Extending the Interval between Full-Range Mass Calibrations on Orbitrap Exploris Mass Spectrometers

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ABSTRACT

Purpose: Develop and evaluate a 1-Point Mass Calibration on Orbitrap Exploris that minimizes workflow interruptions.

Methods: Existent full-range mass calibration curves are recalibrated using the integrated EASY-IC ion source, twice weekly over 9 weeks. Residual mass errors are evaluated for infused common reference ion mixes and by Multi-Attribute Method (MAM) and Small Molecule LC-MS applications.

Results: Without requiring any change to the instrument setup, long-term mass drifts of > 25 ppm are effectively eliminated.

INTRODUCTION

While Orbitrap™ mass spectrometers provide excellent mass accuracy, slow drifts require regular –recommended daily– calibration of the full mass range. Alternatively, the mass drift may be corrected for using a reference mass. Latest-generation Orbitrap instruments offer the integrated EASY-IC™ mass correction functionality, which provides sub-ppm mass accuracy for at least 5 days but is limited to mass drifts within ±15 ppm. We developed a 1-Point Mass Calibration on Thermo Scientific™ Orbitrap Exploris™ that extends the validity of full-range mass calibration data to well over one month, and which complements the EASY-IC mass correction. We evaluate this approach for different mass ranges and test it in real-life applications in the fields of high-resolution (HR) MAM and Small Molecule analyses.

MATERIALS AND METHODS

Sample Preparation: Thermo Scientific™ Pierce™ FlexMix™ calibration solution was infused as received. Ammonium hexafluorophosphate (AHFP) was infused as a 1 mg/mL solution in 50% isopropanol. Pierce™ BSA Protein Digest was redissolved in 1 mL 0.1% formic acid (1 pmol/µL BSA digest), vortexed for 10 s, divided into 100 µL aliquots in polypropylene vials, and stored at –20 ºC. Waters mAb Tryptic Digestion Standard (40 µg) was redissolved in 80 µL 0.1% formic acid (500 ng/µL NISTmAb digest), divided into 15 µL aliquots in polypropylene vials, and stored at –20 ºC. For each MAM acquisition batch, fresh BSA digest and NISTmAb digest sample aliquots from the freezer were thawed, vortexed, and spun down before putting them into the autosampler. For the Small Molecule analyses, blank meat muscle matrix (QuEChERS extract in acetonitrile) was spiked with 500 µg/L of each of the sulfonamides (sulfachlorpyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamonomethoxine, sulfamoxole, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisoxazole).

Test Methods: An Orbitrap Exploris MX mass spectrometer with BioPharma option was initially calibrated with FlexMix and AHFP for the full mass range (40–8000 Th). Twice weekly over 9 weeks, mass accuracies were evaluated both using the original calibration curve and after running a 1-Point Mass Calibration. Over the first 6 weeks, subsequently, MAM LC-MS analyses of BSA digest and the NISTmAb digest were run. After 3 months, a subset of the BSA digest experiments was repeated to address the complementarity of the 1-Point Mass Calibration and the EASY-IC mass correction.

Two instrument methods were created within the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software as part of the HR MAM workflow to evaluate the long-term mass drift on the Orbitrap Exploris MX. For BSA digest a 22-min gradient from 2–35% acetonitrile with 0.1% formic acid was used for HPLC separation of 5 µL injections; the scan range was 280–1600 Th at a resolution setting of 120'000. For NISTmAb digest a 105-min gradient from 2–35% acetonitrile with 0.1% formic acid was used for HPLC separation of 2 µL injections; the scan range was 300–1800 Th at a resolution setting of 120'000.

Likewise, an Orbitrap Exploris 240 was initially calibrated with FlexMix, and Small Molecule LC-MS experiments using Runstart EASY-IC mass correction were performed over 2 weeks. Starting from day 5, a daily 1-Point Mass Calibration was run in between sequences. In brief, a 17-min gradient from 0–95% methanol with 0.1% formic acid was used for HPLC separation of 2 µL injections. Additionally, a FAIMS Pro Duo unit was installed for robustness reasons. The Full Scan mass range was 120–1000 Th at a resolution setting of 30'000.

Data Analysis: FlexMix and AHFP mass errors were extracted from the evaluation results.

For BSA digest data evaluation the mass accuracies of 9 specific peptides were extracted from the acquired data files using a MAM report template within Chromeleon CDS; average mass errors over 19 injections were plotted in Origin. Likewise, for NISTmAb digest data evaluation the mass accuracies of 7 specific peptides were extracted; Box-Whisker plots visualizing per-substance mass error statistics were created in Origin.

For Small Molecule data evaluation, the mass accuracies of 15 sulfonamides (mass range 215–311 Th) as well as the EASY-IC mass correction were extracted from the RAW files using Thermo Scientific™ TraceFinder™. Box-Whisker plots visualizing day-to-day statistics were created in Origin.

RESULTS

Development of the 1-Point Mass Calibration

The Instrument Control Software was extended with a procedure that switches off ionization in the analytical source, turns on the integrated EASY-IC ion source, and conditions it if necessary. The procedure then performs positive and negative mass calibrations on the generated fluoranthene radical ions. This takes 10–70 s depending on how much conditioning the EASY-IC ion source needs (e.g. when it had been idle for several days). The calibrations are combined with the existent mass calibration curves to compensate for any mass drift since creation of the latter; these compensations persist until a new 1-Point or full FlexMix mass calibration is performed.

The main benefit of this dedicated 1-Point Mass *Calibration* is that it requires no changes to the analytical setup. Furthermore, interferences from the analytical source are excluded, so that a much wider reference mass tolerance window (±150 ppm) can be used than for mass *corrections* with EASY-IC or with a user-defined lock mass. Also, the *calibration* results are applied to all subsequent acquisition runs, whereas the mass *correction* options only apply to the acquisition in question and typically add a scan-to-scan overhead.

The ability of the 1-Point Mass Calibration to compensate drifts was verified for the Orbitrap Exploris MX's full mass range with FlexMix and AHFP over 6 weeks, and for low and standard mass range over 3 months (Figure 1). During this period, the Ion Transfer Tube (ITT) was exchanged for a clean one twice, the second time followed by a bake-out. Mass drifts (orange traces) were evaluated using the original calibration curves; on this instrument and under the given workload, the drifts evolved much slower than the Orbitrap Exploris specification of ≤3 ppm RMS/day. After 4 to 5 weeks the mass drifts exceeded 10 ppm RMS so that the Spectral Mass Accuracy Evaluation could no longer check the internal mass errors. Beyond 10 weeks the drifts exceeded –20 ppm and the positive-mode evaluation failed altogether; the drift was ca –27 ppm according to the fluoranthene ion signal.

Figure 1. Positive- and negative-mode mass drifts from the original calibration (orange) and residual mass errors after 1-Point Mass Calibration (green), evaluated for FlexMix and AHFP over up to 14 weeks.
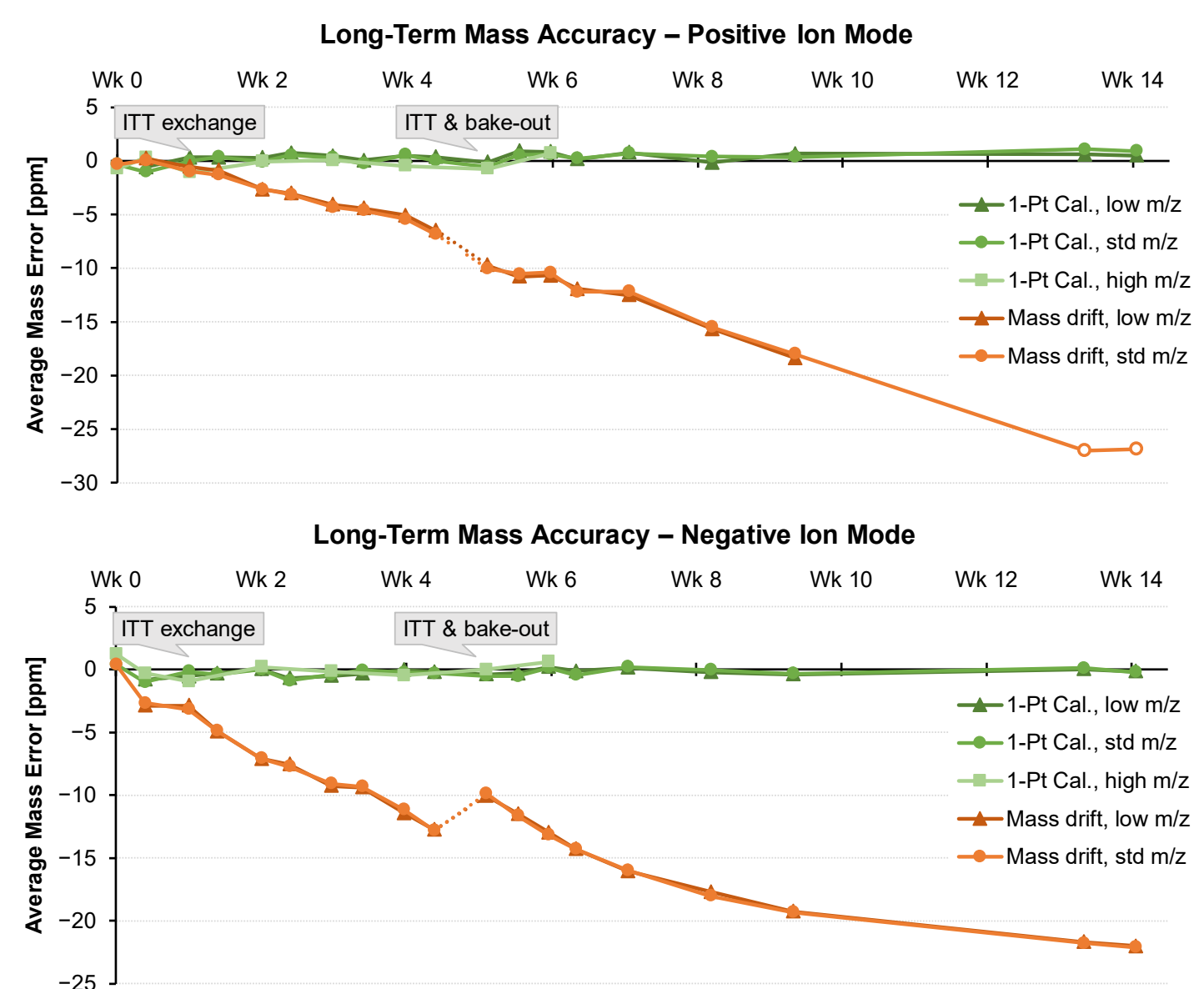

After each 1-Point Mass Calibration, on the other hand, the compensated mass errors (Figure 1, green traces) stayed within ±1¼ ppm for all evaluated mass sub-ranges for over 3 months. The ITT exchanges and even the bake-out did not noticeably deteriorate the mass compensation. Other parameters related to the Orbitrap mass accuracies were monitored as well. The eFT Phase was found to drift insignificantly and stayed well within specifications throughout these 3 months. The maximum deviations in resolution and target dependencies were merely 1.0 ppm and 0.7 ppm, respectively. Thus, besides the 1-Point Mass Calibration to compensate for mass drifts, no further analyzer calibrations need to be regularly performed to warrant good mass accuracy, not even after user maintenance actions.

Multi-Attribute-Method Applications for BSA Protein Digest and mAb Tryptic Digest

The new Orbitrap Exploris MX instrument model is targeted at the MAM QC market, for which stable long-term analytical performance with minimal workflow interruption is key. To validate the performance of the 1-Point Mass Calibration, 9 peptides from BSA digest were monitored over 6 weeks on the same days as the FlexMix evaluations. Figure 2 displays the mass errors, averaged over 19 injections done on each day of evaluation; while there is a slight systematic offset, all masses are accurate to well within a 3-ppm window. Merely 1% of the crude data (not shown) had a maximum absolute mass error of 3.0–3.4 ppm during these 6 weeks.

Figure 2. Mass errors for peptide components in BSA digest sample. Each datapoint is an average mass deviation of 19 injections.
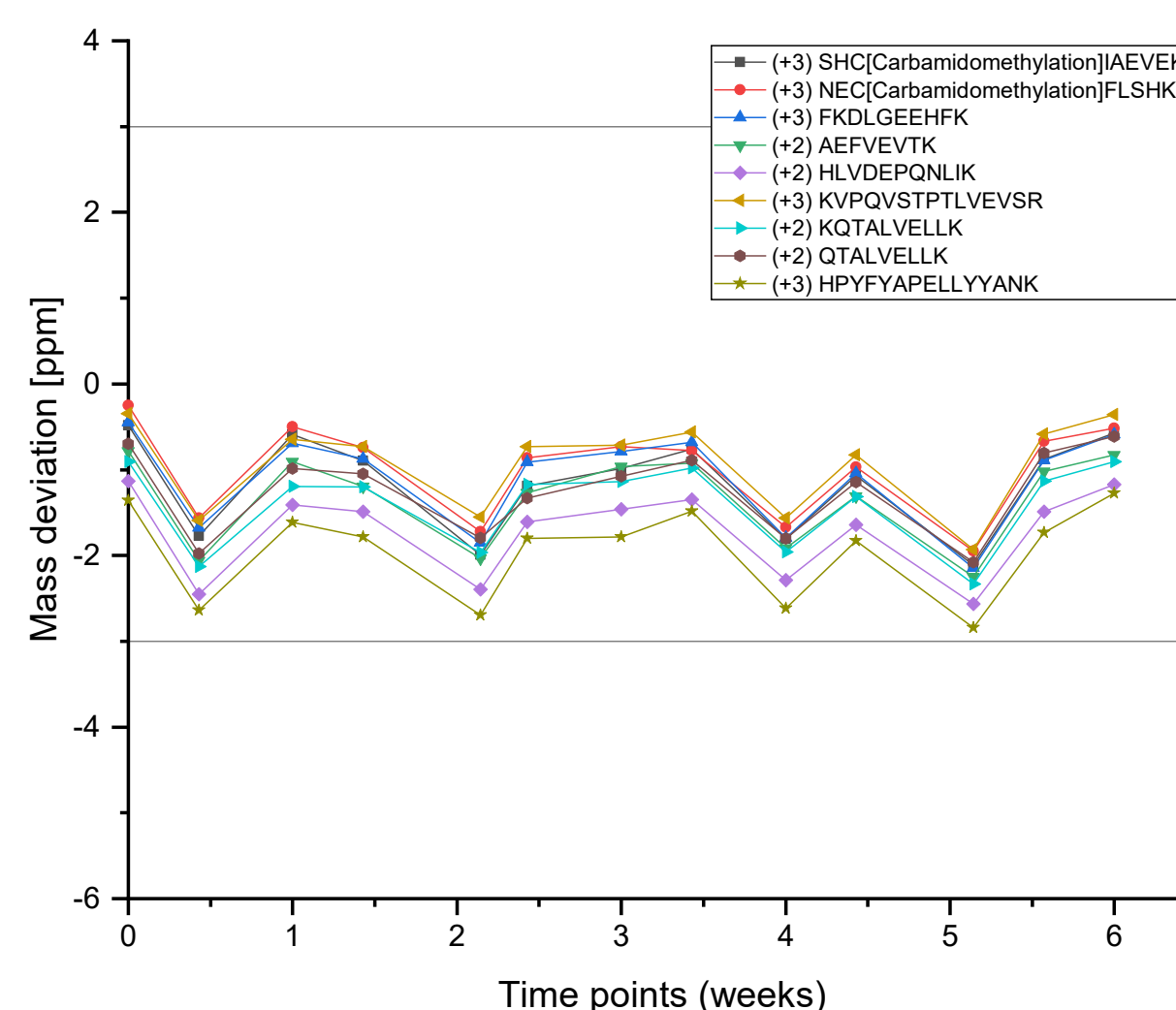
Furthermore, seven peptides from NISTmAb digest were monitored on each day of evaluation over the first 5 weeks. The mass errors appeared to be substance dependent, which may be due to interfering compounds in the LC-MS spectra of this complex sample. Apart from these small systematic offsets, each substance exhibited a rather narrow variation in measured accurate mass throughout the 5 weeks, as illustrated by the Box-Whisker plots in Figure 3.

Figure 3. Per-substance Box-Whisker plots of all mass errors over 5 weeks for each monitored component in NISTmAb digest sample (without any lock mass correction).
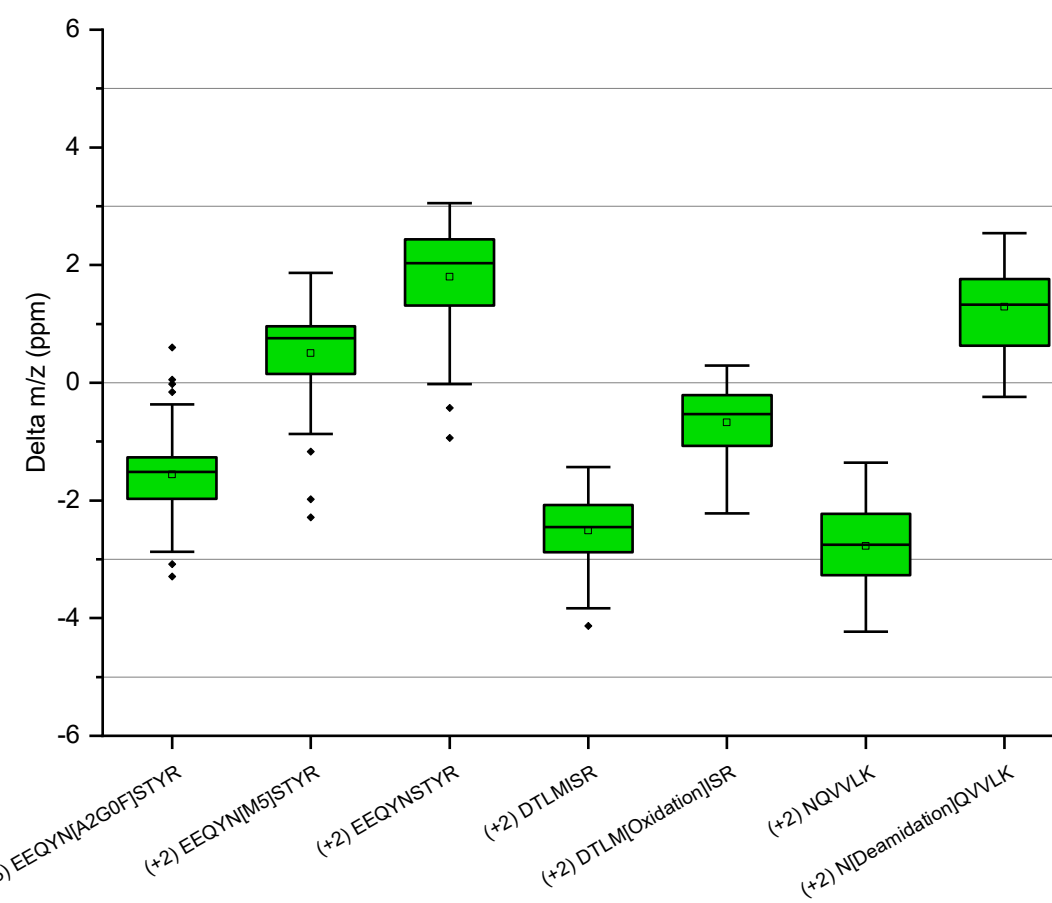
Small Molecule Application for Sulfonamides in Meat Muscle Matrix

Besides the peptide-oriented MAM workflow, we investigated the use of the 1-Point Mass Calibration in Small Molecule applications on an Orbitrap Exploris 240. 15 Sulfonamides were monitored for 14 days with RunStart EASY-IC mass correction; starting at day 5, a daily 1-Point Mass Calibration was performed. For each day, mass deviations for all substances in all injections are pooled into a Box-Whisker plot in Figure 4. All mass errors stay well within 3 ppm over 14 days, as would be expected with the RunStart EASY-IC feature; the 1-Point Mass Calibration does not interfere. On the contrary: Figure 4 also shows how the EASY-IC mass correction (blue trace) got bigger in magnitude as the instrument's mass drift developed over the first 5 days. Then, each 1-Point Mass Calibration (diamonds) brought the EASY-IC mass correction back to near-zero.

Figure 4. Per-day Box-Whisker plots of mass errors for sulfonamides analyzed in meat muscle matrix, and RunStart EASY-IC mass correction (blue). Diamonds mark the 1-Point Mass Calibration that was executed daily starting at Day 5.
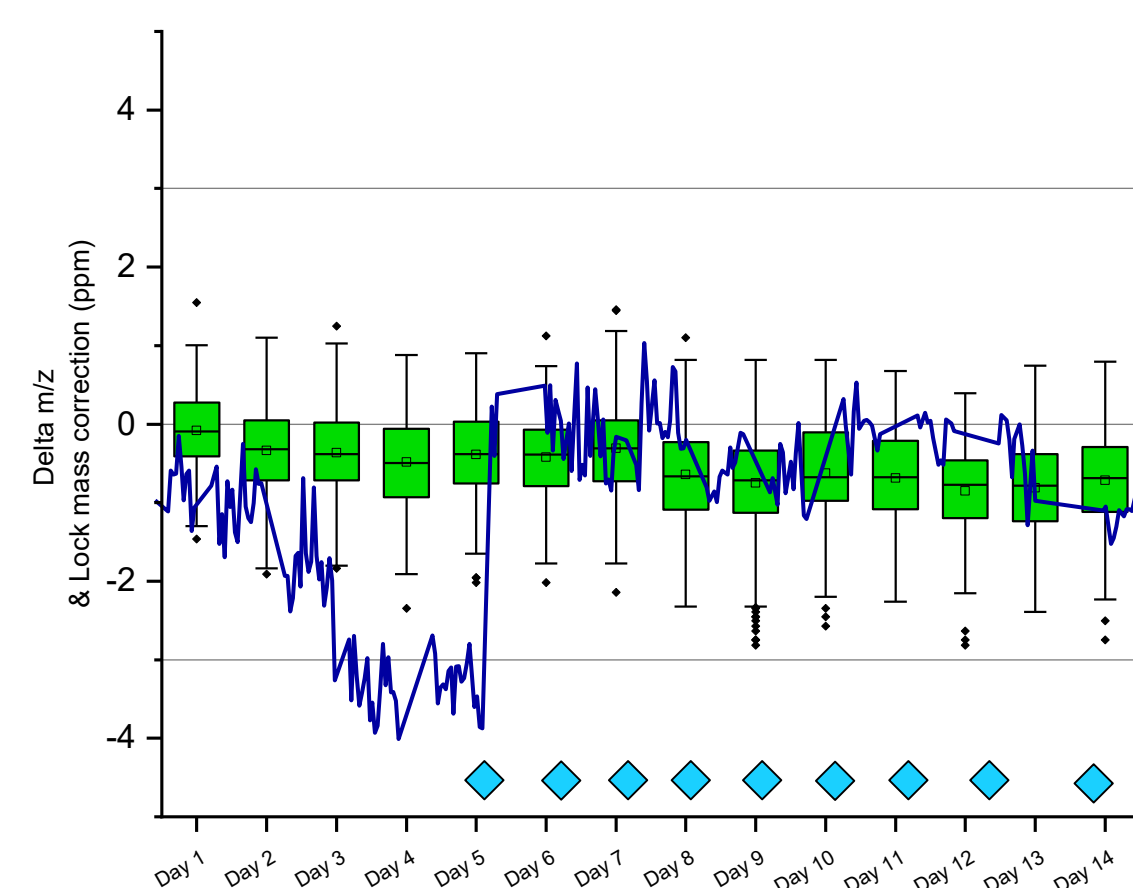

Complementarity of 1-Point Mass Calibration and EASY-IC mass correction

For mass drifts bigger than ±15 ppm, the mass correction with EASY-IC or with a user lock mass is no longer accepted while it cannot be warranted anymore that the correct ion signal was picked as the reference mass. For the Orbitrap Exploris MX in this study, for example, that would have been the case after ca 7 weeks (Figure 1). Given a worst-case mass drift of 3 ppm RMS/day though, this critically large deviation may already be reached after 5 days. The impact of this is exemplified in Figure 5 for the BSA analysis of BSA digest at 3 months after the full mass calibration, when the instrument had developed a mass drift of ca –27 ppm in positive mode.

With the original, 3-month-old mass calibration data (Figure 5, top row), although the acquisition method had the EASY-IC feature turned on, the fluoranthene ion was no longer identifiable with certainty and no actual mass correction was performed. As a result, all peptides were no longer detected in their extracted-ion chromatograms (XICs, using ±5 ppm extraction windows). The 1-Point Mass Calibration compensated for the mass drift and thus, in the subsequent run (Figure 5, middle row), the EASY-IC mass correction was near-zero and did get applied, and all peptide XICs were recovered. Another run with the EASY-IC feature disabled (Figure 5, bottom row) afforded essentially equal mass accuracies, indicating that so shortly after the 1-Point Mass Calibration, the mass drift was still fully compensated for.

Figure 5. MAM analysis of BSA digest 3 months after full mass calibration: exemplary XIC (left) of SHC[Carbamidomethylation]IAEVEK (m/z 536.75820, ±5 ppm extraction window) and zoom-in of the mass spectrum (right) at the apex of elution of this peptide.
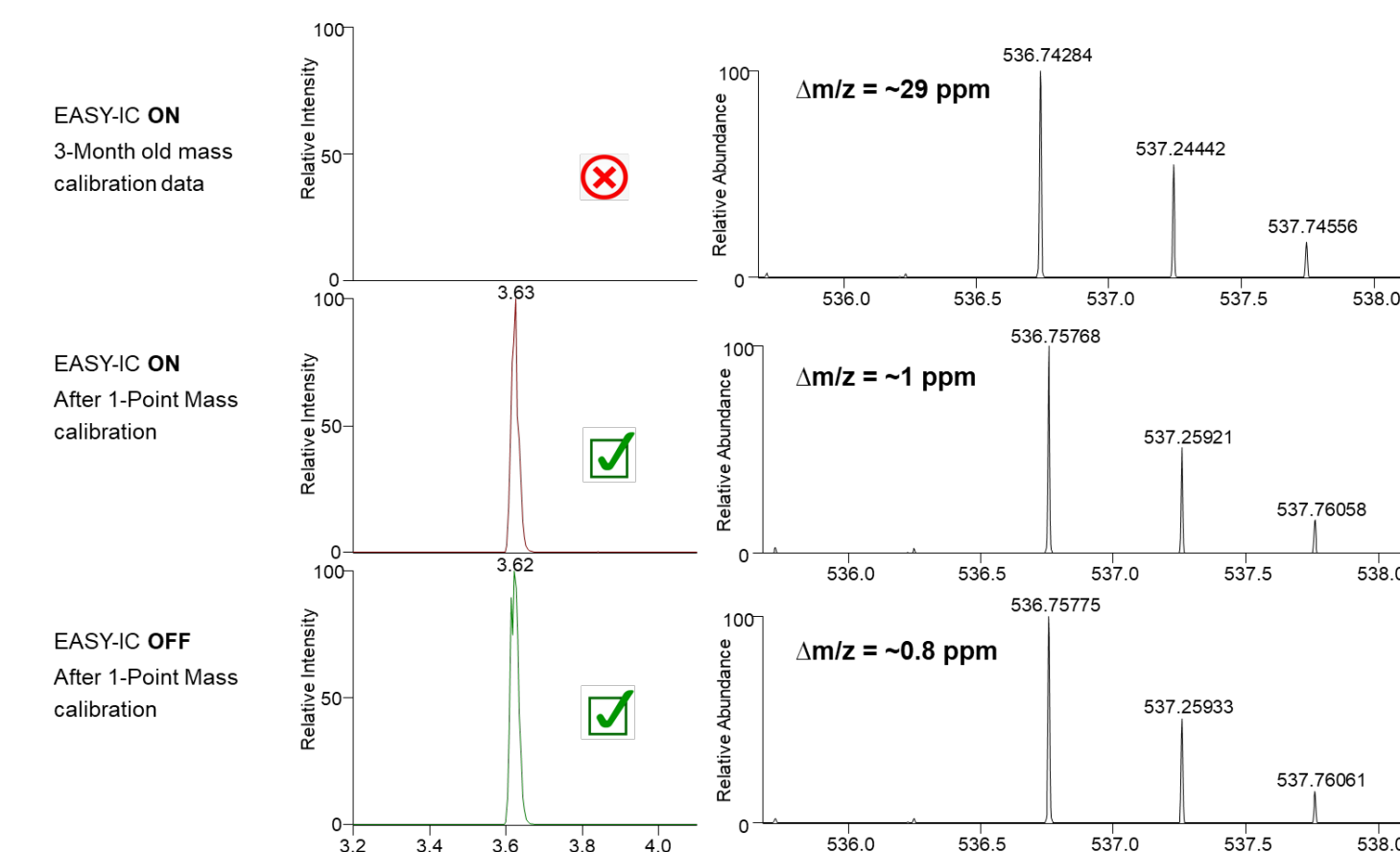
CONCLUSIONS

- A 1-Point Mass Calibration was developed for the Orbitrap Exploris Series that utilizes the integrated EASY-IC ion source to recalibrate the existent full-range mass calibration curves. Both evaluations with reference ion mixes and real-life MAM and Small Molecule applications confirm that mass drifts even beyond 25 ppm get effectively compensated throughout the full mass range.

- The 1-Point Mass Calibration works independently of the analytical ion source, thus requiring minimal workflow interruption. This new functionality is planned to be schedulable, either at regular intervals (e.g. daily) or in a method, directly preceding e.g. a System performance Evaluation Test through which the user can subsequently confirm the mass accuracies.

- The 1-Point Mass Calibration extends the validity of full-range mass calibration data to at least 2 months, also when user maintenance is performed in between. Other mass accuracy-related parameters were found to drift much slower, so that full instrument calibrations with FlexMix can be much less frequent.

- The 1-Point Mass Calibration is complementary to EASY-IC mass correction and user-defined lock mass correction in that it can compensate for much larger mass drifts and that this compensation persists until the next mass calibration. Each 1-Point Mass Calibration effectively resets the mass correction to near-zero and thus extends the applicability of EASY-IC and user lock mass to much longer time intervals.

TRADEMARKS/LICENSING

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