Mycotoxins, toxic secondary metabolites produced by fungi, contaminate a wide range of food commodities. Adverse effects to human and animal health lead the European Union to lay down maximum levels for certain mycotoxin-matrix combinations.[1] LC-ESI-MS has been demonstrated to be a powerful technique for the simultaneous determination of multiple mycotoxins.[2] One significant drawback of the ESI source is its high susceptibility to matrix effects (i.e. the decrease or - more rarely – the increase of the analytical signal of an analyte due to co-eluting matrix constituents). A common approach to deal with matrix effects is the compensation of the signal suppression/enhancement (SSE) through the use of matrix matched standards.

\[
RA = \frac{\text{area}_{\text{sample}}}{\text{area}_{\text{standard solvent}}} \quad \text{SSE} = \frac{\text{area}_{\text{extracted sample}}}{\text{area}_{\text{extracted standard}}}
\]

In everyday practice the calibration curve is constructed from a single lot of a matrix. However, the degree of SSE for an analyte may vary in different lots of the same matrix, which is referred to as relative matrix effect. Evidence for relative matrix effects have already been found for pesticides in apples and mycotoxins in sorghum.[3,4]

Although relative matrix effects seem to be an important aspect in the development of a quantitative LC-MS/MS method, there is a lack of guidance in official documents. According to a FDA workshop on bioanalytical method validation, SSE values of seven different lots of a matrix were measured and the corresponding RSDs calculated.[5]

Relative matrix effects: variation of SSE within different lots of the same matrix

SSE values of seven different lots of the same matrix: RSD >15%; relative matrix effects

This contribution only considers only the importance of relative matrix effects in the analysis of mycotoxins, since the LC-MS method has already been validated according to SANCO document No. 12495/2001 and has yielded 95% satisfactory results (z-score between -2 and 2; n=681) in proficiency testing.[2]

Experimental

RA and SSE values and the corresponding RSDs were determined for 70 compounds in seven matrices (Tab.1) by spiking blank samples and extracts with an appropriate amount of multi-analyte standard. 5 g of sample extracted with 20 mL ACN/H2O/HAc (79:20:1) for 90 min

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Origin</th>
<th>Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>Austria, Norway</td>
<td>16</td>
</tr>
<tr>
<td>Wheat</td>
<td>Afghanistan, Austria, USA</td>
<td>7</td>
</tr>
<tr>
<td>Figs</td>
<td>Turkey</td>
<td>7</td>
</tr>
<tr>
<td>Raisins</td>
<td>Afghanistan, Iran, Turkey</td>
<td>7</td>
</tr>
<tr>
<td>Almonds</td>
<td>Afghanistan, USA</td>
<td>7</td>
</tr>
<tr>
<td>Pistachios</td>
<td>Afghanistan, USA</td>
<td>7</td>
</tr>
<tr>
<td>Walnuts</td>
<td>Afghanistan, Chile, USA</td>
<td>7</td>
</tr>
</tbody>
</table>

Tab.1: Blank-samples were chosen to cover greatest possible diversity (e.g. origin, variety) within a matrix.

Results and Discussion

Fig. 1: Absolute RA and SSE values (top) and corresponding RSDs (bottom). Every blue dot represents one analyte.

Using replicates derived from a single individual sample for method validation leads to an underestimation of measurement uncertainty.

Next to relative matrix effects, other effects (e.g. different extraction efficiency, (destabilisation of the analyte by matrix components) contribute to the overall variation.

Fig. 2: Comparison of measurement uncertainty for 70 analytes in maize. Every blue dot represents one analyte.

Fig. 3: Comparison of individual RA (top) and SSE (bottom) values for two matrix combinations.

Conclusion

- 80-100% of the evaluated analytes exhibit negligible relative matrix effects
- Relative matrix effects: lead to an underestimation of measurement uncertainty can cause a lack of reproducibility should be considered during initial method validation should be included in official guidelines

Outlook:

Quantification of importance of relative matrix effects in the uncertainty budget

References