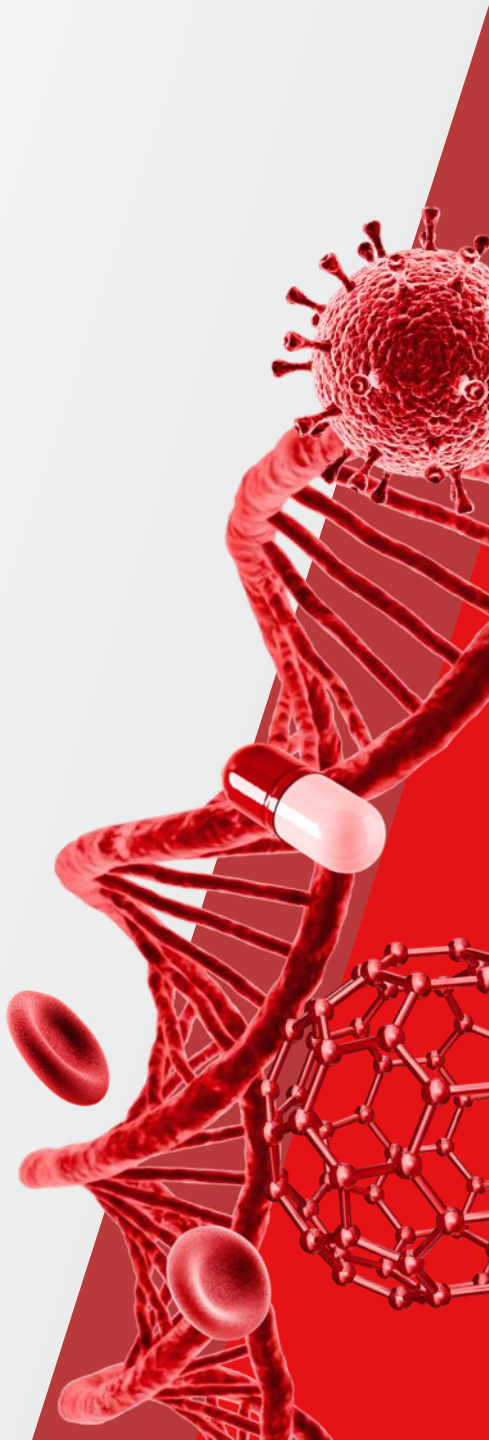
Thermo Scientific™ Orbitrap™ Tribrid™ MS Series: Instrument Control Software v.4.0 Overview

Updated With Defect Fixes in SP1

March-2023



The world leader in serving science

Software Release

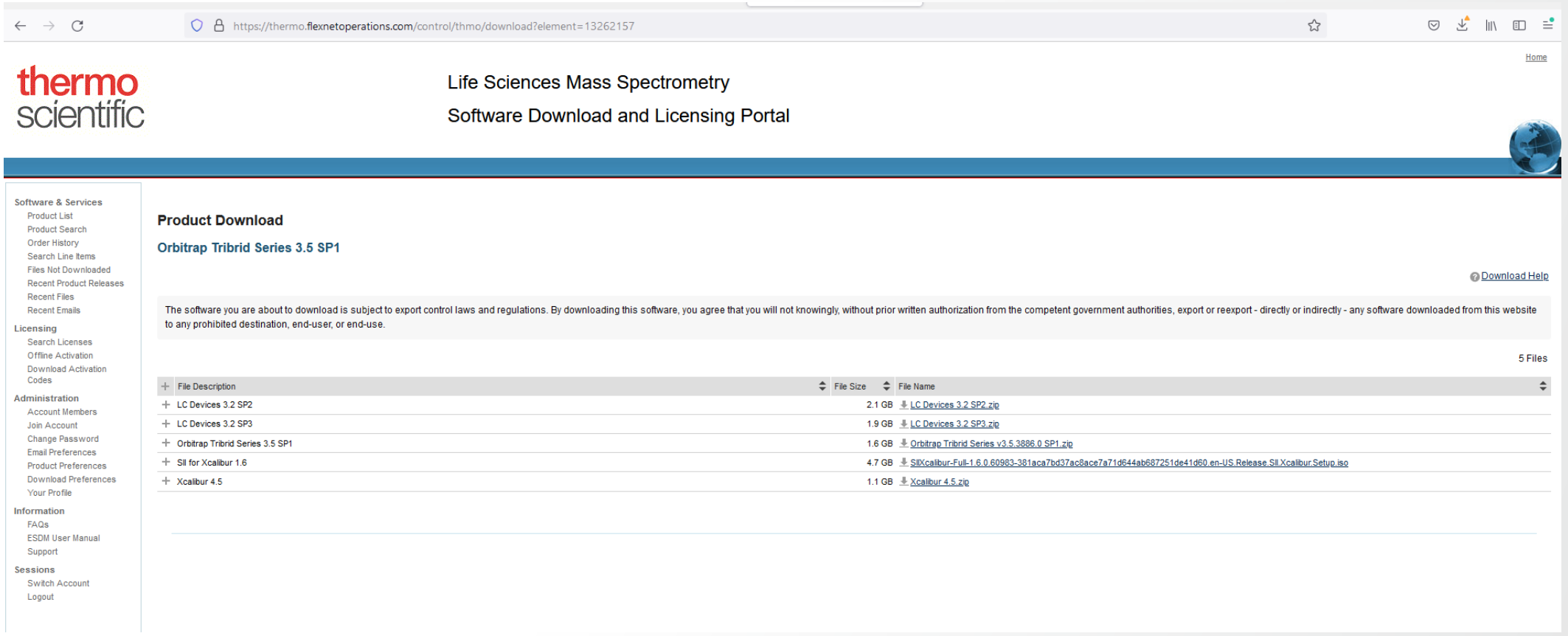
Flexera

Orbitrap Tribrid MS Series ICSW 4.0 SP1 is available to customers using Flexera software distribution site.

Customers new to the Flexera site should use the following link:
https://thermo.flexnetoperations.com/control/thmo/RegisterMemberToAccount

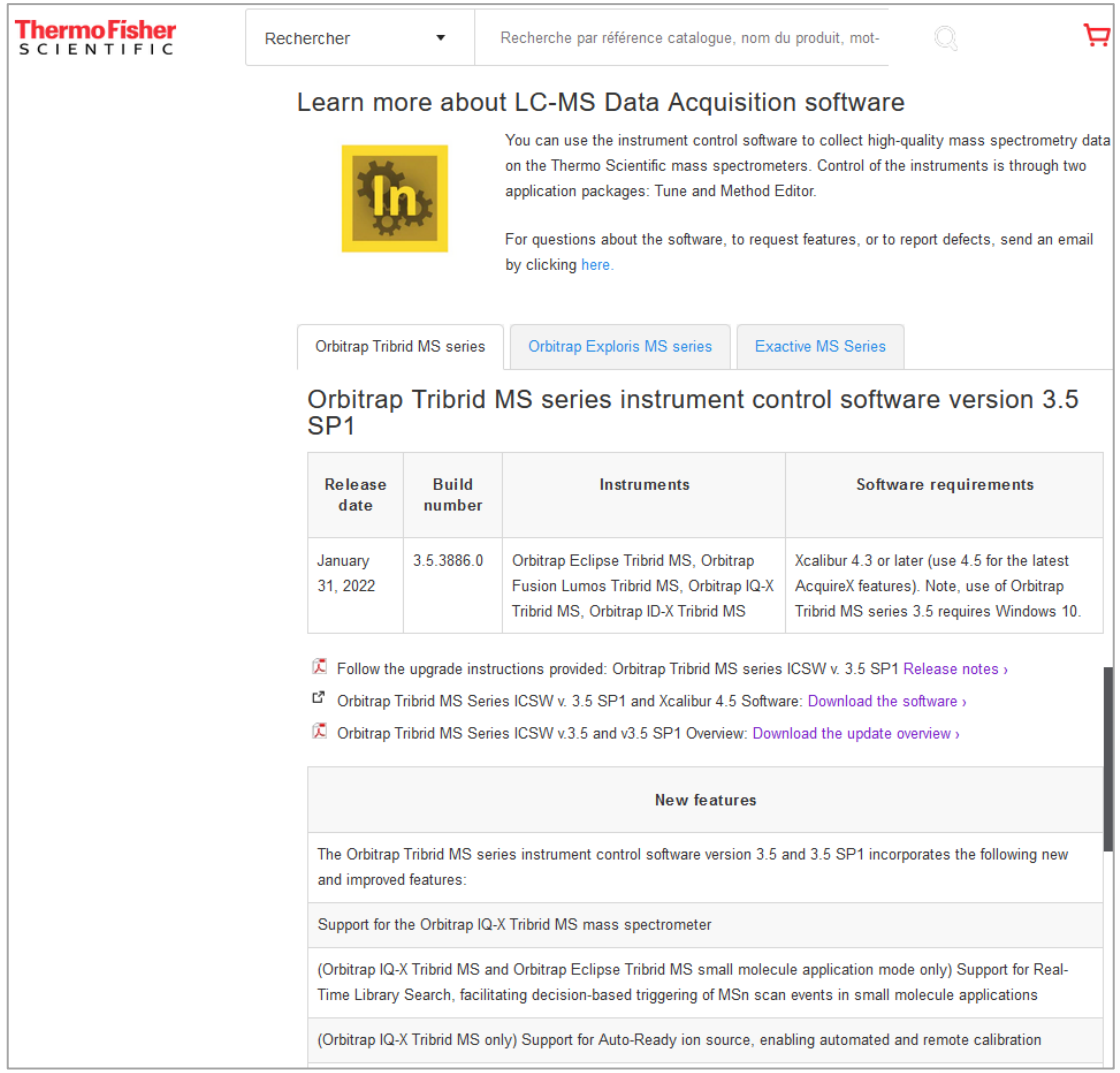
After setting up an account, customers can access the site using the following link:
https://thermo.flexnetoperations.com/control/thmo/login

In the ‘Product List’ page, find ‘Instrument – Orbitrap Tribrid Series’ and identify Orbitrap Tribrid Series 4.0 SP1 in the subfolder.



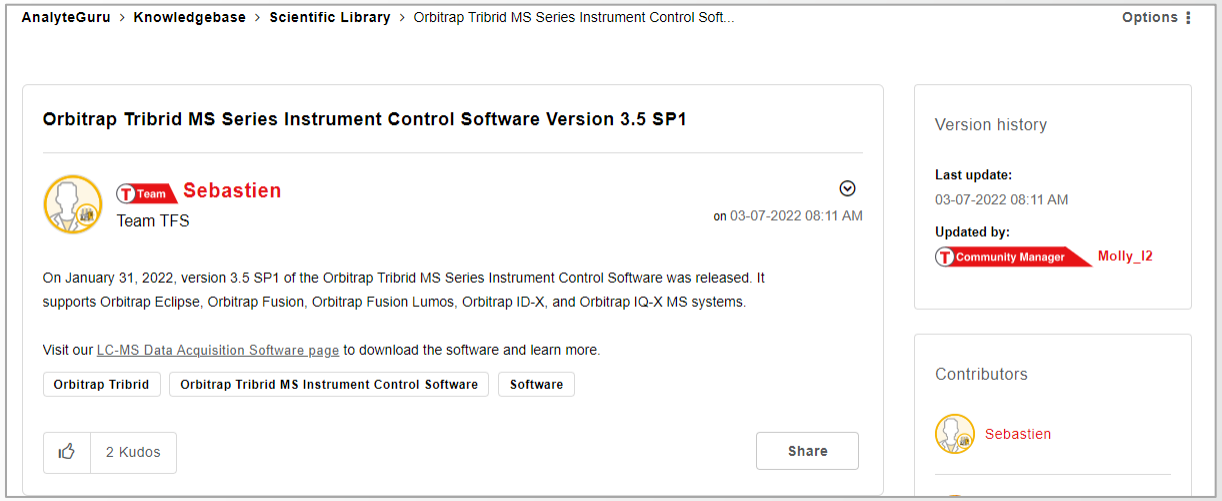
Software Release

Thermofisher.com & AnalyteGuru



- Updates: AnalyteGuru.com

  To receive focused updates, subscribe to the pertinent labels (e.g., *Orbitrap Tribrid MS Instrument Control Software*)



- Information: Thermofisher.com
  - Software information
  - Links for download
  - New Features
  - Known Issues
  - Discovered issues
  - Fixed Defects

Orbitrap Tribrid Series Instrument Control Software v 4.0

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

Thermo Scientific™
Orbitrap Fusion™ MS

Thermo Scientific™
Orbitrap Fusion™ Lumos™ MS

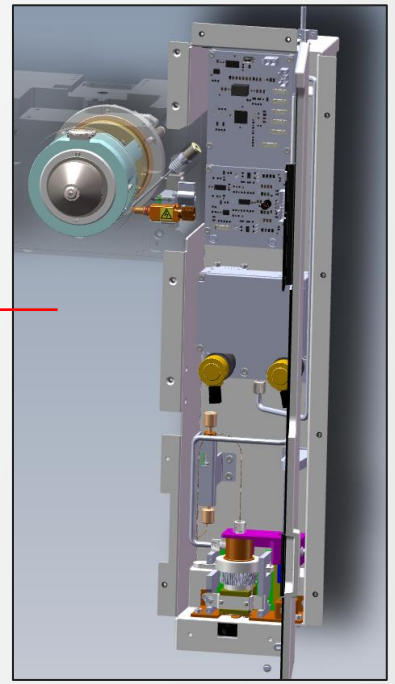
Thermo Scientific™
Orbitrap Eclipse™ MS

Thermo Scientific™
Orbitrap ID-X™ MS

Thermo Scientific™
Orbitrap IQ-X™ MS

Orbitrap Tribrid Series Instrument Control Software v 4.0

NEW Orbitrap Ascend Equipped with Auto-Ready Ion Source



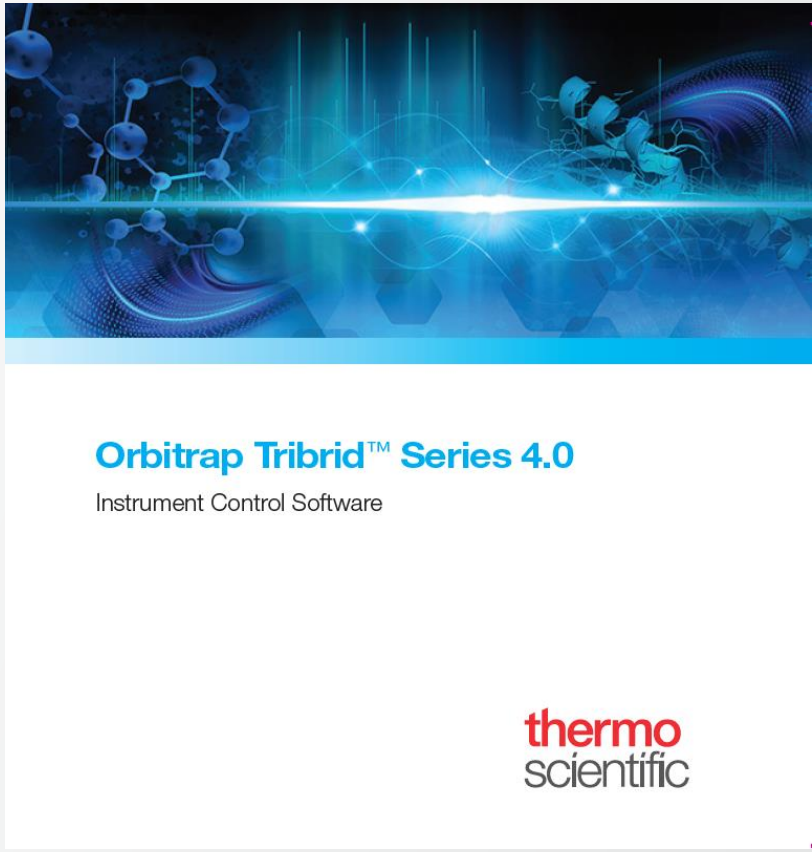
Auto-Ready Ion Source

Thermo Scientific™ Orbitrap Ascend Tribrid™ Mass Spectrometer

New Features And Usability Enhancements Summary

Orbitrap Tribrid Series Instrument Control 4.0

- Support for the Orbitrap Ascend mass spectrometer
- (Orbitrap Ascend, already available for Orbitrap IQ-X) - Support for Auto-Ready ion source
- (Orbitrap Ascend/Eclipse/IQ-X) Real-Time Library Search is now available for both peptide and small molecule application
- (Orbitrap Ascend/Eclipse) New Real-Time Search functionalities.
- Support for the new AcquireX Ab workflow for peptide mapping through the Xcalibur data system 4.6
- Updated accepted mass ranges and isolation ranges across Tribrid models
- Usability enhancements
  - Refined management of tables in DIA scan
  - Expanded multiplexing to up to 20 ions in msx tSIM/tMS2/DIA scans
  - Enabled acquisition with Dynamic Retention Time functionality in combination with FAIMS
  - New/Updated/Modified templates (including CHIMERYS and AcquireX Ab)
- Support for Chromeleon



Resolved Issues in OTS 4.0 SP1

- **Resolved Issues between OTS 4.0 and OTS 4.0 SP1**

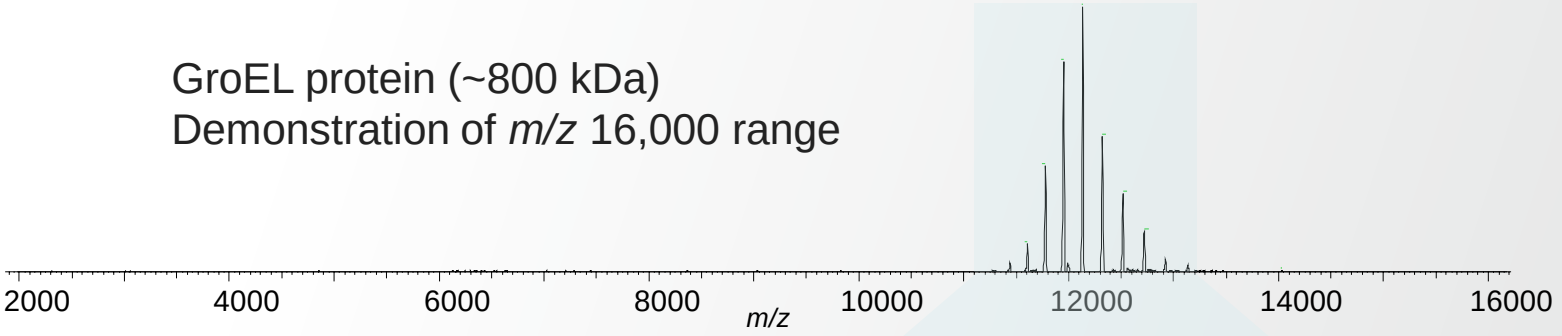
- Addressed an issue in which injection time may oscillate during LC-MS runs of intact proteins on Orbitrap Ascend system
- Addressed an issue in which Discharge Pressure Check fails due to readback error on Orbitrap Fusion ETD system
- Modified accepted range for energy dependency on Orbitrap Ascend system
- Addressed incorrect application of isolation waveforms in MS^n experiments when ion trap isolation is used for more than one stage
- Addressed an issue in which new changes in “Peak Selection and Threshold Settings” properties are not properly synchronized across multiple Real-Time Search filters after re-opening a method previously saved with “Use Common Peak Selection and Threshold Settings” option selected

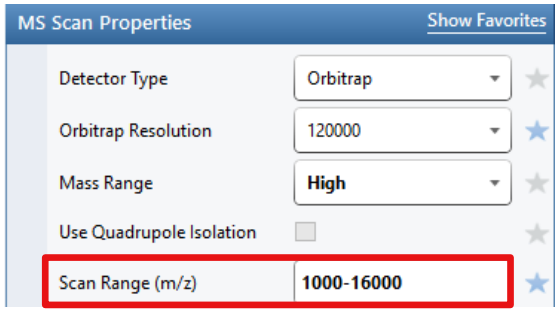
Support for Orbitrap Ascend Mass Spectrometer

Extended High Mass Range up to m/z 16,000

- Availability
  - HMR^n+ License required
  - Compatible with FTMS scans and advanced ion manipulation techniques including ETD, PTCR, UVPD
  - Parent isolation up to 8,000 *m/z* at MS2 and MSn levels

GroEL protein (~800 kDa)
Demonstration of *m/z* 16,000 range

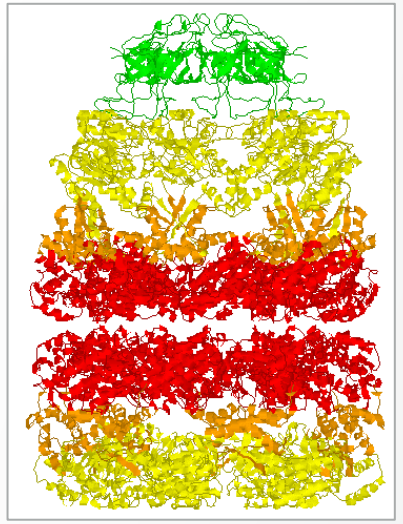




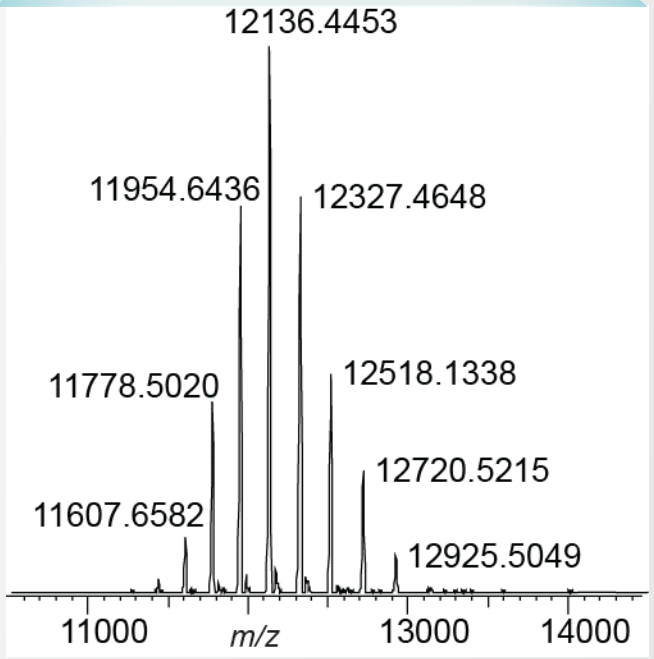
Specify the m/z range over which the ion trap or Orbitrap mass analyzer detects ions.
Range: 100–16000

Learn more...

The allowed scan range (in m/z units) depends on the selected mass range.
• Normal range: 50–2000
• High range:
100–4000 (Ion trap detector)
100–6000 (Orbitrap detector)
100-6000; 500-8000; 1000-16000 (HMR^n+ License Orbitrap Detector)
Tip: As the system optimizes transfer of ions for the defined mass range, it is recommended to restrict the scan range to the region of interest, using a first mass 1 m/z lower than the lightest ion of interest. In addition to the application mode-based defaults, the system templates have recommended application-specific scan ranges.



GroEL structure

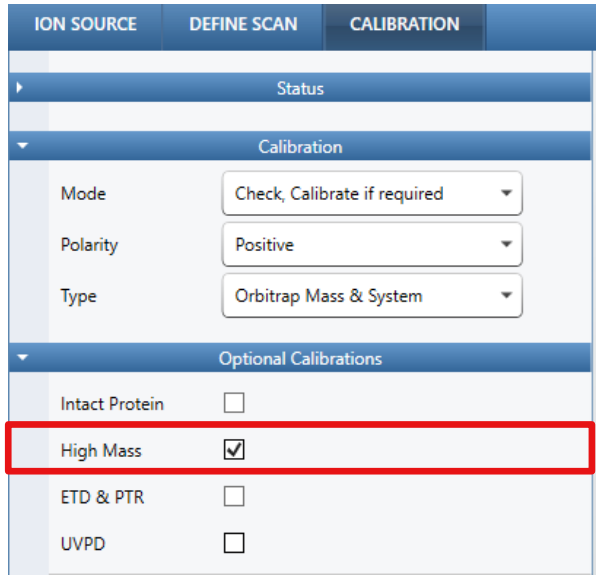


Support for Orbitrap Ascend Mass Spectrometer

Extended High Mass Range up to m/z 16,000 (HMR^n+ License Required)

- Orbitrap Ultra High Mass calibration
  - Run from Calibration Tab or Diagnostics
- Orbitrap Ultra High Mass calibration (AHFP)
  - Run from Diagnostics
  - Provides the best mass accuracy

- Running 'High Mass' optional calibration in Calibration tab overrides previous Ultra High Mass calibration with AHFP (if any)
- Orbitrap Ultra High Mass calibrations are also available for Orbitrap Eclipse with HMR^n License



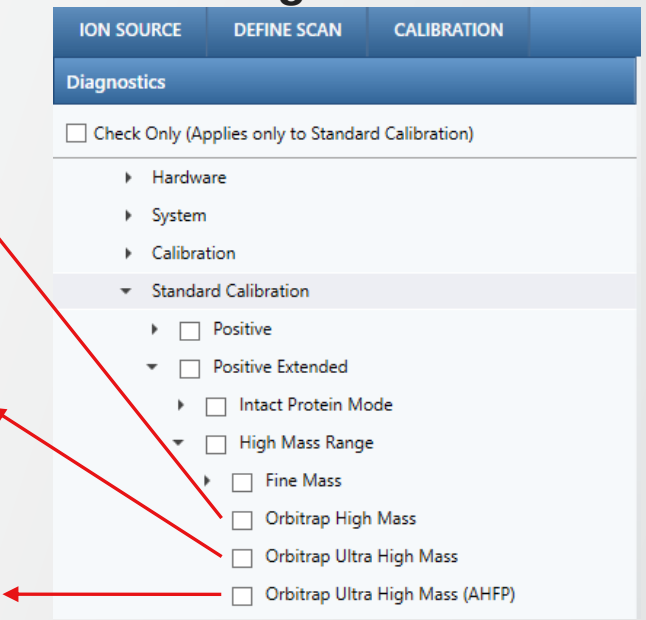
Calibration Tab

Orbitrap High Mass: This procedure runs the Orbitrap calibration in the high mass range up to m/z 4000 (use of FlexMix calibration solution)

Orbitrap Ultra High Mass: This procedure runs the Orbitrap calibration in the ultra high mass range above m/z 4000 (use of FlexMix calibration solution)

Orbitrap Ultra High Mass (AHFP): This procedure runs the Orbitrap calibration in the ultra high mass range above m/z 4000 (use of ammonium hexafluorophosphate – AHFP – solution)

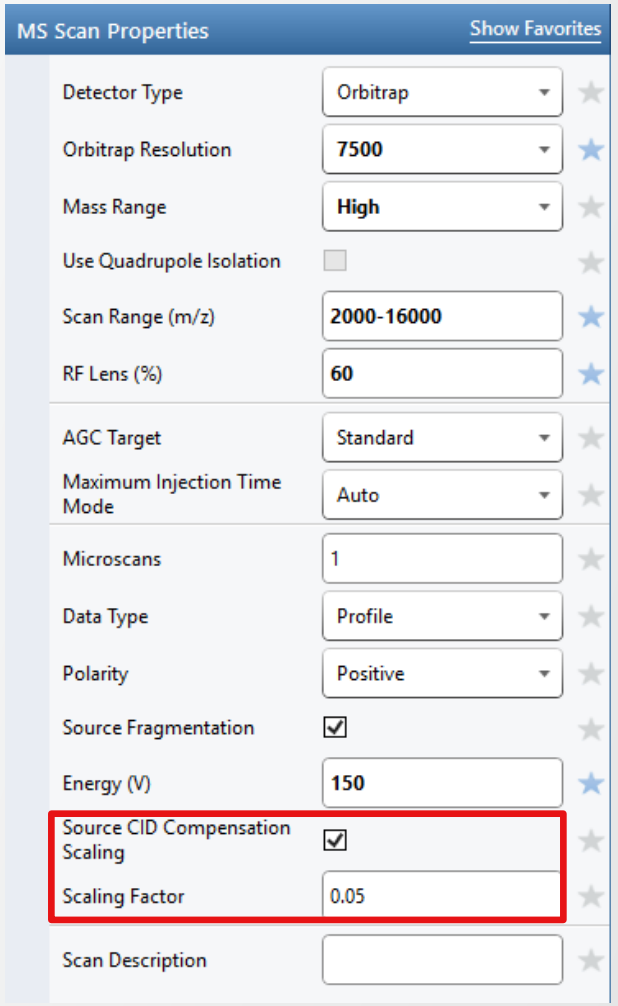
Diagnostics



Support for Orbitrap Ascend Mass Spectrometer

Source Compensation to Maintain Proper Transmission of Ions During Native Protein Analysis

- Enable proper transmission of ions through the front-end of the instrument when operating in Intact Protein Mode / High Pressure Mode and with Source CID enabled for ion declustering
- Moved from Diagnostics to Tune and Method Editor
- Conditions
  - HMR^n+ option activated
  - Application Mode set to Intact Protein
  - Pressure Mode set to High Pressure
  - Source Fragmentation applied
- Also available for Orbitrap Eclipse (with HMR^n option activated)
- Default Source CID Compensation Scaling Factor
  - Orbitrap Eclipse: 0.2
  - Orbitrap Ascend: 0.05
    (lower factor thanks to optics improvements translates into a better mass range)



Support for Orbitrap Ascend Mass Spectrometer

Improved Ion Management Through the Front Ion Routing Multipole Before the C-trap

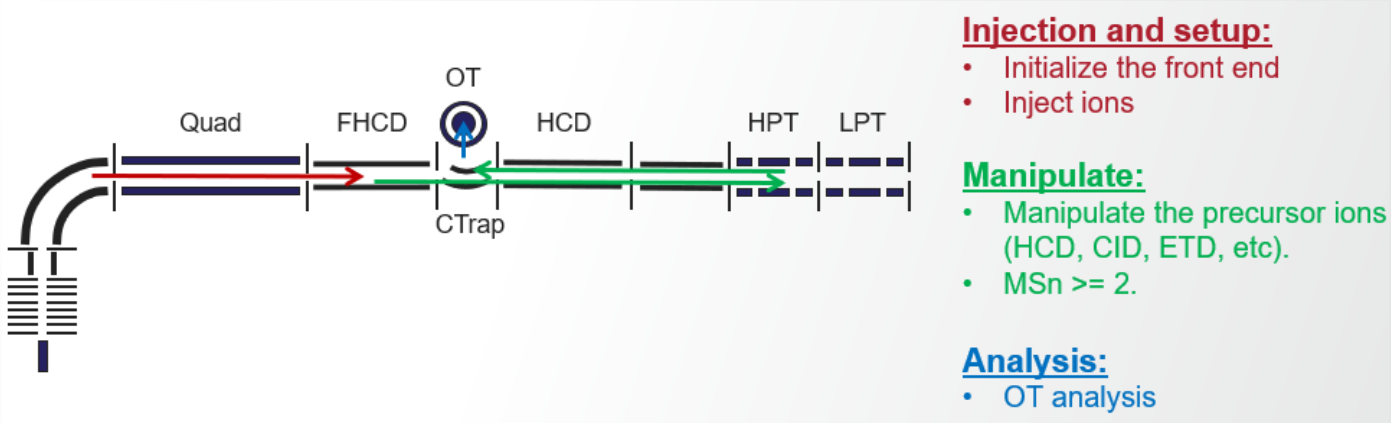
- Usage of Ion Routing Multipoles
  - Front Ion Routing Multipole is used for ion trapping/routing/HCD MS^2 (when isolating with quadrupole)
  - Back Ion Routing Multipole is for ion routing to OT of CID, ETD, UVPD, PTCR and MS^n HCD products

- Benefits

Additional FTMS ion accumulation time or increased FTMS scan rates at the same accumulation time

- Ion injection into the front IRM: allows ion injection while CTrap ejection, OT detection, ion manipulation, etc. are on-going
- The benefits increase with the complexity of the scan: CID, ETD, PTCR, UVPD, MSn

- Impact on acquisition method parameters
  - Transparent in general
  - Increased default Maximum Injection Time (Max parallelizable IT): 'Transient time - 5 ms' (instead of 'Transient time - 10 ms')

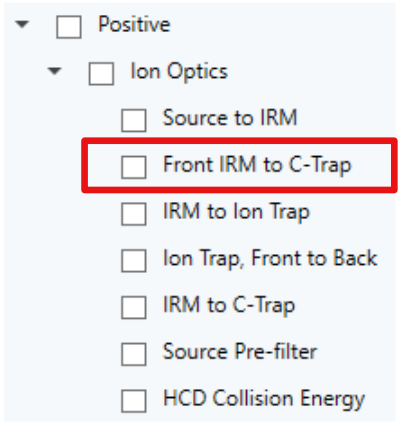


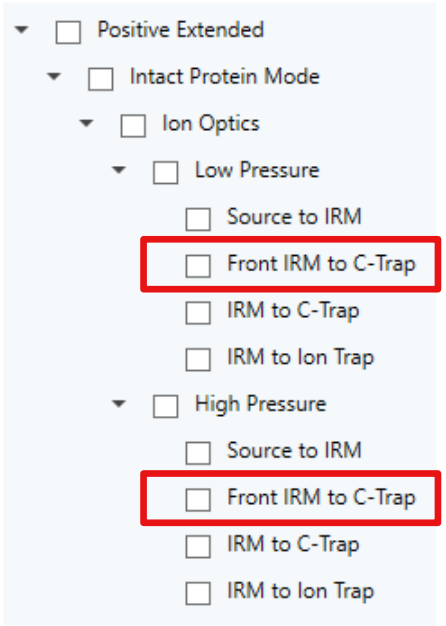
Support for Orbitrap Ascend Mass Spectrometer

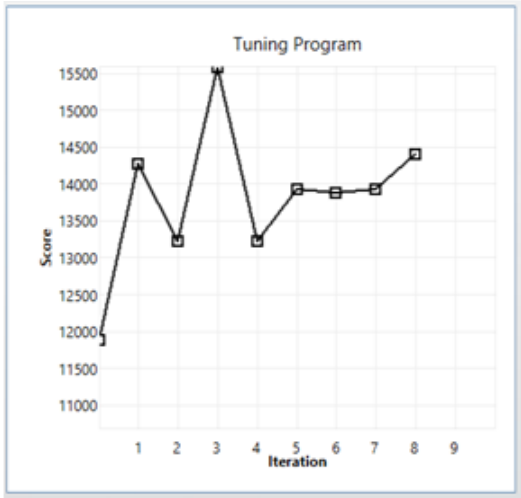
New calibrations to support the IRM before the CTrap

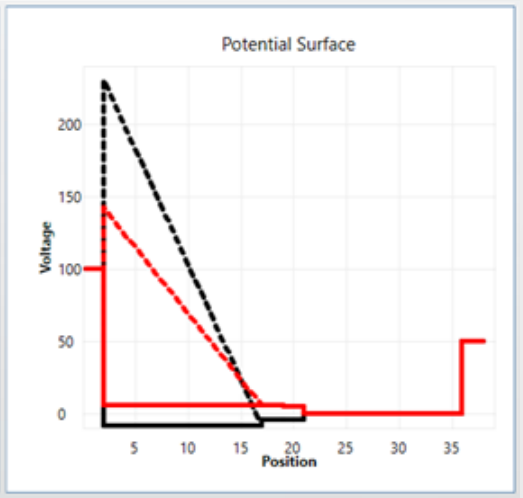
The FHCD<->CTrap transfer calibrations follows the same form as the CTrap<->HCD transfer calibration:

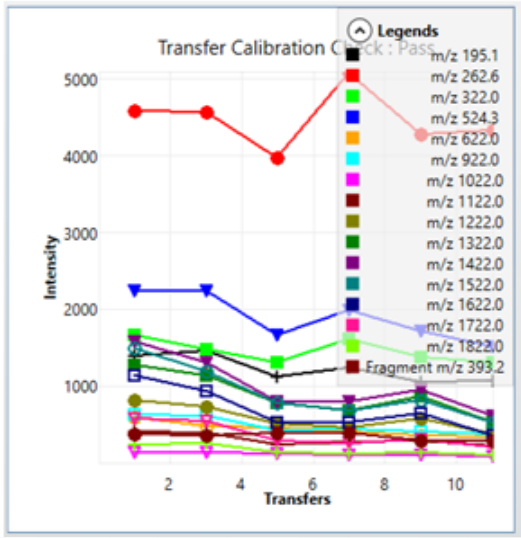
- An evolutionary algorithm that tunes up the critical devices.
- Flavors exist for positive mode, negative mode, low pressure, and high pressure

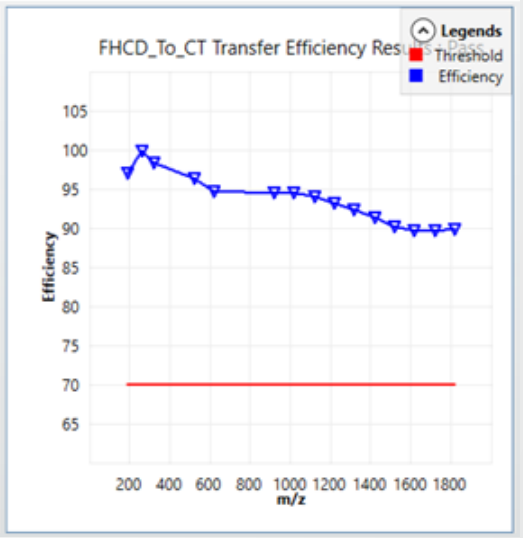












Support for Orbitrap Ascend Mass Spectrometer

New / Renamed Orbitrap Resolution Settings

Resolving power	Max Parallelizable Injection Time
7.5K	11 ms
15K	27 ms
22.5K	43 ms
30K	59 ms
45K	91 ms
60K	123 ms
120K	251 ms
240K	507 ms
480K	1019 ms

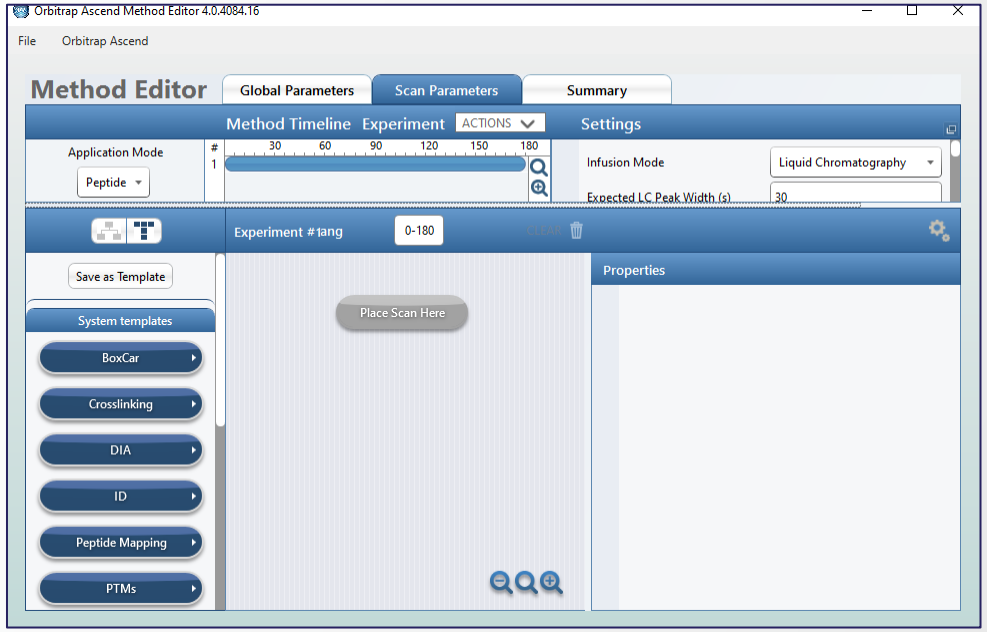
- Available for all scan types (ME and Tune)
- More Flexibility in Short LC Gradient Analysis
- Alignment with Exploris (*e.g.*, 45k vs. 50k RP – note transient length is the same)

Support for Orbitrap Ascend Mass Spectrometer

Impact of Hardware Changes on Acquisition Parameters

- Recommendations
  - RF lens (%): 40 for Small Molecule application mode
  - RF lens (%): 60 for Peptide and Intact Protein application modes
  - HCD Collision Energy (%): change from 30 to 25 for Peptide application Mode (unless other value specifically needed)

- Changes included in Ascend templates



Auto-Ready Ion Source

Support Expanded to Orbitrap Ascend

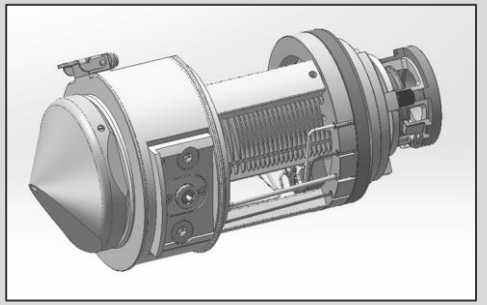
- Challenge
  - Time dedicated to instrument setup must be minimized
  - Instrument must remain well calibrated over time

- Solution
  - Built-in Auto-Ready Ion Source simplifies maintenance with weekly pre-scheduled, remote, and one-click calibration

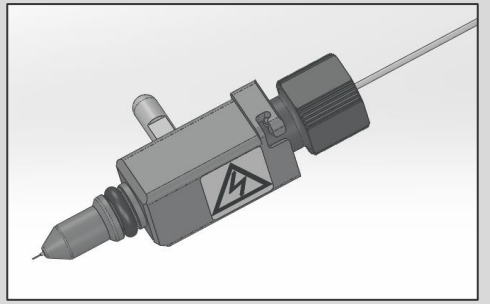
- Supported Models
  - Orbitrap Ascend *NEW*
  - Orbitrap IQ-X

Auto-Ready Calibration Source

Its own ion transfer tube



Dedicated emitter

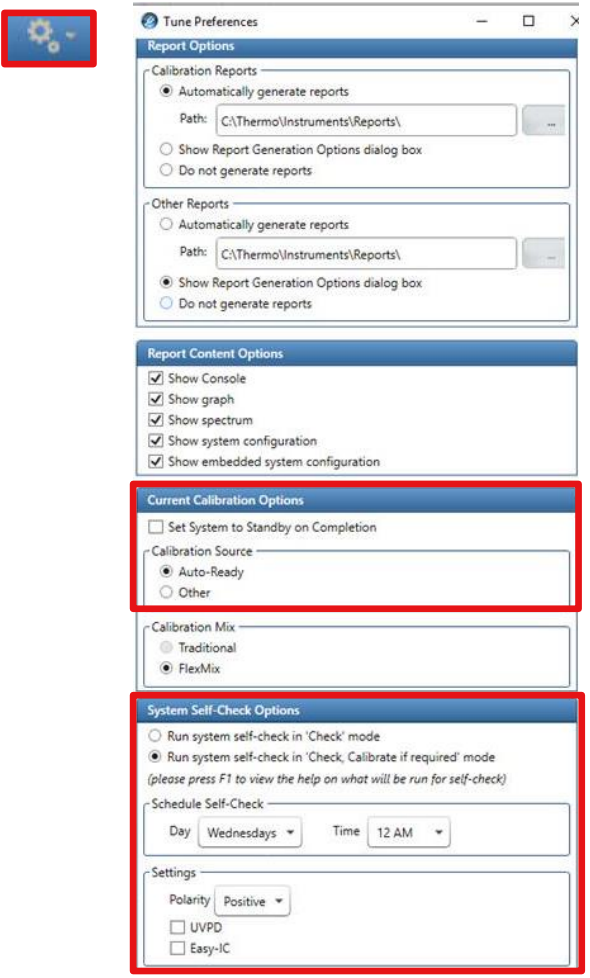


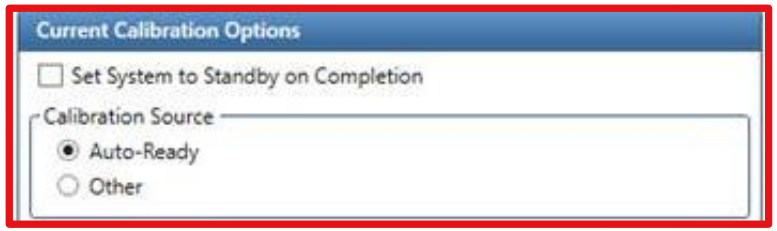
Robust delivery system

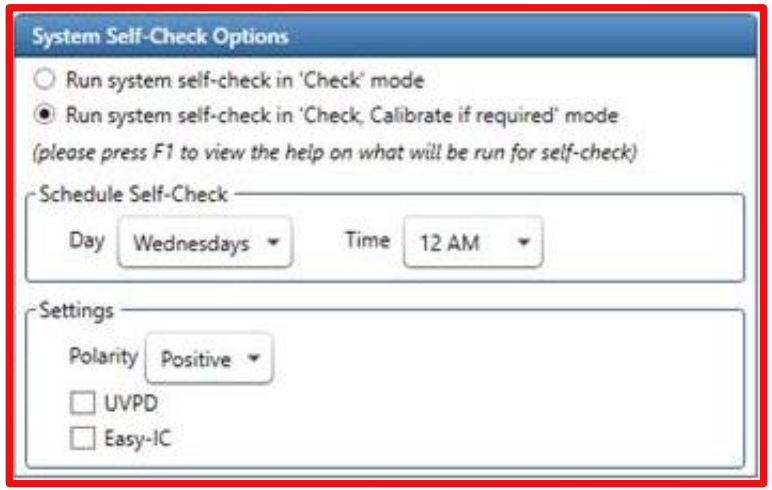
Auto-Ready Ion Source

Setting up Auto-Calibration

Under Tune Preferences







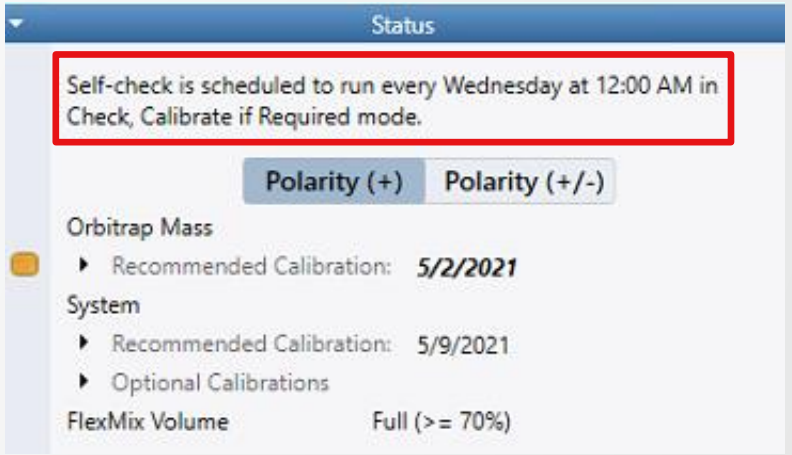
- Current Calibration Options
  - "Auto-Ready" is set as Calibration Source by default (auto and manual modes)
  - Select "Other" to calibrate with syringe pump (manual mode only)
  - Auto-Ready Ion Source only uses pre-filled FlexMix vials

- 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

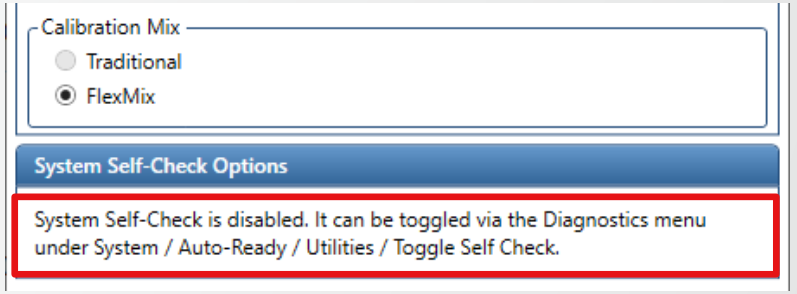
Auto-Ready Ion Source

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)

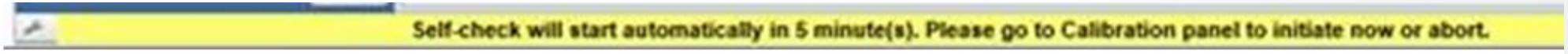


- Self-Check procedure is deferred and subsequently run if
  - There is an acquisition in progress (acquisition sequence or tune recording) at the time of scheduled Self-Check
  - Acquisition queue gets empty within the 24 hours of scheduled Self-Check (check every 5 min)

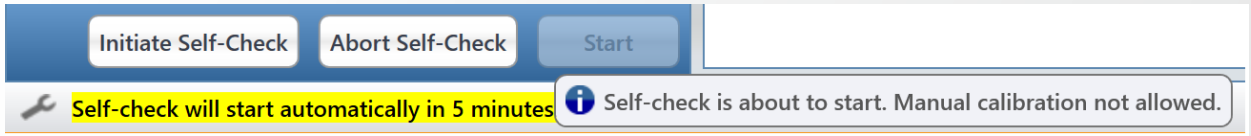
Auto-Ready Ion Source

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

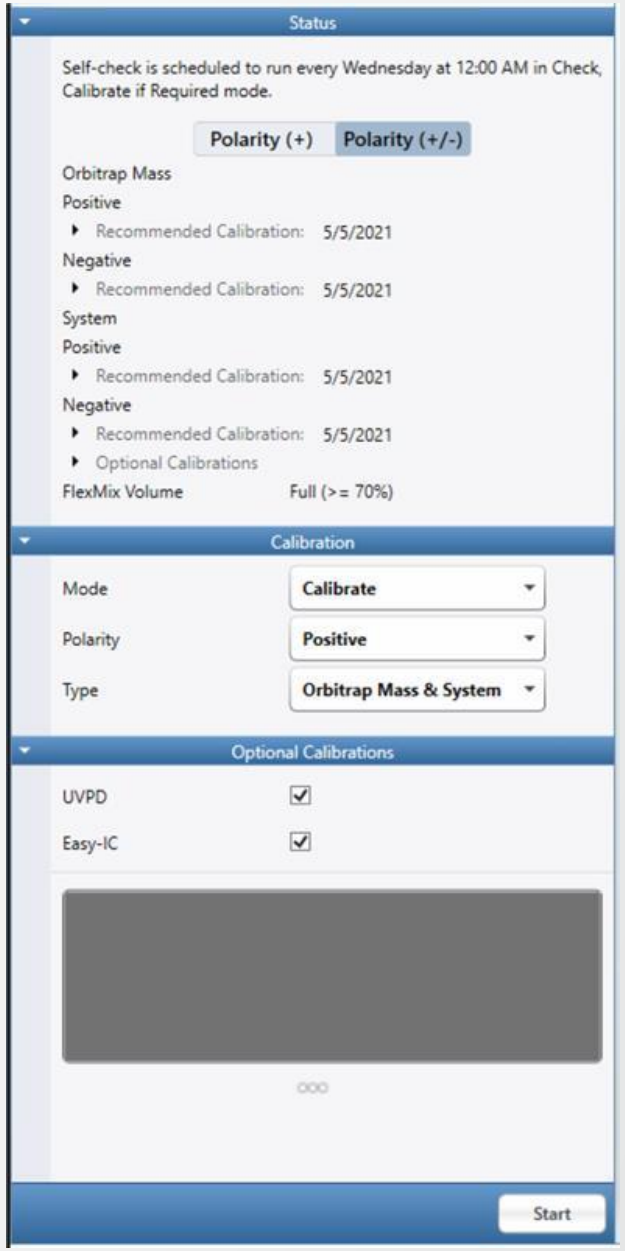


- Self-Check execution
  - Start after 5-min preparation has elapsed
  - Instrument is switched to « On » if currently in standby
  - Calibration UI selection updated to match Self-Check settings (mode, polarity, and optional calibrations)
  - During Self-Check execution
    - Tune operations are disabled, 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

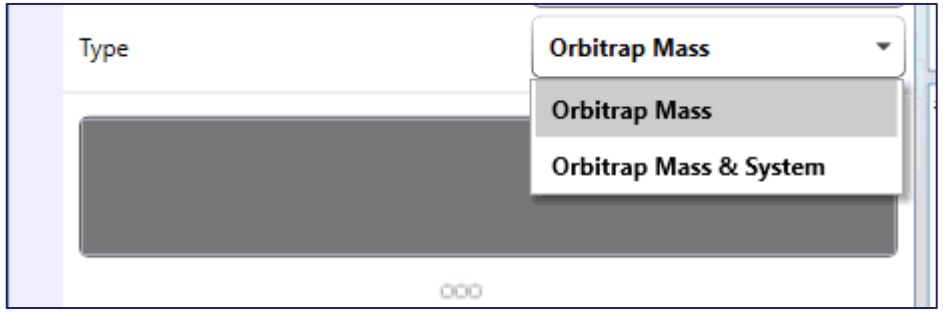
- « Manual » conventional one-click calibration
  - Can be performed at any time by pressing « Start » in Calibration tab
  - All conventional calibration modes and types are available
- Auto-Ready Ion Source is used for « Manual » one-click calibration as long as it is defined as the Calibration Source 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

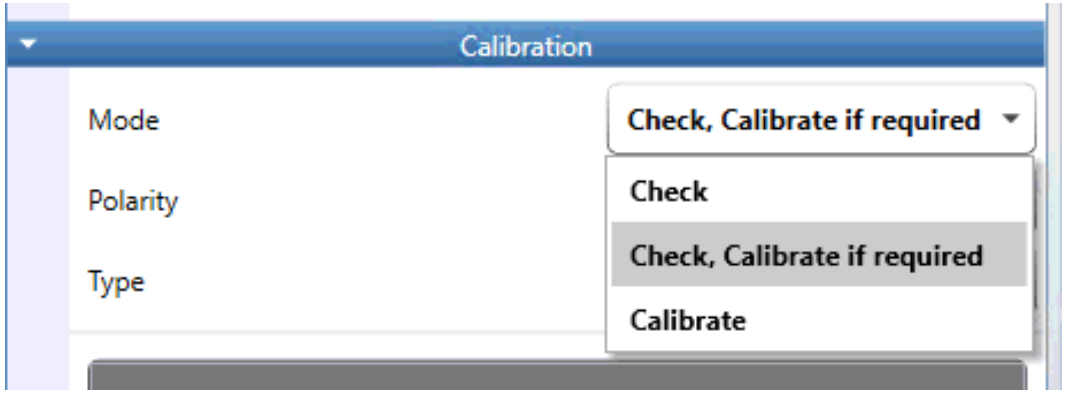


- Types: **Orbitrap Mass** or **Orbitrap Mass & System**
- Selecting Orbitrap Mass triggers the following calibrations:
  - multiplier
  - Orbitrap mass
- Selecting Orbitrap Mass & System triggers the following calibration set, in order:
  - Ion Optics
  - Ion Trap
  - Quadrupole
  - pAGC
  - Orbitrap 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



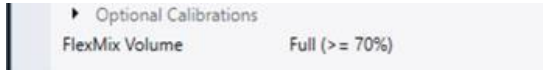
- Modes: “Check”, “Check, calibrate if required”, or “Calibrate”
  - A spray check and calibrant purity evaluation are automatically run prior to any calibration set
- “Check, calibrate if required” operates as follows:
  - Any failed checks are automatically calibrated (as well as dependent calibrations)
  - 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

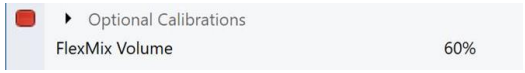
Same as with Conventional source

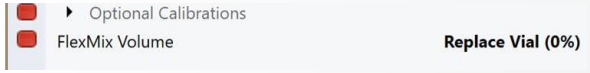
Auto-Ready Ion Source

Calibration Tab

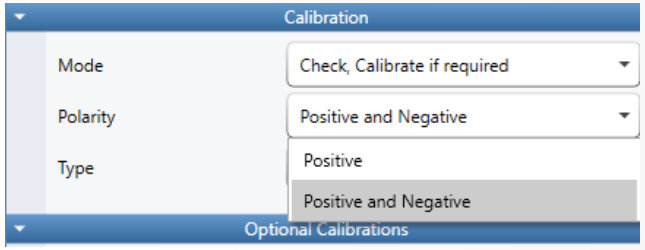
- Calibration status are displayed for polarity (+) or (+/-)
- FlexMix level is indicated in status panel
  - “Full (>=70%)” when >=2.8 mL
  - 5% decrement starting at 70%
  - 10% when >=400 µL
  - “Replace Vial (0%)” when <=125 µl
  - Red indicator is displayed when 0% - start button is disabled, hover text : ‘Replace Vial’
  - Orange indicator is displayed when 10% - 5%
  - No color indicator when above 10%

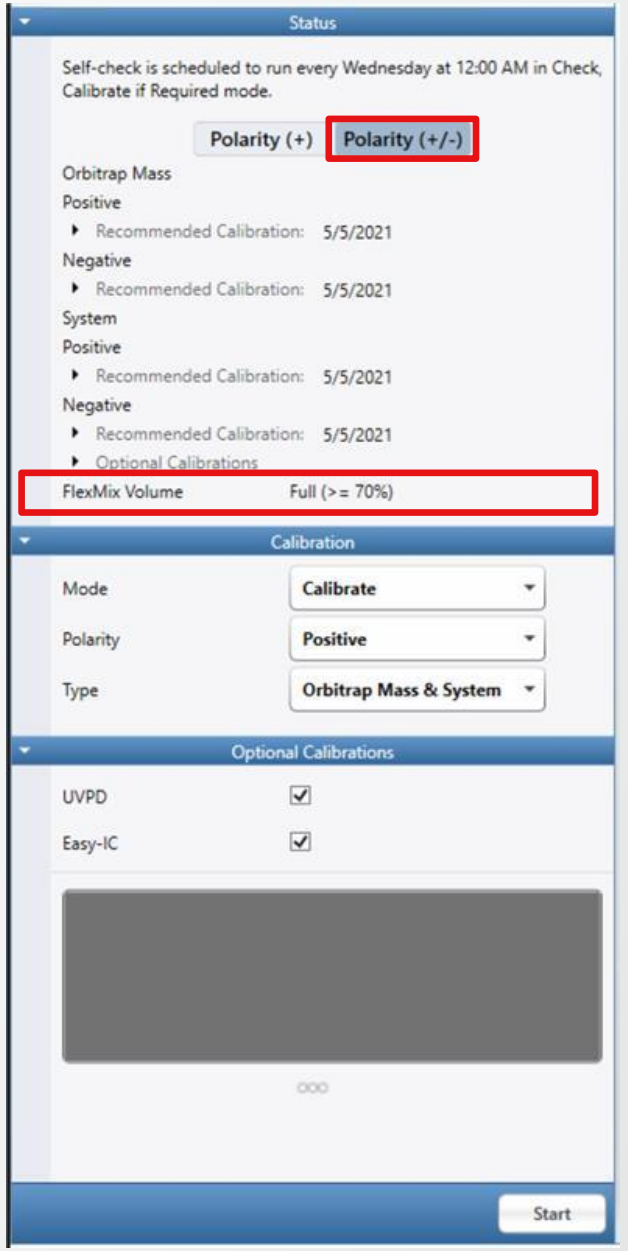






- Calibration Polarity:
  - Positive
  - Positive and Negative





Auto-Ready Ion Source

Calibration Results – Self-Correction And Recommendations

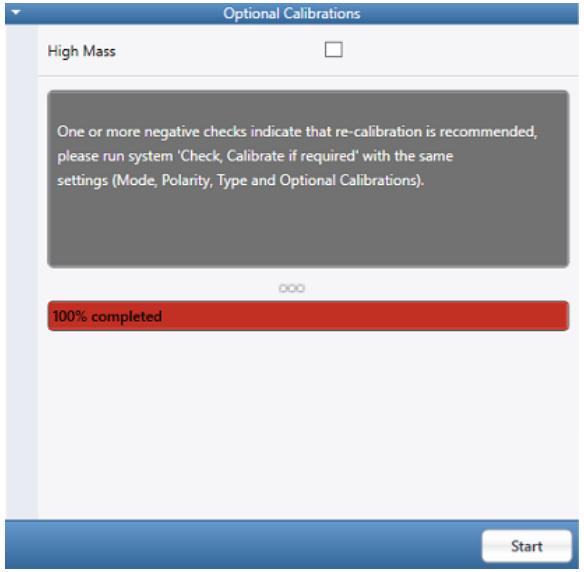
- Upon any calibration failure, two procedure checks are run before the result is reported:
  1. Spray stability check
     - If spray check fails, system will attempt to self-correct spray issue for 5 min. Once stable spray is re-established, the sequence will resume starting from the procedure that originally failed
     - If spray cannot be recovered, the check is aborted and the following notifications appears:

       ***Spray is unstable. Please perform following tasks and retry**:*
       - *Confirm that the calibrant vial has not run out of liquid. If necessary, install a new bottle and run the 'New Vial Installed' diagnostic*
       - *Run the 'Flush Lines' diagnostic (under 'Auto-Ready') to clear any residual air bubbles from the lines*
       - *Run the 'Calibrate Spray Voltage" diagnostic (under 'Auto-Ready') to ensure the spray voltage is set properly*
       - *Re-seat the fittings between (a) the flow sensor and the sprayer and (b) the flow sensor and the pump*
     - 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:

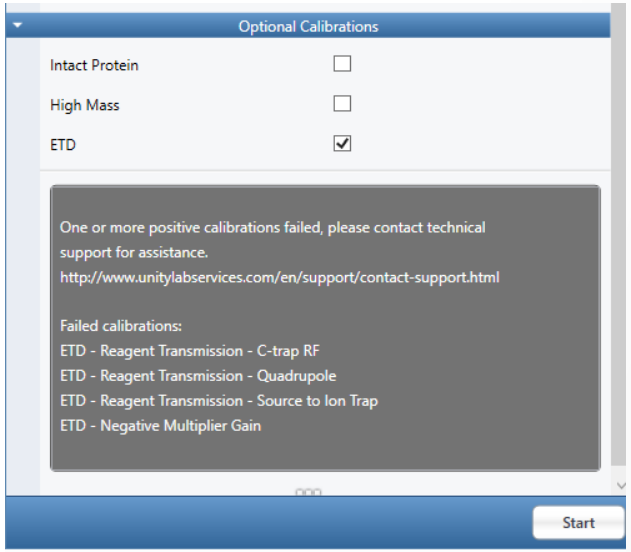
       Calibration solution contaminated or degraded. Please perform following tasks and retry:
       - *Run the 'Flush Lines' diagnostic (under 'Auto-Ready') in attempt to clear any contamination buildup from the lines*
       - *Change the calibrant bottle to a fresh one*

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.

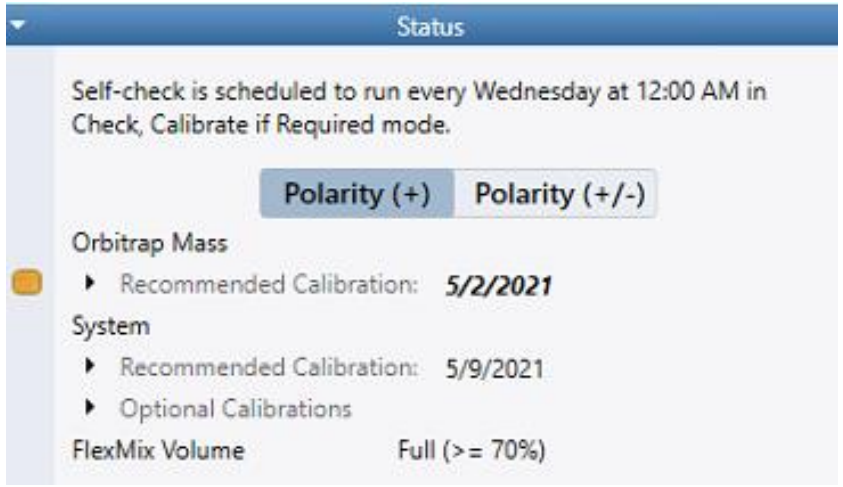


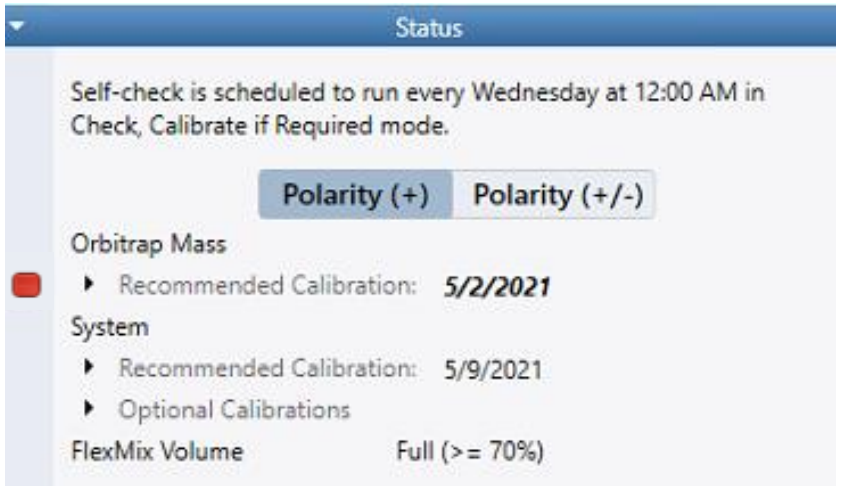
- If the calibration is run in ‘Check, Calibrate if required” or “Calibrate” mode and fails, but both procedure checks pass, a true calibration failure is recorded.

Same as with Conventional source

Auto-Ready Ion Source

Calibration Results – ‘Orange’ vs ‘Red’ Status

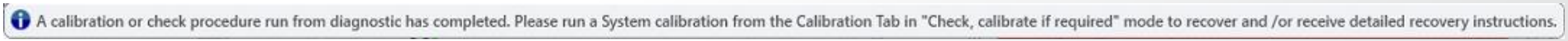




- Status will show an orange indicator light when:
  - A procedure failure (spray stability or calibrant purity evaluation) is recorded during a calibration procedure
  - A single calibration is run from diagnostics
  - A single check is run from diagnostics and fails
  - A procedure is aborted

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

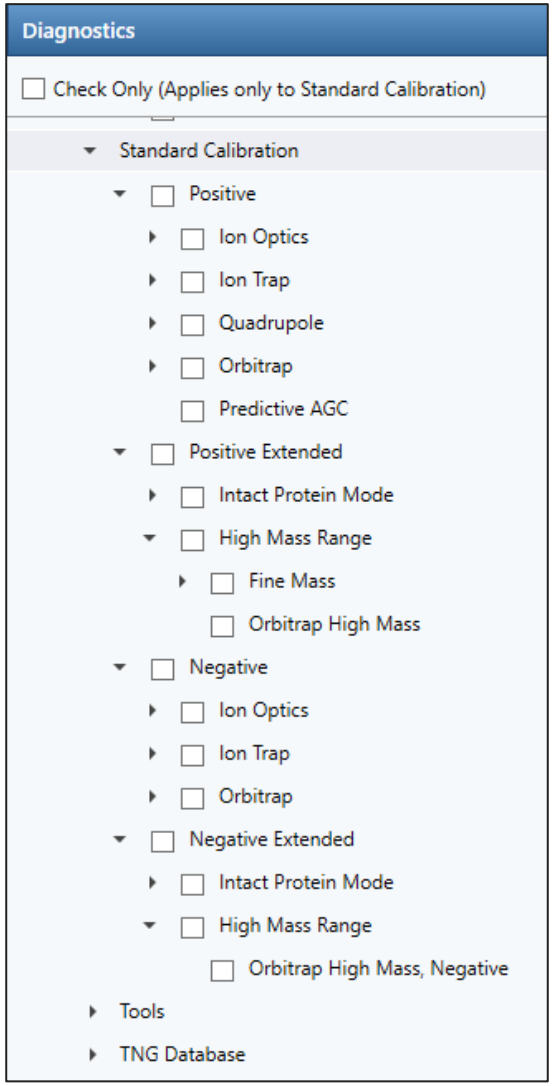
- Hovering over indicator light will provide explanation of state



Same as with Conventional source

Auto-Ready Ion Source

Calibration Procedures in Diagnostics



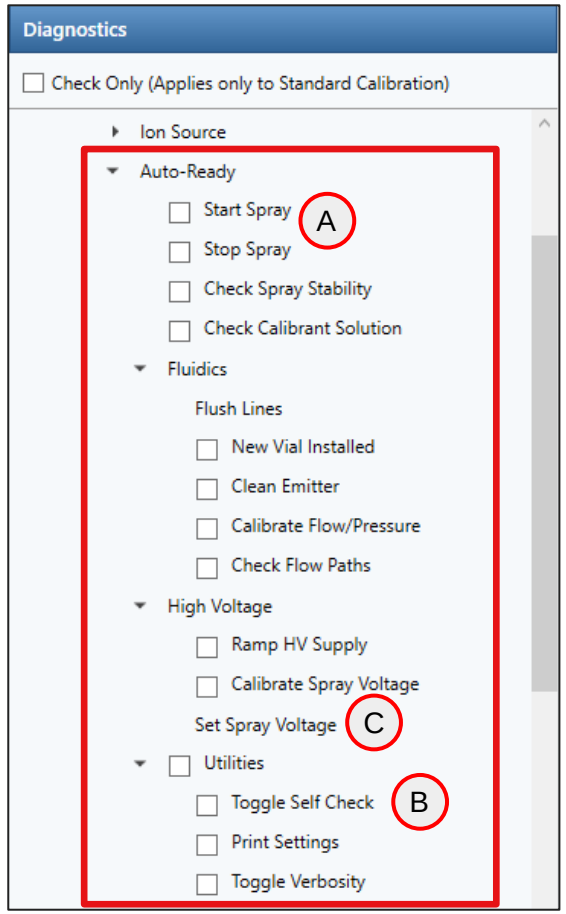
- Calibration tree can be found in the customer diagnostics pane under "Standard Calibration"
  - Individual calibrations may be checked or run
  - As the system requires calibrations to be run in the proper order as a set and some calibrations affect dependent calibrations, calibrating from the diagnostics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

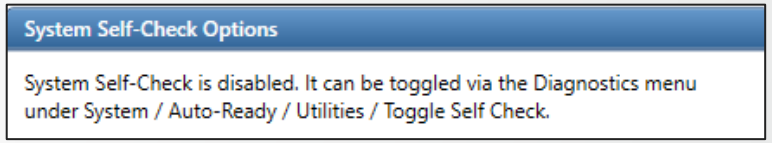
Diagnostics & Readbacks



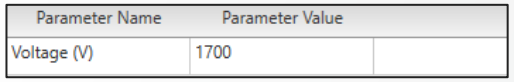
- Auto Ready Ion Source Diagnostics

A FlexMix infusion using Auto Ready must be manually started to run a diagnostic that requires it

B Toggle available to enable / disable Self-Check

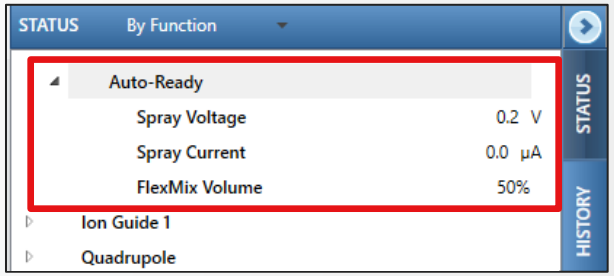


C 'Set Spray Voltage' diagnostics enables a specific value to be manually set, as an alternative to 'Calibrate Spray Voltage'



NEW

- Auto Ready Ion Source Readbacks

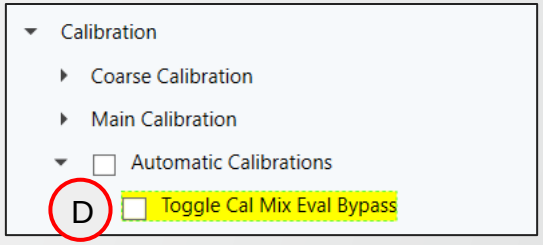


- Calibration Diagnostics

D Toggle available to turn off Calmix evaluation (spray check and calibrant purity evaluation) when calibration run from Calibration Tab (not only for Auto Ready)

(Note: it is recommended keeping evaluation On)

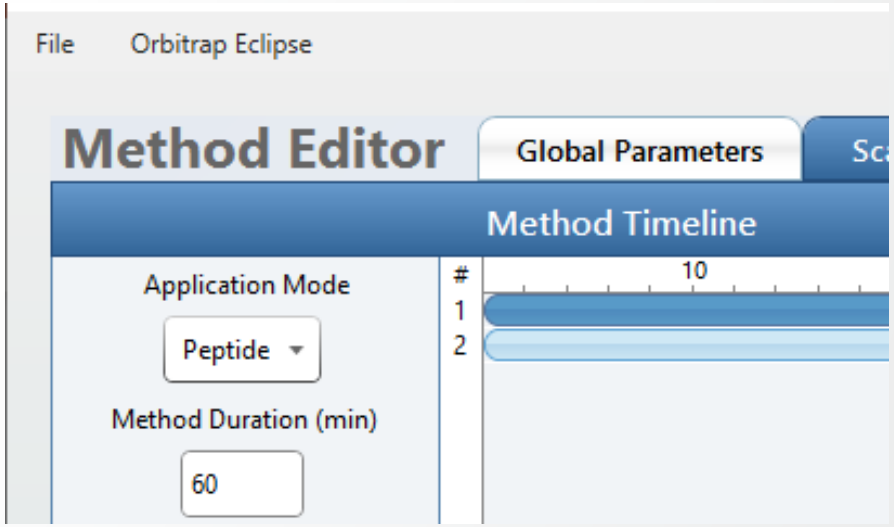
NEW



Support for Real-Time Library Search

New Functionalities to Improve / Expand Data-Driven Acquisition Schemes

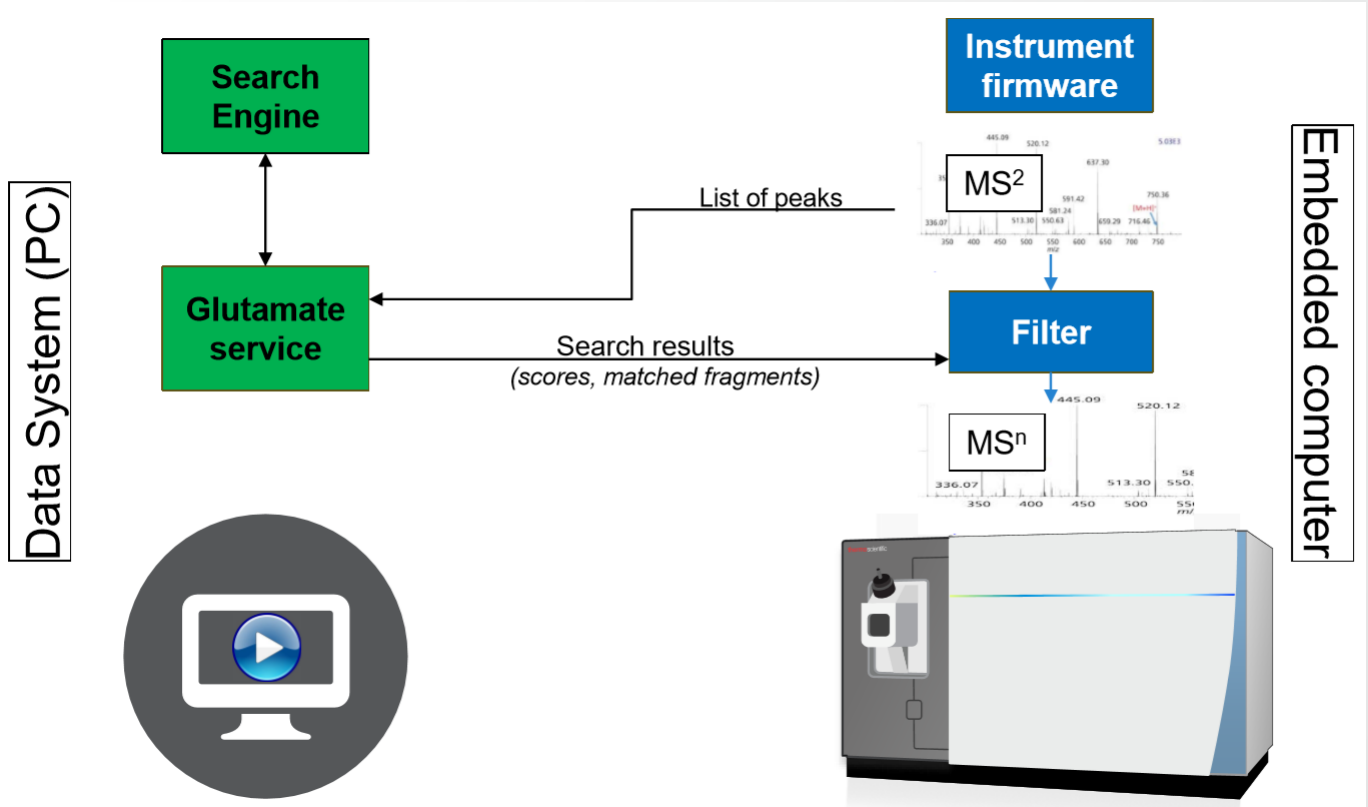
- Supported Models
  - Orbitrap Ascend *NEW*
  - Orbitrap Eclipse
  - Orbitrap IQ-X
- Supported Application Modes
  - Peptide *NEW*
  - Small Molecule
- Novelties *NEW*
  - Structure Filter Properties
  - New searching and filtering functionalities
  - Supported Acquisition scheme (compatible scans, placement constraint, combination of filters)



Support for Real-Time Library Search

Background Services & Communication

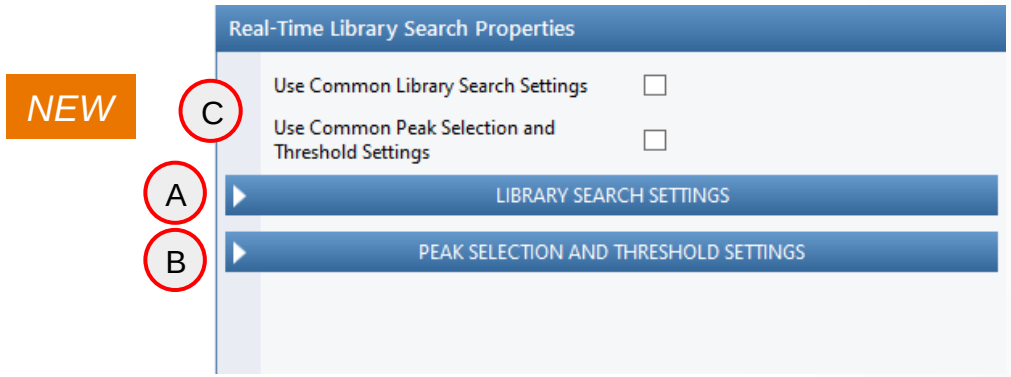
- Glutamate backend service used to process each generated MS2 scan
- Search results returned based on RTLS filter parameters
- Triggering of MSn scan events according to search results, method scheme, and filter thresholds

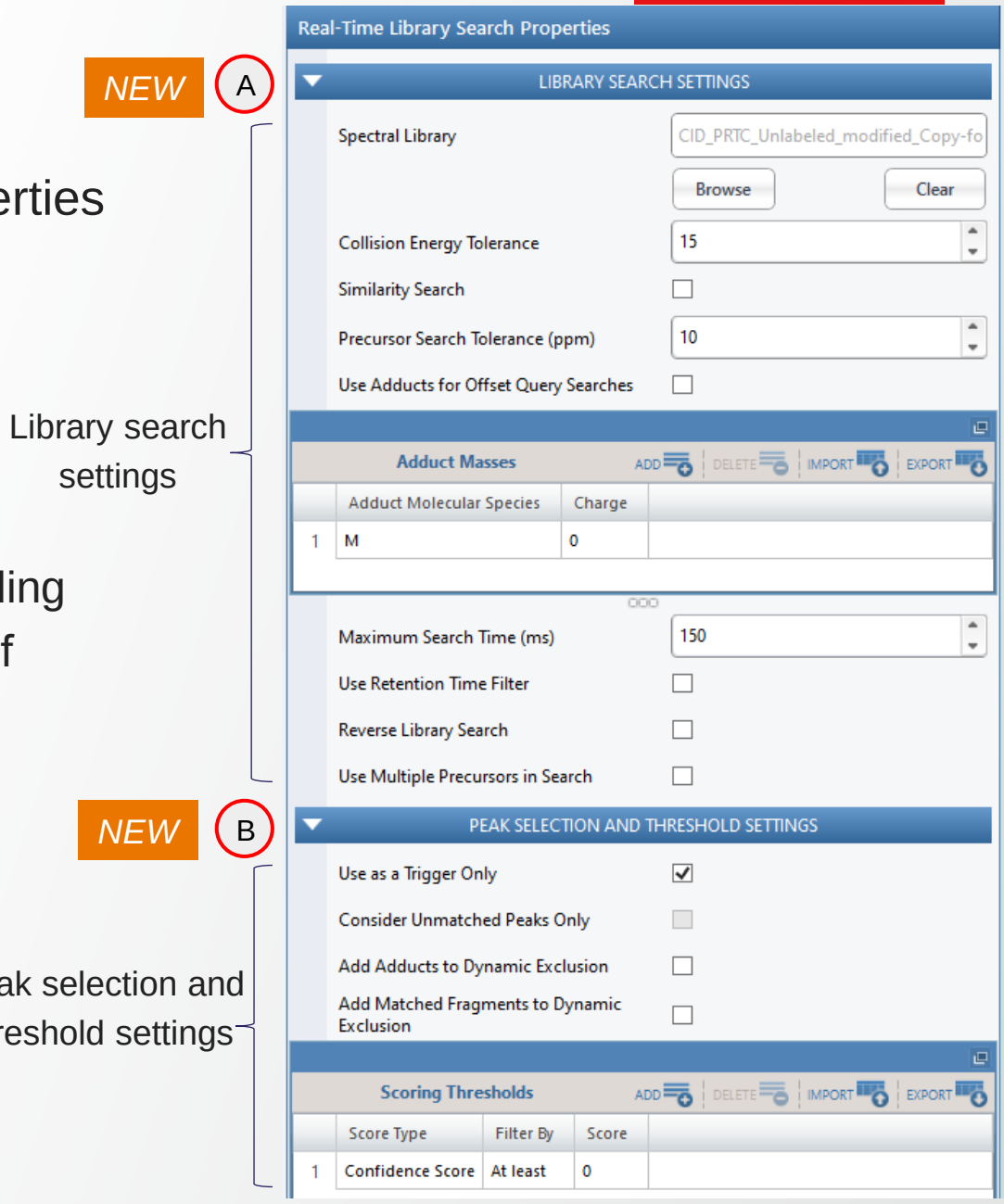


Support for Real-Time Library Search

Filter Overview

- Two expandable / collapsible sections of RTLS filter properties
  - A • Library Search Settings
  - B • Peak Selection and Threshold Settings
- Settings defined in each section can be
  - Specific to each instance of the filter
  - C • Synchronized across other instances of the filter if corresponding ‘Use Common … Settings’ control is enabled (only displayed if several instances)





Support for Real-Time Library Search

Configurable Library Search Settings

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

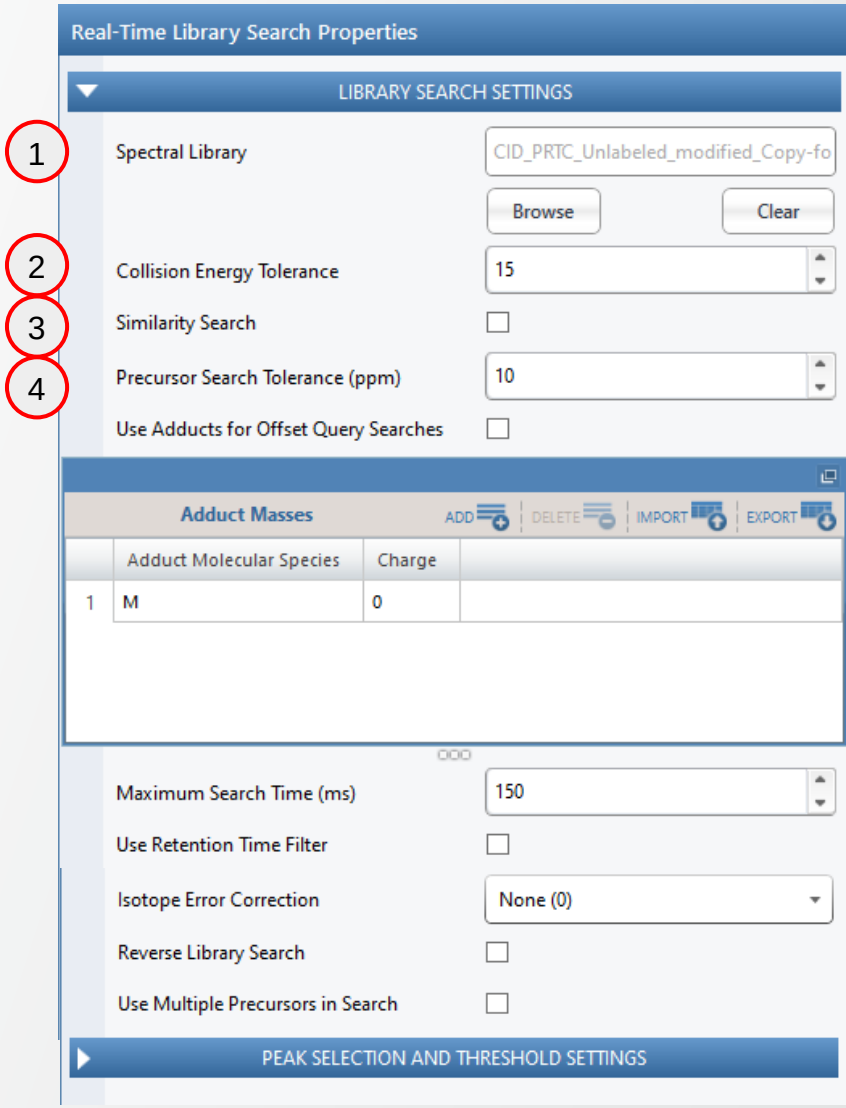
  (Note: Spectral Libraries must be in .db format)

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

- Similarity Search (3) *NEW*
  - Similarity search is performed instead of an identity search
  - Spectral library search does not take into account precursor m/z information
  - When enabled, Precursor Search Tolerance is not displayed as it is not relevant

- Precursor Search Tolerance (ppm) (4)
  - In ppm, +/- value for filtering the spectral library candidates during search
  - Applies to the MS2 event preceding RTLS placement

(Note: Fragment Search Tolerance is hardcoded and set to +/- 0.5 m/z units for IT and +/- 10 ppm for OT)



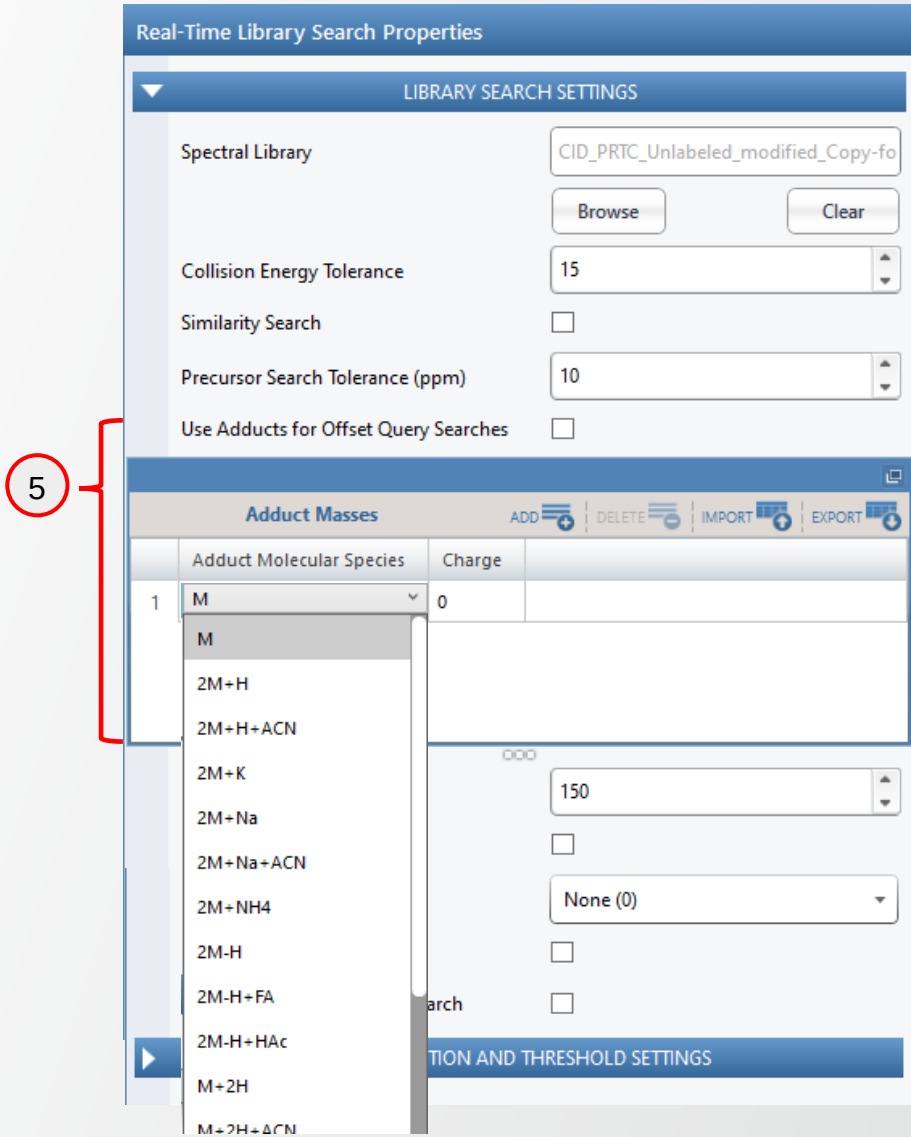
Support for Real-Time Library Search

Configurable Library Search Settings

- Use Adducts for Offset Query Searches (5)
  - Adduct Offset Query functionality may help to identify adducted compounds if an entry does not exist for that molecular species in the database (only unadducted form present in the library).

- Adduct Masses (table) (5)
  - Prepopulated list of adduct ions (taken 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)

- Only available in Small Molecule application mode



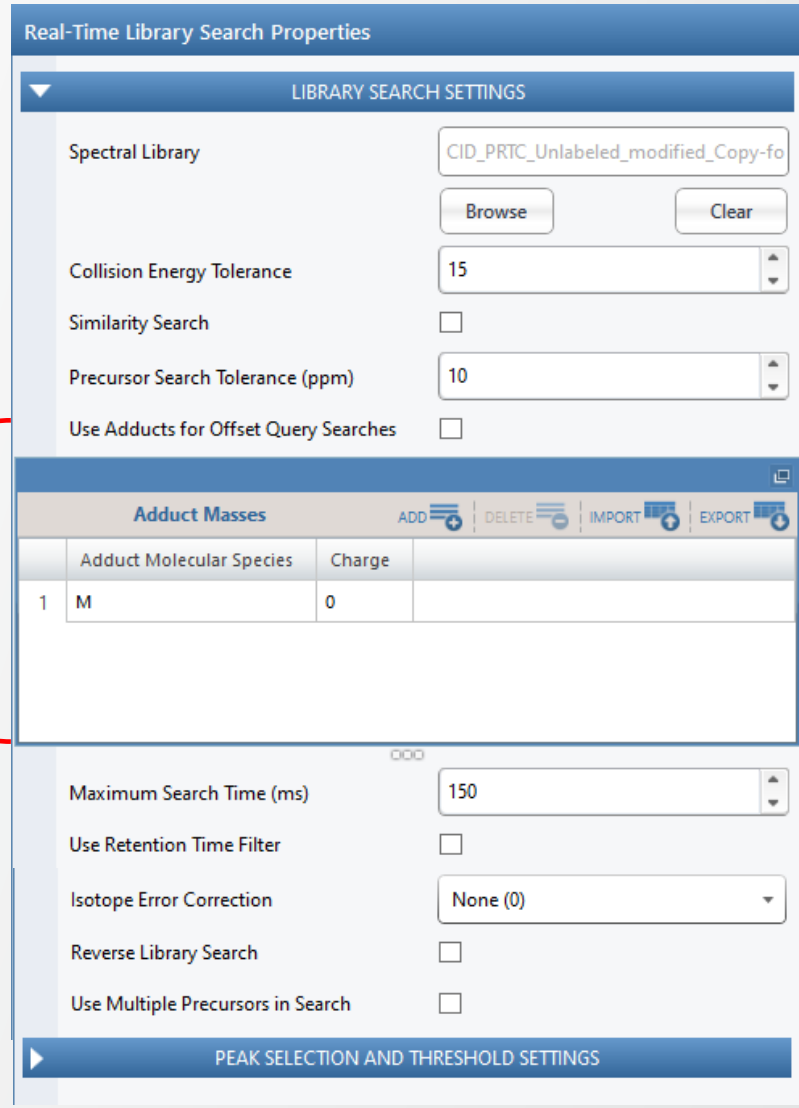
Support for Real-Time Library Search

Configurable Library Search Settings

- Special Adduct Masses (definition) (5)
  - "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)

- Only available in Small Molecule application mode



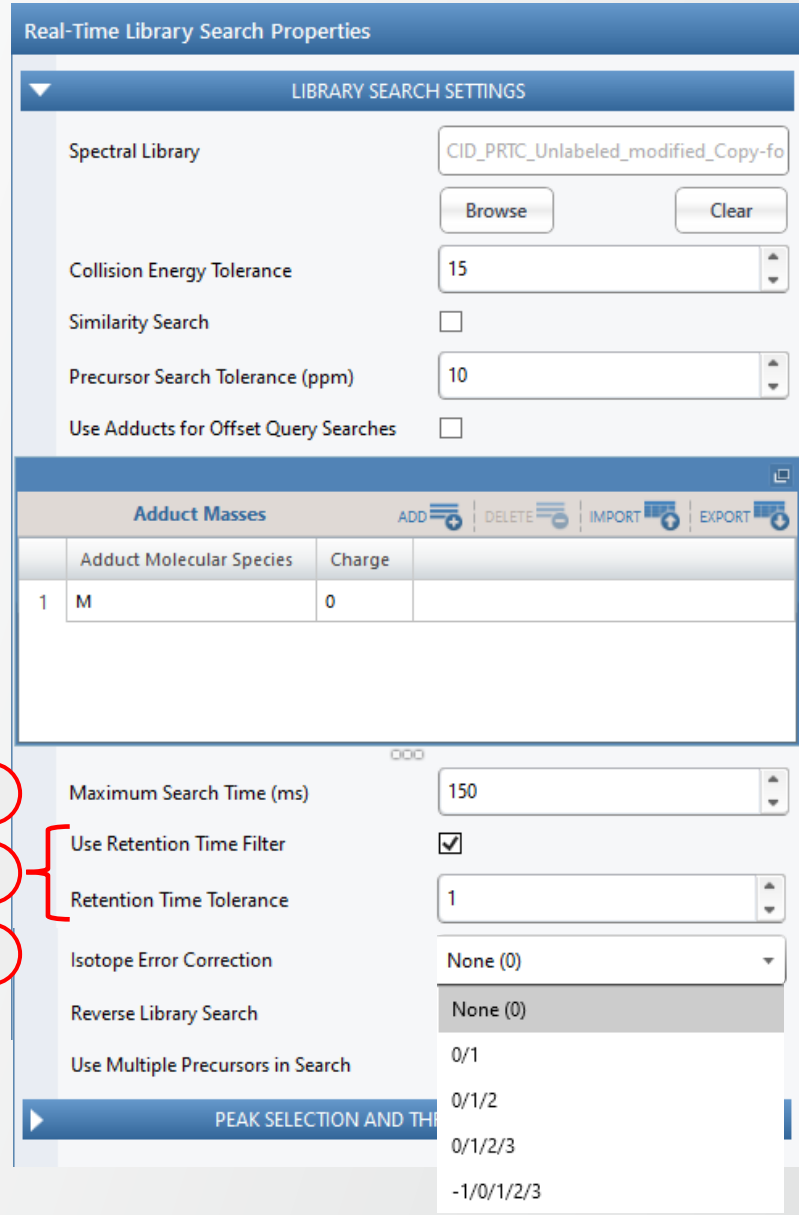
Support for Real-Time Library Search

Configurable Library Search Settings

- Maximum Search Time (ms) (6)
  - 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

- Retention Time Filtering (7) *NEW*
  - Use Retention Time Filter: spectral library candidates are filtered by the current retention time in the run (with tolerance)
  - Retention Time Tolerance: in min, +/- value for filtering the spectral library candidates based on current retention time in the run

- Isotope Error Correction (Peptide application mode only) (8) *NEW*
  - Spectral library search is performed according to the experimental precursor m/z (defined as monoisotopic peak) but also with preset C13 offsets (if any)
  - May overcome incorrect monoisotopic peak assignment

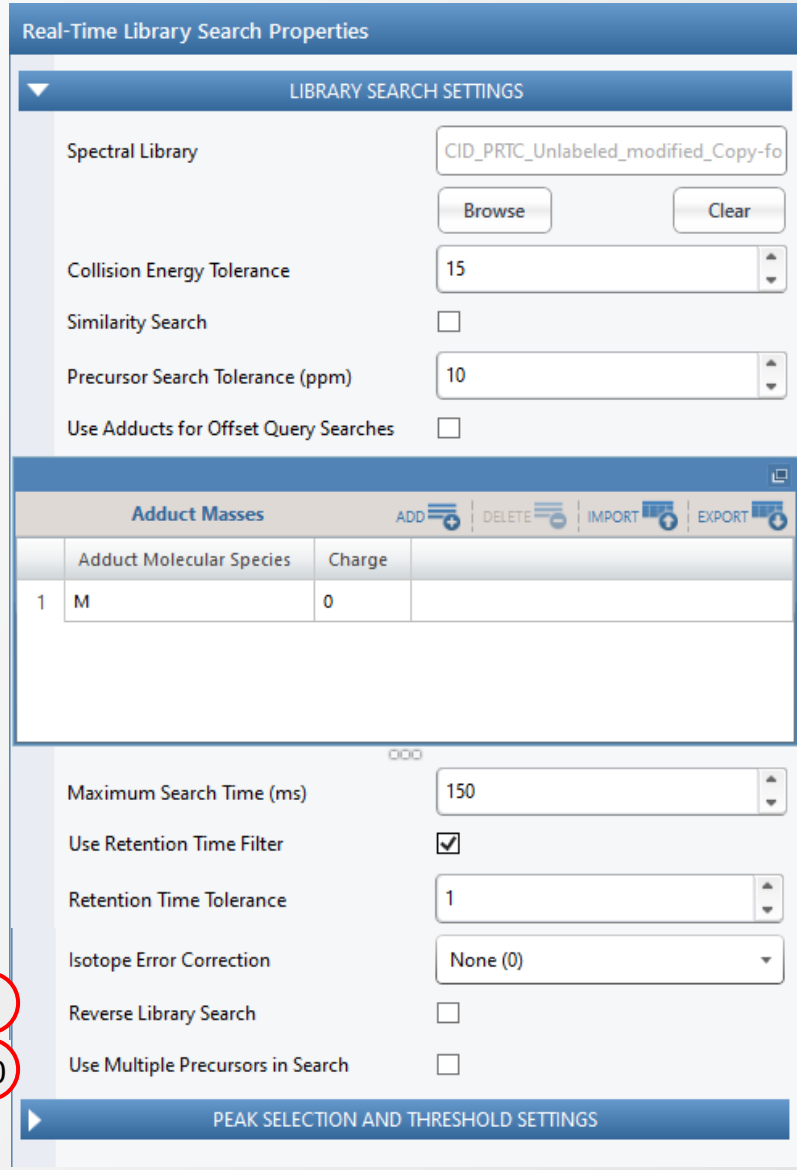


Support for Real-Time Library Search

Configurable Library Search Settings

- Reverse Library Search (9) *NEW*
  - Reversed library search is performed instead of “default” forward search.
  - Forward search : query spectrum is searched against each library spectrum with score negatively affected by peaks present in the query spectrum and not in the library spectrum.
  - Reverse search: each library spectrum is searched against the query spectrum with score negatively affected by peaks present in the library spectrum and not in the query spectrum, but the presence of additional peaks in the query spectrum has no effect on score.

- Use Multiple Precursors in Search (10) *NEW*
  - Product spectrum is searched against multiple precursors and all passing matches are returned.
  - MS1 data available within the same experiment: multiple searches of the product spectrum is performed using each precursor found in the isolation window.
  - MS1 data unavailable within the same experiment: searches are performed with precursor search tolerance set (overridden) to the width of the isolation window.



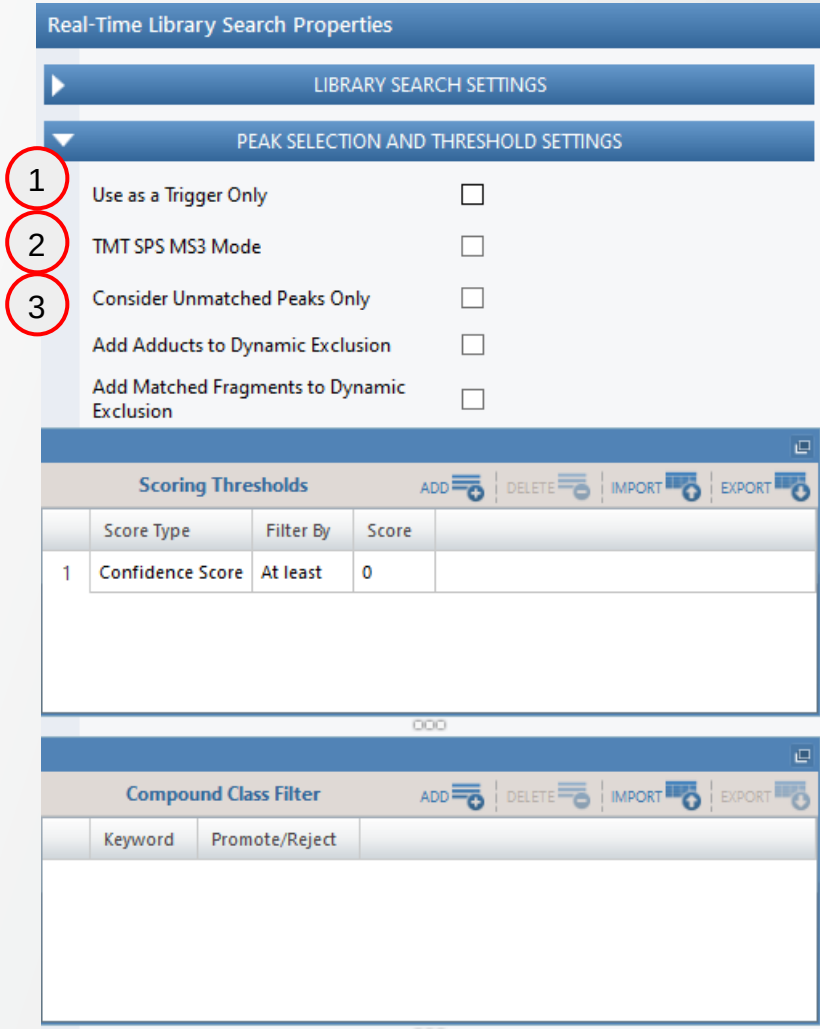
Support for Real-Time Library Search

Configurable Peak Selection and Threshold Settings

- 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 (as long as Consider Unmatched Peaks Only is not selected)

- TMT SPS MS3 Mode (Peptide application mode only) (2) *NEW*
  - When enabled, pass only matched peaks containing a free amine (Lysines or N-terminus) which would carry TMT modification
  - When used in combination with a SPS-MS3 method, SPS peaks that have a high probability of containing TMT tag are prioritized
  - Requirements: properly annotated library, Use as a Trigger Only and Consider Unmatched Peaks Only controls not selected

- Consider Unmatched Peaks Only (3) *NEW*
  - When enabled, pass only unmatched peaks for next scan
  - Requirements: Use as a Trigger Only and TMT SPS MS3 Mode controls not selected



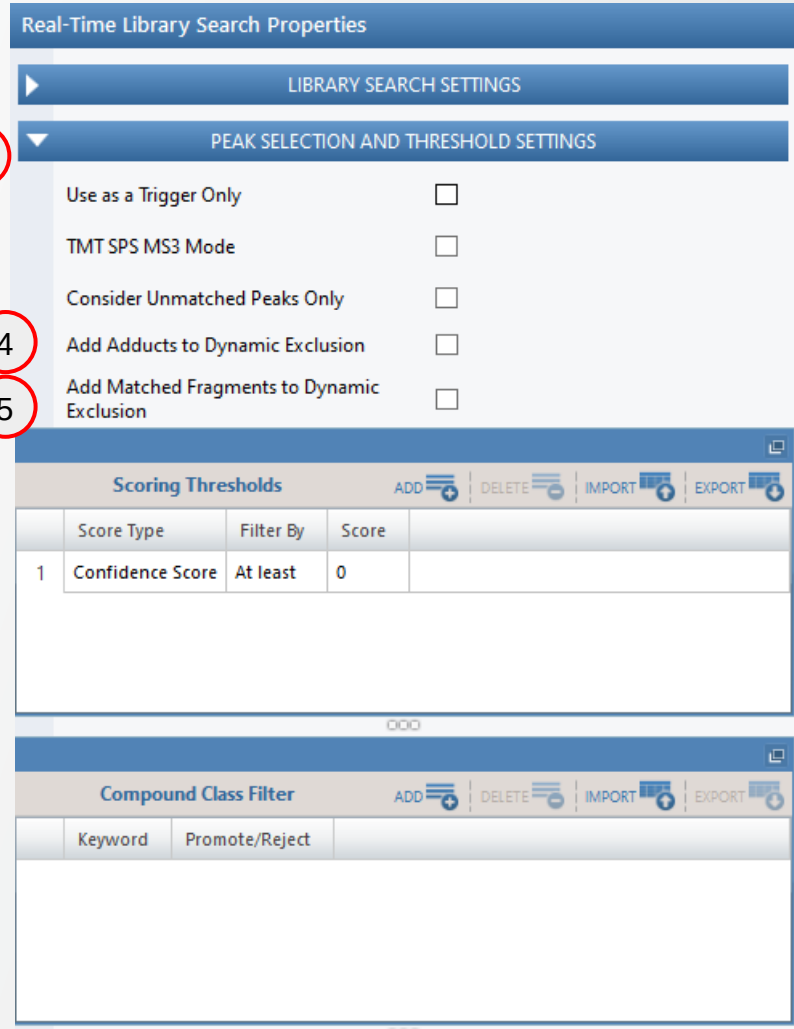
Support for Real-Time Library Search

Configurable Peak Selection and Threshold Settings

- Add Adducts to Dynamic Exclusion (Small molecule application mode only) (4)
  - 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

- Add Matched Fragments to Dynamic Exclusion (5) *NEW*
  - When enabled, matched fragments are added to the dynamic exclusion list to prevent MS2 triggering of known fragments.

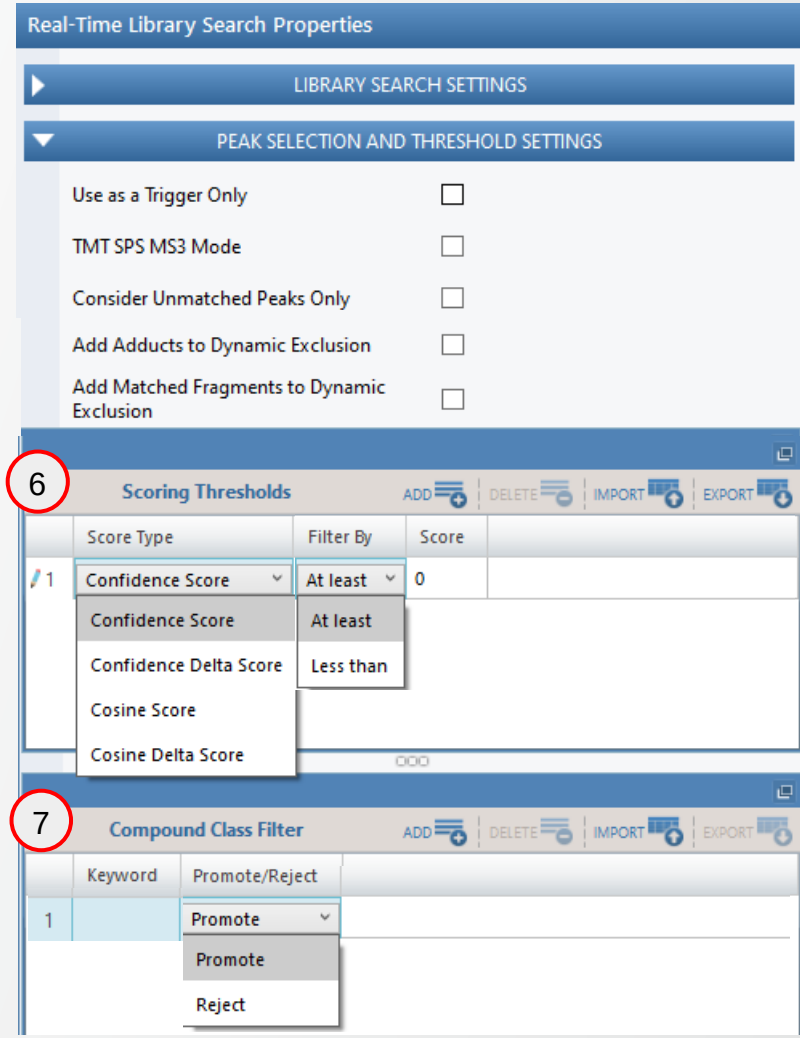


Support for Real-Time Library Search

Configurable Peak Selection and Threshold Settings

- Scoring Thresholds (6)
  - Spectra pass through the filter if the scores satisfy the thresholds
  - 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”, <)

- Compound Class Filter (7)
  - Promote/Reject specific compound classes based on keyword
  - Compound class annotation can be defined within mzVault library management app.
  - 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”, it will pass the filter
    - If a scan top hit meets thresholds, but is “rejected”, it will not pass the filter

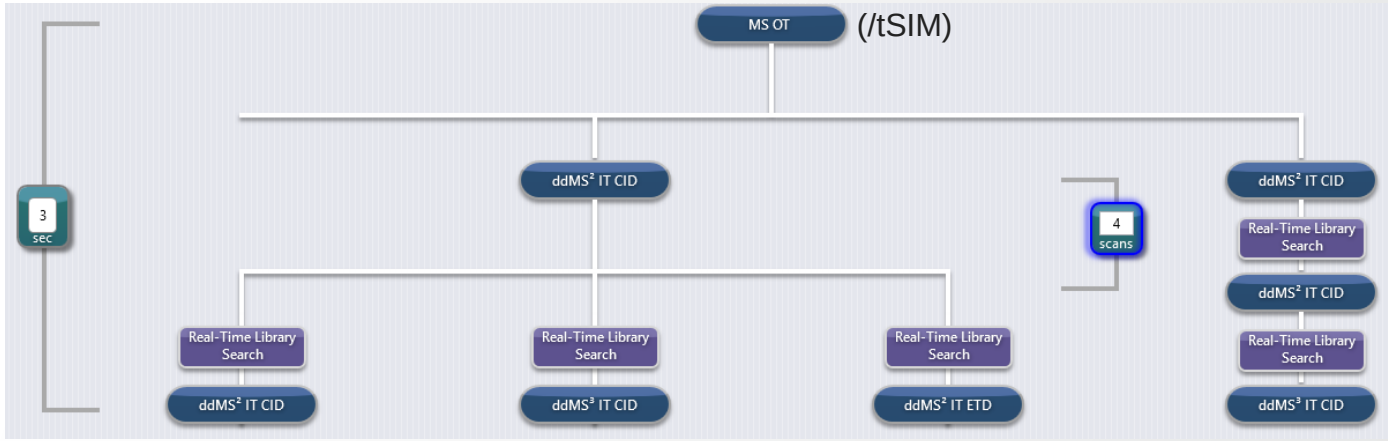


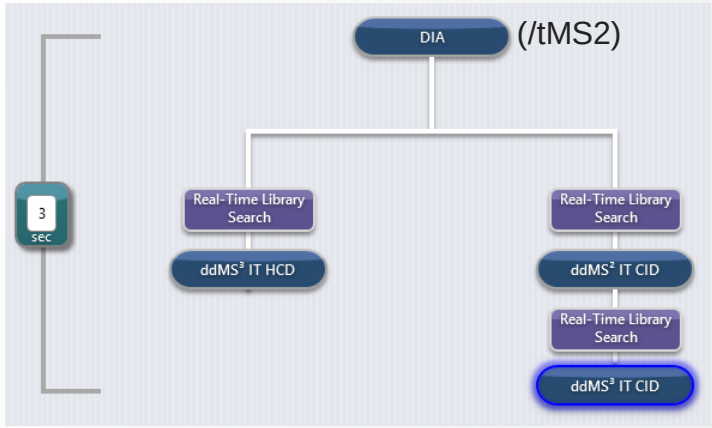
Formula	CAS ID	InChi Key	Compound Class
C8H9NO4	82826		Endogenous Metabolites
C7H8N4O2	58559		Endogenous Metabolites Therapeutics/Prescription Drugs
C73H108O12	6683198		Industrial Chemicals

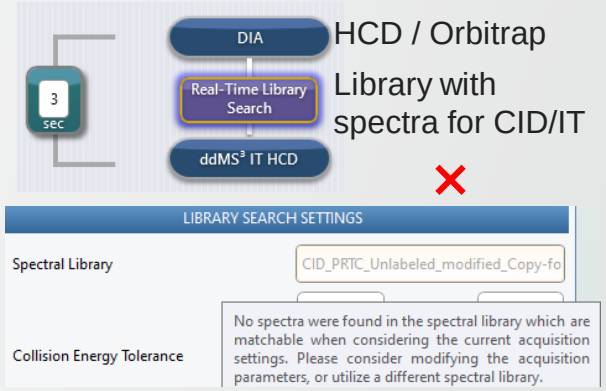
Support for Real-Time Library Search

Supported Acquisition Schemes

- Multiple RTLS filters can be placed in the experiment under multiple MS2 nodes *NEW*
- Multiple RTLS filters can use different Library Search / Peak Selection and Threshold Settings *NEW*
- RTLS supports polarity switching and mixed-polarity modes *NEW*
- RTLS can be placed under a DIA/tMS2 node *NEW*
  (Note: Mandatory to allow DIA-ddMSn acquisition scheme. Other allowed filters under DIA node are 'Precursor Selection Range', 'Precursor Ion Exclusion', and 'Isobaric Tag Loss Exclusion')
- MS2 scan above RTLS filter shall use settings compatible with Spectral library content (Activation and Detector Types)







Real-Time Library Search – Based Acquisition Method

Application Examples

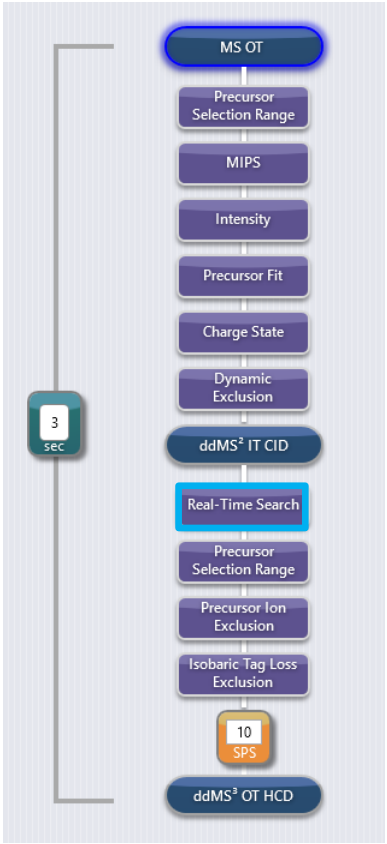
- List of examples presented
  - Alternative or Complementary to RTS-Based Acquisition schemes
  - Advanced Elucidation of Chemical Structures
  - Enhanced Localization of PTM (e.g., phosphorylation)
  - Internal Standard Triggered Data Acquisition Schemes
  - Hybrid DIA / Internal Standard Triggered Data Acquisition Schemes

- Examples illustrate some possible new acquisition schemes enabled by the new features

- Presented method examples have not been fully tested

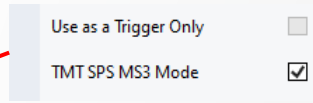
Real-Time Library Search – Based Acquisition Method

Application Example: Alternative or Complementary to RTS-Based Acquisition schemes

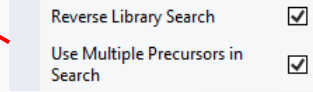


TMT SPS MS3 with RTS

⚠ Proper execution of the methods depends on the Library search sp
(library size, Precursor Search Tolerance, etc.)



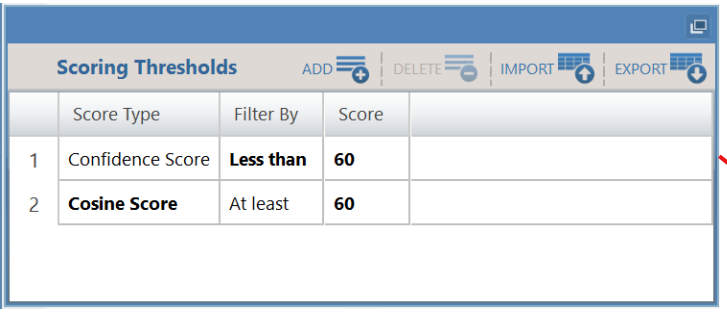
Optional:

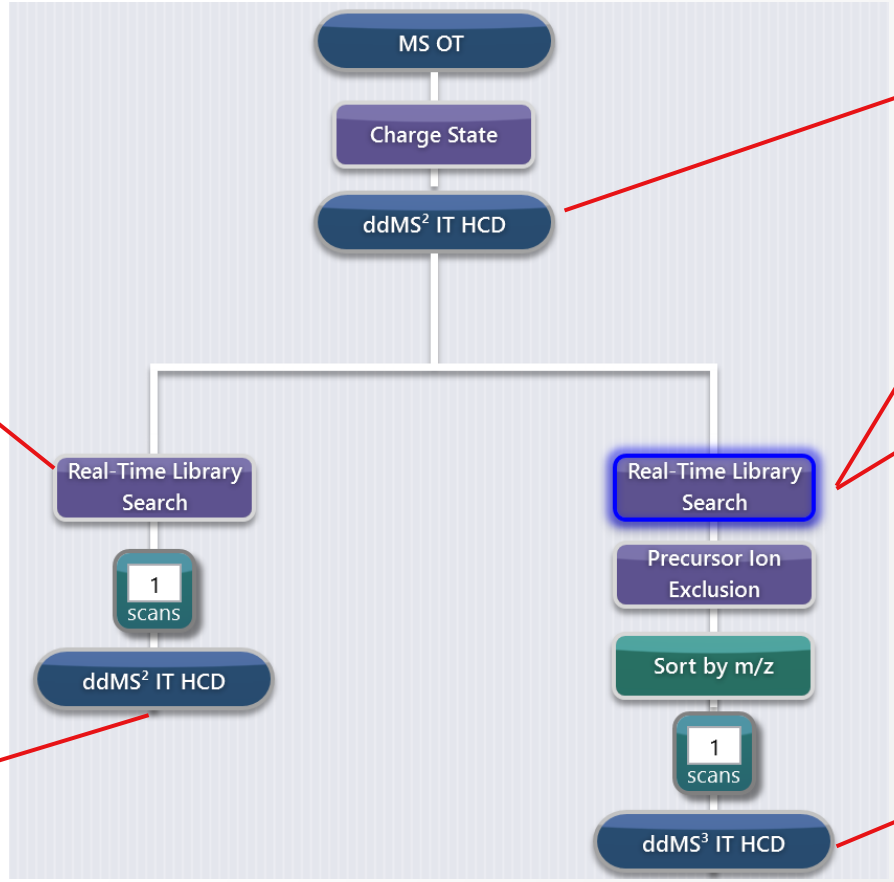


Real-Time Library Search – Based Acquisition Method

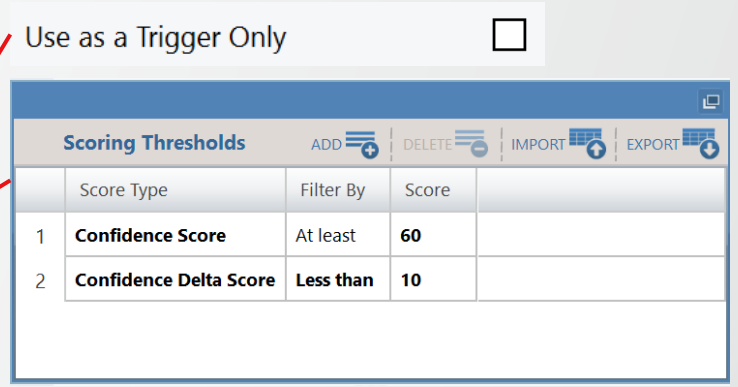
Application Example: Advanced Elucidation of Chemical Structures

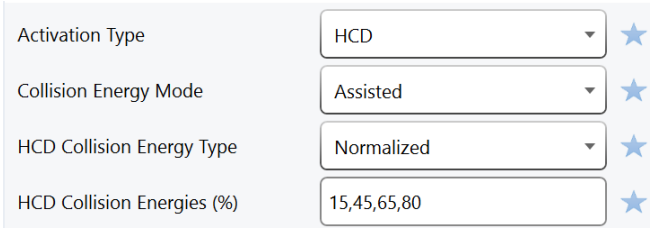
- No further acquisition for unambiguous & confident id. of the cmpds (high cosine / confidence / confidence delta)
- Optimization of CE if match appears under-fragmented (high cosine / low confidence)
- MS3 acquisition on high m/z fragments for compounds with ambiguous scores





HCD@45



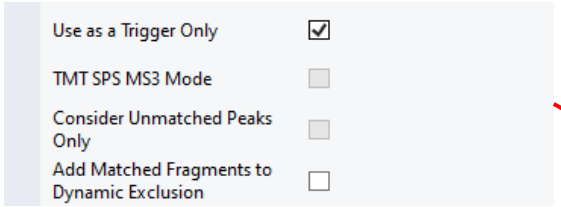


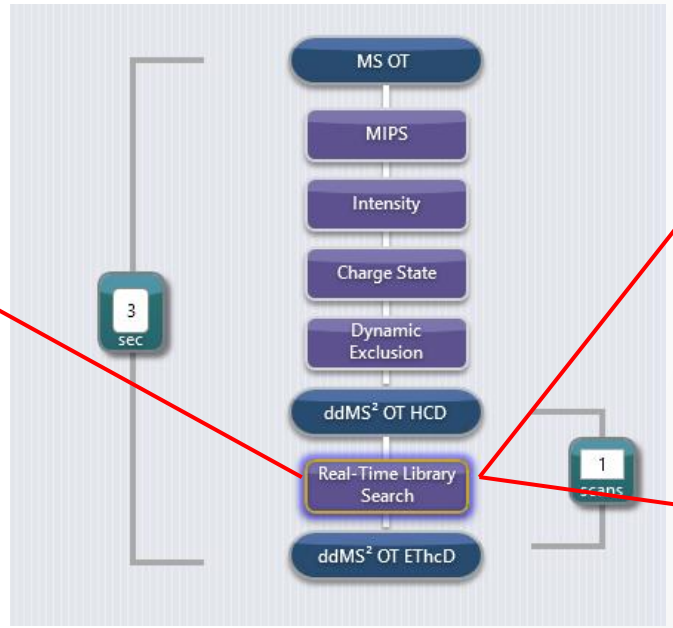
Aim to remove ambiguity by fragmentation of high m/z RTLS matched ions

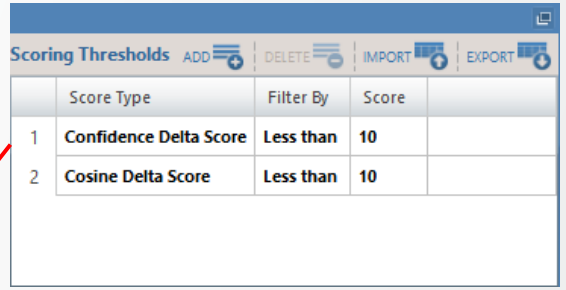
Real-Time Library Search – Based Acquisition Method

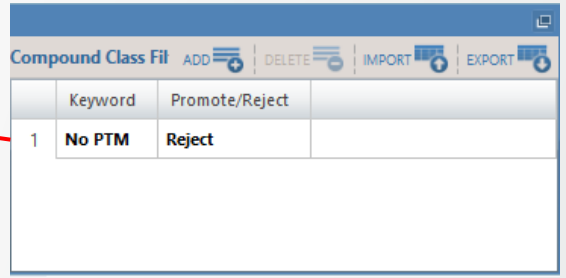
Application Example: Enhanced Localization of PTM (e.g., phosphorylation)

- No further acquisition for peptides without PTM
- No further acquisition for peptides with confident localization of PTM (high cosine / confidence delta)
- Alternative fragmentation for peptides with ambiguous PTM localization
- Needed: ref. spectra for the peptides with different PTM localization and Compound Class properly annotated





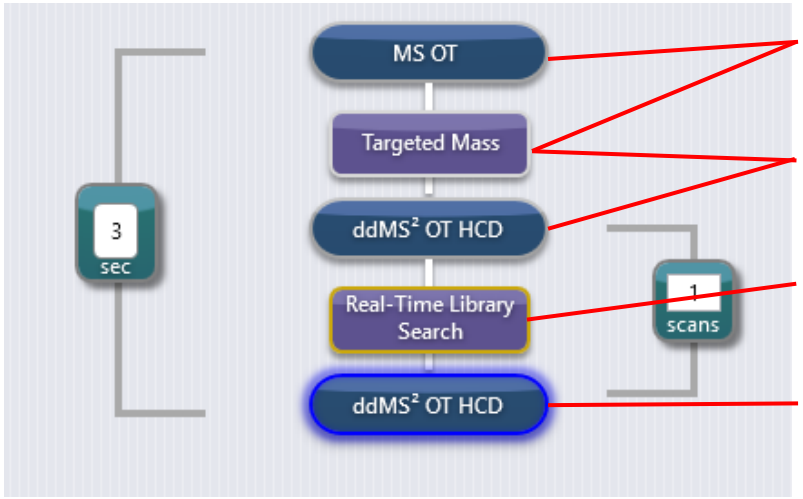




Real-Time Library Search – Based Acquisition Method

Application Example: Internal Standard Triggered Data Acquisition Schemes

- Simplified implementation of SureQuant & Tomahaq methods (switch from multi-branch to single-branch method)
- Improve usability and mitigate method preparation effort



SureQuant

Monitoring IS prec (stable isotopically labeled pep. / shTMTpro labeled pep.)

Fast MS/MS (e.g., low res./IT) on candidate precursor ions

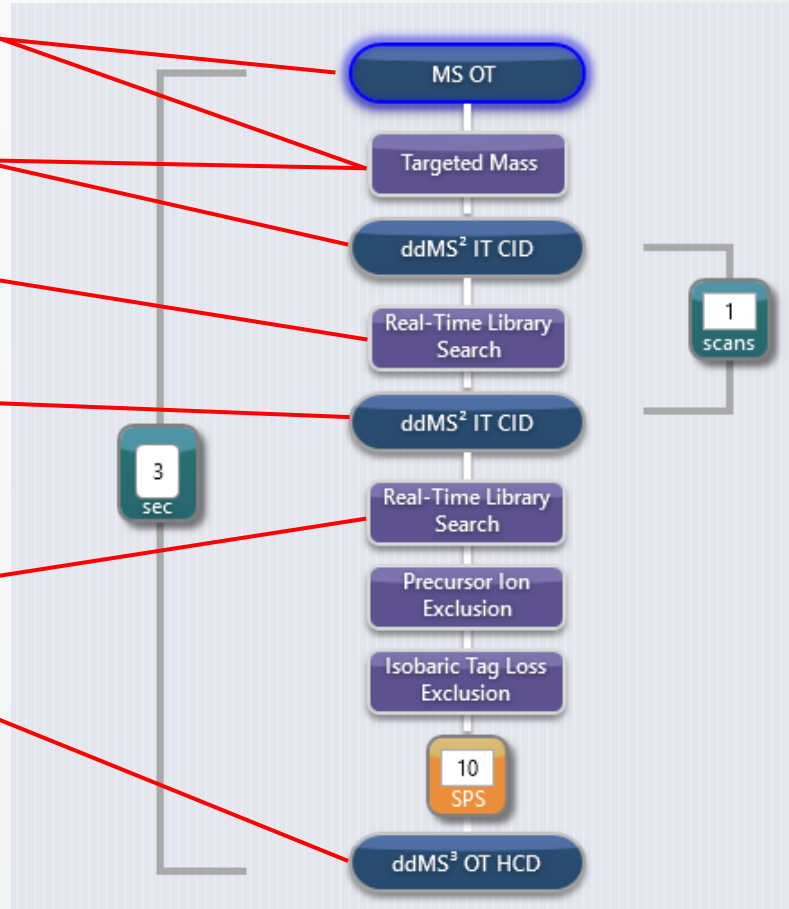
Spectral library search experimental vs. ref spectra: Identification of IS

High-quality MS/SM (e.g., high res./IT) on corresponding endogenous peptide triggered by IS identification

Spectral library search experimental vs. ref spectra: Identification of ENDO

High-quality TMT SPS MS3 on ENDO triggered by its MS2-based detection

Tomahaq



Real-Time Library Search – Based Acquisition Method

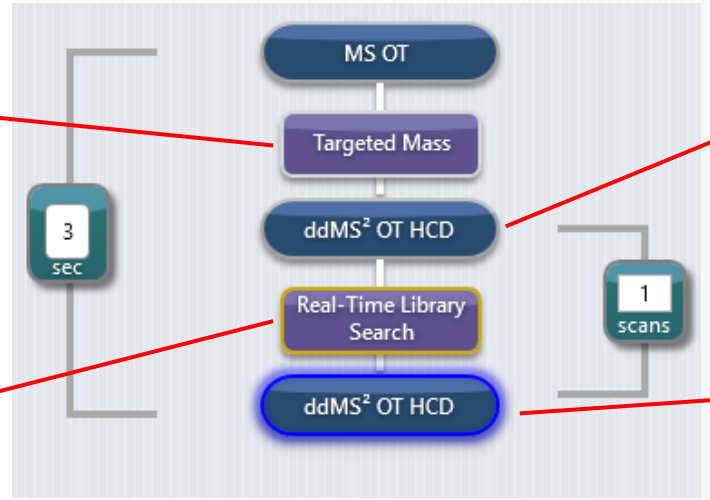
Application Example: Internal Standard Triggered Data Acquisition Schemes

- New “Isotopic Variant Derived Offset” option (“Isolation Offset”) property enables peptide/precursor-specific definition of shifted isolation window for bottom triggered ddMS2 (e.g., from SIL to ENDO peptide though RTLS)
- Needed: Reference spectra properly annotated with relevant Tags (i.e., “massOffset”)

SureQuant

ADD | DELETE | IMPORT | EXPORT

	Compound	Formula	Adduct	m/z	z	Intensity Threshold
1	SSAAPPPPPR[+10.0084]			493.7683	2	1.0e5
2	GISNEGQNASIK[+8.0142]			613.3167	2	1.0e5
3	HVLTSIGEK[+8.0142]			496.2867	2	1.0e5
4	DIPVPKPK[+8.0142]			451.2834	2	1.0e5



Isolation Offset	Off
Detector Type	Orbitrap
Orbitrap Resolution	7500

Isolation Offset	Isotopic Variant Derived Offset
Reported Mass	Offset Mass
Detector Type	Orbitrap
Orbitrap Resolution	60000

PEAK SELECTION AND THRESHOLD SETTINGS

Use as a Trigger Only	☑
TMT SPS MS3 Mode	☐

Compound List

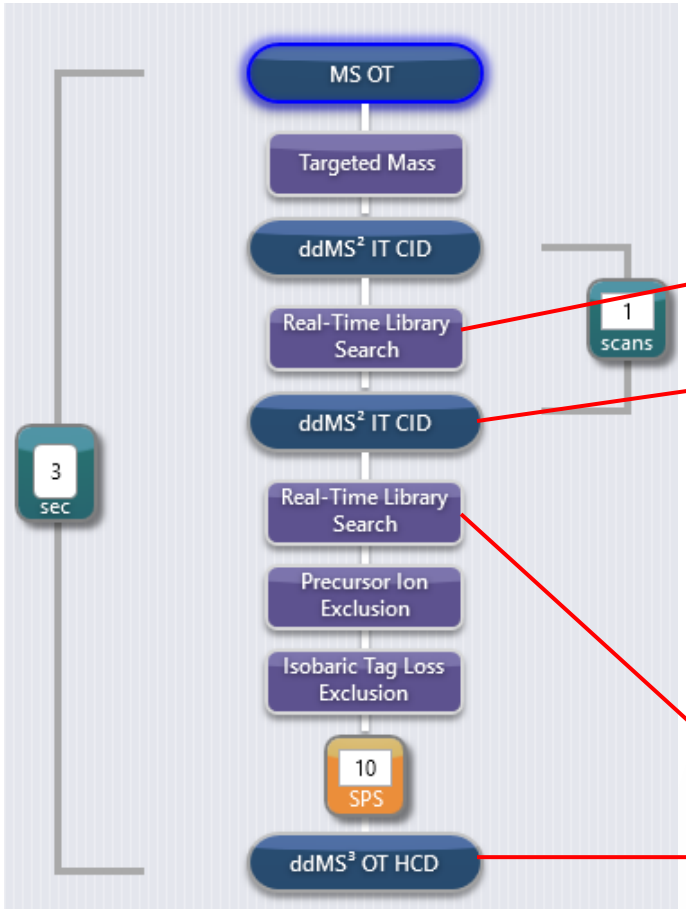
	Compound	Compound Class	Peptide Seq	Tag
▶	SSAAPPPPPR/2		SSAAPPPPPR	massOffset:-10.0084
	GISNEGQNASIK/2		GISNEGQNASIK	massOffset:-8.0142
	HVLTSIGEK/2		HVLTSIGEK	massOffset:-8.0142
	DIPVPKPK/2		DIPVPKPK	massOffset:-8.0142
	IGDYAGIK/2		IGDYAGIK	massOffset:-8.0142

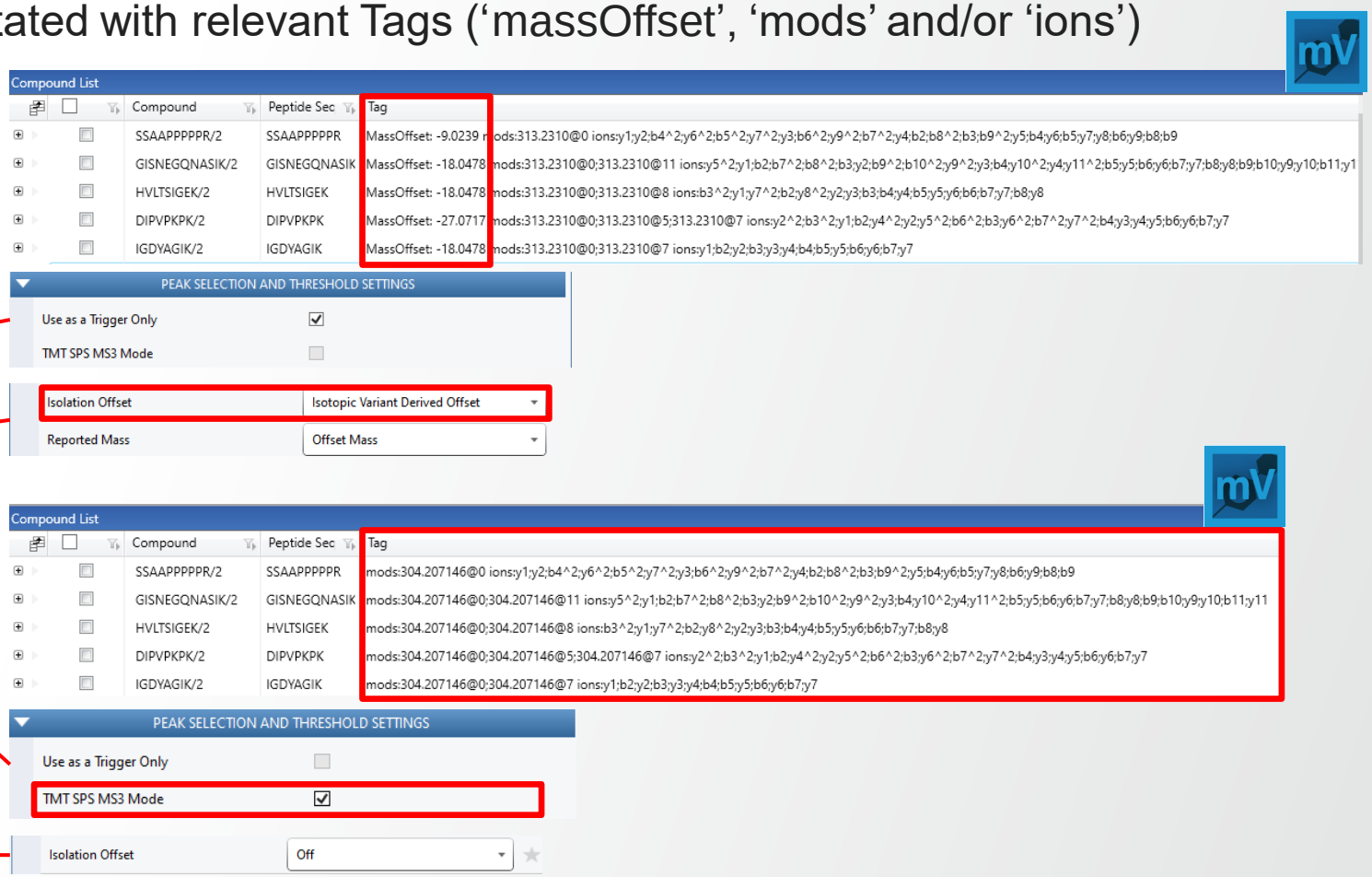
Real-Time Library Search – Based Acquisition Method

Application Example: Internal Standard Triggered Data Acquisition Schemes

- New “Isotopic Variant Derived Offset” option (“Isolation Offset”) property enables peptide/precursor-specific definition of shifted isolation window for bottom triggered ddMS2 (e.g., from IS to ENDO peptide though RTLS)
- Needed: Reference spectra properly annotated with relevant Tags (‘massOffset’, ‘mods’ and/or ‘ions’)

Tomahaq





Real-Time Library Search – Based Acquisition Method

Application Example: Hybrid DIA / Internal Standard Triggered Data Acquisition Schemes

- High quality MS2 acquisition on predefined Endogenous peptides triggered by Internal Standard peptides (SIL) detection from DIA data (SureQuant-like)
- DIA data support proteome profiling (data quality depending on how acquisition time distributed between DIA/SQ)
- New “Isotopic Variant Derived Offset” option (“Isolation Offset”) property enables peptide/precursor-specific definition of shifted isolation window for bottom triggered ddMS2 (e.g., from SIL to ENDO peptide though RTLS)
- Needed: Reference spectra properly annotated with relevant Tags

Hybrid DIA/SureQuant

Real-Time Library Search – Spectral Libraries

Preparation of Spectral Libraries in mzVault Compatible Format (.db)

- Generation of local experimental spectral libraries
- Conversion of existing spectral libraries
- Local copies of mzCloud MS2 fragmentation libraries (small molecules only)

mzVault Format Spectral Library

Real-Time Library Search – Spectral Libraries

Preparation of Spectral Libraries in mzVault Compatible Format (.db)

- Generation of local experimental spectral libraries (mainly illustrated for small molecules but similar with peptides)
  - Acquire MS2 data on compounds of interest (standards)
  - Create the library
  - Add compounds (and metadata)
  - Import the raw file(s)
  - Add MS2 spectra to the compounds
  - Add metadata
  - Save the library

In mzVault

mzVault Format Spectral Library

Real-Time Library Search – Spectral Libraries

Preparation of Spectral Libraries in mzVault Compatible Format (.db)

- Generation of local experimental spectral libraries (mainly illustrated for small molecules but similar with peptides)
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  - Save the library

In mzVault

mzVault Format Spectral Library

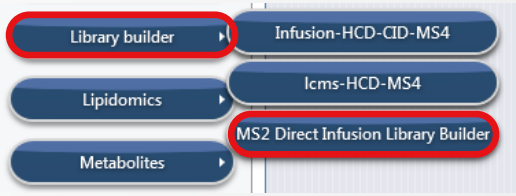
- Conversion of existing spectral libraries

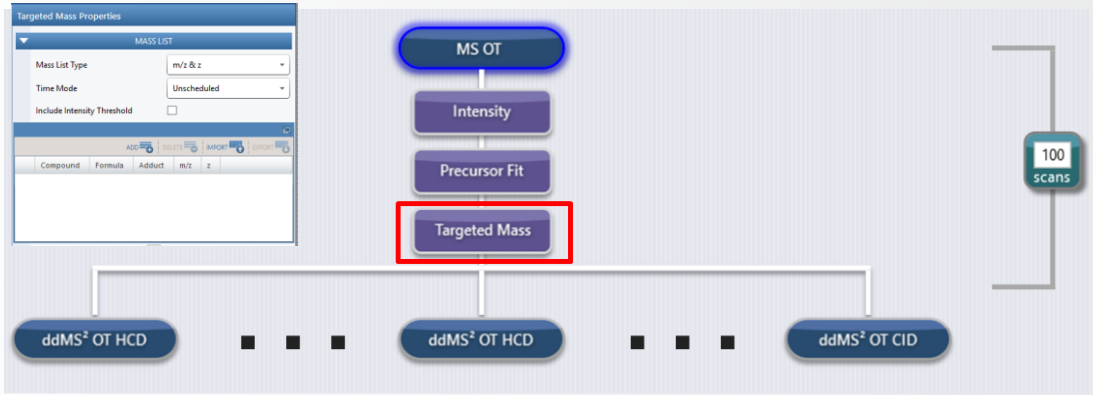
- Local copies of mzCloud MS2 fragmentation libraries (small molecules only)

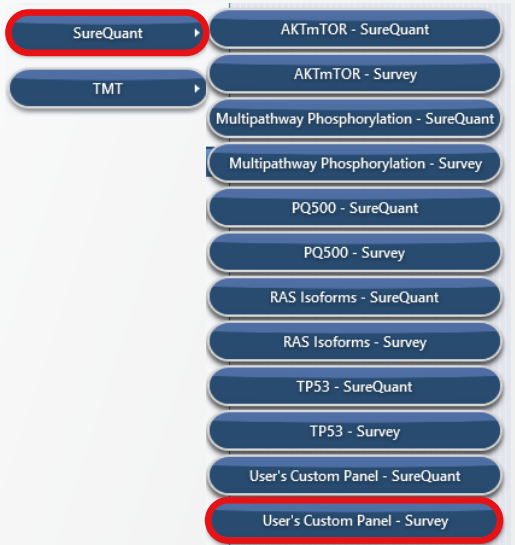
Generation of Local Experimental Spectral Libraries

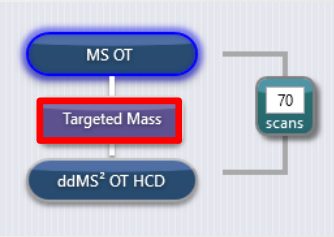
Acquiring MS2 data on Compounds of Interest (Standards)

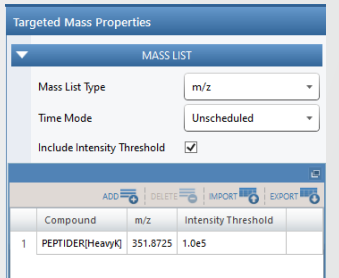
- Provided system templates can be used as models
- Small Molecule MS/MS Library Builder template
  - Analyses are performed as direct infusion or with an LC method (with the need for chrom. peak ≥ 6s)
  - M/z value (or m/z & z) of the compound of interest are included in Targeted Mass filter
  - MS/MS 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)
- Peptide “User’s Custom Panel – SureQuant” template
  - Analyses are performed with an LC method and single HCD or CID nCE
  - M/z value (or m/z & z) of the compound of interest are included in Targeted Mass filter







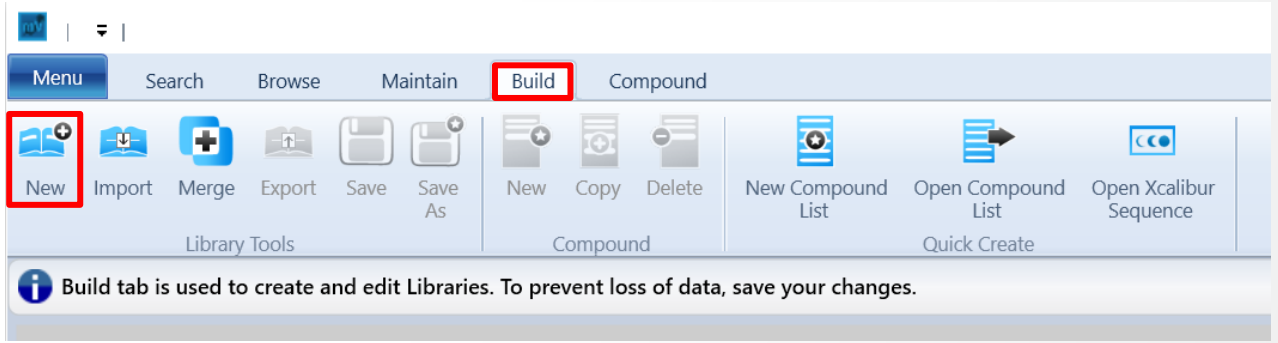


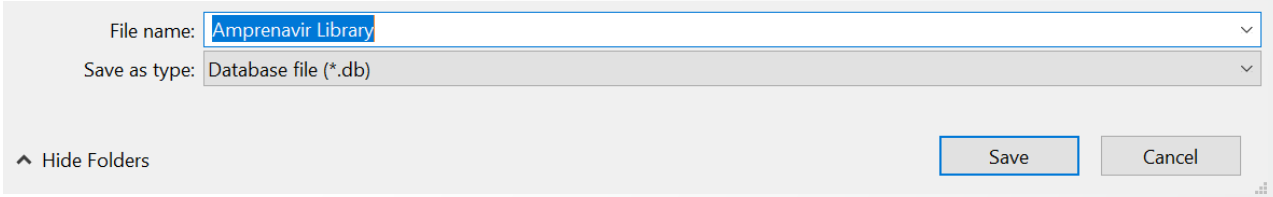


Generation of Local Experimental Spectral Libraries

Creating the Library

- mzVault can be downloaded from https://thermo.flexnetoperations.com/
- When you open mzVault, go to “Build” tab and select “New”
- Choose a name for your library and hit “Save”

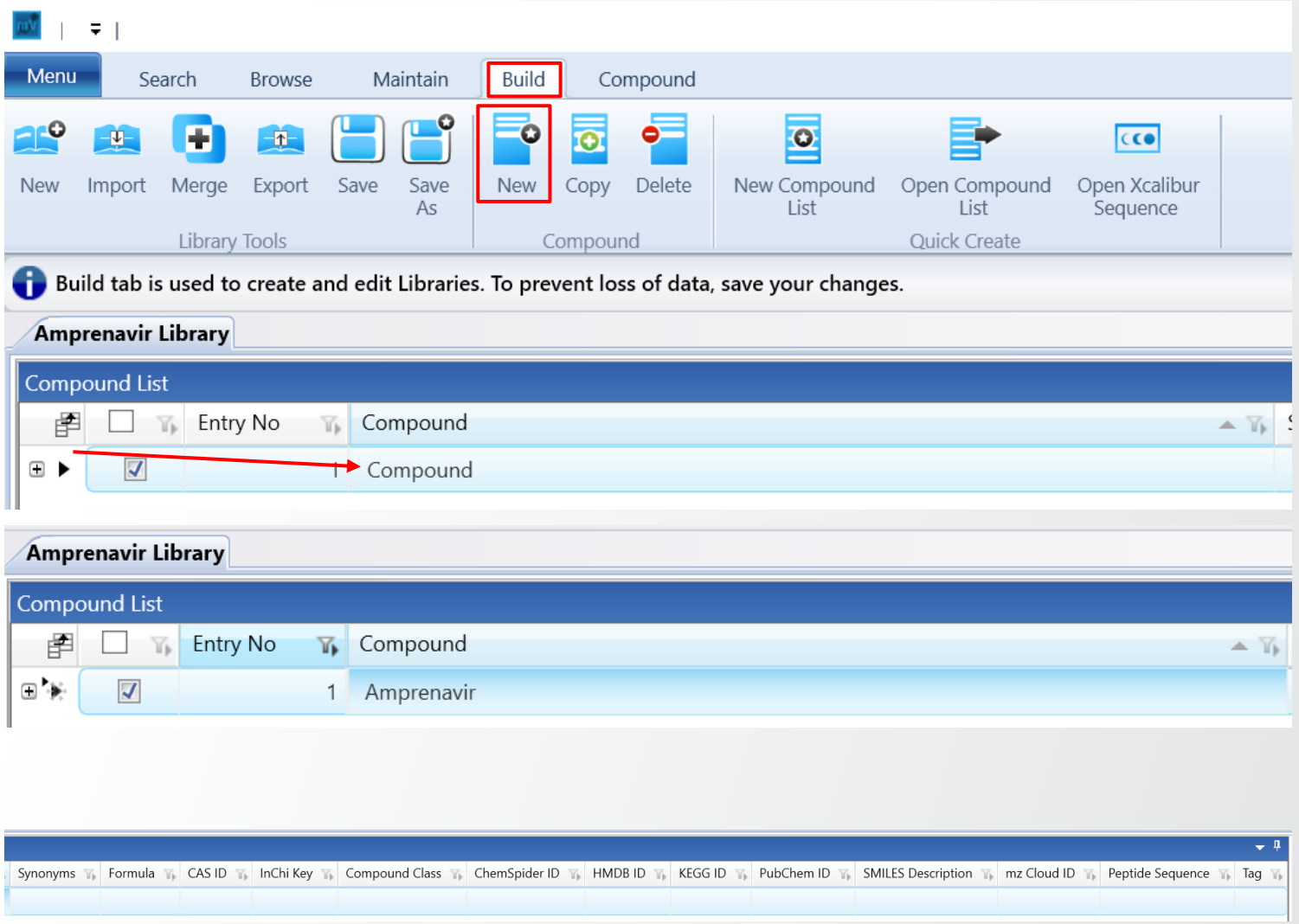




Generation of Local Experimental Spectral Libraries

Adding Compounds and Metadata

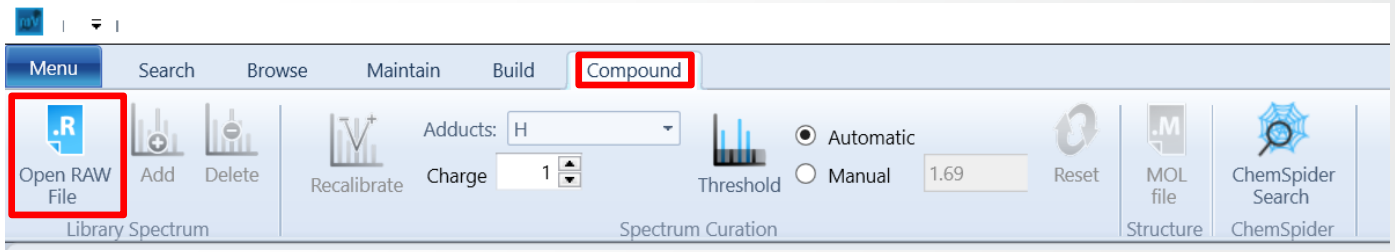
- Under the “Build” tab select “New” under the compound section
- Double-click in the compound field and enter the name of your compound
- There are several other fields to fill in metadata if desired, including “Compound Class”

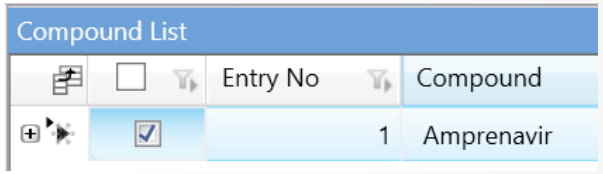


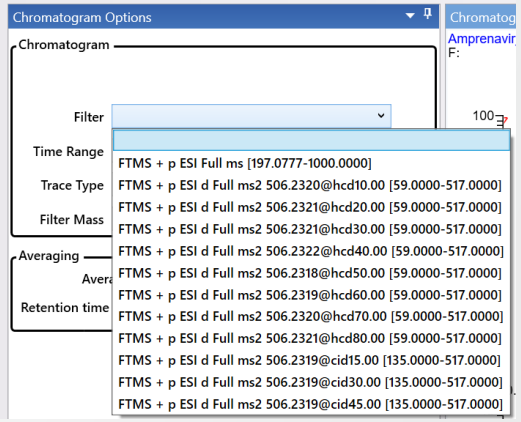
Generation of Local Experimental Spectral Libraries

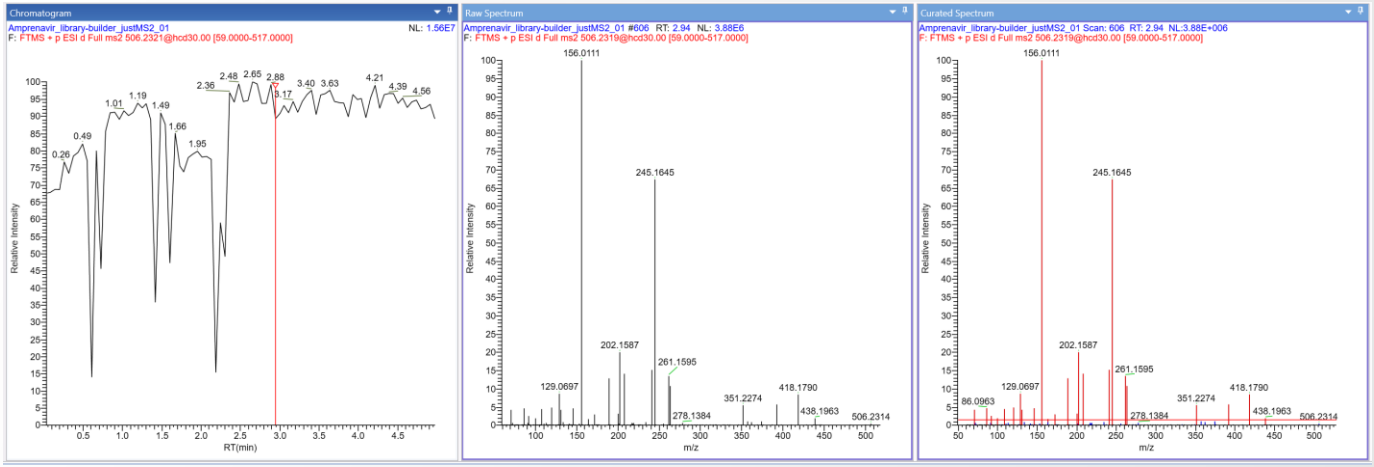
Importing a Raw File

- Under the “Compound” tab, select “Open RAW File”
- To assign a spectra to a compound, select the compound then select the desired filter under “Chromatogram Options”
- Select the scan in the “Chromatogram” window
- The “Raw Spectrum” and “Curated Spectrum” windows show all fragments and all fragments above the relative intensity threshold respectively





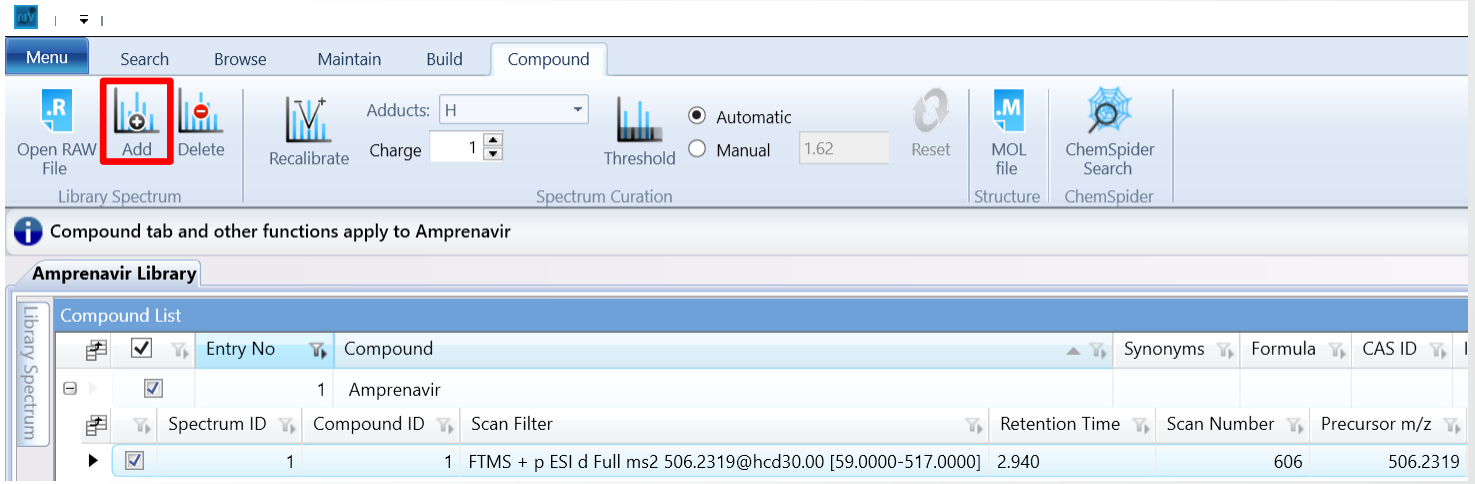




Generation of Local Experimental Spectral Libraries

Adding MS2 Spectra to the Compounds

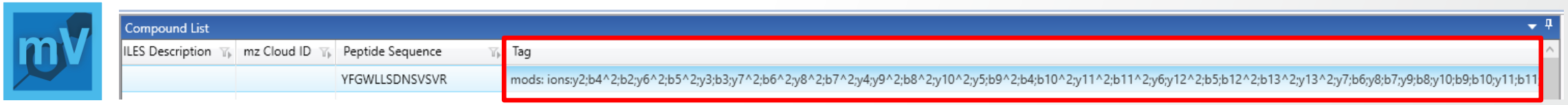
- To add a spectra to the library select “Add”
- Repeat the process for all the compounds of the library



Generation of Local Experimental Spectral Libraries

Manually Adding Metadata

- For spectra requiring additional metadata (modification information, ion annotation, isolation mass offset directives) manually add this data to the new compound “Tag” field in mzVault.



- The Tag field will become editable upon double-clicking the textbox.

- Available metadata directives for RTLS include
  - Modification information - “mods:”
  - Ion annotation - “ions:”
  - Isolation mass offset - “massOffset:”

Generation of Local Experimental Spectral Libraries

Available ‘Tag’ Metadata Inputs and Expected Formats

- Modifications (“mods:”)
  - If the ion annotation string is not provided, matched ion types can be annotated by the presence of the peptide sequence, charge state, and this “mods:” information.
  - Expected Format: “mods:57.0214637236@2;57.0214637236@5;15.99491@6”
  - ***NB***: If multiple mods are provided for the same AA position, they will be summed. Index 0 is the peptide N-terminus, and *n*+1 is the C-terminus.

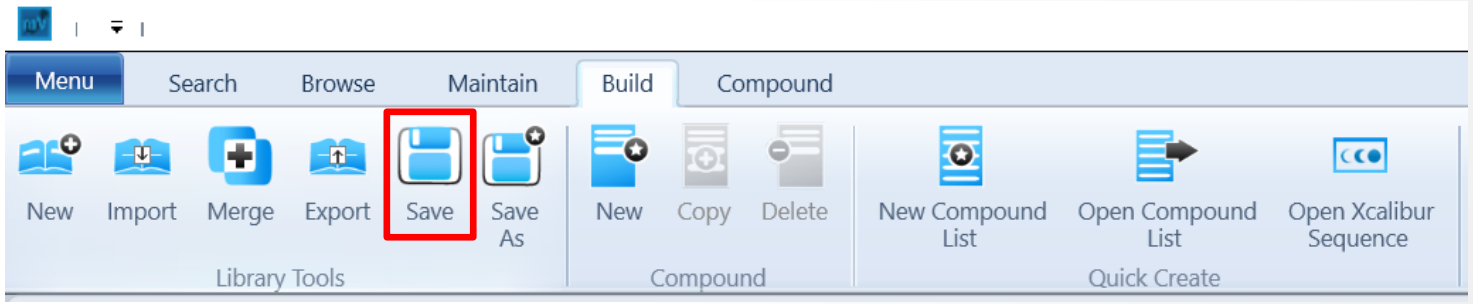
- Fragment Ion Annotation (“ions:”)
  - Expected Format: “ions:y1^3;b2^2;b1;y2^2;y1;b4^3;b3^2;y3^2;b5^3” or just “ions:y1;b2;b1;y2;y1;b4;b3;y3;b5”
  - ***NB***: The number of ion annotations in this string (separated by semicolons) **MUST** match the number of ions in the spectral library entry

- Isolation Mass Offset (“massOffset:”)
  - Isolation mass offset, in Daltons, for follow-up scans. Enabled when “Isotopic Variant Derived” Isolation Offset is selected from the TNG Method Editor drop down.
  - Expected Format: “massOffset:8.213” or “massOffset:-10.001”

Generation of Local Experimental Spectral Libraries

Saving the Library

Select “Save” under the “Build” tab



Real-Time Library Search – Spectral Libraries

Preparation of Spectral Libraries in mzVault Compatible Format (.db)

- Generation of local experimental spectral libraries

- Conversion of existing spectral libraries
  - Prepare reference spectra entries in MassBank Record Format (*.mb)
  - Create the library
  - Import MassBank Records
  - Add metadata
  - Save the library

In mzVault

mzVault Format Spectral Library

- Local copies of mzCloud MS2 fragmentation libraries (small molecules only)

Conversion of Existing Spectral Libraries

Preparing Reference Spectra Entries in MassBank Record Format (*.mb)

- The formal definition of the Massbank Record format is available at https://github.com/MassBank/MassBank-web/blob/main/Documentation/MassBankRecordFormat.md
- Custom spectral library entries and their associated metadata may be imported as MassBank record entries
- Each spectrum should be placed in a single .mb (MassBank record entry) file
- The accumulated chunk of MassBank record entries can be imported into a new mzVault (.db) library
- Accessory metadata (modification information, isolation mass offset directives, ion annotations) may be manually embedded in the mzVault ‘Tag’ field, but is not importable from the massbank records

Conversion of Existing Spectral Libraries

Preparing Reference Spectra Entries in MassBank Record Format (*.mb)

RTLS relevant mzVault parsed Mass Bank library fields

Required MassBank Fields	mzVault database Field	Description
CH$NAME:	**Compound**	**Peptide mode requires the format of "PEPTIDEK/2" (unmodified sequence and charge state separated by a slash)**
CH$COMPOUND_CLASS:	**Compound Class**	**Optional for RTLS, used for "Promote" and "Reject" table**
AC$MASS_SPECTROMETRY: ION_MODE IONIZATION	**Ionization Mode**	**ESI/NSI/HESI/APCI/EI/CI**
AC$MASS_SPECTROMETRY: ION_MODE	**Polarity**	**"Negative" or "Positive"**
AC$MASS_SPECTROMETRY: COLLISION_ENERGY	**Collision Energy**	
MS$FOCUSED_ION PRECURSOR_M/Z	**Precusormass**	
AC$INSTRUMENT_TYPE:	**Mass Analyzer**	**"LC-ESI-FT" for FTMS^2, "LC-ESI-IT" for ITMS^2**
AC$MASS_SPECTROMETRY	**Fragmentation Mode**	**HCD, CID, ETD, UVPD**
AC$CHROMATOGRAPHY: RETENTION_TIME	**Retention Time**	**Optional, retention time info (in minutes)**
PK$NUM_PEAK		**Must match number of peaks in spectrum**
PK$PEAK		**Spectral peaks**

Conversion of Existing Spectral Libraries

Preparing Reference Spectra Entries in MassBank Record Format (*.mb)

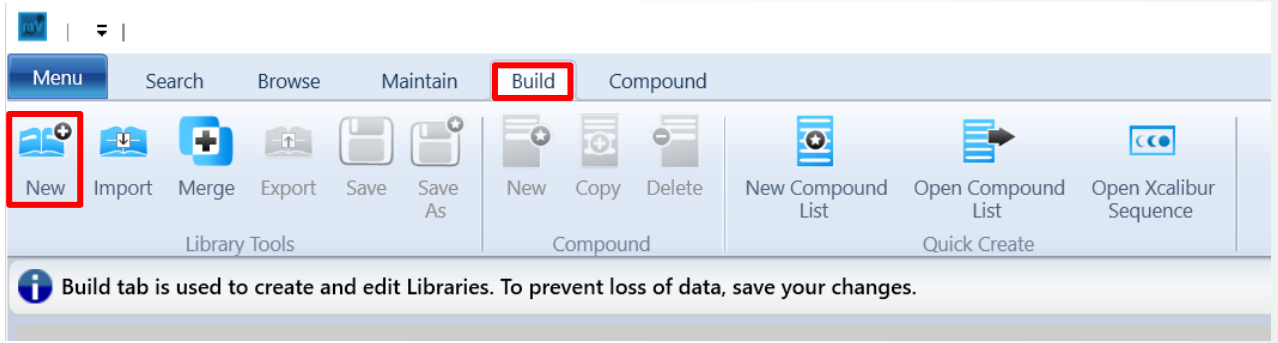
Example Minimal Massbank Record

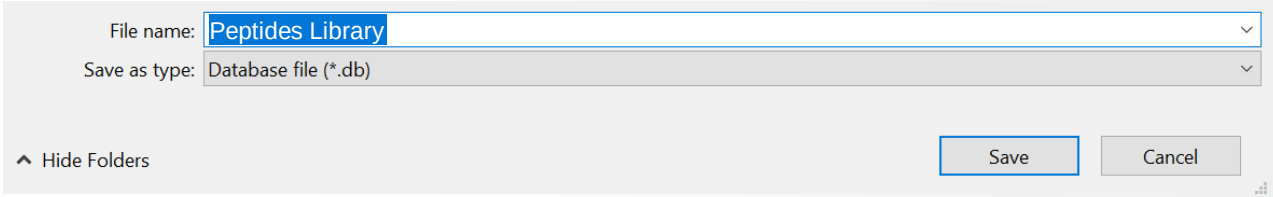
```
CH$NAME: PEPTIDEK/2
CH$COMPOUND_CLASS: Target Peptide
AC$INSTRUMENT_TYPE: LC-ESI-IT
AC$MASS_SPECTROMETRY: MS_TYPE MS2
AC$MASS_SPECTROMETRY: IONIZATION ESI
AC$MASS_SPECTROMETRY: ION_MODE POSITIVE
AC$MASS_SPECTROMETRY: FRAGMENTATION_MODE HCD
AC$MASS_SPECTROMETRY: COLLISION_ENERGY 30 % (nominal)
AC$CHROMATOGRAPHY: RETENTION_TIME 2.5 min
MS$FOCUSED_ION: PRECURSOR_M/Z 500.0000
PK$NUM_PEAK: 13
PK$PEAK: m/z int.
 147.1128082 0.079904221
 276.1553955 0.227184266
 227.1026306 0.506824255
 391.1823425 0.116141349
 504.2664185 0.085744232
 425.203064 0.01078412
 605.3140869 0.100236773
 538.2871704 0.00356305
 702.3668213 1
 351.6870728 0.153104335
 653.3140869 0.048432171
 831.4094238 0.072682858
 782.3566895 0.002288357
//
```

Conversion of Existing Spectral Libraries

Creating the Library in mzVault

- mzVault can be downloaded from https://thermo.flexnetoperations.com/
- When you open mzVault, go to “Build” tab and select “New”
- Choose a name for your library and hit “Save”



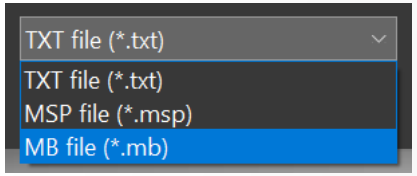


Conversion of Existing Spectral Libraries

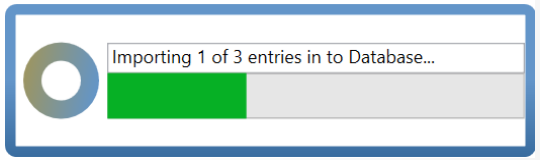
Importing Massbank Records

- Accumulate all relevant massbank record entry files into a single directory
- Follow the directions for mzVault “Creating a Library”
- Click the “Import” option

- On the file selection window, change the filetype drop down to match the “.mb” MassBank record entry format



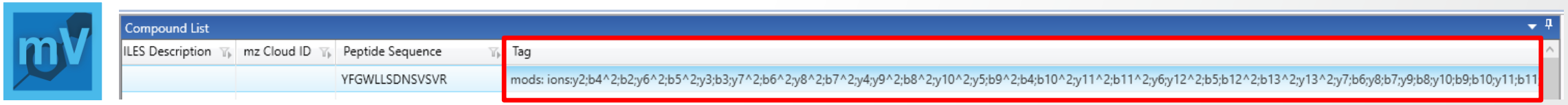
- Select all the files in the directory that you wish to import, and allow mzVault to build the spectral library



Conversion of Existing Spectral Libraries

Manually Adding Metadata

- For spectra requiring additional metadata (modification information, ion annotation, isolation mass offset directives) manually add this data to the new compound “Tag” field in mzVault.



- The Tag field will become editable upon double-clicking the textbox.

- Available metadata directives for RTLS include
  - Modification information - “mods:”
  - Ion annotation - “ions:”
  - Isolation mass offset - “massOffset:”

Conversion of Existing Spectral Libraries

Available ‘Tag’ Metadata Inputs and Expected Formats

- Modifications (“mods:”)
  - If the ion annotation string is not provided, matched ion types can be annotated by the presence of the peptide sequence, charge state, and this “mods:” information.
  - Expected Format: “mods:57.0214637236@2;57.0214637236@5;15.99491@6”
  - ***NB***: If multiple mods are provided for the same AA position, they will be summed. Index 0 is the peptide N-terminus, and *n*+1 is the C-terminus.

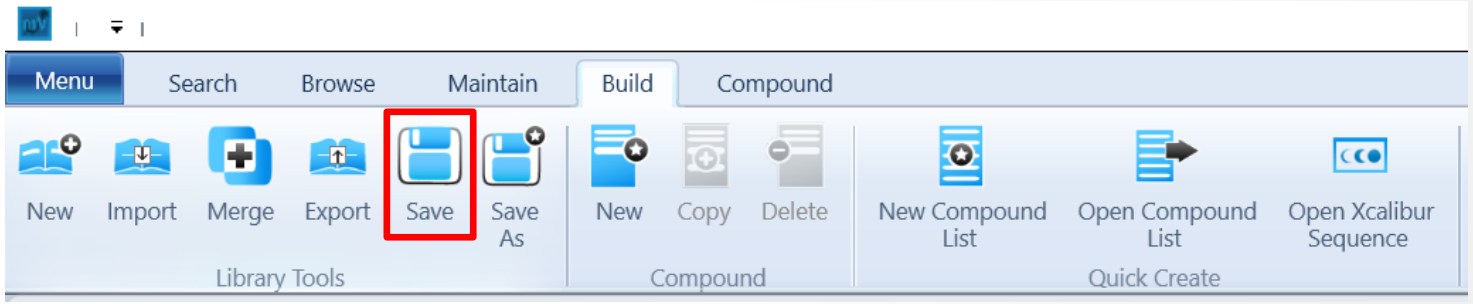
- Fragment Ion Annotation (“ions:”)
  - Expected Format: “ions:y1^3;b2^2;b1;y2^2;y1;b4^3;b3^2;y3^2;b5^3” or just “ions:y1;b2;b1;y2;y1;b4;b3;y3;b5”
  - ***NB***: The number of ion annotations in this string (separated by semicolons) **MUST** match the number of ions in the spectral library entry

- Isolation Mass Offset (“massOffset:”)
  - Isolation mass offset, in Daltons, for follow-up scans. Enabled when “Isotopic Variant Derived” Isolation Offset is selected from the TNG Method Editor drop down.
  - Expected Format: “massOffset:8.213” or “massOffset:-10.001”

Conversion of Existing Spectral Libraries

Saving the Library

Select “Save” under the “Build” tab



Real-Time Library Search – Spectral Libraries

Preparation of Spectral Libraries in mzVault Compatible Format (.db)

- Generation of local experimental spectral libraries
- Conversion of existing spectral libraries
- Local copies of mzCloud MS2 fragmentation libraries (small molecules only)
  - Download spectral libraries from Flexera (https://thermo.flexnetoperations.com/)

Local Copies of mzCloud MS2 Fragmentation Libraries

Downloading Spectral Libraries From Flexera

- Local copies of the mzCloud spectral libraries (updated yearly) can be downloaded from Flexera (https://thermo.flexnetoperations.com/) for eligible customers.

- The mzCloud spectral libraries are available with certain purchases of Compound Discoverer and TraceFinder.

- For more information, visit the mzCloud Mass Spectral Library page.

Real-Time Library Search – Outlook

Upcoming Communication

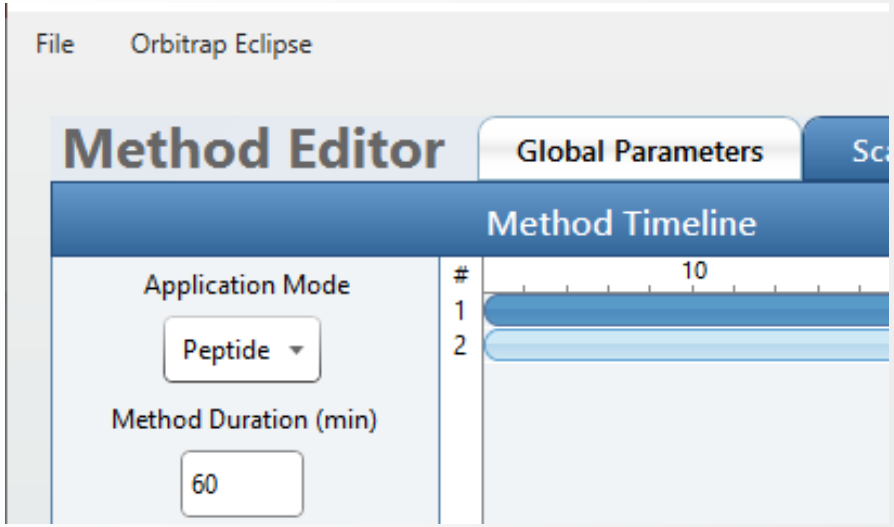
- Guidance for the preparation of RTLS-based acquisition methods (including parameters)
- Tool(s) and process to support automated generation of spectral libraries

→ Subscribe to label *Orbitrap Tribrid MS Instrument Control Software* on AnalyteGuru.com to received focused updates, including Real-Time Library Search related topics

Support for Real-Time Search

New Functionalities to Improve / Expand Data-Driven Acquisition Schemes

- Supported Models
  - Orbitrap Ascend *NEW*
  - Orbitrap Eclipse
- Supported Application Modes
  - Peptide
- Novelties *NEW*
  - Structure Filter Properties
  - New ‘Use Multiple Precursors in Search’ functionalities
  - Supported Acquisition scheme (compatible scans, placement constraint, combination of filters)

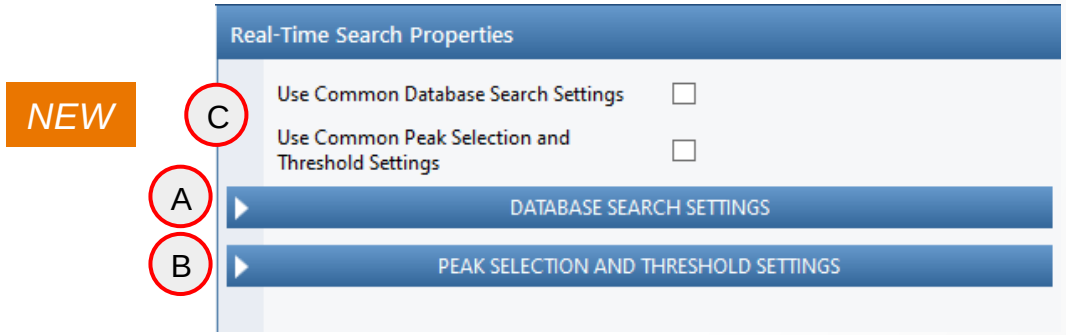


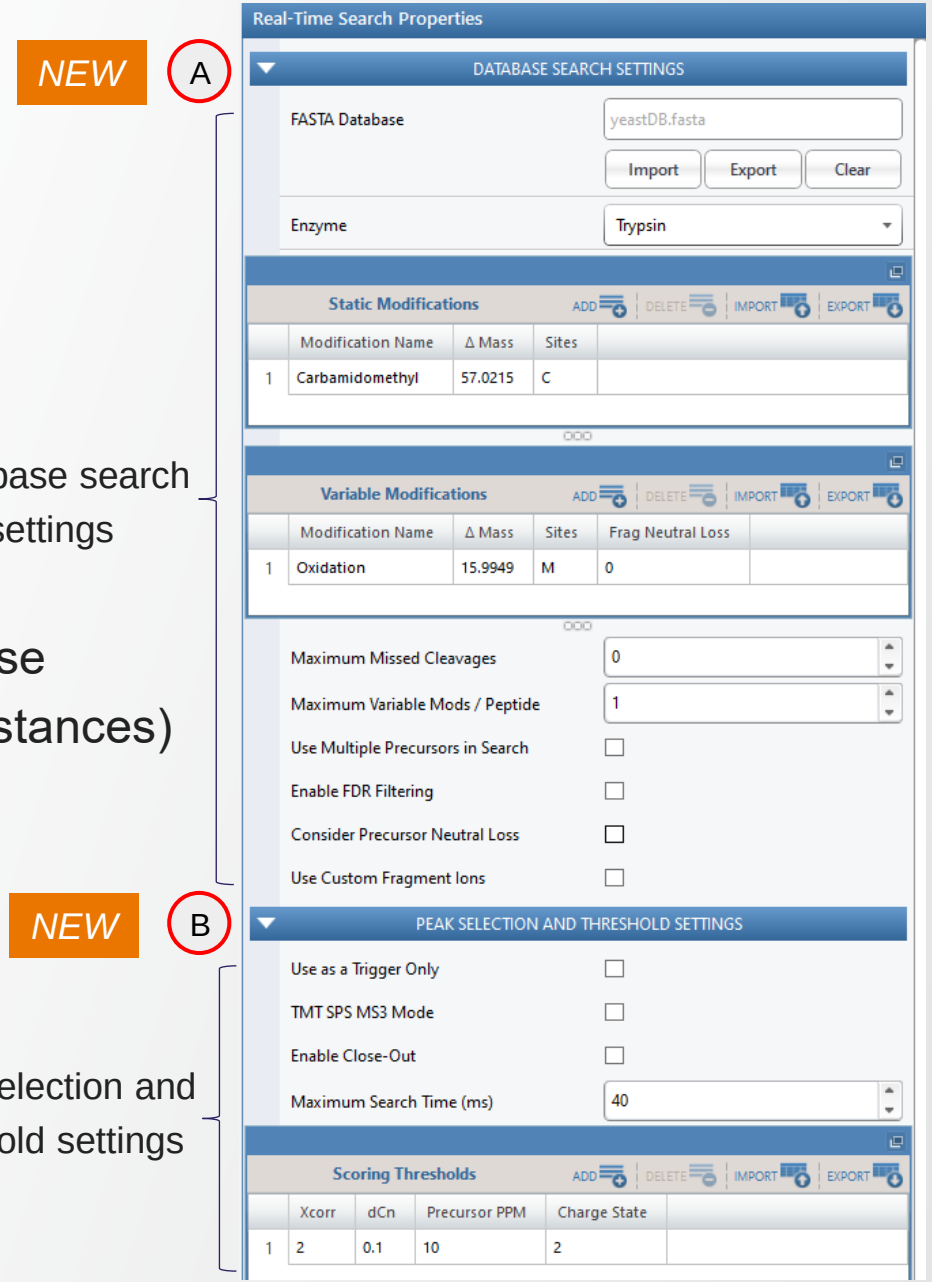
Support for Real-Time Search

Filter Overview

- Two expandable / collapsible sections of RTS filter properties
  - A • Database Search Settings
  - B • Peak Selection and Threshold Settings

- Settings defined in each section can be
  - Specific to each instance of the filter
  - C • Synchronized across other instances of the filter if corresponding ‘Use Common … Settings’ control is enabled (only displayed if several instances)

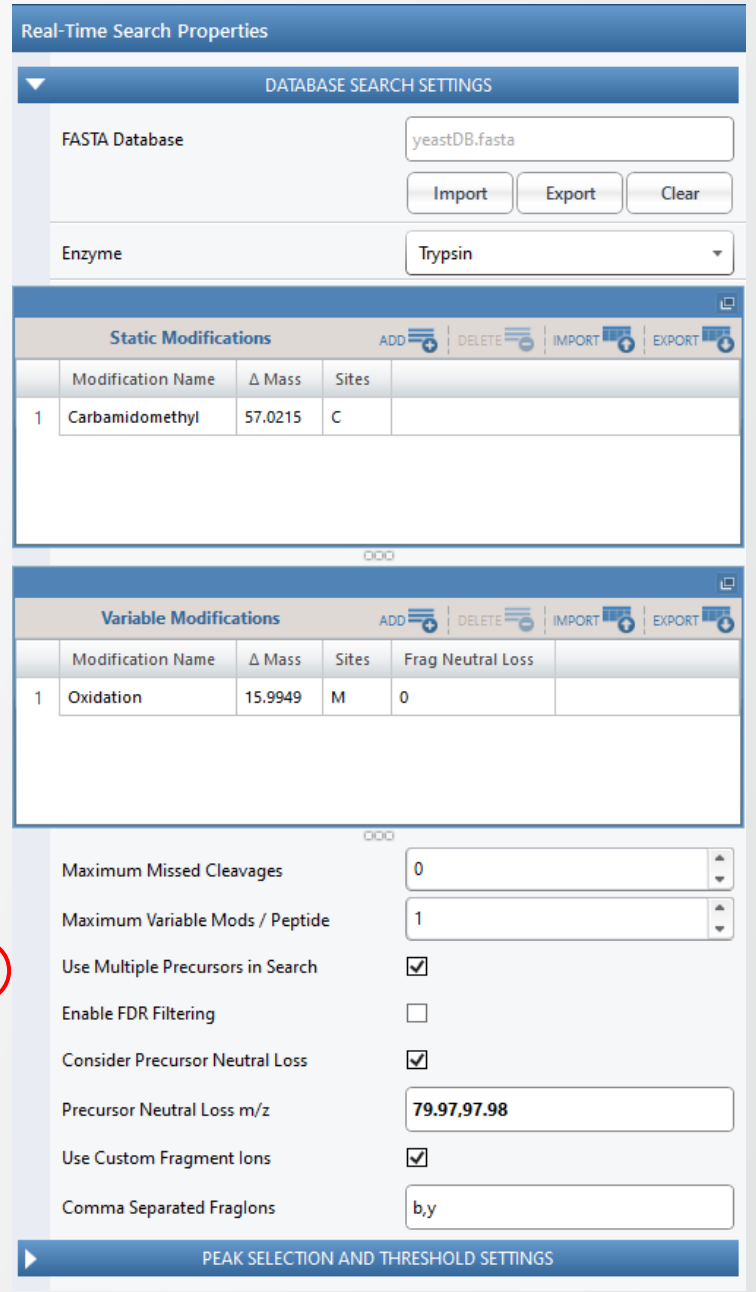




Support for Real-Time Search

Configurable Database Search Settings

- Use Multiple Precursors in Search (1) *NEW*
  - Product spectrum is searched against multiple precursors and all passing matches are returned.
  - MS1 data available within the same experiment: multiple searches of the product spectrum is performed using each precursor found in the isolation window.
  - MS1 data unavailable within the same experiment: searches are performed with precursor search tolerance set (overridden) to the width of the isolation window.
  - The benefit of the option is more limited than for Real-Time Library search, especially when combined with Reverse Library Search option.



Support for Real-Time Search

Supported Acquisition Schemes

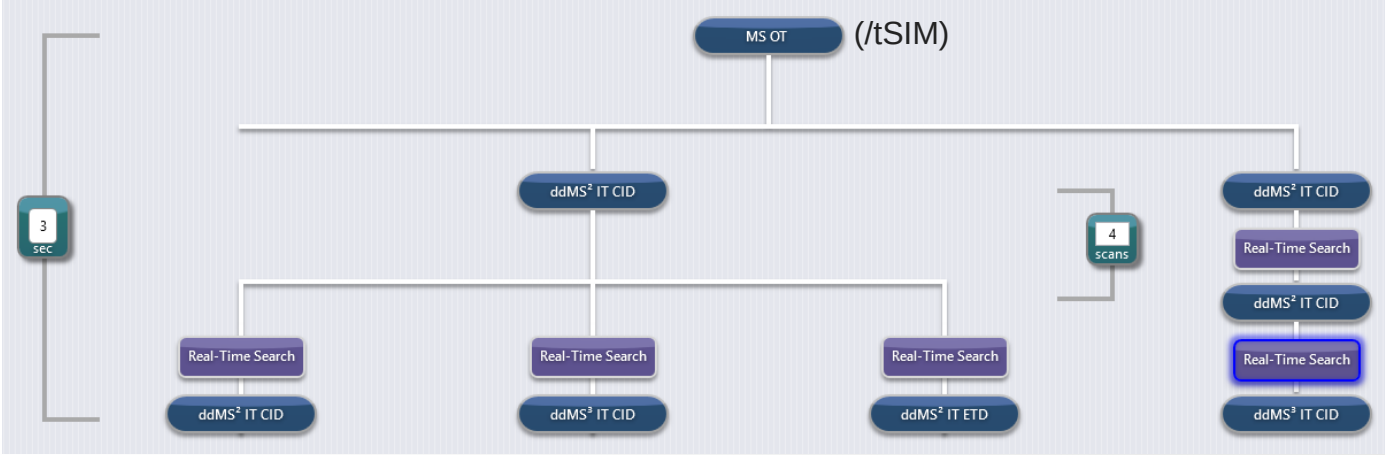
- Multiple RTS filters can be placed in the experiment under multiple MS2 nodes *NEW*

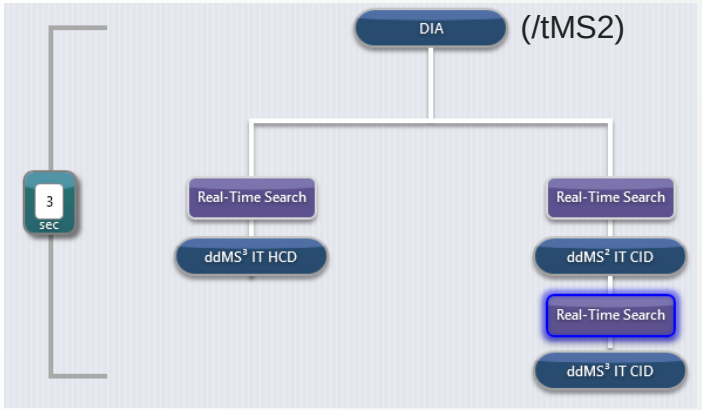
- Multiple RTS filters can use different Database Search / Peak Selection and Threshold Settings *NEW*

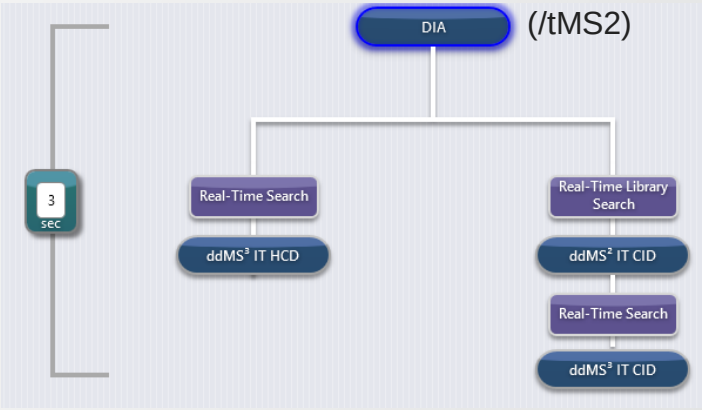
RTS can be placed under a DIA/tMS2 node *NEW*

(Note: Mandatory to allow DIA-ddMSn acquisition scheme. Other allowed filters under DIA node are 'Precursor Selection Range', 'Precursor Ion Exclusion', and 'Isobaric Tag Loss Exclusion')

- RTS and RTLS filters can be combined within a single experiment (and method), but not in series under a single MS2 node *NEW*



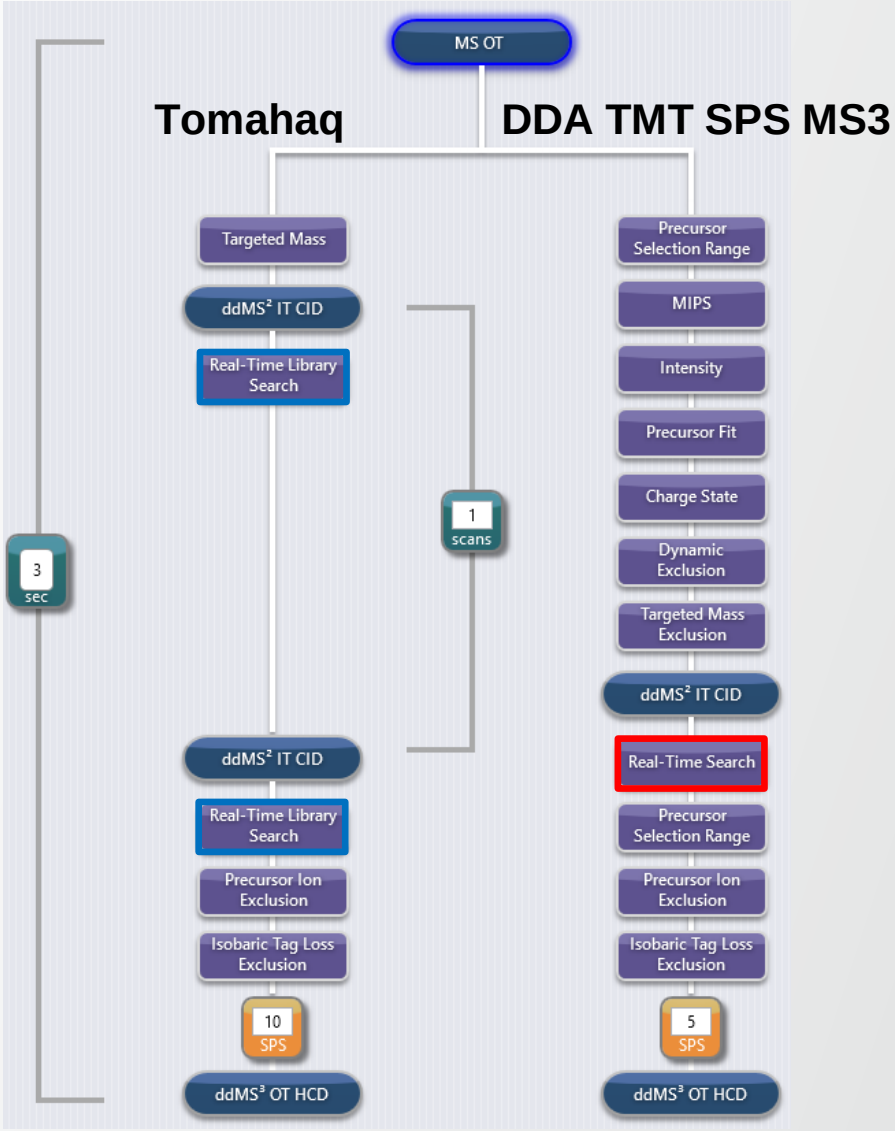




RTS / RTLS – Based Acquisition Method

Application Example: Hybrid DDA TMT SPS MS3 / TDA Tomahaq Method

- Combined Discovery and Targeted method
- First branch dedicated to Tomahaq-like acquisition
- Second branch dedicated to DDA TMT SPS MS3 acquisition
- Combination of RTS / RTLS filters across branches
- Tomahaq-like acquisition branch
  - New “Isotopic Variant Derived Offset” option (“Isolation Offset”) property enables peptide/precursor-specific definition of shifted isolation window for bottom triggered ddMS2 (e.g., from IS to ENDO peptide though RTLS)
  - Needed: Reference spectra in library properly annotated with relevant Tags

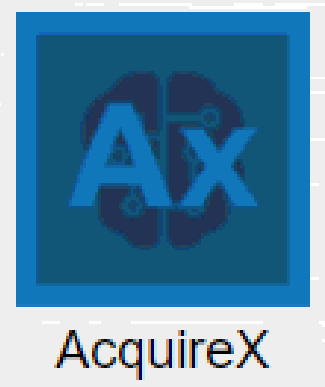


AcquireX Data Acquisition Workflow Enhancements

New Features

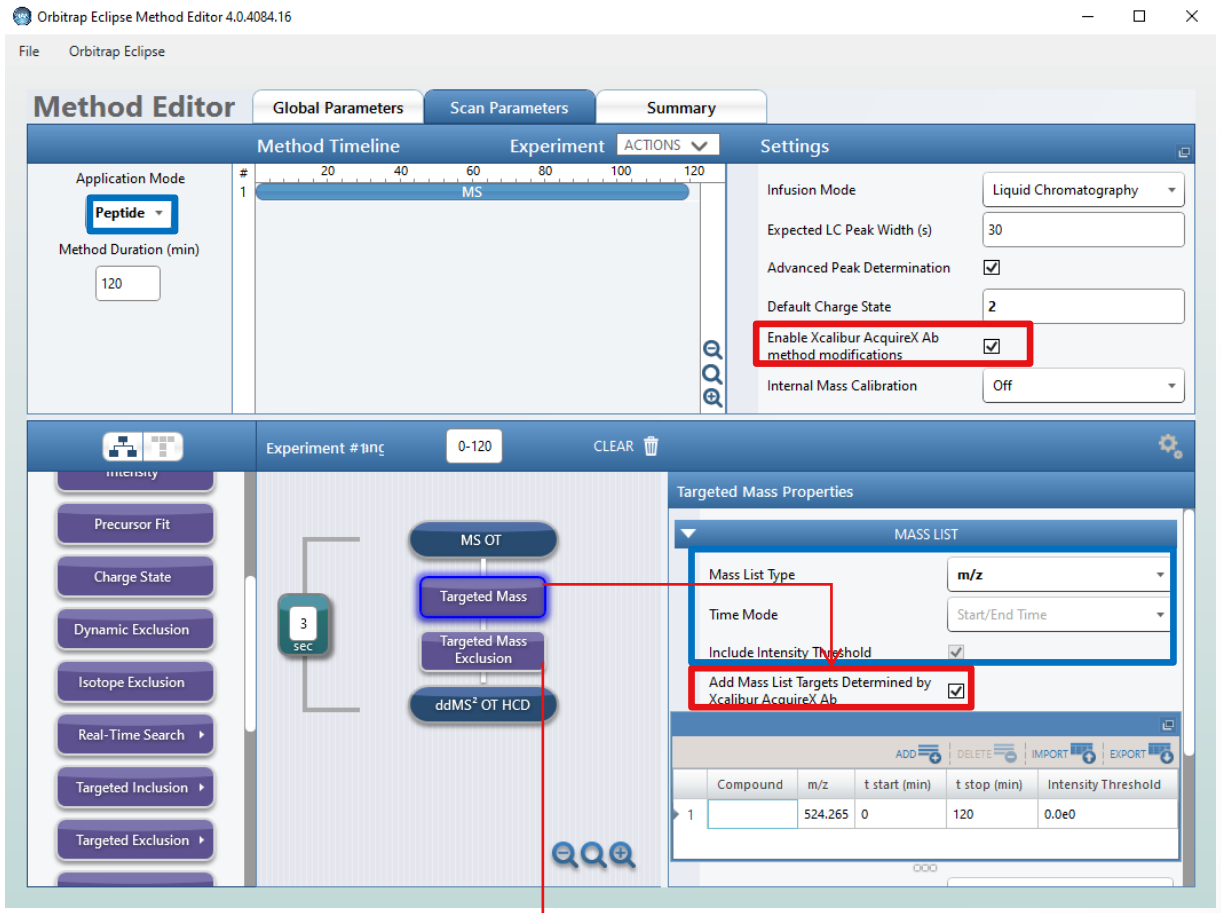
- New intelligent data acquisition workflow for Biopharma applications: AcquireX Ab
  - Available with Xcalibur 4.6 in Peptide Application Mode of Orbitrap Fusion, Fusion Lumos, Eclipse, and Ascend MS systems
  - One AcquireX Ab workflow: Custom Workflow

- New AcquireX workflow for Small Molecule applications: Custom Workflow
  - Available with Xcalibur 4.6 in Small Molecule Application Mode of all Tribrid models
  - Custom Workflow replaces Advanced Deep Scan Workflow

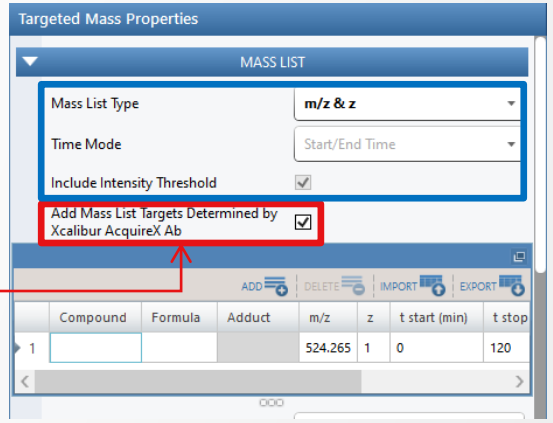


New AcquireX Ab Workflow for Peptide Mapping

New Features in Method Editor



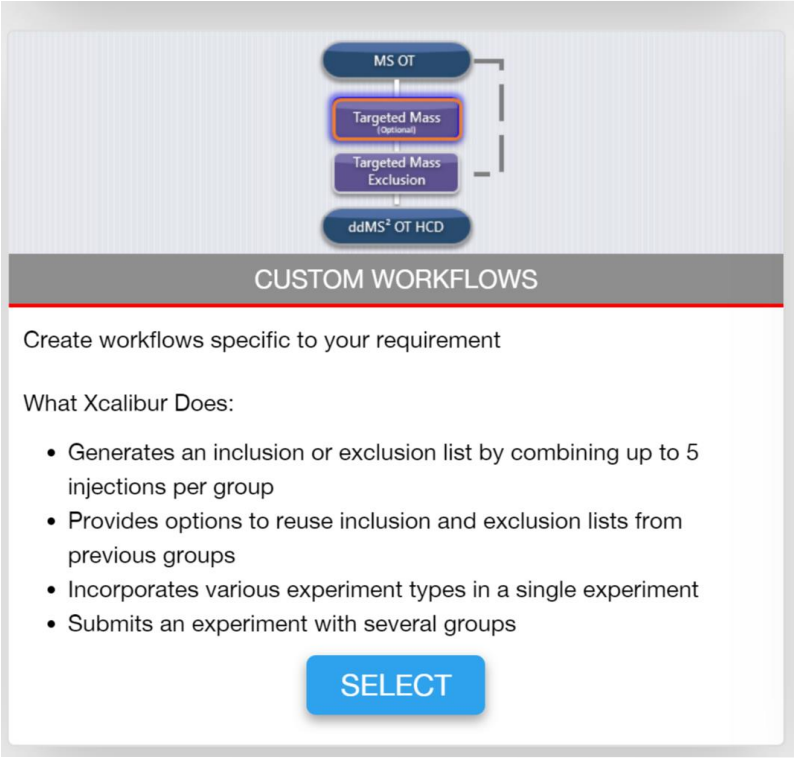
- AcquireX Ab workflows introduced with Xcalibur 4.6
- AcquireX Ab available in Peptide Application Mode
- Controls implemented in
  - Global Settings
  - Targeted Mass and Targeted Mass Exclusion filters
- Constraints
  - Time Mode = Start/End Time
  - Include Intensity Threshold = TruePrecursor Mass Range
  - Mass List Type = m/z OR m/z & z



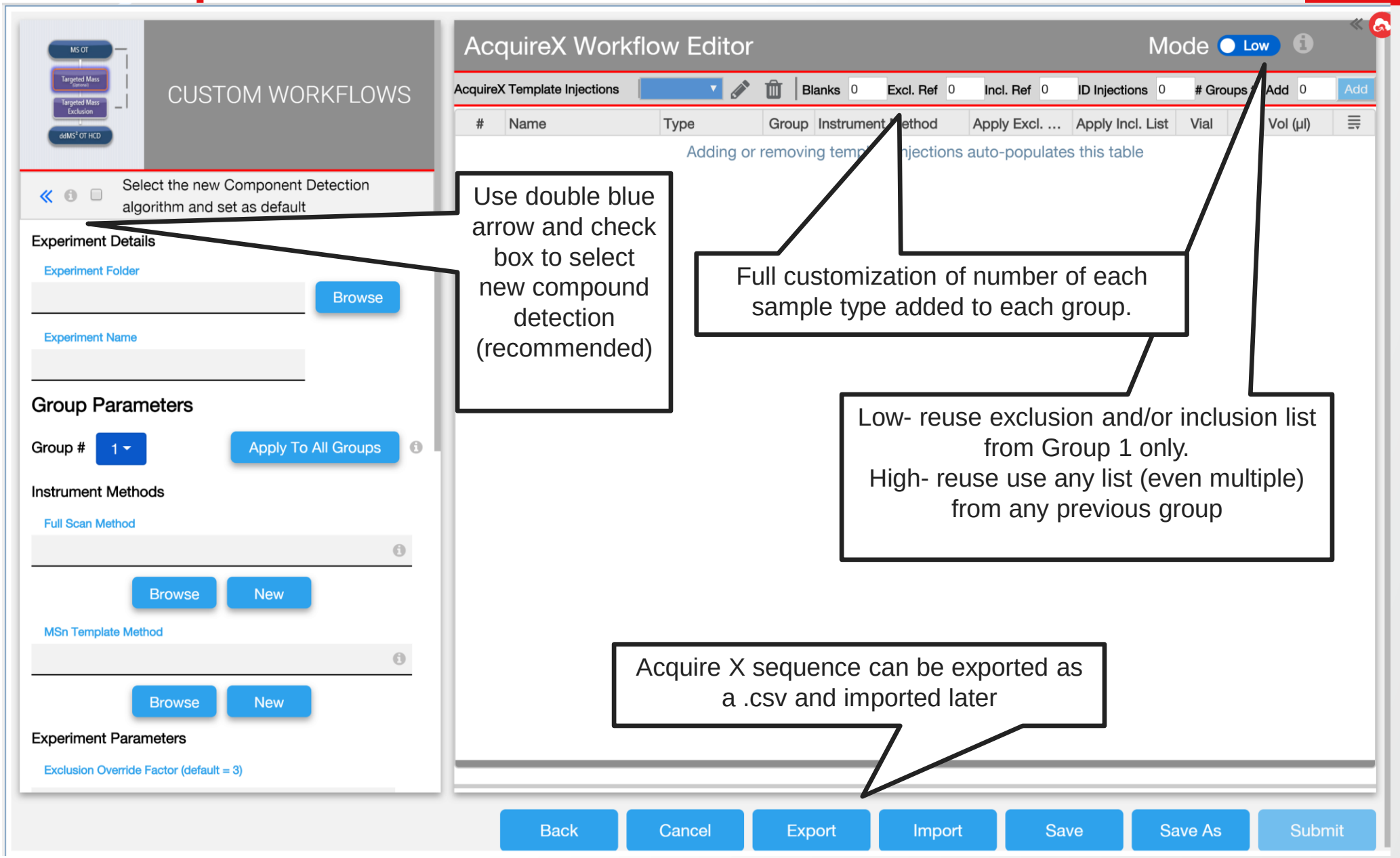
Xcalibur: Acquire X Custom Workflows

Small molecules

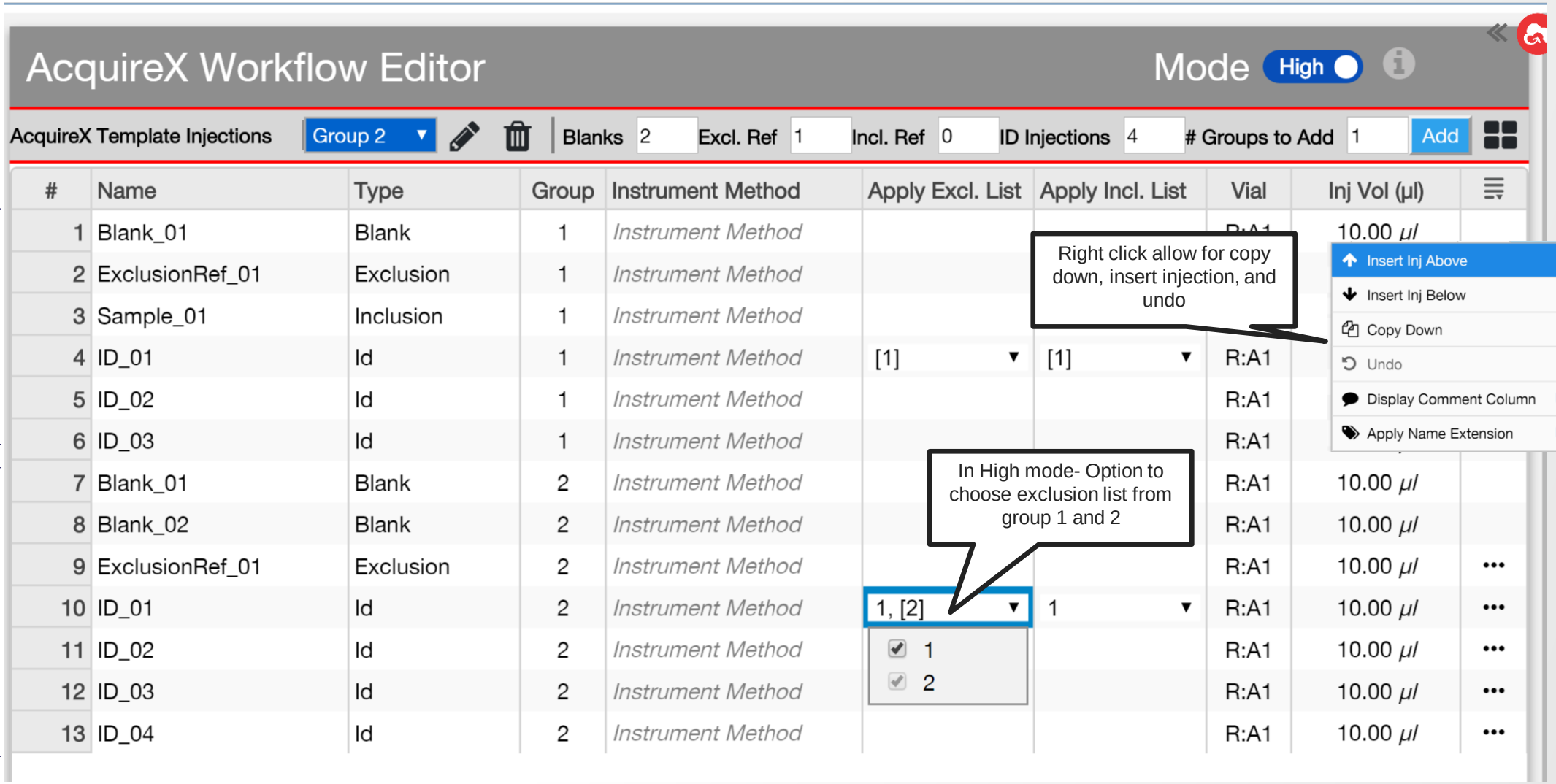
- Advanced Deep Scan has been replaced by Custom Workflows
  - Allows for the use of multiple groups in a single workflow
  - Reuse and combine inclusion and exclusion lists from previous groups
  - Reminder: option to use new component detection from Thermo Scientific™ Compound Discoverer™ software



Xcalibur: Acquire X Custom Workflows



Xcalibur: Acquire X Custom Workflows



Xcalibur: Acquire X Ab Custom workflows

Peptide and protein workflows

- New workflow for peptides/proteins, and other biopharma applications
- 1 workflow called “Custom Ab Workflow”
- Component detection is based on Thermo Scientific™ Biopharma Finder™ software Mass Analyzer algorithm
- Use MSn methods for exclusion/inclusion generation
- Reuse and combine exclusion and inclusion lists from previous groups

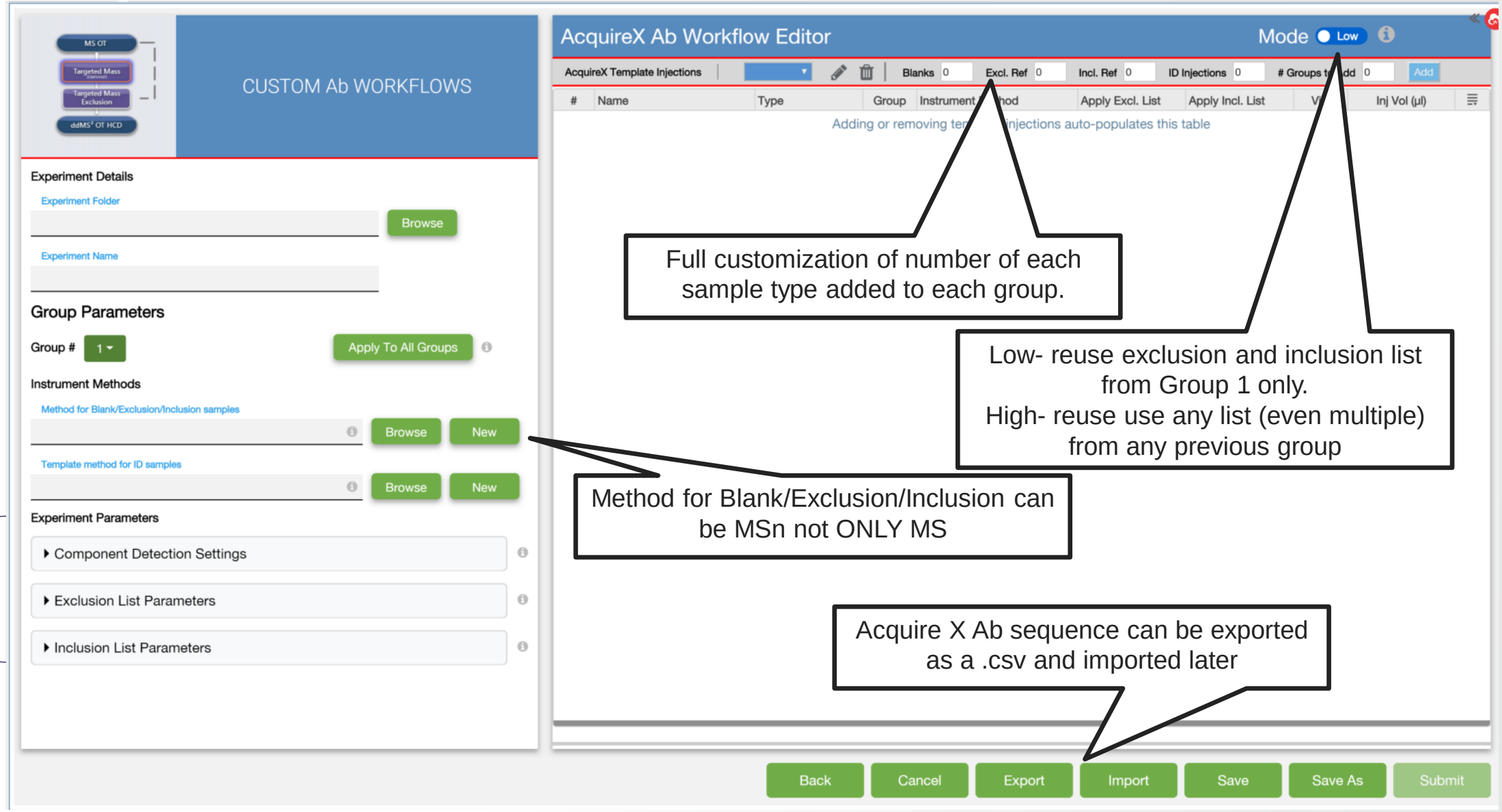
CUSTOM Ab WORKFLOWS

Create peptide mapping workflows specific to your requirement

What Xcalibur Does:

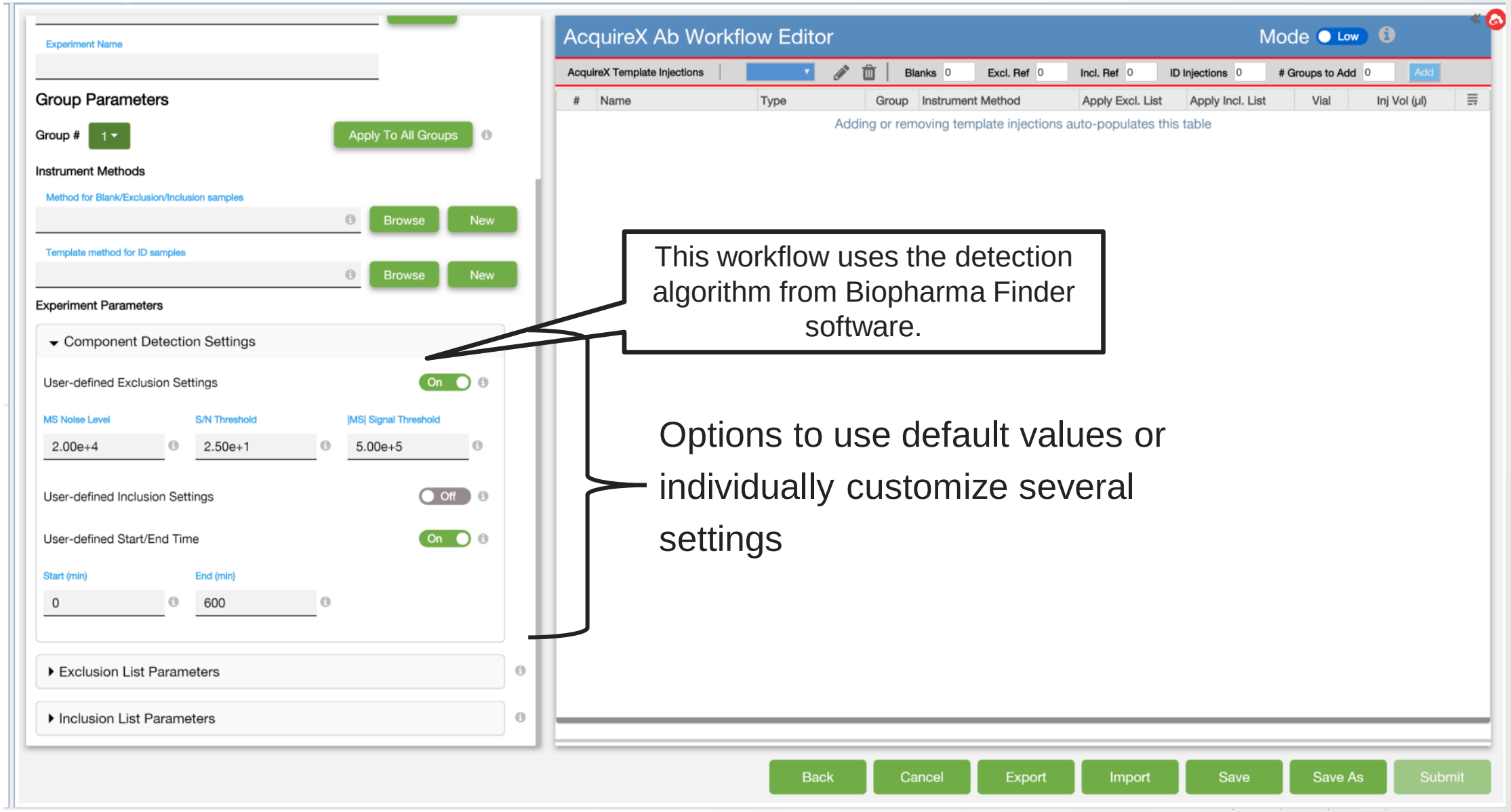
- Creates one exclusion list per group to reduce background fragmentation in your ID runs
- Creates one inclusion list per group to fragment more relevant precursor ions in multiple ID injections
- Injects ID samples iteratively for groups with an inclusion list until all ions in the inclusion list are fragmented or a user-defined number of ID injections is reached
- Injects ID samples iteratively for groups without an inclusion list until all ions in the sample are fragmented or a user-defined number of ID injections is reached
- Provides options to reuse inclusion and exclusion lists from previous groups
- Submits an experiment with several groups

Xcalibur: Acquire X Ab Custom Workflows



Fully customizable detection parameters

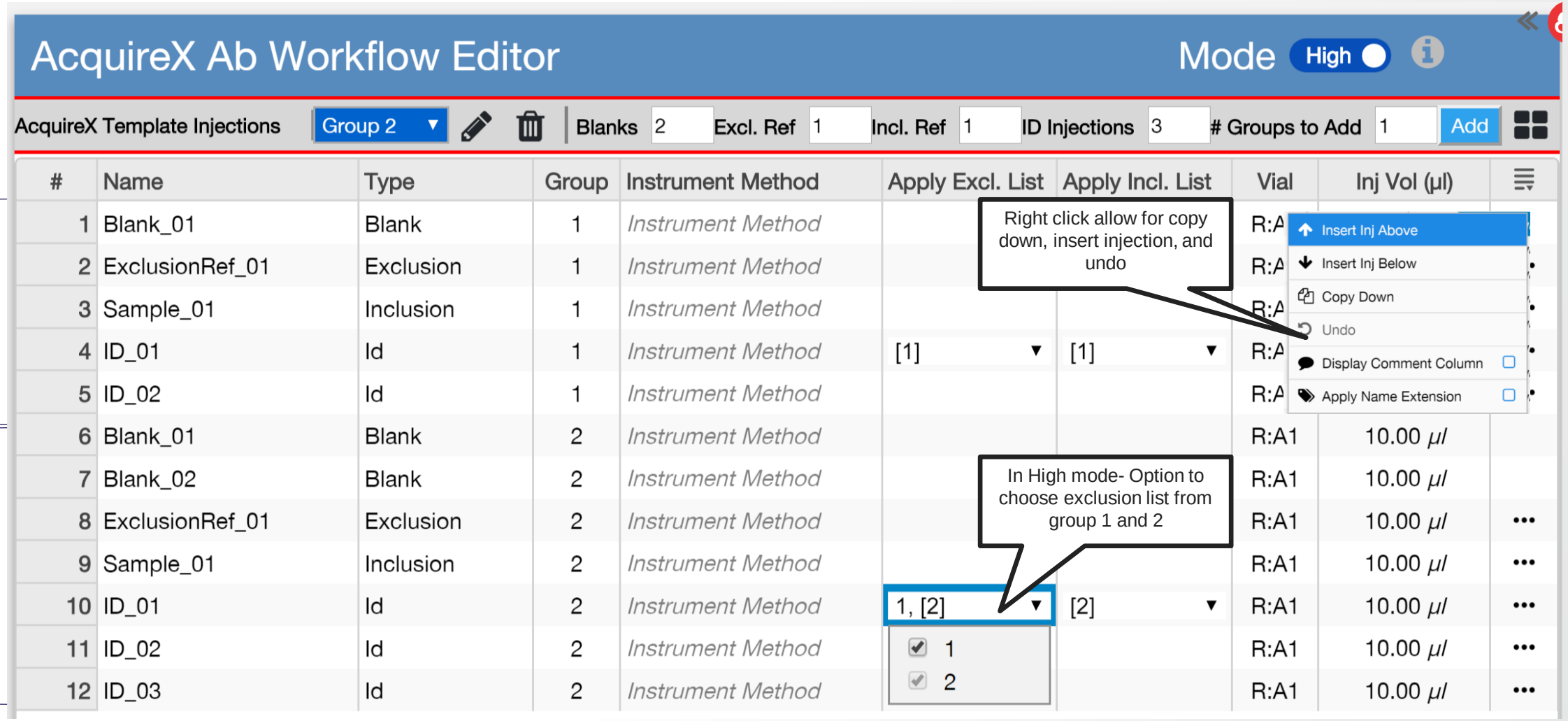
Xcalibur: Acquire X Ab Custom Workflows



This workflow uses the detection algorithm from Biopharma Finder software.

Options to use default values or individually customize several settings

Xcalibur: Acquire X Ab Custom Workflows



Scan and Isolation Ranges Accepted in the Different Scan Types

Update for all Orbitrap Tribrid Models

- Accepted ranges for Orbitrap Ascend *NEW*
- Minor corrections for earlier launched products

Mass Range	Detector	Scan Range Orbitrap Model			
		ID-X, IQ-X	Fusion, Fusion Lumos	Eclipse	Ascend
Normal	Orbitrap & Ion Trap MS1 MS2 and higher	50 – 2000 40 – 2000	50 – 2000 40 – 2000	50 – 2000 40 – 2000	50 – 2000 40 – 2000
High	Orbitrap	n/a	100 – 6000	100 – 6000	100 – 6000
	Ion Trap	n/a	100 – 4000	100 – 4000	100 – 4000
High with HMR^n license	Orbitrap	n/a	n/a	100 – 6000 500 – 8000	n/a
	Ion Trap	n/a	n/a	100 – 4000	n/a
High with HMR^n+ license	Orbitrap	n/a	n/a	n/a	100 – 6000 500 – 8000 1000 – 16000
	Ion Trap	n/a	n/a	n/a	100 – 4000

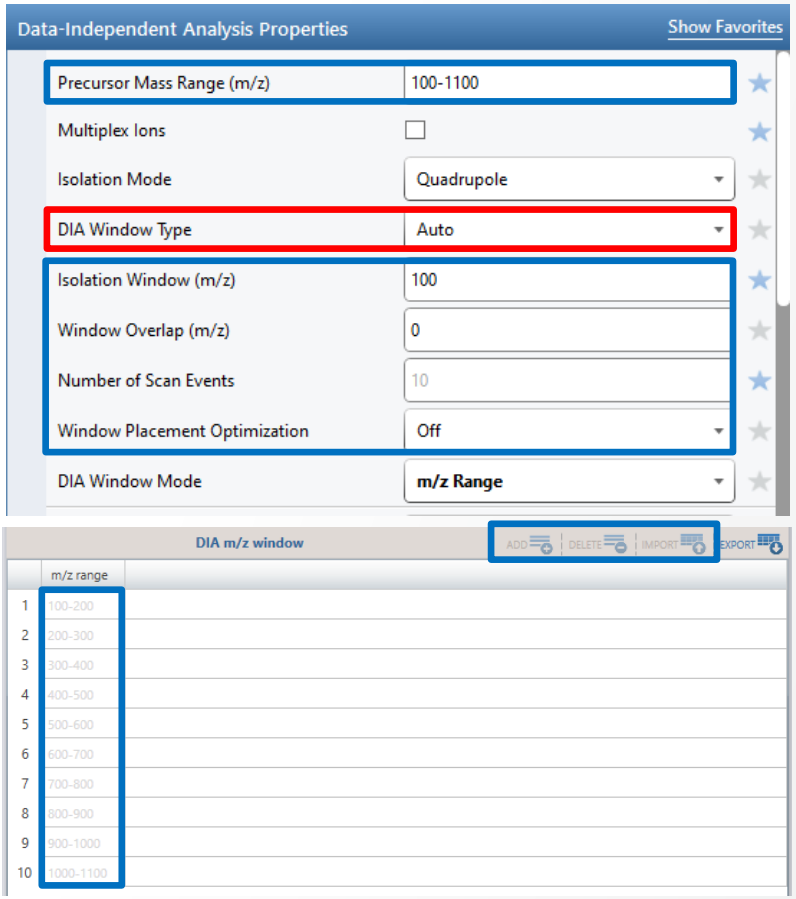
License	Scan Type	Detector	Isolation	Isolation Range Orbitrap Model			
				ID-X, IQ-X	Fusion, Fusion Lumos	Eclipse	Ascend
No License	tSIM	Orbitrap	Quadrupole	50 – 2000	50-3500	50 – 2000	50 – 2000
			Ion Trap	50 – 2000	50-4000	50 – 4000	50 – 4000
		Ion Trap	Quadrupole	50 – 2000	50-3500	50 – 2000	50 – 2000
			Ion Trap	50 – 2000	50-4000	50 – 4000	50 – 4000
	tMS2	Orbitrap	Quadrupole	50 – 2000	50-3500	50 – 2000	50 – 2000
			Ion Trap	50 – 2000	50-4000	50 – 4000	50 – 4000
		Ion Trap	Quadrupole	50 – 2000	50-3500	50 – 2000	50 – 2000
			Ion Trap	50 – 2000	50-4000	50 – 4000	50 – 4000
HMR^n	tSIM	Orbitrap	Quadrupole	n/a	n/a	50 – 2000	n/a
			Ion Trap	n/a	n/a	50 – 8000	n/a
		Ion Trap	Quadrupole	n/a	n/a	50 – 2000	n/a
			Ion Trap	n/a	n/a	50 – 4000	n/a
	tMS2	Orbitrap	Quadrupole	n/a	n/a	50 – 2000	n/a
			Ion Trap	n/a	n/a	50 – 8000	n/a
		Ion Trap	Quadrupole	n/a	n/a	50 – 2000	n/a
			Ion Trap	n/a	n/a	50 – 8000	n/a
HMR^n+	tSIM	Orbitrap	Quadrupole	n/a	n/a	n/a	50 – 2000
			Ion Trap	n/a	n/a	n/a	50 – 8000
		Ion Trap	Quadrupole	n/a	n/a	n/a	50 – 2000
			Ion Trap	n/a	n/a	n/a	50 – 4000
	tMS2	Orbitrap	Quadrupole	n/a	n/a	n/a	50 – 2000
			Ion Trap	n/a	n/a	n/a	50 – 8000
		Ion Trap	Quadrupole	n/a	n/a	n/a	50 – 2000
			Ion Trap	n/a	n/a	n/a	50 – 8000

Management of Tables in DIA Scan

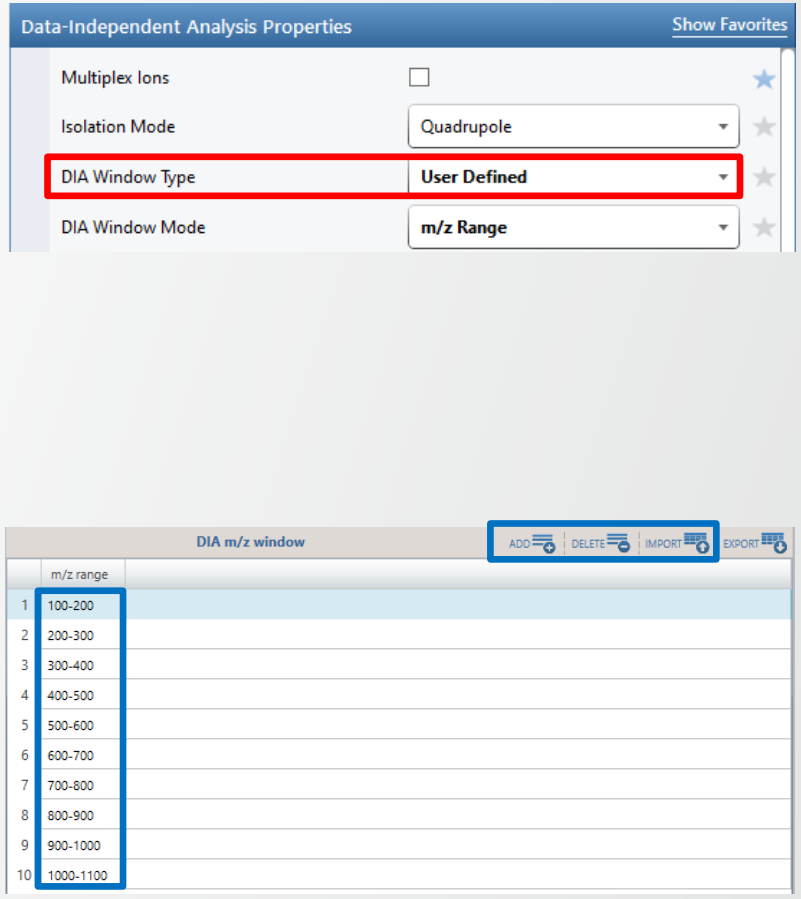
New DIA Window Type

- DIA Window Type = Auto
  - DIA windows calculated from
    - Precursor Mass Range
    - Isolation Window
    - Window Overlap
  - DIA windows in Table cannot be
    - Modified by direct typing
    - Modified by adding / deleting rows
    - Modified by importing files
- DIA Window Type = User Defined
  - DIA windows defined by
    - Direct typing
    - Adding / deleting rows
    - Importing files

DIA Window Type = Auto



DIA Window Type = User Defined

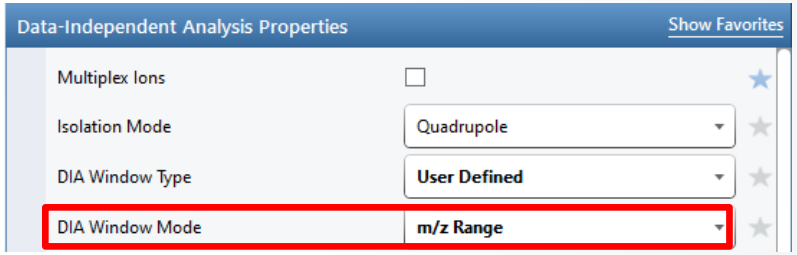


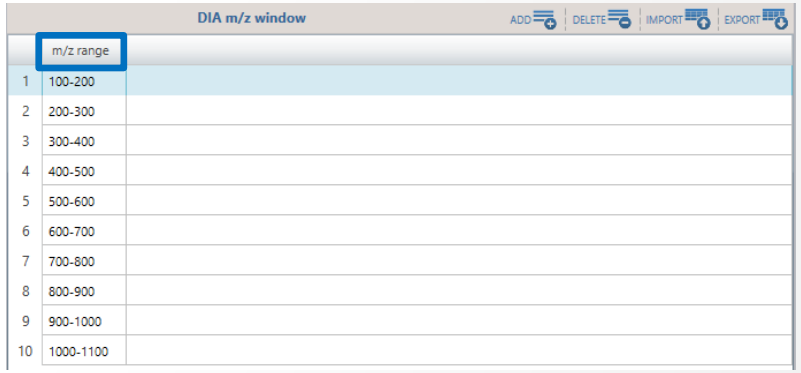
Management of Tables in DIA Scan

New DIA Window Mode

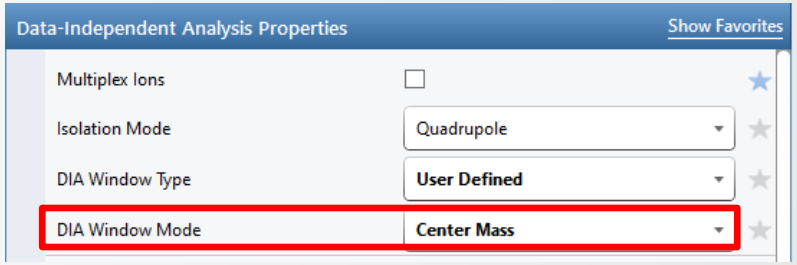
- DIA Window Mode = m/z Range
  - One column in Table
    - m/z Range
- DIA Window Mode = Center Mass
  - Two columns in Table
    - Center Mass
    - Window Width
- DIA Windows are converted when switching between the two modes
- The two DIA Window Modes are compatible with the two DIA Window Types

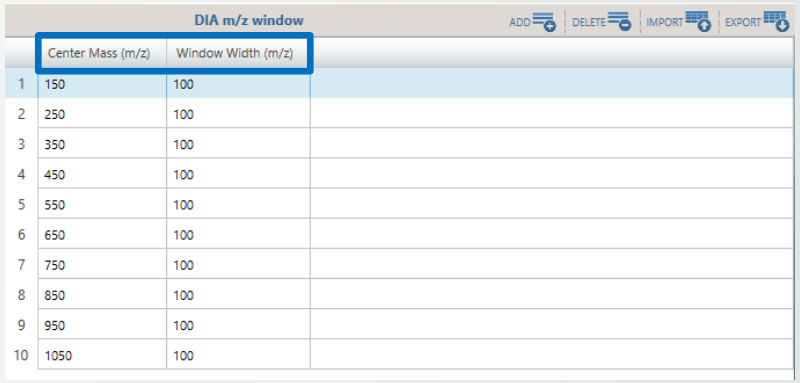
DIA Window Mode = m/z Range





DIA Window Mode = Center Mass

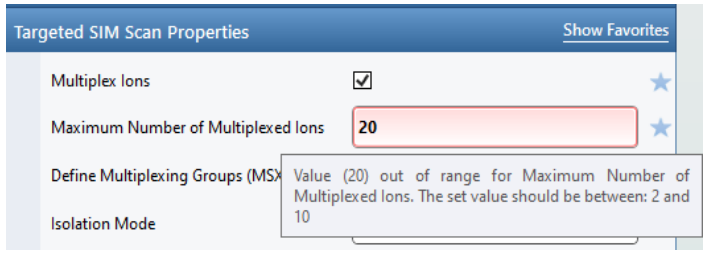


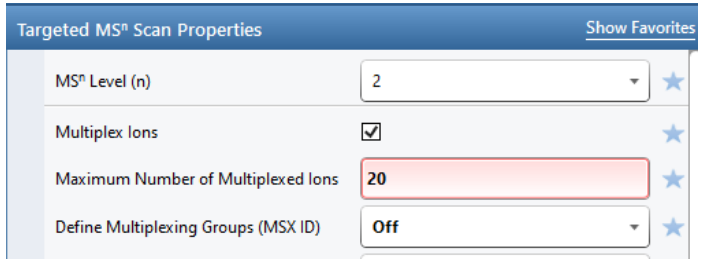


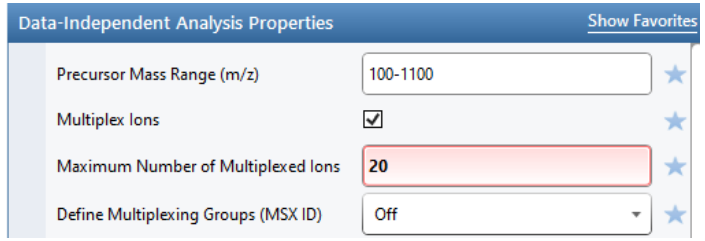
Multiplexing in tSIM / tMS2 / DIA Scans

Expanded Multiplexing to up to 20 Ions

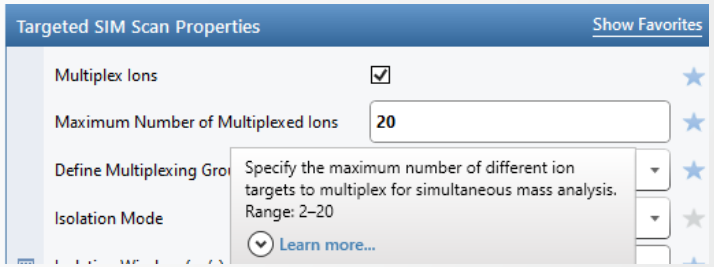
Orbitrap Tribrid Series ICSW 3.5

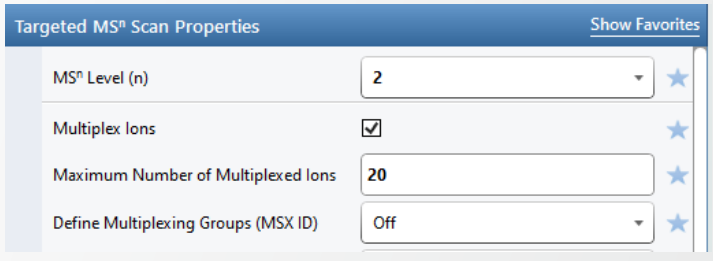


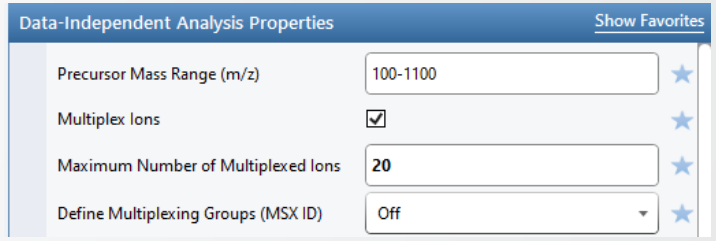




Orbitrap Tribrid Series ICSW 4.0





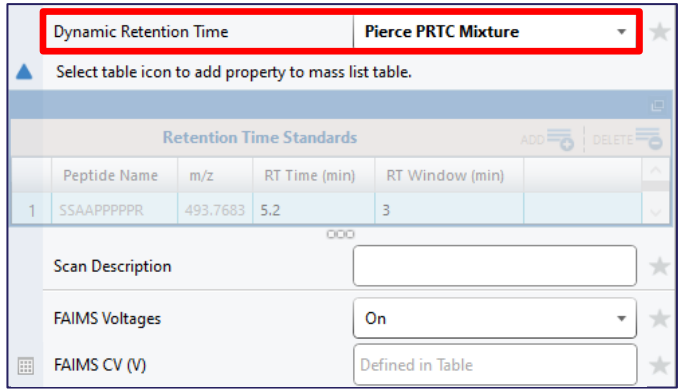


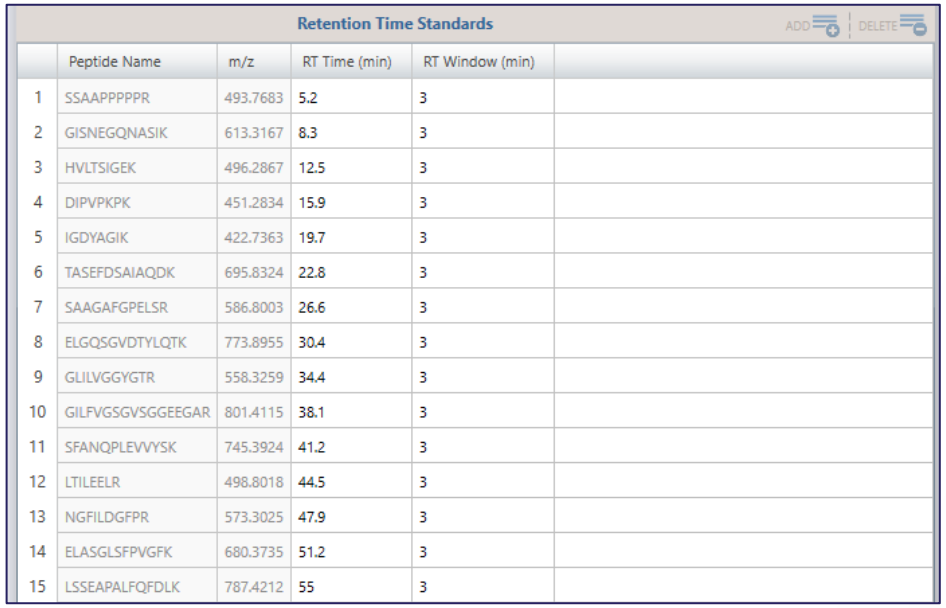
Note: When using Stepped Collision Energy Mode in tMS2 / DIA, multiplexing is limited to 10 ions

tMS2 Acquisition with Dynamic Retention Time

Enabled Definition of PRTC Peptide-Specific FAIMS CV

Orbitrap Tribrid Series ICSW 3.5

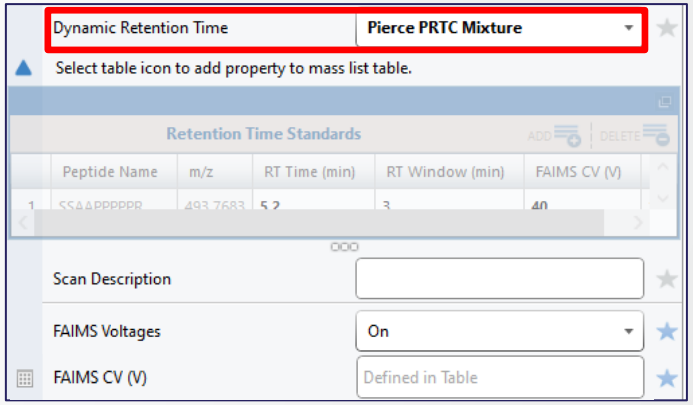


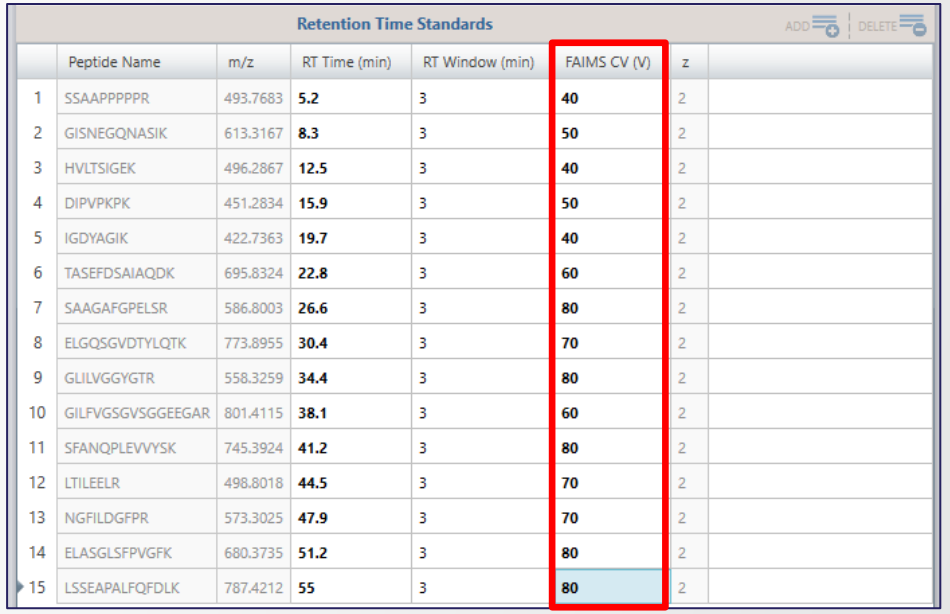


Retention Time Standards

	Peptide Name	m/z	RT Time (min)	RT Window (min)
1	SSAAPPPPPR	493.7683	5.2	3
2	GISNEGQNASIK	613.3167	8.3	3
3	HVLTSIGEK	496.2867	12.5	3
4	DIPVPKPK	451.2834	15.9	3
5	IGDYAGIK	422.7363	19.7	3
6	TASEFDSAIAQDK	695.8324	22.8	3
7	SAAGAFGPELSR	586.8003	26.6	3
8	ELGQSGVDTYLQTK	773.8955	30.4	3
9	GLILVGGYGTR	558.3259	34.4	3
10	GILFVGSGVSGGEEGAR	801.4115	38.1	3
11	SFANQPLEVVYSK	745.3924	41.2	3
12	LTILEELR	498.8018	44.5	3
13	NGFILDGFPR	573.3025	47.9	3
14	ELASGLSFPVGFK	680.3735	51.2	3
15	LSSEAPALFQFDLK	787.4212	55	3

Orbitrap Tribrid Series ICSW 4.0





Retention Time Standards

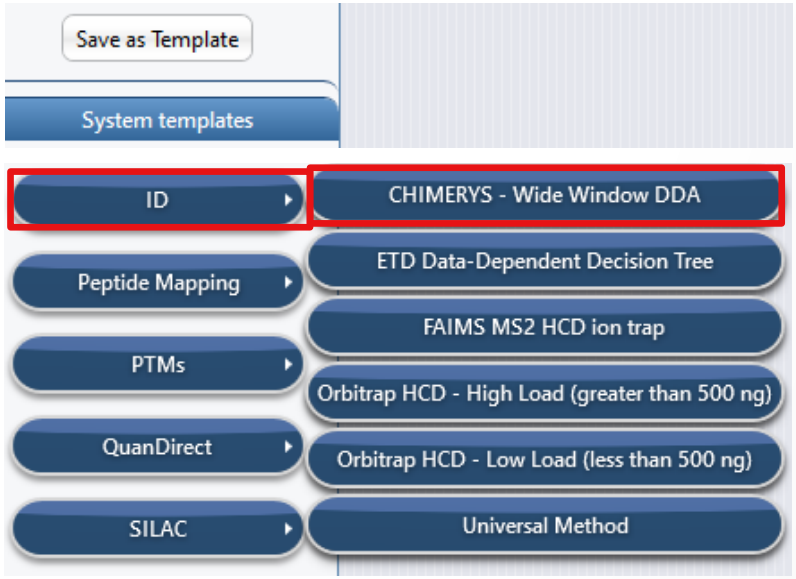
	Peptide Name	m/z	RT Time (min)	RT Window (min)	FAIMS CV (V)	z
1	SSAAPPPPPR	493.7683	5.2	3	40	2
2	GISNEGQNASIK	613.3167	8.3	3	50	2
3	HVLTSIGEK	496.2867	12.5	3	40	2
4	DIPVPKPK	451.2834	15.9	3	50	2
5	IGDYAGIK	422.7363	19.7	3	40	2
6	TASEFDSAIAQDK	695.8324	22.8	3	60	2
7	SAAGAFGPELSR	586.8003	26.6	3	80	2
8	ELGQSGVDTYLQTK	773.8955	30.4	3	70	2
9	GLILVGGYGTR	558.3259	34.4	3	80	2
10	GILFVGSGVSGGEEGAR	801.4115	38.1	3	60	2
11	SFANQPLEVVYSK	745.3924	41.2	3	80	2
12	LTILEELR	498.8018	44.5	3	70	2
13	NGFILDGFPR	573.3025	47.9	3	70	2
14	ELASGLSFPVGFK	680.3735	51.2	3	80	2
15	LSSEAPALFQFDLK	787.4212	55	3	80	2

OTS 4.0 ICSW System Templates

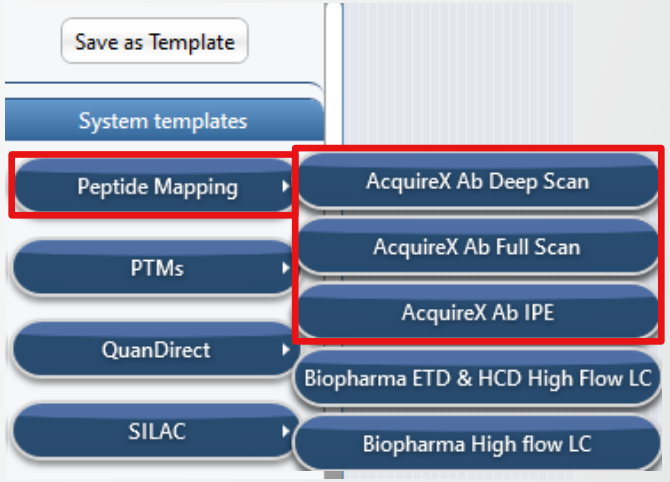
New and Updated Templates

- Ascend templates
- Chimerys templates (Peptide Application Mode)
- AcquireX Ab templates (Peptide Application Mode)
- Updated / corrected templates to improve methods and address inconsistencies

Chimerys



AcquireX Ab



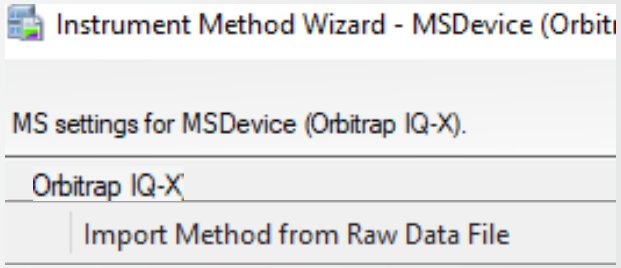
OTS 4.0 ICSW and Chromeleon CDS Software

LC-MS Data Acquisition Under Chromeleon

- **The OTS 4.0 driver** has been tested for use with Chromeleon CDS 7.2.10 MUf and CDS 7.3.1 MUa software.

- The Chromeleon Driver Compatibility matrix reflects the tested compatibility for combinations of Orbitrap Tribrid Series ICSW versions, Chromeleon software versions, and instrument models. For more information and to view the compatibility matrix, sign on to https://support.thermoinformatics.com/downloads/default.aspx, and then select **Chromeleon > Chromeleon > Related Drivers > Driver Compatibility Matrix**.

- Improvements : Method Editor menu bar is now available and allow import of method from raw data file.



- Defect fixes: The previous issue observed with Orbitrap Tribrid Series 3.5 SP2 ICSW, which prevented Workstation Method Editor to be launched without Foundation installed and therefore the use of the MS client driver on Chromeleon enterprise systems using Terminal Server / Citrix clients, has been fixed.

Thank you

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