SLOVENSKI STANDARD
SIST EN 1528-1:1998

01-november-1998

Živila, ki vsebujejo maščobe - Določevanje pesticidov in polikloriranih bifenilov (PCB) - 1. del: Splošna navodila

Fatty food - Determination of pesticides and polychlorinated biphenyls (PCBs) - Part 1: General

Fettreiche Lebensmittel - Bestimmung von Pestiziden und polychlorierten Biphenylen (PCB) - Teil 1: Allgemeines

Aliments gras - Dosage des pesticides et des polychlorobiphényles (PCB) - Partie 1: Généralités

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ICS:

67.050	Splošne preskusne in analizne metode za živilske proizvode	General methods of tests and analysis for food products

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EN 1528-1

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English version

Fatty food - Determination of pesticides and polychlorinated biphenyls (PCBs) - Part 1 : General

Aliments gras - Dosage des pesticides et des polychlorobiphényles (PCB) - Partie 1 : Généralités

Fettreiche Lebensmittel - Bestimmung von Pestiziden und polychlorierten Biphenylen (PCB) - Teil 1: Allgemeines

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Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

The European Standards exist 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.

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CEN

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

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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of an identical text or by endorsement, at the latest by May 1997, and conflicting national standards shall be withdrawn at the latest by May 1997.

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

This European Standard consists of the following Parts:

Part 1 "General" presents the scope of the standard and describes general considerations with regard to reagents, apparatus, gas chromatography etc., applying to each of the analytical methods selected.

Part 2 "Extraction of fat, pesticides and PCBs, and determination of fat content" presents a range of analytical procedures for extracting the fat portion containing the pesticide and PCB residues from different groups of fat-containing foodstuffs.

Part 3 "Clean-up methods" presents the details of methods A to H for the clean-up of fats and oils or the isolated fat portion, respectively, using techniques such as liquid/liquid partition, adsorption or gel permeation column chromatography.

Part 4 "Determination, confirmatory tests, miscellaneous" gives guidance on some recommended techniques for the determination of pesticides and PCBs in fatty foodstuffs and on confirmatory tests and lists a clean-up procedure for the removal of the bulk of lipids when analysing large quantities of fat.

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 methods selected for inclusion in this standard have been validated and are widely used throughout Europe. Any variation in the methods used should be shown to give comparable results.

1 Scope

This European Standard specifies methods for the determination of residues of pesticides and polychlorinated biphenyls (PCBs) in fatty food.

Each method described in this European Standard is suitable for identifying and quantifying a definite range of those non-polar organochlorine and/or organophosphorus pesticides which occur as residues in fats and oils as well as in the fat portion of fat-containing foodstuffs, both of either animal or vegetable origin. The PCB indicator congeners usually selected for the enforcement of maximum residue limits (MRLs) are determined along with the organochlorine pesticides.

This European Standard contains the following clean-up methods that have been subjected to interlaboratory studies and are adopted throughout Europe:

- Method A: Liquid-liquid partitioning with acetonitrile and clean-up on a Florisil®[1]) column (AOAC) [1]
- Method B: Liquid-liquid partitioning with dimethylformamide and clean-up on a Florisil® column (Specht) [2]
- Method C: Column chromatography on activated Florisil® (AOAC) [3]
- Method D: Column chromatography on partially deactivated Florisil® (Stijve) [4]
- Method E: Column chromatography on partially deactivated aluminium oxide (Greve & Grevenstuk) [5]
- Method F: Gel permeation chromatography (GPC) (AOAC) [6]
- Method G: Gel permeation chromatography (GPC) and column chromatography on partially deactivated silica gel (Specht) [7]
- Method H: High performance gel permeation chromatography (HPGPC) (MAFF) [8]

The applicability of the eight methods A to H for residue analysis of organochlorine pesticides, PCB indicator congeners, and organophosphorus pesticides, respectively, is given in table A.1. Where no + sign is shown, there are no data available in literature, but this does not necessarily exclude the applicability.

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.

ISO 1750
Pesticides and other agrochemicals - Common names

EN 1528-2 : 1996
Fatty food - Determination of pesticides and polychlorinated biphenyls (PCBs) - Part 2: Extraction of fat, pesticides and PCBs, and determination of fat content

EN 1528-3 : 1996
Fatty food - Determination of pesticides and polychlorinated biphenyls (PCBs) - Part 3: Clean-up methods

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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 European Standard and does not constitute an endorsement by CEN of the product named.

EN 1528-4 : 1996
Fatty food - Determination of pesticides and polychlorinated biphenyls (PCBs) - Part 4: Determination, confirmatory tests, miscellaneous

NOTE: See also International Standards concerning the determination of fat content, product sampling and preparation of test sample.

3 Principle

3.1 General

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 described 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 residue and minimum co-extraction of any substances which can give rise to interferences in the determination.

NOTE: Methods for extraction of fat are recommended which are simultaneously applicable for the extraction and determination of fat and the residue analysis in the fat portion.

3.3 Clean-up

Maximum removal of interfering substances with minimal loss of analyte from the sample extract, so as to obtain a solution of the extracted residue in a solvent which is suitable for quantitative examination by the selected method of determination.

3.4 Determination

Gas chromatography (GC) with various detectors, e.g. electron-capture detector (ECD), the thermionic detector (P- or N/P- mode), the flamephotometric detector (FPD), the Hall detector or mass spectrometry (MS) as appropriate.

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 has been exceeded.

4 Reagents

4.1 General

Unless otherwise stated, use reagents of the highest purity (i.e. for residue analysis) and only distilled or demineralized water if possible; if this is not possible, redistil the water, solvents, and reagents used as described in annex B and check their purity (see 4.2). Note that ion-exchange resins used for demineralized water can be a source of interferences. 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. If an interference is encountered in a reagent blank determination then check the purity of all reagents used.

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, dimethylformamide 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 B or the requirements of the different analytical methods 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 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, for example as described in 5.3.9 in EN 1528-3 : 1996.

4.2.5 Standard materials and solutions

Use 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. Examine analytical standards for impurities.

When stored at -20 °C, standard materials are generally stable for at least a year or two. 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 spark proof refrigerator (at about 4 °C), are usually stable for 2 to 3 months.

NOTE 1: 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.

NOTE 2: 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 [9], [10].

4.3 Safety aspects associated with reagents

4.3.1 General

The analysis of pesticide and PCB congener residues in a food matrix includes the use of several hazardous chemicals.

The list given in 4.3.2 shows some appropriate safety precautions which shall be observed at all times.

4.3.2 Pesticides and PCBs

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., the following minimal precautions shall be observed 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 good forced ventilation of non-recirculated air; or wear a respirator of the proper type. If the respirator is used, replace cartridges as recommended, since using a contaminated respirator could be worse than wearing no respirator at all.

b) Keep pesticides and PCBs 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) Clearly label 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 reagents

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. 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 are readily absorbed through 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.

Table 1: Hazardous reagents

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	Not highly flammable. Toxic	Avoid contact with eyes. Avoid breathing the vapours	Use an effective fume removal device
Diethyl ether	Store protected from light. Extremely flammable	Unstable peroxides can form upon long standing or exposure to sunlight in bottles	Use an effective fume removal device. See also note on peroxides
Dimethylformamide	Toxic. Flammable	Avoid contact with skin and eyes. Can react vigorously with halogenated hydrocarbons	Use an effective fume removal device
Ethanol	Flammable		Use an effective fume removal device when heating or evaporating
Ethyl acetate	Flammable, expecially when being evaporated	Irritating to eyes and respiratory tract	Use an effective fume removal device
n-Hexane	Highly flammable		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. Can react vigorously with sodium or potassium hydroxide plus chloroform	Use an effective fume removal device

NOTE: Peroxides form in diethyl ether, dioxane, and other ethers during storage. They are explosive and have to be destroyed chemically 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

Clean glassware shall be used for residue analysis. Hot detergent solution may be used for cleaning but afterwards the glassware shall be well rinsed with distilled water and acetone before drying. When a washing machine is used, rinse the glassware after use with acetone then with water. Wash it in the machine with a 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 paper, glass wool, etc. are not listed in the apparatus subclause 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 about 15 ml, 80 mm to 90 mm long, are required for final concentrations. These are preferably calibrated and may be fitted with micro-Snyder columns [11].

5.2.2 Chromatographic tubes, specially made and with glass or polytetrafluoroethylene (PTFE) stopcocks 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 or with 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 shall not be used for this purpose. Polytetrafluoroethylene (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 plastic 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 residues should be checked periodically. A keeper (e.g. propylene glycol, *n*-undecane or hexadecane) may be used to minimize losses of pesticides in certain cases.

Solvent evaporators (see 5.4.2 to 5.4.4) may be used for concentrating large solvent volumes. For small volumes, the use of a gentle stream of pure, dry nitrogen is more advisable.

5.4.2 Kuderna-Danish evaporator [12] (or equivalent) with or without fractionating column, which is heated on a thermostable water bath.

5.4.3 Rotary film evaporator (commercially available), which requires a source of vacuum, and can be heated up to a temperature of 50 °C.

5.4.4 Rotary vacuum evaporator (commercially available), rotating at speeds up to 1300 min^{-1}, which requires a source of vacuum and which shall have a thermostable water bath.

5.5 Homogenizers

If homogenizers are used, take care to ensure that they are spark proof and kept free from contamination. Check bottom-drive macerators for leaks around the drive. The various seals can be a source of contamination.