EVALUATION OF THE BENTLEY FTS® INFRARED ANALYSER

FTS, manufactured by Bentley Instruments (US) and commercialised by Bentley Instruments SARL in Western Europe, is a mid-infrared spectrophotometer (2-10 µm) for the determination of the different components in milk samples.

This instrument uses a high resolution industrial infrared spectrometer based on Fourier transform (FTIR). The interferometer is referenced by a laser and placed in an anti-vibration, temperature regulated airtight enclosure. The complete infrared spectrum is collected and recorded for each sample. Using a MLR or PLS calibration, and with a high work rate (500 samples / hour), standard components (fat, protein and lactose) and other criteria such as the freezing point (FPD) can be determined.
The apparatus is connected to a computer (software under Windows) that ensures the running of the instrument and the signal treatment.

ACTILAIT-CECALAIT evaluated the analytical and instrumental characteristics of the instrument for the determination of fat, protein and "freezing point". Its basic characteristics: instrumental stability and tracing appear satisfactory. Its linearity is also accurate for the standard concentration ranges. The repeatability and accuracy values, for individual and herd milks are in conformity with the regulatory and normative requirements.

The tests:
The evaluation tests were performed in ACTILAIT – CECALAIT's physico-chemistry laboratory (reference and infrared analyses) from August to October 2008 and concerned fat (equivalent fat filter B), protein (MP) and freezing point (FPD). The stability of the instrument, the contamination between samples, the linearity, the repeatability and the accuracy (MLR calibration) were evaluated.

The appreciation criteria of the estimated parameters were taken from ISO 9622 / IDF 141C:2000 "Guide for the operation of mid-infrared instruments", or from the CNIEL/IE handbook concerning the use of infrared apparatus within the context of milk payment and milk control in France.

The following instrumental parameters were used:
- rate: 500 samples / hour;
- no correction of contamination;
- functioning in combined mode with the FCM cell counter (purge assistance).

1- EVALUATION OF STABILITY

1.1- Procedure

The stability was evaluated by the analysis, in automatic mode, of 4 samples of milk in duplicate every 15 minutes for half a working day, representing 14 measurement cycles.
To evaluate the stability of the instrument, the repeatability and reproducibility were calculated for each analytical criterion and by level (average content of Fat: 20.19; 41.18; 63.38; 83.64, Protein: 20.37; 30.19; 39.65; 59.66, and FPD: 514.5; 516.1; 514.6; 514.6).

1.2- Conclusion

The average daily values of standard deviation of reproducibility SR observed for fat and protein were 0.16 and 0.14 g/L respectively, which is below the limits required in ISO 9622 / IDF 141 standard (SR < L / 2.58 → 0.27 g/kg ; L = limit of control card at 99 % equal to 0.7 g/kg).

As no standardised values exist or in the absence of values in the CNIEL handbook for FPD, it can be noted that the reproducibility values obtained are lower than the standardised value of the reference method ISO 5764 / IDF 108:2003 (R = 6 m°C. → SR lower than 2.3 m°c).

2- EVALUATION OF CONTAMINATION BETWEEN SAMPLES

2.1- Procedure

This criterion was evaluated in automatic analysis mode, by analysing the same cow milk and distilled water according to the sequence: MILK – MILK - WATER - WATER repeated twenty times for the criteria: fat, protein and FPD. The evaluation was carried out on 4 different fat and protein content: (20, 20) for milk 1; (40, 30) for milk 2; (60, 40) for milk 3 and (80, 60) for milk 4.

The contamination level was estimated by the formula:
Tc (%) = [(Σ(Water 1) - Σ(Water 2)) / (Σ(Water 2) - Σ(Water2))] x 100

2.2- Conclusion

The average contamination level for fat, protein and FPD between successive samples is 0.36%, 0.24% and 0.43% respectively, which is lower than the 1% acceptability limit relative to rapid methods for the determination of milk composition for milk payment and milk control. The contamination level also complies with the manufacturer's specifications: Tc<0.5 %.

3- EVALUATION OF LINEARITY

In all cases, volume/volume dilutions were carried out by corrected weighing of density. This corresponds to the principle of quantitative analysis of infrared spectrophotometry and to the French reference measurements. The FTS instrument was calibrated and aligned by the constructor using only CECALAIT's median and high calibration ranges, i.e. from 22 to 92 g/l for fat and from 24 to 66 g/l for protein.

3.1- Fat

3.1.1- Procedure

A range of 14 milk samples from 0 to 125 g/l was prepared by mixing cream and skimmed milk. The range was analysed in automatic mode, in duplicate, in increasing and then decreasing order of fat content.

3.1.2- Results

The Ar/At ratio (Ar and At: amplitude of residues and amplitude of content respectively) is equal to 2.3 %, that is higher than the limit of 2% expressed in ISO 9622 / IDF 141C standard. However, a linear regression in the range from 0 to about 100 g/l enables a linear section to be characterised. Within this range, the Ar/At ratio is equal to 0.6 %, which corresponds to the recommendations of the standard.

3.2- Protein

3.2.1- Procedure

A range of 14 milk samples from 0 to 85 g/l was prepared by mixing the proteic retentate and filtrate obtained by tangantial ultrafiltration (cutoff threshold: 10KD). The range was analysed in automatic mode, in duplicate, in increasing and then decreasing order of protein content.

3.2.2- Results

The Ar/At ratio within the range of concentrations studied is equal to 0.4%, which is in conformity with the recommendations of 2% maximum given in ISO 9622 / IDF 141C standard.

3.3- Conclusion

The linearity of the instrument is satisfactory for fat (0-100 g/l) and protein (0-85 g/l) content. For fat, an adapted mathematical adjustment would be necessary when using this instrument outside this range, either over the entire range studied (0 to 120 g/L) or over a "high" content range, which corresponds to ewes' milk.

4- EVALUATION OF THE CALIBRATION

4.1- Procedure

The evaluation of the calibration for fat and protein, initially installed by the manufacturer, was performed with 13 commercial "median" and "high" infrared standard reference materials (SRM) produced by Actilait-Cecalait in September 2008. Each sample was analysed in duplicate.

4.2- Results

The table below presents the results obtained:

	N	Min-max	Sr	d	Sd	Sl1	Sl3
Fat (g/l)	13	22-53	0,05	0.31	0.15	0.15	0.10
Protein (g/l) Median	13	24-40	0,06	0.21	0.10	0.10	0.08

Table 1: FTS Calibration parameters for fat and protein
N: number of standards, min and max: minimum and maximum values, Sr: standard deviation of repeatability , d and Sd: mean and standard deviation of deviations (instrument –reference), Sl1 and Sl3: residual standard deviation of simple linear regression (reference vs instrument) or multiple (reference vs MG, MP and lactose).

It can be noted that the residual standard deviation of regression is close to the standard deviation of deviations. The residual interactions are not significant.

4.3- Conclusion

The residual standard deviations of the linear regression of 0.15 g/L and 0.10 g/L obtained for fat and protein are in agreement with the recommendations of the CNIEL/IE handbook (below 0.20 and 0.15 g/l respectively), which corresponds to the content in cows' milk.

5- EVALUATION OF REPEATABILITY AND ACCURACY

5.1- Samples

The tests were performed on 125 samples of individual milk from 4 farms in the Jura and 80 samples of herd milk from the Franche-Comté region. Bronopol was added to the samples to give a final concentration of 0.02%.

5.2- Repeatability

5.2.1- Procedure

The repeatability of the instrument was evaluated using all the milk samples (individual and herd milk) for fat and protein, and using herd milk samples for FPD. The quantitative analyses were performed in automatic analysis mode, in duplicate for each set of 10 samples according to the following sequence: (Set 1 rep 1 - Set 1 rep 2 - Set 2 rep 1 - Set 2 rep 2 … Set n rep 1 - Set n rep 2). A control milk was analysed every 30 samples to verify the stability of the analyser.

5.2.2- Results

5.2.2.1- Individual milk

The table below presents the results obtained:

	n	min	max	M	Sx	Sr	Sr (%)	r
Fat (g/l)	125	15.7	50.1	35.13	5.93	0.047	0.13	0.13
Protein (g/l)	125	27.4	41.5	33.83	2.80	0.076	0.22	0.21

Table 2: FTS repeatability parameters for fat and protein in individual milk
n: number of results; min and max: minimum and maximum values, M and Sx: mean and standard deviation of the results; Sr and Sr%: absolute and relative standard deviation of repeatability; r: maximum deviation of repeatability in 95% of cases.

5.2.2.2- Herd milk

	n	min	max	M	Sx	Sr	Sr (%)	r
Fat (g/l)	80	34.6	45.4	39.64	2.00	0.084	0.21	0.23
Protein (g/l)	74	32.4	38.9	35.77	1.65	0.085	0.24	0.24
FPD (m°c x-1)	80	481	522	515.5	5.8	1.40	0.27	3.88

Table 3: FTS repeatability parameters for fat and protein in herd milk
n: number of results; min and max: minimum and maximum values, M and Sx: mean and standard deviation of the results; Sr and Sr%: absolute and relative standard deviation of repeatability; r: maximum deviation of repeatability in 95% of cases.

5.2.3- Conclusion

For fat and protein content and for both types of milk, FTS presents a standard deviation of repeatability (Sr) of 0.065 g/L and 0.08 g/L respectively, in accordance with the recommendations of ISO 9622/IDF 141 C: 2000 standard and the CNIEL/IE handbook (Sr ≤ 0,14 g/l

and r ≤ 0,4 g/l.). Concerning the freezing point (FPD), the standard deviation of repeatability (Sr) obtained is in accordance with the recommendations of the CNIEL/IE handbook (Sr ≤ 2 m°c → r ≤ 5.5 m°c).

5.3- Accuracy

5.3.1- Procedure

The accuracy of the analyser was evaluated using all the milk samples (individual and herd) for fat and protein, and using the herd milk samples for FPD. The quantitative analyses were performed in accordance with the evaluation of repeatability (cf. 5.2.1). For fat and protein, the evaluation concerns the values obtained after calibration of the instrument with commercial SRMs produced by ACTILAIT-CECALAIT (cf §4). For FPD, the instrumental values are from a calibration carried out by the manufacturer.

The following reference methods were used:
- Fat**:** Gerber acido-butyrometric method according to NF V 04-210 (single test and then confirmation if more important residues for the individual milk samples).
- Protein: Amido black method according to NF V 04-216 (test in duplicate).
- Freezing point: Thermistor cryoscopic method according to ISO 5764/IDF 108 (single test).

5.3.2- Results

5.3.2.1 - Fat

The following table and figures present the results obtained:

	INDIVIDUAL MILK	HERD MILK
n	118	79
min (g/l)	15.7	34.6
max (g/l)	47.9	45.4
Y (g/l)	35.24	39.70
X (g/l)	35.03	39.68
Sy (g/l)	5.85	1.90
d (g/l)	-0.21	-0.01
Sd (g/l)	0.38	0.34
Sy,x (g/l)	0.378	0.324
b	1.010	0.949
a	-0.14	2.03

Table 4: FTS accuracy parameters for fat
n, min, max: number of results, minimum and maximum values; Y,X: mean of the results using the reference and instrumental methods; Sy: standard deviation of the results from the reference method; d, Sd: mean and standard deviation of deviations; Sy,x: residual standard deviation; b, a: slope and intercept of the linear regression.

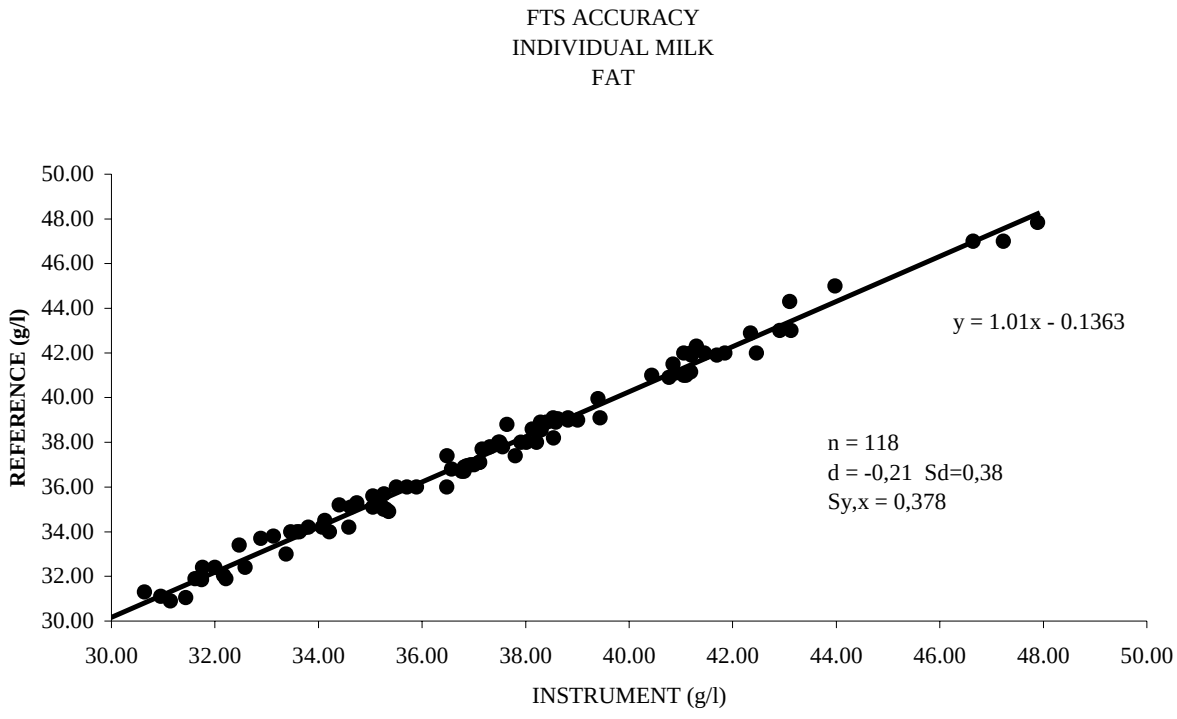

Figure 1: Relation between FTS and reference results for fat in individual milk

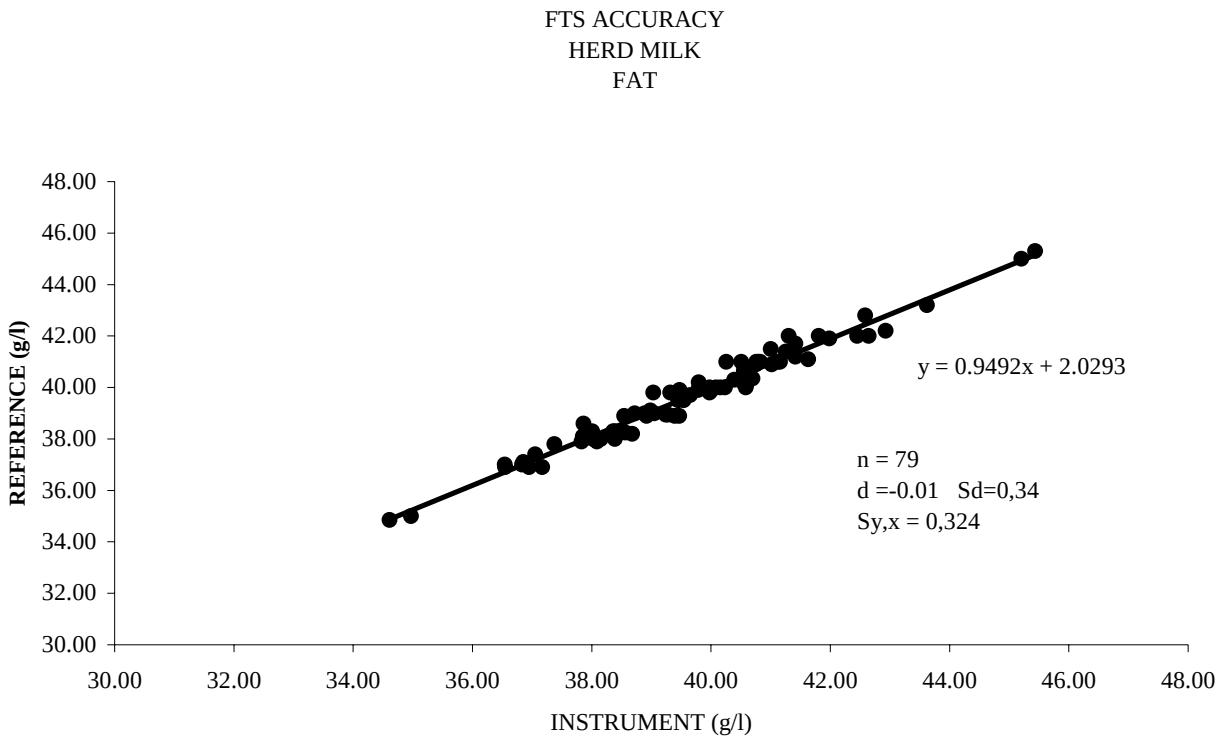

Figure 2: Relation between FTS and reference results for fat in herd milk

It can be noted that:

- Individual milk: mean = –0.21g/l and standard deviation of deviations = 0.38 g/l. The regression slope (-0.14) obtained is not significantly different from zero (P=1%). The residual standard deviation of regression is equal to 0.378 g/l.

- Herd milk: mean = -0.01 g/l and standard deviation of deviations = 0.34 g/l. The regression slope obtained is significantly different from 1.00 (P = 5%) and the intercept is significantly different from zero (P=1%). The residual standard deviation of regression is equal to 0.324 g/l.

5.3.2.2- Protein

The following table and figures present the results obtained.

	INDIVIDUAL MILK	HERD MILK
n	120	74
min (g/l)	27.4	32.4
max (g/l)	41.5	38.9
Y (g/l)	33.88	35.72
X (g/l)	33.79	35.77
Sy (g/l)	2.80	1.65
d (g/l)	-0.09	0.05
Sd (g/l)	0.35	0.22
Sy,x (g/l)	0.349	0.218
b	0.995	0.990
a	0.24	0.32

Table 5: FTS accuracy parameters for protein

n, min, max: number of results, minimum and maximum values; Y,X: mean of the results using the reference and instrumental methods; Sy: standard deviation of the results from the reference method; d, Sd: mean and standard deviation of deviations; Sy,x: residual standard deviation; b, a: slope and intercept of the linear regression.

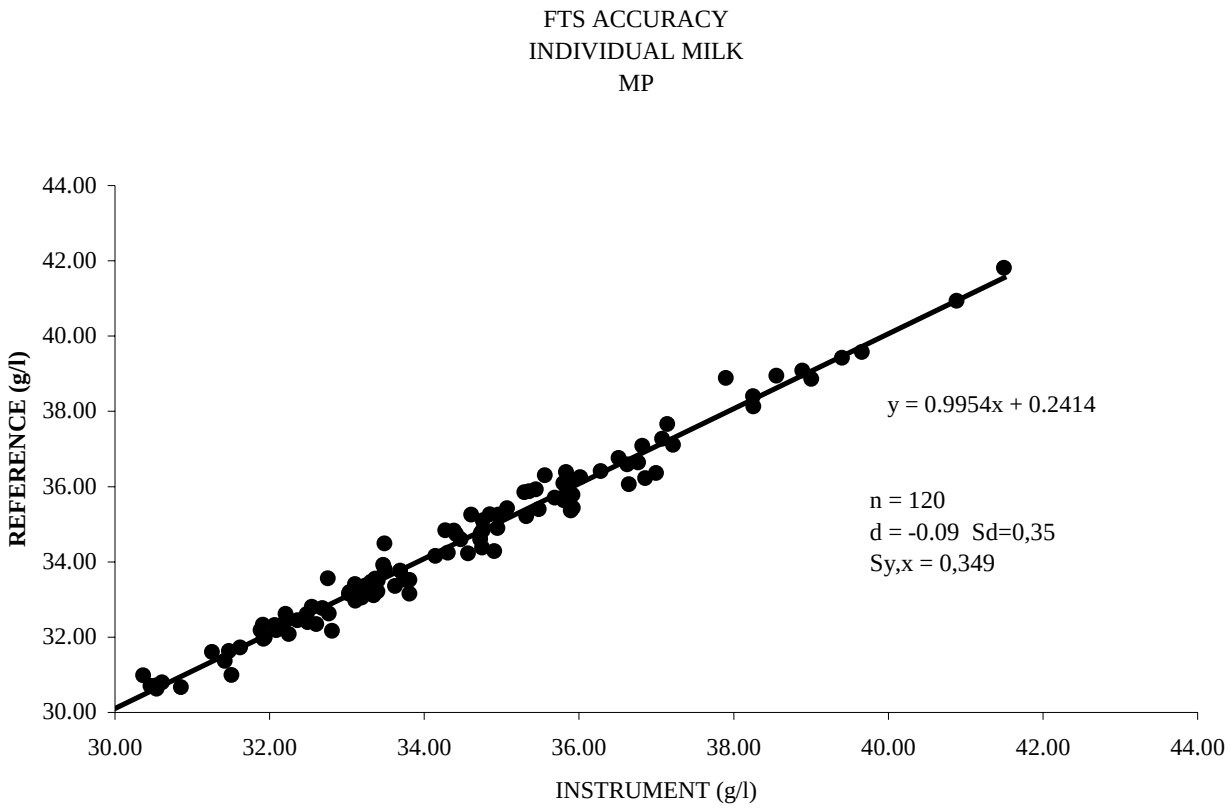

Figure 3: Relation between FTS and reference results for protein in individual milk

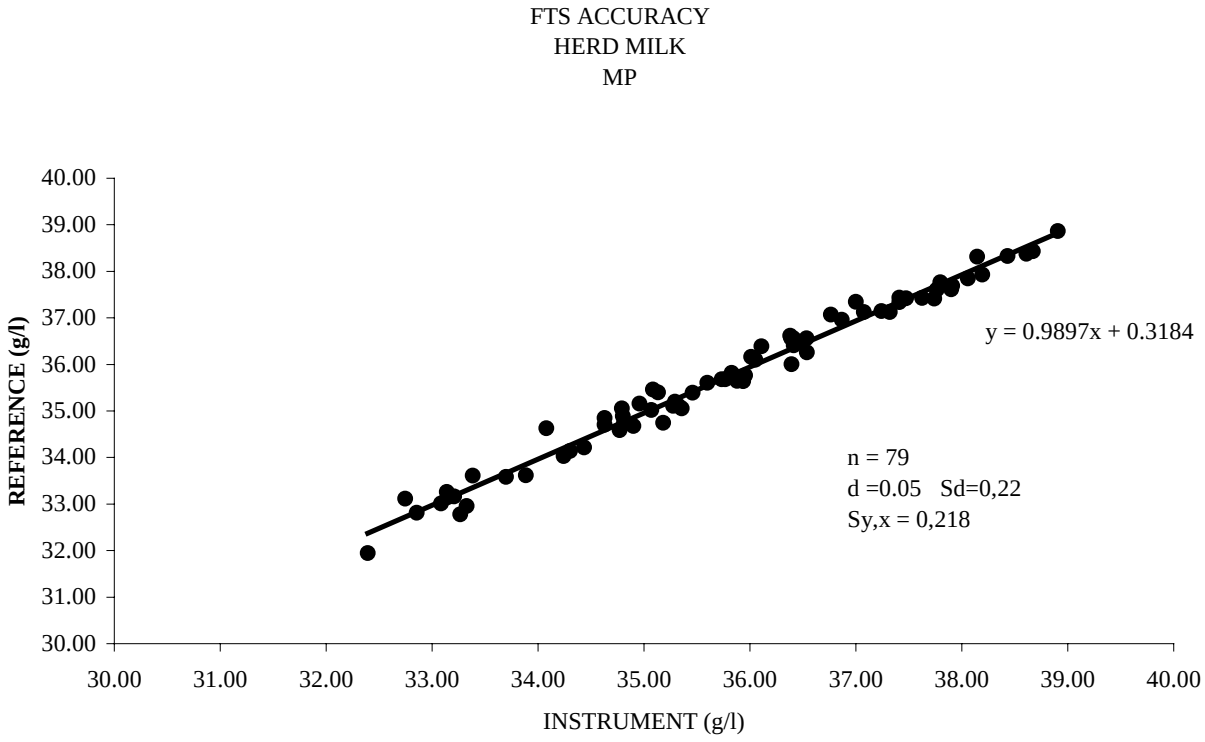

Figure 4: Relation between FTS and reference results for protein in herd milk

It can be noted that:

- Individual milk: mean = -0.09 g/l and standard deviation of deviations = 0.35 g/l. The regression slope obtained is not significantly different from 1.00 (P = 5%) and the intercept is significantly different from zero (P=1%). The residual standard deviation of regression is equal to 0.349 g/l.

- Herd milk: mean = 0.05 g/l and standard deviation of deviations = 0.22 g/l. The regression slope obtained is not significantly different from 1.00 (P = 5%) and the intercept is significantly different from zero (P=1%). The residual standard deviation of regression is equal to 0.218 g/l.

5.3.2.3- FPD

The table and figure below present the results obtained.

	HERD MILK
n	80
min (m°C x-1)	481
Max (m°C x-1)	522
Y (m°C x-1)	521.1
X (m°C x-1)	515.5
Sy (m°C)	5.81
d (m°C x-1)	-5.7
Sd (m°C)	3.1
Sy,x (m°C)	3.03
b	0.853
a	81.5

Table 6: FTS accuracy parameters for FPD

n, min, max: number of results, minimum and maximum values; Y,X: mean of the results using the reference and instrumental methods; Sy: standard deviation of the results from the reference method; d, Sd: mean and standard deviation of deviations; Sy,x: residual standard deviation; b, a: slope and intercept of the linear regression.

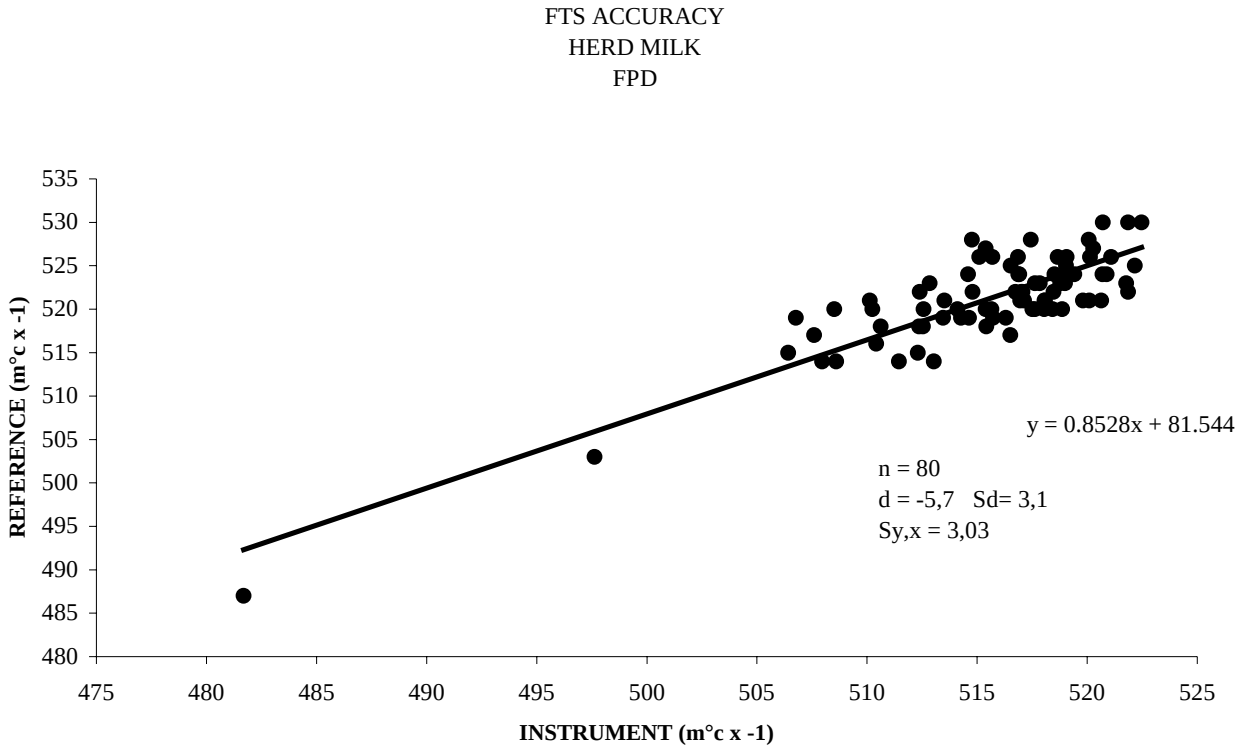

Figure 5: Relation between FTS and reference results for FPD in herd milk

It can be noted that the mean and the standard deviation of deviations are –5.7 (m°C x –1) and 3.1m°C respectively. The regression slope obtained is significantly different from 1.00 (P = 1%) and the intercept is significantly different from zero (P=1%). The residual standard deviation of regression is equal to 3.03 m°C.

5.3.3- Conclusion

Concerning fat, for herd milk samples, the mean deviation and the standard deviation of deviations obtained is in accordance with the recommendations of ISO 9622/IDF 141 C:2000 standard (limits of 0.23 g/l and 0.7 g/l respectively). For individual milk samples, the mean deviation is slightly higher than the tolerance, whereas the standard deviation of deviations is conform (limits of 0.18 g/l and 1 g/l respectively). This exceedance in mean deviation is probably linked to the detailed composition of milk samples taken into account in the validation group.
Concerning protein, for individual and herd milk samples, the means and standard deviation of deviations obtained are in accordance with the recommendations of ISO 9622/IDF 141 C:2000 standard.
Concerning FPD, the standard deviation obtained is 3.0 m°C which enables an accuracy of estimation of +/- 6.0 m°C. The results obtained are in accordance with the specifications of the manufacturer BENTLEY INSTRUMENTS (Sy,x lower than 4 m°C).

GENERAL CONCLUSION

The results obtained concerning fat, protein and freezing point using the Bentley FTS instrument are in conformity with the recommendations of the ISO 9622/IDF 141 C:2000 standard "Guide for the operation of mid-infrared instruments", and the CNIEL/IE handbook for use of infrared instruments within the context of milk payment and milk control in France.

References :

- *Report of evaluation of infrared BENTLEY FTS® analyser - X. QUERVEL, Ph. TROSSAT – Actilait / Cecalait – November 2008.*

- *ISO 9622 / IDF 141C: 2000 standard: Whole milk – Determination of milkfat, protein and lactose content - de la teneur en matière grasse laitière, en protéines et en lactose – Guide for the operation of mid-infrared.*

- *ISO 5764 / IDF 108: 2003 standard: Milk – Determination of freezing point – Thermistor cryoscope method (reference method).*

- *CNIEL/IE handbook for the use of the infrared instruments within the context of milk payment and milk control in France.*

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