ILNAS

Institut luxembourgeois de la normalisation
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ILNAS-EN 15662:2008

Foods of plant origin - Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up

Aliments d'origine végétale - Méthode polyvalente de détermination des résidus des pesticides par CG-SM et CL/SM/SM avec extraction/partition avec de

Pflanzliche Lebensmittel - Bestimmung von Pestizidrückständen mit GC-MS und/oder LC-MS/MS nach Acetonitril-Extraktion/Verteilung und Reinigung mit

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National Foreword

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Foods of plant origin - Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE - QuEChERS-method

Aliments d'origine végétale - Méthode polyvalente de détermination des résidus des pesticides par CG-SM et CL/SM/SM avec extraction/partition avec de l'acétonitrile et nettoyage par SPE dispersés - Méthode QuEchERS

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

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Foreword

This document (EN 15662:2008) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by May 2009, and conflicting national standards shall be withdrawn at the latest by May 2009.

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

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1 Scope

This European Standard describes a method for the analysis of pesticide residues in foods of plant origin, such as fruits (including dried fruits), vegetables, cereals and processed products thereof. The method has been collaboratively studied on a large number of commodity/pesticide combinations.

2 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) 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. Quantification is performed using an internal standard, which is added to the extract after the initial addition of acetonitrile. A brief overview of the method is shown in the flowchart in Annex C.

3 Reagents

3.1 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.

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.

3.2 Water, HPLC quality

3.3 Acetonitrile, HPLC quality

3.4 Methanol, HPLC quality

3.5 Ammonium formate

3.6 Magnesium sulfate, anhydrous, grit, e.g. Fluka No. 63135

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

3.7 Magnesium sulfate, anhydrous, fine powder

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

3.8 Sodium chloride

3.9 Disodium hydrogencitrate sesquihydrate

3.10 Trisodium citrate dihydrate

3.11 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.

3.12 Buffer-salt-mixture for second extraction and partitioning:

Weigh 4 g ± 0,2 g of magnesium sulfate anhydrous (3.6), 1 g ± 0,05 g of sodium chloride, 1 g ± 0,05 g of trisodium citrate dihydrate and 0,5 g ± 0,03 g of disodium hydrogencitrate sesquihydrate into a cup (4.11). These amounts refer to approximately 10 ml water in the sample.

For highly acidic samples (with pH < 3) the pH-value achieved after the addition of buffering salts is usually below 5. To better protect acid labile compounds the pH-value can be elevated by adding 5 mol/l sodium hydroxide solution (3.11): For lemons, limes and currants add 600 µl and for raspberry 200 µl of sodium hydroxide solution directly to the salt mixture.

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. The preparation of the salt mixtures can be enormously facilitated using a sample divider (4.12). The amounts of salts given above are to be used for sample portions containing approximately 10 g water.

3.13 Formic acid solution in acetonitrile, volume fraction φ = 5 ml formic acid/100 ml

Dilute 0,5 ml of formic acid (mass fraction w = > 95 %) to 10 ml with acetonitrile (3.3).

3.14 Primary secondary amin sorbent

For example, Bondesil-PSA® 40 µm Varian No. 12213023[1)].

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

3.15 Graphitised Carbon Black sorbent (GCB), e.g. Supelco Supelclean Envi-Carb® [1)] SPE Bulk Packing, No. 57210U

Other graphitised carbon sorbents may be used, but investigations will be necessary to prove equivalency especially regarding analyte losses.

3.16 Sorption mixture 1: GCB (3.15)/ magnesium sulfate anhydrous fine powder (3.7)-mixture, 1 + 59 mass portions

Mix the two components intensively to form a visually homogeneous mixture.

3.17 Sorption mixture 2: GCB (3.15)/ magnesium sulfate anhydrous fine powder (3.7)-mixture, 1 + 19 mass portions

Mix the two components intensively to form a visually homogeneous mixture.

NOTE It is highly advisable to prepare the sorption mixtures 1 (3.16) and 2 (3.17) in advance and store them in sealable vessels. For the extract clean-up according to 5.4.3 the pre-mixed sorption mixtures 1 or 2 are weighed into the centrifuge tubes (4.4).

1) Bondesil-PSA® is a product supplied by Varian, Inc. (Palo Alto, CA, USA). Envi-Carb is a product supplied by Supelco. 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 the same results.

3.18 C-18-sorbent (Octadecyl-silyl-modified silica gel), Bulk material 50 µm

3.19 Internal standard and quality control standard solutions in acetonitrile, ρ = 10 µg/ml to 50 µg/ml

Table 1 shows a list of potential internal standards (ISTDs) and quality control (QC) standards that may be used in this method. The suggested concentration values (C_{ISTD}) listed refers to the ISTD solutions that should be added at the first extraction step (5.2). An appropriate dilution of this solution ($C_{ISTD}^{cal\ mix}$) should be prepared to be used for the preparation of the standard solutions. For more details see 3.22.

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

Name of the compound	Log P (octanol-water partition coefficient)	Chlorine atoms	Suggested concentration C_{ISTD} [µg/ml][a]	GC				LC	
				ECD	NPD	MSD EI (+)	MSD CI (-)	MS/MS ESI (+)	MS/MS ESI (-)
Potential Internal Standards									
PCB 18	5,55	3	50	+++	-	++	+++	-	-
PCB 28	5,62	3	50	+++	-	++	+++	-	-
PCB 52	6,09	4	50	+++	-	++	+++	-	-
Triphenyl phosphate	4,59	-	20	-	+++	+++	-	+++	-
Tris-(1,3-dichlorisopropyl)-phosphate	3,65	6	50	+++	+++	+++	+++	+++	+
Triphenylmethane	5,37	-	10	-	-	+++	-	-	-
Bis-nitrophenyl urea (nicarbazin)	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[b]	6,83	6	50	+++	-	++	+++	-	-
PCB 153[b]	7,75	6	50	+++	-	++	+++	-	-
Anthracene (or its d10 analogue)[c]	4,45	-	100	-	-	++	-	-	-

a Exemplary concentrations of the ISTD solutions to be added to the test samples in 5.2, use acetonitrile as solvent.

b Recoveries of PCB 138 and 153 drop as lipid amount in the sample increases, recoveries of those two compounds exceeding 70 % indicate that no unacceptable partitioning losses have occurred even for the most lipophilic pesticides.

c Recoveries of anthracene exceeding 70 % will indicate that no unacceptable losses of pesticides with high carbon affinity have occurred during dispersive SPE with GCB.

3.20 Pesticide stock solutions

Prepare individual stock solutions of analytical standards at concentrations that are sufficient to allow the preparation of complex pesticide working solutions (3.21) that are used for the preparation of standard solutions.

Usually, store stock solutions at ≤ -18 °C. Check the stability of stock solutions during storage regularly [2]. In some cases the addition of acids or bases can be helpful to enhance stability and extend the acceptable

storage period. Before withdrawing any aliquot from this solution redissolve any precipitation that may have occurred.

3.21 Pesticide working solutions

Because of the broad applicability of this method and due to the partly divergent pH-stability of pesticides, more than one working solution each containing one or more pesticides can be needed to cover the entire pesticide spectrum of interest. These are prepared by mixing together defined volumes of the required pesticide stock solutions (3.20) and appropriately diluting them with acetonitrile. The pesticide concentrations in these mixtures should be sufficient to allow the preparation of the required matrix matched standards (see 3.22.2) with moderate dilution of the blank sample extract (e.g. less than 20 %).

Usually, store pesticide working solutions at ≤ -18 °C. Check the stability of pesticides contained in these mixtures during storage regularly [2]. In some cases the addition of acids or bases can be helpful to enhance stability and extend acceptable storage times.

3.22 Standard solutions (calibration mixtures)

3.22.1 Solvent-based standards

Solvent-based standards are prepared by mixing known volumes of the pesticide working solutions ($V_{pest}^{cal\ mix}$ see 3.21) and the ISTD solution ($V_{ISTD}^{cal\ mix}$ see 3.19) and filling up to volume with acetonitrile.

The volume of the ISTD solution to be employed ($V_{ISTD}^{cal\ mix}$) will depend on the volume of the standard solution to be prepared ($V^{cal\ mix}$) and should be such to ensure an ISTD concentration similar to that in the sample test solutions (5.3, 5.4).

EXAMPLE If 1 ml solvent-based standard is prepared the volume of ISTD solution to be added should contain a mass of ISTD ($m_{ISTD}^{cal\ mix} = C_{ISTD}^{cal\ mix} x\ V_{ISTD}^{cal\ mix}$) which is 10-fold smaller than the mass of ISTD added to the test portions in 5.2.3, where 10 ml of acetonitrile are used for extraction. It is thus indicated to appropriately dilute the concentration of internal standard solution (in this case $C_{ISTD}^{cal\ mix}$ = 0,1 × C_{ISTD}). Then the same pipette volume can be used to add ISTDs to spike test samples and for the preparation of standard solutions. Table 2 shows exemplarily the ratio of the ISTD mass that should be added to the test portions (5.2.3) and the standard solutions (3.22).

The preparation of multiple standard solutions covering a broad concentration range will allow the construction of a calibration curve (see 6.2).

NOTE A pesticide concentration of 1 µg/ml correlates to a residue level of 1 mg/kg when a 10 g sample is employed (e.g. samples with water content > 30 %) or 2 mg/kg when 5 g sample is employed (e.g. cereals).

3.22.2 Matrix-matched standards

Prepare matrix-matched standards in the same way as solvent-based standards, however, instead of pure acetonitrile use extracts of blank samples (prepared as described in 5.1 to 5.4, but without ISTD addition). To minimize errors caused by matrix induced effects during chromatography, it is best to choose similar commodities (e.g. apple for apple samples, carrot for carrot samples, etc.). Should the dilution of the blank sample extract upon addition of the pesticide working solutions exceed 20 %, a volume adjustment may be necessary to avoid errors caused by differences in the matrix-induced enhancement effect between sample extract and matrix-matched standard.

The stability of pesticides in matrix-matched standards can be lower than that of standards in pure acetonitrile and has to be checked more thoroughly.