
**Vegetable fats and oils —
Isomeric diacylglycerols —
Determination of relative amounts
of 1,2- and 1,3-diacylglycerols**

*Corps gras d'origine végétale — Diacylglycérols isomériques —
Détermination des teneurs relatives en 1,2- et 1,3-diacylglycérols*



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Foreword

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Vegetable fats and oils — Isomeric diacylglycerols — Determination of relative amounts of 1,2- and 1,3- diacylglycerols

1 Scope

This International Standard specifies the determination of the degree of isomerization of diacylglycerols in vegetable fats and oils. 1,2-Diacylglycerols are transformed to the more stable 1,3-isomers during storage or due to acidic catalysed reaction.

The mass fraction of 1,2-diacylglycerols can be used as a quality criterion for vegetable fats and oils.

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*

3 Terms and definitions

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

3.1

degree of isomerization

mass fraction of the peak areas of all 1,2-diacylglycerols (C_{32} , C_{34} , C_{36}) relative to the sum of the peaks of all diacylglycerols (C_{32} , C_{34} , C_{36}).

NOTE The mass fraction is expressed as a percentage to one decimal place.

4 Principle

A miniaturized column chromatography on a silica gel column is used to separate the isomeric diacylglycerols as the more polar fraction from the major part of other lipids. The peak areas of 1,2- and 1,3-isomers are determined by gas chromatography after silylation. Only C_{32} -, C_{34} - and C_{36} -diacylglycerols are taken into account.

5 Reagents

WARNING — Attention is drawn to the regulations which specify the handling of hazardous substances. Technical, organizational and personal safety measures shall be followed.

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent purity.

5.1 Silica gel 60¹⁾, for column chromatography, (0,063 to 0,100 mm).

5.1.1 Silica gel 60, with a moisture content, $w_{\text{H}_2\text{O}} = 5$ % mass fraction.

Activate the silica gel by heating overnight at (160 ± 5) °C. After heating, place the silica gel in a desiccator for cooling and then transfer the silica gel to a stoppered flask. Add 5 % mass fraction of water and shake until no lumps can be seen and the powder flows freely (1 h in an automatic shaking machine). Store the conditioned silica gel overnight before use.

5.2 Cotton wool, defatted.

5.3 Reference substances²⁾.

5.3.1 Dilaurin (1,3-dilauroyl-*sn*-glycerol).

5.3.2 Dipalmitin (1,2- and 1,3-dipalmitoyl-*sn*-glycerol, mixed isomers, $w \approx 99$ % mass fraction).

5.3.3 Distearin (1,2- and 1,3-distearoyl-*sn*-glycerol, mixed isomers, $w \approx 99$ % mass fraction).

5.3.4 Diolein (1,2- and 1,3-dioleoyl-*sn*-glycerol, mixed isomers, $w \approx 99$ % mass fraction).

5.4 Reference solutions:

For the reference substances dipalmitin and distearin, prepare a solution in toluene (mass concentration, $\rho = 1$ mg/ml).

5.5 Isooctane (2,2,4-trimethylpentane).

5.6 Diethyl ether, eluent.

5.7 Diisopropyl ether.

5.8 Solvent mixture: isooctane/diisopropyl ether [volume fraction $\varphi(\text{isooctane}) = 85$ ml/100 ml and $\varphi(\text{diisopropyl ether}) = 15$ ml/100 ml].

5.9 1-Methylimidazole.

5.10 *N*-Methyl-*N*-(trimethyl-silyl)heptafluorobutyramide (MSHFBA).

5.11 Silylating reagent: 50 µl 1-methylimidazole in 1 ml of MSHFBA.

5.12 Toluene.

6 Apparatus

Usual laboratory equipment and, in particular, the following.

6.1 Analytical balance, capable of weighing to the nearest 0,001 g and displaying 0,000 1 g.

1) Merck Art.-No 612-1484 or 613-2623 (www.merck.de) are examples of suitable products available commercially. 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.

2) A suitable supplier is Sigma-Aldrich (www.sigmaaldrich.com). This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this supplier.

6.2 Desiccator, for storing adsorbents after heating.

6.3 Pipette tip ³⁾, capacity 5 ml, length approx. 150 mm.

6.4 Pointed flask, capacity 10 ml, with ground-glass neck and rounded bottom.

6.5 Beaker, capacity 10 ml.

6.6 Rotary evaporator.

6.7 Gas chromatograph for capillary columns, split injector, flame ionization detector (FID) and suitable integration system.

Do not use on-column or splitless injectors, as this results in isomerization of 1,2-diacylglycerols.

6.8 Fused silica capillary column, for gas chromatography (internal diameter, 0,25 mm or 0,32 mm, length 12 m, 15 m, 30 m or 60 m) coated with 5 % diphenyl-, 95 % dimethylpolysiloxane, 0,1 µm film thickness.

Other columns of similar polarity and selectivity may also be used.

7 Sample

7.1 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 5555 ^[1].

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

7.2 Preparation of test sample

Prepare the test sample in accordance with ISO 661.

8 Procedure

8.1 Preparation of the silica gel chromatography column

Insert a small stopper of cotton wool (5.2) approx. 5 mm high, in the lower part of a pipette tip (6.3) followed by 1 g silica gel 60 (5.1). Cover the silica layer with a 5 mm high stopper of cotton wool. Compress the filling by slight pressing with a flat ended rod.

8.2 Separation of the fraction containing non polar lipids

8.2.1 Weigh, to the nearest 0,1 mg, about 100,0 mg of the test sample into a beaker (6.5) and add 1 ml of toluene (5.12).

8.2.2 Transfer the test portion on to the column, carefully purging the flask with 1 ml solvent mixture (5.8). Wash the column with two 3,5 ml portions of the solvent mixture (5.8). Rinse the end of the pipette tip with solvent mixture (5.8) and discard the solvent.

3) VWR/Merck Art.-No 1.15101.1000 (www.merck.de) is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.

8.2.3 Elute the diacylglycerols with two 3,5 ml portions of diethyl ether (5.6) and collect the eluate in a pointed flask (6.4).

8.2.4 Remove the solvent from the eluate to about 1 ml with a rotary evaporator (6.6) at 20 °C. Transfer the remaining solution into a reaction vial. Blow off the solvent in the reaction vial with a stream of nitrogen.

8.3 Preparation of trimethylsilyl ethers (silylation)

8.3.1 Add 200 µl of the silylation reagent (5.11) to the reaction vial containing the diacylglycerols, seal and allow the mixture to react for 20 min at room temperature.

8.3.2 After silylation, add 1 ml acetone and use 1 µl to 2 µl of the solution for the gas chromatography.

8.4 Gas chromatography

The conditions in Table 1 for the gas chromatograph have been found to give useful chromatograms.

Table 1 — Gas chromatographic conditions

Function	Condition
Capillary GC column	Restek RTX5 ⁴⁾ , 60 m; internal diameter 0,25 mm, film thickness 0,1 µm
Injection volume	1 µl (split 1:50)
Carrier gas	Hydrogen at 2 ml/min, constant flow
Gas for FID	Hydrogen at 33 ml/min and air at 420 ml/min
Injector temperature	340 °C
Detector temperature	340 °C
Temperature oven	240 °C; maintain for 1 min, heat at 10 °C/min to 320 °C, maintain for 10 min

Optimize the temperature programme and the velocity of the carrier gas flow so that chromatograms similar to Figure A.1 are obtained. Test the separation with silylated diacylglycerol fractions.

8.5 Identification of diacylglycerol isomers

Prepare a solution of dipalmitin (1,2-C₃₂, 1,3-C₃₂) (5.3.2), distearin (1,2-C₃₆, 1,3-C₃₆) (5.3.3), and diolein (1,2-C₃₆, 1,3-C₃₆) (5.3.4), each with a mass concentration, $\rho = 1$ mg/ml toluene. Add 100 µl of this solution to a derivatization vial. Blow off the solvent in the reaction vial with a stream of nitrogen and silylate.

Do not add acetone to the reference solutions.

9 Result of the determination

9.1 Identification of diacylglycerols

To identify the 1,2- and 1,3-diacylglycerols in the test portion, determine the retention times of the reference standards.

4) Example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.

NOTE The retention times of the diacylglycerols containing unsaturated fatty acids can differ slightly from those of the corresponding saturated fatty acids. For the purpose of this method, it is assumed that the response factors of all diacylglycerols are equal.

9.2 Determination of the peak areas of 1,2- and 1,3- diacylglycerols in the oil

Calculate the percentage mass fraction, $w_{1,2}$, of the 1,2-diacylglycerols as follows:

$$w_{1,2} = \frac{A_{1,2}}{\sum A} \times 100$$

where

$A_{1,2}$ is the peak area of all 1,2-diacylglycerols (C_{32} , C_{34} , C_{36}) present in the test portion;

$\sum A$ is the sum of the peak areas of the individual 1,2- and 1,3-diacylglycerols (C_{32} , C_{34} , C_{36}).

Report the result to one decimal place.

10 Precision of the method

10.1 Interlaboratory test

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

10.2 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, shall in not more than 5 % of cases exceed the value of r given in Table B.1.

10.3 Reproducibility

The absolute difference between two single test results, obtained with the same method on identical test material in different laboratories by different operators using different equipment, shall in not more than 5 % of cases exceed the value of R given in Table B.1.

11 Test report

The test report shall contain at least the following information:

- all information necessary for the complete identification of the sample;
- the sampling method used, if known;
- the test method used, with reference to this International Standard;
- all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents occurred when performing the method, which may have influenced the test result(s);
- the test result obtained;
- if the repeatability has been checked, the final quoted result obtained.

Annex A
(informative)

Example of a typical chromatogram

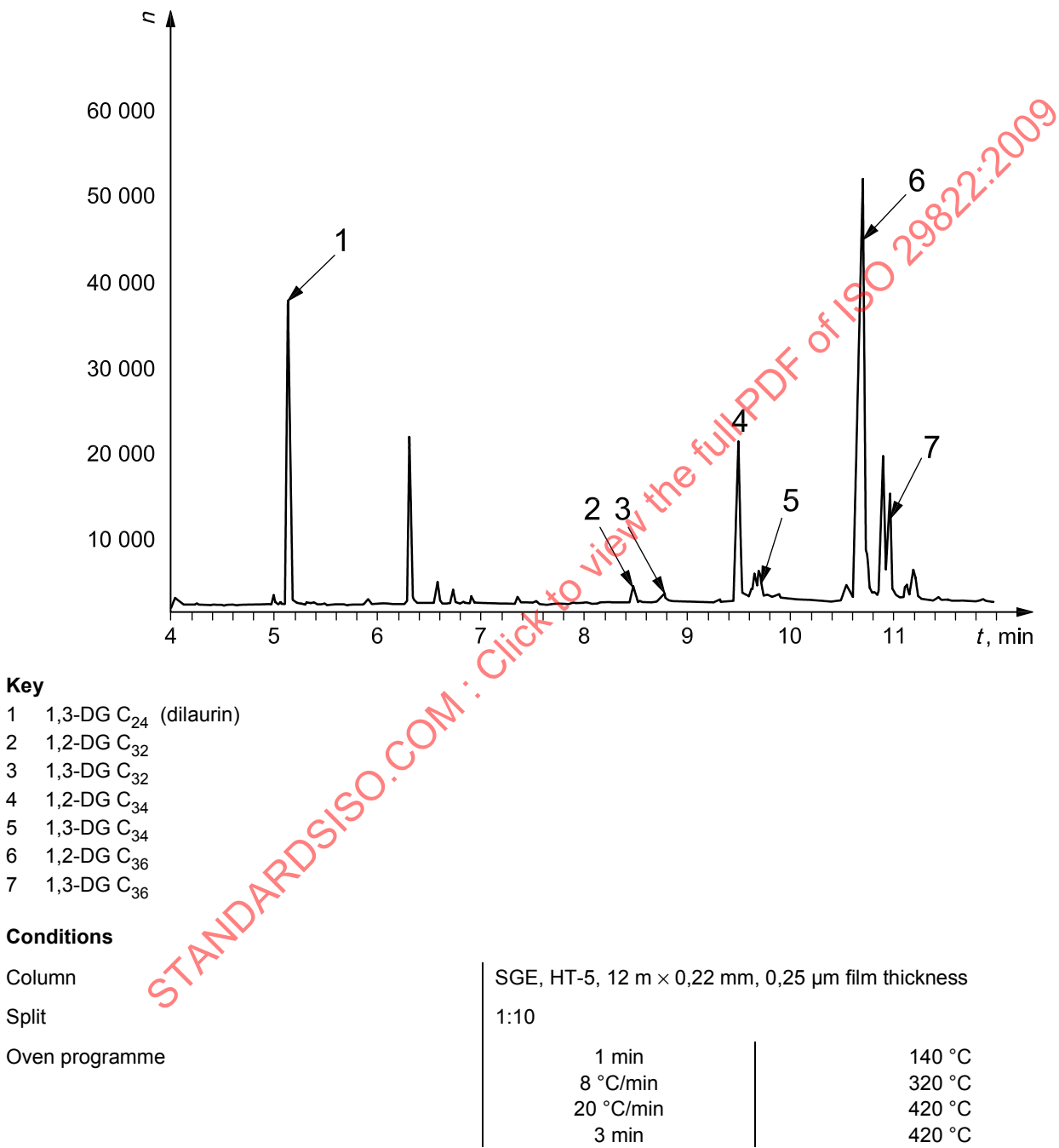


Figure A.1 — Gas chromatogram obtained from an olive oil

Annex B (informative)

Results of an interlaboratory test

The precision of the method is the result of an interlaboratory study organized by the Joint Committee for the Analysis of Fats, Oils, Fat Products, Related Products and Raw Materials (DIN/DGF) on an international basis. The study was carried out in 2005 on five samples, evaluated according to DGF Standard Method A-II [4], ISO 5725-1:1994 [2] and ISO 5725-2:1994 [3]. In this test, 19 laboratories from France, Germany and Italy participated.

Table B.1 — Summary of statistical results for olive oils

Sample	A	B	C	D	E
Number of participating laboratories, n_p	19	19	19	19	19
Number of laboratories retained after eliminating outliers, n_p	16	19	18	19	19
Number of individual test results of all laboratories on each sample, n_z	32	38	36	38	38
Mean value, $\bar{w}_{1,2}$, %	42,18	34,91	60,27	55,44	35,14
Repeatability standard deviation, s_r , %	0,51	0,76	1,24	1,63	0,72
Repeatability coefficient of variation, $CV(r)$, %	1,2	2,2	2,0	2,9	2,0
Repeatability limit, r, ($= 2,8 s_r$), %	1,42	2,14	3,46	4,55	2,00
Reproducibility standard deviation, s_R , %	1,56	1,60	2,15	2,19	2,95
Reproducibility coefficient of variation, $CV(R)$, %	3,7	4,6	3,6	4,0	8,4
Reproducibility limit, R, ($= 2,8 s_R$), %	4,36	4,48	6,02	6,14	8,27