

INTERNATIONAL UNION OF PURE
AND APPLIED CHEMISTRY

APPLIED CHEMISTRY DIVISION

COMMISSION ON OILS, FATS AND DERIVATIVES*

**STANDARD METHODS FOR THE
ANALYSIS OF OILS, FATS AND
DERIVATIVES**

6th Edition

1st Supplement: Part 6 (1982)

**SECTION II. OILS AND FATS
SECTION III. GLYCERINES**

Prepared for publication by

A. HAUTFENNE

Université Catholique de Louvain
Louvain-la-Neuve, Belgium

*Membership of the Commission during the period 1981-83 is as follows:

Chairman: D. FIRESTONE (USA); *Vice-Chairman:* M. NAUDET (France); *Secretary:* A. HAUTFENNE (Belgium); *Titular Members:* Ö. LEVIN (Sweden); J. POKORNY (Czechoslovakia); H. WESSELS (FRG); *Associate Members:* T. ASAHARA (Japan); J. L. BEARE-ROGERS (Canada); A. DIEFFENBACHER (Switzerland); E. FEDELI (Italy); J. GRACIAN TOUS (Spain); E. KURUCZ (Hungary); T. MØLLER (Denmark); W. POCKLINGTON (UK); W. PRINS (Netherlands); M. TEUPEL (FRG); A. VISAPÄÄ (Finland); G. ZWERENZ (Austria); *National Representatives:* G. BAZAN (Argentina); A. JOHNSON (Australia); B. JACOBSBERG (Belgium); R. LAGO (Brazil); D. CHOBANOV (Bulgaria); A. TULLOCH (Canada); G. HØLMER (Denmark); A. GAD (Egypt); R. LINKO (Finland); J. P. WOLFF (France); G. OSTERMANN (FRG); V. KAPOULAS (Greece); N. BRINGI (India); M. MCCARTHY (Ireland); T. HASHIMOTO (Japan); P. W. HENDRIKSE (Netherlands); F. SHORLAND (New Zealand); W. ZWIERZYKOWSKI (Poland); R. STOICA (Romania); D. CARR (South Africa); M. GASSIOT MATAS (Spain); R. OHLSON (Sweden); H. BRÜSCHWEILER (Switzerland); R. PERIN (Turkey); K. WILLIAMS (UK); A. WALKING (USA); V. RUBAJLO (USSR).

2.311 DETERMINATION OF ERUCIC ACID

1. SCOPE

This Standard describes a method for the determination of the erucic acid (cis 13-docosenoic acid) content of animal and vegetable oils and fats.

2. FIELD OF APPLICATION

This Standard is applicable to animal and vegetable oils and fats even if they contain cetoleic acid (cis 11-docosenoic acid, a cis-isomer of docosenoic acid which occurs in fish oils), and to hydrogenated oils and fats which may contain both cis- and trans-isomers of docosenoic acid.

3. PRINCIPLE

Conversion of the oils or fats into the methyl esters of the constituent fatty acids, followed by separation of the methyl esters by low-temperature-argentation thin-layer chromatography and quantitative determination of the separated methyl esters by gas-liquid chromatography.

4. APPARATUS

- 4.1. 50 ml beakers
- 4.2. 100 and 500 ml round-bottomed flasks
- 4.3. Small pointed-bottom glass tubes
- 4.4. Distillation device for diethyl ether equipped with a nitrogen stream
- 4.5. Columns, glass, length about 200 mm, internal diameter about 10 mm, with filter of glass wool or sintered-glass. Alternatively, small funnels with sintered-glass filters
- 4.6. Oven regulated at $100 \pm 2^\circ\text{C}$
- 4.7. Apparatus for thin-layer chromatography to include, in particular :
 - 4.7.1. Glass plates, 20 x 20 cm
 - 4.7.2. Applicator, for depositing solutions in the form of a narrow band or streak on thin-layer chromatography plates
 - 4.7.3. Developing tank, with lid
- 4.8. Deep-freeze unit, capable of maintaining developing tank and contents at a temperature of minus 20°C to minus 25°C
- 4.9. Ultra-violet lamp
- 4.10. Apparatus for gas-liquid chromatography, preferably with an electronic integrator, as described in method 2.302 (Gas-Liquid Chromatography of Fatty Acid Methyl Esters)

5. REAGENTS

- 5.1. n-Hexane, analytical reagent quality
- 5.2. Toluene, analytical reagent quality
- 5.3. Methanol, 50 per cent aqueous solution (V/V)
- 5.4. Diethyl ether, freshly distilled, free from peroxides and residue
- 5.5. Development solvent. Mixture of toluene (5.2) and n-hexane (5.1) 90 : 10 (V/V)
- 5.6. Silica powder, containing gypsum as binder, of suitable quality for thin-layer chromatography
- 5.7. Silica gel, of suitable quality for column chromatography

- 5.8. Methyl erucate, 5 mg/ml solution in hexane
Dissolve 50 mg methyl erucate in a few ml of hexane (5.1) and dilute to 10 ml with hexane.
- 5.9. Silver nitrate, 200 g/l aqueous solution
Dissolve 24 g silver nitrate in water and make up to 120 ml with water.
- 5.10. Methyl tetracosanoate, 0.25 mg/ml solution in hexane (internal standard)
Dissolve 25 mg methyl tetracosanoate in a few ml of hexane (5.1) and dilute to 100 ml with hexane.
- 5.11. 2',7'-dichlorofluorescein, 0.5 g/l solution in 50 per cent aqueous methanol (5.3)
Dissolve 50 mg of 2',7'-dichlorofluorescein in 100 ml of 50 per cent aqueous methanol (5.3) by warming and stirring.

6. PROCEDURE

6.1. Preparation of fatty acid methyl esters.

Take a representative sample of about 400 mg of the oil or fat and prepare a solution of the fatty acid methyl esters in hexane (5.1) (about 20 to 50 mg/ml) by one of the methods described in method 2.301 (Preparation of Fatty Acid Methyl Esters).

6.2. Thin-layer chromatography.

6.2.1. Preparation of plates.

Place 60 g silica powder (5.6) in a 500 ml round-bottomed flask (4.2) with 120 ml of silver nitrate solution (5.9) and shake for one minute to obtain a fully homogeneous slurry. Spread the slurry over the plates (4.7.1) in the normal manner to give a layer thickness of approximately 0.5 mm. This quantity of slurry should be sufficient for the preparation of five plates. Allow the plates to partially air-dry (preferably by leaving them in the dark for about 30 minutes). Fully dry and activate the plates by placing them in an oven (4.6) maintained at $100 \pm 2^\circ\text{C}$ for 2.5 hours. Activation at 110°C for 1 hour may be found satisfactory provided the plates do not darken as a result.

Use the plates as soon as possible after activation.

Score lines through the coating 10 mm from the sides and the top of each plate before use - this has the effect of reducing edge effects during the development of the plates.

6.2.2. Application of methyl esters.

Using the applicator (4.7.2) deposit 50 μl of the solution of methyl esters obtained according to 5.1. in a narrow streak about 50 mm long, at least 40 mm from the side of the plate and 10 mm from the bottom. Apply in a similar way 100 μl of a solution containing equal volumes of the prepared solution of methyl esters and the methyl erucate solution (5.8). Particular care is necessary during the application of solutions because of the fragile nature of the coating. (Note : If desired, 50 μl of the methyl erucate solution may be applied to the plate as above to assist in identifying the methyl erucate band after development : see Figure). After the application of the methyl esters the bottom edge of the plate may be stood in diethyl ether (5.4) until the ether ascends to about 5 mm above the area of sample application. This will concentrate the methyl esters in a narrow band.

6.2.3. Development of plates.

Pour the development solvent (5.5) into the tank (4.7.3) to a depth of about 5 mm and place the tank, complete with lid, in the deep-freeze cabinet (4.8) at minus 22°C , or as near to this temperature as possible. (In certain cases it may be advantageous to line the tank with filter paper in contact with the developing solvent). After two hours place the plate carefully in the tank and allow the solvent to ascend to about one-half to two-thirds of the height of the plate.

Remove the plate, allowing the solvent to drain off, and evaporate under the fume-cupboard the residual solvent from the plate with a stream of nitrogen.

Replace the plate in the tank and allow the solvent to ascend to the top of the plate.

Remove the plate and, as previously, dry with a stream of nitrogen.

Spray the plate with 2',7'-dichlorofluorescein solution (5.11). View the plate under ultra-violet light (4.9) and locate the band containing the methyl erucate derived from the sample by reference to the intensified band obtained from the sample to which methyl erucate has been added. (See Figure).

6.2.4. Separation of the methyl ester fractions.

Scrape off with great care the band containing the methyl erucate derived from the sample into a beaker (4.1) taking care to avoid losses (Fraction 1). Similarly remove the layer located above and below the methyl erucate band. This layer will contain the methyl esters of the remainder of the fatty acids and must be transferred to a separate beaker (4.1) (Fraction 2). Add 1.0 ml of the methyl tetracosanoate standard solution (5.10) and 10 ml

diethyl ether (5.4) to each beaker. Stir and transfer the contents of the beakers to separate columns or funnels (4.5) each containing about 1 g silica gel (5.7). Extract the methyl esters using three or four 10 ml portions of diethyl ether (5.4). Collect the filtrates in 100 ml round-bottomed flasks (4.2). Evapore each filtrate to a small volume using a gentle stream of nitrogen (4.4). Transfer the solutions to small pointed-bottom glass tubes (4.3). Remove all the solvent under the fume-cupboard by evaporation with a stream of nitrogen in such a way that the methyl esters concentrate at the bottom of the tubes. Dissolve the methyl esters in about 20-50 μ l of hexane (5.1).

6.3. Gas-liquid chromatography.

6.3.1. Follow the procedure described in method 2.302 and inject in the apparatus (4.10) 1-2 μ l of the solutions of methyl esters obtained from Fraction 1, (which contains the methyl erucate derived from the sample), and from Fraction 2 (which contains the remainder of the fatty acid methyl esters).

6.3.2. Obtain from the gas-liquid chromatographic analysis the following data :

(i) for Fraction 1 :

- a) methyl erucate peak area (A_e)
- b) internal standard peak area (A_{i1})
- c) total methyl ester peak areas excluding the internal standard peak area (A_1)

(ii) for Fraction 2 :

- a) internal standard peak area (A_{i2})
- b) total methyl ester peak areas excluding the internal standard peak area (A_2)

7. EXPRESSION OF RESULTS

7.1. The erucic acid content of the sample (expressed in terms of its methyl ester as a percentage of the total fatty acid methyl esters prepared from the sample) is given by the formula :

$$A_{i1} \frac{A_e}{\left[\frac{A_1}{A_{i1}} + \frac{A_2}{A_{i2}} \right]} \times 100$$

where

A_e , A_1 , A_2 , A_{i1} and A_{i2} are the peak areas referred to in 6.3.2, corrected as necessary by use of calibration factors.

For practical purposes the value for methyl erucate given by the above formula is equivalent to the level of erucic acid expressed as a percentage of the total level of fatty acids in the sample.

7.2. If peak areas are obtained in percentages the values for A_1 and A_2 may be calculated as follows :

$$A_1 = 100 - A_{i1}$$

$$A_2 = 100 - A_{i2}$$

7.3. The method of calculation (7.1) assumes that the level of tetracosanoic acid in the sample is negligible. If significant amounts of this acid are shown to be present the value for methyl tetracosanoate (A_{i2}) obtained from the chromatogram of Fraction 2 must be reduced to :

$$A_{i2} - A_{i3}$$

where

$$A_{i3} = \frac{A_{it} \times A_p}{A_{pt}}$$

and

A_{i3} = peak area of methyl tetracosanoate derived from the sample and which forms part of the peak area attributed to the internal standard in the chromatogram of Fraction 2,

A_p = peak area of methyl palmitate obtained from Fraction 2,

A_{it} = peak area of methyl tetracosanoate obtained from a chromatogram of the total fatty acids (as methyl esters) in the sample, the methyl esters being prepared and chromatographed according to methods 2.301 and 2.302,

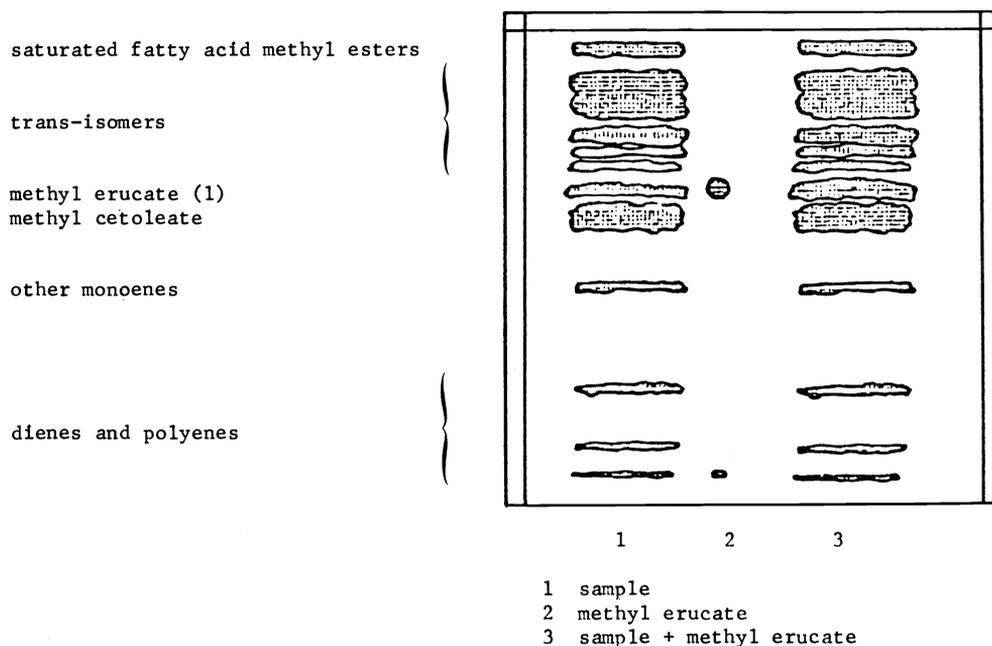
A_{pt} = peak area of methyl palmitate obtained from the chromatogram of the total fatty acids as referred to in A_{it} above,

8. REPEATABILITY

The difference between the results of two determinations carried out simultaneously or in rapid succession on the same sample, by the same analyst, under the same conditions, shall not exceed 0.5 g per 100 g of sample or 10 per cent of the determined value, whichever is the greater.

FIGURE

Typical thin-layer chromatogram showing the separation of the methyl esters of erucic acid, cetolic acid and trans-isomers of docosenoic acid



(1) The fraction designated methyl erucate will usually contain methyl esters of other monoenoic acids but should be free of methyl cetoleate.

II. OILS AND FATS

1982

2.312 DETERMINATION OF LINOLEIC ACID

1. SCOPE

This Standard describes a method for the determination of linoleic acid (cis-cis-9,12-octadecadienoic acid) in edible fats and oils. (Note 1).

2. FIELD OF APPLICATION

The method is applicable to animal and vegetable oils and fats intended for food use and in oils and fats isolated from food products.

3. PRINCIPLE

Determination of the total amount of polyunsaturated fatty acids having a cis-cis-1,4-pentadienoic acid structure in one portion of the sample by an enzymatic method.
 Determination of the total octadecadienoic acids and other polyunsaturated fatty acids in a further portion of the sample by gas-liquid chromatography.
 Calculation of the amount of linoleic acid in the sample from the value obtained by the enzymatic method, using a factor derived from the proportion of the total octadecadienoic fatty acids to the total polyunsaturated fatty acids as determined by gas-liquid chromatography.

4. APPARATUS

For the enzymatic analysis : see method 2.209.
 For the gas-liquid chromatography : see methods 2.301 and 2.302.

5. REAGENTS

For the enzymatic analysis : see method 2.209.
 For the gas-liquid chromatography : see methods 2.301 and 2.302.

6. PROCEDURE

- 6.1. Determine enzymatically the amount of polyunsaturated fatty acids having a cis-cis-1,4-pentadienoic structure in one portion of the sample according to method 2.209 and express the results as g fatty acids per 100 g of sample.
- 6.2. Using a further portion of the sample, prepare the fatty acid methyl esters according to method 2.301 and determine by gas-liquid chromatography the peak area for the total octadecadienoic acids and the peak areas for the remaining polyunsaturated fatty acids, as described in 6.2.1 of method 2.302.

7. EXPRESSION OF RESULTS

The amount of linoleic acid, expressed as g per 100 g of sample, is given by the formula :

$$P \times \left(\frac{A_1}{A_1 + A_2 + A_3 + \dots} \right)$$

where

P is the amount of polyunsaturated fatty acids having a cis-cis-1,4-pentadienoic acid structure, determined according to method 2.209 and expressed as g per 100 g of sample,

A₁ is the area of the octadecadienoic acid peak,

A₂, A₃ ... are the areas of the polyunsaturated fatty acids, other than octadecadienoic acid, determined according to method 2.302 and measured with the same unit as A₁. (Note 2).

8. NOTES

1. The method determines the total amount of octadecadienoic acids containing the cis-cis-1,4-pentadienoic structure but, except under unusual circumstances, this amount is the linoleic acid content.
2. The method of calculation is based on the assumption that in each polyunsaturated fatty acid peak the proportion of isomers not having a cis-cis-1,4-pentadienoic structure is the same. Since the polyunsaturated fatty acids present in most processed fats and oils are predominantly octadecadienoic acids, the effect of any variations from this proportion will be minimal.

II. OILS AND FATS

1932

2.323 SOLID CONTENT DETERMINATION IN FATS BY NMR
(LOW RESOLUTION NUCLEAR MAGNETIC RESONANCE)1. SCOPE

This Standard describes methods for the determination of solid content in fats by low resolution nuclear magnetic resonance. These methods are divided in :

- a) Methods for routine use without special temperature pretreatment
- b) Methods for routine use with special temperature pretreatment
- c) Methods for specific applications.

2. FIELD OF APPLICATION

This Standard is applicable to animal and vegetable fats, hydrogenated or not.

a) Methods for routine use without special thermal pretreatment

Applicable to fats which do not need a stabilization of their crystalline form - fats used in margarine shortening and confectionery fats industries.

b) Methods for routine use with special thermal pretreatment

Applicable to fats showing a high polymorphism and, by this way, able to crystallize in an unstable form - cocoa butter and similar fats.

c) Methods for specific applications

Applicable to all types of fats, which are brought through a particular thermal pretreatment to an absolute stable crystalline form in equilibrium with the liquid phase. Suitable for investigatory or development work.

3. DEFINITION

The solid content of a fat, determined by low resolution nuclear magnetic resonance (NMR) equals 100 times :

- either the ratio between the intensity of the free decay signal caused by the hydrogen nuclei belonging to the glycerides in solid state and the intensity of the free ce signal caused by all the hydrogen nuclei belonging to the glycerides in solid state as well as in liquid state, when the determination is performed with pulsed NMR,
- or the complement to one of the ratio between the integration value of the signal obtained from the hydrogen nuclei belonging to the glycerides in liquid state and the integration value of the signal which would be given by all the hydrogen nuclei of the sample if this last would be liquid at the temperature of measurement, when the determination is performed with continuous wave NMR.

4. PRINCIPLE

Measurement with a low resolution (pulsed or continuous wave) NMR apparatus of the solid content in a partly crystallized fat or fat mixture after solidification under carefully prescribed conditions.

5. MEASURING CONDITIONS IN NMR

According to the principle of NMR spectrometry used (pulsed NMR or continuous wave NMR) measuring conditions are different :

a) Methods for routine use without special pretreatment

Used in pulsed NMR parallel measurements, in continuous wave NMR serial measurements. (Note 11).

b) Methods for routine use with special pretreatment

Same as a).

c) Methods for specific applications

Used for both NMR serial measurements.

6. THERMAL PRETREATMENTS OF SAMPLES

As the obtained results are dependent of the conditions of the thermal pretreatment of the sample, every modification of these conditions modifies the results.

6.1. Principle

After complete melting of samples in order to eliminate any crystal germs, solidify them, then bring them to measurement temperatures in rigorously defined and reproducible conditions.

6.2. Methods a) for routine use without special thermal pretreatment

6.2.1. Apparatus

- 6.2.1.1. Measuring tubes, nominal 2 ml, corresponding to the NMR spectrometer which will be used later. Tubes should be selected in order that their external diameter does not vary more than ± 0.25 mm of the mean value.
- 6.2.1.2. Aluminium blocks with holes bored in them. The diameter of the holes must not be greater by more than 0.4 mm than the mean external diameter of the measurement tubes. The depth of the holes should be such that the level of the fat is 10 mm below the outside surface of the block. The thickness of the metal under the holes and the distance between the edge of a peripheral hole and the nearest lateral face should be 10 mm. The distance between the axes of two neighbouring holes should be 7 mm greater than the diameter of the holes. Blocks must be in equal number, with thermostatic baths to be used later.
- 6.2.1.3. Metal racks (2). The depth of the racks must be such as the level of the fat will be 5 mm below the surface of the bath in which rack will be placed.
- 6.2.1.4. Water bath maintained at 80°C.
- 6.2.1.5. Water bath regulated at 60°C.
- 6.2.1.6. Water baths regulated at each of the measurement temperature (parallel measurements) or water baths (2) which may be regulated between 5°C and 60°C (serial measurements).
- 6.2.1.7. Non-freezing liquid bath regulated at 0°C.

Baths with regulated temperature (6.2.1.5 - 6.2.1.6 - 6.2.1.7) have to be equipped with :

- a thermostatic control capable of keeping the temperature of the liquid at the desired value to the nearest 0.1°C,
- an efficient stirring device,
- a thermometer accurate to 0.05°C,
- a convenient support for aluminium blocks such as the outside surface of the blocks is 5 mm above the level of the liquid. A similar support must exist also in the 80°C bath.

6.2.2. Procedure

6.2.2.1. Parallel measurements (Note 11)

Melt the fat at 80°C in the water bath (6.2.1.4) and if necessary filter. Homogenise the sample thoroughly and fill as many tubes (6.2.1.1) as there are measurement temperatures with the quantity of fat needed by the measurement method.

Place each sample tube containing the completely melted fat after reheating to 80°C in a rack (6.2.1.3) in the 60°C water bath (6.2.1.5). Keep the tubes in this bath for at least 5 minutes.

Transfer the tubes to the second rack located in the 0°C bath (6.2.1.7). Keep the tubes in this bath for exactly 60 + 2 minutes.

Remove one after the other, the tubes from the 0°C bath, dry them quickly and place them, at the rate of one per temperature, into the blocks (6.2.1.2) which are in the baths (6.2.1.6) regulated at the different measurement temperatures.

30 minutes after having put each sample tube into a block, remove each tube successively, transfer it as quickly as possible into the sample holder of the NMR apparatus and make the measurement. (Note 1).

6.2.2.2. Serial measurements (Note 11)

Melt the fat at 80°C in the water bath (6.2.1.4) and if necessary filter. Homogenise the sample thoroughly and fill a sample tube (6.2.1.1) with the quantity of fat needed by the method of measurement.

Place the sample tube, containing the completely melted fat after reheating to 80°C, in the rack (6.2.1.3) of the 60°C water bath (6.2.1.5). Keep the tube in this bath for at least 30 minutes.

After this time, transfer the sample tube to the second rack placed in the 0°C bath (6.2.1.7). Keep the sample tube in this bath for exactly 60 ± 2 minutes. Remove the sample tube from the 0°C bath, dry it quickly avoiding warming up, and place it in a hole of an aluminium block (6.2.1.2) set in a water bath (6.2.1.6) regulated at the lowest measurement temperature. 30 minutes after having put the sample tube into the block, remove it, transfer it as quickly as possible into the sample holder of the NMR apparatus and make the measurement. Place then this sample tube into a hole of an aluminium block setted in the second water bath regulated at the second measurement temperature. After 30 minutes remove the tube from this bath and make the NMR measurement. Place then the sample tube into a hole of the block setted in the first water bath, temperature of which has been elevated meanwhile at the third measurement temperature, and so on until the highest measurement temperature has been attained. (Note 2).

6.3. Methods b) for routine use with special thermal pretreatment

6.3.1. Apparatus

As in 6.2.1, adding :

6.3.1.1. Water bath regulated at $26^\circ\text{C} \pm 0.1^\circ\text{C}$.

6.3.2. Procedure

For parallel measurements as well as for serial measurements (Note 11), follow the modes of operation described under 6.2.2 with the following modifications :

after the sample has been kept at 60°C for a convenient time :

- maintain it for 90 minutes + 5 minutes in the bath (6.2.1.7) regulated at 0°C
- maintain it for 40 hours + 0.5 hour in the bath (6.3.1.1) regulated at 26°C
- maintain it for 90 minutes + 5 minutes in the bath regulated at 0°C
- maintain it, finally, for 60 minutes in a bath (6.2.1.6) at each of the measurement temperatures which have been chosen.

6.4. Methods c) for specific applications

6.4.1. Apparatus

6.4.1.1. Measurement tubes as described under 6.2.1.1.

6.4.1.2. Aluminium blocks (3) with holes bored in them, having the same characteristics as those described under 6.2.1.2.

6.4.1.3. Water baths (2) which can be regulated between 5 and 75°C.

6.4.1.4. Non-freezing liquid bath regulated at $-15^\circ\text{C} \pm 1^\circ\text{C}$.

These baths must show the same characteristics as the ones described under 6.2.1.6.

6.4.2. Procedure

Introduce into a measuring tube (6.4.1.1) the quantity of fat, formerly melted and if necessary filtered, required by the measurement method which will be used later.

Place one aluminium block (6.4.1.2) into each of the water baths (6.4.1.3) and into the non-freezing liquid bath (6.4.1.4).

Regulate the temperature of one of the water baths to 70°C.

Place the tube containing the test sample into one of the holes of the block. Maintain at 70°C for two hours.

Transfer the tube into the block placed in the -15°C bath and maintain at -15°C for two hours.

Regulate the temperature of one of the water baths at the temperature of the flow point (U tube method - Note 3), or not less than 0.25°C below this temperature. Maintain at this temperature for one hour.

Transfer the tube to the block placed in the bath regulated at -15°C, and maintain at this temperature for one hour.

Regulate one of the water baths at 5°C below the first measurement temperature. Transfer the tube to the block placed in this bath and maintain overnight.

Regulate the second water bath at the first measurement temperature. Transfer the tube to the block of this bath, and after 45 minutes at least, proceed with the NMR measurement.

Place then the sample tube in the block of the first water bath which has formerly been regulated to the second measurement temperature. After 45 minutes at least, proceed with the NMR measurement.

Place after that the tube in the block of the second water bath, the temperature of which has been elevated to the third measurement temperature. And so on until the highest measurement temperature has been attained. (Note 2 - Note 4).

In the case of continuous wave NMR measurement, raise the temperature of the available bath to approximately 15°C (round value) above the melting point of the sample, transfer the tube to the block of this bath, and after 45 minutes at least proceed with the NMR measurement.

7. DETERMINATION OF THE SOLID CONTENT WITH PULSED NMR

7.1. Principle

The intensity of the relaxation signal due to the nuclei of the hydrogen atoms belonging to glycerides which are solid at the temperature of measurement and the one of the relaxation signal due to the nuclei of the hydrogen atoms belonging to all the glycerides as well solid as liquid at this same temperature, are deducted from the responses given by the NMR spectrometer 10 μ sec. and 70 μ sec. after the pulse, correcting the signal after 10 μ sec. by an empirical factor determined statistically.

7.2. Apparatus

- 7.2.1. Low resolution pulsed NMR apparatus meeting the following characteristics :
- magnet the field of which is sufficiently homogeneous to ensure that the half life time of the magnetisation of a reference sample of fluid oil is longer than 1000 μ sec.,
 - a dead time plus pulse width smaller than 10 μ sec.,
 - a microswitch which starts the measurements automatically on insertion of the sample tube,
 - a trigger time which is adjustable,
 - a probe head in the magnet gap suitable for sample tubes of 10 mm diameter,
 - a digital voltmeter output for direct measurement.
- Preferentially, the instrument should be equipped with a computer performing automatically 3 successive measurements at 10 μ sec. and 70 μ sec., and the calculation of the mean solid content, which then is directly read on the digital voltmeter. Measuring time is then equal to 6 seconds. (Note 5).
It is recommended to maintain the working zone at a mean temperature between the extreme temperatures of measurement.
In the case of solid content determinations on cocoa butter and similar fats, the computer has to be modified to respect a 6 seconds interval between two successive measurements. In this case the value read is the result of only one determination.

- 7.2.2. Glass measurement tubes adapted to the NMR apparatus meeting the following characteristics :
- external diameter 10.0 + 0.25 mm
 - wall thickness 0.9 + 0.05 mm
 - length 150 mm

- 7.2.3. 10 ml volumetric flasks.

7.3. Reagents

- 7.3.1. Carbone tetrachloride, pure.
- 7.3.2. Liquid oil (olive oil).
- 7.3.3. Calibration samples (2) corresponding to closely defined solid contents, approximately 35 and 70 per cent (supplied by the manufacturer).

7.4. Procedure

7.4.1. Calibration of the apparatus

7.4.1.1. Preliminary adjustment of the apparatus

Proceed with the adjustment of the apparatus (7.2.1) according to the directions of the manufacturer.

After having introduced an empty measurement tube (7.2.2) into the magnetic field of the apparatus, ensure that no significant indication appears upon the digital voltmeter display after measurements at 10 μ sec. and 70 μ sec.

After having introduced into the magnetic field a measurement tube containing 2 ml of liquid oil (7.3.2), adjust the amplitude of the output signal :

- not exceeding 75 per cent of the highest value that can be displayed on the analogue voltmeter, if a computer has to be used,
- between 85 and 90 per cent of this same highest value, if measurements are to be made independently after 10 μ sec. and 70 μ sec.

7.4.1.2. Verification of the linearity of the response

Prepare three liquid oil solutions containing respectively 2.5, 5 and 7.5 ml of liquid oil (7.3.2) in volumetric flasks (7.2.3) and make up to 10 ml with carbon tetrachloride (7.3.1). (Note 6).

Into a measuring tube, introduce 2 ml of the first solution, then measure the response of the apparatus after 10 μ sec. and 70 μ sec.. Make 5 consecutive readings for each duration, using the automatic repetition device.

Empty, rinse and dry the tube.

Repeat the operation with each of the other two solutions as well as with the liquid oil

using one and the same tube.

Let $R_{0.25}$, $R_{0.50}$, $R_{0.75}$ and R_1 be the mean values of each series of measurements both at 10 $\mu\text{sec.}$ and 70 $\mu\text{sec.}$. (Note 7).

Construct the representative straight line of the values of R as a function of the concentration of the oil in the solution introduced in the tube.

This straight line should pass through the origin. If this is not the case, check the adjustment of the apparatus.

7.4.1.3. Adjustment of the computer (for the apparatus which have one)

Place alternatively into the magnetic field each of the two calibration samples. Change the position of the adjusting potentiometer as to obtain an analogue voltmeter reading which is as close as possible of the expected solid content.

Proceed by successive approximations until 25 measurements for each of the two calibration tubes do not give values which differ by more than 0.3 for each of the expected values. (Note 8).

Lock the adjusting potentiometer. (Note 9).

7.4.1.4. Determination of the correction factor (if a computer is not used)

Make for each of the two calibration samples (7.3.3) 25 readings at 10 $\mu\text{sec.}$ and 25 $\mu\text{sec.}$ readings at 70 $\mu\text{sec.}$ under the conditions defined above, every reading being made 2 sec. after the preceding one. Take the mean value of each of these 4 series of measurements. Make sure that the extreme values do not differ by more than ± 0.5 from the mean value for each series.

Calculate the value of the F factor from the relation :

$$F = \frac{S \cdot L_{70}}{(100 S)(L_{10} - L_{70})}$$

where

L_{10} is the mean response at 10 $\mu\text{sec.}$ and L_{70} the mean response at 70 $\mu\text{sec.}$ for a solid content S.

Take the mean value for the values found for the two standards. This mean value should be between 1 and 2.

7.4.2. NMR measurement

7.4.2.1. Preparation of the sample

In a measurement tube (7.2.2), place 2 ml of the fat to be studied, which has been first melted and filtered if necessary. Treat this tube under the conditions of the chosen thermal pretreatment.

7.4.2.2. Actual NMR measurement

7.4.2.2.1. Method a) for routine use without special temperature pretreatment

When the tube has been maintained for the required time at the temperature of the measurement, remove it from the block and introduce it as quickly as possible into the sample holder of the magnet and make :

- if a computer is used, 1 reading of a total time equal to 6 seconds,
- if a computer is not used, 3 readings at 10 $\mu\text{sec.}$ and 3 readings at 70 $\mu\text{sec.}$, each pulse being made 2 seconds after the preceding one.

7.4.2.2.2. Method b) for routine use with special thermal pretreatment

The interval between two consecutive pulses must be at least 6 seconds.

7.4.2.2.3. Method c) for special applications

Follow without modifications the procedure described under 7.4.2.2.1.

7.4.3. Calculation

7.4.3.1. Use of a computer

The solid content of the sample at the measurement temperature is read directly on the analogue voltmeter display.

Make two parallel tests and take the mean value.

7.4.3.2. Use of readings at 10 $\mu\text{sec.}$ and 70 $\mu\text{sec.}$

The solid content of the sample at the measurement temperature is given by the formula :

$$S \% = 100 \frac{(E_{10} - E_{70}) F}{E_{70} + (E_{10} - E_{70}) F}$$

where

E_{10} is the response of the apparatus after 10 μ sec.,

E_{70} is the response of the apparatus after 70 μ sec.,

F is the empirical factor determined statistically, according to 7.4.1.4.

Make two parallel tests and take the mean value.

7.4.4. Number of samples that can be examined simultaneously

The number of samples that can be examined simultaneously depends on the method used.

7.4.4.1. Method a) for routine use without special thermal pretreatment

Since only 20 tubes can be examined simultaneously, the number of samples depends on the number of temperatures.

7.4.4.2. Method b) for routine use with special thermal pretreatment

Same as 7.4.4.1.

7.4.4.3. Method c) for specific applications

The number of samples is practically unlimited.

8. DETERMINATION OF THE SOLID CONTENT WITH CONTINUOUS WAVE NMR

8.1. Principle

The signal due only to the nuclei of the hydrogen atoms belonging to glycerides which are liquid at the measurement temperature is integrated and the value found is compared to that which would be obtained if the sample would be totally liquid at this measurement temperature. This is obtained by studying parallel to the sample, a liquid reference oil at all temperatures of measurement and at a temperature which is distinctly greater than the melting point of the sample.

8.2. Apparatus

8.2.1. Low resolution continuous wave NMR apparatus meeting the following characteristics :

- magnet equipped with a supplementary frequency modulation unit,
- magnet equipped with an adapter for tubes of 12 mm diameter,
- magnet placed in an insulated container, the air temperature of which can be regulated to 0.2°C in the whole range of temperature to be studied, being blown continuously at the base of the adapter,
- radio frequency level which is adjustable,
- integration device with an integration time about 30 to 45 seconds, with automatic repetition of the integration. (Note 10).

8.2.2. Glass measurement tubes, adapted to the NMR apparatus meeting the following characteristics :

- external diameter 12.0 \pm 0.3 mm,
- wall thickness 0.6 mm approximately,
- length higher than 45 mm.

8.3. Reagents

8.3.1. Reference liquid oil

This oil must remain liquid in the whole range of temperatures. Olive oil, refined and eventually winterized, is to be used preferentially.

8.3.2. Relaxed water

Manganese chloride ($MnCl_2$), 1.25 g/l solution in distilled water.

8.4. Procedure

8.4.1. Calibration of the apparatus

8.4.1.1. Preliminary adjustment

The chamber of the apparatus (8.2.1) being at ambient temperature, carry out the adjustment of the apparatus according to the directions of the manufacturer.

After introducing into the magnetic field an empty measurement tube, and engagement of the integration device, no significant indication should appear on the display of the digital voltmeter.

8.4.1.2. Determination of the maximum utilisable energie of the radiofrequency field

For correct estimation of solid contents, the radio saturation level, for olive oil, must be approximately 2 per cent and in no case should exceed 4 per cent. This can be obtained with a convenient adjustment of the radiofrequency level. Since each kind of NMR apparatus uses a different method to adjust the radiofrequency level and to obtain a given saturation level, a general procedure cannot be described. Follow in each particular case the instructions given by the manufacturer and ensure that saturation level for olive oil is correct.

8.4.1.3. Verification of the linearity of the response

Weigh 0.4 - 0.8 - 1.2 - 1.6 g of reference liquid oil (8.3.1) to the nearest 0.010 g into 4 measurements tubes, taking care that no drops of oil adhere to the walls of the tube above its working part.

Measure the response of the apparatus for the 4 tubes using the maximum utilisable radiofrequency level. Make for each tube 5 successive integrations and take the mean value. The coefficient of variation for repeated readings of the signal of a same tube should not exceed 0.35 per cent.

Let $R_{0.4}$, $R_{0.8}$, $R_{1.2}$, $R_{1.6}$ be these mean values.

Construct the representative straight line for the values of R as a function of the weight of the oil in each tube.

This straight line should pass through the origin. If this is not the case, recheck the adjustment of the apparatus.

8.4.2. NMR measurement

8.4.2.1. Preparation of the sample

Place about 1.6 to 1.7 g of the fat to be studied which has been previously melted and filtered if necessary, in a measurement tube (8.2.2).

Prepare in the same conditions a control tube using a similar quantity of the reference liquid oil (8.3.1).

Treat simultaneously these two tubes under the conditions of thermal treatment chosen.

8.4.2.2. Actual NMR measurement

8.4.2.2.1. Method a) for routine use without special thermal pretreatment

Regulate to 60°C temperature the air which is blown in the base of the sample holder. When equilibrium is reached, remove the control tube from the 60°C bath (6.2.1.5) in which it should have been kept for at least 30 minutes. Dry it rapidly, and transfer it rapidly into the sample holder. Check the resonance. Measure the response of the apparatus using the maximum utilisable radiofrequency. Make two integrations and take the mean value. Let T_L be this mean value.

Transfer the control tube into the rack placed in the 0°C bath (6.2.1.7).

Remove the sample tube from the 60°C bath, dry it rapidly, transfer it rapidly into the sample holder, and measure the response E_L of the apparatus in the same conditions as above. Transfer then the sample tube into the rack of the 0°C bath.

Raise the temperature of air blown into the chamber to the first measurement temperature.

When the control tube has been kept, first 60 + 2 minutes at 0°C, then 30 minutes at the first measurement temperature, introduce it rapidly into the sample holder, check the resonance and measure the response T_T of the apparatus in the same conditions as previously. Transfer then the control tube tube to the block placed in the bath regulated at the second measurement temperature.

After the sample tube has also been kept for 60 + 2 minutes at 0°C and 30 minutes at the first measurement temperature, introduce it into the sample holder and measure the response E_T of the apparatus. Transfer the sample tube to the block placed in the bath regulated at the second measurement temperature.

Raise the temperature of air which is blown into the chamber at the second measurement temperature and the temperature of the first water bath to the third measurement temperature and so on.

8.4.2.2.2. Method b) for routine use with special thermal pretreatment

After the measurement at 60°C, follow the special procedure for solidification and thermal treatment described under 6.3.2, and after that proceed as above 8.4.2.2.1.

8.4.2.2.3. Method c) for specific applications

Regulate the temperature of air which is blown in the base of the sample holder at the first measurement temperature, during which time the sample is kept at this temperature. After the minimum required time, remove the control tube from the block, transfer it rapidly into the sample holder, and check the resonance. Measure the response of the apparatus using the maximum utilisable radiofrequency level. Make three successive integrations and take the mean value. Let T_T be this mean value. Transfer the control

tube into the bath regulated to the second measurement temperature. Remove from the first bath the sample tube and measure the response E_T of the apparatus in the same conditions as above. Transfer the sample tube to the block of the second bath. Raise the temperature of the air which is blown into the chamber at the second measurement temperature and the temperature of the first bath to the third measurement temperature, and so on.

After every determination at each measurement temperature have been performed raise the temperature of a bath and that of air blown into the chamber to approximately 15°C above the melting temperature of the sample. Measure at this temperature the responses E_L and T_L for the sample and the control tubes.

8.4.3. Calculation

The solid content of the sample at temperature T is given by the formula :

$$S \% = 100 \left(1 - \frac{T_L \cdot E_T}{E_L \cdot T_T} \right)$$

where

T_T is the response of the apparatus for the control tube at temperature T,

E_T is the response of the apparatus for the sample tube at temperature T,

T_L is the response of the apparatus for the control tube at temperature L where the sample is liquid,

E_L is the response of the apparatus for the sample tube at temperature L where the sample is liquid.

Make two parallel tests and take the mean value.

The following details should be indicated on the results sheet :

- radiofrequency level that has been used,
- audiofrequency level that has been used,
- saturation for olive oil.

8.4.4. Number of samples that can be examined simultaneously

The number of samples that can be examined simultaneously depends on the method used.

8.4.4.1. Method a) for routine use without special thermal pretreatment

According to the integration and transfer times, it is necessary to shift samples one from another for 1 minute and 30 seconds.

Consequently the number of samples that can be examined simultaneously cannot exceed 12 (plus control); this necessitates the temperature of the blown air and the temperature of a water bath to be raised to the desired values in less than 10 minutes.

8.4.4.2. Method b) for routine use with special thermal pretreatment

Same as 8.4.4.1.

8.4.4.3. Method c) for specific applications

Sample number practically unlimited.

9. NOTES

1. It may happen, for technical reasons (insufficient number of thermostatic baths available in particular) that it is not possible to use the process of parallel measurements. The same result can be obtained, but after a much longer time, by using the so called "consecutive measurements".
Prepare a number of identical samples equal to the number of measurement temperatures. Solidify the first sample, heat it to the first measurement temperature, make the NMR measurement. Solidify (after a suitable interval of time to avoid overlapping of the operations) the second test sample, heat it to the second measurement temperature and make the NMR measurement, and so on until all the measurements have been made at all the required temperatures.
2. If the bath should reach a temperature higher than that required do not try to reduce the process. Note this temperature and take the measurements without making modifications.
3. Determination of the flow point (method of Finck and Kleinhert, using the Tottoli melting point apparatus, e.g. manufactured by Büchi).
Use U tubes with 3 mm inside diameter and 80 mm length of the arms with a 10 mm distance between the axes. Introduce a quantity of solid fat into one of the arms so as to form a plug of 10 mm height. Maintain in a freezer at -15°C for 30 minutes. Press the plug of fat to 1 cm from the bottom of the U tube. Place it in the Tottoli apparatus and

increase the temperature of the silicone oil bath at a rate of 0.5°C/min. until this plug descends clearly under its own weight. Take the corresponding temperature as the flow point.

4. There is no disadvantage in interrupting the series of measurements between two consecutive temperatures provided that the tube is maintained in the bath regulated at the last measurement temperature during the interruption.
5. A simplified apparatus of the type Minispec Bruker P 20 i may be used.
6. The volumes of oil to use for preparing solutions should not be measured with a pipette, but using volumetric flasks which can be rinsed out.
7. Equivalent results can be obtained using mixtures of a 100 per cent solid fat (linseed oil hardened to melting point 70°C) and pure olive oil containing respectively 25, 50, 75, 100 per cent (V/V) of liquid.
8. This procedure also allows the linearity of response of the apparatus to be checked rapidly.
9. The reading of the adjusting potentiometer has, in general, no correlation with the correction factor.
10. A simplified apparatus of the type Newport Quantity Analyser MKI may be used.
11. Important

Solid contents below 20°C obtained according to the parallel and the serial mode of operation may differ. The mode of operation used should therefore be mentioned in the report on the test.

III. GLYCERINES

1982

3.101 DETERMINATION OF THE RELATIVE DENSITY 20/20 IN AIR AND OF THE
CONVENTIONAL MASS PER UNIT VOLUME1. SCOPE AND FIELD OF APPLICATION

This Standard describes a method for the determination of the relative density 20/20 in air and of the conventional mass per unit volume of industrial glycerines. (Note 1). The methods are suitable for any solution of glycerol in water. The relative density 20/20 in air is intended primarily for purified glycerines.

2. DEFINITIONS

The relative density 20/20 in air of glycerines is the ratio of the mass in air of a given volume of the glycerines at 20°C to that of the same volume of water at 20°C. (Note 2). The conventional mass per unit volume of glycerines is the quotient of its mass in air by its volume. It is expressed in g per ml or in kg per cubic meter. The conventional mass per unit volume of a given substance varies with temperature.

3. PRINCIPLE

Calibration of the pycnometer by measurement of the conventional mass of the water contained in the pycnometer at a given temperature.

Measurement of the conventional mass of the sample contained in the pycnometer at the same temperature.

Calculation of the relative density 20/20 in air by dividing the conventional mass of the sample by the conventional mass of an equal volume of water when both of them are measured at 20°C.

Calculation of the volume of the pycnometer at a given temperature from the conventional mass of the water contained in the pycnometer at that temperature and from the table of conventional mass per unit volume of water at different temperatures. (Appendix 1).

Calculation of the conventional mass per unit volume of the sample at a given temperature by dividing the conventional mass of the sample at that temperature by the volume of the pycnometer at the same temperature.

4. APPARATUS

4.1. Pycnometer, Gay-Lussac type or any other suitable pycnometer, preferably of capacity about 50 ml, whether or not equipped with a thermometer graduated in 0.1°C

4.2. Water bath regulated at the required temperature $\pm 0.1^\circ\text{C}$

5. REAGENTS

5.1. Water, distilled, freshly boiled and then cooled to a temperature slightly below the required temperature

5.2. Acetone

6. PROCEDURE

Carry out each weighing to the nearest 0.1 mg.

Bring the substance (water or glycerine) to a temperature slightly below the required temperature.

Empty the pycnometer (4.1). Rinse it with acetone (5.2). Dry it.

After 2 to 3 minutes weigh it empty but with its stopper or thermometer.

Fill it to the half way up the neck with the substance, avoiding the formation of bubbles.

Insert the stopper or thermometer. Rinse the outside with water (5.1). Immerse the pycnometer in the water-bath (4.2) at the temperature of measurement to a depth half way up the ground portion of the neck, leaving it there for at least 30 minutes. While keeping the pycnometer in the water-bath, remove the excess liquid so that the level is exactly

flush with the end of the capillary tube.

Withdraw the pycnometer from the water-bath and hold it for 1 minute in a stream of water at a temperature below the required temperature.

Dry the outside of the pycnometer with a lint-free cloth. Leave to cool if necessary and wipe with a cloth lightly soaked in acetone.

After 2-3 minutes, reweigh the pycnometer. Calculate, by difference, the conventional mass of substance (water or glycerine) it contains. (Note 3).

7. EXPRESSION OF RESULTS

7.1. Relative density 20/20 in air.

The relative density 20/20 in air ($d_{20/20}$) is given by the formula :

$$d_{20/20} = \frac{m_2}{m_1}$$

where

m_1 is the conventional mass, in g, of water (5.1) contained in the pycnometer (4.1) at 20°C, as determined in 6,

m_2 is the conventional mass, in g, of glycerine contained in the pycnometer at 20°C, as determined in 6.

7.2. Mass per unit volume at temperature t.

The mass per unit volume at temperature t (ρ_t), in g/ml, is given by the formula :

$$\rho_t = \frac{m_3}{V_t}$$

where

m_3 is the conventional mass of glycerine, in g, contained in the pycnometer at temperature t, as determined in 6,

V_t is the volume of the pycnometer at temperature t, in ml. (Note 4).

If the mass per unit volume has to be expressed in kg/m³ (SI units), the value found has to be multiplied by 10³.

Express the results to three decimal places.

8. PRECISION

8.1. Repeatability.

Duplicate determination on the same day by the same analyst using the same apparatus should not differ by more than 0.0002.

8.2. Reproducibility.

Single determinations made in different laboratories should not differ by more than 0.0005.

9. NOTES

1. The relative density 20/20 in air is in current use for the qualification of purified glycerines. It is a measure for its glycerol content.

To convert the relative density in air to glycerol content, the table of Bosart and Snoddy has been included. (Appendix 2).

The conventional mass per unit volume of glycerines is used to convert volumes to masses (not taking into account buoyancy due to air).

2. The expression "relative density" (d) and "specific gravity" are equivalent. When the term "relative density" includes the symbol 20/4, the measurement is carried out at 4°C for the reference substance (water) and at 20°C for the substance to be examined (glycerines).

The conversion $d_{20/20}$ to $d_{20/4}$ may be calculated using the following formula :

$$d_{20/4} = d_{20/20} \times 0.99823$$

3. If it is necessary to carry out numerous determinations, it may be practical not to calibrate the pycnometer at each measurement, but merely to check that its mass and volume remain constant with time.
4. The volume of the pycnometer (4.1) at a given temperature t (V_t), in ml, is given by the formula :

$$V_t = \frac{m_t}{\rho_{t, H_2O}}$$

where

m_t is the conventional mass, in g, of water (5.1) contained in the pycnometer (4.1) at temperature t , as determined in 6,

ρ_{t, H_2O} is the conventional mass per unit volume of water at temperature t , in g/ml. (Appendix 1).

If the pycnometer is calibrated at 20°C the volume of the pycnometer at t °C can be calculated using the formula :

$$V_t = V_{20} [1 + \alpha (t - 20)]$$

where

V_t is the volume of the pycnometer at t °C, in ml,

V_{20} is the volume of the pycnometer at 20°C, in ml,

α is the cubic expansion coefficient of the glass of the pycnometer, in °C⁻¹.

Some values of α :

Boro-silicate glass D50 : $10 \times 10^{-6} \text{ } ^\circ\text{C}^{-1}$

Boro-silicate glass G20 : $16 \times 10^{-6} \text{ } ^\circ\text{C}^{-1}$

Sodium glass : $15\text{-}30 \times 10^{-6} \text{ } ^\circ\text{C}^{-1}$

APPENDIX 1

WATER

Conventional mass per unit volume (ρ_{t, H_2O}) - Temperature (t)

(From Draft Proposal ISO/DP 6883 - Doc. ISO/TC 34/SC 11 N 186)

t (°C)	ρ_{t, H_2O} (g/ml)	t (°C)	ρ_{t, H_2O} (g/ml)	t (°C)	ρ_{t, H_2O} (g/ml)
15	0.99807	32	0.99399	49	0.98746
16	0.99791	33	0.99367	50	0.98701
17	0.99774	34	0.99334	51	0.98656
18	0.99756	35	0.99300	52	0.98609
19	0.99737	36	0.99265	53	0.98563
20	0.99717	37	0.99230	54	0.98515
21	0.99696	38	0.99193	55	0.98467
22	0.99674	39	0.99156	56	0.98419
23	0.99651	40	0.99119	57	0.98369
24	0.99627	41	0.99080	58	0.98319
25	0.99601	42	0.99041	59	0.98269
26	0.99575	43	0.99001	60	0.98213
27	0.99548	44	0.98960	61	0.98166
28	0.99520	45	0.98918	62	0.98114
29	0.99491	46	0.98876	63	0.98061
30	0.99461	47	0.98834	64	0.98007
31	0.99431	48	0.98790	65	0.97953

APPENDIX 2

GLYCERINES

Relative density in air - Glycerol concentration (m/m)

(From AOCS Book of Official and Tentative Methods)

(Method Ea 7-50 - Bosart and Snoddy Table)

Glycerol concentration	relative density in air			
	15/15°C.	15.5/15.5°C.	20/20°C.	25/25°C.
per cent				
100	1.26557	1.26532	1.26362	1.26201
99	1.26300	1.26275	1.26105	1.25945
98	1.26045	1.26020	1.25845	1.25685
97	1.25785	1.25760	1.25585	1.25425
96	1.25525	1.25500	1.25330	1.25165
95	1.25270	1.25245	1.25075	1.24910
94	1.25005	1.24980	1.24810	1.24645
93	1.24740	1.24715	1.24545	1.24380
92	1.24475	1.24450	1.24280	1.24115
91	1.24210	1.24185	1.24020	1.23850
90	1.23950	1.23920	1.23755	1.23585
89	1.23680	1.23655	1.23490	1.23320
88	1.23415	1.23390	1.23220	1.23055
87	1.23150	1.23120	1.22955	1.22790
86	1.22885	1.22855	1.22690	1.22520
85	1.22620	1.22590	1.22420	1.22255
84	1.22355	1.22325	1.22155	1.21990
83	1.22090	1.22055	1.21890	1.21720
82	1.21820	1.21790	1.21620	1.21455
81	1.21555	1.21525	1.21355	1.21190
80	1.21290	1.21260	1.21090	1.20925
79	1.21015	1.20985	1.20815	1.20655
78	1.20740	1.20710	1.20540	1.20380
77	1.20465	1.20440	1.20270	1.20110
76	1.20190	1.20165	1.19995	1.19840
75	1.19915	1.19890	1.19720	1.19565
74	1.19640	1.19615	1.19450	1.19295
73	1.19365	1.19340	1.19175	1.19025
72	1.19090	1.19070	1.18900	1.18755
71	1.18815	1.18795	1.18630	1.18480
70	1.18540	1.18520	1.18355	1.18210
69	1.18260	1.18240	1.18080	1.17935
68	1.17985	1.17965	1.17805	1.17660
67	1.17705	1.17685	1.17530	1.17385
66	1.17430	1.17410	1.17255	1.17110
65	1.17155	1.17130	1.16980	1.16835
64	1.16875	1.16855	1.16705	1.16560
63	1.16600	1.16575	1.16430	1.16285
62	1.16320	1.16300	1.16155	1.16010
61	1.16045	1.16020	1.15875	1.15735
60	1.15770	1.15745	1.15605	1.15460
59	1.15490	1.15465	1.15325	1.15185
58	1.15210	1.15190	1.15050	1.14915
57	1.14935	1.14910	1.14775	1.14640
56	1.14655	1.14635	1.14500	1.14365
55	1.14375	1.14355	1.14220	1.14090
54	1.14100	1.14080	1.13945	1.13815
53	1.13820	1.13800	1.13670	1.13540
52	1.13540	1.13525	1.13395	1.13265
51	1.13265	1.13245	1.13120	1.12995

Glycerol concentration	relative density in air			
	15/15°C.	15.5/15.5°C.	20/20°C.	25/25°C.
per cent				
50	1.12985	1.12970	1.12845	1.12720
49	1.12710	1.12695	1.12570	1.12450
48	1.12440	1.12425	1.12300	1.12185
47	1.12165	1.12150	1.12030	1.11915
46	1.11890	1.11880	1.11760	1.11650
45	1.11620	1.11605	1.11490	1.11380
44	1.11345	1.11335	1.11220	1.11115
43	1.11075	1.11060	1.10950	1.10845
42	1.10800	1.10790	1.10680	1.10575
41	1.10525	1.10515	1.10410	1.10310
40	1.10255	1.10245	1.10135	1.10040
39	1.09985	1.09975	1.09870	1.09775
38	1.09715	1.09705	1.09605	1.09510
37	1.09445	1.09435	1.09335	1.09245
36	1.09175	1.09165	1.09070	1.08980
35	1.08905	1.08895	1.08805	1.08715
34	1.08635	1.08625	1.08535	1.08455
33	1.08365	1.08355	1.08270	1.08190
32	1.08100	1.08085	1.08005	1.07925
31	1.07830	1.07815	1.07735	1.07660
30	1.07560	1.07545	1.07470	1.07395
29	1.07295	1.07285	1.07210	1.07135
28	1.07035	1.07025	1.06950	1.06880
27	1.06770	1.06760	1.06690	1.06625
26	1.06510	1.06500	1.06435	1.06370
25	1.06250	1.06240	1.06175	1.06115
24	1.05985	1.05980	1.05915	1.05860
23	1.05725	1.05715	1.05655	1.05605
22	1.05460	1.05455	1.05400	1.05350
21	1.05200	1.05195	1.05140	1.05095
20	1.04935	1.04935	1.04880	1.04840
19	1.04685	1.04680	1.04630	1.04590
18	1.04435	1.04430	1.04380	1.04345
17	1.04180	1.04180	1.04135	1.04100
16	1.03930	1.03925	1.03885	1.03850
15	1.03675	1.03675	1.03635	1.03605
14	1.03425	1.03420	1.03390	1.03360
13	1.03175	1.03170	1.03140	1.03110
12	1.02920	1.02920	1.02890	1.02865
11	1.02670	1.02665	1.02640	1.02620
10	1.02415	1.02415	1.02395	1.02370
9	1.02175	1.02175	1.02155	1.02135
8	1.01935	1.01930	1.01915	1.01900
7	1.01690	1.01690	1.01675	1.01660
6	1.01450	1.01450	1.01435	1.01425
5	1.01210	1.01205	1.01195	1.01185
4	1.00965	1.00965	1.00955	1.00950
3	1.00725	1.00725	1.00720	1.00710
2	1.00485	1.00485	1.00480	1.00475
1	1.00240	1.00240	1.00240	1.00235

III. GLYCERINES

1932

3.102 MEASUREMENT OF COLOUR IN HAZEN UNITS

(Platinum - cobalt scale)

1. SCOPE AND FIELD OF APPLICATION

This Standard describes a method for the visual measurement of the colour of clear, slightly coloured industrial glycerines.

This Standard is applicable only to glycerines in which the colour-producing bodies present have light absorption characteristics nearly identical with those of the reference platinum-cobalt colour standards. (Note 1).

2. DEFINITION

The colour of glycerines is equal to one Hazen unit when -by visual measurement- it is judged the same as that of a solution obtained by diluting 500 times the standard solution prepared as indicated below. (Notes 2, 3).

3. PRINCIPLE

The colour of the glycerines is matched against the colour of a standard solution defined in Hazen units.

4. APPARATUS

4.1. 250 ml beaker

4.2. 100 ml and 1 litre volumetric flasks

4.3. 1, 5, 10 and 20 ml graduated pipettes

4.4. Nessler tall-form colour comparison tubes, 100 ml capacity, provided with ground-on, optically clear, glass caps. The graduation mark should be between 275 and 295 mm above the bottom of the tube. In practice the tubes should be selected and used in sets that possess the same depth of solution (within 2 to 3 mm) when filled to the mark.

4.5. Colour comparator

4.5.1. A colour comparator rack constructed to permit visual comparison of light longitudinally transmitted through the Nessler tubes.

The comparator should be constructed so that white light is passed either through or reflected off a white glass plate and directed vertically with equal intensity through the tubes.

The colour comparator should be shielded so that no light can enter the tubes horizontally.

4.5.2. Light source, daylight type fluorescent tube.

or

4.6. A colour comparator provided with permanent platinum-cobalt colour discs, in conjunction with the appropriate Nessler tubes.

4.7. Spectrophotometer

4.8. Cells, path-length 1 cm

5. REAGENTS

5.1. Hydrochloric acid ($\rho = 1.19$ g/ml approx.)

5.2. Cobalt chloride hexahydrate ($\text{CoCl}_2 \cdot 6 \text{H}_2\text{O}$), analytical reagent quality

5.3. Potassium chloroplatinate (K_2PtCl_6), analytical reagent quality

6. PROCEDURE

6.1. Preparation of the platinum-cobalt colour standards.

6.1.1. Preparation of the stock solution (500 Hazen units).

Weigh, to the nearest 0.001 g, 1.245 g of potassium chloroplatinate (5.3) and 1.000 g of cobalt chloride (5.2). Transfer quantitatively to a beaker (4.1). Dissolve with water and 100 ml of hydrochloric acid (5.1). Pour quantitatively into a 1 litre volumetric flask (4.2). Dilute to the mark with distilled water. Mix.

The absorbance of this solution measured with the spectrophotometer (4.7) in a cell (4.8) against distilled water must fall within the limits given below :

Wavelength nm	Absorbance
430	0.110 - 0.120
455	0.130 - 0.145
480	0.105 - 0.120
510	0.055 - 0.065

(Note 4).

6.1.2. Preparation of the colour standards.

Prepare 20 colour standards having 5 to 100 Hazen units in steps of 5, by transferring successively by pipette (4.3) 1, 2, ..., 20 ml of the stock solution (6.1.1) to 100 ml volumetric flasks (4.2).

Dilute to 100 ml with distilled water. Mix.

The number of Hazen units expressing the colour of the solutions are respectively 5, 10, ..., 100; each ml of the stock solution corresponds to an increment of five in the colour standard number. (Note 5).

6.2. Measurement.

Introduce 100 ml of sample into a Nessler tube (4.4), passing the sample through a filter if it has any visible turbidity. Cap the tube. Place it in the comparator (4.5 or 4.6). Introduce the colour standards (100 ml) into Nessler tubes. Cap the tubes. Place them one after the other in the comparator until the best match between the sample and the standard colours are obtained.

7. EXPRESSION OF RESULTS

Report as the colour of the sample, the Hazen unit number of the standard that most nearly matches the samples. If the colour lies midway between two standards, report the darker of the two. (Note 6).

If differences in hue preclude a definite match of sample and standard, report the range over which an apparent match is obtained and report the sample as "off-hue".

8. NOTES

1. The measurement of colour in Hazen units is in current commercial use for the evaluation of purified glycerine.
2. Hazen, APHA, Pt-Co colour units represent the same colour values.
3. The standard solution may be replaced by permanent colour discs with Pt-Co standard glasses.
4. The stock solution (500 Hazen units) should be stored in the dark in stoppered glass bottles. Under these conditions the stock solution is stable for 1 year.
5. The colour standards, although stable for at least 1 month, shall preferably be prepared fresh.
6. Colour standards may be prepared in smaller steps if the colour of the sample admits of a closer match than can be obtained with standards differing by five units.

III. GLYCERINES

1982

3.122 DETERMINATION OF THE ACIDITY AND THE SAPONIFICATION EQUIVALENT

1. SCOPE AND FIELD OF APPLICATION

This Standard describes a method for the determination of the acidity and the saponification equivalent of purified glycerines.

2. DEFINITION

The acidity is a measure of the content of fatty acids; it is expressed in millimoles of sodium hydroxide required to neutralise the acids present in 100 g of glycerine. The saponification equivalent is a measure of the content of fatty acids and esters; it is expressed in millimoles of sodium hydroxide required to neutralise the acids and to saponify the esters present in 100 g of glycerine.

3. PRINCIPLE

3.1. Acidity

Neutralisation of an aqueous solution of the test portion with a standardised solution of sodium hydroxide in the presence of phenolphthalein as indicator.

3.2. Saponification equivalent

Further to the acidity determination, sodium hydroxide solution is added until it is in excess.
Boiling. Titration of this excess with standardised hydrochloric acid solution.

4. APPARATUS

- 4.1. 250 ml conical flasks made of alkali-resistant glass
- 4.2. Reflux condensers to fit the flasks (4.1)
- 4.3. Stoppers equipped with a soda-lime tube to fit the flasks (4.1)
- 4.4. Bottle provided with a soda-lime tube

5. REAGENTS

- 5.1. Distilled water, freshly boiled and subsequently cooled in a bottle (4.4)
- 5.2. Sodium hydroxide, 0.1 N aqueous solution, accurately standardised
- 5.3. Hydrochloric acid, 0.1 N aqueous solution, accurately standardised
- 5.4. Phenolphthalein, 10 g/l solution in 95 per cent (V/V) ethanol

6. PROCEDURE

Weigh 50 g of the well mixed sample into a flask (4.1). Add 50 ml of cooled water (5.1). Add 0.5 ml of phenolphthalein (5.4). Carry out a blank test at the same time using 90 ml of cooled water (5.1).
If the solution is alkaline, adjust to neutrality with the hydrochloric acid solution (5.3). If the solution is acidic, titrate with the sodium hydroxide solution (5.2) to the first permanent pink colour.
Add a further quantity of the sodium hydroxide solution (5.2) until a total of 20.0 ml has been added. Connect the flask to the reflux condenser (4.2). Heat to boiling. Boil for 5 minutes. Allow to cool somewhat.
Wash down the condenser with a little water (5.1).
Disconnect the flask. Close it with a stopper (4.3). Cool.
Titrate with the hydrochloric acid solution (5.3).

7. EXPRESSION OF RESULTS

The acidity expressed in millimoles of sodium hydroxide required to neutralise the acids present in 100 g of glycerine is given by the formula :

$$\frac{100 \times V \times T_1}{m}$$

where

V is the number of ml of the sodium hydroxide solution (5.2) used for the determination,
T₁ is the exact normality of the sodium hydroxide solution (5.2) used,
m is the mass, in g, of the sample.

The saponification equivalent expressed in millimoles of sodium hydroxide required to neutralise the acids and to saponify the esters present in 100 g of glycerine, is given by the formula :

$$\frac{100 \times (V_1 - V_2) \times T_2}{m}$$

where

V₁ is the number of ml of the hydrochloric acid solution (5.3) used for the titration of the blank test,
V₂ is the number of ml of the hydrochloric acid solution (5.3) used for the titration of the sample,
T₂ is the exact normality of the hydrochloric acid solution (5.3) used,
m is the mass, in g, of the sample.

III. GLYCERINES

1982

3.138 DETERMINATION OF THE SULPHATED ASH CONTENT

1. SCOPE AND FIELD OF APPLICATION

This Standard describes a method for the determination of the sulphated ash content. This Standard is applicable to purified glycerine.

2. DEFINITION

The sulphated ash content of purified glycerine is the quantity of sulphated ash, expressed as a percentage (m/m), determined by the present method.

3. PRINCIPLE

Combustion of the test portion. Moistening of the residue with concentrated sulphuric acid. Heating to expel the acid and to burn off all the combustible material. Weighing of the sulphated residue.

4. APPARATUS

4.1. Platinum dish, 70 to 90 mm diameter and 40 to 50 mm deep

4.2. Desiccator

4.3. Muffle furnace, regulated at $840 \pm 10^\circ\text{C}$

5. REAGENT

5.1. Sulphuric acid ($\rho = 1.84 \text{ g/ml}$)

6. PROCEDURE

Heat the dish (4.1) for a few minutes in the furnace (4.3) regulated at $840 \pm 10^\circ\text{C}$. Allow to cool to room temperature in a desiccator (4.2).

Weigh to the nearest 0.001 g.

Weigh, to the nearest 0.05 g, about 50 g of the sample in the dish.

Gently heat the dish containing the weighed sample over a small flame until glycerol vapours are evolved, avoiding spattering.

Ignite the vapours. Allow the sample to burn quietly in a place free from draughts, without further application of heat.

Allow the dish to cool.

Moisten the residue with 0.5 ml of sulphuric acid (5.1). Remove the excess acid by heating until the white fumes disappear and combustible material is burned away.

Repeat this operation.

Place the dish for 5 minutes in the muffle furnace regulated at $840 \pm 10^\circ\text{C}$.

Allow the dish to cool in a desiccator to room temperature.

Weigh the dish (4.1) to the nearest 0.001 g.

7. EXPRESSION OF RESULTS

The sulphated ash content, expressed as a percentage (m/m), is given by the formula :

$$\frac{100 (m_1 - m_0)}{m}$$

where

m_0 is the mass, in g, of the empty dish (4.1),

m_1 is the mass, in g, of the dish containing the sulphated ash,

m is the mass, in g, of the test portion.