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Part 12 : 1996
ISO 10633-1 :
1995

Methods for

Analysis of oilseed residues

Part 12. Determination of glucosinolates content by high-performance liquid chromatography

ICS 67.200.20

Committees responsible for this British Standard

The preparation of this British Standard was entrusted to Technical Committee AW/2, Oilseeds and residues, upon which the following bodies were represented:

FOSFA International
Grain and Feed Trade Association
Leatherhead Food Research Association
Ministry of Agriculture, Fisheries and Food
National Farmers' Union
National Institute of Agricultural Botany
Seed Crushers' and Oil Processors' Association
Tropical Growers' Association
United Kingdom Agricultural Supply Trade Association

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Contents

National foreword

This Part of BS 4325 has been prepared by Technical Committee AW/2 and is identical with ISO 10633-1 : 1995 *Oilseed residues — Determination of glucosinolates content — Part 1 : Method using high-performance liquid chromatography*, published by the International Organization for Standardization (ISO) and in the preparation of which the United Kingdom played a full part.

Cross-references

International standard	Corresponding British Standard
Normative references	
ISO 771 : 1977	BS 4325 *Methods for analysis of oilseed residues* Part 1 : 1978 *Determination of moisture and volatile matter content* (Technically equivalent)
EN ISO 3696 : 1995 ISO 3696 : 1987	BS EN ISO 3696 : 1995 *Water for analytical laboratory use. Specification and test methods.* (Identical)
ISO 5502 : 1992	BS 4325 *Methods for analysis of oilseed residues* Part 9 : 1992 *Preparation of test samples* (Identical)
EN ISO 9167-1 : 1995 ISO 9167-1 : 1992	BS EN ISO 9167 *Rapeseed — Determination of glucosinolates content* Part 1 : 1995 *Method using high-performance liquid chromatography* (Identical)
Informative references	
ISO 5500 : 1986	BS 6606 : 1987 *Methods for sampling oilseed residues* (Identical)
ISO 5725 : 1986[1)]	BS 5497 *Precision of test methods* Part 1 : 1987 *Guide for the determination of repeatability and reproducibility for a standard test method by inter-laboratory tests* (Identical)

Additional information. It is recommended that the test sample (clause **7**) be washed with dichloromethane before continuing with the determination of glucosinolate content (clause **8**). This is to remove any seed treatment chemicals i.e. pesticides which may be present in the sample and which may deactivate the enzyme 'sulfatase' (**4.8**). Some pesticides, such as diquat that are used on rapeseed, are not soluble in hexane and will still be in the residue after solvent extraction.

Compliance with a British Standard does not of itself confer immunity from legal obligations.

[1)] ISO 5725 : 1986, to which informative reference is made in the text, has been superseded by ISO 5725-1 : 1994, ISO 5725-2 : 1994, ISO 5725-3 : 1994, ISO 5725-4 : 1994 and ISO 5725-6 : 1994 which are identical with BS ISO 5725 *Accuracy (trueness and precision) of measurement methods and results*, BS ISO 5725-1 : 1994 *General principles and definitions*, BS ISO 5725-2 : 1994 *Basic methods for the determination of repeatability and reproducibility of a standard measurement method*, BS ISO 5725-3 : 1994, *Intermediate measures of the precision of a standard measurement method*, BS ISO 5725-4 : 1994 *Basic methods for the determination of the trueness of a standard measurement method*, and BS ISO 5725-6 : 1994 *Use in practice of accuracy values*.

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.

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.

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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

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.*

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.

If it is desired to express the result relative to a specified moisture and volatile matter content w_S [e.g. w_S = 9 % (m/m)], multiply the result obtained for dry matter (as above) by

$$\frac{100-w_S}{100}$$

9.2 Response factors

The following response factors shall be used.

NOTE 7 These response factors have been determined experimentally and have been fixed by consensus between the various laboratories who took part in the tests; they may need to be supplemented or revised in due course.

1	Desulfoglucoiberin	1,07
2	Desulfoprogoitrin	1,09
3	Desulfoepi-progoitrin	1,09
4	Desulfosinigrin	1,00
5	Desulfoglucoraphanin	1,07
6	Desulfogluconapoleiferin	1,00
7	Desulfoglucoalyssin	1,07
8	Desulfogluconapin	1,11
9	Desulfo-4-hydroxyglucobrassicin	0,28
10	Desulfoglucobrassicanapin	1,15
11	Desulfoglucotropaeolin	0,95
12	Desulfoglucobrassicin	0,29
13	Desulfogluconasturtiin	0,95
14	Desulfo-4-methoxyglucobrassicin	0,25
15	Desulfoneoglucobrassicin	0,20
16	Other desulfoglucosinolates	1,00

9.3 Calculation on the total glucosinolate content

The total glucosinolate content, expressed in micromoles per gram of dry matter of the product, is equal to the sum of the contents of each glucosinolate (the corresponding peak area of which is greater than 1 % of the sum total of the peak areas).

If the difference between the total glucosinolate content results using both concentrations satisfies the requirements for repeatability (see 10.1), there is no contamination of the internal standard. In this case, take as the result the arithmetic mean of the two determinations.

If the requirements for repeatability are not satisfied, repeat the procedure on two other test portions and perform a blank test (8.5) omitting the internal standard solution. The contaminant area is subtracted from the internal standard area to give the true area of the internal standard used in 9.1. Take as the result the arithmetic mean of the results of the two determinations.

Glucotropaeolin (A.2) may be used as the internal standard solution in 8.2.1.

10 Precision

Details of the interlaboratory test to determine the precision of the method are summarized in annex B.

10.1 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, should not be greater than 10 % of the arithmetic mean of the two results with a minimum value of 1 µmol/g.

10.2 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, should not be greater than 20 % of the arithmetic mean of the two results with a minimum value of 2 µmol/g.

11 Test report

The test report shall specify

- the method in accordance with which sampling was carried out,
- the method used,
- the test result(s) obtained, and
- if the repeatability has been checked, the final quoted result obtained.

It shall also mention all operating details not specified in this part of ISO 10633, or regarded as optional, together with details of any incidents which may have influenced the test result(s).

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

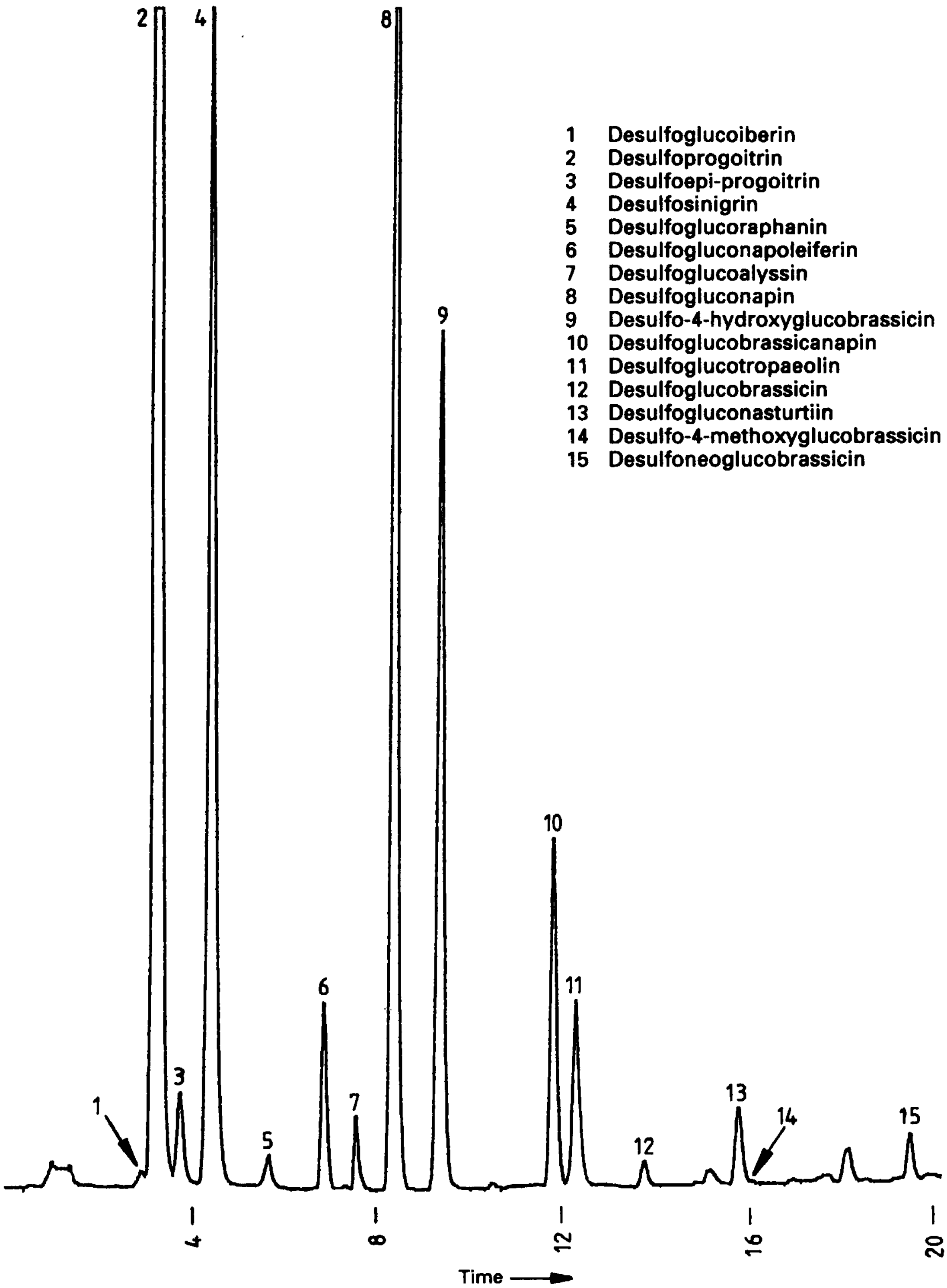

Figure 1 — Example of a typical chromatogram

Annex A
(normative)

Preparation of reagents

A.1 Sinigrin monohydrate

A.1.1 Sinigrin monohydrate, 5 mmol/l solution

Dissolve 207,7 mg of potassium allylglucosinolate monohydrate in water in a 100 ml one-mark volumetric flask and dilute to the mark with water.

The solution thus prepared may be stored in a refrigerator at approximately 4 °C for up to a week or in a freezer at – 18 °C for a period of 6 months.

A.1.2 Sinigrin monohydrate, 20 mmol/l solution

Dissolve 831,0 mg of potassium allylglucosinolate monohydrate in water in a 100 ml one-mark volumetric flask and dilute to the mark with water.

The solution thus prepared may be stored in a refrigerator at approximately 4 °C for up to a week or in a freezer at – 18 °C for a period of 6 months.

A.1.3 Purity check

Use one or more of the following three tests:

- HPLC analysis using the method specified in ISO 9167-1;
- analysis of the intact sinigrin by HPLC (ion-pair technique);
- analysis of the desulfated and silylated sinigrin by gas chromatography.

For each test, the chromatogram shall show only one major peak representing at least 98 % of the total peak area.

Confirm the purity by determining the quantity of glucose released after hydrolysis with myrosinase (thioglucoside glucohydrolase; EC 3.2.3.1). Measure the glucose by enzymatic means using a commercially available test kit. Take into account any free glucose present. This is determined in the same way but without addition of myrosinase. The molar concentration of released glucose should be at least 98 % of the molar concentration of the sinigrin solution tested.

A.2 Glucotropaeolin

NOTE 8 Glucotropaeolin is sometimes difficult to separate from other natural minor glucosinolates.

A.2.1 Glucotropaeolin, 5 mmol/l solution

Dissolve 223,8 mg of glucotropaeolin in water in a 100 ml one-mark volumetric flask and dilute to the mark with water.

The solution thus prepared may be stored in a refrigerator at approximately 4 °C for up to a week or in a freezer at – 18 °C for a period of 6 months.

A.2.2 Glucotropaeolin, 20 mmol/l solution

Dissolve 895,0 mg of glucotropaeolin in water in a 100 ml one-mark volumetric flask and dilute to the mark with water.

The solution thus prepared may be stored in a refrigerator at approximately 4 °C for up to a week or in a freezer at – 18 °C for a period of 6 months.

A.2.3 Purity check

Check the purity in accordance with the procedure described in A.1.3.

A.2.4 Response factors

Verify that the response factors of glucotropaeolin, in comparison with sinigrin, correspond to those indicated in 9.2.

A.3 Sulfatase

A.3.1 Preparation of ion-exchange columns

Cut five Pasteur pipettes (5.9) 7 cm above the neck and place a glass wool plug (5.8) in the neck. Place the pipettes vertically on a stand and add to each a sufficient quantity of ion-exchange resin (4.7.1) such that, once the water has drained off, a volume of 500 µl of resin is obtained.

Pour 1 ml of the imidazole formate solution (4.4) into each pipette and rinse twice with 1 ml portions of water.

A.3.2 Purification

Weigh, to the nearest 0,1 mg, 25 mg of *Helix pomatia* type H1 sulfatase (4.8). Dissolve it in 2,5 ml of water and transfer 500 µl of this solution to each of the columns prepared in A.3.1. Wash each column with 1,5 ml of water and discard the effluent. Then add

1,5 ml of 0,2 mol/l sodium acetate solution (4.3) and collect the eluates from the five columns in a test tube.

Concentrate the eluates by filtration using an immersion filter[7)] until approximately 100 µl of liquid remains (sulfatase with a molar mass higher than 5 000 is not removed). Add 2,5 ml of water and concentrate once more by filtration until approximately 100 µl of liquid remains. Dilute to 2,5 ml with water and store the purified sulfatase in a freezer at − 18 °C (in small amounts in order to allow defrosting only of the amount necessary for immediate use).

A.3.3 Test of sulfatase activity

A.3.3.1 Preparation of a 0,15 mmol/l sinigrin solution, buffered to pH 5,8.

Prepare the following three solutions as follows:

a) transfer 1 ml of acetic acid to a 500 ml one-mark volumetric flask and dilute to the mark with water;

b) transfer 1 ml of ethylene diamine to a 500 ml one-mark volumetric flask and dilute to the mark with water;

c) mix 73 ml of solution a) with 40 ml of solution b) and adjust the mixture to pH 5,8 using solution a) or solution b) as appropriate.

Pour 3 ml of the 5 mmol/l sinigrin solution (A.1.1) into a 100 ml one-mark volumetric flask and dilute to the mark with solution c).

A.3.3.2 Test of activity

Using a pipette, transfer 2 ml of the buffered sinigrin solution (A.3.3.1) into the reference and measuring cells of a double-beam spectrometer (5.3), adjusted to a wavelength of 228 nm with a cell temperature of 30 °C. At time $t = 0$, add 50 µl of purified sulfatase (A.3.2) to the measuring cell and immediately switch on the recorder. Stop the recorder when the absorbance no longer varies (A_e). Plot the tangent to the point $t = 0$ and measure its gradient $\Delta A/\Delta t$.

The activity unit of the sulfatase corresponds to the production of 1 µmol/min of desulfated sinigrin at 30 °C and pH 5,8.

The activity of the solution tested, expressed in units of activity per millilitre of sulfatase solution, is equal to

$$\frac{\Delta A}{\Delta t} \times \frac{V}{\Delta \varepsilon} \times \frac{1\,000}{50} \times 10^6$$

where

$\Delta A/\Delta t$ is the gradient of the tangent at point $t = 0$, in absorbance units per minute;

V is the volume, in litres, of the reacting medium (i.e. $2{,}05 \times 10^{-3}$ litres);

$\Delta \varepsilon$ (approximately 1 500 $l \cdot mol^{-1} \cdot cm^{-1}$) is the difference between the molar extinction coefficients of sinigrin and desulfosinigrin at 228 nm; i.e.

$$\Delta \varepsilon = \frac{A_e}{l \times c}$$

where

A_e is the difference between the absorbance at equilibrium of the desulfated sinigrin and the absorbance at time $t = 0$;

l is the optical cell path length, in centimetres (i.e. 1 cm);

c is the concentration of desulfated sinigrin at equilibrium, in moles per litre.

Hence:

$$c = \frac{0{,}15 \times 10^{-3} \times 0{,}95 \times 2}{2{,}05} \text{ mol/l}$$

$$= 1{,}39 \times 10^{-3} \text{ mol/l}$$

where 0,95 is the yield at equilibrium of the desulfation of the sinigrin.

Alternatively, the activity of the sulfatase may be calculated using the following simplified formula, where the activity is given by the expression

$$\frac{\Delta A \times 5{,}7}{\Delta t \times A_e}$$

A.3.4 Dilution

Using a pipette, transfer 1 ml of purified sulfatase (A.3.2) to a 10 ml one-mark volumetric flask. Dilute to the mark with water and mix.

Divide the solution into small quantities and store in a freezer at − 18 °C.

7) Millipore PLGC 11K25 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.

Annex B
(informative)

Results of an interlaboratory test

An interlaboratory test carried out in 1992 by the International Organization for Standardization gave the statistical results (evaluated in accordance with ISO 5725[8]) shown in table B.1. Eleven laboratories participated and each carried out two determinations on each sample. There were no outliers.

Table B.1 — Determination of glucosinolate content in oilseeds

Sample	A	A	A	A	A	B	B	B	B	B	C	C	C	C	C
	TOT	PRO	GNA	4OH	GBN	TOT	PRO	GNA	4OH	GBN	TOT	PRO	GNA	4OH	GBN
Mean value (µmol/g dry matter)	13,4	7,8	2,7	0,9	0,8	47,5	27,4	12,3	2,2	2,6	5,9	2,6	1,4	0,4	0,4
Standard deviation of repeatability, s_r	0,41	0,31	0,10	0,08	0,03	1,39	1,18	0,83	0,21	0,16	0,21	0,12	0,07	0,03	0,02
Coefficient of variation of repeatability (%)	3,09	3,94	3,67	9,27	3,66	2,92	4,31	6,78	9,22	6,21	3,61	4,56	5,39	8,88	6,75
Repeatability, $s_r \times 2{,}83$	1,2	0,9	0,3	0,2	0,1	3,9	3,3	2,3	0,6	0,5	0,6	0,3	0,2	0,1	0,1
Standard deviation of reproducibility, s_R	1,11	0,73	0,33	0,28	0,15	3,71	2,48	1,80	1,19	0,47	0,86	0,29	0,25	0,15	0,18
Coefficient of variation of reproducibility (%)	8,33	9,38	12,27	31,85	17,82	7,81	9,06	14,67	53,31	17,83	14,44	11,37	18,02	42,05	48,73
Reproducibility, $s_R \times 2{,}83$	3,1	2,1	0,9	0,8	0,4	10,4	6,9	5,1	3,3	1,3	2,4	0,8	0,7	0,4	0,5

TOT: Total glucosinolates
PRO: Progoitrin
GNA: Gluconapin
4OH: 4-Hydroxyglucobrassicin
GBN: Glucobrassicanapin

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

List of references

See national foreword.

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