



BSI Standards Publication

**Foodstuff — Quick Method for the Analysis
of Multiple Highly Polar Pesticides and their
Metabolites in Foodstuff Involving Extraction
with Acidified Methanol and Measurement
by LC- or IC-MS/MS (QuPPE-Method)**

National foreword

This British Standard is the UK implementation of EN 18032:2025.

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 committee manager.

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English Version

**Foodstuff - Quick Method for the Analysis of Multiple
Highly Polar Pesticides and their Metabolites in Foodstuff
Involving Extraction with Acidified Methanol and
Measurement by LC- or IC-MS/MS (QuPPE-Method)**

Produits alimentaires - Méthode rapide pour l'analyse
de plusieurs pesticides hautement polaires et de leurs
métabolites dans les aliments impliquant une
extraction avec du méthanol acidifié et une analyse par
LC- ou CI-SM/SM (méthode QuPPE)

Lebensmittel - Schnellmethode zur Bestimmung
mehrerer hochpolarer Pestizide und ihrer Metaboliten
in Lebensmitteln nach Extraktion mit angesäuertem
Methanol und Messung mittels LC- oder IC-MS/MS
(QuPPE-Methode)

This European Standard was approved by CEN on 11 August 2025.

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European foreword

This document (EN 18032:2025) has been prepared by Technical Committee CEN/TC275 “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of an identical text or by endorsement, at the latest by April 2026, and conflicting national standards shall be withdrawn at the latest by April 2026.

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1 Scope

This document specifies a procedure for the analysis of residues of highly polar pesticides and metabolites, which are not amenable to common multiresidue methods, in various food commodities of plant and animal origin, including fruits, vegetables, cereals, pulses, oily seeds, nuts, milk, liver and honey. The method was developed at the EURL-SRM hosted at CVUA Stuttgart [1], [2], [3] and has been collaboratively studied on a large number of commodity/pesticide combinations. Guidelines for calibration are outlined in CEN/TS 17061:2019.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

CEN/TS 17061:2019, *Foodstuffs - Guidelines for the calibration and quantitative determination of pesticide residues and organic contaminants using chromatographic methods*

SANTE/11312/2021v2,¹ *Analytical quality control and method validation procedures for pesticide residues analysis in food and feed*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in SANTE/11312/2021v2 apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp/>
- IEC Electropedia: available at <https://www.electropedia.org/>

4 Principle

Residues are extracted from the homogeneous test portion following water adjustment and addition of acidified methanol. In the case of fruits and vegetables, the mixture is centrifuged, filtered and directly analysed by LC-MS/MS or IC-MS/MS. In the case of cereals, pulses, nuts, oily seeds and foods of animal origin, EDTA is added for the complexation of metal ions, such as calcium and magnesium, which positively affects the analysis of certain compounds (e.g. glyphosate and AMPA). As such commodities also contain a substantial content of protein, they are additionally diluted with acetonitrile to precipitate proteins. Samples with high lipid content are subjected to dispersive SPE with C18-sorbent. Various LC- and IC-MS/MS methods, allowing simultaneous analysis of different combinations of pesticides, are provided in this document. Quantification is performed employing isotope-labelled analogues of the target analytes as internal standards (IL-ISs), so far these are available. When adding IL-ISs directly to the test portion at the beginning of the procedure, they compensate for any factors having an influence on recovery-rates, such as volume-deviations and analyte losses during sample preparation. Matrix-effects during measurement are also corrected. The use of IL-ISs, ensures good accuracy and reproducibility. Quantification without IL-ISs is possible, but careful water adjustment and other approaches for addressing recovery losses and matrix effects are required in this case. The analytical procedure entails few working steps and involves little material consumption. A brief overview of the method is shown in the flowcharts within the “On-line Supplement” linked in Annex C. Abbreviations and symbols are listed in Annex D.

¹ Version 2 of 2021. Implemented as of 01/01/2024.

Available at: <https://www.eurl-pesticides.eu/docs/public/tmpl article.asp?CntID=727>

5 Preparation and storage of the samples

5.1 General considerations

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 (portion-to-portion) 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, otherwise, extraction shall involve supplementary comminution, e.g. through a homogenizing device (A.2.2) or grinding aids (e.g. metal balls (A.2.3)).

5.2 Laboratory sample

A laboratory sample is the sample arriving to the laboratory for analysis and should ideally be sampled according to international sampling protocols [4], [5]. A laboratory sample that is extensively spoiled or degraded should not be analysed. Samples associated with a shelf life should be analysed within their stated shelf life. If possible, process 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 processed without delay, it should be stored under appropriate conditions to keep it fresh and to minimize deterioration.

If the laboratory sample is in a state that does not require milling prior to analysis (e.g. juices, milk, and cereal flour), stir or shake the sample well and then withdraw the analytical test portions directly. Where the homogeneity of the sample is, however, not sufficient or the extraction of residues is expected to be significantly compromised due to the presence of larger particles, intensive comminution should be performed using appropriate means.

5.3 Treatment of laboratory samples prior to milling

For preparation of the analytical sample, take only the portion of the laboratory sample to which the maximum residue levels apply [6], [7]. If a reduction of the laboratory sample is required, out of practical reasons, it shall be carried out in a way ensuring representativeness. This, for example, applies when samples are made up of larger units, and the capacity of the available mixer is too small to process the required number of units in one go. In this case, parts of the sample can be used, i.e. wedge-shaped sections (e.g. melons) or cross sections (e.g. cucumbers) that include the skin (outer surface). Opposite sections from each unit (e.g. quarters) should then be used for mixing (see e.g. [8]). For samples of small units (e.g. small fruits such as berries, legumes, cereals), the sample shall be thoroughly mixed before taking any aliquot for further processing.

Any parts that would cause difficulties with the homogenization process may be removed prior to milling (e.g. in the case of stone fruits, the stones). The mass of the sample before and after removing the interfering sample portions shall be recorded. Precautions should be taken to avoid any losses of juice or flesh. The sample obtained in this way is referred to as the test sample. Calculation of the residue shall be based on the mass of the original test sample (including the stones and assuming that these parts are residue free).

In case of cryogenic comminution using dry ice, cutting the samples coarsely (e.g. 3 × 3 cm) with a knife and putting them into the freezer (e.g. at ≤ -18 °C overnight) prior to comminution facilitates processing.

5.4 Sample homogenization

5.4.1 General considerations

For fresh products (e.g. fruits and vegetables), homogenization at ambient or refrigerator temperature is possible, but cryogenic milling is to be preferred in most cases, as it minimizes losses of susceptible analytes, and usually results in smaller particle sizes and a higher degree of homogeneity. In the case of fresh, non-frozen products, keep the time gap between homogenization and extraction as short as possible to minimize degradation of susceptible target analytes.

Cryogenic milling (additionally assisted by dry ice or liquid nitrogen (A.1.12)) typically further improves homogenization of commodities with tough skins (e.g. tomatoes or grapes). When test samples are processed at low temperatures, take measures to minimize the condensation of humidity on the sample. Residual carbon dioxide should be allowed to sufficiently dissipate, so that its contribution to the sample weight will be negligible. For the homogenization using liquid nitrogen, the sample material (e.g. entire units or coarsely cut units) is immersed into a suitable plastic container (e.g. PP or polystyrene) containing liquid nitrogen. When completely frozen, it is transferred into a powerful knife mill and ground until a fine powder is obtained. The frozen powder should be transferred quickly into a storage container. Make sure that all liquid nitrogen is evaporated before tightly closing the storage container and placing it into the freezer.

For dry commodities, the aim of milling is to produce particle sizes that are < 1 mm. Smaller particle sizes will facilitate extraction. However, be aware that friction during milling can cause very high temperatures. Cryo-milling involving addition of liquid nitrogen or dry ice helps to keep temperatures low. The use of ultra-centrifugal mills with ring-filters helps to limit the maximum particle size. Milling by a knife mill usually results in a broad particle distribution. A two-step milling typically helps to reduce the average particle size further. When dealing with dry commodities of high oil content, extensive milling with a knife mill and milling with ultra-centrifugal mills often leads to the formation of a paste due to the release of liquid oil. Cryo-milling is to be preferred for such samples. Homogenates of dry commodities should be optimally also stored in the freezer, although analyte stability under ambient conditions is typically much better than in liquid homogenates.

NOTE Upon comminution (including the coarse cutting mentioned above) of non-frozen high moisture content samples (e.g. fruits and vegetables) matrix-juices are released that can accelerate the degradation of certain susceptible pesticides with which they come in contact. This includes pesticides located on the skin of products, which get exposed to fruit juices when coarsely cut pieces (later used for cryo-milling) are mixed in a bag.

5.4.2 Preparation of homogenates of dried fruit and similar commodities

Dried fruits and similar commodities (≈ 15 % to 40 % moisture-content) can be processed as a slurry or in deep frozen state without addition of water. For the processing as a slurry, weigh 500 g of frozen dried fruits, add X g of cold water (A.1.1) (see Table 1 and Table B.1) and homogenize the mixture using a strong mixer (A.2.1), if possible, with addition of dry ice (A.1.12) to prevent or slow-down any chemical and enzymatic reactions. For extractions according to E3 (see Table 2), weigh Y g of homogenate (see Table 1 and Table B.1 corresponding to 5 g sample). Alternatively, immerse the sample material into a plastic or polystyrene container containing liquid nitrogen. When completely frozen, transfer it into a powerful knife mill and grind until a fine powder is obtained. Do not mill for too long and quickly transfer the frozen powder into a storage container and place it into the freezer to avoid that it becomes clumpy and more difficult to handle.

Table 1 — Dried fruits/water proportion during homogenization

Moisture content of product	Amount of sample	Water amount added	Weight of analytical portion (Y g; corresponding to 5 g of original dry sample)
%	g	X g	g
≈15 to < 25	500	900	14
25 to < 35	500	850	13,5
≥ 35 to 40	500	800	13

Freeze-dried fruit and vegetables (typical moisture content 0,3 % to 4 %) are homogenized with a high speed knife mill (A.2.1), preferably after adding dry ice or liquid nitrogen (A.1.12) to keep the material cool. Thereof, 2 g sample is employed for analysis (as in the case of other dry extract-rich commodities such as spices and dried herbs).

5.5 Analytical test portion

One or more individual test portions, each sufficient for one analysis, should be taken from the sample homogenate, prepared as described above. If the test sample was stored prior to weighing the analytical portions and it is noticed, that the homogeneity of the test sample has been compromised during storage, the test sample shall be mixed again before taking test portions, to ensure that homogeneity has been re-established.

If fresh products are homogenized in a non-frozen state, the test portions should be weighed and analysed immediately after milling, unless it could be demonstrated, that no significant degradation of target analytes occurs in the time period between comminution and start of analysis. If the test portions cannot be analysed directly, they shall be frozen until required. Keep in mind that in such non-frozen liquid homogenates a separation of flesh and juice can occur and potentially increase portion-to-portion variability. Thus stir the homogenate well before weighing.

6 Procedure

The extraction is described in modules E1 to E7 (Table 2). Depending on the commodity, the centrifuged raw extracts are either filtered and directly subjected to the measurement modules M1 to M5 (Table 5) or cleaned-up prior to measurement by one or more of the clean-up modules C1 to C4 (Table 3). A dilution module DL (Table 4) prior to measurement is either indicated or recommended, but this also depends on the sensitivity of the instrumentation used. The residue concentrations in the samples can be calculated using the quantification approaches described under Q1 to Q6 (see Table 6 with reference to calibration procedures in CEN/TS 17061:2019). For the application of the Q-Modules a decision tree can be found under [13]. Further calibration/quantification procedures can be found under CEN/TS 17061:2019.

Preferred combinations of modules for a multitude of raw and processed commodities, concerning the extraction of samples and clean-up of raw extracts are listed in Table B.1.

Table 2 — Extraction modules

Module ^a	Short description ^b	Preferred application ^c and restrictions	Examples / Remarks ^c
E1	Extraction of 10 g sample without water addition	Commodities with high water content (≥ 80 %)	Fruit, vegetables, juices
E2	Extraction of 10 g sample after addition of, e.g. I. Two ml water II. 2,5 ml water III. Three ml water IV. 3,5 ml water	Commodities with average water content (>40 % to < 80 %)	I. Potato, parsley II. Banana, horseradish, fresh peas, ginger III. Avocado, olive IV. Garlic
E3	Extraction of e.g. 13,5 g homogenate that was prepared with the addition of water, see Table 1	Commodities with low water content (≈ 15 % to 40 %)	Dried fruits and similar products (e.g. jams)
E4	Extraction of 5 g honey after addition of 7,5 ml water	Honey	
E5a	Extraction of 2 g or 5 g sample after addition of 10 ml water	Commodities with very low water content (< 15 %). Restrictions: Not recommended for pesticides and metabolites containing a phosphonic acid moiety (e.g. glyphosate, AMPA, MPPA) see E5b.	<u>Low lipid content:</u> Herbs, Refined cereals, whole grain cereals, pseudo cereals, pulses, skimmed milk powder. <u>High lipid content:</u> Oilseeds, nuts, spices, milk powder, infant formula.
E5b	Extraction of 2 g or 5 g sample after addition of 9 ml water, 1 ml 10 % EDTA, 0,1 ml formic acid	Commodities with very low water content (< 15 %). Recommended for pesticides and metabolites containing a phosphonic acid moiety (e.g. glyphosate, AMPA, MPPA).	

Module ^a	Short description ^b	Preferred application ^c and restrictions	Examples / Remarks ^c
E6a	Extraction of 10 g sample after addition of, e.g. I. 1,0 ml water II. 1,5 ml water III. 2,0 ml water IV. 2,5 ml water V. 3,0 ml water	Commodities of animal origin with water content (>30 %). Restrictions: Not recommended for pesticides and metabolites containing a phosphonic acid moiety (e.g. glyphosate, AMPA, MPPA) see E6b.	I. Skimmed milk (low lipid content) II. Whole fat milk III. Kidney, fish IV. Egg, muscle V. Liver In the case of processed meat products increase the amount of water (e.g.: in the case of cured ham with 50 % moisture add 5 ml of water or 4/1/0,1 ml of water/10 % EDTA/formic acid to 10 g sample).
E6b	Extraction of 10 g sample after addition of water/10 % EDTA / formic acid, e.g. as follows: I. 0/1/0,1 ml II. 0,5/1/0,1 ml III. 1/1/0,1 ml IV. 1,5/1/0,1 ml V. 2/1/0,1 ml	Commodities of animal origin with water content (>30 %). Recommended for pesticides and metabolites containing a phosphonic acid moiety (e.g. glyphosate, AMPA, MPPA).	
E7	Extraction of 5 g fat sample assisted by mechanical aids (e.g. metal balls)	Fats (lipids that have a non-liquid, e.g. solid, waxy or pasty consistency at room temperature)	Fats of animal and plant origin
^a The modules are described in Annex A. ^b The indication “without water addition” refers to procedures where IL-IS is added before any aliquotation of the extract. Where IL-IS is not used or where it is added to an aliquot of the extract, water adjustment is indicated up to a total volume of 10 ml (sample water + added water) to reduce the bias. ^c Water contents of the various commodities can be found in Table B.1 under column V and VI. For commodities where a freeze-out step and/or a cold centrifugation is recommended after the extraction step column VIII of the same table contains the coding “F-O” (see also the Recommendations under 3.1).			

Table 3 — Clean-up modules

Module ^a	Description	Preferred applications	Examples
C0	No clean-up	Most fruits and vegetables	
C1	Freeze-out for the precipitation of poorly soluble co-extractives or to assist filtration	Commodities, of which the extracts show difficulties in filtration or contain co-extractives that precipitate at low temperatures (e.g. lipids, sugars, proteins)	Raw extracts of e.g. pineapples, potatoes, cereal flours (especially if finely milled), avocado, olives, strawberries
C2	Dispersive SPE with C18-sorbent	Commodities containing high lipid and low protein content	Avocado, olives
C3a	Precipitation with acetonitrile for removal of proteins and other co-extractives with limited solubility in acetonitrile	Commodities containing high protein content	Refined cereals, pseudo cereals, pulses, skimmed milk powder
C3b	Precipitation with acetonitrile combined with C18 sorbent for removal of proteins, lipids and other co-extractives with limited solubility in acetonitrile	Commodities containing high protein and high lipid content	Oilseeds, nuts, cereals, liver, kidney, whole fat milk, muscle
C4	Ultrafiltration using cut-off filters of 5 kDa or 10 kDa	Commodities with high protein content	Oilseeds, nuts, pulses, cereals, pseudo cereals, liver, kidney, whole fat milk, muscle
^a	The modules are described in Annex A.		

Table 4 — Dilution modules - exemplary

Module DLx ^a	Description of dilution ^b	Circumstances (indicative) ^c	Examples / Recommendations ^d
DL1	Undiluted extracts	• Measurement of undiluted extracts possible with satisfactory performance • Analyte sensitivity does not allow dilution, but matrix effects (compared to calibration) are negligible (<ca. ± 20 %) or sufficiently compensated (e.g. via IL-IS or matrix matching)	
DL2	2-fold dilution ($V_1 + V_2$, 1 + 1)	• Considerable (non-negligible) matrix effects (e.g. between ± 20 % and ± 40 %), that are not sufficiently compensated otherwise (e.g. via IL-IS or matrix matching) • See also general goals and preconditions below	• DL2 prior to analysis by M3a or M3b of sample extracts of E5b and E6b (with EDTA) following C3a or C3b
DLx 5 to 10 e.g. x = 5 (DL5); x = 10 (DL10)	5-fold dilution ($V_1 + V_2$, 1+4) 10-fold ($V_1 + V_2$, 1+9)	• Strong matrix effects (e.g. between ± 40 % and ± 90 %) that are not sufficiently compensated otherwise (e.g. via IL-IS or matrix matching) • Heavy matrix load in extract, that affects performance or robustness of measurement • See also general goals and preconditions below	• DL10 prior to analysis of chlorate, perchlorate, phosphonic acid, by M3b • DL5 when using IC-MS/MS (M4 or M5) • DL5 prior to analysis by M3a or M3b of sample extracts of E5b and E6b (with EDTA) following C3a or C3b
DLx > 10 e.g. x = 50 (DL50)	50-fold dilution ($V_1 + V_2$, 1+49)	• Very strong matrix effects (e.g. > ± 90 %) that are not sufficiently compensated otherwise (e.g. via IL-IS or matrix matching) • Heavy matrix load in extract, that affects performance or robustness of measurement • See also general goals and preconditions below	• DL50 prior to analysis of bromide (high MRLs and high background levels)
^a The modules are described in Annex A. The module number “x” indicates the dilution factor and serves the documentation of the procedure. Any dilution factor can be used as long as preconditions (see list after Table 4) are met. ^b Dilutions can be accomplished by mixing a certain volume of the extract (V_1) with a certain volume of a water-miscible solvent (V_2), which may be a LC-mobile phase eluent (see A.6). Dilution can be part of the LC- or IC-injection process. In case the dilution induces a precipitation, filtration with syringe filter (A.2.11) is recommended after dilution. Dilutions with blank sample extracts are also possible and sometimes reasonable (e.g. for keeping matrix effects similar). ^c Matrix effects are defined as in the SANTE document SANTE/11312/2021; e.g. a signal suppression by 90 % is expressed as –90 % ^d Dilution factors are exemplary and may be individually adjusted considering measurement sensitivity, analyte concentration as well as the purpose of analysis (i.e. screening or quantitative).			

When applying Table 4, general goals of dilution are to:

- reduce matrix effects,
- reduce retention time shifts,
- improve peak shapes,
- improve chromatographic separation from interfering compounds,
- obtain a signal within the calibration range/ linear range,
- reduce exposure of measurement instrument to matrix (increase long term robustness and reduce maintenance intervals).

Thereby, general preconditions for dilution are that:

- the signal intensities of analyte(s) and IL-IS(s) remain satisfactory after dilution,
- any shifts of matrix effects are adequately addressed by the calibration approach.

Table 5 — Measurement modules

Module ^a	Instrument details	Exemplary Analytes	Exemplary Columns
M1	LC-MS/MS HILIC phase ESI neg. mode	Glyphosate, Glufosinate, Fosetyl, Chlorate, Perchlorate, etc.	APPC ^b Torus DEA ^b Raptor Polar X ^d Obelisc N ^e Trinity Q1 ^c Luna Polar Pesticides ^f
M2	LC-MS/MS HILIC phase ESI pos. mode	Chlormequat, Mepiquat, Cyromazine, Matrine, Nicotine, etc.	Amide BEH ^b Obelisc R ^e Trinity P1 ^c
M3	LC-MS/MS Porous graphitic carbon phase ESI neg. mode	Glyphosate, Glufosinate, Fosetyl, Chlorate, Perchlorate, etc.	Hypercarb ^c
M4	IC-MS/MS Anion exchange phase ESI neg. mode	Glyphosate, Glufosinate, Fosetyl, Chlorate, Perchlorate, etc.	AS19 ^c AS24 ^c

Module ^a	Instrument details	Exemplary Analytes	Exemplary Columns
M5	IC-MS/MS Cation exchange column ESI pos. mode	Chlormequat, Mepiquat, Cyromazine, Matrine, Nicotine, etc.	CS17 ^c

^a The modules are described in Annex A.

^b APC, Torus DEA, Amide BEH are the trade names of products supplied by WATERS. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN or CENELEC of the product named. Equivalent products may be used if they can be shown to lead to the same results.

^c Trinity Q1, Trinity P1, Hypercarb, AS19, AS24, CS17 are the trade names of products supplied by Thermo. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN or CENELEC of the product named. Equivalent products may be used if they can be shown to lead to the same results.

^d Raptor Polar X is the trade name of a product supplied by Restek. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN or CENELEC of the product named. Equivalent products may be used if they can be shown to lead to the same results.

^e Obelisc N, Obelisc R are the trade names of products supplied by Sielc. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN or CENELEC of the product named. Equivalent products may be used if they can be shown to lead to the same results.

^f Luna Polar Pesticides is the trade name of a product supplied by Phenomenex. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN or CENELEC of the product named. Equivalent products may be used if they can be shown to lead to the same results.

Table 6 — Quantification modules

Module ^g	Description	Circumstances		Effects			Preconditions, Remarks and Examples	Reference within CEN/TS 17061:2019
		Matching blank matrix used ^a	IL-IS (added at beginning of procedure) ^b	This approach... corrects for poor absolute recovery ^c	corrects for matrix effects ^d	reduces impact of interactions in injector ^e		
Q1a	Quantification using calibration based on external standards in solvent (solvent-based without IL-IS)	No	No	No	No	No	Preconditions: As this approach does not correct for recovery or matrix effects, absolute recovery rates shall be sufficient and matrix effects should be negligible or sufficiently reduced via dilution. Example: Bromide (where blank matrices can virtually not be found and no IL-IS is available)	6.4.2 to 6.4.5
Q1b	Quantification using calibration based on external standards in solvent and IL-IS (solvent-based using IL-IS^f)	No	Yes	Yes	Yes	No	Preconditions: The absence of matrix (in the calibration solutions) does not dramatically affect the chromatographic behaviour of the target analyte. Example: Chlorate in milk (where blank matrices can virtually not be found but IL-IS is available)	6.5.3
Q2a	Quantification using calibration based on external standards in extracts of generic residue-free samples (preferably with low extract load), e.g. cucumber, tomato without the use of IL-IS (matrix-based without IL-IS)	No	No	No	No	Yes	Example: Where a lab analyses multiple types of samples within the same batch making it practically difficult to prepare matrix-matched standards	6.4.2 to 6.4.5

Module ^g	Description	Circumstances		Effects			Preconditions, Remarks and Examples	Reference within CEN/TS 17061:2019
		Matching blank matrix used ^a	IL-IS (added at beginning of procedure) ^b	This approach...				
				corrects for poor absolute recovery ^c	corrects for matrix effects ^d	reduces impact of interactions in injector ^e		
Q2b	Quantification using calibration based on external standards in extracts of generic residue-free samples (preferably with low extract load), e.g. cucumber, tomato and IL-IS (matrix-based using IL-IS^f)	No	Yes	Yes	Yes	Yes	Example: Where a lab analyses multiple types of samples within the same batch making it practically difficult to prepare matrix-matched standards	6.5.3
Q3a	Quantification using calibration based on external standards in extracts of residue-free samples of the same matrix type (matrix-matched without IL-IS)	Yes	No	No	Yes	Yes	Precondition: The absolute recovery rate of the analyte should be sufficient (as this approach does not correct for recovery losses).	6.4.2 to 6.4.5
Q3b	Quantification using calibration based on external standards in extracts of residue-free samples of the same matrix type, with IL-IS (matrix-matched using IL-IS^f)	Yes	Yes	Yes	Yes	Yes		6.5.3
Q4a	Quantification using external standards spiked on sample portions of residue-free samples i.e. quantification by calibration of the entire procedure (<u>preferably using a matching matrix</u>) (procedural without IL-IS)	(Yes) Matching matrix preferable	No	(Yes) Inherent if matching matrix used	(Yes) Inherent if matching matrix used	Yes	Precondition: A matching matrix should be used to correct for recoveries and matrix effects (as no IL-IS is used). Remark: Be aware that some analytes show variable absolute recoveries even between different samples of the same matrix type (e.g. different types of rice).	6.7

Module ^g	Description	Circumstances		Effects			Preconditions, Remarks and Examples	Reference within CEN/TS 17061:2019
		Matching blank matrix used ^a	IL-IS (added at beginning of procedure) ^b	This approach...				
				corrects for poor absolute recovery ^c	corrects for matrix effects ^d	reduces impact of interactions in injector ^e		
Q4b	Quantification using external standards spiked on sample portions of residue-free samples i.e. quantification by calibration of the entire procedure (using IL-IS and preferably using a <u>matching matrix</u>) (procedural using IL-IS^f)	(Yes) Matching matrix preferable	Yes	Yes	Yes	Yes	Remark: The IL-IS corrects for both matrix effects and recovery rates and should be added at the beginning of the procedure (procedural IL-IS) both to test portion of the sample to be analysed and the portions used for procedural calibration. If the same type of matrix is used, matrix effects and recovery rates are additionally corrected via matrix-matching and the procedural approach. If a matching matrix is not available then a matrix with a similar impact on recovery is to be preferred.	6.7
Q5a/b	Quantification using standard addition to the final extract (optionally using IL-IS if available). If no IL-IS is used, Module Q5a applies and if IL-IS is used, Module Q5b applies. For more information see decision tree under [13]. (standard additions on extract aliquots)	No (the identical matrix is used)	(Yes) Optional	No	Yes (inherent)	Yes	Any IS can be used here. The use of IL-IS will not correct for recovery. This approach is to be used for analytes having satisfactory absolute recovery rates, but unacceptable matrix effects not properly addressed by other means. It is assumed, that each extract aliquot contains the whole residue originally contained in the matrix fraction represented by this aliquot. It is thus important to make sure to properly supplement water (Table B.1), so that the total volume of the extract is 20 mL water.	6.6.2

Module ^g	Description	Circumstances		Effects			Preconditions, Remarks and Examples	Reference within CEN/TS 17061:2019
		Matching blank matrix used ^a	IL-IS (added at beginning of procedure) ^b	This approach...				
				corrects for poor absolute recovery ^c	corrects for matrix effects ^d	reduces impact of interactions in injector ^e		
Q6a/b	Quantification using standard addition to separate test portions of the sample (optionally using IL-IS if available). If no IL-IS is used, Module Q6a applies and if IL-IS is used, Module Q6b applies. For more information see decision tree under [13]. (standard additions on sample portions)	No (the identical matrix is used)	(Yes) Optional	Yes (inherent)	Yes (inherent)	Yes	Example: cases where a matching matrix, that could be used for calibration, is not available and correction of result recovery is needed.	6.6.3
^a Matching blank matrices with negligible levels of the target analyte (=that could be used for preparing calibration standards) are readily available and used “Yes” or not available “No”.								
^b “Yes” indicates that a suitable IL-IS is available and used at the beginning of the procedure (before any aliquoting). “No” indicates that an IL-IS is not available or available but not used.								
^c Where recovery losses during extraction are not negligible, procedures marked with “Yes” are recommended. Where correction for recovery is solely based on the use of IL-IS, the IL-IS needs to be added to the samples at the beginning of the procedure (before any aliquoting).								
^d Where matrix effects are not negligible, procedures marked with “Yes” are recommended.								
^e Where the chromatographic performance is compromised in absence of (any) matrix (e.g. due to interactions of the analyte with the injector surfaces), procedures marked with “Yes” are recommended.								
^f In case of low recovery rates, the IL-IS should be added at the beginning of the procedure (= procedural IL-IS).								
^g The modules are described in Annex A. For the application of the Q-Modules, a decision tree can be found under [13].								

7 Evaluation of results

7.1 Identification

For identification, refer to the latest version of SANTE/11312/2021: *Analytical quality control and method validation procedures for pesticide residues analysis in food and feed*. The identification process includes the collection of a series of evidences, which will together solidify the decision about the identity of the analyte. The process includes the comparison of retention times and peak shapes of the analytes to be identified in the sample and a co-injected IL-IS and/or a reference standard of the native compound as well as a comparison of the relative abundance (i.e. signal-ratio) of one or more pairs of recorded MS/MS mass-transitions with those of a reference standard. In case of further doubts, supporting evidence can be obtained by re-analysing the extract by a different chromatographic separation system (column, solvent).

Matrices causing very strong signal suppression on analyte signals increase the likelihood of false-negative results. The use of IL-IS helps to estimate matrix effects (e.g. signal suppression). In case of very strong suppressions (stronger than e.g. -70 %), check for the feasibility of detecting the analyte at the LOQ to minimize the risk of a false-negative result. For this, overspike the sample extract with the target analyte at the LOQ and/or a higher level, and (re-) inject. Increase the LOQ if needed.

7.2 Calibration and quantification

The measuring systems used (e.g. LC-MS/MS) shall be calibrated according to the requirements of CEN/TS 17061:2019 taking into account the provisions of the analytical quality control and method validation procedures document, SANTE/11312/2021. Table 6 shows quantification modules (Q1 to Q6) that are specifically recommended for calibration and quantification if certain circumstances and preconditions are met. A decision tree aimed at facilitating the prioritization of quantification modules can be found in [13].

Where a certain analyte shows recovery rates, that do not meet the accepted performance criteria (e.g. see SANTE/11312/2021), calibration approaches addressing this aspect should be used. Adding the IL-IS at the beginning of the procedure is the most convenient approach. In absence of IL-ISs, the approach of standard additions to sample portions (Q6) or the approach of procedural calibration (Q4a) are to be used (see also Table 6).

The concentration range of calibration solutions should encompass the residue concentrations of the target analytes in the sample extracts.

Calibration not involving IL-IS: Where no adequate IL-IS is employed and the impact of matrix effects during measurement is substantial or unknown, calibration approaches should ensure compensation of matrix-effects. In the opposite case, the generated results should only be regarded as a first estimate (semiquantitative). Where relevant residue concentrations are detected (e.g. suspected MRL exceedances), a more precise analysis is needed. This can involve various approaches, such as the use of matrix-matched calibration standards (Q3a), standard additions (Q5 and Q6, with the latter also correcting for recovery losses) or procedural calibration (Q4a). It is further important to make sure that the total volume of the raw extract prior to any dilution is as close as possible to 20 ml (corresponds to a sample mass concentration of 0,5 g/ml extract, if the test portion is 10 g). This shall be done by adjusting the water content according to Annex B.

Calibration involving IL-IS: Using IL-ISs typically obviates the need for matrix-matching, but matrix-based calibrations (Q2 to Q6) are often preferable to solvent-based ones, as the absence of matrix-components can lead to peak distortions due to interactions of various analytes with surfaces within the chromatographic system.

For quantification purposes, it is of foremost importance that the ratio between the absolute IL-IS amount added to the sample prior to extraction (or to the isolated aliquot of the sample extract) and the absolute IL-IS amount added to the calibration standard solutions (or at least the ratio of the two amounts) is known as it is used in calculations.

IL-IS can alternatively be added to an aliquot of the sample extract (e.g. 1 ml), that is transferred to the injection vial. In this way, the required IL-IS amount is reduced by a factor of e.g. 20, which lowers the costs of the analysis. This still ensures that matrix effects are reliably compensated and retention time shifts are reliably detected. This approach, however, does not correct for deviations in the extract volume and for any analyte losses that occur before the IL-IS addition (e.g. during extraction and clean-up). In case of major deviations of the extract volume and in the case of analytes, that are known to show low or variable absolute recoveries, the results obtained in this way (late IL-IS addition) should be considered semiquantitative and re-analysis with the addition of a suitable IL-IS before extraction should be conducted before reporting quantitative results.

7.3 Calculation of residue levels

7.3.1 Calculation of residue concentrations using external calibration without IL-ISs

The residue level of the identified analyte A in the sample in mg/kg (w_A) is calculated according to Formula (1), using the mass concentration of the sample in the final extract ($\rho_{\text{final extract}}^{\text{sample}}$) and the mass concentration of the analyte in the final extract (ρ_A , see quantification modules):

$$w_A = \frac{\rho_A}{\rho_{\text{final extract}}^{\text{sample}}} \quad (1)$$

where

ρ_A is the mass concentration of the identified analyte A in the analysis solution (final extract) in $\mu\text{g/ml}$, see respective references to CEN/TS 17061:2019, 6.4.2 to 6.4.5, under Q1 to Q6;

$\rho_{\text{final extract}}^{\text{sample}}$ is the mass concentration of the sample in the final extract in g/ml.

The mass concentration of the sample in the final extract is calculated as follows by Formula (2):

$$\rho_{\text{final extract}}^{\text{sample}} = \frac{m_{\text{sample}}}{V_{\text{ex}}} \quad (2)$$

where

m_{sample} is the mass of the analytical test portion of the sample;

V_{ex} is the volume of the extract after extraction or clean-up. Provided that the water content of the sample has been adjusted as shown in Table B.1, the extract volume (in ml) can be assumed being 20 ml (or 40 ml in case of a clean-up step according to modules C3a or C3b, involving a twofold dilution of the sample extract with acetonitrile).

As no IL-IS is used, the volume of the extract (V_{ex}) shall be known. Where dilution has taken place (module DL), the dilution factor (F_{dilution}) is to be considered, see Formula (3). Dilutions applied both to sample extract and calibration solutions alike are not to be considered in the dilution factor. The mass concentration of the sample in the final extract $\rho_{\text{final extract}}^{\text{sample}}$ (see also quantification modules under A.7) is calculated by Formula (4):

$$F_{\text{dilution}} = \frac{V_A + V_B}{V_A} \quad (3)$$

$$\rho_{\text{final extract}}^{\text{sample}} = \frac{m_{\text{sample}}}{V_{\text{ex}}} \times \frac{1}{F_{\text{dilution}}} = \frac{m_{\text{sample}}}{V_{\text{ex}}} \times \frac{V_A}{(V_A + V_B)} \quad (4)$$

where

V_A is the volume of the raw extract aliquot employed for dilution (in ml);

V_B is the volume of solvent added for dilution of V_A (in ml);

F_{dilution} is the dilution factor concerning dilutions of the final extract (excluding dilutions equally applied to the calibration standards).

Using the measured peak area of pesticide in the sample (y_A), determine the concentration of the analyte in the final extract (ρ_A) as described in CEN/TS 17061:2019, 6.4.2 to 6.4.5. Calculate the mass fraction w_A of the analyte in the sample by using Formula (1).

7.3.2 Calculation of residue concentrations using external calibration and IL-IS

The following calculation approach requires that the ratio of the IL-IS masses added to the test portions and to the calibration standard(s) $\frac{m_{\text{sample}}^{\text{IL-IS}}}{m_{\text{Cal}}^{\text{IL-IS}}}$ is known and constant. By keeping the IL-IS constant throughout the calibration levels, the peak ratio $\frac{y_A}{y_{\text{IL-IS}}}$ of each calibration level can be plotted against the absolute mass of the analyte $m_{\text{Cal}}^{\text{analyte}}$, rather than the ratio $\frac{m_{\text{Cal}}^{\text{analyte}}}{m_{\text{Cal}}^{\text{IL-IS}}}$ (the $m_{\text{Cal}}^{\text{IL-IS}}$ is set as 1). See also CEN/TS 17061:2019, 6.5.3.

The mass fraction w_A of the analyte in the sample, in milligram per kilogram, is calculated with Formula (5):

$$w_A = \frac{\left(\frac{y_A}{y_{\text{IL-IS}}} - c \right)}{b} \cdot \frac{1}{m_{\text{sample}}} \cdot \frac{m_{\text{sample}}^{\text{IL-IS}}}{m_{\text{Cal}}^{\text{IL-IS}}} \quad (5)$$

where

y_A is the peak area of the analyte in the final extract (in counts);

$y_{\text{IL-IS}}$ is the peak area of the IL-IS in the final extract (in counts);

c is the intercept of the linear calibration function;

b is the slope of the linear calibration function;

m_{sample} is the mass of test portion (in g);

$m_{\text{sample}}^{\text{IL-IS}}$ is the mass of the IL-IS added to the sample prior to extraction (irrelevant as eliminated);

$m_{\text{Cal}}^{\text{IL-IS}}$ is the mass of the IL-IS in the calibration standards (irrelevant as eliminated).

In case the IL-IS is added to an aliquot of the final extract, m_{sample} shall be exchanged by $m_{\text{sample-AL}}$, which is derived using Formula (6):

$$m_{\text{sample-AL}} = \frac{m_{\text{sample}}}{V_{\text{ex}}} \times \frac{1}{F_{\text{dilution}}} = \frac{m_{\text{sample}}}{V_{\text{ex}}} \times \frac{V_A}{(V_A + V_B)} \quad (6)$$

where

$m_{\text{sample-AL}}$ is the sample mass represented in the aliquot used to add the IL-IS (in g)

With any dilution undertaken after the addition of IL-IS to a defined aliquot are not to be considered in the dilution factor.

7.3.3 Calculation of residue concentration using the standard additions approach

The standard additions approach is the method of choice where no appropriate IL-IS is available. This approach typically compensates better for matrix effects than a matrix-matched calibration (CEN/TS 17061:2019, 6.6).

In case of the standard additions to the final extract, determine the concentration of the analyte ρ_A in the final extract using a linear regression graph of peak areas versus spiked concentrations and the mass of the sample represented by the used aliquot of the final extract, as described in CEN/TS 17061:2019, 6.6.2. Calculate the mass fraction w_A of the analyte in the sample by using Formula (1). The assumption is made, that the total volume of the sample extract is exactly 20 ml (or 40 ml in case of a clean-up step according to modules C3a or C3b, involving a twofold dilution with acetonitrile), provided that the water content of the sample has been adjusted as shown in Table B.1.

In case of standard additions to multiple test portions, determine the mass of the analyte in the weighted sample using a linear regression graph of peak areas vs. spiked analyte masses as described in CEN/TS 17061:2019, 6.6.3. The mass fraction of the analyte in the sample is the quotient of the mass of the analyte m_A in the test portion and the mass of the test portion m_{sample} .

If the signals of the standard addition extracts are outside of the linear LC-MS/MS detection range, the solutions should be diluted.

With the standard additions approach, the sought analyte concentration is determined using linear extrapolation. Therefore, it is important that the analyte has linear detection properties in the investigated calibration range.

7.3.4 Calculation of residue levels of complex residue definitions

In the case of complex legal residue definitions some components need to be expressed as defined in the residue definition by using conversion factors (Cf) derived from the molecular weights. An exemplary calculation for glufosinate is shown below.

$C_{\text{Glufosinate (sum)}}$

$$\begin{aligned} &= C_{\text{Glufosinate}} + C_{\text{N-Acetyl-Glufosinate}} \times C_{f(\text{N-Acetyl-Glufosinate} \rightarrow \text{Glufosinate})} + C_{\text{MPPA}} \times C_{f(\text{N-Acetyl-Glufosinate} \rightarrow \text{Glufosinate})} \\ &= C_{\text{Glufosinate}} + C_{\text{N-Acetyl-Glufosinate}} \times (\text{Mw}_{\text{Glufosinate}} / \text{Mw}_{\text{N-Acetyl-Glufosinate}}) + C_{\text{MPPA}} \times (\text{Mw}_{\text{Glufosinate}} / \text{Mw}_{\text{MPPA}}) \\ &= C_{\text{Glufosinate}} + C_{\text{N-Acetyl-Glufosinate}} \times (181,13 / 223,16) + C_{\text{MPPA}} \times (181,16 / 152,09) \\ &= C_{\text{Glufosinate}} + C_{\text{N-Acetyl-Glufosinate}} \times 0,811 + C_{\text{MPPA}} \times 1,191 \end{aligned}$$

8 Validity of the method

The method was validated through inter-laboratory tests for various analyte/matrix combinations. The validation data shown in [2], [3] and [13] were generated within the framework of several interlaboratory validation studies. Laboratories were asked to conduct validation experiments according to a detailed analytical protocol and to submit their data. The analytes and IL-ISs needed were provided to the laboratories by the organizers while the blank matrices were purchased and homogenized by the laboratories themselves. The type of matrices to be used in these studies was fixed. Overall, three separate validation rounds were conducted, each with 10 to 20 participating laboratories. It was considered essential, that a substantial number of Analyte/Matrix/Level combinations has been successfully validated by at least 8 laboratories.

Sufficient validity of the method for a certain analyte-matrix combination was assumed where at least three laboratories with at least five recovery replicates per level, achieved (IL-IS-corrected) recovery rates in the range of 80 % to 120 % and a relative standard deviation under repeatability conditions (RSD) of ≤ 20 %. The validation results can be found in [13] in aggregated form and under [12] in a detailed form. The limits of quantification depend on the analyte, the matrix and the sensitivity of the analytical devices used. Table 7 shows for which combinations of analytes and commodity groups (classification, see SANTE/11312/2021) validation data fulfilling the above criteria have been generated by the present method.

Additional validation data on this method or variants of this method can be found within [12].

The method can be used for checking the compliance of food of plant and animal origin with MRLs by enforcement controls or company own checks (see Table 8 and Table 9).

Table 7 — Overview of available validation data generated within the frame of inter-laboratory validation studies (successful validation by ≥ 3 laboratories with $n = 5$ for 2 levels)

Analyte	Commodity group (codes as used in SANTE/11312/2021)						
	High water content (1)	High acid content and high water content (2)	High oil content and very low water content (4a)	High oil content and intermediate water content (4b)	High starch and/or protein content and low water and fat content (5)	Meat (muscle) and Seafood (7)	Milk and milk products (8)
AMPA	x	x	x		x	x	x
Bromide	x	x		x	x		x
Chlorate	x	x		x	x		x
Chlormequat	x	x		x	x		x
Cyanuric acid		x					
Cyromazine	x	x		x	x		x
Daminozide	x	x		x	x		x
Ethephon	x	x	x		x	x	x
Fosetyl-Al	x	x	x		x	x	x
Glufosinate	x	x	x		x	x	x
Glyphosate	x	x	x		x	x	x
HEPA	x	x	x		x		x
Maleic hydrazide	x	x					
Melamine	x	x		x			

Analyte	Commodity group (codes as used in SANTE/11312/2021)						
	High water content (1)	High acid content and high water content (2)	High oil content and very low water content (4a)	High oil content and intermediate water content (4b)	High starch and/or protein content and low water and fat content (5)	Meat (muscle) and Seafood (7)	Milk and milk products (8)
Mepiquat	x	x		x	x		x
MPPA	x	x	x		x	x	x
N-Acetyl-Glufosinate	x	x	x		x	x	x
N-Acetyl-Glyphosate	x	x	x		x	x	x
Nereistoxin	x	x		x	x		x
Perchlorate	x	x		x	x		x
Phosphonic acid	x	x			x		x
Trimethyl-sulfonium	x	x		x	x		x

Table 8 — Overview of currently applying MRLs for commodities of plant origin and comparison to the lowest successfully validated levels (LSVLs) within the inter-laboratory study, status as of DATE [11]

Compound	High Water/ Acid content			Dry - Low Fat			Dry- High Fat		
	LSVL ^a mg/kg	N ^b	Lowest MRL mg/kg	LSVL ^a mg/kg	N ^b	Lowest MRL mg/kg	LSVL ^a mg/kg	N ^b	Lowest MRL mg/kg
AMPA ^c	0,05	12		0,1	5		0,1	6	
Bromide	0,1	10	20 /5 (berries)	10	9	50			20
Chlorate	0,01	12	0,05	0,02	12	0,05			0,05
Chlormequat (Cl)	0,01	10	0,01	0,01	10	0,01			0,01
Cyanuric acid ^c	0,05	5		0,1	1		0,1	1	
Cyromazine	0,01	10	0,01	0,01	7	0,02			0,01
Daminozide	0,01	8	0,06	0,01	5	0,06			0,1
Ethephon	0,01	10	0,05	0,02	4	0,05	0,02	6	0,1
Fosetyl	0,01	12	2 (for sum)	0,02	5	2 (for sum)	0,02	6	2 (for sum)
Glufosinate	0,03	9	0,03 (for sum)	0,06	5	0,03 (for sum)	0,06	7	0,03 (for sum)
Glyphosate	0,05	11	0,1	0,1	6	0,1	0,1	7	0,1
HEPA ^c	0,02	10		0,04	5		0,04	7	
Maleic hydrazide	0,02	4	0,2	0,04	1	0,2	0,04	1	0,2
Melamine ^c	0,01	8		0,01	7				
Mepiquat (Cl)	0,01	10	0,02	0,01	10	0,02			0,05

Compound	High Water/ Acid content			Dry - Low Fat			Dry- High Fat		
	LSVL ^a mg/kg	N ^b	Lowest MRL mg/kg	LSVL ^a mg/kg	N ^b	Lowest MRL mg/kg	LSVL ^a mg/kg	N ^b	Lowest MRL mg/kg
MPPA	0,02	11	See glufosinate	0,04	6	See glufosinate	0,04	6	See glufosinate
N-Acetyl- Glufosinate	0,02	12	See glufosinate	0,04	7	See glufosinate	0,04	7	See glufosinate
N-Acetyl- Glyphosate ^c	0,05	9		0,1	4		0,1	6	
Nereistoxin ^c	0,01	9		0,01	7				
Perchlorate ^d	0,01	11		0,02	11				
Phosphonic acid	0,1	6	1,5	0,2	5	1,5			1,5
Trimethyl- sulfonium	0,01	11	0,05	0,01	10	0,05			0,05
^a Lowest Successfully Validated Level in interlaboratory studies. ^b Number of laboratories contributing validation results. ^c Not legally relevant compound (not included in the residue definition for enforcement purposes). ^d Legally considered a contaminant.									

Table 9 — Overview of currently applying MRLs for commodities of animal origin and comparison to the lowest successfully validated levels (LSVLs) within the inter-laboratory study, status as of [11]

Compound	Liver			Milk			Kidney		
	LSVL ^a mg/kg	N ^b	Lowest MRL mg/kg	LSVL ^a mg/kg	N ^b	Lowest MRL mg/kg	LSVL ^a mg/kg	N ^b	Lowest MRL mg/kg
AMPA ^c	0,05	8		0,05	8		0,05	3	
Bromide			0,05	5	7	0,05			0,05
Chlorate			0,05	0,01	6	0,1			0,05
Chlormequat (Cl)			1,5/0,15 (poultry)	0,01	9	0,5			1,5/0,15 (poultry)
Cyanuric acid ^c	0,05	2		0,05	2		0,05	2	
Cyromazine			0,05	0,01	9	0,01			0,01
Daminozide			0,06	0,01	8	0,06			0,06
Ethephon	0,01	6	0,4/0,08 (poultry)	0,01	7	0,05	0,01	2	0,4/0,08 (poultry)
Fosetyl	0,01	7	0,5 (for sum)	0,01	7	0,5 (for sum)	0,01	3	0,5 (for sum)
Glufosinate	0,03	7	3/0,1 (poultry) (for sum)	0,03	8	0,03 (for sum)	0,03	3	3/0,1 (poultry) (for sum)

Compound	Liver			Milk			Kidney		
	LSVL ^a mg/kg	N ^b	Lowest MRL mg/kg	LSVL ^a mg/kg	N ^b	Lowest MRL mg/kg	LSVL ^a mg/kg	N ^b	Lowest MRL mg/kg
Glyphosate	0,05	8	0,05/0,2 (bovine)	0,05	8	0,05	0,05	4	0,05/ 0,1 (poultry)/ 0,5 (swine)/ 2 (bovine)
HEPA ^c				0,02	8		0,02	1	
Maleic hydrazide	0,02	2	0,1/0,02 (poultry)	0,02	1	0,07			2/ 0,02 (poultry)
Melamine ^c				0,01	8				
Mepiquat (Cl)			0,05(poultry)/ 0,07 (swine)/0,5	0,01	9	0,07			0,05 (poultry) / 0,07 (swine)/ 0,8
MPPA	0,02	8	See glufosinate	0,02	8	See glufosinate	0,02	5	See glufosinate
N-Acetyl- Glufosinate	0,02	8	See glufosinate	0,02	8	See glufosinate	0,02	4	See glufosinate
N-Acetyl- Glyphosate ^c	0,05	6		0,05	7		0,05	2	
Nereistoxin ^c				0,01	8				
Perchlorate ^d				0,01	6				
Phosphonic acid			0,5 (poultry, swine)/0,9	0,1	5	0,4			7/ 0,5 (poultry)
Trimethyl- sulfonium			0,05	0,01	9	0,1			0,05
^a Lowest Successfully Validated Level in interlaboratory studies. ^b Number of laboratories contributing validation results. ^c Not legally relevant compound (not included in the residue definition for enforcement purposes). ^d Legally considered a contaminant.									

9 Test report

The test report shall contain at least the following:

- all information necessary for the identification of the sample;
- a reference to this document (including its year of publication);
- the results and the units in which the results have been expressed;
- date and type of sampling procedure (if possible);
- date of receipt of sample in the laboratory;
- date of test;
- any particular observations made in the course of the test;
- any operations not specified in the method or regarded as optional which might have affected the results.

10 Additional information on analysis

10.1 Extraction Time

For fruit and vegetables, a shaking time of 1 min is usually sufficient. In the case of grain and other dry samples, it is important to make sure that such samples are finely ground to improve the accessibility of the residues to the extraction solvent. Furthermore, an extraction time of at least 15 min is necessary for these matrices. In the case of insufficiently comminuted test samples or if there are indications that the extraction yield of the analytes is insufficient, the extraction should be extended in time and / or supported by mechanical means (e.g. shaking machine (A.2.8), high-performance dispersing device (A.2.2)). When using a high-performance dispersing device, after adding 10 ml of acidified methanol and the IL-IS, the dispersing element is dipped into the sample and homogenized for approx. 2 min at high speed. Since the IL-IS is already in the sample, there is no need to rinse off the sample residue adhering to the dispersing element. However, the dispersing element shall always be cleaned before its use for the next sample. Shaking or soaking times should not exceed 60 min to minimize degradation of unstable analytes.

10.2 Scaling

In this document, certain masses are specified in the extraction modules E1 to E7 for the sample weight. Both extraction and clean-up can be scaled up or down independently. When scaling down the extraction (i.e. the weight of the test portion), it is of foremost importance to make sure that the portion-to-portion variability remains acceptable. Up-scaling or taking the mean value of repetitive analyses is necessary if the degree of homogeneity is insufficient.