

ThermoFisher
S C I E N T I F I C

High Resolution MS in Forensic Toxicology Screening

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Thermo Fisher Scientific

The world leader in serving science

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

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 MS² and or MSⁿ data
- Screening applications utilize different types of mass spectrometers
 - **Ion Traps** : MS and MSⁿ 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 MS² experiments for the Thermo Scientific™ Q-Exactive™ Plus. **Full Scan followed by 4 vDIA events for the Thermo Scientific™ Q-Exactive Focus.**

Triple Quadrupole is great tool! but ?

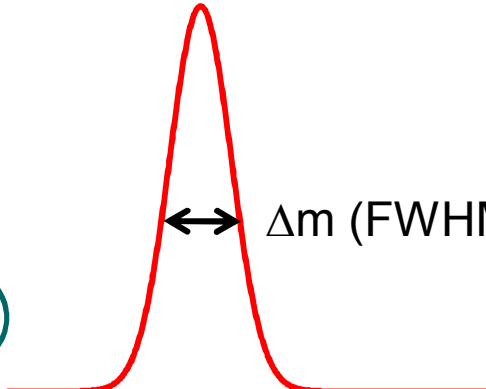
- It is only targeted!
- Selectivity provided by tandem MS/MS (SRM transition needed)
- False positives are reality!
- Need to setup instrument (SRM) before analysis
- Realistic breakpoint is 200-300 compounds in a run
- Time consuming data processing

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

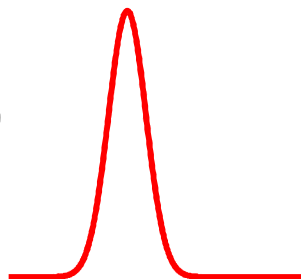
Mass Resolution FWHM

- Resolution**

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


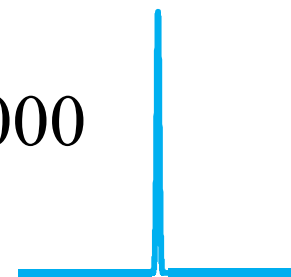
- Quadrupole MS

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



- Orbitrap (HRAM) MS

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



How Accurate Is Your Mass?

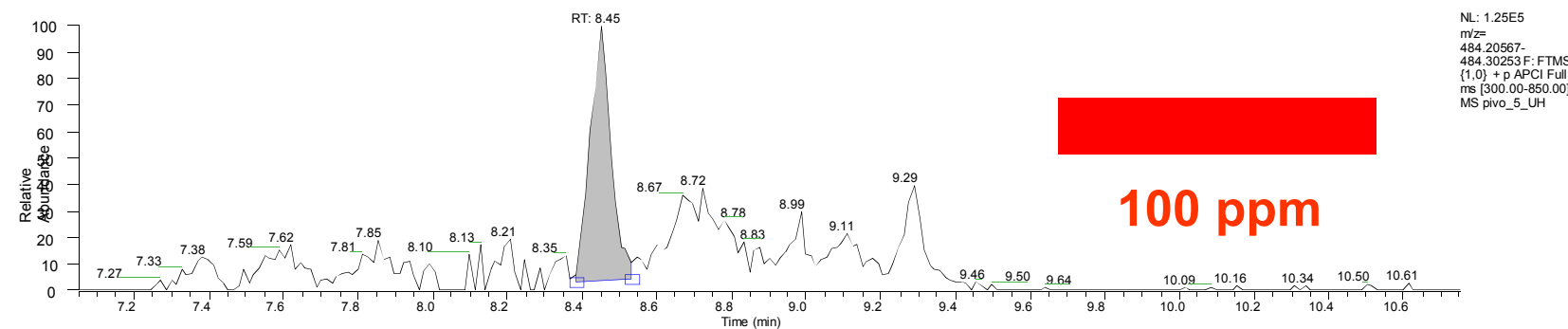
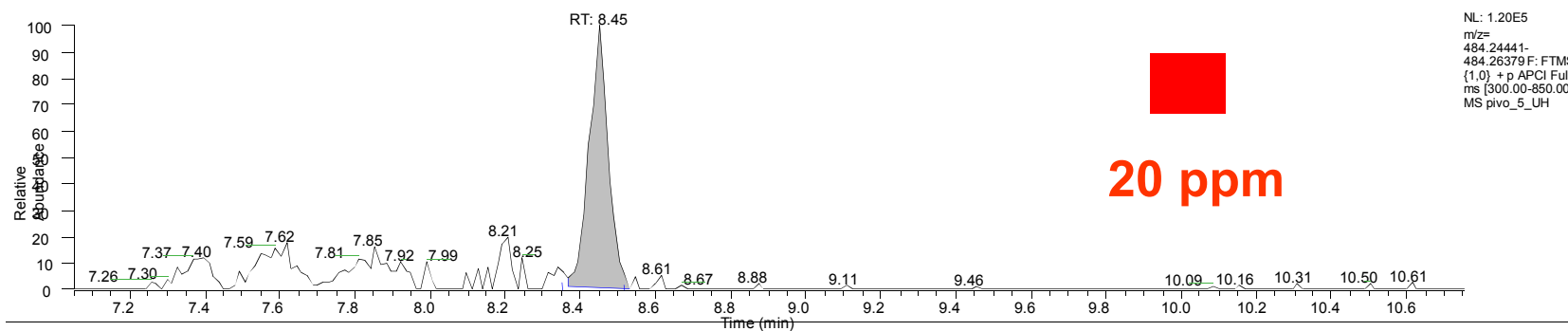
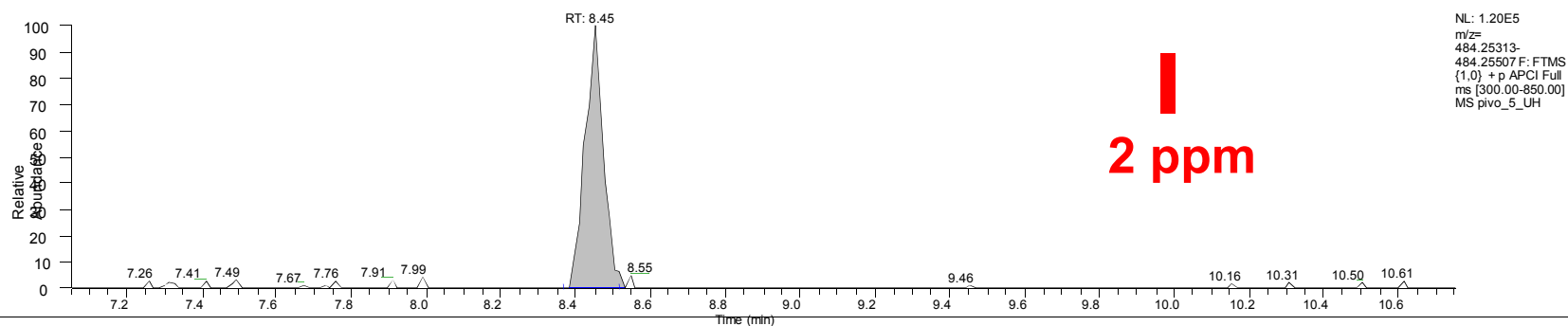
- Mass accuracy

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

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

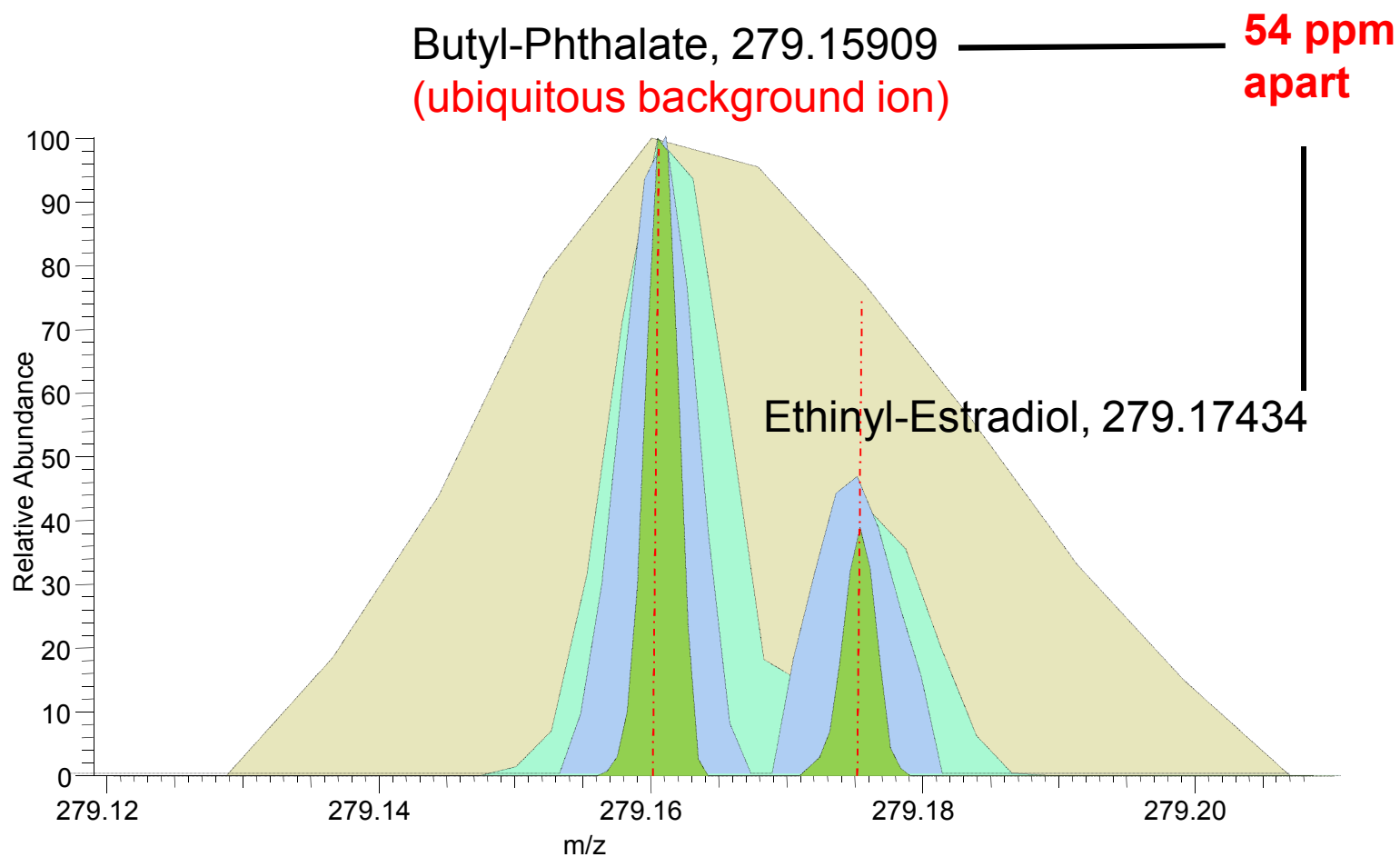
- Orbitrap MS
TOF MS $\Delta m / z = \frac{500.10314 - 500.10214}{500.10314} \cdot 10^6 = 2 ppm$

Selectivity Increases With Higher Mass Accuracy

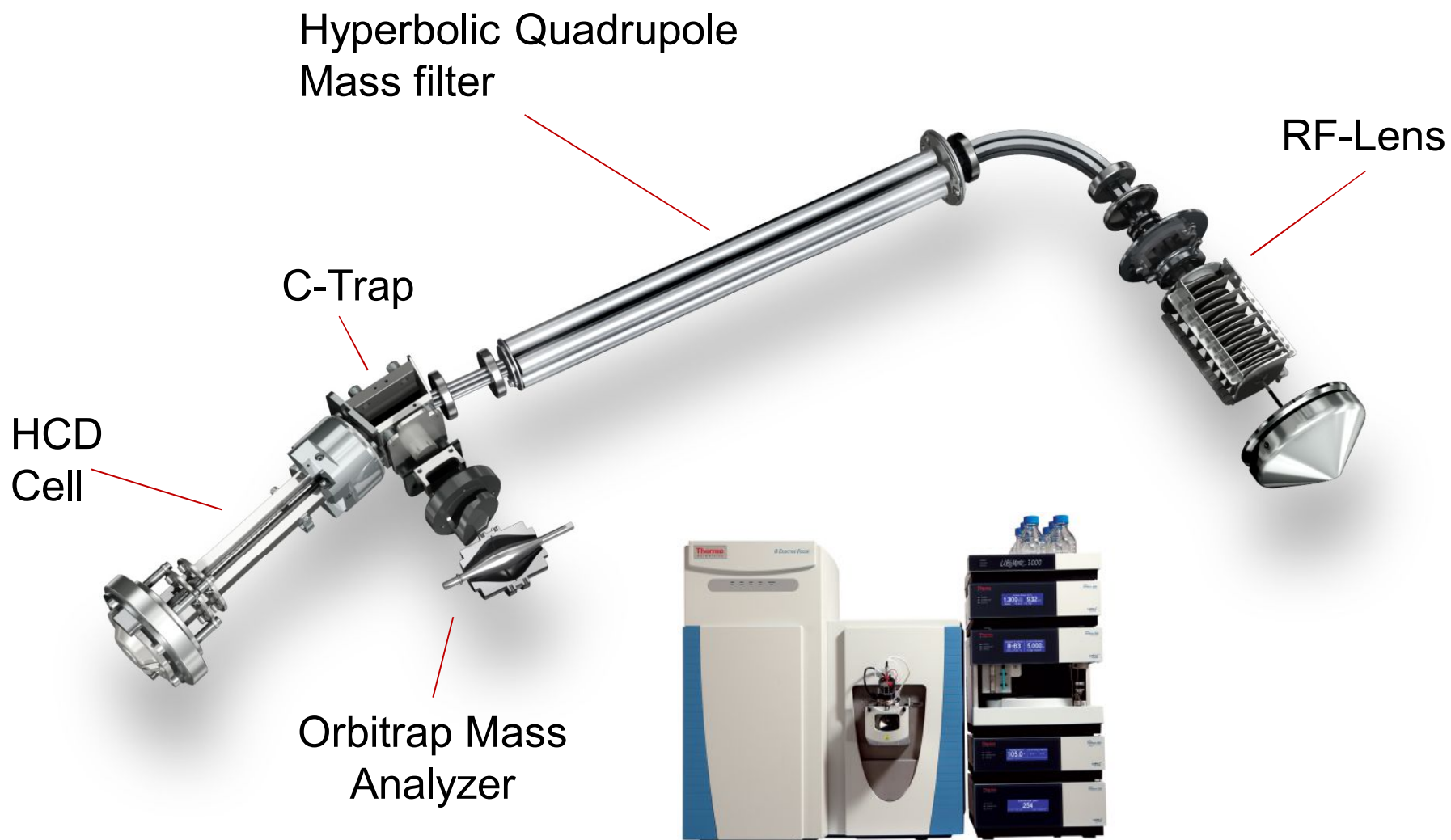


Specificity = Resolution + Mass Accuracy

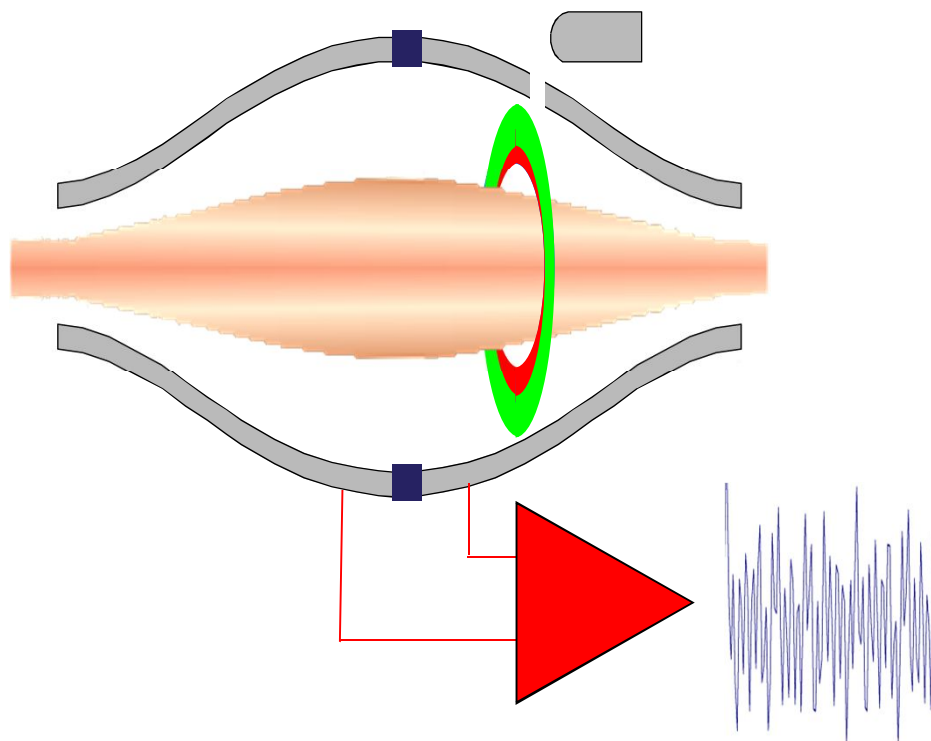
Resolution: 10k, 30k, 50k, 100k



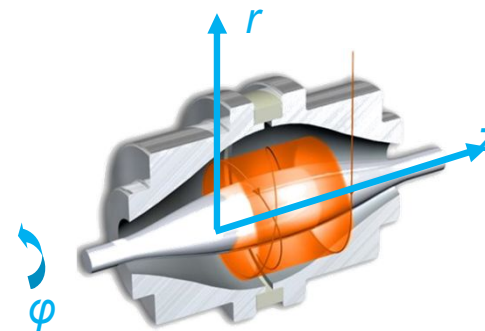
Q Exactive MS - a 3D view



Principle of Orbitrap MS Operation



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



Hyper-logarithmic potential distribution:
"ideal Kingdon trap"

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

Characteristic frequencies:

- Frequency of rotation ω_ϕ
- Frequency of radial oscillations ω_r
- Frequency of axial oscillations ω_z

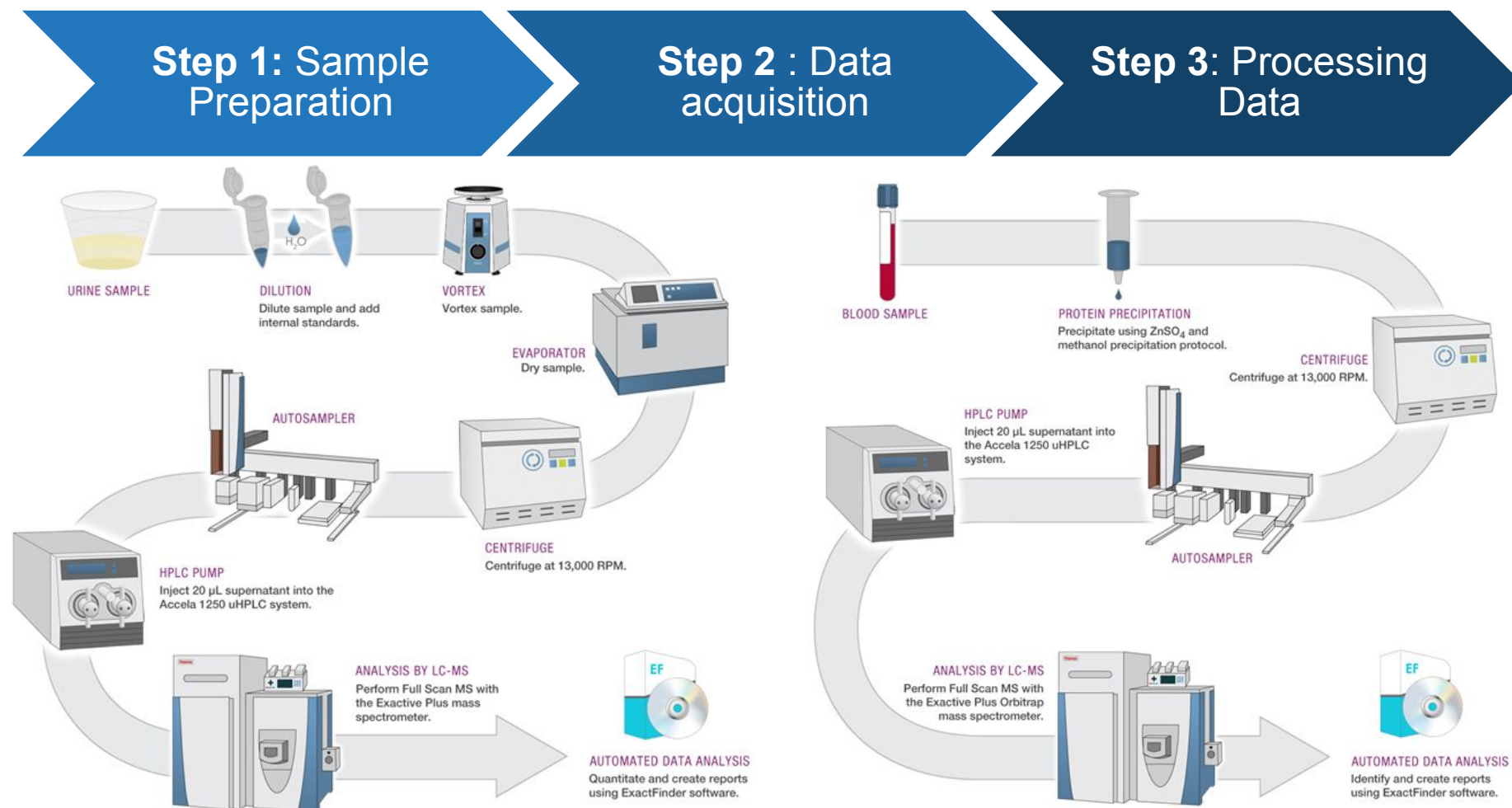
$$\omega_\phi = \frac{\omega_z}{\sqrt{2}} \sqrt{\left(\frac{R_m}{R}\right)^2 - 1} \quad \omega_r = \omega_z \sqrt{\left(\frac{R_m}{R}\right)^2 - 2}$$

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

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

Orbitrap technology – Workflow examples



Q Exactive Focus - Acquisition approaches

- 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 MS² mode. If the ion has a low intensity it is probable that it won't be selected for MS² 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

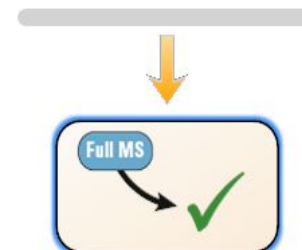
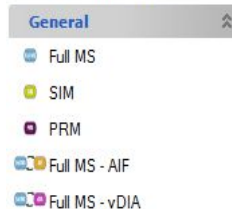
Full MS/ All Ion Fragmentation – vDIA*

- 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

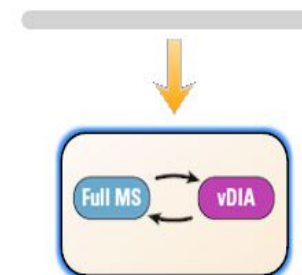
PRM (Parallel Reaction Monitoring)

- 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

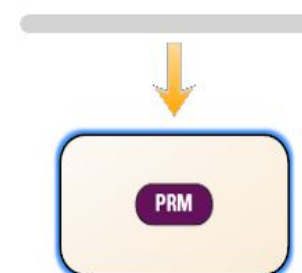
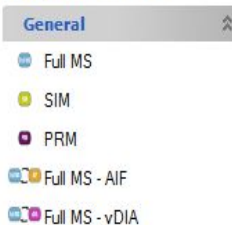
Experiments



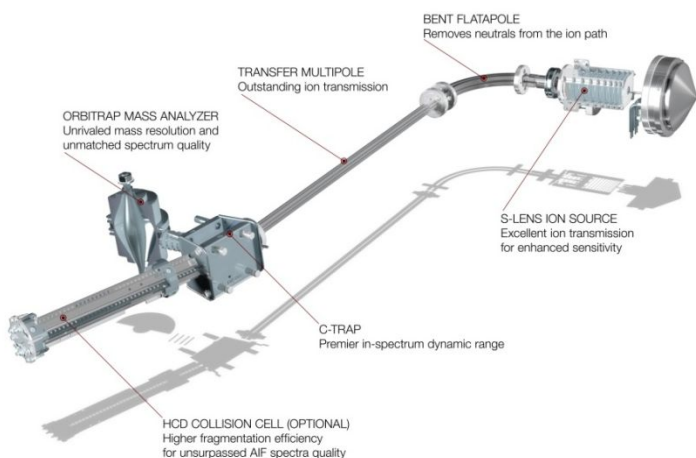
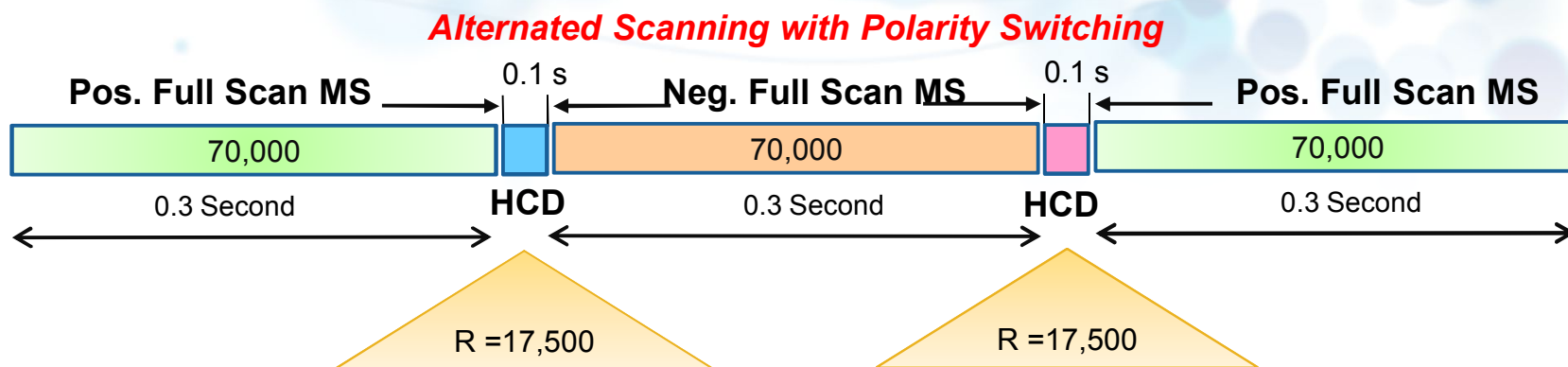
Experiments



Experiments



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 μ m

• Injection volume 20 μ L

• 30 mn or 15 mn Gradient

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**

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 MS²
- Same theme as TraceFinder
- Security

Get Results

Quick, Effortless, Accurate



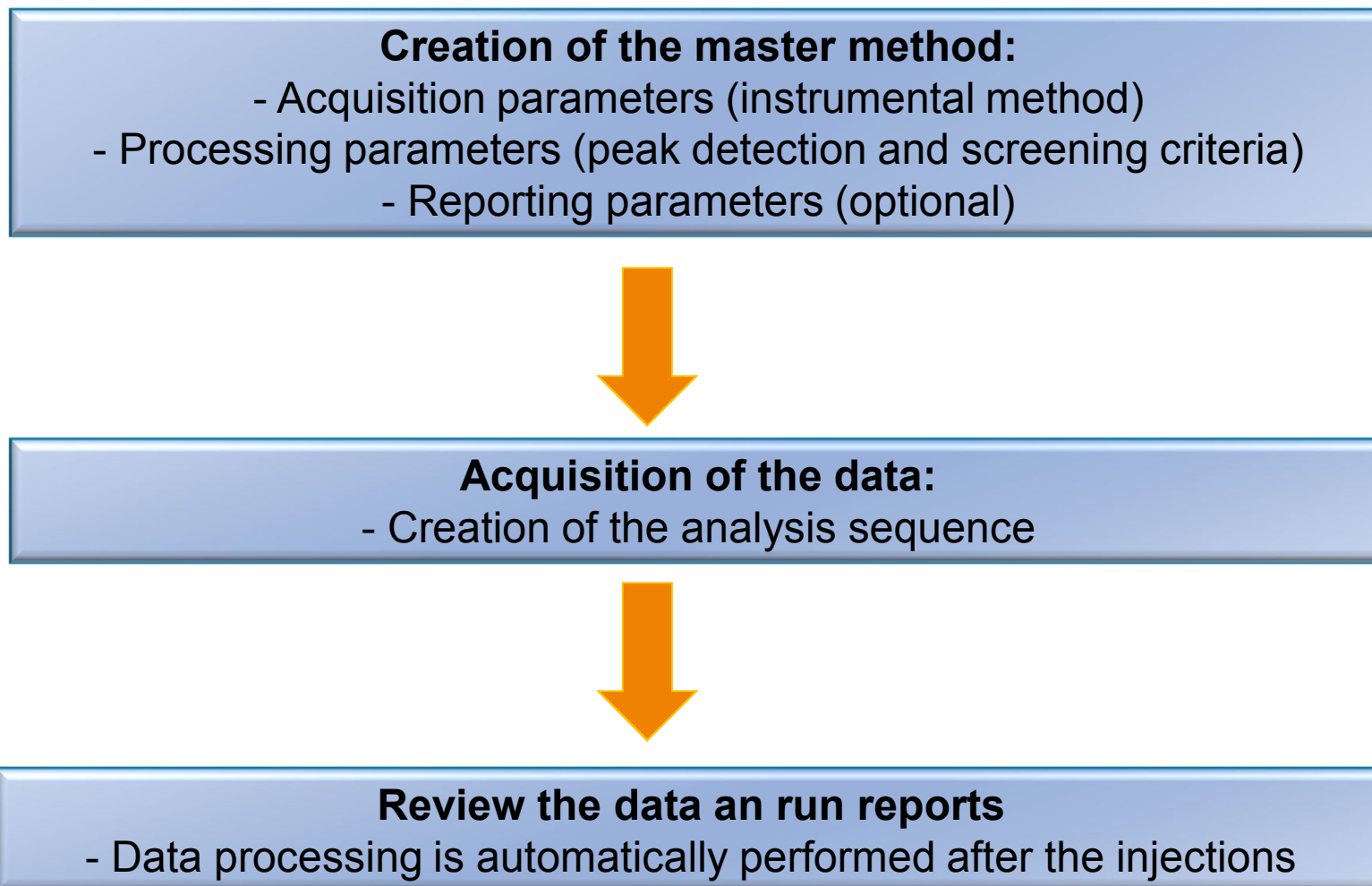
ToxFinder 1.0

Targeted Screening Software

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by copyright law and international treaties as
described in Help About.

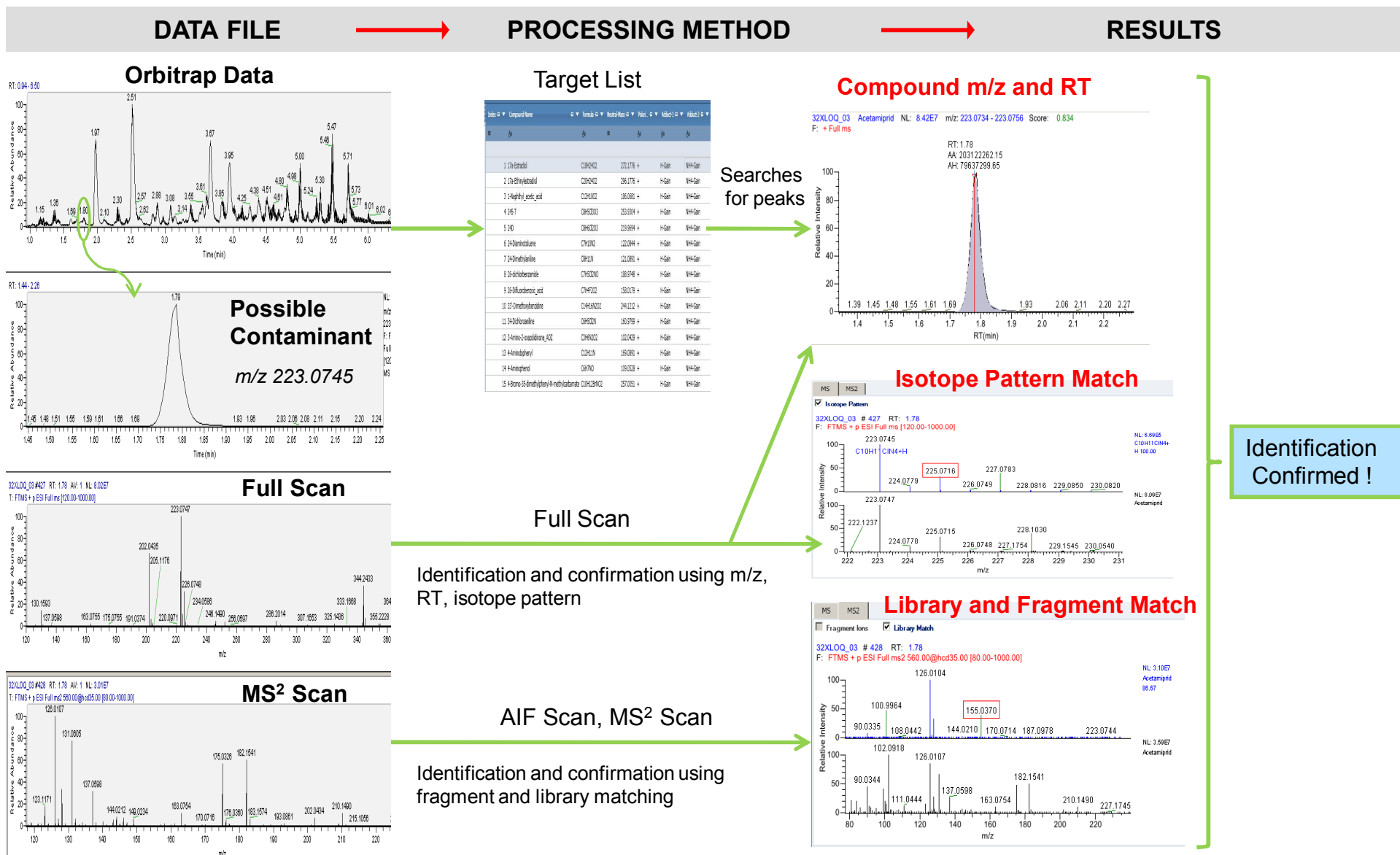
Thermo
SCIENTIFIC

TraceFinder : Screening workflow



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

Targeted / Untargeted Screening workflow



Data review - flags

The table results: flag code

The screenshot displays the Thermo TraceFinder LC interface. The main window shows a table of results for 'Screening_Example*'. The table has columns for 'Flag', 'MZ', 'IP', 'FI', 'Compound Name', 'Formula', 'Confirmed', 'm/z (Expected)', and 'm/z (Apex)'. The 'Flag' column contains green circles, yellow triangles, and red squares. Two callout boxes provide explanations for these flags. A blue box highlights the 'Flag', 'MZ', 'IP', and 'FI' columns. A blue arrow points from this box to the left callout. Another blue arrow points from the 'Confirmed' column to the right callout.

Flag	MZ	IP	FI	Compound Name	Formula	Confirmed	m/z (Expected)	m/z (Apex)
●	●	●	●	11-hydroxy-delta-9-THC	C21H30O3	2 out of 2	331.22677	331.22647
●	●	●	●	6-Monoacetylmorphine	C19H21NO4	2 out of 2	328.15433	328.15414
●	●	●	●	Amilofide	C17H27N3O4S	2 out of 2	370.1795	370.17929
●	●	●	●	Amilofide	C20H23N	2 out of 2	278.19033	278.19037
●	●	●	●	Amilofide	C20H25CIN2O5	2 out of 2	409.15248	409.15234
●	●	●	●	Antioyrine	C11H12N2O	2 out of 2	189.10224	189.10234
●	●	●	●	Aterolol	C14H22N2O3	2 out of 2	267.17032	267.17017
●	●	●	●	Atropine	C17H23NO3	2 out of 2	290.17507	290.17502
●	●	●	●	Benzocaine	C9H11NO2	2 out of 2	166.08625	166.08629
●	●	●	●	Benzoylgonine	C16H19NO3	2 out of 2	290.13868	290.13867
●	●	●	●	Biperiden	C21H29NO	2 out of 2	312.23219	312.23196
●	●	●	●	Buprenorphine	C29H41NO4	2 out of 2	468.31084	468.3107

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

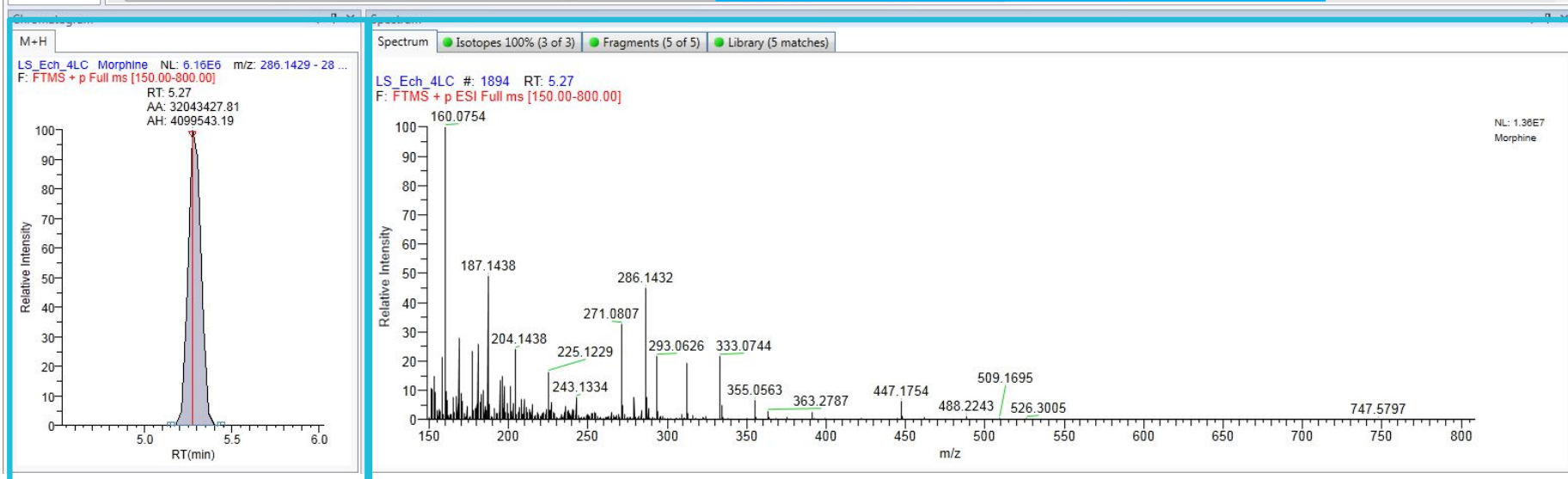
Data review – Chromatogram and spectrum

Data Review - Screening batch

Sam...	Compounds	Flag	MZ	RT	IP	LS	Compound Name	Formula	Adduct	Confirmed	Measured Area	m/z (Expected)	m/z (Delta ppm)	RT (Expected)	RT (Delta)	Isotopic Pattern Sco
1	CDB_Tox_dec2012_PR						Cafeine	C8H10N4O2	M+H	3 out of 3	8.4338E09	195.0876	-1.1912	5.90	0.64	100
							Morphine	C17H19NO3							0.27	100
							Sotalol	C12H20N2O							0.27	100
							Acebutolol	C18H28N2O							0.18	100
							Amiloride	C6H8ClN7O							0.41	100
							Carbamazepine epoxi	C15H12N2O							-0.39	36
							Carteolol	C16H24N2O							0.71	100
							Colchicine	C22H25NO6							0.46	34
							Cortisol_D3_exp	C21H27D3O							-0.21	100

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 profile, the fragmentation spectrum or the library spectrum comparison



The chromatogram view

The spectrum view

Data review – isotopic pattern

Data Review - Screening batch

Sam...	Compounds	Flag	Select	MZ	RT	IP	LS	Compound Name	Formula	Adduct	Confirmed	Measured Area	m/z (Expected)	m/z (Delta (ppm))	RT (Expected)	RT (Delta)	Isotopic Pattern Sco
1	CDB_Tox_dec2012_PR	●	✓	●	●	●	●	Cafeine	C8H10N4O2	M+H	3 out of 3	8.4338E09	195.0876	-1.1912	5.90	0.64	100
2		●	✓	●	●	●	●	Morphine	C17H19NO3	M+H	3 out of 3	3.2043E07	286.1438	-2.0323	5.00	0.27	100
3		●	✓	●	●	●	●	Sotalol	C12H20N2O3S	M+H	3 out of 3	2.5118E07	273.1267	-2.013	5.80	0.27	100
4		●	✓	●	●	●	●	Acebutolol	C18H28N2O4	M+H	2 out of 3	8.2429E08	337.2122	-2.1461	7.50	0.18	100
5		●	✓	●	●	●	●	Amiloride	C6H8CIN7O	M+H	1 out of 3	1.8732E08	230.0552	-1.9875	5.50	0.41	100
6		●	✓	●	●	●	●	Carabamazepine epoxi	C15H12N2O2	M+H	0 out of 3	1.2625E06	253.0972	0.2519	7.60	-0.39	36
7		●	✓	●	●	●	●	Carteolol	C16H24N2O3	M+H	2 out of 3	1.1641E09	293.186	-1.6514	6.20	0.71	100
8		●	✓	●	●	●	●	Colchicine	C22H25NO6	M+H	0 out of 3	9.0536E06	400.1755	-2.7052	7.80	0.46	34
9		●	✓	●	●	●	●	Cortisol_D3_exp	C21H27D3O5	M-H	1 out of 3	4.9355E07	364.2198	-1.1522	8.20	-0.21	100

Chromatogram
M+H
LS_Ech_4LC Morphine NL: 6.16E6 m/z: 286.1429 - 28...
F: FTMS + p Full ms [150.00-800.00]
RT: 5.27
AA: 32043427.81
AH: 4099543.19

Spectrum
Spectrum ● Isotopes 100% (3 of 3) ● Fragments (5 of 5) ● Library (5 matches)
LS_Ech_4LC
F: FTMS + p ESI Full ms [150.00-800.00]
Scan #: 1882-1918 RT: ...

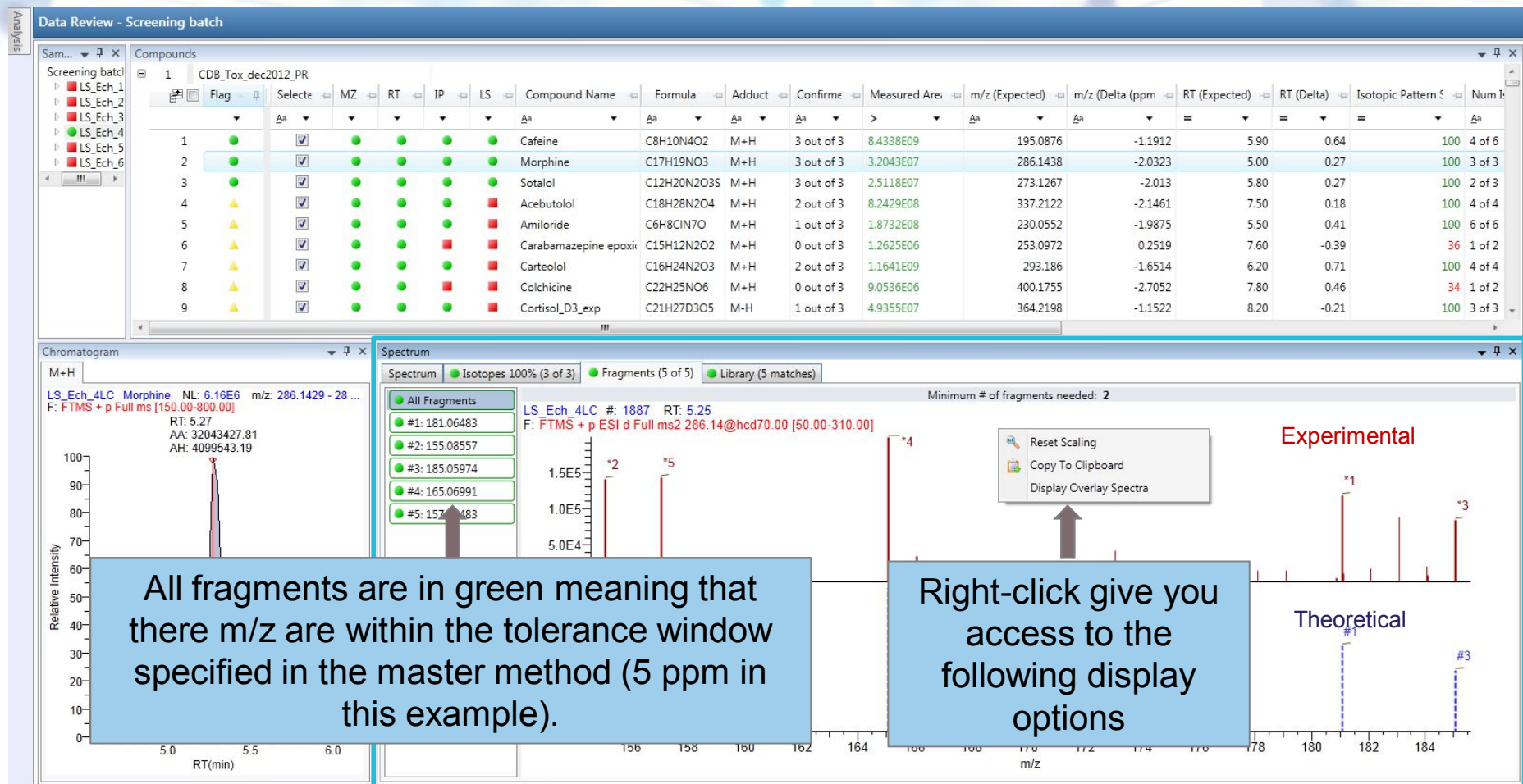
Right-click give you access to the following display options:

- Reset Scaling
- Copy To Clipboard
- Display Overlay Spectra
- Hide Resolution Label
- Show Noise Label

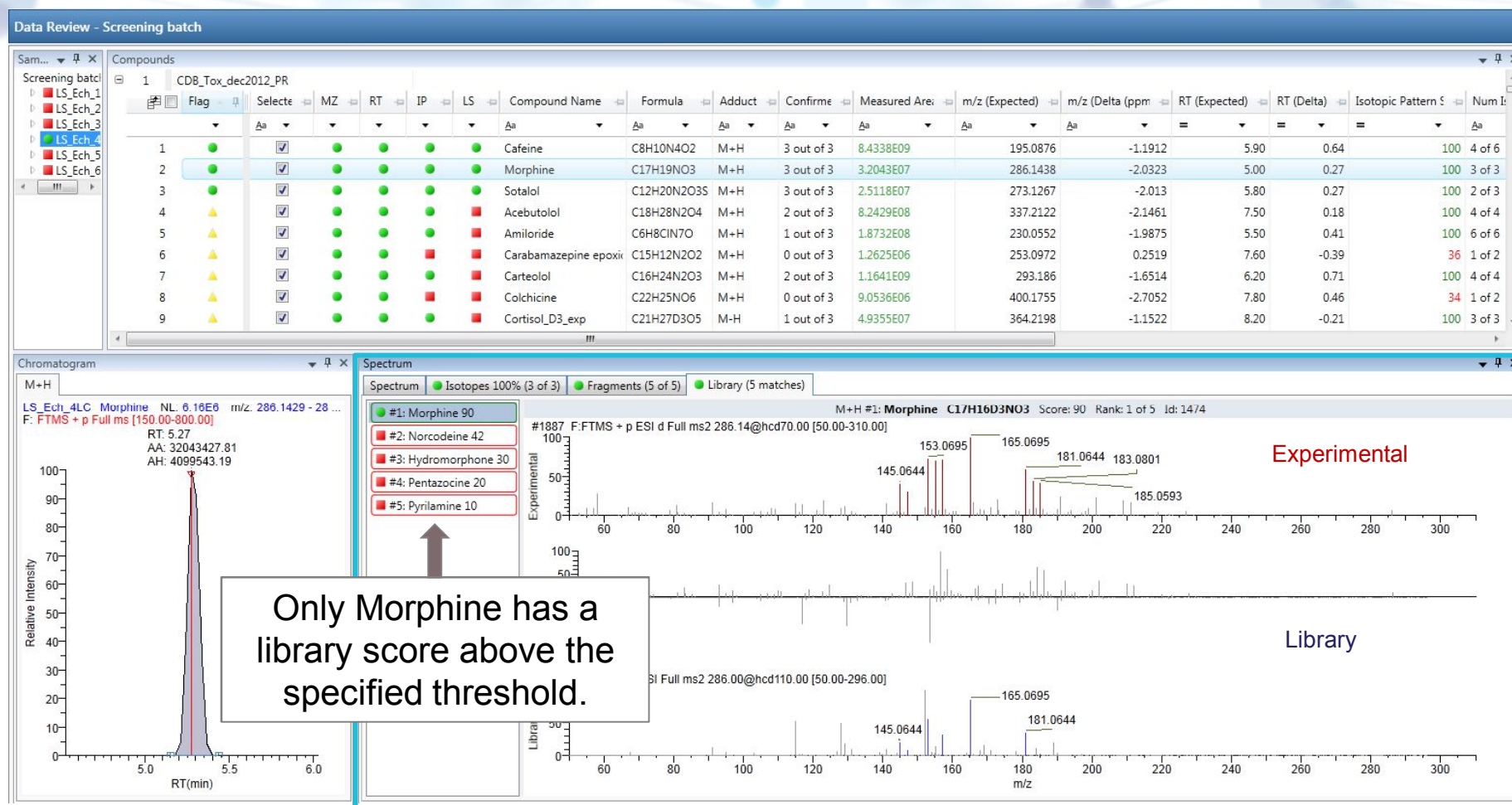
All isotopes are in green meaning that they fit with the predicted spectrum in terms of mass and intensity (according to the criteria of the master method)

Isotopic Pattern Identify Confirm Fit Threshold (%) 80
Allowed Mass Deviation (ppm) 5
Allowed Intensity Deviation (%) 15
Use Internal Mass Calibration

Data review - Fragments



Data review – library comparison



Screening – Excel Export of a table

Thermo TraceFinder LC

Real time status | User: valerie.thibert | ? | ⚙️
Waiting for Device Initialization

File View Tools Help

New
Open
Save
Print batch

Export data to
Recent Files
Exit

Target Screening

Report View

Local Method

Acquisition
Screening
Processing
Peak Detection
Reports

Acquisition
Analysis
Method Development

Data Review - 20150310

Samples	Compounds	File	MZ	IP	FI	LS	Compound Name	Lib Match Name	Formula
							Bromazepam	N/A	C14H10BrN3O
							Cetirizine	N/A	C21H25ClN2O3
							Citalopram	N/A	C20H21FN2O
							Clobazam	N/A	C16H13ClN2O2
							Clonazepam	N/A	C15H10ClN3O3
							propoxypl	N/A	C22H29NO2
							pam	N/A	C16H13ClN2O
							nhydramin	N/A	C17H21NO
							amine	N/A	C17H22N2O
							lopram	N/A	C20H21FN2O

Data Review Export

Filename: C:\TraceFinderData\32\Projects\Screening\20150310.xlsx

File Format
 Excel CSV

Sheet Layout
 Multiple Worksheets Single Worksheet

Data To Export
 Export selected rows (visible columns only)
 Export all batch data

Export Cancel

Relative Intensity

RT(min) m/z

NL: 7.74E8
Diazepam

39
13.0711

0 700 800 900 1000

Report View

Reporting - 20150310

Template

- Target Screening High Density Sample Report
- Target Screening High Density Sample Report 2
- Target Screening Summary Report
- Targeted Screening _ VT

Rules

Sheet Name	Rules
Sheet1	EachSample
Sheet2	EachSample

Design New Preview PDF Excel CSV Print Generate

Customize your reports

Generate a csv, excel or PDF report

Preview a report

Report Preview - Target Screening Summary Report.xlsx

Report Ty	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
Target Screening Summary Report																	
1																	
2	Batch:	20150310					Lab Name:	Default Laboratory									
3	Method:	Screening-dd_AccPheHex_20150310					Assay:	Assay name									
4	Inst. Method:	Screening-dd_AccPheHex_20150310					User:	userqex2									
5																	
6	Sample ID	Raw File	Sample Type	Vial Position	Acquisition Date												
7		Melange_std_tugml_01	Unknown	GE2	3/10/2015 2:47:23 PM												
8																	
9	C:\Thermo\TraceFinder\3.2\General\Databases\Screening_AccPheHex_20150310.cdb																
10	Found	Confirmed	Target Name	+/-	Area	RT (Meas)	Formula	Expected m/z	Measured m/z	Delta m/z	Isotopic Pattern Score (%)	Num Isotopes Matched	Library Match	Library Score (%)	Fragments Found	Adducts	
11	●	0 out of 2	19-Norandrosterone	+	2.58E+05	0.52	C18H28O2	277.21621	277.21664	1.57	20	1 of 2	N/A	N/A	0	Hydrogen**	
12	●	0 out of 2	19-Noretiocholanolone	+	2.58E+05	0.52	C18H28O2	277.21621	277.21664	1.57	20	1 of 2	N/A	N/A	0	Hydrogen**	
13	●	0 out of 2	4-Aminobiphenyl	+	1.75E+07	0.52	C12H11N	170.09643	170.09679	2.10	33	1 of 3	N/A	N/A	0	Hydrogen**	
14	●	0 out of 2	4-Butoxyphenylacetic aci	+	3.45E+05	0.52	C12H16O3	209.11722	209.11717	-0.23	35	1 of 2	N/A	N/A	0	Hydrogen**	
15	●	1 out of 2	Alpha-Thujone	+	8.24E+06	0.52	C10H16O	153.12739	153.12767	1.83	97	2 of 2	N/A	N/A	0	Hydrogen**	
16	●	0 out of 2	Dihydrotestosterone	+	5.97E+06	0.52	C19H30O2	291.23186	291.23187	0.04	70	2 of 3	N/A	N/A	0	Hydrogen**	

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

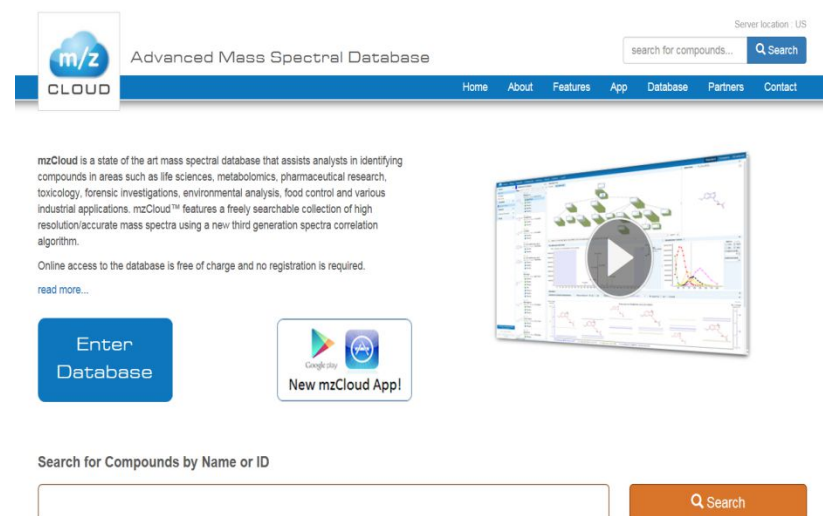
Compound Groupings	Unique Entries	Total Spectra
Environmental and Food Safety	1,634	8,906
Clinical Research and Forensic Toxicology	926	4,630

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

- A novel mass database/library of **MS/MS** and **MSⁿ** 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/>



The screenshot shows the mzCloud website interface. At the top, there is a navigation bar with links for Home, About, Features, App, Database, Partners, and Contact. A search bar is located in the top right corner. Below the navigation bar, there is a main content area with a large blue button labeled "Enter Database" and a "New mzCloud App!" button. A search bar is also present in the main content area, labeled "Search for Compounds by Name or ID".

6,321 (+61)
compounds

9,985 (+78)
trees

1,923,641 (+8,811)
spectra

697,276 (+1,632)
QM models

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Views

- Standard
- Compare
- Structures

Libraries

- Reference Library

Search

- Spectrum
- Tree
- Structure
- Monoisotopic Mass
- Peak
- Precursor
- Name

Search Results

Tools

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Reference Library

Filter Quick search - Name or ID or Mol. Mass

Results for 'sulfa' x

No: 537
Parathion
Monoiso. Mass: 291.03303

- Thermo ESI CID HCD MS⁵
- Thermo ESI HCD MS²
- Eawag ESI HCD MS²

No: 592
S-Adenosylhomocysteine
Monoiso. Mass: 384.12159

- Thermo ESI CID HCD MS⁴
- Thermo ESI CID HCD MS³

No: 651
Sulfadoxine
Monoiso. Mass: 310.07358

- Thermo ESI CID HCD MS⁵
- Thermo ESI HCD MS²
- Thermo ESI HCD MS²
- Thermo ESI CID HCD MS⁵
- Thermo ESI HCD MS²

No: 693
Didodecyl-3,3-thiodipropionate (DLTDP)
Monoiso. Mass: 514.40558

- Thermo ESI HCD MS²

No: 801
Glucosamine 6-sulfate
Monoiso. Mass: 259.03619

record count 582

Spectral Tree

Filtered Recalibrated

3/11 FT HCD 30 NCE, 18.665 eV MS2 311.08 Scan #4 3/11

Recalibrated Spectrum

FTMS + ESI ms2 311.0809@hcd30.00 [50.00-321.08]

MS¹ [M+H]⁺ m/z 311.08085 HCD 30: 1W 1

MS²

Structure C₁₂H₁₄N₄O₄S

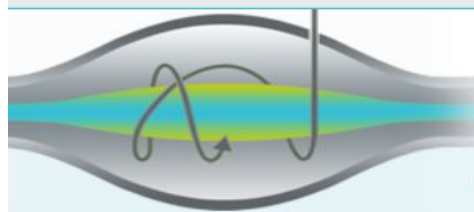
Precursor Structure C₁₂H₁₅N₄O₄S⁺

m/z 311.08085

Blue Structure: Heuristic Prediction
Brown Structure: Quantum Chemical Prediction

Metadata

Quantum Chemical Annotations



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