was estimated on raw data, before applying the PLS coefficients.

The results show that the manufacturer's linearity adjustement is satisfactory for the whole range of fat and protein tested. However, it should be optimized by using a 3-order polynomial, for high level milk, for instance ewe's milk at the end of lactation (fat > 100g/l).

❹ REPEATABILITY

Repeatability was evaluated by automatic analysis of 140 individual milk samples and 55 herd milks, with fat ranging from 16 to 73 g/l and protein from 26 to 48 g/l. Only individual milks were preserved with 0.02% bronopol.

Each set of 10 samples was analysed in duplicate. The stability of the analyser was checked during the tests. Both methods of calibration were used.

The results are given in table 1, page 4, in « La Lettre de CECALAIT ».

Whatever the calibration used, the repeatability values comply with IDF standard 141B specifications, ie **Sr = 0.14 g/l and r = 0.4 g/l.**

For the FPD, Sr is below the limit given for the cryoscopic reference method, Sr = 1.4 m°C.

❺ ACCURACY

Accuracy was evaluated, as in ❹, by duplicate (not consecutive) automatic analysis of :

- 112 individual milk samples, preserved with 0.02% bronopol, for milk recording purpose,
- 55 herd milks, for milk payment purpose.

Reference methods used were the official methods for milk payment, ie :

- the Gerber method for fat,
- the Amido Black method for protein,

and the cryoscopic plateau seeking freezing point determination (IDF standard 108B).

For fat and protein, the instrument was calibrated using :

- « traditional PLS » from a set of 13 recombined milk samples, following the technique described by O. LERAY (1989),
- «P.L.S. Spectrum», made by Foss, without adjustement with local milks.

« PLS spectrum » was also used for FPD.

Accuracy was estimated by using :

- the mean bias to the reference values (*moyennes des écarts*),
- the standard deviation of the differences (*écarts types des écarts*),
- the residual standard deviation (Sy,x),
- the equations of the estimated linear regressions,

Tables 2 and 3, page 5, in « La Lettre de CECALAIT » show the results on individual and herd milks.

NB : (1) the values are not reported, because the range measured was too small.
For FPD evaluation in herd milks,5 samples were spiked with water (up to 3%) to enlarge the range tested.

For fat, the mean biases are :

- **- 0.25 g/l** and **+ 0.28 g/l,** in « traditional PLS calibration »,
- + **1.07 g/l** and **+ 0.98 g/l**, in « PLS Spectrum calibration »,

respectively for individual and herd milks.

The slopes are not significatively different from 1.00 for herd milks, but are so, whatever the calibration, for individual milks. The residual standard deviations are :

- **0.667** and **0.292,** in « traditional PLS calibration »,
- **0.654** and **0.282**, in « PLS Spectrum calibration »,

respectively for individual and herd milks.

For protein, the mean biases are :

- **- 0.01 g/l** and **– 0.08 g/l,** in « traditional PLS calibration »,
- **+ 0.94 g/l** and **+0.91 g/l**, in « PLS Spectrum calibration »,

respectively for individual and herd milks.

The slopes are significatively different from 1,00 whatever the calibration, for individual milks, and for herd milks using « PLS Spectrum ». It is not significatively different from 1,00 for herd milks, using « traditional PLS ». The residual standard deviations are :

- **0.340** and **0.132,** in « traditional PLS calibration »,
- **0.346** and **0.124**, in « PLS Spectrum calibration »,

respectively for individual and herd milks.

However, these deviations remain very small and still comply with users wishes.

For FPD, the residual standard deviation is about **6 m°C,** for individual milks and about **2.8 m°C,** for herd milks. So the estimation precision for these milks is about **± 5.6 m°C**.

In conclusion, for fat and protein, the analytical characteristics of Milkoscan 6000 comply with the limits fixed in IDF standard 141, ie residual standard deviation of 1.0 g/kg for individual milks and 0.7 g/kg for herd milks.
In «traditional PLS» calibration, the bias is slightly over +/- 0.15 g/kg for fat. It may come from the delay –about a month- between the preparation of the calibration samples and the analysis of herd milks. In « PLS Spectrum » calibration, mean biases are higher. This may come from the manufacturer's calibration, which was performed without local milks. A new calibration using local milk samples, as required in IDF standard 128 is recommended for fat, protein and FPD.

For FPD, the accuracy found here complies with the values specified by FOSS, ie Sy,x < 4 m°C for herd milks.

❻ GENERAL CONCLUSION

The analytical characteristics of Milkoscan 6000 : instrumental stability, carry-over effect, linearity, repeatability, accuracy, have all been found satisfactory. They comply with the requirements of milk payment purposes.
For FPD, using Milkoscan 6000 may be an economical way to screen milks before performing a cryoscopic analysis.

For abbreviations and bibliography, please, see pages 6 & 7 in La Lettre de CECALAIT