BS EN 12393-1:2013

BSI Standards Publication

Foods of plant origin — Multiresidue methods for the determination of pesticide residues by GC or LC-MS/MS

Part 1: General considerations

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National foreword

This British Standard is the UK implementation of EN 12393-1:2013. It supersedes BS EN 12393-1:2008 which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee AW/275, Food analysis - Horizontal methods.

A list of organizations represented on this committee can be obtained on request to its secretary.

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Aliments d'origine végétale - Méthodes multirésidus de détermination de résidus de pesticides par CPG ou CL-SM/SM - Partie 1: Généralités

Pflanzliche Lebensmittel - Multiverfahren zur Bestimmung von Pestizidrückständen mit GC oder LC-MS/MS - Teil 1: Allgemeines

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Foreword

This document (EN 12393-1:2013) 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of an identical text or by endorsement, at the latest by May 2014, and conflicting national standards shall be withdrawn at the latest by May 2014.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 12393-1:2008.

The following significant technical changes have been made:

a) separation of analytes by liquid chromatography with MS/MS-detection in methods N and P;

b) incorporation of information on GC-MS/MS detection;

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

d) editorial updating of the document according to references, etc.

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 selected analytical 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 3.1.

1 Scope

This European Standard gives general considerations for the determination of pesticide residues in foods of plant origin.

Each method specified in this European Standard is suitable for identifying and quantifying a definite range of those organohalogen, and/or organophosphorus and/or organonitrogen pesticides which occur as residues in foodstuffs of plant origin.

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

— method M: Extraction with acetone and liquid-liquid partition with dichloromethane/light petroleum, if necessary clean-up on Florisil® [1)] [1], [2], [3];

— method N: Extraction with acetone, liquid-liquid partition with dichloromethane or cyclohexane/ethyl acetate and clean-up with gel permeation and silica gel chromatography [4], [5];

— method P: Extraction with ethyl acetate and, if necessary, clean-up with gel permeation chromatography [6].

The applicability of the three methods M, N and P for residue analysis of organohalogen, organophosphorus and organonitrogen pesticides, respectively, is given for each method.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 12393-2:2013, *Foods of plant origin — Multiresidue methods for the determination of pesticide residues by GC or LC-MS/MS — Part 2: Methods for extraction and clean-up*

EN 12393-3:2013, *Foods of plant origin — Multiresidue methods for the determination of pesticide residues by GC or LC-MS/MS — Part 3: Determination and confirmatory tests*

3 Principle

3.1 General

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 always be clearly documented and demonstrated to give valid results.

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 given in 3.2 to 3.5.

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 this product.

Quality control procedures for pesticide residue analysis, e.g. published by the European Commission [7], should be followed in its updated versions. The principles laid down in this guidance document such as initial method validation, on-going performance verification and calibration should be followed carefully in order to ensure a proper use of different combinations of separation and detection methods. Also, further information such as in [8] might be helpful.

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

3.3 Clean-up

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

3.4 Determination

Gas chromatography (GC) with selective detectors may be used: electron-capture detection (ECD) for organohalogen, thermionic detector (NPD, P-mode or N/P mode) for organophosphorus and organonitrogen compounds and flame-photometric detector (FPD) for organophosphorus and organosulfurous pesticides. Hall detector (ECHD), atomic emission detector (AED) and mass spectrometry (MS) and tandem mass spectrometry may also be used for a large class of pesticides. As alternative, liquid chromatography (LC) with MS-MS-detection has been proven to be successful.

3.5 Confirmation

Procedures to confirm the identity and quantity of observed residues should be used, particularly in those cases where it would appear that the maximum residue limit (MRL) has been exceeded.

3.6 Use of internal standards

An internal standard may be added in a constant amount to samples, the blank and calibration standards. This substance can be used for checking critical points of methods (e.g. volatile or unstable compounds) and/or for quantification purposes, if appropriate. This is done to correct for the loss of analyte during sample preparation and sample clean-up or to check reproducibility of final determination steps by GC or LC. The internal standard should be preferably a compound that has very similar physico-chemical and chemical properties compared to the analyte.

4 Reagents

4.1 General

Use reagents of purity suitable for pesticide residue analysis and check their purity (see 4.2). If required, purify water and solvents used, e.g. as described in Annex A, and check their purity (see 4.2). 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.

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 under the same conditions as used in the method. The chromatogram should not show any interfering impurity.

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 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 A or the requirements of the different analytical methods. Extract the salts 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 the purity 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. The chromatogram should not show any interfering impurity. Check the activity of adsorbents regularly as described in the methods M to P (see EN 12393-2).

4.2.5 Standard materials and solutions

Use standard 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 for prolonged periods of time. Examine analytical standards for impurities.

When stored at -20 °C, standard materials are generally stable for at least a year. To allow equilibration, it is recommended to 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 freezer (at about -20 °C), are usually stable for 6 months, but the influence of repeated uses has to be checked.

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. Therefore, the use of polytetrafluoroethylene (PTFE) screw-cap flasks is recommended for the storage of stock and standard solutions.

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 [7], [8].

4.3 Safety aspects associated with reagents

4.3.1 General

The analysis of pesticide residues in a food matrix includes the use of several hazardous chemicals. Safety precautions as given in 4.3.2 and 4.3.3 shall be observed at all times.

4.3.2 Pesticides

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

b) Keep pesticides 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) Label clearly 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 organisation'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 (e.g. use of spark-proof refrigerators or freezers). When working with flammable solvents, 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 can easily be absorbed through the 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.

The use of hazardous solvents mentioned in the Montreal Protocol [9] (such as chlorinated solvents) should be minimized as far as possible.

Table 1 — Hazardous reagents, their effects and ways of containment

Name of reagent	Problem	Comment	Solution
Acetone	Highly flammable	Forms explosive peroxides with oxidising 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	Toxic	Avoid contact with eyes and avoid breathing vapours.	Use an effective fume removal device.
Diethyl ether	Extremely flammable	Unstable peroxides can form upon long standing or exposure to sunlight in bottles.	Store protected from light. Use an effective fume removal device. See also the warning on peroxides.
Ethanol	Flammable		Use an effective fume removal device when heating or evaporating.
Ethyl acetate	Flammable, especially when being evaporated	Irritating to eyes and respiratory tract.	Use an effective fume removal device.
n-Hexane	Highly flammable Toxic		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.	Use an effective fume removal device.

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

Thoroughly clean glassware shall be used for residue analysis.

There is a risk of carry-over of pesticides through use of glassware and other laboratory equipment. This should be taken into account, particularly when employing laboratory washing machines. Hot detergent solution (with no interfering compounds) may be used for cleaning, but afterwards the glassware shall be well rinsed with distilled water and acetone before drying. Before being washed in a washing machine, the glassware shall be rinsed with acetone, then with water. Wash it in the machine with 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, watch glasses, pipettes, filter papers, glass wool, glass rods and glass beads, etc. are not listed in the apparatus clause 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 approximately 15 ml, 80 mm to 90 mm long, are required for final concentrations.

These are preferably calibrated and may be fitted with micro-Snyder® [2] columns [10].

5.2.2 Chromatographic tubes, which should be specially made and have glass or PTFE stop-cocks 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 and 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 should not be used for this purpose. 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 can lead to contamination. Glass or PTFE stoppers are necessary. Similarly, do not use separating funnels with plastics 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 residue should be checked periodically. A keeper (e.g. propylene glycol, n-undecane or hexadecane) can be used to minimize losses of pesticides in certain cases.

Solvent evaporators can be used for concentrating large solvent volumes (for small volumes, the use of a gentle stream of pure, dry nitrogen is more advisable), such as:

5.4.2 Kuderna-Danish evaporator [3] [11] (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 approximately 50 °C.

5.4.4 Rotary vacuum evaporator (commercially available) which rotates at speeds up to 1 300 min^{-1}, which requires a source of vacuum and which has a thermostable water bath.

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2) Micro-Snyder® columns 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.

3) The Kuderna-Danish evaporator 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 this product.

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.

5.6 Centrifuges

Centrifuges shall be explosion proof and shall have centrifuge tubes in which several hundred millilitres of emulsions can be spun at rotational frequencies of 2 000 min^{-1} to 4 000 min^{-1} or more.

5.7 Gas chromatography

Gas chromatography apparatus is described in EN 12393-3:2013, 4.2.

5.8 Liquid chromatography

Liquid chromatography apparatus is described in EN 12393-3:2013, 4.3.

6 Procedure

6.1 General

Operators should thoroughly familiarize themselves with the method before starting analyses: reagent blanks shall be performed and established as being satisfactory. Also spiked recovery experiments over a broad range of levels including the maximum residue limit (MRL) should be carried out and found to be satisfactory (see Clause 9). Additionally, an appropriate reference material should be analysed whenever it is available.

Quality control procedures for pesticide residue analysis, e.g. published by the European Commission [7], should be followed in its updated versions.

Also further information such in [8] might be helpful.

It is not always possible to complete analyses in a day and sometimes it is necessary to store sample extracts overnight. In such a case, ensure that the sample extracts in the form of a solution in an anhydrous solvent are stored either:

a) in a refrigerator (at about 4 °C) in a well stoppered vessel in the dark; or

b) in the dark under deep freeze conditions of approximately -20 °C.

Where sample extracts are stored overnight, check sample extracts to ensure that they are stable during overnight storage.

Do not interrupt clean-up steps, such as column chromatography, etc.

6.2 Small scale procedures

If, in some cases, extractions of residues are carried out only with small amounts of sample, solvents and materials (small scale procedure), choose a compatible small scale procedure for clean-up.

If however, the results obtained by a small scale procedure show that the residues approach or exceed the maximum residue limit, it is advisable to choose the second extraction and clean-up procedure for confirmation with larger amounts of sample, solvents and materials.

6.3 Preparation and storage of the samples

6.3.1 General

Sample processing and storage procedures should be demonstrated to have no significant effect on the residues present in the test sample (sometimes also called “analytical sample”). Processing should also ensure that the test sample is homogeneous enough so that sub-sampling variability is acceptable. If a single analytical portion is unlikely to be representative of the test sample, larger or replicate portions shall be analysed, to provide a better estimate of the true value. The degree of comminution should support a quantitative residue extraction.

6.3.2 Laboratory sample

A laboratory sample that is wholly or extensively spoiled or degraded should not be analysed. When possible, prepare laboratory samples immediately after arrival and in any event, before any significant physical or chemical changes have taken place. If a laboratory sample cannot be prepared without delay, it should be stored under appropriate conditions to keep it fresh and to avoid deterioration. Generally, laboratory samples should not be stored longer than three days before preparation. Dried or similarly processed samples should be analysed within their stated shelf life.

6.3.3 Partly-prepared test sample

For preparation of the partly-prepared test sample take only the portion of the laboratory sample to which the maximum residue level applies. No further plant-parts may be removed.

The reduction of the laboratory sample shall be carried out in such a way that representative portions are obtained (e.g. by sub-division into four and selection of opposite quarters). For samples of small units (e.g. small fruits such as berries, legumes, cereals), the sample shall be thoroughly mixed before weighing out the partly prepared test sample. When the samples are made up of larger units, take wedge-shaped sections (e.g. melons) or cross sections (e.g. cucumbers) that include the skin (outer surface) from each unit [12].

6.3.4 Test sample

From each partly prepared test sample, any parts that would cause difficulties with the homogenisation process should be removed. In the case of stone fruits, the stones shall be removed. A record of the plant-parts that have been removed shall be kept. Precautions should be taken to avoid any losses of juice or flesh. This is the test sample. Calculation of the residue shall be based on the mass of the original test sample (including the stones).

Where the homogeneity of the test sample is not sufficient or the extraction of residues may be significantly compromised due to large particle sizes, intensive comminution should be performed using appropriate means. This is possible at ambient temperature, if separation of flesh and juice or degradation of target pesticides does not occur to a significant extent. Comminution of samples in a frozen state can significantly reduce losses of chemically labile analytes and usually results in smaller particle sizes and thus achieve a higher degree of homogeneity. Cutting the samples coarsely (e.g. 3 cm x 3 cm) with a knife and putting them into the freezer (e.g. -18 °C overnight) prior to comminution facilitates processing. Processing can be also assisted and improved by cryogenic milling (using dry ice or liquid nitrogen) by keeping the temperature below 0 °C. Especially in the case of fruits and vegetables, cryogenic milling is much more effective at homogenising commodities that have tough skins (e.g. tomatoes or grapes) compared to milling at ambient temperature. Given the fact that non-systemic pesticides, often predominantly occur on the skin, cryogenic milling significantly reduces sub-sampling variability. When processing test samples at low temperatures condensation caused by high humidity should be avoided. Residual carbon dioxide should be allowed to sufficiently dissipate so that its contribution to weigh of the sample will be negligible.

6.3.5 Test portion

Individual test portions, each sufficient for one analysis, should be abstracted from the comminuted test sample. These test portions should be analysed immediately. If test portions cannot be analysed directly, the test sample or the test portions shall be frozen until required. If test portions are taken from test samples after being stored frozen, the test samples shall be mixed before taking test portions to ensure that homogeneity has been re-established.

6.4 Extraction

For the extraction of non-fatty food, blending with an appropriate solvent is used in most cases. If possible, let the test portions thaw with extraction solvents. Each period of blending should take at least 2 min.

EN 12393-2 presents some suitable extraction procedures.

6.5 Clean-up

In addition to the residues, the extracts obtained in accordance with the methods in EN 12393-2, contain any co-extracted substances which can interfere in the analysis. To purify the crude extracts, several methods may be used, including liquid-liquid partition, adsorbent column chromatography and gel permeation chromatography.

EN 12393-2 specifies the details of methods M, N and P for the clean-up of foods of plant origin.

Each liquid/liquid partition should be performed in a separating funnel with a 2 min shake with occasional release of the pressure by opening the stop-cock 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. Organic solutions containing pesticide residues should not remain in contact with anhydrous sodium sulfate for more than 30 min, since this can result in compound losses.

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 a rather volatile (e.g. 1,7-dibromoheptane or pentachlorobenzene) and a less volatile indicator compound (e.g. 1,2,3,4-tetrachloronapthalene or isodrin [4)]) is advised. Use the rather volatile substance as an indicator of possible losses of pesticides during the evaporation stage by comparison of its peak area or height with the peak area or height of the less volatile indicator compound. An added indicator compound can also be used as an internal standard material identification (relative retention time) and quantification purposes.

Unless so specified, evaporations of organic solvent solutions should not be allowed to go to complete dryness since this can lead to pesticide losses.

7 Determination

For determination of pesticide residues, GC or LC is used in most cases.

A suitable GC system, preferably equipped with separate heaters for injector, detector and column ovens, shall be used. The facility to inject directly on to the GC column is often of advantage. Although the choice of

4) = 1, 2, 3, 4, 10,10' Hexachloro - 1, 4, 4', 5, 8, 8' - hexahydro - 1,4 - endo - 5, 8 - endodimethano-naphthalene.

the different parts of the GC system is a matter for the experience of the analyst, the following general recommendations are made.

Various types of detectors have been proved to be most useful for the determination of organohalogen, organophoshorus, and organonitrogen pesticides.

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 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 EN 12393-3:2013, Annex A.

8 Confirmatory tests

Analyses for confirmation of the identity and quantity of observed pesticide residues should be performed, particularly in those cases in which it appears that the maximum residue limit (MRL) has been exceeded.

The methods described in this European Standard permit the residue to be identified from the retention times of the compounds on the GC columns; at least two columns of different polarities should be used. The procedures listed in EN 12393-3 as high performance liquid chromatography (HPLC), GC of oxidation and other conversion products, and similar techniques, are of value. Results obtained using mass spectrometry (MS) present the most definitive evidence for confirmation/identification purposes.

EN 12393-3 gives some recommended techniques for confirmation of results.

9 Evaluation of results

9.1 Calculation

The accuracy and precision from replicate determinations should fall within a range, which is acceptable in accordance to the quality control procedures for pesticide residue analysis, published by the European Commission [7]. Generally, the mean of recoveries from replicate determinations should fall within the range of 70 % up to 120 %, whereas for routine analysis, a range of 60 % up to 140 % is acceptable with a relative standard deviation less than or equal to 20 %. These values may change and the current version of the quality control procedures for pesticide residue analysis, e.g. published by the European Commission [7], should be followed in its updated versions.

Under some circumstances (depending on the pesticides, their levels, and matrices), this range cannot be achieved.

Calculate the concentration of pesticide residues in the sample from the ratio of the response of sample and standard or standard series. Express this concentration on the portion of the laboratory sample to which the maximum residue level applies.

NOTE For example in the case of stone fruits, the mass of the stones are taken into account for the calculation, but the analysis is performed without these.

In cases where one or more residues approach or exceed the MRL, analyse at least one additional test portion.

9.2 Precision

The precision of the analytical method should be evaluated in accordance with the requirements of ISO 5725 [13]. Some general criteria, based on experience, are given in [7].

9.3 Practical limit of determination

Theoretically, the practical limit of determination in the sample concerned is defined as that concentration of the pesticide residue in milligrams per kilogram, which would correspond, on a chromatogram of an extract of the sample, to the lowest measurable peak area or height, with an acceptable degree of confidence in the result.

The practical limit of determination depends on the degree of purification, the nature of the analyte and the instrument used for the final determination. Since these conditions cannot be laid down exactly, the practical limit of determination should be established for each method and in each laboratory. As a general rule, the practical limit of determination for a residue of pesticide should be at most one-tenth of its maximum residue limit (MRL). If, however, the maximum residue limit is 0,05 mg/kg or less, a practical limit of determination one-fifth of this value is sufficient, except when the maximum residue limit is set at or about the limit of determination.

9.4 Expression of results

Express the pesticide content according to current legislation concerning foods of plant origin. Do not correct the mean concentration for the percentage recovery of the pesticide residue. Where no residue approaches or exceeds the maximum residue limit, report the value found from a single determination.

State the mean concentration and each result obtained as a mass fraction in mg/kg. If matrix blank values did occur, they should be reported separately without correcting the mean concentrations of the residue.

If required, results should be qualified with uncertainty data in accordance to the quality control procedures for pesticide residue analysis [7].

10 Test report

The test report shall contain at least the following data:

— all information necessary for the identification of the sample;

— a reference to this European Standard;

— the results and the units in which they have been expressed;

— date and type of sampling procedure (if known);

— date of receipt of sample in the laboratory;

— date of test;

— measurement uncertainty, if it has been required;

— any particular points observed in the course of the test;

— any operations not specified in the method or regarded as optional which might have affected the results.

Annex A
(informative)

Purification of solvents and reagents

- Acetone: Distil over glass beads.
- Acetonitrile: Mix 4 l of acetonitrile with 1 ml of orthophosphoric acid and 30 g of phoshorus pentoxide in a round-bottomed glass flask. Add glass beads and distil at 81 °C to 82 °C (do not allow the temperature to exceed 82 °C).
- Cyclohexane: Distil over sodium hydroxide pellets.
- Dichloromethane: Distil over glass beads.
- Diethyl ether: Distil over glass beads.
- Ethanol: Distil over glass beads.
- Ethyl acetate: Distil over glass beads.
- *n*-Hexane: Distil over sodium hydroxide pellets.
- Iso-octane: Distil over sodium hydroxide pellets.
- Light petroleum: Distil over potassium hydroxide or sodium hydroxide pellets.
- Methanol: Distil over glass beads.
- Sodium chloride: Heat at 500 °C for at least 4 h and allow to cool in a desiccator.
- Sodium sulfate: Heat at 500 °C for at least 4 h and allow to cool in a desiccator.

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