SLOVENSKI STANDARD
SIST EN 1528-3:1998

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Živila, ki vsebujejo maščobe - Določevanje pesticidov in polikloriranih bifenilov (PCB) - 3. del: Metode čiščenja

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

Fettreiche Lebensmittel - Bestimmung von Pestiziden und polychlorierten Biphenylen (PCB) - Teil 3: Reinigungsverfahren

Aliments gras - Dosage des pesticides et des polychlorobiphényles (PCB) - Partie 3: Méthodes de purification

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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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English version

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

Aliments gras - Dosage des pesticides et des polychlorobiphényles (PCB) - Partie 3 : Méthodes de purification

Fettreiche Lebensmittel - Bestimmung von Pestiziden und polychlorierten Biphenylen (PCB) - Teil 3 : Reinigungsverfahren

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CEN

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

Central Secretariat: rue de Stassart,36 B-1050 Brussels

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of an identical text or by endorsement, at the latest by May 1997, and conflicting national standards shall be withdrawn at the latest by May 1997.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

The residues to be analysed in this European Standard are associated with the fat portion of the samples. In addition to the residues, the extracts obtained in accordance with EN 1528-2 : 1996 or in accordance with the following methods contain material including fats and other lipids, which could interfere in the analysis. To purify the crude extracts or the fats and oils to be analysed, several methods may be used.

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® 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]

1 Scope

This Part of EN 1528 specifies 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. The applicable usage of the methods A to H is given in detail in each method described.

NOTE: See also EN 1528-4 which lists a clean-up procedure for the removal of the bulk of lipids when analysing large quantities of fat.

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 1528-1 : 1996
Fatty food - Determination of pesticides and polychlorinated biphenyls (PCBs) - Part 1: General

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

3 Principle

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

4 General

Each liquid/liquid partition shall be performed in a separating funnel with a 2 min shake with occasional release of the pressure by opening the stopcock with the funnel inverted. If vigorous shaking produces very stable emulsions, gentle shaking for longer periods may be preferable. Emulsions may be broken by adding 1 ml to 2 ml of saturated sodium chloride or sodium sulfate solution, by warming under the hot water tap or by centrifuging.

When separating layers, leave any emulsified interface with the portion to be re-extracted or discarded.

The activity of the Florisil® used for column chromatography in this standard shall be checked at regular intervals and, if necessary, shall be properly adjusted as described in the methods concerned.

NOTE 1: Florisil®[1]) is widely used as an adsorbent in residue analysis because of its high capacity for binding lipids. Its activity, however, can vary considerably, depending on the batch of manufacturing and on the shipping and storage conditions.

The rate of elution of chromatographic columns is usually specified but should generally be in the range of 1 ml/min to 5 ml/min.

In the analysis of organochlorine pesticides, at this stage of the procedure the addition of a known quantity of the rather volatile pentachlorobenzene (or 1,7-dibromoheptane) and a less volatile indicator compound (e.g. 1, 2, 3, 4-tetrachloronaphthalene or isodrin[2])) is recommended. Use pentachlorobenzene as an indicator of possible losses of pesticides during the evaporation stage by comparison of its peak area (height) with the peak area (height) of the less volatile indicator compound. An added indicator compound can also be used as an internal standard material for identification (relative retention time) and quantification purposes. With the electron capture detector (ECD), however, coextractives may give peaks (positive and negative) emerging with the same retention time as the internal standard material.

Unless otherwise specified, evaporation of organic solvent solutions should not be allowed to go to complete dryness as this can result in compound losses.

NOTE 2: A bibliography relating to the methods is included (see annex B).

5 Method A: Liquid-liquid partitioning with acetonitrile and clean-up on a Florisil® column (AOAC) [1]

5.1 Applicability

This method has been shown to be applicable for the determination of 17 organochlorine pesticides and metabolites, the PCB indicator congeners and 6 organophosphorus pesticides (in special cases), as given in annex A of EN 1528-1 : 1996.

5.2 Principle

Extraction of the residues, together with the fat, from the sample by one of the procedures described in EN 1528-2 : 1996. Concentration of the extract almost to dryness, redissolving in light petroleum and partitioning of the residues into acetonitrile. After diluting the acetonitrile with an excess of water, partitioning of the residues into light petroleum. Chromatography of the concentrated organic phase on a Florisil® column using light petroleum/diethyl ether for elution. Concentration of the eluates for examination by GC.

5.3 Reagents and materials

All reagents and materials used shall be suitable for the analysis of residues of pesticides and PCBs and shall be in accordance with clause 4 of EN 1528-1 : 1996. If purification is necessary, the procedures given in annex A are appropriate.

5.3.1 Acetonitrile.

5.3.2 Light petroleum, having a boiling range from 40 °C to 60 °C.

5.3.3 Acetonitrile (5.3.1), saturated with light petroleum (5.3.2).

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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.

[2]) 1, 2, 3, 4, 10, 10'-Hexachloro-1, 4, 4', 5, 8, 8'-hexahydro-1,4-endo-5,8-endodimethanonaphthalene

5.3.4 Diethyl ether, peroxide-free. Distil and stabilize with 2,0 % of its volume with absolute ethanol.

5.3.5 Eluting mixture A: light petroleum (5.3.2) and diethyl ether (5.3.4) 94:6 (V/V).

5.3.6 Eluting mixture B: light petroleum (5.3.2) and diethyl ether (5.3.4) 85:15 (V/V).

5.3.7 Eluting mixture C: light petroleum (5.3.2) and diethyl ether (5.3.4) 50:50 (V/V).

5.3.8 Sodium sulfate, granular, anhydrous. Heat at 500 °C or 550 °C for at least 4 h, allow to cool and store in a stoppered bottle.

5.3.9 Florisil®, 150 µm to 250 µm (60 mesh to 100 mesh). Activate by heating at 650 °C for 4 h, immediately transfer into a tightly stoppered container and store in the dark. Before use, heat at 130 °C for at least 5 h and allow to cool in a desiccator.

Test each batch of Florisil® from time to time as follows.

Pass 1 ml of a standard *n*-hexane solution containing 0,1 mg/l each of lindane, heptachlor, aldrin, heptachlor epoxide and dieldrin and 0,3 mg/l of endrin through the adsorption column. Elute and concentrate as described in 5.5.3. Determine the recovery by gas chromatography.

The Florisil® is satisfactory if lindane, heptachlor, aldrin and heptachlor epoxide are eluted quantitatively in the eluting mixture A (5.3.5) and dieldrin and endrin are eluted quantitatively in the eluting mixture B (5.3.6).

5.3.10 Sodium chloride solution, saturated. Before preparing the solution, heat the sodium chloride at 500 °C for at least 4 h.

5.4 Apparatus

Usual laboratory apparatus and, in particular, the following:

5.4.1 Separating funnels, capacity 125 ml and 1000 ml, with ground stoppers and PTFE stop-cocks.

5.4.2 Chromatographic tube, 25 mm outer diameter (o. d.), 50 mm long, with PTFE stop-cock and a sintered glass disk or a glass wool plug.

5.4.3 Chromatographic tube, 22 mm internal diameter (i. d.), 300 mm long, with PTFE stop-cock and a sintered glass disk or a glass wool plug.

5.4.4 Kuderna-Danish evaporator, capacity 500 ml, with attached graduated collection tube, or equivalent.

5.4.5 2-ball-micro-Snyder column, or **micro-Vigreux-column**.

5.5 Procedure

5.5.1 Extraction of fat, pesticides and PCBs

General methods shall be carried out in accordance with EN 1528-2 : 1996.

5.5.2 Acetonitrile/light petroleum partitioning

Weigh up to 3 g of fat into a 125 ml separating funnel (5.4.1) and add light petroleum (5.3.2) so that the total volume of fat and light petroleum is 15 ml. Add 30 ml of acetonitrile saturated with light petroleum (5.3.3), shake vigorously for 1 min, allow to separate and drain the acetonitrile layer into a 1000 ml separating funnel containing 650 ml of water, 40 ml of the saturated sodium chloride solution (5.3.10) and 100 ml of light petroleum (5.3.2). Extract the light petroleum layer in the 125 ml funnel with three additional 30 ml portions ofd acetonitrile saturated with light petroleum (5.3.3) and shake vigorously for 1 min each time.

Combine all extracts in the 1000 ml funnel. Hold the separating funnel in a horizontal position and mix thoroughly for 30 s to 45 s. Allow the layers to separate and drain the aqueous layer into a second 1000 ml separating funnel. Add 100 ml of light petroleum to the second funnel, shake vigorously for 15 s and allow to separate. Discard the aqueous layer, combine the light petroleum layer with that in the original separating funnel and wash with two 100 ml portions of water. Discard the washings and draw off the light petroleum layer through a chromatographic tube (5.4.2) containing anhydrous sodium sulfate (5.3.8) into a 500 ml Kuderna-Danish evaporator (5.4.4). Rinse the funnel and then the column with three portions of about 10 ml of light petroleum. Evaporate the combined extracts and rinses to about 10 ml in a Kuderna-Danish evaporator (5.4.4) for transfer to the Florisil® column.

5.5.3 Florisil® column chromatography

Prepare the chromatographic column (5.4.3) containing 10 cm, after settling, of activated Florisil® (5.3.9), topped with 1 cm anhydrous sodium sulfate. Pre-wet the column with 40 ml to 50 ml of light petroleum. Place a Kuderna-Danish evaporator (5.4.4) fitted with a graduated collection tube under the column to collect the eluate. Transfer the solution derived from 5.5.2 on to the column, letting it pass through at not more than 5 ml/min. Rinse the container twice with 5 ml portions of light petroleum, pour the rinsings on to the column, rinse the walls of the tube with additional small portions of light petroleum and elute at about 5 ml/min with 200 ml of eluting mixture A (5.3.5). Change the receivers and elute with 200 ml of eluting mixture B (5.3.6) at 5 ml/min. Change the receivers and elute with 200 ml of eluting mixture C (5.3.7) at 5 ml/min.

Concentrate each eluate separately to a suitable volume in the Kuderna-Danish evaporator (5.4.4). If a volume of less than 5 ml is required, use a 2-ball micro-Snyder or micro-Vigreux column (5.4.5). Should further clean-up be necessary, carry this out on a second, freshly prepared Florisil® column.

The first eluate contains the organochlorine compounds listed in annex A of EN 1528-1 : 1996, except dieldrin and endrin which are contained in the second eluate. Organophosphorus compounds could occur in all three eluates.

5.6 Determination

Determination shall be carried out in accordance with clause 7 of EN 1528-1 : 1996 and clause 4 of EN 1528-4 : 1996.

5.7 Evaluation of results

The results shall be evaluated in accordance with clauses 9 to 11 of EN 1528-1 : 1996.

5.8 Test report

The results of the tests shall be reported in accordance with clause 12 of EN 1528-1 : 1996.

6 Method B: Liquid-liquid partitioning with dimethylformamide and clean-up on a Florisil® column (Specht) [2]

6.1 Applicability

This method has been shown to be applicable for the determination of 18 organochlorine pesticides and metabolites, the PCB indicator congeners and 7 organophosphorus pesticides, as given in annex A of EN 1528-1 : 1996. It is suitable for the determination of very low residue concentrations because very well purified extracts are obtained. However, the rigorous clean-up procedure takes a correspondingly long time to complete.

6.2 Principle

Extraction of the residues, together with the fat, from the sample by one of the procedures described in EN 1528-2 : 1996. Concentration of the extract almost to dryness, redissolving in light petroleum and partitioning of the residues into dimethylformamide. After addition of sodium sulfate solution, further partitioning of the residues into light petroleum. Chromatography of the concentrated organic phase on a Florisil® column, using elution with light petroleum/diethyl ether for the organochlorine compounds and with light petroleum/ethyl acetate for the organophosphorus pesticides. Concentration of the eluates for examination by GC.

6.3 Reagents and materials

All reagents and materials used shall be suitable for the analysis of residues of pesticides and PCBs and shall be in accordance with clause 4 of EN 1528-1 : 1996. If purification is necessary, the procedures given in annex A are appropriate.

6.3.1 Light petroleum, having a boiling range from 40 °C to 60 °C.

6.3.2 Light petroleum (6.3.1), saturated with dimethylformamide (6.3.3).

6.3.3 Dimethylformamide.

6.3.4 Dimethylformamide (6.3.3), saturated with light petroleum (6.3.1).

6.3.5 Diethyl ether, peroxide-free.

6.3.6 Ethyl acetate.

6.3.7 *n*-hexane.

6.3.8 Eluting mixture I: light petroleum (6.3.1) and diethyl ether (6.3.5) 94:6 (V/V).

6.3.9 Eluting mixture II: light petroleum (6.3.1) and ethyl acetate (6.3.6) 6:4 (V/V).

6.3.10 Florisil®, 150 μm to 250 μm (≙ 60 mesh to 100 mesh).

Heat at 550 °C for at least 2 h, allow to cool, and store in a tightly stoppered container. Prior to use, heat the Florisil® at 130 °C for at least 5 h, allow to cool in a desiccator, and then add five parts of distilled water to 95 parts of the adsorbent (m/m). Shake this mixture for at least 20 min, and then store it in a tightly stoppered container for at least 10 h.

6.3.11 Sodium sulfate, anhydrous, heated at 550 °C for at least 2 h.

6.3.12 Sodium sulfate solution, 2 g/100 ml.

6.4 Apparatus

Usual laboratory apparatus and, in particular, the following:

6.4.1 Chromatographic tube, 20 mm i. d., 40 cm to 50 cm long, with PTFE stopcock and a sintered glass disk.

6.4.2 Rotary evaporator with evaporation flasks of capacity 500 ml and a waterbath capable of being controlled between 20 °C and 50 °C.

6.4.3 Separating funnels, capacity 500 ml and 250 ml, with ground stoppers and PTFE stop-cocks.

6.5 Procedure

6.5.1 Extraction of fat, pesticides and PCBs

General methods shall be carried out in accordance with EN 1528-2 : 1996.

6.5.2 Dimethylformamide/light petroleum partitioning

Dissolve the fat or oil or the sample extract (containing 2 g to 5 g of fat) in 25 ml of light petroleum saturated with dimethylformamide (6.3.2) and transfer the solution to a 250 ml separating funnel (6.4.3). Rinse the sample container with small portions of a previously measured volume of 75 ml of dimethylformamide (6.3.4). Add the remainder of the dimethylformamide to the separating funnel, and shake vigorously for 1 min. Drain the dimethylformamide phase, and again extract the light petroleum phase with 10 ml of dimethylformamide (6.3.4).

Transfer the combined dimethylformamide phases to a 500 ml separating funnel, and add 200 ml of sodium sulfate solution (6.3.12). Extract successively with one 40 ml and three 25 ml aliquot portions of light petroleum (6.3.2) for 1 min each time. Wash the combined light petroleum phases with 10 ml of water, dry over sodium sulfate (6.3.11), filter through a cotton wool plug, add 5 ml of *n*-hexane, and concentrate in a rotary evaporator to approximately 5 ml.

6.5.3 Florisil® column chromatography

About half-fill the chromatographic tube containing a sintered glass disk (6.4.1) or a glass wool plug with light petroleum, and sprinkle in 30 g of Florisil® (6.3.10) in small portions through a funnel, keeping the stop-cock partly open and gently tapping the column. Use only columns free from visible inclusions of air. Cover the Florisil® with an approximately 2 cm layer of sodium sulfate and allow the light petroleum to drain to 2 mm above the top of the column packing.

Transfer the solution derived from 6.5.2 on to the column. Allow the solution to percolate to a level of 1 mm to 2 mm above the top of the column. Rinse the flask with small portions of a previously measured volume of 200 ml of eluting mixture I (6.3.8), add the rinsings to the column, and also let them percolate to a level of 1 mm to 2 mm above the top of the column. Elute the column with the remainder of the total 200 ml aliquot portion of eluting mixture I, at a flow rate of approximately 5 ml/min. Add 5 ml of *n*-hexane to the eluate, concentrate it in a rotary evaporator to approximately 5 ml, quantitatively rinse with *n*-hexane into a volumetric flask or graduated test tube, and dilute to a definite volume, e.g. 10 ml, with *n*-hexane (eluate I).

If, in addition to the organochlorine compounds, it is required also to determine the organophosphorus pesticides, stop the elution just before the last traces of eluting mixture I have entered into the column packing.

Change the receiver, and continue to elute with 300 ml of eluting mixture II (6.3.9). Concentrate the second eluate in a rotary evaporator to approximately 5 ml, quantitatively rinse with ethyl acetate into a volumetric flask or graduated test tube, and dilute to a definite volume, e.g. 10 ml, with ethyl acetate (eluate II).

6.6 Determination

Determination shall be carried out in accordance with clause 7 of EN 1528-1 : 1996 and clause 4 of EN 1528-4 : 1996.

6.7 Evaluation of results

The results shall be evaluated in accordance with clauses 9 to 11 of EN 1528-1 : 1996.

6.8 Test report

The results of the tests shall be reported in accordance with clause 12 of EN 1528-1 : 1996.

7 Method C: Column chromatography on activated Florisil® (AOAC) [3]

7.1 Applicability

This method has been shown to be applicable for the determination of 4 organochlorine pesticides and metabolites (p, p'-TDE, p, p'-DDE, p, p'-DDT and dieldrin) and the PCB indicator congeners in fish samples, as given in annex A of EN 1528-1 : 1996.

7.2 Principle

Extraction of the residues, together with the fat, from the fish sample with light petroleum, concentration of the extract to a small volume. Chromatography of the solution on a Florisil® column using light petroleum/diethyl ether for elution. Concentration of the eluate for examination by GC.

7.3 Reagents and materials

All reagents and materials used shall be suitable for the analysis of residues of pesticides and PCBs and shall be in accordance with clause 4 of EN 1528-1 : 1996. If purification is necessary, the procedures given in annex A are appropriate.

7.3.1 Light petroleum, having a boiling range from 40 °C to 60 °C.

7.3.2 Diethyl ether, peroxide-free. Distil and stabilize with 2,0 % of its volume with absolute ethanol.

7.3.3 Eluting mixture A: light petroleum (7.3.1) and diethyl ether (7.3.2) 94:6 (V/V).

7.3.4 Eluting mixture B: light petroleum (7.3.1) and diethyl ether (7.3.2) 85:15 (V/V).

7.3.5 Sodium sulfate, granular, anhydrous, heated at 550 °C for at least 2 h.

7.3.6 Florisil®, 190 µm to 250 µm (≙ 60 mesh to 80 mesh).

Activate by heating at 650 °C for 4 h, immediately transfer into a tightly stoppered container and store in the dark. Before use, heat at 130 °C for at least 5 h and allow to cool in a desiccator.

Test each batch of Florisil® from time to time as follows.

Pass 1 ml of a standard *n*-hexane solution containing 0,1 mg/l each of lindane, heptachlor, aldrin, heptachlor epoxide and dieldrin and 0,3 mg/l of endrin through the adsorption column (7.5.2). Elute and concentrate as described in 7.5.2. Determine the recovery by gas chromatography.

The Florisil® is satisfactory if lindane, heptachlor, aldrin and heptachlor epoxide are eluted quantitatively in the eluting mixture A (7.3.3) and dieldrin and endrin are eluted quantitatively in the eluting mixture B (7.3.4).

7.3.7 Boiling aid, e.g. carborundum.

7.4 Apparatus

Usual laboratory apparatus and, in particular, the following:

7.4.1 Chromatographic tube, 10 mm i. d., 30 cm long, with PTFE stop-cock and a coarse fritted disk.

7.4.2 Kuderna-Danish evaporator, capacity 125 ml, with attached graduated 10 ml collection tube, or equivalent.

7.4.3 High speed blender, fitted with a leak-proof glass jar and an explosion proof motor or homogenizer.

7.4.4 Centrifuge, explosion proof, provided with glass tubes or blender cups of capacity about 200 ml, in which the tubes can be spun at rotational frequencies of about 1000 min^{-1} to 3000 min^{-1}.

7.4.5 Snyder column

7.5 Procedure

7.5.1 Extraction of fat, pesticides and PCBs

Weigh 20 g of the thoroughly ground and mixed sample into the blender cup. Moisten 40 g of sodium sulfate (7.3.5) with light petroleum (7.3.1) and add to the sample. Mix the sample, using a stirring rod, let it stand for 20 min, and mix again. Add 100 ml of light petroleum to the sample and blend for 1 min to 2 min. Centrifuge the balanced sample cups for 1 min to 2 min at approximately 2000 min^{-1} to obtain a clear light petroleum extract. Place a glass wool plug in a funnel, overlay it with 20 g of sodium sulfate, and place the funnel in a 250 ml volumetric flask. Decant the light petroleum extract through the sodium sulfate into the volumetric flask. Mix the sample again with a stirring rod, add 100 ml of light petroleum, and extract as before. Dilute to the 250 ml volume mark with light petroleum. Transfer a 25 ml aliquot portion to a tared 100 ml flat-bottom flask. Place the flask on the boiling water bath, remove the solvent and allow to cool. Weigh the flask and determine the concentration of the fat in the fish sample.

For fish containing less than 10 % of fat, transfer a 25 ml aliquot portion of the 250 ml volumetric flask to a 125 ml Kuderna-Danish evaporation flask. For fish containing more than 10 % fat, take an aliquot portion containing not more than 200 mg fat. Add several granules of a boiling aid (7.3.7) and concentrate to approximately 3 ml on the boiling water bath. Allow it to cool and remove the Snyder column (7.4.5). Rinse the evaporation flask with two 1 ml portions of light petroleum and, using only air current, concentrate the sample to 3 ml ready for transfer to the Florisil® column.

7.5.2 Florisil® column chromatography

To the chromatographic tube (7.4.1), add 4 g of Florisil® (7.3.6) and top up with a 2 cm layer of sodium sulfate (7.3.5). Mark the column 1 cm above the sodium sulfate layer and pre-wet with 20 ml to 25 ml of light petroleum (7.3.1).

As the solvent level reaches the mark, place a 125 ml evaporation flask (7.4.2) under the column. Transfer the solution derived from 7.5.1 onto the column (7.3.4), wash the tube with 1 ml of light petroleum and add wash to the column. Ensure the solvent level does not go below the mark. Temporarily close the stop-cock if necessary. Add 35 ml of eluting mixture A (7.3.3) and elute the PCBs and DDT and its analogs.

When the solvent level reaches the mark, change the evaporation flask, and add 35 ml of eluting mixture B (7.3.4) to elute compounds such as dieldrin and endrin. Add several granules of boiling aid to each evaporation flask, attach the Snyder column (7.4.5), and carefully concentrate it on the water bath. Allow the evaporation flask to cool, remove the Snyder column and evaporate the solvent under a gentle stream of nitrogen to an appropriate volume for GC determination. Fractions containing mixtures of PCBs and chlorinated compounds such as DDE may require additional separation techniques.

7.6 Determination

Determination shall be carried out in accordance with clause 7 of EN 1528-1 : 1996 and clause 4 of EN 1528-4 : 1996.

7.7 Evaluation of results

The results shall be evaluated in accordance with clauses 9 to 11 of EN 1528-1 : 1996.

7.8 Test report

The results of the tests shall be reported in accordance with clause 12 of EN 1528-1 : 1996.