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FOURTH COMMISSION DIRECTIVE
of 5 December 1972
establishing Community methods of analysis for the official control of feedingstuffs

(73/46/EEC)

(OJ L 83, 30.3.1973, p. 21)

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►M1	Commission Directive 81/680/EEC of 30 July 1981	L 246	32	29.8.1981
►M2	Commission Directive 92/89/EEC of 3 November 1992	L 344	35	26.11.1992
►M3	Commission Directive 98/54/EC of 16 July 1998	L 208	49	24.7.1998

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FOURTH COMMISSION DIRECTIVE

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establishing Community methods of analysis for the official control of feedingstuffs

(73/46/EEC)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community;

Having regard to the Council Directive of 20 July 1970 [1] on the introduction of Community methods of sampling and analysis for the official control of feedingstuffs, as amended by the Directive No 72/275/EEC of 20 July 1972 [2], and in particular Article 2 thereof;

Whereas that Directive requires that official controls of feedingstuffs be carried out using Community methods of sampling and analysis for the purpose of checking compliance with requirements arising under the provisions laid down by law, Regulation or administrative action concerning the quality and composition of feedingstuffs;

Whereas Directives No 71/250/EEC of 15 June 1971 [3], No 71/393/EEC of 18 November 1971 [4] and No 72/199/EEC of 27 April 1972 [5] have already established a number of Community methods of analysis; whereas the progress of work since then makes it advisable to adopt a fourth set of methods;

Whereas the measures provided for in this Directive are in accordance with the Opinion of the Management Committee for Feedingstuffs;

HAS ADOPTED THIS DIRECTIVE:

Article 1

The Member States shall require that analyses for official controls of feedingstuffs as regards moisture contents of animal and vegetable fats and oils and magnesium and crude fibre contents of feedingstuffs be carried out according to the methods described in Annex I to this Directive.

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Article 2

The Member States shall require that analyses for official controls of feedingstuffs as regards their contents of retinol (vitamin A), ►M3 __________ ◄ be carried out according to the methods described in Annex II to this Directive.

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Article 3

The Member States shall, not later than 1 January 1974, bring into force the laws, regulations or administrative provisions necessary to comply with this Directive. They shall forthwith notify the Commission thereof.

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(1) OJ No L 170, 3. 8. 1970, p. 2.
(2) OJ No L 171, 29. 7. 1972, p. 39.
(3) OJ No L 155, 12. 7. 1971, p. 13.
(4) OJ No L 279, 20. 12. 1971, p. 7.
(5) OJ No L 123, 29. 5. 1972, p. 6.

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Article 4

This Directive is addressed to the Member States.

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ANNEX I

1. DETERMINATION OF MOISTURE IN ANIMAL AND VEGETABLE FATS AND OILS

1. Purpose and Scope

This method makes it possible to determine the water and volatile substances content of animal and vegetable fats and oils.

2. Principle

The sample is dried to constant weight at 103 ºC. The loss in mass is determined by weighing.

3. Apparatus

3.1. Flat-bottomed dish, of a corrosion-resistant material, 8 to 9 cm in diameter and approximately 3 cm high.

3.2. Mercury thermometer with a strengthened bulb and expansion tube at the top end, graduated from approximately 80 ºC to at least 110 ºC, and approximately 10 cm in length.

3.3. Sand bath or electric hot-plate.

3.4. Desiccator, containing an efficient drying agent.

3.5. Analytical balance.

4. Procedure

Weigh out to the nearest mg approximately 20 g of the homogenized sample into the dry, weighed dish (3.1) containing the thermometer (3.2). Heat on the sand bath or hot-plate (3.3), stirring continuously with the thermometer, so that the temperature reaches 90 ºC in about 7 minutes.

Reduce the heat, watching the frequency with which bubbles rise from the bottom of the dish. The temperature must not exceed 105 ºC. Continue to stir, scraping the bottom of the dish, until bubbles stop forming.

In order to ensure complete elimination of moisture, reheat several times to 103 ºC ± 2 ºC, cooling to 93 ºC between successive heatings. Then leave to cool to room temperature in the desiccator (3.4) and weigh. Repeat this operation until the loss in mass between two successive weighings no longer exceeds 2 mg.

N.B. An increase in the mass of the sample after repeated heating indicates an oxidation of the fat, in which case calculate the result from the weighing carried out immediately before the mass began to increase.

5. Calculation of results

The moisture content, as a percentage of the sample, is given by the following formula:

$$(M_1 - M_2) \cdot \frac{100}{M_o}$$

where:

M_o = mass, in grammes, of the test sample;

M_1 = mass, in grammes, of the dish with its contents before heating;

M_2 = mass, in grammes, of the dish with its contents after heating.

Results lower than 0·05 % must be recorded as ‘lower than 0·05 %’.

Repeatability

The difference in moisture between the results of two parallel determinations carried out on the same sample must not exceed 0·05 %, in absolute value.

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2. DETERMINATION OF MAGNESIUM

— by atomic absorption spectrophotometry —

1. Purpose and Scope

This method makes it possible to determine the quantity of magnesium in feeding stuffs. It is particularly appropriate for determining magnesium contents lower than 5 %.

2. Principle

The sample is ashed and dissolved in dilute hydrochloric acid. If it contains no organic substances, it is dissolved directly in dilute hydrochloric acid. The solution is diluted and the magnesium content determined by atomic absorption spectrophotometry at 285·2 nm, by comparison with standard solutions.

3. Reagents

3.1. Hydrochloric acid a.p. d: 1·16.

3.2. Concentrated hydrochloric acid a.p. d: 1·19.

3.3. Magnesium ribbon or wire, or magnesium sulphate heptahydrate, dried at room temperature.

3.4. Strontium salt solution (chloride or nitrate) at 2·5 % (w/v) strontium (= 76·08 g $SrC1_2 \cdot 6H_2O$ a.p., or 60·38 g $Sr (NO_3)_2$ a.p.).

3.5. Standard magnesium solution: weigh out to the nearest mg 1 g magnesium (3.3) which has previously had its oxide coating carefully removed, or the corresponding quantity (10·143 g) of magnesium sulphate heptahydrate (3.3). Place in a 1 000 ml graduated flask, add 80 ml hydrochloric acid (3.1), leave to dissolve and make up to 1 000 ml with water. 1 ml of this solution contains 1·000 mg magnesium.

4. Apparatus

4.1. Platinum, silica or porcelain ashing crucibles.

4.2. Thermostatically controlled electric muffle furnace.

4.3. Atomic absorption spectrophotometer.

5. Procedure

5.1. *Preparation of the sample solution*

5.1.1. *Feeding stuffs composed exclusively of mineral substances*

Weigh out to the nearest mg 5 g of the sample into a 500 ml graduated flask with 250 to 300 ml water. Add 40 ml hydrochloric acid (3.1), bring to the boil and keep the liquid gently boiling for 30 minutes. Leave to cool, make up to volume with water, mix and filter into a dry beaker through a dry pleated filter. Discard the first 30 ml of the filtrate. In the presence of silica, treat 5 g of sample with a sufficient quantity (15—30 ml) of hydrochloric acid (3.2), evaporate to dryness on a water bath and transfer to an oven at 105 ºC for one hour. Proceed as from the third sentence of 5.1.2.

5.1.2. *Feeding stuffs composed predominantly of mineral substances*

Weigh out to the nearest mg 5 g of the sample into a crucible and ash at 550 ºC in the muffle furnace until an ash which is free from carbonaceous particles is obtained, and leave to cool. In order to eliminate silica, add to the ash a sufficient quantity (15—30 ml) of hydrochloric acid (3.2), evaporate to dryness on a water bath and transfer to an oven at 105 ºC for one hour. Treat the residue with 10 ml hydrochloric acid (3.1) and transfer to a 500 ml graduated flask using warm water. Leave to cool and make to volume with water. Mix and filter into a dry beaker through a dry pleated filter. Discard the first 30 ml of the filtrate.

5.1.3. *Feedingstuffs composed predominantly of organic substances*

Weigh out to the nearest mg 5 g of the sample into a crucible and ash at 550 ºC in the muffle furnace until an ash which is free from carbonaceous particles is obtained. Treat the ash with 5 ml hydrochloric acid (3.2), evaporate to dryness on a water bath and then dry for one hour in the oven at 105 ºC in order to render the

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silica insoluble. Treat the ash with 5 ml hydrochloric acid (3.1), transfer to a 250 ml graduated flask using warm water, bring to the boil, leave to cool and make up to volume with water. Mix and filter into a dry beaker through a dry pleated filter. Discard the first 30 ml of the filtrate.

5.2. *Measurement by atomic absorption*

By diluting the standard solution (3.5) with water, prepare at least 5 reference solutions of increasing concentration, corresponding to the optimal measuring range of the spectrophotometer. Add to each solution 10 ml strontium salt solution (3.4) and then make up the volume to 100 ml with water. Dilute with water one aliquot part of the filtrate obtained from 5.1.1, 5.1.2 or 5.1.3, so as to obtain a magnesium concentration which is within the limits of concentration of the reference solutions. The hydrochloric acid concentration of this solution must not exceed 0·4 N. Add 10 ml strontium salt solution (3.4) and then make up the volume to 100 ml with water. Measure the absorption of the solution to be determined and of the reference solutions at 285·2 nm.

6. **Calculation of results**

Calculate the quantity of magnesium in the sample by relation to the reference solutions. Express the result as a percentage of the sample.

Repeatability

The difference between the results of two parallel determinations carried out on the same sample must not exceed 5 %, in relative value.

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3. DETERMINATION OF CRUDE FIBRE

1. **Purpose and scope**

This method makes it possible to determine fat-free organic substances in feedingstuffs which are insoluble in acid and alkaline media and are conventionally described as crude fibre.

2. **Principle**

The sample, defatted where necessary, is treated successively with boiling solutions of sulphuric acid and potassium hydroxide of specified concentrations. The residue is separated by filtration on a sintered-glass filter washed, dried, weighed and ashed within a range of 475 to 500 ºC. The loss of weight resulting from ashing corresponds to the crude fibre present in the test sample.

3. **Reagents**

3.1. Sulphuric acid, c = 0,13 mol/l.

3.2. Anti-foaming agent (e.g. n-octanol).

3.3. Filter aid (Celite 545 or equivalent), heated at 500 ºC for four hours (8.6).

3.4. Acetone.

3.5. Light petroleum boiling-range 40 to 60 ºC.

3.6. Hydrochloric acid, c = 0,5 mol/l.

3.7. Potassium hydroxide solution, c = 0,23 mol/l.

4. **Apparatus**

4.1. Heating unit for digestion with sulphuric acid or potassium hydroxide solution, equipped with a support for the filter crucible (4.2) and provided with an outlet tube with a tap to the liquid outlet and vacuum, possibly with compressed air. Before use each day preheat the unit with boiling water for five minutes.

4.2. Glass filter crucible with fused sintered glass filter plate pore size 40-90 µm. Before first use, heat to 500 ºC for a few minutes and cool (8.6).

4.3. Cylinder of at least 270 ml with a reflux condenser, suitable for boiling.

4.4. Drying oven with thermostat.

4.5. Muffle furnace with thermostat.

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4.6. Extraction unit consisting of a support plate for the filter crucible (4.2.) and with a discharge pipe with a tap to the vacuum and liquid outlet.

4.7. Connecting rings to assemble the heating unit (4.1), crucible (4.2) and cylinder (4.3) and to connect the cold extraction unit (4.6) and crucible.

5. Procedure

Weigh out to the nearest 0,001 g, 1 g of the prepared sample and place it in the crucible (4.2), (see observations 8.1, 8.2 and 8.3) and add 1 g of filter aid (3.3).

Assemble the heating unit (4.1) and the filter crucible (4.2), then attach the cylinder (4.3) to the crucible. Pour 150 ml of boiling sulphuric acid (3.1) into the assembled cylinder and crucible and if necessary add a few drops of anti-foaming agent (3.2).

Bring the liquid to the boil within 5 ± 2 minutes and boil vigorously for exactly 30 minutes.

Open the tap to the discharge pipe (4.1) and, under vacuum, filter the sulphuric acid through the filter crucible and wash the residue with three consecutive 30 ml portions of boiling water, ensuring that the residue is filtered dry after each washing.

Close the outlet tap and pour 150 ml boiling potassium hydroxide solution (3.7) to the assembled cylinder and crucible and add a few drops of anti-foaming agent (3.2). Bring the liquid to boiling point within 5 ± 2 minutes and boil vigorously for exactly 30 minutes. Filter and repeat the washing procedure used for the sulphuric acid step.

After the final washing and drying, disconnect the crucible and its contents and reconnect it to the cold extraction unit (4.6). Apply the vacuum and wash the residue in the crucible with three consecutive 25 ml portions of acetone (3.4) ensuring that the residue is filtered dry after each washing.

Dry the crucible to constant weight in the oven at 130 ºC. After each drying cool in the desiccator and weigh rapidly. Place the crucible in a muffle furnace and ash to constant weight at 475 ºC to 500 ºC for at least 30 minutes.

After each heating cool first in the furnace and then in the desiccator before weighing.

Carry out a blank test without the sample. Loss of weight resulting from ashing must not exceed 4 mg.

6. **Calculation of results**

The crude fibre content as a percentage of the sample is given by the expression:

$$\frac{(b - c) \times 100}{a}$$

where

a = mass of sample in g;

b = loss of mass after ashing during the determination, in g;

c = loss of mass after ashing during the blank test, in g.

7. **Repeatability**

The difference between two parallel determinations carried out on the same sample must not exceed:

— 0,3 in absolute value for crude fibre contents lower than 10 %,

— 3 % relative to the higher result, for crude fibre contents equal to or greater than 10 %.

8. **Observations**

8.1. Feedingstuffs containing more than 10 % crude fat must be defatted prior to analysis with light petroleum (3.5). Connect the filter crucible (4.2) and its contents to the cold extraction unit (4.6) and apply vacuum and wash the residue with three consecutive 30 ml portions of light petroleum, ensuring that the residue is dry. Connect the crucible and its contents to the heating unit (4.1) and continue as described under 5.

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8.2. Feedingstuffs containing fats which cannot be extracted directly with light petroleum (3.5) must be defatted as shown in 8.1 and defatted once more after boiling with acid.

After boiling with acid and the subsequent washing connect the crucible and its contents to the cold extraction unit (4.6) and wash three times with 30 ml acetone followed by three further washings with 30 ml portions of light petroleum. Filter under vacuum until dry and continue the analysis as described under 5, beginning with potassium hydroxide treatment.

8.3. If the feedingstuffs contain over 5 % of carbonates, expressed as calcium carbonate, connect the crucible (4.2) with the weighed sample to the heating unit (4.1). Wash the sample three times with 30 ml hydrochloric acid (3.6). After each addition let the sample stand for about one minute before filtering. Wash once with 30 ml water and then continue as described under 5.

8.4. If an apparatus in the form of a stand is used (several crucibles attached to the same heating unit) no two individual determinations on the same sample for analysis may be carried out in the same series.

8.5. If after boiling it is difficult to filter the acidic and basic solutions, use compressed air through the discharge pipe of the heating unit and then continue filtering.

8.6. The temperature for ashing should not be higher than 500 ºC in order to extend the lifetime of the glass filter crucibles. Care must be taken to avoid excessive thermal shock during heating and cooling cycles.

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ANNEX II

1. DETERMINATION OF RETINOL (VITAMIN A)

1. Purpose and Scope

This method makes it possible to determine the quantity of retinol (Vitamin A) in feedingstuffs, concentrates and premixes. The lower limit of the determination is 10 000 IU/kg for highly pigmented feeds and 4 000 IU/kg for others [1]. Products are classified in two groups, according to their presumed retinol content:

Group A: contents lower than 200 000 IU/kg;

Group B: contents equal to or greater than 200 000 IU/kg.

2. Principle

The sample is hydrolised when hot with a potassium hydroxide solution in an ethanol medium and in the presence of an antioxidant or in a nitrogen atmosphere. The mixture is extracted with 1,2-dichlorethane. The extract is evaporated to dryness and treated with light petroleum. The solution is chromatographed on a column of aluminium oxide (forGroup B products, chromatography is only required in certain cases). For Group A products the retinol is determined by spectrophotometry at 610 nm after development of a coloured complex according to the Carr-Price reaction; for Group B products by spectrophotometry in the UV at 325 nm.

3. Reagents

(a) *used for analysing products of Groups A and B*

3.1. 96 % (v/v) ethanol.

3.2. 10 % (w/v) sodium ascorbate solution a.p., or

3.3. Purified nitrogen.

3.4. 50 % (w/v) potassium hydroxide solution a.p.

3.5. Potassium hydroxide solution 1 N a.p.

3.6. Potassium hydroxide solution 0·5 N a.p.

3.7. 1,2-dichlorethane a.p.

3.8. Light petroleum, boiling range: 30—50 ºC. If necessary, purify as follows: stir 1 000 ml light petroleum with 20 ml lots of concentrated sulphuric acid until the acid remains colourless. Remove the acid and wash the light petroleum successively with 500 ml water, twice with 250 ml of 10 % (w/v) sodium hydroxide solution and three times with 500 ml water. Remove the aqueous layer, dry the light petroleum for 1 hour over active carbon and anhydrous sodium sulphate, filter and distil.

3.9. Aluminium oxide, standardized according to Brockmann: ash for 8 hours at 750 ºC, cool in a desiccator and keep in a brown glass bottle fitted with a ground-glass stopper. Before use in chromatography moisten as follows: place in a brown glass bottle 10 g aluminium oxide and 0·7 ml water, seal with a stopper, reheat for 5 minutes in a boiling water bath while shaking. Leave to cool. Verify the activity of the aluminium thus prepared by subjecting a known quantity of retinol (3.17) (ca. 500 IU) to the procedure of 5.3 and 5.4 and checking recovery.

3.10. Basic aluminium oxide, degree of activity 1 (Woelm, Merck or equivalent).

3.11. Pure diethyl ether. Remove peroxides and traces of water by chromatography on a column of basic aluminium oxide (3.10). (25 g aluminium oxide per 250 ml diethyl ether.)

3.12. Light petroleum solutions (3.8) at 4, 8, 12, 16 and 20 % (v/v) diethyl ether (3.11).

3.13. Sodium sulphide solution 0·5 molar in 70 % (v/v) glycerine, prepared from sodium sulphide a.p.

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[1] 1 IU = 0·3 µg of retinol.

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(b) *used exclusively for analysing Group A products*

3.14. Crystallizable benzene a.p.

3.15. Chloroform a.p. Remove the ethanol, phosgene and traces of water by chromatography on a column of basic aluminium oxide (3.10) (50 g aluminium oxide per 200 ml chloroform; it is advisable to chromatograph the first 50 ml of the eluate a second time).

3.16. Carr-Price reagent: stir approximately 25 g antimony trichloride a.p. (kept in a desiccator) with 100 ml chloroform (3.15) until the solution is saturated. A slight deposit of antimony trichloride causes no problem. Add 2 ml acetic anhydride a.p. Keep in a refrigerator in a brown glass bottle with ground-glass stopper. The solution keeps for several weeks.

3.17. Retinol — standardized spectrophotometrically.

(c) *used exclusively for analysing Group B products*

3.18. Isopropanol, for chromatography.

4. **Apparatus**

4.1. Water bath.

4.2. Vacuum evaporation apparatus with round flasks of different capacities.

4.3. Glass chromatography tubes (length: 300 mm; internal diameter: about 13 mm).

4.4. Spectrophotometer with 10 mm cells. Measurements in the UV require silica cells.

4.5. UV lamps suitable for 365 nm.

5. **Procedure**

N.B. All operations must be carried out away from direct light, if necessary in brown glass equipment.

5.1. *Test Sample*

From the finely divided sample, take a test sample proportional to the presumed retinol content, thus:

0·1—1·0 g for concentrates (contents greater than 20 000 IU/g);

3·0—5·0 g for premixes (contents of between 400 and 20 000 IU/g);

10—20 g for mineral mixtures;

30 g for Group A products.

Immediately place the test sample in a 500 ml flask with a ground-glass stopper.

5.2. *Hydrolysis and extraction* [1]

Add successively to the test sample 40 ml ethanol (3.1), 2 ml sodium ascorbate solution (3.2) [2], 10 ml potassium hydroxide solution (3.4) and 2 ml sodium sulphide solution (3.13).

Heat for 30 minutes at 70—80 ºC under a reflux condenser and then leave to cool under a stream of water. Add 50 ml ethanol (3.1) and 100 ml 1,2-dichlorethane (3.7) (taken with a pipette). Shake vigorously and then decant the supernatant liquid into a decanting container. Add to the container 150 ml potassium hydroxide solution (3.5), shake for 30 seconds and leave to stand until the layers are separated. Collect the dichlorethane layer (lower layer) in a decanting container, add 40 ml potassium hydroxide solution (3.6), shake for 10 seconds and leave to stand until the layers are separated. Collect the dichlorethane layer in a decanting container and wash 6—8 times with 40 ml lots of water until free of alkali (phenolphthalein test). Collect the dichlorethane layer and remove the last traces of water using strips of filter paper.

(1) For milk feeds and products with a tendency to agglomerate or swell, *double* the quantity of the reagents shown in the first and second paragraphs of 5.2.

(2) Sodium ascorbate need not be added when hydrolysis is carried out in a nitrogen atmosphere.

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Evaporate to dryness an aliquot part of the solution under vacuum and on the water bath at 40 ºC. Rapidly treat the residue with 5 ml light petroleum (3.8).

For Group A products, chromatograph as shown in 5.3.1.

For Group B products, transfer the solution to a 50 ml graduated flask, make up to volume with light petroleum (3.8), mix and measure the optical density as shown in 5.4.2.

5.3. *Chromatography*

5.3.1. *Group A products*

Fill a chromatography tube (4.3) to a height of 200 mm with 10 g aluminium oxide (3.9) previously slurried with light petroleum (3.8). Place in the tube the solution obtained in 5.2 and immediately add 20 ml light petroleum (3.8). Elute successively with 10 ml lots of the light petroleum solutions at 4, 8, 12, 16 and 20 % diethyl ether (3.12) under pressure or partial vacuum, the rate of flow being 2 to 3 drops per second.

The carotene is eluted first [1]. The retinol is generally eluted with the light petroleum solution at 20 % diethyl ether (3.12). The elution is followed under UV light (brief irradiation of the column with the mercury lamp). The fluorescent zone of the retinol is clearly separated from the yellow xanthophyll zones following it. Collect the eluate fraction containing the retinol in an Erlenmeyer flask.

5.3.2. *Group B products*

Chromatography must only be carried out if the optical density measurements obtained in 5.4.2 do not conform to the requirements given in 5.4.2.

If chromatography proves necessary, place in the chromatography column an aliquot part of the solution in the light petroleum obtained in 5.2, containing approximately 500 IU of retinol, and chromatograph as shown in 5.3.1.

5.4. *Measurement of the optical density*

5.4.1. *Group A products*

Evaporate to dryness under vacuum the eluate containing the retinol obtained in 5.3.1. Treat the residue with 2 ml benzene (3.14). Take 0·3 ml of this solution and add 3 ml of the Carr-Price reagent (3.16). A blue colouring develops. Measure the optical density with the spectrophotometer at 610 nm exactly 30 seconds after the reaction has begun. Determine the retinol content by reference to a standard curve obtained from benzene solutions of increasing retinol-standard concentrations treated with Carr-Price reagent (2 to 16 IU retinol-standard (3.17) per 0·3 ml benzene (3.14) +3 ml Carr-Price reagent (3.16)). The standard curve must be checked regularly and frequently using the standard and a freshly prepared Carr-Price reagent solution.

5.4.2. *Group B products*

Take an aliquot part of the solution in light petroleum obtained in 5.2 containing approximately 200 IU retinol. Evaporate to dryness under vacuum and treat the residue with 25 ml isopropanol (3.18). Measure the optical density in the spectrophotometer at 325, 310 and 334 nm. The absorption maximum is located at 325 nm. The retinol content of the solution is calculated as follows:

$$E_{325} \cdot 18{\cdot}30 = \text{IU of retinol/ml}$$

However, the ratio of the optical densities

$$E_{310}: E_{325} \text{ and } E_{334}: E_{325}$$

must be 6 : 7 = 0·857.

If one of these ratios differs appreciably from this value (< 0·830 or > 0·880), the measurement of the optical densities must be preceded by chromatography in accordance with the method

[1] Carotene content may be determined by optical density measurement at 450 nm; $E\,\frac{1\,\%}{1\text{ cm}} = 2\,600$

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given in 5.3.2. If the measurement of the optical densities carried out after chromatography shows that the abovementioned ratios still differ appreciably from the value of 0·857 (< 0·830 or > 0·880), the determination must be carried out in accordance with the method given for Group A products.

6. **Calculation of results**

Calculate the retinol content of the sample taking into account the weight of the test sample and the dilutions carried out in the course of analysis. Express the results in IU of retinol per kg of feedingstuff or per kg of concentrate or premix.

Repeatability

The difference between the results of two parallel determinations carried out on the same sample must not exceed:

— 20 %, in relative value, for retinol contents lower than 75 000 IU/kg;
— 15 000 IU for contents between 75 000 and 150 000 IU/kg;
— 10 %, in relative value, for contents between 150 000 and 250 000 IU/kg;
— 25 000 IU for contents between 25 000and 500 000 IU/kg;
— 5 %, in relative value, for contents greater than 500 000 IU/kg.

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