



BSI Standards Publication

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

National foreword

This British Standard is the UK implementation of CEN/TS 17062:2017.

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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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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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 $\sigma = 5$ 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. 12213023¹⁾.

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 $\rho = 10 \mu\text{g/ml}$ to $100 \mu\text{g/ml}$.

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 $\leq -18^\circ\text{C}$. 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 (ρ_A^{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 A₁:

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

4.15 Mobile phase B₁:

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

4.16 Mobile phase A₂:

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

4.17 Mobile phase B₂:

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

4.18 Mobile phase A₃:

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

4.19 Mobile phase B₃:

Methanol/water 9+1 (V/V) with 5 mmol/l ammonium formate, $\rho = 0,315$ 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 μ l to 100 μ l, 200 μ l to 1 000 μ l 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.