Go Beyond

with technology and intelligence

Orbitrap ID-X Mass Spectrometer

Product introduction and application solutions

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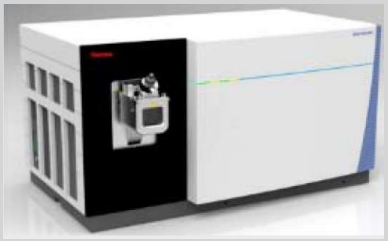
Technical Sales Manager LSMS

Sofia, October 25th 2018

Tribrid MS Product Series

Orbitrap ID-X



- Mass range: 50 – 6000 m/z
- Mass accuracy: < 1 ppm (internal cal)
- Max. resolution: 500.000
- Max. scan speed: 30 Hz OT, 40 Hz IT
- Method templates
- Assisted CE
- AcquireX
- Dissociation modes: sCID, CID, HCD, ETD, EThcD, UVPD

NEW

Fusion

- Mass range: 50 – 6000 m/z
- Mass accuracy: < 1 ppm (internal cal)
- Max. resolution: 500.000
- Max. scan speed: 30 Hz OT, 40 Hz IT
- Method templates
- Assisted CE
- AcquireX
- Segmented quadrupole
- Intact protein mode
- Dissociation modes: sCID, CID, HCD, ETD

Orbitrap Fusion Lumos

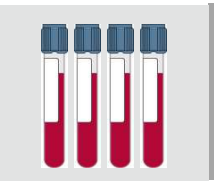
- Mass range: 50 – 6000 m/z
- Mass accuracy: < 1 ppm (internal cal)
- Max. resolution: 500.000 (optional 1M)
- Max. scan speed: 30 Hz OT, 40 Hz IT
- Method templates
- Assisted CE
- AcquireX
- Segmented quadrupole
- Intact protein mode
- UVPD
- Dissociation modes: sCID, CID, HCD, ETD, EThcD, UVPD

Small Molecule Unknown Analysis

Challenges

- Structural Diversity
- Background Interference
- Sample Limitations
  - Detection Limit
  - Dynamic Range
  - Spectral Density
- MS^2 is Insufficient
- MS^n is Difficult to Setup and Analyze

Applications



Metabolomics

Metabolite, Degradant and Impurity Identification

Extractables and Leachables Identification

Thermo Scientific Orbitrap ID-X Tribrid Mass Spectrometer

Break your bottlenecks with technology and intelligence

Built on Tribrid Architecture

Revolutionary Orbitrap MS dedicated for small molecule analysis

Collect more meaningful data, not just more data

AquireX automated novel acquisition workflow allowing for comprehensive sample interrogation

Match analysis requirements with speed, simplicity, and flexibility

MS^n library generating tool allows to create your own spectral library and m/z Logic for improved coverage and confidence

Annotate compounds using MS^n and HCD/CID fragmentation to demystify unknowns

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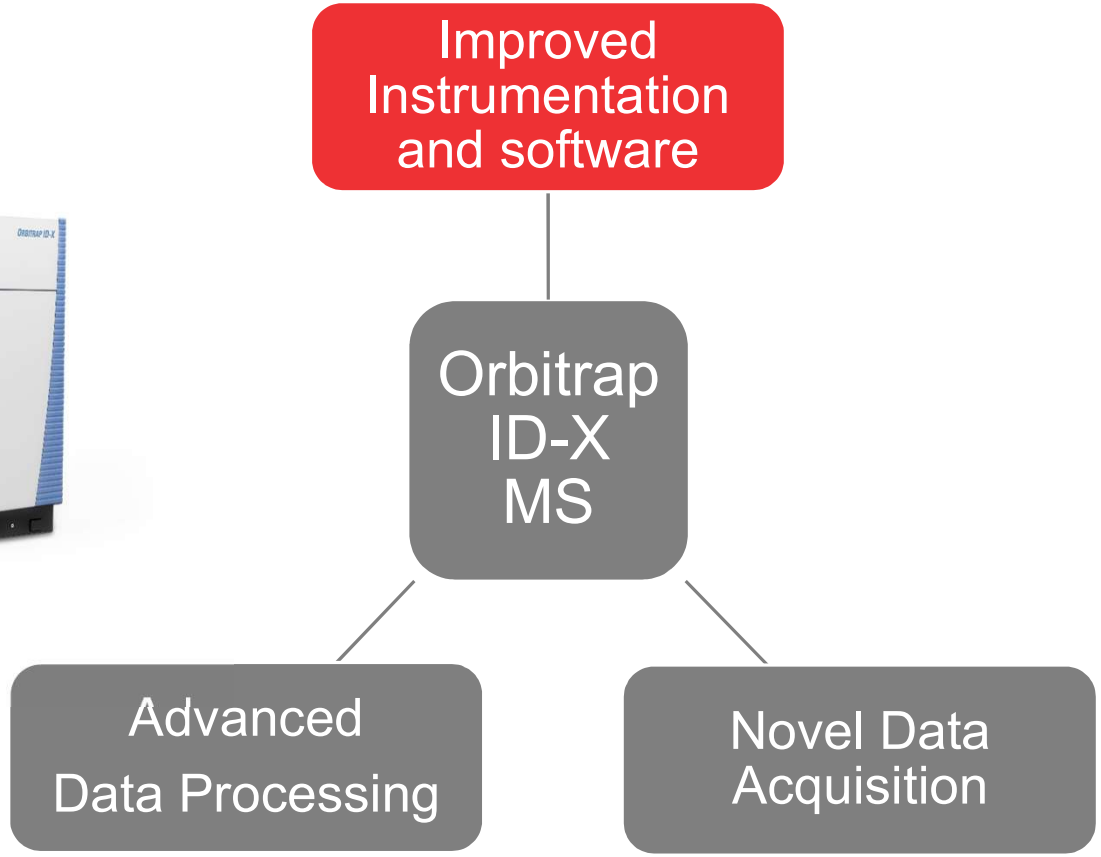
Small Molecule Dedicated Mass Spectrometer on Thermo Scientific Tribrid Platform

Thermo Scientific™ Orbitrap ID-X™ Tribrid™ Mass Spectrometer System



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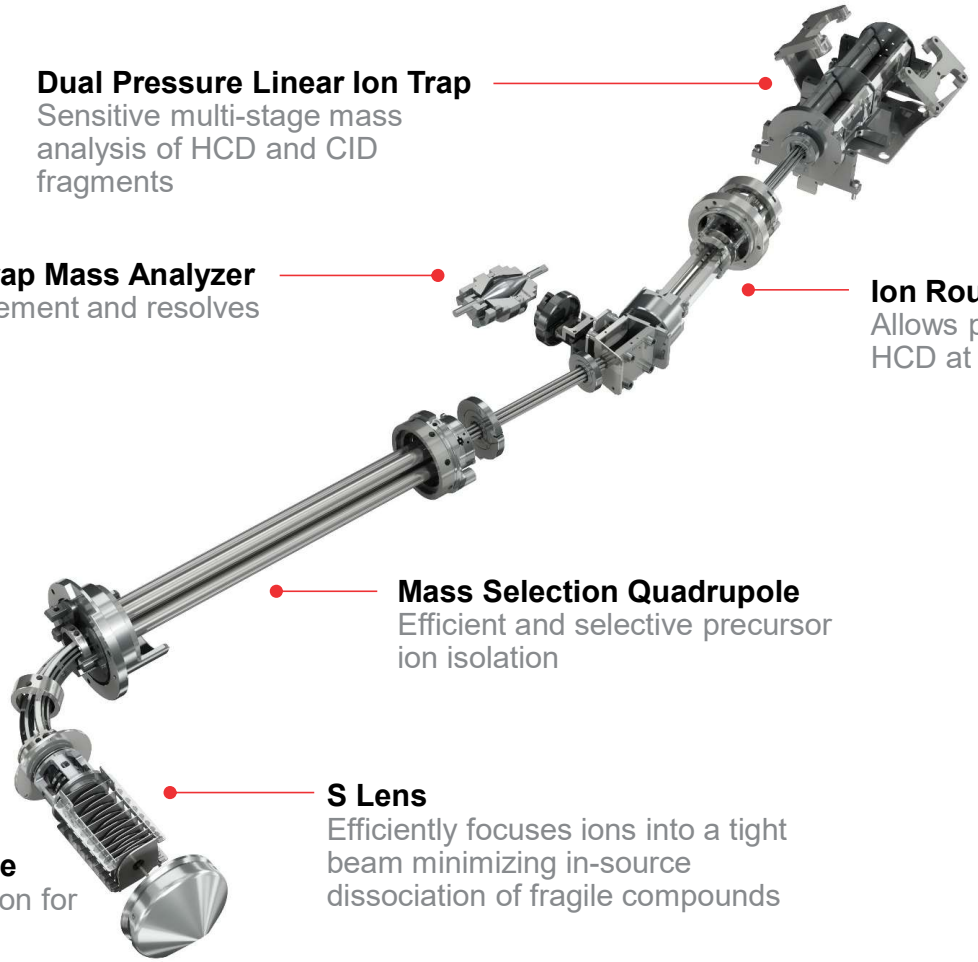
Orbitrap ID-X – What is inside?



Dual Pressure Linear Ion Trap
Sensitive multi-stage mass analysis of HCD and CID fragments

Ultra High Field Orbitrap Mass Analyzer
Accurate mass measurement and resolves isobaric species

Ion Routing Multipole
Allows parallel analysis HCD at any fragmentation level

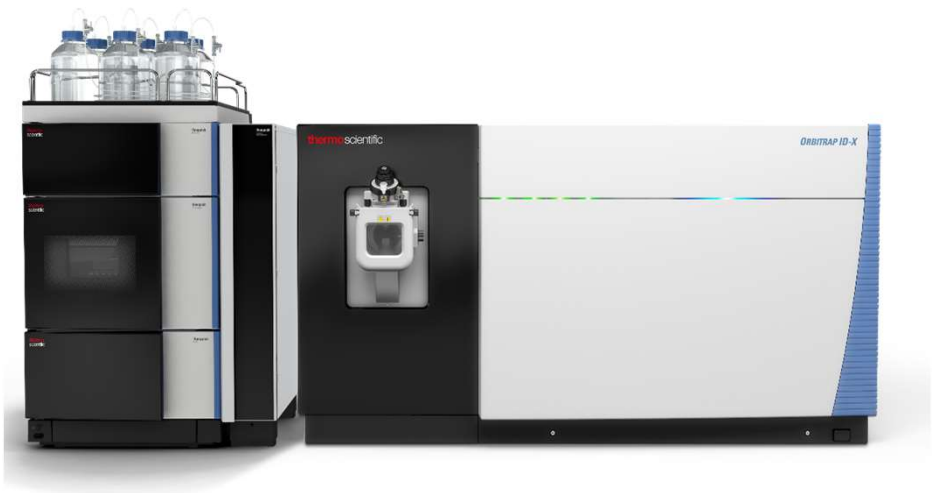
Mass Selection Quadrupole
Efficient and selective precursor ion isolation

Active Beam Guide
Prevents neutrals and high velocity clusters from entering mass selecting quadrupole

S Lens
Efficiently focuses ions into a tight beam minimizing in-source dissociation of fragile compounds

Optional Easy IC Ion Source
Generates internal calibrant ion for real-time mass calibration

Orbitrap ID-X Tribrid Mass Spectrometer Instrument Features



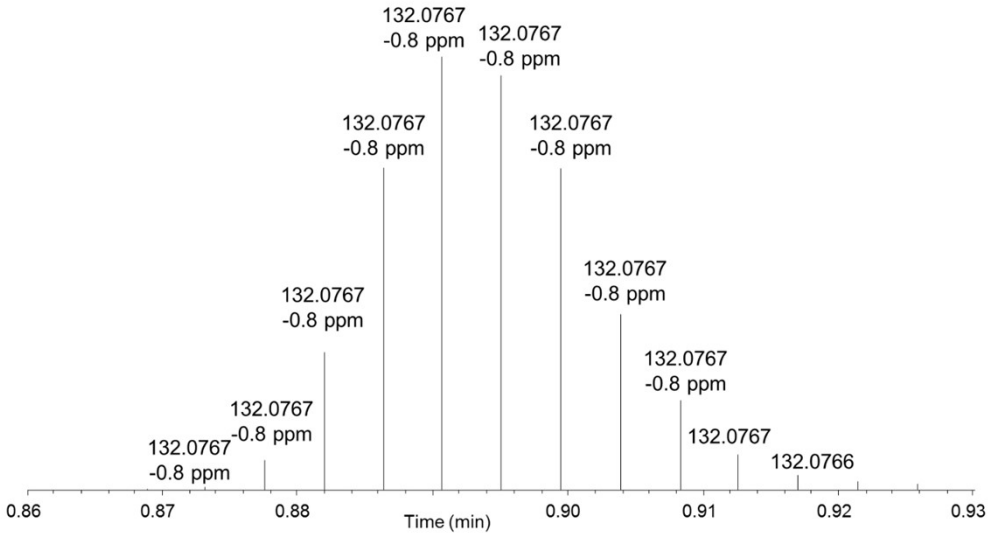
Max Resolution	500,000 at *m/z* 200
Scan Rate OTMS^2	30 Hz
Scan Rate ITMS^2	40 Hz
Quad Mass Selection	Precursor isolation to 0.4 amu
Ion Trap MS^n	Up to MS^{10}
Mass Accuracy	3 ppm external, 1ppm internal
Dissociation	CID, HCD

Instrument Improvements

- **Thermo Scientific™ OptaMax™ NG** ion source for enhanced usability and robustness
- **Streamlined calibrations** with improved mass calibration for ions with *m/z* <200
- **User interface** and default parameters optimized for small molecule analysis
- **Expansive library** of application specific small molecule methods
- **Assisted CE**, allowing for real-time collision energy optimization
- **Library Builder** method for the acquisition of high-quality MS^n spectral trees for local library generation

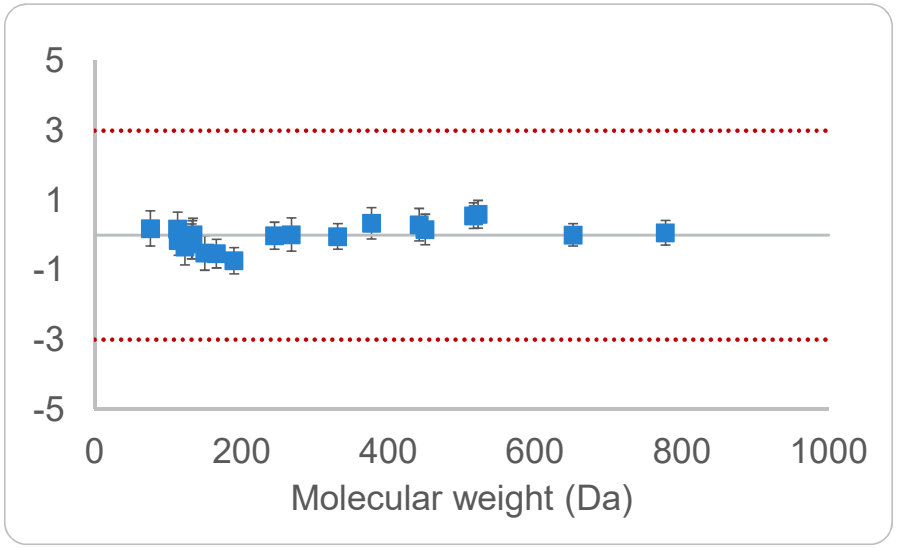
New Calibration Routine: Improved Mass Stability for Low *m/z* Ions

Excellent Scan-To-Scan Mass Measurement Accuracy



Sub-ppm mass measurement accuracy for Creatine (theoretical MH^+ 132.0768 Da) detected over the LC-MS elution profile

Excellent Run-To-Run Mass Measurement Accuracy



LC-MS mass measurement accuracy for a mixture of 24 small molecule standards conducted over 72 hours

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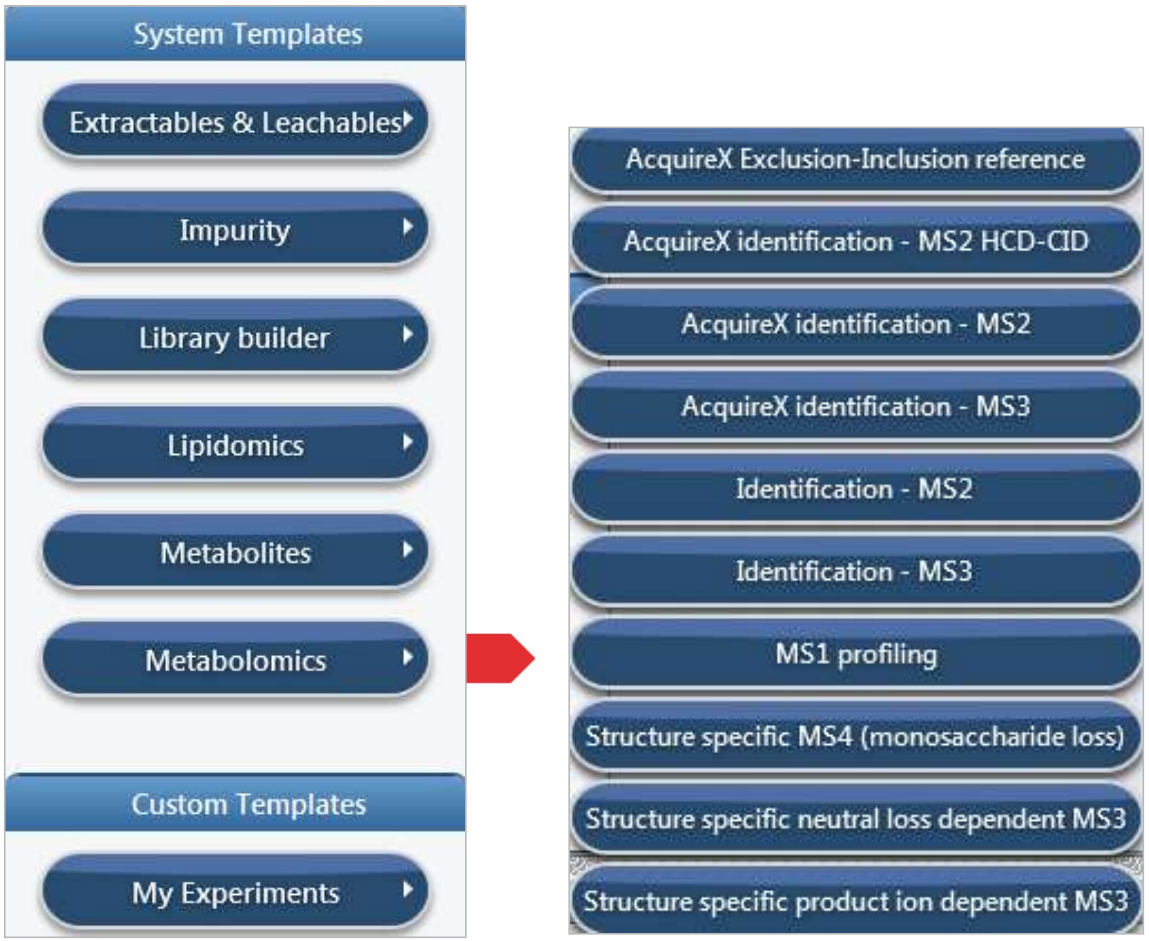
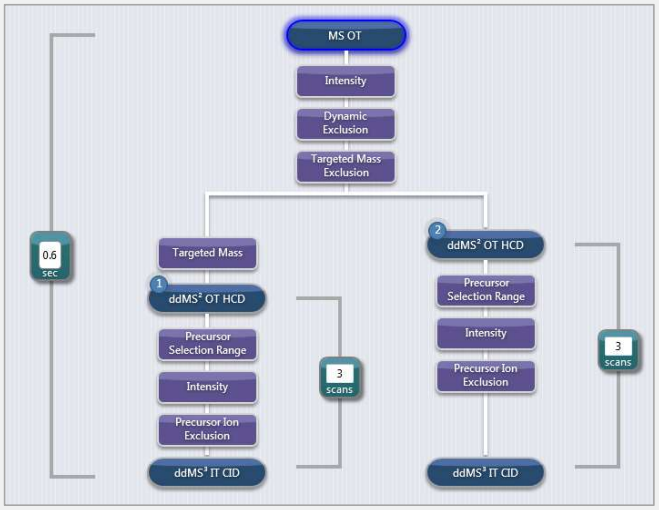
Pre-defined Method Templates for Getting Started



One-click Method Set-up

- Expansive library of application specific method templates
- Easy to use methods covering advanced workflows for small molecule profiling, identification and characterization



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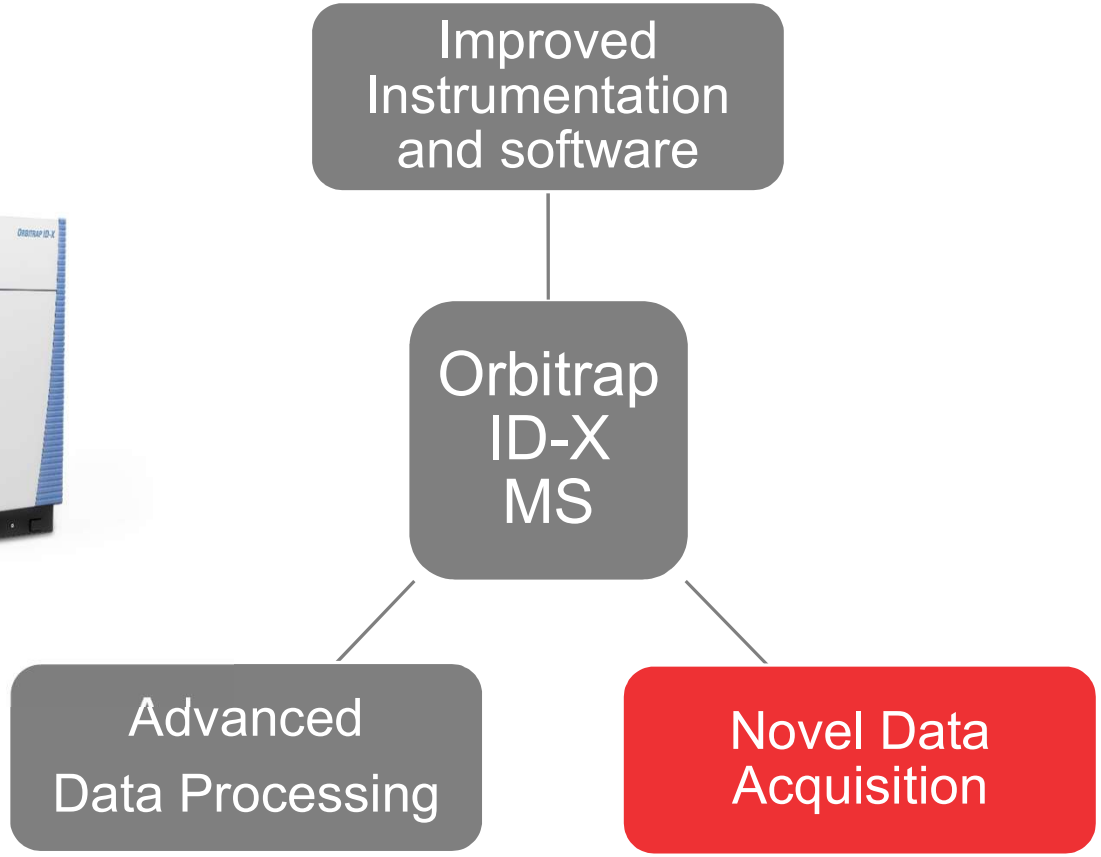
Small Molecule Dedicated Mass Spectrometer on Thermo Scientific Tribrid Platform

Thermo Scientific™ Orbitrap ID-X™ Tribrid™ Mass Spectrometer System



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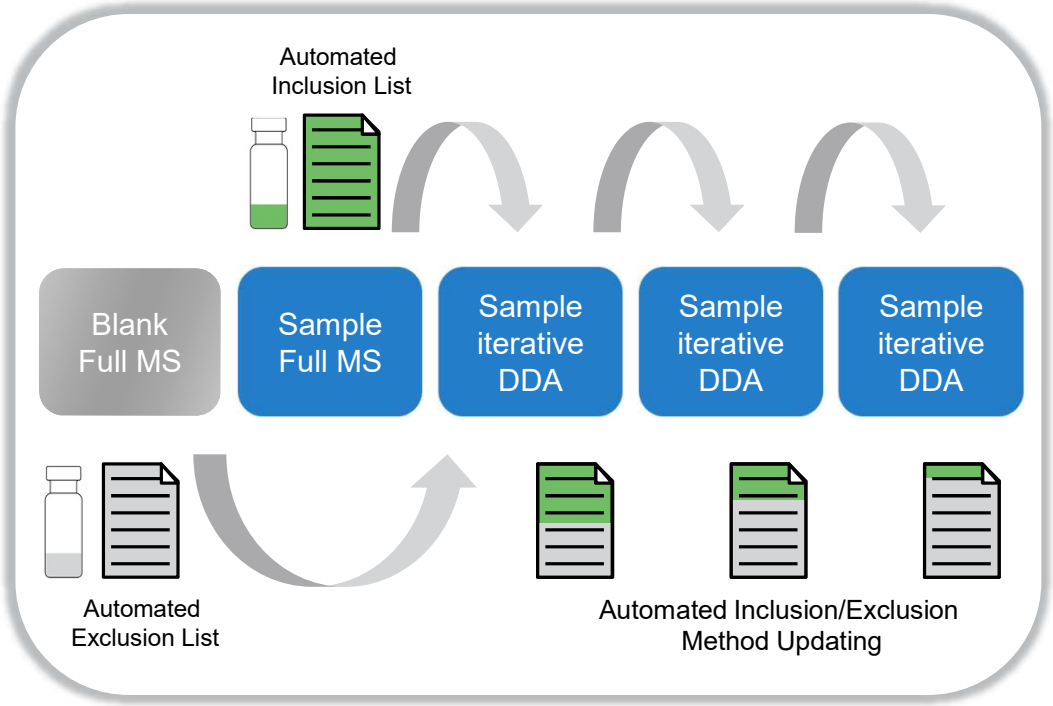
How To Get MS^n On Everything?

- Current LC-MS^n workflows require the user to strike a balance between spectral quality, specificity, and the breadth of analyte coverage
  - DDA MS^n analysis: Provides un-paralleled sensitivity and specificity, but at the cost of reduced sample coverage
  - DIA MS/MS: Extensive sample coverage but with greatly reduced specificity and selectivity. Incompatible with MS^n
- With AcquireX we break this interdependency between spectral quality and sample coverage.

AcquireX: Fully Automated All-inclusive Data Acquisition Workflow



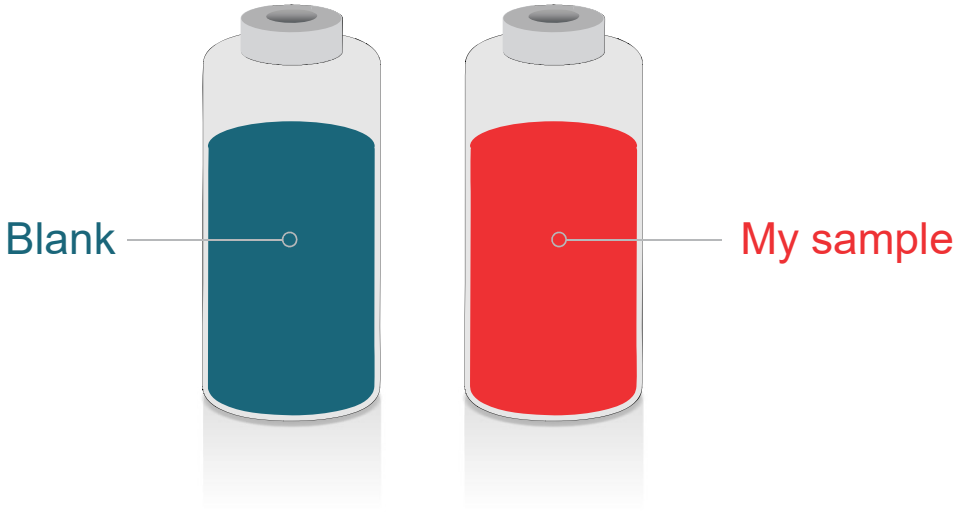
Automated Interrogation of All Compounds

- Automated exclusion of background ions
- Automated inclusion of sample compounds
- Automated inclusion/exclusion list method updating
- Iterative data informed re-injections for exhaustive MS^n profiling

AcquireX : How it works?

The challenge…

- I need to generate high quality MS^n spectra on **all** components in my sample
- My sample is too complex and not all features are fragmented
- Duty cycle is often wasted triggering on background features
- Manually excluding background and including relevant features is too laborious



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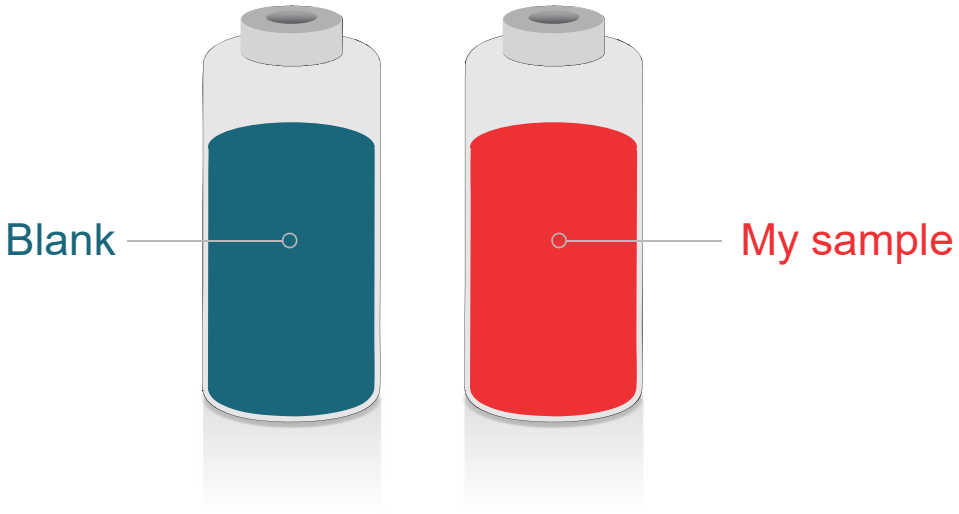
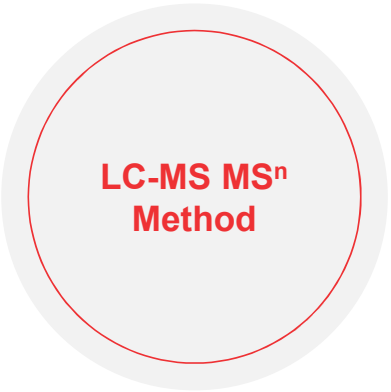
AcquireX : How it works?

How do I analyze all of the features in my sample using a data dependent MS^n method?

In an automated manner?





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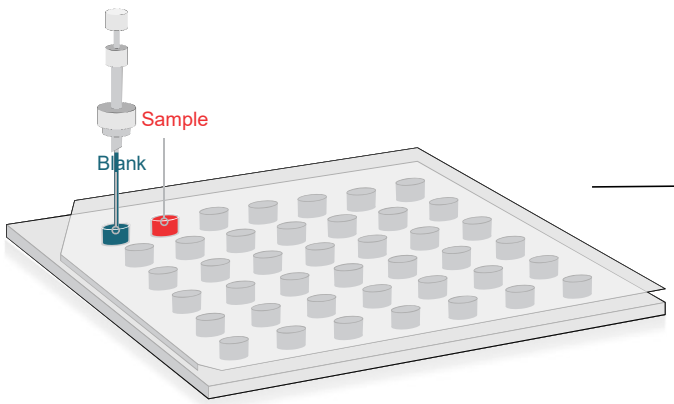
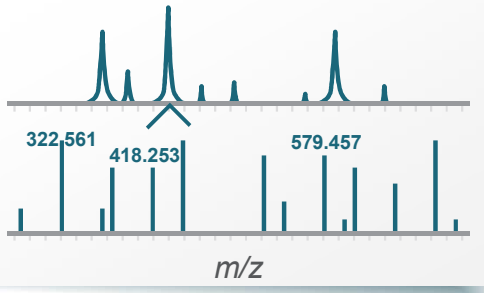
AcquireX : How it works?

Step 1: LC-MS analysis of background (blank)





EXCLUSION LIST

322.561
418.253
579.457
.....
.....

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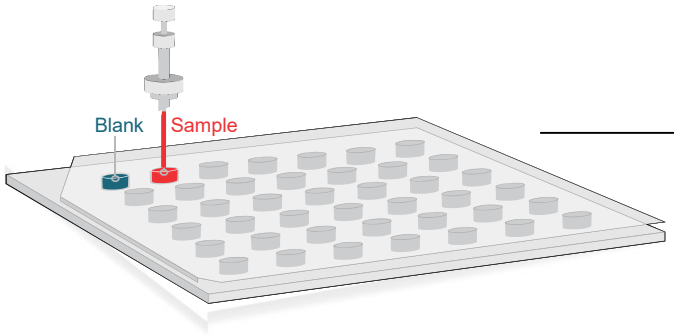
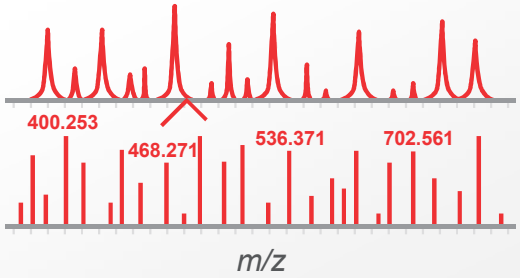
AcquireX : How it works?

Step 2: Full scan LC-MS analysis of the sample





INCLUSION LIST

400.253
468.271
536.371
702.561
.....

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AcquireX : How it works?

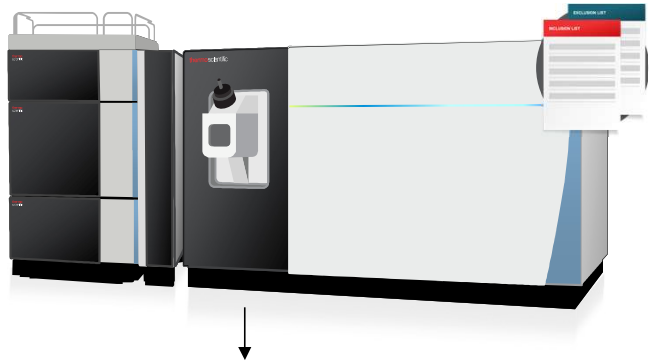
EXCLUSION LIST

322.561
418.253
579.457
.....
.....

INCLUSION LIST

400.253
468.271
536.371
702.561
.....

Step 3: Update the method and run the sample



INCLUSION LIST

400.253
468.271
~~536.371~~
~~702.561~~
.....

EXCLUSION LIST

322.561
418.253
579.457
536.371
702.561

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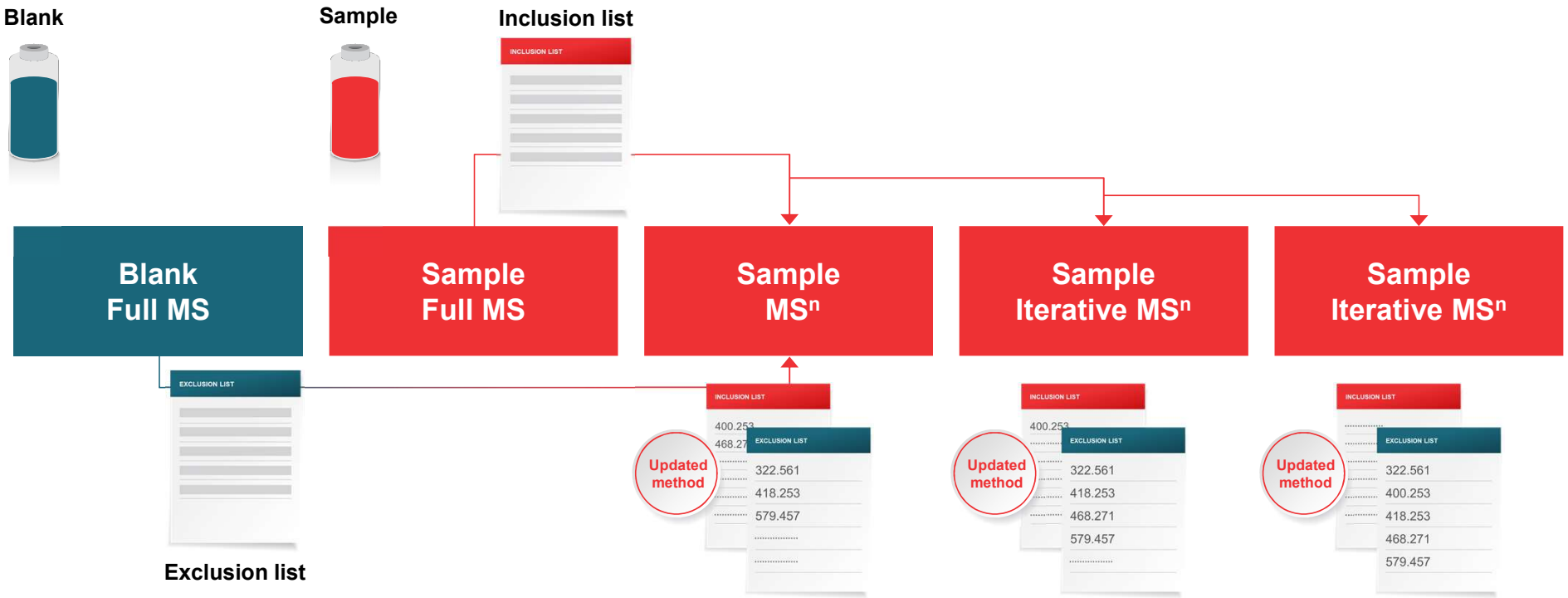
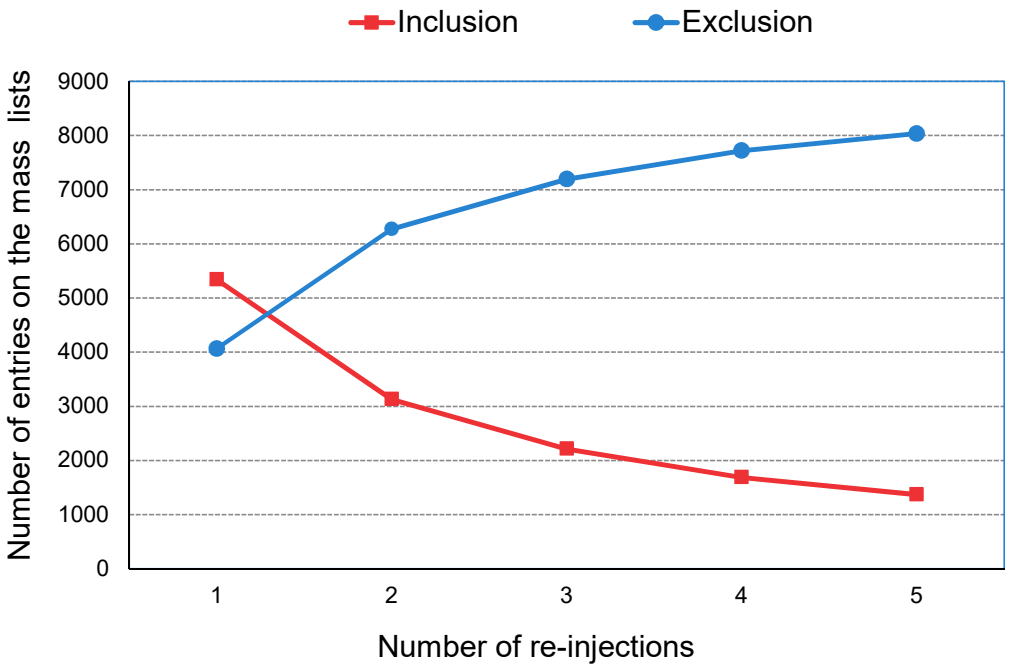
AcquireX : How it works?

AcquireX Workflow



Automatically updated run-to-run inclusion/exclusion lists

AcquireX: Automated Method Updating



LC-MS analysis of NIST SRM 1950 metabolites in frozen human plasma. The instrument automatically updates the inclusion and exclusion lists during successive iterative AcquireX re-injections, such that exclusion list continues to grow, while inclusion list declines with each additional re-injection

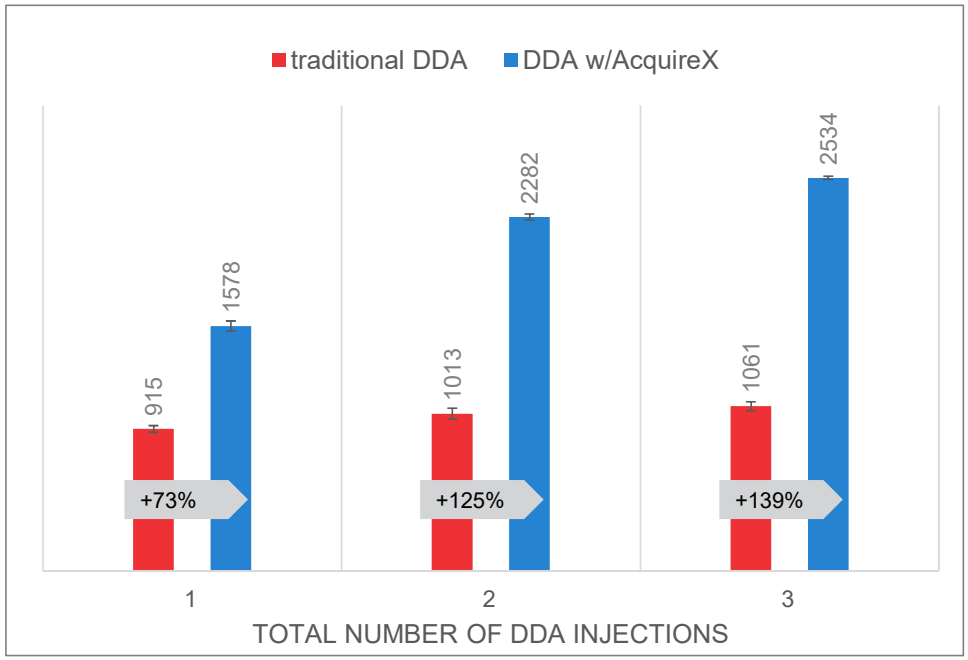
Ntai et al. ThP 564

Deep Scan Analysis

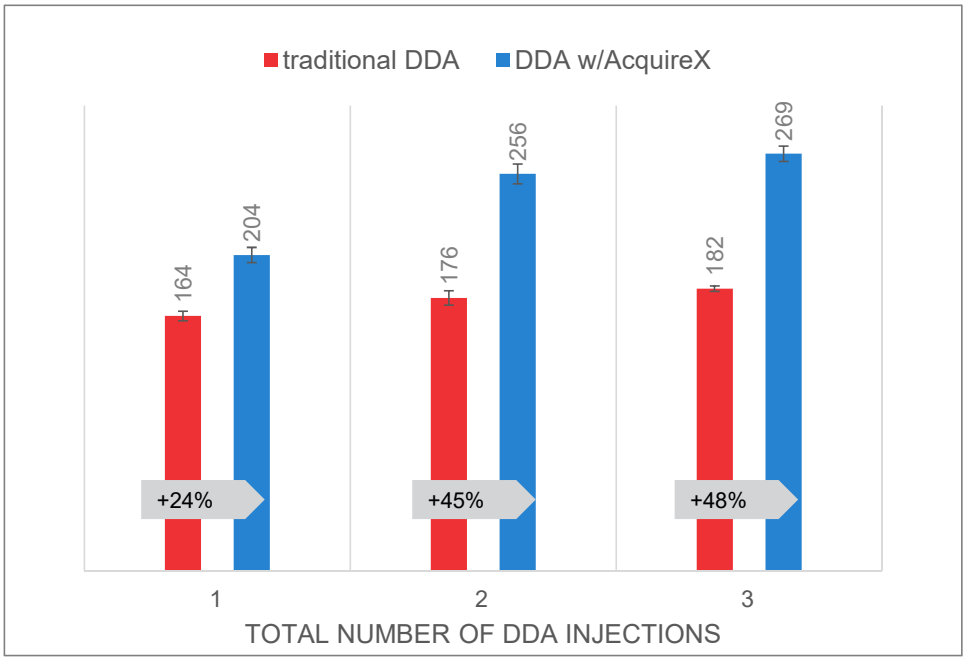
- 2 µl of NIST SRM 1950 plasma extract was analyzed using 2.1mm x 150 mm Hypersil Gold column
- Automated generation of exclusion list with >4000 features
- Automated generation of inclusion list with >5000 features
- Inclusion list size decreases with each re-injection while exclusion list size increases

AcquireX: More Confident Identifications with Increased Productivity

Increased Number of MS features with Fragmentation Spectra



Increased Number of mzCloud Spectral Matches Using Compound Discoverer 3.0 software



Comparison of three re-injections of the **extracted human plasma** using traditional DDA and DDA with AcquireX showing more than 2x increase in the number of compounds with fragmentation spectra (left) and almost 50% gain in confidently identified compounds (right) when DDA with AcquireX is used

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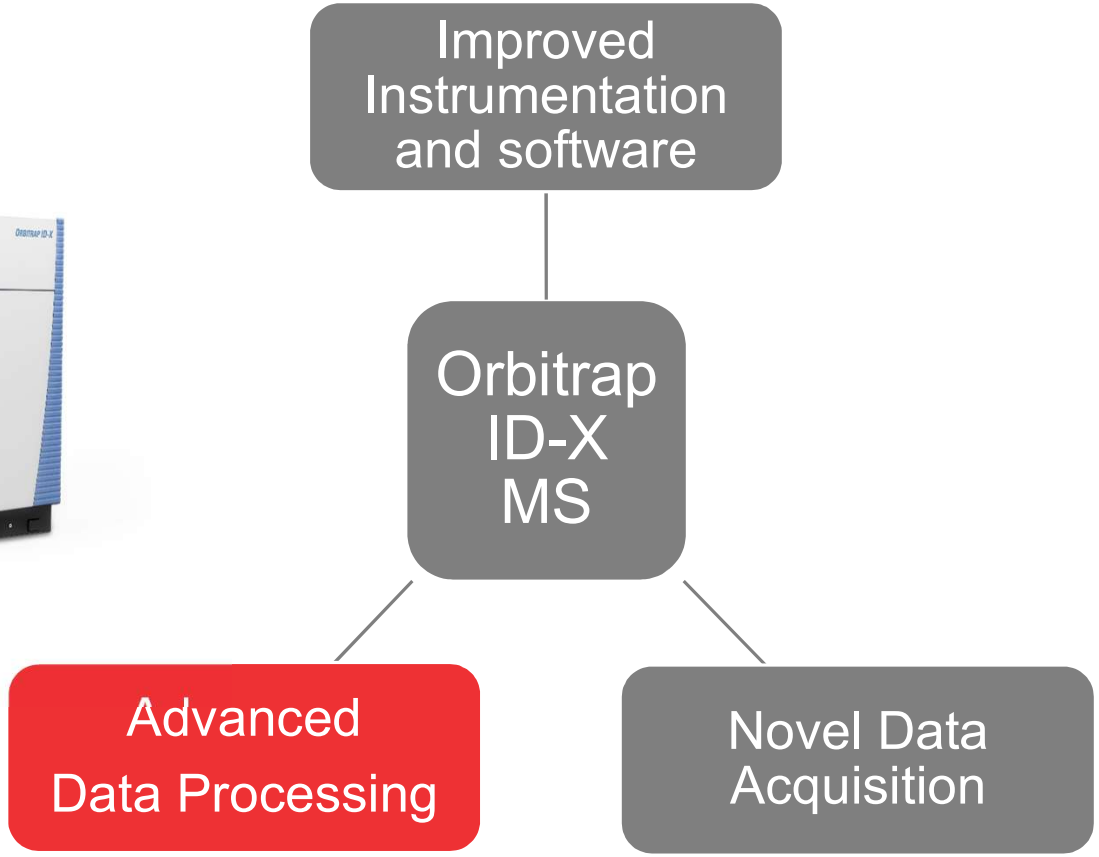

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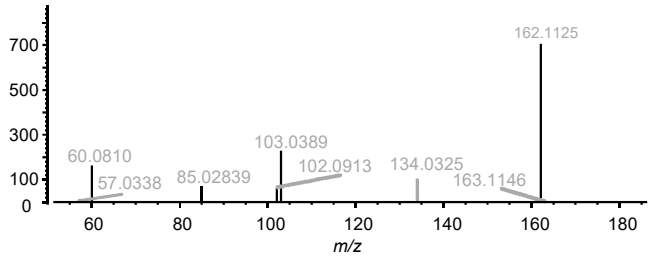
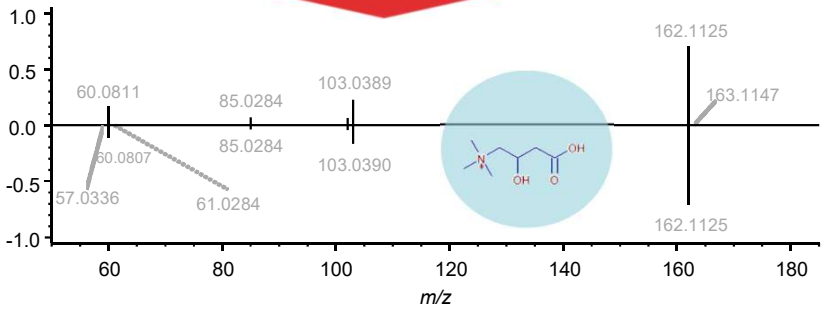
Small Molecule Data Processing Challenges

Ideally

MS^2 of unknown compound



Spectral Library Search

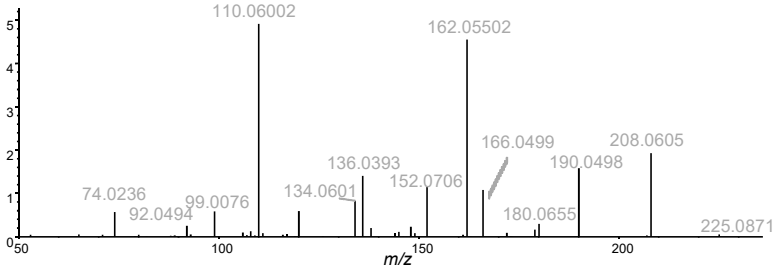


Reference data found in spectral library

→ **Compound identified**

But what if …

MS^2 of unknown compound



Spectral Library Search

NO reference data found in spectral library

Database Search
(HMDB,KEGG,BioCyc,PubChem)

441 hits

(based on predicted elemental composition)

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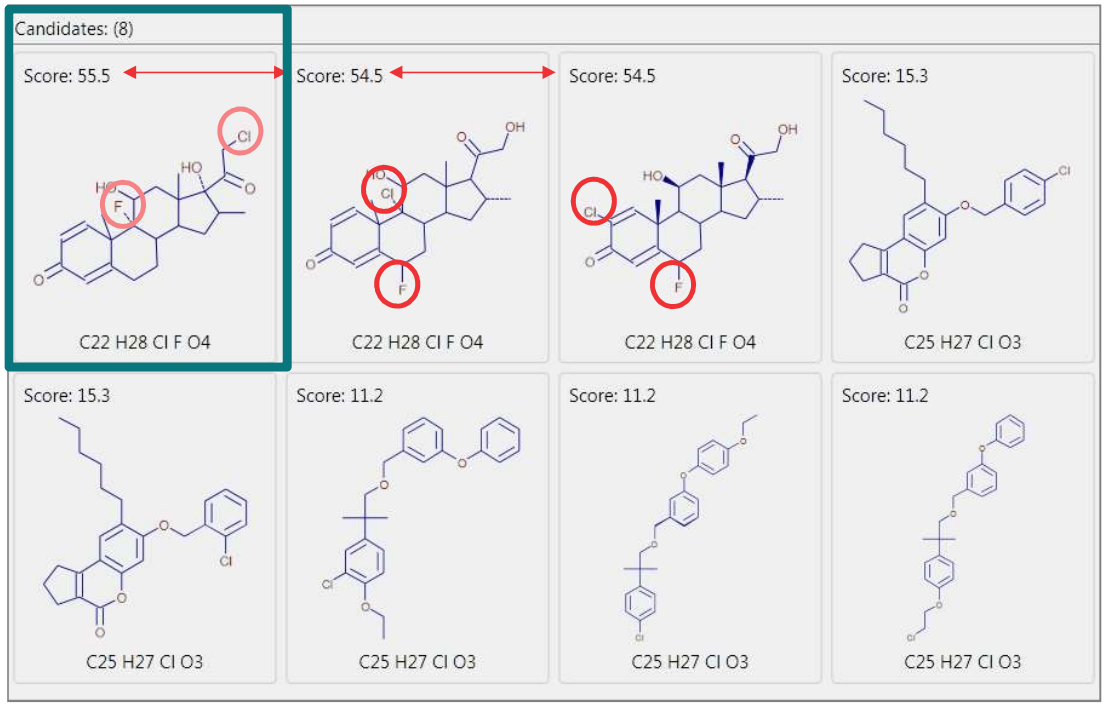
mzLogic: Ranking for Putative Structure Candidates

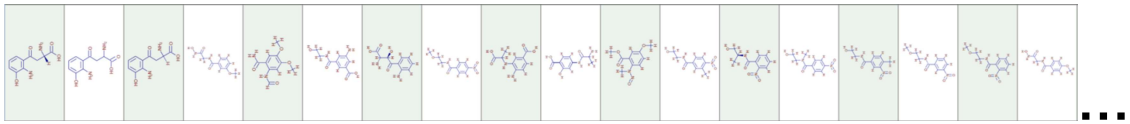
mzLogic Structure Ranking



Compound Discoverer mzLogic workflow

- **Step 1**: For a given elemental composition identifies candidate structures (i.e. using ChemSpider)
- **Step 2**: Performs a loose tolerance similarity search (in mzCloud) of the candidate structures from step 1 to retrieve mzCloud spectra that correspond to the substructures of the candidate structures
- **Step 3**: Maximum Common Substructure Search (MCSS) ranks the candidate structures from step 1 based on the match of the substructure library spectra retrieved in step 2 and the experimental data

mzLogic: Allows Ranking Putative Candidate Structures

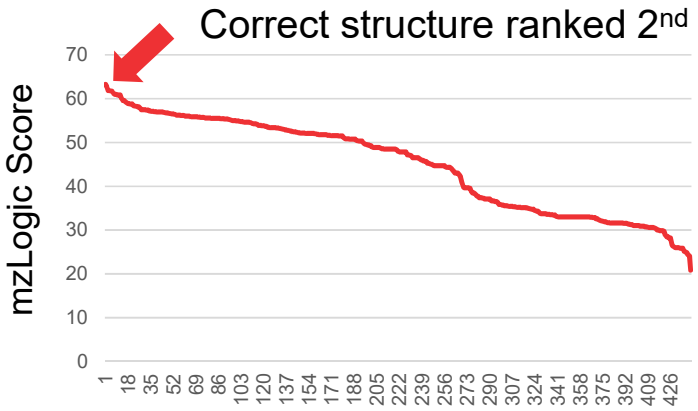


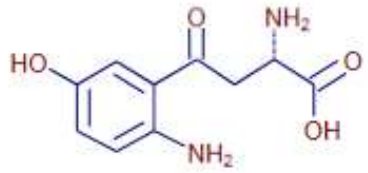
441 putative candidates for identification

Curated spectral library HCD/CID MS^n (2.8M spectra)

mzLogic in Compound Discoverer 3.0 software
With the new mzLogic algorithm, you can use the extensive fragmentation spectral information in mzCloud to rank-order putative database results

With the new mzLogic algorithm, you can use the extensive fragmentation spectral information in mzCloud to rank-order putative database results





3-hydroxy-kynurenine

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mzCloud

mzCloud – Advanced Mass Spe

https://www.mzcloud.org

Server location : EU

search for compounds... Search

m/z CLOUD

Advanced Mass Spectral Database

Home About Features App Database Partners Contact

mzCloud is a state of the art mass spectral database that assists analysts in identifying compounds in areas such as life sciences, metabolomics, pharmaceutical research, toxicology, forensic investigations, environmental analysis, food control and various industrial applications. mzCloud™ features a freely searchable collection of high resolution/accurate mass spectra using a new third generation spectra correlation algorithm.

Online access to the database is free of charge and no registration is required.

read more...

Enter Database

Google play

New mzCloud App!

Current mzCloud Statistics

Number of compounds: 8,261

Number of spectral trees: 12,464

	Raw	Filtered	Recalibrated
Positive	9,006	9,006	9,006
Negative	3,458	3,458	3,458

Number of spectra: 2,816,374

	Raw	Filtered	Recalibrated
Positive	1,300,563	481,209	481,219
Negative	315,537	118,923	118,923

Search for Compounds by Name or ID

Search

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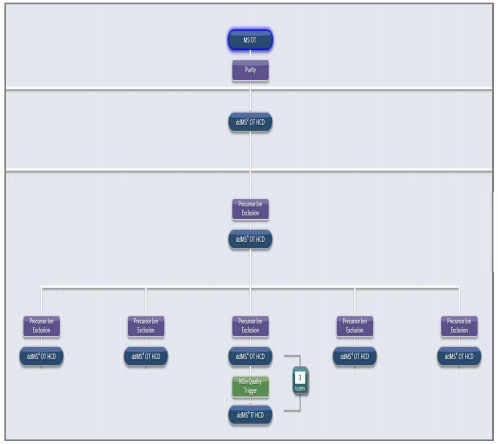
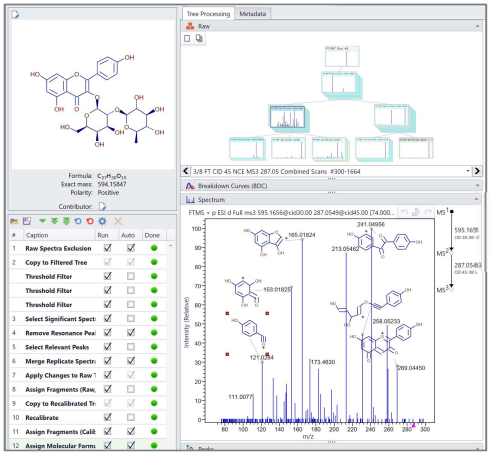
New MS^n Library Builder Template

MS^n Data Acquisition



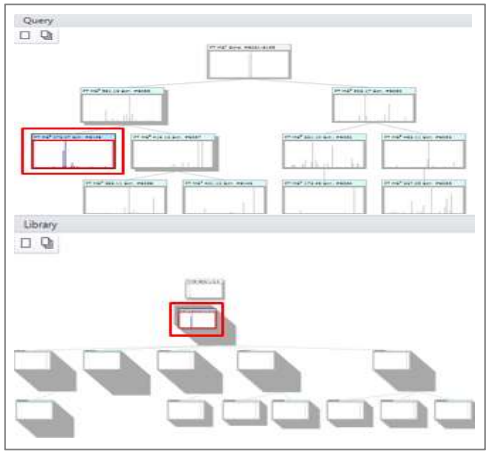
Ion tree spectra generation using the MS^n library builder method template (Infusion or LC-MS)

MS^n Library Creation



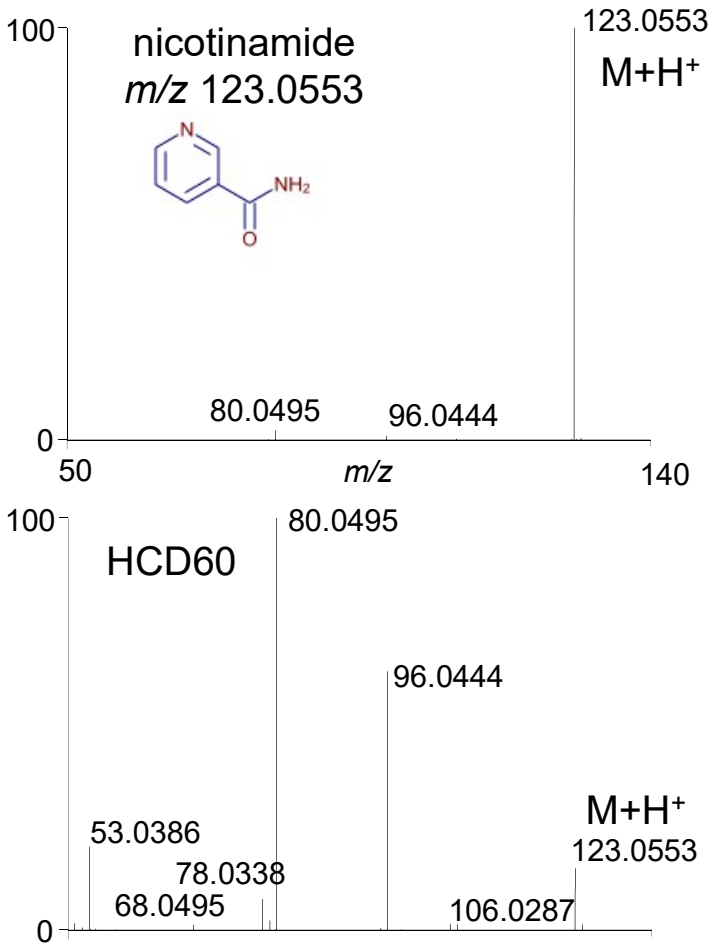
Ion tree MS^n spectra curation and local library generation using Mass Frontier 8.0 software

Apply MS^n Library For Unknown ID



The local MS^n library can be searched for unknown small molecule identification using Mass Frontier 8.0 software

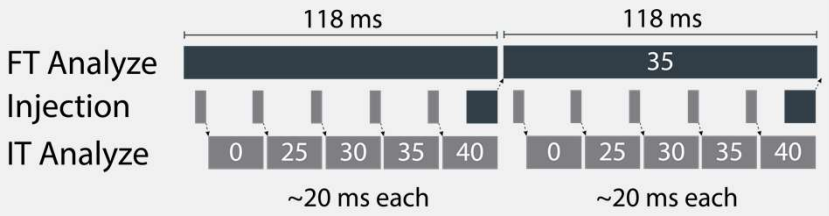
Assisted CE: Compound Specific Real-Time Collision Energy Optimization



Suboptimal Fragmentation Using HCD20

Optimal Fragmentation Achieved Using Assisted CE (Corresponds to 60% NCE)

Assisted CE Determination



- Instrument collects hidden ion trap scans to generate break-down curves per compound
- Optimal collision energy is ascertained based on specified precursor depletion threshold
- FTMS analytical scan is collected using the optimal collision energy

Compound Annotation using Mass Frontier to demystify unknowns

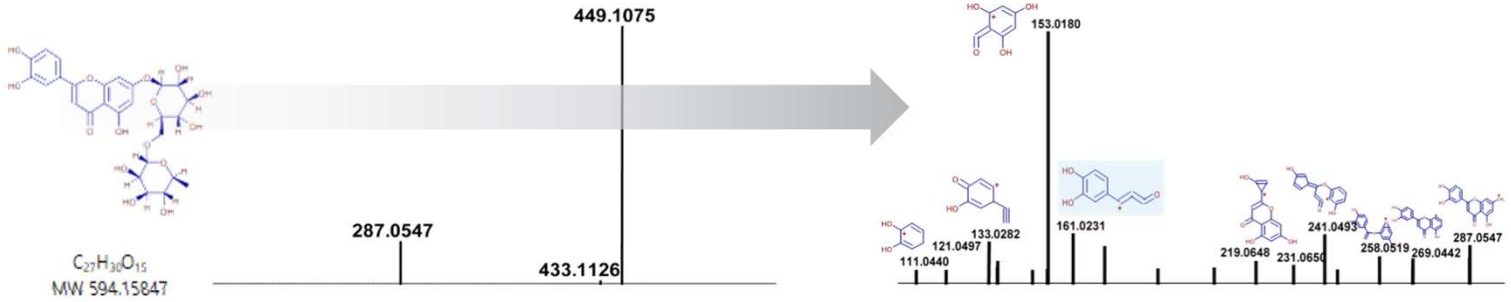
Structure relevant fragment ion information of MS^3 enables isomer identification

MSMS results indistinguishable without spectra from standards

MS^3 results easily distinguish the isomers

Kaempferol 3-O-β - rutinoside

Luteolin 7-rutinoside



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Orbitrap ID-X MS for Small Molecule Applications

- Untargeted Metabolomics
- Targeted Metabolomics
- Extractables and Leachables
- Identification of phytochemicals and other natural products
- Drug Metabolism
- Lipidomics
- Impurity Identification
- Any other complex small molecule application

- Proven Tribrid™ Architecture
- Predefined Method Templates
- AcquireX
- mzLogic
- Assisted CE
- MS^n Library Builder Template
- Compound Annotation
- Powerful Software Packages

Match Analysis Requirements With Speed, Simplicity and Flexibility

Novel approach to leverage MS^n data to find best candidate structures

Compound Discoverer Software

Efficiently extract high-confidence insights from information-rich small molecule HRAM data

Serves as a hub to seamlessly connect users to the tools they need to analyze productively and confidently

new

mzCloud Mass Spectral Library

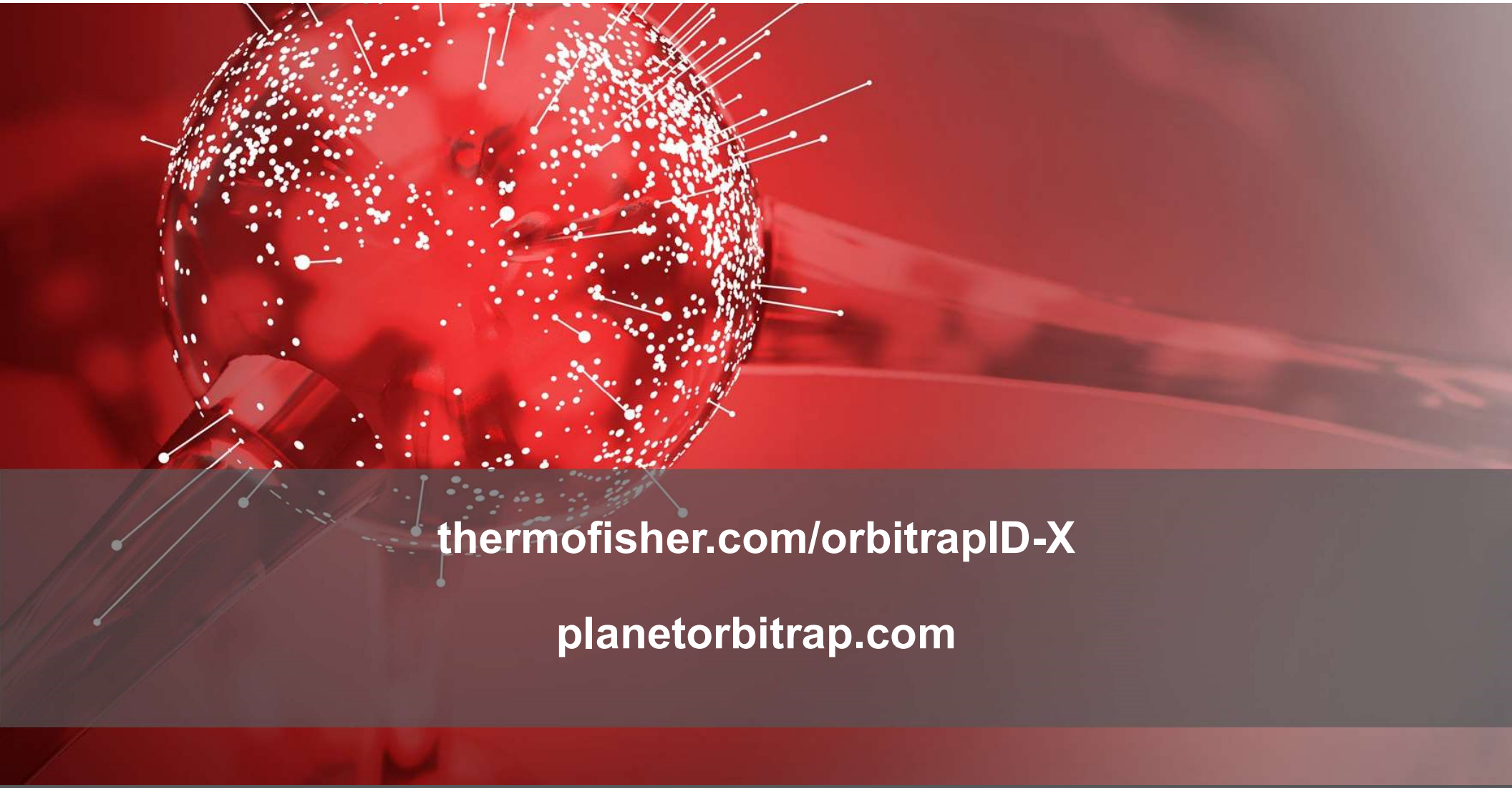
Rank search more **effectively** with industry leading online spectra fragmentation library

mzLogic Algorithm

From 1000's of candidates and hours of work to **fast automated logical analysis**

MassFrontier Software

Supports MS^n spectral ion trees searching and enables custom libraries



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