SLOVENSKI STANDARD
SIST EN 1528-4:1998

01-november-1998

Živila, ki vsebujejo maščobe - Določevanje pesticidov in polikloriranih bifenilov (PCB) - Določevanje, potrditveni preskusi, drugo

Fatty food - Determination of pesticides and polychlorinated biphenyls (PCBs) - Determination, confirmatory tests, miscellaneous

Fettreiche Lebensmittel - Bestimmung von Pestiziden und polychlorierten Biphenylen (PCB) - Teil 4: Verfahren zur Bestimmung und Absicherung, Verschiedenes

Aliments gras - Dosage des pesticides et des polychlorobiphényles (PCB) - Partie 4: Détermination, essais de confirmation, divers

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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 4 : Determination, confirmatory tests, miscellaneous

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Fettreiche Lebensmittel - Bestimmung von Pestiziden und polychlorierten Biphenylen (PCB) - Teil 4 : Verfahren zur Bestimmung und Absicherung, Verschiedenes

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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of an identical text or by endorsement, at the latest by May 1997, and conflicting national standards shall be withdrawn at the latest by May 1997.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 Part of EN 1528 gives guidance on some recommended techniques for the determination of pesticides and polychlorinated biphenyls (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.

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

3 General

The methods described in this Part of EN 1528 permit the residues present to be provisionally identified and quantified, by gas chromatographic methods using selective detectors.

All positive results require confirmation of identity and quantity.

The procedures listed for confirmation such as alternative GC columns, alternative GC detectors, thin-layer chromatography (TLC), high performance liquid chromatography (HPLC), column fractionation, derivatization, spectral measurements, etc., are all of value. Results obtained using mass spectrometry (MS) present definitive evidence for confirmation/identification purposes.

4 Determination

4.1 Gas chromatography

4.1.1 General

A suitable GC system, preferably equipped with separate heaters for injector, detector and column ovens, should be used. Although the choice of the different parts of the GC system is a matter for the experience of the analyst, the following general recommendations are made.

The detectors should be properly adjusted, according to the manufacturers' instructions. Variations in detector sensitivity should be checked periodically by verifying the linearity of the calibration curves using standard solutions of pesticides.

The quantification unit of the gas chromatographic system needs to include an integration system which permits the calculation not only of peak heights but also of peak areas.

It has been found in practice that equivalent results can be achieved despite the adoption of different GC conditions, and different makes of instruments. On the other hand, specifying standard GC parameters does not in any way guarantee that the quality of the results generated will be identical.

For typical GC conditions, see annex B.

4.1.2 Columns

Either packed or capillary columns may be used.

When packed columns are to be used, then glass columns of lengths between 1,5 m and 3 m and of internal diameter (i. d.) 2 mm to 6 mm, are recommended, however, they are not suitable for the separation of PCB congeners.

A robust, inert support should be used: materials such as Gaschrom Q, Chromosorb W/HP, Anachrom Q in 125 μm to 150 μm (100 to 120 mesh), 150 μm to 190 μm (80 mesh to 100 mesh) or 190 μm to 250 μm (60 to 80 mesh) ranges have been successfully employed.[1])

A variety of stationary phases and stationary phase mixtures have been used successfully for a variety of residue analyses. For example, the following types are most frequently used:

Hydrocarbon:	Apiezon L
Methylsilicones:	DC-11, DC-200, OV-1, OV-101, SP-2100, SE-30
Methylphenylsilicones:	OV-17, OV-25, OV-61, SP-2250, SE-52, SE-54
Trifluoropropylmethylsilicones:	QF-1, OV-210, SF-2401
Phenylcyanopropylmethylsilicones:	DB-1301, DB-1701, OV-225, XE-60
Polyethylene glycol:	Carbowax 20 M[1]).

Stationary phases should be coated onto the support with care, the ratio depending on the support/phase combination chosen. Newly filled columns should be conditioned for at least 24 h at a temperature near the maximum recommended operating temperature with the type of stationary phase used and should then be tested for their efficiency and selectivity at the required operating temperature using standard mixtures of pesticides. The end of the column should always be disconnected from the detector during conditioning.

Pure, dry nitrogen (oxygen-free, especially when using an electron capture detector (ECD)), or an argon/methane mixture (in the case of a pulsed ECD), should be used as carrier gas for packed columns. The flow rate depends on the size and type of column used. Generally, gas flow rates should be controlled as accurately as possible. Molecular sieve filters should be installed for all gas supplies and regenerated regularly.

Finally, GC conditions (column length, stationary phase type, injector, detector and column temperatures, gas flow rates, etc.) should be such that the separation of the pesticides and PCBs likely to be present is as complete as possible.

Capillary GC has a separation power superior to that of packed columns. This technique is recommended especially in the case of complex extracts.

Fused silica columns having an internal diameter of 0,20 mm to 0,35 mm and a length of between 20 m and 60 m have proved particularly satisfactory because of their separation efficiency, service life and mechanical properties. Wide-bore columns having an internal diameter of 0,5 mm to 0,8 mm may also be useful in some cases. The following stationary phases are frequently used as coatings:

SE-30	(equivalent to OV-1, DB-1, CP Sil 5, BP-1, SPB-1, etc.);
SE-54	(equivalent to DB-5, CP Sil 8, BP-5, SPB-5, etc);
OV-17	(equivalent to OV-11, OV-22, SP-2250, DC-710, DB 608, etc.);
DB 1301	(equivalent to DB-624);
DB-1701	(equivalent to OV-1701, CP Sil 19-CB, BP-10, SPB-7, etc.);
OV 225	(equivalent to DB-225, SIL 43-CB, SPB-2330, etc.);
WAX	(equivalent to DB-WAX, CP-WAX-52-CB, Carbowax 20 M, etc.)[2]).

A test for separation efficiency of capillary columns, is given in 7.2 of EN 1528-1 : 1996.

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[1]) Gaschrom Q, Chromosorb W/HP, Anachrom Q, Apiezon L, DC-11 ... Carbowax 20 M are examples of suitable products available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of these products.

[2]) SE-30 ... Carbowax 20 M are examples of suitable products available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of these products.

4.1.3 Injection techniques

Various injection techniques are useful such as:

a) Grob splitless injection

b) On-column injection

c) Programmed Temperature Vaporization (PTV) injection

The applicability of these techniques depends on the apparatus used and on special requirements.

4.2 Preliminary tests

Determine the linear dynamic range of detector response under the actual GC conditions used by injecting dilute standard solutions.

Inject into the gas chromatograph an appropriate volume (between 1,0 µl and 10 µl, depending on the system) of the purified extracts obtained according to the analytical method used. The chromatogram so obtained should enable both the identity and the approximate concentration of the compounds present in the extracts to be established.

4.3 Determination

Make sure that all measurements are performed within the linear dynamic range of the system.

Prepare at least two standard solutions of the pesticides or PCB congeners identified in the solvent to be used for the final extract (usually light petroleum or *n*-hexane). Their concentrations should encompass the probable concentration expected in the final extracts. Then inject equal volumes of the final extracts obtained and of the two or more standard solutions into the gas chromatograph. It is essential that the injection of the purified portions of the sample extracts is preceded and followed by injection of the standard solutions.

Measure the peak areas or peak heights. The results obtained from any two injections of the same standard solution should not differ more than approximately 5 % from each other. Inclusion of an internal standard is useful (see clause 4 of EN 1528-3 : 1996).

It is necessary to ensure that the standard materials and samples are dissolved in the same solvent, otherwise varying evaporation profiles will result which could lead to changes in the retention times and peak areas or heights. For example, increases in peak heights of 35 % have been observed for PCB congeners on changing from iso-octane to toluene.

The contents of individual PCB congeners should not be added together to obtain the total PCBs since such a value is meaningless. There is also no point in carrying out other extrapolations to a fictitious total content of PCBs (e.g. calculated as Clophen® A 60[3]) since these are generally based on the incorrect assumption that the PCB distribution pattern in the sample is exactly the same as that of the industrial PCB commercial product.

A determination is only possible if the mean of recoveries from multiple determinations for the substance concerned is in the range 70 % to 110 % for individual determinations. Compliance with this condition has to be checked periodically by repeated measurements of recovery from samples containing known additions of the relevant standard material.

5 Confirmatory tests [1]

5.1 General

When analyses are performed for regulatory purposes it is especially important that confirmatory tests are carried out before reporting adversely on samples containing residues of pesticides not usually associated with that commodity or where maximum residue limits (MRLs) appear to have been exceeded. Contamination of samples with non-pesticidal chemicals occurs from time to time and in some chromatographic methods these compounds can have similar properties to pesticides and could therefore be misidentified as such. Examples in gas chromatography include the responses of ECD to phthalate esters and of phosphorus-specific detectors to compounds containing sulfur.

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[3]) Clophen® A 60 is an example of a mixture of PCBs that were formerly available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product.

Confirmatory tests can be divided into two types: quantitative tests are necessary when MRLs appear to be exceeded whilst qualitative confirmation of identity is also needed in these cases, and when atypical residues are encountered. Qualitative tests can involve chemical reactions or separations where some loss of the residue occurs. Particular problems occur in confirmation when MRLs are set at or about the limit of determination.

The need for confirmatory tests can depend upon the type of sample or its known history. In many substrates, certain residues are nearly always found. For a series of samples of similar origin it could only be necessary to confirm the identity of residues in the initial samples. Similarly, when it is known that a particular pesticide has been applied to the sample material there could be little need for confirmation of identity, although a random proportion of samples should be confirmed. Where control samples are available, these should be used to check the presence of possible interfering substances.

In quantitative confirmation at least one alternative procedure should be used and the lower result reported. In qualitative confirmation, an alternative technique using different physiochemical properties is desirable.

The necessary steps to positive identification are a matter of judgement for the analyst and particular attention should be paid to the choice of a method which will eliminate the effect of interfering compounds. The chosen method will depend upon the availability of suitable apparatus and expertise within the testing laboratory.

As guidance to the analyst a number of alternative procedures for confirmation are given in 5.2 to 5.9.

5.2 Alternative GC columns

The results obtained in the primary analysis should be quantitatively and qualitatively confirmed using at least one alternative column containing a stationary phase of different polarity. The quantitative results obtained should be within 20 % of the primary analysis and the lower figure should be reported, since the higher figure could have been enhanced by interference from co-extracted material. Further quantitative confirmation is required if the results differ by more than 20 %, except when the MRL is set at or about the limit of determination when a variation of up to 100 % would be acceptable.

In choosing the alternative column material, consideration should be given to separating any other pesticide or PCB residues or interfering compounds known to have retention times on the primary column identical to that of the residue detected. The alternative column may be a packed column or, preferably, a capillary column whose differing resolving power can be utilized. Whilst the use of an alternative gas-chromatographic column might not always give positive confirmation it will often quickly disprove a suspected identity. In either case further confirmation is required to identify the residue.

5.3 Alternative GC detectors

When pesticides containing several chemical elements are present, detectors showing enhanced response to these elements may be used. Detectors such as flame photometric (sulfur, phosphorus and tin), alkali flame ionization (phosphorus and nitrogen) and coulometric/conductivity (nitrogen, sulfur and halogens) can give valuable additional information on residues. The sulfur/phosphorus response ratio obtained by using a flame photometric detector can give useful information in the case of phosphorothioates.

5.4 Thin-layer chromatography (TLC)

In some instances, confirmation of gas chromatographic findings is most conveniently achieved by TLC. Identification is based on two criteria, Rf value and visualization reaction. The scientific literature contains numerous references to the technique. A IUPAC Report on Pesticides [2] reviews the technique and serves as a convenient introduction. The quantitative aspects of thin-layer chromatography are, however, limited. A further extension of this technique involves the removal of the area on the plate corresponding to the Rf of the compound of interest followed by elution from the layer material and further chemical or physical confirmatory analysis.

A solution of the standard pesticide should always be spotted on the plate alongside the sample extract to obviate any problems of non-repeatability of Rf. Over-spotting of extract with standard pesticide can also give useful information. The advantages of TLC are speed, low cost and applicability to heat sensitive materials; disadvantages include (usually) lower sensitivity than GC and frequent need for a more efficient clean-up. In some countries problems can be encountered when high humidity or high temperature cause lack of repeatability.

5.5 High performance liquid chromatography (HPLC)

HPLC can often be used advantageously for the confirmation of residues initially found by gas chromatography or by other techniques and can be in certain circumstances the preferred quantitative technique. Post- or pre-column derivatization, and/or use of different detectors, are further options available to the analyst, especially when heat-sensitivity or low volatility make the compound to be analysed less amenable to gas chromatography.

5.6 Column fractionation

The order of elution from chromatographic columns used for cleaning up sample extracts can help to verify the identity of a compound. Thus an element of confirmation can be built in to the extraction and clean-up procedure.

5.7 Derivatization

5.7.1 Chemical reactions

Small scale chemical reactions resulting in degradation, addition or condensation products of pesticides, followed by re-examination of the products by chromatographic techniques, have frequently been used. The reactions result in products possessing different retention times and/or detector response from those of the parent compound. A sample of standard pesticide should be treated alongside the suspected residue so that the results from each can be directly compared. A fortified extract should also be included to prove that the reaction has proceeded in the presence of co-extracted sample material. A review of chemical reactions which have been used for confirmatory purposes has been published [3]. Chemical reactions have the advantages of being fast and easy to carry out, but it is possible that specialized reagents will need to be purchased and/or purified.

5.7.2 Physical reactions

A useful technique is the photochemical alteration of a pesticide residue to give one or more products with a reproducible chromatographic pattern [4]. A sample of standard pesticide and fortified extract should always be treated in an exactly similar manner. Samples containing more than one pesticide residue can give problems in the interpretation of results. In such cases pre-separation of specific residues may be carried out using TLC, HPLC or column fractionation prior to reaction.

5.7.3 Other methods

Many pesticides are susceptible to degradation/transformation by enzymes. In contrast to normal chemical reactions, these processes are very specific and generally consist of oxidation, hydrolysis or de-alkylation [5]. The products possess different chromatographic characteristics from the parent pesticide and may be used for confirmatory purposes if compared with reaction products using standard pesticides.

5.8 Mass spectrometry (MS)

Results obtained using MS present definitive evidence for confirmation/identification purposes [6], [7]. Where the apparatus is available it is usually the confirmatory technique of choice. There are two principal methods of introducing samples into the instrument. The preferred method utilizes gas chromatographic separation prior to introduction into the mass spectrometer. This allows full mass spectral analysis of the peak observed during the primary analysis. Alternatively, samples may be introduced using the direct insertion probe technique. This method may be used in conjunction with TLC or HPLC when these have been used as initial confirmatory procedures. Residues separated by these techniques are isolated and subjected to MS.

To increase sensitivity, particularly with fast scanning quadrupole instruments, techniques known as single and multiple ion detection have been used. A sufficient number of fragment ions have to be selected to ensure unambiguous identification. Increased sensitivity with respect to the molecular ion can be obtained by using chemical ionization in place of electron-impact. As mass spectrometers are generally sensitive at the nanogram level some extracts from primary gas chromatographic analysis might require concentration before mass spectrometric analysis, particularly when electron-capture detectors have been used for quantification. In some cases additional clean-up will be necessary, particularly if full spectra are to be obtained.

Problems can be encountered with heat-sensitive compounds during MS and particular care has to be taken when coupling gas chromatographs to mass spectrometers. As there is almost no differential response to compounds in MS complications can arise in the presence of co-eluting contaminants.