Non-fatty food — Determination of N-methylcarbamate residues —

Part 1: HPLC-method with SPE clean-up

The European Standard EN 14185-1:2003 has the status of a British Standard

ICS 67.060; 67.080.01

BSi
British Standards

National foreword

This British Standard is the official English language version of EN 14185-1:2003.

The UK participation in its preparation was entrusted to Technical Committee AW/-/3, Horizontal analysis, which has the responsibility to:

— aid enquirers to understand the text;

— present to the responsible international/European committee any enquiries on the interpretation, or proposals for change, and keep the UK interests informed;

— monitor related international and European developments and promulgate them in the UK.

A list of organizations represented on this committee can be obtained on request to its secretary.

Cross-references

The British Standards which implement international or European publications referred to in this document may be found in the BSI Catalogue under the section entitled “International Standards Correspondence Index”, or by using the “Search” facility of the BSI Electronic Catalogue or of British Standards Online.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

Compliance with a British Standard does not of itself confer immunity from legal obligations.

This British Standard was published under the authority of the Standards Policy and Strategy Committee on 6 May 2003

Summary of pages

This document comprises a front cover, an inside front cover, the EN title page, pages 2 to 16, an inside back cover and a back cover.

The BSI copyright date displayed in this document indicates when the document was last issued.

Amendments issued since publication

Amd. No.	Date	Comments

ISBN 0 580 41780 8

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 14185-1

April 2003

ICS 67.060; 67.080.01

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.

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.

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

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

Ref. No. EN 14185-1:2003 E

Contents

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].

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-phthaldialdehyde (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, ρ = 0,05 µg/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 µg/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, ρ = 0,05 µg/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 µg/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

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 °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 ±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.

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.

Pass the eluate from the HPLC analytical column through a post-column reactor as in 5.8.1, containing a post-column hydrolysis column as in 5.8.4. Hold the reactor at a temperature of 120 °C.

To the eluate from the hydrolysis column, add OPA reagent (4.19) at a flow-rate of 0,1 ml/min via a low-dead volume T-piece.

Pass the mixture through a fluorescence detector. The excitation and emission wavelengths are set at 340 nm and 455 nm, respectively.

NOTE Different alternative HPLC operating conditions are possible, if they can be shown to lead to the same results. For alternative HPLC operating conditions, see annex B.

9 Calculation of the results

For identifying any N-methylcarbamate present, compare the retention times of the peaks obtained for the sample test solution with those obtained by mixing and diluting pesticide stock solutions (4.23) as appropriate.

For the quantification of an identified N-methylcarbamate, use standard solutions (4.24) of this compound which contain suitable concentrations, e.g. between 0,005 µg/ml and 0,05 µg/ml. Follow the procedure described in 8.4. In the chromatogram, measure the peak heights (or peak areas) obtained for the compound and the internal standard trimethacarb and calculate the quotient of both values. For a calibration graph, plot the amounts of the compound contained in 1 ml each of the standard solution on the abscissa and the corresponding quotients on the ordinate.

For the peak obtained for the identified compound from the sample test solution, calculate the quotient as described above and read off the mass present in the sample test solution from the calibration graph. Calculate the mass fraction w, in milligrams per kilogram sample material, using equation (1):

$$w = \frac{x}{m} \qquad (1)$$

where

x is the mass of the compound read from the calibration graph, in micrograms;

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

10 Confirmatory tests

Analyses for confirmation of the identity and quantity of observed pesticide residues should be performed, particularly in those cases in which it appears that the maximum residue limit (MRL) has been exceeded.

Coupled gas chromatography-mass spectrometry (GC-MS) is a specific method to confirm the identity and the quantity of N-methylcarbamates. For those compounds which are not amenable to gas chromatography, techniques using liquid chromatography-mass spectrometry (LC-MS) or liquid chromatography-photodiode array detection (LC-DAD) are appropriate.

11 Precision, Repeatability and Reproducibility

Details for the interlaboratory test on the precision of the method are summarized in annex A. The values derived from the interlaboratory test according to ISO 5725 may not be applicable to analyte concentration ranges and matrices other than as given in Table 1 and annex A.

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 absolute differences between two single test results on identical test material reported by two laboratories will exceed the reproducibility limit R in not more than 5 % of the cases.

Table 1 — Precision data

Compound		Sample				
		green pepper			apple	
Carbaryl	x mg/kg	0,098	0,780	0,980	0,084	0,890
	r mg/kg	0,013	0,096	0,222	0,005	0,270
	R mg/kg	0,045	0,340	0,340	0,070	0,390
Carbofuran	x mg/kg	0,094	0,770	0,930	0,084	0,890
	r mg/kg	0,022	0,093	0,089	0,007	0,280
	R mg/kg	0,036	0,510	0,370	0,047	0,320
Methiocarb	x mg/kg	0,083	0,680	0,880	0,080	0,730
	r mg/kg	0,015	0,112	0,108	0,010	0,063
	R mg/kg	0,052	0,340	0,450	0,063	0,720
Methiocarb sulfoxide	x mg/kg	0,082	0,610	0,830	0,083	0,830
	r mg/kg	0,023	0,148	0,166	0,014	0,142
	R mg/kg	0,058	0,530	0,740	0,049	0,220
Methomyl	x mg/kg	0,094	0,710	0,910	0,088	0,860
	r mg/kg	0,015	0,085	0,076	0,007	0,250
	R mg/kg	0,053	0,290	0,420	0,015	0,250
Oxamyl	x mg/kg	0,090	0,680	0,840	0,080	0,820
	r mg/kg	0,016	0,133	0,175	0,007	0,280
	R mg/kg	0,062	0,410	0,380	0,022	0,280
Propoxur	x mg/kg	0,100	0,740	0,890	0,092	0,880
	r mg/kg	0,027	0,107	0,240	0,006	0,270
	R mg/kg	0,064	0,500	0,570	0,024	0,360

12 Test report

The test report shall contain at least the following information:

all information necessary for the identification of the sample;

a reference to this European Standard;

the results and the units in which they have been expressed;

date of sampling and type of sampling procedure, if known;

date of receipt of sample in the laboratory;

date of test;

any particular points observed in the course of the test;

any operations not specified in the method or regarded as optional which might have affected the results.

Annex A
(informative)

Precision Data

In accordance with ISO 5725, the following parameters have been defined in an interlaboratory study. The tests were conducted by 8 European laboratories under the direction of the Inspectorate for Health Protection, Alkmaar, Amsterdam and Groningen, The Netherlands.

Table A.1 — Precision data for Carbaryl

Sample	green pepper	green pepper	green pepper	apple	apple
Year of interlaboratory test	1998	1998	1998	1998	1998
Number of samples	16	16	16	16	16
Number of laboratories	8	8	8	8	8
Number of laboratories retained after eliminating outliers	8	8	8	7	8
Number of outliers	0	0	0	1	0
Number of accepted results	16	16	16	14	16
Mean value ($\bar{x}$) mg/kg	0,098	0,780	0,980	0,084	0,890
Repeatability standard deviation (s_r) mg/kg	0,005	0,034	0,079	0,002	0,100
Repeatability relative standard deviation (RSD_r) %	4,7	4,4	8,0	2,2	11,0
Repeatability limit (r) mg/kg	0,013	0,096	0,222	0,005	0,270
Reproducibility standard deviation (s_R) mg/kg	0,016	0,120	0,120	0,025	0,140
Reproducibility relative standard deviation (RSD_R) %	16	16	12	30	15
Reproducibility limit R mg/kg	0,045	0,340	0,340	0,070	0,390
Horrat value (Ho_R)	0,70	0,96	0,75	1,29	0,92

Table A.2 — Precision data for Carbofuran

Sample	green pepper	green pepper	green pepper	apple	apple
Year of interlaboratory test	1998	1998	1998	1998	1998
Number of samples	16	16	16	16	16
Number of laboratories	8	8	8	8	8
Number of laboratories retained after eliminating outliers	7	8	8	7	8
Number of outliers	1	0	0	1	0
Number of accepted results	14	16	16	14	16
Mean value ($\bar{x}$) mg/kg	0,094	0,770	0,930	0,084	0,890
Repeatability standard deviation (s_r) mg/kg	0,008	0,033	0,032	0,003	0,100
Repeatability relative standard deviation RSD_r) [%	8,0	4,3	3,4	3,0	11,0
Repeatability limit (r) mg/kg	0,022	0,093	0,089	0,007	0,280
Reproducibility standard deviation (s_R) mg/kg	0,013	0,18	0,13	0,017	0,11
Reproducibility relative standard deviation (RSD_R) %	14	24	14	20	13
Reproducibility limit R mg/kg	0,036	0,510	0,370	0,047	0,320
Horrat value (Ho_R)	0,61	1,45	0,86	0,86	0,80

Table A.3 — Precision data for Methiocarb

Sample	green pepper	green pepper	green pepper	apple	apple
Year of interlaboratory test	1998	1998	1998	1998	1998
Number of samples	16	16	16	16	16
Number of laboratories	8	8	8	8	8
Number of laboratories retained after eliminating outliers	7	7	7	6	6
Number of outliers	1	1	1	2	2
Number of accepted results	14	14	14	12	12
Mean value ($\bar{x}$) mg/kg	0,083	0,680	0,880	0,080	0,730
Repeatability standard deviation (s_r) mg/kg	0,005	0,040	0,039	0,004	0,023
Repeatability relative standard deviation (RSD_r) %	6,3	5,9	4,4	4,4	3,1
Repeatability limit (r) mg/kg	0,015	0,112	0,108	0,010	0,063
Reproducibility standard deviation (s_R) mg/kg	0,019	0,120	0,160	0,023	0,260
Reproducibility relative standard deviation (RSD_R) %	22	18	18	28	35
Reproducibility limit R mg/kg	0,052	0,340	0,450	0,063	0,720
Horrat value (Ho_R)	0,94	1,06	1,10	1,20	2,08

Table A.4 — Precision data for Methiocarb sulfoxide

Sample	green pepper	green pepper	green pepper	apple	apple
Year of interlaboratory test	1998	1998	1998	1998	1998
Number of samples	16	16	16	16	16
Number of laboratories	8	8	8	8	8
Number of laboratories retained after eliminating outliers	8	7	8	8	7
Number of outliers	0	1	0	0	1
Number of accepted results	16	14	16	16	14
Mean value ($\bar{x}$) mg/kg	0,082	0,610	0,830	0,083	0,830
Repeatability standard deviation (s_r) mg/kg	0,008	0,053	0,059	0,005	0,051
Repeatability relative standard deviation (RSD_r) %	10,0	9,0	7,1	6,1	6,1
Repeatability limit (r) mg/kg)	0,023	0,148	0,166	0,014	0,142
Reproducibility standard deviation (s_R) mg/kg	0,021	0,19	0,27	0,018	0,08
Reproducibility relative standard deviation (RSD_R) %	26	31	32	21	10
Reproducibility limit R mg/kg	0,058	0,530	0,740	0,049	0,220
Horrat value (Ho_R)	1,12	1,80	1,94	0,90	0,61

Table A.5 — Precision data for Methomyl

Sample	green pepper	green pepper	green pepper	apple	apple
Year of interlaboratory test	1998	1998	1998	1998	1998
Number of samples	14	14	14	14	14
Number of laboratories	7	7	7	7	7
Number of laboratories retained after eliminating outliers	7	7	6	6	7
Number of outliers	0	0	1	1	0
Number of accepted results	14	14	12	12	14
Mean value ($\bar{x}$) mg/kg	0,094	0,710	0,910	0,088	0,860
Repeatability standard deviation (s_r) mg/kg	0,005	0,030	0,027	0,003	0,090
Repeatability relative standard deviation (RSD_r) %	5,6	4,3	3,0	3,0	11,0
Repeatability limit (r) mg/kg	0,015	0,085	0,076	0,007	0,250
Reproducibility standard deviation (s_R) mg/kg	0,019	0,10	0,15	0,006	0,090
Reproducibility relative standard deviation (RSD_R) %	20	15	17	6,2	11
Reproducibility limit R mg/kg	0,053	0,290	0,420	0,0153	0,250
Horrat value (Ho_R)	0,88	0,89	1,05	0,27	0,67

Table A.6 — Precision data for Oxamyl

Sample	green pepper	green pepper	green pepper	apple	apple
Year of interlaboratory test	1998	1998	1998	1998	1998
Number of samples	14	14	14	14	14
Number of laboratories	7	7	7	7	7
Number of laboratories retained after eliminating outliers	7	7	7	6	7
Number of outliers	0	0	0	1	0
Number of accepted results	14	14	14	12	14
Mean value ($\bar{x}$) mg/kg	0,090	0,680	0,840	0,080	0,820
Repeatability standard deviation (s_r) mg/kg	0,006	0,048	0,062	0,003	0,100
Repeatability relative standard deviation (RSD_r) %	6,5	7,0	7,4	3,1	12,0
Repeatability limit (r) mg/kg	0,016	0,133	0,175	0,007	0,280
Reproducibility standard deviation (s_R) mg/kg	0,022	0,150	0,140	0,008	0,100
Reproducibility relative standard deviation (RSD_R) %	25	22	16	10	12
Reproducibility limit R mg/kg	0,062	0,410	0,380	0,022	0,280
Horrat value (Ho_R)	1,09	1,29	0,98	0,43	0,73

Table A.7 — Precision data for Propoxur

Sample	green pepper	green pepper	green pepper	apple	apple
Year of interlaboratory test	1998	1998	1998	1998	1998
Number of samples	16	16	16	16	16
Number of laboratories	8	8	8	8	8
Number of laboratories retained after eliminating outliers	8	8	8	6	8
Number of outliers	0	0	0	2	0
Number of accepted results	16	16	16	12	16
Mean value ($\bar{x}$) mg/kg	0,100	0,740	0,890	0,092	0,880
Repeatability standard deviation (s_r) mg/kg	0,010	0,038	0,080	0,002	0,090
Repeatability relative standard deviation (RSD_r) %	10,0	5,1	10,0	2,4	11,0
Repeatability limit (r) mg/kg	0,027	0,107	0,240	0,006	0,270
Reproducibility standard deviation (s_R) mg/kg	0,023	0,180	0,200	0,009	0,130
Reproducibility relative standard deviation (RSD_R) %	23	24	23	9	14
Reproducibility limit R mg/kg	0,064	0,500	0,570	0,0239	0,360
Horrat value (Ho_R)	1,02	1,44	1,41	0,39	0,86

Annex B
(informative)

Alternative HPLC operating conditions

The separation of N-methylcarbamates can also be performed with the following HPLC operating conditions:

Analytical column: 150 mm long, 4,6 mm inner diameter, packed with Inertsil ODS-80A (5 µm)[2)].

Guard column: 10 mm long, 4,0 mm inner diameter, packed with Inertsil ODS-2 (5 µm)[2)].

Mobile phase D: acetonitrile (4.8).

Mobile phase E: acetonitrile/sodium acetate trihydrate solution [$\rho(CH_3COONa \cdot 3H_2O)$ = 161 mg/l] 10/90 (*v*/*v*).

Apply the following binary gradient programme, at a flow-rate of 0,85 ml/min:

Linearly from 0 % of mobile phase D (i.e. 100 % mobile phase E) to 56 % of mobile phase D during 20 min, then linearly to 78 % of mobile phase D in 1 min, and finally 78 % of mobile phase D for 5 min.

As an alternative, a sodium hydroxide solution can be used for the hydrolysis of the N-methylcarbamates, see [2] and [5]. To the eluate from the HPLC analytical column, add sodium hydroxide solution (0,05 mol/l) with a flow rate of 0,5 ml/min in a 0,5 ml reaction coil in the column reactor held at a temperature of 100 °C. To the effluent of the coil, add OPA reagent (4.19) with a flow rate of 0,1 ml/min via a low-dead volume T-piece.

NOTE A complete post-column LC-system for the analysis of N-methylcarbamate pesticides using this approach is commercially available.

---

2) Inertsil is a trade name of a product supplied by GL-Science, Tokyo, Japan. 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.

Bibliography

ISO 1750, Pesticides and other agrochemicals — Common names.

ISO 5725, Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests.

[1] Analytical Methods for Pesticide Residues in Foodstuffs, 6th Edition 1996. Inspectorate for Health Protection, Ministry of Public Health, Welfare and Sport, The Hague/Ryswyk, The Netherlands.

[2] de Kok, A., Hiemstra, M., and Vreeker, C. P.: Improved clean-up method for the multi-residue analysis of N-methylcarbamates in grains, fruits and vegetables by means of HPLC with postcolumn reaction and fluorescence detection. Chromatographia **24**, 469–476 (1987).

[3] de Kok, A., Hiemstra, M., and Vreeker, C. P.: Optimisation of the postcolumn hydrolysis reaction on solid phases for the routine high-performance liquid chromatographic determination of N-methylcarbamate pesticides in food products. J. Chromatogr. **507**, 459–472 (1990).

[4] de Kok, A., and Hiemstra, M.: Optimisation, automation and validation of the solid-phase extraction clean-up and on-line chromatographic determination of N-methylcarbamates in fruits and vegetables. J. Assoc. Off. Anal. Chem. **75**, 1063–1072 (1992).

[5] Krause, R. T.: Liquid chromatographic determination of N-methylcarbamate insecticides and metabolites in crops. II. Analytical characteristics and residue findings. J. Assoc. Off. Anal. Chem. **68**, 734–741 (1985).

blank

BS EN
14185-1:2003

BSI — British Standards Institution

BSI is the independent national body responsible for preparing British Standards. It presents the UK view on standards in Europe and at the international level. It is incorporated by Royal Charter.

Revisions

British Standards are updated by amendment or revision. Users of British Standards should make sure that they possess the latest amendments or editions.

It is the constant aim of BSI to improve the quality of our products and services. We would be grateful if anyone finding an inaccuracy or ambiguity while using this British Standard would inform the Secretary of the technical committee responsible, the identity of which can be found on the inside front cover. Tel: +44 (0)20 8996 9000. Fax: +44 (0)20 8996 7400.

BSI offers members an individual updating service called PLUS which ensures that subscribers automatically receive the latest editions of standards.

Buying standards

Orders for all BSI, international and foreign standards publications should be addressed to Customer Services. Tel: +44 (0)20 8996 9001.
Fax: +44 (0)20 8996 7001. Email: orders@bsi-global.com. Standards are also available from the BSI website at ~~http://www.bsi-global.com~~.

In response to orders for international standards, it is BSI policy to supply the BSI implementation of those that have been published as British Standards, unless otherwise requested.

Information on standards

BSI provides a wide range of information on national, European and international standards through its Library and its Technical Help to Exporters Service. Various BSI electronic information services are also available which give details on all its products and services. Contact the Information Centre. Tel: +44 (0)20 8996 7111. Fax: +44 (0)20 8996 7048. Email: info@bsi-global.com.

Subscribing members of BSI are kept up to date with standards developments and receive substantial discounts on the purchase price of standards. For details of these and other benefits contact Membership Administration. Tel: +44 (0)20 8996 7002. Fax: +44 (0)20 8996 7001.
Email: membership@bsi-global.com.

Information regarding online access to British Standards via British Standards Online can be found at ~~http://www.bsi-global.com/bsonline~~.

Further information about BSI is available on the BSI website at ~~http://www.bsi-global.com~~.

BSI
389 Chiswick High Road
London
W4 4AL