SLOVENSKI STANDARD
SIST-TS CEN/TS 17062:2017

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Hrana rastlinskega izvora - Večelementna metoda za določanje ostankov pesticidov v rastlinskih oljih z LC-MS/MS

Foods of plant origin - Multimethod for the determination of pesticide residues in vegetable oils by LC-MS/MS

Pflanzliche Lebensmittel - Multiverfahren zur Bestimmung von Pestizidrückständen in pflanzlichen Ölen mit LC-MS/MS

Aliments d'origine végétale - Multiméthode de détermination des résidus de pesticides dans les huiles végétales par CL-SM/SM

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English Version

Foods of plant origin - Multimethod for the determination of pesticide residues in vegetable oils by LC-MS/MS

Aliments d'origine végétale - Multiméthode de détermination de la teneur en résidus de pesticides dans les huiles végétales par CL-SM/SM

Pflanzliche Lebensmittel - Multiverfahren zur Bestimmung von Pestizidrückständen in pflanzlichen Ölen mit LC-MS/MS

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COMITÉ EUROPÉEN DE NORMALISATION
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European foreword

This document (CEN/TS 17062:2017) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

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1 Scope

This Technical Specification describes a method for the analysis of pesticide residues in plant oils (fat content > 90 %, water content < 5 %). It has been validated in an interlaboratory test with olive oil. However, laboratory experiences are available also for other kind of oils such as sunflower seed oil, sesame oil, flax seed oil, rape seed oil, grape seed oil, thistle oil and pumpkin seed oil.

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.

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

3 Principle

The homogeneous sample is extracted with acetonitrile. After centrifugation, an aliquot of the organic phase is cleaned-up by dispersive solid phase extraction (D-SPE; sorbents PSA and C18). To separate co-extracted fat a freeze-out step of the acetonitrile phase can be applied. After clean up an additional centrifugation step is performed. The 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 used for LC-MS/MS analysis. A scheme of the procedure is given in Annex C.

NOTE In contrast to the method described in EN 15662 [1], this procedure does not include any addition of water.

4 Reagents

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

4.1 Water, HPLC quality.

4.2 Acetonitrile, HPLC quality.

4.3 Methanol, HPLC quality.

4.4 Acetic acid.

4.5 Ammonium formate.

4.6 Formic acid solution in acetonitrile, volume concentration σ = 5 ml formic acid/100 ml :

Dilute 5 ml of formic acid (mass fraction $w \geq 95$ %) to 100 ml with acetonitrile (4.2).

4.7 Primary secondary amin sorbent (PSA), e.g. Bondesil-PSA® 40 µm Agilent 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.

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

4.9 Internal standard and quality control standard solutions in acetonitrile, mass concentration ρ = 10 µg/ml to 100 µg/ml.

Table 1 shows a list of potential internal standards (ISTDs) and quality control (QC) standards that may be used in this method.

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

Compound	Log P (octanol-water partition coefficient)	Suggested concentration C_{ISTD} [µg/ml]	MS/MS ESI (+)	MS/MS ESI (-)
Tris-(1,3-dichlorisopropyl)-phosphate	3,65	10	+++	+
Linuron-D6	3,00	10	++	
Carbofuran-D3	1,80	10	++	
Chlorpyrifos-D10	4,70	10	+++	
Bis-nitrophenyl urea (nicarbazin)	3,76	10	-	+++

4.10 Primary pesticide standards:

Use standards of known purity, only.

4.11 Pesticide stock solutions:

Prepare individual stock solutions of analytical standards at concentrations that are sufficient to allow the preparation of complex pesticide working solutions 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.

4.12 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 (4.11) and appropriately diluting them with acetonitrile. The

1) Bondesil-PSA® is a product supplied by Agilent. This information is given for the convenience of users of this European Technical Specification and does not constitute an endorsement by CEN of the product named. Equivalent products may be used if they can be shown to lead to the same results.

pesticide concentrations in these mixtures should be sufficient to allow the preparation of the required matrix matched standards (4.13.2) with moderate dilution of the blank sample extract (e.g. less than 20 %).

Usually, pesticide working solutions should be stored at low temperature in the dark. Check the stability of pesticides contained in these mixtures during storage regularly [2] and adapt the storing conditions accordingly. In some cases the addition of acids or bases can be helpful to enhance stability and extend acceptable storage times.

4.13 Standard solutions (calibration mixtures):

4.13.1 Solvent-based standards:

Prepare solvent-based standards by mixing known volumes of the pesticide working solutions (4.12) and make up to volume with acetonitrile. The preparation of solvent based calibration mixtures with different analyte concentrations ($\rho_{\text{A}}^{\text{cal}}$) and identical internal standard concentrations ($\rho_{\text{ISTD}}^{\text{cal}}$) is necessary to create a calibration graph.

The concentration of the internal standards in the calibration mixtures ($\rho_{\text{ISTD}}^{\text{cal}}$) shall be equivalent to the concentration of the internal standard in the sample extracts, as the internal standards are added after extraction.

The quotient $V_{\text{ISTD}}^{\text{cal}} / V_{\text{Std}}$ from the volume used ($V_{\text{ISTD}}^{\text{cal}}$) shall be equivalent to the solution of the internal standard (4.9) and the final volume of the calibration standards (V_{Std}) shall be equivalent to the quotient $V_{\text{ISTD}} / V_{\text{Aliquot}}$ (see 6.1). If 60 µl ISTD solution (4.9) are added to 6 ml of aliquot of the centrifugate, 6 ml of standard solution shall be spiked with 60 µl of ISTD solution. If other volumes of calibration standards are used, the addition of ISTD solution shall be adjusted.

NOTE A pesticide concentration of 1 µg/ml correlates to a residue level of 5 mg/kg when a 2 g test portion is employed.

4.13.2 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 (samples, where no pesticides have been found with this method). The extract is prepared as described in Clause 5 (but without ISTD addition). To minimize errors caused by matrix induced effects during chromatography, it is best to choose similar commodities (e.g. olive oil for olive oil samples etc.).

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.

4.14 Mobile phase A_1:

Ammonium formate solution in water for HPLC, ρ = 0,315 g ammonium formate / 1 000 ml, substance concentration c = 5 mmol/l.

4.15 Mobile phase B_1:

Ammonium formate solution in methanol for HPLC, ρ = 0,315 g ammonium formate / 1 000 ml, c = 5 mmol/l.

4.16 Mobile phase A_2:

Acetic acid solution in water, add 0,1 ml of glacial acetic acid to 1 000 ml of water.

4.17 Mobile phase B_2:

Acetic acid solution in acetonitrile, add 0,1 ml of glacial acetic acid to 1 000 ml of acetonitrile.

4.18 Mobile phase A_3:

Methanol/water 2+8 (*V/V*) with 5 mmol/l ammonium formate, ρ = 0,315 g ammonium formate / 1 000 ml.

4.19 Mobile phase B_3:

Methanol/water 9+1 (*V/V*) with 5 mmol/l ammonium formate, ρ = 0,315 g ammonium formate / 1 000 ml.

4.20 Cotton wool.

5 Apparatus

Usual laboratory apparatus and, in particular, the following:

5.1 Automatic pipettes, suitable for handling volumes of 10 µl to 100 µl, 200 µl to 1 000 µl and 2 ml to 10 ml.

NOTE Instead of the latter, 10 ml graduated glass pipettes can be used alternatively.

5.2 Single use centrifuge tubes with screw caps, 50 ml

EXAMPLES

a) 50 ml centrifuge tubes made of poly-tetrafluoroethylene with screw caps; or

b) disposable 50 ml polypropylene centrifuge tubes with screw caps

5.3 Polypropylene-single use tubes with screw caps, 10 ml or 12 ml

5.4 Centrifuges, suitable for the centrifuge tubes employed in the procedure (6.2.2 and 6.2.3) and capable of achieving at least 1 000 *g*.

5.5 10 ml solvent-dispenser for acetonitrile, for use with the acetonitrile reservoir bottle.

5.6 Injection vials, 1,5 ml, suitable for LC autosampler, if necessary with micro-inserts.

5.7 Vibration device, e.g. Vortex (used for recovery studies).

5.8 Freezer, > 60 l, ≤ –18 °C.

5.9 LC-MS/MS system, equipped with electrospray ionization (ESI) interface (see Annex A).

6 Procedure

6.1 Extraction

Transfer a representative test portion of 2 g (m_{sample}) of the homogenous sample into a 50 ml centrifuge tube (5.2). Add 10 ml of acetonitrile (4.2 V_{ex}). Close the tube and shake vigorously for 1 min. Centrifuge for 5 min with at least 1 000 *g* for better separation of the phases.

Transfer an aliquot of the acetonitrile phase $V_{Aliquot}$ (e.g. 6 ml extract) into a tube with screw cap (5.3). Add a defined volume (V_{ISTD}) of the ISTD solution (4.9). The volume corresponds to 1 % of the aliquot volume (e.g. 60 µl ISTD solution to 6 ml acetonitrile phase).

6.2 Clean-up

6.2.1 General

The two different clean-up methods described in 6.2.2 and 6.2.3 were successfully validated and may be used alternatively.

6.2.2 Clean-up with amino-sorbent and silica-based reversed phase sorbent

Transfer an aliquot of 4 ml of the acetonitrile phase (6.1) into a Polypropylene single use tube (5.3) already containing 100 mg of PSA (4.7) and 100 mg of C18 sorbent (4.8). Close the tube, shake vigorously for 30 s and centrifuge (5 min at ≥ 1 000 g). Immediately isolate and acidify the clear extract as described in 6.2.4.

In case residues with acetic groups (e.g. phenoxy carboxylic acids) shall be determined, a second aliquot of the centrifuged extract from 6.1 is filled into an injection vial and analysed directly with LC-MS(/MS) to avoid losses of acidic groups by PSA clean-up.

25 mg PSA and 25 mg C18 sorbent are needed per ml of extract.

6.2.3 Freezing-out of co-extracted fat and clean-up with amino-sorbent

Store an aliquot of the extract from 6.1 containing the internal standard for at least 1,5 h at ≤ - 18 °C to freeze out most of the fat in the extract. For separation of the latter filter the extract over cotton wool (4.20). Take 4 ml from the cold and fat separated solution for dispersive SPE.

Transfer an aliquot of 4 ml of the acetonitrile phase into a Polypropylene single use tube (5.3) already containing 100 mg of PSA (4.7). Close the tube, shake vigorously for 30 s and centrifuge (5 min at ≥ 1 000 g). Immediately isolate and acidify the clear extract as described in 6.2.4.

If residues with acetic groups shall be determined, transfer a second aliquot into an injection vial and analyse directly with LC-MS(/MS) to avoid losses of acidic groups with PSA clean-up.

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. 25 mg PSA sorbent are needed per ml of extract.

6.2.4 Extract stabilization

Transfer an aliquot of 3 ml of the cleaned-up extract from 6.2.2 or 6.2.3 into a screw cap storage vial (5.3), taking care to avoid sorbent particles of being carried over, and slightly acidify by adding 30 µl of a 5 % formic acid solution in acetonitrile (4.6). Transfer the pH-adjusted extract into auto-sampler vials and use it for liquid chromatographic analysis. Store the residual extract in a refrigerator to be used if necessary.

For 1 ml extract 10 µl of the formic acid solution (4.6) are necessary.

6.3 Determination by liquid chromatography with tandem mass spectrometry (LC-MS/MS)

Inject the sample extracts derived from 6.2.2 to 6.2.4 and standard solutions (4.13) into the LC instruments in an appropriate sequence. This may involve bracketing of the sample extracts with the calibration solutions.

The measurement may be performed using various instruments, instrument parameters and columns. Some instrument parameters and columns are listed in Annex A. These conditions have been shown to provide satisfactory results, but are provided as examples, only.

For some gradient/column combinations it is necessary to mix the extract with water or the aqueous mobile phase to achieve a sufficient separation of the analytes.

NOTE If extracts are diluted with water or aqueous mobile phases it is important to avoid that non-polar parts of the extract precipitate or emulsions occur. This could lead to losses of lipophilic analytes. In this case an injection applying an injector programme can be helpful (see A.4).

The chromatographic conditions as outlined in Annex A have been shown to be satisfactory.

Suitable experimental conditions of LC-MS/MS measurements are outlined in CEN/TR 15641 [3]. Nevertheless, individual tuning of the compounds on the instrument that is used for measurement usually provides better sensitivities.

7 Evaluation of results

7.1 Identification and quantification

For the identification of residues in the final extract, use relative retention time ratio against the ISTD ($Rt_{(A)}/Rt_{(ISTD)}$) obtained from the same run. Check positive results by comparing the intensity ratios between the SIM masses (m/z) or SRM transitions of the analyte. The expected intensity ratios can be determined with the standard solutions. If the ratios of the samples and the standards have a variation of more than 20 %, the rules of EU Quality Control Procedures have to be followed [2]. According to these procedures positive results shall be ensured by using additional measures, e.g. additional SIM masses or SRM transitions or other chromatographic conditions (column, eluents).

For calibration and for checking the linearity of detection of each substance, plot the peak area ratio or peak height ratio of pesticide and internal standard $y_A^{cal} / y_{ISTD}^{cal}$ (if an internal standard is used) versus the concentration ratio of the analyte against the ISTD ($\rho_A^{cal} / \rho_{ISTD}^{cal}$) in the standard solution (4.13). If no internal standards are used, plot the peak areas or peak height y_A^{cal} against the concentration of the analyte ρ_A^{cal}.

The calibration area shall be adapted to the residue concentration and should not exceed a decimal power. Possibly more calibration graphs shall be established using the standard solution. The calibration function is selected according to CEN/TS 17061:2017, 4.2.1.

For a first estimation of the residue level or for the verification of absence of residues, solvent based standards (4.13.1) can be used. They can also be used for quantification, if it was shown that no enhancement or suppression of the analyte signal through matrix occurs. If relevant residue levels are observed (e.g. with possible MRL violation) matrix matched standard shall be preferred for exact quantification.

7.2 Calculation of residue concentrations using the internal standard

The determination of the concentration of the analyte ρ_A in the final extract is performed by using the measured peak area ratio or peak height ratio from pesticide and internal standard y_A/y_{ISTD} in the sample as described in CEN/TS 17061. The mass fraction w of the analyte in the sample, in milligram per kilogram, is calculated with Formula (1):

$$w = \frac{\rho_A \times V_{ex}}{m_{Sample}} \quad (1)$$