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SWEDISH STANDARDS INSTITUTE

Foods of plant origin – Multiresidue methods for the determination of pesticide residues by GC or LC-MS/MS – Part 3: Determination and confirmatory tests

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Europastandarden EN 12393-3:2013 gäller som svensk standard. Detta dokument innehåller den officiella engelska versionen av EN 12393-3:2013.

Denna standard ersätter SS-EN 12393-3:2008, utgåva 2.

The European Standard EN 12393-3:2013 has the status of a Swedish Standard. This document contains the official version of EN 12393-3:2013.

This standard supersedes the Swedish Standard SS-EN 12393-3:2008, edition 2.

Upplysningar om sakinnehållet i standarden lämnas av SIS, Swedish Standards Institute, telefon 08-555 520 00. Standarder kan beställas hos SIS Förlag AB som även lämnar allmänna upplysningar om svensk och utländsk standard.

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Denna standard är framtagen av kommittén för Livsmedel och foder, SIS/TK 435.

Har du synpunkter på innehållet i den här standarden, vill du delta i ett kommande revideringsarbete eller vara med och ta fram andra standarder inom området? Gå in på www.sis.se - där hittar du mer information.

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 12393-3

November 2013

ICS 67.050

Supersedes EN 12393-3:2008

English Version

Foods of plant origin - Multiresidue methods for the determination of pesticide residues by GC or LC-MS/MS - Part 3: Determination and confirmatory tests

Aliments d'origine végétale - Méthodes multirésidus de détermination de résidus de pesticides par CPG ou CL-SM/SM - Partie 3: Détermination et essais de confirmation

Pflanzliche Lebensmittel - Multiverfahren zur Bestimmung von Pestizidrückständen mit GC oder LC-MS/MS - Teil 3: Verfahren zur Bestimmung und Absicherung

This European Standard was approved by CEN on 21 September 2013.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

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 CEN-CENELEC Management Centre has the same status as the official versions.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

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Foreword

This document (EN 12393-3:2013) 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 2014, and conflicting national standards shall be withdrawn at the latest by May 2014.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN 12393-3:2008.

This document will supersede EN 12393-3:2008 with the following significant technical changes:

a) introduction of the LC-MS/MS as a recommended technique for the determination of pesticide residues;

b) deletion of method L as no longer in use;

c) deletion of old Annex B with considerations concerning MS confirmation;

d) addition of a new Annex B with suitable GC-MS/MS operating conditions;

e) addition of new Annex C with typical LC-MS/MS operating conditions.

EN 12393, *Foods of plant origin — Multiresidue methods for the determination of pesticide residues by GC or LC-MS/MS*" is divided into 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 M, N and 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 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, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey 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 European 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 Clause 3.

1 Scope

This European Standard gives guidance on some recommended techniques for the determination of pesticide residues in foods of plant origin 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

Not applicable.

3 General

The methods specified in this European Standard permit identification and quantification of pesticide residues by gas chromatography using selective detectors or liquid chromatography with tandem-mass spectrometric detector (LC-MS/MS).

All relevant results require confirmation of identity and quantity.

The procedures listed for confirmation such as alternative GC columns, alternative GC detectors, high-performance liquid chromatography (HPLC), column fractionation, derivatisation, 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 General

4.1.1 Identification

A number of parameters can be employed to determine the identity of an analyte present in the sample extract. This includes:

a) retention time of the analyte in question (RT) or, even better, the retention time ratio against the ISTD (Rt(A)/Rt(ISTD)) obtained from the same run (the simultaneous use of columns of different polarity improves this type of identification);

b) in case of MS or MS/MS detection, the relative abundance of simultaneously recorded signals (in general 3 ions are required in MS applications and 2 SRM transitions in MS/MS);

c) the application of high resolution mass spectrometry;

d) in case of MS with electron impact ionisation the comparison of the full scan mass spectrum of a suspected peak (when indicated after subtraction of background) with spectral libraries;

e) the quantification of equivalent amounts with different specific GC detectors, such as electron capture (ECD), nitrogen-phosphorous (NPD) or flame photometric (FPD) detector.

The parameters obtained for the analyte to be identified in the sample extract are compared with those obtained for the pesticides in the calibration solution(s). Should a higher degree of certainty be required for the confirmation of the analyte identity, additional measures may be necessary, such as the use of different chromatographic separation conditions or the evaluation of additional m/z or SRM-transitions. The occurrence of several stable isotopes of certain elements (e.g. Cl, Br, S) may be very helpful to identify substances by MS techniques.

For more information about the required identification criteria, see [1].

4.1.2 Quantification

For quantification, a chromatographic system calibrated with an sufficient number of appropriately distributed calibration points has to be used. The precision of calibration has to fulfil minimum requirements. Make sure that all the measurements are performed within the calibrated range of the system. In exceptional cases only, single-level calibration may be used. It has to be checked that the response of analytes present in complex mixtures does not differ from the response of separate analytes. Mixtures of isomers, degradation products and derivatives of analytes may require special conditions during calibration.

For calibration, either standards in solvents or standards prepared in blank matrix (matrix-matched standards) may be used. If matrix effects during GC injection or atmospheric pressure ionisation cannot be excluded, the use of matrix-matched standards or, even better, a quantification by standard addition has to be preferred. To detect instable detector response or such errors, which influence the amount of the analyte in the final extract, one or more internal standards should be added either to extracts or before extraction. To consider specific losses of individual analytes or their matrix effects, stable isotope labelled standards (if available) may be added to the sample before extraction.

All signals automatically identified by software tools may be considered as potential pesticide residues. However, any final quantification of relevant pesticide residues should be based on visual inspection of chromatograms. Before this European Standard can be used to quantify pesticides which are not tested before, a complete initial method validation is required. In all other cases, an on-going performance verification is sufficient to demonstrate the accuracy of the analytical method in a given laboratory.

For more information about the required quantification criteria, see [1] in its current version.

4.2 Gas chromatography (GC)

4.2.1 General

The detectors (see EN 12393-1:2013, 3.4) 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 measurement may be performed using various instruments, instrument parameters and columns. Some suitable instrument parameters and columns are listed in Annexes A and B.

For suitable experimental conditions of GC-MS measurement, see [2].

For suitable experimental conditions of GC-MS/MS measurement, see [3].

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

4.2.2 GC columns

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, hydrogen or helium should be used as carrier gas. The flow rate depends on the size and type of column used. Generally, ensure that gas flow rates are controlled as accurately as possible. Gas purification filters should be installed for all gas supplies and replaced 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.

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:

— Methyl polysiloxane	equivalent to SE-30, OV-1, OV-101, DB-1, SPB-1, BP-1, HP-1, ULTRA-1, RTx-1, AT-1, CPSil-5, etc.
— Methyl 5 % phenyl polysiloxane	equivalent to SE-54, OV-23, DB-5, SPB-5, BP-5, HP-5MS, ULTRA 2, RTx-5, CPSil-8, VF-5ms, etc.
— Methyl 50 % phenyl polysiloxane	equivalent to OV-17, DB-17, SPB-7, BP-10, HP-17, RTx-17, AT-50, etc.
— 6 % Cyanopropylphenyl 94 % methyl polysiloxane	equivalent to DB-1301, RTx-1301, HP-1301, etc.
— Methyl 7 % cyanopropyl 7 % phenyl polysiloxane	equivalent to DB-1701, CPSil-19, RTx-1701, AT-1701 OV-1701, CP-SIL-19-CB, BP-10, SPB-7, etc.
— 50 % Cyanopropyl-phenyl 50 % dimethyl polysiloxane	equivalent to SP-2330, CP-Sil 43 CB, OV-225, Rtx-225, BP-225, 007-225, etc.
— Polyethylene glycol	equivalent to DB-Wax, Supelcowax 10, Super-ox, CPWax-52, Stabilwax, BP-20, HP-20M, AT-Wax, etc.

4.2.3 Injection techniques

Various injection techniques are useful such as split/splitless injection or programmed temperature vaporisation (PTV) injection.

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

4.2.4 GC determination

The measurement may be performed using various columns, instruments, acquisition parameters and GC detectors. Widely used specific detectors are electron capture (ECD), nitrogen-phosphorous (NPD) and flame photometric (FPD) detectors. Nowadays, GC is more often combined with single stage or tandem mass spectrometers (MS or MS/MS). Some instrument parameters and columns are listed in Annexes A and B.