Lot-to-lot variation and its impact on the accuracy of LC-MS based multi-mycotoxin analysis

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Introduction

Multi-mycotoxin determination is based on LC-ESI-MS/MS in combination with an extraction procedure that recovers a broad range of analytes [1]. In most cases, raw extracts are diluted and injected with limited or even no sample clean-up, i.e. "*dilute and shoot*", as clean-up steps would remove some of the analytes for further analysis. Compromised sample preparation and LC-MS conditions might lead to incomplete extraction recovery (R_E) and signal suppression/enhancement (SSE) result in a method bias, which is expressed as apparent recovery (R_A). To calculate the concentration of a mycotoxin in the sample, the response of the sample is compared to the response of a calibration standard and, if necessary, corrected for R_A:

$$c_{mycotoxin} = \frac{area_{mycotoxin\ in\ sample}}{area_{mycotoxin\ in\ standard}} * \frac{1}{R_A}$$

The calculated concentration of the analyte needs to be associated with the expanded measurement uncertainty (U):

$$c_{mycotoxin} \pm U$$

For a result that is corrected for R_A, the relative standard uncertainty associated with R_A (u_{RA}) needs to be accounted for in the estimation of the relative expanded measurement uncertainty (U_r). In everyday practice, u_{RA} is estimated based on replicate analysis of a single lot of a matrix. However, due to the heterogeneous nature of a matrix, R_A may vary for different lots of the same matrix *i.e.* "*lot-to-lot variation*". Although the lot-to-lot variation caused different SSE for mycotoxins in different lots of the same matrix (*e.g.* sorghum [2]), its effect on the measurement uncertainty remains unstudied.

Hypothesis:

Neglecting lot-to-lot variation during method validation can lead to an underestimation of u_{RA}

Objective:

Estimation of the contribution of lot-to-lot variation to U_r

This study presents the first calculation of U_r for the determination of mycotoxins in food and feed considering the lot-to-lot variation, and differs significantly from studies which evaluated U_r under repeatability conditions of a single lot of a matrix.

Experimental

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

Extraction:

5 g of sample were extracted with 20 mL ACN/H_2O/HAc (79:20:1) for 90 min

Dilution:

Supernatant was diluted (1:1) with ACN/H_2O/HAc (20:79:1)

LC-ESI-MS/MS:

Agilent 1290 HPLC - Phenomenex Gemini C18, 150 x 4.6 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)

Calculation of RA

$$R_A = \frac{area_{spiked\ sample}}{area_{neat\ solvent\ standard}}$$

Estimation of the contribution of lot-to-lot variation to the methods accuracy [3]

$u_{r,RA}$ was calculated as the RSD from replicate analysis of the R_A value of one lot ($u_{r,RA_{single\ lot}}$) and from the R_A values of seven different lots ($u_{r,RA_{lot-to-lot}}$). The contribution of lot-to-lot variation to R_A was evaluated by comparing $u_{r,RA_{single\ lot}}$ to $u_{r,RA_{lot-to-lot}}$. U_r was calculated for each analyte from the relative standard uncertainty of the within-laboratory precision ($u_{r,wL}$) and $u_{r,RA}$. $u_{r,wL}$ was calculated as the RSD of R_A values of the same lot measured over a long time interval. The contribution to U_r was evaluated by comparing U_r calculated based on a single lot ($U_{r,single\ lot}$) to $U_{r,lot-to-\ lot}$ where the lot-to-lot variation is considered as an error source.

$$u_{r,RA_{single\ lot}} = RSD(R_A\ of\ 7\ aliquots\ of\ 1\ lot) \qquad U_{r,single\ lot} = 2 * \sqrt{u_{r,wL}^2 + u_{r,RA_{single\ lot}}^2}$$

$$u_{r,RA_{lot-to-lot}} = RSD(R_A\ of\ 1\ aliquot\ of\ 7\ lots) \qquad U_{r,lot-to-\ lot} = 2 * \sqrt{u_{r,wL}^2 + u_{r,RA_{lot-to-lot}}^2}$$

Results

Contribution of lot-to-lot variation to the uncertainty of the apparent recovery

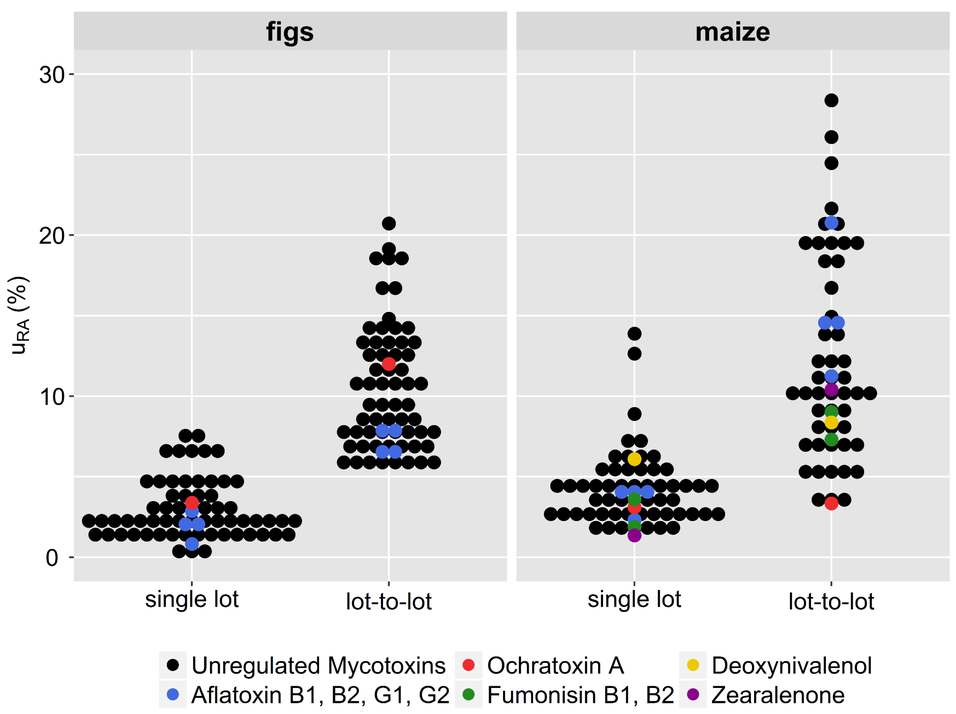

Fig. 1: Comparison of the uncertainty of the method bias RA (u_{RA}) calculated as the relative standard deviation of the RA values of seven aliquots of a single lot of a matrix ($u_{RA,single\ lot}$) and one aliquot of seven different lots of a matrix ($u_{RA,lot-to-lot}$), respectively. The evaluation was carried out for 66 mycotoxins in figs and maize [3].

Contribution of lot-to-lot variation to the relative expanded measurement uncertainty

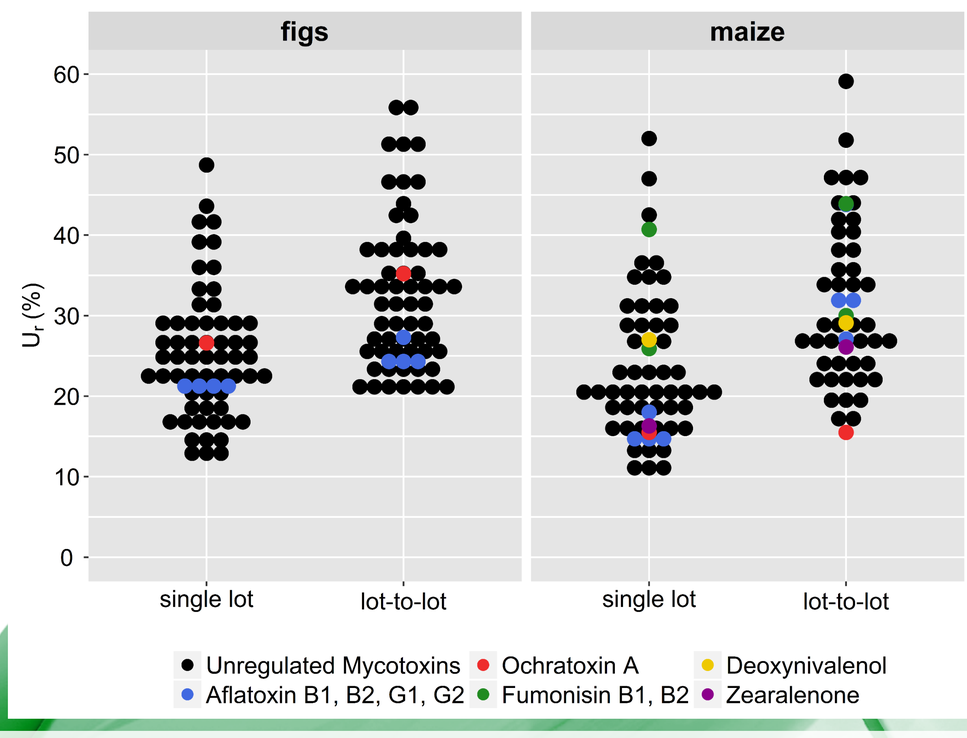

Fig. 2: Relative expanded measurement uncertainty (U_r) for 66 mycotoxins in figs and maize [3]. $U_{r,single\ lot}$ was evaluated from a single lot of a matrix and does not account for the lot-to-lot variation. $U_{r,lot-to-\ lot}$ was evaluated based on seven different lots of a matrix and accounts for the lot-to-lot variation.

Discussion

The contribution of lot-to-lot variation to the accuracy of an LC-MS based multi-mycotoxin assay

In both matrices, the lot-to-lot variation contributed to u_{RA} either due to differences in analyte recovery or relative matrix effects. Thus method validation that is based on a single lot might lead to overoptimistic uncertainties. Relevant validation guidelines call for the evaluation of RE, SSE and RA. However, it is often not specified whether these performance parameter have to be evaluated based on a single lot or different lots of a matrix. In extreme cases, analytes that might pass validation based on a single lot might fail validation when the lot-to-lot variation is considered.

The increase in u_{RA} caused by the lot-to-lot variation was shown to lead to a higher expanded measurement uncertainty. Therefore, the consideration of the lot-to-lot variation leads to a more realistic estimate of the uncertainty associated with the measurement result and should be required by the official guidelines on mycotoxin analysis.

References

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Conclusion

- Lot-to-lot variation can contribute to u_{RA} and thus to U_r.
- The major contribution of lot-to-lot variation to u_{RA} were differences in R_E in figs and differences in SSE (relative matrix effects) in maize.
- Considering lot-to-lot variation during method validation leads to a more realistic estimate of the expanded measurement uncertainty
- For the described multi-mycotoxin assay we propose a fit-for-purpose U_r of 50 %, independent of the concentration of the analyte.

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