



The Latest and Greatest in LC/MS

Achieving the utmost
information from today's
complex analyses

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Manager

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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 μ l w/o mixer*))
 - a) with Fixed loop (20 μ l*) injection
 - b) with Flow through (80 μ l*) injection
3. More robustness for lowest carry over with 1290 Infinity Autosampler
< 0.002 % Chlorhexidine**

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



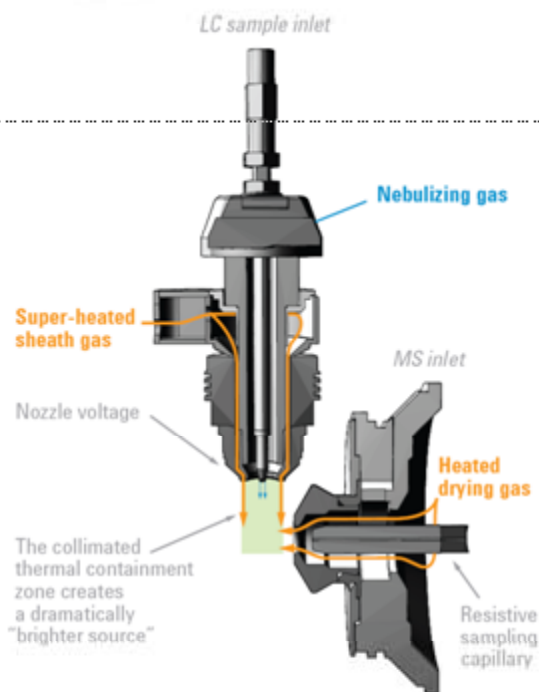
Agilent Technologies

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)



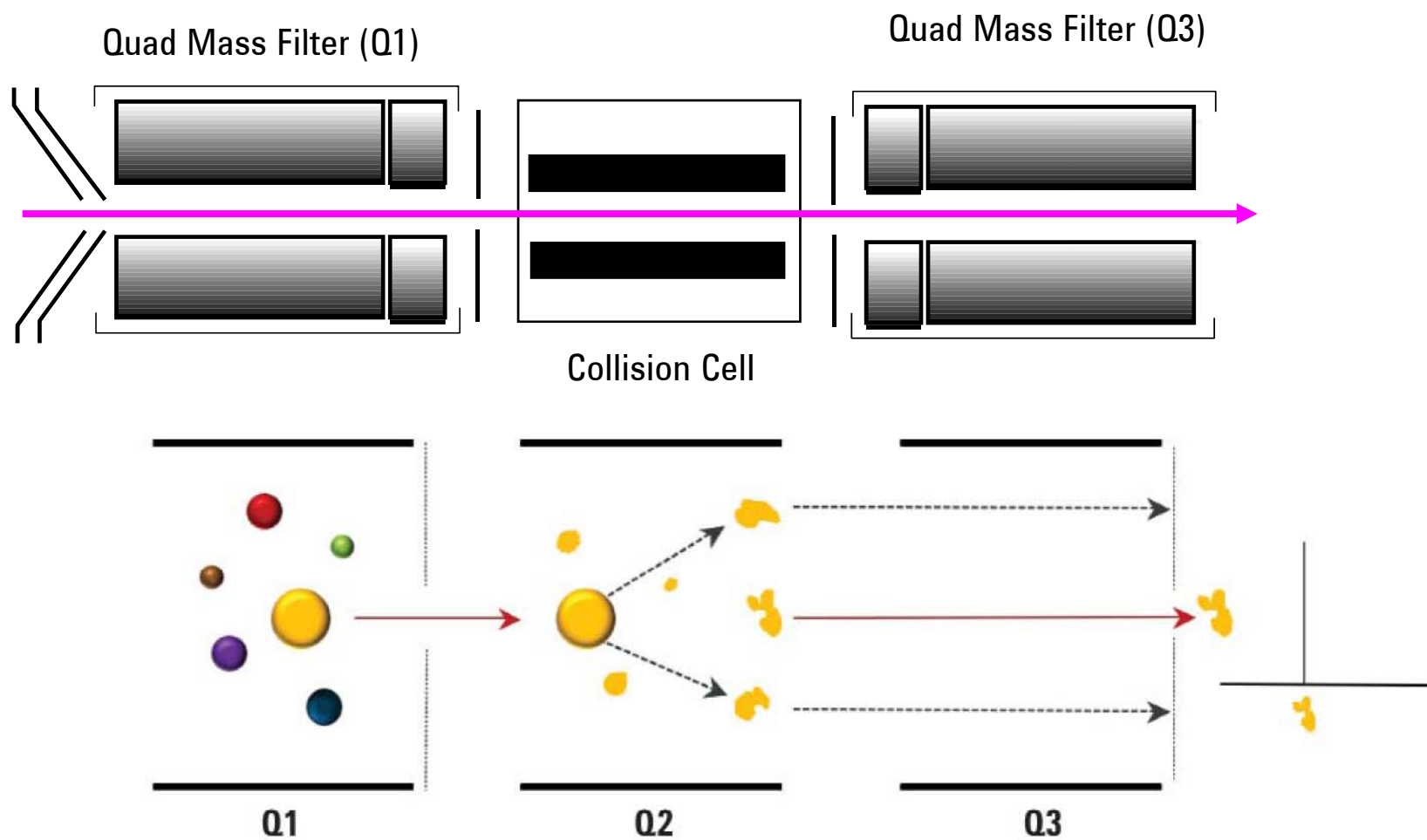
Agilent's new 6430 Triple Quad LC/MS System



- 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)

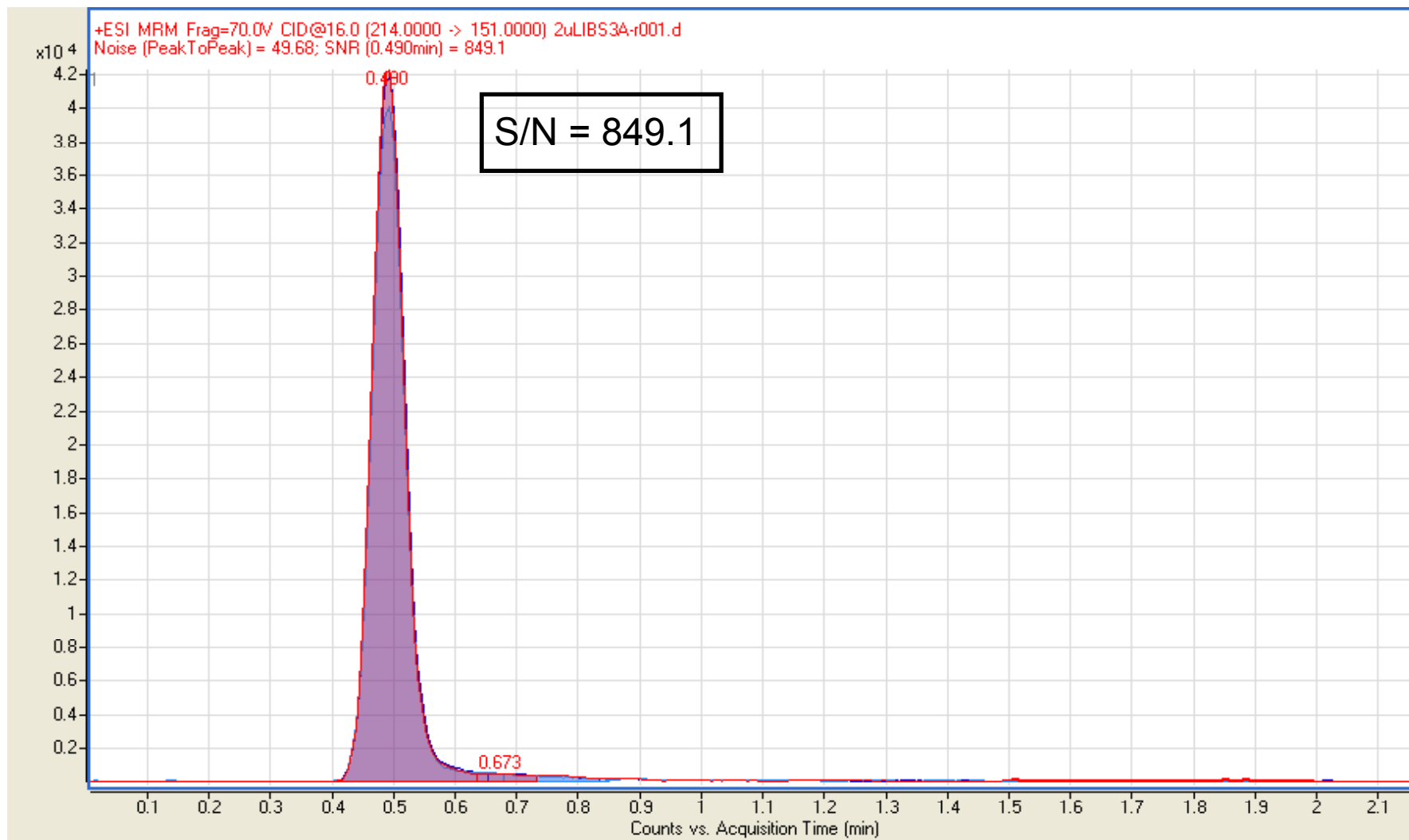


MRM Scanning: the power of the QQQ



Selectivity

MRM transition 214→151



Food Safety – Toxins and Toxicity

Pesticides – already in excess of 1600 compounds

Herbicides

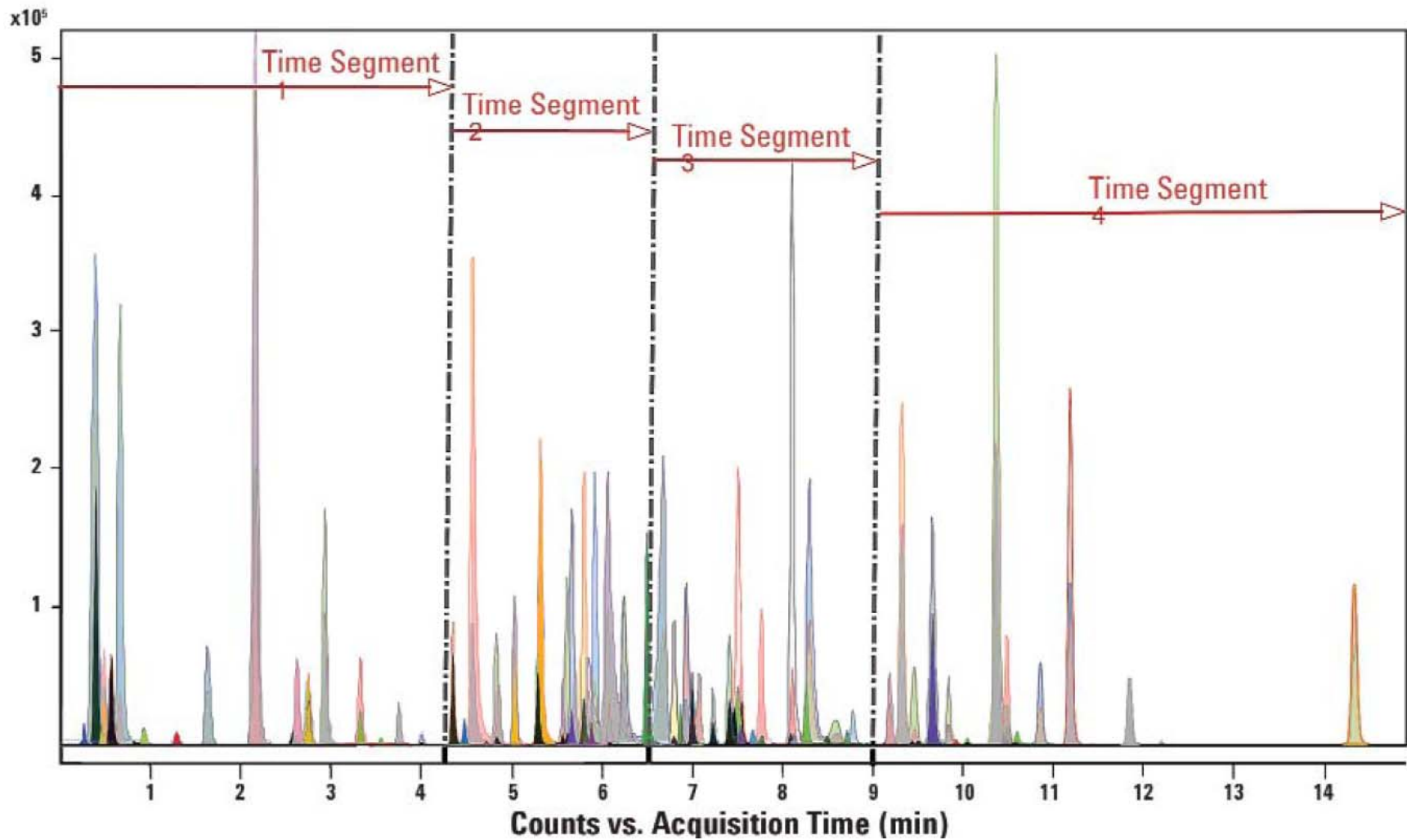
Natural Toxins – e.g. aflatoxins, ochratoxin, patulin

Food Additives

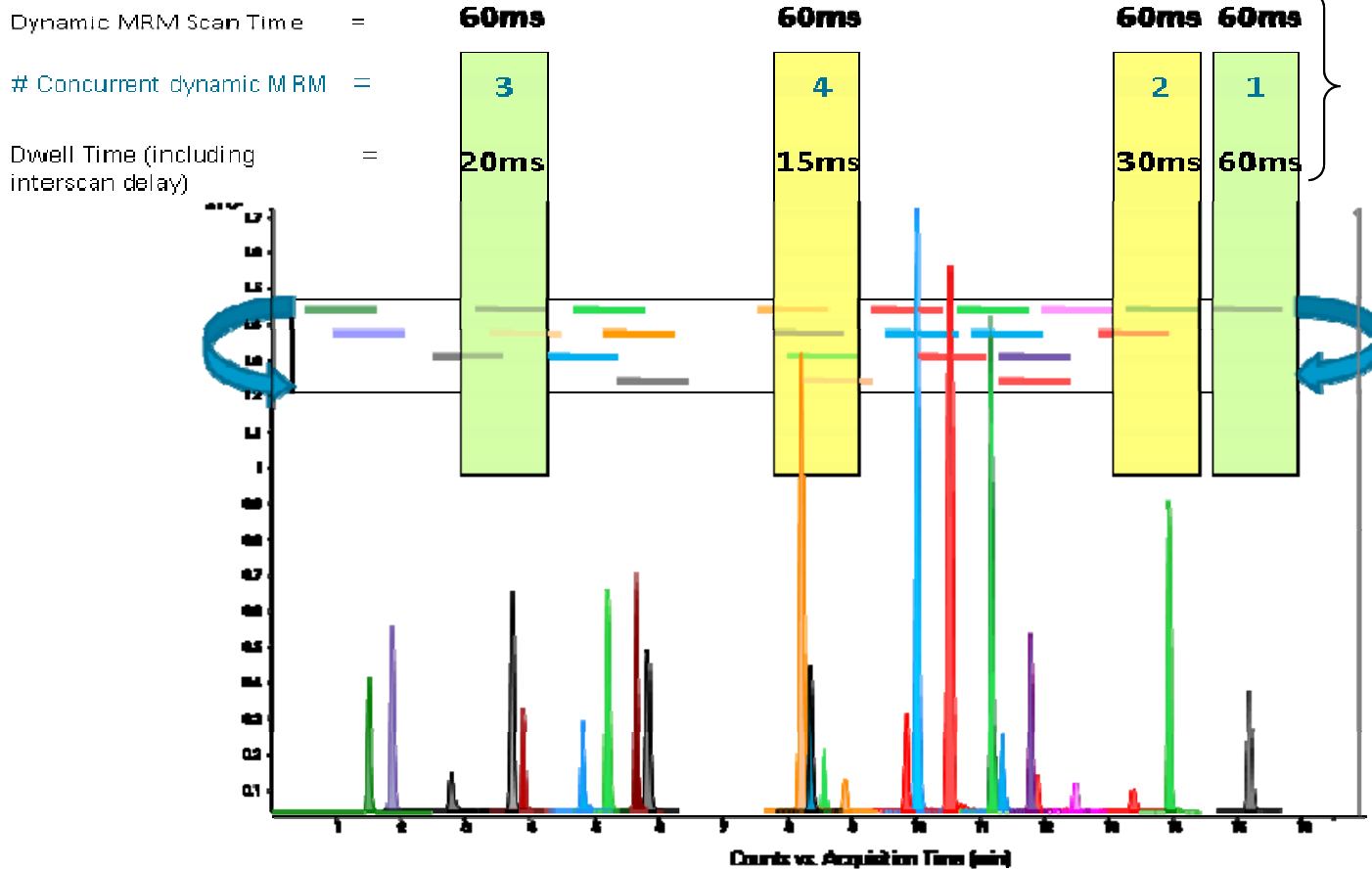
- Antimicrobial
- Antiprotozoal
- Antimicrobial Growth promoters
- Anabolic Growth promoters
- Food Dyes



Approach #1: Time Segments



New Solution: Dynamic MRM



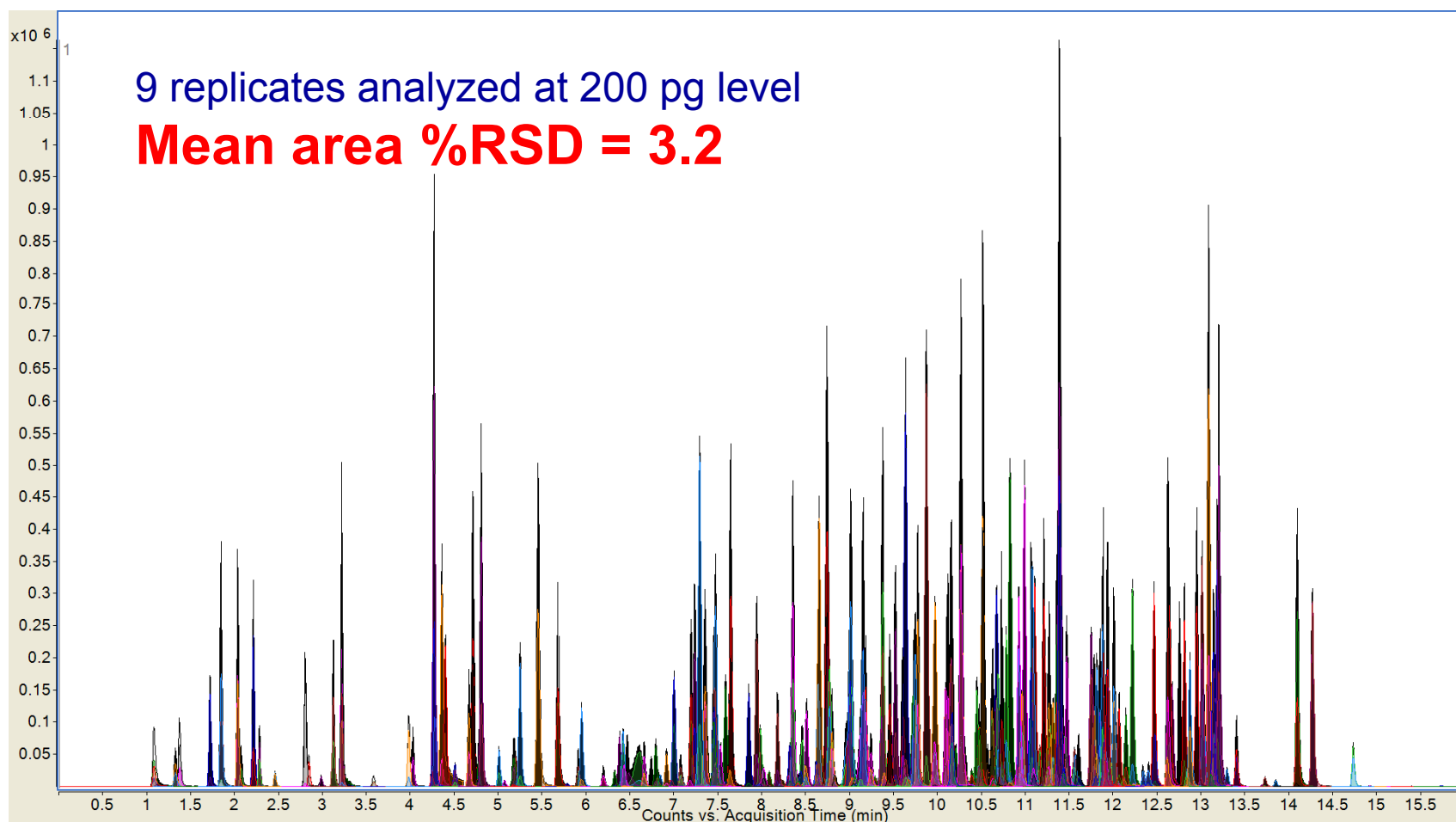
of MRMs will vary:
Dwell times are adjusted dynamically to keep scan time constant for best quant data quality.

MRMs chosen based on analyte RT window

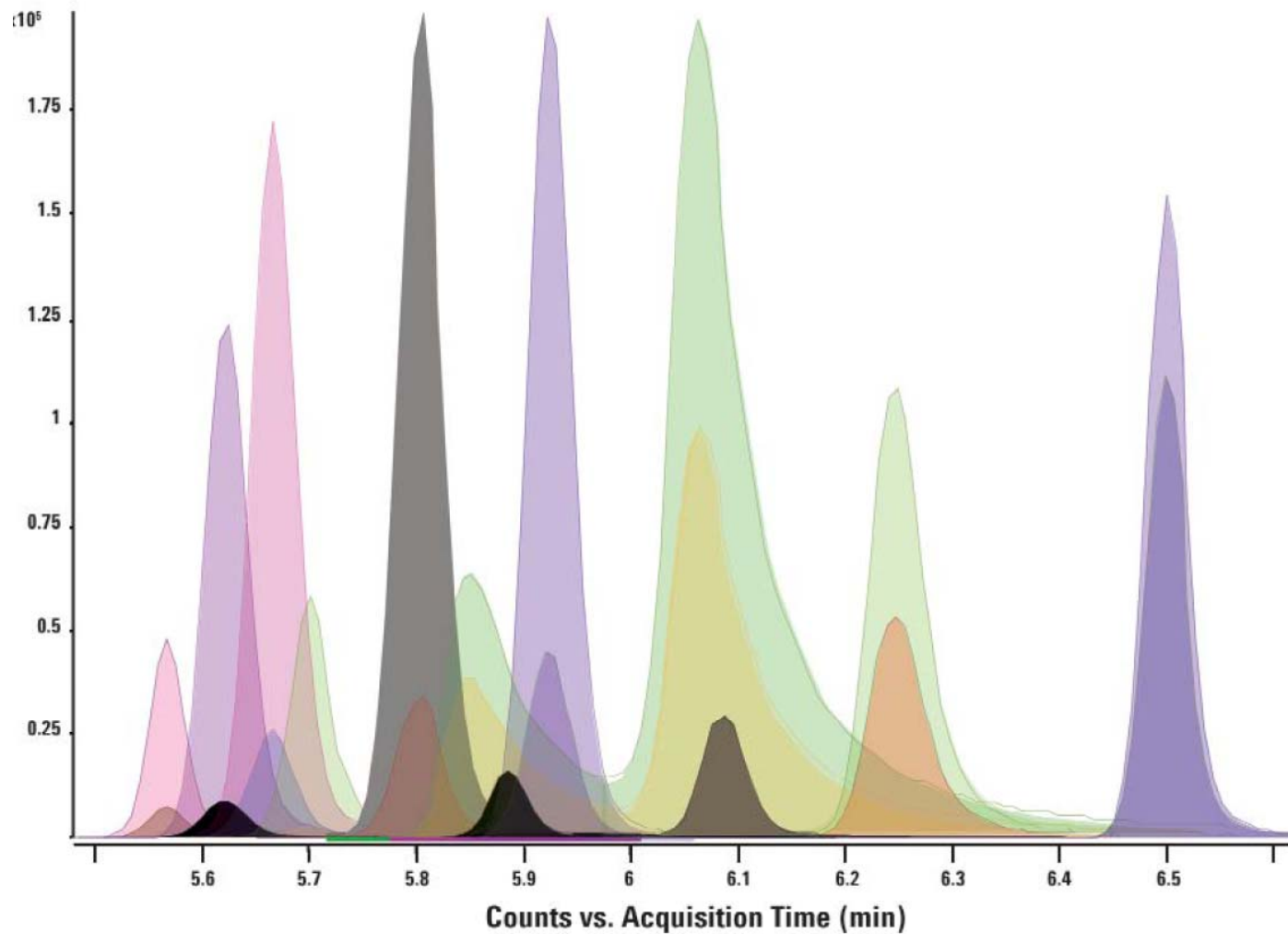
On average, the number of MRMs at any time are many fewer than with time segment methods, allowing much faster MS cycle times.

Analysis of 600 Pesticide DMRM Transitions in 15 min.

Agilent 1290 Infinity LC + 6430 Triple Quad

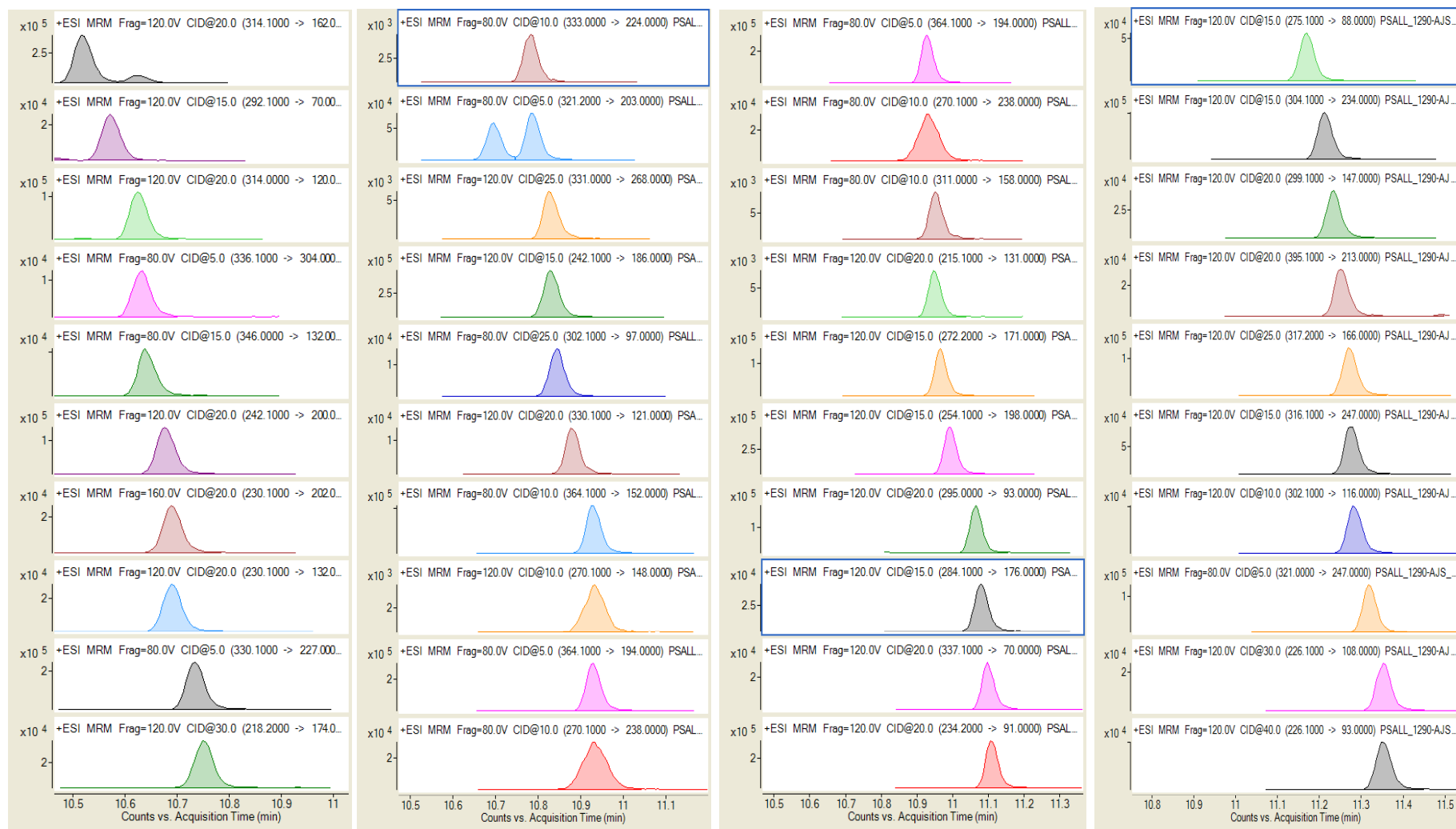


Peak Capacities are very high with UHPLC 1 minute window: 11 pesticides



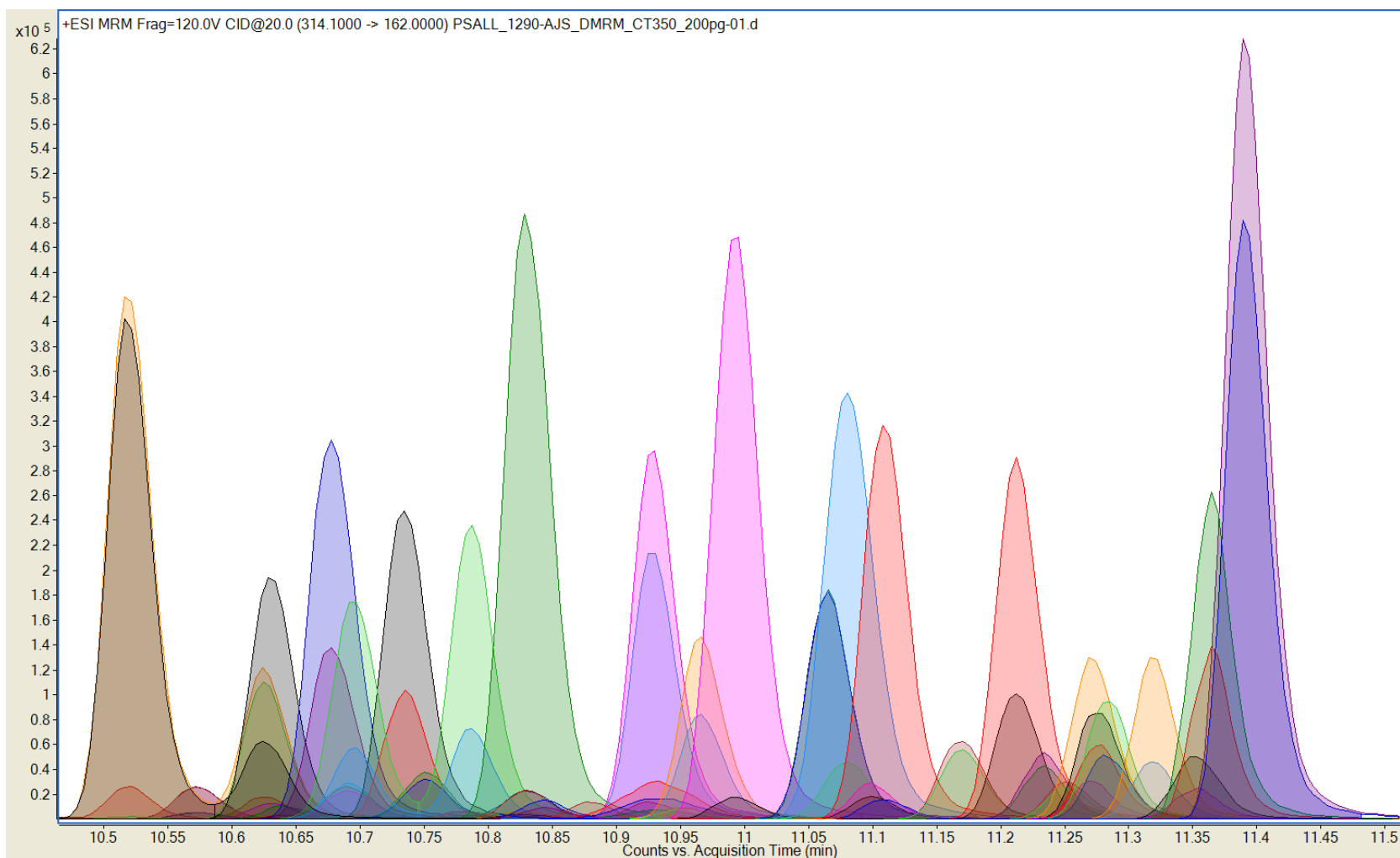
Dynamic MRM Targets the Analyte when it Elutes

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

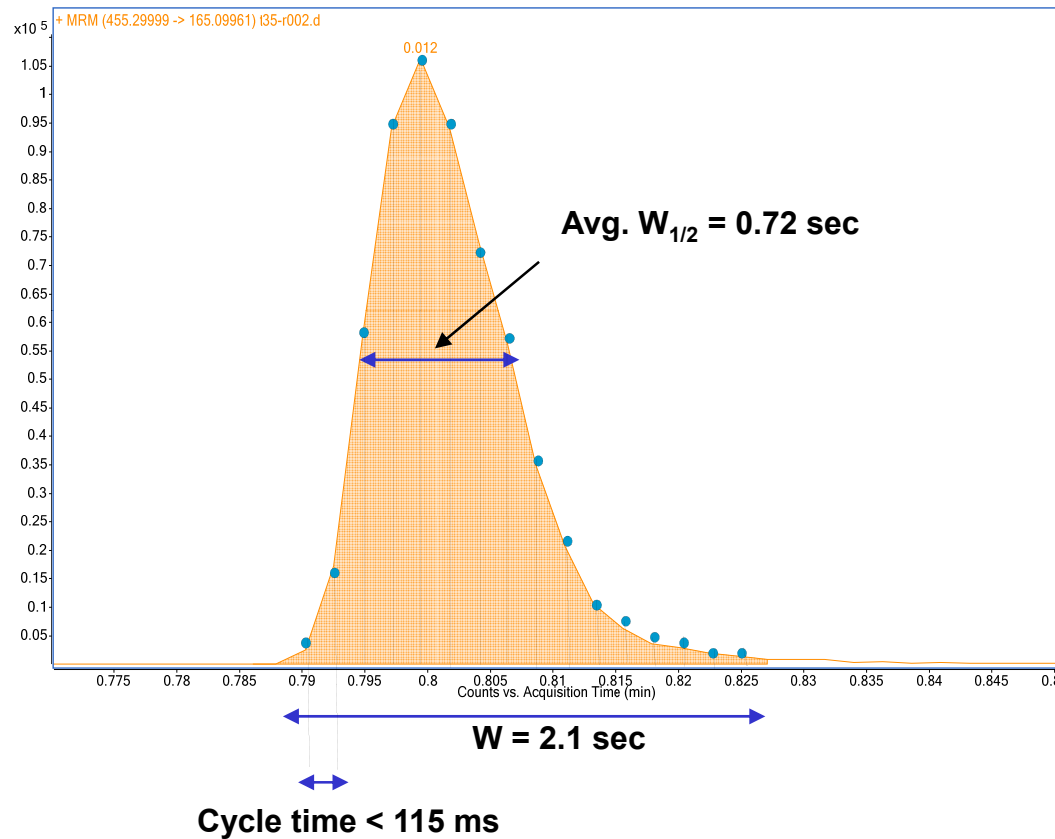


Peak Capacities are very high with UHPLC

40 Extracted Ion Transitions in 1 minute

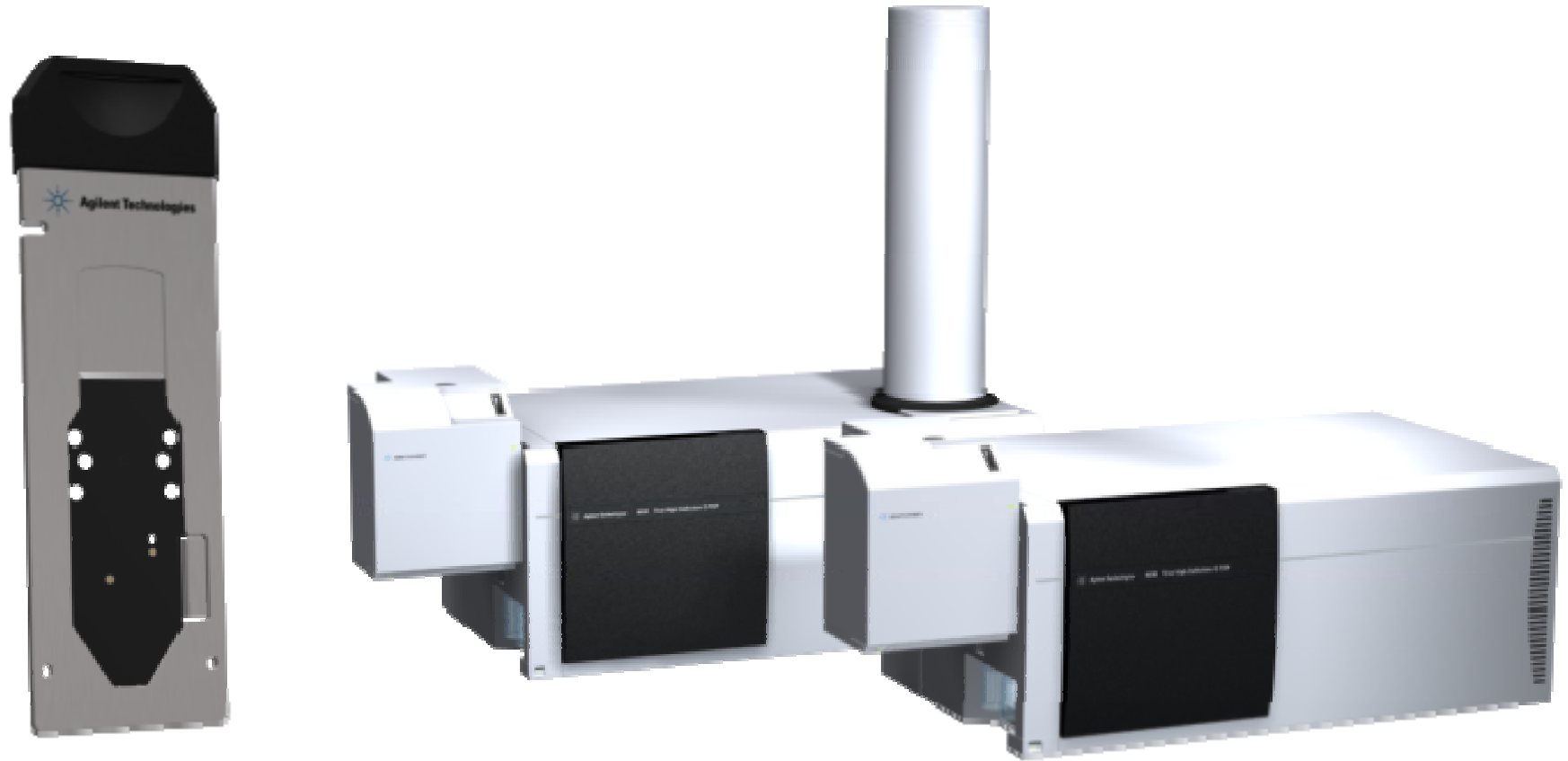


Fast Polarity switching and fast MRMs with 6430

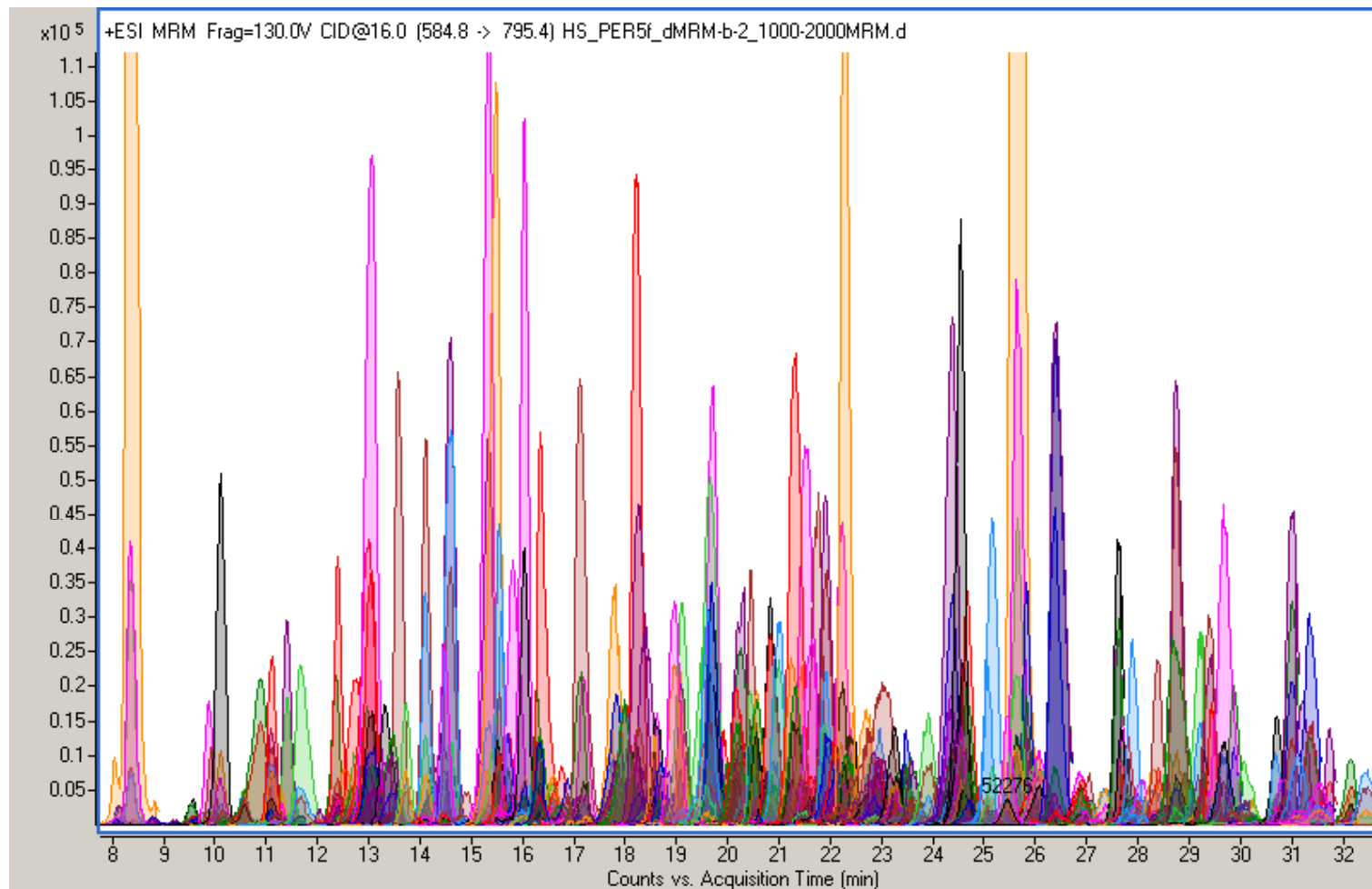


The verapamil peak eluted in two seconds from a sub-2-micron column at pressures exceeding a **1,000 bar** with Agilent's new **1290 Infinity** LC System. The verapamil ion transition from 455 to 165 was monitored more than fifteen times across the peak with multiple injections demonstrated a peak area 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/MS 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

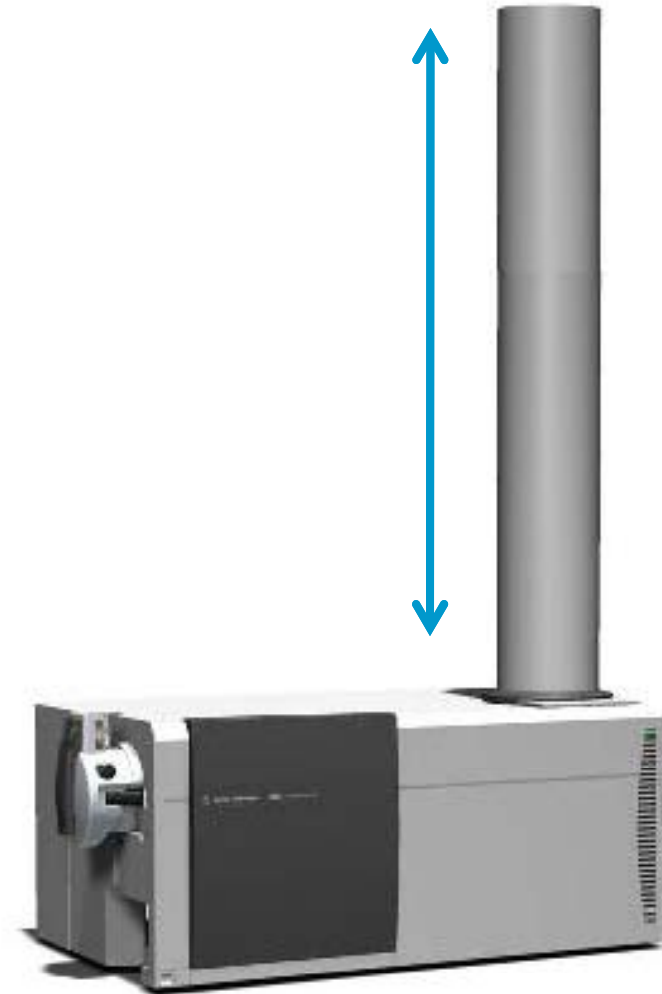
5 decades dynamic range

40,000 resolving power

20 Spectra/sec

Excellent Linearity and Isotopic Fidelity

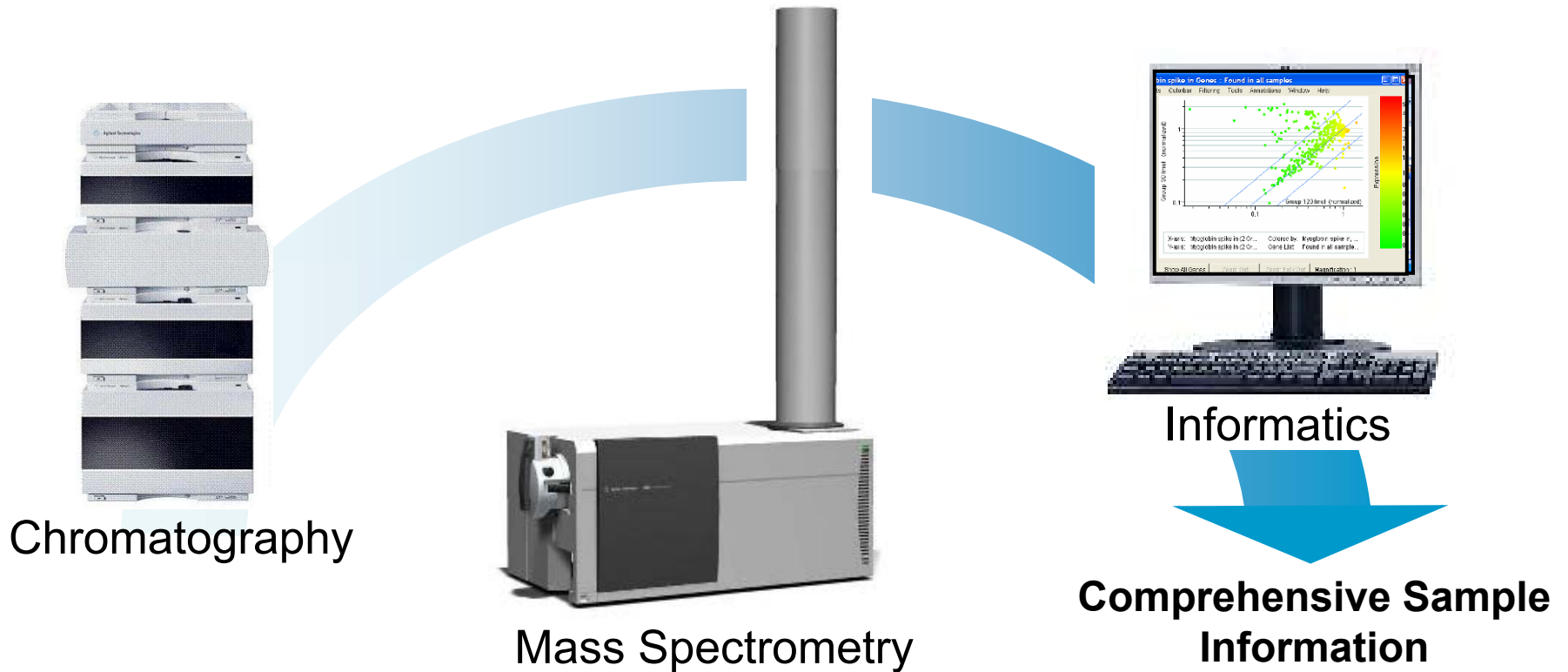
Supports Agilent Jet Stream and HPLC-Chip



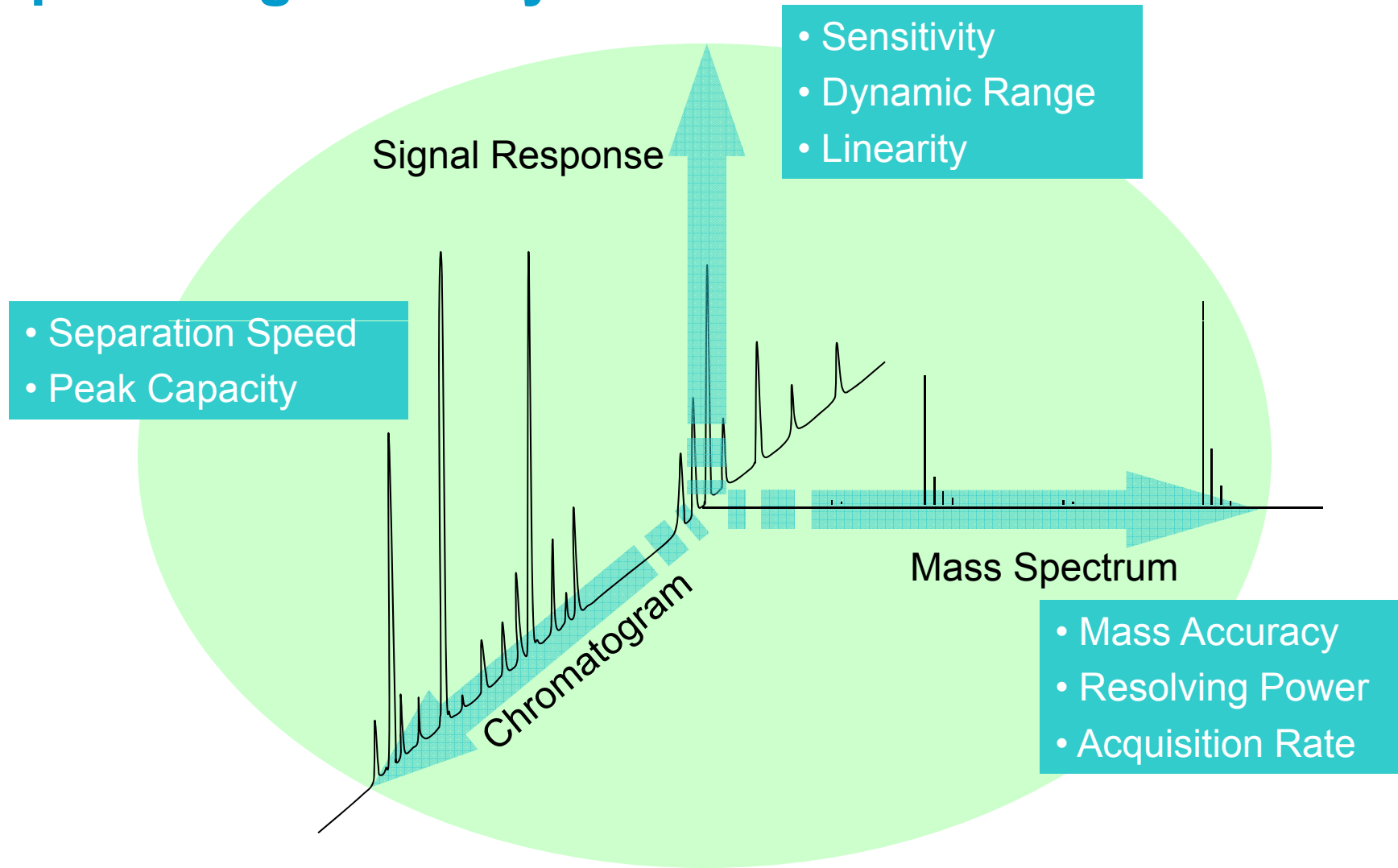
What is Ultra High Definition?

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

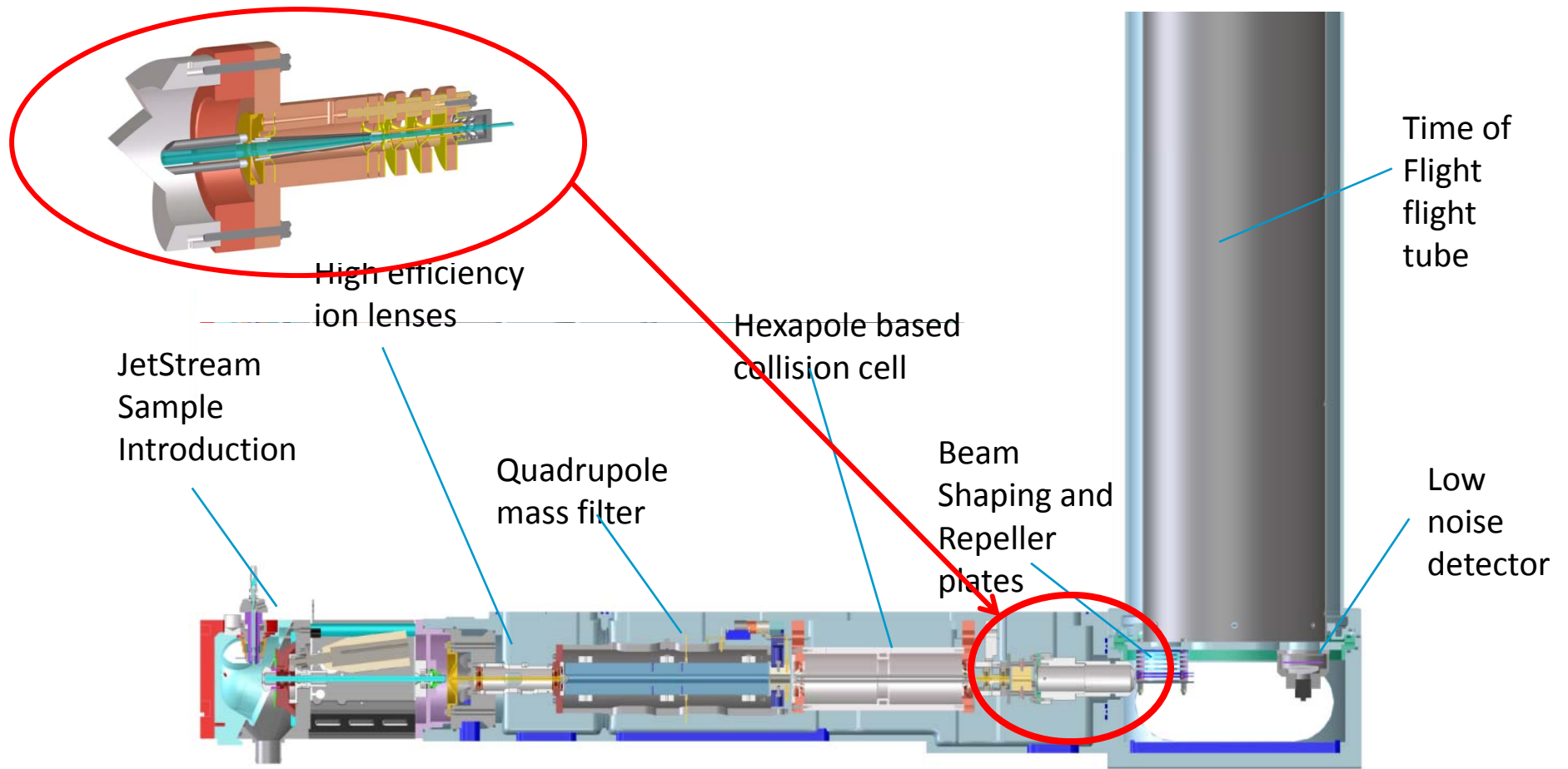
Ultra High Definition LC/MS achieves the **Maximum Qualitative and Quantitative Information possible.**



Ultra High Definition Optimizing all Analytical Dimensions

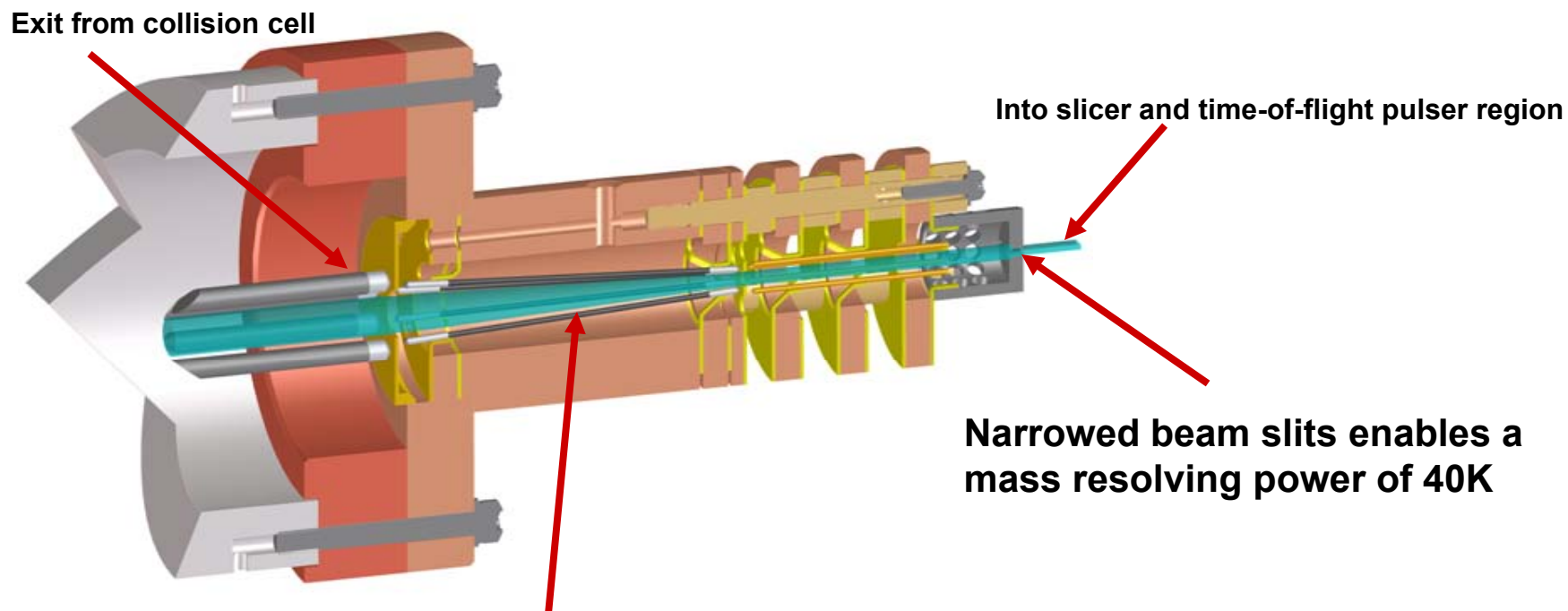


6540 QTOF: New Technologies



Ion Beam Compression (IBC)* Technology Drives Higher Resolution

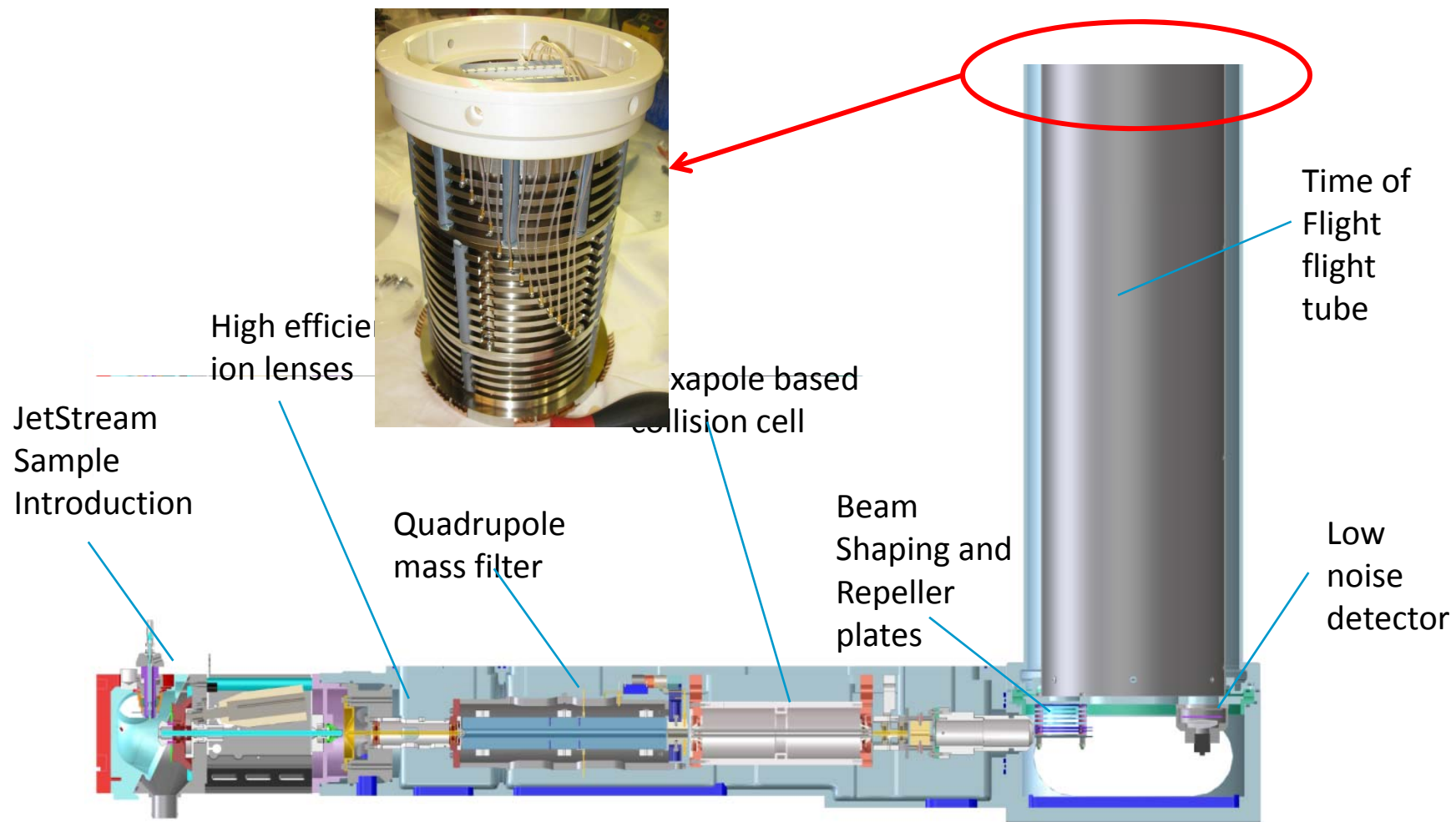
Active Ion Beam Compression simultaneously maximizes ion transmission and reduces beam divergence



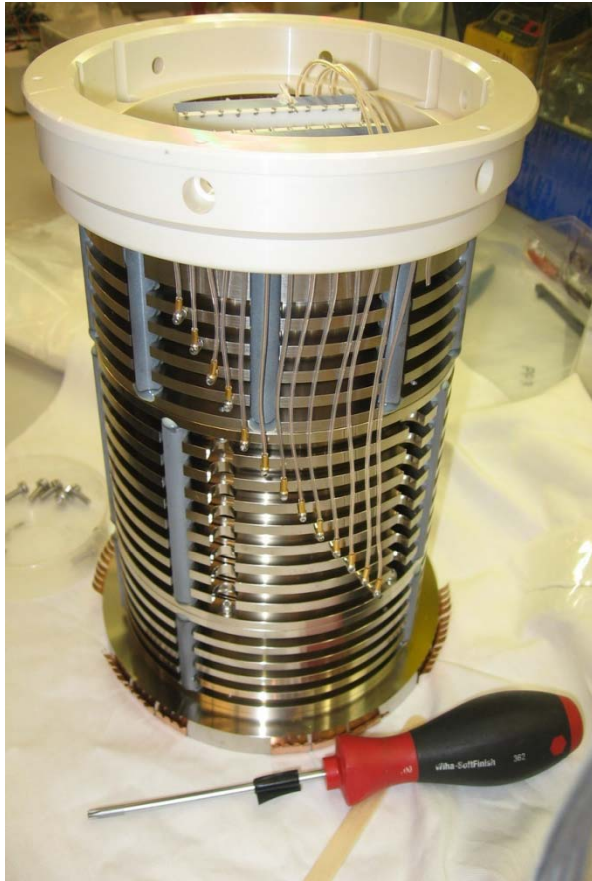
Active Ion Beam Compression is achieved with Agilent's Axial Ion Acceleration Technology applied to a tapered ion guide design.

*** Patent pending**

6540 QTOF: New Technologies



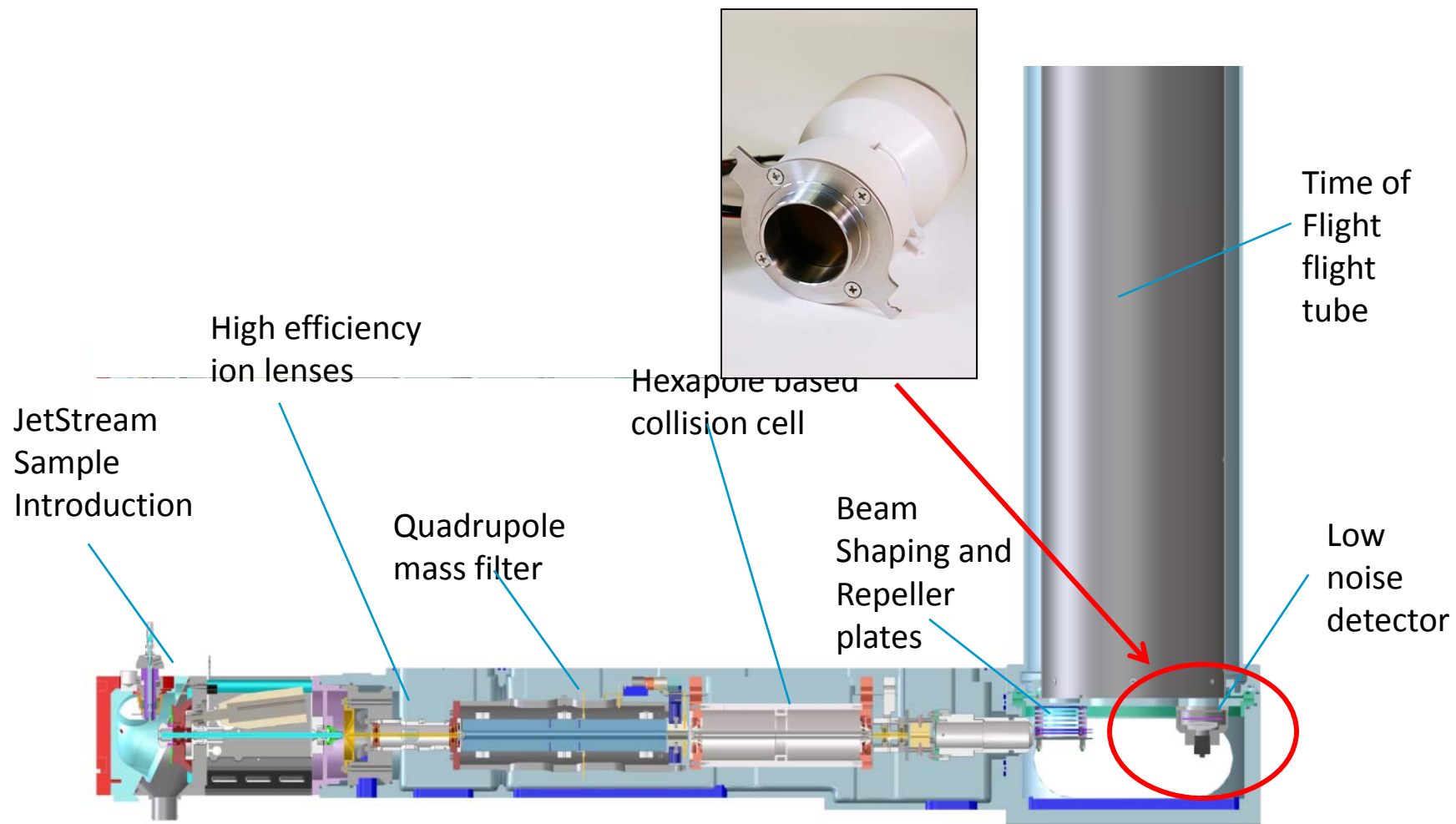
Enhanced Mirror Technology (EMT)



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**

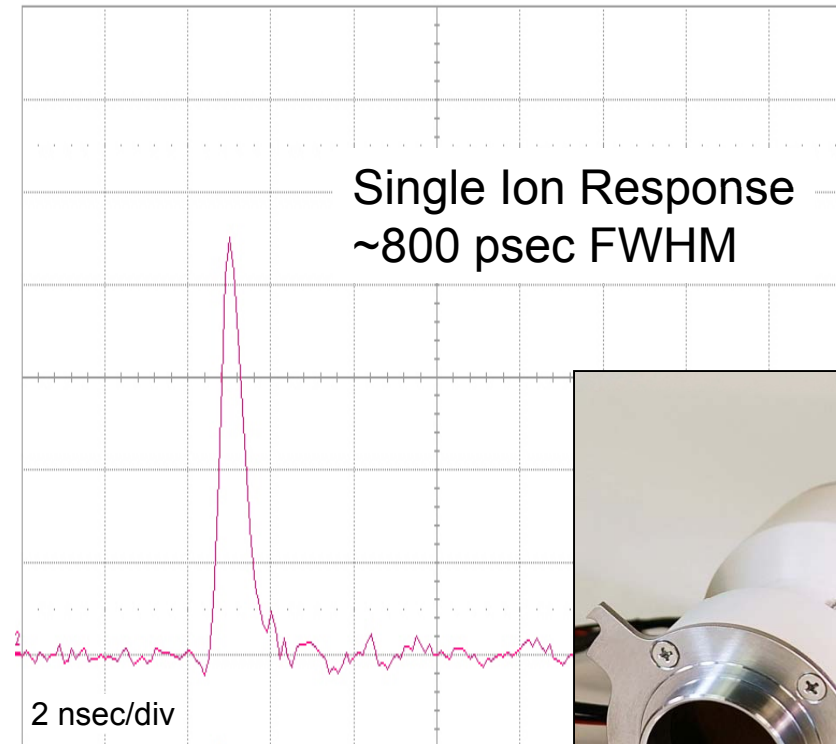
6540 QTOF: New Technologies



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

Resolution improvement with new detector

Performance characteristics

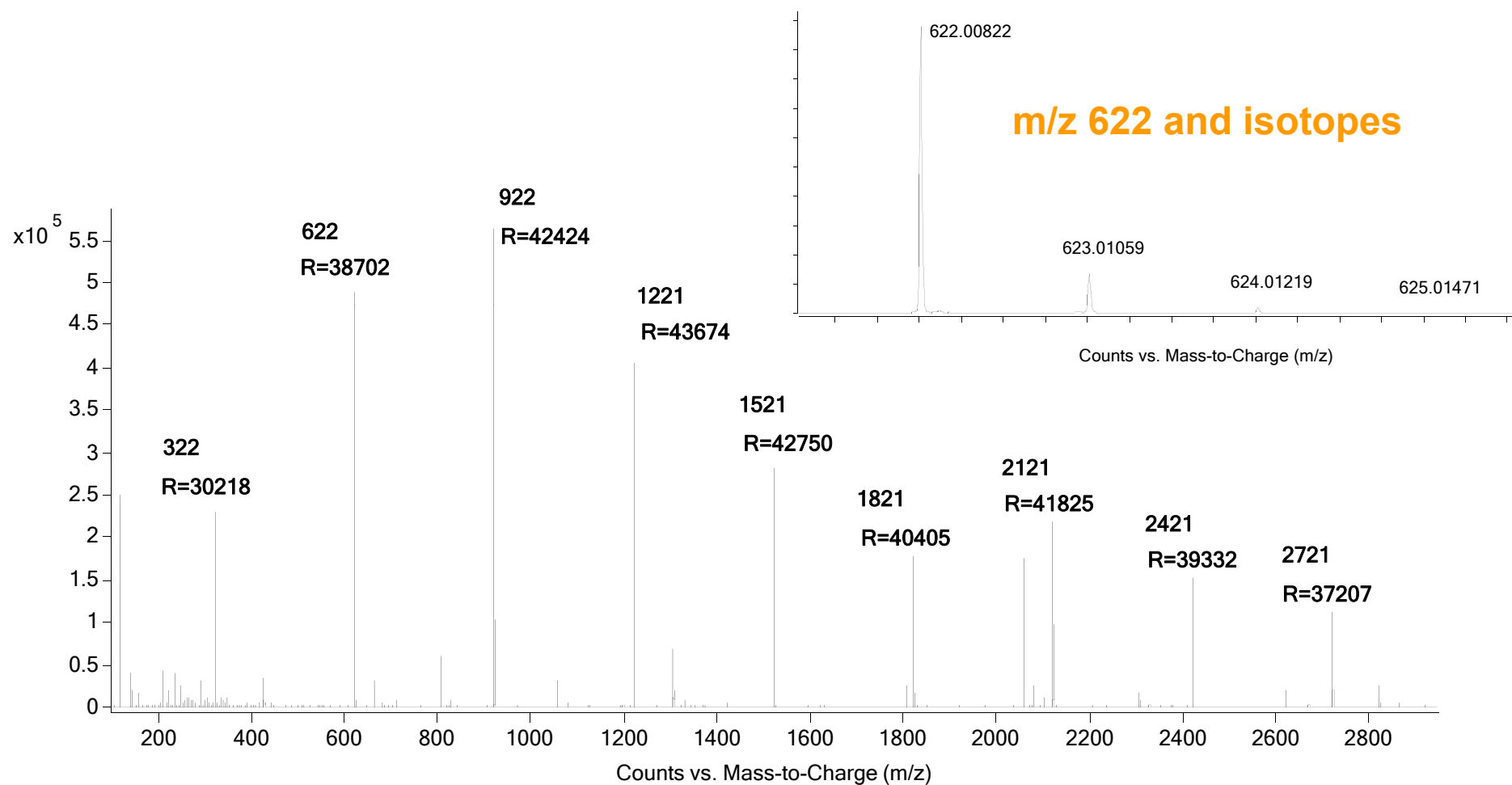
- Pulse width less than 1 nanosecond
- Resolution in 2GHz -extended dynamic range mode over 30k on high mass
- Large increase in resolution for low mass ions
- Maintain 5 orders of magnitude linear dynamic range

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

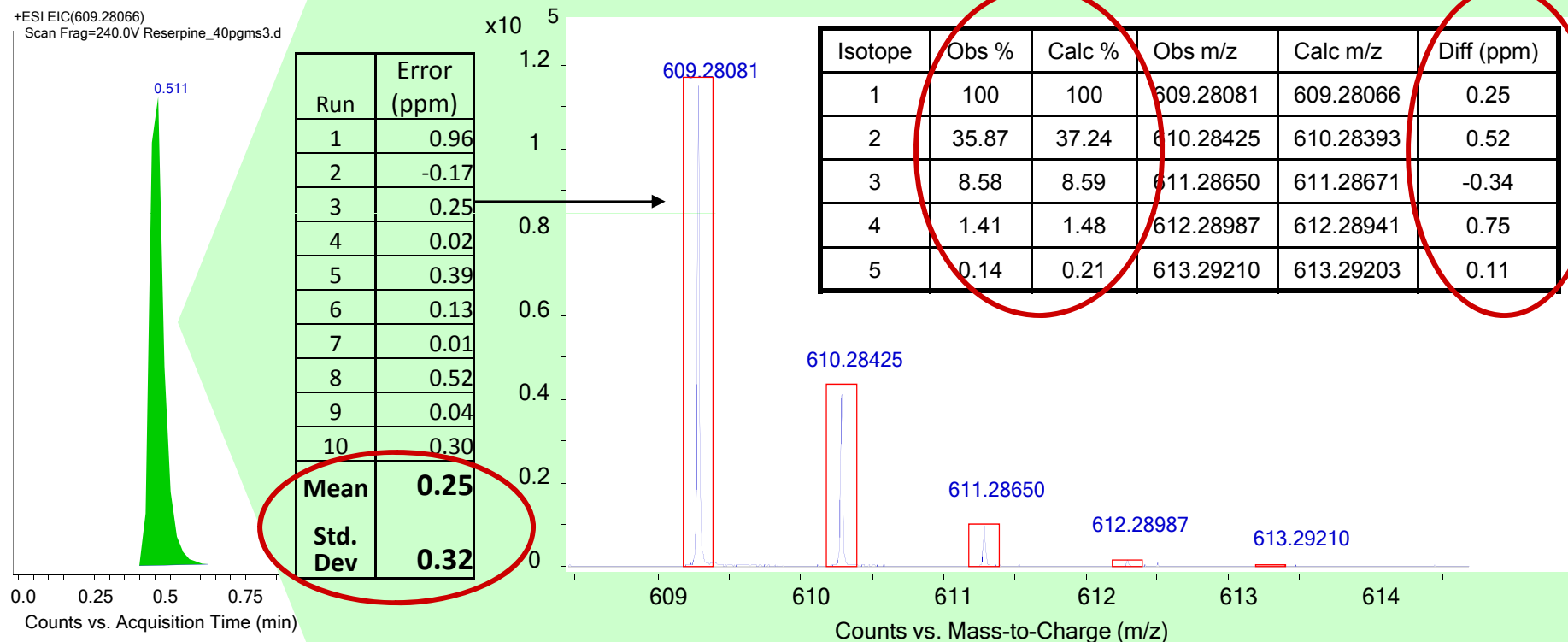


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6540 Ultra High Definition QTOF

Mass Accuracy – Repetitive Injections

40pg reserpine on-column, 10 injections

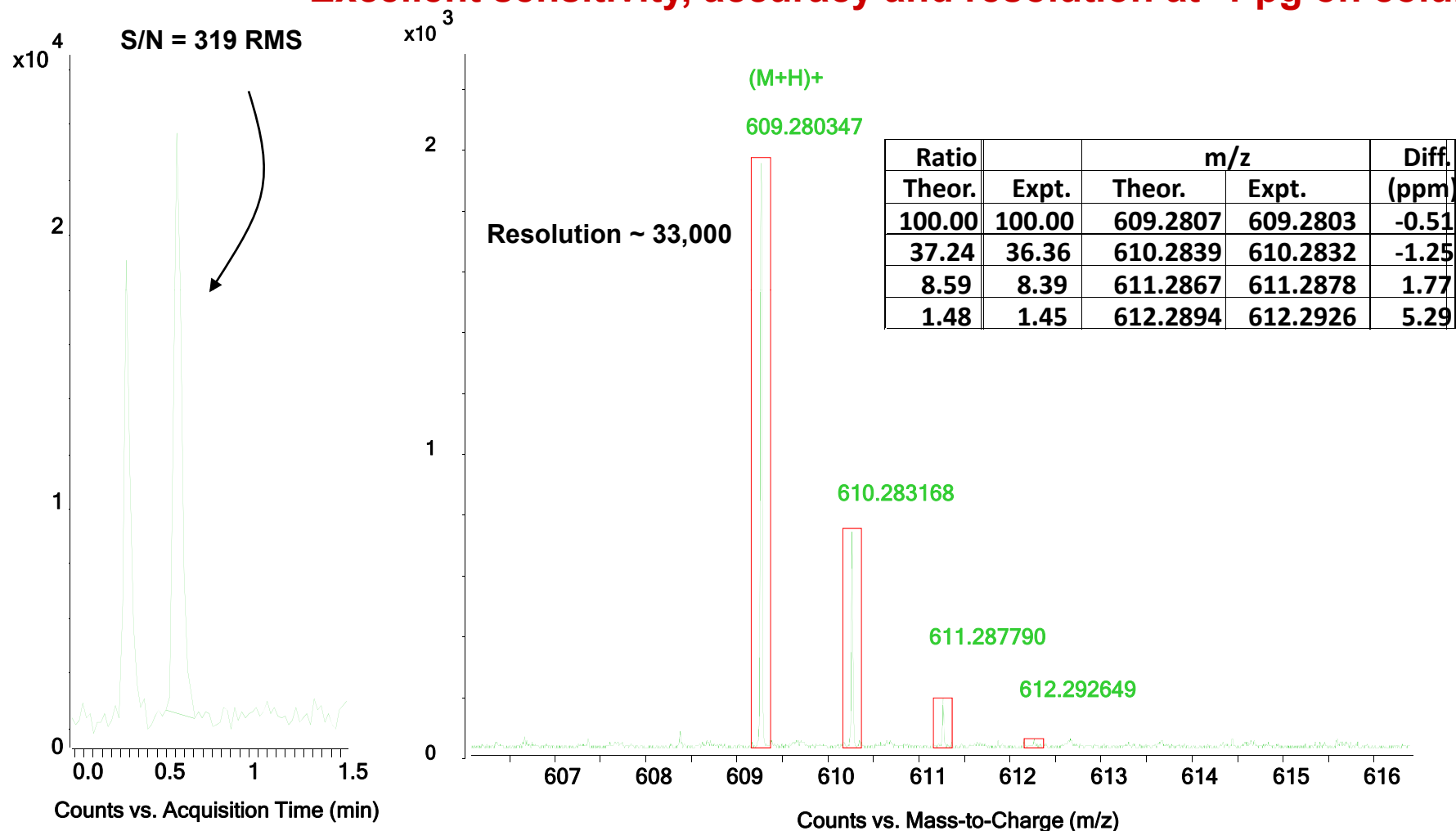


250 ppb mass accuracy calibration and very accurate isotopic ratios

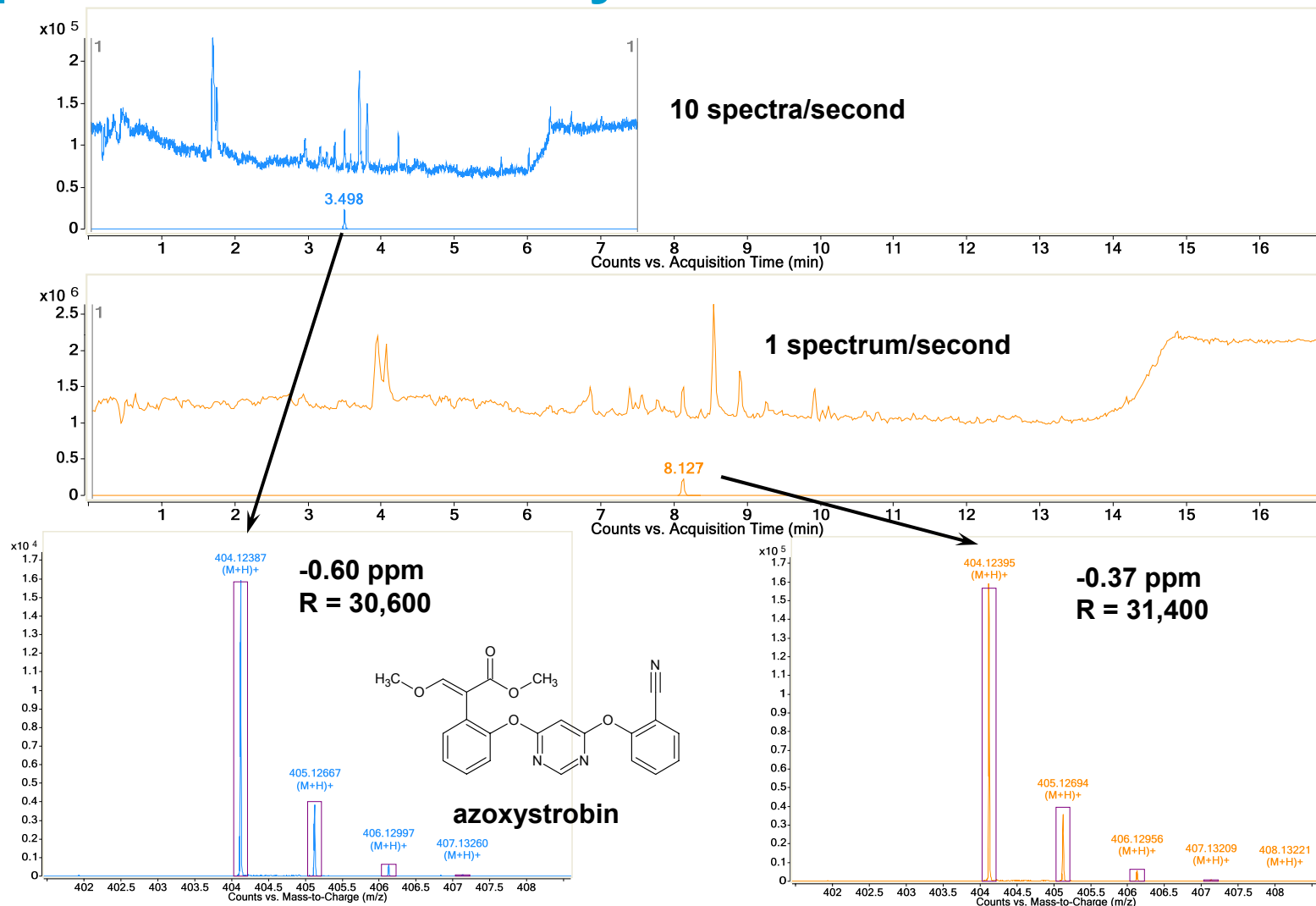
6540 Ultra High Definition QTOF

Sensitivity – Full Scan MS Mode- 1 picogram

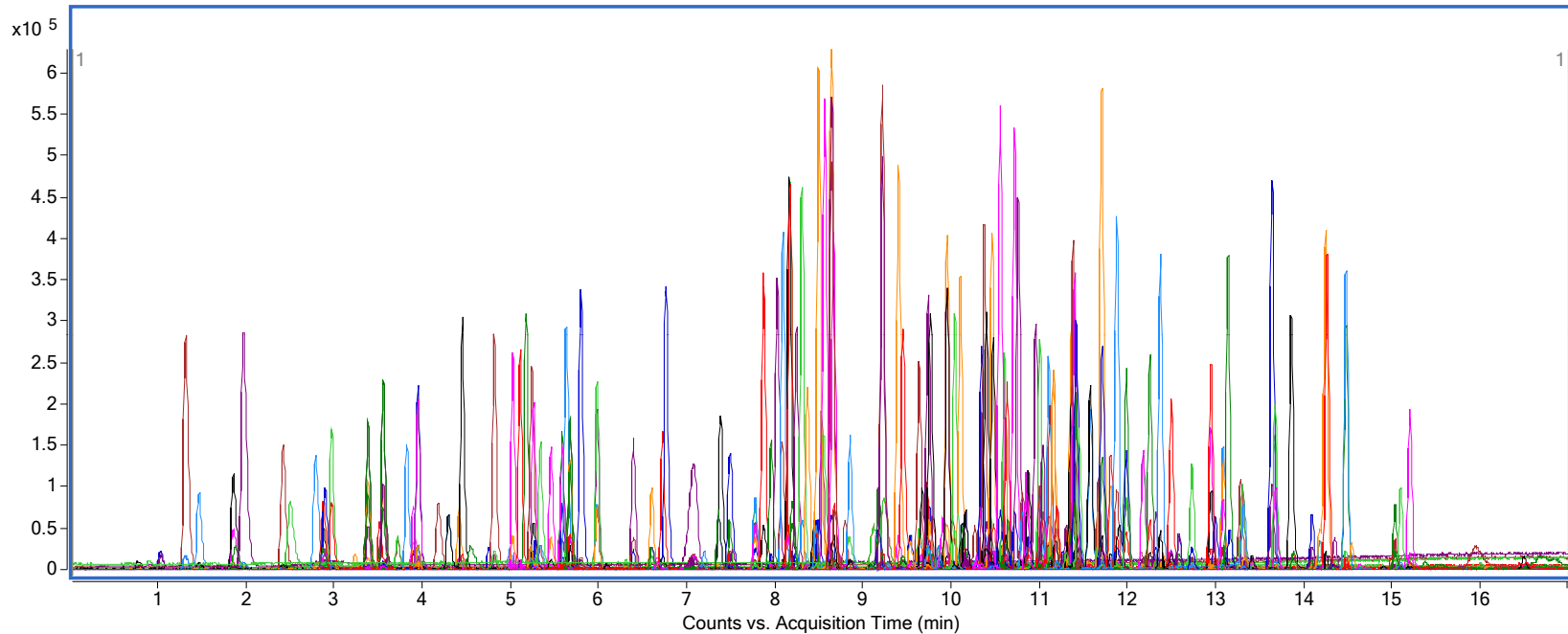
Excellent sensitivity, accuracy and resolution at 1 pg on column



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 2 nd 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

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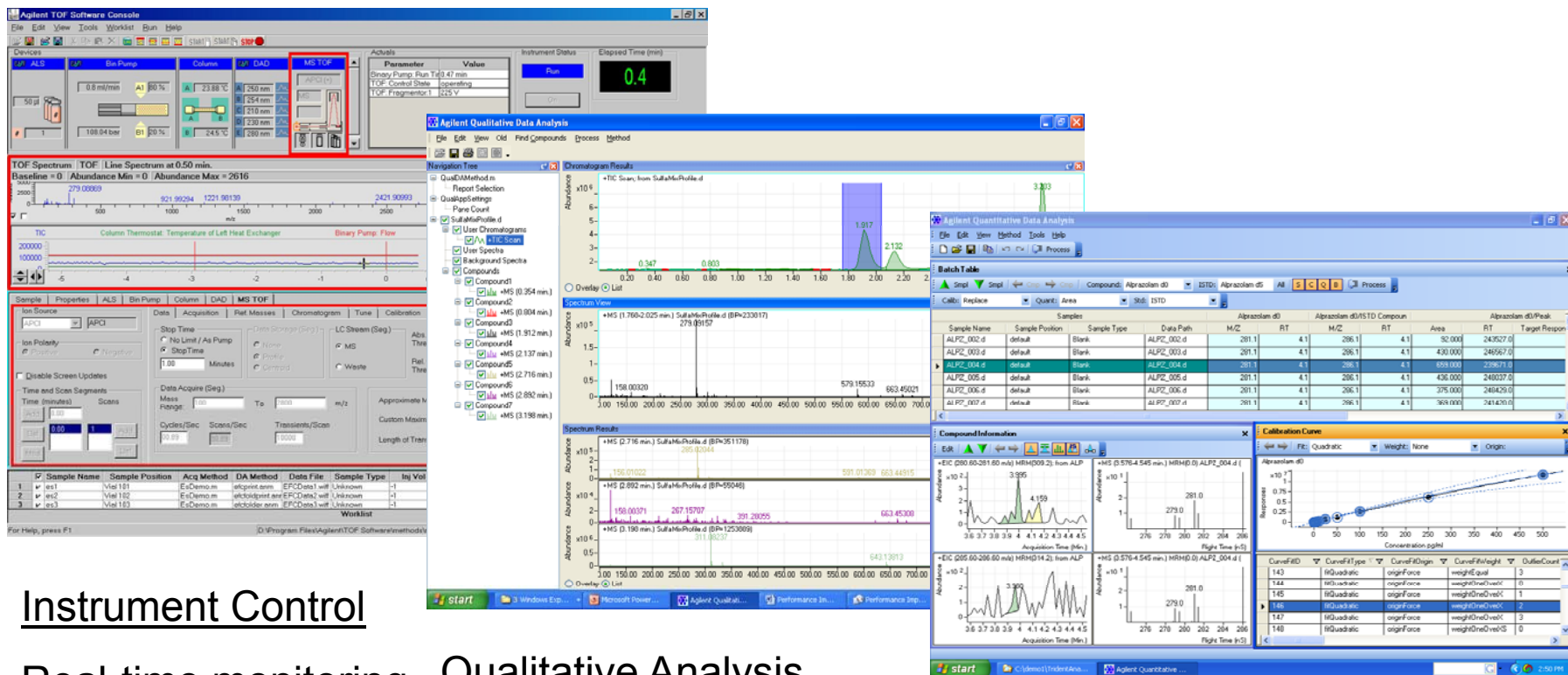
www.agilent.com/chem



Agilent Technologies

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



Agilent Technologies

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



METLIN Metabolite Database



Agilent METLIN Personal Metabolite Database

Powerful database searching for easier metabolite identification

- Identifying 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



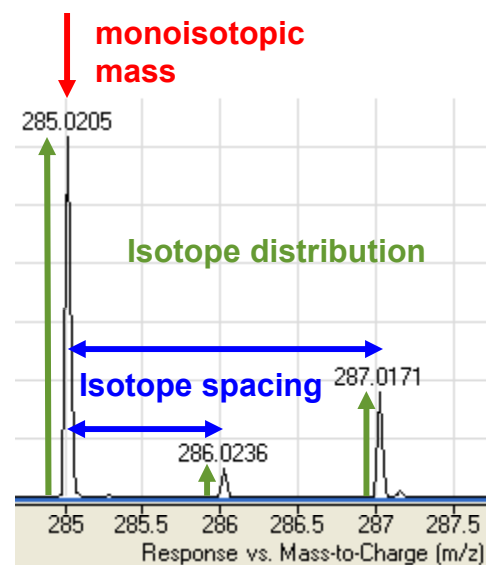
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Agilent Personal Compound Database

Accurate mass and optional RT databases (AMRT)

Name	Formula	Mass Submitted	Mass	Delta Mass (ppm)	RT Submitted	RT (min)	CAS
Mipaflox	C8H16FN2OP	182.09843					371-86-9
Codlemone	C12H22O	182.16707					33956-49-9
Acephate	C4H10NO3PS	183.01190					30560-19-1
Deisopropylbrometon	C7H13N5O	183.11201					13365-86-1
Hydroxysimazine	C7H13N5O	183.11201					2599-11-3
Sebutylazine-desethyl-2-OH	C7H13N5O	183.11201					33124-63-9
Terbutylazine-desethyl-2-OH	C7H13N5O	183.11201					66753-06-8

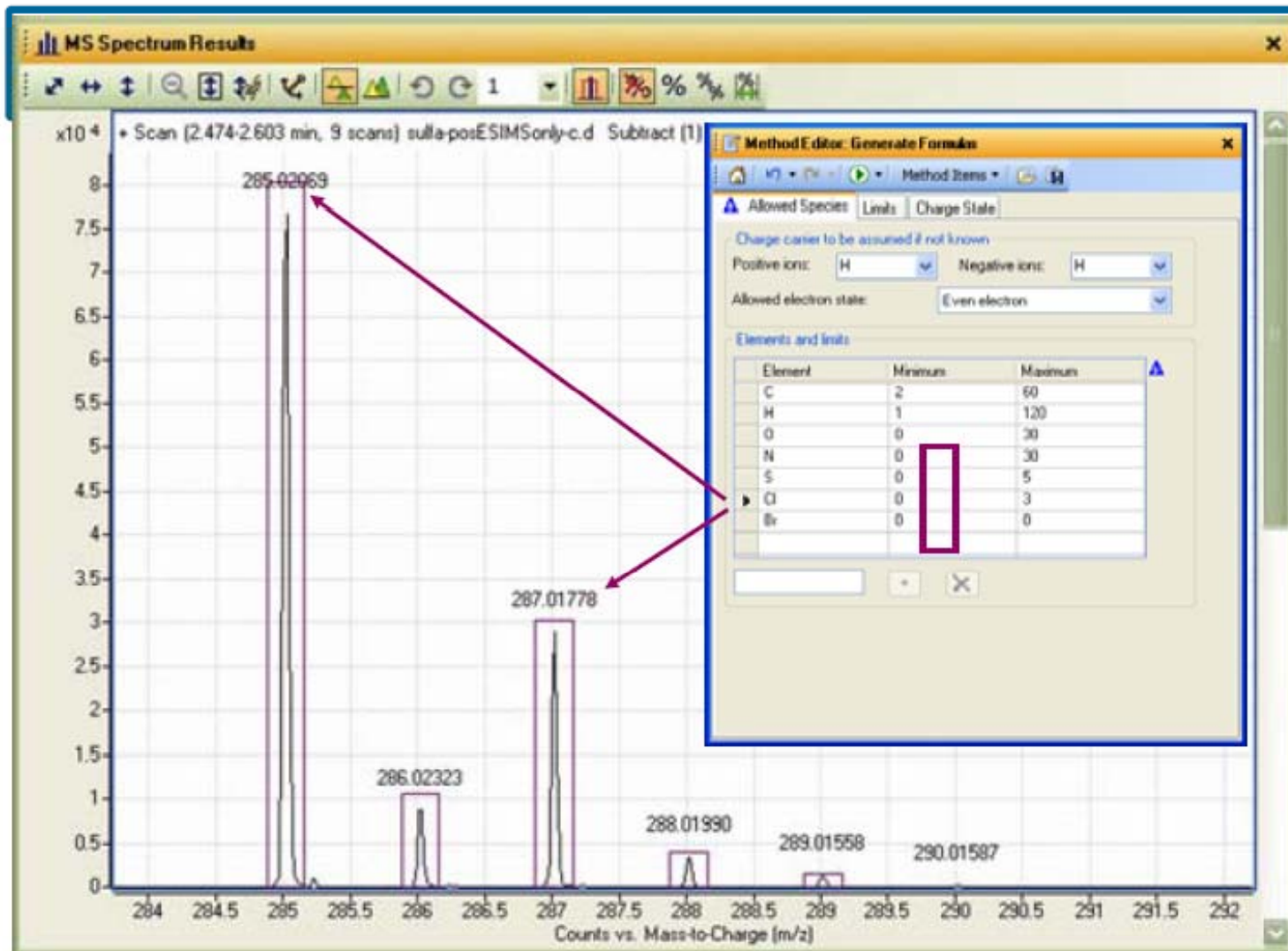
Scoring based on



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 Personal Compound Database and Library - D:\MassHunter\databases\Demo.cdb

File Edit View Database Links Help

Find Spectra

Single Search Batch Search Batch Summary Edit Compounds Spectral Search Browse Spectra **Edit Spectra**

Observed spectra

	Precursor Ion	Collision Energy	Ion Polarity	Ionization Mode	Instrument Type
	267.17	10	Positive	ESI	QTOF
	267.17	20	Positive	ESI	QTOF
▶	267.17	40	Positive	ESI	QTOF

Compound spectra

	Precursor Ion	Collision Energy	Ion Polarity	Ionization Mode	Instrument Type
	103.12298	10	Positive	ESI	QTOF
▶	103.12298	20	Positive	ESI	QTOF
	103.12298	40	Positive	ESI	QTOF

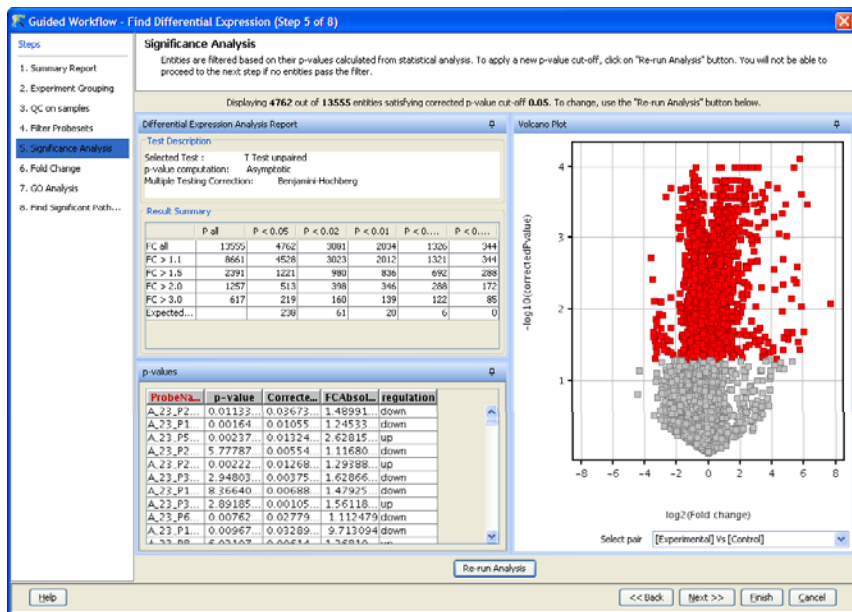
Add Spectra
Delete Spectra

Single Search Results: 9 hits

	Compound Name	Formula	Mass	RT (min)	CAS	ChemSpider
▶	Cadaverine	C5H14N2	102.11570		462-94-2	267
	Metformin	C4H11N5	129.10145		657-24-9	3949
	Caffeine	C8H10N4...	194.08038		58-08-2	2424
	Sulfamethoxazole	C10H11N...	253.05211		723-46-6	
	Atenolol	C14H22N...	266.16304		29122-68-7	2162
	Sulfamethizole	C9H10N4...	270.02452		144-82-1	
	Sulfamethazine	C12H14N...	278.08375		57-68-1	
	Sulfachloropyridazine	C10H9Cl...	284.01347		80-32-0	
	Sulfadimethoxine	C12H14N...	310.07358		122-11-2	

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

The screenshot shows the MassProfiler Pro 2.0 - LCMS software interface. The main window is titled "MassProfiler Pro 2.0 - LCMS" and has a menu bar with "Project", "Search", "View", "Tools", "Annotations", "Windows", and "Help". Below the menu bar is a toolbar with various icons. The interface is divided into several panes:

- Project Navigator:** Located on the left, it shows a tree view of the project structure. It includes "LCMS Demo" (containing "Experiments" with "LCMS" and "GCMS" sub-items), "LCMS" (containing "Samples", "Interpretations" with "All Samples", "Treatment (Non-averaged)", and "Treatment"), "Analysis" with "All Entities" and "Filtered on Flags [P, M]", and "My Favorites". Below this is a "GCMS" section with a similar structure.
- Workflow:** Located on the right, it contains a list of workflow steps categorized into "Experiment Setup", "Quality Control", "Analysis", "Class Prediction", and "Results Interpretat...".

Two white text boxes with red arrows pointing to the Project Navigator and Workflow panes provide additional information:

- The first box, pointing to the "LCMS" and "GCMS" items in the Project Navigator, contains the text: "Project can contains multiple experiment types => combine GC/MS and LC/MS !!!".
- The second box, pointing to the Workflow pane, contains the text: "Workflow navigator for ease-of-use".

The status bar at the bottom right of the window shows "48M of 57M".

New Guided Workflow for Experiment Analysis

Guided Workflow - Find Differential Expression (Step 3 of 8)

Steps

1. Summary Report
2. Experiment Grouping
3. QC on samples
4. Filter Abundance
5. Significance Analysis
6. Fold Change
7. GO Analysis
8. Find Significant Path...

QC on samples

Sample quality can be assessed by examining the values in the PCA plot and other experiment specific quality plots. To remove a sample from your experiment, select the sample from any of the views and click on the Add/Remove button. If a sample is removed, re-summation of the remaining samples will be performed.

Displaying 14 out of 14 samples retained in the analysis. To change, use the "Add/Remove Samples" button below.

Samples	Treatment
010408-002	Untreated
010408-004	Untreated
010408-005	Untreated
010408-006	Untreated
010408-007	Untreated
010408-008	Untreated
010408-011	Untreated
010408-013	Treated
010408-014	Treated
010408-016	Treated
010408-018	Treated
010408-019	Treated
010408-020	Treated
010408-021	Treated

3D PCA Scores

Legend - 3D PCA Scores

Color by Treatment

- Treated
- Untreated

Description

Algorithm: Principal Components Analysis

Parameters:

- Column indices = [1-14]
- Pruning option = [numPrincipalComponents, [4]]
- Mean centered = true
- Scale = true
- 3-D scores = true
- PCA on = Columns

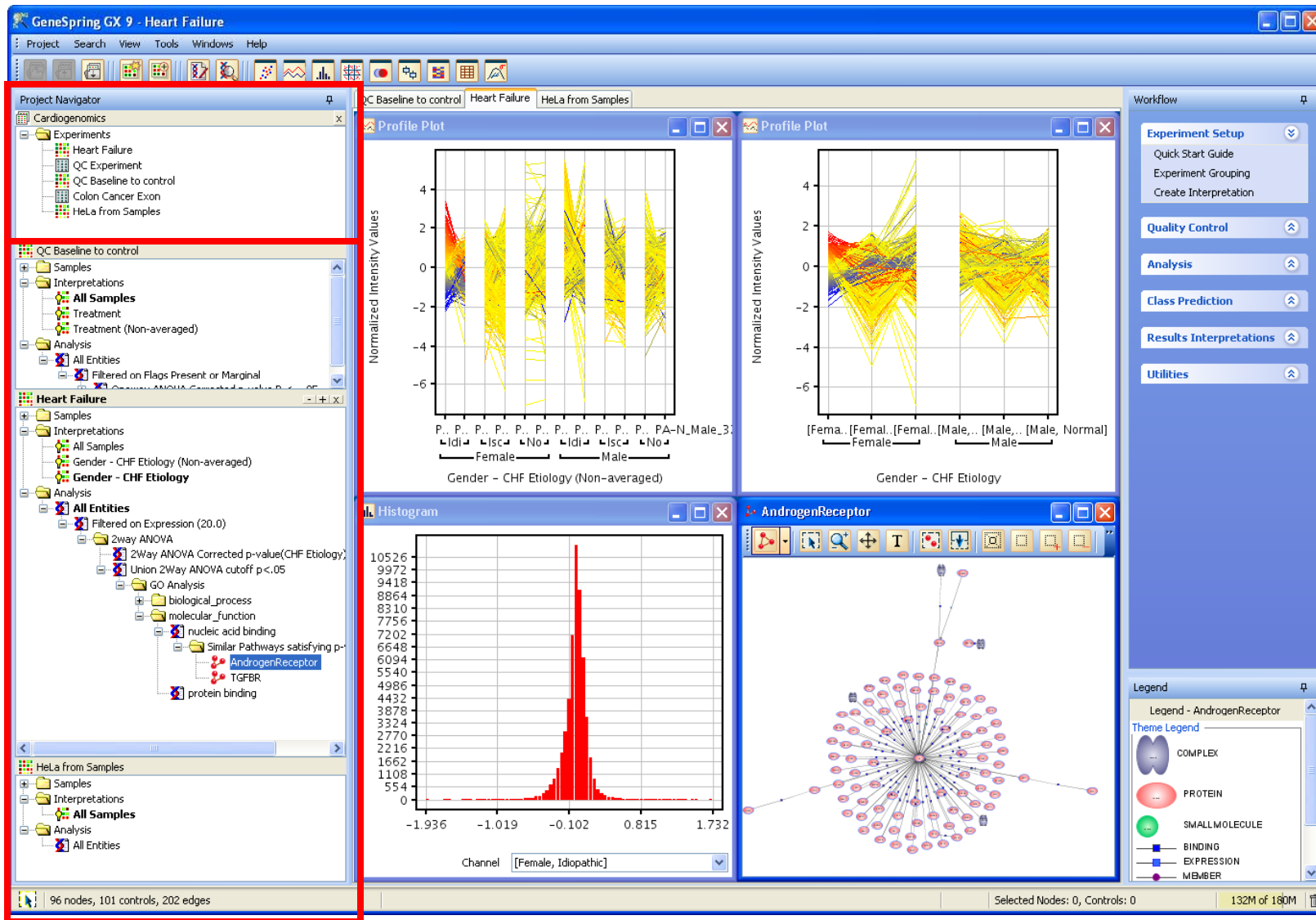
Step-by-Step Wizard with Instructions

Perfect for Novices !!!

Add/Remove Samples

Help << Back Next >> Finish Cancel

Hierarchical Experiment and Data Organization



The Compound ID Browser – Built Into MPP

MS Spectrum Results
Cpd 2: Sulfachloropyridazine: +ESI Scan (3.374-3.440 min, 5 scans)...

MS Peaks One: + Scan (3.374-3.440 min) Sub - Sulfa_Grad1_Pos_04.d

m/z	Ion	Abund	Abund%	Charge	Sat	Diff (ppm)
285.0215	(M+H) ⁺	1251689	100	1		2.73
286.0239	(M+H) ⁺	161676	12.92	1		2.57
287.0189	(M+H) ⁺	479243	38.29	1		3.5
288.0208	(M+H) ⁺	56210	4.49	1		1.97
289.016	(M+H) ⁺	20009	1.6	1		1.08
290.0158	(M+H) ⁺	2018	0.16	1		-5.79

Structure Viewer
Structure: NC1=CC=C(S(=O)(=O)NC2=CC=NC=C2Cl)C=C1

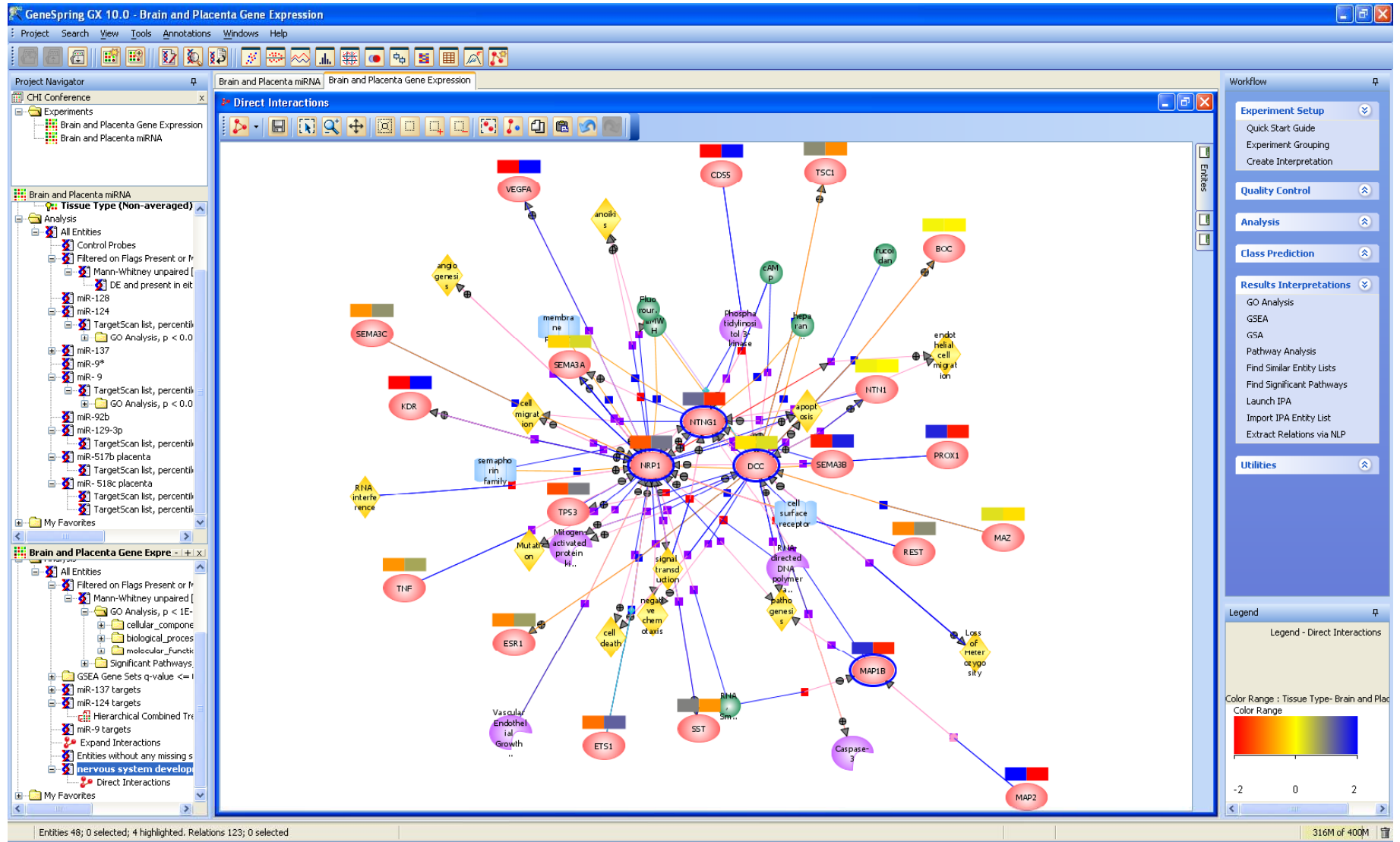
Compound List

Show	Cpd	Name	RT	Mass	Mass (Tgt)	Diff (Tgt, ppm)	Algorithm	Formula (Tgt)	Score	Area	Height	Vol	Abund
<input checked="" type="checkbox"/>	1	Sulfamethazine	2.861	278.0844	278.0837	2.27	Find By Formula	C12H14N4O2S	97.82	19378433	5226683		3059610
<input checked="" type="checkbox"/>	4	Sulfamethizole	3.059	270.0251	270.0245	2.14	Find By Formula	C9H10N4O2S2	98.48	8954916	3074781		1697803
<input checked="" type="checkbox"/>	2	Sulfachloropyridazine	3.407	284.0143	284.0135	2.78	Find By Formula	C10H9ClN4O2S	97.36	8890215	3449561		1251689
<input checked="" type="checkbox"/>	3	Sulfadimethoxine	3.854	310.0745	310.0736	2.94	Find By Formula	C12H14N4O4S	96.15	17316311	6823625		3448982

Annotations:

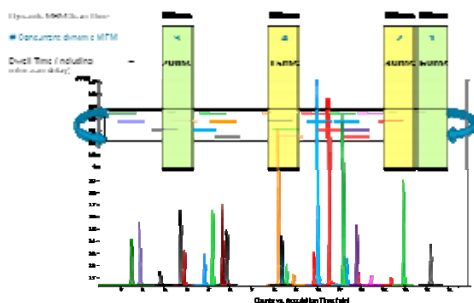
- Cmpd Mass Spectrum w/ MFG "Boxes"**: Points to the mass spectrum plot.
- Identified Ion species**: Points to the MS Peaks One table.
- MS/MS spectra (not shown here)**: Points to the MS Peaks One table.
- Optional structures**: Points to the chemical structure in the Structure Viewer.
- Compounds with mismatched "best" hits e.g. from DB search and MFG, are flagged red in the compound table for resolution**: Points to the red text in the Compound List table.
- Compound List – selection changes Cmpd Mass Spectrum, peak list and structure (cmpd-centric navigation)**: Points to the Compound List table.
- For LC/MS + GC/MS !**: A red box containing this text.

Overlay Networks w/ Expression Data/Conditions



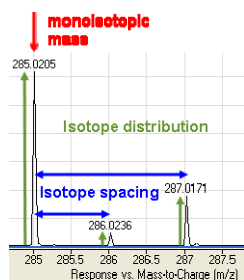
Driving Applications Forward

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

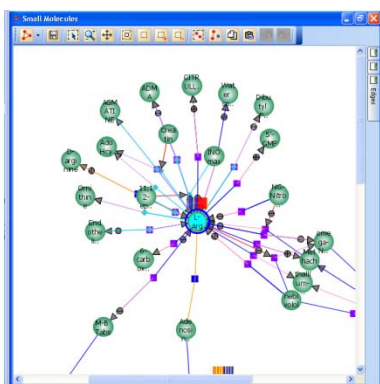


High Throughput Targeted Screening

- 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