Hay for livestock feeding – Method validation

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Background:
Hay and grass may account for up to 100% of the feed for dairy and beef cattle. Thus hay may be of high importance for the pesticide residue exposure of livestock. Hay was therefore chosen as test material for EUPCT-CF12 carried in January/February 2018. Method performance using QuEChERS according to EN 15662 and dSPE employing three different kits (Table 1) were studied for a selection of analytes (Figure 1). Using 1 gram of sample QuEChERS (EN 15662) performed equally well or better than when modifying the method by using one of the three other dSPE kits. 402 pesticides and metabolites of pesticides were therefore validated on hay using QuEChERS extraction according to EN 15662 and analysis by LC-MSMS and GC-MSMS. The pesticides and metabolites validated are listed in Table 3. The validation was performed in accordance with the requirements outlined in SANTE/11813/20173.

Analytical procedure:

Homogenisation of test material
Chopping and Milling (sieve size 0.1 mm)

Extraction procedure employed for the validation
- Weigh 1 g hay (milled to flour)
- Add a ceramic homogenizer and 10 g water and shake briefly.
- Add 4 g MgSO4, 1 g NaCl, 1 g Na2SO4, citratehydrate and 0.5 g Na2H citrate sesquihydrate. Shake vigorously for 1 min.
- Centrifuge for 10 min at 4500 rpm

Transfer the supernatant to a 15 ml tube and store in the freezer (-20°C)
- Centrifuge for 5 min. at 4500 rpm
- Add 2 ml of the supernatant to a 15 ml tube containing 150 mg PSA and 900 mg MgSO4 and shake for 30 seconds.
- Centrifuge for 5 min. at 4500 rpm

To an aliquot add 10 µl/mg of 5% formic acid solution in acetonitrile. Dilute the extract 1:1 with acetonitrile and analyse by LC- and GC-MSMS

Analytical setup
QuEChERS: 5 µl injection on a TG-5SILMS (30mx0.25 mm ID, 0.5 µm film thickness) using QuEChERS method
GC-MSMS: 5 µl injection on a TG-5SILMS (30mx0.25 mm ID, 0.5 µm film thickness)

GC-MSMS: 5 µl injection on a TG-5SILMS (30mx0.25 mm ID, 0.5 µm film thickness) column, detection in MRM mode with 70 eV ionisation, source temp at 180°C and transfer line at 250°C.

LC-MSMS: 1 µl injection separated on a Acquity UPLC, BEH C18 (1.7 μm, 2 x 100 mm) column. Gradient elution going from 98% water ± 0.1% formic acid and 0.02% ammonium hydroxide solution to 98% methanol in 10 min. Ionisation in both positive and negative mode and detection in MRM mode.

Results:
Overall results for validation performed on hay using QuEChERS (EN 15662) with sample size reduced to 1 gram. * recoveries for chlorthalonil were 47%.

<table>
<thead>
<tr>
<th>LOQ (mg/kg)</th>
<th>Recoveries</th>
<th>RSD%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.025</td>
<td>0.05</td>
<td>0.5</td>
</tr>
<tr>
<td>Range</td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>GC-MSMS</td>
<td>219 39 28 59(47) 128 93 2-28 10</td>
<td>296</td>
</tr>
<tr>
<td>LC-MSMS</td>
<td>214 58 25 68-120 87 1-20 7</td>
<td>297</td>
</tr>
</tbody>
</table>

Total no. validated compounds excluding duplicates 402

Conclusion:
QuEChERS (EN 15662) gave satisfactory extraction of analytes from hay (Figure 1, Table 2). In total 402 pesticides and metabolites of pesticides were validated. 296 were GC-MSMS amenable compounds and 297 were LC-MSMS amenable. An LOQ of 0.025 mg/kg were obtained for majority of the pesticides and metabolites (see Table 2)

From inspection of GC-MS full scan chromatogram the hay extract obtained with QuEChERS (EN 15662) was found to be relatively low compared to the amount of co-extract observed for oat (Figure 2).