SLOVENSKI STANDARD
SIST-TS CEN/TS 17743:2022

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Živila - Določevanje ostankov pesticidov z ekstrakcijo z etil acetatom z uporabo GC- in LC-MS/MS (SweEt)

Foodstuff - Determination of pesticide residues by ethyl acetate extraction using GC- and LC-MS/MS (SweEt)

Lebensmittel - Bestimmung von Pestizidrückständen mit Ethylacetatextraktion durch GC- und LC-MS/MS (SweEt)

Produits alimentaires - Dosage des résidus de pesticides par extraction à l'acétate d'éthyle par GC- et LC-MS/MS (SweEt)

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English Version

Foodstuff - Determination of pesticide residues by ethyl acetate extraction using GC- and LC-MS/MS (SweEt)

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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European foreword

This document (CEN/TS 17743:2022) 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 document specifies a method for the analysis of pesticide residues in foods of plant and of animal origin by ethyl acetate extraction using GC- and LC-MS/MS (SweEt).

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 17061:2019, *Foodstuffs - Guideline for the calibration and quantitative determination of chromatographic methods for the determination of pesticide residues and organic contaminants*

3 Terms and Definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

4 Principle

The method is based on extraction with ethyl acetate followed by determination with liquid chromatography (LC) and gas chromatography (GC). No clean-up is needed for low fat/oil fruit, vegetables and cereals or honey. However, for commodities with high fat/oil content, ethyl acetate/cyclohexane is used for extraction. Furthermore, a clean-up step using gel permeation chromatography (GPC) is needed. The animal products with low fat/oil content are extracted with ethyl acetate followed by clean-up using Primary Secondary Amine (PSA) and C18[1] as sorbents. For extraction of cereals, acidified ethyl acetate is used, which makes it possible to analyze acidic pesticides such as phenoxy acids.

Low fat/oil fruits, vegetables and honey: The homogenous sample is extracted with ethyl acetate after addition of $NaHCO_3$. After extraction, Na_2SO_4 is added to remove water. The sample extract is centrifuged and filtered prior to injection to GC-MS/MS and LC-MS/MS.

Cereals: The grinded sample is extracted after water addition with acidified ethyl acetate (5.21). After extraction, Na_2SO_4 is added to remove water. The sample extract is centrifuged and filtered prior to injection to GC-MS/MS and LC-MS/MS.

Animal origin: The homogenous sample is extracted using ethyl acetate or ethyl acetate + cyclohexane ($V_1 + V_2$, 1 + 1). After extraction, Na_2SO_4 is added to remove water. The choice of extraction solvent depends on the fat/oil content of the sample. Samples with a fat/oil content of a mass fraction ≤ 10 % are extracted with ethyl acetate and purified with the sorbents PSA and C18 whereas for samples with higher fat/oil content ethyl acetate + cyclohexane ($V_1 + V_2$, 1 + 1) followed by GPC is used. The sample extract is finally filtered prior to injection to GC-MS/MS and LC-MS/MS.

[1] Octadecylsilyl chemically bonded silica gel.

5 Reagents

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

5.1 Water, HPLC quality.

5.2 Methanol, HPLC quality.

5.3 Acetonitrile, HPLC quality.

5.4 Ethyl acetate, pesticide residue grade.

5.5 Cyclohexane, pesticide residue grade.

5.6 Toluene, pesticide residue grade.

5.7 Acetic acid, purity greater than mass fraction ≥ 98 %.

5.8 Ammonia, mass fraction of 25 % NH_3 (approximately 13,4 mol/l).

5.9 Formic acid, mass fraction of 98 % to 100 %.

5.10 Sulphuric acid, mass fraction of 95 % to 97 %.

5.11 Sodium hydroxide.

5.12 Sodium sulphate, Na_2SO_4, water free, p.a.

5.13 Sodium hydrogen monocarbonate, $NaHCO_3$, water free, p.a.

5.14 BioBeads® S-X3 resin, 38 µm to 75 µm (200 to 400 mesh)[2].

5.15 Primary secondary amino sorbent (PSA).

EXAMPLE Bondesil-PSA® 40 µm Agilent No 12213023[3]. Other amino sorbents may be used, but investigations may be necessary to prove equivalency especially regarding analyte losses and pH of the final extracts.

5.16 C18 sorbent[1]

EXAMPLE Bondesil-C18® 40 µm, Agilent[3].

2 BioBeads®-S-X3 resin, 38 µm to 75 µm (200 to 400 mesh) is the trademark of a product supplied by Bio-Rad. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN or CENELEC of the product named. Equivalent products may be used if they can be shown to lead to the same results.

3 Bondesil-PSA® 40 µm Agilent No 12213023 and Bondesil-C18® 40 µm are the trademarks of products supplied by Agilent. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN or CENELEC of the product named. Equivalent products may be used if they can be shown to lead to the same results.

5.17 Ammonium formate solution (50 mM), pH 4,1.

Add 1,92 ml ± 0,02 ml 98 % formic acid (26 mol/l; 5.9) to 900 ml ± 5 ml water. Adjust the pH between 4,1 and 4,15 with ammonia (5.8). Dilute 9 parts buffer solution with 1 part water.

The buffer solution is stable up to 3 months at +4 °C.

5.18 Mobile phase A, 10 mmol/l ammonium formate, pH 4,0 to 4,2.

Add 200 ml ± 2 ml (volumetric glass) of 50 mmol/l buffer (5.17) to 800 ml ± 5 ml of water (5.1).

The buffer solution is stable up to 3 months at +4 °C.

5.19 Mobile phase B, Methanol (5.2).

5.20 Wash solution for HPLC, acetonitrile + water, V_1 + V_2, 80 + 20.

Add 200 ml ± 2 ml water (5.1) to 800 ml ± 5 ml acetonitrile (5.3).

5.21 Acidified ethyl acetate for extraction, volume fraction of 1 % acetic acid.

Mix 495 ml of ethyl acetate (5.4) with 5 ml acetic acid (5.7).

5.22 Sodium hydroxide, 5 mol/l.

Dissolve 20 g of NaOH (5.11) in approximately 80 ml of water (5.1) and dilute to 100 ml.

5.23 Sodium chloride, NaCl.

5.24 Sulphuric acid, H_2SO_4, 2,5 mol/l.

Mix 7 ml of H_2SO_4 (5.10) with 50 ml water (5.1).

5.25 Primary pesticide standards.

Primary standards are purchased with purity >95 %.

5.26 Pesticide stock solutions.

Stock solutions are prepared in the most suitable solvent with regards to their solubility and stability.

5.27 Intermediate pesticide working solutions.

Intermediate standard solutions of pesticide mixtures (40 µg/ml) are prepared in methanol, acetonitrile or toluene.

5.28 Pesticide calibration solutions.

Working solutions used for calibration on LC-MS/MS in fruit and vegetables are prepared in blank matrix (generic matrix) with low suppression, e.g. banana or carrot. By doing so the matrix effect can be compensated and the stability of pesticides improved as well.

Working solutions for the calibration on GC-MS/MS are prepared in generic matrix, e.g. cucumber with no or little enhancement of the signal. True matrix matched working solutions are always used for the calibration on both types of analytical techniques for cereals and products of animal origin.

The findings in fruit and vegetables above the maximum residue level (MRL) are always confirmed against true matrix matched standards.

5.29 GPC eluent and extraction solvent for commodities with high fat/oil content, ethyl acetate + cyclohexane (V1 + V2, 1 + 1).

6 Apparatus

Usual laboratory apparatus and, in particular, the following:

6.1 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.

6.2 Centrifuge tubes with screw caps, 114 mm × 28 mm, polypropylene (50 ml).

6.3 Volumetric cylinder for ethyl acetate (25 ml).

6.4 Syringes, e.g. 10 ml disposable syringes.

6.5 Syringe filters, 0,20 µm pore size, polytetrafluoroethylene (PTFE) for filtration of final extracts.

6.6 Injection vials, 1,5 ml suitable for LC and GC auto-samplers.

6.7 Analytical balance, accuracy 0,1 mg.

6.8 Laboratory balance, accuracy 0,01 g.

6.9 Centrifuge.

6.10 Ultrasonic bath.

6.11 Instrument for GPC, equipped with a column, 25 mm internal diameter, 50 cm length, and 5 ml sample loop.

6.12 Mechanical shaker (300 r/min) or a high-speed mechanical shaker, for example from Spex®SamplePrep[4] shaking ≥ 1 500 r/min**.**

6.13 Rotavapor or similar for evaporation.

6.14 Nitrogen evaporator with thermostated water bath.

6.15 Vibration device, e.g. Vortex.

6.16 LC-MS/MS system, see Annex A.

6.17 GC-MS/MS system, see Annex A.

[4] A high-speed mechanical shaker from Spex®SamplePrep is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of those products.

7 Procedure

7.1 Sample preparation

7.1.1 Fruit and vegetables

Weigh a test portion of 10,0 g ± 0,1 g (m_{sample}) of the homogenous laboratory sample into a 50 ml centrifuge tube (6.2). For a recovery test (QC sample), add 50 µl to 250 µl of standard solution (5.27) at this stage. Wait for 5 min before adding 3,0 g ± 0,1 g of sodium hydrogen monocarbonate ($NaHCO_3$, 5.13) and add 20,0 ml ± 0,1 ml ethyl acetate (V_{ex}, 5.4). Shake vigorously by hand for approximately 30 s and thereafter place the tubes with a mechanical shaker at 300 r/min for 15 min, alternatively at 1 500 r/min for 3 min (6.12). Add 10,0 g ± 0,5 g of sodium sulphate (Na_2SO_4, 5.12) and shake the sample for 1 min. Centrifuge the tubes for 3 min at 3 200 *g* (6.9). Filter the crude extract using a 0,2 µm PTFE syringe filter (6.5). The final extract has a concentration of 0,50 g/ml sample in ethyl acetate and is analyzed directly by GC- and LC-MS/MS (6.16, 6.17).

For dried fruit (water content about 20 % or less) and honey, water should be added prior to the extraction. Weigh e.g. 10 g of sample, add 15 ml ± 0,1 ml cold water, shake for 5 min and extract as above.

NOTE: To improve phase separation for extracted honey samples, 1,00 g ± 0,05 g of NaCl (5.23) should be added prior to centrifugation, i.e. after extraction in combination with the addition of Na_2SO_4 (5.12).

7.1.2 Cereals

Weigh 5,0 g ± 0,1 g grinded, dry sample (m_{sample}) in a 50 ml centrifuge tube (6.2), add 10 ml ± 1 ml water (5.1) and shake to wet the sample. Add 10,0 ml ± 0,1 ml acidified ethyl acetate (V_{ex}, 5.21), and shake the mixture vigorously by hand or with a vibration device (6.15) for 30 s in order to get the sample homogeneously mixed with the solvent and thereafter for 15 min with a mechanical shaker at 300 r/min, alternatively for 5 min at 1 500 r/min (6.12). Add 10,0 g ± 0,5 g Na_2SO_4 (5.12) and shake with a vibration device (6.15), alternatively with a mechanical shaker (1 500 r/min, 6.12) for 30 s. Centrifuge the tubes for 3 min at 3 200 *g* (6.9). Filter the crude extract using a 0,2 µm PTFE syringe filter (6.5). The final extract has a concentration of 0,50 g/ml sample in solvent and is analyzed directly by GC- and LC-MS/MS (6.16, 6.17).

NOTE: For the analysis of esters of phenoxy acids or conjugates the sample is hydrolyzed with 300 µL sodium hydroxide (5 mol/l, 5.11) in order to release covalently bound compounds. Shake for 1 min and let the sample stand for 30 min at room temperature. Neutralize the sample by 300 µL sulphuric acid (2,5 mol/l, 5.10) and extract with acidified ethyl acetate as described above.

7.1.3 Products of animal origin with the fat/oil content ≤ 10 %

Weigh 5,0 g ± 0,1 g sample (m_{sample}) in a 50 ml centrifuge tube (6.2) and add 10,0 ml ± 0,1 ml ethyl acetate (V_{ex}, 5.4). Shake vigorously by hand or with a vibration device (6.15) for approximately 30 s and thereafter for 15 min with a mechanical shaker at 300 r/min, alternatively for 3 min at 1 500 r/min (6.12). Add 5,0 g ± 0,1 g Na_2SO_4 (5.12) and shake with a vibration device or shake for 30 s with a mechanical shaker at 1 500 r/min (6.12). Centrifuge the tubes for 3 min at 3 200 *g* (6.9). Filter the crude extract using a 0,2 µm PTFE syringe filter (6.5). For each sample, add 0,20 g ± 0,01 g PSA and 0,20 g ± 0,01 g of C18 to a 2 ml sample tube followed by 1 ml of the filtered crude extract between vigorous shaking. Centrifuge at 3 200 r/min (6.9) for 3 min at room temperature. The final extract has a concentration of 0,50 g/ml sample in ethyl acetate and is analyzed directly by GC- and LC-MS/MS (6.16, 6.17).