The Latest and Greatest in LC/MS

Achieving the utmost information from today's complex analyses

Robin Philp

LC/MS Solutions Development Manager

Agilent Technologies
Singapore

1290 Infinity LC and MS

- The perfect front end for LC/MS

1. Highest power range (1200 bar x 2 ml) for highest separation power per time
   - Speed for higher throughput
   - Resolution for higher data quality (ion suppression)

2. Lowest delay volume* for fastest gradients
   (pump delay volume (10 µl w/o mixer*)
   a) with Fixed loop (20 µl*) injection
   b) with Flow through (80 µl*) injection

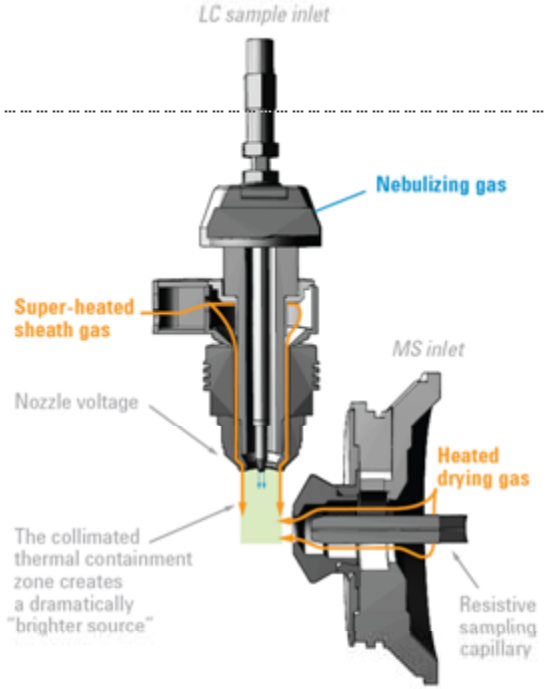
3. More robustness for lowest carry over with 1290 Infinity Autosampler
   < 0.002 % Chlorhexidine**

** Delay Volume: 10 % step gradient Definition, preliminary Data*

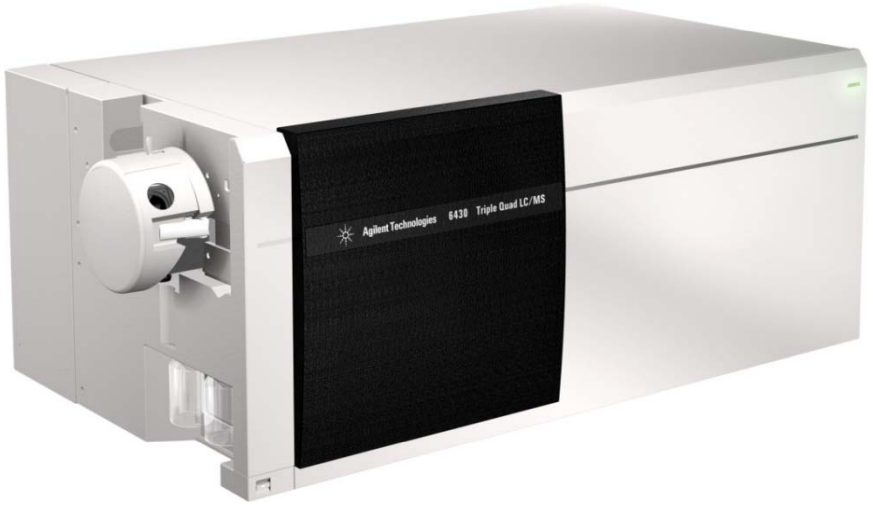
1,000th Agilent Triple Quad sold in March 2009

6460 – With JetStream Technology: The World's Most Sensitive QQQ

- Sensitivity: 1pg reserpine 1000:1
- Mass range: 5 – 3000 m/z
- Minimum dwell time: 1msec
- Polarity switching: 30msec
- Scan rate: 5200u/sec
- Number of MRM: 40000
- Dynamic MRM: 4000
- Mass resolution: 0.5 Da (manual tune)

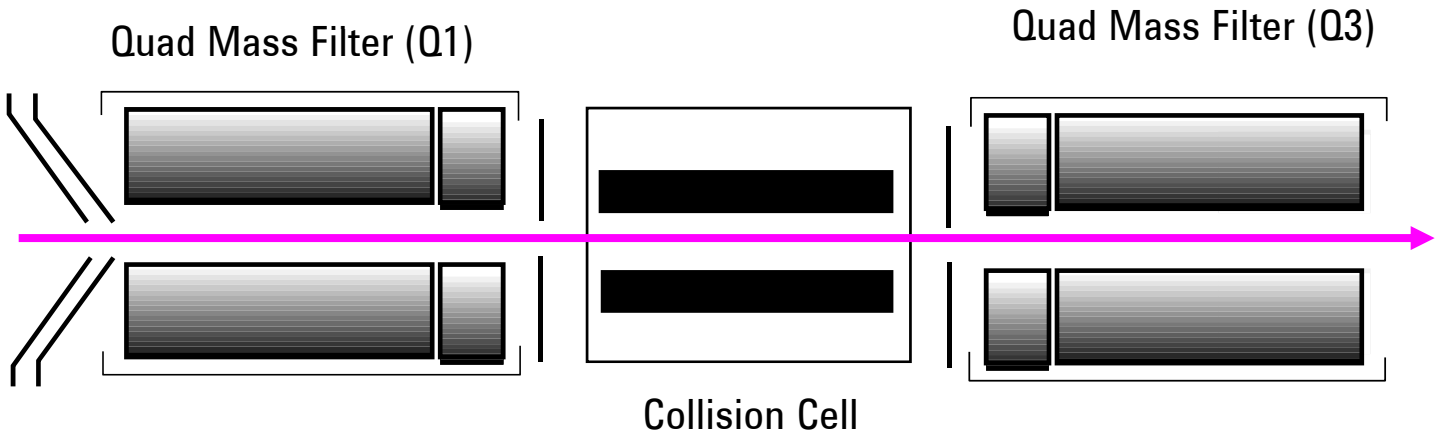


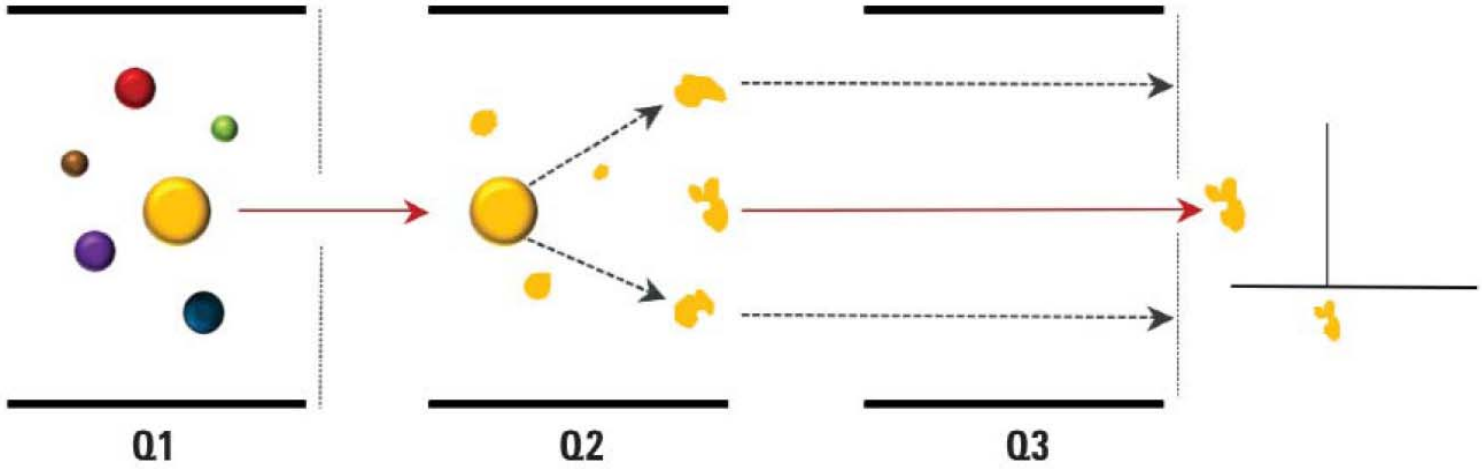
Agilent's new 6430 Triple Quad LC/MS System



- Sensitivity: 1pg reserpine 300:1
- Mass range: 5 – 2250 m/z
- Minimum dwell time: 1msec
- Polarity switching: 30msec
- Scan rate: 5200u/sec
- Number of MRM: 19800
- Dynamic MRM: 4000
- Mass resolution: 0.5 Da (manual tune)

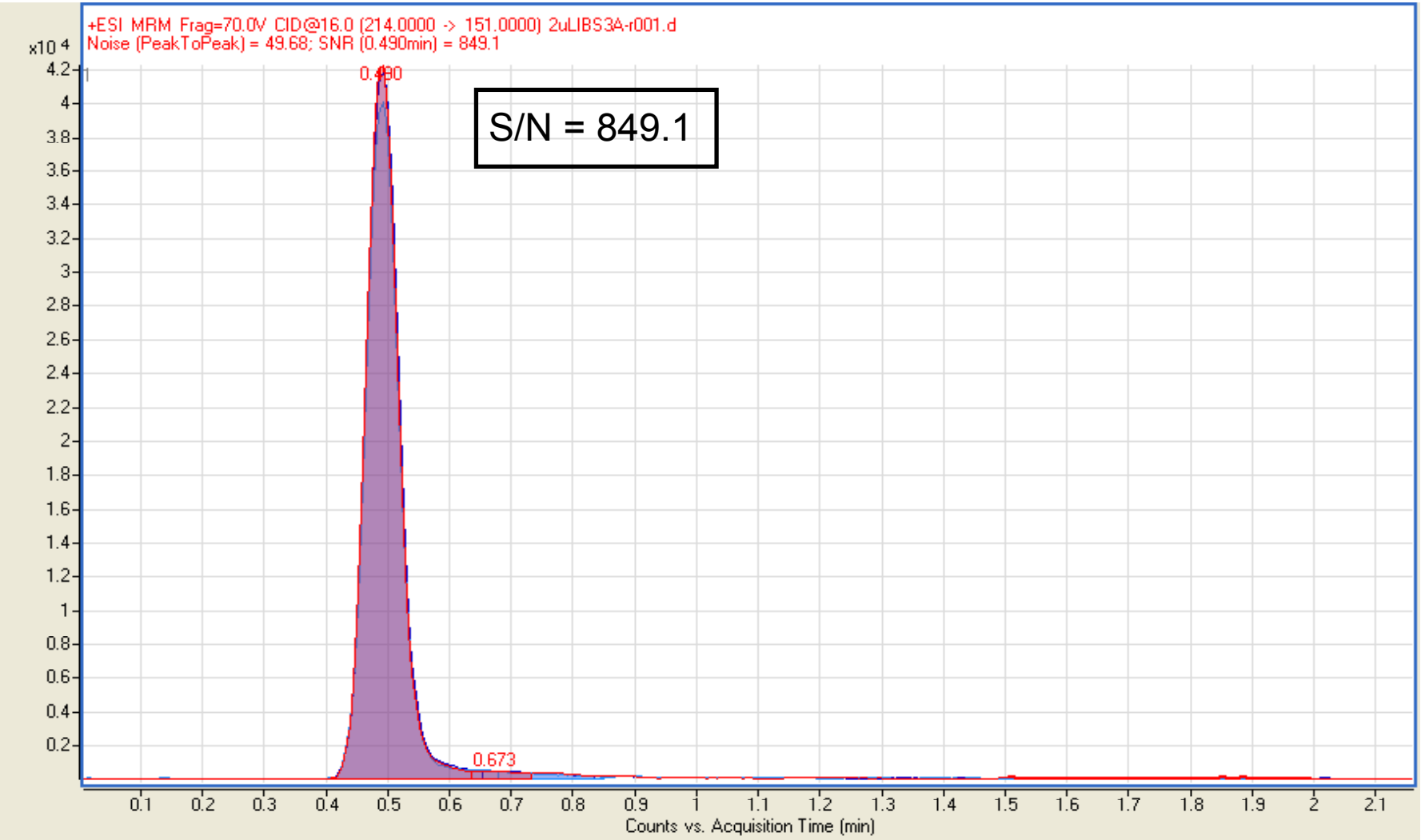
MRM Scanning: the power of the QQQ





Selectivity

MRM transition 214→151



Food Safety – Toxins and Toxicity

Pesticides – already in excess of 1600 compounds

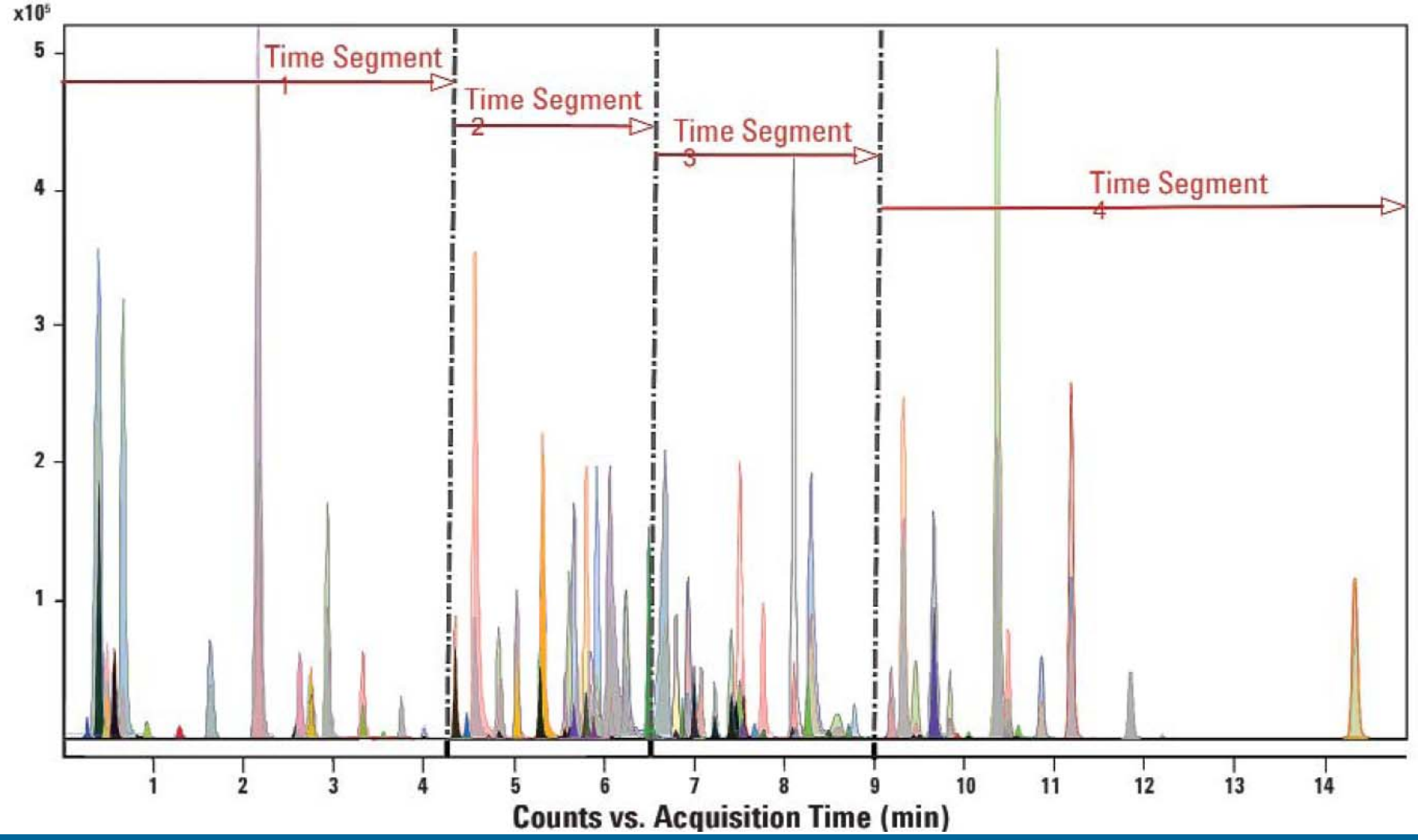
Herbicides

Natural Toxins – e.g. aflatoxins, ochratoxin, patulin

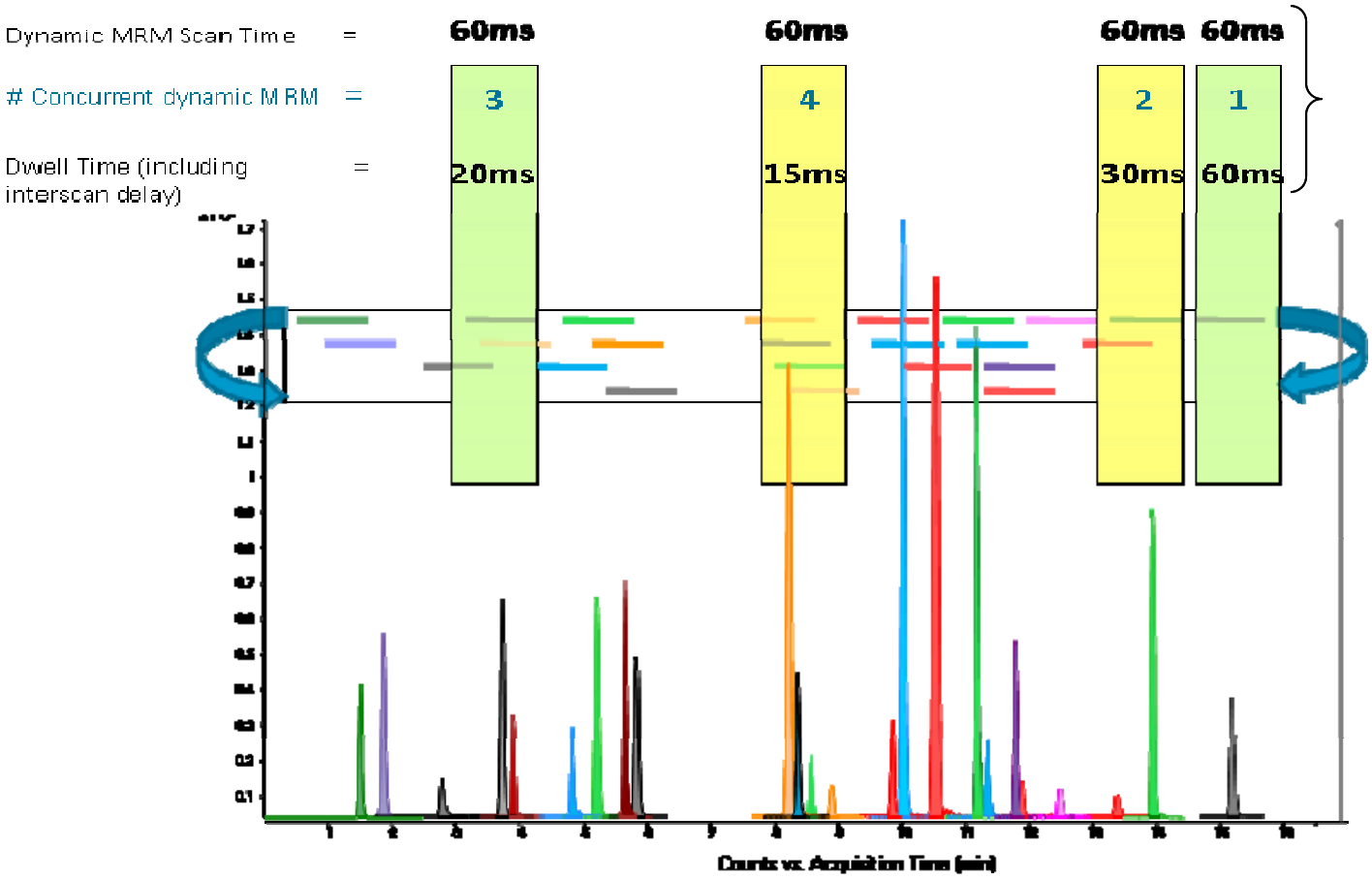
Food Additives

- Antimicrobial
- Antiprotozoal
- Antimicrobial Growth promoters
- Anabolic Growth promoters
- Food Dyes

Approach #1: Time Segments



New Solution: Dynamic MRM



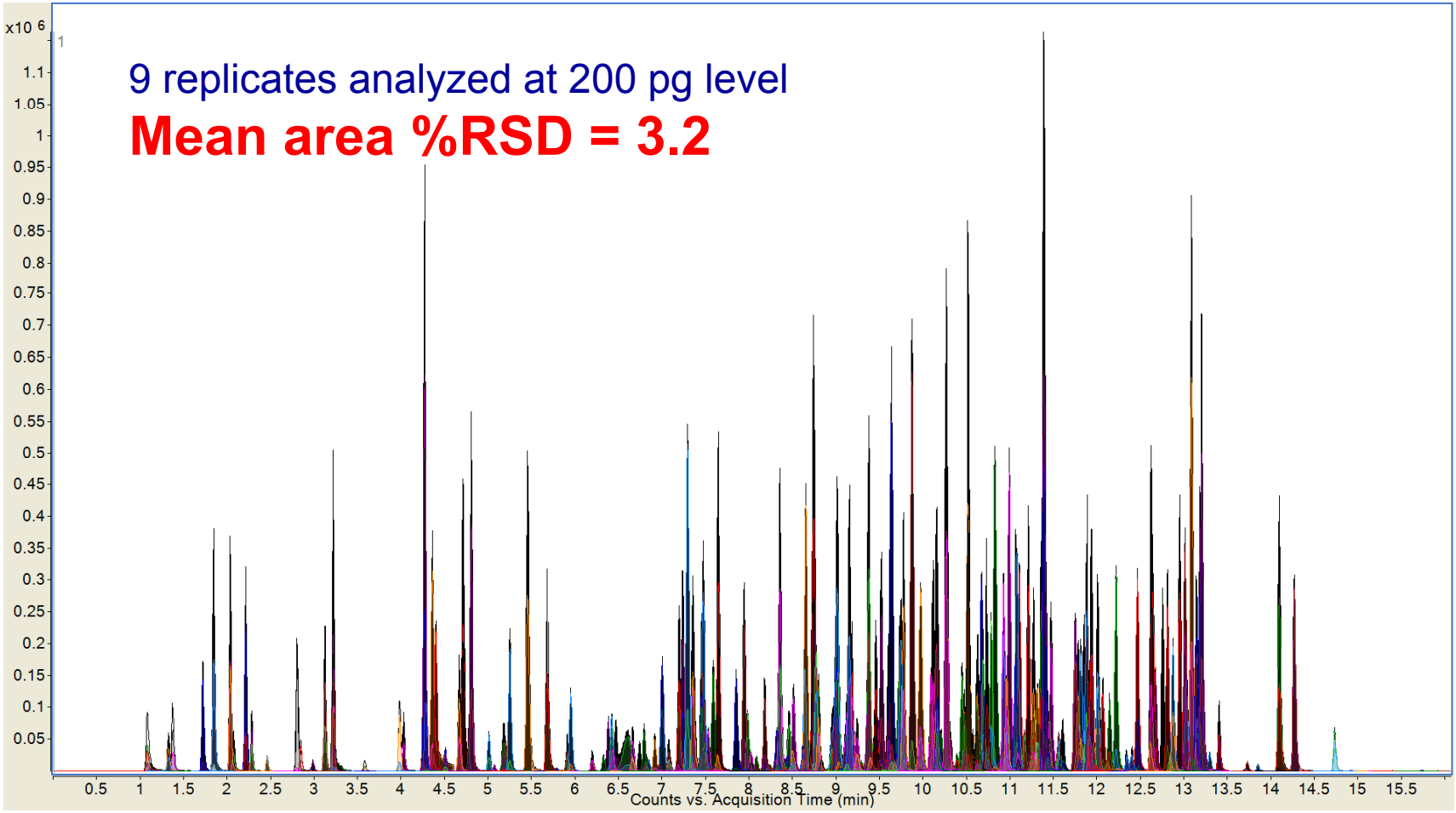
# of MRMs will vary: Dwell times are adjusted dynamically to keep scan time constant for best quant data quality.

MRMs chosen based on analyte RT window

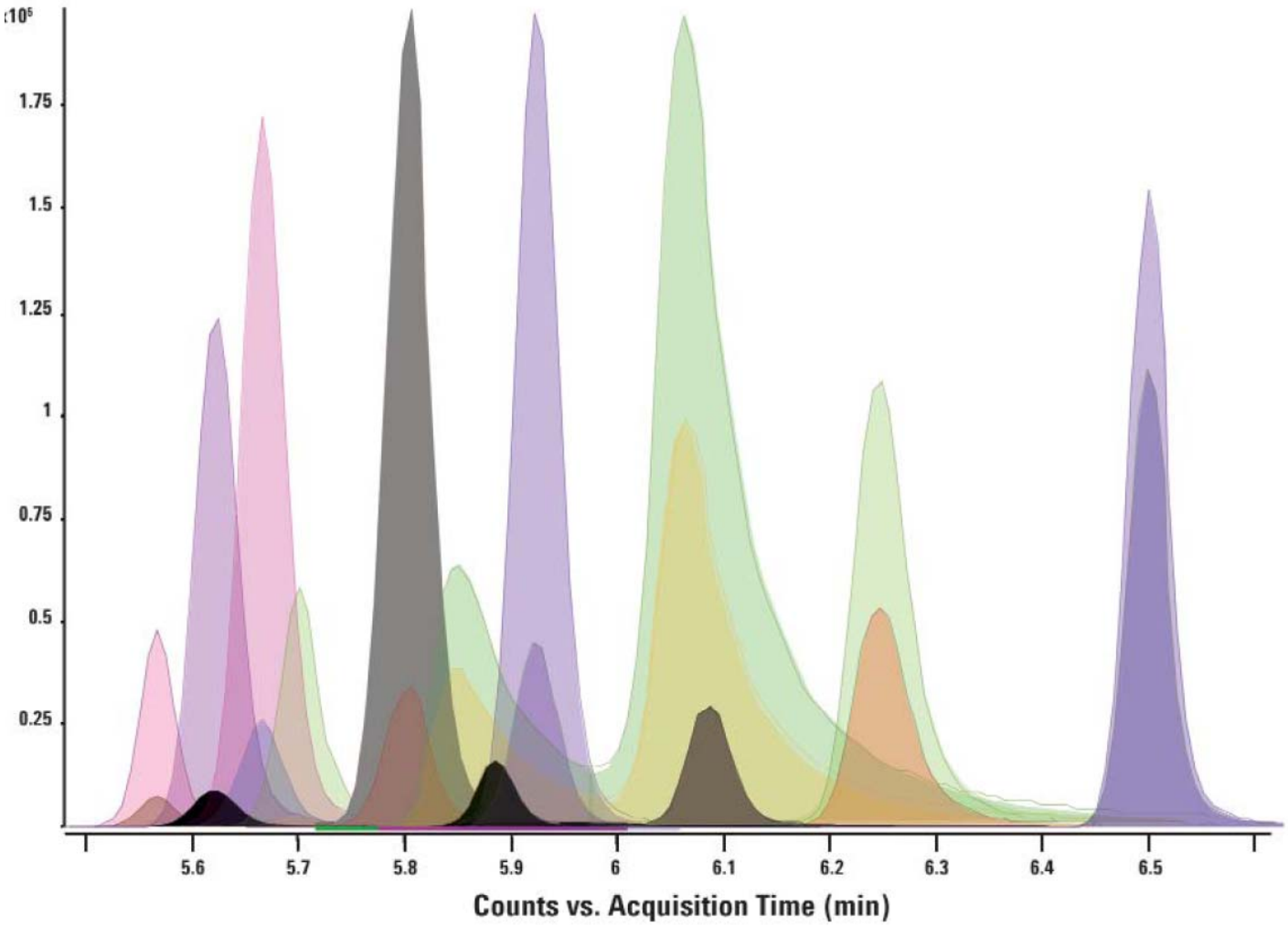
On average, the number of MRMs at any time are many fewer than with time segment methods, allowing much faster MS cycle times.

Analysis of 600 Pesticide DMRM Transitions in 15 min.

Agilent 1290 Infinity LC + 6430 Triple Quad

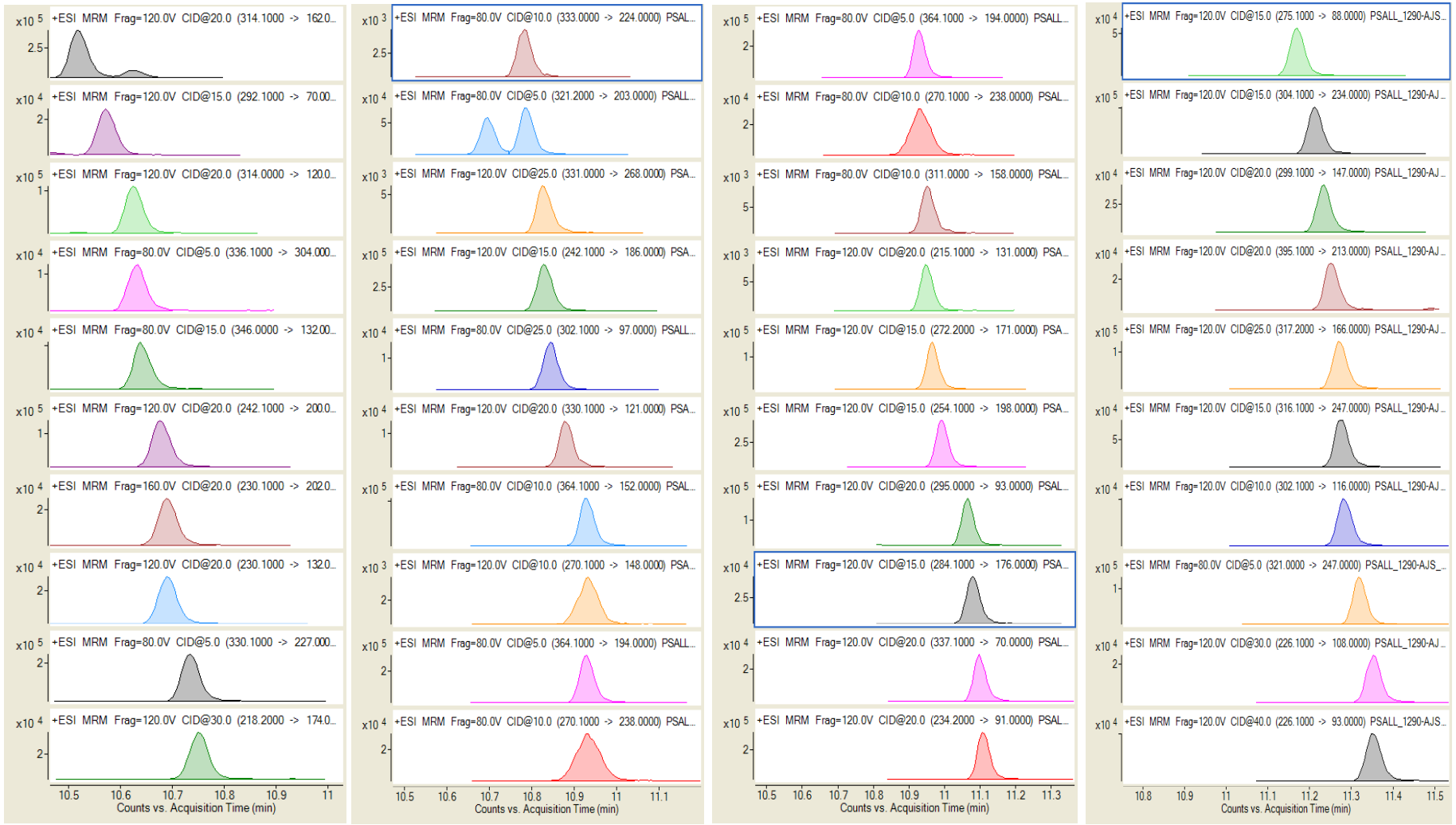


Peak Capacities are very high with UHPLC
1 minute window: 11 pesticides



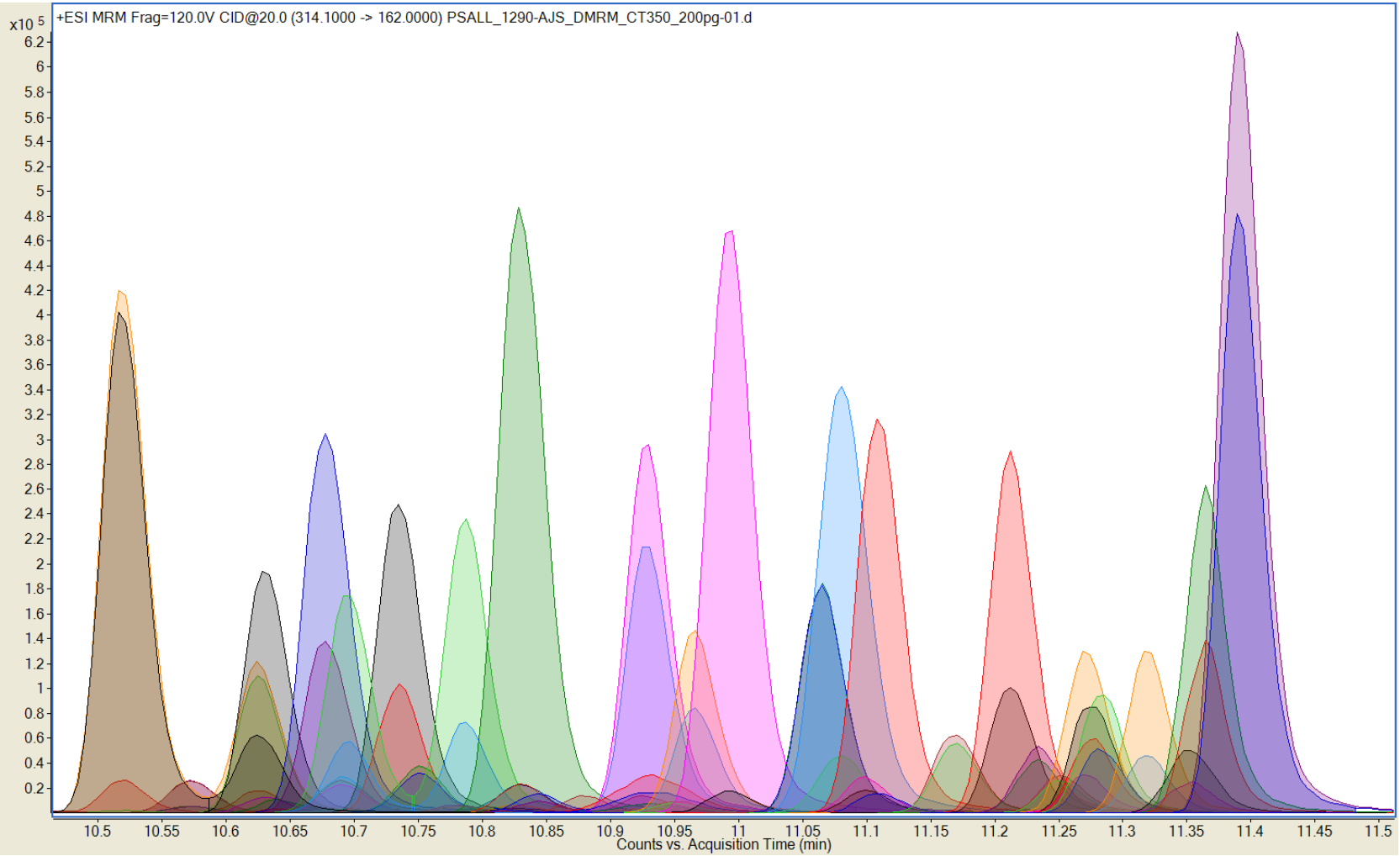
Dynamic MRM Targets the Analyte when it Elutes

EIC's of 40 Pesticides Eluting in a 1 min. RT Window

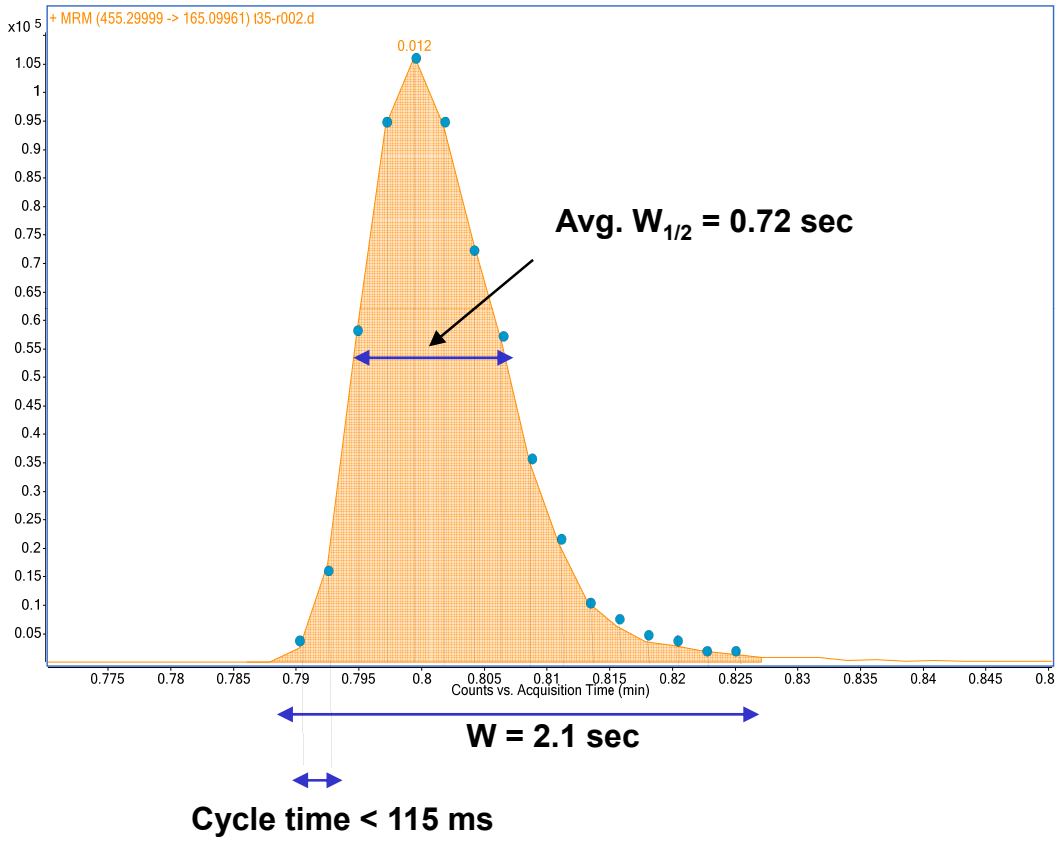


Peak Capacities are very high with UHPLC

40 Extracted Ion Transitions in 1 minute

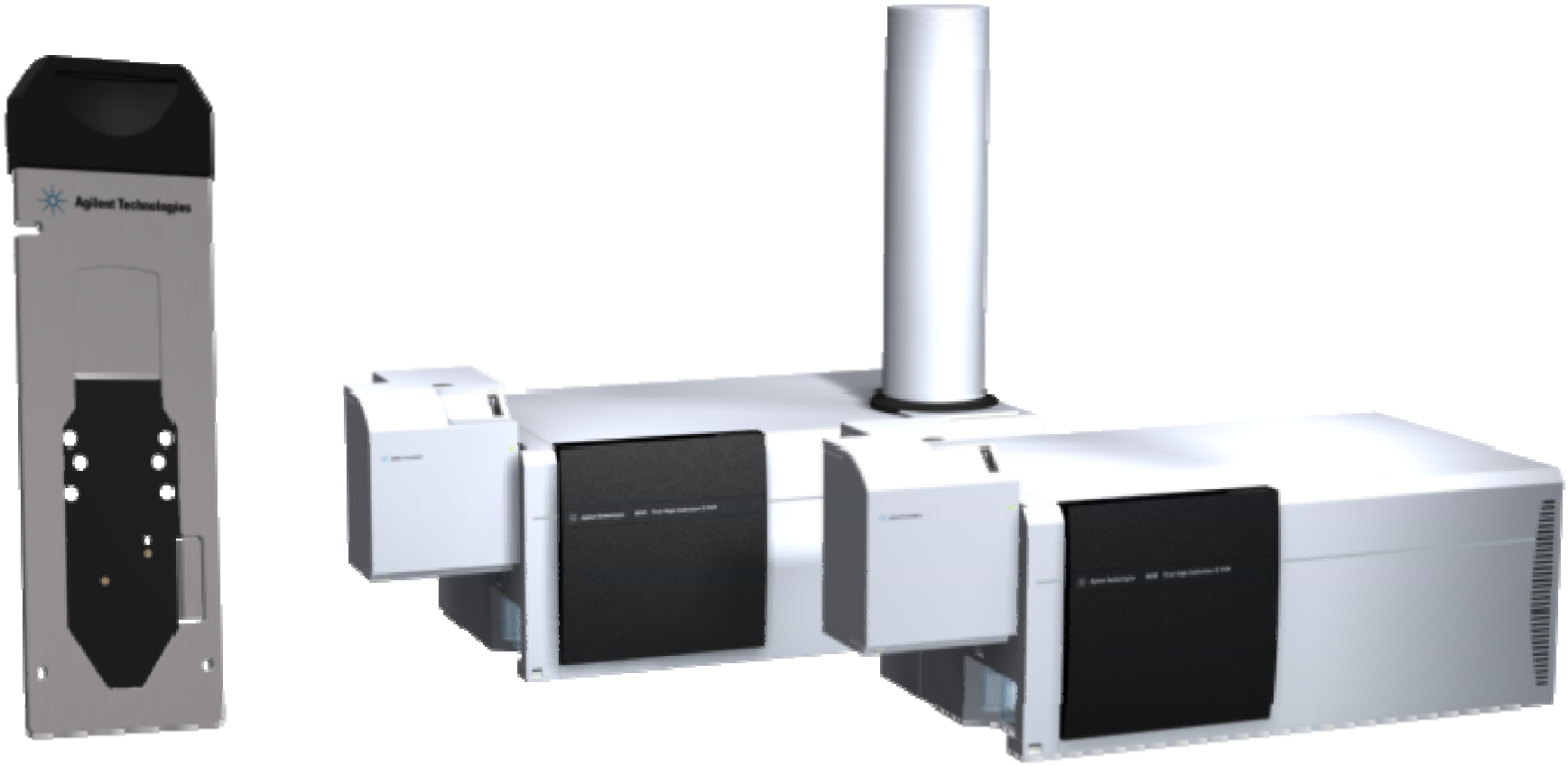


Fast Polarity switching and fast MRMs with 6430

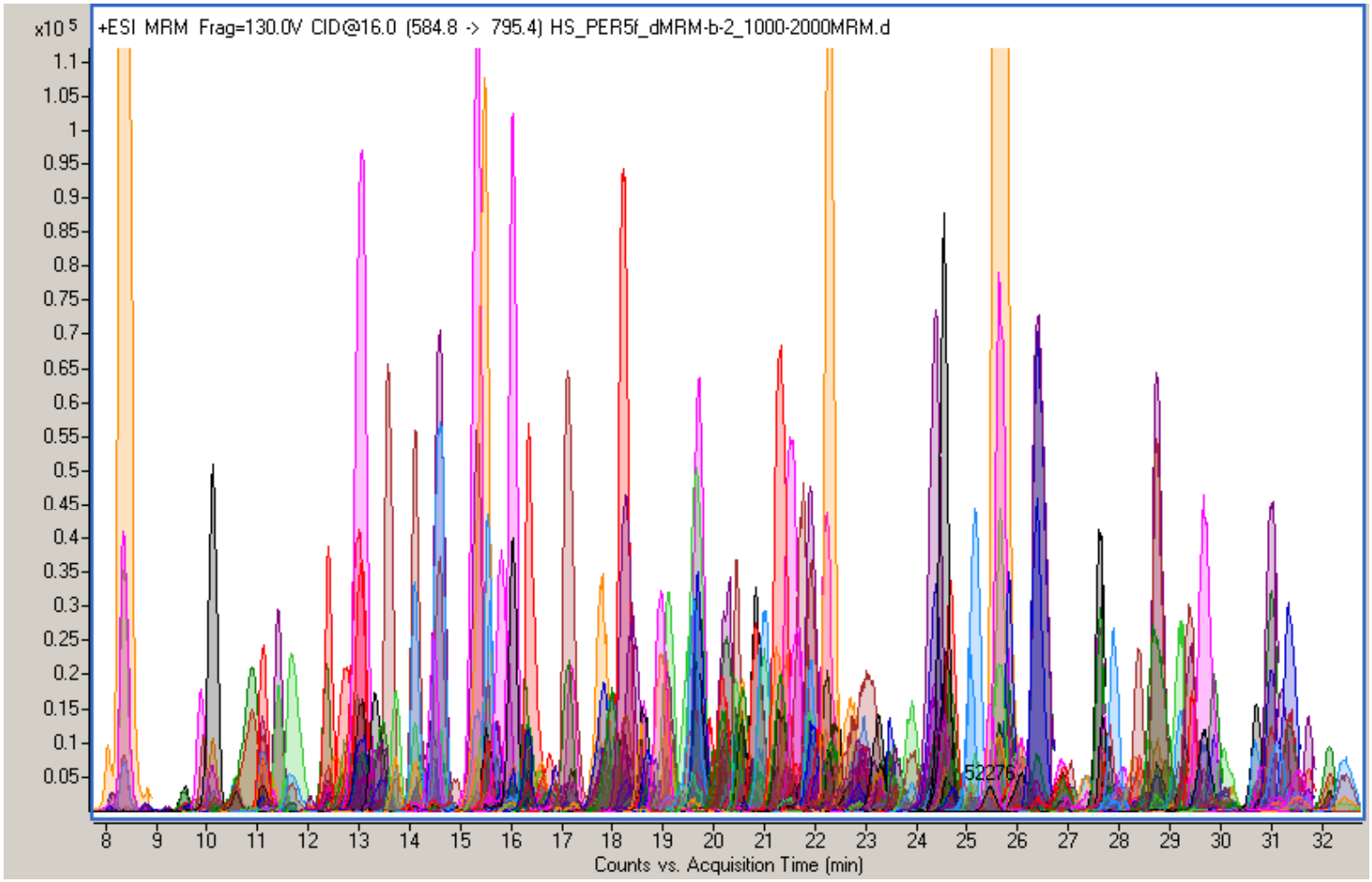


The verapamil peak eluted in two seconds from a sub-2-micron column at pressures exceeding a **1,000 bar** with Agilent's new **1290 Infinity** LC System. The verapamil ion transition from 455 to 165 was monitored more than fifteen times across the peak with multiple injections demonstrated a peak area RSD of 5%. **The cycle time for six ion transitions and positive/negative polarity** switching **was less than 115 milliseconds.**

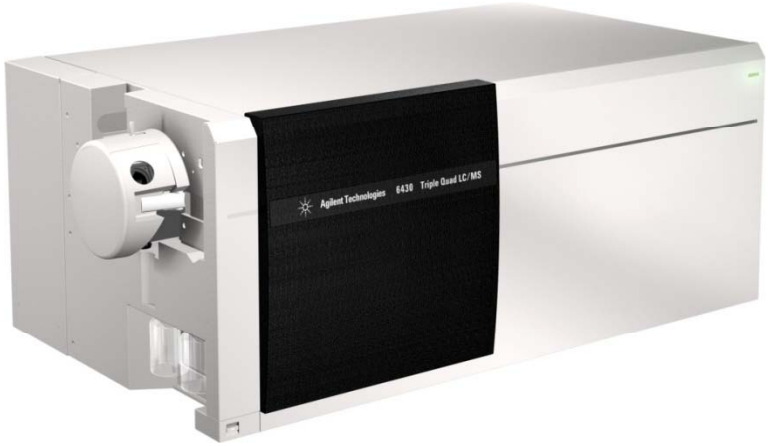
Agilent 1200 HPLC-Chip/MS is Compatible with All Agilent 6000-series MS Systems



2,000 DMRM transitions for tryptic peptides in depleted human plasma with 6400 Series QQQ



Key 6430 Applications



Food Safety and Environmental Monitoring: Food safety applications are the largest LC-Triple Quad market for Agilent. The higher sensitivity, faster MRMs, and integrated Dynamic MRM will make either the 6430 or the 6460 the perfect choice for all customers.

CRO Market: Customers looking for a sensitive, robust, and easy-to-use LC/QQQ system for both pharmaceutical and environmental applications.

Peptide Quant and Biomarker Validation: This market requires both the highest sensitivity and excellent reproducibility in a very complex matrix such as serum or plasma. This application is best served with the ChipCube which makes the 6430 the best choice.

Agilent's New 6540 Ultra High Definition Accurate Mass Q-TOF

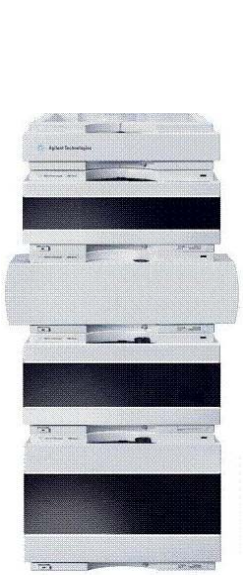
Exceptional accurate mass, sensitivity, dynamic range and resolution … perfect match for 1290 Infinity UHPLC

- 500 ppb mass accuracy
- femtogram sensitivity
- 5 decades dynamic range
- 40,000 resolving power
- 20 Spectra/sec
- Excellent Linearity and Isotopic Fidelity
- Supports Agilent Jet Stream and HPLC-Chip

What is Ultra High Definition?

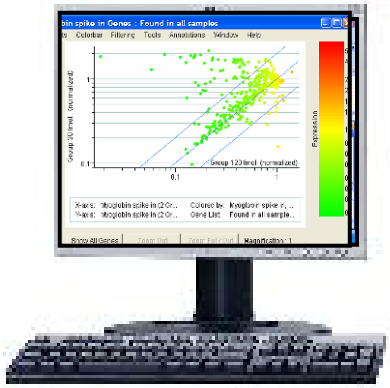
Ultra High Definition is the combination of uncompromising performance in **All Dimensions** of the analytical measurement.

Ultra High Definition LC/MS achieves the **Maximum Qualitative and Quantitative Information possible**.



Chromatography

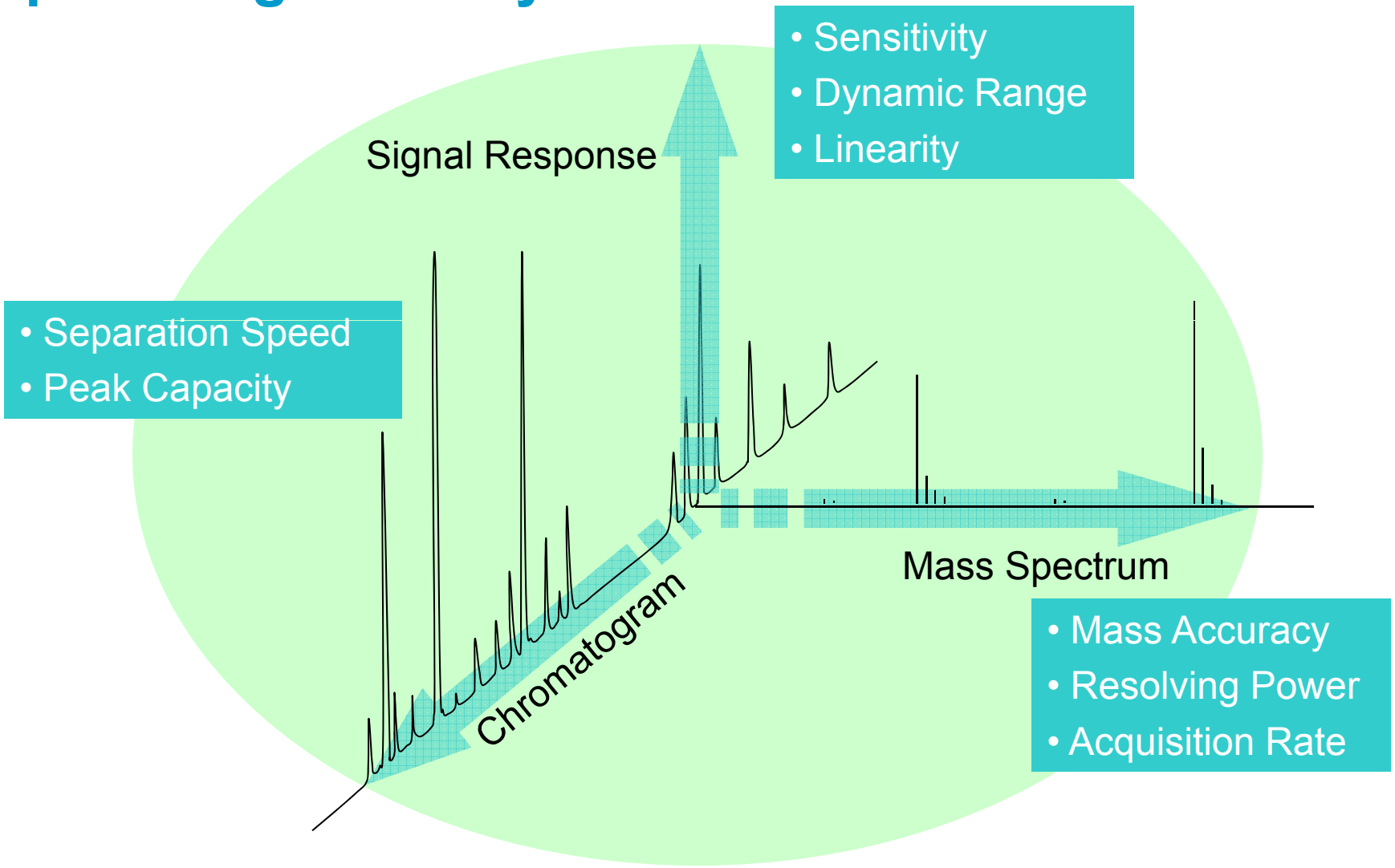
Mass Spectrometry



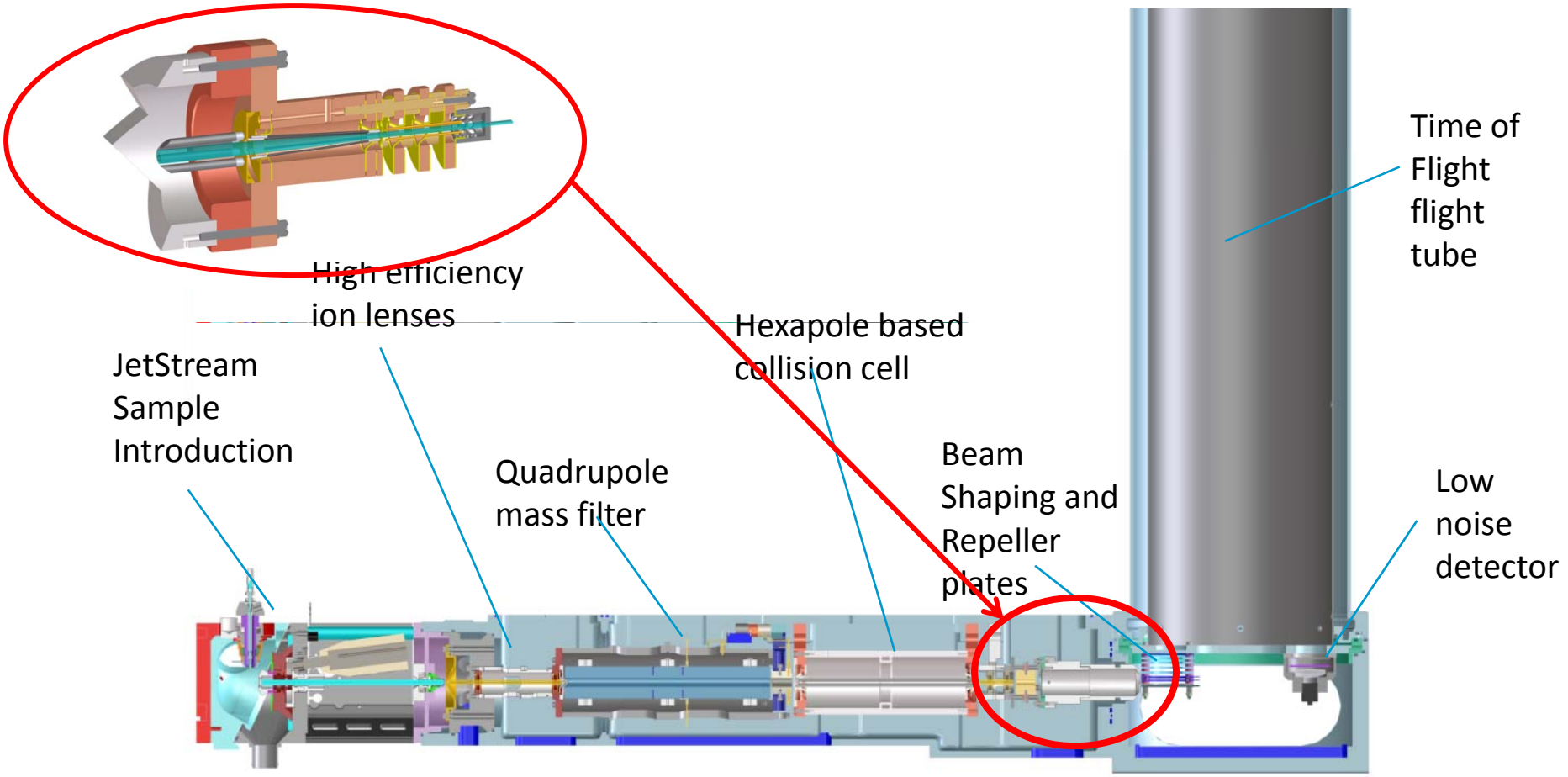
Informatics

Comprehensive Sample Information

Ultra High Definition
Optimizing all Analytical Dimensions

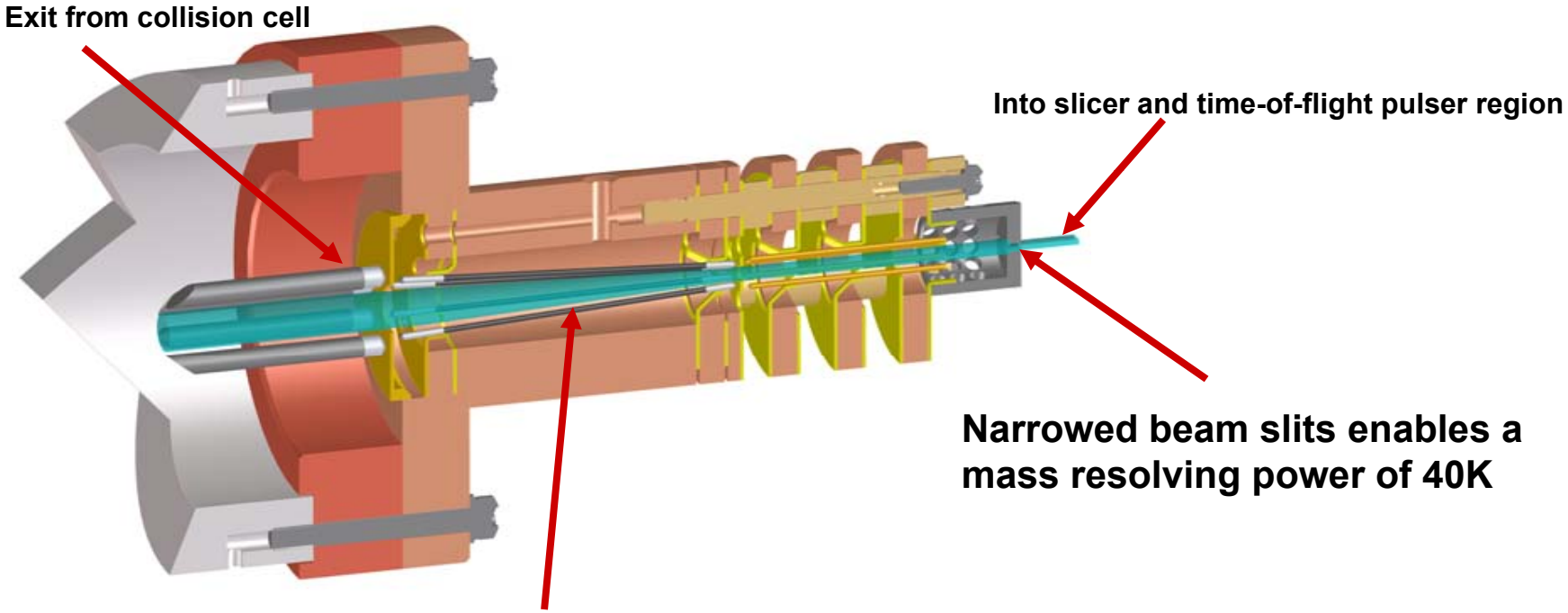


6540 QTOF: New Technologies



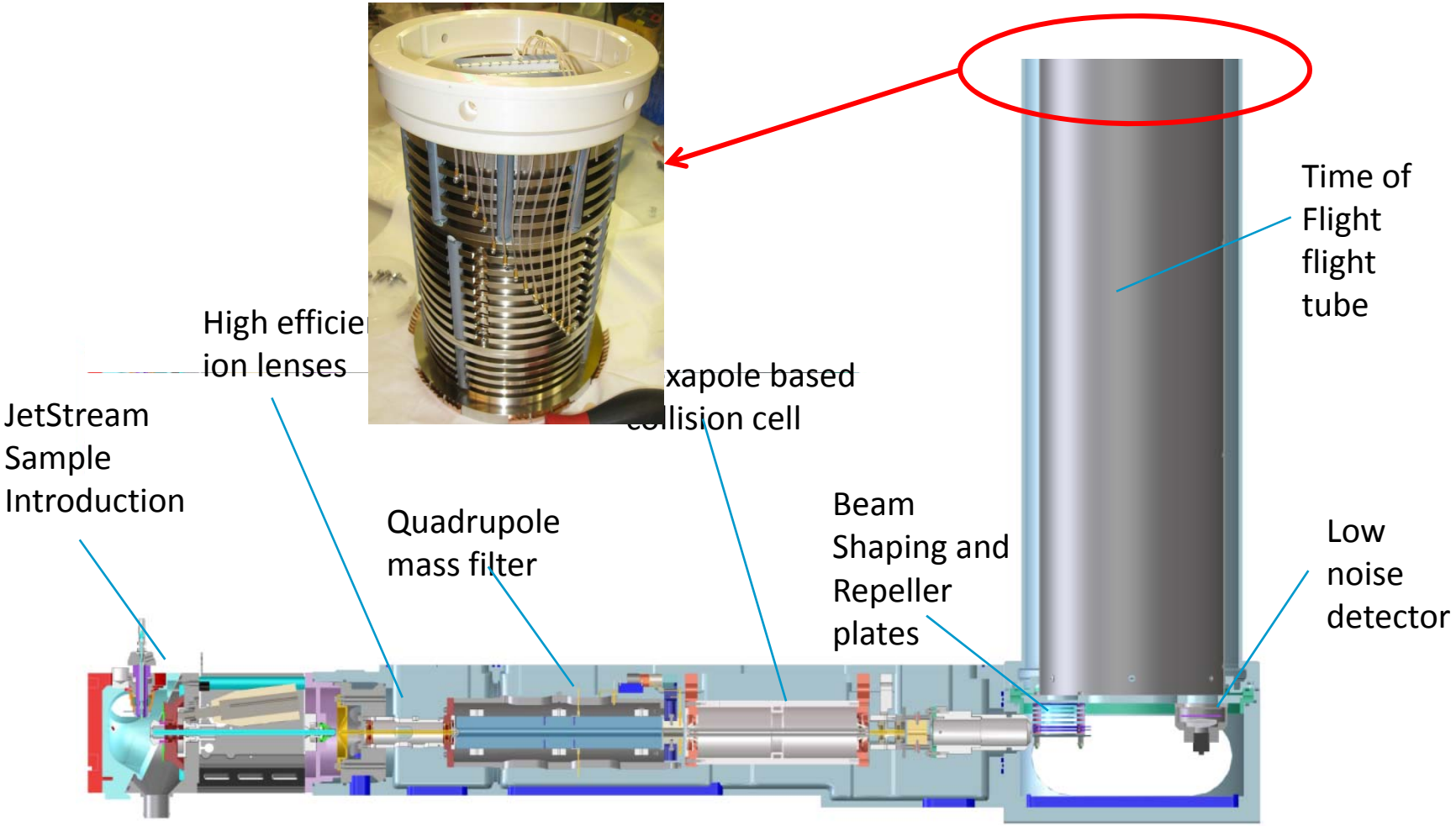
Ion Beam Compression (IBC)* Technology Drives Higher Resolution

Active Ion Beam Compression simultaneously maximizes ion transmission and reduces beam divergence



*** Patent pending**

6540 QTOF: New Technologies

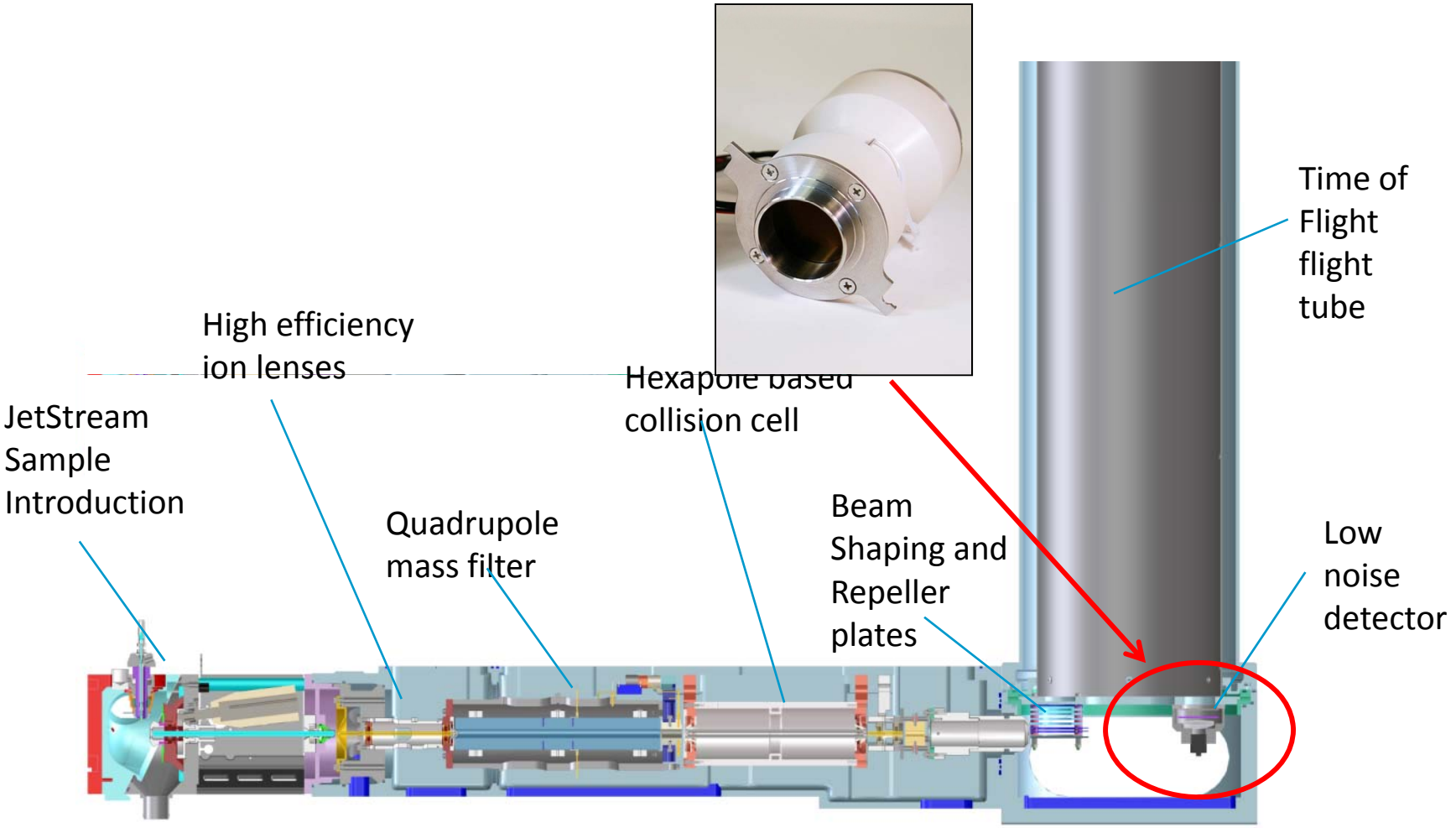


Enhanced Mirror Technology (EMT)

Enhanced Mirror Technology minimizes variation and distribution of ion arrival time and energies

- **2nd order time focusing**
- **Deeper electrostatic ion mirror**
- **35% longer flight tube**

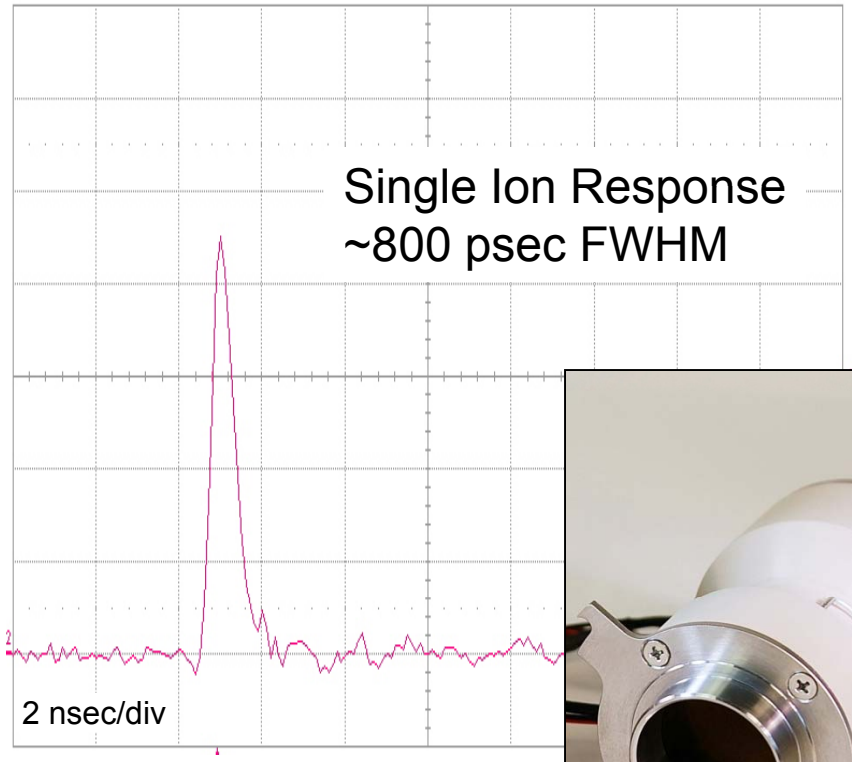
6540 QTOF: New Technologies



Ultra High Speed Detector For Research Grade Performance

New Bipolar TOF Detector

- New ultra fast and high efficiency scintillator
- New ultra fast response PMT design continues the tradition of high dynamic range and detector lifetime
- Developed by Photonis with Agilent TOF Technology
- Specifically enhances Resolution in 2Ghz Ext. Dynamic Range Mode



Making Research Grade Performance possible in a Benchtop Format

New Photonis Fast Bipolar Detector

Performance characteristics

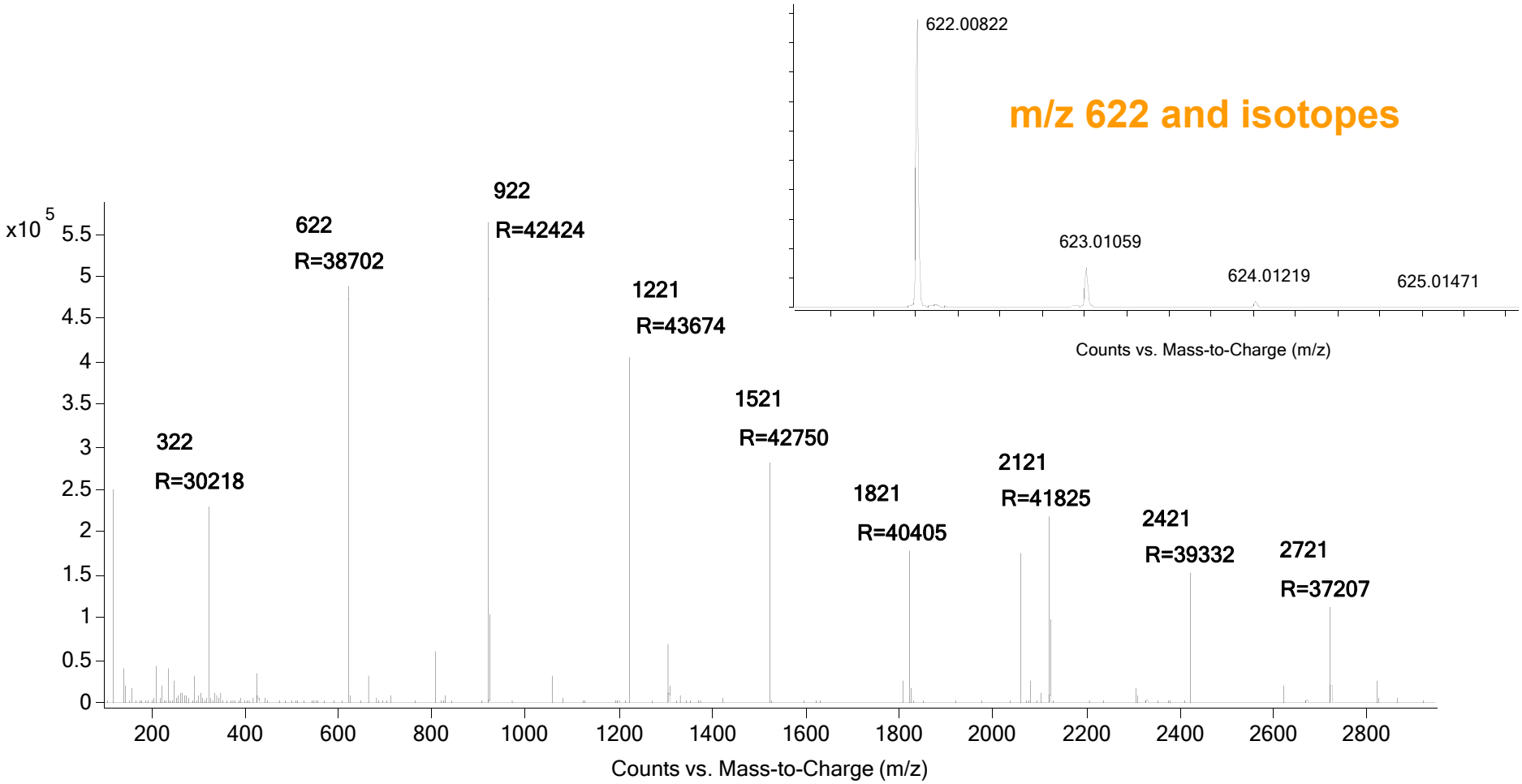
- Pulse width less than 1 nanosecond
- Resolution in 2GHz -extended dynamic range mode over 30k on high mass
- Large increase in resolution for low mass ions
- Maintain 5 orders of magnitude linear dynamic range

Resolution improvement with new detector

m/z	Old detector	New detector
112.9935	8207	14313
301.98828	13090	21669
601.93257	17261	25811
1033.88433	20582	27940
1333.82243	21878	28254
1633.75861	23364	29495
1933.69346	24761	30815
2233.62762	25551	31124

6540 Ultra High Definition QTOF

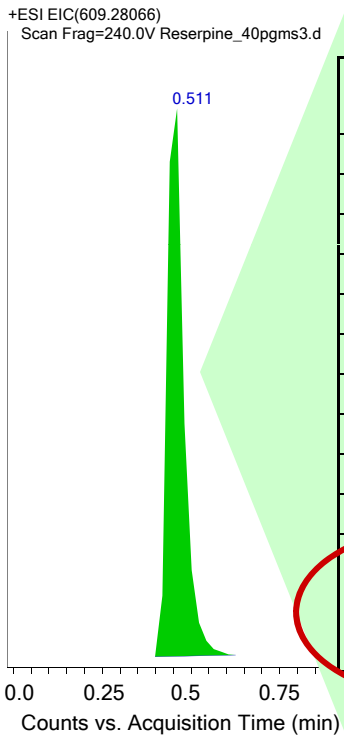
Maintaining Resolving Power – Across the Mass Range



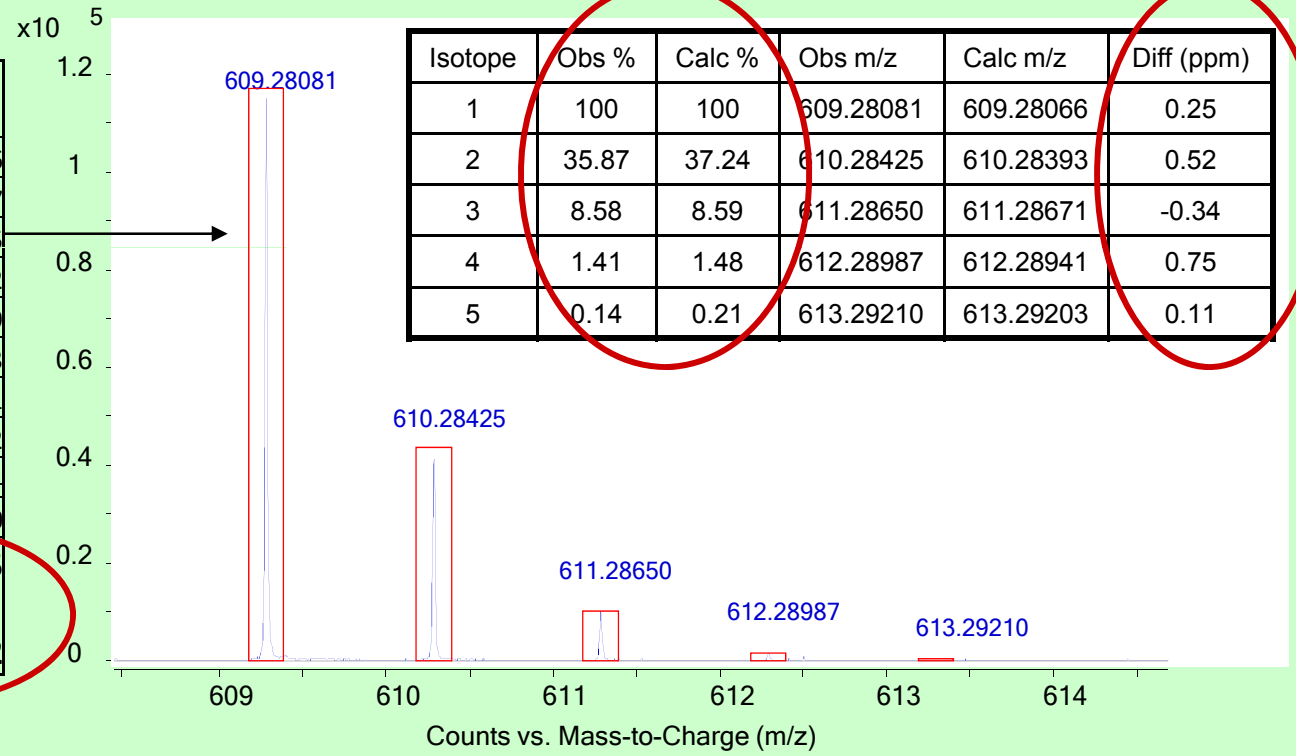
Scan Rate Independent

6540 Ultra High Definition QTOF
Mass Accuracy – Repetitive Injections

40pg reserpine on-column, 10 injections



Run	Error (ppm)
1	0.96
2	-0.17
3	0.25
4	0.02
5	0.39
6	0.13
7	0.01
8	0.52
9	0.04
10	0.30
Mean	**0.25**
Std. Dev	**0.32**



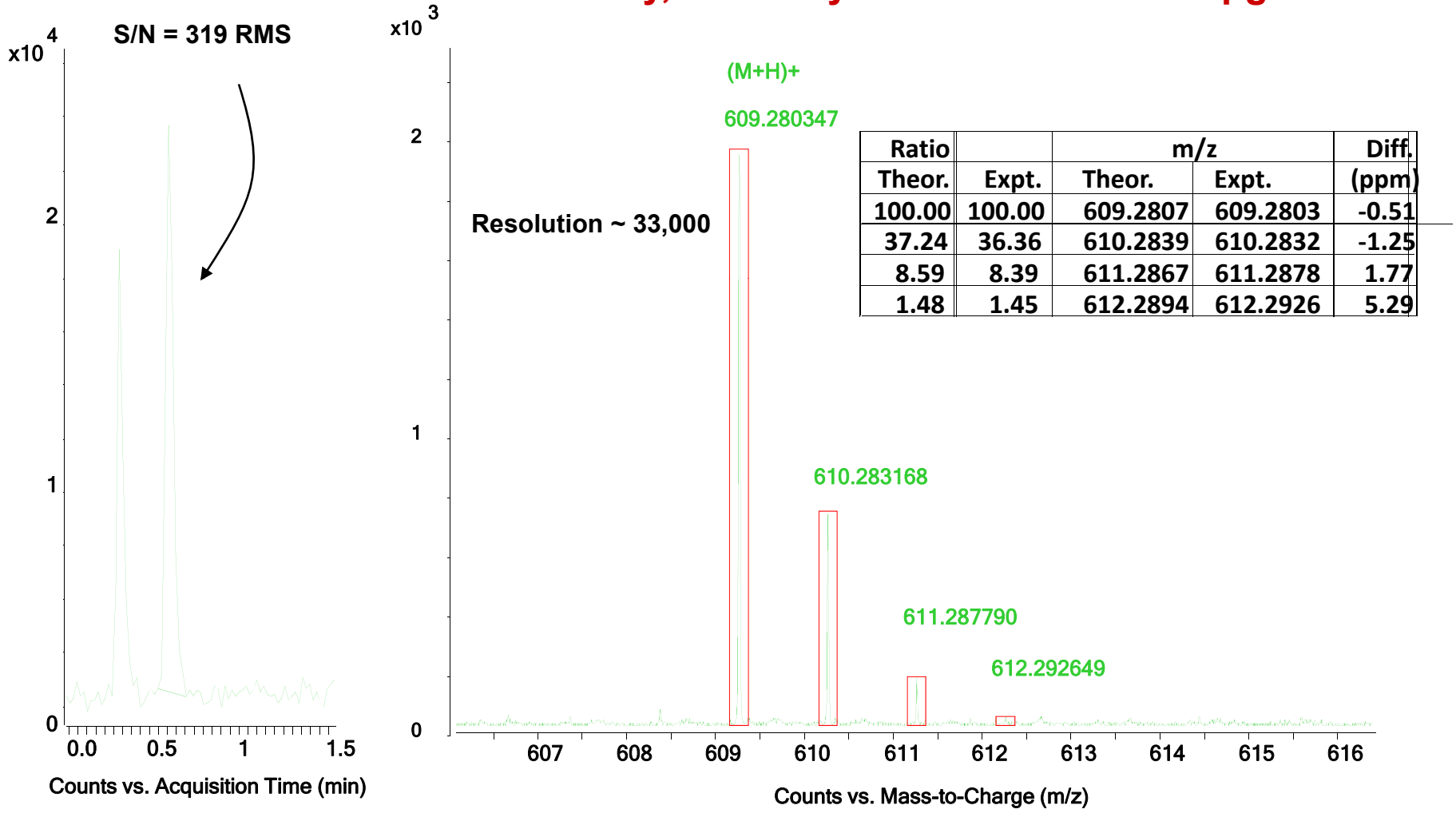
Isotope	Obs %	Calc %	Obs m/z	Calc m/z	Diff (ppm)
1	100	100	609.28081	609.28066	0.25
2	35.87	37.24	610.28425	610.28393	0.52
3	8.58	8.59	611.28650	611.28671	-0.34
4	1.41	1.48	612.28987	612.28941	0.75
5	0.14	0.21	613.29210	613.29203	0.11

250 ppb mass accuracy calibration and very accurate isotopic ratios

6540 Ultra High Definition QTOF

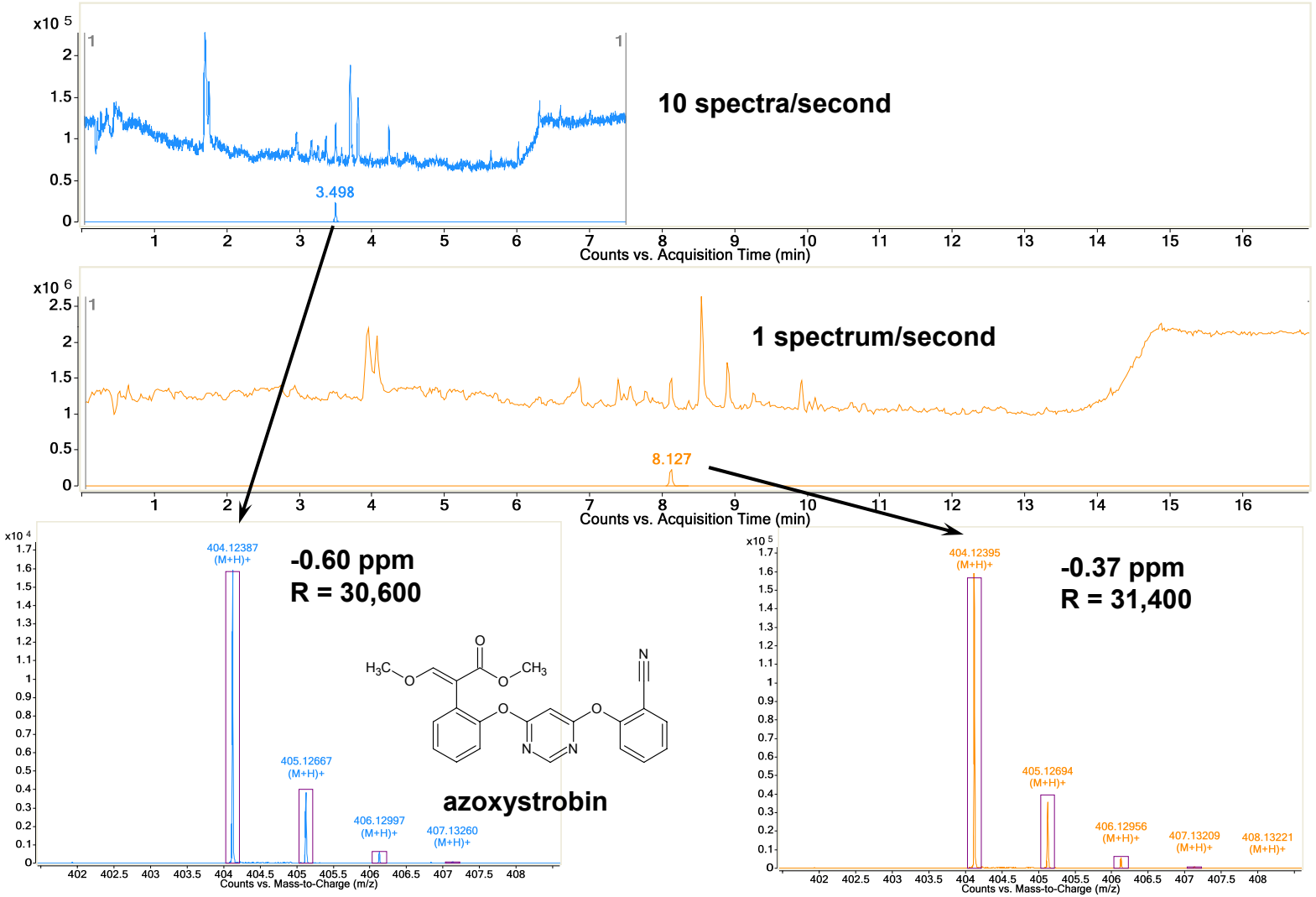
Sensitivity – Full Scan MS Mode- 1 picogram

Excellent sensitivity, accuracy and resolution at 1 pg on column

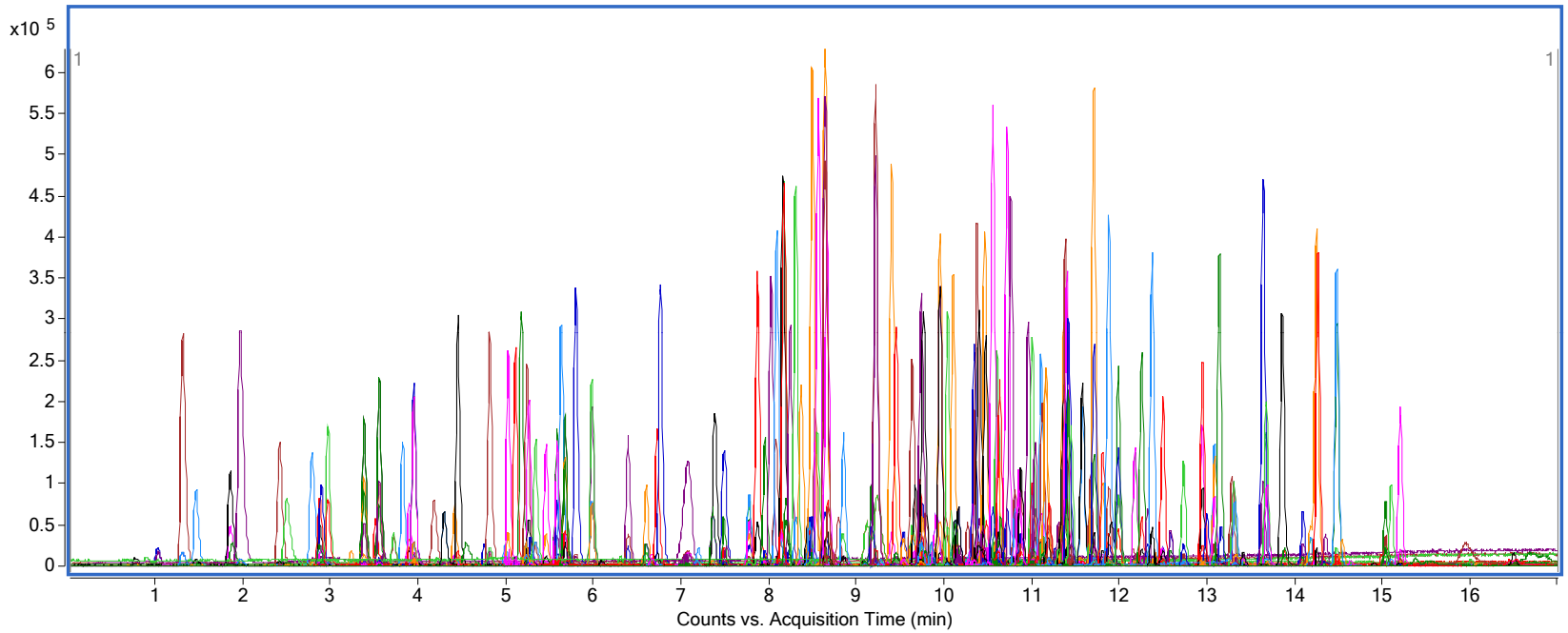


Ratio		m/z		Diff.
Theor.	Expt.	Theor.	Expt.	(ppm)
100.00	100.00	609.2807	609.2803	-0.51
37.24	36.36	610.2839	610.2832	-1.25
8.59	8.39	611.2867	611.2878	1.77
1.48	1.45	612.2894	612.2926	5.29

Mass Accuracy And Mass Resolution And Analysis Speed Simultaneously



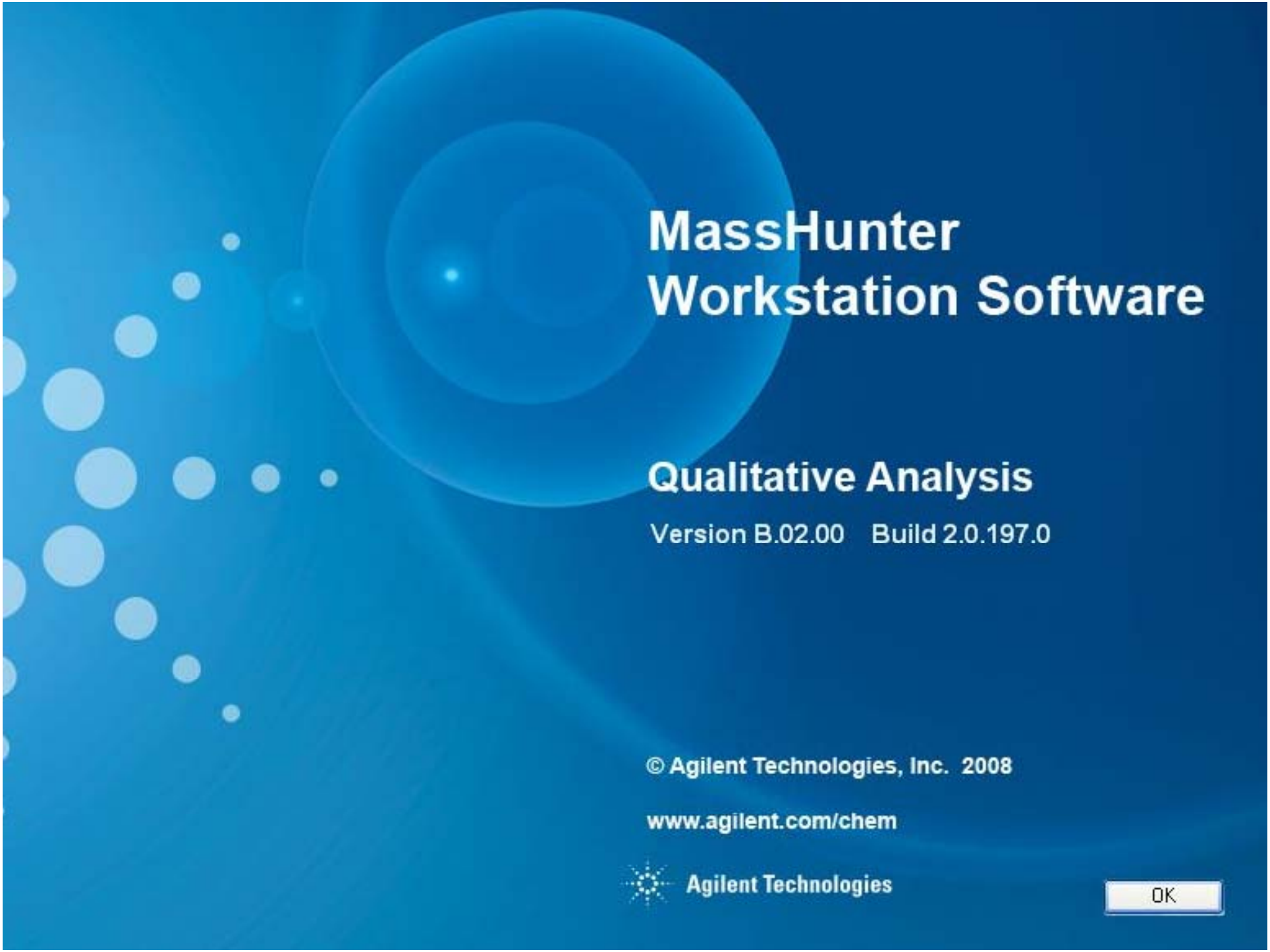
17 minute analysis – 224 Pesticides
6540 QTOF/1290 Infinity uHPLC



3 Hz Data Acquisition Speed
50pg on-column

6540 Ultra High Definition Q-TOF
Key Design Enhancements

Feature	Advantage	Benefit
Ion Beam Compression- compresses ion beam into dense layer with uniform energy	Minimize sensitivity loss while passing through narrow slit	Greater mass resolution without sensitivity loss
Enhanced Mirror Technology- deeper mirror with 2nd order focusing	Minimize distribution of ion arrival time and energy	Greater mass resolution
Longer Flight Tube- 35% increase in length	Added length provides more time for ions to resolve	Greater mass resolution
Fast Bipolar Detector – 2X faster than previous version	Greater mass resolution in extended dynamic range mode	ID more compounds in complex mixtures- metabolomics
Fine tune Internal Reference Based Calibration	2-3 fold improvement in mass accuracy	Greater confidence for compound ID


MassHunter
Workstation Software
Qualitative Analysis
Version B.02.00 Build 2.0.197.0
© Agilent Technologies, Inc. 2008
www.agilent.com/chem
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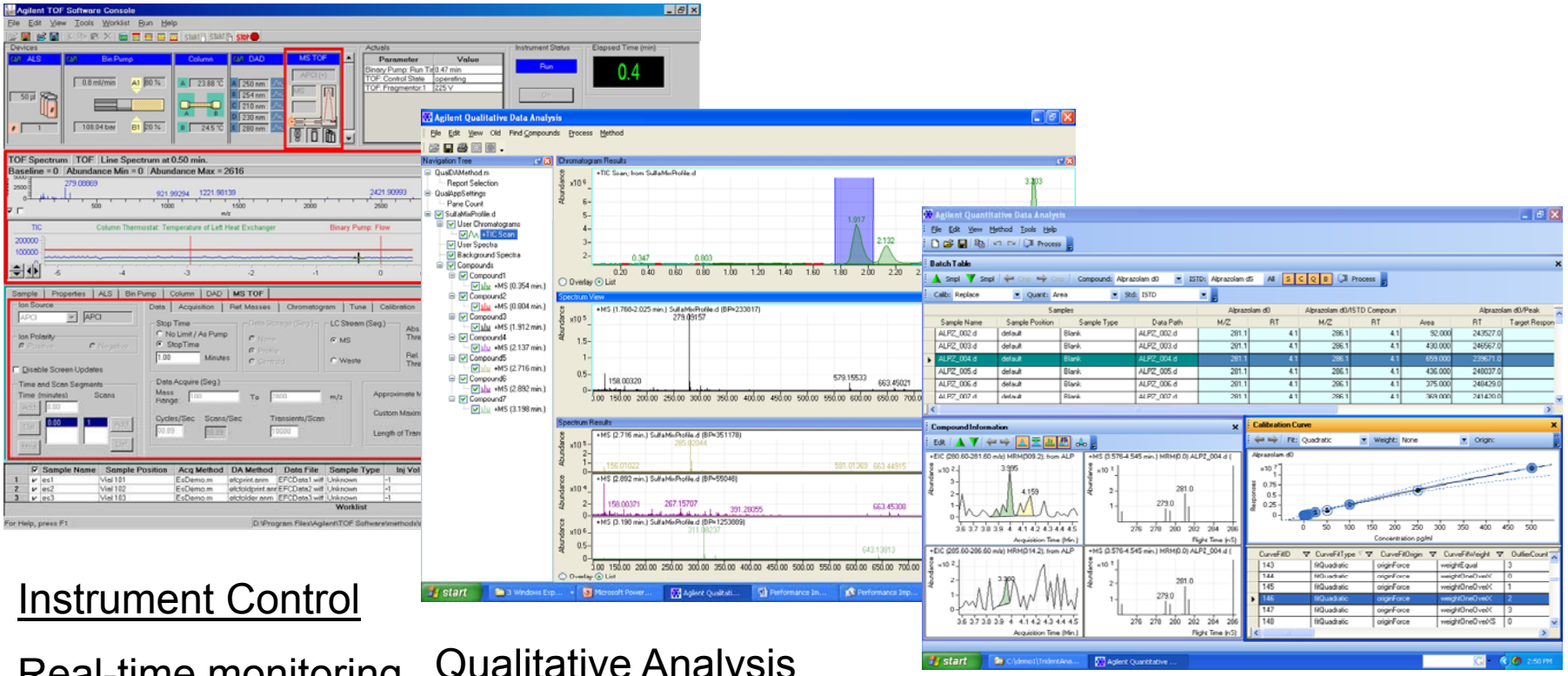
OK

MassHunter Software



Instrument Control

Real-time monitoring

Method set-up

Autotune

Qualitative Analysis

Chromatographic results

Spectral results

Find compounds

Quantitative Analysis

User filters

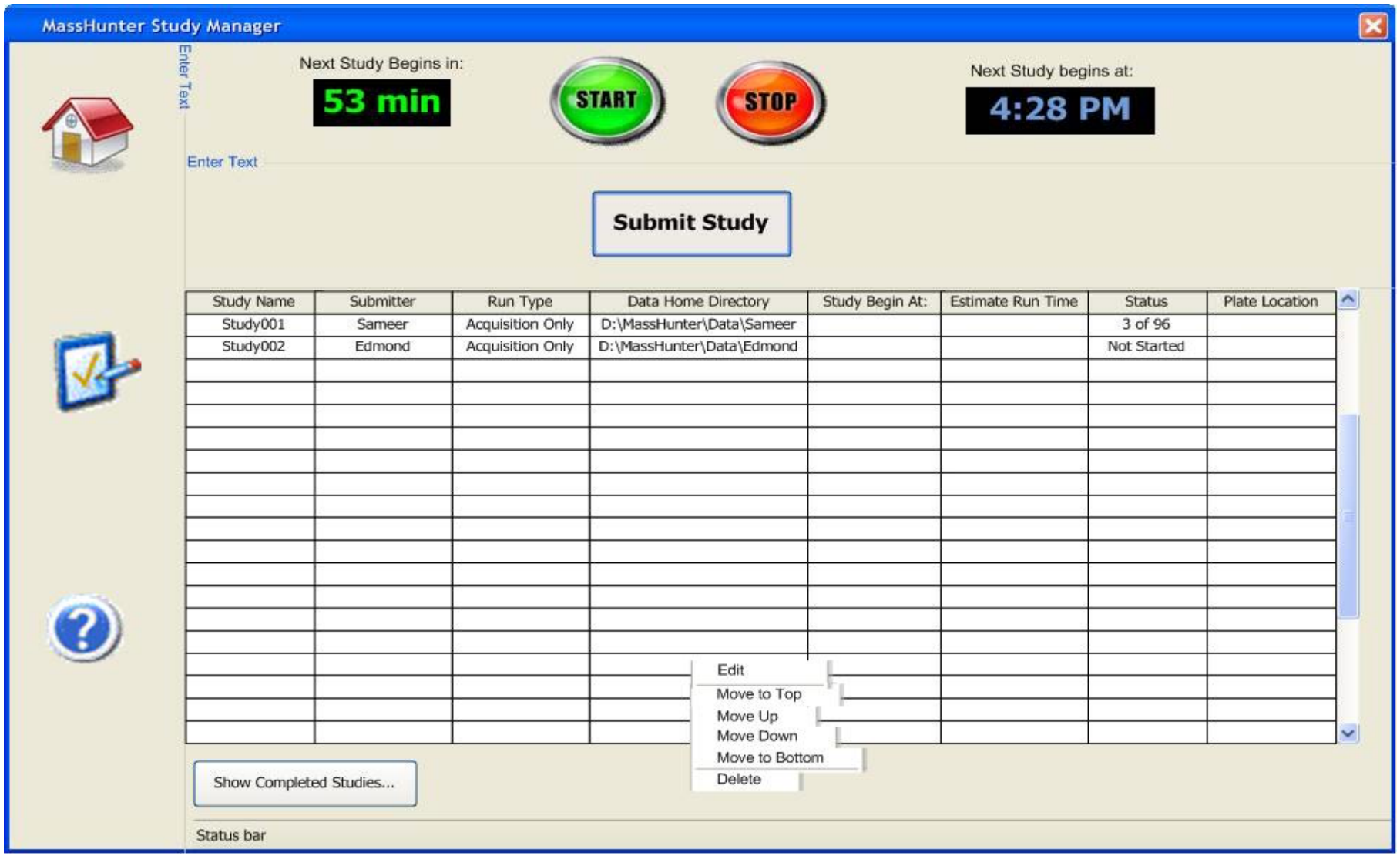
Compound results

Calibration curve

MassHunter: Key New Highlights

- MRM Optimizer – B.02.00 All new QQQ and upgradable
- Dynamic MRM
- Peptide Optimizer – aimed at the validation of biomarkers
- New Pharma workflows – Pharmacokinetics and ADME
  - Electronic input of study information
  - Automated quantitation of results
  - Logical data review (by compound group in early ADME studies)
  - Electronic export of data for use in other applications
  - MassHunter Study Manager

MassHunter Study Manager



METLIN Metabolite Database

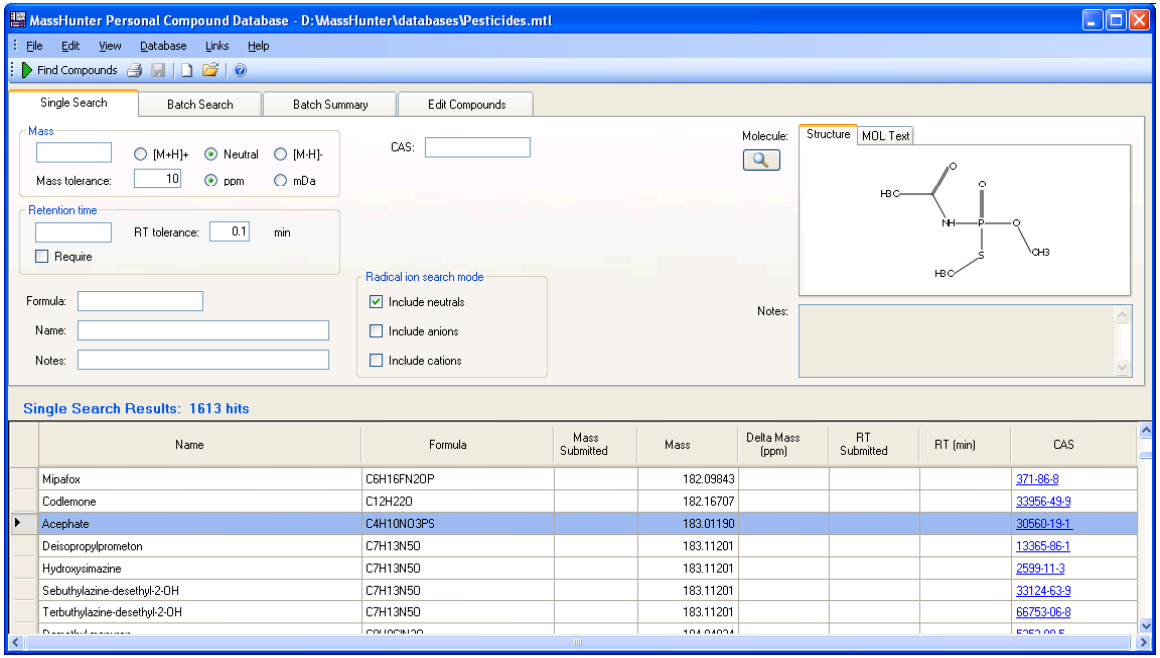
Agilent METLIN Personal Metabolite Database

Powerful database searching for easier metabolite identification

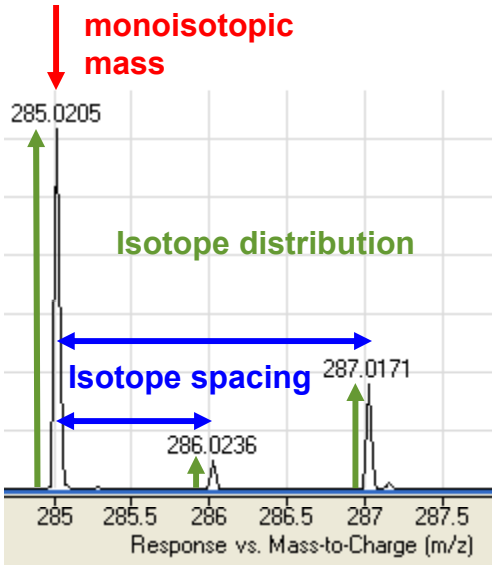
- Indentifying metabolites is a key step in untargeted metabolomic experiments
- Metabolite database searches using accurate-mass MS information can greatly improve metabolite identification by significantly narrowing the list of possibilities
- The METLIN metabolite database compiled by the Scripps Research Institute is one of the most comprehensive in the world today

Agilent Personal Compound Database

Accurate mass and optional RT databases (AMRT)



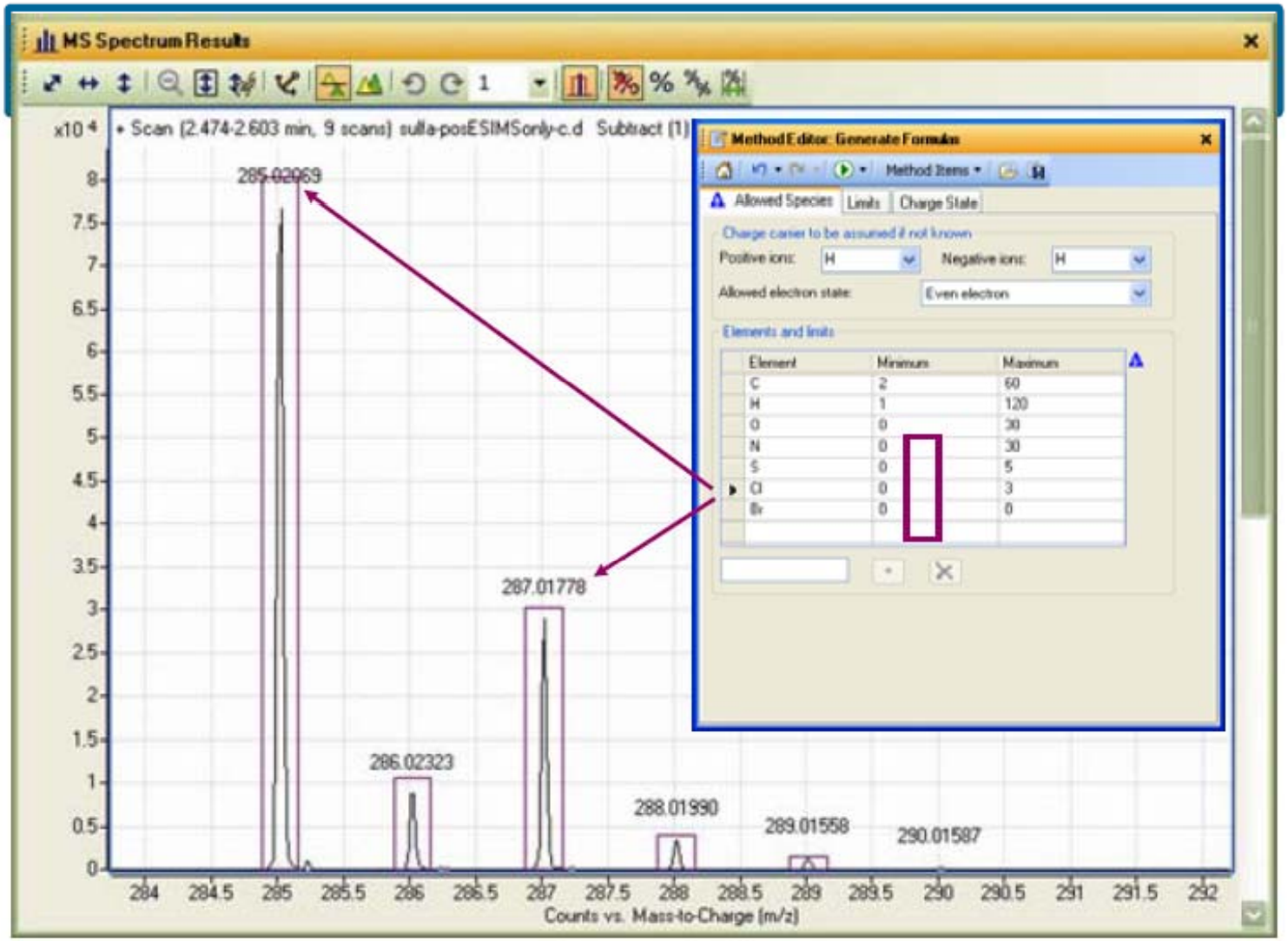
Scoring based on



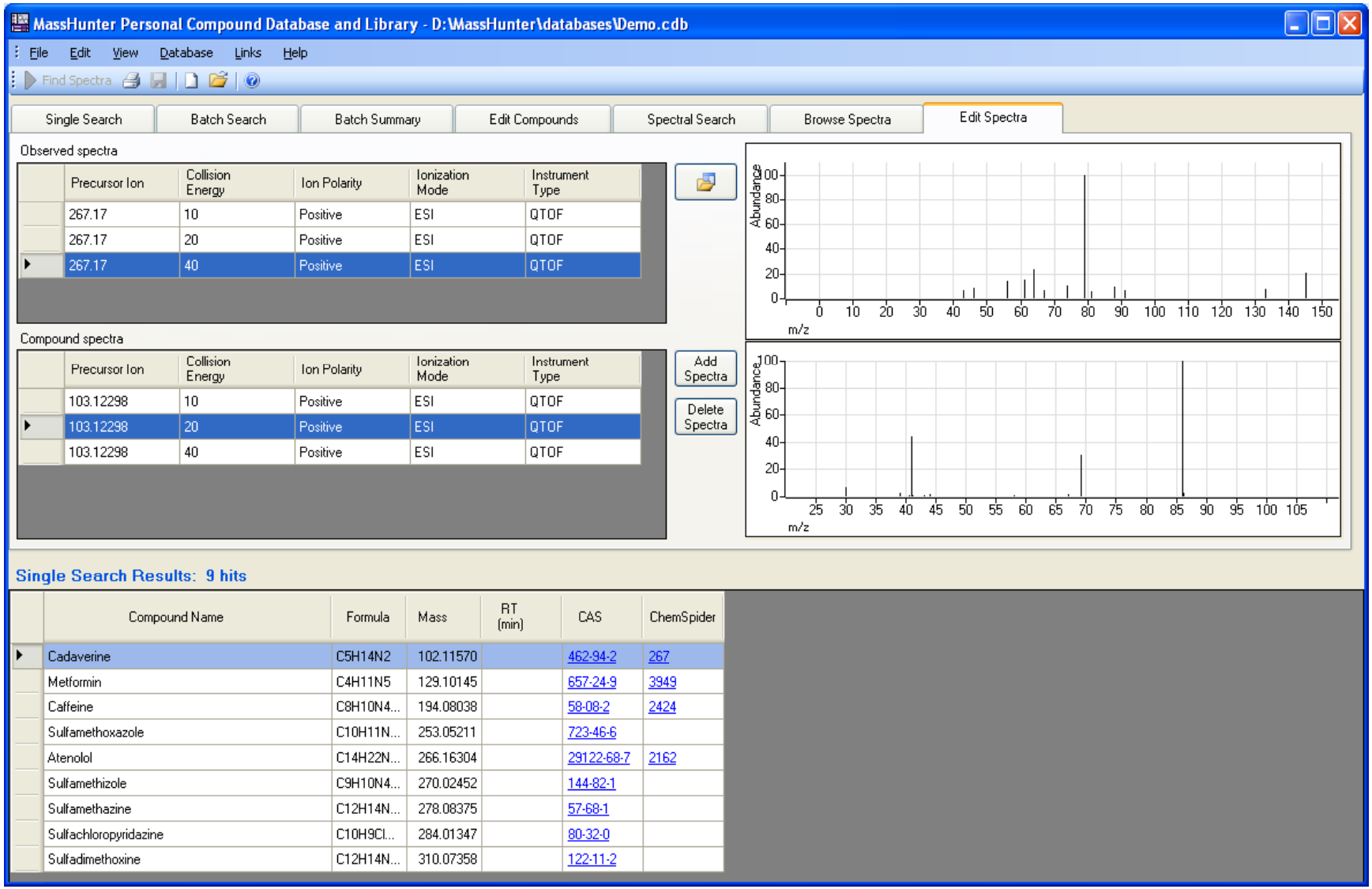
New for TOF based screening

- Agilent provided database of 23000 endogenous metabolites (METLIN)
- Agilent just released Personal Pesticide database (1600 pesticides)
- Agilent provided forensics database (7000)
- Agilent provided lipids database (9000)
- Custom databases

Molecular Formula Generator

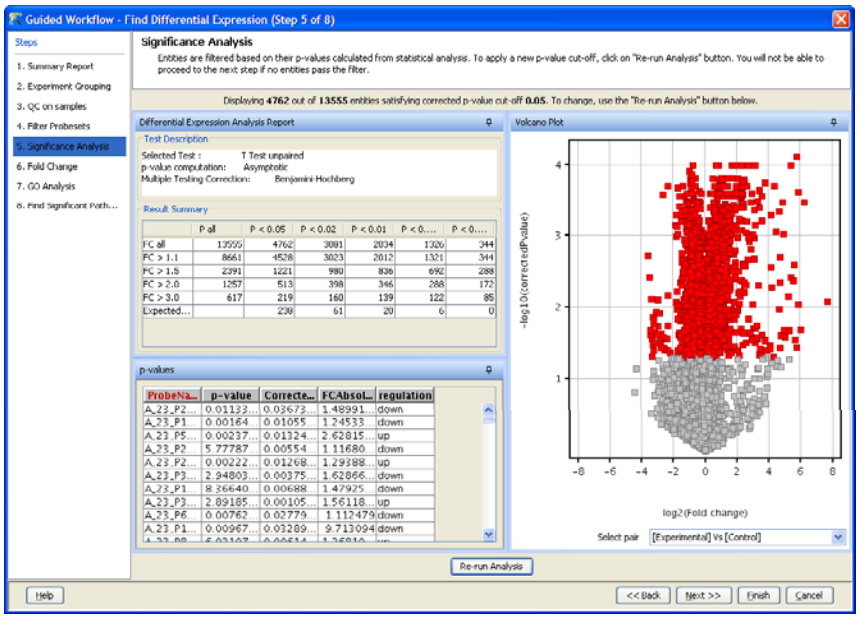


User Interface for PCDL (Q-TOF only initially)



MassHunter Mass Profiler Professional B.02.00 (MPP)

Differential Analysis Software Solution for Mass Spectral Data



Agilent Mass Profiler Professional (MPP)

Completely new user interface with dramatically improved usability

- Workflow navigator and “Guided Workflow” for novices !
- Designed specifically for mass spec based differential analysis

Significantly improved workflow for more speed and better accuracy

- Seamless integration of GC/MS and LC/MS data in one experiment.
- Unified new compound exchange file format (.cef) for streamlined data flow.
- New ID Browser for cmpd identification, annotation + browsing of MS detailed results
- Better annotation for LC/MS and GC/MS data (e.g. cmpd names, MS/MS spectra)
- New 2-pass recursive extraction to improve statistics by finding missed features

New optional integrated Pathway Analysis Module

- For Metabolomics and Proteomics Studies!
- Use of curated pathways (web) or 15 Agilent provided networks (NLP of literature)

Four levels of software solutions for the widest range of applications.

- Mass Profiler (Std) for entry-level differential analysis of 2 samples or sample sets.
- Mass Profiler Professional for sophisticated differential analysis of ≥ 2 sample sets.
- Optional Pathway Analysis module for metabolomics and proteomics studies
- Upgrade to GeneSpring MS “Add-on” to GeneSpring GX for Systems Biology studies!

MPP Target Applications

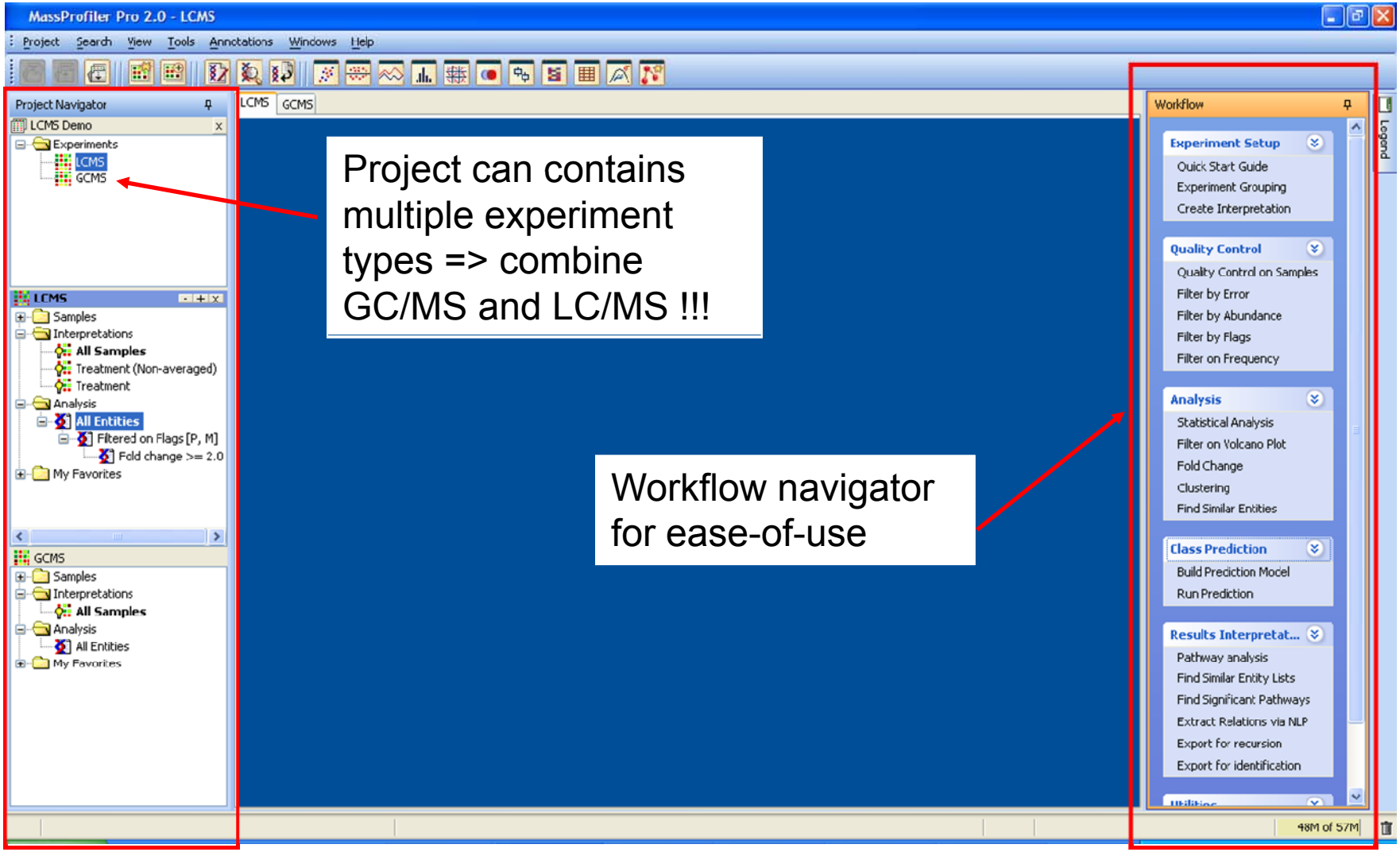
Metabolomics + Proteomics

- Biomarker Discovery
- Together with Genomics in Systems Biology (GeneSpring platform)
- Environmental Metabolomics
- Plant Metabolomics to create more resistant species

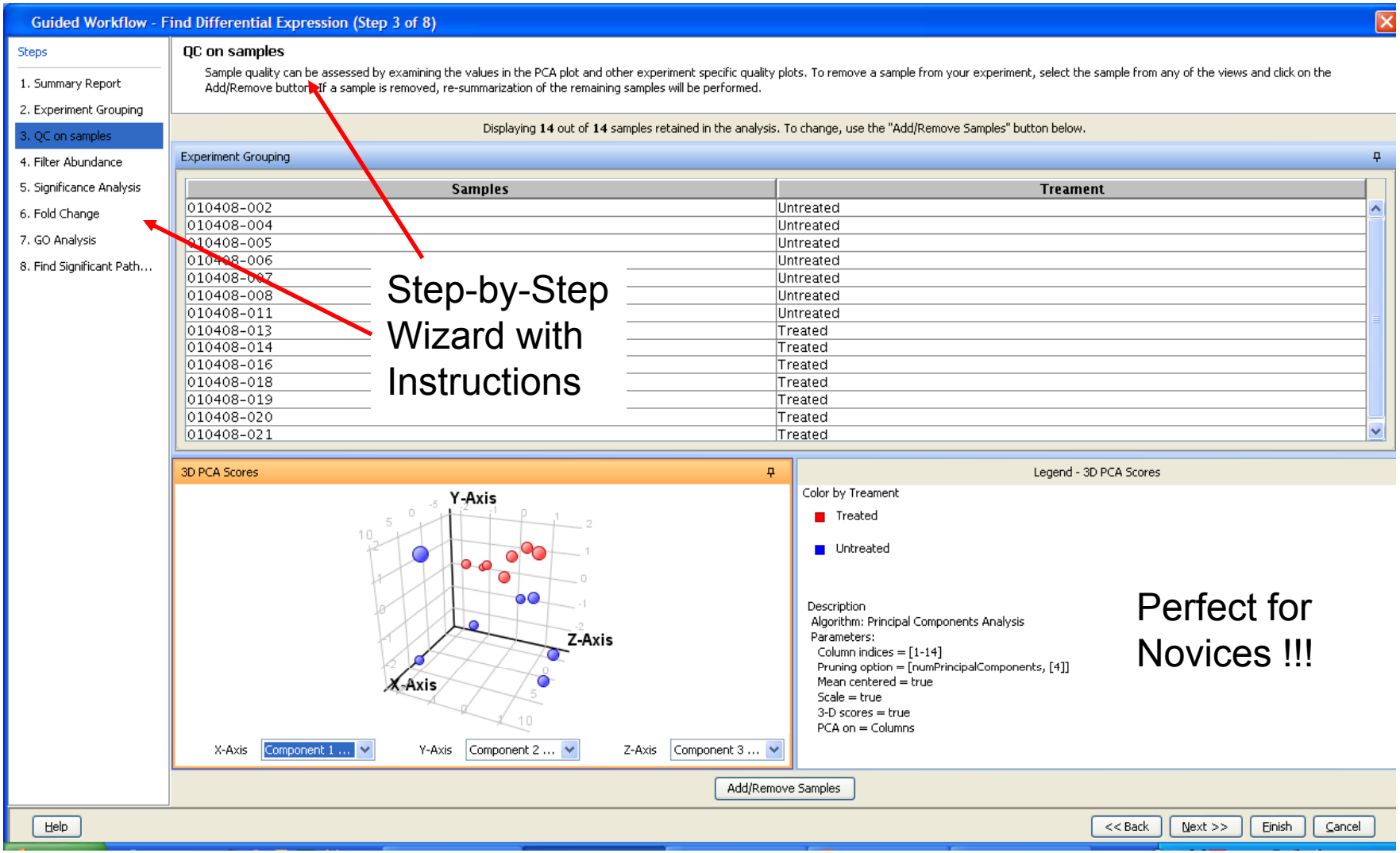
General mass spec based differential analysis

- Food, beverages, flavors and fragrances
  - Adulteration and fraud (natural products)
  - Origin determination and authenticity
  - ID of compounds and degradents affecting taste, texture, smell
- Degradation Studies
- Optimization of complex manufacturing processes of products
- Follow blood metabolome of athletes over time
- Compare water from different wells, locations in a river

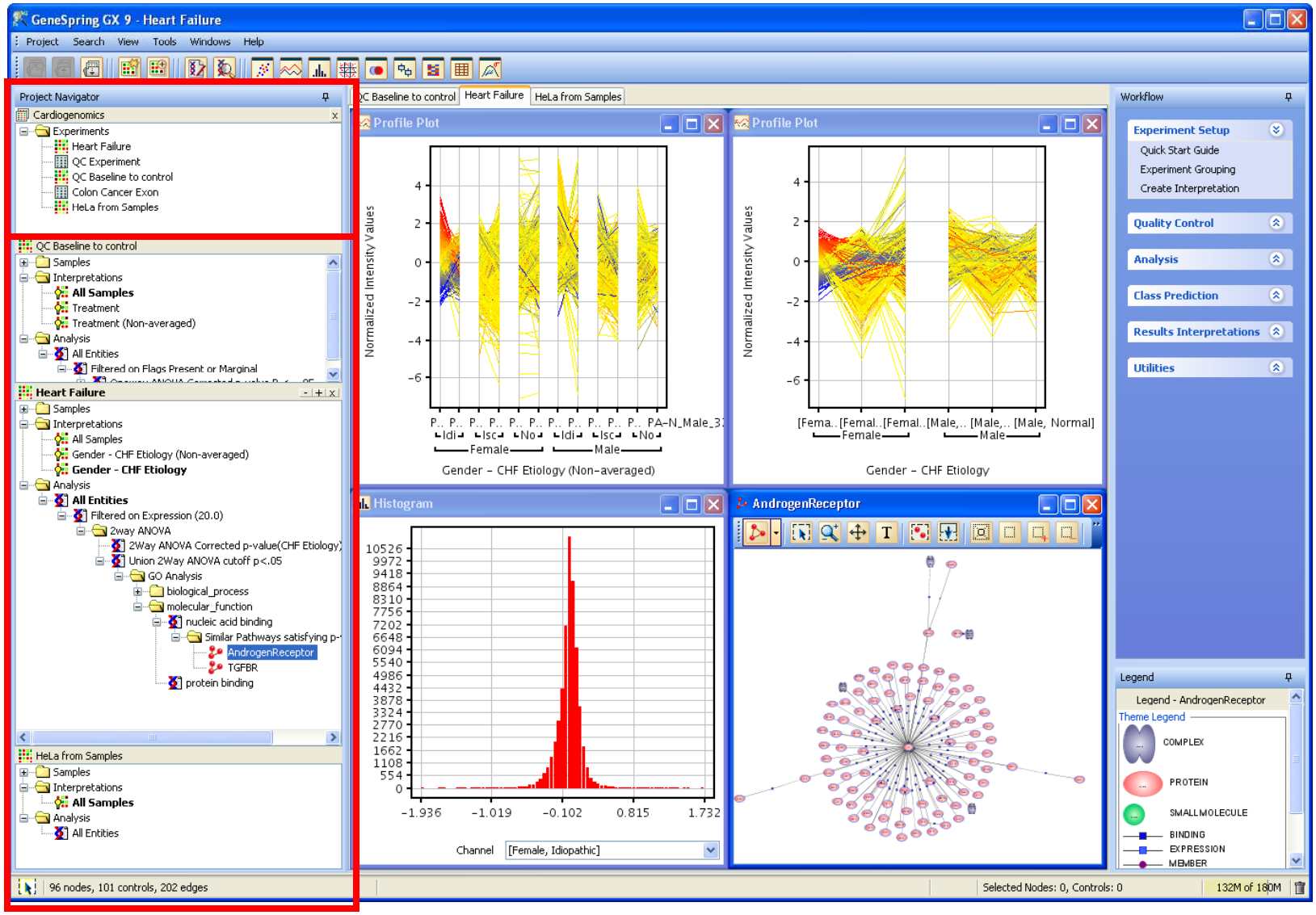
Mass Profiler Professional: New User Interface



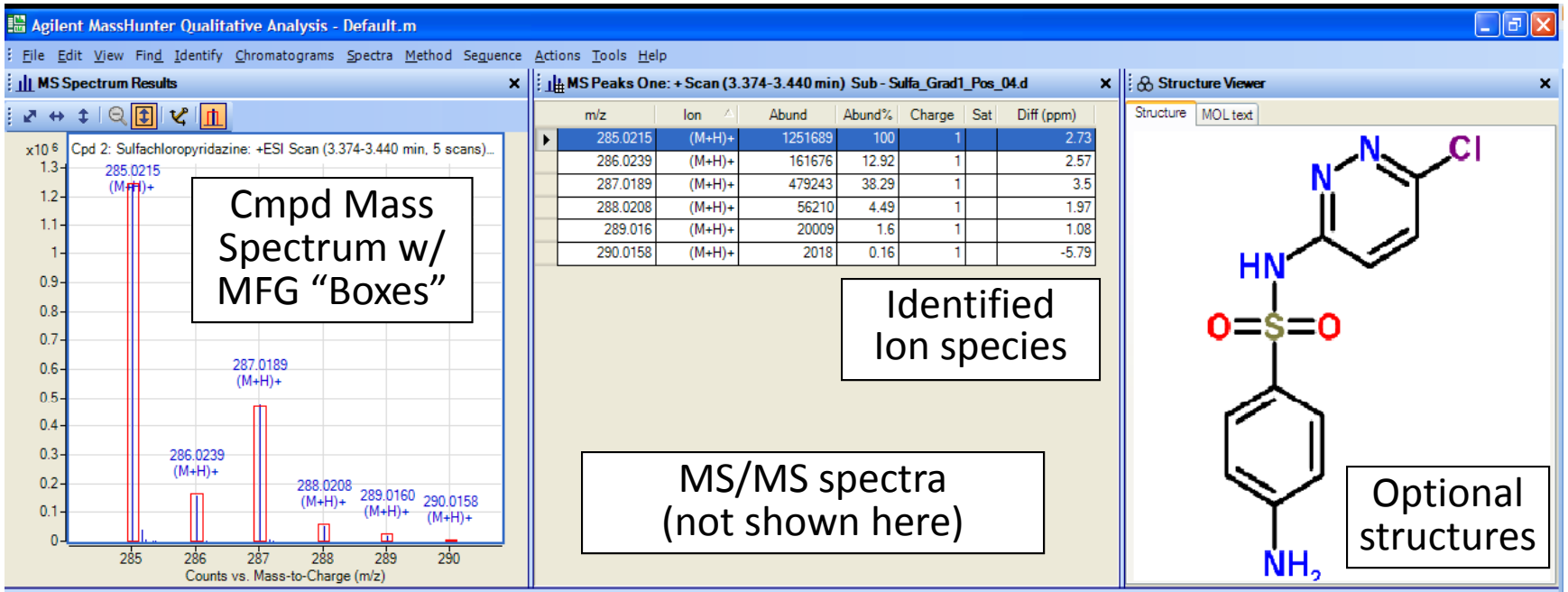
New Guided Workflow for Experiment Analysis



Hierarchical Experiment and Data Organization

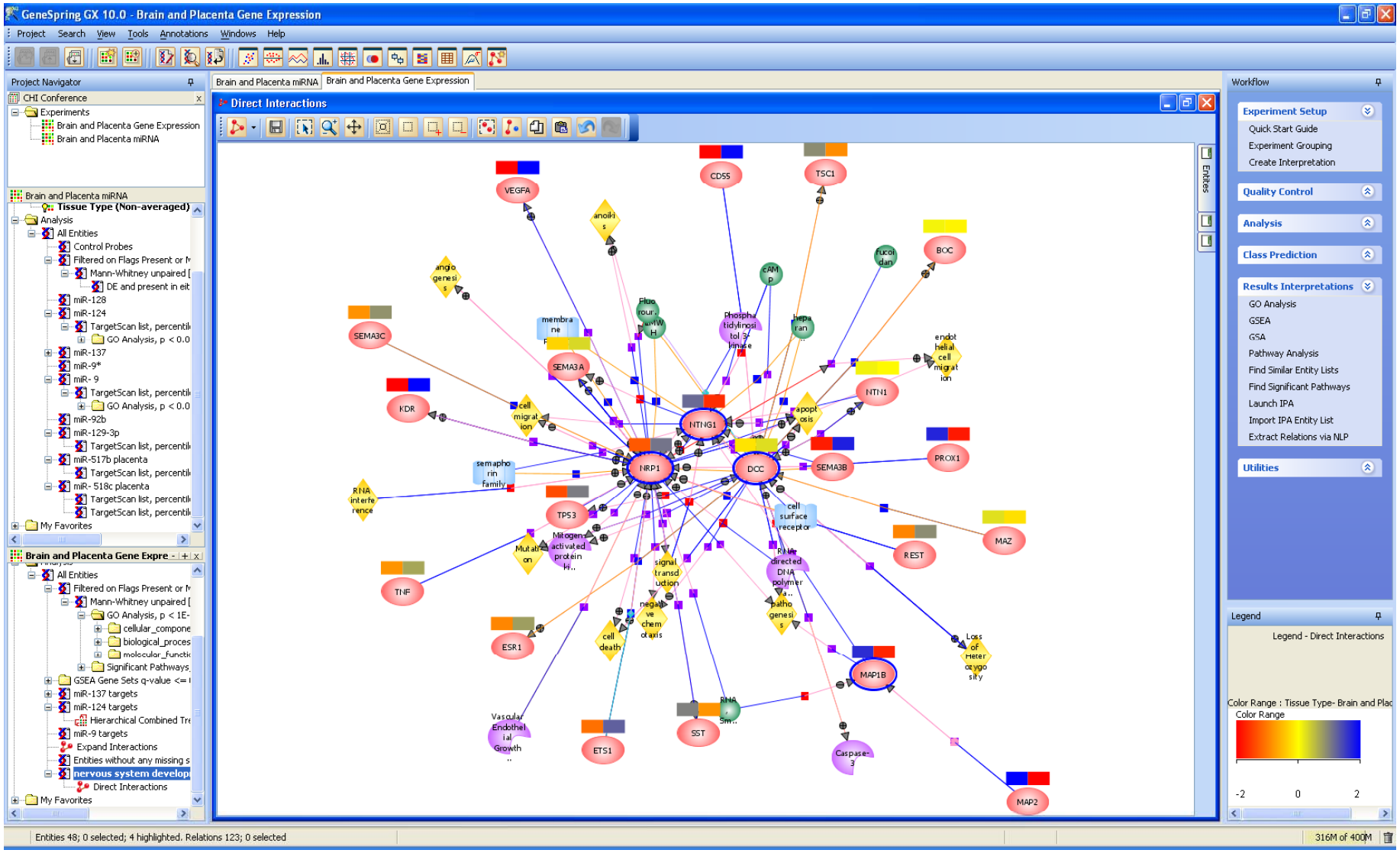


The Compound ID Browser – Built Into MPP



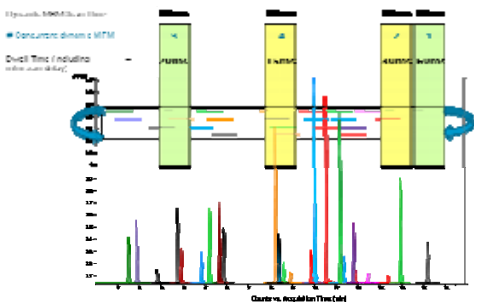
For LC/MS + GC/MS !

Overlay Networks w/ Expression Data/Conditions



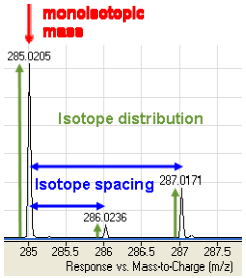
Driving Applications Forward

New MassHunter Software Tools Enable Huge Productivity Gains



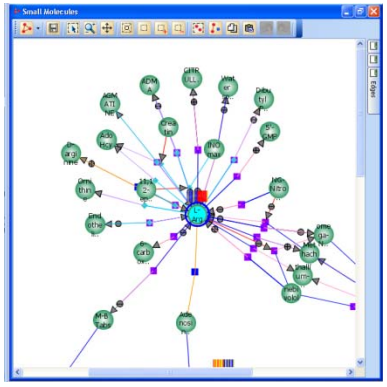
High Throughput Quantitation

- MH Optimizer quickly and easily optimizes MS/MS signal
- Dynamic MRM methods deliver robust assays faster
- Easy export to WATSON LIMS
- Fast flexible Custom Reporting is 10X faster



High Throughput Targeted Screening

- Personal Compound Databases (PCD) use accurate mass and RT
- Available for:
  - Metabolomics 23,000 compounds
  - Pesticides 1,600
  - Toxicology/Forensics 7,000
- New MS/MS Library Searching Capability



Proteomics / Metabolomics & Non-targeted Screening

- New Mass Profiler Professional
  - Intuitive workflows
  - Powerful statistical tools rendered easy-to-apply
- Pathway Architect for direct biochemical pathway interrogation

CLEARLY BETTER

Thank You

Agilent LC/MS, GC/MS, and ICP-MS Products

Agilent Technologies