Confident Quantitation

Any compound, any matrix, any user.

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Ensuring Customer Success

Thermo Scientific™ TSQ Altis™ Triple Quadrupole MS and Thermo Scientific™ TSQ Quantis™ Triple Quadrupole MS

Introduction to Thermo Scientific TSQ Altis MS and TSQ Quantis MS

Performance: Sensitivity, Selectivity (H-SRM)

TSQ Altis MS

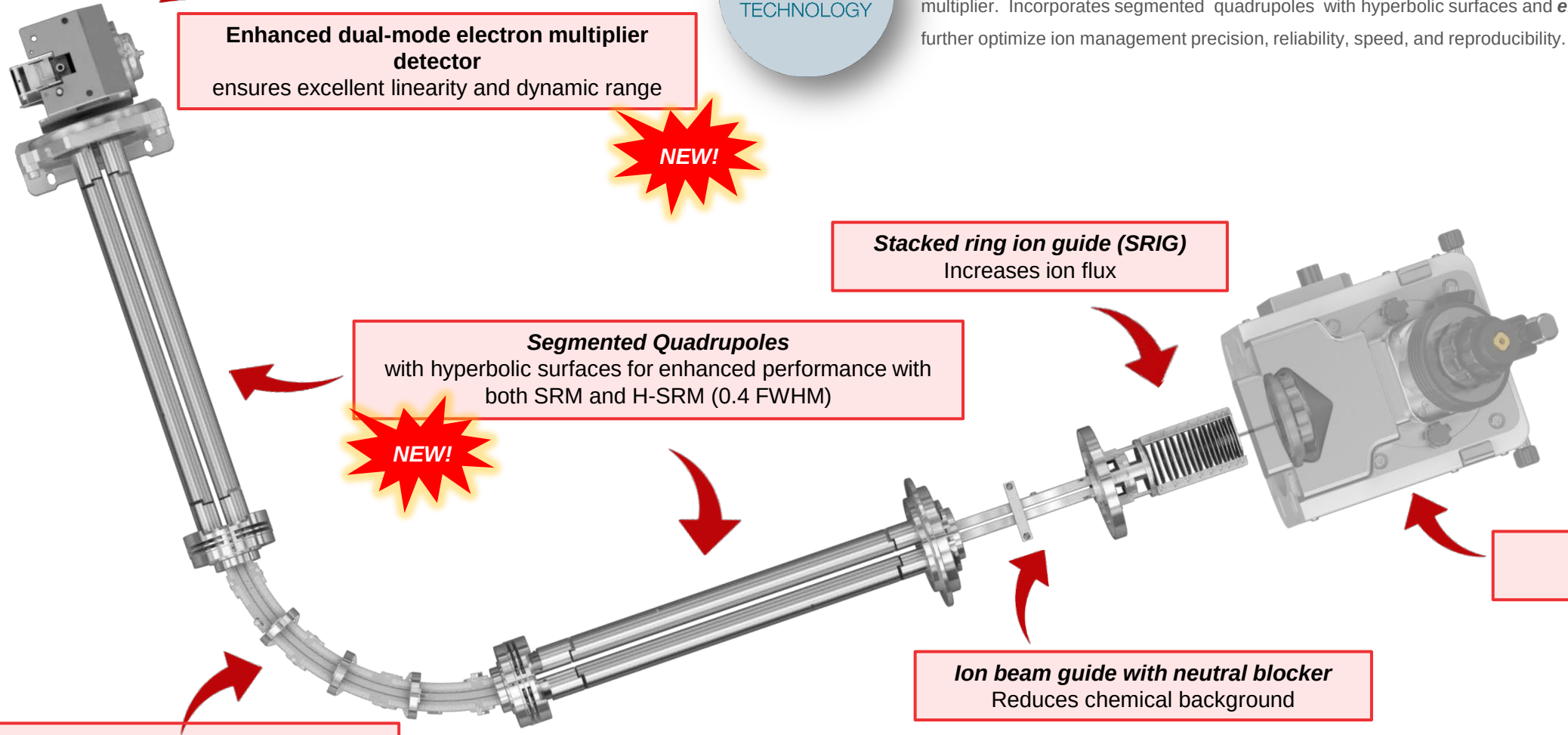
TSQ Quantis MS

	TSQ Altis MS *High-end*	TSQ Quantis MS *Mid-tier*
Mass Range	5-2000	5-3000
SRM/sec	600	600
Selectivity (H-SRM)	0.2 Da FWHM	0.4 Da FWHM
Sensitivity (HESI Reserpine 1 pg)	500,000:1	150,000:1
Targeted Market	Omics, Research, Pharma/Biopharma, Clinical Research and Forensic Toxicology	Environmental and Food Safety, Clinical Research, and Forensic Toxicology

Robustness, Reproducibility, Speed, Ease-of-Use, Flexibility

Thermo Scientific TSQ Quantis MS: Unprecedented Robustness, Day After Day

Active Ion Management Plus (*AIM+*) - The next step in precision design delivers the ultimate in ion management, inception to detection, from the OptaMax™ ion source housing to the enhanced electron multiplier. Incorporates segmented quadrupoles with hyperbolic surfaces and ***enhanced RF Electronics*** to further optimize ion management precision, reliability, speed, and reproducibility.



Enhanced dual-mode electron multiplier detector
ensures excellent linearity and dynamic range

NEW!

Stacked ring ion guide (SRIG)
Increases ion flux

Segmented Quadrupoles
with hyperbolic surfaces for enhanced performance with both SRM and H-SRM (0.4 FWHM)

NEW!

OptaMax™ NG
APCI ready

NEW!

Ion beam guide with neutral blocker
Reduces chemical background

Active collision cell with axial DC field
facilitates more SRMs/sec

Thermo Scientific TSQ Altis MS: Sensitivity with Robustness, No Compromises

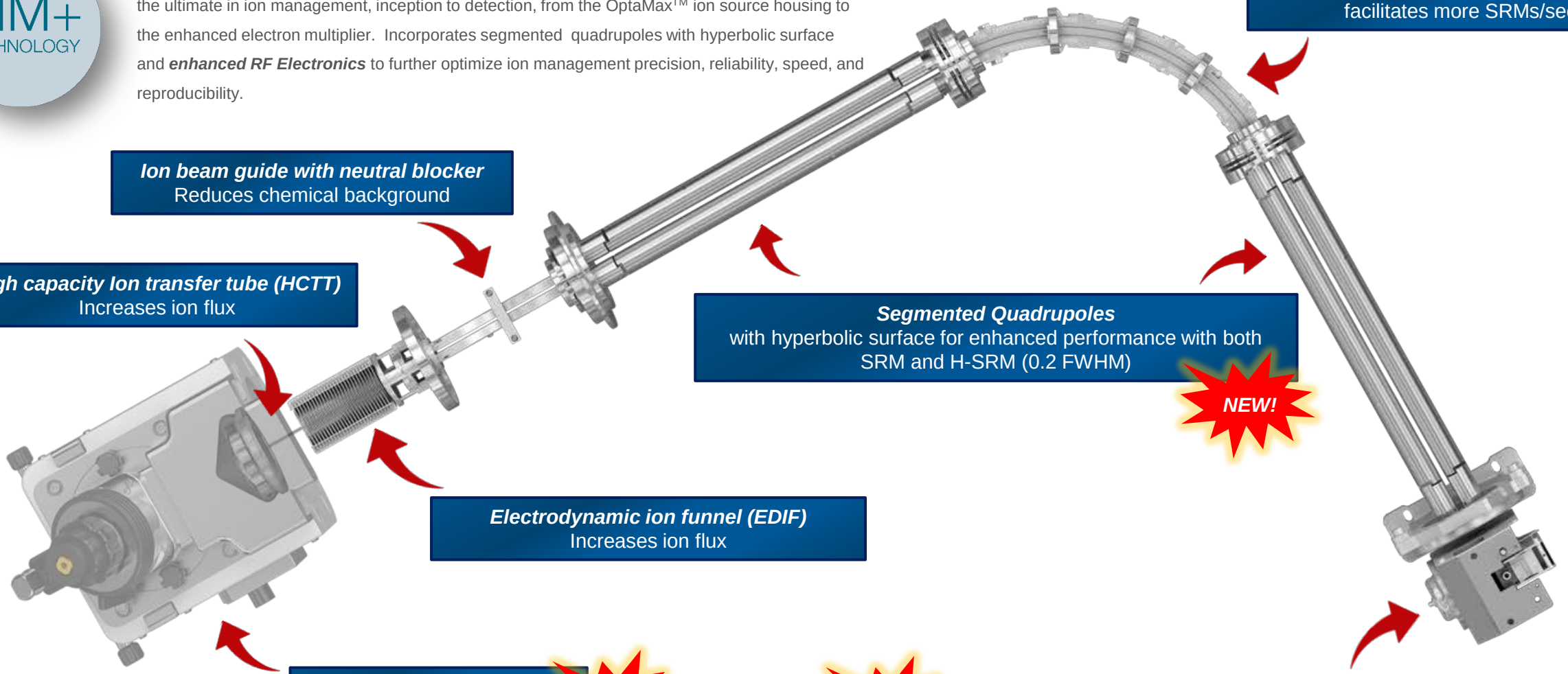
AIM+ TECHNOLOGY

Active Ion Management Plus (*AIM+*) - The next step in ***precision design*** delivers the ultimate in ion management, inception to detection, from the OptaMax™ ion source housing to the enhanced electron multiplier. Incorporates segmented quadrupoles with hyperbolic surface and ***enhanced RF Electronics*** to further optimize ion management precision, reliability, speed, and reproducibility.

Active collision cell with axial DC field
facilitates more SRMs/sec

Ion beam guide with neutral blocker
Reduces chemical background

High capacity Ion transfer tube (HCTT)
Increases ion flux



Segmented Quadrupoles
with hyperbolic surface for enhanced performance with both SRM and H-SRM (0.2 FWHM)

NEW!

Electrodynamic ion funnel (EDIF)
Increases ion flux

OptaMax™ NG
APCI ready

NEW!

NEW!

Enhanced dual-mode electron multiplier detector
Ensures excellent linearity and dynamic range

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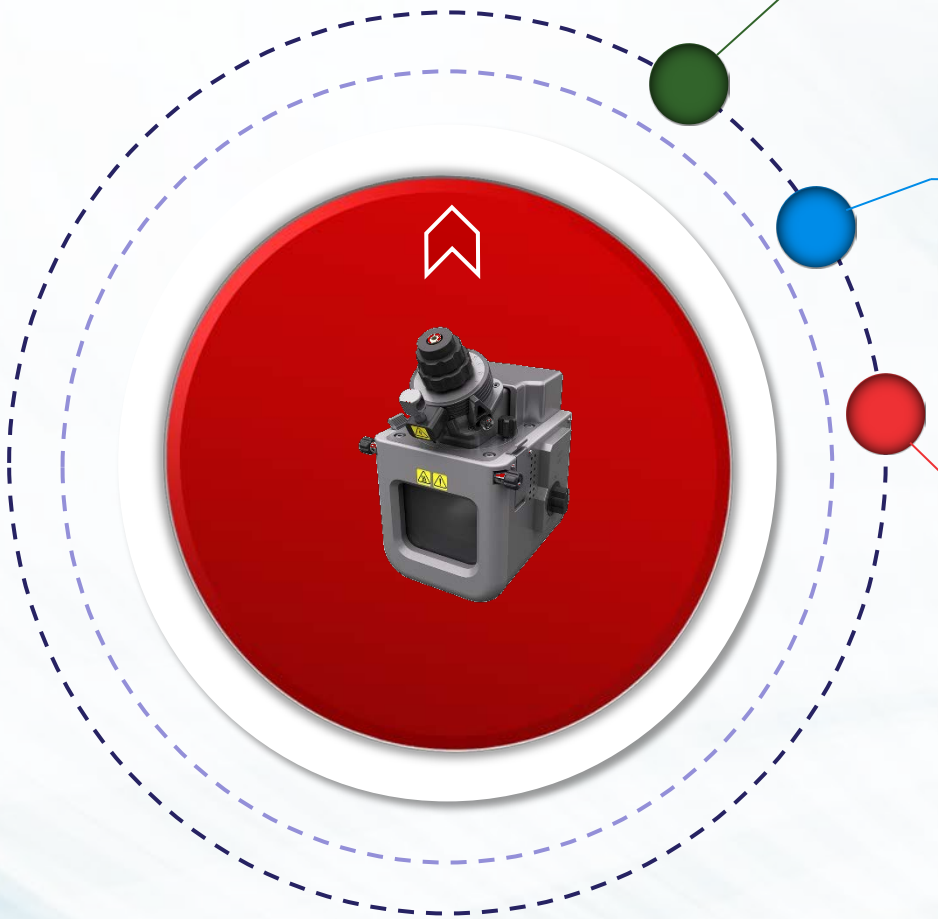
OptaMax NG Source Housing

Benefits: Reliable and consistent performance with improved usability!



Re-designed APCI discharge assembly

- Built-in to every source (separate APCI sprayer required for APCI mode)
- Re-designed on/off switch (to improve usability)

Re-designed HESI Sprayer

- Needle adjustment is “set and forget”
- Tool available to help the user to correctly set needle protrusion

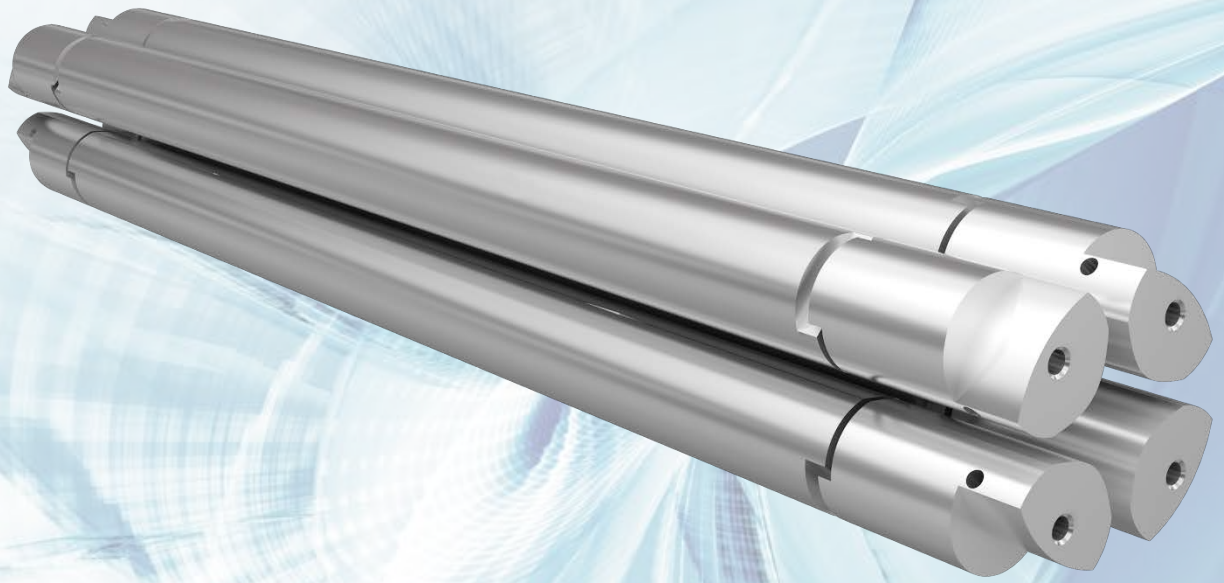
Usability and Consistency

- Vertical adjustment moved to the side for easier access
- Improved sprayer alignment and stability
- New finer threads on HESI and APCI sprayers to make installation easier

Segmented Quadrupoles

Benefits: Increased Sensitivity (more significant at higher mass range)
Flat tuning for consistent and robust performance

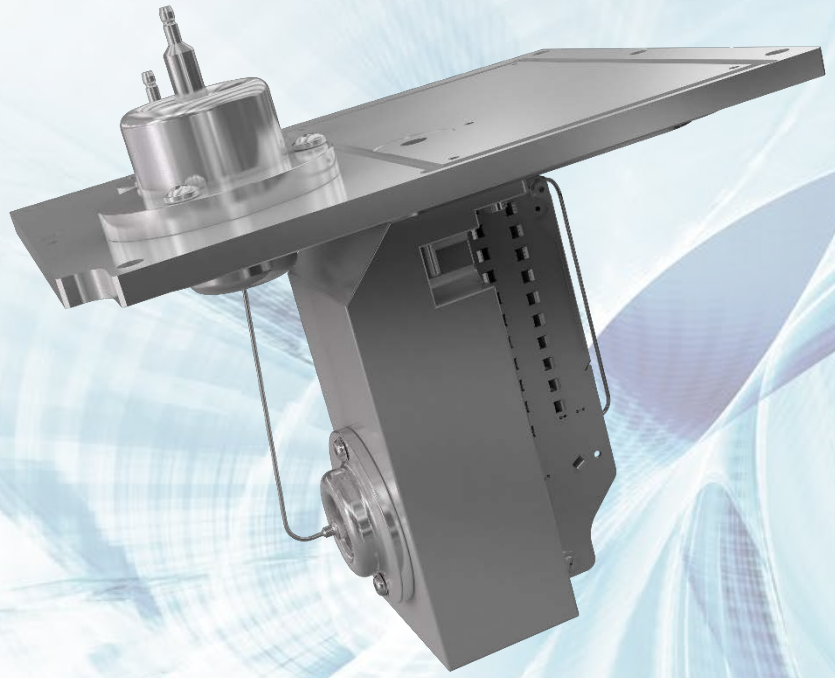
- The use of RF only pre-filters (segments) between the entrance lens and the quadrupole minimizes the effects of fringe fields, leading to improved transmission (and therefore sensitivity) at unit and higher resolution.
- Hyperbolic surface enhances peak shape, increasing transmission at higher resolutions and masses.
- With the RF only pre-filter, the tuning of several lenses is flat across mass range allowing the voltage to be set and not tuned. This helps reducing the complexity of the tune and making the systems more consistent.



Detector

Benefits: Increased electron multiplier lifetime. Increased Uptime!

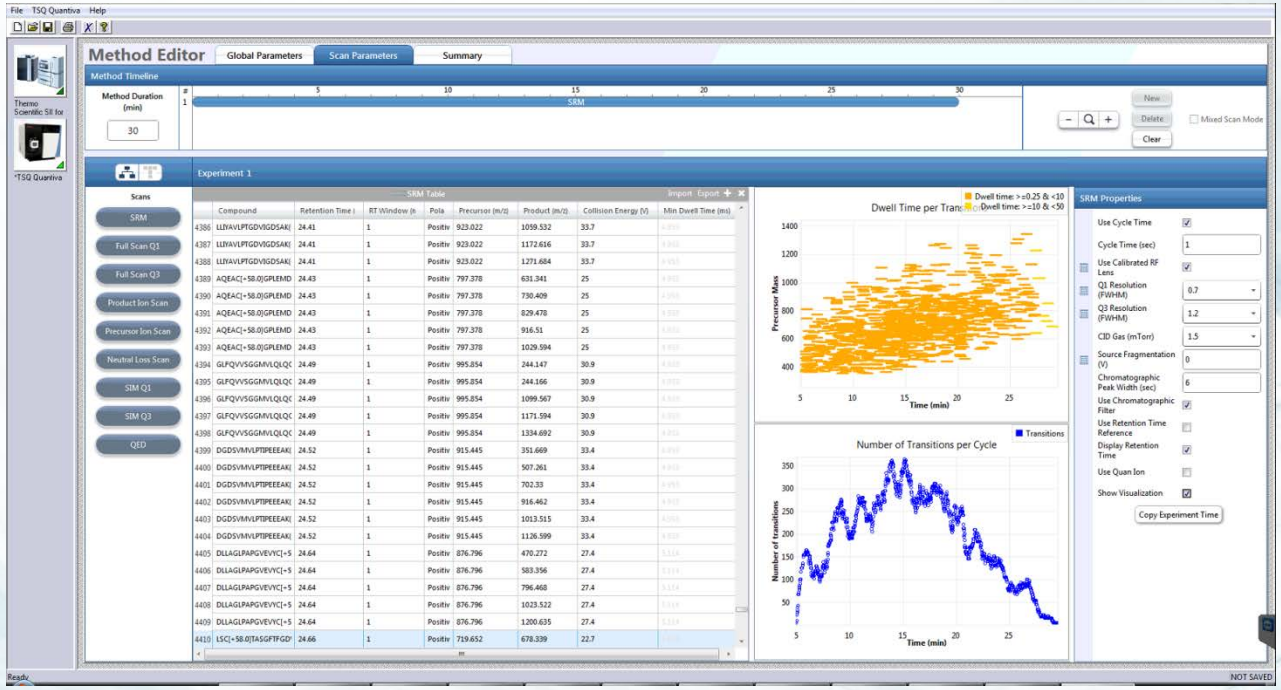
- Increased number of dynodes (21) for extended lifetime.
- Improved electron multiplier calibration routine.
- Reduced number of service visits leading to more uptime.
- Maintaining excellent linearity and dynamic range



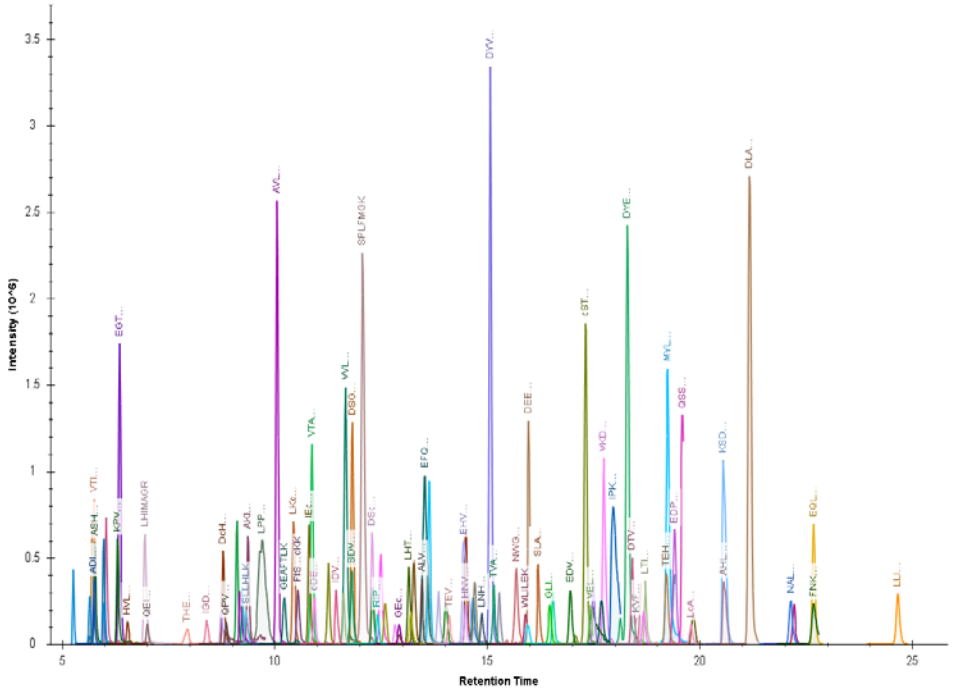
RF Circuitry

Benefits: More compounds in the same run or longer dwells on existing method

- New main RF/DC electronics
- Analyze more compounds in the same time window or better Quantitation results with better ion statistics (more scans across your chromatographic peak)
- Up to **600 SRM/sec**



965 peptides, 4762 transitions, 1 min RT window,
35 minute method



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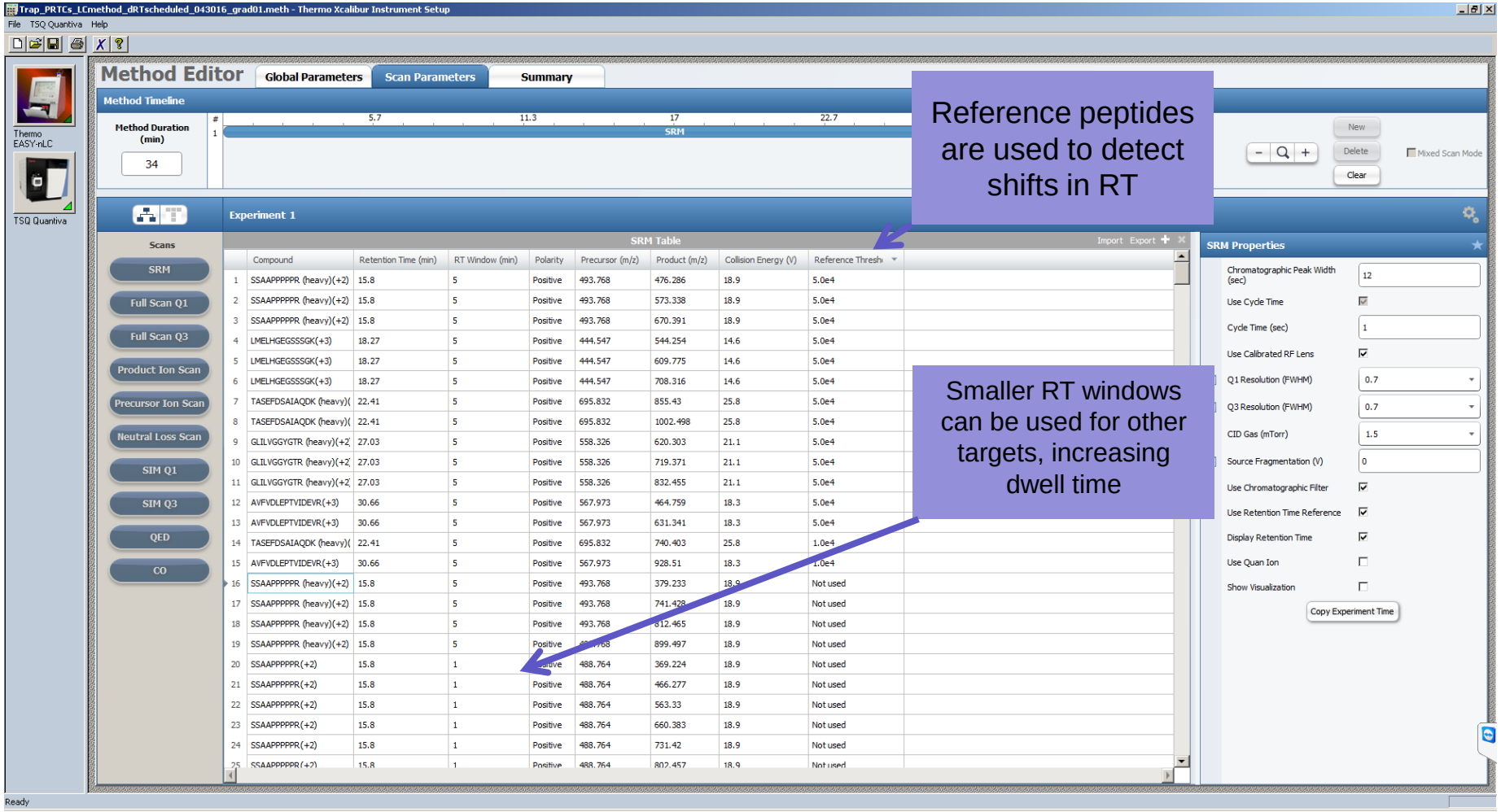
What do Thermo Scientific™ QqQs have to make them easier to use?

- Dynamic Retention Time Adjustment: on-the-fly RT window shifts to accommodate targeted workflows



- Result: More peaks detected without being cut-off or missed; less manual method updating

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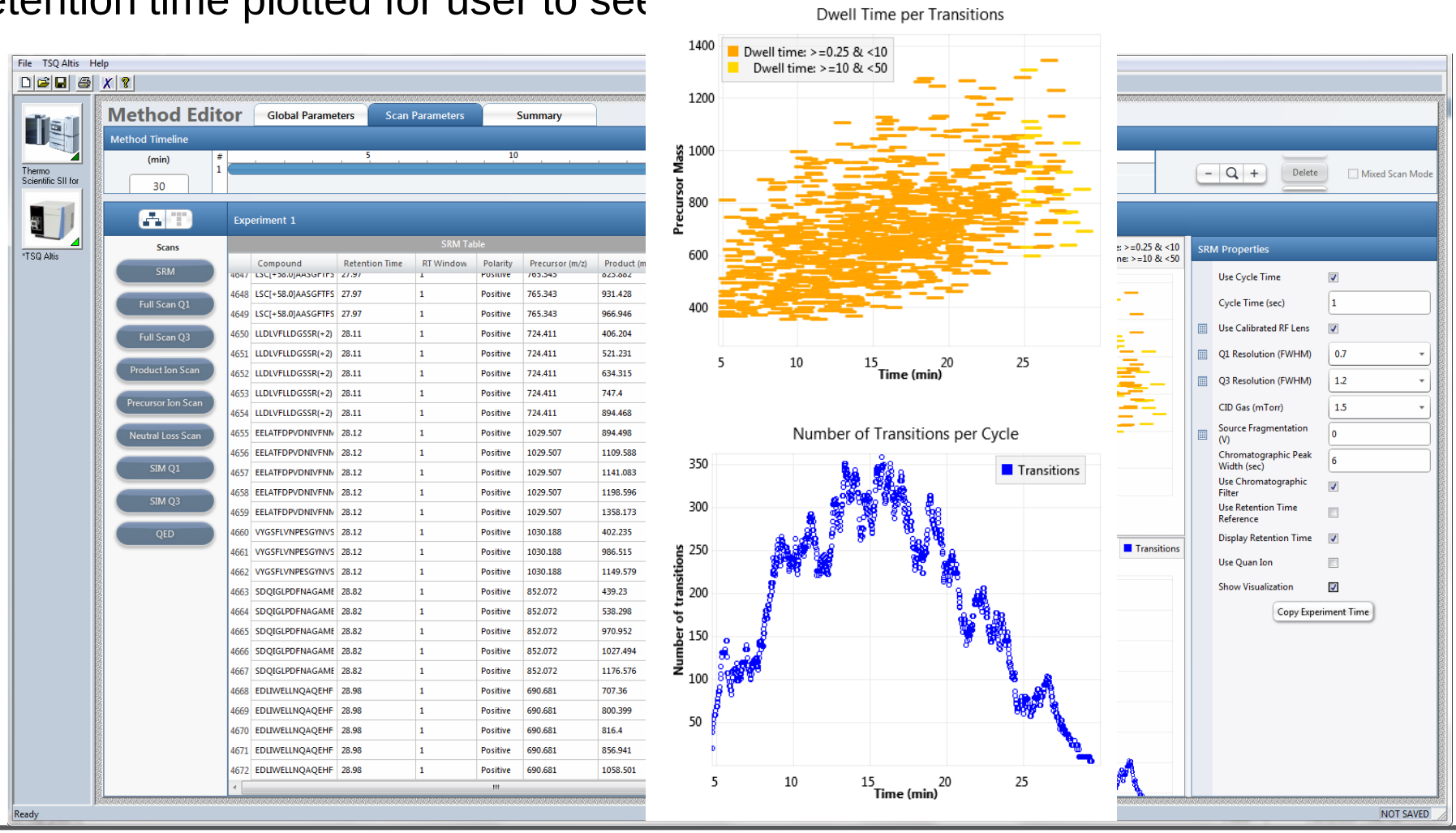
Interactive SRM Visualization

- Minimum dwell times shown, so adjustments can be made before data are generated!
- Number of transitions vs retention time plotted for user to see
- BONUS:
  - It is interactive!!

Take home message:

- Visualization allows the user to adjust the method BEFORE it is run, resulting in better data!
- Dwell times are shown, before poor-quality data can be generated!



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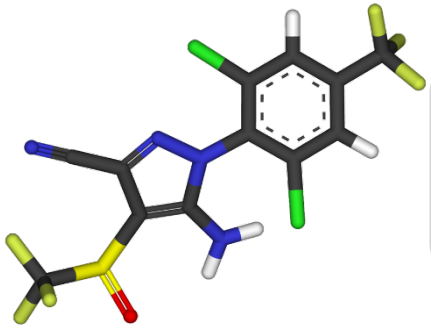
Any compound, any matrix, any user.

Robust
Consistent
Reliable

Fipronil in Eggs

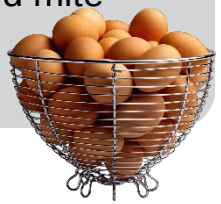


What is Fipronil?

Fipronil is an insecticide commonly used against fleas, ticks, cockroaches, ants, termites, etc. It kills insects by disrupting their CNS and is used in various insect-control products for both agricultural and domestic use

Why in Eggs?

Fipronil was mixed with Dega-16, used for red mite treatment commonly found in poultry farms.



Should I be worried?

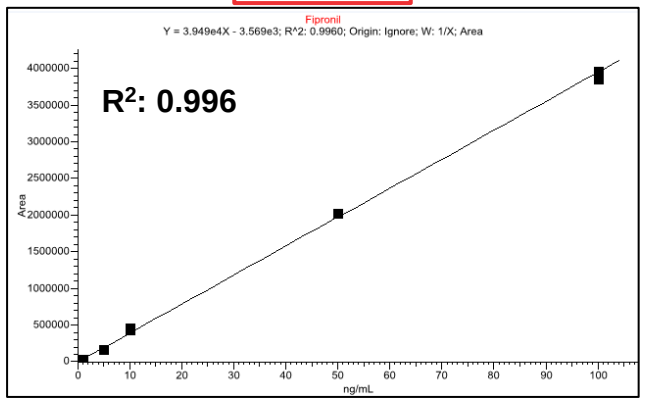
Ingestion of large amounts can lead to kidney, liver, and thyroid damage. The amounts typically present in eggs are very low, however, it is a huge safety concern.

What's Needed?

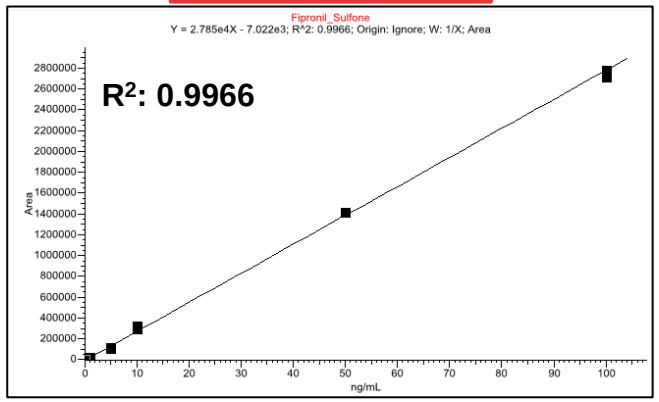
Robust, sensitive, reliable analysis and quantitation of Fipronil and Fipronil Sulfone in eggs

Matrix Matched Calibration for UHPLC-MS/MS on a Thermo Scientific TSQ Quantis MS

Fipronil



Fipronil sulfone



Compound name	Recovery (%) 0.5 µg/kg spike level	Recovery (%) 1 µg/kg spike level	Recovery (%) 5 µg/kg spike level
Fipronil (434.9> 322.9)	104	89	99
Fipronil sulfone (450.9> 415.0)	99	95	102

Compound name	LOD [µg/kg]	LOQ [µg/kg]	Repeatability (%) 0.5 µg/kg spike level	Repeatability (%) 5 µg/kg spike level
Fipronil (434.9> 322.9)	0.1	0.5	8.5	6.1
Fipronil sulfone (450.9> 415.0)	0.1	0.5	7.7	6.4

Ion Ratio Calculations	Fipronil (434.9> 249.9)	Fipronil (434.9> 398.84)	Fipronil sulfone (450.9> 282.00)	Fipronil sulfone (450.9> 243.84)
Ion ratio* (%) Standard 100 ng/ml	24.4	13.1	78.1	23.6
Ion ratio* (%) Spike 0.5 µg/kg	26.0	14.8	83.4	29.4
Ion ratio repeatability (RSD %)* 0.5 µg/kg spike level	9.1	16.3	4.4	18.9
Ion ratio repeatability* (RSD %) 5 µg/kg spike level	4.4	4.5	1.8	5.8

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Reproducibility and Long-term Stability Test

Rapid analysis of fipronil and fipronil sulfone in eggs by liquid chromatography and triple quadrupole mass spectrometry

Authors
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Thermo Fisher Scientific, Special Solutions Center, Dreieich, Germany

Keywords
Fipronil, fipronil sulfone, eggs, LC-MS, Accucore aQ, TSQ Quantis, UltiMate 3000 RSLC

Goal

Develop a quick and simple method for the determination of fipronil and fipronil sulfone in eggs using an in-house modified QuEChERS acetonitrile extraction protocol and LC-MS/MS determination.

Introduction

Recently, it was reported that millions of eggs contaminated with the insecticide fipronil have been distributed to more than 17 countries.[1] On July 20th 2017, it was made public that in some cases the pesticide fipronil was mixed with another formulation and sprayed on chickens against ticks, fleas and lice.[1] As the determined levels were in some cases substantially higher (up to 1.2 mg/kg) than the EU MRL of 0.005 mg/kg for the sum of fipronil and fipronil sulfone,[2,3] there is a demand for quick and efficient methods for the determination of both substances in egg matrix and potentially in chicken meat.

This brief presents a quick and simple method for the determination of fipronil and fipronil sulfone in eggs using an in-house modified QuEChERS acetonitrile extraction protocol.

Experimental

Sample preparation

Egg samples purchased in a local store were extracted using the procedure described in Figure 1.

Sample preparation consumables

- 50 mL conical sterile polypropylene centrifuge tubes, P/N 339652
- 15 mL conical sterile polypropylene centrifuge tubes; P/N 339650
- Thermo Scientific™ HyperSep™ dispersive SPE Mylar pouch 4000 mg magnesium sulfate and 1000 mg NaCl, 50 pk, P/N 60105-340
- Magnesium sulfate, 99%, for analysis, anhydrous; 12196721

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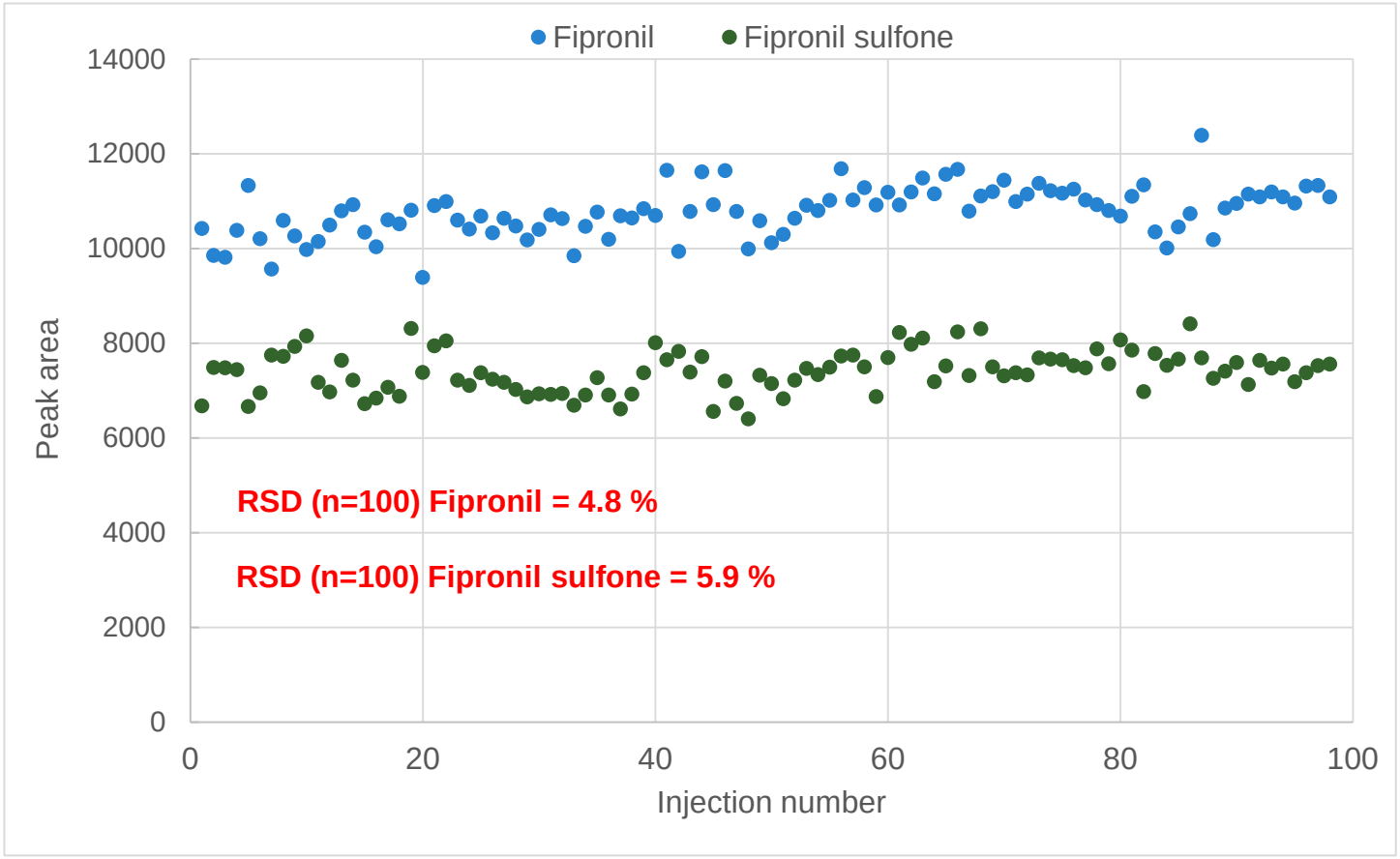

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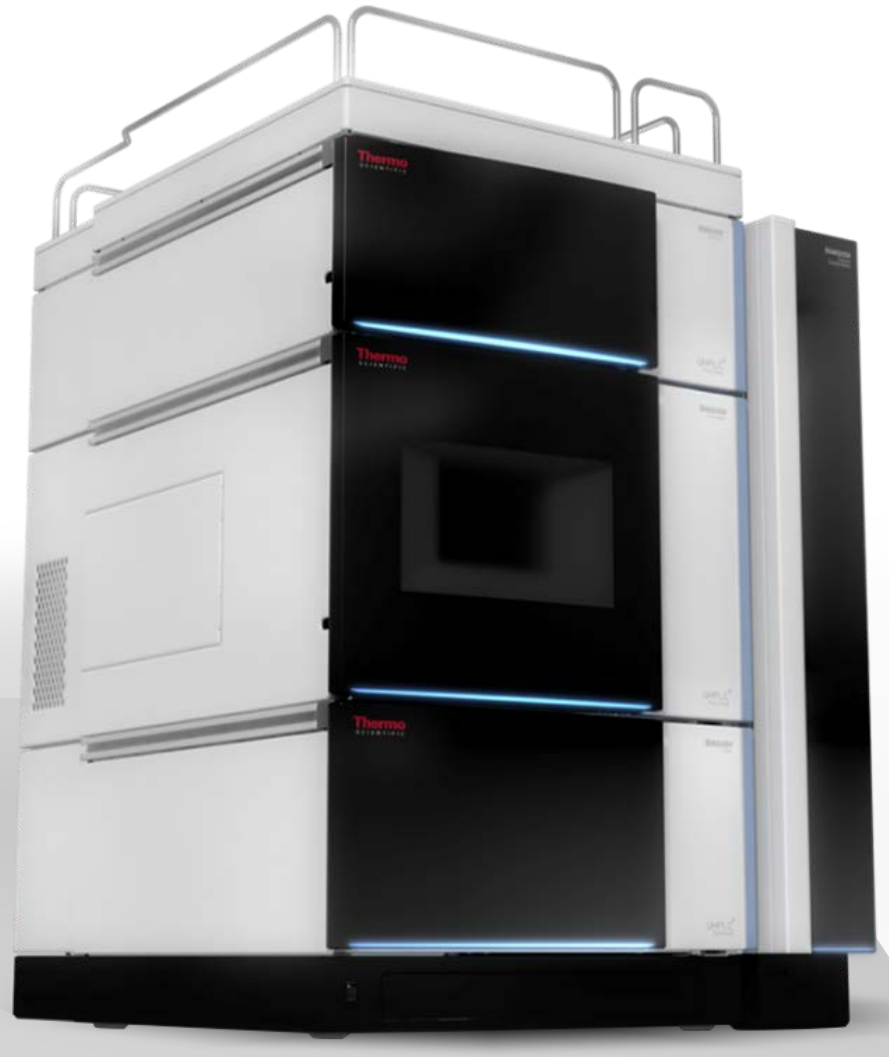
LC-MS/MS Quantitation of ~100 Drugs of Abuse in Urine in Under Two Minutes on the Thermo Scientific™ TSQ Quantis™ MS

Why High-throughput LC-MS/MS for Drugs of Abuse Analyses?

- Large number of samples
  - Need to reduce analysis times
- Reduce false positives (immunoassays)
  - LC-MS/MS has high selectivity
- Reduce costs
  - Multi-class drugs of abuse require multiple immunoassays
  - Still may require LC-MS/MS confirmation
- Thermo Scientific™ Vanquish™ Horizon UHPLC and TSQ Quantis™ MS can meet these requirements

Thermo Scientific Vanquish Horizon UHPLC



Vanquish Horizon UHPLC Platform:

- Maximize UHPLC separations with 1500 bar (22,000 psi) pump pressure limit
- Unmatched retention time performance via parallel dual piston principle
- Ultra-low Gradient Dead Volume (35 uL) for faster separations
- Viper-based, tool-free fluidic connections
- Biocompatible, iron-free flow path
- Sample pre-compression for better injection reproducibility and longer column lifetimes
- Standard AS capacity: 4 racks (216 vials); expandable Charger module for up to 20 well plates)
- New column thermostatting technology
- Removable doors for easy access

Experimental Design – Liquid Chromatography

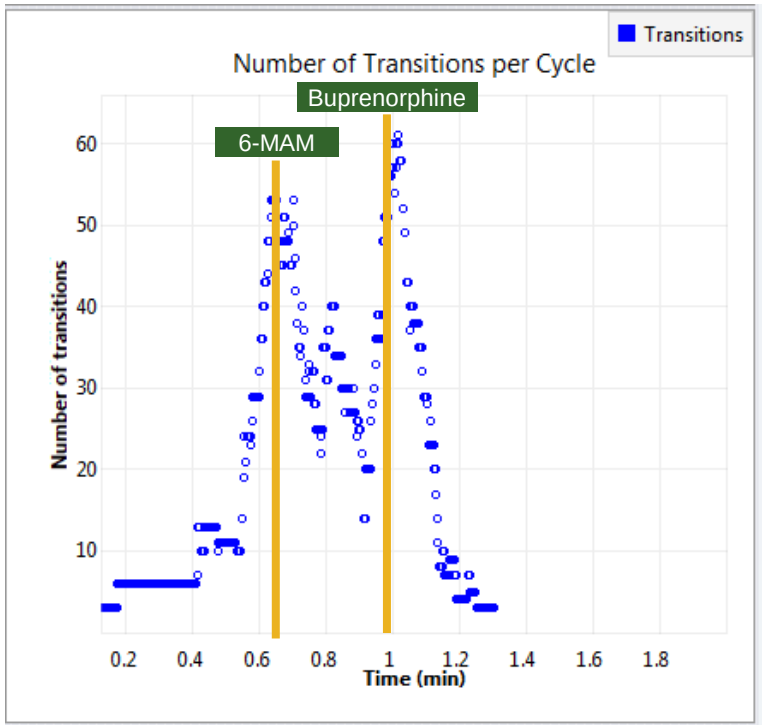
- Thermo Scientific™ Vanquish™ Horizon UHPLC
  - Column: 2.1 x 50 mm, 1.9 um Hypersil Gold AQ
  - Column Temp: 40 C
  - Mobile Phase: [A] H_2O + 0.1% HCOOH; [B] ACN + 0.1% HCOOH
  - Flow Rate: 1.0 mL/min (no split)
  - Gradient: see table
  - Injection Volume: 2 uL

No	Time	Flow [ml/min]	%B	Curve
1	0.000	Run		
2	0.000	1.000	0.0	5
3	0.400	1.000	22.5	5
4	0.950	1.000	75.2	5
5	1.450	1.000	75.2	5
6	1.470	1.000	0.0	5
7	1.490	1.000	0.0	5
8	1.510	1.200	0.0	5
9	2.150	1.200	0.0	5
10	2.200	1.000	0.0	5

Note: total LC runtime is 2.2 minutes for ~100 drugs of abuse

Experimental Design – Mass Spectrometry

- Thermo Scientific™ TSQ Quantis™ MS
  - Ionization Mode: HESI, Positive ion mode
  - MS Acquisition Mode: Selective Reaction Monitoring (SRM) – see # Transitions vs. RT below
  - Cycle time: 0.15 s
  - Quad Isolation (Q1,Q3) = Unit (0.7 Da FWHM)



Note: elution of 6-MAM & Buprenorphine occur at the times of highest # SRM transitions (i.e., during lowest dwell times)

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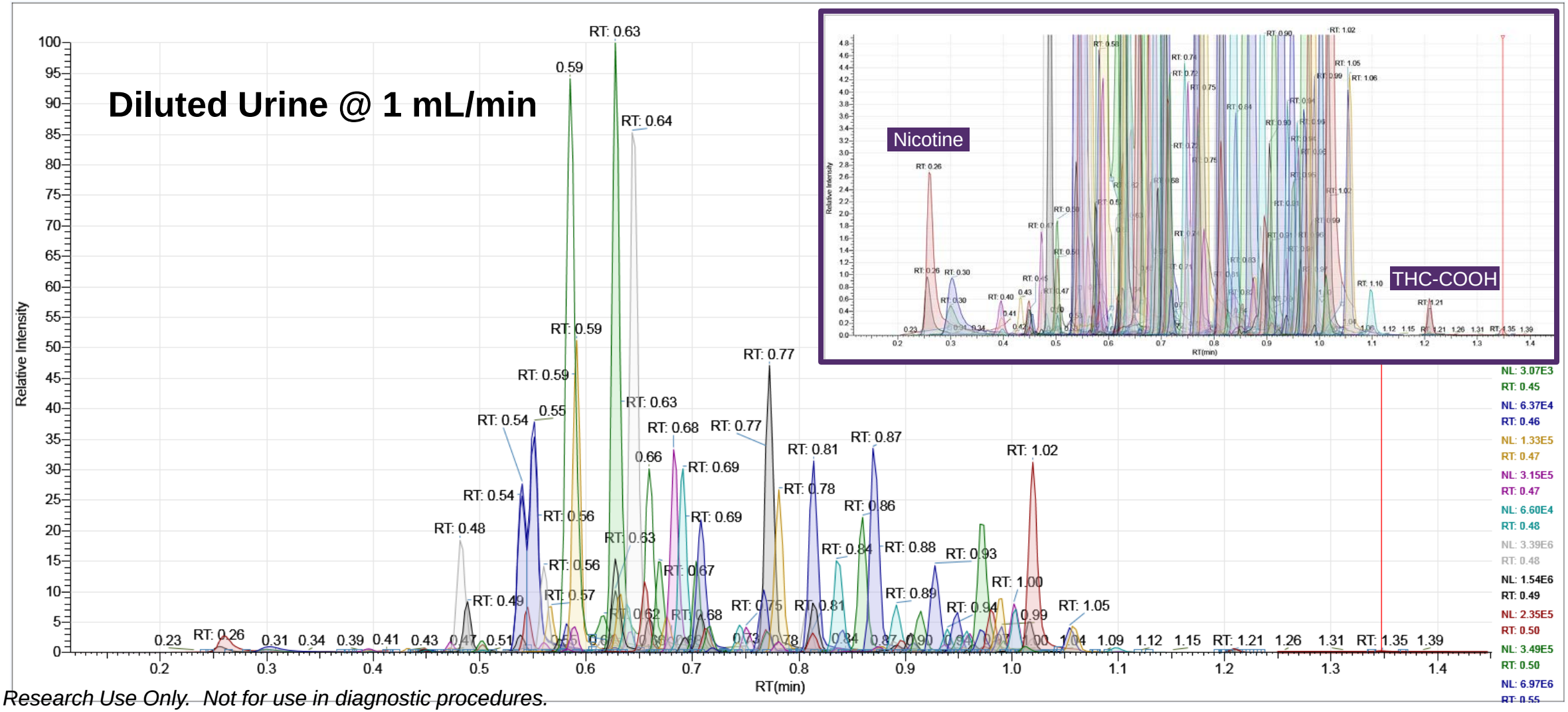
~100 Drugs of Abuse on Thermo Scientific TSQ Quantis MS



For Research Use Only. Not for use in diagnostic procedures.

SRM chromatograms of ~100 drugs of abuse in under 1.3 minutes [THC-COOH elutes at 1.21 min, inset]

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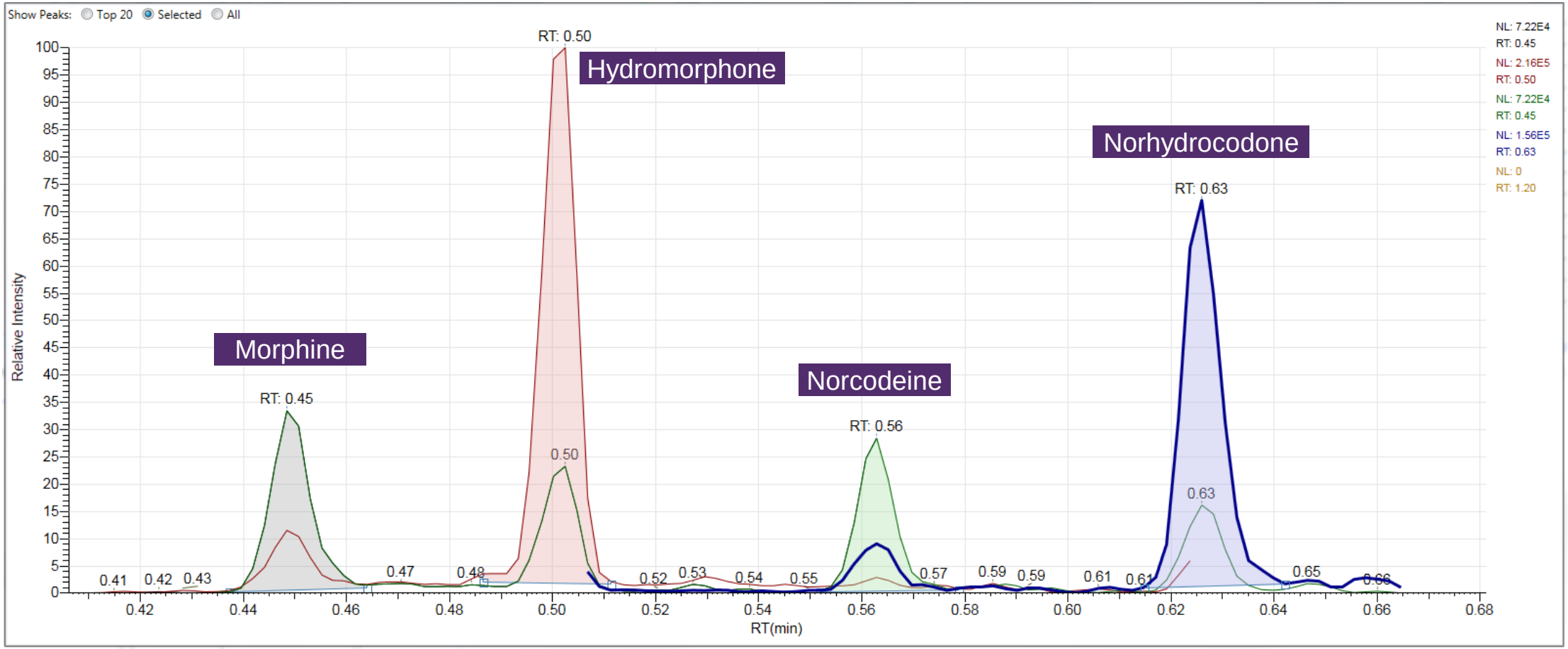
~100 Drugs of Abuse on Thermo Scientific TSQ Quantis MS

Separation of opiate isomers @ m/z 286



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Opiate isomers at m/z 286 are well separated in under 12 s [typical LC peak = 1.2 s wide]

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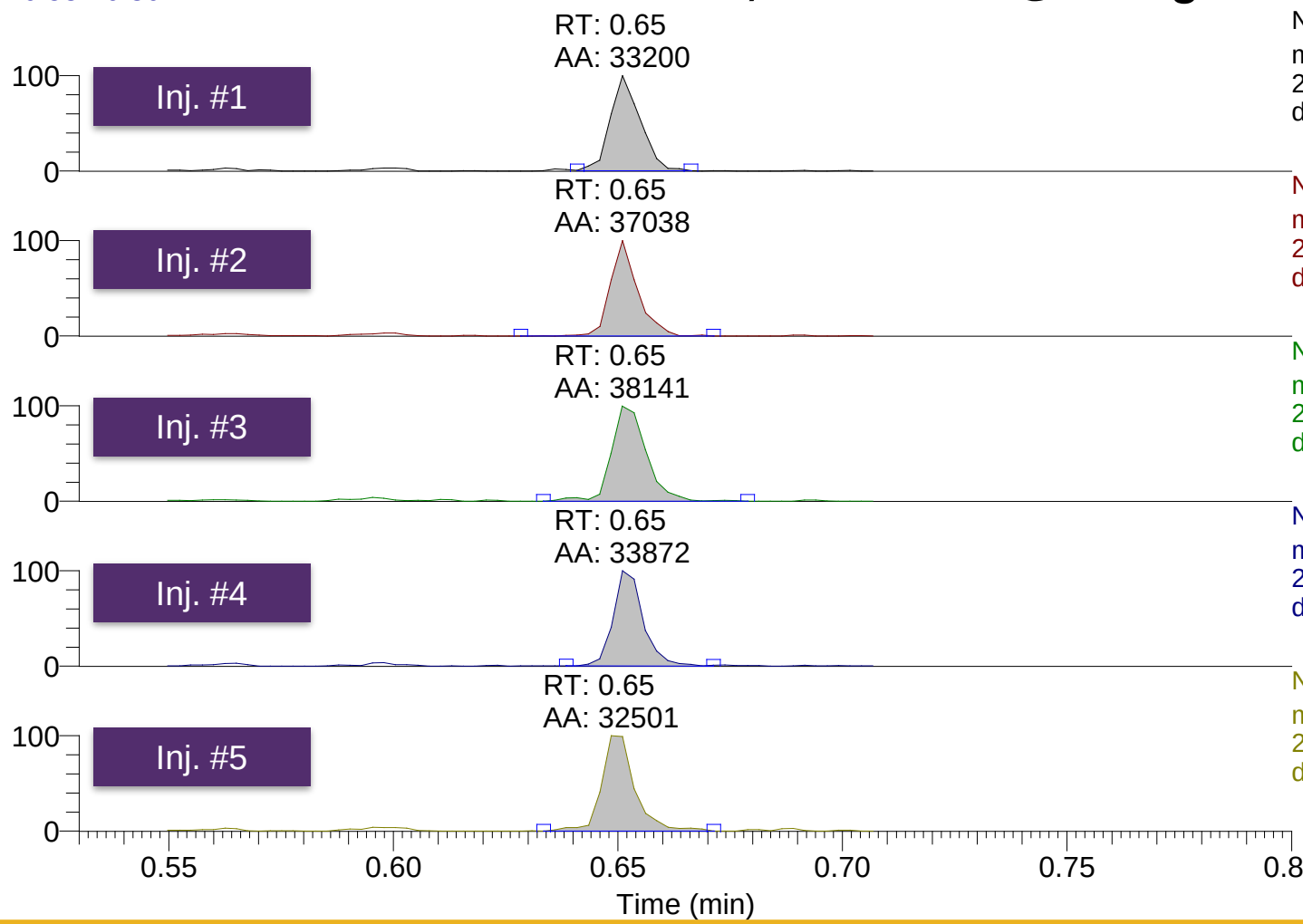
~100 Drugs of Abuse on Thermo Scientific TSQ Quantis MS

6-MAM, Calibrator @ 10 ng/mL



NL: 7.17E4
m/z= 165.02-165.12 F: + c ESI SRM ms2 328.150 [165.069-165.071, 211.079-211.081, 212.069-212.071, 253.109-253.111] MS ICIS doa_urine_assay_2x_dilution_2021

NL: 8.76E4
m/z= 165.02-165.12 F: + c ESI SRM ms2 328.150 [165.069-165.071, 211.079-211.081, 212.069-212.071, 253.109-253.111] MS ICIS doa_urine_assay_2x_dilution_2022

NL: 7.08E4
m/z= 165.02-165.12 F: + c ESI SRM ms2 328.150 [165.069-165.071, 211.079-211.081, 212.069-212.071, 253.109-253.111] MS ICIS doa_urine_assay_2x_dilution_2023

NL: 7.26E4
m/z= 165.02-165.12 F: + c ESI SRM ms2 328.150 [165.069-165.071, 211.079-211.081, 212.069-212.071, 253.109-253.111] MS ICIS doa_urine_assay_2x_dilution_2024

NL: 6.26E4
m/z= 165.02-165.12 F: + c ESI SRM ms2 328.150 [165.069-165.071, 211.079-211.081, 212.069-212.071, 253.109-253.111] MS ICIS doa_urine_assay_2x_dilution_2025

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- 1:2 dilution of 10 ng/mL 6-MAM in urine, 2 uL injections [%CV = 8.5%];
- Dwell Time = 1.63 ms (50 simultaneous SRM transitions w/ 0.15 s SRM Cycle Time)

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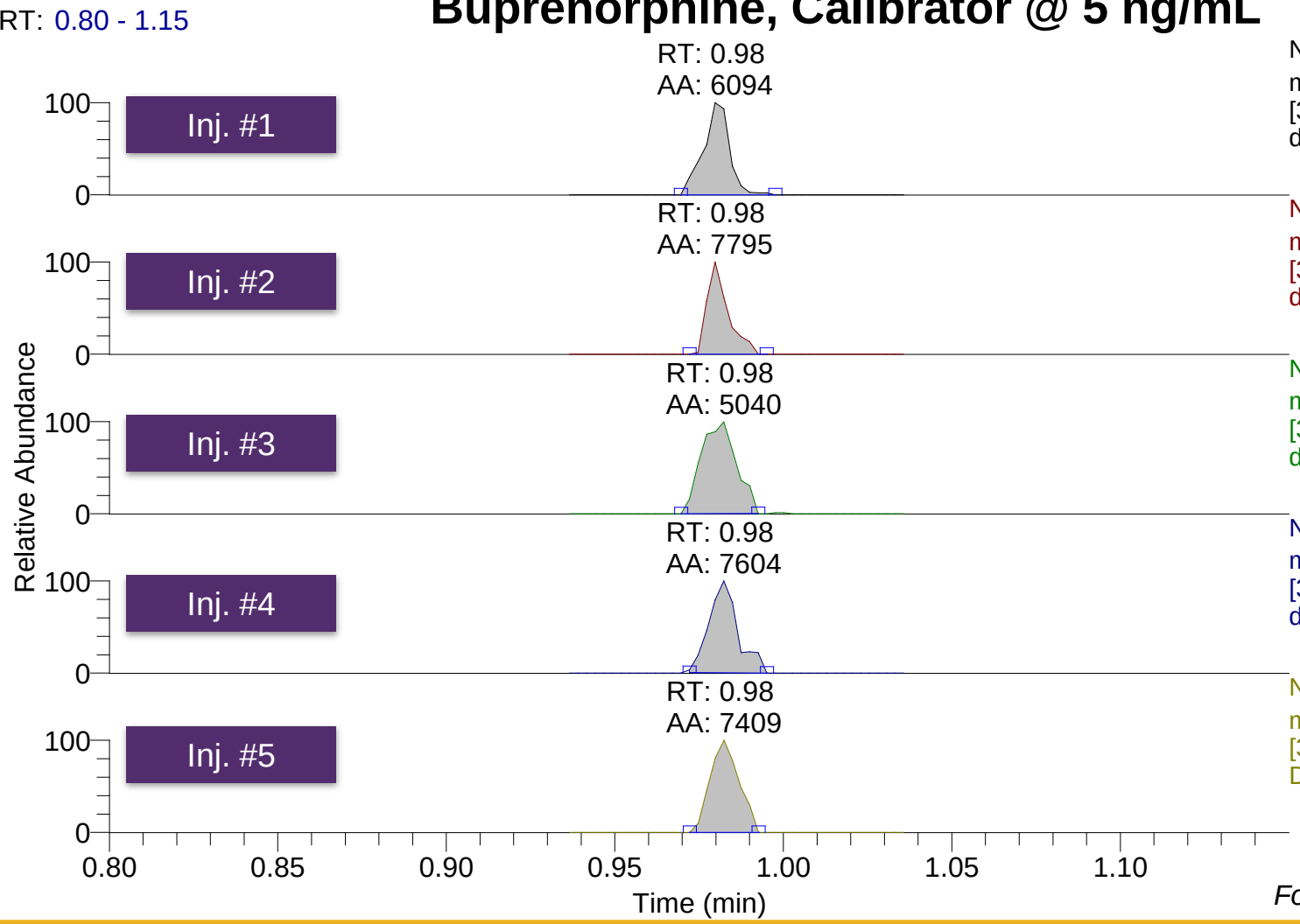
~100 Drugs of Abuse on Thermo Scientific TSQ Quantis MS

Buprenorphine, Calibrator @ 5 ng/mL



NL: 1.13E4
m/z= 396.16-396.26 F: + c ESI SRM ms2 468.310 [396.209-396.211, 414.259-414.261] MS ICIS doa_urine_assay_2x_dilution_2016

NL: 1.80E4
m/z= 396.16-396.26 F: + c ESI SRM ms2 468.310 [396.209-396.211, 414.259-414.261] MS ICIS doa_urine_assay_2x_dilution_2017

NL: 6.86E3
m/z= 396.16-396.26 F: + c ESI SRM ms2 468.310 [396.209-396.211, 414.259-414.261] MS ICIS doa_urine_assay_2x_dilution_2018

NL: 1.28E4
m/z= 396.16-396.26 F: + c ESI SRM ms2 468.310 [396.209-396.211, 414.259-414.261] MS ICIS doa_urine_assay_2x_dilution_2019

NL: 1.23E4
m/z= 396.16-396.26 F: + c ESI SRM ms2 468.310 [396.209-396.211, 414.259-414.261] MS ICIS DoA_Urine_Assay_2x_Dilution_2020

For Research Use Only. Not for use in diagnostic procedures.

- 1:2 dilution of 5 ng/mL Buprenorphine in urine, 2 uL injections [%CV = 16.5%];
- Dwell Time = 0.82 ms (60 simultaneous SRM transitions w/ 0.15 s SRM Cycle Time)

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Sensitive, Robust, Reproducible Peptide Quantitation Workflows

Targeted Peptide Quantitation Workflow Solutions with Thermo Scientific™ TSQ Altis™ MS

Why Choose a Triple Quad for Peptide Quantitation?

- They’re FAST!
- They’re cost effective for targeted work!
- They are DESIGNED TO TARGET your analytes!!
- They are simple to use for targeted analyses!!!
- Once method development is done, DATA ANALYSIS is EASIER!!!
- Sensitivity and robustness of the QqQ platform is well demonstrated!!

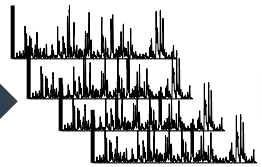
Transitioning Discovery Data to Targeted Data on the Thermo Scientific TSQ Altis MS

- A workflow from HRAM to QqQ Targeted Peptide Quantitation

1 week (work by Dr. David Sarracino)

Pooled, fractionated, and/or individual samples

Data Dependent Analysis (DDA)



Protein ID characterization QE-HF Orbitrap MS

Proteome Discoverer

Skyline

Consolidated Spectral Library Creation

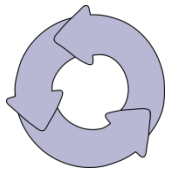
- Protein name, accession number
- Spectral Library example
- Peptide *m/z*, z
- Top ranked transitions
- Optimized CE
- Retention time with LC conditions

SRM selection and refinement

2 days

- Spectral Libraries can be additive
- SRM parameters can be stored
- RTs can be stored
- Targeted methods can be generated from cumulative databases

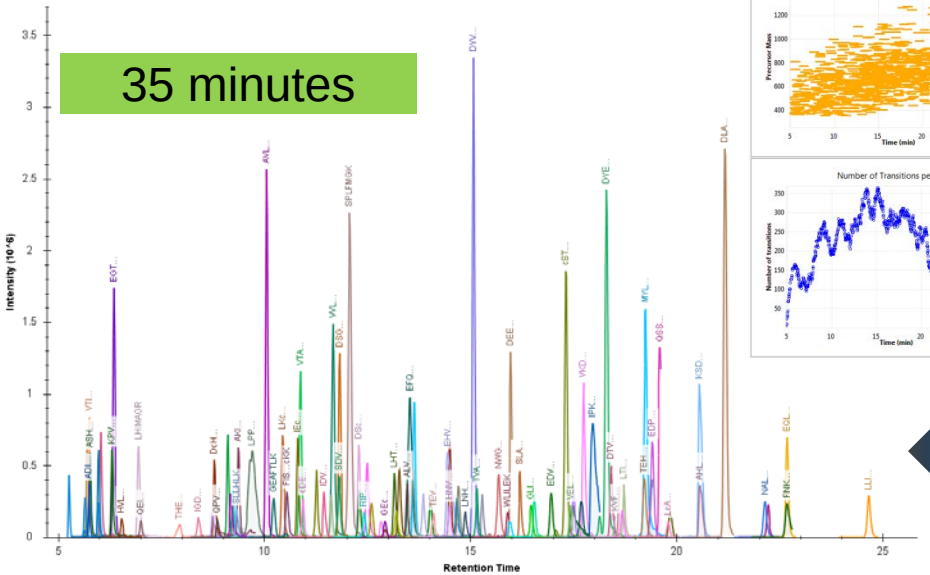
Iterative Empirical Determination



- **iRT Prediction**
- **Retention Time Scheduling**
- **Collision Energy Optimization**
- **Final Method**

1 day

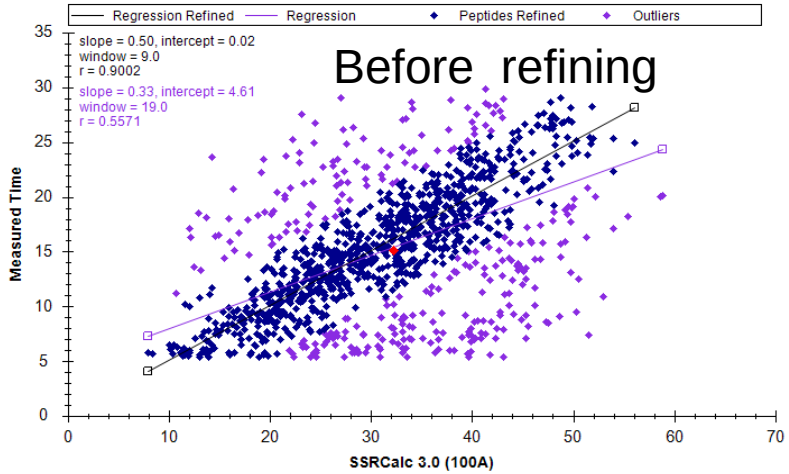
35 minutes

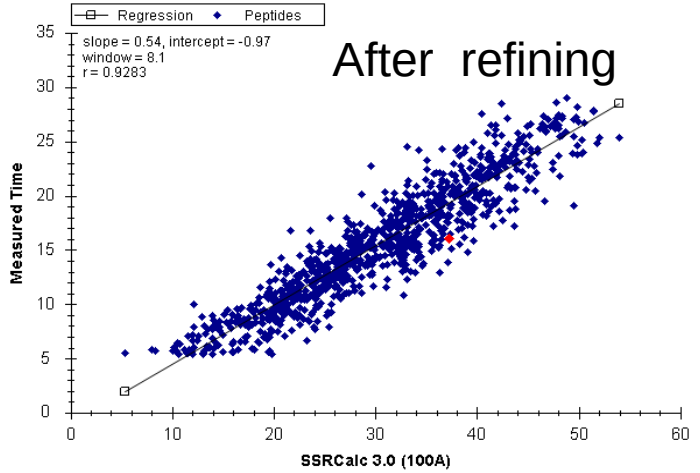


ThermoFisher SCIENTIFIC

SRM Refinement and Selection for 950 peptides from spectral libraries

- Human proteome was digested *in silico* and compared to spectral libraries
- ~1200 tryptic peptides matched a spectral library hit
- 53 untargeted methods set up for SRM refinement
  - 2 charge states for most peptides
  - 8-10 transitions per precursor
  - ~20,000 transitions
  - 31 hours of acquisition time
  - 10-15 hours of analysis time
- Result:
  - 950 plasma peptides + 15 PRTC peptides
  - 4762 transitions in a 35 minute run
  - RT scheduling with 1 min windows; dRT so no RT updates necessary

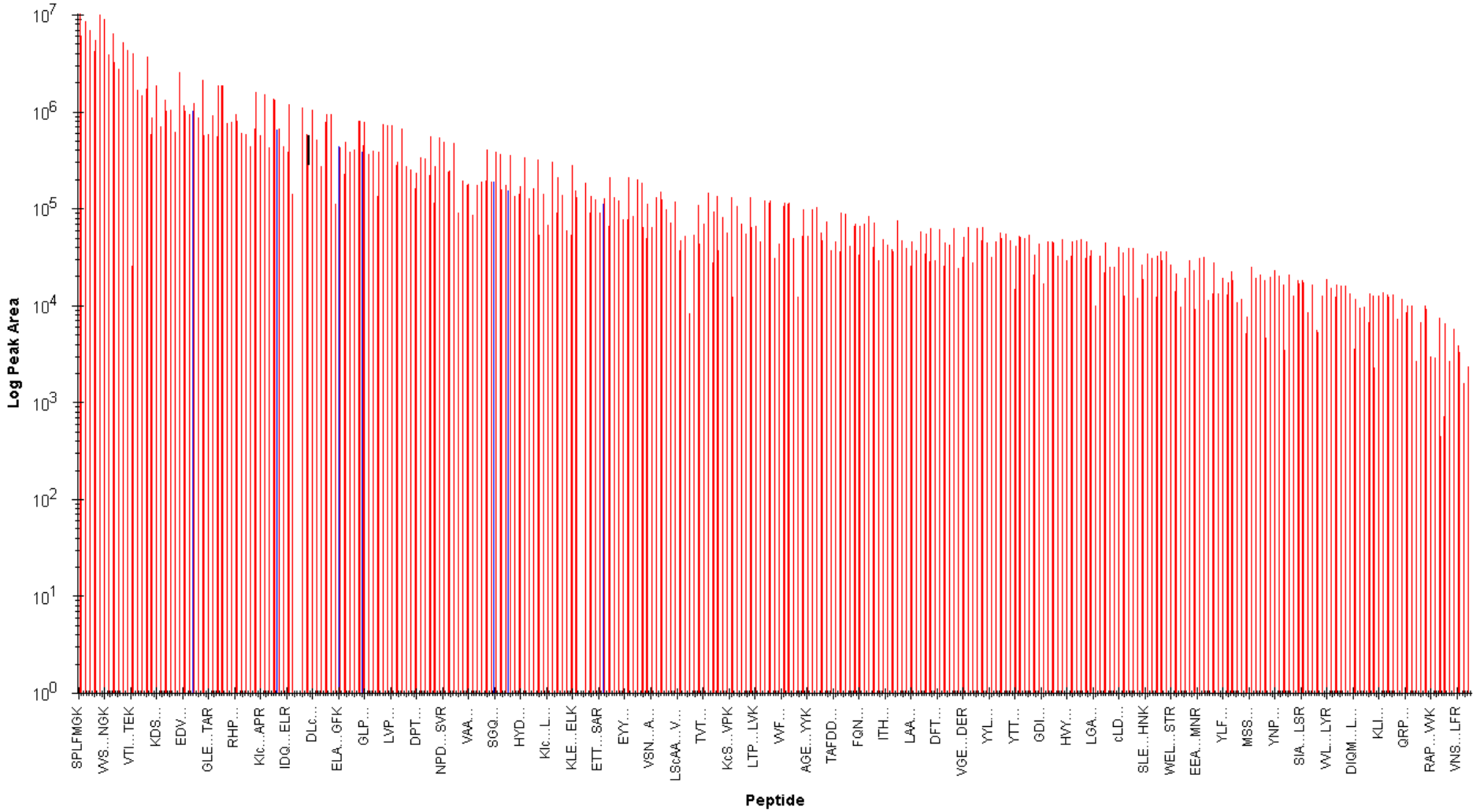




ThermoFisher SCIENTIFIC

Peaks detected over 3-fold range intensity from digested neat plasma

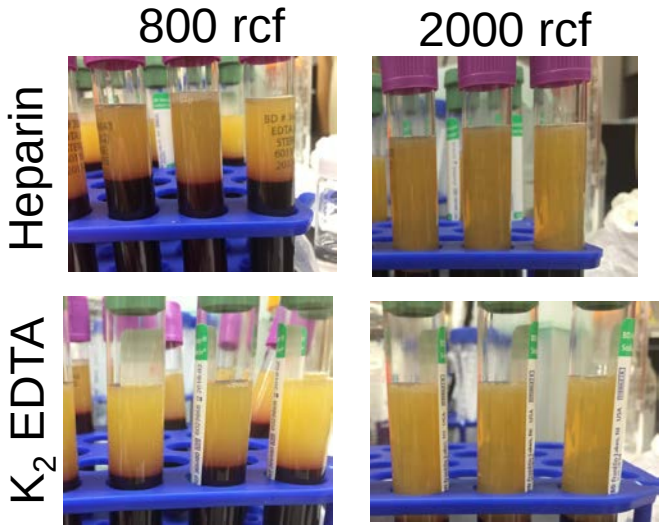
- Peptides ID'd in Q Exactive HF analysis of fractionated plasma (48 BRP fx)
- Intensity ranking for each peptide on the Altis spans ~3 log
- Blue bars indicate PRTC peptides spiked in (5 fmol/uL plasma digest; 1 ug/uL plasma concentration)



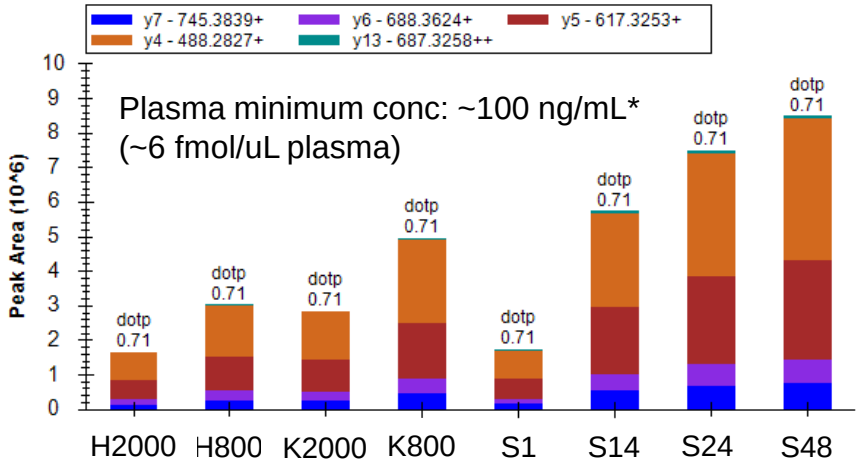
ThermoFisher SCIENTIFIC

Detection of changes in peptide intensity due to plasma/serum processing

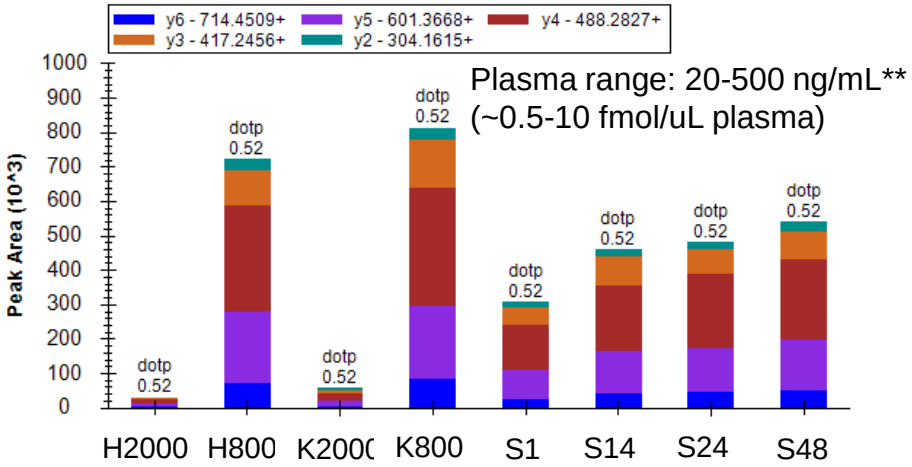
Vacutainer and centrifugation



Hemoglobin subunit A (HBA1) VGAGAGEYGAEALER

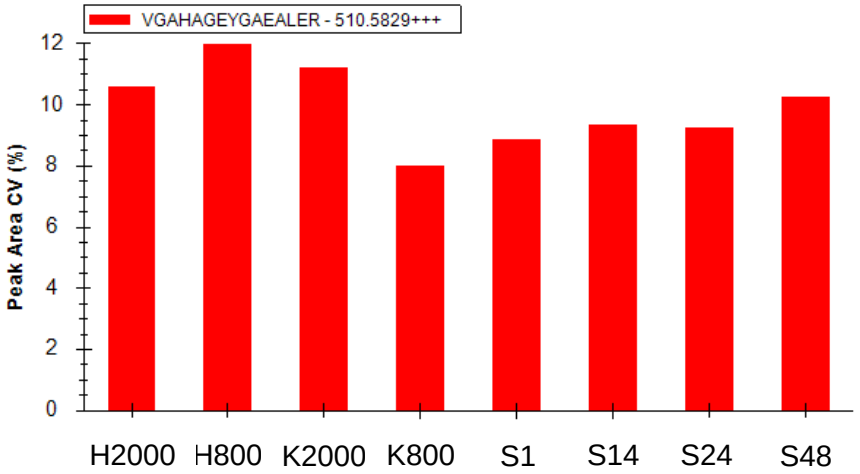


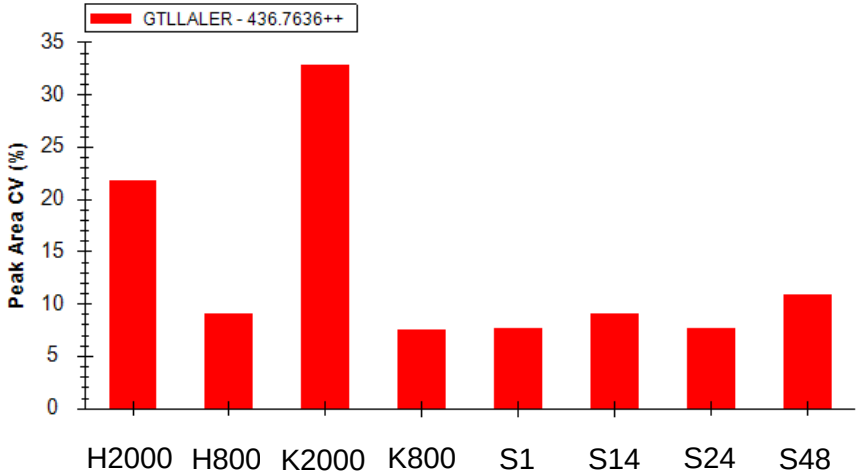
Thrombospondin-1 (THBS1) GTLLALER



Serum Time Course

Whole blood spun after
1, 14, 24, 48 hours on bench,
then digested according to
protocol





* http://www.abcam.com/human-hemoglobin-subunit-alpha-elisa-kit-ab219049.html#description_images_2

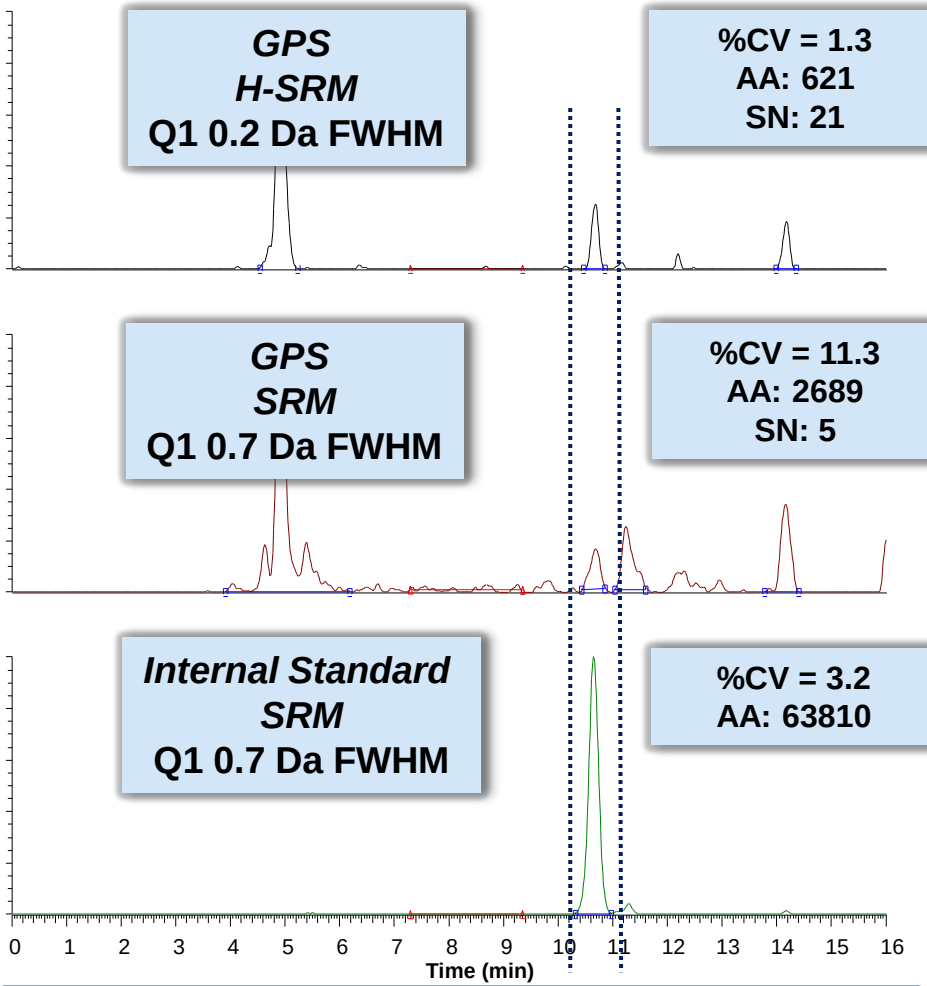
** Peterson et al 2010, Am. J. Hematol

n = 5 for all samples

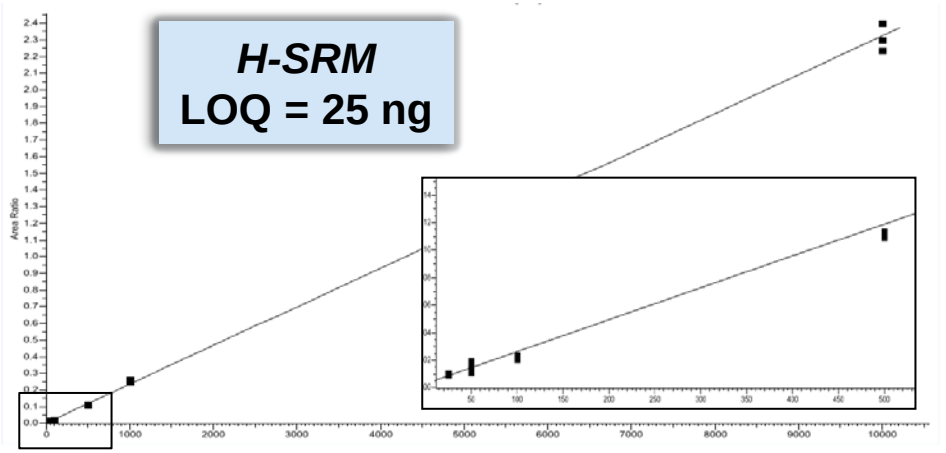
ThermoFisher SCIENTIFIC

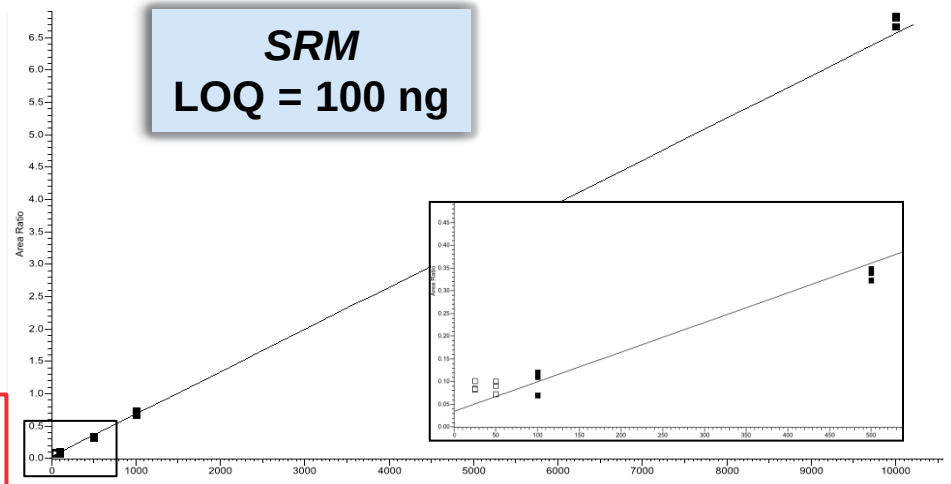
Superior Sensitivity with H-SRM (0.2 Da FWHM) – GPSVFPLAPSSK

GPSVFPLAPSSK - Peptide from monoclonal antibody hinge region



25 ng GPSVFPLAPSSK and *IS*





Experimental Details

LC : Ultimate NCS-3500RS
Flow rate: 25µL/min
Solvent A: 2% ACN in H_2O w/0.1% FA
Solvent B: 5% H_20 in ACN w/0.1% FA

Professor Jun Qu
University of Buffalo

ThermoFisher SCIENTIFIC

Confident Quantitation – Any compound, Any Matrix, Any User

Team Confident Quantitation

- Dr. Mary Blackburn (Sr. Platform Manager, TSQ)
- Dr. Claudia Martins (Product Manager, Triple Quadrupole MS)
- Dr. Alan Atkins (Technical Trainer)
- Dr. Sue Abbatiello (Product Specialist)
- Dr. Neloni Wijeratne (Product Specialist)
- Dr. Eric Huang (Product Specialist)
- Dr. Deb Bhattacharyya (Sr. Marketing Manager, Triple Quadrupole MS)

Confident
Quantitation

Any compound, any matrix, any user.

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