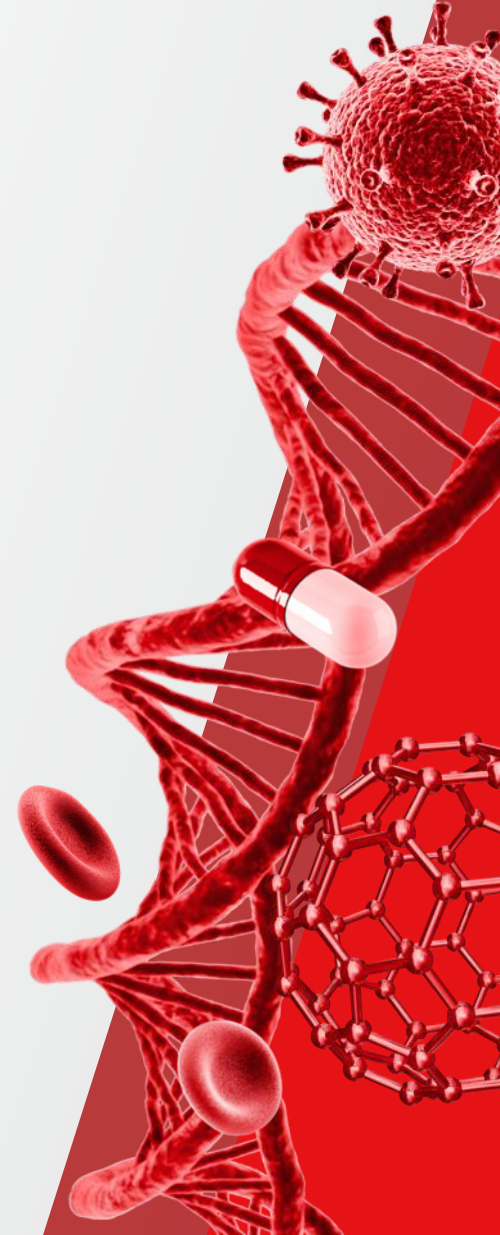


Thermo Scientific™ Orbitrap™ Tribrid™ MS Series: Instrument Control Software v.4.0 Overview

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.

The screenshot shows a web browser window with the URL <https://thermo.flexnetoperations.com/control/thmo/download?element=13262157>. The page header includes the ThermoFisher Scientific logo and the text "Life Sciences Mass Spectrometry Software Download and Licensing Portal". A navigation menu on the left lists various options under "Software & Services", "Licensing", "Administration", and "Information". The main content area is titled "Product Download" and "Orbitrap Tribrid Series 3.5 SP1". A disclaimer states: "The software you are about to download is subject to export control laws and regulations. By downloading this software, you agree that you will not knowingly, without prior written authorization from the competent government authorities, export or reexport - directly or indirectly - any software downloaded from this website to any prohibited destination, end-user, or end-use." Below the disclaimer is a table with 5 files for download:

File Description	File Size	File Name
+ LC Devices 3.2 SP2	2.1 GB	LC Devices 3.2 SP2.zip
+ LC Devices 3.2 SP3	1.9 GB	LC Devices 3.2 SP3.zip
+ Orbitrap Tribrid Series 3.5 SP1	1.6 GB	Orbitrap Tribrid Series v3.5.3886.0 SP1.zip
+ SII for Xcalibur 1.6	4.7 GB	SIIforXcalibur-Full-1.6.0.60983-381aca7bd37ac8ace7a71d644ab687251de41d60.en-US.Release.SII.Xcalibur.Setup.iso
+ Xcalibur 4.5	1.1 GB	Xcalibur 4.5.zip

Thermofisher.com & AnalyteGuru

ThermoFisher Scientific

Rechercher Recherche par référence catalogue, nom du produit, mot-

Learn more about LC-MS Data Acquisition software

You can use the instrument control software to collect high-quality mass spectrometry data on the Thermo Scientific mass spectrometers. Control of the instruments is through two application packages: Tune and Method Editor.

For questions about the software, to request features, or to report defects, send an email by clicking [here](#).

Orbitrap Tribid MS series Orbitrap Exploris MS series Exactive MS Series

Orbitrap Tribid MS series instrument control software version 3.5 SP1

Release date	Build number	Instruments	Software requirements
January 31, 2022	3.5.3886.0	Orbitrap Eclipse Tribid MS, Orbitrap Fusion Lumos Tribid MS, Orbitrap IQ-X Tribid MS, Orbitrap ID-X Tribid MS	Xcalibur 4.3 or later (use 4.5 for the latest AcquireX features). Note, use of Orbitrap Tribid MS series 3.5 requires Windows 10.

Follow the upgrade instructions provided: Orbitrap Tribid MS series ICSW v. 3.5 SP1 [Release notes](#)

Orbitrap Tribid MS Series ICSW v. 3.5 SP1 and Xcalibur 4.5 Software: [Download the software](#)

Orbitrap Tribid MS Series ICSW v.3.5 and v3.5 SP1 Overview: [Download the update overview](#)

New features

The Orbitrap Tribid MS series instrument control software version 3.5 and 3.5 SP1 incorporates the following new and improved features:

- Support for the Orbitrap IQ-X Tribid MS mass spectrometer
- (Orbitrap IQ-X Tribid MS and Orbitrap Eclipse Tribid MS small molecule application mode only) Support for Real-Time Library Search, facilitating decision-based triggering of MSn scan events in small molecule applications
- (Orbitrap IQ-X Tribid MS only) Support for Auto-Ready ion source, enabling automated and remote calibration

- Updates: [AnalyteGuru.com](https://www.analyteguru.com)
- To receive focused updates, subscribe to the pertinent labels (e.g., *Orbitrap Tribid MS Instrument Control Software*)

AnalyteGuru > Knowledgebase > Scientific Library > Orbitrap Tribid MS Series Instrument Control Soft...

Orbitrap Tribid MS Series Instrument Control Software Version 3.5 SP1

Sebastien Team TFS on 03-07-2022 08:11 AM

On January 31, 2022, version 3.5 SP1 of the Orbitrap Tribid MS Series Instrument Control Software was released. It supports Orbitrap Eclipse, Orbitrap Fusion, Orbitrap Fusion Lumos, Orbitrap ID-X, and Orbitrap IQ-X MS systems.

Visit our [LC-MS Data Acquisition Software page](#) to download the software and learn more.

Orbitrap Tribid Orbitrap Tribid MS Instrument Control Software Software

2 Kudos Share

Version history

Last update: 03-07-2022 08:11 AM

Updated by: Molly_I2

Contributors

Sebastien

- Information: [Thermofisher.com](https://www.thermofisher.com)
 - Software information
 - Known Issues
 - Links for download
 - Discovered issues
 - New Features
 - 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



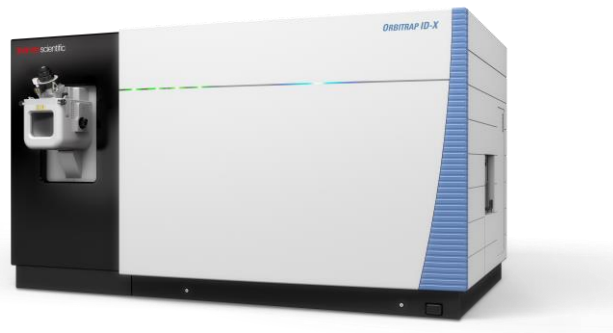
Thermo Scientific™
Orbitrap Fusion™ MS



Thermo Scientific™
Orbitrap Fusion™ Lumos™ MS



Thermo Scientific™
Orbitrap Eclipse™ MS



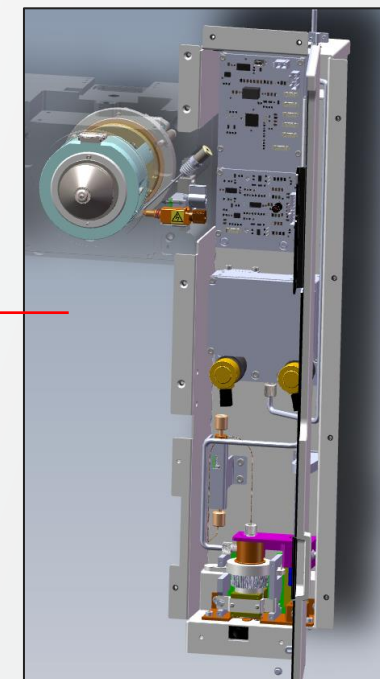
Thermo Scientific™
Orbitrap ID-X™ MS



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



Orbitrap Tribrid™ Series 4.0

Instrument Control Software

thermo
scientific

Resolved Issues in OTS 4.0 SP1

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

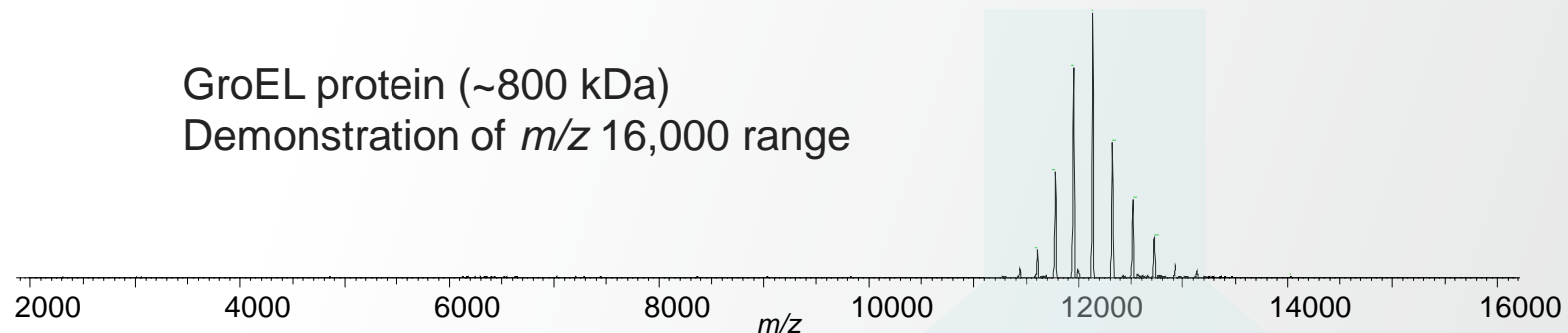
- Addressed an issue in which injection time may oscillate during LC-MS runs of intact proteins on Orbitrap Ascend system
- Addressed an issue in which Discharge Pressure Check fails due to readback error on Orbitrap Fusion ETD system
- Modified accepted range for energy dependency on Orbitrap Ascend system
- Addressed incorrect application of isolation waveforms in MSⁿ 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
 - HMRⁿ+ 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 MS_n levels

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



MS Scan Properties		Show Favorites
Detector Type	Orbitrap	★
Orbitrap Resolution	120000	★
Mass Range	High	★
Use Quadrupole Isolation	<input type="checkbox"/>	★
Scan Range (m/z)	1000-16000	★

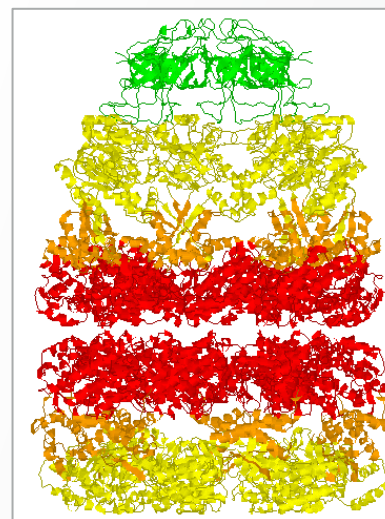
Specify the m/z range over which the ion trap or Orbitrap mass analyzer detects ions.
Range: 100-16000

[Learn more...](#)

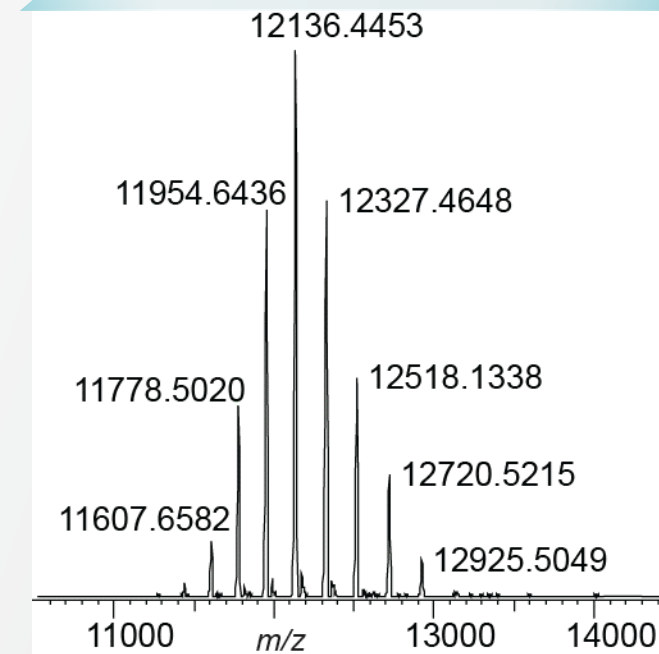
The allowed scan range (in m/z units) depends on the selected mass range.

- Normal range: 50-2000
- High range:
100-4000 (Ion trap detector)
100-6000 (Orbitrap detector)
100-6000; 500-8000; 1000-16000 (HMRⁿ+ 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 (HMRⁿ+ 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 HMRⁿ License

ION SOURCE	DEFINE SCAN	CALIBRATION
Status		
Calibration		
Mode	Check, Calibrate if required	
Polarity	Positive	
Type	Orbitrap Mass & System	
Optional Calibrations		
Intact Protein	<input type="checkbox"/>	
High Mass	<input checked="" type="checkbox"/>	
ETD & PTR	<input type="checkbox"/>	
UVPD	<input type="checkbox"/>	

Calibration Tab

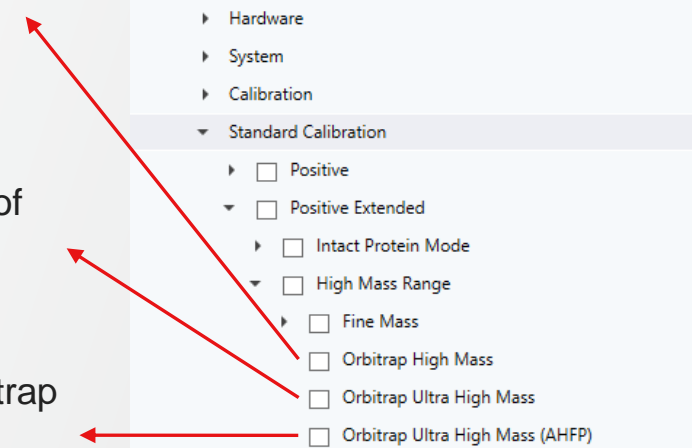
Orbitrap High Mass: This procedure runs the Orbitrap calibration in the high mass range up to m/z 4000 (use of FlexMix calibration solution)

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

ION SOURCE	DEFINE SCAN	CALIBRATION
Diagnostics		
<input type="checkbox"/> Check Only (Applies only to Standard Calibration)		
▶ Hardware		
▶ System		
▶ Calibration		
▼ Standard Calibration		
▶ <input type="checkbox"/> Positive		
▼ <input type="checkbox"/> Positive Extended		
▶ <input type="checkbox"/> Intact Protein Mode		
▼ <input type="checkbox"/> High Mass Range		
▶ <input type="checkbox"/> Fine Mass		
▶ <input type="checkbox"/> Orbitrap High Mass		
▶ <input type="checkbox"/> Orbitrap Ultra High Mass		
▶ <input type="checkbox"/> Orbitrap Ultra High Mass (AHFP)		



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
 - HMRⁿ+ option activated
 - Application Mode set to Intact Protein
 - Pressure Mode set to High Pressure
 - Source Fragmentation applied
- Also available for Orbitrap Eclipse (with HMRⁿ 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)

MS Scan Properties		Show Favorites
Detector Type	Orbitrap	★
Orbitrap Resolution	7500	★
Mass Range	High	★
Use Quadrupole Isolation	<input type="checkbox"/>	★
Scan Range (m/z)	2000-16000	★
RF Lens (%)	60	★
AGC Target	Standard	★
Maximum Injection Time Mode	Auto	★
Microscans	1	★
Data Type	Profile	★
Polarity	Positive	★
Source Fragmentation	<input checked="" type="checkbox"/>	★
Energy (V)	150	★
Source CID Compensation Scaling	<input checked="" type="checkbox"/>	★
Scaling Factor	0.05	★
Scan Description		★

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 MS² (when isolating with quadrupole)
 - **Back Ion Routing Multipole** is for ion routing to OT of CID, ETD, UVPD, PTCR and MSⁿ HCD products

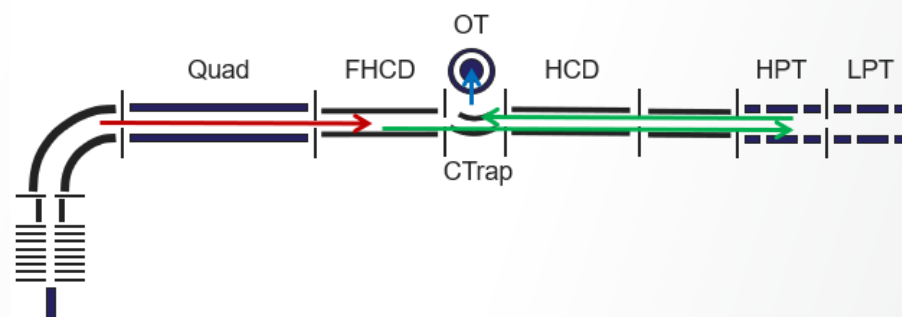
- Benefits

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, MS_n

- 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')



Injection and setup:

- Initialize the front end
- Inject ions

Manipulate:

- Manipulate the precursor ions (HCD, CID, ETD, etc).
- MS_n >= 2.

Analysis:

- OT analysis

Support for Orbitrap Ascend Mass Spectrometer

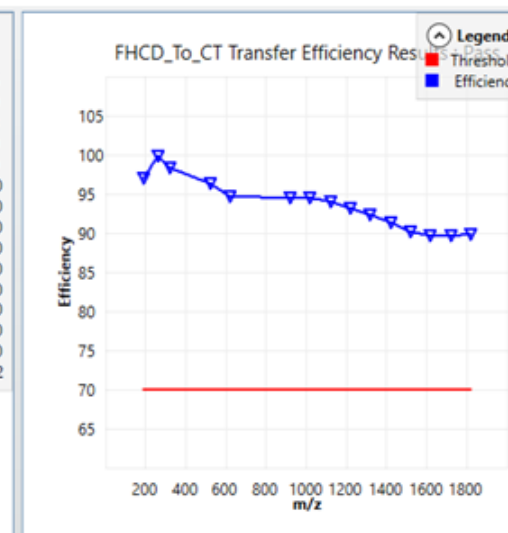
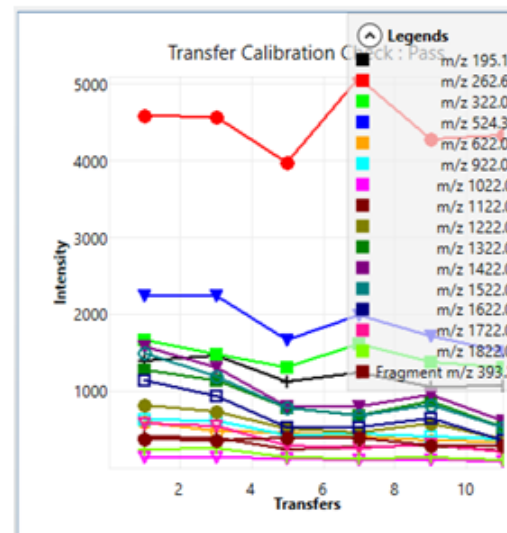
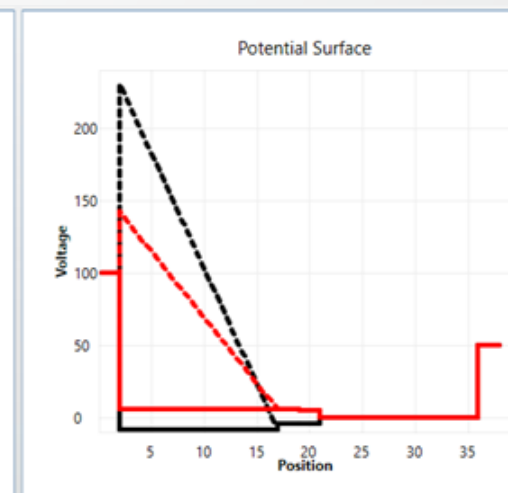
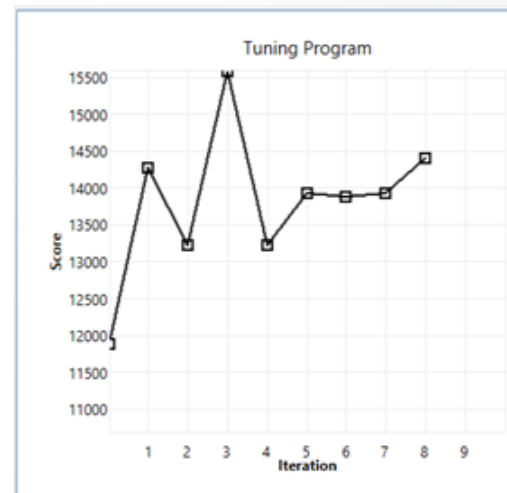
New calibrations to support the IRM before the CTrap

The FHCD \leftrightarrow CTrap transfer calibrations follows the same form as the CTrap \leftrightarrow HCD transfer calibration:

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

Positive
 Ion Optics
 Source to IRM
 Front IRM to C-Trap
 IRM to Ion Trap
 Ion Trap, Front to Back
 IRM to C-Trap
 Source Pre-filter
 HCD Collision Energy

Positive Extended
 Intact Protein Mode
 Ion Optics
 Low Pressure
 Source to IRM
 Front IRM to C-Trap
 IRM to C-Trap
 IRM to Ion Trap
 High Pressure
 Source to IRM
 Front IRM to C-Trap
 IRM to C-Trap
 IRM to Ion Trap



Support for Orbitrap Ascend Mass Spectrometer

New / Renamed Orbitrap Resolution Settings

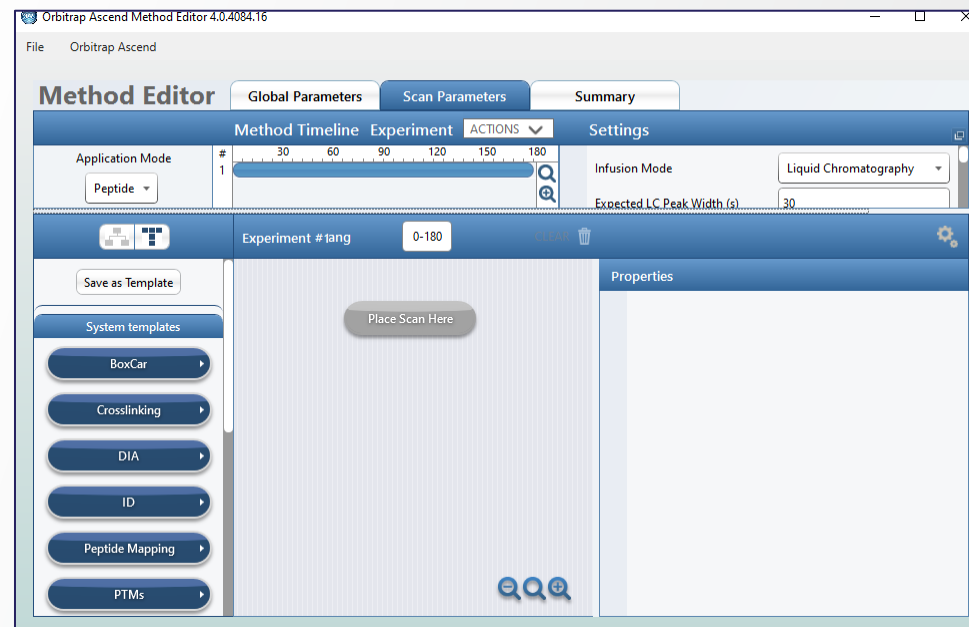
Resolving power	Max Parallelizable Injection Time
7.5K	11 ms
15K	27 ms
22.5K	43 ms
30K	59 ms
45K	91 ms
60K	123 ms
120K	251 ms
240K	507 ms
480K	1019 ms

- 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



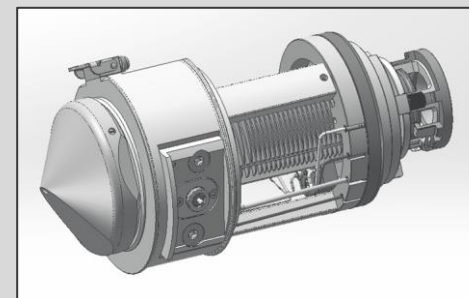
Auto-Ready Ion Source

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

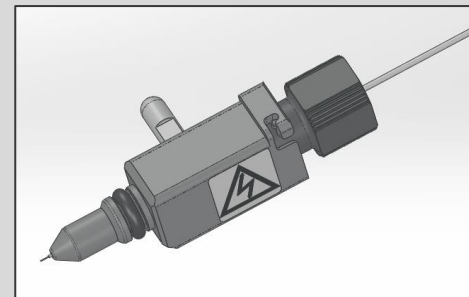
Its own ion transfer tube



Robust delivery system



Dedicated emitter



Auto-Ready Ion Source

Setting up Auto-Calibration

Under Tune Preferences



Tune Preferences

Report Options

Calibration Reports

Automatically generate reports
Path: C:\Thermo\Instruments\Reports\

Show Report Generation Options dialog box
 Do not generate reports

Other Reports

Automatically generate reports
Path: C:\Thermo\Instruments\Reports\

Show Report Generation Options dialog box
 Do not generate reports

Report Content Options

Show Console
 Show graph
 Show spectrum
 Show system configuration
 Show embedded system configuration

Current Calibration Options

Set System to Standby on Completion

Calibration Source

Auto-Ready
 Other

Calibration Mix

Traditional
 FlexMix

System Self-Check Options

Run system self-check in 'Check' mode
 Run system self-check in 'Check, Calibrate if required' mode
(please press F1 to view the help on what will be run for self-check)

Schedule Self-Check

Day: Wednesdays Time: 12 AM

Settings

Polarity: Positive

UVPD
 Easy-IC

Current Calibration Options

Set System to Standby on Completion

Calibration Source

Auto-Ready
 Other

System Self-Check Options

Run system self-check in 'Check' mode
 Run system self-check in 'Check, Calibrate if required' mode
(please press F1 to view the help on what will be run for self-check)

Schedule Self-Check

Day: Wednesdays Time: 12 AM

Settings

Polarity: Positive

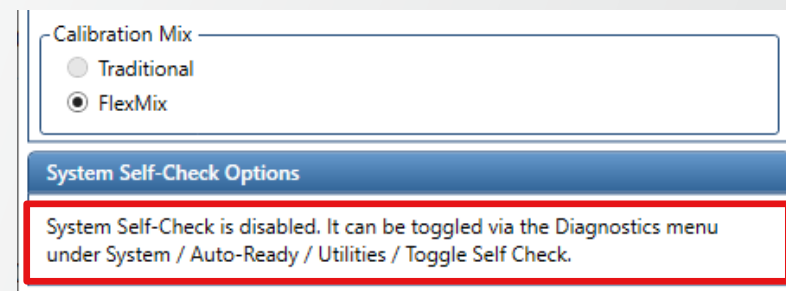
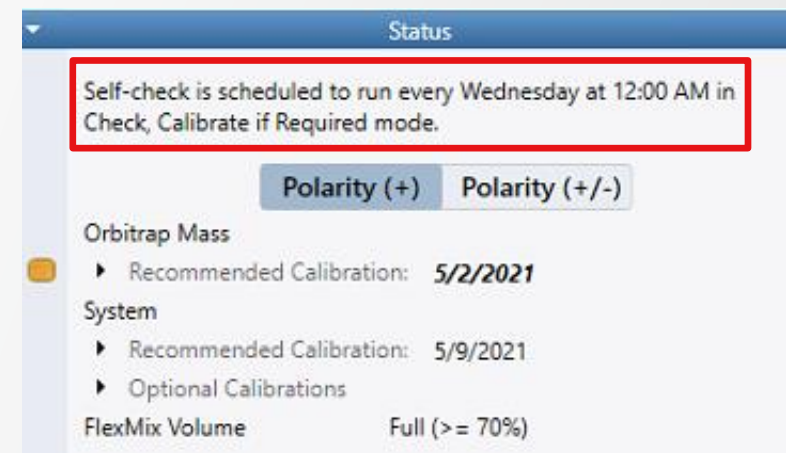
UVPD
 Easy-IC

- 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

Status

Self-check is scheduled to run every Wednesday at 12:00 AM in Check, Calibrate if Required mode.

Polarity (+) Polarity (+/-)

Orbitrap Mass

Positive

▶ Recommended Calibration: 5/5/2021

Negative

▶ Recommended Calibration: 5/5/2021

System

Positive

▶ Recommended Calibration: 5/5/2021

Negative

▶ Recommended Calibration: 5/5/2021

▶ Optional Calibrations

FlexMix Volume Full (>= 70%)

Calibration

Mode Calibrate

Polarity Positive

Type Orbitrap Mass & System

Optional Calibrations

UVPD

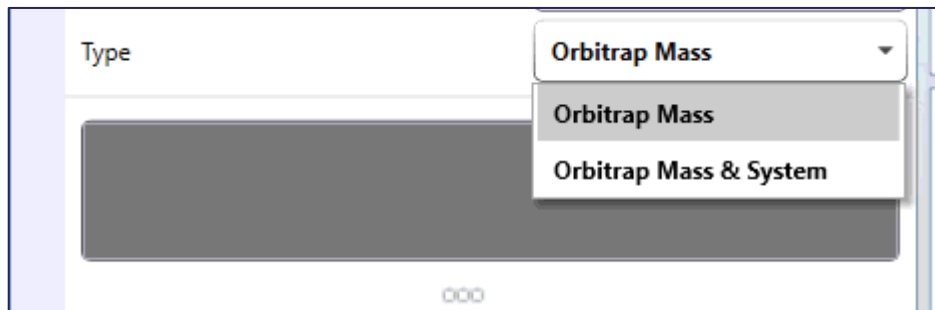
Easy-IC

Start

Same as with Conventional source

Auto-Ready Ion Source

Calibration Tab - Calibration Types

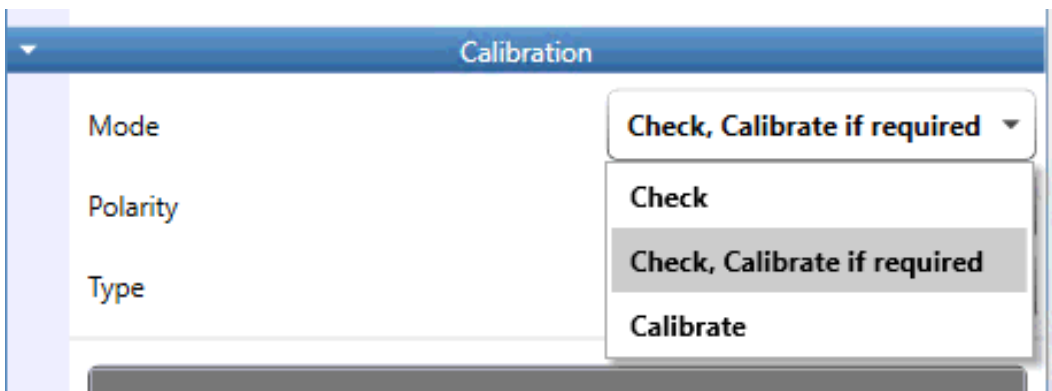


- Types: **Orbitrap Mass** or **Orbitrap Mass & System**
- Selecting Orbitrap Mass triggers the following calibrations:
 - multiplier
 - Orbitrap mass
- Selecting Orbitrap Mass & System triggers the following calibration set, in order:
 - Ion Optics
 - Ion Trap
 - Quadrupole
 - pAGC
 - Orbitrap mass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



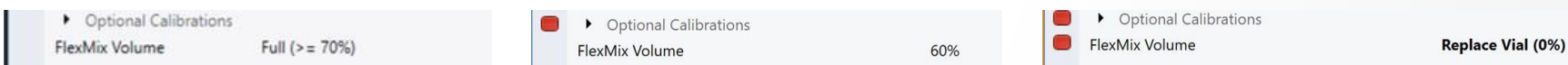
- Modes: “Check”, “Check, calibrate if required”, or “Calibrate”
 - A spray check and calibrant purity evaluation are automatically run prior to any calibration set
- “Check, calibrate if required” operates as follows:
 - Any failed checks are automatically calibrated (as well as dependent calibrations)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

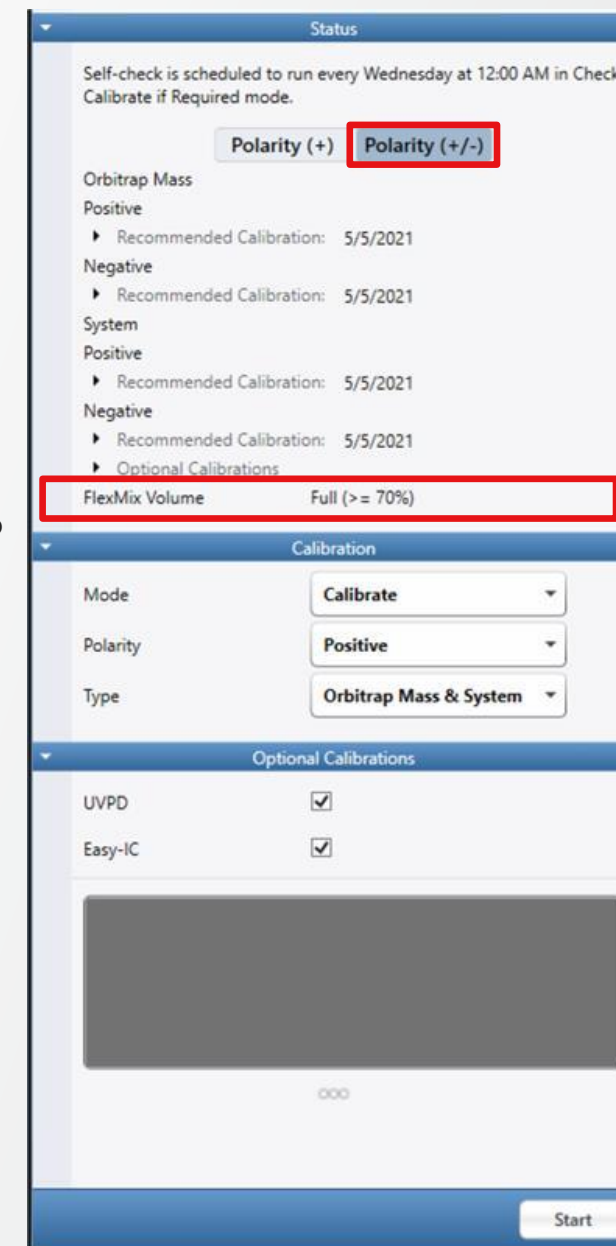
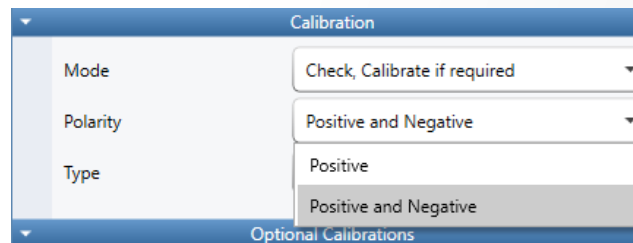
Auto-Ready Ion Source

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:

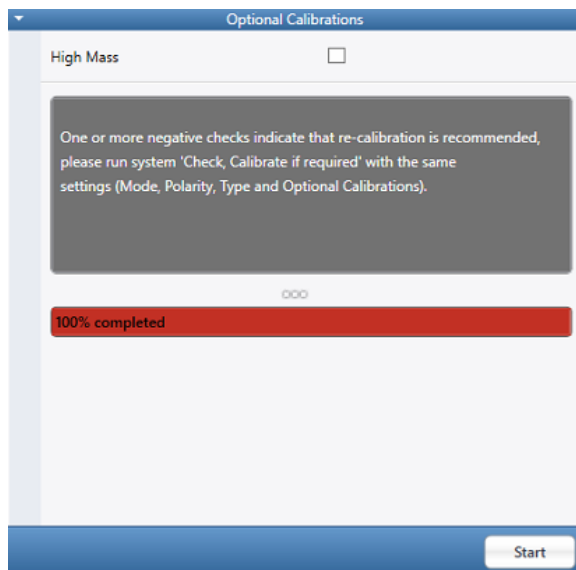
 - *Confirm that the calibrant vial has not run out of liquid. If necessary, install a new bottle and run the 'New Vial Installed' diagnostic*
 - *Run the 'Flush Lines' diagnostic (under 'Auto-Ready') to clear any residual air bubbles from the lines*
 - *Run the 'Calibrate Spray Voltage' diagnostic (under '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:

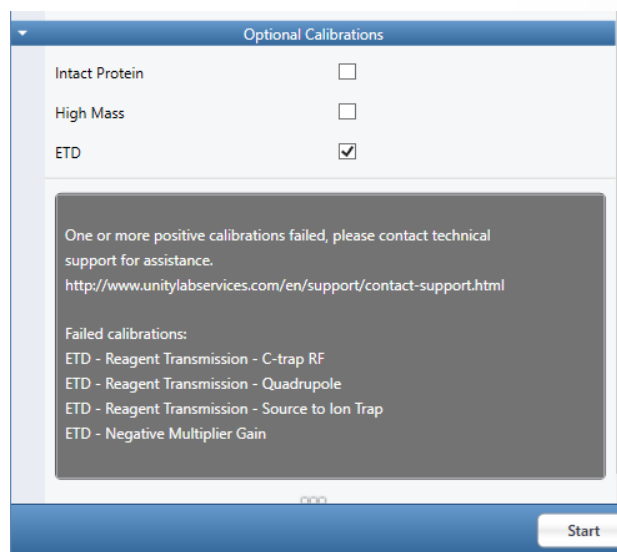
 - *Run the 'Flush Lines' diagnostic (under 'Auto-Ready') in attempt to clear any contamination buildup from the lines*
 - *Change the calibrant bottle to a fresh one*

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

The screenshot shows the 'Status' page of the instrument. At the top, it states 'Self-check is scheduled to run every Wednesday at 12:00 AM in Check, Calibrate if Required mode.' Below this, there are two buttons: 'Polarity (+)' and 'Polarity (+/-)'. Under the 'Orbitrap Mass' section, there is an orange indicator light and the text 'Recommended Calibration: 5/2/2021'. The 'System' section shows 'Recommended Calibration: 5/9/2021' and 'Optional Calibrations'. The 'FlexMix Volume' is shown as 'Full (>= 70%)'.

The screenshot shows the 'Status' page of the instrument. At the top, it states 'Self-check is scheduled to run every Wednesday at 12:00 AM in Check, Calibrate if Required mode.' Below this, there are two buttons: 'Polarity (+)' and 'Polarity (+/-)'. Under the 'Orbitrap Mass' section, there is a red indicator light and the text 'Recommended Calibration: 5/2/2021'. The 'System' section shows 'Recommended Calibration: 5/9/2021' and 'Optional Calibrations'. The 'FlexMix Volume' is shown as 'Full (>= 70%)'.

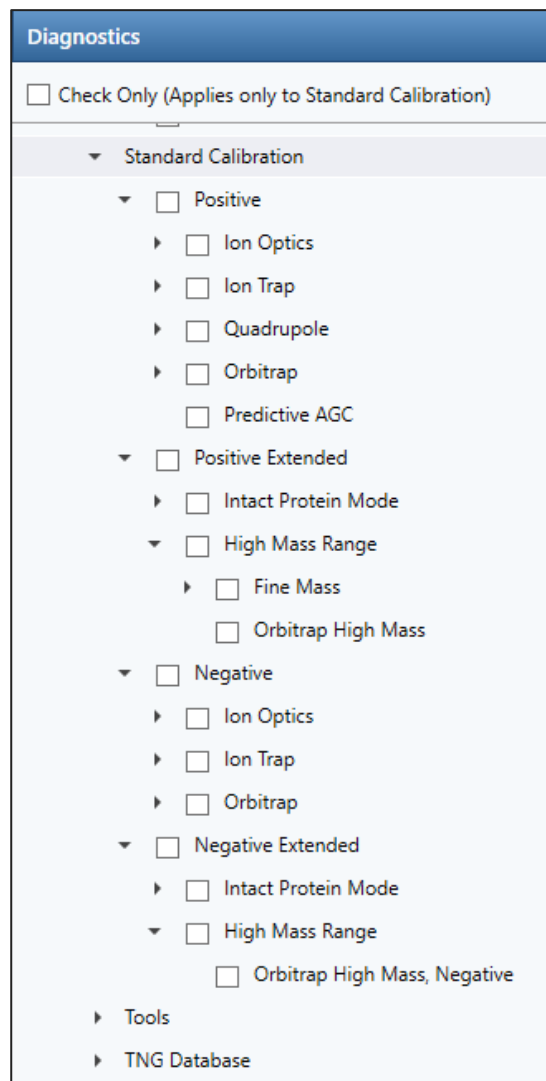
- Status will show an orange indicator light when:
 - A procedure failure (spray stability or calibrant purity evaluation) is recorded during a calibration procedure
 - A single calibration is run from diagnostics
 - A single check is run from diagnostics and fails
 - A procedure is aborted
- Status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

A calibration or check procedure run from diagnostic has completed. Please run a System calibration from the Calibration Tab in "Check, calibrate if required" mode to recover and /or receive detailed recovery instructions.

Same as with Conventional source

Auto-Ready Ion Source

Calibration Procedures in Diagnostics



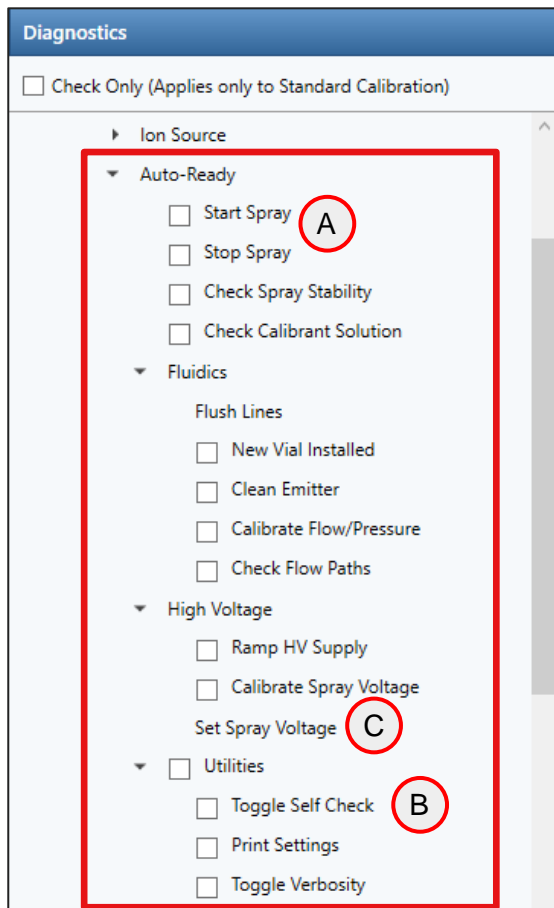
- Calibration tree can be found in the customer diagnostics pane under “Standard Calibration”
 - Individual calibrations may be checked or run
 - As the system requires calibrations to be run in the proper order as a set and some calibrations affect dependent calibrations, calibrating from the diagnostics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

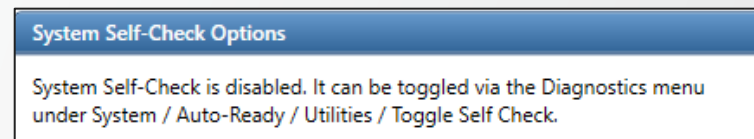
Auto-Ready Ion Source

Diagnostics & Readbacks



- Auto Ready Ion Source Diagnostics

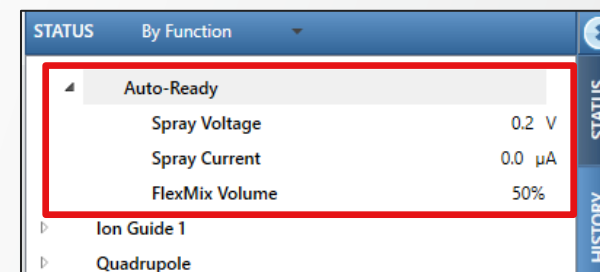
- (A) FlexMix infusion using Auto Ready must be manually started to run a diagnostic that requires it
- (B) Toggle available to enable / disable Self-Check
- (C) 'Set Spray Voltage' diagnostics enables a specific value to be manually set, as an alternative to 'Calibrate Spray Voltage'



Parameter Name	Parameter Value
Voltage (V)	1700

NEW

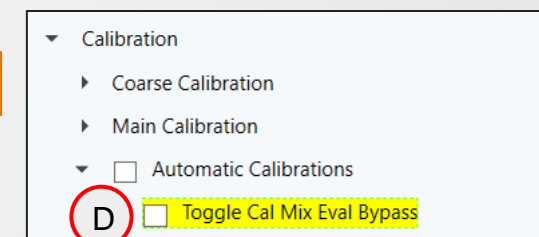
- Auto Ready Ion Source Readbacks



- 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)

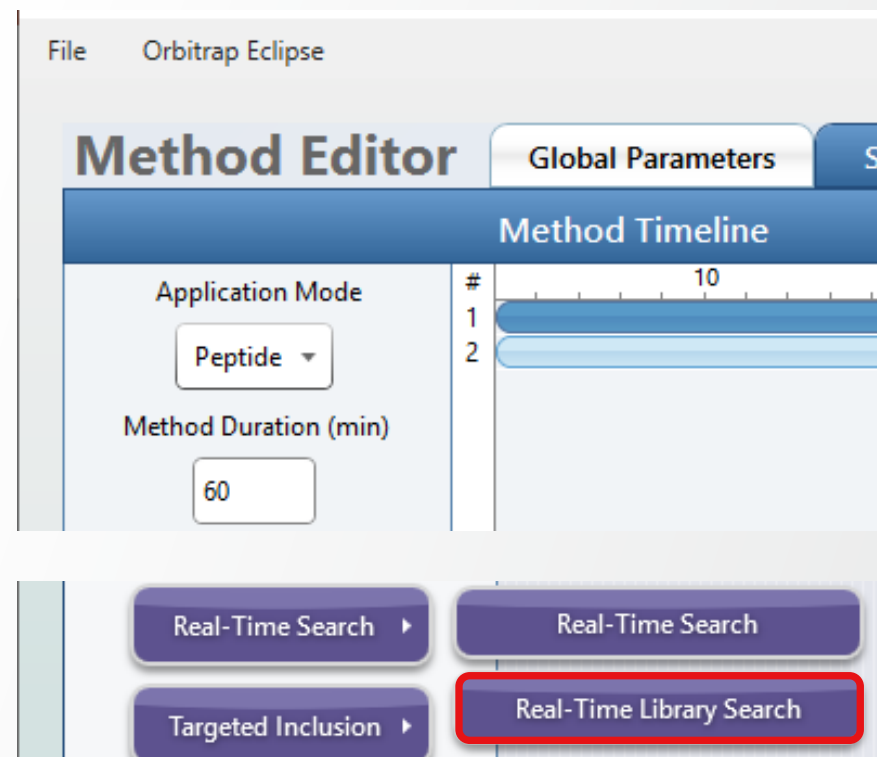
NEW



Support for Real-Time Library Search

New Functionalities to Improve / Expand Data-Driven Acquisition Schemes

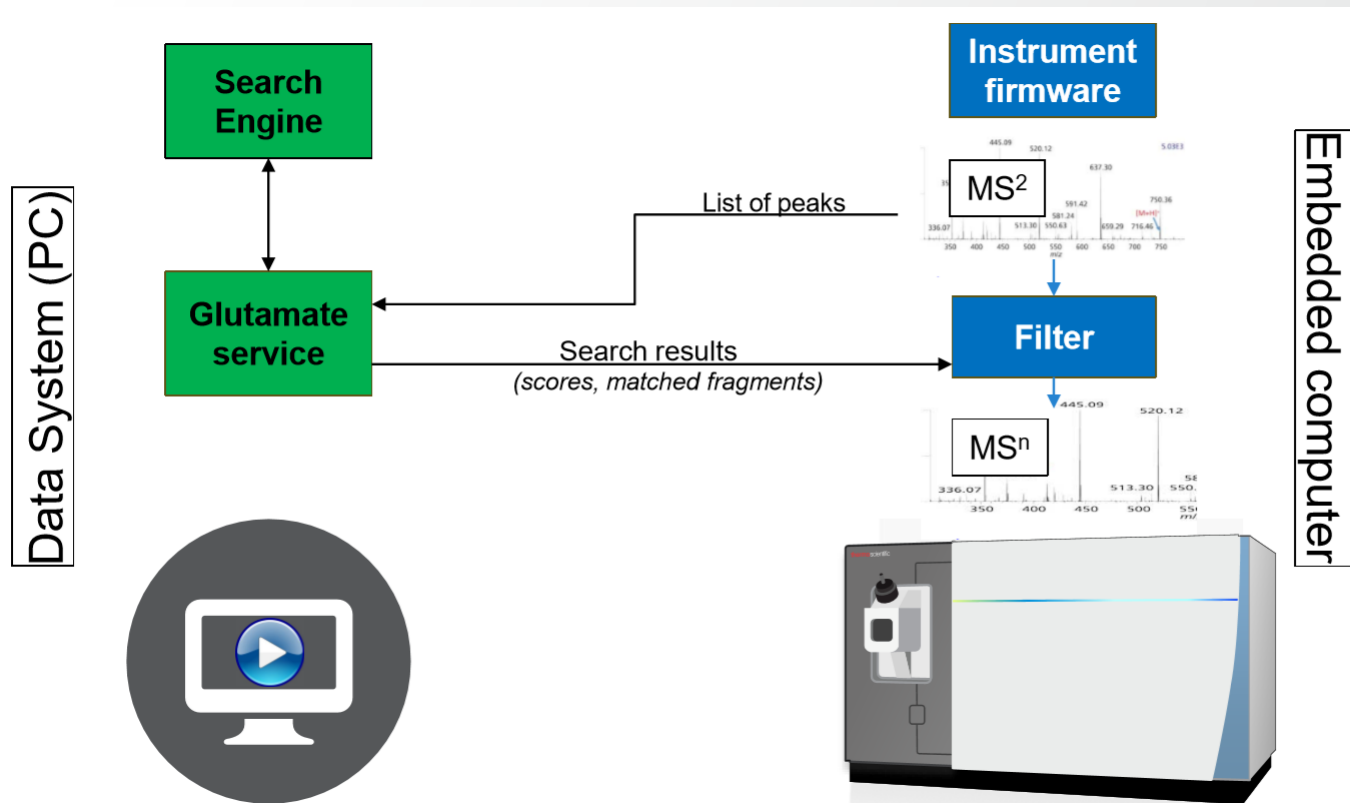
- Supported Models
 - Orbitrap Ascend **NEW**
 - Orbitrap Eclipse
 - Orbitrap IQ-X
- Supported Application Modes
 - Peptide **NEW**
 - Small Molecule
- Novelties **NEW**
 - Structure Filter Properties
 - New searching and filtering functionalities
 - Supported Acquisition scheme (compatible scans, placement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



Support for Real-Time Library Search

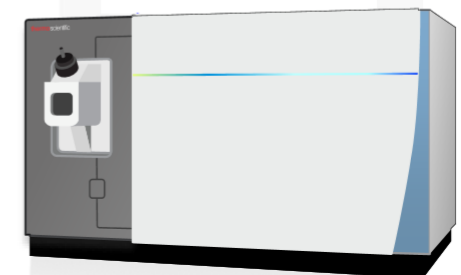
Background Services & Communication

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



Data System (PC)

Embedded computer



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)

NEW

Real-Time Library Search Properties

Use Common Library Search Settings

Use Common Peak Selection and Threshold Settings

A LIBRARY SEARCH SETTINGS

B PEAK SELECTION AND THRESHOLD SETTINGS

NEW

A

Library search settings

Real-Time Library Search Properties

LIBRARY SEARCH SETTINGS

Spectral Library CID_PRTC_Unlabeled_modified_Copy-fo

Browse Clear

Collision Energy Tolerance 15

Similarity Search

Precursor Search Tolerance (ppm) 10

Use Adducts for Offset Query Searches

Adduct Masses

	Adduct Molecular Species	Charge	
1	M	0	

Maximum Search Time (ms) 150

Use Retention Time Filter

Reverse Library Search

Use Multiple Precursors in Search

PEAK SELECTION AND THRESHOLD SETTINGS

Use as a Trigger Only

Consider Unmatched Peaks Only

Add Adducts to Dynamic Exclusion

Add Matched Fragments to Dynamic Exclusion

Scoring Thresholds

	Score Type	Filter By	Score	
1	Confidence Score	At least	0	

NEW

B

Peak selection and threshold settings

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)

Real-Time Library Search Properties

LIBRARY SEARCH SETTINGS

1 Spectral Library CID_PRTC_Unlabeled_modified_Copy-fo
Browse Clear

2 Collision Energy Tolerance 15

3 Similarity Search

4 Precursor Search Tolerance (ppm) 10
Use Adducts for Offset Query Searches

Adduct Masses ADD DELETE IMPORT EXPORT

	Adduct Molecular Species	Charge
1	M	0

Maximum Search Time (ms) 150

Use Retention Time Filter

Isotope Error Correction None (0)

Reverse Library Search

Use Multiple Precursors in Search

PEAK SELECTION AND THRESHOLD SETTINGS

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

The screenshot displays the 'Real-Time Library Search Properties' dialog box. The 'LIBRARY SEARCH SETTINGS' section includes fields for Spectral Library (CID_PRTC_Unlabeled_modified_Copy-fo), Collision Energy Tolerance (15), Similarity Search (unchecked), Precursor Search Tolerance (ppm) (10), and Use Adducts for Offset Query Searches (unchecked). The 'Adduct Masses' table is highlighted with a red bracket and a circled '5'. The table has columns for Adduct Molecular Species and Charge. The first row shows 'M' and '0'. A dropdown menu is open for the first row, listing various adduct species: M, 2M+H, 2M+H+ACN, 2M+K, 2M+Na, 2M+Na+ACN, 2M+NH4, 2M-H, 2M-H+FA, 2M-H+HAc, M+2H, and M+2H+ACN. Below the table, there are fields for a numerical value (150) and a dropdown menu (None (0)).

	Adduct Molecular Species	Charge
1	M	0

5

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) 5
 - 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

Real-Time Library Search Properties

LIBRARY SEARCH SETTINGS

Spectral Library: CID_PRTC_Unlabeled_modified_Copy-fo
Browse Clear

Collision Energy Tolerance: 15

Similarity Search:

Precursor Search Tolerance (ppm): 10

Use Adducts for Offset Query Searches:

Adduct Masses

	Adduct Molecular Species	Charge	
1	M	0	

Maximum Search Time (ms): 150

Use Retention Time Filter:

Isotope Error Correction: None (0)

Reverse Library Search:

Use Multiple Precursors in Search:

PEAK SELECTION AND THRESHOLD SETTINGS

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

Real-Time Library Search Properties

LIBRARY SEARCH SETTINGS

Spectral Library: CID_PRTC_Unlabeled_modified_Copy-fo
Browse Clear

Collision Energy Tolerance: 15

Similarity Search:

Precursor Search Tolerance (ppm): 10

Use Adducts for Offset Query Searches:

Adduct Masses

	Adduct Molecular Species	Charge	
1	M	0	

ADD DELETE IMPORT EXPORT

6 Maximum Search Time (ms): 150

7 Use Retention Time Filter:

7 Retention Time Tolerance: 1

8 Isotope Error Correction: None (0)

Reverse Library Search: None (0)

Use Multiple Precursors in Search: 0/1

PEAK SELECTION AND TH

0/1/2
0/1/2/3
-1/0/1/2/3

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. 9
 - MS1 data unavailable within the same experiment: searches are performed with precursor search tolerance set (overridden) to the width of the isolation window. 10

Real-Time Library Search Properties

LIBRARY SEARCH SETTINGS

Spectral Library CID_PRTC_Unlabeled_modified_Copy-fo

Collision Energy Tolerance 15

Similarity Search

Precursor Search Tolerance (ppm) 10

Use Adducts for Offset Query Searches

Adduct Masses ADD DELETE IMPORT EXPORT

Adduct	Adduct Molecular Species	Charge	
1	M	0	

Maximum Search Time (ms) 150

Use Retention Time Filter

Retention Time Tolerance 1

Isotope Error Correction None (0)

Reverse Library Search

Use Multiple Precursors in Search

PEAK SELECTION AND THRESHOLD SETTINGS

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

Real-Time Library Search Properties

LIBRARY SEARCH SETTINGS

PEAK SELECTION AND THRESHOLD SETTINGS

- 1** Use as a Trigger Only
- 2** TMT SPS MS3 Mode
- 3** Consider Unmatched Peaks Only
- Add Adducts to Dynamic Exclusion
- Add Matched Fragments to Dynamic Exclusion

Scoring Thresholds

	Score Type	Filter By	Score	
1	Confidence Score	At least	0	

Compound Class Filter

Keyword	Promote/Reject	
---------	----------------	--

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) 4
 - 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.

Real-Time Library Search Properties

LIBRARY SEARCH SETTINGS

PEAK SELECTION AND THRESHOLD SETTINGS

- Use as a Trigger Only
- TMT SPS MS3 Mode
- Consider Unmatched Peaks Only
- Add Adducts to Dynamic Exclusion 4
- Add Matched Fragments to Dynamic Exclusion 5

Scoring Thresholds ADD DELETE IMPORT EXPORT

	Score Type	Filter By	Score	
1	Confidence Score	At least	0	

Compound Class Filter ADD DELETE IMPORT EXPORT

Keyword	Promote/Reject
---------	----------------

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”, \geq) 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

Real-Time Library Search Properties

LIBRARY SEARCH SETTINGS

PEAK SELECTION AND THRESHOLD SETTINGS

Use as a Trigger Only

TMT SPS MS3 Mode

Consider Unmatched Peaks Only

Add Adducts to Dynamic Exclusion

Add Matched Fragments to Dynamic Exclusion

6 Scoring Thresholds

Score Type	Filter By	Score
1 Confidence Score	At least	0
Confidence Score	At least	
Confidence Delta Score	Less than	
Cosine Score		
Cosine Delta Score		

7 Compound Class Filter

Keyword	Promote/Reject
1	Promote
	Promote
	Reject

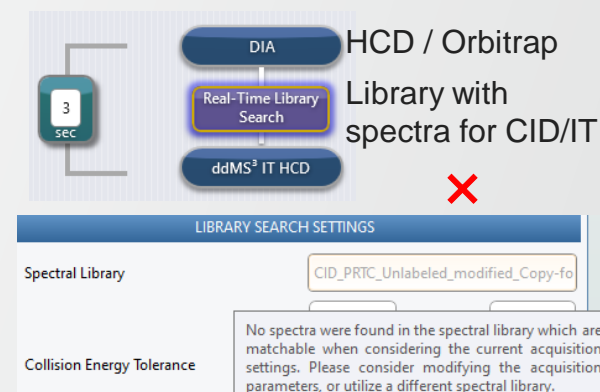
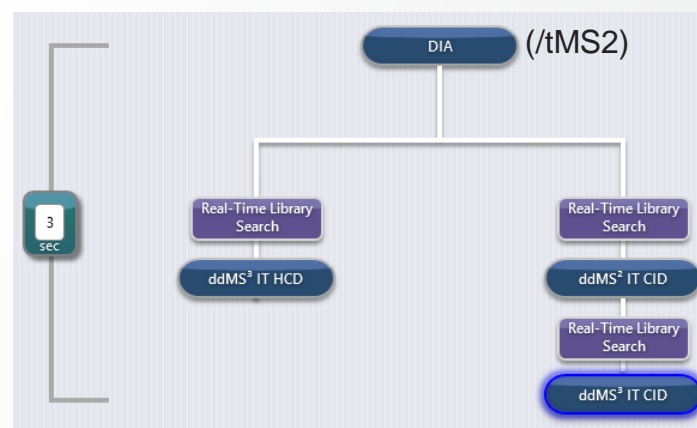
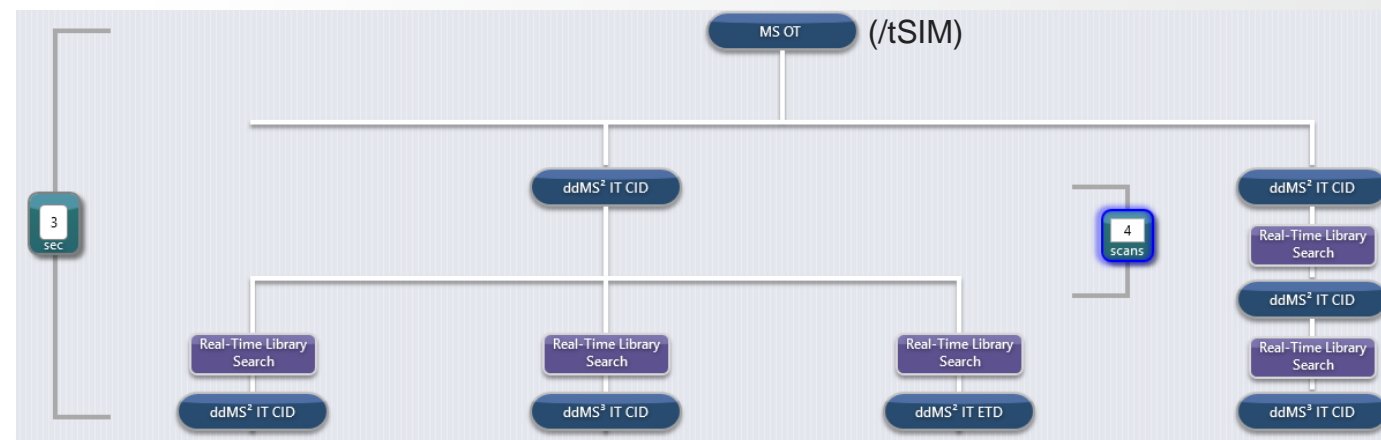
Formula	CAS ID	InChi Key	Compound Class
C8H9NO4	82826		Endogenous Metabolites
C7H8N4O2	58559		Endogenous Metabolites Therapeutics/Prescription Drugs
C73H108O12	6683198		Industrial Chemicals



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)



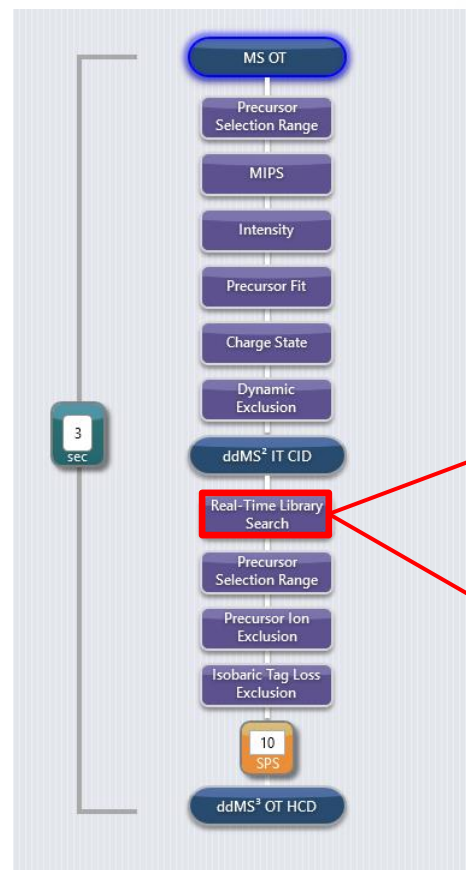
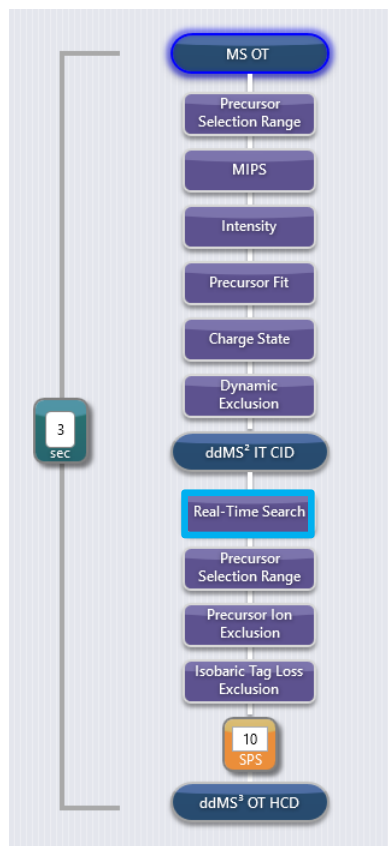
Real-Time Library Search – Based Acquisition Method

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



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

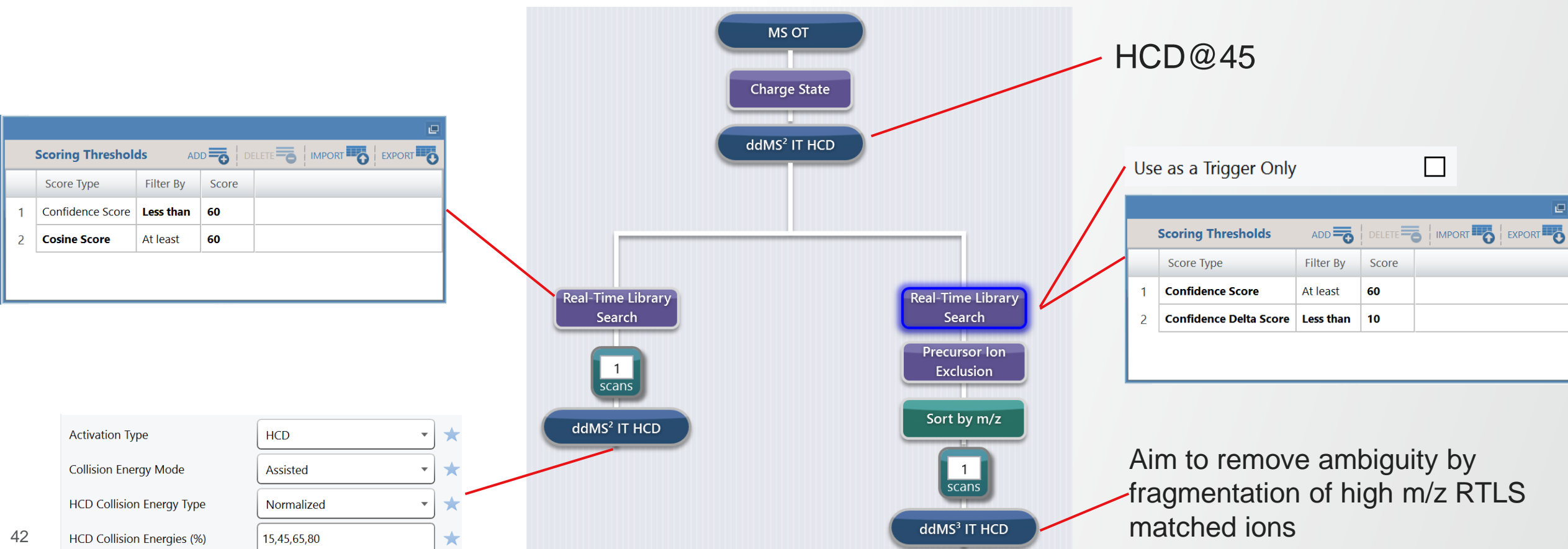
Use as a Trigger Only	<input type="checkbox"/>
TMT SPS MS3 Mode	<input checked="" type="checkbox"/>
Optional:	
Reverse Library Search	<input checked="" type="checkbox"/>
Use Multiple Precursors in Search	<input checked="" type="checkbox"/>

TMT SPS MS3 with RTS

Real-Time Library Search – Based Acquisition Method

Application Example: Advanced Elucidation of Chemical Structures

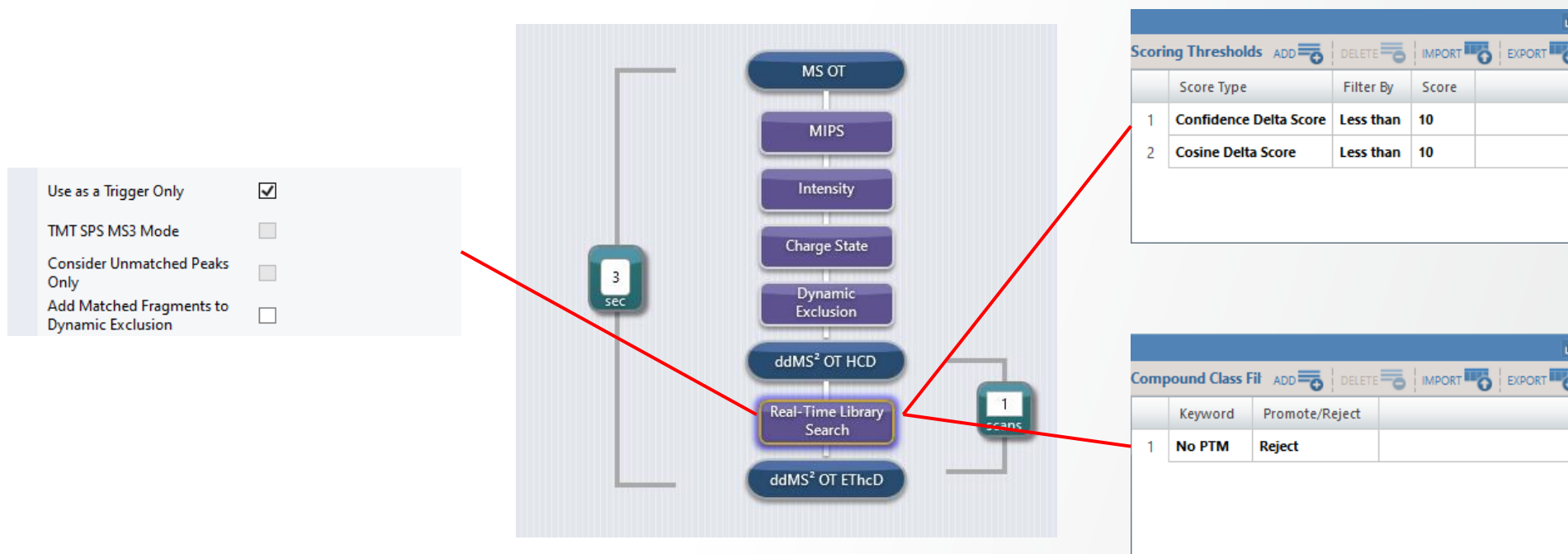
- No further acquisition for unambiguous & confident id. of the cmpds (high cosine / confidence / confidence delta)
- Optimization of CE if match appears under-fragmented (high cosine / low confidence)
- MS3 acquisition on high m/z fragments for compounds with ambiguous scores



Real-Time Library Search – Based Acquisition Method

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

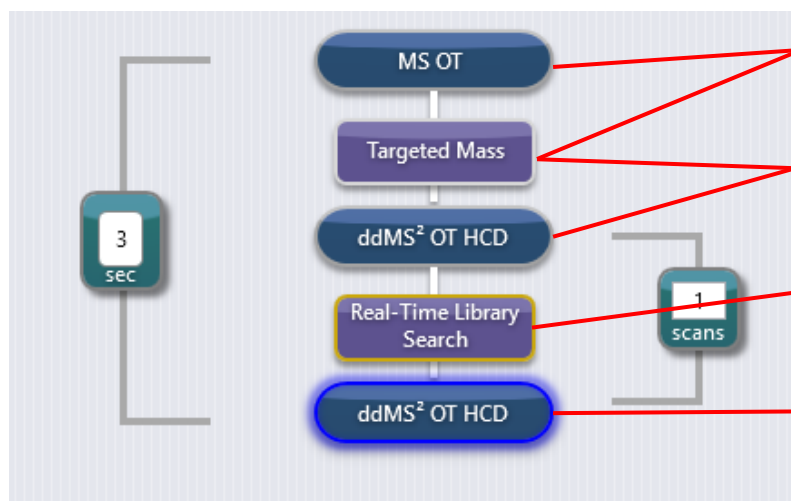
- No further acquisition for peptides without PTM
- No further acquisition for peptides with confident localization of PTM (high cosine / confidence delta)
- Alternative fragmentation for peptides with ambiguous PTM localization
- Needed: ref. spectra for the peptides with different PTM localization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

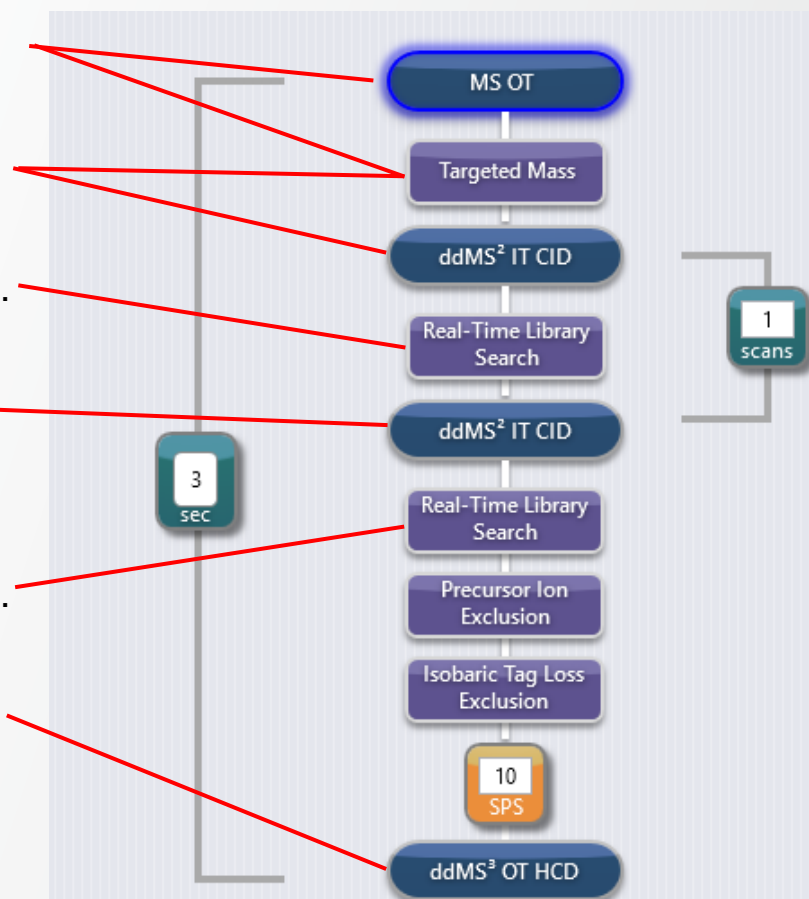
Spectral library search experimental vs. ref spectra: Identification of IS

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

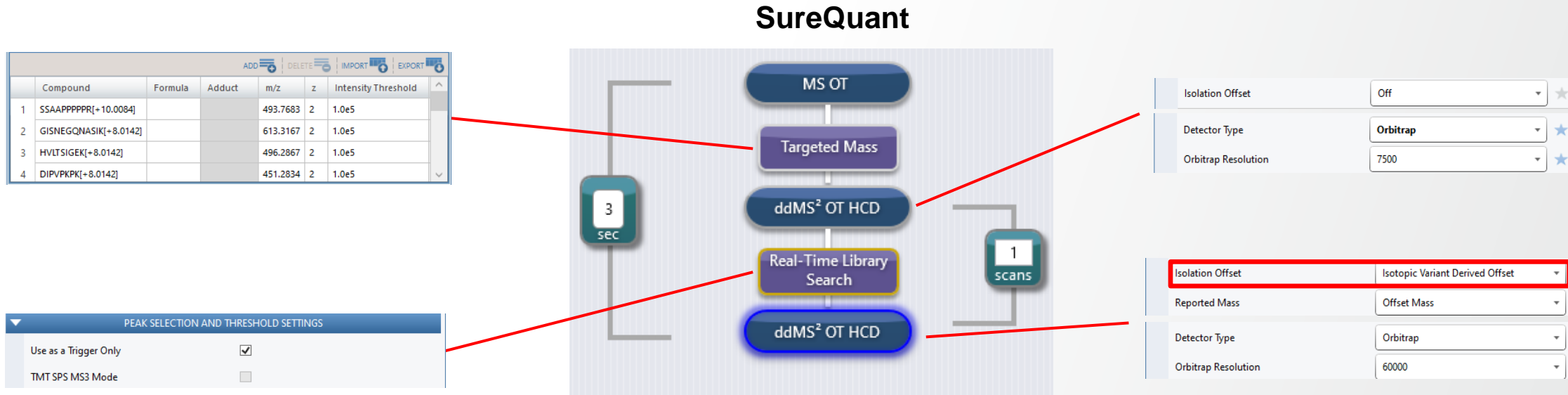
Tomahaq



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”)



Compound List

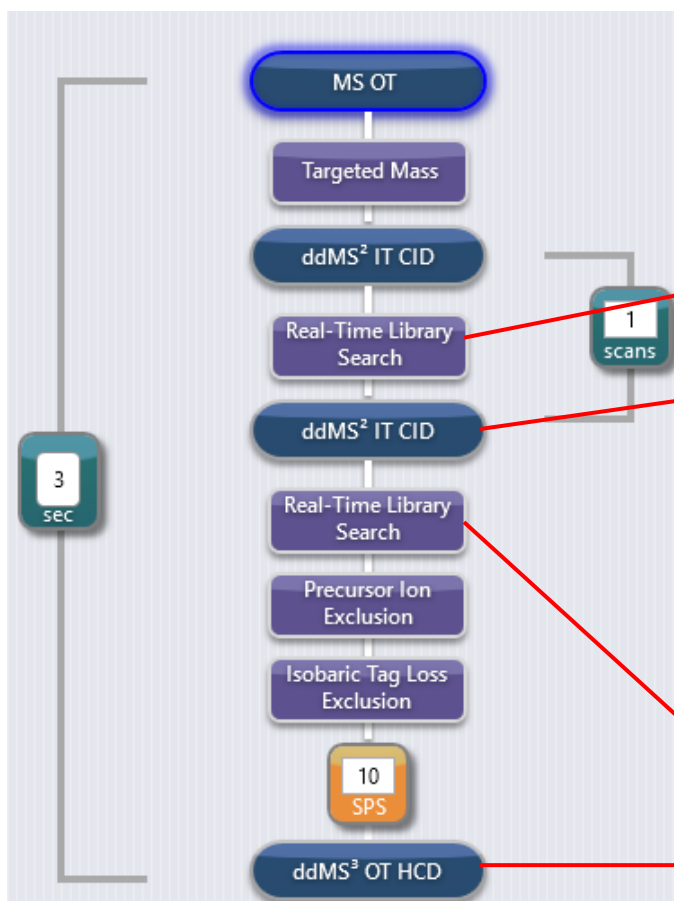
Compound	Compound Class	Peptide Seq	Tag
SSAAPPPPPR/2		SSAAPPPPPR	massOffset:-10.0084
GISNEGQNASIK/2		GISNEGQNASIK	massOffset:-8.0142
HVLTSIGEK/2		HVLTSIGEK	massOffset:-8.0142
DIPVPKPK/2		DIPVPKPK	massOffset:-8.0142
IGDYAGIK/2		IGDYAGIK	massOffset:-8.0142

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



Compound	Peptide	Sec	Tag
SSAAPPPPPR/2	SSAAPPPPPR		MassOffset: -9.0239 mods:313.2310@0 ionsy1;y2;b4^2;y6^2;b5^2;y7^2;y3;b6^2;y9^2;b7^2;y4;b2;b8^2;b3;b9^2;y5;b4;y6;b5;y7;y8;b6;y9;b8;b9
GISNEGQNASIK/2	GISNEGQNASIK		MassOffset: -18.0478 mods:313.2310@0;313.2310@11 ionsy5^2;y1;b2;b7^2;b8^2;b3;y2;b9^2;b10^2;y9^2;y3;b4;y10^2;y4;y11^2;b5;y5;b6;y6;b7;y7;b8;y8;b9;b10;y9;y10;b11;y1
HVLTSIGEK/2	HVLTSIGEK		MassOffset: -18.0478 mods:313.2310@0;313.2310@8 ionsb3^2;y1;y7^2;b2;y8^2;y2;y3;b3;b4;y4;b5;y5;y6;b6;b7;y7;b8;y8
DIPVPPPK/2	DIPVPPPK		MassOffset: -27.0717 mods:313.2310@0;313.2310@5;313.2310@7 ionsy2^2;b3^2;y1;b2;y4^2;y2;y5^2;b6^2;b3;y6^2;b7^2;y7^2;b4;y3;y4;y5;b6;y6;b7;y7
IGDYAGIK/2	IGDYAGIK		MassOffset: -18.0478 mods:313.2310@0;313.2310@7 ionsy1;b2;y2;b3;y3;y4;b4;b5;y5;b6;y6;b7;y7

PEAK SELECTION AND THRESHOLD SETTINGS

Use as a Trigger Only

TMT SPS MS3 Mode

Isolation Offset

Reported Mass

Compound	Peptide	Sec	Tag
SSAAPPPPPR/2	SSAAPPPPPR		mods:304.207146@0 ionsy1;y2;b4^2;y6^2;b5^2;y7^2;y3;b6^2;y9^2;b7^2;y4;b2;b8^2;b3;b9^2;y5;b4;y6;b5;y7;y8;b6;y9;b8;b9
GISNEGQNASIK/2	GISNEGQNASIK		mods:304.207146@0;304.207146@11 ionsy5^2;y1;b2;b7^2;b8^2;b3;y2;b9^2;b10^2;y9^2;y3;b4;y10^2;y4;y11^2;b5;y5;b6;y6;b7;y7;b8;y8;b9;b10;y9;y10;b11;y11
HVLTSIGEK/2	HVLTSIGEK		mods:304.207146@0;304.207146@8 ionsb3^2;y1;y7^2;b2;y8^2;y2;y3;b3;b4;y4;b5;y5;y6;b6;b7;y7;b8;y8
DIPVPPPK/2	DIPVPPPK		mods:304.207146@0;304.207146@5;304.207146@7 ionsy2^2;b3^2;y1;b2;y4^2;y2;y5^2;b6^2;b3;y6^2;b7^2;y7^2;b4;y3;y4;y5;b6;y6;b7;y7
IGDYAGIK/2	IGDYAGIK		mods:304.207146@0;304.207146@7 ionsy1;b2;y2;b3;y3;y4;b4;b5;y5;b6;y6;b7;y7

PEAK SELECTION AND THRESHOLD SETTINGS

Use as a Trigger Only

TMT SPS MS3 Mode

Isolation Offset

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

The diagram illustrates the Hybrid DIA/SureQuant workflow and its configuration. The workflow consists of three main steps: DIA, Real-Time Library Search, and ddMS² OT HCD. The Real-Time Library Search step is highlighted with a red box and a red arrow pointing to the settings panel on the right. The settings panel shows the following configuration:

- Reverse Library Search:
- Use Multiple Precursors in Search:
- PEAK SELECTION AND THRESHOLD SETTINGS:
 - Use as a Trigger Only:
 - TMT SPS MS3 Mode:
- Isolation Offset: Isotopic Variant Derived Offset
- Reported Mass: Offset Mass
- Detector Type: Orbitrap
- Orbitrap Resolution: 60000

The Compound List table below shows the results of the search, with the Tag column highlighted in red:

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

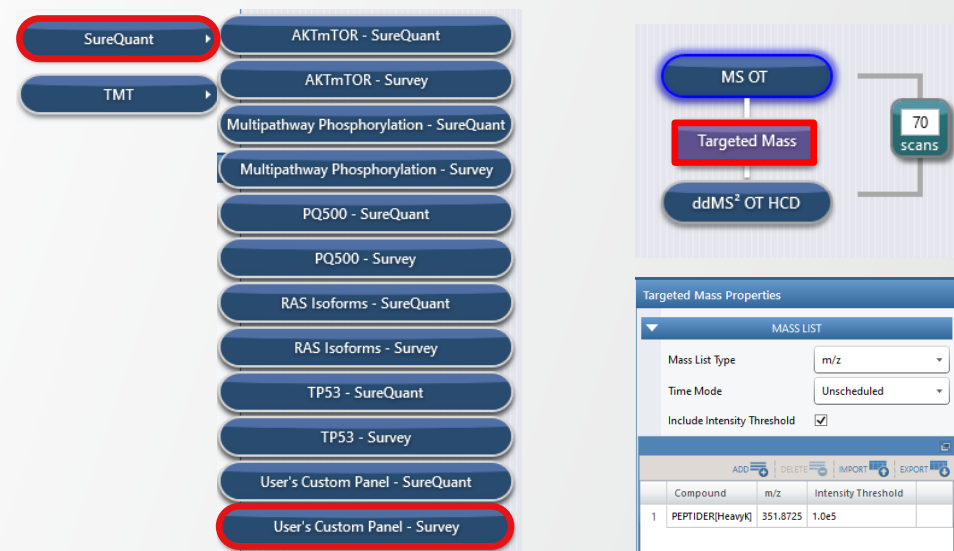
In mzVault



Generation of Local Experimental Spectral Libraries

Acquiring MS2 data on Compounds of Interest (Standards)

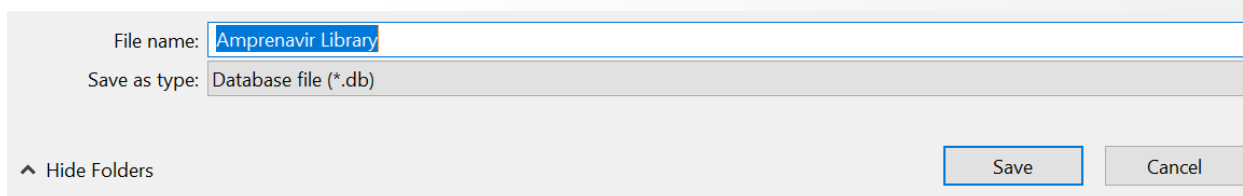
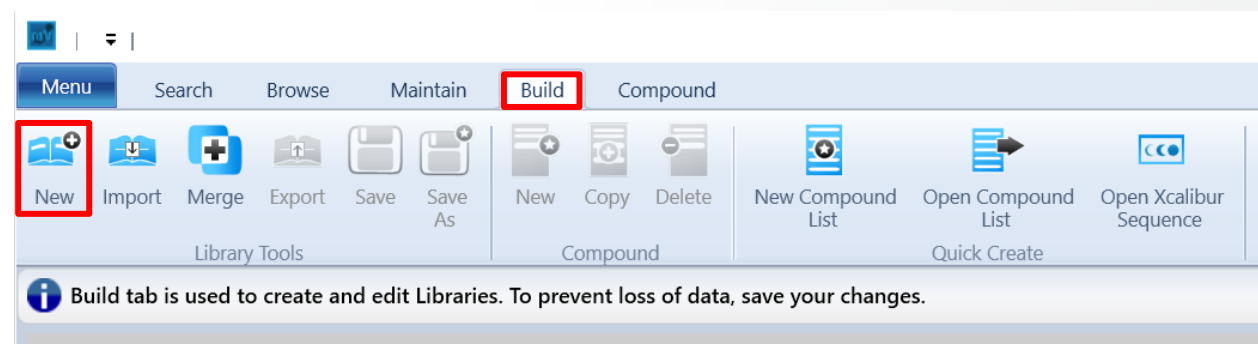
- Provided system templates can be used as models
- Small Molecule MS/MS Library Builder template
 - Analyses are performed as direct infusion or with an LC method (with the need for chrom. peak $\geq 6s$)
 - M/z value (or m/z & z) of the compound of interest are included in Targeted Mass filter
 - MS/MS 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”

The screenshot displays the software interface for building a library. The 'Build' tab is selected, and the 'New' button is highlighted. Below the toolbar, a message states: "Build tab is used to create and edit Libraries. To prevent loss of data, save your changes." The main area shows the 'Amprenavir Library' with a 'Compound List' table. The table has columns for 'Entry No' and 'Compound'. A red arrow points to the 'Compound' field in the first row, which contains the text 'Compound'. Below this, the same table is shown with the 'Compound' field filled with 'Amprenavir' and the 'Entry No' field containing '1'. At the bottom, a table of metadata fields is visible, including Synonyms, Formula, CAS ID, InChi Key, Compound Class, ChemSpider ID, HMDB ID, KEGG ID, PubChem ID, SMILES Description, mz Cloud ID, Peptide Sequence, and Tag.

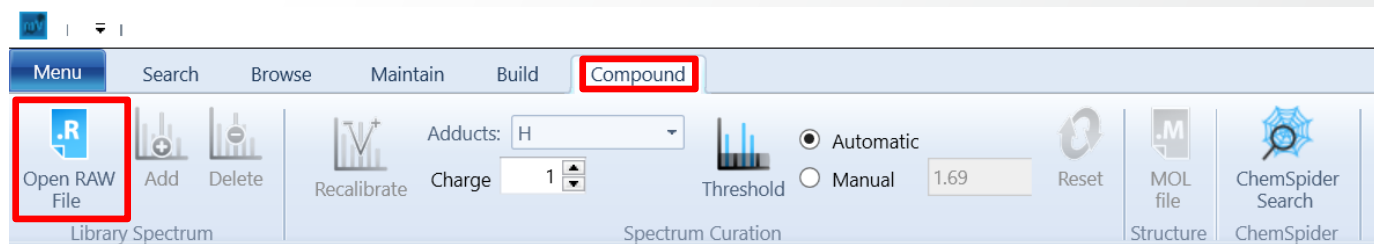
Entry No	Compound
	Compound
1	Amprenavir

Synonyms	Formula	CAS ID	InChi Key	Compound Class	ChemSpider ID	HMDB ID	KEGG ID	PubChem ID	SMILES Description	mz Cloud ID	Peptide Sequence	Tag

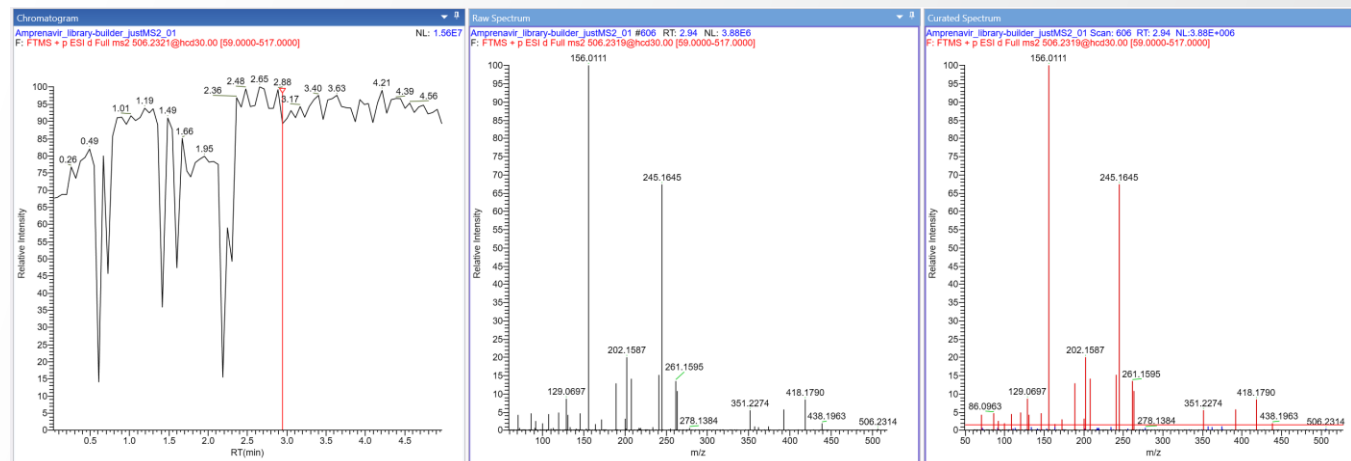
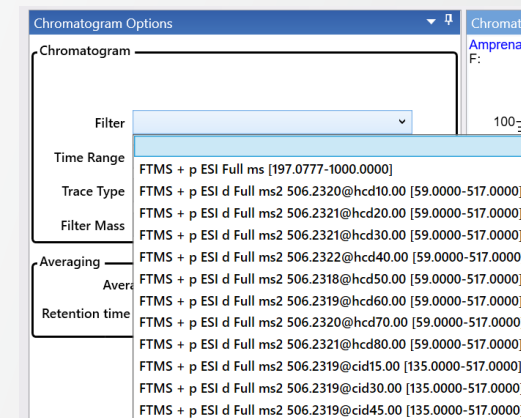
Generation of Local Experimental Spectral Libraries

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



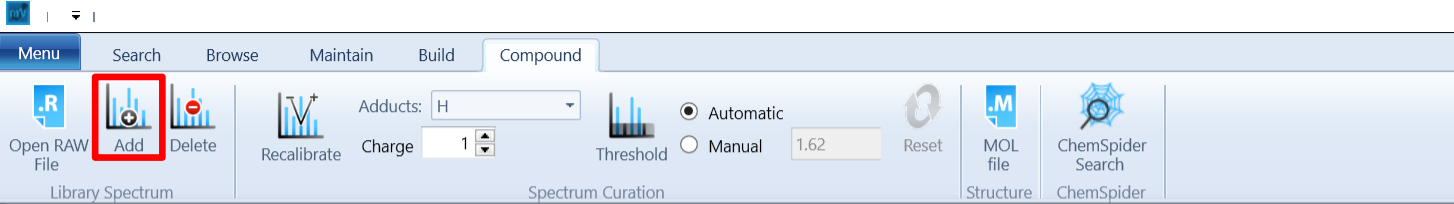
Compound List	
Entry No	Compound
1	Amprenavir



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



The screenshot shows the software interface with the 'Compound' tab selected. In the 'Library Spectrum' section, the 'Add' button is highlighted with a red box. Other buttons include 'Open RAW File', 'Delete', 'Recalibrate', 'Adducts: H', 'Charge: 1', 'Threshold', 'Automatic', 'Manual', 'Reset', 'MOL file', and 'ChemSpider Search'.

Compound tab and other functions apply to Amprenavir

Amprenavir Library

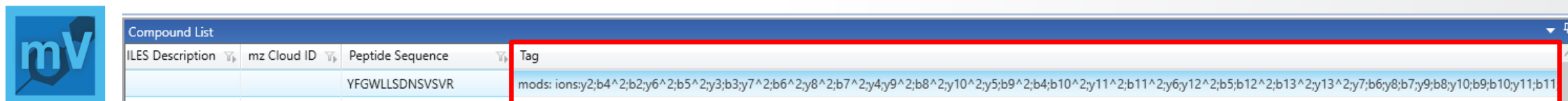
Library Spectrum

Compound List						
Entry No	Compound	Synonyms	Formula	CAS ID		
1	Amprenavir					
Spectrum ID	Compound ID	Scan Filter	Retention Time	Scan Number	Precursor m/z	
1	1	FTMS + p ESI d Full ms2 506.2319@hcd30.00 [59.0000-517.0000]	2.940	606	506.2319	

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.



ILES Description	mz Cloud ID	Peptide Sequence	Tag
		YFGWLLSDNSVSVR	mods: ions:y2;b4^2;b2;y6^2;b5^2;y3;b3;y7^2;b6^2;y8^2;b7^2;y4;y9^2;b8^2;y10^2;y5;b9^2;b4;b10^2;y11^2;b11^2;y6;y12^2;b5;b12^2;b13^2;y13^2;y7;b6;y8;b7;y9;b8;y10;b9;b10;y11;b11

- 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

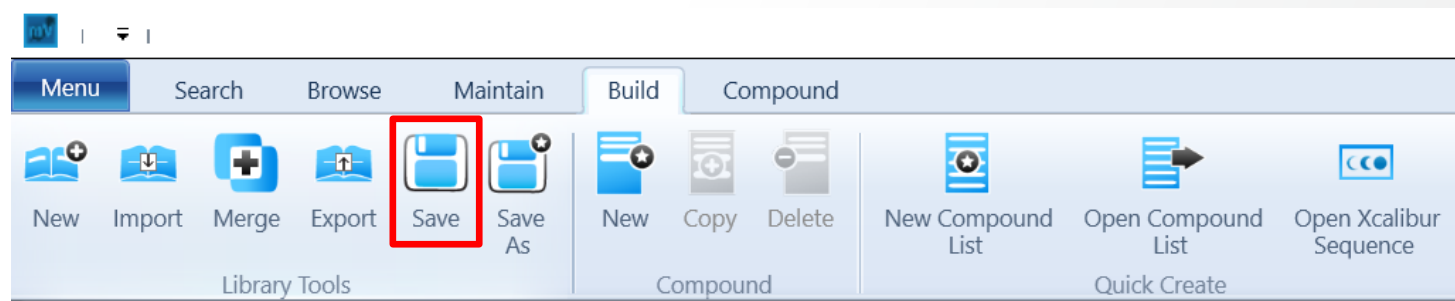
Available 'Tag' Metadata Inputs and Expected Formats

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

In mzVault



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)

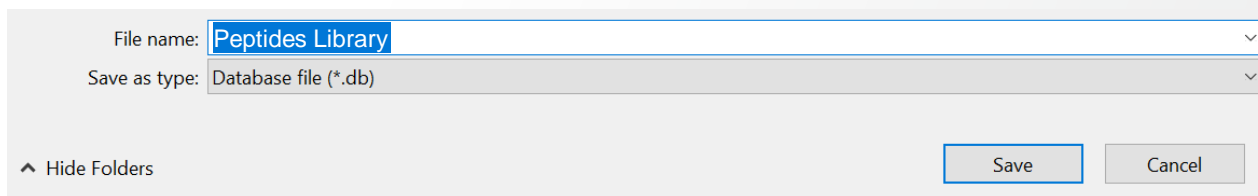
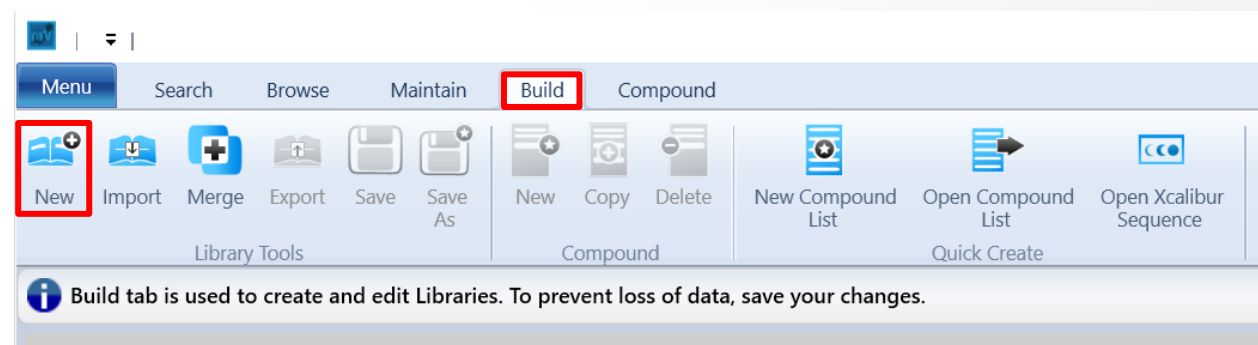
Example Minimal Massbank Record

```
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

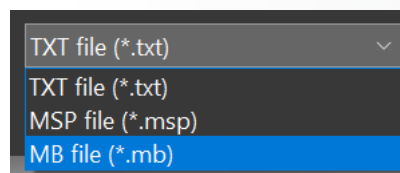
- Follow the directions for mzVault “Creating a Library”



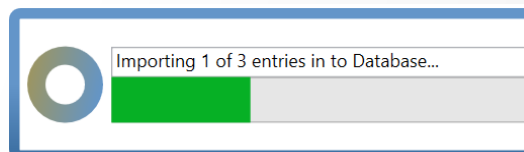
- Click the “Import” option



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



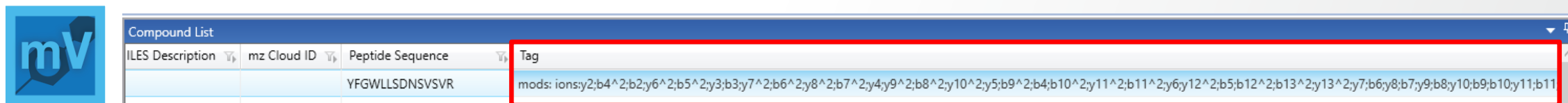
- Select all the files in the directory that you wish to import, and allow mzVault to build the spectral library



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.



ILES Description	mz Cloud ID	Peptide Sequence	Tag
		YFGWLLSDNSVSVR	mods: ions:y2;b4^2;b2;y6^2;b5^2;y3;b3;y7^2;b6^2;y8^2;b7^2;y4;y9^2;b8^2;y10^2;y5;b9^2;b4;b10^2;y11^2;b11^2;y6;y12^2;b5;b12^2;b13^2;y13^2;y7;b6;y8;b7;y9;b8;y10;b9;b10;y11;b11

- 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

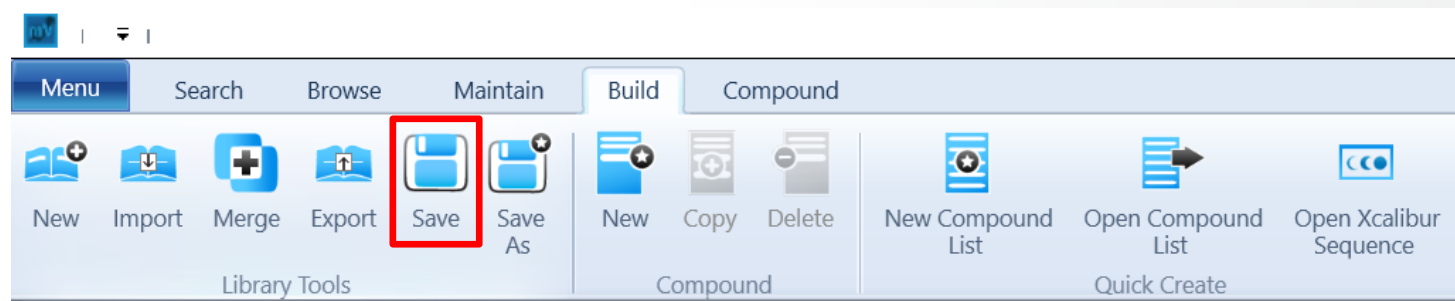
Available 'Tag' Metadata Inputs and Expected Formats

- 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

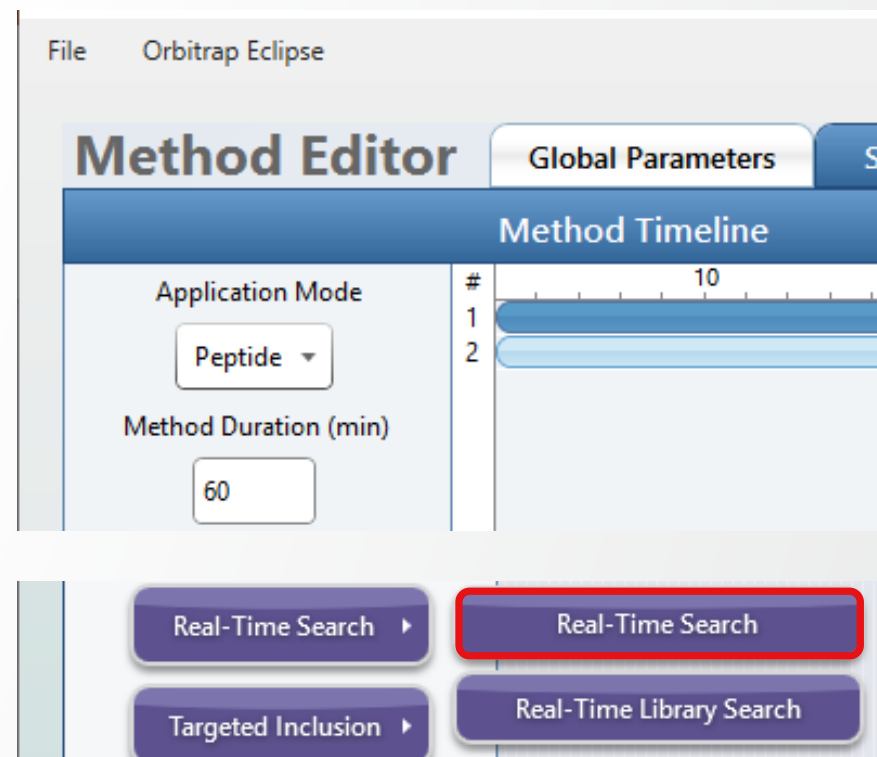
Upcoming Communication

- 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](https://www.analyteguru.com) to receive 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)

NEW

Real-Time Search Properties

Use Common Database Search Settings

Use Common Peak Selection and Threshold Settings

(A) DATABASE SEARCH SETTINGS

(B) PEAK SELECTION AND THRESHOLD SETTINGS

NEW (A)

Database search settings

Real-Time Search Properties

DATABASE SEARCH SETTINGS

FASTA Database: yeastDB.fasta

Enzyme: Trypsin

Static Modifications

Modification Name	Δ Mass	Sites
1 Carbamidomethyl	57.0215	C

Variable Modifications

Modification Name	Δ Mass	Sites	Frag Neutral Loss
1 Oxidation	15.9949	M	0

Maximum Missed Cleavages: 0

Maximum Variable Mods / Peptide: 1

Use Multiple Precursors in Search:

Enable FDR Filtering:

Consider Precursor Neutral Loss:

Use Custom Fragment Ions:

PEAK SELECTION AND THRESHOLD SETTINGS

Use as a Trigger Only:

TMT SPS MS3 Mode:

Enable Close-Out:

Maximum Search Time (ms): 40

Scoring Thresholds

Xcorr	dCn	Precursor PPM	Charge State
1 2	0.1	10	2

(B)

Peak selection and threshold settings

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.

1

Real-Time Search Properties

DATABASE SEARCH SETTINGS

FASTA Database

Enzyme

Static Modifications

ADD DELETE IMPORT EXPORT

	Modification Name	Δ Mass	Sites	
1	Carbamidomethyl	57.0215	C	

Variable Modifications

ADD DELETE IMPORT EXPORT

	Modification Name	Δ Mass	Sites	Frag Neutral Loss	
1	Oxidation	15.9949	M	0	

Maximum Missed Cleavages

Maximum Variable Mods / Peptide

Use Multiple Precursors in Search

Enable FDR Filtering

Consider Precursor Neutral Loss

Precursor Neutral Loss m/z

Use Custom Fragment Ions

Comma Separated FragIons

PEAK SELECTION AND THRESHOLD SETTINGS

Support for Real-Time Search

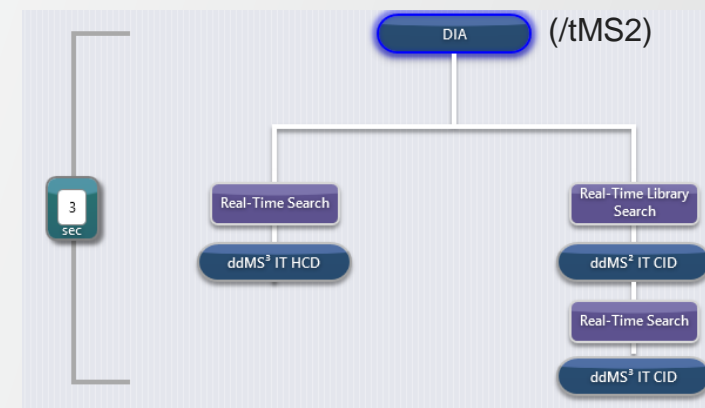
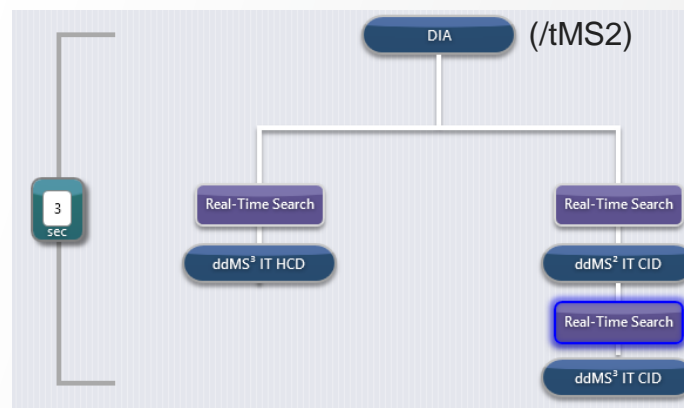
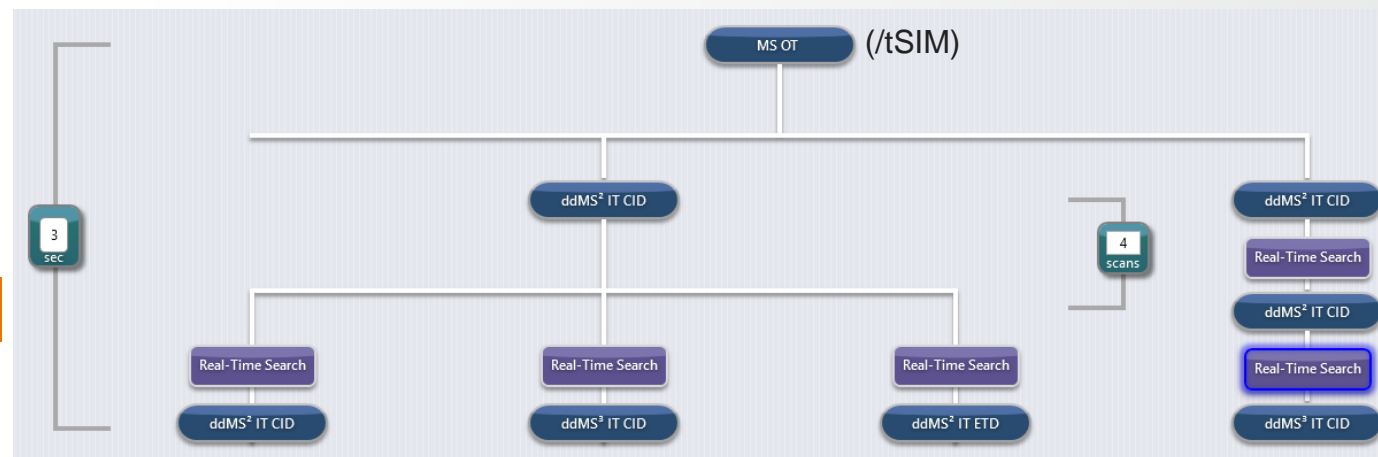
Supported Acquisition Schemes

- 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')

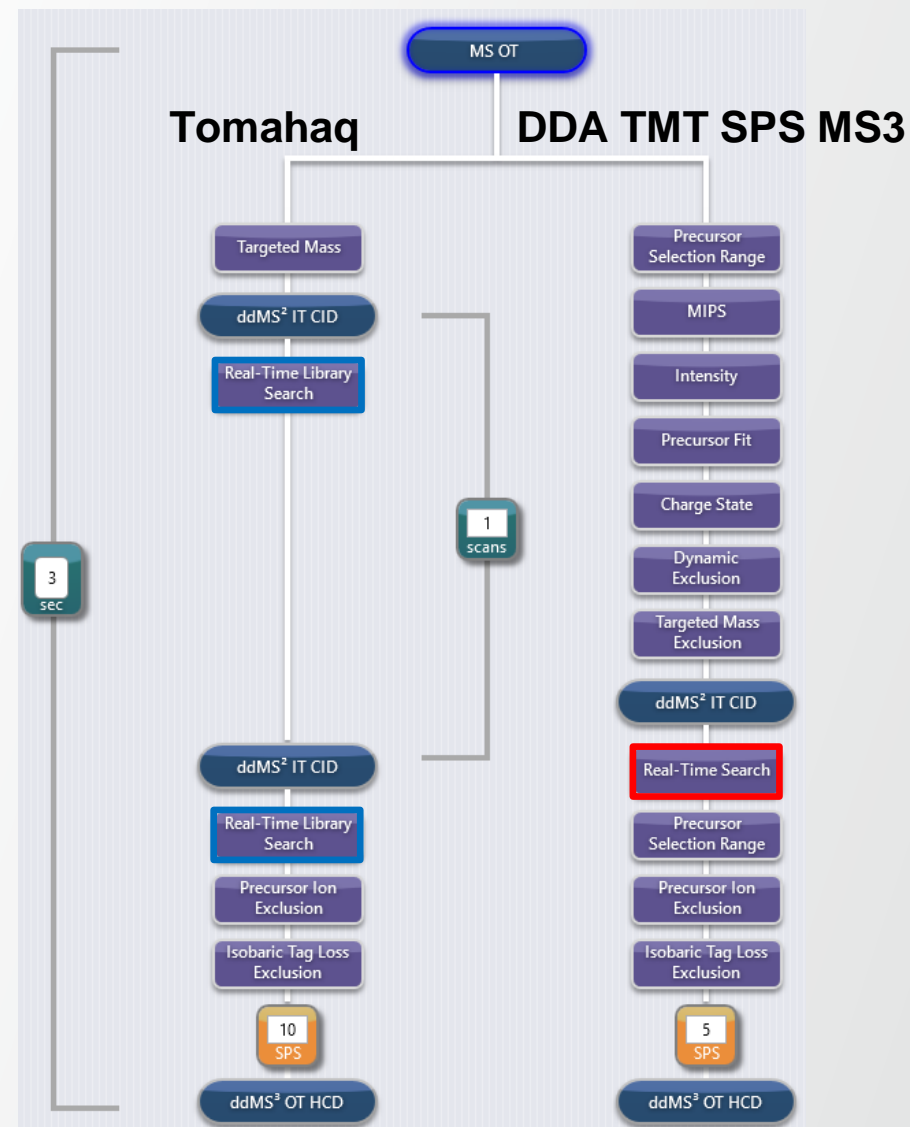
- RTS and RTLS filters can be combined within a single experiment (and method), but not in series under a single MS2 node **NEW**



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



AcquireX Ab



AcquireX

New AcquireX Ab Workflow for Peptide Mapping

New Features in Method Editor

The screenshot shows the Orbitrap Eclipse Method Editor 4.0.4084.16. The 'Application Mode' is set to 'Peptide'. The 'Method Duration (min)' is 120. The 'Method Timeline' shows a single MS scan from 0 to 120 minutes. The 'Settings' panel includes 'Infusion Mode' (Liquid Chromatography), 'Expected LC Peak Width (s)' (30), 'Advanced Peak Determination' (checked), 'Default Charge State' (2), 'Enable Xcalibur AcquireX Ab method modifications' (checked), and 'Internal Mass Calibration' (Off). The 'Targeted Mass Properties' panel shows 'Mass List Type' (m/z), 'Time Mode' (Start/End Time), 'Include Intensity Threshold' (checked), and 'Add Mass List Targets Determined by Xcalibur AcquireX Ab' (checked). A table below shows one target:

Compound	m/z	t start (min)	t stop (min)	Intensity Threshold
1	524.265	0	120	0.0e0

- 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

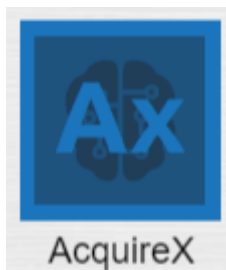
This close-up shows the 'Targeted Mass Properties' panel with 'Mass List Type' set to 'm/z & z'. The 'Include Intensity Threshold' is checked, and 'Add Mass List Targets Determined by Xcalibur AcquireX Ab' is checked. A table below shows one target:

Compound	Formula	Adduct	m/z	z	t start (min)	t stop
1			524.265	1	0	120

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



The screenshot shows a software interface for creating custom workflows. At the top, a flowchart displays a sequence of steps: 'MS OT', 'Targeted Mass (Optional)', 'Targeted Mass Exclusion', and 'ddMS² OT HCD'. The 'Targeted Mass (Optional)' step is highlighted with a red border. Below the flowchart, a grey header reads 'CUSTOM WORKFLOWS'. Underneath, the text 'Create workflows specific to your requirement' is followed by the heading 'What Xcalibur Does:'. A bulleted list describes the software's capabilities: generating inclusion or exclusion lists, reusing lists from previous groups, incorporating various experiment types, and submitting experiments with multiple groups. A blue 'SELECT' button is positioned at the bottom of the interface.

MS OT

Targeted Mass (Optional)

Targeted Mass Exclusion

ddMS² OT HCD

CUSTOM WORKFLOWS

Create workflows specific to your requirement

What Xcalibur Does:

- Generates an inclusion or exclusion list by combining up to 5 injections per group
- Provides options to reuse inclusion and exclusion lists from previous groups
- Incorporates various experiment types in a single experiment
- Submits an experiment with several groups

SELECT

Xcalibur: Acquire X Custom Workflows

The screenshot shows the AcquireX Workflow Editor interface. On the left, there is a sidebar with 'CUSTOM WORKFLOWS' and a list of workflow types: MS-OT, Targeted Mass, Targeted Mass Exclusion, and ddMS⁺-OT-HCD. Below this is a section for 'Experiment Details' with fields for 'Experiment Folder' and 'Experiment Name', each with a 'Browse' button. The 'Group Parameters' section includes a 'Group #' dropdown set to '1' and an 'Apply To All Groups' button. Under 'Instrument Methods', there are three sections: 'Full Scan Method', 'MSn Template Method', and 'Experiment Parameters' (with 'Exclusion Override Factor (default = 3)').

The main area is titled 'AcquireX Workflow Editor' and shows a table for 'AcquireX Template Injections'. The table has columns: #, Name, Type, Group, Instrument Method, Apply Excl. ..., Apply Incl. List, Vial, and Vol (µl). Above the table, there are controls for 'Blanks 0', 'Excl. Ref 0', 'Incl. Ref 0', 'ID Injections 0', and '# Groups Add 0'. A note above the table states: 'Adding or removing template injections auto-populates this table'. At the bottom of the interface are buttons for 'Back', 'Cancel', 'Export', 'Import', 'Save', 'Save As', and 'Submit'.

Callouts provide the following information:

- Use double blue arrow and check box to select new compound detection (recommended)
- Full customization of number of each sample type added to each group.
- Low- reuse exclusion and/or inclusion list from Group 1 only.
High- reuse use any list (even multiple) from any previous group
- Acquire X sequence can be exported as a .csv and imported later

Xcalibur: Acquire X Custom Workflows

Group 1
"Deep Scan"

Group 2
"Iterative precursor exclusion"

AcquireX Workflow Editor

Mode **High**

AcquireX Template Injections **Group 2** | Blanks 2 | Excl. Ref 1 | Incl. Ref 0 | ID Injections 4 | # Groups to Add 1 | **Add**

#	Name	Type	Group	Instrument Method	Apply Excl. List	Apply Incl. List	Vial	Inj Vol (µl)
1	Blank_01	Blank	1	Instrument Method			R:A1	10.00 µl
2	ExclusionRef_01	Exclusion	1	Instrument Method			R:A1	10.00 µl
3	Sample_01	Inclusion	1	Instrument Method			R:A1	10.00 µl
4	ID_01	Id	1	Instrument Method	[1]	[1]	R:A1	10.00 µl
5	ID_02	Id	1	Instrument Method			R:A1	10.00 µl
6	ID_03	Id	1	Instrument Method			R:A1	10.00 µl
7	Blank_01	Blank	2	Instrument Method			R:A1	10.00 µl
8	Blank_02	Blank	2	Instrument Method			R:A1	10.00 µl
9	ExclusionRef_01	Exclusion	2	Instrument Method			R:A1	10.00 µl
10	ID_01	Id	2	Instrument Method	1, [2]	1	R:A1	10.00 µl
11	ID_02	Id	2	Instrument Method			R:A1	10.00 µl
12	ID_03	Id	2	Instrument Method			R:A1	10.00 µl
13	ID_04	Id	2	Instrument Method			R:A1	10.00 µl

Right click allow for copy down, insert injection, and undo

In High mode- Option to choose exclusion list from group 1 and 2

- Insert Inj Above
- Insert Inj Below
- Copy Down
- Undo
- Display Comment Column
- Apply Name Extension

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



 A flowchart titled 'CUSTOM Ab WORKFLOWS' showing a sequence of steps: MS OT, Targeted Mass (Optional), Targeted Mass Exclusion, and ddMS² OT HCD. A dashed line connects the 'Targeted Mass (Optional)' and 'Targeted Mass Exclusion' steps.

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

The screenshot shows the 'AcquireX Ab Workflow Editor' interface. On the left, there's a sidebar with 'CUSTOM Ab WORKFLOWS' and a navigation menu including 'MS OT', 'Targeted Mass', 'Targeted Mass Exclusion', and 'ddMS² OT HCD'. Below this are sections for 'Experiment Details' (with fields for 'Experiment Folder' and 'Experiment Name'), 'Group Parameters' (with 'Group #' set to 1 and an 'Apply To All Groups' button), and 'Instrument Methods' (with 'Browse' and 'New' buttons for different sample types). At the bottom left, 'Experiment Parameters' are listed as 'Component Detection Settings', 'Exclusion List Parameters', and 'Inclusion List Parameters'. A bracket on the far left labels these as 'Fully customizable detection parameters'. The main area is a table titled 'AcquireX Template Injections' with columns for '#', 'Name', 'Type', 'Group', 'Instrument Method', 'Apply Excl. List', 'Apply Incl. List', 'V', and 'Inj Vol (µl)'. Above the table are controls for 'Blanks', 'Excl. Ref', 'Incl. Ref', 'ID Injections', and '# Groups to Add'. A 'Mode' dropdown is set to 'Low'. At the bottom, there are buttons for 'Back', 'Cancel', 'Export', 'Import', 'Save', 'Save As', and 'Submit'. Five callout boxes provide additional information: 1. 'Full customization of number of each sample type added to each group.' points to the 'Blanks', 'Excl. Ref', 'Incl. Ref', and 'ID Injections' fields. 2. 'Low- reuse exclusion and inclusion list from Group 1 only. High- reuse use any list (even multiple) from any previous group' points to the 'Apply Excl. List' and 'Apply Incl. List' columns. 3. 'Method for Blank/Exclusion/Inclusion can be MSn not ONLY MS' points to the 'Instrument Method' column. 4. 'Acquire X Ab sequence can be exported as a .csv and imported later' points to the 'Export' and 'Import' buttons. 5. A large bracket on the left side encompasses the 'Experiment Parameters' section and is labeled 'Fully customizable detection parameters'.

Xcalibur: Acquire X Ab Custom Workflows

Experiment Name

Group Parameters

Group # 1 Apply To All Groups

Instrument Methods

Method for Blank/Exclusion/Inclusion samples Browse New

Template method for ID samples Browse New

Experiment Parameters

Component Detection Settings

User-defined Exclusion Settings On

MS Noise Level 2.00e+4 S/N Threshold 2.50e+1 [MS] Signal Threshold 5.00e+5

User-defined Inclusion Settings Off

User-defined Start/End Time On

Start (min) 0 End (min) 600

Exclusion List Parameters

Inclusion List Parameters

AcquireX Ab Workflow Editor Mode Low

AcquireX Template Injections Blanks 0 Excl. Ref 0 Incl. Ref 0 ID Injections 0 # Groups to Add 0 Add

#	Name	Type	Group	Instrument Method	Apply Excl. List	Apply Incl. List	Vial	Inj Vol (µl)
Adding or removing template injections auto-populates this table								

Back Cancel Export Import Save Save As Submit

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

AcquireX Ab Workflow Editor

Mode **High** ⓘ

AcquireX Template Injections **Group 2** ✎ 🗑️ | Blanks 2 Excl. Ref 1 Incl. Ref 1 ID Injections 3 # Groups to Add 1 **Add** 🗄️

#	Name	Type	Group	Instrument Method	Apply Excl. List	Apply Incl. List	Vial	Inj Vol (μl)	
1	Blank_01	Blank	1	Instrument Method			R:A		
2	ExclusionRef_01	Exclusion	1	Instrument Method			R:A		
3	Sample_01	Inclusion	1	Instrument Method			R:A		
4	ID_01	Id	1	Instrument Method	[1]	[1]	R:A		
5	ID_02	Id	1	Instrument Method			R:A		
6	Blank_01	Blank	2	Instrument Method			R:A1	10.00 μl	
7	Blank_02	Blank	2	Instrument Method			R:A1	10.00 μl	
8	ExclusionRef_01	Exclusion	2	Instrument Method			R:A1	10.00 μl	⋮
9	Sample_01	Inclusion	2	Instrument Method			R:A1	10.00 μl	⋮
10	ID_01	Id	2	Instrument Method	1, [2]	[2]	R:A1	10.00 μl	⋮
11	ID_02	Id	2	Instrument Method	<input checked="" type="checkbox"/> 1 <input checked="" type="checkbox"/> 2		R:A1	10.00 μl	⋮
12	ID_03	Id	2	Instrument Method			R:A1	10.00 μl	⋮

Right click allow for copy down, insert injection, and undo

In High mode- Option to choose exclusion list from group 1 and 2

Each group can have a different number to sample types

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	50 – 2000	50 – 2000	50 – 2000
		40 – 2000	40 – 2000	40 – 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

Data-Independent Analysis Properties Show Favorites

Precursor Mass Range (m/z) 100-1100

Multiplex Ions

Isolation Mode Quadrupole

DIA Window Type Auto

Isolation Window (m/z) 100

Window Overlap (m/z) 0

Number of Scan Events 10

Window Placement Optimization Off

DIA Window Mode m/z Range

DIA m/z window ADD DELETE IMPORT EXPORT

	m/z range
1	100-200
2	200-300
3	300-400
4	400-500
5	500-600
6	600-700
7	700-800
8	800-900
9	900-1000
10	1000-1100

DIA Window Type = User Defined

Data-Independent Analysis Properties Show Favorites

Multiplex Ions

Isolation Mode Quadrupole

DIA Window Type User Defined

DIA Window Mode m/z Range

DIA m/z window ADD DELETE IMPORT EXPORT

	m/z range
1	100-200
2	200-300
3	300-400
4	400-500
5	500-600
6	600-700
7	700-800
8	800-900
9	900-1000
10	1000-1100

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

Data-Independent Analysis Properties Show Favorites

Multiplex Ions ★

Isolation Mode Quadrupole ★

DIA Window Type User Defined ★

DIA Window Mode m/z Range ★

DIA m/z window ADD DELETE IMPORT EXPORT

	m/z range
1	100-200
2	200-300
3	300-400
4	400-500
5	500-600
6	600-700
7	700-800
8	800-900
9	900-1000
10	1000-1100

DIA Window Mode = Center Mass

Data-Independent Analysis Properties Show Favorites

Multiplex Ions ★

Isolation Mode Quadrupole ★

DIA Window Type User Defined ★

DIA Window Mode Center Mass ★

DIA m/z window ADD DELETE IMPORT EXPORT

	Center Mass (m/z)	Window Width (m/z)
1	150	100
2	250	100
3	350	100
4	450	100
5	550	100
6	650	100
7	750	100
8	850	100
9	950	100
10	1050	100

Multiplexing in tSIM / tMS2 / DIA Scans

Expanded Multiplexing to up to 20 Ions

Orbitrap Tribrid Series ICSW 3.5

Targeted SIM Scan Properties Show Favorites

Multiplex Ions ★

Maximum Number of Multiplexed Ions ★

Define Multiplexing Groups (MSX ID) ★

Isolation Mode

Orbitrap Tribrid Series ICSW 4.0

Targeted SIM Scan Properties Show Favorites

Multiplex Ions ★

Maximum Number of Multiplexed Ions ★

Define Multiplexing Groups (MSX ID) ★

Isolation Mode [Learn more...](#) ★



Targeted MSⁿ Scan Properties Show Favorites

MSⁿ Level (n) ★

Multiplex Ions ★

Maximum Number of Multiplexed Ions ★

Define Multiplexing Groups (MSX ID) ★

Targeted MSⁿ Scan Properties Show Favorites

MSⁿ Level (n) ★

Multiplex Ions ★

Maximum Number of Multiplexed Ions ★

Define Multiplexing Groups (MSX ID) ★

Data-Independent Analysis Properties Show Favorites

Precursor Mass Range (m/z) ★

Multiplex Ions ★

Maximum Number of Multiplexed Ions ★

Define Multiplexing Groups (MSX ID) ★

Data-Independent Analysis Properties Show Favorites

Precursor Mass Range (m/z) ★

Multiplex Ions ★

Maximum Number of Multiplexed Ions ★

Define Multiplexing Groups (MSX ID) ★

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

Dynamic Retention Time Pierce PRTC Mixture

Select table icon to add property to mass list table.

Retention Time Standards				
	Peptide Name	m/z	RT Time (min)	RT Window (min)
1	SSAAPPPPPR	493.7683	5.2	3

Scan Description

FAIMS Voltages On

FAIMS CV (V) Defined in Table



Orbitrap Tribrid Series ICSW 4.0

Dynamic Retention Time Pierce PRTC Mixture

Select table icon to add property to mass list table.

Retention Time Standards					
	Peptide Name	m/z	RT Time (min)	RT Window (min)	FAIMS CV (V)
1	SSAAPPPPPR	493.7683	5.2	3	40

Scan Description

FAIMS Voltages On

FAIMS CV (V) Defined in Table

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	SAAGAFGPPELSR	586.8003	26.6	3
8	ELGQSGVDTYLQTK	773.8955	30.4	3
9	GLILVGGYGTR	558.3259	34.4	3
10	GILFVGSVSGGEEGAR	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

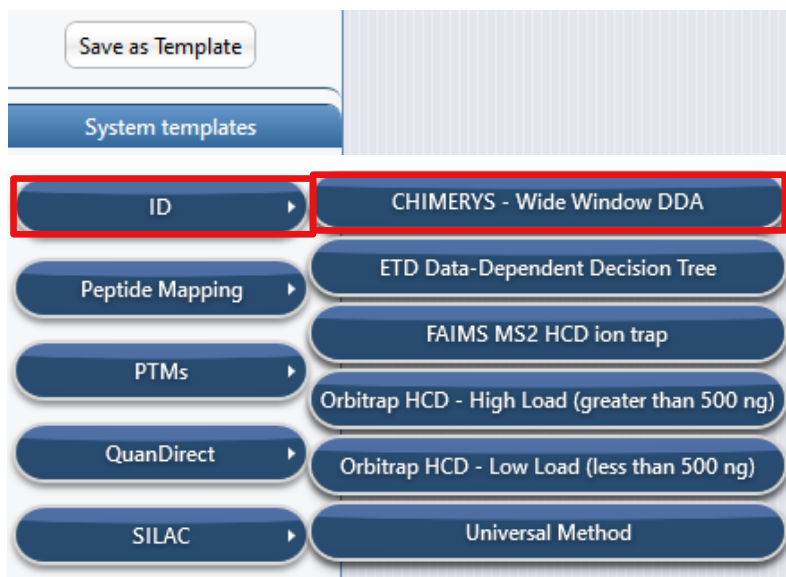
Retention Time Standards						
	Peptide Name	m/z	RT Time (min)	RT Window (min)	FAIMS CV (V)	z
1	SSAAPPPPPR	493.7683	5.2	3	40	2
2	GISNEGQNASIK	613.3167	8.3	3	50	2
3	HVLTSIGEK	496.2867	12.5	3	40	2
4	DIPVPKPK	451.2834	15.9	3	50	2
5	IGDYAGIK	422.7363	19.7	3	40	2
6	TASEFDSAIAQDK	695.8324	22.8	3	60	2
7	SAAGAFGPPELSR	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	GILFVGSVSGGEEGAR	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

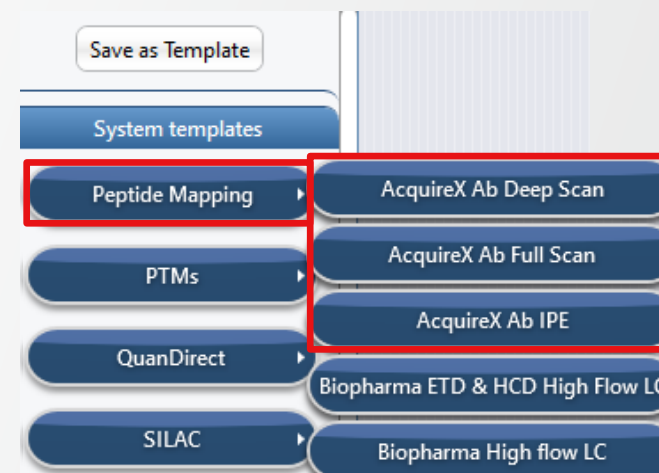
New and Updated Templates

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

Chimerys



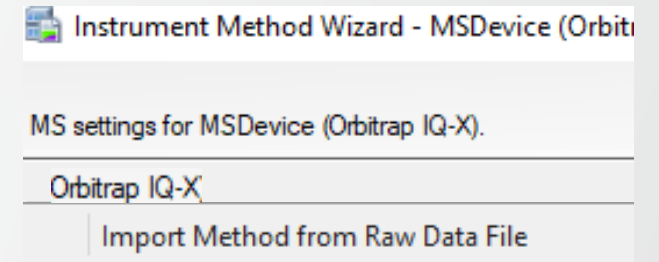
AcquireX Ab



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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