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Origins and Mitigation of Unwanted Dissociation of Fragile Analyte Ions in Compact Quadrupole Orbitrap Mass Spectrometers

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ABSTRACT

Purpose: Develop dedicated tuning of compact quadrupole Orbitrap instruments for fragile analytes.

Methods: Diagnostic manipulation of ion optics in low-flow infusion; high-flow LC-MS XICs.

Results: Discerned MS1 dissociation in the S-Lens/Funnel, in the Inject Filter, and upon trapping. Implemented a Mild Trapping mode for reduced MS1 dissociation.

INTRODUCTION

Metabolomics, lipidomics, and related fields often deal with fragile analyte ions, whose identification and quantitation are hampered by unwanted dissociation inside the mass spectrometer. 1) Analyte signal intensity is reduced, affecting the LOD and LOQ. 2) The fragment ions, generated on MS1 level, lead to unproductive scans in data-dependent acquisition (DDA). 3) Some fragment ions have the same composition as other analytes of interest, leading to false identifications even on high-resolution accurate-mass (HRAM) instruments. Here we distinguish critical regions where MS1 dissociation may occur in the Thermo Scientific™ Orbitrap Exploris™ series of compact quadrupole–Orbitrap™ HRAM instruments. We screen hardware settings influencing the extent of dissociation and evaluate a milder tuning parameter set to deal with very fragile samples.

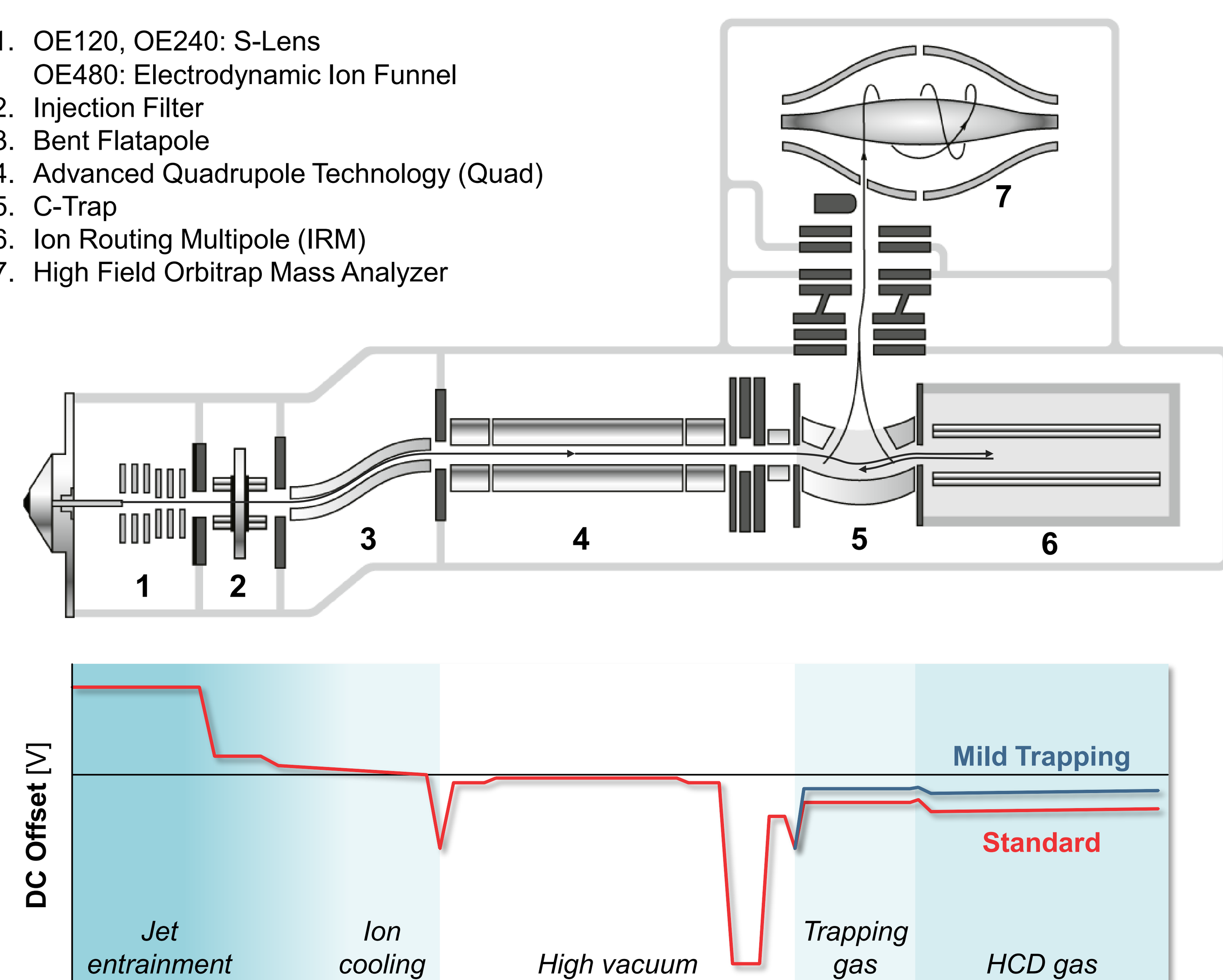
MATERIALS AND METHODS

Samples: Fragile ion mix of isoleucine (Ile)/phenylalanine (Phe)/adenosine/sodium glycodeoxycholate (GDC); 100 µg/L mix of 24 metabolites; 2 µg/L mix of 8 small molecules.

Test Methods: The fragile ion mix was infused at 3 µL/min on the Orbitrap Exploris 120 and Orbitrap Exploris 480 mass spectrometers. 10-µScan SIMs and MSXs at 5 Th isolation and 80–460 Th analytical window were recorded at 15k resolution, using a fixed inject time matching 1e5 target, while manipulating ion optical parameters. Thermo Scientific™ Pierce™ FlexMix™ Calibration Solution was infused at 5 µL/min; the HCD isolation width was 5 Th for Ultramark and 2 Th for MRFA.

The metabolite mix was studied on a Thermo Scientific™ Vanquish™ Flex UHPLC–Orbitrap Exploris 240 setup with Thermo Scientific™ Hypersil GOLD™ C18 column (150 × 2.1 mm, 1.9 µm); 0.3 mL/min gradient elution with MeOH/H_2O + 0.1% FA, S-Lens RF Level 70; FullMS *m/z* 67–1000 at 120k resolution. The small-molecule mix was studied at 0.5 mL/min gradient elution; tSIM on *m/z* 110–1300 at 1.5 Th isolation, 60k/30k resolution.

Figure 1. A) The Orbitrap Exploris ion optical path; B) standard and Mild Trapping DC tuning profiles, including vacuum regions.



Data Analysis: Ion optics manipulations and development of the mild tuning parameter set were performed in the Service Diagnostic Tool. LC-MS acquisitions were run through Thermo Scientific™ Xcalibur™ software. RAW files were processed in Qual Browser (signal intensities) or Thermo Scientific™ TraceFinder™ software (LC-MS peak areas) to determine MS1 fragment:Analyte ratios and Mild Trapping:Standard-tuning relative transmissions.

DIAGNOSIS OF MS1 DISSOCIATION ORIGINS

In the widened SIM spectra of the fragile analytes, MS1 fragment contributions must originate from after the isolating Quad, i.e. in the C-Trap and/or IRM (**Figure 1**). Up-front contributions follow from comparison with analyte + fragment MSX scans without inject filtering, with the S-Lens/Funnel RF forced from Level 70/40 (not tuned for fragile analytes) to 20 Vpp, and in standard operation. **Figure 2** shows that different analyte ions mainly undergo MS1 dissociation in distinct regions of the instrument: Ile as well as Phe and adenosine (not shown) do so mostly upon trapping, whereas GDC is susceptible to RF heating in the Injection Filter and in the S-Lens/Funnel.

Figure 2. MS1 dissociation origins for fragile analytes (* RF Levels *not* tuned).

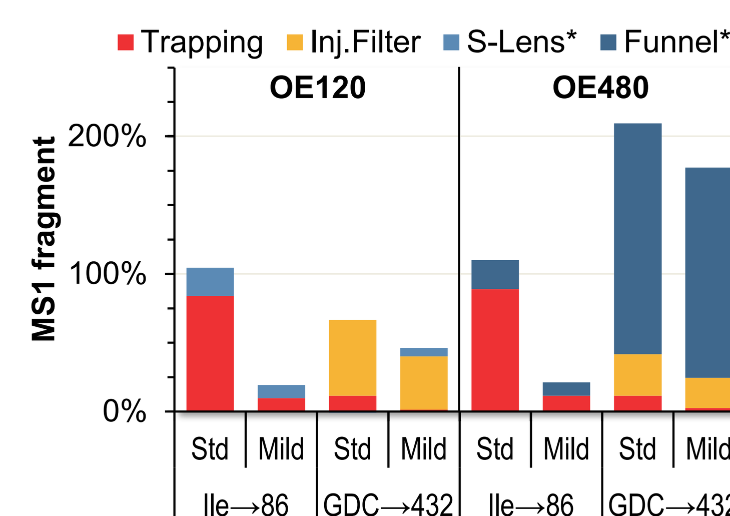
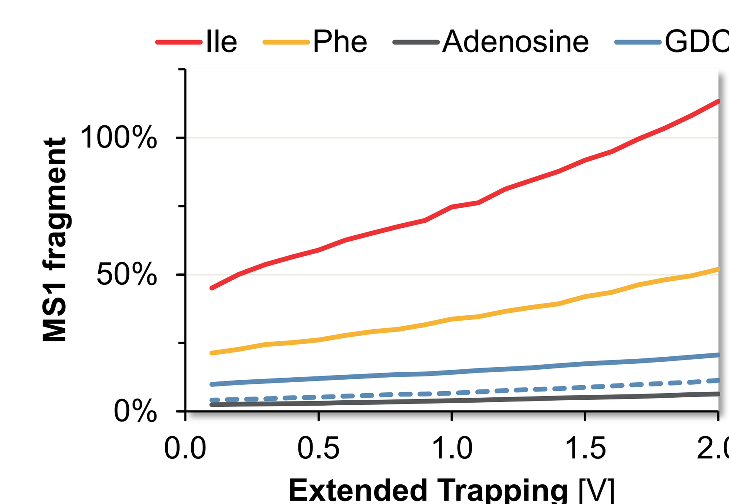


Figure 3. MS1 dissociation depends on Extended Trapping offset.

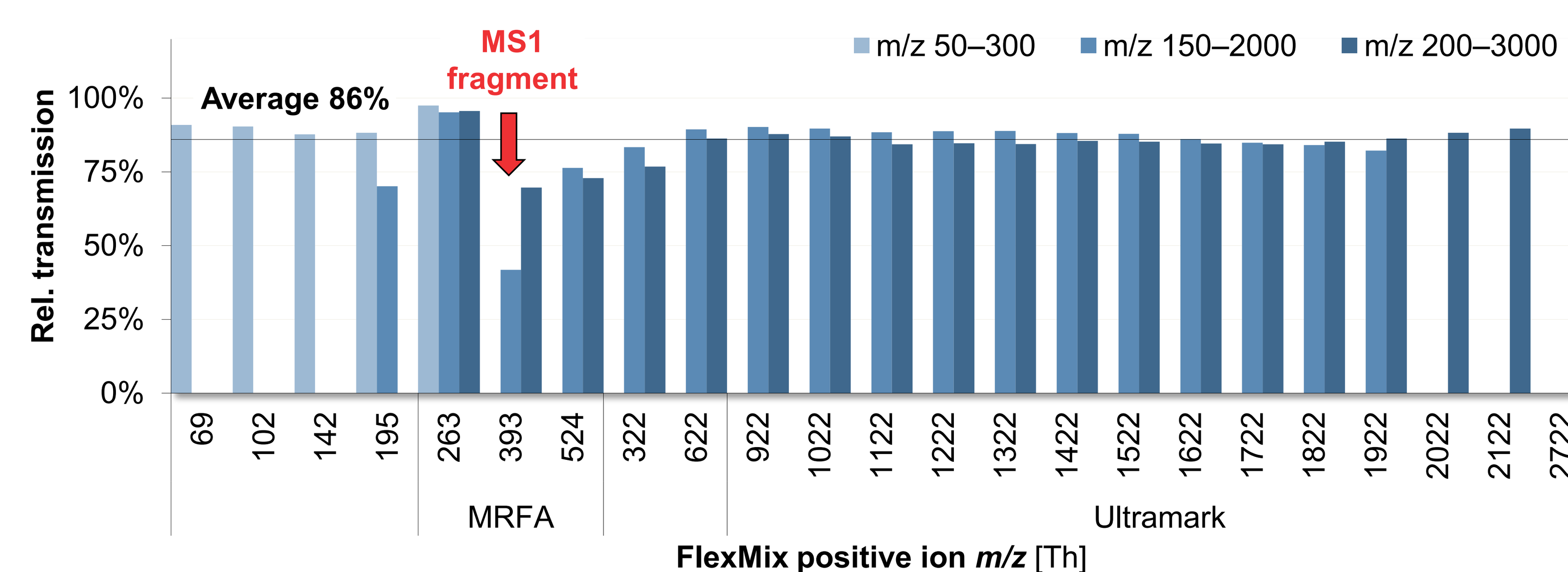


While the user can influence dissociation in the S-Lens/Funnel through the RF Level (by balancing with intensity), and in the Injection Filter through the isolation width or the scan mode, the trapping parameters are not tunable. For the standard DC tuning profile (**Figure 1**) the ions, having been kinetically cooled in the Bent Flatapole and sent through the Quad in high vacuum, enter the gas-containing C-Trap and IRM with considerable velocity and thus may get collisionally activated. We scanned the ion optical devices to assess the potential of reducing MS1 dissociation. Both the Extended Trapping offset (**Figure 3**) and CLT GND affect the fragment-to-analyte ratio. Based on these findings, we implemented a Mild Trapping mode that shifts all DC offsets for the operation of C-Trap and IRM closer to that of the Bent Flatapole. As included in **Figure 2**, this mode indeed affords much smaller contributions of trapping-induced MS1 dissociation than the standard tuning.

EVALUATION OF MILD TRAPPING

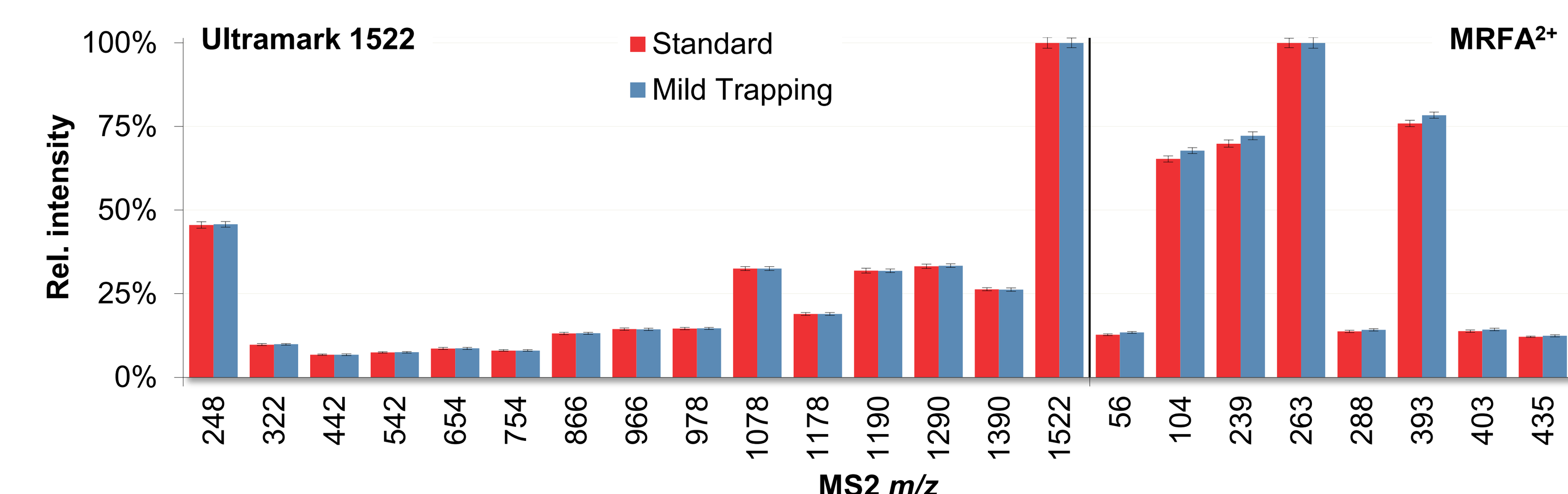
We compared FlexMix FullMS intensities with mild vs standard tuning parameters (**Figure 4**). MS1 dissociation of $[MRFA{\cdot}H_3O{\cdot}H]^{2+}$ into $[RFA{\cdot}H_3O]^{+}$ (*m/z* 263→393) is notably reduced and also its 1+ charge state is slightly suppressed. All other, stable ions have similar relative transmission, indicating an average penalty of 14% in this experiment; some analytes may experience a higher penalty.

Figure 4. Mild Trapping reduces MS1 dissociation, accompanied with a small intensity loss.



HCD product ion ratios stay essentially unaltered with the mild tuning parameters (**Figure 5**); that is, Mild Trapping does not affect e.g. DDA experiments.

Figure 5. MS2 spectral distributions are not affected.



HIGH-FLOW LC-MS APPLICATION

Mild Trapping drastically reduces MS1 dissociation in LC-MS analysis of the metabolite mix (**Figure 6**). Note that GDC dissociates much less than in the infusion SIMs in general, while the contribution from RF heating in the Inject Filter is much smaller in FullMS. In LC-MS tSIM analysis of the small-molecule mix (**Figure 7**), the peak area of the fragile methylmalonate is more than doubled thanks to suppressed dissociation, but in this case the stable analytes suffer ca. 25% loss.

Figure 6. Mild Trapping suppresses MS1 dissociation in LC-MS of metabolites.

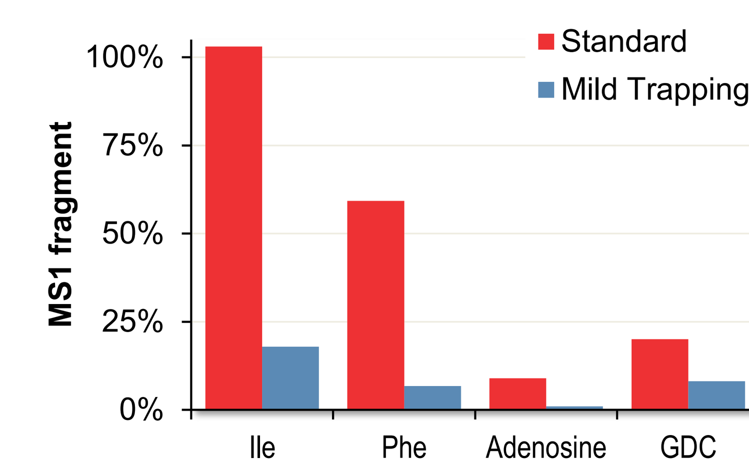
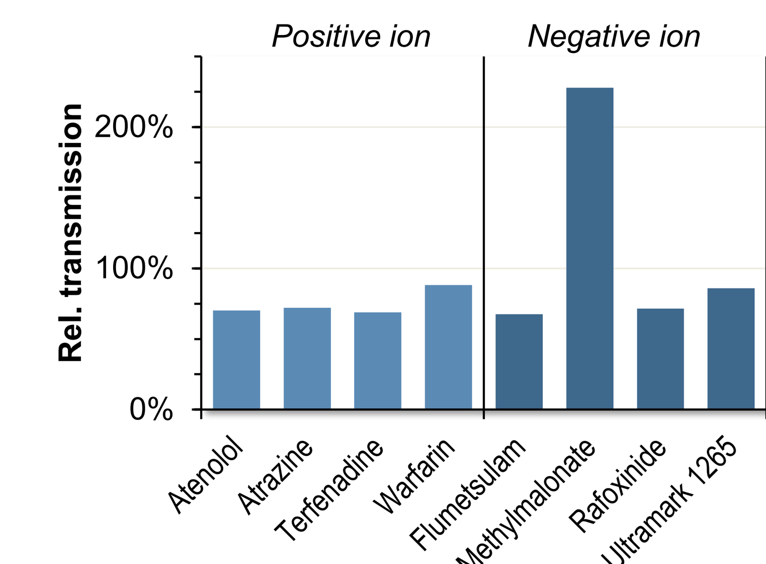


Figure 7. Mild Trapping improves LC-MS tSIM peak areas of fragile analytes.



CONCLUSIONS

- Fragile analytes suffer unwanted MS1 dissociation in gas-containing regions of the mass spectrometer. Some ions are prone to fragment due to RF heating (S-Lens/Funnel, Injection Filter), while other ions dissociate due to collisions upon trapping in the C-Trap–Ion Routing Multipole.
- Mild Trapping reduces ion velocity at trapping, suppressing this fragmentation contribution. As a result, false identifications will be reduced and fragile analyte intensities are improved.
- A penalty in stable-ion transmission of 14–25% is observed, apparently depending on the analysis method and/or the analyte. HCD spectral distributions are unaltered.

TRADEMARKS/LICENSING

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PO65791-EN 0422S

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