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Non fatty foods - Determination of benzimidazole fungicides carbendazim, thiabendazole and benomyl (as carbendazim) - Part 3: HPLC method with liquid/liquid-partition clean up

Fettarme Lebensmittel - Bestimmung der Benzimidazol-Fungizide Carbendazim, Thiabendazol und Benomyl (als Carbendazim) - Teil 3: HPLC-Verfahren mit Reinigung durch Flüssig/Flüssig-Verteilung
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Aliments non gras - Détermination des benzimidazoles antifongiques : le carbendazime, le thiabendazole et le bénomyl en tant que carbendazime - Partie 3: Méthode CLHP avec purification par séparation liquide/liquide
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67.050	Spošne preskusne in analizne metode za živilske proizvode	General methods of tests and analysis for food products
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EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN 14333-3

October 2004

ICS 67.080.01

English version

**Non fatty foods - Determination of benzimidazole fungicides
carbendazim, thiabendazole and benomyl (as carbendazim) -
Part 3: HPLC method with liquid/liquid-partition clean up**

Aliments non gras - Détermination des benzimidazoles
antifongiques : le carbendazime, le thiabendazole et le
bénomyl en tant que carbendazime - Partie 3: Méthode
CLHP avec purification par séparation liquide/liquide

Fettarme Lebensmittel - Bestimmung der Benzimidazol-
Fungizide Carbendazim, Thiabendazol und Benomyl (als
Carbendazim) - Teil 3: HPLC-Verfahren mit Reinigung
durch Flüssig/Flüssig-Verteilung

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

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This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

CEN members are the national standards bodies of 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.



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Foreword

This document (EN 14333-3:2004) 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.

EN 14333-3:2004 (E)**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 not determined with the method.

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 partitioned with hydrochloric acid solution; the aqueous phase is made alkaline and partitioned with ethyl acetate. The organic layer is evaporated and the residue is dissolved in the HPLC mobile phase. 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 Ethyl acetate**3.4 Methanol**

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

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

3.7 Sodium sulfate, anhydrous

3.8 Hydrochloric acid solution, ρ (HCl) = 0,1 mol/l

3.9 Alkaline solution, pH 13,4

Dissolve 33 g of anhydrous sodium acetate, 200 g of sodium chloride and 40 g of sodium hydroxide in 1 l of water.

3.10 Phosphate buffer solution, pH 7,2 to 7,5

Dissolve 2 g of dipotassium hydrogen phosphate trihydrate and 0,5 g of potassium dihydrogen phosphate in 1 l of water.

3.11 Mobile phase for HPLC: Methanol (3.4) / phosphate buffer solution (3.10) 55 + 45 (V/V).

Prior to use, filter the mixture through a membrane filter (4.7).

3.12 Carbendazim stock solution, ρ (carbendazim) = 10 mg/100 ml

In a 50 ml volumetric flask, weigh 5 mg, to the nearest 0,1 mg, of carbendazim. Add 5 ml of the hydrochloric acid solution (3.8) and allow the flask to stand in an ultrasonic bath for 5 min to 10 min. Dilute the solution with 40 ml of water, allow the flask to stand again in the ultrasonic bath for 10 min and dilute to the mark with water.

3.13 Thiabendazole stock solution, ρ (thiabendazole) = 50 mg/100 ml in methanol (3.4)

3.14 Standard solutions

Dilute the carbendazim stock solution (3.12) or the thiabendazole stock solution (3.13) as appropriate with the mobile phase for HPLC (3.11).

4 Apparatus

4.1 General

Usual laboratory equipment and in particular the following:

4.2 Food chopper

4.3 High speed blender or homogenizer

4.4 Separatory funnels, capacity 100 ml

4.5 Rotary evaporator, with a water bath

4.6 High performance liquid chromatograph, equipped with

4.6.1 Pumping system, an injection valve for 50 μ l, 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, e.g. 250 mm long, 4,6 mm inner diameter, packed with ODS-120T[®], TSK-GEL[®] 1), particle size 5 μ m.

4.7 Membrane filter, pore size 0,45 μ m, suitable for water and methanolic solutions

4.8 Glass fibre filter, 90 mm diameter

4.9 Syringe filter, pore size 0,45 μ m, suitable for water and methanolic solutions

1) TSK-GEL[®] is a trade name of a product supplied by Tosoh Biosep, 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.

EN 14333-3:2004 (E)**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 sample 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 150 ml (V_1) of ethyl acetate (3.3) and 3 ml sodium hydroxide solution (3.5) and homogenize the mixture for 30 s. Add 30 g of sodium sulfate (3.7) 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.

5.2.2 Lemons, limes and plums

Proceed as described in 5.2.1, but add 6,0 ml sodium hydroxide solution (3.5) instead of 3,0 ml.

5.2.3 Juices

Check which volume (x ml) of diluted sodium hydroxide solution (3.6) 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 (3.5) and homogenize the mixture for 30 s. Proceed as described in 5.2.1.

5.3 Liquid-liquid partitioning

Transfer an aliquot portion of 50 ml (V_2) of the solution derived from 5.2 to a separatory funnel (4.4). Add 10 ml of hydrochloric solution (3.8), shake the funnel for 2 min and allow the layers to separate. Drain the lower aqueous layer to a second separatory funnel and repeat the extraction of the upper organic layer twice, using 10 ml and 5 ml of hydrochloric acid solution, respectively. Collect all aqueous layers in the second separatory funnel and discard the organic layer.

To the combined aqueous layers, add 5 ml of the alkaline solution (3.9) and 15 ml of ethyl acetate and shake the funnel for 2 min. Allow sufficient time for the layers to separate and discard the lower aqueous layer. Shake the upper organic layer with 10 ml of water and discard the aqueous layer. Concentrate the upper organic layer to approximately 2 ml in a rotary evaporator (4.5) with the water bath temperature set at 35 °C and evaporate the remaining ethyl acetate using a gentle stream of nitrogen.

To the residue, add 5 ml \pm 0,2 ml (V_3) of the mobile phase for HPLC (3.11) and mix well.

5.4 HPLC measurement

Filter the solution derived from 5.3 through a syringe filter (4.9) and inject 50 μ l of this sample test solution into the HPLC system (4.6), applying a flow rate of 1,0 ml/min of the mobile phase (3.11). For quantitation, inject also the same volume of appropriately diluted standard solutions (3.14).

Pass the HPLC column eluate first through a UV detector set at 285 nm and, if both detectors are used, 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 6 min for carbendazim and 8,5 min for thiabendazole.

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

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):

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

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;
- V_1 is the volume of ethyl acetate used for extraction (5.2), in millilitres;
- V_2 is the aliquot portion of V_1 taken for partitioning (5.3), in millilitres;
- V_3 is the volume of the final sample test solution obtained from step 5.3, in millilitres;
- m is the mass of the test portion, in grams.

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7 Confirmatory tests

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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.

The results can also be confirmed by using part 1 or part 2 of this European Standard. For thiabendazole, gas chromatographic analysis with mass spectrometric detection may also be applied.

In some cases, particularly with acidic commodities, low recoveries are obtained with thiabendazole. Therefore, recoveries should be checked regularly.

8 Precision

8.1 General

Details of an interlaboratory test of the precision of the method according to ISO 5725-2 are summarized in Annex A. The values derived from the interlaboratory test may not be applicable to analyte concentration ranges and matrices other than given in Annex A.

8.2 Repeatability

The absolute difference between two single test results found on identical test material by one operator using the same apparatus within the shortest feasible time interval will exceed the repeatability limit r in not more than 5 % of the cases.

The values are listed in Table 1: