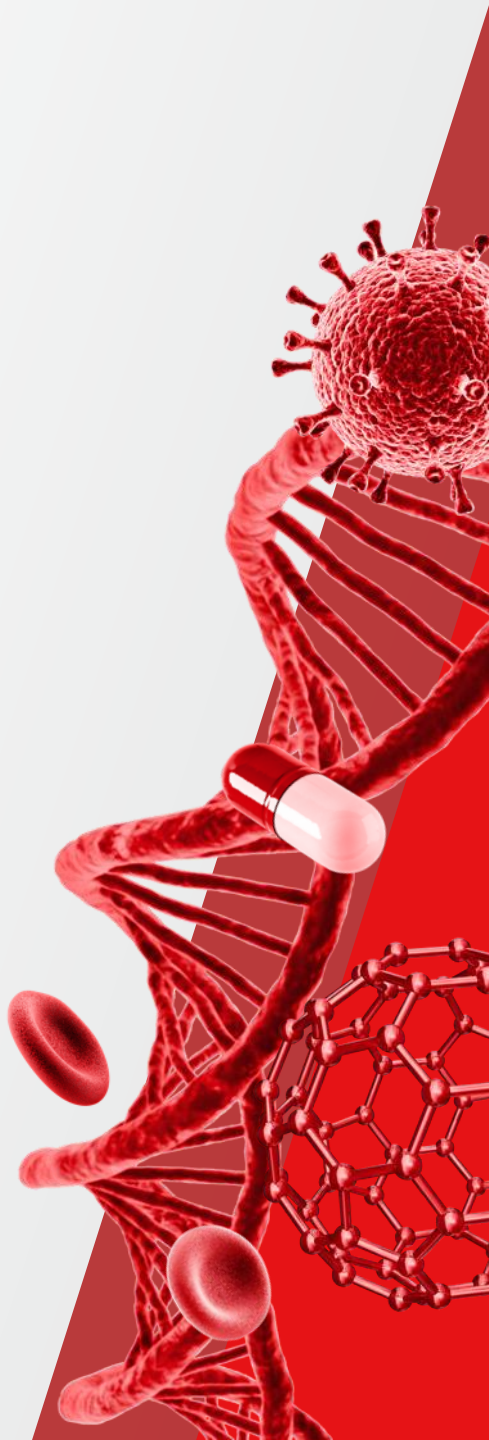
Thermo Scientific™ Orbitrap™ Tribrid™ MS Series: Instrument Control Software v.3.5 Overview



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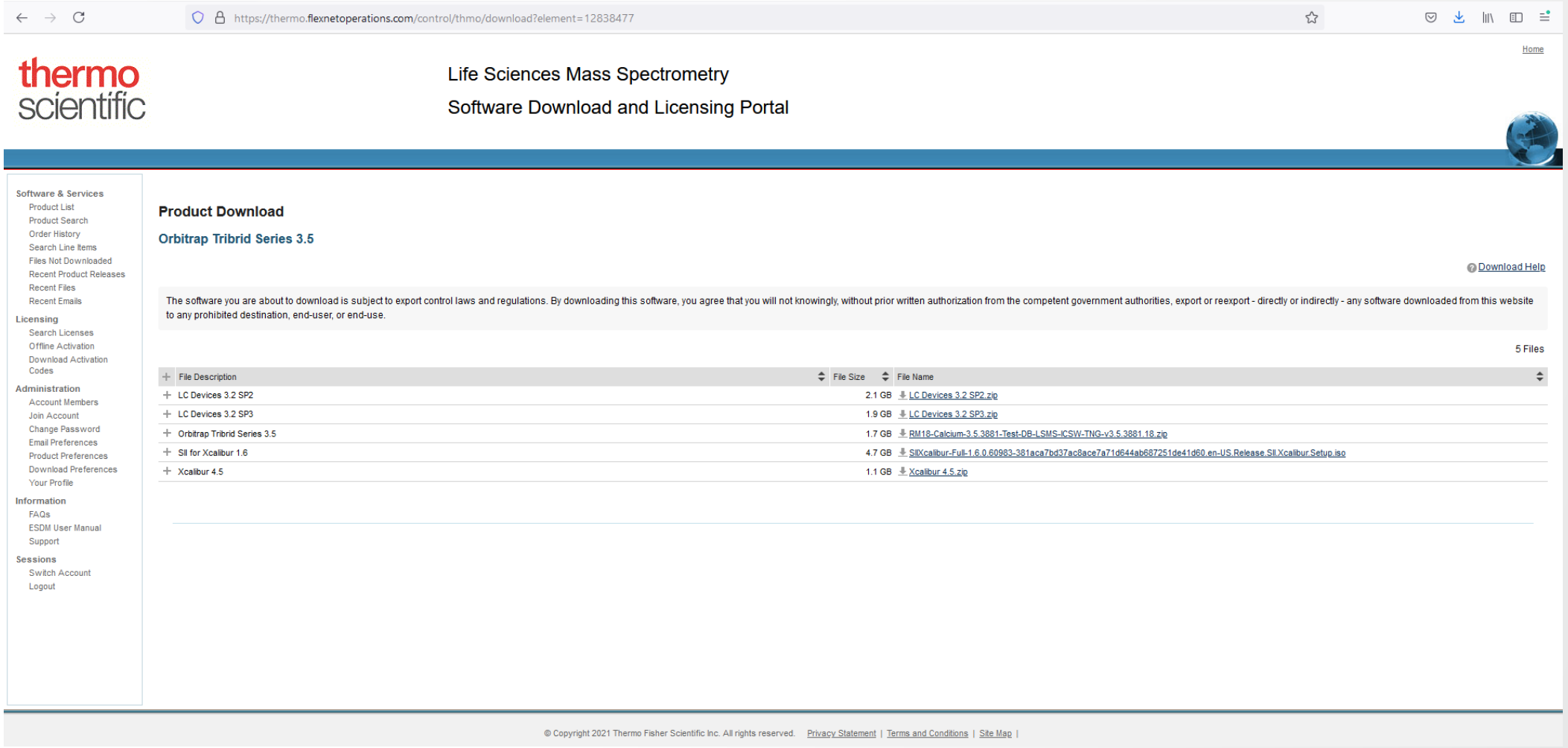
planet.orbitrap@thermofisher.com | July-2021

Software Release

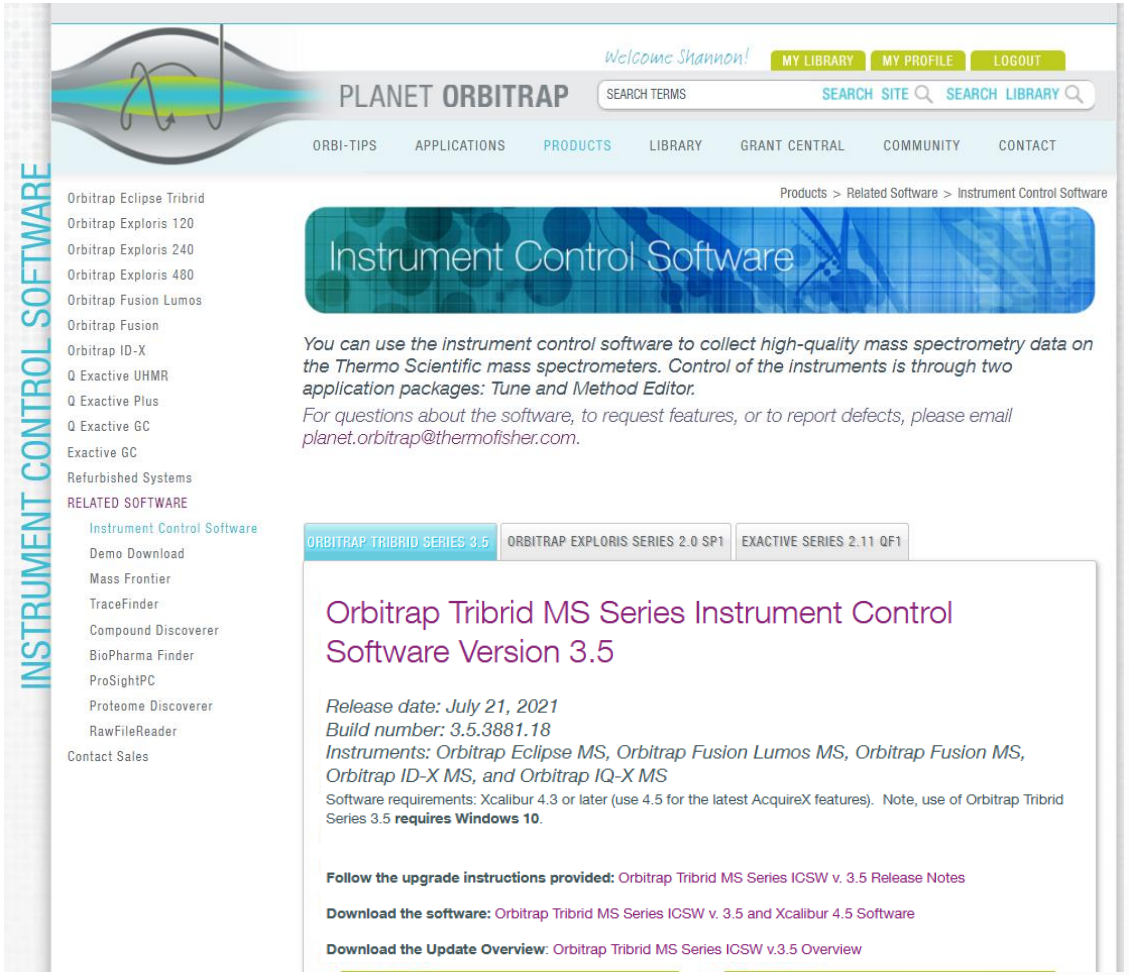
Flexera

Download: [Orbitrap Tribrid MS Series ICSW v. 3.5 and Xcalibur 4.5 Software]



Software Release

Planet Orbitrap



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Orbitrap Tribrid Series Instrument Control Software v 3.5

Orbitrap ID-X, Orbitrap Fusion, Orbitrap Fusion Lumos, and Orbitrap Eclipse MS Systems

Thermo Scientific™
Orbitrap ID-X™ MS

Thermo Scientific™
Orbitrap Fusion™ MS

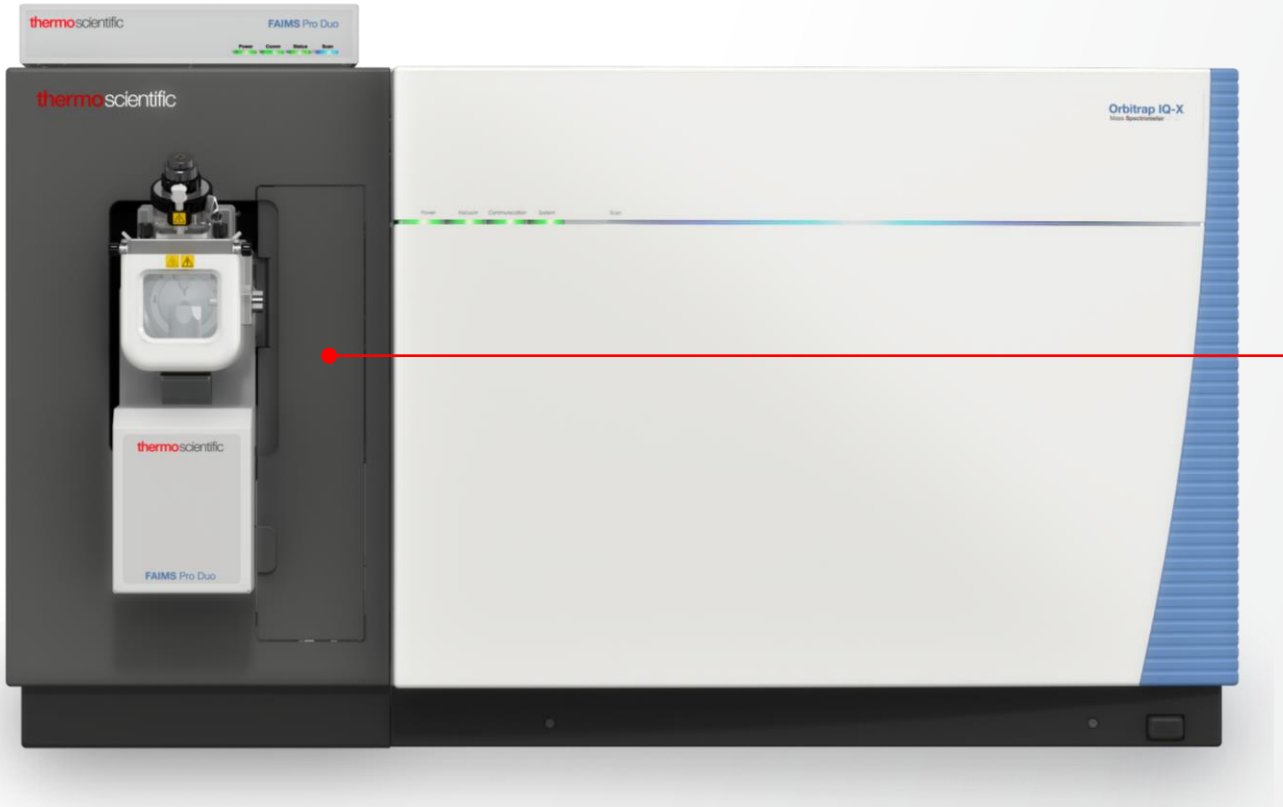
Thermo Scientific™
Orbitrap Fusion™ Lumos™ MS

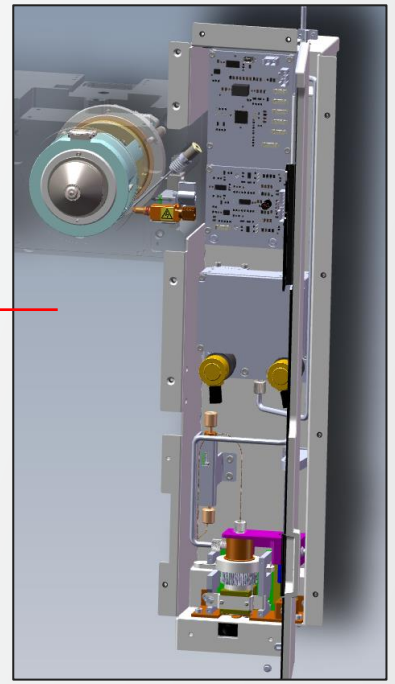
Thermo Scientific™
Orbitrap Eclipse™ MS

Orbitrap Tribrid Series Instrument Control Software v 3.5

Orbitrap IQ-X Equipped with Auto-Ready Ion Source, FAIMS Pro Duo Interface

FAIMS Pro Duo Interface





Auto-Ready Ion Source

Thermo Scientific™ Orbitrap IQ-X™ Tribrid™ Mass Spectrometer

New Features And Usability Enhancements Summary

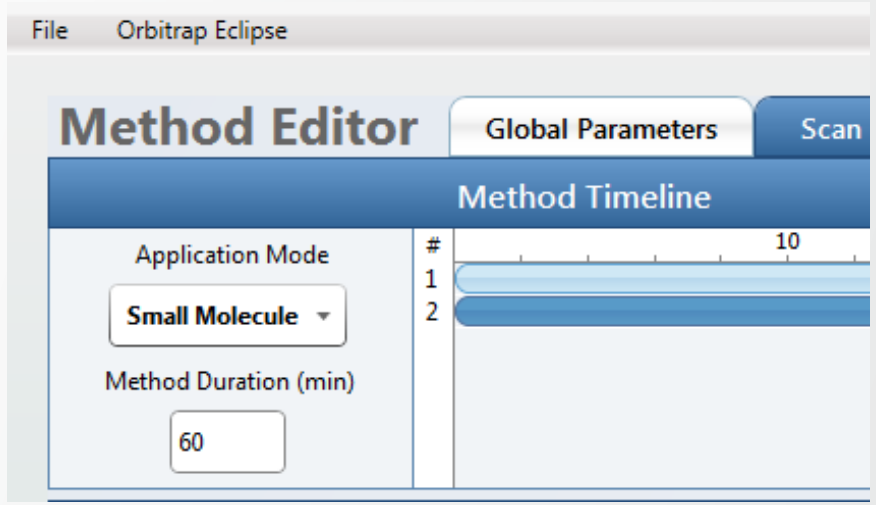
Orbitrap Tribrid Series Instrument Control 3.5

- Support for the Orbitrap IQ-X mass spectrometer
- Orbitrap IQ-X and Eclipse (small molecule mode) - Support for Real-Time Library Search
- Xcalibur 4.5 - Support for new AcquireX workflow and more flexible parameter settings
- Orbitrap IQ-X - Support for Auto-Ready ion source
- Support for FAIMS Pro Duo Interface
- Optimized FAIMS CV post-switching delay time
- Total Carrier Gas Flow succeeds FAIMS User Gas to enable lower flow rates for improved nanospray stability
- Advanced Peak Determination (APD) is now standard for all Orbitrap Tribrid products and benefits from application mode specific optimization
- Support for Extended low mass range m/z 40 in MSn scans
- Mild Trapping option available to reduce MS1 fragmentation of labile compounds
- Usability enhancements
  - Support for absolute and normalized HCD collision energies
  - Support for target specific HCD collision energy in Targeted Mass Filter Table
- Support for Chromeleon

Support for Real-Time Library Search

Real-time Acquisition Decisions for Improved Structure Elucidation of Small Molecules

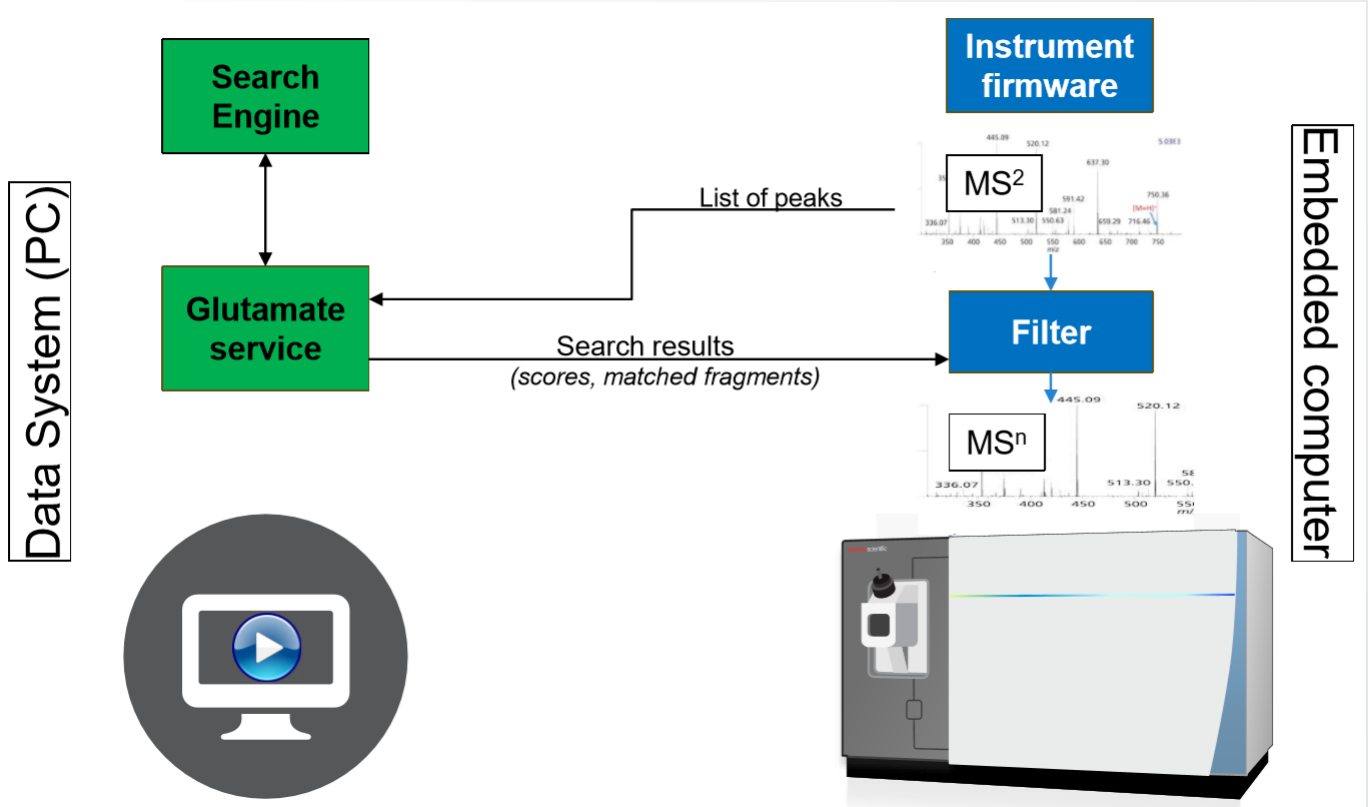
- Challenge
  - Elucidation of chemical structures is one of the most difficult challenges in analytical chemistry
  - MS2 is often insufficient for structure assignment
  - MSn can help, but requires experience to set up
- Solution
  - On-the-fly MS2 spectral matching against custom mzVault libraries or local copies of mzCloud libraries for decision-based triggering of MSn scan events
- Availability
  - Orbitrap IQ-X
  - Orbitrap Eclipse (small molecule application mode)



Support for Real-Time Library Search

Background Services & Communication

- Glutamate backend service used to process each generated MS2 scan
- Search results returned based on RTLS filter parameters
- Triggering of MSn scan events according to search results, method scheme, and filter thresholds



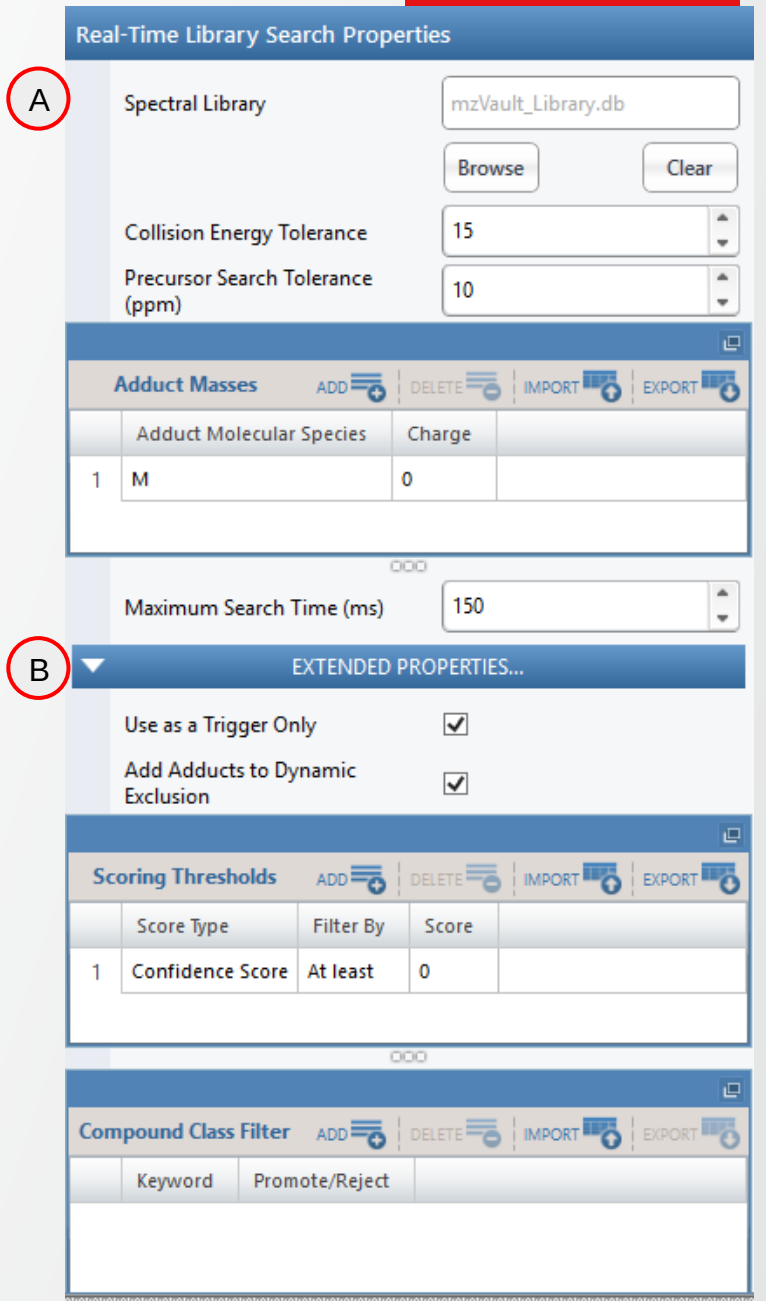
Support for Real-Time Library Search

Filter Overview

- Two sections of RTLS filter properties
  - Global parameters (A)
  - Filter instance specific “Extended Properties” (B)

- Global parameters (A)
  - Synchronized across all instances of the filter
  - Impact general search behaviors

- Extended properties (B)
  - Filter instance specific within the method decision tree
  - Controlled independently for each filter and can lead to specific outcomes and complex decisions



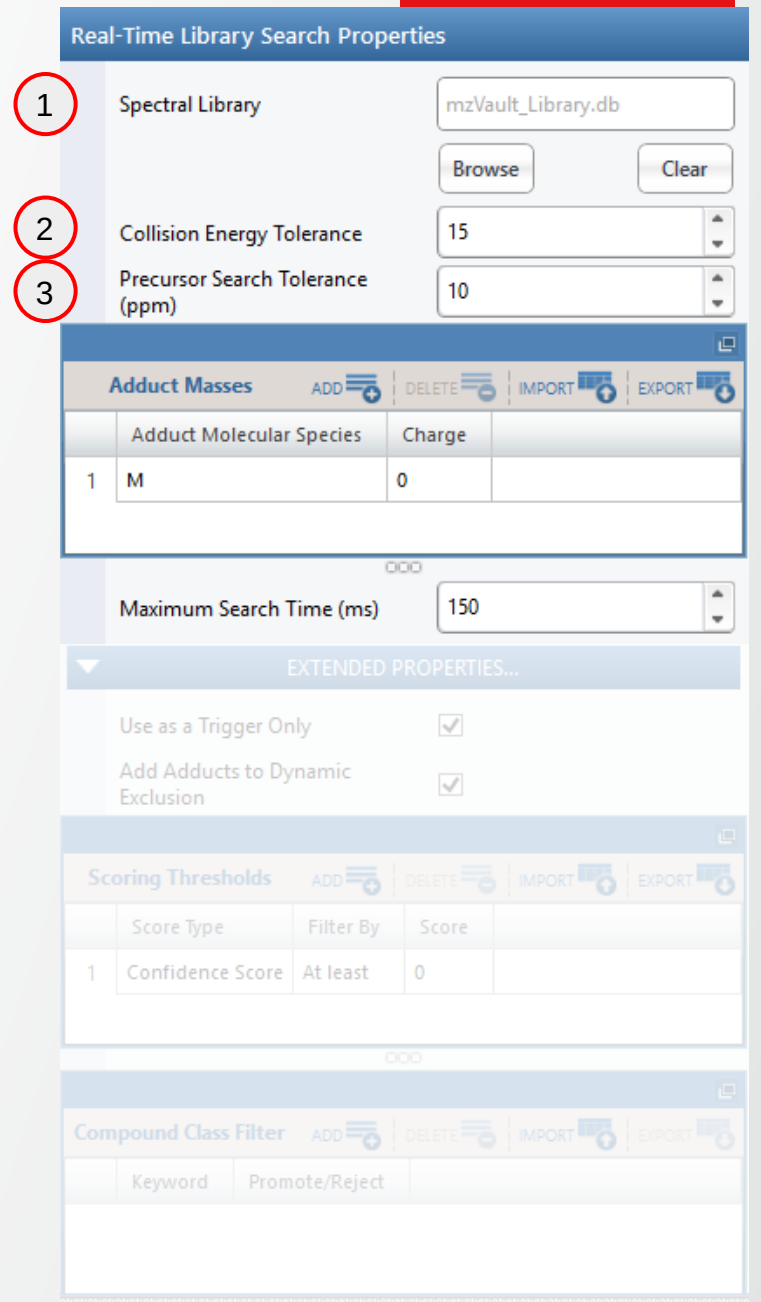
Support for Real-Time Library Search

Configurable (Global) Parameters within RTLS Filter

- Spectral Library (1)
  - Reference to a path on disk – library not embedded in .meth file
  - Custom mzVault spectral libraries or local copies of mzCloud MS2 libraries (in .db format)

- Collision Energy Tolerance (2)
  - +/- value for filtering the spectral library candidates during search
  - Applies to the MS2 event preceding RTLS placement

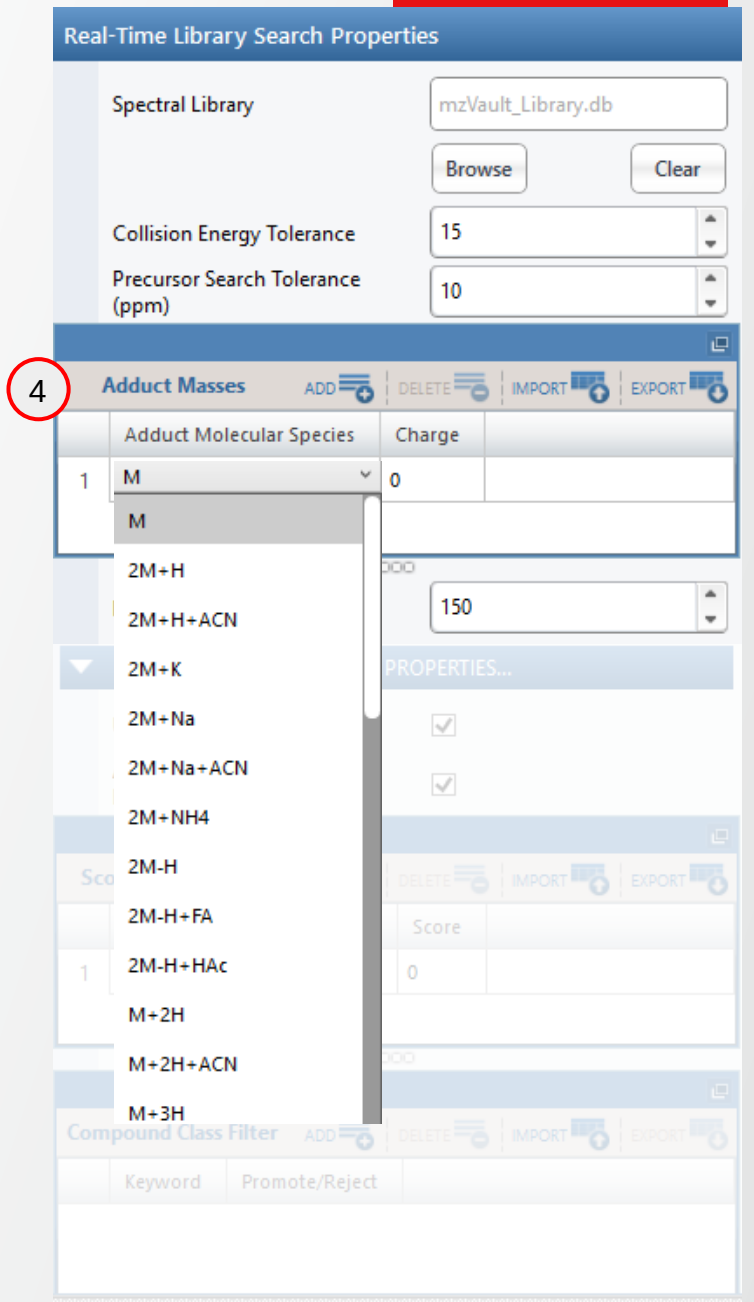
- Precursor Search Tolerance (ppm) (3)
  - In ppm, +/- value for filtering the spectral library candidates during search
  - Applies to the MS2 event preceding RTLS placement



Support for Real-Time Library Search

Configurable (Global) Parameters within RTLS Filter

- Adduct Masses (settings) 4
  - Adduct Offset Query functionality may help to identify adducted compounds if an entry does not exist for that molecular species in the database.
  - Prepopulated list of adduct ions from Compound Discoverer
  - When selected, auto-populates with default charge state for consideration (but is user editable)
  - Adducts can be limited to a single charge state or applied to all charges (give 0 as charge state)

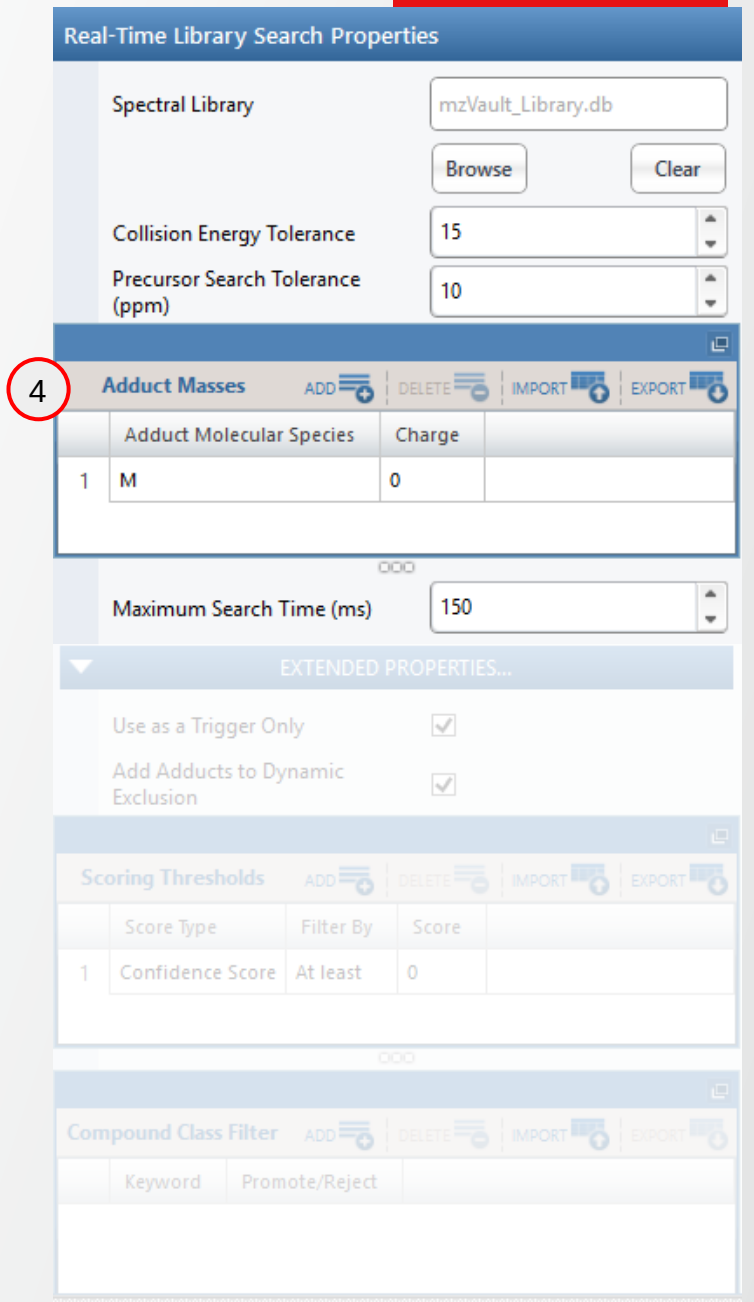


Support for Real-Time Library Search

Configurable (Global) Parameters within RTLS Filter

- Special Adduct Masses (definition) (4)
  - "M" – this is equivalent to $[M+zH]^{z+}$ or $[M-zH]^{z-}$
  - "M" at Charge "0" would apply to protonated and deprotonated forms across all charge states

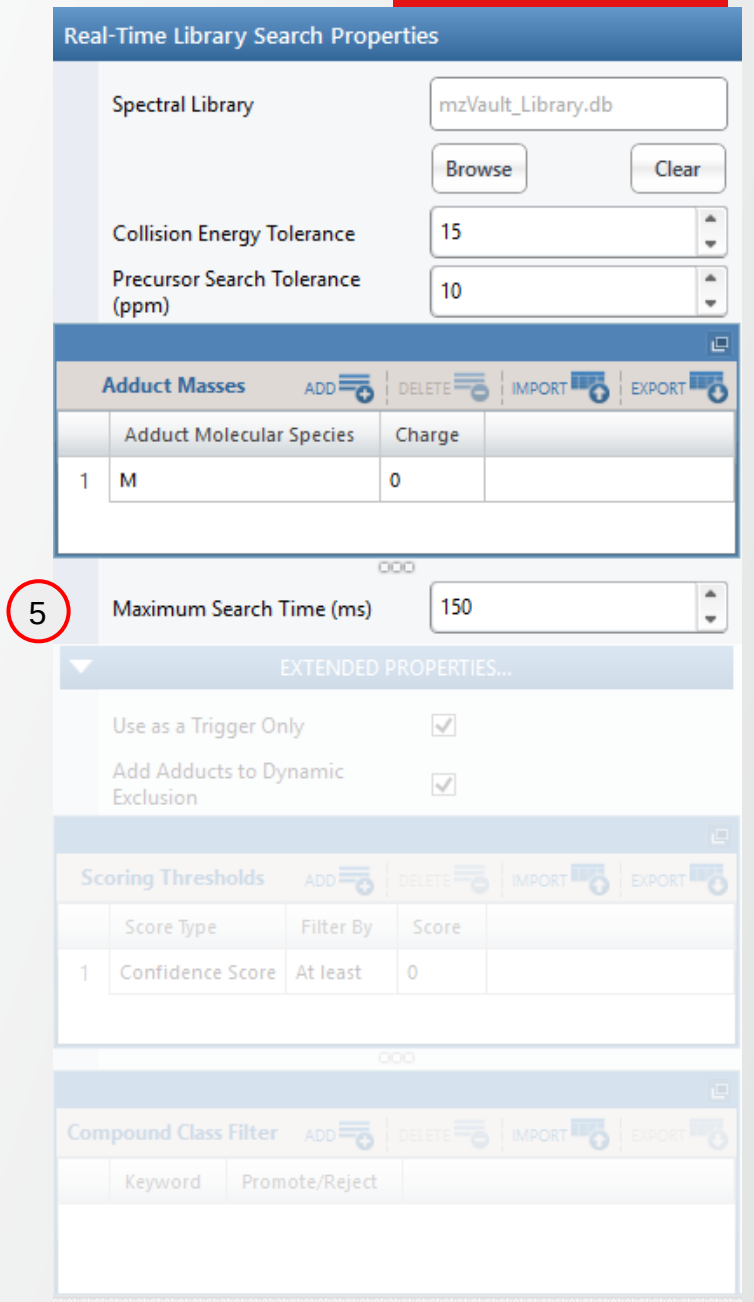
- Adduct Masses Validation
  - Duplicate adducts are allowed at different charge states (warning displayed if full duplication)
  - Adduct at all charges (Charge "0") will give warning if same adduct is added at a specific charge
  - Warning displayed if a charge is entered not matching the settings of "Charge State" filter (possibly included upstream in the method branch)



Support for Real-Time Library Search

Configurable (Global) Parameters within RTLS Filter

- Maximum Search Time (ms) (5)
  - Searches are executed serially and can abort after user defined duration is expired (returning empty results)
  - Normal instrument acquisition continues even while searches are executing



Support for Real-Time Library Search

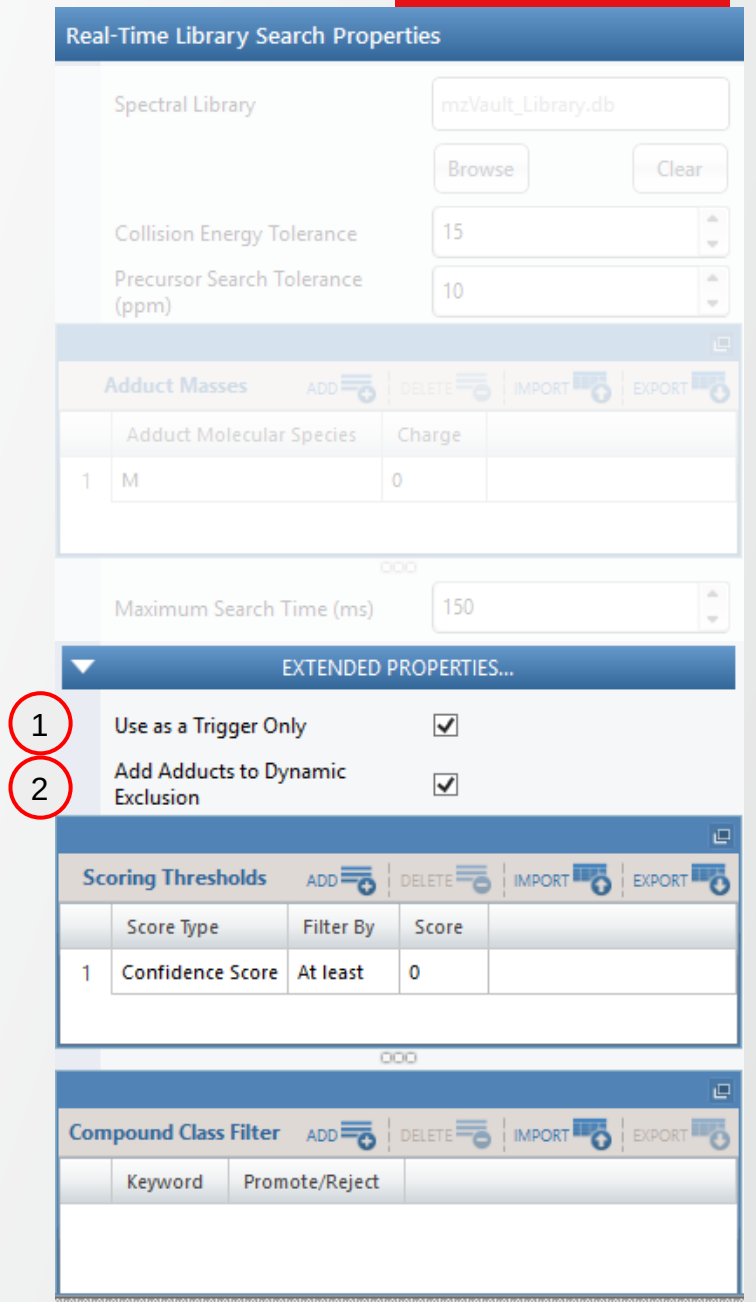
Configurable Parameters within Extended Properties Section

- Use as a Trigger Only (1)
  - When enabled, pass **all peaks** (whole spectrum) for next scan
  - When disabled, pass only **matched peaks** for next scan

- Add Adducts to Dynamic Exclusion (2)
  - When the scoring thresholds (below) are satisfied, if this option is enabled, the calculated hypothetical adducted *m/z* values are added to dynamic exclusion

(Note: Monoisotopic peak and 3x C13 isotopic peaks are considered, regardless of the elemental composition of the analyte identified)

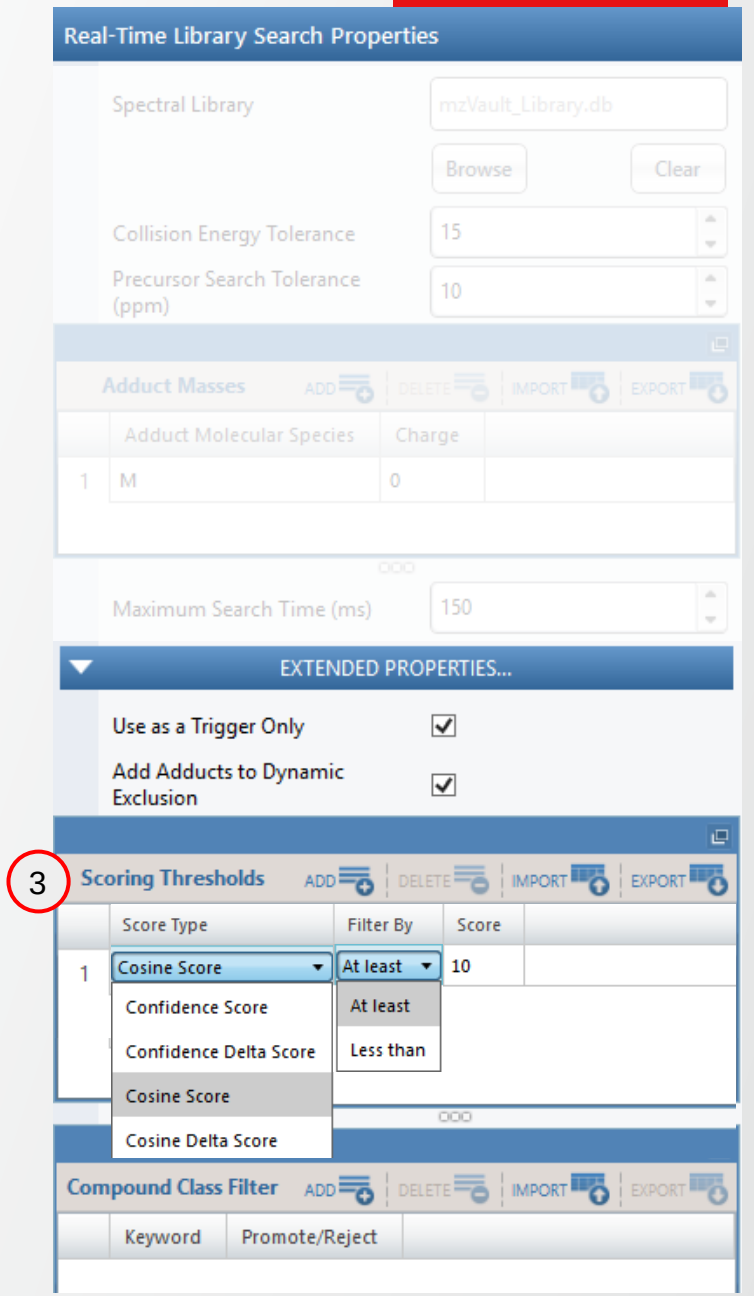
  - The duration and *m/z* tolerance of the dynamic exclusion is taken from the dynamic exclusion filter of the method as defined by the user



Support for Real-Time Library Search

Configurable Parameters within Extended Properties Section

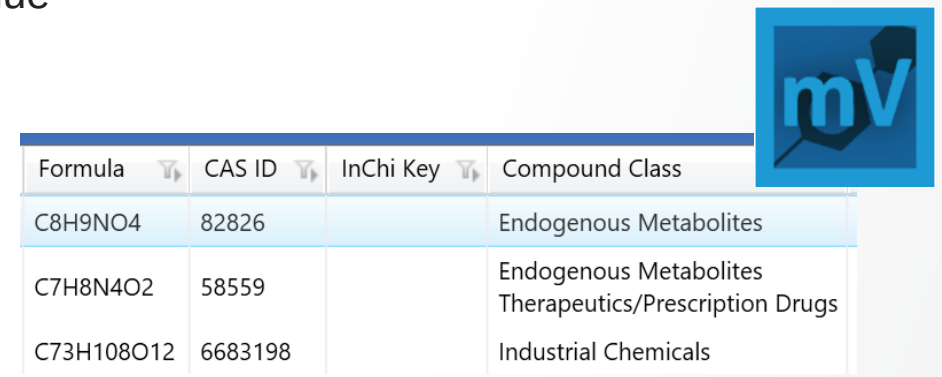
- Scoring Thresholds (3)
  - Two HighChem scores exposed: Cosine Score & Confidence Score
  - Delta scores between the 1st and 2nd best hit
  - Score thresholds can be set to require a minimum (“At least”, >=) or a maximum value (“Less than”, <)
  - Thresholds are separately configured for each instance of RTLS filter and can lead to different scan outcomes
  - Duplicated score thresholds trigger a validation error



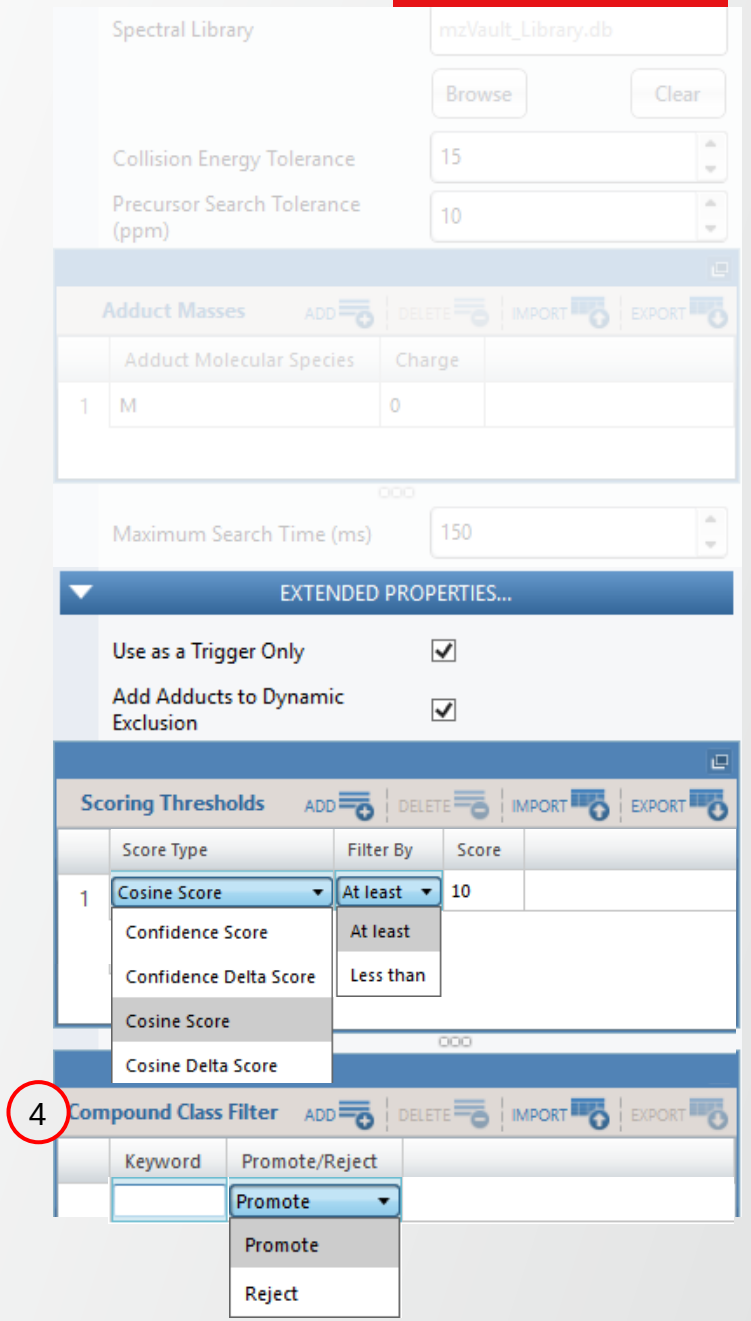
Support for Real-Time Library Search

Configurable Parameters within Extended Properties Section

- Compound Class Filter (4)
  - Promote/Reject specific compound classes based on keyword
  - Compound class annotation can be defined within mzVault library management application
  - Like for the standard peptide RTS filter, the Compound Class Filter items which are “promoted” or “rejected” will override the user defined thresholds
    - If a scan top hit does not meet thresholds, but is promoted by the compound class filter, it will continue
    - If a scan’s top hit does meet thresholds, but is rejected by the compound class filter, it will not continue



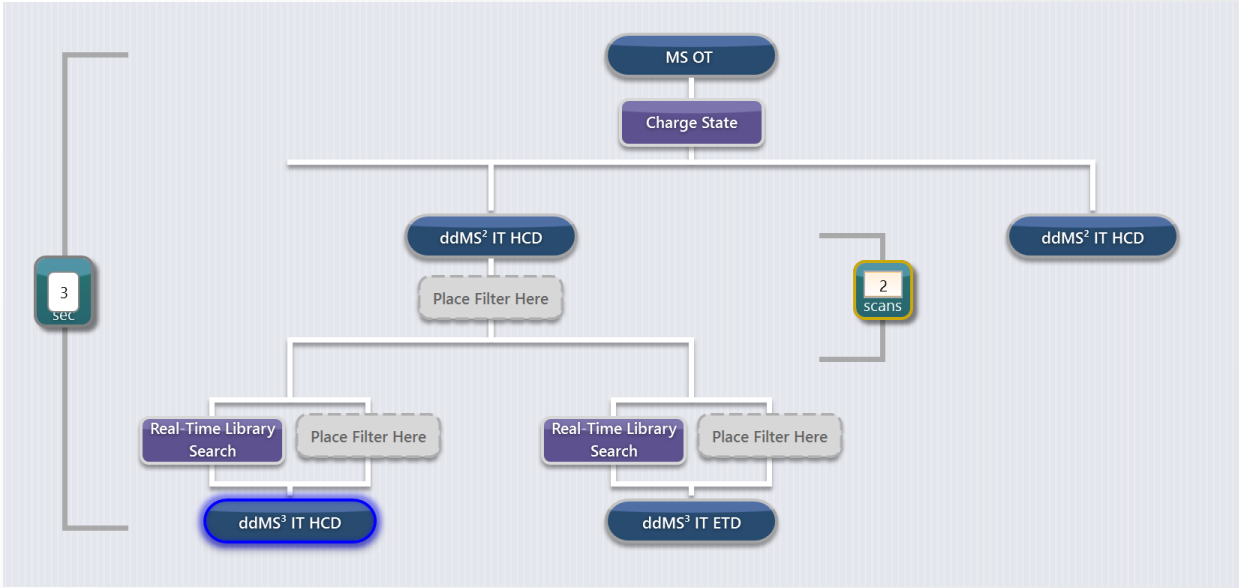
Formula	CAS ID	InChi Key	Compound Class
C8H9NO4	82826		Endogenous Metabolites
C7H8N4O2	58559		Endogenous Metabolites Therapeutics/Prescription Drugs
C73H108O12	6683198		Industrial Chemicals



Support for Real-Time Library Search

RTLS Filter Placement Constraints

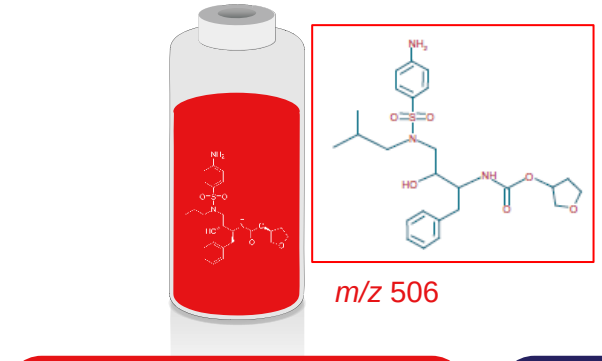
- RTLS filters can only be placed under a single MS2 node, but can be present in multiple copies.
- RTLS currently supports a single polarity per method
- RTLS currently cannot be placed after a tMSn node



Real-Time Library Search – Based Acquisition Method

Application: MET-IQ - Intelligent mass spectrometry for small molecules

- Identification and characterization of unknown compounds, chemically similar to known compounds of interest
- Metabolomics applications, identification of various environmental contaminants, unknown extractables and leachables as well as emerging clinical toxins



m/z 506

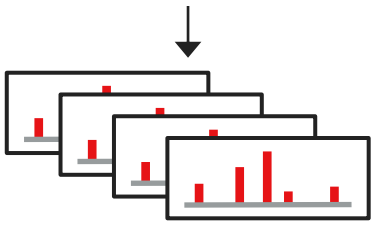
Known compound

Unknown related compounds

Compare MS^2 spectra to library in real-time

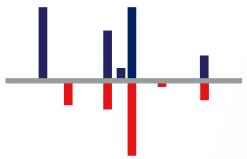
If similarity match, trigger MS^3

Characterize unknown compounds using MS^2 & MS^3

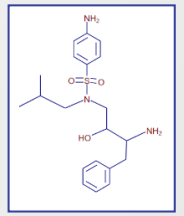


Build a spectral library

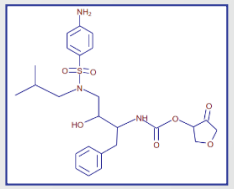
Experimental MS^2 of an unknown



Library MS^2 of the known compound



m/z 408



m/z 520

Real-Time Library Search – Based Acquisition Method

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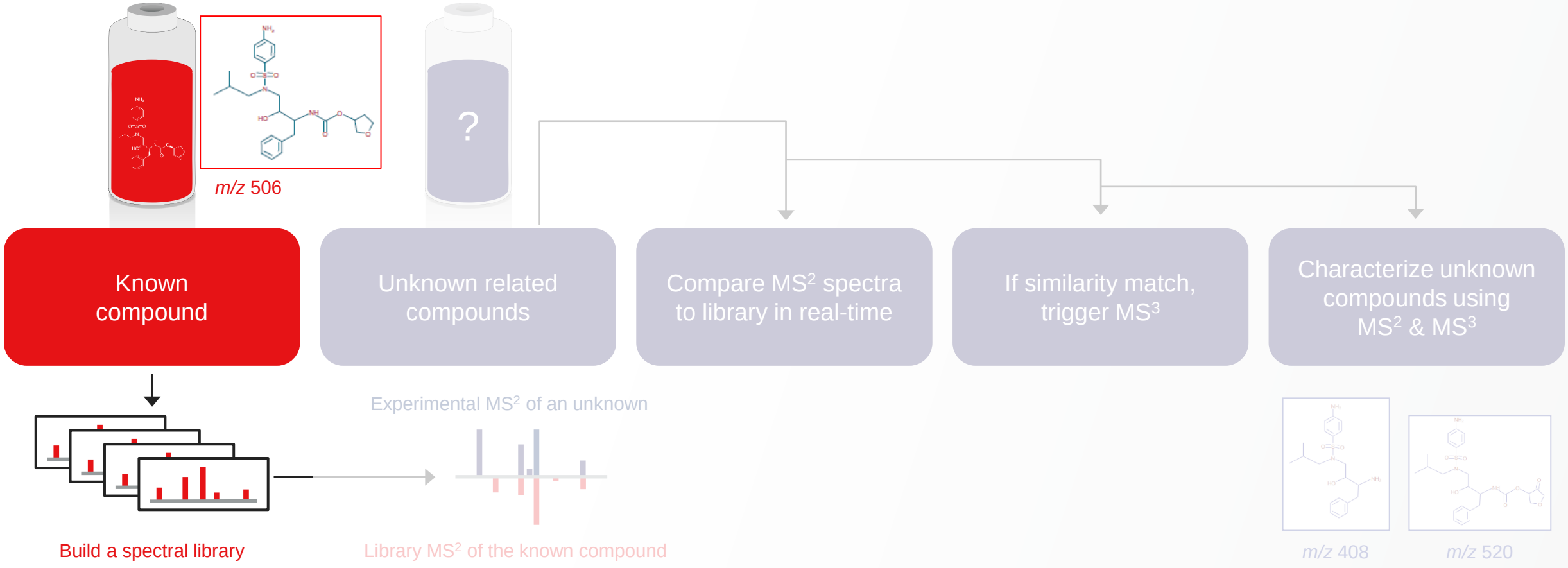
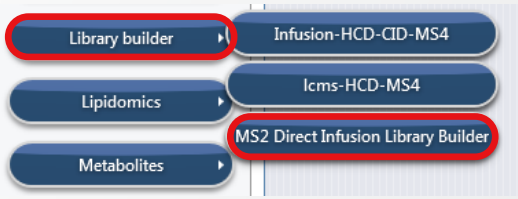
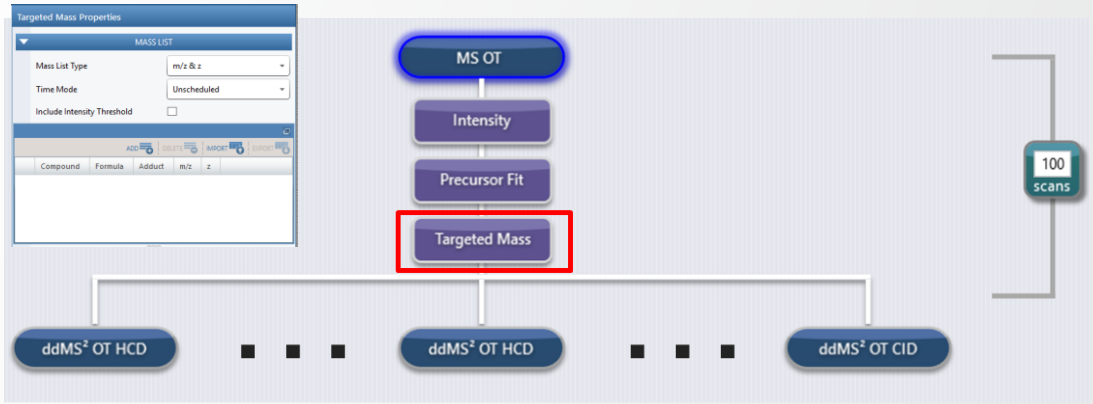


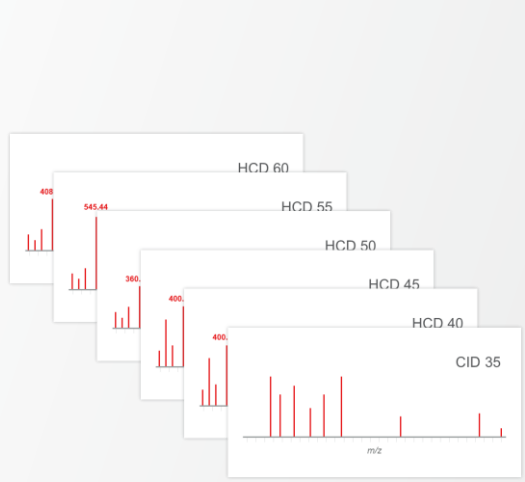
Real-Time Library Search – Based Acquisition Method

Generation of Spectral Libraries for known compounds

- Build a new method from the new preset MS/MS Library Builder template to create the spectral library for the compound of interest
  - Analyses are performed as direct infusion or with an LC method (with the need for chrom. peak ≥ 6s)
  - M/z value (or m/z & z) of the compound of interest are included in Targeted Mass filter
  - MS/MS acquisition is performed with HCD at nCE 10,20, 30,…, 80 and with CID at nCE 15, 30, and 45 (11 total MS2 scan events)
- Data are processed with mzVault (can be downloaded from https://thermo.fexnetoperations.com/)







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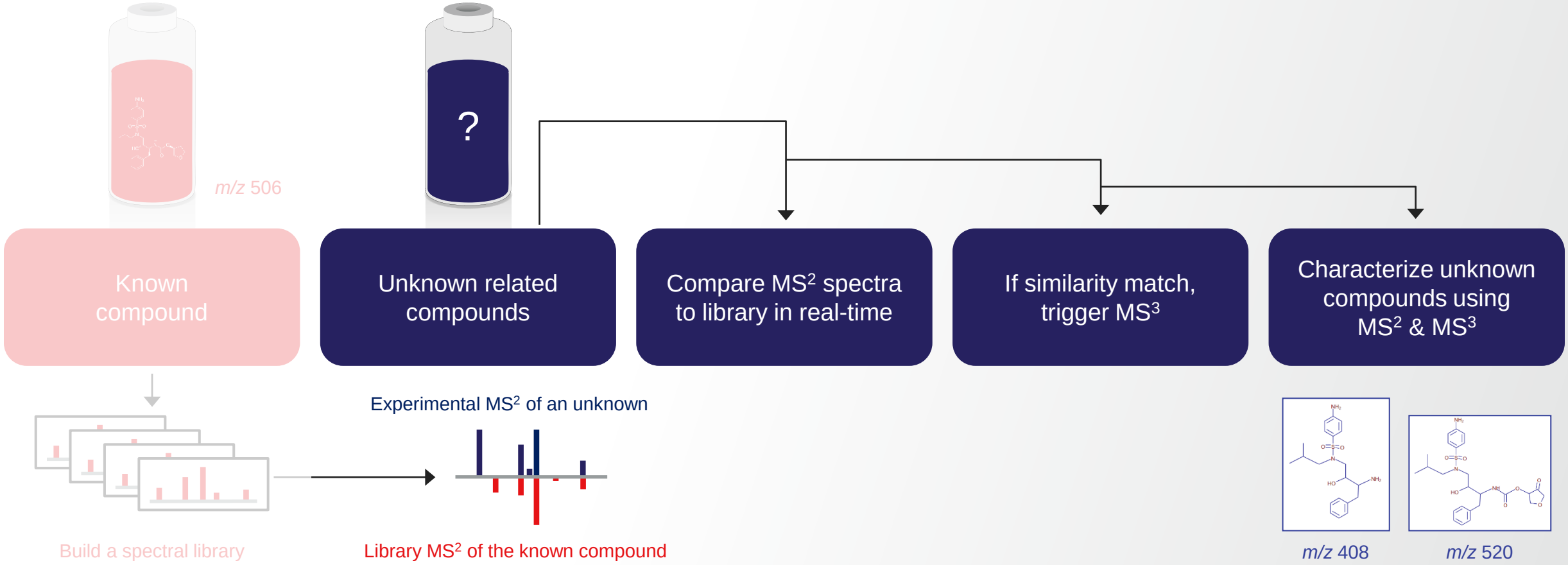


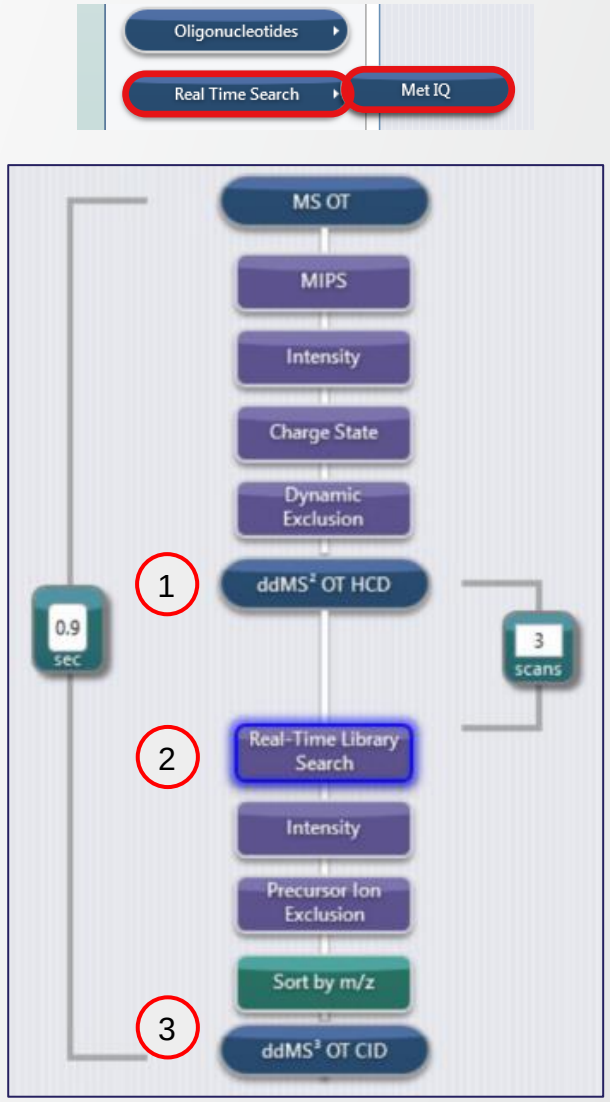
Real-Time Library Search – Based Acquisition Method

Application: MET-IQ - Intelligent mass spectrometry for small molecules

- Build a new method from the preset Met-IQ method template to interrogate the samples of unknown related compounds (transformational/metabolized products)

- During LC-MS/MS analyses of the samples

1. MS2 profiling of the samples containing unknown related compounds is performed
2. MS2 spectra are compared to spectral library in real-time (using broad precursor ion m/z search tolerance)
3. MS3 are triggered only on compounds for which spectra are similar to the spectra of the compound of interest, prioritizing the selection of ions with high m/z (more informative)



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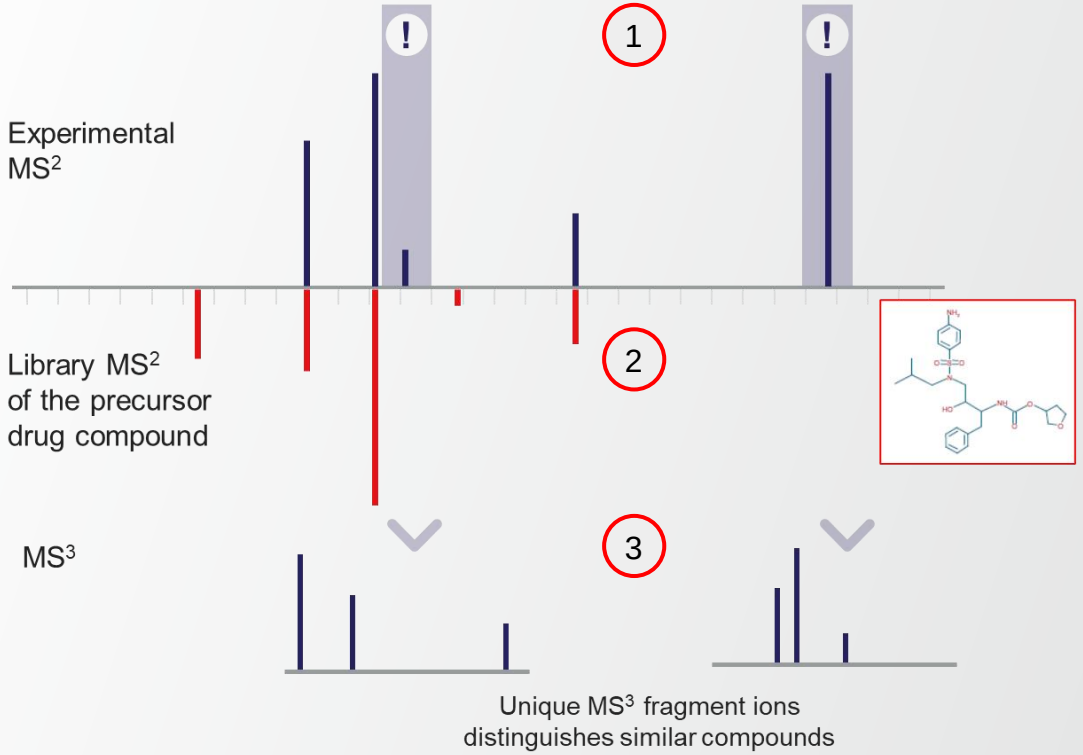
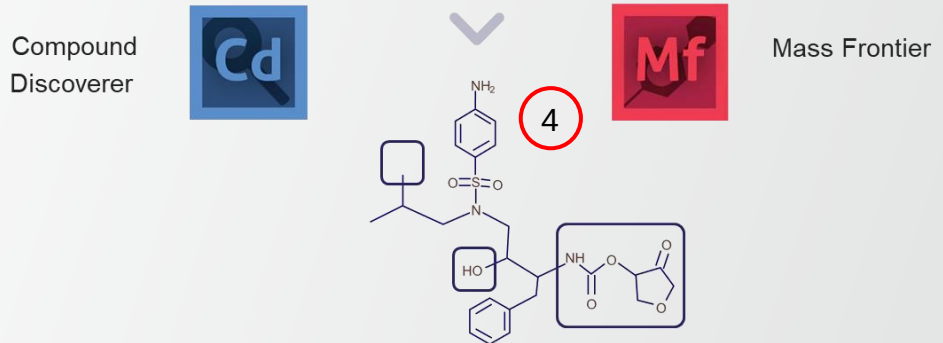


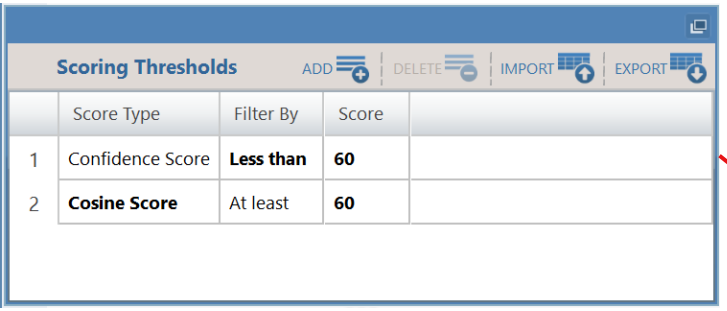
- Post-acquisition data processing 4
- Using Mass Frontier and Compound Discoverer
- Enabling further annotation of MS2 and MS3 spectra generated by MET-IQ
- Allowing the identification of unknown related compounds (transformational/metabolized products)

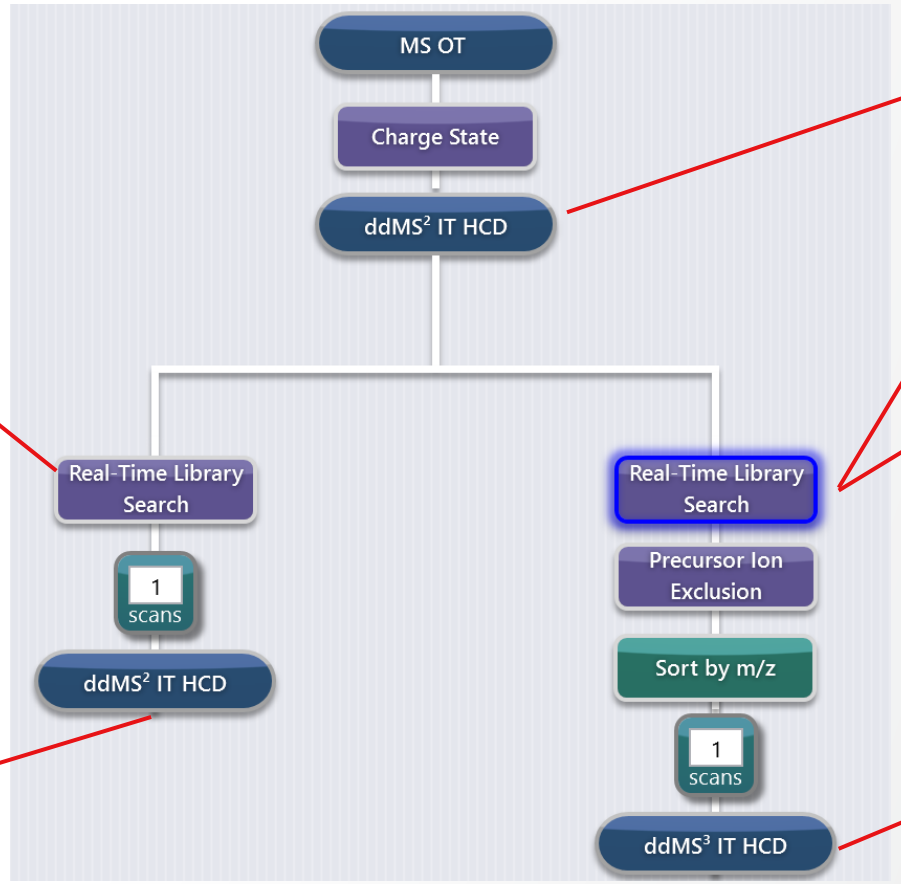


Real-Time Library Search – Based Acquisition Method

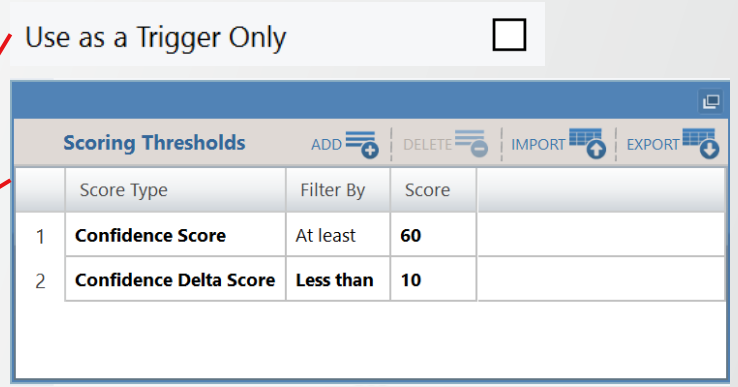
Application: Advanced elucidation of chemical structures

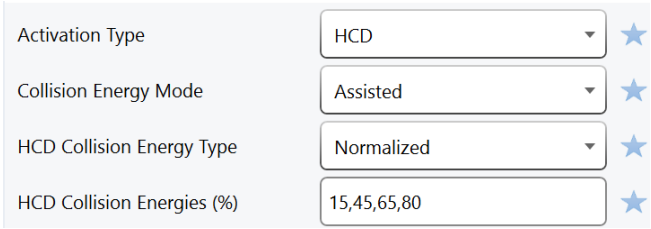
- No further acquisition for unambiguous & confident id. of the cmpds (high cosine / confidence / confidence delta)
- Optimization of CE if match appears under-fragmented (high cosine / low confidence)
- MS3 acquisition on high m/z fragments for compounds with ambiguous scores





HCD@45





Aim to remove ambiguity by fragmentation of high m/z RTLS matched ions

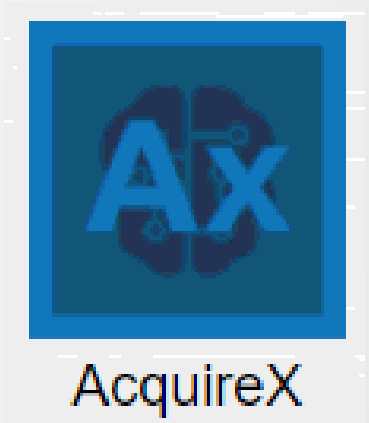
AcquireX Data Acquisition Workflow Enhancements

New Features

- Challenge
  - Higher coverage and throughput is required to expedite studies including 100s of samples
  - The ability to control multiple different matrix backgrounds is necessary in large scale studies

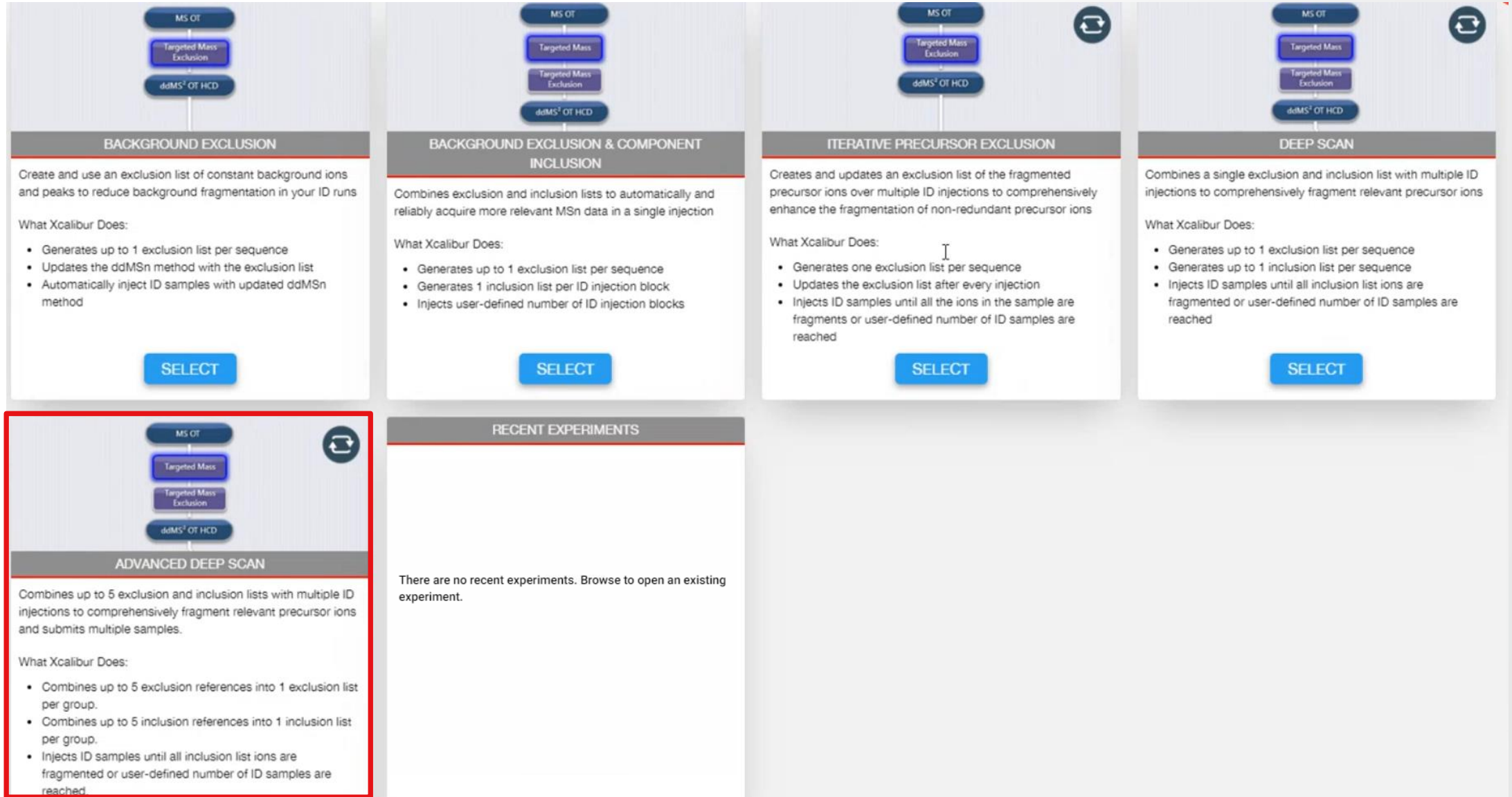
- Solution
  - New « Advanced Deep Scan » workflow to provide more flexibility in studies execution
  - New functionalities to improve user experience

- Availability
  - Xcalibur 4.5
  - All Tribrid models (small molecule application)



AcquireX Data Acquisition Workflow Enhancements

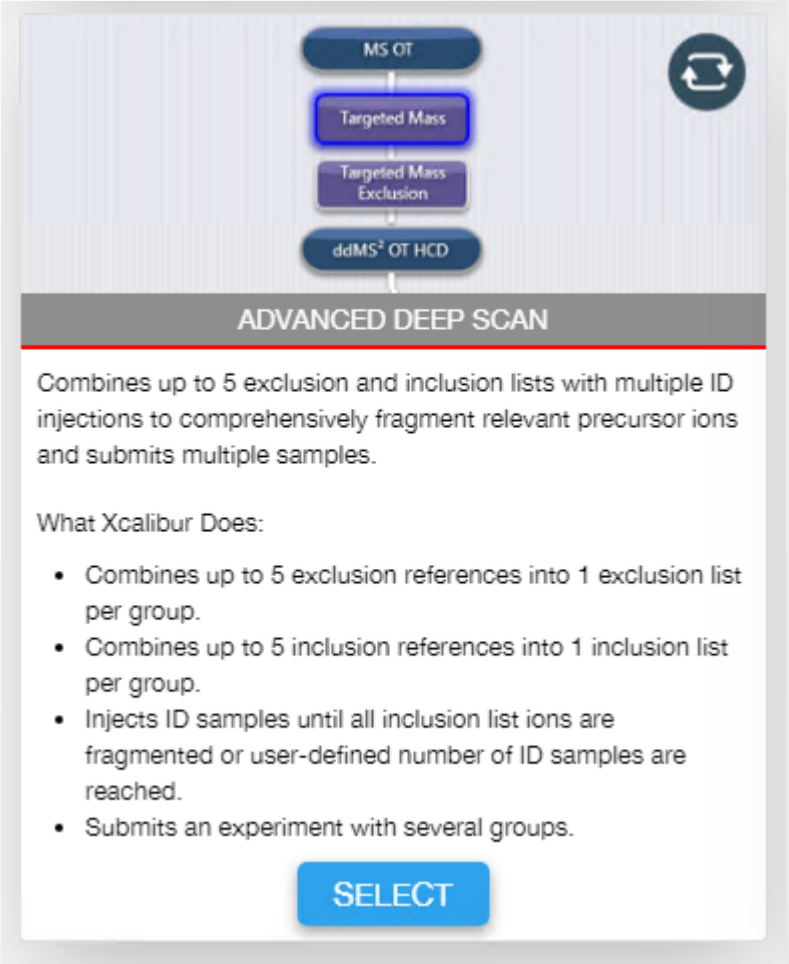
New « Advanced Deep Scan » Workflow to Provide More Flexibility in Studies Execution



AcquireX Data Acquisition Workflow Enhancements

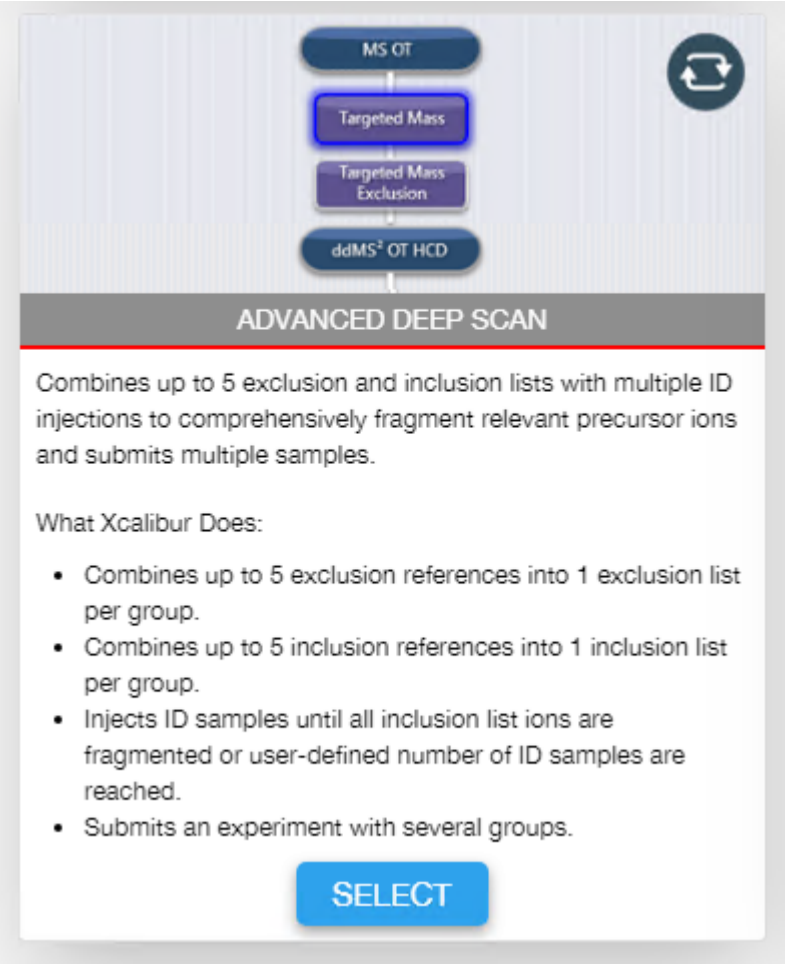
New « Advanced Deep Scan » Workflow to Provide More Flexibility in Studies Execution

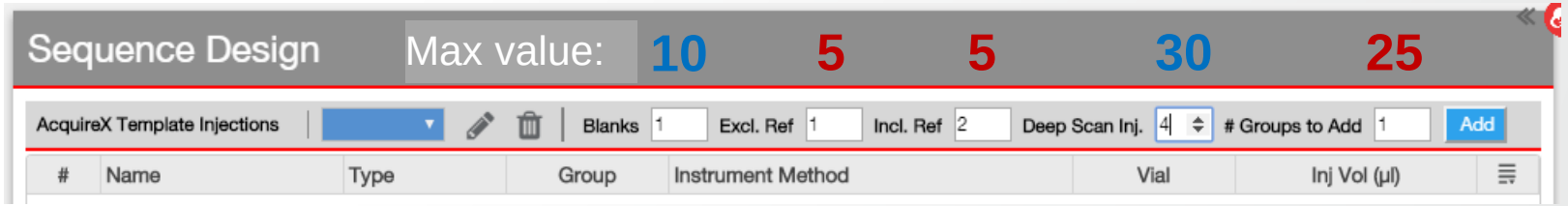
- Submit several samples defined by groups with different instrument methods in the same experiment
- Combine up to 5 inclusion or exclusion lists that are applied to the subsequent id injections
- Add wash/blank samples anywhere in the sequence
- Group overview facilitated in Read-only table



AcquireX Data Acquisition Workflow Enhancements

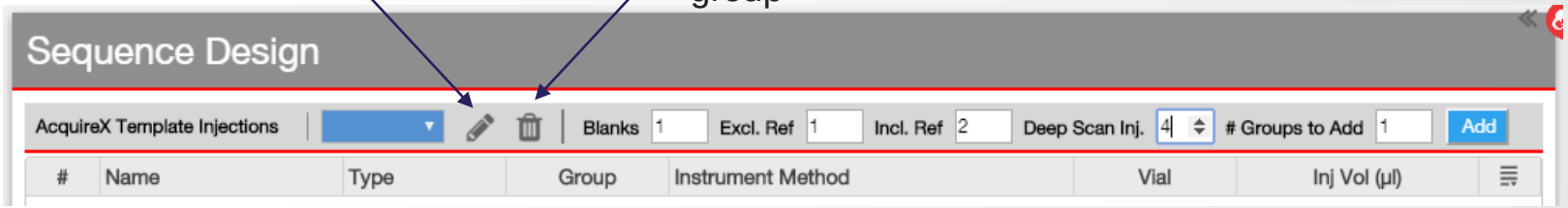
Advanced Deep Scan – Sequence Design





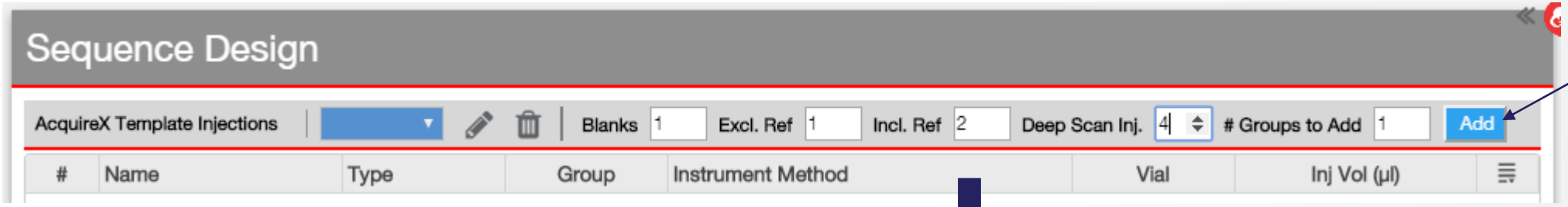
Edit a specific group

Delete a specific group

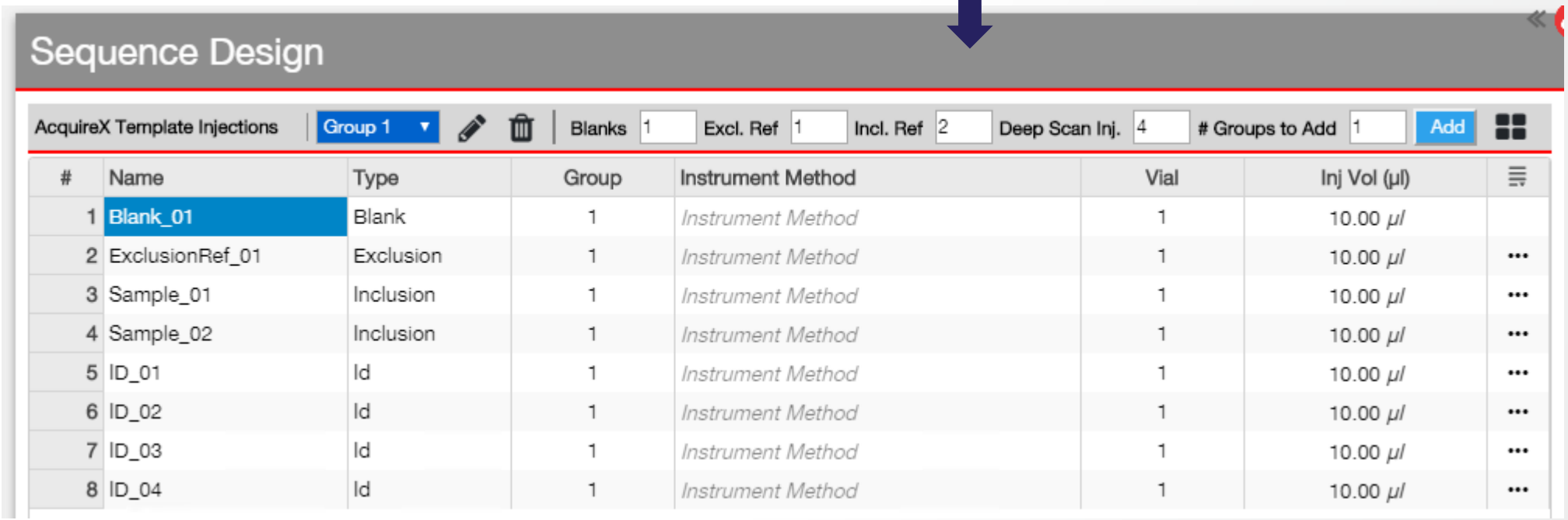


AcquireX Data Acquisition Workflow Enhancements

Advanced Deep Scan – Adding a Group in the Sequence

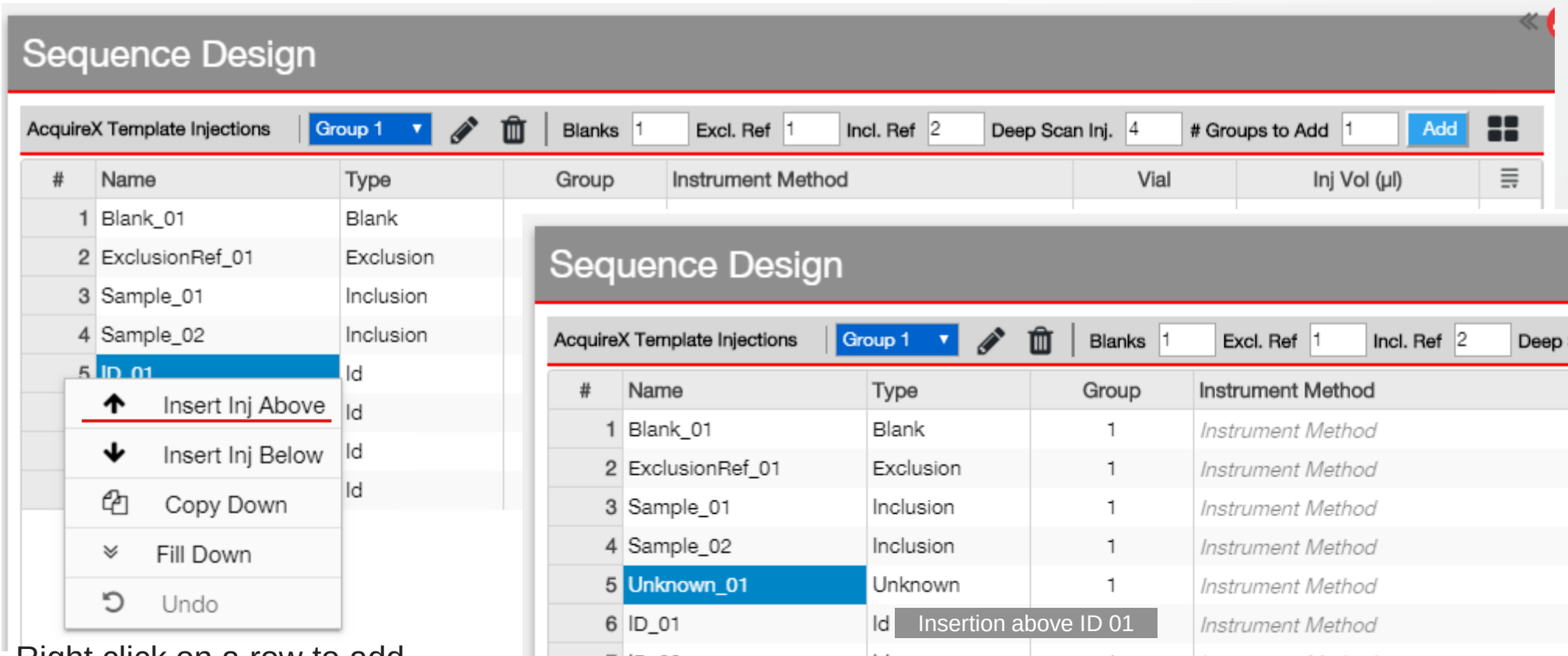


To add one or several groups



AcquireX Data Acquisition Workflow Enhancements

Advanced Deep Scan – Inserting a wash/blank sample in the Sequence



Right click on a row to add wash/blank samples

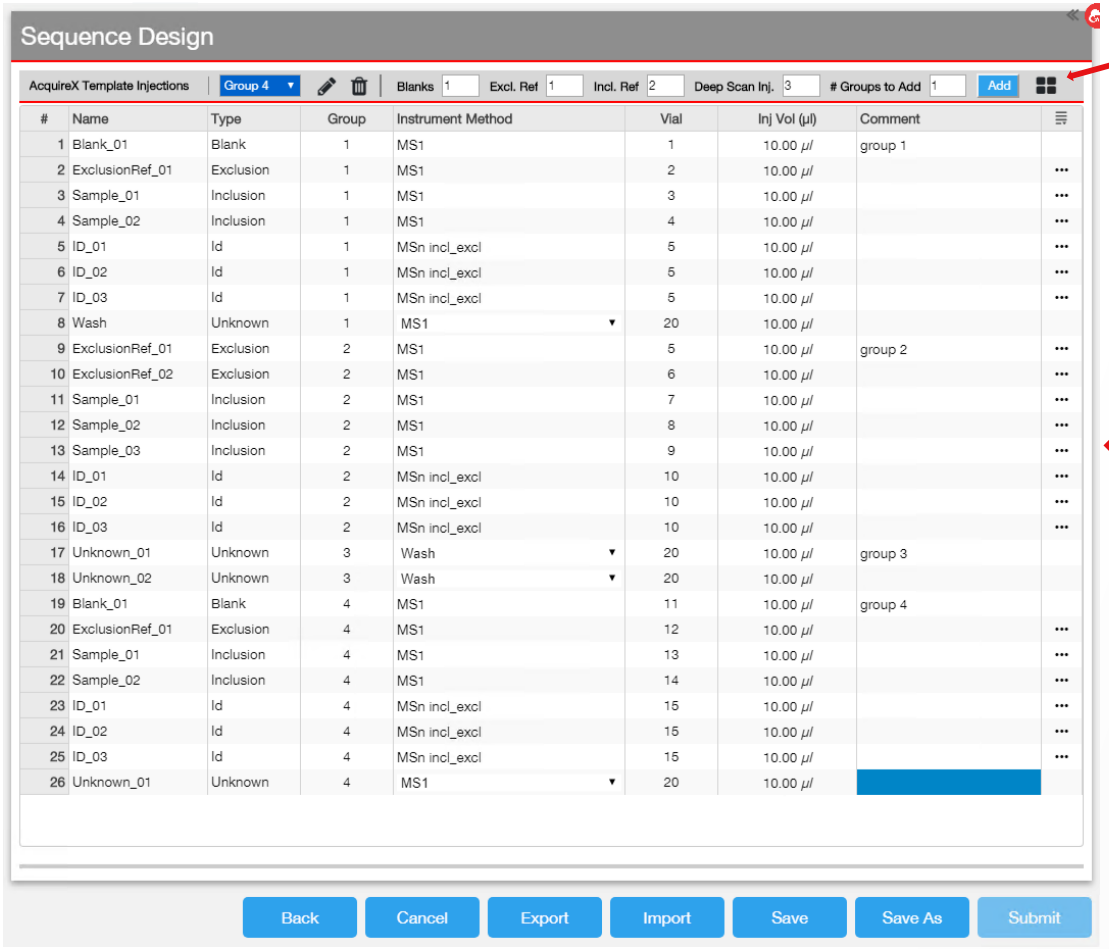
AcquireX Data Acquisition Workflow Enhancements

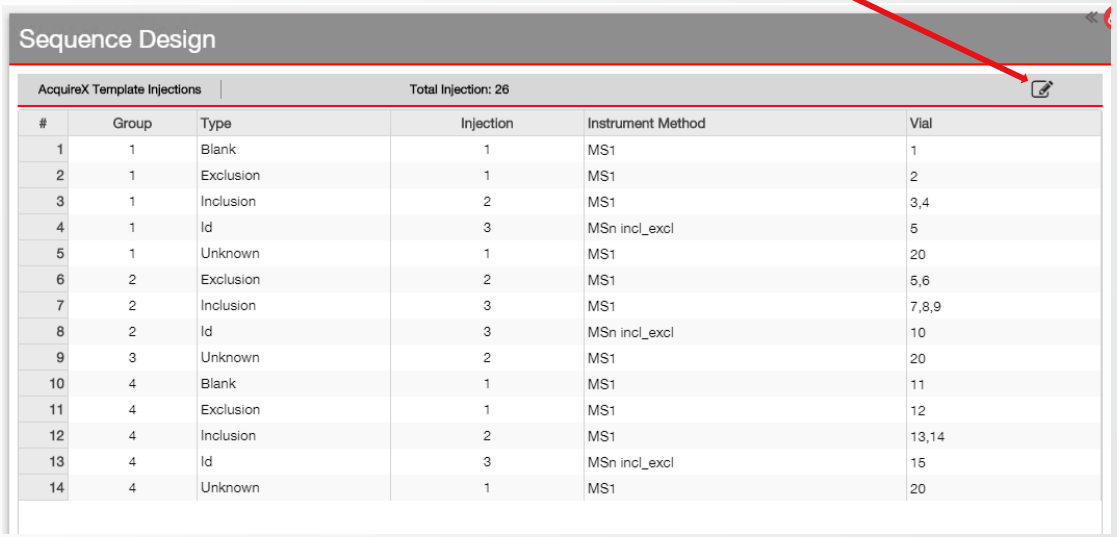
Advanced Deep Scan – Group Overview

Editable table

Icons to toggle between the view

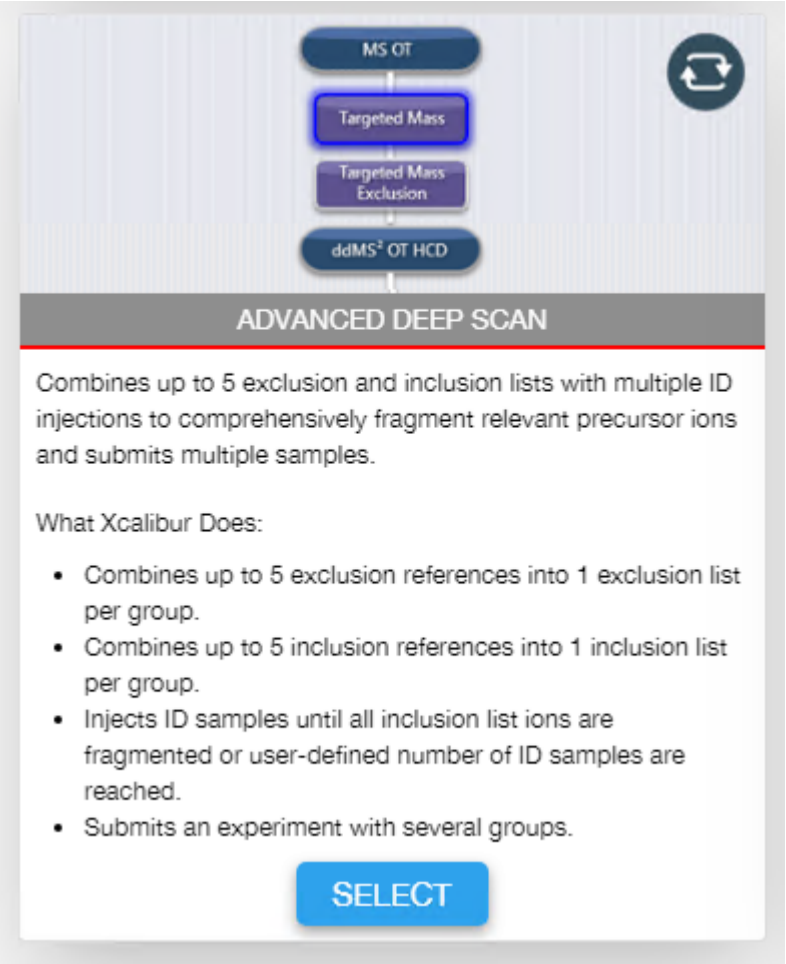
Read-only table

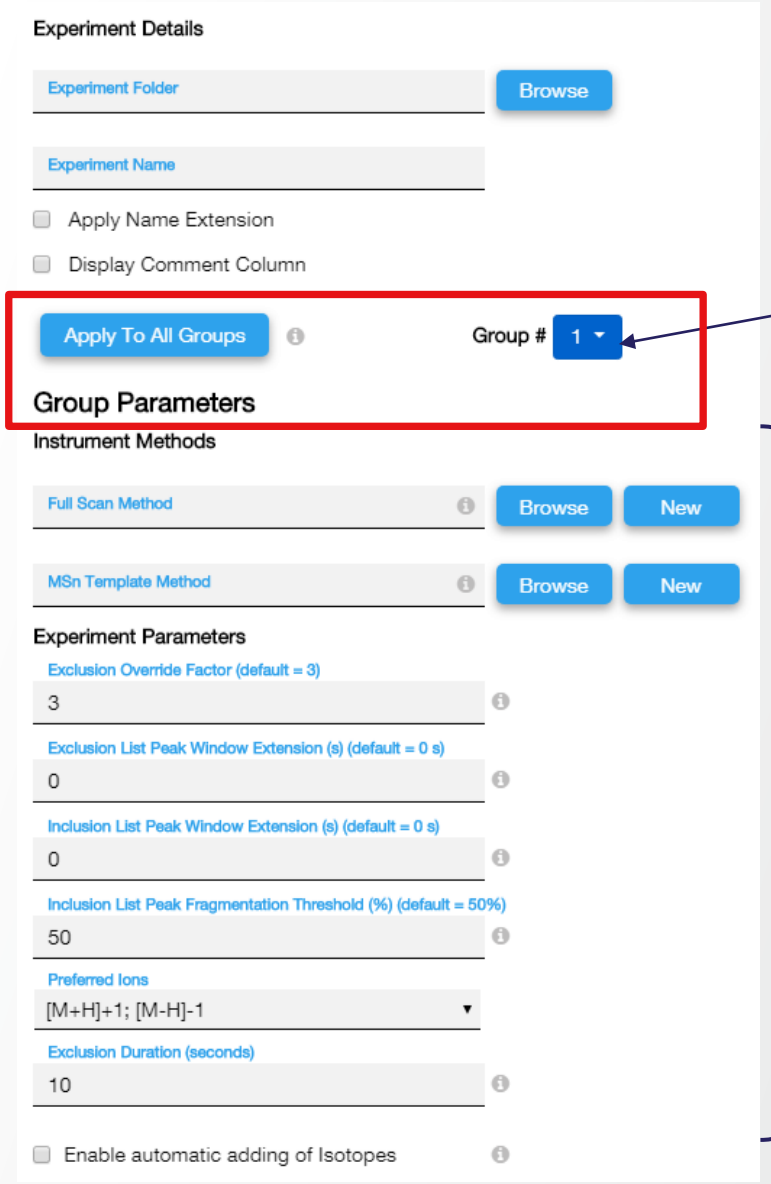




AcquireX Data Acquisition Workflow Enhancements

Advanced Deep Scan – User-Defined Parameters



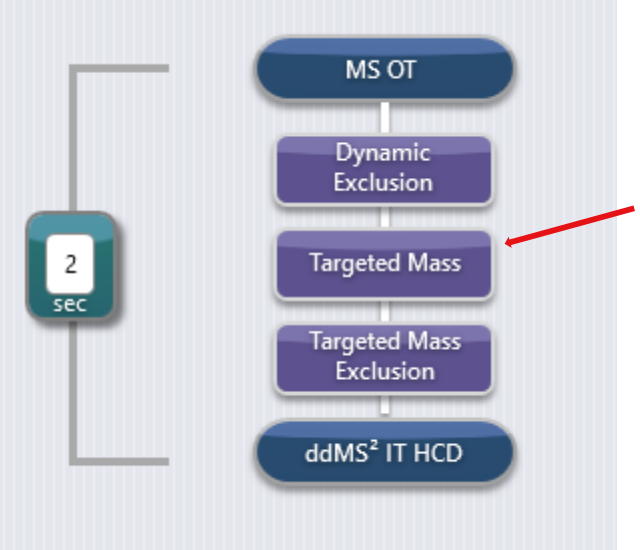


Each group can have different experiment parameters

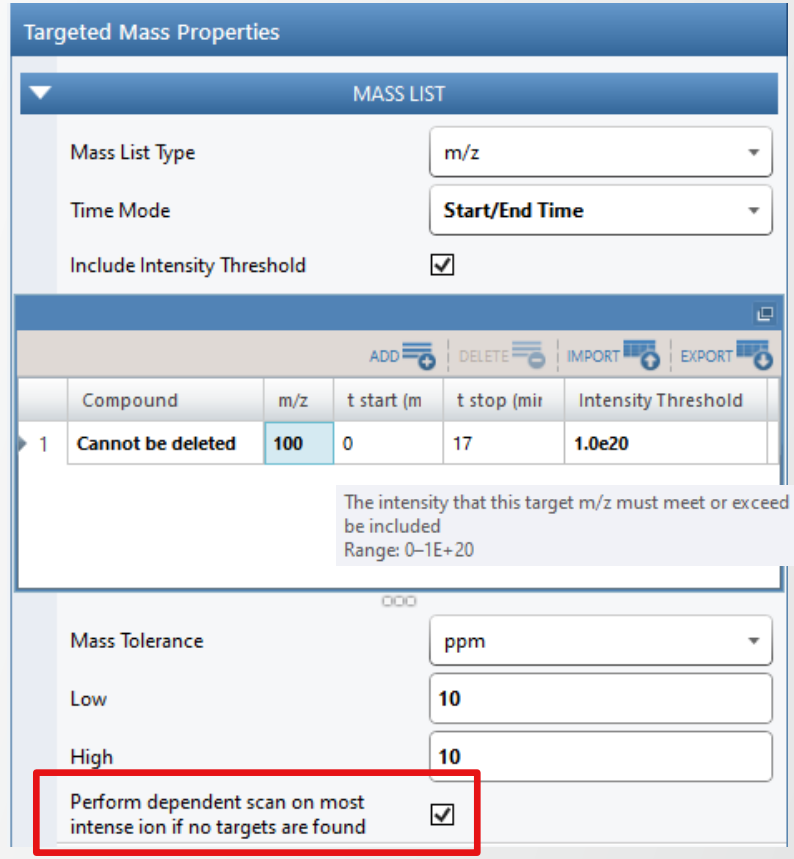
Same as Xcalibur 4.4

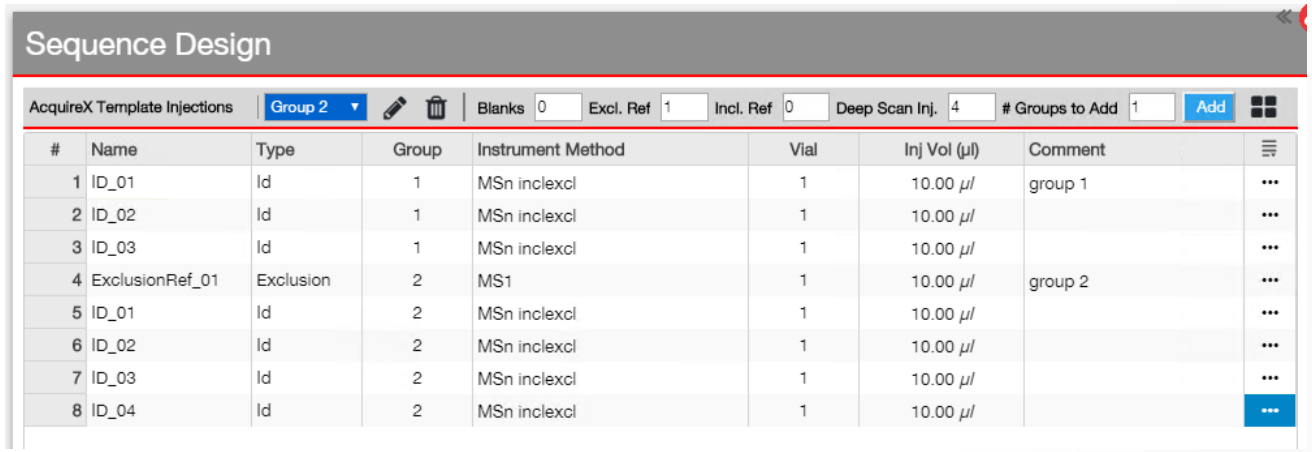
AcquireX Data Acquisition Workflow Enhancements

Advanced Deep Scan to Submit several “Iterative Precursor Exclusion” samples



For a deep scan experiment an inclusion list is required in the method.





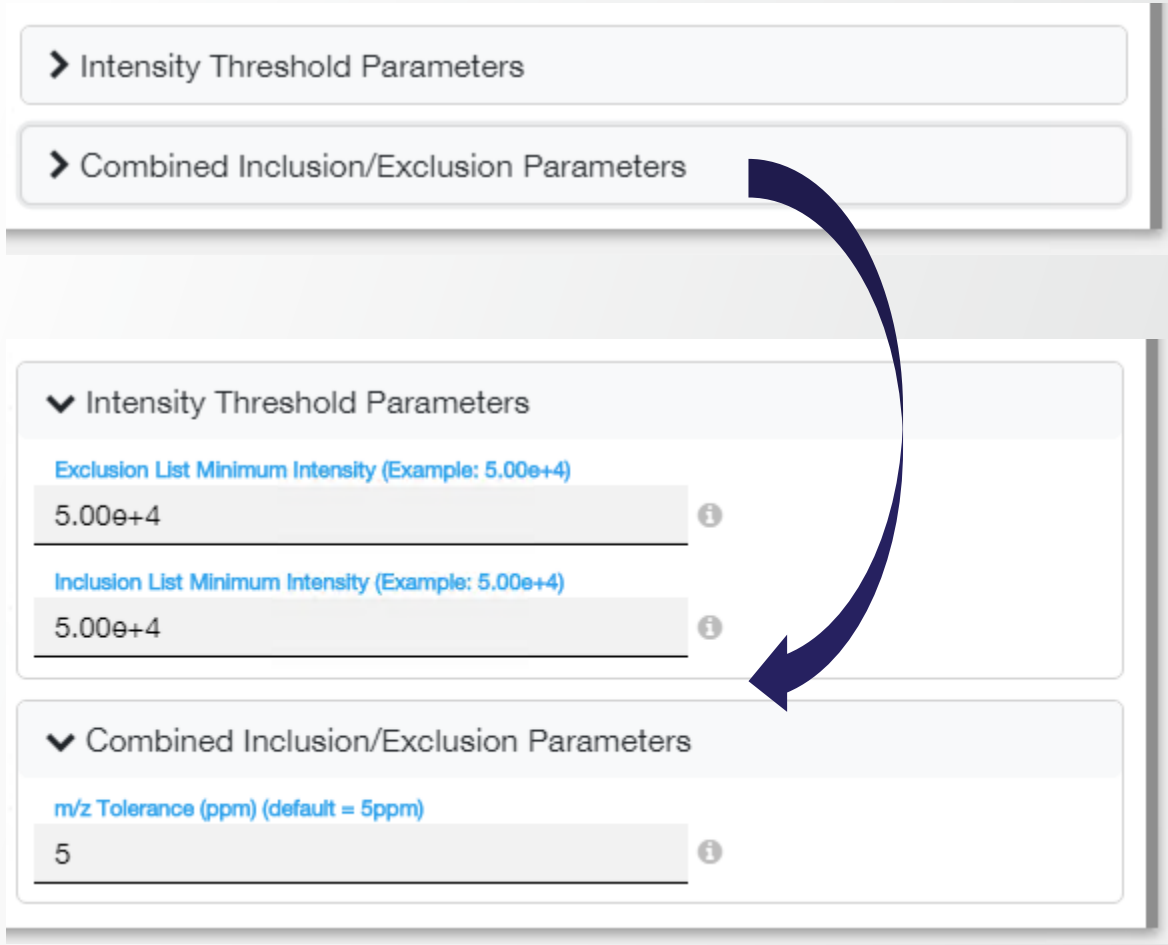
- After ID1, all of the targeted ions will be added to the exclusion list.
- If the inclusion list is not empty, the next ID run will start
- After the number of ID runs are acquired, the next group will be submitted.

AcquireX Data Acquisition Workflow Enhancements

New Functionalities to Improve User Experience

- User-defined minimum intensity for inclusion and exclusion lists for all existing and new AcquireX experiments
- Import/Export Acquirex Experiment from the Advanced Deep Scan, Deep Scan, and Iterative Precusors Exclusion workflows as a .csv file
- New column available to add comments
- Copy/fill down for sample name, vial position and injection volume
- Option to skip to the next queued sequence automatically if a processing error occurs
- Parallel acquisition and processing to save time and reduce solvent consumption

New parameters in Xcalibur 4.5



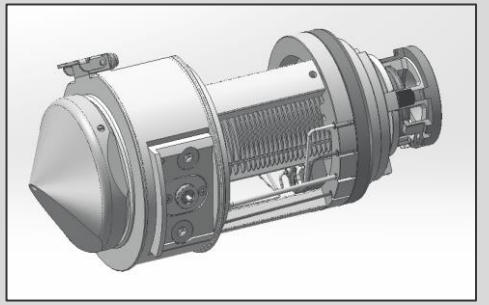
Auto-Ready Ion Source

New Features

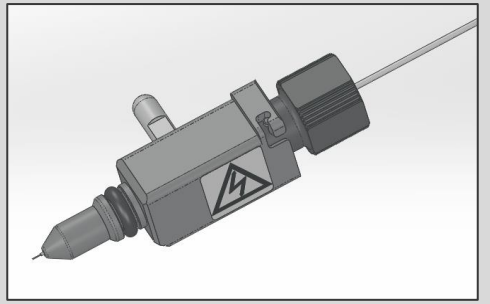
- Challenge
  - Time dedicated to instrument setup must be minimized
  - Instrument must remain well calibrated over time
- Solution
  - Built-in Auto-Ready Ion Source simplifies maintenance with weekly pre-scheduled, remote, and one-click calibration
- Availability
  - Orbitrap IQ-X

New Auto-Ready Calibration Source

Its own ion transfer tube



Dedicated emitter

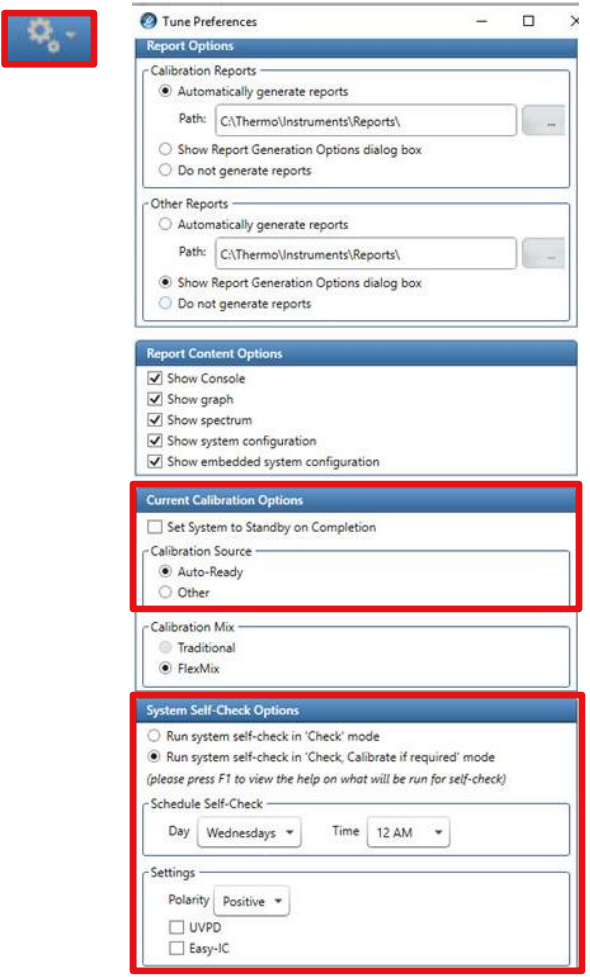


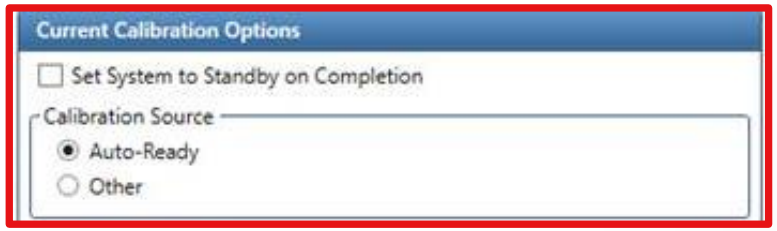
Robust delivery system

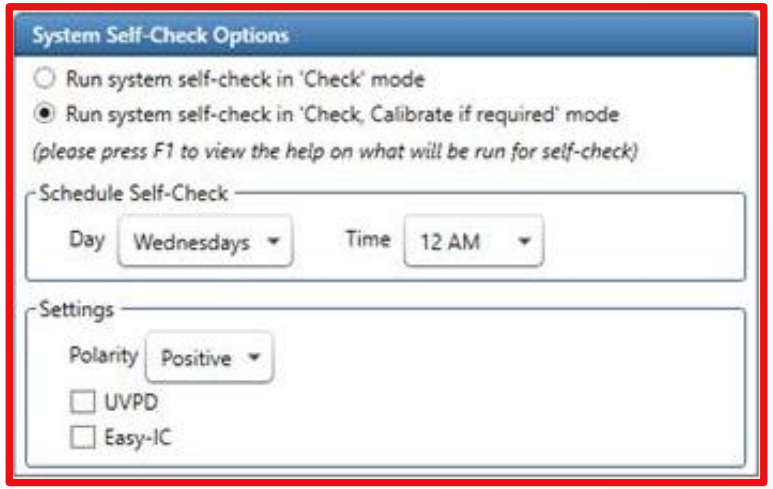
Auto-Ready Ion Source

Setting up Auto-Calibration

Under Tune Preferences







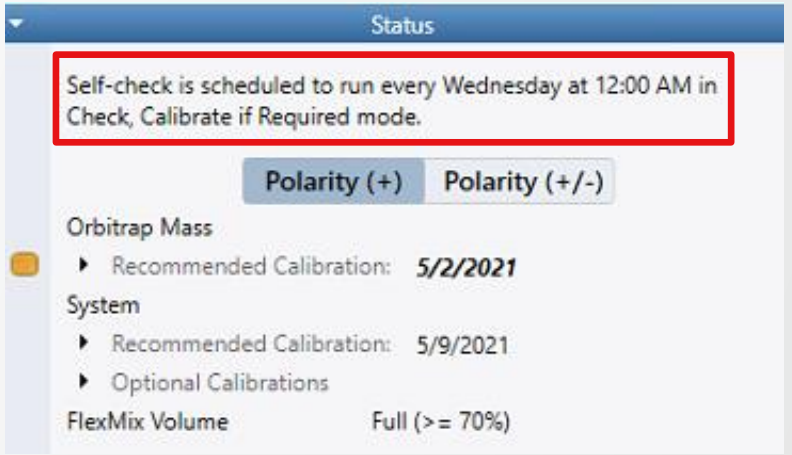
- Current Calibration Options
  - "Auto-Ready" is set as Calibration Source by default (auto and manual modes)
  - Select "Other" to calibrate with syringe pump (manual mode only)
  - Auto-Ready Ion Source only uses pre-filled FlexMix vials

- System Self –Check Options
  - Self-Check can be set to « Check » or « Check, Calibrate if required » (and systematically set to « Orbitrap Mass & System » calibration type)
  - Day and time for Self-Check is defined
  - The polarity (*« Positive » or « Positive & Negative »*) and optional calibrations used for Self-Check are defined in Settings section

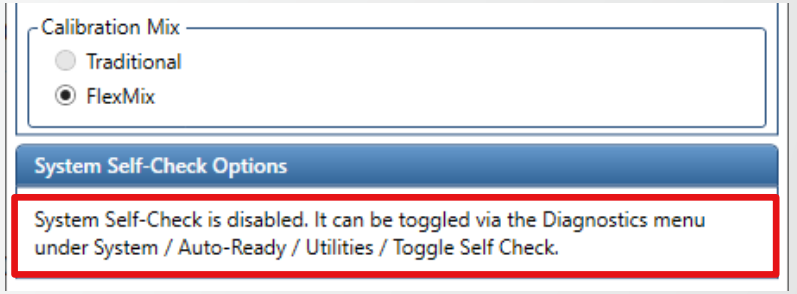
Auto-Ready Ion Source

Self-Check Pre-Conditions

- Self-Check settings
  - They are indicated in status pane of calibration tab



- Self-Check (Auto-Calibration) procedure is pursued only if
  - The instrument is in standby or scanning (“On”)
  - Auto-Ready is current Calibration Source in Tune Preferences
  - Self-Check is enabled in diagnostics (notification in Tune Preferences Self-Check is disabled)



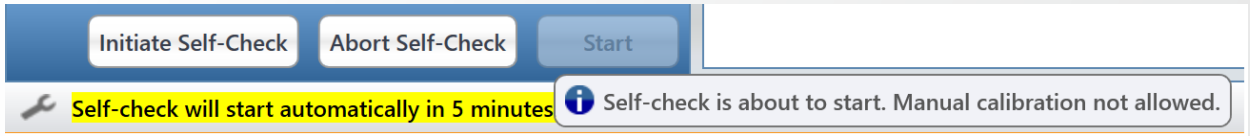
- Self-Check procedure is deferred and subsequently run if
  - There is an acquisition in progress (acquisition sequence or tune recording) at the time of scheduled Self-Check
  - Acquisition queue gets empty within the 24 hours of scheduled Self-Check (check every 5 min)

Auto-Ready Ion Source

Self-Check Procedure

- 5-min delay Self-Check preparation
  - Notification displayed in Tune bottom panel that Self-Check is about to start; text updated every minute

  - Same information displayed in notification area of calibration panel
  - Show « Initiate Self-Check » / « Abort Self-Check » buttons and disable « Start » button in calibration panel

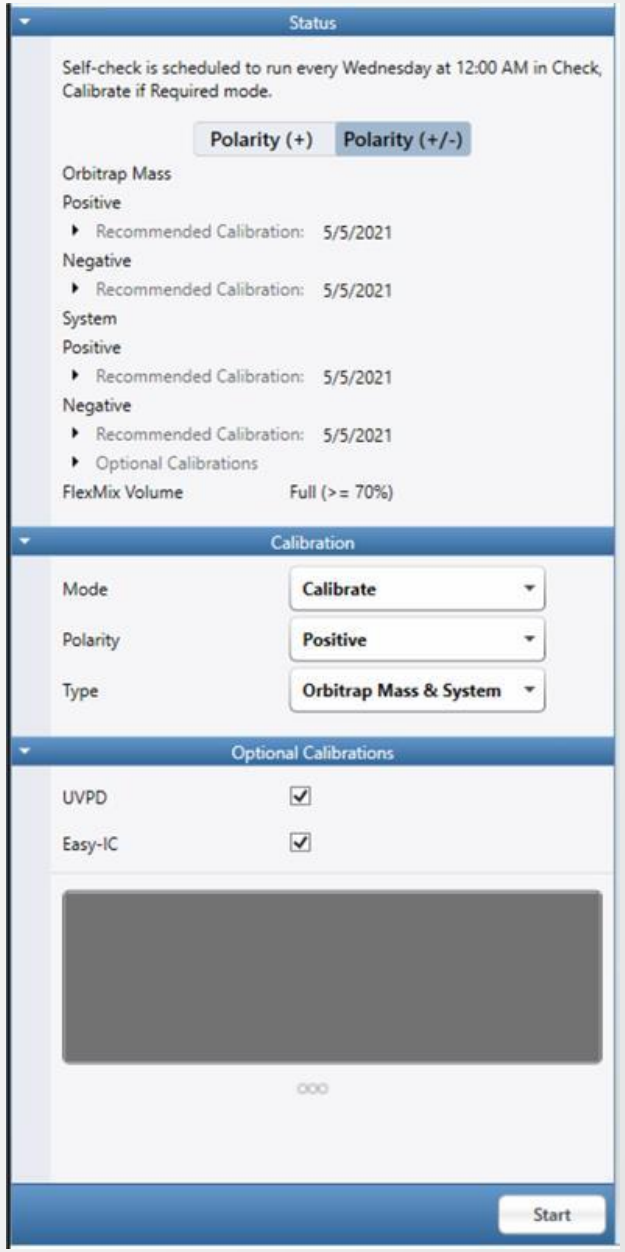


- Self-Check execution
  - Start after 5-min preparation has elapsed
  - Instrument is switched to « On » if currently in standby
  - Calibration UI selection updated to match Self-Check settings (mode, polarity, and optional calibrations)
  - During Self-Check execution
    - Tune operations are disabled, procedure can be aborted by pressing relevant button (aborted Self-Check not run until next scheduled check)
    - Self-Check running status is displayed (progress bar, notification panel)

Auto-Ready Ion Source

Calibration Tab

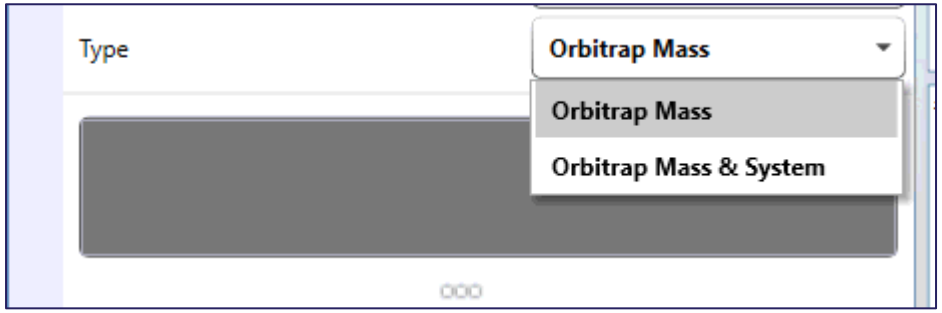
- « Manual » conventional one-click calibration
  - Can be performed at any time by pressing « Start » in Calibration tab
  - All conventional calibration modes and types are available
- Auto-Ready Ion Source is used for « Manual » one-click calibration as long as it is defined as the Calibration Source in Tune Preferences
- Status pane displays calibration sets with recommended re-calibration dates
  - Dates become bolded/italicised when due date = today or day in past
- Caret opens to display last calibration dates
- Under System, last calibration dates can be displayed for optional calibrations



Same as with Conventional source

Auto-Ready Ion Source

Calibration Tab - Calibration Types

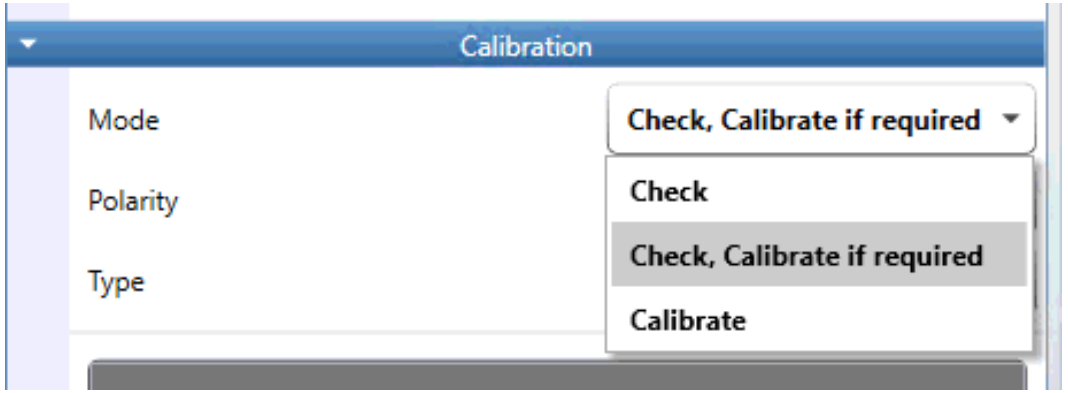


- Types: **Orbitrap Mass** or **Orbitrap Mass & System**
- Selecting Orbitrap Mass triggers the following calibrations:
  - multiplier
  - Orbitrap mass
- Selecting Orbitrap Mass & System triggers the following calibration set, in order:
  - Ion Optics
  - Ion Trap
  - Quadrupole
  - pAGC
  - Orbitrap mass
- If Orbitrap Mass & System selected, “Optional Calibrations” are exposed (based on configuration & polarity* selected)

Same as with Conventional source

Auto-Ready Ion Source

Calibration Tab - Calibration Modes



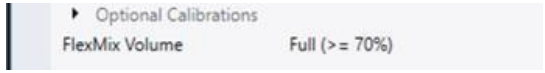
- Modes: “Check”, “Check, calibrate if required”, or “Calibrate”
  - A spray check and calibrant purity evaluation are automatically run prior to any calibration set
- “Check, calibrate if required” operates as follows:
  - Any failed checks are automatically calibrated (as well as dependent calibrations)
  - The system automatically runs calibrations that have expired (bypass check), i.e., *Multiplier gain* after 7 days and all remaining calibrations after 28 days
  - The system automatically runs Orbitrap mass calibration

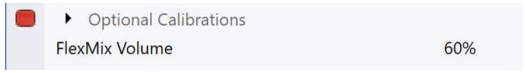
Same as with Conventional source

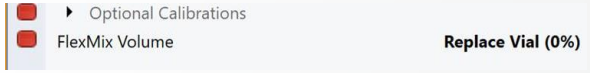
Auto-Ready Ion Source

Calibration Tab

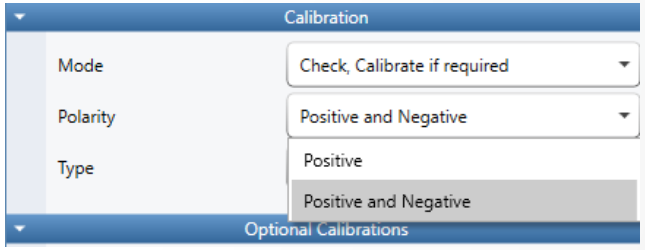
- Calibration status are displayed for polarity (+) or (+/-)
- FlexMix level is indicated in status panel
  - “Full” when >= 3.75ml (>=75%)
  - 0% when <= 125ul
  - 70% when >= 3.50ml
  - 5% decrement starting at 70%
  - “Replace Vial” when 0%
  - Red indicator is displayed when 0% - start button is disabled, hover text indicates ‘Replace Vial’
  - Orange indicator is displayed when 10% - 1%
  - No color indicator when above 10%

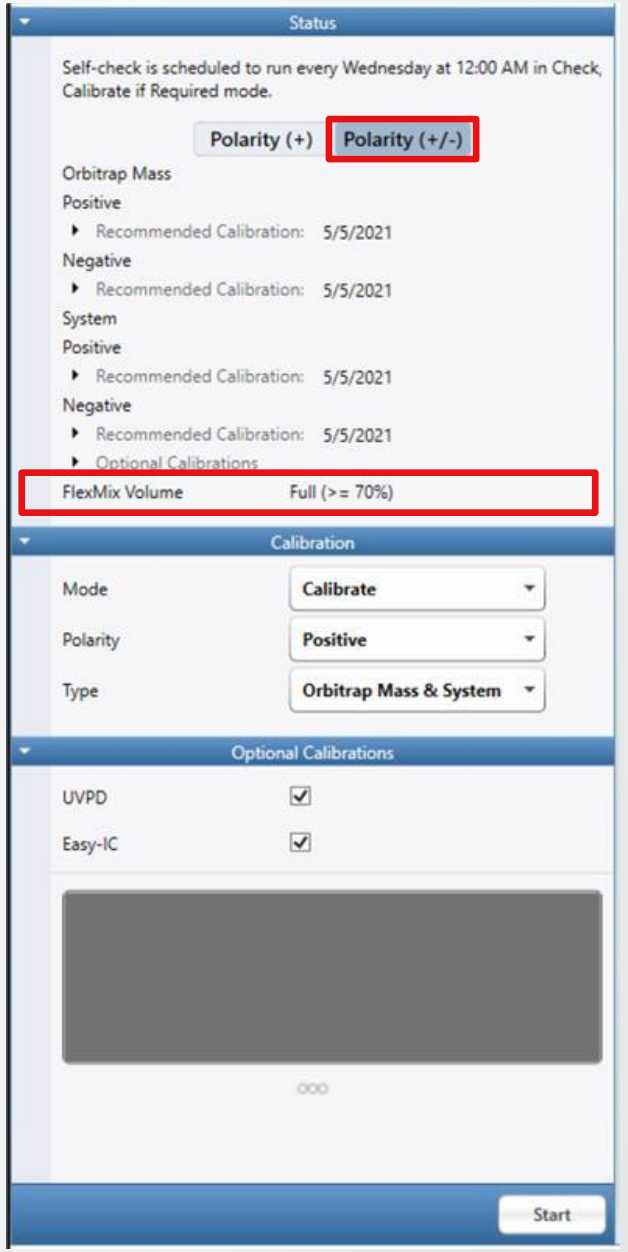






- Calibration Polarity:
  - Positive
  - Positive and Negative





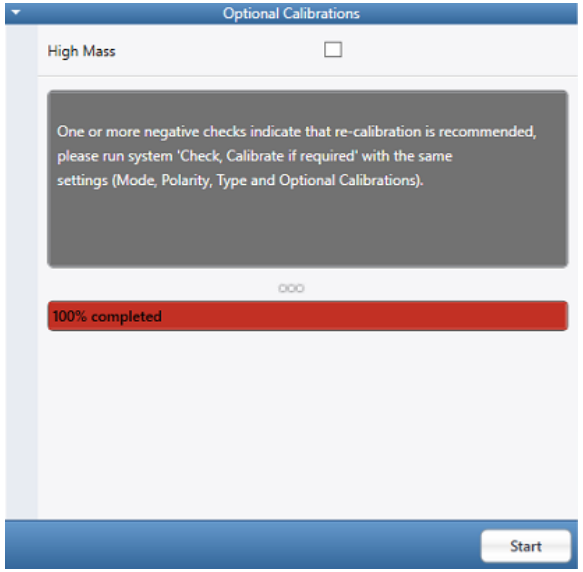
Auto-Ready Ion Source

Calibration Results – Self-Correction And Recommendations

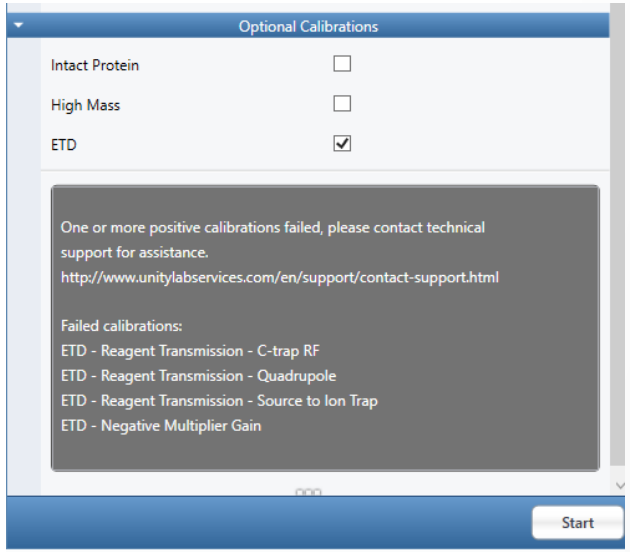
- Upon any calibration failure, two procedure checks are run before the result is reported:
  1. Spray stability check
     - If spray check fails, system will attempt to self-correct spray issue for 5 min. Once stable spray is re-established, the sequence will resume starting from the procedure that originally failed
     - If spray cannot be recovered, the check is aborted and the following notifications appears: “A <procedure> failed due to spray instability. Please see below for recovery recommendations.”
       - “A <procedure> failed due to spray instability. Please perform following tasks and retry:
       - -- Run the 'Flush Calibration Source Lines' diagnostic in attempt to clear any residual air bubbles from the lines
       - -- Re-seat the fittings between the calibrant pump and the sprayer
       - -- Run the 'Calibration Source HV Calibration' diagnostic to ensure the spray voltage is set properly
     - If spray check passes, the second procedure check is run.
  2. Calibrant purity evaluation
     - If cal mix evaluation fails, system will attempt to recover acceptable calibrant purity by running one flush cycle of calibration source lines
     - If acceptable cal mix purity cannot be recovered, the procedure is aborted and the following notifications appears: “A <procedure> failed due to calibrant contamination or degradation. Please see below for recovery recommendations.”
       - “A <procedure> failed due to calibrant contamination or degradation. Please perform following tasks and retry:
       - -- Run the 'Flush Calibration Source Lines' diagnostic in attempt to clear any contamination buildup from the lines
       - -- Change the calibrant bottle to a fresh one

Auto-Ready Ion Source

Calibration Results – Procedure vs Calibration Failure



- If the ‘Check-only’ routine is failed, but procedure checks pass, the system will recommend the user run in “Check, Calibrate if required” mode.

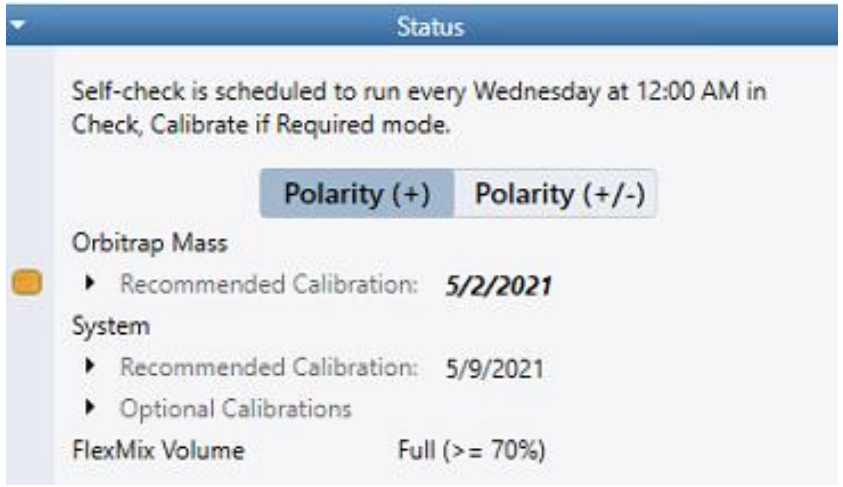


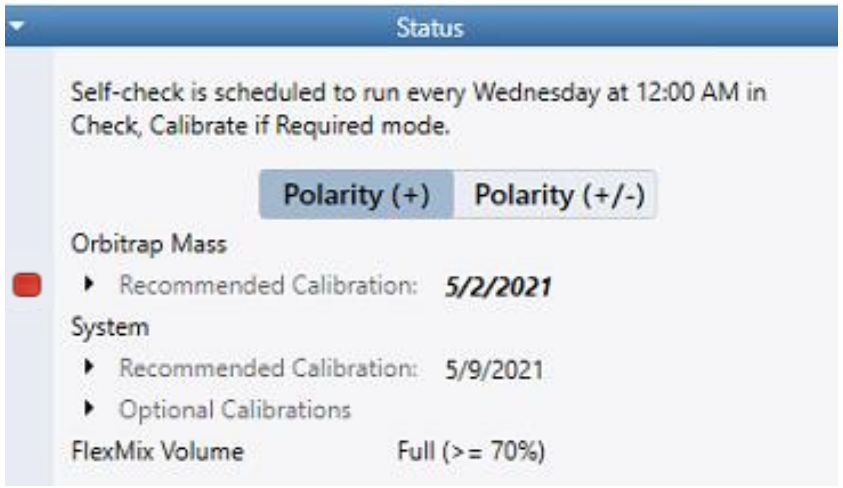
- If the calibration is run in ‘Check, Calibrate if required” or “Calibrate” mode and fails, but both procedure checks pass, a true calibration failure is recorded.

Same as with Conventional source

Auto-Ready Ion Source

Calibration Results – ‘Orange’ vs ‘Red’ Status

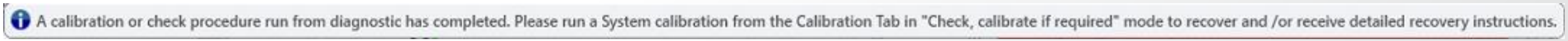




- Status will show an orange indicator light when:
  - A procedure failure (spray stability or calibrant purity evaluation) is recorded during a calibration procedure
  - A single calibration is run from diagnostics
  - A single check is run from diagnostics and fails
  - A procedure is aborted

- Status will show a red indicator light when:
  - A true calibration failure is recorded (calibration fails and both procedure checks pass)

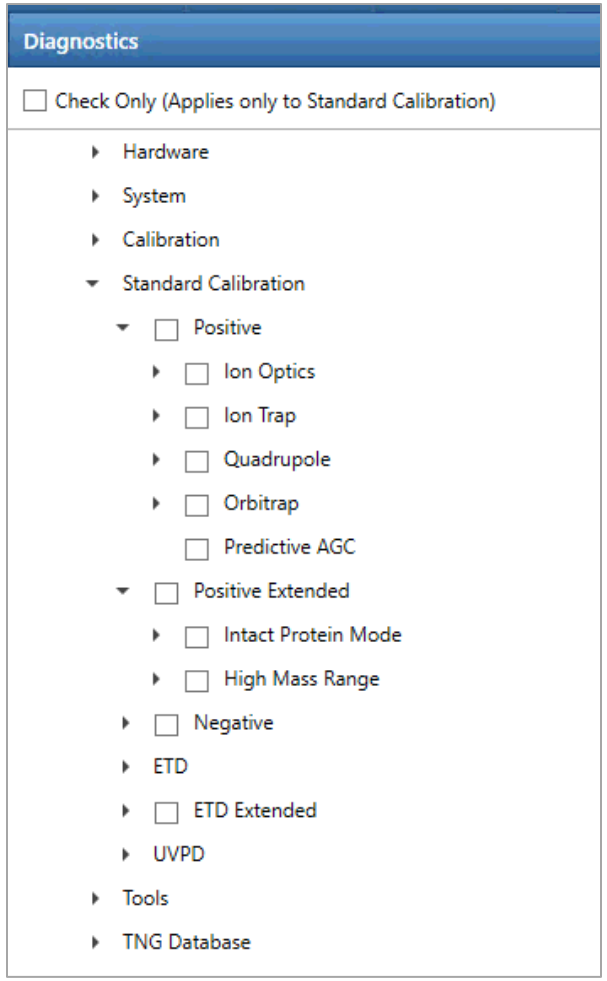
- Hovering over indicator light will provide explanation of state



Same as with Conventional source

Auto-Ready Ion Source

Calibration Procedures in Diagnostics



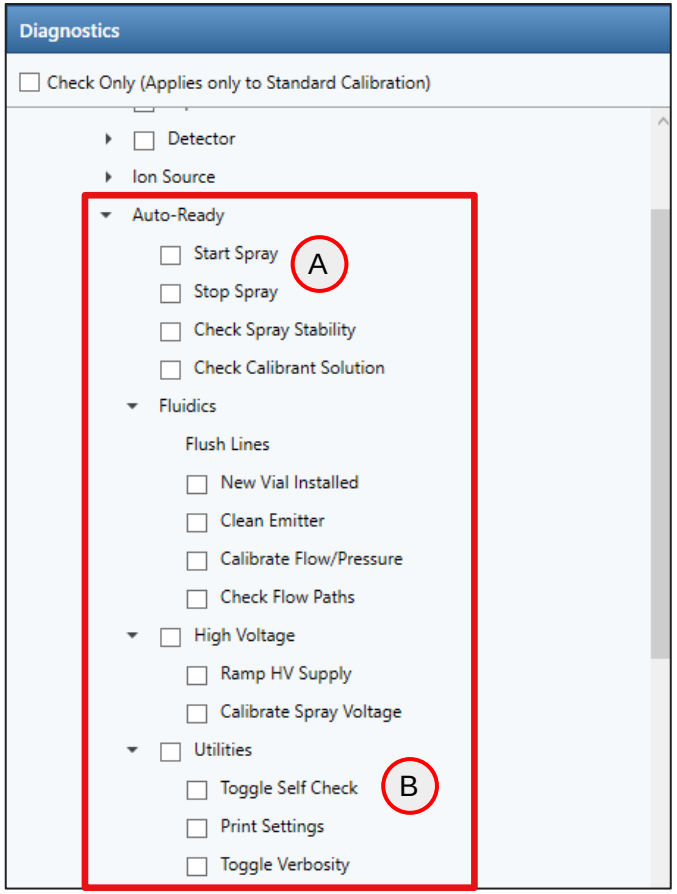
- Calibration tree can be found in the customer diagnostics pane under "Standard Calibration"
  - Individual calibrations may be checked or run
  - As the system requires calibrations to be run in the proper order as a set and some calibrations affect dependent calibrations, calibrating from the diagnostics pane will have the following affect on the status panel in the calibration pane:

Result in Diagnostics	Impact on Status Panel
Check passes	No impact
Check fails	Associated set ORANGE
Calibration passes	Associated set ORANGE
Calibration fails	Associated set ORANGE

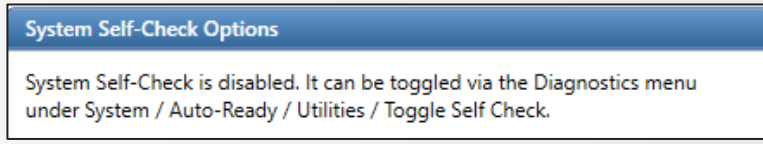
Same as with Conventional source

Auto-Ready Ion Source

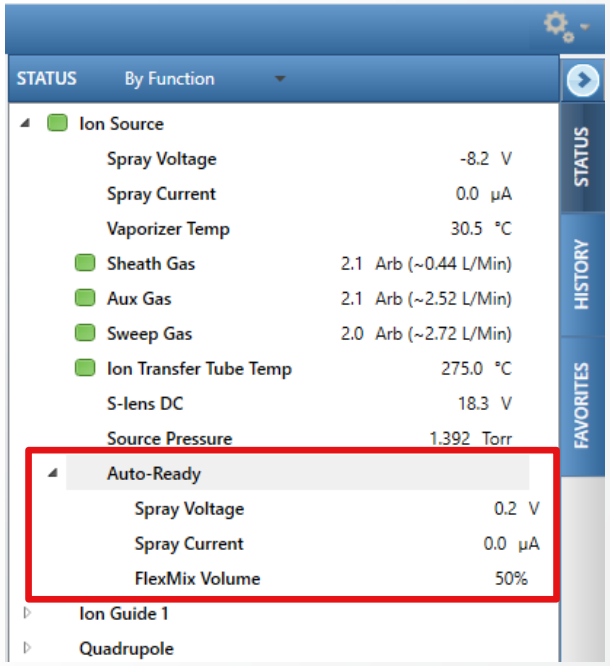
Diagnostics & Readbacks



- Auto Ready Ion Source Diagnostics
  - FlexMix infusion using Auto Ready Ion Source must be manually started to run a diagnostic that requires it (A)
  - Toggle available to enable / disable Self-Check (B)



- Auto Ready Ion Source Readbacks



Improvements in FAIMS Support

New Features and Integration with Orbitrap Tribrid Series ICSW 3.5

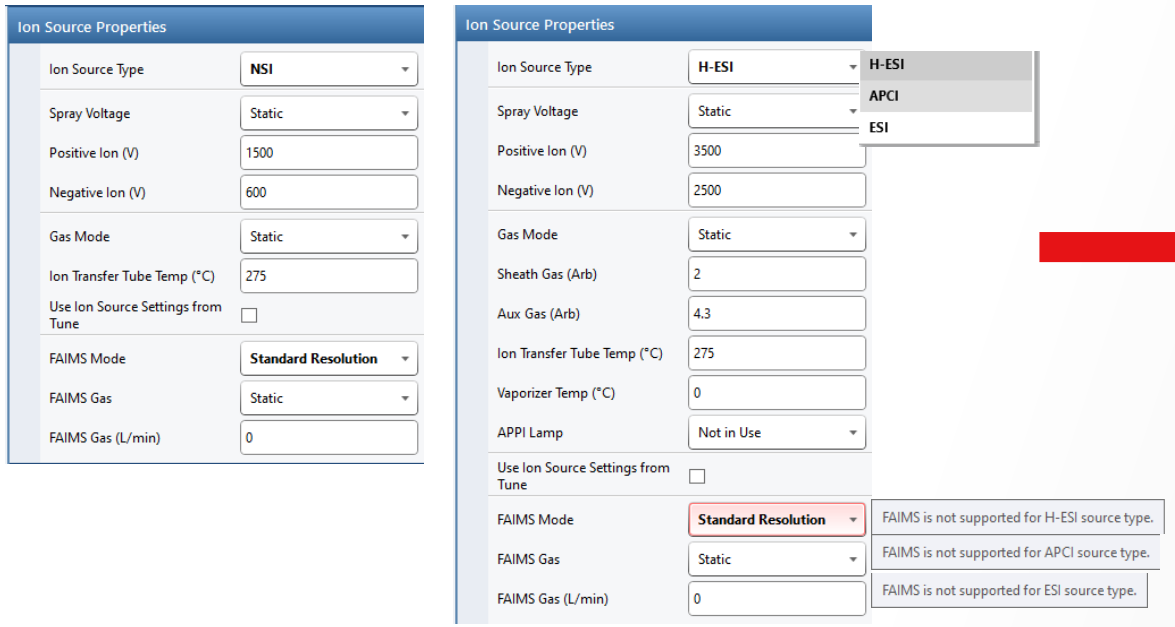
- The new FAIMS Pro Duo Interface extends FAIMS application to a wide range of chromatographic flow rates
- Compensation Voltage (CV) switching time has been further optimized for targeted applications
- Management of Carrier Gas flow has been modified to facilitate the optimization of spray stability in nanoflow LC applications
- Availability
  - All Tribrid models
- New features aligned with Orbitrap Exploris Series (OES) ICSW 3.1

Improvements in FAIMS Support

FAIMS Pro Duo Interface

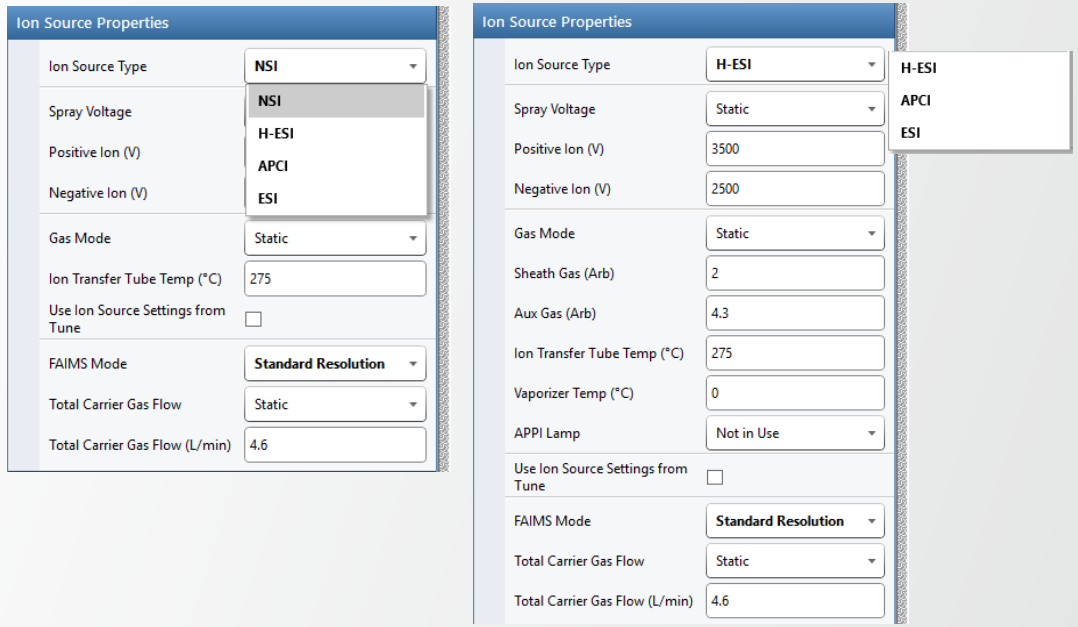
- FAIMS Application Over a Wide Range of Chromatographic Flow Rates
  - Analyses with FAIMS Pro Duo Interface possible with the OptaMax NG ion source at flow rates up to 1 mL/min
  - FAIMS with HESI / APCI ion source type supported by Instrument Control Software

Orbitrap Tribrid Series ICSW 3.4



FAIMS only supported for NSI source type

Orbitrap Tribrid Series ICSW 3.5



FAIMS supported for ALL source types

Improvements in FAIMS Support

Optimized FAIMS CV Post-switching Delay Time for more Productive Targeted Acquisition

- Time necessary between the application of two consecutive CV settings to wait for the completion of the ion transfer through the FAIMS interface
- Previous implementation (ICSW 3.4 and earlier)
  - CV switching time dependent on ion tranfer tube (ITT) geometry

  MS with round bore ITT: 40 ms (e.g., Orbitrap Fusion)

  MS with high capacity ITT: 25 ms (e.g., Orbitrap Eclipse)
- New implementation (ICSW 3.5)
  - CV switching time dependent on ion tranfer tube geometry and magnitube of CV change
  - Fixed switching for CV steps above 2V and shorter time for smaller CV steps (following linear interpolant)

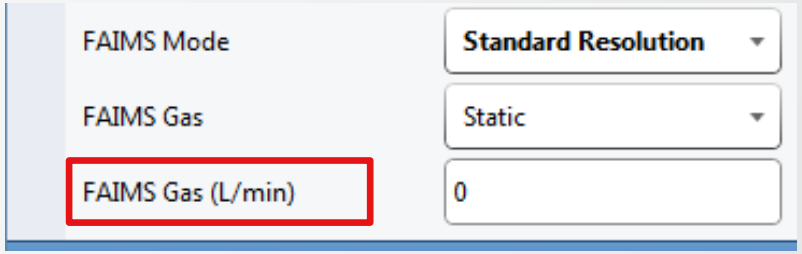
ITT Geometry	CV switching time		Instruments
	CV step ≥ 2V	CV step ≤ 1V	
Round bore ITT	40 ms	< 25 ms	Orbitrap Fusion, Orbitrap ID-X, Orbitrap IQ-X
High capacity ITT	25 ms	< 15 ms	Orbitrap Fusion Lumos, Orbitrap Eclipse

Improvements in FAIMS Support

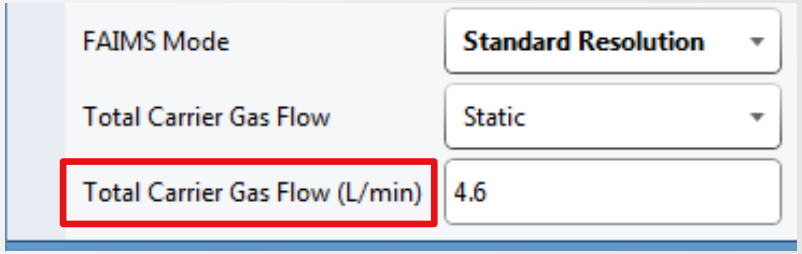
Modified Management of Carrier Gas Flow To Address Spray Stability Issues at Nanoflow LC Rates

- FAIMS carrier gas flow needs to be user settable within a defined range to address spray stability issue
- Implementation in ICSW 3.4 and earlier based on two parameters controlling a single gas flow / line
  - Carrier Gas Flow - hidden parameter taking a fixed value depending on ion tranfer tube (ITT) geometry
  - FAIMS Gas (or FAIMS User Set Gas) – User defined parameter accessible in Method Editor and Tune Page
- New implementation in ICSW 3.5
  - Carrier Gas Flow and FAIMS Gas are merged into a single parameter « Total Carrier Gas Flow » accessible in Method Editor and Tune
  - User settable ranges for Total Carrier Gas Flow
    - Round bore ITT: 3.5-7.7 L/min (default value: 4.6 L/min)
    - High capacity ITT: 0.7-4.3 L/min (default value: 1.2 L/min)
  - Total Carrier Gas Flow value for methods converted from ICSW 3.4 (or earlier)
    - Same ITT geometry: Default Total Carrier Gas Flow value + Previous User Set Gas value
    - Different ITT geometry: Default Total Carrier Gas Flow value

ICSW 3.4



ICSW 3.5

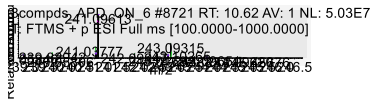


Advanced Peak Determination (APD)

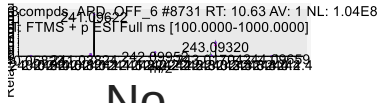
Broaden and Improved Implementation across Orbitrap Tribrid Family

- Aligned with Orbitrap Exploris Series ICSW 3.1
- Now Standard for all Orbitrap Tribrid products
- Application mode specific versions of peak annotation algorithm

Improved peak annotation of small molecules, e.g., halogenated compounds, reducing MS2 triggering on isotopes

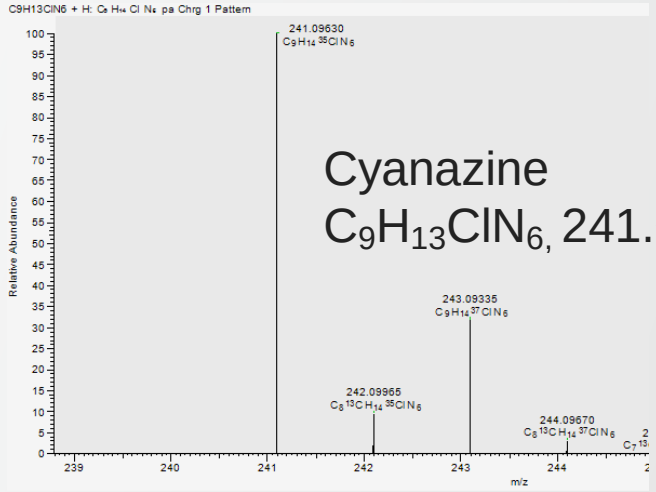


APD



No
APD

APD OFF→ A1, A2 isotopes
MS/MS triggered

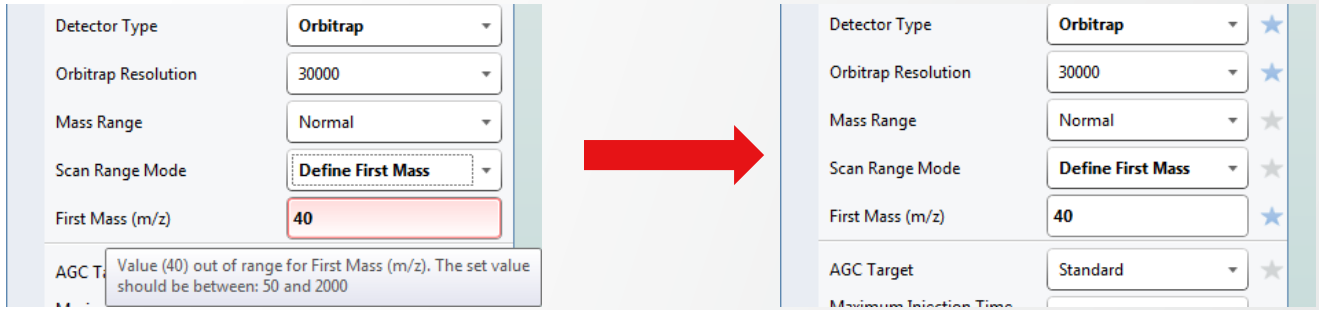


Cyanazine
$C_9H_{13}ClN_6$, 241.09630

Support for Extended Low Mass Range m/z 40 in MS^n Scans

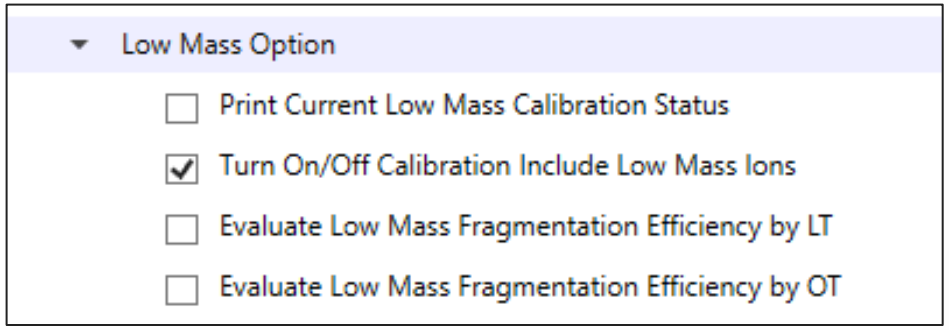
Enabling Identification of Additional Fragment Ions for Chemical Structure Elucidation

- Available for all Tribrid models
- Applied only to MS^n scans (no MS1)
- Applied to Orbitrap and Ion Trap scans



ICSW 3.4 *ICSW 3.5*

- Modified calibrations including ions below m/z 50. Application controlled by toggle in Diagnostics
  - Accessible under System>Low Mass Option>Turn On/Off Calibration Include Low Mass Ions
  - « On » by default for Orbitrap IQ-X. « Off » for all other Tribrid
  - List of required calibrations after turning on the option displayed in Diagnostics report

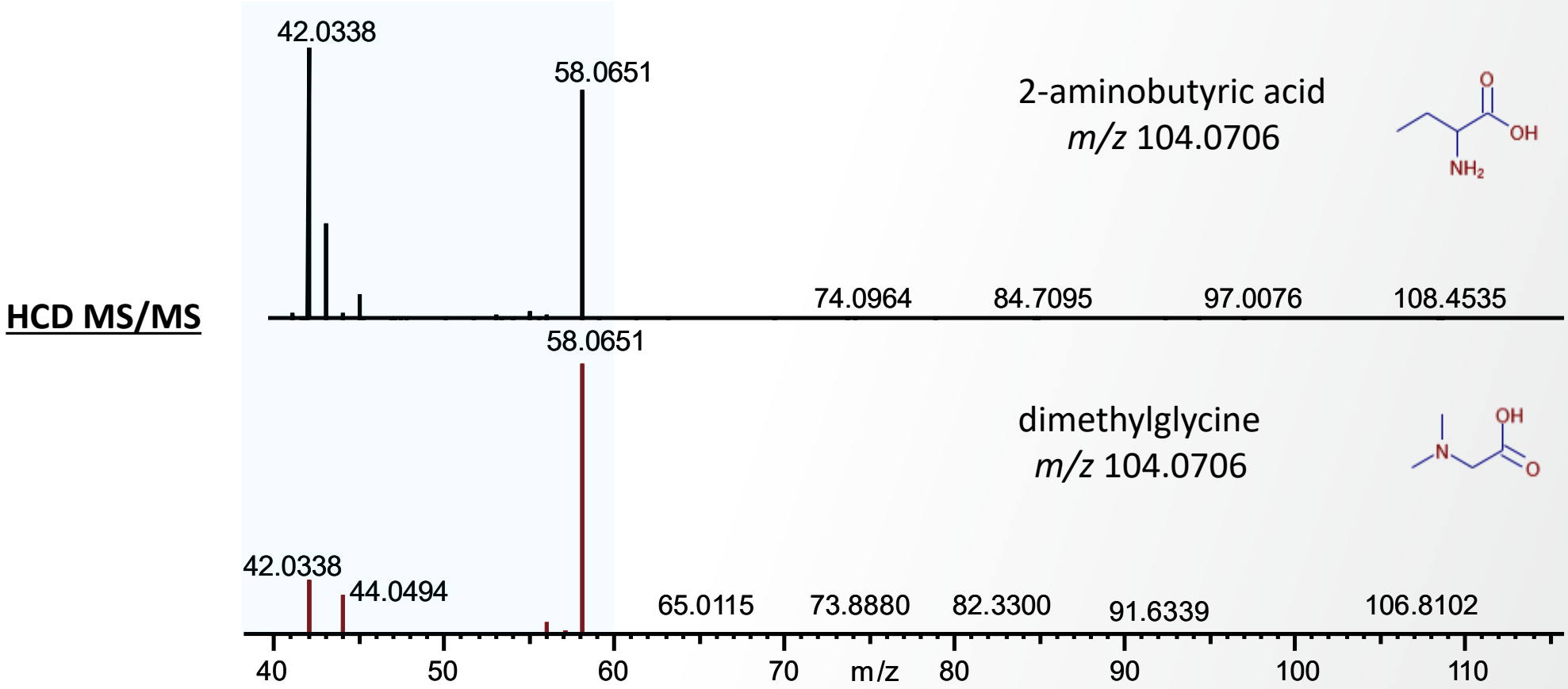


Turn On/Off Calibration Include Low Mass Ions

```
Calibration is including 40-50 m/z ions
The instrument need to be recalibrated with the following order to get the best low mass performance
Please run the following calibrations:
1. Main calibration - Optics - Multipole RF Amplitude Twice
2. Main calibration - Ion Trap - Resolution Normal Mass Range - All Scan Speed
3. Standard calibration - Ion Trap - Mass - All Scan Speed
4. Main calibration - Optics - CTrap RF
5. Standard calibration - Orbitrap - Mass
```

Support for Extended Low Mass Range m/z 40 in MS^n Scans

Enabling Identification of Additional Fragment Ions for Chemical Structure Elucidation



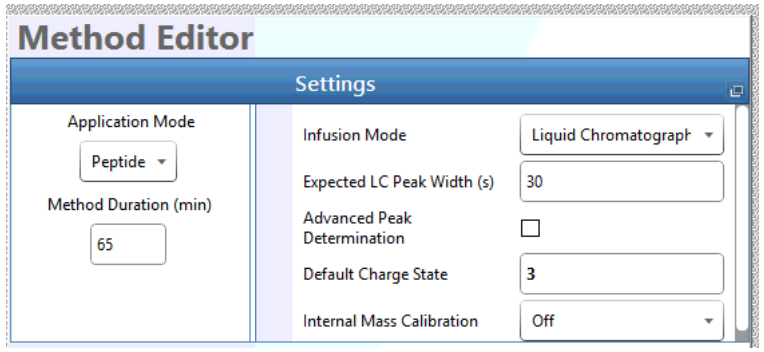
Fragments in mass range *m/z* 40-60 provide fragmentation ions distinguishing the two isomers

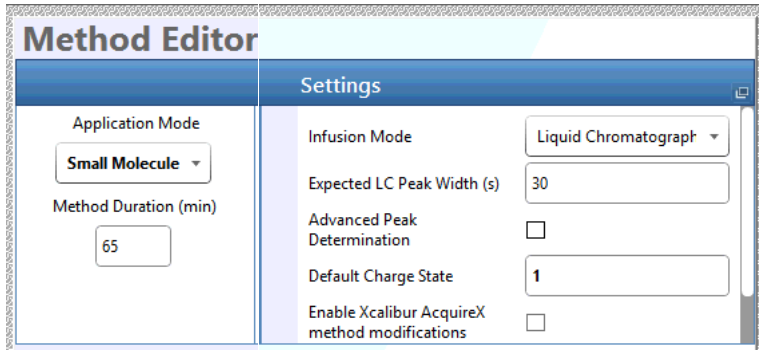
Support for Mild Trapping

Enabling Reduced Fragmentation of Fragile and Labile Compounds

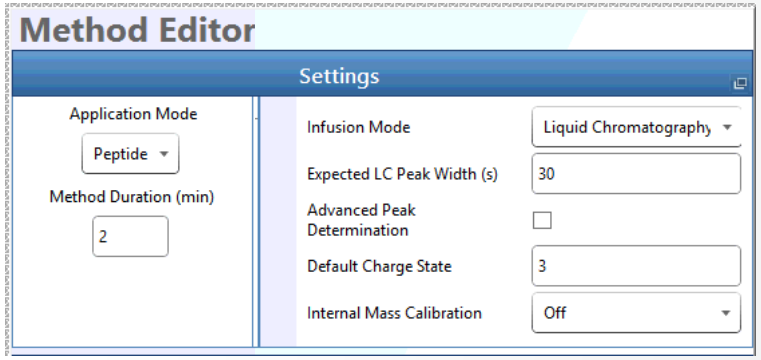
- Aligned with OES ICSW 3.1; Available for all Tribrid models (small molecule application mode)
- New checkbox in Method Editor / toggle in Tune Diagnostics

ICSW 3.4

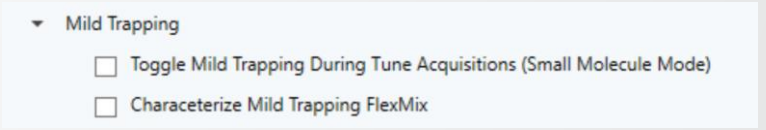




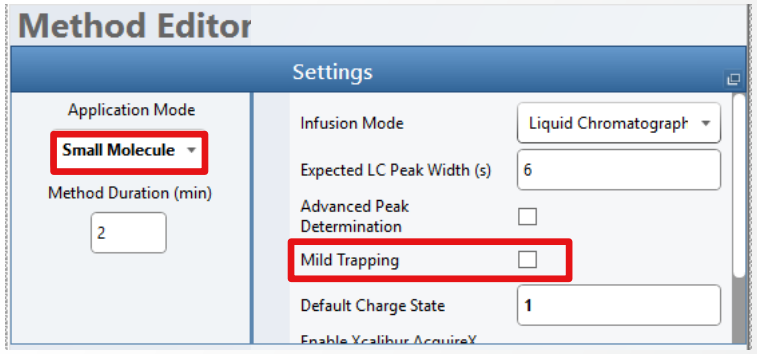
ICSW 3.5



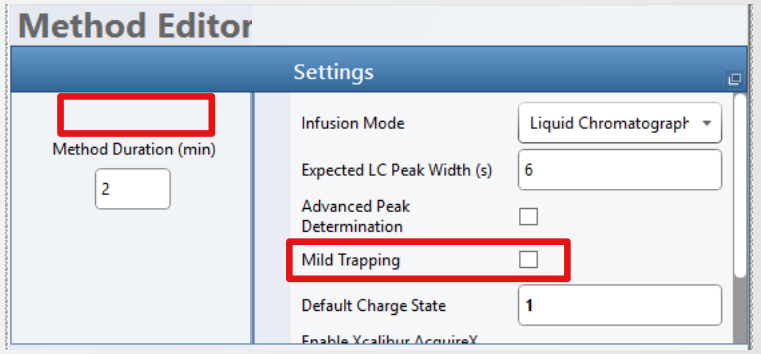
Diagnostics Toggle in Tune



Orbitrap Fusion, Fusion Lumos, Eclipse



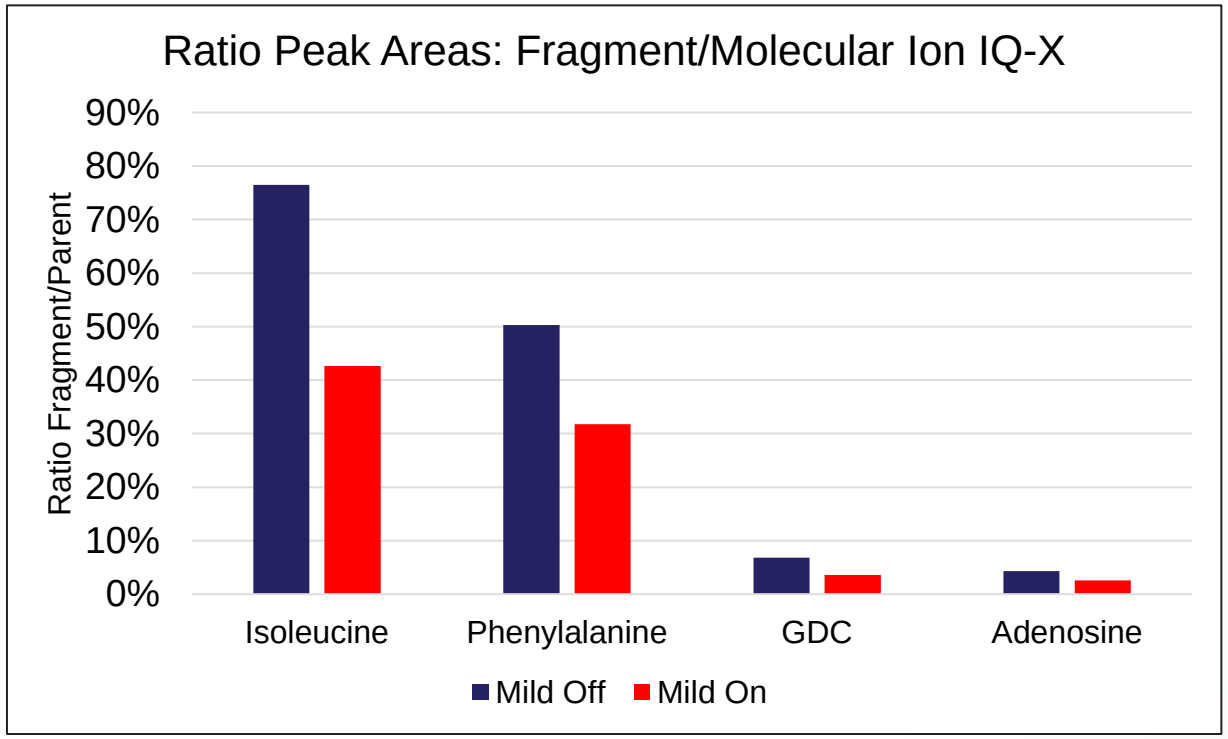
Orbitrap ID-X, IQ-X

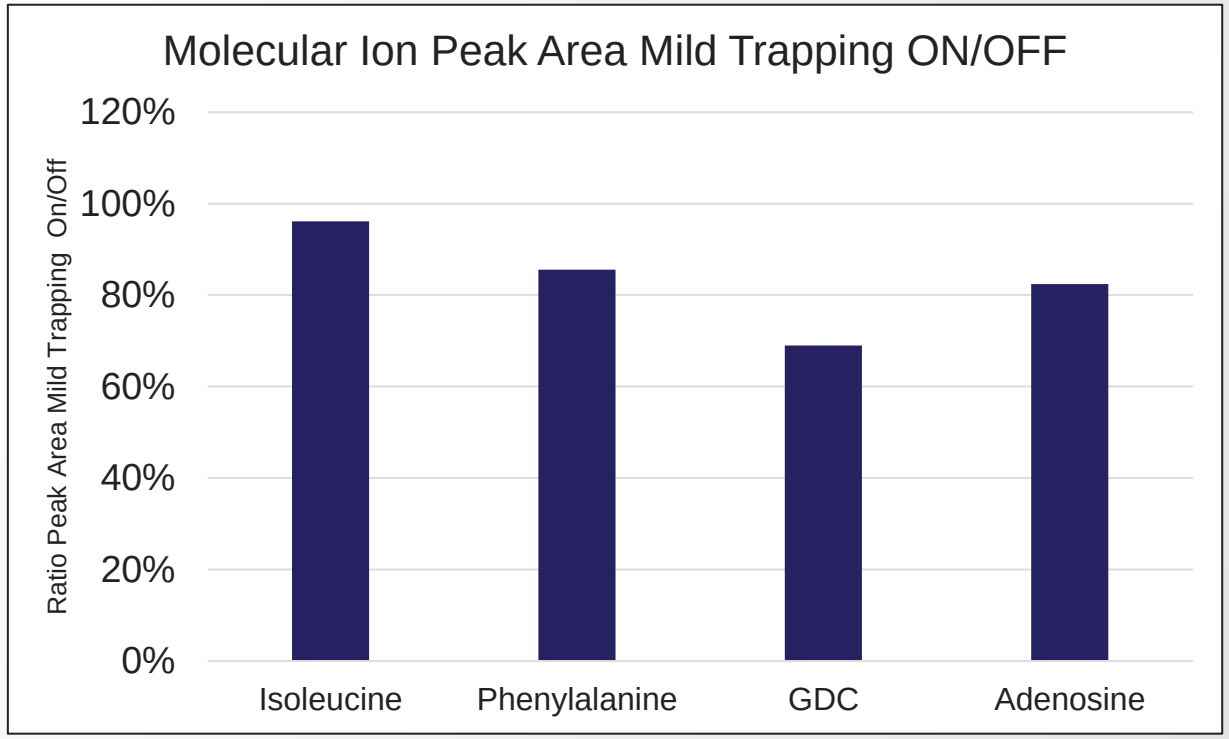


Support for Mild Trapping

Enabling Reduced Fragmentation of Fragile and Labile Compounds

- Injection energy reduced upon injection into the IRM
- Significant decrease in fragmentation of most fragile compounds (up to 50%)
- Moderate decrease in overal signal (≈ 20%)



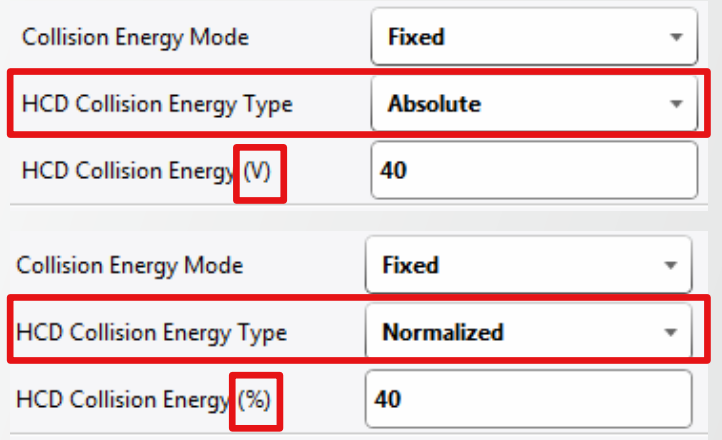


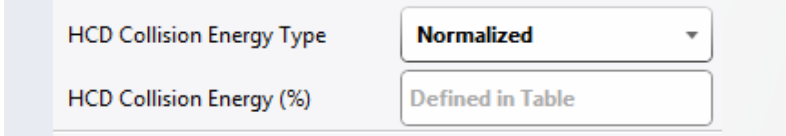
Support for Absolute and Normalized HCD Collision Energy

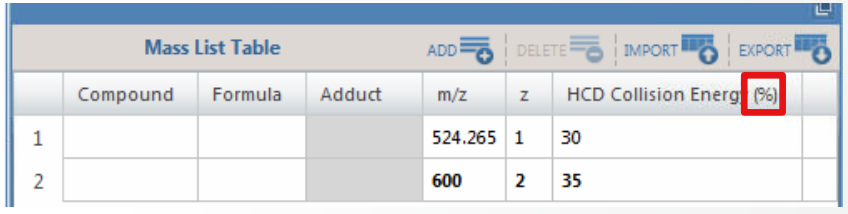
Enabling Expanded Control on Compound Fragmentation

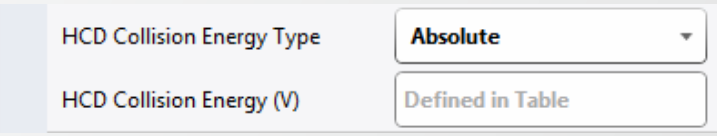
- Aligned with Orbitrap Exploris Series ICSW 3.1

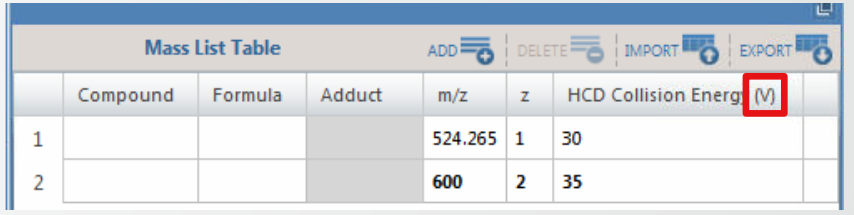
- New parameter in Method editor (and Tune/Define Scan) – HCD Collision Energy Type
  - Available as a property of MSn scan type (e.g., ddMS2, tMS2, or DIA)
  - Drive the definition of Absolute or Normalized value in HCD Collision Energy Field (reflected by displayed unit)
  - Control the type of HCD CE value used in Mass list table for targeted or directed acquisition but cannot be used for the definition of target specific HCD CE type







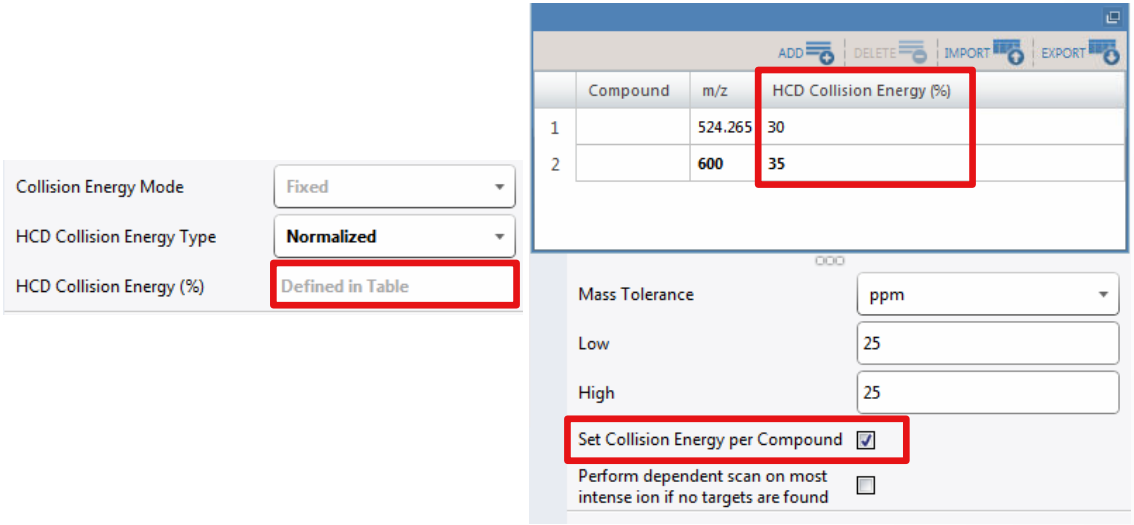


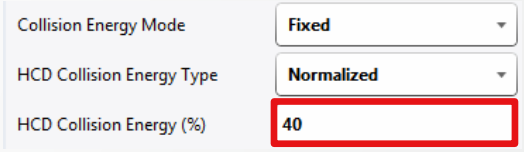


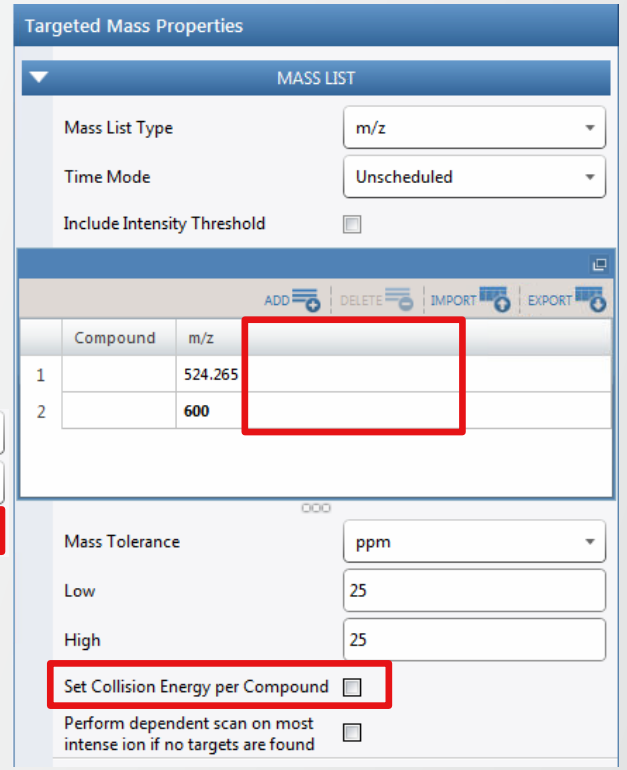
Support for Target Specific HCD CE in Targeted Mass Filter Table

Enabling More Flexibility in Directed Acquisition (DDA With Inclusion List)

- Aligned with Orbitrap Exploris Series ICSW 3.1
- New option in Method editor– Set Collision Energy per Compound
  - Available as a property of Targeted Mass Filter for HCD Activation Type
  - Further flexibility through possible combination with
    - Normalized or Absolute HCD CE
    - « Perform dependent scan on most intense ion if no targets are found »







Support for Target Specific HCD CE in Targeted Mass Filter Table

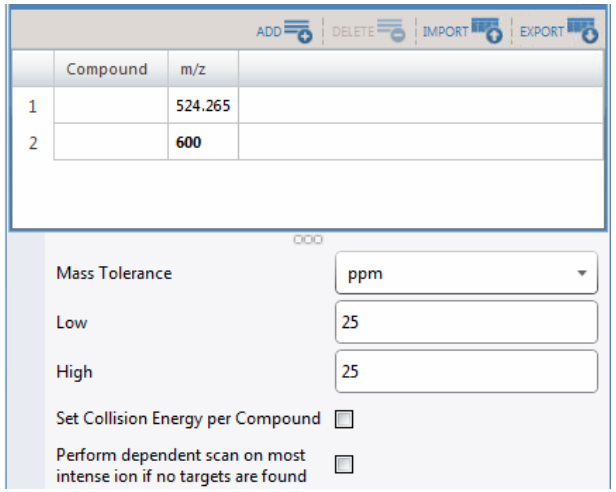
Enabling More Flexibility in Directed Acquisition (DDA With Inclusion List)

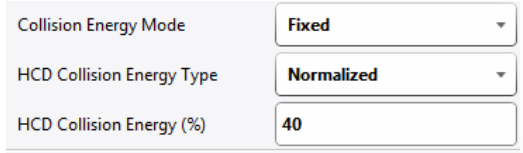
Collision Energy Application		Case 1	Case 2	Case 3	Case 4
Set Collision Energy per Compound		FALSE ☐	FALSE ☐	TRUE ☒	TRUE ☒
Perform dependent scan on most intense Ions if no targets are found		FALSE ☐	TRUE ☒	FALSE ☐	TRUE ☒
Collision Energy Mode	**Targets (Table)**	Fixed OR Stepped for all targets	Fixed OR Stepped for all (targets & additional ions)	Fixed for all targets (greyed out)	Fixed for all (targets & additional ions) (greyed out)
	Additional Ions	n.a.		n.a.	
HCD Collision Energy Type	**Targets (Table)**	Absolute OR Normalized for all targets	Absolute OR Normalized for all (targets & additional ions)	Absolute OR Normalized for all targets	Absolute OR Normalized for all (targets & additional ions)
	Additional Ions	n.a.		n.a.	
HCD Collision Energy Value	**Targets (Table)**	In Scan Property: 1 (frozen) value applied to all targets OR 2-5 (frozen) values applied to all targets	In Scan Property: 1 (frozen) value OR 2-5 (frozen) values applied to all (targets & additional ions)	In Scan Property: "Defined in Table" -> In Table: 1 individual value per target	In Table: 1 individual value per target
	Additional Ions	n.a.		n.a.	In Scan Property: 1 (frozen) value applied to all additional ions

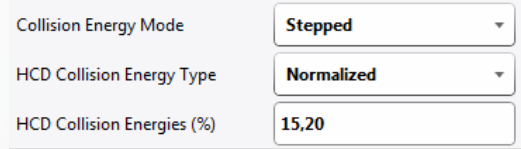
Support for Target Specific HCD CE in Targeted Mass Filter Table

Enabling More Flexibility in Directed Acquisition (DDA With Inclusion List)

Collision Energy Application		Case 1	Case 2	Case 3	Case 4
Set Collision Energy per Compound		FALSE ☐	FALSE ☐	TRUE ☒	TRUE ☒
Perform dependent scan on most intense Ions if no targets are found		FALSE ☐	TRUE ☒	FALSE ☐	TRUE ☒
Collision Energy Mode	**Targets (Table)**	Fixed OR Stepped for all targets	Fixed OR Stepped for all (targets & additional ions)	Fixed for all targets (greyed out)	Fixed for all (targets & additional ions) (greyed out)
	Additional Ions	n.a.		n.a.	
HCD Collision Energy Type	**Targets (Table)**	Absolute OR Normalized for all targets	Absolute OR Normalized for all (targets & additional ions)	Absolute OR Normalized for all targets	Absolute OR Normalized for all (targets & additional ions)
	Additional Ions	n.a.		n.a.	
HCD Collision Energy Value	**Targets (Table)**	In Scan Property: 1 (frozen) value applied to all targets OR 2-5 (frozen) values applied to all targets	In Scan Property: 1 (frozen) value OR 2-5 (frozen) values applied to all (targets & additional ions)	In Scan Property: "Defined in Table" -> In Table: 1 individual value per target	In Table: 1 individual value per target
	Additional Ions	n.a.		n.a.	In Scan Property: 1 (frozen) value applied to all additional ions







Support for Target Specific HCD CE in Targeted Mass Filter Table

Enabling More Flexibility in Directed Acquisition (DDA With Inclusion List)

Collision Energy Application		Case 1	Case 2	Case 3	Case 4
Set Collision Energy per Compound		FALSE ☐	FALSE ☐	TRUE ☒	TRUE ☒
Perform dependent scan on most intense Ions if no targets are found		FALSE ☐	TRUE ☒	FALSE ☐	TRUE ☒
Collision Energy Mode	**Targets (Table)**	Fixed OR Stepped for all targets	Fixed OR Stepped for all (targets & additional ions)	Fixed for all targets (greyed out)	Fixed for all (targets & additional ions) (greyed out)
	Additional Ions	n.a.		n.a.	
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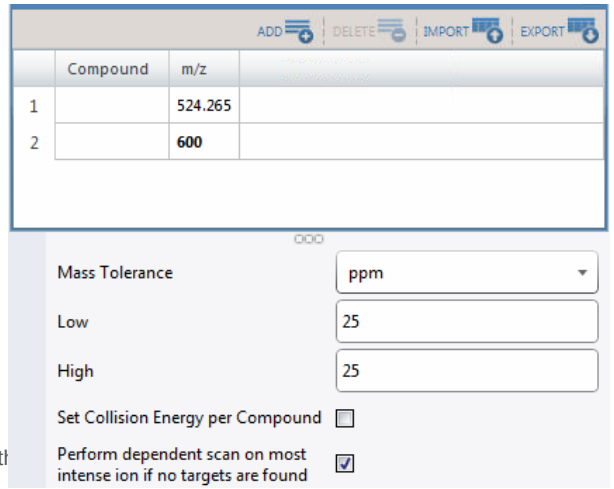
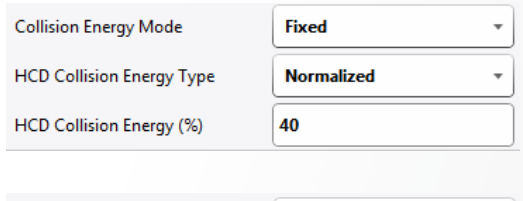
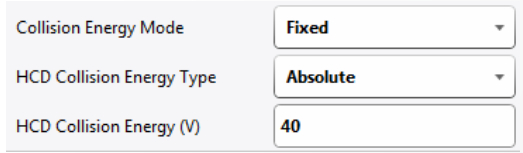
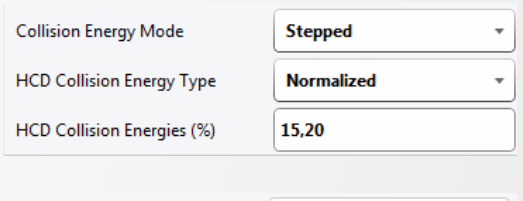
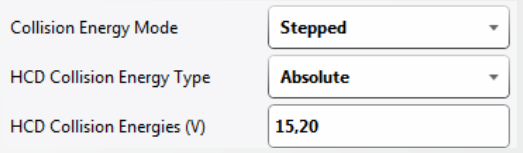
	Additional Ions	n.a.		n.a.	
HCD Collision Energy Value	**Targets (Table)**	In Scan Property: 1 (frozen) value applied to all targets OR 2-5 (frozen) values applied to all targets	In Scan Property: 1 (frozen) value OR 2-5 (frozen) values applied to all (targets & additional ions)	In Scan Property: "Defined in Table" -> In Table: 1 individual value per target	In Table: 1 individual value per target
	Additional Ions	n.a.		n.a.	In Scan Property: 1 (frozen) value applied to all additional ions







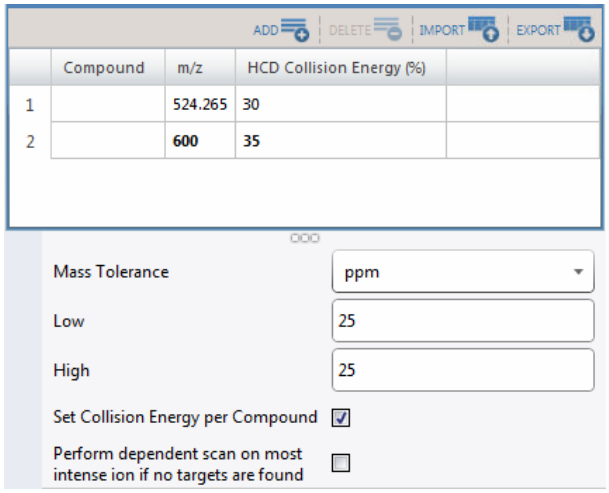


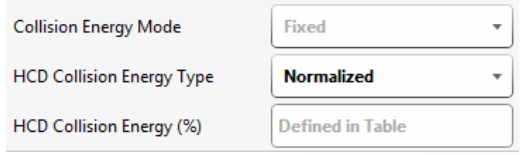


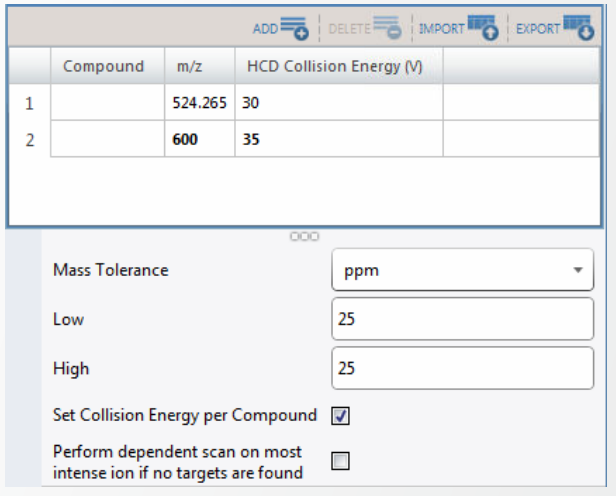
Support for Target Specific HCD CE in Targeted Mass Filter Table

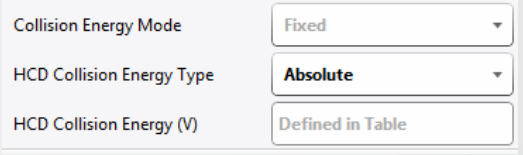
Enabling More Flexibility in Directed Acquisition (DDA With Inclusion List)

Collision Energy Application		Case 1	Case 2	Case 3	Case 4
Set Collision Energy per Compound		FALSE ☐	FALSE ☐	TRUE ☒	TRUE ☒
Perform dependent scan on most intense Ions if no targets are found		FALSE ☐	TRUE ☒	FALSE ☐	TRUE ☒
Collision Energy Mode	**Targets (Table)**	Fixed OR Stepped for all targets	Fixed OR Stepped for all (targets & additional ions)	Fixed for all targets (greyed out)	Fixed for all (targets & additional ions) (greyed out)
	Additional Ions	n.a.		n.a.	
HCD Collision Energy Type	**Targets (Table)**	Absolute OR Normalized for all targets	Absolute OR Normalized for all (targets & additional ions)	Absolute OR Normalized for all targets	Absolute OR Normalized for all (targets & additional ions)
	Additional Ions	n.a.		n.a.	
HCD Collision Energy Value	**Targets (Table)**	In Scan Property: 1 (frozen) value applied to all targets OR 2-5 (frozen) values applied to all targets	In Scan Property: 1 (frozen) value OR 2-5 (frozen) values applied to all (targets & additional ions)	In Scan Property: "Defined in Table" -> In Table: 1 individual value per target	In Table: 1 individual value per target
	Additional Ions	n.a.		n.a.	In Scan Property: 1 (frozen) value applied to all additional ions





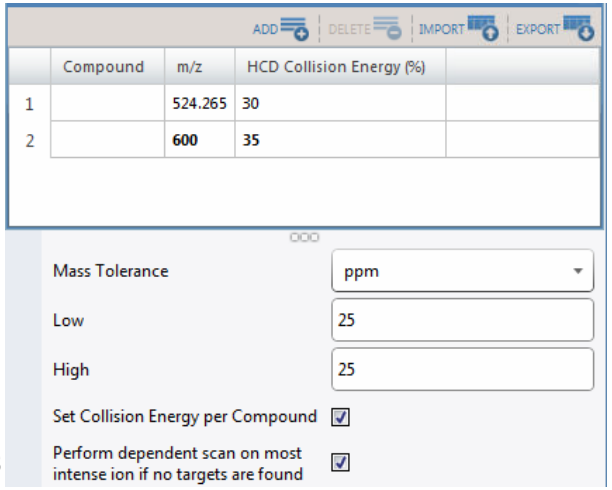


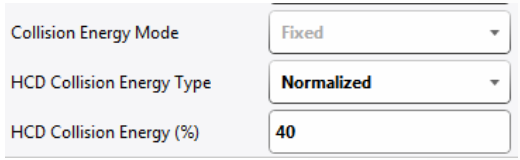


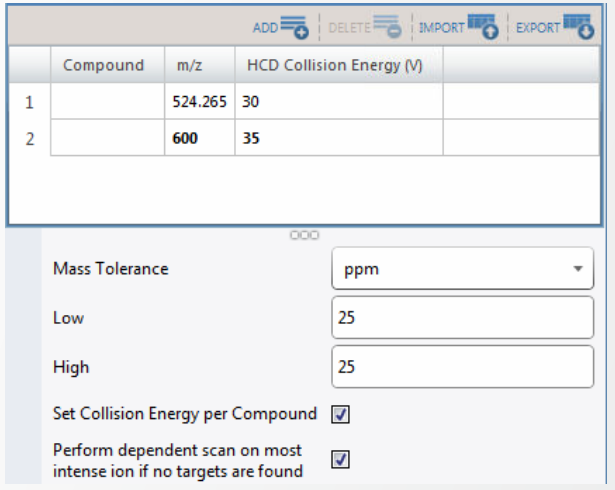
Support for Target Specific HCD CE in Targeted Mass Filter Table

Enabling More Flexibility in Directed Acquisition (DDA With Inclusion List)

Collision Energy Application		Case 1	Case 2	Case 3	Case 4
Set Collision Energy per Compound		FALSE ☐	FALSE ☐	TRUE ☒	TRUE ☒
Perform dependent scan on most intense Ions if no targets are found		FALSE ☐	TRUE ☒	FALSE ☐	TRUE ☒
Collision Energy Mode	**Targets (Table)**	Fixed OR Stepped for all targets	Fixed OR Stepped for all (targets & additional ions)	Fixed for all targets (greyed out)	Fixed for all (targets & additional ions) (greyed out)
	Additional Ions	n.a.		n.a.	
HCD Collision Energy Type	**Targets (Table)**	Absolute OR Normalized for all targets	Absolute OR Normalized for all (targets & additional ions)	Absolute OR Normalized for all targets	Absolute OR Normalized for all (targets & additional ions)
	Additional Ions	n.a.		n.a.	
HCD Collision Energy Value	**Targets (Table)**	In Scan Property: 1 (frozen) value applied to all targets OR 2-5 (frozen) values applied to all targets	In Scan Property: 1 (frozen) value OR 2-5 (frozen) values applied to all (targets & additional ions)	In Scan Property: "Defined in Table" -> In Table: 1 individual value per target	In Table: 1 individual value per target
	Additional Ions	n.a.		n.a.	In Scan Property: 1 (frozen) value applied to all additional ions







Support for Chromeleon

Enabling Orbitrap Tribrid Series ICSW 3.5 to run Under Chromeleon 7.2.10 MUd

- Aligned with Orbitrap Exploris Series ICSW 3.1
- Chromeleon CDS software can substitute Xcalibur to control Orbitrap Tribrid MS with GMP compliance with audit trail compatibility, allowing:
  - Mass spectrometer to be Tuned
  - Acquisition methods to be prepared
  - Acquisition to be performed from Tune page
  - Series of analyses to be run (Sequences)
- Fully tested with Orbitrap IQ-X and using Foundation 3.1 SP8
- Limitations
  - AcquireX functionalities are not supported by Chromeleon
  - Tune page needs to be opened from Chromeleon when Chromeleon is installed

Thank you

https://planetorbitrap.com/instrument-control-software