

Methods for

Analysis of oilseed residues —

**Part 8: Determination of total
isothiocyanate content and
vinylthiooxazolidone content**

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Committees responsible for this British Standard

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- British Food Manufacturing Industries Research Association
- Department of Trade and Industry (Laboratory of the Government Chemist)
- FOSFA International
- Grain and Feed Trade Association
- Institute of Biology
- International Association of Seed Crushers
- Intervention Board for Agricultural Produce
- Ministry of Agriculture, Fisheries and Food
- National Farmers' Union
- National Institute of Agricultural Botany
- National Resources Institute
- Royal Society of Chemistry
- Seed Crushers' and Oil Processors' Association
- Tropical Growers' Association

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National foreword

This Part of BS 4325 has been prepared under the direction of the Agriculture and Food Standards Policy Committee. It supersedes BS 4325-8:1984, which is withdrawn. It is identical with ISO 5504:1992 *Oilseed residues — Determination of total isothiocyanate content and vinylthiooxazolidone content* 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
ISO 734:1979	BS 4325 <i>Methods for analysis of oilseed residues</i> Part 4:1982 <i>Determination of hexane extract (or light petroleum extract), called “oil content”</i> (Identical)
ISO 5500:1986	BS 6606:1987 <i>Methods for sampling oilseed residues</i> (Identical)
ISO 5502:1992	BS 4325 <i>Methods for analysis of oilseed residues</i> Part 9:1992 <i>Preparation of test samples</i> (Identical)

At present, there are no corresponding British Standards for ISO 9167-1:1992 and ISO 9167-2 (to be published) but, as these references are supplied for information only, the validity of this British Standard is not affected.

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Summary of pages

This document comprises a front cover, an inside front cover, pages i and ii, pages 1 to 8, an inside back cover and a back cover.

This standard has been updated (see copyright date) and may have had amendments incorporated. This will be indicated in the amendment table on the inside front cover.

1 Scope

This International Standard specifies a method for the determination of the total isothiocyanate (ITC) content and 5-vinylthiooxazolidone (VTO) content produced by the enzymic hydrolysis of glucosinolates in oilseed residues of the *Brassica* family.

Under the operating conditions described, the method does not allow determination of free isothiocyanates and free vinylthiooxazolidone.

It has been concluded from the results of inter-laboratory tests that, owing to the accuracy of the method, it is not suitable for use on products having contents of less than

2 $\mu\text{mol/g}$ of ITC

6 $\mu\text{mol/g}$ of VTO

Annex A gives, for information, a method of determining the isothiocyanate content by argentimetry. This method is not applicable, however, to oilseed residues of the *Brassica* family having low glucosinolates contents.

NOTE 1 For rapeseeds, two methods for the determination of glucosinolates are given in ISO 9167-1 and ISO 9167-2.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were 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 editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 734:1979, *Oilseeds residues — Determination of hexane extract (or light-petroleum extract), called "oil content"*.

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

3 Principle

After removal of the fats and oil (de-fatting), if necessary, and drying of the oilseed residue, enzymatic hydrolysis of the glucosinolates, extraction of isothiocyanates (ITC) with dichloromethane or chloroform and of vinylthiooxazolidone (VTO) with diethyl ether.

Determination of ITC by gas chromatography and of VTO by ultraviolet spectrometry.

4 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of at least equivalent purity.

4.1 Reagents for determination of ITC

4.1.1 *Dichloromethane*, or, failing this, *chloroform*.

WARNING — Take special precautions when handling chloroform. Avoid prolonged exposure to chloroform by, for example, carrying out all operations, so far as possible, in a fume cupboard.

4.1.2 *n-Hexane*, or, failing this, *light petroleum* (boiling range 40 °C to 60 °C).

4.1.3 *Buffer solution*, of pH 7, commercially available, or, for example, a solution prepared as follows.

Measure 35,3 ml of 0,1 mol/l citric acid monohydrate ($\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$) solution (21,01 g/l solution) into a 200 ml one-mark volumetric flask and dilute to the mark with a 0,2 mol/l solution of disodium hydrogen phosphate dihydrate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$) (35,61 g/l solution). Check the pH and, if necessary, adjust.

4.1.4 *Enzyme source*, myrosidase (thioglucosidase), available commercially.

NOTE 2 It is also possible to use an enzyme source prepared from white mustard seed (*Sinapis alba* L.)¹⁾, as follows.

Finely grind the white mustard seed so that at least 80 % will pass through a sieve of aperture size 280 μm .

Remove the oil and fat from the grindings using *cold* hexane or, failing this, *cold* light petroleum (4.1.2), carrying out the extraction using a method which will permit extraction until not more than 2 % of oil remains in the product and without the temperature exceeding 30 °C. For example, use a double-walled Soxhlet apparatus, or carry out extractions by grinding with hexane in a microgrinder cooled by running water. Remove traces of solvent at ambient temperature, preferably using a slight current of air.

Store the enzyme source thus obtained at 4 °C in a hermetically sealed glass bottle. Under these conditions, it can be kept for about 6 weeks, but it is preferable to use a freshly prepared enzyme source.

It is recommended that a blank test be performed to ensure that the enzyme source does not contain ITC in quantities which could significantly affect the results.

4.1.5 *Butyl isothiocyanate*, 2 mmol/l standard solution in dichloromethane or chloroform (4.1.1).

It is preferable to prepare a 20 mmol/l standard stock solution [230,4 mg of butyl isothiocyanate in 100 ml of dichloromethane or chloroform (4.1.1)] and to prepare a 10^{-1} dilution before use.

The dilution factor may be modified depending on the assumed ITC content of the product.

¹⁾ Use seed in which more than 85 % of the grains germinate in less than 72 h and which is less than 2 years old.