

**Non-fatty food – Determination of  
N-methylcarbamate residues –  
Part 1: HPLC-method with SPE clean-up**

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English version

**Non-fatty food - Determination of N-methylcarbamate residues -  
Part 1: HPLC-method with SPE clean-up**

Aliments non gras - Dosages des résidus de N-  
méthylcarbamates - Partie 1: Méthode par CLHP avec  
purification SPE

Fettarme Lebensmittel - Bestimmung von N-  
Methylcarbamat-Rückständen - Teil 1: HPLC-Verfahren mit  
Reinigung durch Festphasenextraktion

This European Standard was approved by CEN on 20 February 2003.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Management Centre or to any CEN member.

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 Management Centre has the same status as the official versions.

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EUROPEAN COMMITTEE FOR STANDARDIZATION  
COMITÉ EUROPÉEN DE NORMALISATION  
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## Foreword

This document (EN 14185-1:2003) 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of an identical text or by endorsement, at the latest by October 2003, and conflicting national standards shall be withdrawn at the latest by October 2003.

Annexes A and B are informative.

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

## 1 Scope

This European Standard specifies a high performance liquid chromatographic (HPLC) method for the determination of N-methylcarbamate pesticides in cereals, fruits and vegetables.

The method has been validated by collaborative study for carbaryl, carbofuran, methiocarb, methomyl, oxamyl and propoxur parent compounds and for methiocarb sulfoxide in green peppers and apples at levels between 0,08 mg/kg and 0,9 mg/kg.

No collaborative data are available for the performance of the method in the determination of other significant metabolites although it is known that the method will not work for oxamyl and methomyl oximes.

## 2 Normative references

This European Standard incorporates, by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text, and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies (including amendments).

EN ISO 3696, *Water for analytical laboratory use — Specification and test methods (ISO 3696:1987)*.

## 3 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. Optionally, this extract may be cleaned up by solid phase extraction (SPE) using a cartridge packed with aminopropyl-bonded silica. In the extract 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. For further information on this method, see [1] to [4].

**EN 14185-1:2003 (E)****4 Reagents****4.1 General**

Unless otherwise specified, use reagents of recognized analytical grade, preferably for HPLC and pesticide residue analysis, and distilled water for cleaning of glassware or water of at least grade 1 as defined in EN ISO 3696.

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

**4.2 Safety aspects associated with reagents**

**WARNING — Many pesticides are toxic by various routes of exposure, especially in concentrated form. When working with these pesticides consult safety data sheets of the manufacturer for information.**

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.

**4.3 Acetone****4.4 Acetic acid****4.5 Dichloromethane****4.6 Light petroleum, boiling range 40 °C to 60 °C****4.7 Methanol****4.8 Acetonitrile****4.9 Solvent mixture**

Acetonitrile (4.8) / acetic acid (4.4) 99,9 + 0,1 (V/V)

**4.10 Water, purified by a LC-grade water purification system****4.11 Sodium acetate****4.12 o-Phthaldialdehyde****4.13 Sodium tetraborate, anhydrous****4.14 2-Mercaptoethanol****4.15 Trimethacarb****4.16 Mobile phase A**

Acetonitrile (4.8) / water (4.10) 20 + 80 (V/V), containing 2,5 mmol/l sodium acetate (4.11). Prior to use, filter the mobile phase A with gentle suction through a membrane filter (5.10).

**4.17 Mobile phase B**

Methanol (4.7) / water (4.10) 20 + 80 (V/V), containing 2,5 mmol/l sodium acetate (4.11). Prior to use, filter the mobile phase B with gentle suction through a membrane filter (5.10).

#### 4.18 Mobile phase C

Acetonitrile (4.8) / water (4.10) 60 + 40 (V/V), containing 2,5 mmol/l sodium acetate (4.11). Prior to use, filter the mobile phase C with gentle suction through a membrane filter (5.10).

#### 4.19 OPA reagent

In a 1 000 ml volumetric flask, dissolve 3,8 g of sodium tetraborate (4.13) in approximately 900 ml of water (4.10). Add a solution of 250 mg of o-phthalaldehyde (4.12) in 10 ml of acetonitrile (4.8). Next, add 0,1 ml of 2-mercaptoethanol (4.14) and dilute to 1 000 ml with water.

The solution is stable for approximately one week.

#### 4.20 SPE eluting mixture (optional)

Dichloromethane (4.5) / methanol (4.7) 99 + 1 (V/V).

#### 4.21 Internal standard solution, $\rho = 0,05 \mu\text{g/ml}$ .

Dissolve 10 mg of trimethacarb (4.15) in 10 ml of acetonitrile (4.8) and dilute 100  $\mu\text{l}$  of this solution to 100 ml in a volumetric flask with solvent mixture (4.9) to give dilution A (1  $\mu\text{g/ml}$ ). In a second volumetric flask, dilute 5 ml of dilution A to 100 ml with acetonitrile (4.8) / water (4.10) 20 + 80 (V/V).

#### 4.22 Standard materials

N-methylcarbamate pesticides such as aldicarb, bendiocarb, bufencarb, butocarboxim, carbanolate, carbaryl, carbofuran, cloethocarb, dioxacarb, dithiocarb, ethidimuron, ethiofencarb, fenobucarb, isoprocarb, methiocarb, methomyl, oxamyl, promecarb, propoxur, thiofanox, the metabolite 3-hydroxy-carbofuran and the sulfoxide and sulfone metabolites of aldicarb, butocarboxim, ethiofencarb, methiocarb and thiofanox.

#### 4.23 Pesticide stock solutions, $\rho = 0,05 \mu\text{g/ml}$ .

Dissolve 10 mg of a standard material (4.22) in 10 ml of acetonitrile (4.8) and dilute 100  $\mu\text{l}$  of this solution to 100 ml in a volumetric flask with solvent mixture (4.9) to give dilution A (1  $\mu\text{g/ml}$ ). In a second volumetric flask, dilute 5 ml of dilution A to 100 ml with acetonitrile (4.8) / water (4.10) 20 + 80 (V/V).

#### 4.24 Pesticide standard solutions

Prepare appropriate standard solutions by diluting suitable amounts of pesticide stock solutions (4.23) with internal standard solution (4.21) / water (4.10) 20 + 80 (V/V).

#### 4.25 Keeper solution

Mix 2 g of ethylene glycol with 8 ml of acetone (4.3).

### 5 Apparatus

#### 5.1 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.

#### 5.2 Chopper

#### 5.3 Homogenizer or high speed blender

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**5.4 Centrifuge**, provided with polytetrafluoroethylene tubes of capacity 200 ml, and capable of producing a rotational speed of at least 4 000 min<sup>-1</sup>.

**5.5 Water bath**, capable of being maintained at 50 °C or 60 °C

**5.6 SPE cartridges**, packed with 100 mg aminopropyl-bonded silica, particle size 40 µm (e.g. Bond-Elut<sup>®1</sup>) (optional)

**5.7 Device for eluting SPE cartridges** (5.6) with suction (optional)

NOTE Apparatus for automated SPE elution is commercially available [4].

**5.8 High performance liquid chromatograph**, equipped with

**5.8.1** Ternary pumping system with six-port injection valve with a 100 µl sample loop, a post-column system (consisting of a column reactor, a low-dead volume T-piece and a pulse-free reagent pump), a fluorescence detector and a quantification unit with an integrating system.

**5.8.2** HPLC guard column, stainless steel cartridge, 10 mm long, 4,0 mm inner diameter (e.g. LiChroCART<sup>®1</sup>), packed with Superspher<sup>®1</sup>) 60 RP-8 (particle size 4 µm).

**5.8.3** HPLC analytical column, stainless steel cartridge, 250 mm long, 4,0 mm inner diameter (e.g. LiChroCART<sup>®1</sup>), packed with Superspher<sup>®1</sup>) 60 RP-8 (particle size 4 µm).

**5.8.4** Post-column hydrolysis column, stainless steel, 50 mm long, 4,0 mm inner diameter, packed with Aminex<sup>®1</sup>) A 27 (15 µm).

**5.9 Ultrasonic bath**

**5.10 Membrane filters**, pore size 0,45 µm

## 6 Sampling

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

## 7 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 limit 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.

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1) Bond-Elut<sup>®</sup> is a trade name of a product supplied by Analytichem International, Harbor City, CA, USA. LiChroCART<sup>®</sup> and Superspher<sup>®</sup> are trade names of products supplied by Merck, Darmstadt, Germany. Aminex<sup>®</sup> is a trade name of a product supplied by Bio-Rad, Hercules, CA, USA. 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.