Confident Quantitation

Any compound, any matrix, any user.

ThermoFisher SCIENTIFIC

Tomorrow’s Quantitation with Triple Quadrupole MS

Confident Quantitation

1. An Introduction to the TSQ portfolio
2. Features and Benefits
3. Robust Solution

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Confident Quantitation

1. An Introduction to the TSQ portfolio
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PERFORMANCE

Environmental and Food Safety
Clinical Research
Pharma QA/QC

TSQ Fortis

- Mass Range m/z 5 – 3000
- Max Resolution ***0.4 FWHM***
- Max 30,000 transitions per run
- Polarity Switching < 20 msec
- Dynamic interscan time
- 600 SRM/sec
- TNG software
- Chromeleon support
- ***50,000:1 S/N***

Food Safety
Pharma
Clinical Research
Forensic Toxicology

TSQ Quantis

- Mass Range m/z 5 – 3000
- Max Resolution ***0.4 FWHM***
- Max 30,000 transitions per run
- Polarity Switching < 20 msec
- Dynamic interscan time
- 600 SRM/sec
- TNG software
- Chromeleon support
- ***150,000:1 S/N***

Pharma/Biopharma
Environmental and Food Safety
Omics

TSQ Altis

- Mass Range m/z 5 – 2000
- Max Resolution ***0.2 FWHM***
- Max 30,000 transitions per run
- Polarity Switching < 20 msec
- Dynamic interscan time
- 600 SRM/sec
- TNG software
- Chromeleon support
- ***500,000:1 S/N***

VALUE

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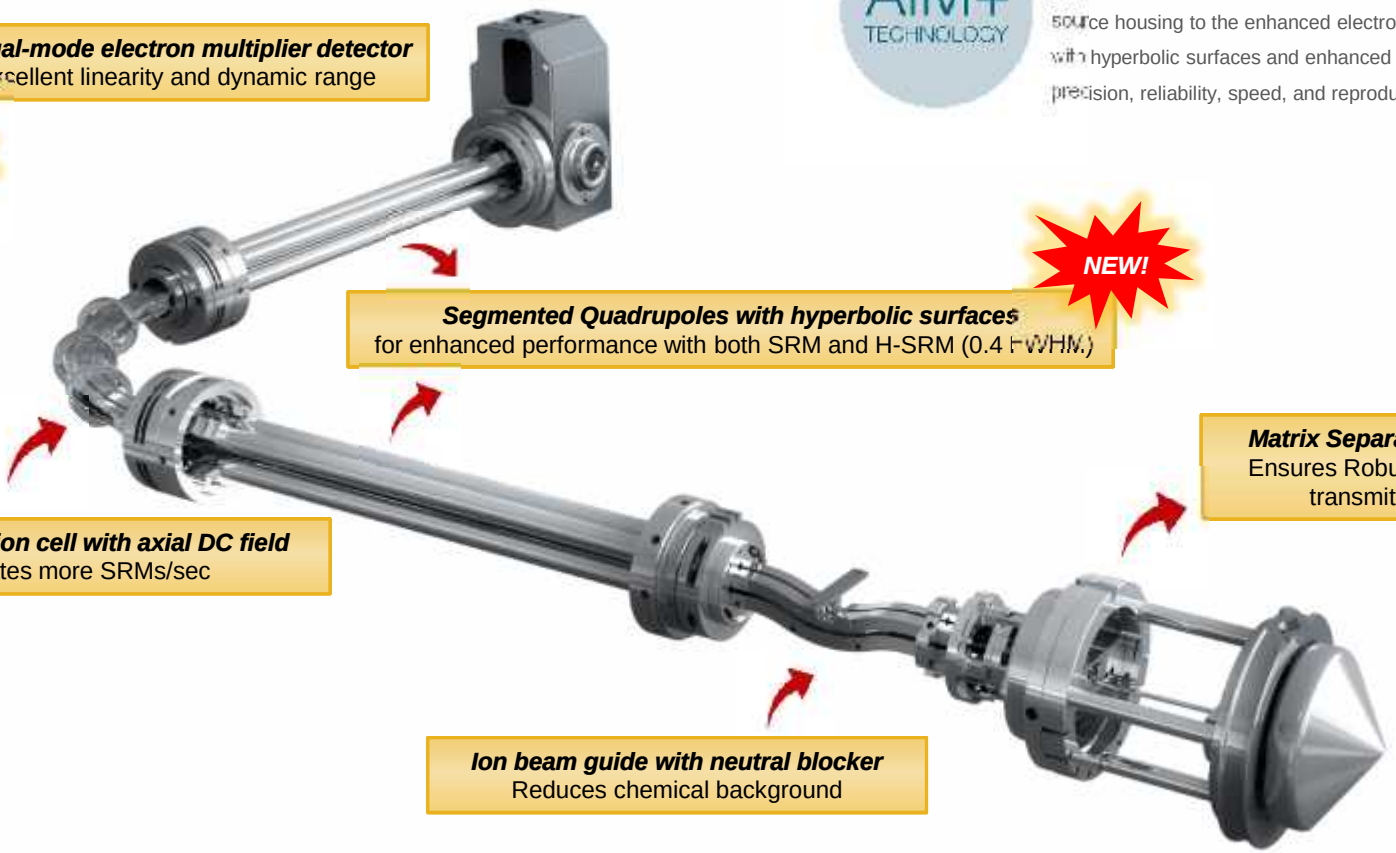
TSQ Fortis: Affordable Productivity, For Everyone

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!



NEW!

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

NEW!

Matrix Separator Ion Guide (MSIG)
Ensures Robustness while efficiently transmitting the ion beam

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

Ion beam guide with neutral blocker
Reduces chemical background

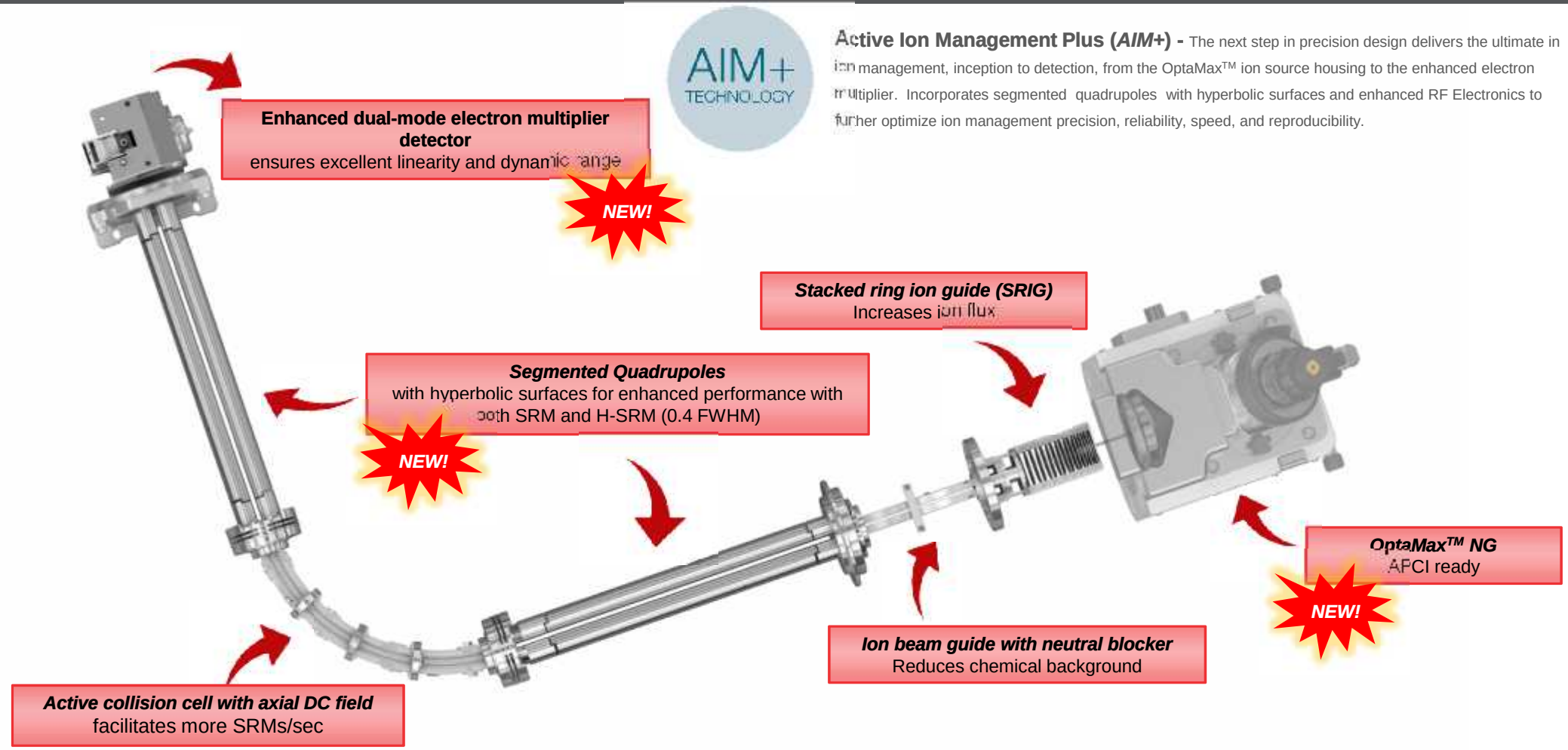
NEW!

OptaMax™ NG
APCI ready

ThermoFisher SCIENTIFIC

TSQ Quantis: Confidence, 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.



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TSQ Altis: 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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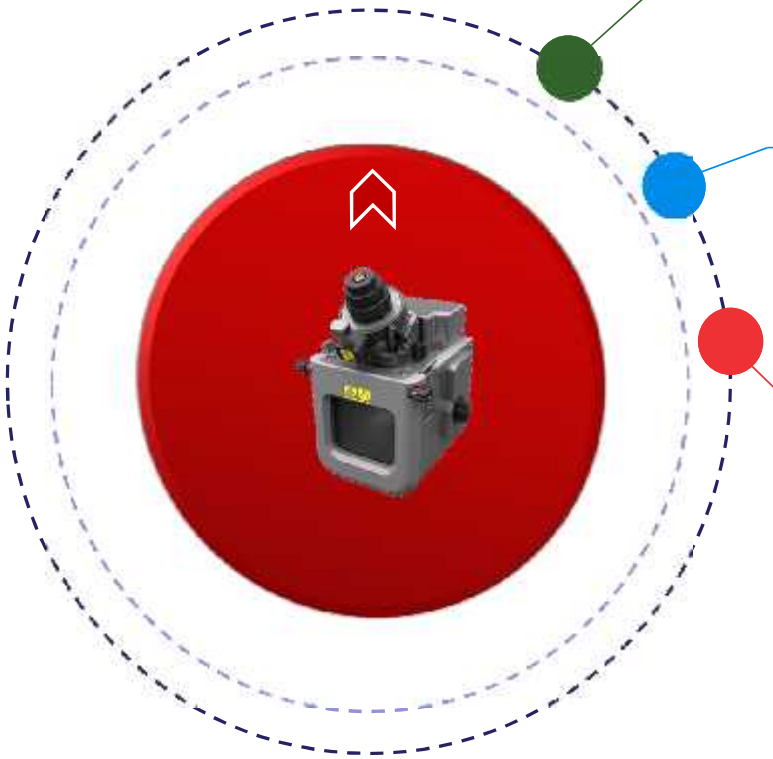
OptaMax NG Source Housing

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 no longer possible during acquisition (locked position)
- Tool available to help the user to correctly set needle protrusion

Usability and Consistency

- Vertical adjustment moved to the side for easier access
- New drain insert with improved latching and locating pin to prevent rotation
- 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

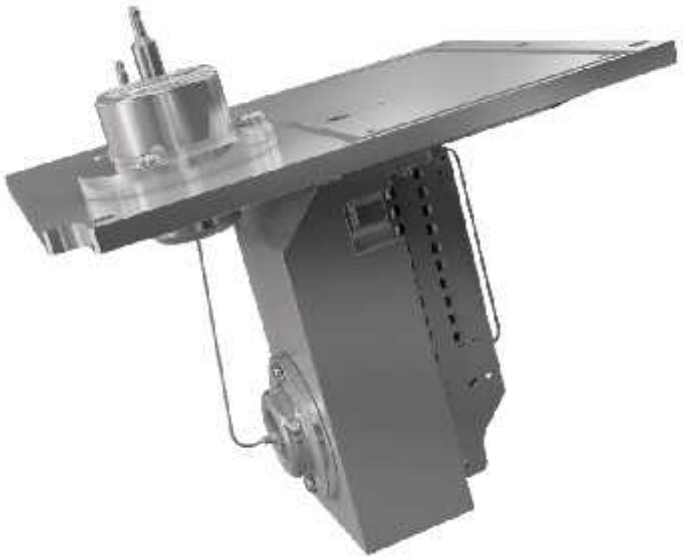
- The use of RF only pre-filters (segments) between the entrance lens and the quadrupole minimizes the effects of fringe fields, leading to improved transmission (and therefore sensitivity) at unit and higher resolution.

- 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!

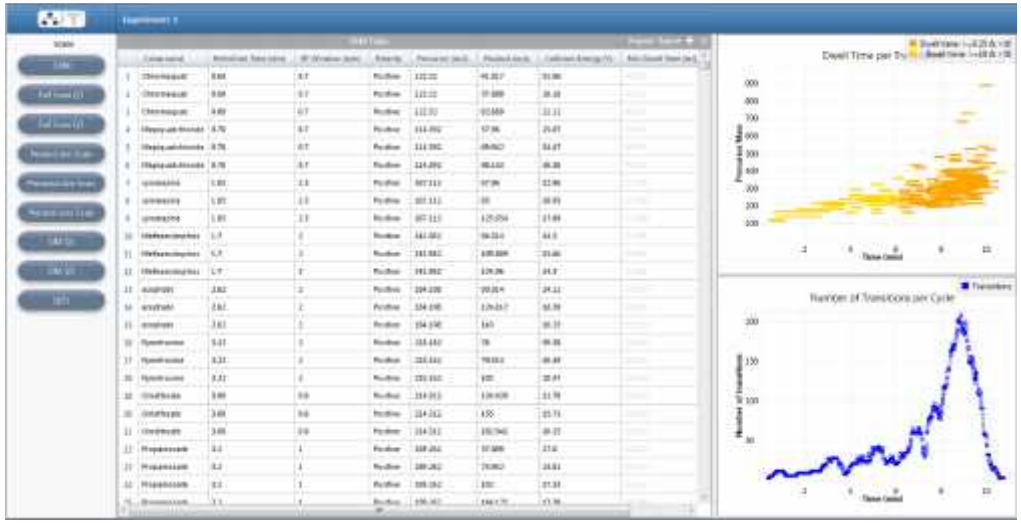
- Increased number of dynodes (21) for extended lifetime.
- Improved electron multiplier calibration routine.
- Excellent linearity and dynamic range across the mass range.
- Reduced number of service visits leading to more uptime.



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**



Confident Quantitation

1. Introduction to TSQ Fortis - Critical Features
2. Features and Benefits
3. Robust Solution – Content

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Features that Enable Every Analytical Laboratory

Targeted Quantitaton Workflows

Robustness

Consistency in day to day performance, sample to sample results, and user to user productivity

Speed

would enable higher throughput, faster analysis of complex mixtures

Sensitivity

Best-in-class sensitivity for all molecule types regardless of matrix complexity

Resolution

Unusual for QqQs, however, significant benefits for complex mixtures, large molecules in complex matrices

What Makes the new Triple Quads Robust?

Robustness

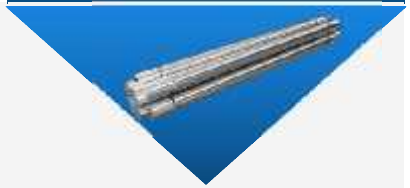
The ability to perform at the expected level (LOD, LOQ, MRL) under adverse conditions (complex samples, limited sample preparation) for the desired period of time without maintenance.

OptaMax NG	ITT/Sweep Cone	MSIG	Ion Beam Guide
			
Source housing	**Ion Transfer Tube and Sweep Cone**	**Matrix Separator Ion Guide**	**Ion beam guide with neutral blocker**
		TSQ Fortis only!	
Reliable and consistent performance with improved usability!	*No need to break vaccum for basic maintenance!*	*Eliminates neutrals while ensuring efficient transmission and sensitivity*	*Blocks neutrals - keeps the ion path clean and reduces chemical background!*

What Makes the new Triple Quads Consistent?

Consistency

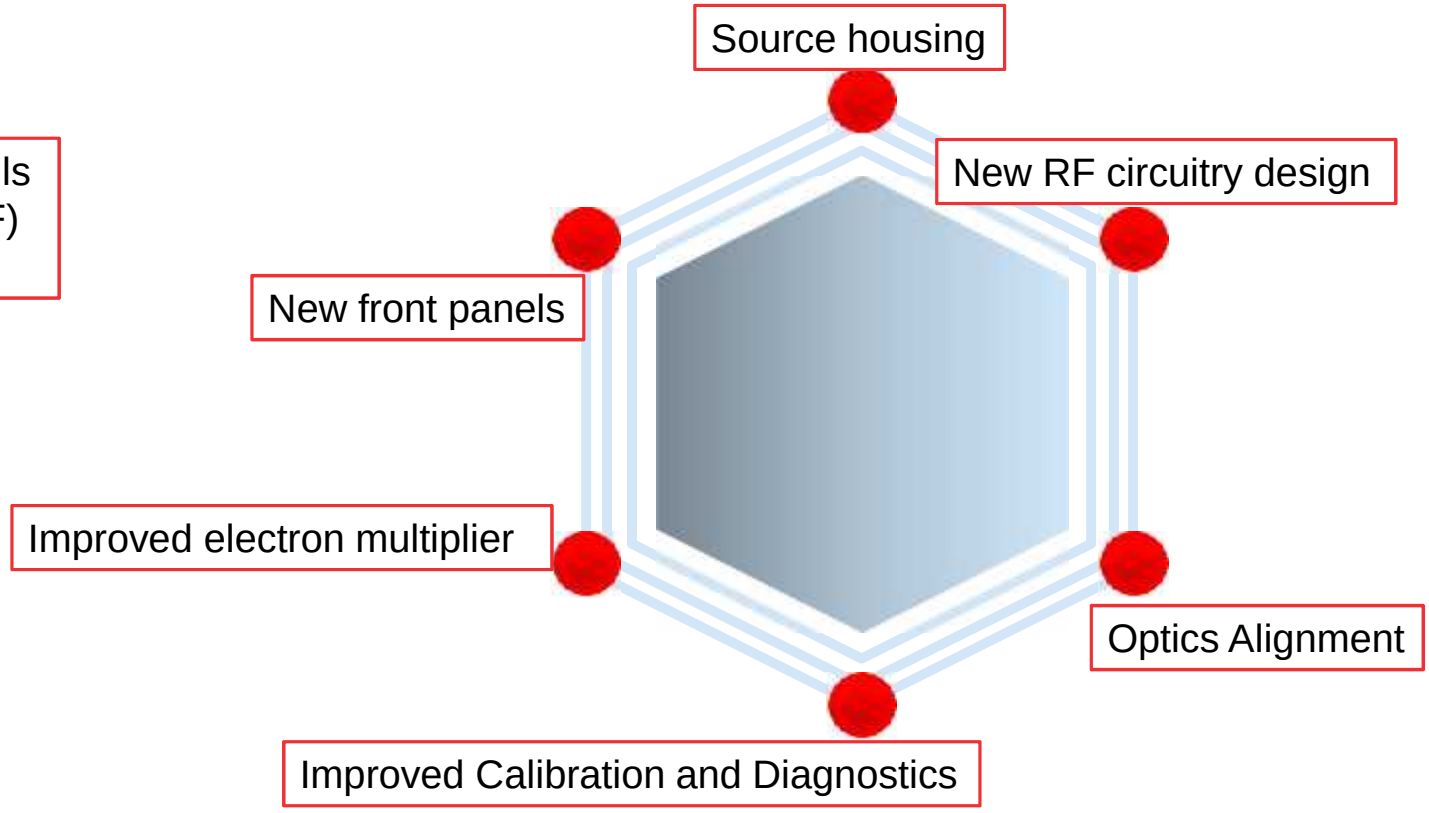
The ability to report equivalent results every time independently of the system or the user (day-to-day and system-to-system)

OptaMax NG	Optics	Segmented Ouads
		
Re-designed Source Housing	**Improved Optics Alignment**	**Segmented Quads with hyperbolic surfaces**
Reliable and consistent performance with improved usability!	*More consistent performance from system-to-system!*	*Flat tuning across the mass range!*

What Makes the new Triple Quads Reliable?

Reliability

Increased time between service calls
(mean time between failure- MTBF)
Increased uptime

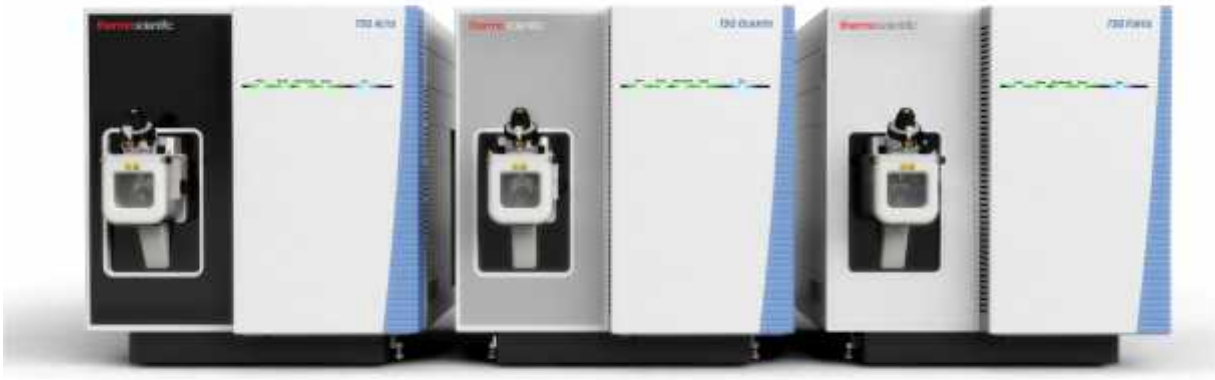


Factors Contributing to Enhanced Speed

Speed

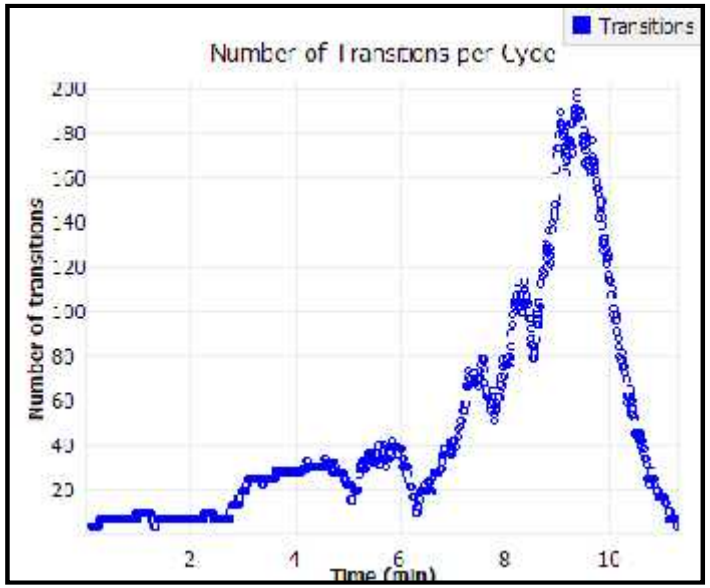
Critical feature when targeting more analytes in the same method or when increasing throughput by reducing the analysis time.

- **Active Collision Cell with axial DC field**
  - 90°cell design for noise reduction
- **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**

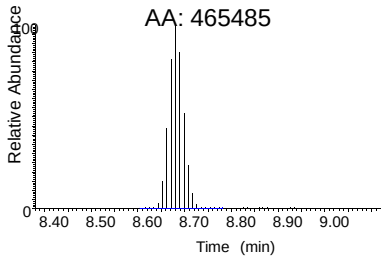


Pesticides in Leek: Robust, Reliable, Fast Quantitation Workflows

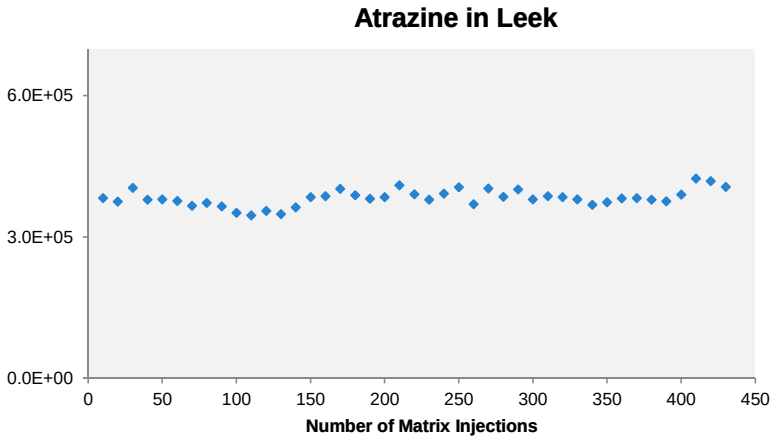
Increase Productivity - Monitoring more compounds per unit time!
Excellent Quantitative Performance at Lower Dwell Times!
Robust data enables Maximizing Uptime!



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

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Sensitivity

Sensitivity

The ability to accurately and precisely detect and/or quantify an analyte

Different criteria can be used to establish a limit of quantitation.

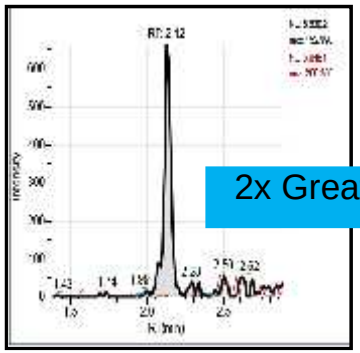
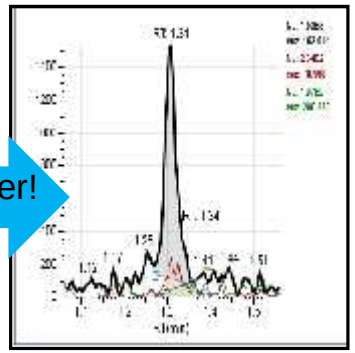
- **Multiple Product Ions** – typically one quantifier ion and multiple confirming ions
- **Excellent precision** of replicate measurements at reporting level
- **Excellent accuracy** at reporting level
- **Extended Linearity**

Active Ion Management Plus (*AIM+*)

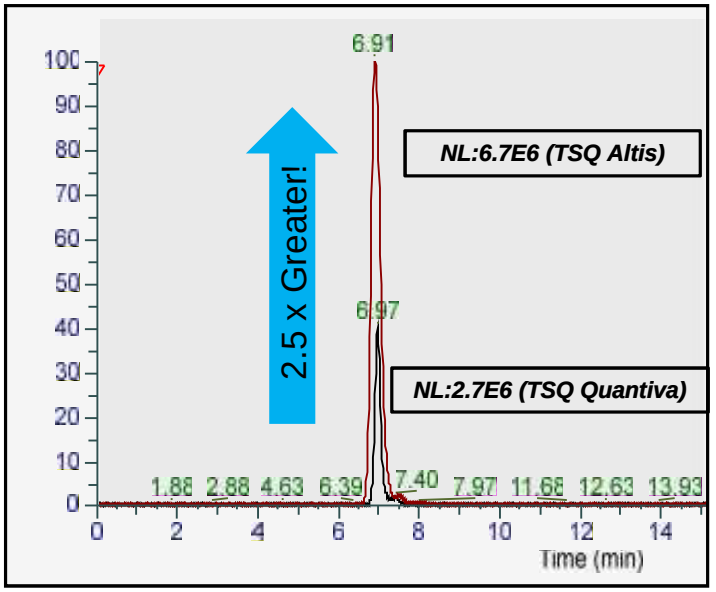
The next step in precision design delivers the ultimate in ion management, inception to detection, from the OptaMax NG source housing to the enhanced electron multiplier. Incorporates segmented quadrupoles and enhanced RF Electronics to further optimize ion management precision, reliability, speed, and reproducibility.

TSQ Altis: Demonstration of sensitivity for lipidomics and metabolomics

LPA 17:0 @ 0.01 ng/mL

TSQ Quantiva	TSQ Altis
	
RSD 3.4% (n=3)	***RSD 0.9% (n=3)***

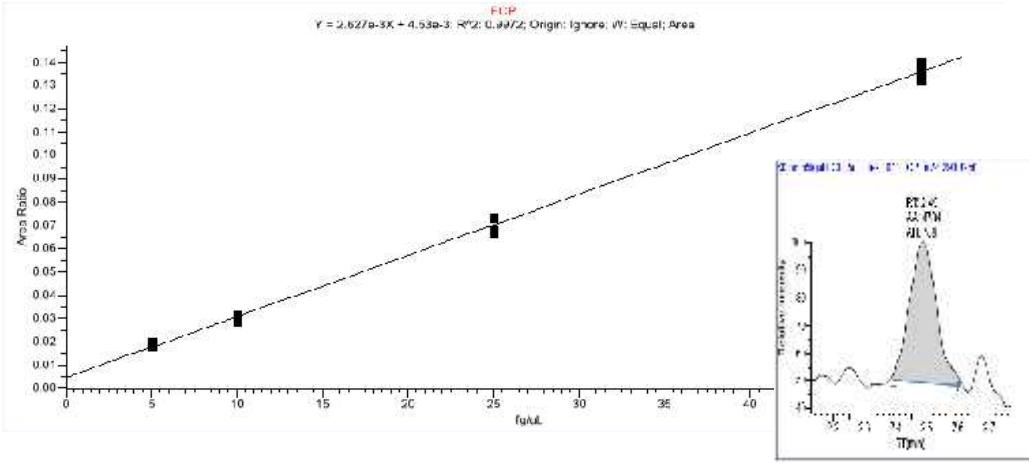
Glutamic Acid



LPA at 0.01 ng/mL with RSD below 1%! 2 x greater response on TSQ Altis! Confirming Ion detected on TSQ Altis!
A 2.5 fold response increase was observed when transferring the metabolomics assay from TSQ Quantiva to TSQ Altis.

TSQ Altis: Sensitivity, Precision and Dynamic Range

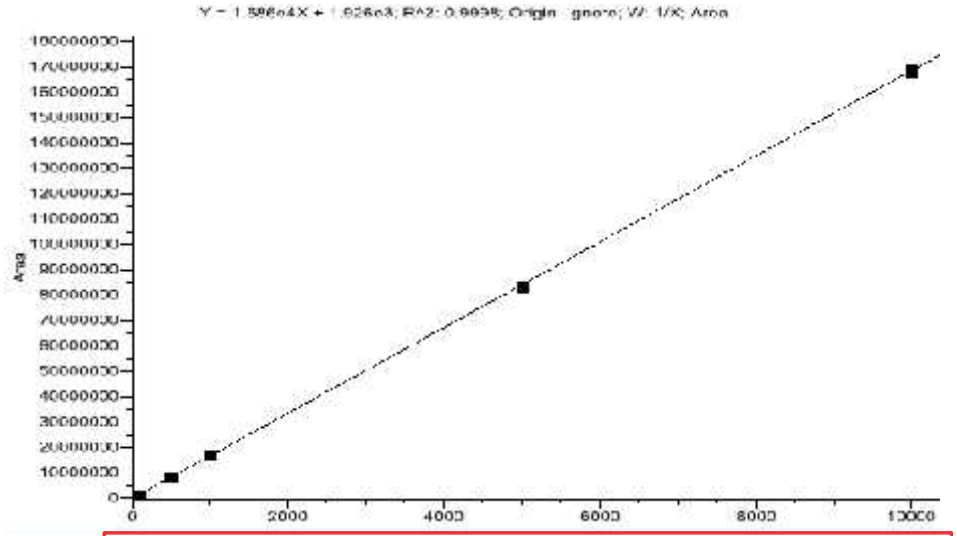
Superior Sensitivity and Reproducibility - Fluticasone Propionate in matrix!
25 fg on column with 4.2% CV!



UHPLC : Thermo Scientific™ Vanquish™ Flex Binary UHPL 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: 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 UPLC 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

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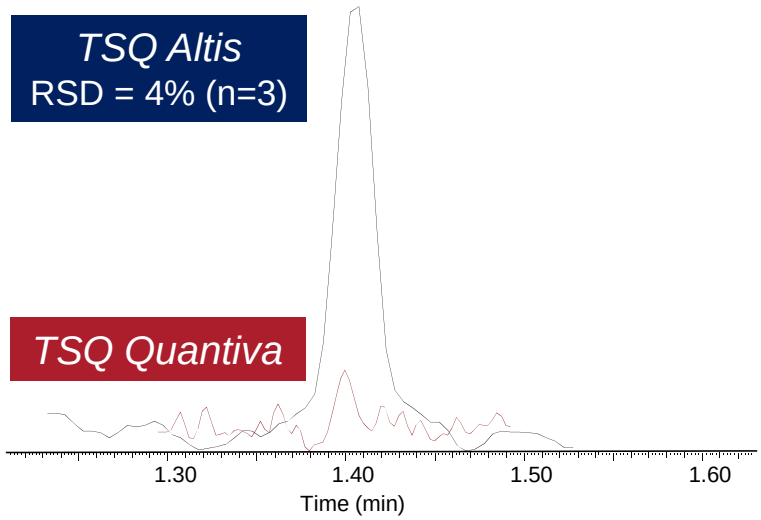
TSQ Altis: Quantitation of Therapeutic Drugs in Plasma

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 TSQ Quantiva

Application Note 64977

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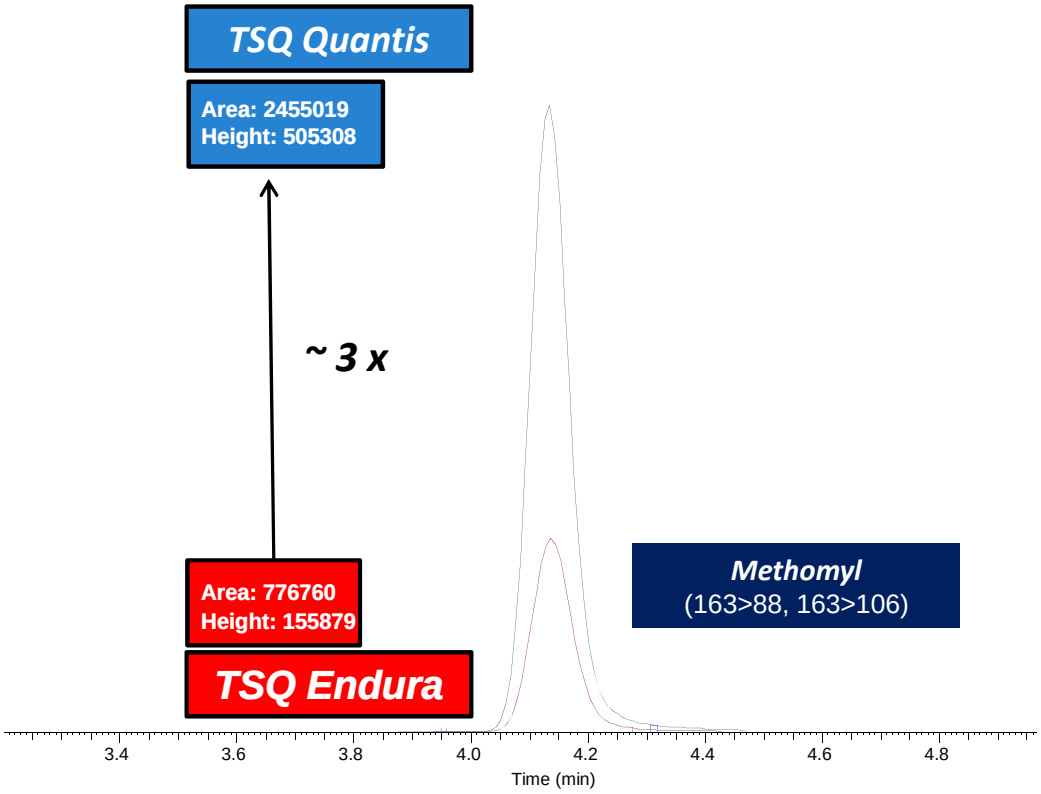
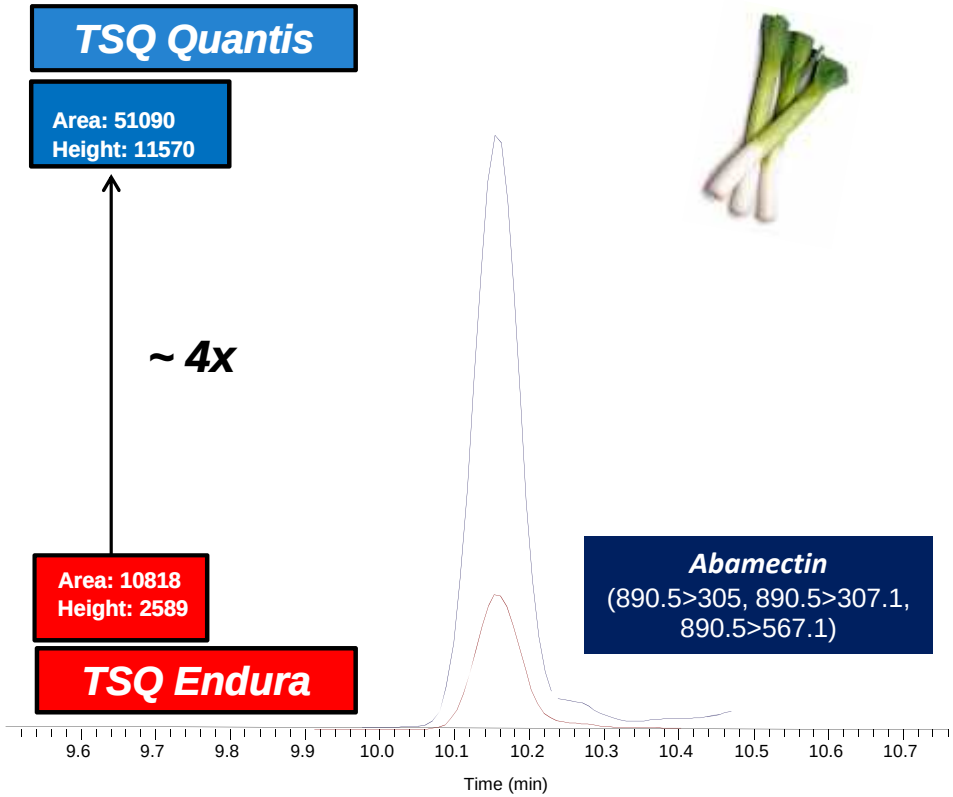
TSQ Quantis: Sensitivity Improvement for Pesticide Residue Analysis



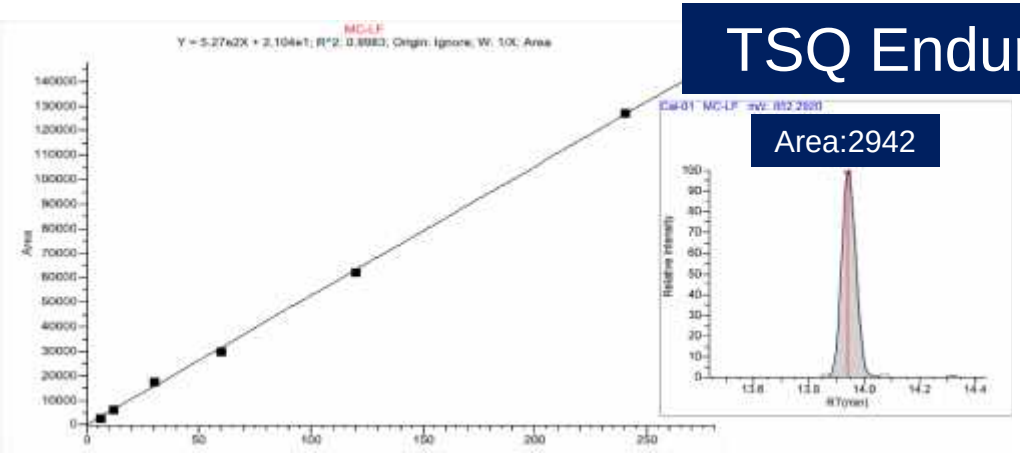


3 x average sensitivity improvement across mass range!

Application Note 64971

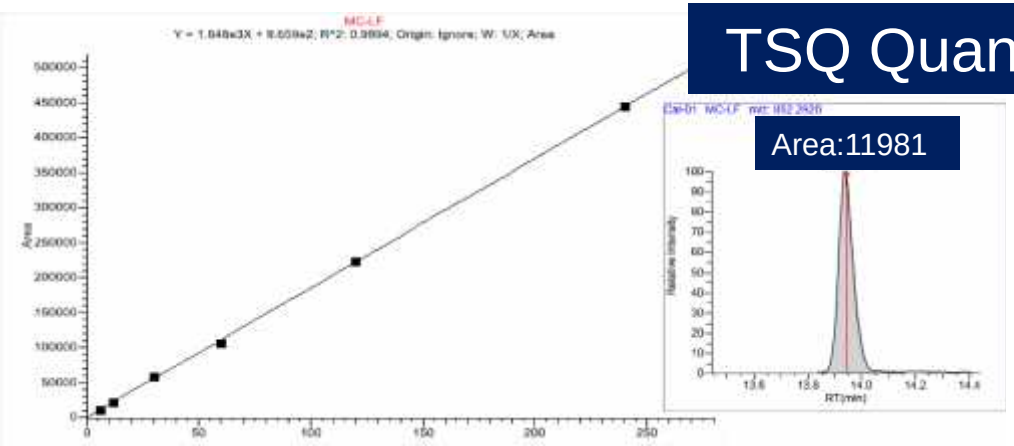
ThermoFisher SCIENTIFIC

TSQ Quantis: Sensitivity in Regulatory Methodology (EPA 544)



TSQ Endura

Area:2942



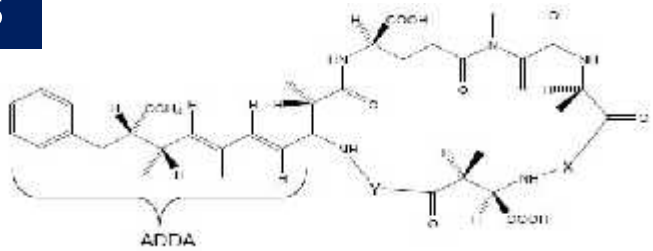
TSQ Quantis

Area:11981

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

EPA 544 released in February 2015

Algae blooms in Western Lake Erie



~ 4 x average sensitivity improvement over TSQ Endura

Application Note 64968

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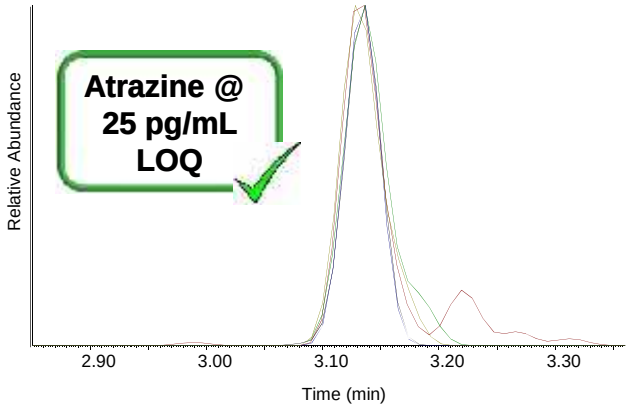
How to demonstrate sensitivity? Which system is right for you?

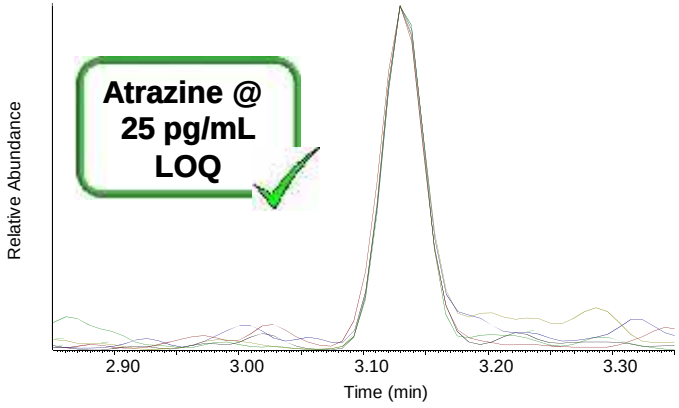
Sensitivity is a lot more than peak area! It should be demonstrated as a precise and accurate measurement!

TSQ Fortis



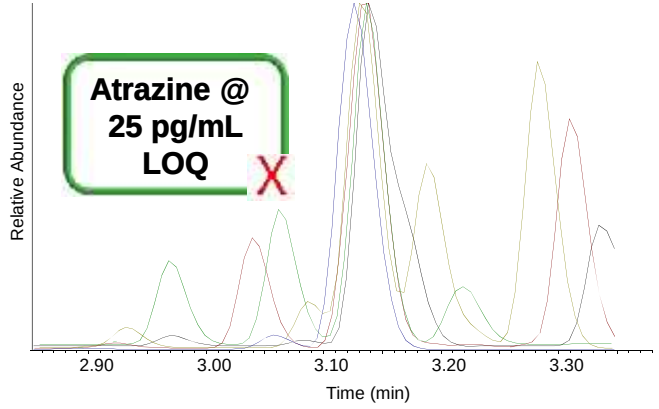
RSD = 3% (n=5)

TSQ Endura



RSD = 4% (n=5)

TSQ Quantum Access Max



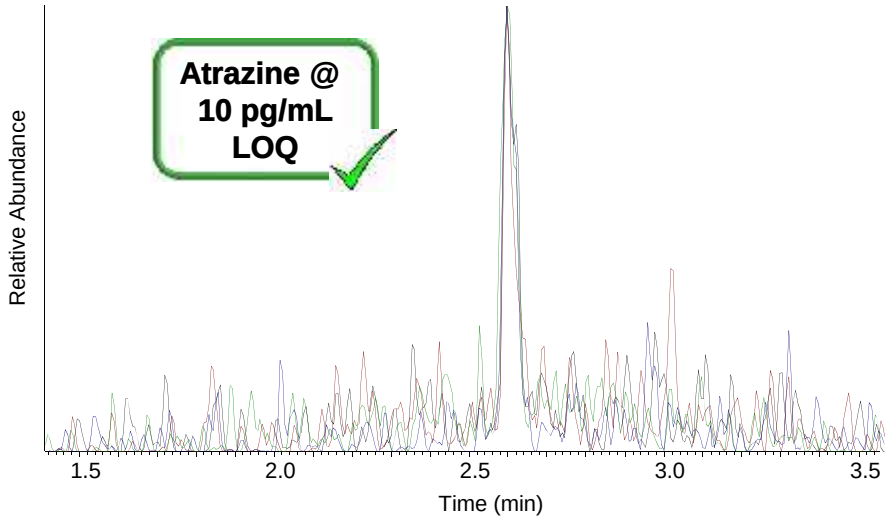
RSD = 24% (n=5)

What to expect when evaluating Limits of Quantitation based on precision and accuracy?

TSQ Quantis **> 3x** ***TSQ Endura*** **≥ (2x)** ***TSQ Fortis*** **> 3-5x** ***TSQ Quantum Access Max***

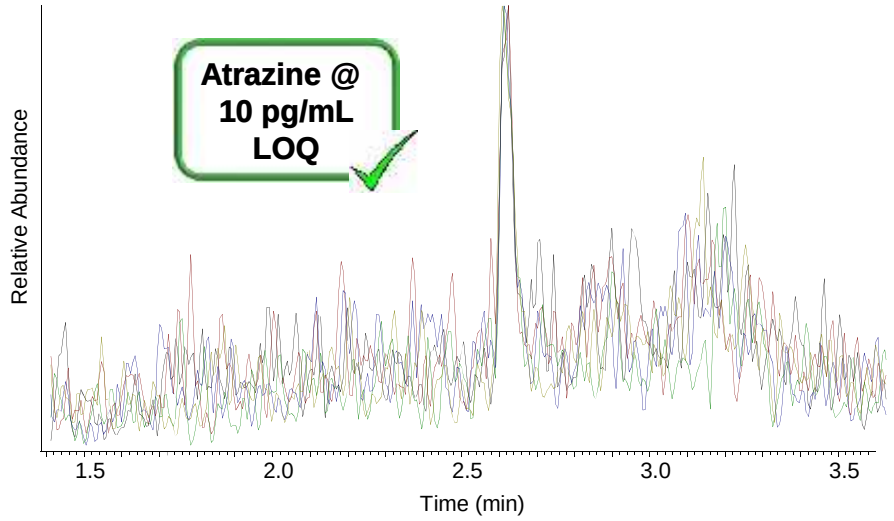
How to demonstrate sensitivity? Which system is right for you?

TSQ Quantis



RSD = 5% (n=5)

TSQ Quantiva



RSD = 5% (n=5)

What to expect when evaluating Limits of Quantitation based on precision and accuracy?

TSQ Altis > 2-3x TSQ Quantiva ≥ (2x) TSQ Quantis

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Specificity

Resolution

The ability to filter the *m/z* of interest and obtain a better signal to noise ratio in complex matrices.

New segmented quadrupoles with hyperbolic surfaces

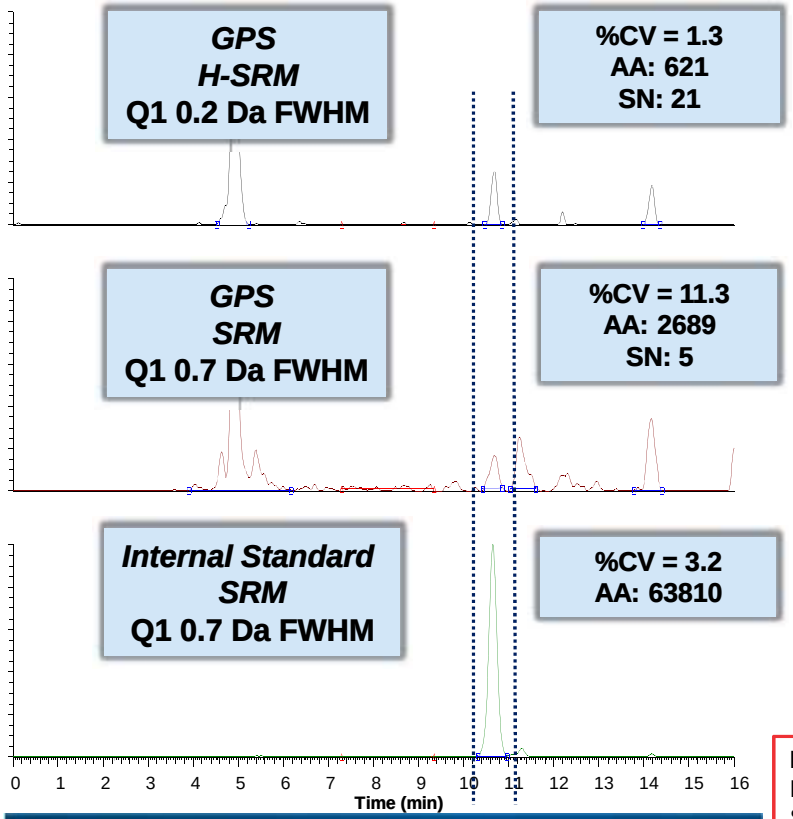
TSQ Altis – 0.2 Da FWHM

TSQ Quantis – 0.4 Da FWHM

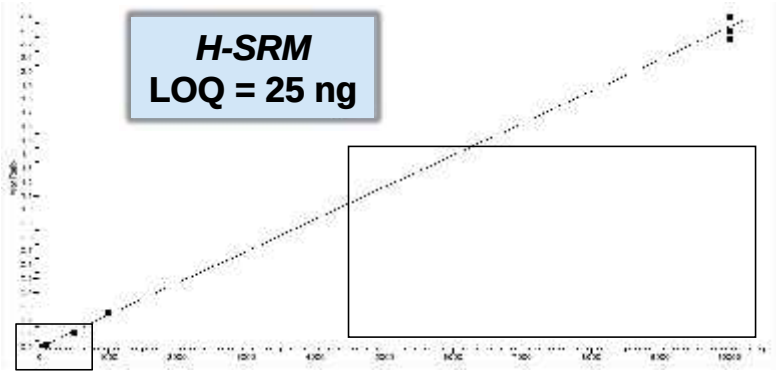
TSQ Fortis – 0.4 Da FWHM

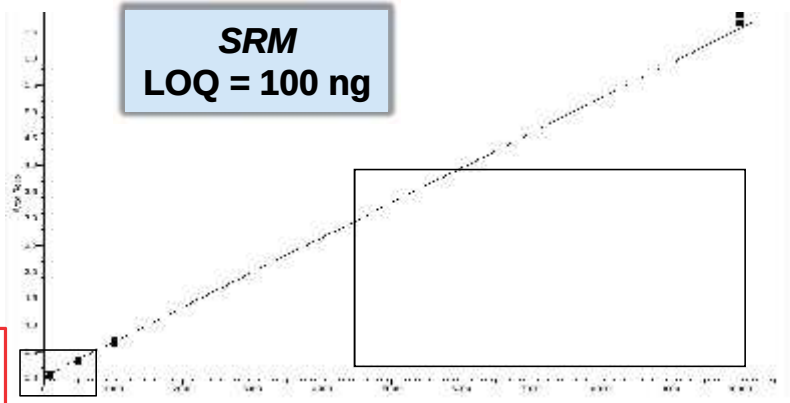
Superior Sensitivity with H-SRM (0.2 Da FWHM) – GPSVFPLAPSSK

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

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Ease of Use: Simple Maintenance

Simple user maintenance for additional uptime

5 min operation!
No need to break vacuum!

OptaMax NG

Re-designed source housing

NEW!

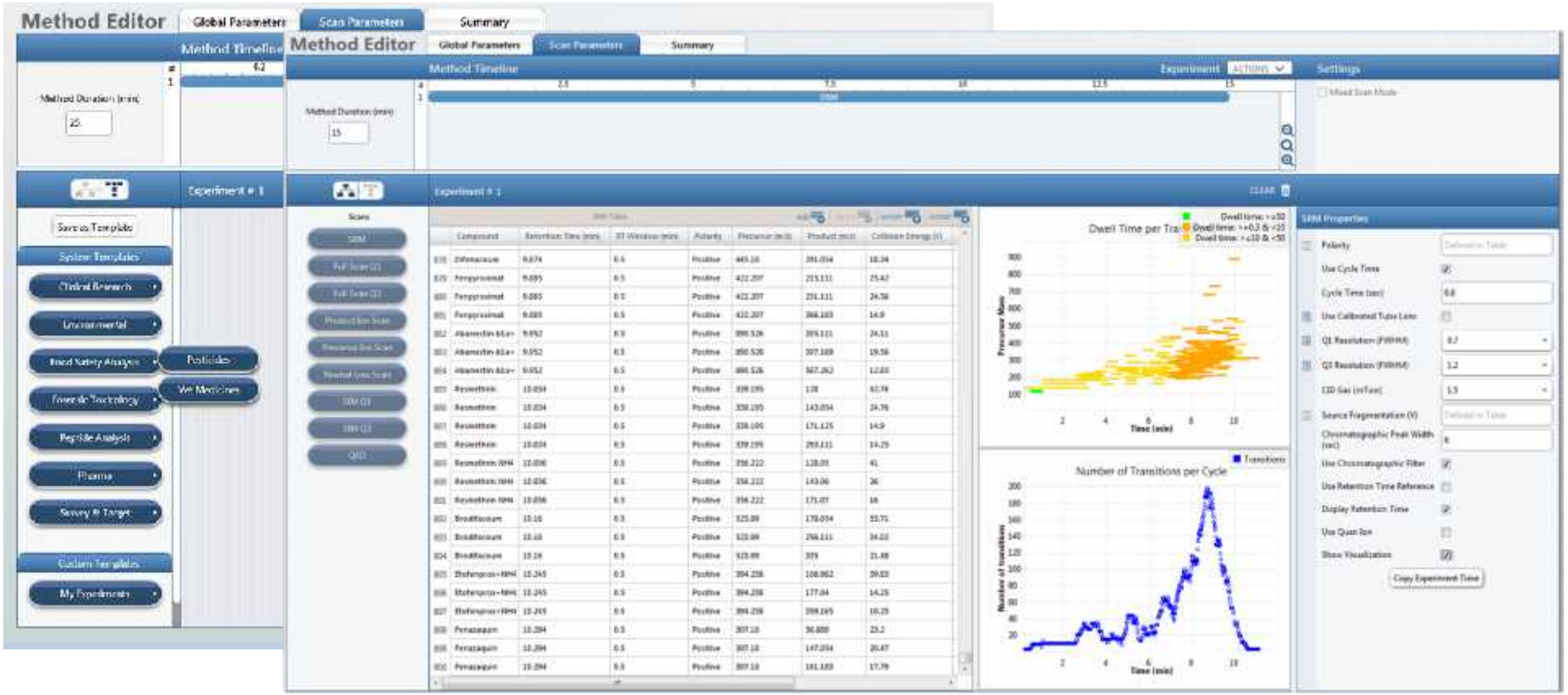
New tool available to ensure consistent positioning of HESI needle

ITT/Sweep Cone

Ion transfer tube and sweep cone

No need to break vaccum for basic maintenance
5 minute Operation!

Ease of Use: Method Setup made easy with Method Templates



Recommended conditions available to the user:
Optimized conditions specific for the MS model used

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Instrument Method Templates available in the latest ICSW – version 3.1

- **Clinical Research**
  - *Vitamin D*
  - *Steroids*
  - *Immunossuppressants*
- **Forensic Toxicology**
  - *ETG/ETS*
- **Food Safety**
  - *Pesticides*
  - *Veterinary Medicines*
- **Environmental**
  - *Perchlorate*
  - *HAAs*
  - *EPA 544*
  - *EPA 545*
  - *PFCs*
- **Pharma QA/QC**
  - *Phenylephrine*

TSQ Fortis

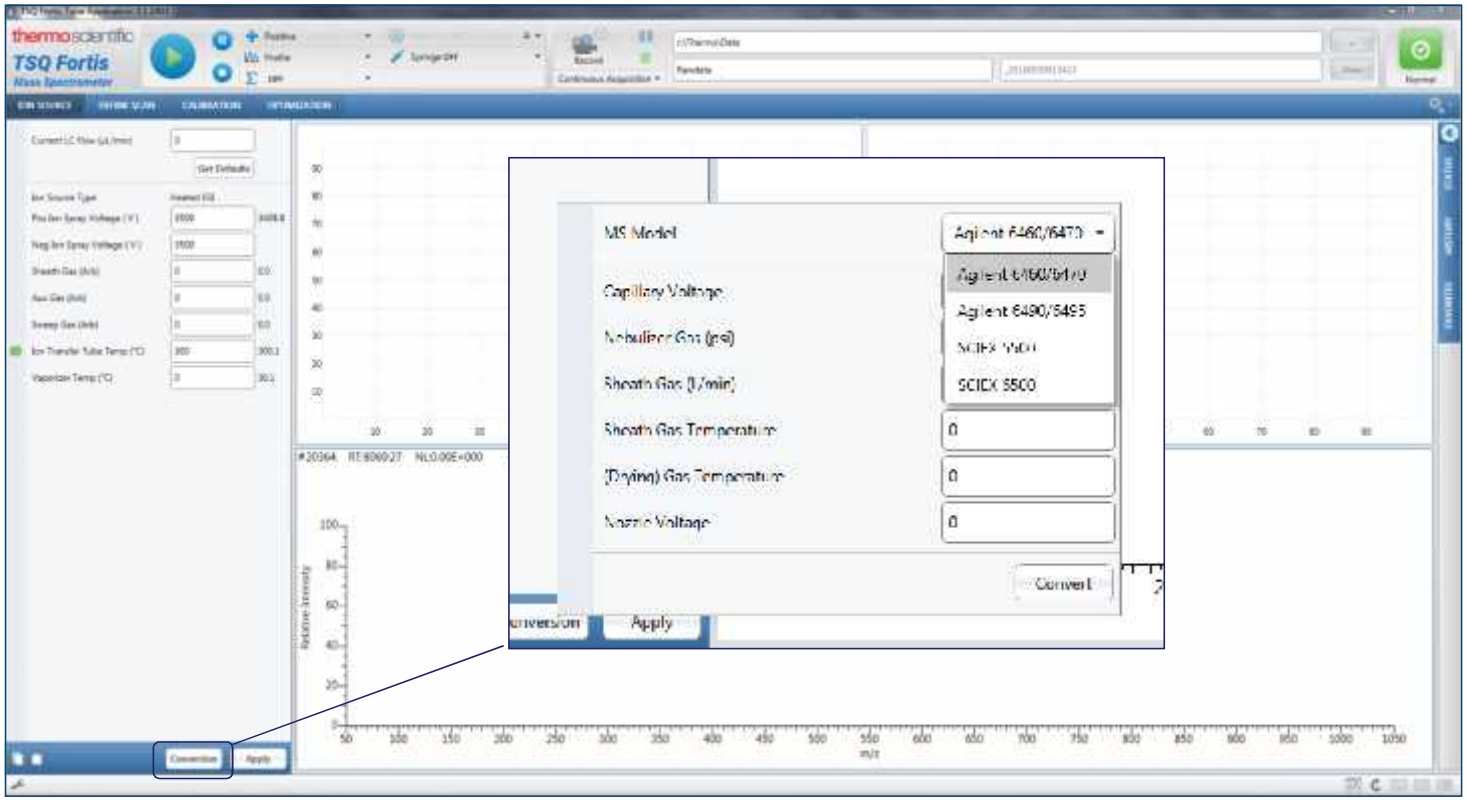
- **Clinical Research**
  - *Steroids*
  - *Immunossuppressants*
- **Forensic Toxicology**
  - *Drugs of Abuse*
  - *ETG/ETS*
- **Food Safety**
  - *Pesticides*
  - *Veterinary Medicines*
- **Environmental**
  - *HAAs*
  - *EPA 544*
  - *EPA 545*
- **Pharma**
  - Phenylephrine
  - Pharma Panel
- **Peptide Analysis**
  - *PRTC Heavy*
  - *PRTC Hela*

TSQ Quantis

- **Clinical Research**
  - *Steroids*
  - *Immunossuppressants*
- **Food Safety**
  - *Pesticides*
  - *Veterinary Medicines*
- **Environmental**
  - *PFCs*
- **Pharma**
  - Fluticasone
  - Pharma Panel
- **Peptide Analysis**
  - *PRTC Heavy*
  - *PRTC Hela*

TSQ Altis

Ease of Use: Conversion Tool



Automated Conversion Tool – conversion of source parameters from our competitors into TSQ parameters

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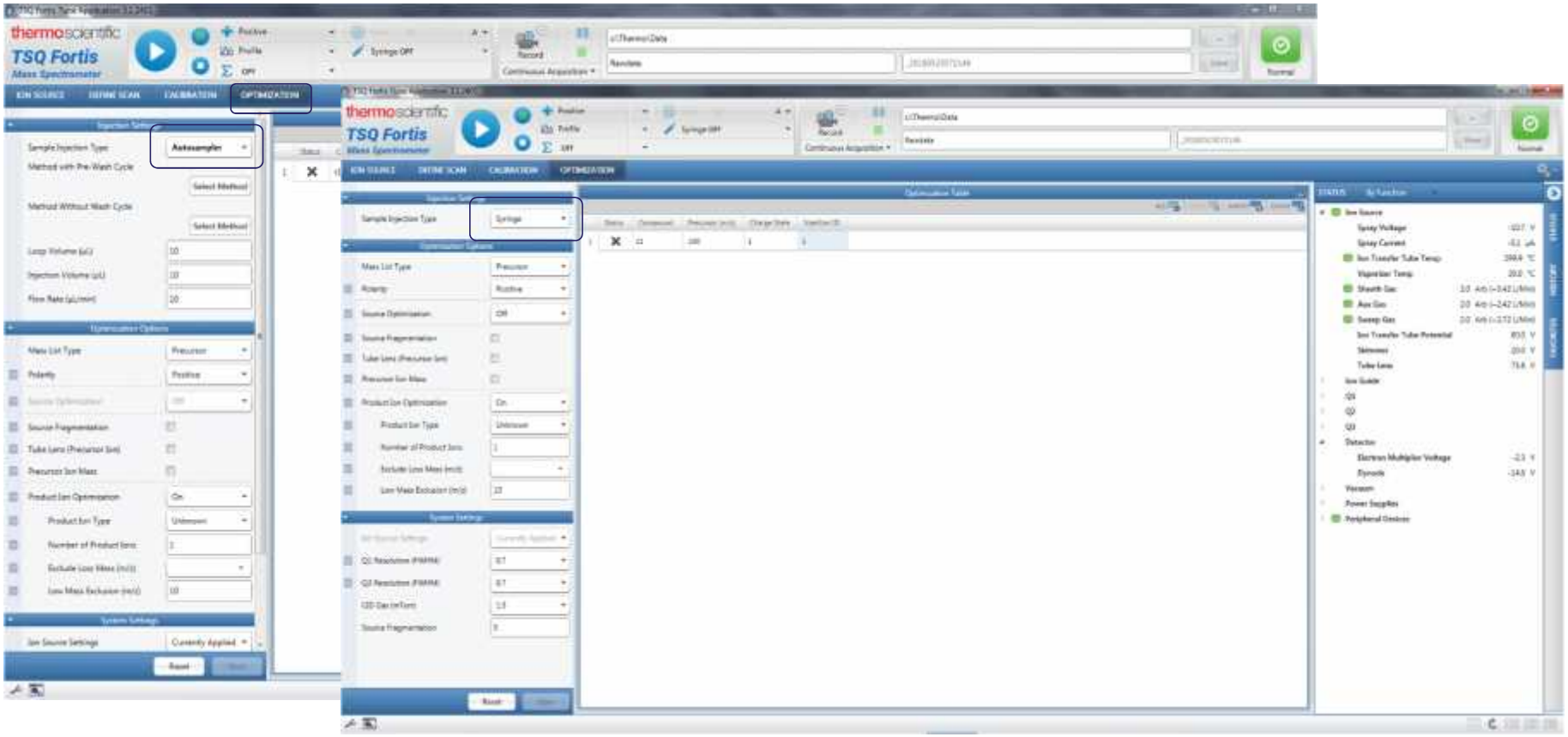
Ease of Use: Compound Optimization



Automated Compound Optimization Tool – Optimization of SRM parameters via infusion or Autosampler!

ThermoFisher SCIENTIFIC

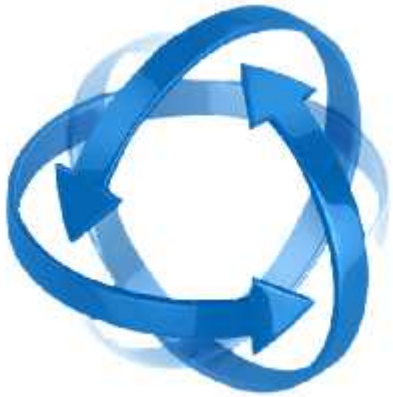
Flexibility

- **LC Options**
  - **High Sample Throughput**
    - Multi channel – Transcend and Prelude
    - CTC PAL 3 – reduced time between injections, barcode reading
  - **High Performance LC**
    - UHPLC – Vanquish Horizon and Vanquish Flex

- **MS Ion Sources**
  - H-ESI
  - APCI
  - OptaMax – APCI ready (only need to change the sprayer – 2 min operation)
  - OptaMax Duet – Automated HESI/APCI methods (no need to change sprayer)
  - APPI
  - Easy spray source and flex for nanoLC applications

NEW!

- **Software Options**
  - TraceFinder Software
    - Method development to automated report generation – offering comprehensive solution for every target application
  - Chromeleon
    - Data acquisition and processing
  - Skyline
    - Compatibility with TraceFinder



Confident Quantitation

1. Introduction to TSQ Fortis - Critical Features
2. Features and Benefits
3. **Robust Solution**

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Markets and Opportunities

Environmental Safety

MS is widely used as a detection tool. But, presence of polar compounds either require IC separation, or a time consuming derivatisation step.

Clinical Research

In Clinical Research (RUO), many analytical methods are used. But, MS is gaining popularity owing to speed, selectivity, and sensitivity

Food Safety

LC-MS is a popular choice to address an ever increasing list of analytes across multiple types of complex matrices. Selectivity, sensitivity, robustness play critical role

Pharma/ Biopharma

From high throughput screening to QA/QC based quantitation, from targeted quan in discovery Bioanalysis to DMPK – robust, reliable MS based detection is critical for confident quantitation

Targeted Proteomics

Ability to develop sensitive and selective quantitation assays for multiple peptides from several protein digests ensure LC-MS/MS as a platform of choice

Environmental Safety – Critical Challenges

Increased Confidence for Environmental Safety

Ultimate robustness with outstanding data quality, comprehensive and reliable workflows that ensures every laboratory achieve their business and scientific goals with ease

- **Be Prepared to Address Challenges, Everyday**
  - Robust, reproducible, reliable workflows for quantitation of hundreds of contaminants in a variety of matrices
  - High quality results enabling every environmental safety laboratory
- **Address Productivity Goals with Workflow Solutions**
  - **Speed** – analyze more compounds/injection or reduce overall run times
  - **Robustness** – less maintenance, increased uptime
  - **Ease of use** – easy method setup, data review and customized reporting
  - **Sensitivity** – detect contaminants with class leading sensitivity

Demonstration of Sensitivity - Environmental

Detection of haloacetic acids according to the EPA guidelines

Ion Chromatography

Workhorse for every analytical lab analyzing polar molecules
Spans high-pressure and address cost/sample challenges

TSQ Fortis MS

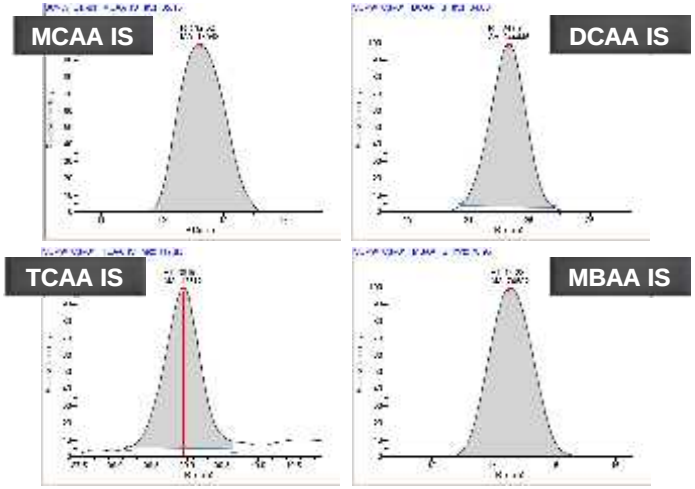
Robustness offering unmatched productivity with best-in-class sensitivity
Addresses critical quantitation challenges for every analytical laboratory

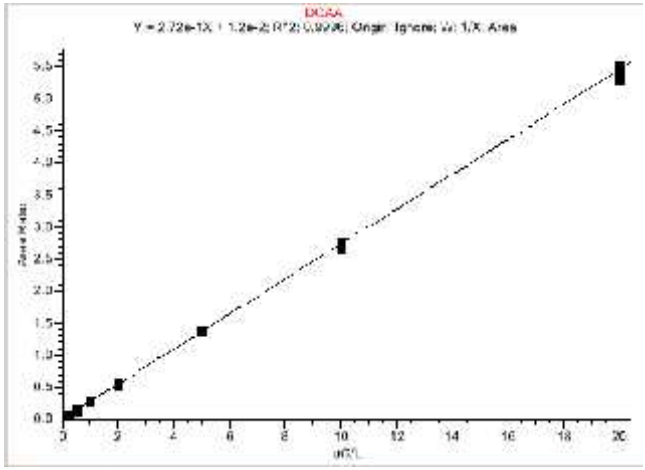
TraceFinder Software 4.1

One software for multiple LC-MS platform

Application Note 65196

Compound	MDL (µg/L)
MCAA	0.03
MBAA	0.03
DCAA	0.02
DBAA	0.02
BCAA	0.03
TCAA	0.06
BDCAA	0.05
DBCAA	0.15
TBAA	0.15
Dalapon	0.03
Bromate	0.02





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Perchlorate in Water

Best-in-class sensitivity with exceptional robustness

Ion Chromatography

Workhorse for every analytical lab analyzing polar molecules

Spans high-pressure and address cost/sample challenges

TSQ Fortis MS

Robustness offering unmatched productivity with best-in-class sensitivity

Addresses critical quantitation challenges for every analytical laboratory.

TraceFinder Software 4.1

One software for multiple LC-MS platform

One software from method development to report generation

Application Note 65201

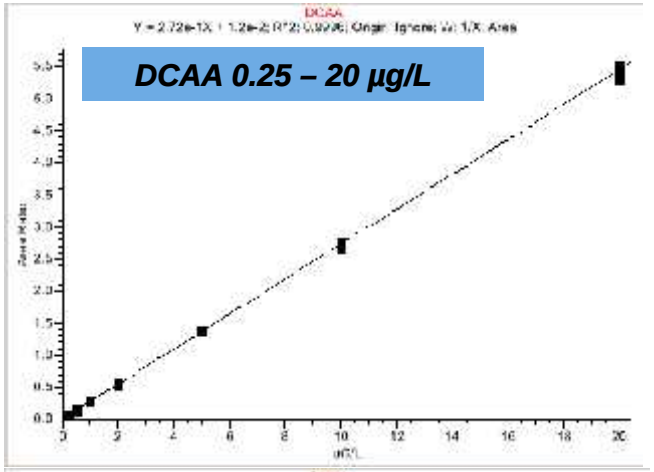
ThermoFisher SCIENTIFIC

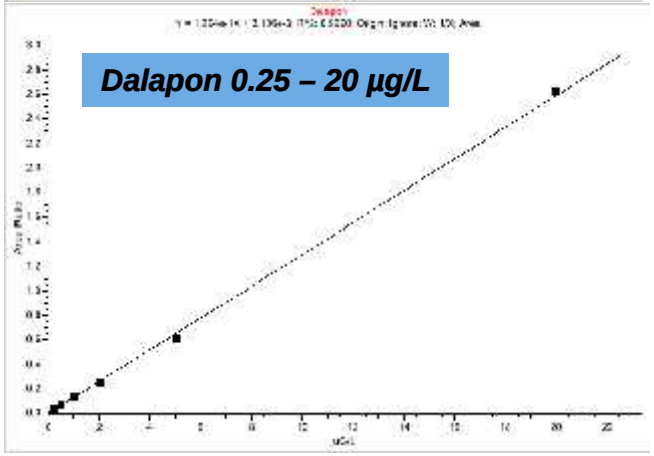
TSQ Fortis (& Ion Chromatography): Environmental Analysis

Haloacetic Acids by IC-MS/MS

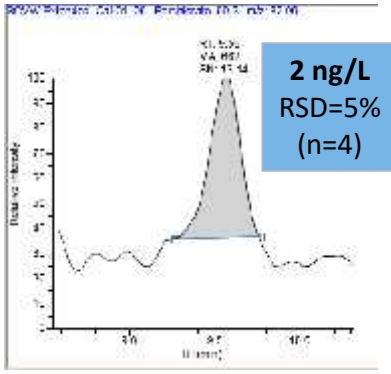
Compound	MDL (µg/L)
MCAA	0.03
MBAA	0.03
DCAA	0.02
DBAA	0.02
BCAA	0.03
TCAA	0.06
BDCAA	0.05
DBCAA	0.15
TBAA	0.15
Dalapon	0.03
Bromate	0.02

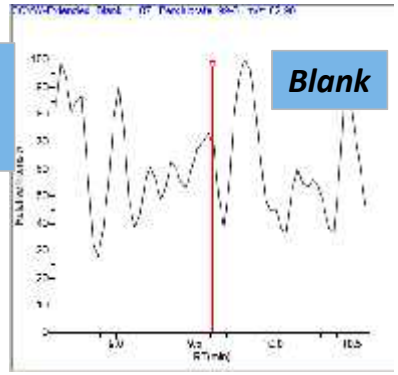
Application Note 65196

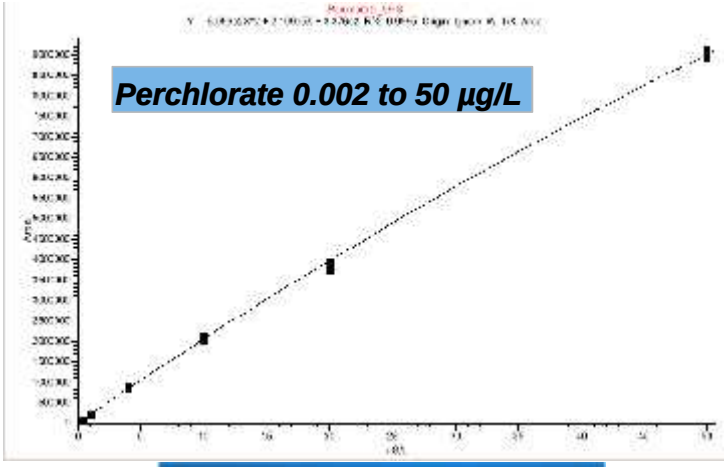




Perchlorate by IC-MS/MS



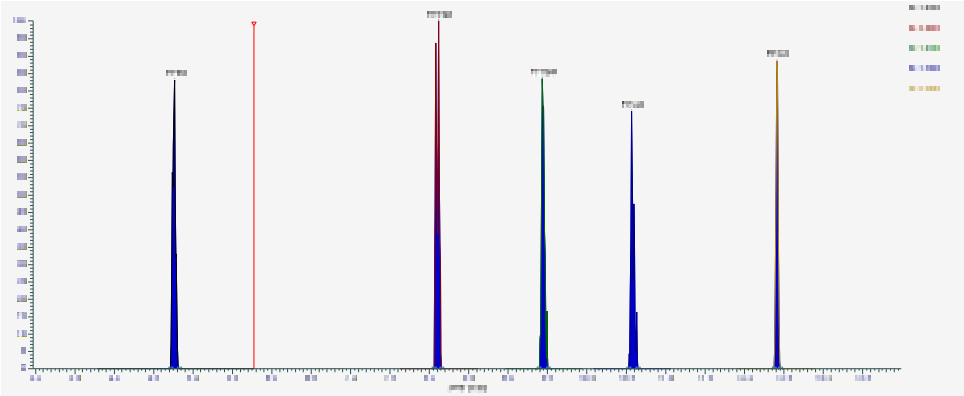




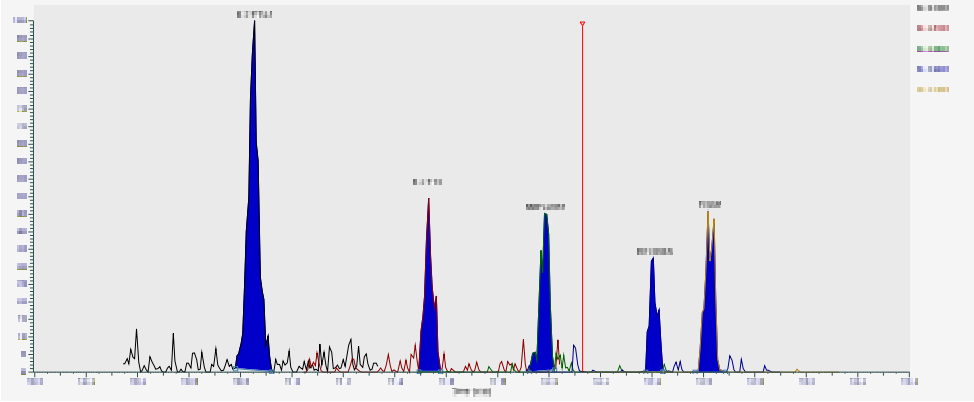
Application Note 65201

ThermoFisher SCIENTIFIC

TSQ Quantis: Separation of Perfluoronated Alkyl Substances, 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 Flex Binary UHPLC system

Delay Column: 3.0 x 50 mm, 5 um BDS Hypersil C8
Analytical Column: 2.1x100 mm, 2.6 um Accucore C18
Column Temp: 30 C
Mobile Phase: [A] H_2O + 10 mM Am. Acetate; [B] MeOH
Injection Volume: 3 uL

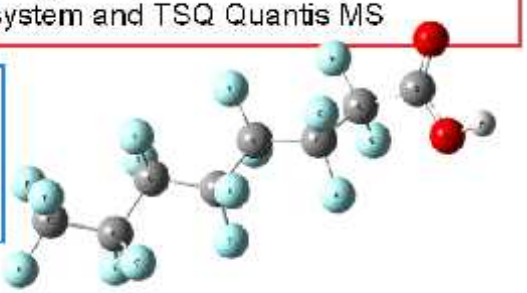
TSQ Quantis MS

Ionization Mode: HESI, Negative ion mode
MS Acquisition Mode: Selective Reaction Monitoring (SRM) Cycle time: 0.45 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



TSQ Altis: Confident Quantitation of challenging analytes in environmental matrices

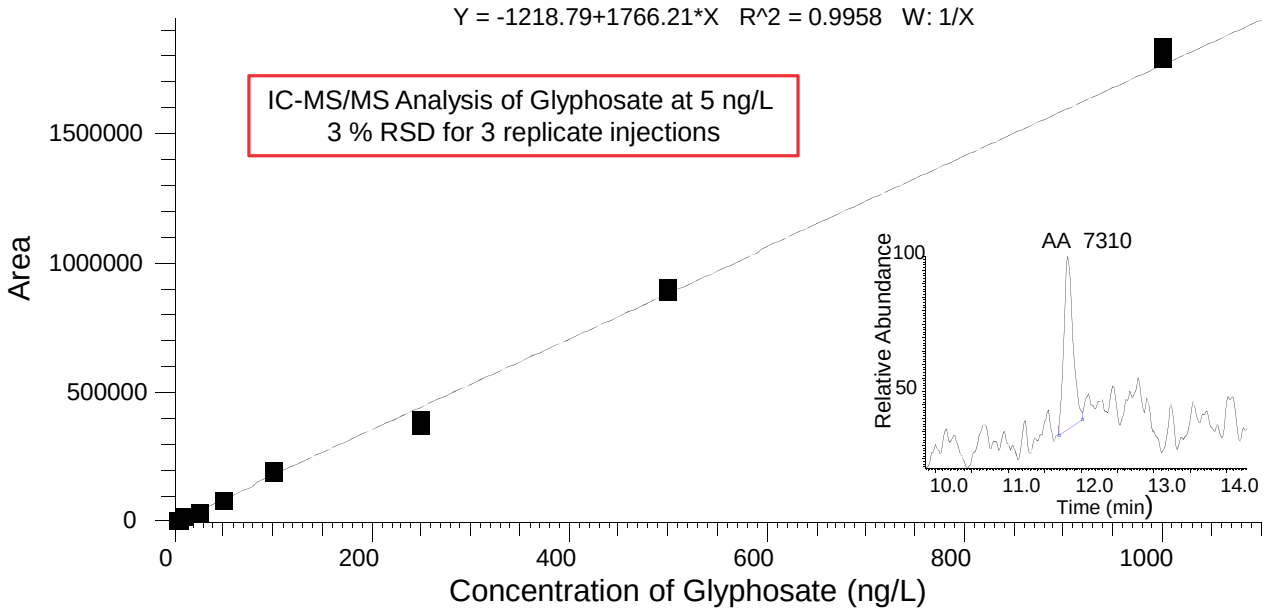
IC-MS/MS solutions for environmental analysis
Quantitation of **Glyphosate** at **5 ng/L**, **3% RSD**

Experimental Details

ICS 5000+
Flow rate: 0.3 mL/min
Eluent Source: Eluent Generator
Mobile Phase: KOH

MS: TSQ Altis

Software: TraceFinder Software 4.1



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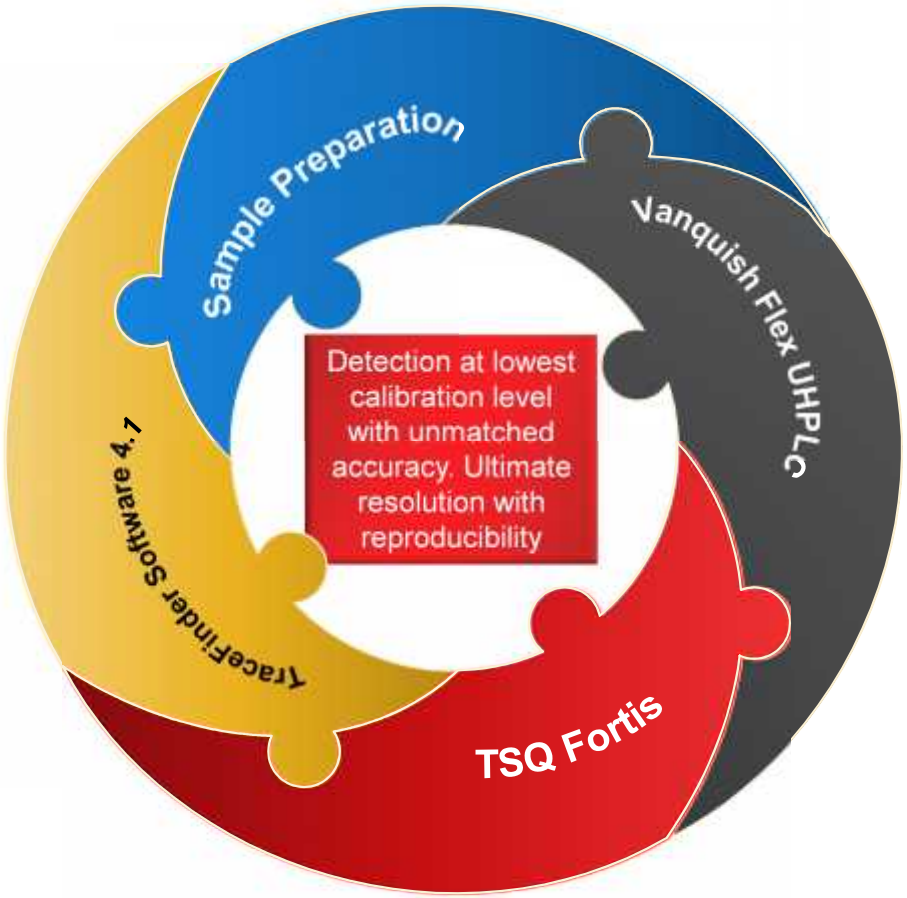
Food Safety

Confident Quantitation for Food Safety
Ultimate robustness with selectivity, reproducibility enabling comprehensive workflows to ensure every food safety laboratory

- **Be Prepared to Address Challenges, Everyday**
  - Robust, reproducible, reliable workflows for quantitation of hundreds of contaminants in a variety of matrices
  - Address upcoming analytical demands of increased number of contaminants with methods having shorter run times

- **Address Productivity Goals with Workflow Solutions**
  - **Speed** – analyze more compounds/injection or reduce overall run times
  - **Robustness** – less maintenance, increased uptime
  - **Ease of use** – easy method setup, data review and customized reporting
  - **Sensitivity** – detect contaminants with class leading sensitivity

Pesticide Quantitation with Fortis



Sample Prep:

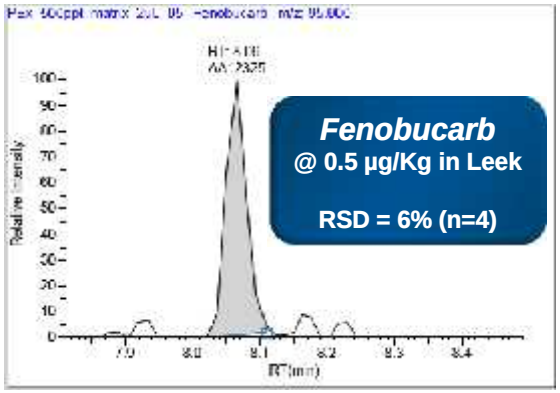
- QuEChERS based

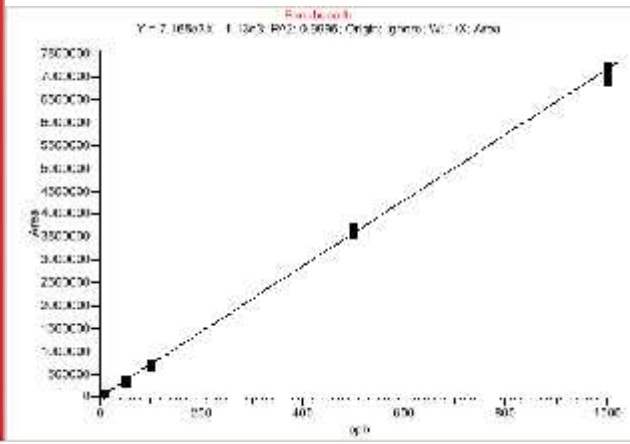
LC Conditions:

- Thermo Scientific™ Accucore™ aQ column (2.1x100x2.6 m)
- 2 µL injection
- Run Time 15 min

MS Conditions:

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





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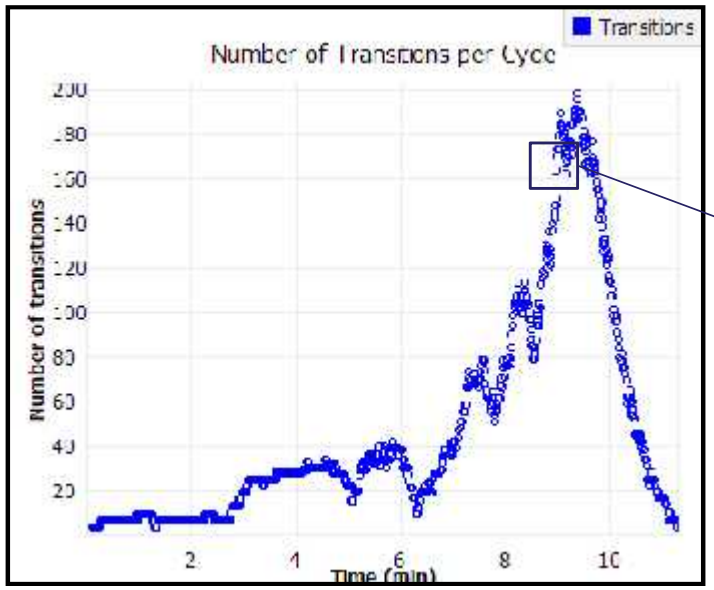
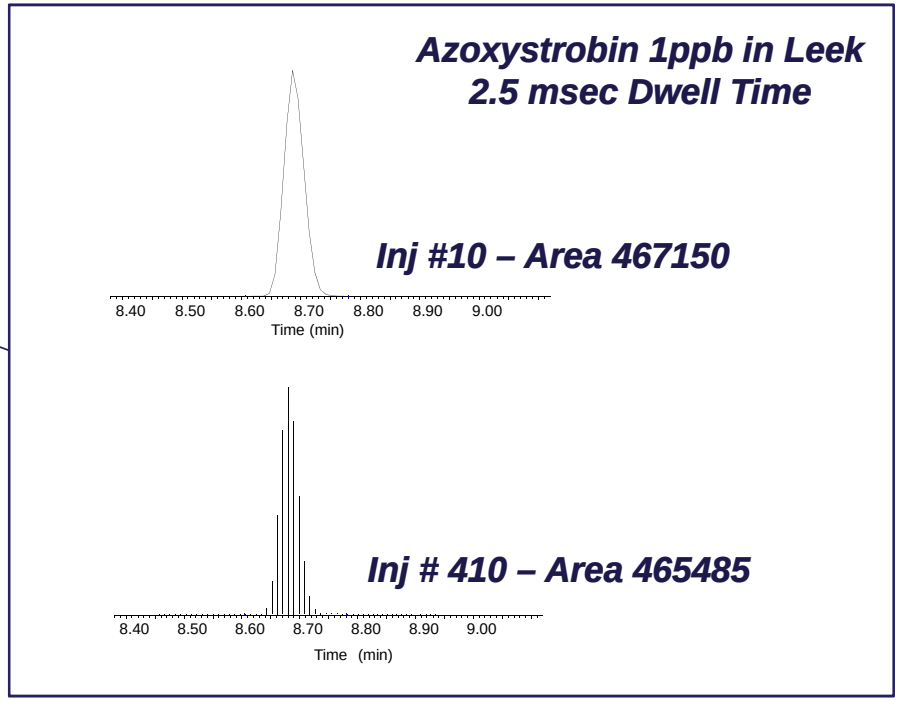
TSQ Platform: Robust, Reliable, Fast Quantitation Workflows

Excellent Quantitative Performance at Lower Dwell Times!



SRM Visualization from Instrument Control Software 3.0 displaying the number of transitions per unit time



Application Note 64971

~ 160 Transitions Monitored Simultaneously with Polarity Switching. Excellent Reproducibility (% RSD 2.3) below the MRL

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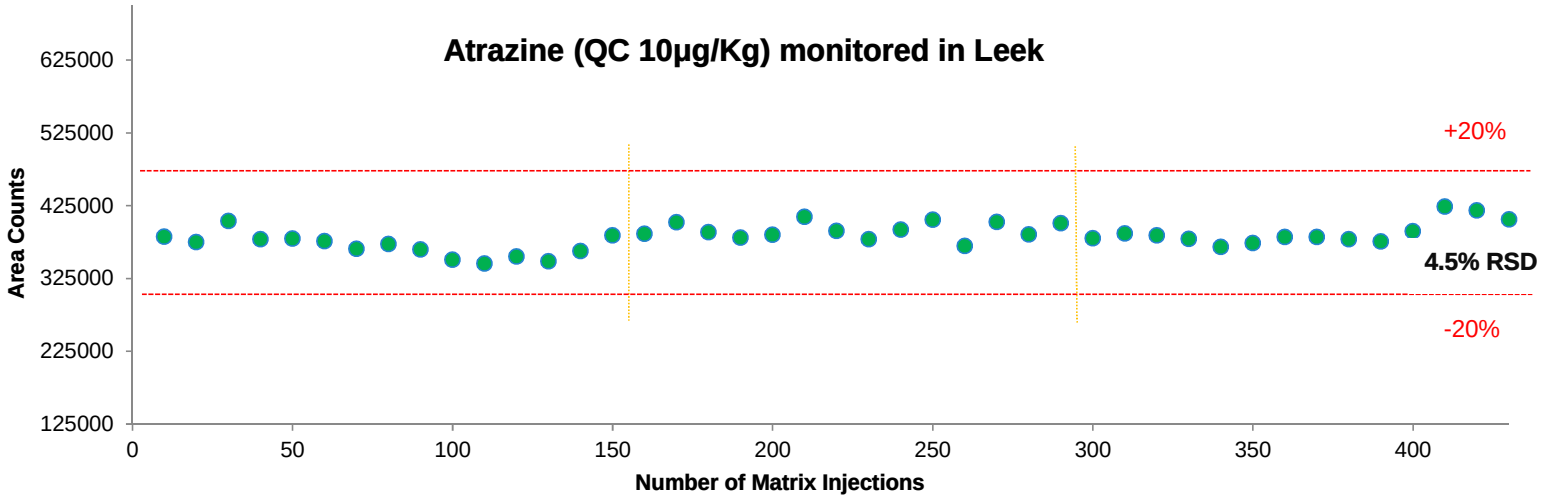
TSQ Quantis: Demonstration of Robustness – Food Safety

Atrazine QC monitored in leek for more than 400 injections with 4.5% RSD . Red lines represent ± 20% response at 10 µg/Kg. Yellow lines show the time the system was placed in standby mode for 12h to demonstrate consistent performance after standby period



FOOD SAFETY

Application Note 64971

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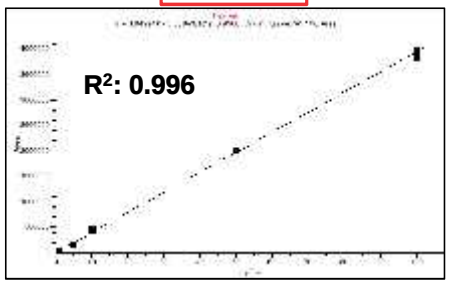
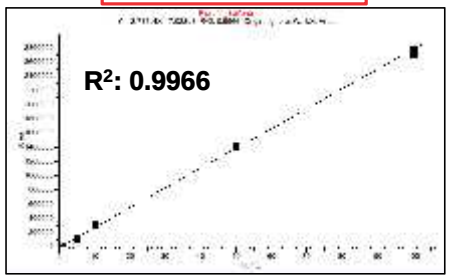
TSQ Quantis: Fipronil Quantitation- Matrix Matched Calibration for UHPLC-MS/MS

Fipronil



Fipronil sulfone

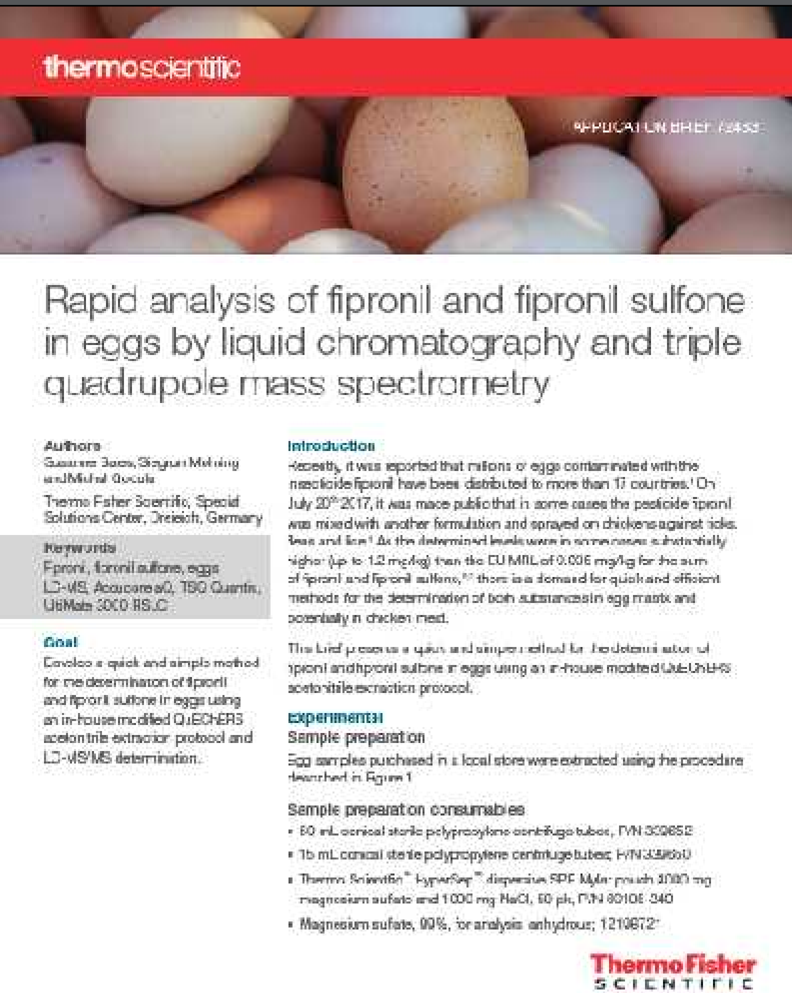


Compound name	Recovery (%) 0.5 µg/kg spike level	Recovery (%) 1 µg/kg spike level	Recovery (%) 5 µg/kg spike level
Fipronil	104	89	99
Fipronil sulfone	99	95	102

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 72453

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

Authors
Susanne Boos, Siegrun Mohring and Michal Godula

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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.[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] there is a demand for quick and efficient methods for the determination of both substances in egg matrix and potentially in chicken meat.

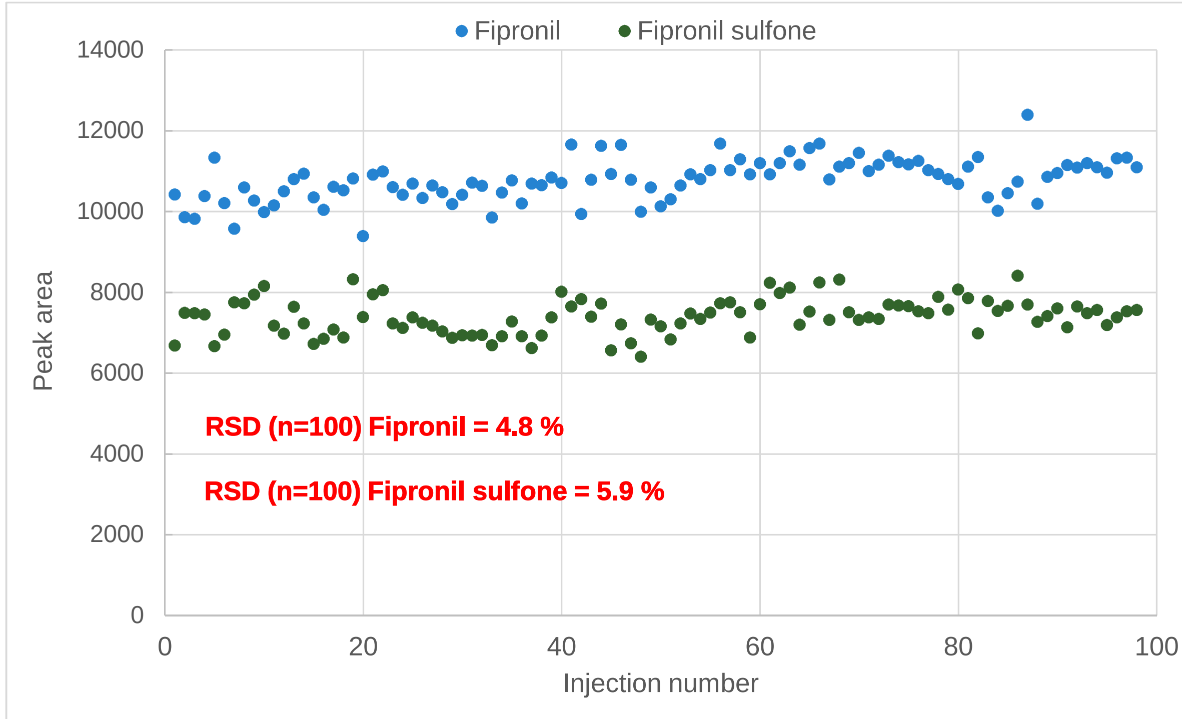
This brief presents a quick and simple method for the determination of fipronil and fipronil sulfone in eggs using an in-house modified QuEChERS acetonitrile extraction protocol.

Experimental

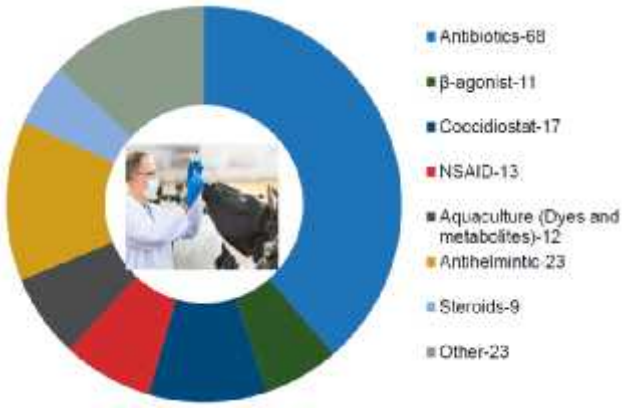
Sample preparation
Egg samples purchased in a local store were extracted using the procedure described in Figure 1.

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; 1210872

ThermoFisher SCIENTIFIC



Vet Drugs Analysis and Quantitation



- Multi-class veterinary drugs analysis showing
  - Fit for purpose Thermo Scientific™ Accucore™ RP-MS 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

Sample Prep:

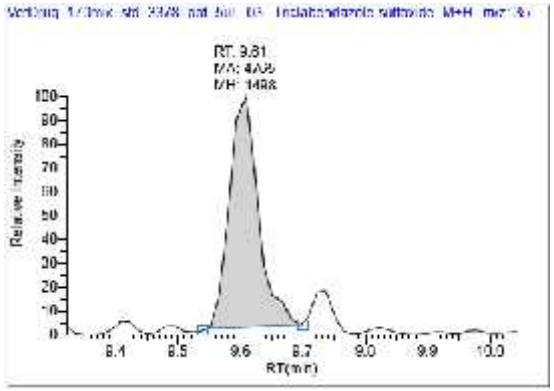
- QuEChERS based approach

LC Conditions:

- Thermo Scientific™ Accucore™ RP-MS (2.1x100x2.6 um)
- 5 µL injection

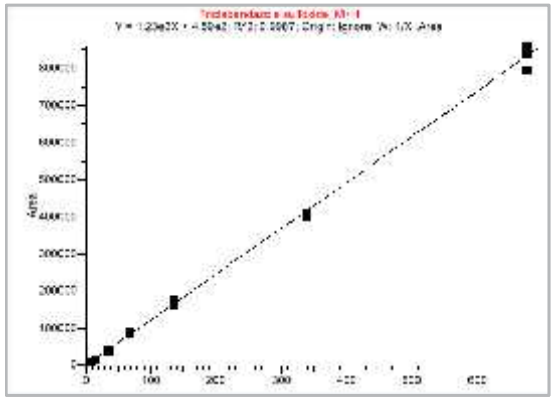
MS Conditions:

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



Triclabendazole Sulfoxide
@ 3.4 µg/Kg in beef extract

RSD = 9% (n=3)



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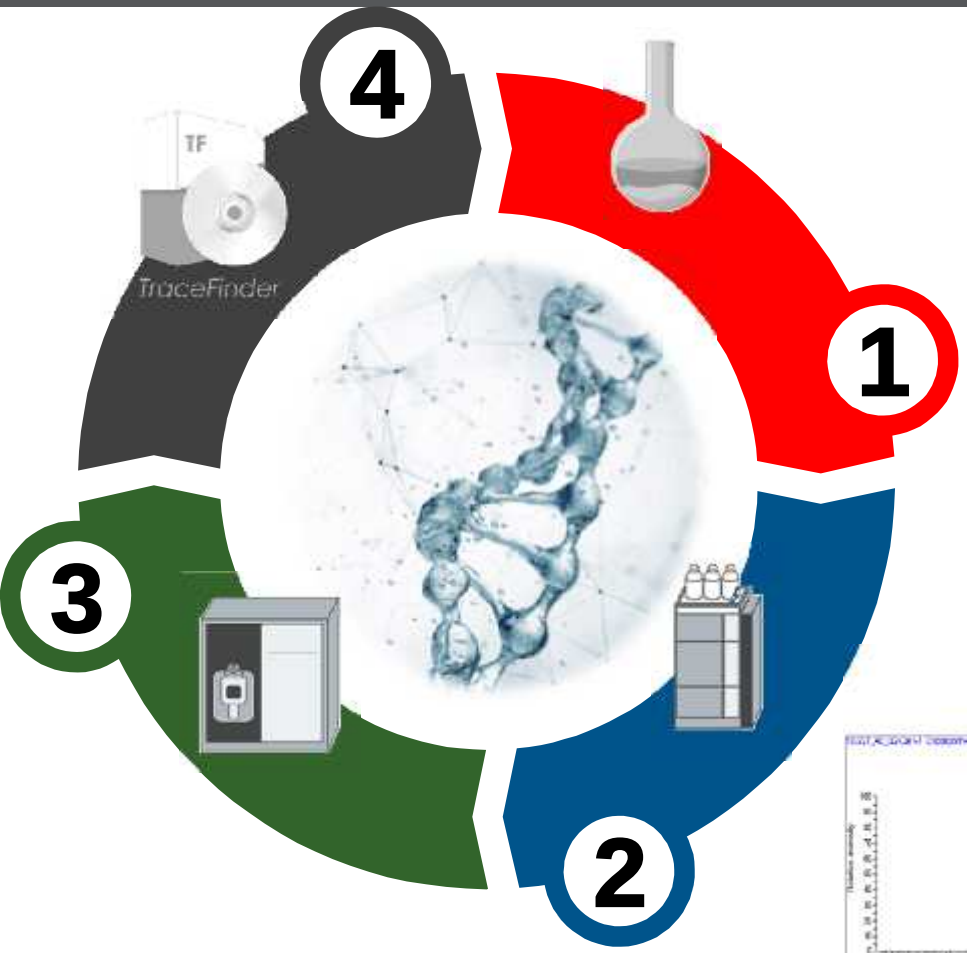
Clinical Research

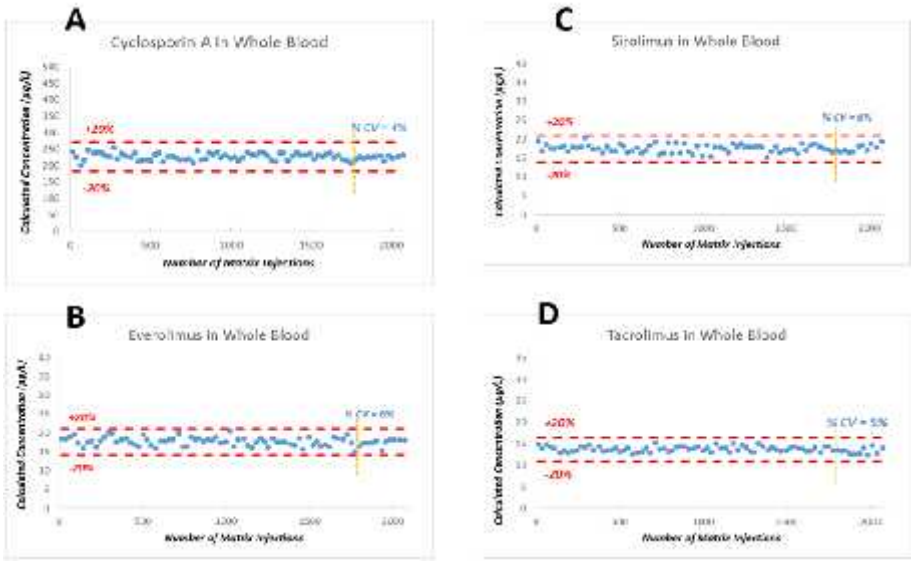
Confident Quantitation for Clinical Research and Forensic Toxicology
Address high throughput capabilities with robust, reliable data addressing sensitivity requirements to enable every laboratory achieve their business and scientific goals

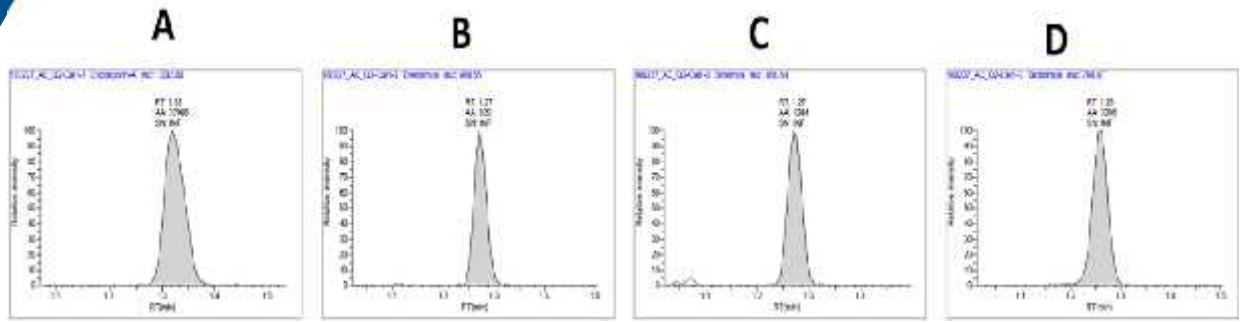
- **Be Prepared to Address Challenges, Everyday**
  - Robust, reproducible, reliable methods for fast quantification critical analytes in biological matrices
- **Address Productivity Goals with Workflow Solutions**
  - **Speed** – analyze more compounds/injection or reduce overall run times
  - **Robustness** – less maintenance, increased uptime
  - **Ease of use** – easy method setup, data review and customized reporting
  - **Sensitivity** – detect contaminants with class leading sensitivity

TSQ Fortis: Immunosuppressants in Whole Blood



~2000+ injections showing %CV ≤ 6%





TSQ Fortis: Demonstration of Robustness – Clinical Research

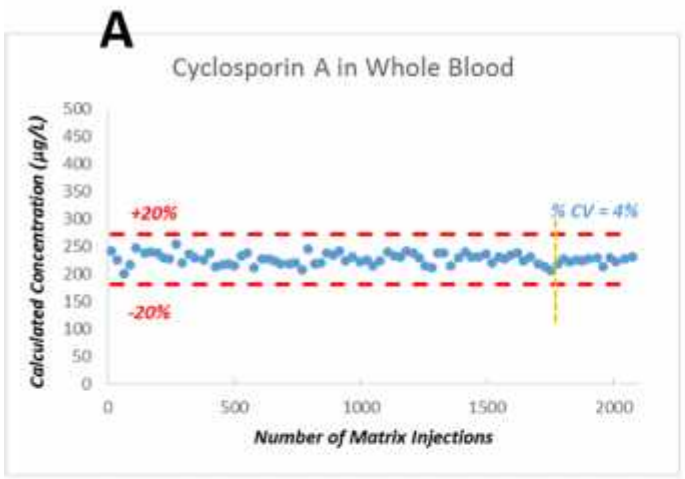
Immunosuppressants

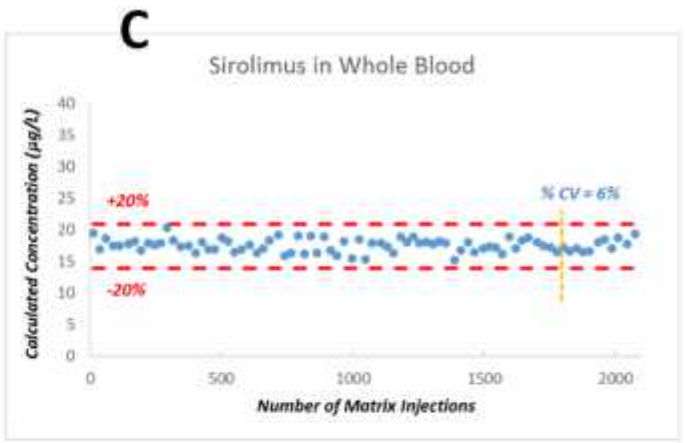
+2000 injections over 6 days (CV%≤ 6%)

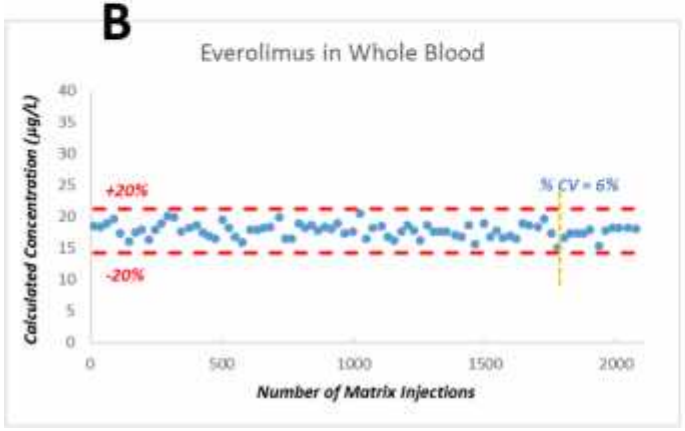
Red lines represent ± 20% of calculated amounts (ng/mL)

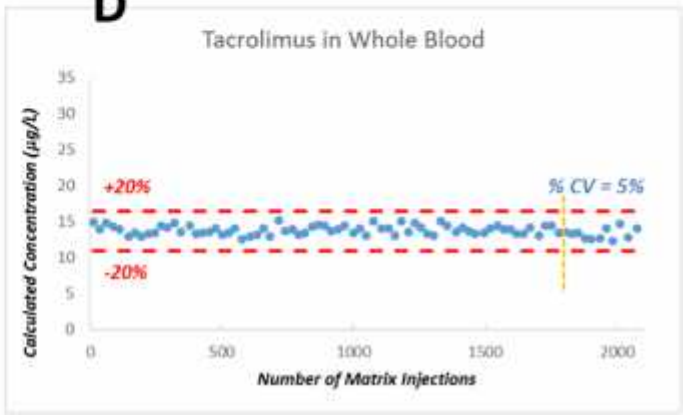
Yellow line represent the period in which the ion transfer tube was cleaned (user basic maintenance ~5 min operation) to demonstrate consistent performance before and after user maintenance.

Technical Note 65206

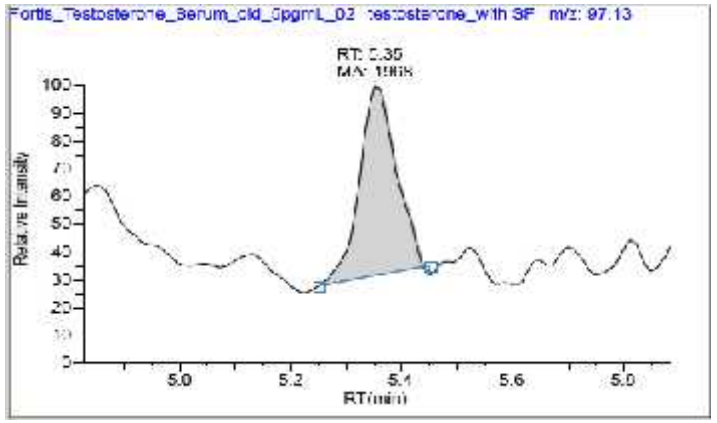


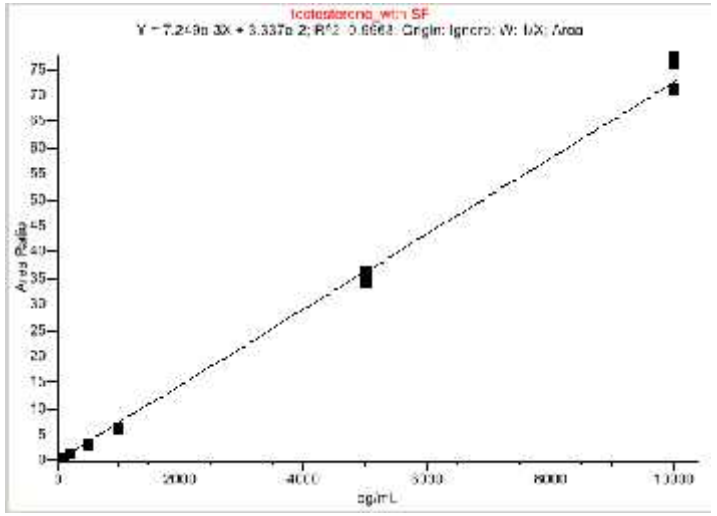




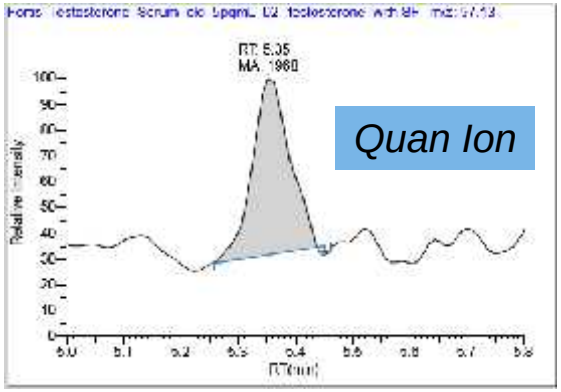


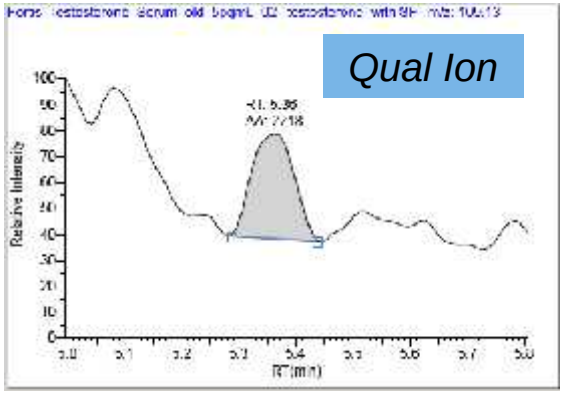
TSQ Fortis: Testosterone in Plasma

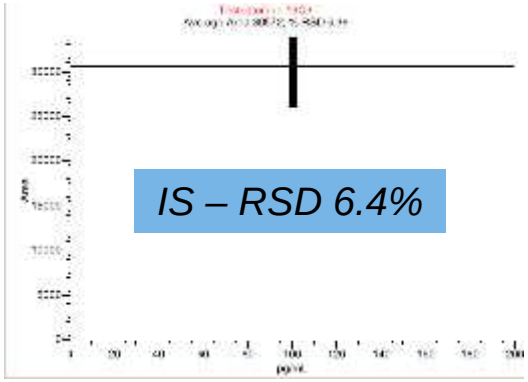




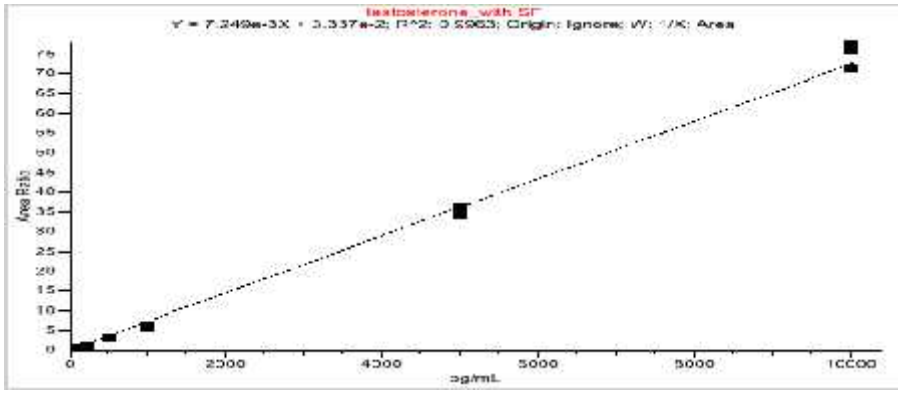
TSQ Fortis: Clinical Research – Testosterone in plasma







5 pg/mL in plasma (RSD = 2.3%)



Technical Brief 65207

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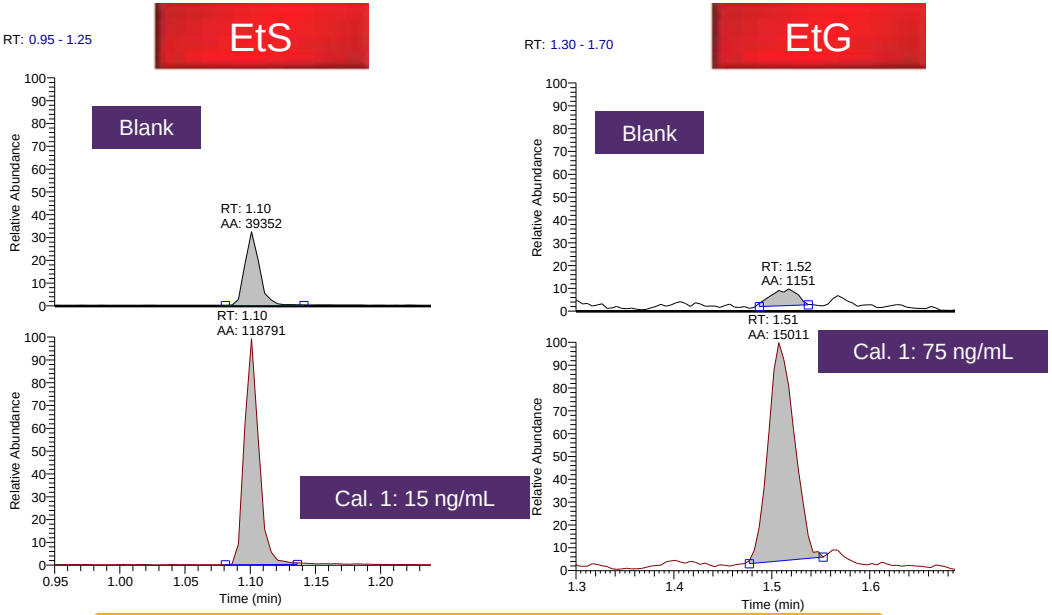
Example SRM Chromatograms – EtS & EtG



EtS & EtG show measurable amounts in Blank Urine samples

Thermo Scientific™ Vanquish™ Horizon UHPLC system

Column: 3.0 x 100 mm, 3 um Syncronis AQ
Column Temp: 30 C
Mobile Phase: [A] H_2O + 0.1% HAc; [B] MeOH
Flow Rate: 0.5 mL/min
Gradient: see table
Injection Volume: 10 uL (EtG/EtS); 5 uL (Barbs)

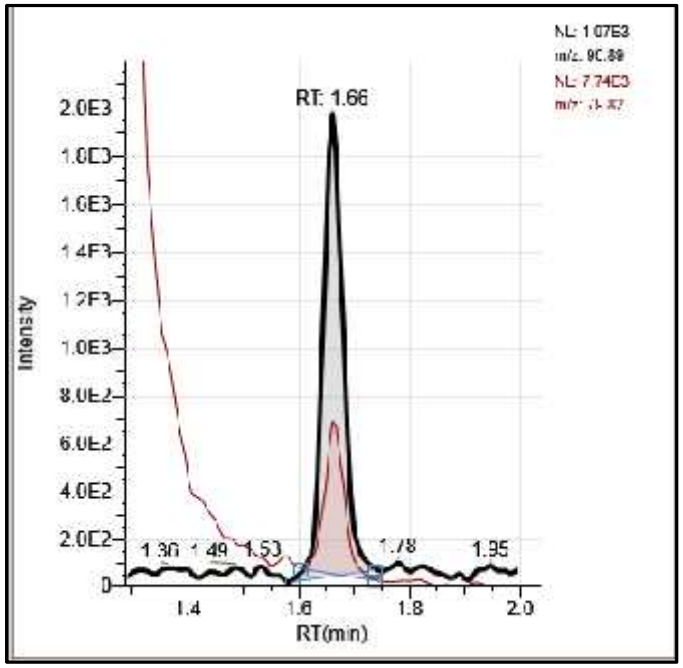
TSQ Quantis Triple Quadrupole MS

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)

- 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.

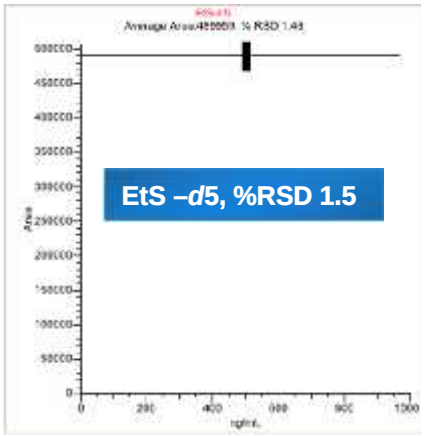
TSQ Quantis: Robust Quantitation of ETG and ETS

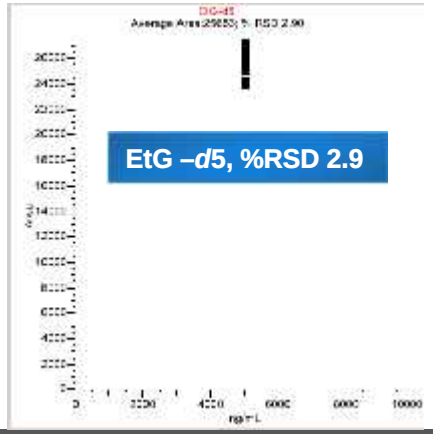
Excellent Precision for the most challenging quantitative assays



50 pg on column for Ethyl Sulfate in Urine

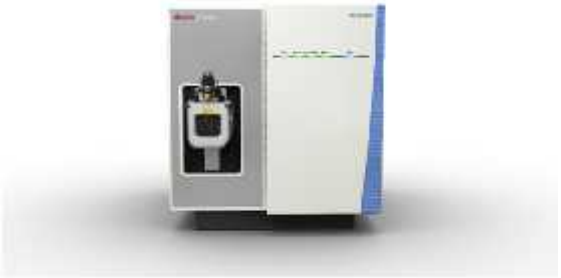
Technical Note 64970



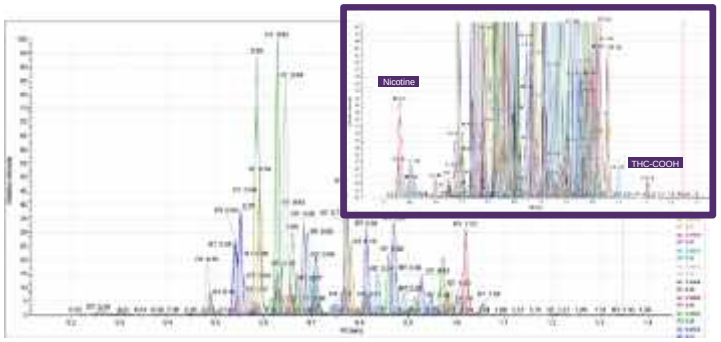


Experimental Details

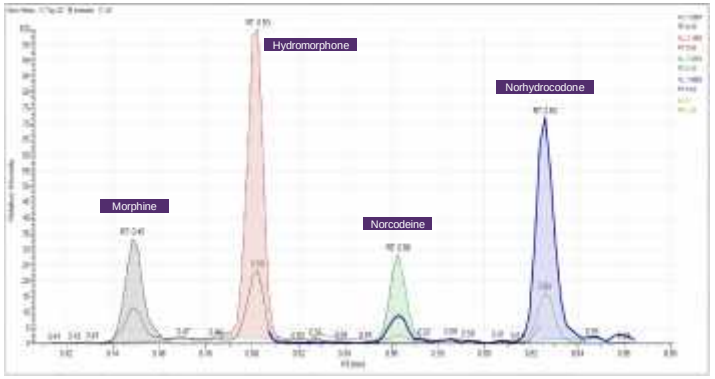
UHPLC : Vanquish Flex Binary
Flow rate: 0.4mL/min
Solvent A: 0.1%FA in H_2O
Solvent B: 0.1%FA in MeOH



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



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



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

Vanquish Horizon UHPLC System

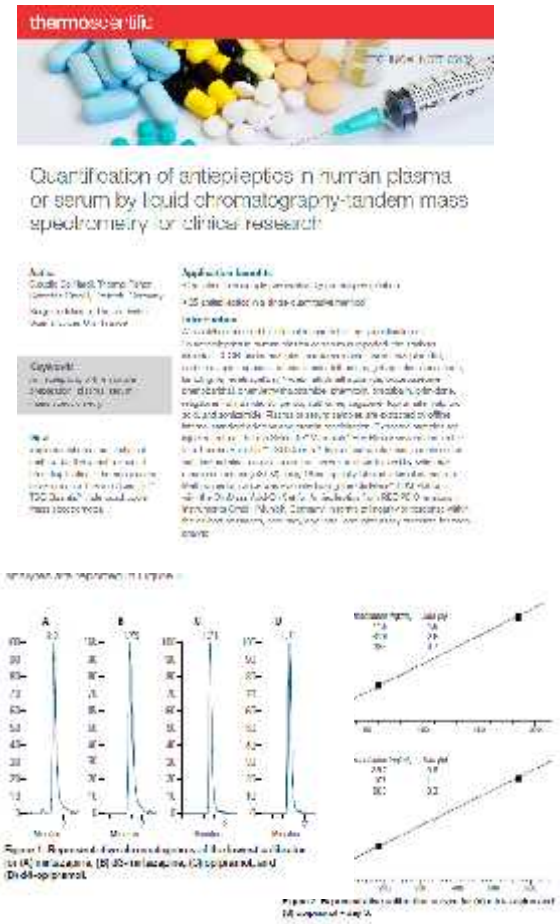
Column: 2.1 x 50 mm, 1.9 um Hypersil Gold AQ
Column Temp: 40 C
Mobile Phase: [A] H_2O + 0.1% HCOOH; [B] ACN + 0.1% HCOOH
Flow Rate: 1.0 mL/min (no split)
Gradient: see table
Injection Volume: 2 uL

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)

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.

Enabling Clinical Research Applications



- Quantitation workflows for a range of analytes
- Antidepressants, antimycotics, benzodiazepines, antiepileptics in human plasma
- Low LLOQs achieved for each analyte in complex matrix condition in an easy-to-implement workflow solution

TSQ Fortis and TSQ Quantis – Enabling Clinical Research

Productivity
Address high productivity demands for every clinical research applications with unmatched speed

Ease-of-Use
Achieve quality data regardless of user expertise with exemplary ease

Sensitivity
Address best-in-class sensitivity that addresses all requirements while improving price-performance ratio of the workflow solution

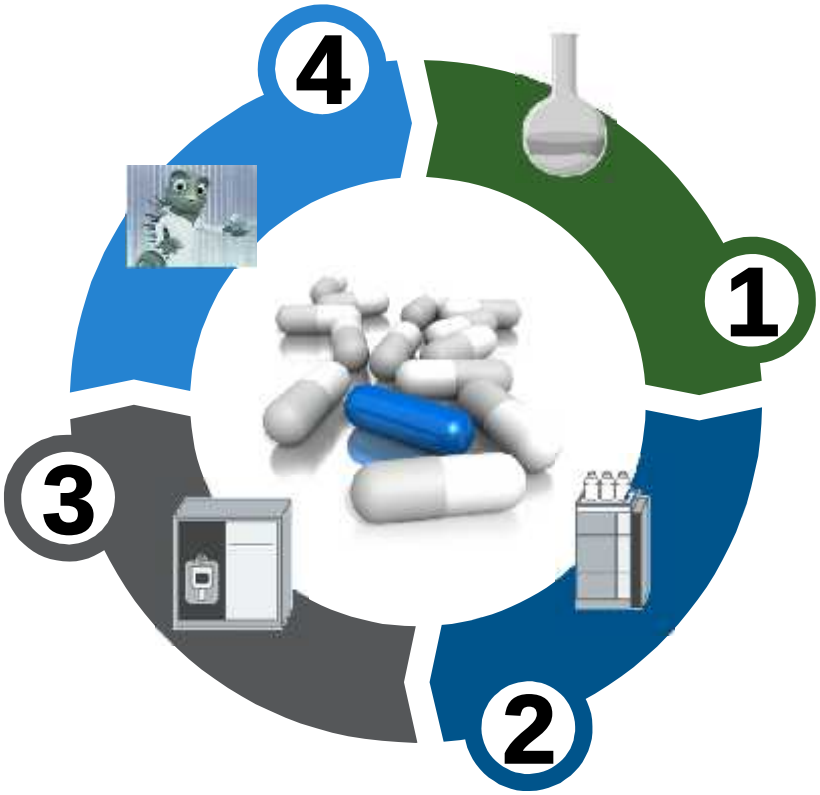
Robustness
High robustness offered by the triple quadrupole ensures very low variability for thousands of injections

Pharmaceutical Applications

Confident Quantitation for Pharmaceutical QA/QC Applications
Achieve robust, reliable, and reproducible assays of drug candidates in water and biological matrices with easy-to-implement quantitative solutions

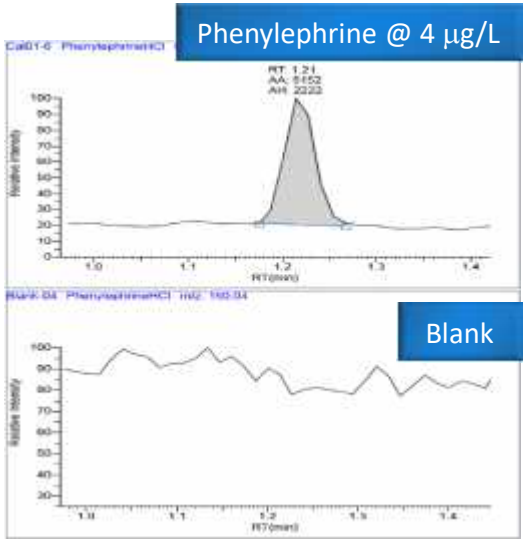
- **Be Prepared to Address Challenges, Everyday**
  - Robust, reproducible, reliable methods for fast quantification of drug candidates
  - Address both small and large molecule types
- **Address Productivity Goals with Workflow Solutions**
  - **Robustness** – less maintenance, increased uptime
  - **Ease of use** – easy method setup, data review and customized reporting
  - **Speed** – analyze more compounds/injection or reduce overall run times
  - **Sensitivity** – detect contaminants with class leading sensitivity

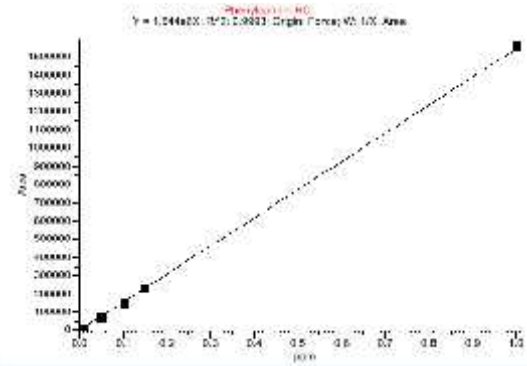
TSQ Fortis: Demonstration of Sensitivity – Pharma QA/QC



- Demonstration of sensitivity, precision, accuracy and robustness for Pharma QA/QC.
- The same quantitation workflow can also be transferred to quantify Phenylephrine in biological matrices

Application Note 65200





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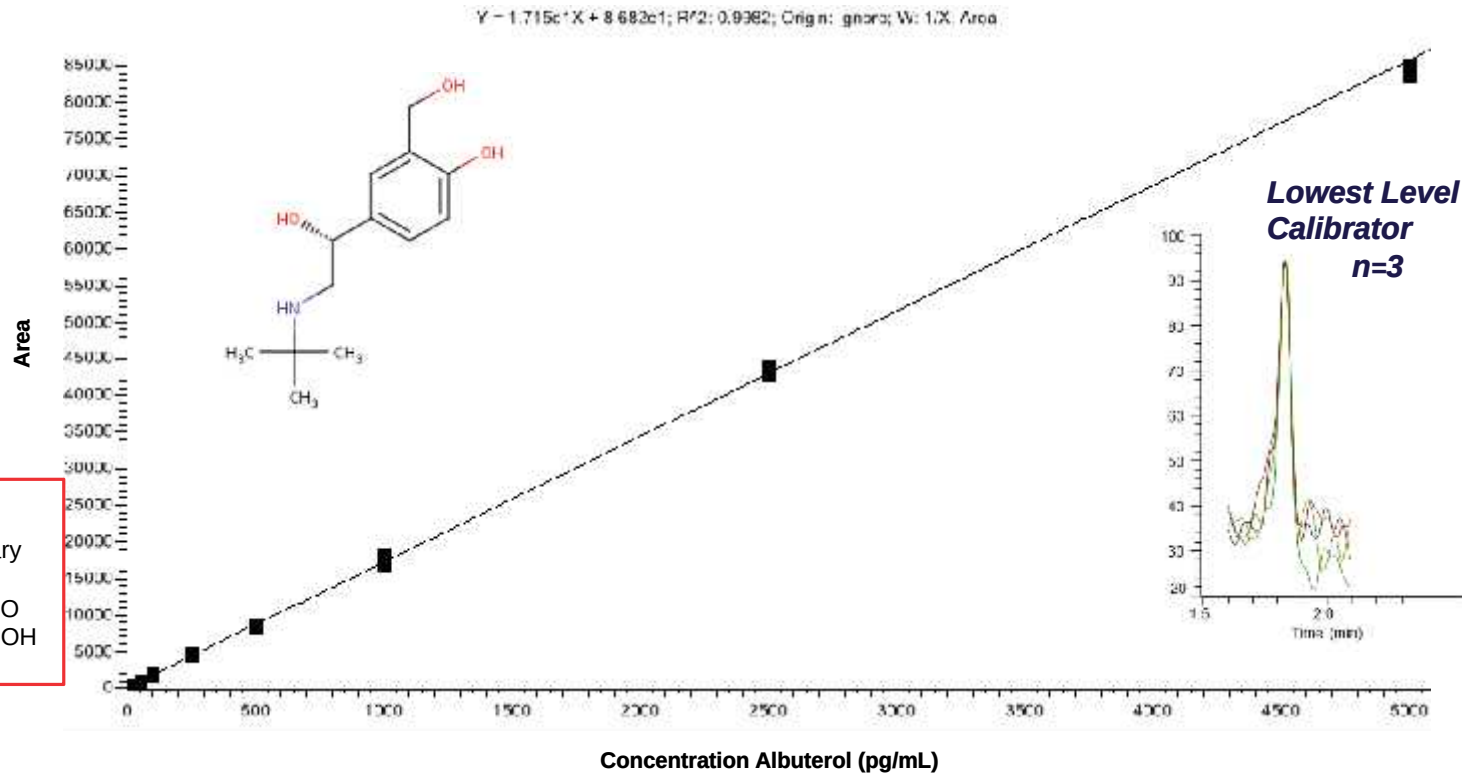
TSQ Quantis: Sensitive Quantitation of Albuterol

Excellent Linearity and Reproducibility across the Dynamic Range



Experimental Details

UHPLC : Thermo Scientific™ Vanquish™ Flex Binary system Flow rate: 0.5 mL/min
Solvent A: 0.1% FA 4 mM ammonium formate in H_2O
Solvent B:0.1% FA 4 mM ammonium formate in MeOH

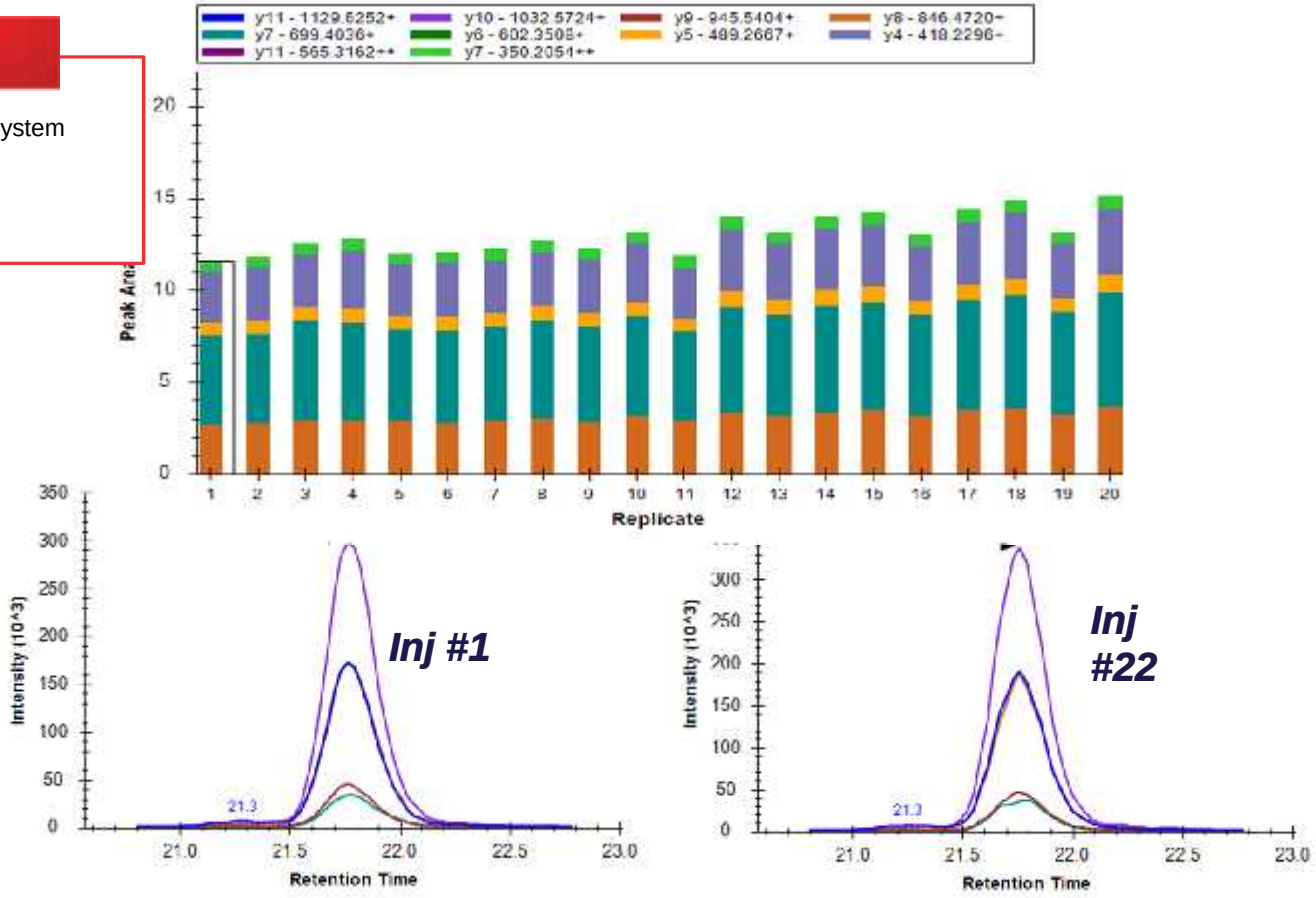
ThermoFisher SCIENTIFIC

Peptide Quantitation with TSQ Quantis Triple Quadrupole MS

Experimental Details

UHPLC : Thermo Scientific™ Easy nLC 1200 system
Flow rate: 300 nL/min
Solvent A: 2%ACN 0.1%FA in H_2O
Solvent B: 10%H_2O 0.1 %FA in ACN

Consistent Ion Ratios Across Wide Mass Range for Confident Peptide Quantitation



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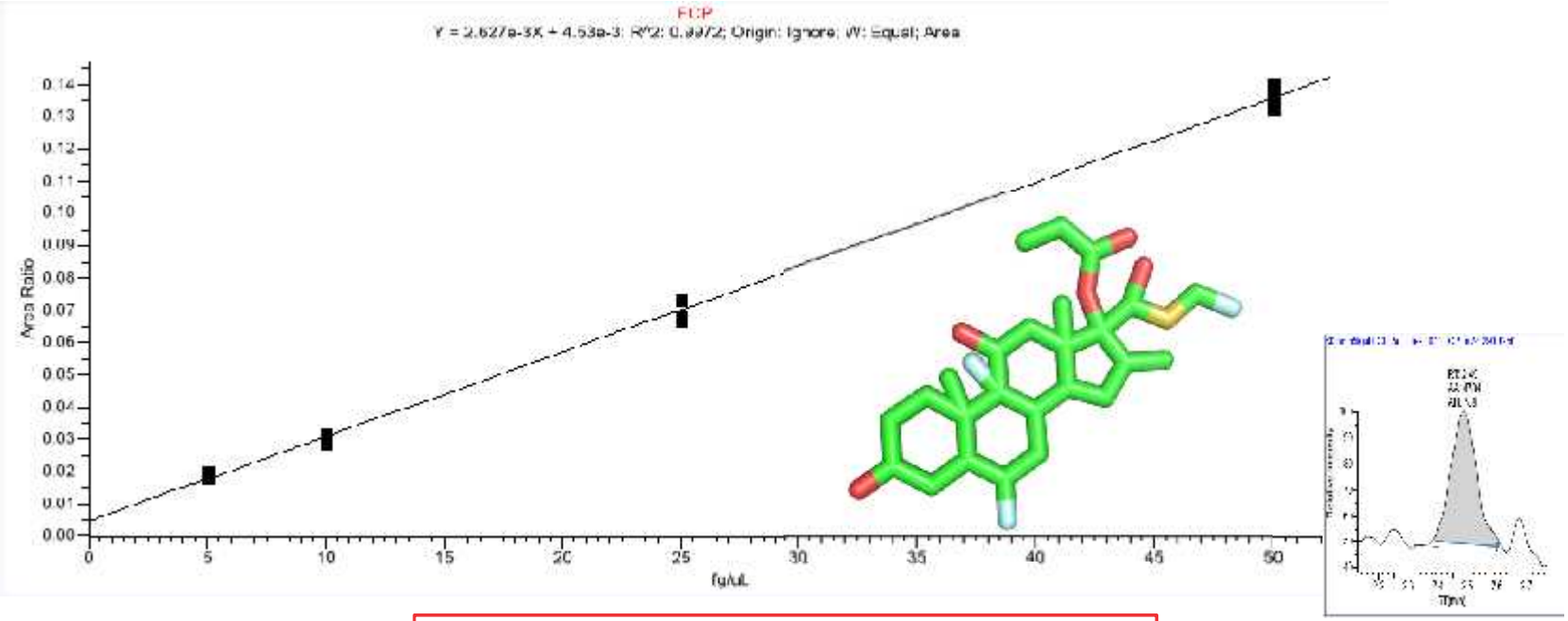
TSQ Altis: Fluticasone Propionate

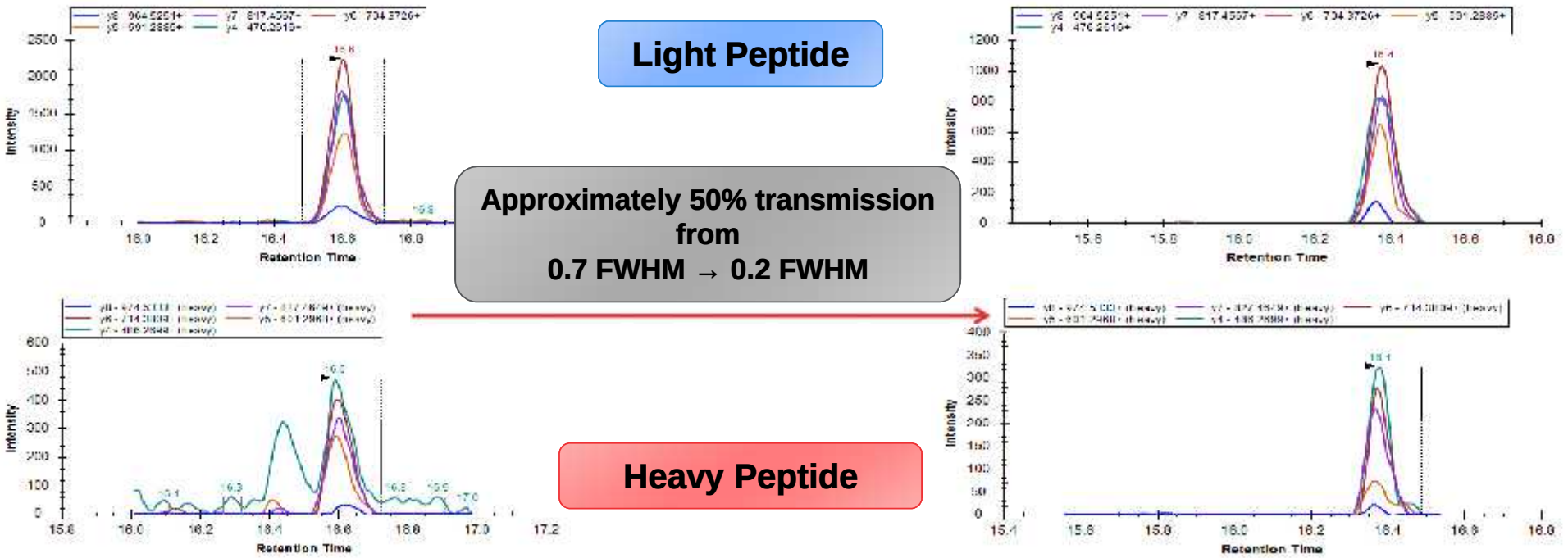
Superior Sensitivity and Reproducibility - Fluticasone Propionate in matrix!
25 fg on column with 4.2% CV!



Experimental Details

UHPLC : Thermo Scientific™ Vanquish™ Flex Binary 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: TraceFinder Software 4.1

TSQ Altis: Protein Quantitation – Benefits of Higher Selectivity and Sensitivity



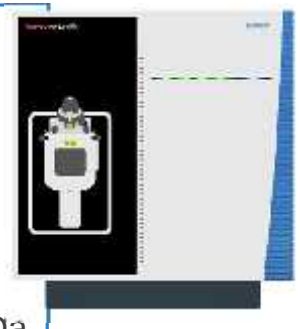
Experimental Details

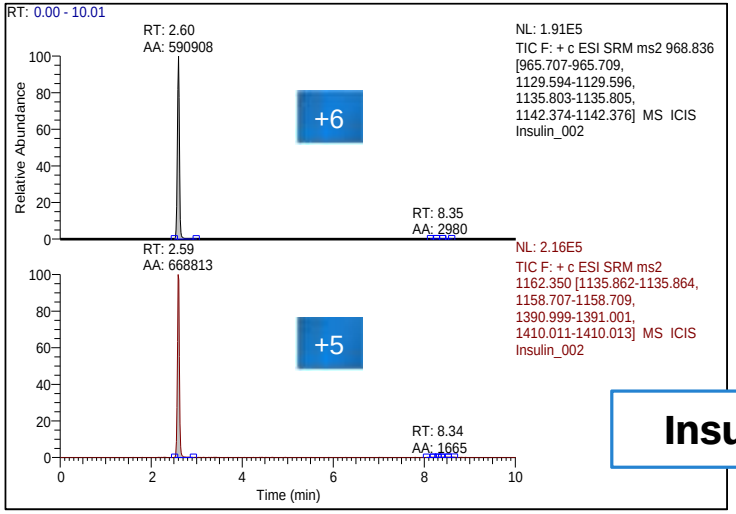
LC : Thermo Scientific™ Easy nLC 1200
Flow rate: 300nL/min
Solvent A: 2%ACN with 0.1%FA in H_2O
Solvent B: 10%H_2O with 0.1 %FA in ACN

Quantitation of Mixture of Large Molecules

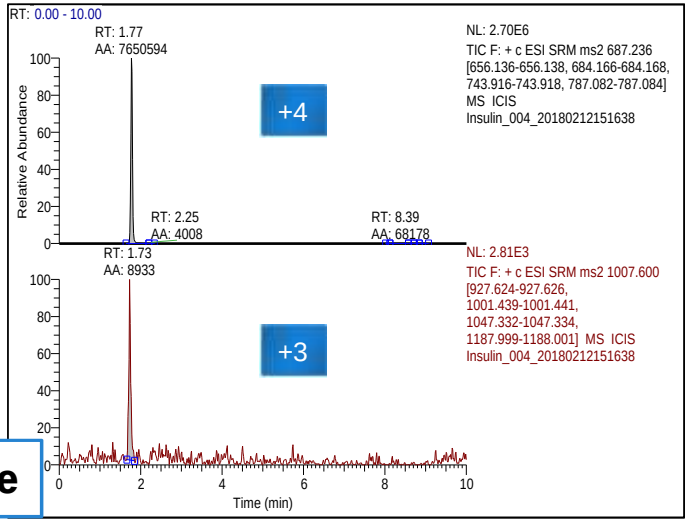
- Column: 2.1 x 50 mm, 1.5 um Accucore Vanquish C18
- Column Temp: 60 C
- Mobile Phase: [A] H_2O + 0.1% Formic Acid; [B] ACN + 0.1% Formic Acid
- Injection Volume: 10 uL
- Sample Temp: 10 C

- Ionization Mode: HESI, Positive ion modes
- MS Acquisition Mode: Selective Reaction Monitoring (SRM) – see table below
- Cycle Time: 0.8 s
- Quad Isolation (Q1,Q3) = Unit (1.2 Da FWHM)





Insulin



C-Peptide

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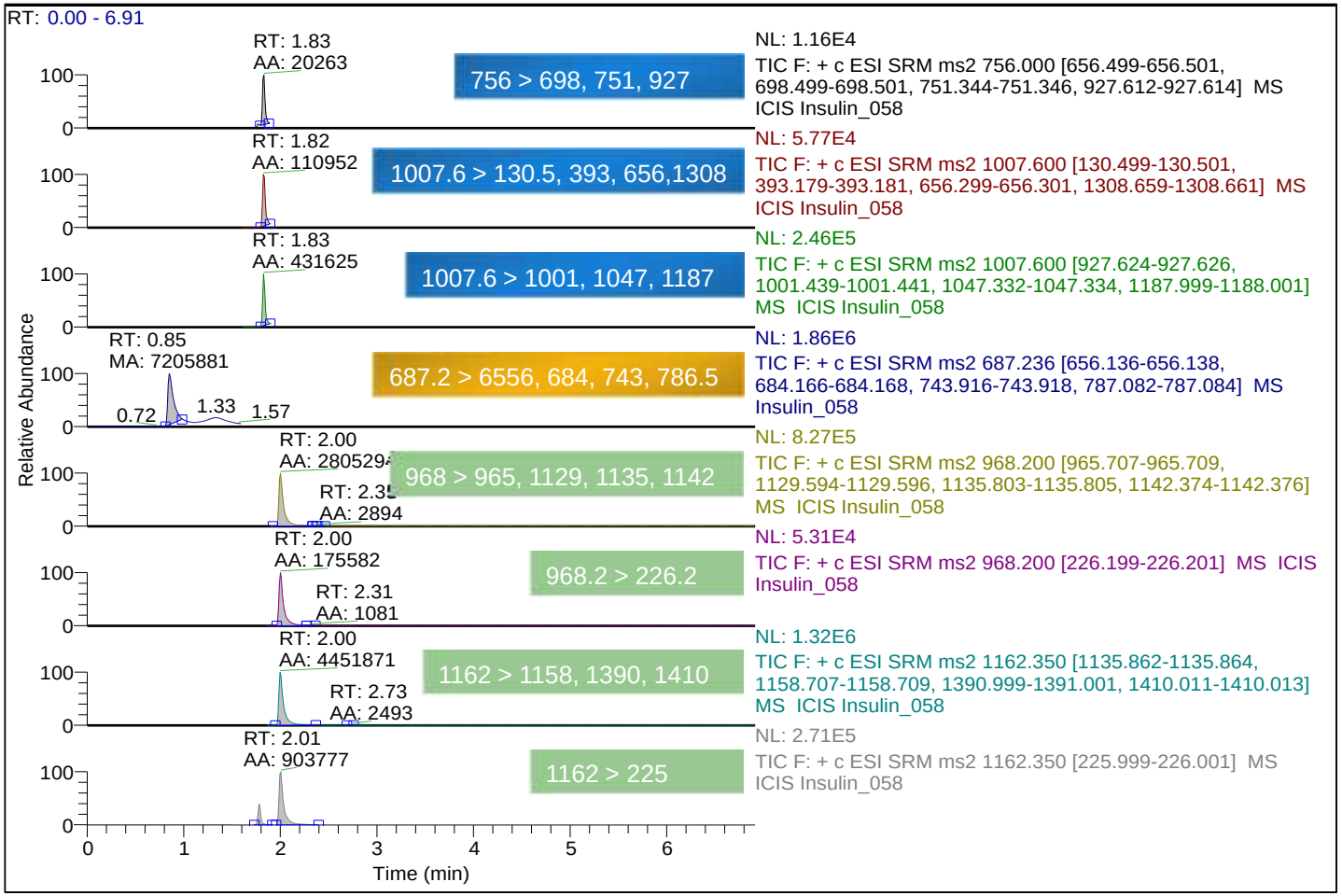
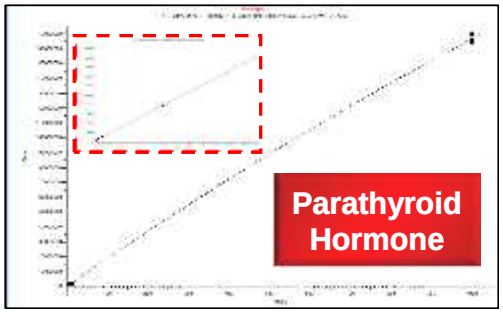
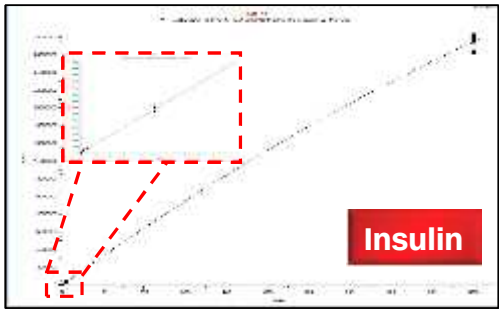
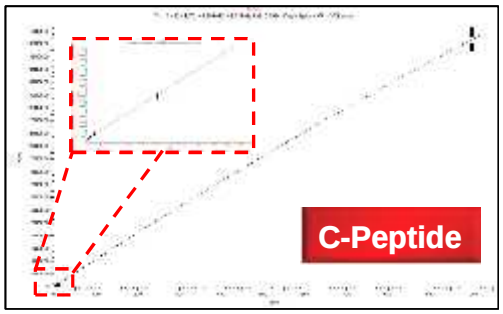
Optimized LC/MS – C-Peptide, Parathyroid Hormone, and Insulin









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Confident Quantitation – Today and Tomorrow

TSQ Altis

TSQ Quantis

TSQ Fortis