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Milk-based infant foods — Determination of fat content — Röse-Gottlieb gravimetric method (Reference method)

Aliments à base de lait pour enfants en bas âge — Détermination de la teneur en matière grasse — Méthode gravimétrique Röse-Gottlieb (Méthode de référence)

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Foreword

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Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

International Standard ISO 8381 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, in collaboration with the International Dairy Federation (IDF) and the Association of Analytical Chemists (AOAC) and will also be published by these organizations.

Users should note that all International Standards undergo revision from time to time and that any reference made herein to any other International Standard implies its latest edition, unless otherwise stated.

Milk-based infant foods — Determination of fat content — Röse-Gottlieb gravimetric method (Reference method)

0 Introduction

This International Standard has been prepared within the framework of producing a series of reference methods, which are harmonized to the greatest possible extent, for the gravimetric determination of the fat content of milk, milk products and milk-based foods. These methods are based on either the Röse-Gottlieb (RG), or the Weibull-Berntrop (WB) or the Schmid-Bondzynski-Ratzlaff (SBR) principle.

For this International Standard, dealing with milk-based infant foods containing no or not more than 5 % (*m/m*) (in the dry matter) of starch or dextrin, or vegetable, fruit, meat, etc., a method based on the RG principle has been chosen because

- a) for products containing a high percentage of lactose and other carbohydrates, such as milk-based infant foods, an RG procedure with dissolution in ammonia is, in principle, preferable to a method employing a hydrochloric acid digestion (Schmid-Bondzynski-Ratzlaff or Weibull-Berntrop method). This preference is based on the fact that in the acid digestion, ether-soluble substances and a great quantity of charred material that impedes the extraction are formed from the carbohydrates;
- b) many types of infant foods are milk-based and contain no or not more than a few per cent of starch or dextrin; therefore they can be examined by the RG method. In the case of products containing more than 5 % (*m/m*) (in the dry matter) of starch or dextrin, or vegetable, fruit, meat, etc., recourse has to be made to a method utilizing the Weibull-Berntrop principle¹⁾.

The details of the determination are based on the revised RG method for milk specified in ISO 1211²⁾, comprising a number of modifications to improve the precision of the method. An explanation of these modifications is given in the introduction to ISO 1211.

1 Scope and field of application

This International Standard specifies the reference method for the determination of the fat content of liquid, concentrated and dried milk-based infant foods containing no or not more than 5 % (*m/m*) (in the dry matter) of starch or dextrin, or vegetable, fruit, meat, etc. (Malto-dextrins without higher molecular mass dextrins, which are often present in infant foods, do not disturb the RG extraction even when present in high percentages.)

NOTE — If the product does not dissolve completely in ammonia owing to the presence of more than a few per cent of starch or dextrin, or to the presence of hard lumps, or if it contains free fatty acids in significant quantities, the result of the determination will be too low. In the case of such products, recourse should be made to a method utilizing the Weibull-Berntrop principle¹⁾.

2 References

ISO 707, *Milk and milk products — Methods of sampling*.

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

3 Definition

fat content of milk-based infant foods: All the substances determined by the method specified in this International Standard.

It is expressed as a percentage by mass.

1) See ISO 8262-1, *Milk products and milk-based foods — Determination of fat content by the Weibull-Berntrop gravimetric method (Reference method) — Part 1: Infant foods*.

2) ISO 1211, *Milk — Determination of fat content — Gravimetric method (Reference method)*.

4 Principle

Extraction of an ammoniacal ethanolic solution of the liquid, diluted or dissolved test portion with diethyl ether and light petroleum, removal of the solvents by distillation or evaporation, and determination of the mass of the substances extracted which are soluble in light petroleum. (This is usually known as the Röse-Gottlieb principle.)

5 Reagents

All reagents shall be of recognized analytical grade and shall leave no appreciable residue when the determination is carried out by the method specified. The water used shall be distilled water or water of at least equivalent purity.

To test the quality of the reagents, carry out a blank test as specified in 8.3. Use an empty fat-collecting vessel, prepared as specified in 8.4, for mass control purposes (see 10.1). The reagents shall leave no residue greater than 0,5 mg.

If the residue of the complete reagent blank test is greater than 0,5 mg, determine the residue of the solvents separately by distilling 100 ml of the diethyl ether and light petroleum respectively. Use an empty control vessel to obtain the real mass of residue, which shall not exceed 0,5 mg.

Replace unsatisfactory reagents or distil the solvents if they do not meet this requirement.

5.1 Ammonia solution, containing approximately 25 % (m/m) of NH_3 , $\rho_{20} \approx 910 \text{ g/l}$.

NOTE — If ammonia solution of this concentration is not available, a more concentrated solution of known concentration may be used (see 8.5.2).

5.2 Ethanol, or ethanol denatured by methanol, at least 94 % (V/V).

(See 10.5.)

5.3 Congo-red solution.

Dissolve 1 g of Congo-red in water and dilute to 100 ml.

NOTE — The use of this solution, which allows the interface between the solvent and aqueous layers to be seen more clearly, is optional (see 8.5.4). Other aqueous colour solutions may be used provided that they do not affect the result of the determination.

5.4 Diethyl ether, free from peroxides (see 10.3) and containing no or not more than 2 mg/kg of antioxidants and complying with the requirements for the blank test (see the introductory paragraphs to clause 5, and also 10.1 and 10.4).

5.5 Light petroleum, having any boiling range between 30 and 60 °C.

5.6 Mixed solvent, prepared shortly before use by mixing equal volumes of the diethyl ether (5.4) and the light petroleum (5.5).

6 Apparatus

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

Usual laboratory equipment, and in particular

6.1 Analytical balance.

6.2 Centrifuge, in which the fat-extraction flasks or tubes (6.6) can be spun at a rotational frequency of 500 to 600 min^{-1} to produce an acceleration of 80g to 90g at the outer end of the flasks or tubes.

NOTE — The use of the centrifuge is optional but recommended (see 8.5.7).

6.3 Distillation or evaporation apparatus, to enable the solvents and ethanol to be distilled from the fat-collecting flasks or to be evaporated from beakers and dishes (see 8.5.10 and 8.5.14) at a temperature not exceeding 100 °C.

6.4 Drying oven, electrically heated, with ventilation port(s) fully open, capable of being maintained at $102 \pm 2 \text{ °C}$ throughout the working space. The oven shall be fitted with a suitable thermometer.

6.5 Water-baths, capable of being maintained at the following temperatures:

40 to 60 °C (see 8.1.1);

30 to 40 °C (see 8.1.2);

40 to 50 °C (see 8.5.1);

60 to 70 °C (see 8.5.3).

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

NOTE — It is also possible to use **fat-extraction tubes** (or **flasks**) with **siphon** or **wash-bottle fittings**, but the procedure is then different and is that specified in the annex. The long inner limb of the fitting may have a hooked end if desired.

The flasks (or tubes, see the note) shall be provided with good quality bark corks or stoppers of other material (for example silicone rubber or PTFE¹⁾) unaffected by the reagents used. Bark corks shall be washed with the diethyl ether (5.4), kept in water at 60 °C or more (but not boiling) for at least 15 min, and shall then be allowed to cool in the water so that they are saturated when used.

1) Polytetrafluoroethylene

6.7 Rack, to hold the fat-extraction flasks (or tubes) (see 6.6).

6.8 Wash bottle, suitable for use with the mixed solvent (5.6). A plastic wash bottle shall not be used.

6.9 Fat-collecting vessels, for example boiling flasks (flat-bottomed) of capacity 125 to 250 ml, conical beakers of capacity 250 ml, or metal dishes.

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

6.10 Boiling aids, fat-free, of non-porous porcelain or silicon carbide, or glass beads (optional in the case of metal dishes).

6.11 Measuring cylinders, of capacities 5 and 25 ml.

6.12 Pipettes, graduated, of capacity 10 ml.

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

7 Sampling

See ISO 707.

All liquid, viscous or pasty laboratory samples shall be kept at a temperature of 2 to 4 °C from the time of sampling to the time of commencing the procedure. In the case of a sealed can or bottle, store it unopened at a temperature below 20 °C.

8 Procedure

NOTE — The alternative procedure using fat-extraction tubes with siphon or wash-bottle fittings (see the note to 6.6) is described in the annex.

8.1 Preparation of the test sample

8.1.1 Liquid products

Shake and invert the container. Open the container, pour the product slowly into a second container (provided with an airtight lid) and mix by repeated transfer, taking care to incorporate in the sample any fat or other constituent adhering to the wall and ends of the first container. Finally transfer the product as completely as possible to the second container. Close this container.

If necessary, condition the unopened container in the water bath (6.5) at 40 to 60 °C. Remove and shake the container vigorously every 15 min. After 2 h, remove the container, dry the outside with a tissue and allow to cool to room temperature. Remove the lid or cap entirely and thoroughly mix the contents by stirring with a spoon or spatula. (If fat separates out, do not test the sample.) Transfer the product as completely as possible to the second container. Close this container.

8.1.2 Viscous or pasty products

Open the container and thoroughly mix the contents with a spoon or spatula. If possible, use an up-and-down rotary movement in such a way that the top layers and the contents of the lower corners of the container are moved and mixed. Take care to incorporate in the sample any fat or other constituent adhering to the wall and ends of the container. Transfer the product as completely as possible to a second container (provided with an airtight lid). Close this container.

If necessary, condition the unopened container in the water bath (6.5) at 30 to 40 °C. Remove the container, dry the outside with a tissue and open it. Scrape out all product adhering to the interior of the container, transfer to a dish large enough to permit thorough stirring, and mix until the whole mass is homogeneous. Transfer the product as completely as possible to a second container as above. Close this container.

8.1.3 Dried products

Mix thoroughly by repeatedly rotating and inverting the container. If necessary, transfer all of the laboratory sample to a suitable airtight container of adequate capacity to allow this operation to be carried out.

8.2 Test portion

Mix the test sample (8.1) by gently stirring (in the case of viscous, pasty or dried products) or by gently inverting the container three or four times (in the case of liquid products) and immediately weigh to the nearest 1 mg, directly or by difference, into a fat-extraction flask (6.6), 1,5 to 10 g of the test sample, corresponding to 1,0 to 1,5 g of dry matter.

The test portion shall be delivered as completely as possible into the lower (small) bulb of the extraction flask (6.6).

8.3 Blank test

Carry out a blank test simultaneously with the determination, using the same procedure and same reagents, but replacing the dissolved test portion (see 8.5.1) by 10 ml of water (see 10.2).

8.4 Preparation of fat-collecting vessel

Dry a vessel (6.9) containing a few boiling aids (6.10) in the oven (6.4), controlled at 102 ± 2 °C, for 1 h (see note 1).

Allow the vessel to cool (protected from dust) to the temperature of the weighing room (glass vessel for at least 1 h, metal dish for at least 0,5 h) (see note 2).

Using tongs (6.13) (to avoid, in particular, temperature variations), place the vessel on the balance and weigh to the nearest 0,1 mg.

NOTES

1 Boiling aids are desirable to promote gentle boiling during the subsequent removal of solvent, especially in the case of glass vessels; their use is optional in the case of metal dishes.

2 The vessel should not be placed in a desiccator, to avoid insufficient cooling or unduly long cooling times.

8.5 Determination

8.5.1 If necessary, add water at 65 ± 5 °C to the test portion to obtain a total volume of 10 to 11 ml, and so as to wash the test portion into the small bulb of the flask. Shake gently with slight warming in a water bath (6.5) controlled at 40 to 50 °C until the product is completely dispersed. Cool in running water.

8.5.2 Add 1,5 to 2 ml of the ammonia solution (5.1), or an equivalent volume of a more concentrated ammonia solution (see the note to 5.1), and mix thoroughly with the dispersed test portion in the small bulb of the flask. After the addition of the ammonia, continue the determination without delay.

8.5.3 Heat the flask at 65 ± 5 °C in the water bath (6.5) for 15 to 20 min with occasional shaking (optional in the case of liquid products) and then cool to laboratory temperature.

8.5.4 Add 10 ml of the ethanol (5.2) and mix gently but thoroughly by allowing the contents of the flask to flow backward and forward between the two bulbs; avoid bringing the liquid too near to the neck of the flask. If desired, add 2 drops of the Congo-red solution (5.3).

8.5.5 Add 25 ml of the diethyl ether (5.4), close the flask with a cork (see 6.6) saturated with water or with a stopper wetted with water, and shake the flask vigorously, but not excessively (in order to avoid the formation of persistent emulsions), for 1 min with the flask in a horizontal position and the small bulb extending upwards, periodically allowing the liquid in the large bulb to run into the small bulb. If necessary, cool the flask in running water, then carefully remove the cork or stopper and rinse it and the neck of the flask with a little of the mixed solvent (5.6), using the wash bottle (6.8), so that the rinsings run into the flask or into the prepared fat-collecting vessel (see 8.4).

8.5.6 Add 25 ml of the light petroleum (5.5), close the flask with the rewetted cork or rewetted stopper (by dipping in water), and shake the flask gently for 30 s as described in 8.5.5.

8.5.7 Centrifuge the closed flask for 1 to 5 min at a rotational frequency of 500 to 600 min^{-1} (see 6.2). If a centrifuge 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.

8.5.8 Carefully remove the cork or stopper and rinse it and the inside of the neck of the flask with a little of the mixed solvent so that the rinsings run into the flask or into the fat-collecting vessel.

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 decantation of solvent.

NOTE — In figures 1 and 2, one of the three types of flasks as specified in ISO 3889 is shown, but this does not imply any preference over the other types.

8.5.9 Holding the extraction flask by the small bulb, carefully decant as much as possible of the supernatant layer into the prepared fat-collecting vessel (see 8.4) containing a few boiling aids (6.10) in the case of flasks (optional with metal dishes), avoiding decantation of any of the aqueous layer (see figure 2).

8.5.10 Rinse the outside of the neck of the extraction flask with a little of the mixed solvent, collecting the rinsings in the fat-collecting vessel and taking care that the mixed solvent does not spread over the outside of the extraction flask.

If desired, the solvent or part of the solvent may be removed from the vessel by distillation or evaporation as described in 8.5.14.

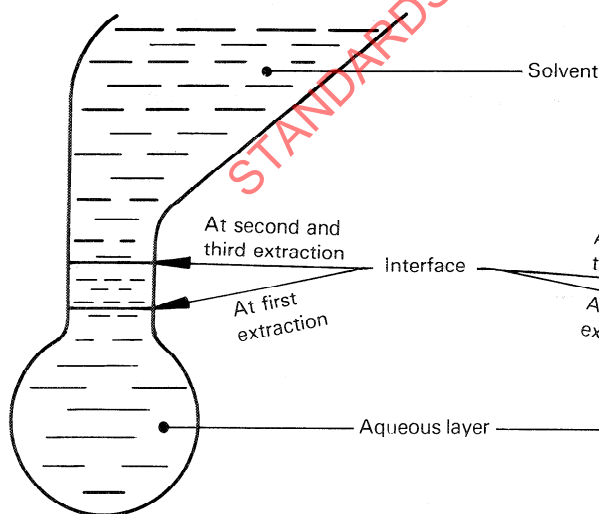


Figure 1 — Before decantation
(8.5.8, 8.5.12, 8.5.13)

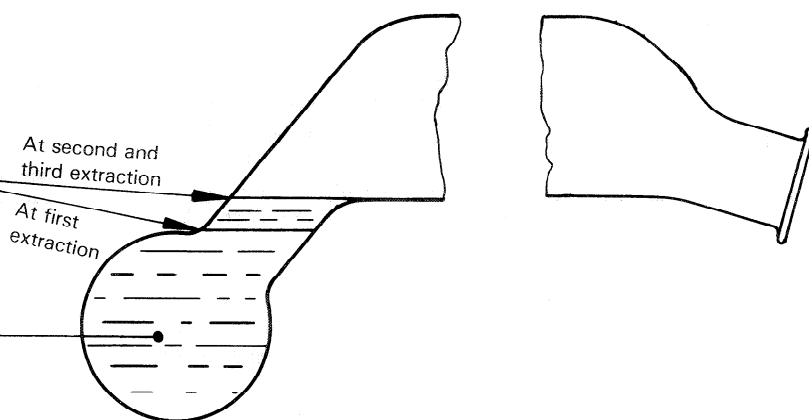


Figure 2 — After decantation
(8.5.9, 8.5.12, 8.5.13)

8.5.11 Add 5 ml of the ethanol (5.2) to the contents of the extraction flask, using the ethanol to rinse the inside of the neck of the flask and mix as described in 8.5.4.

8.5.12 Carry out a second extraction (without the addition of ethanol) by repeating the operations described in 8.5.5 to 8.5.10 inclusive, but using only 15 ml of the diethyl ether (5.4) and 15 ml of the light petroleum (5.5); use the ether to rinse the inside of the neck of the extraction flask.

If necessary, raise the interface to slightly above the middle of the stem of the flask (see figure 1) to enable the final decantation of solvent to be as complete as possible (see figure 2).

8.5.13 Carry out a third extraction (without the addition of ethanol) by again repeating the operations described in 8.5.5 to 8.5.9 inclusive, but using only 15 ml of the diethyl ether (5.4) and 15 ml of the light petroleum (5.5); use the ether to rinse the inside of the neck of the extraction flask.

If necessary, raise the interface to slightly above the middle of the stem of the flask (see figure 1) to enable the final decantation of solvent to be as complete as possible (see figure 2).

NOTE — The third extraction should be omitted for products having fat contents of less than 3 % (*m/m*) in the dry matter.

8.5.14 Remove the solvents (including ethanol) as completely as possible from the flask by distillation, or from the beaker or dish by evaporation (see 6.3), rinsing the inside of the neck of the flask with a little of the mixed solvent (5.6) before commencing the distillation.

8.5.15 Heat the fat-collecting vessel (flask placed on its side to allow solvent vapour to escape) for 1 h in the drying oven (6.4), controlled at 102 ± 2 °C. Remove the fat-collecting vessel from the oven, allow to cool (not in a desiccator, but protected from dust) to the temperature of the weighing room (glass vessel for at least 1 h, metal dish for at least 0,5 h) and weigh to the nearest 0,1 mg.

Do not wipe the vessel immediately before weighing. Place the vessel on the balance using tongs (6.13) (to avoid, in particular, temperature variations).

8.5.16 Repeat the operations described in 8.5.15 until the mass of the fat-collecting vessel decreases by 0,5 mg or less, or increases, between two successive weighings. Record the minimum mass as the mass of the fat-collecting vessel and extracted matter.

8.5.17 Add 25 ml of the light petroleum to the fat-collecting vessel in order to verify whether or not the extracted matter is wholly soluble. Warm gently and swirl the solvent until all the fat is dissolved.

If the extracted matter is wholly soluble in the light petroleum, take the mass of fat as the difference between the final mass of the vessel containing the extracted matter (see 8.5.16) and its initial mass (see 8.4).

8.5.18 If the extracted matter is not wholly soluble in the light petroleum, or in case of doubt, extract the fat completely from the vessel by repeatedly washing with warm light petroleum.

NOTE — National legislation may mandatorily prescribe such an extraction, either in general or in particular cases.

Allow any trace of insoluble material to settle and carefully decant the light petroleum without removing any insoluble material. Repeat this operation three more times, using the light petroleum to rinse the inside of the neck of the vessel.

Finally, rinse the outside of the top of the vessel with mixed solvent so that the solvent does not spread over the outside of the vessel. Remove light petroleum vapour from the vessel by heating the vessel for 1 h in the drying oven (6.4), controlled at 102 ± 2 °C, allow to cool and weigh, as described in 8.5.15 and 8.5.16.

Take the mass of fat as the difference between the mass determined in 8.5.16 and this final mass.

9 Expression of results

9.1 Method of calculation and formula

The fat content, expressed as a percentage by mass, is equal to

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

where

m_0 is the mass, in grams, of the test portion (8.2);

m_1 is the mass, in grams, of the fat-collecting vessel and extracted matter determined in 8.5.16;

m_2 is the mass, in grams, of the prepared fat-collecting vessel (see 8.4), or, in the case of undissolved material, of the fat-collecting vessel and insoluble residue determined in 8.5.18;

m_3 is the mass, in grams, of the fat-collecting vessel used in the blank test (8.3) and any extracted matter determined in 8.5.16;

m_4 is the mass, in grams, of the prepared fat-collecting vessel (see 8.4) used in the blank test (8.3), or, in the case of undissolved material, of the fat-collecting vessel and insoluble residue determined in 8.5.18.

Report the result to the nearest 0,01 % (*m/m*).

9.2 Precision

NOTE — The values for repeatability and reproducibility are expressed for the 95 % probability level and were derived from the results of an interlaboratory trial carried out in accordance with ISO 5725¹⁾.

1) ISO 5725, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests*.

9.2.1 Repeatability

The difference between two single results found on identical test material by one analyst within a short time interval should not exceed the following values:

- for products having a fat content of more than 5 % (m/m):
0,2 g of fat per 100 g of product
- for products having a fat content of 5 % (m/m) or less:
0,1 g of fat per 100 g of product
- for liquid products:
0,05 g of fat per 100 g of product

9.2.2 Reproducibility

The difference between two single and independent results found by two operators working in different laboratories on identical test material should not exceed the following values:

- for products having a fat content of more than 5 % (m/m):
0,4 g of fat per 100 g of product
- for products having a fat content of 5 % (m/m) or less:
0,2 g of fat per 100 g of product
- for liquid products:
0,1 g of fat per 100 g of product

10 Notes on procedure

10.1 Blank test to check the reagents

In this blank test, a 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 reagent. This 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 the formula in 9.1) of the control vessel shall 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 control vessel, shall not exceed 0,5 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 fresh anhydrous butterfat. If necessary, distil solvents in the presence of 1 g of anhydrous butterfat per 100 ml of solvent. Solvents treated in this way should only be stored for short periods following distillation.

10.2 Blank test carried out simultaneously with the determination

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 of the balance room and any temperature difference between the fat-collecting vessel and the balance room at the two weighings (8.4 and 8.5.16 or 8.5.18).

Under favourable conditions (low value in the blank test on reagents, equable temperature of the balance room, sufficient cooling time for the fat-collecting vessel), the value will usually be less than 0,5 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 for a value of more than 2,5 mg are applied, this fact should be mentioned in the test report (clause 11).

If the value obtained in this blank test regularly exceeds 0,5 mg, the reagents should be checked if this has not been recently done. Any impure reagent or reagents traced should be replaced or purified (see the introductory paragraphs to clause 5, and also 10.1).

10.3 Test for peroxides in diethyl ether

To test for peroxides, add 1 ml of a freshly prepared 100 g/l potassium iodide solution to 10 ml of the 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 either layer.

Other suitable methods of testing for peroxides may be used.

To ensure that diethyl ether (without antioxidants) is free, and is maintained free, from peroxides, treat the ether as follows at least 3 days before it is to be used.

Cut zinc foil into strips that will reach at least half-way up the bottle containing the ether, using approximately 80 cm² of foil per litre of ether.

Before use, completely immerse the strips of foil for 1 min in a solution containing 10 g of copper(II) sulfate pentahydrate (CuSO₄·5H₂O) and 2 ml of concentrated [98 % (m/m)] sulfuric acid per litre. Wash the strips gently but thoroughly with water, place the wet copper-plated strips in the bottle containing the ether, and leave the strips in the bottle.

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

10.4 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 direct use for reference purposes.

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

10.5 Ethanol

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

11 Test report

The test report shall show the method used and the result obtained. It shall also mention all operating conditions not specified in this International Standard, or regarded as optional, as well as any circumstances likely to have influenced the results. The blank value ($m_3 - m_4$, see 9.1) shall be reported if it exceeds 2,5 mg.

The test report shall include all the information necessary for the complete identification of the sample.

Bibliography

- [1] HOSTETTLER, H. *IDF Report No. 51* (1957).

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