SLOVENSKI STANDARD
oSIST prEN 15662:2017

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Hrana rastlinskega izvora - Večelementna metoda za določanje ostankov pesticidov z uporabo analize na osnovi GC- in LC- po delitvi in izpiranju acetonitrila z disperzivno SPE - Metoda QuEChERS

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

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

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

Ta slovenski standard je istoveten z: **prEN 15662**

ICS:

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

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This draft European Standard is submitted to CEN members for enquiry. It has been drawn up by the Technical Committee CEN/TC 275.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
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European foreword

This document (prEN 15662:2017) has been prepared by Technical Committee CEN/TC 275 "Food analysis - Horizontal methods", the secretariat of which is held by 275.

This document is currently submitted to the CEN Enquiry.

This document will supersede 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 FprCEN/TR 17063).

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.

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, 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 FprCEN/TR 17063. Guidelines for calibration are outlined in FprCEN/TS 17061.

2 Normative references

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

FprCEN/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 amin 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 determinative 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 extract after the initial addition of acetonitrile, but this is not mandatory. Details for calibration, see FprCEN/TS 17061 on calibration.

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 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. Generally, laboratory samples should not be stored longer than 3 days before preparation. 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 may 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. 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. This is the test sample. Calculation of the residue shall be based on the mass of the original test sample (including the stones).

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 test portions are taken from test samples after being stored frozen, the test samples 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).

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. Approved 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	A test portion of 10 g is alkalized with (a) 0,6 ml or (b) 0,2 ml sodium hydroxide solution (5 mol/l) and then extracted with acetonitrile	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, yam, parsnip (b) Bread, fresh dates, chestnuts
E4	A test portion of 13,5 g is homogenized after the addition of water and then 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 %)	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 (>45 % 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 (<10 %) 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 C 2 to C 5, analysis of extracts with low matrix-load	Cucumber, apples, sufficiently diluted raw-extracts
C1	Freezing-out of co-extracted fat and waxes	Removal of co-extracted fat (even in combination with further clean-up steps, e.g. C 2, C 3, C 5)	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 E 5 (e.g. cereal grain and products thereof) and E 7 (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	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/CI/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 from. 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	Examples
Q1	Quantification using external standards in solvent	Determinations where matrix-effects are assumed to be negligible	see FprCEN/TS 17061:2017, 4.3 and 4.4.2 to 4.4.5
Q2	Quantification using external standards in matrix	Determinations where matrix-effects shall be considered	see FprCEN/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 FprCEN/TS 17061:2017, 4.5 and 4.5.1
Q4	Quantification using standard addition to the final extract	Determinations where matrix effects shall be considered and suitable blank matrices are not available	see FprCEN/TS 17061:2017, 4.3, 4.5, 4.5.1 and 4.5.2
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 FprCEN/TS 17061:2017, 4.6.1
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 FprCEN/TS 17061:2017, 4.6.2
Q7	Quantification by calibration of the entire procedure	Determinations where matrix-effects shall be considered or incomplete extractions of the analyte occur	see FprCEN/TS 17061:2017, 4.7

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

Commodity	Extraction	Description[a]	Clean-up	Description[b]	Clean-up (altern.)	Description[b]
Apple juice	E1	10 g / 0 ml	C2	PSA[b] 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		
Apricots juicer	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	E3 [a]	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	C5 [b]	PSA 25 +GCB 7,5[c]		
Bean seeds (fresh)	E3 [a]	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	E2 [b]	10 g / NaOH 2	C2	PSA 25		
Blackberries freeze-dried	E7	2 g / 10 ml	C2	PSA 25	C3 [a]	**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	C3[a]	**PSA 50**
Bread (34 % to 43 % water)	E3 [b]	10 g / 4,5 ml	C2	PSA 25		
Breadfruits (70 % water)	E3 a	10 g / 2,5 ml	C2	PSA 25		
Broccoli	E1	10 g / 0 ml	C2	PSA 25		
Butterhead lettuce	E1	10 g / 0 ml	C5 [a]	PSA 25 + GCB 2,5		
Carrot	E1	10 g / 0 ml	C5 [a]	PSA 25 + GCB 2,5		
Carrots	E1	10 g / 0 ml	C5 [a]	PSA 25 + GCB 2,5		
Carrots freeze-dried	E7	2 g / 10 ml	C2	PSA 25	C5 [a]	**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		

Commodity	Extrac-tion	Description[a]	Clean-up	Description[b]	Clean-up (altern.)	Description[b]
Celeriacs/turnip rooted celeries	E1	10 g / 0 ml	C5 [a]	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	C5 [b]	**PSA 25 + GCB 7,5**
Cereal flour	E5	5 g / 10 ml	C1 + C3 [a]	Freeze out + PSA 50	C4	**PSA 25 + C18 25**
Cereal grain	E5	5 g / 10 ml	C1 + C3 [a]	Freeze out + PSA 50	C4	**PSA 25 + C18 25**
Cereal semolina	E5	5 g / 10 ml	C 1 + C 3[a]	Freeze out + PSA 50	C4	**PSA 25 + C18 25**
Cereals flakes	E5	5 g / 10 ml	C1 + C3 [a]	Freeze out + PSA 50	C4	**PSA 25 + C18 25**
Cherries	E1	10 g / 0 ml	C2	PSA 25		
Chestnuts (45 % to 52 % water)	E3 [b]	10 g / 4,5 ml	C2	PSA 25		
Chinese cabbages	E1	10 g / 0 ml	C2	PSA 25		
Chives	E1	10 g / 0 ml	C5 [b]	PSA 25 + GCB 7,5		
Chives freeze-dried	E7	2 g / 10 ml	C2	PSA 25	C5 [b]	**PSA 25 + GCB 7,5**
Coconut fresh	E6	5 g / 6 ml	C2	PSA 25		
Coffee beans	E7	2 g / 10 ml	C3 [b]	PSA 75		
Coriander	E1	10 g / 0 ml	C5 [b]	PSA 25 + GCB 7,5		
Corn freeze-dried	E7	2 g / 10 ml	C2	PSA 25		
Corn fresh	E3 [a]	10 g / 2,5 ml	C2	PSA 25		
Courgettes	E1	10 g / 0 ml	C2	PSA 25		
Cress	E1	10 g / 0 ml	C5 [b]	PSA 25 + GCB 7,5		
Cucumber	E1	10 g / 0 ml	C2	PSA 25		
Cultivated fungi	E1	10 g / 0 ml	C2	PSA 25		
Currants	E2 [a]	10 g / NaOH 1	C2	PSA 25		
Currants freeze-dried	E7	2 g / 10 ml	C2	PSA 25		
Currants juice	E2 [a]	10 g / NaOH 1	C2	PSA 25		
Dates (dried)	E4	500 g / 850 ml	C2	PSA 25		
Dates (fresh, 50 % to 60 % water)	E3 [b]	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	C5 [a]	PSA 25 + GCB 2,5		
Figs dried	E4	500 g / 850 ml	C2	PSA 25		

Commodity	Extrac-tion	Description[a]	Clean-up	Description[b]	Clean-up (altern.)	Description[b]
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		
Ginkgo seeds (55 % water)	E3 [b]	10 g / 4,5 ml	C2	PSA 25		
Globe artichokes	E1	10 g / 0 ml	C2	PSA 25		
Gooseberrys	E2 [b]	10 g / NaOH 2	C2	PSA 25		
Grape leaves	E3 [a]	10 g / 2,5 ml	C2	PSA 25		
Grape leaves	E1	10 g / 0 ml	C5 [b]	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		
Herbs dried	E7	2 g / 10 ml	C2	PSA 25	C5 [b]	**PSA 25 + GCB 7,5**
Honey	E5	5 g / 10 ml	C2	PSA 25		
honeydew melon	E1	10 g / 0 ml	C2	PSA 25		
Horseradish	E3 [a]	10 g / 2,5 ml	C2	PSA 25		
Jackfruit (74 % water)	E3 [a]	10 g / 2,5 ml	C2	PSA 25		
Kales	E1	10 g / 0 ml	C5 [b]	PSA 25 + GCB 7,5		
Kiwi	E1	10 g / 0 ml	C2	PSA 25		
Plantain	E3 [a]	10 g / 2,5 ml	C2	PSA 25		
Kohlrabi	E1	10 g / 0 ml	C2	PSA 25		
Lamb's lettuces	E1	10 g / 0 ml	C5 [b]	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	E2 [a]	10 g / NaOH 1	C2	PSA 25		
Lemons	E2 [a]	10 g / NaOH 1	C1 + C2	Freeze out + PSA 25		
Lentils (dried)	E5	5 g / 10 ml	C2	PSA 25		
Lime juice	E2 [a]	10 g / NaOH 1	C2	PSA 25		
Limes	E2 [a]	10 g / NaOH 1	C1 + C2	Freeze out + PSA 25		
Lotus roots (79 % water)	E3 [a]	10 g / 2,5 ml	C2	PSA 25		
Lotus seeds	E3 [a]	10 g / 2,5 ml	C2	PSA 25		
Lotus seeds dried (14 % water)	E5	5 g / 10 ml	C2	PSA 25		