INTERNATIONAL STANDARD

ISO 23291

IDF 248

First edition
2020-01

Milk and milk products — Guidelines for the application of in-line and on-line infrared spectrometry

Lait et produits laitiers — Lignes directrices pour l'application de la spectrométrie infrarouge en ligne

Reference numbers
ISO 23291:2020(E)
IDF 248:2020(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

International Dairy Federation
Silver Building • Bd Auguste Reyers 70/B
B-1030 Brussels
Phone: +32 2 325 67 40
Fax: +32 2 325 67 41
Email: info@fil-idf.org
Website: www.fil-idf.org

Published in Switzerland

Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee 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.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

IDF (the International Dairy Federation) is a non-profit private sector organization representing the interests of various stakeholders in dairying at the global level. IDF members are organized in National Committees, which are national associations composed of representatives of dairy-related national interest groups including dairy farmers, dairy processing industry, dairy suppliers, academics and governments/food control authorities.

ISO and IDF collaborate closely on all matters of standardization relating to methods of analysis and sampling for milk and milk products. Since 2001, ISO and IDF jointly publish their International Standards using the logos and reference numbers of both organizations.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. IDF shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

This document was prepared by the IDF *Standing Committee on Statistics and Automation* and ISO Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by ISO and IDF.

The work was carried out by the IDF/ISO Action Team (S12) of the *Standing Committee on Statistics and Automation* under the aegis of its project leaders, Dr S. Holroyd (NZ) and Dr A. Larsen (DK).

Milk and milk products — Guidelines for the application of in-line and on-line infrared spectrometry

1 Scope

This document gives guidelines for using infrared spectrometry in in-line and on-line applications for dairy processing. These applications are distinct to those covered in ISO 21543 | IDF 201.

It is applicable, but not limited, to:

— the determination of protein, fat and total solids in liquid milk and milk products using mid and near infrared spectrometry;

— the determination of protein, fat and moisture in solid or semi-solid products, such as milk powder, and butter and liquid dairy streams using near infrared spectrometry.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
in-line analysis
analysis of a product line where the sensor probe interfaces directly with the product stream being measured, or a reflectance measurement through an optical window into the product stream

3.2
on-line analysis
analysis of a product line where the sensor probe interfaces indirectly with the product stream being measured by way of a bleed loop, automated grab sampler or other means of subsampling

3.3
at-line analysis
analysis of a product where the instrument is physically remote to the product stream being measured and the sample is manually introduced to the instrument

Note 1 to entry: While not covered in this document, this definition is added here in order to distinguish this type of spectometric analysis from in- and on-line apparatus.

3.4
near infrared instrument
NIR instrument
proprietary apparatus utilizing wavelengths within the range 400 nm to 2 500 nm or 25 000 cm^{-1} to 4 000 cm^{-1} (both visible and NIR range) or 12 820 cm^{-1} to 4 000 cm^{-1} (NIR range only) which, when used under certain conditions, estimates mass fractions or other parameters of use

3.5
mid infrared instrument
MIR instrument
proprietary apparatus utilizing wavelengths in the range 4 000 cm^{-1} to 400 cm^{-1}, which, when used under the conditions specified in this document, estimates the mass fractions or other parameters of use specified in Clause 1

4 Principle

A laboratory in-line or on-line instrument is installed according to the manufacturer's guidelines for the type of process under measurement. Absorbance within the wavelength regions mentioned above is measured by transmission, reflectance and a combination of both, or by attenuated total reflectance (ATR). The resulting spectral information is transformed to constituent concentrations or constituent values with other units by calibration models developed by representative samples from the population to be tested.

5 Apparatus

5.1 Infrared instrument, based on diffuse reflectance or transmittance measurement in the near (400 nm to 2 500 nm or 25 000 cm^{-1} to 4 000 cm^{-1} or 12 820 cm^{-1} to 4 000 cm^{-1}) or mid (4 000 cm^{-1} to 400 cm^{-1}) wavelength region or segments of this or at discrete wavelengths. The optical operation principle may be dispersive (e.g. grating monochromators), interferometric or diode-array based. The instrument should be provided with an appropriate diagnostic test system for testing photometric instrument noise, wavelength accuracy and wavelength precision (for scanning spectrophotometers). The instrument shall be able to optically view into the product stream with an appropriate interface. There are many commercial such devices with a wide variety of technology depending on specific applications.

6 Installation and sampling considerations

6.1 General

Any installation shall cover both the integrity of the infrared instrument as well as interface with the process stream. Key aspects for the integrity of the instrument include:

— protection from cleaning regimes;

— isolation from vibration, dust and other environmental contaminants;

— an appropriate temperature regime for the specific instrument;

— many regions have strict protocols for materials in contact with human food products, such as Regulation (EC) No 1935/2004[3] or US equivalent 3A, and this is an important consideration for probes or cell construction.

A reliable, stable and consistent sampling interface is the key to successful use of in-line and on-line spectrometry. The following aspects are important.

— The ability to sample a representative flow of product. This can be ascertained by experimentation as well as an understanding of the fluid dynamics of the process flow.

— The ability to be consistently cleaned to the same level as the rest of the installation for good grade hygiene. For liquid product streams, this may mean that the probe is cleaned by the regular cleaning in place protocols. For powder, an air jet or similar can be necessary to remove sample prior to each measurement. Experimentation is often required to determine the most effective cleaning protocol for a specific environment.

— Stability over time. The interface shall not be altered by changes in plant or process as these can impact spectral quality and thus predictive performance of the instrument.

— Pipe, flow direction, sampling valve position and other technical considerations.

A critical component of any in-line or on-line system is how it samples from the process stream. The following key aspects shall be considered:

— the relationship between sample and spectra;

— the probe: optical interface, location and type;

— sampling and sample handling and timing, especially afterwards until the sample is analysed by the reference method;

— combinations and composition ranges of major and minor sample components: analytes (total solids, fat and protein) and non-analytes (component that can interfere with the results);

— seasonal, geographic and genetic effects on milk composition;

— different processing conditions, the design of production line and the speed of the process flow;

— the temperature, pressure and homogeneity;

— the turbulent/laminar flow, air entrapment or foaming of the process stream.

The infrared measurements and reference analyses shall be performed on the same test sample in order to minimize effects related to sampling uncertainty. It is suggested that sampling should take place in a steady-state situation, meaning there is very little fluctuation in sample composition in the piping at the moment of sampling. With in-line analysis, this means that a spectrum shall be taken preferably with a manual signalling option actually on the instrument housing that will flag the moment a manual sample is taken. This serves to accurately record the time of the sample collection and match it with the corresponding spectra.

An understanding of the process time constants is also important to assess to ensure alignment between the reference sample and spectroscopic measurement. The infrared measurements and the initiation of reference analyses should also be performed with a minimum time lag (preferably less than one day) and should reflect the stability of the matrix. The handling and treatment of samples from the time they are taken until their analysis by the reference method is also important: it shall be consistent and maintain the integrity of the sample. For example, milk powder may be cooled for a specific period prior to reference analysis. It is important this is similar for all samples used to build and validate the reference set and in routine use.

Sampling procedures should be respected uniformly during the calibration, validation and monitoring steps and not changed over time. The ability to obtain a representative sample from the process and link it closely to an infrared spectrum is important. Effective sampling will vary depending on the nature of the process stream. In addition, as it is not normally a single sample that gets laboratory tested for fat, protein and solids but sub-samples, the ability to sub-sample effectively should also be considered.

The sampling point should be defined properly. Samples should be collected in such a way that they represent the same or similar portion of sample that was measured by the infrared instrument. Considerations such as homogeneity of the process stream are important and it can be challenging to ensure a consistent representative sample is obtained in all process conditions. It is important to accurately record the time of the sample collection and match it with the corresponding spectra.

6.2 In-line analysis systems

An in-line system shall be set up so that a truly representative flow of material passes across the optical interface, and that there is no build-up of either material or contamination on the optical surface of the

probe. There are many commercial probes for in-line systems depending upon the type of matrix being sampled. When a probe type is being selected, the following considerations are important:

— an appropriate optical interface, given process stream, range of environment conditions and matrix variation over time;

— the compatibility of the probe with the instrument and the fibre optical connection to the instrument;

— the ability to change/remove the probe if required;

— the cost of installation;

— the location of the probe in relation to the presence of a sample valve where a corresponding sample for reference testing is taken.

6.3 On-line analysis systems

For on-line systems, the considerations differ as they shall reliably extract a representative sample of material from the process stream. The extraction system shall be able to be kept clean and remain reliable and consistent over an extended period. The types of commercially available extraction systems differ depending on the process stream matrix. Furthermore, the samples should be collected and measured over a certain period of time to ensure inclusion of time-dependent effects. This design will improve the ruggedness and give a more even performance of the calibration over the entire analyte concentration range.

7 Types of process flows

7.1 General

Each type of process flow has differing considerations regarding the instrument set up.

7.2 Liquids

Liquid in-line analysers are installed in an upstream flow. In this way, the liquid present in the pipe will apply a pressure to the measurement point, so it is sure there is no air. The sampling point, for calibration analysis and monitoring, should be placed at the measuring point or as close as possible downstream. A possibility to indicate the sampling time and duration should be provided. A sanitary sampling device shall be used.

7.3 Semi-solids

With process streams, such as butter and cream or cottage cheese, effective placement and operation of in-line probes for measurement is very important. Care shall be taken that the probe interfaces with a representative flow of product and no build up or material impedes measurement. With heterogeneous materials, such as cream or cottage cheese, it is also important that an appropriate reference sample volume can be taken from the line and correctly handled so as to provide an accurate reference measurement.

7.4 Powders

Milk and protein powders are also a challenging process stream to accurately measure in-line. Probes may be positioned so as to ensure they interface with a flow of fresh powder. The probes themselves may also be designed in such a way as to catch a powder sample and then remove it after measurement via clean compressed air, so the powder is stationary during measurement with no air gaps that could introduce non-uniformity. In other cases, powder may be scanned through a window with renewing powder flow. The sampling point should be as close to the probe as possible to retrieve reference/calibration samples.

8 Calibration and validation

It is noted that calibration and validation is a complex area and many aspects are common to both at-line as well as in-line or on-line instruments. These are well covered in ISO 21543 | IDF 201.

The instrument shall be calibrated before it is used. Because of the complex nature of the infrared wavelength region[2], the instrument shall be calibrated on a series of process samples with corroborating laboratory reference tests or similar analytical results. It is noted that when the process is statistically under control, recognized secondary methods such as at-line NIR/Fourier-transform infrared spectroscopy (FTIR) can also be used to develop and periodically check the performance of calibration models. However, it is strongly recommended that the bulk of the spectra used for calibration are measured under in-line or on-line process conditions. An in-line instrument that is located at-line or in a laboratory may be used with care to extend the range of calibration samples, but these should not comprise the bulk of the calibration set. This will ensure spectral variation inherent to the process is adequately captured in the calibration spectra set. Due to the nature of NIR spectra, typically an NIR instrument will require a greater number of calibration samples than a MIR instrument, but this can vary depending on the sample matrix type and the specific compositional parameter being calibrated. If a secondary instrument is used as reference for the calibration and validation work, then it is essential to ensure it is in adequate control. When using chemical analysis as reference analysis, it is highly recommended to use duplicate analyses instead of a single analysis.

If the required agreement and precision cannot be obtained by a single calibration, then the application area can be split up into static or dynamic sub-areas, each with an associated calibration, to fulfil the requirements. These would ideally be selected automatically when required in conjunction with changes in processing conditions. A nonlinear modelling approach could also be used. Here, an identification step could be used to know which sample/product is currently to be measured. A model selection by a trigger from the process control system can also be useful adjunct to ensure the appropriate models are in use for different products.

With in-line instruments, there is often a challenge to obtain sufficient variation in sample composition. Ideally, deliberate process variation can be used to obtain an appropriate sample range. If this is not possible, samples taken from start up or during processing changes can yield greater variation, provided care is taken that they are truly representative of the process.

Calibration models developed for in-line instruments should have good performance in terms of agreement and precision as described in ISO 21543 | IDF 201. In some cases, where the reaction time of the process is much longer than the in-line instrument measurement time, a plot of moving average of results can be very helpful to increase the performance of the instrument reading. When averaging can increase accuracy of the instrument reading or trending, the results can be used in a better way to control the process. However, even more important is to have good performance with respect to the repeatability in order to detect small variations in the production. It is also possible to use in-line infrared measurement for trend control. In cases of a biased reading, the results can still be used to control the process based on trending information.

Validation should be accomplished by checking the performance of the installation and associated calibration using an independent test set, preferably sampled after the calibration period but also covering a similar amount of process variation, if possible. This data can be analysed as described in ISO 21543 | IDF 201 and the calibration adjusted if required. Following calibration adjustment, re-validation should be carried out. A validation report specifying the following information should also be prepared:

— all the information necessary for complete identification of the samples (e.g. date, sample type);

— the laboratory test method(s) used, with reference to the relevant International Standards and including a laboratory reference error;

— any unusual circumstances that could have influenced the results;

— the test results obtained;

— the root mean square error of prediction (RMSEP);

— the root mean square error of cross validation (RMSECV).

9 Long-term monitoring and calibration adjustment

The calibration shall be evaluated both when implemented and over time in order to assess the number of outliers and observe any product changes not represented in the calibration. Outliers can be identified in a number of ways. There is a range of statistical techniques for assessing outliers: these are software dependent and set a threshold above which samples are classified as outliers. These could be true process outliers or alternatively simply indicate a sample that captures normal variability. Their inclusion can be an effective method of expanding the calibration.

Before adding samples identified as potential outliers, it should be considered if the samples bring value or error to the calibration. The start-up of a process or analysis at the beginning of a batch process can have measurements that are not valuable to the calibration since the product can be very heterogeneous and thus may not be representative for the analyte of interest.

For in-line analysis applications, outliers can be detected regularly due to insufficient sample presentation (e.g. air bubbles, incidences when there is no sample or a sample partly visible during instrument scanning time). Single results detected as outliers do not require confirmation by means of a reference analysis method.

The frequency of checking the instrument calibrations should be sufficient to ensure the calibration is operating under steady control with respect to systematic (bias or slope/intercept) and random [standard error of prediction (SEP)] deviations from the reference method. The calibration surveillance sampling frequency depends on the number of products produced, but five to ten calibration surveillance samples per week is in most cases sufficient to monitor the performance properly. The number of surveillance samples will also depend on the size of the bias to be detected. The smaller the bias shift, the more samples can be needed to meet the required level of significance. When implementing a bias, it is important to ensure that the value used truly reflects a significant systematic difference between the predicted result and reference values. To this end, assessing multiple results over time alongside an assessment of result significance by means of classic statistical methods ensures that a bias adjustment based on a short-term erroneous result is not implemented.

An appropriate response with regards to an observed bias is important. A bias is simply an arithmetic correction factor applied to results in order for them to more closely align with reference results. The application of a bias that is large in relation to the range of results and/or fluctuates significantly on a daily or weekly basis can indicate that recalibration is required or that there are other issues impacting performance.

Calibration surveillance samples should be collected randomly to reduce the possibility to implement a systematic error in the surveillance. To ensure the entire product concentration range and all product types are included in the calibration surveillance, a selective sampling can be added to the random selected calibration surveillance samples.

Monitoring is part of the internal control plan. The trueness monitoring of the calibration model ensures that the method performs according to performance established during the validation step. This requires the analysis of production samples by infrared and by reference method according to a predefined monitoring plan.

Each instrument will have different protocols for its short-term and long-term stability. For short-term stability, it is suggested that a plot of day-to-day performance test data is useful. This data or some subset of it may be averaged to provide an indication of longer-term stability, but overall a defined plan that affords a rapidly assessed measure of instrument health is important.

Sample type, handling and condition as well as the frequency should be proposed.

