Lot-to-lot variation in LC-MS based multi-mycotoxin determination and its contribution to the measurement uncertainty

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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]. The conditions for extraction, chromatographic separation and detection cannot be optimal for each of the target analytes. Incomplete extraction recovery (RE) and signal suppression/enhancement (SSE) result in a method bias, which is expressed as apparent recovery (RA). 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 RA:

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

The calculated concentration of the analyte needs to be associated with the expanded measurement uncertainty ($U_{k=2}$):

$$c_{mycotoxin} \pm U_{k=2}$$

The most important inconsistency in analytical practice concerns the evaluation of the bias and whether or not a bias correction is applied [2].

For a result that is corrected for RA, the uncertainty associated with RA (u_{RA}) needs to be accounted for in the estimation of $U_{k=2}$. In everyday practice, u_{RA} is estimated based on replicate analysis of a single lot of a matrix. However, due to heterogeneous nature of a matrix, RA 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 rice [3] and sorghum [4], its effect on the measurement uncertainty remains unstudied.

Hypothesis:

The lot-to-lot variation leads to an increase of the measurement uncertainty.

Objective:

Evaluation of the effect of the lot-to-lot variation on the measurement uncertainty.

This study presents the first calculation of the measurement uncertainty of 66 mycotoxins in maize and figs under the consideration of the lot-to-lot variation, and differs significantly from assays which were evaluated based on 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

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

Calculation of the measurement uncertainty

$U_{k=2}$ was calculated for each analyte from the within-laboratory precision (u_{wL}) and u_{RA}. $u_{r,wL}$ was calculated as the RSD of RA values of the same lot measured over a long time interval (7 sample in 7 weeks). $u_{r,RA}$ was calculated as the RSD from replicate analysis of the RA value of one lot ($u_{r,RA_{1\ lot}}$) and from the RA values of 7 different lots ($u_{r,RA_{7\ lots}}$). To evaluate the effect of the lot-to-lot variation on the measurement uncertainty, U calculated based on a single lot ($U_{r,single\ lot}$) was compared to $U_{r,lot-to-lot}$ where the lot-to-lot variation is considered as an error source.

$$U_{r,single\ lot} = 2 * \sqrt{u_{r,wL}^2 + u_{r,RA_{1\ lot}}^2}$$

$$U_{r,lot-to-lot} = 2 * \sqrt{u_{r,wL}^2 + u_{r,RA_{7\ lots}}^2}$$

Results

Influence of the lot-to-lot variation on the measurement uncertainty of a multi-mycotoxin method

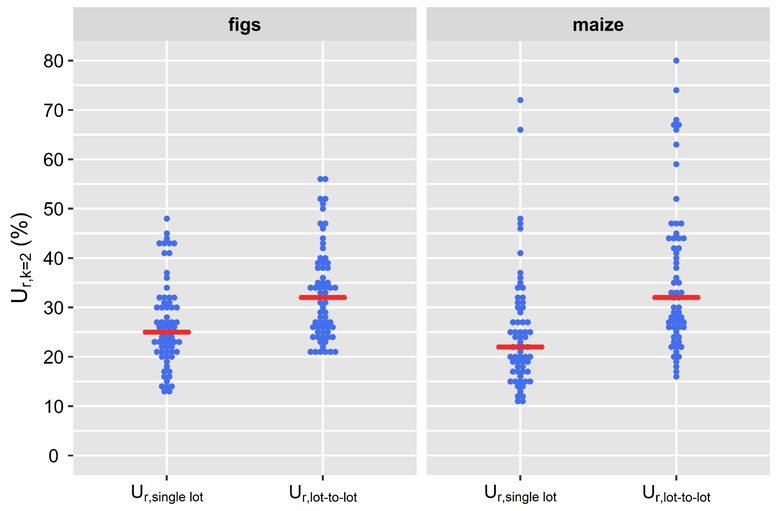

Fig. 1: Comparison of the relative expanded measurement uncertainty ($U_{r,k=2}$) neglecting the lot-to-lot variation ($U_{r,single\ lot}$) to $U_{r,lot-to-lot}$ which accounted for the lot-to-lot variation for 66 mycotoxins (blue) in figs and maize. Median value is indicated in red.

figs
- Increase of median $U_{r,k=2}$ from 25 % to 32 %
- Mainly due to differences in RE

maize
- Increase of median $U_{r,k=2}$ from 22 % to 32 %
- Mainly due to differences in SSE
  = relative matrix effects

Influence of the lot-to-lot variation on the measurement uncertainty of regulated mycotoxins

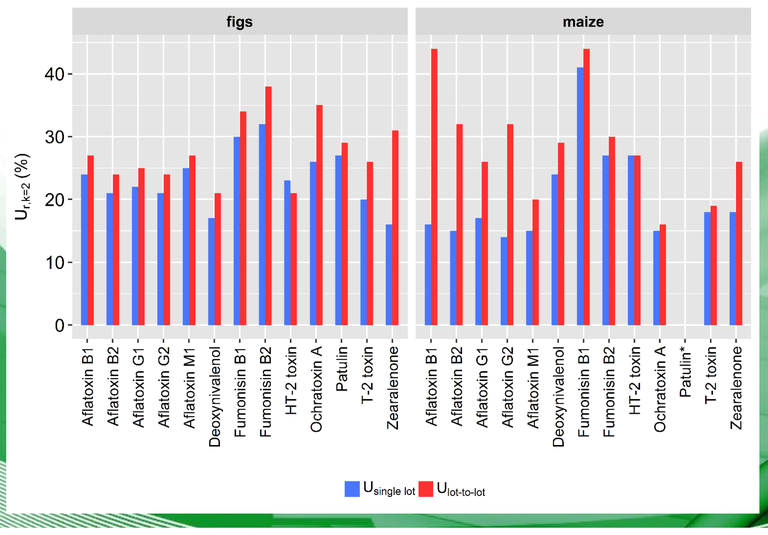

Fig. 2: Comparison $U_{r,single\ lot}$ (blue) to $U_{r,lot-to-lot}$ (red) for the regulated mycotoxins in figs and maize.* = not evaluated.

figs
- Increase of U for all regulated MT, except HT-2 toxin
- Especially for ochratoxin, T-2 toxin and zearalenone

maize
- Increase of U for all regulated MT, except HT-2 toxin
- Especially for the aflatoxins and zearalenone

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Discussion

Role of lot-to-lot variation in the estimation of accuracy

The increase of $U_{k=2}$ due to the lot-to-lot variation implies that method validation based on replicate analysis of a single sample leads to an underestimation the measurement uncertainty. Using different lots of a matrix for method validation accounts for the inhomogeneity within a matrix and gives a more realistic estimation of the accuracy of a method. Ideally, the lots used in method validation would be representative for the whole variation that can occur within the matrix being studied. Even though 7 different lots may not cover the whole intra-matrix variation, the likelihood of detecting a decrease in accuracy due to the lot-to-lot variation dramatically increases. Therefore, clearly written instructions on method validation based on different lots of a matrix should be included in the official guidelines as has already be done by the FDA for bioanalytical method validation [5].

Reduction strategies

The performed experiments provide an estimation of the increase of $U_{k=2}$ due to the lot-to-lot variation, but did not aim to reduce or eliminate it. In the case of the presented multi-mycotoxin method, changes in the extraction procedure and quantitation strategy are not feasible as it has been carefully optimized for routine analysis of a broad range of analytes. Isotopically labeled standards, if commercially available and economically feasible, spiked before or after extraction would compensate for differences in RA and SSE, respectively. To reduce effect of the lot-to-lot variation, separate calibration on a variety level (e.g. red and white sorghum [3]; peeled, brown and red Arborio rice [4]) can be performed. For analytes occurring at a higher mass fraction (*e.g* fumonisins and deoxynivalenol), sample extracts could be diluted, which is known to lead to a decrease of SSE [6]. For reliable quantitation close to a critical level, *e.g.* the legal limit, standard addition should be considered.

Conclusion

- The measurement uncertainty was calculated for 66 mycotoxins in figs and maize.
- The consideration of the lot-to-lot variation as an error source lead to increase of $U_{r,k=2}$ from 25 % to 32 % in figs and 22 % to 32 % in maize.
- Estimation of $U_{k=2}$ based on different lots of a matrix gives a more realistic estimation of the accuracy. Detailed instruction should be included in the official guidelines.
- To reduce the effect of the lot-to-lot variation, the use isotopically labeled standards, separate calibration on a variety level, dilution of the sample extracts and standard addition should be considered.

Acknowledgement

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