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 It supersedes BS EN 12393-2:2008 which is withdrawn.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of an identical text or by endorsement, at the latest by May 2014, and conflicting national standards shall be withdrawn at the latest by May 2014.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.

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.

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.

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.

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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.

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)

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 $\mathbf{M_1}$ or $\mathbf{M_2}$**. 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.

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):

$$R = \frac{(V_{\mathrm{Ex}} + f_1 \times V_{\mathrm{water}}) \times V_{\mathrm{end}} \times W_{\mathrm{St}} \times F_{\mathrm{A}}}{V_{\mathrm{R1}} \times V_{\mathrm{i}} \times m \times F_{\mathrm{St}}} \qquad (1)$$

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;

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

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.

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 g6). 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.

solution to 2 ml on a solvent evaporator (6.3.2). Remove the last traces of solvent with a gentle stream of nitrogen.

6.4.2.2 Partition with cyclohexane/ethyl acetate

To the homogenate derived from 6.4.1.2 or 6.4.1.3 add 35 g sodium chloride (6.2.18) and exactly 100 ml of GPC eluting mixture (6.2.6) to the same container and homogenize it again for 1 min. When the phases are clearly separated, collect the upper organic phase. In case of insufficient or delayed phase separation (more than 30 min) centrifuge the mixture. Measure out exactly 200 ml (V_{R1}) of the organic phase in a graduated cylinder and filter this volume through a cotton-wool plug (6.2.24) layered with approximately 100 g sodium sulfate (6.2.19) in a funnel. Collect the filtrate in a 500-ml round-bottomed flask and rinse the graduated cylinder and the funnel four times each with approximately 20 ml GPC eluting mixture. Concentrate the combined filtrate to a watery residue (not to dryness) using the solvent evaporator (6.3.2).

6.4.3 Clean-up by gel permeation chromatography

6.4.3.1 General

In cases where extracts without GPC are clean enough to obtain chromatograms free of significant interferences from the matrix the GPC step may be omitted. However, validation data according to 6.8 have been obtained with GPC. Alternative types and dimensions of GPC columns than described under 6.3.4 may be used if conditions are adapted accordingly and if they can be shown to lead to equivalent results.

6.4.3.2 Packing gel permeation column

Allow the Bio-Beads® (approximately 50 g) to swell overnight in the GPC eluting mixture (6.2.6). Then pour the suspension all at once into the column (capacity of approximately 180 ml). As soon as the gel bed has settled (free from air bubbles) to a level of approximately 32 cm, insert the plunger, lower it down to the bed level, and screw it into place. If the gel bed sinks to a still lower level after prolonged operation, the plunger shall be adjusted accordingly (observe manufacturer's instructions).

6.4.3.3 Checking elution volumes

For each gel permeation column before the first use, the elution conditions shall be checked on several analytes of the lower and upper elution volume range (see Table 3), and on appropriate crude extracts. To do so, load the sample loop with crude extracts or a mixture of standard solutions, elute as described in step 6.4.3.4 and determine by means of a suitable analytical method whether the added analytes are completely recovered or whether interferences are caused by non-separated impurities. The same check shall be carried out on analytes after they have been in use for a lengthy period.

NOTE It has been shown that some matrices can introduce randomized adsorption effect of certain analytes on Bio-Beads® resin and can cause false negative or false positive results.

6.4.3.4 Clean-up of crude extracts

To the concentrated residue of a crude extract obtained in 6.4.2.1 or 6.4.2.2 add exactly 7,5 ml of ethyl acetate and dissolve by gentle swirling. Add approximately 5 g of salt mixture (6.2.20) for binding the remaining water, swirl again, and add exactly 7,5 ml of cyclohexane to yield a total volume of 15,0 ml (V_{R2}). Shake for approximately 20 s, allow the salt mixture to settle and filter through a fast flow-rate filter paper, and inject 5 ml of the filtrate (V_{R3}) into one of the sample loops of the gel permeation chromatograph.

Elute the gel permeation column with the GPC eluting mixture at a flow rate of 5,0 ml/min. The instrument switches of the gel permeation chromatograph are adjusted according to the checking of the elution volumes. The settings depend on the target pesticides. Typical settings are as follows (see also Table 3):

- dump switch to 18 min to discard 90 ml;

- collect switch to 15 min to collect 75 ml;
- wash switch to 2 min for column rinse with 10 ml.

Concentrate the collect volume to approximately 1 ml on a solvent evaporator (rotate slowly, immerse flask only slightly), pipette into a ground-stoppered graduated test tube, rinse the evaporator flask with ethyl acetate and dilute with ethyl acetate to a volume of 5,0 ml (V_{R4}). This addition of ethyl acetate shall not be omitted on any account in order to ensure complete dissolution of the residue.

For the determination of individual pesticide residues, the instrument can be set to a smaller collect volume, matched to the respective analyte, according to the values given in Table 3.

6.4.4 Additional clean-up for GC determination: Chromatography on a silica gel minicolumn

6.4.4.1 Preparation of column

Pack the chromatographic column (6.3.5) in the following order: glass wool plug (6.2.23), 1,0 g of deactivated silica (6.2.22) gel, 5 mm to 10 mm layer of sodium sulfate (6.2.19), glass wool plug. Before use, rinse the column with 5 ml of *n*-hexane and discard the eluate. As soon as the *n*-hexane has drained to the top of the silica gel, put the sample solution onto the top of the column, in accordance with 6.4.4.3.

6.4.4.2 Checking separation efficiency of silica gel

To the column pre-washed in 6.4.4.1, add 1,0 ml of a solution containing 0,05 µg/ml of HCB, 0,10 µg/ml of lindane, 0,20 µg/ml of heptachlor epoxide, 0,25 µg/ml of α-endosulfan, 0,25 µg/ml of dieldrin and 1,25 µg/ml of endosulfan sulfate in *n*-hexane. Provided the activity of the silica gel is correctly adjusted, the added analytes are present after elution as described in 6.4.4.3 and electron capture gas-chromatographic analysis in the following fractions:

- eluate 1: HCB (100 %), lindane (100 %), heptachlor epoxide (partial amount), α-endosulfan (partial amount);
- eluate 2: Heptachlor epoxide (residual amount), α-endosulfan (residual amount), endosulfan sulfate (95 % to 100 %), dieldrin (100 %).

6.4.4.3 Fractionation of sample extract

Pipette 2,5 ml (V_{R5}) of the solution derived from 6.4.3.4 into a long-neck round-bottomed flask and add 5 ml of isooctane. Carefully evaporate to 1 ml (on no account to dryness) on a solvent evaporator (rotate slowly, immerse flask only a little). If the solution still contains ethyl acetate, add again isooctane and repeat evaporation.

Pipette the solution remaining after evaporation onto the silica gel column pre-washed in 6.4.4.1 and rinse with approximately 1 ml of *n*-hexane. Then rinse the flask with 2 ml of eluent 1 (6.2.13), and, as soon as the hexane has drained from the top of the column packing, add the rinsing to the column. From this point, collect the eluate in a graduated test tube. Then elute with a further 6 ml of eluent 1, and fill the receiver with eluent 1 to a volume of 10 ml (V_{end}). The resultant solution represents eluate 1.

Rinse the flask used for evaporation of the GPC eluate with 2 ml of toluene (eluent 2) (6.2.14). Then add this rinsing to the column. Collect the solution in a second graduated test tube and elute with 6 ml of toluene. Fill the receiver with toluene to a volume of 10 ml (V_{end}) to give eluate 2. Continue chromatography by the same procedure performed consecutively with eluent 3 (6.2.15), eluent 4 (6.2.16) and acetone (eluent 5) (6.2.17). Each time, rinse the flask with 2 ml, elute with a further 6 ml and dilute the eluate to a volume of 10 ml (V_{end}) to give eluates 3, 4 and 5. An example of the distribution of the analytes among the different eluates is shown in Table 3. The pesticides that can be purified by this method are listed in Table 3.

Table 3 — Elution volume ranges in gel permeation chromatography and the distribution of the analytes in the eluates from silica gel column chromatography

Analytes	GPC elution volume range	Silica gel eluates [s]				
	ml	1 [t]	2 [u]	3 [v]	4 [w]	5 [x]
3,4,5-Trimethacarb	100 to 140	0	0	5	0	0
Acephate	115 to 145	0	0	0	0	5
Acetamiprid	120 to 220	0	0	0	0	5
Aclonifen	115 to 145	0	5	0	0	0
Acrinathrin [a]	70 to 120	4	1	0	0	0
Alachlor	125 to 150	0	0	5	0	0
Aldrin	120 to 150	5	0	0	0	0
Ametryn	115 to 190	0	0	1	3	0
Amidithion	115 to 145	0	0	0	4	3
Anilazine [b]	105 to 135	0	0	5	0	0
Anthraquinone	145 to 185	0	2	4	0	0
Atrazine	110 to 135	0	0	4	3	0
Azinphos-ethyl	130 to 160	0	0	5	0	0
Azinphos-methyl	145 to 180	0	0	4	0	0
Azoxystrobin	120 to 155	0	0	0	5	0
Bendiocarb	130 to 160					
Benfluralin	100 to 130	5	0	0	0	0
Bensulfuron-methyl	100 to 150					
Benthiavalicarb-isopropyl [a]	60 to 110	0	0	0	5	0
Benzoylprop-ethyl	125 to 150	0	3	3	0	0
Bifenox	115 to 150	0	3	3	0	0
Bifenthrin [a]	090 to 120	0	5	0	0	0
Binapacryl	100 to 130	0	5	0	0	0
Bitertanol	100 to 130	0	0	0	4	2
Boscalid	105 to 130	0	0	5	0	0
Bromacil [c]	105 to 140	0	0	0	5	0
Bromophos	120 to 150	4	2	0	0	0
Bromophos-ethyl	110 to 140	5	1	0	0	0
Bromopropylate	095 to 135	0	0	3	3	1
Bromoxynil octanoate	120 to 150	0	5	1	0	0
Buprofezin [a]	90 to 120	0	0	4	0	0
Butocarboxim-sulfoxide	120 to 160					
Butoxycarboxim	105 to 140					
Camphechlor (Toxaphene)	110 to 150	5	1	0	0	0

Table 3 *(continued)*

Analytes	**GPC elution volume range**	**Silica gel eluates** [s]				
	ml	1[t]	2[u]	3[v]	4[w]	5[x]
Captafol[d]	120 to 150	0	0	5	0	0
Captan[d]	120 to 150	0	0	5	0	0
Carbaryl	125 to 170	0	0	5	0	0
Carbendazim	135 to 200					
Carbofuran	125 to 155	0	0	5	0	0
Carbophenothion	120 to 140	0	3	0	0	0
Carbophenothion-methyl	120 to 160	0	4	0	0	0
Chinomethionat	170 to 200	0	1	4	0	0
(Quinomethionate)	170 to 200	0	1	4	0	0
Chlorbenside[e]	120 to 155	0	0	1	0	0
Chlorbenside sulfone	130 to 160	0	0	5	0	0
α-Chlordane	110 to 140	5	0	0	0	0
γ-Chlordane	100 to 130	5	0	0	0	0
Chlorfenapyr[a]	085 to 105	0	5	0	0	0
Chlorfenprop-methyl	125 to 150	0	5	0	0	0
Chlorfenson	120 to 150	1	5	0	0	0
Chlorfenvinphos	110 to 140	0	0	4	3	0
Chlorfluazuron[a]	80 to 110					
Chloridazon	130 to 155	0	0	0	4	1
Chlormephos	115 to 145	3	3	0	0	0
Chlorobenzilate	100 to 135	0	0	4	2	1
Chloroneb	145 to 170	0	5	0	0	0
Chloropropylate	100 to 135	0	0	4	2	0
Chlorsulfuron	110 to 150					
Chlorothalonil	125 to 165	0	5	0	0	0
Chlorotoluron	115 to 150	0	0	0	5	2
Chloroxuron	130 to 155	0	0	1	5	0
Chlorpropham	110 to 135	0	2	4	0	0
Chlorpyrifos	110 to 140	2	4	0	0	0
Chlorpyrifos-methyl	120 to 150	1	4	0	0	0
Chlorthal-dimethyl	135 to 160	0	5	1	0	0
Chlorthiophos	115 to 155	0	4	0	0	0
Cinosulfuron	100 to 150					
Clodinafop-propargyl	100 to 125	0	0	5	0	0

Table 3 *(continued)*

Analytes	GPC elution volume range	Silica gel eluates [s]				
	ml	1 [t]	2 [u]	3 [v]	4 [w]	5 [x]
Clofentezine [a]	85 to 190					
Clomazone	115 to 145	0	0	5	0	0
Cloquintocet-1-mexyl	105 to 130	0	0	2	4	0
Coumaphos	135 to 165	0	0	5	0	0
Crotoxyphos	105 to 145	0	0	0	4	0
Crufomate	100 to 140	0	0	0	3	4
Cyanazine	110 to 135	0	0	0	4	0
Cyanofenphos	115 to 145	0	2	4	0	0
Cyanophos	115 to 150	0	0	4	0	0
Cycloate	125 to 150	0	0	5	1	0
Cyfluthrin [a]	090 to 120	0	5	0	0	0
λ-Cyhalothrin [a]	090 to 110	0	5	0	0	0
Cymoxanil [f]	110 to 130	0	0	0	5	0
Cypermethrin	100 to 135	0	5	0	0	0
Cyproconazole	100 to 120					
Cyprodinil	105 to 135	0	0	5	0	0
o,p'-DDD	110 to 140	5	0	0	0	0
p,p'-DDD	100 to 140	5	0	0	0	0
o,p'-DDE	120 to 150	5	0	0	0	0
p,p'-DDE	120 to 150	5	0	0	0	0
o,p'-DDT	120 to 150	5	0	0	0	0
p,p'-DDT	110 to 140	5	0	0	0	0
DEF [g]	115 to 135	0	0	5	1	0
Deltamethrin	100 to 135	0	5	0	0	0
Demeton-S-methyl	125 to 155	0	0	0	0	0
Demeton-S-methyl sulfone	120 to 160	0	0	0	2	3
Demeton-S sulfone[h]	115 to 140	0	0	0	3	3
Demeton-S sulfoxide [i]	140 to 170	0	0	0	0	3
N-Desethyl-pirimiphos-methyl	120 to 155	0	0	1	5	0
Desmedipham	100 to 120					
Dialifos	110 to 140	0	3	3	0	0
Di-allate	120 to 150	0	4	1	0	0
Diazinon	105 to 135	0	0	5	0	0
Dichlobenil	125 to 155	1	5	0	0	0
Dichlofenthion	110 to 140	3	3	0	0	0

Table 3 *(continued)*

Analytes	GPC elution volume range	Silica gel eluates [s]				
	ml	1 [t]	2 [u]	3 [v]	4 [w]	5 [x]
Dichlofluanid [j]	100 to 140	0	3	3	0	0
p,p'-Dichlorobenzophenone [k]	125 to 155	0	5	0	0	0
Dichlorvos	115 to 140	0	0	1	3	0
Diclofop-methyl	135 to 165	0	0	5	0	0
Dicloran	105 to 145	0	5	0	0	0
Dicofol [l]	100 to 150	2	4	0	0	0
Dicrotophos	130 to 160	0	0	0	0	5
Dieldrin	120 to 150	0	5	0	0	0
Diethofencarb	105 to 130	0	0	5	0	0
Difenoconazol	110 to 140	0	0	0	3	3
Diflubenzuron	110 to 135					
Diflufenican	105 to 125	0	0	5	0	0
Dimefox	120 to 155	0	0	0	0	5
Dimethachlor	135 to 165	0	0	4	2	0
Dimethoate	120 to 150	0	0	0	3	3
Dimetilan	120 to 180					
Dimoxystrobin [a]	85 to 160	0	0	3	3	0
Dinitramine [m]	105 to 130	4	1	0	0	0
Dinobuton	110 to 140	0	4	2	0	0
Dinocap	100 to 120	0	5	0	0	0
Dioxathion	110 to 140	0	3	3	1	0
Diphenylamin	130 to 160	0	5	0	0	0
Disulfoton [n]	115 to 150	0	2	0	0	3
Disulfoton sulfone	110 to 140	0	0	5	0	0
Disulfoton sulfoxide	120 to 150	0	0	0	0	5
Ditalimfos	120 to 150	0	0	4	1	0
Diuron	115 to 135					
Edifenphos	130 to 160	0	0	4	0	0
α-Endosulfan	110 to 150	2	4	0	0	0
β-Endosulfan	110 to 150	0	5	0	0	0
Endosulfan sulfate	100 to 140	0	5	0	0	0
Endrin	130 to 160	0	5	0	0	0
EPN	135 to 160	0	5	0	0	0
Ethiofencarb-sulfone	110 to 170					
Ethion	100 to 140	0	5	0	0	0

Table 3 *(continued)*

Analytes	**GPC elution volume range**	**Silica gel eluates** [s]				
	ml	1 [t]	2 [u]	3 [v]	4 [w]	5 [x]
Ethofumesate	110 to 135	0	0	3	0	0
Ethoprophos	120 to 155	0	0	4	1	0
Etrimfos	105 to 140	0	0	5	0	0
Famophos	125 to 155	0	0	5	0	0
Famoxadon	95 to 115	0	0	5		0
Fenamidone	105 to 140	0	0	4	1	0
Fenamiphos	105 to 140	0	0	0	4	2
Fenarimol	125 to 150	0	0	0	4	0
Fenazaquin	105 to 145	0	0	3	2	0
Fenchlorphos	120 to 150	4	2	0	0	0
Fenhexamid	100 to 140	0	0	1	4	1
Fenitrothion	120 to 150	0	4	0	0	0
Fenoxycarb	120 to 145	0	0	4	0	0
Fenpiclonil	100 to 130	0	0	3	3	0
Fenpropathrin	100 to 120	0	5	1	0	0
Fenpropimorph	90 to 120	0	0	4	2	0
Fenson	130 to 160	0	5	0	0	0
Fensulfothion	120 to 150	0	0	0	3	3
Fenthion	130 to 160	0	3	0	0	0
Fenvalerate	105 to 135	0	4	1	0	0
Fluazifop-P-butyl	105 to 130	0	0	5	0	0
Flubenzimine [j] [a]	085 to 120	0	5	0	0	0
Fluchloralin	100 to 120	5	1	0	0	0
Flucythrinat [a]	090 to 120	0	5	0	0	0
Fludioxonil [a]	090 to 120	0	0	5	0	0
Fluoroglycofen-ethyl [a]	090 to 115	0	0	5	0	0
Fluotrimazole	100 to 140	0	0	4	2	0
Fluquinconazol	095 to 125	0	0	3	3	0
Flurtamone [a]	085 to 105	0	0	0	5	0
Flusilazol	105 to 135	0	0	0	4	2
Flutriafol	115 to 135	0	0	0	3	3
Fluvalinate	095 to 120	0	5	0	0	0
Folpet	140 to 180	0	3	4	0	0
Fonofos	120 to 150	0	4	1	0	0
Formothion	120 to 150	0	0	4	1	0
Fuberidazole [m]	120 to 160	0	0	0	5	1

Table 3 *(continued)*

Analytes	GPC elution volume range	Silica gel eluates [s]				
	ml	1 [t]	2 [u]	3 [v]	4 [w]	5 [x]
Furathiocarb	105 to 135	0	0	5	1	0
Genite	135 to 165	0	5	0	0	0
Haloxyfop-ethoxyethyl	70 to 110					
Haloxyfop-methyl	100 to 160					
α-HCH	120 to 150	5	0	0	0	0
ß-HCH	100 to 130	5	0	0	0	0
δ-HCH	100 to 130	5	0	0	0	0
ε-HCH	105 to 135	5	0	0	0	0
Heptachlor	110 to 140	5	0	0	0	0
cis-Heptachlor epoxide	125 to 155	3	3	0	0	0
trans-Heptachlor epoxide	125 to 155	3	3	0	0	0
Heptenophos	120 to 150	0	0	1	4	0
Hexachlorobenzene	140 to 165	5	0	0	0	0
Hexaconazol	105 to 135	0	0	0	4	2
Hexaflumuron [a]	70 to 110	0	0	5	1	0
Hexythiazox	120 to 140	0	0	4	0	0
Imazalil [i m]	120 to 150	0	0	0	0	5
Imidacloprid	120 to 160	0	0	0	1	1
Indoxacarb	95 to 120	0	0	5	0	0
Iodofenphos	120 to 150	4	2	0	0	0
Ioxynil	125 to 155	0	5	1	0	0
Iprodione	115 to 145	0	0	5	1	0
Iprovalicarb [a]	085 to 110	0	0	0	5	0
Isobenzan	105 to 140	5	0	0	0	0
Isocarbamid	130 to 165	0	0	0	1	5
Isodrin	120 to 150	5	0	0	0	0
Isopropalin	110 to 135	5	0	0	0	0
Isoproturon	115 to 135					
Isoxaflutole	100 to 140	0	0	5	0	0
Iodfenphos	120 to 150	4	2	0	0	0
δ-Keto-endrin	135 to 165	3	4	0	0	0
Kresoxim-methyl	120 to 155	0	0	5	0	0
Lenacil	130 to 160	0	0	0	5	0
Leptophos	120 to 150	5	1	0	0	0
Lindane (γ-HCH)	110 to 140	5	0	0	0	0

Table 3 *(continued)*

Analytes	GPC elution volume range	Silica gel eluates [s]				
	ml	1 [t]	2 [u]	3 [v]	4 [w]	5 [x]
Linuron	120 to 140	0	0	4	1	0
Malaoxon	110 to 140	0	0	0	4	0
Malathion	110 to 140	0	0	4	0	0
MCPA-(2-butoxyethyl) ester [f]	115 to 145	0	0	5	0	0
Mecarbam	105 to 145	0	0	4	0	0
Mefenpyr-diethyl	100 to 130	0	0	5	0	0
Mepanipyrim	110 to 140	0	0	5	0	0
Mepronil	110 to 140	0	0	5	0	0
Mephosfolan	140 to 170	0	0	0	2	4
Merphos [o]	125 to 145	5	0	0	0	0
Metalaxyl	115 to 150	0	0	0	5	1
Metazachlor	125 to 150	0	0	4	2	0
Metconazol	85 to 115	0	0	0	4	1
Methabenzthiazuron	150 to 180	0	0	0	5	0
Methacrifos	125 to 165	0	0	4	0	0
Methamidophos	120 to 150	0	0	0	0	4
Methidathion	130 to 165	0	0	4	0	0
Methiocarb	120 to 150	0	0	4	0	0
Methomyl	95 to 175	0	0	0	5	0
Methoprotryne	115 to 140	0	0	0	4	1
Methoxychlor	125 to 155	0	5	0	0	0
Metolachlor	130 to 160	0	0	5	1	0
Metolcarb						
Metrafenon [a]	90 to 140	0	0	5	0	0
Metribuzin	125 to 150	0	0	3	1	0
Mevinphos	120 to 150	0	0	0	5	0
Mirex	130 to 160	5	0	0	0	0
Monocrotophos	115 to 140	0	0	0	0	5
Monolinuron	125 to 150	0	0	4	2	0
Morphothion	130 to 170	0	0	0	5	0
Naled [p]	115 to 155	0	0	4	1	0
Napropamid	135 to 165	0	0	2	4	0
Nicosulfuron	100 to 150					
Nitralin	115 to 145	0	1	5	0	0
Nitrofen	135 to 165	2	5	0	0	0
Nitrothal-isopropyl	105 to 135	0	1	4	1	0

Table 3 *(continued)*

Analytes	**GPC elution volume range**	**Silica gel eluates** [s]				
	ml	1 [t]	2 [u]	3 [v]	4 [w]	5 [x]
Nuarimol	130 to 155	0	0	0	5	0
Octachlorodipropyl ether(S 421)	110 to 130	5	0	0	0	0
Omethoate	140 to 160	0	0	0	0	5
Oxadiazon	115 to 145	0	0	5	0	0
Oxadixyl	145 to 175	0	0	0	5	0
Oxamyl	140 to 165	0	0	0	3	0
Oxychlordane (Octachlor epoxide)	100 to 160	5	0	0	0	0
Oxydemeton-methyl [i]	135 to 165	0		0	0	0
Paraoxon	110 to 140	0	0	1	4	0
Paraoxon-methyl	140 to 170	0	0	1	4	1
Parathion	110 to 140	0	4	1	0	0
Parathion-methyl	120 to 150	0	4	1	0	0
Penconazol	110 to 140	0	0	0	4	2
Pendimethalin	125 to 155	1	5	0	0	0
Pentachloroaniline	110 to 140	5	0	0	0	0
Pentachloroanisole	125 to 160	5	0	0	0	0
Pentachlorobenzene	125 to 165	5	0	0	0	0
Permethrin	115 to 145	0	5	1	0	0
Perthane	110 to 140	5	1	0	0	0
Phenkapton	115 to 145	3	3	0	0	0
Phenmedipham	100 to 120	0	0	3	3	1
Phenthoate	115 to 150	0	1	4	0	0
Phorate [q]	115 to 145	0	2	0	0	0
Phosalone	110 to 140	0	0	4	0	0
Phosphamidon	110 to 145	0	0	0	1	5
Phoxim	120 to 150	0	5	0	0	0
Picoxystrobin	100 to 130	0	0	5	0	0
Piperonyl butoxide [f]	100 to 130	0	0	4	1	0
Pirimicarb	130 to 170	0	0	0	5	0
Pirimiphos-ethyl	100 to 135	0	0	4	0	0
Pirimiphos-methyl	105 to 145	0	0	4	0	0
Prochloraz	120 to 150	0	0	0	4	2
Procymidone	120 to 150	0	0	5	0	0
Profenofos	130 to 155	0	0	4	1	0
Profluralin	100 to 125	5	0	0	0	0

Table 3 *(continued)*

Analytes	**GPC elution volume range**	**Silica gel eluates** [s]				
	ml	1 [t]	2 [u]	3 [v]	4 [w]	5 [x]
Promecarb	110 to 135					
Propachlor	125 to 150	0	0	5	0	0
Propanil	105 to 130	0	0	4	2	0
Propham	100 to 180	0	0	5	0	0
Propiconazole	120 to 150	0	0	0	4	2
Propoxur	110 to 130	0	0	4	2	0
Propyzamide	95 to 125	0	0	4	0	0
Proquinazid	95 to 160	0	0	4	1	0
Prosulfocarb	120 to 145	0	2	3	0	0
Prosulfuron	80 to 120					
Prothiofos	105 to 145	5	0	0	0	0
Pyrazophos	110 to 140	0	0	5	0	0
Pyrethrin [m]	100 to 130	0	0	5	1	0
Pyridaben	95 to 125	0	0	4	0	0
Pyrifenox	125 to 165	0	0	2	4	0
Pyrimethanil	140 to 170	0	0	5	0	0
Pyriproxyfen	105 to 135	0	2	3	0	0
Quinalphos	115 to 155	0	0	4	0	0
Quinmerac	115 to 145					
Quinoxyfen	110 to 140	0	0	5	0	0
Quintozene	135 to 165	0	0	4	0	0
Quizalofop-P-ethyl	110 to 140					
Rabenzazole [m]	120 to 160	0	0	5	0	0
Resmethrine [f]	100 to 130	0	5	0	0	0
Salithion	125 to 165	0	5	0	0	0
Silthiofam	90 to 120	0	0	5	0	0
Simazine	95 to 135	0	0	2	4	0
Spinosyn A (Spinosad)	75 to 110					
Spirodiclofen [a]	80 to 120	0	1	4	0	0
Spiroxamin [r] [a]	85 to 105	0	0	0	0	4
Strobane T	125 to 160	5	0	0	0	0
Sulfotep	100 to 130	0	4	1	0	0
Sulprofos	115 to 155	0	3	0	0	0
Tebuconazol [a]	90 to 120	0	0	0	3	3
Tebufenpyrad	105 to 145	0	0	5	0	0

Table 3 *(continued)*

Analytes	**GPC elution volume range**	**Silica gel eluates** [s]				
	ml	1 [t]	2 [u]	3 [v]	4 [w]	5 [x]
Tebutam	110 to 150	0	0	2	3	0
Teflubenzuron [a]	90 to 115	0	0	5	0	0
Tecnazene	130 to 160	5	0	0	0	0
Terbacil	120 to 145	0	0	0	5	0
Terbufos	125 to 155	0	3	0	0	0
Terbuthylazin	105 to 130	0	0	4	2	0
Terbutryn	115 to 175	0	0	1	2	0
2,3,4,5-Tetrachloronitrobenzene	130 to 160	5	0	0	0	
Tetrachlorvinphos	120 to 140	0	0	4	1	0
Tetradifon	120 to 150	0	5	0	0	0
Tetramethrin	120 to 150	0	0	5	0	0
Tetrasul	125 to 155	5	0	0	0	0
Thiabendazol	130 to 160	0	0	1	4	2
Thiacloprid	110 to 180					
Thiamethoxam	100 to 140	0	0	0	1	4
Thifensulfuron-methyl	110 to 150					
Thiodicarb	130 to 180	0	0	0	5	1
Thiofanox-sulfone	120 to 150	0	0	0	4	1
Thiofanox-sulfoxide	110 to 150					
Thionazin	120 to 150	0	1	4	0	0
Tolclofos-methyl	130 to 165	0	5	0	0	0
Tolylfluanid [j]	105 to 135	0	3	3	0	0
Triadimefon	100 to 130	0	0	3	3	0
Triadimenol	100 to 130	0	0	0	4	2
Tri-allate	120 to 150	0	5	0	0	0
Triamiphos	125 to 160	0	0	0	0	4
Triasulfuron	100 to 150					
Triazophos	120 to 140	0	0	4	1	0
Triazoxide	165 to 195	0	0	0	5	0
Trichlorfon [i]	100 to 140	0	0	0	0	4
Trichloronat	110 to 140	5	1	0	0	0
Trifloxystrobin [a]	90 to 110	0	0	5	0	0
Triflumizol [a]	85 to 105	0	0	0	5	0
Trifluralin	100 to 130	5	0	0	0	0
Triflusulfuron-methyl [a]	85 to 190					
Triforin						

Table 3 *(continued)*

Analytes	**GPC elution volume range**	**Silica gel eluates** [s]				
	ml	1 [t]	2 [u]	3 [v]	4 [w]	5 [x]
Triticonazol	100 to 125	0	0	0	4	1
Vinclozolin	100 to 130	0	4	1	0	0
Zoxamid [a]	85 to 125	0	0	5	0	0

NOTE 1 Figures in the table indicate recovery rates: 5 = more than 90 %; 4 = approximately 60 % to 90 %; 3 = approximately 30 % to 60 %; 2 = approximately 10 % to 30 %; 1 = less than 10 %; 0 = not recovered.

NOTE 2 There are some substances such as Ametryn, Clofentezine, Triflusulfuron-methyl, Triazoxide, etc. which can elute very late and thus occur in the following runs if ‘collect’ and ‘wash’ volumes are chosen too low.

[a] It has to be considered that this substance elutes very early in GPC (near to the dump volume) and thus losses of recovery can be observed.

[b] Extract the analytical sample with the addition of potassium acetate; for GC, inject an aliquot portion of the sample solution with the addition of acetic acid.

[c] Peak height depending on solvent.

[d] Gas chromatographic column has to be well conditioned.

[e] When chromatographed on silica gel, an additional peak will be observed with a longer GC retention time.

[f] Analysed by GC/MS.

[g] Also from Merphos.

[h] Inject standard solution within 2 min after injection of sample solution.

[i] Improved recovery when eluted with further 8 ml of acetone.

[j] Extract the test portion with the addition of citric acid and oxalic acid.

[k] Degradation product of dicofol.

[l] Degradation to p,p'-dichlorobenzophenone.

[m] For sample preparation, proceed with complete exclusion of light.

[n] Following chromatography on silica gel, disulfoton sulfoxide is observed in eluate 5.

[o] Partly oxidized to DEF.

[p] When chromatographed on silica gel, varying recoveries are observed.

[q] Partly decomposed during chromatography on silica gel.

[r] Eluate 5 needs 12 ml acetone for complete eluation of the analyte.

[s] Silica gel eluates: If no data are listed, the substances are usually determined directly from the GPC-extract

[t] Eluate 1 *n*-hexane-toluene 65+35 (V/V).

[u] Eluate 2 toluene.

[v] Eluate 3 toluene-acetone 95+5 (V/V).

[w] Eluate 4 toluene-acetone 80+20 (V/V).

[x] Eluate 5 acetone.

6.5 Gas chromatography

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

Inject an aliquot portion (V_i) of the eluates obtained in 6.4.4.3 into the gas chromatograph. For analyses with the mass spectrometric detector, the flame photometric detector and the thermionic detector an aliquot portion (V_i) of the solution obtained in 6.4.3.4 (V_{R4}) also can be generally injected into the gas chromatograph. In this case, where no mini silica gel column is applied, V_{end} and V_{R5} are not applicable in Formula (3).

6.6 Liquid chromatography with tandem-mass spectrometric detection (LC-MS/MS)

Use a LC-MS/MS system suitable for determining pesticide residues as described in EN 12393-3.

In order to inject an aliquot portion (V_i) of the eluate obtained in 6.4.3.4 (V_{R4}) into the LC-MS/MS the GPC eluate has to be reconstituted in a solvent mixture suitable for HPLC. Thereby, the dilution factor of the solvent exchange step depends on the sample weight-in in order to guarantee a constant matrix concentration in the final extract to be injected into the HPLC. Validation data were obtained with a matrix concentration of 0,585 g/ml. To obtain such matrix concentration, the dilution scheme is depending from the sample mass. Examples for different sample mass are given as follows.

Thus, for a test portion of **25 g,** take an aliquot of 0,5 ml (V_{R5}) from GPC eluate obtained in 6.4.3.4 (V_{R4}) and evaporate to dryness using a solvent evaporator. Add 0,5 ml of methanol and carefully dissolve the residue by using an ultrasonic bath, but avoid evaporation or heating of methanol. After complete re-dissolvation of the dry reminder add 0,5 ml of a 0,1 % acetic acid solution (6.2.12) and mix well. The resulting dilution factor (V_{end}/V_{R5}) in the obtained 1 ml (V_{end}) of final extract corresponds to 2.

For a test portion of **50 g,** take an aliquot of 0,5 ml (V_{R5}) from GPC eluate obtained in 6.4.3.4 (V_{R4}) and evaporate to dryness using asolvent evaporator. Add 1,0 ml of methanol and carefully dissolve the residue by using an ultrasonic bath, but avoid evaporation or heating of methanol. After complete re-dissolvation of the dry reminder add 1,0 ml of a 0,1 % acetic acid solution (6.2.12) and mix well. The resulting dilution factor (V_{end}/V_{R5}) in the obtained 2 ml (V_{end}) of final extract corresponds to 4.

For a test portion of **100 g,** take an aliquot of 0,5 ml (V_{R5}) from GPC eluate obtained in 6.4.3.4 (V_{R4}) and evaporate to dryness using a solvent evaporator. Add 2,0 ml of methanol and carefully dissolve the residue by using an ultrasonic bath, but avoid evaporation or heating of methanol. After complete re-dissolvation of the dry reminder add 2,0 ml of a 0,1 % acetic acid solution (6.2.12) and mix well. The resulting dilution factor (V_{end}/V_{R5}) in the obtained 4 ml (V_{end}) of final extract corresponds to 8.

NOTE Depending on the sensitivity of the LC-MS/MS system used, the appropriate dilution factor might vary.

6.7 Calculation of residues

The residue R, expressed in milligrams per kilogram, of an identified analyte is calculated from Formula (2):

$$R = \frac{F_A \times W_{St} \times f_2}{F_{St} \times V_i \times m} \tag{2}$$

in which

$$f_2 = \frac{V_{end} \times f_3 \times V_{Ex} \times V_{R2} \times V_{R4}}{V_{R1} \times V_{R3} \times V_{R5}} \tag{3}$$

where

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

V_{Ex} is the volume, in millilitres, of acetone and water added in extraction step 6.4.1.2 or 6.4.1.3, plus water contained in the sample, in millilitres;

f_3 is the factor considering the volume contraction from mixing acetone with the water (sum of added water and the water present in the test portion) in 6.4.2.1 and additionally losses of acetone in the water phase during partition in 6.4.2.2. The typical values of f_3 are 0,98 for 6.4.2.1 and 0,95 for 6.4.2.2;

V_{R1} is the portion of volume V_{Ex} used for partition in 6.4.2, in millilitres;

V_{R2} is the volume of solution of evaporation residue prepared for gel permeation chromatography by method described in 6.4.3.4, in millilitres;

V_{R3} is the portion of volume V_{R2} injected for gel permeation chromatography (volume of sample loop), in millilitres;

V_{R4} is the volume of solution obtained after gel permeation chromatography by procedure described in 6.4.3.4, in millilitres;

V_{R5} is the portion of volume V_{R4} used for chromatography in 6.4.4.3, or used for re-dissolvation step in 6.6, in millilitres;

V_{end} is the final volume of eluate solution obtained in 6.4.4.3, or the final volume obtained in 6.6 after re-dissolvation, in millilitres;

V_i is the portion of volume V_{end} or volume V_{R4} injected into the gas chromatograph or of volume V_{end} injected into the liquid chromatograph, respectively; in microlitres;

W_{St} is the amount of analyte injected with standard solution, in nanograms;

F_A is the peak area obtained from V_i;

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

6.8 Collaborative studies

Couples of matrices and pesticides which have been submitted to collaborative studies [7] are presented in Table 4.

Table 4 — Matrices and pesticides tested with method N

	carrot	potato	savoy cabbage	spinach	tomato	cucumber	citrus fruits	cereals	nuts, avocado	yellow peas	raisins
3,4,5-Trimethacarb						x	x	x			x
Acephate				+							
Acetamiprid						x	x	x			x
Aclonifen					+		+	+	+		
Acrinathrin						x	x	x	x		
Aldrin						x					
Ametryn						x	x	x	x		
Atrazin						x	x	x			x
Azinphos-methyl						x		x			
Azoxystrobin					+		+	+	+		x
Bendiocarb						x	x	x			x
Bensulfuron-methyl						x	x	x			
Benthiavalicarb-isopropyl						x	x		x		x
Bifenthrin						x		x			
Bitertanol						x		x			
Boscalid						+	+	+	+		
Bromacil						x		x			
Bromophos	+	+			+						
Bromopropylate				+	+						
Bupirimat						x		x			
Buprofezin						x		x	x		
Butocarboxim-sulfoxide						x			x		x
Butoxycarboxime						x	x	x			x
Captan					+						
Carbaryl						x	x	x	x		x
Carbendazim						x	x	x			
Carbofuran						x		x	x		
Chinomethionat						x	x	x	x		
Chlorfenapyr						+	+	+	+		
Chlorfenprop-methyl						+	+	+	+		

Table 4 *(continued)*

	carrot	potato	savoy cabbage	spinach	tomato	cucum-ber	citrus fruits	cereals	nuts, avocado	yellow peas	raisins
Chlorfenvinphos						x	x	x	x		
Chlorfluazuron									x		
Chloridazon						x		x			
Chlorsulfuron						x		x			x
Chlorothalonil				+							
Chlorpropham		+									
Chlorpyrifos				+	+						
Chlozolinat						x		x			
Cinosulfuron						x	x	x			x
Clodinafop-propargyl					+		+	+	+		
Clofentenzine							x				
Clomazone					+		+	+	+		x
Cloquintocet-1-mexyl					+		+	+	+		
Cycloat						x		x			
Cyhalothrin						x					
Cyfluthrin						x					
Cypermethrin						x		x			
Cyproconazol						x		x	x		x
Cyprodinil					+		+	+	+		
o, p'-DDE	+										
p, p'-DDE	+			+							
o, p'- DDT	+										
p, p'-DDT	+				+						
Deltamethrin						x					
Demeton-S-methylsulfon						x	x	x			x
Desmedipham							x				
Diazinon	+		+							+	
Dichlobenil						x		x			
Dichlofluanid	+										
Diclofop-methyl						x	x	x	x		
Dicloran	+										
Dichlorvos						x		x			
Dicofol				+	+						

Table 4 *(continued)*

	carrot	potato	savoy cabbage	spinach	tomato	cucumber	citrus fruits	cereals	nuts, avocado	yellow peas	raisins
Dieldrin	+	+	+	+	+					+	
Diethofencarb					+		+	+	+		x
Difenoconazole					+		+	+	+		
Diflubenzuron							x				x
Diflufenican					+		+	+	+		x
Dimethachlor						x	x	x	x		
Dimethoat						x		x			
Dimetilan						x	x	x			x
Dimoxystrobin						x	x	x	x		
Diphenylamin					+		+	+	+		
Disulfoton						x		x			
Ditalimfos						x	x	x	x		
Diuron						x		x	x		x
α-endosulfan					+						
β-endosulfan				+	+						
Endosulfan sulfate				+	+						
Endrin					+						
Ethiofencarb-sulfone							x	x			x
Ethion					+						
Ethofumesate					+		+	+	+		
Famoxadone						x	x	x	x		
Fenamidone						+	+	+	+		
Fenarimol				+							x
Fenazaquin					+		+	+	+		x
Fenhexamid						x	x				x
Fenitrothion	+		+								
Fenoxycarb					+		+	+	+		x
Fenpiclonil					+		+	+	+		
Fenpropathrin				+							
Fenpropimorph						x	x	x	x		
Fenvalerat						x					
Fluazifop-P-butyl						x		x			x
Flucythrinat						x					
Fludioxonil					+		+	+	+		

Table 4 *(continued)*

	carrot	potato	savoy cabbage	spinach	tomato	cucumber	citrus fruits	cereals	nuts, avocado	yellow peas	raisins
Fluoroglycofen-ethyl					+		+	+	+		
Fluquinconazole					+		+	+	+		
Flurtamone						+	+	+	+		
Flusilazol						x		x			
Flutriafol					+		+	+	+		
Fluvalinat						x					
Folpet				+							
Furathiocarb							x				
Haloxyfop-Ethoxyethyl						x					
Haloxyfop-methyl						x					
HCB	+	+		+	+					+	
α-HCH					+						
ß-HCH					x						
Lindane (γ-HCH)	+	+	+	+	+					+	
Heptachlor						x					
Heptachlor epoxide	+		+								
Hexaconazol						x		x			
Hexaflumuron						x					
Hexythiazox								x	x		
Imazalil								x			
Imidacloprid						x	x	x			x
Indoxacarb						+	+	+	+		
Iprodione				+							
Iprovalicarb						+	+	+	+		x
Isoproturon						x	x	x			x
Isoxaflutole								x			
Jodfenphos						x		x			
Kresoxim-methyl					+		+	+	+		x
Linuron						x	x	x			x
Malathion				+						+	
Mecarbam				+							
Mefenpyr-diethyl					+		+	+	+		x
Mepanipyrim						x	x	x	x		
Mepronil						x	x	x	x		

Table 4 *(continued)*

	carrot	potato	savoy cabbage	spinach	tomato	cucumber	citrus fruits	cereals	nuts, avocado	yellow peas	raisins
Metalaxyl						x	x	x			x
Metazachlor						x					
Metconazole					+		+	+	+		
Methabenzthiazuron						x		x			
Methacrifos						x			x		
Methamidophos						x		x			
Methidathion						x		x			
Methiocarb						x	x	x	x		x
Methomyl						x	x		x		x
Methoxychlor						x					
Metolachlor						x	x	x			x
Metolcarb						x	x	x	x		x
Metrafenon						x	x		x		
Metribuzin						x		x	x		
Mevinphos						x		x			
Monocrotophos						x		x			x
Napropamide					+		+	+	+		x
Nicosulfuron											x
Nuarimol					+		+	+	+		
Oxadixyl						x		x			x
Oxamyl								x			
Oxydemeton-methyl						x					x
Parathion	+		+	+	+						
Parathion-methyl						x		x			
PCB 28						x		x			
PCB 138						x		x			
Penconazol						x		x			x
Pendimathalin						x					
Permethrin				+							
Phenmedipham						x		x	x		x
Phosalone	+			+						+	
Phosphamidon						x					
Picoxystrobin						+	+	+	+		
Pirimicarb						x		x	x		x
Pirimiphos-methyl		+		+	+					+	

Table 4 *(continued)*

	carrot	potato	savoy cabbage	spinach	tomato	cucum-ber	citrus fruits	cereals	nuts, avocado	yellow peas	raisins
Prochloraz								x			
Procymidone					+						
Profenofos						x		x			
Promecarb						x		x			x
Propham		+									
Propiconazol						x		x			
Propoxur						x	x	x	x		x
Propyzamid						x	x	x	x		
Proquinazid						x	x	x	x		
Prosulfocarb					+		+	+	+		
Prosulfuron							x				x
Pyrazophos						x		x			
Pyridaben						+	+	+	+		
Pyrifenox					+		+	+	+		
Pyrimethanil						x	x	x			x
Pyriproxyfen						+	+	+	+		
Quinalphos						x		x			
Quinmerac						x					x
Quinoxyfen						x	x	x	x		
Quintozene				+						+	
Quizalofop-ethyl											x
Silthiofam						+	+	+	+		
Simazin						x		x			
Spinosyn A (Spinosad)								x			
Spirodiclofen						x	x	x	x		
Spiroxamine						+	+	+	+		
Sulfotep						x		x			
Tebuconazol						x	x	x	x		x
Tebufenpyrad					+		+	+	+		x
Tebutam						x		x			
Teflubenzuron									x		
Terbacil						x		x			
Terbuthylazin						x		x			
Tetradifon					+						

Table 4 *(continued)*

	carrot	**potato**	**savoy cabbage**	**spinach**	**tomato**	**cucumber**	**citrus fruits**	**cereals**	**nuts, avocado**	**yellow peas**	**raisins**
Thiabendazol								x			
Thiacloprid						x	x	x			x
Thiamethoxam						x	x	x			x
Thifensulfuron-methyl						x		x			
Thiodicarb											x
Thiofanox-sulfone						x		x			
Thiofanox-sulfoxide						x		x			
Tolclofos-methyl				+							
Triadimefon						x		x			
Triadimenol						x		x			
Triallat						x					
Triasulfuron							x	x			x
Triazophos						x		x			
Trichloronat						x					
Trifloxystrobin						+	+	+	+		
Triflumizol					x		x	x	x		
Trifluralin						x					
Triflusulfuron-methyl						x		x			
Triforin									x		x
Triticonazole					+		+	+	+		
Vinclozolin	+	+		+	+						
Zoxamide						+	+	+	+		

Key

+ = data obtained from proficiency tests;

x = data obtained from inter-laboratory method validation studies.

Validation data from the study which was the basis for [7]; individual recovery data listed in [7].

6.9 Applicability

The pesticides that can be analysed by this method are listed in Table 3. Crops and foods on which the method was tested are listed in Table 5.

Table 5 — Crops and foods tested with Method N

Apples	Coffee, raw	Nuts	Spices
Avocados	Cucumbers	Onions	Spinach
Bananas	Curly kale	Peaches	Strawberries
Beans	Currants, red	Peanuts	Sugar beets
Beer	Grapes	Pears	Sweet peppers
Carrots	Head cabbage	Peas	Tea and tea-like products
Cauliflower	Hops	Pineapples	Tobacco
Cereals	Kohlrabi	Plums	Tomatoes
Cherries	Lettuce	Potatoes	Wine
Citrus fruit	Melons	Raisins	
Cacao products	Must	Savoy cabbage	

7 Method P: Extraction with ethyl acetate, and if necessary, clean-up by gel permeation chromatography

7.1 Principle

A portion of the chopped and homogenized sample is extracted with ethyl acetate together with sodium hydrogen carbonate and sodium sulfate in a sample tube. After shaking and ultrasonication, the sample tube is centrifuged. An aliquot portion of the crude extract can be injected directly without clean-up into a gas chromatograph or liquid chromatograph, or, if necessary, purified by gel permeation chromatography on Bio-Beads®S-X3 using a mixture of cyclohexane and ethyl acetate as eluent. The eluate is concentrated for examination by GC or LC.

7.2 Reagents

7.2.1 General

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

7.2.2 Ethyl acetate 7

7.2.3 Sodium sulfate

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

7.2.4 Cyclohexane

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

7.2.6 Bio-Beads® S-X3, 38 µm to 75 µm (200 mesh to 400 mesh)

7.2.7 Sodium hydrogen carbonate

7.3 Apparatus

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

7.3.1 Homogenizer

7.3.2 Solvent evaporator, with water bath

7.3.3 Instrument for GPC, e.g. GPC Autoprep® 1001 or 1002, equipped with chromatographic column, 25 mm internal diameter, 50 cm length, and 5 ml sample loops (V_{R1}); 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 7.4.3.1

7.3.4 Centrifuge tubes, e.g. of polypropylene with screw caps

7.3.5 Centrifuge

7.3.6 Syringes, e.g. 10 ml disposable syringes, and Syringe filters, PTFE 0,20 µm pore size

7.4 Procedure

7.4.1 Preparation of the sample

Chop the sample and homogenate (7.3.1) carefully.

7.4.2 Extraction

Weigh 10 g (m) of the prepared sample into a centrifuge tube (7.3.4), add 3 g of sodium hydrogen carbonate (7.2.7), a suitable amount of at least 10 g of sodium sulfate (7.2.3) in order to absorb the sample water (see Table A.1 for water content) and 20 ml (V_{Ex}) of ethyl acetate. Shake vigorously for 1 min and ultrasonicate for 3 min.

If the sample's degree of comminution is insufficient or the residues do not readily extract from the matrix, the extraction time may be prolonged (e.g. to 20 min using a mechanical shaker) or assisted by a high-speed disperser (e.g. Ultra-Turrax®).

Centrifuge (7.3.5) for 3 min at 3 000 *g*. Isolate and filtrate the crude extract through a 0.20 µm PTFE filter (7.3.6).

In some cases when interferences occur, typically for high fat commodities such as avocado, clean-up by GPC is recommended.

7.4.3 Clean-up by gel permeation chromatography (if required)

7.4.3.1 Packing gel permeation column

Allow the Bio-Beads® (7.2.6) (approximately 50 g) to swell overnight in the GPC eluting mixture (7.2.5). Then pour the suspension all at once into the column (capacity of approximately 180 ml). As soon as the gel bed has settled (free from air bubbles) to a level of approximately 32 cm, insert the plunger, lower it down to the bed level, and screw into place. If the gel bed sinks to a still lower level after prolonged operation, the plunger shall be adjusted accordingly (observe manufacturer's instructions).

7.4.3.2 Checking elution volumes

For each gel permeation column before the first use, the elution conditions shall be checked on several analytes of the lower and upper elution volume range, and on appropriate crude extracts. To do so, load the sample loop with a mixture of standard solutions or crude extracts, elute as described in 7.4.3.3 and determine by means of a suitable analytical method whether the added analytes are completely recovered or whether interferences are caused by non-separated impurities. The same check shall be carried out on columns after they have been in use for a lengthy period.

7.4.3.3 Clean-up of crude extracts

To 5,0 ml (V_{R1}) of ethyl acetate extract (7.4.2) add 5,0 ml (V_{R2}) of cyclohexane. Shake for approximately 20 s, filter through a fast flow rate filter paper and inject 5 ml of the filtrate (V_{R3}) into one of the sample loops of the gel permeation chromatograph.

Elute the gel permeation column with the GPC eluting mixture at a flow rate of 5,0 ml/min. The instrument switches of the gel permeation chromatograph are adjusted according to the checking of the elution volumes. Usually the setting is as follows:

— dump switch to	16 min to discard 80 ml;
— collect switch to	22 min to collect 110 ml;
— wash switch to	2 min for column rinse with 10 ml.

Concentrate the collected volume to approximately 1 ml on a solvent evaporator (rotate slowly, immerse flask only slightly), pipette into a ground-stoppered graduated test tube, rinse the evaporator flask with ethyl acetate and dilute with ethyl acetate to a volume of 2,5 ml (V_{end}). This addition of ethyl acetate shall not be omitted on any account in order to ensure complete dissolution of the residue.

If analytes are analysed which elute early in GPC, the use of a crude extract or a wider GPC collect fraction is necessary.

7.5 Gas chromatography

Use a suitable gas chromatographic system as described in EN 12393-3 and inject an aliquot of the extract from 7.4.2 or 7.4.3.3.

7.6 Liquid chromatography

Use a suitable liquid chromatographic system as described in EN 12393-3 and inject an appropriate aliquot of e.g. 2 µl or less of the extract from 7.4.2 or 7.4.3.3.

7.7 Calculation of results

The residue R, expressed in milligrams per kilogram, of an identified analyte is calculated from Formula (4):

$$R = \frac{F_{\mathrm{A}} \times W_{\mathrm{St}} \times V_{\mathrm{EX}} \times f_4}{F_{\mathrm{St}} \times V_{\mathrm{i}} \times m} \qquad (4)$$

in which either f_4 is 1 (if 7.4.3 is not required) or f_4 is calculated by Formula (5) in the case of clean-up of crude extracts by GPC:

$$f_4 = \frac{(V_{\mathrm{R1}} + V_{\mathrm{R2}}) \times V_{\mathrm{end}}}{V_{\mathrm{R1}} \times V_{\mathrm{R3}}} \qquad (5)$$

where

F_{A} is the peak area obtained from V_{i};

W_{St} is the amount of compound injected with standard solution, in nanograms;

V_{Ex} is the volume, in millilitres, of ethyl acetate added in extraction step 7.4.2;

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

V_{i} is the portion of volume injected into the gas chromatograph or the liquid chromatograph, respectively, in microlitres;

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

V_{R1} is the portion of volume V_{Ex} used in 7.4.3.3, in millilitres;

V_{R2} is the volume of cyclohexane added to V_{R1} in 7.4.3.3, in millilitres;

V_{end} is the final volume obtained in 7.4.3.3 after re-dissolvation, in millilitres;

V_{R3} is the portion of volume V_{R1} + V_{R2} injected for gel permeation chromatography (volume of sample loop), in millilitres.

7.8 Proficiency testing data

All couples of the following matrices and pesticides were proven by proficiency tests without clean-up:

Matrices: Leek, mandarin.

Pesticides: See Table 6.

Table 6 — Pesticides proven by proficiency tests without clean-up

Aldicarb	Ethion	Methidathion	Pyriproxyfen
Azinphos-methyl	Fenpropathrin	Methomyl	Spinosyn A
Carbendazim	Imazalil	Orthophenylphenol	Spinosyn D
Chlorpyrifos	Imidacloprid	Oxamyl	Thiabendazole
Deltamethrin	Indoxacarb	Pendimethalin	Thiacloprid
Diazinon	Kresoxim-methyl	Phosalone	Tolylfluanid
Dimethoate	Malathion	Prochloraz	Triflumuron
EPN	Methamidophos	Prothiofos	

7.9 Applicability

Crops and foods on which the method was tested are listed in Table 7.

Table 7 — Crops and foods tested with Method P

Apples	Carrots	Head cabbage	Oranges	Strawberries
Avocados	Cucumbers	Leeks	Peaches	Tomatoes
Bananas	Grapes	Lettuce		

The pesticides and metabolites listed in Table 8 can be analysed with GC- or LC-MS/MS by this method.

Table 8 — Pesticides and metabolites that can be analysed with GC- or LC-MS/MS by Method P

Abamectin
Acephate
Acetamiprid
Acibenzolar-S-methyl
*Aclonifen**
Acrinathrin **
Aldicarb
Aldicarb-sulfone
Aldicarb-sulfoxide
Aminocarb
Aspon
Atrazine
Atrazine-desethyl
Atrazine-desisopropyl
Azinphos-ethyl
Azinphos-methyl
Azoxystrobin
Benalaxyl
Bendiocarb
Benfuracarb
Bentazone
Bifenthrin
Binapacryl
Biphenyl
Bitertanol
Boscalid
Bromophos
Bromophos-ethyl
Bromopropylate
Bromuconazole
Bupirimate
Buprofezin
Butocarboxim
Butocarboxim-sulfoxide
Butoxycarboxim
Butralin
Cadusafos
Carbaryl
Carbendazim
Carbofuran
Carbofuran-3OH
Carbophenothion
Carbosulfan
Carfentrazone-ethyl
Chinomethionat
Chlordane, cis-
Chlordane, trans-
Chlordimeform
Chlorfenapyr
Chlorfenson
Chlorfenvinphos
Chlorfluazuron
Chlormephos
Chlorobenzilate
Chlorobromuron
Chloropropylate
Chlorothalonil
Chlorpropham
Chlorpyrifos
Chlorpyrifos-methyl
Chlorpyrifos-O-Analogue
Chlorthal-dimethyl
Chlozolinate
Clofentezine
Clomazone
Clothianidin
Coumaphos
Cyanazin
Cyanofenfos
Cyanofos
Cyazofamid
Cyfluthrin
Cyfluthrin, beta-
Cypermethrin
Cyproconazole
Cyprodinil
Danifos
DEET
Deltamethrin
Demeton
Demeton-S-methyl
Demeton-S-methyl-sulfone
Demeton-S-methyl-sulfoxide
Desmethyl pirimicarb
Desmetryn
Dialifos
Diazinon
Dichlobenil
Dichlofluanid
Dichloroaniline, 3,5-
Dichlorvos
Dicloran
Dicrotophos
Dieldrin
Diethofencarb
Difenoconazole
Diflubenzuron
Dimethoate
Dimethomorph
Dinobuton
Dinocap
Dinoseb
Dinoterb
Dioxathion
Diphenamid
Diphenylamine
Disulfoton
Disulfoton-Sulfone
Disulfoton-sulfoxide
DMSA
DMST
DNOC
Endosulfan, alpha-
Endosulfan, beta-
Endosulfansulfate
Endrin
EPN
Epoxiconazole
Esfenvalerate
Ethiofencarb
Ethiofencarb-sulfone
Ethiofencarb-sulfoxide
Ethion
Ethofumesate
Ethoprophos
Etofenprox
Etrimfos
Famoxadone
Fenamiphos
Fenamiphos-Sulfone
Fenamiphos-Sulfoxide
Fenarimol
Fenazaquin
Fenbuconazole
Fenchlorphos
Fenhexamid
Fenitrothion
Fenoxycarb
Fenpiclonil
Fenpropathrin
Fenpropimorph
Fenpyroximate
Fenson
Fensulfothion
Fensulfothion-oxon
Fensulfothion-oxon-sulfone
Fensulfothion-sulfone
Fenthion
Fenthion-oxon
Fenthion-oxon-sulfone
Fenthion-oxon-sulfoxide
Fenthion-sulfone
Fenthion-sulfoxide
Fenvalerate
Fipronil
Fluacrypyrim
Fluazifop-P-butyl
Fluazinam
Flucythrinate
Fludioxinil
Fludioxonil
Flufenoxuron
Flumetralin
Fluquinconazole
Flusilazole
Flutriafol
Fonofos
Formothion
Fosthiazate
Furalaxyl
Furathiocarb
Haloxyfop
Haloxyfop-Ethoxyethylester
Haloxyfop-Methyl

Table 8 *(continued)*

HCH, alpha-
HCH, beta-
HCH, delta-
HCH, gamma-
Heptachlor
Heptachlor epoxide
Heptenophos
Hexachlorobenzene
Hexaconazole
Hexazinone
Hexythiazox
Imazalil
Imidacloprid
Indoxacarb
Iprodione
Iprovalicarb
Isofenphos
Isofenphos-methyl
Isoprocarb
Isopropalin
Isoproturon
Isoxaben
Jodfenphos
Kresoxim-methyl
Lambda-Cyhalothrin**
Leptophos
Linuron
Lufenuron
Malaoxon
Malathion
Mecarbam
Mepanipyrim
Mephosfolan
Metaflumizone
Metalaxyl
Metconazole
Methabenzthiazuron
Methamidophos
Methiocarb
Methiocarb-sulfone
Methiocarb-sulfoxide
Methomyl
Methoxychlor
Methoxyfenozide
Metidathion
Metribuzin

Mevinphos
Monocrotophos
Myclobutanil
Napropamide
Nitrofen
Omethoate
Orthophenylphenol
Oxadixyl
Oxamyl
Oxamyl-Oxime
Paclobutrazol
Paraoxon
Paraoxon-Methyl
Parathion
Parathion-methyl
Penconazole
Pencycuron
Pendimethalin
Pentachloroaniline
Pentachloroanisole
Pentachlorobenzene
Permethrin
Phenmedipham
Phenothrin
Phenthoate
Phorate
Phorate-O-Analogue
Phorate-Sulfone
Phorate-Sulfoxide
Phosalone
Phosmet
Phosmet oxon
Phosphamidon
Phoxim
Piperonyl Butoxide
Pirimicarb
Pirimiphos-Ethyl
Pirimiphos-methyl
Prochloraz
Procymidone
Profenofos
Promecarb
Propamocarb
Propaquizafop
Propargite
Propetamphos

Propham
Propiconazole
Propoxur
Propyzamide
Prosulfocarb
Prothioconazole-desthio
Prothiofos
Pymetrozine
Pyraclofos
Pyraclostrobin
Pyrazophos
Pyridaben
Pyridaphenthion
Pyrifenox
Pyrimethanil
Pyriproxyfen
Quinalphos
Quinoxyfen
Quintozene
Quizalofop
Simazine
Spinosyn A
Spinosyn D
Spiroxamine
Sulfentrazone
Sulfotep
tau-Fluvalinate
TCNB, 2,3,4,5-
Tebuconazole
Tebufenozide
Tebufenpyrad *Tecnazene*
Teflubenzuron
Tefluthrin
TEPP
Tepraloxydim
Terbufos
Terbufos Sulfone
Terbufos Sulfoxide
Terbufos-O-sulfone
Terbufos-oxon
Terbufos-oxon-sulphoxid
Terbuthylazine
Terbutryn
Tetrachloranilin, 2,3,5,6-
Tetrachlorvinphos
Tetraconazole

Tetradifon
Tetrasul
Thiabendazole
Thiacloprid
Thiametoxam
Thiodicarb
Thiometon
Thiometon-sulfone
Thiometon-sulfoxide
Thionazin
Thiophanate-methyl
Tolclofos-methyl
Tolylfluanid
Triadimefon
Triadimenol
Triamiphos
Triazamate
Triazofos
Trichlorfon
Trichloronat
Trichlorophenol, 2,4,6-
Trifloxystrobin
Triflumizole
Triflumuron
Trimethacarb, 2,3,5-
Triticonazole
Vamidothion
Vamidothion-sulfoxide
Vinclozolin
Zoxamide

* Substances shown in italics have been evaluated using GC and all other using LC.

** It has to be considered that this substance elutes very early in GPC (near to the dump volume) and thus losses of recovery can be observed.

Annex A
(informative)

Average water content of crops and foods

Table A.1 — Average water content of crops and foods

Crops and foods	Average water content (in g/100 g)
Cocoa powder	5
Cereals, spices (herbs), coffee (raw), tea, tea-like products	10
Bananas, horse radish	75
Peas, currants(black), potatoes, parsley, grapes	80
Pineapples, apples, pears, currants(red), cherries, oranges, plums, chives	85
Cauliflower, beans (green), broccoli, strawberries, grapefruit, curly kale, kohlrabi, melons, carrots, must, pepper, peaches, mushrooms, red beet, red cabbage, spinach, head cabbage, lemons, sugar beet (edible root), onions	90
Witloof chicory, endives, cucumbers, radish, rhubarb, lettuce, celeriac, asparagus, tomatoes	95

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