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Animal and vegetable fats and oils — Detection and identification of a volatile organic contaminant by GC/MS

Corps gras d'origines animale et végétale — Détection et identification d'un contaminant organique volatil par CPG/SM

Reference number
ISO 15303:2001(E)

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

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 International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 15303 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

Annex A of this International Standard is for information only.

Animal and vegetable fats and oils — Detection and identification of a volatile organic contaminant by GC/MS

1 Scope

This International Standard specifies a method for the detection and identification of a volatile organic contaminant in edible oils.

It is applicable to the identification of volatile industrial chemicals in both crude and refined edible oils that are suspected of being contaminated. It also enables determination of the concentration of the contaminant.

This International Standard is not applicable to the determination of the concentration of chemicals that may react with the edible oil or with one of its natural components. In these cases, the presence of the contaminant may sometimes be established on a qualitative basis. Also, this International Standard is not applicable to non-volatile chemicals.

This method has been shown to be applicable for the identification of the following compound classes:

- saturated halogenated hydrocarbons;
- unsaturated halogenated hydrocarbons;
- esters; aldehydes; alcohols; amines; ketones; ethers;
- cyclic and aromatic compounds;
- nitrogen compounds;
- acrylates; etc.

The method has been evaluated for concentrations in the range of 1 mg/kg to 10 mg/kg.

2 Normative reference

The following normative document contains provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent edition of the normative document indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

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

3 Principle

A deuterated reference compound with a GC retention time close to that of the suspected contaminant is added to the oil at a concentration close to that of the suspected contaminant. A sample of the oil is then introduced into a thermal cold-trapping inlet of a GC/MS apparatus. Volatile components evaporate from the oil and are held in the cold trap. The trap is then flash-heated to 160 °C and the chemicals released from the trap are swept into the GC/MS for analysis.

4 Reagents

Use only reagents of recognized analytical grade, and distilled or demineralized water or water of equivalent purity.

4.1 Standard reference compound, corresponding to suspected contaminating compound (99 % pure).

4.2 Methanol, Analar grade.

4.3 Fully deuterated internal standards of benzene, ethyl benzene or naphthalene (99 % pure).

4.4 Helium, chemically pure grade.

4.5 Refined, bleached, deodorized (RBD) groundnut oil, or a similar stable vegetable liquid oil, known to be free of industrial chemicals related to the analyte under consideration.

5 Apparatus

Usual laboratory apparatus and, in particular, the following.

5.1 Capillary gas chromatograph.

5.2 Mass spectrometer.

5.3 Thermal desorption cold-trapping device[1].

5.4 Capillary column, of length 50 m, methyl polysiloxane with OV101 (or equivalent) stationary phase, 0,5 µm film thickness and 0,32 mm internal diameter.

5.5 Gas chromatograph/mass spectrometer, operating under the following conditions.

a) Thermal desorption cold-trapping temperature programme (at injection):

   — thermal cold trap oven 160 °C;

   — thermal cold trap below −40 °C.

   The trap is held at a temperature below −40 °C for 5 min and is then flash-heated to 160 °C. The GC temperature programme is started simultaneously with the flash-heating of the trap. The trap is held at 160 °C for 3 min.

b) GC temperature programme:

   — initial temperature 50 °C for 5 min;

   — increase at 7,5 °C per min;

   — final temperature 250 °C for 5 min.

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[1] The Chrompack Purge and Trap System 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 the product.

c) MS conditions:

— scan	one scan per second or faster;
— source	70 eV, 200 °C, 100 µA, 4 kV.

d) Gas flows:

— column	1 ml/min helium;
— split during cold trapping	5 ml/min.

5.6 Vortex mixer.

6 Procedure

6.1 Preparation of standard and blank

6.1.1 Use a refined, bleached and deodorized vegetable oil [e.g. groundnut (4.5)] as a carrier and as a blank.

6.1.2 Make up the standard (4.1) (i.e. a pure chemical of known composition, corresponding to that of the suspected contaminant) in a concentrated form (e.g. 100 mg/kg) in the carrier oil (6.1.1). This is the stock solution. Dilute this stock solution with carrier oil to the required concentration (usually in the range 1 mg/kg to 10 mg/kg). The standard solution should be of a similar concentration to the contaminant in the sample.

6.1.3 Dilute the suspect oil, if necessary, with the carrier oil (4.5) so that the concentration of the suspected contaminant is likely to be in the range 1,0 mg/kg to 10 mg/kg, i.e. the established linear range for quantification with this method.

6.2 Preparation of internal standards

Prepare a solution of deuterated benzene, ethyl benzene or naphthalene (4.3) in methanol (4.2) at a concentration of 0,1 g/l (0,1 µg/µl) as needed. The internal standard chosen should be the one having the closest GLC retention time to that of the analyte.

6.3 Preparation of analytical sample

Accurately weigh 1,00 g of the oil under test. Add 5,0 µl of internal standard solution in methanol (4.2), selected according to 6.2. Mix on the vortex mixer (5.6).

Repeat with all samples, standard and blank. For best results, allow to stand overnight to allow equilibration.

6.4 Determination by GC/MS analysis

The following conditions have been found to be satisfactory.

Pack an empty thermal cold-trapping (TCT) tube with clean glass wool to a length of about 3 cm to 4 cm. Take approximately 10 mg of sample and place in the tube. If the observed concentration of the contaminant exceeds 10 mg/kg, following analysis of a 10 mg portion of oil, repeat the determination with a sample size of 2,5 mg. For still larger concentrations, it is recommended that the sample be accurately diluted with the blank oil (6.1.1) until the concentration range is 1 mg/kg to 10 mg/kg (see 6.1.3).

Pre-cool the cold trap to below –20 °C, and continue cooling to below –40 °C. Fit the tube containing the oil in the TCT oven and start the TCT and GC/MS programmes.

6.5 Identification

Compare the GC retention time and mass spectrum of the analyte with those of the standard. Identification requires a good match of both spectra and GC retention times.

Quantification is performed by using reconstructed ion chromatograms of selected ions from the analyte and the internal standard and integrating their areas. This method is used to minimize interference from co-elutants.

7 Calculation and expression of results

7.1 Calculation

The peak area of the analyte ion divided by the peak area of the internal standard ion is the response ratio R:

$$R = \frac{K \cdot C}{C_{\mathrm{is}}}$$

where

K is a constant;

C is the concentration of analyte ion;

C_{is} is the concentration of internal standard.

$$R_{\mathrm{SAM}} = \frac{K \cdot C_{\mathrm{SAM}}}{C_{\mathrm{is}}}$$

where

R_{SAM} is the value of R for the sample;

C_{SAM} is the concentration of analyte ion in the sample.

Since C_{is} is a constant by definition:

$$R_{\mathrm{SAM}} = F \cdot C_{\mathrm{SAM}}$$

where the factor F is

$$F = \frac{K}{C_{\mathrm{is}}}$$

Similarly, the value of R for the standard is:

$$R_{\mathrm{STD}} = F \cdot C_{\mathrm{STD}}$$

where C_{STD} is the concentration of analyte ion in the standard.

Therefore

$$\frac{c_{\mathrm{SAM}} \cdot F}{c_{\mathrm{STD}} \cdot F} = \frac{R_{\mathrm{SAM}}}{R_{\mathrm{STD}}}$$

$$c_{\mathrm{SAM}} = \frac{R_{\mathrm{SAM}} \cdot c_{\mathrm{STD}}}{R_{\mathrm{STD}}}$$

7.2 Expression of results

Carry out duplicate determinations. Report the identity of the contaminant and the mean of the two duplicate values obtained for its concentration, provided the repeatability limit in 8.2 is satisfied. Otherwise, repeat the determination on two further test portions. If this time the difference again exceeds 0,6 mg/kg, take as the result the arithmetic mean of the four determinations, provided that the maximum difference between the individual results does not exceed 1,0 mg per kilogram of oil.

Report the result to one decimal place.

8 Precision

8.1 Interlaboratory tests

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

8.2 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 0,6 mg/kg.

8.3 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 1,43 mg/kg.

NOTE This method has been ring tested with 1,1,1-trichloroethane, 2-ethyl hexyl acrylate, dicylopentadiene, tetrachloroethylene, *N*,*N*-ethylmethylaniline, *N*,*N*-dimethylaniline, hexan-2-ol, benzene, cumene and ethyl acrylate (see bibliographic references).

Annex A
(informative)

Results of interlaboratory tests

A.1 General

Two interlaboratory tests were carried out on the determination of organic chemical contaminants in edible oils. The first of these was carried out in 1993 and is reported in Table A.1. The second was carried out in 1994 and is reported in Table A.3.

NOTE It was agreed at the meeting of ISO/TC 34/SC 11 held in April 1996 that in view of the specialized equipment needed for this work, the results of these international interlaboratory tests would be judged to be acceptable notwithstanding the limited number of participants and results obtained. It should be noted that the protocol concerned was not in agreement with ISO 5725:1986 or ISO 5725-1:1994.

A.2 First interlaboratory trial, 1993

Number of laboratories invited to participate: 21 from 10 countries.

Number of laboratories submitting results: 7 from 5 countries.

Participants were provided with eight edible oils labelled sample A to H. In order to simulate actual shipping practice in which the nature of the last three cargoes, and thus that of the potential contaminant, is known, the analysts were told that the potential contaminant could be one of three compounds. Details of the samples are given in Table A.1. Statistical results are given in Table A.2.

This method is suitable for the detection of the presence of a known contaminant in an oil or fat. However, the precision data obtained during the collaborative trials were not considered satisfactory to confirm the low levels of the contaminant as required by the trade and ISO.

Table A.1 — Samples for first test on edible oil cargoes

Sample code	Supposed list of previous cargoes[a]	Spiked concentration in oil mg/kg
A	Dichloromethane 1,2-Dibromoethane 1,1,1-TRICHLOROETHANE	1,12
B	2-ETHYL HEXYL ACRYLATE Methyl acrylate Dipentene	1,25
C	Methyl *t*-butyl ether DICYCLOPENTADIENE Furfural	1,29
D	Trichloroethylene TETRACHLOROETHYLENE 2-Furaldehyde	1,03
E	*N*,*N*-ETHYLMETHYLANILINE 5-Ethyl-2-methylpyridine *N*,*N*-Dimethyl aniline	1,04
F	Pyridine *N*,*N*-DIMETHYLANILINE Nitrotoluene	1,10
G	Butanal HEXAN-2-OL Di-isobutyl ketone	1,04
H	Cumene Pyridine BENZENE	1,04

[a] Chemicals written in capital letters are those actually contained in the oil, but this information was not given to the analysts until after the test.

Table A.2 — Statistical analysis of the results of first interlaboratory trial

	Sample							
	A	B	C	D	E	F	G	H
No. of laboratories	7	7	7	7	7	7	7	7
Mean observed concentration (mg/kg)	1,11	1,32	1,53	1,05	1,58	1,18	0,83	0,91
Spiked concentration (mg/kg)	1,12	1,25	1,29	1,03	1,04	1,10	1,04	1,04
Number of accepted results for the repeatability determination	2	2	3	2	3	2	2	3
Repeatability standard deviation, s_r (mg/kg)	0,15	0,08	0,12	0,08	0,13	0,04	0,11	0,04
Repeatability coefficient of variation (%)	13,7	5,6	15,9	6,6	19,4	3,5	14,3	4,0
Repeatability limit, r (2,83 s_r)	0,43	0,23	0,62	0,21	0,51	0,11	0,31	0,11
Number of accepted results for the reproducibility determination	7	6	7	6	5	6	6	7
Reproducibility standard deviation, s_R (mg/kg)	0,64	0,57	1,09	0,29	1,80	0,43	0,44	0,18
Reproducibility coefficient of variation (%)	58,2	42,2	79,0	29,7	13,5	36,8	53,0	19,6
Reproducibility limit, R (2,83 s_R)	1,82	1,61	3,09	0,81	5,09	1,22	1,25	0,51
Range of results (mg/kg)	0,58 to 2,50	0,58 to 2,10	0,57 to 3,50	0,68 to 1,30	0,40 to 4,73	0,4 to 1,7	0,17 to 1,37	0,6 to 1,10

NOTE Although it is not statistically accurate to take a mean of values for r and R when these are obtained on different samples, it was considered to be an acceptable compromise in estimating the precision of the method overall in the present case. It can be calculated from this table that the mean repeatability limit (r) is 0,36, whilst the mean reproducibility limit (R) is 2,38.

A.3 Second interlaboratory trial, 1994

Number of laboratories invited to participate: 16 from 7 countries.

Number of laboratories submitting results: 9 from 5 countries.

Participants were provided with three edible oils labelled sample I to K. In order to simulate actual shipping practice in which the nature of the last three cargoes, and thus that of the potential contaminant, is known, the analysts were told that the potential contaminant would be one of three compounds. Details of the samples are given in Table A.3. Statistical results are given in Table A.4.

Table A.3 — Samples for second test on edible oil cargoes

Sample code	List of supposed previous cargoes[a]	Spiked concentration in oil mg/kg
I	2-Ethyl hexyl acrylate ETHYL ACRYLATE Vinyl acetate	1,03
J	*N,N*-Dimethylaniline Epichlorhydrin 1,3-Dichloropropen	
K	CUMENE Tetrachloroethylene *N,N*-Ethylmethylaniline	1,25

[a] Chemicals written in capital letters are those actually added to the RBD edible seed oil, but this information was not given to the analysts until after the test. J was a blank.

Table A.4 — Statistical analysis of the results of second interlaboratory trial

	Sample (m/z)				
	I (55)	K (105)	K (105)	K (120)	K (120)
	Internal standard (m/z)				
	Benzene (84)	Ethylbenzene (98)	Ethylbenzene (116)	Ethylbenzene (98)	Ethylbenzene (116)
No. of laboratories	9	9	8	8	8
No. of accepted results	7	8	8	6	7
Mean (mg/kg)	1,19	0,94	0,99	0,92	0,97
Repeatability standard deviation, s_r (mg/kg)	0,48	0,07	0,08	0,04	0,08
Repeatability coefficient of variation (%)	40,0	7,7	8,5	4,15	8,7
Repeatability limit, r (2,83 s_r)	1,3	0,2	0,24	0,11	0,24
Reproducibiliy standard deviation, s_R (mg/kg)	0,50	0,50	0,51	0,52	0,52
Reproducibility coefficient of variation (%)	42	53,25	51,2	56,8	53,5
Reproducibility limit, R (2,83 s_R)	1,4	1,4	1,4	1,47	1,47
Range of results (mg/kg)	0,5 to 2,75	0,16 to 1,69	0,15 to 1,66	0,1 to 1,47	0,09 to 1,67

NOTE Although it is not statistically accurate to take a mean of values of r and R when these are obtained on different samples, it was considered to be an acceptable compromise in estimating the precision of the method overall in the present case. It can be calculated from the results in this table that the mean repeatability limit (r) is 0,61, whilst the mean reproducibility limit (R) is 1,43.

Bibliography

[1] ISO 5725:1986, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests*

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

[3] Leatherhead Food Research Association. *Research Reports*, No. 708 (June 1993)

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