Autonomous mass calibration of benchtop Orbitrap mass spectrometers ensures continuous long-term operation and reliable drug compounds quantification

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ABSTRACT

Purpose: Evaluate a One-Point Mass Self-Calibration that enables continuous long-term operation of Orbitrap Exploris mass spectrometers.

Methods: Thermo Scientific™ Orbitrap Exploris™ mass spectrometers are calibrated on a daily and weekly basis using an autonomous mass calibration. Drug compounds are spiked into crashed plasma and quantified down to sub-ppt levels. Mass deviations are monitored for long-term operations.

Results: Reliable drug compounds quantification is demonstrated down to 50 ppt with mass errors of < 3 ppm during long-term operation without changing the analytical setup.

INTRODUCTION

While Orbitrap mass spectrometers provide excellent mass accuracy, slow drifts require regular calibration of the full mass range with a mix of calibrants. Additionally, the mass drift may be corrected by using a lock mass providing sub-ppm mass accuracy. However, this lock mass correction cannot handle large mass drifts.

This study introduces a self-calibration mode based on a One-Point Mass Calibration which extends the validity of the full-range mass calibration. This self-calibration enables fully autonomous operation of the mass spectrometer for one month while maintaining the highest data quality, mass accuracy and reproducibility. This approach is evaluated for the quantification of drug compounds spiked into crashed bovine plasma.

MATERIALS AND METHODS

Sample Preparation: Lyophilized bovine plasma was reconstituted by adding 10 mL of water. The plasma was crashed by adding 30 mL of acetonitrile, vortexed for 30 seconds, and centrifuged for 5 min at 3,000 rpm. The supernatant was removed and diluted at a ratio of 1:1 with water. The diluted plasma was spiked with an internal standard spiking solution (Fentanyl-D5, Fluoxetine-D6, and Imipramine-D3), resulting in a final concentration of 0.5 ng/mL of matrix blank solution with internal standard. Stock solutions of the drug compounds Flecainide, Fluoxetine, and Imipramine were diluted with methanol, reconstituted plasma, and internal standard spiking solution to create an analyte spiking solution at a concentration of 5,000 ng/mL for the analytes and 0.5 ng/mL for the internal standards. Further diluting the analyte spiking solution with the matrix blank solution led to a full dilution series of 15 calibration levels to cover a concentration range from 500 ng/mL (500 ppb) down to 0.01 ng/mL (10 ppt).

Test Methods: Two Orbitrap Exploris mass spectrometers were initially calibrated with Thermo Scientific™ Pierce™ FlexMix™ calibration solution to ensure the validity of the full-range mass and system calibration. Two additional full-range mass and system calibrations were performed on the Orbitrap Exploris MX between week 3 and week 4, and between week 7 and week 8 extending the validity of the full-range mass and system calibration. In the preferences of the Orbitrap Exploris instrument control software, the mass self-calibration option (**Figure 1**) was enabled and set to a daily basis for the Orbitrap Exploris 120 and to a weekly basis for the Orbitrap Exploris MX. Afterwards, each Orbitrap mass spectrometer was connected to a Thermo Scientific™ Vanquish™ Horizon UHPLC system.

Four LC-MS methods were created within the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software; two Full Scan methods and two targeted MS2 methods (tMS2). The RunStart EASY-IC option was enabled for each of the two methods applying a mass correction to every RAW file acquired. For the Full Scan methods, the scan range was 200-800 m/z at an Orbitrap resolution of 120,000.

For the tMS2 methods, the quadrupole isolation width was 2 m/z at an Orbitrap resolution of 60,000. For all instrument methods a 4-minute gradient from 5-95% mobile phase B (A: water with 10 mM ammonium formate; B: acetonitrile with 0.1% formic acid) was used for HPLC separation of 5 µL injections. Each calibration level was injected five times to the mass spectrometer.

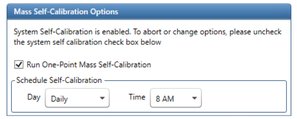

Figure 1. Mass self-calibration options to enable and schedule an autonomous One-Point Mass Self-Calibration within the preferences of the Orbitrap Exploris instrument control software.

Data Analysis: For data evaluation, the linearity, dynamic range, and lower limit of quantitation (LLOQ) of the drug compounds were determined from the acquired RAW files using a pre-defined Chromeleon CDS template. Likewise, the mass accuracies of the drug compounds were extracted from the RAW files using Chromeleon CDS. Box-Whisker plots showing mass deviation over time were created in Microsoft Excel.

RESULTS

Quantification of drug compounds

All calibration curves passed the linear fit score of $R^2 > 0.990$. The LLOQ values were determined at 50 ppt for all drug compounds in the Full Scan and the tMS2 scan mode on both mass spectrometers. These LLOQ values resulted in a linear dynamic range of 50 ppt to 500 ppb for each drug compound. On the Orbitrap Exploris MX, which has run over a time period of 8 weeks in total, the linearity showed consistent results across the whole 8 weeks of measurements (**Figure 2**).

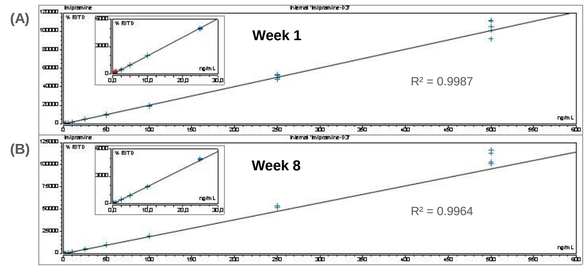

Figure 2. (A) Calibration curve for Imipramine taken from the 1st week of measurements on the Orbitrap Exploris MX. (B) Calibration curve for Imipramine taken from the 8th week of measurements on the Orbitrap Exploris MX.

Evaluation of mass deviations

The instrument control software was extended with an autonomous calibration procedure, so called *One-Point Mass Self-Calibration*, which is based on the One-Point Mass Calibration procedure. The main benefit of this mass calibration is, that it requires no changes to the analytical setup.

The autonomous procedure allows to run a scheduled One-Point Mass Calibration at a defined cadence (*Daily* or *Weekly*). The scheduled mass calibration happens when the mass spectrometer is in an idle state for at least 5 minutes, in *On Mode* or *Standby Mode*. It takes up to 70 s to run the mass self-calibration. The autonomous procedure generates a self-calibration report (**Figure 3**) and updates the *master.cal* file in terms of the last successful mass calibration, extending the validity of the mass calibration.

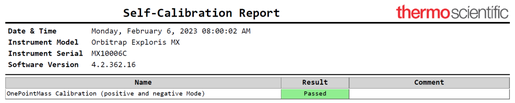

Figure 3. Automatic generated Self-Calibration report after performing a scheduled One-Point Mass Self-Calibration.

On the Orbitrap Exploris MX, the mass self-calibration was scheduled for each Monday over a time period of 8 weeks. Every following Thursday a full calibration curve of the drug compounds was recorded in Full Scan mode; the RunStart EASY-IC option was enabled. No calibration curve was run in week 3. The mass deviations of the drug compounds were monitored (n = 65 data points per compound per week) and stayed well within 3 ppm over the full 8 weeks (**Figure 4**). The lowest mass error observed was -1.40 ppm, the highest mass error observed was 1.37 ppm.

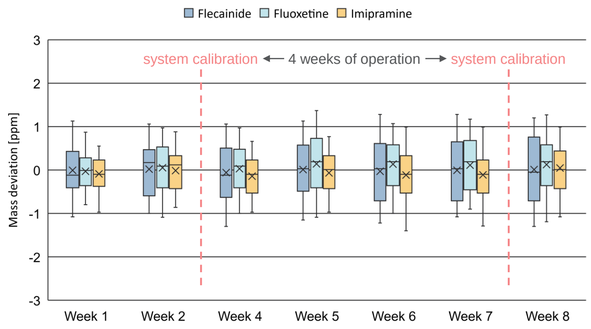

Figure 4. Box-Whisker plot of all mass deviations (n = 65 data points per compound per week) over 8 weeks for each drug compound monitored on the Orbitrap Exploris MX mass spectrometer using Full Scan mode (with RunStart EASY-IC option enabled). Additional system calibrations were applied between week 3 and week 4, and between week 7 and week 8 extending the validity of the full-range mass and system calibration.

On the Orbitrap Exploris 120, the mass self-calibration was scheduled on daily basis over 12 continuous days. A full calibration curve was recorded each day in Full Scan mode and tMS2 scan mode; the RunStart EASY-IC option was disabled for the days 1 to 6 but enabled for the days 7 to 12. The mass deviations of the drug compounds (n = 65 data points per compound per day for each scan mode) stay well within 3 ppm over the 12 days. During the first 6 days the lowest mass error observed was -1.46 ppm, the highest mass error observed was 2.72 ppm. For the days 7 to 12 the lowest mass error observed was -1.15 ppm, the highest mass error observed was 2.1 ppm. **Figure 5** shows the mass deviations over the 12 days of measurements using the tMS2 scan mode.

While there is no mass correction applied in first 6 days, Box-Whisker plot **(B)** illustrates the benefit of enabling the RunStart EASY-IC option for the days 7 to 12.

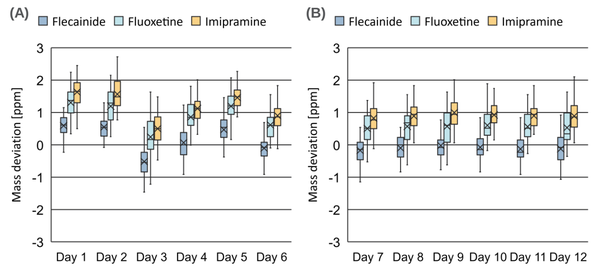

Figure 5. Box-Whisker plots of all mass deviations (n = 65 data points per compound per day) over 12 continuous days for each drug compound monitored on the Orbitrap Exploris 120 mass spectrometer using tMS2 scan mode. Plot (A) illustrates the mass deviations for the days 1 to 6. Plot (B) illustrates the days 7 to 12 with RunStart EASY-IC option enabled.

CONCLUSIONS

All drug compounds show consistent mass accuracy and linearity of detection over a time period of 8 weeks. In both scan modes, Full Scan and tMS2, the compounds of interest are reliable quantified down to 50 ppt in crashed bovine plasma.

The One-Point Mass Self-Calibration extends the validity of the full-range mass calibration and delivers accurate mass measurement below 3 ppm for all compounds and all concentrations without the need to infuse a calibration solution.

The One-Point Mass Self-Calibration only takes a few minutes and can be executed daily or weekly in combination with RunStart EASY-IC, to ensure the highest data quality and reliability.

The One-Point Mass Self-Calibration enables continuous long-term operation of the mass spectrometer with no change to the analytical setup, thus optimizing the instrument uptime and productivity.

REFERENCES

Erik P.A. Couzijn, et al.: Extending the Interval between Full-Range Mass Calibrations on Orbitrap Exploris Mass Spectrometers (PO66109 EN0921S)

TRADEMARKS/LICENSING

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