INTERNATIONAL STANDARD

ISO 10633-1

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Oilseed residues — Determination of glucosinolates content —

Part 1: Method using high-performance liquid chromatography

Tourteaux de graines oléagineuses — Dosage des glucosinolates —

Partie 1: Méthode par chromatographie en phase liquide à haute performance

Reference number
ISO 10633-1:1995(E)

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.

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.

International Standard ISO 10633-1 was prepared by Technical Committee ISO/TC 34, *Agricultural food products,* Subcommittee SC 2, *Oleaginous seeds and fruits.*

ISO 10633 consists of the following parts, under the general title *Oilseed residues — Determination of glucosinolates content*:

— *Part 1: Method using high-performance liquid chromatography*

— *Part 2: Method using X-ray fluorescence spectrometry*

Annex A form an integral part of this part of ISO 10633. Annex B is for information only.

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4.5 Internal standard

Use either sinigrin monohydrate (potassium allylglucosinolate monohydrate, $M_r = 415{,}49$) (see A.1) or, for rapeseed in which sinigrin is present naturally, glucotropaelin (potassium benzylglucosinolate, $M_r = 447{,}52$) (see A.2).

See annex A for details of the preparation and purity check of these reagents.

4.6 Mobile phases

4.6.1 Eluant A: water filtered through a 0,45 µm filter and purified by passing through an activated charcoal cartridge system[1], or water of equivalent purity.

4.6.2 Eluant B: acetonitrile, HPLC grade, 20 % (*V/V*) solution in water that has been purified and passed through a 0,45 µm filter. The concentration may be modified in relation to the column used.

4.7 Ion-exchange resin

4.7.1 DEAE Sepharose CL-6B[2], sold as a commercial ready-to-use suspension, or an equivalent product.

4.7.2 DEAE Sephadex A25[2] **suspension**

Mix 10 g of DEAE Sephadex A25 resin (or equivalent) in excess 2 mol/l acetic acid solution. Leave to settle. Add 2 mol/l acetic acid until the volume of the supernatant liquid is equal to the volume of the sediment.

4.8 Sulfatase, *Helix pomatia* type H1 (EC 3.1.6.1)[3].
Purify, test and dilute the sulfatase in accordance with the methods described in A.3.1 to A.3.4.

5 Apparatus

Usual laboratory apparatus and, in particular, the following.

5.1 High-performance liquid chromatograph, capable of gradient elution and of maintaining a column temperature of 30 °C, connected to an **ultraviolet detector** capable of measurements at a wavelength of 229 nm.

5.2 Chromatography column for HPLC, type C_{18} or C_8, of particle size less than or equal to 5 µm, for example[4]:

Lichrosorb RP 18 column, ≤ 5 µm (150 mm × 4,6 mm);

Spherisorb ODS2 column, ≤ 5 µm (250 mm × 4 mm; 250 mm × 5 mm);

Novapak C_{18} column, ≤ 4 µm (150 mm × 4 mm);

Lichrospher RP8 column, ≤ 5 µm (125 mm × 4 mm);

Nucleosil C_{18} column, ≤ 5 µm (200 mm × 4 mm).

The performance of the column should be checked regularly, preferably using a reference sample of rapeseed desulfoglucosinolate[5]. In particular, the column shall not degrade 4-hydroxyglucobrassicin, an important and relatively unstable glucosinolate.

New columns shall be subjected to preliminary conditioning in accordance with the manufacturer's instructions so that reproducible results can be obtained.

5.3 Double-beam spectrometer, capable of operating in the ultraviolet region of the spectrum, and at a controlled temperature of 30 °C, equipped with **quartz cells** of 1 cm optical path and a **recording system.**

5.4 Microgrinder, for example a coffee mill.

5.5 Centrifuge, suitable for use with the tubes (5.6) and capable of obtaining a centrifugal acceleration of 5 000 *g*.

5.6 Polypropylene tubes, of 6 ml capacity.

5.7 Water bath, or other heating apparatus, capable of being maintained at 75 °C ± 1 °C.

1) The Norganic Millipore system is an example of a suitable product available commercially. This information is given for the convenience of users of this part of ISO 10633 and does not constitute an endorsement by ISO of this product.

2) DEAE Sepharose and Sephadex are examples of suitable products available commercially. This information is given for the convenience of users of this part of ISO 10633 and does not constitute an endorsement by ISO of these products.

3) Sulfatase S-9626 (from Sigma Chemicals) with an activity of 16 600 units/g is an example of a suitable product available commercially. This information is given for the convenience of users of this part of ISO 10633 and does not constitute an endorsement by ISO of this product.

4) The products mentioned are examples of suitable products available commercially. This information is given for the convenience of users of this part of ISO 10633 and does not constitute an endorsement by ISO of these products.

5) Reference samples of oilseed desulfoglucosinolate may be obtained from the Community Reference Bureau (Brussels).

5.8 Glass wool

Oilseed residues — Determination of glucosinolates content —

Part 1:
Method using high-performance liquid chromatography

1 Scope

This part of ISO 10633 specifies a method for the determination of the content of the different glucosinolates in crucifer oilseeds.

NOTES

1 This method does not determine glucosinolates which are substituted on the glucose molecule, but these compounds are of little importance in commercial rapeseed.

2 This method allows determination of intact glucosinolates. However, it does not identify and quantify the products formed from the degradation of glucosinolates during preparation of the meal. Therefore, the antinutritional effects of these degradation products cannot be taken into consideration.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this part of ISO 10633. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this part of ISO 10633 are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 771:1977, *Oilseed residues — Determination of moisture and volatile matter content.*

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods.*

ISO 5502:1992, *Oilseed residues — Preparation of test samples.*

ISO 9167-1:1992, *Rapeseed — Determination of glucosinolates content — Part 1: Method using high-performance liquid chromatography.*

3 Principle

Extraction of glucosinolates in a methanol solution, then purification and enzymatic desulfation on ion-exchange resins. Determination using reverse-phase high-performance liquid chromatography (HPLC) with gradient elution and ultraviolet detection.

4 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and water complying with the specifications for grade 2 of ISO 3696.

4.1 Methanol, HPLC grade, 70 % (*V/V*) solution.

4.2 Sodium acetate, 0,02 mol/l solution at pH 4,0.

4.3 Sodium acetate, 0,2 mol/l solution.

4.4 Imidazole formate, 6 mol/l solution.

Dissolve 204 g of imidazole in 113 ml of formic acid in a 500 ml one-mark volumetric flask. Dilute to the mark with water.

8.4.4 Place a tube (5.6) under the column to collect the eluate.

Elute the obtained desulfoglucosinolates with two 1 ml portions of water, allowing the water to drain after each addition.

8.4.5 Mix the eluate well. If not used immediately for chromatography, store the eluate in the dark in a freezer at – 18 °C for a week.

8.5 Blank test

If required (see 9.3), carry out a blank test using the same procedure on a test portion taken from the same test sample, but omitting the sinigrin internal standard solution in order to detect and quantify any sinigrin present in the test portion.

8.6 Chromatography

8.6.1 Adjustment of apparatus

Adjust the chromatograph (5.1) as follows:

— flowrate of the mobile phase (4.6): depends on the nature of the column (see 8.6.2) and generally is of the order of 1 ml/min;

— temperature of the column (5.2): 30 °C;

— detection wavelength: 229 nm.

8.6.2 Determination

Operating in accordance with the instructions for the apparatus, inject into the chromatograph not more than 50 µl of the desulfoglucosinolate solution obtained in 8.4.4.

Use an elution gradient appropriate to the column employed.

NOTES

5 The following elution gradients are given as examples.

a) For a Lichrosorb RP18 column, ⩽ 5 µm (150 mm × 4,6 mm):

— 100 % of eluant A (4.6.1) for 1 min;

— a linear elution gradient over 20 min until 0 % of eluant A and 100 % of eluant B (4.6.2) are obtained;

— a linear elution gradient over 5 min until 100 % of eluant A and 0 % of eluant B are obtained;

— 100 % of eluant A for 5 min to establish equilibrium.

b) For a Lichrospher RP8 column, ⩽ 5 µm (125 mm × 4 mm):

— 100 % of eluant A over 2 min 30 s;

— a linear elution gradient over 18 min until 0 % of eluant A and 100 % of eluant B are obtained;

— 100 % of eluant B for 5 min;

— a linear elution gradient over 2 min until 100 % of eluant A and 0 % of eluant B are obtained;

— continue for 5 min to establish equilibrium.

6 The gradient profiles may be modified to give optimum separations according to the columns used.

8.6.3 Examination of chromatograms

Take into account only those peaks having an area greater than 1 % of the total sum of the peak areas.

The order of elution of the peaks with a type C_{18} column and a suitable elution gradient (see the examples given in 8.6.2) is generally as shown in figure 1.

9 Expression of results

9.1 Calculation of the content of each glucosinolate

The content of each glucosinolate, expressed in micromoles per gram of dry matter of the product, is equal to

$$\frac{A_g}{A_s} \times \frac{n}{m} \times \frac{K_g}{K_s} \times \frac{100}{100 - w}$$

where

A_g is the peak area, in integrator units, corresponding to the desulfoglucosinolate under consideration;

A_s is the peak area, in integrator units, corresponding to the internal standard used (desulfosinigrin or desulfoglucotropaeolin);

K_g is the response factor of the desulfoglucosinolate under consideration (9.2);

K_s is the response factor of the internal standard used (desulfosinigrin or desulfoglucotropaeolin);

m is the mass, in grams, of the test portion;

n is the quantity, in micromoles, of internal standard added to the tube in 8.2 (sinigrin or glucotropaeolin);

w is the moisture and volatile matter content, expressed as a percentage by mass, of the test sample.

5.8 Glass wool

5.9 Pasteur pipettes, 150 mm long, and a suitable **stand** or any other appropriate device.

6 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 part of ISO 10633. A recommended sampling method is given in ISO 5500[6].

7 Preparation of test samples

Reduce the laboratory sample in accordance with ISO 5502 to obtain the required size of test sample. Grind if necessary.

Take a sample of this and determine the moisture and volatile matter content in accordance with ISO 771.

If the result is less than 10 % (*m/m*), this value will be used for the calculation (9.1). Continue immediately with the determination of glucosinolate content (clause 8) using the test sample without further treatment.

If the moisture and volatile matter content is found to be in excess of 10 % (*m/m*), dry the test sample using a current of air at approximately 45 °C, then redetermine the content. Continue this process until a moisture and volatile matter content of less than 10 % (*m/m*) is obtained. This final value is used for the calculation. Continue immediately with the determination of glucosinolate content (clause 8) using the dried test sample.

8 Procedure

NOTE 3 If it is required to check whether the repeatability requirement is met, carry out two single determinations in accordance with 8.1 to 8.4 and 8.6 under repeatability conditions.

8.1 Test portion

Label two tubes (5.6) A and B and transfer to each a test portion of 100 mg, weighed to the nearest 0,1 mg, of the prepared test sample (clause 7).

8.2 Extraction of glucosinolates

8.2.1 Place the tubes in the water bath (5.7) set at 75 °C and leave for 1 min. Add 2 ml of boiling methanol solution (4.1) and then immediately add

— to tube A, 200 µl of 5 mmol/l internal standard solution (A.1.1); and

— to tube B, 200 µl of 20 mmol/l internal standard solution (A.1.2).

NOTE 4 See 4.5 for the use of an alternative internal standard solution.

8.2.2 Continue heating at 75 °C for a further 10 min, shaking the tubes at regular intervals. Mix the contents of each tube and then centrifuge at 5 000 *g* for 3 min. Transfer the supernatant liquid from each tube to two other tubes (5.6) labelled A′ and B′.

8.2.3 Add, to each of the two tubes A and B containing the solid residue, 2 ml of boiling methanol (4.1) and reheat in the water bath (5.7) set at 75 °C for 10 min, shaking the tubes at regular intervals.

Centrifuge for 3 min and then add the supernatant liquid from the tubes A and B, respectively, to the tubes A′ and B′, respectively, containing the supernatant liquids retained in 8.2.2.

8.2.4 Adjust the volume of the combined extracts in the tubes A′ and B′ to approximately 5 ml with water and mix.

These extracts, if stored in the dark in a freezer at –18°C, may be kept for 2 weeks.

8.3 Preparation of ion-exchange columns

Cut the required number of Pasteur pipettes (5.9), i.e. two pipettes per sample, so as to leave a volume of 1,2 ml above the neck and place a glass wool plug (5.8) in the neck of each pipette. Place the pipettes vertically on a stand.

Transfer 0,5 ml of a well-mixed suspension of ion-exchange resin (4.7.2) to each pipette and allow to settle and drain.

Rinse the pipettes with 2 ml of the imidazole formate (4.4) and then twice with 1 ml portions of water.

8.4 Purification and desulfation

8.4.1 Carry out the following operations for each combined extract.

8.4.2 Transfer 1 ml of the extract (8.2.4) to a prepared column (8.3) without disturbing the resin surface and allow to drain. Add two 1 ml portions of the sodium acetate buffer (4.2), allowing the buffer to drain after each addition.

8.4.3 Add to the column 75 µl of diluted, purified sulfatase solution (4.8). Leave to act overnight at ambient temperature.

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