Improved metabolome coverage and increased confidence in unknown identification through novel automated acquisition strategy combining sequential injections and MS^n

Anas Kamleh, PhD

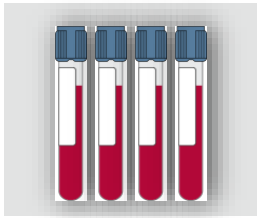
Nordic Metabolomics Society meeting, Örebro, 28/08/2018

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

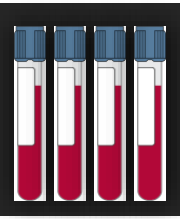
Extractables and Leachables Identification

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



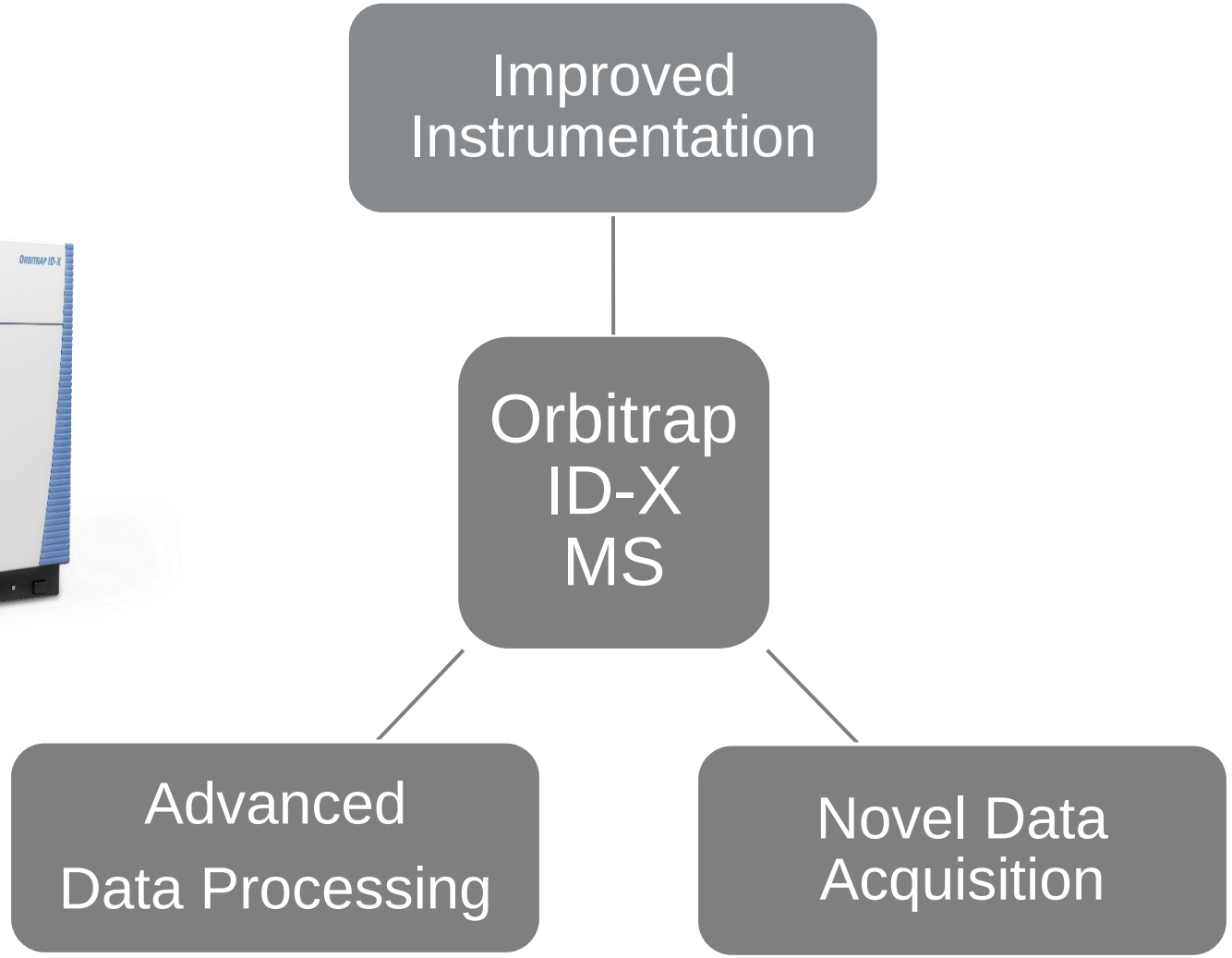
Metabolomics

Metabolite, Degradant and Impurity Identification

Extractables and Leachables Identification

Small Molecule Dedicated Mass Spectrometer on Thermo Scientific Tribrid Platform

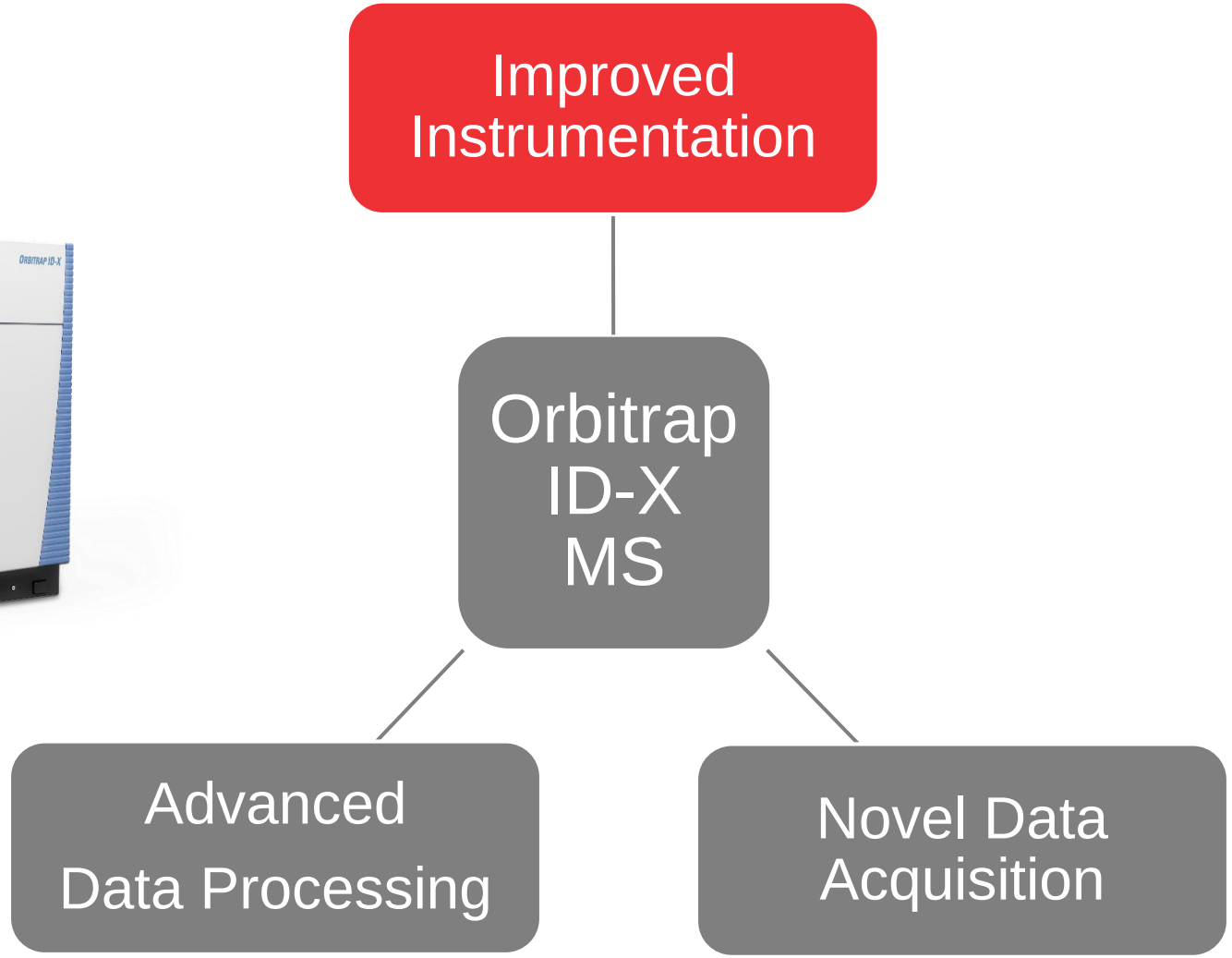
Thermo Scientific™ Orbitrap ID-X™ Tribrid™ Mass Spectrometer System



ThermoFisher SCIENTIFIC

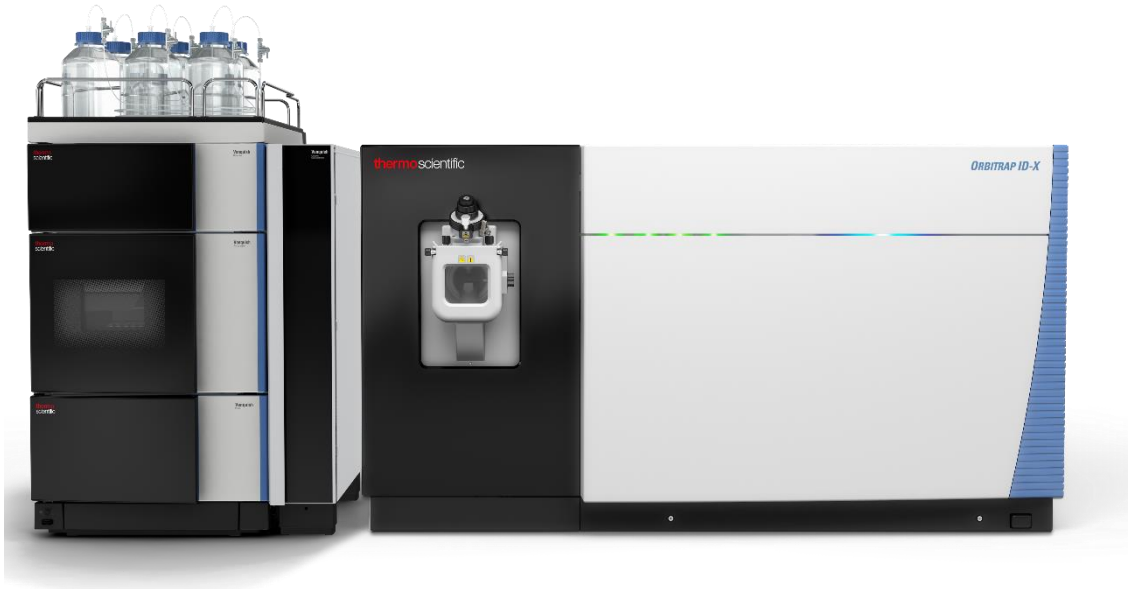
Small Molecule Dedicated Mass Spectrometer on Thermo Scientific Tribrid Platform

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



ThermoFisher SCIENTIFIC

Orbitrap ID-X Tribrid Mass Spectrometer Instrument Features



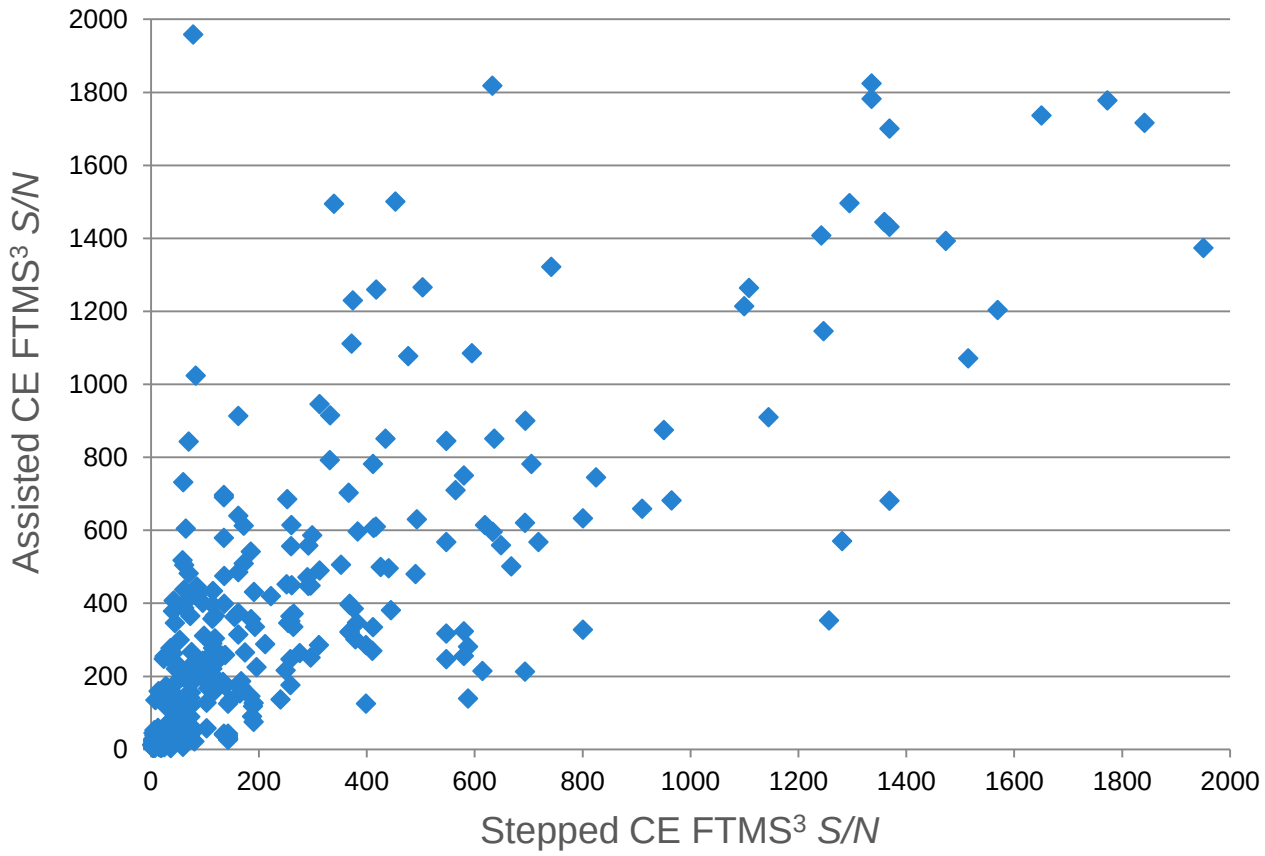
Max Resolution	**500,000** at *m/z* 200
Scan Rate OTMS^2	30 Hz
Scan Rate ITMS^2	40 Hz
Quad Mass Selection	Precursor isolation to 0.4 amu
Ion Trap MS^n	Up to **MS^{10}**
Mass Accuracy	3 ppm external, **1** ppm internal
Dissociation	**CID, HCD**

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 collection** 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

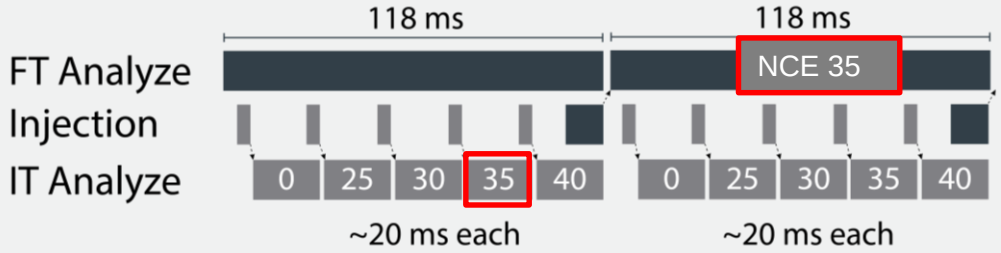
Assisted CE Allows Maximizing MS^n Spectral Quality

Assisted CE Improves MS^n *S/N*



Comparison of $FTMS^3$ signal to noise for LC-MS runs with Assisted CE vs. Stepped CE shows higher number of fragment ions with increased *S/N* resulting from Assisted CE

Assisted CE Determination



- Instrument collects hidden ion trap scans to generate break-down curves per compound in parallel with acquisition of the preceding FTMS scan
- Optimal collision energy is ascertained based on specified precursor depletion threshold
- $FTMS^2$ analytical scan is collected using the optimal collision energy

Bailey et al. ThP 831

ThermoFisher SCIENTIFIC

Small Molecule Dedicated Mass Spectrometer on Thermo Scientific Tribrid Platform

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

Improved Instrumentation

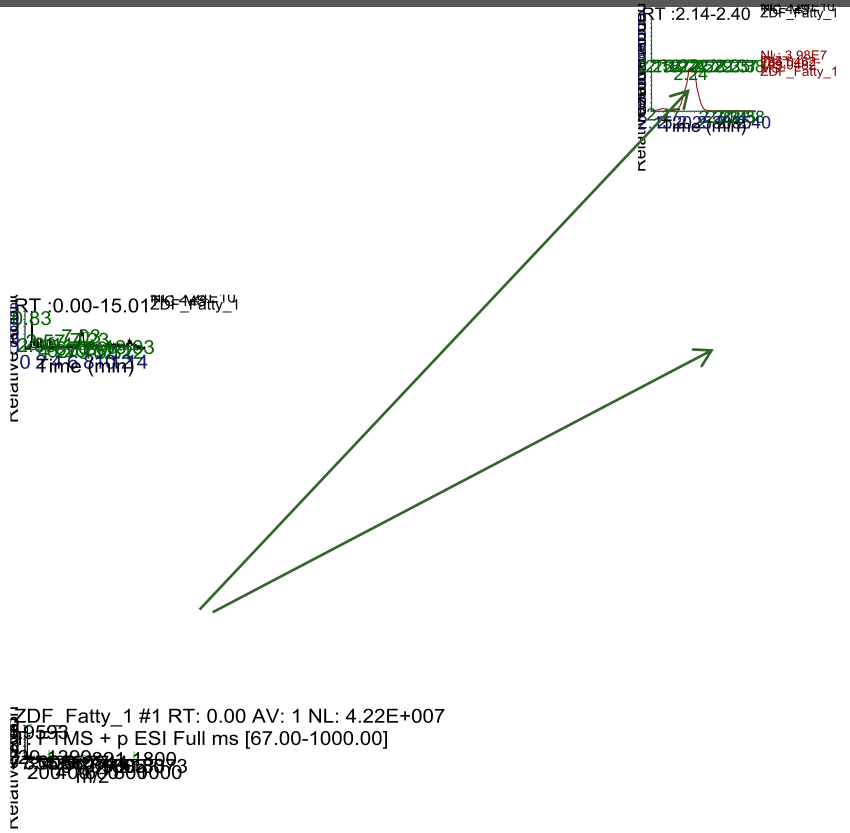
Orbitrap ID-X MS

Advanced Data Processing

Novel Data Acquisition

ThermoFisher SCIENTIFIC

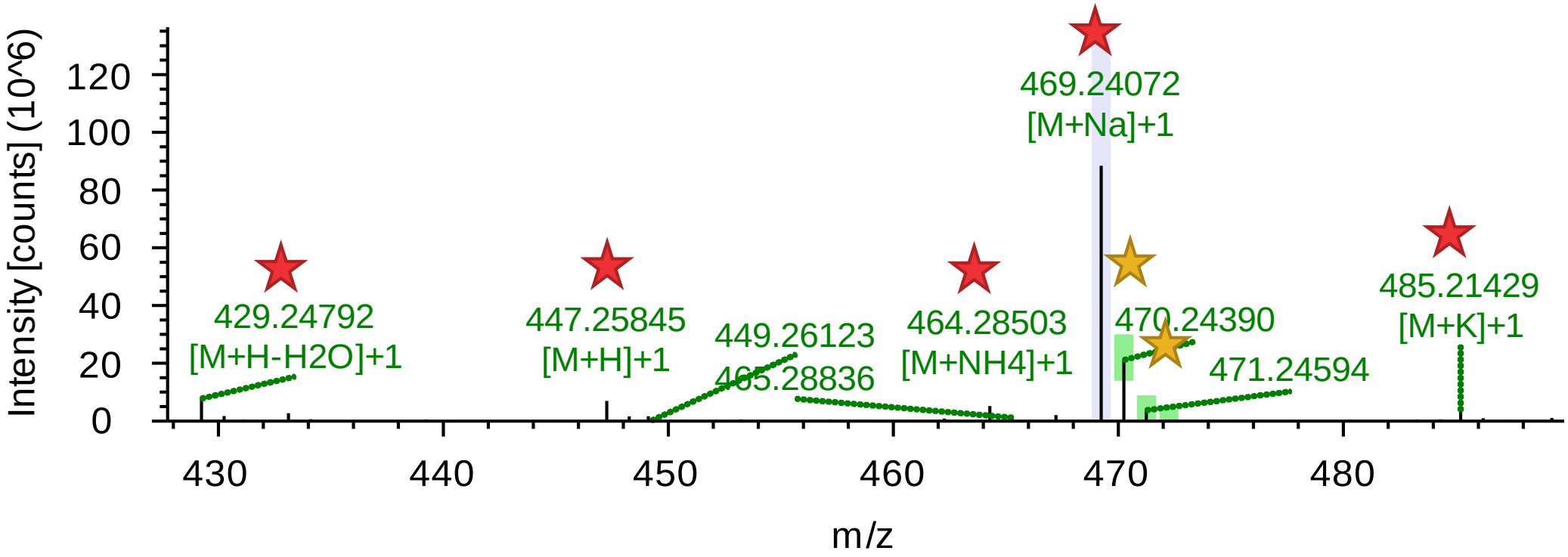
Unknown analysis of complex samples



	Scan Num	m/z	Intensity	Relative	Segment N
1	429	293.0978	42376652	100	1
2	429	177.0071	34436436	81.26276	1
3	429	116.9858	30508254	71.99307	1
4	429	193.0472	20951336	49.44075	1
5	429	84.95967	18056726	42.61008	1
6	429	134.9965	17734342	41.84932	1
7	429	157.0837	15091724	35.6133	1
8	429	149.0122	14569577	34.38114	1
9	429	102.9702	14347303	33.85662	1
10	429	135.1015	12689367	29.94424	1
11	429	132.1019	10999948	25.95757	1
12	429	114.0913	10636511	25.09993	1
13	429	371.1009	8655578	20.42535	1
14	429	102.1277	8600961	20.29646	1
15	429	229.141	8251913	19.47278	1
16	429	118.0861	7597958	17.92958	1
17	429	115.0366	6710113	15.83446	1
18	429	188.0918	6661077	15.71874	1
244	429	235.0489	544108.6	1.283982	1

Electrospray ionization produces multiple ions per compound

12162_serum_asthma_female_52_28, #1379, RT=7.317 min, FTMS (+)
C22 H38 O9 as [M+Na]+1

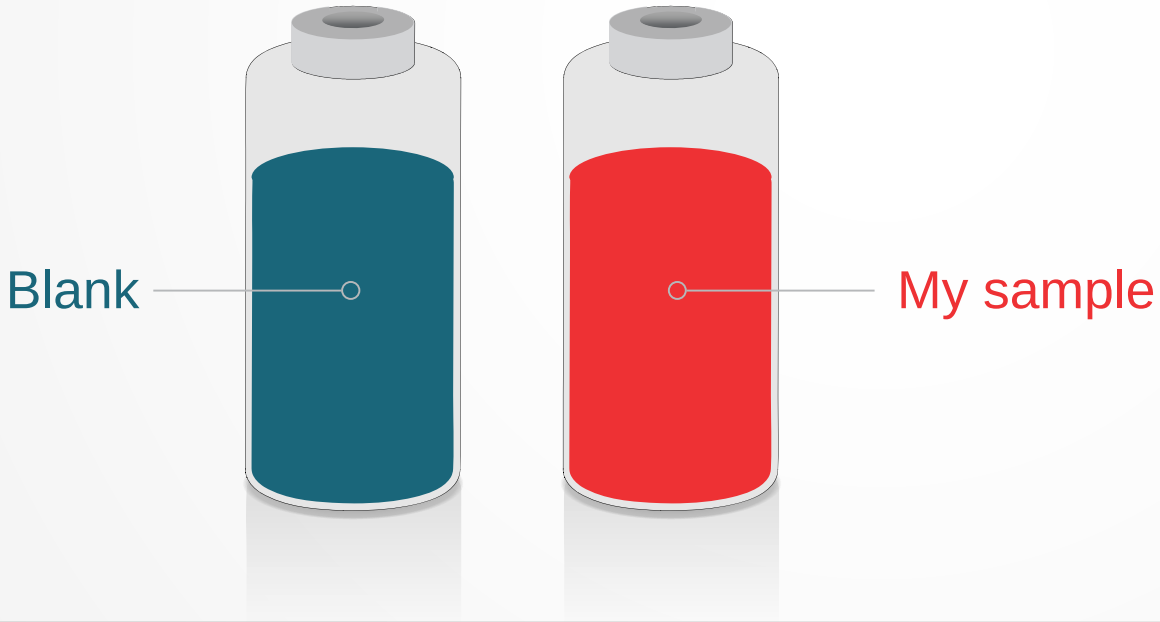


AcquireX

Automated acquisition workflow for comprehensive sample interrogation

This is my challenge!

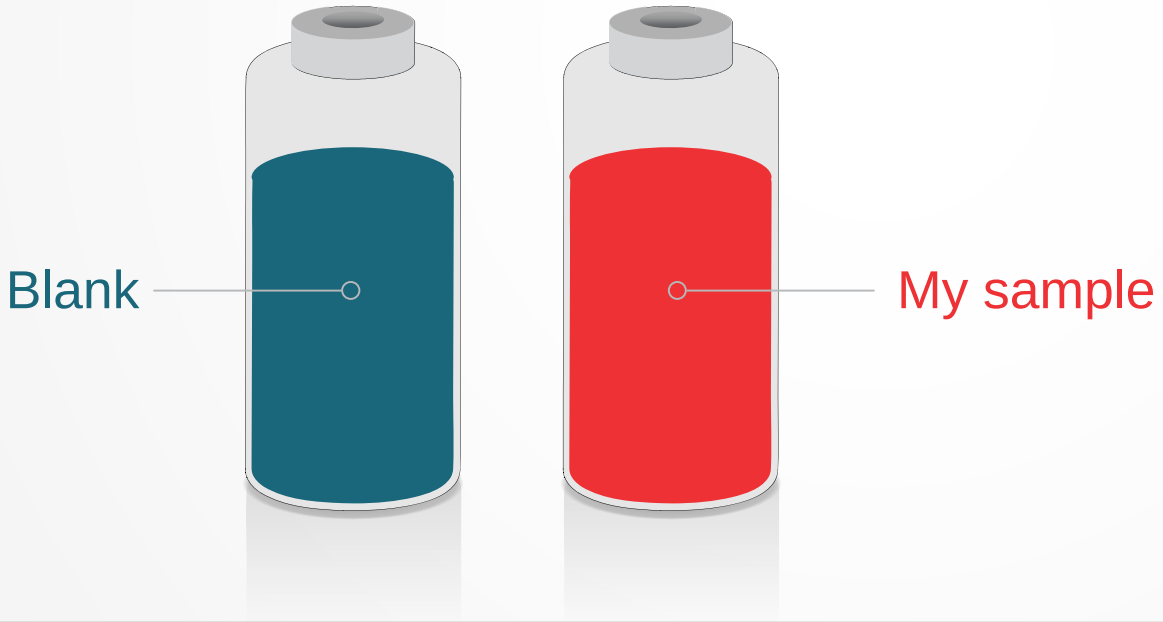
- I need to generate **high quality** MS^n spectra on **all** components in my sample
- My sample is too complex and not all features are fragmented
- Duty cycle is often wasted triggering on background features
- Manually excluding background and including relevant features is too laborious



LC-MS MS^n Method

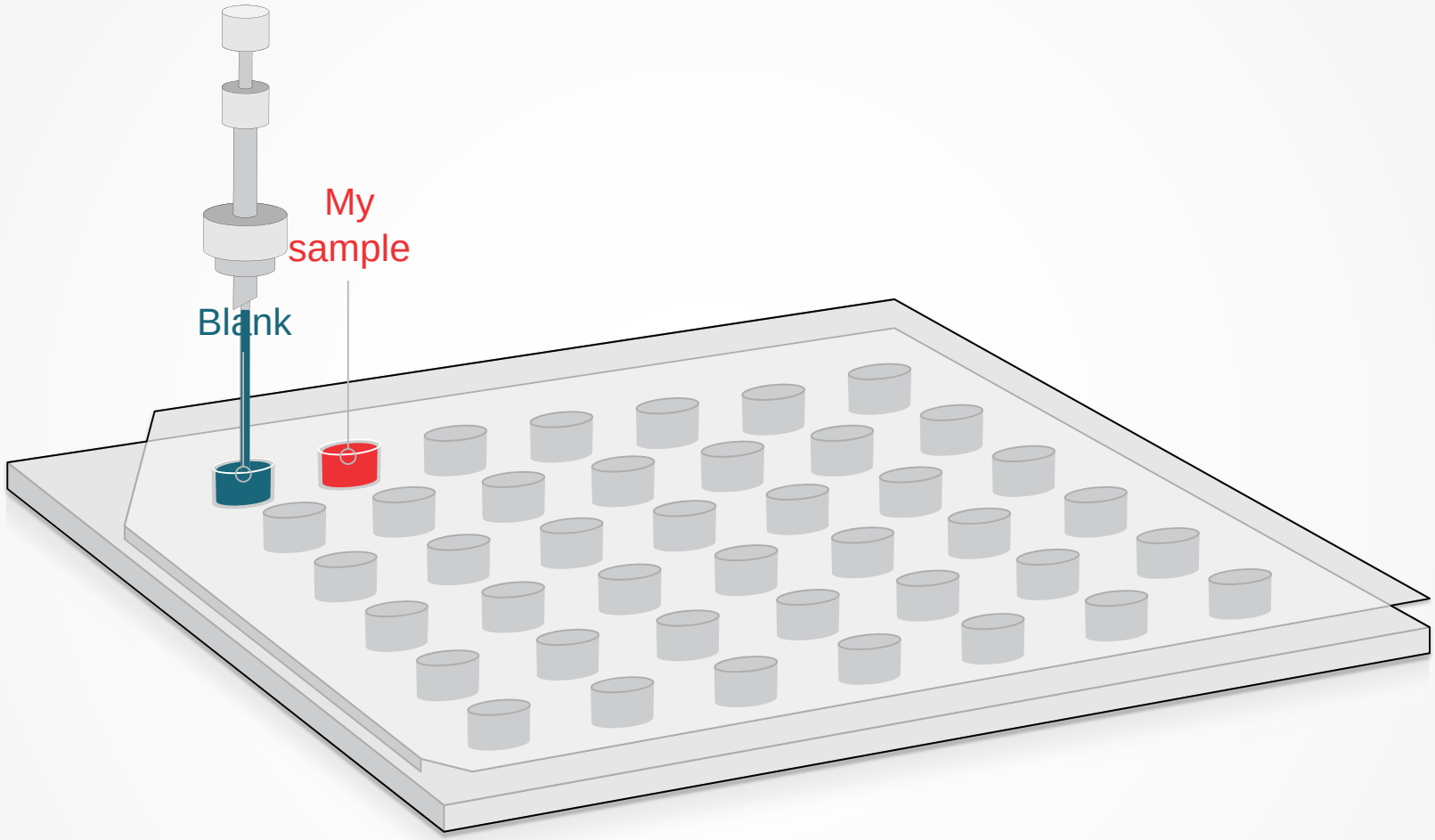
How do I analyze all of the features in my sample using a data-dependent MS^n method?

In an automated manner?



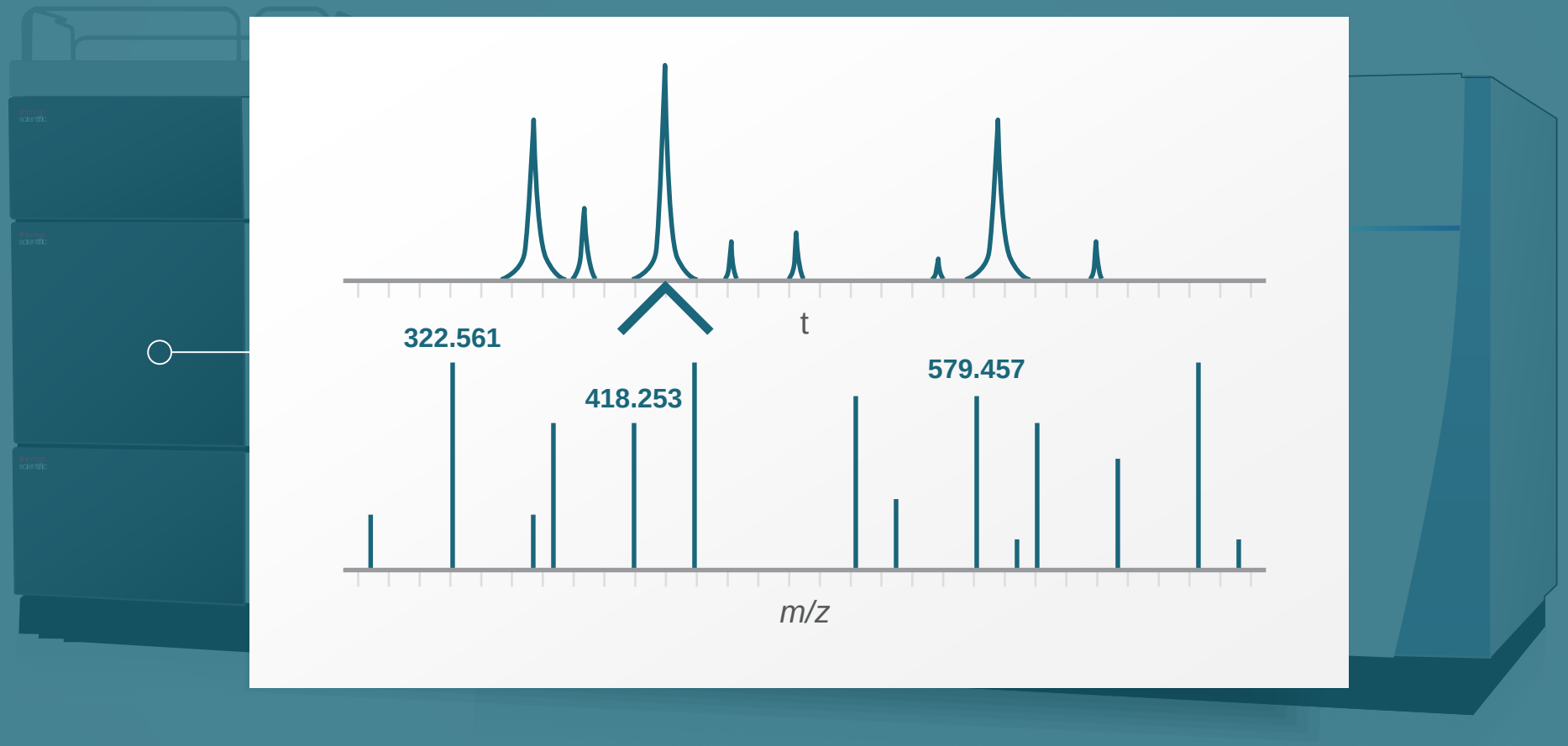
LC-MS MS^n Method

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

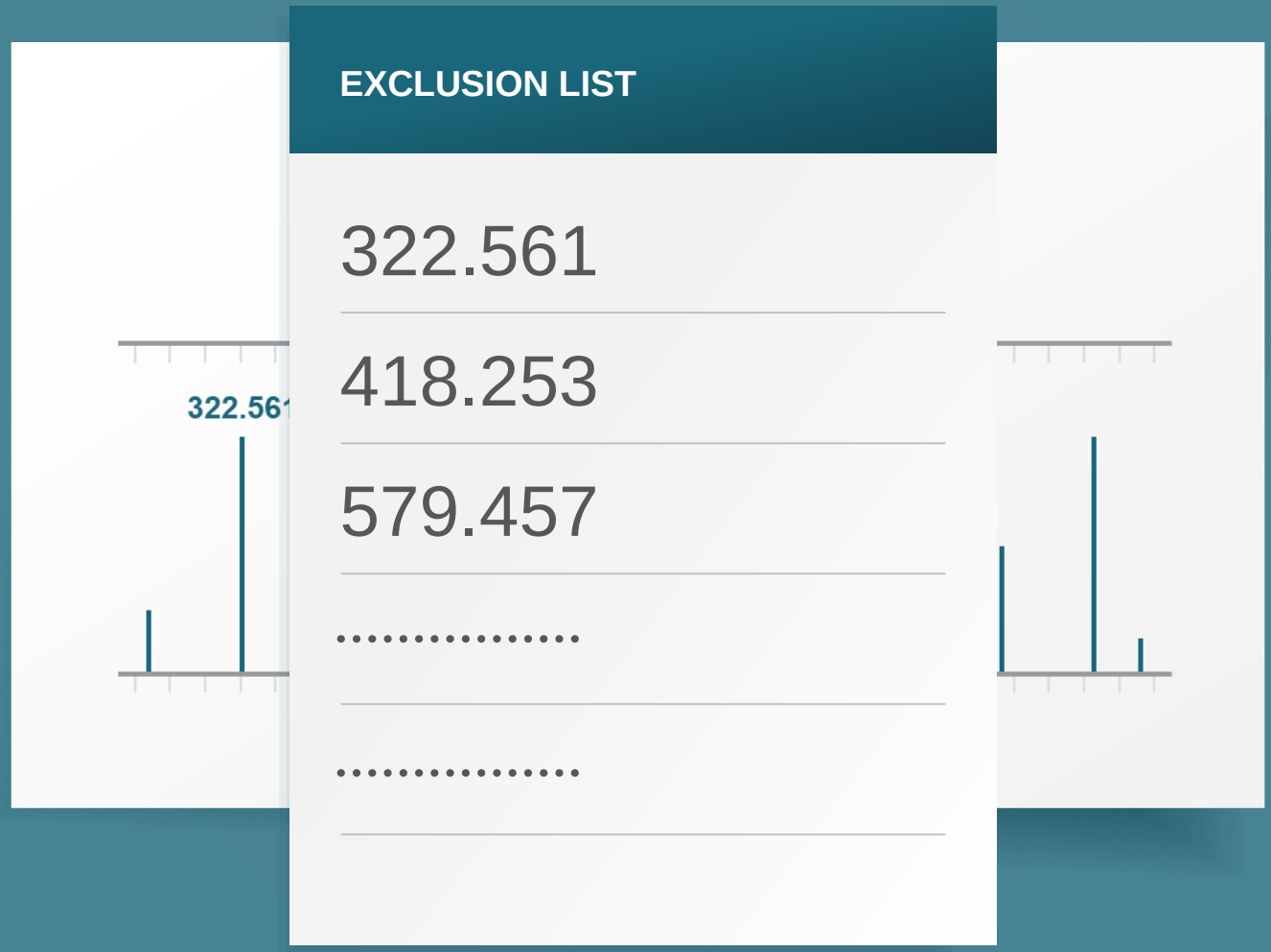


Step 1: LC-MS analysis of background (blank)

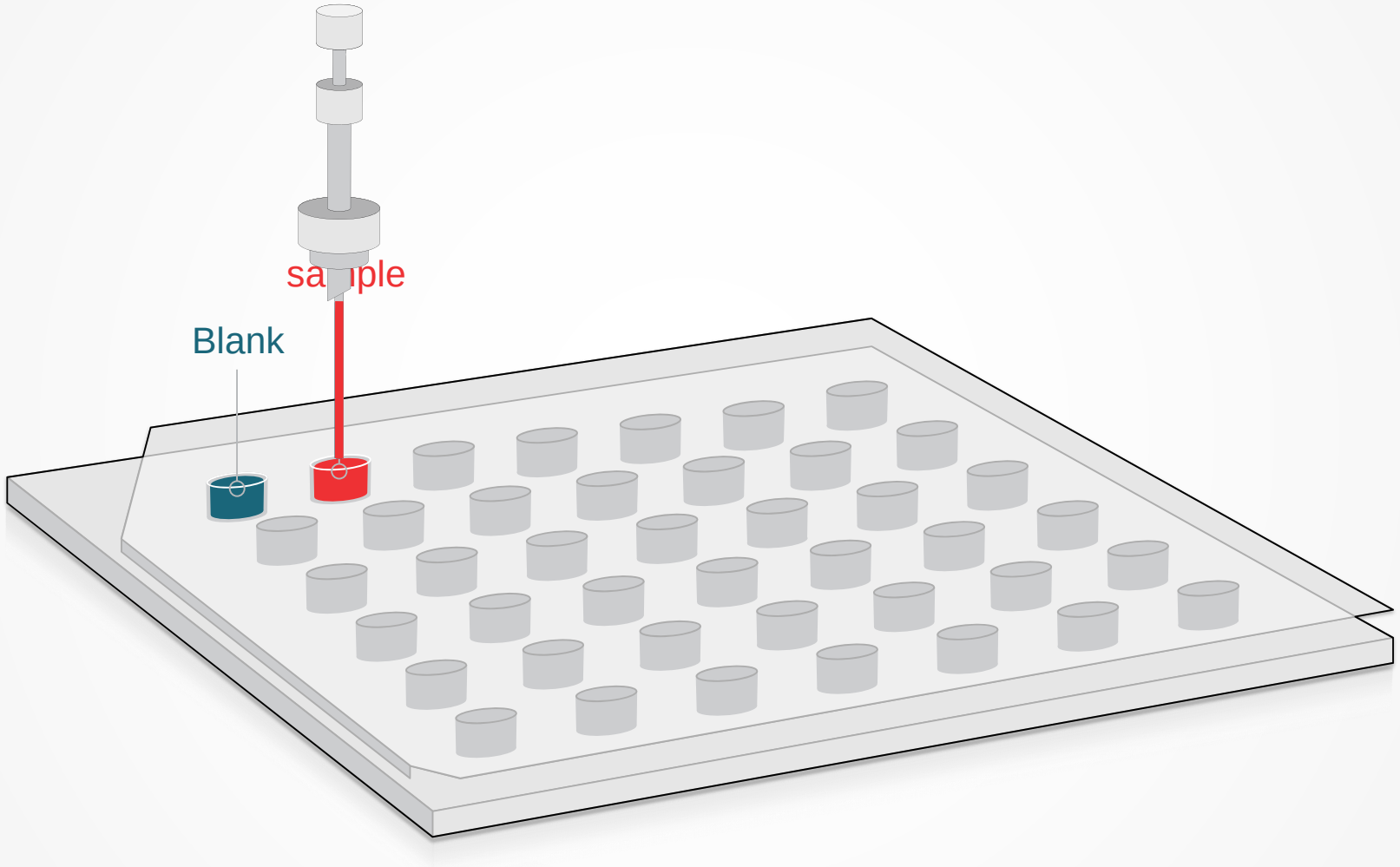
Step 1: LC-MS analysis of background (blank)



Step 2: Automatic exclusion list generation from the blank LC-MS

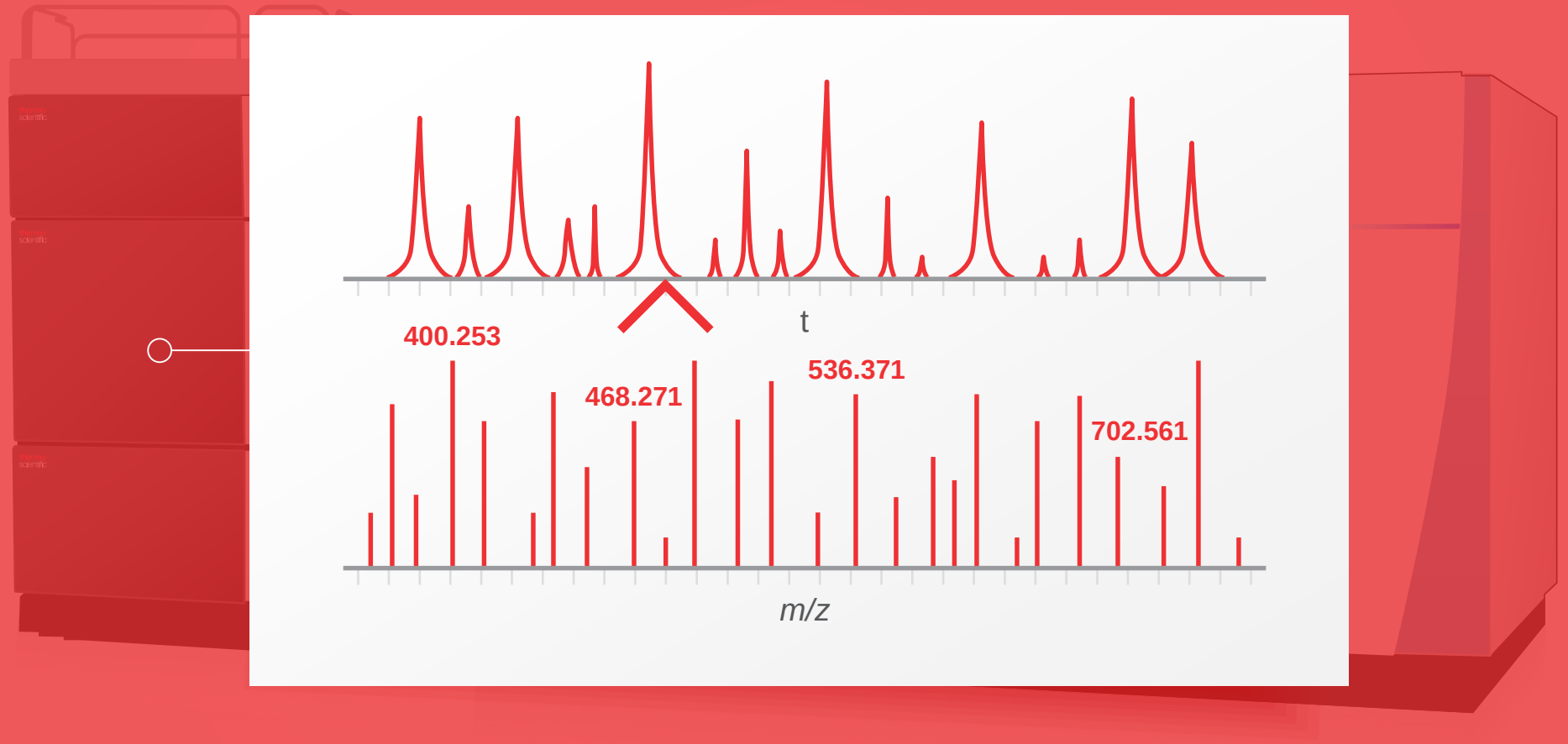


Step 3: Full scan LC-MS analysis of my sample

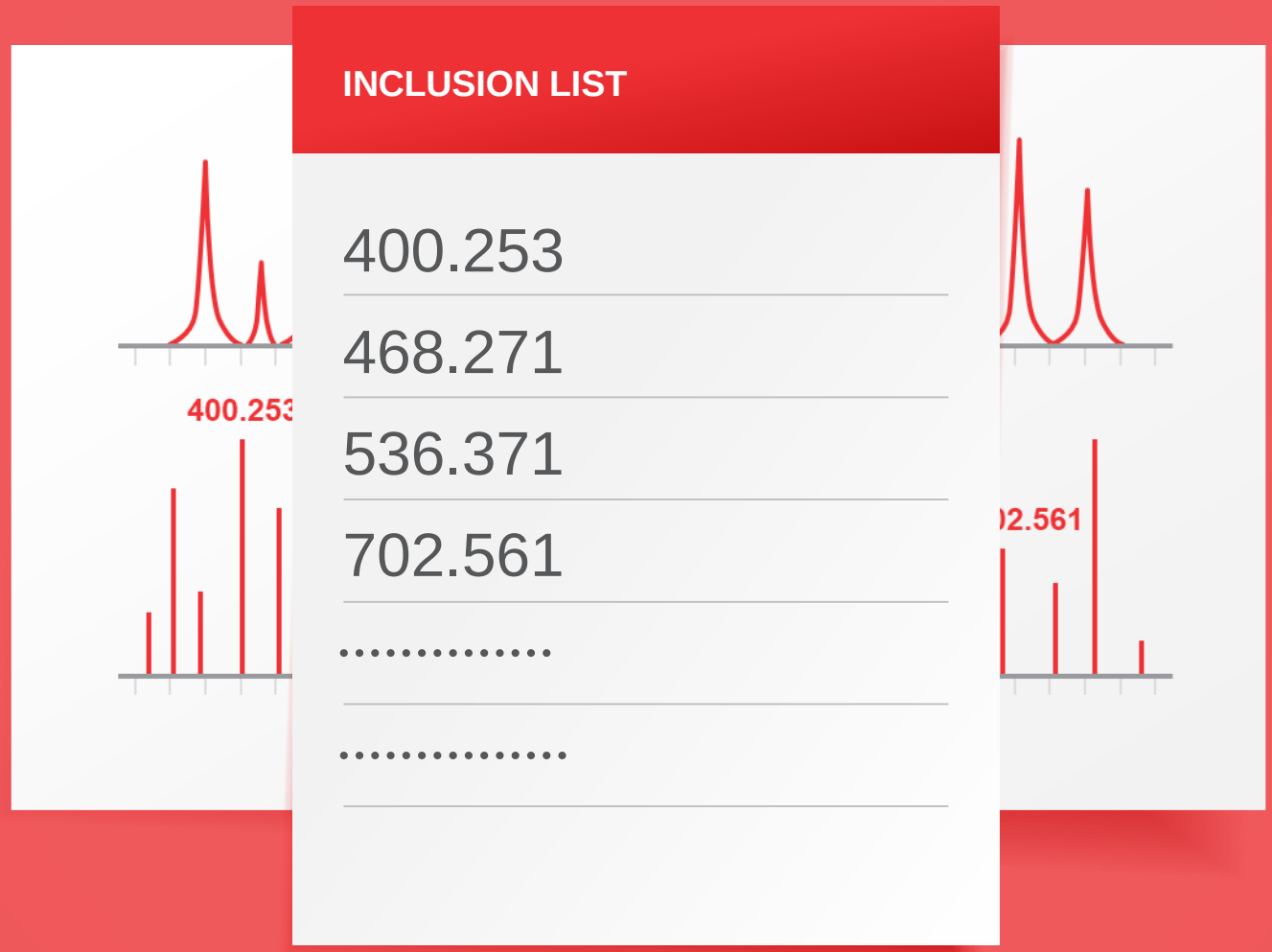


Step 3: Full scan LC-MS analysis of my sample

Step 3: Full scan LC-MS analysis of my sample



Step 4: Automatic inclusion list generation from my sample



Step 5: Automatic method update with exclusion and inclusion lists

EXCLUSION LIST

322.561

418.253

579.457

.....

.....

INCLUSION LIST

400.253

468.271

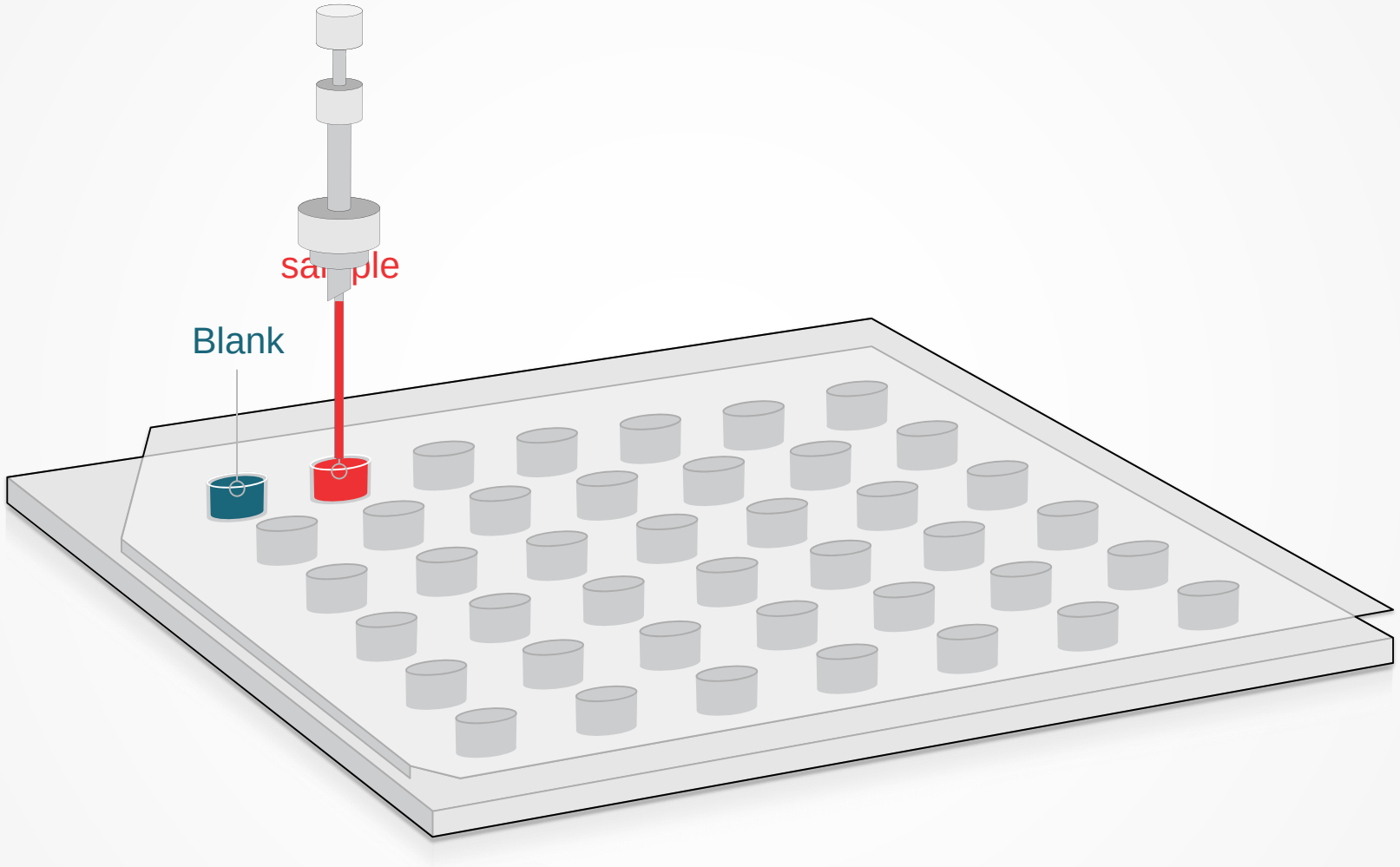
536.371

702.561

.....

.....

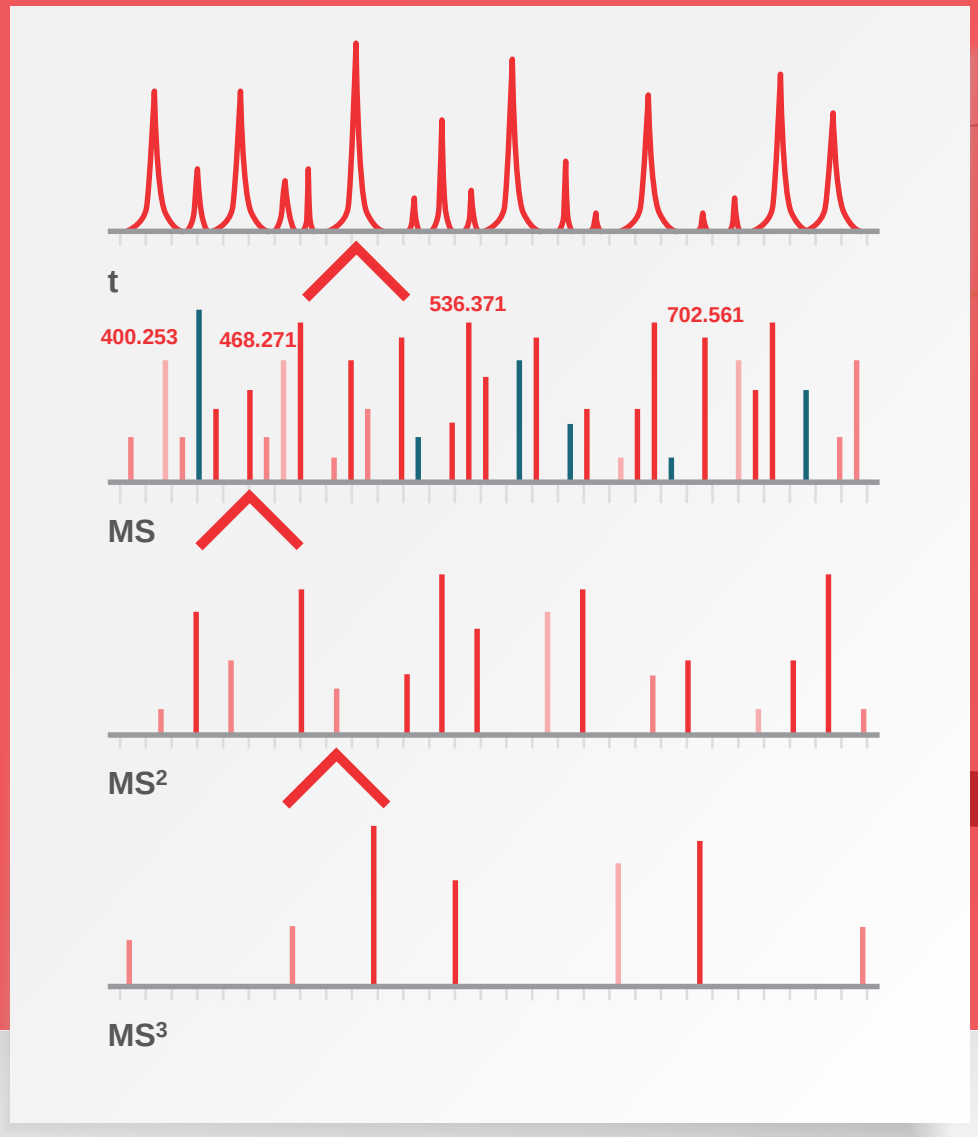
Step 6: First automated LC-MS^n analysis using the updated inclusion and exclusion lists



Step 6: First automated LC-MS^n analysis using the updated inclusion and exclusion lists

Step 7: Updating the lists automatically

Moving triggered features from inclusion to exclusion list



INCLUSION LIST

400.253
468.271
536.371
702.561
.....

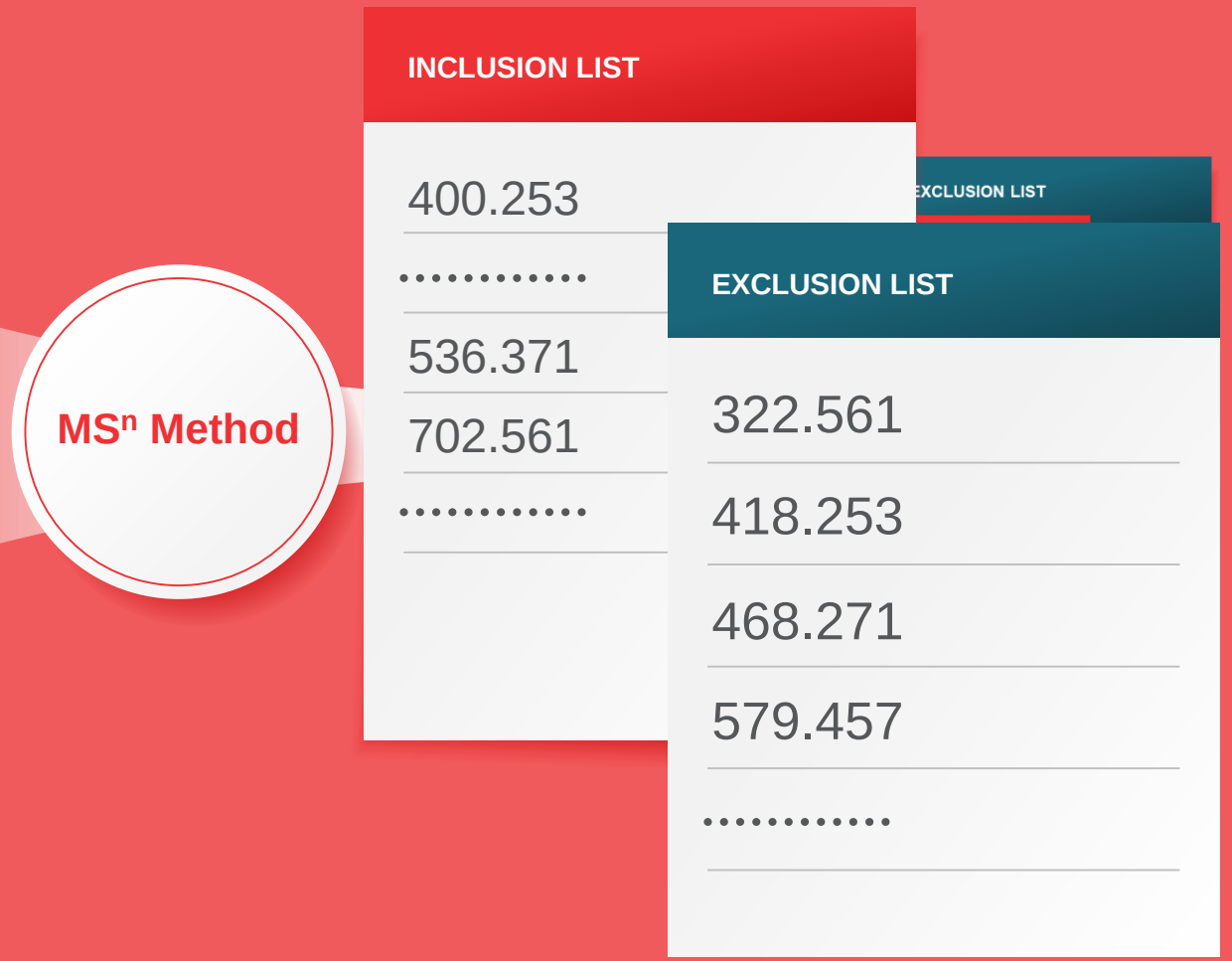
EXCLUSION LIST

322.561
418.253
468.271
579.457
.....

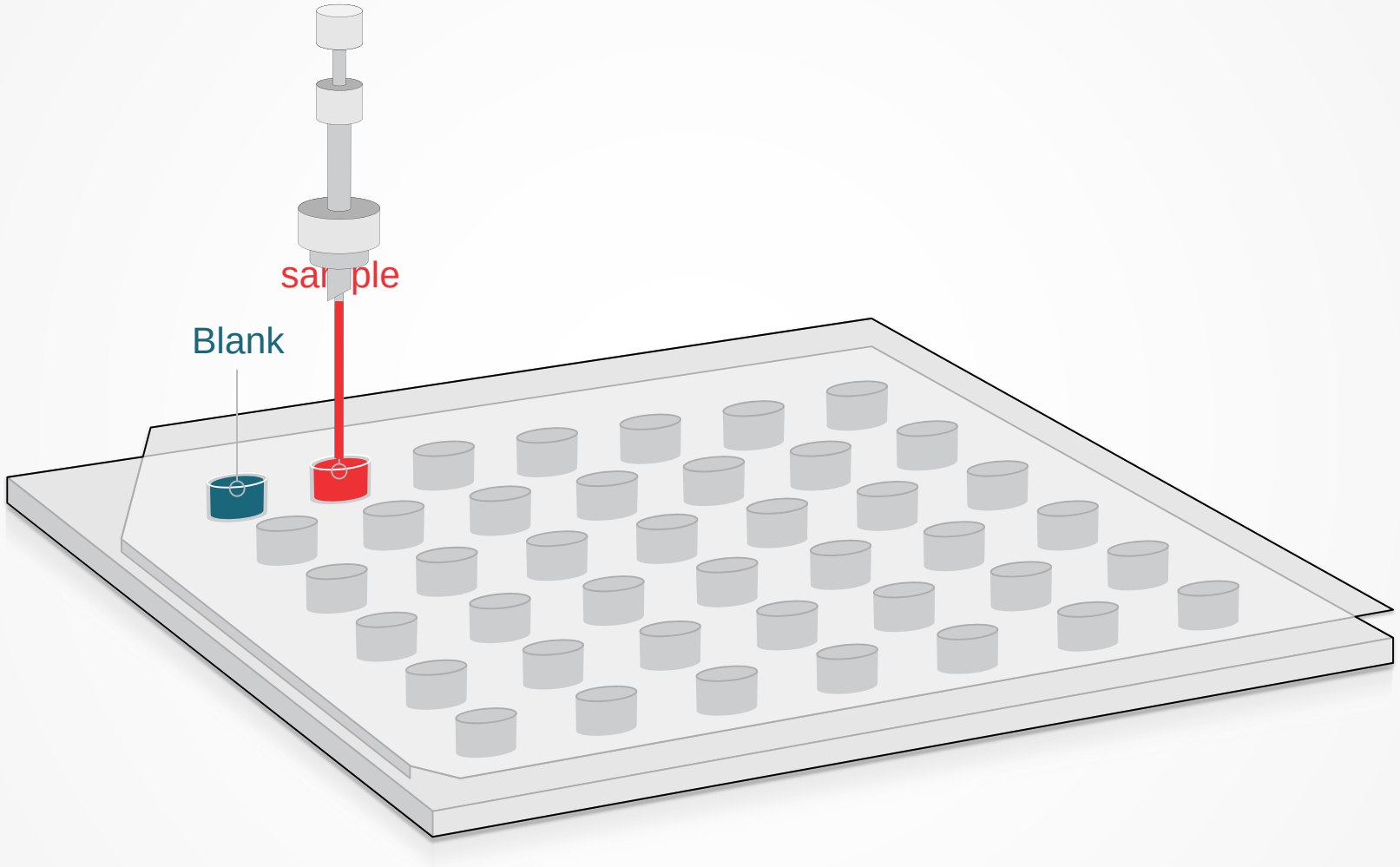
thermo scientific

Step 8: Updating the MS method automatically

with the current inclusion and exclusion lists



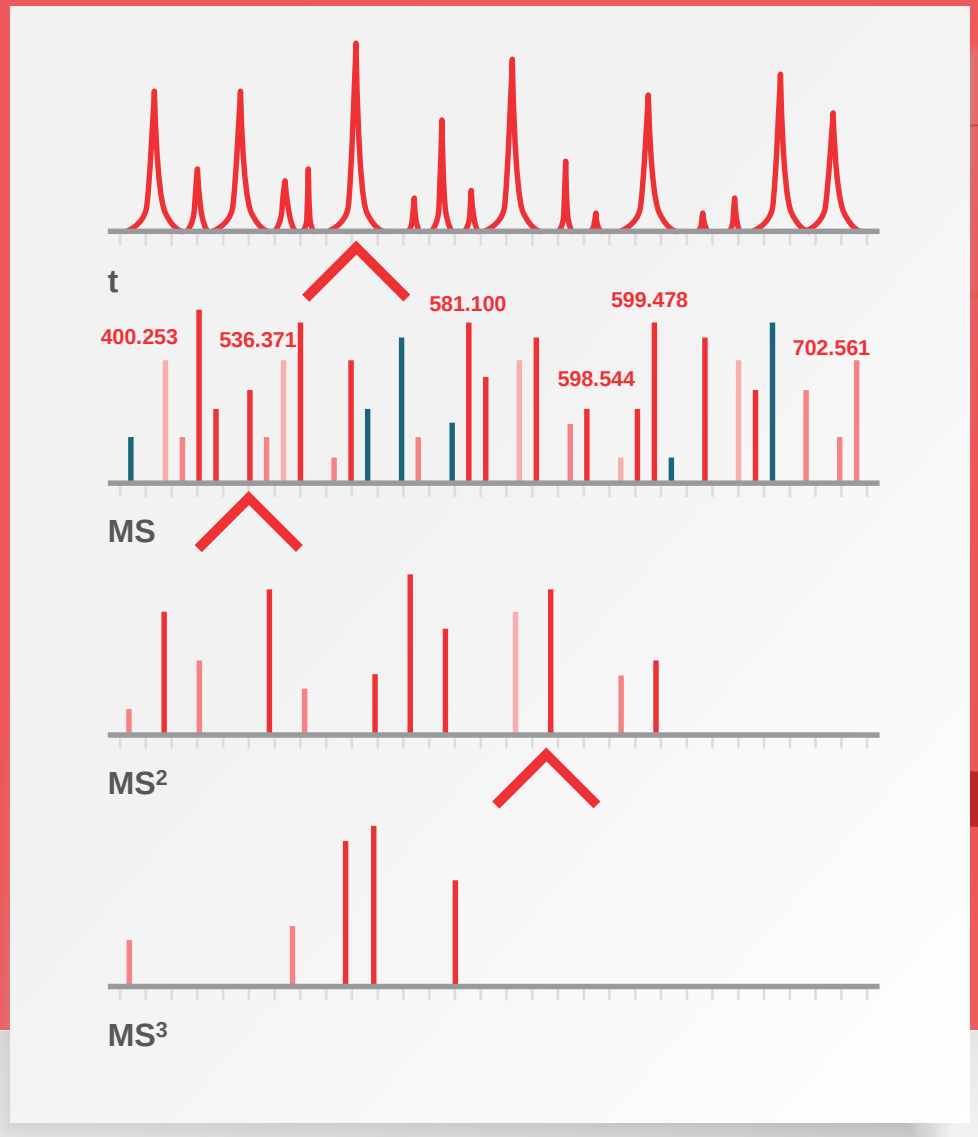
Step 9: Second automated LC-MS^n analysis using the updated inclusion and exclusion lists



Step 9: Second automated LC-MS^n analysis using the updated inclusion and exclusion lists

Step 10: Updating the lists automatically

Moving newly triggered features from inclusion to exclusion list



INCLUSION LIST

400.253
536.371
581.100
598.544
599.478
702.561

EXCLUSION LIST

322.561
418.253
468.271
536.371
579.457

thermo scientific

Repeat steps 9-10 until all of the features on the inclusion list are sampled

Injection 4

INCLUSION LIST

598.544

599.478

EXCLUSION LIST

322.561

400.253

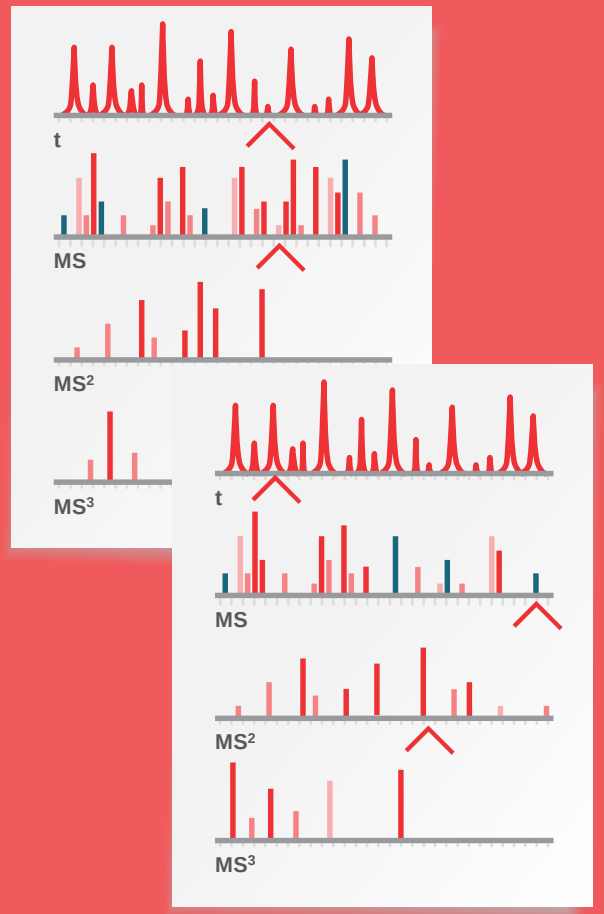
418.253

468.271

536.371

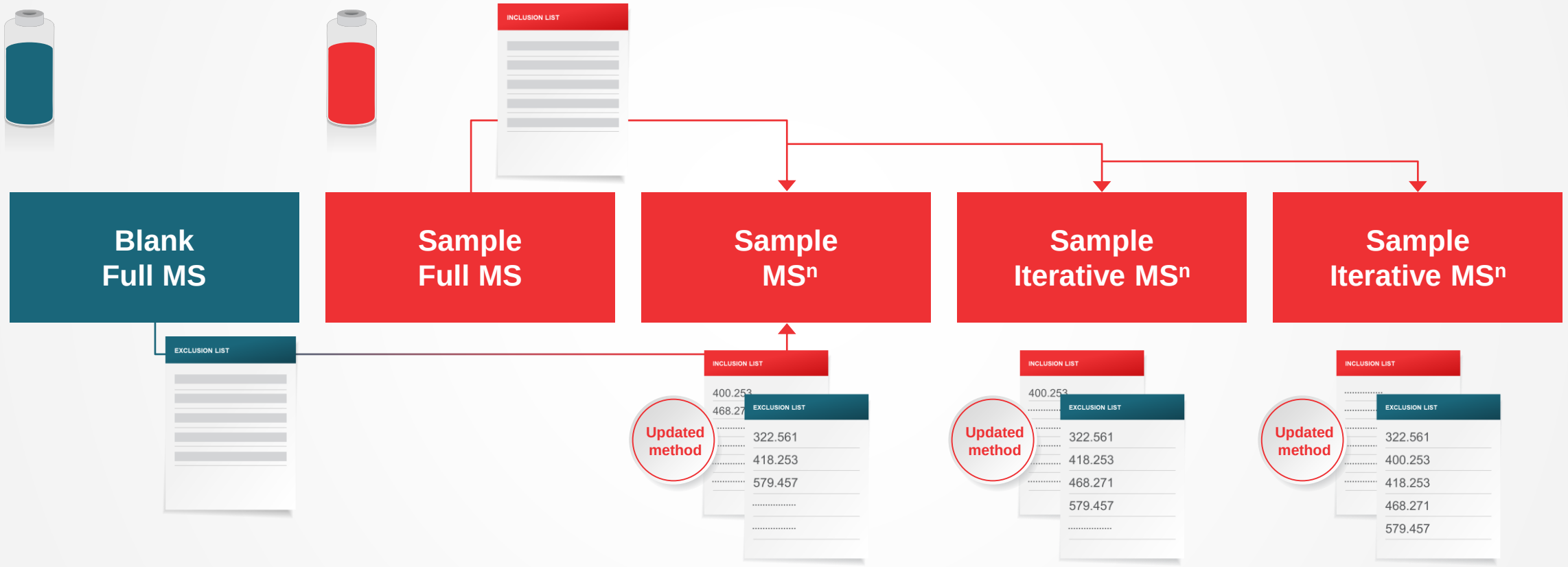
579.457

581.100



thermo scientific

AcquireX: Comprehensive MS^n analysis of all of the features in the sample

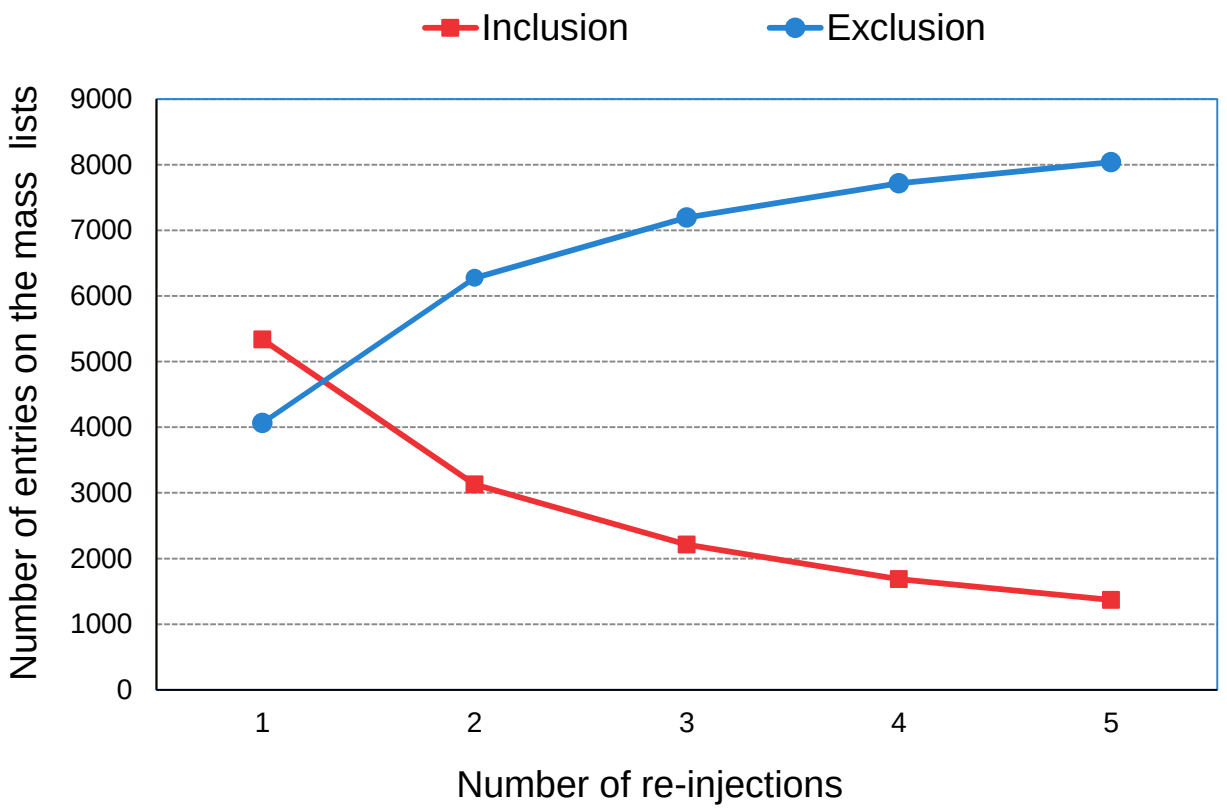


Automatically updated run-to-run inclusion/exclusion lists

Collect more meaningful data, not just more data: www.thermofisher.com/AcquireX

thermo scientific

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

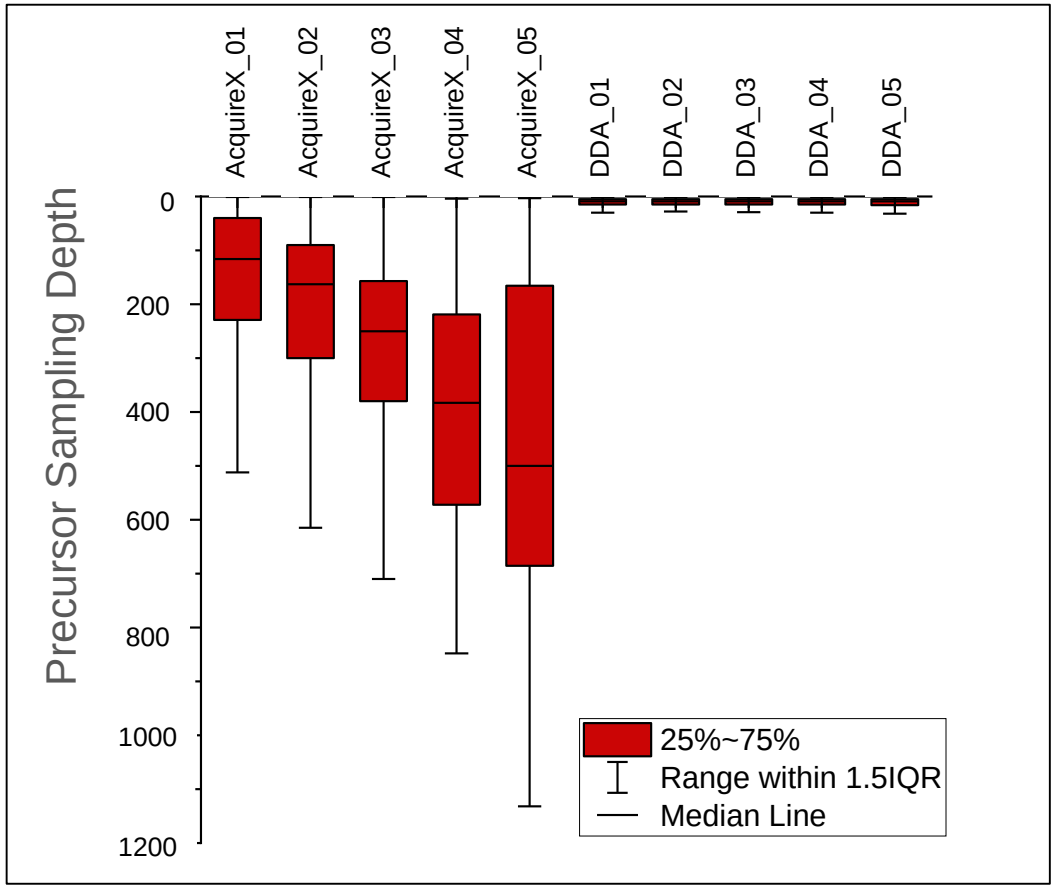
Ntai et al. ThP 564

Deep Scan Analysis

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

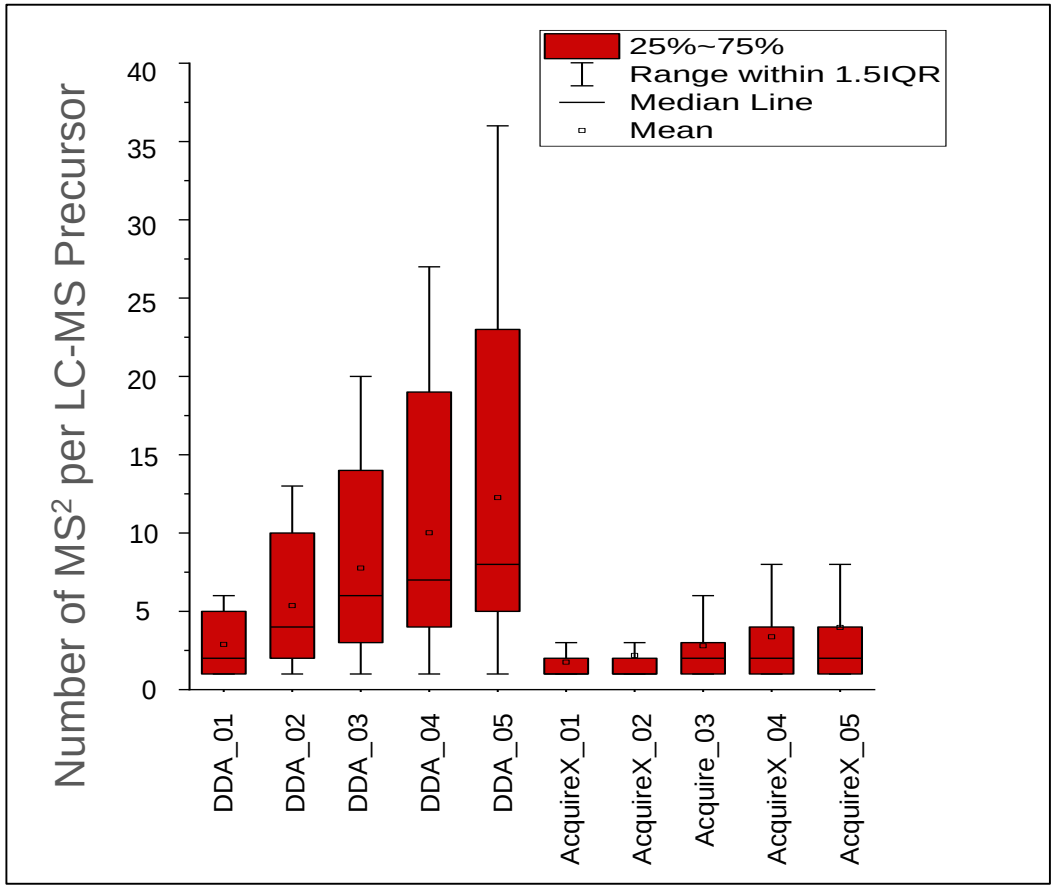
AcquireX: Deeper Interrogation of Sample

Increased Precursor Sampling Depth



Comparison of traditional DDA and AcquireX re-injections shows increased coverage with the AcquireX acquisitions

……With Less Redundancy



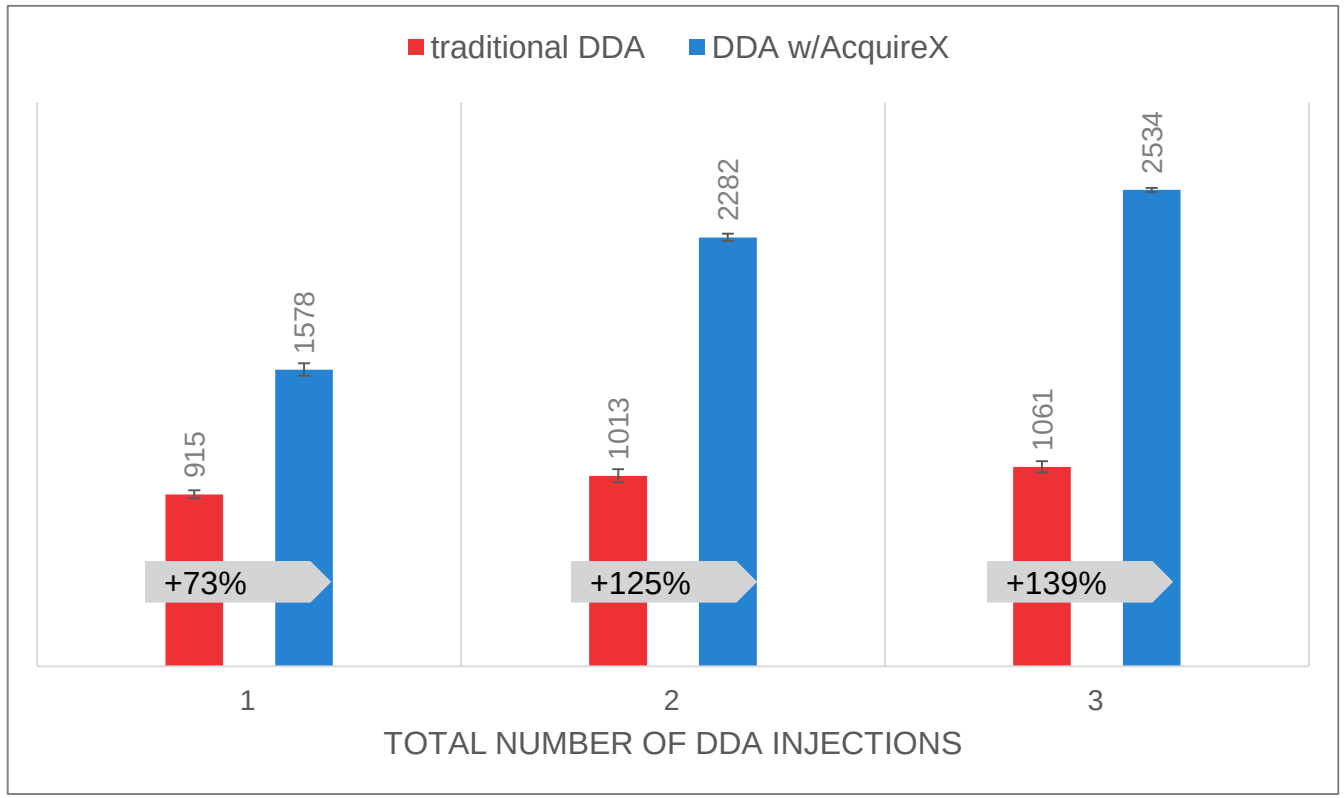
Comparison of traditional DDA and AcquireX re-injections shows less redundancy in total number of fragmentation spectra per compound during the AcquireX acquisitions

McAlister et al. MP 120

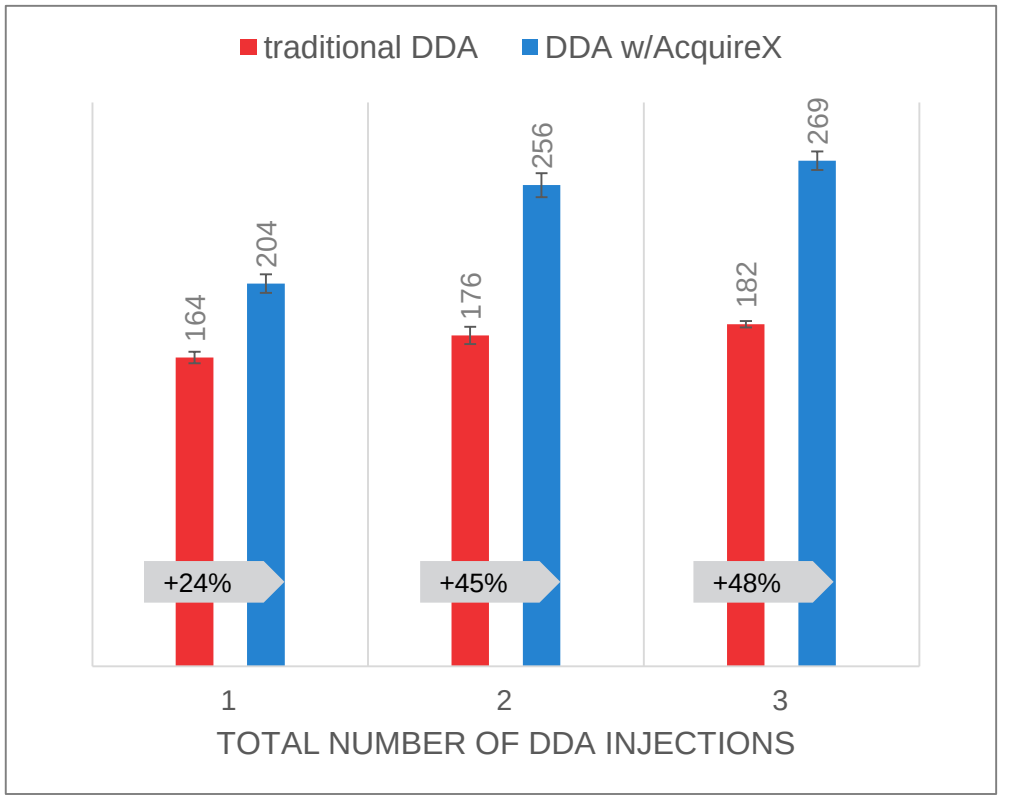
ThermoFisher SCIENTIFIC

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

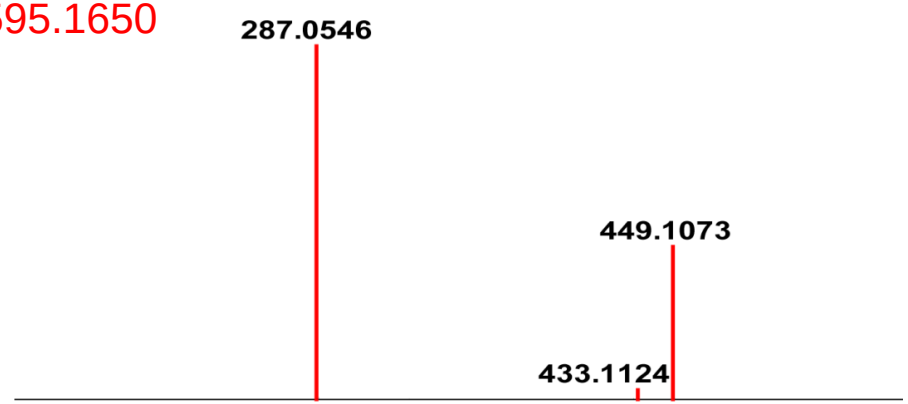


Comparison of three re-injections of the **extracted human plasma** using traditional DDA and DDA with AcquireX showing more than 2x increase in the number of compounds with fragmentation spectra (left) and almost 50% gain in confidently identified compounds (right) when DDA with AcquireX is used

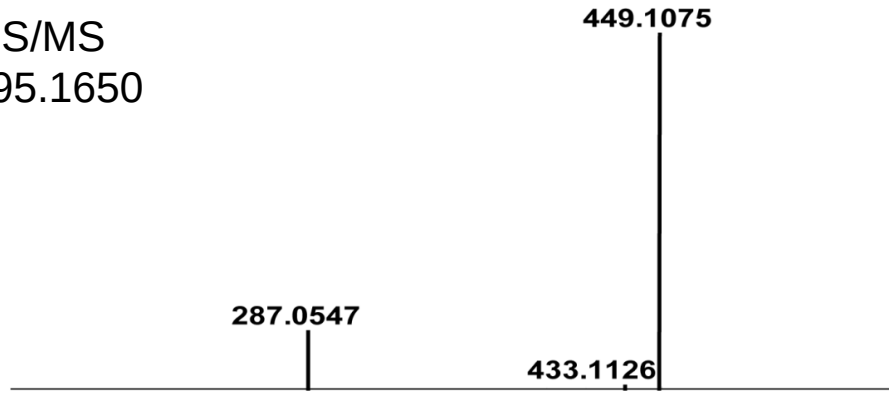
MS^n Based Structural Characterization of Flavonoid Isomers

Indistinguishable by MS^2

MS/MS
595.1650

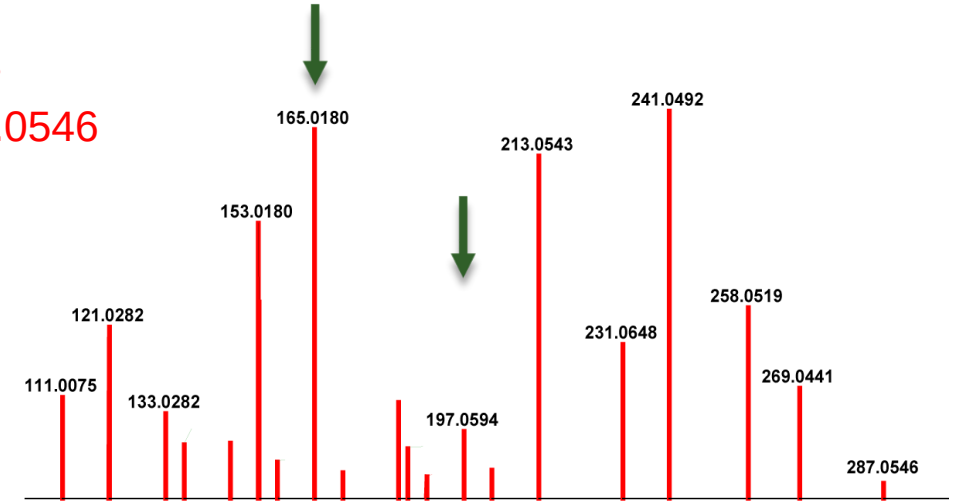


MS/MS
595.1650

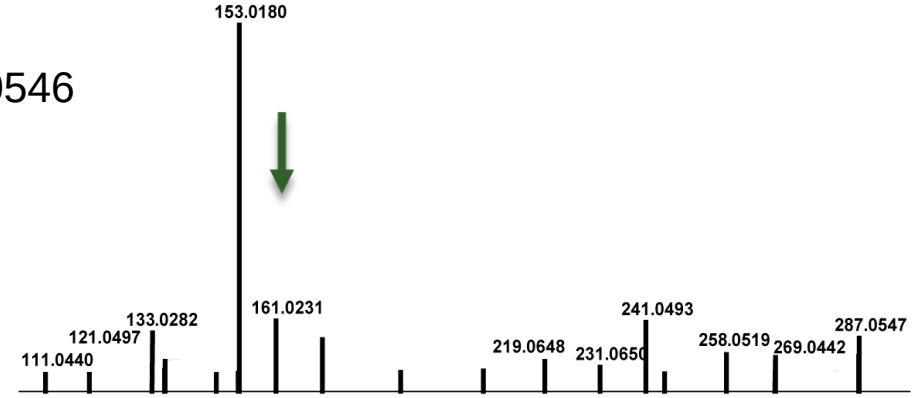


Distinguishable by MS^3

MS^3
287.0546

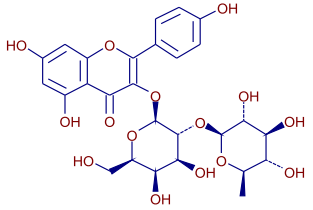


MS^3
287.0546

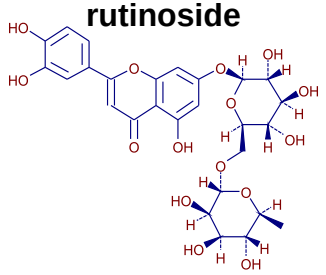


Isomers

Kaempferol 3-O-ß -rutinoside



Luteolin 7-rutinoside

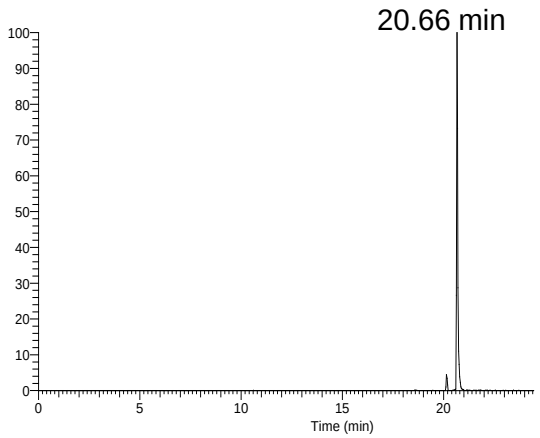


ThermoFisher SCIENTIFIC

Complete Characterization of Co-eluting Isomeric Lipids Using MS^3

MS^1 → MS^2 → MS^3

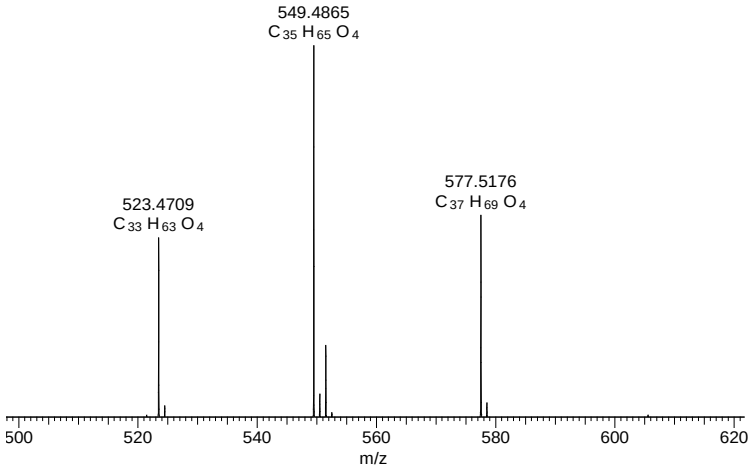
882.7541



TG 48:1 $[M+NH_4]^+$

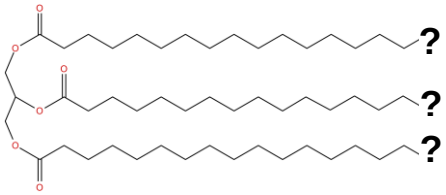
$C_{51}H_{96}O_6$

Triglyceride
Total 48 acyl carbons and 1 double bond

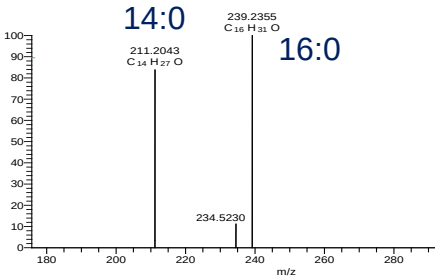


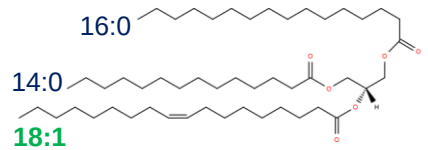
Mixture of neutral losses

18:1 FA + NH_3
16:0 FA + NH_3
14:0 FA + NH_3
+ more…



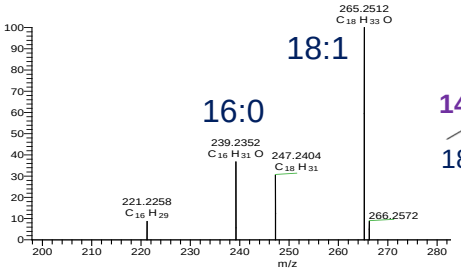
523.4709
NL 18:1

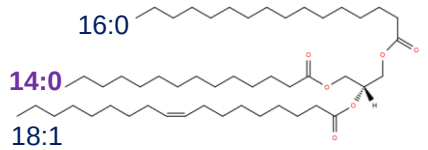




Isomer 1

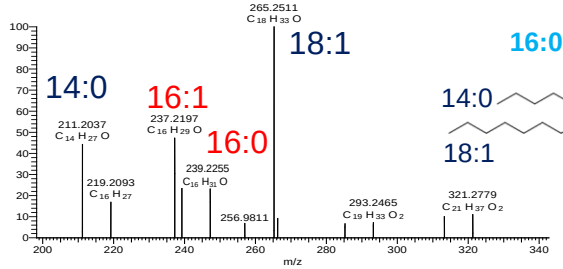
577.5176
NL 14:0

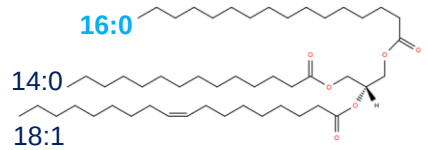




Isomer 1

549.4685
NL 16:0

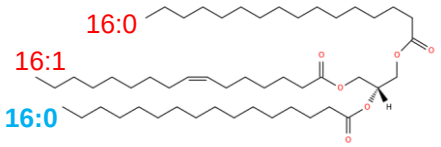




Isomer 1

TG 14:0_18:1_**16:0**
TG 16:0_16:1_**16:0**

Two isomers



Isomer 2

ThermoFisher SCIENTIFIC

Small Molecule Dedicated Mass Spectrometer on Thermo Scientific Tribrid Platform

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

Improved Instrumentation

Orbitrap ID-X MS

Advanced Data Processing

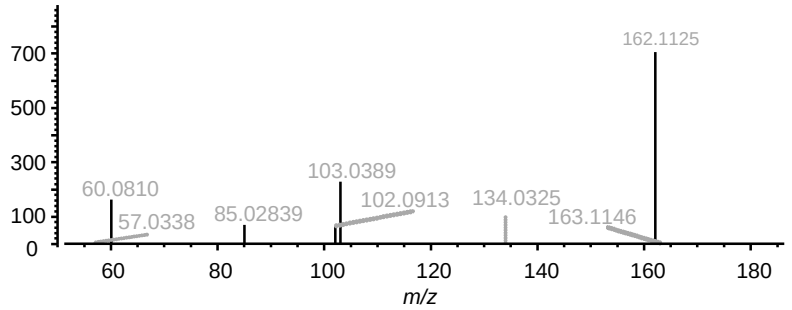
Novel Data Acquisition

ThermoFisher SCIENTIFIC

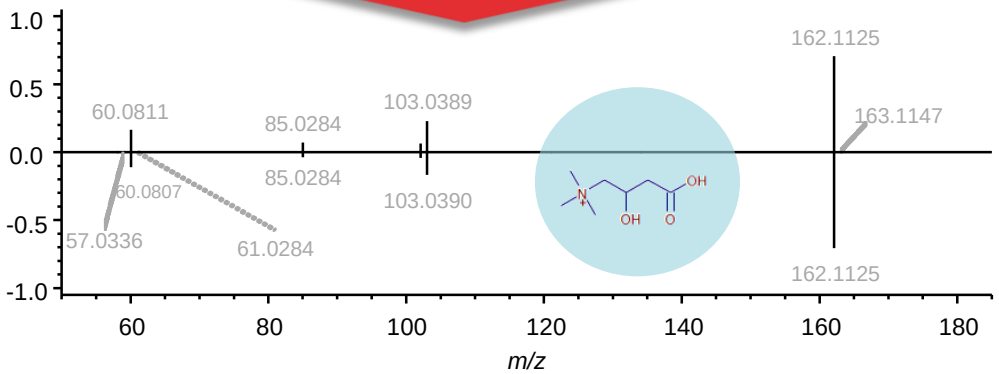
Small Molecule Data Processing Challenges

Ideally

MS^2 of unknown compound



Spectral Library Search

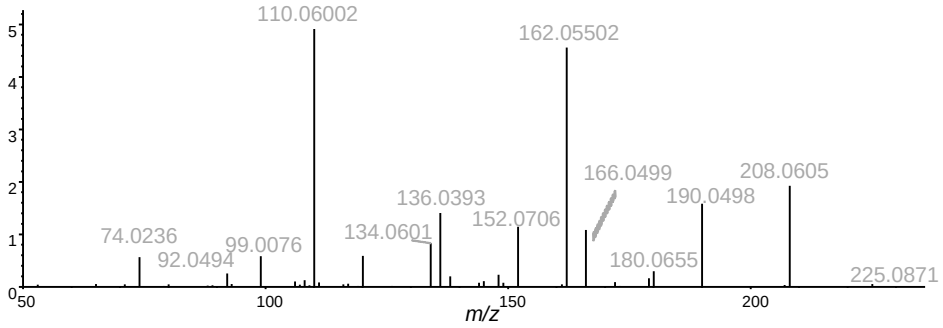


Reference data found in spectral library

→ **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 formula)

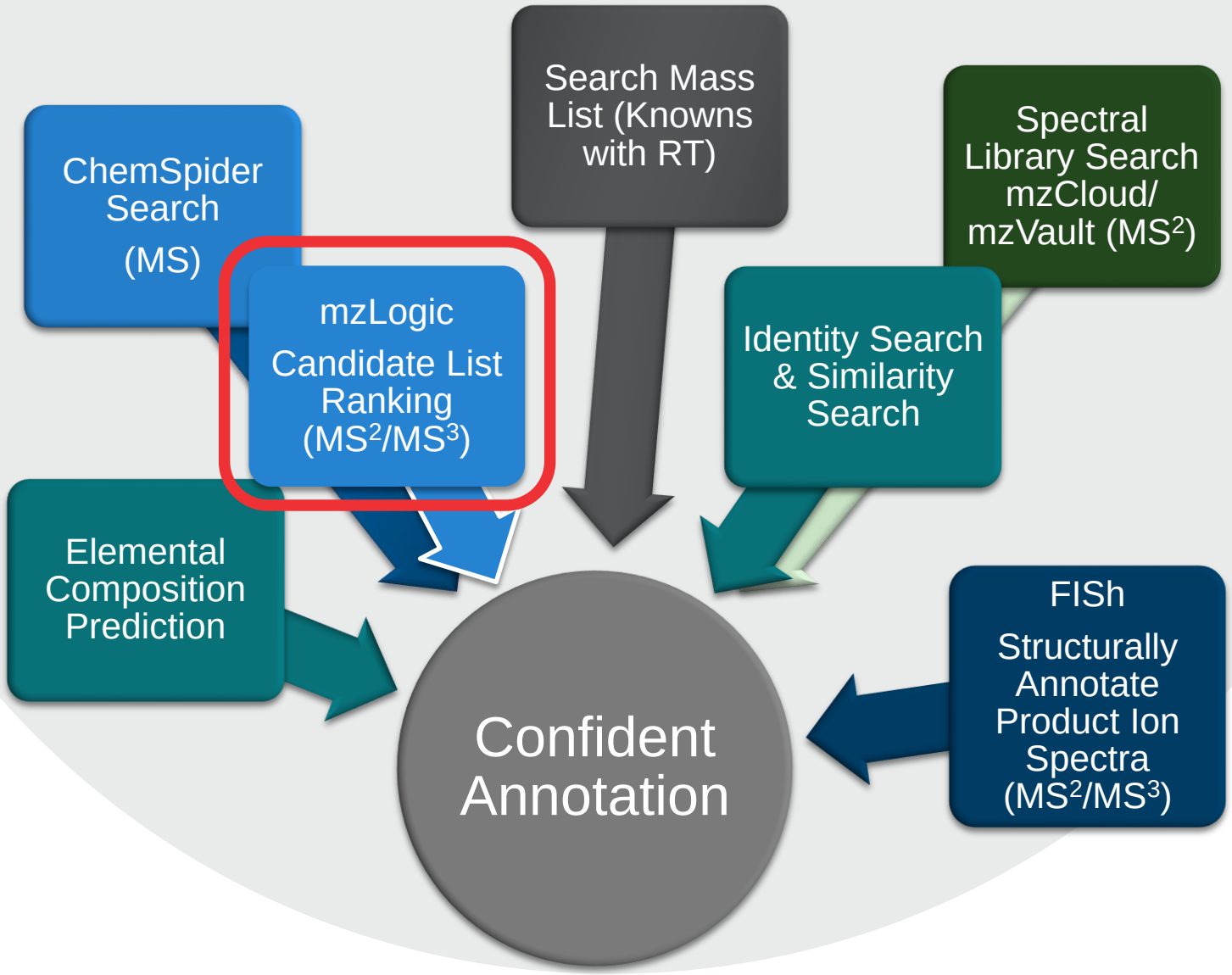
ThermoFisher SCIENTIFIC

Thermo Scientific Compound Discoverer 3.0 Software: Confident Compound Annotation

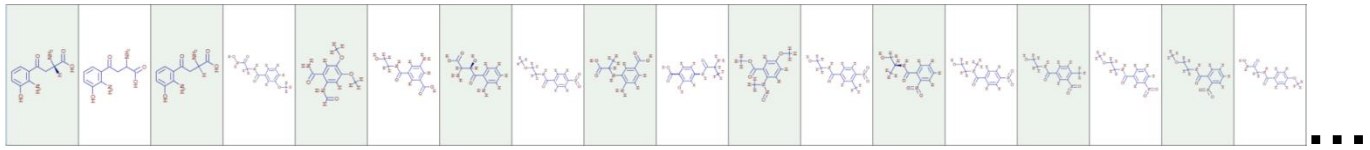
Diverse Chemical Space

new **mzLogic algorithm**

Rank-ordering of putative structures



mzLogic: Allows Ranking Putative Candidate Structures



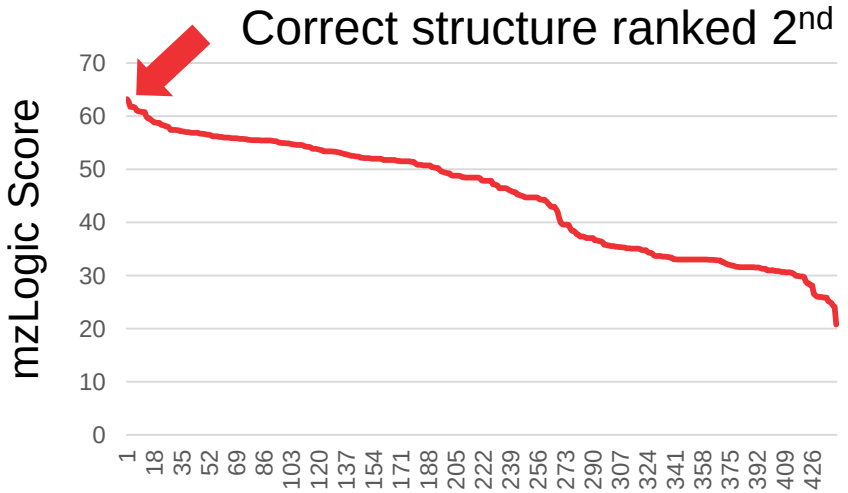
441 putative candidates for identification

mzCloud™

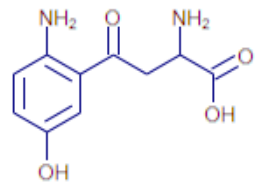
Curated spectral library
HCD/CID/MS^n 2.8M spectra

mzLogic in Compound Discoverer 3.0 software

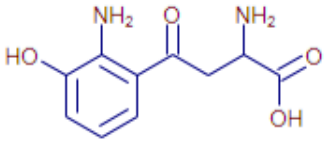
With the new mzLogic algorithm, you can use the extensive fragmentation spectral information in mzCloud to rank-order putative database results



#1 5-hydroxy-kynurenine



#2 3-hydroxy-kynurenine



ThermoFisher SCIENTIFIC

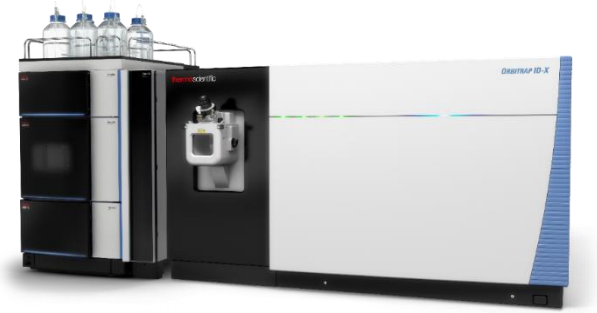
Acknowledgements

Thermo Scientific

Alexander Makarov
Amanda Souza
Andreas Huhmer
August Specht
Balaram Barange
Beena Raman
Caroline Ding
Chris Mullen
Dat Lam
David Peak
Derek Bailey
Edward Gonzalez
Edward Denisov
Eugen Damoc
Evett Kruka
George DeShong
Graeme McAlister
Helene Cardasis
Howard Tran
Iain Mylchreest
Iman Mohtashemi
Ioanna Ntai
Ipsita Basu
Jack Cunniff
Jacob Schwartz
Jean Jacques Dunyach
Jenny Berryhill
Jesse Canterbury
Jonathan Josephs
Jonathan Mcnally
Karen Johnson
Kate Comstock
Ken Miller
Kien Le
Linda Lin
Lisa Thomas
Manish Doshi
Marcia Mendonca
Mark Hardman
Mark Sanders
Maroun El Khoury
Martin Zeller
Mary Blackburn
Mike Senko
Milla Neffling
Neal Borelli
Nick Duczak
Nigama Ekkad
Paul Hendricks
Phil Remes
Priya Rajamani
Rajesh Raina
Ralf Tautenhahn
Randy Porch
Ray Chen
Reiko Kiyonami
Rich Klein
Rick Carberry
Rodney Mccoy
Romain Huguet
Ryo Komatsuzaki
Sally Webb
Scott Peterman
Sean Malynn
Seema Sharma
Shannon Eliuk
Stephanie Samra
Susan Yung
Terry Olney
Thomas Moehring
Tim Stratton
Tony Ziberna
Tran Howard
Uma Kota
Vlad Zabrouskov
Wei Wei
Wilfried Voorhorst
Willy Bjorklund

Collaborators

Daniel Gachotte (Corteva Agriscience)
Fenghe Qiu (Boehringer Ingelheim)
Fuad Nasir (Washington University)
Gary Patti (Washington University)
Jeff Gilbert (Dow AgroSciences)
Jesse Balcer (Dow AgroSciences)
Kevin Cho (Washington University)
Mark Viant (Univ of Birmingham)
Martin Jones (Univ of Birmingham)
Shuguang Ma (Genentech)
Warwick Dunn (Univ of Birmingham)
William Nash (Univ of Birmingham)
Yelena Adelfinskaya (Dow AgroSciences)



ThermoFisher SCIENTIFIC

thermofisher.com/orbitrapID-X

ThermoFisher SCIENTIFIC