ANALYTICAL QC DOCUMENT
SANTE/11813/2017

MAIN CHANGES
Editorial improvements throughout the entire document
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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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.
SANTE 11945/2015
Residuals

New
SANTE/11813/2017
Back-calculated concentration
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.
$$\text{Residual} = \left( \frac{C_{\text{measured}} - C_{\text{true}}}{C_{\text{true}}} \right) \times 100$$
Residual = \frac{(A_{\text{measured}} - A_{\text{true}}) \times 100}{A_{\text{true}}}

A_{\text{measured}}

A_{\text{true}}
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.

<table>
<thead>
<tr>
<th>Glosary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residuals</td>
</tr>
<tr>
<td>The residuals are the deviations of the measurement values from their values predicted by the regression line.</td>
</tr>
</tbody>
</table>
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%.

<table>
<thead>
<tr>
<th>Glosary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deviation of back-calculated concentration</td>
</tr>
<tr>
<td>Deviation of back-calculated concentration (%) = ((C_{\text{measured}} - C_{\text{true}}) \times 100 / C_{\text{true}})</td>
</tr>
</tbody>
</table>
# Identification requirements (for HRAMS)

## Table 4. Identification requirements for different MS techniques

<table>
<thead>
<tr>
<th>MS detector / characteristics</th>
<th>Typical systems (examples)</th>
<th>Acquisition</th>
<th>Requirements for identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unit mass resolution</td>
<td>quadrupole, ion trap, TOF</td>
<td>full scan, limited m/z range, SIM</td>
<td>3 ions</td>
</tr>
<tr>
<td>MS/MS</td>
<td>triple quadrupole, ion trap, Q-trap, Q-TOF, Q-Orbitrap</td>
<td>selected or multiple reaction monitoring (SRM, MRM), mass resolution for precursor-ion isolation equal to or better than unit mass resolution</td>
<td>2 product ions</td>
</tr>
<tr>
<td>Accurate mass measurement</td>
<td>High resolution MS: (Q-)TOF (Q-)Orbitrap FT-ICR-MS sector MS</td>
<td>full scan, limited m/z range, SIM, fragmentation with or without precursor-ion selection, or combinations thereof</td>
<td>2 ions with mass accuracy ≤ 5 ppm&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>combined single stage MS and MS/MS with mass resolution for precursor-ion isolation equal to or better than unit mass resolution</td>
<td>2 ions:&lt;br&gt;1. molecular ion, (de)protonated molecule or adduct ion with mass acc. ≤ 5 ppm&lt;sup&gt;a&lt;/sup&gt; plus&lt;br&gt;1. MS/MS product ion&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>MS detector/Characteristics</th>
<th>Typical systems (examples)</th>
<th>Acquisition</th>
<th>Requirements for identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution</td>
<td></td>
<td></td>
<td>minimum number of ions</td>
</tr>
<tr>
<td>Unit mass resolution</td>
<td>Single MS quadrupole, ion trap, TOF</td>
<td>full scan, limited m/z range, SIM</td>
<td>3 ions</td>
</tr>
<tr>
<td></td>
<td>MS/MS</td>
<td>selected or multiple reaction monitoring (SRM, MRM), mass resolution for precursor-ion isolation equal to or better than unit mass resolution</td>
<td>2 product ions</td>
</tr>
<tr>
<td>Accurate mass measurement</td>
<td>High resolution MS: (Q-)TOF (Q-)Orbitrap FT-ICR-MS sector MS</td>
<td>full scan, limited m/z range, SIM, fragmentation with or without precursor-ion selection, or combinations thereof</td>
<td>2 ions with mass accuracy ≤ 5 ppm[^a, b, c]</td>
</tr>
</tbody>
</table>
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.
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 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.
EURLs Pesticide Residues

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 fulfilled. Exceedances of the MRL must be supported by acceptable individual recovery results (from the same batch) 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 recommended to use standard addition or isotopically labelled internal standards for calibration, for all cases of MRL exceedances.
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 $\text{RSD}_r \leq 20\%$, 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 ($\text{RSD}_{\text{WR}}$), which may be determined from on-going QC-data in routine analyses, should be $\leq 20\%$, excluding any contribution due to sample heterogeneity.
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 ongoing QC-data in routine analyses, should be ≤ 20%, excluding any contribution due to sample heterogeneity.
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.
## Validation parameters and criteria

<table>
<thead>
<tr>
<th>Parameter</th>
<th>What/how</th>
<th>Criterion</th>
<th>Cross reference to AQC document</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity/linearity</td>
<td>Linearity check from five levels</td>
<td>Residuals &lt; ±20%</td>
<td>C14-C19</td>
</tr>
<tr>
<td>Matrix effect</td>
<td>Comparison of response from solvent standards and matrix-matched standards</td>
<td>(±20%)</td>
<td>C22-C24</td>
</tr>
<tr>
<td>LOQ</td>
<td>Lowest spike level meeting the method performance criteria for trueness and precision</td>
<td>≤ MRL</td>
<td>G6</td>
</tr>
<tr>
<td>Specificity</td>
<td>Response in reagent blank and blank control samples Identification criteria</td>
<td>&lt; 30% of RL</td>
<td>C42</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Section D, Table 4</td>
</tr>
<tr>
<td>Trueness (bias)</td>
<td>Average recovery for spike levels tested</td>
<td>70-120%</td>
<td>G4, G6</td>
</tr>
<tr>
<td>Precision (RSD_r)</td>
<td>Repeatability RSD_r for spike levels tested</td>
<td>≤ 20%</td>
<td>G6</td>
</tr>
<tr>
<td>Precision (RSD_WR)</td>
<td>Within-laboratory reproducibility, derived from on-going method validation / verification</td>
<td>≤ 20%</td>
<td>G6</td>
</tr>
<tr>
<td>Robustness</td>
<td>Average recovery and RSD_WR, derived from on-going method validation / verification</td>
<td>See above</td>
<td>G2, G6</td>
</tr>
</tbody>
</table>
### Table 5. Validation parameters and criteria

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

* In case of more than 20% signal suppression or enhancement, matrix-effects need to be addressed in calibration (C22-C30)
### Annex A. Commodity groups and representative commodities

<table>
<thead>
<tr>
<th>Commodity groups</th>
<th>Typical commodity categories</th>
<th>Typical representative commodities</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. High water content</td>
<td>Forage crops</td>
<td>Grasses, alfalfa, clover, rape, fresh sugar beets, Kale/cabbage</td>
</tr>
<tr>
<td></td>
<td>Brassica vegetables</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Silage</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaves of root and vegetables</td>
<td></td>
</tr>
<tr>
<td>2. High acid content and high water content</td>
<td>Fruit pomace</td>
<td></td>
</tr>
<tr>
<td>3. High sugar and low water content</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4a. High oil content and very low water content</td>
<td>Oil seeds cake or meal</td>
<td></td>
</tr>
<tr>
<td>4b. High oil content and intermediate water content</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. High starch and/or protein content and low water and fat</td>
<td>Cereal grain and pulse, incl. cereal</td>
<td>Field bean, dried broad bean, dried haricot bean (yellow, white/navy, brown, speckled), lentils, Wheat, rye, barley and oat, Grasses</td>
</tr>
<tr>
<td>content and low water and fat content</td>
<td>composite feed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pulses</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Straw</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hay</td>
<td></td>
</tr>
<tr>
<td>6. “Difficult or unique commodities”</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Meat and Seafood</td>
<td>Animal origin based composite feed</td>
<td>Feed for fish farms</td>
</tr>
<tr>
<td>8. Milk and milk products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Eggs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Fat from food of animal origin</td>
<td>Fat based composite feed</td>
<td>Fat content above 15%</td>
</tr>
<tr>
<td>Commodity groups</td>
<td>Typical commodity categories within the group(^{140})</td>
<td>Typical representative commodities within the category</td>
</tr>
<tr>
<td>-----------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>1. High water content</td>
<td>Forage crops [Brassica vegetables] Leaves of root and tuber vegetables Root and tuber Silage</td>
<td>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})</td>
</tr>
<tr>
<td>2. High acid content and high water content</td>
<td></td>
<td>By-products and food waste such as Citrus pomace (^{10,15})</td>
</tr>
<tr>
<td>3. High oil/fat content and very low water content</td>
<td>Oil seeds, oil fruits, their products and by products Fat/oil of vegetable and animal origin</td>
<td>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</td>
</tr>
<tr>
<td>4. Intermediate oil content and low water content</td>
<td>Oil seed cake and meal</td>
<td>Olive, rape, sunflower, cotton-seed, soybeans cake or meal</td>
</tr>
<tr>
<td>Commodity groups</td>
<td>Typical commodity categories within the group(^{14})</td>
<td>Typical representative commodities within the category</td>
</tr>
<tr>
<td>---------------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>5. High starch and/or protein content and low water and fat content(^{141})</td>
<td>Cereal grains, their products, by-products and food waste</td>
<td>Barley, oat, maize, rice, rye, spelt, triticale and wheat kernels, flakes, middlings, hulls and bran.</td>
</tr>
<tr>
<td></td>
<td>Legume seeds</td>
<td>Bread, brewers’ and distillers’ grains</td>
</tr>
<tr>
<td></td>
<td>By-products and food waste</td>
<td>Cereal based composite feed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dried beans, peas, lentils</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Seed hulls</td>
</tr>
<tr>
<td>6. “Difficult or unique commodities”(^{12})</td>
<td>Straw</td>
<td>Barley, oat, maize, rice, rye and wheat straw</td>
</tr>
<tr>
<td></td>
<td>Hay</td>
<td>Grasses</td>
</tr>
<tr>
<td></td>
<td></td>
<td>By-products and food waste such as potato protein and fatty acid distillate</td>
</tr>
<tr>
<td>7. Meat and Seafood</td>
<td>Animal origin based composite feed</td>
<td>Fish meal</td>
</tr>
<tr>
<td>8. Milk and milk products</td>
<td>Milk</td>
<td>Milk replacer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>By-products and food waste such as whey(^{15})</td>
</tr>
</tbody>
</table>
### Glossary

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deviation of back-calculated concentration</td>
<td>Deviation of calculated concentration of the calibration standards by the calibration function from the true concentrations.</td>
</tr>
<tr>
<td></td>
<td>[ \text{Deviation of \text{back-calculated concentration (%)} = \frac{(C_{\text{measured}} - C_{\text{true}})}{C_{\text{true}}} \times 100} ]</td>
</tr>
<tr>
<td>Mass extraction window (MEW)</td>
<td><strong>Width</strong> 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.</td>
</tr>
</tbody>
</table>
Thank you for your attention