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Milk and liquid milk products — Guidelines for the application of mid-infrared spectrometry

Lait et produits laitiers liquides — Lignes directrices pour l'application de la spectrométrie dans le moyen infrarouge

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2. www.iso.org/directives

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The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF). It is being published jointly by ISO and IDF.

This second edition of joint ISO 9622|IDF 141 cancels and replaces the first edition (ISO 9622:1999), which has been technically revised.

Foreword

IDF (the International Dairy Federation) is a non-profit organization representing the dairy sector worldwide. IDF membership comprises National Committees in every member country as well as regional dairy associations having signed a formal agreement on cooperation with IDF. All members of IDF have the right to be represented on the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO in the development of standard methods of analysis and sampling for milk and milk products.

The main task of Standing Committees is to prepare International Standards. Draft International Standards adopted by the Standing Committees are circulated to the National Committees for endorsement prior to publication as an International Standard. Publication as an International Standard requires approval by at least 50 % of IDF National Committees casting a vote.

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ISO 9622|IDF 141 was prepared by the International Dairy Federation (IDF) and Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by IDF and ISO.

All work was carried out by an ISO-IDF Project Group on Guidance on the application of mid-infrared spectrometry, of the Standing Committee on *Statistics and Automation (SCSA),* under the aegis of its project leaders, Mr. P. Sauvé (CA) and Mr. H. van den Bijgaart (NL).

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Milk and liquid milk products — Guidelines for the application of mid-infrared spectrometry

1 Scope

This International Standard gives guidelines for the quantitative compositional analysis of milk and liquid milk products, such as raw milk, processed milk, cream and whey, by measurement of the absorption of mid-infrared radiation.

Additional built-in instrument features, such as a conductivity sensor, can improve the performance in the determination of compositional parameters and allow for the estimation of other parameters.

The guidelines specified are applicable to the analysis of cow's milk. The guidelines are also applicable to the analysis of milk of other species (goat, ewe, buffalo, etc.) and derived liquid milk products, provided adequate calibrations are generated for each application and adequate control procedures are in place.

The application is limited to lower viscosity products that can be pumped through the flow system of the analyser and to analytes that do not result in optical saturation at the specific wavelengths being utilized.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable to its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 8196|IDF 128 (all parts), *Milk — Definition and evaluation of the overall accuracy of indirect methods of milk analysis*

ISO 8968-1|IDF 20-1, *Milk — Determination of nitrogen content — Part 1: Kjeldahl method*

ISO 8968-2|IDF 20-2, *Milk — Determination of nitrogen content — Part 2: Block-digestion method (Macro method)*

ISO 8968-5|IDF 20-5, *Milk — Determination of nitrogen content — Part 5: Determination of protein-nitrogen content*

NOTE Other normative documents can apply depending on the specific application or calibration of the automated analyser.

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 8196|IDF 128 (all parts), and the following apply.

3.1
spectral calibration
spectrum calibration model
calibration based on combination of absorbance signals at several (>2) wavelengths in the mid-infrared region or signals from other sensors, mathematically optimized to arrive at the best estimate for the parameter of interest

3.2
slope and intercept calibration
simple linear regression coefficients as established from a least-squares regression of optimized instrument readings against results as obtained with physico-chemical reference methods

4 Principle

After pretreatment and homogenization, where required, the sample is measured with an infrared spectrometer that records the quantity of radiation absorbed in transmittance at specific wavelengths in the mid-infrared region. The spectral data are transformed into estimates of constituent concentrations or other physico-chemical parameters through calibration models developed on representative samples from the population to be tested. For some parameters, i.e. freezing point equivalents, signals from additional installed sensors may be fed to the calibration model.

5 Principal characteristics of infrared instruments

The signals at the relevant wavelengths may be produced using either a Fourier-transformed interferogram or by using optical filters. Instruments and applied calibration models may differ with respect to the number of specific wavelengths used in estimating the parameters of interest.

An infrared instrument is a proprietary apparatus which, when used under the conditions defined in this International Standard, provides estimates of compositional and other parameters in milk and liquid milk products.

6 Factors affecting the measurements

6.1 Instrument factors

6.1.1 Repeatability

To check instrument repeatability, analyse a uniform representative sample a minimum of 12 times in succession. The first two replicate results are discarded to minimize carry-over effects. The calculated repeatability should meet with the repeatability limits for the concerned parameter and sample matrix.

6.1.2 Zero stability

To monitor zero stability, a blank sample (water or zero solution) is analysed periodically during routine use of the instrument. Drift should be relatively small and random with respect to direction (±), such that cumulative drift is minimal. A plot of the zero drift vs time is an effective way to track instrument stability.

NOTE Certain instruments are factory set to auto-correct the zero at regular intervals. It is intended that operators review these automatic corrections to ensure that cumulative drift is not excessive.

6.1.3 Homogenization

To check the efficiency of the homogenizer, make two consecutive analyses, firstly with an unhomogenized whole milk sample, and secondly with the same whole milk sample after it has been homogenized through the instrument's homogenizer. When the average of five replicate fat readings is found, the difference among these five replicate fat readings shall not exceed 0,04 % for a milk sample containing a mass fraction of 4,0 % of milk fat. To calculate the appropriate pass/fail criteria for milk fat concentrations other than 4,0 %, multiply the actual fat content by 0,01 to obtain the new criteria.

NOTE 1 This procedure is only applicable to instruments in which the homogenized discharge can be isolated and collected.

NOTE 2 For applications involving sample matrices with higher levels of fat (i.e. raw cream), it is advisable to check homogenization efficiency with a representative high fat sample. Specific parameters for homogenizer performance depend upon the matrix.

NOTE 3 Instrument readings for every milk fat component (e.g. individual fatty acids or groups of fatty acids) is dependent on the effectiveness of homogenization. Different wavelengths used in calibration models result in unequal sensitivity to homogenizer efficiency and possibly larger relative effects than for fat. When measuring such milk fat components, it is intended that the homogenizer efficiency test be performed for these components, and the difference is not intended to exceed the limit of repeatability for the component.

CAUTION — The results of this test can be misleading, as an instrument in which the homogenizer does not work at all gives very little difference between the first and the second run.

An alternative procedure is to obtain an unhomogenized as well as a homogenized portion of the same milk, either by collecting raw and processed milk from the same tank at a dairy plant or by producing smaller volumes by means of a bench-top or pilot-plant homogenizer. Then measure both the unhomogenized and the same homogenized milk and compare the difference in results to the above-mentioned pass/fail criterion.

The assumption is that the homogenization efficiency of the external homogenizer is good. That can be verified by particle size analysis of the homogenized milk. A reasonable fat globule size distribution is characterized by a d (0,9) of 1,4 µm to 1,5 µm [d (0,9) means that 90 % of the milk fat globules has a diameter of less than d].[17]

Some instruments allow the user to monitor a homogenization index value to track the performance of the homogenizer. The manufacturer's guidelines should be followed.

Monitoring of instrument repeatability can also provide valuable information with respect to the state of the homogenizer. If repeatability on homogenized milk is satisfactory, whereas the repeatability on raw milk is poor (more than twice the variation), the homogenizer is likely not performing at an acceptable level.

6.1.4 Linearity

NOTE 1 The linearity check described in this subclause applies only to the measurement of major components in milk. Linearity checks for other applications, particularly for higher fat products or for parameters other than the major constituents, will differ. It is intended that the manufacturer's guidelines be followed in these cases.

NOTE 2 Linearity can be assessed on either a mass/mass basis or a mass/volume basis. Since the instrument cuvette holds a specific volume of sample, it is most ideal to assess linearity on a mass/volume basis. In either case, linearity solutions are prepared by accurately weighing fractions. To assess linearity on a volume basis, it is intended that accurate density measurements be conducted and appropriate conversions be calculated.

NOTE 3 It is critical, prior to assessing linearity, to confirm that the instrument homogenizer is functioning appropriately (see 6.1.3).

To check the linearity for each of the major components, make up at least 10 solutions of known concentration, which cover the typical range for the specific component. The following solutions are recommended.

a) Homogenized cream with a mass fraction of fat of 8 %, diluted with skimmed milk or zero solution to check the linearity for the determination of the fat content. If homogenized cream at this fat level is unavailable, unhomogenized cream may also be used providing the instrument homogenizer is functioning at an acceptable level (see 6.1.3).

b) UF skimmed milk retentate diluted with ultrafiltrate to check the linearity for the determination of the protein content. Alternatively, whey protein concentrate, sodium caseinate, calcium propionate, skim milk powder or evaporated skim milk diluted with distilled water may also be used. The stock solution should contain a mass fraction of approximately 5,5 % of protein.

c) A solution of 60 g/l of lactose monohydrate, diluted with water or a milk mineral solution[14] to check the linearity for the determination of the lactose content.

Using a stock solution, which has a concentration at the upper end of the typical range, serial dilutions can be made as follows in Table 1:

Table 1 — Serial dilutions

Part of stock solution	Part of diluent	Relative concentration
100	0	1,0
90	10	0,9
80	20	0,8
70	30	0,7
60	40	0,6
50	50	0,5
40	60	0,4
30	70	0,3
20	80	0,2
10	90	0,1
0	100	0,0

The concentrations of the solutions should be in regular increments from zero to the desired upper limits of instrument readings.

Analyse each sample in triplicate, average the results and calculate the linear regression equation $y = bx + a$. Apply linear regression with the expected values per sample on the x-axis and the measured values per sample on the y-axis. Calculate the residuals $e_i = y_i - (bx_i + a)$ from the regression. Plot the residuals e_i (y-axis) versus the expected values (x-axis) in a graph. A visual inspection of the data points usually yields sufficient information about the linearity of the signal. Any outlying residual should be deleted and the calculation process be repeated with the remaining data before applying the further test.

When observed, the curving can be expressed by the ratio, r, by using Formula (1):

$$r = \frac{(e_{max} - e_{min})}{(M_{max} - M_{min})} \times 100 \quad (1)$$

where

e_{max} is the numerical value of the maximum residual from the regression;

e_{min} is the numerical value of the minimum residual from the regression;

M_{max} is the numerical value of the upper measured value for the set of samples concerned;

M_{min} is the numerical value of the lower measured value for the set of samples concerned.

The ratio, r, should be less than 2 %. In case this value is superseded, better performance may be obtained by making separate calibrations for distinct ranges.

Eventually, adjust the linearity of the instrument response for the component in accordance with the manufacturer's instructions. See also Reference [18].

NOTE Alternatively, it is possible to combine a linearity check with the slope and intercept calibration.

6.1.5 Carry-over

Carry-over is defined as the residual volume of the previous sample as a percentage of the total volume of the instrument cell after a single pumping sequence of a sample through the instrument cell.

Internal factors/issues affecting carry-over include pump settings, flow system deficiencies and compensation factors. External factors affecting carry-over include transfer from the stirrer and pipette.

To assess carry-over for the complete system, including carry-over from the eventually applied automatic sampling system, run the samples from 20 separate vials using the complete system.

To assess carry-over for the flow system alone, run the samples manually, thereby wiping the pipette clean between cycles.

To check the carry-over, analyse 20 consecutive samples of water and whole (Be cautious with raw milk; it has to be homogenous.) milk, using the sequence: water, water, milk, milk, water, water, etc., and record for each sample of water and milk, the readings for each of the major compositional parameters.

Calculate for each parameter, the water-to-milk, E_W, and the milk-to-water carry-over, E_M, by using Formula (2) and (3):

$$E_w = \frac{(m_2 - m_1)}{(m_2 - w_2)} \times 100 \quad (2)$$

$$E_M = \frac{(w_1 - w_2)}{(m_2 - w_2)} \times 100 \quad (3)$$

where

w_1 is the sum of the first water readings (Nos. 1 + 5 + 9 + 13 + 17);

w_2 is the sum of the second water readings (Nos. 2 + 6 + 10 + 14 + 18);

m_1 is the sum of the first milk readings (Nos. 3 + 7 + 11 + 15 + 19);

m_2 is the sum of the second milk readings (Nos. 4 + 8 + 12 + 16 + 20).

The calculated carry-over values, E_W and E_M, shall be less than ±1 %.

NOTE It is intended that carry-over be assessed using this technique on the major milk components only.

6.1.6 Water vapour within the instrument

Variations in humidity of the air within the optical unit of the instrument result in variations in the optical zero and calibration. Replace the absorbent (silica gel) before it starts to change colour at the minimum interval specified by the manufacturer. The ambient conditions within certain laboratories might require changes that are more frequent.

6.2 Physico-chemical and biological factors

6.2.1 Milk composition

The signal obtained at each wavelength is the result of absorption by all components, including water.

When applying a spectrum calibration model for a specific component, the consequences of variations in other components may generally be accommodated for in the calibration model. Residual interaction detected can stem from insufficient variation of component concentrations in the spectral calibration sample set.

With traditional calibrations based on absorbance signals at preset wavelengths (MLR calibrations), it is necessary to apply intercorrection in order to accommodate for variations in concentration of the other components. The so-called intercorrection coefficients are specific to each wavelength and each type of instrument.

Check for any residual interaction of major components and, if necessary, adjust the intercorrection coefficients at intervals according to procedures specified by the instrument manufacturer or reference material provider.

Any independent addition of the pure component to milk should not result in a significant shift in the results of the other components, other than expected from the dilution by the added component. For the main components, this can be achieved as follows:[17]

a) add to a milk sample:

   — cream of the same milk;

   — a weighed amount m_{cas} of dried caseinate or milk protein to raise the protein content with about 1 g/100 g;

   — a weighed amount m_{lac} of dried lactose to raise the lactose content with about 1 g/100 g;

b) analyse the original samples and the fortified samples in at least quadruplicate;

c) calculate the means of replicates and, subsequently, the interaction biases using:

$$d_{y/x} = \frac{(Y_{Hx} - Y_{Lx} \cdot f_x)}{(H_x - L_x)} \quad (4)$$

where

Y_{Lx} and Y_{Hx} are the mean concentrations measured for the interfered component Y before and after the addition of the interfering component, respectively;

L_x and H_x are the mean low and high concentrations measured for the interfering component X before and after its addition, respectively;

f_x is the dilution factor for the added component X: fat $f_F = 1 - 0{,}011 \cdot (H_F - L_F)$, protein $f_P = 1 - 0{,}008 \cdot m_{cas}$ and lactose $f_L = 1 - 0{,}006 \cdot m_{lac}$

For the three major components, six combinations should be tested: F/P, F/L, P/F, P/L, L/F, L/P.

For milk sample populations showing large concentration ranges (e.g. individual milks), residual interaction biases should lay within ±0,02.

Procedures and modified milk samples for checking and adjusting intercorrection coefficients are more extensively described elsewhere.[15][16][17]

The intercorrection coefficients should be checked whenever any major part of the instrument, for instance the source, the detector or optical deck is serviced or changed.

6.2.2 Fatty acid composition

With traditional calibrations based on absorbance signals at preset wavelengths (MLR calibrations), the variations in the fatty acid composition of milk (mean molecular mass and degree of unsaturation) can influence significantly the relationship between the results of the reference method and the infrared measurements. When compositional variations occur (for example seasonal variation, regional differences or different species), it may be necessary to modify the calibration of the instrument.

NOTE With spectral calibrations it is possible to reduce the impact of seasonal and regional variation by incorporating these types of variation in the calibration sample set.

6.2.3 Lipolysis

The liberation of fatty acids by the action of lipase can change the instrument's readings. For example when applying an instrument using traditional MLR calibrations, an increase in the lipolysis index of 1 milliequivalent per 100 g of fat, as measured by the BDI method (ISO/TS 22113|IDF/RM 204), changes the instrument's signal for fat by −0,022 % at 5,7 µm (filter A) and by +0,006 % at 3,5 µm (filter B) for a sample containing a mass fraction of fat of 3,5 %.

An increase in the lipolysis index of 1 milliequivalent per 100 g of fat, as measured by the BDI method, changes the instrument's signal for protein at 6,5 µm by + 0,013 % for a test sample containing a mass fraction of protein of 3,0 %.

6.2.4 Physical condition of milk fat

If part of the milk fat appears on the surface in an oiled-off condition, the test sample pumped by the instrument would not be representative of the fat content of the sample. Oiled-off samples shall, therefore, be avoided. Care should be taken to re-incorporate cream layers sticking to the walls of vessels and caps.

6.2.5 Variation in non-protein-nitrogen (NPN)

The IR protein determination is merely based on absorption of infrared energy by the peptide bonds of the protein molecules, whereas with filter instruments, the components of the NPN fraction hardly contribute to the instrument signal at the wavelengths where protein is measured. An instrument may be calibrated to produce a protein nitrogen (in accordance with ISO 8968-5|IDF 20-5) or a total nitrogen (in accordance with ISO 8968-1|IDF 20-1 or ISO 8968-2|IDF 20-2) based protein estimate measured by the Kjeldahl method.

When, with traditional MLR calibrations, the choice is made to use a protein calibration based on total nitrogen, it is assumed that the NPN content of the milk samples used to calibrate the instrument is constant from sample to sample within each calibration set and from set to set. If the NPN varies from sample to sample within the calibration set, distortions of the slope adjustment of the corrected signal on the protein result can cause a larger standard deviation of difference between the Kjeldahl total nitrogen (TN) reference method and the instrument, resulting in reduced accuracy. Consequently, with protein calibration on total nitrogen, variation in the average NPN level from one calibration set to another might require re-adjustment of the calibration.[13]

6.2.6 Variation in citric acid

Citric acid absorbs energy at 6,5 µm, i.e. where protein is typically determined. Variation in citric acid content consequently needs to be compensated for through the protein calibrations.

6.2.7 pH

In raw milk samples with lowered pH, an influence can be observed on the instrumental readings for fat, protein, lactose, urea and freezing point. Below pH 6,4, readings for protein content and freezing point tend to be significantly lower, whereas those for fat and lactose tend to be higher than the values obtained at pH 6,7.[10]

6.2.8 Preservatives

Preservatives can influence the IR response as well as the results of reference method analysis. These effects can be different for different components and may vary between instrument configurations. It is therefore important that these specific effects be examined before implementing any kind of sample preservation in a calibration scheme.

Preservative effects may be assessed by comparing IR results on preserved and unpreserved portions of the same samples. That can be done using blank samples (water) as well as milk samples. It is important to note that preservative biases can differ for liquid and solid (tablet) preservatives having the same active ingredient. Be also aware of the small dilution effect with added preservatives.

If preservative biases are identified, their impact on IR results may be minimized by ensuring that the instrument is calibrated using samples preserved in the same way as the routine test samples. In such case, the reference method shall be performed on milk samples without preservative, unless it has been previously demonstrated that preservative addition does not modify the results of the reference method either through chemical properties or volume.

6.2.9 Product-matrix effects

In more complex products, there can be absorbing groups not related to the specific analyte that is being tested. For example, added fat or protein from other sources might not be covered by the calibration for a pure milk matrix; sugar added to ice-cream mix would result in an inflated estimate of the lactose content of the product.

7 Calibration of the instrument

7.1 Objective

It is essential to adjust the instrument's signal so that for each level of concentration of the component being measured, the instrument reading is closely approximate to the value given by the reference method.

If reference methods are not available or not feasible, alternative methods may be used, provided they are adequately validated.

Because infrared instruments have different calibration systems, no specific procedure can be given. The manufacturer shall supply the laboratories with the means to adjust the instrument to comply with the requirements given in this International Standard. Persons performing calibrations should be familiar with the statistical principles behind the calibration algorithm used.

7.2 Spectrum calibration models

The accuracy and robustness of spectrum calibration models are dependent on the strategies used for sample selection and calibration. Developed calibration models are only valid for samples covered by the domain of the calibration samples. The first step in calibration development is therefore to define the application, e.g. sample type and concentration range. The instrument should be calibrated on a series of natural samples. When calibration samples are selected, care should be taken to ensure that all major factors affecting the accuracy of calibration are covered within the limits of the defined application area. These include the following:

a) variability in combinations and ranges of the compositional and other parameters;

b) seasonal, geographic and genetic variability in the compositional and other parameters.

The calibration may be performed using different techniques, e.g. multiple linear regression (MLR), multivariate algorithms such as partial least square regression (PLS), locally weighted regression (LWR) or artificial neural networks (ANN). The optimal technique may be assessed from cross-validation, where models are subsequently developed on parts of the data and tested on other parts[19]. Additional information may be obtained from testing on an independent test set.

An important issue is the determination of the optimal number of variables (in MLR) or factors (in multivariate calibrations). If too few variables or factors are used, an under-fitted solution is obtained, which means that the model is not large enough to capture the important variability in the data. If too many variables or factors are used, an over-fitted solution may be obtained, where much of the redundancy in the infrared data are modelled. Both cases can result in poor predictions on future samples. Generally, the best solution is the one giving the lowest root-mean-square error of cross-validation (RMSECV) with the fewest variables or factors.

The reference results should be plotted against predicted values obtained by cross-validation. The plot should be examined for outliers. The plot should also be investigated for regions with different levels of

prediction accuracy, random or systematic, which can indicate the need for more calibration samples or a segmentation of the calibration region.

When calibration models have been developed, they should be validated on an independent test set, preferably sampled after the calibration period. The test set should cover all variations in the sample population and should contain at least 25 samples. The results obtained on the independent test set are plotted, reference against infrared and residuals against reference to give a visual impression of the performance of the calibration. After proper handling of eventual outliers, the standard error of prediction (SEP) should not be significantly larger than RMSECV. If this is not the case, the calibration set should be expanded to include more samples. In all cases when a new calibration is developed on an expanded calibration set, the validation process should be repeated on a new independent test set. If necessary, expansion of the calibration set should be repeated until acceptable results are obtained on an independent test set.

It is advised to involve chemometric expertise for development and evaluation of spectrum calibration models.

NOTE Spectral calibrations can be transferred to other instruments of the same type, provided spectrum standardization is applied by adequate mathematical procedures.

7.3 Core settings

Core settings vary depending on the instrument, optical system and application. Core settings may be achieved through spectrum standardization and spectral calibration.

Core settings with a traditional calibration include:

a) gain settings;

b) linearity settings;

c) intercorrection factors.

7.4 Checking the slope and intercept

7.4.1 General

Once the spectrum calibration models or the core settings are established and verified (see Clause 6), regular validation and, if necessary, adjustment of slope and intercept should be used to fine tune the instrument performance and to account for changes in instrument or sample matrix factors.

NOTE It is intended that spectral calibrations or core settings preferably result in operational slope and intercept values close to 1 and 0, respectively.

7.4.2 Samples

Collect a certain number of samples representative of the total sample population being tested by the instrument, and whose composition varies regularly over the entire range of values of each component being measured. Normally, for the major milk components, the number of such samples should exceed 8. More samples may be required for minor or non-traditional components.

The samples should show no sign of physical deterioration and be preserved with the preservative normally used by the laboratory in routine test samples. Samples containing more than 10^6 somatic cells per millilitre should be discarded.

An alternative method, using modified milk samples without interrelationships between major components, is described.[15][16][17]

7.4.3 Analyses

Analyse the individual samples in duplicate using the reference methods to give the results y_i, and in triplicate using the instrument to be calibrated to give the results x_i.

7.4.4 Calculations

Calculate the arithmetic means, x and y, of the replicates for each individual sample and plot the values obtained (x and y) on a graph to check that no outliers are present. If necessary, repeat the analyses.

For each component, determine the equation of regression: $y = bx + a$, the mean bias $\left(\bar{d}\right)$ and the residual standard deviation, s_{yx}, from the regression. For cow's milk, the value s_{yx} should not exceed 0,06 % for each major component. Limit values for minor components will differ.

In case either the calculated regression coefficient, b, differs significantly from the operational slope or the calculated regression coefficient, a, differs significantly from the operational intercept, recalibrate the instrument in accordance with the manufacturer's instructions.

Moreover, the calibration should be checked whenever any major part of the instrument (cell, homogenizer and optical deck) is serviced or changed.

8 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 707|IDF 50.[1]

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Sample bottles should be fit for use, i.e. to transfer test samples from the point of sampling to the laboratory without loss or damage.

Care is to be exercised that sample bottles are leakproof and that a proper empty volume is left. A too large empty volume can facilitate churning; a too small empty volume can cause problems with mixing.

9 Determination

Follow the instructions given by the manufacturer for the measurement of milk samples. Prior to analysis, raw milk samples should be heated to (40 ± 2) °C and mixed gently by inversion.

10 Checking daily short-term stability of the instrument

10.1 General

Check, by analysing regularly one or more control (pilot) samples, that the results remain within accepted tolerances, assuming that no change of the major physico-chemical characteristics of the control material occurs during storage. This test is useful, not only for checking the instrument's stability during a working day, but also from day to day between two subsequent slope and intercept checks; see 7.4.

10.2 Preparation and storage of control samples

Select a batch of the concerned matrix of average value for the concerned parameter and prepare carefully, under constant agitation, as many subsamples as required for one or more working days. Keep subsamples with a suitable preservative at (4 ± 2) °C. Note that the preservative should be added to the

bulk sample prior to splitting. Good quality preserved pasteurized or UHT milk can be stored safely for 2 weeks. Homogenized milk may be used only if the homogenization efficiency is checked separately.

NOTE To verify the uniformity of subsamples, select at random a minimum of 10 subsamples from the batch. Measure the subsamples as a series of single determinations on an IR instrument. Calculate the standard deviation for the fat results. If this is below 0,015 % mass fraction, the uniformity is acceptable. Alternatively, a limit based on the standard deviation of sample s_s, s_s being estimated from n replicates per sample ($n \geq 2$), can be applied:

$$s_s = \left[S_d 2 - \left(S_r 2 / n \right) \right]^{1/2} \quad (5)$$

10.3 Analysis of control samples

Analyse control samples on a regular basis. A separate control sample should be analysed before and after analysis of routine test samples and at least three times per hour during continuous analysis.

Whenever a control sample result exceeds tolerances, the reliability of test results on all samples since the previous valid control sample, should be called into question.

10.4 Monitoring the analytical procedure

In order to monitor the quality of the whole analytical procedure including the instrument's stability, set up a control chart in accordance with ISO 8196-2|IDF 128-2.

10.5 Re-adjustment of instrument settings

10.5.1 Procedure

Action should be taken when

a) the cumulative arithmetic mean, m, is for two consecutive measurements of the control sample outside the same (upper or lower) confidence belt, indicating that the instrument is drifting; this deviation should normally be in the same direction as the deviations of the individual results outside the corresponding individual line, or

b) if in three or four cases, the individual control results fall near or outside the upper or lower limit for individual results, indicating a poor repeatability of the instrument due to the poor quality of the milk sample.

10.5.2 Quality control

In each case, first check the quality of the control milk sample by analysing, at least three times, a new sample of the same control milk assumed to be in good condition. If the quality of the control milk is poor, replace it.

If the quality of the control milk sample is satisfactory, purge and clean the measurement cell, zero-set the instrument and run again, at least three times, the control milk. If the corresponding results fall within the limits, it may be assumed that the detected drift was compensated for by the zero adjustment. Analysis may then be resumed.

If the results again fall outside the limits, evaluate whether this is caused by a random error (that is, poor instrument repeatability) or a systematic error (that is, inappropriate instrument calibration). In each case, stop and check the appropriate instrument functions (see Clause 6) and, if necessary, re-adjust the calibration (see 7.4). When completed, resume analysis and begin a new control chart.

Participation in proficiency testing is recommended.