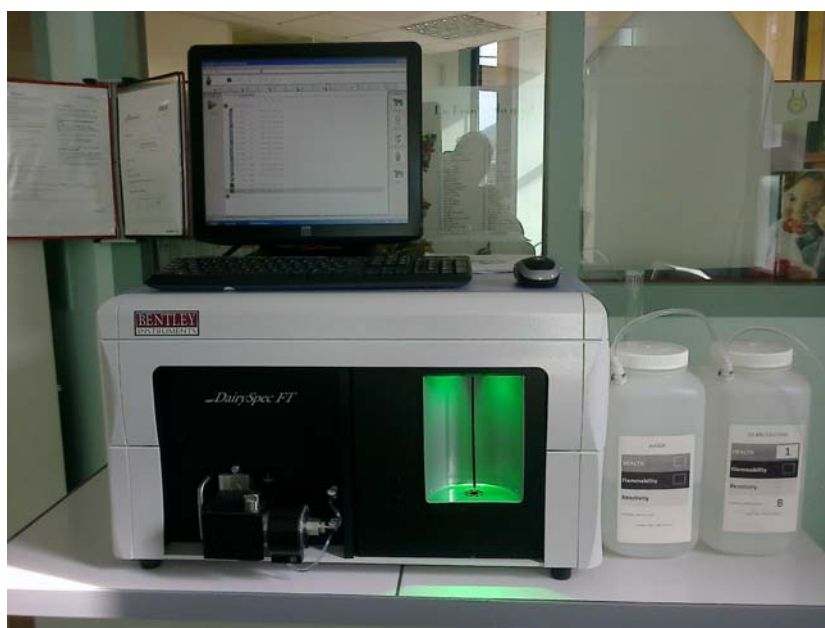


EVALUATION OF THE BENTLEY DAIRYSPEC FT® INFRARED ANALYSER

The DairySpec FT is an infrared spectrophotometer (650 to 4000 cm⁻¹) manufactured by Bentley Instruments (USA), and commercialised in France by Bentley Instruments SARL. It is used for the determination of the composition components in milk and liquid dairy products samples (fat, protein, lactose, dry matter, urea, freezing point...).

This instrument uses a mono-bundle with high resolution industrial interferometer Fourier transform-based infrared system (FTIR). The complete infrared spectrum (standard resolution 8 cm⁻¹) collected enables MLR or PLS calibrations, necessary for the quantification of the major or minor components.

The apparatus is computer controlled with a software, which ensures the signal treatment. The back-up of the analysed samples spectrum enables a posteriori re-treatment of the signal. It is possible to equip the instrument with an auto-sampler and to combine it with the "Somacount" somatic cells counter, to achieve an analytical rate of 300 samples / hour.



The tests:

The evaluation tests were performed in ACTALIA Cecalait's physico-chemistry laboratory (reference and infrared analyses) from June to September 2014. After preliminary tests of stability, linearity and calibration performed for dry matter (MS), fat (MG) and protein (MP), the repeatability and accuracy on milk, cream and proteic retentate were evaluated.

The appreciation criteria of the estimated parameters were taken from either ISO 9622/IDF 141: 2013 "Guidelines for the application of mid-infrared spectrometry" and ISO 8196-3/IDF 128-3: 2009 "Milk - Definition and evaluation of the overall accuracy of alternative methods of milk analysis - Part 3", or from the CNIEL/IE PROC IR v06 handbook concerning the use of infrared apparatus within the context of milk payment and milk control in France. A comparison in relation to the manufacturer specifications is also realised.

The instrument was configured for a rate of 60 samples / hour for cream and retentate, 120 samples / hour for milk and non correction of contamination.

A. PRELIMINARY TESTS

A.1 – Evaluation of the stability

The stability was evaluated by the analysis, in automatic mode, of 3 samples of milk at 3 different rates every 15 minutes during a work half-day, representing 20 measurement cycles. The repeatability and reproducibility were calculated for each analytical criterion and by level.

Results:

The following tables 1 to 3 present the results obtained:

	M (g/l)	Sr (g/l)	Sr (%)	SR (g/l)	SR (%)	r (g/l)	R (g/l)
1	19.91	0.04	0.18%	0.04	0.18%	0.10	0.10
2	40.24	0.04	0.10%	0.04	0.10%	0.11	0.11
3	82.61	0.09	0.11%	0.09	0.11%	0.25	0.25

Table 1: DairySpec FT stability criteria for fat

	M (g/l)	Sr (g/l)	Sr (%)	SR (g/l)	SR (%)	r (g/l)	R (g/l)
1	20.06	0.03	0.13%	0.03	0.13%	0.07	0.07
2	30.24	0.03	0.10%	0.03	0.10%	0.08	0.08
3	58.38	0.06	0.10%	0.06	0.10%	0.17	0.17

Table 2: DairySpec FT stability criteria for protein

	M (g/l)	Sr (g/l)	Sr (%)	SR (g/l)	SR (%)	r (g/l)	R (g/l)
1	99.24	0.05	0.05%	0.05	0.05%	0.14	0.14
2	128.41	0.09	0.07%	0.09	0.07%	0.24	0.24
3	193.54	0.17	0.09%	0.17	0.09%	0.49	0.49

Table 3: DairySpec FT stability criteria for dry matter

M: mean; Sr and SR (Sr% and SR%): standard deviation of absolute repeatability and reproducibility (and relative); r and R: maximum deviation of repeatability and reproducibility in 95 % of cases.

The daily mean values of standard deviation of reproducibility (SR) for fat and protein are below the limits required in ISO 8196-3/IDF 128-3 ($SR \leq 0.29$ g/l for median range or $SR \leq 0.58$ g/l for high range).

As no standardised or regulatory values exist for dry matter, it can be noted that the reproducibility standard deviation SR is lower than the standardised value of the ISO 6731/IDF 21 : 2010 reference method ($R = 2.1$ g/l ; $SR = 0.74$ g/l).

A.2 – Evaluation of linearity

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.

For fat, a range of 12 milk samples from 0 to 100 g/l was prepared by mixing cream and skimmed milk. This range was analysed 5 times in the descending order of the fat rates.

The Ar/At ration (Ar and At: amplitude of residues and amplitude of contents respectively) is equal to 1.6 %, that is in accordance with the limit of 2 % expressed in ISO 9622/IDF 141 standard.

3 ranges of optimal (0-20g/l: Ar/At = 0.36 %, 20-70g/l: Ar/At = 0.42 % and 70-100 g/l: Ar/At = 1.56 %) are also identified. Within the 0-20 and 20-70 g/l ranges, the Ar/At ratios are in accordance with the specifications of the ISO 8196-3/IDF 128-3 standard (limit 1 %).

For protein, a range of 12 milk samples from 0 to 130 g/l was prepared by mixing proteic retentate and filtrate obtained by tangential ultrafiltration (cutoff threshold: 10 KD). The range was analysed 5 times in the descending order of the fat rates.

The Ar/At ration within the range studied is equal to 1.3 %, which is in conformity with the recommendations of 2 % maximum given in ISO 9622/IDF 141 standard. The Ar/At ratios within the 0-110 g/l (Ar/At: 0.85 %), 0-40 g/l (Ar/At: 0.18 %) and 40-110 g/l (Ar/At: 0.64 %) ranges are also in accordance with the specification of the 8196-3/IDF 128-3 standard (limit 1 %).

The linearity of this instrument is therefore satisfactory for fat (range from 0 to 100 g/l) and protein (range from 0 to 130 g/l).

A.3 – Evaluation of the calibration

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 (SRMs) produced by ACTALIA Cecalait in July and September 2014. Each sample was analysed in duplicate.

Results:

The results are presented in the following table:

	N	Min-max	Sr	d	Sd	SI1	SI3
Fat (g/l)-Median	13	22.3 53.7	0.032	0.098	0.194	0.103	0.094
Protein (g/l) -Median	13	24.4 39.9	0.036	-0.045	0.196	0.048	0.029
Dry matter (g/l) -Median	13	104.9 150.8	0.087	-0.159	0.445	0.429	/
Fat (g/l)-High	13	59.6 91.8	0.118	-0.292	0.257	0.262	0.261
Protein (g/l) -High	13	45.2 66.6	0.035	1.575	0.250	0.039	0.037
Dry matter (g/l) -High	13	162.7 212.9	0.086	0.293	0.510	0.531	/

Table 4: DairySpec FT calibration criteria for fat, protein and dry matter

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); SI1 and SI3: residual standard deviation of simple linear regression (reference vs instrument) or multiple (reference vs fat, protein and lactose)

Conclusion:

The residual standard deviations of the linear regression obtained for fat and protein are in conformity with the recommendations of the CNIEL/IE handbook (respectively lower than 0.25 and 0.15 g/l for the "median" range, corresponding to the cow milk contents, and respectively lower than 0.50 and 0.30 g/l for the "high" range, corresponding to ewe milk contents).

B. EVALUATION OF REPEATABILITY AND ACCURACY**B.1 – The samples**

The test were performed on:

- ◆ for milk: 40 samples of tank milk from the Rhône-Alpes region.
 - ◆ for cream: 20 samples of pasteurised cream (obtained by cream skimming) from the Rhône-Alpes region.
 - ◆ for proteic retentate: 25 samples of proteic retentate and permeate (obtained by ultrafiltration of skim milk) from the Bretagne region. Many "artificial" samples were realised by mixing retentate and permeate to obtain a variability of protein rates.
- Bronopol at 0,02 % final was added to all the samples.

B.2 – Procedure

The repeatability and accuracy of the instrument were evaluated for fat, protein and dry matter in milk, fat and dry matter in cream, and protein and dry matter in retentate. The quantitative analyses were performed in duplicate for each sample. A control milk was analysed at the beginning and the end of each set to verify the stability of the instrument.

The evaluation concerns the values obtained after calibration and adjustment of the instrument with commercial SRMs produced by ACTALIA Cecalait for the milk samples, and after calibration and adjustment of the instrument by the manufacturer for the cream and retentate samples.

The following reference methods were used:

- Fat: Gerber acido-butyrometric method according to NF V 04-210: 2000 for milk and NF V 04-263: 1997 for cream (single tests and then confirmation if more important residues).
- Protein: Amido black method according to NF V 04-216: 2011 (test in duplicate –volume/volume dilution of samples according to annex A for the retentate).
- Dry matter: drying method according to ISO 6731/IDF 21: 2010 (single test).

B.2 – Results

B.2.1 – Milk

The following tables and figures present the results obtained:

	n	min	max	M	Sx	Sr	Sr (%)	r
Fat (g/l)	40	38.0	41.8	39.650	0.860	0.022	0.06	0.061
Protein (g/l)	40	31.4	33.3	32.277	0.573	0.023	0.07	0.064
Dry matter (g/l)	40	127.0	130.7	128.958	1.006	0.057	0.04	0.159

Table 1: DairySpec FT repeatability criteria for fat, protein and dry matter in tank milk samples

n: number of results; *min et 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 on 95 % of cases.

For fat and protein, the standard deviation of repeatability is in conformity with the recommendations of the ISO 8196-3/IDF 129-3 standard and the CNIEL/IE handbook (Sr ≤ 0.14 g/l and r ≤ 0.4 g/l).

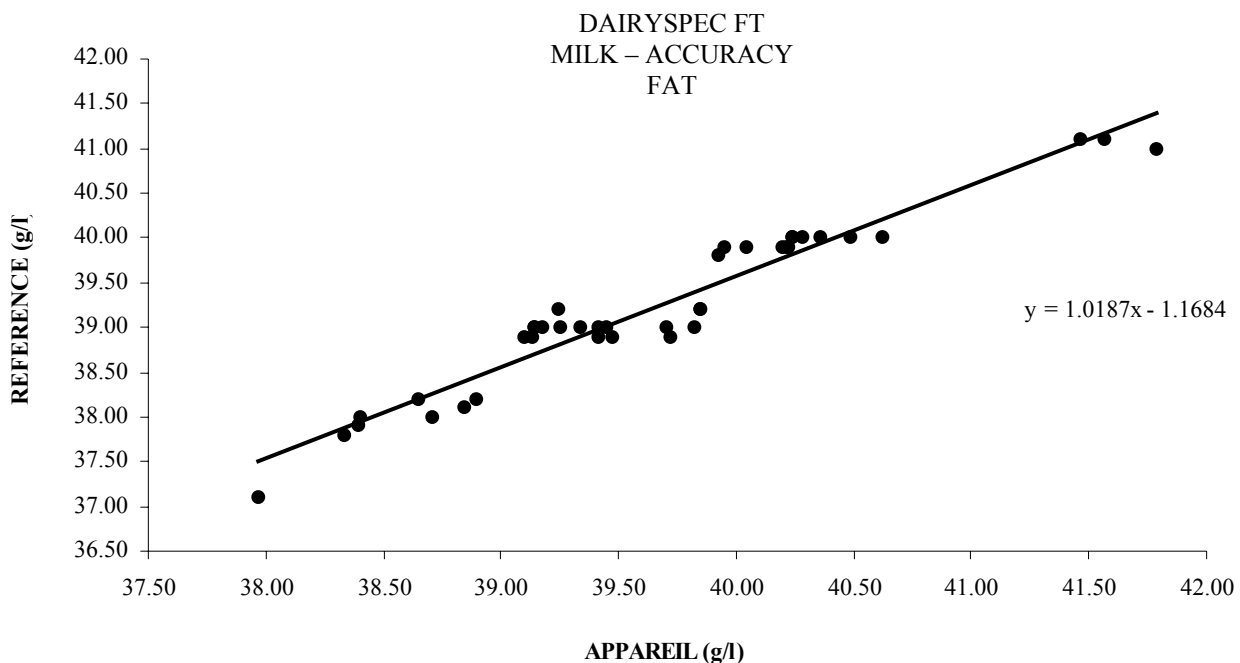
For dry matter, as no values exist in standards or CNIEL/IE handbook, it can be noted that the standard deviation of repeatability obtained is lower than the limits of the ISO 6731/IDF 21 : 2010 reference method (r = 1 g/l - Sr = 0,36 g/l).

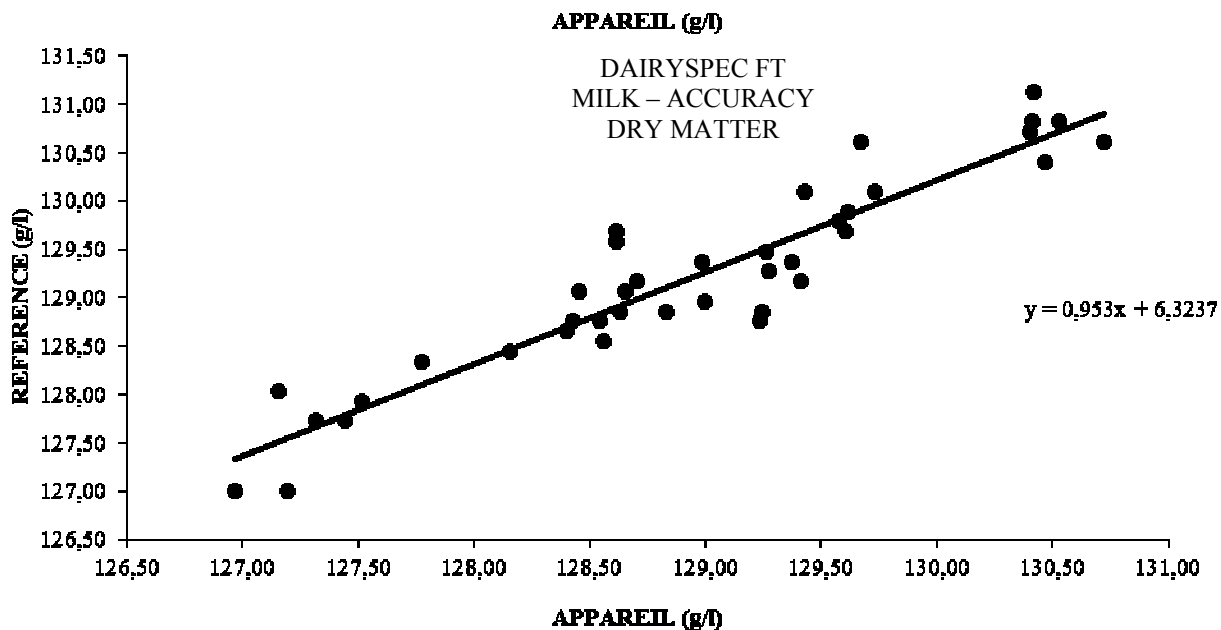
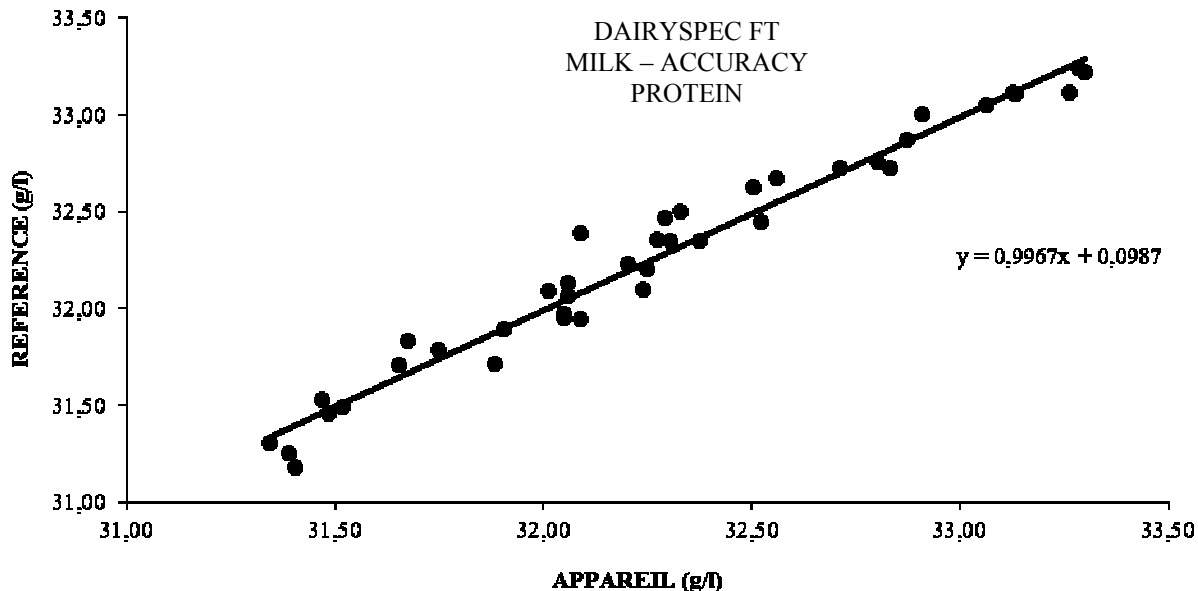
The relative standard deviations of repeatability are also in accordance with the specifications (limit at 0.5 % for all the parameters) of the manufacturer.

	n	min (g/l)	max (g/l)	Y (g/l)	Sy (g/l)	d (g/l)	Sd (g/l)	Sy,x (g/l)	Sy,x (%)	b	a
Fat	40	37.10	41.10	39.223	0.907	0.427	0.232	0.235	0.60	1.019	-1.17
Protein	40	31.18	33.24	32.270	0.581	0.007	0.107	0.108	0.34	0.997	0.10
Dry matter	40 (39)	127.00 (127.00)	131.33 (131.12)	129.255 (129.202)	1.065 (1.024)	-0.297 (-0.268)	0.398 (0.357)	0.403 (0.359)	0.31 (0.28)	0.982 (0.951)	2.67 (6.32)

Table 2: DairySpec FT accuracy criteria for fat, protein and dry matter in tank milk samples. For fat and dry matter, the reference results obtained in m/m units have been converted in m/v by the 1.03 g/ml coefficient.

n, min, max: number of results, minimum and maximum values; *Y, X*: mean results using the reference and instrumental method; *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.





Figures 1, 2 and 3: Relation between DairySpec FT and reference for fat, protein and dry matter in tank milk samples.

It can be noted that:

↳ For fat: the mean and the standard deviation of deviations are respectively equal to 0.43 and 0.23 g/l. The regression slope (1.019) is not significantly different from 1.00 (P = 5 %). The residual standard deviation of regression is equal to 0.24 g/l.

↳ For protein: the mean and the standard deviation of deviations are respectively equal to 0.01 and 0.11 g/l. The regression slope (0.997) is not significantly different from 1.00 (P = 5 %). The residual standard deviation of regression is equal to 0.11 g/l.

↳ For dry matter: the mean and the standard deviation of deviations are respectively equal to -0.27 and 0.36 g/l. The regression slope (0.953) is not significantly different from 1.00 (P = 5 %). The residual standard deviation of regression is equal to 0.36 g/l.

For fat and protein, the residual standard deviations (respectively 0.24 and 0.11 g/l) are in accordance with the recommendations of the ISO 8196-3/IDF 129-3 standard (limit: 0.7 g/l).

For dry matter, as no standard exists, the residual standard deviation obtained (0.36 g/l) enables an accuracy of estimation of +/- 0.72 g/l (risk at 5 %).

The relative residual standard deviations are also in accordance with the specifications (limit at 1 % for all the parameters) of the manufacturer.

B.2.2 – Crème

The following tables and figures present the results obtained:

	n	min	max	M	Sx	Sr	Sr (%)	r
Fat (g/100 g)	20	26.4	44.8	40.182	5.242	0.038	0.09	0.104
Dry matter (g/100 g)	20	33.5	51.0	46.529	4.971	0.042	0.09	0.117

Table 3: DairySpec FT repeatability criteria for fat, protein and dry matter in cream samples

n: number of results; *min* and *max*: minimum and maximum values; *M* and *Sx*: mean and standard deviations of the results; *Sr* and *Sr%*: absolute and relative standard deviation of repeatability; *r*: maximum deviation of repeatability in 95 % of cases.

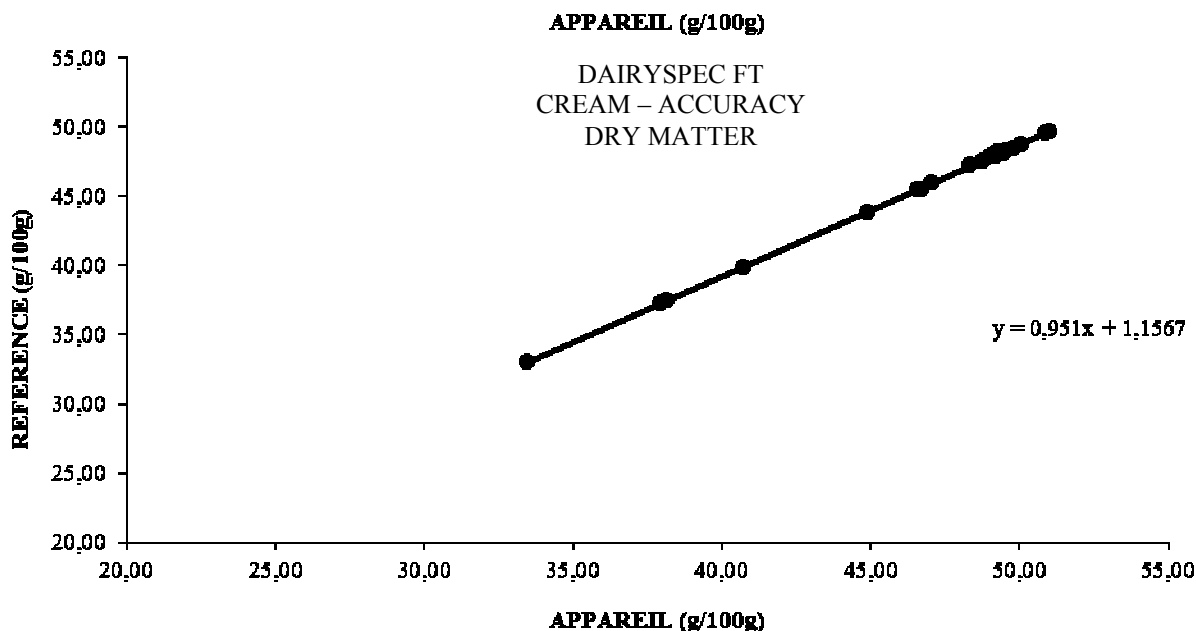
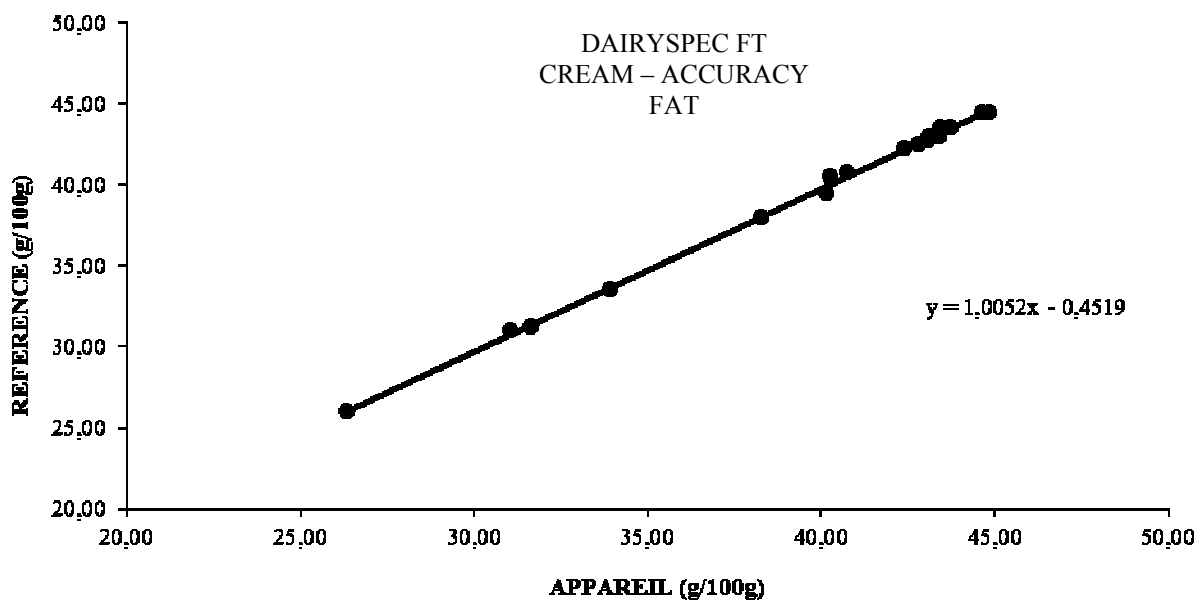
As no standardised or regulatory values exist for fat and dry matter, it can be noted that the standard deviations of repeatability are lower than the limits of the NF V 04-263 : 1997 and ISO 6731/FIL 21 : 2010 reference methods (respectively 0.14 g/100 g and 0.07 g/100 g).

The relative standard deviation of repeatability are also in accordance with the specifications (limit at 0.5 % for all the parameters) of the manufacturer.

	n	min (g/100g)	max (g/100g)	Y (g/100g)	Sy (g/100g)	d (g/100g)	Sd (g/100g)	Sy,x (g/100g)	Sy,x (%)	b	a
Fat	20	26.0	44.5	39.938	5.273	0.245	0.197	0.201	0.50	1.005	-0.45
Dry matter	20	33.0	49.7	45.404	4.728	1.125	0.256	0.079	0.17	0.951	1.16

Table 4: DairySpec FT accuracy criteria for fat and dry matter in cream samples

n, *min*, *max*: number of results, minimum and maximum values; *Y,X*: mean results using the reference and instrumental method; *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.



Figures 4 and 5: Relation between DairySpec FT and reference results for fat and dry matter in cream samples

It can be noted that:

↳ **For fat:** the mean and the standard deviation of deviations are respectively equal to 0.25 and 0.20 g/100 g. The regression slope (1.005) is not significantly different from 1.00 (P = 5 %). The residual standard deviation of regression is equal to 0.20 g/l.

↳ **For dry matter:** the mean and the standard deviation of deviations are respectively equal to 1.13 and 0.26 g/100 g. The regression slope (0.951) is significantly different from 1.00 (P = 1 %). The residual standard deviation of regression is equal to 0.08 g/100 g.

As no standardised or regulatory values exist for fat and dry matter, it can be noted that the residual standard deviations obtained (respectively 0.20 and 0.08 g/100 g) enables accuracies of estimation respectively equal to 0.40 and 0.16 g/100 g (risk at 5 %).

The relative residual standard deviations obtained are in accordance with the specifications (limit at 1 % for all the parameters) of the manufacturer.

B.2.3 – Retentate

The following tables and figures present the results obtained:

	n	min	max	M	Sx	Sr	Sr (%)	r
Protein (g/l)	25	87,3	110,1	98,353	7,002	0,111	0,11	0,309
Dry matter (g/l)	25	145,9	169,1	157,157	6,805	0,145	0,09	0,401

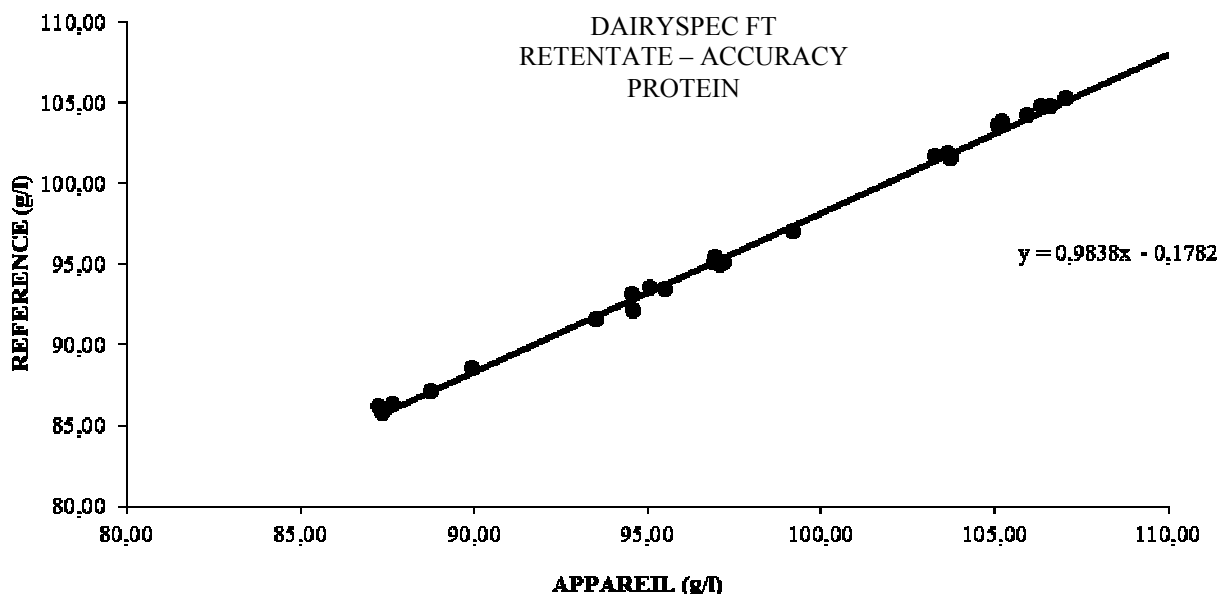
Table 5: DairySpec FT repeatability criteria for protein and dry matter in proteic retentate samples
n: number of results; min and max: minimum and maximum values; M and Sx: mean and standard deviation of the results; Sr and Sr %: absolute and relative standard deviation of repeatability; r: maximum deviation of repeatability in 95 % of cases.

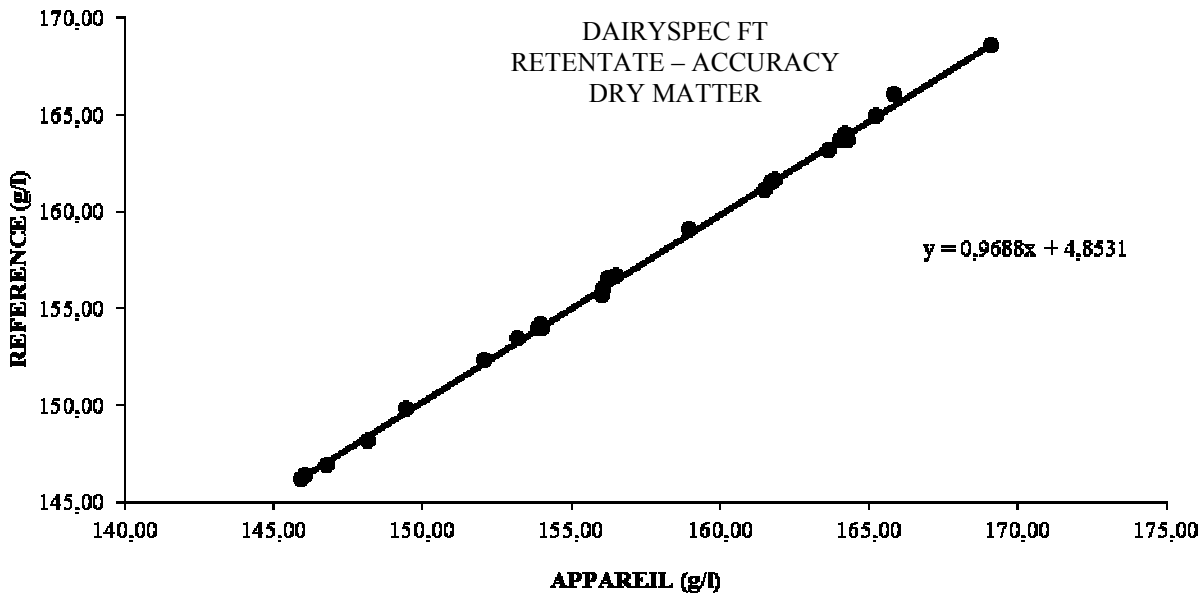
As no standards exist for protein and dry matter, it can be noted that the standard deviations of repeatability obtained are lower or close to the NF V 04-216 : 2011 et ISO 6731/IDF 21: 2010 reference methods limits (respectively 0.37 g/l and 0.11 g/l).

The relative standard deviation of repeatability are also in accordance with the specifications (limit at 0.5 % for all the parameters) of the manufacturer.

	n	min (g/l)	max (g/l)	Y (g/l)	Sy (g/l)	d (g/l)	Sd (g/l)	Sy,x (g/l)	Sy,x (%)	b	a
MP	25	85.79	107.56	96.581	6.897	1.772	0.356	0.345	0.36	0.984	-0.18
MS	25	146.2	168.6	157.099	6.595	0.058	0.295	0.209	0.13	0.969	4.85

Table 6: DairySpec FT accuracy criteria for protein and dry matter in proteic retentate
n, min, max: number of results, minimum and maximum values; Y,X: mean results using the reference and instrumental method; 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.





Figures 6 and 7: Relation between DairySpec FT and reference results for protein and dry matter in proteic retentate

It can be noted that:

↳ **For protein:** the mean and the standard deviation of deviations are respectively equal to 1.77 and 0.36 g/l. The regression slope (0.984) is not significantly different from 1.00 (P = 5 %). The residual standard deviation of regression is equal to 0.35 g/l.

↳ **For dry matter:** the mean and the standard deviation of deviations are respectively equal to 0,06 and 0.30 g/l. The regression slope (0.969) is significantly different from 1.00 (P = 5 %). The residual standard deviation of regression is equal to 0.21 g/l.

As no standardised or regulatory values exist for fat and dry matter, it can be noted that the residual standard deviations obtained (respectively 0.36 and 0.21 g/l) enables accuracies of estimation respectively equal to +/-0.72 and 0.42 g/l (risk at 5 %).

The relative residual standard deviations obtained are in accordance with the specifications (limit at 1 % for all the parameters) of the manufacturer.

CONCLUSION GENERALE

The results obtained for fat and protein in milk are in accordance with the recommendations of the ISO 8196-3/IDF 129-3 standard and the CNIEL/IE PROC IR v06 handbook, within the context of milk payment and milk control in France.

Despite the absence of standard criteria for the other parameters (dry matter in milk, fat and dry matter in cream, protein and dry matter in retentate), the results obtained are in conformity with the specifications of the manufacturer.