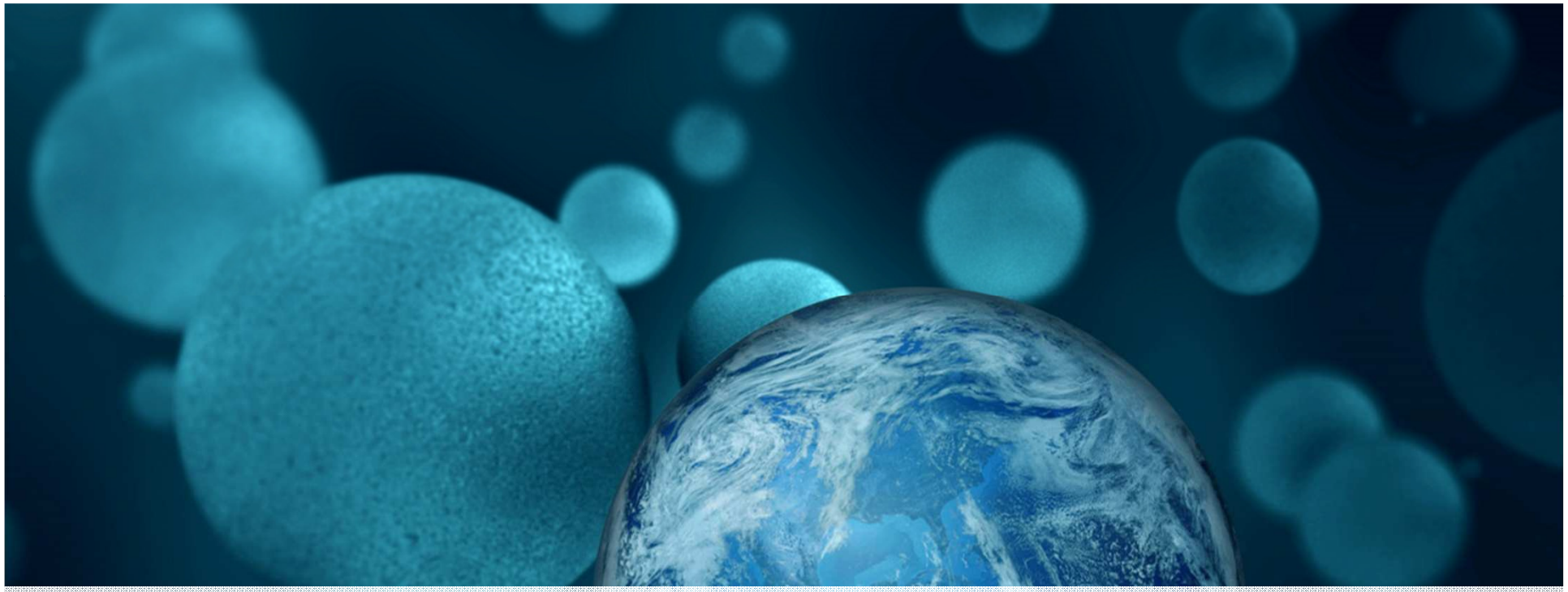

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Forensic Toxicology WorkFlow

Gerhard Stadlmann
Sales Manager HPLC, LCMS EMEA

Summary

- Liquid Chromatography

- Mass Spectrometry

- Softwares
- Applications
- Trendy Techniques

TraceFinder™
The Power to Know

ToxFinder 1.0
Targeted Screening Software

m/z CLOUD

iRC Pro

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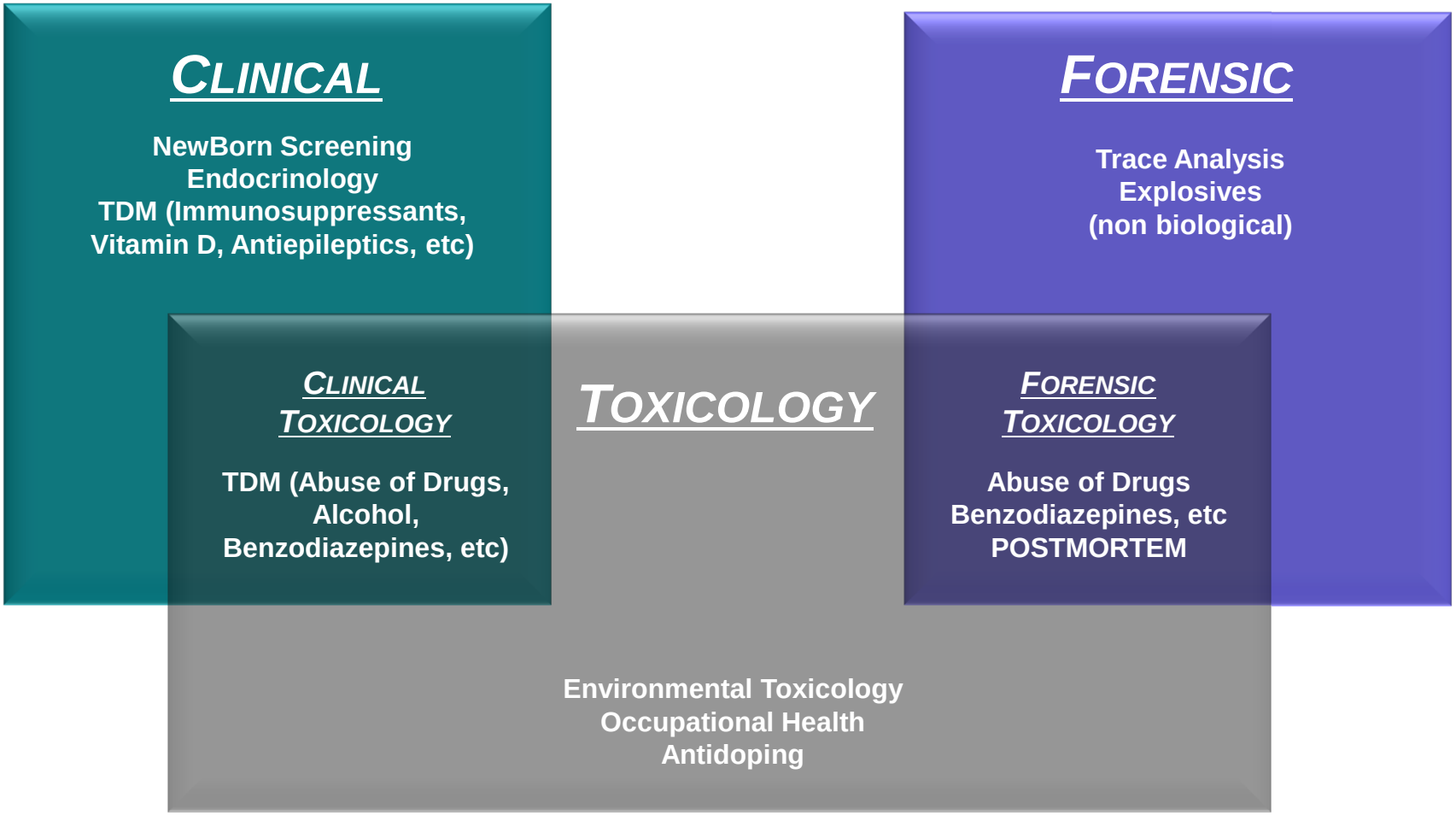
Clinical and Forensic - A Lot of Overlapping Areas



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Customers Expectations/Requirements

I want to perform targeted screening with database/library searching	• I want to be able to screen samples using a database of at least 1000 compounds • I want to be able to use offline and online spectral libraries
I want to identify unknown and need retrospective analysis	• I want to search for totally unknown compounds in addition to targeted screening • I need to retrospectively interrogate a sample searching for new/unexpected compounds
I want to do quantitation	• I want ultimate sensitivity • I want to quantify small concentrations in heavy matrices • I want to quantify more than 1000 compounds in a run

Screening Approaches – Some Definitions

Targeted Screening **“Search for what you know”**	• Compound search after targeted acquisition using a specific list of compounds • Detection parameters need to be numerous and selective to avoid false positives
Untargeted Screening **“Search for what you suspect”**	• Compound search following acqusition based on most intense signals NOT on a list of compounds • Identification based on compound databases and/or spectral libraries
Unknown Screening **“Search for the rest”**	• Search of compounds with an totally untargeted acquisition • Putative identification typically followed by structural elucidation

Screening Workflow

Sample Preparation → **Data acquisition** → **Data Processing**

- Related to sample matrix (urine, blood, serum, plasma, etc)

CLEAN ↓ / EASY ↑

Filtration
Dilute-and-Shoot
Protein Precipitation
Solid Phase Extraction
TurboFlow
Liquid Liquid Extraction
Supported Liquid Extraction

- Ion Trap

- Triple Quadrupole

- High Resolution MS

- TraceFinder
- Compound databases
- Spectral libraries

- mzCloud
- Compound Discoverer

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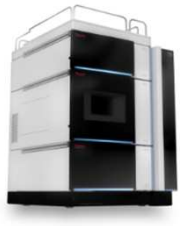
Front Ends and Sample Preparation

Liquid Chromatography

Ultimate 3000
Flexibility



Vanquish
High-end UHPLC

- Require offline sample preparation
- Suitable for multi-channel solutions

TurboFlow

Transcend II
Flexibility

Prelude
Robustness

- Require minimal or no sample preparation
- Suitable for multi-channel solutions

Paperspray

Velox 360
Simplicity

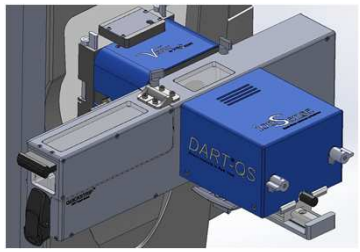
- NO sample preparation
- Simple to use
- Fast

Third Party Vendors Ion Sources

- Paper Spray (Prosolia)
  - No sample preparation

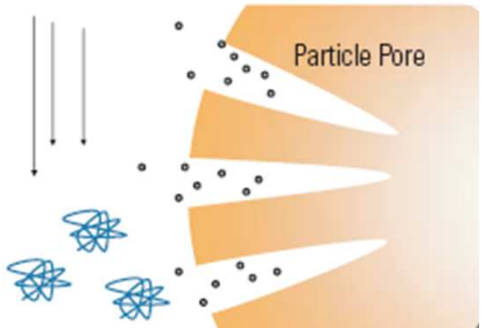
- LDTD (Phytronix)
  - Some sample preparation

- DART (IonSense, Inc.)
  - Some or no sample preparation



TurboFlow for Online Sample Extraction

- Online sample extraction that adds less than 2 minutes to the normal LC runtime
- Allows for the direct injection of complex matrices
- A cleaner sample reaches the mass spec
  - less background noise → better sensitivity
  - longer LC column lifetime
  - cleaner mass spec → less routine cleaning
- You can inject more (up to 100µL)
  - better sensitivity



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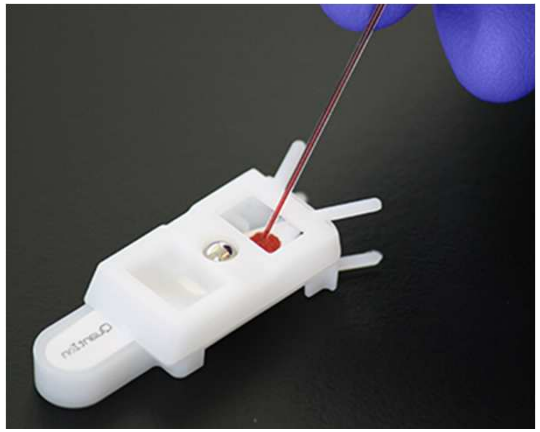
Paperspray – No Sample Preparation



http://www.prosolia.com/resources/videos/velox-360

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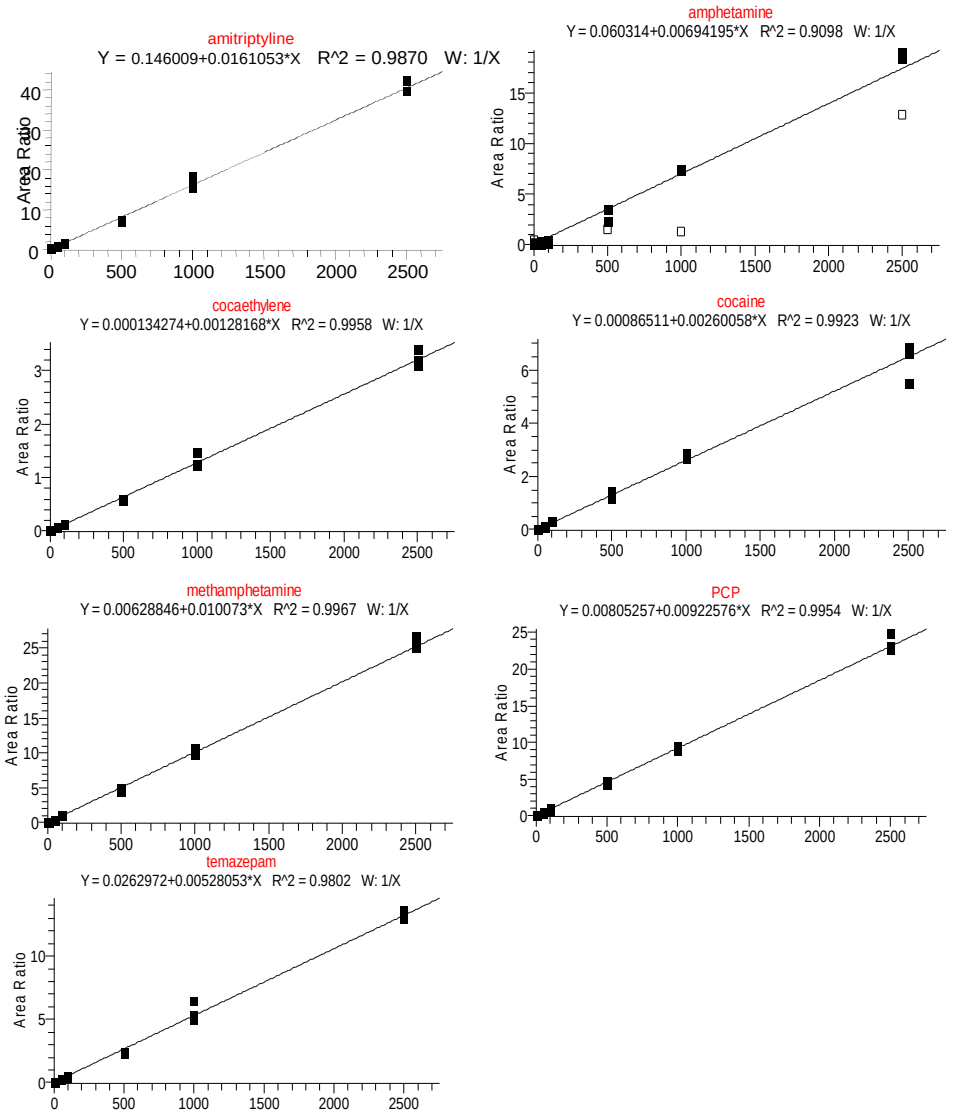
Paperspray on TSQ Quantiva – DoA Quantification in Urine

- LOQs in the 0.5 to 50 ng/mL
- LODs in the 0.5-1 ng/mL
- No sample preparation
- No chromatography

Amitriptyline
Amphetamine
Cocaethylene
Cocaine
Codeine
Methamphetamine
PCP
Temazepam



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Mass Spectrometers – A Leading 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

Triple Quadrupoles for Targeted Screening and Quantitation

TSQ Endura

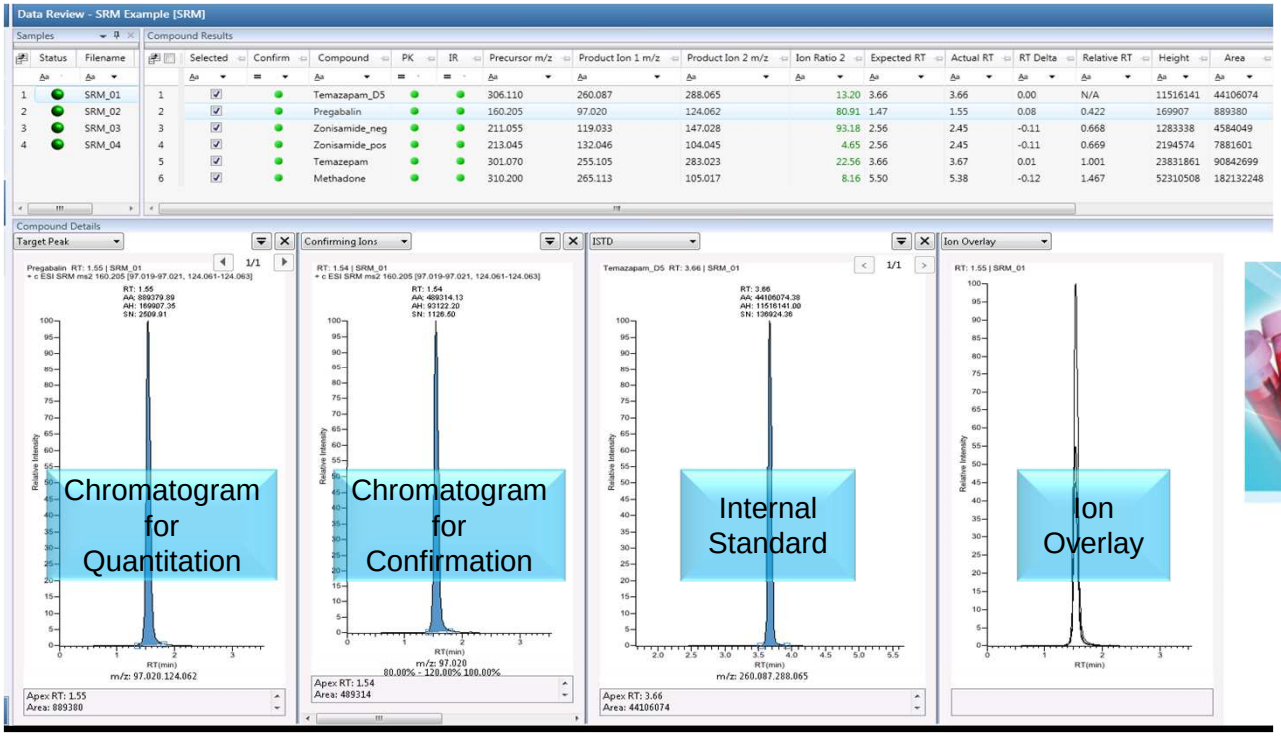
Extreme Quantitative Value

- Best-in-class performance
- Unprecedented usability
- Exceptional robustness

TSQ Quantiva

Extreme Quantitative Performance

- Attogram sensitivity
- Unprecedented usability
- Exceptional robustness



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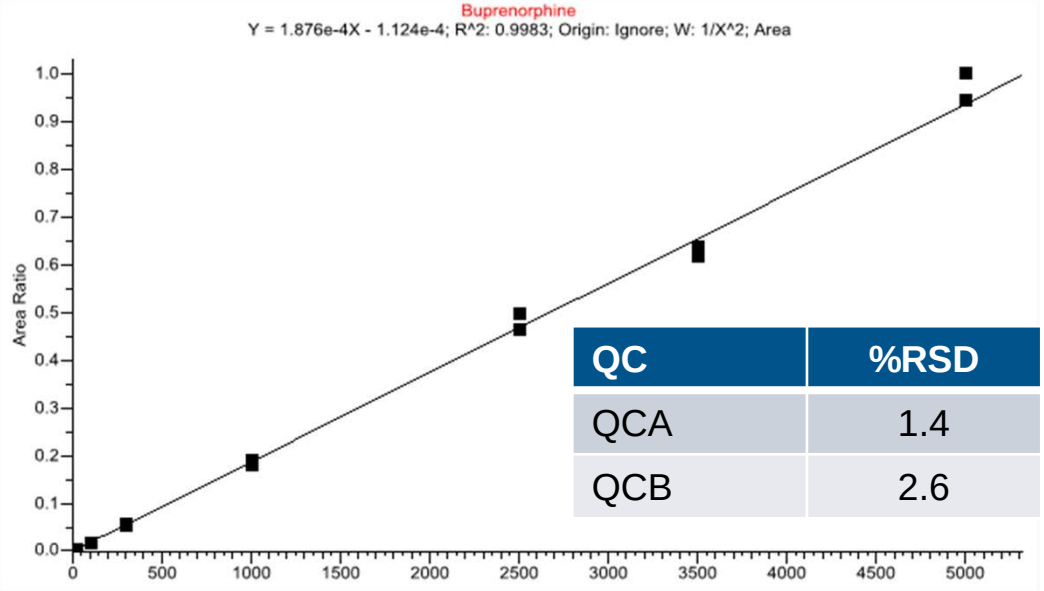
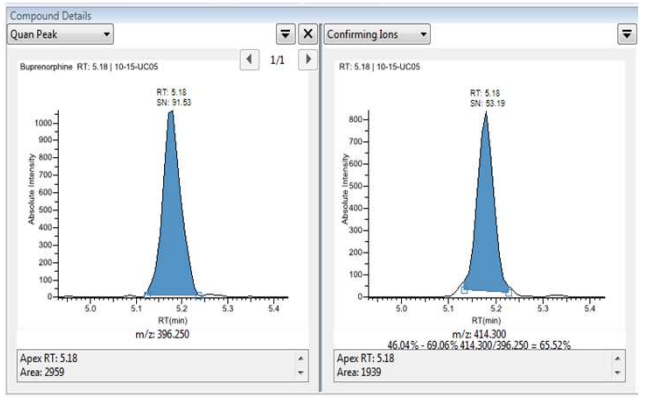
Triple Quadrupoles for Quantitation

- Quantitation of 72 Drugs of Abuse on Urine on TSQ Quantiva
  - Simply dilute-and-shoot
  - Polarity switching
  - One SRM for quantitation and one or two for confirmation
  - Calibration range 5 to 5000 ng/mL



QC	%RSD
QCA	1.4
QCB	2.6

QUANTIFIER QUALIFIER



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Why Q Exactive and Q Exactive Focus MS for Forensic Labs?

Versatile lab of the future

- Targeted screening
- Untargeted screening
- Unknown screening
- Quantitative confirmation

Ultimate performance

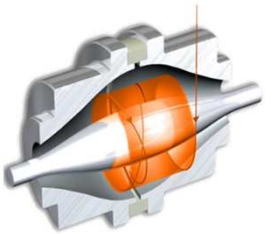
- High sensitivity
- High selectivity
- High Resolution & Mass Accuracy
- Polarity switching

Ease of use

- Simple calibration, holds for days
- No need for optimization
- No compound-specific setup
- Robust platform, low maintenance

Affordable Focus version

- Price comparable to high end Triple Quad



Your High Resolution Accurate Mass lab of the future

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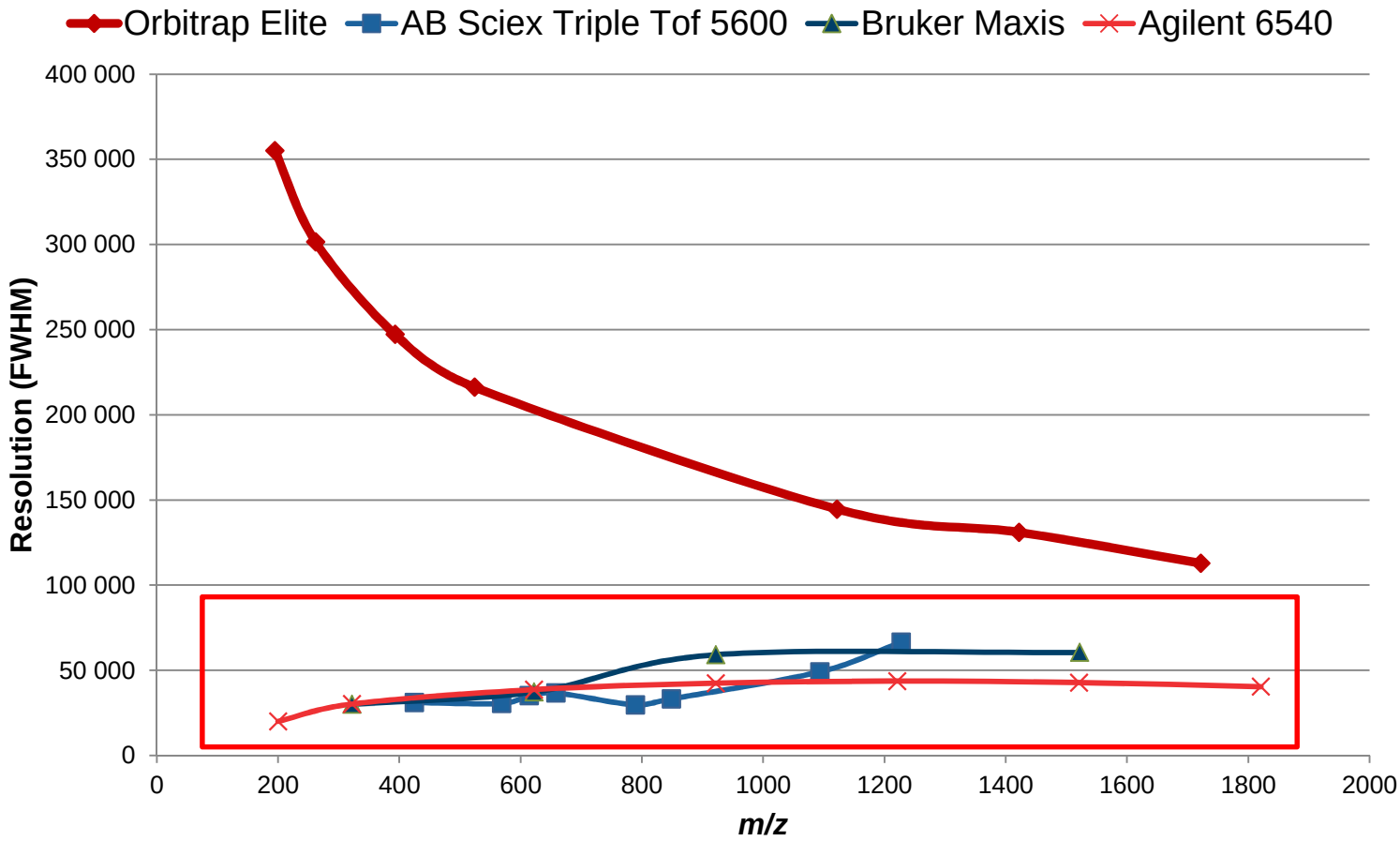
Resolution vs. Competition



- *Outperforming High Res QTofs by a factor >5*
- *2013 Fusion by a magnitude*

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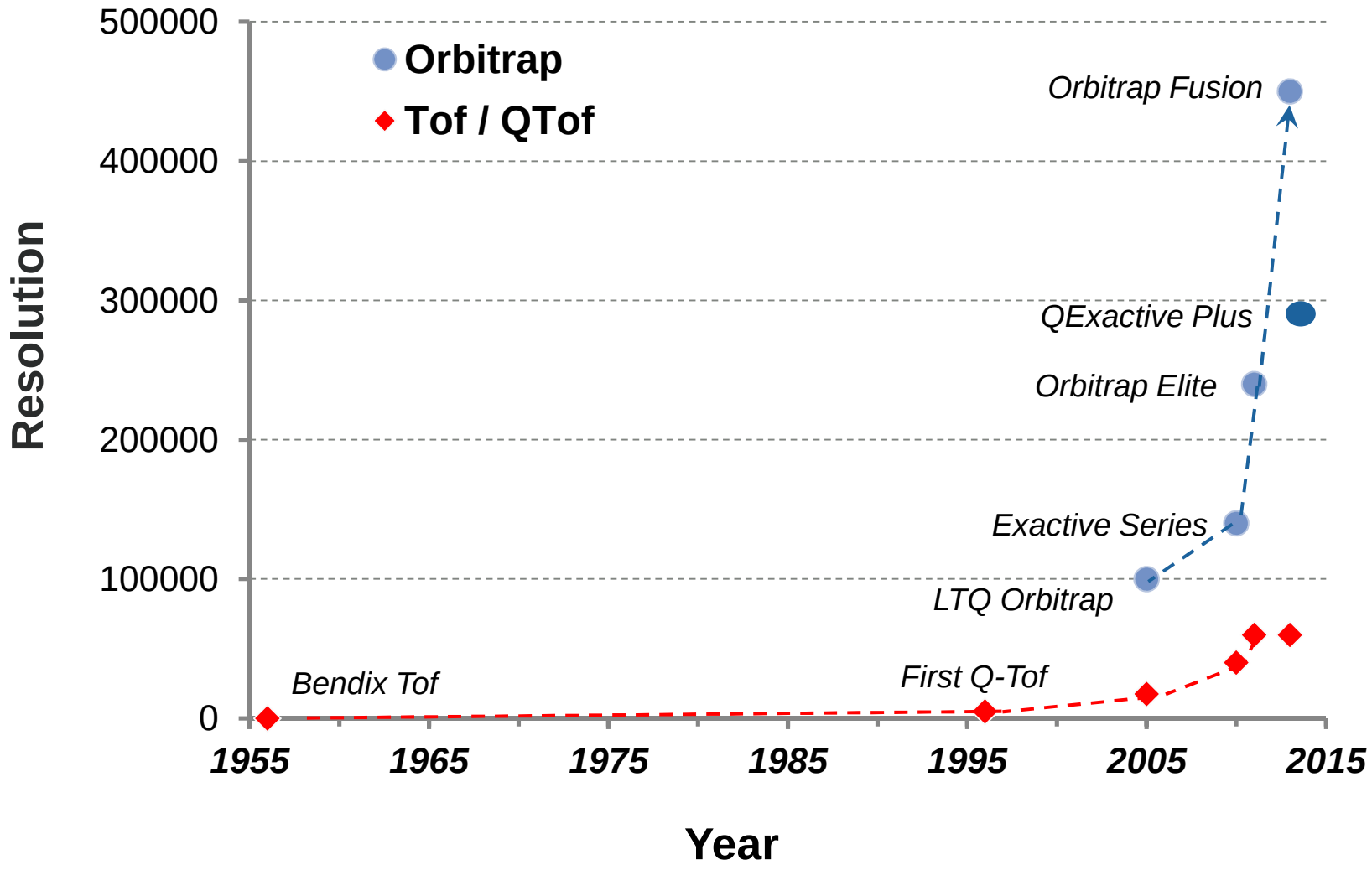
Race for Resolution – Q Exactive *vs.* Q-Tof



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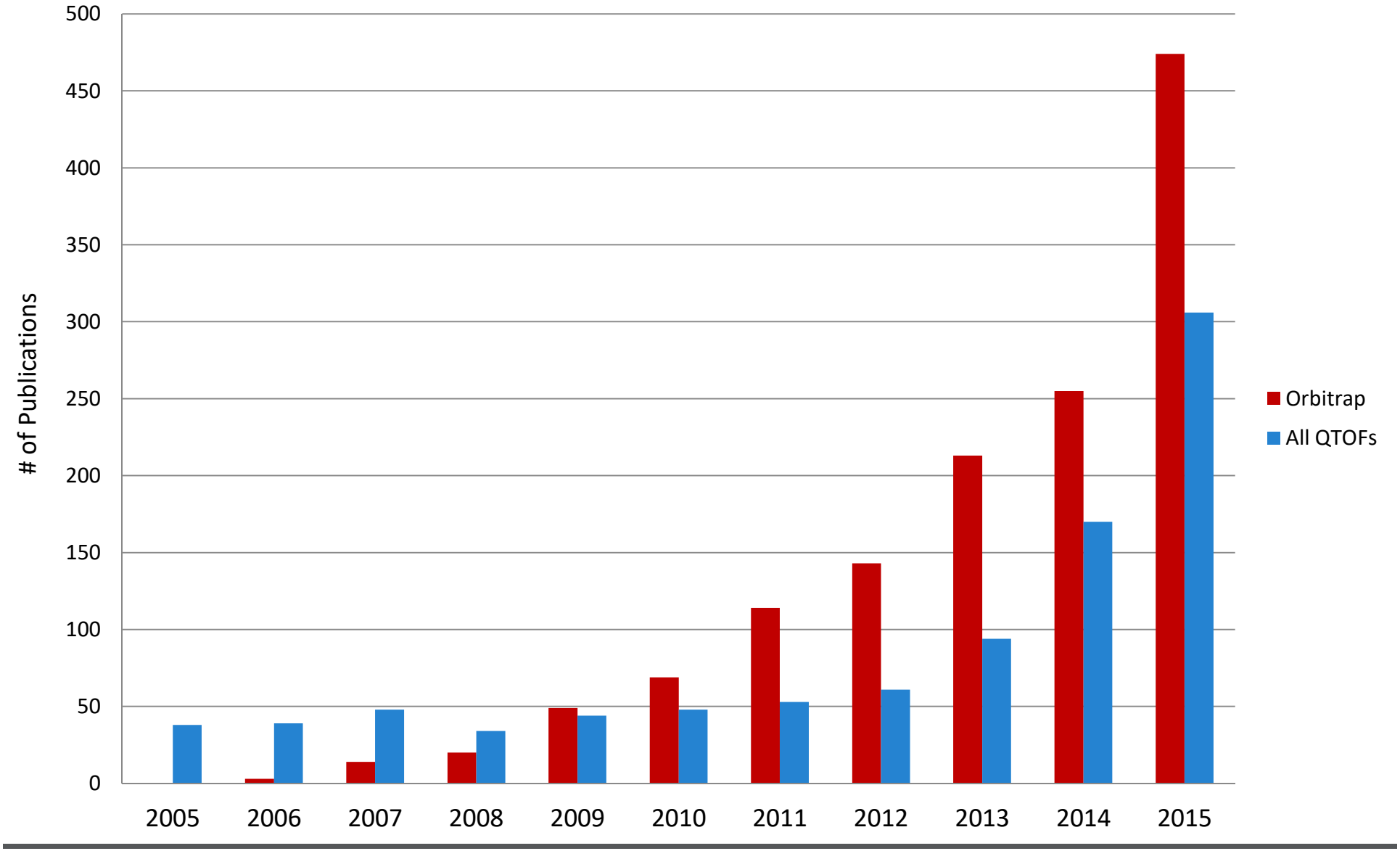
Nature and Science Family of Publications: Orbitrap vs Competition



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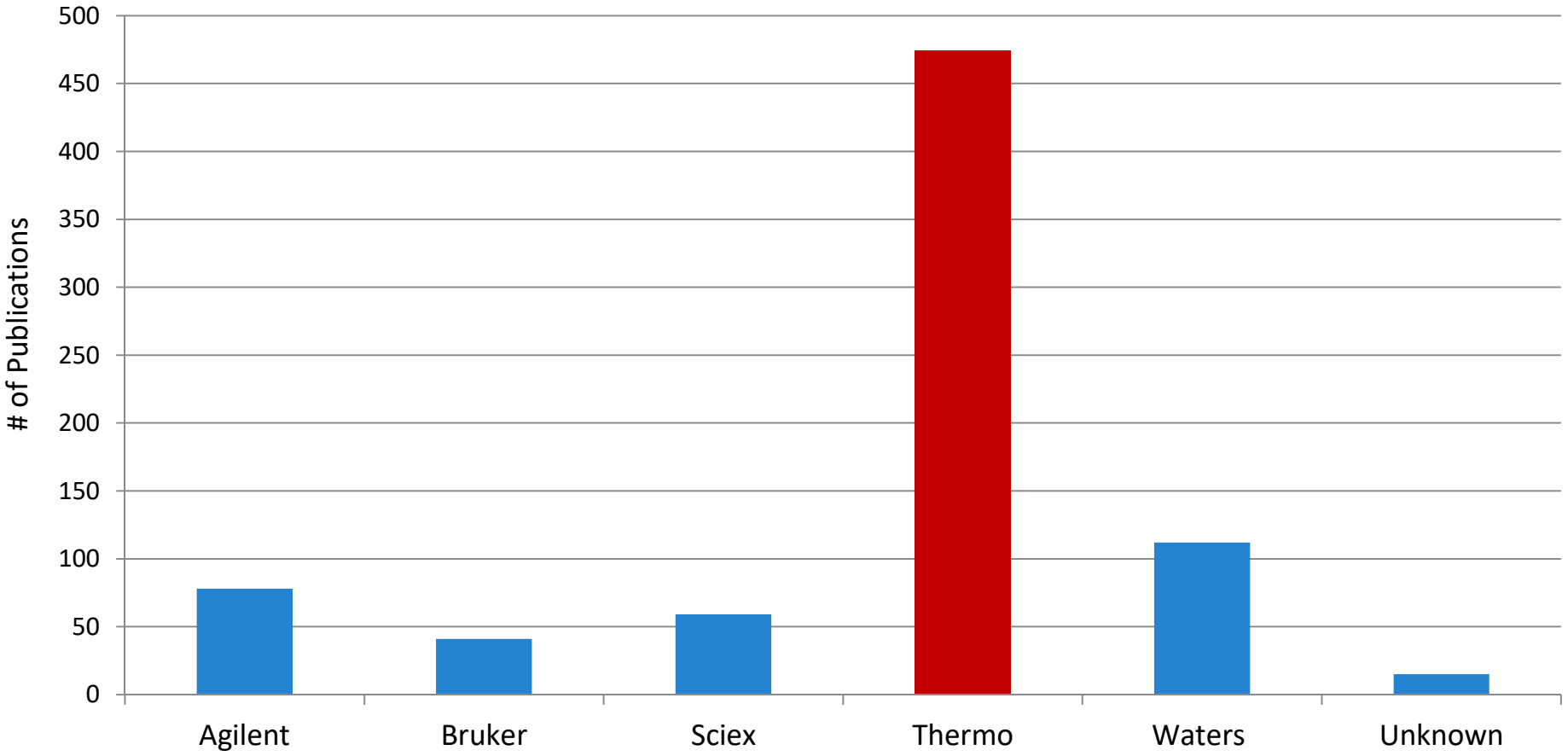
Nature and Science Family of Publications 2015: Orbitrap vs QTOFs



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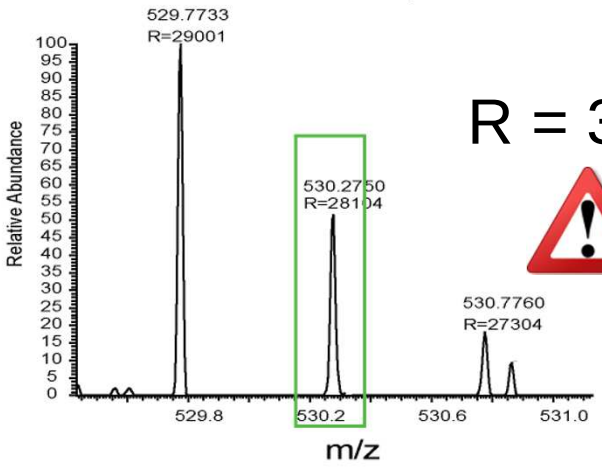
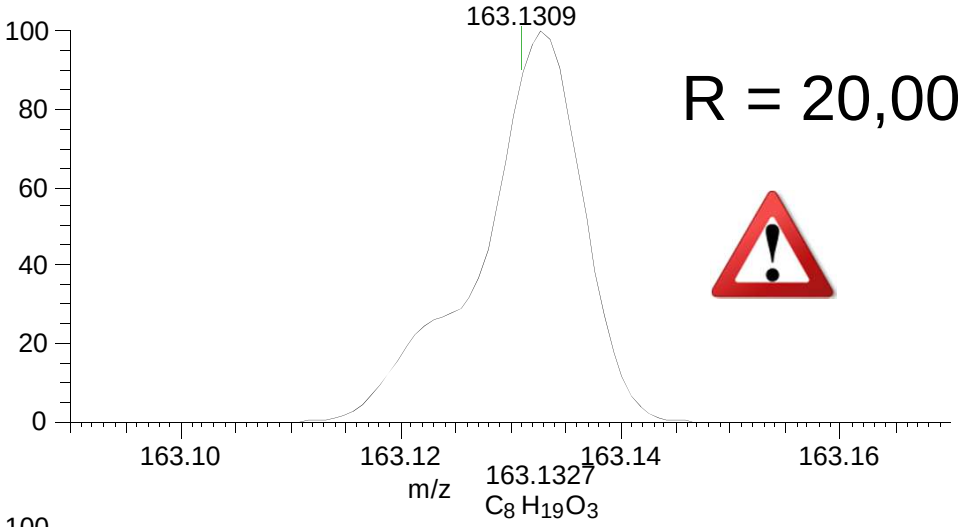
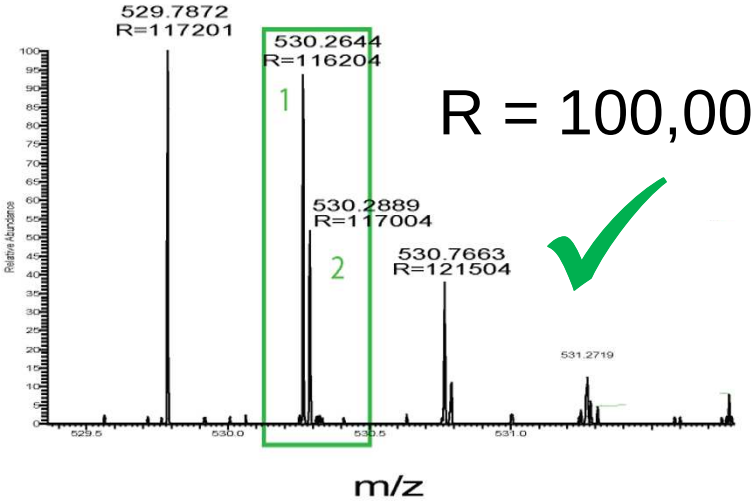
1st Rule of High Resolution Accurate Mass Quantitation



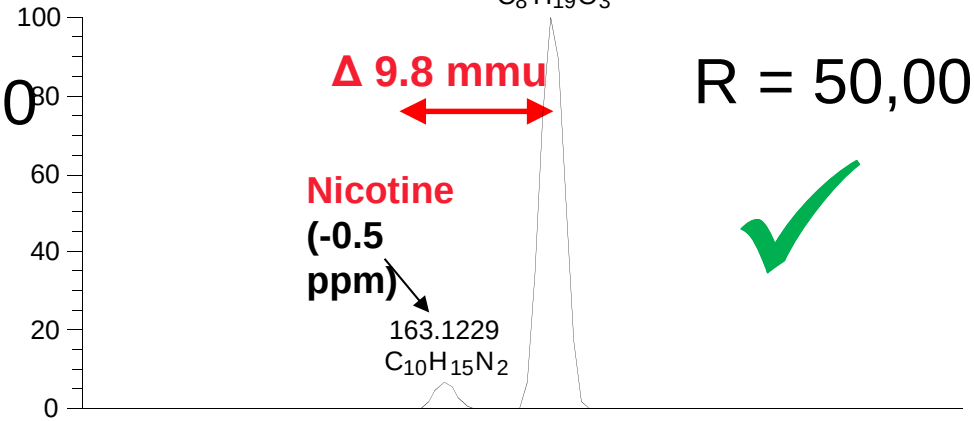
R = 30,000



R = 20,000



R = 100,000



R = 50,000

You can't quantify, what you can't detect.

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Benefits of Using Q Exactive for Screening

Resolving power	Separate target compounds from interference
Mass stability and mass accuracy	Calibration maintained for days to weeks Unrivaled scan to scan mass accuracy and precision
Fast polarity switching	Quick positive-negative switch maintaining accurate mass/charge determination
No trade-off between resolution and sensitivity	Both resolution and sensitivity are retained
Minimum scan to scan variance	Excellent signal/noise, no need for averaging
Experiment flexibility	Easy to use, a plug and play device

Screening Approach Using a Q Exactive

Fragmentation types:

1. **“Confirmational MS2” for targeted screening**

   Triggers all compounds of a specific list

2. **“Discovery MS2” for untargeted screening**

   Triggers compounds having the highest intensity

3. **“AIF” or “vDIA” MS2 for unknown screening**

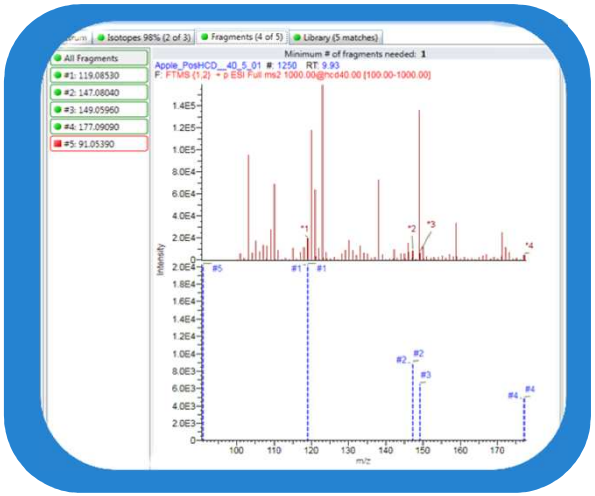
   Fragments everything

TraceFinder Software for Data Acquisition and Processing

Routine Quan

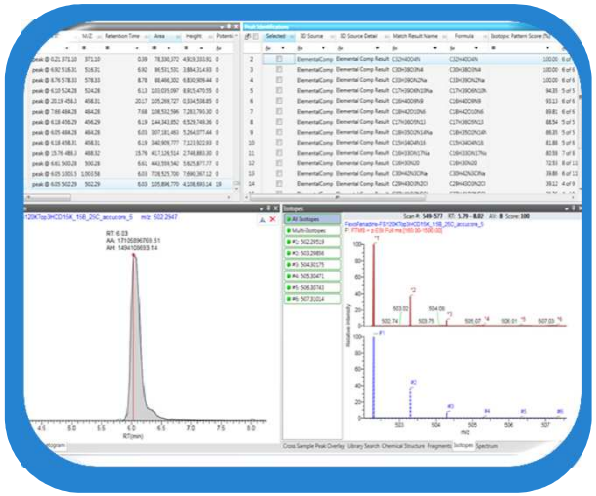
- Rugged and robust quantitation for multiple markets, across LC and GCMS systems

Targeted and Untargeted Screening



- Provides multiple forms of confirmation for targeted screening applications (spectral library, MS/MS2 database, isotopic pattern confirmation)

Unknown Screening



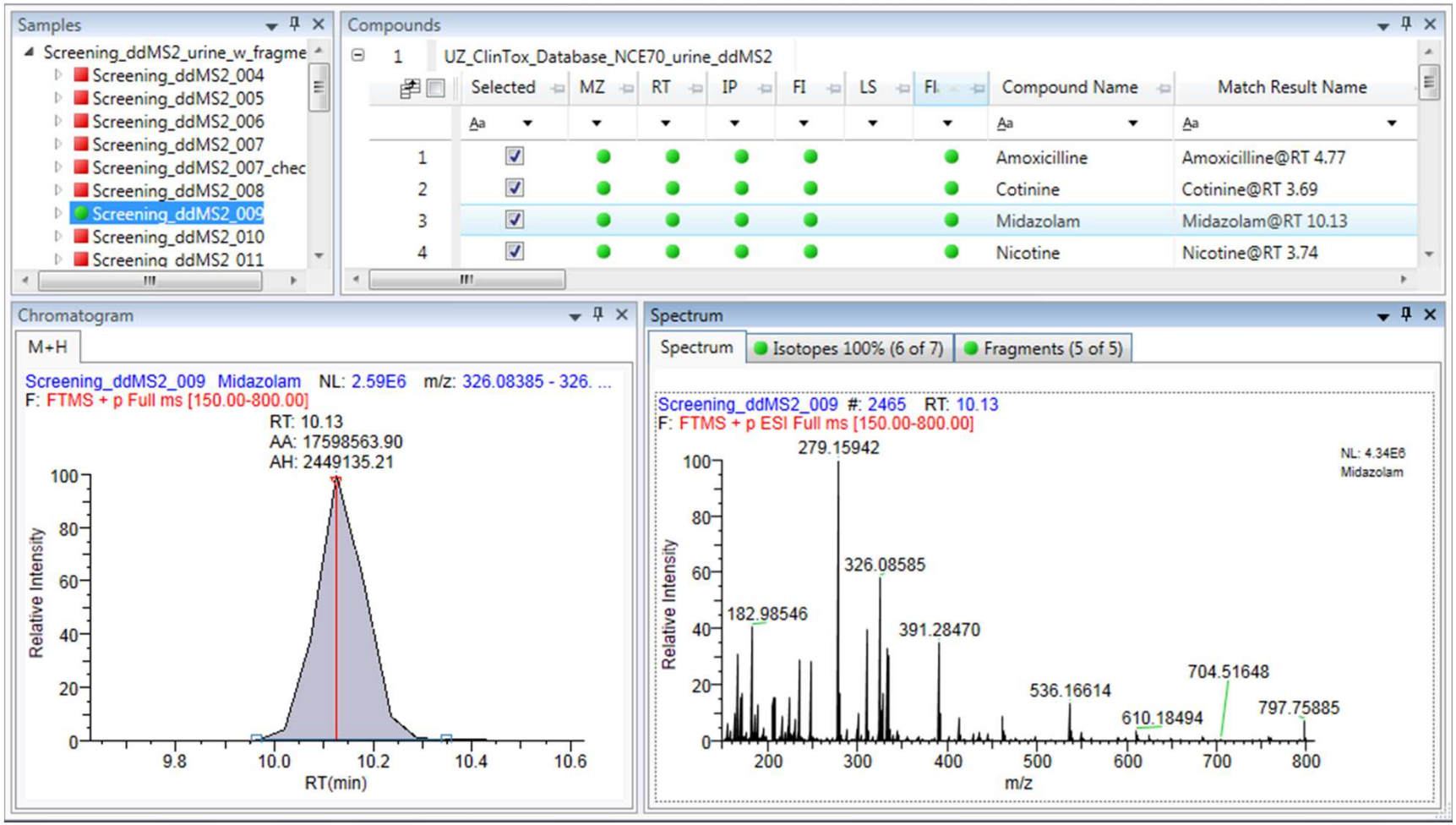
- New in 3.3, allows for screening for unknowns in conjunction with quan or targeted screening

Providing a complete quantitation and screening workflow for routine applications

TraceFInder WorkFlow for Targeted / Untargeted Screening

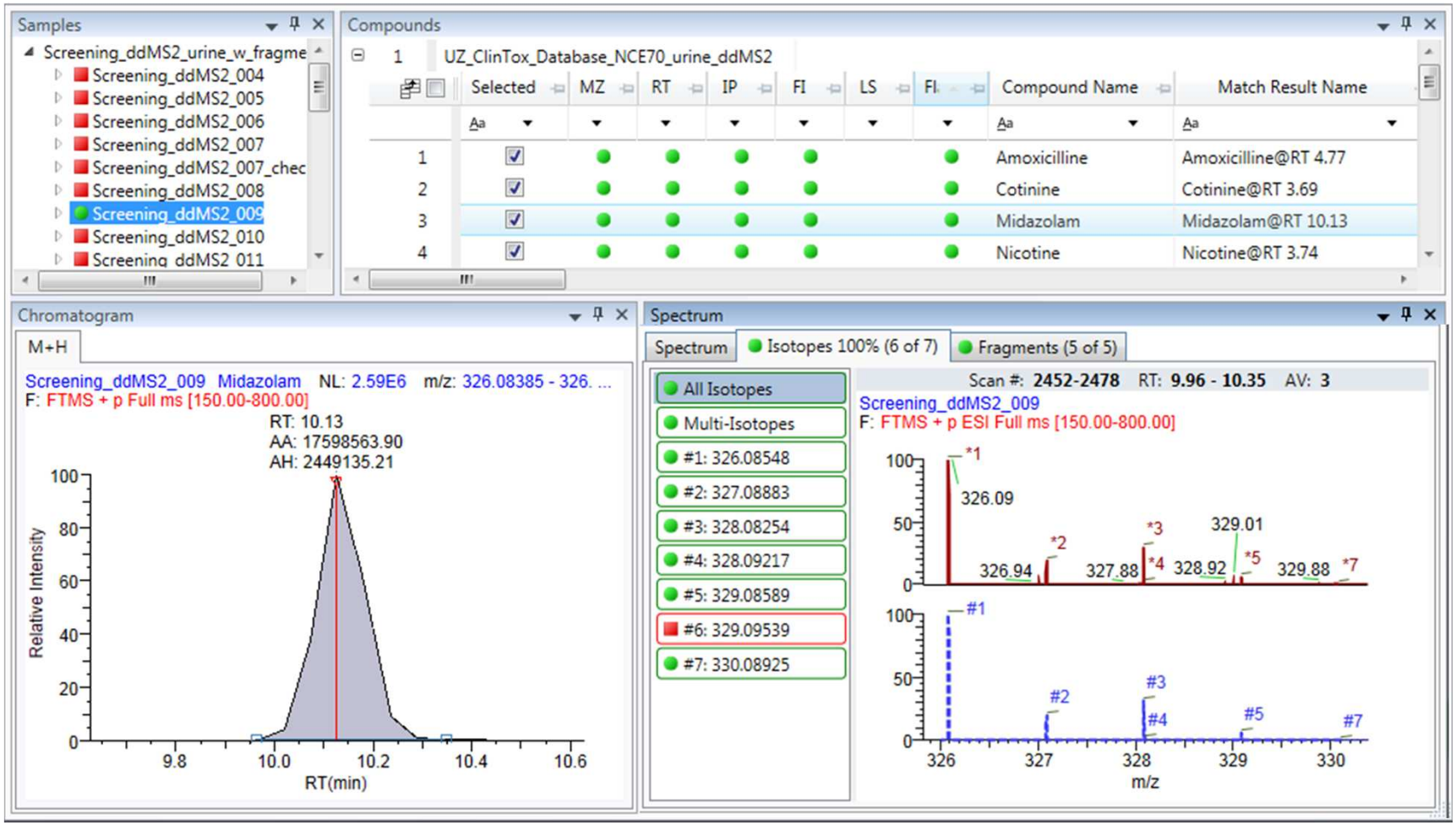
1. Identifies chromatographic peaks based on parent mass from a specific database
2. Confirms identification based on retention time in the database
3. Compares theoretical and experimental isotopic pattern for the parent
4. Compares theoretical and experimental mass spectrum of the parent using a specific spectral library
5. Further confirms based on mass of fragments reported in the database

TraceFinder and Targeted / Untargeted Screening



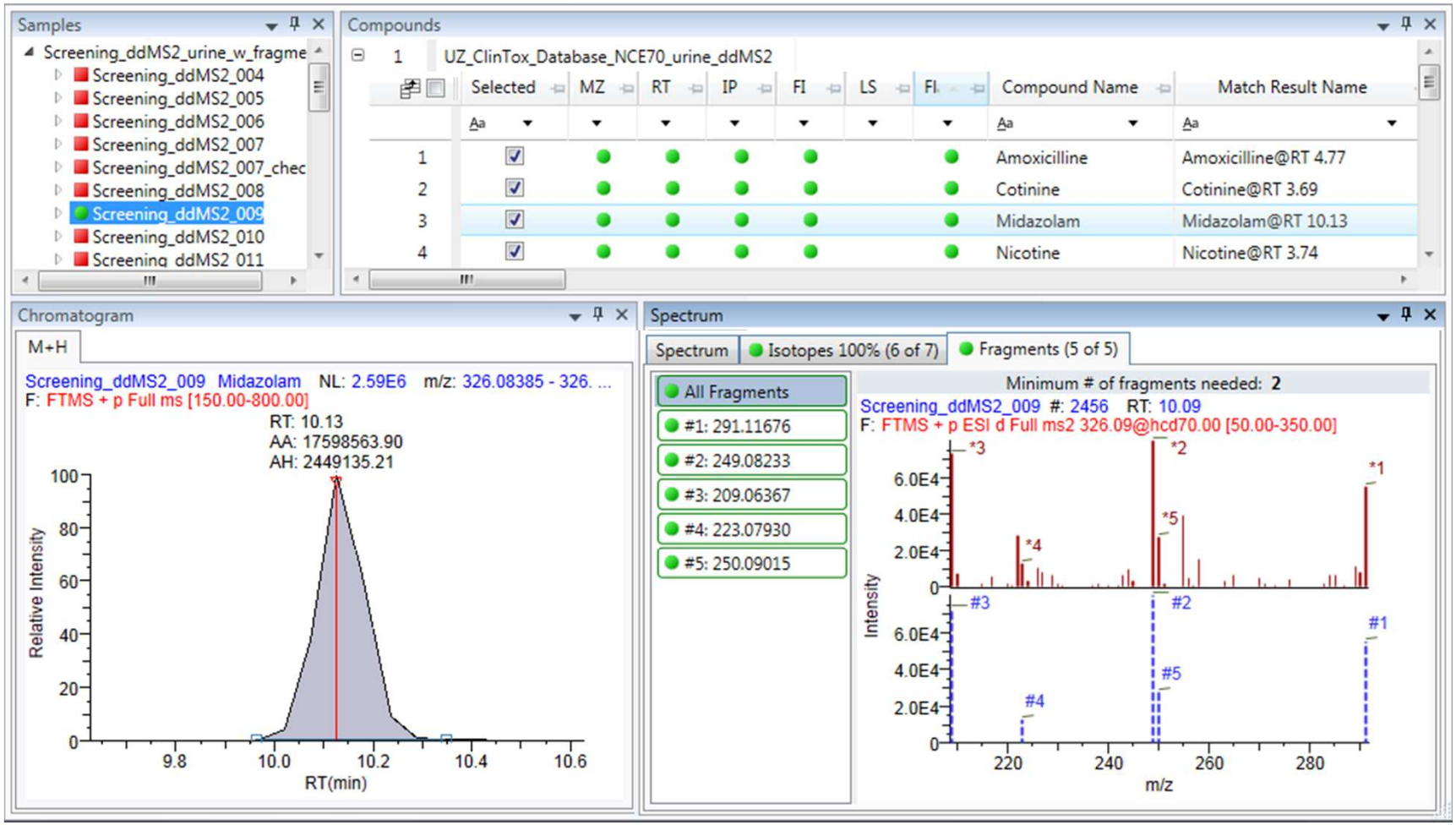
- multiple forms of confirmation: spectral library, MS/MS2 database, isotopic pattern confirmation

TraceFinder and Targeted / Untargeted Screening



- multiple forms of confirmation: spectral library, MS/MS2 database, isotopic pattern confirmation

TraceFinder and Targeted / Untargeted Screening



- multiple forms of confirmation: spectral library, MS/MS2 database, isotopic pattern confirmation

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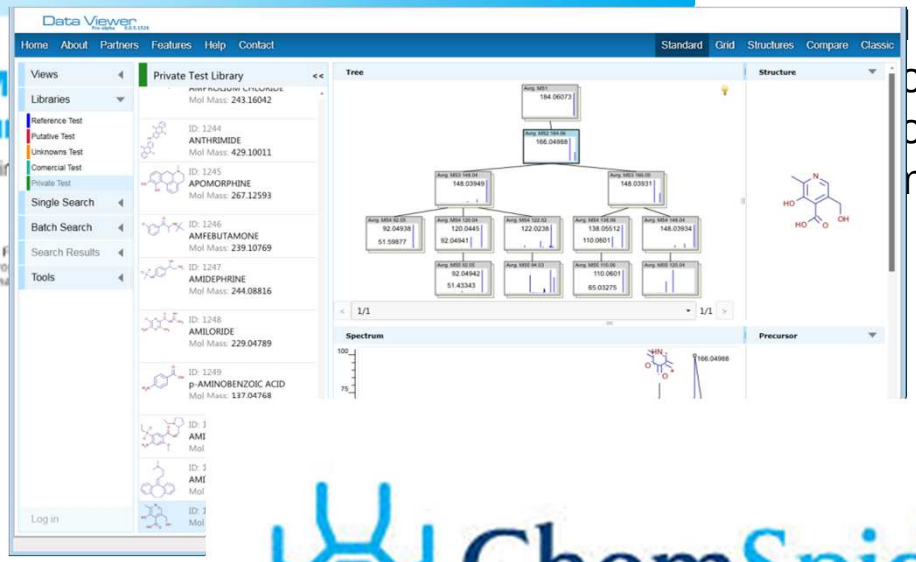
New in TraceFinder 3.3 – Unknown Screening

1. Detect all chromatographic peaks above user specified threshold
2. Identify detected peaks using local database
3. For peaks not identified in step #2 propose molecular formula based on accurate mass and isotopic pattern
4. Search proposed molecular formulas with ChemSpider databases
5. Confirm obtained ChemSpider hit by comparing theoretical fragmentation to experimental fragmentation

The Importance of Databases and Spectral Libraries



HRAM M
Compou
Providing the ulti

4,427 com

Over 900 unique compounds of interest to the clinical research and forensic toxicology communities:

- Prescription drugs
- and industrial toxins
- of abuse
- of interest for monitoring research

https://www.mzcloud.org/

A novel mass library/database
nance enhancing drugs
of fragmentation mass spectra
(**MS/MS** and **MS^n** spectra)

Structural info for compounds
ey are not represented in
y through identification of
ctures

A free chemical structure database providing fast text and structure search access to over 43 million structures from hundreds of data sources.

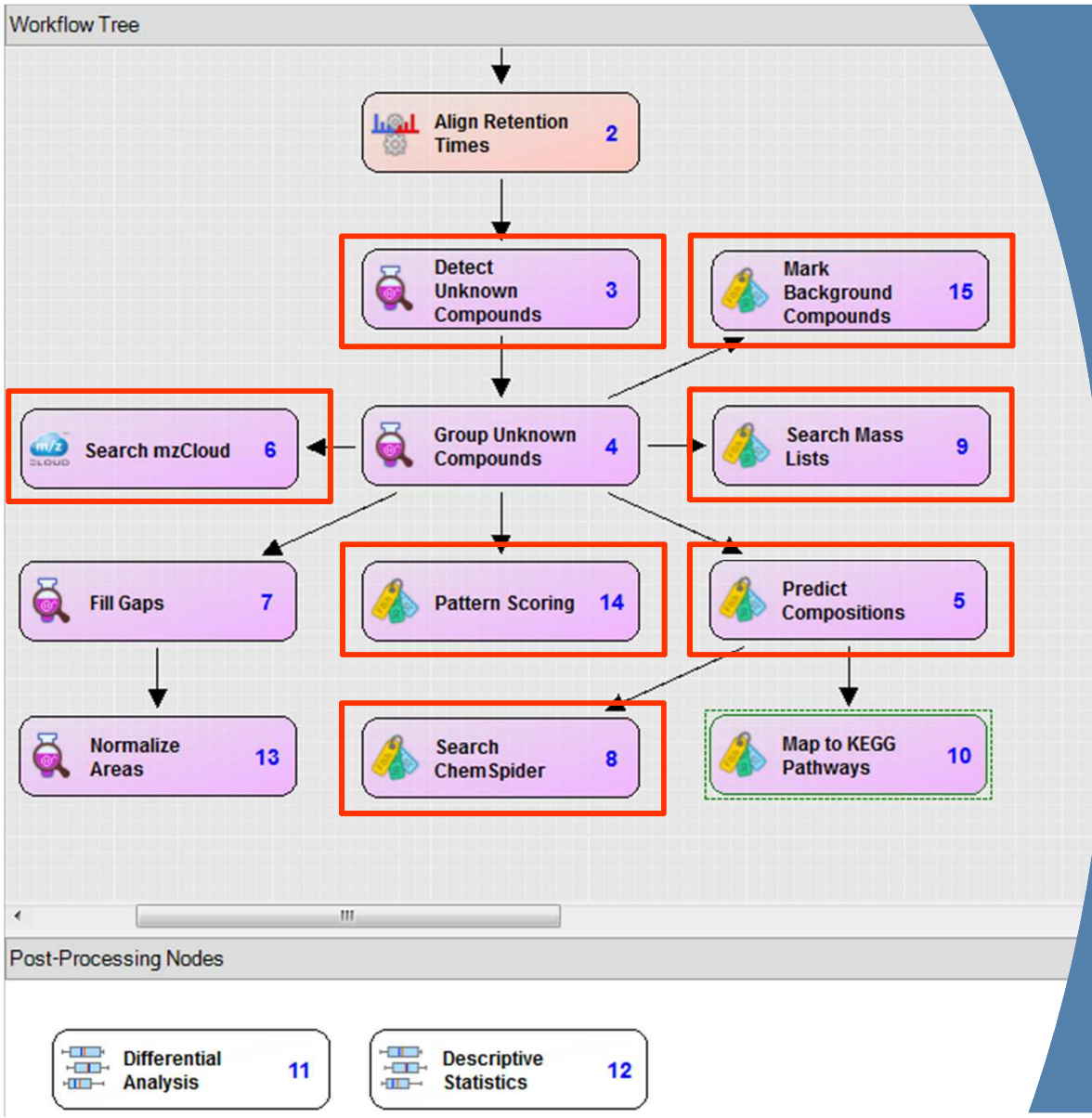
ChemSpider
The free chemical database

ThermoFisher SCIENTIFIC

Compound Discoverer 2.0

		
TraceFinder Targeted Profiling	m/z Cloud Compound Identification	Compound Discoverer Untargeted Discovery Complete small molecule research and structure identification in a Next Generation platform

Compound Discoverer 2.0



- Unknown compound detection
- Automatic background filtering
- Composition prediction
- Mass list search
- Pattern Scoring
- Search mzCloud
- Search ChemSpider

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Unknown Screening

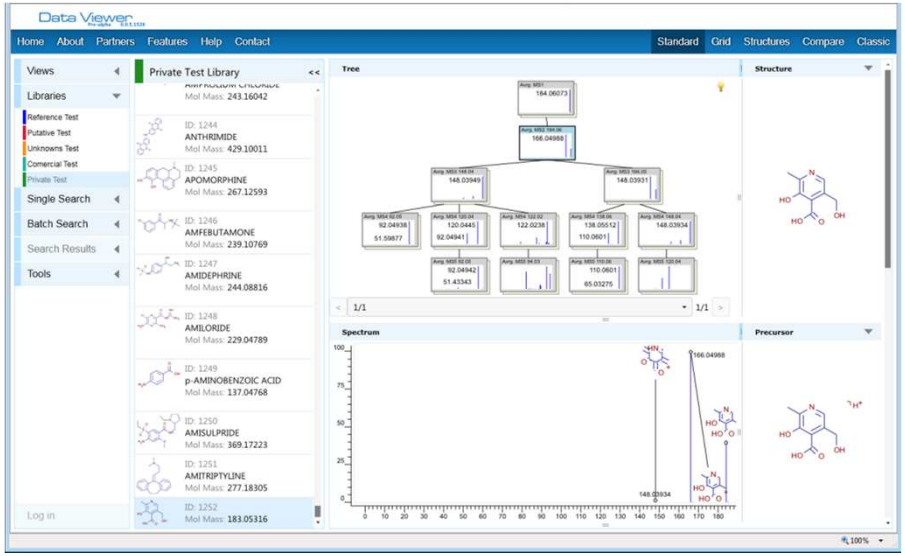
- Targeted Screening using dabatase/library is one approach
  - 10 new Street drugs every week – Impossible to keep database up-to-date.
  - Need for an alternative approach

- Impossible to use ChemSpider only.
  - 28 millions of structures
  - Codeine $C_{18}H_{21}NO_3$: 4902 structures in ChemSpider

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

- https://www.mzcloud.org/



Manually Curated Data

Compounds	Trees	Spectra	Anotations	Peaks	
1 821	2 489	134 087	669 190	219 394 860	more ...

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Thermo
SCIENTIFIC
Transform Your Science