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Oilseed residues — Determination of free residual hexane

Tourteaux de graines oléagineuses — Dosage de l'hexane résiduaire libre

Reference number
ISO 9289:1991(E)

Foreword

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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.

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Introduction

The amount of hexane which can be desorbed rapidly from an oilseed residue by heating is not necessarily equal to the total amount of residual hexane determined in accordance with ISO 8892:1987, *Oilseed residues — Determination of total residual hexane.*

The "free residual hexane" is the amount of hexane desorbed by direct heating of the oilseed residue without a preceding humidification of the sample.

The difference between the total residual hexane and the free residual hexane depends on several factors. In general, it increases with decreasing humidity of the oilseed residue examined, i.e. with increasing dryness of the oilseed residue.

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Oilseed residues — Determination of free residual hexane

1 Scope

This International Standard specifies a method for the determination of the free residual hexane content in oilseed residues after extraction with hydrocarbon-based solvents.

2 Normative reference

The following standard contains provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the edition indicated was valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 5500:1986, *Oilseed residues — Sampling*.

3 Definition

For the purposes of this International Standard, the following definition applies.

free residual hexane: Proportion of volatile hydrocarbons, referred to generally as hexane, remaining in oilseed residues after extraction with hydrocarbon-based solvents and which is desorbed directly by heating at 80 °C without the addition of water.

It is expressed as milligrams of *n*-hexane per kilogram of sample.

4 Principle

Desorption of hexane by heating at 80 °C in a closed vessel after addition of an internal standard. Determination of the hexane content in the head-space by gas chromatography using capillary or packed columns.

5 Reagents and materials

All reagents shall be of recognized analytical grade unless otherwise stated.

5.1 Technical hexane or **light petroleum**, with a composition similar to that used in the industrial extraction of oilseeds or, failing these, ***n*-hexane**.

NOTE 1 It is recommended that technical hexane be used for the calibration. This reagent usually contains more than 50 % of *n*-hexane and consists predominantly of C_6 isomers.

5.2 Internal standard: use either 5.2.1 or 5.2.2.

NOTE 2 If the technical hexane used for the extraction or calibration contains appreciable amounts of cyclohexane, *n*-heptane should be used as the internal standard.

5.2.1 Cyclohexane.

5.2.2 *n*-Heptane.

5.3 Carrier gas, e.g. hydrogen, nitrogen, helium, etc., thoroughly dried and containing less than 10 mg/kg of oxygen.

5.4 Auxiliary gases.

5.4.1 Hydrogen, 99,9 % pure, containing no organic impurities.

5.4.2 Air, containing no organic impurities.

5.5 Calibration oilseed residues, of the same origin as the sample to be analysed and having a low hexane content. If the hexane content is too high, reduce it by spreading the residue in a thin layer and leaving it to stand in the open air for several hours.

Commercial oilseed residues usually have a moisture content of 12 % (*m*/*m*) to 14 % (*m*/*m*). In the case of samples having a different moisture content, it is necessary to carry out the calibration with oilseed residues having the same moisture content as the sample.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Gas chromatograph, with flame ionization detector and an integrator and/or recorder, equipped with either

a) a packed steel column approximately 2 m long and of internal diameter approximately 3,2 mm, packed with an acid-washed diatomaceous earth support of particle size 150 µm to 180 µm (Chromosorb P NAW 60/80 mesh1) is suitable), and coated with 10 % squalane or methylpolysiloxane (SE 301) is suitable), or

b) a capillary column, approximately 30 m long and of 0,3 mm internal diameter, coated with methylpolysiloxane (SE 301) is suitable) having a film thickness of 0,2 µm.

The injector and detector temperature shall be set at approximately 120 °C and the oven temperature at approximately 40 °C. The carrier gas pressure shall be set at approximately 0,3 bar (30 kPa).

If a capillary column is used, the apparatus shall have a 1/100 split injection system.

NOTE 3 For analyses in series, it is recommended to use a gas chromatograph having an automatic sample injection system, combined with a heating bath.

6.2 Heating bath, fitted with a rack to hold the flasks (6.4), capable of being set at 80 °C ± 2 °C and of maintaining the set temperature constant to ± 0,1 °C.

NOTE 4 For continuous use, glycerol is recommended as the heating liquid.

6.3 Gas syringe, of 1 ml capacity.

6.4 Septum flasks, of 20 ml ± 2 % capacity.

6.5 Septa, inert to hexane, of approximately 3 mm thickness, made of a material such as nitrile rubber (e.g. Perbunan1)), or butyl rubber with a polytetrafluoroethylene or polychloroprene seam (e.g. Neoprene1)).

Ensure that the septa used will produce a hermetic seal after crimping.

NOTE 5 The septa often have a very high mechanical resistance; if it is thought, therefore, that the needle of the gas syringe may be damaged by using it to perforate septa, perforate them with a pin before taking the sample from the head-space. Reuse of septa is not recommended.

6.6 Metallic foil caps, for example made of aluminium.

6.7 Crimping pliers, for closing the septum flasks.

6.8 Liquid syringes, of 10 µl capacity.

7 Sampling and sample storage

Sampling shall have been carried out in accordance with ISO 5500. It is essential that loss of hexane from the sample be prevented.

The laboratory sample shall fill a hermetically sealed container (preferably a crimped metal box) and shall be stored at 4 °C. Plastic containers shall not be used.

The determination of residual hexane shall be carried out as soon as the container has been opened.

8 Procedure

8.1 Calibration

8.1.1 Weigh, to the nearest 0,1 g, 5 g of the calibration oilseed residues (5.5) into each of five septum flasks (6.4). Seal each flask with a septum (6.5), cover with a foil cap (6.6) and crimp using the pliers (6.7).

8.1.2 Add, using a syringe (6.8), the volume of solvent (5.1) specified in table 1 to four of the five flasks. Do not add solvent to the fifth flask.

Table 1 — Content of solvent (5.1) in the calibration samples

Flask	1	2	3	4
Volume of solvent added (µl)	1	2	4	7
Free hexane content (mg/kg)	134	268	536	938

NOTE 6 If samples with hexane contents greater than the highest value shown in table 1 have to be tested, the volumes of solvent used for the calibration have to be increased accordingly.

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1) Chromosorb P NAW 60/80, SE 30, Perbunan and Neoprene 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.

8.1.3 Allow the flasks to stand at room temperature for 24 h.

NOTE 7 In the case of a shorter period, it is not certain that the technical hexane has been completely absorbed by the oilseed residue and, furthermore, that the sorption-desorption equilibrium of the technical hexane between the residue and the vapour phase has been reached.

8.1.4 At the end of this time add, by means of a syringe (6.8), 5 µl of the internal standard (5.2.1 or 5.2.2) to each of the five septum flasks through the septum.

8.1.5 At intervals of 15 min, place one septum flask after the other in the heating bath (6.2), set at 80 °C ± 2 °C. The flasks shall be immersed in the heating liquid up to the level of the foil cap.

8.1.6 After heating each of the flasks for exactly 60 min, take, by means of the gas syringe (6.3) previously heated to between 50 °C and 60 °C, exactly 1 ml of the gaseous phase from the head-space, without removing the flasks from the heating bath. Quickly inject into the chromatograph the gaseous phase so removed.

8.1.7 From the chromatogram corresponding to the flask to which no technical hexane was added, calculate the hexane content A_c, expressed as a percentage of the total peak areas.

8.1.8 From each of the chromatograms corresponding to each of the flasks to which technical hexane was added, calculate the calibration factor F using the formula

$$F = \frac{w_h \times A_{is}}{(A_t - A_c - A_{is}) \times w_{is}}$$

where

A_c is the hexane content calculated in 8.1.7;

A_{is} is the content of the internal standard in the calibration oilseed concerned, expressed as a percentage of the total peak areas;

A_t is the total hydrocarbons content, including the internal standard, in the calibration oilseed residue concerned, expressed as a percentage of the total peak areas;

NOTES

8 Hydrocarbons which usually make up technical hexane are 2-methylpentane, 3-methylpentane, methylcyclopentane, cyclohexane, etc. in addition to about 50 % *n*-hexane.

9 Do not include peaks due to the oxidation products, some of which may be present in significant amounts.

w_h is the free hexane content of the calibration oilseed residue concerned (see table 1), expressed in milligrams per kilogram;

w_{is} is the content of the internal standard in the calibration sample, expressed in milligrams per kilogram, i.e. 680 for *n*-heptane or 780 for cyclohexane.

Calculate the mean calibration factor $\overline{F}$ from the results of the four calibration samples.

$\overline{F}$ shall be equal to 1,0 ± 0,1.

8.2 Determination

8.2.1 Weigh, to the nearest 0,1 g, 5 g of the laboratory sample into a septum flask (6.4). Seal the flask immediately with a septum (6.5), cover with a foil cap (6.6) and crimp using the pliers (6.7).

These operations shall be performed rapidly.

8.2.2 Inject 5 µl of the internal standard (5.2.1 or 5.2.2) into the septum flask through the septum using a syringe (6.8), and place the flask in the heating bath (6.2), set at 80 °C ± 2 °C, for exactly 60 min. The flasks shall be immersed in the heating liquid up to the level of the foil cap.

8.2.3 Using the gas syringe (6.3), previously heated to between 50 °C and 60 °C, take exactly 1 ml of the gaseous phase from the head-space, without removing the flask from the heating bath. Quickly inject into the chromatograph the gaseous phase so removed.

8.3 Number of determinations

Carry out two determinations on the same laboratory sample.

9 Expression of results

The free residual hexane content w, in milligrams per kilogram, is given by the formula

$$w = \frac{(A'_t - A'_{is}) \times \overline{F} \times w'_{is}}{A'_{is}}$$

where

A'_{is} is the content of the internal standard in the sample, expressed as a percentage of the total peak areas;

A'_t is the total hydrocarbons content, including the internal standard, of the sample, expressed as a percentage of the total peak areas;

$\overline{F}$ is the mean calibration factor determined in 8.1.7;

w'_{is} is the content of the internal standard in the sample, expressed in milligrams per kilogram, i.e. 680 for *n*-heptane or 780 for cyclohexane.

Take as the final result the arithmetic mean of the two determinations provided that the repeatability requirement is met. If the repeatability requirement is not met, disregard the results and carry out two new determinations on test portions taken from the same test sample.

10 Precision

An inter-laboratory test, carried out at the international level in which 13 laboratories participated, each of which carried out two determinations on each sample, gave the statistical results (evaluated in accordance with ISO 5725[2]) shown in table 2.

11 Test report

The test report shall specify the method used and the results obtained. It shall also mention all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the results.

The test report shall include all information necessary for the complete identification of the sample.

Table 2 — Statistical results of the inter-laboratory test

Sample	Sunflower residue	Colza (rape) residue	
		moisture content 8,8 % (*m*/*m*)	same sample rehumidified, moisture content 12 % (*m*/*m*)
Number of laboratories retained after eliminating outliers	12	11	12
Mean free residual hexane content (mg/kg)	178	624	599
Standard deviation of repeatability, s_r (mg/kg)	12	17	33
Coefficient of variation of repeatability (%)	6,7	2,7	5,5
Repeatability, 2,83 s_r (mg/kg)	34	48	94
Standard deviation of reproducibility, s_R (mg/kg)	55	129	125
Coefficient of variation of reproducibility (%)	31	21	21
Reproducibility, 2,83 s_R (mg/kg)	156	364	353

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

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