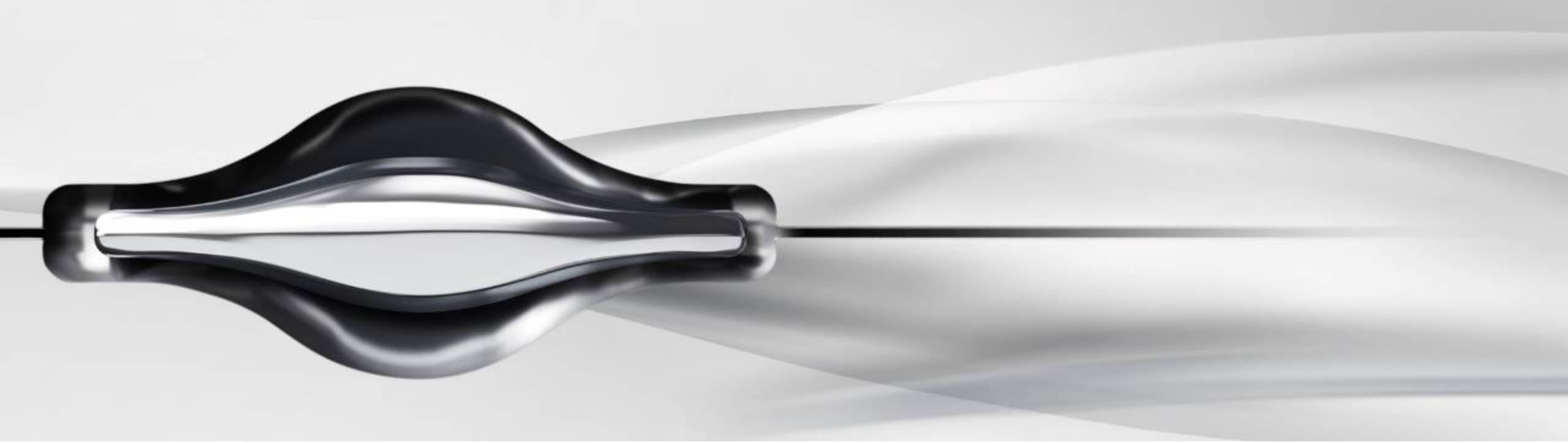


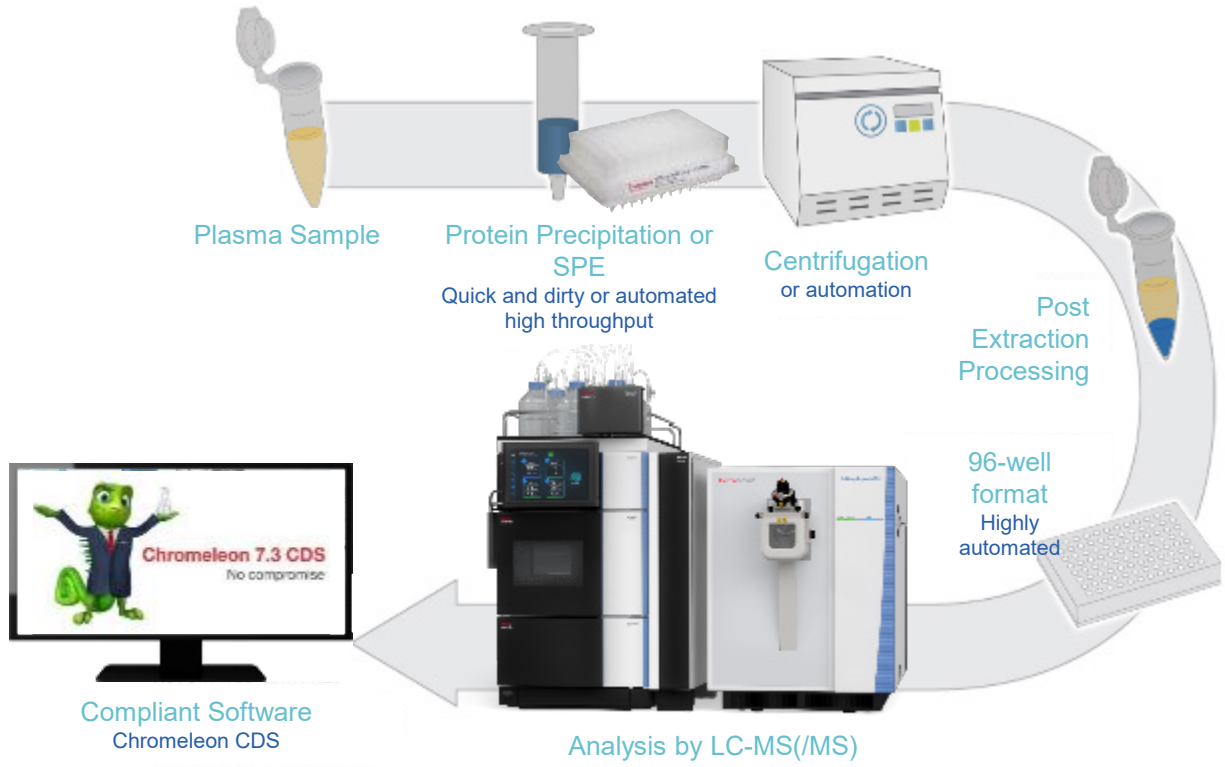
ThermoFisher SCIENTIFIC

Stay Ahead in Pharmaceutical Analysis with Orbitrap™

Jason Goh
Regional Lead Application Specialist, LCMS

Bioanalytical Analysis | Analytical Needs

- Flexibility for both small and large molecules
- Speed for quick turnaround times
- Robustness, easy maintenance, and operational simplicity
- High sensitivity and selectivity
- GLP regulatory pre-clinical studies to support early and late stage clinical development projects.
- Fully validated assay to provide reliable quantitation of analytes and biomarkers in various biological matrices
- Effectively managing costs but still maintaining productivity & quality



ThermoFisher SCIENTIFIC

Bioanalysis MSMS and HRMS Portfolio

Triple Quadrupole MS

Thermo Scientific™
TSQ Altis™ MS

Thermo Scientific™
TSQ Quantis™ MS

Thermo Scientific™
TSQ Fortis™ MS

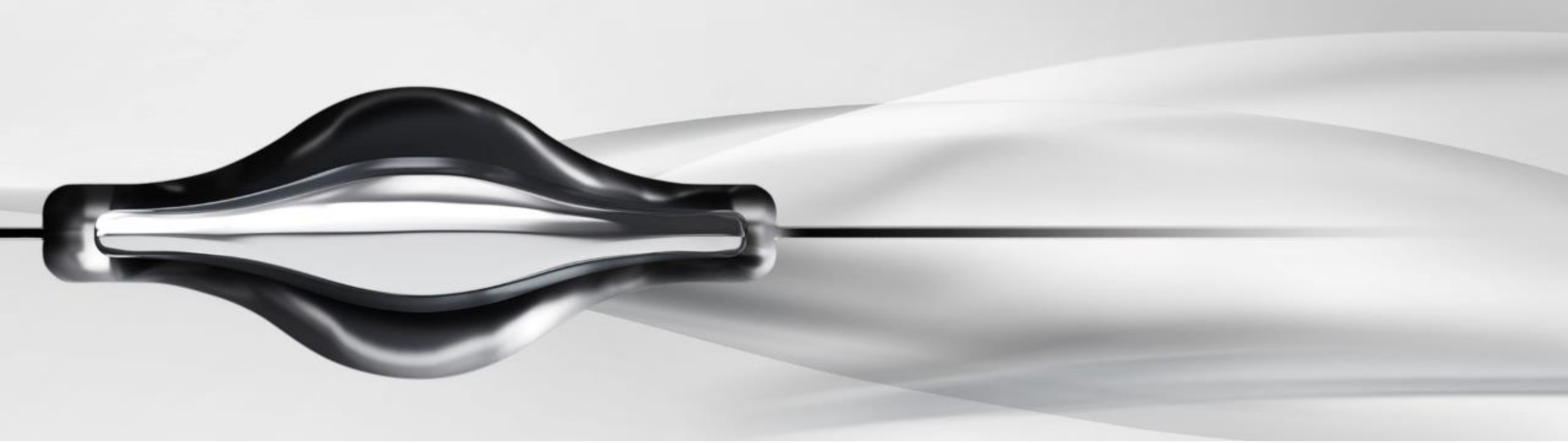
HRAM Orbitrap MS

Orbitrap
Exploris 240 MS
with BioPharma Option

Q Exactive Plus MS

Orbitrap
Exploris 120 MS

ThermoFisher SCIENTIFIC



ThermoFisher SCIENTIFIC

Small Molecule Quantitative BioAnalysis - Orbitrap Exploris 120 Mass Spectrometer

Quantitative Bioanalysis of Small Molecules

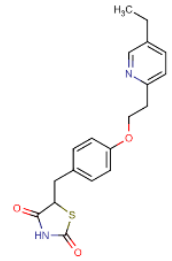
Goal

- Demonstrate capabilities of Orbitrap Exploris 120 mass spectrometer for quantitative bioanalysis of small molecules in plasma and blood
- Show simple method setup with high specificity.
- Highlight Improvements in scan speed whilst maintaining high mass accuracy.
- Show excellent sensitivity and linear range from crude sample preparation.

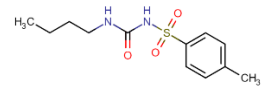
Analytes, samples and comparative data supplied by Xenogesis a drug discovery CRO

XenoGesis

Pioglitazone
MW: 356.1190



Tolbutamide
MW: 270.1038

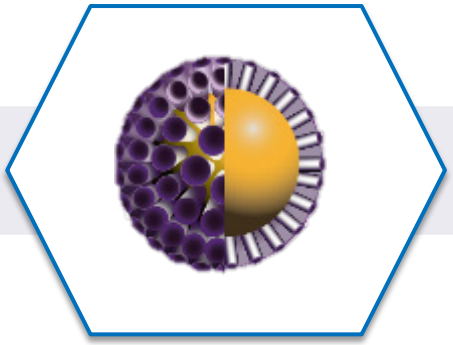


Confidential Client Compounds

Compound A	MW:	267.9735
Compound B	MW:	461.1084
Compound C	MW:	401.1198
Compound D	MW:	477.2740
Compound E	MW:	489.2740

Thermo Scientific™ Vanquish ™ Horizon UHPLC System

Thermo Scientific™ Accucore™ Biphenyl column, 50 x 2.1 mm 2.6 µm



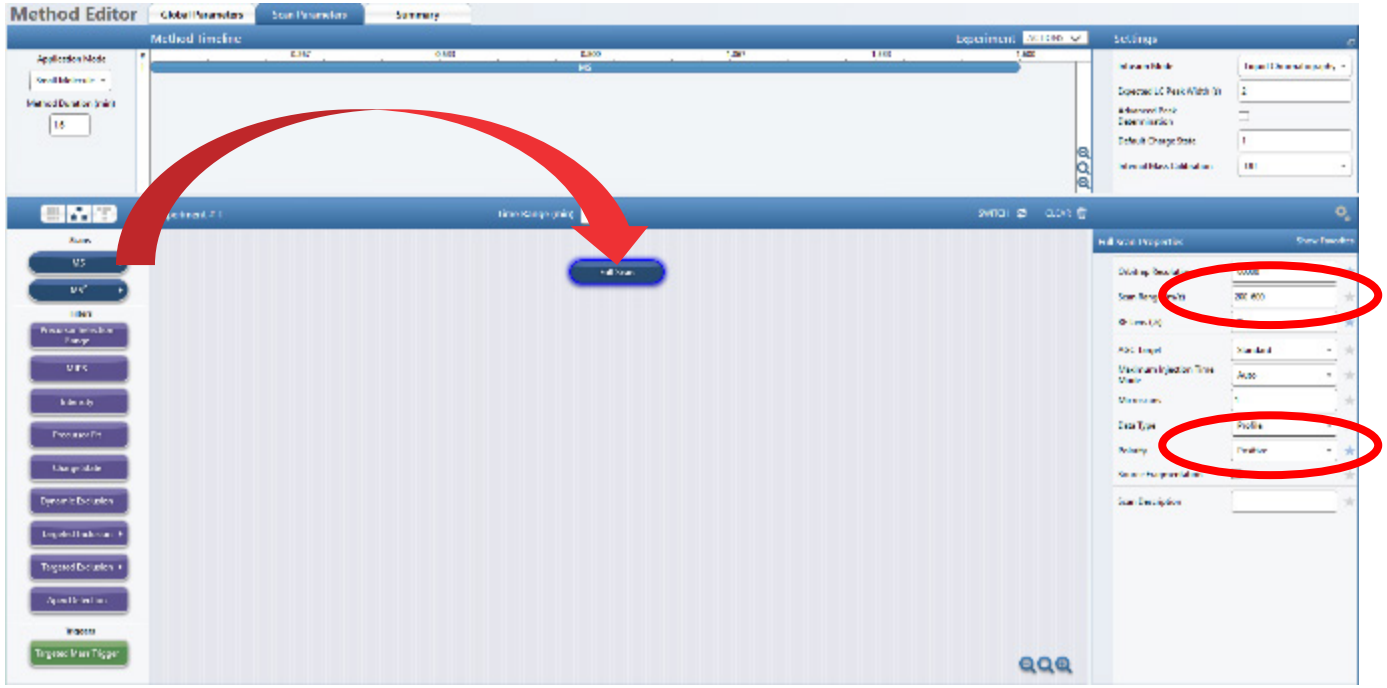
Orbitrap Exploris 120 MS

Chromeleon 7.3 CDS

ThermoFisher SCIENTIFIC

Simple Method Development

- **Easy method setup** - Full Scan methods can easily be set up with just a few clicks. No need to optimize each compound as in typical SRM acquisitions



Click and drag experiment

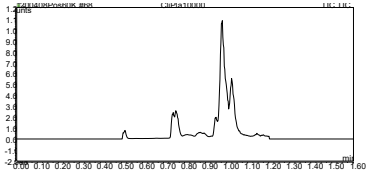
Set Mass range

Select polarity

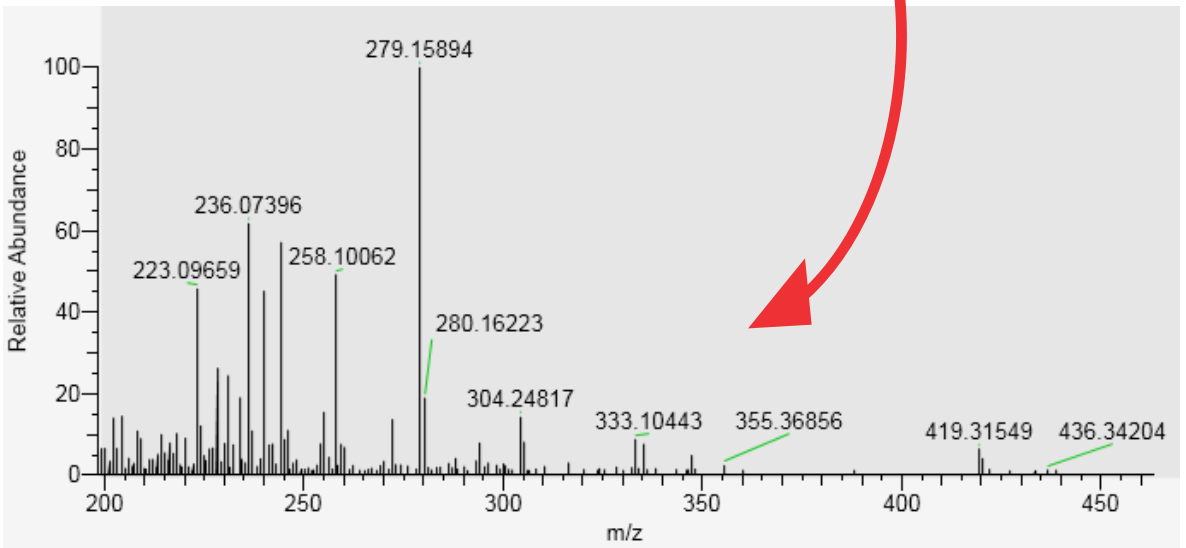
ThermoFisher SCIENTIFIC

Simple Method Development

- **Easy method setup** - Full Scan methods can easily be set up with just a few clicks. No need to optimize each compound as in typical SRM acquisitions
- Full scan data is kept and can be **retrospectively mined** for other compounds later.



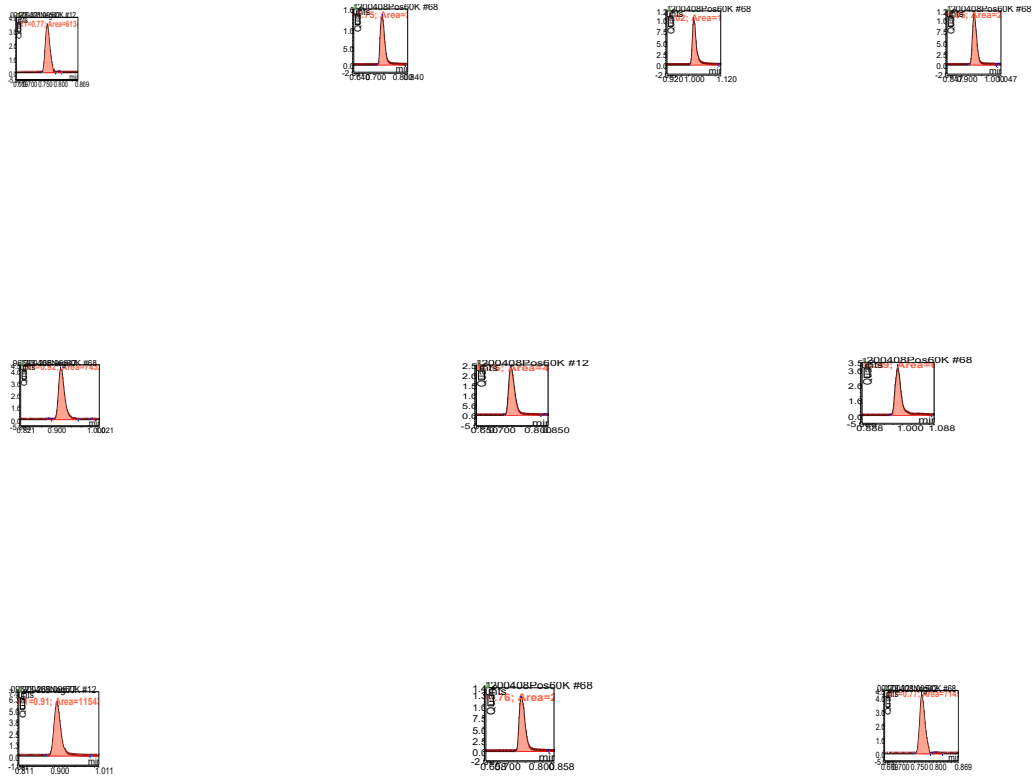
FS *m/z* 200-600
Blood extract



ThermoFisher SCIENTIFIC

Simple Method Development

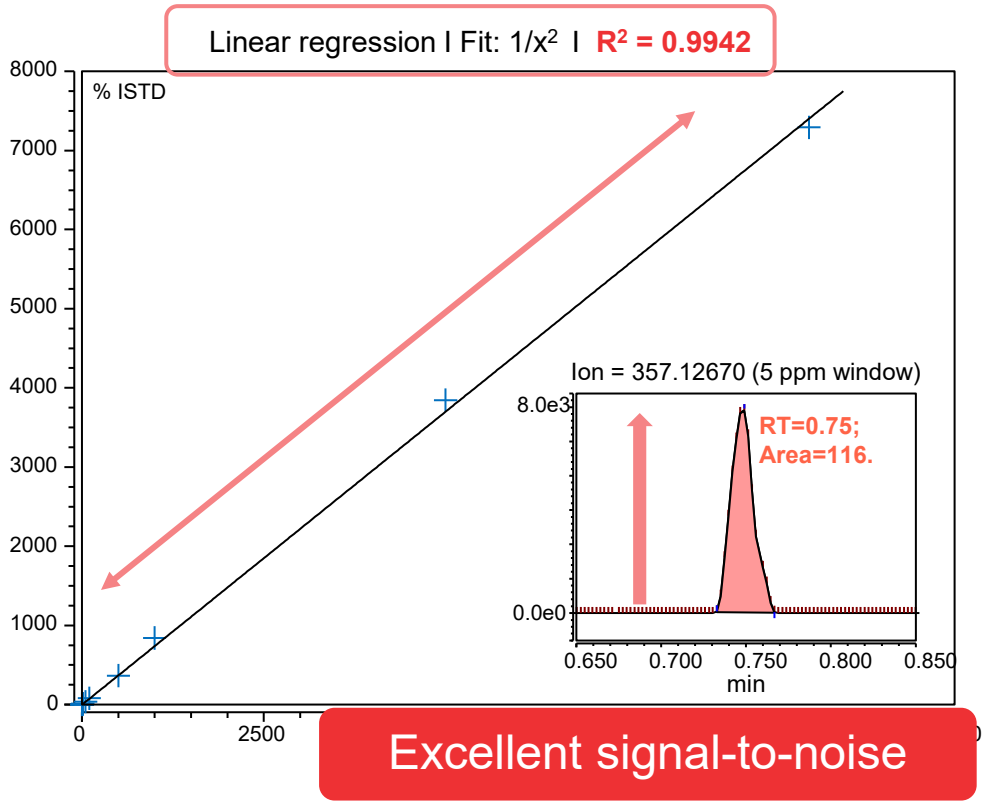
- **Easy method setup** - Full Scan methods can easily be set up with just a few clicks. No need to optimize each compound as in typical SRM acquisitions
- Full scan data is kept and can be **retrospectively mined** for other compounds later.
- Compounds of interest can be extracted and processed very easily. Multiple components can be processed in minutes and **templates within Chromeleon** used to make data interpretation simple.



Quantitative Performance

- **Excellent linear range** was observed with regression greater than 0.99 for each compound.

Pioglitazone Full Scan 60k Positive



Excellent signal-to-noise

ThermoFisher SCIENTIFIC

Quantitative Performance

- **Excellent linear range** was observed with regression greater than 0.99 for each compound.
- Acceptable accuracy was achieved through the **5-orders of magnitude** concentration range with excellent sensitivity.

Pioglitazone Full Scan 60k Positive

5 orders of magnitude linear range

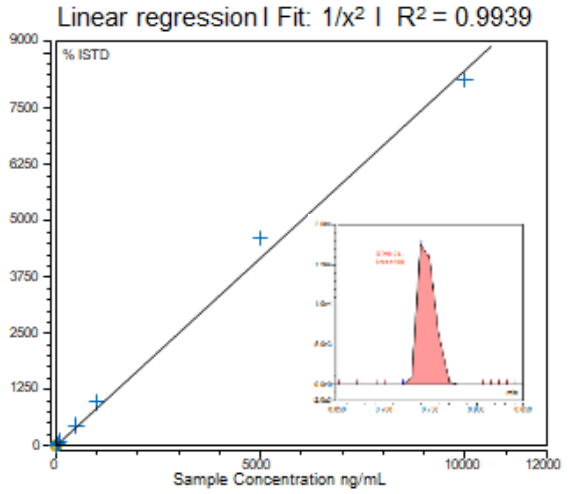
On Column (pg)	Sample (ng/mL)	Pioglitazone (% bias)
0.033	0.1	7.0
0.17	0.5	-13.9
0.33	1	-14.5
1.7	5	-6.3
3.3	10	11.0
17	50	-3.0
33	100	6.5
167	500	-0.7
333	1000	14.2
1667	5000	4.9
3333	10000	-0.6

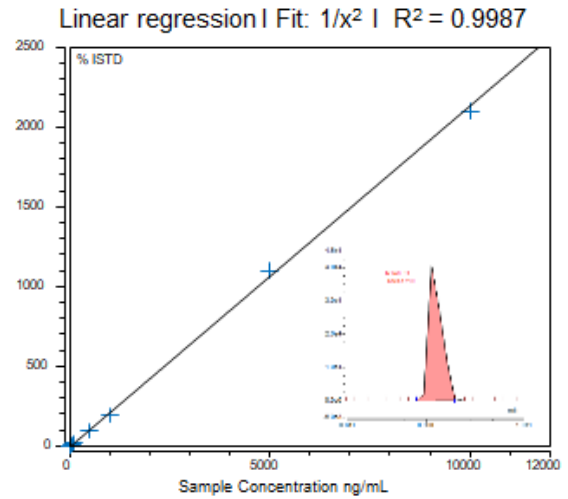
33 femtogram on column

Quantitative Performance

- **Excellent linear range** was observed with regression greater than 0.99 for each compound.
- Acceptable accuracy was achieved through the **5-orders of magnitude** concentration range with excellent sensitivity.
- Fast polarity switching provides **more information** for scouting runs meaning less analysis time.

Pioglitazone (+) and Tolbutamide (-) Full Scan 30k +/- switching



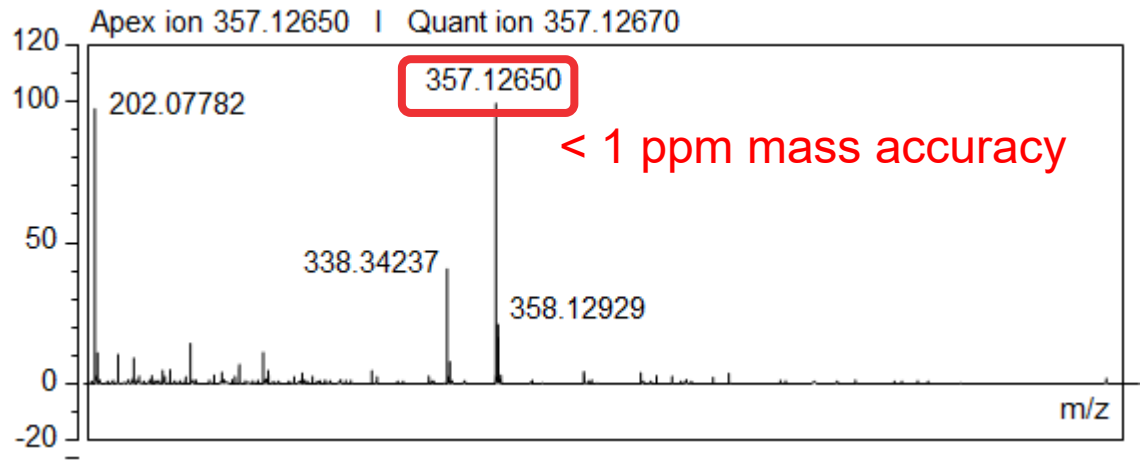


On Column (pg)	Sample (ng/mL)	Pioglitazone (% bias)	Tolbutamide (% bias)
0.17	0.5	11.5367	-
0.33	1	3.5678	-
1.7	5	-2.1068	-10.982
3.3	10	11.5568	26.1573
17	50	-5.8101	-5.6688
33	100	7.2102	4.5346
167	500	1.2058	-8.9456
333	1000	7.5154	-8.4511
1667	5000	2.4095	4.2017
3333	10000	-9.7444	-0.8462

Quantitative Performance

- **Excellent linear range** was observed with regression greater than 0.99 for each compound.
- Acceptable accuracy was achieved through the **5-orders of magnitude** concentration range with excellent sensitivity.
- Fast polarity switching provides **more information** for scouting runs meaning less analysis time.
- **Mass accuracy was excellent** at <1ppm across all compounds and all concentration levels

Type	Level	Vial	Volume	RT	Expected RT	Quant Ion	Mass Accuracy
		Pos'n	µL	min	min	m/z	ppm
Calibration Standard	1	R:A1	1.00	0.75	0.75	357.12670	0.37
Calibration Standard	2	R:A2	1.00	0.74	0.75	357.12670	-0.49
Calibration Standard	3	R:A3	1.00	0.74	0.75	357.12670	0.37
Calibration Standard	4	R:A4	1.00	0.74	0.75	357.12670	-0.06
Calibration Standard	5	R:A5	1.00	0.75	0.75	357.12670	0.03
Calibration Standard	6	R:A6	1.00	0.74	0.75	357.12670	0.11
Calibration Standard	7	R:A7	1.00	0.74	0.75	357.12670	0.20
Calibration Standard	8	R:A8	1.00	0.74	0.75	357.12670	-0.06
Calibration Standard	9	R:A9	1.00	0.74	0.75	357.12670	-0.32
Calibration Standard	10	R:B1	1.00	0.75	0.75	357.12670	-0.32
Calibration Standard	*11*	*R:B2*	*1.00*	*0.75*	*0.75*	*357.12670*	*-0.57*



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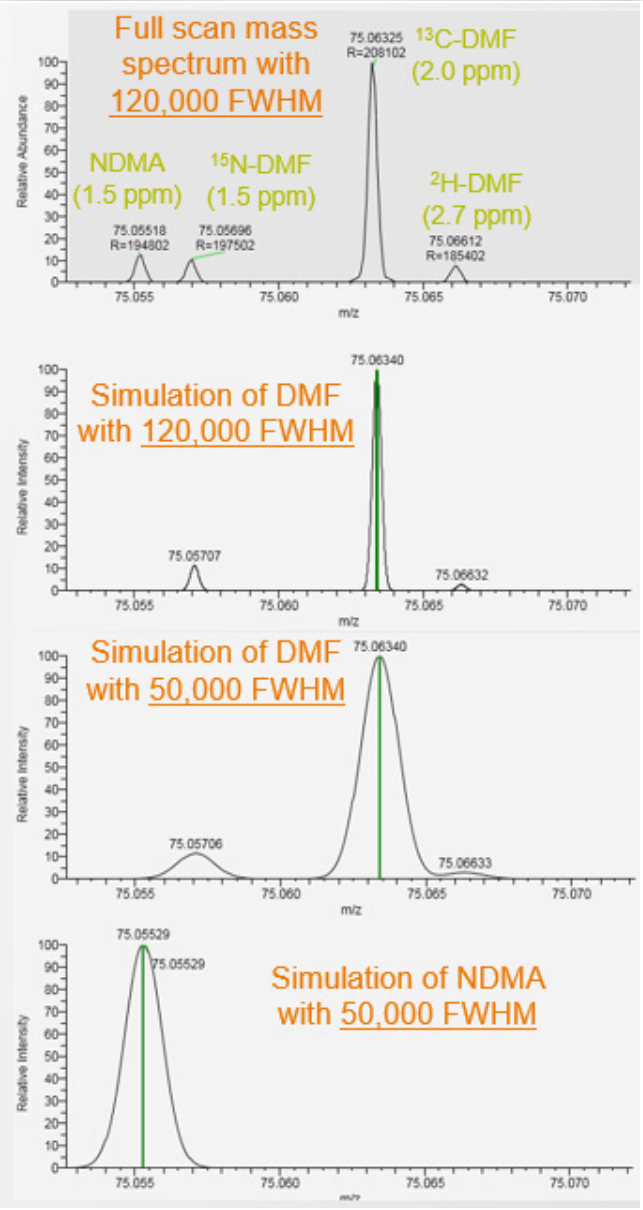
Quantitative Performance

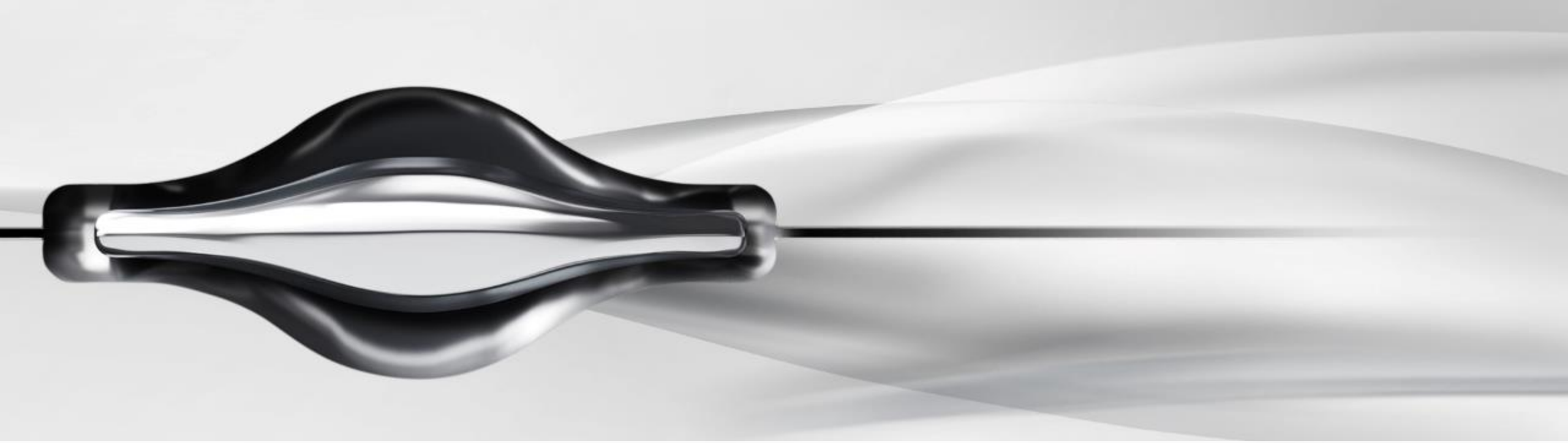
- **Excellent linear range** was observed with regression greater than 0.99 for each compound.
- Acceptable accuracy was achieved through the **5-orders of magnitude** concentration range with excellent sensitivity.
- Fast polarity switching provides **more information** for scouting runs meaning less analysis time.
- **Mass accuracy was excellent** at <1ppm across all compounds and all concentration levels
- **High mass resolution** needed to separate target analyte with matrix interferences for accurate quantitation



ThermoFisher SCIENTIFIC

Summary | Quantitative Bioanalysis Using Orbitrap Exploris 120 MS

- **Operational simplicity** for mass spectrometer setup and acquisition under full scan conditions
- **Wide linear dynamic** range providing analytical flexibility and less reanalysis of samples
- **Exceptional sensitivity** for trace analysis
- **Excellent mass accuracy and resolution** allowing confident analysis



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Small Molecule Metabolite Identification - Thermo Scientific Orbitrap Exploris 240

Small Molecule Pharma Qual/Quan HRAM MS Portfolio

Capability/Performance

Routine Quan/Qual HRAM Orbitrap MS

- Highest resolution: 120K
- Easy to operate / maintain
- Small footprint
- Resolution and Mass Accuracy
- Fast scan speed/polarity switch

Orbitrap Exploris 120 Mass Spectrometer

"We like Q Exactive so much and want to replace existing systems with Q Exactive"
Hai-Ling Sun (DMPK, Gilead sciences)

Proven technology

- Highest resolution: 280K
- Well recognized orbitrap platform
- Versatile for small mol and peptide/protein analysis

Q Exactive Plus Mass Spectrometer

Orbitrap Exploris 240 Mass Spectrometer

Versatile & Robust HRAM workhorse

- Highest resolution: 240K
- Easy to operate / maintain
- Small footprint
- Resolution and Mass Accuracy
- Fast scan speed/polarity switch
- Deeper coverage (AcquireX)
- Versatile for small mol and peptide/protein analysis

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

Ultimate confidence for small mol ID and structure elucidation

- Highest resolution: 1M
- Ultimate flexibility and capability for data acquisition
- Intelligent data acquisition (Decision tree)
- Deeper coverage (AcquireX)
- Rich structure information with Multistage multi-dissociation technologies (CID&HCD)
- More confidence in proposed structure (MS^n)

"Orbitrap is the top choice for small molecule structure analysis!"
Ben Jiang (PKDM, Amgen)

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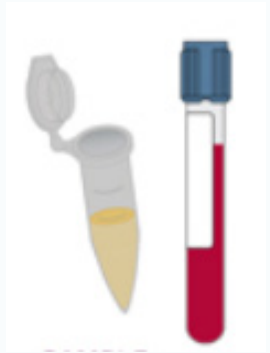
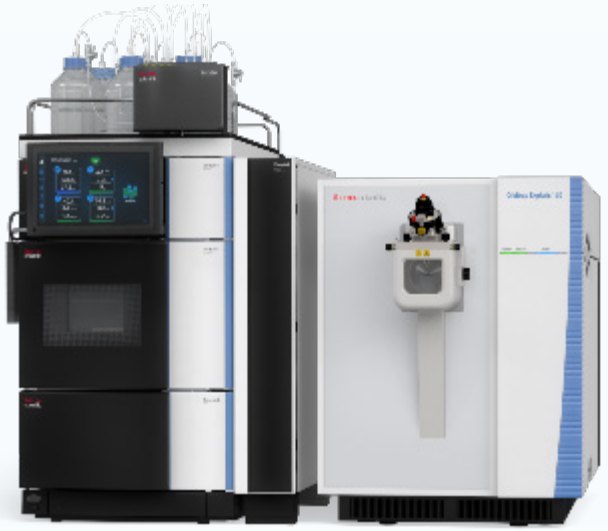
Metabolite Identification and Quantification Workflow

Goal: Understanding drug metabolism by conducting studies to identify and characterize all of major metabolites of the test drug and specific enzymes responsible for its metabolism; to evaluate the impacts of the metabolites on safety and efficacy of the drug; and to utilize the drug's metabolism information to maximize its intellectual property.

Biological samples
in vitro and *in vivo* studies

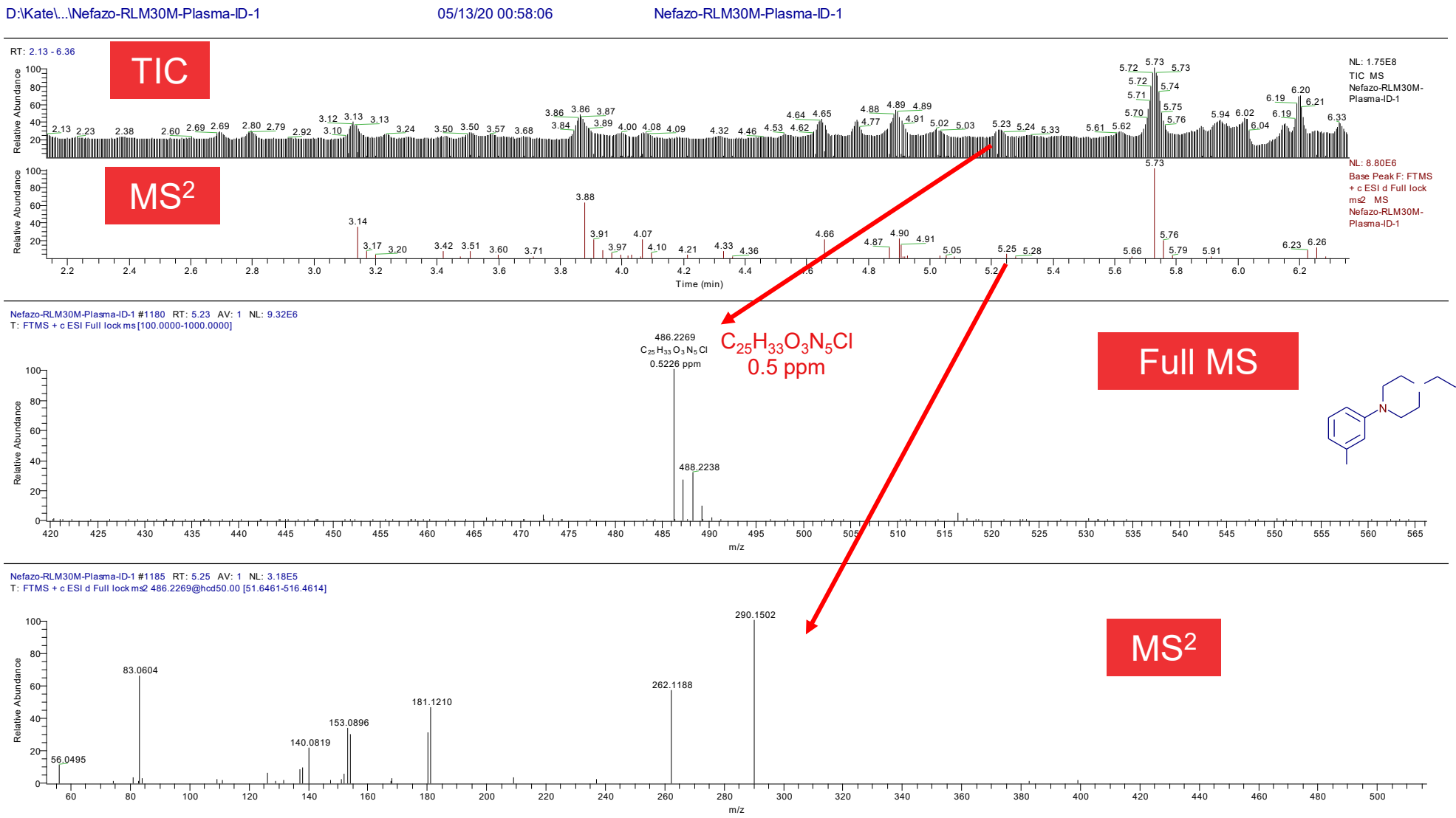


UHPLC-HRMS for data acquisition

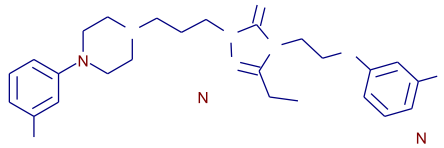


Data processing for metabolite ID and relative quantification

Nefazodone Metabolite in Rat Plasma



Proposed structure



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Compound Discoverer Software

Thermo Scientific™ Compound Discoverer™ Software

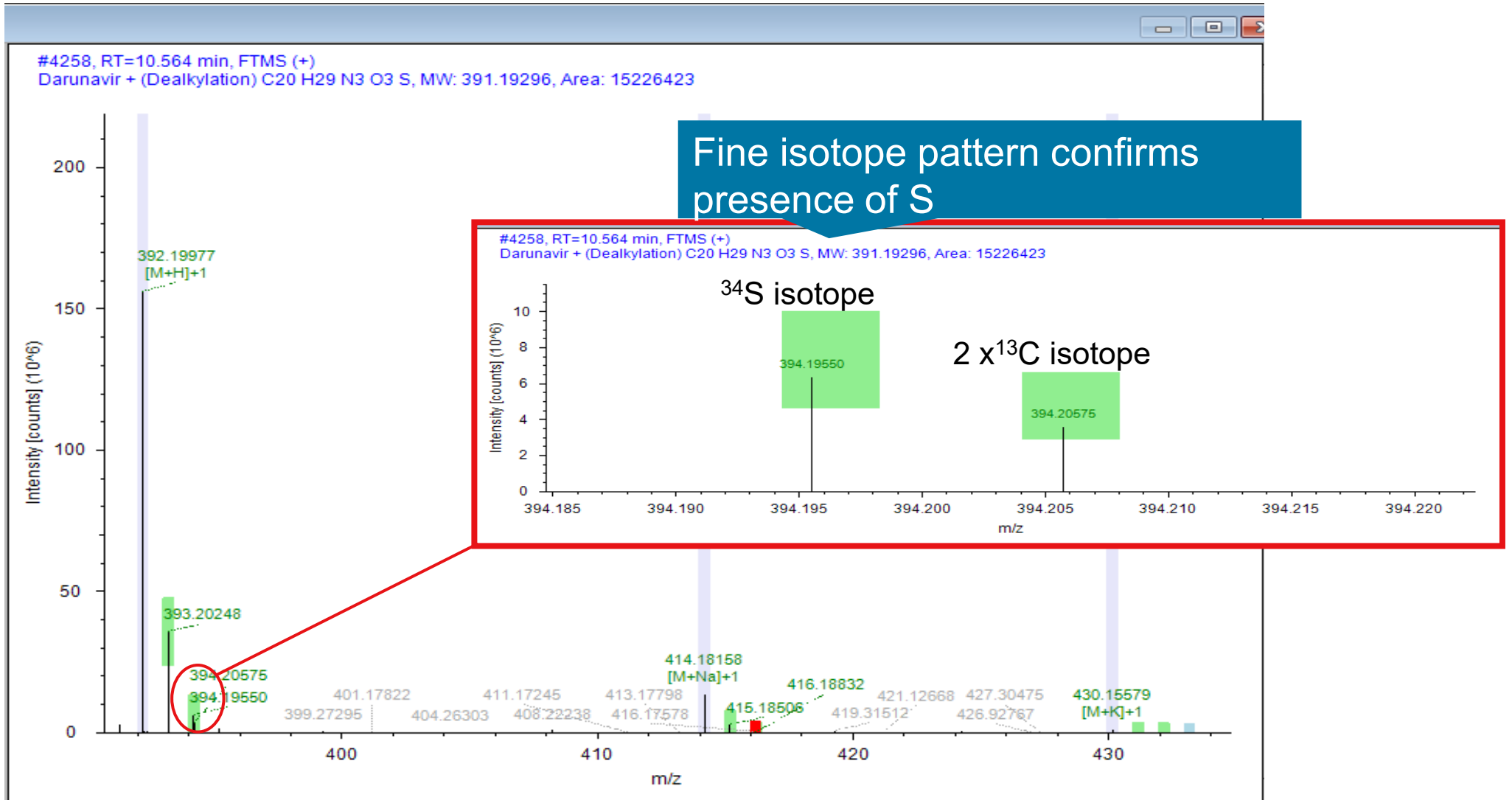
Complete small molecule structure identification in a **Next Generation** platform

Compound Discoverer provides customers with a complete software platform for **small molecule research**.

Compound Discoverer addresses small molecule identification needs and workflows including untargeted metabolomics, metabolism, impurities, degradant, extractables and leachables, environmental, food safety, clinical research and forensic toxicology.

For Research Use Only. Not for use in diagnostic procedures.

Fine Isotope Pattern Match and Visualization - More Confident ID



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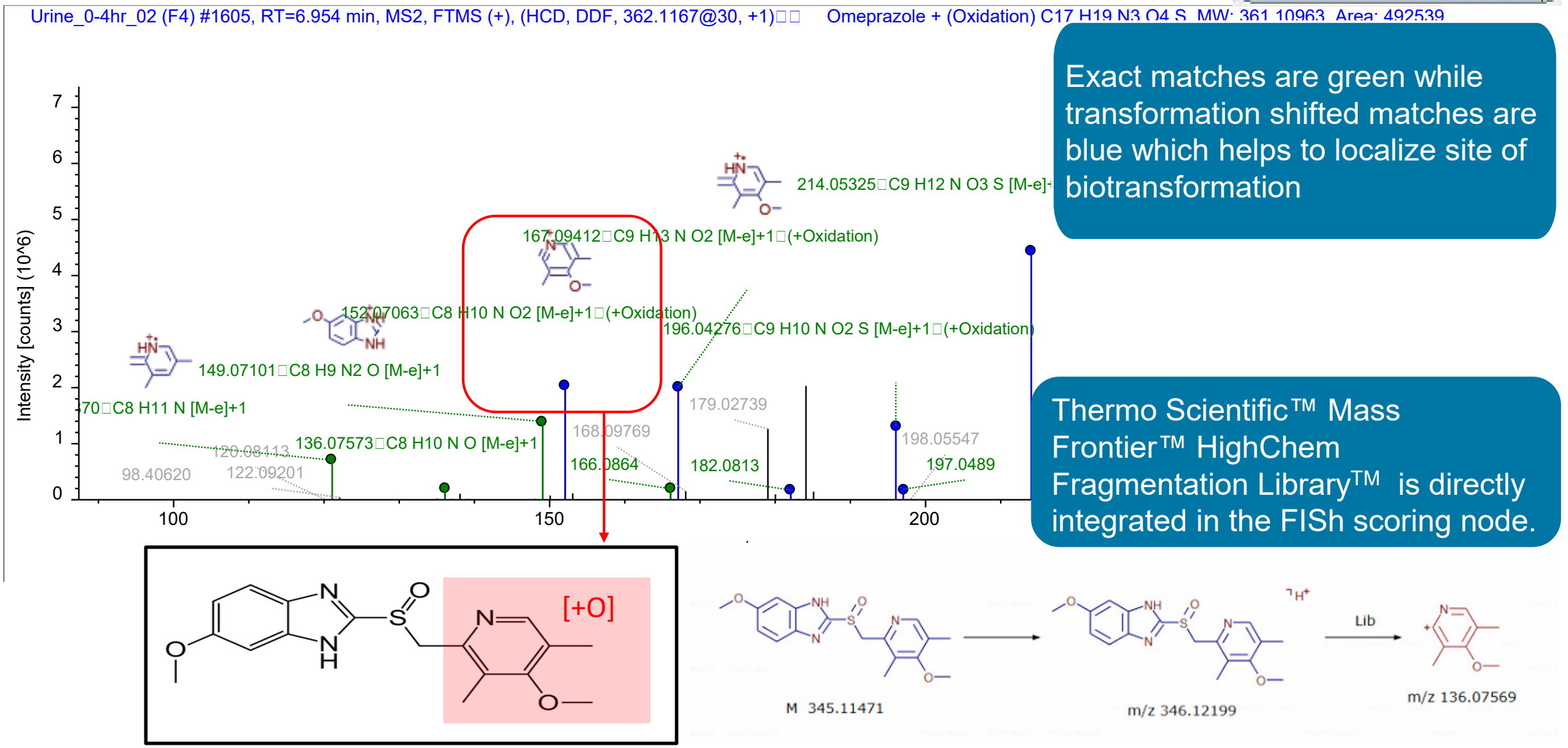
Automatic Fragment Structure Annotations - FISh Scoring Node

FISh Scoring



Exact matches are green while transformation shifted matches are blue which helps to localize site of biotransformation

Thermo Scientific™ Mass Frontier™ HighChem Fragmentation Library™ is directly integrated in the FISh scoring node.

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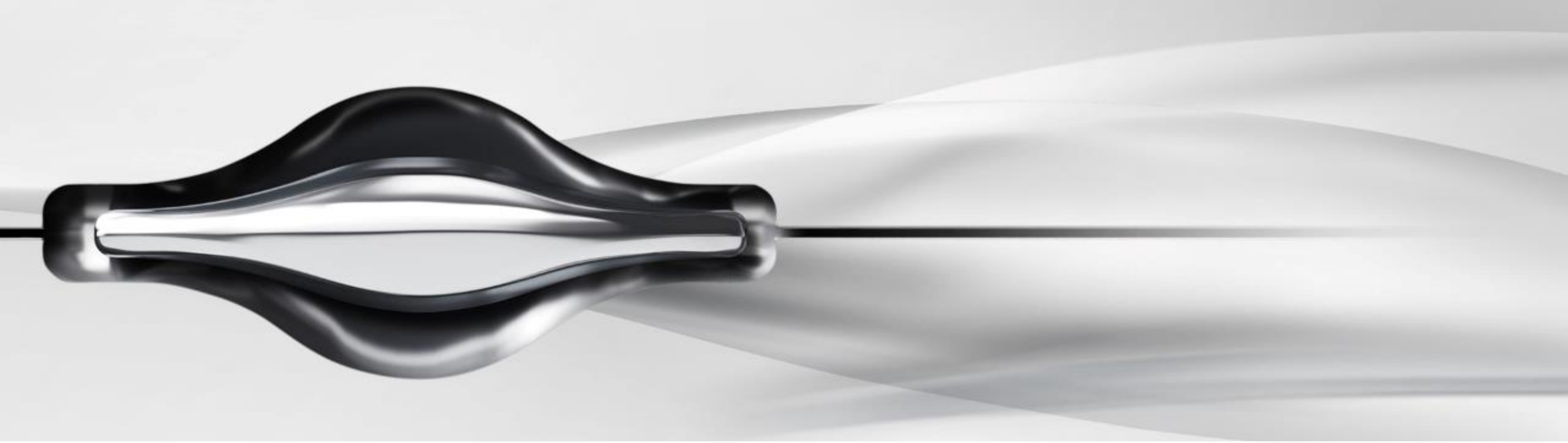

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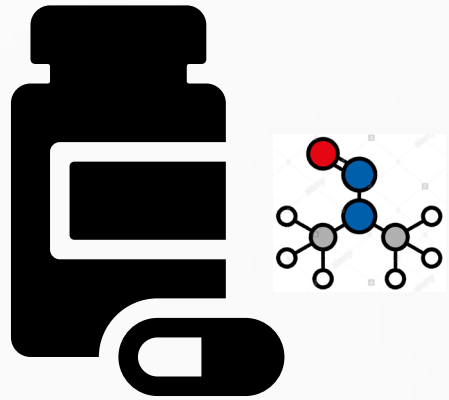
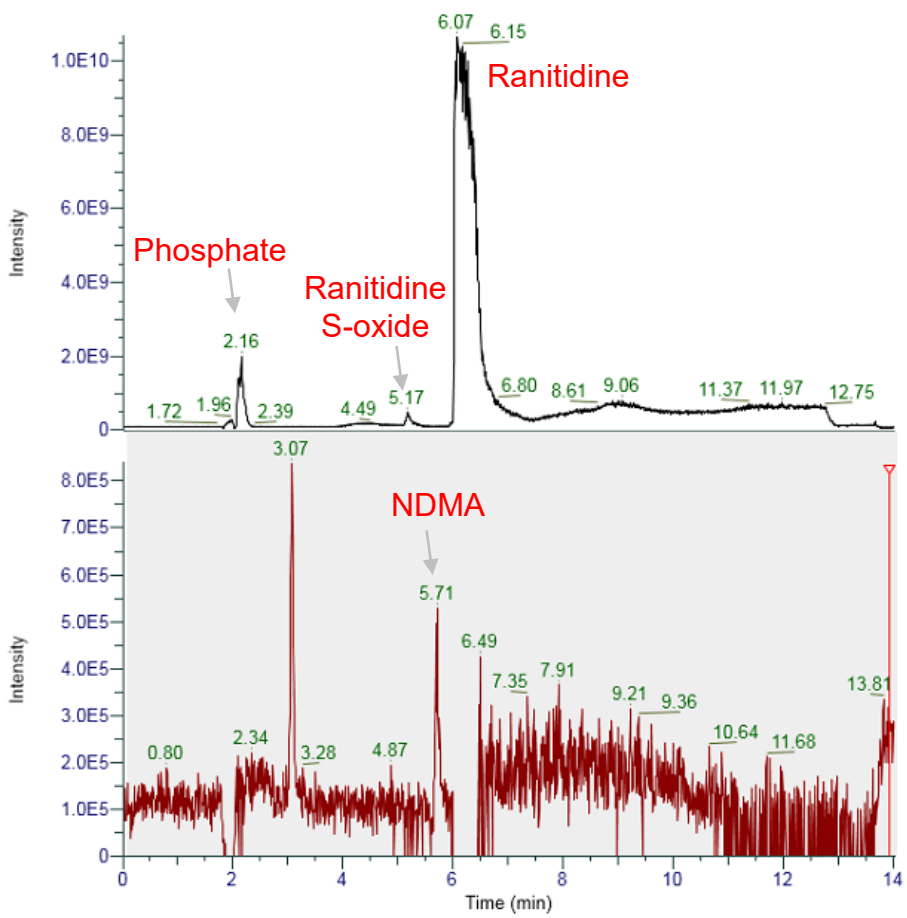
Trace Impurity Analysis - Thermo Scientific Orbitrap Exploris 240



High Resolution Full Scan for Robust Method Development

To develop a robust LCMS method for trace impurity analysis in drug samples, it is important to have a full overview of the drug to ensure minimum matrix interference with target impurities (eq. NDMA). This is achieved via high-resolution full scan mode in Orbitrap Exploris 120 or 240.

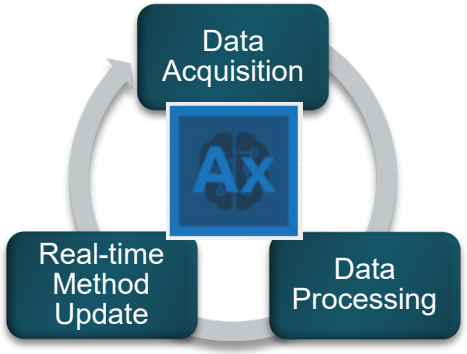


HRMS Full Scan - **Base Peak Chromatogram (BPC)**

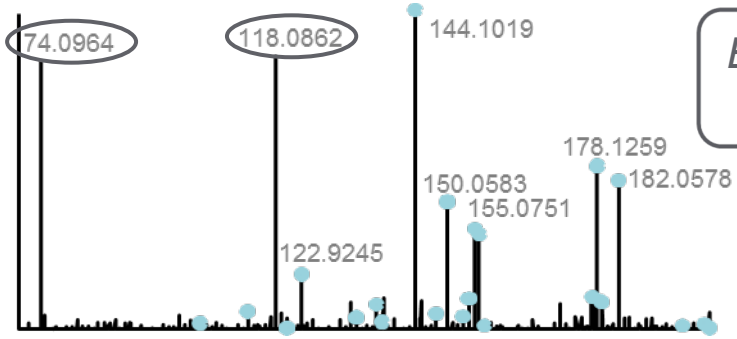
HRMS Full Scan - **Extraction Ion Chromatogram (XIC) of NDMA**

Intelligent Data Acquisition - AcquireX Enables Increased Untargeted Compound Coverage

Never fragment the same ion twice by automatically updating the exclusion list over multiple iterative injections for obtaining exhaustive fragmentation of all small molecules in the study.



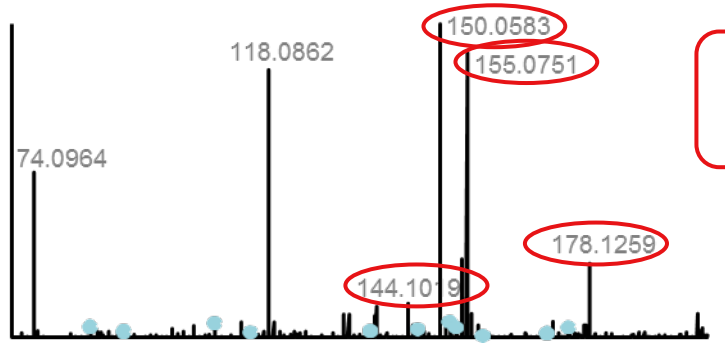
Injection 1 MS/MS



Background ions excluded

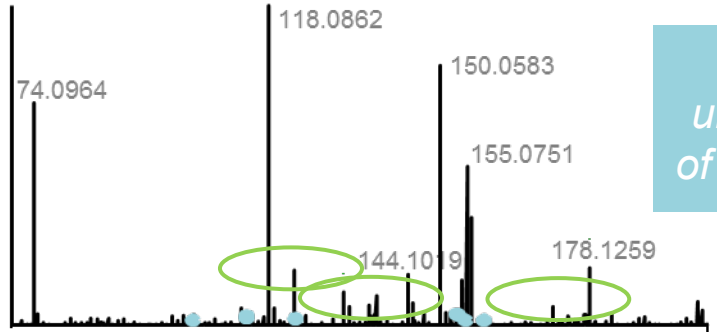
Precursors fragmented

Injection 2 Iterative MS/MS



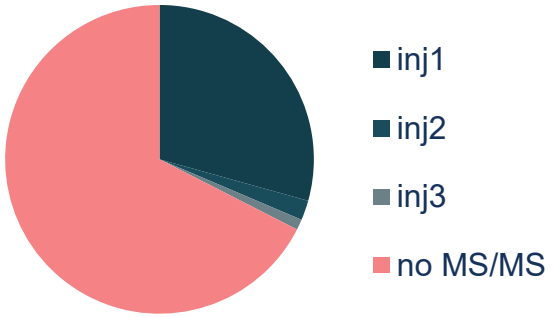
Precursors not re-fragmented

Injection 3 Iterative MS/MS

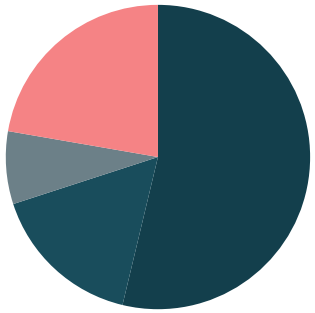


Fragment more unique precursors of lower abundance

Traditional DDA



AcquireX Deep Scan



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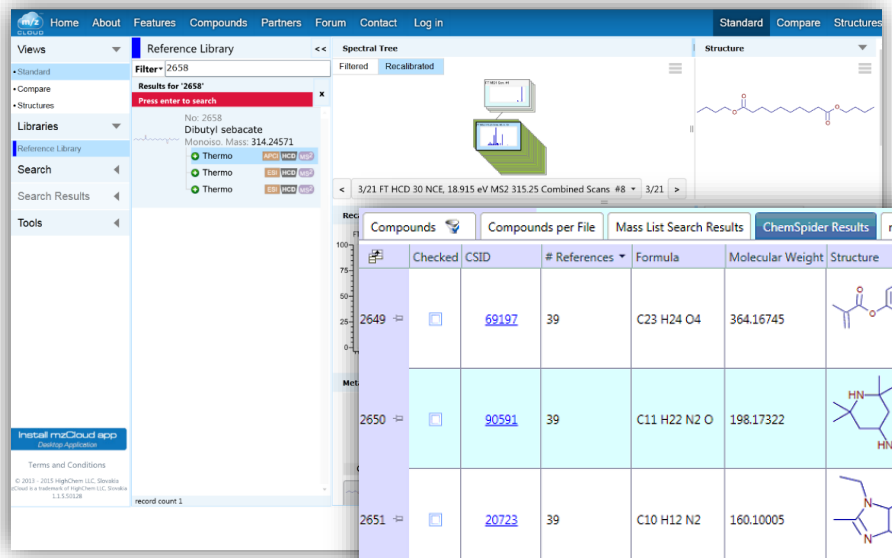
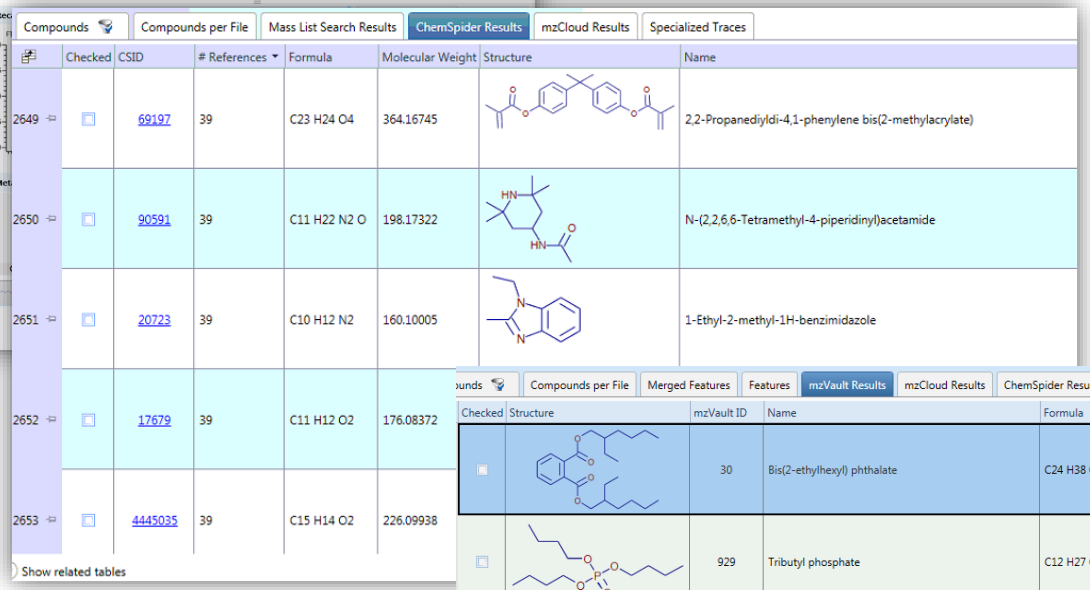
Parallel Identification through Multiple Reference Sources

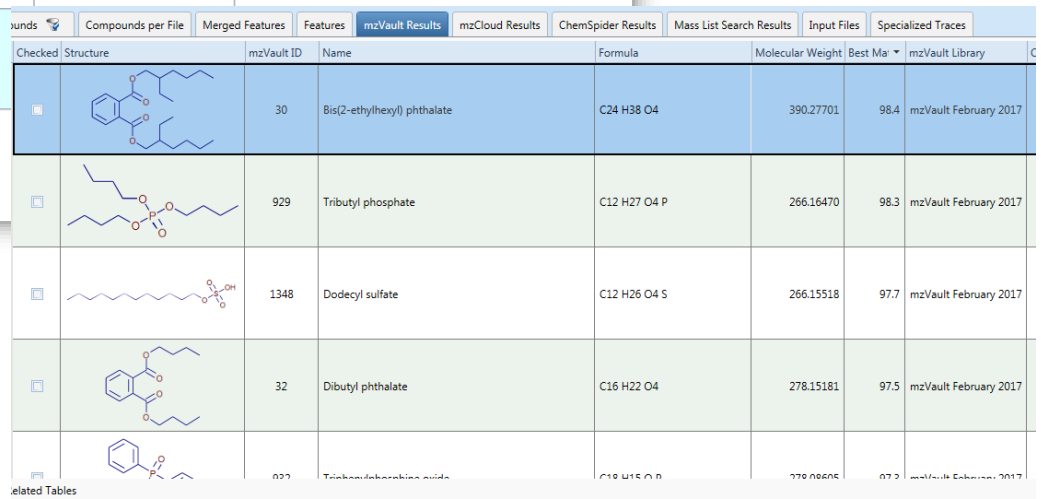
mzCloud online spectral library



ChemSpider



mzVault



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Summary | Metabolite and Trace Impurity ID Using Orbitrap Exploris 240 MS

- **Operational simplicity** for mass spectrometer setup, calibration, and method transfer
- **High performance:** High resolution, mass accuracy (EASY-IC source), sensitivity, scan speed, polarity switching, dynamic range
- **In-depth untargeted compounds coverage** with AcquireX data acquisition allowing confident analysis
- **Powerful small molecules identification workflow** with Compound Discoverer data interpretation software

Thank you

Any Questions?