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