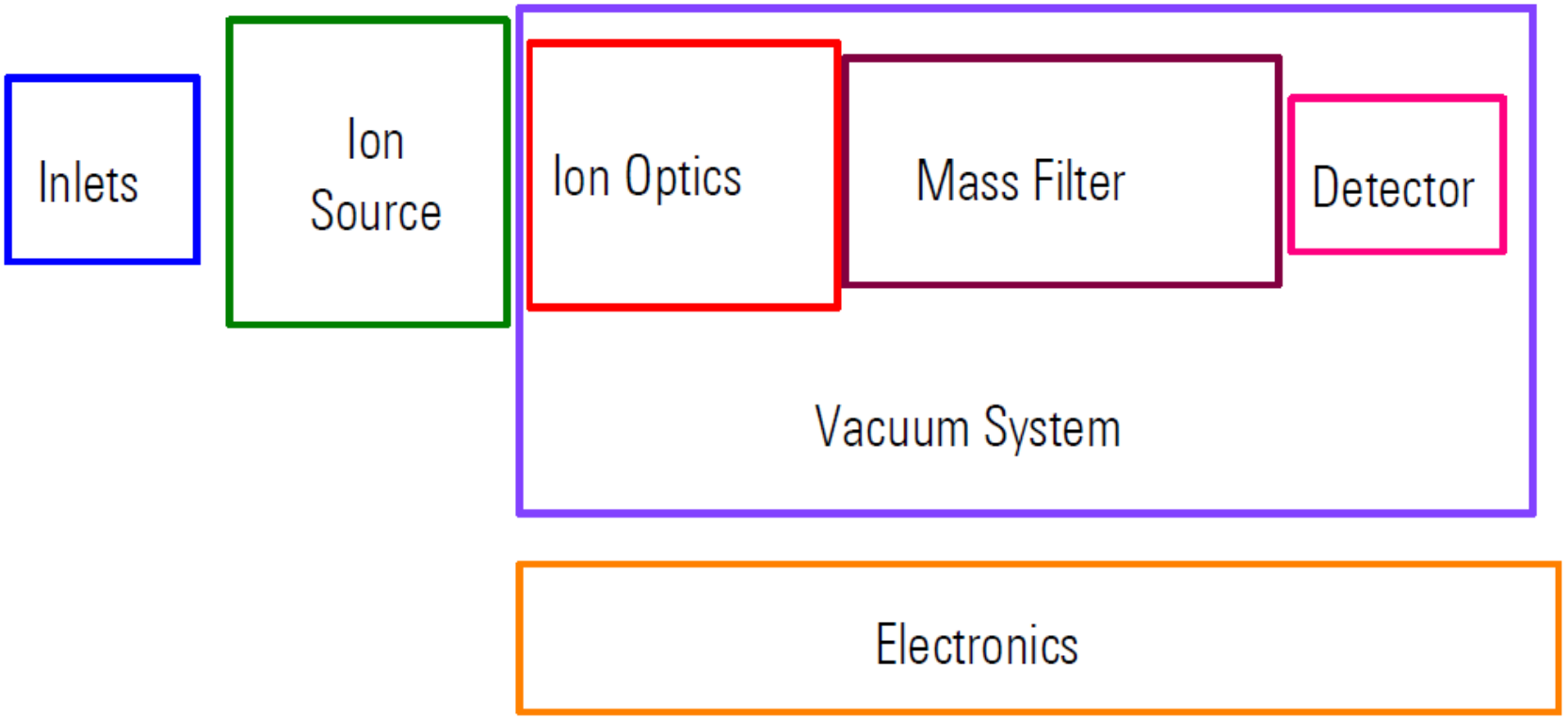
Agilent 6000 LC-MS systems

dr. Jan Srbek, HPST

Functional Block Diagram



LC/MS Ion Sources for 6000 family

Widest choice of sources from any manufacturer

- Electrospray (ESI)
- JetStream (JS-ESI)
- Nanoelectrospray (nanoESI)
- APCI
- APPI
- PDF-MALDI
- Dual ESI and nanoESI sources for TOF and Q-TOF

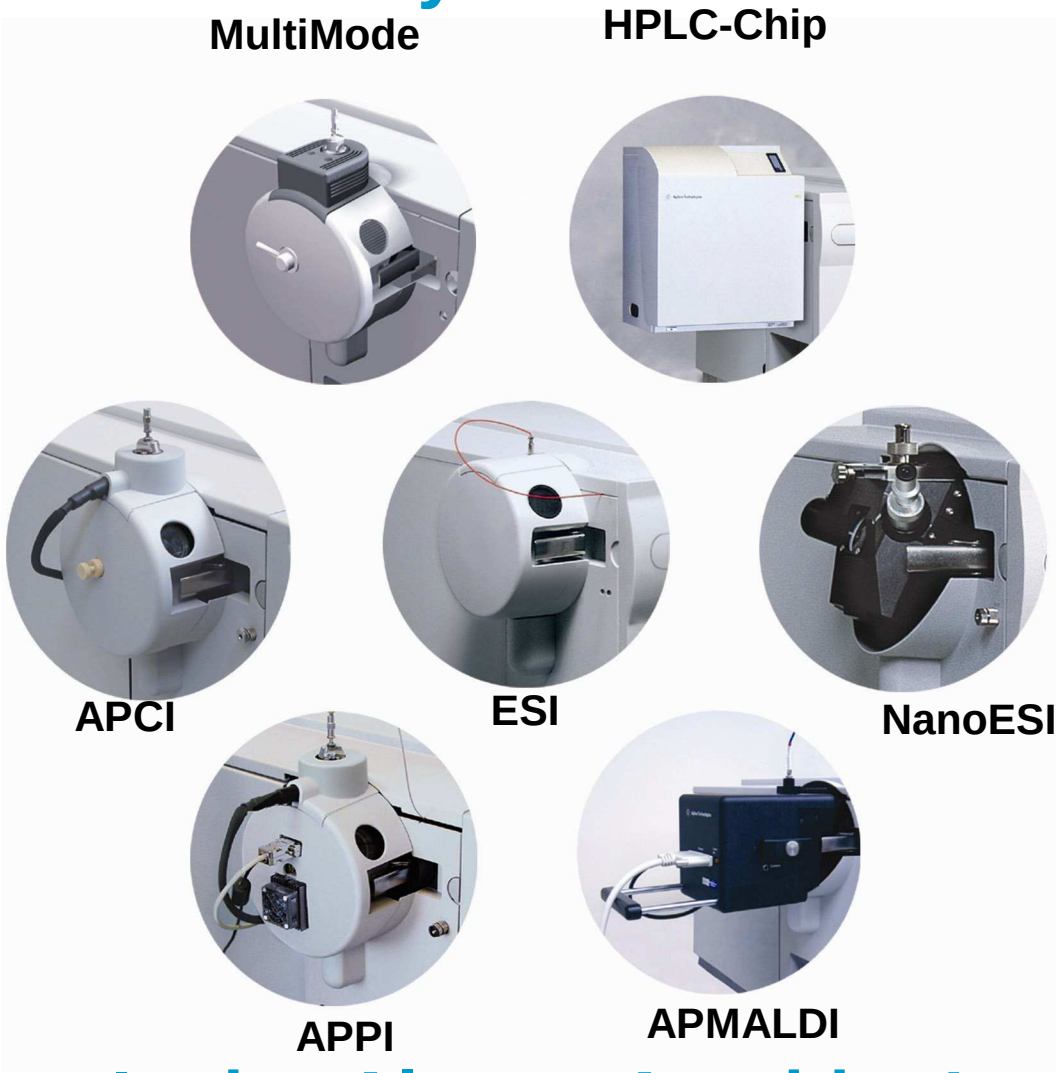
MultiMode Source

- ESI and APCI combined in one source
- Simultaneous operation

HPLC-Chip/MS

- Nanoelectrospray made easy
- Reproducible results
- Sample processing on chip

- Most sources are interchangeable between MS platforms



Ionize Almost Anything!

6100B Single Quad LC/MS Family
SIM/SCAN, universal LC detector

6490 Triple Quadrupole using iFunnel technology

Agilent Technologies

…We need to captures much more ions

Jet Stream sampling

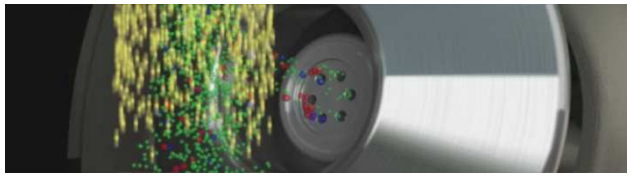
- Thermal confinement of ESI plume
- Efficient desolvation to create gas phase ions
- Creates an ion rich zone

Hexabore Capillary

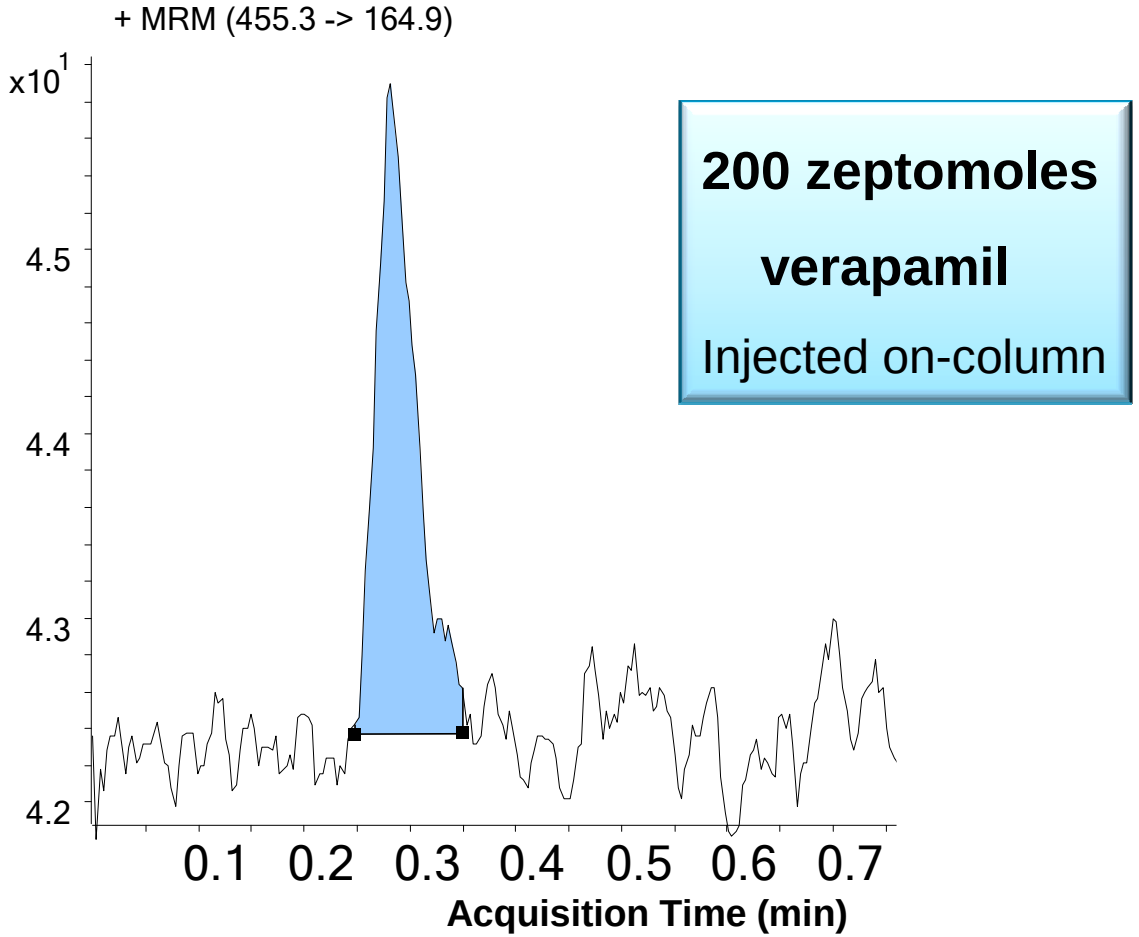
- 6 capillary inlets
- Samples 10X more ion rich gas from the source
- Captures the majority of the gas from the source region

Dual Ion Funnel

- Removes the gas but captures the ions
- Removes neutral noise
- Extends turbo pump life



iFunnel system achieves Zeptomolar Detection Limits…



200 zeptomoles

verapamil

Injected on-column

Triple Quadrupole working modes, MS/MS level, both quads can be in SIM/SCAN

Product ion scan – SIM/SCAN

Precursor ion scan – SCAN/SIM

Neutral loss/gain…

But over 90% of applications uses

SRM (MRM) – SIM/SIM

Still the most sensitive solution for quantitative applications

MassHunter Optimizer

Automated MRM Method Development Software for 6400 series Triple Quad LC/MS

Compound-specific optimization for MRM experiments

- Selection and optimization of precursor ion and fragment ions
- Support of optimization with multiple methods (e.g. pos/neg)
- Creation of a compound database with optimized results

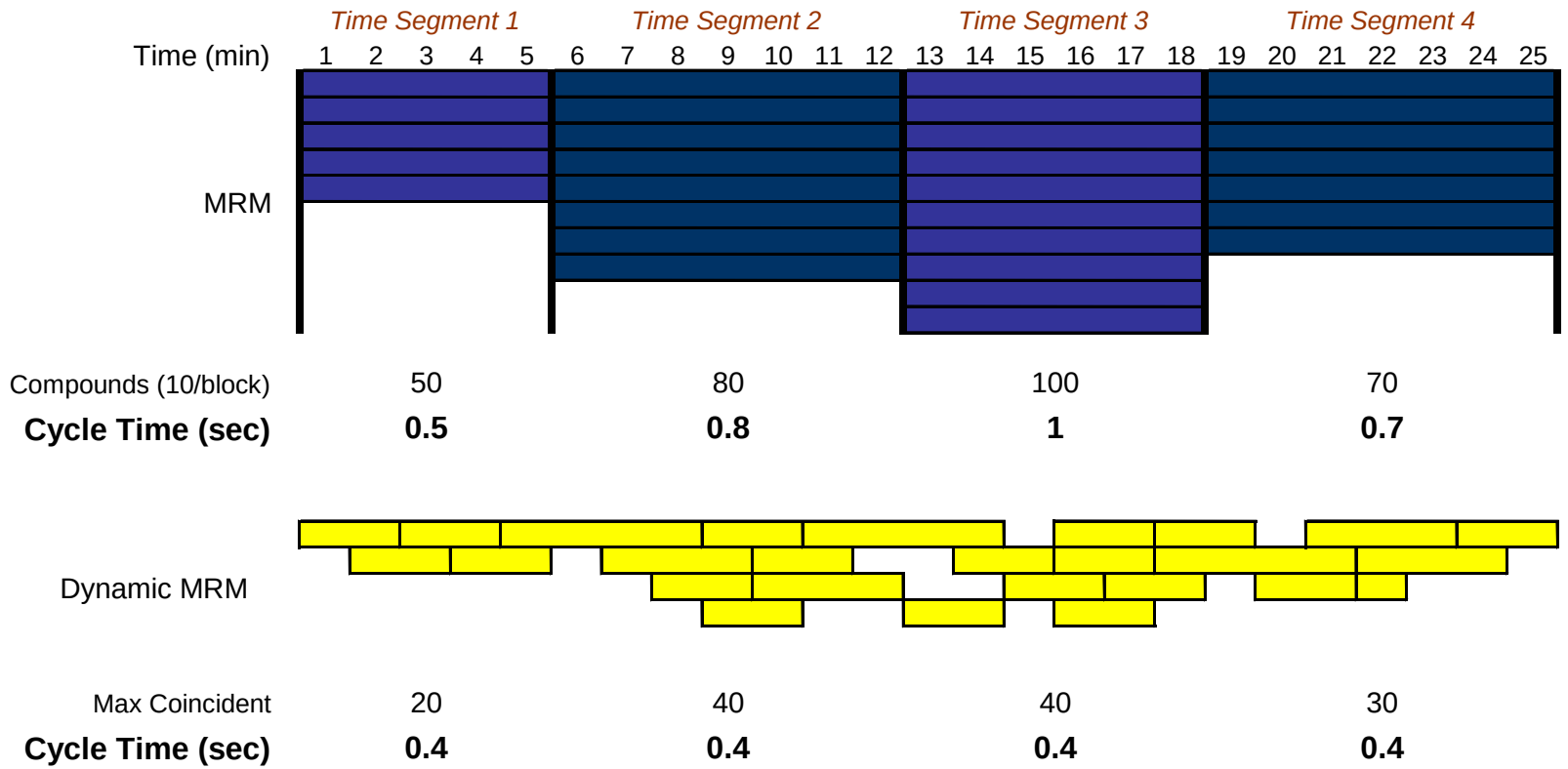
WITHOUT Optimizer:

- Manual optimization for multiple compounds
  => WEEKS of tedious interactive work!

WITH Optimizer:

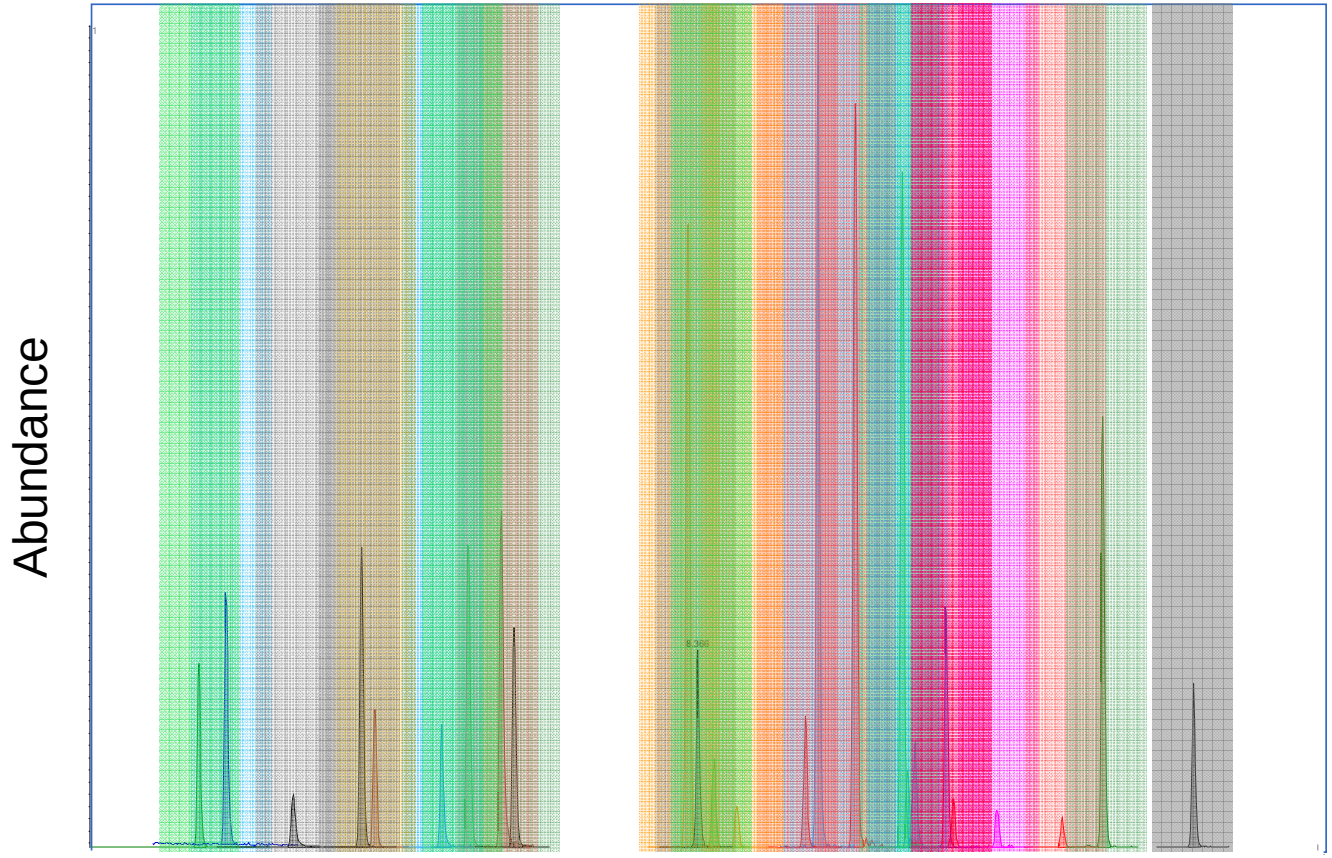
- Optimization can be fully automated
  => One or two days unattended work!

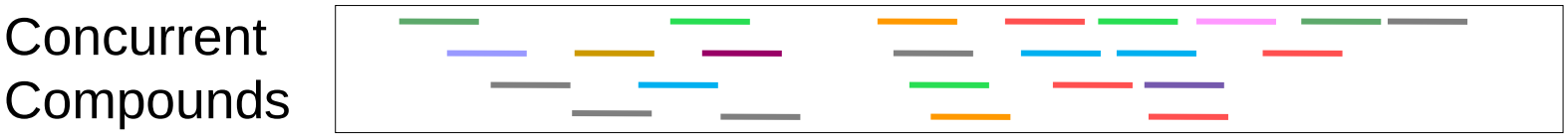
MassHunter “Dynamic MRM”
Allows 10,000 MRMs per analysis!



Cycle time remains constant maintaining equal number of data points across all peaks – dwell time is automatically optimised to achieve this

Dynamic MRM Simulation

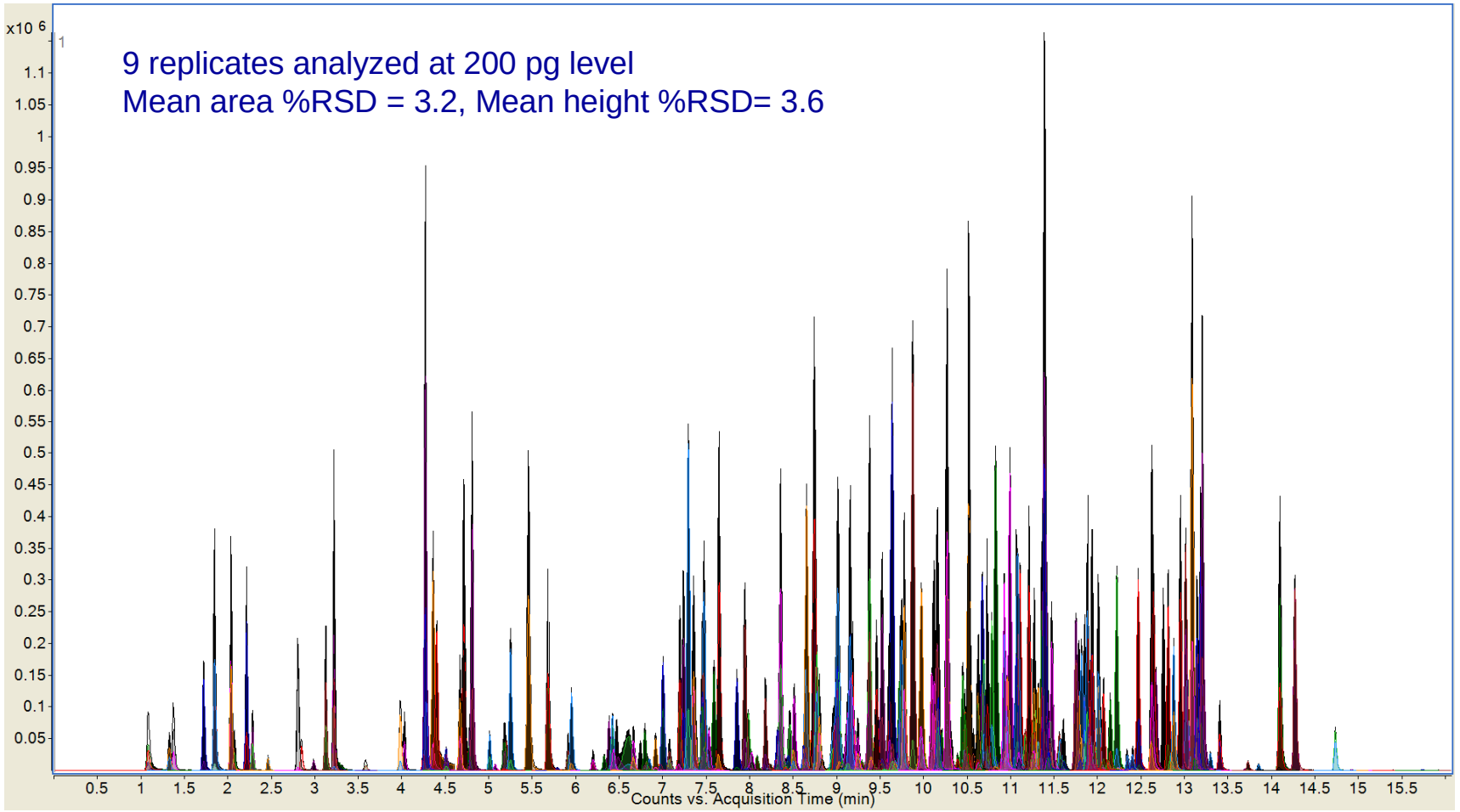




Dwell time of each compound optimised depending on number of eluting compounds so total cycle time is constant

Analysis of 300 Compounds in 15 min.

Agilent 1290 Infinity LC + 6460A Triple Quad with Agilent Jet Stream Technology
~600 Dynamic MRM Transitions



iFunnel technology in mass spectrometry

- new, more efficient ion transport
- increase in sensitivity
- simplifies sample prep
- reduction of the noise
- compact design

6490 Triple Quadrupole LC/MS System with iFunnel Technology

Qualitative MS and MS/MS instruments
TOFs & Q-TOFs

Benefits of TOF Technology Applied to Non-Targeted Profiling Applications

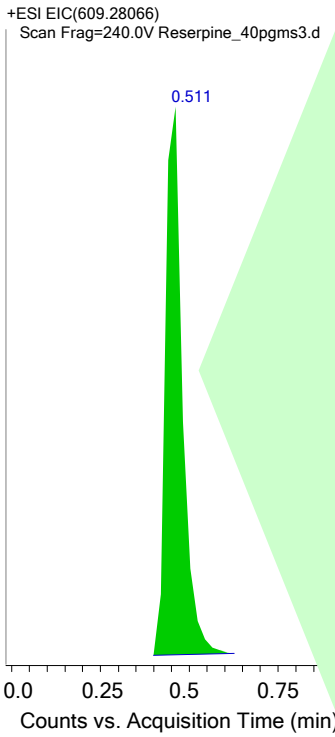
- Full Spectral Acquisition
  - All the Data – All the time: Find and identify unexpected compounds
  - Accurate Mass Database Searching and Reporting (PCD)
- Automatic TOF Instrument Set-up
  - Full Auto-tune facility for ease of use across complete mass range
  - No instrument or compound optimisation required – minimal method development
- Fast, Sensitive, Full Scan Data Acquisition
  - Up to 40 Hz data acquisition rate with no loss of resolution or mass accuracy
  - Allows ultra high resolution chromatographic separations
  - Picogram sensitivity

Keys to Success for Profiling Applications

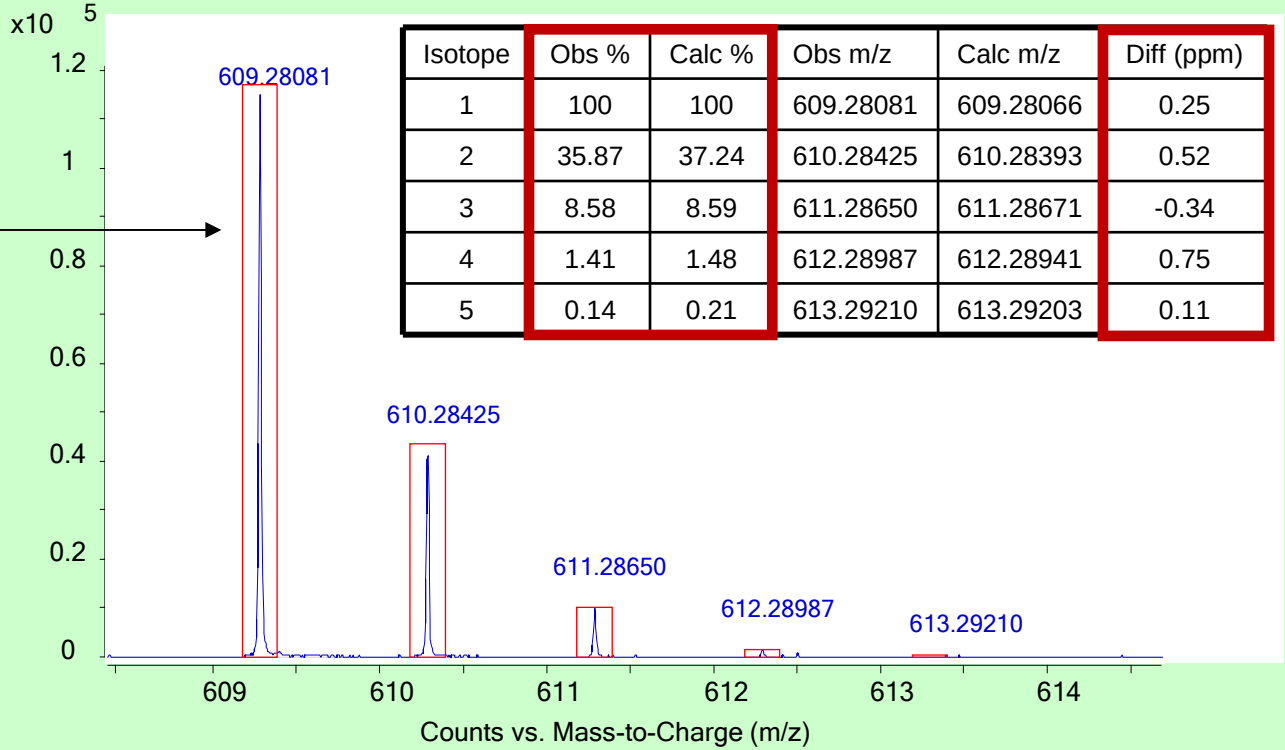
- Sensitivity
  - Low picogram
- Resolution
  - Chromatographic resolution (separation in time)
  - Spectral resolution (m/z separation)
- Dynamic Range
  - Important in identifying major and minor components in the same sample
- Accuracy
  - Chromatographic accuracy (retention time stability)
  - Spectral accuracy (MS and MSMS)
- Data Mining Tools
  - Find the compounds and identify

6540 Ultra High Definition QTOF

Typical Mass Accuracy better than 500 ppb with accurate isotope ratios



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**

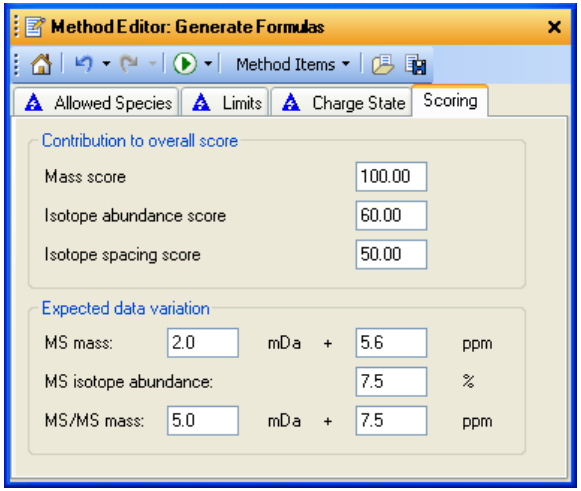


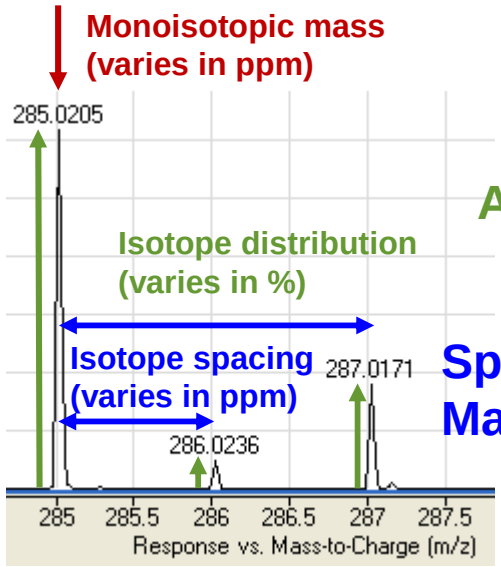
Isotope	Obs %	Calc %	Obs m/z	Calc m/z	Diff (ppm)
1	100	100	609.28081	609.28066	0.25
2	35.87	37.24	610.28425	610.28393	0.52
3	8.58	8.59	611.28650	611.28671	-0.34
4	1.41	1.48	612.28987	612.28941	0.75
5	0.14	0.21	613.29210	613.29203	0.11

Mass accuracy of each isotope and isotope ratios are used for determination of Molecular Formula

Molecular Formula Generation (MFG) Algorithm
Use All Available Information

Scoring based on





Mass Match +

Abund. Match +

Spacing Match =

Overall Score

MS Formula Results: Cpd 2: C10H9ClN4O2S

m/z	Ion	Formula	Abundance
285.021	(M+H)+	C10 H10 N4 O2 S	24506.1

Best	Formula (M)	Calc m/z	Score	Cross Score	Mass	Calc Mass	Diff (ppm)	Abs Diff (p	Spacing Matc	Abund Matc	Mass Match	m/z	DBE
☑	C10 H9 Cl N4 O2 S	285.0208	99.55		284.0137	284.0135	-0.71	0.71	99.19	99.26	99.69	285.021	8
☐	C7 H12 N2 O6 S2	285.021	77.28		284.0137	284.0137	0.01	0.01	99.54	1.93	100	285.021	3
☐	C7 H13 Cl N4 O2 S2	285.0241	75.57		284.0137	284.0168	11.12	11.12	99.87	83.87	46.22	285.021	3

Without use of isotope information, the first hit would have been incorrect!

Wide Dynamic Range:
Co-eluting Metabolites with Parent Drug

Five decades of response in a single scan

verapamil

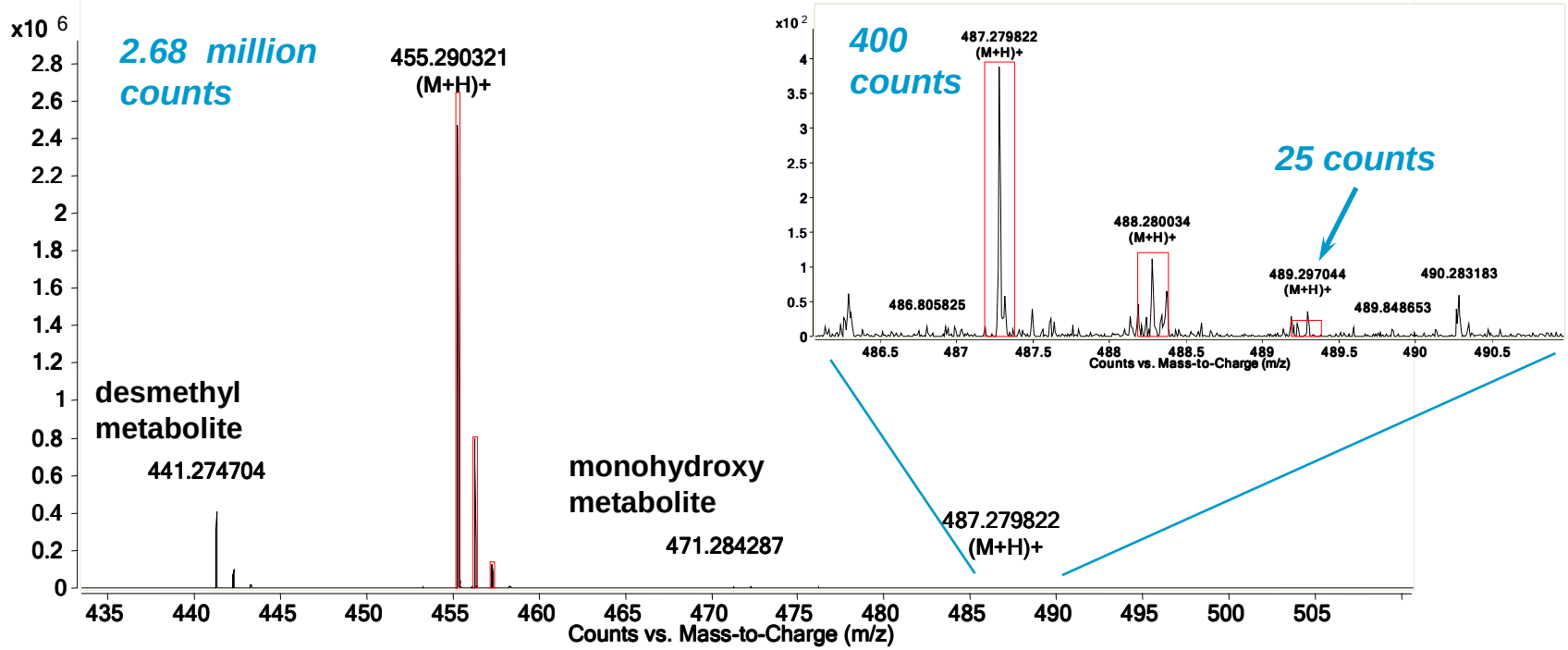
Best	Formula (M)	Ion Formula	Score
✓	C27 H38 N2 O4	C27 H39 N2 O4	99.43

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
✓	C27 H38 N2 O6	C27 H39 N2 O6	80.18

Calc m/z	Diff (ppm)	Mass Match	Abund Match	Spacing Match
487.280263	1.03	98.88	84.39	37.71



Metabolite Identification Schemes

ID compounds of interest

- Fiehn GC/MS Metabolomics Library
- METLIN AMRT Database (LC/MS)
- Molecular Formula Generation (MFG)
- Accurate mass MS/MS confirmation
- METLIN MS/MS library

ID of “true unknown”

- Acquire targeted MS/MS on organic synthesized compounds (based on hypothesized structure)
- Create spectral library
- Isolate compound and do NMR (needs time, cost and money)

METLIN Personal Database

Metabolite-specific database for LCMS metabolomics research

- Developed with Scripps Institute
- Contains ~23,000 compounds
  - ~8000 lipids from LipidMaps
- Manual and batch searches
  - Query based on Empirical formula or accurate mass & retention time
- Customizable
  - Add compounds
  - Assign chromatographic retention times to metabolites
  - Create subset databases
  - Add MS/MS spectra