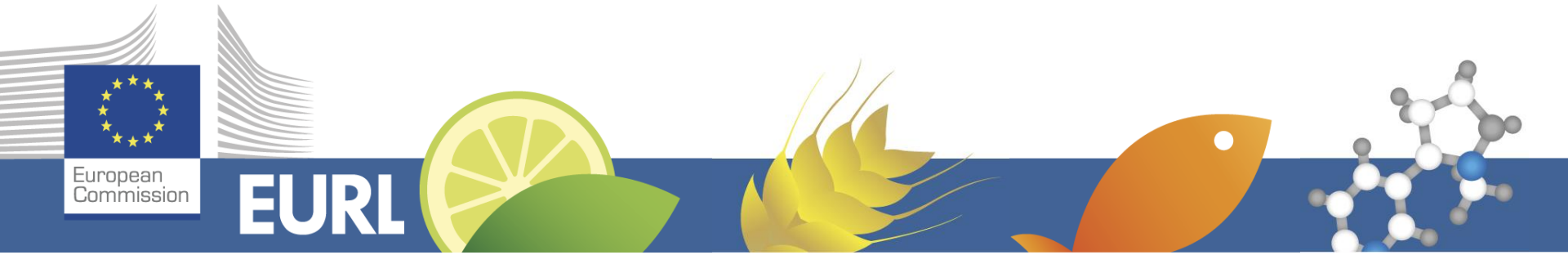
ANALYTICAL QC DOCUMENT SANTE/11813/2017

MAIN CHANGES



1

Editorial improvements throughout the entire document

2

SANTE 11945/2015

A2 This document is complementary and integral to the requirements in ISO/IEC 17025.

A3 The glossary (Appendix D) should be consulted for definitions and explanation of terms used in the text.

In accordance with Article 12 of Regulation (EC) No. 882/2004, laboratories designated for official control of pesticide residues must be accredited to ISO/IEC 17025. According to Article 11 of Regulation (EC) No. 882/2004, analytical methods used in the context of official controls shall comply with relevant European Union rules or with internationally recognised rules or protocols or, in the absence of the above, with other methods fit for the intended purpose or developed in accordance with scientific protocols. Where the above does not apply, validation of analytical methods may further take place within a single laboratory according to an internationally accepted protocol.

According to Article 28 of Regulation (EC) No. 396/2005, technical guidelines dealing with the specific validation criteria and quality control procedures in relation to analytical methods for the determination of pesticide residues may be adopted in accordance with the procedure referred to in Article 45(2) of this regulation. The present document includes mutually acceptable scientific rules for official pesticide residue analysis within the EU as agreed by all Member States of the European Union and constitutes a technical guideline in the sense of article 28 of Regulation (EC) No. 396/2005. It should thus be consulted during audits and accreditations of official pesticide residue laboratories according to ISO/IEC 17025.

New SANTE/11813/2017

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3

SANTE 11945/2015

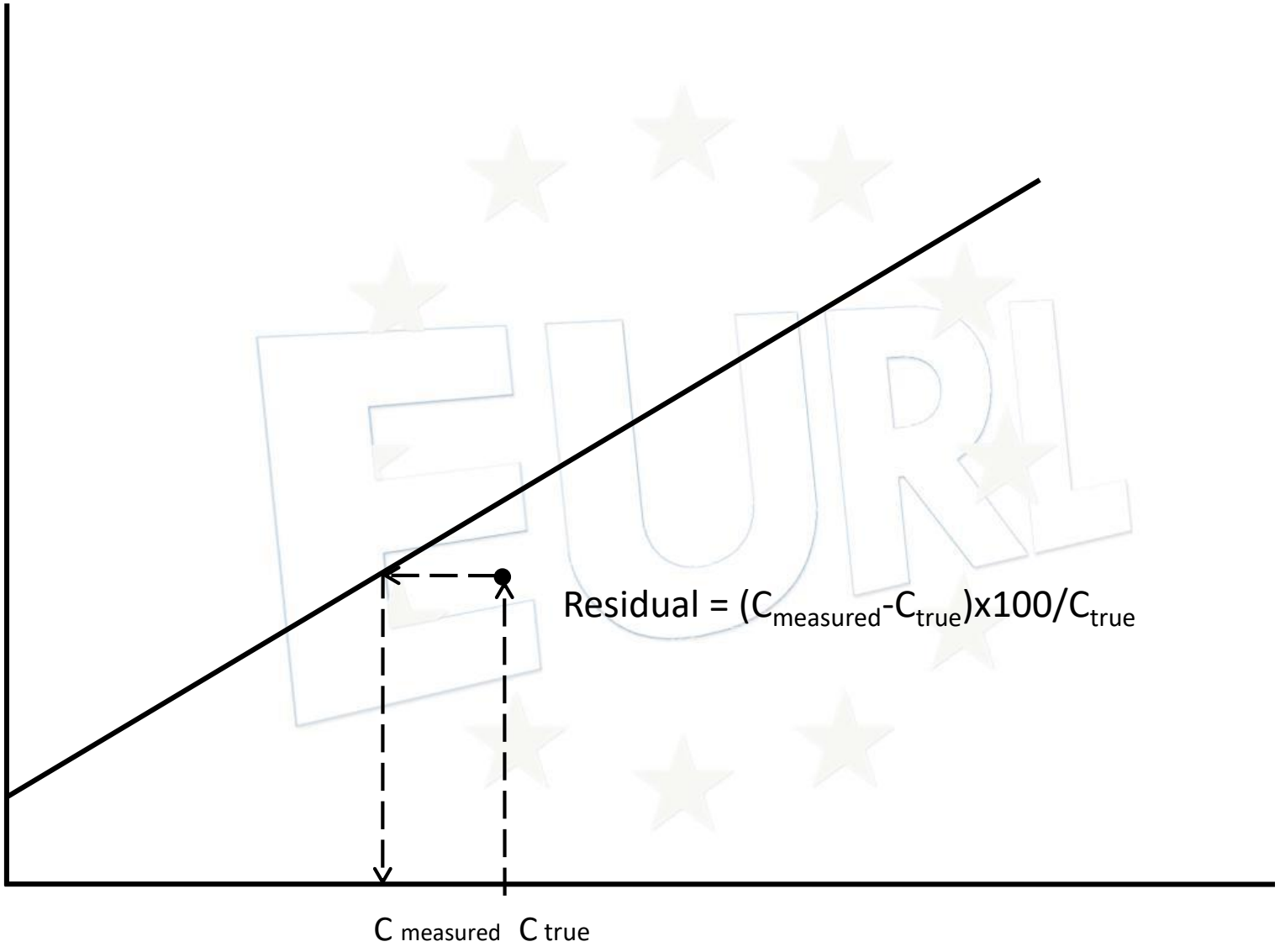
Residuals

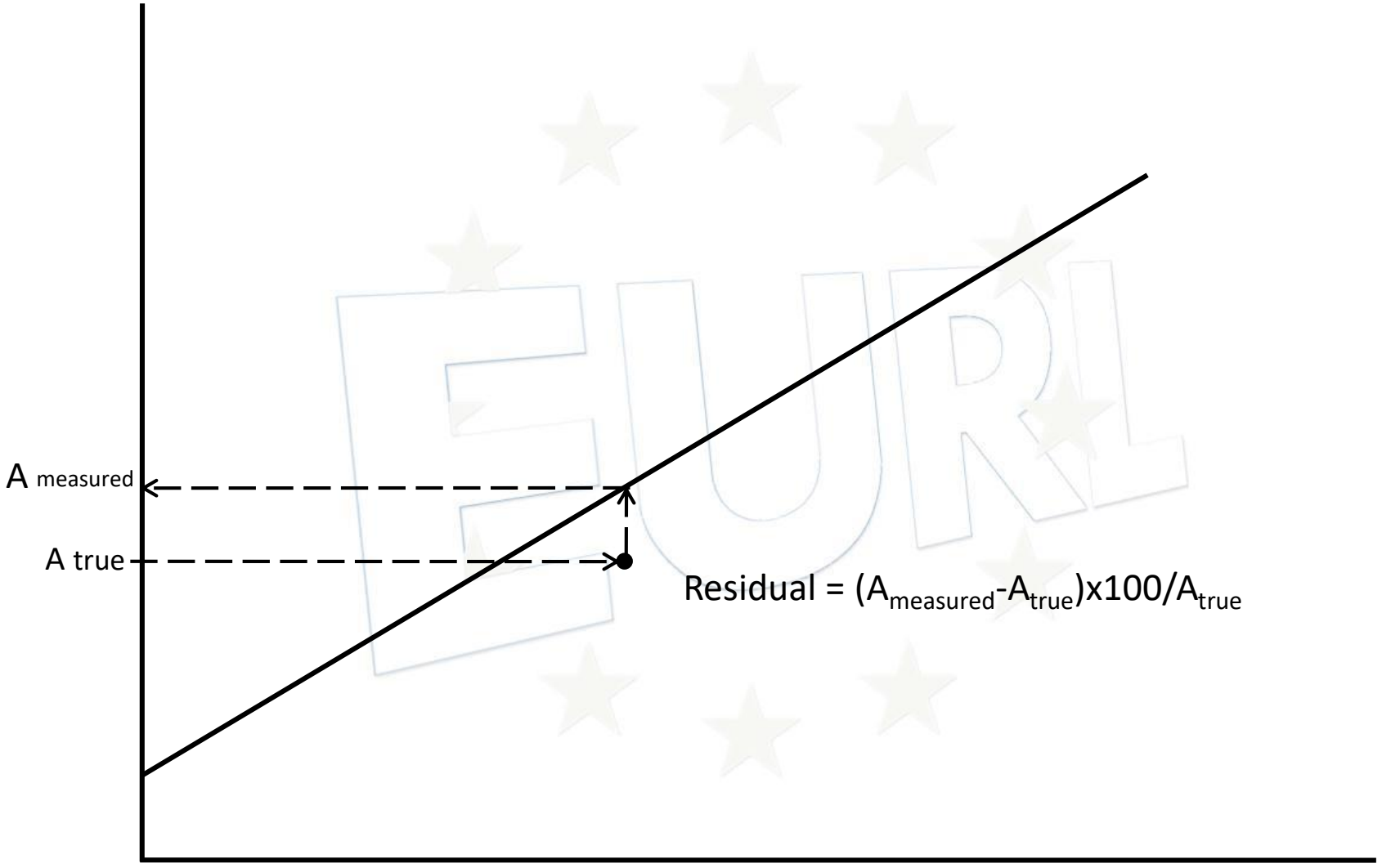
New SANTE/11813/2017

Back-calculated concentration

SANTE/11945/2015
Residuals

C17 Multi-level calibration (three or more concentrations) is preferred. An appropriate calibration function must be used and the calibration curve should not be forced through the origin without justification. The fit of the calibration function must be plotted and inspected visually and/or by calculation of the residuals, avoiding over-reliance on correlation coefficients, to ensure that the fit is satisfactory within the concentration range of the pesticides detected. If individual residuals deviate by more than ±20% from the calibration curve in the relevant region, an alternative calibration function must be used. In general, the use of weighted linear regression (1/x) is recommended, rather than linear regression.


Residual = (C measured-C true)x100/C true
C measured
C true


A measured
A true
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SANTE/11945/2015
Residuals

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Glosary

Residuals	The residuals are the deviations of the measurement values from their values predicted by the regression line.

SANTE/11813/2017
Back-calculated concentration

C17 Multi-level calibration (three or more concentrations) is preferred. An appropriate calibration function must be used (e.g. linear, quadratic, with or without weighing). The **deviation of the back-calculated concentrations of the calibration standards** from the true concentrations, **using the calibration curve** in the relevant region should not be more than ±20%.

Glosary

Deviation of back-calculated concentration	Deviation of calculated concentration of the calibration standards by the calibration function from the true concentrations Deviation of back-calculated concentration (%)= ($C_{measured}$ – C_{true})x100/C_{true}

4

SANTE/11945/2015
Identification requirements (for HRAMS)

Table 4. Identification requirements for different MS techniques[2]

MS detector / characteristics	Typical systems (examples)	Acquisition	Requirements for identification	
			minimum number of ions	other
Unit mass resolution	quadrupole, ion trap, TOF	full scan, limited m/z range, SIM	3 ions	S/N ≥ 3[e] Analyte peaks in the extracted ion chromatograms must fully overlap. Ion ratio within **±30% (relative)** of average of calibration standards from same sequence
MS/MS	triple quadrupole, ion trap, Q-trap, Q-TOF, Q-Orbitrap	selected or multiple reaction monitoring (SRM, MRM), mass resolution for precursor-ion isolation equal to or better than unit mass resolution	2 product ions	
Accurate mass measurement	High resolution MS: (Q-)TOF (Q-)Orbitrap FT-ICR-MS sector MS	full scan, limited m/z range, SIM, fragmentation with or without precursor-ion selection, or combinations thereof	2 ions with mass accuracy ≤ 5 ppm[a,b,c]	
		combined single stage MS and MS/MS with mass resolution for precursor-ion isolation equal to or better than unit mass resolution	2 ions: 1 molecular ion, (de)protonated molecule or adduct ion with mass acc. ≤ 5 ppm[a,c] *plus* 1 MS/MS product ion[d]	

[a] preferably including the molecular ion, (de)protonated molecule or adduct ion
[b] including at least one fragment ion
[c] < 1 mDa for m/z < 200
[d] no specific requirement for mass accuracy
[e] in case noise is absent, a signal should be present in at least 5 subsequent scans

SANTE/11813/2017
Identification requirements (for HRAMS)

MS detector/Characteristics		Acquisition	Requirements for identification	
Resolution	**Typical systems (examples)**		**minimum number of ions**	**other**
Unit mass resolution	Single MS quadrupole, ion trap, TOF	full scan, limited m/z range, SIM	3 ions	S/N ≥ 3[d)] Analyte peaks from both product ions in the extracted ion chromatograms must fully overlap. Ion ratio from sample extracts should be within **±30% (relative)** of average of calibration standards from same sequence
	MS/MS triple quadrupole, ion trap, Q-trap, Q-TOF, Q-Orbitrap	selected or multiple reaction monitoring (SRM, MRM), mass resolution for precursor-ion isolation equal to or better than unit mass resolution	2 product ions	
Accurate mass measurement	High resolution MS: (Q-)TOF (Q-)Orbitrap FT-ICR-MS sector MS	full scan, limited m/z range, SIM, fragmentation with or without precursor-ion selection, or combinations thereof	2 ions with mass accuracy ≤ 5 ppm[a, b, c)]	S/N ≥ 3[d)] Analyte peaks from precursor and/or product ion(s) in the extracted ion chromatograms must fully overlap. Ion ratio: see D12

5

SANTE/11945/2015

D10 The variability of ion ratios should preferably be determined from calibration standards during initial method validation and subsequently as part of the on-going QC procedure during routine analysis. In justified cases, these data may be used to set performance-based criteria, for individual analytes, rather than applying the generic criterion given in Table 4.

SANTE/11813/2017
Identification requirements

6

D11 As long as sufficient sensitivity and selectivity are obtained for both ions, and responses are within the linear range, ion ratios in unit mass resolution MS/MS have shown to be consistent[3] and should not deviate more than 30% (relative) from the reference value.

D12 For accurate mass measurement / high resolution mass spectrometry, the variability of ion ratios is not only affected by S/N of the peaks in the extracted ion chromatograms, but may also be affected by the way fragment ions are generated, and by matrix. For example, the range of precursor ions selected in a fragmentation scan event ('all ions', precursor ion range of 100 Da, 10 Da, or 1 Da) results in different populations of matrix ions in the collision cell which can affect fragmentation compared to solvent standards. Furthermore, the ratio of two ions generated in the same fragmentation scan event tends to yield more consistent ion ratios than the ratio of a precursor from a full scan event and a fragment ion from a fragmentation scan event. For this reason, no generic guidance value for ion ratio can be given. Due to the added value of accurate mass measurement, matching ion ratios are less critical, however, they should be used as indicative. Deviations exceeding 30% should be further investigated and judged with care.

7

SANTE/11945/2015 - Calculation of results (Paragraph E3)

In general, residues data do not have to be adjusted for recovery when the mean recovery is within the range of 70-120%. If residues data are adjusted for recovery, then this must be stated in the report. Exceedances of the MRL must be supported by individual recovery results (from the same batch) within the range of the mean recovery (70-120%) ± 2 x RSD, at least for the repeat confirmatory analyses. If a recovery within this range cannot be achieved, enforcement action is not necessarily precluded, but the risk of relatively poor accuracy must be taken into account. It is then highly recommended to correct for recovery, preferably by using standard addition or isotopically labelled standards, for all cases of MRL exceedances.

SANTE/11813/2017
Calculation of results (Paragraph E3)

Residues results do not have to be adjusted for recovery when the mean recovery is within the range of 80-120% and the criteria of 50% expanded measurement uncertainty is fullfilled. Exceedances of the MRL must be supported by acceptable individual recovery results (from the same batch) 7at least for the repeat confirmatory analyses. If a recovery within this range cannot be achieved, enforcement action is not necessarily precluded, but the risk of relatively poor accuracy must be taken into account. It is then recommended to use standard addition or isotopically labelled internal standards for calibration, for all cases of MRL exceedances.

8

SANTE/11945/2015
Method performance

G6 A quantitative analytical method should be demonstrated at both initial and extended validation stages, as being capable of providing acceptable mean recovery values at each spiking level and for at least one representative commodity from each of the relevant commodity groups (see Annex A). Acceptable mean recoveries are those within the range 70–120%, with an associated repeatability $RSD_r \leq 20\%$, for all analytes within the scope of a method. The LOQ is the lowest spike level of the validation meeting these method performance acceptability criteria. In certain cases and typically with multi-residue methods, recoveries outside this range may be accepted. Exceptionally, where recovery is low but consistent (i.e. demonstrating good precision) and the basis for this is well established (e.g. due to analyte distribution in a partitioning step), a mean recovery below 70% may be acceptable. However, a more accurate method should be used, if practicable. Within-laboratory reproducibility (RSD_{wR}), which may be determined from on-going QC-data in routine analyses, should be ≤ 20%, excluding any contribution due to sample heterogeneity.

SANTE/11813/2017
Method performance

G6 A quantitative analytical method should be demonstrated at both initial and extended validation stages, as being capable of providing acceptable mean recovery values at each spiking level and for at least one representative commodity from each of the relevant commodity groups (see Annex A and Table 5). Acceptable mean recoveries from initial validation are those within the range 70–120%, with an associated repeatability RSDr ≤ 20%, for all analytes within the scope of a method. The LOQ is the lowest spike level of the validation meeting these method performance acceptability criteria. Recovery rates outside the range of 70-120% can be accepted if they are consistent (RSD ≤ 20%) and the basis for this is well established (e.g. due to analyte distribution in a partitioning step) ,but the mean recovery should not be lower than 30 % or above 140 %. However, in these cases a correction for recovery is required or a more accurate method should be used, if practicable. Within-laboratory reproducibility (RSDwR), which may be determined from on-going QC-data in routine analyses, should be ≤ 20%, excluding any contribution due to sample heterogeneity.

9

SANTE/11813/2017
Method performance

G7 The validation must also be used to verify the ability of the method to identify the analyte according to the requirements specified in section D. In justified cases, the validation data may be used to set performance-based criteria, for individual analytes, rather than applying the generic criterion given in Table 4.

10

SANTE/11945/2015
Validation parameters and criteria

Table 5. Validation parameters and criteria

Parameter	What/how	Criterion	Cross reference to AQC document
Sensitivity/linearity	Linearity check from five levels	Residuals < ±20%	C14-C19
Matrix effect	Comparison of response from solvent standards and matrix-matched standards	(±20 %)	C22-C24
LOQ	Lowest spike level meeting the method performance criteria for trueness and precision	≤ MRL	G6
Specificity	Response in reagent blank and blank control samples Identification criteria	< 30% of RL	C42 Section D, Table 4
Trueness (bias)	Average recovery for spike levels tested	70-120%	G4,G6
Precision (RSD_r)	Repeatability RSD_r for spike levels tested	≤ 20%	G6
Precision (RSD_{wR})	Within-laboratory reproducibility, derived from on-going method validation / verification	≤ 20%	G6
Robustness	Average recovery and RSD_{wR}, derived from on-going method validation / verification	See above	G2, G6

SANTE/11813/2017

Table 5. Validation parameters and criteria

Parameter	What/how	Criterion	Cross reference to AQC document
Sensitivity/linearity	Linearity check from five levels	Deviation of back-calculated concentration from true concentration ≤±20%	C14-C19
Matrix effect	Comparison of response from solvent standards and matrix-matched standards	*	C22-C24
LOQ	Lowest spike level meeting the method performance criteria for trueness and precision	≤ MRL	G6
Specificity	Response in reagent blank and blank control samples	≤30% of RL	C42
Trueness (bias)	Average recovery for each spike level tested	70-120%	G3,G6
Precision (RSD_r)	Repeatability RSD_r for each spike leveltested	≤ 20%	G3, G6
Precision (RSD_{wR})	Within-laboratory reproducibility, derived from on-going method validation / verification	≤ 20%	G3, G6
Robustness	Average recovery and RSD_{wR}, derived from on-going method validation / verification	See above	G6, C40-C44
Ion ratio	Check compliance with identification requirements for MS techniques	Table 4	Section D
Retention time		±0.1 min.	D2

* in case of more than 20% signal suppression or enhancement, matrix-effects need to be addressed in calibration (C22-C30)

11 SANTE/11945/2015 Annex A. Commodity groups and representative commodities

Feed

Commodity groups	Typical commodity categories	Typical representative commodities
1. High water content	Forage crops Brassica vegetables Silage Leaves of root and vegetables	Grasses, alfalfa, clover, rape, fresh sugar beets Kale/cabbage
2. High acid content and high water content	Fruit pomace	
3. High sugar and low water content	-	
4a. High oil content and very low water content	Oil seeds cake or m	
4b. High oil content and intermediate water content	-	
5. High starch and/or protein content and low water and fat content	Cereal grain and pr thereof, incl. cereal composite feed Pulses Straw Hay	Field bean, dried broad bean, dried haricot bean (yellow, white/navy, brown, speckled), lentils Wheat, rye, barley and oat Grasses
6. "Difficult or unique commodities"	-	
7. Meat and Seafood	Animal origin based composite feed	Feed for fish farms
8. Milk and milk products	-	
9. Eggs	-	
10. Fat from food of animal origin	Fat based composite feed	Fat content above 15%

SANTE/11813/2017

Commodity groups	*Typical commodity categories within the group*[14]	*Typical representative commodities within the category*
1. High water content	Forage crops Brassica vegetables Leaves of root and tuber vegetables Root and tuber Silage	Grasses, Alfalfa, Clover, Rape Kale/Cabbage Sugar beet leaves and tops Sugar beet and fodder beet roots, carrots, potatoes Maize, clover, grasses By-products and food waste such as apple pomace, tomato pomace, potato peels, flakes and pulp, sugar beet pulp, molasses[15]
2. High acid content and high water content		By-products and food waste such as Citrus pomace [10,15]
3. High oil/fat content and very low water content	Oil seeds, oil fruits, their products and by products Fat/oil of vegetable and animal origin	Cottonseed, linseed, rapeseed, sesame seed, sunflower seed, seed, soybeans Palm oil, rapeseed oil, soya bean oil, fish oil, fatty acid distillate Compound feed with high lipid content
4. Intermediate oil content and low water content	Oil seed cake and meal	Olive, rape, sunflower, cotton-seed, soybeans cake or meal

SANTE/11813/2017

Commodity groups	*Typical commodity categories within the group*[14]	*Typical representative commodities within the category*
5. High starch and/or protein content and low water and fat content [14]	Cereal grains, their products, by-products and food waste	Barley, oat, maize, rice, rye, spelt, triticale and wheat kernels, flakes, middlings, hulls and bran. Bread, brewers' and distillers' grains Cereal based composite feed
	Legume seeds By-products and food waste	Dried beans, peas, lentils Seed hulls
6. "Difficult or unique commodities"[12]	Straw Hay	Barley, oat, maize, rice, rye and wheat straw Grasses
		By-products and food waste such as potato protein and fatty acid distillate
7. Meat and Seafood	Animal origin based composite feed	Fish meal
8. Milk and milk products	Milk	Milk replacer By-products and food waste such as whey [15]

12

SANTE/11813/2017
Glossary

Deviation of back-calculated concentration	Deviation of calculated concentration of the calibration standards by the calibration function from the true concentrations Deviation of back-calculated concentration (%)= ($C_{measured}$ – C_{true})x100/C_{true}
Mass extraction window (MEW)	**Width** of the mass range around the exact mass used to obtain the extraction ion chromatograms, e.g. exact mass ± 1 mDa or exact mass ± 5 ppm.

Thank you for your attention