

Welcome to Agilent's Lunch & Learn at IMSC 2009

**CLEARLY
BETTER**

LC/MS · GC/MS · ICP-MS · CE/MS



Agilent Lunch & Learn

Monday 13:00-14:00

Introducing the new Agilent 6540 and 6538 Ultra High Definition (UHD) Accurate-Mass Q-TOF LC/MS Systems

- 1290 Infinity UHPLC
- Fast, Accurate, High Resolution Q-TOF
- Using Peak Capacity to Improve Screening and Complex Sample Profiling

Ken Imatani & Pat Perkins, Agilent, CA

Agilent Hospitality Suite Monday

Time: 18:15-21:00

Room: Danzig

Agilent Elemental Bingo

Starts at 19:00

Great prizes to be won!

Drinks and Snacks will be provided.



Enter & Win

We will be running daily competitions during IMSC 2009, ask a member of staff for more details!

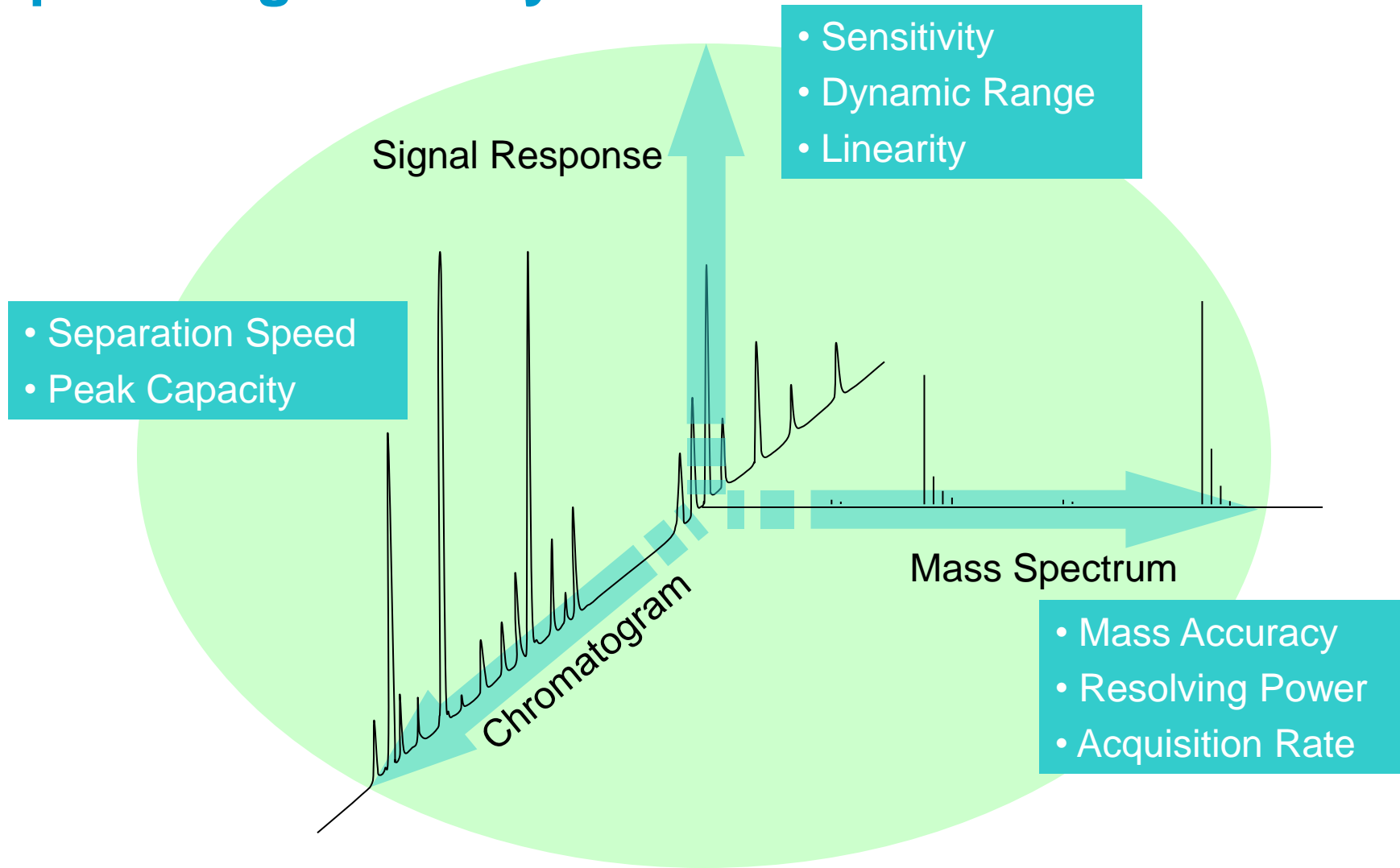


Happy Hour

Daily from 16:00-18:00
at the Agilent booth (B)

Ultra High Definition

Optimizing all Analytical Dimensions



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

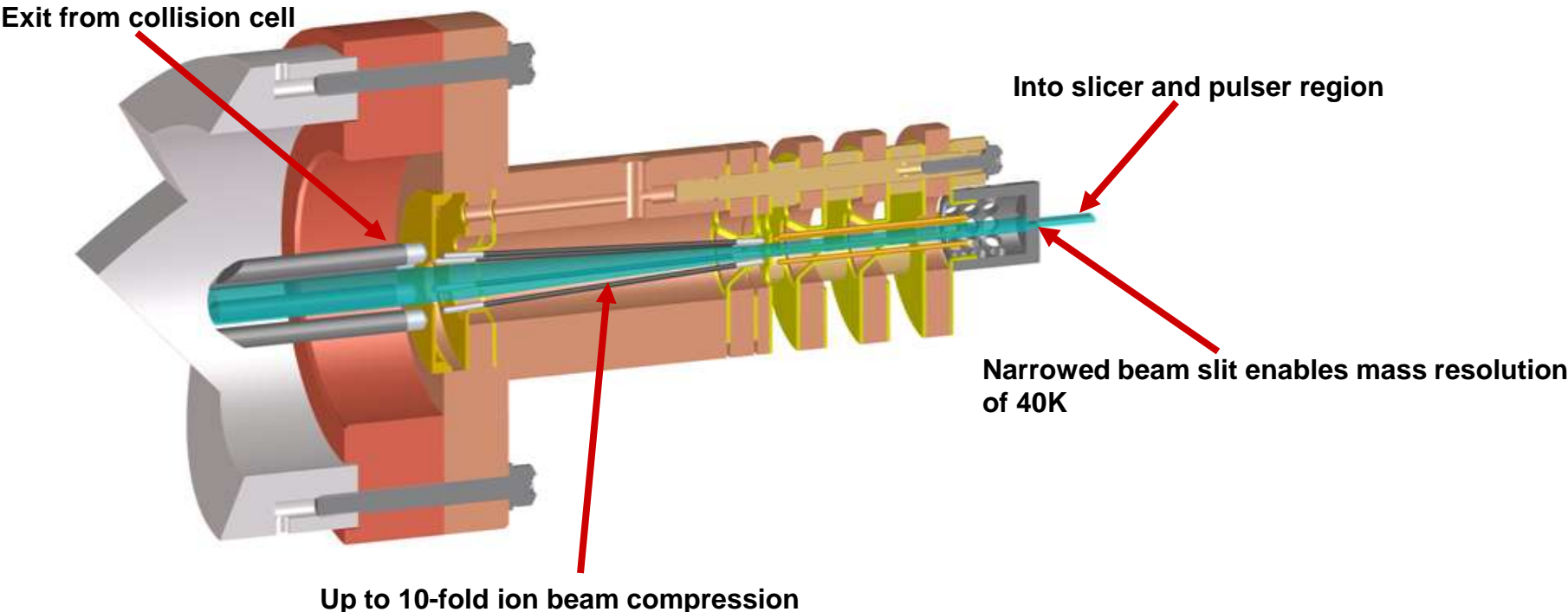
Exceptional accurate mass, sensitivity, dynamic range and resolution ... perfect match for 1290 Infinity UHPLC

- **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**



Ion Beam Compression (IBC)* Technology Drives Higher Resolution

Compressed and cooled ion beam ensures the best sensitivity performance in high resolution mode

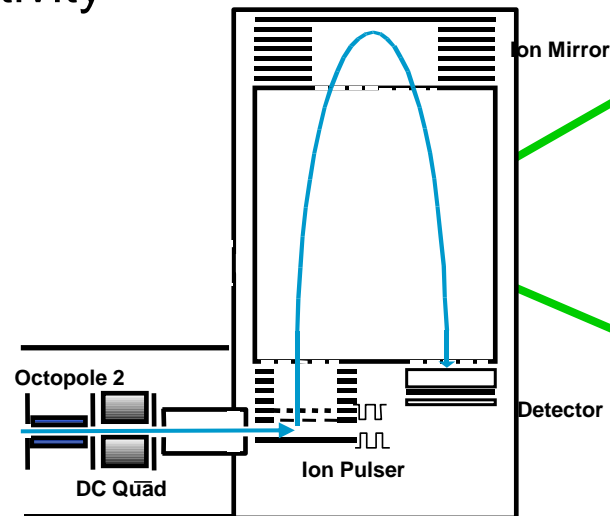


* Patent pending

Enhanced Ion Flight Tube and Mirror Technology

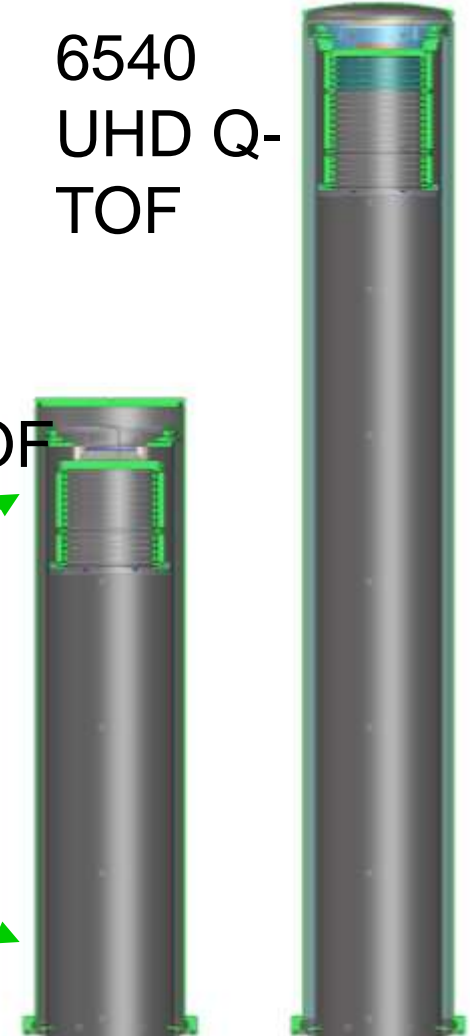
Stable, Sensitive, High Resolution

- 1ppm/C Expansion Coefficient for Inner Flight Tube virtually eliminates calibration drift due to flight tube elongation.
- 2nd Order Temporal Focusing Ion Mirror uses high transmission Harp Grid for maximum sensitivity



6530
Q-TOF

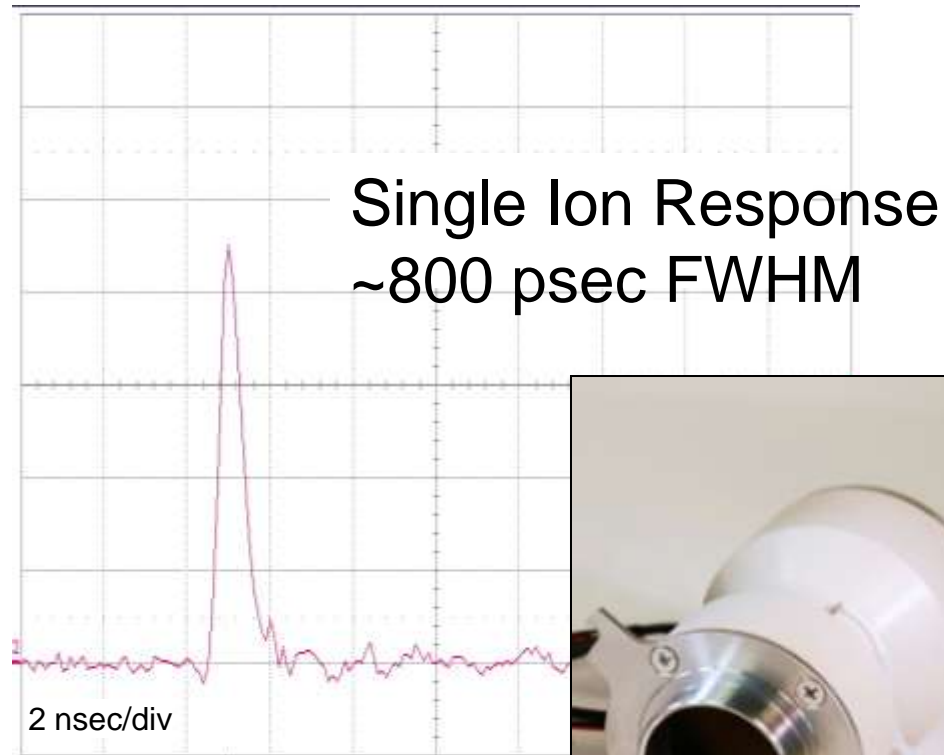
6540
UHD Q-
TOF



Next Generation – Ultra High Speed Detector

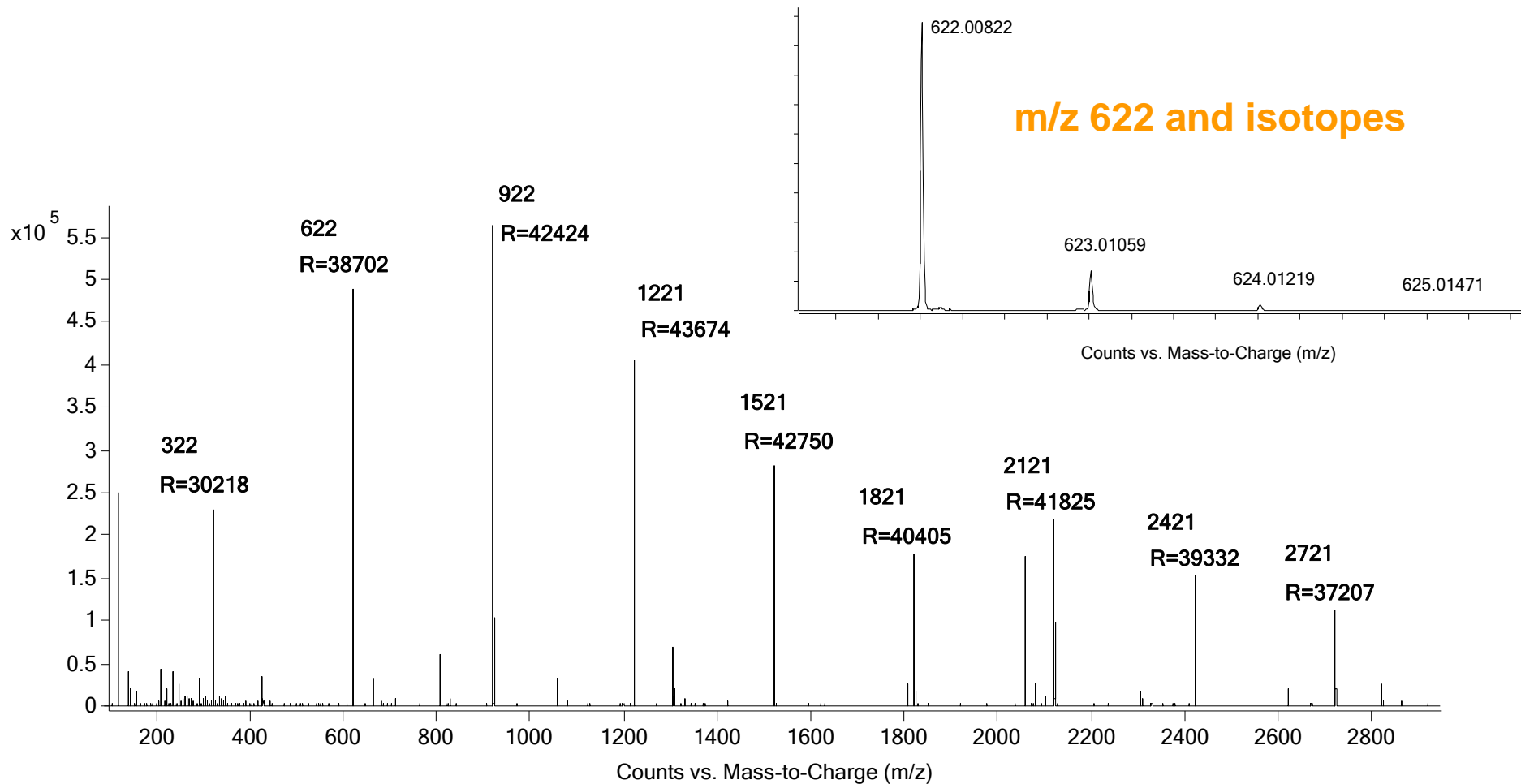
New Bipolar TOF Detector

- 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



6540 Ultra High Definition QTOF

Maintaining Resolving Power – Across the Mass Range



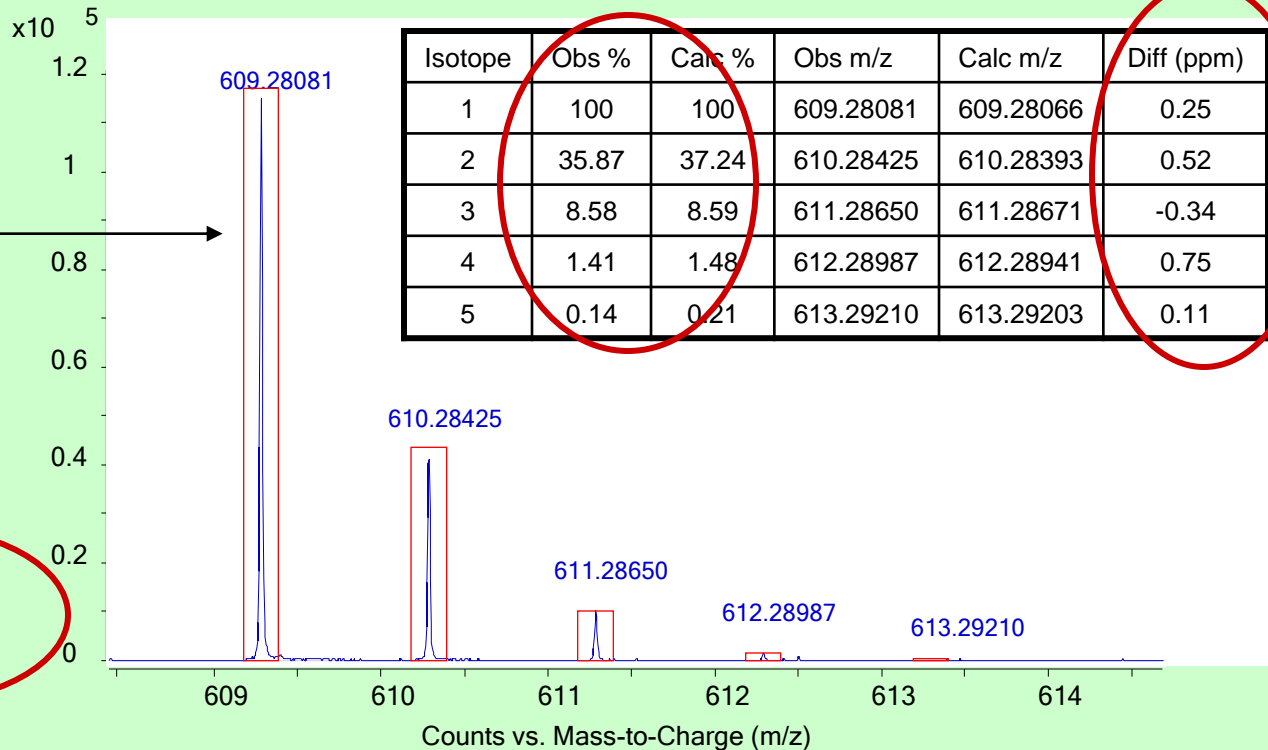
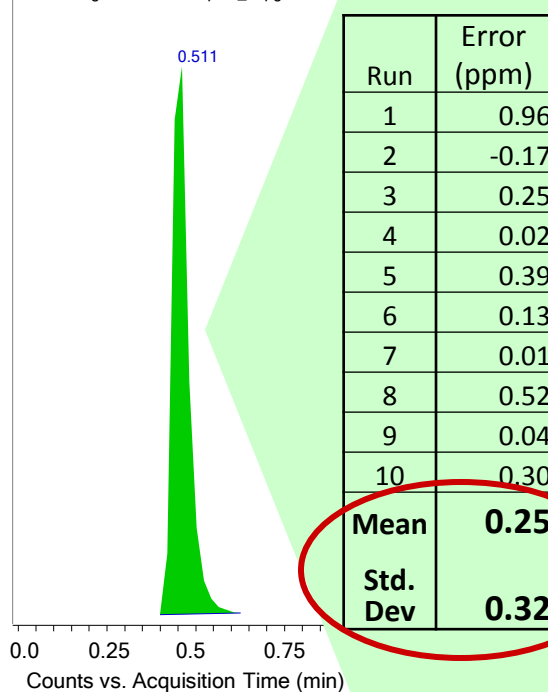
Scan Rate Independent

6540 Ultra High Definition QTOF

Mass Accuracy – Repetitive Injections

40pg reserpine on-column, 10 injections

+ESI EIC(609.28066)
Scan Frag=240.0V Reserpine_40pgms3.d

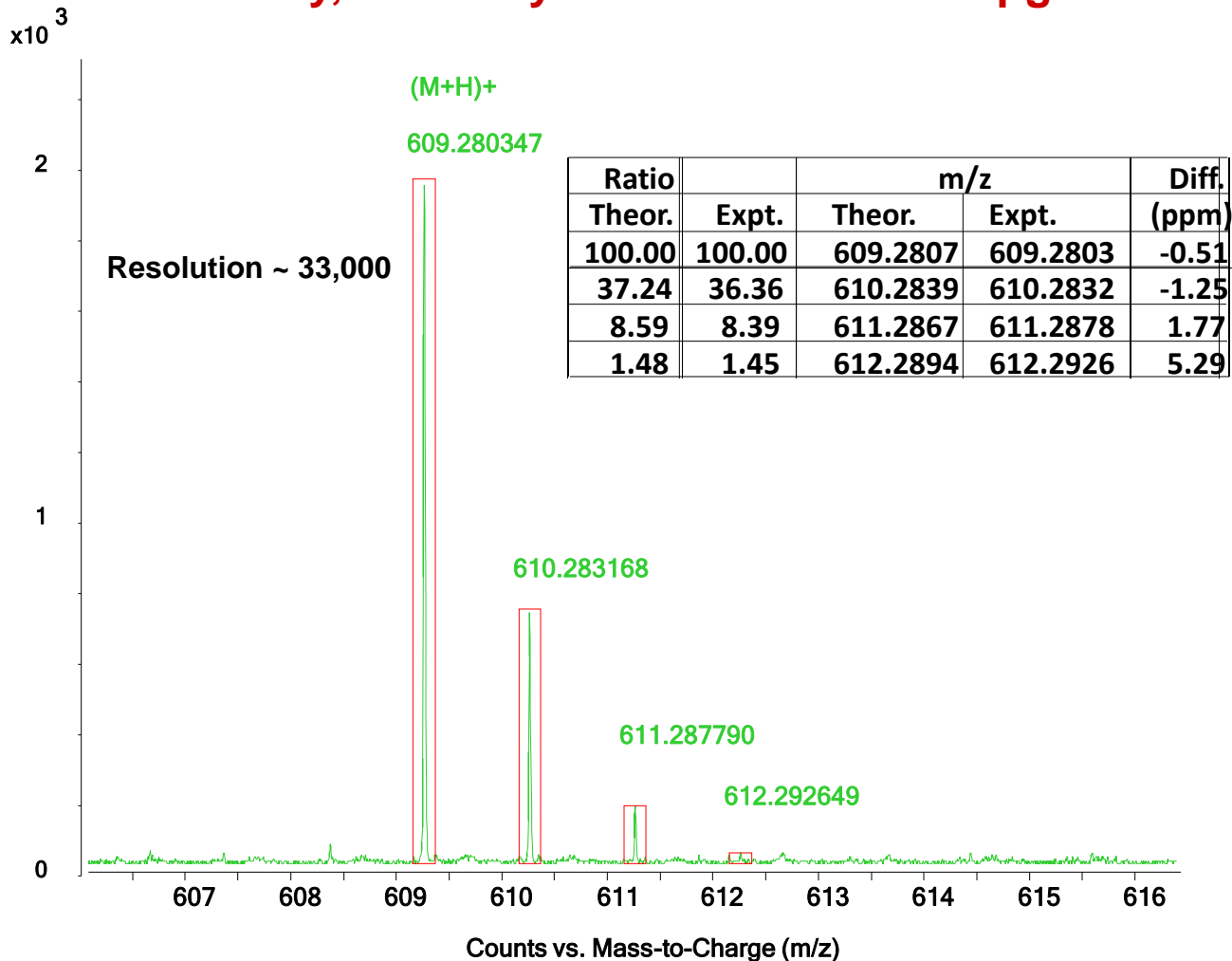
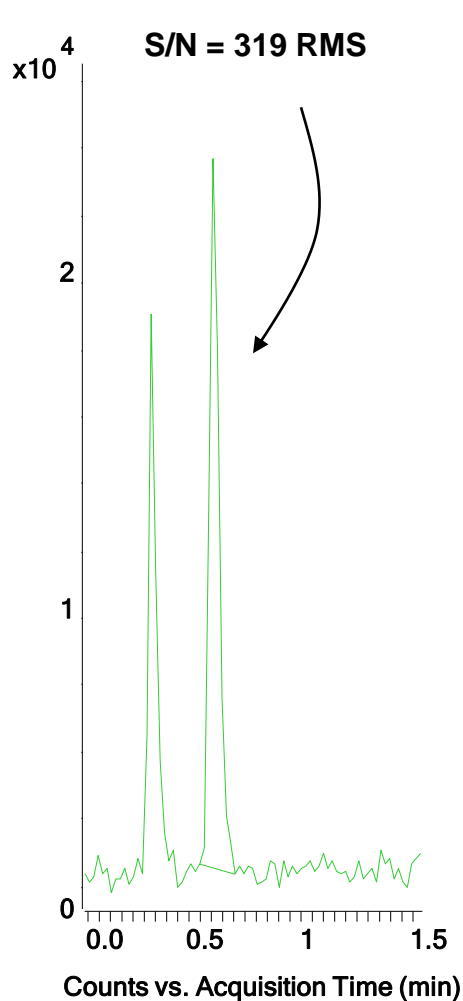


250 ppb mass accuracy calibration and very accurate isotopic ratios

6540 Ultra High Definition QTOF

Sensitivity – Full Scan MS Mode- 1 picogram

Excellent sensitivity, accuracy and resolution at 1 pg on column



Topics

- Example: Pesticides in Foods
- Example: Drug metabolite identification
- Example: Peptide Mapping

Pesticides Screening in Foods

- **Accurate-Mass Databases for Comprehensive Screening of Pesticide Residues in Food by Fast Liquid Chromatography Time-of-Flight Mass Spectrometry**, Milagros Mezcua, Octavio Malato, Juan F. García-Reyes, Antonio Molina-Díaz, and Amadeo R. Fernández-Alba, *Anal. Chem.*, **2009**, *81* (3), 913-929
DOI: 10.1021/ac801411t
- Method for screening 297 pesticides in foods with Agilent 1100 HPLC, 4.6 x 50 mm XDB-C18, 1.8 μ m column, and TOF-MS detection
- Component identification using accurate mass/retention time (AMRT) matching against a database
- 0.6 mL/min flow rate, 12 minute gradient, approximately 23 minutes run time
- Comparable method performance when tested on 60 commercial produce samples (QuEChERS protocol sample prep) and compared with results from QQQ [note: 45/60 samples tested positive!]
- ***Identified several pairs of “isobaric coeluting species” (ICS) which have similar retention times and accurate mass values for their adduct ions or fragments***

Focused AMRT Databases (Personal Compound Databases—PCD)

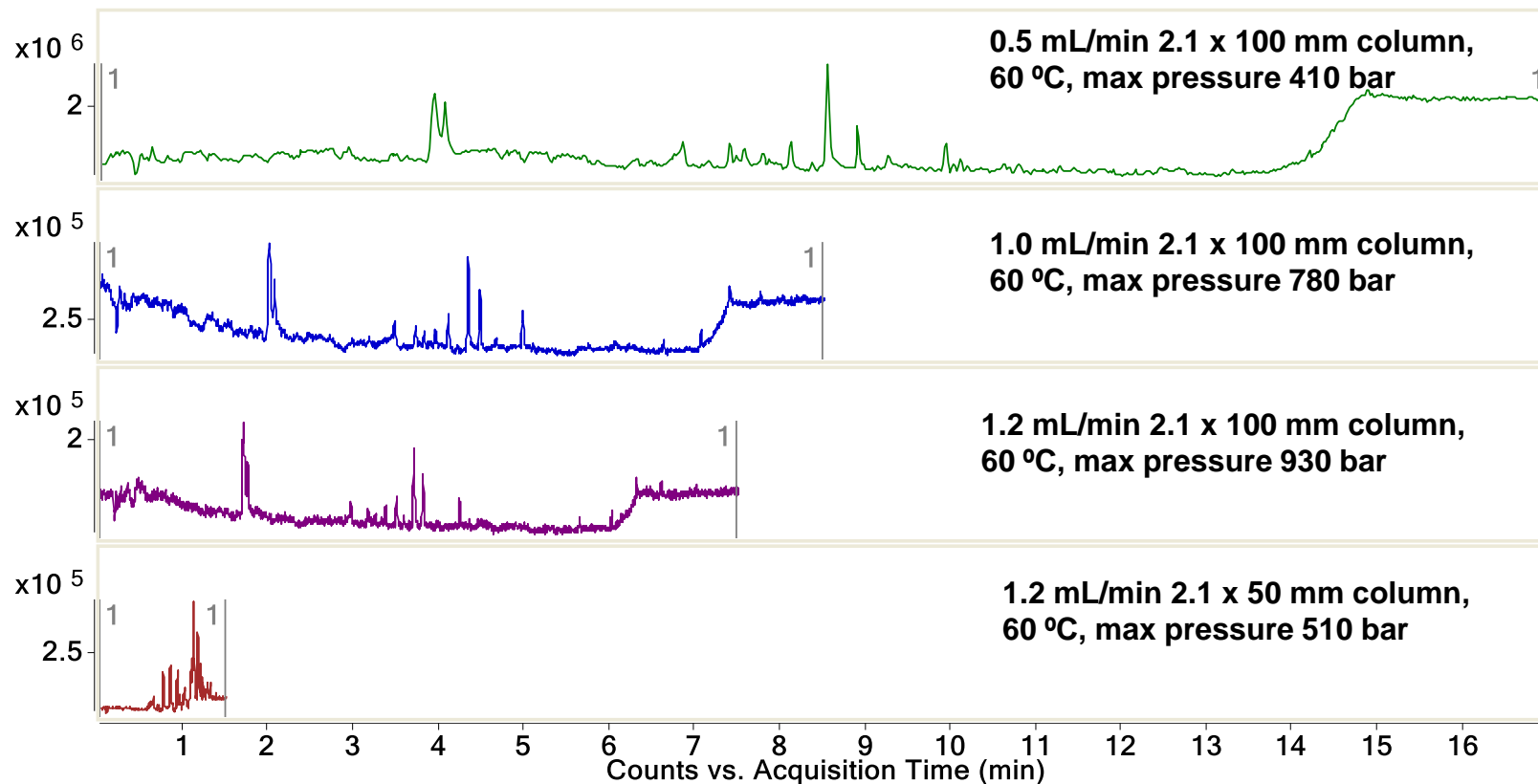
- Pesticides, Part # (G6854AA):
 - 1600 analyte content (formulae & accurate mass)
 - Structures
 - CAS Numbers & Links to external website (pubchem)

- Forensic & Toxicology, Part # (G6855AA):
 - ~7000 analytes content (formulae & accurate mass)
 - Structures
 - CAS Numbers & Links to external website (pubchem)

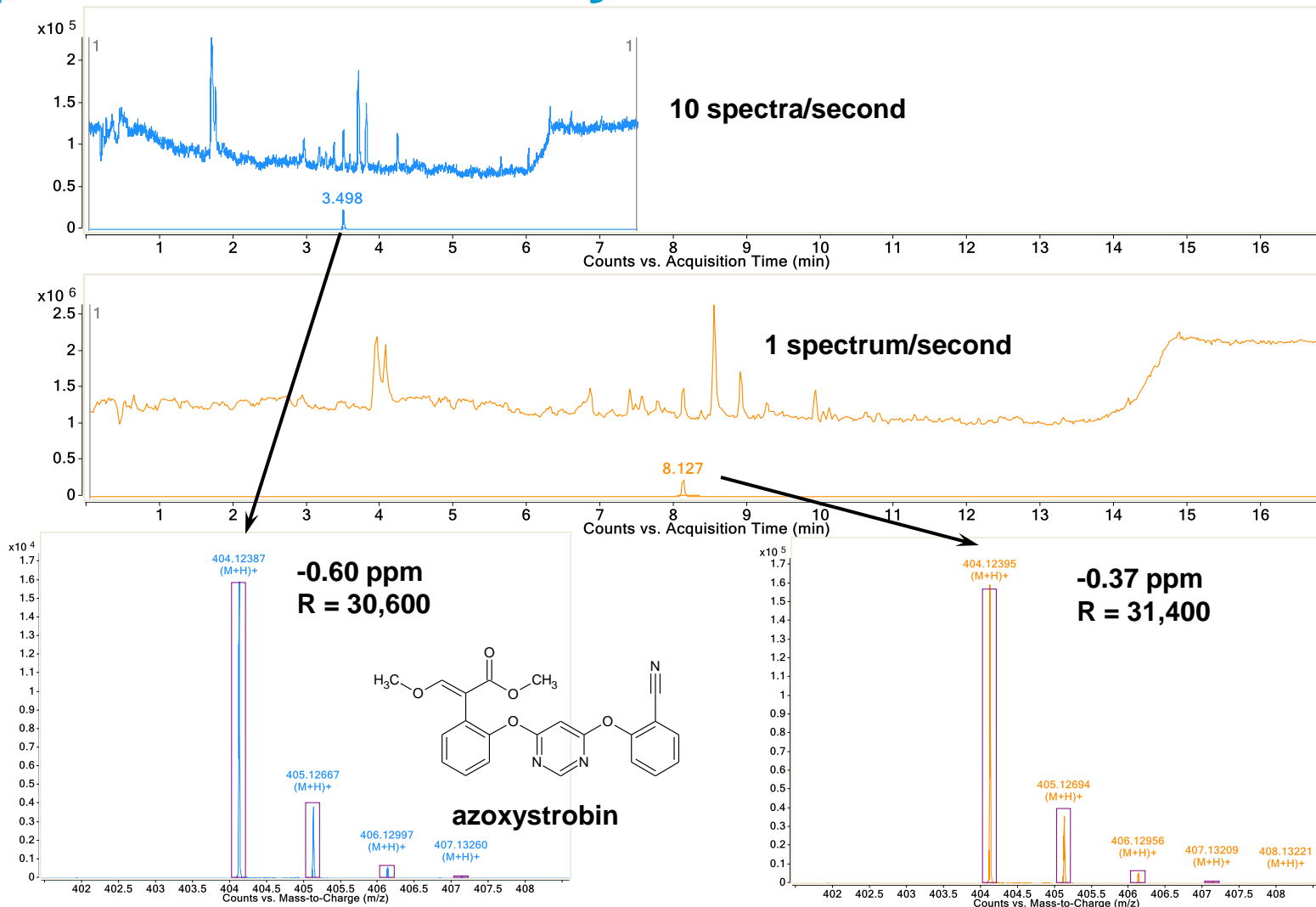
Starting Conditions for Pesticides Analysis

- Configuration
- 1290 Infinity UHPLC, 6540 QTOF with Agilent jet stream technology
- Eclipse Plus C18 column, 2.1 x 50 mm or 100 mm, 1.8 μm
- Analysis conditions
- A = 0.1% formic acid in water, B = 0.1% formic acid in ACN; various flow rates (1.2, 1.0, 0.5 mL/min); 60 °C column temperature
- Gradient = 5% B to 95% B in various times (1.5, 3, 7 , 8.5, 17 minutes)
- 3200 m/z maximum mass, high resolution mode
- MS mode: 100-1000 m/z , various acquisition rates (10, 5, 3, 1 scans/second)

RRLC → UHPLC

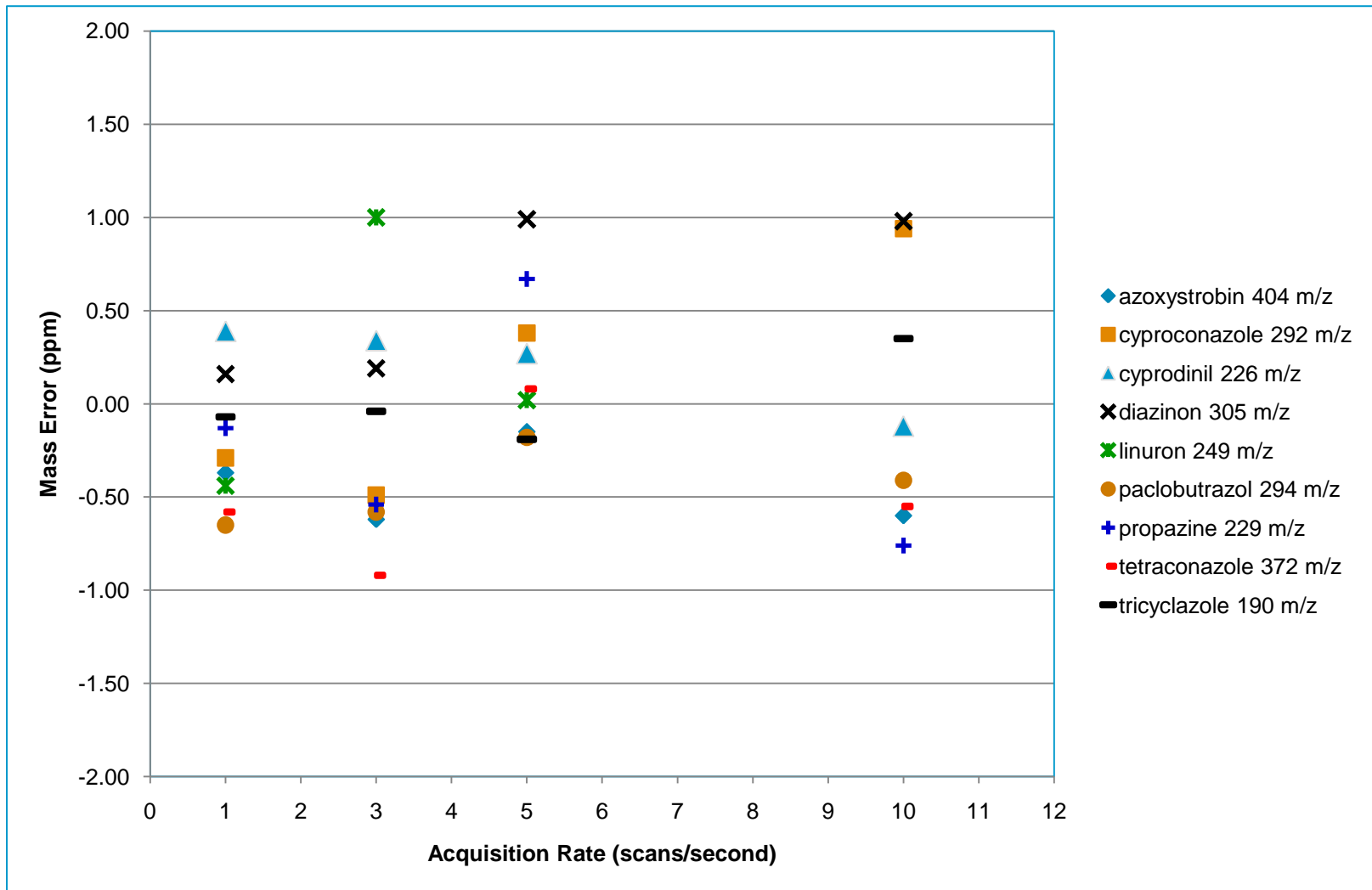


Mass Accuracy and Mass Resolution and Analysis Speed Simultaneously



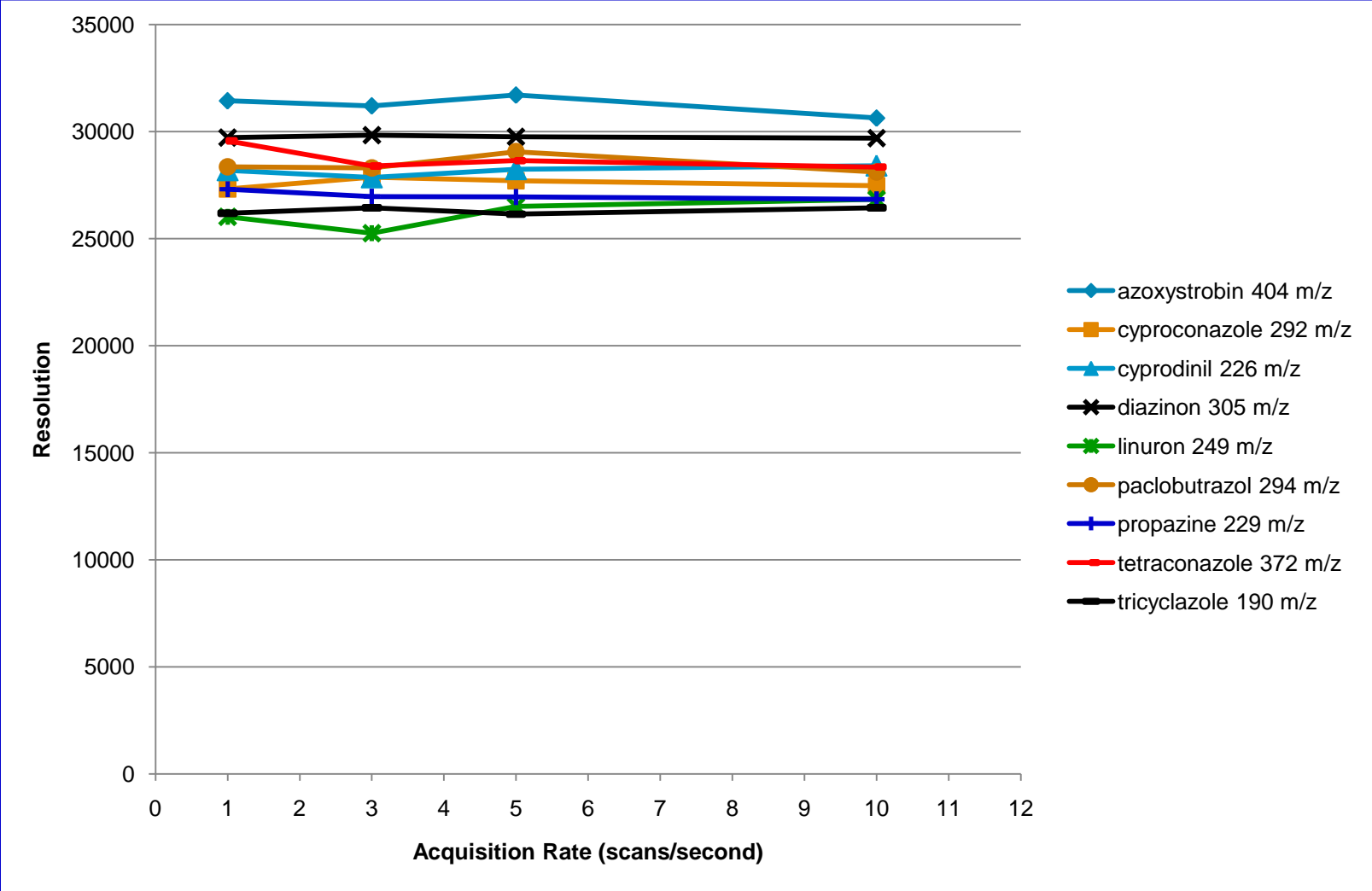
Mass Accuracy is Invariant with Acquisition Rate

Pesticides Example



MS Resolution is Invariant with Acquisition Rate

Pesticides Example

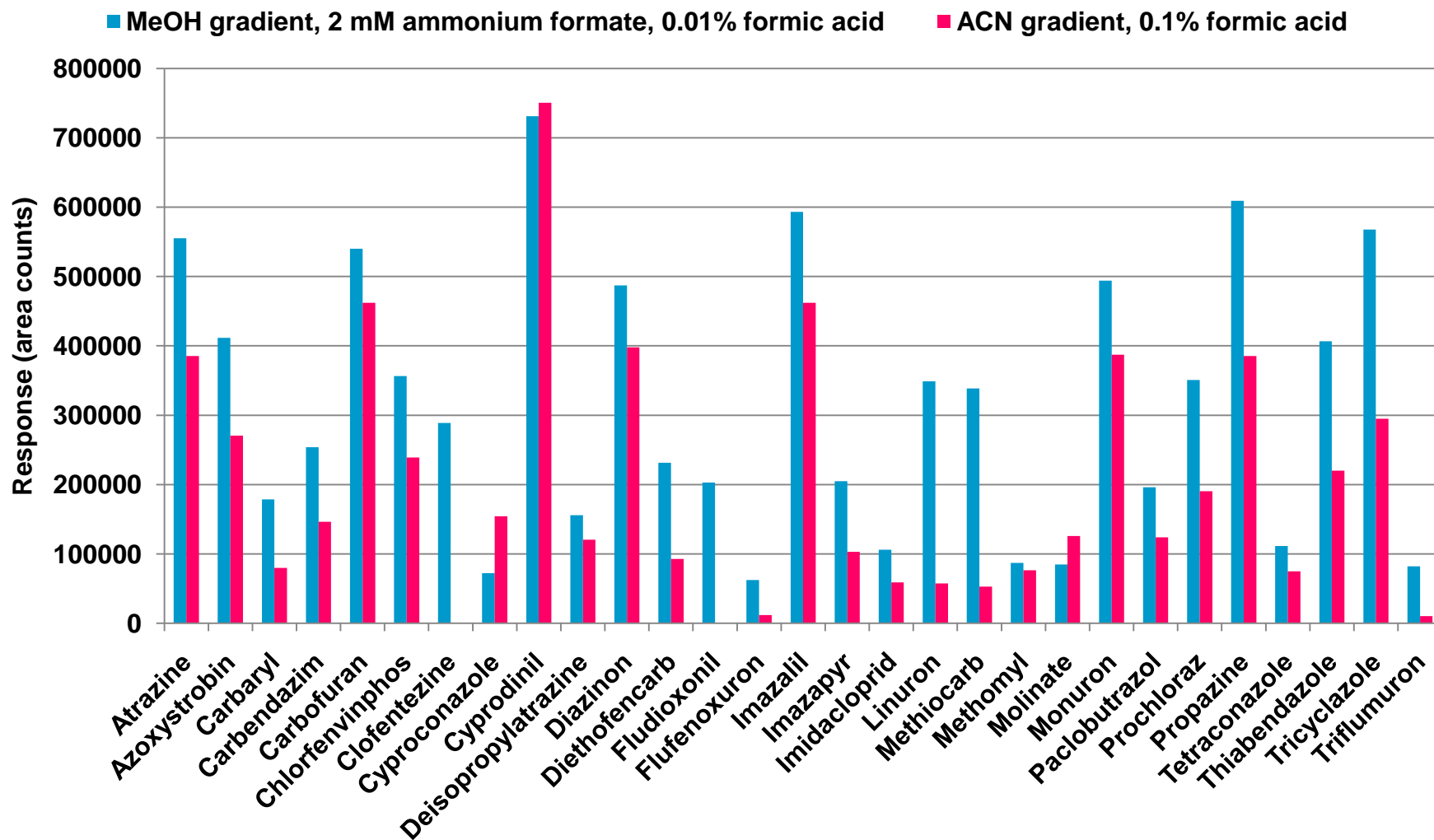


Methanol Mobile Phase for Pesticides Analysis

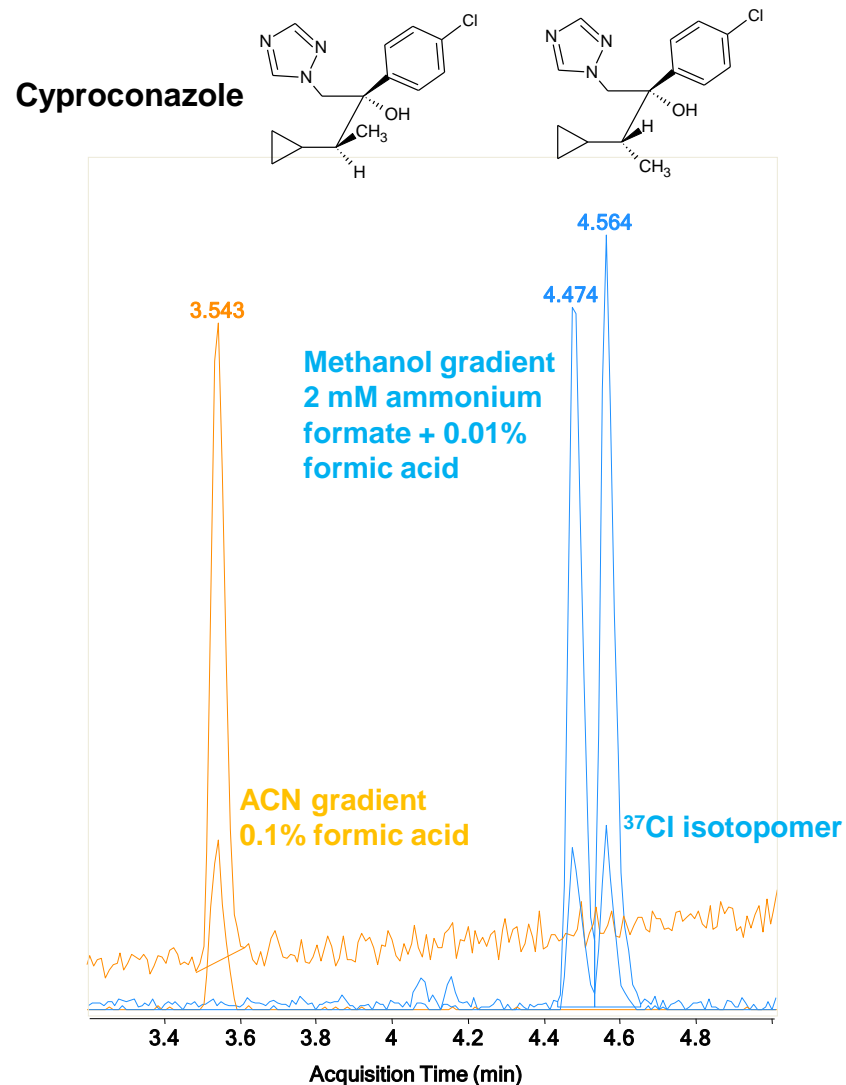
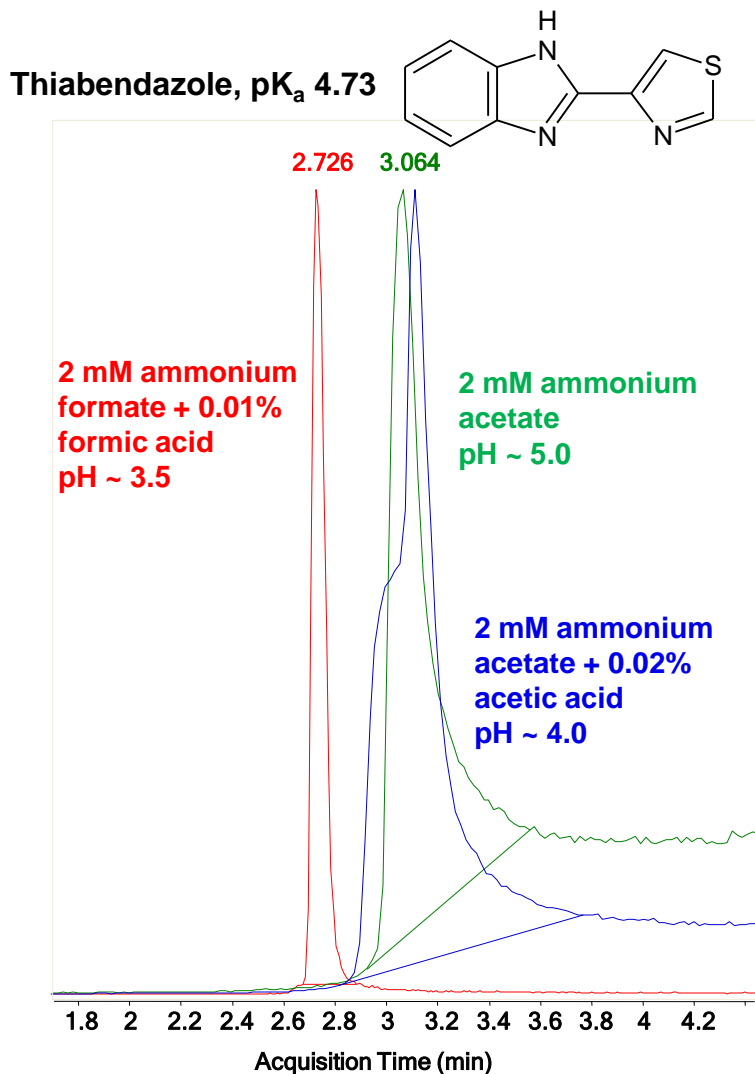
- Configuration
- 1290 Infinity UHPLC, 6540 QTOF with Agilent jet stream technology
- Eclipse Plus C18 column, 2.1 x 50 mm, 1.8 μm
- Analysis conditions
- A = 2 mM ammonium formate, 0.01% formic acid in water, B = 2 mM ammonium formate, 0.01% formic acid in methanol; flow rate 0.8 mL/min, 40 °C column temperature
- Gradient = 5% B to 95% B in 3 min, stop time 4 min (standard) or 10 min (sample with matrix)
- 1700 m/z maximum mass, high resolution mode
- MS mode: 100-1000 m/z , 3 scans/second)

Chamberlain, P., Hall, T., Zavitsanos, P. Agilent Technologies application note 5989-1688EN, January 2005.

Better Response with Methanol Mobile Phase

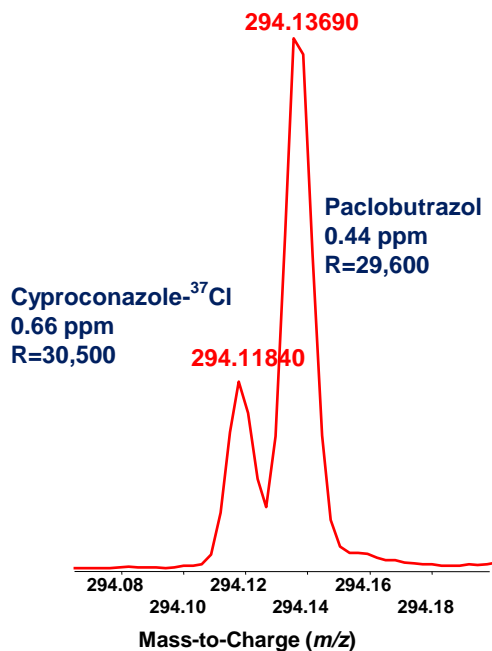


Chromatographic Observations using Standards Same Instrument and Column



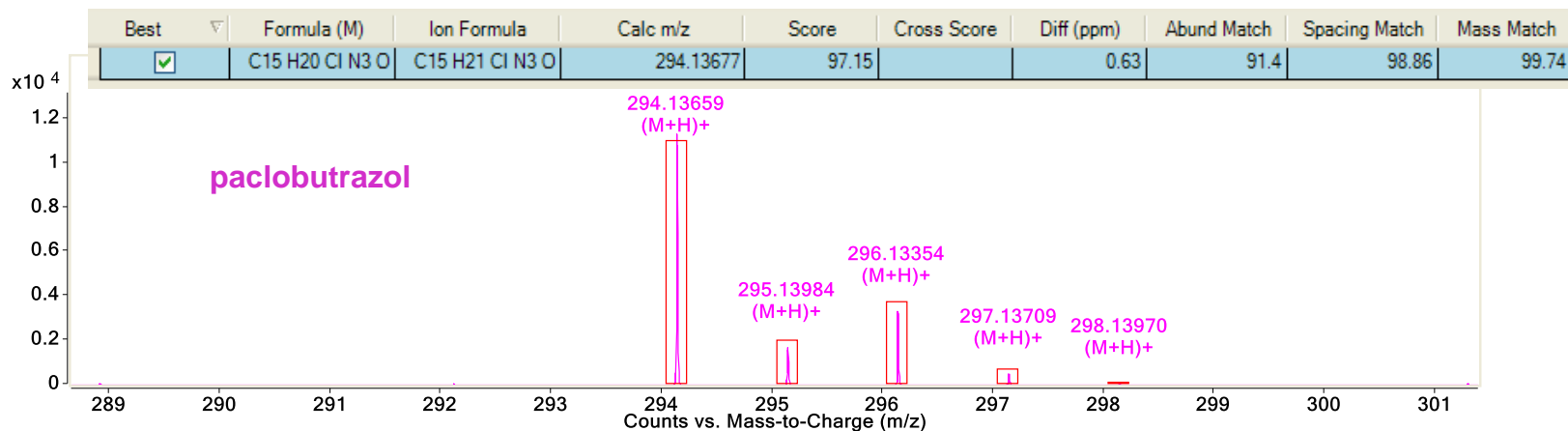
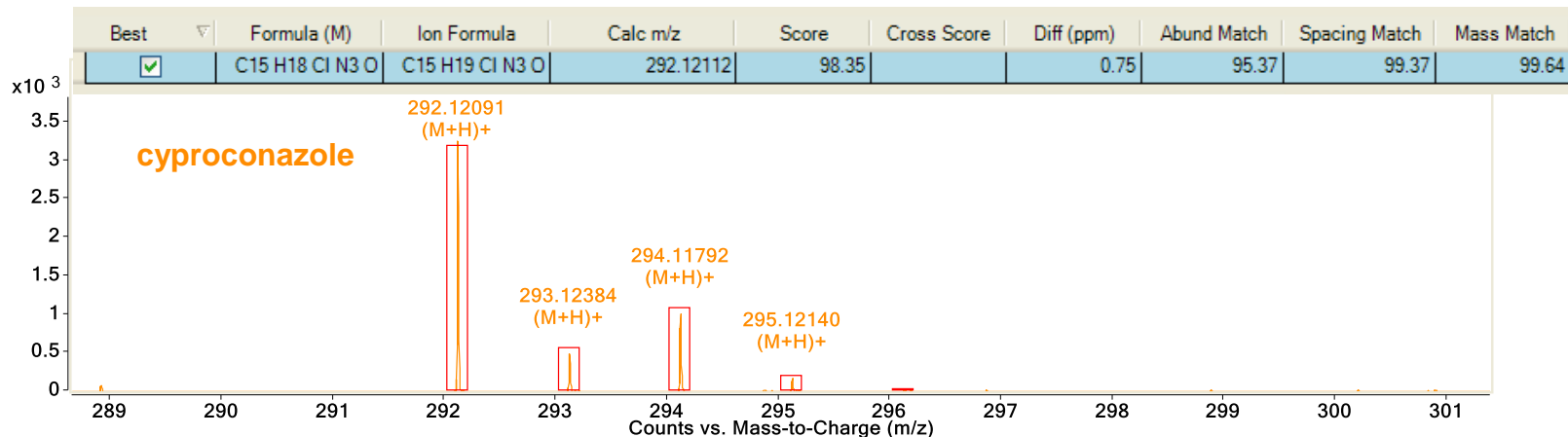
Isobaric Co-eluting Species

Example: 63 ppm Mass Difference (18 mDa)



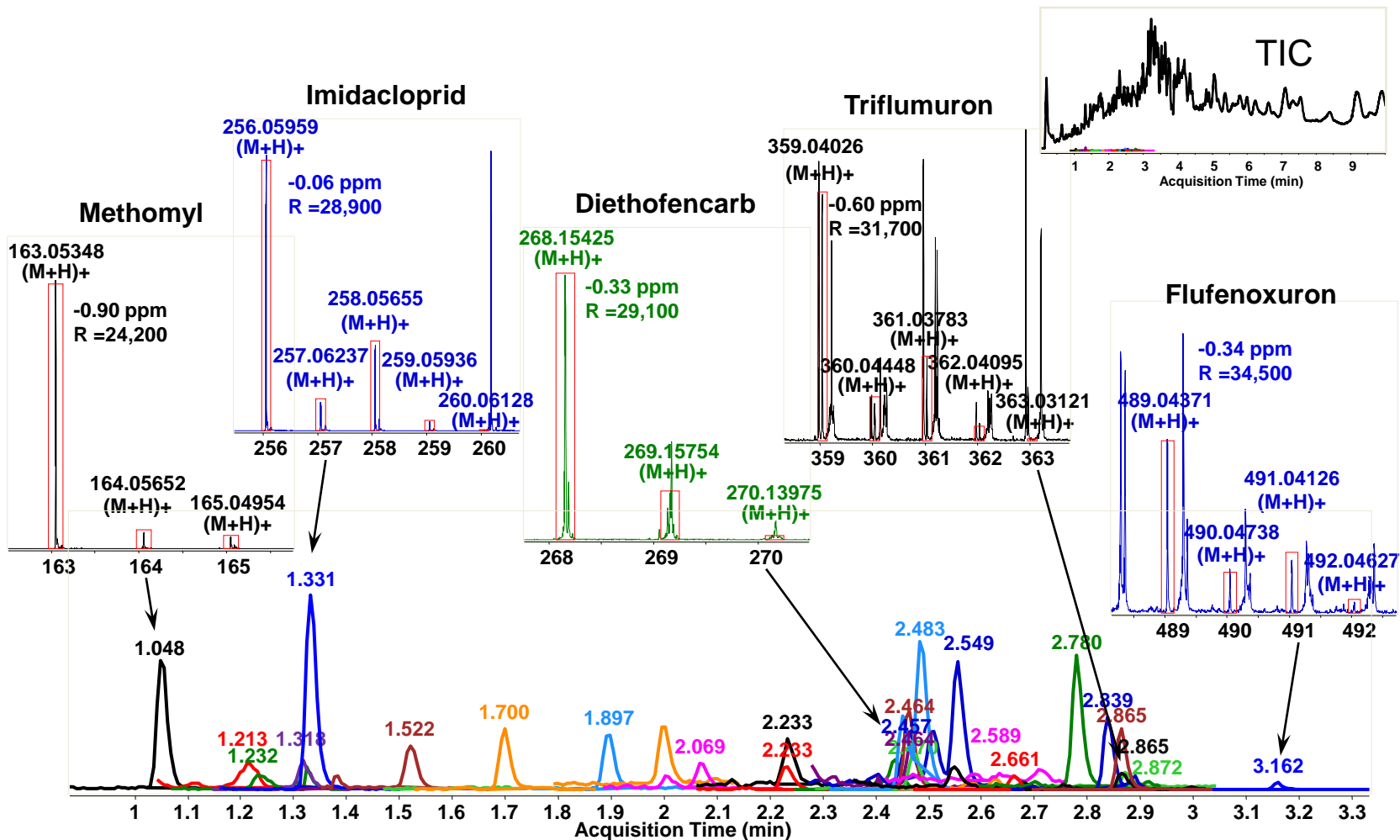
Isobaric Co-eluting Pair	<i>m/z</i>	ppm Difference	Differentiate by		
			RT	No fragment	<i>m/z</i>
Tricyclazole Propazine	190.0433 190.0667	123	X (1.3 min)		X
Methiocarb Diethofencarb fragment	226.0896 226.1074	79		X	X
Diethofencarb fragment Cyprodinil	226.1074 226.1339	117	X (0.53 min)	X	X
Cyproconazole - ³⁷ Cl Paclobutrazol	294.1182 294.1368	63			X
Clofentezine- ³⁷ Cl Diazinon	305.0618 305.1083	300			X
Chlorfenvinphos Triflumuron	358.9768 359.0405	177			X
Tetraconazole Azoxystrobin fragment	372.0288 372.0979	186		X	X

Identify These Pesticides By Isotopic Pattern And Mass



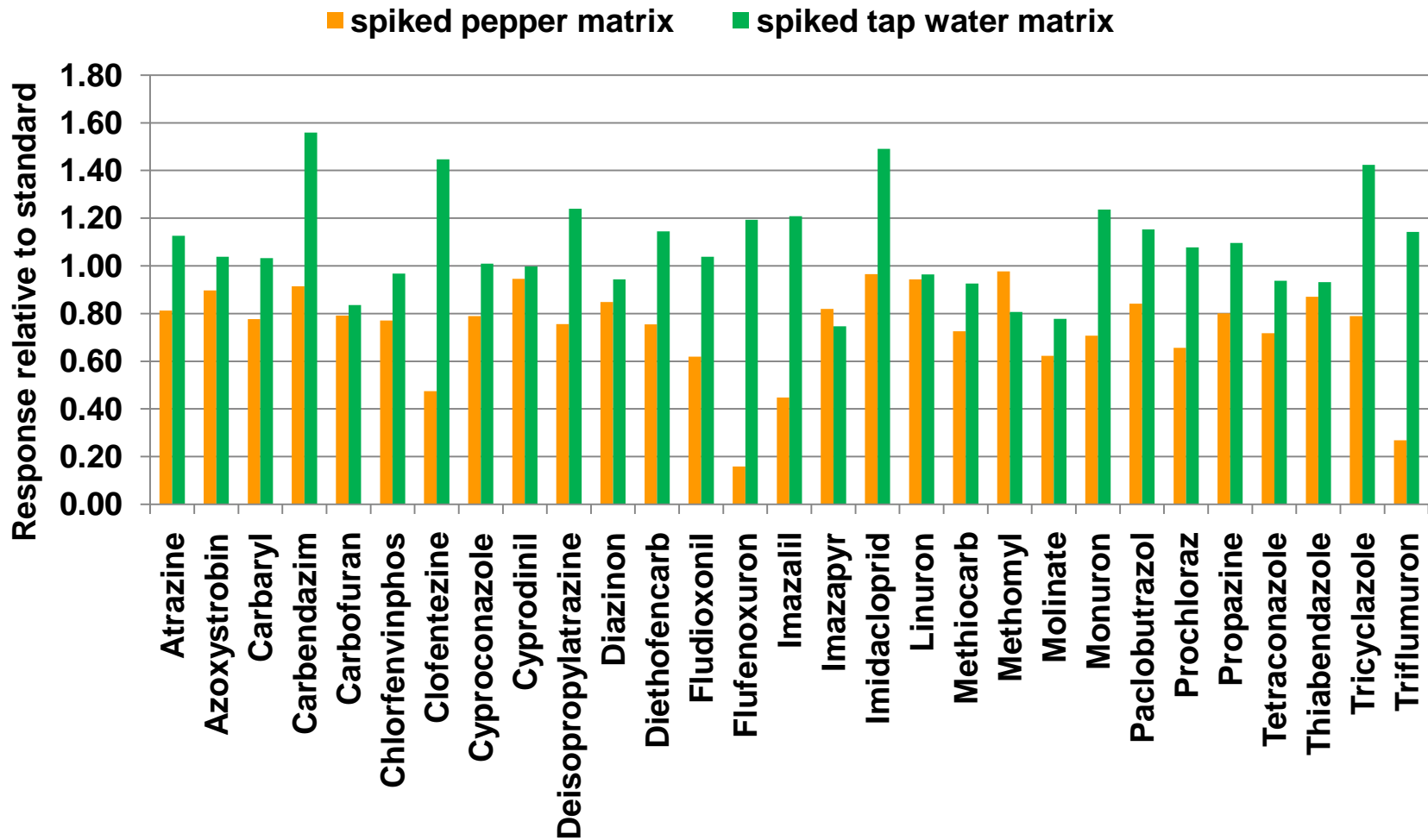
Pesticides in Pepper Matrix, 10 ppb

3 Scans/sec Acquisition Rate



Matrix Suppression Effect

Response of Standard = 1

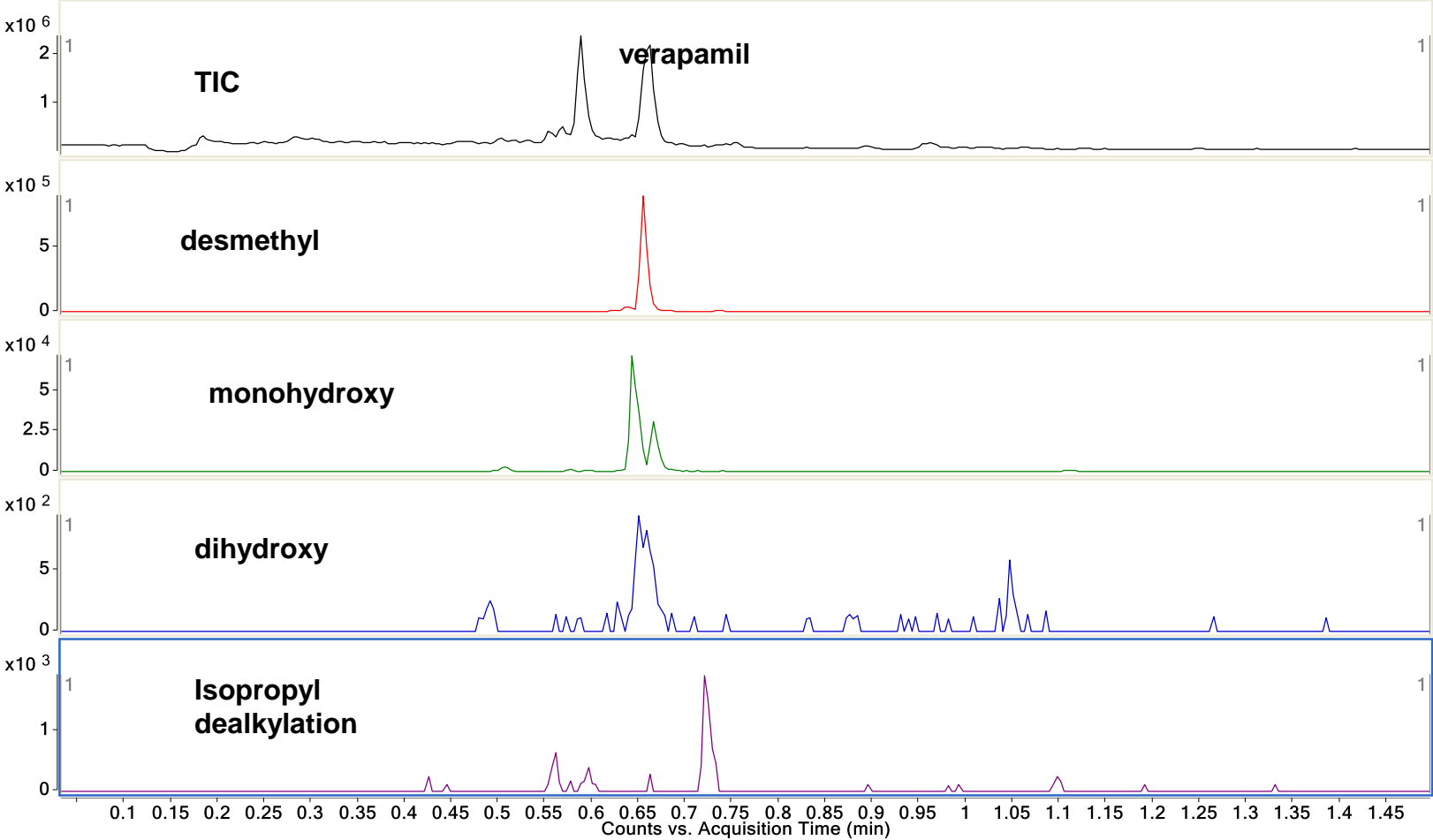


Metabolite ID

Experimental Conditions for Verapamil Metabolite ID Analysis

- Configuration
- 1290 Infinity UHPLC, 6540 QTOF with Agilent jet stream technology
- Eclipse Plus C18 column, 2.1 x 50 mm, 1.8 μm
- Analysis conditions
- A = 0.1% formic acid in water, B = 0.1% formic acid in ACN; 1.2 mL/min; 60 °C column temperature
- Gradient = 5% B to 95% B in 1.0 minute, stoprun 1.5 minutes, postrun 0.5 minutes
- 1700 m/z maximum mass, extended dynamic range mode
- MS mode: 100-1000 m/z , 3 scans/second

Verapamil: Major and Minor Metabolites (Phase I)



Coeluting Metabolites with Parent Drug: Need Wide Dynamic Range

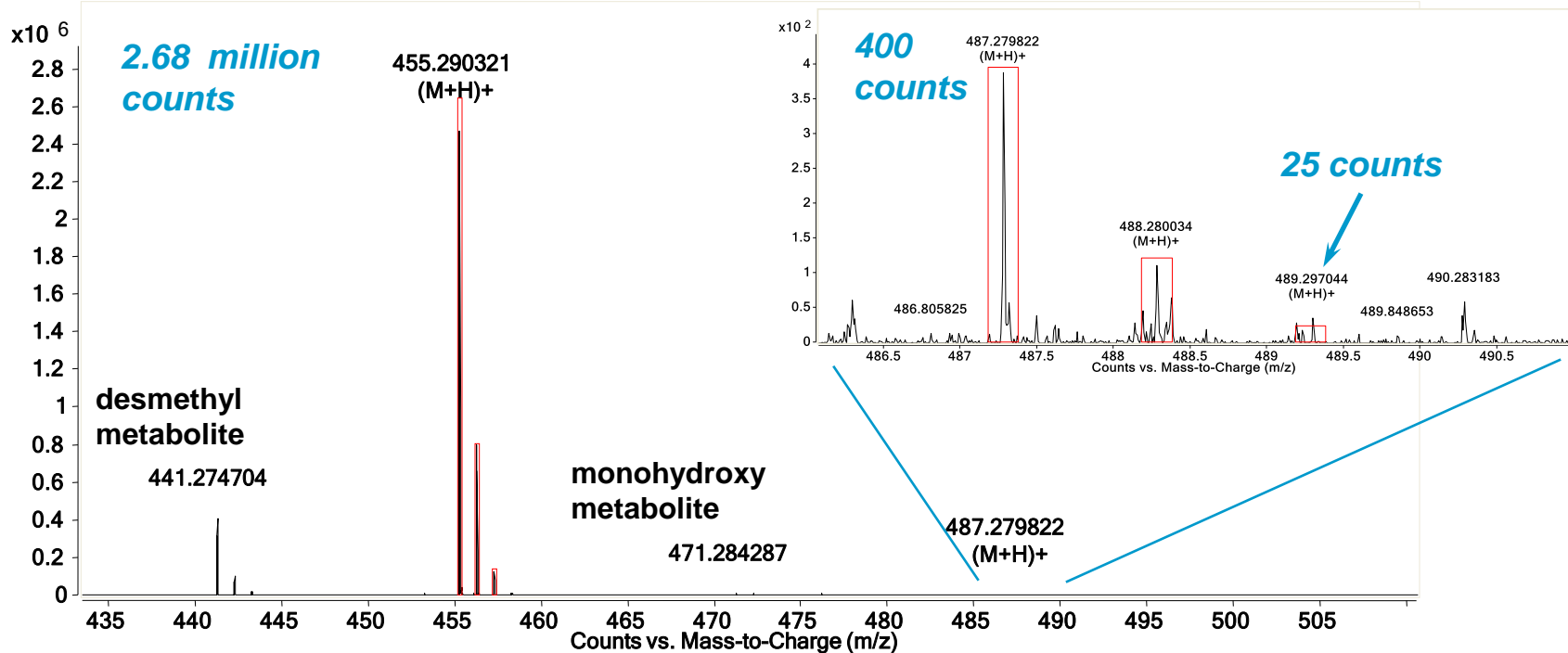
Five decades of response in a single scan

verapamil

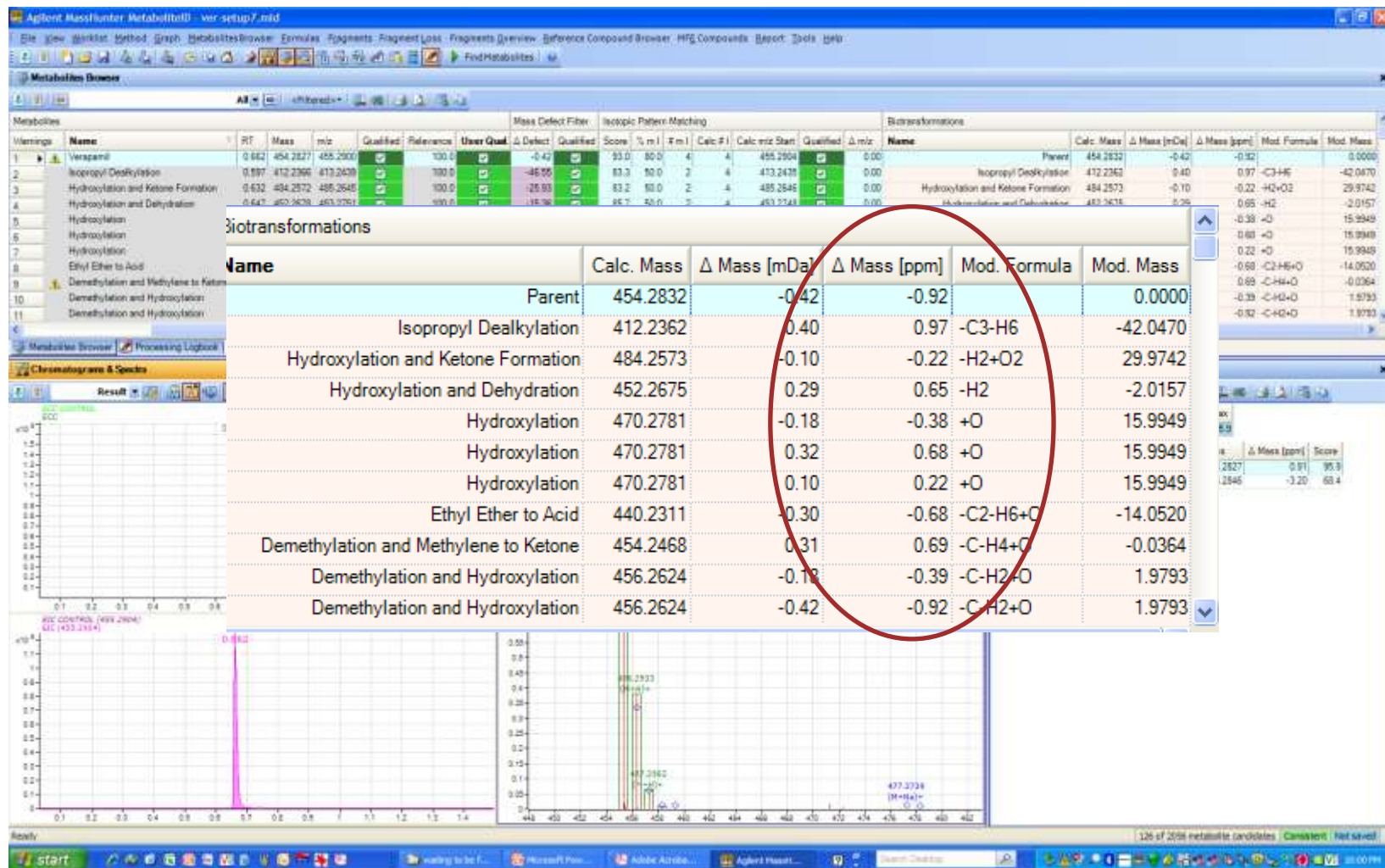
Best	Formula (M)	Ion Formula	Score	
<input checked="" type="checkbox"/>	C ₂₇ H ₃₈ N ₂ O ₄	C ₂₇ H ₃₉ N ₂ O ₄	99.43	
Calc m/z	Diff (ppm)	Mass Match	Abund Match	Spacing Match
455.290434	0.25	99.94	98.27	99.83

dihydroxy metabolite of verapamil

Best	Formula (M)	Ion Formula	Score	
<input checked="" type="checkbox"/>	C ₂₇ H ₃₈ N ₂ O ₆	C ₂₇ H ₃₉ N ₂ O ₆	80.18	
Calc m/z	Diff (ppm)	Mass Match	Abund Match	Spacing Match
487.280263	1.03	98.88	84.39	37.71

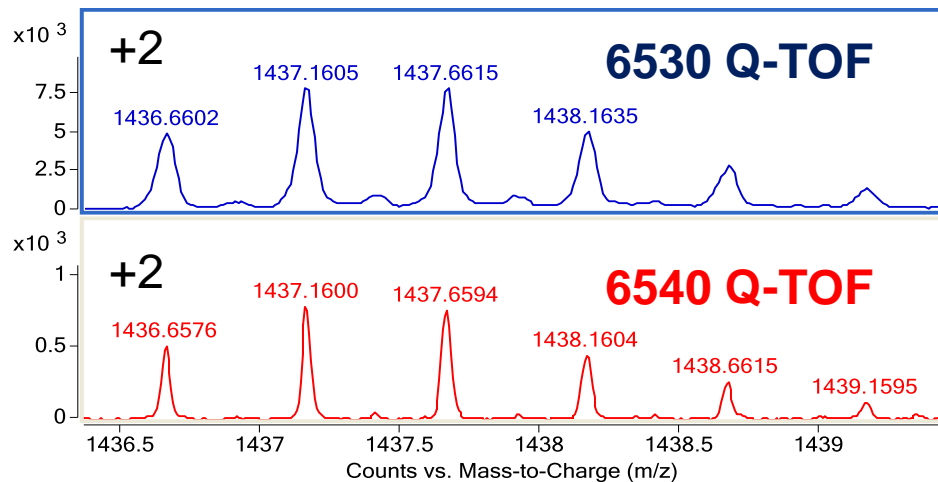


Metabolite ID Results

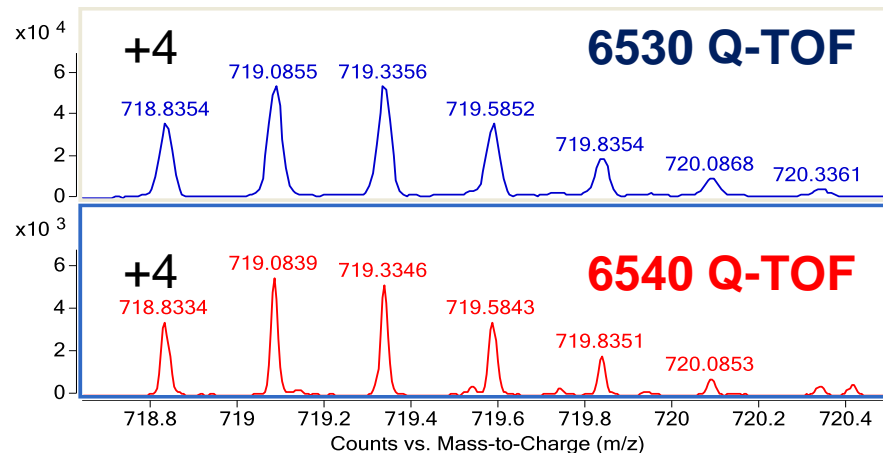
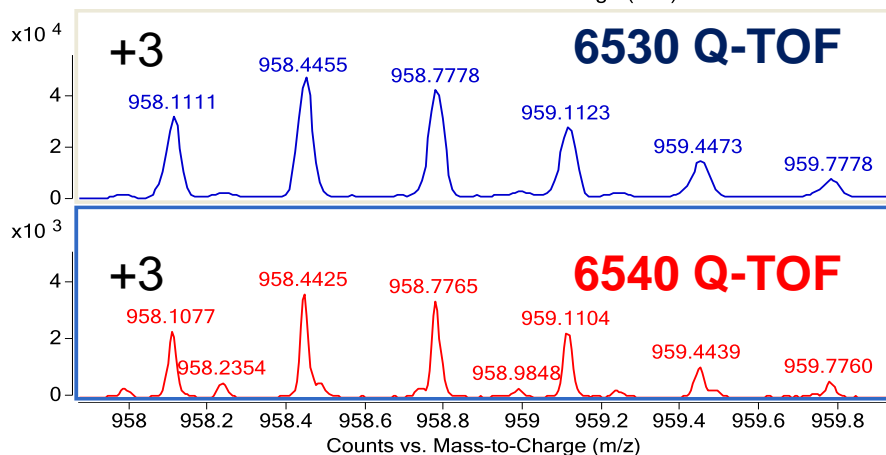


Rapid Peptide Mapping

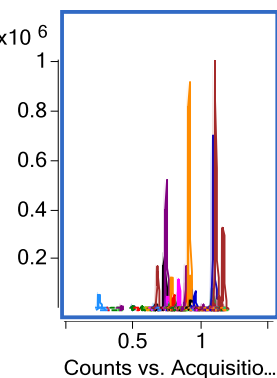
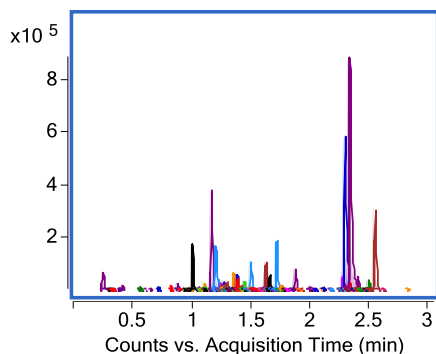
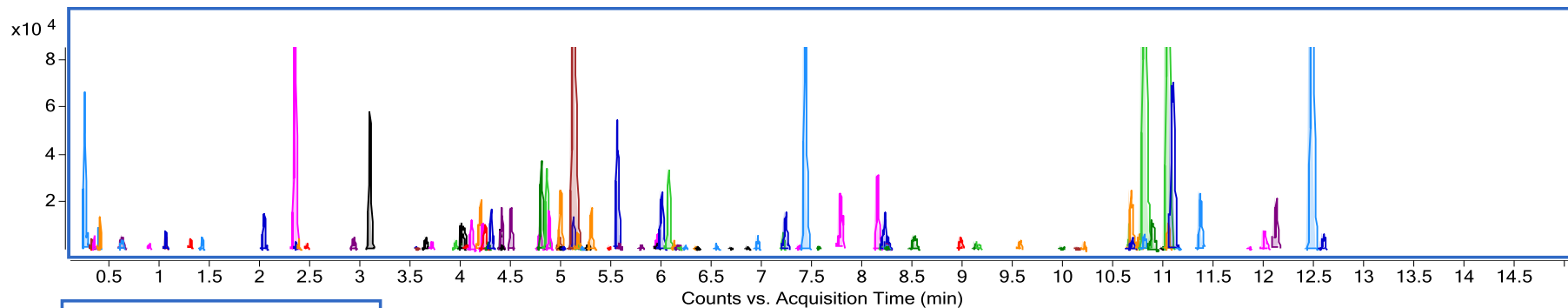
6530 vs. 6540: Comparison of Peptide Mass Spectra



CCTKPESERM PCTEDYLSLILNR

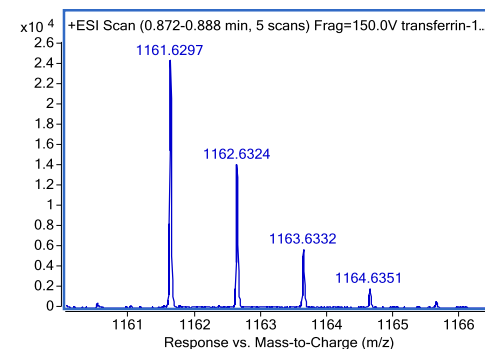
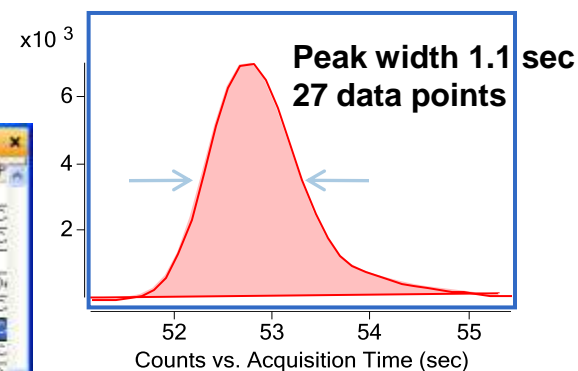


Peptide Mapping: Serotransferrin



RT	Mass	Height	Sequence	Tgl. Seq. Mass	Match Diff (p)	P
0.24	374.2279	54092	TVR	374.2276	0.39	
0.615	1339.5832	7181	NYELLCGDNTR	1339.5827	0.43	1°C
0.732	1580.7624	1990	KENFEVLKDGTR	1580.7617	0.44	1°C
0.489	374.2279	17157	TVR	374.2276	0.45	
1.153	3953.0081	95195	AISNNEADAVTLGGILWYEAQLK.P.	3953.0058	0.56	2°D
0.342	476.242	2710	IMK	476.2417	0.62	2°C
0.879	1160.6231	36631	ATCVELIHK	1160.6223	0.68	1°C
0.746	1435.714	17908	CLVEKGDVAFVK	1435.713	0.74	3°C
0.562	374.2168	11293			0.76	1°D
0.905	1157.5815	4548			1.04	
0.827	2124.0408	1604			1.15	2°D
0.789	1435.7148	22523			1.3	3°C
1.084	2538.2537	2254	TVGGKELDPIWELLNMAHAEHPK	2538.2533	1.32	2°D
0.673	2323.119	963	CLASIAKKTYDSYLGDDYVRI	2323.1155	1.54	1°C
1.003	2280.0896	7062	KTYDSYLGDDYVRAMTNLR	2280.0845	1.79	
0.876	1754.6787	3995	ITMFRAGDVAFAK	1754.6781	1.94	1°D

0.68 ppm



Summary

- Unmatched chromatographic peak capacity and resolving power—can even resolve diastereomers when parameters are adjusted appropriately
- Superb MS mass accuracy and resolution—invariant with acquisition rate
- Sophisticated identification software and algorithms—handle complex samples, overlapping isotopic patterns
- Sum (product?) is greater than the parts
- Run faster chromatography for higher throughput
 - MS can keep up without sacrificing resolution
 - MS can keep up without sacrificing mass accuracy
 - MS has the dynamic range to handle coeluting peaks
 - Software has the power to identify components of complex chromatograms and spectra
 - Result: more information and results per analysis and per unit time