Raise the Bar

**ThermoFisher**
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# Environmental and Food Safety

**Sofia, October 2018**

**Simonas Rudys**
**Technical Sales Manager**
**Emerging Markets EMEA**
**Chromatography and Mass Spectrometry**

The world leader in serving science

## Global Scale

- Approximately 70,000 employees globally
- More than $20 billion in annual revenue
- Unparalleled commercial reach

## Unmatched Depth

- Leading innovative technologies
- Deep applications expertise
- Premier laboratory productivity partner

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thermo scientific | applied biosystems | invitrogen | fisher scientific | unity lab services

**Our Mission: To enable our customers to make the world healthier, cleaner and safer**

*Proprietary & Confidential*

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# Our Business Segments

## Analytical Instruments

**Mass Spectrometry**
Q-Exactive HF mass spectrometer

**Chromatography**
Vanquish UHPLC

**Electron Microscopy**
Titan S/TEM

**Chemical Analysis**
Gemini handheld analyzer

## Life Science Solutions

**Clinical Oncology**
Next Gen Sequencing

**Genetic Sciences**
QuantStudio Dx R qPCR

**Biosciences**
Life Science Reagents

**Human Identification**
GlobalFiler PCR Amplification Kit

**Reproductive Health**
Microarrays

**BioProduction**
Cell Culture Reagents

## Specialty Diagnostics

**Clinical Diagnostics**
Oral-Eze Oral Fluid Collection System

**ImmunoDiagnostics**
ImmunoCAP Allergy and EliA Autoimmunity Tests

**Microbiology**
Antimicrobial Susceptibility Testing Solutions

**Pathology**
Rotary Microtome

**Transplant Diagnostics**
NXType High Resolution Genotyping

## Laboratory Products and Services

**Lab Equipment**
Sorvall WX+ Ultracentrifuge

**Enterprise and Instrument Services**

**Chemicals**

**Distribution and BioPharma Services**

**Lab Consumables**
E1 ClipTip Pipette system

**$20 B Revenue**

- 22%
- 26%
- 36%
- 16%

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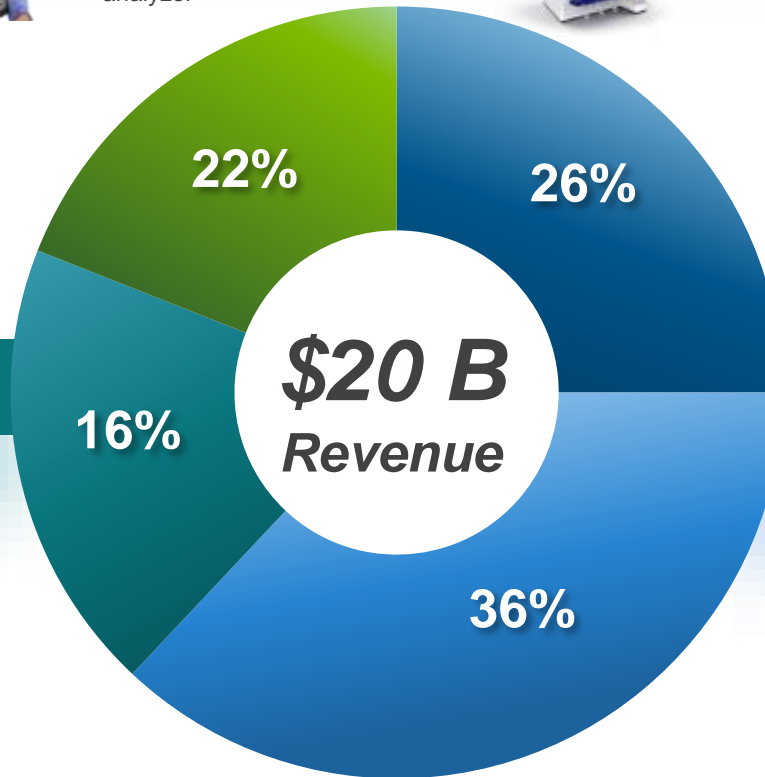
# A Laboratory Solutions Powerhouse

**Software and Services**

Comprehensive services

Multi-vendor support

Thermo Scientific™ Dionex™ Chromeleon™ CDS, LIMS

**Consumables**

IC, LC, GC columns

Vials, septas, fittings

Solvents and standards

**Applications Expertise**

**Instruments**

GC, LC, IC

LC multiplexing

SQ, QQQ, Ion Trap,

Orbitrap, Hybrids

AA, ICP, ICP-MS, IRMS

**Expanded depth and scale offer unequaled portfolio breadth**

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# Thermo Scientific LC Portfolio Overview

| HPLC Systems | | UHPLC Systems | |
|---|---|---|---|
| **Routine Analysis** | **Low-Flow** | **Flexibility, More Performance and Method Development** | **Research & HT** |

**Vanquish Duo Workflows**

**Vanquish Horizon**
- High pressure binary solvent mixing
- Industry-leading precision and accuracy
- Two thermostatting modes
- Unmatched detection sensitivity
- Biocompatible
- Integration of multiple detection technologies

**UltiMate 3000 RSLCnano**

**EASY-nLC 1200**
- UHPLC systems for Nano/Cap/Micro range
- Ideal front-end for proteomics applications

**Vanquish Flex**
- High pressure binary and low pressure quaternary solvent mixing options
- Two thermostatting modes
- Biocompatible
- Integration of multiple detection technologies

**UltiMate 3000 RS/BioRS**
- Specialty workflow support
- Binary and Quaternary UHPLC systems

**UltiMate 3000 SD**
- Workhorse for standard HPLC applications

**UltiMate 3000 Basic Automated**
- Highly economic & reliable

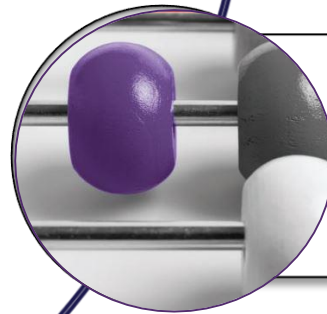| **620 bar** | **800-1200 bar** | **Up to 1000 bar** | **1500 bar** |
|---|---|---|---|

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## Dual LC

- A two channel LC system with standard footprint

## Tandem LC/LC-MS

- System for efficient detector utilization

## Inverse Gradient

- System for advanced quantification capabilities

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# Target Markets of Vanquish Duo Workflows

## Standard LC & LC-MS

Flow path 1

| Analytical Gradient Application 1 | Recondi-tioning | Analytical Gradient Application 1 | Recondi-tioning |

**Value**
Standard instrumentation
Easy to use
**Limitation**
Idle time limits productivity
Low throughput
CAD quantification of unknowns

**Target Market**

## Vanquish Duo for Dual LC

Flow path 1

Flow path 2

| Analytical Gradient Application 1 | Recondi-tioning |
| Analytical Gradient Application 1 | Recondi-tioning |

Throughput increase
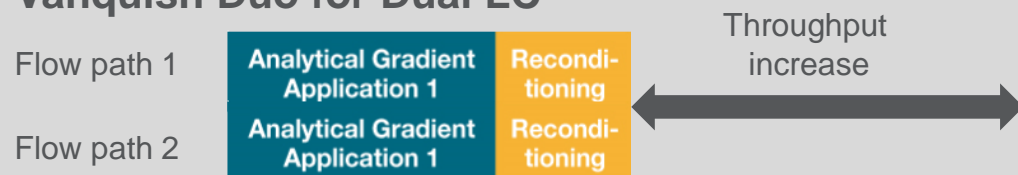
**Value**
Doubled sample throughput
Run complementary assays for better sample knowledge
Bench space savings

**Target Market**

## Vanquish Duo for Tandem LC or LC-MS

Flow path 1

Flow path 2

| Analytical Gradient | Analytical Gradient |
| Recondi-tioning | Recondi-tioning |

Throughput increase
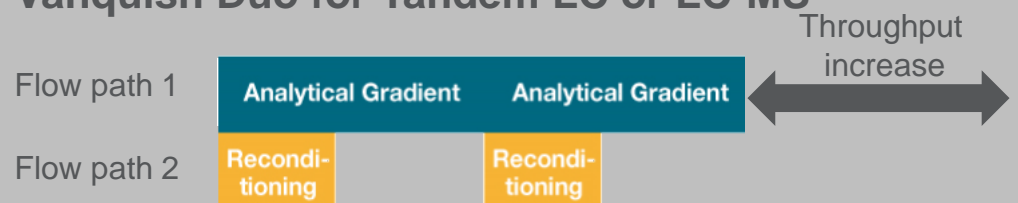
**Value**
Increase LC-MS productivity by 50% with only 5% higher costs (LC-QQQ)

**Target Market**

## Vanquish Duo for Inverse Gradient

Flow path 1

Flow path 2

| Analytical Gradient | Recondi-tioning | Analytical Gradient | Recondi-tioning |

**Value**
Standard-free quantitation of unknown compounds
Do not miss peaks

**Target Market**

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# Thermo Scientific Vanquish Duo System Benefits

- Faster from sample to result
- Higher sample throughput
- Efficient bench space utilization
- Improved return on investments
- Streamlined workflows by intelligent software
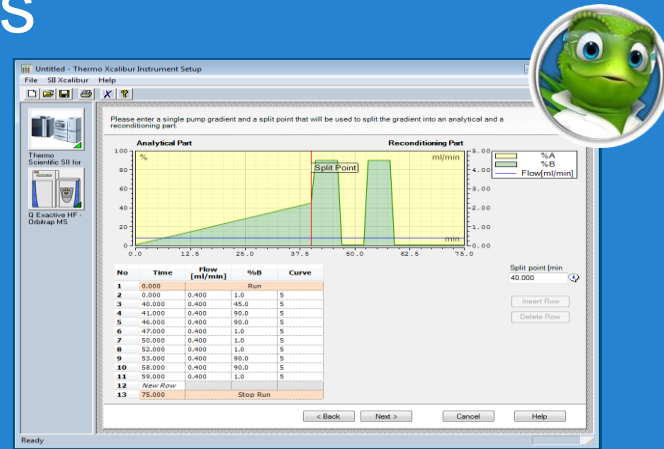- Optimized quantification with Charged Aerosol Detector

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S C I E N T I F I C

## Vanquish Duo

3 workflows requiring different instrument configurations

### For all workflows



Workflow kits

**+**



Chromeleon Tools
e.g.wizards or method translation

Vanquish Duo for
Dual LC

Vanquish Duo for
Tandem LC/LC-MS

Vanquish Duo for
Inverse Gradient

*Improved ROI by extended Productivity for each workflow*

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## Thermo Scientific™ Vanquish™ Flex Dual Pump



- 2 ternary pumps in one housing
- 1000 bar (15,000 psi) version
- Delivers 2 completely independent gradients

## Thermo Scientific™ Vanquish™ Dual Split Samplers



- 2 injection units in one autosampler
- 1000 and 1500 bar (15,000 or 22,000 psi) version
- Independent flow paths

**Thermo Fisher**
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# Thermo Scientific™ Vanquish™ Duo System for Dual LC

**2 x** Binary Pump H

or

**2 x** Binary Pump F

or

**1 x** Dual Pump F

**Dual** Sampler

**2 x** column compartment

or

or

**1 x** column compartment

**Any two of:**

Optical Detector

Charged Aerosol Detector

Mass Spec

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Dual LC

| | | | | |
|---|---|---|---|---|
| Flow Path 1 | Analytical Gradient Application 1 | Recondi-tioning | Analytical Gradient Application 1 | Recondi-tioning |
| Flow Path 2 | Analytical Gradient Application 1 | Recondi-tioning | Analytical Gradient Application 1 | Recondi-tioning |

Dual LC

| | | | | |
|---|---|---|---|---|
| Flow Path 1 | Analytical Gradient Application 1 | Recondi-tioning | Analytical Gradient Application 1 | Recondi-tioning |
| Flow Path 2 | Analytical Gradient Application 2 | Recondi-tioning | Analytical Gradient Application 2 | Recondi-tioning |

## Run two identical columns

## Run your complementary
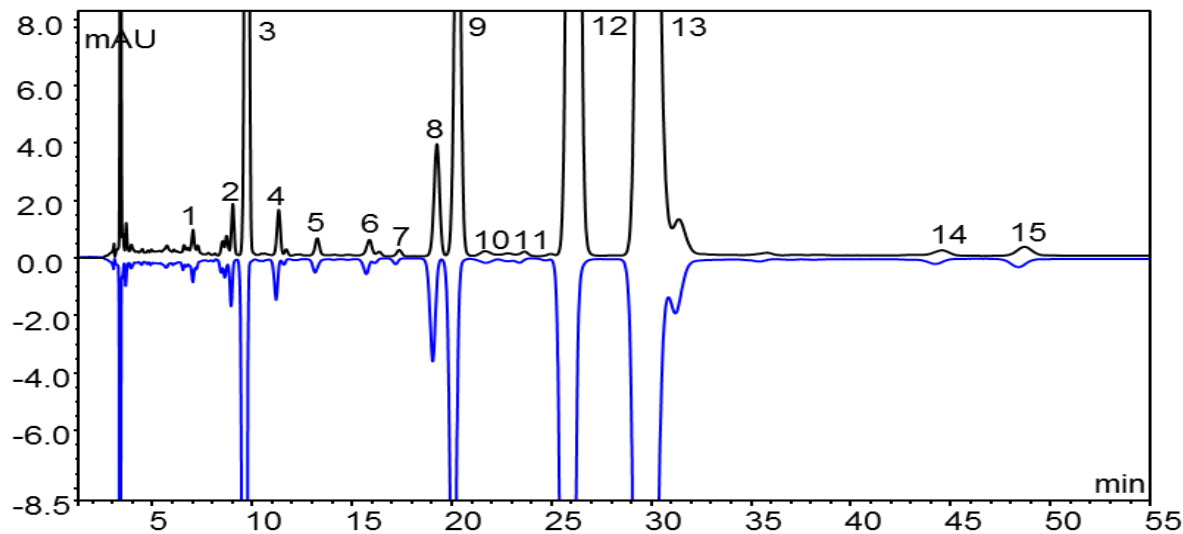


Be finished in
half the time
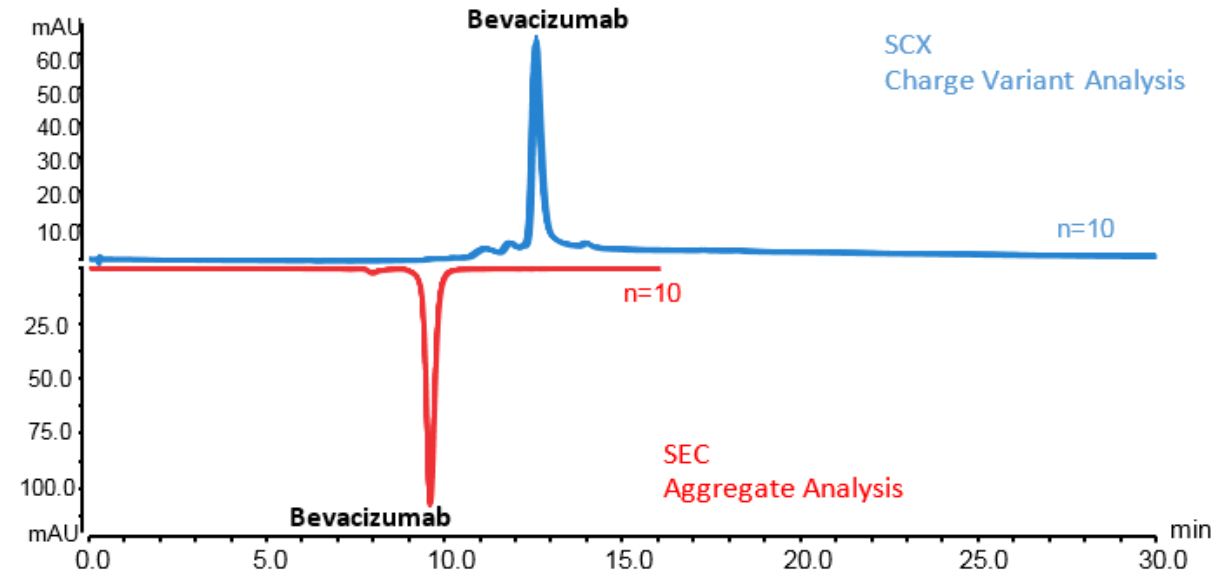
Get more information
out of every sample

**Finished in half the time**

**Efficient characterization**

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# Thermo Scientific Vanquish Duo
# for Tandem LC or LC-MS



*Proprietary & Confidential*

**High investments in modern instrumentation**

**Not fully utilizing instrumentation**

Standard LC / LC-MS

| Analytical Gradient | Recondi-tioning | Analytical Gradient | Recondi-tioning | Analytical Gradient | Recondi-tioning |
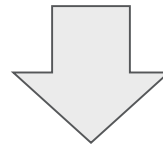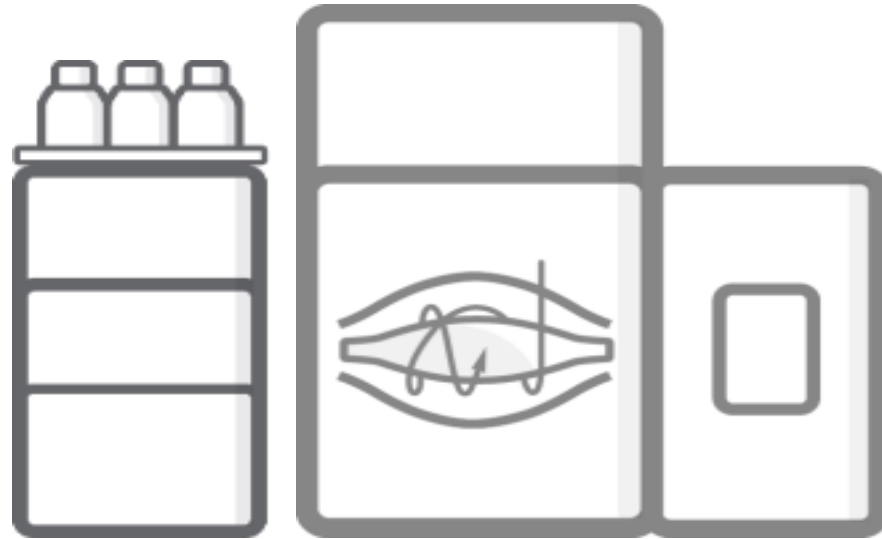
**Time not acquiring meaningful data**

**Asked to maximze the instrument outcome**

**2 x** Binary Pump H

or

**2 x** Binary Pump F

or

**1 x** Dual Pump F

**Single** Sampler

**1 x** column compartment

Any Optical Detector

Charged Aerosol Detector

Any Mass Spec

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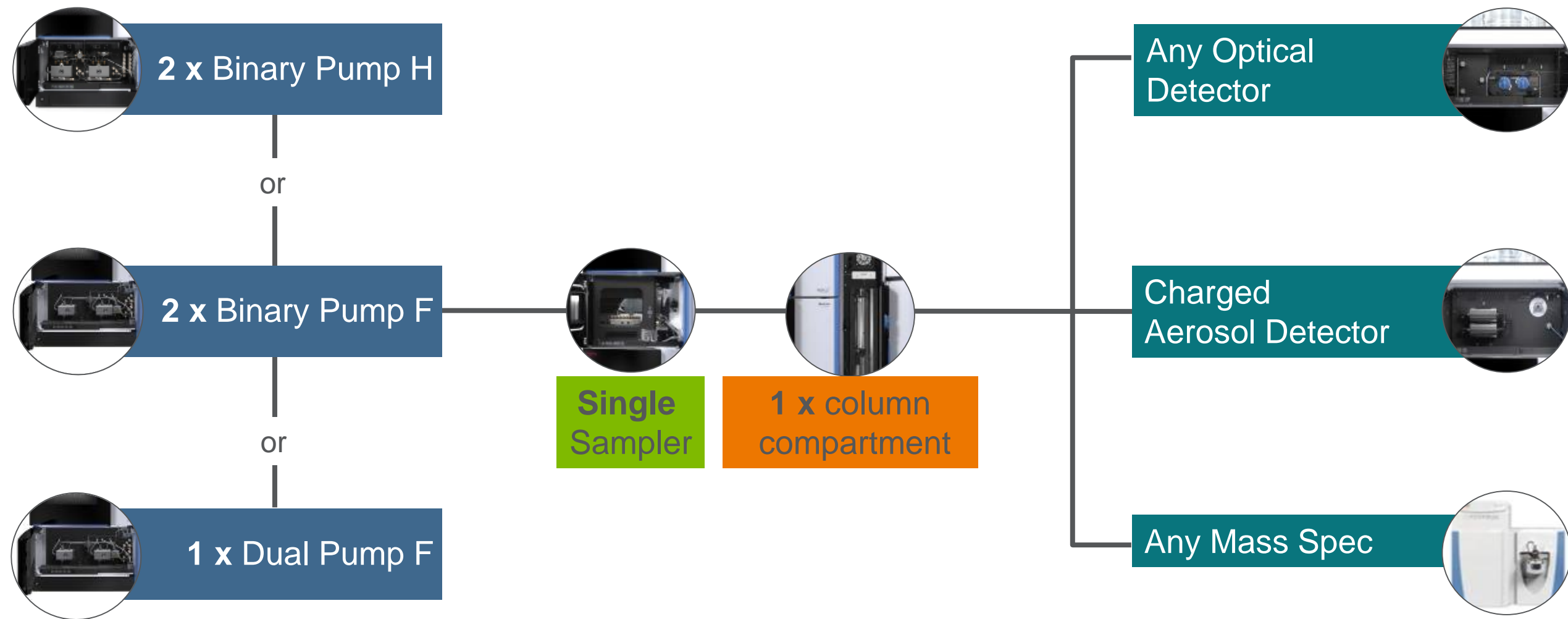
## Advanced column wash without sacrificing throughput
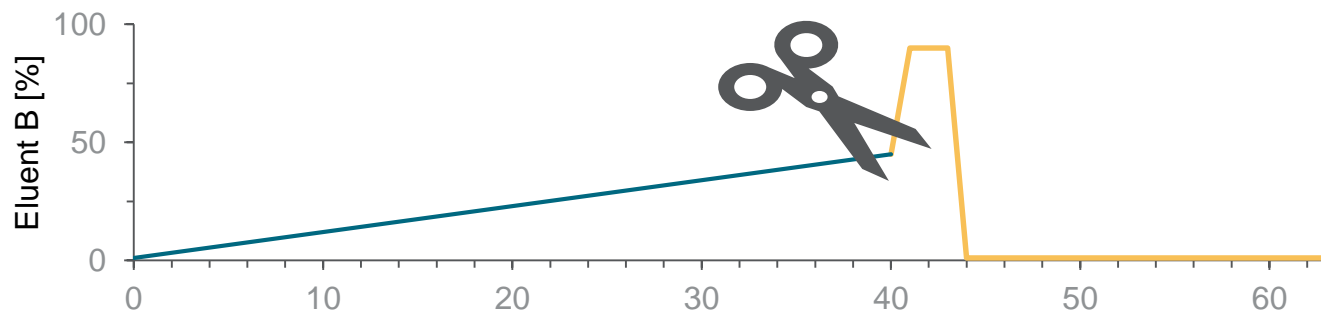


Standard gradient profile
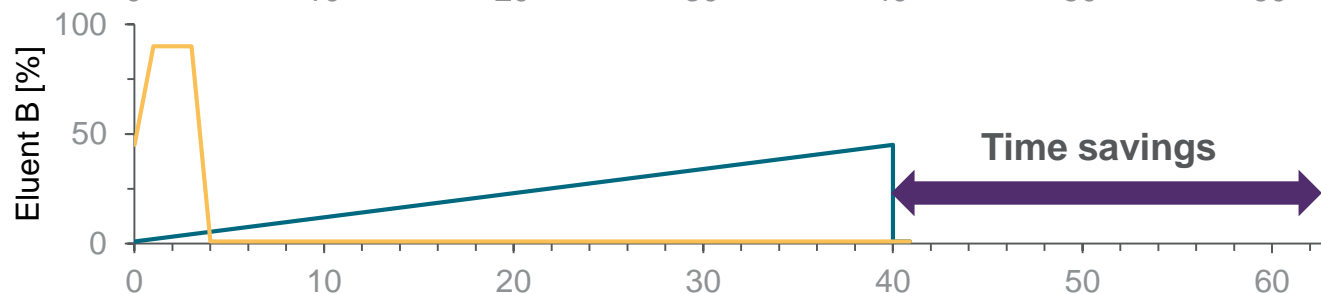
**Tandem mode allows for time savings**
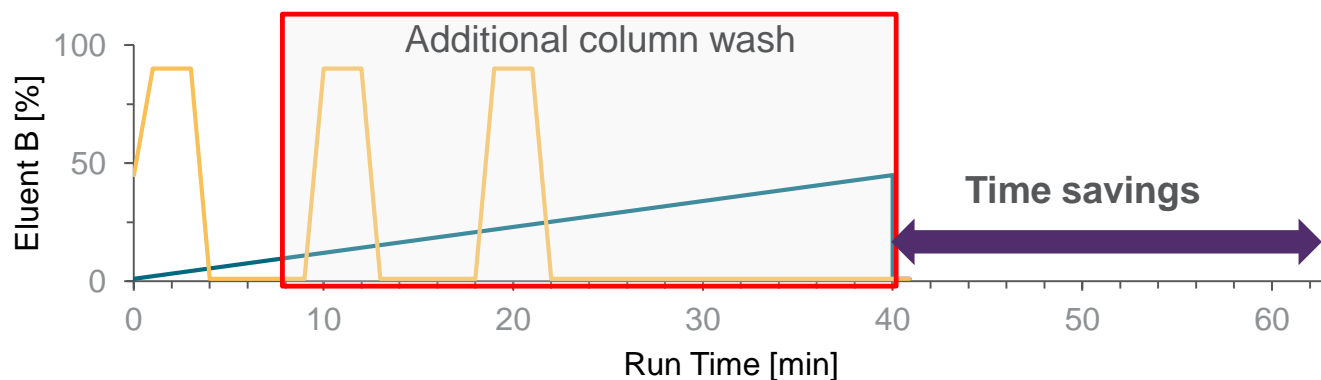
**Extra column washing steps without sacrificing throughput**

# Thermo Scientific Vanquish Duo
# for Inverse Gradient



Standard LC

| Pump 1 | Analytical Gradient | Recondi-tioning | Analytical Gradient | Recondi-tioning | Analytical Gradient | Recondi-tioning |

Inverse Gradient Method

| Pump 1 Pump 2 | Analytical Gradient | Recondi-tioning | Analytical Gradient | Recondi-tioning | Analytical Gradient | Recondi-tioning |

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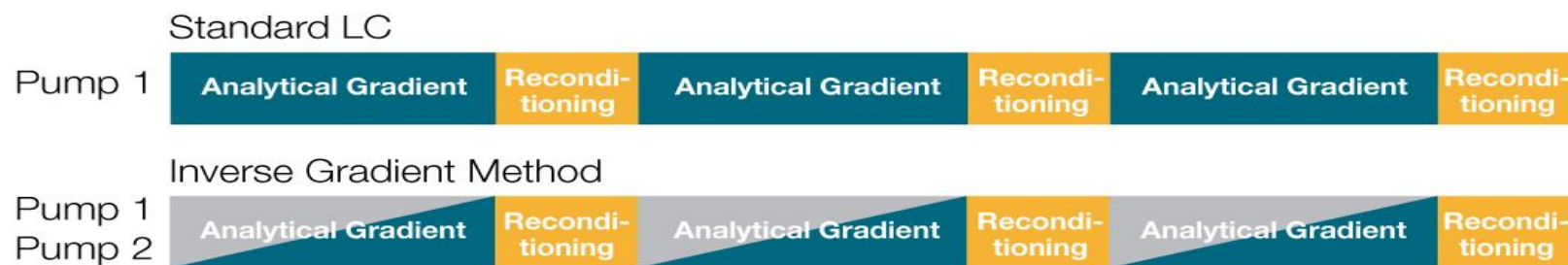
- Detection of substances without a chromophore

- Consistent analyte response independent of chemical structure

- Dynamic range up to four orders of magnitude and highest sensitivity

- Relative quantification using virtually any standard
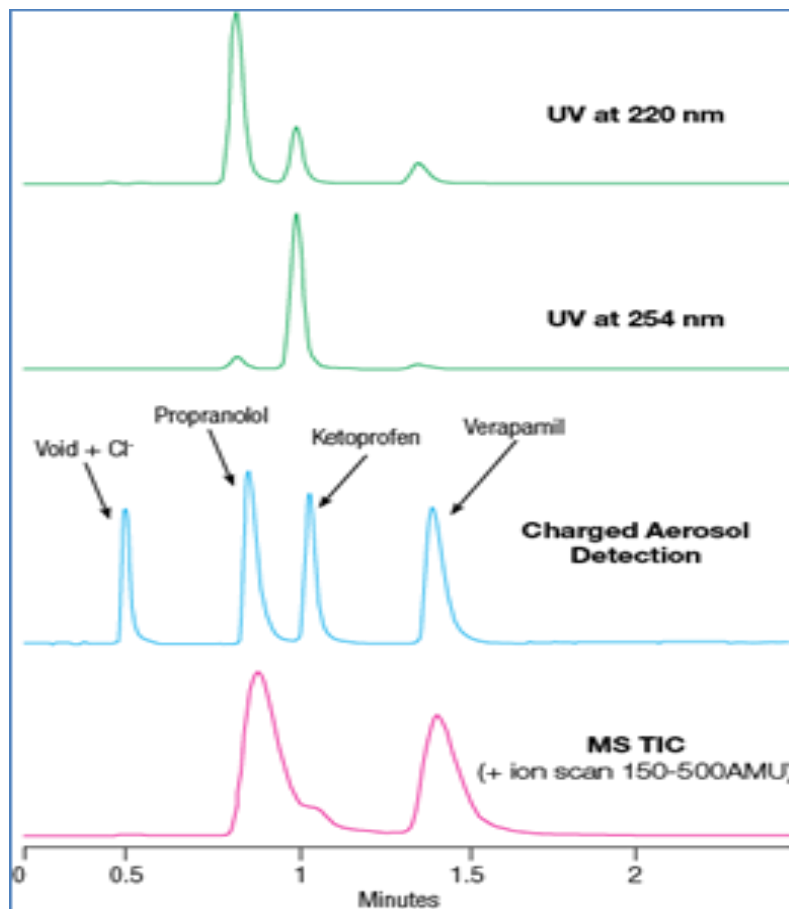


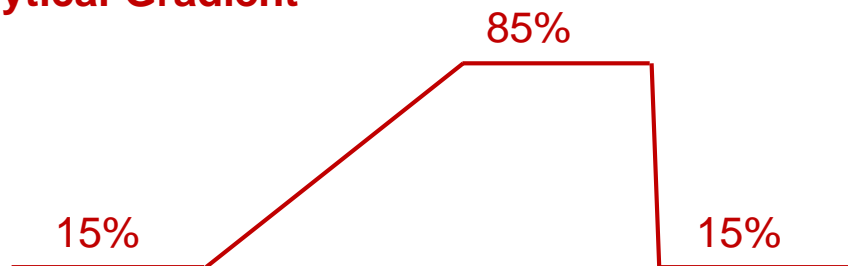Comparison of Charged Aerosol Detection to UV and MS

*Saving costs for additional detectors or assays*

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**2 x** Binary Pump H

or

**2 x** Binary Pump F

or

**1 x** Dual Pump F

**Single** Sampler

**1 x** column compartment

Optional Optical Detector

Charged Aerosol Detector

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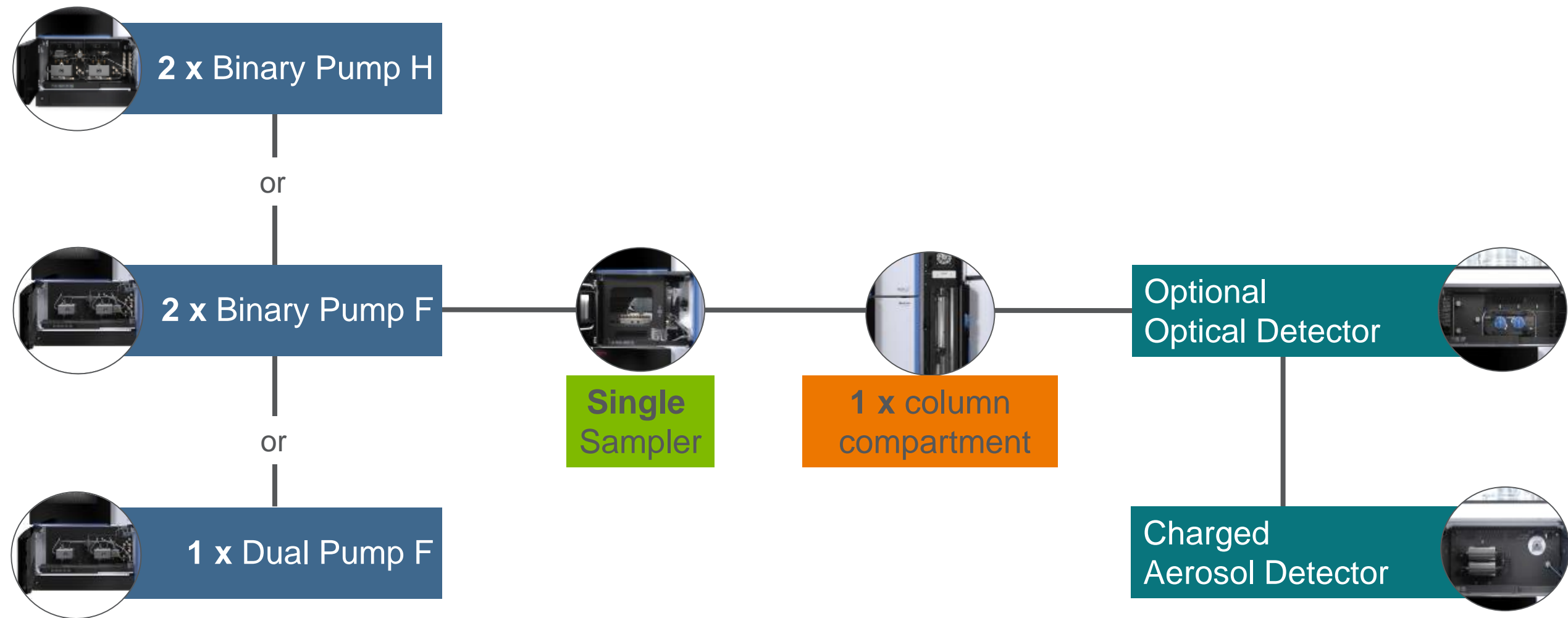
# Inverse Gradient Compensation for Uniform Response

**Analytical Gradient**

85%

15%    15%
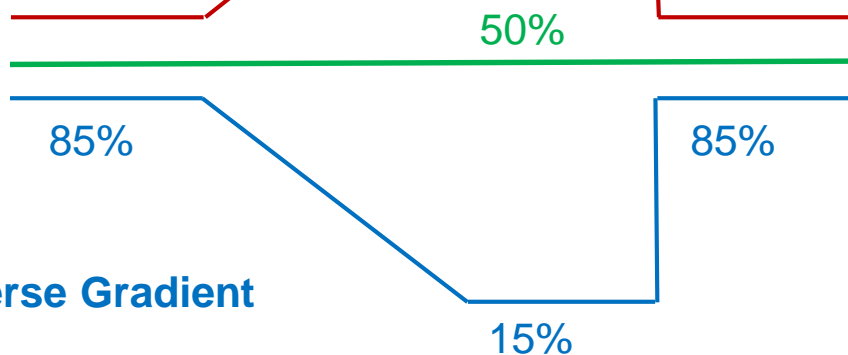
**Eluent at detector**

**Conventional Gradient Elution**
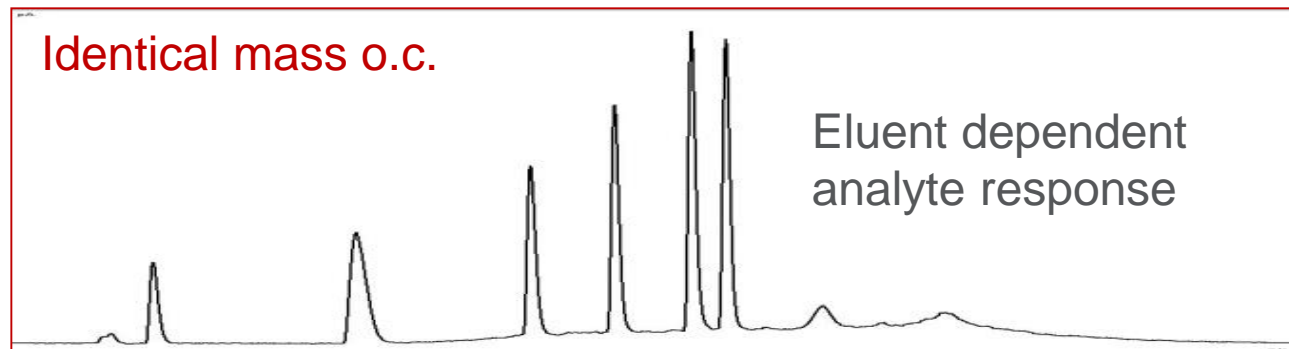
Identical mass o.c.

Eluent dependent analyte response

**Analytical Gradient**

85%

15%    15%

50%

85%    85%

15%

**Inverse Gradient**

**Eluent at detector**

**Inverse Gradient Compensation**

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# Workflow Positioning
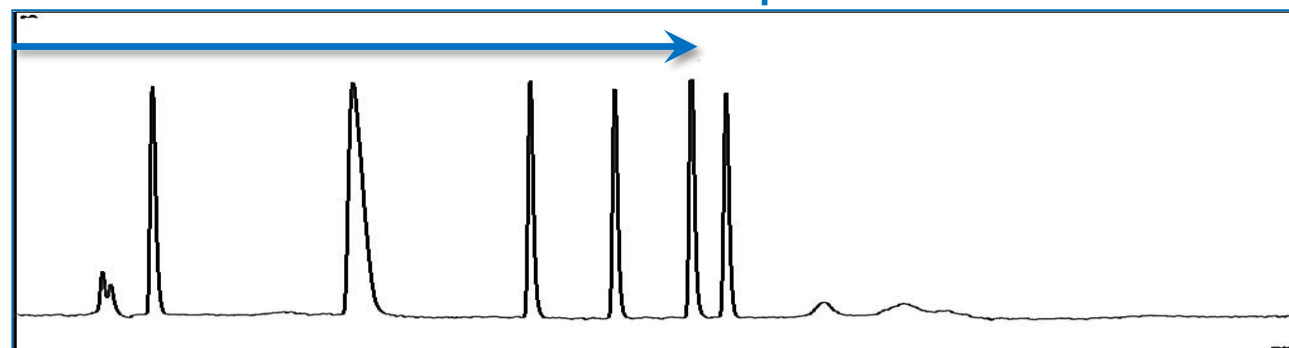
| | Vanquish Duo for Dual LC | Vanquish Duo for Tandem LC or LC-MS | Vanquish Duo for Inverse Gradient |
|---|---|---|---|
| **Improved Return on Investment** | ✓ Double throughput or increase sample knowledge | ✓ Increased detector utilization | ✓ Reliable quantitation |
| **Saves bench space** | ✓ | ✓ | ✓ |
| **Easy to use** | ✓ | ✓ | ✓ |
| **Doubled sample throughput** | ✓ | ✗ | ✗ |
| **Run complementary assays** | ✓ | ✗ | ✗ |
| **Increased throughput for LC-MS** | ✗ | ✓ | ✗ |
| **Reliably quantify unknown** | ✗ | ✗ | ✓ |

# New Sample Detection Portfolio

## Integrated detector options

| Detector | Advantages |
|---|---|
| Thermo Scientific™ Vanquish™ Horizon Diode Array Detector | Outstanding signal to noise performance |
| **NEW** Thermo Scientific™ Vanquish™ Flex Diode Array Detector | Leading linearity, robustness and smooth MS integration |
| Thermo Scientific™ Vanquish™ Flex Variable Wavelength Detector | Supports a wide operating range |
| Thermo Scientific™ Vanquish™ Flex Fluorescence Detector | High selectivity and sensitivity |
| Thermo Scientific™ Vanquish™ Horizon/Flex Charged Aerosol Detector | Offers sensitive, universal detection with a nearly uniform response |
| **NEW** Thermo Scientific™ ISQ™ EC/EM Mass Spectrometer | Single quadrupole MS for robust and easy mass detection |

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**Ultra**
**High**
# *Productivity*
**Liquid**
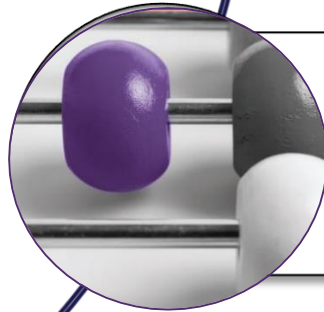**Chromatography**

## Dual LC

- A two channel LC system with standard footprint

## Tandem LC or LC-MS

- System for efficient detector utilization

## Inverse Gradient

- System for advanced quantification capabilities

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# Thermo Scientific Portfolio of Mass Spectrometry

## Non-targeted analysis

- Metabolomics
- Proteomics
- Lipidomics
- Bioanalysis
- Clinical Research/ Precision Medicine

- Biomarker Discovery
- Proteomics
- Metabolomics
- Lipidomics
- Metabolism

**Orbitrap HRAM**

## Quantitative

**Applied Markets**

For the scientific rigor you demand

**Research Markets**

## Qualitative

**MS, MS$^n$**

- Food Safety
- Environmental
- Clinical Research/ Forensic Toxicology

- PTM Analysis
- Proteomics
- Forensic Toxicology

## Targeted analysis

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# 2018 LSMS Hardware Portfolio

## Triple Quadrupoles
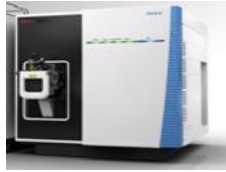
**TSQ Altis**
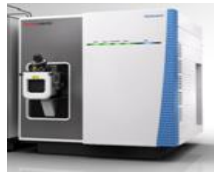
**TSQ Quantis**

**TSQ Fortis** — *NEW*

## Q Exactives
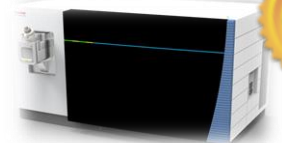
**Q Exactive HF-X** — *NEW*

**Q Exactive HF**

**Q Exactive (Plus)**

**Q Exactive Focus**

## Hybrids

*NEW*

**Options: UVPD, 1M, APD**
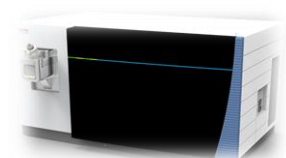
**Orbitrap Fusion Lumos**

**Orbitrap Fusion**

**Orbitrap Elite**

**Thermo Fisher**
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**PERFORMANCE**

Environmental and Food Safety
Clinical Research
Pharma QA/QC

**NEW**

**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

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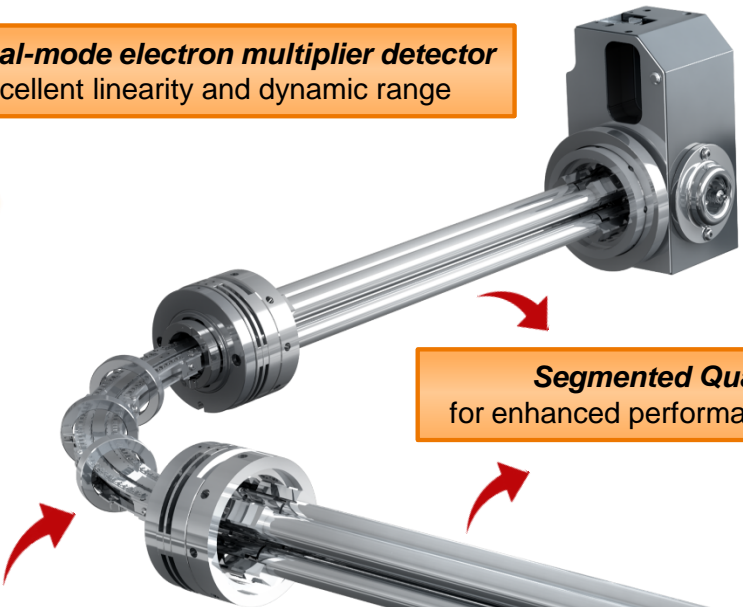
***Segmented Quadrupoles with hyperbolic surfaces***
for enhanced performance with both SRM and H-SRM (0.4 FWHM)

**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

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**Benefits: Increased Robustness while efficiently transmitting the ion beam and ensuring the required sensitivity**

- A mass dependent potential is applied to focus the ions from the ion guide region toward the opening of the matrix separator.

- The off-axis design of the matrix separator enables the separation of neutrals from the ion beam, leading to increased robustness and therefore increased uptime.

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# Segmented Quadrupoles

**Benefits:** Increased Sensitivity, 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.

*New segmented quadrupoles with hyperbolic surfaces*

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Competition



Thermo Scientific

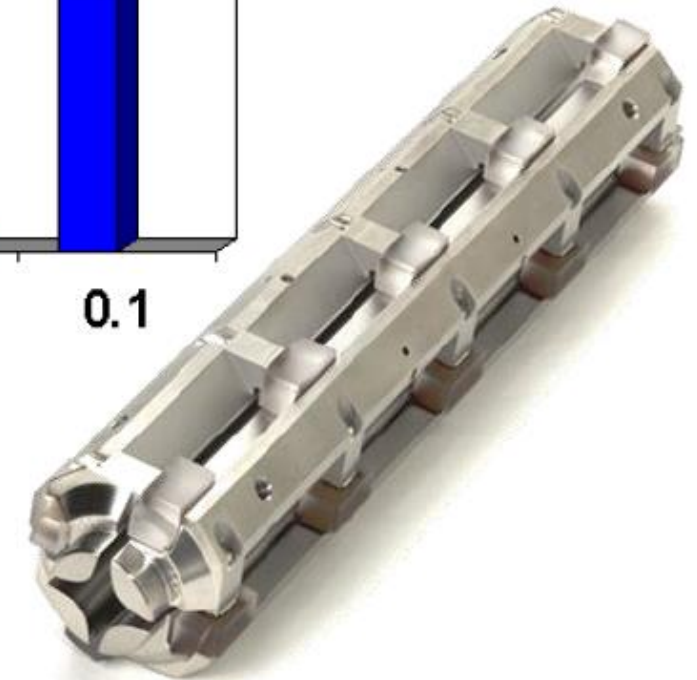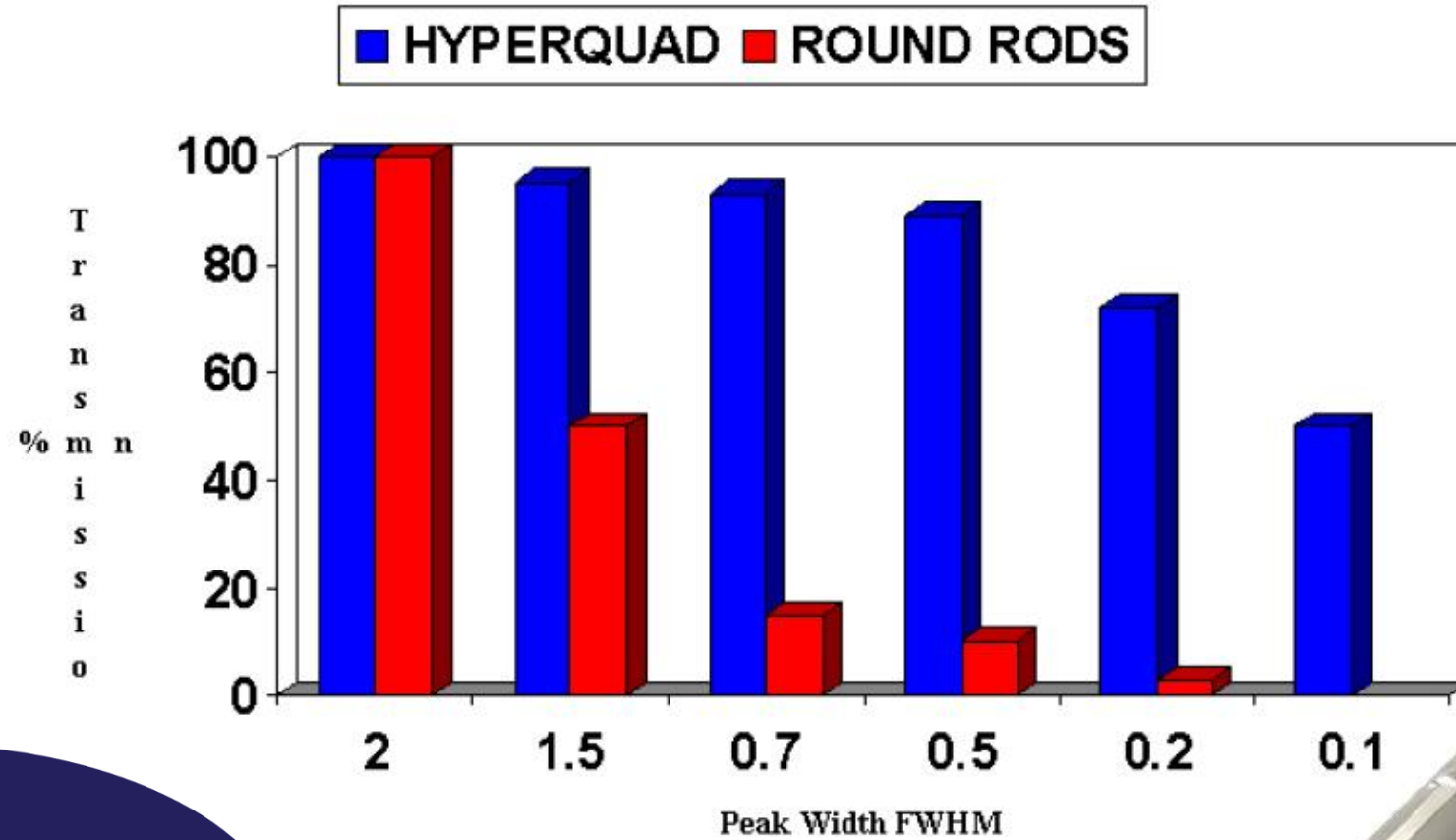ThermoFisher
SCIENTIFIC

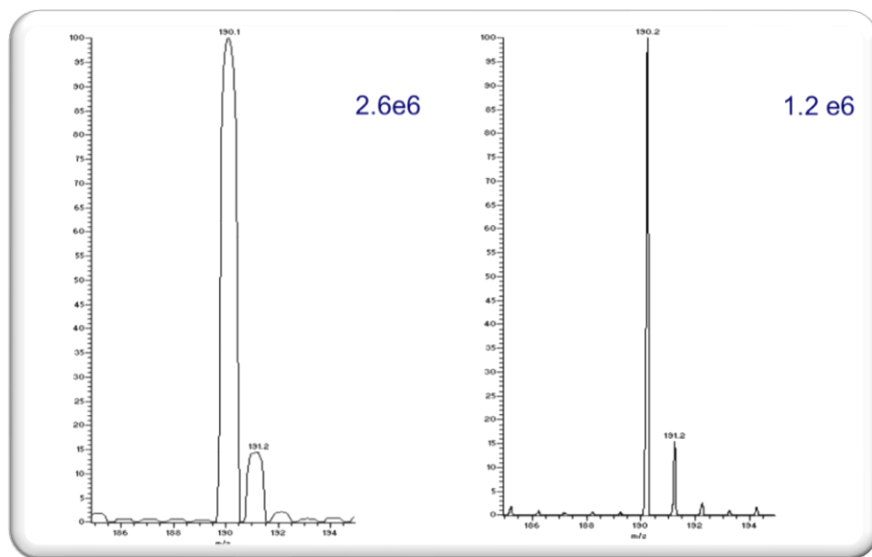# Effect of Peak Width On Transmission



TSQ Altis – 0.2 Da FWHM

TSQ Quantis – 0.4 Da FWHM
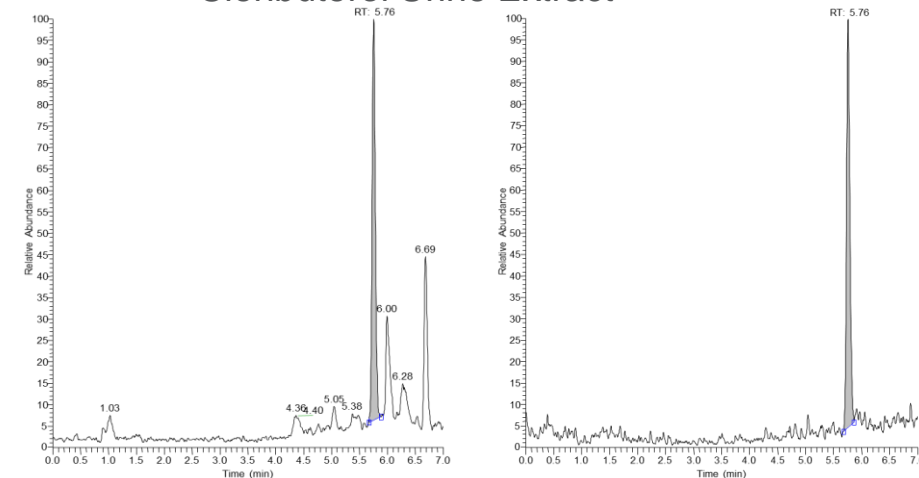
TSQ Fortis – 0.4 Da FWHM

# Hyperquads, Precursor Isolation and H-SRM

- **Enhanced isolation and stability of ion(s)**
  - H-SRM – Improved isolation of precursor ion(s)
  - More Selectivity – isolate only ion(s) of interest
  - **Benefit** - Improved signal to noise
  - **Benefit** More Confidence – less false positives
  - **Benefit** Higher Quality data

Clenbuterol Urine Extract



Unit Resolution 0.7 Da FWHM          H-SRM 0.2 Da FWHM



Improved isolation of precursor without significant loss of signal

None Detected

Chloramphenicol Detected and Quantified
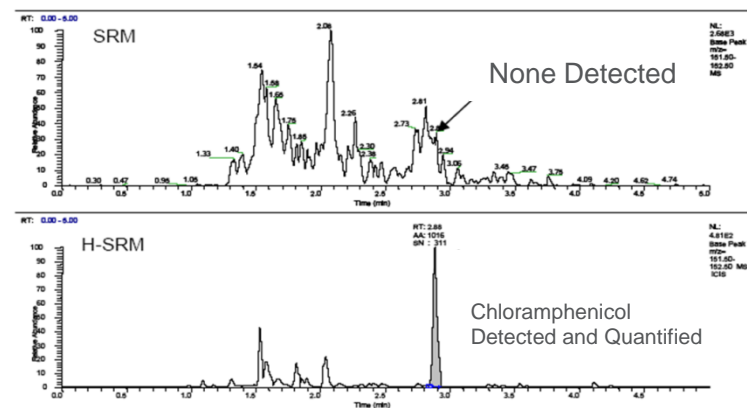
Figure 1. SRM vs. H-SRM for Chloramphenicol (321→152) of a 0.03 µg/kg fortified pork kidney sample.

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# 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** |
| *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!* |
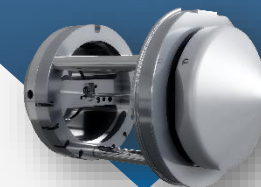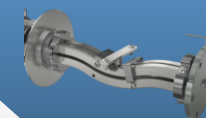
*TSQ Fortis only!*

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# What Makes the new Triple Quads Consistent?
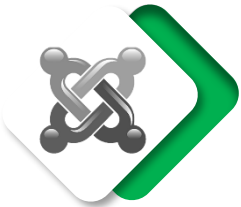
## Consistency

The ability to report equivalent results every time independently of the system or the user (day-to-day and system-to-system)
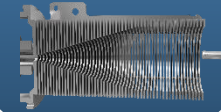
| OptaMax NG | Optics | Segmented Quads |
|---|---|---|
|  |  |  |
| **Re-designed Source Housing** | **Improved Optics Alignment** | **Segmented Quads with hyperbolic surfaces** |
| *Reliable and consistent performance with improved usability!* | *More consistent performance from system-to-system!* | *Flat tuning across the mass range!* |

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# TSQ Fortis: Demonstration of Robustness – Clinical Research

## Immunosuppressants

+2000 injections over 6 days (CV%≤ 6%)
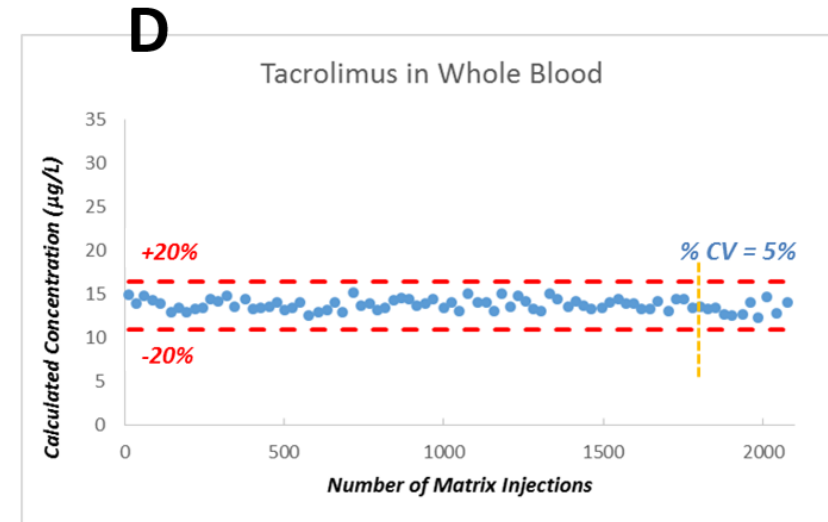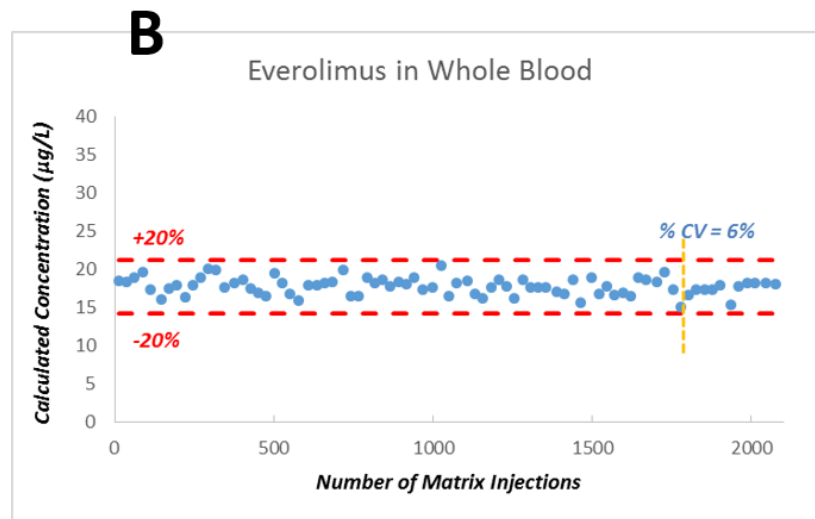
Red lines represent ± 20% of calculated amounts (ng/mL)

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

**A** — Cyclosporin A in Whole Blood
+20% / -20%, % CV = 4%

**B** — Everolimus in Whole Blood
+20% / -20%, % CV = 6%

**C** — Sirolimus in Whole Blood
+20% / -20%, % CV = 6%

**D** — Tacrolimus in Whole Blood
+20% / -20%, % CV = 5%

For Research Use Only

ThermoFisher
SCIENTIFIC

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



**Atrazine (QC 10µg/Kg) monitored in Leek**

+20%

4.5% RSD

-20%

Area Counts

Number of Matrix Injections
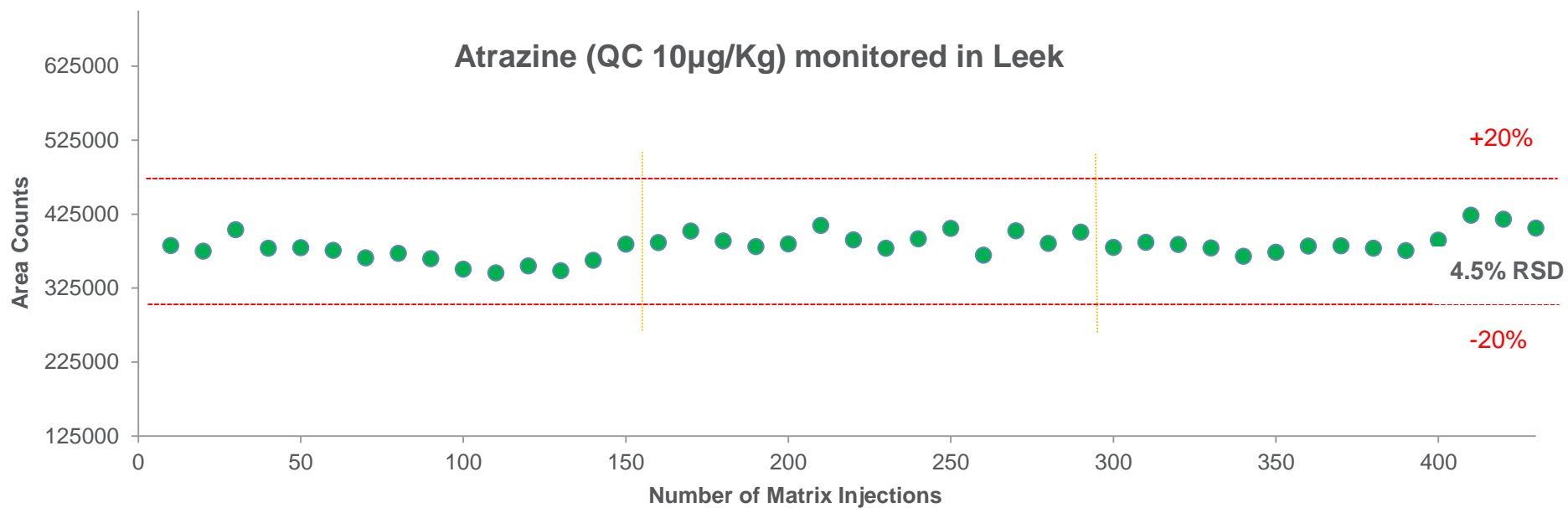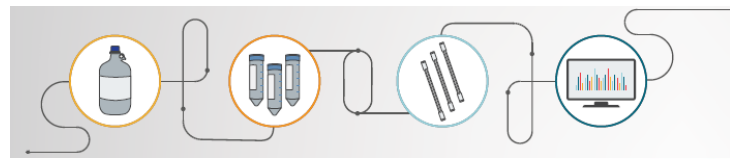
Application Note 64971

**Thermo Fisher**
SCIENTIFIC
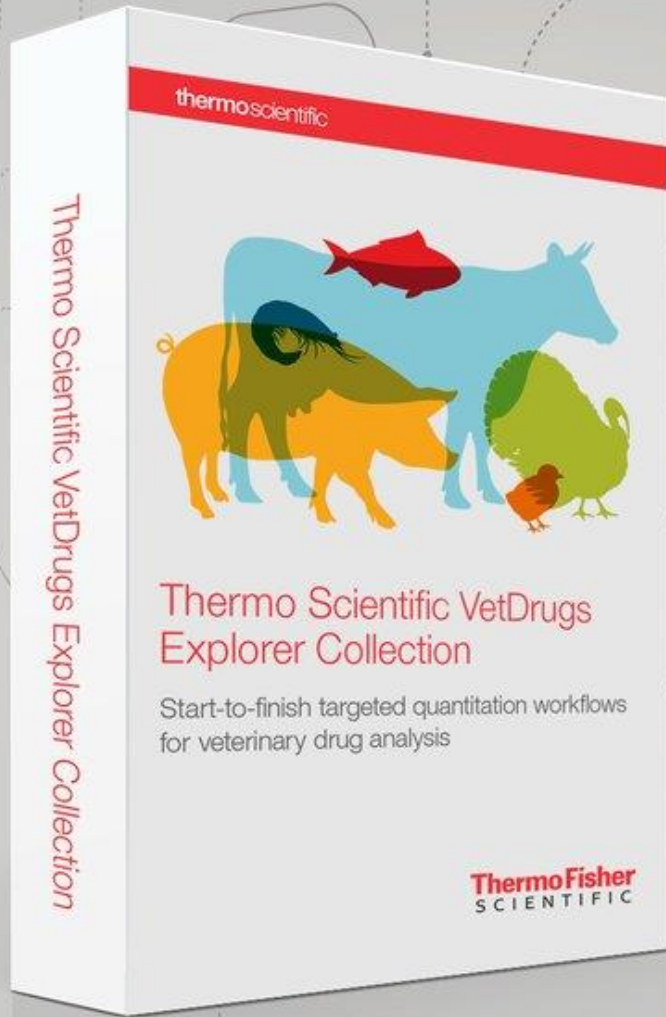
Multi-class Veterinary Drug Screening and Quantitation with a Comprehensive Workflow

## Several single-class methods



Stability of multi-component mixtures
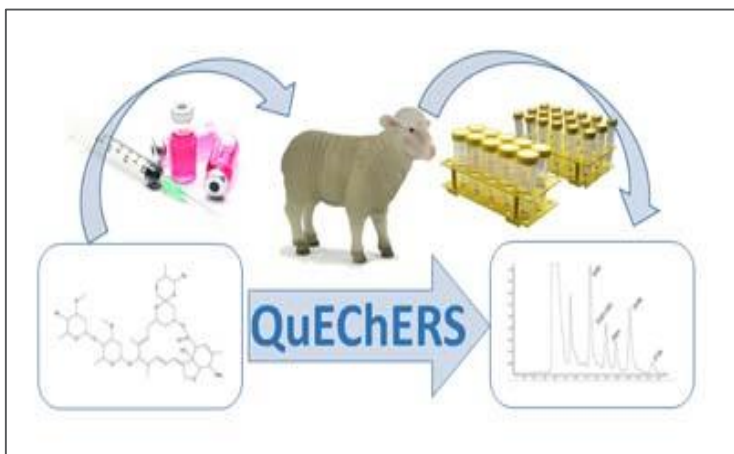


Generic sample prep with good recoveries + RSDs

## One multi-class method



Wide range of chemical classes+ MRLs
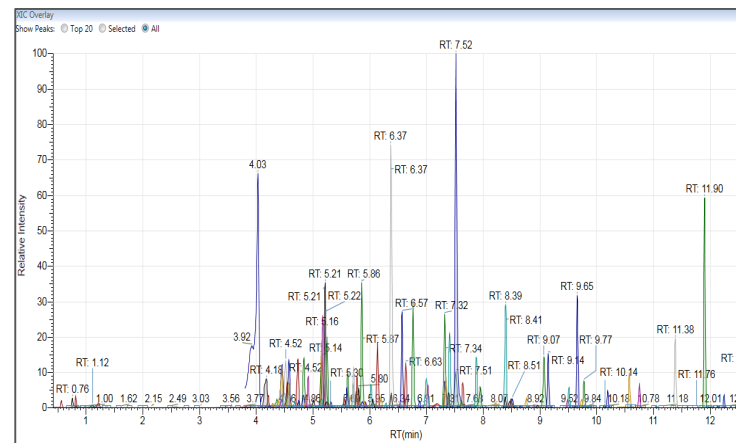


Inert LC system and column for wide pKa range and good peak shape

QuEChERs
Sample Prep
& Columns
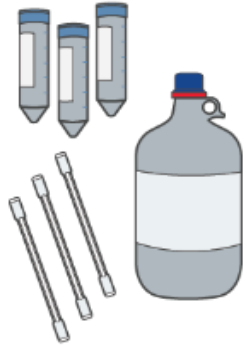
UHPLC

Sensitive
QQQ
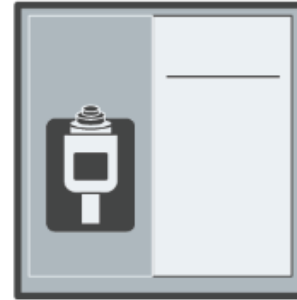
Complete
Methods with SRM,
compound database
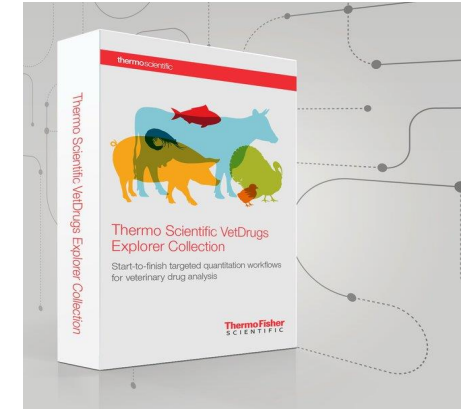
**QuEChERS
Sample
Preparation**
(Consumables +
procedure)

**UHPLC Chromatographic
Separation**
(Inert LC pathway + Column)

**QQQ Detection**
(Robust system with
sensitivity to meet global
MRLs over diverse cmpd
classes)

**Data Processing
&
Reporting**

ThermoFisher
SCIENTIFIC

# Technical Highlights of a Comprehensive Workflow

| Feature | Detail | Comment |
|---|---|---|
| # of Compounds | 170+ Analytes with acquisition and master processing method | Compound data base with optimized SRMs for easy set-up |
| Matrices | Muscle meat (cattle), milk, and salmon (fillet) | Demonstrates broad applicability of the multi-class method approach |
| QC and Analytical Standards | QC Check-20 cmpds<br>Standards- 170 compounds | Ability to analyze 'System Performance Check' |
| Sample Preparation | Generic QuEChERs extraction w/simple clean-up | Detailed procedure required for applicable matrices |
| Column | Thermo Scientific™ Accucore™ VDX 100mm x 2.1 x 2.6 um | Ensure performance across wide polarity range |
| UHPLC-MS system | Thermo Scientific™ TSQ Altis™ Triple Quadrupole MS<br>Thermo Scientific™ Vanquish™ Flex Binary pump | Includes detailed User guide for proper installation and system check-out |
| Software | Thermo Scientific™ TraceFinder™ Software 4.1 SP5 | Unify all aspects of data handling from sample acquisition to report |

Compound Classes

Bar chart showing compound class counts:
- Anthelmintic: 24
- Antibiotic (b-lactam): 12
- Antibiotic (macrolide): 8
- Antibiotic (quinolone): 9
- Antibiotic (sulfonamide): 16
- Antibiotic (tetracycline): 7
- Antibiotic other: 14
- Aquaculture (Dye): 14
- Coccidiostat: 12
- Insecticide: 5
- metabolites: 16
- NSAID: 10
- Sedative: 6
- Steroid: 6
- β-agonist: 13

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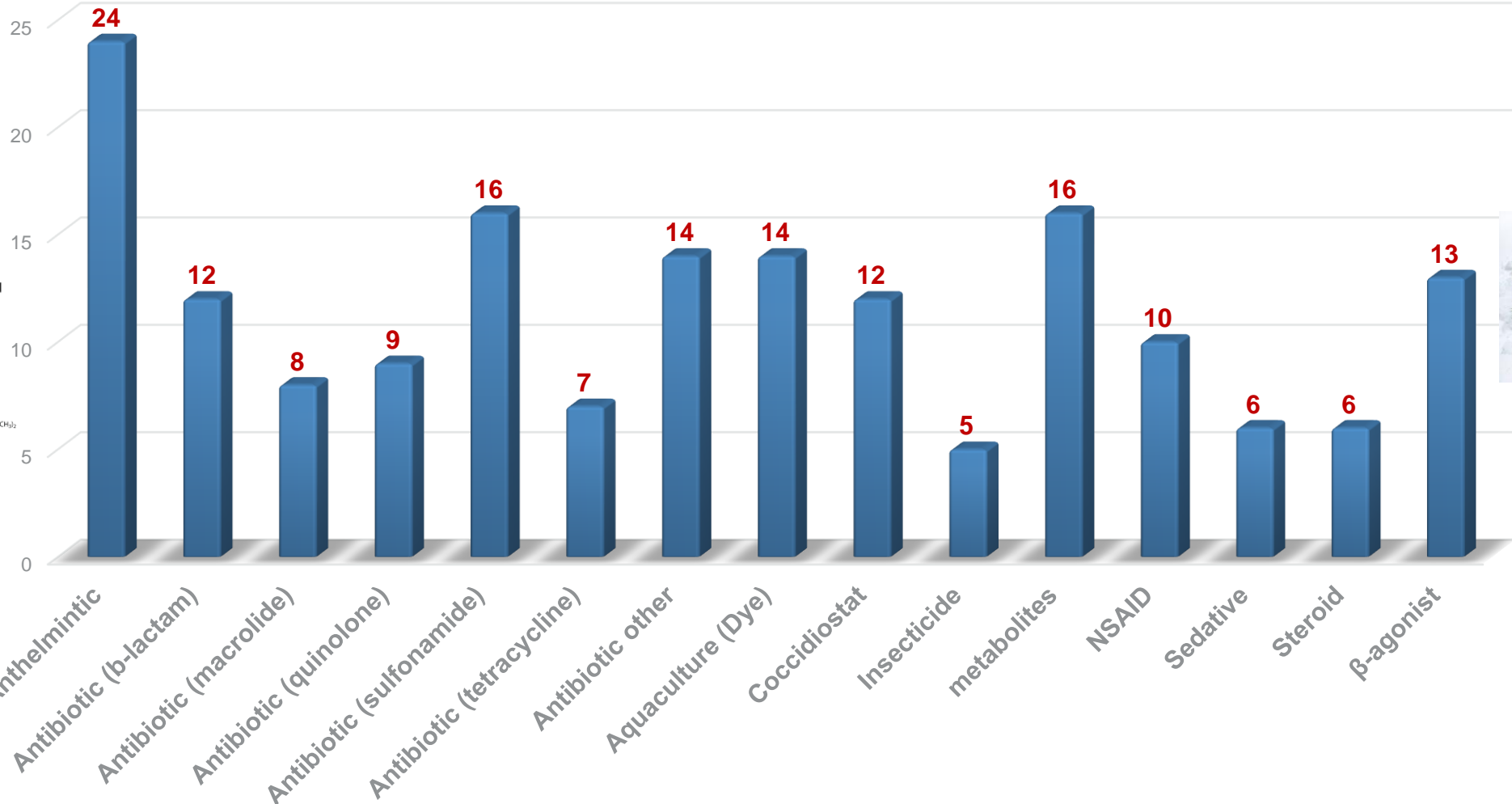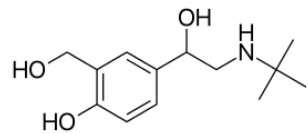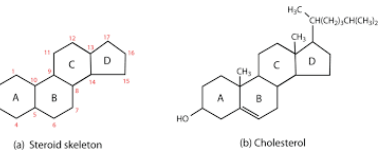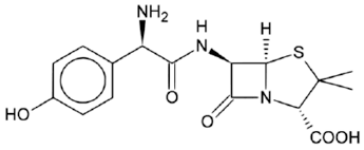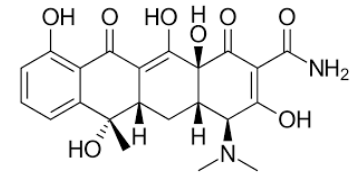
- **QuEChERS based approach**
  - EDTA/$NH_4$ oxalate solution and acetonitrile
  - Sample homogenised until fully dispersed
  - Sodium sulphate added before centrifugation
  - Dispersive SPE (CEC-$C_{18}$) clean-up
  - Add 1 mL H2O to 3mL extract, filter, inject

- **LC conditions**
  - Thermo Scientific™ Accucore™ VDX Column
    2.1 x 100 x 2.6 um
  - MP A: 0.05% formic acid
  - MP B: 0.05% formic acid, 5% H2O in 1:1 MeOH:MeCN
  - 2 uL injection

- **Acquire Data on TSQ Altis**
  - *Use pos/neg switching*
  - Comprehensive CDB with all optimized SRMs

Analytica Chimica Acta 637 (2009) 68–78

Contents lists available at ScienceDirect

**Analytica Chimica Acta**

ELSEVIER

journal homepage: www.elsevier.com/locate/aca

The development and validation of a multiclass liquid chromatography tandem mass spectrometry (LC–MS/MS) procedure for the determination of veterinary drug residues in animal tissue using a QuEChERS (QUick, Easy, CHeap, Effective, Rugged and Safe) approach

George Stubbings*, Timothy Bigwood

*Central Science Laboratory, Sand Hutton, York, YO41 1LZ, UK*

**ThermoFisher**
SCIENTIFIC

**Features**

- Solid core particle- high resolution separations
- Column chemistry selectivity similar to C18 columns
- Optimized for MS detection
- Low column bleed
- Optimized for low tailing
- *Robust against matrix extracts*
- Particle size: 2.6 µm

ThermoFisher
SCIENTIFIC
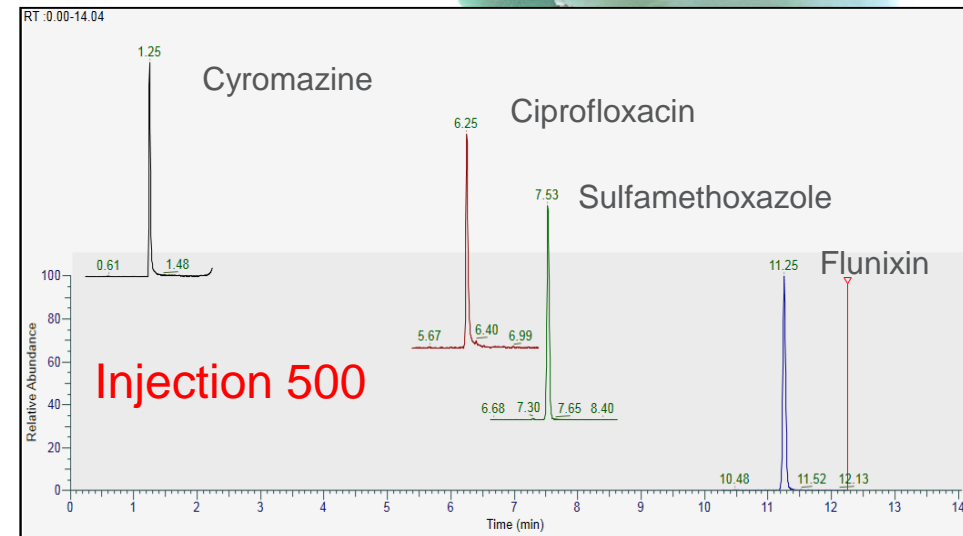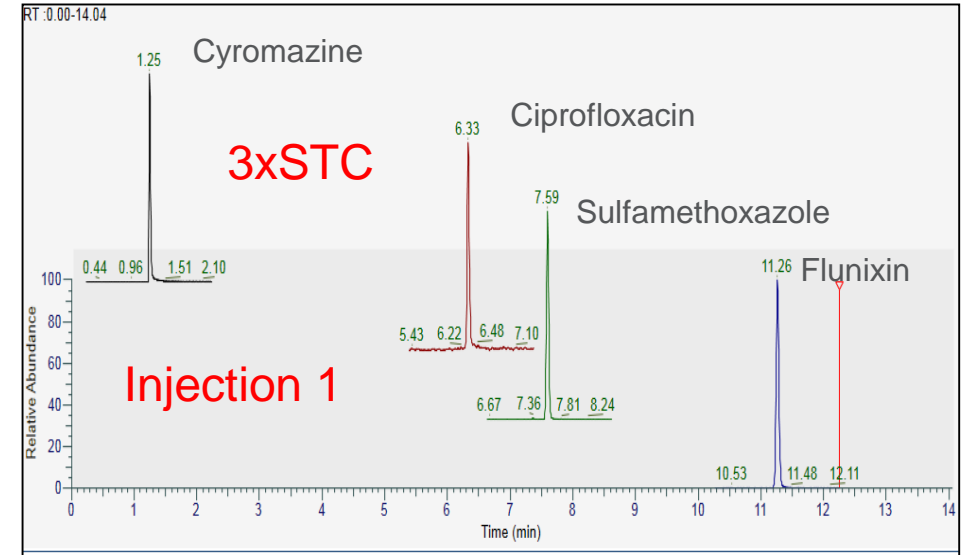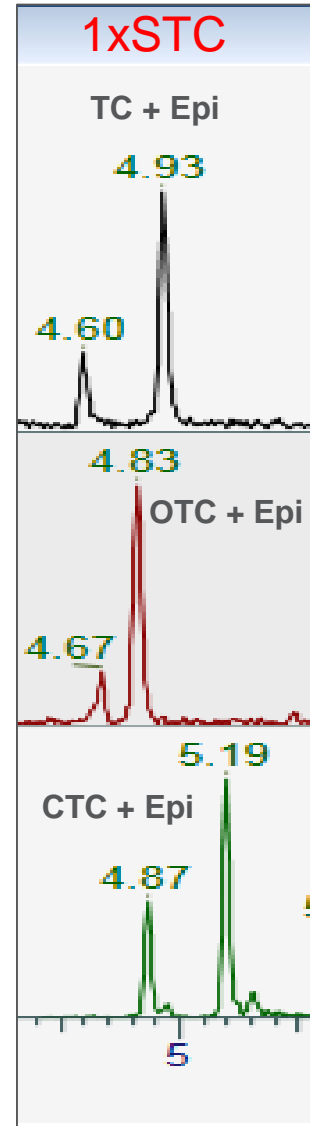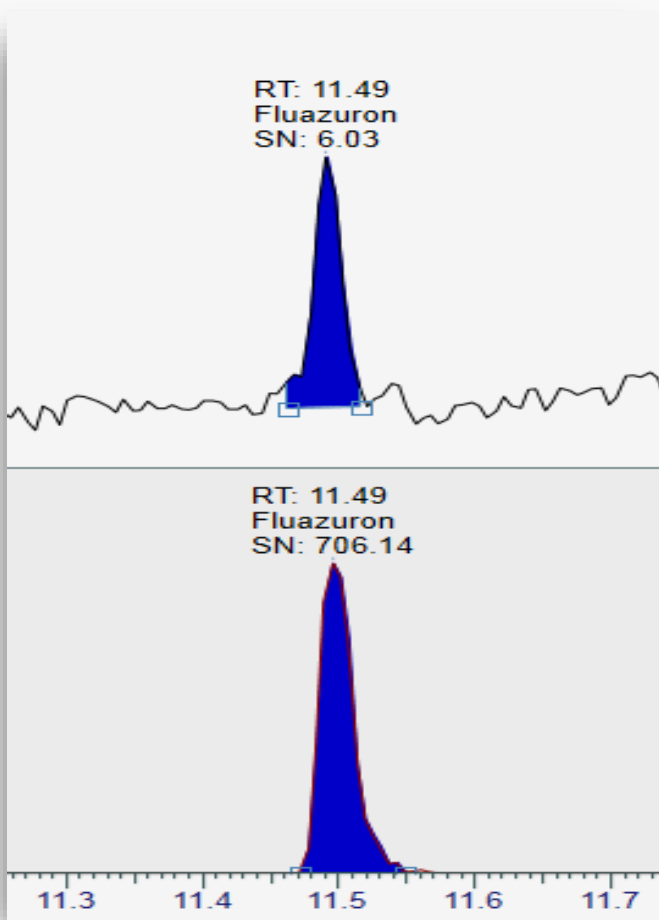
SRM, 0.7 FWHM

H-SRM, 0.2 FWHM

Advantage of H-SRM for Fluazuron in salmon fillet extract: Noise is significantly reduced allowing Improved signal-to-noise (selectivity) against the matrix with high ion transmission
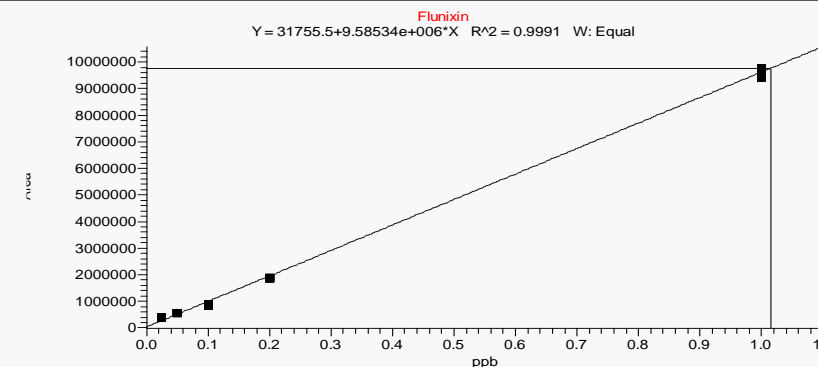
Calibration curve for Flunixin in salmon fillet matrix using H-SRM.

$Y = 31755.5 + 9.58534e+006*X$   $R^2 = 0.9991$   W: Equal

| Compound | MDL (0.7 SRM) | MDL (0.2 HSRM) |
|---|---|---|
| Derquantel | 0.42 | 0.04 |
| Fenbendazole Sulfone | 0.33 | 0.05 |
| Flunixin | 0.38 | 0.04 |
| Hydroxyipronidazole | 0.62 | 0.08 |
| Robenidine | 0.33 | 0.05 |
| Teflubenzuron | 4.47 | 0.47 |

Advantage of H-SRM for improved method detection limits (MDLs) for select compounds in a salmon fillet extract

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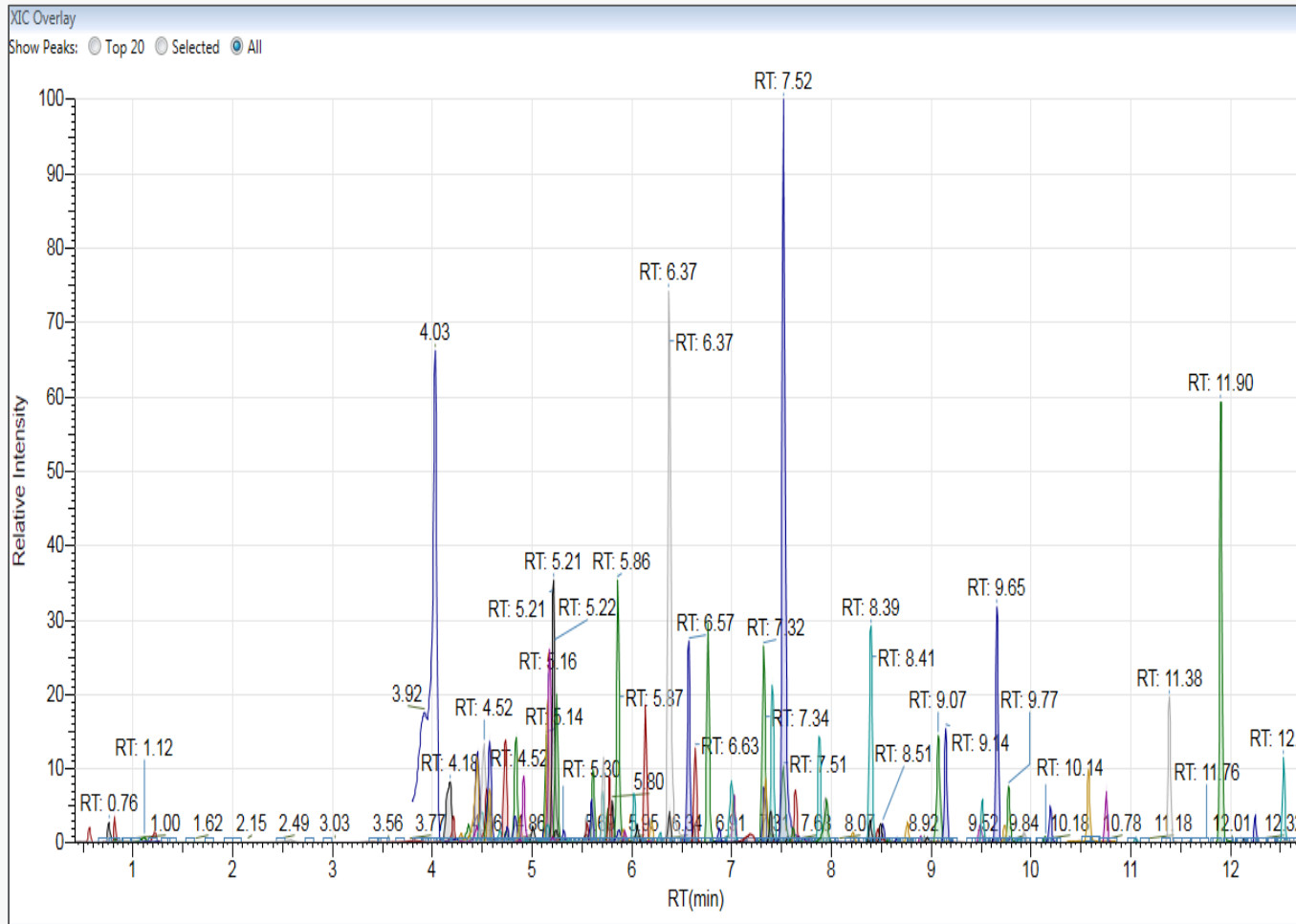
TIC of all SRMs @ 1xSTC in TraceFinder software
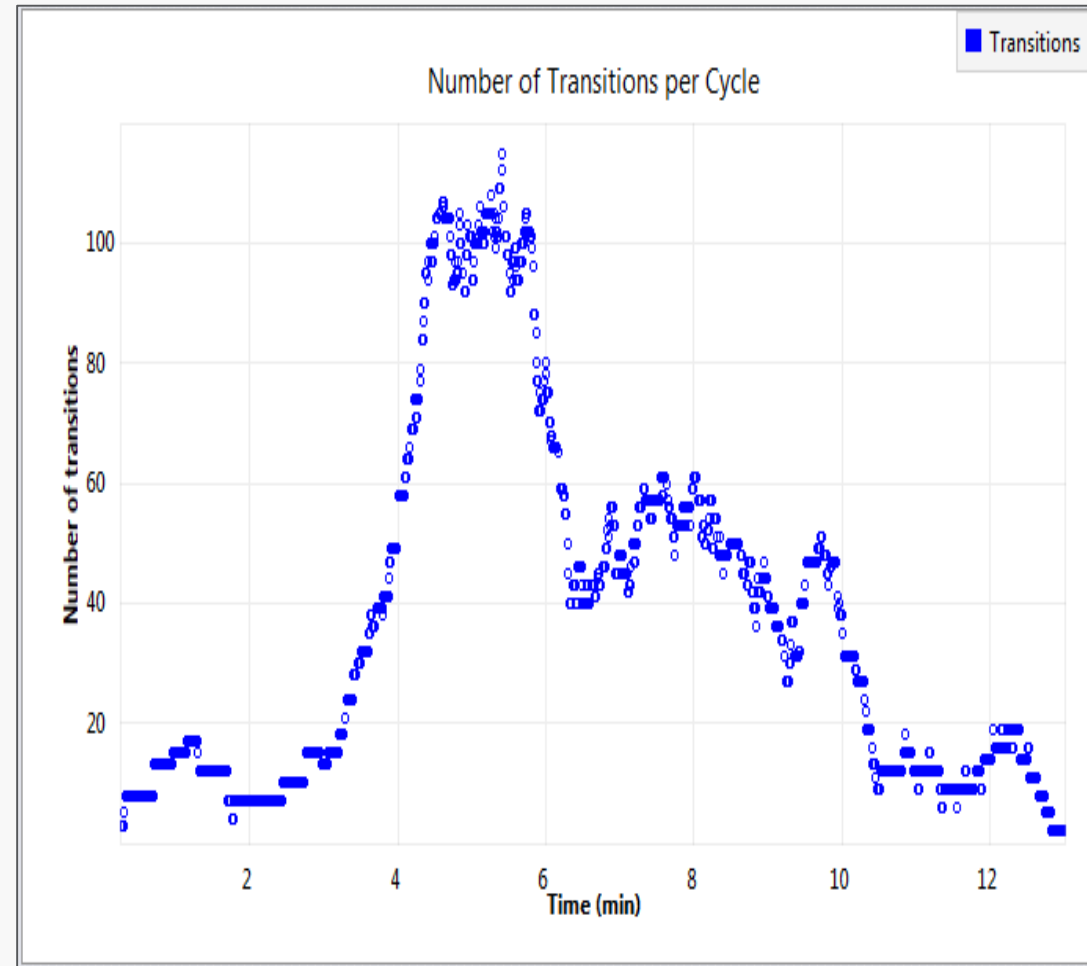
The distribution of 546 SRMs are easily visualized in the instrument software tune page

**Thermo Fisher**
S C I E N T I F I C
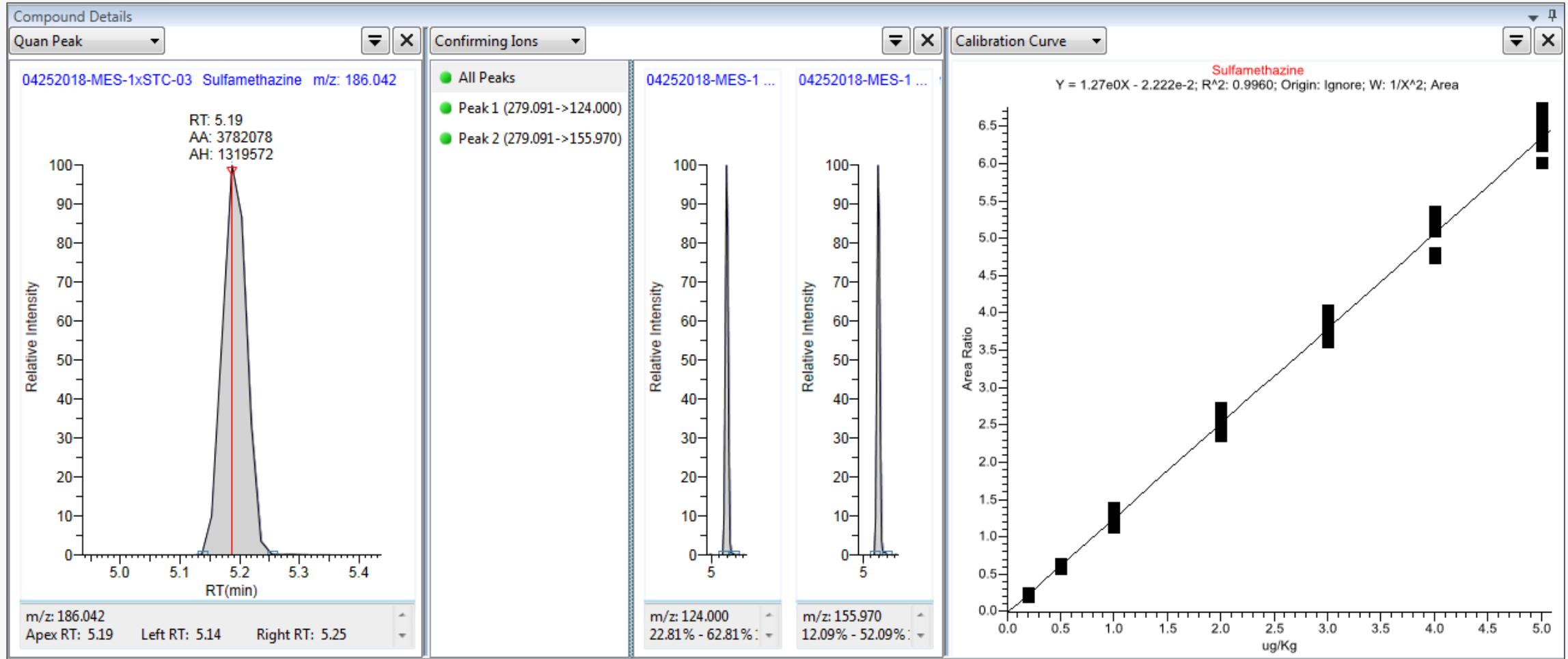
Sulfamethazine in bovine extract at 1× STC, with screening range from 10-250 ng/g.
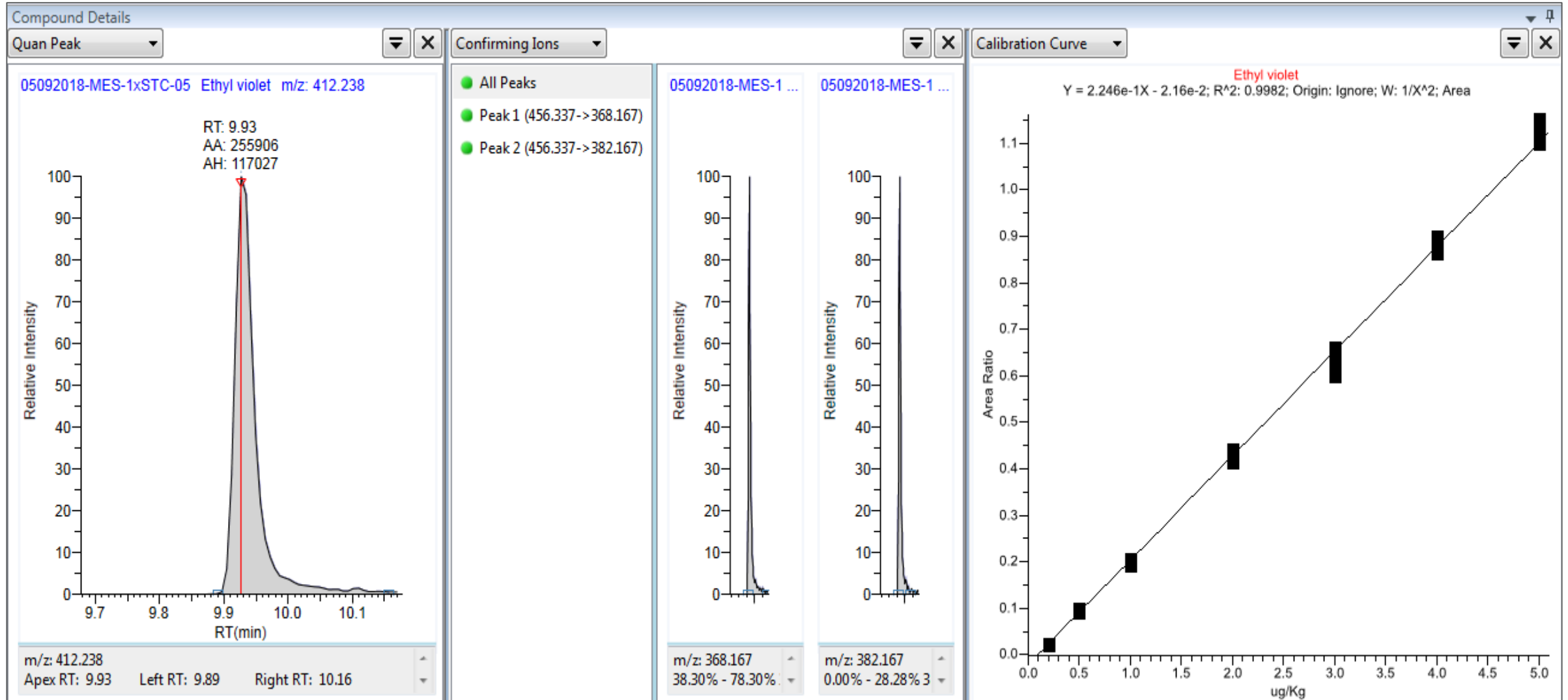
Ethyl Violet in salmon extract @ 1×STC, with a screening range from 0.2–5 ng/g.

# Compound Class - Average Calculated MDL (ng/g)

## Average Method Detection Limit (MDL) by Class

ng/g (ppb)

Milk  Bovine Muscle  Salmon Fillet

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```
32
Sulfadiazine
Concen:      3.198
RT:          5.87              Scan#      5223
Delta RT:    -0.06
Lab File:    0928_MES_STC_3_6
Acq:         9/29/2017 3:56:43 AM
Tgt Ion:     155.97            Resp:      5444880
Ion          Ratio     Lower      Upper
155.97       NaN
92.07        59.41%    50.20%     75.30%
108.00       56.31%    44.74%     67.10%
Scan Filter:  + c ESI SRM ms2 251.060 [92.070-92.072, 107.999-108.00
```
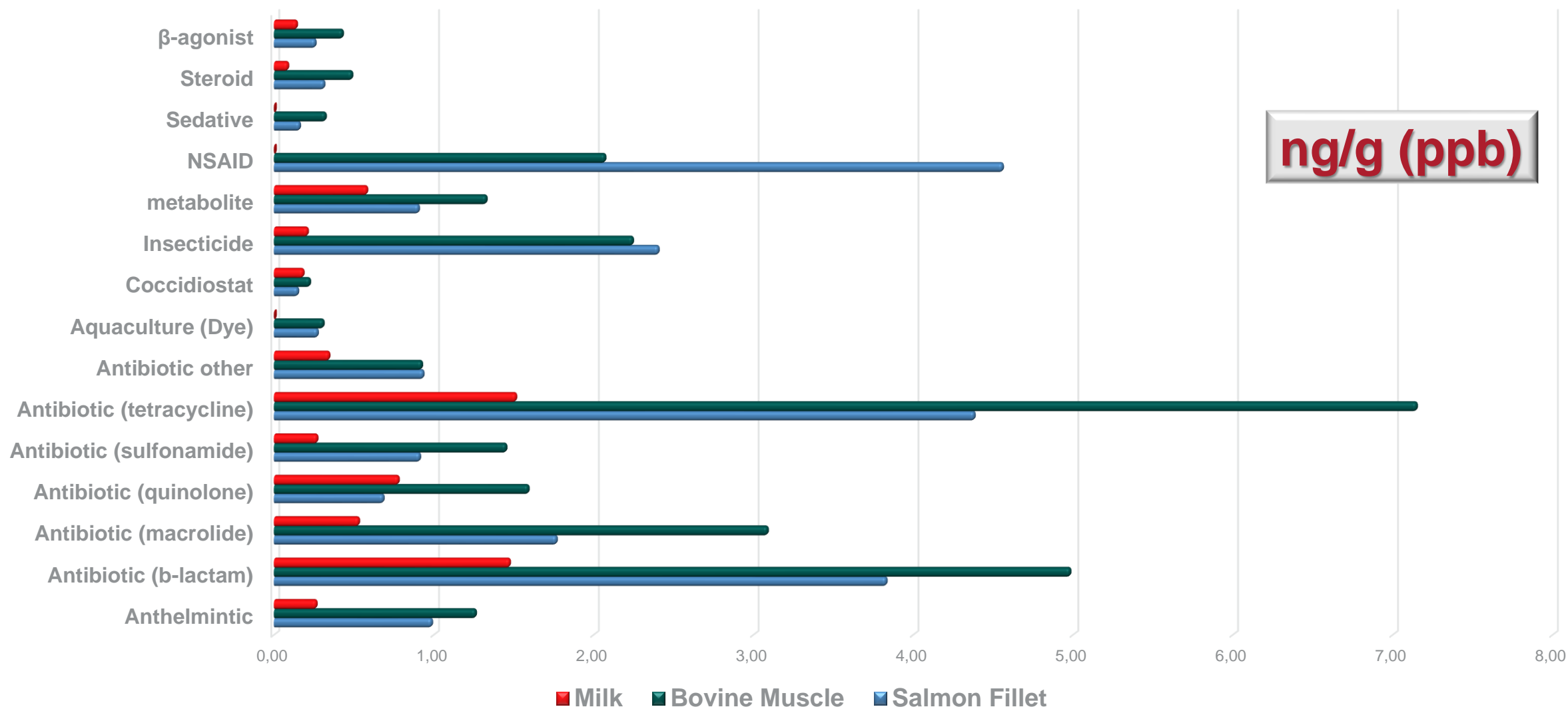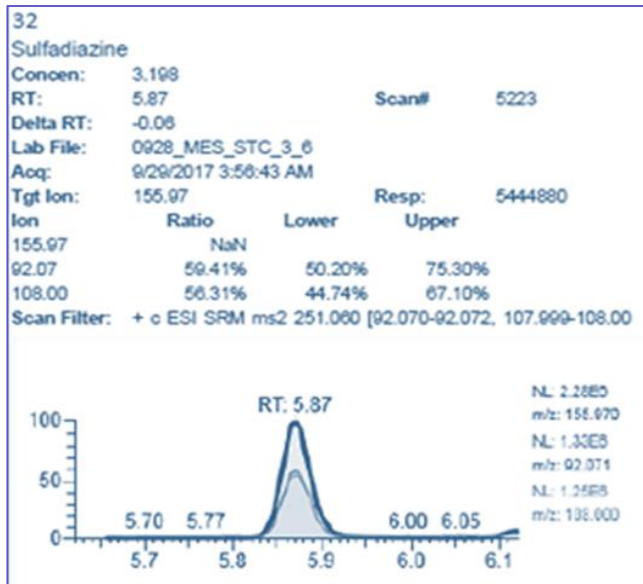
**Validate precision on 4 separate LC/MS/MS Systems to ensure robustness and method transferability**

**Calculate Absolute % Recovery Based upon a 'post spike' at 3 x STC**

**Calculate MDL @ or below a cut-off (Lowest xSTC factor at or below 15% RSD)**

**Calculate %RSDs at each level to check precision**

**Prepare Matrix Extracted Spike (MES) Replicates at 0.2 - 5 x STC (Establish 'Calibration Line' for screening)**

**Establish Screen Target Concentration (STC) Level - Levels typically ¼ to 1/3 the MRL**

**Thermo Fisher**
S C I E N T I F I C

# Retention Time Stability Across 4 LC/MS/MS Systems

- Validation included 4 different LC/MS/MS systems: 3 Thermo San Jose Labs and 1 at Iowa State Lab
- Representative compounds throughout the gradient RT range show excellent stability in matrix (Salmon Fillet shown), with excellent agreement between different systems (different columns, 3 LCs, and MS systems).

| Compound | Exp RT | RT System 1 | RT System 2 | RT System 3 | RT System 4 | AVE | SDEV | %RSD |
|---|---|---|---|---|---|---|---|---|
| Cyromazine | 0.83 | 0.82 | 0.82 | 0.85 | 0.82 | 0.83 | 0.0150 | 1.81 |
| Dimetridazole | 4.16 | 4.07 | 4.11 | 4.15 | 4.12 | 4.11 | 0.0330 | 0.80 |
| Sulfamethoxazole | 5.84 | 5.80 | 5.81 | 5.86 | 5.79 | 5.82 | 0.0311 | 0.53 |
| Oxyphenbutazone | 8.52 | 8.49 | 8.49 | 8.53 | 8.49 | 8.50 | 0.0200 | 0.24 |
| Triclabendazole | 10.21 | 10.19 | 10.22 | 10.24 | 10.21 | 10.22 | 0.0208 | 0.20 |
| Closantel | 11.88 | 11.91 | 11.91 | 11.92 | 11.93 | 11.92 | 0.0096 | 0.08 |
| Monensin | 12.21 | 12.23 | 12.25 | 12.27 | 12.26 | 12.25 | 0.0171 | 0.14 |

*Proprietary & Confidential*

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# Example Data : Salmon Matrix- Precision at STC Levels Across 4 LC/MS/MS Systems

| Compound | Lowest Global MRL | STC | System 1 | System 2 | System 3 | System 4 | Average | SDEV | %RSD |
|---|---|---|---|---|---|---|---|---|---|
| Amoxicillin | 50 | 25 | 24.4 | 24.8 | 25.0 | 27.3 | 25.4 | 1.31 | 5.2 |
| Chlortetracycline | 200 | 100 | 87.3 | 97.1 | 86.4 | 104.0 | 93.7 | 8.39 | 9.0 |
| Danofloxacin | 100 | 50 | 50.9 | 49.8 | 47.3 | 52.3 | 50.1 | 2.09 | 4.2 |
| Dicloxacillin | 300 | 150 | 144.5 | 148.2 | 147.7 | 152.2 | 148.2 | 3.15 | 2.1 |
| Difloxacin | 300 | 150 | 150.0 | 149.2 | 145.6 | 159.3 | 151.0 | 5.80 | 3.8 |
| Doxycycline | 10 | 10 | 9.2 | 9.1 | 9.2 | 10.1 | 9.4 | 0.47 | 5.0 |
| Enrofloxacin | 100 | 50 | 50.2 | 49.4 | 55.0 | 52.3 | 51.7 | 2.51 | 4.9 |
| Flumequine | 500 | 200 | 200.8 | 201.9 | 201.5 | 199.3 | 200.9 | 1.17 | 0.6 |
| Oxolinic Acid | 50 | 25 | 28.2 | 29.3 | 27.5 | 25.8 | 27.7 | 1.48 | 5.3 |
| Oxytetracycline | 200 | 100 | 102.2 | 96.4 | 94.6 | 101.1 | 98.6 | 3.65 | 3.7 |
| Penicillin G | 50 | 25 | 23.6 | 24.0 | 21.6 | 25.4 | 23.6 | 1.57 | 6.7 |
| Sarafloxacin | 10 | 3 | 3.0 | 3.0 | 2.9 | 3.1 | 3.0 | 0.06 | 2.0 |
| Sulfadoxine | SUM 100 | 10 | 9.1 | 8.7 | 9.4 | 10.1 | 9.3 | 0.59 | 6.3 |
| Tetracycline | 200 | 100 | 98.8 | 98.3 | 91.7 | 100.4 | 97.3 | 3.81 | 3.9 |
| Thiamphenicol | 50 | 25 | 23.6 | 24.0 | 22.2 | 26.5 | 24.1 | 1.80 | 7.5 |
| Tilmicosin | 50 | 25 | 27.5 | 28.0 | 25.4 | 23.2 | 26.0 | 2.21 | 8.5 |
| Trimethoprim | 50 | 25 | 21.8 | 18.7 | 24.0 | 22.0 | 21.6 | 2.19 | 10.1 |
| Tylosin | 100 | 50 | 46.8 | 47.5 | 45.9 | 47.7 | 47.0 | 0.80 | 1.7 |

Chromatography with mass spectrometric detection



only
**GC**
amenable

both
**GC and LC**
amenable

only
**LC**
amenable

**IC**
amenable
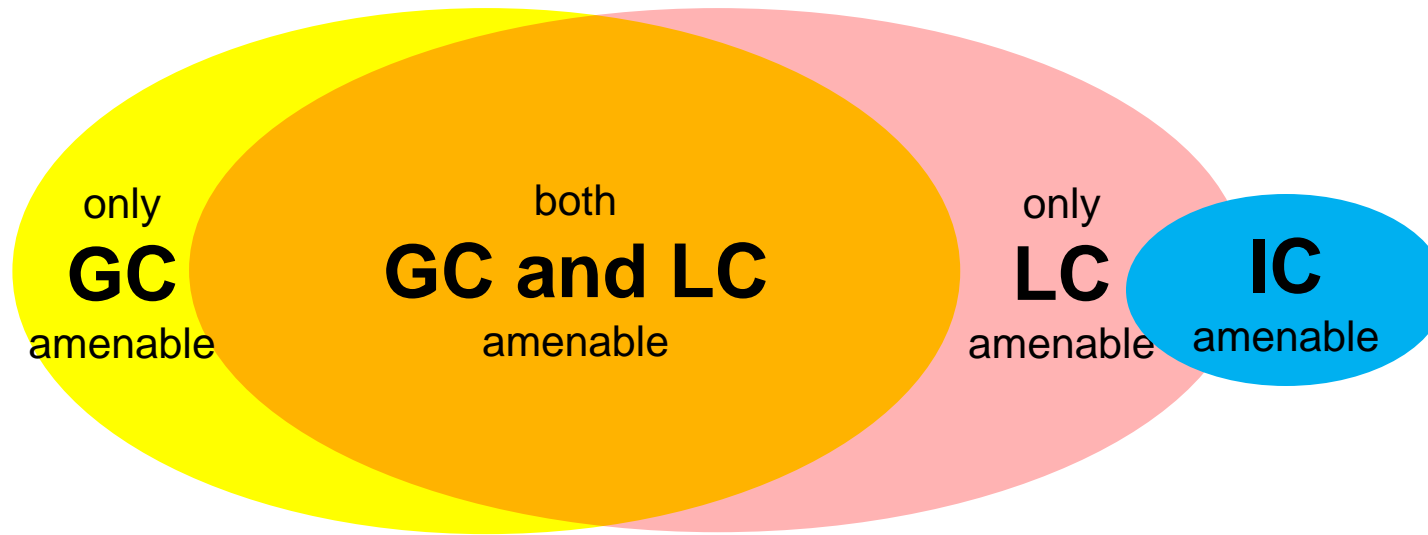
**Current golden standard:**
Targeted quantitative measurement by LC-MS/MS and GC-MS/MS
Multi-residue methods, ~150–250 analytes/method

**Emerging:**
Non-targeted measurement by LC and GC + full scan MS
for better coverage of pesticide scope and easier measurement
IC for easier separation of polar substances

**Thermo Fisher**
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# Sample preparation/analyte detection strategy

**Clean-up efficiency**

Simple extraction

LLE

Dispersive SPE

GPC

SPE

IAC

**Detection selectivity**

HRAM-MS/MS

MS/MS

LC-MS

HPLC-FLD

GC-MS, ECD

HPLC-UV

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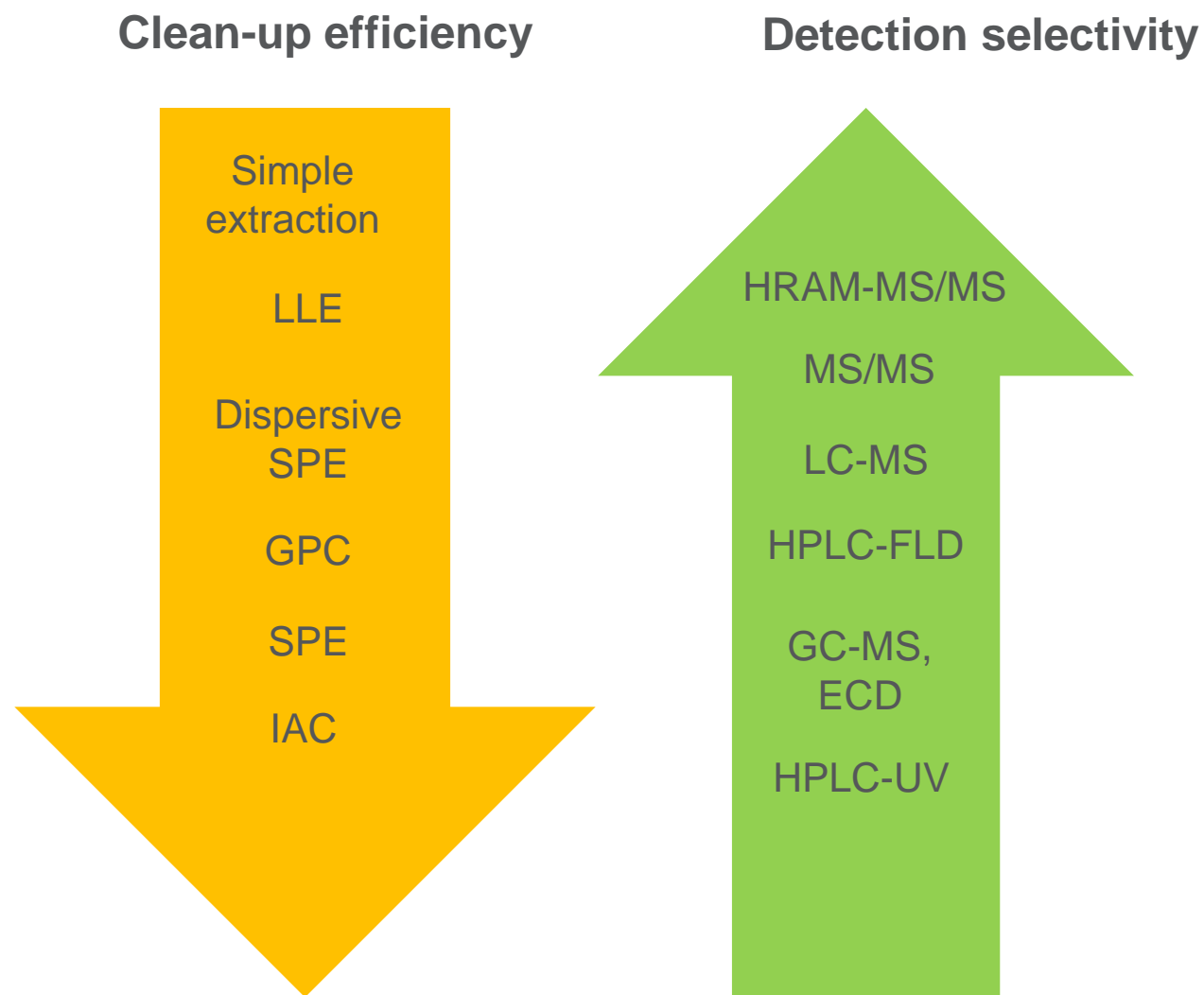
**■ Extraction**

Acetonitrile, Ethyl acetate, Methanol...

**■ Clean-up**

GPC, SPE, LLE, LC
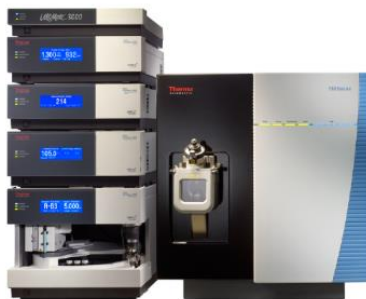
**■ Determination**

GC, LC, GC-MS, LC-MS, GC-MS/MS, LC-MS/MS...

Mostly replaced by **QuEChERS** today

Thermo Scientific™ QuEChERS™ method

**Thermo Fisher**
S C I E N T I F I C

# Improving QuEChERS extraction tips & tricks:

- **Dry food (cereals/dried food, < 25 % water content):**
  - addition of water to enable adequate partitioning and reducing interaction of pesticides with matrix

- **Food containing fat/wax (avocado/oil):**
  - after extraction step add a freezing out step and transfer supernatant to clean-up tube
  - more clean-up might be needed of raw extract (PSA+C18)

- **Food containing complex matrix (tea/spices)**
  - additional clean-up with GCB might be necessary (potential loss of planar structure pesticides like thiabendazole)

- **Acidic food (citrus):**
  - adjust pH (5-5.5) to increase recovery (e.g. citrate buffering salts in QuEChERS extraction tube) and reduce coextraction of matrix interefrences (Note: acid labile compounds require higher pH 8)

- **QuEChERS clean-up tube additives:**

## Product Selection

| Matrix Type | Examples | Sorbent Requirements |
| --- | --- | --- |
| General Matrices | Apples<br>Cucumber<br>Melon | Magnesium Sulfate, PSA |
| Fatty Matrices | Milk<br>Cereals<br>Fish | Magnesium Sulfate, PSA, C18 |
| Pigmented Matrices | Lettuce<br>Carrot<br>Wine | Magnesium Sulfate, PSA,<br>C18, GCB |
| High Pigmented Matrices | Spinach<br>Red Peppers | Magnesium Sulfate, PSA,<br>C18, GCB |

**Click here for more information**

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Sample

How much of a compound?

What analytes are present?

What is different?

Targeted Acquisition

FullScan Approaches – Non Targeted Acquisition

Triple Quadrupole MS/MS

Tribrids
Fusion Lumos

Q Exactive Family

**ThermoFisher**
SCIENTIFIC
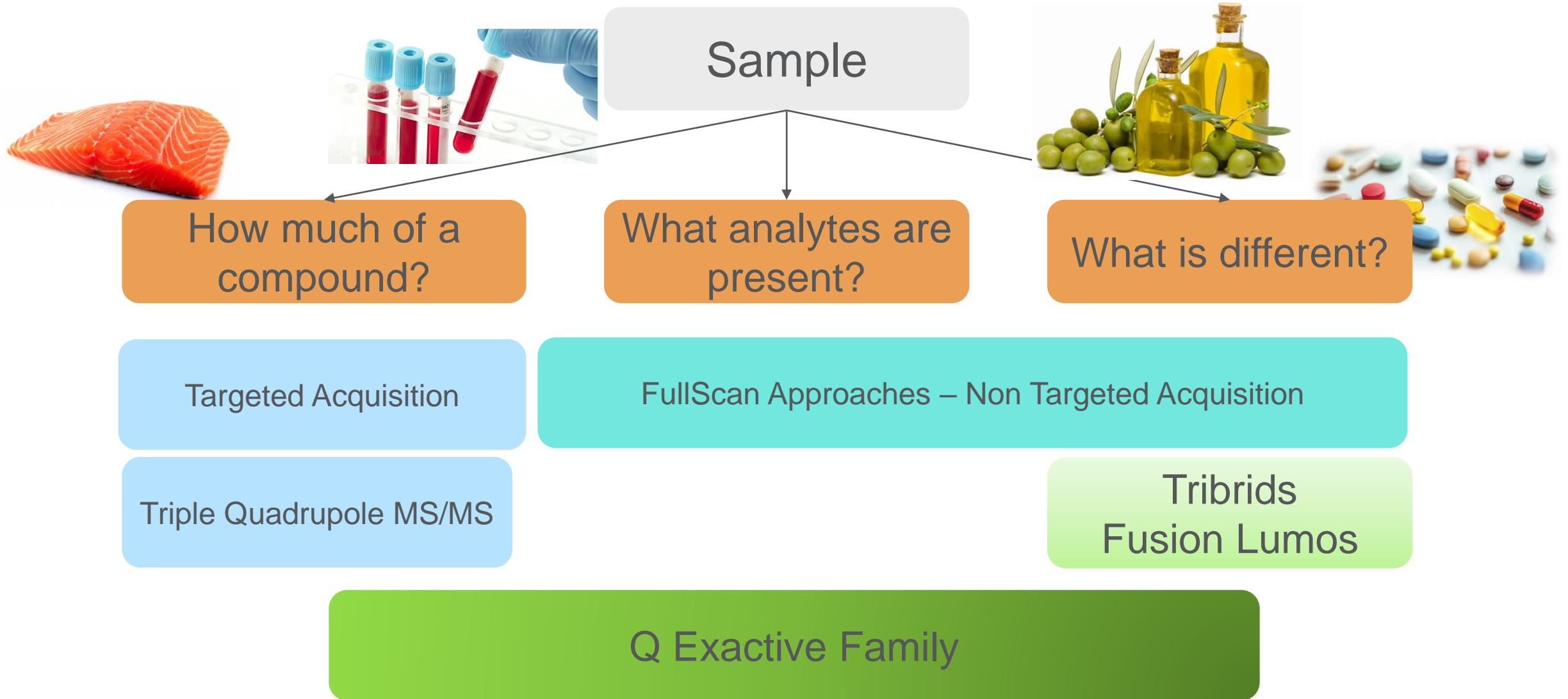
| | Targeted Screening | Untargeted Screening | Unknown Screening |
|---|---|---|---|
| **SEARCH FOR:** | **what you know** | **what you suspect** | **all the rest** |
| **HOW YOU SEARCH:** | Compound search after targeted acquisition; **uses specific compound list** | Compound search follows acqusition based on most intense signals **NOT on a list of compounds** | Search of compounds with a **totally untargeted acquisition** |
| **ADVISORY:** | Detection parameters need to be numerous & selective to avoid false positives | Identification based on compound databases and/or spectral libraries | Putative identification typically followed by structural elucidation |

# Exactive Series Product Portfolio

**Increased Performance** →

**Exactive Plus (EMR)**

- Orbitrap analyzer
- Mass Range  m/z 50 - 6000
- Mass Range EMR: 300 - 20,000
- Mass Accuracy: <1ppm
- Mass Resolution >140,000
- Scan Speed up to 12 Hz
- Octapole transfer for MS and AIF

**Q Exactive Focus**

- Orbitrap analyzer
- Mass Range  m/z 50 - 3000
- Mass Accuracy <1ppm
- Max. Mass Resolution >70,000
- Scan speed up to   12Hz MS and MS/MS
- Polarity switching
- Hyperbolic quadrupole: MS, ddHCD, AIF, SIM, PRM, vDIA
- Multiplexing SIM only
- Refined workflows  for applied markets

**Q Exactive**

- Orbitrap analyzer
- Mass Range  m/z 50 - 6000
- Mass Accuracy <1ppm
- Max. Mass Resolution >140,000
- Scan speed up to  12Hz MS and MS/MS
- Polarity switching
- Hyperbolic quadrupole: MS, ddHCD, AIF, SIM, PRM, DIA
- Spectral Multiplexing

**Q Exactive Plus**

- Orbitrap analyzer
- Mass Range m/z 50 - 6000
- Mass Accuracy <1ppm
- Max. Mass Resolution >140,000
- Scan speed up to  12Hz MS and MS/MS
- Advanced Quadrupole Technology (AQT)
- Advanced Active Beam Guide (AABG)
- Polarity switching
- Segmented quadrupole: MS, ddHCD, AIF, SIM, PRM, DIA
- Spectral Multiplexing
- PRM dyn-RT correction
- Opt. Intact Protein Mode
- Opt. Enh Res.Mode (280k)
- Optional BioPharma Mode  mass range up to 8000

**Q Exactive HF**

- Ultra High Field Orbitrap analyzer
- Mass Range m/z 50 - 6000
- Mass Accuracy <1ppm
- Max. Mass Resolution >240,000
- Scan speed up to   18Hz MS and MS/MS
- Advanced Quadrupole Technology (AQT)
- Advanced Active Beam Guide (AABG)
- Polarity switching
- Segmented quadrupole: MS, ddHCD, AIF, SIM, PRM, DIA
- Spectral Multiplexing
- PRM dynamic RT correction
- Opt. Intact Protein Mode
- Optional BioPharma Mode  mass range up to 8000

**Q Exactive HF-X**

- Ultra High Field Orbitrap analyzer
- Mass Range  m/z 50 - 6000
- Mass Accuracy <1ppm
- Max. Mass Resolution >240,000
- Scan speed up to   40Hz MS and MS/MS
- High capacity transfer tube
- Electrodynamic ion funnel
- Advanced Quadrupole Technology (AQT)
- Advanced Active Beam Guide (AABG)
- Polarity switching
- Segmented quadrupole:  MS, ddHCD, AIF, SIM,  PRM, DIA
- Spectral Multiplexing
- PRM dynamic RT correction
- Advanced ddHCD algorithm
- Optional BioPharma Mode  mass range up to 8000      with Intact Protein settings

**ThermoFisher SCIENTIFIC**
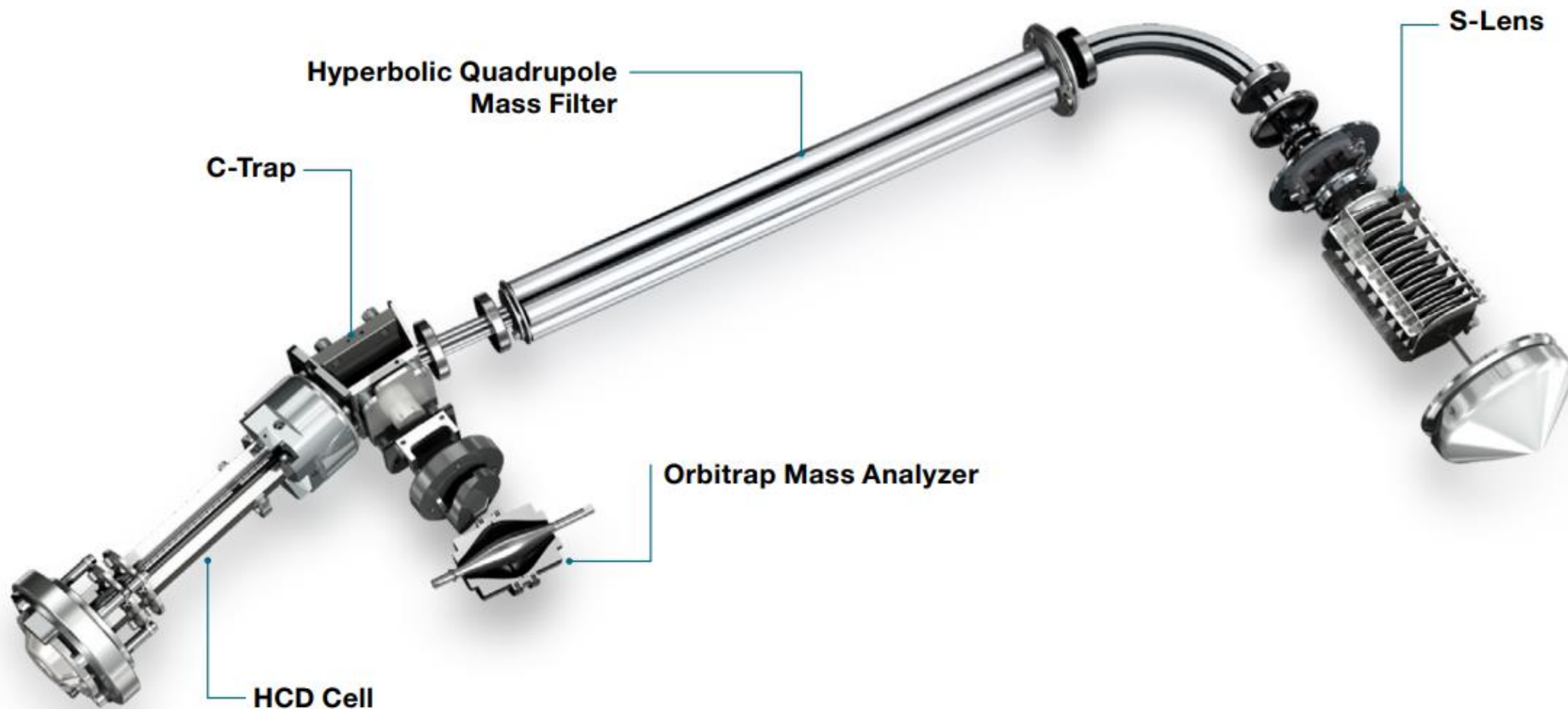
# Benefits of Using Orbitrap Technology

**Resolving Power**

Separate target compounds from interference

**Mass stability and mass accuracy**

Calibration lasts days to weeks because of unrivaled scan:scan mass accuracy & precision

**Fast polarity switching**

Quick positive-negative switch maintaining accurate mass/charge determination

**No trade-off between resolution and sensitivity**

Both resolution and sensitivity are retained

**Minimum scan to scan variance**

Excellent signal/noise, no need for averaging

**Experiment flexibility**

Easy to use, a plug and play device

All your screening and quantitation applications in One platform

**ThermoFisher**
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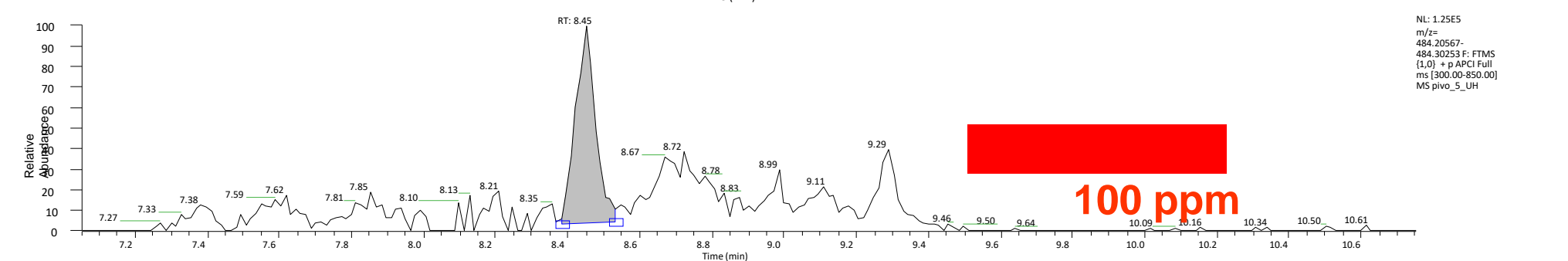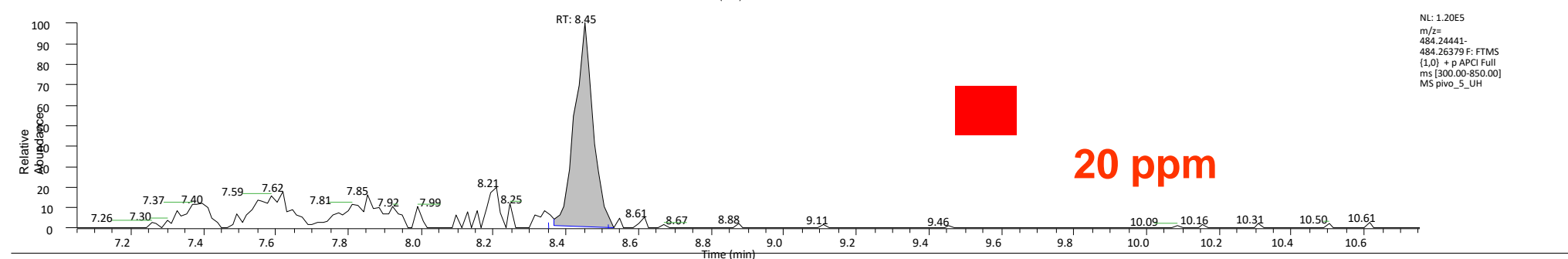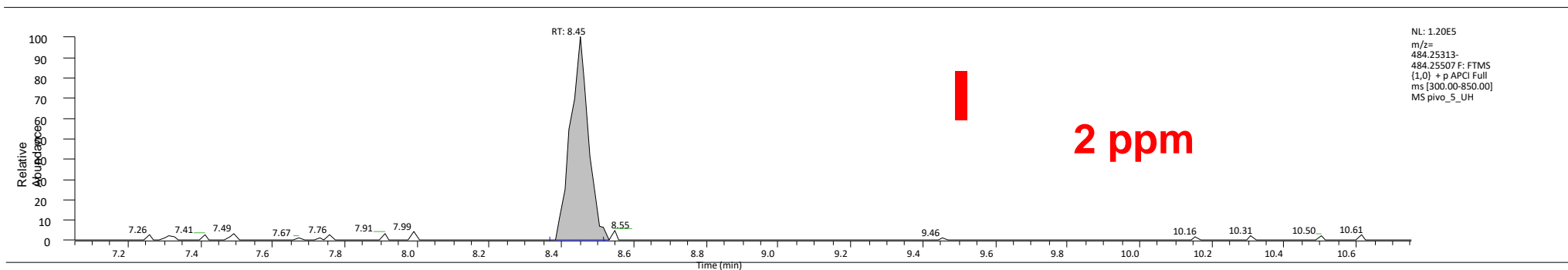
*Zachariasova M et al, Anal. Chim. Acta

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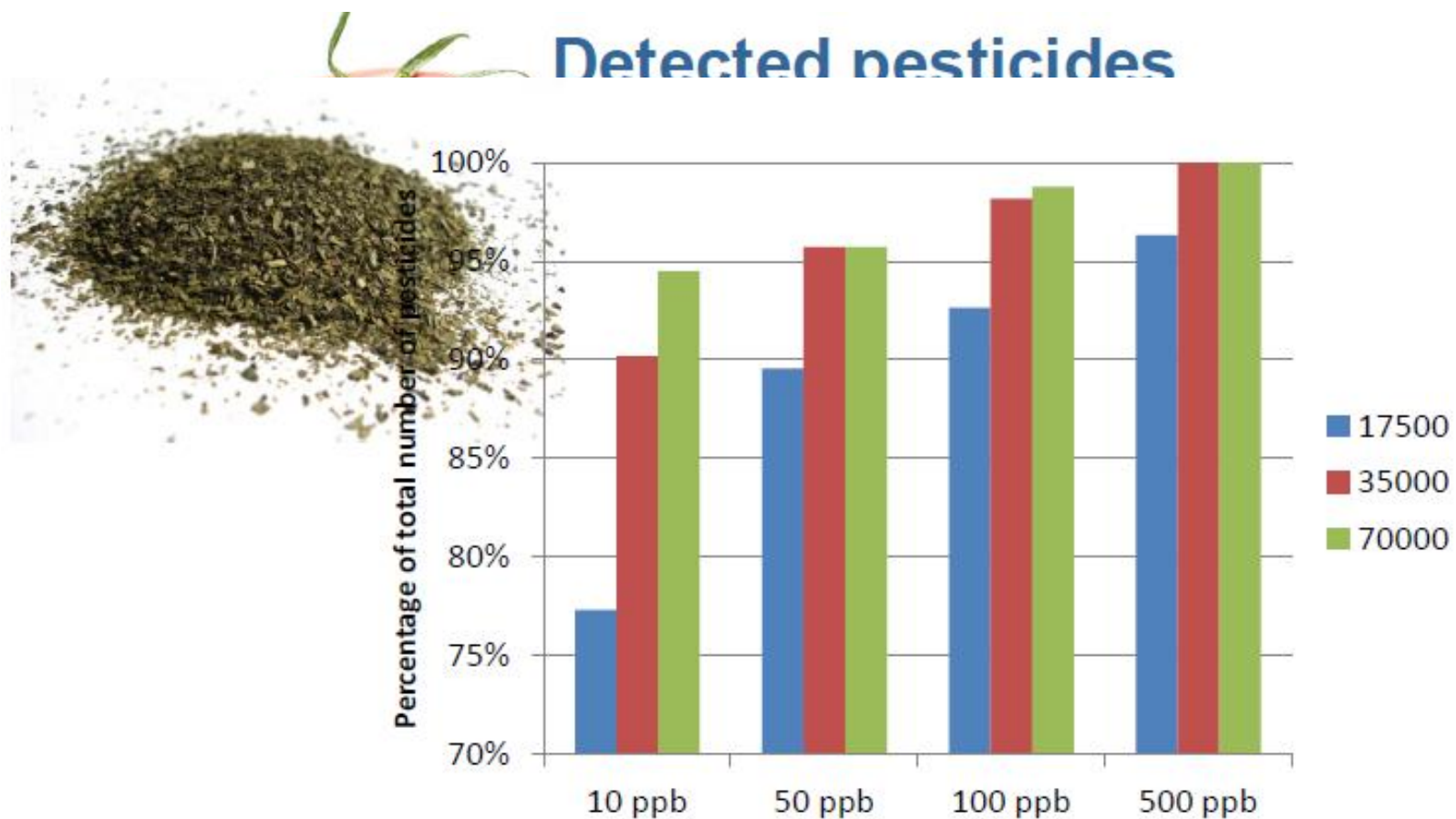
RAFA 2013: Łukasz Rajski, María del Mar Gómez Ramos, Amadeo R. Fernández-Alba; EURL for Pesticide Residues in Fruits and Vegetables. Pesticide Residue Research Group. University of Almeria, Spain. e-mail: amadeo@ual.es

## Full MS or targeted SIM/ddMS2

- Post-acquisition - extracted ion chromatograms of parent ions of interest
- Relies on high resolution for selectivity
- Useful for less complex background
- No method development/preparation needed

## Full MS/ All Ion Fragmentation – vDIA*

- Post-acquisition - extracted ion chromatograms of parent ions of interest
- Scheduled target (inclusion) list (Rt, *m/z*)
- Minimum method development (e.g., predefine parent ions, tr)
- Also for screening purposes

## PRM (Parallel Reaction Monitoring)

- Post-acquisition – extracted ion chromatograms of parent -> fragment transitions acquired
- Scheduled target list (Rt, *m/z*, collision energy)
- Most sensitive and selective even in highly complex matrices

*vDIA method not available in the United States of America*
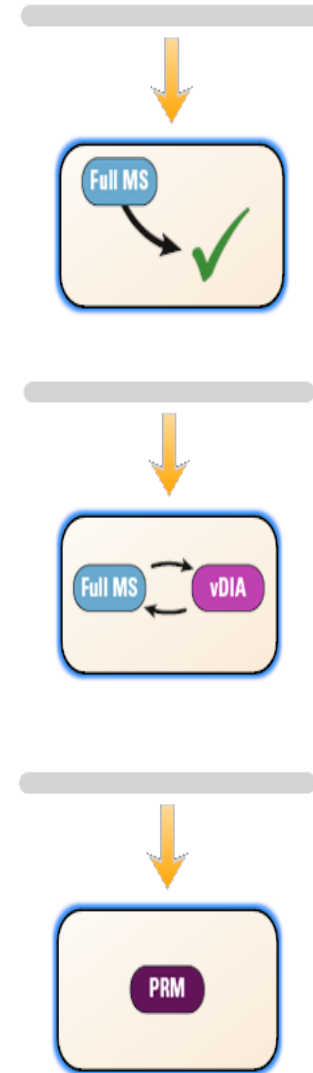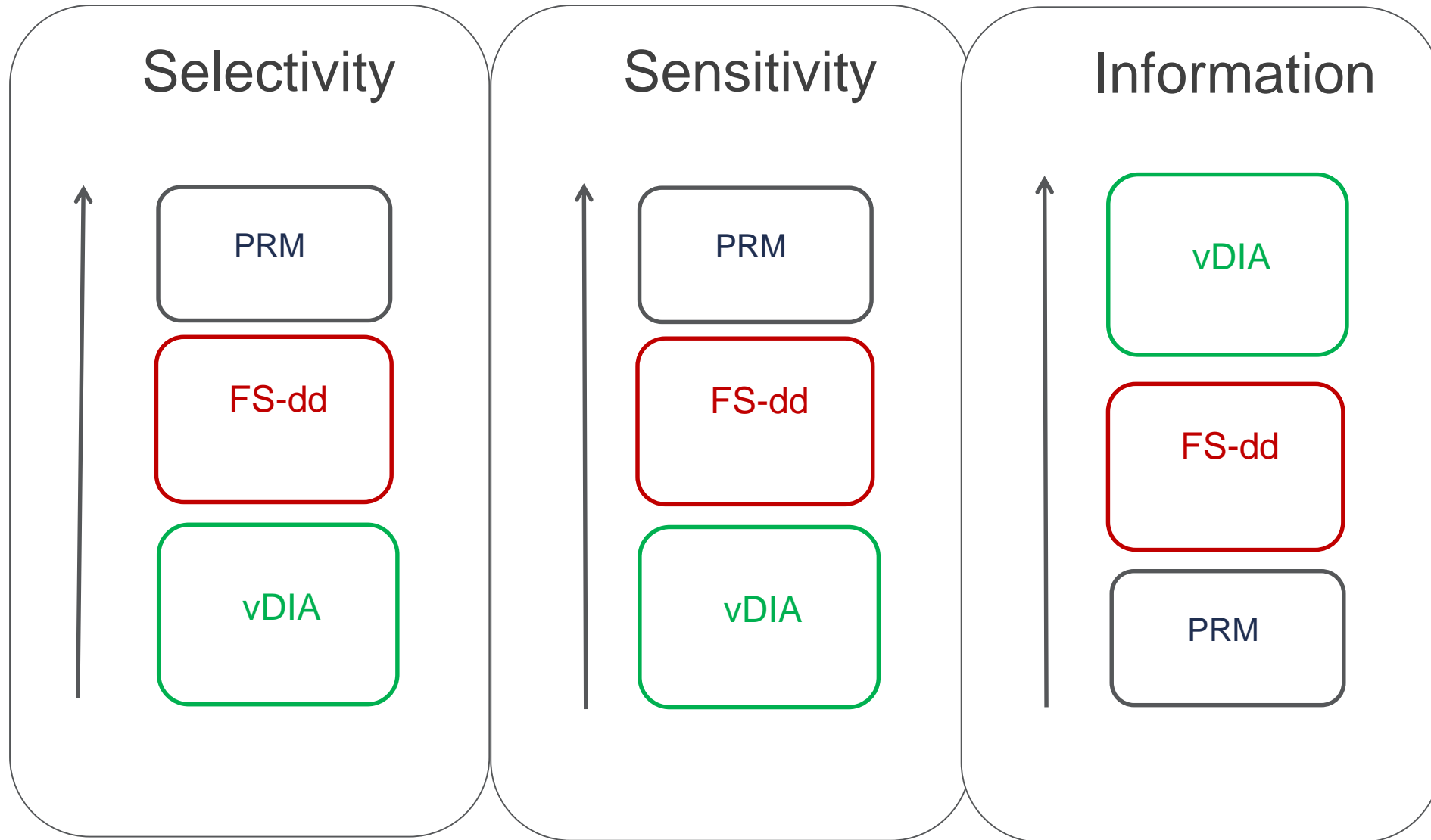
# Orbitrap MS: Unmatched Resolution
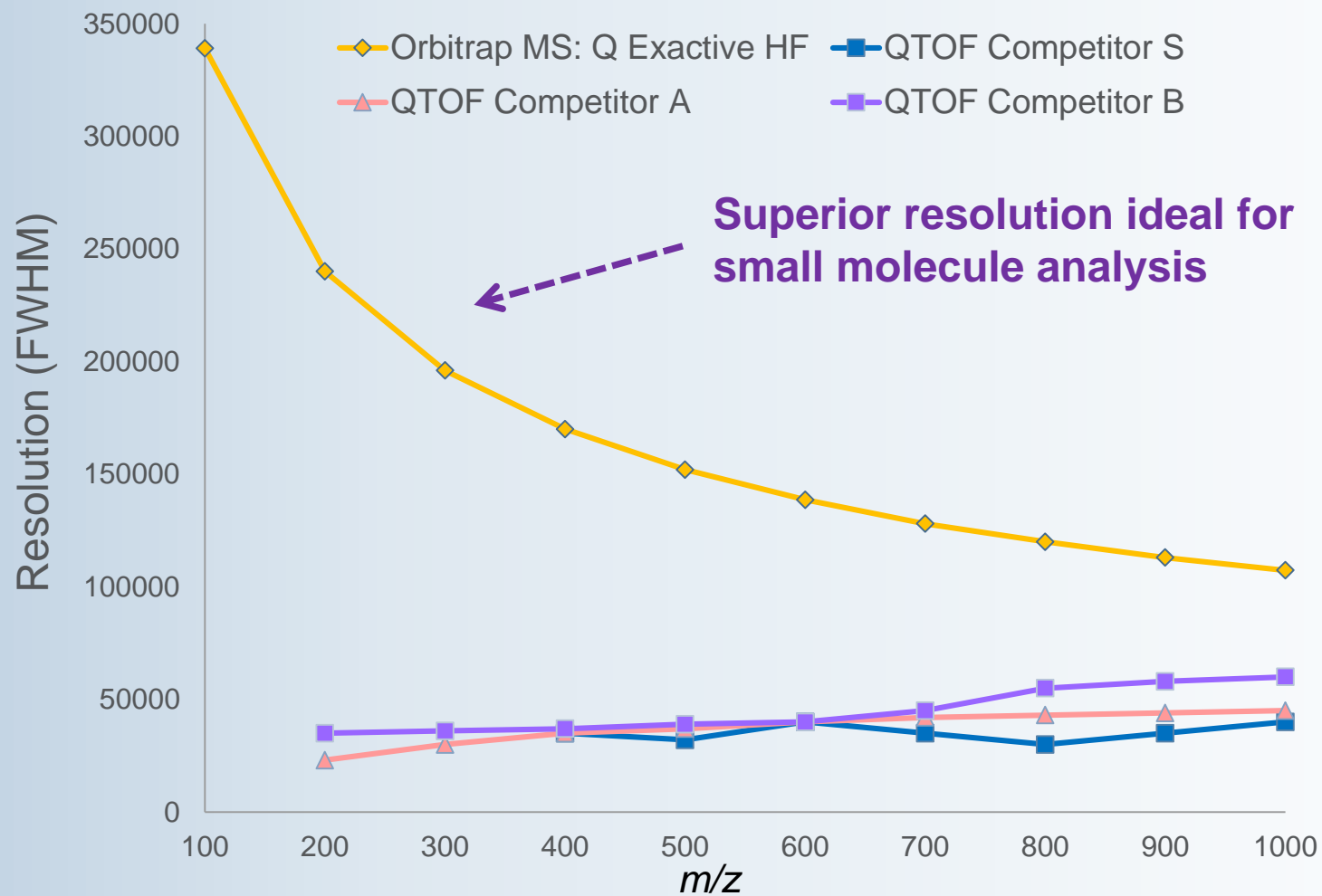
**Benefits of high resolution for small molecule analysis**

- Differentiate similar masses in complex matrix

- Isobaric species

- Fine isotopic pattern

**Superior resolution ideal for small molecule analysis**

Chart legend:
- Orbitrap MS: Q Exactive HF
- QTOF Competitor S
- QTOF Competitor A
- QTOF Competitor B

Y-axis: Resolution (FWHM) — 0, 50000, 100000, 150000, 200000, 250000, 300000, 350000

X-axis: *m/z* — 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000

**High resolution and mass accuracy increase confidence of unknown identification**

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# Analysis of Polar Pesticides by IC-MS/MS

## IC vs LC arguments

| Matrix | Ion Chromatography | | Liquid Chromatography | |
|---|---|---|---|---|
| | Food | Water | Food | Water |
| Sample preparation | Extraction (QuPPe) | None (filtration) | Extraction (QuPPe) (optional FMOC* derivatization) | FMOC* Derivatization (SPE cleanup) (automation possible) |
| Separation quality | Excellent | Excellent | Poor (Hypercarb) | Very good (Reversed Phase) |
| LOQs | 1-10 ppb | 5 -10 ppt | 1 – 10 ppb | 5 – 10 ppt |
| MS/MS | **TSQ Endura/Quantis | **TSQ Quantiva/Altis | **TSQ Quantiv/Altis (Endura not suitable) | **TSQ Quantiva/Altis |
| Matrix tolerance | Very good | Very good | Limited | Good |
| Time consumption | Low | Low | Low - medium | Medium – High (automation possible) |

*FMOC derivatization applicable only for Gly, AMPA, Glu!
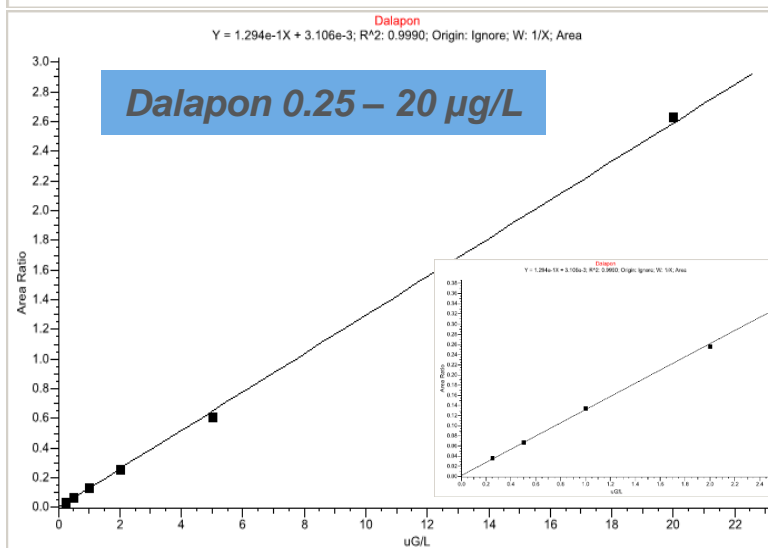**Thermo Scientific™ TSQ™ Endura/Quantis, Thermo Scientific™ TSQ™ Quantiva/Altis™

**Thermo Fisher**
S C I E N T I F I C

# 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 |



DCAA
Y = 2.72e-1X + 1.2e-2; R^2: 0.9996; Origin: Ignore; W: 1/X; Area

**DCAA 0.25 – 20 µg/L**



Dalapon
Y = 1.294e-1X + 3.106e-3; R^2: 0.9990; Origin: Ignore; W: 1/X; Area

**Dalapon 0.25 – 20 µg/L**

**Application Note 65196**

## Perchlorate by IC-MS/MS



SCVW-Extended_Cal-01_05  Perchlorate_99-3  m/z: 82.90

RT: 9.55
MA: 662
SN: 13.14

**2 ng/L**
RSD=5%
(n=4)

SCVW-Extended_Blank_1_07  Perchlorate_99-3  m/z: 82.90

**Blank**



Perchlorate_99-3
Y = -6.099e2X^2 + 2.109e5X + 3.376e2; R^2: 0.9995; Origin: Ignore; W: 1/X; Area

**Perchlorate 0.002 to 50 µg/L**

**Application Note 65201**

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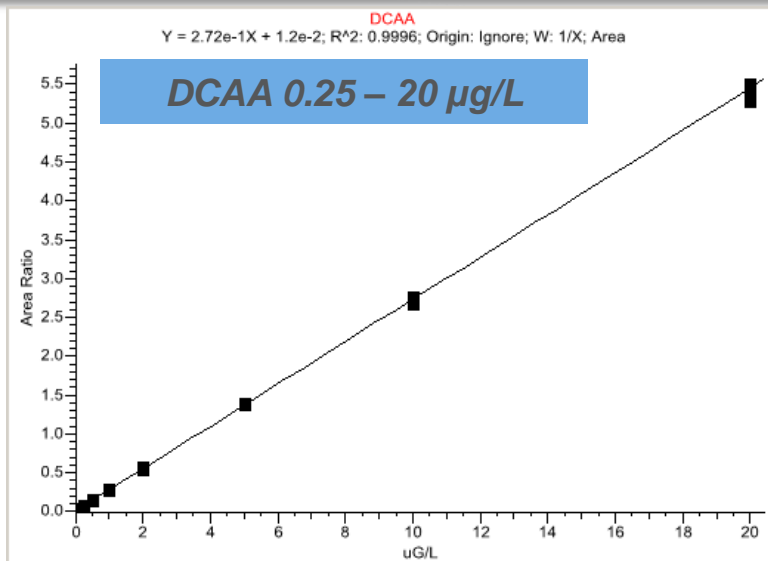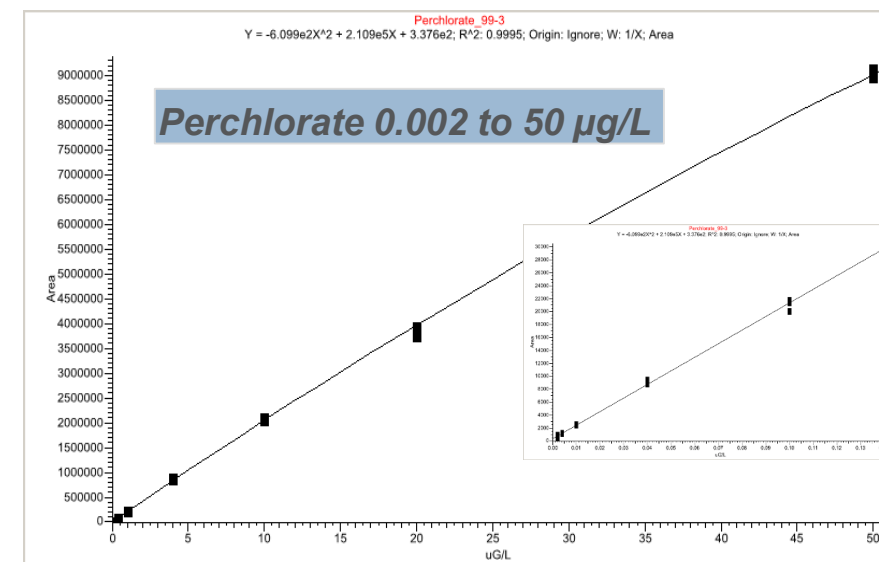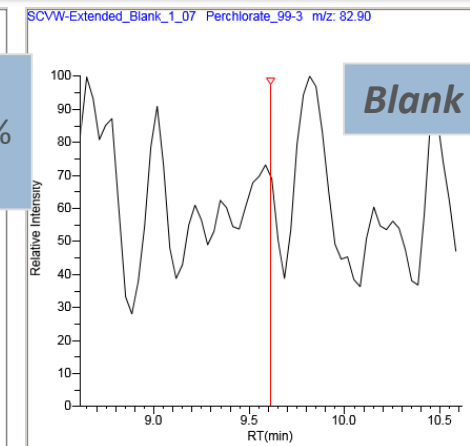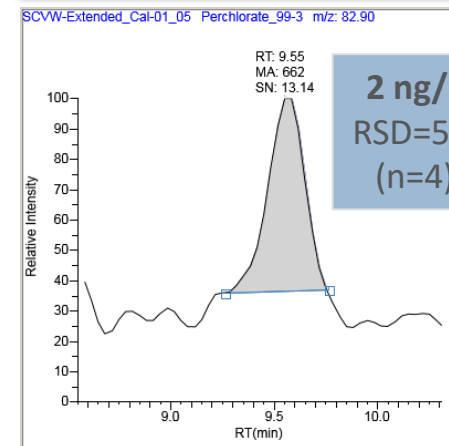
**IC-MS/MS solutions for environmental analysis**
**Quantitation of Glyphosate at 5 ng/L, 3% RSD**

## 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

$Y = -1218.79 + 1766.21*X$   $R^2 = 0.9958$   W: 1/X

IC-MS/MS Analysis of Glyphosate at 5 ng/L
3 % RSD for 3 replicate injections

AA 7310

Relative Abundance

Time (min)

Area

Concentration of Glyphosate (ng/L)

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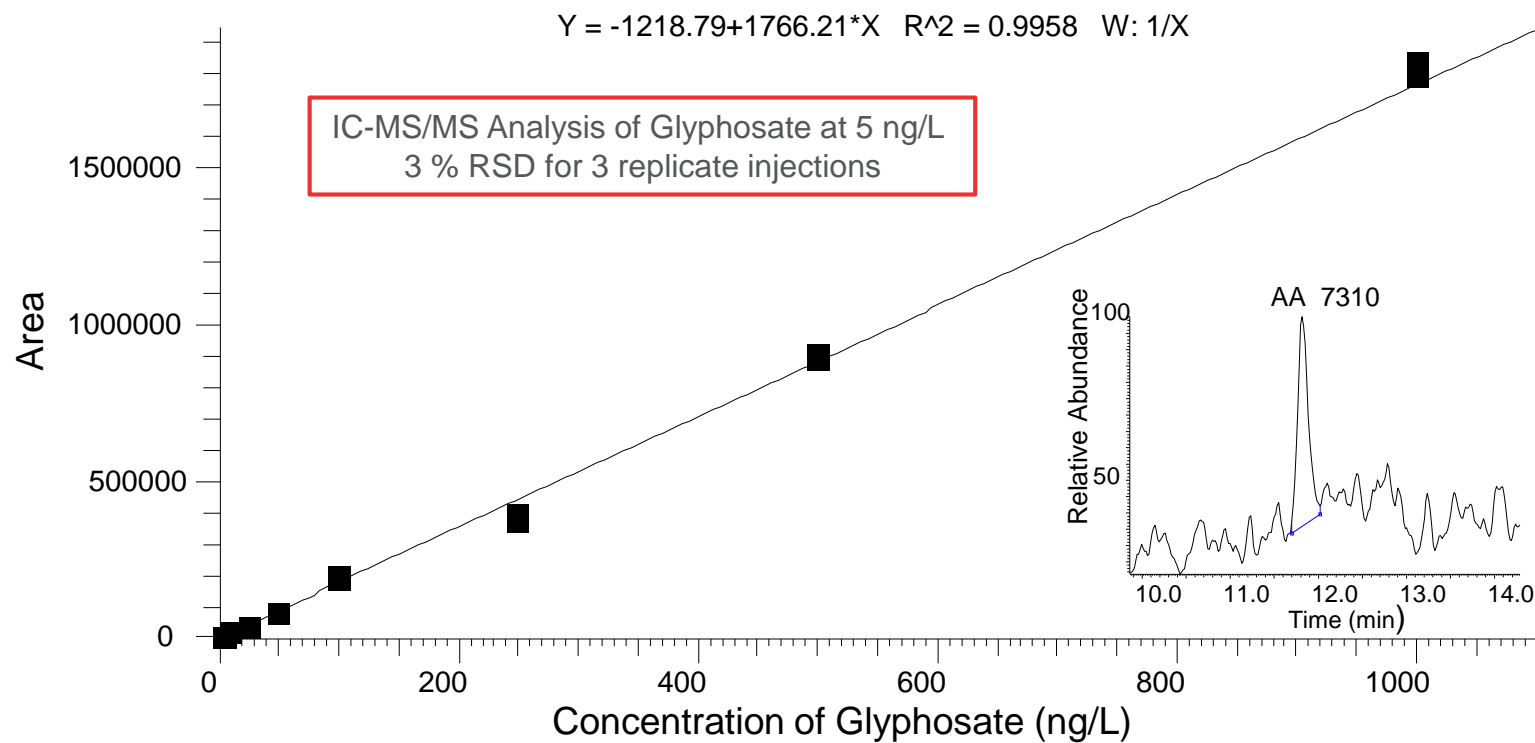
# Polar pesticides in fruits and vegetables by IC-MS/MS Instrumental Configuration

- IC-System: Dionex Integrion HPIC

  - Eluent Source: Dionex EGC 500 KOH

  - Suppressor: ASRS 300

- Autosampler: AS – AP Dionex Autosampler

- Mass Spectrometer: TSQ Quantiva

| Column: | AS24 (2 x 250 mm) |
|---|---|
| Guard Column: | AG24 (2 x 50 mm) |
| Eluent: | KOH |
| Column Temperature: | 30 ºC |
| Flow rate: | 0.3 ml/min |
| Make-up flow: | 0.1 ml/min |
| Make-up solvent: | Isopropanol (0.1 mL/min) |
| Run time: | 22 min |
| Injection volume: | 10 µL |
| Gradient KOH: | |

| No | Time | Concentration [mM] | Curve |
|---|---|---|---|
| 1 | {Initial Tim | Instrument Setup | |
| 2 | {Initial Tim | 22.00 | 5 |
| 3 | 0.000 | Run | |
| 4 | 0.000 | 22.00 | 5 |
| 5 | 4.100 | 22.00 | 5 |
| 6 | 7.000 | 25.00 | 5 |
| 7 | 7.100 | 40.00 | 5 |
| 8 | 9.500 | 60.00 | 5 |
| 9 | 12.000 | 80.00 | 5 |
| 10 | 14.500 | 80.00 | 5 |
| 11 | 15.000 | 100.00 | 5 |
| 12 | 17.000 | 100.00 | 5 |
| 13 | 17.100 | 22.00 | 5 |
| 14 | 19.900 | 22.00 | 5 |
| 15 | 20.000 | 22.00 | 5 |
| 16 | 22.000 | 22.00 | 5 |
| 17 | New Row | | |
| 18 | 22.000 | Stop Run | |

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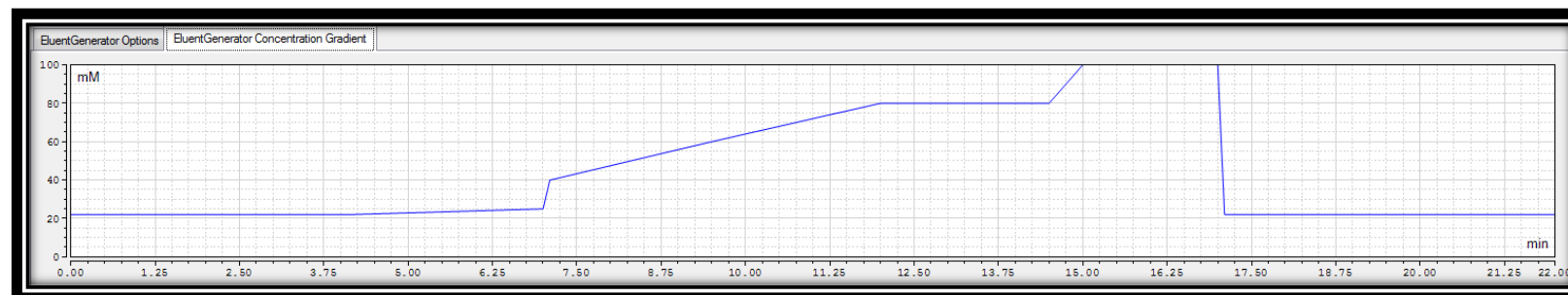
| Compound | LOD (µg/kg) | LOQ (µg/kg) |
|---|---|---|
| Phosphonic acid | 0.50 | 1.0 |
| Fosetyl | 0.03 | 0.1 |
| AMPA | 0.50 | 1.0 |
| Glufosinate | 0.50 | 1.0 |
| N-acetyl-glufosinate | 0.25 | 0.75 |
| Ethephon-hydroxy | 0.25 | 0.75 |
| N-acetyl-AMPA | 0.10 | 0.50 |
| MPPA | 0.10 | 0.50 |
| Ethephon | 0.25 | 0.75 |
| Glyphosate | 0.03 | 0.10 |

| Compound | LOD (µg/kg) | LOQ (µg/kg) |
|---|---|---|
| Phosphonic acid | 0.50 | 1.00 |
| Fosetyl | 0.03 | 0.10 |
| AMPA | 0.50 | 1.00 |
| Glufosinate | 0.25 | 0.75 |
| N-acetyl-glufosinate | 0.25 | 0.75 |
| Ethephon-hydroxy | 0.03 | 0.10 |
| N-acetyl-AMPA | 0.03 | 0.10 |
| MPPA | 0.03 | 0.10 |
| Ethephon | 0.25 | 0.75 |
| Glyphosate | 0.03 | 0.10 |

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IC:Dionex Integrion™
MS:TSQ Quantiva™

Column: AS24 (2 x 250 mm)
Guard Column: AG24 (2 x 50 mm)
Eluent: KOH
Injection volume: 100 µL
Column Temperature: 21 ºC
Flow rate: 0.3 ml/min
Make-up flow: 0.1 ml/min
Make-up solvent: $CH_3OH$

| Time (min) | Concentration of KOH in eluent (mM) |
|---|---|
| 0 | 25 |
| 0.2 | 25 |
| 11 | 80 |
| 11.1 | 100 |
| 12.5 | 100 |
| 12.6 | 25 |
| 17.0 | 25 |

| Component | Matrix | LOD [ppt]] | LOQ [ppt] |
|---|---|---|---|
| Fosetyl | Drinking | 2.5 | 5 |
| | Bottled | 1 | 2.5 |
| | Surface | 2.5 | 5 |
| Glufosinate | Drinking | 5 | 10 |
| | Bottled | 5 | 10 |
| | Surface | 5 | 10 |
| AMPA | Drinking | 5 | 10 |
| | Bottled | 5 | 10 |
| | Surface | 5 | 10 |
| Clopyralid | Drinking | 10 | 50 |
| | Bottled | 5 | 10 |
| | Surface | 5 | 10 |
| Glyphosate | Drinking | 10 | 50 |
| | Bottled | 10 | 50 |
| | Surface | 10 | 50 |

- **Sample preparation**
- **Chromatography**
- **Mass Spectrometry**
- **Trace Elemental**
- **IRMS**
- **Consumables**
- **Services**
- **Informatics**