SLOVENSKI STANDARD
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Živila brez maščob - Multirezidualne metode za določevanje ostankov pesticidov s plinsko kromatografijo - 3. del: Določevanje in potrditveni preskusi

Non-fatty foods - Multiresidue methods for the gas chromatographic determination of pesticide residues - Part 3: Determination and confirmatory tests

Fettarme Lebensmittel - Multiverfahren zur gaschromatographischen Bestimmung von Pestizidrückständen - Teil 3: Verfahren zur Bestimmung und Absicherung

Aliments non gras - Méthodes multirésidus de détermination par chromatographie en phase gazeuse de résidus de pesticides - Partie 3: Détermination et essais de confirmation

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Non-fatty foods - Multiresidue methods for the gas chromatographic determination of pesticide residues - Part 3: Determination and confirmatory tests

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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.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
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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of an identical text or by endorsement, at the latest by April 1999, and conflicting national standards shall be withdrawn at the latest by April 1999.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 European Standard 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 confirmations tests" gives some recommended techniques for the qualitative and the 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 [1] are appropriate to improve method performance, see clause 3.

1 Scope

This European Standard gives guidance on some recommended techniques for the determination of pesticide residues in non-fatty foods and on confirmatory tests.

The identity of any observed pesticide residue is confirmed, particularly in those cases in which it would appear that the maximum residue limit has been exceeded.

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 12393-1 1998 Non-fatty foods - Multiresidue methods for the gas chromatographic determination of pesticide residues - Part 1: General considerations

3 General

The methods described in this European Standard permit the residues present provisionally to be 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 the most definitive evidence for confirmation/identification purpose.

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.

4 Determination

4.1 Gas chromatography (GC)

4.1.1 General

A suitable GC system, preferably equipped with separate heaters for injector, detector and column ovens, shall be used. The facility to inject directly on the GC column is generally of advantage. 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 apparatus should include an integration system which permits the calculation not only of peak heights but also 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 guarantee that the quality of the results generated will be identical.

For typical GC conditions, see annex A.

4.1.2 GC columns

4.1.2.1 General

Either packed or capillary columns can be used. Capillary columns have a separation power superior to that of packed columns. The capillary technique is recommended especially in the case of complex extracts.

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 (oxygen-free) and dry (water-free) nitrogen, 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, ensure that gas flow rates are controlled as accurately as possible. Molecular sieve filters should be installed for all gas supplies and regenerated regularly.

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

4.1.2.2 Packed columns

Glass columns, of length between 1 m and 3 m and of internal diameter (i.d.) 2 mm to 6 mm, are recommended.

A robust, inert support should be used: materials such as Gaschrom Q®, Chromosorb W/HP®, Anachrom Q® in 60 mesh/80 mesh (190 µm to 250 µm), 80 mesh/100 mesh (150 µm to 190 µm) and 100 mesh/120 mesh (125 µm to 150 µm) 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[2]
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[2]

Stationary phases should be coated onto the support with care, the ratio depending on the support/phase combination chosen.

4.1.2.3 Capillary columns

Fused silica columns having an internal diameter of 0,20 mm to 0,35 mm and a length of between 10 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[3]	(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 etc.)
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, Wax-52-CB, Carbowax 20M[2] etc.)

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.

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[1] Gaschrom Q®, Chromosorb W/HP® , Anachrom Q®, 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.

[2] Apiezon L, ..., Carbowax 20M 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.

[3] SE-30... Carbowax 20M 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.

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

4.1.4 Detectors

See 3.4 in EN 12393-1:1998.

4.2 Preliminary tests

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

Inject into the gas chromatograph an appropriate volume (between 1,0 µl and 10,0 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 the measurements are performed within the linear dynamic range of the system. Prepare at least two standard solutions of the identified pesticide in the same solvent as used in the final extract. Their concentrations should encompass the probable concentration expected in the final extract. 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 injections of the purified portions of the sample extracts are preceded and followed by injection of the standard solutions.

Measure the peak areas or peak heights. The results obtained from any two successive injections of the same standard solution should not differ more than approximately 10 % from each other. Inclusion of an internal standard is useful.

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.

A quantitative determination is possible if the mean of recoveries from replicate determinations falls within the range of 70 % up to 110 %, with a relative standard deviation less or equal to 20 %. 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

5.1 General

When analyses are performed for regulatory purposes, it is especially important that confirmatory tests for identification and quantification of the residues are carried out before reporting adversely on samples containing residues of pesticides not usually associated with that commodity or where MRLs appear to have been exceeded [2]. 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 electron-capture detectors to phthalate esters and of phosphorus specific detectors to compounds containing sulfur.

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 may 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, it is not necessary to confirm the 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 can 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 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.