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COMMISSION ON OILS, FATS AND DERIVATIVES

STANDARD METHODS FOR THE ANALYSIS OF OILS, FATS AND DERIVATIVES

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SECTION II: OILS AND FATS

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2.209. DETERMINATION OF POLYUNSATURATED FATTY ACIDS WITH A CIS,CIS 1,4-PENTADIENOIC STRUCTURE

1. SCOPE

This Standard describes an enzymatic method for the determination of polyunsaturated fatty acids with a cis,cis 1,4-pentadienoic structure, i.e. containing the structure

$$-\underset{\text{cis}}{CH = CH} - CH_2 - \underset{\text{cis}}{CH = CH} -$$

2. FIELD OF APPLICATION

This Standard is applicable to the animal and vegetable oils and fats containing fatty acids with a cis,cis 1,4 pentadienoic structure, and especially those having an $\omega 6$ and $\omega 3$ unsaturation (linoleic and linolenic series).

It is not applicable to the fatty acids of the $\omega 8$ and $\omega 9$ series, and to the branched chain fatty acids.

In the case of hydrogenated oils, it is necessary to take into account the percentage content of any initially conjugated dienoic fatty acids.

3. PRINCIPLE

Saponification of the test portion by an alcoholic potassium hydroxide solution at ambient temperature. Liberation of the fatty acids by an hydrochloric acid solution. Addition of a lipoxidase solution. Measurement of the absorption at about 235 nm, and determination of the polyunsaturated fatty acid content by comparison with a standard curve.

4. APPARATUS

4.1. 100 ml volumetric ground-necked flasks, with stoppers
4.2. 1, 10 and 20 ml pipettes
4.3. 1 and 10 ml graduated pipettes
4.4. 10 ml test tubes
4.5. Laboratory centrifuge, with centrifuge tubes
4.6. U.V. spectrophotometer
4.7. 1 cm quartz cells to fit the spectrophotometer 4.6.
4.8. Water bath

5. REAGENTS

5.1. n-hexane, analytical reagent quality
5.2. Hydrochloric acid, 0.5 N aqueous solution
5.3. Potassium hydroxide, 10 N stock aqueous solution :
Dissolve 65 g of potassium hydroxide (86 per cent KOH) in about 80 ml of distilled water. Cool and make up to 100 ml.
5.4. Potassium hydroxide, 0.5 N alcoholic solution :
Dilute 5 ml of the 10 N stock solution (5.3.) to 100 ml with ethanol, 95 per cent (V/V). This solution should be freshly prepared.
5.5. Potassium borate buffer 1.0 M (pH = 9.0) :
Dissolve 61.9 g of boric acid (H_3BO_3), analytical reagent quality, and 25.0 g of potassium hydroxide in about 800 ml of distilled water with heating and stirring. Allow

to cool at room temperature, then check the pH and, if necessary, adjust the pH to 9.0 with hydrochloric acid or potassium hydroxide solution. Dilute to 1000 ml with distilled water.

5.6. Potassium borate buffer 0.2 M (pH = 9.0) :
Dilute 200 ml of the solution (5.5.) to 1000 ml with distilled water.

5.7. Lipoxidase stock solution :
Dissolve an amount of the enzyme equivalent to about 650 000 units of activity in 10 ml of ice cold 0.2 M potassium borate buffer (5.6.) (Note 1).

5.8. Dilute lipoxidase solution :
Mix 2 ml of the stock solution (5.7.) with 8 ml of ice cold 0.2 M borate buffer (5.6.).

5.9. Inactivated lipoxidase solution :
Transfer a few ml of dilute lipoxidase solution (5.8.) into a test tube, taking care that no droplet of the solution should adhere to the wall of the test tube. Immerse the test tube into a boiling-water bath (4.8.) for at least 5 min. The surface of the enzyme solution should be well below the surface of the water bath.

5.10. Reference oil, with a high and accurately known content of polyunsaturated fatty acids

5.11. Nitrogen, purity 99.5 per cent minimum

6. PROCEDURE

6.1. Saponification (Note 2)

Weigh accurately, to within 0.1 mg, 50 - 200 mg of the oil or fat to be analysed (depending on the expected amount of polyunsaturated fatty acids) (Note 3) into a volumetric flask A (4.1.). Add, with a pipette (4.2.) 10 ml of the alcoholic potassium hydroxide solution (5.4.), displace the air in the flask with nitrogen (5.11.) and stopper the flask. Store the flask in darkness, and allow the saponification to proceed for 4 h or longer, with intermittent shaking of the flask to mix its contents.

If the fat has a melting point above room temperature, it is preferable to warm the flask and contents (after stoppering) on a water bath (4.8.) at about 50°C for a few minutes in order to speed up the saponification.

It is recommanded to analyse a sample of oil with an accurately known content of polyunsaturated fatty acids (5.10.) in parallel with the assay, in order to check the procedure.

After saponification is completed, add from suitable pipettes (4.2.) 20 ml of 1.0 M potassium borate buffer (5.5.) and 10 ml of the hydrochloric acid solution (5.2.). Make up to volume (100 ml) with distilled water. Stopper the flask and mix its contents by gently turning the flask upside down a few times, keeping foaming to a minimum (Note 4). If necessary, readjust the volume to 100 ml after mixing.

With a 1 ml pipette (4.2.) transfer 1 ml of the contents of the flask A into another flask B (4.1.) previously flushed with nitrogen (5.11.). When the sample is expected to contain very low amounts of polyunsaturated fatty acids, transfer 2 - 4 ml into the flask B rather then 1 ml. Avoid the transfer of any foam from the outside of the pipette. Add with a 20 ml pipette (4.2.) 20 ml of the 1.0 M potassium borate buffer (5.5.) and dilute to 100 ml with distilled water. Stopper the flask and mix its contents, keeping foaming at a minimum (Note 4).

If a precipitate or turbidity is formed in flask A, place a few ml of the mixture into a centrifuge tube (4.5.) and spin down the precipitate for about 15 min. Transfer 1 ml of the clarified solution into the flask B instead of 1 ml of the contents of the flask A.

6.2. Measurement

Into a first test tube (4.4.) (blank) transfer with a 1 ml graduated pipette (4.3.) 0.1 ml of the inactivated lipoxidase solution (5.9.) and into each of two other test tubes (4.4.) place 0.1 ml of the dilute lipoxidase solution (5.8.). Then add 3 ml of the solution contained in the flask B to each of these 3 test tubes. Shake the tubes to ensure homogeneous solutions. Let the tubes stand for 20 - 30 min (Note 5).

After this time transfer the contents of the tubes into quartz cells (4.7.), and with the spectrophotometer (4.6.) measure the absorbance at the maximum around 235 nm, using the solution containing the inactivated lipoxidase solution as a blank for the zero adjustment.

Take as the result the mean value of the absorbances of the two cells. If the measured values deviate from each other abnormally, repeat the measurement (6.2.).

6.3. Calibration curve

Weigh accurately in a flask A (4.1.), to within 0.1 mg, an amount of the reference oil (5.10.) equivalent to about 100 mg of polyunsaturated fatty acids. Saponify according to the first part of 6.1.

Make up the volume to 100 ml with distilled water, then transfer 10 ml to a second flask B (4.1.). Add 10 ml of 1.0 M potassium borate buffer (5.5.) and make up to volume with distilled water.

From this flask B, transfer with the 10 ml graduated pipette (4.3.) 1, 2, 4, 6, 8 and 10 ml respectively into volumetric flasks C (4.1.) and make up each flask to volume with 0.2 M potassium borate buffer (5.6.).

Proceed according to 6.2. and plot the mean values of the absorbances against the contents of polyunsaturated fatty acids in flasks C. Draw the best straight line through the points plotted; this straight line should pass through the origin.

7. EXPRESSION OF RESULTS

The polyunsaturated fatty acid content (Note 6), in per cent (m/m), is given by the formula :

$$\frac{100 \times a}{m}$$

where :
a is the mass, in mg, of polyunsaturated fatty acids read on the calibration curve against the mean value of the absorbance according to 6.2.
m is the mass, in mg, of the test portion.

If a volume other 1 ml has been withdrawn from flask A, the appropriate factor must be introduced.

8. NOTES

1 - Use an enzyme preparation with an activity of at least 50 000 units per mg. Preparations of low specific activity may give rise to too low absorbances. Preparations of very high specific activity give no better results than those of activities from 50 000 - 100 000 units per mg.

In the freeze-dried state, the enzyme is stable for several years when kept at a temperature of -18°C or below. The stock solution (5.7.) may be kept at -18°C or below for a considerable length of time.

2 - Alternative saponification

Weigh accurately, to within 0.1 mg, 50 - 200 mg of the oil or fat to be analysed (Note 3) (depending of the expected amount of polyunsaturated fatty acids) into a volumetric flask A (4.1.). Add hexane (5.1.) to dissolve the sample, then dilute to 100 ml with hexane, and mix. Transfer with a 1 ml pipette (4.2.) 1.0 ml of the hexane solution into another flask B (4.1.) previously flushed with nitrogen (5.11.). When the sample is expected to contain very low amounts of polyunsaturated fatty acids, transfer 2 - 4 ml into the flask B rather than 1 ml. Completely evaporate the hexane under a gentle flow of nitrogen.

To the solvent free sample in flask B, add 2 ml of potassium hydroxide solution (5.4.) and stopper the flask. Leave the flask in the dark and allow the saponification to proceed for 4 h or longer.

After saponification is complete, add from the suitable pipettes (4.2.) 20 ml of 1.0 M potassium borate buffer (5.5.) and 2 ml of hydrochloric acid solution (5.2.). Dilute to 100 ml with distilled water and mix (Note 4).

A slight turbidity of this solution will not interfere with the following measurement.

3 - When the amount of sample is equivalent to 10 - 80 mg of polyunsaturated fatty acids, the measured absorbance at the maximum will be in the range 0.07 - 0.6.

4 - After saponification a dilute solution of soap is obtained. The concentration of the soap in the flask A is about 1 mg/ml, and in the flask B of about 10 µg/ml.

Soaps are enriched in the foam with a concomitant depletion of the bulk of the solution. If foam is adhering to the pipette when the solution is transferred from one flask to another, this may cause transference of an unknown excess of fatty acids.

5 - The handling of the contents of the test tubes is important. After the initial mixing of the contents, no further mixing shall be done. Further mixing results in increased optical density, both in the blank solution and in those to be measured. Also it is generally not possible to check if a measured value has been read correctely if the solution has been

emptied from the cell, and this has to be filled once again. The reason for the increase in optical density on handling of the solutions is not understood. It means, however, that each laboratory must adopt a standardized procedure for handling the solutions and then must keep to this routine.

6 - The unit in which the amount of polyunsaturated fatty acids is expressed depends on how they are determined in the oils used for constructing the calibration curve. If gas-liquid chromatography is used, the percentage of polyunsaturated fatty acids in the sample is expressed as a percentage of the methyl esters of the fatty acids.

II. OILS AND FATS 1980

2.606. DETERMINATION OF POLYETHYLENE TYPE POLYMERS

FOREWORD

Polyethylene type polymers are non-fat impurities, which have originated from packaging materials. The presence of these "plastics polymers" may cause severe trouble in fat processing as they give rise to deposits and blockages of pipes, valves,.. and in soap manufacture they may produce fibres marblings,...

1. SCOPE

This Standard describes three methods for the determination of polyethylene type polymers in oils and fats, viz one reference method and two alternatives methods (Appendices A and B)

2. FIELD OF APPLICATION

This Standard is applicable to technical oils and fats, which contain more than 50 mg/kg (50 ppm) of polyethylene.

3. PRINCIPLE

After an acid treatment to decompose soaps, dissolution of the sample in chloroform and filtration through a sintered crucible provided with a mat of filter aid.
Washing the crucible with its contents, drying and weighing.
Extraction of the polyethylene from the insoluble matter by nearly boiling tetrachloroethylene. Then :

In the reference method (indirect gravimetric procedure) drying the crucible with its contents and weighing again.

In the method of Appendix A (direct gravimetric procedure) precipitation of the polyethylene from the tetrachloroethylene extract by cold methanol, filtration, drying and weighing.

In the method of Appendix B (infrared procedure) measurement of the absorbance at 2860 cm^{-1} of the tetrachloroethylene extract after prior concentration to a specified volume.

4. APPARATUS

Plastics apparatus shall not be used

4.1. 600 and 1000 ml beakers
4.2. 250 and 1000 ml filter flasks
4.3. Sintered crucibles n° 3 or n° 4 of at least 50 ml capacity
4.4. Desiccator
4.5. Stirrer
4.6. Oven, regulated at 103 $\pm$ 2°C

and for Appendix A :

4.7. Evaporating dish
4.8. Clamp-type filter (vacuum or pressure type) with glass-fibre filter mat
4.9. Glass-fibre filter paper

and for Appendix B :

4.10. 50 and 100 ml bottles, with screw tops lined with aluminium foil
4.11. 100 ml measuring cylinder
4.12. 10 ml hypodermic syringe, glass, with a connector for secure attachment (e.g. Luer Lock fitting)
4.13. Infrared spectrophotometer, suitable for quantitative measurements
4.14. 1.0 mm NaCl cells
4.15. Heater for sample-cell, capable of maintaining the sample-cell at a temperature of 70 to 80°C
4.16. Cell compartment heater :
Any suitable electrically heated commercial hair-dryer, or commercial cell-heater, may be used.
4.17. Oven, electrically heated, ventilated and controlled over a temperature range of 80 to 90°C (polyethylene commence to separate from solution at about 70°C, and at temperatures above 90°C the NaCl plates are liable to fracture).
4.18. Distillation apparatus

5. REAGENTS

5.1. Methanol, containing not more 0.5 per cent (m/m) of water
5.2. Acetone, analytical reagent quality
5.3. Chloroform, analytical reagent quality
5.4. Tetrachloroethylene, analytical reagent quality
For Appendix B requirements are :
a) absorbance at the analytical frequencies
Fill a 1 mm cell with the tetrachloroethylene and scan the spectrum from 4000 cm^{-1} to 1250 cm^{-1}.
If $A_{2860} - A_{2650}$ exceeds 0.12, wash the tetrachloroethylene with water (1 part of water to 4 parts of tetrachloroethylene) and dry with anhydrous sodium sulphate, filter and repeat the scanning procedure.
If $A_{2860} - A_{2650}$ still exceeds 0.12, the batch of tetrachloroethylene is not suitable for use.
The absorbance at 2650 cm^{-1} should not exceed 0.05.

b) effect of heat
Heat two approximately 25 ml portions of the tetrachloroethylene to a temperature of 105°C, one portion for 2 h and the other for 15 min. Using 1 mm cells controlled at a temperature of 70 to 80°C, with the portion heated for 15 min in the reference beam, scan the spectrum from 4000 cm^{-1} to 1250 cm^{-1}.
If $A_{2860} - A_{2750}$ exceeds 0.01, the batch of tetrachloroethylene in unsuitable for use.
5.5. Hydrochloric acid, ethanolic solution :
Mix 1 volume of hydrochloric acid (ρ = 1.19) with 9 volumes of ethanol 95 per cent (V/V)
5.6. Diatomaceous earth filter aid, e.g. acid washed kieselguhr

and for Appendix B :

5.7. Sodium sulphate, anhydrous
5.8. Polyethylene - sample of "unfilled" sheet
5.9. Tallow, free from polyethylene
5.10. Stearic acid, minimum purity 99 per cent as determinated by gas-liquid chromatography (method 2.302.)

6. PROCEDURE

WARNING : Avoid prolonged contact of reagents with the skin. Do not inhale the vapours. Perform all operations under a suitable fume-hood. A fume-hood fitted with an extraction fan is required when the procedure of Appendix B is used.

6.1. Preparation of the test sample

Heat the sample of oil or fat to 110 to 120°C. Stir the sample for 3 - 4 min to ensure complete homogenization.

6.2. Test portion

Weigh about 100 g, to within 0.1 g (Note 1), of the oil or fat prepared according to 6.1. into a 1000 ml beaker (4.1.).

6.3. Acid treatment

Add 75 ml of the hydrochloric acid ethanolic solution (5.5.) to the test portion. Cover the beaker and stir with the stirrer (4.5.) for 5 min at 60 - 70°C.

Cool to below 35°C, then add 270 ml of chloroform (5.3.) and mix until the oil or fat is dissolved (the solution may not be clear). Add 1.0 g of the filter aid (5.6.) suspended in 30 ml of chloroform (5.3.) (to avoid the formation of lumps).

6.4. Determination

Prepare a filter mat by suspending about 0.5 g of filter aid (5.6.) in 30 ml of chloroform (5.3.) and filtering through the sintered glass crucible n° 3 (4.3.). Using suction if necessary, filter the contents of the beaker (stirred immediatly before filtration) into a 1000 ml filter flask (4.2.).

Rinse the beaker with about 50 ml of chloroform and pour the rinsings slowly through the sintered crucible under suction, keeping the chloroform level in the crucible about 5 mm above the "solids" layer, then repeat the rinsing with 50 ml of methanol (5.1.).

Repeat the washing procedure, rinsing the crucible alternately with chloroform and methanol (50 ml portions) until a total of 150 ml of chloroform and 100 ml of methanol has been used. Continue suction until crucible is dry.

Wash the under side of the crucible with chloroform. Wash the contents of the crucible twice with 25 ml of acetone (5.2.) using suction in order to remove absorbed water. Suck air through the crucible for 1 min.

When the crucible appears to be dry, place it in an oven (4.6.) at a temperature of 103 ± 2°C for 15 - 30 min.

Allow the crucible to cool in a desiccator (4.4.) for 30 min and weigh. Repeat the drying and weighing to constant weight (m_1).

Warm the crucible and its contents to 100 - 105°C and wash the contents of the crucible with 25 ml of boiling tetrachloroethylene (5.4.) without suction if possible, and using a clean dry 250 ml filter flask (4.2.) to collect the filtrate (Note 3).

Wash four times more with further 25 ml portions of boiling tetrachloroethylene and suck air through the crucible for 2 min, collecting all the filtrates if the determination is to be completed by either the direct gravimetric procedure (Appendix A) or the infrared procedure (Appendix B).

Wash the crucible with 50 ml of acetone (5.2.) to remove traces of tetrachloroethylene and suck air through the filter for 2 min.

Dry the crucible at 103 ± 2°C. Cool in a desiccator (4.4.) for 30 min and weigh. Repeat the washings with boiling tetrachloroethylene and with acetone to constant weight (m_2).

7 EXPRESSION OF RESULTS (Reference method)

The polyethylene content, in mg per kg (ppm) is given by the formula :

$$\frac{m_1 - m_2}{m_o} \times 10^6$$

where :
m_o is the mass, in g, of the test portion
m_1 is the mass, in g , of the crucible with insolubles before the extraction
m_2 is the mass, in g, of the crucible with insolubles after the extraction.

8. PRECISION

The standard deviation of this indirect gravimetric procedure is 20 mg/kg (at a level of 200 mg/kg of polyethylene).

9. NOTES

1 - For samples expected to contain more than 500 mg/kg of polyethylene, the test portion may be reduced to 50 g.

2 - If the direct gravimetric procedure (Appendix A) or the infrared procedure (Appendix B) is to be used, a clamp-type filter (4.8.) may be substituted for the crucible if preferred, otherwise a crucible must be used.

3 - If a clamp-filter is used :
Carefully cut off the outer part of the glass-fibre filter mat that may have absorbed fat, and extract the inner part with 75 ml of boiling tetrachloroethylene by refluxing for 10 min in a conical flask.

Preheat the clamp-filter to 100 - 105°C and filter the boiling tetrachloroethylene extract through a glass-fibre filter paper (4.9.).

Carry out the washing with two 25 ml portions of boiling tetrachloroethylene.

APPENDIX A - DETERMINATION BY DIRECT GRAVIMETRIC PROCEDURE

A 1. PROCEDURE

Transfer quantitatively the hot tetrachloroethylene filtrate obtained in 6.4. into a 600 ml beaker (4.1.) and add 150 ml of cold (0 - 5°C) methanol (5.1.) swirling gently to mix the liquids. Allow to stand for at least 1 h.

Using a clamp-filter (4.8.) filter the solution through a glass-fibre paper (4.9.) previously dried for 30 min at 103 ± 2°C and weighed to within 0.0002 g after storage in a desiccator (4.4.).

Wash beaker and filter four times with 20 ml portions of methanol (5.1.), suck dry and draw air through the filter for 2 min.

Carefully remove the paper from the funnel, place it in a tared evaporating dish (4.7.) and dry it in an oven (4.6.) at 103 ± 2°C. Allow to cool in a desiccator (4.4.) and weigh the paper and its contents. Repeat the drying and weighing until constant weight is attained (Note 4).

A 2 EXPRESSION OF RESULTS

The polyethylene content, in mg/kg (ppm), is given by the formula :

$$\frac{m_2 - m_1}{m_o} \times 10^6$$

where :
m_o is the mass, in g, of the test portion

m_1 is the mass, in g, of the filter paper

m_2 is the mass, in g, of the filter paper and its contents.

A 3 PRECISION

The standard deviation of this direct gravimetric procedure is 23 mg/kg (at a level of 200 mg/kg of polyethylene).

A 4 NOTE

4 - Alternately a sintered crucible n° 3 provided with a mat of 0.5 g of filter aid, or a sintered crucible n° 4, may be used for the filtration.

APPENDIX B - DETERMINATION BY INFRARED PROCEDURE

B 1. CALIBRATION OF THE INFRARED SPECTROPHOTOMETER

B 1.1. Instrument conditions

Set the infrared spectrophotometer (4.13.) according to the makers' instructions as to obtain optimum performance at the required analytical wave-lengths. The analytical peaks are the CH_2 band at 2860 cm^{-1}, the fatty acid and glyceride carbonyl bands at about 1750 cm^{-1} and 1715 cm^{-1} respectively.

The spectra are measured in the ranges respectively of 3100 cm^{-1} to 2700 cm^{-1} and of 1825 cm^{-1} to 1500 cm^{-1}.

B 1.2. Cell checks

Check the path-length of the sample-cell (4.14.) every 25 scans. The path-length may increase due to erosion of the salt plates by hot solvent. Repolish the cell plates and re-built the cell if the path-length increases by more than 0.02 mm.

The sample-cell may be slowly contaminated by polyethylene plating out on to the surface; this is inhibited by maintaining the cell hot throught the test. Check for contamination by filling both the sample-cell and the reference-cell with tetrachloroethylene and scanning through the range 3000 cm^{-1} to 2800 cm^{-1}. If the absorbance at 2860 cm^{-1} is greater than 0.005 the cell should be cleaned by filling it with hot tetrachloroethylene and placing in the oven at a temperature of 90°C for 15 min. Rinse the cell with tetrachloroethylene and rescan. Repeat until the absorbance is satisfactory.

B 1.3. Calibration of the infrared spectrophotometer with polyethylene

Weigh the following amounts of polyethylene (5.8.) and place in 100 ml sample bottles (4.10) : 0.0125g, 0.0250 g, 0.0375 g and 0.0625 g.

Pipette 50 ml of tetrachloroethylene (5.4.) into each bottle, screw on the foil-lined cap tightly, and without shaking place in the oven (4.17) at a temperature of 105°C. Keep a supply of tetrachloroethylene in the oven. Keep the hypodermic syringe (4.12.) and the sample-cells (4.14.) in an oven at a temperature of 85 - 90°C.

Remove each bottle in turn after 10 min, retighten the screw cap and shake vigorously for 15 s. Replace in the oven for 15 min and then shake again for 15 s. All the polyethylene must be in solution. Keep the bottles in the oven for a maximum of 1 h until the cells have been filled.

Remove the reference-cell from the oven, fill with tetrachloroethylene and place it in the heated cell compartment (Note 5).

Take the most dilute calibration solution from the oven, and fill the sample-cell using the hypodermic syringe. Immediately rinse the syringe with hot tetrachloroethylene and return it to the oven at 80 to 90°C. Place the sample-cell in the instrument and scan the spectrum. Adjust the background to about 95 per cent at 3000 cm^{-1} and scan from 3100 cm^{-1} to 2600 cm^{-1}.

Place the reference-cell in the oven controlled at a temperature of 90°C.

Drain the sample-cell, and rinse it with hot (80°C) tetrachloroethylene, using at least 5 ml in a continuous wash from the hypodermic syringe. Drain the cell and the syringe. Pump air through the cell from the syringe. Reheat the cell and the syringe for 3 min in the oven at a temperature of 90°C.

Check that the reference-cell is full of tetrachloroethylene, top up if necessary and replace it in the spectrophotometer.

Repeat the procedure for the three remaining calibration solutions of polyethylene.

Determine the net absorbance for each solution ($A = A_{2860} - A_{3000}$) and divide this by the cell thickness to obtain A/mm.

Plot a calibration graph of A/mm against polyethylene concentration, using the following table :

Mass of polyethylene calibration sample	Equivalent concentration of polyethylene
0.0125 g	50 mg/kg
0.0250 g	100 mg/kg
0.0375 g	150 mg/kg
0.0625 g	250 mg/kg

This table applies only for 100 g of oil or fat in a final solution of 20 ml of tetrachloroethylene as used in the determination of the unknown sample.

The graph should be a straight line through the origin.

B 1.4. Glyceride correction

Weigh, to the nearest 0.001 g, 0.100 g of tallow (5.9.) and 0.100 g of stearic acid (5.10.) into a tared beaker, then add 100 ml of tetrachloroethylene (5.4.) from a measuring cylinder (4.11.) and dissolve the tallow and stearic acid.

Fill the sample-cell (heat is not required).

Scan the spectrum from 3100 cm^{-1} to 2600 cm^{-1} and from 2000 cm^{-1} to 1500 cm^{-1}.

Measure the net absorbance at 2860 cm^{-1} using the absorbance at 3000 cm^{-1} as the base-point, and at 1750 and 1715 cm^{-1} using the absorbance at 1900 cm^{-1} as the base-point.

Calculate the glyceride correction factor F by the formula :

$$F = \frac{A_{2860}}{A_{1750} + A_{1715}}$$

B 2. PROCEDURE

Evaporate the hot tetrachloroethylene filtrate obtained in 6.4. to about 20 ml using a distillation apparatus (4.18.).

Transfer the solution quantitatively into a 50 ml bottle (4.10.) and continue the evaporation to dryness.

Pipette 20 ml of tetrachloroethylene (5.4.) into the bottle, rew on the foil-lined cap tightly and heat in an oven (4.6.) at 103 ± 2°C for 15 min.

Fill the heated sample-cell with the hot tetrachloroethylene solution and scan the spectrum in the analytical wave-length regions 2800 cm^{-1} and 1750 cm^{-1} (Note 5).

Read the net absorbance at 2860 cm^{-1} and at 1715 cm^{-1} and 1750 cm^{-1} if there are peaks at these frequencies. Ignore differences of less than 0.01 in absorbance.

B 3. EXPRESSION OF RESULTS

Calculate the corrected absorbance A at 2860 cm^{-1} by the formula :

$$A = A_{2860} - F\ (A_{1715} + A_{1750})$$

where :
A_{2860}, A_{1750} and A_{1715} are the net absorbances at the wave-lengts quoted.
F is the glyceride factor established according to B 1.4.

Divide the corrected absorbance by the cell path-length to obtain A/mm and read the polyethylene content, in mg/kg, from the calibration graph prepared as described in B 1.3.

B 4. PRECISION

The standard deviation of this infrared procedure is 36 mg/kg (at a level of 200 mg/kg of polyethylene).

B 5. NOTE

5 - The cell should be heated to a temperature above 70°C to prevent undue cooling; this prevents plating out of the polyethylene on the salt plates and also helps to prevent fracture of the plates.