BS EN ISO 12779:2013

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Lactose — Determination of water content — Karl Fischer method

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National foreword

This British Standard is the UK implementation of EN ISO 12779:2013. It is identical to ISO 12779:2011. It supersedes BS ISO 12779:2011, which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee AW/5, Chemical analysis of milk and milk products.

A list of organizations represented on this committee can be obtained on request to its secretary.

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Lactose - Determination of water content - Karl Fischer method (ISO 12779:2011)

Lactose - Détermination de la teneur en eau - Méthode de Karl Fischer (ISO 12779:2011)

Lactose - Bestimmung des Wassergehaltes - Karl-Fischer-Verfahren (ISO 12779:2011)

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Foreword

The text of ISO 12779:2011 has been prepared by Technical Committee ISO/TC 34 “Food products” of the International Organization for Standardization (ISO) and has been taken over as EN ISO 12779:2013 by Technical Committee CEN/TC 302 “Milk and milk products - Methods of sampling and analysis” the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by November 2013, and conflicting national standards shall be withdrawn at the latest by November 2013.

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Lactose — Determination of water content — Karl Fischer method

1 Scope

This International Standard specifies a method for the determination of the water content of lactose by Karl Fischer (KF) titration.

2 Principle

The test sample is directly titrated with a commercially available two-component Karl Fischer reagent. The water content is calculated from the amount of reagent used.

Titration at a temperature of approximately 40 °C gives shorter titration times and sharper end points. Only at this or higher temperatures is the use of a one-component Karl Fischer reagent (3.1) recommended.

3 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and only distilled or demineralized water or water of equivalent purity. Avoid absorption of moisture from the environment.

3.1 Karl Fischer (KF) reagent[1]. The KF reagent is a commercially available pyridine-free two-component reagent consisting of a titration component (3.2) and a solvent component (3.3). The titration component (3.2) is a methanolic solution of iodine and the solvent component (3.3) is a methanolic solution of sulfur dioxide and an appropriate base (e.g. imidazole).

Alternatively, a pyridine-free one-component KF reagent can also be used which only consists of a titration component. The titration component (3.2) is a solution of iodine, sulfur dioxide and an appropriate base (e.g. imidazole) in a suitable solvent (e.g. diethylene glycol monoethyl ether). The solvent component (3.3) is methanol (3.5). This combination of a one-component titration reagent and methanol is only recommended if the titration is carried out at a temperature of approximately 40 °C or higher.

3.2 Titration component. The titration component of the KF reagent (3.1) shall have a water equivalent of approximately 2 mg/ml of water.

A KF reagent with a water equivalent of approximately 5 mg/ml of water is also possible if a KF apparatus with a burette of capacity 5 ml is used (4.1).

3.3 Solvent component. The solvent component of the KF reagent as specified in 3.1.

3.4 Water standard, $w(H_2O)$ = 10 mg/g.

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[1] Hydranal®-Titrant 2/Hydranal®-Solvent from Sigma-Aldrich and apura® Titrant 2/apura® Solvent from Merck are examples of commercially available two-component systems. Hydranal®-Composite 2 from Sigma-Aldrich and apura® CombiTitrant 2 from Merck are examples of commercially available one-component reagents. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO and IDF of these products.

For the determination of the water equivalent of the titration component, use a commercially available liquid water standard containing 10 mg/g of water.

3.5 **Methanol** (CH_3OH), with a mass fraction of water of not more than 0,05 %.

4 Apparatus

Usual laboratory apparatus and, in particular, the following. Equipment shall be completely dry when used.

4.1 **Karl Fischer (KF) apparatus**. Use an automatic volumetric KF apparatus, preferably with a burette of capacity 10 ml.

The burette should be of capacity 5 ml if a KF reagent with a water equivalent of approximately 5 mg/ml of water is used (see 3.2).

For quicker titrations at approximately 40 °C, use a double-jacketed titration vessel with a water inlet and outlet to carry out the titration.

4.2 **Water bath**, capable of maintaining a water temperature of 40 °C ± 5 °C under thermostatic control, with a pump for heating the double-jacketed titration vessel of the KF apparatus (4.1).

NOTE The use of a water bath with thermostatic control is only necessary if the titrations are carried out at temperatures above room temperature.

4.3 **Analytical balance**, capable of weighing to the nearest 1 mg, with a readability of 0,1 mg.

4.4 **Disposable syringes**, of capacity 5 ml or 10 ml, for quantifying the water standard (3.4).

4.5 **Glass weighing spoon**, for dosing the test sample.

5 Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

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

6 Procedure

6.1 KF apparatus drift

6.1.1 Determine the drift of the KF apparatus (4.1) every day and/or when the working conditions change as follows.

Add to the titration vessel of the KF apparatus (4.1) approximately 30 ml of solvent component (3.3). Pre-titrate the solvent component. Then carry out a titration over 5 min (without addition of a test sample).

If determinations (6.5) are carried out at 40 °C, heat the vessel and its contents using the water bath (4.2) to 40 °C before pre-titrating the solvent component and carrying out the titration without addition of a test sample.

IMPORTANT — During each titration, the amount of solvent component used shall completely cover the electrodes of the KF apparatus.

6.1.2 Calculate the KF apparatus drift, q_V, in millilitres per minute, by using Equation (1):

$$q_V = \frac{V_\mathrm{d}}{t} \tag{1}$$

where

V_d is the volume, in millilitres, of the titration component (3.2) in the titration (6.1.1);

t is the numerical value, in minutes, of the time.

Alternatively, calculate the KF apparatus drift, q_m, in milligrams of water per minute, by using Equation (2):

$$q_m = \frac{V_\mathrm{d}\ \rho_\mathrm{e}}{t} \tag{2}$$

where ρ_e is the water equivalent (6.2) of the titration component (3.2), in milligrams of water per millilitre, used in the titration (6.1.1).

6.2 Water equivalent of the titration component of the KF reagent

6.2.1 Titration

To calculate the water equivalent of the titration component, ρ_e, accurately carry out the titration in this subclause and the calculation step (6.2.2) five times.

Add approximately 30 ml solvent component (3.3) to the titration vessel of the KF apparatus (4.1) (see also 6.1.1) and pre-titrate the solvent component.

NOTE The water equivalent of the titration component does not depend on temperature. It is therefore not necessary to heat the titration vessel for water equivalent determinations. If determinations (6.5) are carried out at 40 °C, the water equivalent can also be determined at 40 °C. In that case, the vessel and its contents are heated to 40 °C using the water bath (4.2) before carrying out titrations.

Into a disposable syringe (4.4), take up between 0,6 g to 0,9 g of the water standard (3.4) (an amount of between 6 mg and 9 mg water). Weigh the syringe and its contents, recording the mass to the nearest 0,1 mg.

It is also possible to take up more than 0,6 g to 0,9 g of the water standard and to use the same contents of the syringe for several titrations, but it is then important to carry out the titrations immediately one after the other. In this case, approximately 0,6 g to 0,9 g of the water standard should also be used for each titration, the exact amount being calculated each time by weighing the syringe and its remaining contents after each injection.

NOTE Only for very experienced laboratories is it possible to use a small amount (approximately one drop from a syringe) of pure water instead of a commercial water standard. Even slight weighing errors lead to massive deviations of the result since they have an effect of 100 %.

Add the amount of water standard in the syringe to the titration vessel. Weigh the empty syringe again, recording its mass to the nearest 0,1 mg. Subtract the mass of the empty syringe from that of the syringe with the water standard before addition of the water standard to the vessel to obtain the net mass of the water standard added to the titration vessel. Record the net mass, $m_{\mathrm{H_2O}_i}$, to the nearest 0,1 mg, of added water standard for each titration (i = 1 … 5).

Carry out a titration while using as shut-off criterion the 10 s stop delay time mentioned in 6.3. Record, for i = 1 … 5, the volume, $V_{\rho_{\mathrm{e},i}}$, in millilitres, of the titration component used.

Do not perform more than three titrations on one portion of solvent component.

After three titrations on one portion, empty the titration vessel and carry out further titrations by again adding to the titration vessel of the KF apparatus approximately 30 ml solvent component, pre-titrating the solvent component and proceeding in the same way as before.

6.2.2 Calculation

Calculate the water equivalent of the titration component, $\rho_{e,i}$, in milligrams of water per millilitre, by using Equation (3):

$$\rho_{e,i} = \frac{m_{H_2O_i} \, w_{H_2O,s}}{V_{\rho_{e,i}}} \qquad (3)$$

where

$m_{H_2O_i}$ is the mass, in grams, of the water standard used for titration i (6.2.1);

$w_{H_2O,s}$ is the water mass fraction, in milligrams per gram, of the water standard;

$V_{\rho_{e,i}}$ is the volume, in millilitres, of the titration component (3.2) in titration i (6.2.1).

6.2.3 Mean value

Calculate the water equivalent of the titration component, ρ_e, expressed in milligrams of water per millilitre, as the mean value of the five (i = 1 … 5) single titrations (6.2.1) by using Equation (4):

$$\rho_e = \frac{\sum_{i=1}^{5} \rho_{e,i}}{5} \qquad (4)$$

6.2.4 Expression of water equivalents

Express the (mean) value of the water equivalent of the titration component to three decimal places.

6.3 Shut-off criterion

According to the features of the apparatus used, the stop criterion shall either be a stop delay time of 10 s or a stop drift slightly above the measured drift (6.1).

6.4 Preparation of the test sample

Homogenize the test sample by mixing the powder thoroughly.

6.5 Determination

Add approximately 30 ml of solvent component (3.3) to the titration vessel of the KF apparatus (4.1). If a one-component KF reagent is used (3.1) or if shorter titration times are preferred, heat the vessel and its contents using the water bath (4.2) to approximately 40 °C.

Charge a glass weighing spoon (4.5) with approximately 0,3 g test sample (6.4). Weigh the spoon and the test portion, recording the mass to the nearest 0,1 mg.

Add the test portion to the titration vessel by quickly opening and closing the septum stopper. Weigh the empty weighing spoon again, recording its mass to the nearest 0,1 mg. Subtract the mass of the spoon from that of the test portion and spoon to obtain the net mass, m, recorded to the nearest 0,1 mg, of the test portion.

Carry out a titration and record the volume, V, in millilitres, of titration component. Do not perform more than two titrations on one portion of the solvent component.

If the titrator does not automatically calculate the results (see 7.1), record the titration time, t.

It is recommended that several replicates be carried out for each sample to obtain more accurate results. In that case, record for each replicate, i, the individual portion mass, m_i, the volume of titration component, V_i, and the titration time, t_i.

7 Calculation and expression of results

7.1 Calculation

Appropriate automatic calculation by the titrator may also be used, if available.

7.1.1 Calculate the water content of the test sample, $w_{\mathrm{H_2O}}$, expressed as percentage by mass, by using either Equation (5) or (6):

$$w_{\mathrm{H_2O}} = \frac{\left(V - q_V\ t\right)\rho_{\mathrm{e}}}{m} \times 100\ \% \tag{5}$$

$$w_{\mathrm{H_2O}} = \frac{V\ \rho_{\mathrm{e}} - q_m\ t}{m} \times 100\ \% \tag{6}$$

where

V is the volume, in millilitres, of titration component (6.5);

q_V is the numerical value of the drift, in millilitres per minute (6.1.2);

q_m is the numerical value of the drift, in milligrams of water per minute (6.1.2);

t is the titration time, in minutes (6.5);

ρ_{e} is the water equivalent of the titration component, in milligrams of water per millilitre (6.2.3);

m is the mass, in milligrams, of the test portion (6.5).

7.1.2 If n replicates for a sample have been carried out, calculate for each of the replicates, i, the individual water content of the test sample, $w_{\mathrm{H_2O},\,i}$, as a percentage mass fraction, by using either Equation (7) or (8):

$$w_{\mathrm{H_2O},\,i} = \frac{\left(V_i - q_V\ t_i\right)\rho_{\mathrm{e}}}{m_i} \times 100\ \% \tag{7}$$

$$w_{\mathrm{H_2O},\,i} = \frac{V_i\ \rho_{\mathrm{e}} - q_m\ t_i}{m_i} \times 100\ \% \tag{8}$$

where

V_i is the volume, in millilitres, of the titration component for replicate i (6.5);

t_i is the titration time, in minutes, for replicate i (6.5);

m_i is the mass, in milligrams, of the test portion for replicate i (6.5);

i is the number of the replicate, $i = 1 \dots n$.

7.1.3 Calculate for the n replicates the mean value of the water content, $\bar{w}_{\mathrm{H_2O}}$, as a percentage mass fraction, of the test sample by using Equation (9):

$$\bar{w}_{\mathrm{H_2O}} = \frac{\sum_{i=1}^{n} w_{\mathrm{H_2O},\,i}}{n} \tag{9}$$

7.2 Expression of test results

Express the test results to two decimal places.

8 Precision

8.1 Interlaboratory test

The values for repeatability and reproducibility derived from this interlaboratory test were determined in accordance with ISO 5725-1[2] and ISO 5725-2[3].

The values for the repeatability and the reproducibility limit are expressed for the 95 % probability level and may not be applicable to concentration ranges and matrices other than those given. Details of the interlaboratory test[4] on the precision of the method are given in Annex A.

8.2 Repeatability

The absolute difference between two individual single test results, obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, shall in not more than 5 % of cases be greater than 0,15 %.

8.3 Reproducibility

The absolute difference between two individual single test results, obtained with the same method on identical test material in different laboratories with different operators using different equipment, shall in not more than 5 % of cases be greater than 0,16 %.

9 Test report

The test report shall contain at least the following information:

a) all information necessary for the complete identification of the sample;

b) the sampling method used, if known;

c) the test method used, with reference to this International Standard (ISO 12779|IDF 227:2011);

d) all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

e) the test result(s) obtained;

f) if the repeatability has been checked, the final quoted result obtained.

Annex A
(informative)

Interlaboratory trial

An international collaborative test involving 15 laboratories was carried out on eight samples (four samples in blind duplicates) of lactose from Germany. Three laboratories did not respect the instructions and two showed significant differences as compared to the others, one with high duplicate ranges, the other with large bias. They were therefore excluded from the statistical evaluation. Observed homogeneity in individual precision values allows the calculated mean (overall) precision values r = 0,15 g/100 g and R = 0,16 g/100 g, which are recommended for use.

Table A.1 shows the combined results for the identical samples. The values were calculated on the basis of the mean values of the single samples reported by each laboratory.

Table A.1 — Results of the interlaboratory test combining identical samples

Parameter	**Sample**				**Mean**
	1 and 4	2 and 7	3 and 6	5 and 8	
Number of laboratories after eliminating outliers	10	10	10	10	—
Mean value, %	4,805	5,100	5,140	5,022	—
Repeatability standard deviation, s_r, %	0,053	0,045	0,062	0,049	0,053
Coefficient of variation of repeatability, %	1,1	0,9	1,2	1,0	1,0
Repeatability limit, r = 2,8 s_r, %	0,148	0,125	0,174	0,137	0,147
Reproducibility standard deviation, s_R, %	0,060	0,045	0,065	0,059	0,058
Coefficient of variation of reproducibility, %	1,2	0,9	1,3	1,2	1,1
Reproducibility limit, R = 2,8 s_R, %	0,168	0,125	0,182	0,164	0,161

Bibliography

[1] ISO 707|IDF 50, *Milk and milk products — Guidance on sampling*

[2] ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

[3] ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

[4] ISENGARD, H.-D., HASCHKA, E., MERKH, G. Lactose — Determination of water content by Karl Fischer titration — Interlaboratory collaborative study. *Bull. Int. Dairy Fed.* 2011, (450)

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