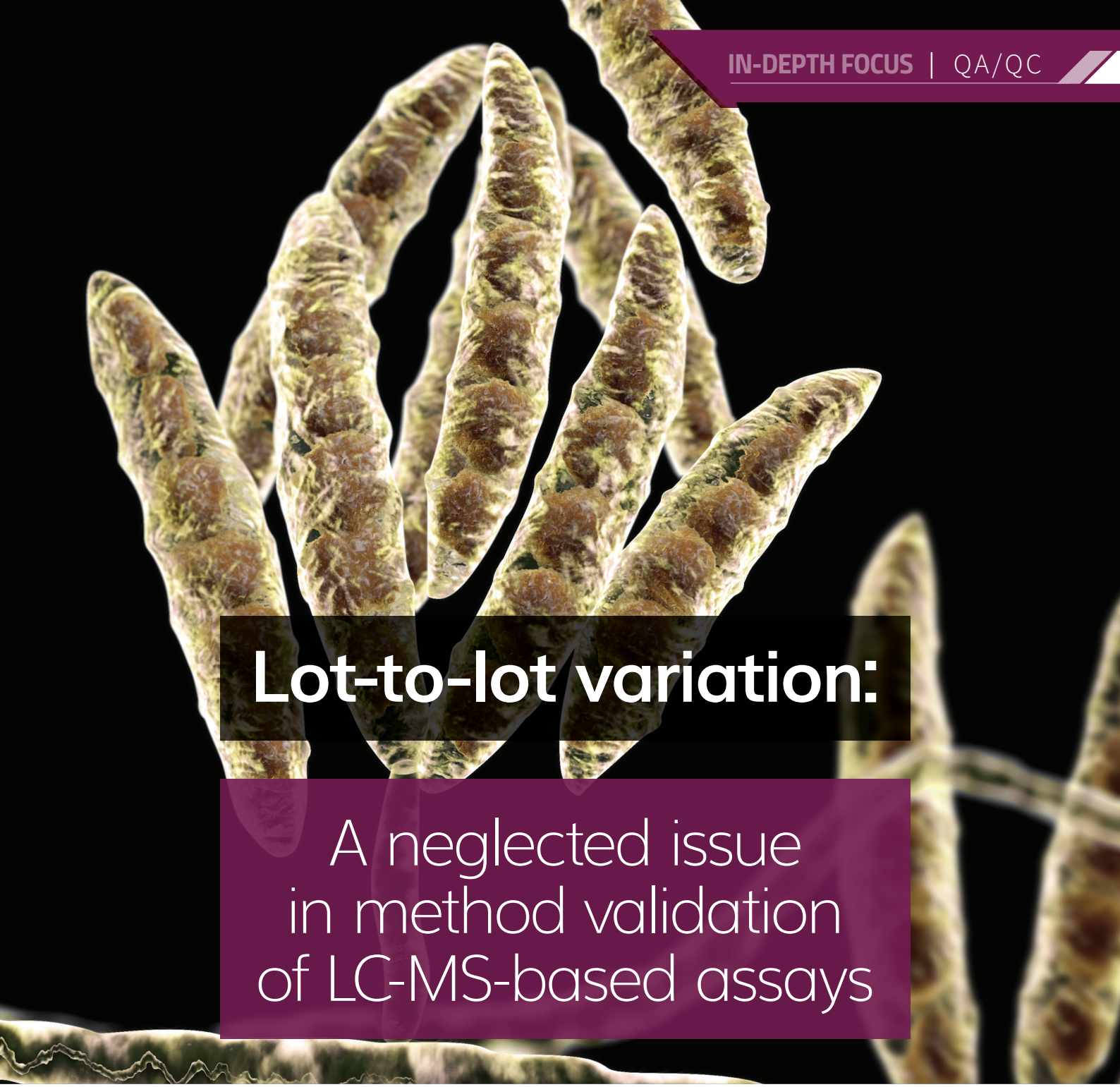

Lot-to-lot variation:

A neglected issue in method validation of LC-MS-based assays

Food analysis, particularly the determination of contaminants and residues, is often based on LC-MS methods in combination with external solvent-based or matrix-matched calibration. The performance of such methods is typically evaluated by in-house validation from replicate analysis of a single lot of a matrix. However, different lots of a matrix might have different extraction recovery factors (R_E) or signal suppression/enhancement (SSE) effects, resulting in lot-to-lot variation. Therefore, failing to consider this variation might lead to an underestimation of the uncertainty of the measurement result. Here, *David Stadler* and *Rudolf Krska* from the University of Natural Resources and Life Sciences, Vienna, discuss the impact of the lot-to-lot variation on the accuracy of a multi-mycotoxin assay.›

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> *Mycotoxins belong to the category of most feared food contaminants*

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ENSURING food safety has become of increasing concern for food producers, especially due to the complexity of a globalised food-supply chain, increased public awareness and media attention on food quality, as well as – most importantly –potential health implications. Mycotoxins, (toxic secondary metabolites produced by fungi), can contaminate food commodities either on the field or during storage and belong to the category of most feared food contaminants. The potential health risk associated with a mycotoxin contamination of the food supply has been recognised by regulatory bodies, such as the European Commission (EC), which have imposed maximum levels for major mycotoxins[1,2]. Comprehensive multi-mycotoxin methods, covering several hundred analytes, allow for the simultaneous determination of the whole spectrum of mycotoxins that occur in food and feed chains.

Multi-mycotoxin methods are commonly based on liquid chromatography coupled to electrospray ionisation – tandem mass spectrometry (LC-ESI-MS/MS) in combination with an extraction procedure that recovers a broad range of analytes[3-6]. 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. Quantification is commonly based on external solvent-based or matrix-matched calibration. Stable isotope dilution analysis is limited to mycotoxins for which ^{13}C-labelled isotopologues are available[7-8]. Standard addition would result in multiple injections per sample and is therefore not popular in routine analysis.

In our laboratory, we use external solvent-based calibration, as one calibration curve can be used for the quantification of the analytes in different matrices. For matrix-matched calibration, blank samples are often hard to obtain and a separate calibration curve must be made for each individual matrix. Using external solvent solvent-based calibration, the measured value is obtained by comparing the response of the analyte to the calibration curve and, if necessary, a correction for the method bias is applied. The method bias, expressed as apparent recovery (RA)[9], may be caused by losses during the recovery procedure (RE) or due to matrix effects, expressed as signal suppression/enhancement (SSE).

Method validation is an integral part of good analytical practice and ensures that the analytical procedure is suitable for its intended use. As external quality control schemes, such as proficiency test schemes or certified reference materials (CRMs), are mostly limited to the regulated mycotoxins, proper in-house validation is crucial. In-house validation includes the determination of linearity, R_E, *SSE*, R_A, limit of quantification and measurement uncertainty[3,5,10]. The extraction and LC conditions of multi-mycotoxin assays are optimised for the detection of a diverse set of analytes and not for individual analytes. Compromised extraction and sample work-up conditions may lead to low RE due to the low solubility and/or stability of an analyte during sample preparation. When a '*dilute and shoot*' method is used for the analysis of mycotoxins in complex matrices such as food, matrix effects might occur due to the comparably high amount of co-injected matrix. Therefore, proper validation of R_E, *SSE* and R_A is crucial. The described performance parameters are commonly evaluated based on replicates of a single lot of a matrix. However, different lots (quantity of material known to have uniform characteristics such as origin and variety) of the same matrix may have different R_E, *SSE* and R_A values resulting in '*lot-to-lot variation*'.[5,11-15]. Lot-to-lot variation can lead to a matrix mismatch in the case where the R_A of the lot used for validation differs from the R_A of the analysed lot. [15]. Ignoring the matrix mismatch leads to the introduction of an error of unknown magnitude.[16]. Although large differences in *SSE* have already been observed for mycotoxins in different varieties of sorghum and rice,[5,14], the lot-to-lot variation is often neglected during the validation of multi-mycotoxin assays.

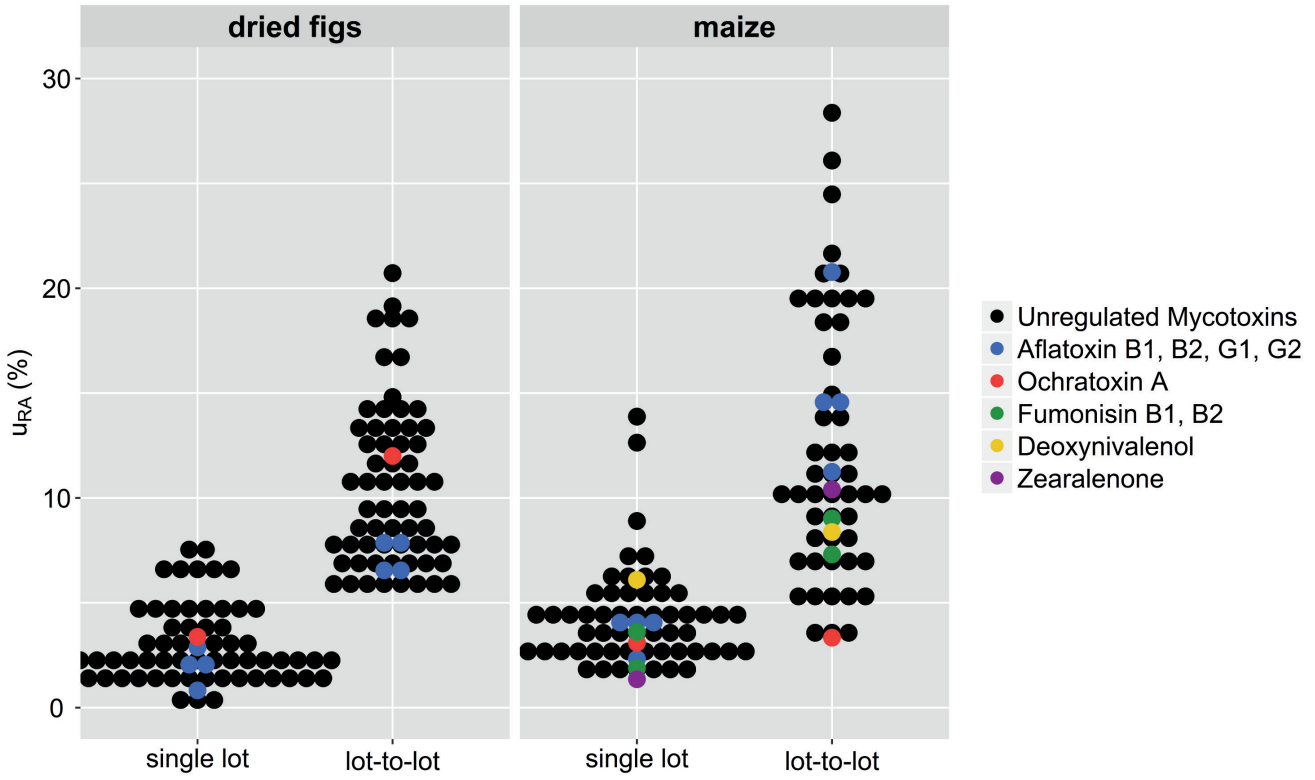
We hypothesised that the lot-to-lot variation, if not considered during method validation, can adversely affect the accuracy of measurement results. Therefore, we determined the impact of lot-to-lot variation on a validated LC-MS MS-based multi-mycotoxin method[3,14,17].

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

In the validation of multi-mycotoxin methods, the evaluation of the lot-to-lot variation is often missing, as they are commonly validated based on replicates of a single lot of a matrix. Therefore, the uncertainty associated with the lot-to-lot variation was estimated for the LC-MS/MS MS-based determination of 60 mycotoxins and fungal metabolites (including all regulated mycotoxins) in dried figs and maize[18].

Seven different lots of a matrix, possessing a diversity that typically occurs within this matrix, were assembled. For dried figs, seven lots differing in specification were bought in local supermarkets. For maize, seven lots differing in origin and variety were collected. A known amount of the analytes was spiked to an aliquot of the individual lots, which did not contain a natural contamination with the analytes under investigation. The R_A values were determined by analysing the spiked samples

FIGURE 1



ABOVE: 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 ($u_{RA,lot\text{-}to\text{-}lot}$), respectively. The evaluation was carried out for 60 mycotoxins (including all regulated mycotoxins[1]) in dried figs and maize.

by using the method under consideration, under repeatability conditions.

The relative standard deviation (RSD) of the R_A values of the seven different lots was used to calculate the uncertainty associated with R_A considering the lot-to-lot variation ($u_{(RA\ lot\text{-}to\text{-}lot)}$), which actually is a combination of the uncertainty of repeatability and the lot-to-lot variation. The uncertainty of the repeatability was calculated from the RSD of the R_A values of seven aliquots of a single lot of a matrix ($u_{(RA,single\ lot)}$). In order to estimate the contribution of the lot-to-lot variation to u_{RA}, $u_{RA,lot\text{-}to\text{-}lot}$ was compared to $u_{RA,single\ lot}$ for 60 analytes in dried figs and maize (***Figure 1***).

The increase of $u_{RA,lot\text{-}to\text{-}lot}$ compared to $u_{RA,single\ lot}$ was caused by different R_A values of the individual lots due to the lot-to-lot variation[18]. In dried figs, the increase was due to different R_E values of the individual lots. This was the case for the regulated mycotoxins aflatoxin B_1, B_2, G_1, G_2 and ochratoxin A.

> *By considering the lot-to-lot variation during method validation, a more realistic estimate of the uncertainty of the measurement result is obtained*

In maize, for most analytes (e.g,. aflatoxins) the increase could be ascribed to differences in *SSE* (relative matrix effects[12]). For the minority of analytes (e.g,. fumonisin B_1, B_2 and zearalenone) the lot-to-lot variation caused different R_E values for the individual lots.

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, such as[15,19,20], call for the evaluation of R_E, *SSE* and R_A. However, it is often not specified whether these performance parameters have toshould 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 may fail validation when the lot-to-lot variation is considered. When a result is corrected for *RE* (e.g,. analysis of patulin and aflatoxins in foodstuffs[21,22]), *SSE or RA*, the uncertainty of correction factor needs to be taken into account for thewhen calculation calculating of the measurement uncertainty. The increase in u_{RA} caused by the lot-to-lot variation was shown to lead to a higher measurement uncertainty.[18]. 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.

Conclusion

In summary, we found that for 60 mycotoxins in figs and maize, the lot-to-lot variation can contribute to the uncertainty of the method, as different lots of a matrix may yield different apparent recovery values. Thus, by considering the lot-to-lot variation during method validation, a more realistic estimate of the uncertainty of the measurement result is obtained. Furthermore, it can be assured that the method delivers reliable results for food samples differing in, for example, origin, variety, composition and processing conditions employed.

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REFERENCES

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