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Animal and vegetable fats and oils — Determination of isolated *trans* isomers by infrared spectrometry

Corps gras d'origines animale et végétale — Détermination, par spectrométrie infrarouge, des isomères trans *isolés*

Reference number
ISO 13884:2003(E)

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

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

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

ISO 13884 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

Animal and vegetable fats and oils — Determination of isolated *trans* isomers by infrared spectrometry

1 Scope

This International Standard specifies a method for the accurate determination of isolated *trans* bonds in natural or processed long-chain fatty acids, fatty acid esters and triglycerides with *trans* isomer levels ⩾ 5 %.

The method is not applicable, or is applicable only with specific precautions, to

- fats and oils containing high levels (over 5 %) of conjugated unsaturation (e.g. tung oil),
- materials containing functional groups which modify the intensity of the C-H deformation about the *trans* double bond [e.g. castor oil containing ricinoleic acid or its geometrical isomer, ricinelaidic acid (12-hydroxy-9-octadecenoic acid)],
- mixed triglycerides having long- and short-chain moieties (such as diacetostearin), or in general,
- any materials containing constituents which have functional groups which give rise to specific absorption bands at or sufficiently close to interfere with the 966 cm^{-1} band of the C-H deformation of the isolated *trans* double bond.

NOTE Dienes, such as *cis-trans* and *trans-trans* dienes, can affect the calibration.

For accurate determinations on materials with *trans* levels below 5 %, a standardized capillary GLC method is recommended (for example, see ISO 15304).

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 661, *Animal and vegetable fats and oils — Preparation of test sample*

ISO 5509, *Animal and vegetable fats and oils — Preparation of methyl esters of fatty acids*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
percentage of *trans* fatty acids
% *trans*
quantity of those substances in the sample, expressed in terms of the mass equivalent of methyl elaidate, divided by the sample mass expressed in grams per 10 ml of solvent

4 Principle

In most naturally occurring vegetable fats and oils, unsaturated constituents contain only isolated (i.e. non-conjugated) double bonds in the *cis* configuration. These *cis* bonds may be isomerized to the *trans* configuration during extraction and processing procedures, due to oxidation, conversion during heating, and/or partial hydrogenation. Animal and marine fats and oils may contain measurable amounts of naturally occurring *trans* isomers. Isolated *trans* bonds in long-chain fatty acids, fatty acid esters and triglycerides may be measured by infrared (IR) spectrometry. An absorption band with a maximum at 966 cm^{-1} (10,3 µm), arising from a C-H deformation about a *trans* double bond, is exhibited in the spectra of all compounds containing an isolated *trans* group. This band is not observed in the spectra of the corresponding *cis* and saturated compounds. Measurement of the intensity of this absorption band under analytically controlled conditions is the basis for a quantitative method for the determination of the isolated *trans* isomer content. For higher accuracy, common interfering absorptions associated with the glycerol backbone of triglycerides and the carboxyl groups of fatty acids must be eliminated by conversion of these samples to their methyl esters prior to analysis.

5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

5.1 Carbon disulfide (CS_2), dry.

WARNING — Prolonged breathing of carbon disulfide vapours is dangerous. This solvent should be handled only under conditions that provide adequate ventilation, preferably under a fume hood.

5.2 Primary standards: methyl elaidate and methyl oleate, with a purity of 99 %[1].

6 Apparatus

6.1 Infrared spectrometer (FT-IR or dispersive), capable of making measurements at 4 cm^{-1} resolution in the spectral range covering 1 050 cm^{-1} to 900 cm^{-1}.

The data-handling capability of the instrument should allow conversion of spectra to absorbance, scale expansion of the x- and y-axes, and readout of wave numbers to the nearest 1 cm^{-1} and of absorbance to the nearest 0,001 AU. FT-IR spectrometers should use TGS or DTGS detectors, or ensure linearity.

6.2 Infrared liquid sampling cells, with NaCl or KBr windows and a fixed optical path length of 1 mm.

For use in null-type instruments, pairs of cells matched to within 0,01 AU are required. In split-beam type instruments with both cells filled with the solvent (5.1), electronic balance of the two beams to within these limits should be attained.

6.3 Volumetric flasks, class A, of capacities 10 ml, 25 ml and 50 ml.

6.4 Pipettes, class A, of capacities 1 ml, 3 ml, 4 ml, 5 ml, 7 ml and 9 ml.

6.5 Disposable Pasteur pipettes, for filling infrared cells with the test sample.

6.6 Analytical balance, with a 60 g capacity, capable of weighing 0,2 g to an accuracy of ± 0,000 1 g.

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1) These are available from Nu-Check-Prep, Inc., Elysian, MN, USA. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products. Equivalent products may be used if they can be shown to lead to the same results.

7 Sampling

Sampling is not part of the method given in this International Standard. A recommended sampling method is given in ISO 5555.

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

8 Preparation of test sample

Prepare the test samples in accordance with ISO 661.

Laboratory samples of solid fats shall be completely melted on a steam bath at a temperature not more than 10 °C above the melting point of the sample. The melted laboratory sample should be completely mixed before taking the test sample. Samples that appear cloudy because of the presence of water should be treated with anhydrous sodium sulfate and filtered before taking the test sample.

9 Procedure

9.1 Preparation of standards

9.1.1 Stocks solutions

Weigh, to the nearest 0,000 1 g, 0,5 g ± 0,01 g of methyl elaidate (5.2) into a 25 ml volumetric flask (6.3). Dilute to volume with solvent (5.1), stopper and mix well. Prepare a second solution of methyl oleate (5.2) in exactly the same manner. These stock solutions have a concentration of 0,020 g/ml. Prepare a 0,002 0 g/ml stock solution of methyl elaidate by pipetting 5,00 ml of the 0,020 g/ml methyl elaidate stock solution into a 50 ml volumetric flask (6.3). Dilute to volume with solvent (5.1), stopper and mix well.

9.1.2 1 %, 4 % and 7 % *trans* standards

Into 10 ml volumetric flasks (6.3), weigh, to the nearest 0,000 1 g, the mass of methyl oleate (5.2) specified in Table 1 to within ± 0,005 g. Pipette (6.4) the corresponding specified volume of the 0,002 g/ml methyl elaidate stock solution (9.1.1) into each of the 10 ml volumetric flasks. Dilute to volume with solvent (5.1), stopper and mix well.

Table 1 — Proportions for 1 %, 4 % and 7 % *trans* standards

Nominal *trans*-isomer content %	**Methyl elaidate solution** ml	**Methyl oleate** g
1	1,00	0,198
4	4,00	0,192
7	7,00	0,186

9.1.3 10 %, 30 %, 50 % and 70 % *trans* standards

Into 10 ml volumetric flasks (6.3), pipette the volume of the 0,020 g/ml methyl elaidate and methyl oleate stock solution (9.1.1) specified in Table 2. Confirm that the pipetted volumes accurately fill the flask up to the 10 ml graduation mark.

Table 2 — Proportions for 10 %, 30 %, 50 % and 70 % *trans* standards

Nominal *trans*-isomer content %	Methyl elaidate solution ml	Methyl oleate solution g
10	1,00	9,00
30	3,00	7,00
50	5,00	5,00
70	7,00	3,00

9.2 Calibration

9.2.1 General

For each standard (9.1.2 and 9.1.3), calculate the exact mass of methyl elaidate diluted in 10 ml of solvent.

Analyse each standard (9.1.2 and 9.1.3) and determine the baseline-corrected infrared absorbance at 966 cm^{-1} as described in 9.2.2 and 9.2.3.

9.2.2 For *trans* content $\leqslant$ 10 %

Using a first-order regression analysis, determine the slope and intercept of the line which best fits the plot of the baseline-corrected absorbance at 966 cm^{-1} for the 1 %, 4 %, 7 % and 10 % *trans* standards (y-axis) as a function of the grams of methyl elaidate per 10 ml of solution (x-axis).

9.2.3 For *trans* content > 10 %

Repeat calibration step 9.2.2 for the 7 %, 10 %, 30 %, 50 % and 70 % *trans* standards.

9.3 Preparation of methyl esters

9.3.1 Convert a 0,5 g to 1,0 g portion of the sample (Clause 8) to methyl esters using the method given in ISO 5509, or any other accepted, standardized method that is appropriate for the type of sample (e.g. references [4], [5] or [6]).

9.3.2 Weigh, to the nearest 0,000 1 g, 0,20 g ± 0,01 g of the prepared (9.3.1), neat methyl esters into a 10 ml volumetric flask (6.3). Dilute to volume with solvent (5.1), stopper and mix well.

For oils and fats containing short-chain fatty acids, care should be taken in the preparation and concentration of the methyl esters to reduce the loss of the more volatile fractions.

9.4 Determination

9.4.1 General

Set up the operating parameters of the infrared spectrometer (6.1) according to the manufacturer's recommendations to collect infrared spectra at 4 cm^{-1} resolution covering the spectral range 1 050 cm^{-1} to 900 cm^{-1}. Conditions employed should be identical for both standards and samples.

Once calibration data have been established, they should be checked periodically to ensure their validity. For highest quantitative accuracy and precision, a primary or secondary reference should be methylated and analysed with each group of samples, then checked to ensure that the measured *trans* level is in agreement with the known or established mean value.

Infrared spectrometers should be checked to ensure that they are operating within the manufacturer's established specifications. Checks should include signal-to-noise as well as photometric and wavelength accuracy.

9.4.2 Single-beam instruments

Fill a clean infrared liquid cell (6.2) with solvent (5.1). Remove any air bubbles, stopper and place in the spectrometer cell holder. Record the single-beam spectrum to be used as a reference (background). Refill the clean cell with the solution obtained in 9.3.2 and record the single-beam spectrum of the sample. Ratio the sample spectrum against the background and convert to absorbance units.

9.4.3 Split- or dual-beam instruments

Fill a clean infrared liquid cell (6.2) with solvent (5.1). Remove any air bubbles, stopper and place in the reference beam cell holder. Fill a second, matched cell (6.2) with the solution obtained in 9.3.2. Remove any air bubbles, stopper and place in the sample beam cell holder. Record the spectrum and convert to absorbance units.

10 Expression of results

10.1 With the spectrum of the sample scale expanded in the region from 1 050 cm^{-1} to 900 cm^{-1}, draw a baseline (XY) tangent to the “feet” of the infrared absorbance band at 966 cm^{-1} as demonstrated in Figure 1. The points in a spectrum between which line XY is drawn will vary with peak absorbance. For accurate results, baselines shall be drawn the same for both standards and samples. For samples with high *trans* content, the baseline should be drawn to the minimum located at 985 cm^{-1}. Occasionally, this minimum may be absent and the point to which the baseline is drawn shall be estimated.

10.2 Determine the baseline-corrected absorbance of the band (A_C) by subtracting the absorbance of the baseline at the peak maximum (A_B) from the absorbance of the peak at 966 cm^{-1} (A_P). The measured position of the peak maximum will vary with instrument and the *trans* content of the sample analysed. The position of the maximum should be established separately for each instrument with the 70 % *trans* standard. This same position should then be used at all concentrations.

10.3 From Figure 1

$$A_C = (A_P - A_B)$$

Using the calibration data for the appropriate *trans* range ($\leqslant$ 10 % or $>$ 10 %), calculate the mass equivalent of methyl elaidate, m_{equiv}, in grams, in the sample (9.3.2) from the following equations:

$$m_{\text{equiv}} = \frac{A_C - a}{b}$$

$$\omega_{\text{trans}} = \frac{m_{\text{equiv}}}{m_t} \times 100\ \%$$

where

A_C is the baseline-corrected absorbance of the band;

a is the intercept;

b is the slope;

ω_{trans} is the % *trans* as methyl elaidate;

m_t is the mass of sample per 100 g of solvent.

10.4 Report results to the nearest 0,1 %.

11 Precision

11.1 Interlaboratory test

Details of an interlaboratory test on the precision of the method are summarized in Annex A. The values derived from this interlaboratory test may not be applicable to concentration ranges and matrices other than those given.

11.2 Repeatability

The absolute difference between two independent single test results, obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases be greater than the repeatability limit r as shown in Annex A.

11.3 Reproducibility

The absolute difference between two single test results, obtained with the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater than the reproducibility limit R as shown in Annex A.

12 Test report

The test report shall specify:

a) all information necessary for the complete identification of the sample;

b) the sampling method used, if known;

c) the test method used, with reference to this International Standard;

d) all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

e) the test result obtained; or

f) if the repeatability has been checked, the final quoted result obtained.

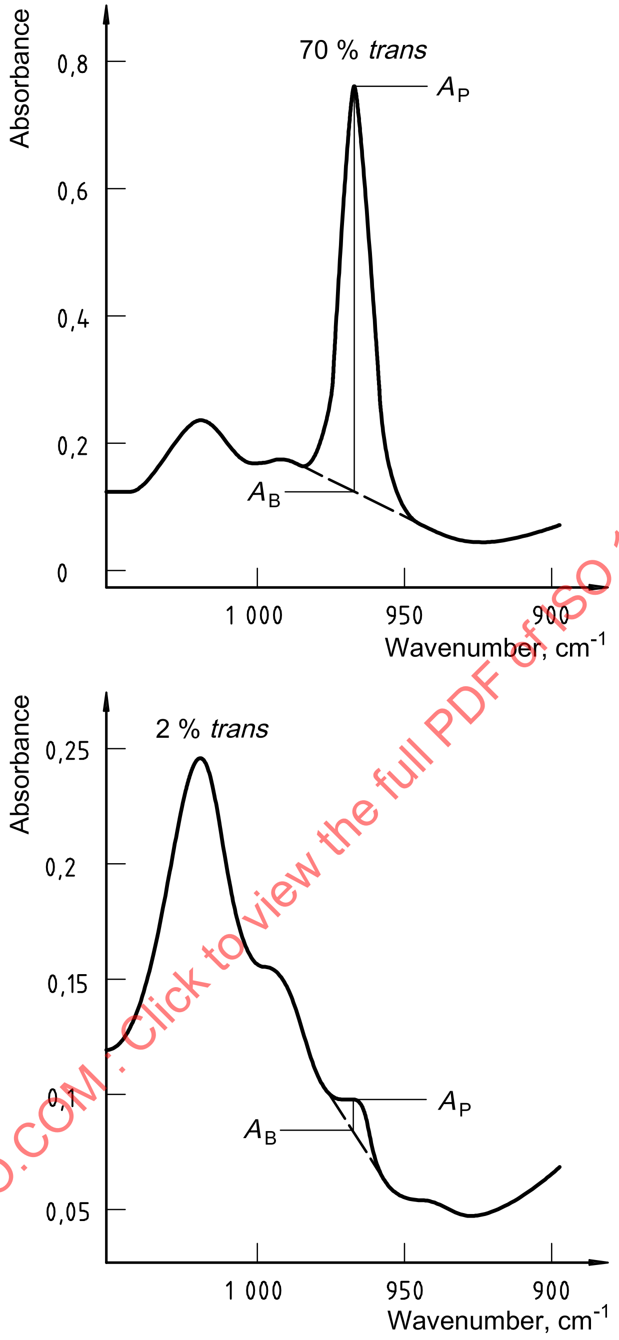

Figure 1 — Infrared spectra of methyl esters containing 70 % and 2 % of the *trans* isomer