

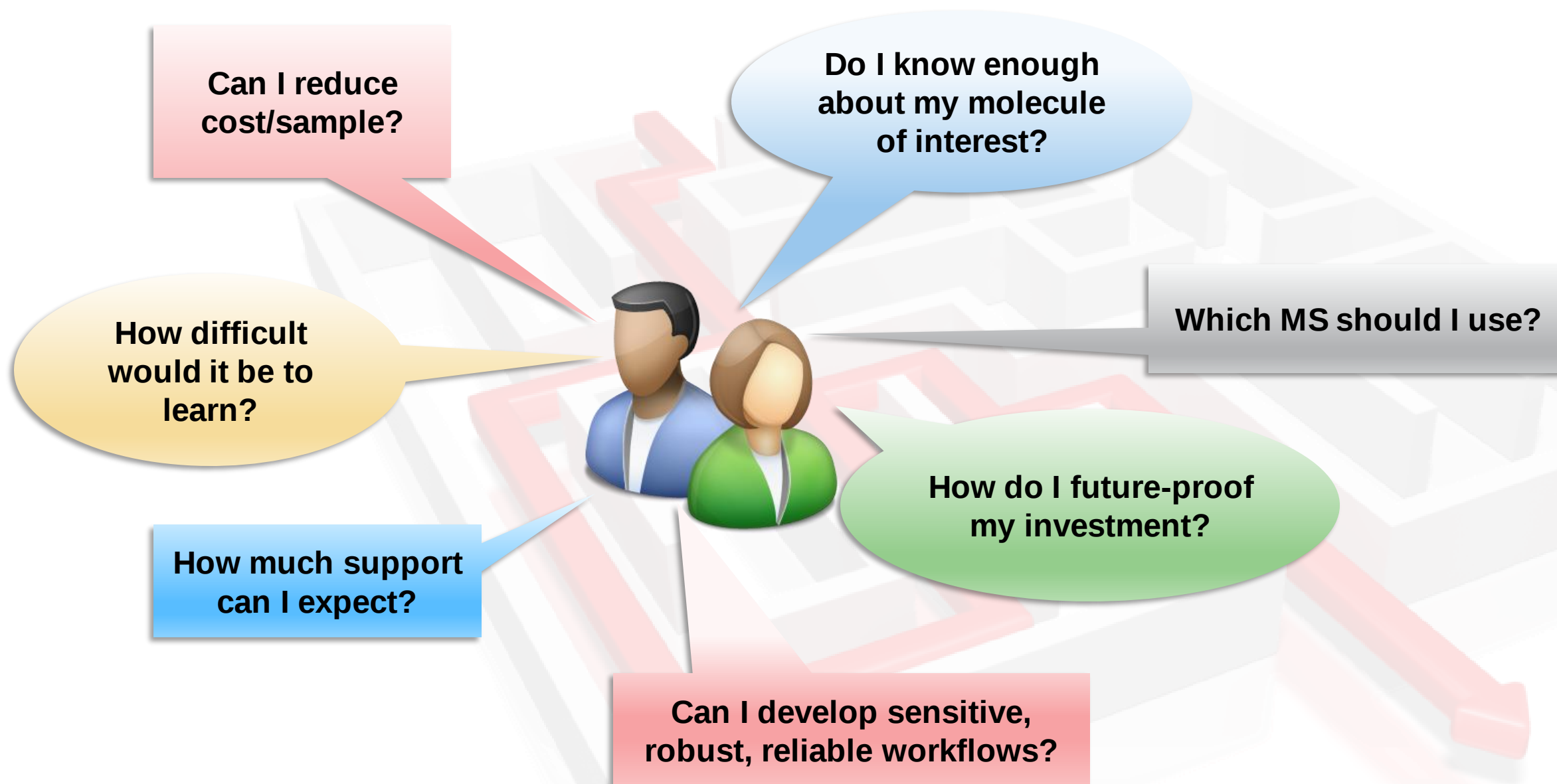


Tomorrow's Quantitation Today: Improving LC-MS Workflows

The world leader in serving science

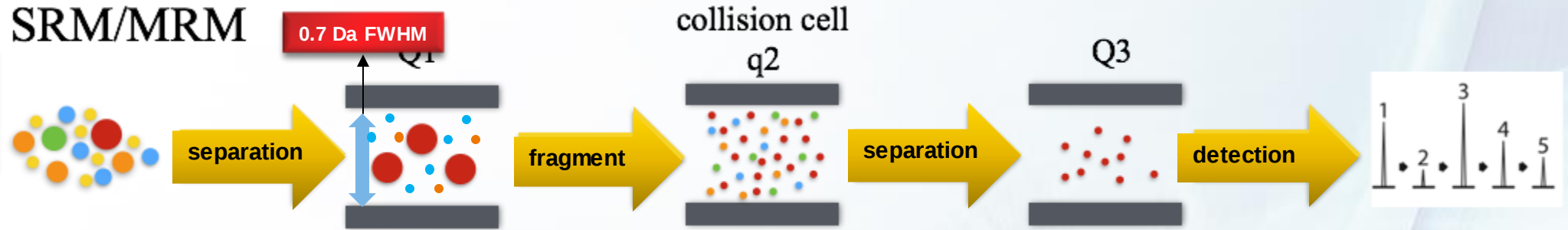


Confident Quantitation for Meeting Expectations

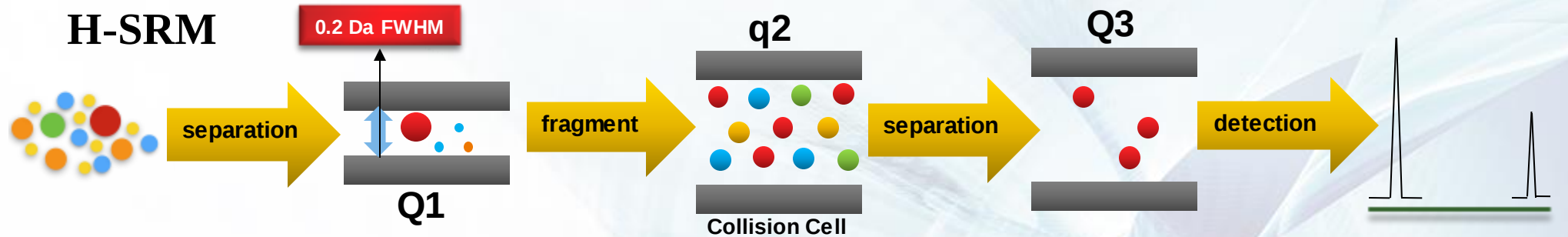


Types of Quantitation: Leverage the Best

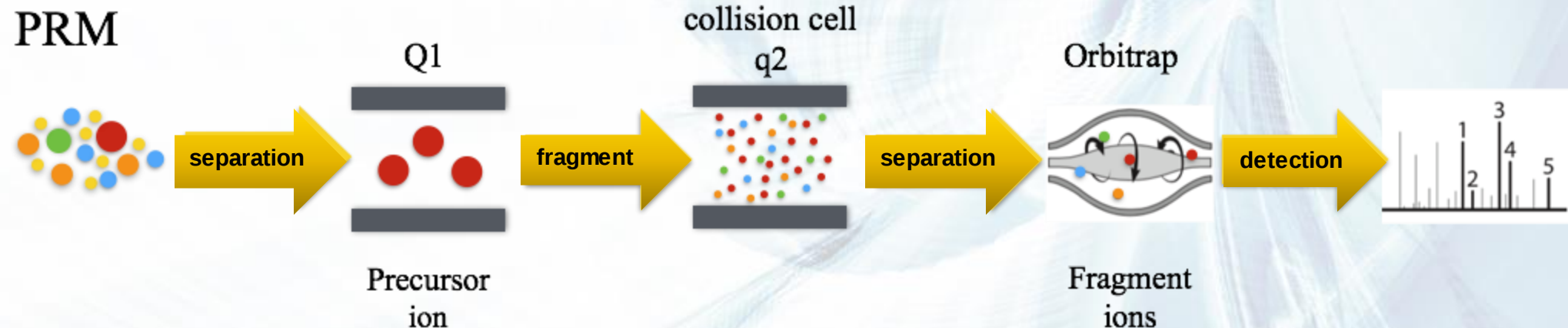
Nominal Mass for
typical targeted
quantitation



Nominal Mass
high resolution
SRM allowing
additional
selectivity with
sensitivity



High Resolution
Accurate Mass for
targeted
quantitation



HRAM or QQQ?

✓ When Triple Quadrupole?

Robustness, reproducibility in targeted quantitation

Most sought after requirement in a routine, targeted quantitation environment for multiple samples and for all molecule types.

Ultimate sensitivity

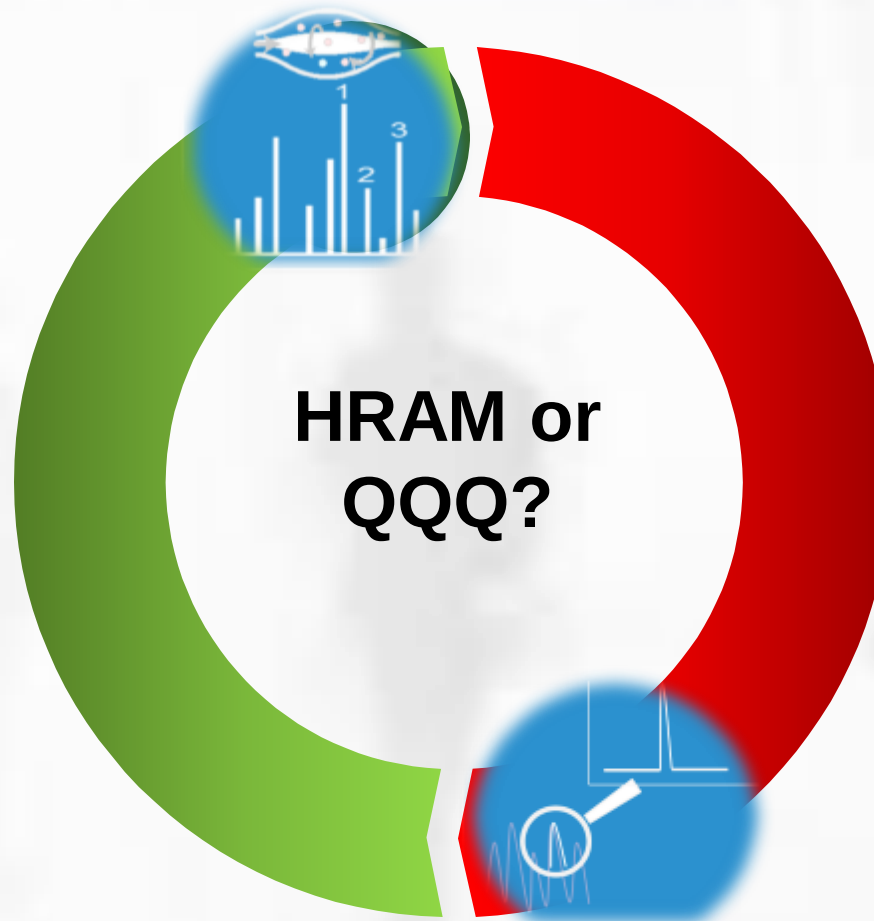
For a host of molecule types in complicated biological matrices.

Reducing cost/sample

Robust, reproducible workflow for multiple sample analysis, every day, week, and months.

From regulated environment to established methods

Easy method development for all molecule types.



✓ When HRAM?

Confirmation of analyte structure

Start with confidence for both identification and confirmation, by accurate mass, isotopic pattern, fragments and RT.

Analysis of unknowns

Addressing the challenge of knowing everything about all the components of your sample.

Retrospective search for new compounds

Leveraging the truly high resolution full scan data to shape your studies during the next step.

Addressing sensitivity requirements

Changing the common notion that HRAM cannot do Quantitation studies.

Sensitivity is not the driving force

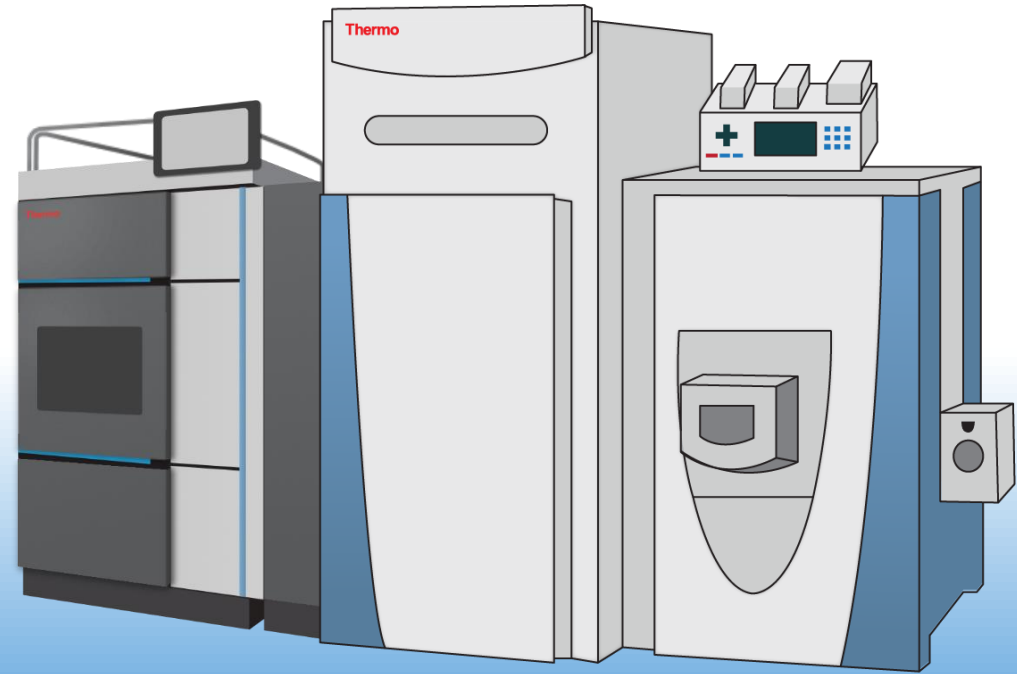
It is important, however, it is not a sample limited situation or they are not looking for low pg/mL.

Orbitrap™ Technology – Highest Quality HRAM Data

Highest resolution available

Best accurate mass capabilities

- Screening and confirmation with a single platform
- Synthetic drug investigation
- Qual/Quan applications
- Screening (targeted and unknown)
- Retrospective analysis
- Small molecule, peptide and protein analysis



THCA - Performance Summary

Synthetic Oral Fluid – HRAM MS/MS
70,000 Resolution, negative ion.

LLOQ: **7.5 pg/mL**

Dynamic range: **7.5 to 300 pg/mL**

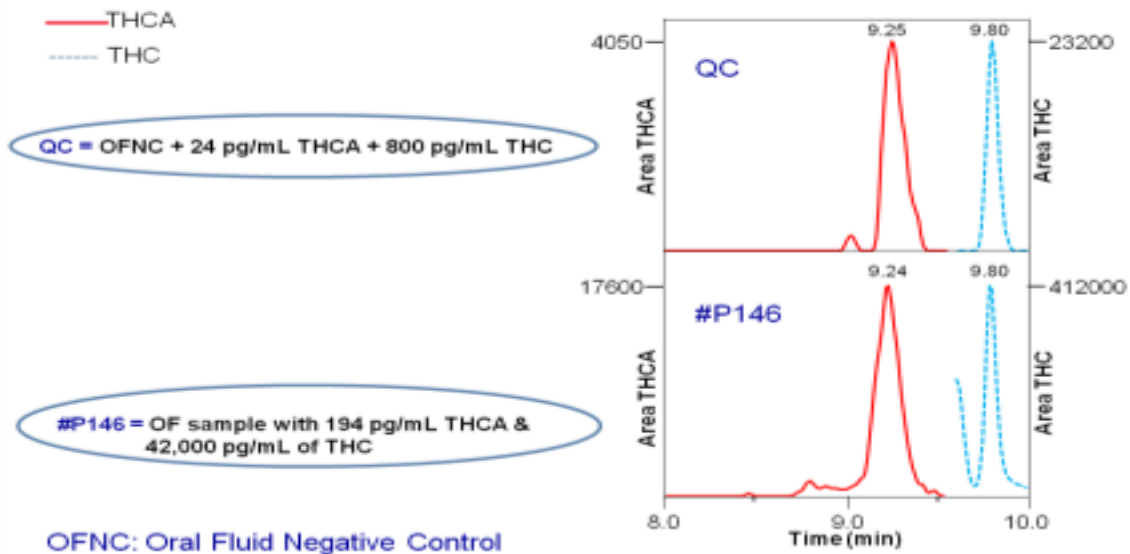
Accuracy: **95.8% to 103.3%**

Precision: **3.7 to 12.5 (intra and inter)**

Carryover: **Not detected**

Ion Suppression: **max 35%**

Simultaneous THCA and THC Analysis



Accuracy, dynamic range and LLOQ

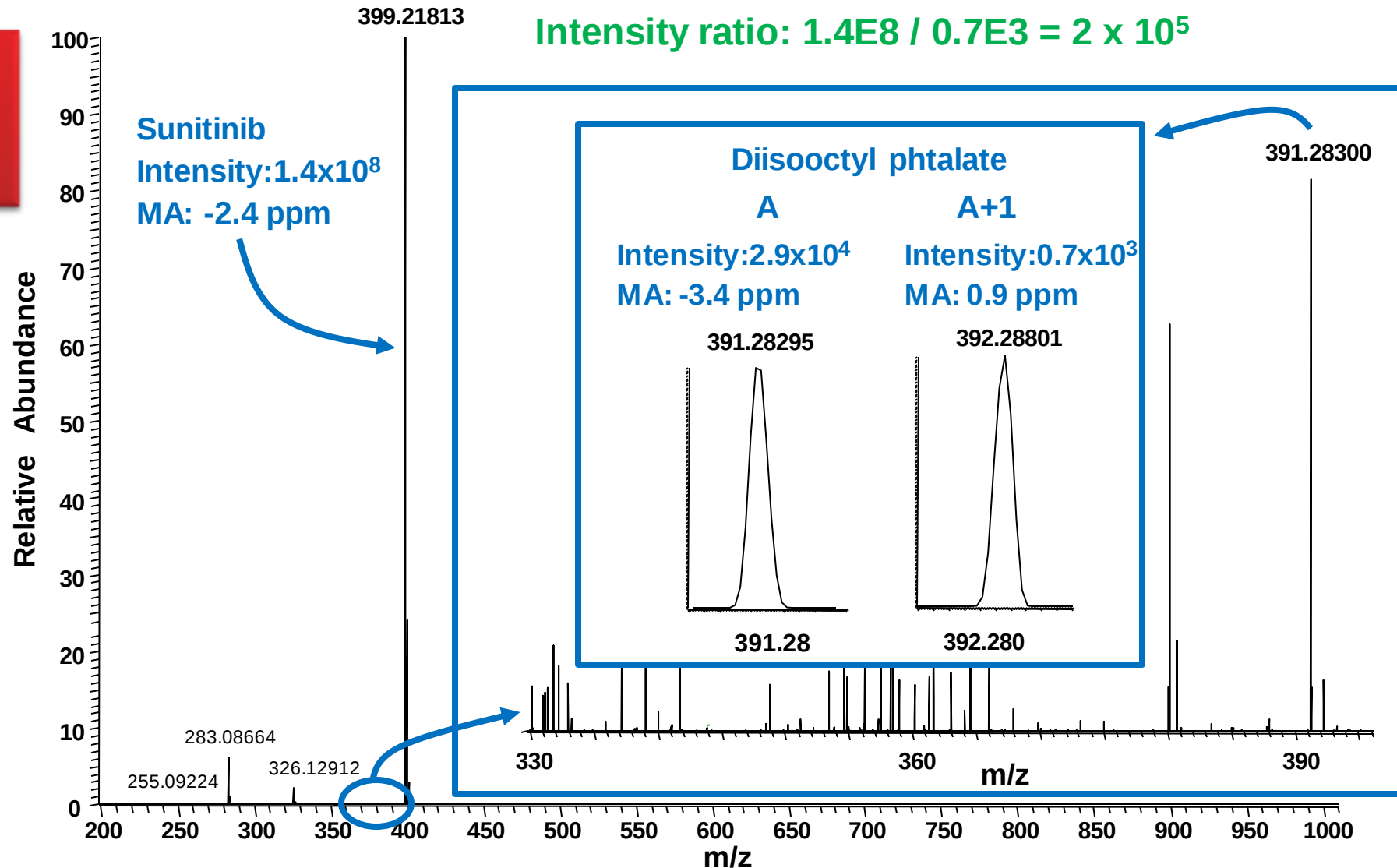
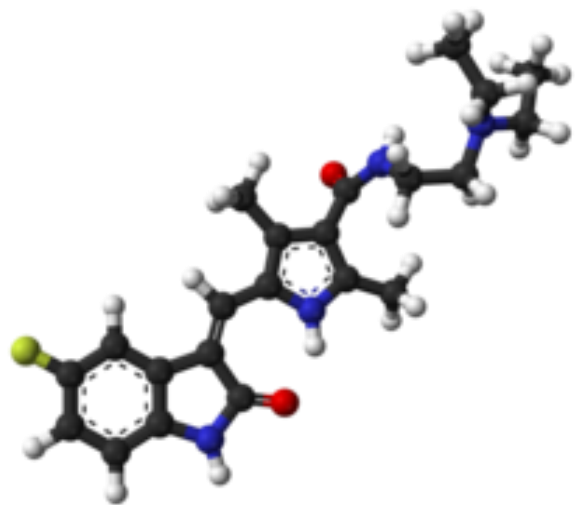
Sample	Specified (pg/mL)	Measured (pg/mL)	Accuracy (%)	Mean Accuracy (%)	Precision (%)
Cal1	7.5	7.26	96.8		
Cal1	7.5	7.30	97.3		
Cal1	7.5	7.80	104.0	99.4	4.07
Cal2	15	16.54	110.3		
Cal2	15	14.00	93.3		
Cal2	15	14.76	98.4	100.7	8.63
Cal3	30	30.13	100.4		
Cal3	30	28.48	94.9		
Cal3	30	27.58	91.9	95.8	4.50
Cal4	75	78.83	105.1		
Cal4	75	77.68	103.6		
Cal4	75	75.81	101.1	103.3	1.97
Cal5	150	141.68	94.5		
Cal5	150	150.90	100.6		
Cal5	150	158.19	105.5	100.2	5.50
Cal6	300	312.20	104.1		
Cal6	300	295.75	98.6		
Cal6	300	287.33	95.8	99.5	4.24

Precision

[Specified] (pg/mL)	Mean Accuracy (%)	Precision (%)
24 (Intra-batch, n=5)	101.0	12.5
24 (Inter-batch, n=15)	96.3	10.8
120 (Intra-batch, n=5)	102.7	5.7
120 (Inter-batch, n=15)	100.1	5.3

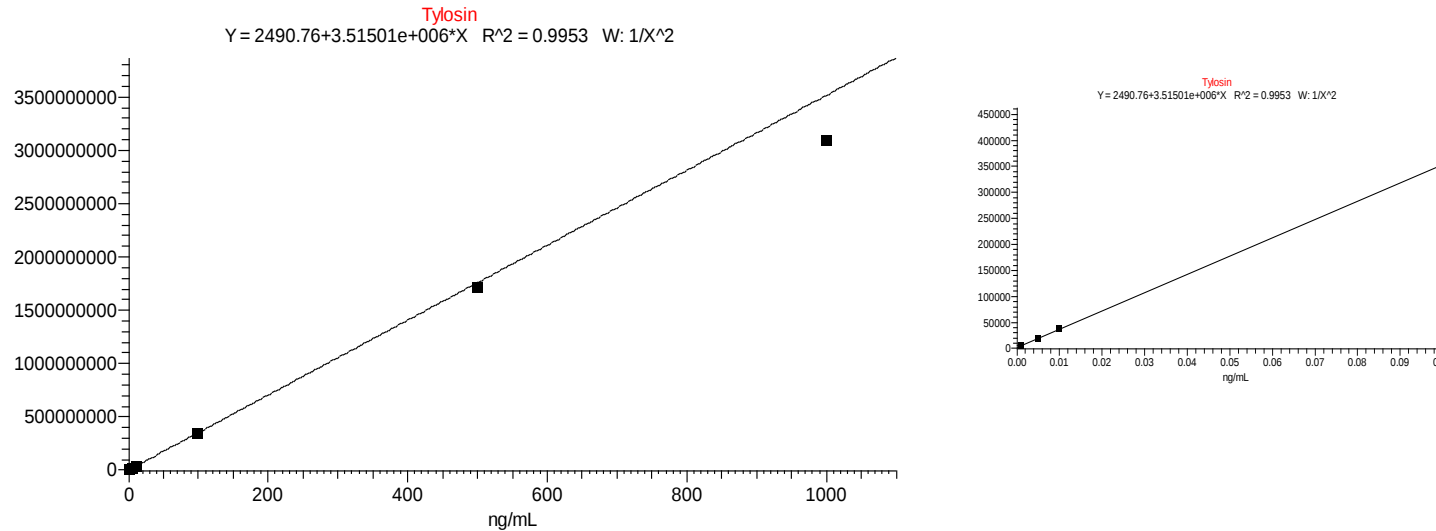
Intrascan Dynamic Range, 5 Orders of Magnitude; HRAM

Sunitinib, the first approved cancer drug by FDA for renal cell carcinoma and gastrointestinal stromal tumor



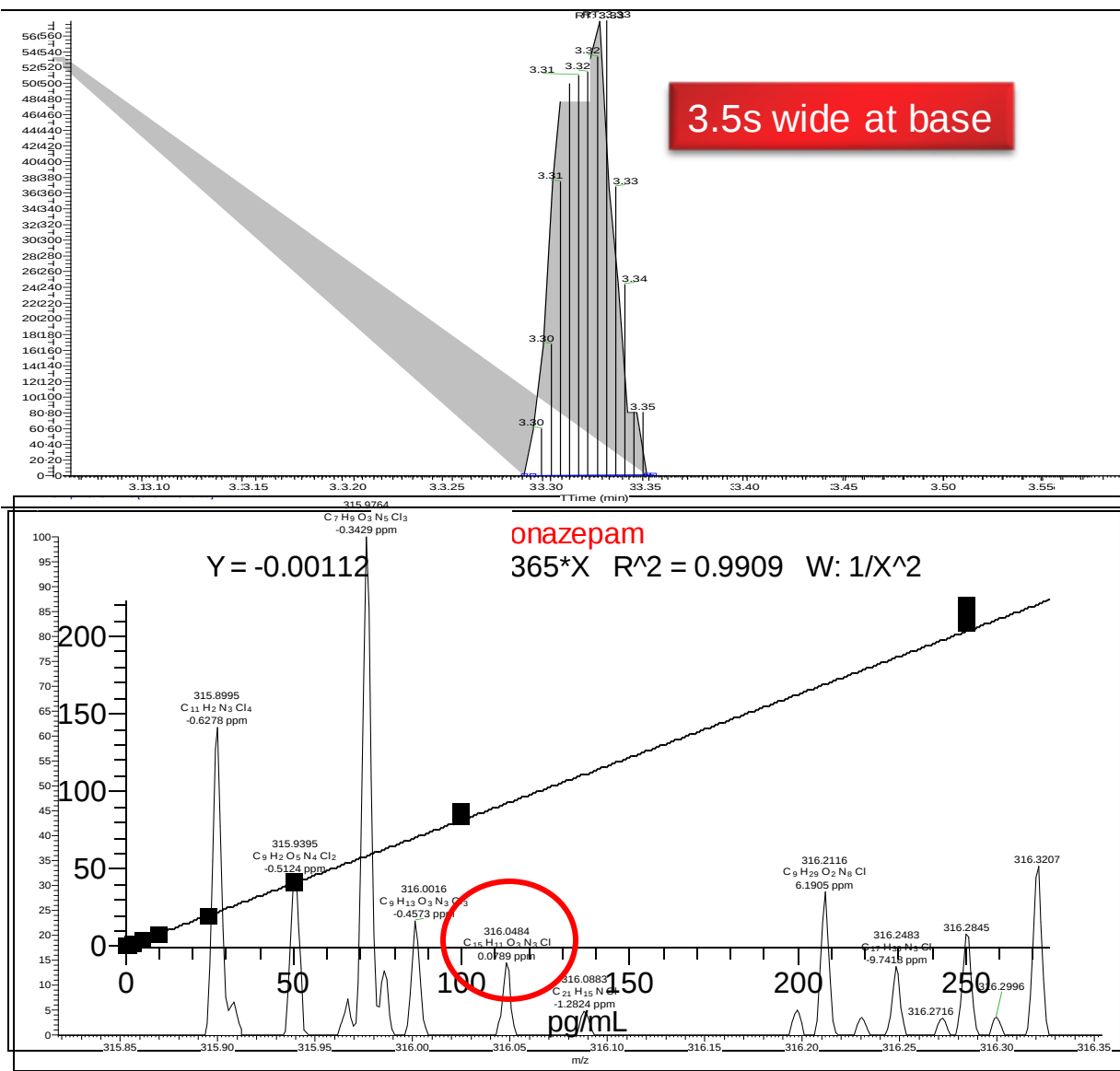
Tylosin: 6 Orders Calibration Range on the Thermo Scientific Q Exactive Focus MS

- Calibration range of Tylosin on the Thermo Scientific™ Q Exactive™ Focus MS could reach 6 orders (1 ppt - 1 ppm)
- We repeated this experiment on another Q Exactive Focus MS, got same results



	File Name	Sample Type	Integration Type	Area	Specified Amount	Calculated Amount	% Diff	Units
1	sim_1ppt01	Standard	Method Settings	6046	0.001	0.001	1.15	ng/mL
2	sim_5ppt01	Standard	Method Settings	18846	0.005	0.005	-6.94	ng/mL
3	sim_10ppt01	Standard	Method Settings	38292	0.010	0.010	1.85	ng/mL
4	sim_100ppt01	Standard	Method Settings	371581	0.100	0.105	5.00	ng/mL
5	sim_1ppb01	Standard	Method Settings	3554296	1.000	1.010	1.05	ng/mL
6	sim_5ppb01	Standard	Method Settings	17635078	5.000	5.016	0.33	ng/mL
7	sim_10ppb01	Standard	Method Settings	39559930	10.000	11.254	12.54	ng/mL
8	sim_100ppb01	Standard	Method Settings	350578977	100.000	99.737	-0.26	ng/mL
9	sim_500ppb01	Standard	Method Settings	1712948617	500.000	487.323	-2.54	ng/mL
10	sim_1000ppb01	Standard	Method Settings	3086844551	1000.000	878.185	-12.18	ng/mL

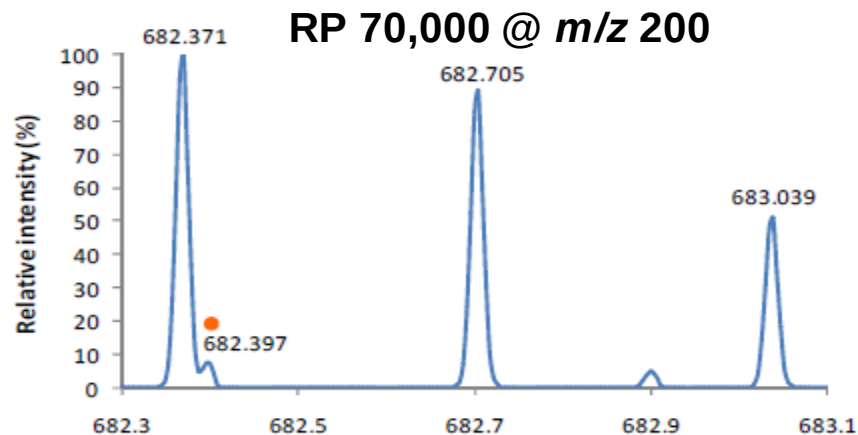
Small Molecule Pharmaceutical Drugs



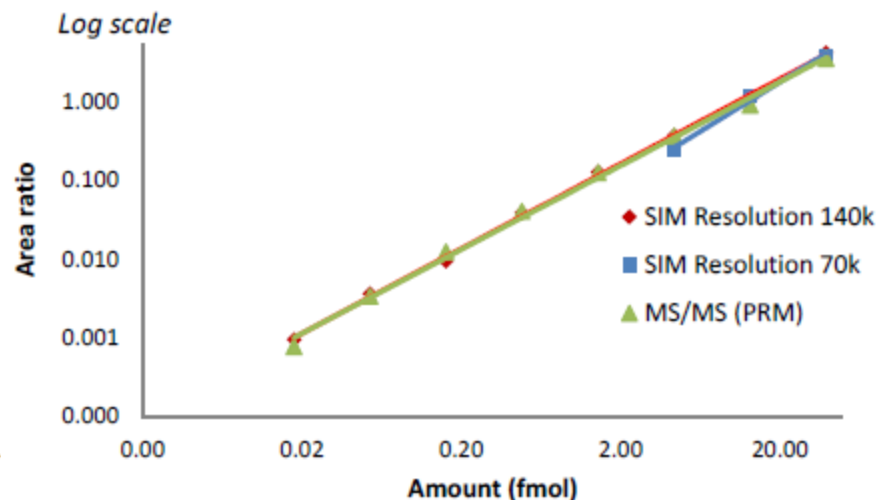
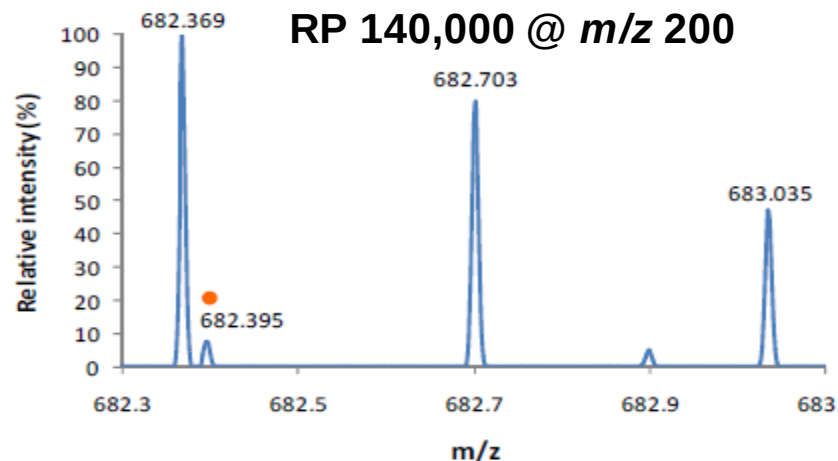
Summary Table			
Compound	LOQ pg/mL	Linear Range pg/mL	Orders of magnitude
Oxycodone	5	5 - 250,000	4.5
Buprenorphine	10	10 - 100,000	4.0
Paroxetine	10	10-250,000	4.2
Ketoconazole	5	5-,100,000	4.2
Clonazepam	5	10-250,000	4.2
Alprazolam	5	5 - 250,000	4.5
Reserpine	5	5-,100,000	4.2

Summary Table			
Compound	LOQ pg/mL	Linear Range pg/mL	Orders of magnitude
Disopyramide	5	5 – 100,000	4.2
Fentanyl	5	5 – 100,000	4.2
Flecainide	5	5 – 50,000	4.0
Imipramine	5	5 – 100,000	4.2
Fluoxetine	5	5 – 100,000	4.2
Diazepam	5	5 – 50,000	4.0

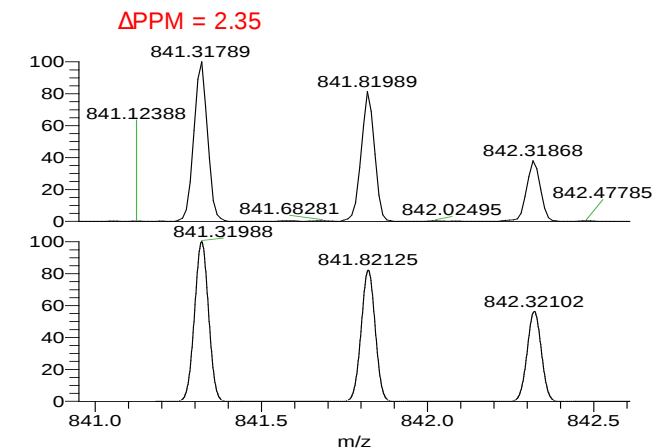
HR/AM: Sensitivity Gain through Resolution (SIM); Peptide Quant



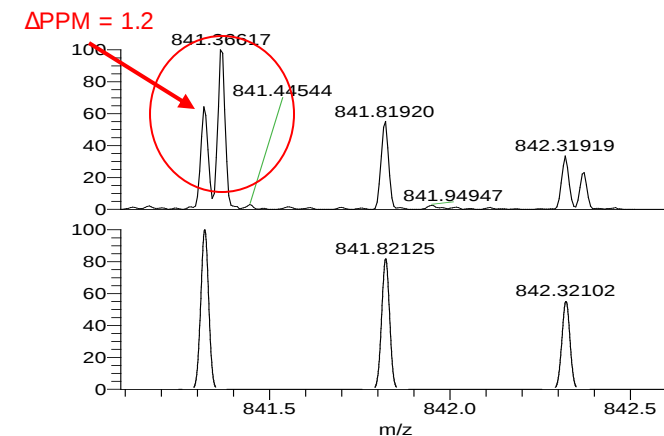
- SDLAVPSELALLK
- m/z 682.40
- Urine digest matrix



Resolution = 35 K



Resolution = 70 K

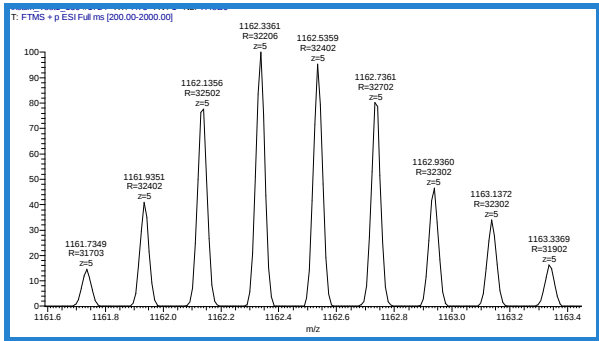
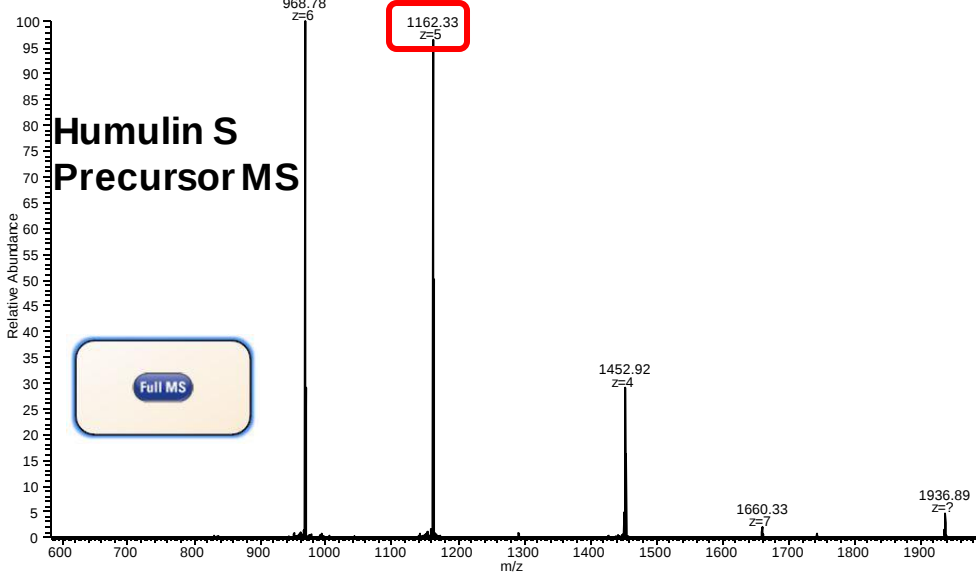


Theoretical Mass $[M+2H]^+ = 841.31987$

Targeted Proteomic Quantification on Quadrupole-Orbitrap Mass Spectrometer, Gallien et al., MCP 2012, O112.019802

Full Scan HRAM MS of Charge States and Isotopes

Insulin_Human IMS2#1428-1445 RT:11.64-11.72AV:3 NL:7.42E8
T:FTMS + p NSI Full ms [500.00-2000.00]



Resolution Setting = 70,000

Full Scan Quantitation Results for Insulin Glargine and Insulin Glulisine

STD Conc (pM)	Mean (5-curves)	Stdev	%CV	Accuracy
0	7.42	1.02		
7.5	10.56	0.95	9.04%	40.80%
15	16378	1.42	8.46%	11.87%
30	28.96	1.12	3.85%	-3.46%
60	58.41	1.61	2.75%	-2.66%
120	115.93	1.96	1.69%	-3.39%
240	232.65	2.80	1.20%	-3.06%
480	473.25	14.41	3.04%	-1.41%
960	963.31	6.47	0.67%	0.34%

Calibration Curve Results

STD Conc (pM)	Mean (3 controls x 5-curves)	Stdevp	%CV	Accuracy
50.00	51.21	1.33	3%	2.43%

Intra-Day Reproducibility

STD Conc (pM)	Mean (3 controls x 5-curves)	Stdevp	%CV	Accuracy
50.00	51.07	0.81	2%	2.15%

Inter-Day Reproducibility

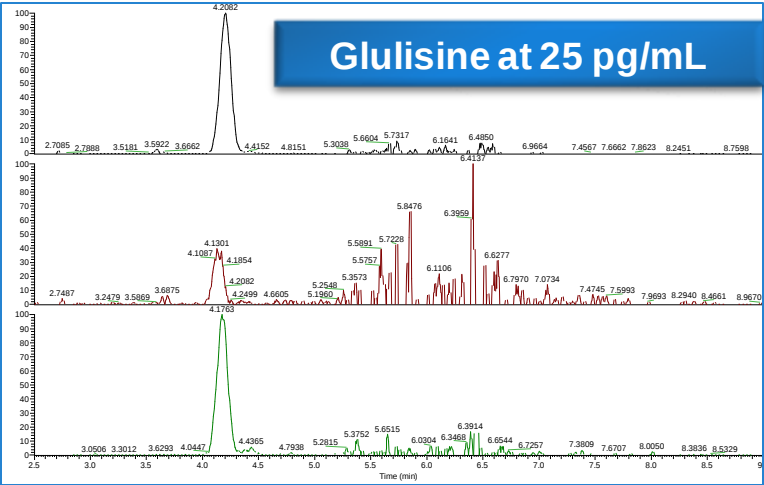
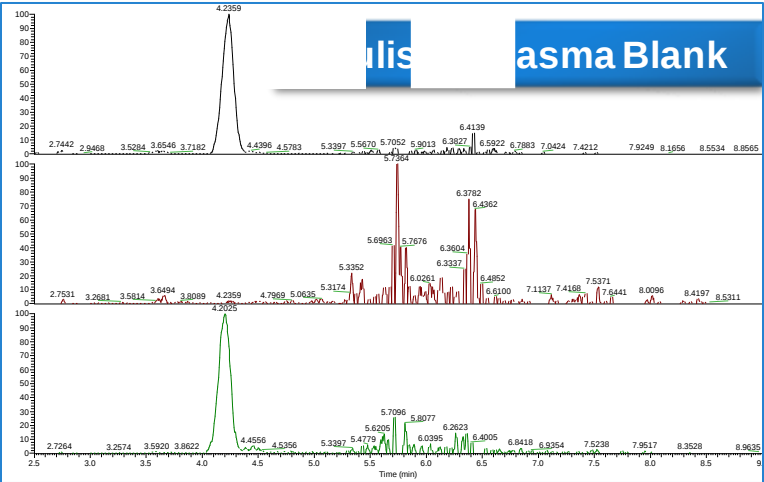
Sample	Spike Conc. (pM)	Exp. Conc. (pM)	Average (pM)	Exp Recovery Conc. (pM)	% Yield
Neat 1	0.00	43.79	44.59		
Neat 2		45.59			
Neat 3		44.38			
Low 1	19.50	65.08	64.11	19.52	100.12%
Low 2		63.65			
Low 3		63.61			
Medium 1	199.50	241.19	237.56	192.97	96.73%
Medium 2		239.80			
Medium 3		231.70			
High 1	919.50	960.91	928.63	884.05	96.14%
High 2		905.35			
High 3		919.64			

Spike and Recovery Study

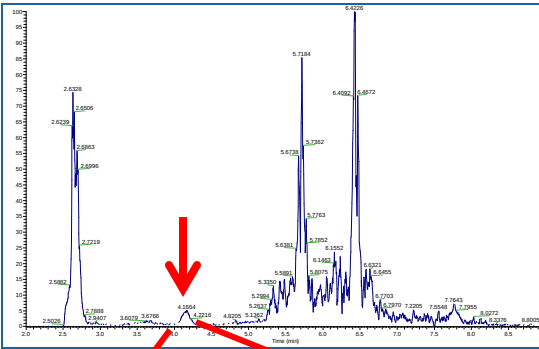
- LOQ = 90pg/mL
- Simplified data acquisition
- Comprehensive coverage
- Large injection volume

Glulisine at 25 pg/mL (4.3 pM)

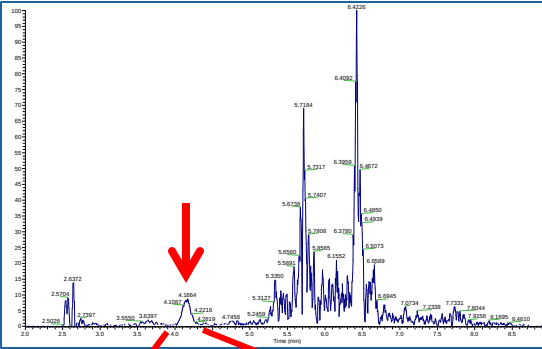
SIM



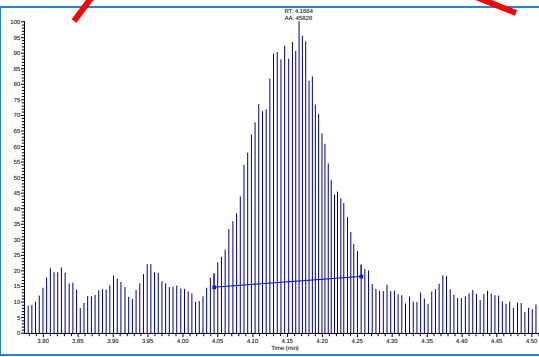
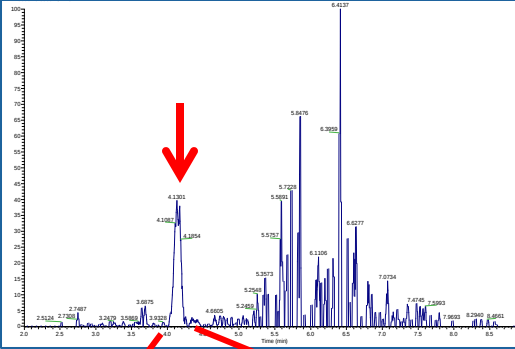
Mass Tolerance = 50 ppm



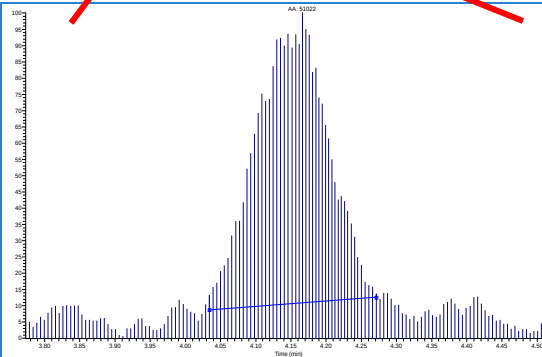
Mass Tolerance = 25 ppm



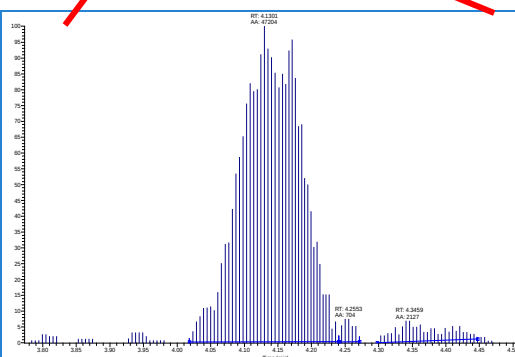
Mass Tolerance = 5 ppm



Mass Tolerance = 50 ppm



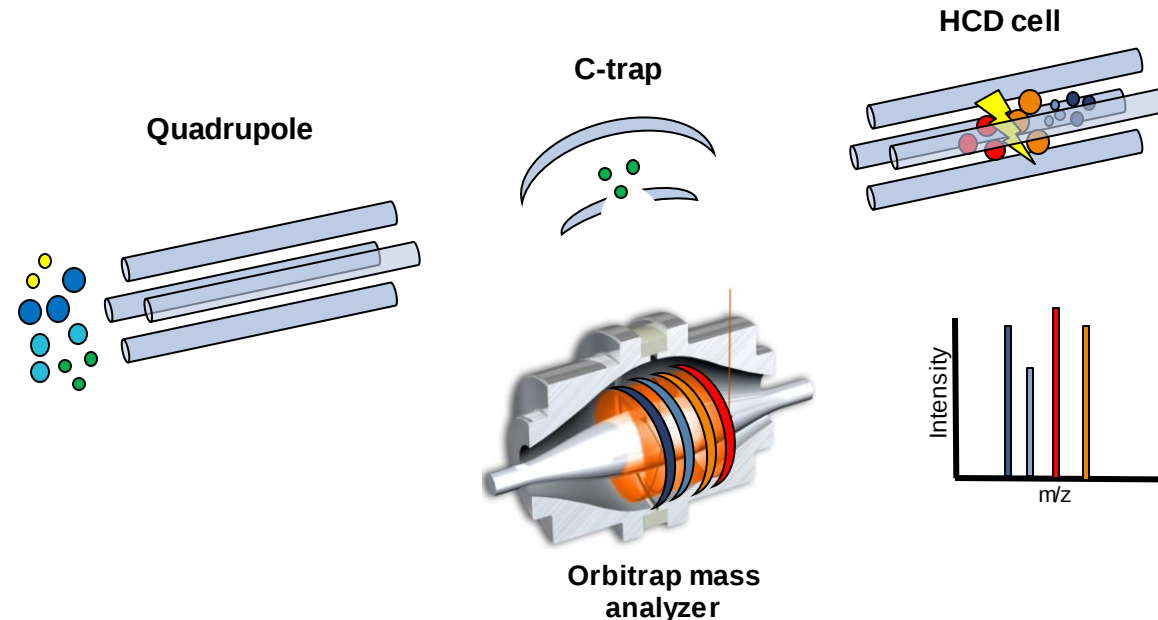
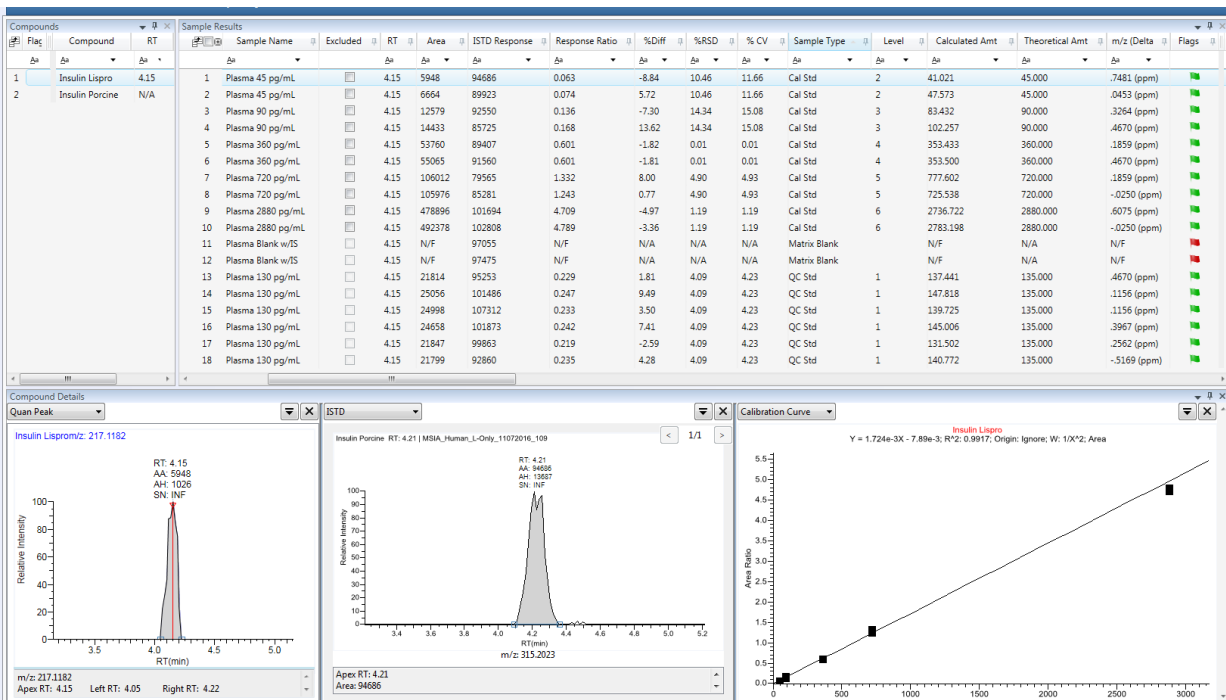
Mass Tolerance = 25 ppm



Mass Tolerance = 5 ppm

Glulisine 35uL Injection Volume

Insulin Lispro at 45pg/mL – PRM

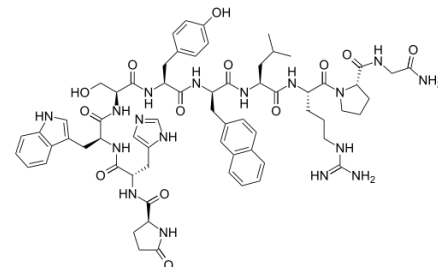
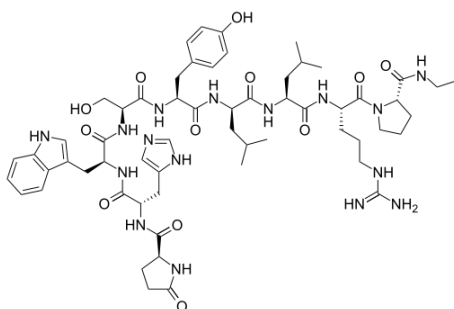


Insulin Analog	Conc. Range pg/mL	Conc. Range pM	Linear Fit (1/x ²)	Mean QC Accuracy (n=6)
Insulin Glulisine	25 - 2,880	4.3 - 496	0.9943	103.7
Insulin Glargine	25 - 2,880	4.3 - 496	0.9955	102.2
Insulin Aspart	45 - 2,880	7.7 - 496	0.9947	98.7
Insulin Detemir	180 - 11,520	30 - 1,986	0.9930	98.8
Insulin Lispro	45 - 2,880	7.7 - 496	0.9917	103.9

Simultaneous detection of all fragment ions
 Specificity from extracting narrow mass tolerances around fragment ions
 Sensitivity from summing the areas of multiple transitions

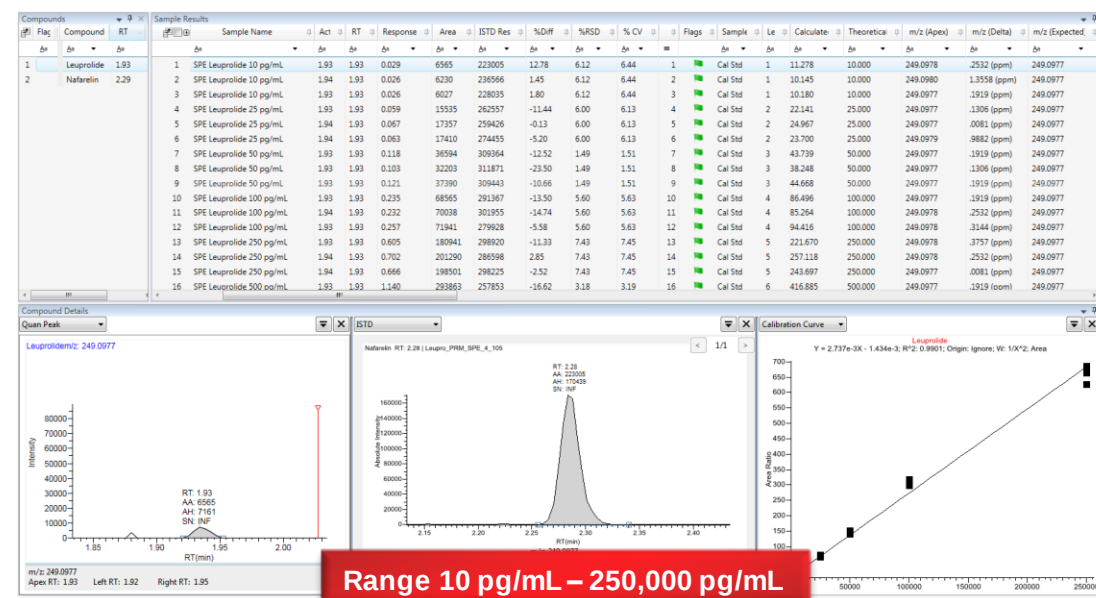
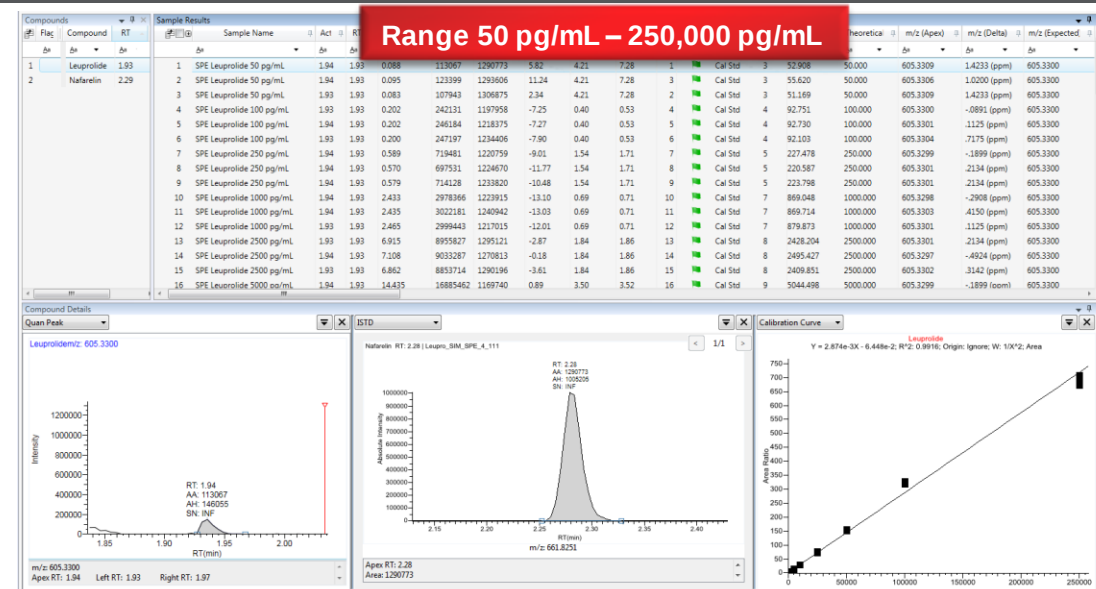
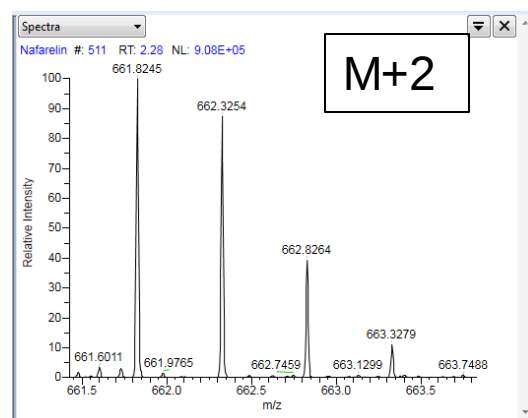
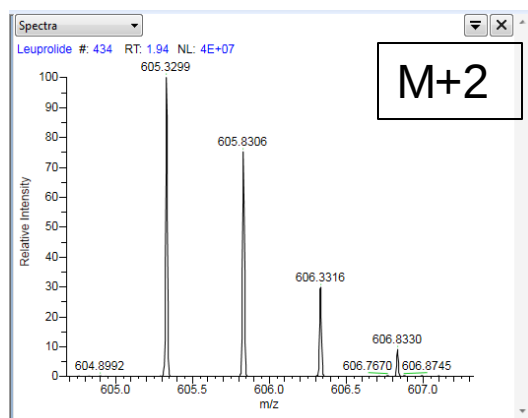
Leuprolide Quantitation – Method Summary

- Sample Prep 300 μ L human plasma – μ Sola HRP SPE
- Q Exactive Focus MS – Thermo Scientific™ Vanquish™ UHPLC system
- Column: Vanquish Hypersil Gold 2.1 x 100 mm, 1.9 μ m
- Injection Volume: 20 μ L
- MS Method: tSIM @ 70k Resolution Setting



Leuprolide MW = 1209

Nafarelin (IS) MW = 1321



Are you using
Quantitation today?

- **Full MS**

- Selectivity comes through resolution
- Parent mass sensitivity, but limited by background
- Minimal method development

Full MS

- **SIM**

- Selectivity comes through resolution
- Ultimate mass sensitivity
- Interference reduction through quad isolation
- Very little optimization needed

i-SIM

- **PRM**

- Highest level of selectivity (resolution & transition)
- Full MS/MS spectrum acquired in a single scan
- Choose transitions to quantitate on post-acquisition

PRM

- The Q Exactive Focus MS offers many different methods for quantitative analysis for different levels of sensitivity and selectivity:
 - SIM – Easy method development
 - Built-in troubleshooting
 - Matrix characterization
 - Highly sensitive and selective
- For all quantitative methods, the high resolution and mass accuracy capabilities of the Q Exactive Focus mass spectrometer provide a strong benefit for both research and routine analysis.
- Orthogonal analysis technique to complement triple quadrupole instrumentation.

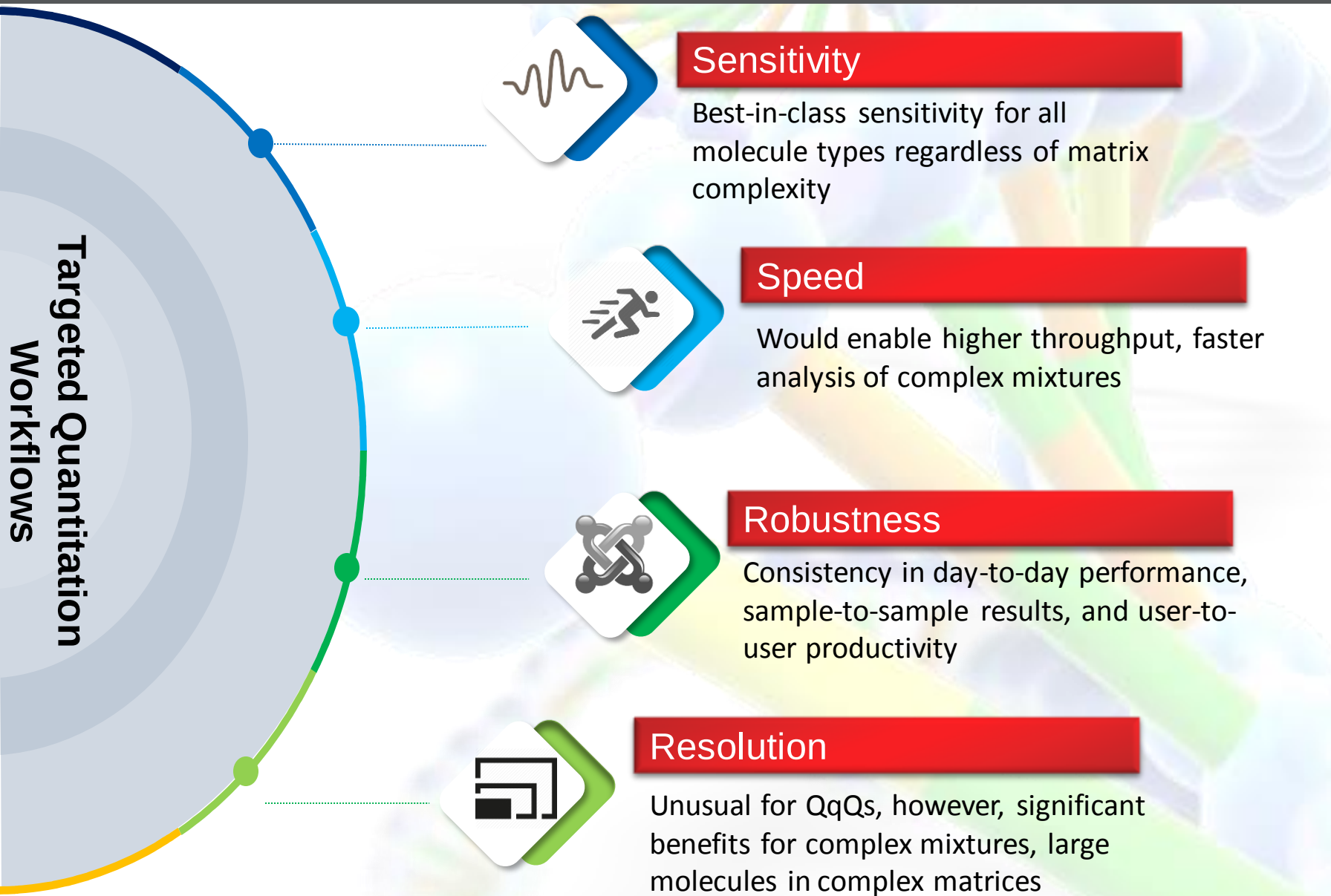
Tomorrow's Quantitation today

To learn more about tomorrow's Quantitation and how it can make a difference in your lab today, visit thermofisher.com/Quantitation

ThermoFisher
SCIENTIFIC

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Expectations from Routine Targeted Quantitation with SRM/H-SRM

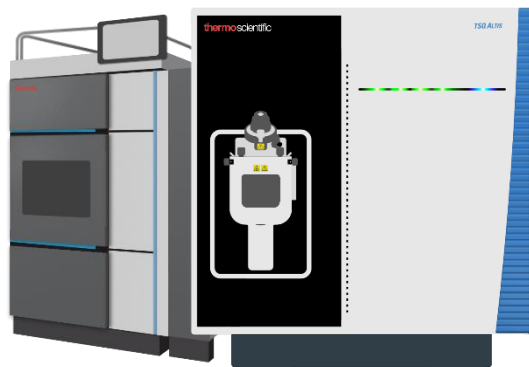


NEW Thermo Scientific TSQ Triple Quadrupole Mass Spectrometers

Thermo Scientific™ TSQ Altis™ Triple Quadrupole Mass Spectrometer

Sensitivity and robustness without compromise

- Ultimate sensitivity and speed
- Increased selectivity with H-SRM
- Unmatched analytical flexibility and reproducibility
- For the most demanding applications



AIM+
TECHNOLOGY

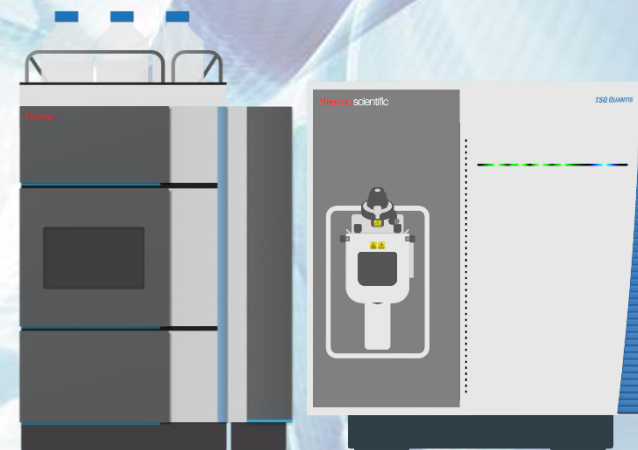
Active Ion Management+

Optimize ion management
precision, reliability, speed, and
reproducibility.

Thermo Scientific™ TSQ Quantis™ Triple Quadrupole Mass Spectrometer

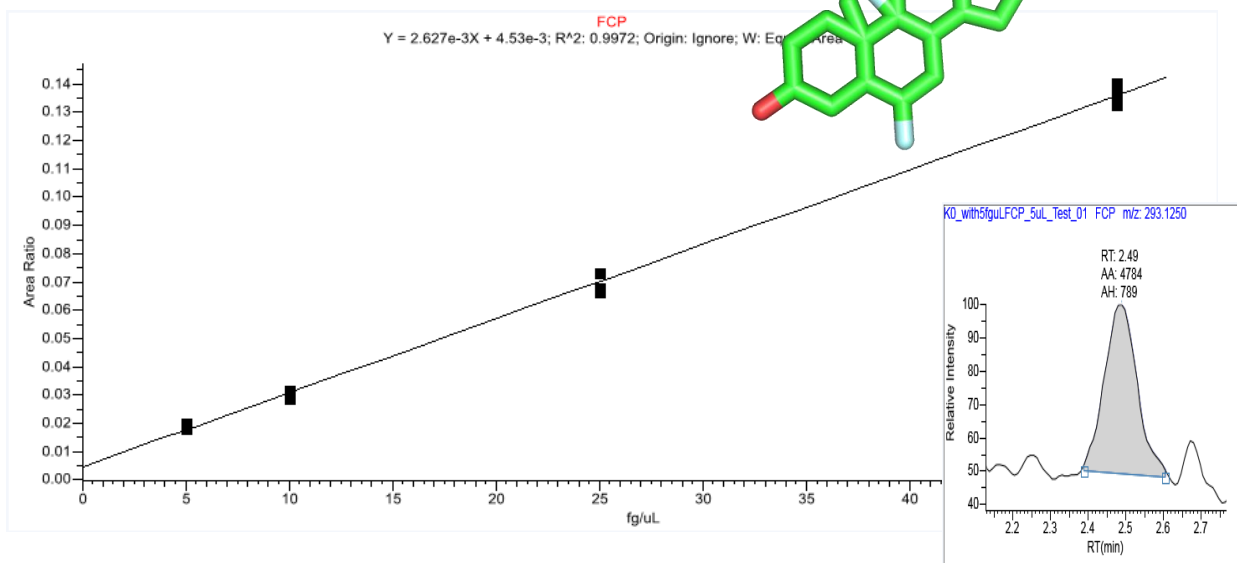
Unprecedented confidence, day after day

- Ultimate robustness
- Best-in-class sensitivity
- Increased selectivity with H-SRM
- Ease of use and maintenance



Sensitivity, Linearity

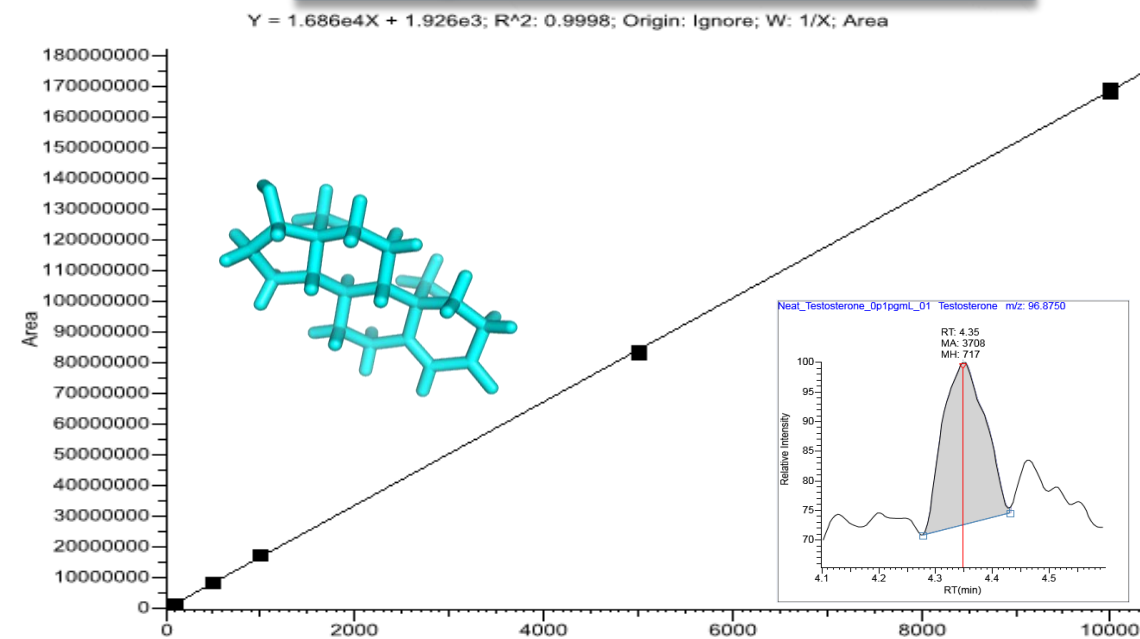
Superior sensitivity and
reproducibility - Fluticasone
Propionate in matrix!
25 fg on column with 4.2% CV!



UHPLC : Thermo Scientific™ Vanquish™ Flex Binary UHPLC System
Flow rate: 0.75 mL/min
Solvent : 75% MeOH:25% H_2O with 0.01% NH_4OH
MS: TSQ Altis Triple Quadrupole MS
Software: Thermo Scientific™ TraceFinder™ Software 4.1

Experimental Details

Superior sensitivity and
reproducibility – Testosterone in
human plasma
5 fg on column with 3% CV!



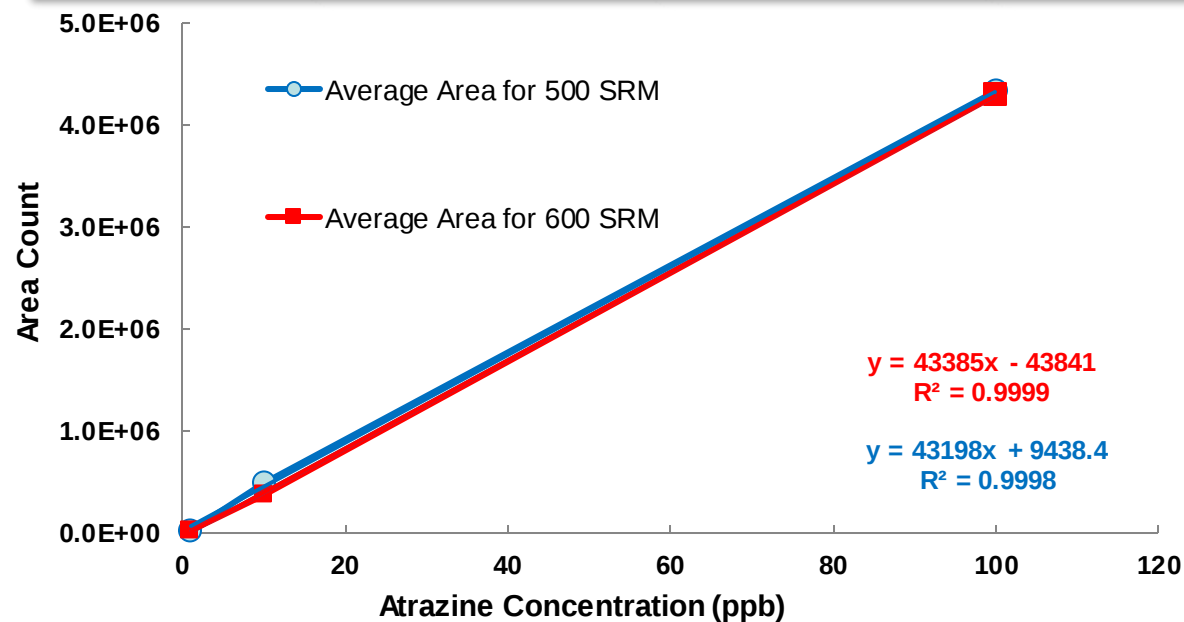
UHPLC : Thermo Scientific™ Vanquish™ Flex Binary UHPLC System
Flow rate: 0.4 mL/min
Solvent A: 0.5 mM NH_4F in Water
Solvent B: MeOH
MS: TSQ Altis Triple Quadrupole MS
Software: TraceFinder Software 4.1

Experimental Details

For Research Use Only

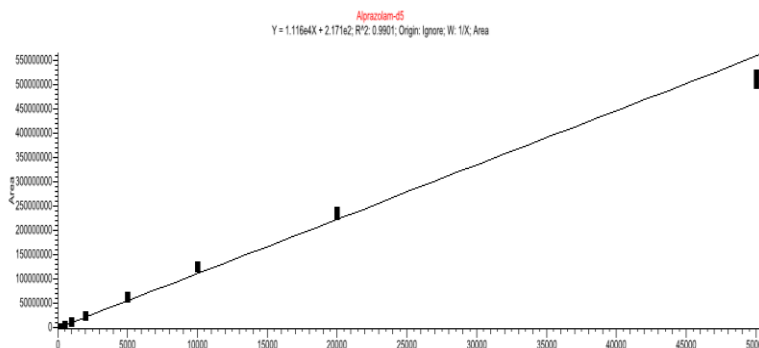
Ensuring High Speed Capabilities

**Equivalent quantitative performance between 500 and 600 SRMs/sec!
Good performance at extremely low dwell times!**



SRMs/Sec	Total Number of Transitions	Dwell Time (mSec)
500	1075	0.769
600	1291	0.437

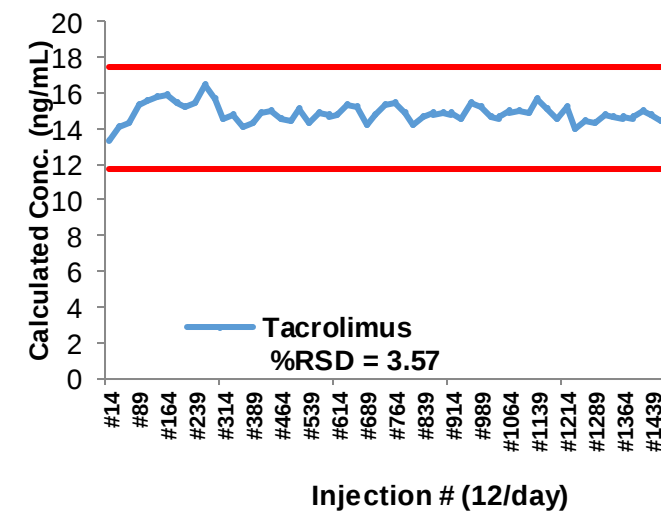
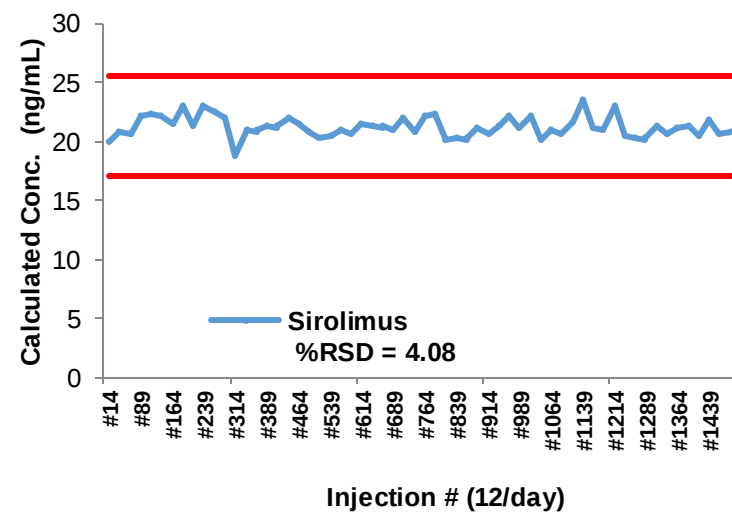
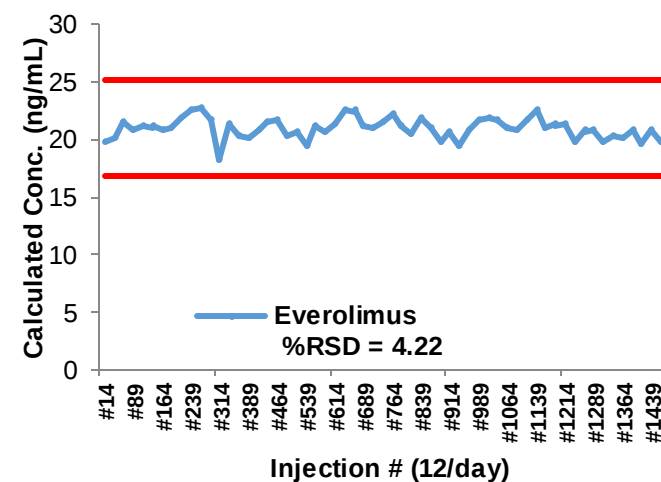
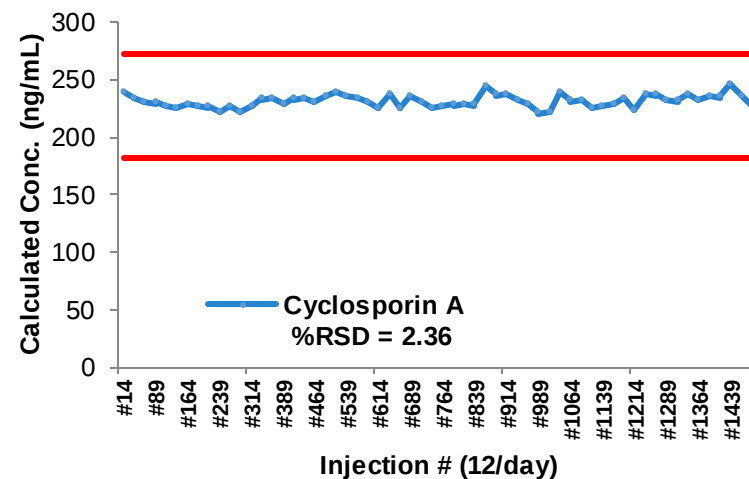
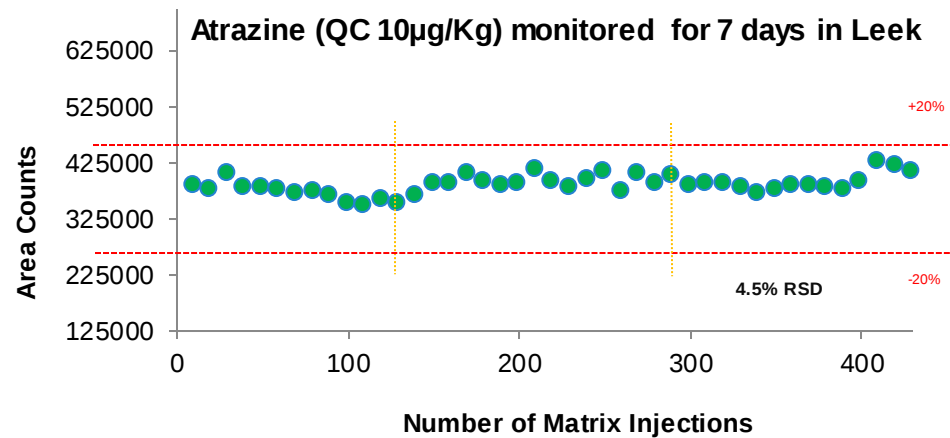
Atrazine Concentration (ppb)	500 SRM/Second		600 SRM/Second	
	Average Area	%CV	Average Area	%CV
1	21682	9	18090	9
10 (MRL)	475465	4	369612	5
100 (MRL)	4326117	1	4296555	1



Alprazolam 0.05 – 50,000 fg/uL (6 orders of magnitude)

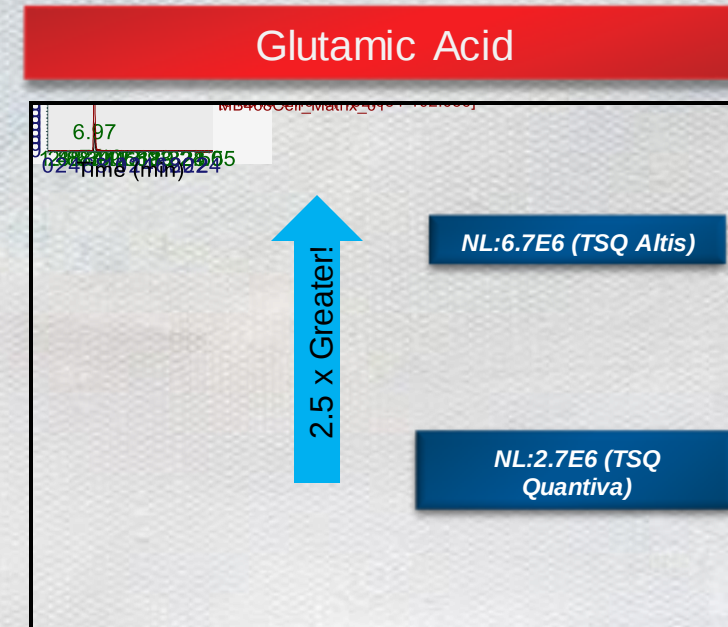
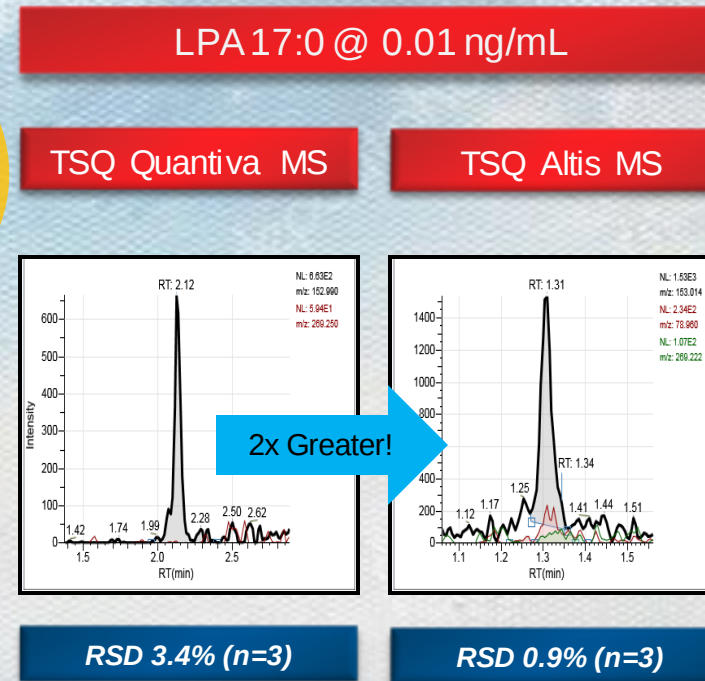
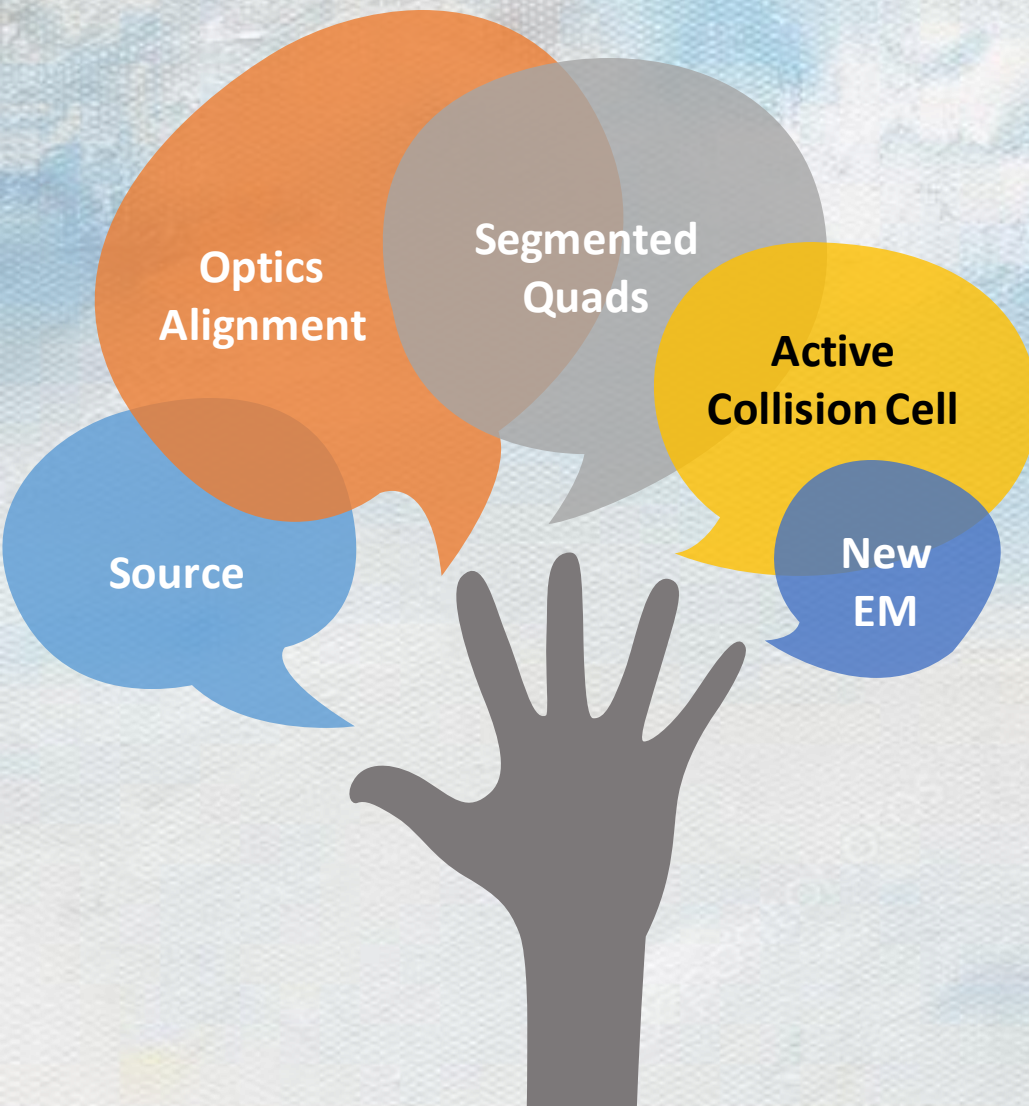
Data acquired on TSQ Altis MS

Ensuring Robustness



For Research Use Only

Five Features that Ensure Increased Sensitivity with Better Reproducibility

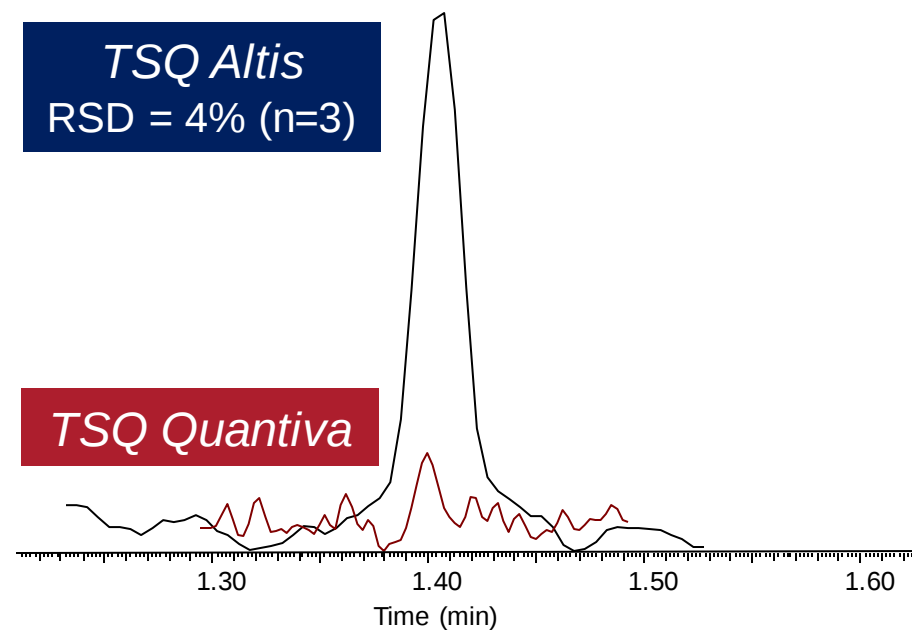


Quantitation of Therapeutic Drugs in Plasma

TSQ Altis Triple Quadrupole Mass Spectrometer

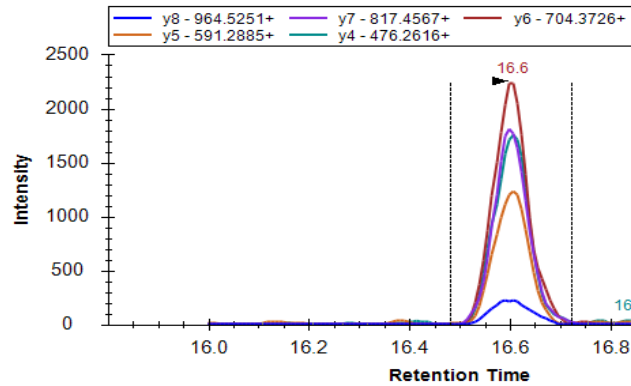
Compound Name	TSQ Quantiva LOQ (pg/mL)	TSQ Altis LOQ (pg/mL)
Desomorphine	5	5
Desmethyldoxepin	10	2.5
Flecainide	2.5	1
Midazolam	5	2.5
Imipramine	10	2.5
Amitriptyline	10	2.5
Fluoxetine	5	5
Diazepam	5	2.5

Quantitation of Desmethyldoxepin in plasma 2.5 pg/mL



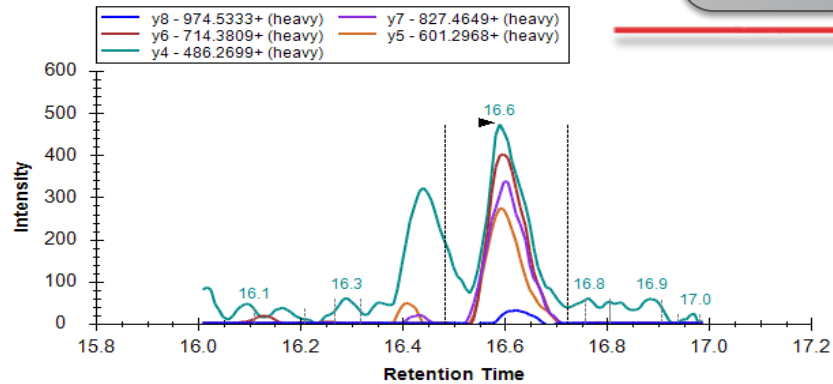
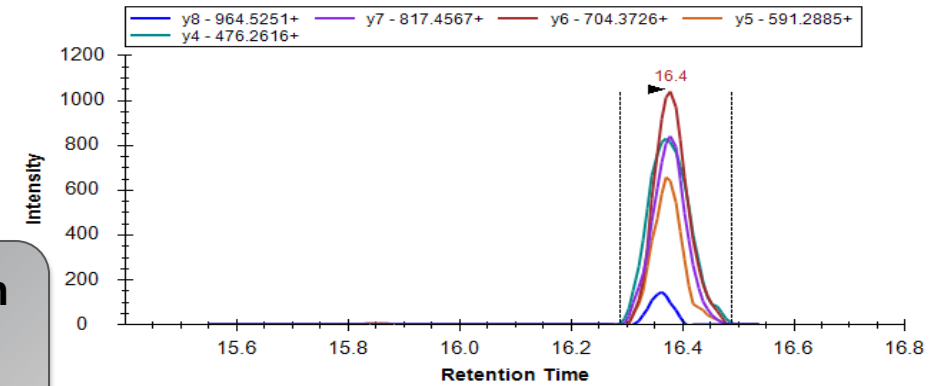
~ 2.5 X average sensitivity improvement over Thermo Scientific™ TSQ Quantiva™ MS

Protein Quantitation – Benefits of Higher Selectivity and Sensitivity

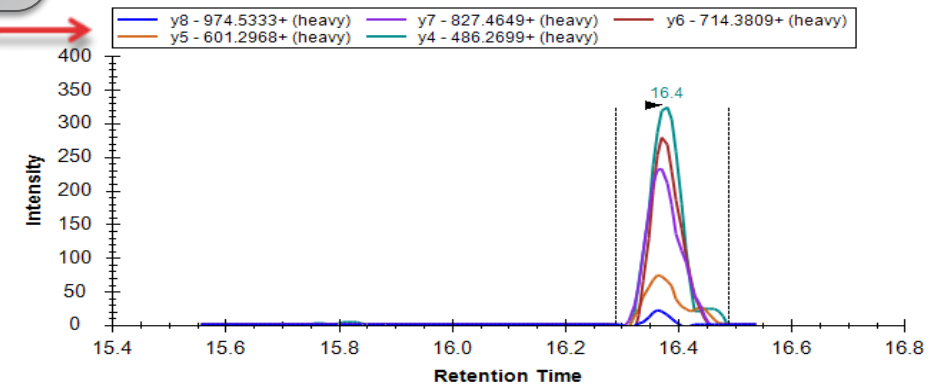


Light Peptide

Approximately 50% transmission
from
0.7 FWHM → 0.2 FWHM



Heavy Peptide



SRM

0.5 fmol NGFILDGFPR in Plasma digest

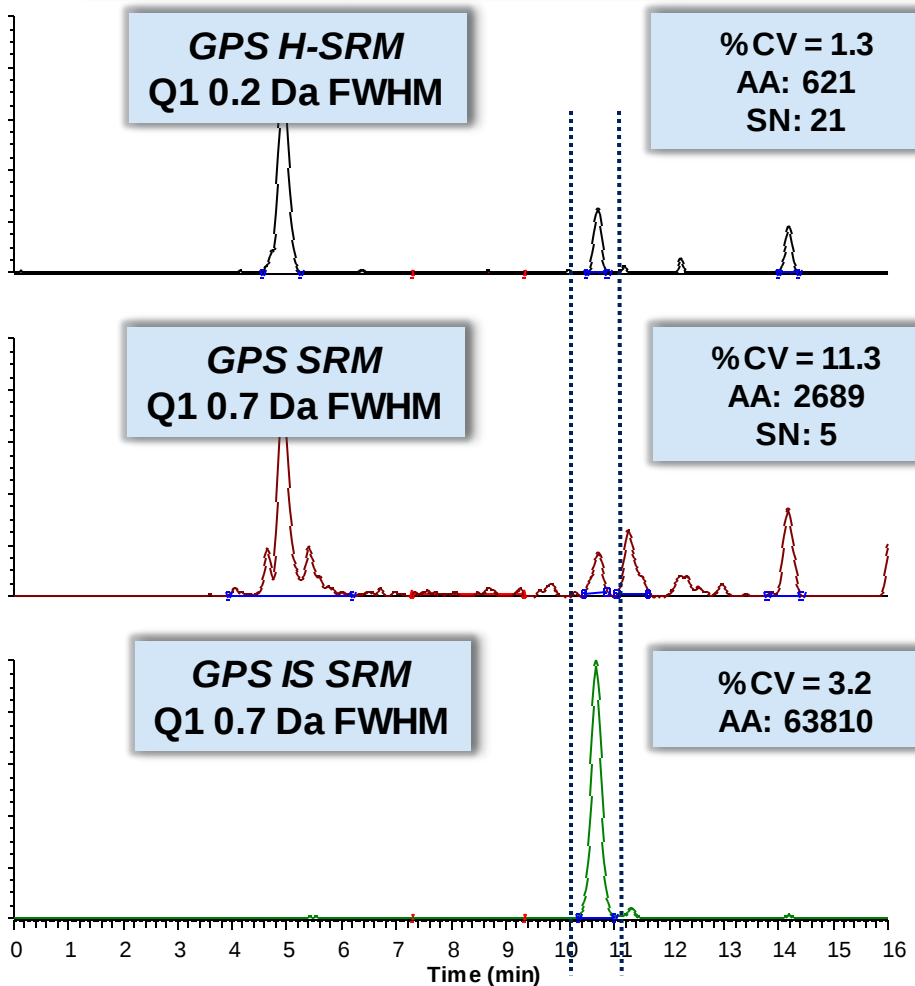
H-SRM

Experimental Details

LC : Thermo Scientific™ EASY-nLC™ 1200 System
Flow rate: 300 nL/min
Solvent A: 2% ACN with 0.1% FA in H₂O
Solvent B: 10% H₂O with 0.1 % FA in ACN

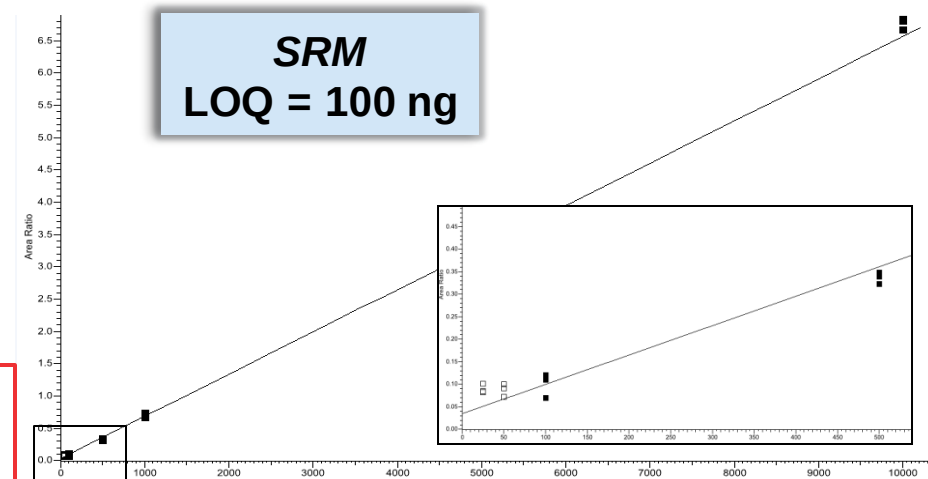
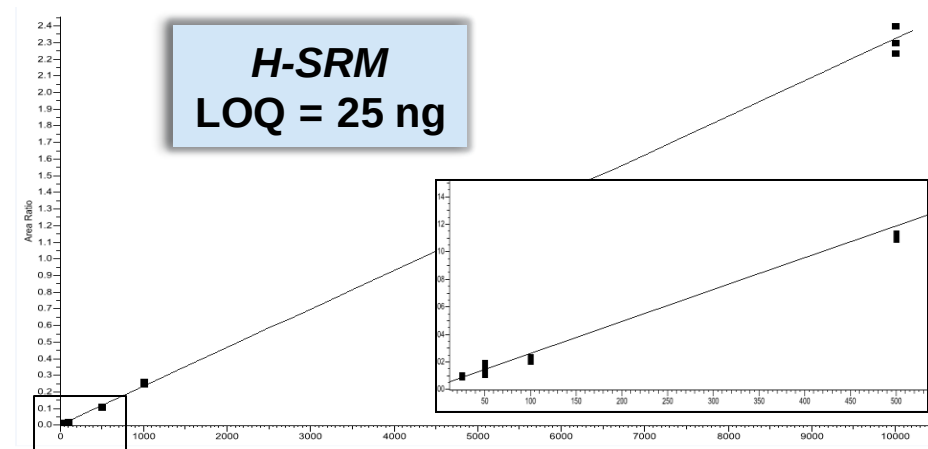
Superior Sensitivity with H-SRM (0.2 Da FWHM) – GPSVFPLAPSSK

GPSVFPLAPSSK - Peptide from monoclonal antibody hinge region



Experimental Details

LC : Thermo Scientific™ Ultimate™
NCS-3500RS
Flow rate: 25 μ L/min
Solvent A: 2% ACN in H₂O w/0.1% FA
Solvent B: 5% H₂O in ACN w/0.1% FA

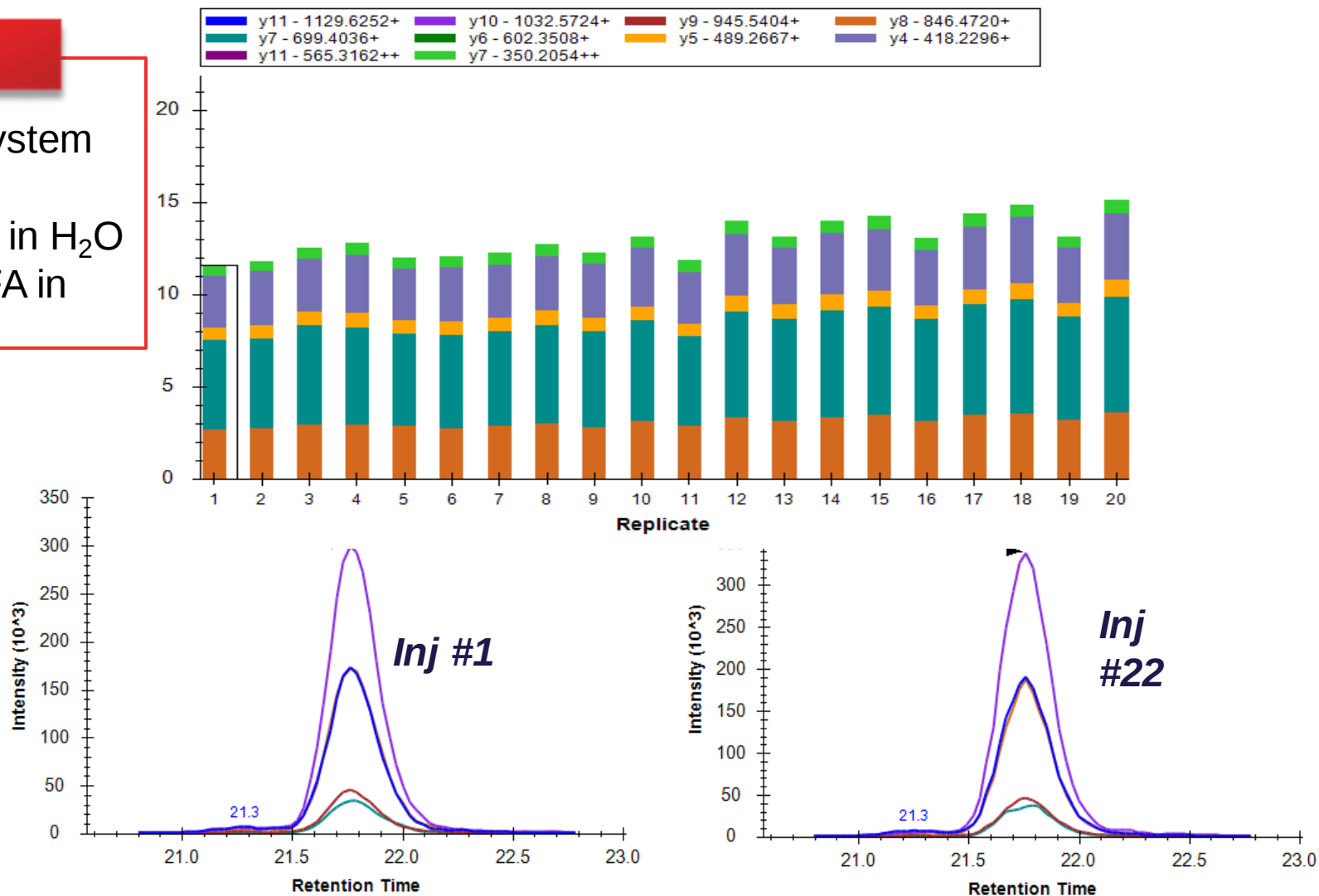


Peptide Quantitation with Thermo Scientific TSQ Quantis Triple Quadrupole MS

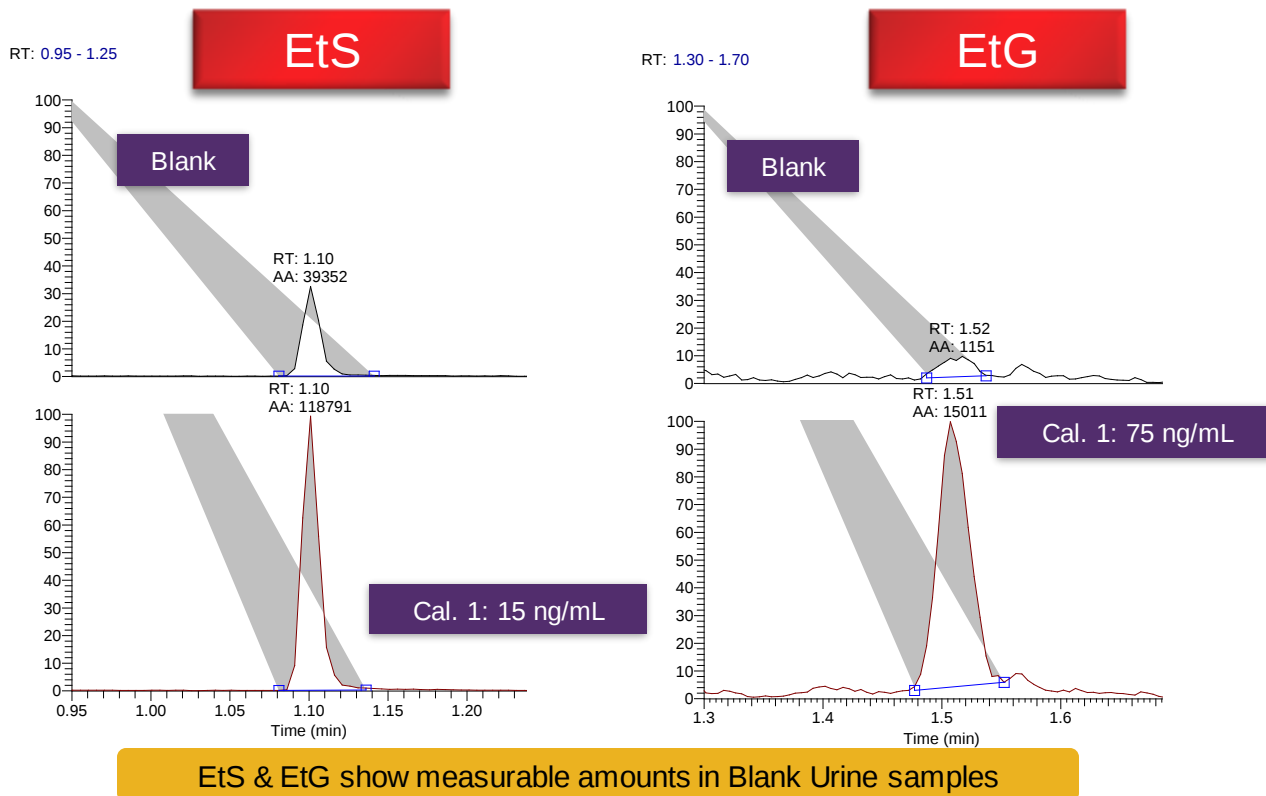
Experimental Details

UHPLC : EASY-nLC 1200 system
Flow rate: 300 nL/min
Solvent A: 2% ACN 0.1% FA in H₂O
Solvent B: 10% H₂O 0.1 % FA in ACN

Consistent ion ratios
across wide mass range
for confident peptide
quantitation



Example SRM Chromatograms – EtS & EtG



- TSQ Quantis MS provides excellent quantitative performance for LC-MS/MS of EtS, EtG and barbiturates in diluted urine in under 4 minutes.
 - LLOQs of 15 ng/mL and 75 ng/mL were achieved for EtS and EtG, respectively, in diluted urine.
 - LLOQs of 200 ng/mL (urine, diluted 1:10) were achieved for 5 barbiturates
- All target compounds can be analyzed with the same LC method.

Thermo Scientific™ Vanquish™ Horizon UHPLC system

Column: 3.0 x 100 mm, 3 um Synchronis AQ
Column Temp: 30 C
Mobile Phase: [A] H₂O + 0.1% HAc; [B] MeOH
Flow Rate: 0.5 mL/min
Gradient: see table
Injection Volume: 10 uL (EtG/EtS); 5 uL (Barbs)

TSQ Quantis Triple Quadrupole MS

Ionization Mode: HESI, Negative ion mode
MS Acquisition Mode: Selective Reaction Monitoring (SRM) – see SRM Table next slide
Cycle time: 0.30 s
Quad Isolation: Q1 = Unit (0.7 Da FWHM); Q3 = Unit or Wide (1.2 Da; Barbs)



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Triple Quadrupole MS Internal Beta Testing: Johns Hopkins Hospital

Goal: Evaluation of TSQ Quantis Triple Quadrupole MS using established TSQ Endura Triple Quadrupole MS method for Immunosuppressants in real donor samples.

Donor	John Hopkins (ng/mL)	San Jose (ng/mL)	(JH-SJ)/Mean
Cyclosporin A-1	1186	1219	2.8%
Cyclosporin A-2	255	273	6.7%
Cyclosporin A-3	51	52.0	1.9%
Cyclosporin A-4	64	64.8	1.2%
Cyclosporin A-5	112	119	5.8%
Sirolimus-1	6.5	9.22	35%
Sirolimus-2	9.8	6.81	-36%
Sirolimus-3	6.3	7.34	15%
Sirolimus-4	18.5	16.3	-13%
Sirolimus-5*	0.5	0.31	-46%
Tacrolimus-1	4.0	4.06	1.4%
Tacrolimus-2	5.9	6.10	3.3%
Tacrolimus-3	2.5	2.95	17%
Tacrolimus-4	11.7	11.9	1.4%
Tacrolimus-5*	1.9	1.84	-3.0%

* Value is Below Limit of Quantitation

- FDA GLP Guidance 2/3 (66.7%) of the values on new system should be within $\pm 20\%$ to validate the method
- 12/15 = 80% Passed. Method transferred successfully!

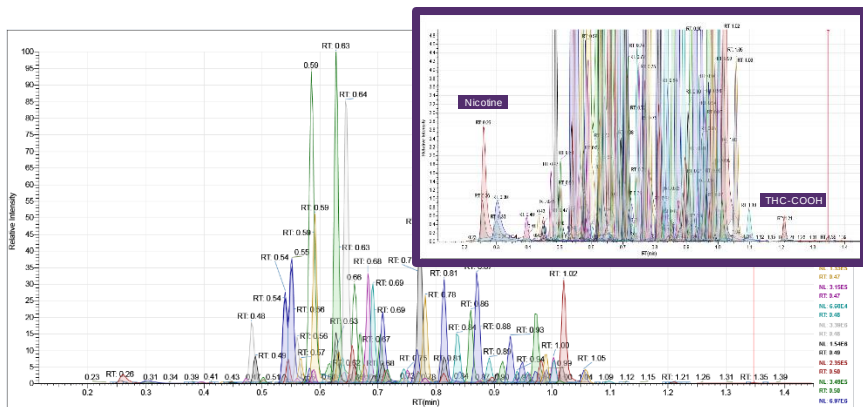
Easy transfer of established method from Thermo Scientific™ TSQ Endura™ Triple Quadrupole MS to TSQ Quantis Triple Quadrupole MS in real world samples!

Experimental Details

UHPLC : Thermo Scientific™ Transcend™ II System
 Flow rate: 0.8 mL/min
 Solvent A: 0.1% FA + 10 mM ammonium formate in H₂O
 Solvent B: 0.1% FA + 10 mM ammonium formate in MeOH

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~100 Drugs of Abuse: Diluted Urine @ 1 mL/min



Vanquish Horizon UHPLC System

Column: 2.1 x 50 mm, 1.9 μ m Hypersil Gold AQ

Column Temp: 40 C

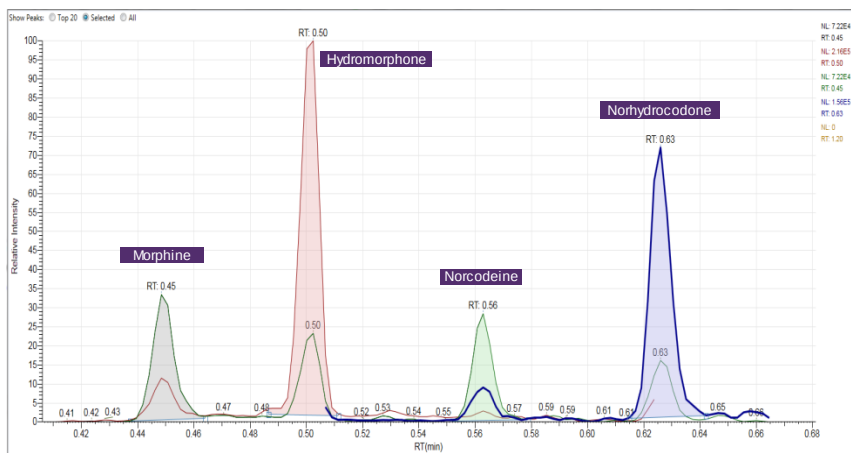
Mobile Phase: [A] H₂O + 0.1% HCOOH; [B] ACN + 0.1% HCOOH

Flow Rate: 1.0 mL/min (no split)

Gradient: see table

Injection Volume: 2 μ L

Sensitivity, acquisition speed and ruggedness of the TSQ Quantis MS, and the reproducibility of the Vanquish Horizon UHPLC system, make the measurement of ~100 drugs of abuse in diluted urine in under 1.5 minutes possible.



TSQ Quantis MS

Ionization Mode: HESI, Positive ion mode

MS Acquisition Mode: Selective Reaction

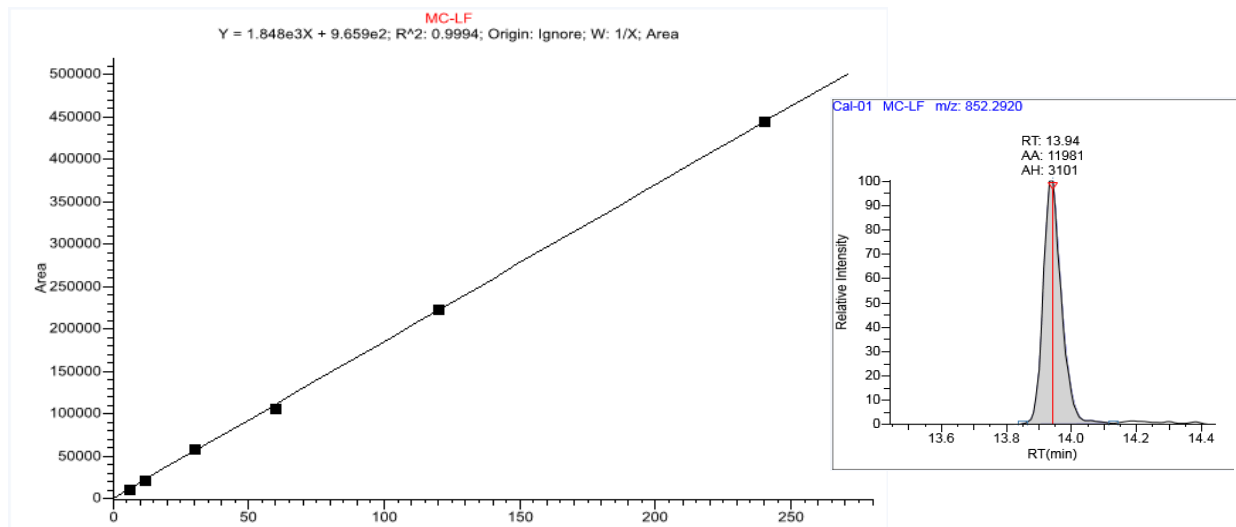
Monitoring (SRM) Cycle time: 0.15 s

Quad Isolation (Q1,Q3) = Unit (0.7 Da FWHM)



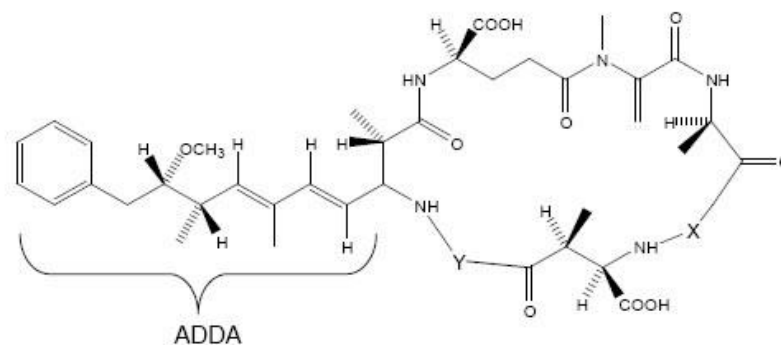
Triple Quadrupole MS Internal Beta Testing: Eurofins Eaton

Goal: Sensitivity and Reliability of TSQ Quantis MS- Regulatory Methodology (EPA 544)



In 2014, half a million people were without drinking water in the state of Ohio due to high toxin levels!

EPA 544 released in February 2015

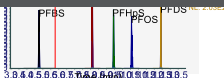


Algae blooms in Western Lake Erie

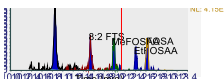


~4X average improvement over TSQ Endura Triple Quadrupole MS

PFAS: Separation of Perfluorinated Alkylsulfonates, 50 pg/mL



PFASs C4 – C10 at 50 pg/mL (0.15 pg on-column); LODs are ~10 pg/mL (0.03 pg O.C.)



C8-PFAS at 50 pg/mL (0.15 pg on-column); LODs are ~10 pg/mL (0.03 pg O.C.)

Vanquish Horizon UHPLC system

Delay Column: 3.0 x 50 mm, 5 μ m BDS Hypersil C8
Analytical Column: 2.1x100 mm, 2.6 μ m Accucore C18
Column Temp: 30 C
Mobile Phase: [A] H₂O + 10 mM Am. Acetate; [B] MeOH
Gradient: see table
Injection Volume: 3 μ L

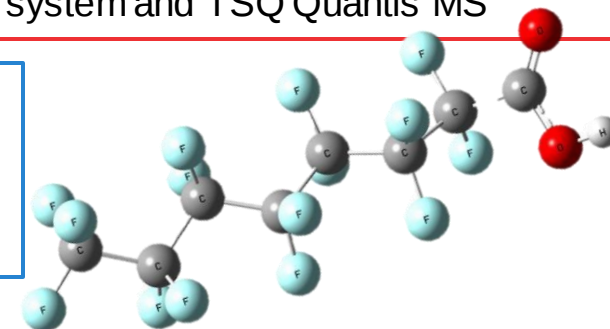
TSQ Quantis MS

Ionization Mode: HESI, Positive ion mode
MS Acquisition Mode: Selective Reaction Monitoring (SRM) **Cycle time: 0.15 s**
Quad Isolation (Q1,Q3) = Unit (0.7 Da FWHM)

PFAS can cause reproductive and developmental, liver and kidney, and immunological issues

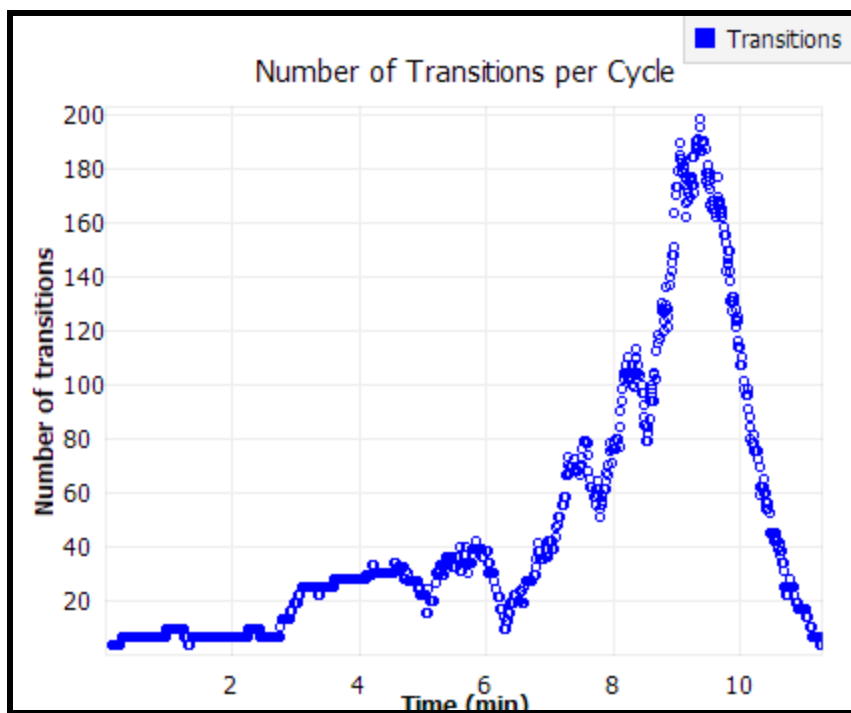
Increased cholesterol levels among exposed populations, low infant birth weights, effects on the immune system, cancer, and thyroid hormone disruption

Robust, reproducible, sensitive quantitative assays for detection and quantitation of PFAS were developed using Vanquish Flex Binary UHPLC system and TSQ Quantis MS

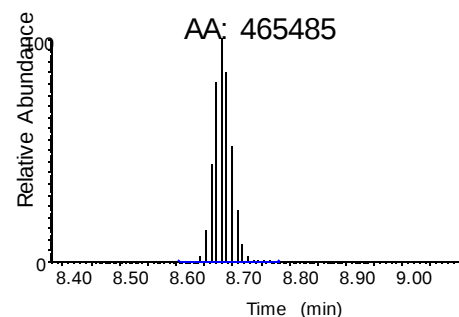


Pesticides in Leek: Robust, Reliable, Fast Quantitation Workflows

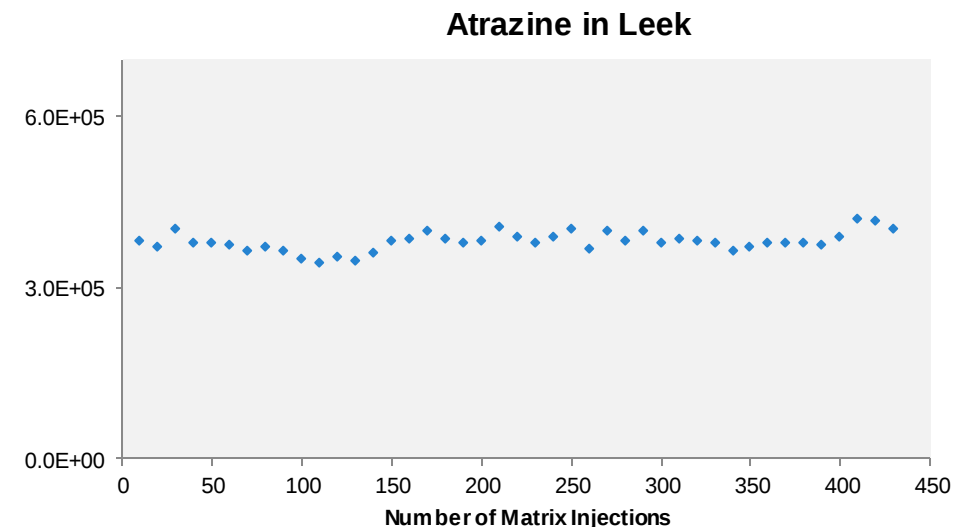
Increase productivity - monitoring more compounds per unit time!
Excellent quantitative performance at lower dwell times!
Robust data enables maximizing uptime!



SRM visualization from instrument control software 3.0 displaying the number of transitions per unit time



Azoxystrobin 1 ppb in Leek
Monitored at 2.5 msec Dwell Time



**~ 160 transitions monitored simultaneously with
polarity switching. Excellent reproducibility (% RSD 2.3) below the MRL.**

IC-MS/MS solutions for environmental analysis Quantitation of **Glyphosate** at **5 ng/L**, **3% RSD**

Experimental Details

Thermo Scientific™ Dionex™ ICS-5000+ HPIC™ System

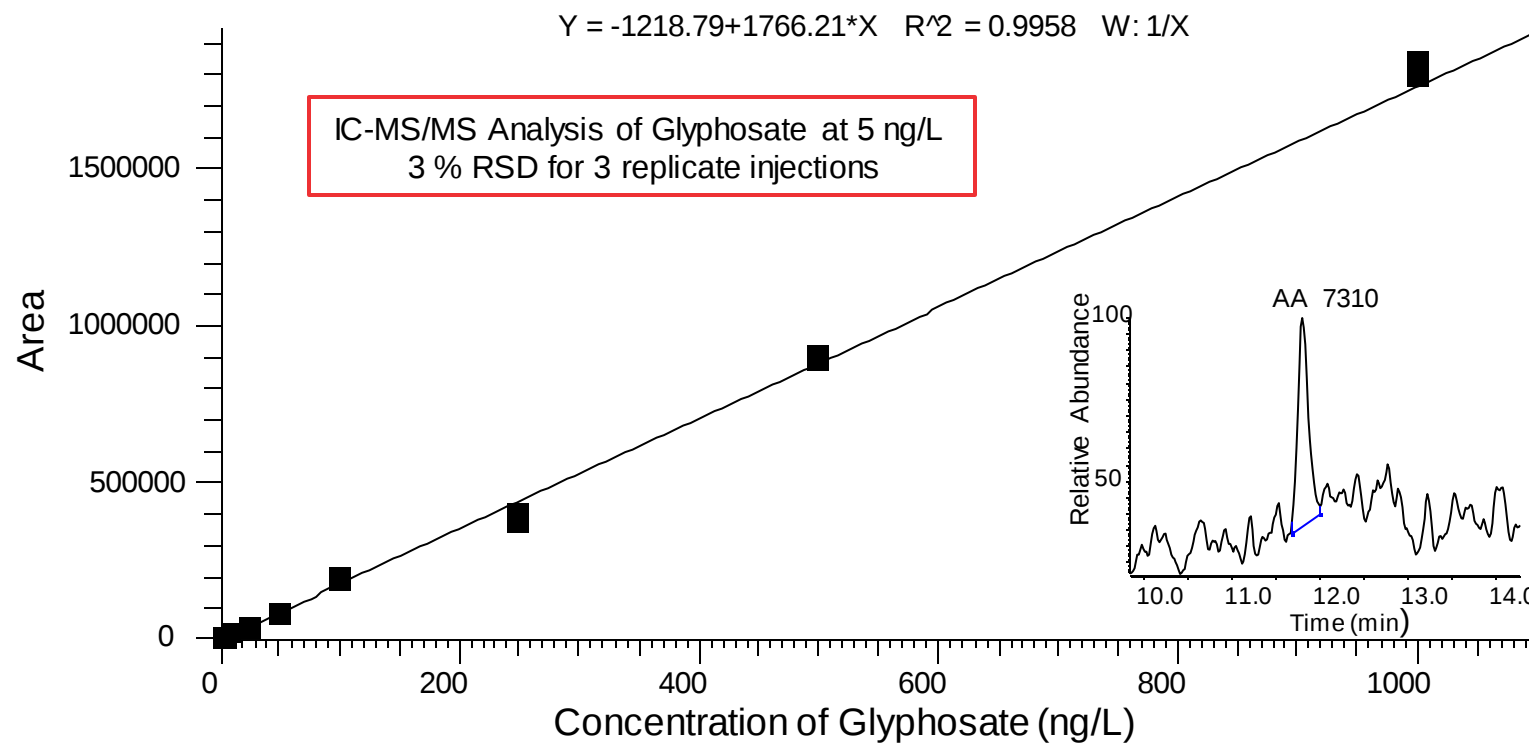
Flow rate: 0.3 mL/min

Eluent Source: Eluent Generator

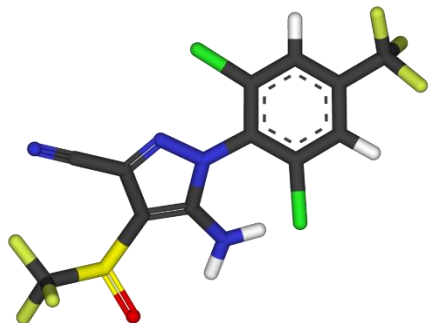
Mobile Phase: KOH

MS: TSQ Altis MS

Software: TraceFinder Software 4.1



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.



The contaminated eggs originated from Dutch farms. They have since been reported in 15 other countries and Hong Kong.



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.

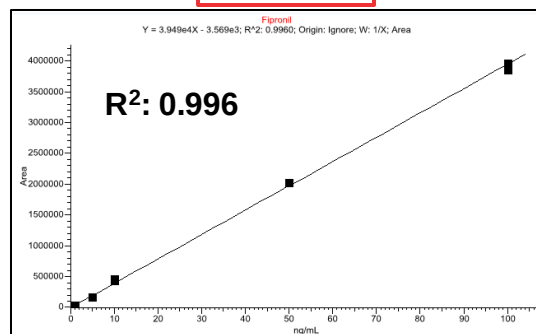
What's Needed?



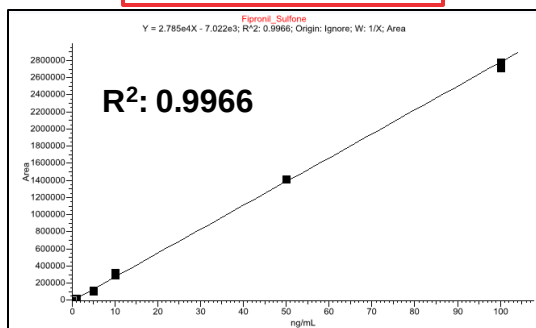
Robust, sensitive, reliable analysis and quantitation of Fipronil and Fipronil Sulfone in eggs

Matrix Matched Calibration for UHPLC-MS/MS

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	104	89	99
Fipronil sulfone	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	0.1	0.5	8.5	6.1
Fipronil sulfone	0.1	0.5	7.7	6.4

Ion Ratio Calculations	Fipronil (SRM 249.96)	Fipronil (SRM 398.845)	Fipronil sulfone (SRM 282.00)	Fipronil sulfone (SRM 243.845)
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

Reproducibility and Long-term Stability Test

thermoscientific

APPLICATION BRIEF 72483

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

Authors

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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.² 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,^{3,4} 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

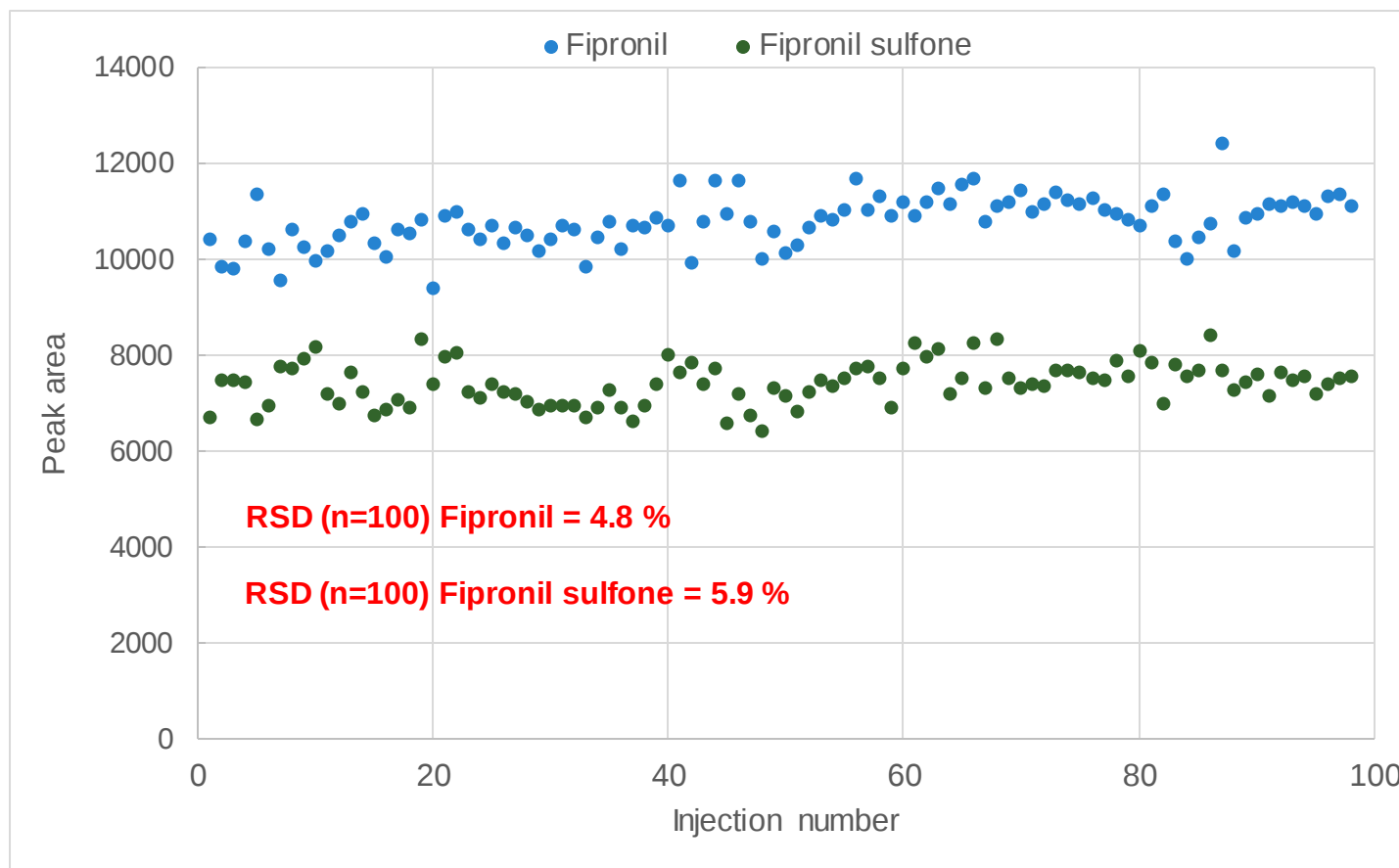
Sample preparation

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

Sample preparation consumables

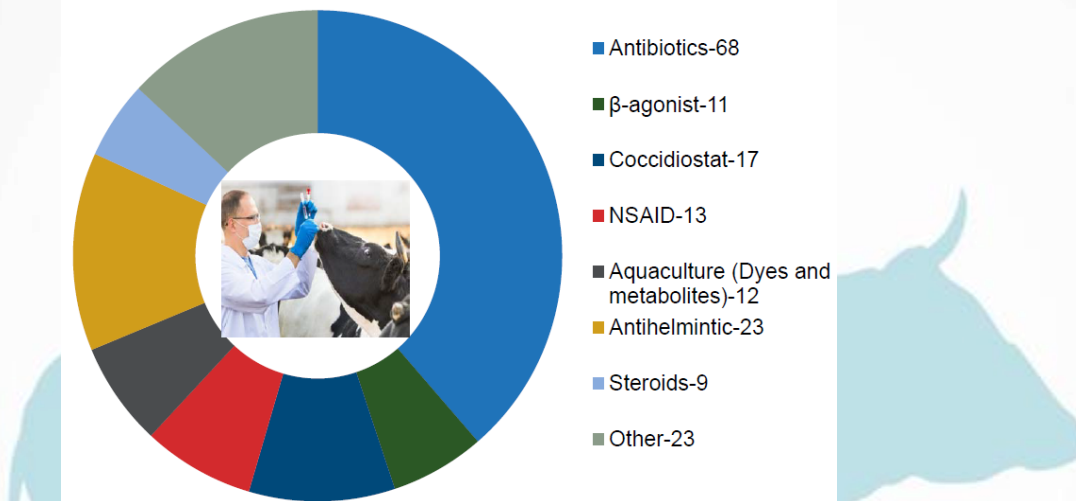
- 50 mL conical sterile polypropylene centrifuge tubes; P/N 330652
- 15 mL conical sterile polypropylene centrifuge tubes; P/N 330650
- Thermo Scientific™ HyperSep™ dispersive SPE Mylar pouch 4000 mg magnesium sulfate and 1000 mg NaCl, 50 pk, P/N 60105-340
- Magnesium sulfate, 99%, for analysis, anhydrous; 12106721

ThermoFisher
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Vet Drugs Analysis and Quantitation

• Compounds and Chemical Classes



• Multi-class veterinary drugs analysis showing

- Fit for purpose Thermo Scientific™ Acclaim™ Polar Advantage II (PA2) reversed-phase column for robust analysis, great peak shape for wide range of compound classes
- Generic QuEChERS extraction applied to bovine, salmon filet, and milk; easy-to-use, low cost, with no extraction concentration
- Robust, sensitive, reproducible results for absolute recovery, precision, and low MDLs for most analytes

• Quantitative Results across Matrices

Sample Prep:

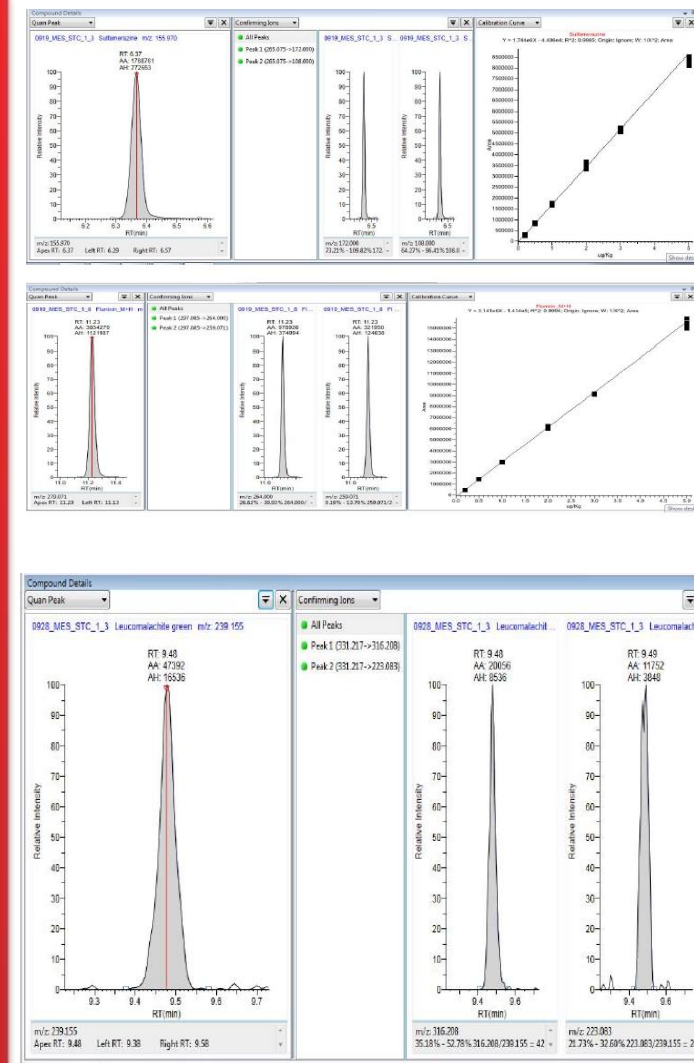
- QuEChERS based approach

LC Conditions:

- Thermo Scientific™ Acclaim™ VANQUISH™ PA2 UHPLC columns (2.1x150x2.2 μm)
- 2 μL injection

MS Conditions:

- TSQ Altis MS (comprehensive database with all optimized SRMs)
- Positive/negative switching



- **Market Specific Software: TraceFinder**

- SRM databases for environmental, food safety, clinical research, and forensic toxicology applications: easy instrument method setup
- Compatible with GC and LC high resolution systems. One software package for different platforms
- Customized reports
- Flexibility on data review – compound or sample centric modes for easy data review. Customized flagging system for easy review of important criteria.

- **Instrument Control Software: Tune 3.0**

- **User**

- Automated Compound Optimization
(via infusion or Autosampler)
- Dynamic Retention Time (dRT)
- Method Conversion Tool (Agilent/Sciex)
- Improved Auto tune and Calibration Routines (Faster with improved reporting)
- Favorites – Easy to setup source settings for calibration

- **Service**

- New/Smarter Diagnostics – better serviceability of the system – improved reporting for service.



HRAM Adoption at Mayo Clinic for Routine Clinical Testing



R J Singh

"Better accuracy and the ability to collect discovery data while simultaneously running clinical assays give HRAM instruments significant advantages compared to triple quadrupoles"

"The HRAM capabilities of the Q Exactive were key to our 26 steroid metabolite assay's development given the structural similarities of the steroid metabolites. The higher resolution and level of confidence provided by HRAM reduces the risk for patients and us from an incorrect result"

"Currently, cost remains a major limiting factor....if money were not an issue, we would like to do all clinical assays on a [HRAM] instrument"



"The Q-Exactive is perfectly positioned to become the next gold standard in clinical/tox quantitative drug analysis"

- Calibration stability
- Higher resolution (compared to TOF)
- Dynamic range, accuracy
- Potential of new and better instruments (higher resolution)"

Quotes taken from Clinical Chemistry article September 2017



Jun Qu
SUNY Buffalo

TESTIMONIALS

“With the new innovations in the Thermo Scientific TSQ Altis MS, my lab can develop quantitative methods for biotherapeutic proteins and target receptors with extreme sensitivity, selectivity, accuracy and precision. This is very exciting for our research since this capacity is very unique”



Sergey Tumanov
Beatson Inst. Cancer Res.

TESTIMONIALS

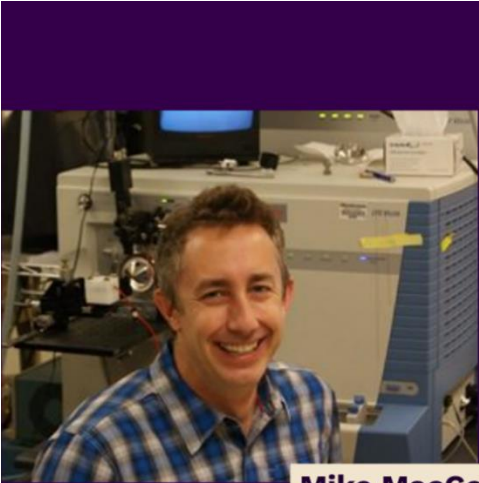
“It was a great pleasure to have a chance to talk to engineers who work on finalizing or testing the prototypes. I find it inspirational when instrumentation prototyping is driven by the input from the researchers”

TESTIMONIALS

“The sensitivity, speed and robustness of the TSQ Altis triple quadrupole MS improves already solid and stable experiments.”



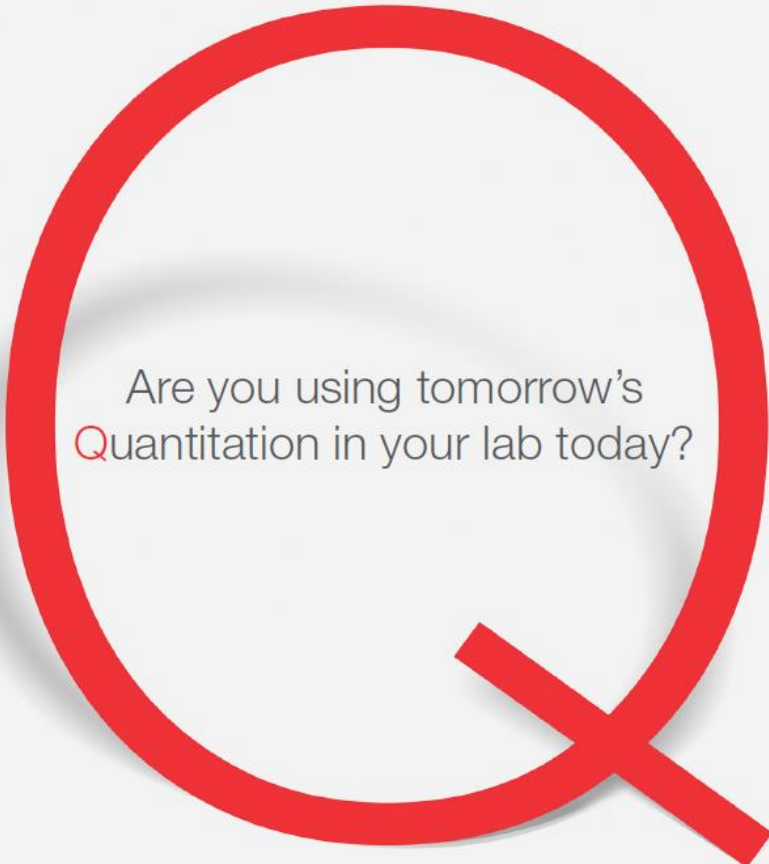
Mike Kinter
Associate Member
Oklahoma Medical Research Foundation



Mike MacCoss
University of Washington

TESTIMONIALS

“Hard to beat the cost per selectivity ratio!!!!”



Are you using tomorrow's
Quantitation in your lab today?

Tomorrow's Quantitation today

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QqQ Quantitation (with SRM and H-SRM) delivers:

Ultimate sensitivity delivered for molecules of all types, matrices, and user expertise.

High-resolution SRM enables additional selectivity, which also ensures increased sensitivity.

Ease-of-use with robust workflow solutions across all applications.

Supreme quantitative performance exhibited by both QqQs, TSQ Altis MS and TSQ Quantis MS.

Join the Fun! *Cache a Chromeleon* Game

- Use your mobile device to complete challenges and earn a Charlie Chromeleon plush toy!
- If you are playing, you have earned points for attending this seminar. Be sure to scan the barcode on the desk outside the door.
- Ask booth staff for more details on how to play.



Please join me in the
Informatics and Chromatography Software
section of our booth where I'll
address additional comments and questions.