Development of a LC-QTOF screening method for pesticides and mycotoxins in cereals

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Introduction: The EURL for Pesticide residues in Cereals and Feeding stuff¹ has today a pesticide screening method using LC-QTOF (Agilent) with a database containing retention times of around 600 pesticides injected into the instrument. Other contaminants in cereals might also be relevant to include e.g. mycotoxins produced by fungi, which can arise at the field or during storage of cereals.

The aim of this project was to include relevant mycotoxins into the current pesticide screening method. In this study extraction and clean up of 25 mycotoxins and other secondary metabolites were investigated simultaneously together with selected pesticides. Recovery and matrix effect during the QuEChERS method were investigated.

Experimental: 25 mycotoxins at relevant concentration levels and 113 pesticides at 0.1 mg/kg were spiked into wheat flour (N=4). Spiked samples and blank samples were cleaned up using the QuEChERS method, and samples were taken after the 4 different steps specified in figure 1: After extraction with water and acetonitrile followed up with centrifugation (step 1), after phase separation with magnesium sulphate (step 2), after a freeze out step to remove lipids (step 3) and finally after PSA clean up (step 4). Matrix matched standards at the four steps using the blank samples and standards in solvent were prepared. Recovery and Matrix-effect was calculated using the following equations:

\[
\text{Recovery} = \frac{\text{Area (spiked sample)}}{\text{Area (matrix standard)}} \times 100\%
\]

\[
\text{Matrix effect} = \frac{\text{Area (matrix standard)}}{\text{Area (pure standard)}} \times 100\%
\]

Discussions: The matrix components during the four steps are illustrated in Figure 2. It shows that more and more matrix-components are removed and the final PSA clean-up step 4 really removes significant amounts of matrix. Analytes are shown in black. Recovery and matrix effect were similar in step 2-4 for most analytes, but some analytes had less matrix-effect after freezing and certainly after the PSA clean-up (Figure 3). However, several of the mycotoxins containing carboxylic acid groups are removed totally during the PSA step 3 (Figure 2). Recovery of the Fumonisins declines already after the freezing step 3. To choose the final sample preparation method a compromise is needed. Some matrix-effect is removed during the freezing step and therefore it was chosen to stop after step 3 before the PSA clean-up step 4, which removes acid analytes. Matrix components and Total Ion Chromatograms of other cereals are shown in Figure 4. It is seen that Oat and Barley has more matrix components than wheat, and therefore more matrix-effect in these cereals are expected.

Conclusions: The QuEChERS method included a freezing step but without PSA clean up was chosen as the best compromise to analyse mycotoxins and pesticides in cereals.

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