Assessment of Relative Matrix Effects for a "Dilute and Shoot" Multi-Mycotoxin LC-MS/MS Method

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Introduction

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 laying 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.

$$RA = \frac{area_{spiked\ sample}}{area_{neat\ solvent\ standard}} \qquad SSE = \frac{area_{spiked\ extract}}{area_{neat\ solvent\ standard}}$$

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 been already 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 accoring to SANCO document No. 12495/2001 and has yielded 93% 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 matrixes (Tab.1) by spiking blank samples and extracts with an apprortiate amount of multi-analyte standard.

Matrix	Origin	Replicates
Maize	Austria, Namibia, Switzerland	7
Wheat	Afghanistan, Austria, Ethopia	16
Figs	Turkey	7
Rasins	Afghanistan, Iran, Turkey	7
Almonds	Afghanistan, USA	7
Pistachios	Afghanistan, USA	7
Walnuts	Afghanistan, Chile, USA	7

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

Sample preparation and LC-ESI-MS/MS analysis scheme

Extraction
5 g of sample extracted with 20 mL ACN/H2O/HAc (79:20:1) for 90 min

Dilution
Supernatant was diluted (1:1) with ACN/H2O/HAc (20:79:1)

LC-ESI-MS
Agilent 1290 HPLC - Phenomenex Gemini C18, 150x4.6 mm, 5 µm
AB SCIEX QTRAP 5500 in scheduled MRM mode
5 µl of diluted raw extract injected in a solvent flow of 1 mL/min, 2 injections (pos/neg)

Results and Discussion

Absolute SSE and RA values and the corresponding RSDs

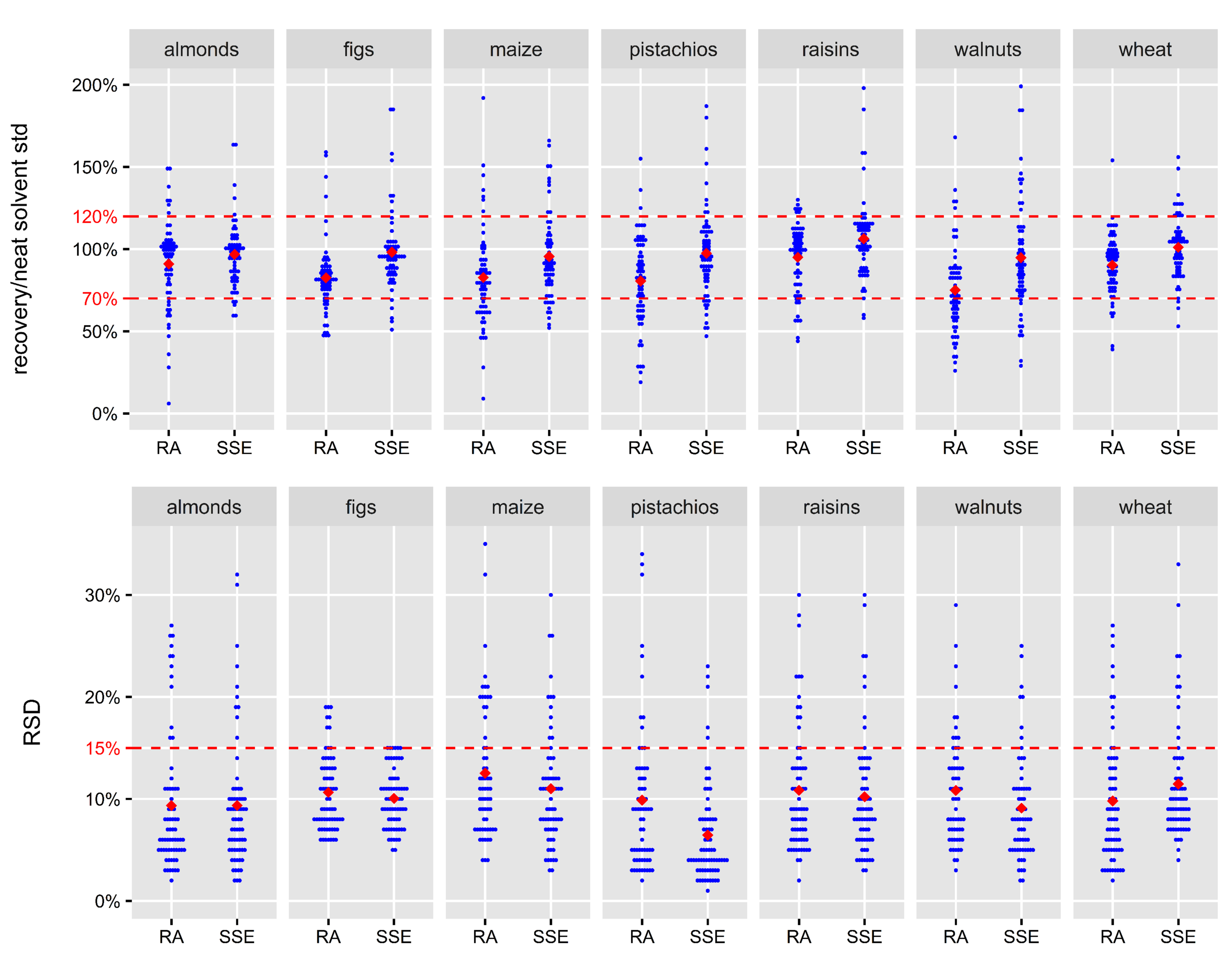

Fig.1: Absolute RA and SSE values (top) and corresponding RSDs (bottom). Every blue dot represents one analyte.

Relevance of relative matrix effects in seven matrixes

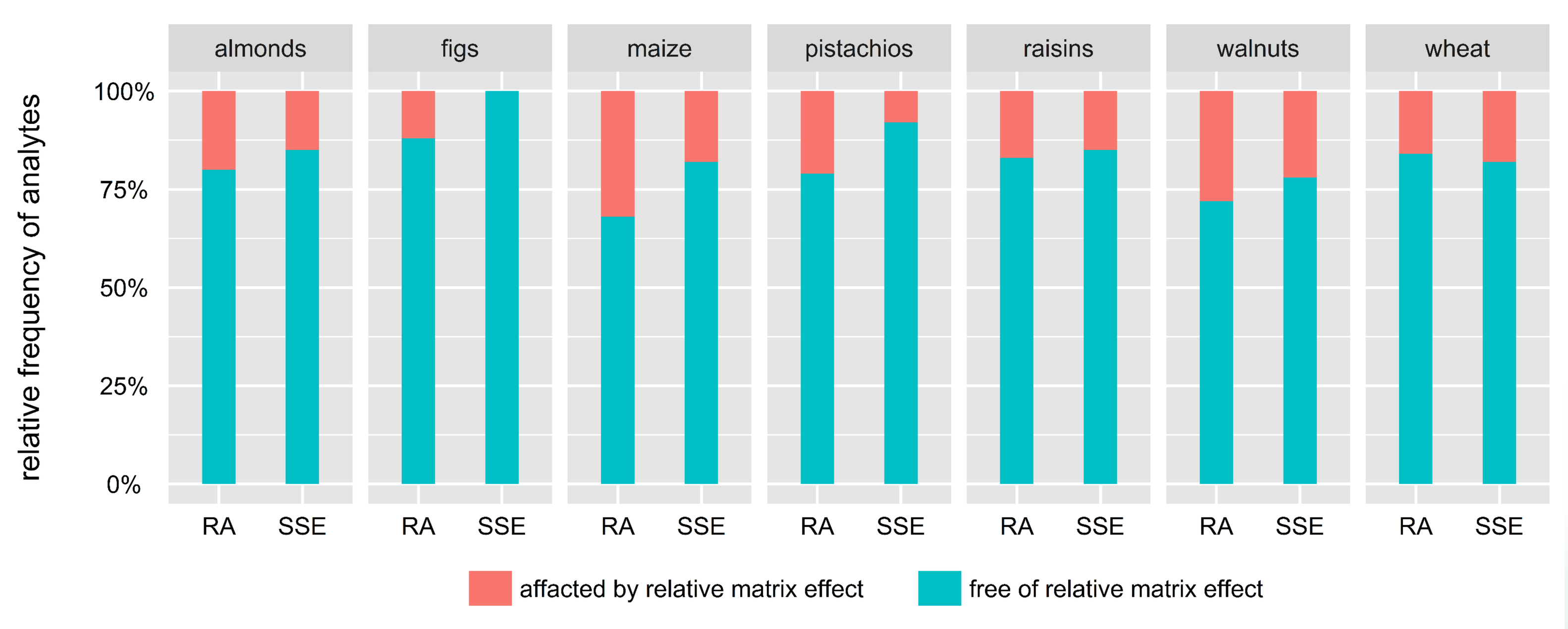

Fig.4: Analyte-matrix combination affected by relative matrix effects and ist importance in the accuracy of the bias.

Underestimation of Uncertainty

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

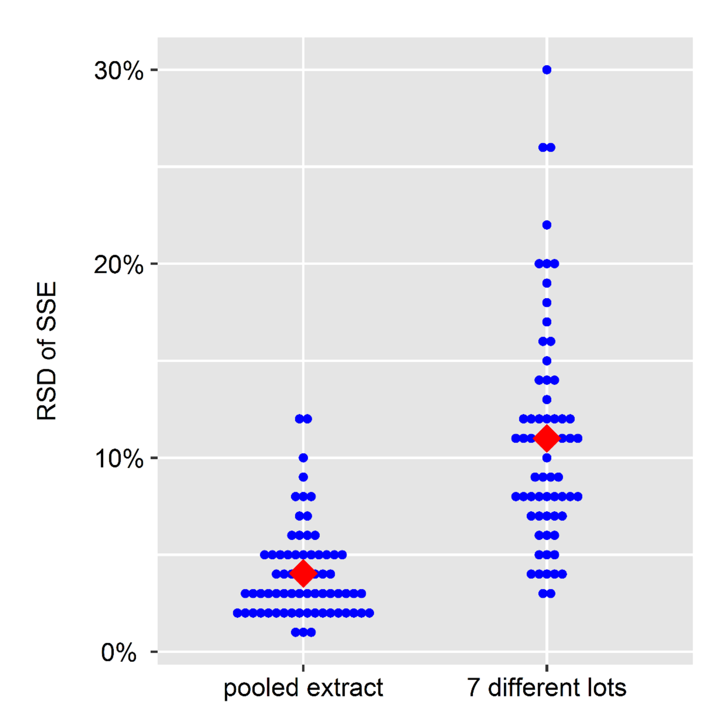

Fig.2: Comparison of measurment uncertainties for 70 analytes in maize. Every blue dot represents one analyte.

Different Sources of Uncertainty

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

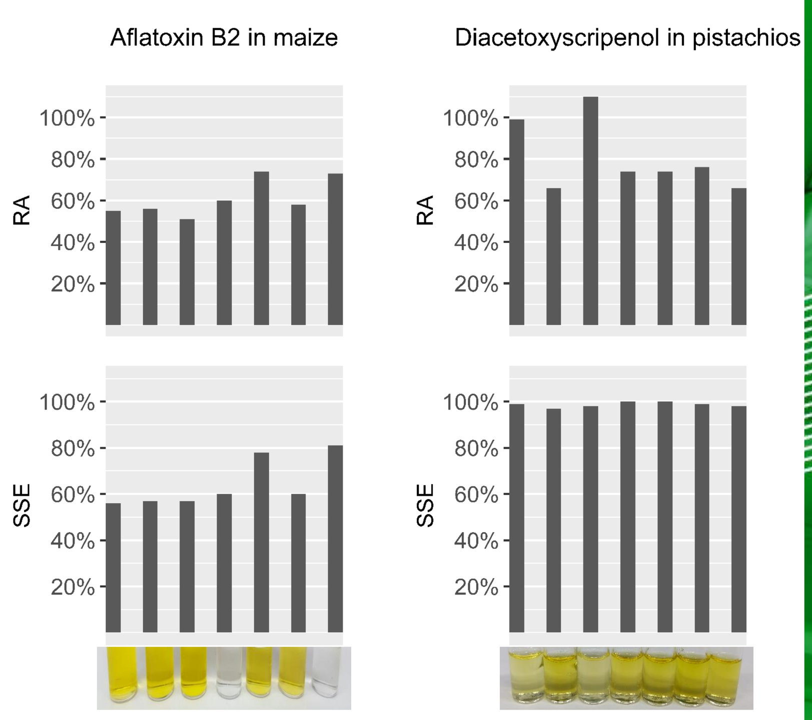

Fig.3: Comparison of individual RA (top) and SSE (bottom) values for two analyte matrix combinations.

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

Acknowledgement

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References

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