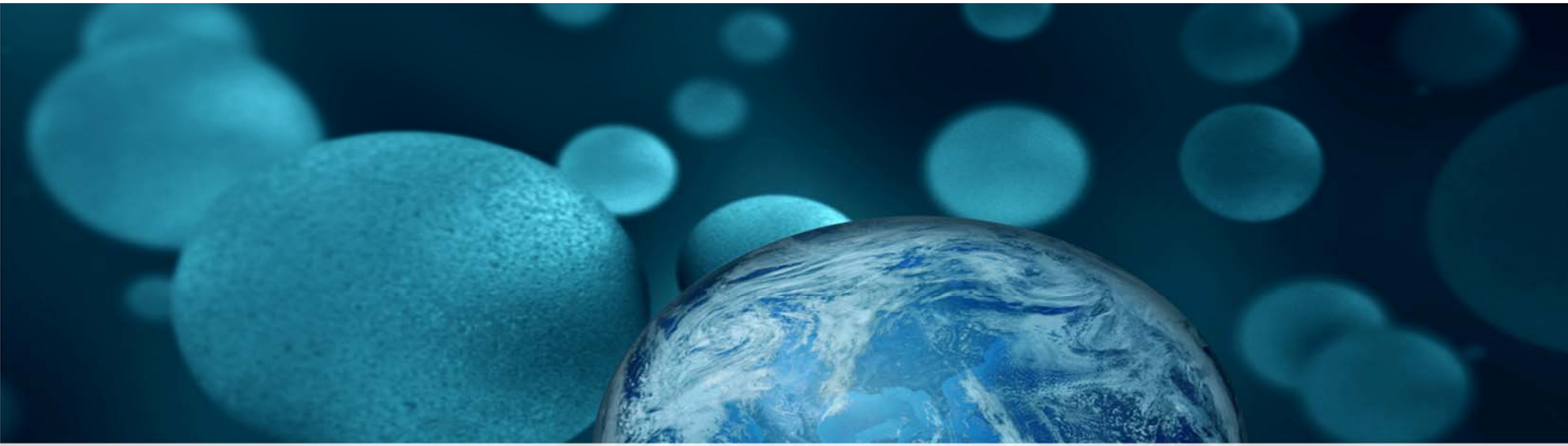


A Comprehensive Multi-Class Veterinary Medicines Method Using A New Best-In-Class Triple Quadrupole Mass Spectrometer

Ed George - Senior Application Scientist - Thermo Fisher Scientific
Danny Chan, George Stubbings, Stuart Adams- Fera Science, UK
Dave Borts and Viet Dang, Iowa State University

Outline

- New Thermo Scientific™ TSQ Quantis™ and TSQ Altis™ Triple Quadrupole MS Systems overview
- Veterinary Medicines Methodology
  - Challenges of multi-class veterinary medicines analysis and integration into a routine testing laboratory
  - Thermo Scientific™ Acclaim™ Column Technology for wide range of VetDrugs
  - Scope of method and results
- Conclusions

Introduction to TSQ Altis and TSQ Quantis

Performance: Sensitivity, Selectivity (H-SRM)

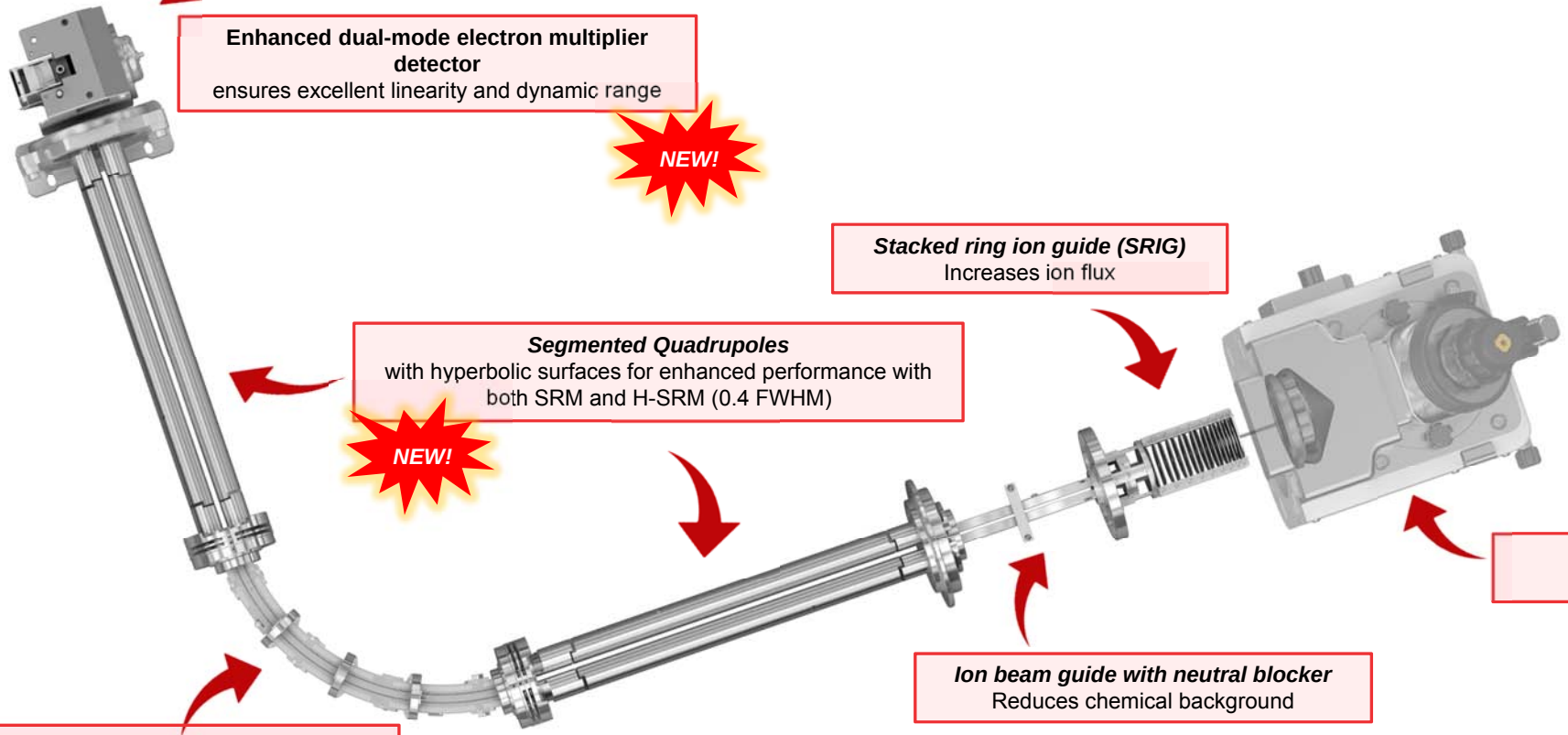
TSQ Altis

TSQ Quantis

	TSQ Altis *High-end*	TSQ Quantis *Mid-tier*
Mass Range	5-2000	5-3000
SRM/sec	600	600
Selectivity (H-SRM)	0.2 Da FWHM	0.4 Da FWHM
Sensitivity (HESI) Reserpine 1 pg	500,000:1	150,000:1

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

Technology in TSQ Quantis: Excellent Robustness, Day After Day



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

NEW!

Stacked ring ion guide (SRIG)
Increases ion flux

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

NEW!

OptaMax™ NG
APCI ready

NEW!

Ion beam guide with neutral blocker
Reduces chemical background

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

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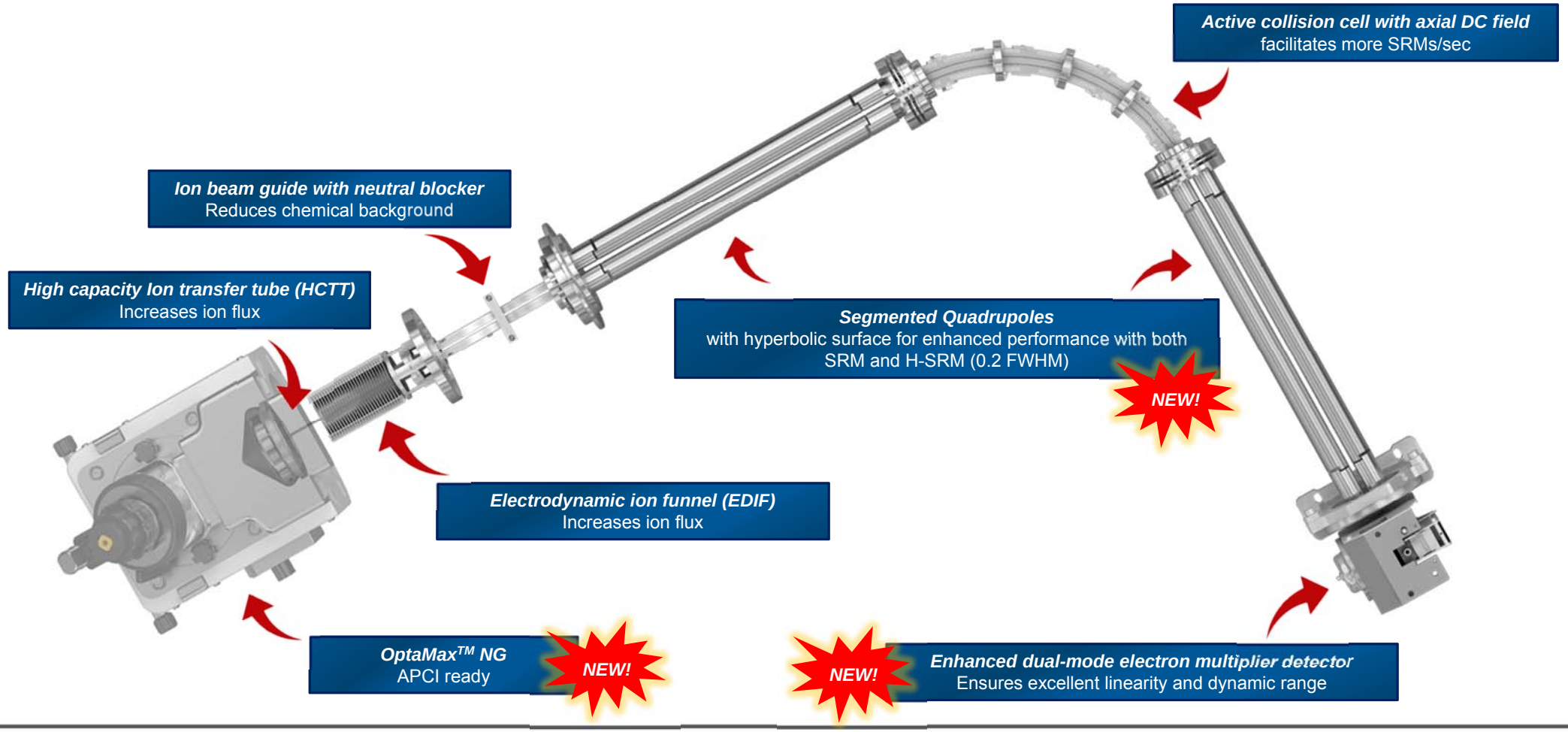
Technology in TSQ Altis: High Sensitivity with Robustness



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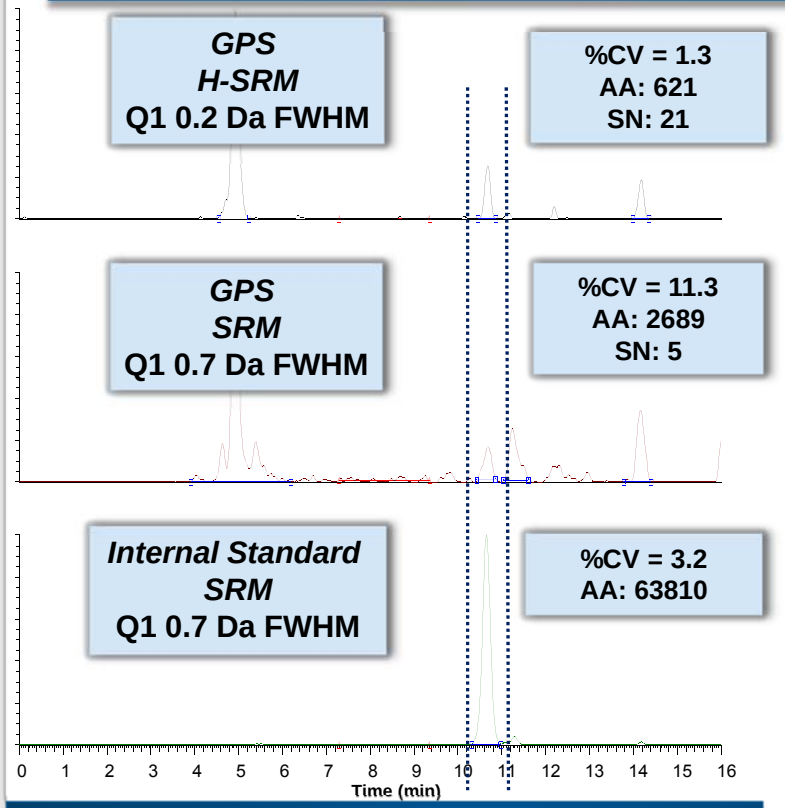
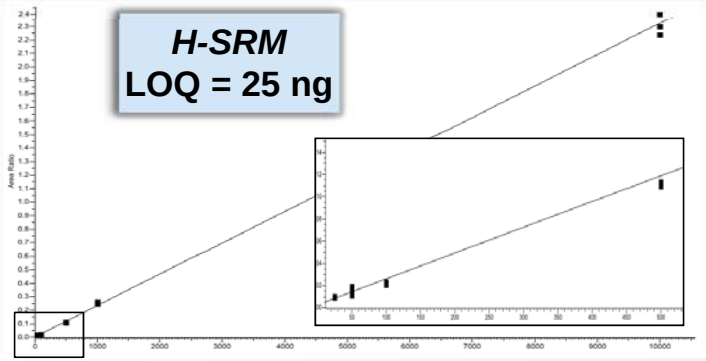
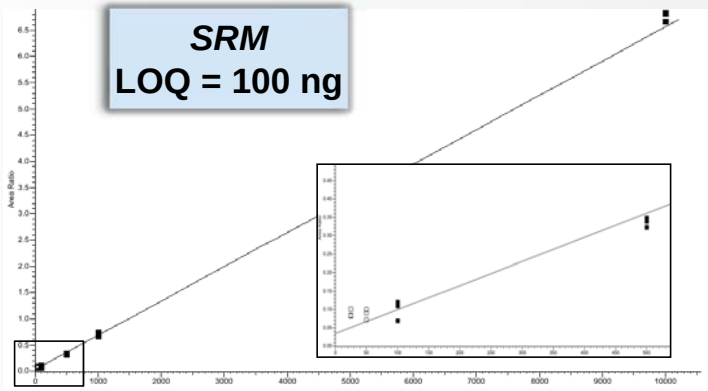
Improved Sensitivity with H-SRM (0.2 Da FWHM) – GPSVFPLAPSSK

GPSVFPLAPSSK - Peptide from monoclonal antibody



25 ng GPSVFPLAPSSK and *IS*





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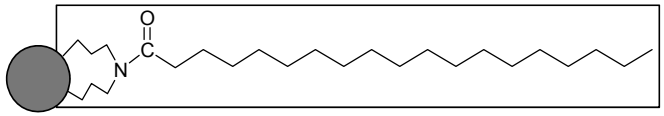
Challenges of Multi-Residue Methods

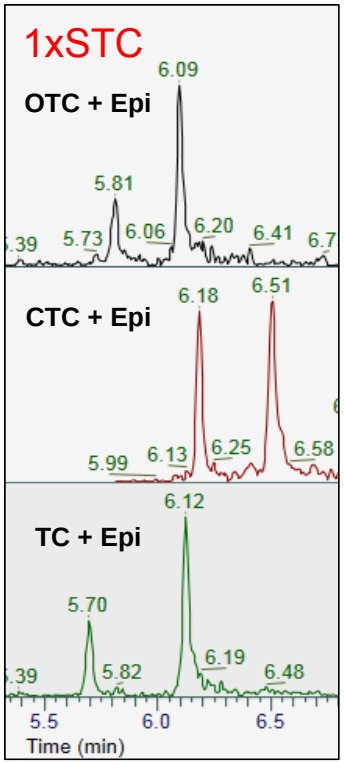
- Generic enough to apply to several different matrices - e.g., meat, fish, dairy
- Stability of Matrix Extracted Spikes (MES) and spiking standards
- Chromatography- Column must handle wide polarity range; be rugged
- Sample prep must minimize loss of analytes and be simple and cost effective
- Single mobile phase for all compounds
- Sufficient sensitivity for certain compounds
- Need for polarity switching
- Accurate quantification
- Identification against guideline criteria
- Can we solve these challenges in a single workflow?

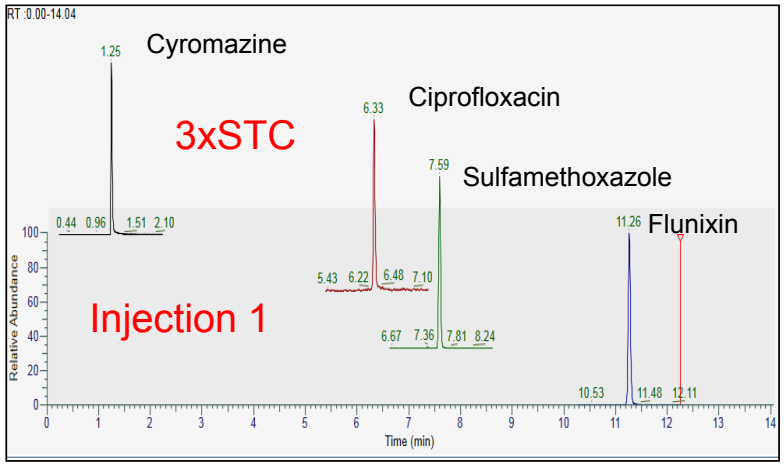
Column-Acclaim Polar Advantage II (PA2)- Robust and Selective for VetDrugs

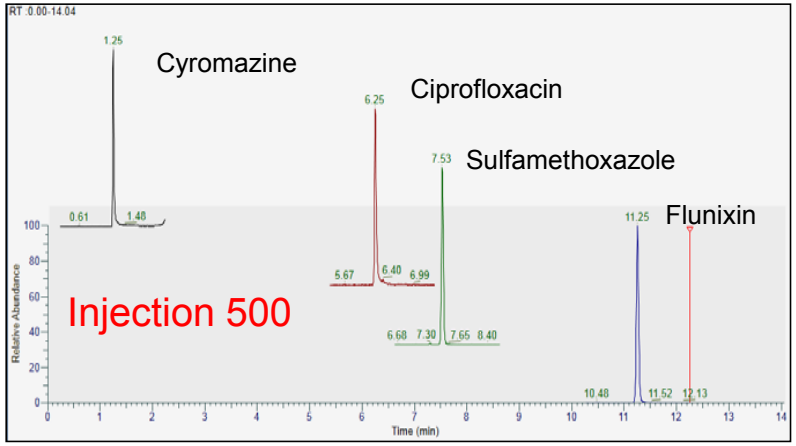


Features

- Unique selectivity- amide embedded group
- Enhanced hydrolytic stability
- 100% aqueous compatibility
- pH Range 1.5 to 10.5
- Low column bleed
- ***Robust against matrix extracts***
- Particle size: 2.2, 3.0 or 4.5-µm
- Advanced surface technology
- Acclaim columns use innovative silane ligands - ensures unique selectivity







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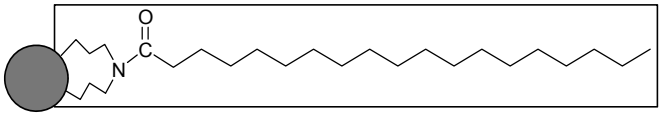
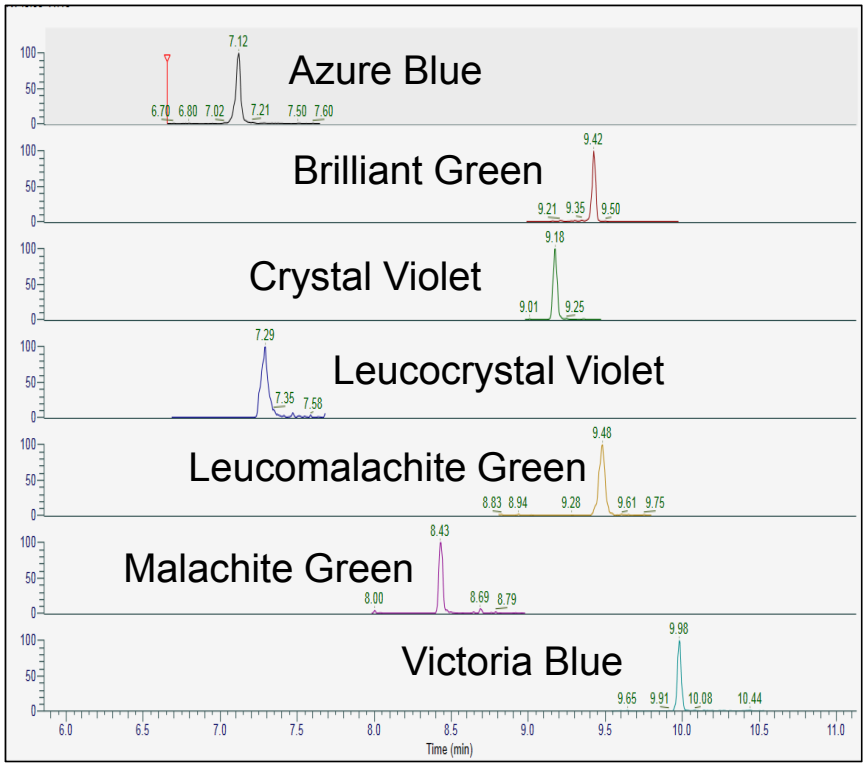
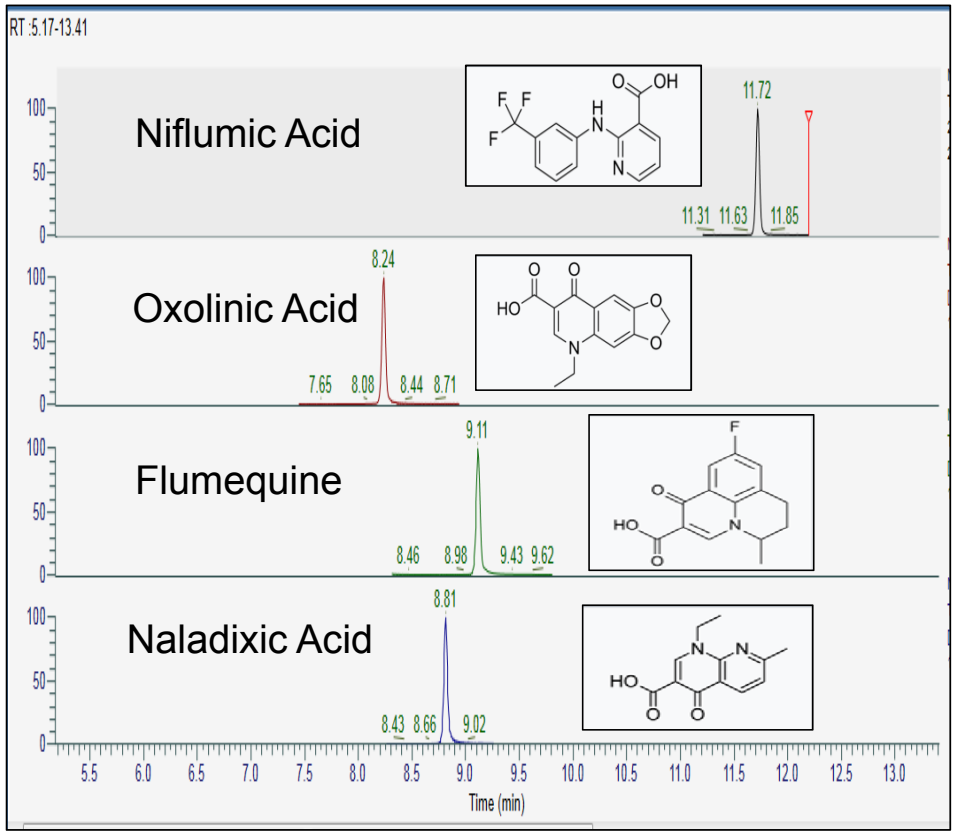
Column-Acclaim Polar Advantage II (PA2)- Robust and Selective for VetDrugs





Dyes- 1xSTC (1ng/g) in Salmon Fillet



Acidic Compounds- 3 x STC in Bovine Muscle

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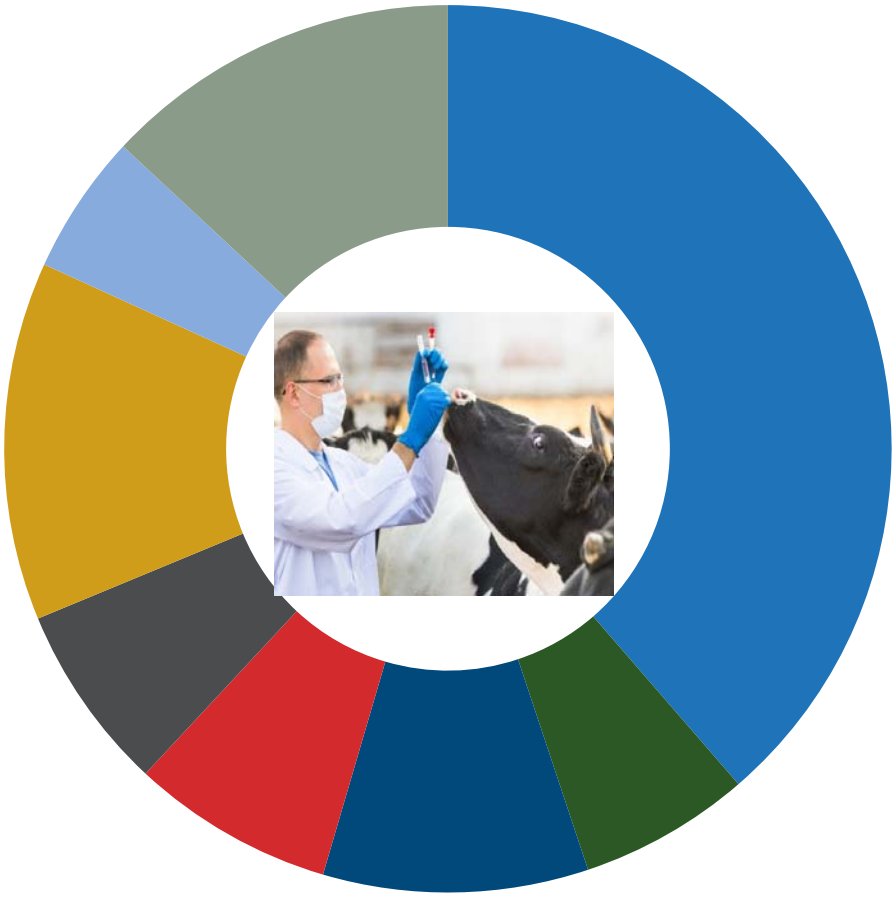
Multi-Residue Method - Overview

- 160+ compounds in 3 matrices: bovine muscle, salmon fillet, and milk (plus addition of labelled internal standards) included in the method from the following classes of veterinary medicines:
  - Cefalosporins, macrolides, penicillins, quinolones, sulfas, tetracyclines, anthelmintics, nitroimidazoles, NSAIDs, sedatives, avermectins and coccidiostats, dyes (applied to fish), steroids (milk)

- Experimental Design:
  - 8 x spikes @ 0.2, 0.5, 1, 3, and 5 x STC = [Screening Target Concentration] for each compound with 2 blanks and one recovery spike per batch
  - Analyze the batches on 3 separate LC/MS/MS systems
  - Use basic elements of the same sample prep applied to all 3 matrices

Compounds Studied and Chemical Classes



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Sample Preparation and LC Conditions

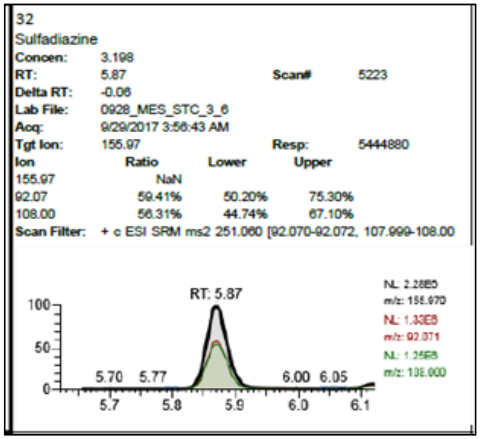
- QuEChERS based approach
  - EDTA/NH_4 oxalate solution and acetonitrile added to sample
  - Sample homogenised until fully dispersed
  - Sodium sulphate added before centrifugation
  - Dispersive SPE (CEC- C_{18}) clean-up
  - Add 1 mL H2O to 3mL extract, filter, inject

- LC conditions
  - Thermo Scientific™ Vanquish™ Acclaim™ PA2, 2.1 x 150 x 2.2 um
  - MP A: 0.05% formic acid + 0.1 mM NH_4F (aq)
  - MP B: 0.05% formic acid in 1:1 MeOH:MeCN
  - 2 uL injection

No	Time	Flow [ml/min]	%B	Curve
1	0.000	Run		
2	0.000	0.400	0.0	5
3	2.200	0.400	0.0	5
4	11.000	0.400	95.0	5
5	13.000	0.500	95.0	5
6	14.400	0.500	95.0	5
7	14.500	0.450	0.0	5
8	16.600	0.400	0.0	5
9	16.600	0.400	0.0	5
10	*New Row*			
11	17.000	Stop Run		

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

Steps for Evaluating Method Performance



Establish Screen Target Concentration (STC) Level
Levels typically ½ the MRL/MRPLs

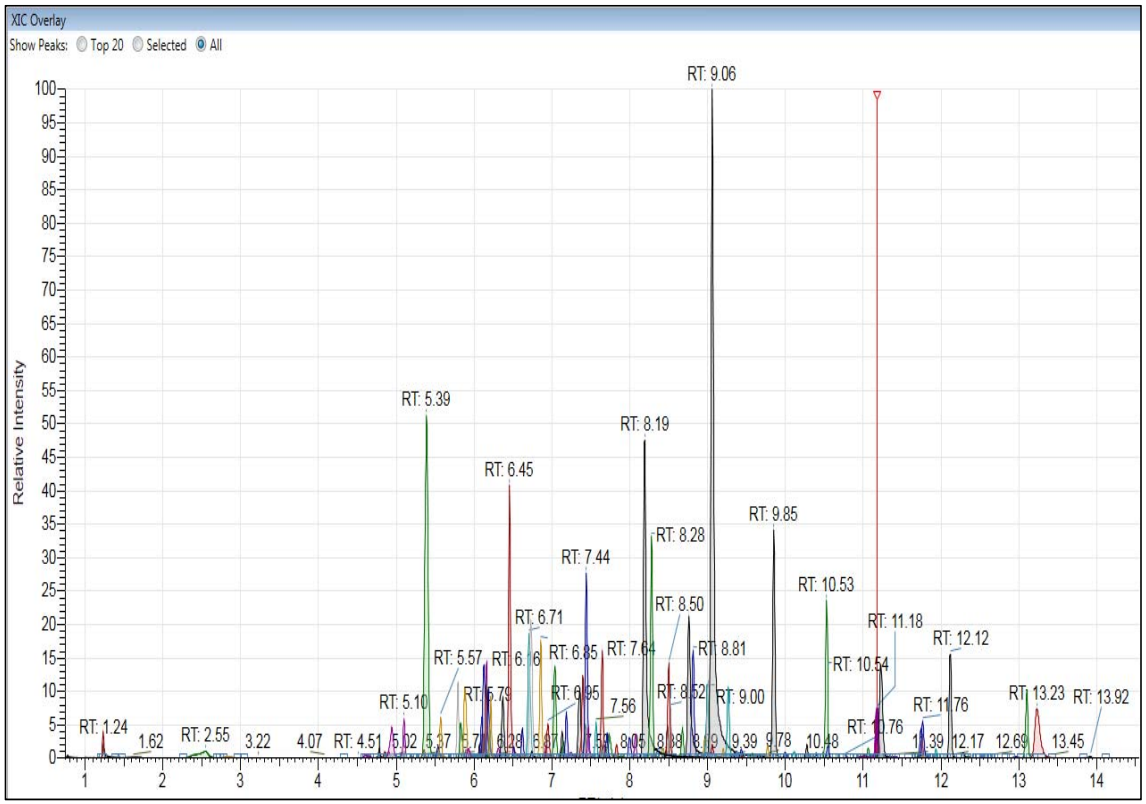
Prepare Matrix Extracted Spike (MES) Replicates at 0.2-5 x STC
(Establish 'Calibration Line' for screening)

Calculate %RSDs at each level to check precision

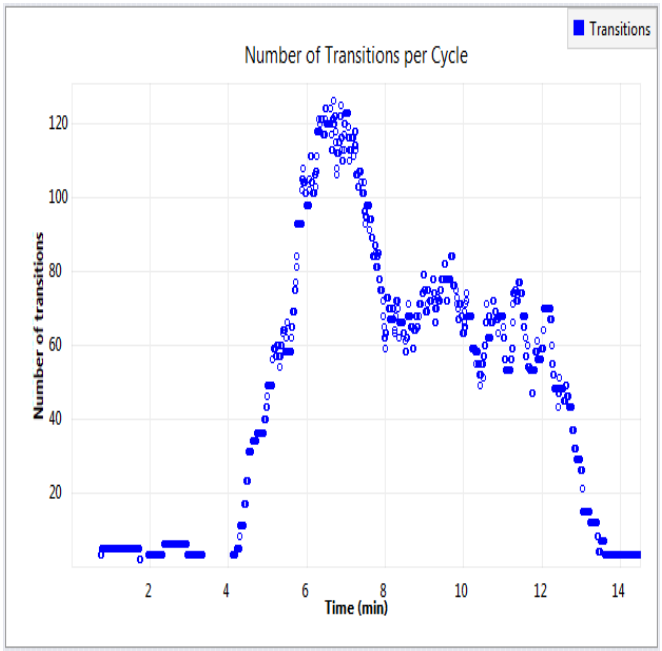
Calculate Absolute % Recovery Based upon
A 'post spike' at 3 x STC [Recovery Std]

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

Extracted SRMs for Multi-Class VetDrugs



Extracted SRMs at 0.5 x STC in MES



TSQ Altis- total of 525 transitions from analysis at left

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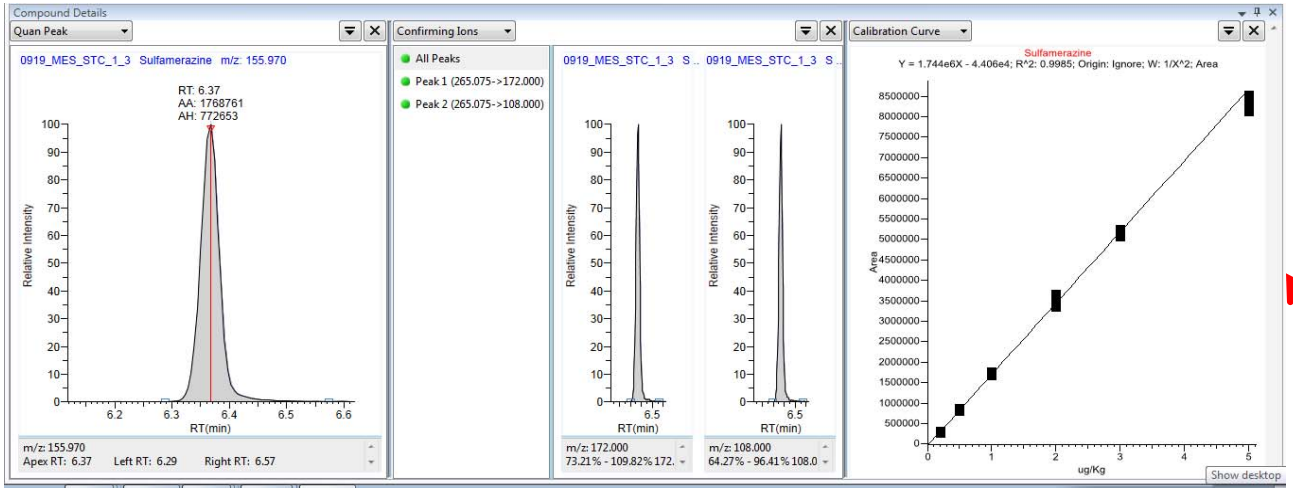
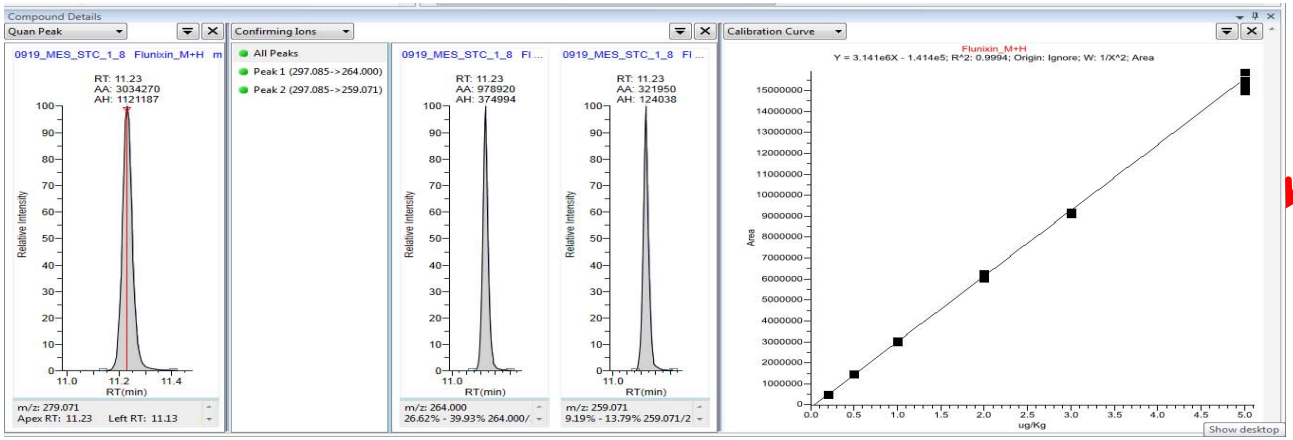
Quantitative Results- 1x to 5x STC- Bovine





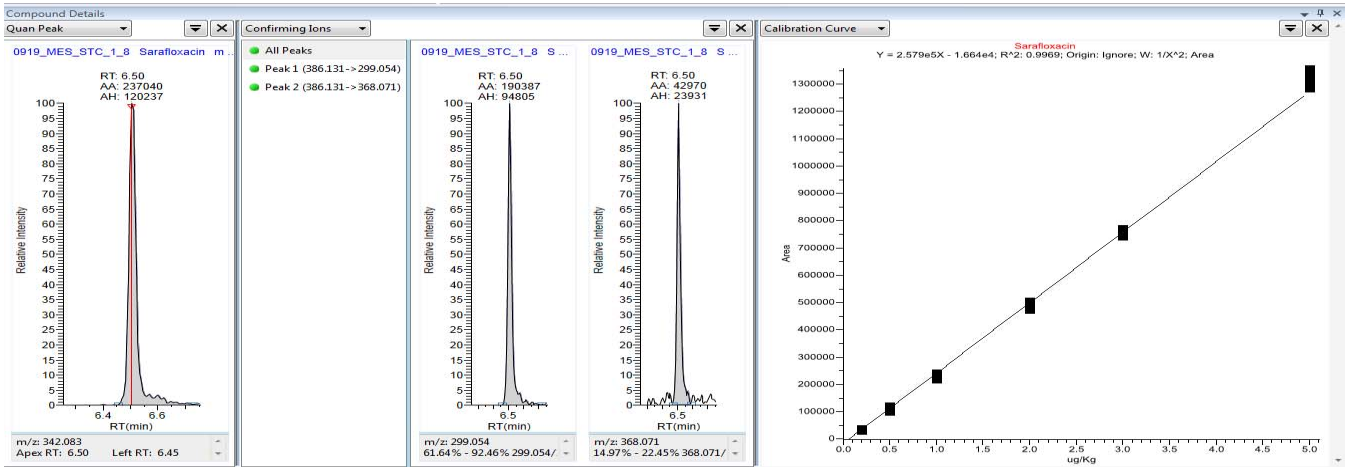
Sulfa Drugs- Sulfamerazine

8 replicates plotted per each point

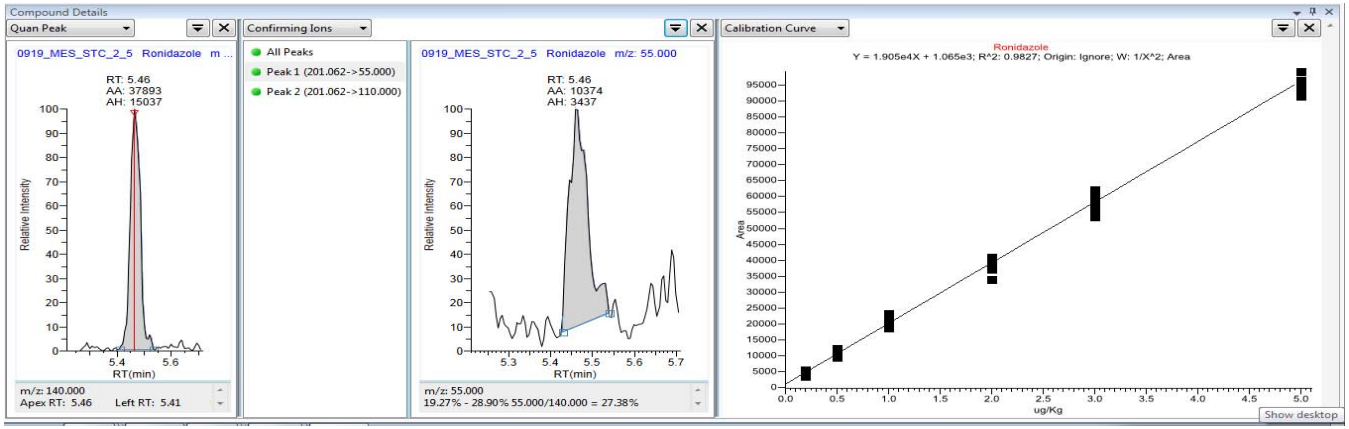
NSAIDs- Flunixin

ThermoFisher SCIENTIFIC

Quantitative Results- 0.2 to 5 x STC- Bovine



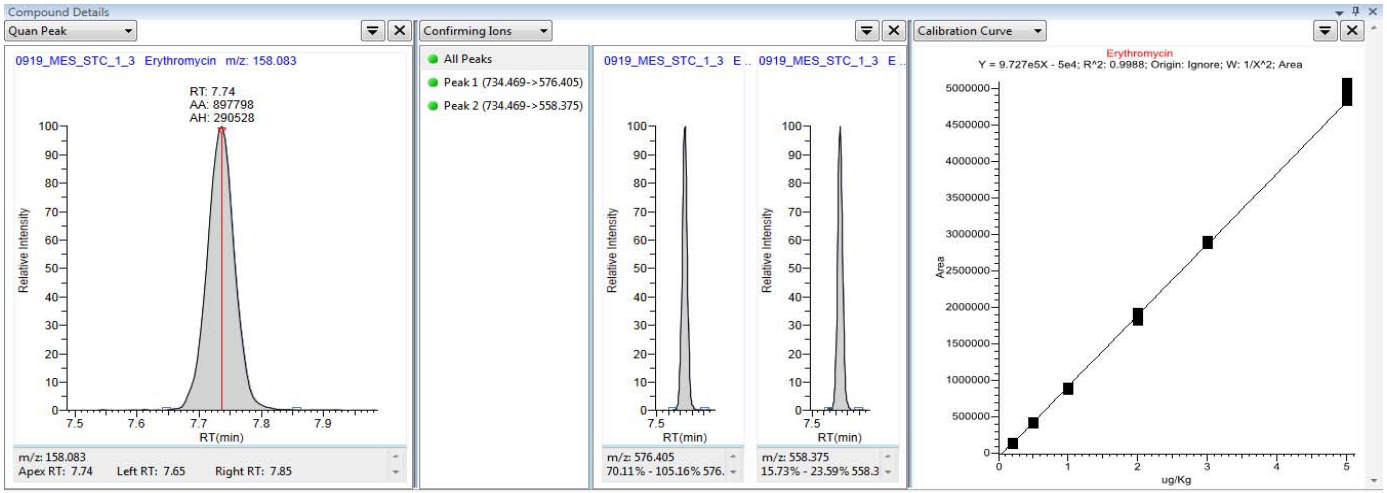
Quinolones-Sarafloxacin



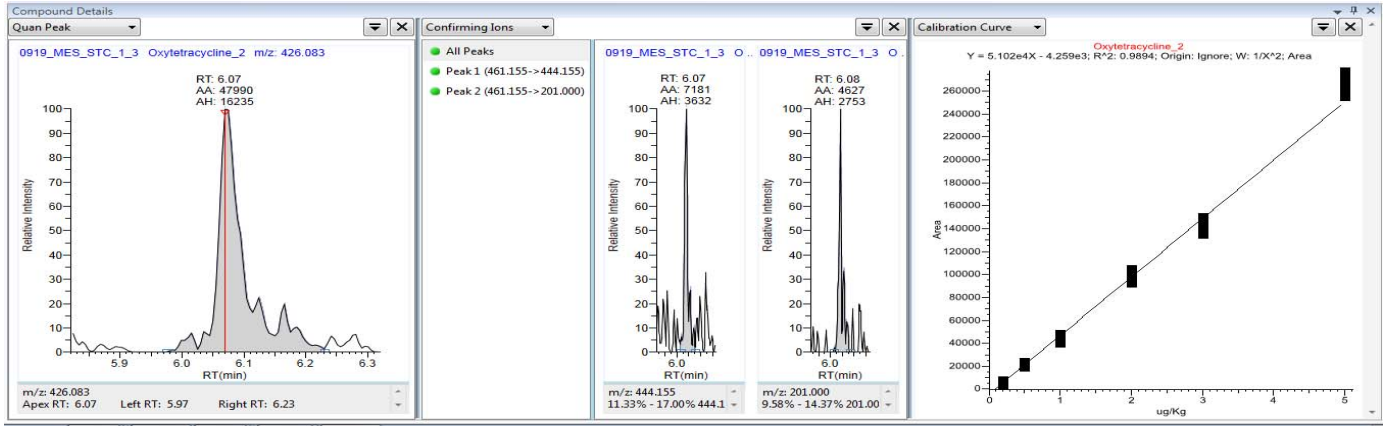
Nitroimidazoles - Ronidazole

ThermoFisher SCIENTIFIC

Quantitative Results- 0.2 to 5 x STC-Bovine



Antibiotics-Erythromycin



Antibiotics-Oxytetracycline

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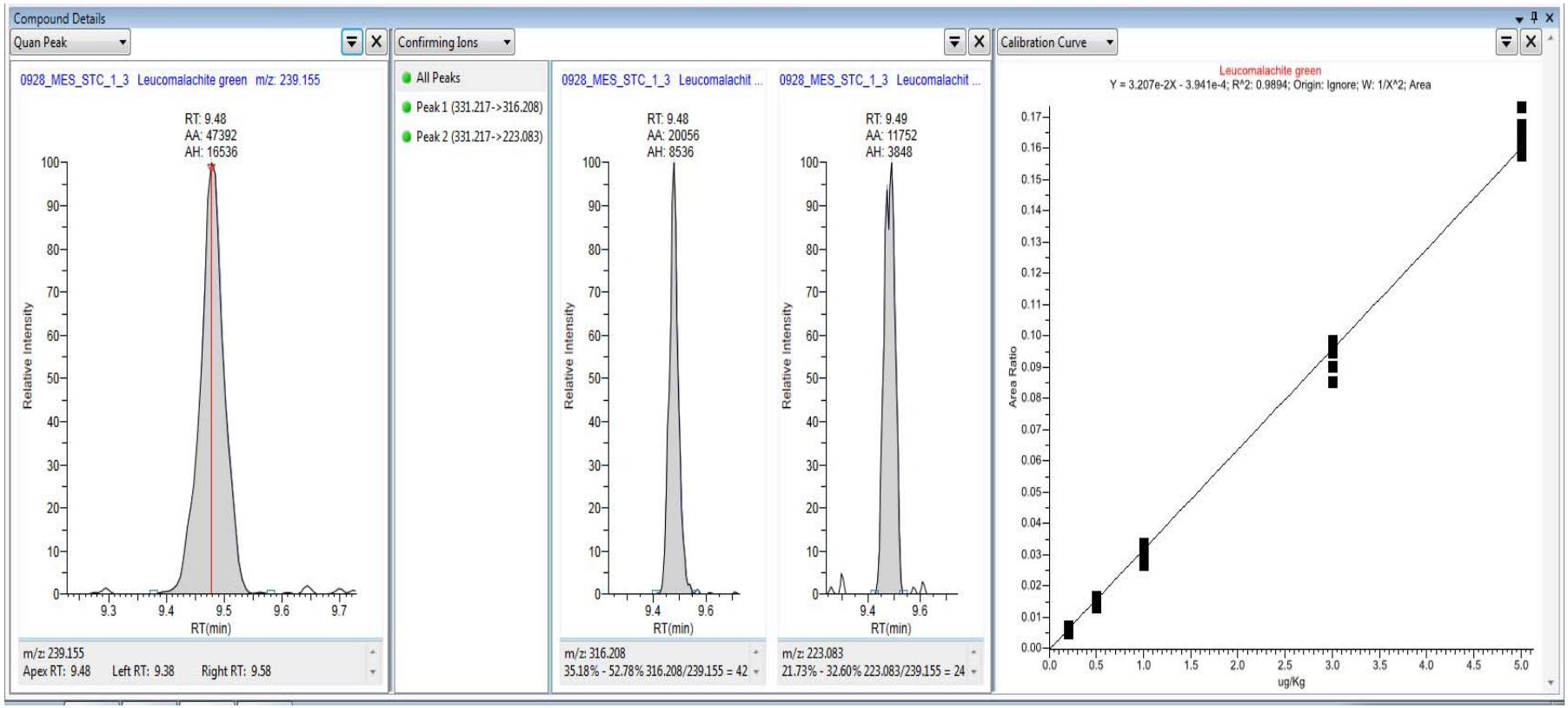
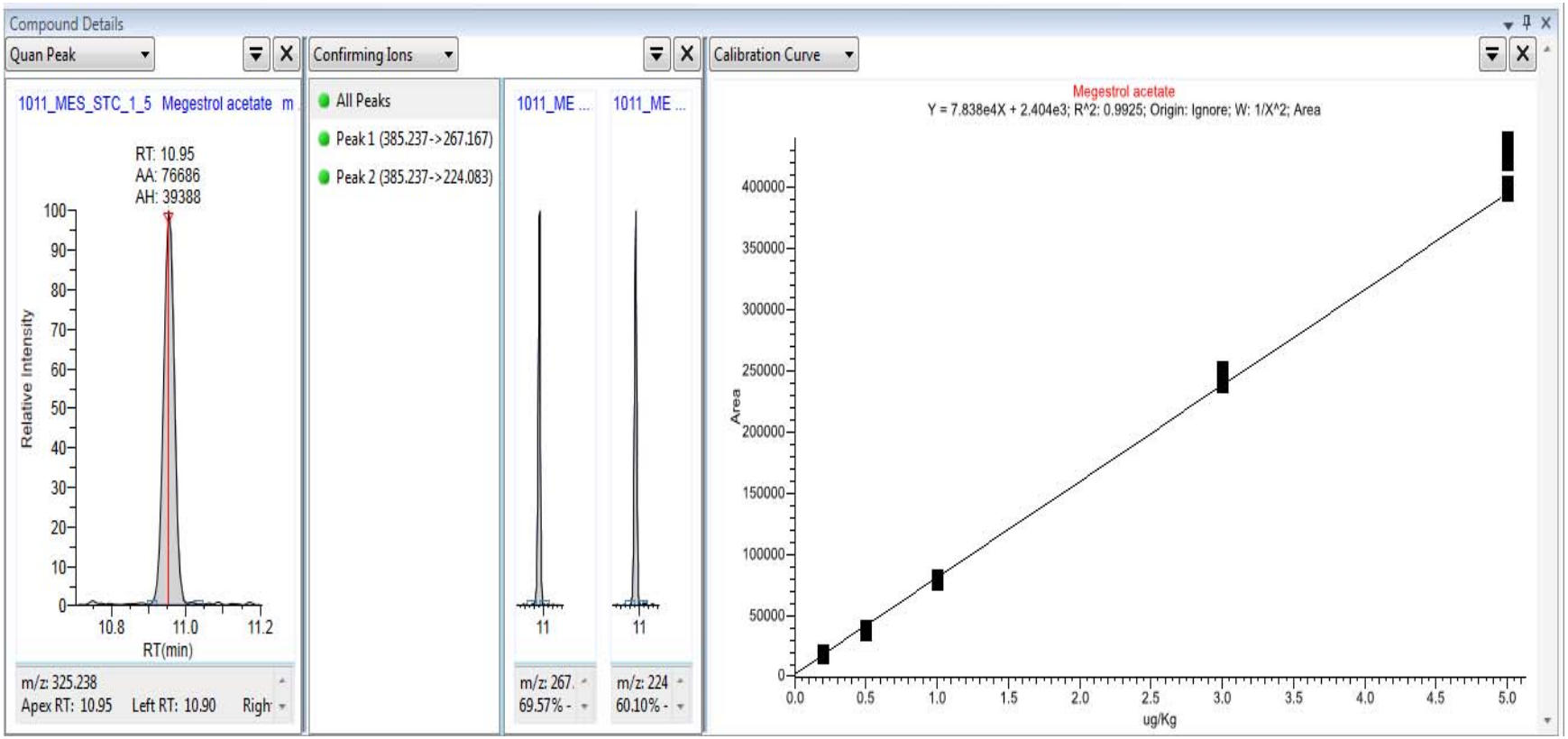
Quantitative Results- 0.2 to 5 x STC-Salmon Fillet



Leucomalachite Green in salmon extract at 1 x STC, with curve representing 0.2-5 ng/g.

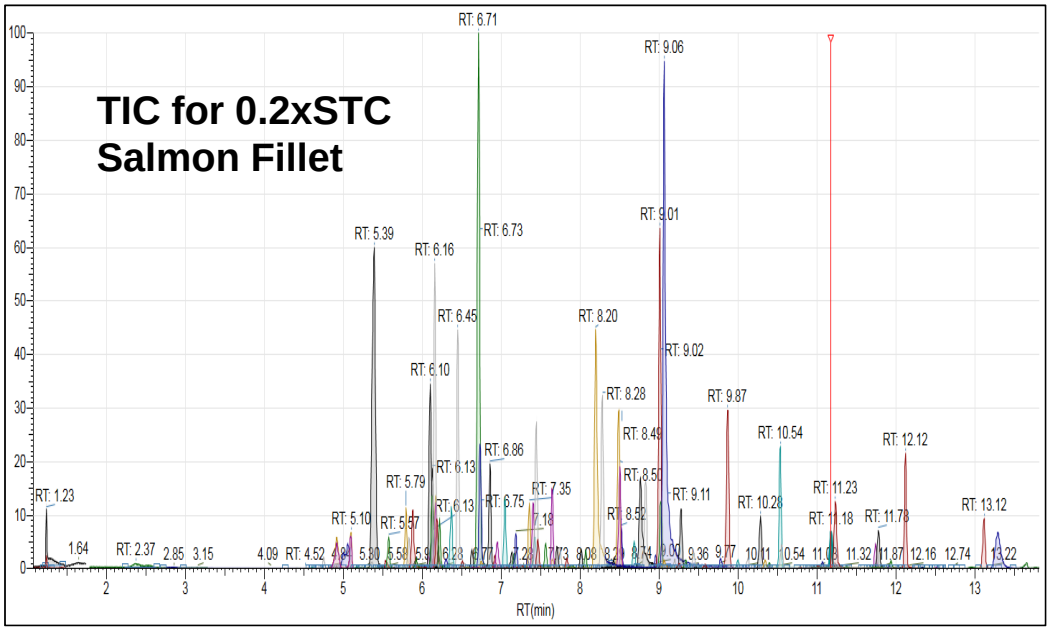
Quantitative Results- 0.2 to 5 x STC-Milk



Steroid hormone Megestrol acetate in milk extract at 1 x STC, with curve representing 0.04-1.0 ng/g

Observed MDLs and % Recoveries in MES

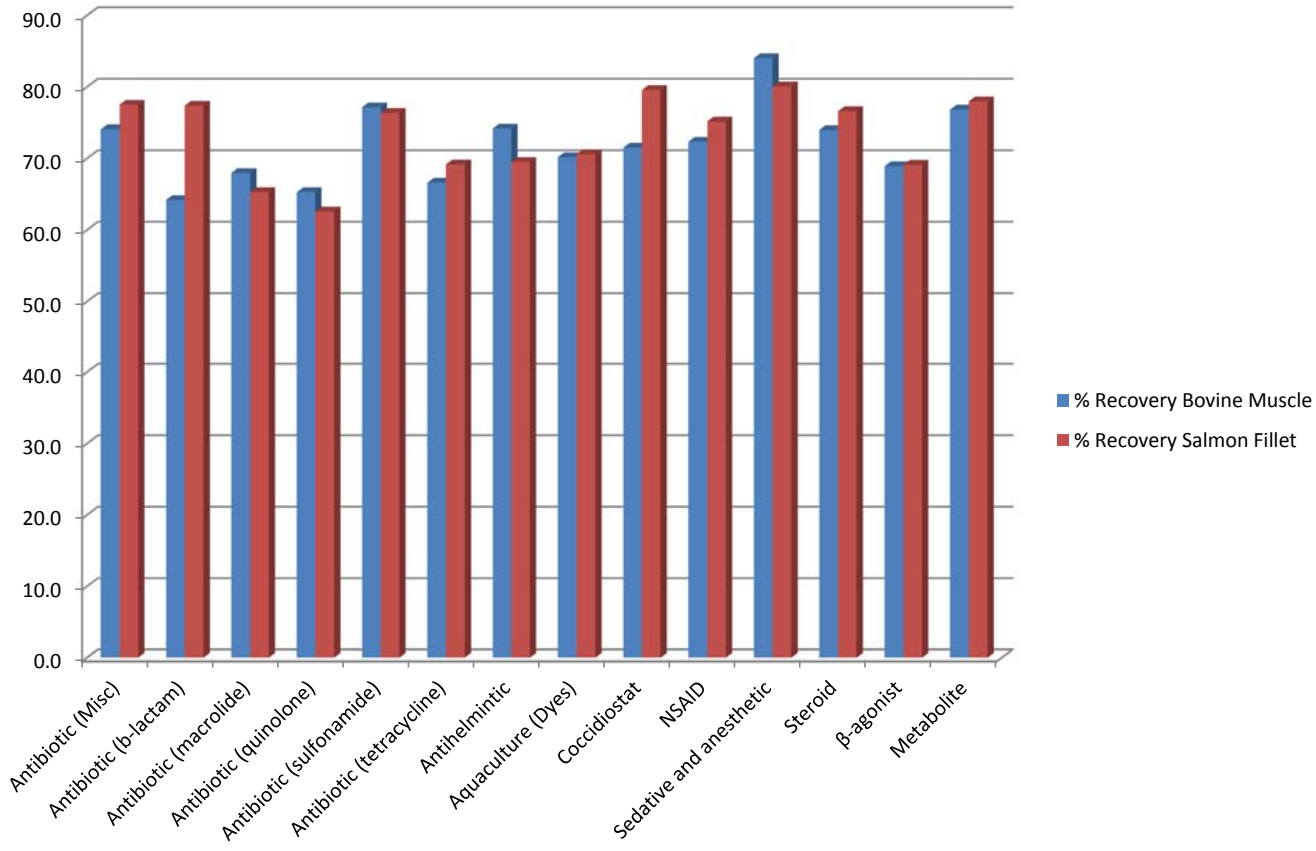
Parameter	Bovine Muscle	Salmon Fillet	Milk*
MDL Average (ng/g)	2.7	3.4	NA
MDL Range (ng/g)	0.01-76	0.01-126	NA
% Recovery-Mean	72.7	73.2	NA
% Recovery Range	39.7-97.5	34.4-101	NA

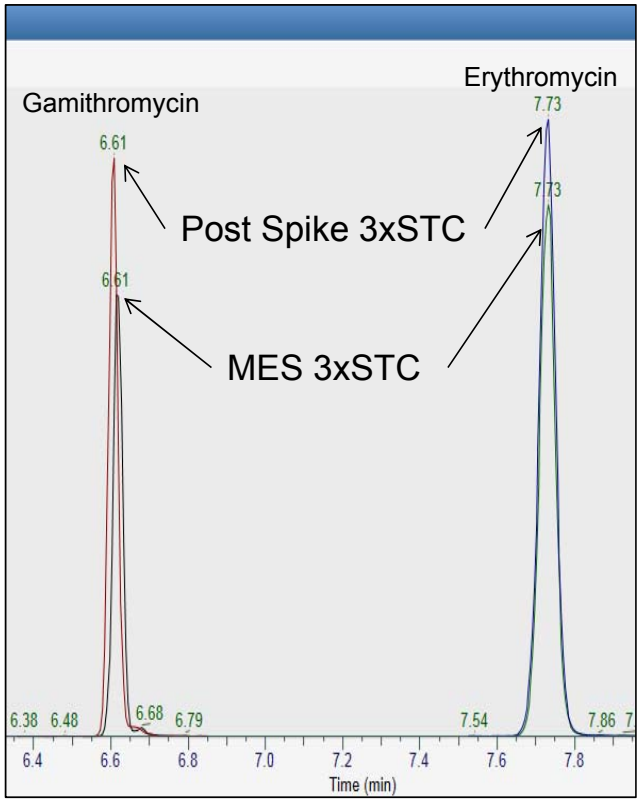


Notes:

- *Milk results pending data reduction
- MDL based on 8 replicate injections (EPA-based Student t calculation)
- Stability of some compounds result in poor precision/higher MDLs, eg. Ampicillin, Penicillin G
- %Recovery is ***absolute recovery*** (no correction) based on comparison with post-spiked MES@ 3xSTC

Compound Class- Average % Recovery (Absolute)





Example comparison of matrix extracted spike vs. post-spike to show absolute recovery from the extraction process

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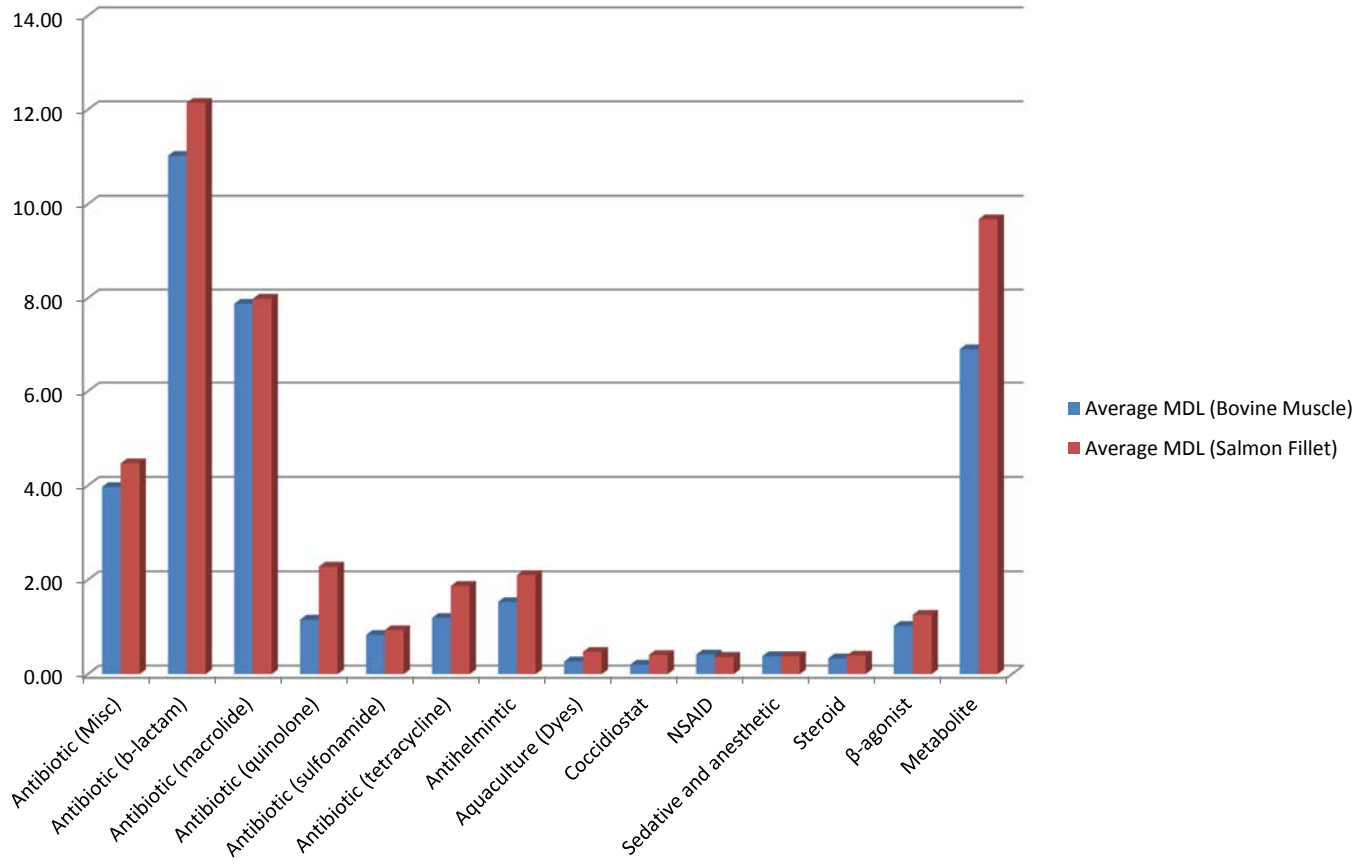
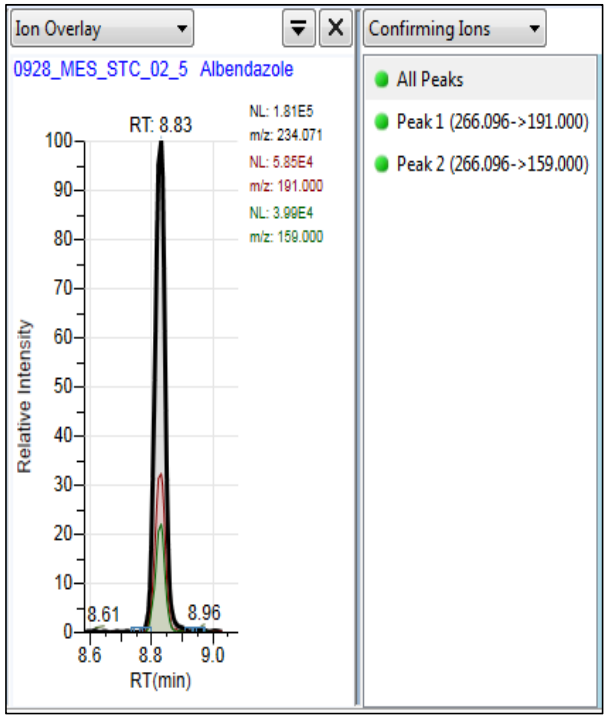
Compound Class- Average Calculated MDL (ng/g)





Example Albendazole in Salmon Fillet
0.2 x STC in Thermo Scientific™
TraceFinder™ software

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Conclusions

- New Thermo Scientific™ TSQ Altis™ and Quantis™ triple quadrupole instruments offer advanced technology and innovative design for robust operation and high sensitivity
- A Multi-class veterinary method has been developed that shows:
  - Fit-for-purpose Acclaim PA2 column for robust analysis, great peak shape for wide range of compound classes
  - Generic QuEChERS extraction applied to bovine, salmon fillet, and milk is easy to use, low cost, with no extract concentration
  - Good results for absolute recovery, precision, and low MDLs for most analytes studied with STC screening range of 0.2 to 5x (Can easily go lower on several analytes)
  - Further optimization of the method on-going with collaborator at Iowa State

Confident Quantitation

Any compound, any matrix, any user.

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