Common Sources of Contaminants Observed When Calibrating an Orbitrap Mass Spectrometer And How to Avoid Them

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ABSTRACT

Purpose: Determine the suitability of a calibration solution to be used for instrument calibration and detail common sources of contamination due to improper storage and handling.

Methods: Samples of calibration solution was purposefully contaminated or degraded by storing in non-standard containers, using improper transfer lines and spray conditions, and installing new hardware. Calibration solutions were evaluated utilizing the Thermo Scientific™ Orbitrap™ Tribrid™ Series instrument control software.

Results: Details on the automated evaluations as well as several common sources of contaminants and preventative measures are provided.

INTRODUCTION

Successful collection of high-quality mass spectrometry data relies on the proper calibration of the mass spectrometer. Although calibration solutions are very stable over time and aim to provide a clean spectrum for optimizing performance, unexpected contamination can occur. When contamination does occur, peaks near the expected calibration peaks can result in poor optimization of the mass spectrometer's operating parameters or a failure to recalibrate the system entirely. The calibration routines used for the Orbitrap Tribrid platform ensure that the observed spectrum is suitable for calibration through an automated Spray Stability and Calmix Evaluation. Here we investigate potential sources of contamination using a commercially available calibration solution due to improper storage, handling, and spray conditions. Additionally, we provide details on the automated evaluation and the new calibration routines in the Orbitrap Tribrid Series instrument control software version 3.4.

MATERIALS AND METHODS

Sample Preparation

Thermo Scientific™ Pierce™ FlexMix™ Calibration Solution was stored in several common containers (PTFE, Eppendorf tubes, glass scintillation vials) in order to examine any changes to the calibration solution. A syringe containing a brass ferrule was disassembled and the ferrule was placed in an Eppendorf tube containing FlexMix for one hour then removed to simulate the effect of leaving the calibration mix in the syringe.

Test Methods

A clean, 500mL syringe was used to infuse FlexMix Calibration solution into a Thermo Scientific™ Orbitrap Eclipse™ Tribrid™ mass spectrometer for each of the prepared samples. A new 0.0025 ID PEEK tubing was installed on the HESI source and a fresh sample of FlexMix was infused. The tan .0025 ID PEEK tubing was replaced with the red .005 ID PEEK tubing and FlexMix was infused at varying spray and flow conditions.

Data Analysis

An Orbitrap Eclipse Tribrid MS operating with Instrument Control Software 3.4 was used to evaluate the suitability of the spray stability and calibration mix for calibrating the system.

RESULTS

Improper Storage Containers

Stored in the proper container, calibration solutions are stable for long periods of time and will remain usable for performing quality calibrations. However, when removed from the original PTFE container and stored in other materials such as glass or plastic, interactions between the solution and container can contaminate the calibration mix.

Figure 1. Stored in a PTFE container, minimal differences between the baseline spectrum (A) and a solution stored at 50° C for three weeks in order to accelerate the degradation of the calibration mix (B).

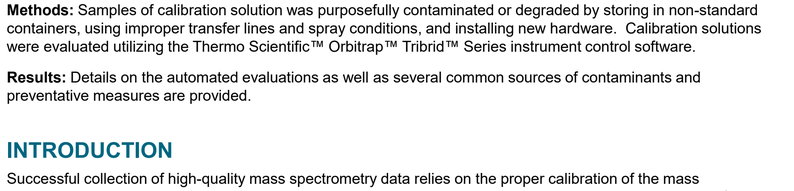


Figure 2. After transferring the calibration solution from a PTFE container (A) to an Eppendorf Tube (B), new peaks are immediately seen across the baseline. After one week (C), the intensity of these peaks double in intensity and are twenty times greater than the PTFE reference.

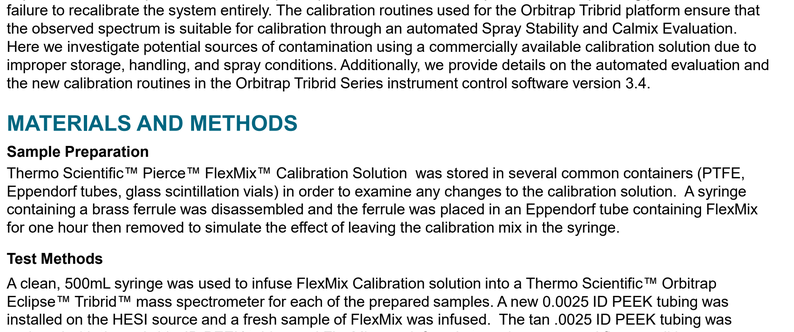
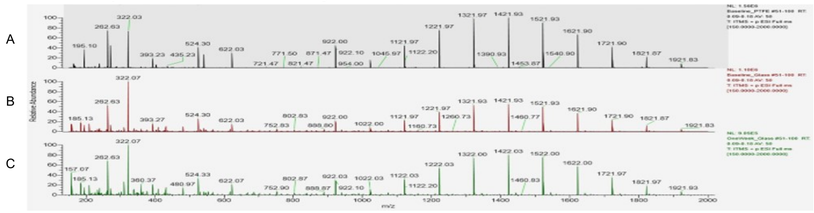


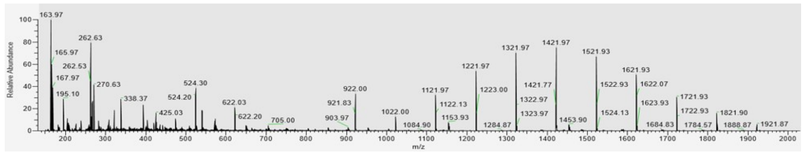
Figure 3. Compared to the spectrum of a standard FlexMix solution (A), solution moved to a glass scintillation vial shows an immediate change in ion intensities and baseline noise (B). After one week of storage in a glass container (C), the total ion current has dropped significantly and the relative abundance of calibration peaks has shifted dramatically.



Extended Storage in Syringe

When left sitting in the syringe, metal ions from the ferrule enter into solution with the calibration mix. The resulting metal complexes can interfere with the expected calibration peaks and disturb the distribution of the calibration spectrum. An overabundance of undesired peaks can result in a failure of the Calmix Evaluation.

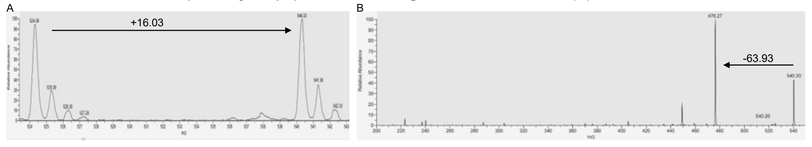
Figure 4. Metal complexes form in solution with the calibration compounds, disturbing the desired spectrum for optimal calibration.



Electrochemistry in Transfer Lines

Increasing the inner diameter and reducing length of the tubing from the grounding union to the HESI sprayer lowers the electrical resistance of the flow path, resulting in changes to the calibration mix due to electrochemistry. This effect increases as the flow rate is lowered and the spray voltage increases.

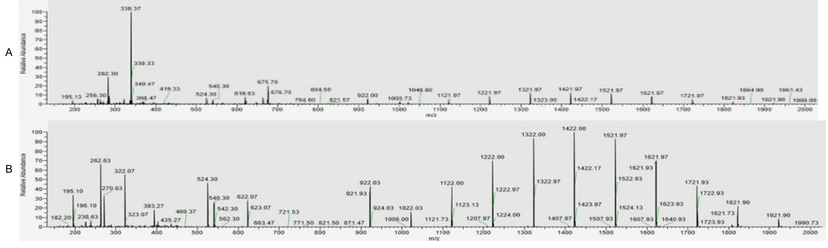
Figure 5. Electrochemistry in the transfer lines results in the oxidation of methionine in the MRFA peptide, causing a shift of 16 m/z in the observed spectrum (A). HCD fragmentation of the peak at 540 m/z shows a loss of 64 m/z, a known pathway for peptides containing oxidized methionine (B).[1]



Background from Fresh Transfer Lines

When first installing new lines to the HESI source, there is invariably contamination introduced that can result in high background signal. This background noise will wash out over time as the calibration solution is infused through the lines.

Figure 6. High background noise is present when first installing new hardware. In particular, erucamide is observed at m/z 338 (A). After infusion of the calibration mix for 20 minutes, the background signal washes out and has minimal interference (B).

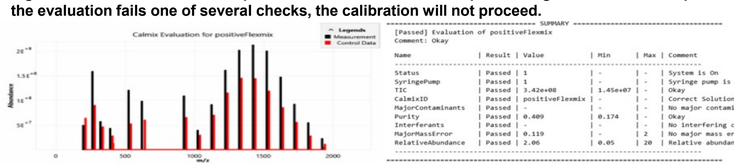


DISCUSSION

The Orbitrap Tribrid Series Instrument Control Software Version 3.4 introduces a new, automated calibration procedure. Unlike previous versions of software that required the user to manually select which calibrations to run, the new procedure organizes calibrations into sets that should be run together in a particular order. Optional calibrations are selectable based on the instrument configuration and polarity. The status window shows information about the calibration status of the system and whether calibration is required.

As the calibration routines can be sensitive to unstable signal and interfering ions near the calibration peaks, a stable, clean calibration spectrum is necessary to achieve the optimal performance of the instrument. Before beginning any calibrations, the Spray Stability and CalMix Evaluation procedures are run automatically. These checks ensure that the spray and calibration solution are acceptable for proceeding with the calibrations Interference from contaminating peaks in the calibration spectrum can also result in poor optimization of the instrument performance. A contamination peak in very close proximity to the target peak may be mistakenly chosen over the desired peak. Oxidation of the MRFA peptide and formation of undesired adducts may reduce the intensity of the calibration peaks relative to the reference spectrum enough that the evaluation fails.

Figure 7. The Calmix Evaluation plots the observed calibration spectrum against a reference spectrum. If the evaluation fails one of several checks, the calibration will not proceed.

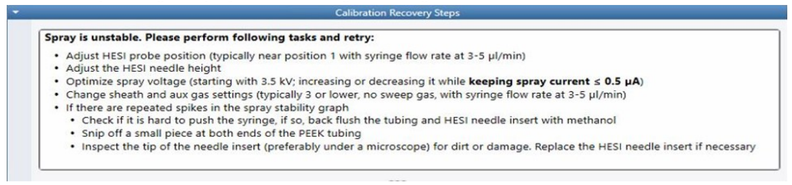


The Calmix Evaluation makes several checks of the observed spectrum against the reference spectrum, starting with:

1. A simple check of the total ion current to be sure that the signal is high enough to calibrate with.
2. Verifying there is a minimal amount of unexplained ions that are not part of the calibration spectrum.
3. Searching in a 10 m/z window around each of the calibration peaks, the evaluation checks that the expected peak is the most intense peak in the window.
4. Finally, a check of the mass error of the calibrants ensures that the system is at least coarse calibrated and ready to proceed with the standard calibrations.

In previous versions of the instrument control software, a failure of these checks was not always distinguishable from a true calibration failure. Now, the calibration procedure differentiates between a procedural failure of the evaluations and true calibration failures. When a procedural failure occurs, recovery steps are provided to assist the user in resolving the issue.

Figure 8. When the Spray Stability or CalMix Evaluation checks fail, recovery steps are provided to assist the user in optimizing the spray and minimizing interfering ions.



CONCLUSIONS

- Stable spray and a clean calibration solution is critical to successful optimization of the mass spectrometer's performance.
- Contamination or degradation observed can be mapped to common errors in calibrant storage and handling. With proper handling, these problems can be avoided.
- While stable when stored in the correct container and used with the recommended transfer lines, improper storage and handling can result in contamination or degradation of the calibration mix.

REFERENCES

1. X. Jiang, J.B. Smith, E.C. Abraham, Identification of a MS-MS fragment diagnostic for methionine sulfoxide, J. Mass Spectrom. 31 (1996) 1309–1310.

TRADEMARKS/LICENSING

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