BS EN 12393-2:2013

BSI Standards Publication

Foods of plant origin — Multiresidue methods for the determination of pesticide residues by GC or LC-MS/MS

Part 2: Methods for extraction and clean-up

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National foreword

This British Standard is the UK implementation of EN 12393-2:2013. It supersedes BS EN 12393-2:2008 which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee AW/275, Food analysis - Horizontal methods.

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

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Foods of plant origin - Multiresidue methods for the determination of pesticide residues by GC or LC-MS/MS - Part 2: Methods for extraction and clean-up

Aliments d'origine végétale - Méthodes multirésidus de détermination de résidus de pesticides par CPG ou CL-SM/SM - Partie 2: Méthodes d'extraction et de purification

Pflanzliche Lebensmittel - Multiverfahren zur Bestimmung von Pestizidrückständen mit GC oder LC-MS/MS - Teil 2: Verfahren zur Extraktion und Reinigung

This European Standard was approved by CEN on 21 September 2013.

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Foreword

This document (EN 12393-2:2013) 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of an identical text or by endorsement, at the latest by May 2014, and conflicting national standards shall be withdrawn at the latest by May 2014.

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 12393-2:2008.

In comparison with EN 12393-2:2008, the following significant technical changes have been made:

a) implementation of liquid chromatography in combination with tandem mass spectrometry (LC-MS/MS) for the quantification and/or confirmation of pesticide residues;

b) incorporation of information on GC-MS/MS;

c) deletion of method L as no longer in use;

d) editorial updating of the document according to references, etc;

e) enlargement of scope of method N concerning number of pesticides and validation data.

EN 12393, *Foods of plant origin – Multiresidue methods for the determination of pesticide residues by GC or LC-MS/MS* is divided into 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 M, N and 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, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

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

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

— method N: Extraction with acetone, liquid-liquid partition with dichloromethane or cyclohexane/ethyl acetate and clean-up with gel permeation and silica gel chromatography [4], [5];

— method P: Extraction with ethyl acetate, and if necessary, clean-up by gel permeation chromatography [6].

This European Standard specifies the details of methods M, N and 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.

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 documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 12393-1:2013, *Foods of plant origin — Multiresidue methods for the determination of pesticide residues by GC or LC-MS/MS — Part 1: General considerations*

EN 12393-3:2013, *Foods of plant origin — Multiresidue methods for the determination of pesticide residues by GC or LC-MS/MS — 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.

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.

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1) Florisil®,is an example of a suitable product available commercially from U.S. Silica company. 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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4 General: Summary of procedures

4.1 Extraction

The extraction procedures are summarized in Table 1.

Table 1 — Extraction procedures

Methods	Mass of samples (M_S) g	Volume of solvent (V_S) ml	Ratio (M_S / V_S) g/ml
M	100	Acetone: 200	½
N	100 [a]	Acetone: 200	½
P	10	Ethyl acetate: 20	½
[a] Only relevant if the water content of the matrix is greater than 70 %.			

4.2 Clean-up

4.2.1 Liquid-liquid partition

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

Table 2 — Liquid-liquid partition

Method	Aliquot portion of extract (A_E) ml	Volume of added water (V_W) ml	Volume of solvent (V_S) ml	Ratio A_E / V_W
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 (method N);

— no added water (method M).

4.2.2 Adsorption column chromatography

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

4.2.3 Gel permeation chromatography with Bio-Beads® S-X3 [2)]

Method N, and, if needed, method P.

2) BioBeads® S-X3 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 Method M: Extraction with acetone and liquid-liquid partition with dichloromethane/light petroleum, if necessary clean-up on Florisil®

5.1 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. Either mixtures of diethyl ether and light petroleum (method M_1) or mixtures of dichlormethane, light petroleum and acetonitrile (method M_2) are used for the elution of analytes from Florisil®. The eluates are 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 12393-1:2013, Clause 4.

5.2.2 Acetone

5.2.3 **Light petroleum,** boiling range 40 °C to 60 °C

5.2.4 Sodium chloride

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

5.2.5 Dichloromethane

5.2.6 Acetonitrile

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 Florisil® (or equivalent), 150 µm to 250 µm (60 mesh to 100 mesh)

Activate by heating at 130 °C to 135 °C for at least 5 h, allow to cool in a desiccator and transfer to an airtight stoppered jar. The adsorbent thus treated keeps its activity only for four days. It can subsequently be reactivated by the same treatment. The activity of the adsorbent should be checked from time to time by eluting pesticide standard materials as described in the method.

5.2.9 Diethyl ether, peroxide-free, containing 2 % (V+V) ethanol

5.2.10 Eluting solvent A: diethyl ether/light petroleum 6+94 (V/V)

5.2.11 Eluting solvent B: diethyl ether/light petroleum 15+85 (V/V)

5.2.12 Eluting solvent C: diethyl ether/light petroleum 50+50 (V/V)

5.2.13 Eluting solvent D: dichloromethane/light petroleum 20+80 (V/V)

5.2.14 Eluting solvent E: dichloromethane/light petroleum/acetonitrile 50+49,65+0,35 (V/V/V)

5.2.15 Eluting solvent F: dichloromethane/light petroleum/acetonitrile 50+48,5+1,5 (V/V/V)

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5.3 Apparatus

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

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

5.3.2 Chromatographic column, with a PTFE stopcock, 22 mm internal diameter, 300 mm long

5.3.3 Solvent evaporator, Kuderna Danish[3)] or equivalent

5.4 Procedure

5.4.1 Preparation of the sample

Chop finely the test sample and mix carefully to obtain homogeneous test portions.

If the water content of the sample is less than 30 %, adjust it to about 80 % by adding water.

NOTE The general water content of some crops and foods is given in Table A.1.

5.4.2 Extraction and partition

Weigh 100 g (m) of the prepared sample into the blender cup (5.3.1) and add 200 ml (V_{Ex}) of acetone. Blend at high speed for 3 min. Transfer the mixture to a Büchner funnel containing a filter paper moistened with acetone, filter under suction into the Büchner flask and measure the volume of the filtrate.

Pour 80 ml (V_{R1}) of filtrate in a 1 l separating funnel with 100 ml of dichloromethane and 100 ml of light petroleum (5.2.3). Shake for 3 min and leave to separate layers. Transfer the lower aqueous layer to a second 1 l separating funnel. Dry upper organic layer from the first separatory funnel by passing through 3 cm of sodium sulfate (5.2.7) supported on washed glass wool in 10 cm funnel collecting in a round-bottomed flask.

Add 7 g of sodium chloride (5.2.4) to the aqueous phase and shake for 30 s until sodium chloride (5.2.4) is dissolved. Add 100 ml of dichloromethane and shake for 3 min. Let the layers separate. Transfer the aqueous phase to a third separating funnel and dry the organic phase again on the same sodium sulfate. Add to the third separating funnel 100 ml of dichloromethane and shake for 3 min, separate and discard the aqueous phase and dry the dichloromethane phase on the same layer of sodium sulfate. Wash the sodium sulfate with 50 ml of dichloromethane and concentrate all organic phases to 2 ml. Add 100 ml of light petroleum and again concentrate to e.g. 2 ml and again until all dichloromethane disappears. Add 20 ml of acetone and reconcentrate to 2 ml (V_{end}). This concentrate may be injected directly into a gas chromatograph equipped with HECD (Hall detector), NPD or FPD (**method M**).

In some cases, a clean-up is recommended for determination by ECD**: methods M_1 or M_2**. For purification, the sample extract is concentrated to 1 ml of acetone (instead of 2 ml) and diluted to a volume of 10 ml with light petroleum.

5.4.3 Clean-up

5.4.3.1 Method M_1

Place a plug of cotton wool in the bottom of the chromatographic column (5.3.2) and fill with light petroleum (5.2.3) on 20 cm. Pour 20 g of Florisil® (5.2.8) and tap along the walls of the column to settle the adsorbent. Cover the top of the adsorbent with 1 cm to 2 cm of sodium sulfate (5.2.7).

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3) The Kuderna-Danish evaporator is an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product.

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Wash the adsorbent with approximately 30 ml of light petroleum. Place the evaporator flask under the column to receive the eluate. Transfer the extract for purification as described in 5.4.2, to the column, allowing it to pass through at a rate of not more than 5 ml/min. Rinse the container with two 5 ml portions of light petroleum, pouring the rinsings onto the column, rinse the walls of the chromatographic column with additional small portions of light petroleum and elute at 5 ml/min with 200 ml eluting solvent A (5.2.10).

Elute further with 200 ml of eluting solvent B (5.2.11) into a separate receiver and finally with 200 ml of solvent C (5.2.12). Concentrate each eluate to a suitable definite volume, e.g. 2 ml (V_{end}) for examination by GC.

5.4.3.2 Method M_2

Place a plug of cotton wool in the bottom of the chromatographic column (5.3.2) and fill with light petroleum (5.2.3) on 20 cm. Pour 20 g of Florisil® (5.2.8) and tap along the walls of the column to settle the adsorbent. Cover the top of the adsorbent with 1 cm to 2 cm of sodium sulfate (5.2.7).

Wash the adsorbent with approximately 30 ml of light petroleum. Place the evaporator flask under the column to receive the eluate. Transfer the extract for purification as described in 5.4.2 to the column, allowing it to pass through at a rate of not more than 5 ml/min. Rinse the container with two 5 ml portions of light petroleum, pouring the rinsings onto the column, rinse the walls of the chromatographic column with additional small portions of light petroleum and elute at 5 ml/min with 200 ml of eluting solvent D (5.2.13).

Elute further with 200 ml of eluting solvent E (5.2.14) into a separate receiver and finally with 200 ml of eluent F (5.2.15). Concentrate each eluate to a suitable definite volume, e.g. 2 ml (V_{end}) for examination by GC.

5.5 Gas chromatography

Use a gas chromatographic system suitable for determining organohalogen, organophosphorus and organo-nitrogen pesticide residues as described in EN 12393-3.

Inject an aliquot portion (V_i) of the eluates obtained in 5.4.2, 5.4.3.1 or 5.4.3.2 into the gas chromatograph.

5.6 Calculation of residues

The residue R, expressed in milligrams per kilogram, of an identified analyte is calculated from Formula (1):

$$R = \frac{(V_{Ex} + f_1 \times V_{water}) \times V_{end} \times W_{St} \times F_A}{V_{R1} \times V_i \times m \times F_{St}} \quad (1)$$

where

V_{Ex} is the volume of acetone added in extraction step 5.4.2;

f_1 is the factor considering the volume contraction from mixing acetone with the water present in the test portion (V_{water}). The typical value of f_1 is 0,90;

V_{water} is the volume of water present in the test portion. Consult reference documents on food composition for average water content. An example of average water content for some crops and vegetables is given in Table A.1;

V_{end} is the final volume of eluate solution obtained in 5.4.2, 5.4.3.1 or 5.4.3.2;

W_{St} is the mass of analyte injected with standard solution;

F_A is the peak area obtained from final extract;

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V_{R1} is the portion of volume V_{Ex} used for partition in 5.4.2;

V_i is the portion of volume V_{end} injected into the gas chromatograph;

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

F_{St} is the peak area obtained from W_{St}.

6 Method N: Extraction with acetone, liquid-liquid partition with dichloromethane or cyclohexane/ethyl acetate, clean-up with gel permeation and silica gel chromatography

6.1 Principle

The chopped test portion is homogenized in acetone, after addition of water, depending on the natural water content of the sample, in order to ensure an acetone/water ratio of 2+1 (V/V). The homogenate is filtered. An aliquot portion of the filtrate is saturated with sodium chloride and diluted with dichloromethane, resulting in separation of excess water. Alternatively, sodium chloride and a mixture of cyclohexane and ethyl acetate are added to the homogenate and the mixture is intensively mixed.

The organic phase is concentrated and cleaned up by gel permeation chromatography (GPC) on Bio-Beads S-X3® (polystyrene gel) using a mixture of cyclohexane and ethyl acetate as eluent. The residue-containing fraction is concentrated, and analysed directly by gas chromatography using a phosphorus/nitrogen selective detector, a flame photometric detector or a mass spectrometer. After solvent exchange, the same fraction can be used for determination by LC-MS/MS. For analysis by electron capture and in some cases also by nitrogen-selective detection, a supplemental clean-up on a small silica gel column may be necessary. In this clean-up step, the pesticides are separated in several fractions thus providing additional leads for identification.

6.2 Reagents

6.2.1 General

All reagents shall be suitable for the analysis of pesticide residues and in accordance with EN 12393-1:2013, Clause 4.

6.2.2 Acetone

6.2.3 Dichloromethane

6.2.4 Ethyl acetate

6.2.5 Cyclohexane

6.2.6 GPC eluting mixture: cyclohexane/ethyl acetate 1+1 (V/V)

6.2.7 n-Hexane

6.2.8 Isooctane

6.2.9 Toluene

6.2.10 Water, for chromatography

6.2.11 Methanol

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6.2.12 Glacial acetic acid, to prepare a 0,1 % acetic acid solution in water

6.2.13 Eluent 1: *n*-hexane / toluene 65+35 (V/V)

6.2.14 Eluent 2: toluene

6.2.15 Eluent 3: toluene / acetone 95+5 (V/V)

6.2.16 Eluent 4: toluene / acetone 80+20 (V/V)

6.2.17 Eluent 5: acetone

6.2.18 Sodium chloride

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

6.2.19 Sodium sulfate, powder

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

6.2.20 Salt mixture: sodium sulfate + sodium chloride 1+1 (w/w)

6.2.21 Celite® 545 [4)]

6.2.22 Silica gel 60 for column chromatography, 63 µm to 200 µm (70 mesh to 230 mesh), deactivated with 1,5 % water

Heat the silica gel for at least 5 h at 130 °C, allow to cool in a desiccator, and store in a tightly stoppered container in the desiccator. To 98,5 g of dried silica gel in a 300 ml conical flask (with ground joint), add 1,5 ml of water dropwise from a burette with continuous swirling. Immediately stopper the flask with ground stopper, shake vigorously for 5 min until all lumps have disappeared, next shake for 2 h on a mechanical shaker, and then store in a tightly stoppered container.

6.2.23 Glass wool, extracted exhaustively with acetone

6.2.24 Cotton-wool, extracted exhaustively with acetone

6.2.25 Bio-Beads® S-X3, 38 µm to 75 µm (200 mesh to 400 mesh), 3 % crosslinked, styrene divinylbenzene beads for size limit chromatography, 2 000 mol weight limit

6.2.26 Filter paper, 6 cm and 13,5 cm diameter, fast flow rate, extracted exhaustively with acetone

6.3 Apparatus

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

6.3.1 High speed blender or homogenizer, with a suitable blender cup

6.3.2 Solvent evaporator, e.g. rotary evaporator for reducing sample extracts and concentration workstation, for preparation of final LC-MS/MS extracts, or similar devices

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4) Celite 545®, (high-purity fluxcalcined diatomaceous silica, especially prepared for chromatography) 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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6.3.3 Ultrasonic bath

6.3.4 Instrument for GPC, e.g. GPC Autoprep 1001 or 1002® [5], equipped with chromatographic column, 25 mm internal diameter, 50 cm length, and 5 ml sample loops **(V_{R3})**; column packing 50 g of Bio-Beads® S-X3 resin, pre-swelled overnight in the GPC eluting mixture, approximately 32 cm column height, packed as described in 6.4.3.2

6.3.5 Chromatographic column, with extended outlet, 7 mm internal diameter, 230 mm long

6.4 Procedure

6.4.1 Extraction

6.4.1.1 General

The average water content of some crops and foods is given in Annex A.

6.4.1.2 Plant material and other foods with a water content exceeding 70 g/100 g

Homogenize 100 g (m) of the comminuted test portion having a water content of x g/100 g with (100 - x) g of water and 200 ml of acetone for 3 min in the blender (6.3.1).

6.4.1.3 Plant material with low water content

Weigh out 10 g to 50 g (m) of the dry or dried matrix having a water content of x g/100 g (for example 25 g to 50 g for dried fruit and dried vegetables; 10 g to 20 g for spices and tea; 50 g for cereal grains). Then add sufficient water to adjust the total water present to 100 g[6]. The amount of water (W) to be added is calculated from the formula $W = 100 - (m \times x)/100$. Blend and let stand for 10 min to 20 min. Next add 200 ml of acetone and homogenize for 3 min.

6.4.2 Partition

6.4.2.1 Partition with dichloromethane

Add 10 g of Celite® 545 and again homogenize for 10 s.

Filter the homogenate derived from 6.4.1.2 or 6.4.1.3 through a fast flow rate filter paper (6.2.26) in a Büchner funnel, with gentle vacuum suction, until more than 200 ml of filtrate is collected.

To avoid losses of solvent due to strong vacuum, it is recommended only to use a low vacuum. Do not allow the filter cake to pull dry.

Measure out 200 ml of filtrate (V_{R1}) in a graduated cylinder, and transfer to a 500 ml separatory funnel. Add 20 g of sodium chloride (6.2.18), and shake vigorously for 3 min. Next add 100 ml of dichloromethane (6.2.3), shake for 2 min, and then let stand for approximately 10 min to allow the phases to separate. Discard the lower aqueous phase. Collect the organic phase in a flask and add approximately 25 g of sodium sulfate (6.2.19), let stand for approximately 30 min with occasional swirling, and then filter through a cotton-wool plug (6.2.24) layered with 3 cm of sodium sulfate in a funnel. Collect the filtrate in a 500 ml round-bottomed flask, and rinse separatory funnel and filter twice with 20 ml portions of ethyl acetate (6.2.4). Concentrate the

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5) GPC Autoprep 1001 or 1002® are examples 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 these products.

6) Consult reference documents on food composition for average water content. An example of average water content for some crops and vegetables is given in Table A.1.

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