

SLOVENSKI STANDARD
SIST EN 12393-2:2009**01-februar-2009****BUXca Yý U**
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Foods of plant origin - Multiresidue methods for the gas chromatographic determination of pesticide residues - Part 2: Methods for extraction and cleanup

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

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

Ta slovenski standard je istoveten z: EN 12393-2:2008

ICS:

67.050	Splošne preskusne in analizne metode za živilske proizvode	General methods of tests and analysis for food products
67.080.01	Sadje, zelenjava in njihovi proizvodi na splošno	Fruits, vegetables and derived products in general

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EUROPEAN STANDARD
NORME EUROPÉENNE
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November 2008

ICS 67.080.01

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English Version

**Foods of plant origin - Multiresidue methods for the gas chromatographic determination of pesticide residues - Part 2:
Methods for extraction and cleanup**

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

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

This European Standard was approved by CEN on 13 September 2008.

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 CEN 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 CEN Management Centre has the same status as the official versions.

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Foreword

This document (EN 12393-2:2008) 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 May 2009, and conflicting national standards shall be withdrawn at the latest by May 2009.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN 12393-2:1998 with following significant technical changes:

- a) deletion of method O: Extraction with acetonitrile, liquid-liquid partition with light petroleum and clean-up on a Florisil[®] column;
- b) addition of an alternative partition step and expansion of the pesticide spectrum in method N: Extraction with acetone, liquid-liquid partition with dichloromethane or cyclohexane/ethyl acetate, clean-up with gel permeation and silica gel chromatography.

This European Standard EN 12393 "Foods of plant origin - Multiresidue methods for the gas chromatographic determination of pesticide residues" 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, Bulgaria, 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 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.

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1 Scope

This European Standard specifies methods for the extraction and clean-up of food samples of plant origin 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 or cyclohexane/ethyl acetate and clean-up with gel permeation and silica gel chromatography [5], [6];
- method P: Extraction 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 food samples of plant origin. 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.

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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.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 12393-1:2008, *Foods of plant origin – Multiresidue methods for the gas chromatographic determination of pesticide residues – Part 1: General considerations*

EN 12393-3, *Foods of plant origin – Multiresidue methods for the gas chromatographic determination of pesticide residues – Part 3: Determination and confirmatory tests*

3 Principles

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.

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.

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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 —Extraction procedures

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

^a Only relevant if the water content of the matrix is greater than 70 %.

4.2 Clean-up

4.2.1 Liquid-liquid partition

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

Table 2 —Liquid-liquid partition

Methods	Aliquot portion of extract	Volume of added water	Volume of solvent	Ratio
	(A E) ml	(V W) ml	(V S) ml	A E / V W
L	50 (= 20 %)	250	50	1/5
M	80	0	200	- ^a
N	200	x ^a	100	- ^a

^a Depends on the water content of the matrix.

Two techniques of liquid-liquid partition are proposed:

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

4.2.2 Adsorption column chromatography

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

4.2.3 Gel permeation chromatography with BioBeads® S-X 3²⁾

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**5.2.1 General**

All reagents shall be suitable for the analysis of pesticide residues and in accordance with EN 12393-1:2008, Clause 4.

5.2.2 Acetone**5.2.3 Dichloromethane****5.2.4 *n*-Hexane****5.2.5 Toluene****5.2.6 Eluting mixture: dichloromethane/toluene/acetone 5+1+1 (V/V/V)****5.2.7 Sodium chloride solution, saturated****5.2.8 Sodium sulfate**

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

5.2.9 Activated charcoal**5.2.10 Silica gel 60 for column chromatography, 63 µm to 200 µm (70 mesh to 230 mesh)****5.2.11 Celite® 545³⁾ (optional)****5.3 Apparatus**

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

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.

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.

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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 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 (5.2.7) 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 (5.2.8) 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.

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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 (5.2.10) in 15 ml of eluting mixture (5.2.6), 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 (5.2.6). 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 studies⁴⁾ are presented in Table 3.

Table 3 — Matrices and pesticides

	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					+	

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.

Table 3 (continued)

	carrot	potato	savoy cabbage	spinach	tomato	yellow pea
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	+	+		+	+	

5.7 Applicability

The following pesticides can be analysed by this method:

Aldrin	Dieldrin	Metribuzin
Ametryn	Dimethachlor	Mevinphos
Atrazine	Dimethoate	Naled
Azinphos-ethyl	Dioxathion	Nitrofen
Azinphos-methyl	Disulfoton	Paraoxon
Aziprotryne	Ditalimfos	Parathion
Bifenthrin	α -Endosulfan	Parathion-methyl
Bromacil	β -Endosulfan	Pendimethalin
Bromophos	Endosulfan sulfate	Permethrin

Bromophos-ethyl	Ethion	Perthane
Bromopropylate	Ethoprophos	Phenkapton
Bupirimate	Etrimfos	Phorate
Captafol	Fenamiphos	Phosalone
Captan	Fenarimol	Pirimiphos-methyl
Carbophenothion	Fenchlorphos	Procymidone
Chlorbenside	Fenitrothion	Profenofos
Chlorfenson	Fenpropathrin	Profluralin
Chlorfenvinphos	Fenson	Prometryn
Chlorfluorenl	Fensulfothion	Propazine
Chlorpropham	Fenthion	Propham
Chlorobenzilate	Fenvalerate	Propyzamide
Chloropropylate	Fluchloralin	Prothiofos
Chlorpyrifos	Flucythrinate	Pyrazophos
Chlorpyrifos-methyl	Fluorodifen	Pyrethrins
Chlorthal	Fluvalinate	Quinalphos
Chlorthiophos	Folpet	Quintozene
Cyanazine	Fonofos	Simazine
Cyanofenphos	Formothion	Sulfotep
Cyanophos	α -HCH	Tecnazene
Cyfluthrin	β -HCH	Terbacil
λ -Cyhalothrin	Heptachlor	Terbufos
Cypermethrin	Heptachlor epoxide	Terbutryn
p,p'-DDD	Heptenophos	Tetrachlorvinphos
o,p'-DDE	Iodofenphos	Tetradifon
p,p'-DDE	Iprodione	Tetramethrin
o,p'-DDT	Isofenphos	Tetrasul
p,p'-DDT	Lindane	Thionazin

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