SLOVENSKI STANDARD

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Živila brez maščob - Multirezidualne metode za določevanje ostankov pesticidov s plinsko kromatografijo - 1. del: Splošna navodila

Non-fatty foods - Multiresidue methods for the gas chromatographic determination of pesticide residues - Part 1: General considerations

Fettarme Lebensmittel - Multiverfahren zur gaschromatographischen Bestimmung von Pestizidrückständen - Teil 1: Allgemeines

Aliments non gras - Méthodes multirésidus de détermination par chromatographie en phase gazeuse de résidus de pesticides - Partie 1: Généralités

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English version

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This European Standard was approved by CEN on 7 September 1998.

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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 Central Secretariat has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and United Kingdom.

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Contents

Foreword

This European Standard 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of an identical text or by endorsement, at the latest by April 1999, and conflicting national standards shall be withdrawn at the latest by April 1999.

This EN 12393 “Non-fatty foods - Multiresidue methods for the gas chromatographic determination of pesticide residues” is divided in three parts:

- Part 1 "General considerations" provides general considerations with regard to reagents, apparatus, gas chromatography, etc., applying to each of the analytical selected methods;

- Part 2 "Methods for extraction and clean-up" presents methods L to P for the extraction and clean-up using techniques such as liquid-liquid partition, adsorption column chromatography or gel permeation column chromatography, etc.;

- Part 3 "Determination and confirmatory tests" gives some recommended techniques for the qualitative and quantitative measurements of residues and the confirmation of the results.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Introduction

This European Standard comprises a range of multi-residue methods of equal status: no single method can be identified as the prime method because, in this field, methods are continuously developing. The selected methods included in this standard have been validated and/or are widely used throughout Europe.

Because these methods can be applied to the very wide range of food commodities/pesticide combinations, using different systems for determination, there are occasions when variations in equipment used, extraction, clean-up and chromatographic conditions are appropriate to improve method performance, see 3.1.

1 Scope

This European Standard gives general considerations for the determination of pesticide residues in non-fatty foods.

Each method described in this European Standard is suitable for identifying and quantifying a definite range of those organohalogen, and/or organophosphorus and/or organonitrogen pesticides which occur as residues in foodstuffs of plant origin.

This European Standard contains the following methods that have been subjected to inter-laboratory studies and/or are adopted throughout Europe:

- method L: Extraction with acetone, liquid-liquid partition with dichloromethane and clean-up on a silica-gel/charcoal column [1];

- method M: Extraction with acetone and liquid-liquid partition with dichloromethane/light petroleum, if necessary clean-up on Florisil®[1] [2, 3, 4];

- method N: Extraction with acetone, liquid-liquid partition with dichloromethane and clean-up with gel permeation and silica gel chromatography [5];

- method O: Extraction with acetonitrile, liquid-liquid partition with light petroleum and clean-up on a Florisil column [6];

- method P: Extraction of organophosphorus compounds with ethyl acetate and, if necessary, clean-up with gel permeation chromatography [7].

The applicability of the five methods L to P for residue analysis of organohalogen, organophosphorus and organonitrogen pesticides, respectively, is given for each method.

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[1] Florisil® is an example of a suitable product available commercially. This information is given for the convenience of users of this standard and does not constitute an endorsement by CEN of this product.

2 Normative references

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

EN 12393-2	1998	Non-fatty foods - Multiresidue methods for the gas chromatographic determination of pesticide residues - Part 2: Methods for extraction and clean-up
EN 12393-3	1998	Non-fatty foods - Multiresidue methods for the gas chromatographic determination of pesticide residues - Part 3: Determination and confirmatory tests

3 Principle

3.1 General

As already described in the introduction, in certain occasions it is possible to improve the method performance by variations in equipment used, extraction, clean-up and chromatographic conditions. Such variations shall be always clearly documented and demonstrated to give valid results.

The methods described in this European Standard are based on a four-stage process (in some cases two stages may be combined, in whole or in part), as given in 3.2 to 3.5.

3.2 Extraction

Extraction of the residues from the sample matrix by the use of appropriate solvents, so as to obtain the maximum efficiency of extraction of the residues and minimum co-extraction of any substances which can give rise to interferences in the determination.

3.3 Clean-up

Removal of interfering materials from the sample extract to obtain a solution of the extracted residue in a solvent which is suitable for determination by the selected method of determination.

3.4 Determination

Gas chromatography (GC) with selective detectors may be used: electron-capture detection (ECD) for organohalogen, thermionic detector (NPD, P-mode or N/P mode) for organophosphorus and organonitrogen compounds and flame-photometric detector (FPD) for organophosphorus and organosulfurous pesticides. Hall detector (ECHD), atomic emission detector (AED) and mass spectrometry (MS) may also be used for a large class of pesticides.

3.5 Confirmation

Procedures to confirm the identity and quantity of observed residues, particularly in those cases where it would appear that the maximum residue limit (MRL) has been exceeded.

4 Reagents

4.1 General

Use reagents of purity suitable for pesticide residue analysis and check their purity (see 4.2). If required, purify water and solvents used, e.g. as described in annex A and check their purity (see 4.2). Purify and periodically activate adsorbents according to the requirements of the different analytical methods; check their purity (see 4.2).

Take every precaution to avoid possible contamination of water, solvents, adsorbents, etc. from plastics and rubber materials.

4.2 Check for purity of reagents

4.2.1 Solvents

Concentrate solvents by the factor involved in the respective method to be used. Test for purity by GC under the same conditions as used in the method. The chromatogram should not show any interfering impurity. Extract or concentrate acetonitrile, acetone, ethyl acetate, hexane, light petroleum and dichloromethane in the same volume as used in the method and examine the resulting solution as above by GC.

4.2.2 Water

Extract 10 parts by volume of water with one part by volume of *n*-hexane or light petroleum, dichloromethane or any other non water miscible solvent used in the method. Separate the organic phase, concentrate by the factor involved in the respective method and test for purity by GC under the same conditions as used in the method. The chromatogram should not show any interfering impurity.

4.2.3 Inorganic salts

Extract inorganic salts, for example sodium chloride, after purification according to annex A or the requirements of the different analytical methods. Extract the salts and any aqueous solution used, with *n*-hexane or light petroleum, dichloromethane or any other non water miscible solvent used in the method. Concentrate the extract by the factor involved in the respective method and test the purity by GC under the same conditions as used in the method. The chromatogram should not show any interfering impurity.

4.2.4 Adsorbents

Elute an amount of adsorbent equal to that used in the analytical method with the corresponding type and volume of solvent or solvent mixture. Concentrate the eluate as indicated in the analytical method and test for purity by GC. The chromatogram should not show any interfering impurity. Check the activity of adsorbents regularly as described in the methods L to P
(see EN 12393-2:1998).

4.2.5 Standard materials and solutions

Use standard materials of at least 95 % purity and traceable quality as standards for residue analysis.

Ensure dilute solutions are prepared and checked frequently, and that standard solutions are stored in glass bottles in a refrigerator and every precaution is taken to avoid possible contamination from plastics or rubber materials. Ensure that the standard solutions are not directly exposed to sunlight or ultraviolet light for prolonged periods of time. Examine analytical standards for impurities.

> NOTE 1: When stored at - 20 °C, standard materials are generally stable for at least a year. To allow equilibration, allow the standards to come up to room temperature before the containers are opened. Stock solutions of concentration 1 mg/ml, if kept in a freezer (at about - 20 °C), are usually stable for 6 months.

> NOTE 2: Changes in volume due to solvent evaporation, for example through the space between a glass stopper and the neck of a flask, can be a source of error. Therefore, the use of polytetrafluoroethylene (PTFE) screw-cap flasks is recommended for the storage of stock and standard solutions.

> NOTE 3: Experience has shown that errors introduced in the preparation, handling and storage of standards and standard solutions are major sources of inaccuracies. Experiences obtained by other national, European and international bodies should be observed [8], [9].

4.3 Safety aspects associated with reagents

4.3.1 General

The analysis of pesticide residues in a food matrix includes the use of several hazardous chemicals. Safety precautions as given in 4.3.2 and 4.3.3 shall be observed at all times.

4.3.2 Pesticides

Many pesticides are extremely toxic by various routes of exposure, especially in their concentrated forms. As an example, the family of organophosphorus pesticides is consistently highly toxic, not only by oral ingestion, but dermally and by inhalation as well. When working with standard materials, standard solutions, etc. observe the following minimal precautions at all times (consult safety data sheets or labels for additional information):

a) perform all laboratory sampling, mixing, weighing, etc., under an effective fume removal device in an area having a good forced ventilation of non-recirculated air; or wear a gas mask of the proper type. If the mask is used, replace cartridges as recommended, since using a contaminated mask could be worse than wearing no mask at all;

b) keep pesticides off the skin. Wear clean protective clothing and non-permeable gloves (such as polyethylene gloves) as necessary. Wash hands thoroughly with soap and water to avoid contaminating food;

c) label clearly all containers with the name and concentration of the appropriate pesticide;

d) study and have readily available information on symptoms of poisoning and first aid treatment for each type of pesticide being handled;

e) consult a physician about preventive measures and antidotes for use in emergencies when pesticide poisoning is suspected;

f) follow your organization's procedures when disposing of waste pesticides. The manufacturer can be contacted for advice on disposal problems;

g) do not enter laboratories working with pesticide residues or other laboratories after handling pesticide formulations until protective clothing and gloves have been removed and hands thoroughly washed with soap and water.

4.3.3 Hazardous solvents

Do not let vapours concentrate to a flammable level in the work area, since it is impossible to eliminate all chance of sparks from static electricity even though electrical equipment is earthed (e.g. use of spark-proof refrigerators or freezers). When working with flammable solvents, use an effective fume removal device to remove these vapours as they are released.

Vapours from certain volatile solvents are highly toxic. Several of these solvents can easily be absorbed through the skin. Use an effective fume removal device to remove vapours of these solvents as they are released.

A list of some hazardous reagents is given in table 1.

The use of hazardous solvents mentioned in the Montreal Agreement (such as chlorinated solvents) should be minimized as far as possible.

Table 1: Hazardous reagents, their effects and ways of containment

Name of reagent	Problem	Comment	Solution
Acetone	Highly flammable	Forms explosive peroxides with oxidizing agents.	Use an effective fume removal device.
Acetonitrile	Toxic	Avoid contact with skin and eyes.	Use an effective fume removal device.
Cyclohexane	Highly flammable		Use an effective fume removal device.
Dichloromethane	Toxic	Avoid contact with eyes and avoid breathing vapors.	Use an effective fume removal device.
Diethyl ether	Extremely flammable	Unstable peroxides can form upon long standing or exposure to sunlight in bottles.	Store protected from light. Use an effective fume removal device. See also the warning on peroxides.
Ethanol	Flammable		Use an effective fume removal device when heating or evaporating.
Ethyl acetate	Flammable, especially when being evaporated	Irritating to eyes and respiratory tract.	Use an effective fume removal device.
n-Hexane	Highly flammable. Toxic		Use an effective fume removal device.
Iso-octane	Highly flammable		Use an effective fume removal device.
Light petroleum	Extremely flammable		Use an effective fume removal device.
Methanol	Flammable. Toxic	Avoid contact with eyes. Avoid breathing the vapours.	Use an effective fume removal device.

SAFETY MEASURES: Peroxides form in diethyl ether, dioxane, and other ethers during storage. They are explosive and have to be destroyed before distillation or evaporation. Exposure to light increases peroxide formation in ethers. Filtration through activated aluminium oxide is reported to be effective in removing peroxides.

5 Apparatus

5.1 Glassware: General

Thoroughly clean glassware shall be used for residue analysis.

There is a risk of carry-over of pesticides through use of glassware and other laboratory equipment. This should be taken into account, particularly when employing laboratory washing machines. Hot detergent solution (with no interfering compounds) may be used for cleaning, but afterwards the glassware shall be well rinsed with distilled water and acetone before drying. Before being washed in a washing machine, the glassware shall be rinsed with acetone, then with water. Wash it in the machine with non-chlorinated detergent, rinse with water and dry. In both cases, verify that the detergent does not leave any interfering impurity. It is also advisable to rinse glassware again with the solvent to be used immediately before use.

Common laboratory glassware or equipment such as beakers, round-bottomed flasks, watchglasses, pipettes, filter papers, glass wool, glass rods and glass beads, etc. are not listed in the apparatus clause of each method in detail.

5.2 Special glassware

5.2.1 Tapered tubes, suitable for evaporation, fitted with 14 mm ground-glass joints and having a capacity of approximately 15 ml, 80 mm to 90 mm long, are required for final concentrations. These are preferably calibrated and may be fitted with micro-Snyder®[2] columns [10].

5.2.2 Chromatographic tubes, which should be specially made and have glass or PTFE stop-cocks are specified in most methods. The tops of the columns should have ground-glass joints to permit attachment of a solvent reservoir or pressure adaptor.

5.3 Auxiliary materials

If necessary, wash filter papers, glass rods and glass beads with pure solvent prior to use. Extract cotton wool, glass wool, quartz wool with *n*-hexane and acetone and any other suitable solvent using a Soxhlet extractor, until sufficiently free from interfering substances.

Solutions are often reduced to a final small volume by passing a stream of nitrogen over them. Rubber or polyvinyl chloride (PVC) tubing should not be used for this purpose. PTFE or nylon tubing usually presents the least risk of contamination.

Do not use ordinary plastics, for example PVC stoppers, in vessels for storing standard materials and solutions as they may lead to contamination. Glass or PTFE stoppers are necessary. Similarly, do not use separating funnels with plastics stoppers or stop-cocks. Replace ordinary plastics stoppers with glass or PTFE stoppers.

5.4 Solvent evaporators

5.4.1 General

Solvent evaporators shall have a thermostable water bath (capable of being controlled between ambient temperature and 100 °C) and preferably a controller for the vacuum.

The effect of the solvent evaporator on the loss of volatile residue should be checked periodically. A keeper (e.g. propylene glycol, n-undecane or hexadecane) can be used to minimize losses of pesticides in certain cases.

Solvent evaporators can be used for concentrating large solvent volumes (for small volumes, the use of a gentle stream of pure, dry nitrogen is more advisable), such as:

5.4.2 Kuderna-Danish[3] **evaporator** [11] (or equivalent) with or without fractionating column, which is heated on a thermostable water bath.

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[2] Micro-Snyder® columns are examples of suitable products available commercially. This information is given for the convenience of users of this standard and does not constitute an endorsement by CEN of these products.