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Highly Accurate Measurements of Ochratoxin A (OTA) in Different Foodstuffs

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The European Union-Reference Laboratory (EU-RL) for Mycotoxins at the Institute of Reference Materials and Measurements of the Joint Research Centers of the European Commission regularly conducts proficiency tests (PT) to asses the measurement capabilities of the National Reference Laboratories of the EU-Member States.

In 2010 the PT consisted of a cereal mix, paprika powder, and green coffee naturally contaminated with OTA, a toxin produced by the moulds Aspergillus ochraceus and Penicillium verrucosum.

Using Exact-Matching Double Isotope Dilution Mass Spectrometry (EMD-IDMS) assigned values [1] for the three materials were determined in our Institute.

In EMD-IDMS a stable-isotope labeled analyte analogue (spike) is blended with the sample (SB). The same spike amount plus a reference material of the analyte is also blended with a comparable analyte-free matrix (CB).

Eq. (1) describes how the mass fraction ($w_{s,i}$) of analyte in the sample is calculated. Aim is to match the ion ratios in sample blend (SB) and calibration blend (CB) as closely as possible (R=R_{SB}/R_{CB}~1) with the result that biases are effectively eliminated.

Therefore EMD-IDMS is considered a "primary ratio method" with a direct link to SI units. [2]

$$w_{s,i} = w_{c,i} \times R \times \frac{m_{c,i} \times m_{Spike,SB}}{m_{Spike,CB} \times m_{smp,i}} \qquad (1)$$

$$\frac{u_c^2(w_{s,i})}{w_{s,i}} = \frac{u^2(w_{c,i})}{w_{c,i}} + \frac{u^2(R)}{R} + \frac{u^2(m_{c,i})}{m_{c,i}} + \frac{u^2(m_{Spike,SB})}{m_{Spike,SB}} + \frac{u^2(m_{Spike,CB})}{m_{Spike,CB}} + \frac{u^2(m_{smp,i})}{m_{smp,i}} \qquad (2)$$

The indices of the weights (m) designate the reference material (c,i), the *spike* in *SB* or *CB*, and the sample (*smp*,*i*). R is the ratio of ion ratios in *SB* over *CB* and $w_{c,i}$ is the mass fraction of he analyte in the reference material. Eq. (1) shows that the Spike purity and mass fraction are of no importance as long as the same spike is used for all measurements.

Eq. (2) describes the combined uncertainty of $w_{s,i}$. Because uncertainties of weights can be negligibly small $u_c(w_{s,i})$ is governed by the uncertainties of the ion ratios and of the reference material. The spike was $^{13}C_{20}$-OTA and the reference material a candidate European RM (ERM-AC 705).

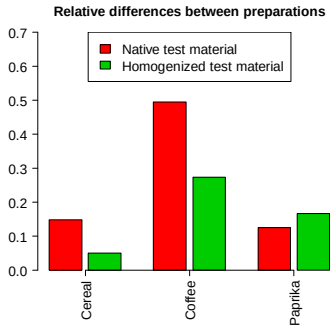
OTA is known for its very heterogeneous distribution in materials and test portions in excess of 10 grams are normally used to circumvent this. Since spike and ERM are costly the amount of test portion per preparation here was limited to 1 gram. Therefore, proper homogenization of the sample becomes paramount. Also, the spike and the RM have to be given sufficient time to equilibrate with the sample.

If all of the above is respected the determined values will have high accuracy.

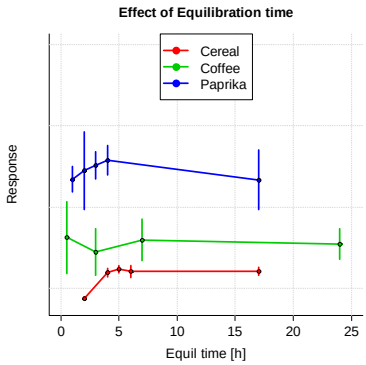
References:

1. Thompson, M., S.L.R. Ellison, and R. Wood, *THE INTERNATIONAL HARMONIZED PROTOCOL...* Pure Appl Chem, 2006. **78**(1): p. 145--196.

2. Mackay, L.G., et al., *High accuracy analysis by isotope dilution mass spectrometry using an iterative exact matching technique.* Accred Qual Assurance, 2003. **8**(5): p. 191-194.



The test materials were further homogenized from their native state with either a mixing mill (Cereal, Paprika) or a mortar mill (Coffee) For the Cereal and the Coffee material a reduction of the between-preparation variability was achieved. For Paprika the additional homogenization was unsuccessful.



Only for the Cereal material a significant effect of the equilibration time of the spike was found. After addition of the spike the cereal, paprika, and coffee samples were left to equilibrate for 5, 4, and 0.5 h, respectively.

Assigned values:

Since the materials were used for a PT our assigned values can be compared to the consensus values of the PT (determined acc. to [1]).

Material	Assigned value	U	k	Consensus value
Cereal	191	9.3	2	189
Coffee	7.1	0.67	2	8
Paprika	13	0.92	2	13.7

The consensus values of the Cereal and Paprika materials fall within the extended uncertainty range around the assigned values.

For Coffee this is not the case. The reason for this might be an undetected bias in the consensus value since almost all the participants of this PT used the same method of analysis.

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