# Principles of Orbitrap Mass Spectrometry


PRESENTED BY

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Product Specialist

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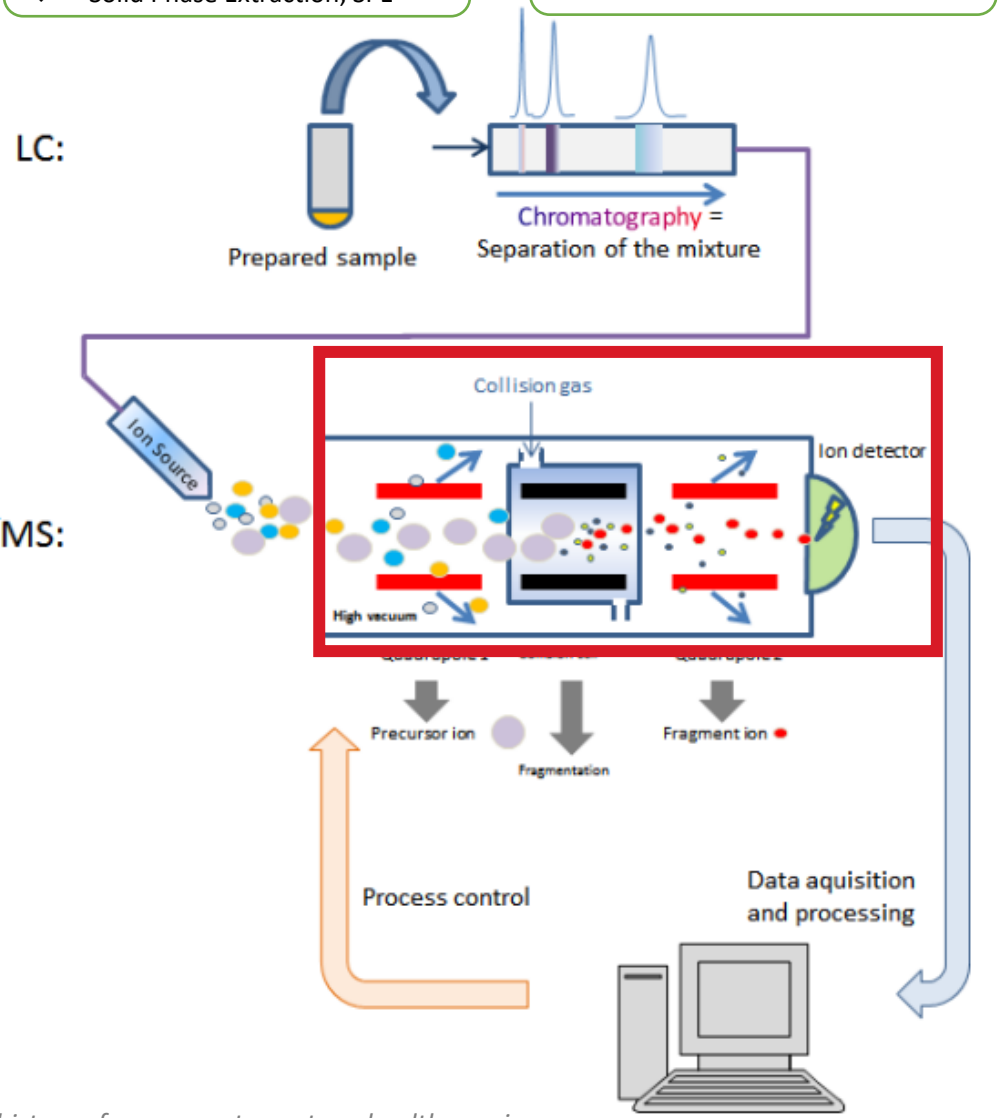
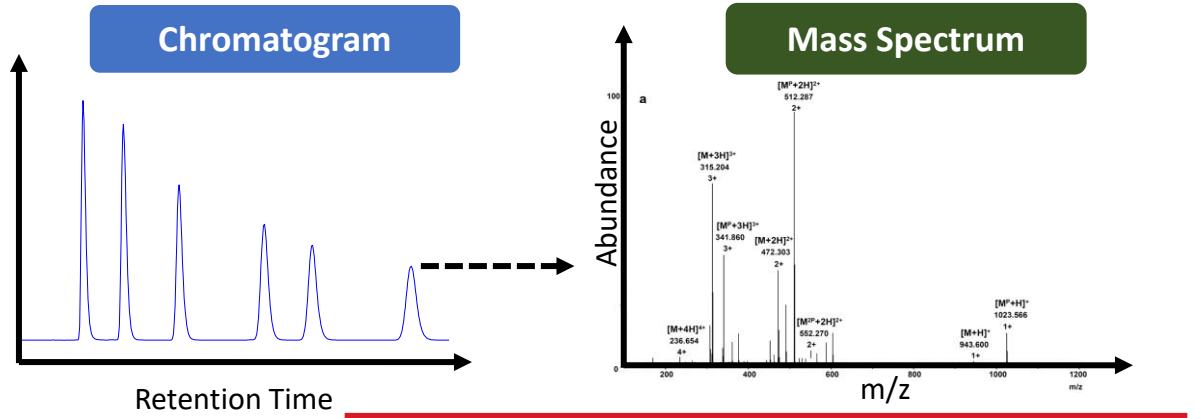
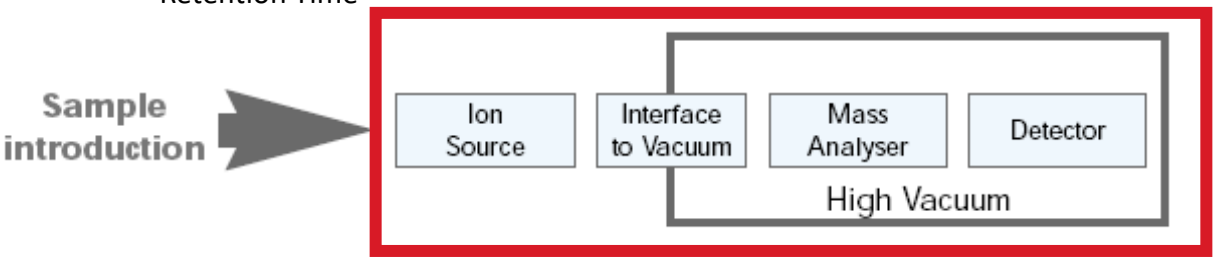
# Workflow & Basic principle of LC-MS/MS

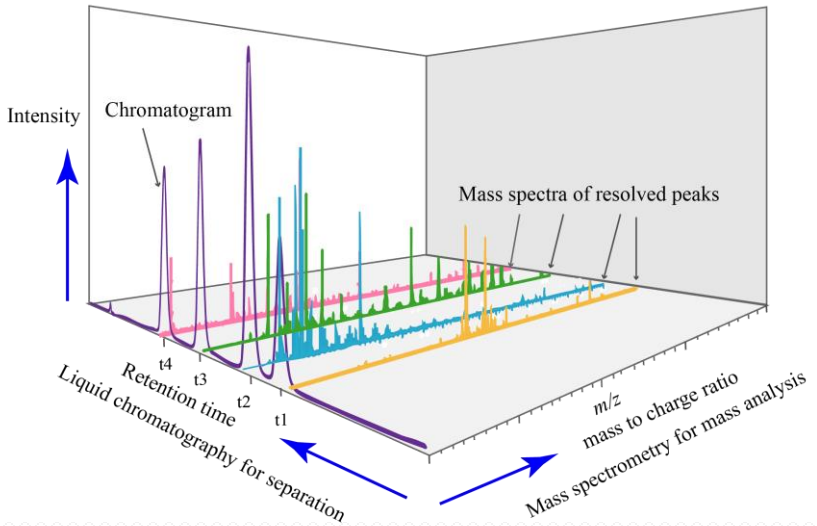
- Liquid-Liquid Extraction; LLE
- Protein Precipitation; PP
- Solid Phase Extraction; SPE

- Stationary Phase; SP = Column
- Mobile Phase; MP = Solvent




*Ref: A brief history of mass spectrometry • healthcare-in-europe.com*

# System Main Workflows

Low-Resolution Mass Spectrometry
- ❖ Ion-Traps
- ❖ Single Quadrupole
- ❖ Triple Quadrupoles

m/z = 1 decimal point

m/z Gabapentin[1] = 172.2

Prepared sample

1x

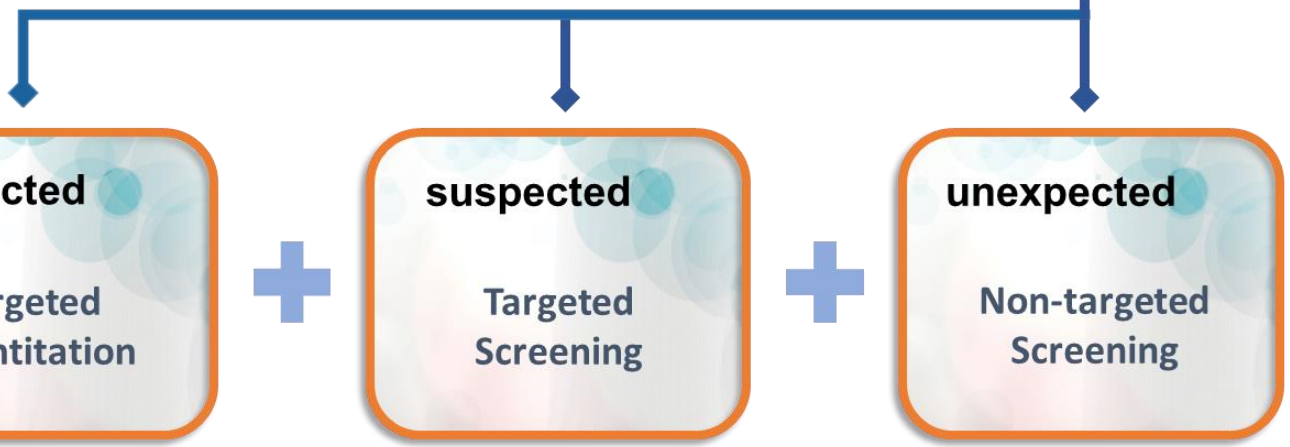
LC-MS/MS

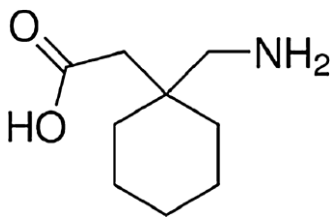
Processing

High-Resolution Mass Spectrometry (HRMS)
- ❖ Time-of-flight (TOF)
- ❖ Orbitrap

m/z = 4 decimal point

m/z Gabapentin[2] = 172.1332




Ref: 1. https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/abs/10.1002/bmc.826
2. https://academic.oup.com/jat/article/44/1/36/5520601?login=false

# Mass Analyzer : Orbitrap™ Technology

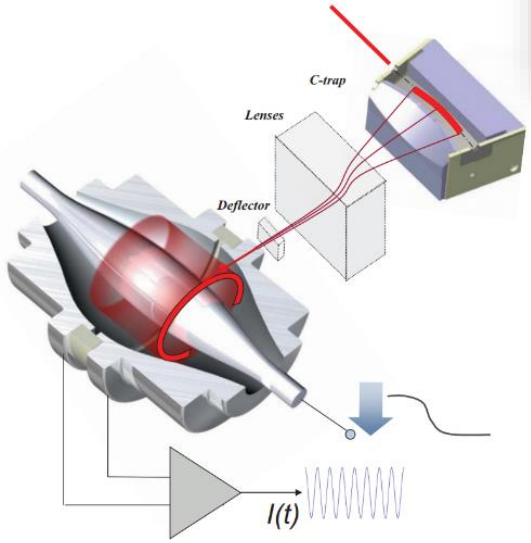
*Anal. Chem.* **2000,** *72,* 1156−1162

## Electrostatic Axially Harmonic Orbital Trapping: A High-Performance Technique of Mass Analysis

**Alexander Makarov***

*HD Technologies Ltd., Atlas House, Simonsway, Manchester, M22 5PP, U.K.*

This work describes a new type of mass analyzer which employs trapping in an electrostatic field. The potential distribution of the field can be represented as a combination of quadrupole and logarithmic potentials. In the absence of any magnetic or rf fields, ion stability is achieved only due to ions orbiting around an axial electrode. Orbiting ions also perform harmonic oscillations along the electrode with frequency proportional to $(m/z)^{-1/2}$. These oscillations are detected using image current detection and are transformed into mass spectra using fast FT, similarly to FT ICR. Practical aspects of the trap design are presented. High-mass resolution up to 150 000 for ions produced by laser ablation has been demonstrated, along with high-energy acceptance and wide mass range.



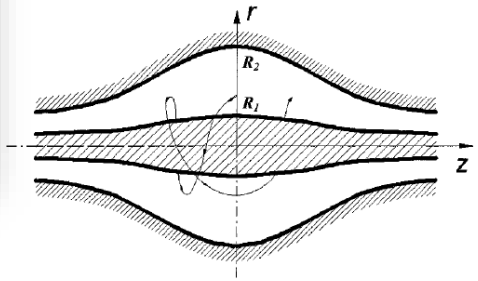
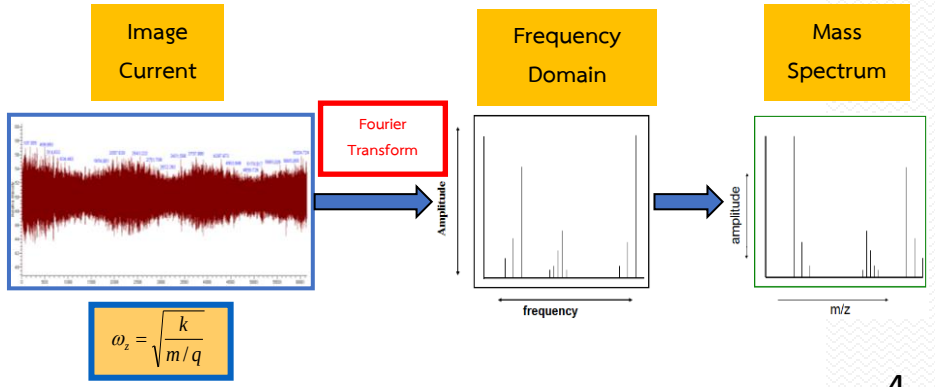
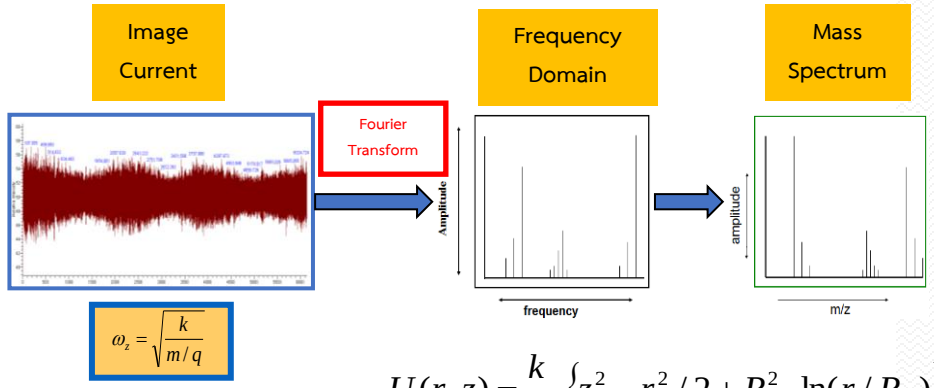




**Figure 1.** Equipotentials of the quadro-logarithmic field and an example of a stable ion trajectory

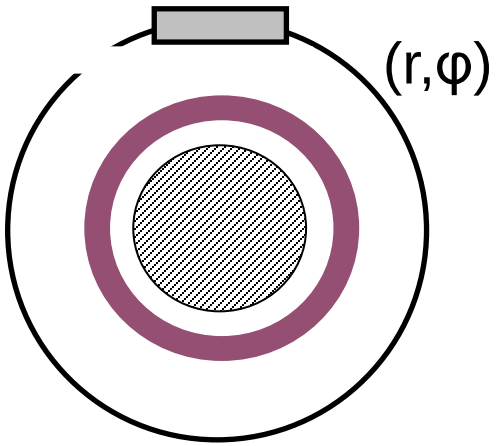


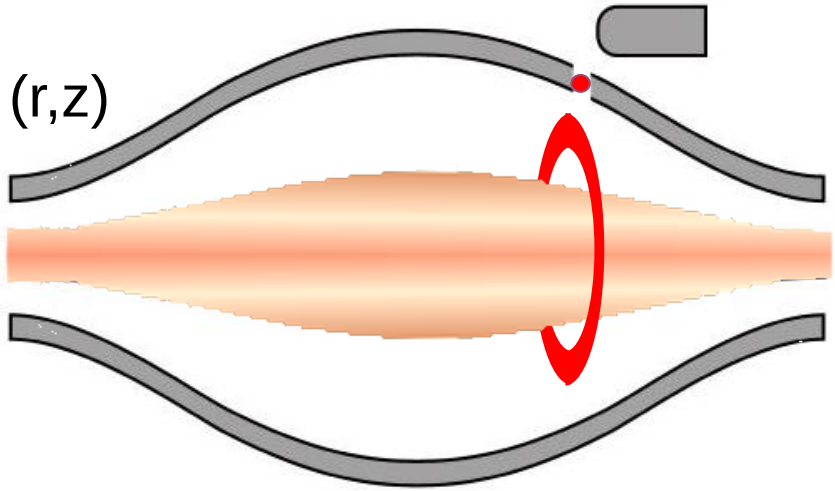
# Mass Analyzer : Orbitrap™ Technology

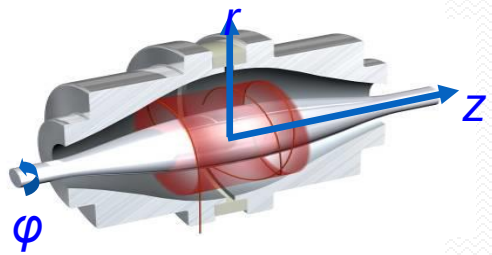
- An ion packet of a selected m/z enters the field.
- Increasing voltage squeezes ions.
- Voltage stabilises and ion trajectories are also stabilized.
- Angular spreading forms a **rotating ring**.
- Ions trapped in an **electrostatic field.**
- Central electrode kept on high voltage.
- Outer electrode is split and able to pick up an **image current** induced by ion packets moving inside the trap.




$$U(r,z) = \frac{k}{2} \cdot \left\{ z^2 - r^2/2 + R_m^2 \cdot \ln(r/R_m) \right\}$$



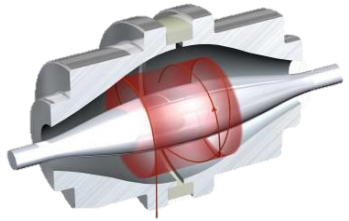

Characteristic frequencies:

- Frequency of rotation $\omega_\varphi$
- Frequency of radial oscillations $\omega_r$
- Frequency of axial oscillations $\omega_z$

A. Makarov, Anal. Chem 2000, 1156-1162

# Orbitrap Exploris Portfolio

**Orbitrap MS** | **Hybrid Orbitrap MS** | **Tribrid Orbitrap MS**



***Orbitrap***

+

***Quadrupole***

+

***Linear Ion Trap***


## *Orbitrap Exploris Series*

*Quadrupole-orbitrap Mass Spectrometer*

- Targeted/non-targeted screening (MS)
- Unknown Identification (MS)
- Confirmation (MS/MS)
- Absolute and Relative Quantitation (MS and/or $MS^2$)

# Orbitrap™ Technology HRAMS

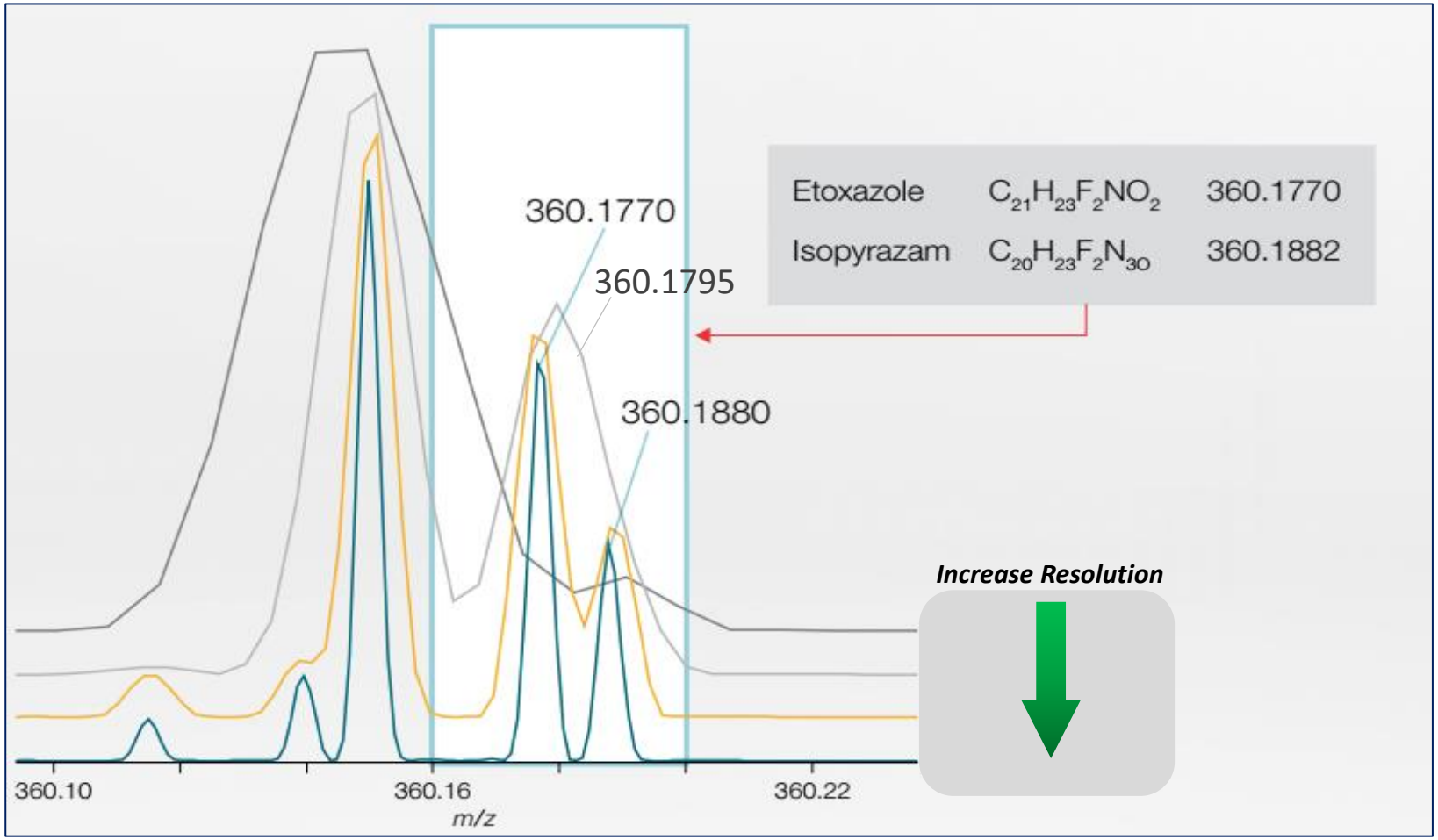
HRAMS = **H**igh **R**esolution **A**ccurate **M**ass **S**pectrometry

**Mass Resolution** = Ability of a mass spectrometer to distinguish

**Mass Accuracy** = The precision of which the mass is measured by the mass spectrometer.

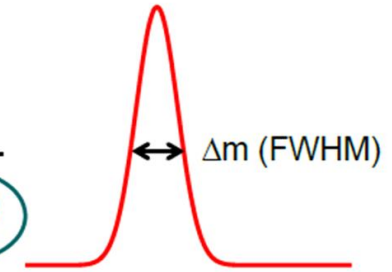
# Why use Orbitrap MS ?

## *High Resolution & High Accuracy*




FWHM = Full Width Half Maximum = Δm

$$R = \frac{m}{\Delta m}$$



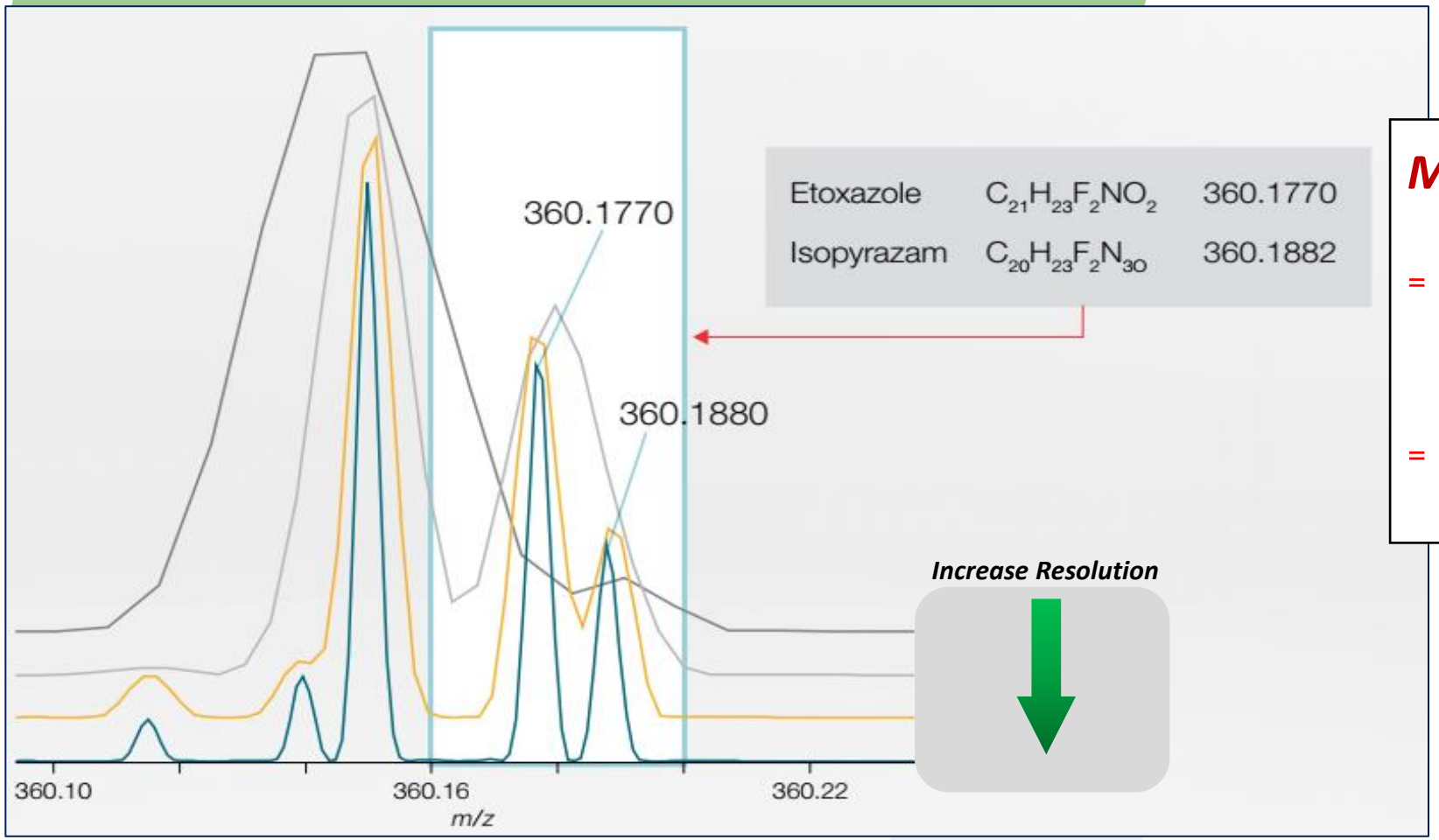

$$R = \frac{360.1795}{0.02000} = 18{,}000$$

$$R = \frac{360.1770}{0.0036} = 100{,}000$$

**Orbitrap Exploris**
Maximum resolution (FWHM): 480,000 at m/z 200

# Why use Orbitrap MS ?

## *High Resolution & High Accuracy*




***Mass accuracy or Mass error (ppm)***

$$= \frac{\text{Measured} - \text{Target monoisotopic mass} \times 10^6}{\text{Target monoisotopic mass}}$$

$$= \frac{360.1880 - 360.1882 \times 10^6}{360.1882} = 0.56 \text{ ppm}$$

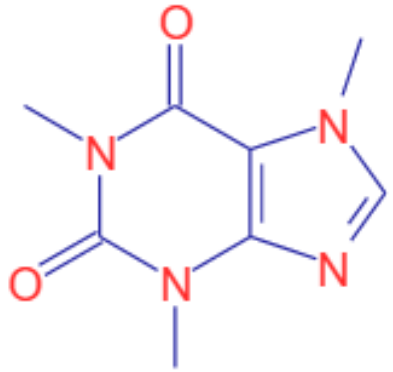
**Orbitrap Exploris**

Mass Accuracy:

<3 ppm for external calibration

<1 ppm for internal calibration

# Mass Resolution & Accuracy



## Caffeine

Molecular formula: $C_8H_{10}O_2N_4$

Precursor adduct: [M+H]+
Precursor formula: $C_8H_{11}O_2N_4$
Precursor m/z: 195.08765

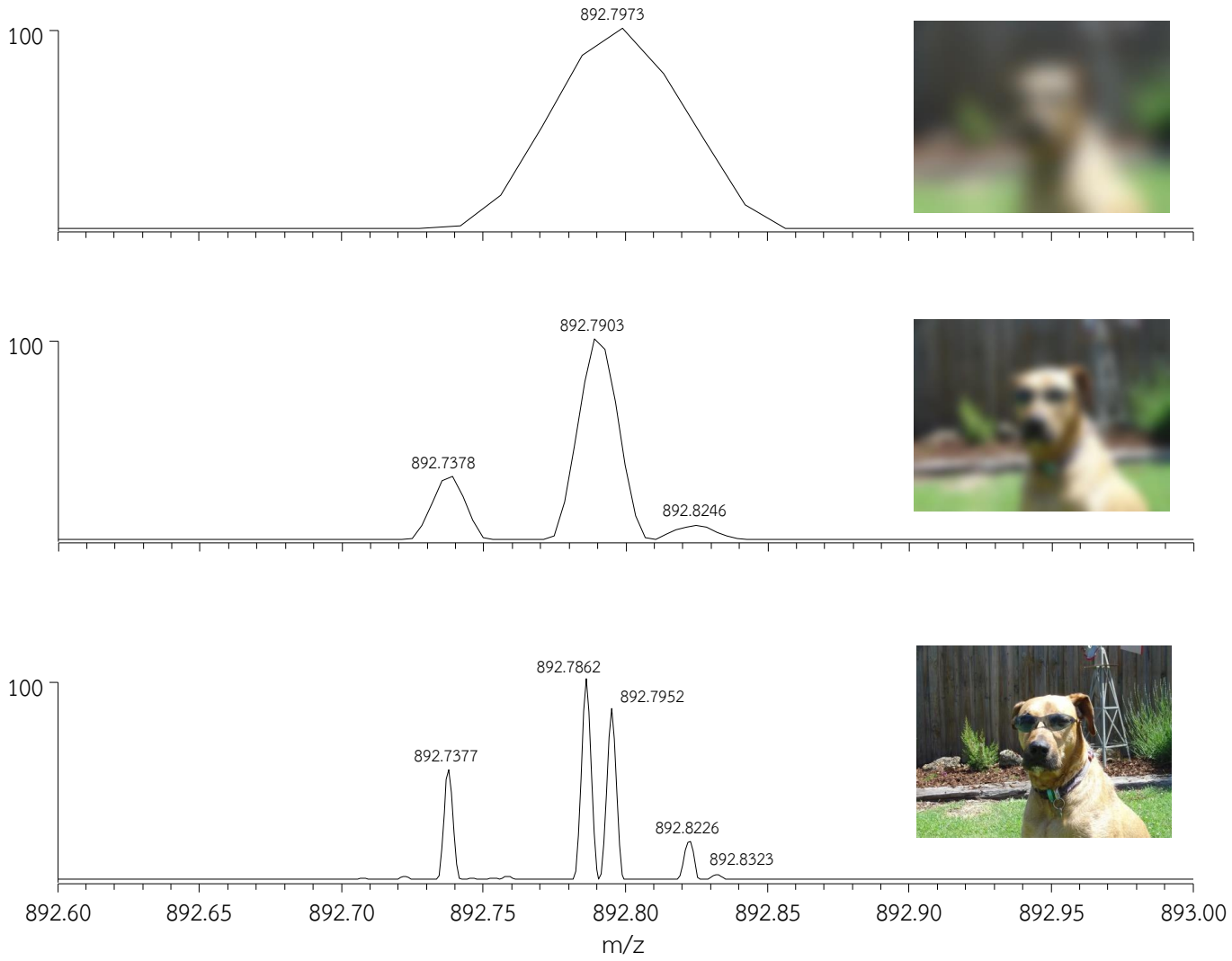
**Specificity = Resolution + Mass Accuracy**

**Main advantage:** the possibility to determine the elemental composition of individual molecular or fragment ions, a powerful tool for the structural elucidation or confirmation.

## Measured m/z = 195.08775

| Precursor Formular | Mass | Mass Accuracy (ppm) |
|---|---|---|
| $C_8H_{11}O_2N_4$ | 195.08765 | 0.502 |
| $C_8H_{19}O^{32}S_2$ | 195.08718 | 2.906 |
| $CH_{11}N_{10}{}^{32}S$ | 195.08834 | -3.012 |
| $C_3H_{12}N_8{}^{35}Cl$ | 195.0868 | 4.887 |
| $C_2H_{12}ON_8P$ | 195.08662 | 5.792 |
| $C_7H_{15}O_6$ | 195.08631 | 7.357 |
| $C_6H_{16}O_3N_2P$ | 195.08931 | -7.973 |
| $C_7H_{16}O_2N_2{}^{35}Cl$ | 195.08948 | -8.878 |
| $C_4H_7N_{10}$ | 195.08497 | 14.266 |
| $C_5H_{15}O_2N_4{}^{32}S$ | 195.09102 | -16.777 |
| $C_{13}H_{11}N_2$ | 195.09167 | -20.119 |
| $C_{11}H_{15}O^{32}S$ | 195.08381 | 20.184 |
| $C_3H_{11}O_4N_6$ | 195.08363 | 21.122 |
| $C_5H_{17}N_4P_2$ | 195.0923 | -23.303 |
| $C_5H_{16}N_4P^{32}S$ | 195.08278 | 25.474 |
| $C_{11}H_{16}OP$ | 195.09333 | -28.593 |
| $C_{12}H_{16}{}^{35}Cl$ | 195.0935 | -29.498 |

# Mass Resolution & Accuracy




- Removing interferences

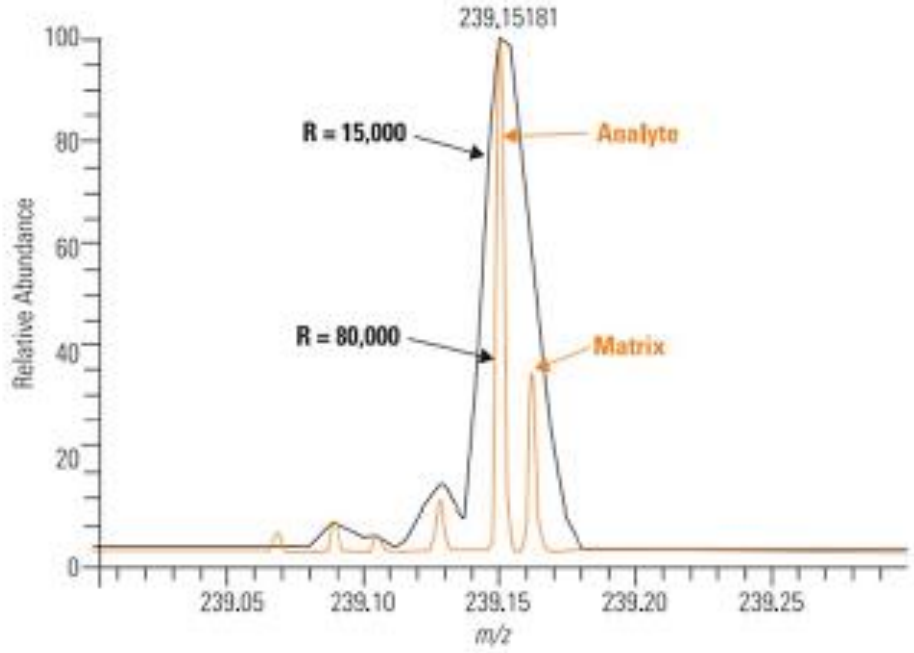
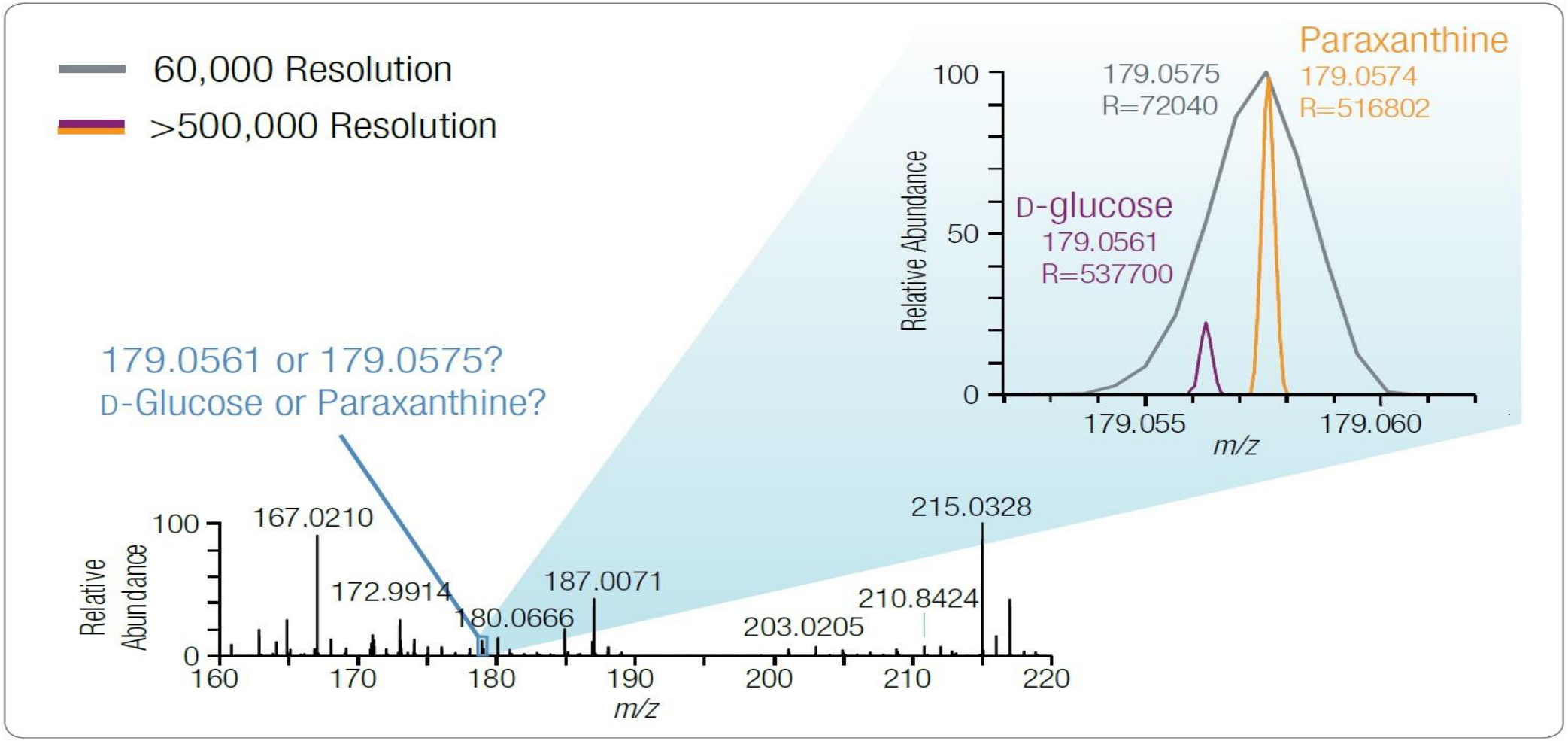



Figure 1: Analysis of the MH+ peak of Pirimicarb at 15,000 and 80,000 resolution.

High resolution is very important for samples with complex matrix (e.g. biological, food), since they will contain a significant number of background ions.

# Mass Resolution & Accuracy

- Isobaric compounds separation

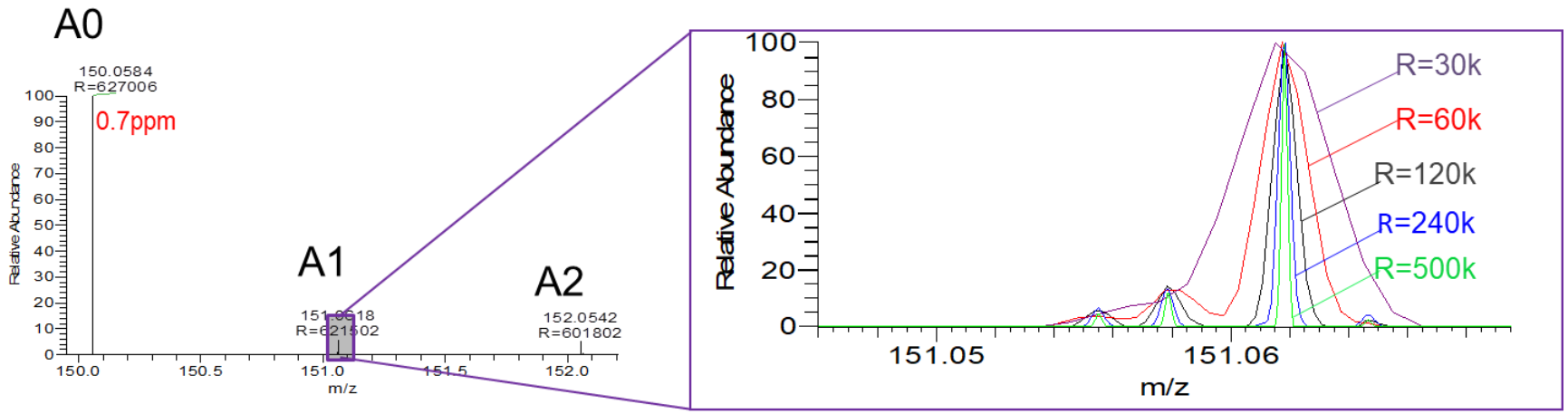


# Mass Resolution & Accuracy

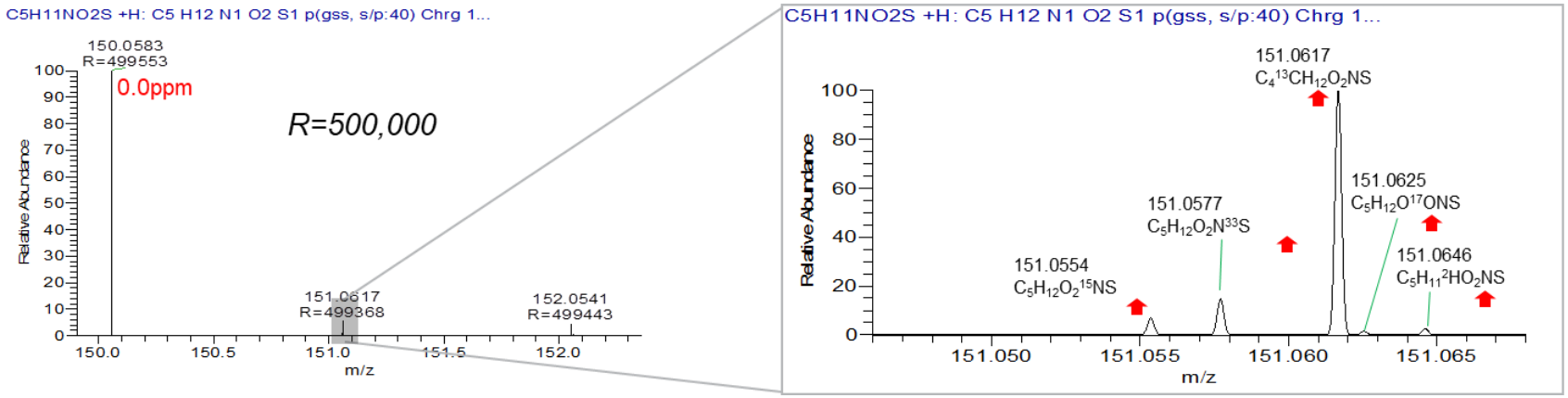
- Fine Isotopic Pattern

L-Methionine ($C_5H_{11}NO_2S$) $[M+H]^+$

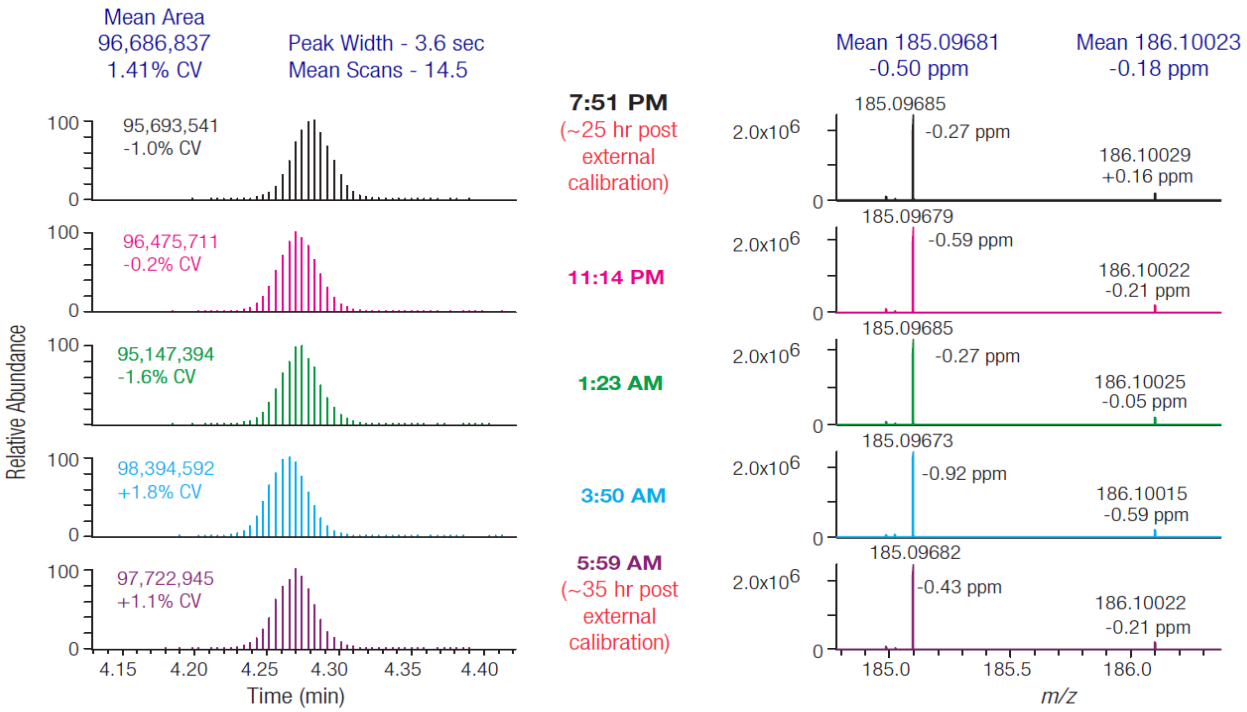
*Observed*




*Simulated*

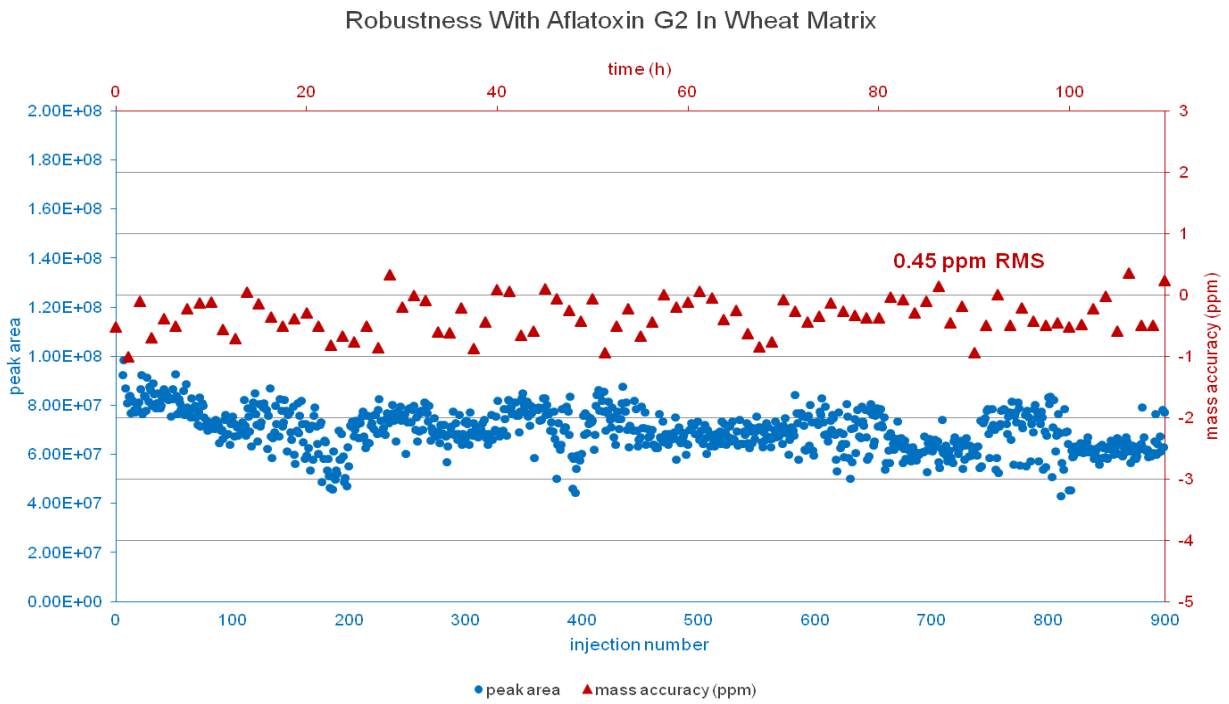


# Stability: Robust and Reproducible HRMS




Ultimate™ 3000 UHPLC coupled to Q Exactive™ : Full Scan MS:

R=70K, $d_5$-Hippuric acid, theoretical m/z 185.0969




Peak intensity (area) variation during 900 injections (blue) with according mass accuracy (peak apex scan, red)

# Orbitrap™ Technology


OptaMax NG
Electrospray
Ion Source

EASY-IC Internal
Calibration Source

Advanced Quadrupole
Technology (AQT)

Independent
Charge Detector

C-Trap

Ion-Routing Multipole

S-Lens

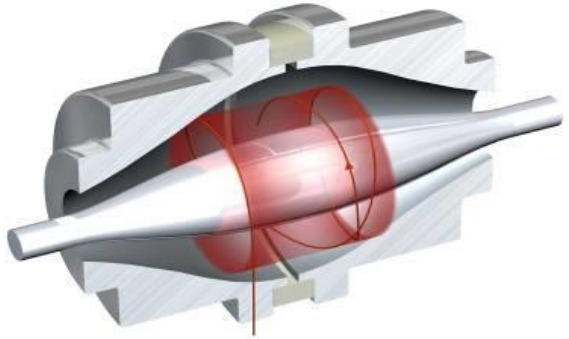
Electrodynamic Ion Funnel
(only in Oribitrap Exploris 480)

Advanced Active
Beam Guide (AABG)
with axial gradient

High Field Orbitrap
Mass Analyzer

# Orbitrap™ Technology

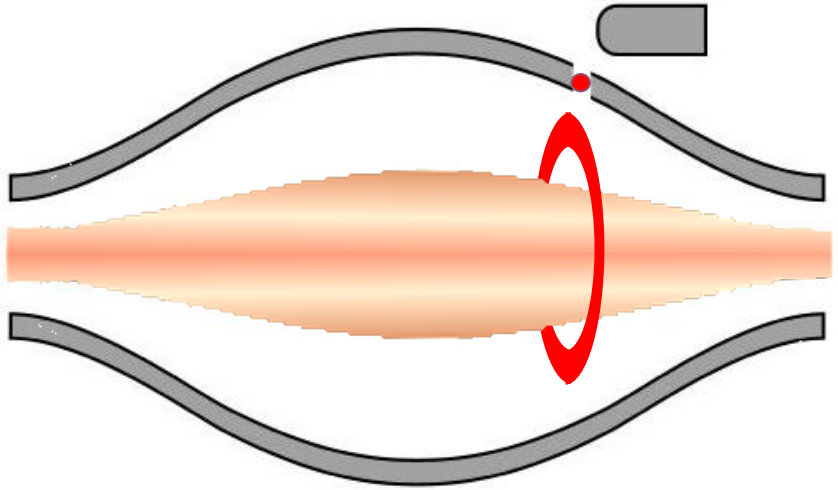
# Resolution vs Sensitivity

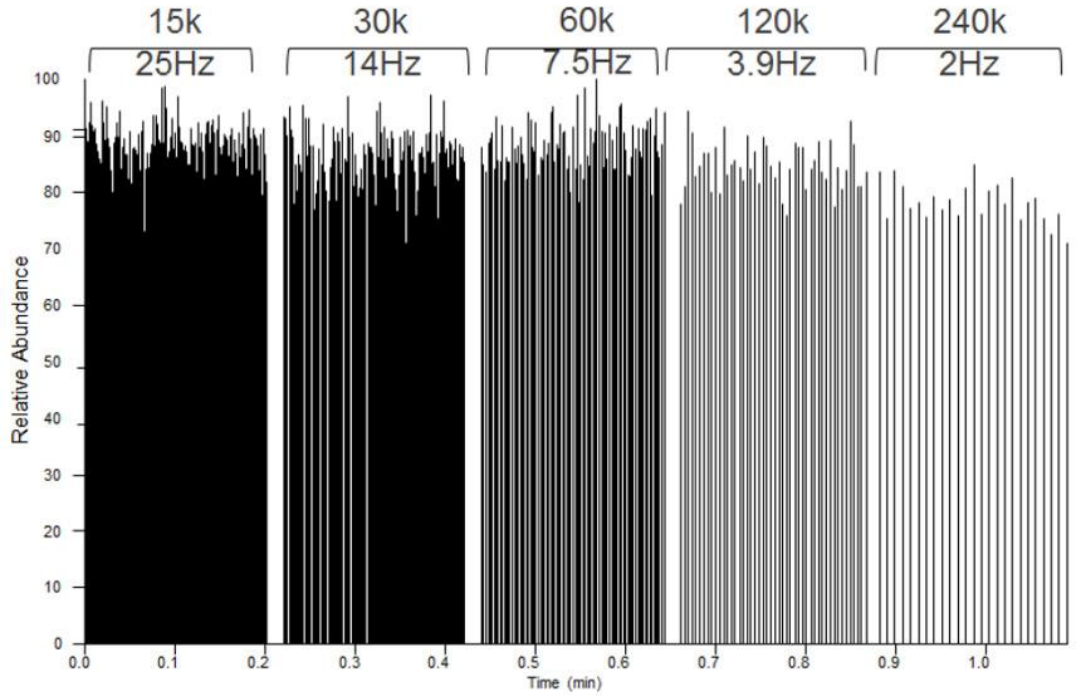


No trade-off between resolution and sensitivity

- No compromise...both resolution and sensitivity are retained
- Analysis of trace compounds with maximum resolution and mass accuracy

(r,z)






Signal intensity does not change with resolution with the Orbitrap detector

# Mass Accuracy

| | |
|---|---|
| Mass Accuracy* | External calibration achieves <3 ppm RMS drift over 24 hours;<br>Internal lock mass calibration achieves <1 ppm RMS drift over 24 hours;<br>EASY-IC achieves <1 ppm RMS drift for at least 5 days |

**Positive Mass Calibration**

## Calibration Report

thermoscientific

| | |
|---|---|
| **Date & Time** | Wednesday, January 26, 2022 09:36:10 AM |
| **Instrument Model** | Orbitrap Exploris 120 |
| **Instrument Serial** | MB10404C |
| **Software Version** | 3.1.279.9 |

| Name | Result | Comment |
|---|---|---|
| Mass Calibration Summary | Passed | |
| Spectral Mass Accuracy Calibration Run Function | Passed | |
| Fine Mass Calibration | Passed | External rms deviation = 0.18 ppm (without lock mass), acceptance limit = 3.00 ppm<br>Internal rms deviation = 0.07 ppm (with lock mass m/z 322.04812), acceptance limit = 1.00 ppm |

### Mass Calibration Summary

| Name | Result | Value | Range | Comment |
|---|---|---|---|---|
| [2022-01-26 09:36] Signal Stability Evaluation (pos) | Passed | – | – | Excellent signal stability: 2.0% RSD! |
| [2022-01-26 09:36] Fine Mass Calibration | Passed | – | – | External rms deviation = 0.18 ppm (without lock mass), acceptance limit = 3.00 ppm<br>Internal rms deviation = 0.07 ppm (with lock mass m/z 322.04812), acceptance limit = 1.00 ppm |
| [2022-01-26 09:36] Spectral Mass Accuracy Calibration Run Function | Passed | – | – | |
| [2022-01-26 09:36] Spectral Mass Accuracy Calibration | Passed | – | – | |
| Type | | – | – | Calibration |

# Mass Accuracy

| | |
|---|---|
| Mass Accuracy* | External calibration achieves <3 ppm RMS drift over 24 hours;<br>Internal lock mass calibration achieves <1 ppm RMS drift over 24 hours;<br>EASY-IC achieves <1 ppm RMS drift for at least 5 days |

**Positive Mass Calibration (7 Days later)**

## Calibration Report

thermoscientific

| | |
|---|---|
| **Date & Time** | Wednesday, February 2, 2022 09:56:53 AM |
| **Instrument Model** | Orbitrap Exploris 120 |
| **Instrument Serial** | MB10404C |
| **Software Version** | 3.1.279.9 |

| Name | Result | Comment |
|---|---|---|
| Mass Check Summary | Passed | |

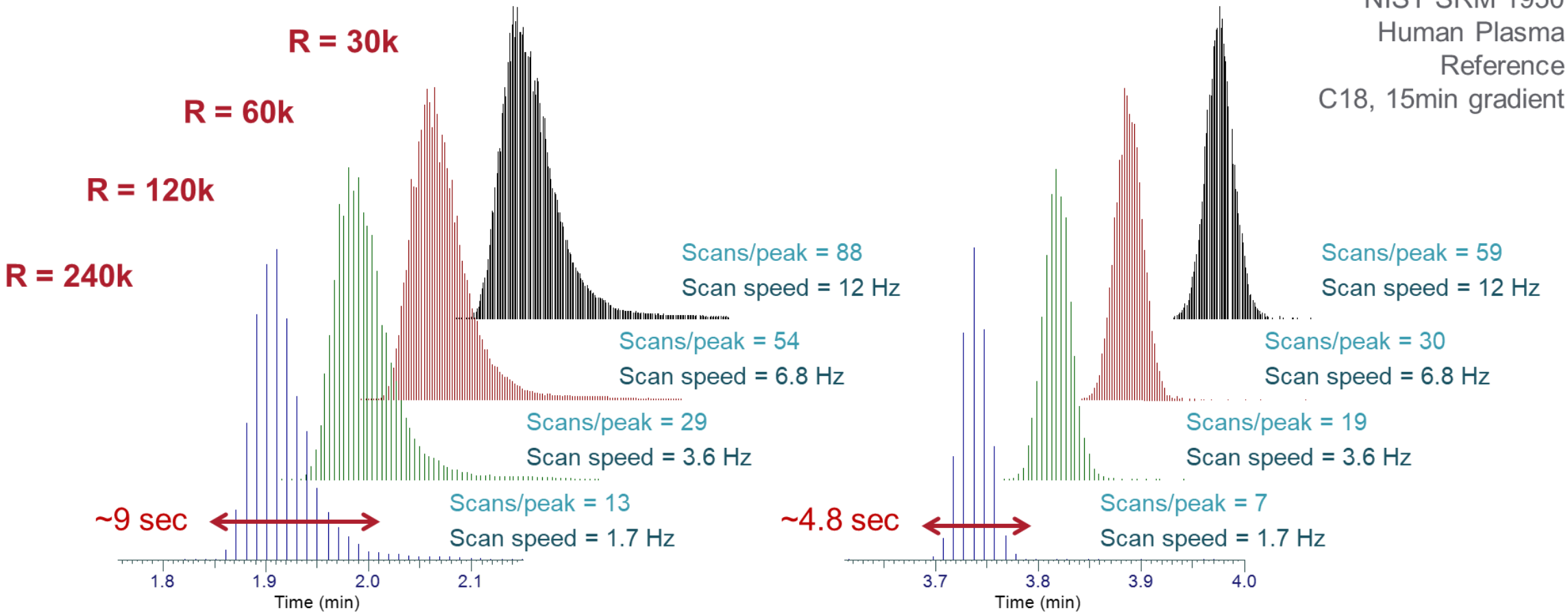
### Mass Check Summary

| Name | Result | Value | Range | Comment |
|---|---|---|---|---|
| [2022-02-02 09:56] Signal Stability Evaluation (pos) | Passed | – | – | Good signal stability: 4.9% RSD! |
| [2022-02-02 09:57] eFT Phase Check | Passed | – | – | Delta t0=0.01 +/- 0.01 |
| [2022-02-02 09:57] Spectral Mass Accuracy Check | Passed | – | – | External Calibration Error rms = 1.3 ppm (Acceptance limit = 3.0 ppm). Internal Calibration Error rms = 0.2 ppm (Acceptance limit = 1.0 ppm). |

Ext. calibration 0.8 ppm -> 1.3 ppm
Int. calibration 0.07 ppm -> 0.2 ppm

# Resolution vs Scan Speed

NIST SRM 1950
Human Plasma
Reference
C18, 15min gradient

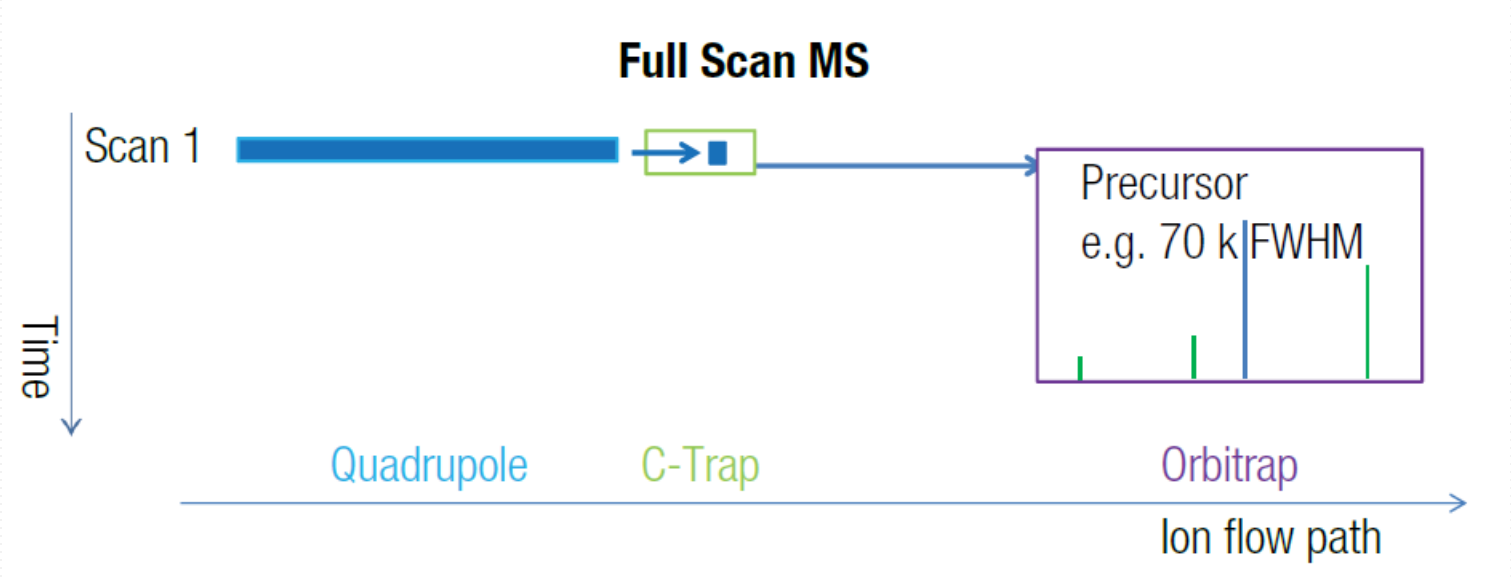


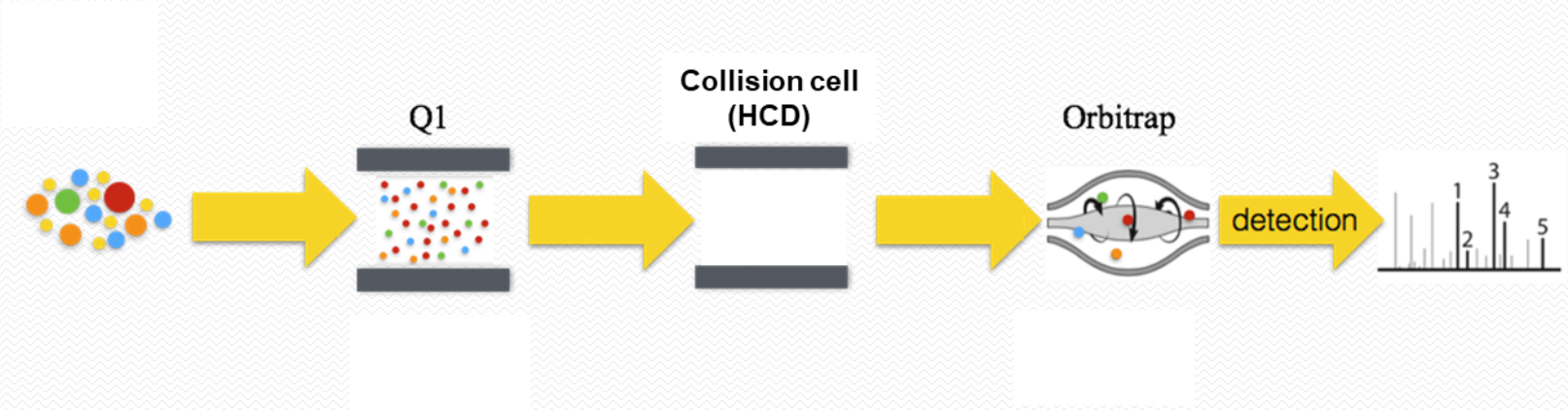

Acetylcarnitine
$C_9H_{17}NO_4$, *m/z* 204.1230

5-HIAA
$C_{10}H_9NO_3$, *m/z* 192.0655

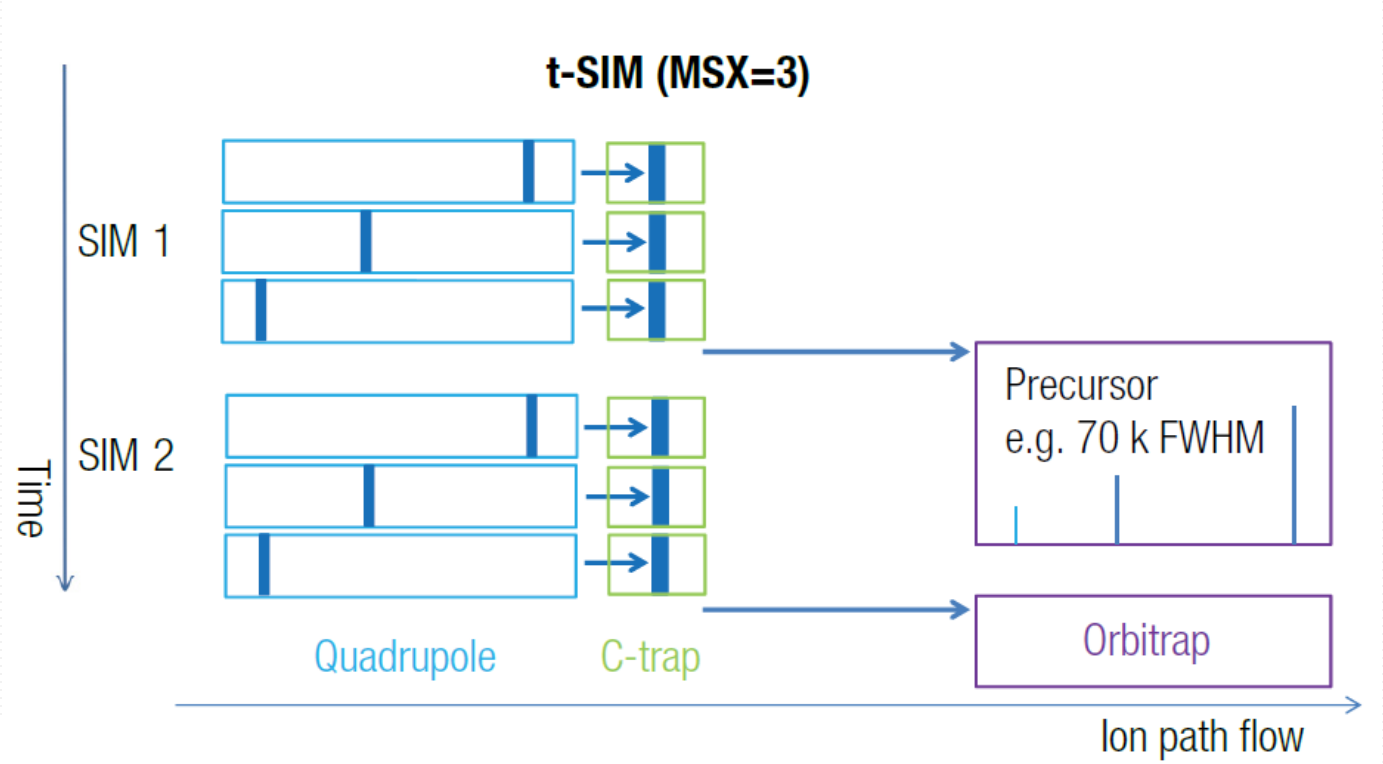
# Orbitrap™ :
# Modes of Acquisition

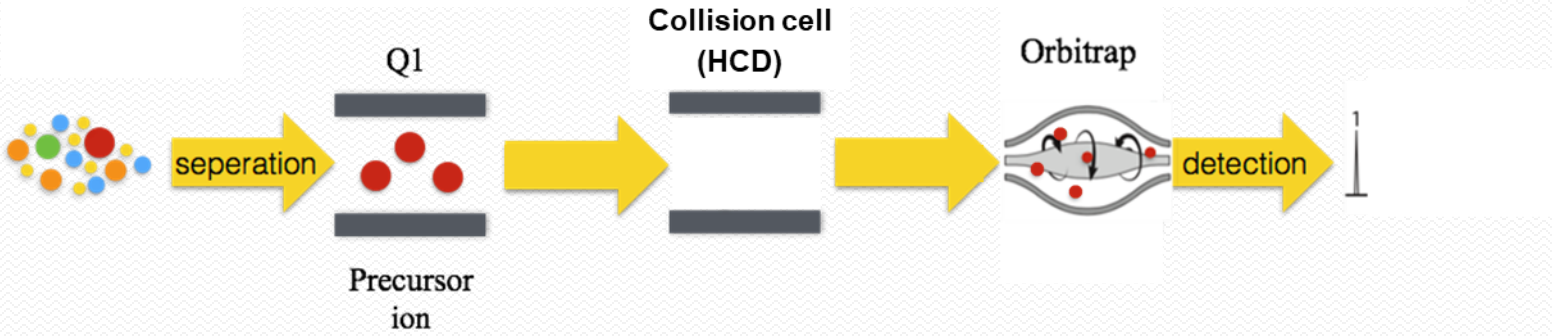
# Full Scan


Full Scan MS
Scan 1
Precursor
e.g. 70 k FWHM
Time
Quadrupole
C-Trap
Orbitrap
Ion flow path

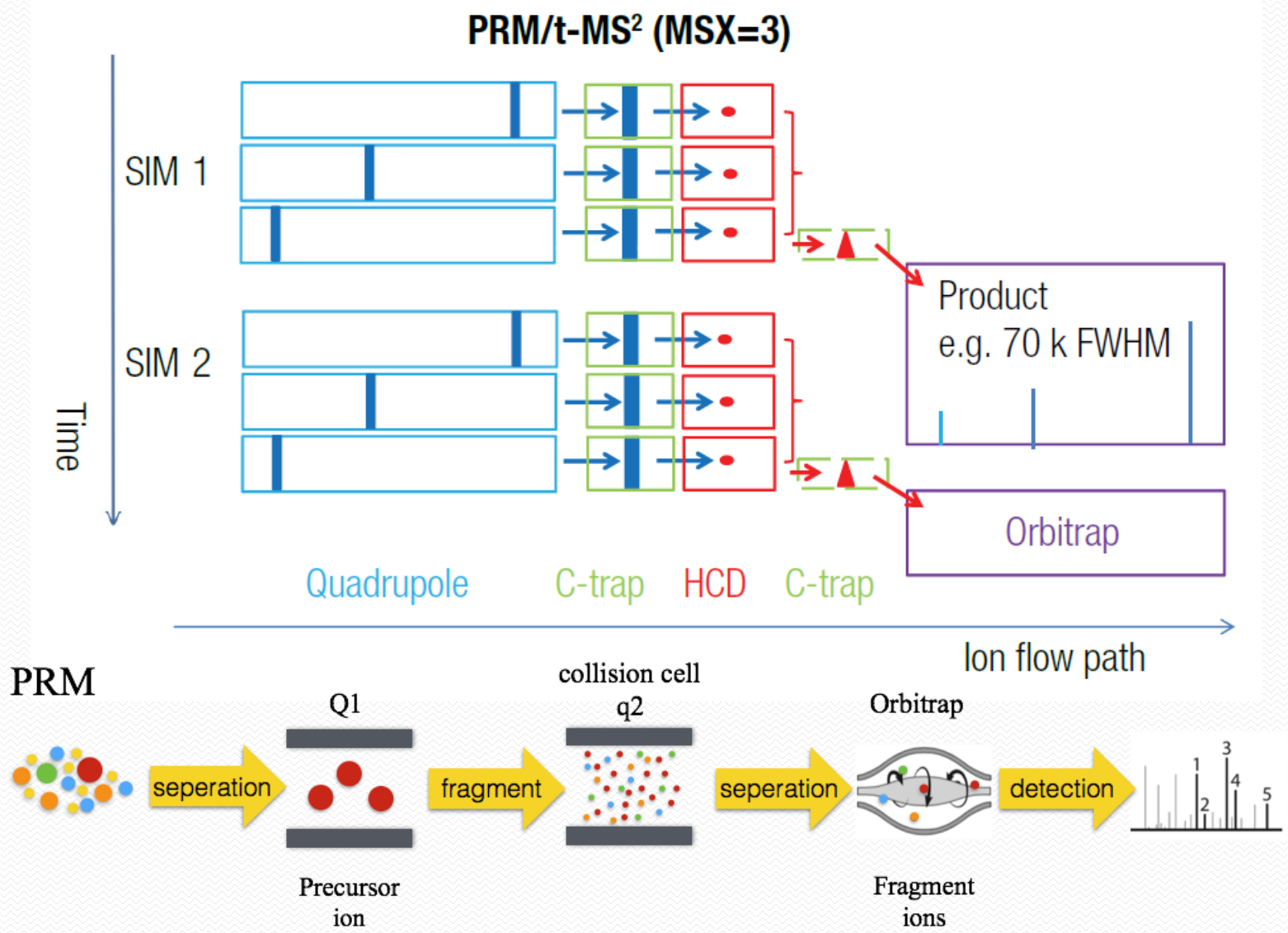

Q1
Collision cell
(HCD)
Orbitrap
detection
1
2
3
4
5

# SIM: Selected Ion Monitoring



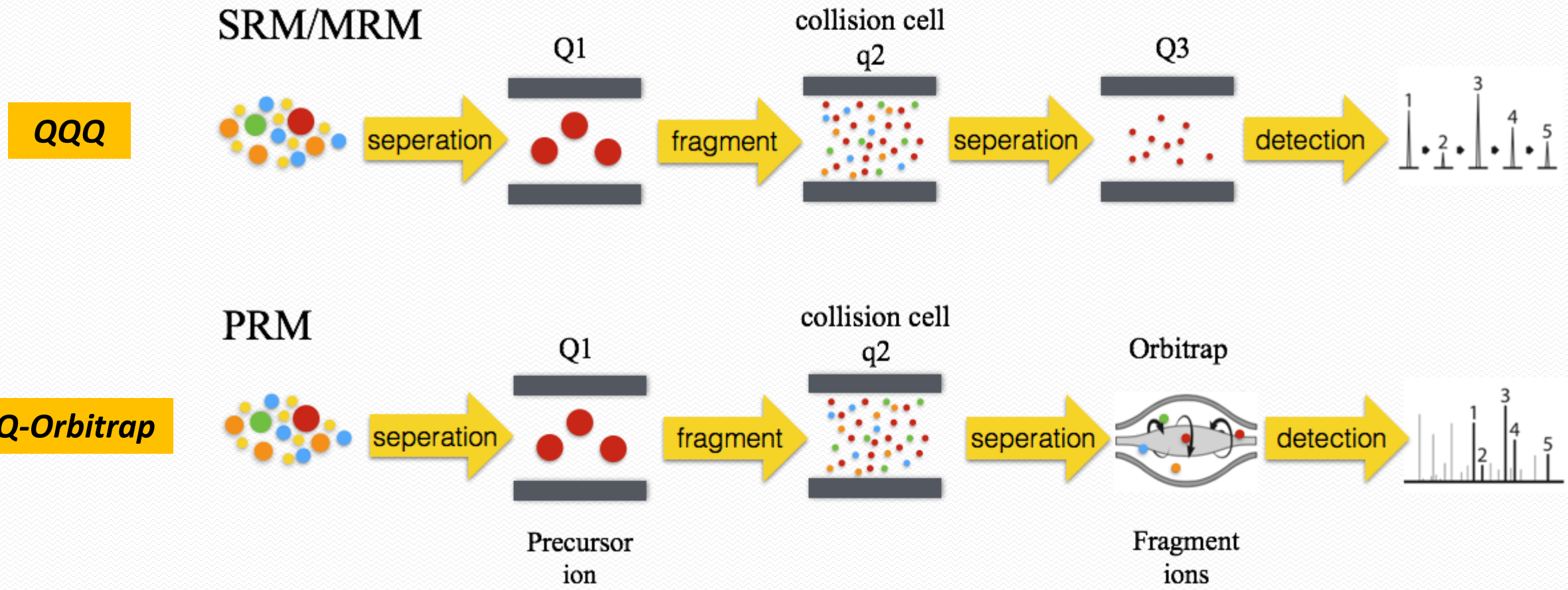
# PRM: Parallel Reaction Monitoring (Product ion scan)



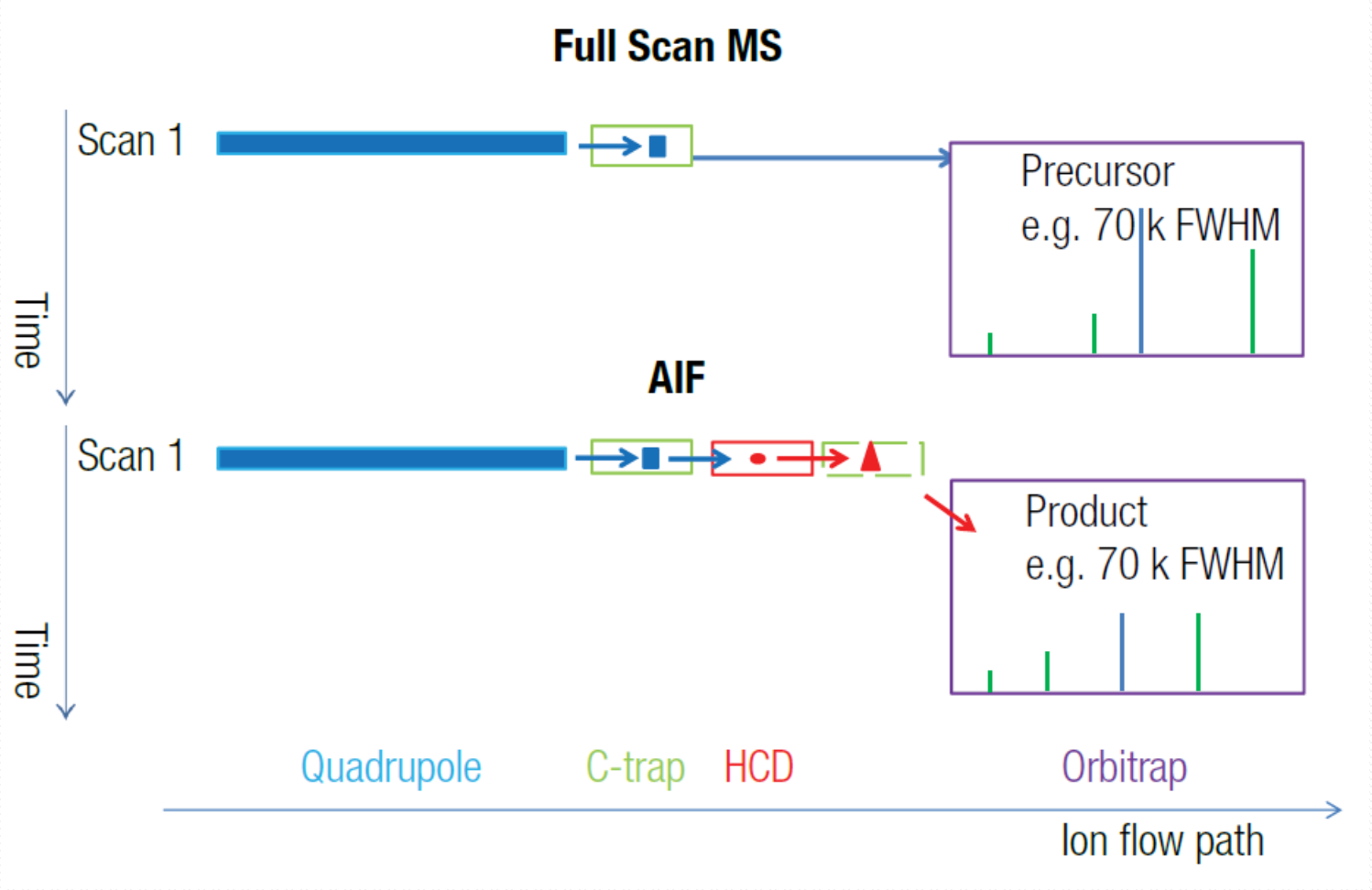

HCD: higher energy collisional dissociation
HCD collision gas: ultra-high-purity nitrogen

Retrospective analysis

# PRM: Parallel Reaction Monitoring

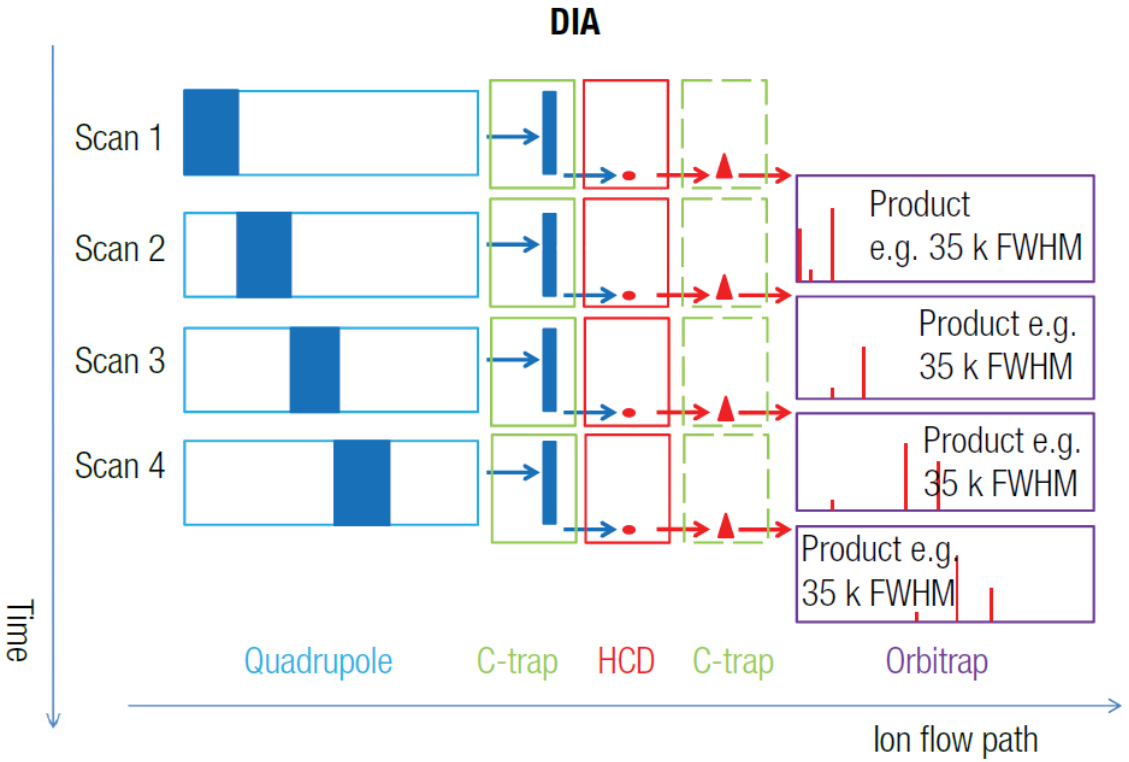


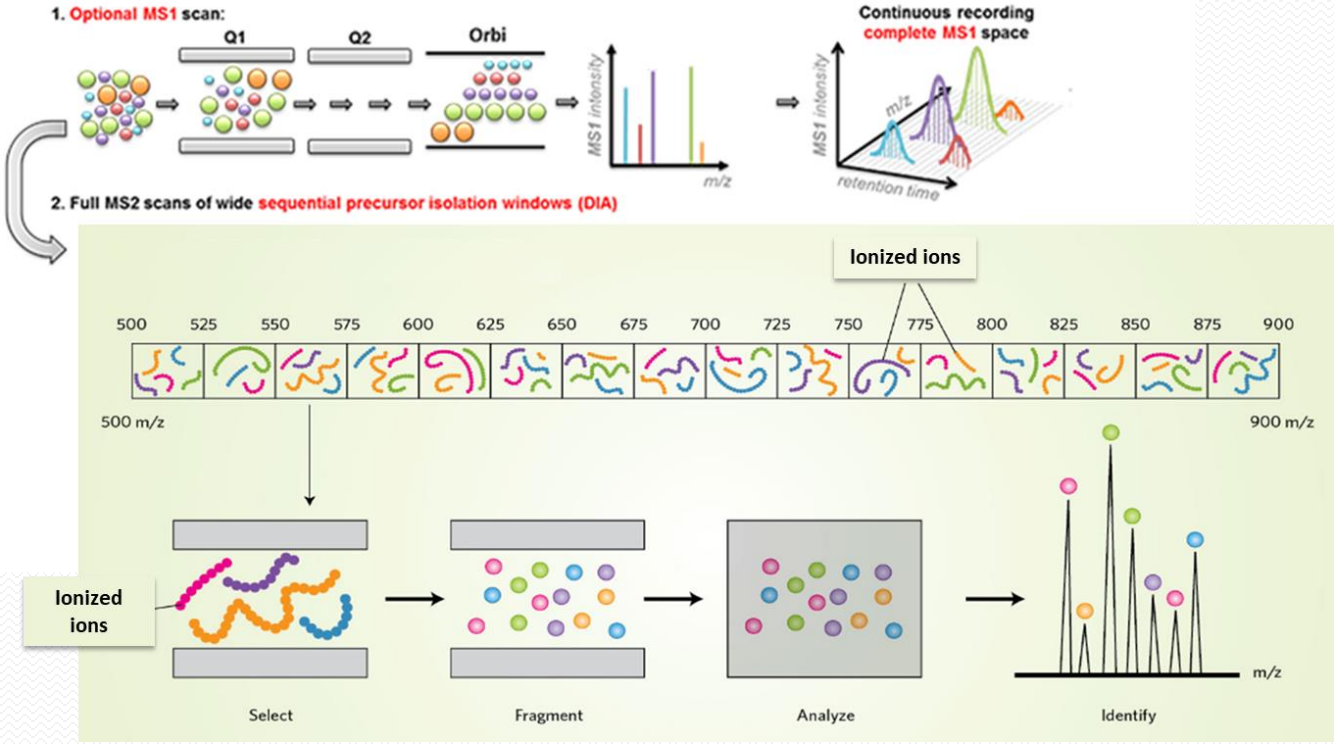
# AIF: All Ion Fragmentation

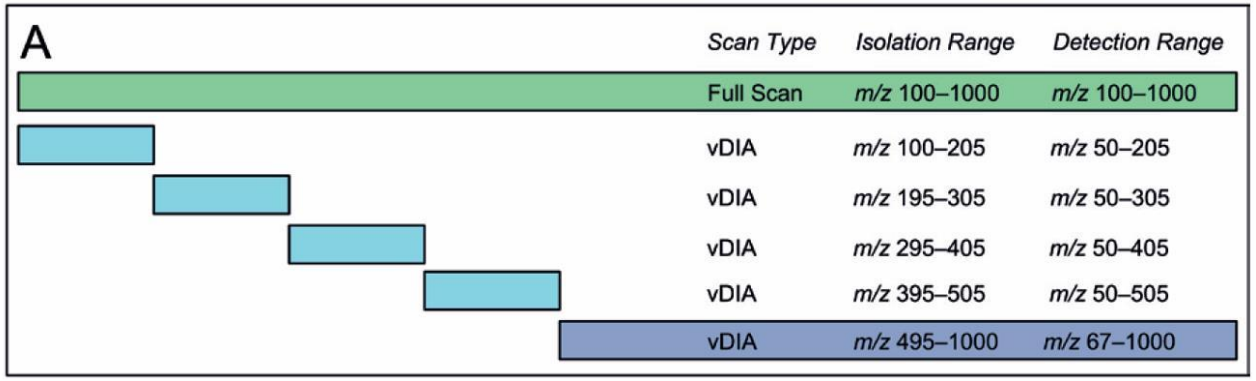



HCD: higher energy collisional dissociation
HCD collision gas: ultra-high-purity nitrogen

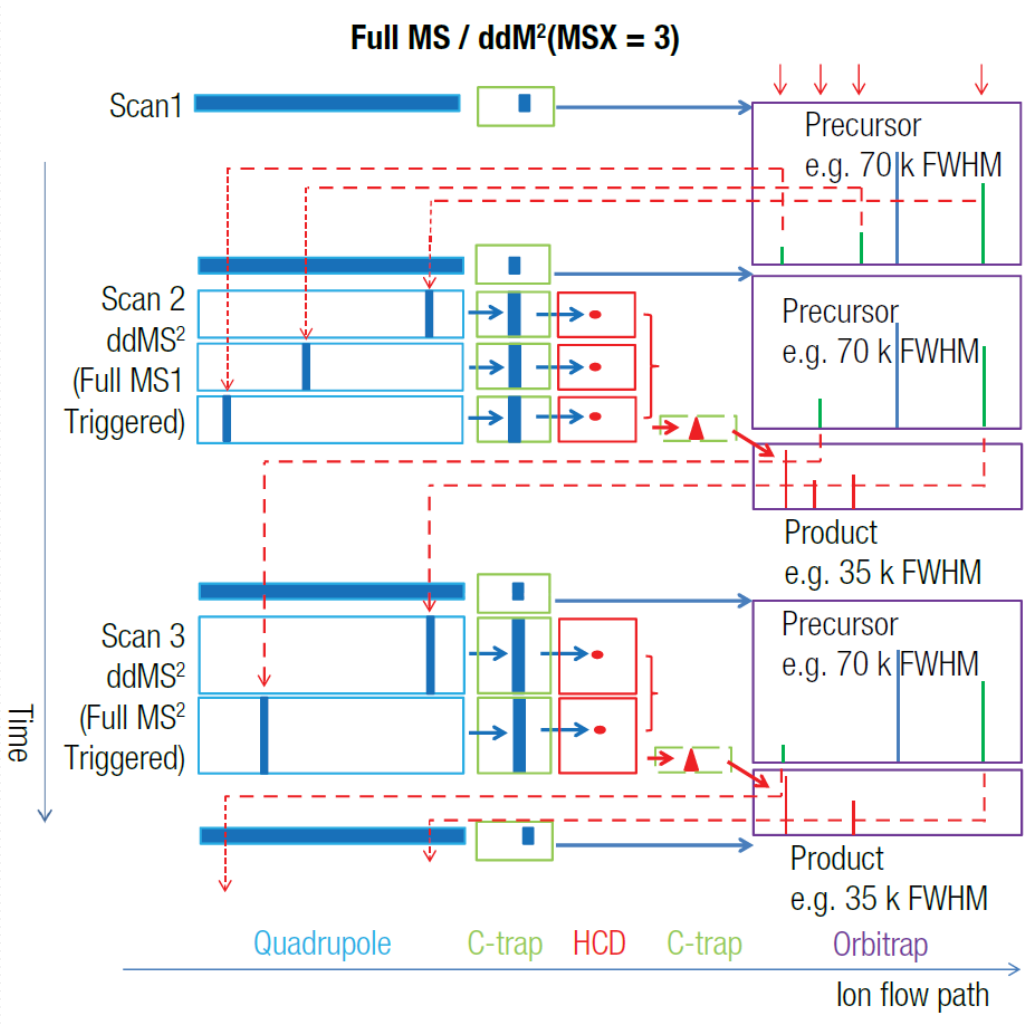
# (v)DIA: (variable) Data Independent Acquisition

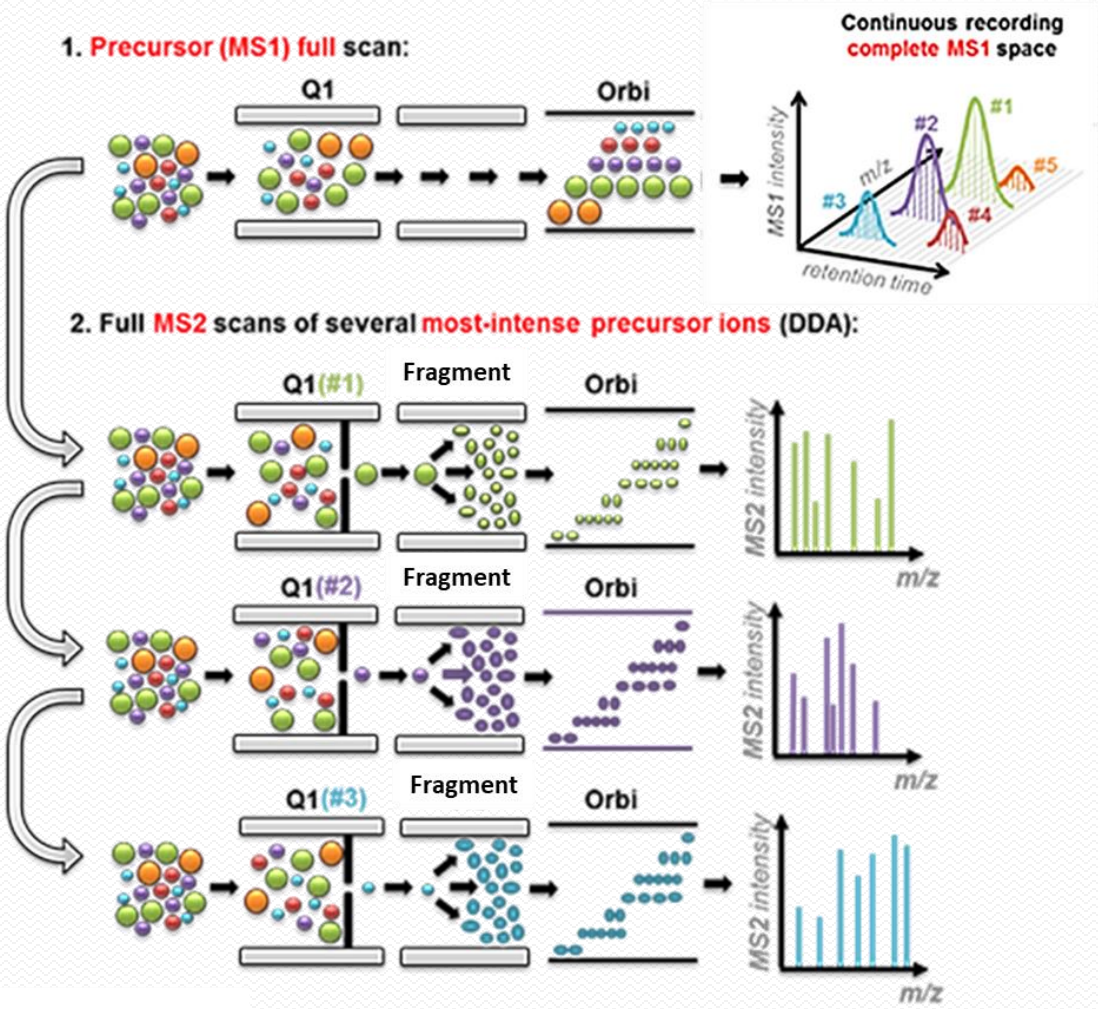


# DDA: Data Dependent Acquisition (ddMS$^2$)



# Orbitrap Applications Universe

Vitamin D in plasma

Antibiotics in water

Omics Research

Pharmaceuticals Clinical Panels

Drugs in Dried Blood Spots

Peptides Spiked into Plasma for Quantitation

Plant Sciences

Microbiomes

Pesticides in Foods

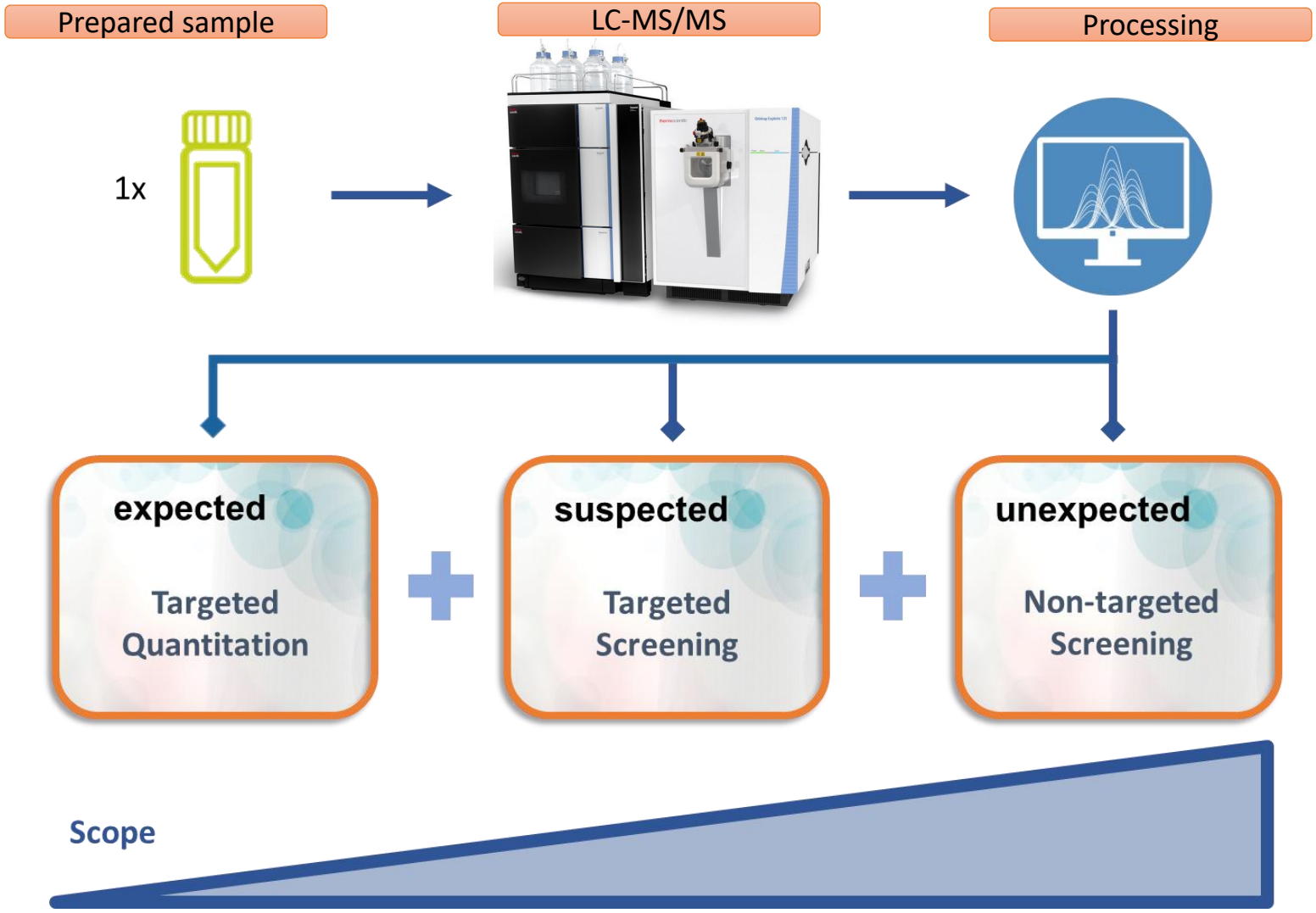
Illicit Drug Screening And Doping

Unknown Screening For Banned Pesticides

Environmental Testing

Forensic Toxicology

# System Main Workflows




High-Resolution Mass Spectrometry (HRMS)

- Orbitrap

m/z = 4 decimal point

# Targeted Quantitation

## Fast LC-HRAM-MS quantification of 36 antidepressants in human plasma using the Orbitrap Exploris 120 mass spectrometer for clinical research

### Samples preparation

Samples of 50 µL plasma
⇩
protein precipitated using 100 µL acetonitrile containing the internal standards
⇩
vortex-mixed and kept at room temperature for 5 min.
⇩
vortex-mixed again
⇩
centrifuged
⇩
The supernatant was transferred to a clean vial
⇩
2 µL were injected onto the LC-MS system.

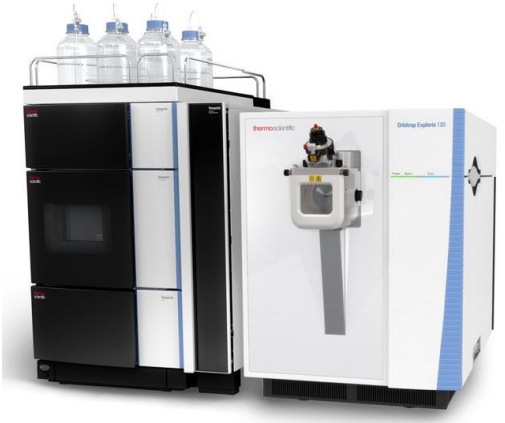
### LC-HRMS Condition

**Liquid chromatograph**

| | |
|---|---|
| Model: | Vanquish Flex Binary UHPLC |
| Mobile phase A: | 5 mM ammonium formate + 0.1% formic acid in water |
| Mobile phase B: | 5 mM ammonium formate + 0.1% formic acid in methanol |
| Column: | Thermo Scientific™ Hypersil GOLD™ Phenyl 2.1 × 100 mm (1.9 µm) |
| Column temperature: | 40 °C |
| Flow rate: | 0.5 mL/min. |
| Run time: | 6.5 min |

**Mass Spectrometer**

| | |
|---|---|
| Model: | Orbitrap Exploris 120 |
| Acquisition mode: | Full Scan |
| Resolution: | 60,000 |
| Scan range: | 100–500 m/z |
| Ionization mode: | HESI, positive |



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# Targeted Quantitation

| Compound name | Formula | Exact mass $[M+H]^+$ (*m/z*) | Internal standard name | Exact mass $[M+H]^+$ (*m/z*) |
|---|---|---|---|---|
| Agomelatine | $C_{15}H_{17}NO_2$ | 244.1332 | Agomelatine-$d_3$ | 247.1520 |
| Atomoxetine | $C_{17}H_{21}NO$ | 256.1696 | Atomoxetine-$d_3$ | 259.1884 |
| Bupropion | $C_{13}H_{18}ClNO$ | 240.1150 | Bupropion-$d_9$ | 249.1715 |
| Citalopram | $C_{20}H_{21}FN_2O$ | 325.1711 | Citalopram-$d_6$ | 331.2087 |
| Clomethiazole | $C_6H_8ClNS$ | 162.0139 | Threo-Dihydrobupropion-$d_9$ | 251.1871 |
| Desmethylcitalopram | $C_{19}H_{19}FN_2O$ | 311.1554 | Desmethylcitalopram-$d_3$ | 314.1743 |
| Desmethylfluoxetine | $C_{16}H_{16}F_3NO$ | 296.1257 | Desmethylfluoxetine-$d_5$ | 301.1571 |
| Desmethylmiansarine | $C_{17}H_{18}N_2$ | 251.1543 | Reboxetine-$d_5$ | 319.2065 |
| Desmethylmirtazapine | $C_{16}H_{17}N_3$ | 252.1495 | Mirtazapine-$d_3$ | 269.1840 |
| Desmethylsertraline | $C_{16}H_{15}Cl_2N$ | 292.0654 | Desmethylsertraline-$d_4$ | 296.0905 |
| Dosulepin | $C_{19}H_{21}NS$ | 296.1468 | Dosulepin-$d_3$ | 299.1656 |
| Duloxetine | $C_{18}H_{19}NOS$ | 298.1260 | Duloxetine-$d_7$ | 305.1700 |
| Ery-dihydro-bupropion | $C_{13}H_{20}ClNO$ | 242.1306 | Threo-Dihydrobupropion-$d_9$ | 251.1871 |
| Fluoxetine | $C_{17}H_{18}F_3NO$ | 310.1413 | Fluoxetine-$d_5$ | 315.1727 |
| Fluvoxamine | $C_{15}H_{21}F_3N_2O_2$ | 319.1628 | Fluvoxamine-$d_3$ | 322.1816 |
| Guanfacine | $C_9H_9Cl_2N_3O$ | 246.0195 | Tramadol-$d_6$ | 270.2335 |
| Hydroxybupropion | $C_{13}H_{18}ClNO_2$ | 256.1099 | Hydroxybupropion-$d_6$ | 262.1475 |
| Methylphenidate | $C_{14}H_{19}NO_2$ | 234.1489 | Methylphenidate-$d_9$ | 243.2054 |
| Mianserin | $C_{18}H_{20}N_2$ | 265.1699 | Mianserin-$d_3$ | 268.1888 |
| Milnacipran | $C_{15}H_{22}N_2O$ | 247.1805 | Milnacipran-$d_{10}$ | 257.2433 |
| Mirtazapine | $C_{17}H_{19}N_3$ | 266.1652 | Mirtazapine-$d_3$ | 269.1840 |
| Moclobemide | $C_{13}H_{17}ClN_2O_2$ | 269.1051 | Moclobemide-$d_8$ | 277.1554 |

Examples of targeted compounds, internal standards, chemical formulas, and exact masses of the protonated ion [M+H]+

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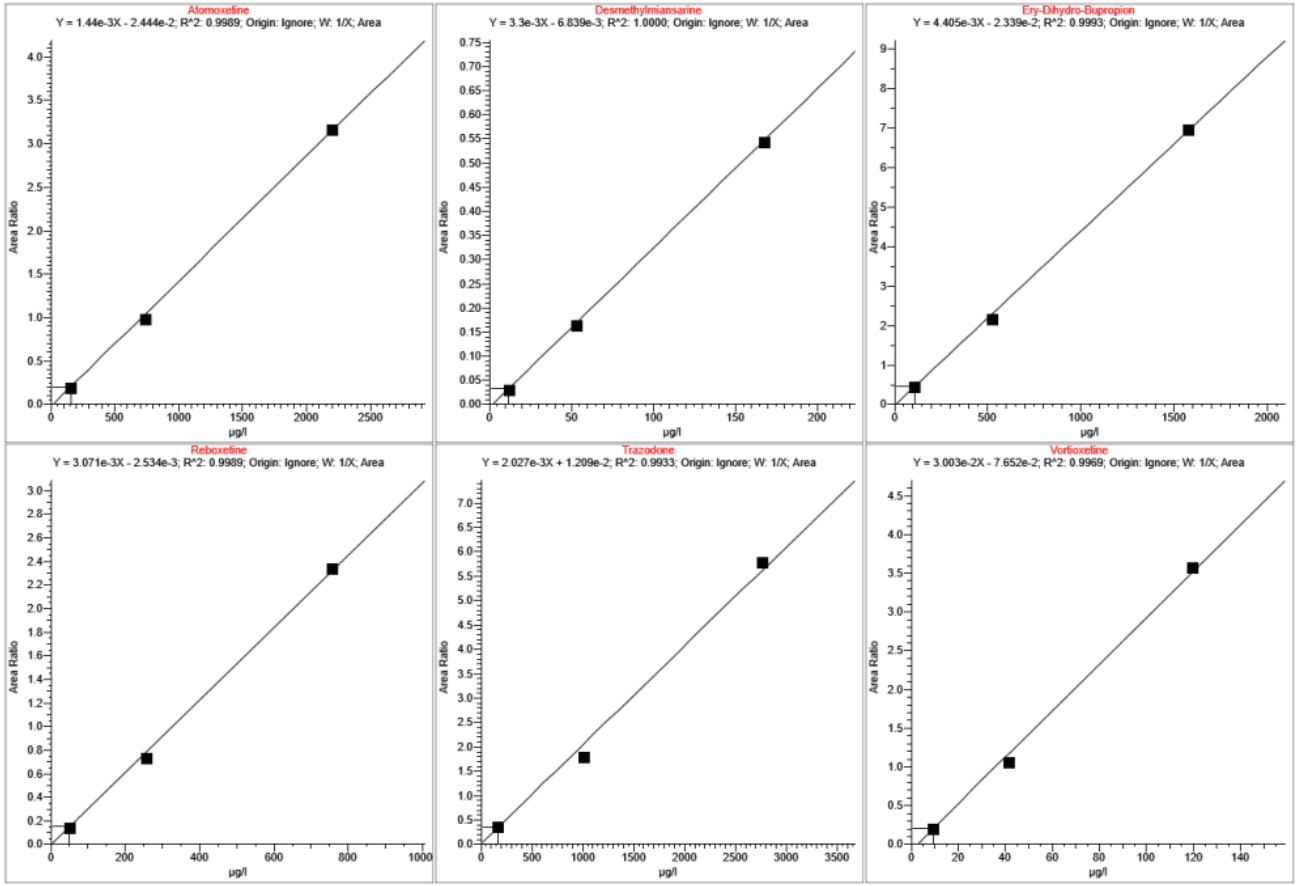
# Targeted Quantitation

| Compound name | Retention time (min) | Concentration (µg/L) | | |
|---|---|---|---|---|
| | | L1 | L2 | L3 |
| Agomelatine | 3.76 | 5.09 | 122 | 727 |
| Atomoxetine | 3.46 | 151 | 738 | 2190 |
| Bupropion | 2.36 | 11.6 | 54.1 | 157 |
| Citalopram | 3.28 | 16.5 | 86.4 | 259 |
| Clomethiazole | 2.75 | 146 | 2011 | 6773 |
| Desmethylcitalopram | 3.29 | 18.5 | 94.9 | 279 |
| Desmethylfluoxetine | 3.84 | 42.7 | 214 | 656 |
| Desmethylmiansarine | 3.19 | 11.8 | 52.7 | 167 |
| Desmethylmirtazapine | 2.26 | 13.2 | 65.0 | 197 |
| Desmethylsertraline | 4.09 | 12.4 | 62.5 | 191 |
| Dosulepin | 3.72 | 16.8 | 83.3 | 244 |
| Duloxetine | 3.79 | 18.5 | 92.5 | 284 |
| Ery-dihydro-bupropion | 2.54 | 105 | 520 | 1568 |
| Fluoxetine | 3.84 | 37.8 | 196 | 595 |
| Fluvoxamine | 3.73 | 34.9 | 185 | 558 |
| Guanfacine | 2.21 | 0.911 | 4.90 | 15.0 |
| Hydroxybupropion | 2.28 | 145 | 712 | 2045 |
| Methylphenidate | 2.17 | 3.80 | 18.4 | 51.8 |
| Mianserin | 3.18 | 10.3 | 55.0 | 168 |
| Milnacipran | 2.41 | 29.2 | 148 | 435 |
| Mirtazapine | 2.29 | 12.0 | 62.0 | 184 |
| Moclobemide | 2.00 | 156 | 841 | 2250 |
| Nefazodone | 4.14 | 34.9 | 176 | 491 |
| O-Desmethyltramadol | 1.78 | 83.8 | 422 | 1186 |
| O-Desmethylvenlafaxine | 1.98 | 37.4 | 189 | 554 |
| Opipramol | 3.73 | 41.5 | 205 | 611 |
| Paroxetine | 3.83 | 19.0 | 96.1 | 299 |
| Reboxetine | 3.42 | 48.0 | 254 | 753 |
| Ritalinic acid | 1.97 | 24.6 | 116 | 372 |
| Sertraline | 4.07 | 4.62 | 79.3 | 310 |
| Tianeptine | 3.49 | 10.2 | 54.5 | 163 |
| Tramadol | 2.10 | 84.5 | 414 | 1138 |
| Tranylcypromine | 3.84 | 7.20 | 36.4 | 108 |
| Trazodone | 2.99 | 161 | 1005 | 2752 |
| Venlafaxine | 2.51 | 22.9 | 120 | 369 |
| Vortioxetine | 4.08 | 8.96 | 41.1 | 119 |

The linearity was good for all compounds in the calibrated range, with a coefficient of determination ($R^2$) above 0.9932

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# Targeted Quantitation

| Analyte | LLOQ (µg/L) |
|---|---|
| Agomelatine | 0.509 |
| Atomoxetine | 15.1 |
| Bupropion | 1.16 |
| Citalopram | 1.65 |
| Clomethiazole | 48.7 |
| Desmethylcitalopram | 1.85 |
| Desmethylfluoxetine | 4.27 |
| Desmethylmiansarine | 1.18 |
| Desmethylmirtazapine | 1.32 |
| Desmethylsertraline | 4.13 |
| Dosulepin | 1.68 |
| Duloxetine | 6.17 |
| Ery-dihydro-bupropion | 10.5 |
| Fluoxetine | 3.78 |
| Fluvoxamine | 3.49 |
| Guanfacine | 0.091 |
| Hydroxybupropion | 14.5 |
| Methylphenidate | 0.380 |

| Analyte | LLOQ (µg/L) |
|---|---|
| Mianserin | 1.03 |
| Milnacipran | 2.92 |
| Mirtazapine | 1.20 |
| Moclobemide | 15.6 |
| Nefazodone | 3.49 |
| O-Desmethyltramadol | 8.38 |
| O-Desmethylvenlafaxine | 3.74 |
| Opipramol | 4.15 |
| Paroxetine | 1.90 |
| Reboxetine | 4.80 |
| Ritalinic acid | 2.46 |
| Sertraline | 0.462 |
| Tianeptine | 1.02 |
| Tramadol | 8.45 |
| Tranylcypromine | 1.44 |
| Trazodone | 32.2 |
| Venlafaxine | 2.29 |
| Vortioxetine | 0.896 |

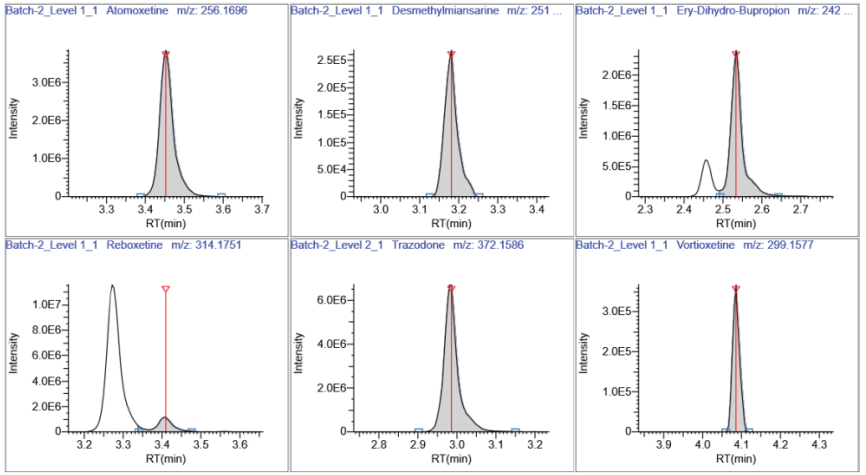



Figure 1. Representative chromatograms at the estimated LLOQ

The lowest concentration of the diluted calibrators that had a mean back-calculated accuracy within 80 to 120% and a precision (CV) better than 20% are presented in the table.

For all compounds, there is a possibility to extend the LLOQ below the lowest calibrator

**Accuracy and precision**

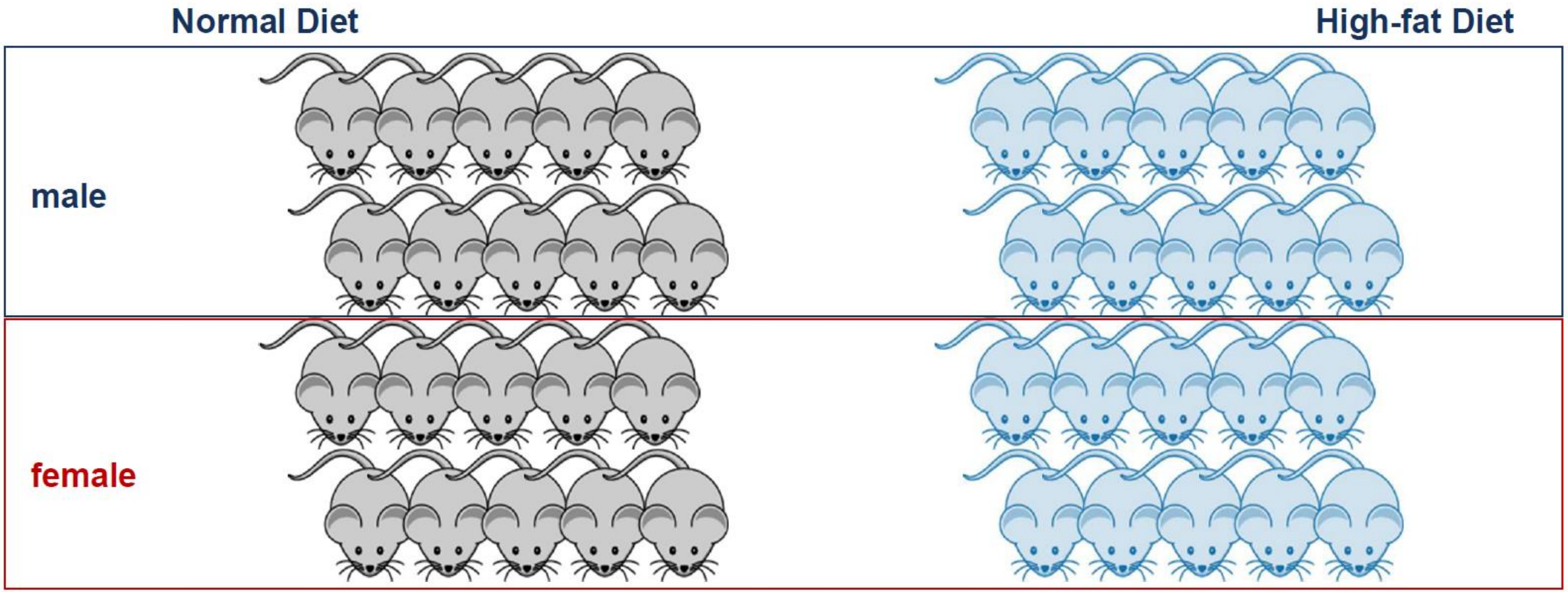
(QC samples (mean, n=5))

- intra-assay accuracy: 85.2-113.9%,
- intraassay precision: <6.5%
- inter-assay accuracy 89.2-114.8%
- inter-assay precision: <6.4%

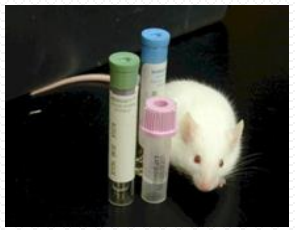
# Metabolomics Case Study:

*Metabolic alterations observed in plasma of mice fed high-fat diet*

# Comparative Study | Diet Induced Changes in Mouse Plasma Metabolome

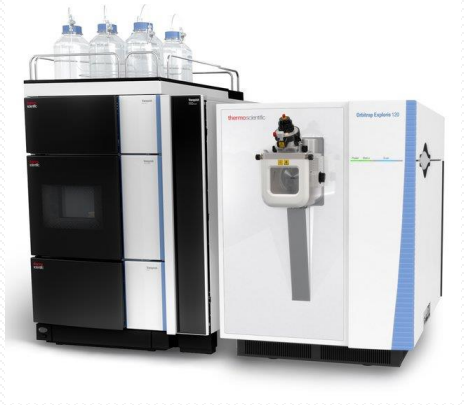


# Untargeted Metabolomics Workflow



Collect Plasma, Urine, Feces, Tissues, *etc.*

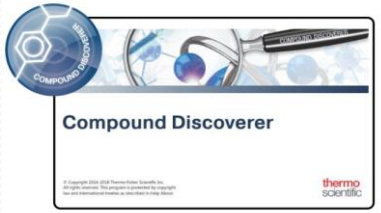
Sample Preparation



Collect High Resolution LC/MS Profiles

Data Processing

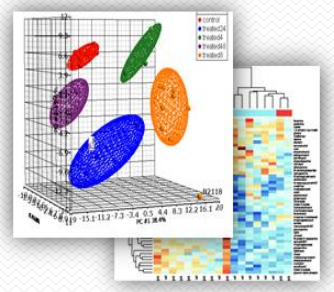
Lists of Compounds

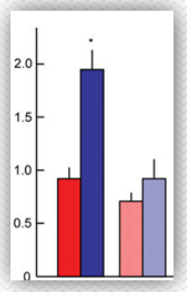

m/z CLOUD

BioCyc

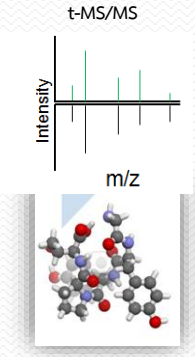
ChemSpider
The free chemical database



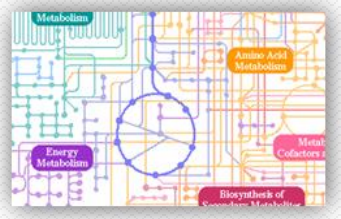
Statistical Analysis




Trends



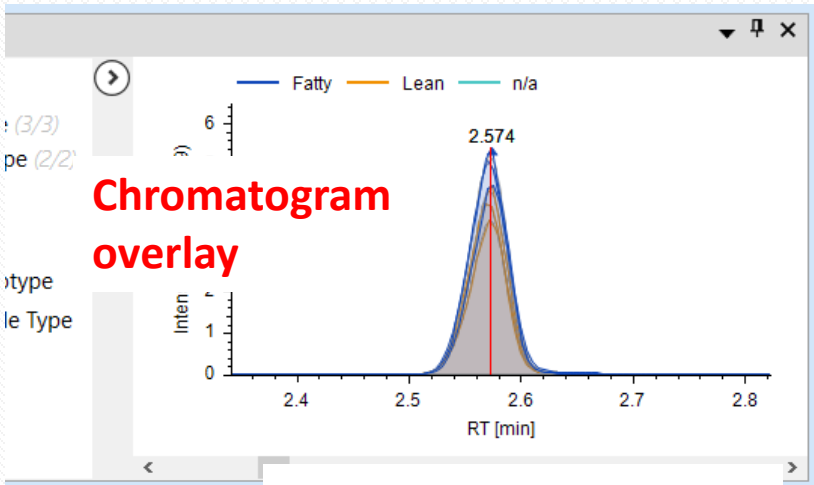

Metabolites

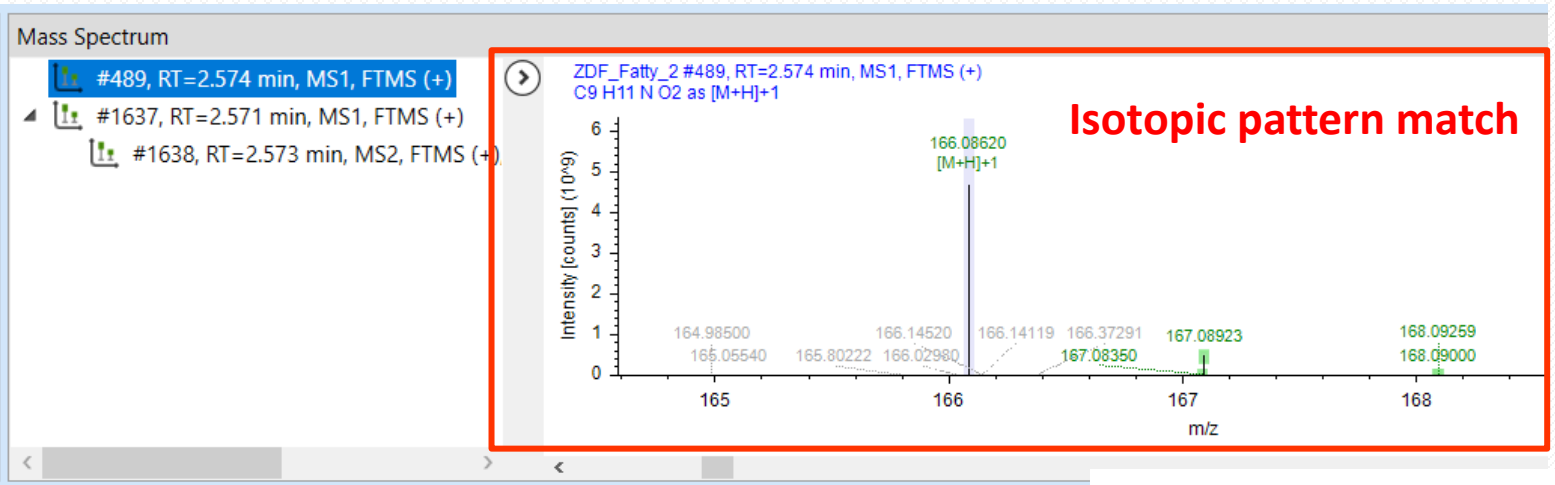



Pathways

Metabolic Pathway

# Compound Discoverer




Elemental composition

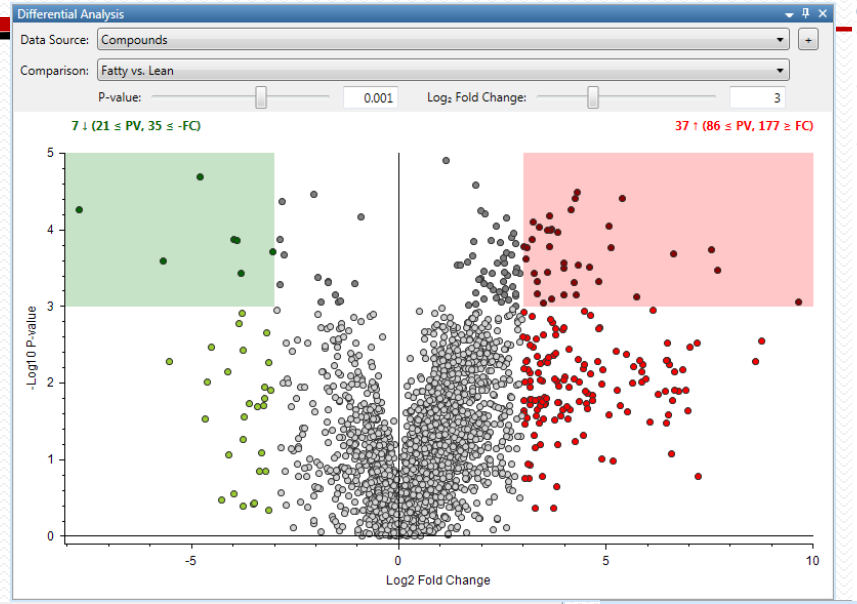
Comparative study

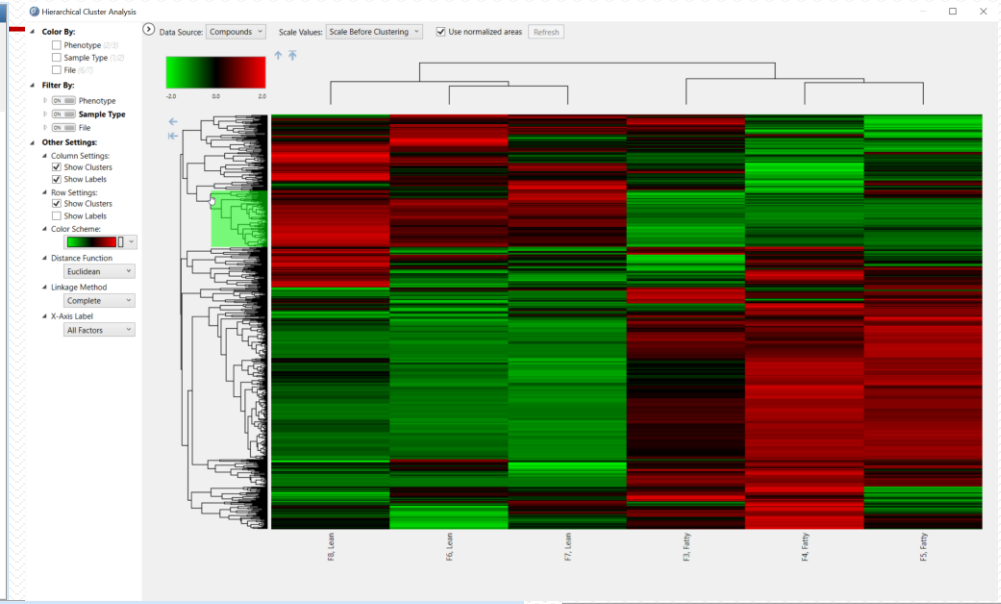
| Name | Formula | Annotation Sc | Molecular Weight | RT [min] | Area (Max.) | # ChemSpi | mzCloud Best Match | MS2 | Group Areas | | Group CV [%] | | Ratio | Log2 Fold Change |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DA9185000 | C12 H10 O S | | 202.04498 | 0.834 | 20360836859 | 1 | | | 1.86e10 | 4.37e9 | 25 | 35 | 4.270 | 2.09 |
| L-Norleucine | C6 H13 N O2 | | 131.09453 | 2.009 | 17671595759 | 18 | 99.7 | | 1.67e10 | 9.63e9 | 17 | 11 | 1.739 | 0.80 |
| Betaine | C5 H11 N O2 | | 117.07877 | 0.862 | 17227484266 | 14 | 98.5 | | 1.70e10 | 1.61e10 | 17 | 11 | 1.056 | 0.08 |
| D-(+)-Tryptophan | C11 H12 N2 O2 | | 204.08974 | 3.113 | 14935877741 | 12 | 99.2 | | 1.18e10 | 1.41e10 | 2 | 9 | 0.838 | -0.26 |
| Creatine | C4 H9 N3 O2 | | 131.06929 | 0.895 | 13731682864 | 2 | 99.9 | | 1.35e10 | 4.24e9 | 20 | 17 | 3.178 | 1.67 |
| L-Phenylalanine | C9 H11 N O2 | | 165.07892 | 2.573 | 13086972275 | 17 | 100.0 | | 1.26e10 | 9.64e9 | 9 | 9 | 1.304 | 0.38 |
| L-Tyrosine | C9 H11 N O3 | | 181.07382 | 1.620 | 12173763807 | 18 | 99.5 | | 8.37e9 | 1.10e10 | 22 | 8 | 0.763 | -0.39 |
| Creatinine | C4 H7 N3 O | | 113.05880 | 0.921 | 11284215174 | 2 | 99.3 | | 1.08e10 | 6.67e9 | 14 | 11 | 1.619 | 0.70 |

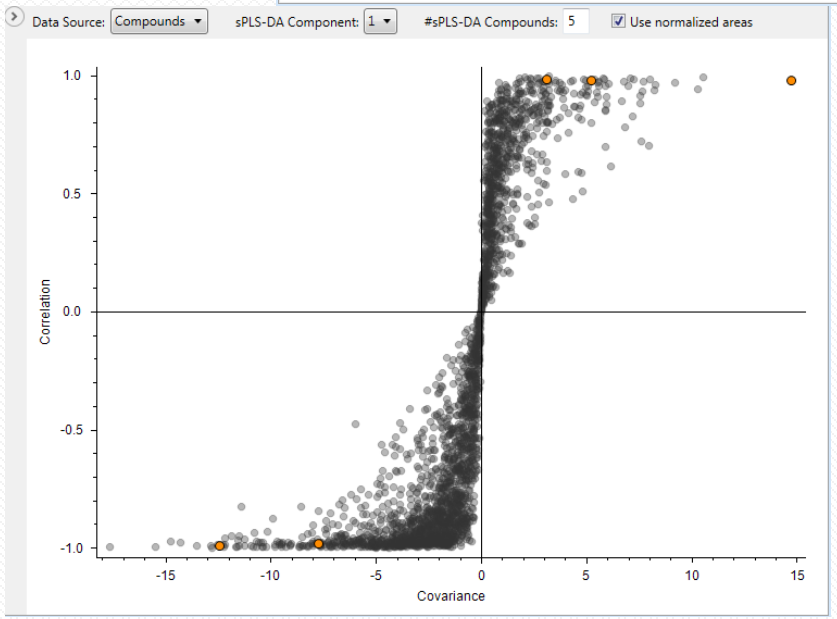
Annotation Source

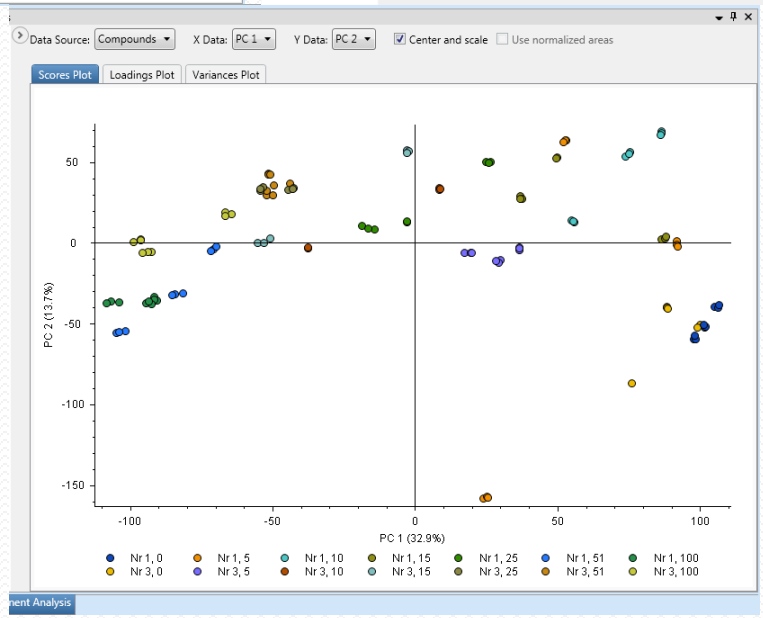
| Compound Match | Formula | Molecular Weig | ΔMass [Da] | ΔMass [ppm] | RDBE | H/C | Rank | # Matched Iso. | # Missed Iso. | # Matched Frag. | SFit [%] | Pattern Cov. [%] | MS Cov. [%] | MSMS Cov. [%] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C9 H11 N O2 | 165.07898 | -0.00006 | -0.38 | 5.0 | 1.2 | 1 | 5 | 0 | 10 | 57 | 100.00 | 100.00 | 99.45 |

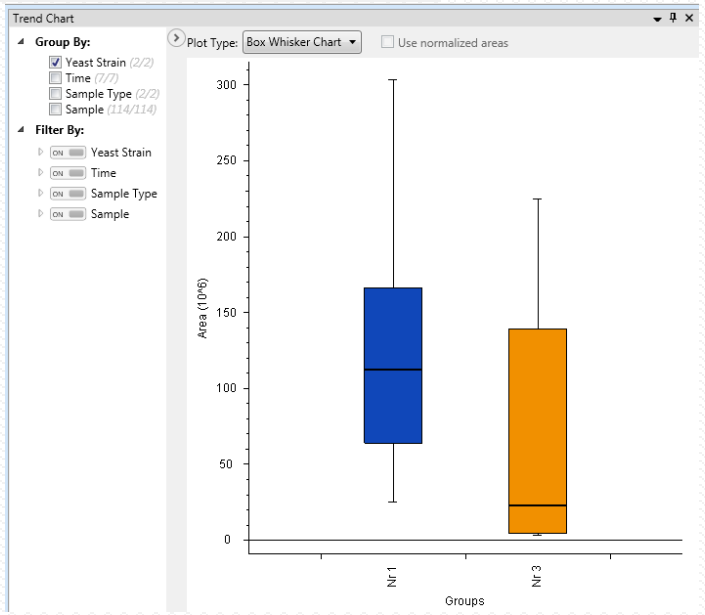
# Compound Discoverer Software: Statistics



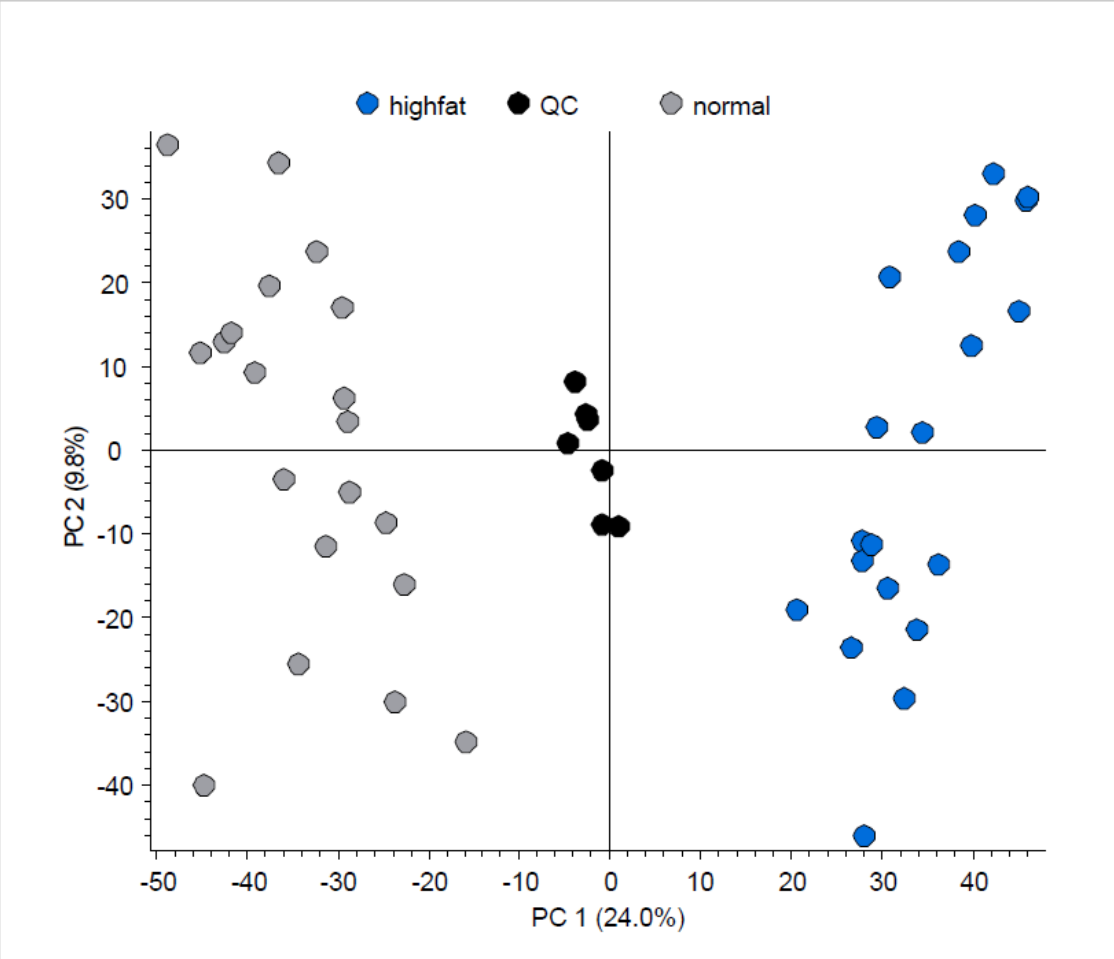



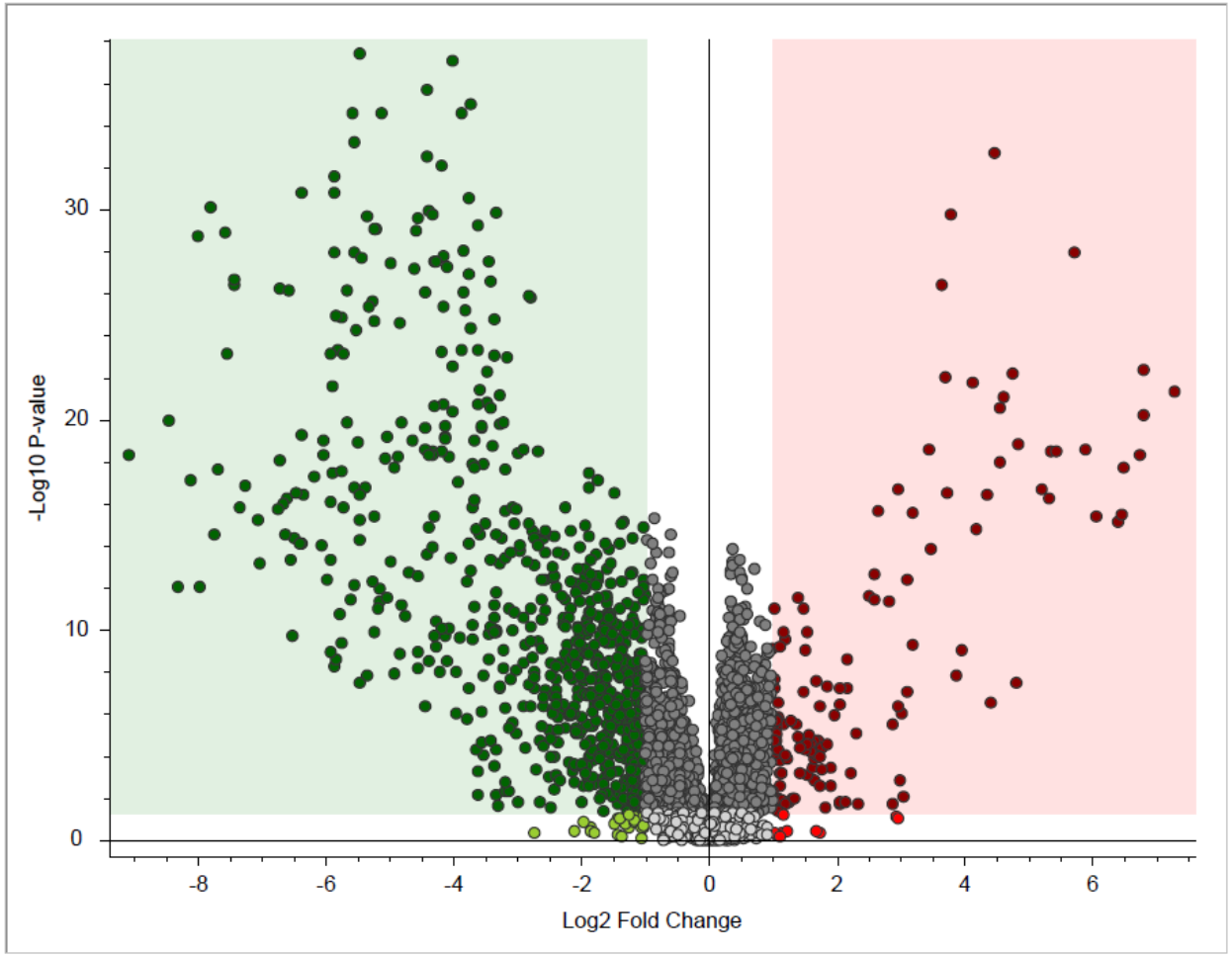



# Differential Analysis




PCA plot




Volcano plot

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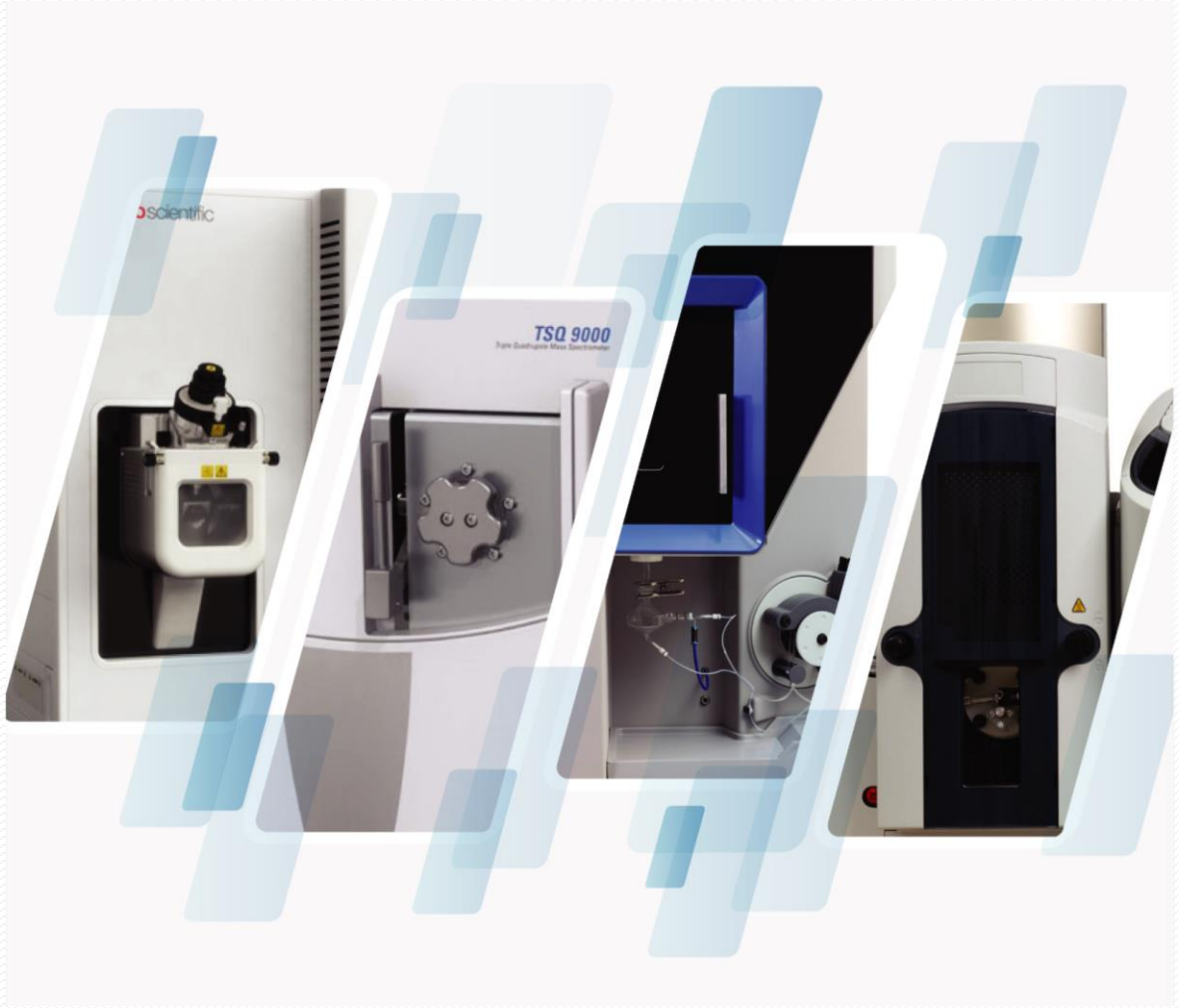


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