BS EN 15662:2018

BSI Standards Publication

Foods of plant origin - Multimethod for the determination of pesticide residues using GC- and LC-based analysis following acetonitrile extraction/partitioning and clean-up by dispersive SPE - Modular QuEChERS-method

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National foreword

This British Standard is the UK implementation of EN 15662:2018. It supersedes BS EN 15662:2008, which is withdrawn.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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Published by BSI Standards Limited 2018

ISBN 978 0 580 94294 5

ICS 67.050

Compliance with a British Standard cannot confer immunity from legal obligations.

This British Standard was published under the authority of the Standards Policy and Strategy Committee on 30 June 2018.

Amendments/corrigenda issued since publication

Date	Text affected

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 15662

May 2018

ICS 67.050

Supersedes EN 15662:2008

English Version

Foods of plant origin - Multimethod for the determination of pesticide residues using GC- and LC-based analysis following acetonitrile extraction/partitioning and clean-up by dispersive SPE - Modular QuEChERS-method

Aliments d'origine végétale - Multiméthode de détermination des résidus de pesticides par analyse CG et CL après extraction/partition avec de l'acétonitrile et purification par SPE dispersive - Méthode modulaire QuEChERS

Pflanzliche Lebensmittel - Multiverfahren zur Bestimmung von Pestizidrückständen mit GC und LC nach Acetonitril-Extraktion/Verteilung und Reinigung mit dispersiver SPE - Modulares QuEChERS-Verfahren

This European Standard was approved by CEN on 27 December 2017.

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European foreword

This document (EN 15662:2018) 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of an identical text or by endorsement, at the latest by November 2018, and conflicting national standards shall be withdrawn at the latest by November 2018.

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

This document supersedes EN 15662:2008.

With the revised version, some amendments and improvements have been taken into consideration, notably:

— the more precise differentiation between feasible modes of operation (Table 1 to Table 5);

— the opportunity to report the applied modes of operation (e.g. extraction or clean-up modules) in a simple way;

— clear indications of approved modes of operation for particular commodities (Table 6);

— the optimization of extraction efficiency by longer extraction time;

— the specification of suitable parameters for the detection with UPLC-MS/MS and GC-MS/MS;

— new approaches for the quantitation of pesticide residues including a simplified procedure for the calculation of residue levels;

— references to the improved validation data for the method (see Table 7 and CEN/TR 17063);

— a list of abbreviations has been added in Annex C.

WARNING — The application of this standard may involve hazardous materials, operations and equipment. This standard does not claim to address all the safety problems associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and to determine the applicability of regulatory limitations prior to use.

According to the CEN-CENELEC Internal Regulations, the national standards organisations 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, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

1 Scope

This European Standard stipulates a method for the analysis of pesticide residues in foods of plant origin, such as fruits (including dried fruits), vegetables (including dried vegetables), cereals and many processed products thereof by using GC, GC-MS(/MS), and/or LC-MS(/MS). The method has been collaboratively studied on a large number of commodity/pesticide combinations. Precision data are summarized in CEN/TR 17063. Guidelines for calibration are outlined in CEN/TS 17061.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

CEN/TS 17061:2017, *Foodstuffs - Guidelines for the calibration and quantitative determination of pesticide residues and organic contaminants using chromatographic methods*

3 Principle

The homogeneous sample is extracted with the help of acetonitrile. Samples with low water content (<80 %) require the addition of water before the initial extraction to get a total of approximately 10 g of water. After addition of magnesium sulfate, sodium chloride and buffering citrate salts, the mixture is shaken intensively and centrifuged for phase separation. An aliquot of the organic phase is cleaned-up by dispersive solid phase extraction (D-SPE) employing bulk sorbents as well as magnesium sulfate for the removal of residual water. Following clean-up with amino-sorbents (e.g. primary secondary amine sorbent, PSA) and if necessary graphitized carbon black (GCB) or octadecylsilane (ODS), extracts are acidified by adding a small amount of formic acid, to improve the storage stability of certain base-sensitive pesticides. The final extract can be directly employed for GC- and LC-based analysis. Suitable detectors for GC analysis are mass-selective detectors (MS or MS/MS) with unit or high mass resolution or other GC detectors, such as flame photometric detector, FPD, and electron capture detector, ECD. For the analysis with LC hyphenations with tandem mass-spectrometry (LC-MS/MS) or high resolution mass-spectrometry are particularly suitable. Quantification may be performed using an internal standard, which is added to the test portion before the first extraction, but this is not mandatory. Details for calibration, see CEN/TS 17061.

4 Preparation and storage of the samples

4.1 General

Sample processing and storage procedures should be demonstrated to have no significant effect on the residues present in the test sample (sometimes also called “analytical sample”). Processing should also ensure that the test sample is homogeneous enough so that portion to portion (sub-sampling) variability is acceptable. If a single analytical portion is unlikely to be representative of the test sample, larger or replicate portions shall be analysed, to provide a better estimate of the true value. The degree of comminution should support a quantitative residue extraction.

4.2 Laboratory sample

A laboratory sample that is wholly or extensively spoiled or degraded should not be analysed. When possible, prepare laboratory samples immediately after arrival and in any event, before any significant physical or chemical changes have taken place. If a laboratory sample cannot be prepared without delay, it should be stored under appropriate conditions to keep it fresh and to avoid deterioration. Dried or similarly processed samples should be analysed within their stated shelf life.

4.3 Partly-prepared test sample

For preparation of the partly-prepared test sample take only the portion of the laboratory sample to which the maximum residue level applies. No further plant-parts shall be removed.

The reduction of the laboratory sample shall be carried out in such a way that representative portions are obtained (e.g. by sub-division into four and selection of opposite quarters). For samples of small units (e.g. small fruits such as berries, legumes, cereals), the sample shall be thoroughly mixed before weighing out the partly-prepared test sample. When the samples are made up of larger units, take wedge-shaped sections (e.g. melons) or cross sections (e.g. cucumbers) that include the skin (outer surface) from each unit [1].

4.4 Test sample

From each partly-prepared test sample, any parts that would cause difficulties with the homogenization process should be removed. In the case of stone fruits, the stones shall be removed. This is the test sample. A record of the plant-parts that have been removed shall be kept. Precautions should be taken to avoid any losses of juice or flesh. Calculation of the residue shall be based on the mass of the original test sample (including the stones where it is necessary).

Where the homogeneity of the test sample is not sufficient or the extraction of residues may be significantly compromised due to large particle sizes, intensive comminution should be performed using appropriate means. This is possible at ambient temperature, if separation of flesh and juice or degradation of target pesticides does not occur to a significant extent. Comminution of samples in a frozen state can significantly reduce losses of chemically labile pesticides and usually results in smaller particle sizes and a higher degree of homogeneity. Cutting the samples coarsely (e.g. 3 cm x 3 cm) with a knife and putting them into the freezer (e.g. −18 °C overnight) prior to comminution facilitates processing. Processing can be also assisted and improved by cryogenic milling (using dry ice or liquid nitrogen) by keeping the temperature below 0 °C. Especially in the case of fruits and vegetables, cryogenic milling is much more effective at homogenizing commodities that have tough skins (e.g. tomatoes or grapes) compared to milling at ambient temperature. Given the fact that non-systemic pesticides often predominantly occur on the skin, cryogenic milling significantly reduces sub-sampling variability. When processing test samples at low temperatures, condensation caused by high humidity should be avoided. Residual carbon dioxide should be allowed to sufficiently dissipate so that its contribution to weigh of the sample will be negligible.

4.5 Test portion

Individual test portions each sufficient for one analysis should be taken from the comminuted test sample. These test portions should be analysed immediately. If test portions cannot be analysed directly, the test sample or the test portions shall be frozen until required. If it is noted that homogeneity of the test sample has been compromised during storage, the test sample shall be mixed before taking test portions to ensure that homogeneity has been re-established.

5 Procedure

Extraction of samples is specified through modules E1 to E9. Extraction is usually followed by a clean-up of the obtained raw extracts using the modules C1 to C5. Clean-up steps may be omitted if interference of matrix load during analysis with chromatographic methods described in modules D1 to D6 is not evident. In some cases clean-up could be replaced by dilution of the raw extracts (module C0). Prior to the determination usually some stabilization of the extracts is performed (module S1). All modules are described in detail in Annex A. Complementary information is given in Annex B.

Tables 1 to 4 contain brief descriptions of the modules as well as application notes and examples of use. For the calculation of residue concentrations in the sample extracts all of the calibration procedures and quantification methods described in options Q1 to Q7 (Table 5) are applicable. Preferred combinations of modules concerning the extraction of samples and clean-up of raw extracts are listed in Table 6 for a multitude of commodities (raw as well as processed).

Table 1 — Extraction (E)

module	Description	Preferred application	Examples
Extraction without hydrolysis			
E1	A test portion of 10 g without any addition of water is extracted with acetonitrile	Plant material and edibles with high water content (≥80 %)	Fruit and vegetables, juices
E2	10 g test portion is extracted by 10 ml acetonitrile after addition of (a) 0,6 ml or (b) 0,2 ml sodium hydroxide solution.	Plant material and edibles with high water content (≥80 %) and high acid content	(a) Lemons, lime, red current (b) raspberry, blackberry
E3	A test portion of 10 g is completed with (a) 2,5 ml or (b) 4,5 ml of water and then extracted with acetonitrile	Plant material and edibles with intermediate water content (> 40 % and < 80 %)	(a) Bananas, root and tuber vegetables (potatoes, yam, parsnip) (b) Bread, fresh dates, chestnuts
E4	Test sample is homogenized with water and a test portion of 13,5 g of the homogenate is extracted with acetonitrile.	Plant material and edibles with low water content (15 % to 40 %)	Dried fruit und similar commodities
E5	A test portion of 5 g is completed with 10 ml of water and then extracted with acetonitrile	Plant material and edibles with very low water content (<15 %) and honey	Cereal grain and products thereof, honey
E6	A test portion of 5 g is completed with 6 ml of water and then extracted with acetonitrile	Plant material and edibles with intermediate water content (>40 % to 80 %) and high matrix load or high oil content (>5 %)	Garlic, avocados
E7	A test portion of 2 g is completed with 10 ml of water and then extracted with acetonitrile	Plant material and edibles with very low water content (<15 %) and high matrix load as well as freeze-dried products	Spices, coffee, tobacco, tea, lentils, freeze-dried fruit
Extraction with hydrolysis			
E8	Hydrolysis of esters and conjugates of acidic pesticides in the slurry of 10 g sample in acetonitrile followed by extraction with acetonitrile (proposed reference test method for alkaline hydrolysis)	Plant material and edibles with neutral or acidic pH and high water content (≥80 %)	Fruit and vegetables, juices, lemons
E9	Hydrolysis of esters and conjugates of acidic pesticides in the slurry of 2 g to 5 g sample in acetonitrile followed by extraction with acetonitrile (proposed reference test method for alkaline hydrolysis)	Plant material and edibles with low water content	Cereal grain and products thereof, garlic, spices, coffee, tobacco, tea, lentils, freeze-dried fruit

Table 2 — Clean-up (C)

Module	Description	Preferred application	Examples
C0	No clean-up	Base-sensitive and acidic pesticides ($pK_a < 5$) that interact with the amino-sorbent (PSA) used in modules C2 to C5, analysis of extracts with low matrix-load	Cucumber, apples, sufficiently diluted raw-extracts
C1	Freezing-out	Removal of co-extracted fat (even in combination with further clean-up steps, e.g. C2, C3, C5)	Oranges, lemons, cereal grain
C2	Dispersive SPE with amino-sorbent (PSA)	Clean-up of raw-extracts prior to the determination of basic and neutral pesticides	Standard-procedure for any commodity not shown separately
C3	Dispersive SPE with a larger amount of amino-sorbent (PSA)	Clean-up of raw-extracts of foods of plant origin with high matrix-load prior to the determination of basic and neutral pesticides	Raw-extracts from modules E5 (e.g. cereal grain and products thereof) and E7 (e.g. coffee, tea, dried herbs, spices)
C4	Dispersive SPE with a mixture of amino-sorbent and silica-based reversed phase sorbent (PSA/ODS)	Simultaneous clean-up of raw-extracts and removal of co-extracted fat	Citrus fruit, cereal grain and products thereof, avocados, olives
C5	Dispersive SPE with a mixture of amino-sorbent and graphitized carbon black (PSA/GCB)	Clean-up of intensely coloured raw-extracts prior to the determination of basic and neutral pesticides	Iceberg lettuce, head lettuce, rocket salad

Table 3 — Extract stabilization (S)

Module	Description	Preferred application	Examples
S0	No extract stabilization	acid-labile analytes	Flazasulfurone, Mesosulfurone, Tribenurone, Triflusulfurone
S1	Extract stabilization with formic acid	base-labile and acid-stable analytes	Majority of analytes

Table 4 — Detection (D)

Module	Description	Preferred application	Examples
D1	LC-MS/MS	Extracts from modules E1 to E9 subsequently cleaned-up with modules C0 to C5	Analytes ionizable by ESI/APCI in extracts from any commodity
D2	LC-HR-MS	Extracts from modules E1 to E9 subsequently cleaned-up with modules C0 to C5	Analytes ionizable by ESI/APCI in extracts from any commodity
D3	GC-MS/MS	Extracts from modules E1 to E7 subsequently cleaned-up with modules C0 to C5	Analytes ionizable by EI/PCI/NCI in extracts from any commodity
D4	GC-MS (incl. ITD and TOF)	Extracts from modules E1 to E7 subsequently cleaned-up with modules C0 to C5	Analytes ionizable by EI/PCI/NCI in extracts from commodities with low matrix-load
D5	GC-FPD	Extracts from modules E1 to E7 subsequently cleaned-up with modules C1 to C5	Organophosphorus and sulfur-containing compounds
D6	GC-ECD	Extracts from modules E1 to E7 subsequently cleaned-up with modules C1 to C5	Organochlorine compounds

The gas chromatographic determination with single quadrupole mass spectrometric detection (preferred in SIM mode), with ion trap detectors and with time-of-flight mass spectrometric detection (independent of the MS resolution) is suited for all analytes. GC-MS analysis without clean-up is only possible if the extracts are highly diluted (module C0).

Table 5 — Quantification (Q)

Option	Description	Preferred application	Reference
Q1	Quantification using external standards in solvent	Determinations where matrix-effects are assumed to be negligible	see CEN/TS 17061:2017, 4.4.2 to 4.4.5
Q2	Quantification using external standards in matrix	Determinations where matrix-effects shall be considered	see CEN/TS 17061:2017, 4.3 and 4.4.2 to 4.4.5
Q3	Quantification using a procedural internal standard and standards in solvent	Determinations where matrix-effects are assumed to be negligible	see CEN/TS 17061:2017, 4.5.2
Q4	Quantification using standard addition to the final extract	Determinations where matrix effects shall be considered and suitable blank matrices are not available	see CEN/TS 17061:2017, 4.6.2
Q5	Quantification using a procedural internal standard and standards in matrix or isotope-labelled internal standards	Determinations where matrix-effects shall be considered for compensation of low recovery	see CEN/TS 17061:2017, 4.3, 4.5.2 and 4.5.3
Q6	Quantification using standard addition to the sample	Determinations where matrix-effects shall be considered without availability of blank (control) samples or incomplete extractions of the analyte occur	see CEN/TS 17061:2017, 4.6.3
Q7	Quantification by calibration of the entire procedure	Determinations where matrix-effects shall be considered or incomplete extractions of the analyte occur	see CEN/TS 17061:2017, 4.7

Table 6 — Preferred combinations of extraction and clean-up modules for particular commodities

Commodity	Extraction (E)	Description (E)[a]	Clean-up (C)	Description (C)[b]	Clean-up (C altern.)	Description (C altern.)[b]
Apple juice	E1	10 g / 0 ml	C2	PSA 25	—	—
Apple pomace	E1	10 g / 0 ml	C2	PSA 25	—	—
Apples	E1	10 g / 0 ml	C2	PSA 25	—	—
Apples, dried	E4	500 g / 850 ml	C2	PSA 25	—	—
Apricots	E1	10 g / 0 ml	C2	PSA 25	—	—
Apricots, dried	E4	500 g / 850 ml	C2	PSA 25	—	—
Apricot juice	E1	10 g / 0 ml	C2	PSA 25	—	—
Asparagus	E1	10 g / 0 ml	C2	PSA 25	—	—
Aubergine	E1	10 g / 0 ml	C2	PSA 25	—	—
Avocado	E6	5 g / 6 ml	C1 + C2	Freeze out + PSA 25	C4	PSA 25 + C18 25
Bananas	E3a	10 g / 2,5 ml	C2	PSA 25	—	—
Bananas, freeze-dried	E7	2 g / 10 ml	C2	PSA 25	—	—
Basil	E1	10 g / 0 ml	C5b	PSA 25 +GCB 7,5	—	—
Bean seeds, fresh	E3a	10 g / 2,5 ml	C2	PSA 25	—	—
Beans, dried	E5	5 g / 10 ml	C2	PSA 25	—	—
Beetroot	E1	10 g / 0 ml	C2	PSA 25	—	—
Blackberries	E2b	10 g / NaOH 2	C2	PSA 25	—	—
Blackberries, freeze-dried	E7	2 g / 10 ml	C2	PSA 25	C3a	PSA 50
Blueberries	E1	10 g / 0 ml	C2	PSA 25	—	—
Blueberries, dried (14 % water)	E4	500 g / 850 ml	C2	PSA 25	—	—
Blueberries, freeze-dried	E7	2 g / 10 ml	C2	PSA 25	C3a	PSA 50
Bread (34 % to 43 % water)	E3b	10 g / 4,5 ml	C2	PSA 25	—	—
Breadfruits (70 % water)	E3a	10 g / 2,5 ml	C2	PSA 25	—	—
Broccoli	E1	10 g / 0 ml	C2	PSA 25	—	—
Carrot	E1	10 g / 0 ml	C5a	PSA 25 + GCB 2,5	—	—
Carrots, freeze-dried	E7	2 g / 10 ml	C2	PSA 25	C5a	PSA 25 + GCB 2,5
Cauliflower	E1	10 g / 0 ml	C2	PSA 25	—	—
Celeriacs/turnip rooted celeries	E1	10 g / 0 ml	C2	PSA 25	C5a	PSA 25 + GCB 2,5
Celery	E1	10 g / 0 ml	C2	PSA 25	—	—
Celery, freeze-dried	E7	2 g / 10 ml	C2	PSA 25	C5b	PSA 25 + GCB 7,5

Commodity	Extraction (E)	Description (E)[a]	Clean-up (C)	Description (C)[b]	Clean-up (C altern.)	Description (C altern.)[b]
Cereal flour	E5	5 g / 10 ml	C1 + C3a	Freeze out + PSA 50	C4	PSA 25 + C18 25
Cereal grain	E5	5 g / 10 ml	C1 + C3a	Freeze out + PSA 50	C4	PSA 25 + C18 25
Cereal semolina	E5	5 g / 10 ml	C 1 + C 3a	Freeze out + PSA 50	C4	PSA 25 + C18 25
Cereals flakes	E5	5 g / 10 ml	C1 + C3a	Freeze out + PSA 50	C4	PSA 25 + C18 25
Cherries	E1	10 g / 0 ml	C2	PSA 25	—	—
Chestnuts (45 % to 52 % water)	E3b	10 g / 4,5 ml	C2	PSA 25	—	—
Chinese cabbages	E1	10 g / 0 ml	C2	PSA 25	—	—
Chives	E1	10 g / 0 ml	C5b	PSA 25 + GCB 7,5	—	—
Chives, freeze-dried	E7	2 g / 10 ml	C2	PSA 25	C5b	PSA 25 + GCB 7,5
Coconut, fresh	E6	5 g / 6 ml	C2	PSA 25	—	—
Coffee beans	E7	2 g / 10 ml	C3b	PSA 75	—	—
Coriander	E1	10 g / 0 ml	C5b	PSA 25 + GCB 7,5	—	—
Corn, freeze-dried	E7	2 g / 10 ml	C2	PSA 25	—	—
Corn, fresh	E3a	10 g / 2,5 ml	C2	PSA 25	—	—
Courgettes	E1	10 g / 0 ml	C2	PSA 25	—	—
Cress	E1	10 g / 0 ml	C5b	PSA 25 + GCB 7,5	—	—
Cucumber	E1	10 g / 0 ml	C2	PSA 25	—	—
Currants	E2a	10 g / NaOH 1	C2	PSA 25	—	—
Currants, freeze-dried	E7	2 g / 10 ml	C2	PSA 25	—	—
Currants juice	E2a	10 g / NaOH 1	C2	PSA 25	—	—
Dates, dried	E4	500 g / 850 ml	C2	PSA 25	—	—
Dates, fresh (50 % to 60 % water)	E3b	10 g / 4,5 ml	C2	PSA 25	—	—
Durian	E6	5 g / 6 ml	C1 + C2	Freeze out + PSA 25	C4	PSA 25 + C18 25
Escaroles/broad-leaved endives	E1	10 g / 0 ml	C5a	PSA 25 + GCB 2,5	—	—
Figs, dried	E4	500 g / 850 ml	C2	PSA 25	—	—
Fungi cultivated	E1	10 g / 0 ml	C2	PSA 25	—	—
Fungi, dried (e.g. Shitake, boletus)	E5	5 g / 10 ml	C2	PSA 25	—	—
Garlic (59 % water)	E6	5 g / 6 ml	C2	PSA 25	—	—
Ginger (79 % water)	E6	5 g / 6 ml	C2	PSA 25	—	—

Commodity	Extraction (E)	Description (E)[a]	Clean-up (C)	Description (C)[b]	Clean-up (C altern.)	Description (C altern.)[b]
Ginkgo seeds (55 % water)	E3b	10 g / 4,5 ml	C2	PSA 25	—	—
Globe artichokes	E1	10 g / 0 ml	C2	PSA 25	—	—
Gooseberrys	E2b	10 g / NaOH 2	C2	PSA 25	—	—
Grape leaves	E3a	10 g / 2,5 ml	C2	PSA 25	—	—
Grape leaves	E1	10 g / 0 ml	C5b	PSA 25 + GCB 7,5	—	—
Grapefruit	E1	10 g / 0 ml	C1 + C2	Freeze out + PSA 25	—	—
Grapes	E1	10 g / 0 ml	C2	PSA 25	—	—
Head brassica	E1	10 g / 0 ml	C2	PSA 25	—	—
Head lettuce	E1	10 g / 0 ml	C5a	PSA 25 + GCB 2,5	—	—
Honey	E5	5 g / 10 ml	C2	PSA 25	—	—
Honeydew melon	E1	10 g / 0 ml	C2	PSA 25	—	—
Horseradish	E3a	10 g / 2,5 ml	C2	PSA 25	—	—
Jackfruit (74 % water)	E3a	10 g / 2,5 ml	C2	PSA 25	—	—
Kales	E1	10 g / 0 ml	C5b	PSA 25 + GCB 7,5	—	—
Kiwi	E1	10 g / 0 ml	C2	PSA 25	—	—
Kohlrabi	E1	10 g / 0 ml	C2	PSA 25	—	—
Lamb's lettuces	E1	10 g / 0 ml	C5b	PSA 25 + GCB 7,5	—	—
Leek	E1	10 g / 0 ml	C2	PSA 25	—	—
Leek, freeze-dried	E7	2 g / 10 ml	C2	PSA 25	—	—
Lemon grass, fresh (71 % water)	E6	5 g / 6 ml	C2	PSA 25	—	—
Lemon juice	E2a	10 g / NaOH 1	C2	PSA 25	—	—
Lemons	E2a	10 g / NaOH 1	C1 + C2	Freeze out + PSA 25	—	—
Lentils, dried	E5	5 g / 10 ml	C2	PSA 25	—	—
Lime juice	E2a	10 g / NaOH 1	C2	PSA 25	—	—
Limes	E2a	10 g / NaOH 1	C1 + C2	Freeze out + PSA 25	—	—
Lotus roots (79 % water)	E3a	10 g / 2,5 ml	C2	PSA 25	—	—
Lotus seeds	E3a	10 g / 2,5 ml	C2	PSA 25	—	—
Lotus seeds, dried (14 % water)	E5	5 g / 10 ml	C2	PSA 25	—	—
Mandarins	E1	10 g / 0 ml	C1 + C2	Freeze out + PSA 25	—	—
Mango	E1	10 g / 0 ml	C5a	PSA 25 + GCB 2,5	—	—

Commodity	Extraction (E)	Description (E)[a]	Clean-up (C)	Description (C)[b]	Clean-up (C altern.)	Description (C altern.)[b]
Mango, freeze-dried	E7	2 g / 10 ml	C2	PSA 25	C5b	PSA 25 + GCB 7,5
Mirabelle	E1	10 g / 0 ml	C2	PSA 25	—	—
Nectarines	E1	10 g / 0 ml	C2	PSA 25	—	—
Olives	E6	5 g / 6 ml	C1 + C2	Freeze out + PSA 25	C4	PSA 25 + C18 25
Onions	E1	10 g / 0 ml	C2	PSA 25	—	—
Oranges	E1	10 g / 0 ml	C1 + C2	Freeze out + PSA 25	—	—
Papaya	E1	10 g / 0 ml	C2	PSA 25	—	—
Parsley	E1	10 g / 0 ml	C5b	PSA 25 + GCB 7,5	—	—
Parsnip	E3a	10 g / 2,5 ml	C2	PSA 25	—	—
Peaches	E1	10 g / 0 ml	C2	PSA 25	—	—
Peaches, freeze-dried	E7	2 g / 10 ml	C2	PSA 25	—	—
Pears	E1	10 g / 0 ml	C2	PSA 25	—	—
Peas, dried	E5	5 g / 10 ml	C2	PSA 25	—	—
Peas, freeze-dried	E7	2 g / 10 ml	C2	PSA 25	—	—
Pepper, freeze-dried	E7	2 g / 10 ml	C2	PSA 25	C5b	PSA 25 + GCB 7,5
Pepper, green, yellow	E1	10 g / 0 ml	C2	PSA 25	—	—
Pepper, red	E1	10 g / 0 ml	C5b	PSA 25 + GCB 7,5	—	—
Peppermint, fresh	E3a	10 g / 2,5 ml	C5b	PSA 25 + GCB 7,5	—	—
Peppermint, fresh (78 % water)	E6	5 g / 6 ml	C2	PSA 25	—	—
Pineapples	E1	10 g / 0 ml	C2	PSA 25	—	—
Pineapples, freeze-dried	E7	2 g / 10 ml	C2	PSA 25	—	—
Plantain	E3a	10 g / 2,5 ml	C2	PSA 25	—	—
Plums	E1	10 g / 0 ml	C2	PSA 25	—	—
Plums, dried	E4	500 g / 850 ml	C2	PSA 25	—	—
Potatoes	E1	10 g / 0 ml	C2	PSA 25	—	—
Potatoes	E3a	10 g / 2,5 ml	C2	PSA 25	—	—
Pumpkins	E1	10 g / 0 ml	C5a	PSA 25 + GCB 2,5	—	—
Quinces	E1	10 g / 0 ml	C2	PSA 25	—	—
Radish	E1	10 g / 0 ml	C2	PSA 25	—	—
Raisins	E4	500 g / 850 ml	C2	PSA 25	—	—
Raspberries	E2b	10 g / NaOH 2	C2	PSA 25	—	—
Red cabbage	E1	10 g / 0 ml	C2	PSA 25	—	—
Rhubarb	E2b	10 g / NaOH 2	C2	PSA 25	—	—
Rhubarb juice	E2b	10 g / NaOH 2	C2	PSA 25	—	—

Commodity	Extraction (E)	Description (E)[a]	Clean-up (C)	Description (C)[b]	Clean-up (C altern.)	Description (C altern.)[b]
Rocket	E1	10 g / 0 ml	C5b	PSA 25 + GCB 7,5	—	—
Romaine lettuce	E1	10 g / 0 ml	C5a	PSA 25 + GCB 2,5	—	—
Rosemary (68 % water)	E6	5 g / 6 ml	C2	PSA 25	—	—
Rosemary, fresh	E3a	10 g / 2,5 ml	C2	PSA 25	—	—
Sage, fresh	E6	5 g / 6 ml	C2	PSA 25	—	—
Sage, fresh	E3a	10 g / 2,5 ml	C5b	PSA 25 + GCB 7,5	—	—
Salsifies	E1	10 g / 0 ml	C2	PSA 25	—	—
Savoy cabbage	E1	10 g / 0 ml	C2	PSA 25	—	—
Shallots	E1	10 g / 0 ml	C2	PSA 25	—	—
Shallots, freeze-dried	E7	2 g / 10 ml	C2	PSA 25	—	—
Spearmint	E1	10 g / 0 ml	C5b	PSA 25 + GCB 7,5	—	—
Spices	E7	2 g / 10 ml	C2	PSA 25	—	—
Spinaches	E1	10 g / 0 ml	C5b	PSA 25 + GCB 7,5	—	—
Strawberries	E1	10 g / 0 ml	C2	PSA 25	—	—
Strawberries, freeze-dried	E7	2 g / 10 ml	C2	PSA 25	C3a	PSA 50
Sweet potatoes	E3a	10 g / 2,5 ml	C2	PSA 25	—	—
Tamarind (31 % water)	E4	500 g / 850 ml	C2	PSA 25	—	—
Taro/eddoe	E3a	10 g / 2,5 ml	C2	PSA 25	—	—
Tea	E7	2 g / 10 ml	C3b	PSA 75	—	—
Thyme, dried	E7	2 g / 10 ml	C2	PSA 25	C5b	PSA 25 + GCB 7,5
Thyme, fresh	E3a	10 g / 2,5 ml	C5b	PSA 25 + GCB 7,5	—	—
Thyme, fresh (65 % water)	E6	5 g / 6 ml	C2	PSA 25	—	—
Tomatoes	E1	10 g / 0 ml	C2	PSA 25	—	—
Tomatoes, dried (14,5 % water)	E4	500 g / 850 ml	C2	PSA 25	—	—
Vegetables	E1	10 g / 0 ml	C2	PSA 25	—	—
Vicia faba (with pods)	E3a	10 g / 2,5 ml	C2	PSA 25	—	—
Wheat sprouts (47 % water)	E3b	10 g / 4,5 ml	C2	PSA 25	—	—
Wine	E1	10 g / 0 ml	C2	PSA 25	—	—
Yams	E3a	10 g / 2,5 ml	C2	PSA 25	—	—

[a] The volume in ml refers to the addition of water; NaOH 1 means addition of 0,6 ml of NaOH (5 mol/l); NaOH 2 means addition of 0,2 ml of NaOH (5 mol/l)

[b] The numbers refer to the weight in mg of PSA (Primary secondary amine sorbent) and/or GCB (Graphitized carbon black) per ml of extract

6 Evaluation of results

6.1 Identification and quantification

A number of parameters can be employed to determine the identity of an analyte present in the sample extract. This includes:

— retention time of the analyte in question (Rt) or, even better, the retention time ratio against the ISTD ($Rt_{(A)}/Rt_{(ISTD)}$) obtained from the same run;

— in case of MS or MS/MS detection, the relative abundance of the recorded masses or transitions respectively (in general 2 SRM transitions are required in MS/MS and 3 ions in MS applications), see also [2], [3], [4]; and

— characteristic peak shape/pattern of the analyte.

The parameters obtained for the analyte to be identified in the sample extract are compared with those obtained for the pesticides in the calibration solution(s). Should a higher degree of certainty be required for the confirmation of the analyte identity, additional measures may be necessary, such as the use of different chromatographic separation conditions or the evaluation of additional m/z or SRM transitions. For more information about the required confirmation criteria (e.g. the recommended maximum tolerances for ion ratios using different MS techniques) see the EU-quality control guidelines described in SANTE/11813/2017 [5]. Table A.1 gives a list of the ISTDs that can be employed. The use of more than one ISTD will provide some backup information.

Use standard solutions to check linearity and to determine the calibration functions for each analyte. The use of matrix-matched standards is to be preferred, however, for a first estimate of the residue level of pesticides in the food or to show their absence, the standard solutions in pure solvent can be used. They can be also used for quantification if preliminary experiments indicate that any suppression or enhancement effects experienced do not significantly affect the results obtained. As soon as relevant residue concentrations are detected (e.g. suspected MRL violations), a more precise determination using matrix-matched standards or the standard addition method should be used.

NOTE Matrix effects influence the response of target analytes in sample extracts compared to the response of standard solutions in pure solvent.

The calibration range should be appropriate to the residue concentrations to be quantified. Thus, it may be necessary to construct more than one calibration graph from the results of calibration measurements.

This standard contains the option to use an internal standard for quantification and identification. Nevertheless, it is still possible to quantify without ISTD. Without ISTD, the volume of the acetonitrile phase is assumed to be identical to the volume of acetonitrile added to the sample (10 ml).

6.2 Calibration

The analytical method is calibrated according to CEN/TS 17061 or the EU-quality control guidelines described in SANTE/11813/2017 [5]. A suitable calibration procedure should be selected from one of the quantification options Q1 to Q7 in A.7.

6.3 Calculation of residue concentration

The mass fraction w_A of each identified analyte depends on the mass concentration of the sample in the final extract $\rho_{\text{sample}}^{\text{final extract}}$ after application of clean-up (modules C0 to C5) and the concentration ρ_A of the substance in this extract (determined according to one of the options Q1 to Q7 given in A.7). It is expressed in mg/kg and is calculated by Formula (1).

$$w_A = \frac{\rho_A}{\rho_{\text{sample}}^{\text{final extract}}} \quad (1)$$

where

ρ_A is the mass concentration of the analyte in the sample extract (option Q, see A.7.1.3, A.7.2.3, A.7.3.3 and A.7.5.3) in µg/ml;

$\rho_{\text{sample}}^{\text{final extract}}$ is the mass concentration of the sample in the final extract (modules C, see A.4.1.3, A.4.2.3, A.4.3.3, etc.), in g/ml.

6.4 Validity of the method

The recoveries obtained from experiments (spiking levels 0,01 mg/kg to 0,25 mg/kg) were usually between 70 % and 110 %.

Interlaboratory method validation studies covered a multitude of analytes using representative commodities (usually cucumber, lemon, wheat flour and raisins). Furthermore extensive individual validation has been carried out. All validation data provided by laboratories are published in the Data Pool of the European Union Reference Laboratories [6].

Validity of the method is confirmed for any specific commodity/pesticide combination if at least two laboratories conducted independently validation studies with the same matrix at two identical fortification levels with at least five replicates per level and obtained a recovery between 70 % and 120 %. Furthermore, the relative standard deviation had to be below or equal 20 % for both spiking levels in each laboratory. CEN/TR 17063 contains all important pesticides, which fulfil these conditions for at least one matrix group.

Table 7 shows the confirmed validation data of the method for specific analytes and commodity groups.

Table 7 — Validation data

No.	Analyte	CAS-No.	Commodity group (see [5])			
			High water content	High water and high acid content	Low water and high starch content	Low water and high sugar content
1	2,4-D	94–75–7	x	x	x	x
2	Acephate	30 560–19–1	x	x	x	x
3	Acetamiprid	135 410–20–7	x	x	x	x
4	Acrinathrin	101 007–06–1	x	x	x	x
5	Aldicarb	116–06–3	x	x	x	x
6	Avermectin B1a	65 195–55–3	x	x	x	x
7	Azinphos-methyl	86–50–0	x	x	x	x
8	Azoxystrobin	131 860–33–8	x	x	x	x
9	Benalaxyl	71 626–11–4	x	x	x	x
10	Bifenthrin	82 657–04–3	x	x	x	x
11	Biphenyl	92–52–4	x	x	x	x
12	Bitertanol	70 585–36–3	x			
13	Boscalid	188 425–85–6	x	x	x	x

No.	Analyte	CAS-No.	Commodity group (see [5])			
			High water content	High water and high acid content	Low water and high starch content	Low water and high sugar content
14	Bromopropylate	18 181–80–1	x	x	x	x
15	Bromoxynil	1 689–84–5	x	x	x	x
16	Bromuconazole	116 255–48–2	x	x	x	x
17	Bupirimate	41 483–43–6	x	x	x	x
18	Buprofezin	69 327–76–0	x	x	x	x
19	Carbaryl	63–25–2	x	x	x	x
20	Carbendazim	10 605–21–7	x	x	x	x
21	Carbofuran	1 563–66–2	x	x	x	x
22	Carbofuran, 3-hydroxy-	16 655–82–6	x	x	x	x
23	Carboxin	5 234–68–4	x	x	x	x
24	Chlorfenapyr	122 453–73–0	x	x	x	x
25	Chlorfenvinphos	470–90–6	x			
26	Chlorpropham	101–21–3	x			
27	Chlorpyrifos (ethyl)	2 921–88–2	x	x		
28	Chlorpyrifos-methyl	5 598–13–0	x	x	x	x
29	Chlorthal-dimethyl	1 861–32–1	x	x	x	x
30	Clofentezine	74 115–24–5	x	x	x	x
31	Clomazone	81 777–89–1	x	x	x	x
32	Clothianidin	210 880–92–5	x	x	x	x
33	Cyazofamid	120 116–88–3	x	x	x	x
34	Cyfluthrin	68 359–37–5	x	x		
35	Cyhalothrin, lambda-	91 465–08–6	x	x		
36	Cymoxanil	57 966–95–7	x	x	x	x
37	Cypermethrin	52 315–07–8	x	x		
38	Cyproconazole	94 361–06–5	x	x	x	x
39	Cyprodinil	121 552–61–2	x	x	x	x
40	DDD, p,p-	72–54–8	x			
41	DDE, p,p-	72–55–9	x			
42	Deltamethrin	52 918–63–5	x			
43	Demeton-S-methyl sulphon	17 040–19–6	x	x	x	x
44	Diazinon	333–41–5	x			
45	Dichlorprop-P	15 165–67–0	x	x	x	x
46	Dichlorvos	62–73–7	x	x	x	x
47	Dicloran	99–30–9	x	x	x	x
48	Dicrotophos	3 735–78–3	x	x	x	x

No.	Analyte	CAS-No.	Commodity group (see [5])			
			High water content	High water and high acid content	Low water and high starch content	Low water and high sugar content
49	Dieldrin	60–57–1	x	x	x	x
50	Diethofencarb	87 130–20–9	x	x	x	x
51	Difenoconazole	119 446–68–3	x	x	x	x
52	Diflubenzuron	35 367–38–5	x	x	x	
53	Dimethoate	60–51–5	x	x	x	x
54	Dimethomorph	110 488–70–5	x	x	x	
55	Diniconazole	83 657–24–3	x	x	x	x
56	Diphenylamine	122–39–4	x	x		x
57	Diuron	330–54–1	x	x	x	x
58	Endosulfan sulfate	1 031–07–8	x	x	x	x
59	Endosulfan, alpha-	33 213–66–0	x	x	x	x
60	Endosulfan, beta-	33 213–65–9	x	x	x	x
61	Epoxiconazol	133 855–98–8	x	x	x	x
62	Ethofumesate	26 225–79–6	x	x	x	x
63	Ethoprophos	13 194–48–4	x	x	x	x
64	Etofenprox	80 844–07–1	x	x	x	x
65	Etridiazol	2 593–15–9	x	x	x	x
66	Famoxadone	131 807–57–3	x	x	x	x
67	Fenamidone	161 326–34–7	x	x	x	x
68	Fenarimol	60 168–88–9	x	x	x	x
69	Fenazaquin	120 928–09–8	x	x	x	x
70	Fenbuconazole	114 369–43–6	x	x	x	x
71	Fenhexamid	126 833–17–8	x	x	x	x
72	Fenitrothion	122–14–5	x	x	x	x
73	Fenoxycarb	79 127–80–3	x	x	x	x
74	Fenpropathrin	64 257–84–7	x	x	x	x
75	Fenpropidin	67 306–00–7	x	x	x	x
76	Fenpropimorph	67 306–03–0	x	x	x	x
77	Fenpyroximate	111 812–58–9	x	x	x	x
78	Fenthion	55–38–9	x	x	x	x
79	Fenvalerate	51 630–58–1	x	x	x	x
80	Fipronil	120 068–37–3	x	x	x	x
81	Flonicamid	158 062–67–0	x	x	x	x
82	Fluazifop	69 335–91–7	x	x	x	x
83	Fluazifop-P	83 066–88–0	x	x	x	x
84	Fludioxonil	131 341–86–1	x	x	x	x

No.	Analyte	CAS-No.	Commodity group (see [5])			
			High water content	High water and high acid content	Low water and high starch content	Low water and high sugar content
85	Flufenoxuron	101 463–69–8	x	x	x	x
86	Fluopicolid	239 110–15–7	x	x	x	x
87	Fluquinconazole	136 426–54–5	x			
88	Flusilazole	85 509–19–9	x	x	x	x
89	Flutolanil	66 332–96–5	x	x	x	x
90	Flutriafol	76 674–21–0	x	x	x	x
91	Fluvalinate	69 409–94–5	x			
92	Formetanate	22 259–30–9	x	x	x	x
93	Haloxyfop-P	95 977–29–0	x	x	x	x
94	Hexachlorobenzene (HCB)	118–74–1	x			
95	Hexaconazole	79 983–71–4	x	x	x	x
96	Hexythiazox	78 587–05–0	x	x	x	x
97	Imazalil	35 554–44–0	x	x	x	x
98	Imidacloprid	138 261–41–3	x	x	x	x
99	Indoxacarb	173 584–44–6	x	x	x	x
100	Iprodione	36 734–19–7	x	x	x	x
101	Iprovalicarb	140 923–17–7	x	x	x	x
102	Isofenphos-methyl	99 675–03–3	x	x	x	x
103	Kresoxim-methyl	143 390–89–0	x	x		
104	Linuron	330–55–2	x	x	x	x
105	Lufenuron	103 055–07–8	x	x		
106	Malaoxon	1 634–78–2	x	x	x	x
107	Malathion	121–75–5	x	x	x	x
108	Mandipropamid	374 726–62–2	x	x	x	x
109	MCPA	94–74–6	x	x	x	x
110	Mepanipyrim	110 235–47–7	x	x	x	x
111	Metalaxyl	57 837–19–1	x	x	x	x
112	Metamitron	41 394–05–2	x	x	x	x
113	Methamidophos	10 265–92–6	x	x	x	x
114	Methidathion	950–37–8	x	x	x	x
115	Methiocarb	2 032–65–7	x	x	x	x
116	Methiocarb-sulfone	2 179–25–1	x		x	x
117	Methiocarb-sulfoxid	2 635–10–1	x	x	x	x
118	Methomyl	16 752–77–5	x	x	x	x
119	Methoxyfenozide	161 050–58–4	x	x	x	x

No.	Analyte	CAS-No.	Commodity group (see [5])			
			High water content	High water and high acid content	Low water and high starch content	Low water and high sugar content
120	Metolachlor (S-)	51 218–45–2	x	x	x	x
121	Metrafenone	220 899–03–6	x	x	x	x
122	Metribuzin	21 087–64–9	x	x	x	x
123	Monocrotophos	6 923–22–4	x	x	x	x
124	Myclobutanil	88 671–89–0	x	x		
125	Omethoate	1 113–02–6	x	x	x	x
126	Oxadiazon	19 666–30–9	x			
127	Oxadixyl	77 732–09–3	x	x	x	x
128	Oxamyl	23 135–22–0	x	x	x	x
129	Paraoxon-methyl	950–35–6	x	x	x	x
130	Parathion	56–38–2	x			
131	Parathion-methyl	298–00–0	x			
132	Penconazole	66 246–88–6	x	x	x	x
133	Pencycuron	66 063–05–6	x	x	x	x
134	Pendimethalin	40 487–42–1	x	x	x	x
135	Permethrin	52 645–53–1	x			
136	Phenmedipham	13 684–63–4	x	x	x	x
137	Phenylphenol, 2-	90–43–7	x	x	x	x
138	Phosalone	2 310–17–0	x			
139	Phosmet	732–11–6	x			
140	Phoxim	14 816–18–3	x	x	x	x
141	Piperonyl butoxide	51–03–6	x	x	x	x
142	Pirimicarb	23 103–98–2	x	x	x	x
143	Pirimicarb, desmethyl-	30 614–22–3	x	x	x	x
144	Pirimiphos-methyl	29 232–93–7	x	x	x	x
145	Procymidone	32 809–16–8	x	x		
146	Profenofos	41 198–08–7	x	x	x	x
147	Propamocarb hydrocloride	24 579–73–5	x	x	x	x
148	Propargite	2 312–35–8	x	x	x	x
149	Propiconazole	60 207–90–1	x	x	x	x
150	Propoxur	114–26–1	x	x	x	x
151	Propyzamide	23 950–58–5	x	x	x	x
152	Prosulfocarb	52 888–80–9	x	x	x	x
153	Prothiofos	34 643–46–4	x			
154	Pymetrozine	123 312–89–0			x	x

No.	Analyte	CAS-No.	Commodity group (see [5])			
			High water content	High water and high acid content	Low water and high starch content	Low water and high sugar content
155	Pyraclostrobin	175 013–18–0	x	x	x	x
156	Pyridaben	96 489–71–3	x	x		
157	Pyrifenox	88 283–41–4	x	x	x	x
158	Pyrimethanil	53 112–28–0	x	x	x	x
159	Pyriproxyfen	95 737–68–1	x	x	x	x
160	Quinalphos	13 593–03–8	x	x	x	x
161	Quinoxyfen	124 495–18–7	x	x	x	x
162	Quintozene (PCNB)	82–68–8	x	x	x	x
163	Spinosyn A	131 929–60–7	x	x	x	x
164	Spinosyn D	131 929–63–0	x	x	x	x
165	Spirodiclofen	148 477–71–8	x	x	x	x
166	Spiroxamine	118 134–30–8	x	x	x	x
167	Tebuconazole	107 534–96–3	x	x	x	x
168	Tebufenozide	112 410–23–8	x	x	x	x
169	Tebufenpyrad	119 168–77–3	x	x	x	x
170	Teflubenzuron	83 121–18–0	x			
171	Tefluthrin	79 538–32–2	x	x	x	x
172	Tepraloxydim	149 979–41–9	x	x	x	x
173	Tetraconazole	112 281–77–3	x	x	x	x
174	Tetradifon	116–29–0	x	x	x	x
175	Tetramethrin	51 384–90–4	x			
176	Thiabendazole	148–79–8	x	x	x	x
177	Thiacloprid	111 988–49–9	x	x	x	x
178	Thiamethoxam	153 719–23–4	x	x	x	x
179	Tolclofos-methyl	57 018–04–9	x	x	x	x
180	Tolylfluanid	731–27–1	x	x		x
181	Triadimefon	43 121–43–3	x	x	x	x
182	Triadimenol	55 219–65–3	x			x
183	Triazophos	24 017–47–8	x			
184	Tricyclazole	41 814–78–2	x			
185	Trifloxystrobin	141 517–21–7	x	x	x	x
186	Triflumizole	68 694–11–1	x	x	x	x
187	Triflumuron	64 628–44–0	x	x	x	x
188	Trifluralin	1 582–09–8	x			
189	Vinclozolin	50 471–44–8	x	x	x	x
190	Zoxamide	156 052–68–5	x	x	x	x

7 Confirmatory tests

A confirmation of quantity involves analysis of a second test portion and is to be performed if the first analysis indicates a suspected limit exceeding residue. More information about the confirmation of identity is given in [5].

8 Precision

Details of the inter-laboratory test of the precision of the method are summarized in CEN/TR 17063. The values derived from the inter-laboratory test may not be applicable to pesticide concentration ranges and matrices other than given in CEN/TR 17063.

9 Test report

The test report shall contain at least the following:

— all information necessary for the identification of the sample;

— a reference to this European Standard;

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

— the date and type of sampling procedure (if possible);

— the date of receipt of sample in the laboratory;

— the date of test;

— any particular observations made in the course of the test;

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

Annex A
(informative)

Description of modules

A.1 Reagents used in all extraction (E) and clean-up (C) and stabilization (S) modules

A.1.1 General and safety aspects:

Unless otherwise specified, use reagents of recognized analytical grade. Take every precaution to avoid possible contamination of water, solvents, sorbents, inorganic salts, etc.

WARNING — The application of this standard may involve hazardous materials, operations and equipment. This standard does not claim to address all the safety problems associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and to determine the applicability of regulatory limitations prior to use.

DISCLAIMER — This standard refers to several trade names products and instruments which are commercially available and suitable for the described procedure. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of the products named. Equivalent products may be used if they can be shown to lead to equivalent results.

A.1.2 Internal standard and quality control standard solutions in acetonitrile, ρ = 10 µg/ml to 50 µg/ml:

Table A.1 shows a list of potential internal standards (ISTDs) and quality control (QC) standard solutions that may be used in this method. The suggested concentration values (C_{ISTD}) listed refer to the ISTD solutions that should be added at the first extraction step. An appropriate dilution of this solution ($C_{\mathrm{ISTD}}^{\mathrm{cal\ mix}}$) should be prepared to be used for the preparation of the standard solutions.

A.1.3 Acetonitrile, HPLC quality.

A.1.4 Magnesium sulfate, anhydrous, grit

Phthalates may be removed in a muffle furnace by heating to 550 °C (e.g. overnight)

A.1.5 Sodium chloride.

A.1.6 Disodium hydrogencitrate sesquihydrate.

A.1.7 Trisodium citrate dihydrate.

A.1.8 Buffer-salt-mixture for second extraction and partitioning:

Weigh 4 g ± 0,2 g of anhydrous magnesium sulfate (A.1.4), 1 g ± 0,05 g of sodium chloride (A.1.5), 1 g ± 0,05 g of trisodium citrate dihydrate (A.1.7) and 0,5 g ± 0,03 g of disodium hydrogencitrate sesquihydrate (A.1.6) into a centrifuge tube (A.2.4). This amount refers to approximately 10 ml water in the sample. Commercially available salt mixtures of identical composition may be used.

NOTE: It is advisable to prepare a sufficient number of buffer-salt-mixtures in advance so that extraction series can be performed quickly without interruption.

A.1.9 Sodium hydroxide solution, substance concentration c = 5 mol/l:

Dissolve 2 g of sodium hydroxide in approximately 5 ml of water and dilute to 10 ml.

A.1.10 Cold water (<4 °C).

A.1.11 Dry ice.

A.1.12 Liquid nitrogen.

A.1.13 Sulfuric acid c = 2,5 mol/l (5N)

Dissolve 25 g of concentrated sulfuric acid (18 mol/l) in 50 ml of water and dilute to 100 ml.

A.1.14 Magnesium sulfate, anhydrous, fine powder, phthalates may be removed in a muffle furnace by heating to 550 °C (e.g. overnight)

A.1.15 Primary secondary amine sorbent

Other amine sorbents may be used, but investigations may be necessary to prove equivalency especially regarding analyte losses and pH value of the final extracts.

A.1.16 C18 sorbent, C18 reversed phase sorbent, Octadecyl, endcapped (ODS ec/C18 ec).

A.1.17 Graphitized Carbon Black sorbent (GCB)

Other graphitized carbon sorbents may be used, but investigations will be necessary to prove equivalency especially regarding analyte losses and pH value of the final extracts.

A.1.18 Formic acid solution in acetonitrile, volume fraction φ = 5 ml formic acid/100 ml:

Dilute 0,5 ml of formic acid (mass fraction $w \geq 95$ %) to 10 ml with acetonitrile (A.1.3).

Table A.1 — Potential internal standards (ISTDs) or quality control (QC) standards

Compound	Log P[a]	Chlor-ine atoms	ρ [b] [µg/ml] C_{ISTD}	GC				LC	
				ECD	NPD	FPD	MS/MS or MSD EI (+)	MS/MS ESI (+)	MS/MS ESI (-)
Potential Internal Standards									
PCB 18 [c,d]	5,55	3	50	+++	-	-	++	-	-
PCB 28 [c,d]	5,62	3	50	+++	-	-	++	-	-
PCB 52 [c,d]	6,09	4	50	+++	-	-	++	-	-
Triphenylphosphate [d]	4,59	-	20	-	+++	+++	+++	+++	-
Tris-(1,3-dichlorisopropyl)-phosphate	3,65	6	50	+++	+++	+++	+++	+++	+
Triphenylmethane[4]	5,37	-	10	-	-	-	+++	-	-
2,4-D $^{13}C6$ (ring)	depends on pH	2	10	-	-	-	-	-	+++
Chlorpyrifos D10 (diethyl D10) [4]	4,7	3	10	+++	+++	+++	+++	+++	-
Diuron D6 (dimethyl D6)	2,9	2	10	-	-	-	-	+++	-
Diazinon D10 (diethyl D10)	3,8	-	20	++	+++	+++	+++	+++	-
Metalaxyl D6 (dimethyl D6)	1,65	0	10	-	++	-	+++	+++	-
N,N´-Bis-4-nitrophenyl) urea (BNPU)[e]	3,76	-	10	-	-	-	-	-	+++
Potential Quality Control Standards (may be contained in the same mixture as the other ISTDs used or added at a different stage of analysis to detect and localize sources of error)									
PCB 138 [d,f]	6,83	6	50	+++	-	-	+++	-	-
PCB 153 [d,f]	7,75	6	50	+++	-	-	+++	-	-
Anthracen (or D10 Anthracen) [g]	4,45	-	100	-	-	-	-	-	-

[a] octanol-water partition coefficient

[b] proposed concentration; solvent: acetonitrile

[c] not appropriate if graphitized carbon black is used during clean-up

[d] not appropriate for extraction of crops with high oil content using module E6

[e] component of nicarbazin

[f] Recovery rates of PCB 138 and 153 decrease with rising oil content of samples. If the recovery of these internal standards is > 70 %, inacceptable losses of fat soluble pesticides did not occur.

[g] If the recovery of anthracen is > 70 %, inacceptable losses planar pesticides during clean-up with graphitized carbon black did not occur.

+++ very good detectable

++ good detectable

+ poor detectable

– not applicable

A.2 Apparatus used in all extraction (E) and clean-up (C) and stabilization (S) modules

Usual laboratory apparatus and, in particular, the following:

A.2.1 Centrifuge tubes with screw caps, 50 ml:

For example: a) 50 ml centrifuge tubes made of poly-tetrafluoroethylene with screw caps, or b) disposable 50 ml polypropylene centrifuge tubes with screw caps.

A.2.2 10 ml solvent-dispenser for acetonitrile; to be employed for dosing of extraction solvent.

A.2.3 Automatic pipettes, suitable for handling volumes of 10 µl to 100 µl, 200 µl to 1 000 µl and 1 ml to 10 ml, or 10 ml graduated glass pipettes.

A.2.4 Polypropylene-single use centrifuge tubes with screw caps, 10 ml or 12 ml.

A.2.5 Shaker, horizontal, vertical or orbital shaker, at least 200 min^{-1}.

A.2.6 Centrifuges, suitable for the centrifuge tubes employed in the procedure (A.2.1, A.2.4) and capable of achieving at least 3000 *g*.

A.2.7 High speed dispersing device, the diameter of the dispersing elements should fit the openings of the centrifuge tubes (A.2.1) used.

A.2.8 Grinding device

A.2.9 Heated shaker, e.g. shaking waterbath or AGYTAX SR1 CP57® [1)]

A.2.10 Freezer, operating at −18 C to −25 °C.

A.2.11 Injection vials, 1,5 ml, suitable for GC and LC autosampler, if necessary with micro-inserts.

A.2.12 Screw capped glass vials, 10 ml or 20 ml, for the storage of excessive amounts of the final extract, if necessary.

A.2.13 Vortex mixer.

A.3 Description of extraction (E) modules

A.3.1 Module E1: Extraction of a 10 g test portion without additional water using acetonitrile

A.3.1.1 Principle

This extraction module is used for plant material and food containing >80 % water, like fruits and vegetables and juices. Table 6 shows the commodities extracted preferably with this extraction module.

1) AGYTAX® is a product supplied by Cirtalab (Spain). This information is given for the convenience of users of this European Standard and do not constitute an endorsement by CEN of the products named. Equivalent products may be used if they can be shown to lead to the same results.

The homogeneous sample is extracted in frozen condition (if possible) with acetonitrile. After addition of magnesium sulfate, sodium chloride and buffering citrate salts (pH 5 to pH 5,5), the mixture is shaken intensively and centrifuged for phase separation. An aliquot of the organic phase is separated and cleaned-up according to clean-up modules (modules C) if necessary.

A.3.1.2 Procedure

A.3.1.2.1 Test portion

Transfer a representative test portion of 10 g ± 0,1 g (m_{sample}) of the comminuted homogenous sample into a 50 ml centrifuge tube (A.2.1).

A.3.1.2.2 Optional ISTD addition

Add a defined small volume of the ISTD solution (A.1.2) ($V_{\text{ISTD}}^{\text{add}}$, e. g. 100 µl) containing one or several of the compounds listed in Table A1 at the concentrations exemplary given (C_{ISTD})

A.3.1.2.3 First extraction

Add 10 ml of acetonitrile (A.1.3) (V_{Ex}). Close the tube and shake samples having room temperature vigorously for 1 min to 3 min or frozen samples for 15 min, using a shaker (A.2.5) if necessary.

Samples should be extracted frozen or while in the process of thawing. If samples are employed for extraction at ambient temperature, it shall be ensured that no significant degradation of the target pesticides occurs. Extended extraction time may be used if shown that it does not affect significantly the recovery rate.

If the sample's degree of comminution is insufficient, the extraction can be assisted by a high-speed disperser (A.2.7). In this case the addition of an ISTD prior to the dispersing step is mandatory. The dispersing element is immersed into the sample/acetonitrile mixture and comminution is performed for about 2 min at high speed. The dispersing element has to be cleaned thoroughly before being used for the next sample to avoid cross-contamination.

A.3.1.2.4 Second extraction step and partitioning

Add the prepared buffer-salt mixture (A.1.8) to the suspension from A.3.1.2.3. Close the tube and immediately shake vigorously for 1 min by hand or 3 min using a shaker (A.2.5) and centrifuge for 5 min at > 3000 *g*.

Without delay separate upper acetonitrile layer and pass the obtained raw extract to the clean-up modules C.

In the presence of water, magnesium sulfate tends to form lumps, which can harden rapidly. This can be avoided, if immediately after the addition of the salt mixture the centrifuge tube is shaken vigorously for few seconds. The 1 min or 3 min extraction of the entire batch may be performed in parallel after the salts were added to all the samples.

A.3.1.3 Calculation

The mass concentration of the sample in the raw extract $\rho_{\text{sample}}^{\text{raw extract}}$ gives the ratio of the amount of the sample, from which the residues are extracted, and the extraction volume. Usually $\rho_{\text{sample}}^{\text{raw extract}}$ is given in g/ml and is calculated by Formula (A.1):

$$\rho_{\text{sample}}^{\text{raw extract}}\left(\text{g}/\text{ml}\right) = \frac{m_{\text{sample}}}{V_{\text{Ex}}} \qquad \text{(A.1)}$$

where

m_{sample} is the mass of test portion, in g;

V_{Ex} is the volume of the organic phase after extraction (usually 10 ml), in ml.

Calculate the mass concentration of ISTD in the raw extract $\rho_{ISTD}^{raw\ extract}$ with Formula (A.2):

$$\rho_{ISTD}^{raw\ extract}\left(\mu g / ml\right) = \frac{\rho_{ISTD}{}^{add} \times V_{ISTD}{}^{add}}{V_{Ex}} \quad (A.2)$$

where

ρ_{ISTD}^{add} is the mass concentration of added ISTD solution, in µg/ml;

V_{ISTD}^{add} is the volume of ISTD added to the sample, in ml;

V_{Ex} is the volume of the organic phase after extraction (usually 10 ml), in ml.

A.3.1.4 Flowchart of module E1

Add 10 ml of acetonitrile to the test portion (10 g ± 0,1 g)

↓

Shake samples having room temperature vigorously for 1 min to 3 min or frozen samples for 15 min

↓

Add buffer-salt mixture and immediately shake vigorously

↓

Centrifuge for 5 min at > 3000 *g*.

↓

Separate upper acetonitrile layer for use in modules C

A.3.2 Module E2: Extraction of a 10 g test portion without additional water after addition of a) 0,6 ml or b) 0,2 ml of 5 mol/l sodium hydroxide solution using acetonitrile

A.3.2.1 Principle

This extraction module is used for acidic plant material and acidic food containing more than 80 % water. For very acidic foods (pH < 3) like lemons 0,6 ml of 5 mol/l sodium hydroxide solution are used, for less acidic like raspberries 0,2 ml. Table 6 shows the commodities extracted preferably with this extraction module.

The homogeneous sample is extracted in frozen condition (if possible) with acetonitrile. After addition of sodium hydroxide solution, magnesium sulfate, sodium chloride and buffering citrate salts (pH 5 to pH 5,5), the mixture is shaken intensively and centrifuged for phase separation. An aliquot of the organic phase is separated and cleaned-up according to clean-up modules (modules C) if necessary.

A.3.2.2 Procedure

A.3.2.2.1 Test portion

Transfer a representative test portion of 10 g ± 0,1 g (m_{sample}) of the comminuted homogenous sample into a 50 ml centrifuge tube (A.2.1).

A.3.2.2.2 Optional ISTD addition

Add a defined small volume of the ISTD solution (A.1.2) ($V_{\text{ISTD}}^{\text{add}}$, e. g. 100 µl) containing one or several of the compounds listed in Table A1 at the concentrations exemplary given (C_{ISTD}).

A.3.2.2.3 First extraction

Add 10 ml of acetonitrile (A.1.3) (V_{Ex}) and a) 0,6 ml (module E2a) or b) 0,2 ml (module E2b) of 5 mol/l sodium hydroxide solution (A.1.9) as given in Table 6. Close the tube and shake samples having room temperature vigorously for 1 min to 3 min or frozen samples for 15 min, using a shaker (A.2.5) if necessary.

Samples should be extracted frozen or while in the process of thawing. If samples are employed for extraction at ambient temperature, it shall be ensured that no significant degradation of the target pesticides occurs. Extended extraction time may be used if shown that it does not affect significantly the recovery rate

If the sample's degree of comminution is insufficient, the extraction can be assisted by a high-speed disperser (A.2.7) In this case the addition of an ISTD prior to the dispersing step is mandatory. The dispersing element is immersed into the sample/acetonitrile mixture and comminution is performed for about 2 min at high speed. The dispersing element has to be cleaned thoroughly before being used for the next sample to avoid cross-contamination.

A.3.2.2.4 Second extraction step and partitioning

Add the prepared buffer-salt mixture (A.1.8) to the suspension from A.3.2.2.3. Close the tube and immediately shake vigorously for 1 min by hand or 3 min using a shaker (A.2.5) and centrifuge for 5 min at > 3000 *g*.

Without delay separate upper acetonitrile layer and pass the obtained raw extract to the clean-up modules C.

In the presence of water, magnesium sulfate tends to form lumps, which can harden rapidly. This can be avoided, if immediately after the addition of the salt mixture the centrifuge tube is shaken vigorously for few seconds. The 1 min or 3 min extraction of the entire batch may be performed in parallel after the salts were added to all the samples.

A.3.2.3 Calculation

The mass concentration of the sample in the raw extract $\rho_{\text{sample}}^{\text{raw extract}}$ gives the ratio of the amount of the sample, from which the residues are extracted, and the extraction volume. Usually $\rho_{\text{sample}}^{\text{raw extract}}$ is given in g/ml and is calculated with Formula (A.3):

$$\rho_{\text{sample}}^{\text{raw extract}}\left(\text{g}/\text{ml}\right)=\frac{m_{\text{sample}}}{V_{\text{Ex}}} \qquad \text{(A.3)}$$

where

m_{sample} is the mass of test portion, in g;

V_{Ex} is the volume of the organic phase after extraction (usually 10 ml), in ml.

The mass concentration of ISTD in the raw extract $\rho_{ISTD}^{raw\ extract}$ is calculated with Formula (A.4):

$$\rho_{ISTD}^{raw\ extract}\left(\mu g / ml\right) = \frac{\rho_{ISTD}^{add} \times V_{ISTD}^{add}}{V_{Ex}} \quad (A.4)$$

where

ρ_{ISTD}^{add} is the mass concentration of added ISTD solution, in µg/ml;

V_{ISTD}^{add} is the volume of ISTD added to the sample, in ml;

V_{Ex} is the volume of the organic phase after extraction (usually 10 ml), in ml.

A.3.2.4 Flowchart of module E2

Add 10 ml of acetonitrile and
a) 0,6 ml or b) 0,2 ml of 5 mol/l sodium hydroxide solution
to the test portion (10 g ± 0,1 g)

↓

Shake samples having room temperature vigorously for 1 min to 3 min or frozen samples for 15 min

↓

Add buffer-salt mixture and immediately shake vigorously

↓

Centrifuge for 5 min at > 3000 *g*.

↓

Separate upper acetonitrile layer for use in modules C

A.3.3 Module E3: Extraction of a 10 g test portion after addition of a) 2,5 ml or b) 4,5 ml of water using acetonitrile

A.3.3.1 Principle

This extraction module is used for plant material and food containing between 40 % and 80 % water, like bananas and fresh dates. Table 6 shows the commodities extracted preferably with this extraction module.

Water is added to the homogeneous sample to obtain a total amount of water of about 10 g in the extraction portion. It is extracted in frozen condition (if possible) with the help of acetonitrile. After addition of magnesium sulfate, sodium chloride and buffering citrate salts (pH 5 to pH 5,5), the mixture is shaken intensively and centrifuged for phase separation. An aliquot of the organic phase is separated and cleaned-up according to clean-up modules (modules C) if necessary.

A.3.3.2 Procedure

A.3.3.2.1 Test portion and addition of water

Transfer a representative test portion of 10 g ± 0,1 g (m_{sample}) of the comminuted homogenous sample into a 50 ml centrifuge tube (A.2.1).

For samples containing between 40 % and 80 % water a sufficient amount of cold water (A.1.10) is added to the sample to obtain a total amount of water of about 10 g in the extraction portion. Depending on the commodity (see Table 6) add a) 2,5 ml (module E3a) or b) 4,5 ml (module E3b) of water.

A.3.3.2.2 Optional ISTD addition

Add a defined small volume of the ISTD solution (A.1.2) ($V_{\text{ISTD}}^{\text{add}}$, e. g. 100 µl) containing one or several of the compounds listed in Table A1 at the concentrations exemplary given (C_{ISTD}).

A.3.3.2.3 First extraction

Add 10 ml of acetonitrile (A.1.3) (V_{Ex}). Close the tube and shake samples having room temperature vigorously for 1 min to 3 min or frozen samples for 15 min, using a shaker (A.2.5) if necessary.

Samples should be extracted frozen or while in the process of thawing. If samples are employed for extraction at ambient temperature, it shall be ensured that no significant degradation of the target pesticides occurs. Extended extraction time may be used if shown that it does not affect significantly the recovery rate.

If the sample's degree of comminution is insufficient, the extraction can be assisted by a high-speed disperser (A.2.7). In this case the addition of an ISTD prior to the dispersing step is mandatory. The dispersing element is immersed into the sample/acetonitrile mixture and comminution is performed for about 2 min at high speed. The dispersing element has to be cleaned thoroughly before being used for the next sample to avoid cross-contamination.

A.3.3.2.4 Second extraction step and partitioning

Add the prepared buffer-salt mixture (A.1.8) to the suspension from A.3.3.2.3. Close the tube and immediately shake vigorously for 1 min by hand or 3 min using a shaker (A.2.5) and centrifuge for 5 min at > 3000 g.

Without delay separate upper acetonitrile layer and pass the obtained raw extract to the clean-up modules C.

In the presence of water, magnesium sulfate tends to form lumps, which can harden rapidly. This can be avoided, if immediately after the addition of the salt mixture the centrifuge tube is shaken vigorously for few seconds. The 1 min or 3 min extraction of the entire batch may be performed in parallel after the salts were added to all the samples.

A.3.3.3 Calculation

The mass concentration of the sample in the raw extract $\rho_{\text{sample}}^{\text{raw extract}}$ gives the ratio of the amount of the sample, from which the residues are extracted, and the extraction volume. Usually $\rho_{\text{sample}}^{\text{raw extract}}$ is given in g/ml and is calculated as with Formula (A.5):

$$\rho_{\text{sample}}^{\text{raw extract}}\left(\text{g / ml}\right) = \frac{m_{\text{sample}}}{V_{\text{Ex}}} \qquad \text{(A.5)}$$

where

m_{sample} is the mass of test portion, in g;

V_{Ex} is the volume of the organic phase after extraction (usually 10 ml), in ml.

The mass concentration of ISTD in the raw extract $\rho_{ISTD}^{raw\ extract}$ is calculated with Formula (A.6):

$$\rho_{ISTD}^{raw\ extract}\left(\mu g / ml\right) = \frac{\rho_{ISTD}^{add} \times V_{ISTD}^{add}}{V_{Ex}} \quad (A.6)$$

where:

ρ_{ISTD}^{add} is the mass concentration of added ISTD solution. in µg/ml;

V_{ISTD}^{add} is the volume of ISTD added to the sample, in ml;

V_{Ex} is the volume of the organic phase after extraction (usually 10 ml), in ml.

A.3.3.4 Flowchart of module E3

Add a) 2,5 ml or b) 4,5 ml of water to the test portion (10 g ± 0,1 g)

↓

Add 10 ml of acetonitrile

↓

Shake samples having room temperature vigorously for 1 min to 3 min or frozen samples for 15 min

↓

Add buffer-salt mixture and immediately shake vigorously

↓

Centrifuge for 5 min at > 3000 *g*.

↓

Separate upper acetonitrile layer for use in modules C

A.3.4 Module E4: Homogenization of the sample with addition of water, extraction of a 13,5 g homogenate using acetonitrile

A.3.4.1 Principle

This extraction module is used for plant material and food containing between 15 % and 40 % water, like dried fruits and similar products. Table 6 shows the commodities extracted preferably with this extraction module.

The sample material is homogenized after addition of water. The homogenate is extracted in frozen condition (if possible) with the help of acetonitrile. After addition of magnesium sulfate, sodium chloride and buffering citrate salts (pH 5 to pH 5,5), the mixture is shaken intensively and centrifuged for phase separation. An aliquot of the organic phase is separated and cleaned-up according to clean-up modules (modules C) if necessary.

A.3.4.2 Procedure

A.3.4.2.1 Homogenizing and Test portion

Add 850 g of cold water (A.1.10) to 500 g of frozen dried fruits and homogenize the mixture (if possible by adding dry ice (A.1.11)) using a sample processing unit (A.2.8).

Transfer a representative test portion of 13,5 g ± 0,1 g (corresponding a test portion of 5 g (m_{sample})) of the homogenate into a 50 ml centrifuge tube (A.2.1).

A.3.4.2.2 Optional ISTD addition

Add a defined small volume of the ISTD solution (A.1.2) ($V_{\text{ISTD}}^{\text{add}}$, e. g. 100 µl) containing one or several of the compounds listed in Table A1 at the concentrations exemplary given (C_{ISTD})

A.3.4.2.3 First extraction

Add 10 ml of acetonitrile (A.1.3) (V_{Ex}). Close the tube and shake samples having room temperature vigorously for 1 min to 3 min or frozen samples for 15 min, using a shaker (A.2.5) if necessary.

Samples should be extracted frozen or while in the process of thawing. If samples are employed for extraction at ambient temperature, it shall be ensured that no significant degradation of the target pesticides occurs. Extended extraction time may be used if shown that it does not affect significantly the recovery rate

If the sample's degree of comminution is insufficient, the extraction can be assisted by a high-speed disperser (A.2.7). In this case the addition of an ISTD prior to the dispersing step is mandatory. The dispersing element is immersed into the sample/acetonitrile mixture and comminution is performed for about 2 min at high speed. The dispersing element has to be cleaned thoroughly before being used for the next sample to avoid cross-contamination.

A.3.4.2.4 Second extraction step and partitioning

Add the prepared buffer-salt mixture (A.1.8) to the suspension from A.3.4.2.3. Close the tube and immediately shake vigorously for 1 min by hand or 3 min using a shaker (A.2.5) and centrifuge for 5 min at > 3000 *g*.

Without delay separate upper acetonitrile layer and pass the obtained raw extract to the clean-up modules C.

In the presence of water, magnesium sulfate tends to form lumps, which can harden rapidly. This can be avoided, if immediately after the addition of the salt mixture the centrifuge tube is shaken vigorously for few seconds. The 1 min or 3 min extraction of the entire batch may be performed in parallel after the salts were added to all the samples.

A.3.4.3 Calculation

The mass concentration of the sample in the raw extract $\rho_{\text{sample}}^{\text{raw extract}}$ gives the ratio of the amount of the sample, from which the residues are extracted, and the extraction volume. Usually $\rho_{\text{sample}}^{\text{raw extract}}$ is given in g/ml and is calculated with Formula (A.7):

$$\rho_{\text{sample}}^{\text{raw extract}}\left(\text{g / ml}\right) = \frac{m_{\text{sample}}}{V_{\text{Ex}}} \qquad \text{(A.7)}$$

where

m_{sample} is the mass of test portion, in g;

V_{Ex} is the volume of the organic phase after extraction (usually 10 ml), in ml.

The mass concentration of ISTD in the raw extract $\rho_{ISTD}^{raw\ extract}$ is calculated with Formula (A.8):

$$\rho_{ISTD}^{raw\ extract}\left(\mu g / ml\right) = \frac{\rho_{ISTD}^{add} \times V_{ISTD}^{add}}{V_{Ex}} \quad \text{(A.8)}$$

where:

ρ_{ISTD}^{add} is the mass concentration of added ISTD solution, in µg/ml;

V_{ISTD}^{add} is the volume of ISTD added to the sample, in ml;

V_{Ex} is the volume of the organic phase after extraction (usually 10 ml), in ml.

A.3.4.4 Flowchart of module E4

Homogenize 850 g of cold water and 500 g frozen dried fruits

↓

Transfer 13,5 g ± 0,1 g of the homogenate into a centrifuge tube and add 10 ml of acetonitrile

↓

Shake samples having room temperature vigorously for 1 min to 3 min or frozen samples for 15 min

↓

Add buffer-salt mixture and immediately shake vigorously

↓

Centrifuge for 5 min at > 3000 *g*.

↓

Separate upper acetonitrile layer for use in modules C

A.3.5 Module E5: Extraction of a 5 g test portion after addition of 10 ml of water using acetonitrile

A.3.5.1 Principle

This extraction module is used for plant material and food containing less than 15 % water, like cereals, cereal products and honey. Table 6 shows the preferred extraction module for the corresponding commodity.

10 ml of water are added to the homogeneous sample which is extracted with acetonitrile. After addition of magnesium sulfate, sodium chloride and buffering citrate salts (pH 5 to pH 5,5), the mixture is shaken intensively and centrifuged for phase separation. An aliquot of the organic phase is separated and cleaned-up according to clean-up modules (modules C) if necessary.

A.3.5.2 Procedure

A.3.5.2.1 Test portion and addition of water

Transfer a representative test portion of 5 g ± 0,05 g (m_{sample}) of the comminuted homogenous sample into a 50 ml centrifuge tube (A.2.1) and add 10 ml of cold water (A.1.10). The effects of extended swelling time should be checked.

A.3.5.2.2 Optional ISTD addition

Add a defined small volume of the ISTD solution (A.1.2) (V_{ISTD}^{add}, e.g. 100 µl) containing one or several of the compounds listed in Table A.1 at the concentrations exemplary given (C_{ISTD}).

A.3.5.2.3 First extraction

Add 10 ml of acetonitrile (A.1.3) (V_{Ex}). Close the tube and shake samples vigorously for 15 min , using a shaker (A.2.5) if necessary. If no shaker is used shake vigorously for 1 min by hand followed by a soaking time of 15 min and shake again for 1 min.

If samples are employed for extraction at ambient temperature, it shall be ensured that no significant degradation of the target pesticides occurs. Extended extraction time may be used if shown that it does not affect significantly the recovery rate.

If the sample's degree of comminution is insufficient, the extraction can be assisted by a high-speed disperser (A.2.7). In this case the addition of an ISTD prior to the dispersing step is mandatory. The dispersing element is immersed into the sample/acetonitrile mixture and comminution is performed for about 2 min at high speed. The dispersing element has to be cleaned thoroughly before being used for the next sample to avoid cross-contamination.

A.3.5.2.4 Second extraction step and partitioning

Add the prepared buffer-salt mixture (A.1.8) to the suspension from A.3.5.2.3. Close the tube and immediately shake vigorously for 1 min by hand or 3 min using a shaker (A.2.5) and centrifuge for 5 min at > 3000 *g*.

Without delay separate upper acetonitrile layer and pass the obtained raw extract to the clean-up modules C.

In the presence of water, magnesium sulfate tends to form lumps, which can harden rapidly. This can be avoided, if immediately after the addition of the salt mixture the centrifuge tube is shaken vigorously for few seconds. The 1 min or 3 min extraction of the entire batch may be performed in parallel after the salts were added to all the samples.

A.3.5.3 Calculation

The mass concentration of the sample in the raw extract $\rho_{sample}^{raw\ extract}$ gives the ratio of the amount of the sample, from which the residues are extracted, and the extraction volume. Usually $\rho_{sample}^{raw\ extract}$ is given in g/ml and is calculated by Formula (A.9):

$$\rho_{sample}^{raw\ extract}\left(\mathrm{g/ml}\right) = \frac{m_{sample}}{V_{Ex}} \qquad \text{(A.9)}$$

where

m_{sample} is the mass of test portion, in g;

V_{Ex} is the volume of the organic phase after extraction (usually 10 ml), in ml.

The mass concentration of ISTD in the raw extract $\rho_{ISTD}^{raw\ extract}$ is according to Formula (A.10):

$$\rho_{ISTD}^{raw\ extract}\left(\mu g / ml\right) = \frac{\rho_{ISTD}^{add} \times V_{ISTD}^{add}}{V_{Ex}} \quad \text{(A.10)}$$

where

ρ_{ISTD}^{add} is the mass concentration of added ISTD solution, in µg/ml;

V_{ISTD}^{add} is the volume of ISTD added to the sample, in ml;

V_{Ex} is the volume of the organic phase after extraction (usually 10 ml), in ml.

A.3.5.4 Flowchart of module E5

Add 10 ml of water to the test portion (5 g ± 0,05 g)

↓

Add 10 ml of acetonitrile

↓

Shake samples vigorously for 15 min by shaker (or 1 min by hand followed by a soaking time of 15 min and again for 1 min)

↓

Add buffer-salt mixture and immediately shake vigorously

↓

Centrifuge for 5 min at > 3000 *g*

↓

Separate upper acetonitrile layer for use in modules C

A.3.6 Module E6: Extraction of a 5 g test portion after addition of 6 ml of water using acetonitrile

A.3.6.1 Principle

This extraction module is used for plant material and food containing between 45 % and 80 % water and with a high matrix load or a high fat content (>5 %), like garlic or avocado. Table 6 shows the commodities extracted preferably with this extraction module.

6 ml of water are added to the homogeneous sample (5 g) to obtain a total amount of water of about 10 g in the extraction portion. It is extracted in frozen condition (if possible) with the help of acetonitrile. After addition of magnesium sulfate, sodium chloride and buffering citrate salts (pH 5 to pH 5,5), the mixture is shaken intensively and centrifuged for phase separation. An aliquot of the organic phase is separated and cleaned-up according to clean-up modules (modules C) if necessary.

A.3.6.2 Procedure

A.3.6.2.1 Test portion and addition of water

Transfer a representative test portion of 5 g ± 0,05 g (m_{sample}) of the comminuted homogenous sample into a 50 ml centrifuge tube (A.2.1) and add 6 ml of cold water (A.1.10).

A.3.6.2.2 Optional ISTD addition

Add a defined small volume of the ISTD solution (A.1.2) ($V_{\text{ISTD}}^{\text{add}}$, e. g. 100 µl) containing one or several of the compounds listed in Table A1 at the concentrations exemplary given (C_{ISTD})

A.3.6.2.3 First extraction

Add 10 ml of acetonitrile (A.1.3) (V_{Ex}). Close the tube and shake samples having room temperature vigorously for 1 min to 3 min or frozen samples for 15 min, using a shaker (A.2.5) if necessary.

Samples should be extracted frozen or while in the process of thawing. If samples are employed for extraction at ambient temperature, it shall be ensured that no significant degradation of the target pesticides occurs. Extended extraction time may be used if shown that it does not affect significantly the recovery rate.

If the sample's degree of comminution is insufficient, the extraction can be assisted by a high-speed disperser (A.2.7). In this case the addition of an ISTD prior to the dispersing step is mandatory. The dispersing element is immersed into the sample/acetonitrile mixture and comminution is performed for about 2 min at high speed. The dispersing element has to be cleaned thoroughly before being used for the next sample to avoid cross-contamination.

A.3.6.2.4 Second extraction step and partitioning

Add the prepared buffer-salt mixture (A.1.8) to the suspension from A.3.6.2.3. Close the tube and immediately shake vigorously for 1 min by hand or 3 min using a shaker (A.2.5) and centrifuge for 5 min at > 3000 g.

Without delay separate upper acetonitrile layer and pass the obtained raw extract to the clean-up modules C.

In the presence of water, magnesium sulfate tends to form lumps, which can harden rapidly. This can be avoided, if immediately after the addition of the salt mixture the centrifuge tube is shaken vigorously for few seconds. The 1 min or 3 min extraction of the entire batch may be performed in parallel after the salts were added to all the samples.

A.3.6.3 Calculation

The mass concentration of the sample in the raw extract $\rho_{\text{sample}}^{\text{raw extract}}$ gives the ratio of the amount of the sample, from which the residues are extracted, and the extraction volume. Usually $\rho_{\text{sample}}^{\text{raw extract}}$ is given in g/ml and is calculated with Formula (A.11):

$$\rho_{\text{sample}}^{\text{raw extract}}\left(\text{g}/\text{ml}\right) = \frac{m_{\text{sample}}}{V_{\text{Ex}}} \qquad \text{(A.11)}$$

where

m_{sample} is the mass of test portion, in g;

V_{Ex} is the volume of the organic phase after extraction (usually 10 ml), in ml.

The mass concentration of ISTD in the raw extract $\rho_{ISTD}^{raw\ extract}$ is according to Formula (A.12):

$$\rho_{ISTD}^{raw\ extract}\left(\mu g / ml\right) = \frac{\rho_{ISTD}^{add} \times V_{ISTD}^{add}}{V_{Ex}} \quad \text{(A.12)}$$

where

ρ_{ISTD}^{add} is the mass concentration of added ISTD solution, in µg/ml;

V_{ISTD}^{add} is the volume of ISTD added to the sample, in ml;

V_{Ex} is the volume of the organic phase after extraction (usually 10 ml), in ml.

A.3.6.4 Flowchart of module E6

Add 6 ml of water to the test portion (5 g ± 0,05 g)

↓

Add 10 ml of acetonitrile

↓

Shake samples having room temperature vigorously for 1 min to 3 min or frozen samples for 15 min

↓

Add buffer-salt mixture and immediately shake vigorously

↓

Centrifuge for 5 min at > 3000 *g*

↓

Separate upper acetonitrile layer for use in modules C

A.3.7 Module E7: Extraction of a 2 g test portion after addition of 10 ml of water using acetonitrile

A.3.7.1 Principle

This extraction module is used for plant material and food with very low water content (<10 %) and with a high matrix load, like spices, coffee, tobacco or tea. It is also used for freeze-dried products, which raw materials contain more than 80 % of water. Table 6 shows the commodities extracted preferably with this extraction module.

10 ml of water are added to the homogeneous sample (2 g) which is extracted with acetonitrile. After addition of magnesium sulfate, sodium chloride and buffering citrate salts (pH 5 to pH 5,5), the mixture is shaken intensively and centrifuged for phase separation. An aliquot of the organic phase is separated and cleaned-up according to clean-up modules (modules C) if necessary.

A.3.7.2 Procedure

A.3.7.2.1 Test portion and addition of water

Transfer a representative test portion of 2 g ± 0,02 g (m_{sample}) of the comminuted homogenous sample into a 50 ml centrifuge tube (A.2.1) and add 10 ml of cold water (A.1.10). The effects of extended swelling time should be checked.

A.3.7.2.2 Optional ISTD addition

Add a defined small volume of the ISTD solution (A.1.2) (V_{ISTD}^{add}, e. g. 100 µl) containing one or several of the compounds listed in Table A.1 at the concentrations exemplary given (C_{ISTD}).

A.3.7.2.3 First extraction

Add 10 ml of acetonitrile (A.1.3) (V_{Ex}). Close the tube and shake samples vigorously for 15 min , using a shaker (A.2.5) if necessary. If no shaker is used shake vigorously for 1 min by hand followed by a soaking time of 15 min and shake again for 1 min.

If samples are employed for extraction at ambient temperature, it shall be ensured that no significant degradation of the target pesticides occurs. Extended extraction time may be used if shown that it does not affect significantly the recovery rate.

If the sample's degree of comminution is insufficient, the extraction can be assisted by a high-speed disperser (A.2.7). In this case the addition of an ISTD prior to the dispersing step is mandatory. The dispersing element is immersed into the sample/acetonitrile mixture and comminution is performed for about 2 min at high speed. The dispersing element has to be cleaned thoroughly before being used for the next sample to avoid cross-contamination.

A.3.7.2.4 Second extraction step and partitioning

Add the prepared buffer-salt mixture (A.1.8) to the suspension from A.3.7.2.3. Close the tube and immediately shake vigorously for 1 min by hand or 3 min using a shaker (A.2.5) and centrifuge for 5 min at > 3000 g.

Without delay separate upper acetonitrile layer and pass the obtained raw extract to the clean-up modules C.

In the presence of water, magnesium sulfate tends to form lumps, which can harden rapidly. This can be avoided, if immediately after the addition of the salt mixture the centrifuge tube is shaken vigorously for few seconds. The 1 min or 3 min extraction of the entire batch may be performed in parallel after the salts were added to all the samples.

A.3.7.3 Calculation

The mass concentration of the sample in the raw extract $\rho_{sample}^{raw\ extract}$ gives the ratio of the amount of the sample, from which the residues are extracted, and the extraction volume. Usually $\rho_{sample}{}^{raw\ extract}$ is given in g/ml and is calculated with Formula (A.13):

$$\rho_{sample}^{raw\ extract}\left(g/ml\right) = \frac{m_{sample}}{V_{Ex}} \qquad (A.13)$$

where

m_{sample} is the mass of test portion, in g;

V_{Ex} is the volume of the organic phase after extraction (usually 10 ml), in ml.

The mass concentration of ISTD in the raw extract $\rho_{sample}^{raw\ extract}$ is calculated with Formula (A.14):

$$\rho_{ISTD}^{raw\ extract}\left(\mu g / ml\right) = \frac{\rho_{ISTD}^{add} \times V_{ISTD}^{add}}{V_{Ex}} \quad (A.14)$$

where

ρ_{ISTD}^{add} is the mass concentration of added ISTD solution, in µg/ml;

V_{ISTD}^{add} is the volume of ISTD added to the sample, in ml;

V_{Ex} is the volume of the organic phase after extraction (usually 10 ml), in ml.

A.3.7.4 Flowchart of module E7

Add 10 ml of water to the test portion (2 g ± 0,02 g)

↓

Add 10 ml of acetonitrile

↓

Shake samples vigorously for 15 min by shaker (or 1 min by hand followed by a soaking time of 15 min and again for 1 min)

↓

Add buffer-salt mixture and immediately shake vigorously

↓

Centrifuge for 5 min at > 3000 *g*

↓

Separate upper acetonitrile layer for use in modules C

A.3.8 Module E8: Extraction of a 10 g test portion without additional water with simultaneous alkaline hydrolysis of alkaline labile esters and conjugates using acetonitrile

A.3.8.1 Principle

This extraction module is used for the determination of acidic pesticides, which residue definitions include beside the free acid also esters and conjugates, which can or have to be hydrolyzed under alkaline condition according to the residue analysis for the MRL setting. This extraction module gives default condition for the hydrolysis of conjugates and esters of 2,4-D, 2,4-DB, clopyralid, dichlorprop(-P), fluazifop(-P), fluroxypyr, haloxyfop and MCPA/MCPB. Most of the relevant esters of the given compounds are practically hydrolyzed quantitatively under the given conditions. This extraction module is actually not applicable on conjugates of 6-OH- and 8-OH-bentazone, dicamba and pyridate. This extraction module is used for plant material and food, for which according to Table 6 the extraction modules E1, E2a or E2b are usually used.

The homogeneous sample is extracted at 40 °C with the help of acetonitrile after addition of 1 mmol sodium hydroxide per g sample for very acidic commodities (usually extract by module E2a, like lemons and lime) or 0,5 mmol sodium hydroxide per g sample for all other commodities. After addition of sulfuric acid (for neutralization), magnesium sulfate, sodium chloride and buffering citrate salts (pH 5 to pH 5,5), the mixture is shaken intensively and centrifuged for phase separation. An aliquot of the organic phase is separated and cleaned-up according if necessary. Clean-up modules C2, C3, C4 and C5 may not be used, as the primary secondary amine sorbent can cause losses of the free acids.

A.3.8.2 Procedure

A.3.8.2.1 Test portion

Transfer a representative test portion of 10 g ± 0,1 g (m_{sample}) of the comminuted homogenous sample into a 50 ml centrifuge tube (A.2.1).

As the acid content of lemons and lime has to be considered for the neutralization, the test portion of very acidic commodities can only be reduced if the volume of the sodium hydroxide solution is reduced proportionally.

A.3.8.2.2 Optional ISTD addition

Add a defined small volume of the ISTD solution (A.1.2) ($V_{\text{ISTD}}^{\text{add}}$, e.g. 100 µl) containing one or several of the compounds listed in Table A.1 at the concentrations exemplary given (C_{ISTD}).

A.3.8.2.3 Hydrolysis and first extraction

Add 10 ml of acetonitrile (A.1.3) (V_{Ex}). Add 2,0 ml of 5 mol/l sodium hydroxide solution (A.1.9) for very acidic commodities (usually extracted by module E2a) or 1,0 ml of 5 mol/l sodium hydroxide solution (A.1.9) for all other commodities. Close the tube and shake vigorously for 30 min at 40 °C using a heated shaker (A.2.9). To neutralize add 1,4 ml of 2,5 mol/l sulfuric acid (A.1.13) for very acidic commodities and 1,0 ml of 2,5 mol/l sulfuric acid (A.1.13) for all other commodities.

If the sample's degree of comminution is insufficient, the extraction can be assisted by a high-speed disperser (A.2.7). In this case the addition of an ISTD prior to the dispersing step is mandatory. The dispersing element is immersed into the sample/acetonitrile mixture and comminution is performed for about 2 min at high speed. The dispersing element has to be cleaned thoroughly before being used for the next sample to avoid cross-contamination.

A.3.8.2.4 Second extraction step and partitioning

Add the prepared buffer-salt mixture (A.1.8) to the suspension from A.3.8.2.3. Close the tube and immediately shake vigorously for 1 min by hand or 3 min using a shaker (A.2.5 or A.2.9) and centrifuge for 5 min at > 3000 g.

Without delay separate upper acetonitrile layer and pass the obtained raw extract to the clean-up modules C0 or C1.

In the presence of water, magnesium sulfate tends to form lumps, which can harden rapidly. This can be avoided, if the centrifuge tube is shaken vigorously for five seconds immediately after the addition of the salt mixture. The 1 min or 3 min extraction of the entire batch may be performed in parallel after the salts were added to all the samples.

A.3.8.3 Calculation

The mass concentration of the sample in the raw extract $\rho_{\text{sample}}^{\text{raw extract}}$ gives the ratio of the amount of the sample, from which the residues are extracted, and the extraction volume. Usually $\rho_{\text{sample}}^{\text{raw extract}}$ is given in g/ml and is calculated with Formula (A.15):

$$\rho_{\text{sample}}^{\text{raw extract}}\left(\text{g / ml}\right)=\frac{m_{\text{sample}}}{V_{\text{Ex}}} \tag{A.15}$$

where

m_{sample} is the mass of test portion, in g;

V_{Ex} is the volume of the organic phase after extraction (usually 10 ml), in ml.

The mass concentration of ISTD in the raw extract $\rho_{\text{ISTD}}^{\text{raw extract}}$ is calculated with Formula (A.16):

$$\rho_{\text{ISTD}}^{\text{raw extract}}\left(\mu\text{g / ml}\right)=\frac{\rho_{\text{ISTD}}^{\text{add}}\times V_{\text{ISTD}}^{\text{add}}}{V_{\text{Ex}}} \tag{A.16}$$

where

$\rho_{\text{ISTD}}^{\text{add}}$ is the mass concentration of added ISTD solution, in µg/ml;

V_{ISTD}^{add} is the volume of ISTD added to the sample, in ml;

V_{Ex} is the volume of the organic phase after extraction (usually 10 ml), in ml.

A.3.8.4 Flowchart of module E8

Add 10 ml of acetonitrile and 2,0 ml (for very acidic commodities) or 1,0 ml of 5 mol/l sodium hydroxide solution to the test portion (10 g ± 0,1 g)

↓

Shake vigorously for 30 min at 40 °C

↓

Neutralize with 1,0 ml (or 1,4 ml for very acidic commodities) of 2,5 mol/l sulfuric acid

↓

Add buffer-salt mixture and immediately shake vigorously

↓

Centrifuge for 5 min at > 3000 *g*.

↓

Separate upper acetonitrile layer for use in modules C0 or C1

A.3.9 Module E9: Extraction of 2 g or 5 g test portions after addition of water with simultaneous alkaline hydrolysis of alkaline labile esters and conjugates using acetonitrile

A.3.9.1 Principle

This extraction module is used for the determination of acidic pesticides, which residue definitions include beside the free acid also esters and conjugates, which can or have to be hydrolyzed under alkaline condition according to the residue analysis for the MRL setting. This extraction module gives default condition for the hydrolysis of conjugates and esters of 2,4-D, 2,4-DB, clopyralid, dichlorprop

(-P), fluazifop (-P), fluroxypyr, haloxyfop and MCPA/MCPB. Most of the relevant esters of the given compounds are practically hydrolyzed quantitatively under the given conditions. This extraction module is actually not applicable on conjugates of 6-OH- und 8-OH-bentazone, dicamba and pyridate. This extraction module is used for plant material and food, for which according to Table 6 the extraction modules E5, E6 or E7 are usually used.

The homogeneous sample is extracted at 40 °C with the help of acetonitrile after addition of 1 mmol sodium hydroxide per g sample for a 5 g test portion and 2,5 mmol sodium hydroxide per g sample for a 2 g test portion. After addition of sulfuric acid (for neutralization), magnesium sulfate, sodium chloride and buffering citrate salts (pH 5 to pH 5,5), the mixture is shaken intensively and centrifuged for phase separation. An aliquot of the organic phase is separated and cleaned-up according if necessary. Clean-up modules C2, C3, C4 and C5 may not be used, as the primary secondary amine sorbent can cause losses of the free acids.

A.3.9.2 Procedure

A.3.9.2.1 Test portion

For commodities, which are usually extracted by module E5 or E6, Transfer a representative test portion of 5 g ± 0,05 g (m_{sample}) and for commodities, which are usually extracted by module E7, 2 g ± 0,02 g (m_{sample}) as representative test portion of the comminuted homogenous sample into a 50 ml centrifuge tube (A.2.1).

A.3.9.2.2 Optional ISTD addition

Add a defined small volume of the ISTD solution (A.1.2) (V_{ISTD}^{add}, e.g. 100 µl) containing one or several of the compounds listed in Table A.1 at the concentrations exemplary given (C_{ISTD}).

A.3.9.2.3 Hydrolysis and first extraction

Add 10 ml of water (A.1.10) for commodities, which are usually extracted by module E5 or E7, or 6 ml of water (A.1.10) for commodities, which are usually extracted by module E6.

Add 10 ml of acetonitrile (A.1.3) (V_{Ex}) and 1,0 ml of 5 mol/l sodium hydroxide solution (A.1.9). Close the tube and shake vigorously for 30 min at 40 °C using a heated shaker (A.2.9). To neutralize add 1,0 ml of 2,5 mol/l sulfuric acid (A.1.13).

If the sample's degree of comminution is insufficient, the extraction can be assisted by a high-speed disperser (A.2.7). In this case the addition of an ISTD prior to the dispersing step is mandatory. The dispersing element is immersed into the sample/acetonitrile mixture and comminution is performed for about 2 min at high speed. The dispersing element has to be cleaned thoroughly before being used for the next sample to avoid cross-contamination.

A.3.9.2.4 Second extraction step and partitioning

Add the prepared buffer-salt mixture (A.1.8) to the suspension from A.3.9.2.3. Close the tube and immediately shake vigorously for 1 min by hand or 3 min using a shaker (A.2.5 or A.2.9) and centrifuge for 5 min at > 3000 g.

Without delay separate upper acetonitrile layer and pass the obtained raw extract to the clean-up modules C0 or C1.

In the presence of water, magnesium sulfate tends to form lumps, which can harden rapidly. This can be avoided, if immediately after the addition of the salt mixture the centrifuge tube is shaken vigorously five seconds. The 1 min or 3 min extraction of the entire batch may be performed in parallel after the salts were added to all the samples.

A.3.9.3 Calculation

The mass concentration of the sample in the raw extract $\rho_{\text{sample}}^{\text{raw extract}}$ gives the ratio of the amount of the sample, from which the residues are extracted, and the extraction volume. Usually $\rho_{\text{sample}}^{\text{raw extract}}$ is given in g/ml and is calculated with Formula (A.17):

$$\rho_{\text{sample}}^{\text{raw extract}}\left(\text{g}/\text{ml}\right)=\frac{m_{\text{sample}}}{V_{\text{Ex}}} \tag{A.17}$$

where

m_{sample} is the mass of test portion, in g;

V_{Ex} is the volume of the organic phase after extraction (usually 10 ml), in ml.

The mass concentration of ISTD in the raw extract $\rho_{\text{ISTD}}^{\text{raw extract}}$ is calculated with Formula (A.18):

$$\rho_{\text{ISTD}}^{\text{raw extract}}\left(\mu\text{g}/\text{ml}\right)=\frac{\rho_{\text{ISTD}}^{\text{add}}\times V_{\text{ISTD}}^{\text{add}}}{V_{\text{Ex}}} \tag{A.18}$$

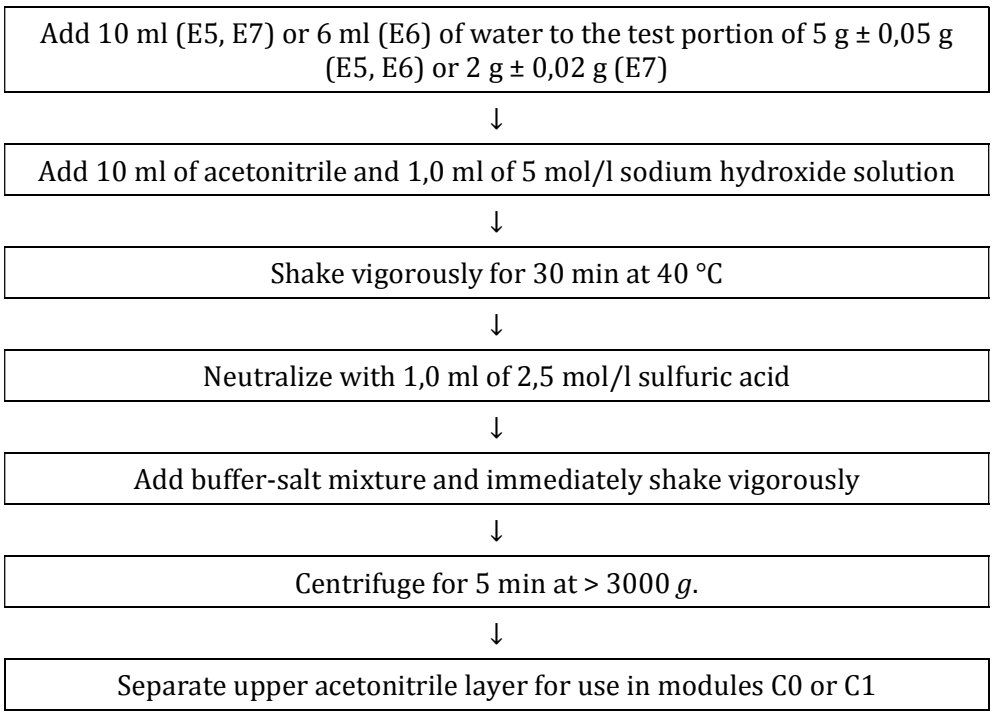
where

$\rho_{\text{ISTD}}^{\text{add}}$ is the mass concentration of added ISTD solution, in µg/ml;

V_{ISTD}^{add} is the volume of ISTD added to the sample, in ml;

V_{Ex} is the volume of the organic phase after extraction (usually 10 ml), in ml.

A.3.9.4 Flowchart of module E9



A.4 Description of clean-up (C) modules

A.4.1 Module C0: No clean-up

A.4.1.1 Principle

This clean-up module is used for extracts obtained by the modules E1 to E9 to determine alkaline labile and acidic pesticides (pK_a<5), which can bind to the primary secondary amine sorbent used in the modules C2 to C5. This clean-up module can also be used for analysis of commodities with a low matrix load. The recommended clean-up modules for the different sample types are given in Table 6.

The raw extract obtained by the modules E1 to E9 passes without clean-up to the extract stabilization (modules S), but it can be diluted if applicable.

A.4.1.2 Procedure

To reduce matrix effects an aliquot of the raw extract (V_1) can be diluted with a defined volume of an appropriate solvent (V_2) to resulting total volume (V_1+V_2) if the sensitivity of the used detection system is sufficient. If extract stabilization is necessary the possibly diluted extract is passed to module S1 without delay.

A.4.1.3 Calculation

The mass concentration of the final extract $\rho_{\text{sample}}^{\text{final extract}}$ gives the ratio of the amount of the sample, from which the residues are extracted, and the extraction volume. Usually $\rho_{\text{sample}}^{\text{final extract}}$ is given in g/ml and is calculated with Formula (A.19):

$$\rho_{\text{sample}}^{\text{final extract}}\left(\text{g}/\text{ml}\right)=\rho_{\text{sample}}^{\text{raw extract}}\times\frac{V_1}{\left(V_1+V_2\right)} \tag{A.19}$$

where:

$\rho_{\text{sample}}^{\text{raw extract}}$ is the mass concentration of the raw extract, in g/ml;

V_1 is the used aliquot of the raw extract, in ml;

V_2 is the added volume of an appropriate solvent, in ml.

The mass concentration of ISTD in the final extract $\rho_{\text{ISTD}}^{\text{final extract}}$ is calculated with Formula (A.20):

$$\rho_{\text{ISTD}}^{\text{final extract}}\left(\mu\text{g}/\text{ml}\right)=\frac{\rho_{\text{ISTD}}^{\text{add}}\times V_{\text{ISTD}}^{\text{add}}}{V_{\text{Ex}}}\times\frac{V_1}{\left(V_1+V_2\right)} \tag{A.20}$$

where:

$\rho_{\text{ISTD}}^{\text{add}}$ is the mass concentration of added ISTD solution, in µg/ml;

V_{ISTD}^{add} is the volume of ISTD added to the sample, in ml;

V_{Ex} is the volume of the organic phase after extraction (usually 10 ml), in ml;

V_1 is the used aliquot of the raw extract, in ml;

V_2 is the added volume of an appropriate solvent, in ml.

A.4.1.4 Flowchart of module C0

Dilute (if applicable) and use in modules S

A.4.2 Module C1: Clean-up by freezing-out of co-extracted fat, wax, sugars

A.4.2.1 Principle

This clean-up module is used for extracts obtained by the modules E2, E5, E6, E8 and E9 to reduce fat in extracts of a high fat content. It can be used in combination with other clean-up modules (C2, C3 and C5). The recommended clean-up modules for the different sample types are given in Table 6.

The raw extract obtained by the modules E2, E5, E6, E8 and E9 is freed from fat by freezing-out. The resulting extract can be clean-up by other clean-up-modules or is used for extract stabilization (modules S).

A.4.2.2 Procedure

Transfer an aliquot of 8 ml of the acetonitrile phase obtained by modules E2, E5 and E6 into a centrifuge tube (A.2.4) and store overnight in a freezer (A.2.10) (for flour, 2 h are sufficient), wherewith the major part of fat and waxes solidify and precipitate. Following a short centrifugation (where necessary), 6 ml of the still cold extract is taken for further clean-up by modules C2, C3 or C5 or for extract stabilization (modules S).

NOTE Freezing out also helps to partly remove some additional sample co-extractives with limited solubility in acetonitrile such as sugars.

To reduce matrix effects an aliquot of the raw extract (V_1) can be diluted with a defined volume of an appropriate solvent (V_2) to resulting total volume (V_1+V_2) if the sensitivity of the used detection system is sufficient.

If extract stabilization is necessary the maybe diluted extract is passed to modules S1 without delay.

A.4.2.3 Calculation

The mass concentration of the final extract $\rho_{\text{sample}}^{\text{final extract}}$ gives the ratio of the amount of the sample, from which the residues are extracted, and the extraction volume. Usually $\rho_{\text{sample}}^{\text{final extract}}$ is given in g/ml and is calculated with Formula (A.21):

$$\rho_{\text{sample}}^{\text{final extract}}\left(\text{g/ml}\right)=\rho_{\text{sample}}^{\text{raw extract}}\times\frac{V_1}{\left(V_1+V_2\right)} \qquad \text{(A.21)}$$

where

$\rho_{\text{sample}}^{\text{raw extract}}$	mass concentration of the raw extract, in g/ml;
V_1	is the used aliquot of the raw extract, in ml;
V_2	is the added volume of an appropriate solvent, in ml.

The mass concentration of ISTD in the final extract $\rho_{\text{sample}}^{\text{final extract}}$ is calculated with Formula (A.22):

$$\rho_{\text{ISTD}}^{\text{final extract}}\left(\mu\text{g/ml}\right)=\frac{\rho_{\text{ISTD}}^{\text{add}}\times V_{\text{ISTD}}^{\text{add}}}{V_{\text{Ex}}}\times\frac{V_1}{\left(V_1+V_2\right)} \qquad \text{(A.22)}$$

where

$\rho_{\mathrm{ISTD}}^{\mathrm{add}}$ is the mass concentration of added ISTD solution, in µg/ml;

V_{ISTD}^{add} is the volume of ISTD added to the sample, in ml;

V_{Ex} is the volume of the organic phase after extraction (usually 10 ml), in ml;

V_1 is the used aliquot of the raw extract, in ml;

V_2 is the added volume of an appropriate solvent, in ml.

A.4.2.4 Flowchart of module C1

Transfer 8 ml of the raw extract into a centrifuge tube and store overnight in a freezer (for flour, 2 h are sufficient)

↓

Centrifugation (where necessary) and take 6 ml of the still cold extract

↓

Dilute (if applicable) and use in modules S

A.4.3 Module C2: Clean-up by dispersive SPE with amine sorbent (PSA)

A.4.3.1 Principle

This clean-up module is used for extracts obtained by the modules E1 to E4 and E6 for the determination of neutral and alkaline pesticides in all commodities not mentioned further. It is also used for citrus fruits extracts after the clean-up by module C1. The recommended clean-up modules for the different sample types are given in Table 6.

The raw extract obtained by the modules E1 to E4 and E6 is cleaned-up by dispersive solid phase extraction (SPE) using primary secondary amine sorbent (PSA). The cleaned-up extract is used for extract stabilization (modules S).

A.4.3.2 Procedure

An aliquot of 6 ml of the acetonitrile phase obtained by the modules E1 to E4 and E6 is transferred into a Polypropylene-single use tube (A.2.4) already containing 150 mg PSA (A.1.15) and 900 mg of magnesium sulfate (A.1.14). Close the tube, shake vigorously for 30 s using a shaker (A.2.5) if necessary and centrifuge for 5 min at > 3000 *g*. Immediately isolate and acidify the clear extract as described in module S if necessary.

For 1 ml of extract 150 mg magnesium sulfate and 25 mg PSA are necessary.

NOTE It is helpful to load the centrifuge tubes with the dispersive SPE sorbents before beginning the extraction procedure needed for one batch of samples.

To reduce matrix effects an aliquot of the raw extract (V_1) can be diluted with a defined volume of an appropriate solvent (V_2) to resulting total volume (V_1+V_2) if the sensitivity of the used detection system is sufficient.

If extract stabilization is necessary the maybe diluted extract is passed to module S1 without delay.

A.4.3.3 Calculation

The mass concentration of the final extract $\rho_{\text{sample}}^{\text{final extract}}$ gives the ratio of the amount of the sample, from which the residues are extracted, and the extraction volume. Usually $\rho_{\text{sample}}^{\text{final extract}}$ is given in g/ml and is calculated with Formula (A.23):

$$\rho_{\text{sample}}^{\text{final extract}}\left(\text{g / ml}\right) = \rho_{\text{sample}}^{\text{raw extract}} \times \frac{V_1}{\left(V_1 + V_2\right)} \tag{A.23}$$

where

$\rho_{\text{sample}}^{\text{raw extract}}$ is the mass concentration of the raw extract, in g/ml;

V_1 is the used aliquot of the raw extract, in, in ml;

V_2 is the added volume of an appropriate solvent, in ml

The mass concentration of ISTD in the final extract $\rho_{\text{ISTD}}^{\text{final extract}}$ is calculated with Formula (A.24):

$$\rho_{\text{ISTD}}^{\text{final extract}}\left(\mu\text{g / ml}\right) = \frac{\rho_{\text{ISTD}}^{\text{add}} \times V_{\text{ISTD}}^{\text{add}}}{V_{\text{Ex}}} \times \frac{V_1}{\left(V_1 + V_2\right)} \tag{A.24}$$

where

$\rho_{\text{ISTD}}^{\text{add}}$ is the mass concentration of added ISTD solution, in µg/ml;

V_{ISTD}^{add} is the volume of ISTD added to the sample, in ml;

V_{Ex} is the volume of the organic phase after extraction (usually 10 ml), in ml;

V_1 is the used aliquot of the raw extract, in ml;

V_2 is the added volume of an appropriate solvent, in ml.

A.4.3.4 Flowchart of module C2

Transfer 6 ml of the raw extract into a centrifuge tube already containing 150 mg PSA and 900 mg of magnesium sulfate

↓

Shake vigorously for 30 s and centrifuge for 5 min at > 3000 *g*

↓

Dilute (if applicable) and use in modules S

A.4.4 Module C3: Clean-up by dispersive SPE with a larger amount of amine sorbent (PSA) a) 50 mg PSA/ml extract or b) 75 mg PSA/ml extract)

A.4.4.1 Principle

This clean-up module is used for extracts obtained by the modules E5 (e.g. for cereals, cereal products, flour) and E7 (e.g. for coffee, tea, dried herbs, spices) to clean-up especially for the determination of neutral and alkaline pesticides in plant material and food with low water content. The recommended clean-up modules for the different sample types are given in Table 6.

The raw extract obtained by the modules E5 and E7 is cleaned-up by dispersive solid phase extraction (SPE) using an increased amount of primary secondary amine sorbent (PSA). The cleaned-up extract is used for extract stabilization (modules S).

A.4.4.2 Procedure

An aliquot of 6 ml of the acetonitrile phase obtained by the modules E5 or E7 is transferred into a Polypropylene-single use tube (A.2.4) already containing a) 300 mg PSA (A.1.15) (module C3a) or b) 450 mg PSA (A.1.15) (module C3b) and 900 mg of magnesium sulfate (A.1.14). Close the tube, shake vigorously for 30 s using a shaker (A.2.5) if necessary and centrifuge for 5 min at > 3000 *g*. Immediately isolate and acidify the clear extract as described in module S if necessary.

For 1 ml of extract 150 mg magnesium sulfate and a) 50 mg PSA or b) 75 mg PSA are necessary.

NOTE: It is helpful to load the centrifuge tubes with the dispersive SPE sorbents before beginning the extraction procedure needed for one batch of samples.

To reduce matrix effects an aliquot of the raw extract (V_1) can be diluted with a defined volume of an appropriate solvent (V_2) to resulting total volume (V_1+V_2) if the sensitivity of the used detection system is sufficient.

If extract stabilization is necessary the maybe diluted extract is passed to module S1 without delay.

A.4.4.3 Calculation

The mass concentration of the final extract $\rho_{\text{sample}}^{\text{final extract}}$ gives the ratio of the amount of the sample, from which the residues are extracted, and the extraction volume. Usually $\rho_{\text{sample}}^{\text{final extract}}$ is given in g/ml and is calculated with Formula (A.25):

$$\rho_{\text{sample}}^{\text{final extract}}\left(\text{g}/\text{ml}\right)=\rho_{\text{sample}}^{\text{raw extract}}\times\frac{V_1}{\left(V_1+V_2\right)} \tag{A.25}$$

where

$\rho_{\text{sample}}^{\text{raw extract}}$ is the mass concentration of the raw extract, in g/ml;

V_1 is the used aliquot of the raw extract, in ml;

V_2 is the added volume of an appropriate solvent, in ml.

The mass concentration of ISTD in the final extract $\rho_{\text{ISTD}}^{\text{final extract}}$ is calculated with Formula (A.26):

$$\rho_{\text{ISTD}}^{\text{final extract}}\left(\mu\text{g}/\text{ml}\right)=\frac{\rho_{\text{ISTD}}^{\text{add}}\times V_{\text{ISTD}}^{\text{add}}}{V_{\text{Ex}}}\times\frac{V_1}{\left(V_1+V_2\right)} \tag{A.26}$$

where

$\rho_{\text{ISTD}}^{\text{add}}$ is the mass concentration of added ISTD solution, in µg/ml;

V_{ISTD}^{add} is the volume of ISTD added to the sample, in ml;

V_{Ex} is the volume of the organic phase after extraction (usually 10 ml), in ml;

V_1 is the used aliquot of the raw extract, in ml;

V_2 is the added volume of an appropriate solvent, in ml.

A.4.4.4 Flowchart of module C3

Transfer 6 ml of the raw extract into a centrifuge tube already containing a) 300 mg PSA or b) 450 mg PSA and 900 mg of magnesium sulfate

↓

Shake vigorously for 30 s and centrifuge for 5 min at > 3000 *g*

↓

Dilute (if applicable) and use in modules S

A.4.5 Module C4: Clean-up by dispersive SPE with a mixture of amine sorbent (PSA) and silica based reverse phase sorbent (C18)

A.4.5.1 Principle

This clean-up module is used for extracts obtained by the modules E2, E5 or E6 to reduce fat and clean-up in one step. It can be used alternatively to the sequential combination of clean-up modules C1 and C2, e.g. for citrus fruits, cereals and cereal products, avocado and olives. The recommended clean-up modules for the different sample types are given in Table 6.

The raw extract obtained by the modules E2, E5 and E6 is cleaned-up by dispersive solid phase extraction (SPE) using a mixture of primary secondary amine sorbent (PSA) and C18 reversed phase sorbent. The cleaned-up extract is used for extract stabilization (modules S).

A.4.5.2 Procedure

An aliquot of 6 ml of the acetonitrile phase obtained by the modules E2, E5 or E6 is transferred into a Polypropylene-single use tube (A.2.4) already containing 150 mg PSA (A.1.15), 150 mg C18 sorbent (A.1.16) and 900 mg of magnesium sulfate (A.1.14). Close the tube, shake vigorously for 30 s using a shaker (A.2.5) if necessary and centrifuge for 5 min at > 3000 *g*. Immediately isolate and acidify the clear extract as described in module S if necessary.

For 1 ml of extract 150 mg magnesium sulfate, 25 mg PSA and 25 mg C18 sorbent are necessary.

NOTE: It is helpful to load the centrifuge tubes with the dispersive SPE sorbents before beginning the extraction procedure needed for one batch of samples.

To reduce matrix effects an aliquot of the raw extract (V_1) can be diluted with a defined volume of an appropriate solvent (V_2) to resulting total volume (V_1+V_2) if the sensitivity of the used detection system is sufficient.

If extract stabilization is necessary the maybe diluted extract is passed to module S1 without delay.

A.4.5.3 Calculation

The mass concentration of the final extract $\rho_{\text{sample}}^{\text{final extract}}$ gives the ratio of the amount of the sample, from which the residues are extracted, and the extraction volume. Usually $\rho_{\text{sample}}^{\text{final extract}}$ is given in g/ml and is calculated with Formula (A.27):

$$\rho_{\text{sample}}^{\text{final extract}}\left(\text{g}/\text{ml}\right) = \rho_{\text{sample}}^{\text{raw extrakt}} \times \frac{V_1}{\left(V_1 + V_2\right)} \qquad \text{(A.27)}$$

where

$\rho_{\text{sample}}^{\text{raw extract}}$ is the mass concentration of the raw extract, in g/ml,

V_1 is the used aliquot of the raw extract, in ml;

V_2 is the added volume of an appropriate solvent, in ml.

The mass concentration of ISTD in the final extract $\rho_{\text{ISTD}}^{\text{final extract}}$ is calculated with Formula (A.28)

$$\rho_{\text{ISTD}}^{\text{final extract}}\left(\mu\text{g}/\text{ml}\right)=\frac{\rho_{\text{ISTD}}^{\text{add}}\times V_{\text{ISTD}}^{\text{add}}}{V_{\text{Ex}}}\times\frac{V_1}{\left(V_1+V_2\right)} \quad \text{(A.28)}$$

where

$\rho_{\text{ISTD}}^{\text{add}}$ is the mass concentration of added ISTD solution, in µg/ml;

V_{ISTD}^{add} is the volume of ISTD added to the sample, in ml;

V_{Ex} is the volume of the organic phase after extraction (usually 10 ml), in ml;

V_1 is the used aliquot of the raw extract, in ml;

V_2 is the added volume of an appropriate solvent, in ml.

A.4.5.4 Flowchart of module C4

Transfer 6 ml of the raw extract into a centrifuge tube already containing 150 mg PSA, 150 mg C18 sorbent and 900 mg of magnesium sulfate

↓

Shake vigorously for 30 s and centrifuge for 5 min at > 3000 *g*

↓

Dilute (if applicable) and use in modules S

A.4.6 Module C5: Clean-up by dispersive SPE with a mixture of amine sorbent (PSA) and graphitized carbon black

A.4.6.1 Principle

This clean-up module is used for extracts obtained by the modules E1 or E7 to clean-up especially for the determination of neutral and alkaline pesticides in extracts of samples with a high content of carotenoids or chlorophyll. The recommended clean-up modules for the different sample types are given in Table 6.

The raw extract obtained by the modules E1 or E7 is cleaned-up by dispersive solid phase extraction (SPE) using a mixture primary secondary amine sorbent (PSA) and graphitized carbon black (GCB). The cleaned-up extract is used for extract stabilization (modules S).

A.4.6.2 Procedure

Transfer an aliquot of 6 ml of the acetonitrile phase obtained by the modules E1 or E7 into a Polypropylene-single use tube (A.2.4) already containing 150 mg of PSA (A.1.15), 900 mg of magnesium sulfate (A.1.14) and a) 15 mg of GCB (A.1.17) (module C5a) or b) 45 mg of GCB (A.1.17) (module C5b). Close the tube, shake vigorously for 30 s using a shaker (A.2.5) if necessary and centrifuge for 5 min at > 3000 *g*. Immediately isolate and acidify the clear extract as described in module S if necessary.

For 1 ml extract 150 mg of magnesium sulfate, 25 mg of PSA and, depending on the sample, a) 2,5 mg of GCB or b) 7,5 mg of GCB are necessary.

NOTE It is helpful to load the centrifuge tubes with the dispersive SPE sorbents before beginning the extraction procedure needed for one batch of samples.

To reduce matrix effects an aliquot of the raw extract (V_1) can be diluted with a defined volume of an appropriate solvent (V_2) to resulting total volume (V_1+V_2) if the sensitivity of the used detection system is sufficient.

If extract stabilization is necessary the maybe diluted extract is passed to module S1 without delay.

A.4.6.3 Calculation

The mass concentration of the final extract $\rho_{\text{sample}}^{\text{final extract}}$ gives the ratio of the amount of the sample, from which the residues are extracted, and the extraction volume. Usually $\rho_{\text{sample}}^{\text{final extract}}$ is given in g/ml and is calculated with Formula (A.29):

$$\rho_{\text{sample}}^{\text{final extract}}\left(\text{g}/\text{ml}\right)=\rho_{\text{sample}}^{\text{raw extract}}\times\frac{V_1}{\left(V_1+V_2\right)} \tag{A.29}$$

where

$\rho_{\text{sample}}^{\text{raw extract}}$ is the mass concentration of the raw extract, in g/ml;

V_1 is the used aliquot of the raw extract, in ml;

V_2 is the added volume of an appropriate solvent, in ml.

The mass concentration of ISTD in the final extract $\rho_{\text{ISTD}}^{\text{final extract}}$ is according to Formula (A.30):

$$\rho_{\text{ISTD}}^{\text{final extract}}\left(\mu\text{g}/\text{ml}\right)=\frac{\rho_{\text{ISTD}}^{\text{add}}\times V_{\text{ISTD}}^{\text{add}}}{V_{\text{Ex}}}\times\frac{V_1}{\left(V_1+V_2\right)} \tag{A.30}$$

where

$\rho_{\text{ISTD}}^{\text{add}}$ is the mass concentration of added ISTD solution, in µg/ml;

V_{ISTD}^{add} is the volume of ISTD added to the sample, in ml;

V_{Ex} is the volume of the organic phase after extraction (usually 10 ml), in ml.

V_1 is the used aliquot of the raw extract, in ml;

V_2 is the added volume of an appropriate solvent, in ml.

A.4.6.4 Flowchart of module C5

Transfer 6 ml of the raw extract into a centrifuge tube already containing 900 mg of magnesium sulfate, 150 mg of PSA and a) 15 mg of GCB or b) 45 mg of GCB

↓

Shake vigorously for 30 s and centrifuge for 5 min at > 3000 *g*

↓

Dilute (if applicable) and use in modules S

A.5 Description of extract stabilization (S) modules

A.5.1 Module S0: No extract stabilization

A.5.1.1 Principle

This module is used for extracts obtained by the modules C0 to C5, if acid-labile pesticides (like flazasulfuron, mesosulfuron, tribenuron, triflusulfuron) are to be determined.

The extract obtained by the modules C0 to C5 is passed to detection modules (modules D) without treatment.

A.5.1.2 Procedure

The extract obtained by the modules C0 to C5 is passed to detection modules (modules D) without treatment. Transfer the extract into autosampler vials (A.2.11) and use for gas- and liquid chromatographic analysis (modules D). Store the residual extract in a refrigerator to be used if necessary.

A.5.1.3 Flowchart of module S0

Transfer the extract into autosampler vials and use for gas- and liquid chromatographic analysis (modules D)

A.5.2 Module S1: Extract stabilization with formic acid

A.5.2.1 Principle

This module is used for extracts obtained by the modules C0 to C5, if acid-stable pesticides are to be determined.

To the extract obtained by the modules C0 to C5 add 10 µl 5 % formic acid solution in acetonitrile per 1 ml are added and then it is passed to detection modules (modules D).

A.5.2.2 Procedure

Transfer 5 ml aliquot of the cleaned-up extract from modules C0 to C5 into a screw cap storage vial (A.2.12), taking care to avoid sorbent particles of being carried over, and slightly acidify by adding 50 µl of a 5 % formic acid solution in acetonitrile (A.1.18) and shake. Transfer the pH-adjusted extract into autosampler vials (A.2.11) and use for gas- and liquid chromatographic analysis (modules D). Store the residual extract in a refrigerator to be used if necessary.

For 1 ml extract 10 µl of the formic acid solution (A.1.18) are necessary.

The effect of the marginal dilution caused by the extract stabilization has not to be considered for the mass concentration of the sample in the final extract.

A.5.2.3 Flowchart of module S1

Transfer 5 ml cleaned-up extract into a screw cup storage vial, add 50 µl of a 5 % formic acid solution in acetonitrile and shake.

↓

Transfer the extract into autosampler vials and use for gas- and liquid chromatographic analysis (modules D)

A.6 Description of detection (D) modules

A.6.1 Module D1: Determination using liquid chromatography with tandem-mass spectrometric detection (LC-MS/MS)

A.6.1.1 Principle

The liquid chromatographic determination with tandem-mass spectrometric detection is suited for all analytes extracted by modules E1 to E9, which are ionizable at atmospheric pressure. A clean-up using one of the modules C1 to C5 is recommended, but not in every case necessary due to the high selectivity of the tandem-mass spectrometric detection.

A.6.1.2 Procedure

Inject solutions from modules S0 or S1 into a liquid chromatographic system, which is hyphenated with a tandem-mass spectrometer via electrospray- or APCI-interface.

A.6.1.3 Apparatus

The measurement may be performed using various instruments, instrument parameters and columns. Some instrument parameters and columns are listed in this module. The following LC-MS/MS operating conditions have been shown to be satisfactory, but they are only examples of experimental conditions on the stated instruments. Variations of these conditions are not deviations from the method.

A.6.1.4 Examples of suitable conditions

A.6.1.4.1 HPLC system 1

For most LC-amenable compounds:

Injector programme	draw 5 µl Mobile phase A1,
	draw 1 µl sample,
	wash needle with acetonitrile
	draw 2 µl Mobile phase A1
	draw 1 µl sample,
	wash needle with acetonitrile
	draw 2 µl Mobile phase A1
	draw 1 µl sample,
	wash needle with acetonitrile

	draw 2 µl Mobile phase A1
	draw 1 µl sample,
	wash needle with acetonitrile
	draw 5 µl Mobile phase A1
Column	Phenomenex Aqua 5 µ C18 125Å, 50 mm × 2 mm
Mobile phase A1	Methanol/water 2+8 (*V/V*) with 5 mmol/l ammonium formate
Mobile phase B1	Methanol/water 9+1 (*V/V*) with 5 mmol/l ammonium formate
Column temperature	20 °C

Table A.2 — Flow rate and elution gradient

Time min	**Flow rate** µl/min	**Mobile phase A1 %**	**Mobile phase B1 %**
0	200	100	0
11	200	0	100
23	200	0	100
25	200	100	0
33	200	100	0

A.6.1.4.2 HPLC system 2

For most LC-amenable compounds:

Column	Waters Acquity UPLC BEH C18, 50 mm x 2,1 mm, particle size 1,7 µm
Mobile phase A1	Methanol/water 2+8 (V/V) with 5 mmol/l ammonium formate
Mobile phase B1	Methanol/water 9+1 (V/V) with 5 mmol/l ammonium formate
Column temperature	room temperature
Injection volume	3 µl

Table A.3 — Flow rate and elution gradient

Time min	**Flow rate** µl/min	**Mobile phase A1 %**	**Mobile phase B1 %**
0	400	100	0
6,1	400	0	100
12,7	400	0	100
13,8	400	100	0
18,2	400	100	0

A.6.1.4.3 HPLC system 3

For acidic compounds:

Column	Zorbax XDB C18, 150 mm x 2,1 mm, particle size 3,5 µm
Mobile phase A2	Acetic acid solution in water, add 0,1 ml glacial acetic acid to 1 000 ml of water
Mobile phase B2	Acetic acid solution in acetonitrile, add 0,1 ml glacial acetic acid to 1 000 ml of acetonitrile
Column temperature	40 °C
Injection volume	5 µl

Table A.4 — Flow rate and elution gradient

Time min	**Flow rate** µl/min	**Mobile phase A2 %**	**Mobile phase B2 %**
0	300	80	20
20	300	0	100
22	300	0	100
22,1	300	80	20
30	300	80	20

A.6.1.4.4 MS/MS system 1

MS/MS instrument	AB Sciex QTRAP 5500
Ion source	Turbo Ion Spray (ESI)

Table A.5 — Ion source and general parameters

Curtain gas	nitrogen, 40 psi	Gas 2 temperature	400 °C
Collision gas	nitrogen, 2 units	Resolution MS 1/ MS 2	0,7 amu (FWHM[a])
Ion spray voltage	5500 V	Dwell time	≥ 3 ms (depending on sensitivity of MRM)
Gas 1	nitrogen, 40 psi		
Gas 2	nitrogen, 40 psi		
[a] FWHM = Full width at half maximum			

A.6.1.4.5 MS/MS system 2

MS/MS instrument	Agilent 6460A
Ion source	Jet Stream Source (ESI)

Table A.6 — Ion source and general parameters

Drying gas flow	6 l/min	Sheath gas temperature	400 °C
Drying gas temperature	325 °C	Nozzle voltage	off
Nebulizer gas pressure	35 psi	Resolution MS 1/ MS 2	unit
Capillary voltage	4000 V	Dwell time	≥ 3 ms (depending on sensitivity of MRM)
Sheath gas flow	12 l/min		

These conditions are given in Agilent Application Note: 5990-4253EN (see Appendix II and III, http://www.chem.agilent.com/Library/applications/5990-4253EN.pdf).

A.6.1.4.6 MS/MS system 3

MS/MS instrument	Waters Xevo TQS
Ion source	Electrospray

Table A.7 — Ion source and general parameters

Curtain gas (cone)	nitrogen, 150 l/h	Desolvation temperature	500 °C to 600 °C
Collision gas	argon, 0,15 ml/min	Resolution MS 1/ MS 2 (FWHM[a])	ca. 0,7 amu
Ion spray voltage	800 V	Dwell time	≥ 3 ms (depending on sensitivity of MRM)
Gas 1 (nebuliser)	7 bar		
Gas 2 (desolvation)	800 l/h to1000 l/h		

[a] FWHM = Full width at half maximum

A.6.1.5 Procedural remarks

For suitable experimental conditions of LC-MS/MS measurement see CEN/TR 15641:2007 “Determination of pesticide residues by LC-MS/MS — Collection of mass spectrometric parameters” [2]. Nevertheless, individual tuning of the compounds on the instrument that is used for measurement usually provides better sensitivities.

For LC-MS/MS analysis reversed phase columns are especially well suited, which tolerate high water content in the gradient elution.

Using particular gradient/column combinations makes it necessary for highly polar compounds to dilute the extract with water or mobile phase A to get sufficient peak shape and chromatographic resolution. It has to be considered in these cases that increasing the water content can cause precipitation of highly lipophilic compounds which can be lost during filtration. Therefore automated dilution of the solutions in the instrument injector is recommended (see A.6.1.4.1.).

In spite of the high selectivity of MS/MS detection it is recommended to observe at least two mass transition of each analyte for a confident identification. It has to be considered in the selection of the number of mass transitions simultaneously detected that the number of measuring points for one peak is at least 10. Therefore the use of automatic algorithms (like “scheduled MRM” or “triggered MRM”) is recommended to measure the analyte-typical mass transitions in a small window around the expected retention time of the analyte.

Within a sequence standard solutions have to be injected regularly, to this see also the requirements of the EU Quality Control Procedures [5].

A.6.2 Module D2: Determination using liquid chromatography with accurate mass spectrometric detection (LC-accurate mass)

A.6.2.1 Principle

The liquid chromatographic determination with accurate mass spectrometric detection is suited for all analytes extracted by modules E1 to E9, which are ionizable at atmospheric pressure. A clean-up using one of the modules C1 to C5 is recommended, but not in every case necessary due to the high selectivity of the accurate mass spectrometric detection.

A.6.2.2 Procedure

Inject solutions from modules S0 or S1 into a liquid chromatographic system, which is hyphenated with an accurate mass spectrometer via electrospray- or APCI-interface.

A.6.2.3 Apparatus

The measurement may be performed using various instruments, instrument parameters and columns. Some instrument parameters and columns are listed in this module. The following LC- accurate mass system operating conditions have been shown to be satisfactory, but they are only examples of experimental conditions on the stated instruments. Variations of these conditions are not deviations from the method.

A.6.2.4 Examples of suitable conditions

A.6.2.4.1 LC system 1

For most LC-amenable compounds:

UHPLC	Agilent 1290 Infinity
Injection volume	3 µl
Column	Agilent Zorbax Eclipse Plus Rapid Resolution HD, 1,8 µm, 100 mm x 2,1 mm
Mobile phase A3	Water with 5 mmol/l ammonium formate and 0,1 % formic acid
Mobile phase B3	Methanol with 5 mmol/l ammonium formate and 0,1 % formic acid
Column temperature	30 °C

Table A.8 — Flow rate and elution gradient

Time min	**Flow rate** µl/min	**Mobile phase A3** %	**Mobile phase B3** %
0	300	80	20
2	300	80	20
15	300	0	100
17	300	0	100
17,1	300	80	20
19	300	80	20

A.6.2.4.2 LC system 2

For most LC-amenable compounds:

HPLC pump	DIONEX Ultimate 3000 RS
Autosampler	DIONEX Ultimate 3000 RS
Injection volume	10 µl
Column	THERMO SCIENTIFIC Acclaim RSLC 120 C18, 2,2 µm – 120 Å – 100 mm x 2,1 mm
With pre-column	WATERS Vanguard Acquity UPLC BEH C18 1,7 µm – 2,1 × 5 mm
Mobile phase A4	Methanol/water 1+9 (*V/V*) with 5 mmol/l ammonium formate and 0,01 % formic acid
Mobile phase B4	Methanol with 5 mmol/l ammonium formate and 0,01 % formic acid
Column temperature	30 °C

Table A.9 — Flow rate and elution gradient

Time min	**Flow rate** µl/min	**Mobile phase A4 %**	**Mobile phase B4 %**
0	200	99	1
0,1	200	99	1
1	200	99	1
3	200	61	39
14	400	0,1	99,9
16	480	0,1	99,9
16,1	480	99	1
19	480	99	1
19,1	200	99	1
20	200	99	1

A.6.2.4.3 LC system 3

HPLC pump	Dionex Ultimate 3000 binary pump HPG-3400RS
Autosampler	Dionex Ultimate 3000 RS - WPS 3000
Column	Thermo Scientific Accucore aQ C18, 100 mm x 2,1 mm, particle size 2,6 µm
Mobile phase A3	5 mmol/l ammonium formate and 0,1 % of formic acid in water
Mobile phase B3	5 mmol/l ammonium formate and 0,1 % of formic acid in methanol
Column temperature	40 °C

Table A.10— Flow rate and elution gradient

Time min	**Flow rate** µl/min	**Mobile phase A3** **%**	**Mobile phase B3** **%**
0	400	80	20
1,0	400	80	20
10,5	400	2	98
14,5	400	2	98
15,0	400	80	20
19,0	400	80	20

A.6.2.4.4 LC system 4

UPLC:	Agilent 1290 Infinity
Injection volume:	5 µl
Column	Dionex Acclaim RSLC 120 C18 (2,2 µm; 120 Å; 2,1 mm x 100 mm)
Mobile phase A5	Milli-Q water with 0,1 % formic acid and 5 mmol/l ammonium hydroxide.
Mobile phase B5	Acetonitrile with 0,1 % formic acid.
Column temperature	30 °C

Table A.11 — Flow rate and elution gradient

Time min	**Flow rate** µl/min	**Mobile phase A5** **%**	**Mobile phase B5** **%**
0	0,2	90	10
1	0,2	90	10
3	0,2	60	40
14	0,4	10	90
16	0,4	10	90
16,1	0,2	90	10
20	0,2	90	10

A.6.2.4.5 LC system 5

HPLC pump	ACQUITY UPLC I-Class BSM
Autosampler	ACQUITY I-Class FTN
Injection volume:	5 µl
Column	Waters Acquity UPLC BEH C18, 100 mm x 2,1 mm, particle size 1,7 µm
Mobile phase A6	Water with 10 mmol/l ammonium acetate
Mobile phase B6	Methanol with 10 mmol/l ammonium acetate
Column temperature	45 °C

Table A.12 — Flow rate and elution gradient

Time min	**Flow rate** µl/min	**Mobile phase A6 %**	**Mobile phase B6 %**
0	0,45	98	2
0,25	0,45	98	2
12,25	0,45	1	99
13,00	0,45	1	99
13,01	0,45	98	2
17,00	0,45	98	2

A.6.2.4.6 Accurate mass system 1

Accurate mass instrument AB Sciex TripleTOF 5600+

Ion source DuoSpray Ion Source (ESI)

Table A.13 — Ion source and general parameters

Ion Source Gas 1	nitrogen, 30 l/min
Ion Source Gas 2	nitrogen, 30 l/min
Curtain Gas	20
Temperature	400 °C
IonSpray Voltage	5000 V

A.6.2.4.7 Accurate mass system 2

Accurate mass instrument BRUKER IMPACT II

Ion source Turbo Ion Spray (ESI)

Table A.14 — Ion source

End plate offset:	500
Capillary voltage:	2500 V
Nebulizer:	31 psi
Dry Gas:	8 l/min
Dry Temp:	200°C

A.6.2.4.8 Accurate mass system 3

Accurate mass instrument Thermo Scientific Q-Exactive

Ion source Heated Electrospray Ionization Source (HESI II) positive ionization

Table A.15— Ion source parameters

Sheath gas flow rate	nitrogen, 45 l/min	Capillary temperature	250 °C
Aux gas flow rate	nitrogen, 4 l/min	S-lens RF level	50
Sweep gas flow rate	nitrogen, 2 l/min	Aux gas heater temperature	230 °C
Spray voltage	3,80 (kV)		

A.6.2.4.9 Accurate mass system 4

Accurate mass instrument 6550 iFunnel QTOF LC/MS, Agilent Technologies

Ion source Agilent Jet stream ESI

Table A.16 — Ion source and general parameters

Drying gas	nitrogen, 13 l/min	Sheat gas temperature	400 °C
Sheat gas Flow	nitrogen, 12 l/min	Collision gas	nitrogen, 2 units
Ion spray voltage	4000 V		

A.6.2.4.10 Accurate mass system 5

Accurate mass instrument Xevo G2-S QTof (YDA 172)

Ion source Z-Spray (ESI)

Table A.17 — Ion source and general parameters

G2-S QTof		
Mode:	ESI (+)	ESI (-)
Analyser mode:	Sensitivity	Sensitivity
Capillary:	1,0 kV	1,5 kV
Source Temp:	120°C	120 °C
Cone gas (Nitrogen):	50 l/h	10 l/h
Sample cone:	40 V	40 V
Desolv Temp:	550 °C	350 °C
Desolv gas:	1000 l/h	900 l/h

A.6.2.5 Procedural remarks

For LC-accurate mass analysis, reversed phase columns are especially well suited, which tolerate high water content in the gradient elution.

Using particular gradient/column combinations makes it necessary for highly polar compounds to dilute the extract with water or mobile phase A to get sufficient peak shape und chromatographic resolution. It has to be considered in these cases that increasing the water content can cause precipitation of highly lipophilic compounds which can be lost during filtration. Therefore automated dilution of the solutions in the instrument injector is recommended.

Simultaneous work in full scan MS and MS/MS is recommended. For detection and identification two or more ions (preferably including the molecular ion) with mass accuracy better than 5 ppm are required. If fragment ion is obtained in MS/MS mode then acceptable mass accuracy is < 10 ppm. For quantitation wider mass extraction window can be used.

Within a sequence standard solutions have to be injected regularly, to this see also the requirements of the EU Quality Control Procedures [5].

A.6.3 Module D3: Determination using gas chromatography with tandem-mass spectrometric detection (GC-MS/MS)

A.6.3.1 Principle

The gas chromatographic determination with tandem-mass spectrometric detection is suited for all analytes extracted by modules E1 to E7, which are volatile without decomposition. A clean-up using one of the modules C1 to C5 is recommended, but is not necessary in every case due to the high selectivity of the tandem-mass spectrometric detection.

A.6.3.2 Procedure

Inject solutions from modules S0 or S1 into a gas chromatographic system, which is hyphenated with a tandem-mass spectrometer via electron impact ion source. The measurement may be performed using various GC-MS/MS instruments, instrument parameters and columns.

A.6.3.3 Apparatus

The following GC-MS/MS operating conditions have been shown to be satisfactory, but they are only examples of experimental conditions on the stated instruments. Variations of these conditions are not deviations from the method.

A.6.3.4 Examples of suitable conditions

A.6.3.4.1 Instrument 1

Instrument	Thermo Fischer Scientific; TSQ Quantum XLS, Trace GC Ultra with PTV
Carrier gas	Helium, 1,0 ml/min, constant flow
Injector	Programmable temperature vaporizer (PTV) with Siltek baffled liner 2mm ID
Injection technique	Splitless, PTV-programmed
Injection	1 µl, final extract into acetone/hexane (1/1, v/v) transferred (see B.10)
Injector temperature programme	0,02 min isothermal at 70 °C, programmed to rise at 720 °C/min to 280 °C, 1,2 min isothermal at 280 °C; rise at 840 °C/min to 320 °C, 6 min isothermal
Injector purge	0,0 min to 1,3 min no purge, 1,3 min to 6,0 min 80 ml/min (clean flow), after 6,0 min 50 ml/min split flow
Column	Fused-silica-capillary with 5 % phenyl/ 95 % dimethylsiloxane, length 20 m, inner diameter 0,18 mm, film thickness 0,18 µm (e.g. Restek Rxi-5Sil MS)
Column temperature	80 °C isothermal for 1,5 min, programmed to rise at 30 °C/min to 210 °C, rise at 20 °C/min to 320 °C, isothermal at 320 °C for

	2 min.
Transfer-line temperature	280 °C
Solvent delay	3,5 min
Ionization	Electron impact, 70 eV, 25 µA
Source temperature	250 °C
Resolution MS1/MS2	0,7 amu (FWHM = Full width at half maximum)
Collision gas	Argon, 1,0 × 10^{-3} Torr

These conditions are given in Thermo Application Note 52027 (see http://www.thermofisher.com.au/Uploads/file/Scientific/Applications/Scientific-Instruments-Automation/Fast-GC-MSMS-Pesticide-Analysis-AN52072.pdf)

A.6.3.4.2 Instrument 2

Instrument	Agilent GC 6890N, Kodiak 800 MS/MS
Carrier gas	Helium, 21 psi, constant pressure
Injector	Programmable temperature vaporizer (PTV)
Injection technique	PTV-programme with solvent delay
Injection	3 µl
Injector temperature programme	0,8 min isothermal at 40 °C, programmed to rise at 720 °C/min to 300 °C, 5 min isothermal
Injector purge	0,0 min to 0,5 min 10 ml/min (vent flow), 0,5 min to 2,5 min no purge, after 2,5 min 50 ml/min purge flow
Column	Fused-silica-capillary with 5 % phenyl/ 95 % dimethylsiloxane, length 30 m, inner diameter 0,25 mm, film thickness 0,25 µm (e.g. J&W HP5 MS)
Column temperature	70 °C isothermal for 2 min, programmed to rise at 25 °C/min to 150 °C, rise at 3 °C/min to 180 °C, rise at 20 °C/min to 280 °C, isothermal at 280 °C for 10 min.
Transfer-line temperature	250 °C
Solvent delay	6,5 min
Ionization	Electron impact, 70 eV
Source temperature	200 °C
Resolution MS1/MS2	0,7 amu (FWHM = Full width at half maximum)
Collision gas	Argon, 1,4 × 10^{-3} Torr

A.6.3.4.3 Instrument 3

The following parameters are an example for using backflush.

Instrument	Agilent GC 6890N, Triplequad 7000
Carrier gas	Helium
Carrier gas programme	Retention time locking for chlorpyrifos-methyl at 16,5 min
Carrier gas pressure during backflush	after 41,87 min: inlet pressure 1psi, backflush pressure 30 psi
Injector	Programmable temperature vaporizer (PTV)
Injection technique	Splitless
Injection	2 µl
Injector temperature programme	0,01 min isothermal at 80 °C, programmed to rise at 500 °C/min to 300 °C, 2 min isothermal
Injector purge	0,0 min to 0,75 min Septum purge flow 3 ml/min, 0,75 min to 2,0 min Purge flow to split vent 30 ml/min, after 2,0 min Gas saver 20 ml/min
Column	Fused-silica-capillary with 5 % phenyl/ 95 % dimethylsiloxane, length 30 m, inner diameter 0,25 mm, film thickness 0,25 µm (e.g. DB5 MS)
Column temperature	70 °C isothermal for 2 min, programmed to rise at 25 °C/min to 150 °C, rise at 3 °C/min to 180 °C, rise at 20 °C/min to 280 °C, isothermal at 280 °C for 10 min, post run (backflush) 3 min at 280 °C
Transfer-line temperature	280 °C
Solvent delay	3,75 min
Ionization	Electron impact, 70 eV
Source temperature	300 °C
Resolution MS1/MS2	1,2 amu each
Quenching gas	Helium, 2,25 ml/min
Collision gas	Nitrogen, 1,5 ml/min
Time filter peak width	0,7 s

A.6.3.5 Procedural remarks

For suitable experimental conditions of GC-MS/MS measurement see CEN/TR 16699 “Foodstuffs - Determination of pesticide residues by GC-MS/MS - Tandem mass spectrometric parameters” [3]. Nevertheless, individual tuning of the compounds on the instrument that is used for measurement usually provides better sensitivities.

For GC-MS/MS analysis nonpolar columns are especially well suited, e.g. with 100 % methylsiloxane or with 5 % phenyl- and 95 % methylsiloxane.

Contamination of the injector and the beginning of the capillary column can cause partial decomposition of some analytes and shift of the retention time. To avoid this, an analyte protectants mixture (AP-mix) containing D-sorbitol (99,5 %), glucono-delta-lactone, Shikimi acid and 3-ethoxy-1,2-propandiole is well suited. For preparation of this mixture 2 g of 3-ethoxy-1,2-propandiole are weighed into a 10 ml volumetric flask, then 2 ml of an 5 % glucono-delta-lactone solution, 1 ml of 5 % D-sorbitol solution and 1 ml of 5 % Shikimi acid solution are added. Solvent is always acetonitrile/water (60/40. v/v). The volumetric flask is filled up with acetonitrile/water (60/40. v/v). Thirty µl of the analyte protectants mixture are added to 1 ml final extract.

Backflush is recommended. At backflush the column flow is reversed after elution of the last relevant analytes, so the less volatile matrix compounds remaining on the column are pressed backwards through the column and they leave it through the split exit. As a consequence, shift of retention time and contamination of the ion source can be minimized.

In spite of the high selectivity of MS/MS detection it is recommended to observe at least two mass transition of each analyte for a confident identification. It has to be considered in the selection of the number of mass transitions simultaneously detected that the number of measuring points for one peak is at least 10. Therefore the use of automatic algorithms (like “scheduled MRM” or “triggered MRM”) is recommended to measure the analyte-typical mass transitions in a small window around the expected retention time of the analyte.

Within a sequence standard solutions have to be injected regularly, to this see also the requirements of the EU Quality Control Procedures [5].

A.6.4 Module D4: Determination using gas chromatography with mass spectrometric detection (GC-MS)

A.6.4.1 Principle

The gas chromatographic determination with single quadrupole mass spectrometric detection (preferred in SIM mode), with ion trap detectors and with time-of-flight mass spectrometric detection (independent of the MS resolution) is suited for all analytes extracted by modules E1 to E7, which are volatile without decomposition after clean-up using one of the modules C1 to C5. GC-MS analysis without clean-up is only possible if the extracts are highly diluted (module C0).

A.6.4.2 Procedure

Inject solutions from modules S0 or S1 into a gas chromatographic system with mass spectrometric detection. The measurement may be performed using various GC-MS instruments, instrument parameters and columns.

A.6.4.3 Apparatus

The following GC-MS operating conditions have been shown to be satisfactory, but they are only examples of experimental conditions on the stated instruments. Variations of these conditions are not deviations from the method.

A.6.4.4 Examples of suitable conditions

A.6.4.4.1 Instrument 1

Instrument	Agilent GC 6890B GC/5973N MSD
Carrier gas	Helium, 2,0 ml/min, constant flow
Injector	Cold injection system KAS (CIS/PTV)
Injection technique	Cold injection with solvent delay
Injection	3 µl
Injector temperature programme	0,8 min isothermal at 50 °C, programmed to rise at 720 °C/min to 280 °C, 5 min isothermal at 280 °C, rise at 720 °C/min to 300 °C, 5 min isothermal at 300 °C
Injector purge	0,0 min to 0,5 min 20 ml/min (vent flow), 0,5 min to 2,0 min no purge, 2,0 min to 6,0 min 47,4 ml/min (purge flow), after 6,0 min Gas saver 20 ml/min
Column	Fused-silica-capillary with 5 % phenyl/ 95 % dimethylsiloxane, length 30 m, inner diameter 0,25 mm, film thickness 0,25 µm (e.g. DB5 MS)
Column temperature	40 °C isothermal for 2,0 min, programmed to rise at 30 °C/min to 220 °C, rise at 5 °C/min to 260 °C, rise at 20 °C/min to 280 °C, isothermal at 280 °C for 15 min.
Transfer-line temperature	280 °C
Solvent delay	4,0 min
Ionization	Electron impact, 70 eV
Source temperature	230 °C

A.6.4.4.2 Instrument 2

Instrument	Agilent GC 6890B GC/5973N MSD
Carrier gas	Helium, 1,0 ml/min, constant flow
Injector	Split/splitless injector with deactivated linear single taper liner
Injection technique	Pulsed splitless, 200 kPa, pulse time 1,0 min
Injection	1 µl
Injector temperature	240 °C
Injector purge	1,5 min after injection (purge time) purge flow to split vent 50 ml/min
Column	Fused-silica-capillary with 5 % phenyl/ 95 % dimethylsiloxane, length 30 m, inner diameter 0,25 mm, film thickness 0,25 µm (e.g. HP5 MS)
Column temperature	70 °C isothermal for 2,0 min, programmed to rise at 25 °C/min to 170 °C, rise at 3 °C/min to 210 °C, rise at 30 °C/min to 290 °C, isothermal at 290 °C for 9 min.
Transfer-line temperature	280 °C
Solvent delay	6,0 min
Ionization	Electron impact, 70 eV
Source temperature	230 °C

A.6.4.5 Procedural remarks

For retention times and typical fragment ions of pesticides see CEN/TR 16468 "Food analysis – Determination of pesticide residues by GC-MS – Retention times and mass spectrometric parameters" [4].

For GC-MS analysis nonpolar columns are especially well suited, e.g. with 100 % methylsiloxane or with 5 % phenyl- and 95 % methylsiloxane. For confirmation of positive findings more polar columns are recommended, e.g. with 50 % phenyl- and 50 % methylsiloxane or with 14 % cyanopropylphenyl- and 86 % methylsiloxane.

Contamination of the injector and the beginning of the capillary column can cause partial decomposition of some analytes and shift of the retention time. To avoid this, an analyte protectants mixture (AP-mix) containing D-sorbitol (99,5 %), glucono-delta-lactone, Shikimi acid and 3-ethoxy-1,2-propandiole is well suited. For preparation of this mixture 2 g of 3-ethoxy-1,2-propandiole are weighed into a 10 ml volumetric flask, then 2 ml of a 5 % glucono-delta-lactone solution, 1 ml of 5 % D-sorbitol solution and 1 ml of 5 % Shikimi acid solution are added. Solvent is always acetonitrile/water (60/40, v/v). The volumetric flask is filled up with acetonitrile/water (60/40, v/v). 30 µl of the analyte protectants mixture are added to 1 ml final extract.

Backflush is recommended. At backflush the column flow is reversed after elution of the last relevant analytes, so the less volatile matrix compounds remaining on the column are pressed backwards through the column and they leave it through the split exit. As a consequence, shift of retention time and contamination of the ion source can be minimized.

For identification of compounds using a single quadrupole mass spectrometer selected ion monitoring (SIM) is more sensitive than scanning the whole mass range. In SIM mode at least three characteristic mass ions are required for a confident identification of the analytes. It has to be considered in the selection of the number of ions simultaneously monitored that the number of measuring points for one peak is at least 10.

For evaluation of results the ion ratios of the selected ions of the compound has to be considered beside retention time and characteristic peak shape/pattern to match in the extract and a standard solution. If a selected ion is interfered in the extract, it may not be considered for evaluation.

If the quantitative evaluation of one analyte peak is interfered by peaks of co-extracted matrix compounds, a standard solution of this analyte in residue-free matrix extract of the same commodity shall be measured for comparison.

Within a sequence standard solutions have to be injected regularly, to this see also the requirements of the EU Quality Control Procedures [5].

A.6.5 Module D5: Determination using gas chromatography with flame photometric detection (GC-FPD)

A.6.5.1 Principle

The gas chromatographic determination with flame photometric detection is suited for all phosphor and sulfur containing analytes from extracts by modules E1 to E7, which are volatile without decomposition after clean-up using one of the modules C1 to C5. GC-FPD analysis without clean-up is only possible if the extracts are highly diluted (module C0).

A.6.5.2 Procedure

Inject solutions from modules S0 or S1 into a gas chromatographic system with flame photometric detection. The measurement may be performed using various GC instruments and columns.

A.6.5.3 Apparatus

The following GC operating conditions have been shown to be satisfactory, but they are only examples of experimental conditions on the stated instruments. Variations of these conditions are not deviations from the method.

A.6.5.4 Examples of suitable conditions

A.6.5.4.1 Instrument 1

Instrument	Agilent GC 5890
Carrier gas	Helium, 8,0 ml/min, constant flow
Injection technique	Split/splitless
Injection technique	60 s splitless, than split ratio 1:10
Injection	3 µl
Injector temperature	250 °C
Column	Fused-silica-capillary with 14 % cyanoproplyphenyl/ 86 % dimethylploysiloxane, length 15 m, inner diameter 0,53 mm, film thickness 1,0 µm (e.g. DB1701)
Column temperature	60 °C isothermal for 2,0 min, programmed to rise at 10 °C/min to 260 °C, isothermal at 260 °C for 10 min.
Detector	FPD with phosphor or sulfur filter
Detector temperature	240 °C
Detector gas	14 ml/min helium, 75 ml/min hydrogen, 120 ml/min air

A.6.5.4.2 Instrument 2

Instrument	Fisons MEGA 5300
Carrier gas:	Helium, 3,0 ml/min, constant flow
Injection technique	Split/splitless
Injection technique	90 s splitless, than split ratio 1:20
Injection	2 µl
Injector temperature	250 °C
Column	Fused-silica-capillary with 50 % phenyl/ 50 % dimethylsiloxane, length 30 m, inner diameter 0,32 mm, film thickness 0,25 µm (e.g. DB-17)
Column temperature	70 °C isothermal for 2,0 min, programmed to rise at 30 °C/min to 130 °C, to rise at 50 °C/min to 250 °C, to rise at 1 °C/min to 260 °C, isothermal at 260 °C for 12 min.
Detector	FPD with phosphor or sulfur filter
Detector temperature:	130 °C
Detector gas:	25 ml/min helium, 100 ml/min hydrogen, 25 ml/min oxygen

For GC-FPD analysis of organophosphorus compounds columns of medium polarity are often used. The FPD is also applicable for analytes containing sulfur or organostannic compounds. Using FPD for quantification of analytes containing sulfur it has to be considered that there is no linear relation between concentration and response.

For splitless injection, the starting temperature of the oven should be about 20 °C below the boiling point of the solvent of the final extract.

Contamination of the injector and the beginning of the capillary column can cause partial decomposition of some analytes and shift of the retention time. The use of an analyte protectants mixture in GC with columns of medium polarity is not known so far.

Within a sequence standard solutions have to be injected regularly, to this see also the requirements of the EU Quality Control Procedures [5].

Backflush is recommended. At backflush the column flow is reversed after elution of the last relevant analytes, so the less volatile matrix compounds remaining on the column are pressed backwards through the column and they leave it through the split exit. As a consequence, shift of retention time and contamination of the ion source can be minimized.

A.6.6 Module D6: Determination using gas chromatography with electron capture detection (GC-ECD)

A.6.6.1 Principle

The gas chromatographic determination with electron capture detection is suited for organochlorine compounds and pyrethroids from extracts by modules E1 to E7, which are volatile without decomposition after clean-up using one of the modules C1 to C5. A solvent exchange of the final extract is highly recommended.

A.6.6.2 Procedure

Inject solutions from module S0 after solvent exchange (e.g. into toluene) into a gas chromatographic system with electron capture detector. The measurement may be performed using various GC instruments and columns.

A.6.6.3 Apparatus

The following GC operating conditions have been shown to be satisfactory, but they are only examples of experimental conditions on the stated instruments. Variations of these conditions are not deviations from the method.

A.6.6.4 Examples of suitable conditions

A.6.6.4.1 Instrument 1

Instrument	Agilent GC 6890
Carrier gas:	Helium, 1,0 ml/min, constant flow
Injector	Split/splitless injector with deactivated linear single taper liner
Injection technique	Pulsed splitless, 200 kPa, pulse time 1,0 min
Injection	1 µl
Injector temperature	240 °C
Injector purge	1,5 min after injection (purge time) purge flow to split vent 50 ml/min

Column	Fused-silica-capillary with 5 % phenyl/95 % dimethylsiloxane, length 30 m, inner diameter 0,25 mm, film thickness 0,25 µm (e.g. HP5 MS)
Column temperature	70 °C isothermal for 2,0 min, programmed to rise at 25 °C/min to 170 °C, rise at 3 °C/min to 210 °C, rise at 30 °C/min to 290 °C, isothermal at 290 °C for 9 min.
Detector	^{63}Ni-ECD
Detector temperature	300 °C
ECD make up gas	Nitrogen, 70 ml/min
ECD anode purge	Nitrogen, 6 ml/min

A.6.6.4.2 Instrument 2

Instrument	Agilent GC 7890A
Carrier gas	Helium, average velocity 35 cm/s at 80 °C
Injector	Split/splitless injector with ultra-inert single taper liner
Injection technique	Splitless
Injection	1 µl
Injector temperature	250 °C
Injector purge	0,5 min after injection (purge time) purge flow to split vent 60 ml/min
Column	Retention gap (deactivated fused silica tubing), length 5 m, inner diameter 0,32 mm connected to fused-silica-capillary with 35 % phenyl/ 65 % dimethylsiloxane, length 30 m, inner diameter 0,32 mm, film thickness 0,25 µm (e.g. DB-35 UI)
Column temperature	80 °C isothermal for 0,5 min, programmed to rise at 26 °C/min to 175 °C, rise at 6,5 °C/min to 235 °C, rise at 15 °C/min to 300 °C, isothermal at 300 °C for 6 min.
Detector	µECD
Detector temperature	340 °C
ECD make up gas	Nitrogen, 30 ml/min

These conditions are given in Agilent Application Note 5990-9735EN (see http://www.chem.agilent.com/Library/applications/5990-9735EN.pdf).

For retention times and relative response of pesticides see CEN/TR 16468 “Food analysis – Determination of pesticide residues by GC-MS – Retention times and mass spectrometric parameters” [4].

For splitless injection, the starting temperature of the oven should be about 20 °C below the boiling point of the solvent of the final extract.

Contamination of the injector and the beginning of the capillary column can cause partial decomposition of some analytes and shift of the retention time. The use of an analyte protectants mixture in GC with columns of medium polarity is not known so far.

Within a sequence standard solutions have to be injected regularly, to this see also the requirements of the EU Quality Control Procedures [5].

Backflush is recommended. At backflush the column flow is reversed after elution of the last relevant analytes, so the less volatile matrix compounds remaining on the column are pressed backwards through the column and they leave it through the split exit. As a consequence, shift of retention time and contamination of the ion source can be minimized.

A.7 Description of quantification (Q) options

A.7.1 Option Q1: Quantification using external standards in solvent

A.7.1.1 Principle

The determination of the concentration of the identified analyte in the final extract is performed by comparison to standards of known concentration in solvent. It is referred to the general remarks on the procedure and calculation of calibration given in CEN/TS 17061:2017, 4.1 and 4.2).

This quantification module is applicable if no variability of signal intensity occurs within a sequence, if no compensation of losses during extraction, clean-up and measurement is necessary and if matrix effects can be excluded (see Table 5).

A.7.1.2 Procedure

Analytical standard solutions of different concentrations are produced by diluting stock solutions using an appropriate solvent. These solutions are chromatographed together with the extracts of the samples. The peak areas (or heights) of the analytes in the standard solutions and the sample extracts are determined. After selection of an appropriate calibration function (see CEN/TS 17061:2017, 4.4.2 to 4.4.5), the concentration of the analyte in the sample extract ρ_A can be calculated.

A.7.1.3 Calculation

The calculation of the residue of the analyte in the sample can be done on the basis of ρ_A as given in 6.3.

A.7.2 Option Q2: Quantification using external standards in matrix

A.7.2.1 Principle

The determination of the concentration of the identified analyte in the final extract is performed by comparison to standards of known concentration in matrix extracts from residue-free material (matrix-matched standards). It is referred to the general remarks on the procedure and calculation of calibration given in CEN/TS 17061:2017, 4.1, 4.2 and 4.3 (for checking for matrix effects).

This quantification option shall be applied if no variability of signal intensity occurs within a sequence, if no compensation of losses during extraction, clean-up and measurement is necessary and if matrix effects cannot be excluded.

A.7.2.2 Procedure

Analytical standard solutions of different concentrations are produced by diluting stock solutions using final extracts of residue-free material. Another approach for the preparation of matrix-matched standards is to evaporate until dryness a known volume of the extracts of residue-free material under a gentle stream of nitrogen and reconstitute with the same volume of the standard in solvent. These solutions are chromatographed together with the extracts of the samples. The peak areas (or heights) of the analytes in the standard solutions and the sample extracts are determined. After selection of an appropriate calibration function (see CEN/TS 17061:2017, 4.4.2 to 4.4.5) the concentration of the analyte in the sample extract ρ_A can be calculated.

A.7.2.3 Calculation

The calculation of the residue of the analyte in the sample can be done on the basis of ρ_A as given in 6.3.

A.7.3 Option Q3: Quantification using a procedural internal standard and standards in solvent

A.7.3.1 Principle

The determination of the concentration of the identified analyte in the final extract is performed by comparison to standards of known concentration in solvent containing a constant amount of procedural internal standard. It is referred to the general remarks on the procedure and calculation of calibration using internal standard given in CEN/TS 17061:2017, 4.5.

This quantification option shall be applied if compensation of losses during extraction and clean-up or of variability in the measurement is necessary and if matrix effects can be excluded.

A.7.3.2 Procedure

Analytical standard solutions of different concentrations are produced by diluting different volumes of stock solutions and a constant volume of the internal standard solution to defined volume using an appropriate solvent. These solutions are chromatographed together with the extracts of the samples. The peak areas (or heights) of the analytes in the standard solutions and the sample extracts are determined. Calculate the concentration of the analyte in the sample extract ρ_A after selection of an appropriate calibration function according to CEN/TS 17061:2017, 4.5.1.

A.7.3.3 Calculation

The calculation of the residue of the analyte in the sample can be done on the basis of ρ_A as given in 6.3.

A.7.4 Option Q4: Quantification using standard addition to the final extract

A.7.4.1 Principle

The determination of the concentration of the identified analyte in the final extract is performed by comparison of the analyte signal in the final extract to the analyte signal of final extract which are spiked with known amounts of the analyte. It is referred to the general remarks on the procedure and calculation given in CEN/TS 17061:2017, 4.6.3.

This quantification option shall be applied if only matrix effects have to be compensated and if an appropriate residue-free material is not available. The application of this option presumes a linear relation between the concentration of the analyte and the response in the detection system.

A.7.4.2 Procedure

Before the measurement known amounts of the analyte which is to be determined are added to aliquots of the final extract. All aliquots are filled up to the same volume with solvent. The different spiked final extracts are chromatographed together with the unspiked extract of the sample. The peak areas (or heights) of the analytes in the unspiked and spiked extracts are determined and plotted (see CEN/TS 17061:2017, 4.6.2). Using the regression line the mass of the analyte in the extract can be determined.

A.7.4.3 Calculation

The calculation of the residue of the analyte in the sample can be done as given in CEN/TS 17061:2017, 4.6.2.

A.7.5 Option Q5: Quantification using a procedural internal standard and standards in matrix or isotope-labelled internal standards

A.7.5.1 Principle

The determination of the concentration of the identified analyte in the final extract is performed by comparison to standards of known concentration in matrix extracts from residue-free material (matrix-matched standards) containing a constant amount of procedural internal standard. It is referred to the general remarks on the procedure and calculation given in CEN/TS 17061:2017, 4.5.

The use of an isotope-labelled internal standard, which differs only in the isotope with the analyte molecule, is a special case. Matrix effects are generally very well compensated here so that it is not necessary to use residue-free material for the standards.

This quantification option shall be applied if losses during extraction and clean-up or variability in the measurement have to be compensated or significant matrix effects occur.

A.7.5.2 Procedure

Analytical standard solutions of different concentrations are produced by diluting different volumes of stock solutions and a constant volume of the internal standard solution to defined volume using matrix extracts from residue-free material. These solutions are chromatographed together with the extracts of the samples. The peak areas (or heights) of the analytes in the standard solutions and the sample extracts are determined. After selection of an appropriate calibration function (see CEN/TS 17061:2017, 4.5.1) the concentration of the analyte in the sample extract ρ_A can be calculated.

If isotope-labelled internal standards are used a constant volume of the isotope-labelled internal standard solution and variable volumes of the native analyte solution in solvent are filled up to a defined volume.

A.7.5.3 Calculation

The calculation of the residue of the analyte in the sample can be done on the basis of ρ_A as given in 6.3.

If isotope-labelled internal standards are used the calculation is simplified in such a way that the knowledge of the extraction solvent volume, dilution factors, matrix effects and so on is not necessary. The mass fraction of the analyte w_A can be obtained directly from then calibration using the known sample weight. A constant volume of the isotope-labelled internal standard solution and variable volumes of the native analyte solution in solvent are filled up to a defined volume. The calculation of the residue of the analyte in the sample can be done as given in CEN/TS 17061:2017, 4.5.2 and 4.5.3.

A.7.6 Option Q6: Quantification using standard addition to the sample

A.7.6.1 Principle

The determination of the concentration of the identified analyte in sample is performed by comparison of the analyte signal in the final extract of the sample to the analyte signals of final extracts of the same sample spiked with known amounts of the analyte. In this case known amounts of the analyte are added to the sample prior to extraction. It is referred to the general remarks on the procedure and calculation given in CEN/TS 17061:2017, 4.6.1.

The application of the option presumes a linear relation between the concentration of the analyte and the response in the detection system. This quantification Option shall be applied if losses of the analyte occur during extraction and clean-up or if variability in the measurement has to be compensated and if an appropriate residue-free material is not available.

A.7.6.2 Procedure

Before the extraction known amounts of the analyte which is to determine are added to test portions of the sample. Afterwards the unspiked and the spiked test portions of the sample pass through the whole analytical procedure. The peak areas (or heights) of the analytes in the unspiked and spiked samples are determined and plotted (see CEN/TS 17061:2017, 4.6.3). Using the regression line the mass of the analyte in the sample can be determined.

A.7.6.3 Calculation

The calculation of the residue of the analyte in the sample can be done as given in CEN/TS 17061:2017, 4.6.3.

A.7.7 Option Q7: Quantification by calibration of the entire procedure

A.7.7.1 Principle

The determination of the concentration of the identified analyte in the final extract is performed by comparison of the analyte signal in the final extract of the sample to the analyte signals of finals extracts of calibration samples. The samples and calibration sample sample pass through the whole analytical procedure. It is referred to the general remarks on the procedure and calculation given in CEN/TS 17061:2017, 4.7.

This quantification option shall be applied if systematic major or lower findings have to be compensated, if matrix effects have to be compensated and if an appropriate residue-free material is not available.

A.7.7.2 Procedure

Before the extraction known amounts of the analyte which is to determine are added to test portions of the calibration sample. Afterwards the unspiked samples and the spiked test portions of the calibration samples pass through the whole analytical procedure. The peak areas (or heights) of the analytes in the unspiked and spiked calibration samples are determined. After selection of an appropriate calibration function (see CEN/TS 17061:2017, 4.4.3 to 4.4.4) the concentration of the analyte in the sample extract ρ_A can be calculated.

A.7.7.3 Calculation

The calculation of the residue of the analyte in the sample can be done on the basis of ρ_A as given in 6.3.

Annex B
(informative)

Complementary information

B.1 General

This method (QuEChERS) was first published in [7] in 2003 and later amended to the present procedure in order to broaden the analyte and matrix spectrum.

B.2 Preparation of the buffer-salt mixture (A.1.8)

If commercially available buffer-salt mixtures are not used, the preparation of several portions of this mixture is helpful using a sample divider.

B.3 Reagents for clean-up

If commercially available centrifuge tubes pre-packed with reagents for clean-up are not used, it is recommended to fill all centrifuge tubes needed for clean-up before extraction. The use of a sample divider is helpful in that case.

B.4 Prolongation of extraction time

The prolongation of the extraction time to ≥ 15 min is needed to obtain better extraction efficiency. When using the originally proposed extraction (shaking) time of 1 min for frozen samples, it was noticed that some pesticides showed low extraction yields. An extraction time of > 60 min should be avoided to reduce decomposition of labile analytes.

B.5 Working without internal standards

To clarify the extent of volume contraction and the influence of matrix components on phase separation, in three laboratories special experiments were conducted. Fifteen different foodstuffs of plant origin (including tea and wheat flour) were spiked with 20 pesticides each. The raw extracts were analysed by LC-MS/MS without clean-up, but after sufficient dilution to exclude matrix effects. It was noticed that the recovery was independent from the volume of the acetonitrile phase (ranging from 7,0 ml to 10,5 ml). The layer between acetonitrile and water phase containing solid plant parts was larger than 5 ml in some cases (e.g. avocado, lemon, tea, wheat flour). Nevertheless, the mean recovery of all pesticides spiked to one matrix in one lab ranged between 77 % and 114 %. Considering the mean value of all three laboratories, except for two matrices (avocado, tea) the mean recovery was in the range 97 % to 103 %. The experiments demonstrated that an internal standard is not needed to correct the extraction volume of 10 ml.

B.6 Scaling

In the extraction modules a fixed mass of the test portion (e.g. 10 g, 5 g or 2 g) is prescribed. This amount is sufficient to prepare 6 ml of raw extract used in the clean-up procedures. The described extraction and clean-up steps are scalable as desired, as long as the amounts of reagents used remain in the same proportion. It should be kept in mind, however, that the smaller the amount of sample employed the higher the sub-sampling variability will be. During validation, each laboratory should thus investigate the typical sub-sampling variability achieved when employing the available comminution devices, using representative samples containing incurred residues.

B.7 Adjustment of pH value

By adding the citrate buffering salts (A.1.8) during extraction most samples obtain pH values between 5 and 5,5. This pH range is a compromise, at which both, the quantitative extraction of acidic herbicides and the protection of alkali labile (e.g. captan, folpet, tolylfluanid) and acid labile (e.g. pymetrozine, dioxacarb) compounds is sufficiently achieved.

Following contact with PSA (A.1.15) the pH of the extracts increases, reaching measured values exceeding the value of 8. This can compromise the stability of base sensitive pesticides (e.g. captan, folpet, dichlofluanid, tolylfluanid, pyridate, methiocarb sulfon, chlorothalonil). If the extracts are acidified quickly to pH 5 the degradation of such compounds is reduced significantly so that storage over several days is possible. At this pH acid-labile pesticides (e.g. pymetrozine, dioxacarb, thiodicarb) are also sufficiently stable over several days.

Only some very sensitive sulfonyl urea herbicides, carbosulfan and benfuracarb have been shown not to be sufficiently protected at pH 5. However, these compounds have been shown to be stable at the pH of the non-acidified extract (after dispersive SPE) over several days. If these compounds are within the scope of analysis an aliquot of the non-acidified extract should be employed for measurement. If the measurement can be performed quickly, the extract at pH 5 may be used as well. It should be noted, however, that the most acidic sulfonylureas can experience losses during PSA clean-up. These can be analysed together with the acidic pesticides directly from the raw extract (6.3 and A.4).

B.8 Recovery studies

For recovery studies e.g. 10 g sample is fortified using 100 µl of a pesticide solution in acetonitrile or acetone. A short vibration using a Vortex mixer (A.2.13) can help to disperse solvent and pesticides well throughout the sample. Fortification using larger volumes of standard solution (e.g. > 200 µl) should be avoided. If this is not possible, a volume compensation should be performed in the blank samples used to prepare matrix matched calibration solutions, to avoid differences in the matrix concentration of the final extract.

B.9 Clean-up with GCB

It has to be taken into account, that some planar pesticides and ISTDs have a great affinity towards the planar structure of GCB. But recovery studies showed that no noteworthy losses occur if the extract, after dispersive SPE with GCB, still maintains some visible amount of chlorophyll or carotenoids. Anthracene (or D10-Anthracen) can be used as QC standards (see Table 1). If the recovery of anthracene is above 70 %, this will also be the case for planar pesticides having the highest affinity towards carbon.

B.10 Concentration of the final extracts and solvent exchange

If large volume injection (3 µl or more) cannot be performed and the desired detection limits of the compounds of interest cannot be achieved, the concentration of the final extracts and, if necessary, a solvent exchange may be considered. If GC-MSD is employed a simple evaporative concentration of the extracts by a factor of four should be sufficient. To achieve this e.g. 4 ml of the acidified extract (pH 5) are transferred into a test tube and reduced to approximately 1 ml at 40 °C using a slight nitrogen flow. Solvent exchange is an option if GC performance using acetonitrile is not satisfactory or if NPD is employed (without PTV-injector). For this, an extract aliquot is evaporated to almost dryness at 40 °C using a slight nitrogen flow and resolved in 1 ml of an appropriate solvent (some droplets of a keeper e.g. dodecane can help to reduce losses of the most volatile compounds). The blank extract (needed for the preparation of calibration solutions) should be treated the same way.

Annex C
(informative)

Abbreviations

The abbreviations used within this document are listed and explained in Table C.1.

Table C.1 — List of abbreviations

Abbreviation	Meaning
APCI	Atmospheric Pressure Chemical Ionization
c	substance concentration
C18	octadecyl-silyl-modified silica gel
c_{ISTD}	concentration of internal standard
$c_{\mathrm{ISTD}}^{\mathrm{cal\ mix}}$	dilution of internal standard solution to generate calibration mixtures
D-SPE	Dispersive Solid Phase Extraction
ECD	Electron Capture Detector
EI	Electron Impact ionisation
ESI	ElectroSpray Ionisation
FPD	Flame Photometric Detector
FWHM	Full width of half maximum
g	9,81 ms^{-2}
GC	Gas Chromatography
GCB	Graphitised Carbon Black sorbent
GC-MS	Hyphenation of Gas Chromatography and Mass Spectrometry
GC-MS/MS	Hyphenation of Gas Chromatography and tandem Mass Spectrometry
HPLC	High Performance Liquid Chromatography
ID	Inner diameter
ISTD	Internal standard
ITD	Ion Trap Detector
LC-HR-MS	Hyphenation of Liquid Chromatography and High Resolution Mass Spectrometry
LC-MS	Hyphenation of Liquid Chromatography and Mass Spectrometry
LC-MS/MS	Hyphenation of Liquid Chromatography and tandem Mass Spectrometry
Log P	Logarithm to the base 10 of octanol-water partition coefficient
MRL	Maximum Residue Level
MRM	Multi Reaction Monitoring
MS	Mass spectrometry
MS/MS	Tandem Mass Spectrometry
MSD	Mass Selective Detector

Abbreviation	Meaning
NaOH	Sodium hydroxide
NCI	Negative Chemical Ionization
NPD	Nitrogen Phosphorus Detector
ODS	Octadecylsilane
PCI	Positive Chemical Ionization
pKa	Negative logarithm to the base 10 of acid dissociation constant (Table 2, C0)
PSA	Primary Secondary Amine sorbent
PTV	Programmable Temperature Vaporizer
QC	Quality Control
Rt	Retention time
$Rt_{(A)}$	Retention time of analyte
$Rt_{(ISTD)}$	Retention time of internal standard
SIM	Selected Ion Monitoring
SPE	Solid Phase Extraction
SRM	Selected Reaction Monitoring
TOF	Time-Of-Flight
UHPLC	Ultra High Performance Liquid Chromatography
UPLC-MS/MS	Hyphenation of Ultra-Performance Liquid Chromatography and tandem Mass Spectrometry
w	mass fraction
w_A	mass fraction of the identified active substance
ρ	mass concentration
ρ_A	mass concentration of active substance in the final extract
ρ_{sample}	mass concentration of the sample in the final extract
φ	volume fraction
V_1	used aliquot of raw extract
V_2	added volume of water or acetonitrile/water
V_{Ex}	volume of the organic phase after extraction
V_{ISTD}^{add}	added volume of internal standard solution
m_{sample}	mass of test portion
ρ_{ISTD}^{add}	mass concentration of added internal standard solution
$\rho_{ISTD}^{raw\ extract}$	mass concentration of internal standard in raw extract
$\rho_{sample}^{final\ extract}$	mass concentration of sample in final extract
$\rho_{sample}^{raw\ extract}$	mass concentration of sample in raw extract

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