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Animal and vegetable fats and oils — Determination of low-boiling halogenated hydrocarbons in edible oils

Corps gras d'origines animale et végétale — Dosage des hydrocarbures halogénés à bas point d'ébullition dans les huiles comestibles

Reference number
ISO 16035:2003(E)

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Contents

Foreword

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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 16035 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 low-boiling halogenated hydrocarbons in edible oils

1 Scope

This International Standard specifies a method for the determination of low-boiling halogenated hydrocarbons by means of static headspace gas chromatography. Halogenated hydrocarbon solvents are ubiquitously distributed toxic compounds. The method is applicable to all edible fats and oils to determine these compounds in the range 0,01 mg/kg to 0,2 mg/kg.

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 661:1989, *Animal and vegetable fats and oils — Preparation of test sample*

3 Terms and definitions

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

3.1
halogenated hydrocarbons content
content of low-boiling halogenated hydrocarbons in the products determined by the method specified in this International Standard

NOTE 1 The halogenated hydrocarbons content is expressed in milligrams per kilogram.

NOTE 2 For the purposes of this International Standard, the term low-boiling halogenated hydrocarbons covers those specified in 5.1.

4 Principle

The contents of halogenated hydrocarbons in the test sample are determined by manual or automatic static headspace gas chromatography of a sample drawn from the vapour space above the heated test sample. A capillary column is used for separation, and detection is carried out by means of an electron capture detector (ECD). An internal standard is used to obtain a quantitative evaluation. For the purpose of calibration, calibration curves are obtained by the addition of various halogenated hydrocarbon calibration solutions to a blank sample.

5 Reagents and materials

WARNING — Attention is drawn to the regulations which prescribe the handling of dangerous matter. Technical, organizational and personal safety measurements shall be followed.

Use only reagents of recognized analytical grade unless otherwise stated.

5.1 Standard substances, of appropriate purity as required by the nature and number of components to be determined, for example, the following.

5.1.1 1,1,1-Trichloroethane, density = 1,34 g/ml.

5.1.2 Tetrachloromethane, density = 1,59 g/ml.

5.1.3 1,1,2-Trichloroethene, density = 1,46 g/ml.

5.1.4 Tetrachloroethene, density = 1,63 g/ml.

5.1.5 2-Bromo-1-chloropropane (internal standard), density = 1,54 g/ml.

5.1.6 Dichloromethane (methylene chloride), density = 1,32 g/ml.

5.1.7 Trichloromethane (chloroform), density = 1,47 g/ml.

5.1.8 Bromodichloromethane, density = 1,98 g/ml.

5.1.9 Dibromochloromethane, density = 2,45 g/ml.

5.1.10 Tribromomethane (bromoform), density = 2,89 g/ml.

5.1.11 1,1,2-Trichloro-1,2,2-trifluoroethane, density = 1,57 g/ml.

5.2 Isooctane, suitable for haloform analysis. Check the isooctane for its halogenated hydrocarbons content.

5.3 Nitrogen (extra purified), **helium** or **hydrogen**, as carrier gas.

5.4 Argon/methane or **nitrogen**, as purge gas for the ECD.

Use in accordance with the manufacturer's instructions.

5.5 Halogenated hydrocarbons stock solutions

Add 10 µl of each of the standard substances (5.1) to be determined to a 100 ml volumetric flask that contains 40 ml of isooctane, using an injection syringe and make up to the mark. However, only add 5 µl of tetrachloromethane since its detection sensitivity is very high, but add 500 µl of dichloromethane since its detection sensitivity is very low.

These stock solutions contain:

- 1,1,1-trichloroethane: 13,40 mg/100 ml;
- tetrachloromethane: 7,95 mg/100 ml;
- 1,1,2-trichloroethene: 14,60 mg/100 ml;
- tetrachloroethene: 16,30 mg/100 ml;
- dichloromethane: 660,00 mg/100 ml;
- trichloromethane: 14,70 mg/100 ml;
- bromodichloromethane: 19,80 mg/100 ml;
- dibromochloromethane: 24,50 mg/100 ml;
- tribromomethane: 28,90 mg/100 ml;
- 1,1,2-trichloro-1,2,2-trifluoroethane: 15,70 mg/100 ml.

5.6 Halogenated hydrocarbons standard solutions I, II, III and IV

Use the stock solution (5.5) to prepare standard solutions (I, II, III, IV) of appropriate concentrations by adding, for example, 200 µl, 400 µl, 1 000 µl and 2 000 µl of the stock solution to 10 ml volumetric flasks that contain a little isooctane, and make up to the mark with isooctane.

The internal standard (5.7) may also be added to the halogenated hydrocarbon standard solutions.

5.7 Internal standard solution (2-bromo-1-chloropropane solution).

Add 200 µl 2-bromo-1-chloropropane to a little isooctane in a 50 ml volumetric flask using an injection syringe and make up to the mark with isooctane (concentration by mass = 308 mg/50 ml).

In order to obtain a suitable dilution, add 400 µl of the above solution to a little isooctane in a 10 ml volumetric flask and make up to the mark with isooctane. The concentration by mass of this solution is 2,464 mg/10 ml.

5.8 Blank solution

Use an oil which is as free as possible from the halogenated hydrocarbons.

6 Apparatus

6.1 Gas chromatograph, suitable for use with a capillary column and fitted with an electron capture detector (ECD) and, if available, an automatic headspace sampler. The conditioning apparatus used for the injection vials shall have a temperature range of at least 80 °C.

It is advantageous to have a facility for heating up to 110 °C in determining the halogenated hydrocarbon contents of fats and oils since higher sensitivities can be achieved. It is also advisable to use an apparatus which makes it possible to establish a constant conditioning time for all the samples.

6.2 Low-polarity glass or **quartz capillary column**, whose separation efficiency shall be high enough to make it possible to separate all the substances shown in the chromatogram in Figure 1.

NOTE A fused silica column of length 50 m and internal diameter 0,32 mm, coated with cross-linked SE 54, film thickness 1 µm, has been found to be suitable.

6.3 Injection vials, of capacities 10 ml or 25 ml, with gas-tight closures and PTFE-faced septa.

Check whether the injection vials and closures emit any halogenated hydrocarbons and store them in a sealed container. Special attention shall be paid to leak tightness if tempering times are long. Leaks are indicated by variations of the peak areas of the internal standard.

6.4 Microsyringes, of capacities 10 µl, 50 µl and 100 µl.

6.5 Gas-tight injection syringe, of capacity 2,5 ml, with needle of wide diameter with lateral orifice for manual injections from the headspace.

6.6 Heating bath or **aluminium block**, with clamps for holding injection vials (6.3), thermostatically maintained at 80 °C.

Glycerol is recommended as heating fluid for continuous operation in a heating bath.

Peak identification

1	dichloromethane	7	bromodichloromethane
2	1,1,2-trichloro-1,2,2-trifluoroethane	8	2-bromo-1-chloropropane (internal standard)
3	trichloromethane	9	dibromochloromethane
4	1,1,1-trichloroethane	10	tetrachloroethene
5	tetrachloromethane	11	tribromomethane (bromoform)
6	1,1,2-trichloroethene		

NOTE Operating conditions are given in 9.1.

Figure 1 — Chromatogram of the standard substances (see 6.2)

7 Sampling

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

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

During the period between sampling and analyses, store the sample in such a way that no change occurs in its content of halogenated hydrocarbons.

8 Preparation of samples

8.1 General

The fact that halogenated hydrocarbons are often present in the laboratory atmosphere and the laboratory apparatus is frequently contaminated with them makes it difficult to test for them in trace concentrations. Steps shall therefore be taken to ensure that contamination is kept as low as possible at all stages in the analysis. In addition, blank solutions (5.8) shall be analysed with every test series (see 8.4)

8.2 Test solutions

Weigh, to the nearest 1,0 mg, approximately 4 g of the sample oil, in duplicate, into two injection vials (6.3). After closing the vials, add 10 µl of the internal standard solution (5.7) and 10 µl of isooctane (5.2) to the sample using a microsyringe (6.4). Carefully shake the vial without wetting the stoppers.

8.3 Calibration solutions

8.3.1 For every series of samples, make up a calibration series using a suitable edible oil (see 8.3.2) and the standard solutions specified in 5.6. Carry out a duplicate determination for each of the four standard concentrations. Prepare the calibration solutions as described in 8.2, but instead of adding 10 µl of solvent (isooctane), add 10 µl of the corresponding standard solution [10 µl of the standard solutions I, II, III and IV are equivalent to an added amount of 20 ng, 40 ng, 100 ng or 200 ng multiplied by the absolute value of the density of the respective halogenated hydrocarbon (see 5.1.1 to 5.1.11)]. In the case of tetrachloromethane, however, multiply by a factor of 0,5 and for dichloromethane multiply by a factor of 50 (see 5.5).

8.3.2 An edible oil which is as free as possible from the halogenated hydrocarbons shall be used for the calibration series. It will therefore be necessary either to keep a suitable edible oil sample in stock or to first analyse the sample test solutions and then select a suitable edible oil sample for making up the calibration series. If the sample used for the calibration series has not already been examined as part of the sample test series, it shall be tested, as such, in duplicate.

8.4 Blank sample

To test for contamination, with each series of samples, also inject from an injection vial which is sealed in the normal manner but does not contain a test sample.

9 Procedure

9.1 Gas chromatography conditions

Since the gas chromatography conditions depend on the design of the equipment, it is not possible to provide information which is generally valid. If the column specified in 6.2 is used with automatic injection, the following parameters have been found suitable:

a) temperature programme:

- 50 °C for 5 min,
- 50 °C to 150 °C at 5 °C/min,
- 150 °C at 5 min, then
- 150 °C to 250 °C at 10 °C/min;

b) injector temperature: 200 °C;

c) detector temperature: 280 °C;

d) temperature of sample heating bath: 80 °C;

e) temperature of injection syringe:

  - 100 °C, or
  - 120 °C if heating bath temperature is 110 °C;

f) split ratio of capillary injector: 1:30;

g) carrier gas: nitrogen (inlet pressure about 800 hPa);

h) purge gas for ECD:

  - argon/methane or nitrogen (30 ml/min).

For automatic sampling by means of a gas overpressure system, the following parameters are additionally recommended:

a) pressure build-up time: 0,5 min;

b) bath temperature: 80 °C (aluminium block);

c) conditioning time: 60 min;

d) injector (needle) temperature: 200 °C;

e) injection time: 5 s.

9.2 Gas chromatography

9.2.1 General

In headspace gas chromatography, it is not only the substance-specific detector response but also the vapour pressure of the compound concerned in the sample matrix which is of critical importance for detection sensitivity. It is therefore essential to maintain precise conditioning of the injection vials and the injection syringe.

9.2.2 Automatic sample injection

Condition the test and calibration solutions, prepared according to 8.2 and 8.3, in the heating appliance of the automatic headspace injector for 60 min. Then make the injection and start the temperature programme on the GC.

9.2.3 Manual sampling

Condition as follows the test solutions and the calibration solutions, prepared according to 8.2 and 8.3. Successively place the injection vials in the heating device (6.6) set at 80 °C. Condition the gas-tight injection syringe in a drying oven at 100 °C. After a conditioning period of the samples of exactly 60 min at 80 °C, press down the piston of the syringe (using safety gloves!) and collect the sample by inserting the needle of the syringe through the septum into the headspace of the injection vial. Move the piston up and down several times to purge the syringe with the gas phase. Then take up to 2,5 ml of the gas phase and inject it into the gas chromatograph.

The samples shall be placed into the heating device in such a way that each sample is maintained for exactly 60 min at the specified temperature.

To increase the sensitivity, the samples may be heated to an even higher temperature.

10 Calculation and expression of results

10.1 Determination of the ratios from the measurement for the sample and internal standard

Determine the ratio Q_H from the peak areas or peak heights using the following equation:

$$Q_H = \frac{A_1}{A_2}$$

where

A_1 is the peak area or peak height of the halogenated hydrocarbon;

A_2 is the peak area or peak height of the internal standard.

10.2 Calculation of regression from calibration solutions

Determine the fraction equivalent to the added internal standard for each of the halogenated hydrocarbon peaks obtained with the calibration solutions specified in 8.3. Calculate Q_Z using the following equation:

$$Q_Z = Q_{E+Z} - Q_E$$

where

Q_{E+Z} is the ratio of the analytical signal of the calibration test solution with the internal standard added;

Q_E is the ratio of the analytical signal of the same calibration test solution without the standard added.

Carry out a regression calculation using the pairs of values obtained for Q_Z and the amount of standard added (see 8.3) and determine the content in nanograms per sample mass for each of the individual halogenated hydrocarbons.

10.3 Calculation of the content of halogenated hydrocarbons

Calculate the content w of the individual halogenated hydrocarbon, in milligrams per kilogram of sample, using the following equation (internal standard method):

$$w = \frac{m_1}{m_0 \times 1000}$$

where

m_1 is the mass of the individual halogenated hydrocarbon (calculated in accordance with 10.2), in nanograms;

m_0 is the sample mass, in grams.

Give the result to the third decimal place.

11 Precision

11.1 Results of 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 using 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 given in Annex A.

11.3 Reproducibility

The absolute difference between two single test results, obtained using 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 given 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 results;

e) the test results obtained or, if the repeatability has been checked, the final result obtained.

Annex A
(informative)

Results of interlaboratory test

A collaborative test was carried out at an international level in 2000/2001 by DIN, in which 14 laboratories participated, each performing two replicates, on five samples, as shown in Table A.1. The statistical results shown in Tables A.2 to A.4. The results were evaluated in accordance with ISO 5725[1].

Table A.1 — Halogenated hydrocarbons added to each sample

Values in milligrams per kilogram

Sample code[a]	Trichloromethane	1,1,1-Trichloroethane	1,1,2-Trichloroethene	Tetrachloroethene	Bromodichloromethane
A	0,000	0,000	0,000	0,000	0,000
B	—	0,023	—	0,053	—
C	0,159	—	—	—	0,054
D	—	0,040	0,176	0,072	—
E	0,031	—	—	—	0,029

[a] Sample A (blank): Refined olive oil (no halogenated hydrocarbons added).

Samples B and C: Extra virgin olive oil.

Samples D and E: Palm olein.

In all, 30 vials of each sample were prepared. Six of these vials were analysed for the homogeneity of the added hydrocarbons. The standard deviations found were from 0,001 mg/kg to 0,007 mg/kg.

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[1] ISO 5725:1994, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by interlaboratory tests* (now withdrawn).

Table A.2 — Results for hydrocarbons found in the blank sample A

Values in milligrams per kilogram

Laboratory No.	Trichloromethane		1,1,1-Trichloroethane		1,1,2-Trichloroethene		Tetrachloroethene		Bromodichloromethane	
	Added HC 0,000		Added HC 0,000		Added HC 0,000		Added HC 0,000		Added HC 0,000	
	value 1	value 2	value 1	value 2	value 1	value 2	value 1	value 2	value 1	value 2
1	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
2	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
3	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
4	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
5	0,000	0,000	0,001	0,002	0,001	0,001	0,000	0,000	0,000	0,000
6	0,002	0,008	0,001	0,006	0,000	0,000	0,001	0,001	0,001	0,001
7	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
8	0,005	0,005	0,005	0,005	0,005	0,005	0,005	0,005	0,005	0,005
9	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
10	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
11	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
12	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
13	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001
14	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Mean	0,001		0,001		0,001		0,001		0,001	

Table A.3 — Statistical results for blank sample A

Parameter	Halogenated hydrocarbon				
	Trichloromethane	1,1,1-Trichloroethane	1,1,2-Trichloroethene	Tetrachloroethene	Bromodichloromethane
Sample identification	A	A	A	A	A
Number of participating laboratories	14	14	14	14	14
Number of laboratories retained after eliminating outliers	14	14	14	14	14
Number of test results in all laboratories	28	28	28	28	28
Added amount	0,000	0,000	0,000	0,000	0,000
Mean (mg/kg)	0,001	0,001	0,001	0,001	0,001
Repeatability standard deviation (s_r)	0,001	0,001	0,000	0,000	0,000
Repeatability relative standard deviation, %	145,0	122,6	0,0	0,0	0,0
Repeatability limit (r)	0,003	0,003	0,000	0,000	0,000
Reproducibility standard deviation (s_R)	0,002	0,002	0,001	0,001	0,001
Reproducibility relative standard deviation, %	252,5	217,3	268,9	268,9	268,9
Reproducibility limit (R)	0,006	0,005	0,004	0,004	0,004

Table A.4 — Statistical results for samples B, C, D and E

Parameter	Halogenated hydrocarbon								
	Trichloro-methane		1,1,1-Trichloro-ethane		1,1,2-Trichloro-ethene	Tetrachloro-ethene		Bromo-dichloro-methane	
Sample identification	C	E	B	D	D	B	D	C	E
Number of participating laboratories	14	14	14	14	14	14	14	14	14
Number of laboratories retained after eliminating outliers	13	13	13	14	13	12	12	13	12
Number of test results in all laboratories	26	26	26	28	26	24	24	26	24
Added amount	0,159	0,031	0,023	0,040	0,176	0,053	0,072	0,054	0,029
Mean (mg/kg)	0,156	0,035	0,024	0,042	0,158	0,055	0,071	0,055	0,029
Repeatability standard deviation (s_r)	0,005	0,005	0,002	0,005	0,005	0,001	0,002	0,002	0,002
Repeatability relative standard deviation, %	2,9	13,7	7,0	11,9	3,5	2,4	2,4	3,4	6,2
Repeatability limit (r)	0,013	0,013	0,005	0,014	0,015	0,004	0,005	0,005	0,005
Reproducibility standard deviation (s_R)	0,021	0,005	0,004	0,007	0,027	0,005	0,005	0,004	0,002
Reproducibility relative standard deviation, %	13,4	13,7	15,7	17,3	17,3	8,4	7,7	7,6	8,2
Reproducibility limit (R)	0,058	0,013	0,011	0,020	0,076	0,013	0,015	0,012	0,007

Bibliography

[1] ISO 661:1989, *Animal and vegetable fats and oils — Preparation of test samples*

[2] ISO 5555:1991, *Animal and vegetable fats and oils — Sampling*

[3] ISO 5725-1:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

[4] ISO 5725-2:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

[5] Collection of official methods under article 35 of the German Federal Foods Act, Food Analysis L 13.04-1: *Determination of low-boiling halogenated hydrocarbons in edible oils*

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