SLOVENSKI STANDARD
SIST EN 12393-2:1999

01-maj-1999

Živila brez maščob - Multirezidualne metode za določevanje ostankov pesticidov s plinsko kromatografijo - 2. del: Metode za ekstrakcijo in čiščenje

Non-fatty foods - Multiresidue methods for the gas chromatographic determination of pesticide residues - Part 2: Methods for extraction and clean-up

Fettarme Lebensmittel - Multiverfahren zur gaschromatographischen Bestimmung von Pestizidrückständen - Teil 2: Verfahren zur Extraktion und Reinigung

Aliments non gras - Méthodes multirésidus de détermination par chromatographie en phase gazeuse de résidus de pesticides - Partie 2: Méthodes d'extraction et de purification

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67.050	Splošne preskusne in analizne metode za živilske proizvode	General methods of tests and analysis for food products

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English version

Non-fatty foods - Multiresidue methods for the gas chromatographic determination of pesticide residues - Part 2: Methods for extraction and clean-up

Aliments non gras - Méthodes multirésidus de détermination par chromatographie en phase gazeuse de résidus de pesticides - Partie 2: Méthodes d'extraction et de purification

Fettarme Lebensmittel - Multiverfahren zur gaschromatographischen Bestimmung von Pestizidrückständen - Teil 2: Verfahren zur Extraktion und Reinigung

This European Standard was approved by CEN on 7 September 1998.

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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.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
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Contents

Foreword

This European Standard 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of an identical text or by endorsement, at the latest by April 1999, and conflicting national standards shall be withdrawn at the latest by April 1999.

This European Standard EN 12393 is divided in three parts:

- Part 1 "General considerations" provides general considerations with regard to reagents, apparatus, gas chromatography etc., applying to each of the analytical selected methods.
- Part 2 "Methods for extraction and clean-up" presents methods L to P for the extraction and clean-up using techniques such as liquid-liquid partition, adsorption column chromatography or gel permeation column chromatography, etc.
- Part 3 "Determination and confirmatory tests" gives some recommended techniques for the qualitative and the quantitative measurements of residues and the confirmation of the results.

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, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Introduction

This European Standard comprises a range of multi-residue methods of equal status: no single method can be identified as the prime method because, in this field, methods are continuously developing. The selected methods included in this standard have been validated and/or are widely used throughout Europe.

Because these methods can be applied to the very wide range of food commodities/pesticide combinations, using different systems for determination, there are occasions when variations in equipment used, extraction, clean-up and chromatographic conditions are appropriate to improve method performance, see clause 3.

1 Scope

This European Standard specifies methods for the extraction and clean-up of non-fatty food samples for quantitative determination of pesticide residues.

Different solvents can be used for this purpose. These pesticide residues are generally associated with other co-extracted compounds which would interfere in the analysis. To purify the crude extracts to be analysed, several techniques can be used.

This standard contains the following extraction and clean-up methods that have been subjected to interlaboratory studies and /or are adopted throughout Europe:

- method L: Extraction with acetone, liquid-liquid partition with dichloromethane and clean-up on a silica gel/charcoal column [1];

- method M: Extraction with acetone and liquid-liquid partition with dichloromethane/light petroleum, if necessary clean-up on Florisil® [1] [2, 3, 4];

- method N: Extraction with acetone, liquid-liquid partition with dichloromethane and clean-up with gel permeation and silica gel chromatography [5];

- method O: Extraction with acetonitrile, liquid-liquid partition with light petroleum and clean-up on a Florisil column [6];

- method P: Extraction of organophosphorus compounds with ethyl acetate, and if necessary, clean-up by gel permeation chromatography [7].

This European Standard specifies the details of methods L to P for the extraction and the clean-up of samples of non-fatty food. Several solvents at different volumes are used for extraction. Techniques of clean-up are listed such as liquid-liquid partition, liquid chromatography on various adsorbents and gel permeation chromatography.

A table providing the couples (matrix/pesticide) which have been submitted to collaborative studies and a list of indicative applicability of the method to different pesticides are given for each method, wherever possible.

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[1] Florisil® is an example of a suitable product available commercially. This information is given for convenience of users of this standard and does not constitute an endorsement by CEN of this product.

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.

EN 12393-1: 1998 Non-fatty foods - Multiresidue methods for the gas chromatographic determination of pesticide residues - Part 1: General considerations

EN 12393-3: 1998 Non-fatty foods - Multiresidue methods for the gas chromatographic determination of pesticide residues - Part 3: Determination and confirmatory tests

3 Principle

As already described in the introduction, in certain occasions it is possible to improve the method performance by variations in equipment used, extraction, clean-up and chromatographic conditions. Such variations shall be always clearly documented and demonstrated to give valid results.

The pesticide residues are extracted from the sample by the use of appropriate solvents, so as to obtain the maximum efficiency of extraction of the pesticide residues and minimum co-extracted substances which can give rise to interferences in the determination. Any interfering materials are removed from the sample extract to obtain a solution of the extracted pesticide residues in a solvent which is suitable for quantitative examination by the selected method of determination.

4 General: Summary of procedures

4.1 Extraction

The extraction procedures are summarized in Table 1.

Table 1

Methods	Mass of samples (M S)	Volume of solvent (V S)	Ratio M S / V S
L	100 g	Acetone: 200 ml	1/2
M	100 g	Acetone: 200 ml	1/2
N	100 g [1)]	Acetone: 200 ml	1/2
O	100 g	Acetonitrile: 200 ml	1/2 [2)]
P	50 g	Ethyl acetate: 100 ml	1/2

1) Only relevant if the water content of the matrix is greater than 70 %.

2) Depends on the water content of the matrix.

4.2 Clean-up

4.2.1 Liquid-liquid partition

The liquid-liquid partition procedures are summarized in Table 2.

Table 2

Methods	Aliquot portion of extract (A E)	Volume of added water (V W)	Volume of solvent (V S)	Ratio A E / V W
L	50 ml (= 20 %)	250 ml	50 ml	1/5
M	80 ml	0 ml	200 ml	- [1]
N	200 ml	x ml [1]	100 ml	- [1]
O	250 ml	600 ml	100 ml	1/3
1) Depends on the water content of the matrix.				

Two techniques of liquid-liquid partition are proposed:

- with added water (methods L, N,O);
- no added water (method M).

4.2.2 Adsorption column chromatography

Methods: L, M, N, O with different adsorbents: silica gel, charcoal, Florisil®, used pure or in mixture.

4.2.3 Gel permeation chromatography with BioBeads® S-X 3[2)]

Method N, and, if needed, method P.

5 Method L: Extraction with acetone, liquid-liquid partition with dichloromethane and clean-up on a silica gel/charcoal column

5.1 Principle

The chopped test portion is homogenized in acetone and the homogenate is filtered. An aliquot portion of the filtrate is diluted with water and extracted with dichloromethane. The organic phase is concentrated and chromatographed on a column of silica gel and activated charcoal. The pesticide residues are eluted with a mixture of dichloromethane, toluene and acetone. The eluate is concentrated for examination by GC.

5.2 Reagents

All reagents shall be suitable for the analysis of pesticide residues and in accordance with clause 4 in EN 12393-1:1998.

5.2.1 Acetone

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2) BioBeads® S-X 3 is an example of a suitable product available commercially. This information is given for convenience of users of this standard and does not constitute an endorsement by CEN of this product.

5.2.2 Dichloromethane

5.2.3 *n*-Hexane

5.2.4 Toluene

5.2.5 Eluting mixture: dichloromethane/toluene/acetone 5/1/1 (V/V/V).

5.2.6 Sodium chloride solution, saturated.

5.2.7 Sodium sulfate

Heat at 500 °C for at least 4 h, allow to cool and store in a stoppered bottle.

5.2.8 Activated charcoal

5.2.9 Silica gel 60 for column chromatography, 63 µm to 200 µm (70 mesh to 230 mesh).

5.2.10 Celite® 545 [3] (optional)

5.3 Apparatus

Usual laboratory equipment in accordance with EN 12393-1:1998 and, in particular, the following:

5.3.1 High speed blender or **homogenizer,** with a suitable blender cup.

5.3.2 Solvent evaporator, with a water bath, capable of being maintained at 40 °C.

5.3.3 Chromatographic column, with a sintered glass disk and a polytetrafluoroethylene (PTFE) stopcock, 25 mm internal diameter, 400 mm long.

5.4 Procedure

5.4.1 Preparation of the sample

Chop the sample into small pieces and mix thoroughly.

5.4.2 Extraction

Weigh 100 g of the coarsely comminuted sample into a 1 l beaker, add 200 ml of acetone and homogenize for approximately 30 s. If necessary, Celite® 545 can be used additionally as a filter

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[3] Celite® 545 is an example of a suitable product available commercially. This information is given for convenience of users of this standard and does not constitute an endorsement by CEN of this product.

aid. Rinse the homogenizer with 50 ml of acetone and reserve the washing for rinsing the beaker and the Büchner funnel later. Filter the homogenate with suction through a moistened round filter paper in a Büchner funnel. Rinse the filter cake with the 50 ml portion of acetone used earlier as washing liquid.

Thoroughly shake the filtrate and measure its volume. Take exactly one-fifth of this filtrate and shake it vigorously for at least 2 min with 250 ml of water, 25 ml of sodium chloride solution (5.2.6) and 50 ml of dichloromethane in a 1 l separatory funnel. If the filtrate is not shaken sufficiently well, the recovery is possibly reduced substantially. Repeat this extraction with 50 ml of dichloromethane. Combine the dichloromethane phases, and dry on 30 g of sodium sulfate (5.2.7) for 30 min. Filter the dried extract through a filter paper. Rinse the flask and filter paper with 30 ml of dichloromethane applied in three portions. Evaporate the filtrate to approximately 2 ml, and remove the last traces of solvent by swirling the flask manually. Dissolve the residue in 10 ml of dichloromethane.

5.4.3 Column preparation

Fill the chromatographic column (5.3.3) with dichloromethane to a level of 1 cm. Slurry 5 g of silica gel (5.2.9) in 15 ml of eluting mixture (5.2.5), and pour the slurry into the column. Drain off the supernatant. Next, thoroughly mix 15 g of silica gel and 1 g of activated charcoal in a 50 ml beaker, and slowly add 35 ml of eluting mixture (Caution: Generation of heat). Do not add more than 35 ml of eluting mixture otherwise the suspension will become separated into phases, resulting in poor passage of active ingredients through the column.

Add the activated charcoal-silica gel mixture onto the silica gel in the chromatographic column, by pouring it through a funnel, at first slowly and then in a gush, at the same time stirring constantly and with the column stopcock open. Use any eluate that has already passed through the column for rinsing the flask. Drain the eluting mixture to a level 2 cm above the packing, and top the column with a total of 5 g of sodium sulfate added in small portions. Next pre-wash the column with 50 ml of eluting mixture.

5.4.4 Clean-up

Transfer the dichloromethane solution derived from 5.4.2 quantitatively to the prepared column, completing the transfer with a total of 5 ml of dichloromethane. Collect the liquid already flowing through the column and the subsequent eluate in a 250 ml round-bottomed flask. Elute the column with 140 ml of eluting mixture (5.2.5). Evaporate the collected eluates to approximately 30 ml.Transfer it to a 50 ml round-bottomed flask and evaporate again to approximately 2 ml. Beforehand, empty the receiver of the rotary evaporator. Do not on any account rotary-evaporate the solution to dryness. Transfer the solution to a graduated test tube and dilute with *n*-hexane to 5,0 ml.

5.5 Gas chromatography

Use a gas chromatographic system suitable for determining organohalogen, organophosphorus and organonitrogen pesticide residues.

5.6 Collaborative studies

Couples of matrices and pesticides which have been submitted to collaborative studies[4)] are presented in Table 3.

Table 3

	carrot	potato	savoy cabbage	spinach	tomato	yellow pea
bromophos	+	+			+	
bromopropylate				+	+	
captan					+	
chlorpropham		+				
chlorpyrifos				+	+	
cypermethrin				+		
o, p'-DDE	+					
p, p'-DDE	+			+		
o, p'-DDT	+					
p, p'-DDT	+				+	
diazinon	+		+			+
dichlofluanid	+					
dicofol				+	+	
dieldrin	+	+	+	+	+	+
α-endosulfan					+	
β-endosulfan				+	+	
endosulfan sulfate				+	+	
endrin					+	
ethion					+	
fenarimol				+		
fenitrothion	+		+			
fenpropathrin				+		
folpet				+		
α-HCH					+	
heptachlor epoxide	+		+			
iprodione				+		
lindane (γ-HCH)	+	+	+	+	+	+
malathion				+		+
mecarbam				+		
parathion	+		+	+	+	
permethrin				+		
phosalone	+			+		+
pirimiphos-methyl		+		+	+	+
procymidone					+	
propham		+				
quintozene				+		+
tetradifon					+	
tolclofos-methyl				+		
vinclozoline	+	+		+	+	

4) For the collaborative studies, the activated charcoal and the silica gel 60, 63 µm to 200 µm (70 mesh to 230 mesh) from the Merck Company were used. This information is given for convenience of users of this standard and does not constitute an endorsement by CEN of these products.

5.7 Applicability

The following pesticides can be analysed by this method:

Aldrin
Ametryn
Atrazine
Azinphos-ethyl
Azinphos-methyl
Aziprotryne
Bifenthrin
Bromacil
Bromophos
Bromophos-ethyl
Bromopropylate
Bupirimate
Captafol
Captan
Carbophenothion
Chlorbenside
Chlorfenson
Chlorfenvinphos
Chlorflurenol
Chlorpropham
Chlorobenzilate
Chloropropylate
Chlorpyrifos
Chlorpyrifos-methyl
Chlorthal
Chlorthiophos
Cyanazine
Cyanofenphos
Cyanophos
Cyfluthrin
λ-Cyhalothrin
Cypermethrin
p,p'-DDD
o,p'-DDE
p,p'-DDE
o,p'-DDT
p,p'-DDT
Deltamethrin
Desmetryn
Dialifos
Diazinon
Dichlobenil
Dichlofenthion
Dichlofluanid
Dichlorvos
Dicofol
Dieldrin
Dimethachlor
Dimethoate
Dioxathion
Disulfoton
Ditalimfos
α-Endosulfan
ß-Endosulfan
Endosulfan sulfate
Ethion
Ethoprophos
Etrimfos
Fenamiphos
Fenarimol
Fenchlorphos
Fenitrothion
Fenpropathrin
Fenson
Fensulfothion
Fenthion
Fenvalerate
Fluchloralin
Flucythrinate
Fluorodifen
Fluvalinate
Folpet
Fonofos
Formothion
α-HCH
ß-HCH
Heptachlor
Heptachlor epoxide
Heptenophos
Iodofenphos
Iprodione
Isofenphos
Lindane
Malaoxon
Malathion
Mecarbam
Metalaxyl
Metazachlor
Methidathion
Methoprotryne
Methoxychlor
Metolachlor
Metribuzin
Mevinphos
Naled
Nitrofen
Paraoxon
Parathion
Parathion-methyl
Pendimethalin
Permethrin
Perthane
Phenkapton
Phorate
Phosalone
Pirimiphos-methyl
Procymidone
Profenofos
Profluralin
Prometryn
Propazine
Propham
Propyzamide
Prothiofos
Pyrazophos
Pyrethrum
Quinalphos
Quintozene
Simazine
Sulfotep
Tecnazene
Terbacil
Terbufos
Terbutryn
Tetrachlorvinphos
Tetradifon
Tetramethrin
Tetrasul
Thionazin
Tolclofos-methyl
Tolylfluanid
Triadimefon
Tri-allate
Triazophos
Trichloronat
Trifluralin
Vinclozolin

Crops and foods on which the method was tested:

Apples	Grapes	Pineapples
Apricots	Head cabbage	Plums
Aubergines	Honey	Potatoes
Beans	Kohlrabi	Radishes (large and small types)
Carrots	Leeks	Red cabbage
Celeriac	Lettuce	Savoy cabbage
Cherries	Mandarin oranges	Spinach
Chillies	Mushrooms	Strawberries
Chinese cabbage	Oranges	Sweet peppers
Corn salad	Parsley	Tomatoes
Cucumbers	Peaches	Witloof chicory
Dandelion	Pears	
Endives	Peas	

6 Method M: Extraction with acetone and liquid-liquid partition with dichloromethane/light petroleum, if necessary clean-up on Florisil®

6.1 Principle

The chopped test portion is homogenized in acetone and the homogenate is filtered. An aliquot portion of the filtrate is extracted with a mixture of light petroleum and dichloromethane and then with dichloromethane. The organic phase can be injected directly without clean up into a gas chromatograph with an appropriate detector or purified on a Florisil® column. The eluates are concentrated for examination by GC.

6.2 Reagents

All reagents shall be suitable for the analysis of pesticide residues and in accordance with clause 4 in EN 12393-1:1998.

6.2.1 Acetone

6.2.2 Light petroleum, boiling range 40 °C to 60 °C.

6.2.3 Sodium chloride

Heat at 500 °C for at least 4 h, allow to cool, and store in a stoppered bottle.

6.2.4 Dichloromethane

6.2.5 Acetonitrile