Multi Residue analysis of pesticide residues in Fruits & Vegetables

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Multi Residue Method / Ethyl acetate Extraction

- 10g sample + 20ml EtOAc + 10g sodium sulfate + 3g NaHCO3
- In Falcon polypropylene conical tube
- Homogenization with Ultra Turrax for 1 min
- Centrifuge at 3300 rpm for 3 min
- Freeze at < -15°C for at least 2 h
- Sample dilution in MeOH and EtOAc for LC-MS/MS and GC-MS/MS measurement respectively (0.2g sample/ml extract)

Introduction: Measurement Uncertainty (MU) as the qualitative indicator of the confidence in the analytical data, describes the range around an experimental result within which the true value is expected to lie within a defined probability. The expanded measurement uncertainty (MU) was calculated following the approach recommended in Appendix C of the SANTE doc. 11945/2015 using the within laboratory reproducibility data and the results obtained from the EU Proficiency tests.

Objectives:
✓ Development and validation of Multi Residue Method for the Pesticide Residues analysis in fruits and Vegetables using LC-MS/MS & GC-MS/MS
✓ To provide evidence that the method is fit for the intended purpose

Validation Data & Results
Validation studies have been carried out in both LC-MS/MS & GC-MS/MS analytical systems. For the LC-MS/MS analysis spiked samples of 6 replicates at 2 different levels were analyzed for in total 193 pesticides in 5 different commodities, while in the GC-MS/MS analysis 6 different commodities were studied for 85 pesticides. The average recoveries of the majority of the compounds were found to be in the range of 70-120% and were characterized by precision lower than 20%.

Estimation of Measurement Uncertainty (MU)

The expanded MU is calculated using the within-laboratory reproducibility relative standard deviation combined with estimates of the method and the laboratory bias using PT data applying the equation:

\[ u' = \sqrt{u'(RSD_{WR})^2 + u'(bias)^2} \]

Within Laboratory Reproducibility
Is calculated using routine recoveries for 2 methods:
LC-MS/MS with 73 pesticides and GC-MS/MS with 72 pesticides

<table>
<thead>
<tr>
<th>EUPT</th>
<th>Pesticides</th>
<th>Lab Result mg/kg</th>
<th>Assigned Value mg/kg</th>
<th>[Bias] 2</th>
<th>Qn</th>
<th>No of results</th>
<th>True</th>
<th>Qn True</th>
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</thead>
<tbody>
<tr>
<td>EUPT - FV18, Spinach</td>
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<td></td>
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<td></td>
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<td></td>
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<tr>
<td>Chlorantraniliprole</td>
<td>2.59E+04</td>
<td>1.96E+00</td>
<td>1.01E+00</td>
<td>3.15</td>
<td>158</td>
<td>90.84E+03</td>
<td>0.0047</td>
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<tr>
<td>Cypermethrin</td>
<td>1.61E+04</td>
<td>1.65E+00</td>
<td>1.57E+00</td>
<td>2.43</td>
<td>151</td>
<td>90.74E+03</td>
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<tr>
<td>Chlorothalonil</td>
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<td>1.02E+00</td>
<td>1.01E+00</td>
<td>5.38</td>
<td>156</td>
<td>90.84E+03</td>
<td>0.0047</td>
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<tr>
<td>Etofenprox</td>
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<td>Malathion</td>
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<tr>
<td>Tebuconazole</td>
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<td>1.01E+00</td>
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<td>1.01E+00</td>
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<tr>
<td>Carbaryl</td>
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<td>90.74E+03</td>
<td>0.0047</td>
<td></td>
</tr>
</tbody>
</table>

Measurement Uncertainty:

| Expended MU: | U' = \sqrt{u'(RSD_{WR})^2 + u'(bias)^2} = 0.068 |

Results & Discussion:
✓ The validation results of the average recovery and precision are within the acceptable range of the SANTE criteria.
✓ The expanded MU of the method was estimated at 35%, lower than the default MU of 50% recommended to be used by the competent authorities in the SANTE guidelines.
✓ The method fits for the purpose to be used

Case of practical application of samples in exceeding MRL

Case 1: Strawberries
Cypermethrin: found value = 0.55 mg/kg, MRL= 0.07 mg/kg, MU=50% Result: 0.55 ± 0.28 The sample exceeds the MRL!

Case 2: Peaches
Carbendazim: found value = 0.31 mg/kg, MRL = 0.2 mg/kg, MU=50% Result: 0.31 ± 0.16 The sample does not exceed the MRL!