ARTICLE

EVALUATION OF THE ADVANCED LACTOSCOPE FTIR™ ANALYSER

The LactoScope FTIR™ is a mid infrared spectrophotometer manufactured by Delta Instruments (Netherlands, Advanced group) and commercialized in France by Humeau Laboratories. It is used for the determination of the principal components in milk and cream.

This instrument uses a mono-bundle Fourier transform-based infrared system (FTIR). The apparatus is connected to a computer that ensures the running and the signal treatment. Two mathematical calculations can be carried out: traditional PLS (MLR) and PLS.

Consumables:

The consumables used are:

- "Zero" solution: water + 0.1% triton
- Clean solution: Decon 90™ 4% aqueous solution.

The tests:

The evaluation tests were performed in ACTILAIT – CECALAIT's physico-chemistry laboratory (reference and infrared analyses) in August and September 2009. These tests concerned the following criteria:

- Preliminary linearity and calibration tests on raw milk: fat, equivalent Filter B (MGb), protein (MP) and dry matter (MS);
- Evaluation of repeatability and accuracy on raw milk: fat, equivalent Filter B, protein, dry matter and freezing point (FPD);
- Evaluation of repeatability and accuracy on cream: fat (MG).

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:
- Manual tests at a rate of about 120 samples / hour;
- No correction of contamination;
- MLR calibration for milk;
- PLS calibration for cream.

A- PRELIMINARY TESTS

A-1- EVALUATION OF LINEARITY

In all cases, volume/volume dilutions were carried out by calibrated weighing of density. This corresponds to the principle of quantitative analysis of infrared spectrophotometry and to the reference measurements in France.

A-1.1- Fat

A-1.1.1- Procedure

A range of 14 milk samples from 0 to 120 g/l was prepared by mixing cream and skimmed milk. The range was analysed in duplicate, in increasing order of fat content.

A-1.1.2- Results

The linearity of the response of the instrument is illustrated in figure 1. The distribution of the deviations from linearity according to the theoretical dilutions of cream and skimmed milk can be observed.

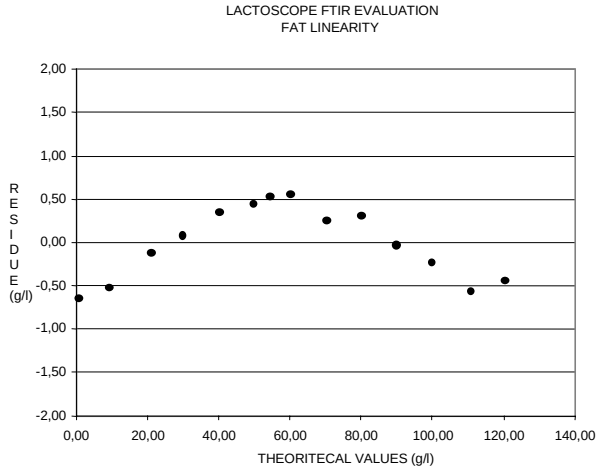
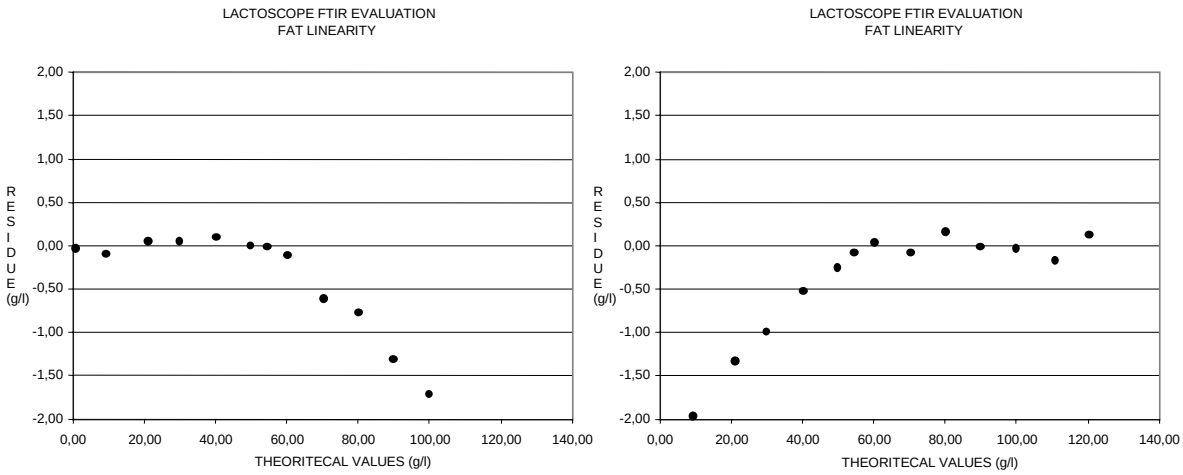


Figure 1: Linearity of LactoScope FTIR for MGb parameter (linear regression 0-120 g/l)

The Ar/At ratio (Ar and At: amplitude of residues and amplitude of content respectively) is 1.0 %, which is in compliance with the 2% limit laid down in ISO 9622 / IDF 141C standard. The ratios of 0.4 and 0.7% were obtained for the specific linear regressions carried out on the ranges 0 to 60 g/l and 60 to about 120 g/l respectively. (figures 2 and 3).



Figures 2 and 3: Linearity of the FTIR LactoScope for fat b parameter (linear regressions 0-60 g/l and 60-120 g/l)

A-1.2- Protein

A-1.2.1- Procedure

A range of 14 milk samples from 0 to 120 g/l was prepared by mixing the proteic retentate and filtrate obtained from tangantial ultrafiltration (cutoff threshold: 10KD). The range was analysed in duplicate, in increasing order of protein content.

A-1.2.2- Results

The linearity of the response of the instrument is illustrated in figure 4. The distribution of the deviations from linearity according to the theoretical dilutions of retentate and filtrate can be observed.

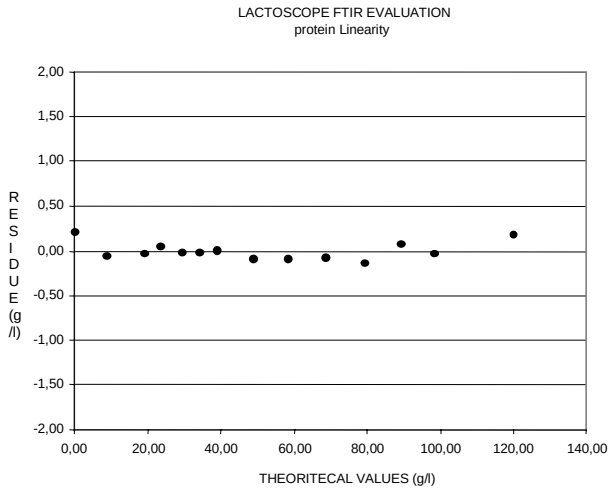


Figure 4: Linearity of FTIR LactoScope for protein parameter (linear regression 0-120 g/l)

The Ar/At ratio in the range of concentrations studied is 0.3%, which is in compliance with the recommendations of maximum 2% laid down in ISO 9622 / IDF 141 standard.

A-1.3- Conclusion

The linearity of the instrument is satisfactory for fat (0-120 g/l) and protein (0-120 g/l) content. For fat, two distinct linearity ranges can be observed: 0 to about 60 g/l and 60 to about 120 g/l.

A-2- EVALUATION OF THE CALIBRATION

A-2.1- Procedure

The calibration for fat and protein initially installed by the manufacturer was evaluated in August 2009 using 13 commercial "median" and "high" infrared standard reference materials (SRM) commercialized by Actilait-Cecalait. Each sample was analysed in duplicate.

A-2.2- Results

The results are shown in the table below:

	N	Min-max	Sr	d	Sd	Sl1	Sl3
Fat b (g/l)	13	22-53	0.02	-0.23	0.18	0.10	0.09
Protein (g/l)	13	24-40	0.07	1.97	0.21	0.04	0.04
Dry matter (g/l)	13	103-151	0.16	-6.85	0.52	0.53	/

Table 1: calibration criteria of FTIR LactoScope for fat b, protein and dry matter parameters

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 for fat and protein, the residual standard deviations of regression (Sl1) are low. The residual interactions are not significant.

A-2.3- Conclusion

The residual standard deviations of regression obtained for fat and protein are very satisfactory. They are in agreement with the recommendations in the CNIEL/IE handbook (below 0.20 and 0.15 g/l respectively, corresponding to the content in cows' milk). This handbook is addressed to interprofessional milk payment and milk control laboratories.

As no standard criteria exist for dry matter, a residual standard deviation of 0.53 g/l can be observed.

B- EVALUATION OF REPEATABILITY AND ACCURACY

B-1- RAW MILK

B-1.1- Samples

The tests were performed on 40 samples of herd milk from the Franche-Comté region. Bronopol was added to the samples to give a final concentration of 0.02%.

B-1.2- Repeatability

B-1.2.1- Procedure

The repeatability of the instrument for fat, protein, dry matter and freezing point was evaluated using all the milk samples. The quantitative analysis of each sample was carried out in consecutive duplicate. A control milk was analysed every 10 samples to verify the stability of the analyser.

B-1.2.2- Results

The following table summarizes the results obtained:

	n	min	max	M	Sx	Sr	Sr (%)	r
Fat b (g/l)	40	34.96	44.95	38.64	2.03	0.03	0.07	0.08
Protein (g/l)	40	29.65	36.23	32.50	1.20	0.04	0.13	0.12
Dry matter (g/l)	40	121.99	133.00	128.08	2.45	0.13	0.10	0.35
Freezing point (m°C x-1)	40	526	538	533	2.7	0.8	0.15	2.27

Table 2: repeatability criteria of FTIR LactoScope for fat b, protein, dry matter and freezing point parameters on herd milk samples
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.

B-1.2.3- Conclusion

The FTIR LactoScope presents a standard deviation of repeatability (Sr) for fat b and protein content in compliance with and distinctly lower than the recommendations in the ISO 9622/IDF 141 C: 2000 standard and the CNIEL/IE handbook (Sr ≤ 0,14 g/l and r ≤ 0,4 g/l.). No standard criteria exist for dry matter, but the standard deviation of repeatability (Sr) obtained is lower than the maximum acceptable limit laid down in IDF 21 (0.26 g/l).
Concerning the freezing point (FPD), the standard deviation of repeatability (Sr) obtained is in compliance with the recommendations of the CNIEL/IE handbook (Sr ≤ 2 m°c → r ≤ 5.5 m°c).

The relative standard deviations of repeatability observed (Sr %) are in line with the manufacturer's recommendations, with a maximum acceptable limit fixed at 0.25%.

B-1.3- Evaluation of accuracy

B-1.3.1- Procedure

The accuracy of the analyser for fat b, protein, dry matter and freezing point was evaluated using all the milk samples. The quantitative analyses were performed in compliance with the evaluation of repeatability (cf. B-1.2).

The evaluation concerns the values obtained for fat b, protein and dry matter after calibration of the instrument using commercial SRMs produced by ACTILAIT-CECALAIT (cf A-2). 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);
- Protein: Amido black method according to NF V 04-216 (test in duplicate);
- Dry matter: drying method according to IDF 21 (single test);
- Freezing point: Thermistor cryoscopic method according to ISO 5764/IDF 108 (single test).

B-1.3.2- Results

The results obtained are noted in the following tables and figures:

	Fat b (g/l)	Protein (g/l)	Dry matter (g/l)	Freezing point (m°C x –1)
n	38	40	39	40
min	34.10	29.63	122.54	514
max	40.90	36.35	134.38	528
Y	37.83	32.60	128.94	520
Sy	1.90	1.24	2.48	3
d	+0.52	-0.10	-0.99	+13
Sd	0.29	0.16	0.36	2,7
Sy,x	0.298	0.160	0.348	2,6
Sy,x %	0.78	0.49	0.27	0.49
b	1.009	1.026	1.045	0.725
a	-0.85	-0.74	-4.75	134

Table 3: accuracy criteria of the FTIR LactoScope for fat b, protein, dry matter and freezing point on raw milk

n, min, max: number of results, minimum and maximum values; Y,X: mean 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.

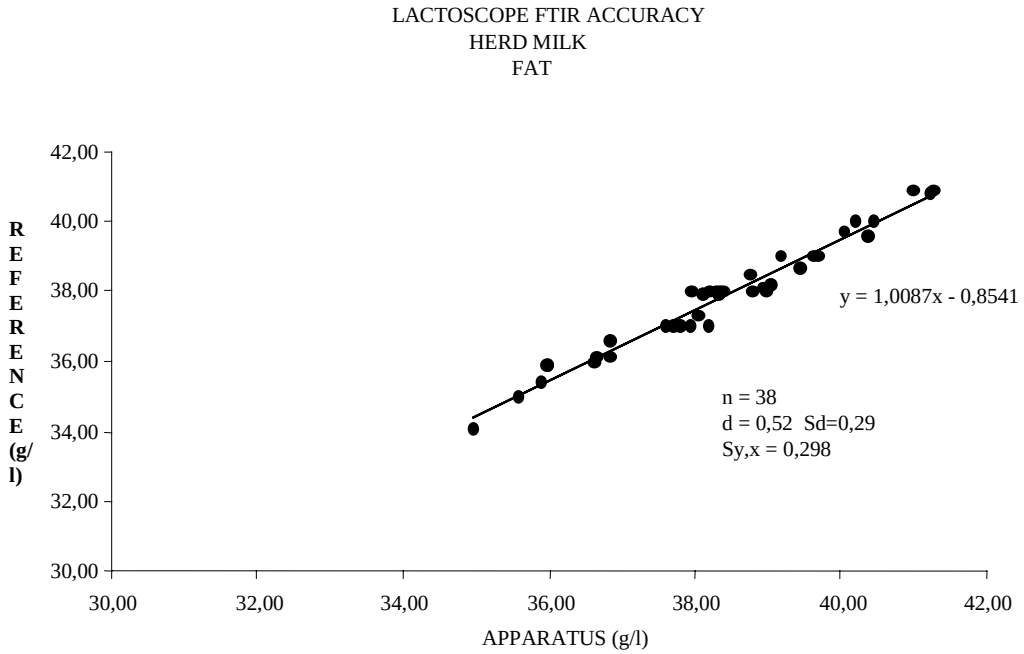


Figure 5: Relationship between the FTIR LactoScope and the reference results for fat b in milk

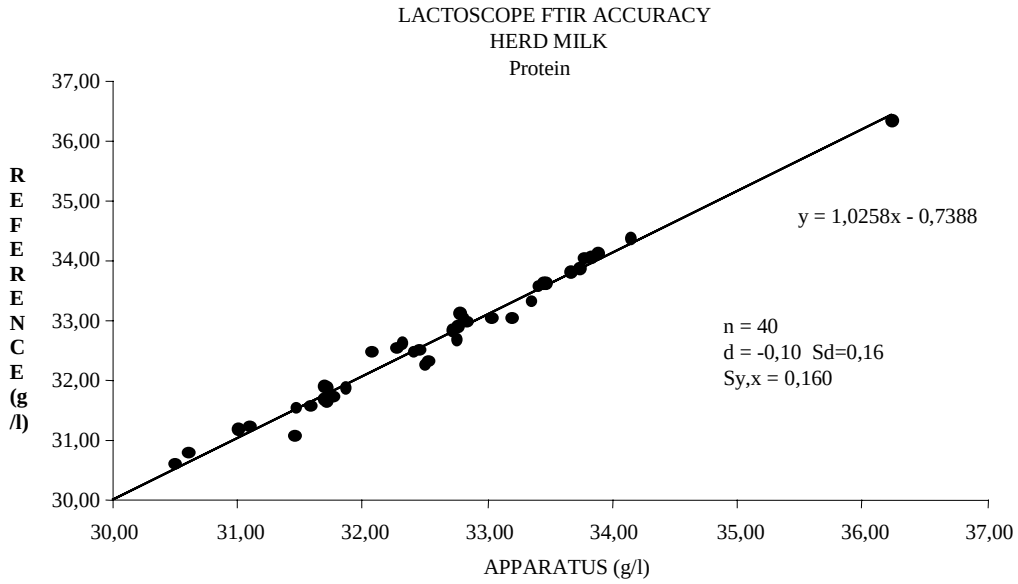


Figure 6: Relationship between the FTIR LactoScope and the reference results for protein in milk

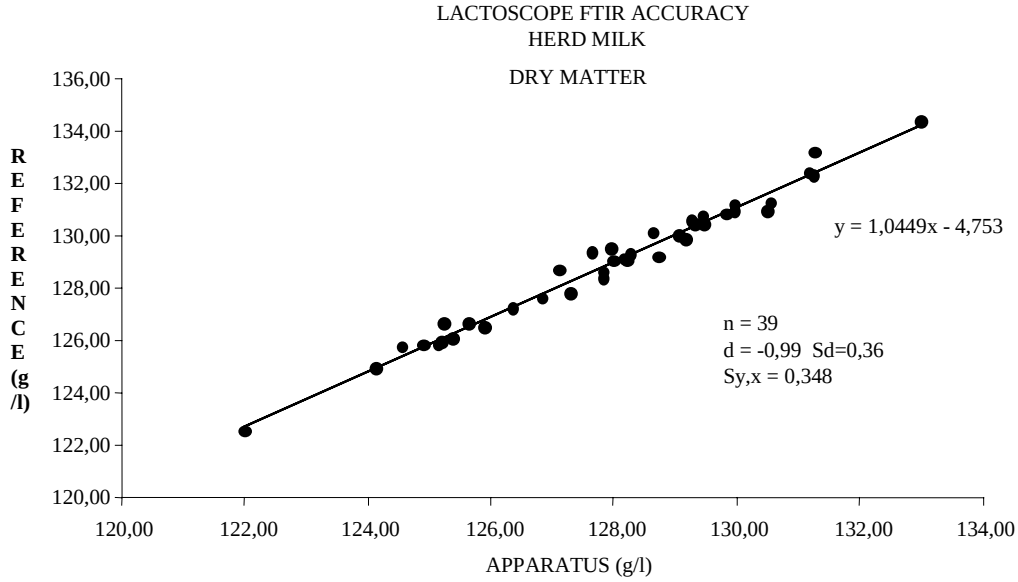


Figure 7: Relationship between the FTIR LactoScope and the reference results for dry matter in milk

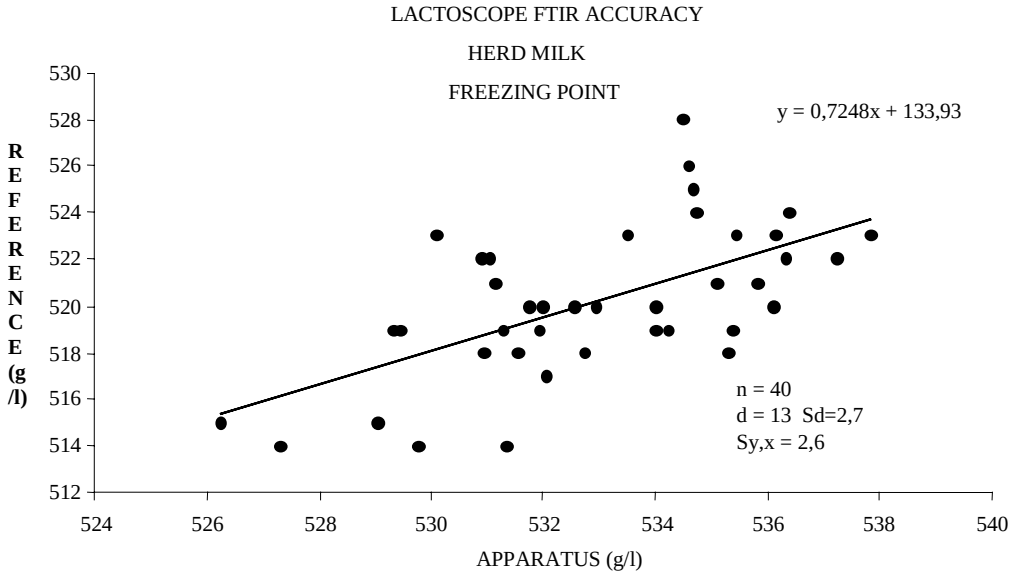


Figure 8 : Relationship between the FTIR LactoScope and the reference results for freezing point in milk

B-1.3.3- Conclusion

The mean and standard deviation of deviations (+0.52 and 0.29 g/l respectively) for fat b are partially in line with the recommendations laid down in ISO 9622/IDF 141 C:2000 standard (limits of 0.17 g/l and 0.7 g/l for a milk blend). The general exceedance of the deviations is due to the composition of the milk samples, which is different to the samples used in the calibration. The regression line obtained is not significantly different from 1 (P = 5%) and the intercept is significantly different from zero (P=1%). The residual standard deviation of regression is equal to 0.298 g/l;

For protein, the mean and the standard deviation of deviations (–0.10 et 0.16 g/l respectively) are in line with the recommendations laid down in ISO 9622/IDF 141 C:2000 standard (same limits as for fat). The regression line obtained is not significantly different from 1 (P = 5%) and the intercept is significantly different from zero (P=1%). The residual standard deviation of regression is equal to 0.160 g/l ;

For dry matter, the mean and the standard deviation of deviations are –0.99 et 0.36 g/l respectively. No standard criteria exist for this component. However, the results obtained enable an accuracy of estimation of +/- 0.70 g/l to be considered after adjustment using representative samples. Indeed, the significant average deviation is, as with fat content, due to the composition of the milk samples, which is different to the samples used in the calibration. The regression line obtained is significantly different from 1 (P = 5%) and the intercept is significantly different from zero (P=1%). The residual standard deviation of regression is equal to 0.348 g/l ;

For the freezing point, the mean and the standard deviation of deviations are +13 and 2.7 (m°Cx-1) respectively. With no standard criteria, the results obtained enable an accuracy of estimation of +/- 5.2 (m°C x –1) to be considered after adjustment using representative samples. The regression line obtained is significantly different from 1 (P = 5%) and the intercept is significantly different from zero (P=1%). The residual standard deviation of regression is equal to 2.6 (m°Cx-1). The significant average deviations and line deviations in relation to 0 and 1 respectively, are due to the absence of previous specific calibrations using representative samples.

Finally, for all the parameters evaluated, the relative residual standard deviations (Sy,x %) are in line with the manufacturer's recommendations, with a maximum acceptable limit fixed at 1%.

B-2- CREAM

B-2.1- Samples

The tests were performed on samples of UHT cream sold in supermarkets and hypermarkets. 20 samples were analysed: 10 were samples as is from the shops and 10 were made up of a mixture of whole cream and skim milk. Bronopol was added to the samples to give a final concentration of 0.02%.

B-2.2- Repeatability

B-2.2.1- Procedure

The repeatability of the instrument for fat was evaluated using all the samples. The quantitative analyses were performed in consecutive duplicate. A control milk was analysed every 10 samples to verify the stability of the analyser.

B-2.2.2- Results

The results obtained are presented in the table below:

	n	min	max	M	Sx	Sr	Sr (%)	r
Fat (g/100g)	20	17.97	31.82	25.82	5.07	0.05	0.19	0.13

Table 4: repeatability criteria FTIR LactoScope for fat on cream

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.

B-2.2.3- Conclusion

With no standard criteria or recommendations from the manufacturer, the FTIR LactoScope presents a standard deviation of repeatability identical to the maximum acceptable limit for the reference method NF EN ISO 2450, that is 0.05 g/100g.

B-2.3- Evaluation of accuracy

B-2.3.1- Procedure

The accuracy of the analyser for fat was evaluated using all the cream samples. The quantitative analyses were performed in compliance with the evaluation of repeatability (cf. B-2.2). The instrumental values are from a calibration carried out by the manufacturer.
The reference method used was the Röse-Gottlieb method according to NF EN ISO 2450 (single test).

B-2.3.2- Results

The results obtained are presented in the tables and figures below:

	n	min	max	Y	Sy	d	Sd	Sy,x	b	a
Global fat (g/100g)	18	17,97	31,82	24,94	4,95	0,85	0,47	0,189	0,954	0,35

Table 5: accuracy criteria of the FTIR LactoScope for fat in cream

n, min, max: number of results, minimum and maximum values; Y,X: mean 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.

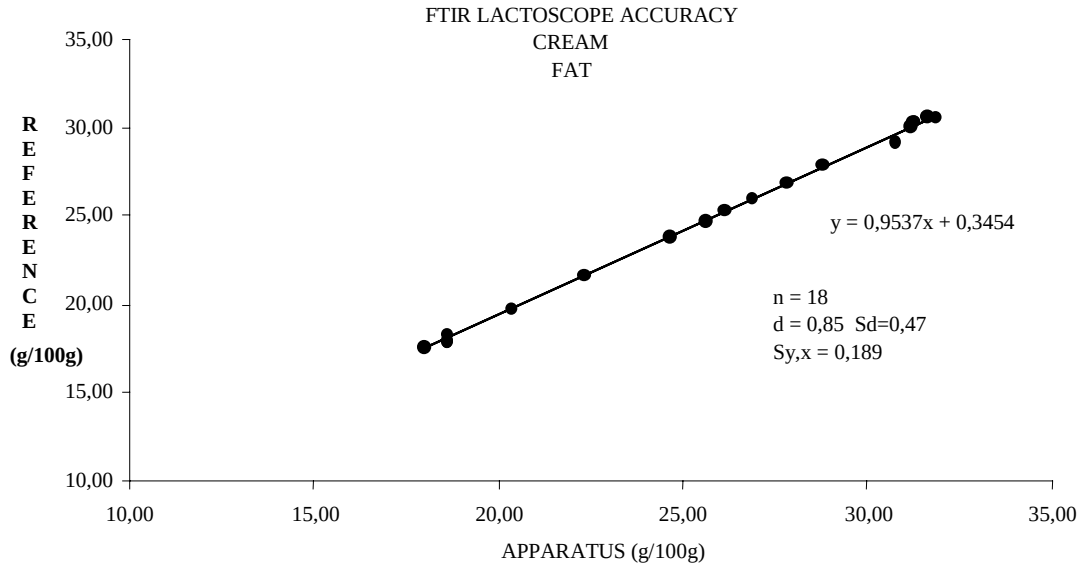


Figure 9: Relationship between the FTIR LactoScope and the reference results for fat content in cream

It can be noted that:
Overall, the mean and the standard deviation of deviations are respectively 0.85 and 0.47 g/100g. The regression line obtained is significantly different from 1 (P = 1%) and the intercept is significantly different from zero (P=1%). The residual standard deviation of regression is equal to 0.189 g/l.
A satisfactory linearity and a residual standard deviation of 0.080 g/100g can be observed for the study carried out on the range of samples made up of a mixture of cream and skim milk.

B-2.3.3- Conclusion

Overall, with no standard criteria or recommendations from the manufacturer, the results obtained concerning fat enable an accuracy of estimation of +/-0.38 g/100g to be considered after adjustment using representative samples.

From the evaluation report of the infrared Advanced LactoScope FTIR™ analyser - X. QUERVEL, Ph. TROSSAT – Actilait / Cecalait – September 2009