

Comparison of Calibration Strategies for Accurate Quantitation of Organic Contaminants in Food: A Case Study of Ochratoxin A in Flour

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Introduction

Analysis of low-level organic contaminants in complex matrices is essential for monitoring global food safety. However, balancing sample throughput with complex experimental designs and/or sample clean-up to best reduce matrix effects is a constant challenge. Multiple strategies exist to mitigate these effects, with internal standard based methods such as isotope dilution mass spectrometry (IDMS) being the most advantageous.

Here, multiple calibration strategies were investigated for the quantitation of ochratoxin A (OTA) in wheat samples by liquid chromatography high resolution mass spectrometry (LC-HRMS) involving classical external calibration approaches as well as various implementations of IDMS. Although these calibration strategies were used for the quantitation of OTA in wheat, these methods can be applied for the quantitation of a variety of other toxins in food.

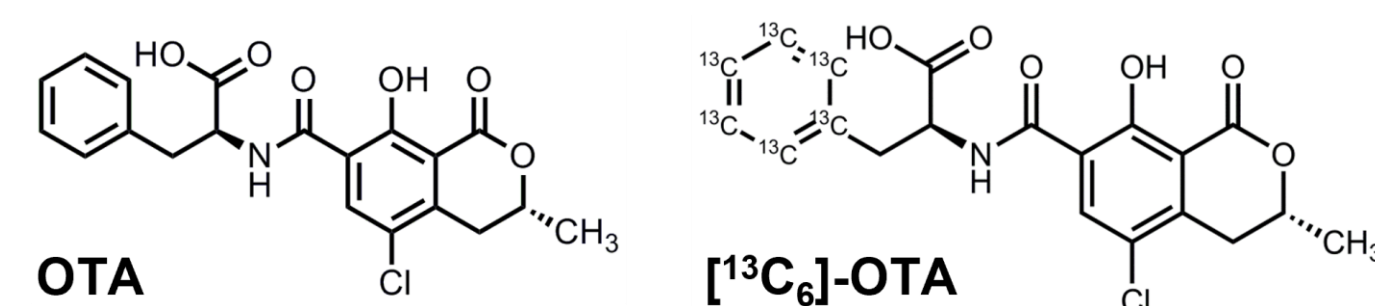
Materials

Certified reference materials (CRMs) are valuable in monitoring food safety and can serve to calibrate instruments, develop and validate quantitation methods, or act as quality control samples. NRC has produced a suite of mycotoxin CRMs to support regulatory testing and promote measurement comparability.

Here, CRMs of OTA (OTAN-1) and/or [¹³C₆]-OTA (OTAL-1) were used to quantitate OTA in wheat samples. The CRM MYCO-1 was used as a quality control (QC) sample to evaluate the accuracy of each method.



NRC mycotoxin CRMs



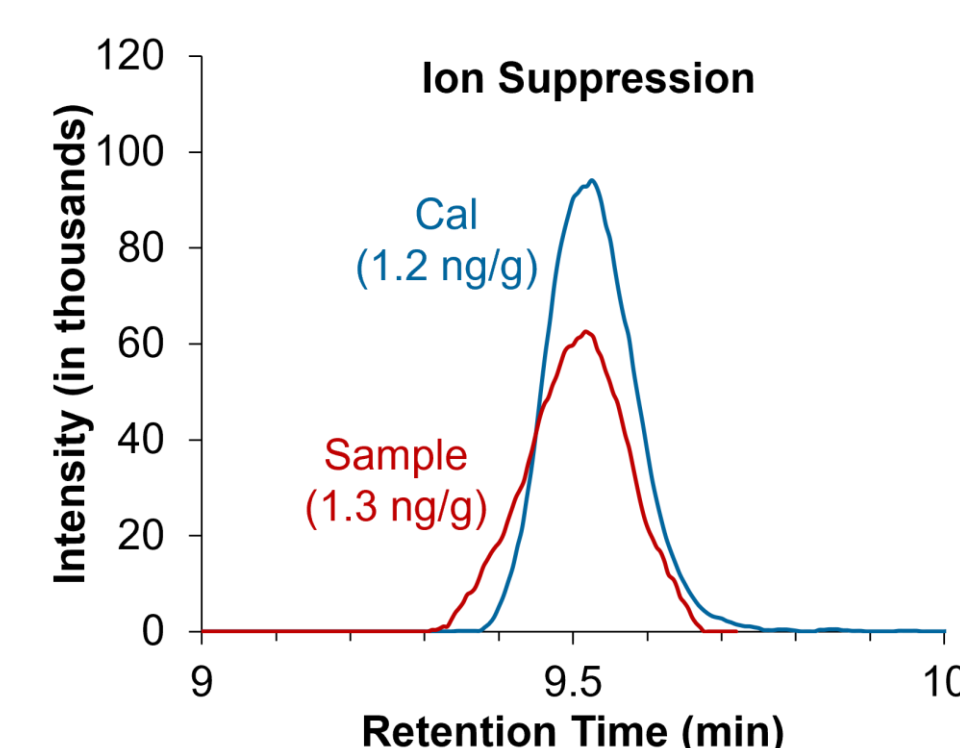
Chemical structures of OTA and [¹³C₆]-OTA

Methods

External Standard Calibration (External Cal)

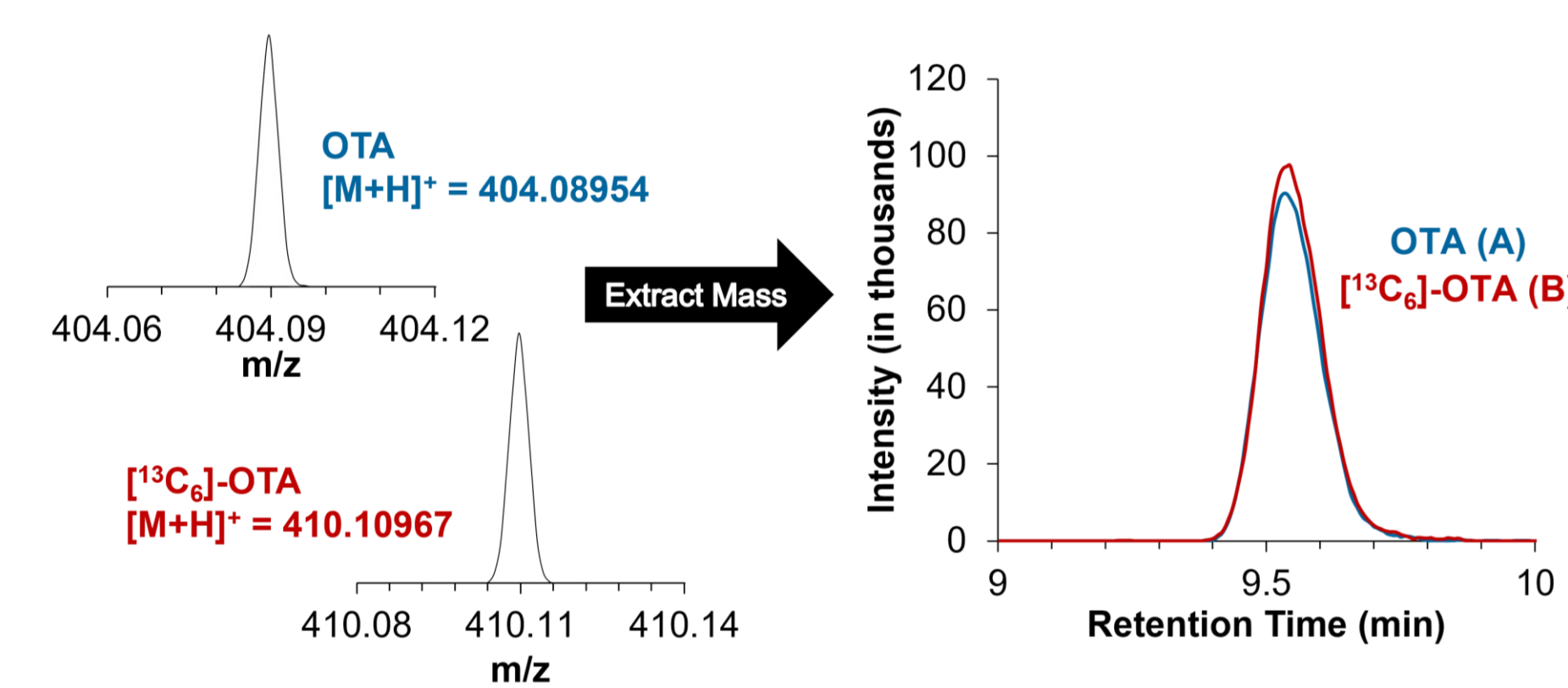
This method requires complete extraction of the analyte or the results must be recovery-corrected. The percent recovery must be determined from an independent experiment typically using a blank matrix which is not always available. Additionally, a clean-up step is usually required to limit matrix effects.

LC-HRMS chromatograms of OTA in calibration standard solution (1.2 ng/g) and MYCO-1 extract (1.3 ng/g) revealing ion suppression



Isotope Dilution Methods

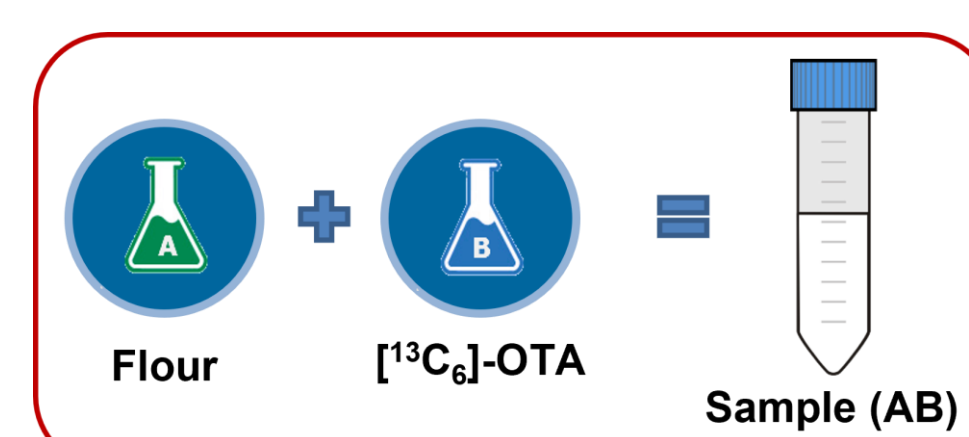
The addition of an isotopically enriched compound to the sample can help to counteract matrix effects. Complete analyte extraction may not be required, however complete equilibration between analyte and the internal standard (IS) must be achieved. Ratios of native analyte peak area to isotope-labelled peak area are used, therefore solvent evaporation of samples is no longer an utmost concern. The IS should be as structurally similar as possible to the analyte to achieve perfect coelution and equal ionization efficiency.



OTA and [¹³C₆]-OTA exhibit similar elution and ionization profiles by LC-MS

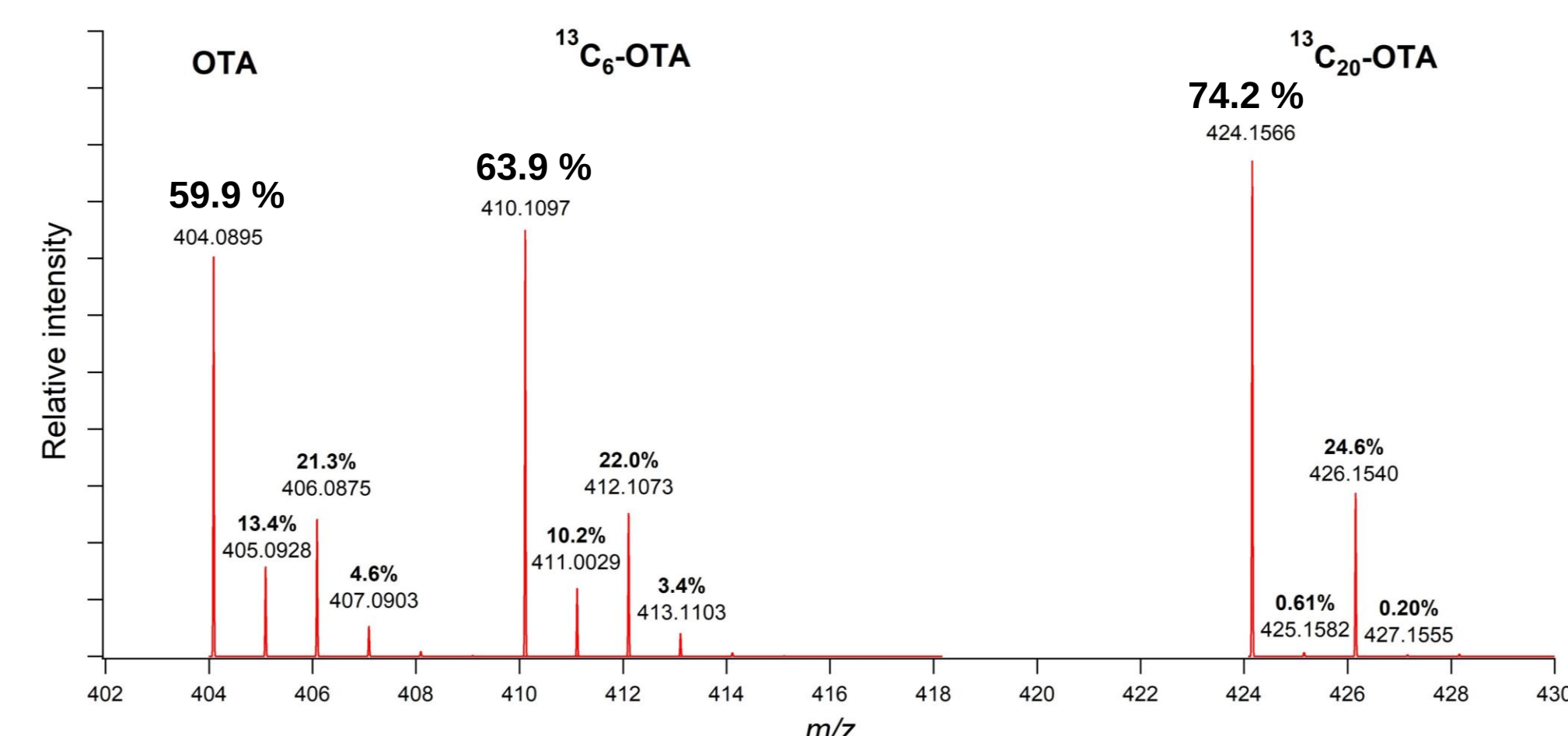
Single Isotope Dilution Mass Spectrometry (ID¹MS)

ID¹MS requires an isotopically enriched standard, with a certified mass fraction, to act as the primary calibrator. A single sample is prepared by spiking a matrix sample with an isotopically enriched standard and the ratio of the native analyte peak area to isotope-labelled peak area is measured (r_{AB}).



Experimental scheme for ID¹MS

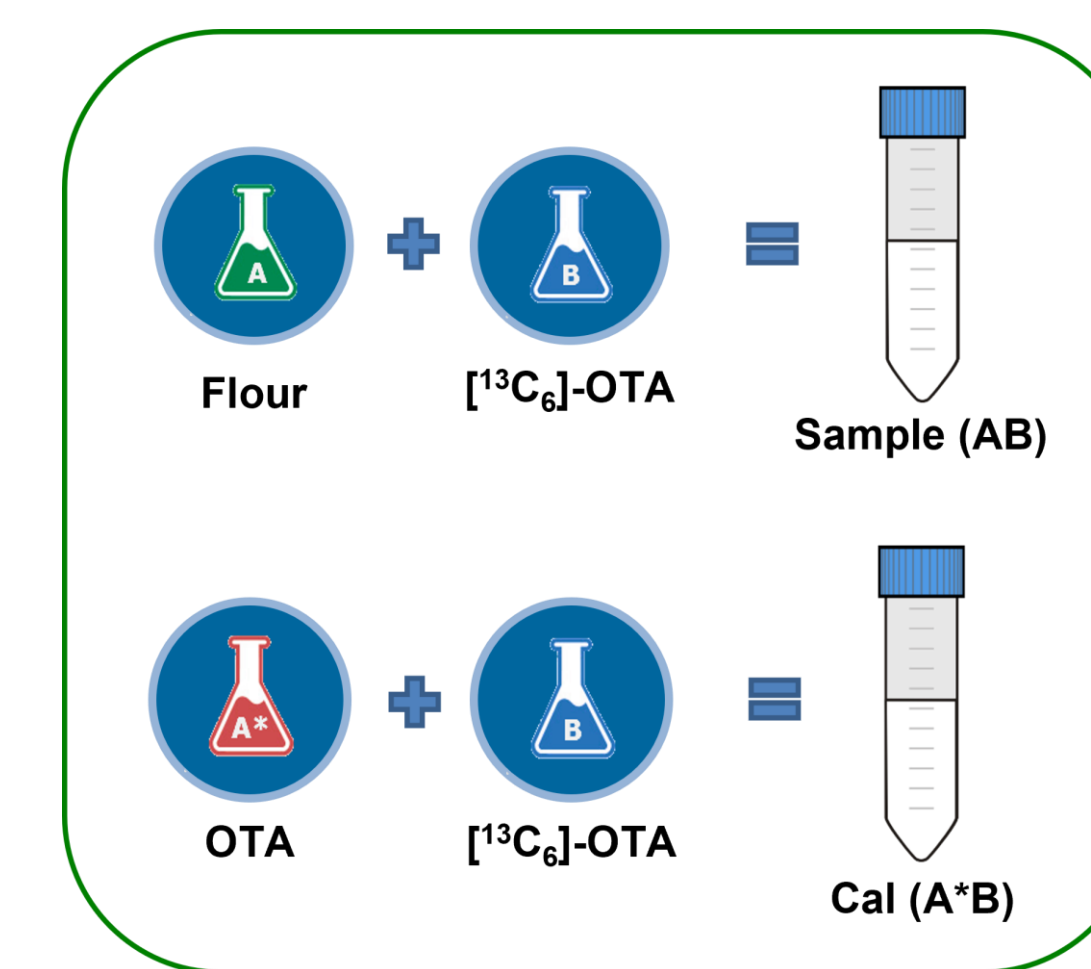
With ID¹MS, one must consider isotopic enrichment bias. The bias originates because the percent abundance of the monoisotopic mass is not constant, but rather varies among unlabelled and their labelled counterparts. This bias is only applicable for ID¹MS and would be even more significant in the case of commonly used fully labelled standards.



Isotopic pattern of native OTA, [¹³C₆]-OTA, and [¹³C₂₀]-OTA. The percent contribution of each isotopologue toward the full isotopic envelope is highlighted

Double Isotope Dilution Mass Spectrometry (ID²MS)

For this method, the concentration of the isotopically enriched standard is no longer required and isotopic enrichment bias is eliminated. Two samples are prepared where the internal standard (IS) is spiked into both the matrix sample and the corresponding native reference standard and the ratio of the native analyte peak area to isotope-labelled peak area is measured in both samples (r_{AB} and $r_{A'B}$).

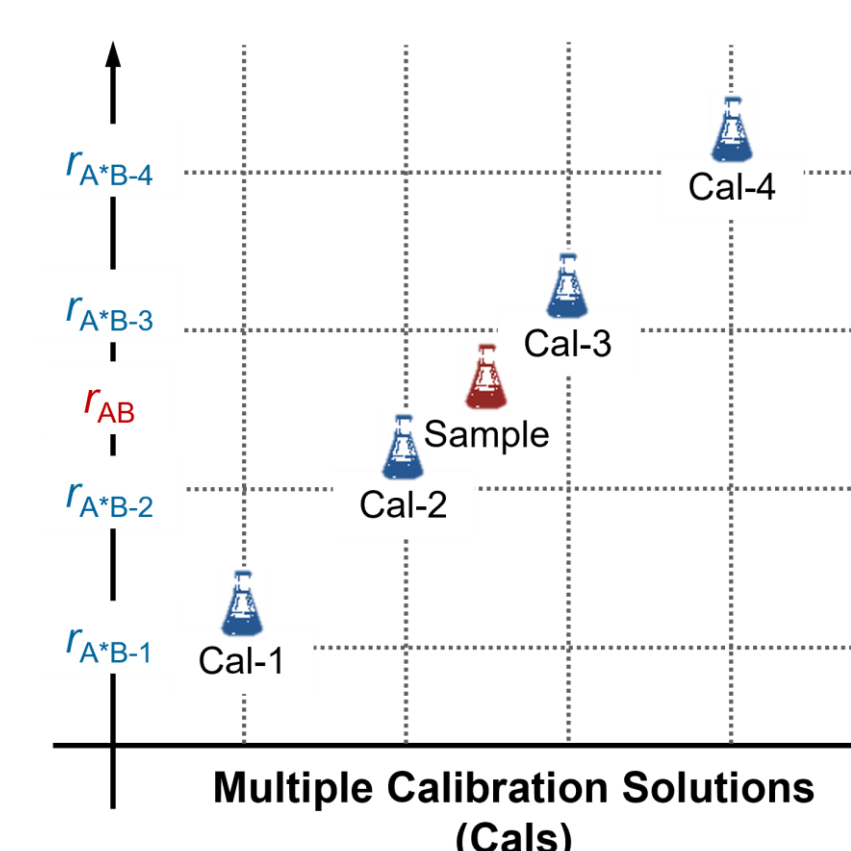


Experimental scheme for ID²MS

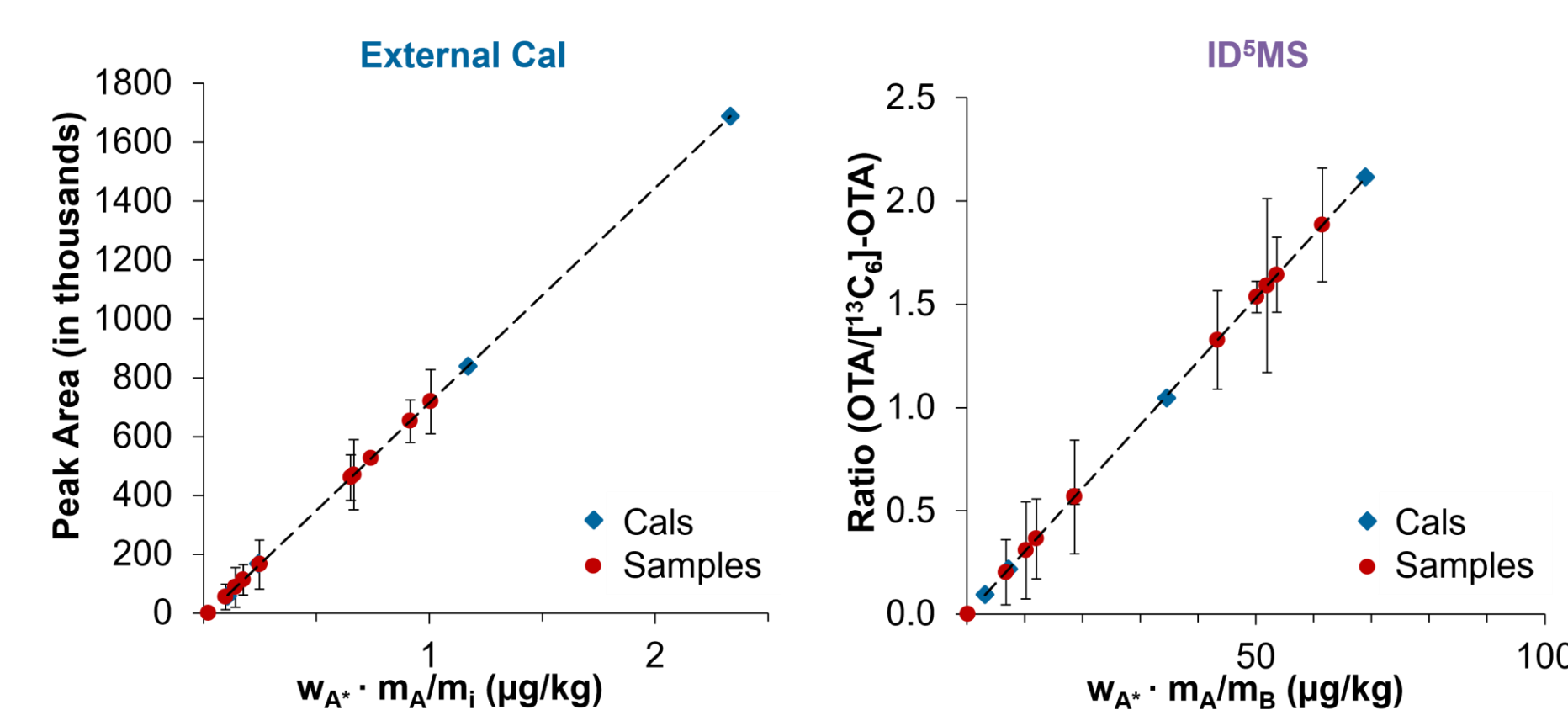
Exact-matching ID²MS can be used to minimize mass bias effects providing a more accurate result. Here, the concentration of the native analyte in the Cal should match the estimated mass fraction of the analyte in the sample. Both are then spiked with an equal amount of IS. Additionally, both solutions are prepared such that the signal ratio of unlabelled to labelled is close to 1:1. Preliminary work is required to first estimate the mass fraction of analyte in each sample and typically an independent Cal would need to be prepared for each sample.

Higher Order Isotope Dilution Mass Spectrometry (IDⁿMS)

A "multi-spike" approach can be used where multiple Cals bracket the samples. The calibration curve range can be tailored to include as many or as few points as desired. Provided the analyte is stable, a single calibration curve can be re-analyzed multiple times allowing the analysis of a large number of samples, maximizing throughput. Cals are prepared with varying amounts of the native standard solution and spiked with the same amount of IS. Results can be determined using a specific isotope dilution equation or a calibration curve can be fitted similar to external standard calibration.



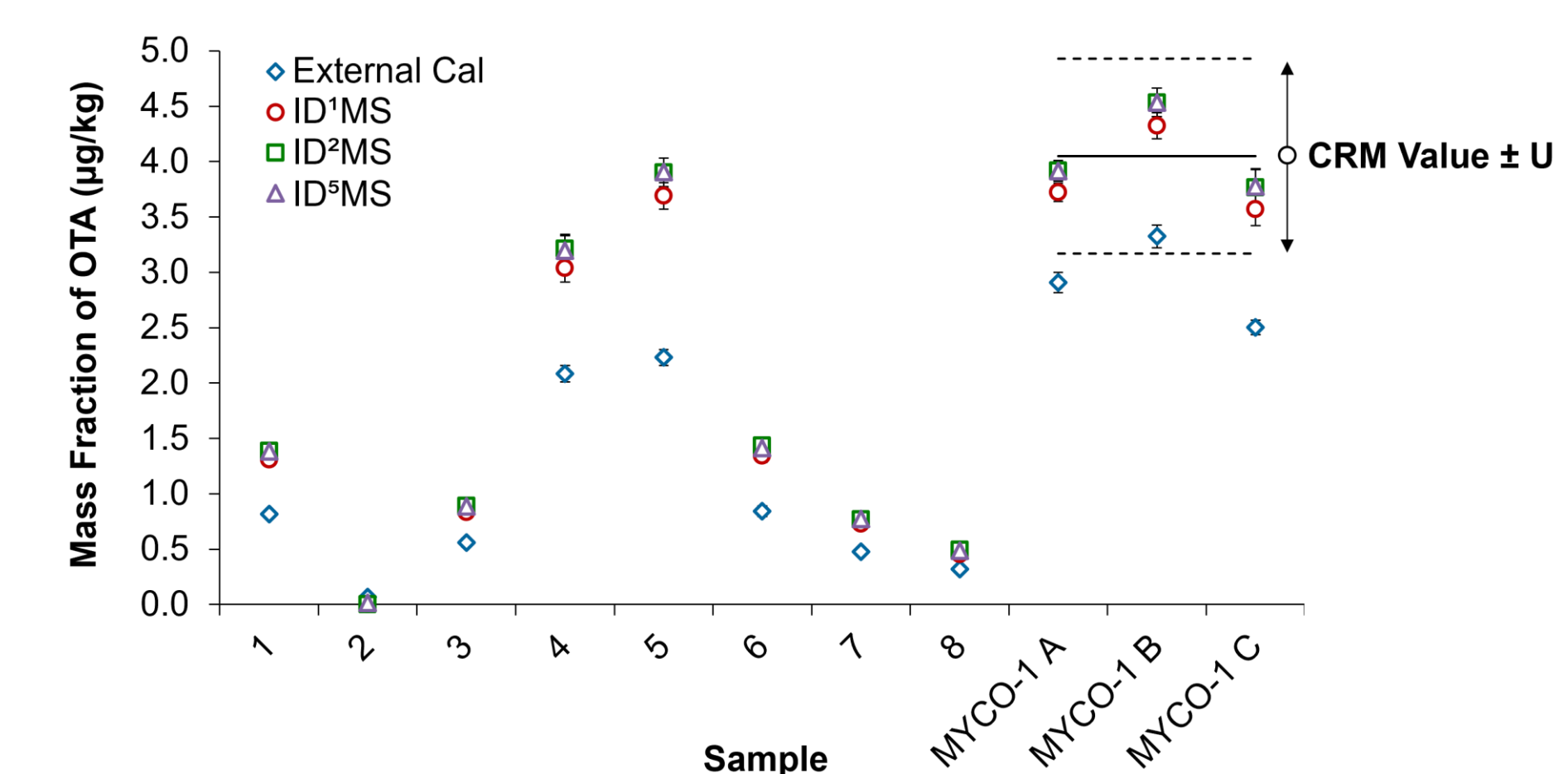
Experimental scheme for ID⁵MS



Calibration curves for the external standard calibration method using peak areas and ID⁵MS method using peak areas ratios show the impact of matrix effects

Results

Results obtained for each method were calculated using the same set of extracted sample to eliminate any homogeneity issues as a potential source for any observed differences. For External Cal, the mass fraction obtained for each sample was much lower than those obtained by IDMS methods. It is evident from the results of MYCO-1 that isotope dilution strategies provide more accurate quantitation than external calibration as they generated results consistent with the certified value. However, the mass fractions obtained by ID¹MS were on average 6 % lower than those obtained by ID²MS and ID⁵MS for all samples. This difference was attributed to the isotopic enrichment bias.



Mass fraction of OTA obtained for each quantitation method

Conclusion

The CRM MYCO-1 facilitated validation of the results and confirmed the entire analytical process. It showed that external standard calibration under represented the mass fraction of OTA due to ion suppression. Isotope dilution methods overcome matrix effects by measuring a ratio of the two isotopic forms of OTA. These methods allowed for accurate quantitation using high-quality, SI-traceable calibration standard CRMs OTAN-1 and OTAL-1. Three different isotope dilution methods were outlined including ID¹MS, ID²MS, and IDⁿMS which can also be applied to other natural toxins in food.

Method	Advantages	Limitations
External Cal	Cost effective (no IS).	Requires complete extraction. Matrix effects.
ID ¹ MS	Minimal sample preparation and analysis time.	Isotopic enrichment bias. Lack of labelled CRMs.
ID ² MS	Only requires native as a CRM. Eliminates isotopic enrichment bias.	Ideal if exact-matching (labour intensive).
ID ⁿ MS	Ideal for measuring samples over wide range.	Most sample preparation.

References

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