SLOVENSKI STANDARD
SIST EN 14185-2:2006

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Nemastna živila – Določevanje ostankov N-metilkarbama – 2. del: Metoda HPLC s kolonskim čiščenjem z diatomejsko zemljo

Non fatty foods - Determination of N-methylcarbamate residues - Part 2: HPLC method with clean-up on a diatomaceous earth column

Fettarme Lebensmittel - Bestimmung von N-Methylcarbamatrückständen - Teil 2: HPLC-Verfahren mit Reinigung auf einer Kieselgur-Säule

Aliments non gras - Dosage des N-méthylcarbamates - Partie 2 : Méthode CLHP avec purification sur colonne de terre de diatomées

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Foreword

This document (EN 14185-2:2006) 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of an identical text or by endorsement, at the latest by November 2006, and conflicting national standards shall be withdrawn at the latest by November 2006.

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, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

1 Scope

This draft European Standard specifies a high performance liquid chromatographic (HPLC) method for the determination of residues of N-methylcarbamate pesticides in fruits and vegetables and is based on the method of Krause [1].

The method has been validated by collaborative study for aldicarb, carbofuran, furathiocarb, methomyl, oxamyl, propoxur and thiodicarb parent compounds and for the metabolites aldicarb sulfoxide, aldicarb sulfone (aldoxycarb) and 3-hydroxy-carbofuran in tomatoes and oranges at levels between 0,04 mg/kg and 0,25 mg/kg.

No collaborative data are available for the performance of the method in the determination of other significant compounds although it is known that the method is unsatisfactory for benfuracarb.

2 Principle

The sample is homogenized with dichloromethane, the homogenate is filtered and the filtrate evaporated. For citrus fruits only, the sample is homogenized with acetonitrile, the filtrate is washed with n-hexane and evaporated. In both cases, the residue is dissolved in an aqueous sodium chloride solution. The solution is transferred to a diatomaceous earth column and the column is eluted with dichloromethane. The eluates are evaporated and the residue is dissolved in methanol. In this solution, the N-methylcarbamates are determined by reversed-phase high-performance liquid chromatography (HPLC) with post-column hydrolysis. The methylamine formed is allowed to react with o-phthaldialdehyde and 2-mercaptoethanol and the derivatives are detected with a fluorescence detector [2].

3 Reagents

3.1 General

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

Label all standard containers with name and purity of the pesticides. For the full chemical names and structures, see ISO 1750.

3.2 Safety aspects

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 to determine the applicability of regulatory limitations prior to use.

3.3 Acetonitrile

3.4 n-Hexane

3.5 Acetonitrile, saturated with n-hexane

In a separating funnel, shake 400 ml of acetonitrile (3.3) with 40 ml of n-hexane (3.4) for 3 min and use the lower layer.

3.6 Dichloromethane[1)]

3.7 Methanol

3.8 Sodium chloride solution, saturated

3.9 Sodium hydroxide solution, c = 0,05 mol/l

NOTE Sodium hydroxide was found to be a possible source of contamination. Suitable products are e.g. Hydrolysis Reagent for Carbamate Pesticide Analysis CB 130[2)] or sodium hydroxyde solution (1 mol/l) for HPCE, Merck no. 102251[3)].

3.10 Filter aid, e.g. Celite 545® [4)]

3.11 Solid support material, Extrelut NT[2)] refill packs

3.12 o-Phthaldialdehyde

3.13 Tetraborate buffer solution, 38,1 g/l sodium tetraborate decahydrate in water

3.14 2-Mercaptoethanol

3.15 Mobile phase A

Water, purified by a LC-grade water purification system. Prior to use, filter it with gentle suction through a membrane filter (4.13).

3.16 Mobile phase B

Acetonitrile (3.3). Prior to use, filter it with gentle suction through a membrane filter (4.13).

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1) Due to environmental reasons, also ethyl acetate can be used, however, only the use of dichloromethane has been tested in the inter-laboratory study, and therefore, the applicability of ethyl acetate has to be proven in an appropriate manner.

2) Hydrolysis Reagent for Carbamate Pesticide Analysis CB 130 and Derivatization Reagent 2 are products supplied by Pickering Laboratories, Mountain View, California, 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.

3) Sodium hydroxide solution for HPCE, Extrelut NT and RP Select B are products supplied by E. Merck, Darmstadt, Germany. 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.

4) Celite 545 is a product supplied by Celite Corp. (World Minerals Inc., Santa Barbara, 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.

3.17 OPA reagent

Dissolve 1 g of o-phthaldialdehyde (3.12) in 10 ml of methanol (3.7) and add 0,4 ml of 2-mercaptoethanol (3.14). In a 1 000 ml volumetric flask, dilute the mixture and make up to the mark with tetraborate buffer solution (3.13). Adjust the pH value of the solution to pH 10. Store under a nitrogen atmosphere.

NOTE Alternatively the "Derivatization Reagent 2"[1)] may be used.

3.18 Standard materials

N-methylcarbamate pesticides such as aldicarb, bendiocarb, bufencarb, butocarboxim, carbaryl, carbofuran, dioxacarb, ethiofencarb, furathiocarb, methiocarb, methomyl, oxamyl, promecarb, propoxur, thiodicarb, thiofanox, trimethacarb, the metabolites 3-hydroxy-carbofuran and 3-keto-carbofuran and the sulfoxide and sulfone metabolites of aldicarb, butocarboxim and thiofanox.

3.19 Pesticide stock solutions, mass concentration ρ = 2,0 µg/ml.

In a 25 ml volumetric flask, dissolve 50 mg of a standard material (3.18) in acetonitrile (3.3) and make up to the mark with acetonitrile.

3.20 Pesticide standard solutions

Prepare appropriate standard solutions by diluting suitable amounts of pesticide stock solutions (3.19) with methanol, e.g. to 1 µg/ml or to 0,1 µg/ml, if necessary.

4 Apparatus

4.1 General

Glassware shall be thoroughly cleaned. Hot detergent solution may be used for cleaning, but afterwards the glassware shall be well rinsed with distilled water and acetone before drying.

Usual laboratory apparatus is used and, in particular, the following.

4.2 Food chopper

4.3 Homogenizer or high speed blender

4.4 Glass bottle, 500 ml

4.5 Rotary evaporator with a water bath, capable of being maintained at 35 °C

4.6 Buchner porcelain funnel, 9,5 cm diameter

4.7 Filtration flask, 1 l

4.8 Glass fibre filter, 9 cm diameter

4.9 Separating funnel, 1 l

4.10 Column filled with diatomaceous earth, applicable for a volume of 20 ml solution, e. g. pre-packed polypropylene columns Extrelut NT 20 [2] (see page 5), Chem Elut 20 ml[5] or Chromabond XTR (70 ml) [6]. Used Extrelut NT 20 columns may be refilled with Extrelut NT refill packs (3.11).

4.11 High performance liquid chromatograph, equipped with

4.11.1 Binary pumping system with six-port injection valve with a 20 µl sample loop, a fluorescence detector and a quantification unit with an integrating system.

4.11.2 HPLC analytical column, stainless steel cartridge, 250 mm long, 4,0 mm inner diameter (e.g. packed with RP Select B [2] (see page 5)) (particle size 5 µm).

4.11.3 Post-column system, consisting of two reaction coils in different thermostats, two low-dead volume T-pieces, and two pulse-free reagent pumps.

NOTE A complete post-column LC-system for the analysis of N-methylcarbamates is commercially available.

4.12 Ultrasonic bath

4.13 Membrane filter, pore size 0,45 µm

5 Sampling

Prepare the laboratory sample according to a generally recommended method of sampling to achieve a representative part of the product to be analyzed.

6 Preparation of the samples

Where possible, carry out the analysis of samples immediately on their arrival in the laboratory. Do not analyse a laboratory sample which is wholly or extensively spoiled.

For analysis take only the portion of the laboratory sample to which the maximum residue level applies. No further plant-parts may be removed. A record of the plant-parts which have been removed shall be kept. The sample thus prepared is the test sample.

If the sample cannot be analysed immediately, store it at 0 °C to 5 °C for no longer than 3 days before analysis.

The reduction of the test sample shall be carried out in such a way that representative portions are obtained (e.g. by division into four and selection of opposite quadrants). When the samples are in small units (e.g. small fruits, legumes, cereals), the test sample shall be thoroughly mixed before weighing out the test portion. When the samples are in larger units, take wedge-shaped sections (e.g. large fruits and vegetables) or cross sections (e.g. cucumbers) which include the outer surface from each unit.

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[5] Chem Elut 20 ml is a product supplied by Varian Inc., Palo Alto, 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.

[6] Chromabond XTR is a product supplied by Macherey-Nagel, Düren, Germany. 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.