SLOVENSKI STANDARD
SIST EN 14333-1:2005

01-januar-2005

Nemastna ģivila - Doloļ evanje benzimidazolnih fungicidov: karbendazim, tiabendazol in benomil (kot karbendazim) - 1. del: Metoda HPLC s ļ iģļ enjem z ekstrakcijo na trdni fazi

Non fatty foods - Determination of benzimidazole fungicides carbendazim, thiabendazole and benomyl (as carbendazim) - Part 1: HPLC method with solid phase extraction clean up

Fettarme Lebensmittel - Bestimmung der Benzimidazol-Fungizide Carbendazim, Thiabendazol und Benomyl (als Carbendazim) - Teil 1: HPLC-Vefahren mit Reinigung durch Festphasenextraktion

Aliments non gras - Détermination des benzimidazoles antifongiques : le carbendazime, le thiabendazole et le bénomyl en tant que carbendazime - Partie 1: Méthode CLHP avec purification par extraction en phase solide

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67.080.01	Sadje, zelenjava in njuni proizvodi na splošno	Fruits, vegetables and derived products in general

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

Management Centre: rue de Stassart, 36 B-1050 Brussels

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Foreword

This document (EN 14333-1:2004) 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of an identical text or by endorsement, at the latest by April 2005, and conflicting national standards shall be withdrawn at the latest by April 2005.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.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

1 Scope

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

When benomyl is present, it is completely degraded to carbendazim and is also determined as carbendazim. Thiophanate-methyl is not determined with the method.

The method has been validated for carbendazim and thiabendazole in an interlaboratory test with homogenates of apples and oranges.

2 Principle

The sample is homogenized with acetone, dichloromethane and light petroleum and the homogenate is centrifuged to yield two layers of the supernatant. An aliquot portion of the upper layer is evaporated to dryness and the residue is dissolved in methanol containing benzimidazole as the internal standard. The solution is cleaned up by solid phase extraction (SPE) using a cartridge packed with diol-bonded silica. In the cartridge eluate, carbendazim and thiabendazole are determined by reversed-phase high performance liquid chromatography (HPLC) with UV or UV and fluorescence detectors.

3 Reagents

3.1 General

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

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 Acetone

3.4 Dichloromethane

3.5 Light petroleum, boiling range 40 °C to 60 °C

3.6 Methanol

3.7 Sodium hydroxide solution, mass concentration ρ (NaOH) = 4,0 g/100 ml

3.8 Ortho-Phosphoric acid, at least ρ (H_3PO_4) = 85 g/100 g

3.9 Phosphoric acid solution: To a 100 ml volumetric flask, transfer 0,6 ml ortho-phosphoric acid (3.8) and dilute to the mark with water.

3.10 SPE eluting mixture: Phosphoric acid solution (3.9) / methanol (3.6) 1 + 1 (*V/V*)

3.11 Phosphate buffer solution (pH 7): In a 500 ml volumetric flask, dissolve 1,76 g of potassium dihydrogen phosphate and 3,63 g of disodium hydrogen phosphate in water and dilute to the mark with water.

3.12 Mobile phase for HPLC: Methanol (3.6) / phosphate buffer solution (3.11) 7 + 3 (*V/V*). Prior to use, filter the mixture with suction through a membrane filter (4.9).

3.13 Carbendazim stock solution, ρ (carbendazim) = 10 mg/100 ml in methanol (3.6)

3.14 Thiabendazole stock solution, ρ (thiabendazole) = 10 mg/100 ml in methanol (3.6)

3.15 Benzimidazole stock solution, ρ (benzimidazole) = 10 mg/100 ml in methanol (3.6)

3.16 Internal standard solution, ρ (benzimidazole) = 0,50 µg/ml

In a 100 ml volumetric flask, dilute 500 µl of the benzimidazole stock solution (3.15) to the mark with methanol (3.6).

3.17 Standard solution, ρ (carbendazim) = 0,25 µg/ml, ρ (thiabendazole) = 0,25 µg/ml, ρ (benzimidazole) = 0,50 µg/ml

Transfer 250 µl of carbendazim stock solution (3.13), 250 µl of thiabendazole stock solution (3.14) and 500 µl of benzimidazole stock solution (3.15) to a 100 ml volumetric flask and dilute to the mark with methanol (3.6) / phosphate buffer solution (3.11) 1 + 1 (*V/V*).

4 Apparatus

4.1 General

Usual laboratory equipment and in particular the following:

4.2 Food chopper

4.3 Homogenizer or high speed blender

4.4 Centrifuge, provided with polytetrafluoroethylene tubes of capacity 250 ml, and capable of producing a rotational speed of at least 4000 min^{-1}

4.5 Water bath, capable of being maintained at 60 °C

4.6 SPE cartridges, packed with 500 mg of the diol-bonded silica Bond Elut 2OH[1)], 40 µm irregular particles

NOTE Several other brands have given unacceptable low recoveries, which is why the brand in this application is critical.

4.7 Device for eluting SPE cartridges (4.6), with suction

NOTE Apparatus for automated SPE elution is commercially available.

4.8 High performance liquid chromatograph, equipped with

4.8.1 Pumping system, an injection valve for 100 µl, a UV detector and a fluoresence detector connected in series and a quantification unit with an integrating system.

4.8.2 HPLC analytical column, stainless steel cartridge, 150 mm long, 6,0 mm inner diameter, packed with Shodex DE-613 [1)](poly alkyl methacrylate), particle size 6 µm.

4.9 Membrane filter, pore size 0,45 µm, suitable for water and methanolic solutions

4.10 Syringe filter, pore size 0,45 µm, for water and methanolic solutions

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

From the test sample (5.1), weigh a test portion of 15 g (m) to the nearest 0,1 g into a centrifuge tube (4.4), add 30 ml of acetone (3.3) and homogenize the mixture for 30 s using the homogenizer (4.3). Add 30 ml of dichloromethane (3.4) and 30 ml of light petroleum (3.5) and continue to homogenize the mixture for 30 s. Centrifuge the tube for 5 min at a rotational speed of 4000 min^{-1}. Decant the upper organic layer into a conical flask and measure its volume.

From this layer (V_1), transfer an aliquot portion of 3 ml (V_2) to a test tube. Place the test tube in a water bath set at 60 °C (4.5) and gently evaporate the solution to near dryness. Allow the remaining solvent to evaporate in air, and dissolve the residue in 2 ml ± 0,1 ml (V_3) of internal standard solution (3.16).

5.3 Solid phase extraction

Attach a SPE cartridge (4.6) to a suitable eluting device (4.7). Aspirate the liquids through the cartridge giving a dropwise flow or a flow of at most 5 ml/min. Pass 2 ml of SPE eluting mixture (3.10) followed by 2 ml of methanol (3.6), and discard the eluates. Transfer an aliquot portion of 1 ml ± 0,05 ml of the solution derived from 5.2 to the SPE cartridge, pass 1 ml of methanol (3.6) through the cartridge and discard the eluate. Dry the cartridge with a stream of air. Next, pass 2,0 ml of SPE eluting mixture (3.10) through the cartridge and immediately start to collect the eluate in a test tube. To the eluate, add 100 µl of sodium hydroxide solution (3.7) and mix.

5.4 HPLC measurement

Filter the solution derived from 5.3 through a syringe filter (4.10) and inject 100 µl of this sample test solution into the HPLC system (4.8). For quantitation, inject also the same volume of the standard solution (3.17). Apply a column oven temperature of 40 °C and a flow rate of 0,75 ml/min of the mobile phase (3.12).

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

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.

NOTE 2 The retention times obtained under these conditions are approximately 8,5 min for the internal standard benzimidazole, 11,5 min for carbendazim and 14 min for thiabendazole.

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1) Bond Elut 2OH is a trade name of a product supplied by Varian Sample Preparation Products, Harbor City, CA, USA. Shodex DE-613 is a trade name of a product supplied by Showa Denko, Japan. These informations are given for the convenience of users of this European Standard and do not constitute an endorsement by CEN of the products named. Equivalent products may be used if they can be shown to lead to the same results.

6 Calculation

For quantitation, use, if possible, several wavelengths for the UV absorption and the fluorescence measurement.

Measure the peak heights or peak areas for carbendazim, thiabendazole and the internal standard benzimidazole obtained from the standard solution (3.17). Calculate the response factor f, using equation (1):

$$f = \frac{C' \times A'_{St}}{C'_{St} \times A'} \quad (1)$$

where

C' is the mass concentration of carbendazim or thiabendazole in the standard solution, in micrograms per millilitre;

C'_{St} is the mass concentration of benzimidazole in the standard solution, in micrograms per millilitre;

A' is the peak height or peak area obtained for carbendazim or thiabendazole;

A'_{St} is the peak height or peak area obtained for benzimidazole.

Measure the peak heights or peak areas for carbendazim, thiabendazole and the internal standard benzimidazole obtained from the sample test solution. Calculate the mass fraction w of carbendazim or thiabendazole, in milligrams per kilogram of sample, using equation (2):

$$w = \frac{A \times C_{St} \times V_1 \times V_3 \times f}{A_{St} \times V_2 \times m} \quad (2)$$

where

A is the peak height or peak area obtained for carbendazim or thiabendazole;

A_{St} is the peak height or peak area obtained for benzimidazole;

C_{St} is the mass concentration of benzimidazole in the internal standard solution, in micrograms per millilitre;

V_1 is the volume of the organic layer obtained from extraction, in millilitres;

NOTE Under the conditions described in 5.2, V_1 has been measured to be 87 ml as an average for several crops. However, each laboratory should check V_1 and use either the average volume for all crops or the specific one for each crop.

V_2 is the aliquot portion of V_1 taken for further processing (5.2), in millilitres;

V_3 is the volume of internal standard solution for dissolving the residue from V_2 (5.2), in millilitres;

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

f is the response factor f obtained from equation (1).

7 Confirmatory tests

Analyses for confirming the identity and quantity of the carbendazim and thiabendazole should be performed, particularly in those cases in which it appears that a maximum residue limit (MRL) has been exceeded.

The identity of carbendazim and thiabendazole can be confirmed by comparing the absorption spectra of sample test solution and standard solution by using a diode array detector.