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SLOVENSKI STANDARD
SIST EN 14333-2:2005

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Non fatty foods - Determination of benzimidazole fungicides carbendazim, thiabendazole and benomyl (as carbendazim) - Part 2: HPLC method with gel permeation chromatography clean up

Fettarme Lebensmittel - Bestimmung der Benzimidazol-Fungizide Carbendazim, Thiabendazol und Benomyl (als Carbendazim) - Teil 2: HPLC-Verfahren mit Reinigung durch Gelpermeationschromatographie

Aliments non gras - Détermination des benzimidazoles antifongiques, le carbendazime, le thiabendazole et le bénomyl en tant que carbendazime - Partie 2: Méthode CLHP avec purification par chromatographie par perméation de gel

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ICS:

67.050	Splošne preskusne in analizne metode za živilske proizvode	General methods of tests and analysis for food products
67.080.01	Sadje, zelenjava in njuni proizvodi na splošno	Fruits, vegetables and derived products in general

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EUROPEAN STANDARD

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This European Standard was approved by CEN on 29 July 2004.

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Foreword

This document (EN 14333-2:2004) has been prepared by Technical Committee CEN/TC 275 "Food analysis - Horizontal methods", the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by April 2005, and conflicting national standards shall be withdrawn at the latest by April 2005.

EN 14333 consists of the following parts, under the general title *Non fatty foods – Determination of benzimidazole fungicides carbendazim, thiabendazole and benomyl (as carbendazim):*

— *Part 1: HPLC method with solid phase extraction clean up*;

— *Part 2: HPLC method with gel permeation chromatography clean up*;

— *Part 3: HPLC method with liquid/liquid-partition clean up*.

WARNING — The use of this standard may involve hazardous materials, operations and equipment. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

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1 Scope

This document specifies a high performance liquid chromatographic method for the determination of the benzimidazole fungicides carbendazim and thiabendazole in fruits, vegetables and processed products.

When benomyl is present, it is completely degraded to carbendazim and is also determined as carbendazim. Thiophanate-methyl is partly decomposed and therefore not quantitatively determined.

The method has been validated for carbendazim and thiabendazole in an interlaboratory test with homogenates of apples, French beans, mushrooms, lemons and fruit based infant food.

2 Principle

The sample is homogenized with ethyl acetate, sodium hydroxide solution and anhydrous sodium sulfate and the homogenate is filtered. An aliquot portion of the ethyl acetate extract is cleaned up by gel permeation chromatography (GPC) on a polystyrene gel using a cyclohexane/ethyl acetate mixture for elution. In the GPC eluate, carbendazim and thiabendazole are determined by high performance liquid chromatography (HPLC) on a normal phase column and with UV or UV and fluorescence detectors.

3 Reagents

3.1 General

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Unless otherwise specified, use reagents of recognized analytical grade, preferably for HPLC and pesticide residue analysis, and only distilled or demineralized water.

3.2 Safety aspects associated with reagents

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Vapours from some volatile solvents are toxic. Several of these solvents are readily absorbed through skin. Use an effective fume hood to remove vapours of these solvents as they are set free. Carbendazim and thiabendazole are toxic; avoid contact with skin and eyes.

3.3 Ethyl acetate

3.4 Cyclohexane

3.5 Sodium sulfate, anhydrous

3.6 Sodium hydroxide solution, mass concentration ρ (NaOH) = 26 g/100 ml

3.7 Diluted sodium hydroxide solution, ρ (NaOH) = 2,6 g/100 ml

3.8 GPC eluting mixture: Cyclohexane (3.4) / ethyl acetate (3.3) 1 + 1 (*V/V*)

3.9 Solvent mixture: dilute 5 ml ethyl acetate (3.3) with cyclohexane (3.4) to 100 ml

3.10 Mobile phase A for HPLC: Cyclohexane (3.4)

3.11 Mobile phase B for HPLC: Cyclohexane (3.4) / isopropanol / methanol 85 + 15 + 5 (*V/V/V*)

To 250 ml of this mixture, add one drop of ammonia solution (25 g/100 g). Prior to use, filter the mixture through a membrane filter (4.7).

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3.12 Mobile phase C for HPLC: Isopropanol

3.13 Carbendazim stock solution, ρ (carbendazim) = 25 mg/100 ml in methanol

3.14 Thiabendazole stock solution, ρ (thiabendazole) = 65 mg/100 ml in acetone

3.15 Standard solutions

Dilute the carbendazim stock solution (3.13) or the thiabendazole stock solution (3.14) with ethyl acetate (3.3) and cyclohexane (3.4) to obtain appropriate dilutions in cyclohexane / ethyl acetate of about 80 + 20 (*V*/*V*) to 75 + 25 (*V*/*V*). When injected into the HPLC system (4.6), the dilutions should contain less than 10 ml/100 ml of methanol and less than 2 ml/100 ml of acetone.

4 Apparatus

4.1 General

Usual laboratory equipment and in particular the following :

4.2 Food chopper

4.3 High speed blender or homogenizer

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4.4 Rotary evaporator with a water bath

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4.5 Instrument for GPC equipped with glass column with two adjustable end pieces, 500 mm long, 10 mm inner diameter, and with at least one sample loop (1 ml), column packing BioBeads®[1] S-X3 resin, pre-swelled overnight in the GPC eluting mixture (3.8) and packed as described in 5.3.1.

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NOTE Apparatus for automated GPC is commercially available.

4.6 High performance liquid chromatograph equipped with

4.6.1 Pumping system, with three solvent reservoirs, an injection valve for 15 µl, a UV detector and a fluorescence detector connected in series and a quantification unit with an integrating system;

4.6.2 HPLC analytical column, stainless steel cartridge, 150 mm long, 4,6 mm inner diameter, packed with a suitable diol-bonded silica for normal phase chromatography, particle size 3 µm.

4.7 Membrane filter, pore size 0,45 µm, suitable for organic solutions

4.8 Glass fibre filter, 90 mm diameter

4.9 Syringe filter, pore size 0,45 µm, suitable for organic solutions

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1) BioBeads ® is a trade name of a product supplied by Bio-Rad Laboratories, Richmond, CA, USA. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of the product named. Equivalent products may be used if they can be shown to lead to the same results.

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5 Procedure

5.1 Preparation of test sample

Prepare a homogenate from the laboratory sample, for example by chopping (4.2), from which a representative test portion is taken.

5.2 Extraction

5.2.1 Commodities except lemons, limes, plums and juices

From the test sample (5.1), weigh a test portion of 75 g (m) to the nearest 0,5 g into the jar of a blender (4.3). Add 200 ml (V_1) of ethyl acetate (3.3) and 3,0 ml sodium hydroxide solution (3.6) and homogenize the mixture for 30 s. Add 40 g of sodium sulfate (3.5) and continue to homogenize the mixture for 2,5 min. Filter the homogenate with gentle suction through a glass fibre filter (4.8) topped with 20 g of sodium sulfate. To the filtrate, add 10 g of sodium sulfate and allow it to stand for 3 min.

From the ethyl acetate solution, take an aliquot portion of 100 ml (V_2) and concentrate it to approximately 1 ml in a rotary evaporator (4.4) with the water bath temperature set at 37 °C. Transfer the concentrate to a 5 ml calibrated test tube, rinsing the flask with ethyl acetate, and dilute the solution to 2,5 ml with ethyl acetate. Rinse the flask again with cyclohexane (3.4), add the rinsing to the test tube and adjust with cyclohexane to a volume of 5 ml (V_3).

5.2.2 Lemons, limes, plums

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Proceed as described in 5.2.1, but add 6,0 ml sodium hydroxide solution (3.6) instead of 3,0 ml.

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5.2.3 Juices

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Check which volume (x ml) of diluted sodium hydroxide solution (3.7) is required to adjust a portion of 7,5 g of the juice to approximately pH 10. Weigh a test portion of 75 g (m) to the nearest 0,5 g into the jar of a blender (4.3). Add 200 ml (V_1) of ethyl acetate (3.3) and x ml of sodium hydroxide solution (3.6) and homogenize the mixture for 30 s. Proceed as described in 5.2.1.

5.3 Gel permeation chromatography

5.3.1 Packing gel permeation column

Allow approximately 9 g of BioBeads S-X3 resin (4.5) to swell overnight in the GPC eluting mixture (3.8). Then pour the suspension all at once into the column. As soon as the gel bed has settled (free from air bubbles) to a level of approximately 40 cm, insert the plunger, lower it down to the bed level and screw it into place. Start the flow of GPC eluting mixture at a low rate and gradually increase it to 1 ml/min. If the gel bed sinks to a still lower level, adjust the plunger accordingly (observe manufacturer's instructions).

NOTE For alternative GPC column, see Annex B.

5.3.2 Checking elution volumes

Load the sample loop with 1 ml of appropriately diluted standard solutions in cyclohexane (3.4) / ethyl acetate (3.3) 1 + 1 (*V/V*), elute as described in 5.3.3 and determine, whether carbendazim and thiabendazole are completely recovered. Collect fractions of 0,5 ml around the expected starting and end points of the elution range.

NOTE In general, the elution range is 20 ml to 27 ml for carbendazim and 24 ml to 30 ml for thiabendazole.

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5.3.3 Clean-up

Filter the solution derived from 5.2 through a syringe filter (4.9) and inject 1 ml (V_4) of the filtrate into the sample loop of the gel permeation chromatograph (4.5). Elute with the GPC eluting mixture (3.8) at a flow rate of 1 ml/min and collect the fraction during the elution ranges for carbendazim and thiabendazole, which were determined in 5.3.2.

Concentrate this fraction to approximately 1 ml in a rotary evaporator (4.4) with the water bath temperature set at 37 °C. Transfer the concentrate to a 3 ml (V_5) volumetric flask, rinsing the evaporation flask with solvent mixture (3.9), and dilute the solution to the mark with solvent mixture.

5.4 HPLC measurement

Filter the solution derived from 5.3.3 through a syringe filter (4.9) and inject 15 µl of this sample test solution into the HPLC system (4.6). For quantitation, inject also the same volume of appropriately diluted standard solutions (3.15).

Apply the following ternary gradient programme, at a flow rate of 0,65 ml/min :

— 88 % mobile phase A (3.10), 10 % mobile phase B (3.11) and 2 % mobile phase C (3.12) for 30 s, then linearly to 78 % mobile phase A, 20 % mobile phase B and 2 % mobile phase C from 30 s to 3 min and linearly to 54 % mobile phase A, 20 % mobile phase B and 26 % mobile phase C from 3 min to 35 min.

The gradient programme may be shortened if no late eluting peaks are expected.

Pass the HPLC column eluate first through a UV detector set at 285 nm and, if both detectors are used, then through the fluorescence detector set at excitation and emission wavelengths of 285 nm and 315 nm, respectively.

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NOTE 1 For UV detection, other suitable wavelengths are 240 nm for carbendazim and 300 nm for thiabendazole. For the fluorescence detection of thiabendazole, the optimum wavelengths are 295 nm for excitation and 350 nm for emission.

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NOTE 2 The retention times obtained under these conditions are approximately 8 min for carbendazim and 15 min for thiabendazole.

NOTE 3 For alternative HPLC operating conditions, see Annex C.

6 Calculation

Measure the peak heights or peak areas obtained for carbendazim and thiabendazole in the sample test solution and the standard solution. Use, if possible, several wavelengths for the UV absorption and the fluorescence measurement.

Calculate the mass fraction w of carbendazim or thiabendazole, in milligrams per kilogram of sample, using equation (1) :

$$W = \frac{A \times C_{St} \times V_1 \times V_3 \times V_5}{A_{St} \times V_2 \times V_4 \times m} \quad (1)$$

where

A is the peak height or peak area obtained from the sample test solution;

A_{St} is the peak height or peak area obtained from the standard solution;

C_{St} is the mass concentration of carbendazim or thiabendazole in the standard solution, in micrograms per millilitre;