ISO 15303 – Animal and vegetable fats and oils – detection and identification of a volatile organic contaminant by GC/MS

Instrument: Varian 3800GC with 1200L Triple Quadruple MS/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.

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;

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.

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:

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

where

K is a constant;

C is the concentration of analyte ion;

C_{is} is the concentration of internal standard.

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

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:

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

where the factor F is

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

Similarly, the value of R for the standard is:

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

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

$$\frac{C_{SAM} \cdot F}{C_{STD} \cdot F} = \frac{R_{SAM}}{R_{STD}}$$

$$C_{SAM} = \frac{R_{SAM} \cdot C_{STD}}{R_{STD}}$$

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).

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)

[4] Leatherhead Food Research Association. *Technical Notes*, No. 110 (April 1994)

[5] Leatherhead Food Research Association. *Research Reports*, No. 742 (February 1997)

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