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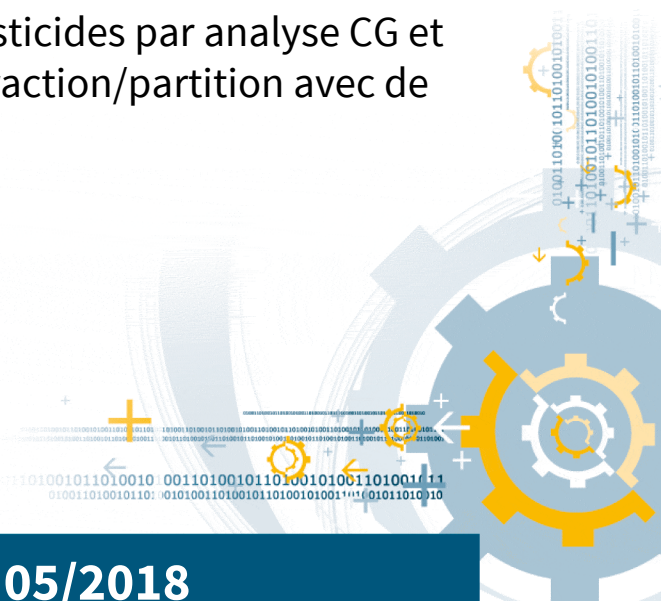
ILNAS-EN 15662:2018

Foods of plant origin - Multimethod for the determination of pesticide residues using GC- and LC-based analysis following acetonitrile

Pflanzliche Lebensmittel - Multiverfahren
zur Bestimmung von
Pestizidrückständen mit GC und LC nach
Acetonitril-Extraktion/Verteilung und

Aliments d'origine végétale -
Multiméthode de détermination des
résidus de pesticides par analyse CG et
CL après extraction/partition avec de

05/2018



National Foreword

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EUROPEAN STANDARD ^{ILNAS-EN 15662:2018} **EN 15662**
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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 GC
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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This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
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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