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EAS 81-3 (2006) (English): Milk powders  
Methods of analysis Part 3:  
Determination of fat content -  
Gravimetric method

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**ISO INSIDE**  
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## **EAST AFRICAN STANDARD**

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**Milk powders — Methods for the analysis — Part 3: Determination of fat content — Gravimetric method (Reference method)**

**EAST AFRICAN COMMUNITY**

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# EAS 81-3:2006

## Foreword

Development of the East African Standards has been necessitated by the need for harmonizing requirements governing quality of products and services in East Africa. It is envisaged that through harmonized standardization, trade barriers which are encountered when goods and services are exchanged within the Community will be removed.

In order to achieve this objective, the Partner States in the Community through their National Bureaux of Standards, have established an East African Standards Committee.

The Committee is composed of representatives of the National Standards Bodies in Partner States, together with the representatives from the private sectors and consumer organizations. Draft East African Standards are circulated to stakeholders through the National Standards Bodies in the Partner States. The comments received are discussed and incorporated before finalization of standards, in accordance with the procedures of the Community.

East African Standards are subject to review, to keep pace with technological advances. Users of the East African Standards are therefore expected to ensure that they always have the latest versions of the standards they are implementing.

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**Dried milk and dried milk products —  
Determination of fat content — Gravimetric  
method (Reference method)**

*Lait sec et produits à base de lait sec — Détermination de la teneur en  
matière grasse — Méthode gravimétrique (Méthode de référence)*



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## 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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 1736 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 5, *Milk and milk products*, in collaboration with the International Dairy Federation (IDF) and AOAC International, and will also be published by these organizations.

This third edition cancels and replaces the second edition (ISO 1736:1985), which has been technically revised.

Annexes A and B of this International Standard are for information only.



# Dried milk and dried milk products — Determination of fat content — Gravimetric method (Reference method)

**WARNING** — The use of this International Standard may involve hazardous materials, operations and equipment. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this standard to establish safety and health practices and determine the applicability of regulatory limitations prior to use.

## 1 Scope

This International Standard specifies the reference method for the determination of the fat content of dried milk and dried milk products. The method is applicable to dried milk with a fat content of 40 % (mass fraction) or more, dried whole, dried partially skimmed, and dried skimmed milk, dried whey, dried buttermilk and dried butter serum.

**NOTE** When the powder contains hard lumps which do not dissolve in ammonia solution or contains free fatty acids in significant quantities, noticeable by a distinct smell, the result of the determination will be too low. With such products recourse should be made to a method using the Weibull-Berntrop principle (see ISO 8262-3).

## 2 Normative reference

The following normative document contains provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, this publication do not apply. However, parties to agreement based on this International Standard are encouraged to investigate the possibility of applying the most recent edition of the normative document indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 3889, *Milk and milk products — Determination of fat content — Mojonnier-type fat extraction flasks.*

## 3 Term and definition

For the purposes of this International Standard, the following term and definition applies.

### 3.1

#### **fat content of dried milk and dried milk products**

mass fraction of substances determined by the procedure specified in this International Standard

**NOTE** The fat content is expressed as a mass fraction, in percent [formerly given as % (*m/m*)].

## 4 Principle

An ammoniacal ethanolic solution of a test portion is extracted with diethyl ether and light petroleum. The solvents are removed by distillation or evaporation. The mass of the substances extracted is determined.

**NOTE** This is usually known as the Röse-Gottlieb principle.

## 5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity.

The reagents shall leave no appreciable residue when the determination is carried out by the method specified (see 9.2.2).

**5.1 Ammonia solution**, containing a mass fraction of  $\text{NH}_3$  of approximately 25 % ( $\rho_{20} = 910 \text{ g/l}$ ).

NOTE If ammonia solution of this concentration is not available, a more concentrated solution of known concentration may be used (see 9.4.2).

**5.2 Ethanol** ( $\text{C}_2\text{H}_5\text{OH}$ ), or ethanol denatured by methanol, containing a volume fraction of ethanol of at least 94 %. (See A.5.)

**5.3 Congo red solution**

Dissolve 1 g of Congo red in water in a 100 ml one-mark volumetric flask (6.14). Dilute to the mark with water.

NOTE The use of this solution, which allows the interface between the solvent and aqueous layers to be seen more clearly, is optional (see 9.4.4). Other aqueous colour solutions may be used provided that they do not affect the result of the determination.

**5.4 Diethyl ether** ( $\text{C}_2\text{H}_5\text{OC}_2\text{H}_5$ ), free from peroxides (see A.3), containing no more than 2 mg/kg of antioxidants, and complying with the requirements for the blank test (see 9.2.2, A.1 and A.4).

NOTE The use of diethyl ether could lead to hazardous situations. Due to expected changes in safety regulations, studies are ongoing to replace diethyl ether by another reagent provided that it does not affect the end result of the determination.

**5.5 Light petroleum**, with any boiling range between 30 °C and 60 °C or, as equivalent, **pentane** ( $\text{CH}_3[\text{CH}_2]_3\text{CH}_3$ ) with a boiling point of 36 °C and complying with the requirements for the blank test (see 9.2.2, A.1 and A.4).

NOTE The use of pentane is recommended because of its higher purity and constant quality.

**5.6 Mixed solvent**

Shortly before use, mix equal volumes of diethyl ether (5.4) and light petroleum (5.5).

## 6 Apparatus

**WARNING** — Since the determination involves the use of volatile flammable solvents, all electrical apparatus employed shall comply with legislation relating to the hazards in using such solvents.

Usual laboratory equipment and, in particular, the following.

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

**6.2 Centrifuge**, capable of holding the fat-extraction flasks or tubes (6.6) and capable of spinning at a rotational frequency of 500  $\text{min}^{-1}$  to 600  $\text{min}^{-1}$  to produce a radial acceleration of 80 *g* to 90 *g* at the outer end of the flasks or tubes.

NOTE The use of the centrifuge is optional but recommended (see 9.4.7).

**6.3 Distillation or evaporation apparatus**, for distilling the solvents and ethanol from the boiling or conical flasks, or evaporating from beakers and dishes (see 9.4.14) at a temperature not exceeding 100 °C.

**6.4 Drying oven**, electrically heated, with ventilation port(s) fully open, capable of being maintained at a temperature of  $102\text{ °C} \pm 2\text{ °C}$  throughout its working space.

The oven shall be fitted with a suitable thermometer.

**6.5 Water bath**, capable of being maintained at a temperature of  $65\text{ °C} \pm 5\text{ °C}$ .

**6.6 Mojonnier-type fat-extraction flasks**, as specified in ISO 3889.

NOTE It is also possible to use fat-extraction tubes, with siphon or wash-bottle fittings, but then the procedure is different. The alternative procedure is given in annex B.

The fat-extraction flasks shall be provided with good quality corks or stoppers of another material [e.g. silicone rubber or polytetrafluoroethylene (PTFE)] unaffected by the reagents used. Bark corks shall be extracted with the diethyl ether (5.4), kept in water at a temperature of  $60\text{ °C}$  or more for at least 15 min, and shall then be allowed to cool in the water so that they are saturated when used.

**6.7 Rack**, for holding the fat-extraction flasks (or tubes) (6.6).

**6.8 Wash bottle**, suitable for use with the mixed solvent (5.6).

A plastics wash bottle shall not be used.

**6.9 Fat-collecting vessels**, such as boiling flasks (flat-bottomed), of capacities 125 ml to 250 ml, conical flasks, of capacity 250 ml, or metal dishes.

If metal dishes are used, they shall be of stainless steel, flat-bottomed with a diameter of 80 mm to 100 mm and a height of approximately 50 mm.

**6.10 Boiling aids**, fat-free, of non-porous porcelain or silicon carbide (optional when metal dishes are used).

**6.11 Measuring cylinders**, of capacities 5 ml and 25 ml.

**6.12 Pipettes**, graduated, of capacity 10 ml.

**6.13 Tongs**, made of metal, for holding flasks, beakers or dishes.

**6.14 Volumetric flask**, one-mark, of capacity 100 ml.

## 7 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 707.

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

Store the samples at a temperature of between  $2\text{ °C}$  and  $6\text{ °C}$  from the time of sampling.

## 8 Preparation of test sample

Thoroughly mix the test sample by repeatedly rotating and inverting the sample container. If necessary, transfer all of the test sample to an airtight container of approximately twice the volume of the test sample to allow this operation to be carried out.

## 9 Procedure

NOTE 1 If it is required to check whether the repeatability limit (11.2) is met, carry out two single determinations in accordance with 9.1 to 9.4.

NOTE 2 An alternative procedure using fat-extraction tubes with siphon or wash-bottle fittings (see note in 6.6) is given in annex B.

### 9.1 Test portion

Mix the test sample (clause 8) by gently stirring or rotating and inverting the container several times. Immediately weigh to the nearest 1 mg, directly or by difference, in a fat-extraction flask (6.6) one of the following test portions:

- a) about 1 g of dried high-fat milk, of dried whole milk or of dried butter serum;
- b) about 1,5 g of dried partially skimmed milk;
- c) about 1,5 g of dried skimmed milk;
- d) about 1,5 g of dried whey;
- e) about 1,5 g of dried buttermilk.

Transfer the test portion as completely as possible into the lower (small) bulb of the fat-extraction flask.

### 9.2 Blank tests

#### 9.2.1 Blank test for method

Carry out a blank test simultaneously with the determination using the same procedure and same reagents, but replacing the dispersed test portion in 9.4.1 by 10 ml of water (see A.2).

If the value obtained in the blank test regularly exceeds 1,0 mg, check the reagents if this has not been recently done (9.2.2). Corrections of more than 2,5 mg should be mentioned in the test report.

#### 9.2.2 Blank test for reagents

To test the quality of the reagents, carry out a blank test as specified in 9.2.1. Additionally use an empty fat-collecting vessel, prepared as specified in 9.3, for mass control purposes. The reagents shall leave no residue greater than 1,0 mg (see A.1).

If the residue of the complete reagent blank test is greater than 1,0 mg, determine the residue of the solvents separately by distilling 100 ml of the diethyl ether (5.4) and light petroleum (5.5), respectively. Use an empty fat-collecting vessel, prepared for control purposes as described above, to obtain the real mass of residue which shall not exceed 1,0 mg.

Very occasionally, the solvents may contain volatile matter which is strongly retained in fat. If there are indications of the presence of such substances, carry out blank tests on all the reagents and for each solvent using a fat-collecting vessel with about 1 g of anhydrous butterfat. If necessary, redistil solvents in the presence of 1 g of anhydrous butterfat per 100 ml of solvent. Use the solvents only shortly after the redistillation.

Replace unsatisfactory reagents and solvents, or redistil solvents.

### 9.3 Preparation of fat-collecting vessel

Dry a fat-collecting vessel (6.9) with a few boiling aids (6.10) in the oven (6.4) set at 102 °C for 1 h.

NOTE 1 Boiling aids are desirable to promote gentle boiling during the subsequent removal of solvents, especially when using glass fat-collecting vessels; their use is optional with metal dishes.

Protect the fat-collecting vessel from dust and allow it to cool to the temperature of the weighing room (glass fat-collecting vessel for at least 1 h; metal dish for at least 30 min).

NOTE 2 To avoid insufficient cooling or unduly long cooling times, the fat-collecting vessel should not be placed in a desiccator.

Use tongs (6.13) to place the fat-collecting vessel on the balance. Weigh the fat-collecting vessel to the nearest 1,0 mg.

NOTE 3 Tongs should preferably be used to avoid, in particular, temperature variations.

## 9.4 Determination

**9.4.1** Carry out the determination without delay.

Add about 10 ml of preheated water at a temperature of  $65\text{ °C} \pm 5\text{ °C}$  to the test portion in the fat-extraction flask (9.1) to obtain a total volume of 10 ml to 11 ml. Use the water to wash the test portion into the small bulb of the fat-extraction flask. Mix thoroughly with the test portion in the small bulb until the test portion is completely dispersed.

**9.4.2** Add 2 ml of ammonia solution (5.1) to the test portion (9.4.1), or an equivalent volume of a more concentrated ammonia solution (see note in 5.1). Mix thoroughly with the test portion in the small bulb of the fat-extraction flask.

**9.4.3** Heat the flask to  $65\text{ °C} \pm 5\text{ °C}$  in the water bath (6.5) for 15 min to 20 min with occasional shaking. Cool in running water to room temperature.

**9.4.4** Add 10 ml of ethanol (5.2). Mix gently but thoroughly by allowing the contents of the fat-extraction flask to flow backwards and forwards between the small and large bulb. Avoid bringing the liquid too near to the neck of the flask. If desired, add 2 drops of the Congo red solution (5.3).

**9.4.5** Add 25 ml of diethyl ether (5.4). Close the fat-extraction flask with a cork saturated with water or with a stopper of other material wetted with water (6.6). Shake the flask vigorously, but not excessively, for 1 min to avoid the formation of persistent emulsions.

While shaking, keep the fat-extraction flask in a horizontal position with the small bulb extending upwards, periodically allowing the liquid to run from the large bulb into the small bulb. If necessary, cool the flask in running water to about room temperature. Carefully remove the cork or stopper and rinse it and the neck of the flask with a little mixed solvent (5.6). Use the wash bottle (6.8) so that the rinsings run into the flask.

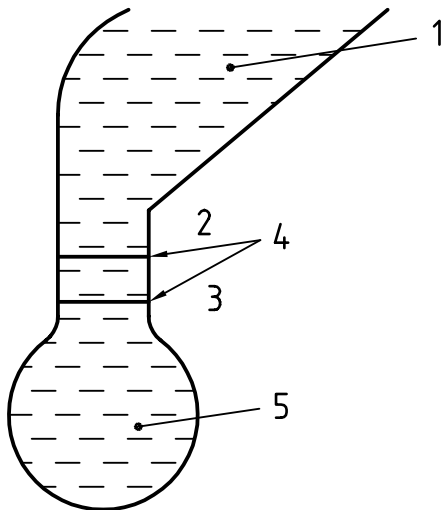
**9.4.6** Add 25 ml of the light petroleum (5.5). Close the fat-extraction flask with the rewetted (by dipping into water) cork or stopper. Mix gently again for 30 s as described in 9.4.4. Proceed with shaking as described in 9.4.5.

**9.4.7** Centrifuge the closed fat-extraction flask for between 1 min and 5 min at a radial acceleration of 80 *g* to 90 *g*. If a centrifuge (6.2) is not available, allow the closed flask to stand in the rack (6.7) for at least 30 min until the supernatant layer is clear and distinctly separated from the aqueous layer. If necessary, cool the flask in running water, to room temperature.

**9.4.8** Carefully remove the cork or stopper and rinse it and the inside of the neck of the fat-extraction flask with a little mixed solvent (5.6). Use the wash bottle (6.8) so that the rinsings run into the flask. If the interface is below the bottom of the stem of the flask, raise it slightly above this level by gently adding water down the side of the flask (see Figure 1) to facilitate the decanting of solvent.

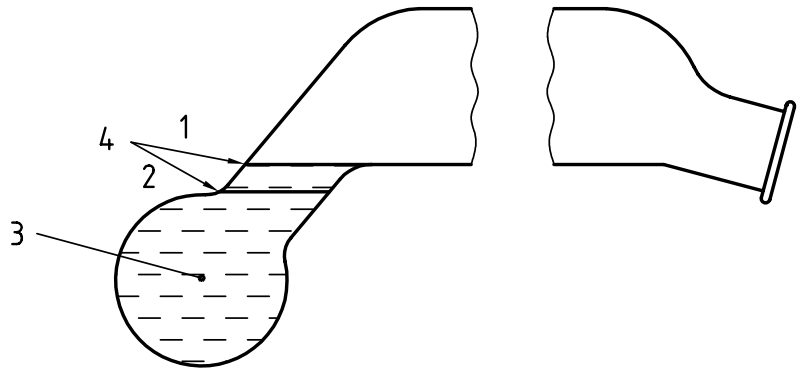
NOTE In Figures 1 and 2, one of the three types of fat-extraction flasks as specified in ISO 3889 has been chosen, but this does not imply any preference over other types.

**9.4.9** Hold the fat-extraction flask by the small bulb and carefully decant as much as possible of the supernatant layer into the prepared fat-collecting vessel (see 9.3) containing a few boiling aids (6.10) in the case of a boiling or conical flask (optional with metal dishes). Avoid decanting any of the aqueous layer (see Figure 2).



- Key**
- 1 Solvent
  - 2 At second and third extraction
  - 3 At first extraction
  - 4 Interface
  - 5 Aqueous layer

**Figure 1 — Before decanting**



- Key**
- 1 At second and third extraction
  - 2 At first extraction
  - 3 Aqueous layer
  - 4 Interface

**Figure 2 — After decanting**

**9.4.10** Rinse the outside of the neck of the fat-extraction flask with a little mixed solvent (5.6). Collect the rinsings in the fat-collecting vessel. Take care that the mixed solvent does not spread over the outside of the fat-extraction flask. If desired, remove the solvent or a part of it from the fat-collecting vessel by distillation or evaporation as described in 9.4.14.

**9.4.11** Add 5 ml of ethanol (5.2) to the contents of the fat-extraction flask. Using the ethanol, rinse the inside of the neck of the flask and mix as described in 9.4.4.

**9.4.12** Carry out a second extraction by repeating the operations described in 9.4.5 to 9.4.9 inclusive. Instead of 25 ml, use only 15 ml of diethyl ether (5.4) and 15 ml of light petroleum (5.5). Using the diethyl ether, rinse the inside of the neck of the fat-extraction flask too.

If necessary, raise the interface slightly to the middle of the stem of the flask by gently adding water down the side of the flask (see Figure 1) to enable the final decanting of solvent to be as complete as possible (see Figure 2).

**9.4.13** Carry out a third extraction without addition of ethanol by again repeating the operations described in 9.4.5 to 9.4.9 inclusive. Again, use only 15 ml of diethyl ether (5.4) and 15 ml of light petroleum (5.5). Using the diethyl ether, rinse the inside of the neck of the fat-extraction flask again.

If necessary, raise the interface slightly to the middle of the stem of the flask by gently adding water down the side of the flask (see Figure 1) to enable the final decanting of solvent to be as complete as possible (see Figure 2).

**NOTE** The third extraction may be omitted for products with a fat content of less than 5 %.

**9.4.14** Remove the solvents (including the ethanol) as completely as possible from the fat-collecting vessel, by distillation if using a boiling or conical flask, or by evaporation if using a beaker or dish (6.3). Rinse the inside of the neck of the boiling or conical flask with a little mixed solvent (5.6) before commencing the distillation.

**9.4.15** Heat the fat-collecting vessel, with the boiling or conical flask placed on its side to allow solvent vapour to escape, for 1 h in the drying oven (6.4) set at 102 °C. Remove the fat-collecting vessel from the oven and immediately verify whether or not the fat is clear. If the fat is not clear, fatty extraneous matter is presumed to be present and the whole procedure shall be repeated. If the fat is clear, protect the fat-collecting vessel from dust and allow the fat-collecting vessel to cool (preferably not in a desiccator) to the temperature of the weighing room (a glass fat-collecting vessel for at least 1 h, a metal dish for at least 30 min).

Do not wipe the fat-collecting vessel immediately before weighing. Use tongs (6.13) to place the fat-collecting vessel on the balance. Weigh the fat-collecting vessel to the nearest 1,0 mg.

**9.4.16** Heat the fat-collecting vessel, with the boiling or conical flask placed on its side to allow solvent vapour to escape, for a further 30 min in the drying oven (6.4) set at 102 °C. Cool and reweigh as described in 9.4.15. If necessary, repeat the heating and weighing procedures until the mass of the fat-collecting vessel decreases by 1,0 mg or less, or increases between two successive weighings. Record the minimum mass as the mass of the fat-collecting vessel and extracted matter.

## 10 Calculation and expression of results

### 10.1 Calculation

Calculate the fat content of the sample using the following equation:

$$w_f = \frac{(m_1 - m_2) - (m_3 - m_4)}{m_0} \times 100 \%$$

where

$w_f$  is the mass fraction of fat in the sample, in percent;

$m_0$  is the mass of the test portion (9.1), in grams;

$m_1$  is the mass of the fat-collecting vessel and extracted matter, determined in 9.4.16, in grams;

$m_2$  is the mass of the prepared fat-collecting vessel (9.3), in grams;

$m_3$  is the mass of the fat-collecting vessel used in the blank test (9.2) and any extracted matter determined in 9.4.16, in grams;

$m_4$  is the mass of the fat-collecting vessel (9.3) used in the blank test (9.2), in grams.

### 10.2 Expression of results

Round the result to two decimal places.

## 11 Precision

### 11.1 Interlaboratory test

Details of an interlaboratory test in accordance with ISO 5725<sup>1)</sup> on the precision of the method have been published (see reference [6]).

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<sup>1)</sup> ISO 5725:1986 (now withdrawn) was used to obtain the precision data.

The values for repeatability and reproducibility limits are expressed for the 95 % probability level and may not be applicable to concentration ranges and matrices other than those given.

### **11.2 Repeatability**

The absolute difference between two independent single test results, obtained using 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, will not in more than 5 % of cases be greater than a mass fraction of:

- 0,20 % for dried high-fat milk and dried whole milk;
- 0,15 % for dried partially skimmed milk and dried buttermilk;
- 0,10 % for dried skimmed milk and dried whey.

### **11.3 Reproducibility**

The absolute difference between two independent single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will not in more than 5 % of cases be greater than a mass fraction of:

- 0,30 % for dried high-fat milk and dried whole milk;
- 0,25 % for dried partially skimmed milk and dried buttermilk;
- 0,20 % for dried skimmed milk and dried whey.

## **12 Test report**

The test report shall specify:

- all information necessary for the complete identification of the sample;
- the sampling method used, if known;
- the test method used, together with reference to this International Standard;
- 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);
- the corrections made, if a value of more than 2,5 mg is obtained in the blank test for the method;
- the test result(s) obtained; or if the repeatability has been checked, the final quoted result obtained.



## Annex A (informative)

### Notes on procedures

#### A.1 Blank test to check the reagents (see 9.2.2)

In this blank test, a fat-collecting vessel for mass control purposes has to be used in order that changes in the atmospheric condition of the balance room or temperature effects of the fat-collecting vessel will not falsely suggest the presence or absence of non-volatile matter in the extract of the reagents. This fat-collecting vessel may be used as a counterweight vessel in the case of a two-pan balance. Otherwise deviations of the apparent mass ( $m_3 - m_4$  in 10.1) of the fat-collecting vessel for control purposes should be considered when checking the mass of the fat-collecting vessel used for the blank test. Hence, the change in apparent mass of the fat-collecting vessel, corrected for the apparent change in mass of the fat-collecting vessel for control purposes, shall show no increase in mass greater than 1,0 mg.

Very occasionally, the solvents may contain volatile matter which is strongly retained in fat. If there are indications of the presence of such substances, carry out blank tests on all the reagents and for each solvent using a fat-collecting vessel with about 1 g of anhydrous butterfat. If necessary, redistil solvents in the presence of 1 g of anhydrous butterfat per 100 ml of solvent. Use the solvents only shortly after redistillation.

#### A.2 Blank test carried out simultaneously with the determination (see 9.2.1)

The value obtained in the blank test, carried out simultaneously with the determination, enables the apparent mass of substances extracted from a test portion ( $m_1 - m_2$ ) to be corrected for the presence of any non-volatile matter derived from the reagents and also for any change of atmospheric conditions in the balance room and some temperature difference between the fat-collecting vessel and the balance room at the two weighings (9.4.16 and 9.3).

Under favourable conditions (low value in the blank test on reagents, equable temperature of the balance room, sufficient cooling time for fat-collecting vessel), the value will usually be less than 1,0 mg and can then be neglected in the calculation in the case of routine determinations. Slightly higher values (positive and negative) up to 2,5 mg are also often encountered. After correction for these values, the results will still be accurate. When corrections of more than 2,5 mg are applied, this should be mentioned in the test report (clause 12).

If the value obtained in this blank test regularly exceeds 1,0 mg, the reagents should be checked, if no recent check has been made. Any impure reagent or reagents traced should be replaced or purified (see 9.2.2 and A.1).

#### A.3 Test for peroxides

To test for peroxides, add 1 ml of a freshly prepared 100 g/l potassium iodide solution to 10 ml of diethyl ether in a small glass-stoppered cylinder which has been previously rinsed with the ether. Shake the cylinder and allow to stand for 1 min. No yellow colour should be observed in the diethyl ether layer.

Other suitable methods of testing for peroxides may be used.

To ensure that the diethyl ether is free, and is maintained free, from peroxides, treat the diethyl ether at least 3 days before it is to be used as follows.

Cut zinc foil into strips that will reach at least half-way up the bottle containing the diethyl ether, using approximately 80 cm<sup>2</sup> of foil per litre of diethyl ether.

Before use, completely immerse the strips of foil for 1 min in a solution containing 10 g of copper(II) sulfate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) and 2 ml of concentrated [98 % (mass fraction)] sulfuric acid per litre. Wash the strips gently but thoroughly with water, place the wet copper-plated strips in the bottle containing the diethyl ether, and leave the strips in the bottle.

Other methods may be used provided that they do not affect the result of the determination.

#### **A.4 Diethyl ether containing antioxidants**

Diethyl ether containing about 1 mg of antioxidants per kilogram is available in some countries, especially for fat determinations. This content does not exclude its use for reference purposes.

In other countries, diethyl ether with higher antioxidant contents, for example up to 7 mg/kg, is available. Such ether should only be used for routine determinations with an obligatory blank test carried out simultaneously with the determination(s) to correct for systematic errors due to the antioxidant residue. For reference purposes, such diethyl ether shall always be distilled before use.

#### **A.5 Ethanol**

Ethanol denatured otherwise than by the addition of methanol may be used, provided that the denaturant does not affect the result of the determination.

## Annex B (informative)

### Alternative procedure using fat-extraction tubes with siphon or wash-bottle fittings

#### B.1 General

If fat-extraction tubes with siphon or wash-bottle fittings are to be used, use the procedure specified in this annex. The tubes shall be provided with good quality cork stoppers as specified for the flasks in 6.6 (see Figure B.1 as an example).

#### B.2 Procedure

##### B.2.1 Preparation of test sample

See clause 8.

##### B.2.2 Test portion

Proceed as specified in 9.1 but using the fat-extraction tubes (See note in 6.6 and Figure B.1).

The test portion shall be delivered as completely as possible to the bottom of the fat-extraction tube.

##### B.2.3 Blank test

See 9.2 and A.2.

##### B.2.4 Preparation of fat-collecting vessel

See 9.3.

##### B.2.5 Determination

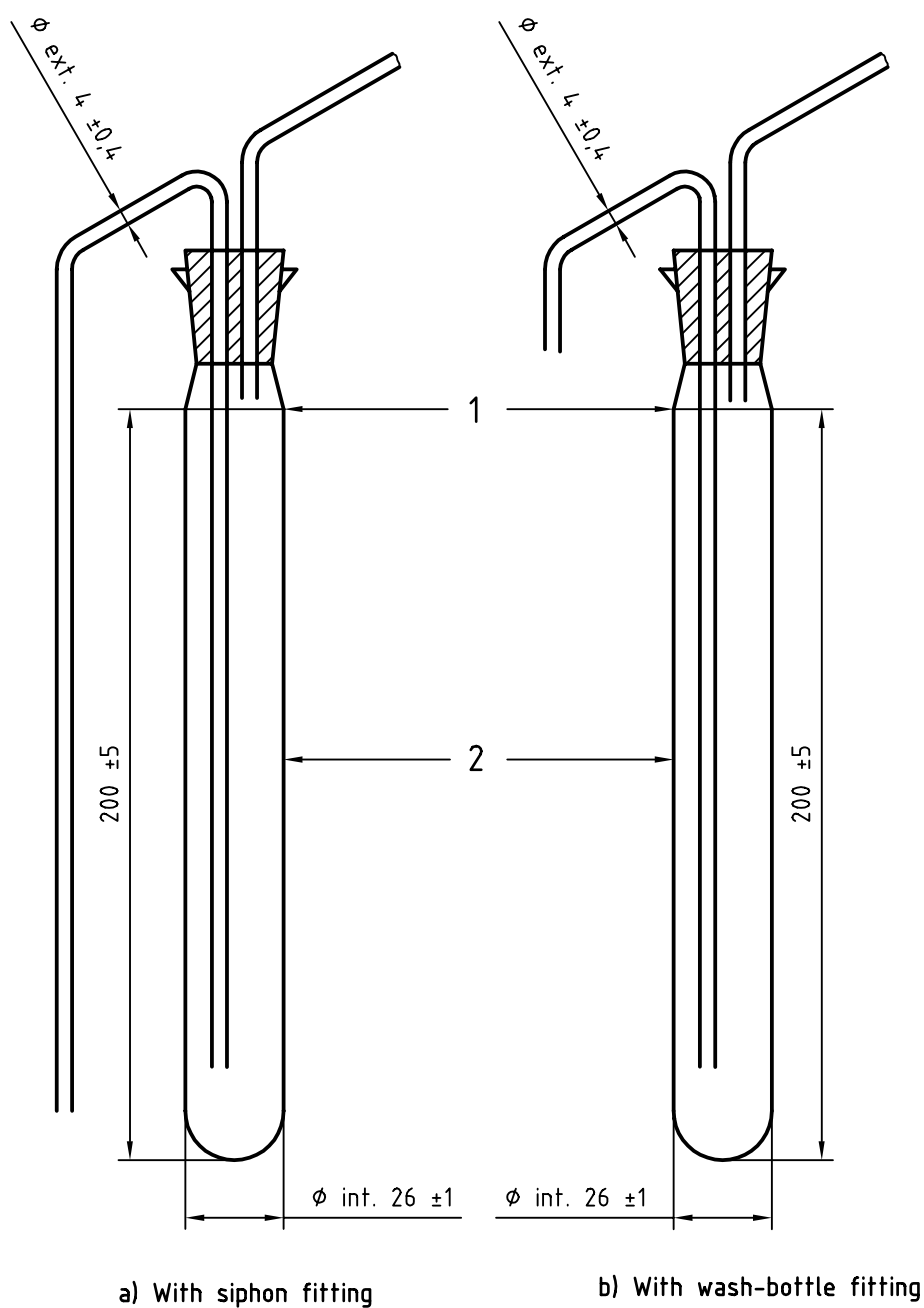
**B.2.5.1** Carry out the determination without delay.

Add 10 ml of water at  $65\text{ °C} \pm 5\text{ °C}$  to the test portion in the fat-extraction tube (B.2.2) so as to wash the test portion on to the bottom of the tube. Mix thoroughly.

**B.2.5.2** Add 2 ml of ammonia solution (5.1) to the test portion in the fat-extraction tube (B.2.2), or an equivalent volume of a more concentrated ammonia solution (see note in 5.1). Mix thoroughly with the pretreated test portion at the bottom of the fat-extraction tube.

**B.2.5.3** Heat the tube at  $65\text{ °C} \pm 5\text{ °C}$  in the water bath (6.5) for 15 min to 20 min with occasional shaking. Cool in running water to room temperature.

**B.2.5.4** Add 10 ml of ethanol (5.2). Mix gently but thoroughly with the mixture at the bottom of the fat-extraction tube. If desired, add 2 drops of the Congo-red solution (5.3).



**Key**

- 1 Capacity to this level with fittings removed  $105 \text{ ml} \pm 5 \text{ ml}$
- 2 Wall thickness  $1,5 \text{ mm} \pm 0,5 \text{ mm}$

**Figure B.1 — Examples of fat-extraction tubes**

**B.2.5.5** Add 25 ml of diethyl ether (5.4). Close the fat-extraction tube with a cork saturated with water or with a stopper of other material wetted with water (6.6). Shake the tube vigorously, but not excessively, with repeated inversions for 1 min, to avoid the formation of persistent emulsions. If necessary, cool the tube in running water. Carefully remove the cork or stopper and rinse it and the neck of the tube with a little mixed solvent (5.6). Use the wash bottle (6.8) so that the rinsings run into the tube.

**B.2.5.6** Add 25 ml of the light petroleum (5.5). Close the fat-extraction tube with the rewetted (by dipping in water) cork or stopper. Shake the tube gently for 30 s, as described in B.2.5.5.

**B.2.5.7** Centrifuge the closed fat-extraction tube for 1 min to 5 min at a radial acceleration of 80 *g* to 90 *g*. If a centrifuge (6.2) is not available, allow the closed tube to stand in the rack (6.7) for at least 30 min until the supernatant layer is clear and distinctly separated from the aqueous layer. If necessary, cool the tube in running water to room temperature.

**B.2.5.8** Carefully remove the cork or stopper and rinse it and the neck of the fat-extraction tube with a little mixed solvent (5.6). Use the wash bottle (6.8) so that the rinsings run into the tube.

**B.2.5.9** Insert a siphon fitting or a wash-bottle fitting into the fat-extraction tube. Push down the long inner limb of the fitting until the inlet is approximately 4 mm above the interface between the layers. The inner limb of the fitting shall be parallel to the axis of the fat-extraction tube.

Carefully transfer the supernatant layer out of the fat-extraction tube into the fat-collecting vessel (see 9.3) containing a few boiling aids (6.10) in the case of boiling or conical flasks (optional with metal dishes). Avoid the transfer of any of the aqueous layer. Rinse the outlet of the fitting with a little mixed solvent, collecting the rinsings in the fat-collecting vessel.

**NOTE** The supernatant layer can be transferred out of the fat extraction-tube by using, for example, a rubber bulb attached to the short stem to apply pressure.

**B.2.5.10** Loosen the fitting from the neck of the fat-extraction tube. Slightly raise the fitting and rinse the lower part of its long inner limb with a little mixed solvent (5.6). Lower and re-insert the fitting and transfer the rinsings to the fat-collecting vessel.

Rinse the outlet of the fitting with a little mixed solvent again, collecting the rinsings in the fat-collecting vessel. If desired, remove the solvent or a part of it from the fat-collecting vessel by distillation or evaporation as described in 9.4.14.

**B.2.5.11** Again loosen the fitting from the neck. Slightly raise the fitting and add 5 ml of ethanol to the contents of the fat-extraction tube. Use the ethanol to rinse the long inner limb of the fitting. Mix as described in B.2.5.4.

**B.2.5.12** Carry out a second extraction by repeating the operations described in B.2.5.5 to B.2.5.10. Instead of 25 ml, use only 15 ml of diethyl ether (5.4) and 15 ml of light petroleum (5.5). Using the diethyl ether, rinse the long inner limb of the fitting during the removal of the fitting from the fat-extraction tube after the previous extraction.

**B.2.5.13** Carry out a third extraction without the addition of ethanol by again repeating the operations described in B.2.5.5 to B.2.5.10. Again, use only 15 ml of diethyl ether and 15 ml of light petroleum. Using the diethyl ether, rinse the long inner limb of the fitting as described in B.2.5.12.

**NOTE** The third extraction may be omitted for milk with a fat content of less than 5 %.

**B.2.5.14** Proceed as described in 9.4.14 to 9.4.16.

## Bibliography

- [1] ISO 707, *Milk and milk products — Guidance on sampling*.
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