
**Hrana, ki ne vsebuje maščobe - Določevanje ostankov N-metil karbamata - 1. del:
Metoda HPLC s čiščenjem SPE**

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

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

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

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General methods of tests and
analysis for food products

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EUROPEAN STANDARD
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**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.

CEN members are the national standards bodies of Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Luxembourg, Malta, Netherlands, Norway, Portugal, Slovakia, Spain, Sweden, Switzerland and United Kingdom.

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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.

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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-phthalaldehyde and 2-mercaptoethanol and the derivatives are detected with a fluorescence detector. For further information on this method, see [1] to [4].

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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

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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 μl 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, clothocarb, 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.

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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 μl 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⁻¹.

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^{®1)}) (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^{®1)}, packed with Superspher^{®1)} 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^{®1)}), packed with Superspher^{®1)} 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^{®1)} 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.

1) Bond-Elut[®] is a trade name of a product supplied by Analytichem International, Harbor City, CA, USA. LiChroCART[®] and Superspher[®] are trade names of products supplied by Merck, Darmstadt, Germany. Aminex[®] 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.

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.

From each test sample, remove those parts which would interfere with the analytical procedure. In the case of stone fruits, the stones may be removed. Care shall be taken that as little as possible of the remainder such as juice or flesh is lost. The basis for the calculation of the residue mass fraction is the mass of the original test sample (with stones).

If samples have to be stored for more than 3 days, they shall be deep-frozen at $-18\text{ }^{\circ}\text{C}$ or lower. To ensure that even after thawing representative samples can be taken, prepare portions of the product which are each sufficient for one analysis.

Chop the test sample and weigh out test portions of masses of 15 g to an accuracy of $\pm 1\%$.

8 Procedure

8.1 General

Prepare reagent and matrix blanks and carry out spiked recovery tests at levels appropriate to the maximum residue limits.

NOTE Workers should thoroughly familiarize themselves with the method before starting the analyses.

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8.2 Extraction

In a centrifuge tube (5.4), homogenize the 15 g (*m*) test portion with 30 ml of acetone (4.3) for 30 s. Add 30 ml of dichloromethane (4.5) and 30 ml of light petroleum (4.6) and continue to homogenize for further 30 s. Centrifuge the tube 5 min at a rotational speed of 4 000 min^{-1} . Decant the upper (organic) layer into an Erlenmeyer flask.

From this layer, transfer an aliquot portion of 2 ml to a test tube and add 50 μl of keeper solution (4.25). Gently evaporate the solvent to near dryness.

8.3 Solid-phase extraction (optional)

Through a SPE cartridge (5.6) attached to a suitable eluting device (5.7), pass 1 ml of dichloromethane (4.5) with suction and discard the eluate. Dissolve the residue derived from 8.2 in 1 ml of dichloromethane. Transfer the solution onto the SPE cartridge, using 0,5 ml of dichloromethane for rinsing, and immediately start to collect the eluate in a test tube. Continue the elution with 1 ml of SPE eluting mixture (4.20), collect this eluate in the same tube and gently evaporate the solvent to near dryness.

NOTE This clean-up step removes a considerable amount of co-extractives from the matrix and avoids interfering deposits on the HPLC guard column.

8.4 HPLC measurement

Dissolve the residue derived from 8.2 or 8.3 in 1 ml of internal standard solution (4.21) with the help of an ultrasonic bath (5.9). Filter the solution through a membrane filter (5.10) and inject 100 μl into the HPLC system (5.8) applying a guard column as e.g. in 5.8.2 and an analytical column as e.g. in 5.8.3.

Apply for example the following ternary gradient program, at a flow rate of 0,75 ml/min:

75 % mobile phase A and 25 % of mobile phase B for 5 min, then linearly from 0 % of mobile phase C to 100 % of mobile phase C during 20 min, finally 100 % of mobile phase C for 5 min.