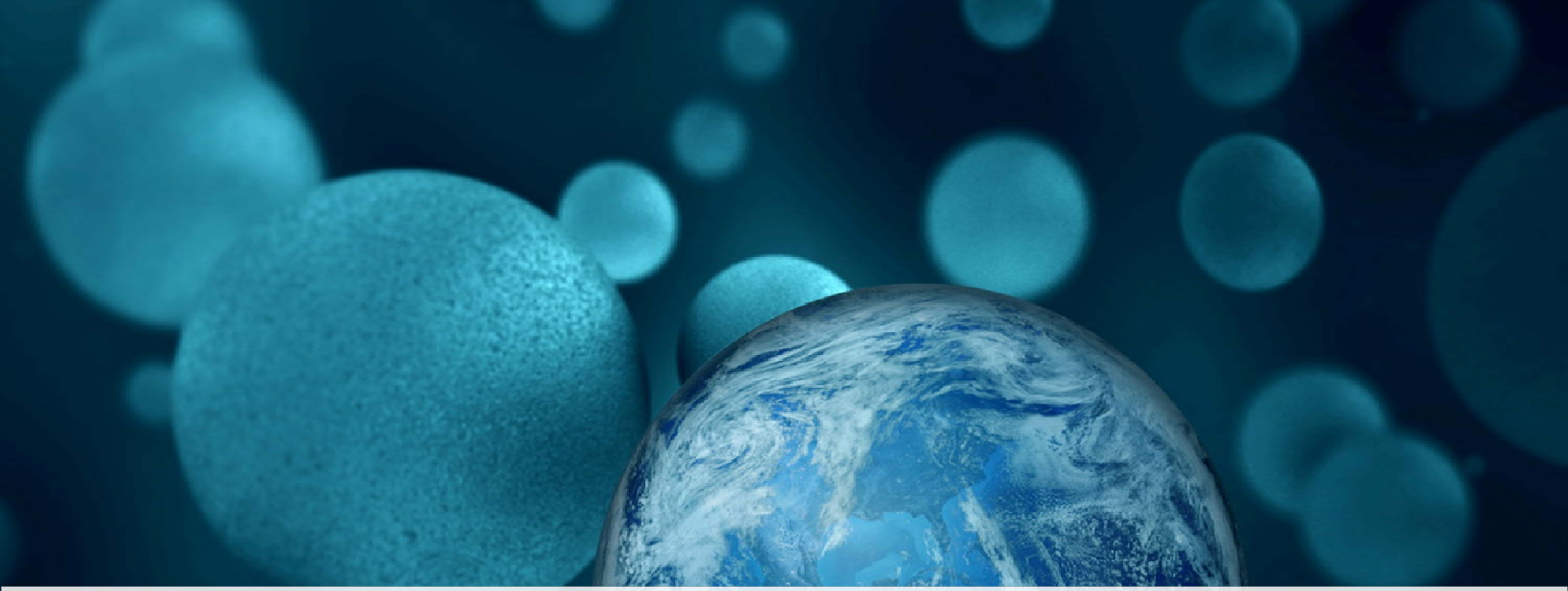

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High Resolution MS in Forensic Toxicology Screening

Osama Abu-Nimreh
CMD Sales Support Specialist
MECEC , Dubai

Screening Approaches in LC/MSMS

- Screening applications are commonly used in forensic and clinical toxicology laboratories.
  - **Targeted screening :** compound is identified and confirmed using databases and/or libraries.
  - **Unknown screening:** no databases and libraries available. Compound is identified using MS^2 and or MS^n data.
- Screening applications utilize different types of mass spectrometers
  - **Ion Traps :** MS and MS^n experiments. Pos/Neg switching
  - **Triple quadrupole :** 2 SRMs/analyte. Confirmation using the Ion Ratio.
  - **HRAM instruments** (OrbiTrap) : Full Scan followed by AIF for the Exactive Plus. Full Scan followed by MS^2 experiments for the Thermo Scientific™ Q-Exactive™ Plus. **Full Scan followed by 4 vDIA events for the Thermo Scientific™ Q-Exactive Focus.**

Screening – General Workflows

Step 1: Sample Preparation

Depending on sample type (*urine, plasma, serum, whole blood*):
-Dilution
-LLE
-SPE
-Online TurboFlow extraction
-Protein precipitation

Step 2 : Data acquisition

Different approaches:
-Ion trap
-Triple quadrupole
-Orbitrap (HRAM MS)

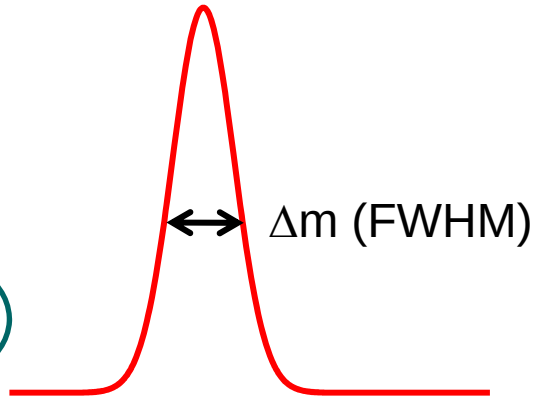
Step 3: Processing Data

TraceFinder
-ToxID
-HRAM screening

Mass Resolution FWHM

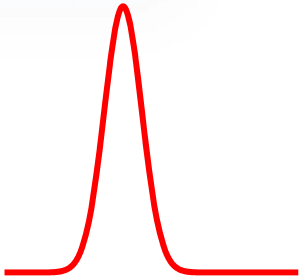
- **Resolution**

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



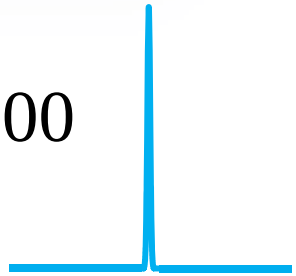
- Quadrupole MS

$$R = \frac{400}{0.4} = 1000$$



- **Orbitrap (HRAM) MS**

$$R = \frac{400}{0.004} = 100000$$



How Accurate Is Your Mass?

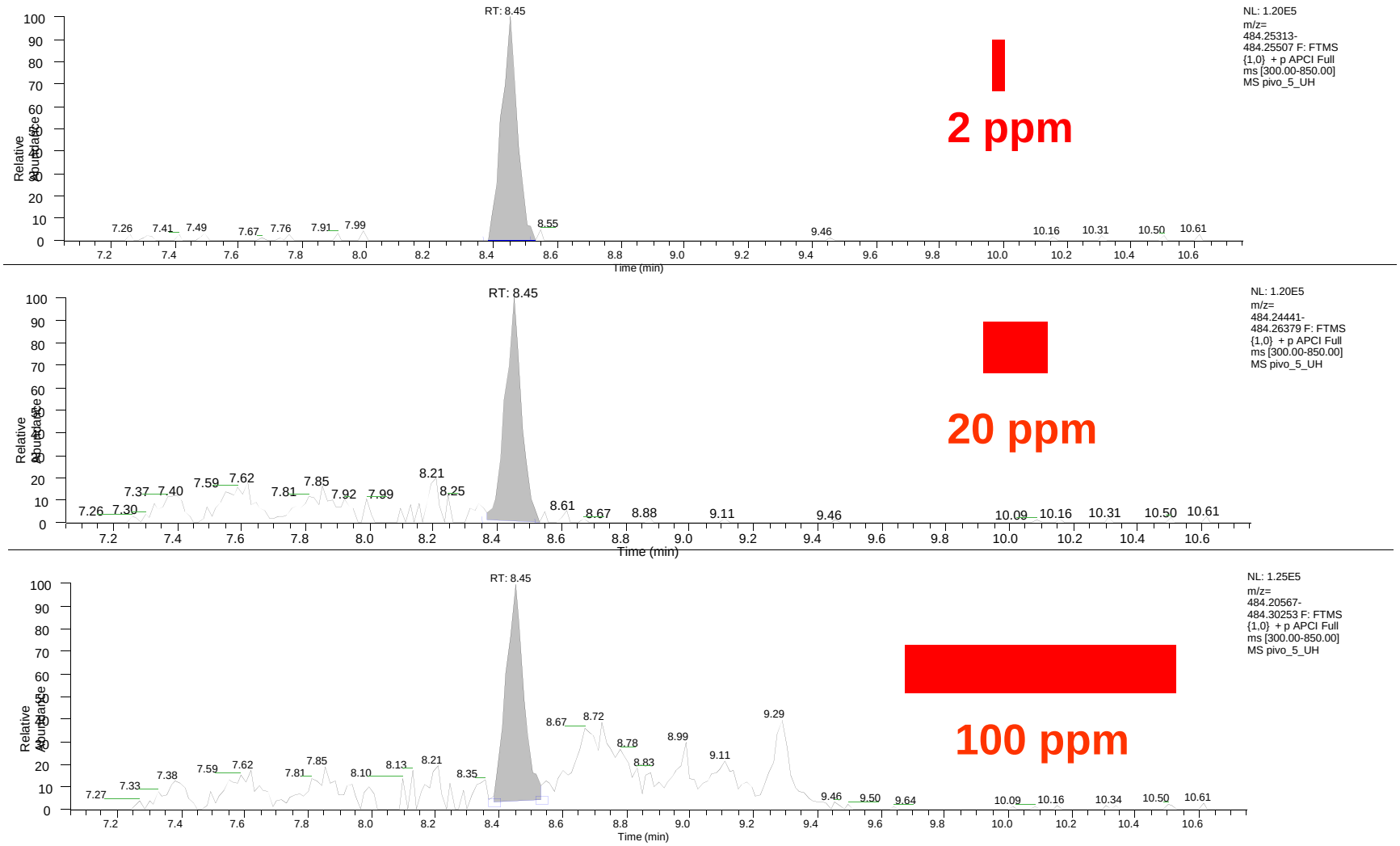
- Mass accuracy

$$\Delta m / z = \frac{m_{meas} - m_{true}}{m_{true}} \cdot 10^6$$

- Quadrupole MS $\quad \Delta m / z = \frac{500.1 - 500.0}{500} \cdot 10^6 = 200 ppm$

- Orbitrap MS
TOF MS $\quad \Delta m / z = \frac{500.10314 - 500.10214}{500.10314} \cdot 10^6 = 2 ppm$

Selectivity Increases With Higher Mass Accuracy



*Zachariasova M et al, Anal. Chim. Acta

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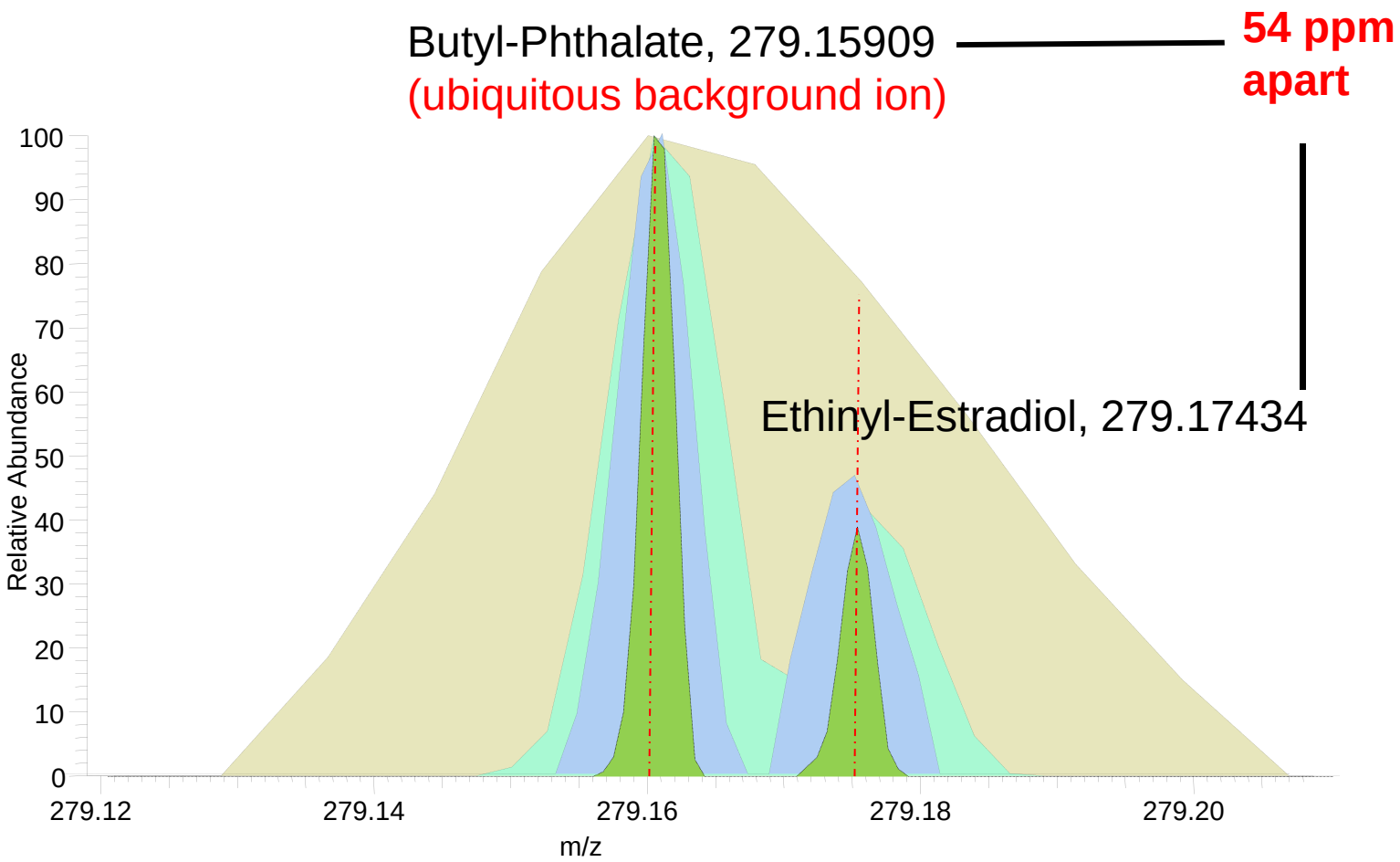
Specificity = Resolution + Mass Accuracy

Resolution: 10k, 30k, 50k, 100k



ThermoFisher SCIENTIFIC

The Industry's Leading Thermo Scientific™ MS Portfolio

Exactive Series MS

Non-targeted Analysis

HR/AM

Tribrid Orbitrap MS

- Metabolomics
- Proteomics
- Bioanalysis

Quantitative

- Food Safety
- Environmental
- Clinical/Toxicology

Applied Markets

Transform Your Science

Research Markets

- Biomarker Discovery
- Proteomics
- Metabolism

Qualitative

- Metabolomics
- PTM Analysis
- Lipidomics

Triple Quads

MS, MS^n

Targeted Analysis

Ion Traps

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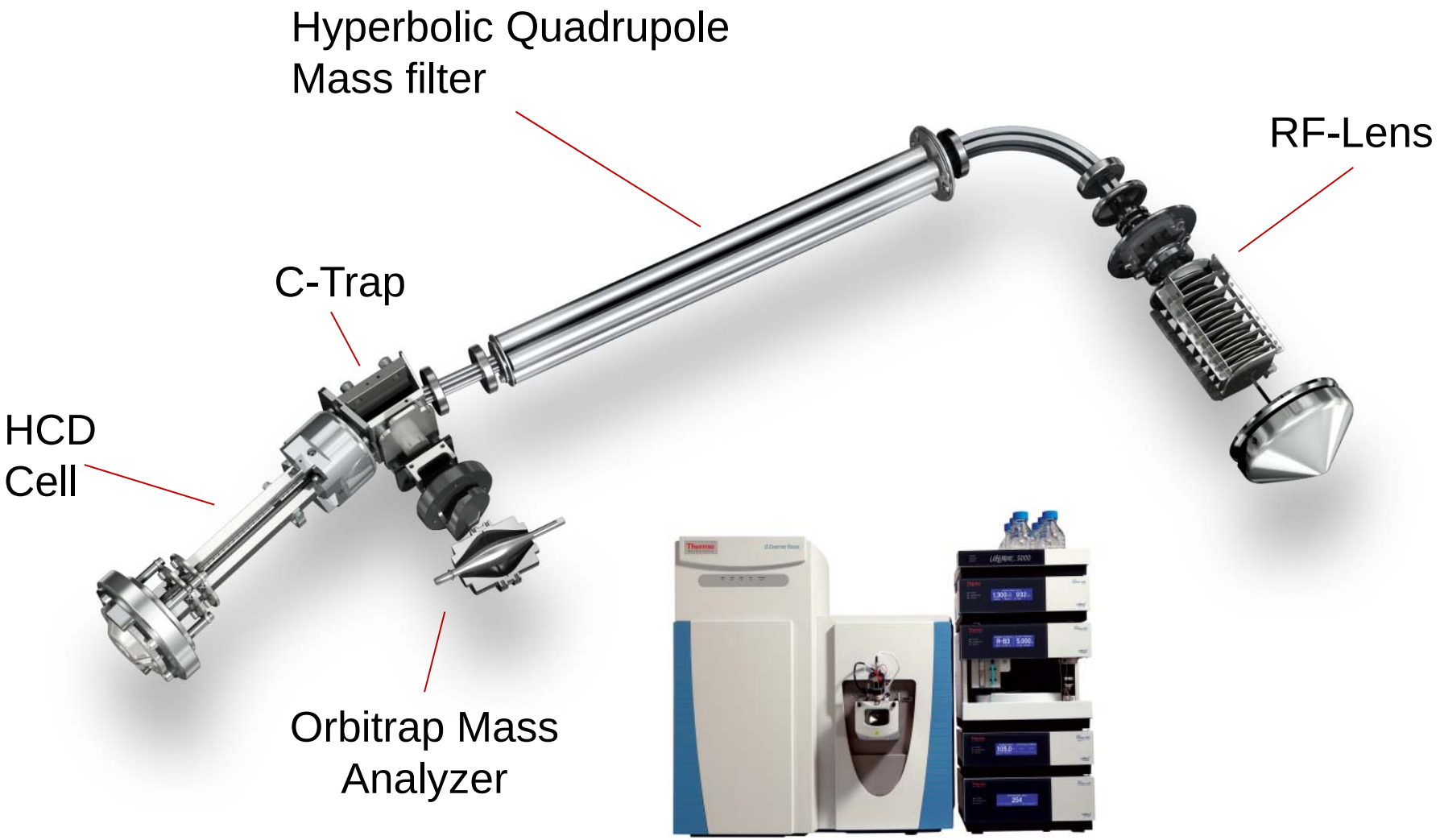
Q Exactive MS - a 3D view



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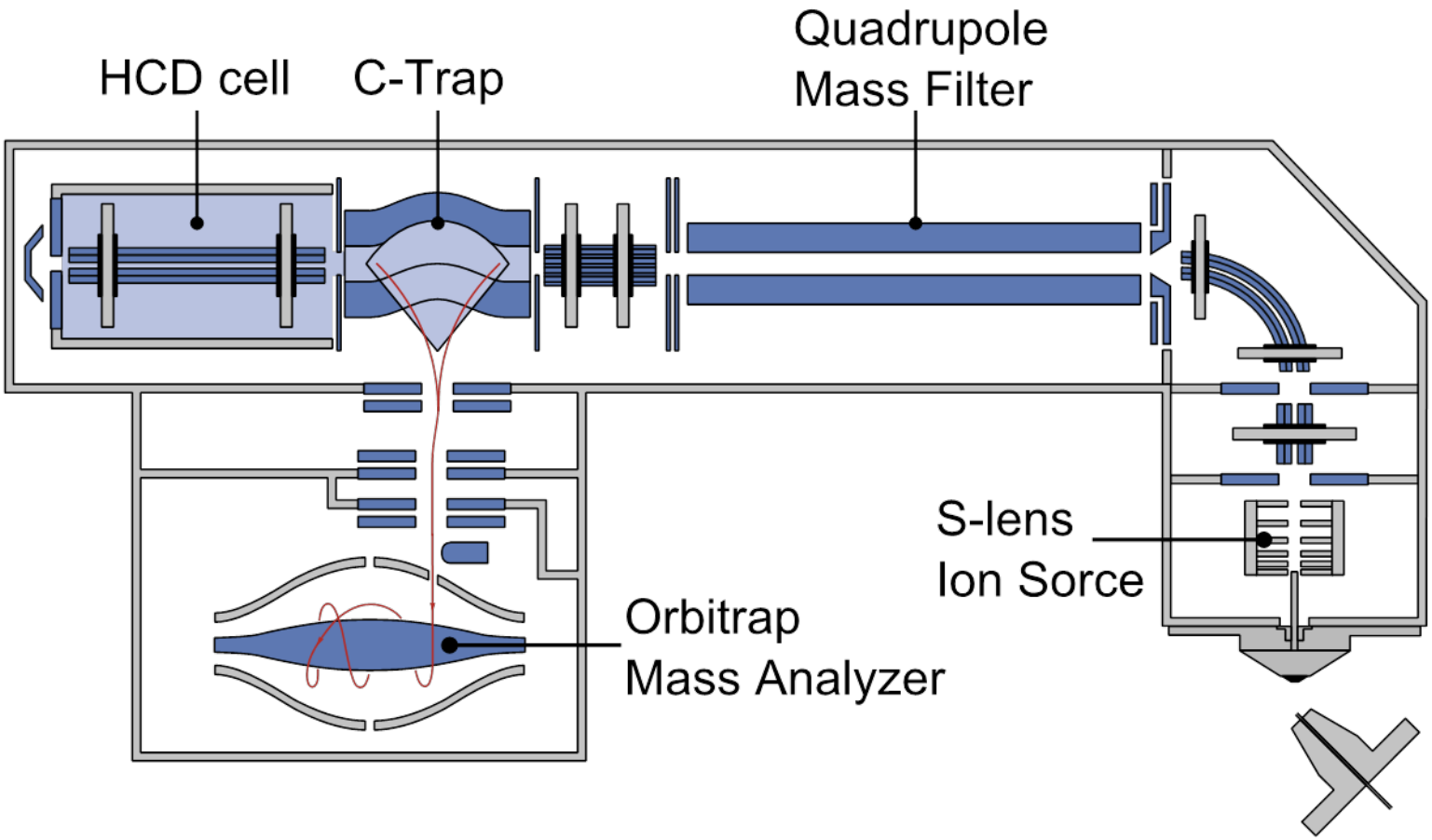
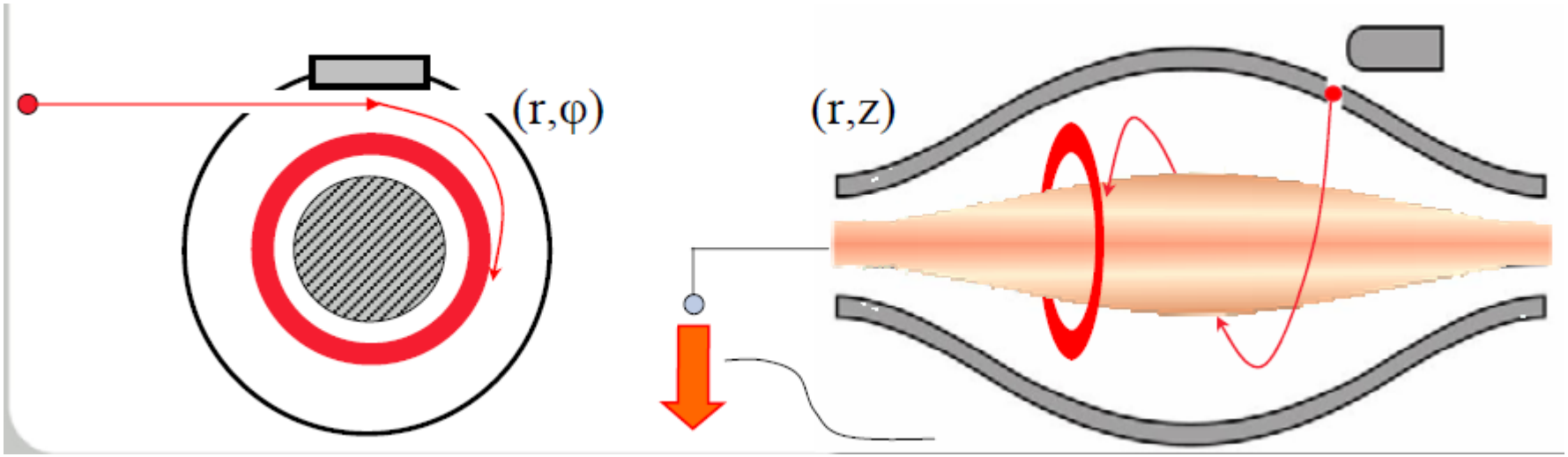
Q Exactive ™ : Hardware



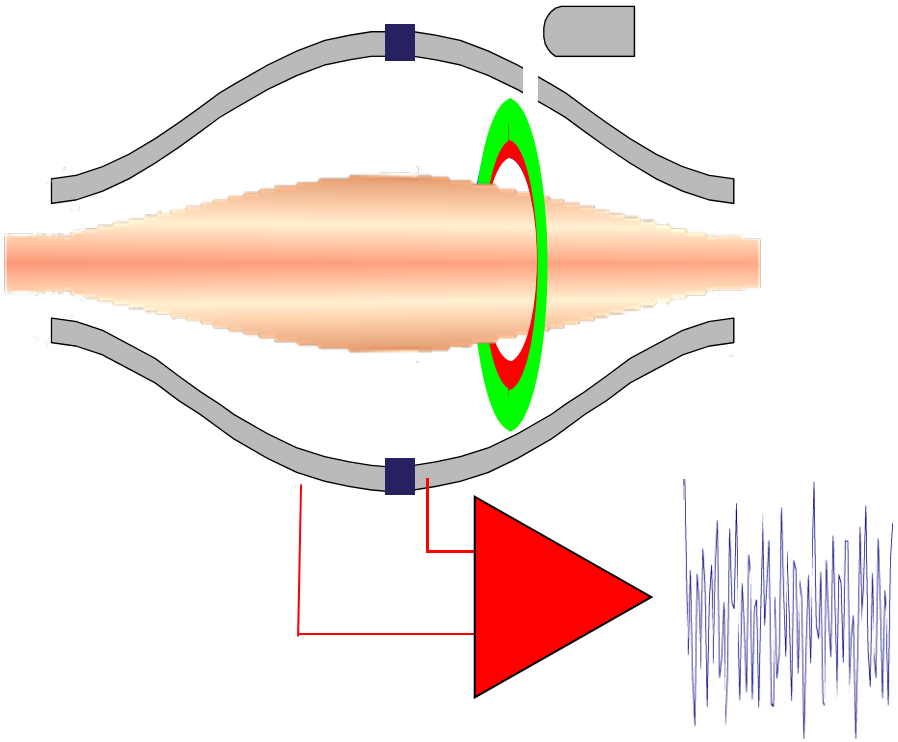
1. Ions are injected through the source
2. …and trapped in the C-trap and squeezed into a smaller cloud
3. …then a voltage pulse across C-trap ejects ions towards the Orbitrap
4. …where they are trapped and detected

Orbitrap - Principle of Operation



- A short ion packet of one m/z from c- trap enters the field tangentially
- C-trap is only used as an ion storage device
- Ions are squeezed towards the central electrode by increasing voltage on the central electrode
- In the axial direction, ions are forced to move away from the narrow gap towards the wider gap near the equator. This initiates axial oscillations
- After the voltage increase stops, ion trajectories become a stable spiral

Orbitrap - Principle of Operation



- Ion m/z separation depends on
  - Frequency of harmonic oscillations and is proportional to sq root of *m/z*
- Three frequencies create oscillations
  - Frequency of rotation
  - Frequency of radial oscillations
  - Frequency of axial oscillations

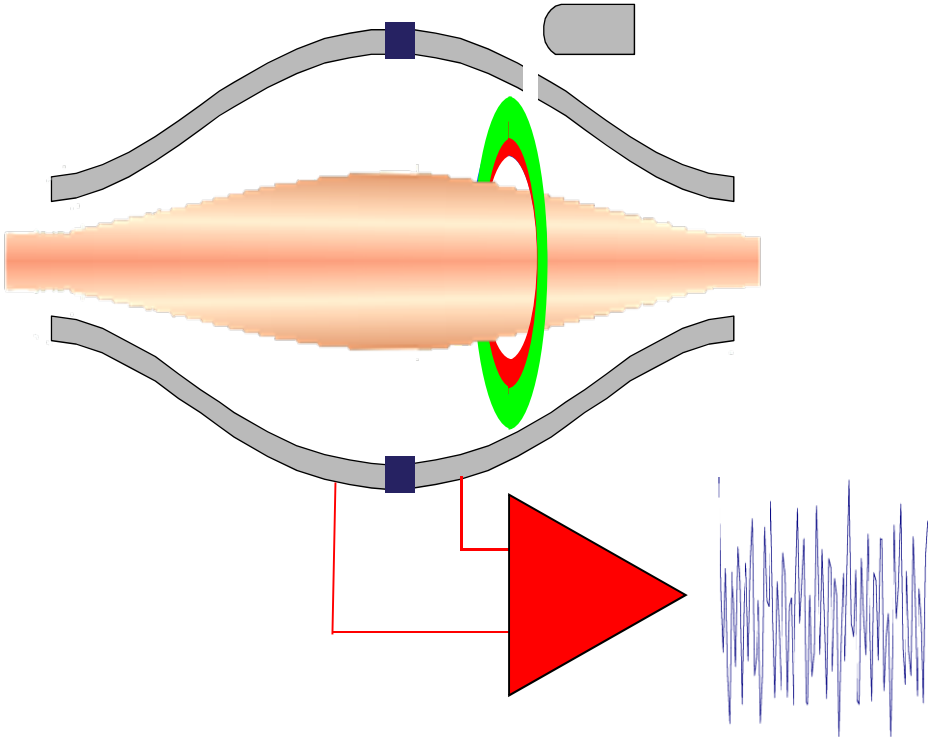
- Resolving power is
  - Inversely proportional to the square root of *m/z*
  - Proportional to acquisition time

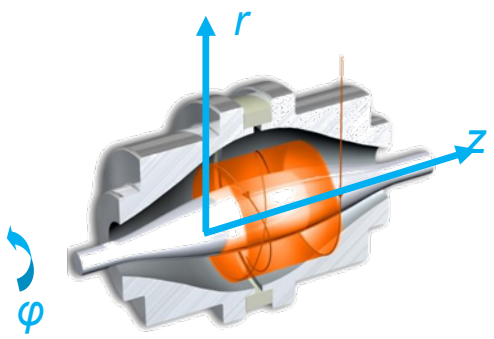
- Sensitivity is independent of acquisition speed.

- Red rings smallest *m/z*; Blue ring larger *m/z*; Green ring largest *m/z*

Makarov A. *Anal. Chem.* 2000, *72*, 1156-1162.

Principle of Orbitrap MS Operation





Hyper-logarithmic potential distribution:
"ideal Kingdon 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 ω_φ
  - Frequency of radial oscillations ω_r
  - Frequency of axial oscillations ω_z

$$\omega_z = \sqrt{\frac{k}{m/q}}$$

$$\omega_\varphi = \frac{\omega_z}{\sqrt{2}} \sqrt{\left(\frac{R_m}{R}\right)^2 - 1} \qquad \omega_r = \omega_z \sqrt{\left(\frac{R_m}{R}\right)^2 - 2}$$

Makarov A. *Anal. Chem.* 2000, *72*, 1156-1162.

Triple Quadrupole is great tool! but ?

- It is only targeted!
- Selectivity provided by tandem MS/MS (SRM transition needed)
- False positives are reality!
- Need to setup instrument (SRM) before analysis
- Realistic breakpoint is 200-300 compounds in a run
- Time consuming data processing

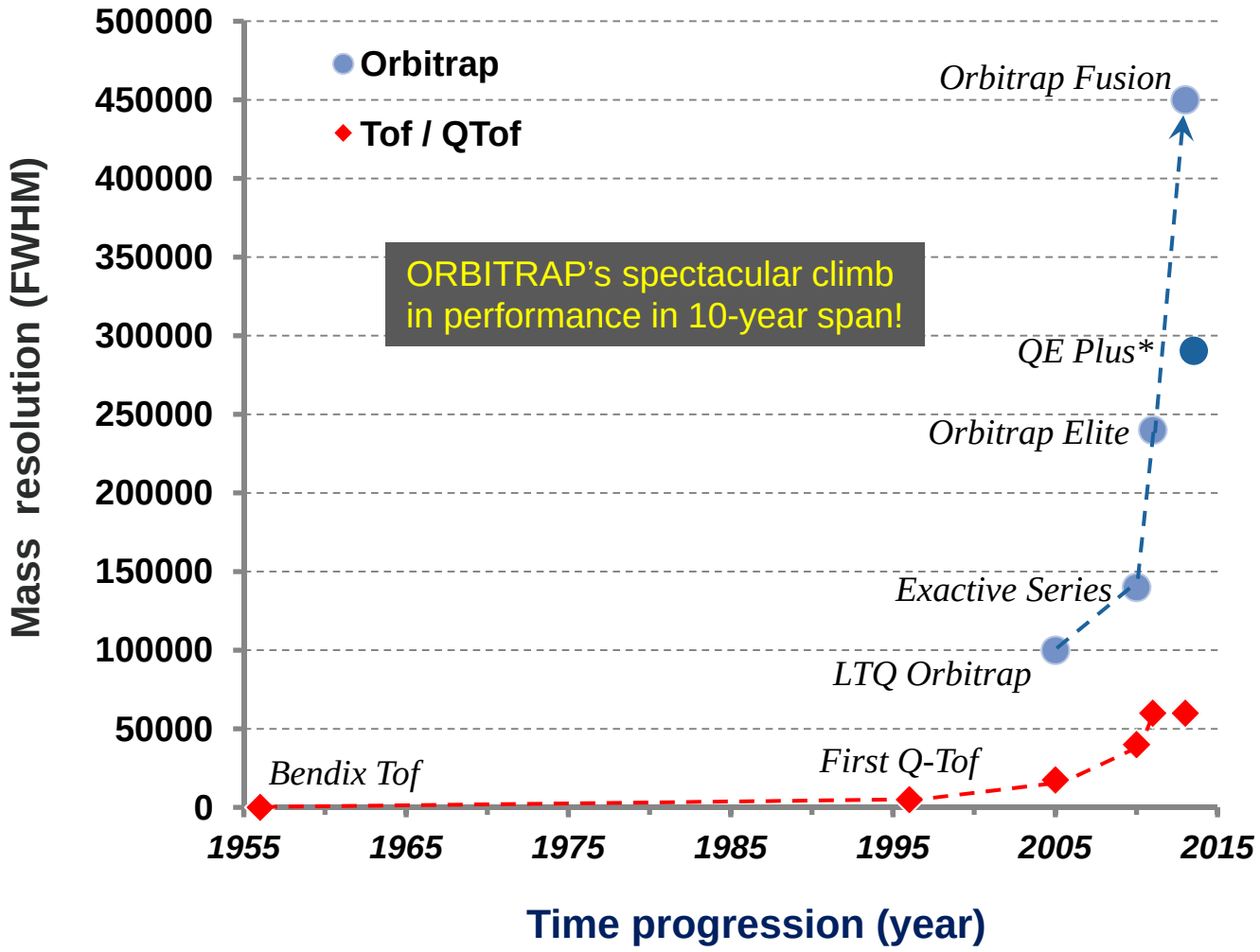
HRAM is a solution!

- Can perform the same level of quantitation as MS/MS
- Selectivity obtained by accurate mass measurement (only m/z needed)
- No false positives!
- No need to setup instrument (SRM) before analysis
- Unlimited number of compounds in a run – perfect for screening
- Automated data processing

Orbitrap Mass Analyzer Features

- Fundamental difference to other HRAM instruments
- Parameter measured is **frequency**, not time/voltage/current
- Resolution allows more accurate *m/z* determination
- Less prone to ambient conditions changes
- Usually stable within <2 ppm during several days
- No need for lock mass in “routine work”
- Small footprint
- Easy to setup

High Resolution MS Technology Race



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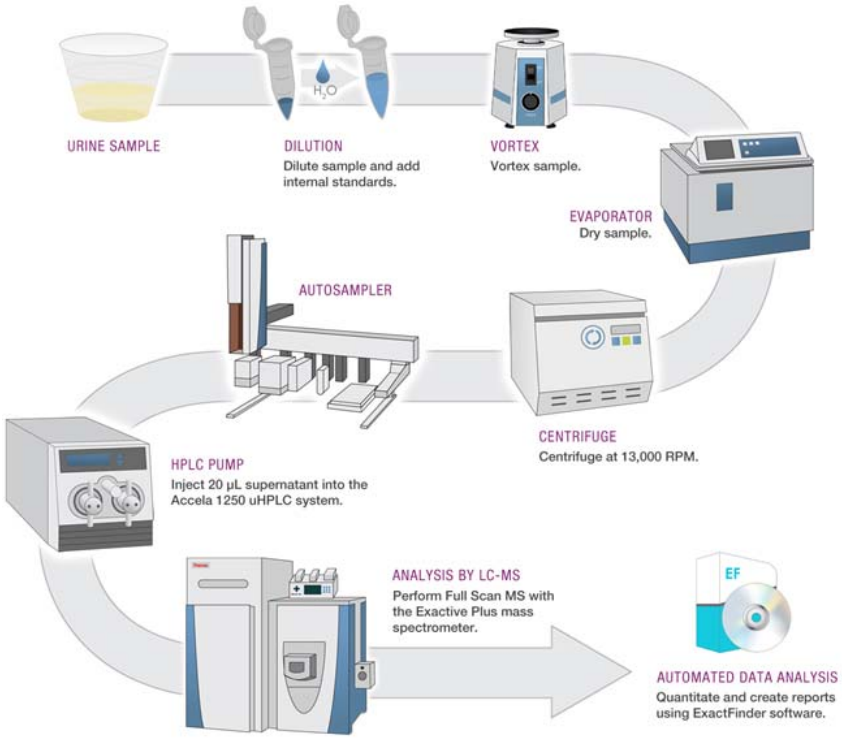
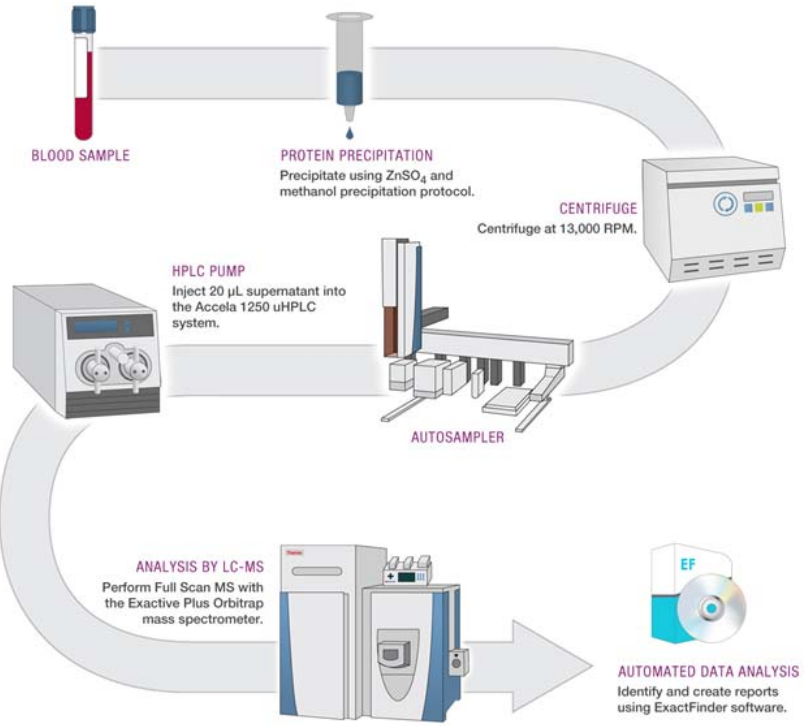
Orbitrap technology – Workflow examples

Step 1: Sample Preparation

Step 2 : Data acquisition

Step 3: Processing Data





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Q Exactive Focus - Acquisition apporaches

- There are 3 approaches possible for screening:
  - **DDE** : Data Dependent Experiment. Here the system selects the more intense ions reported in the Full scan MS spectra to fragment those on MS^2 mode. If the ion has a low intensity it is probable that it won't be selected for MS^2 and therefore not confirmed by the processing software.
  - **AIF** : All Ion Fragmentation.Here the system fragments all the ions present in the MS spectra in the collision cell. Lack of specificity.
  - **DIA** : Data Independent Analysis. Here fragmentation is performed in different mass ranges. It is more specific than AIF but less specific than DDE.

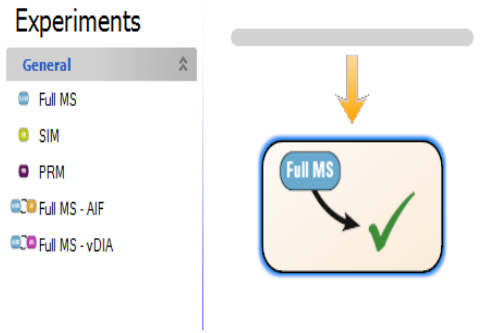
Today we use the two approaches DDE and vDIA for screening purposes, we strongly suggest the vDIA approach for a better fragmentation.

Drug identification based on :
Accurate mass of the parent ion
Accurate mass of the fragment ions
Isotopic pattern
Library match
Chromatographic retention time window

3 ways of Quantitation/Screening for Routine Work

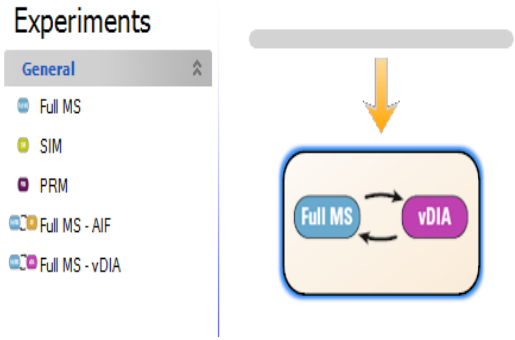
Full MS or targeted SIM/ddMS2

- Post-acquisition - extracted ion chromatograms of parent ions of interest
- Relies on high resolution for selectivity
- Useful for less complex background
- No method development/preparation needed



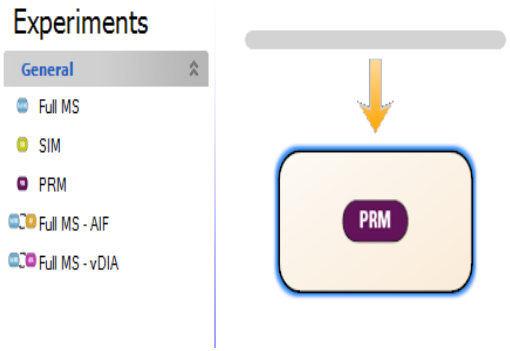
Full MS/ All Ion Fragmentation – vDIA*

- Post-acquisition - extracted ion chromatograms of parent ions of interest
- Scheduled target (inclusion) list (Rt, *m/z*)
- Minimum method development (e.g., predefine parent ions, tr)
- Also for screening purposes



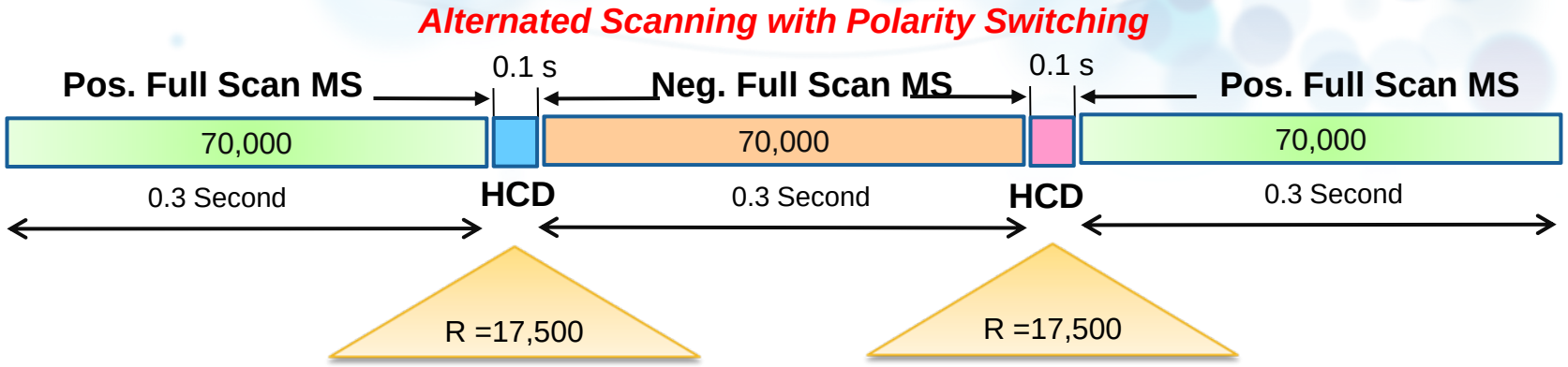
PRM (Parallel Reaction Monitoring)

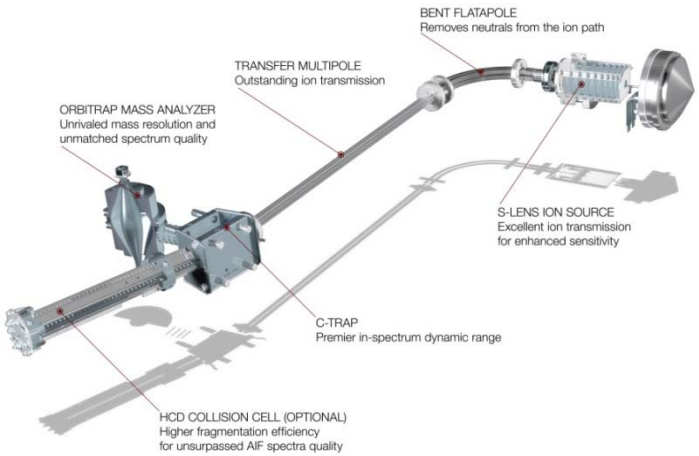
- Post-acquisition – extracted ion chromatograms of parent -> fragment transitions acquired
- Scheduled target list (Rt, *m/z*, collision energy)
- Most sensitive and selective even in highly complex matrices



vDIA method not available in the United States of America

The Screening Method: General features





- LC Method
  - Mobile Phase:
    - A: 10 mM ammonium formate in 0.1% formic acid
    - B: ACN containing 0.1% formic acid
  - **LC column: PFP, 150 x 2.1 mm, 5 µm**
  - Injection volume 20 µL
  - 30 mn or 15 mn Gradient

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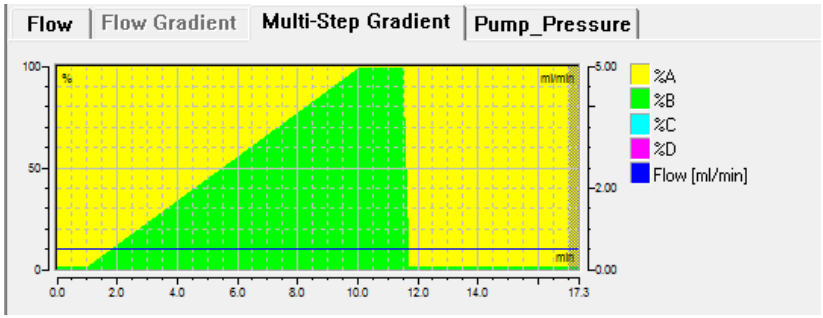
Screening LC conditions

- Mobile Phases
- **A: H_2O, $[NH4]^+$ $[HCOO]^-$ 2mM, 0.1% HCOOH**

  (for 1L of mobile phase A use 1L of water and add 126mg of ammonium formiate and 1mL of formic acid)
- **B: $[NH4]^+$ $[HCOO]^-$ 2mM, MeOH/ACN 50/50, 0.1% HCOOH, 1 % H_2O**

  (for 1L of mobile phase B use 495mL of methanol, 495mL of acetonitrile, 10mL of water, and add 126mg of ammonium formiate and 1mL of formic acid)
- UHPLC Separation
- Accucore Phenyl Hexyl 100 x 2.1 mm, 2.6 µm
- Column Oven : 40° C



	Retention [min]	Flow [ml/min]	%B	%C	%D	Curve
1	0.000	0.500	1.0	0.0	0.0	
2	0.000	0.500	1.0	0.0	0.0	
3	1.000	0.500	1.0	0.0	0.0	
4	10.000	0.500	99.0	0.0	0.0	
5	11.500	0.500	99.0	0.0	0.0	
6	11.700	0.500	1.0	0.0	0.0	
7	17.000	0.500	1.0	0.0	0.0	

Thermo Scientific™ TraceFinder™ 4.1 Software

TraceFinder 4.1

- Easy to use software for all LC MS & GC MS quantitation and screening needs
  - **User security/audit trails**: Individuals or domain groups can be given different levels of access to the system and data
  - **Common confirmations in Quan and Screening workflows: Quantitate the things that you know and screen for suspects in a single method**
  - Screening to quantitation workflows for efficient method development
  - **Enhanced custom reports** with many of the same formula functions as MS Excel for calculations and conditional formatting
  - **Intelligent Sequencing** to save time and samples

Software for Targeted and Non-Targeted Analysis

ThermoFisher SCIENTIFIC

Thermo Scientific™ ToxFinder 1.0 for Simplified Targeted Screening

- Intuitive software for routine semi-quantitation and targeted screening needs in Clinical Research and Forensic Toxicology
- Customizable databases, compound confirmations, data review layouts and reporting
- Experiment specific design for SRM, Full MS – AIF, and Full MS-data dependent MS^2
- Same theme as TraceFinder
- Security

Get Results

Quick, Effortless, Accurate

ToxFinder 1.0

Targeted Screening Software

Thermo SCIENTIFIC

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TraceFinder : Screening workflow

Creation of the master method:
- Acquisition parameters (instrumental method)
- Processing parameters (peak detection and screening criteria)
- Reporting parameters (optional)

Acquisition of the data:
- Creation of the analysis sequence

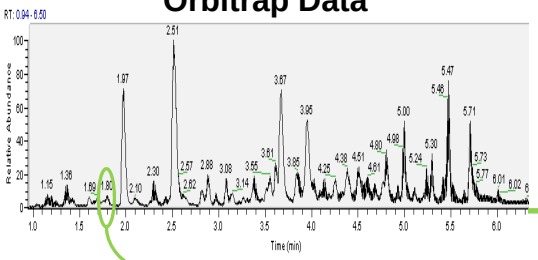
Review the data an run reports
- Data processing is automatically performed after the injections

Note: You can also process data that have already been acquired.

Targeted / Untargeted Screening workflow

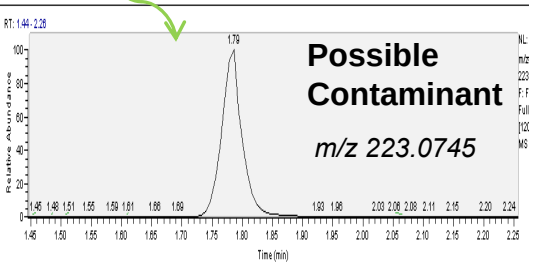
DATA FILE → **PROCESSING METHOD** → **RESULTS**

Orbitrap Data

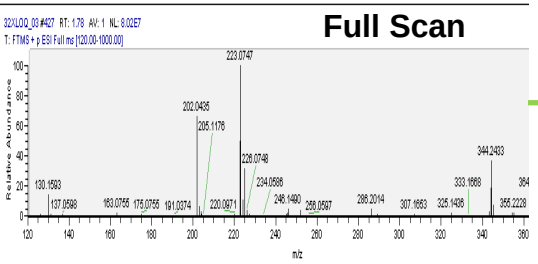


Possible Contaminant

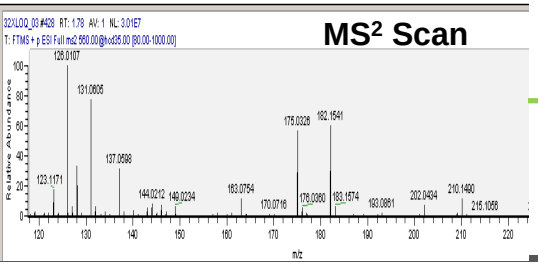
m/z 223.0745



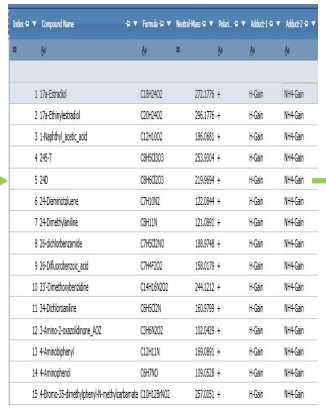
Full Scan



MS² Scan

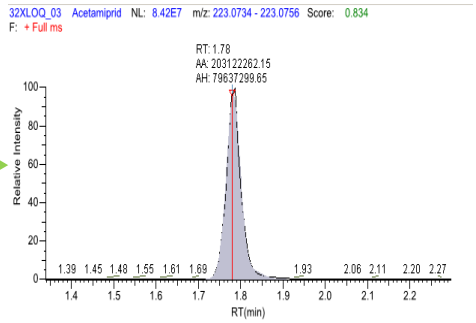


Target List

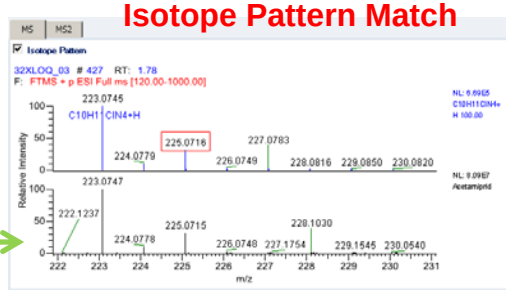


Searches for peaks

Compound m/z and RT



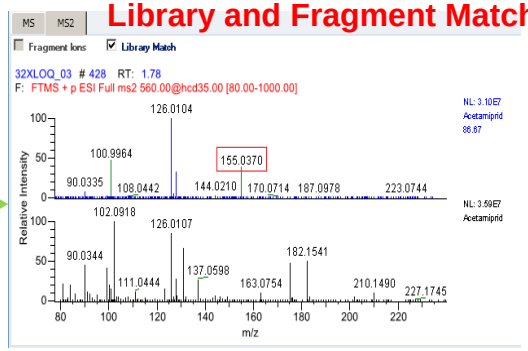
Isotope Pattern Match



Full Scan

Identification and confirmation using m/z, RT, isotope pattern

Library and Fragment Match



AIF Scan, MS² Scan

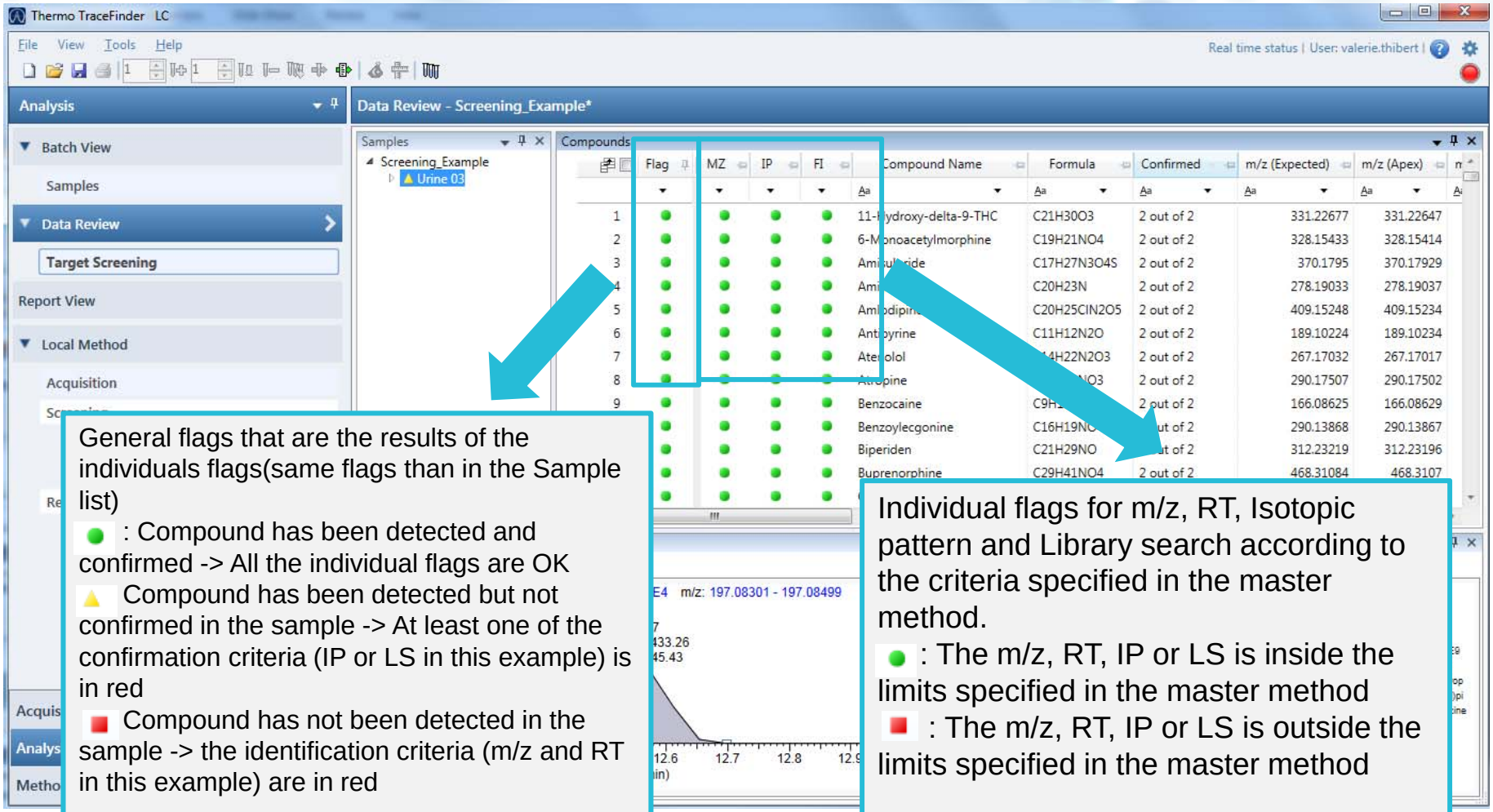
Identification and confirmation using fragment and library matching

Identification Confirmed !
Acetamiprid

ThermoFisher SCIENTIFIC

Data review - flags

The table results: flag code



General flags that are the results of the individuals flags(same flags than in the Sample list)

● : Compound has been detected and confirmed -> All the individual flags are OK

▲ Compound has been detected but not confirmed in the sample -> At least one of the confirmation criteria (IP or LS in this example) is in red

■ Compound has not been detected in the sample -> the identification criteria (m/z and RT in this example) are in red

Individual flags for m/z, RT, Isotopic pattern and Library search according to the criteria specified in the master method.

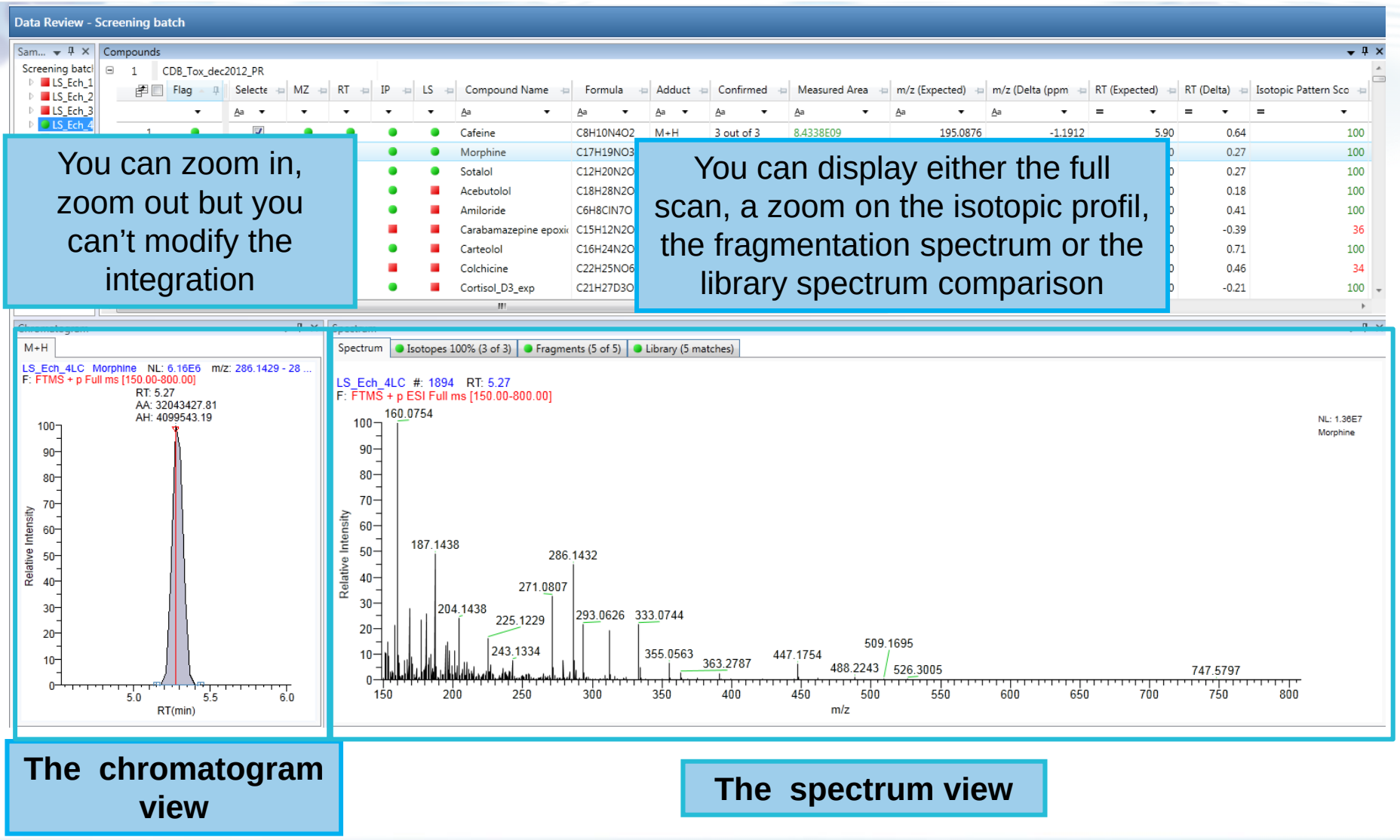
● : The m/z, RT, IP or LS is inside the limits specified in the master method

■ : The m/z, RT, IP or LS is outside the limits specified in the master method

Data review – Chromatogram and spectrum

You can zoom in, zoom out but you can't modify the integration

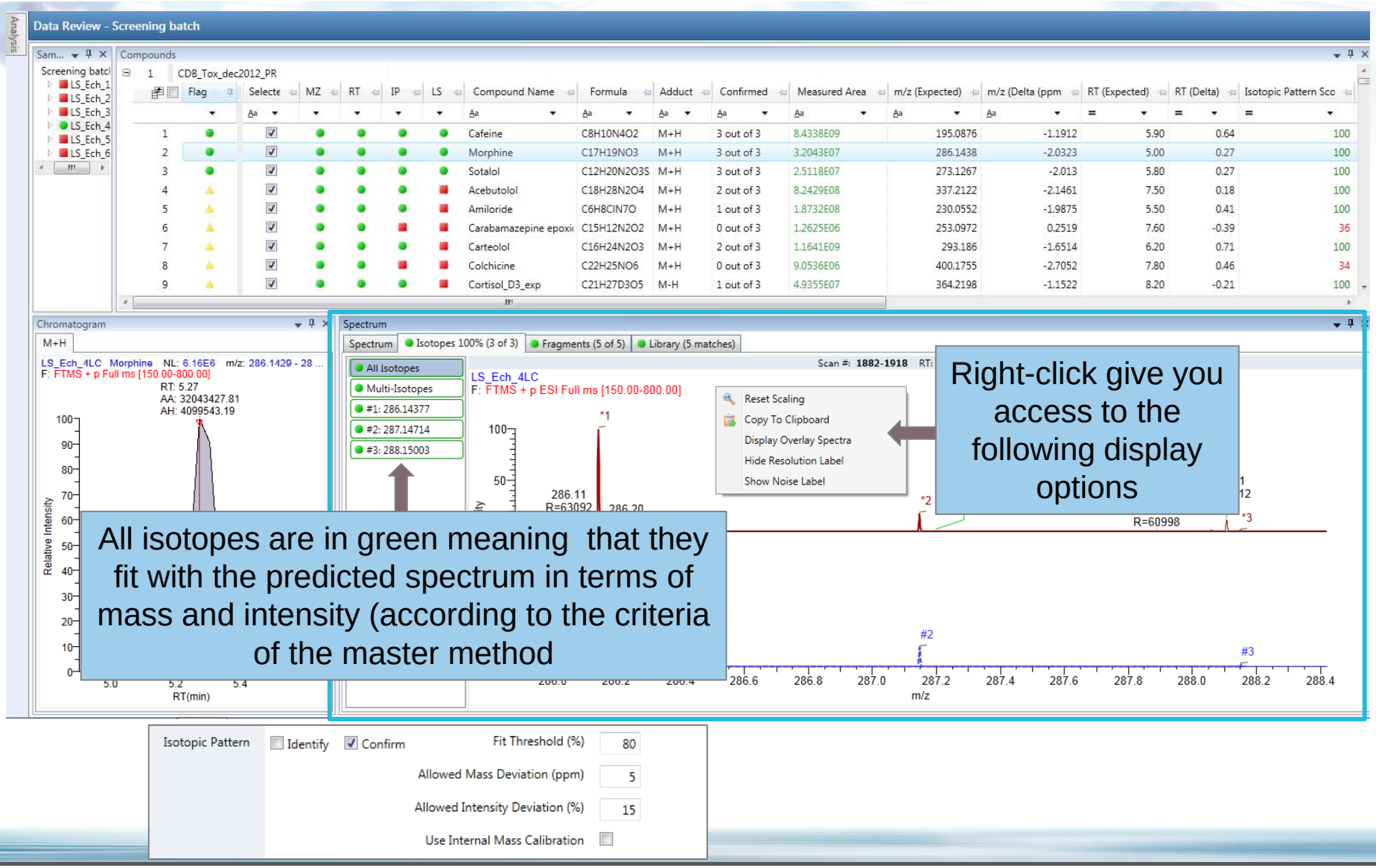
You can display either the full scan, a zoom on the isotopic profil, the fragmentation spectrum or the library spectrum comparison



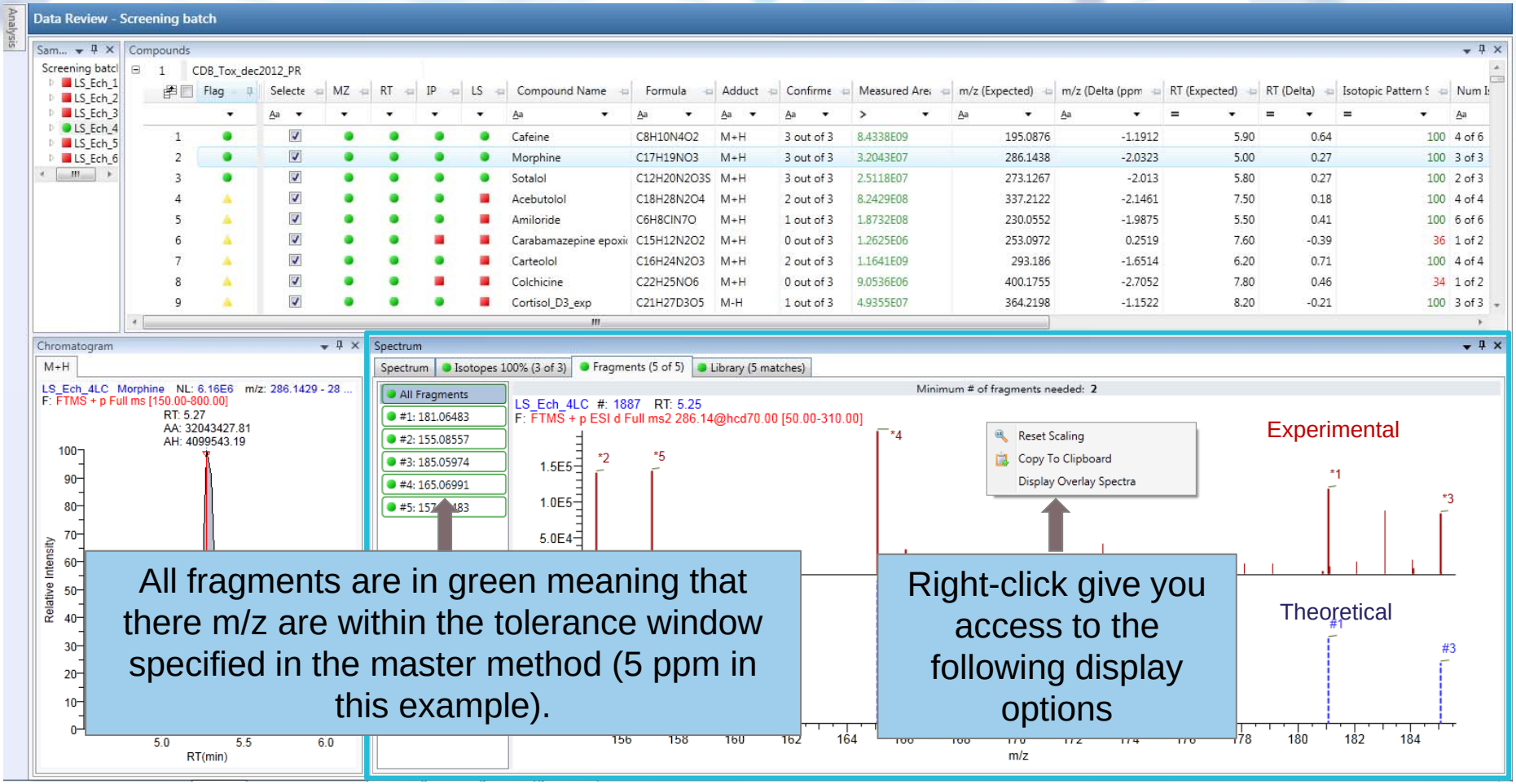
The chromatogram view

The spectrum view

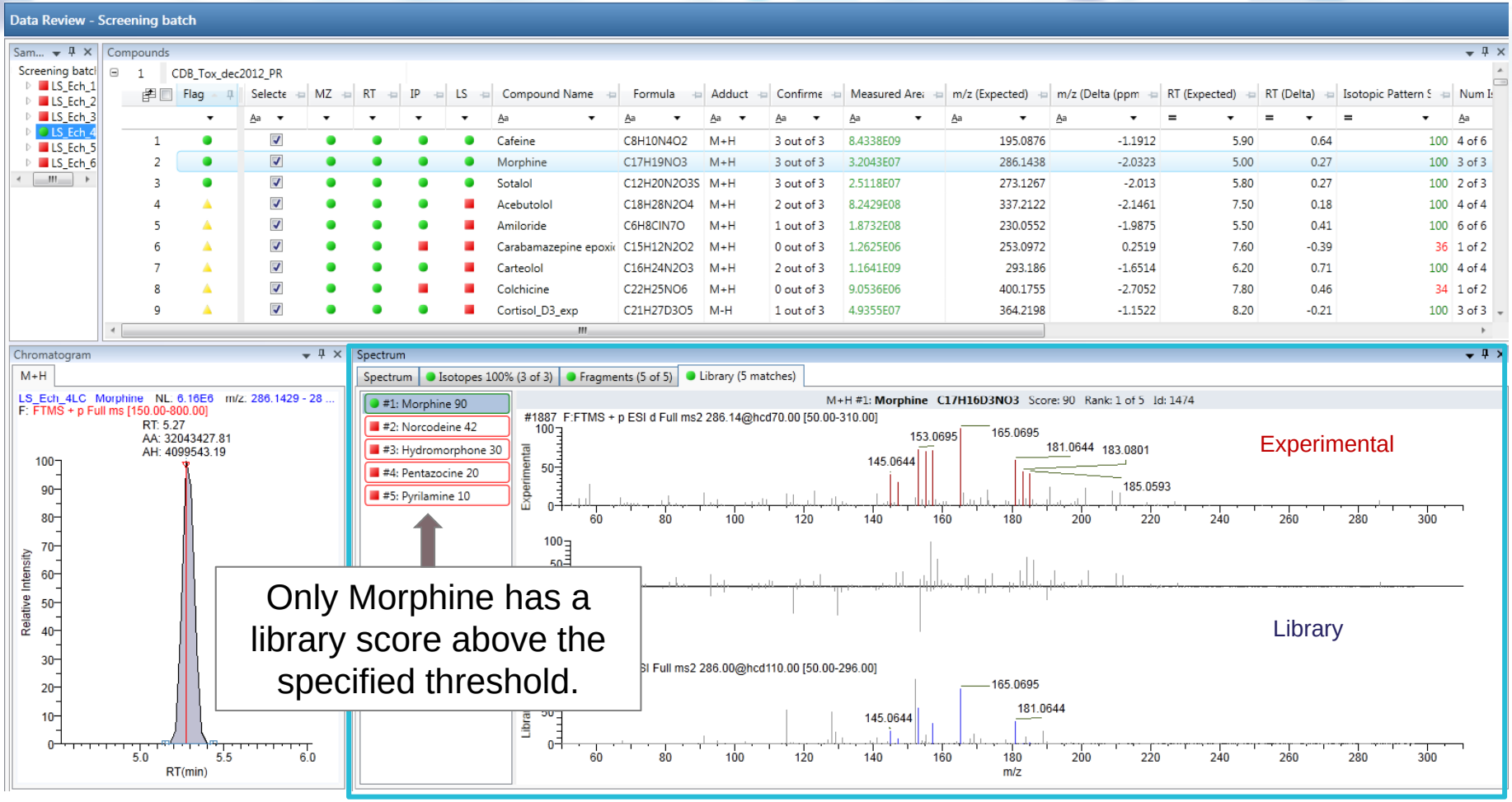
Data review – isotopic pattern



Data review - Fragments

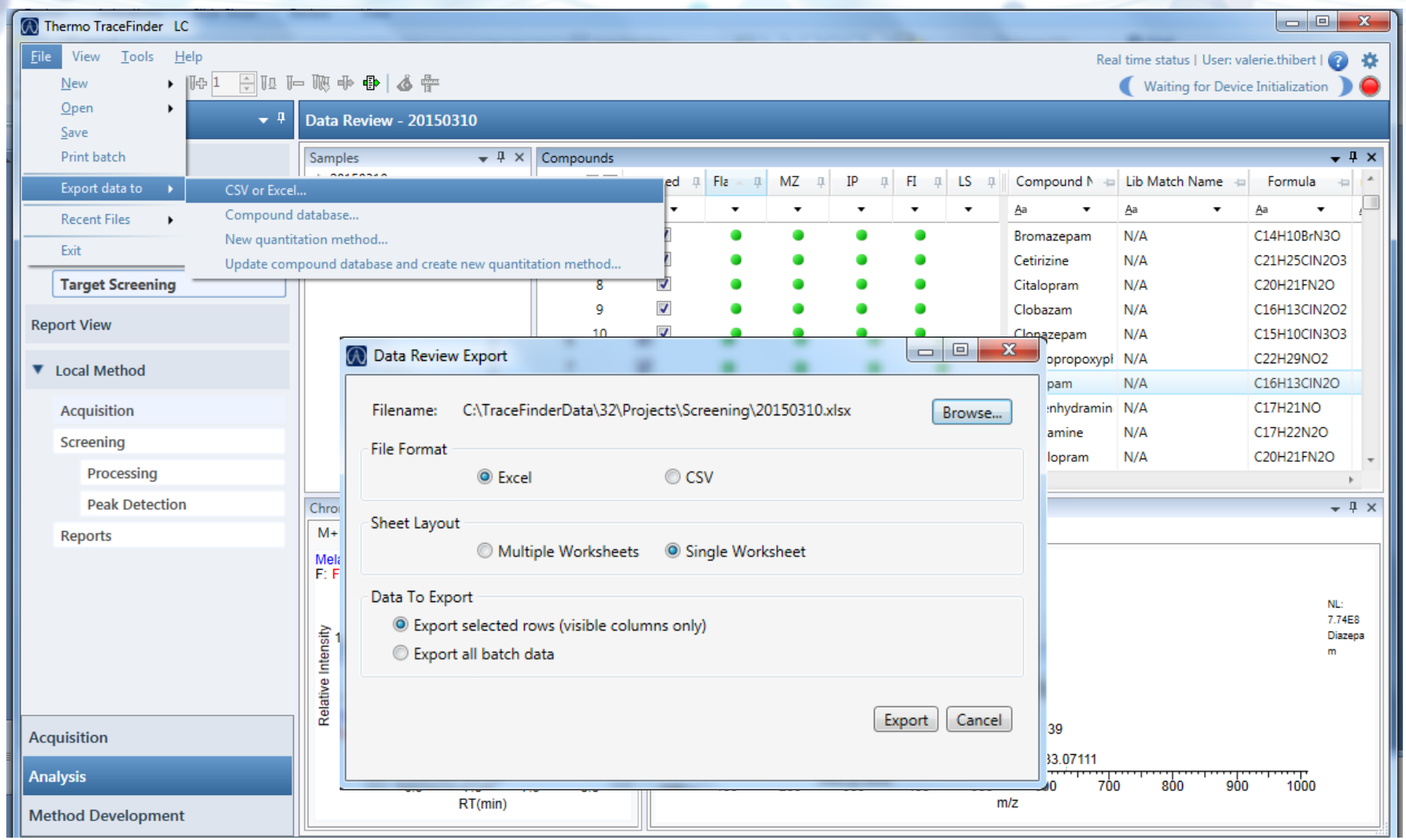


Data review – library comparison

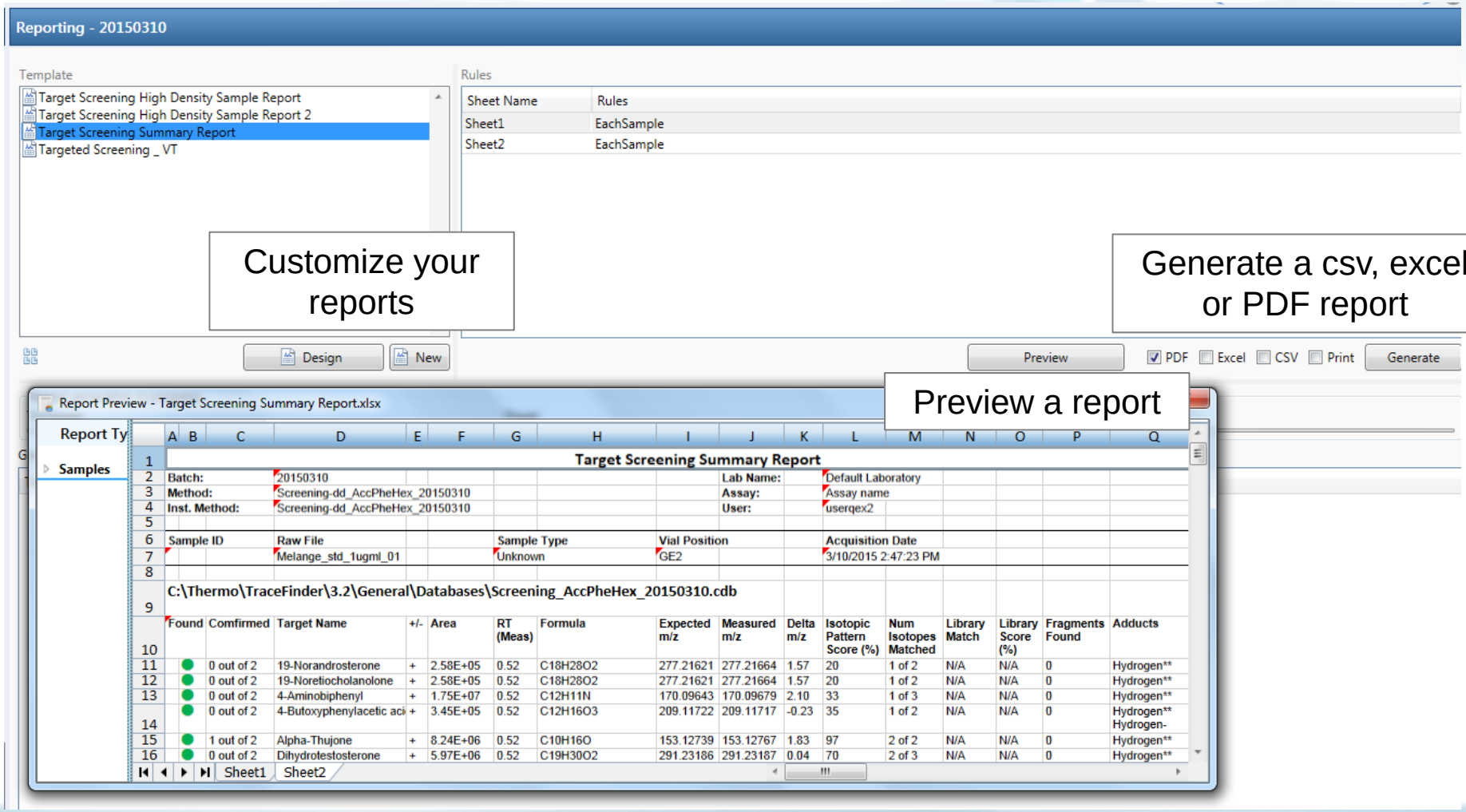


	Flag	Selecte	MZ	RT	IP	LS	Compound Name	Formula	Adduct	Confirme	Measured Area	m/z (Expected)	m/z (Delta (ppm	RT (Expected)	RT (Delta)	Isotopic Pattern S	Num Is
1							Cafeine	C8H10N4O2	M+H	3 out of 3	8.4338E09	195.0876	-1.1912	5.90	0.64	100	4 of 6
2							Morphine	C17H19NO3	M+H	3 out of 3	3.2043E07	286.1438	-2.0323	5.00	0.27	100	3 of 3
3							Sotalol	C12H20N2O3S	M+H	3 out of 3	2.5118E07	273.1267	-2.013	5.80	0.27	100	2 of 3
4							Acebutolol	C18H28N2O4	M+H	2 out of 3	8.2429E08	337.2122	-2.1461	7.50	0.18	100	4 of 4
5							Amiloride	C6H8ClN7O	M+H	1 out of 3	1.8732E08	230.0552	-1.9875	5.50	0.41	100	6 of 6
6							Carabamazepine epoxi	C15H12N2O2	M+H	0 out of 3	1.2625E06	253.0972	0.2519	7.60	-0.39	36	1 of 2
7							Carteolol	C16H24N2O3	M+H	2 out of 3	1.1641E09	293.186	-1.6514	6.20	0.71	100	4 of 4
8							Colchicine	C22H25NO6	M+H	0 out of 3	9.0536E06	400.1755	-2.7052	7.80	0.46	34	1 of 2
9							Cortisol_D3_exp	C21H27D3O5	M-H	1 out of 3	4.9355E07	364.2198	-1.1522	8.20	-0.21	100	3 of 3

Screening – Excel Export of a table



Report View



Industry Leading HRAM Library

Comprehensive HRAM Library and DB created on Thermo Scientific™ Q Exactive™ MS at R 140,000

Searchable in TraceFinder software

Consists of:

- Pesticides, Mycotoxins, Veterinary Drugs, Environmental Contaminates, PFCs
- Clin/Tox (Drugs of Abuse, Therapeutic Drugs, Poisons)
- The new spectra library will include the following: 3 ramped CE @ 20, 30, 40 eV and 2 step collision energies @ 40 with 50% and 70 with 50%
- Will contain RTs, and RRTs using the same group of ISDs for both EFS + Clin/Tox

EFS + Clin/Tox MS/MS Spectra to be available in mzCloud

Compound Groupings	Unique Entries	Total Spectra
Environmental and Food Safety	1,634	8,906
Clinical Research and Forensic Toxicology	926	4,630

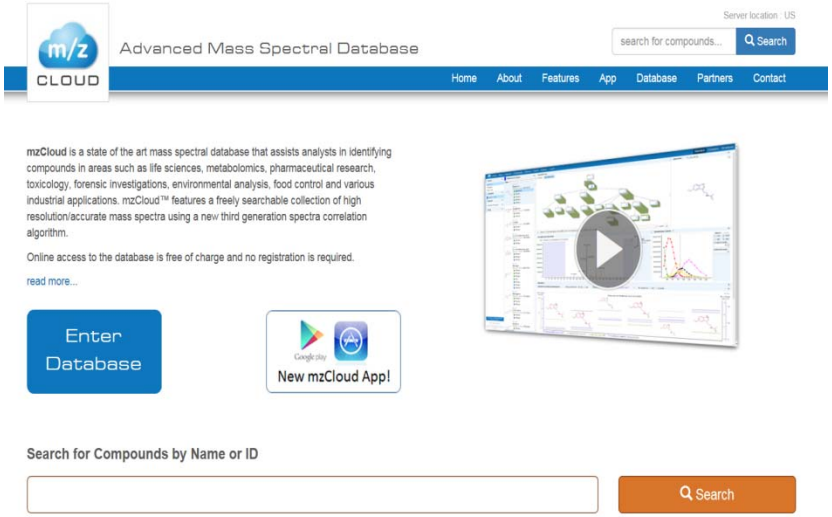
Compound Classes Provided in HRAM MS/MS Libraries

COMPOUND CLASS	
Food Safety and Environmental	**Forensic Toxicology**
Emerging Environmental Contaminants	Drugs of Abuse
Pesticides	Natural and Industrial Toxins
Veterinary Drugs	Prescription Drugs
Mycotoxins	Performance Enhancing Drugs
Perfluorinated Compounds (PFCs)	Other Drug Monitoring Research

mzCloud™

- A novel mass database/library of **MS/MS** and **MS^n** spectra (140.000 FWHM at m/z 200)
- Structural info for compounds even if they are not represented in the library through identification of **substructures**
- Multi-energy, Multi-fragment level, Multi-fragment technique
- Open consortium to establish a large public domain library which our software will link to

- https://www.mzcloud.org/



6,321 (+61) compounds | 9,985 (+78) trees | 1,923,641 (+8,811) spectra | 697,276 (+1,632) QM models | view more statistics

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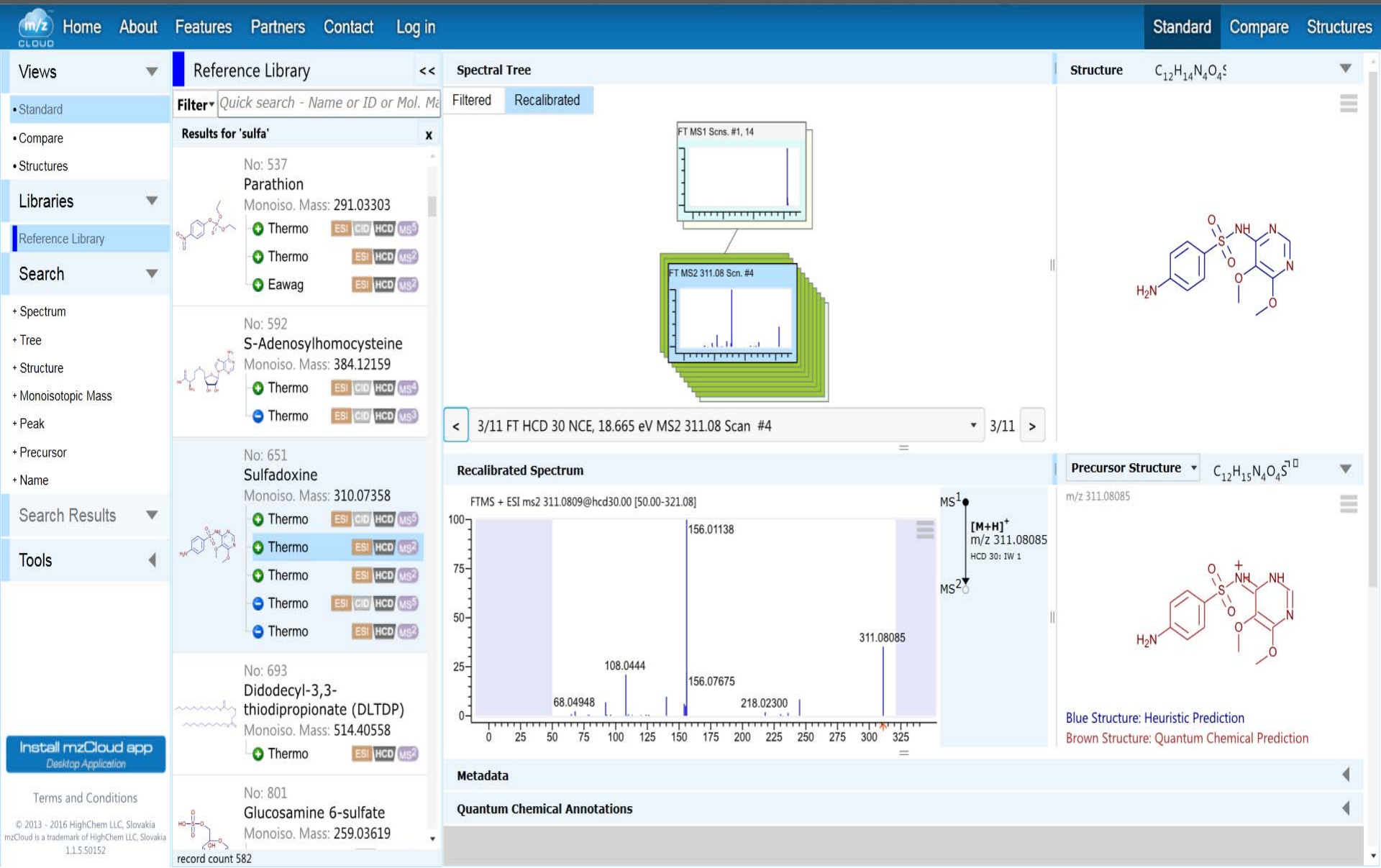

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