# #010 - A Practical Lock-Mass Calibrant Introduction Method for Improved Mass Accuracy and Reduced False Positive Identifications in Non-Targeted Analyses

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## Introduction

- Accurate mass information is critical for identification of unknown compounds in non-targeted analysis (NTA)
- Specifications for mass accuracy error on Q-Exactive: < 3 ppm for external calibration and < 1 ppm for internal/lock-mass calibration
- Lock-mass calibration with background ions:
  - Convenient
  - Ions may change over time
  - Relying on a single calibrant ion can result in no/poor recalibration if the calibrant ion signal is not present (ion suppression is common in foods)

## Non-Targeted Method

Sample Preparation

Spiked Standards: Non-Targeted Standard Quality Control (NTS/QC) mixture; 83 compounds, molecular weight range 126-1110, logP range -8 to 8, diversity in elemental composition
(modified from: Knolhoff A.M., Premo J.H., Fisher C.M. Anal. Chem. 2021, 93, 1596-1603.)

Sample Matrices: Neat Standard and Spiked Arrowroot Biscuit Extract

Extraction with 75:25 ACN with 1%FA: $H_2O$
(Mol, H.G.J., et al. Anal. Chem. 2008, 80, 9450-9459.)

750 µL extract + 250 µL $H_2O$

UPLC Shimadzu Nexera

Column: Kinetex C18, 2.1x150 mm, 1.7 µm, 100 Å

60°C, 0.4 mL/min; A: 0.1% FA in $H_2O$; B: 0.1% FA in ACN

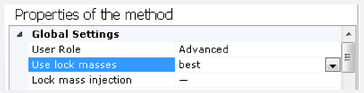
Gradient: 2.5 min hold at 5% B, 25 min gradient to 95% B, 2 min hold at 95% B, 5 min equilibration at 5% B

MS Thermo Q-Exactive

Full Scan: Polarity switching, 70k resolution

MS/MS: MS at 70k resolving power, MS/MS at 17.5k resolving power, DDA (top 10), positive mode only

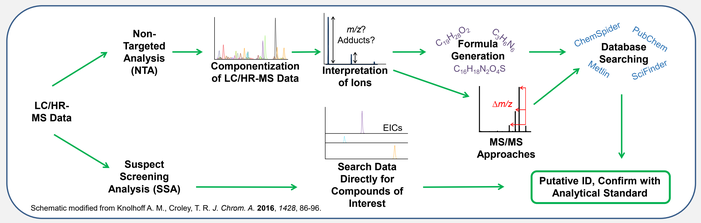
Lock-Mass: "Best" vs. "If all present" = "All"




Processing Software

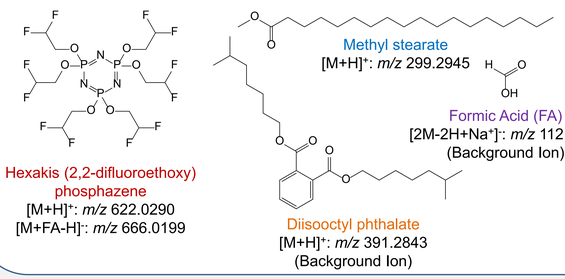
Spectrus Processor/IntelliTarget (ITA); (ACD/Labs)

Compound Discoverer 3.2 (CD, Thermo)
(Knolhoff A.M., Premo J.H., Fisher C.M. Anal. Chem. 2021, 93, 1596-1603.)




## Setup for Calibrant Introduction

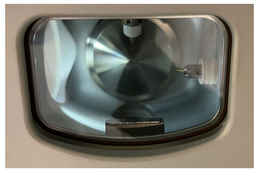
### Calibrants




### Steel Holder and Absorbent Sponges

Sponge dimensions: length ≈ 0.3in, diameter ≈ 0.25in

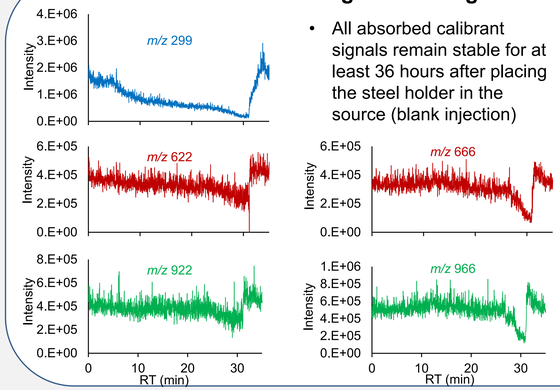
Steel holder dimensions: ~1.25in x ~0.5in and depth ≈ 0.4in

Steel holder 1-2 cm inside the HESI source housing

Bruker calibrant reservoir kit (originally designed for lock-mass correction for the Maxis QTOF series instruments)

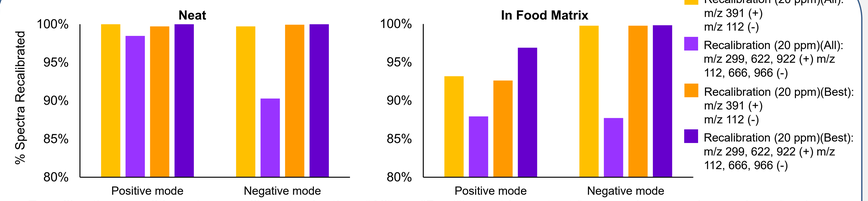
### Calibrant Signals Throughout LC Gradient




- All absorbed calibrant signals remain stable for at least 36 hours after placing the steel holder in the source (blank injection)
- Some background ion signals are stable but not during all queues/runs
- *m/z* 112 used as low *m/z* calibrant with absorbed calibrants

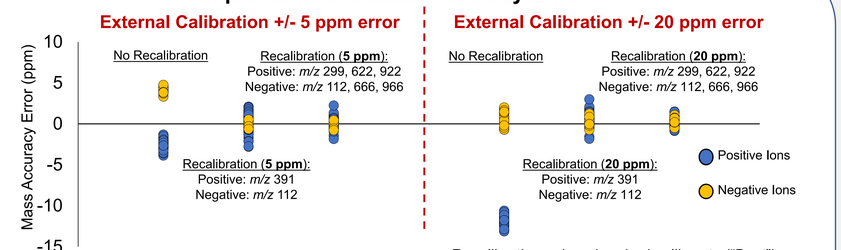
## Results: Improved Data Quality for NTA

### % Spectra Recalibrated



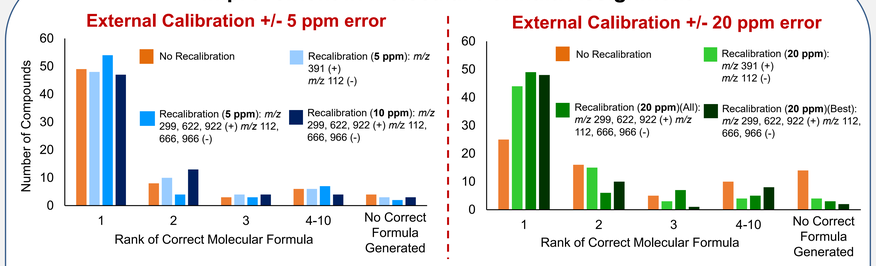

- Recalibration conditions (e.g., calibrant selection; "All" vs. "Best") most important for complex samples such as foods
- Recalibration using absorbed calibrants with "Best" condition resulted in the most spectra recalibrated in a complex food matrix; ~10% more spectra recalibrated in both positive and negative mode with "Best" compared to "All"

### Improvement in Mass Accuracy Error




- Recalibration using background ions ("Best") improved mass accuracy errors to within +/- 1 ppm for >73% of 83 standard compounds in food (with external cal. +/- 5 and +/- 20 ppm)
- Recalibration using absorbed calibrants ("Best") improved mass accuracy errors to within +/- 1 ppm for >96% of 83 standard compounds in food (with external cal. +/- 5 ppm and +/- 10 to 20 ppm)

### Improvement in Molecular Formula Assignment




- Absorbed calibrants at 5 ppm tolerance ("Best") gives most correct rank 1 molecular formula in food extract
- Recalibration at 5 ppm tolerance ("Best") gives more higher rank correct molecular formulae than 10 ppm tolerance (even using background ions)
- Lock-mass recalibration generates ~2x as many correct rank 1 molecular formula when the external calibration is off by +/- 20 ppm (background & absorbed calibrants)
- More rank 1 molecular formula generated using absorbed vs. background calibrants (for "All" and "Best")

## Conclusions

- Co-dosing the two lower *m/z* calibrants on the same sponge under the 5-hole cap and dosing the highest *m/z* calibrant on a separate sponge under the 12-hole cap generated stable signal for all calibrants for at least 36 hrs
- Mass accuracy and molecular formula generation improved with recalibration, providing better data quality for non-targeted analysis and more confident putative identifications
- The use of absorbed lock-mass calibrants can improve mass accuracy errors to within +/- 1 ppm even at an external mass tolerance of 20 ppm
- "All" vs. "Best": "Best" was selected as the optimal recalibration condition for NTA applications
  - Mass accuracy improvement similar
  - "All" generated more top-hit correct molecular formula matches
  - "All" results in ~10% fewer re-calibrated spectra compared to "Best" throughout chromatogram
- Recalibrating with the absorbed calibrants and formic acid dimer improved mass accuracy and molecular formula generation more than recalibrating with the background ions tested