Confident Quantitation

Any compound, any matrix, any user.

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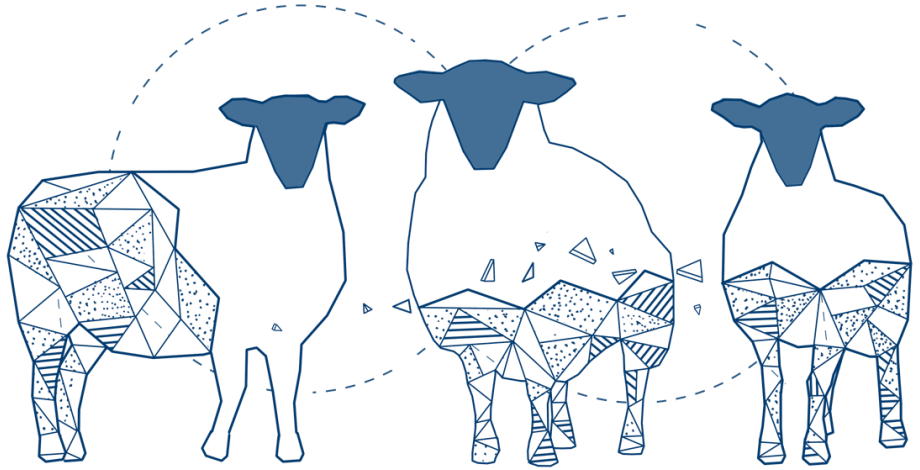
Introduce the next generation technology for veterinary drug residues detection and food authentication

Dr. John Xue

Outline

- Introduction and Challenges
- LC-MS Workflow solutions
- Integration of multi-class analysis into a routine testing lab
- Orbitrap HRAM & Triple Quadrupole for Halal Applications
- Allergen detection and criminal fraud in the Sea food supply chain



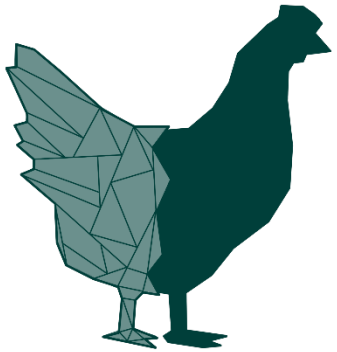
Background to Veterinary Medicines

- What are veterinary medicines?
  - Pharmacologically active compounds which are used to treat and prevent diseases of animals
  - Restore, correct or modify physiological functions by exerting a pharmacological, immunological or metabolic action

- Residues of veterinary medicines in food?
  - Residues or their transformation products can remain in foods after treatment of animals
  - Frequency of residues is very low

- Approvals and usage are highly regulated and monitored

- Concerns over antibiotic resistance from over-use in farming world-wide



Regulation of Veterinary Medicines

- Definition of Maximum Residue Limits (MRLs)
  - Maximum amount of each veterinary medicine that is safely and legally permitted
  - Definition may include metabolites
  - Sub ug/kg to 1000s ug/kg
  - Frequently updated (Commission Regulation 37/2010)

- In the EU specified in Council Regulation 2377/90 (as amended)
  - Annex I - substances for which a full MRL has been fixed
  - Annex II - MRL is not required
  - Annex III - Provisional MRL has been established
  - Annex IV – MRL can not be established because residues of those substances, at whatever limit, constitute a hazard to human health

- United States - US FDA, Code of Federal Regulations, Title 21, Part 556 (21 CFR 556), 2014

EU: Many Compound Classes and Sample Types

Group A:
Substances with anabolic effects
and unauthorised substances

A1: stilbenes and derivatives

A2: Anti thyroid agents; eg. thio uracil

A3: Steroids; eg. boldenone

A4: Resorcyclic acid lactones; eg. Zearalanol

A5 :Beta-agonists; eg. clenbuterol

A6 :Prohibited substances; no MRL eg. chloramphenicol

Group B:
Veterinary drugs and contaminents

B1 : Antibacterials

B2 “other” veterinary drugs
- Anthelmintics (B2a)
- Anticoccidials (B2b)
- Carbamates and pyrethroids (B2c)
- Sedatives (B2d)
- Non-steroidal anti-inflammatory drugs (B2e) eg ibuprofen
- Other pharmacologically active substances (B2f) eg dexamethason

B3: Other substances and environmental contaminants
- Organochlorine compounds including PCBs (B3a)
- Organophosphorus compounds (B3b)
- Chemical elements (B3c)
- Mycotoxins (B3d)
- Dyes (B3e) and
- Others (B3f)

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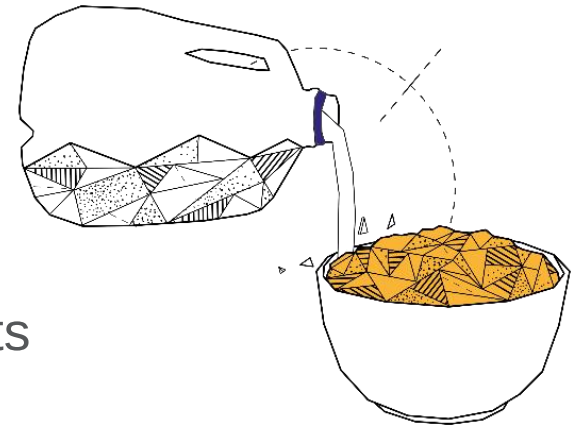
Challenges of Multi-Residue Methods

- 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 preparation must minimize loss of analytes, be simple and cost effective
- Sufficient sensitivity for certain compounds
- Need for polarity switching
- Avoid reporting residue result not actually in sample (False +ve)
- Avoid missing residue result in a sample from not being detected (False -ve)
- Results need to be in compliance with regulations & accreditation requirements

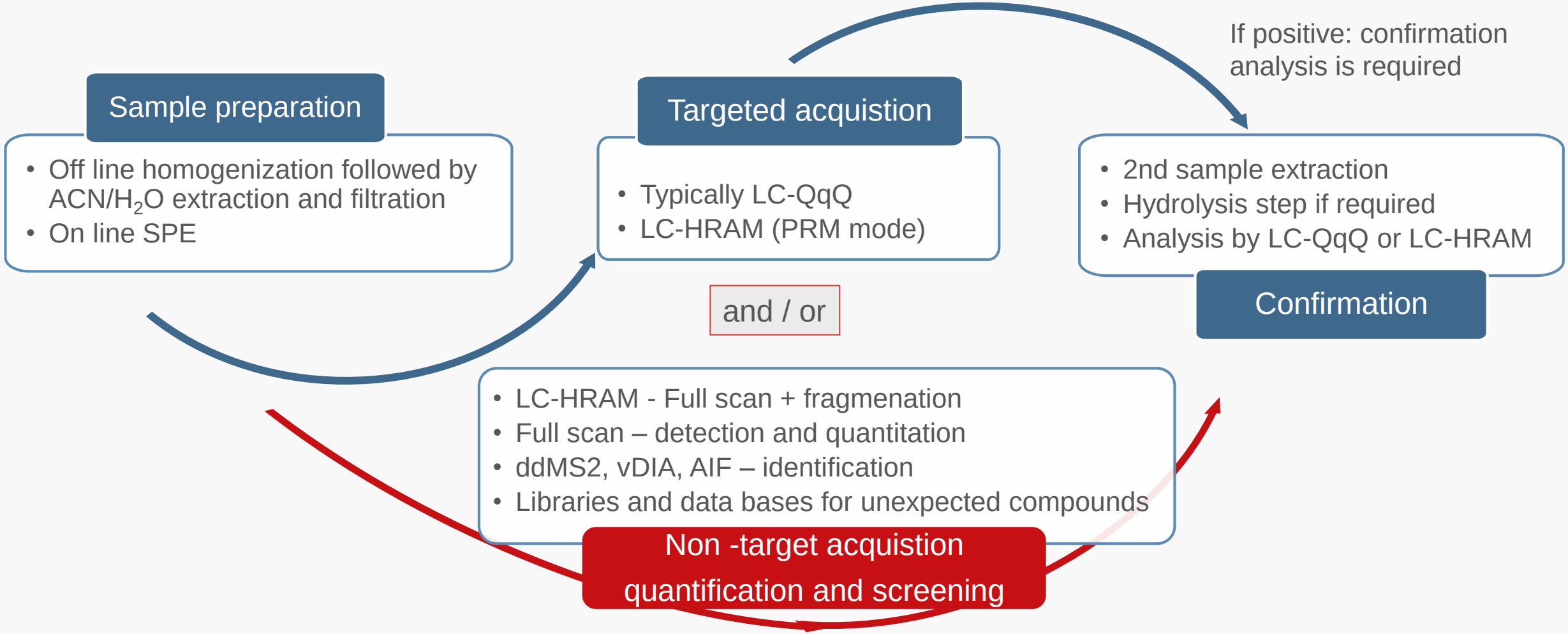


Can we solve these challenges in a single workflow?

Analytical Approaches

- Historically based on class specific methods
- Increase in the use of generic extraction approaches
  - e.g. QuEChERS - based approach - screening as many compounds as possible in a single analysis
  - Broad scope, but less clean-up and lower recoveries for some compounds
- MS based screening approaches accepted in veterinary medicines
  - Validation of screening method based on detectability (CCß)
  - Use of internal standards and matrix-extracted calibrations
  - Low frequency of residues
- In Reality:
  - Many labs use a combination of MS screening and class specific methods

LC-MS Workflow Solutions



- Identification of residues by HRMS is included in the latest US FDA guidelines, but not current EU regulations

vDIA is not available in the U.S.

Confident Quantitation

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

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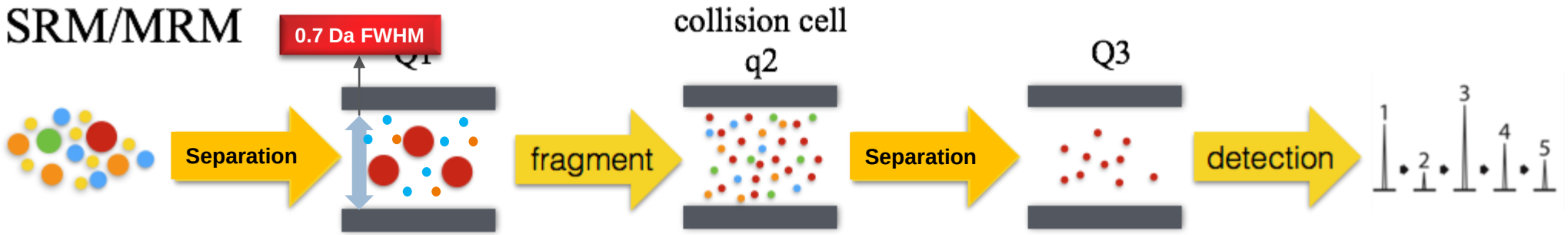
Critical Challenges in Targeted Quantitation

Barriers & Challenges

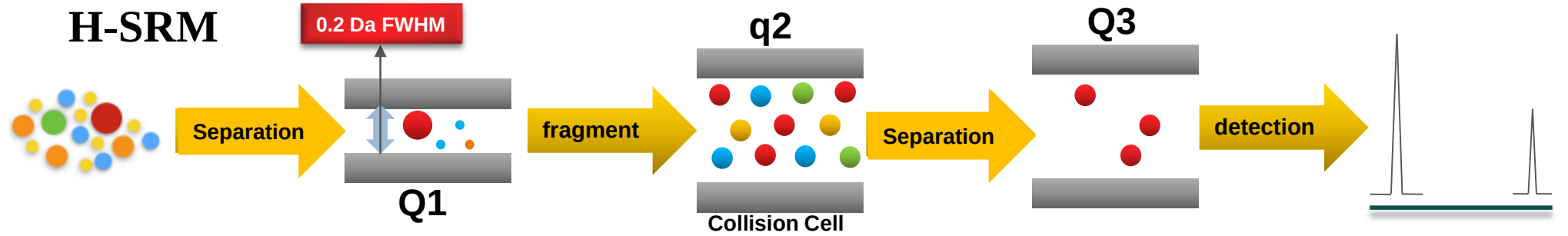
- **Information**
  - Beneficial to have sufficient knowledge about molecule of interest
- **Cost/Sample**
  - Address constant demand to reduce cost/sample
- **Transition to LC-MS/MS**
  - Reduce barriers to using LC-MS/MS
  - Develop robust, reliable, sensitive workflows
- **Right platform for right application**
  - Address critical issues pertaining to choosing the right MS platform
- **New technology/vendor/instrument**
  - Enable transition
- **Protect Investment**
  - Future proofing investment

Types of Quantitation: Leverage the Best

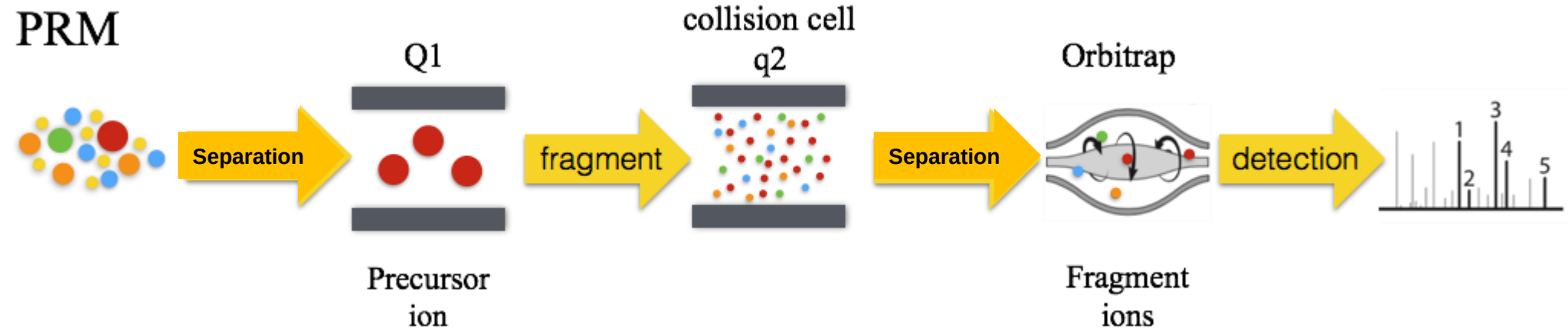
Nominal Mass for Typical targeted quantitation



Nominal Mass high resolution SRM allowing additional selectivity with sensitivity



High Resolution Accurate Mass for targeted quantitation



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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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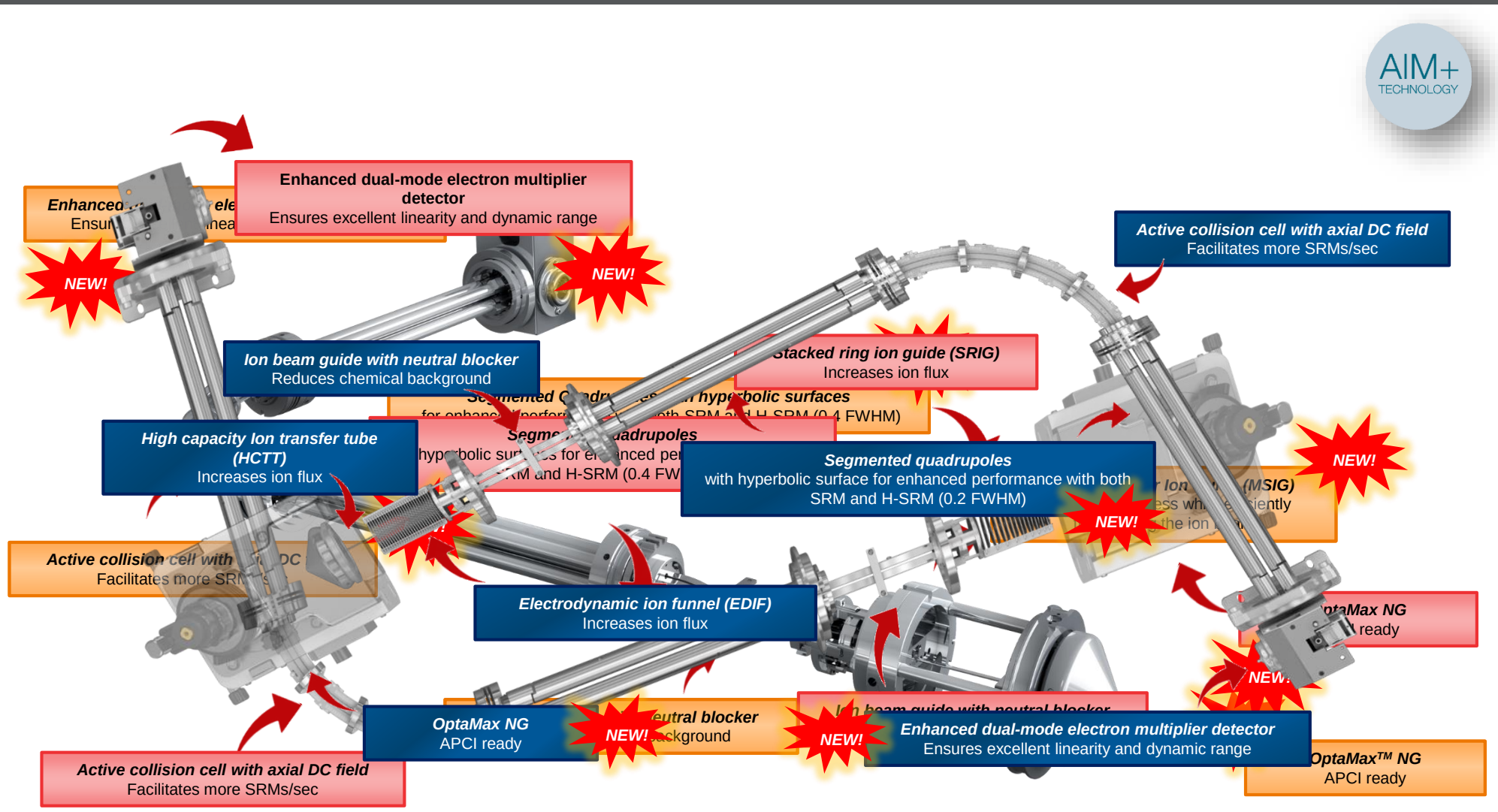
TSQ Fortis Triple Quadrupole MS: Affordable Productivity, For Everyone



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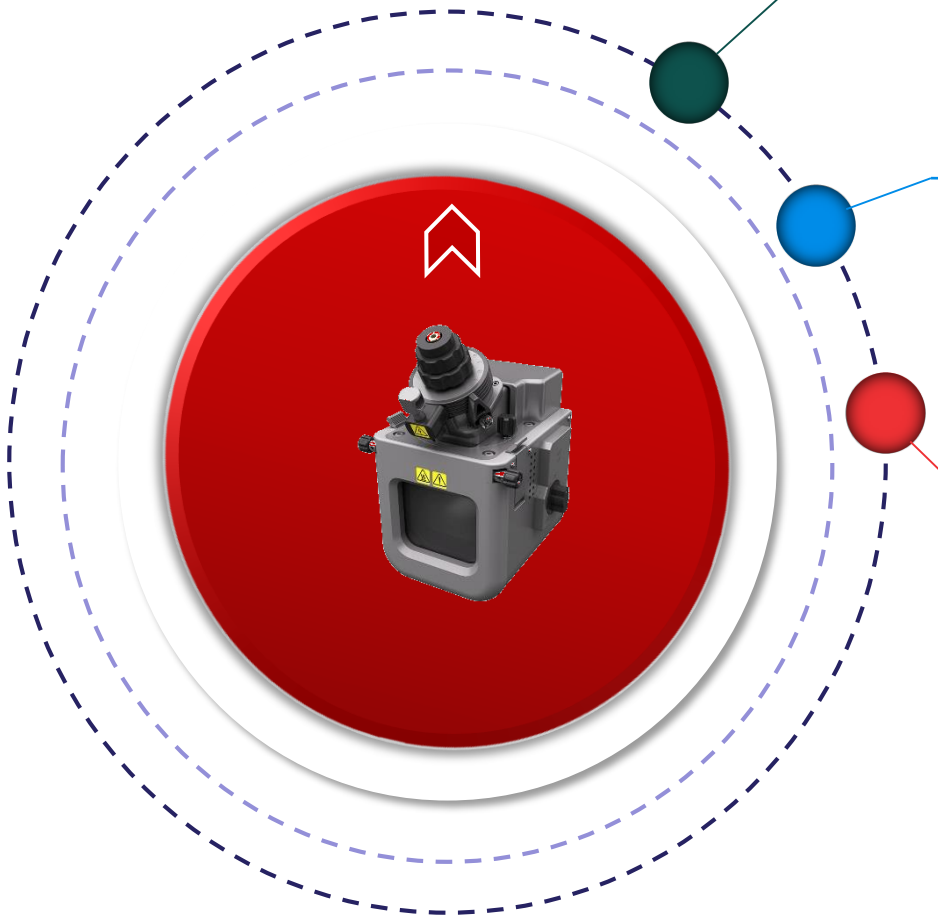
OptaMax NG Source Housing

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

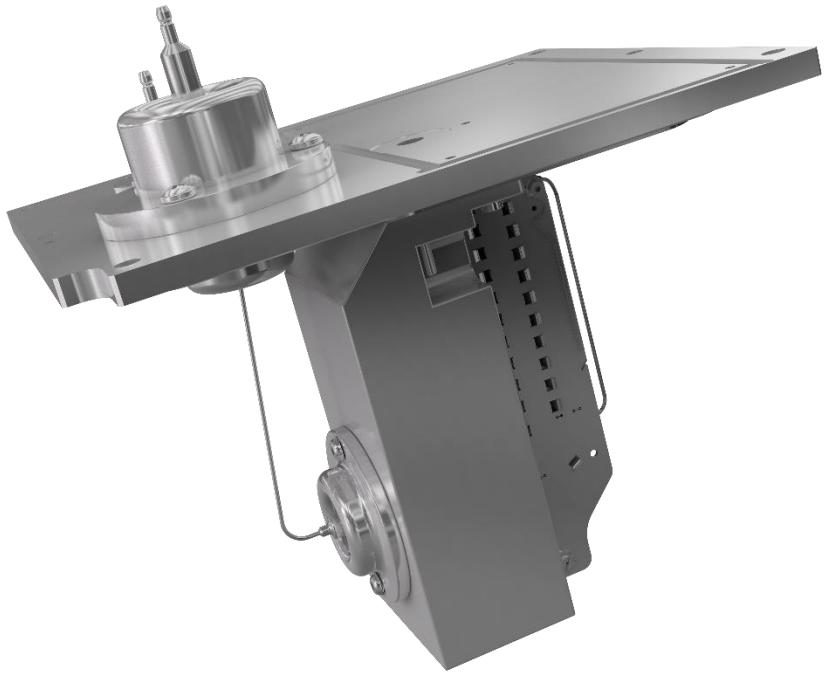
- The use of RF only pre-filters (segments) between the entrance lens and the quadrupole minimizes the effects of fringe fields, leading to improved transmission (and therefore sensitivity) at unit and higher resolution.

- 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.



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1 An Introduction to the TSQ portfolio

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

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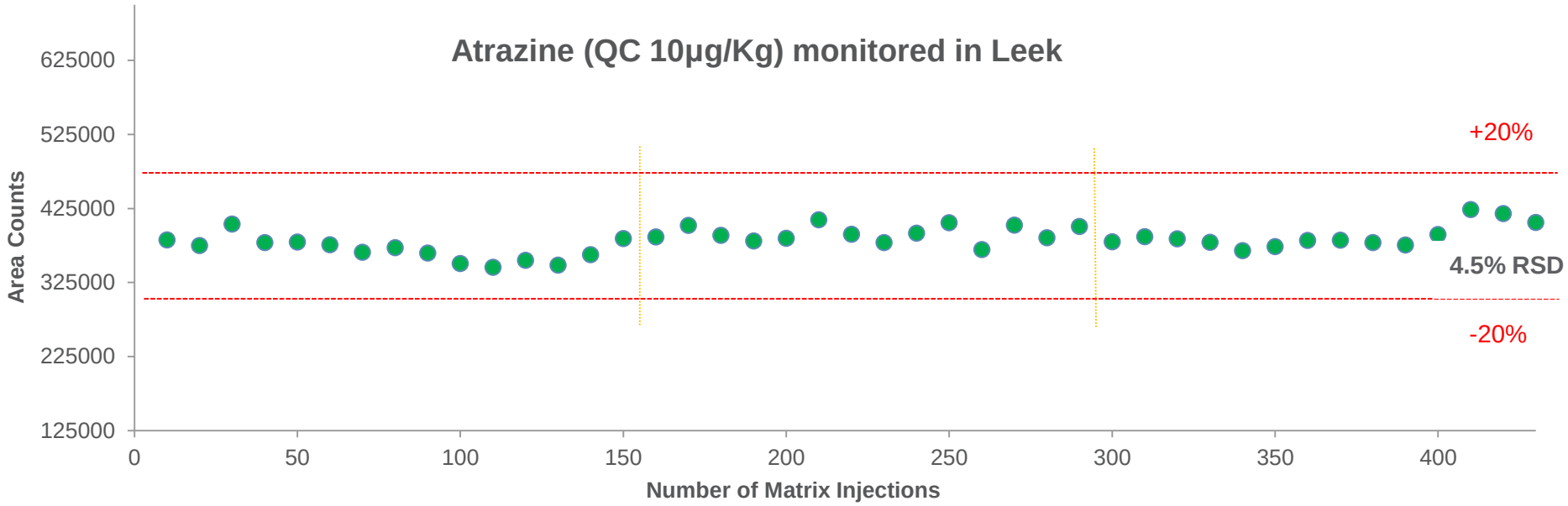
TSQ Quantis: Demonstration of Robustness – Food Safety

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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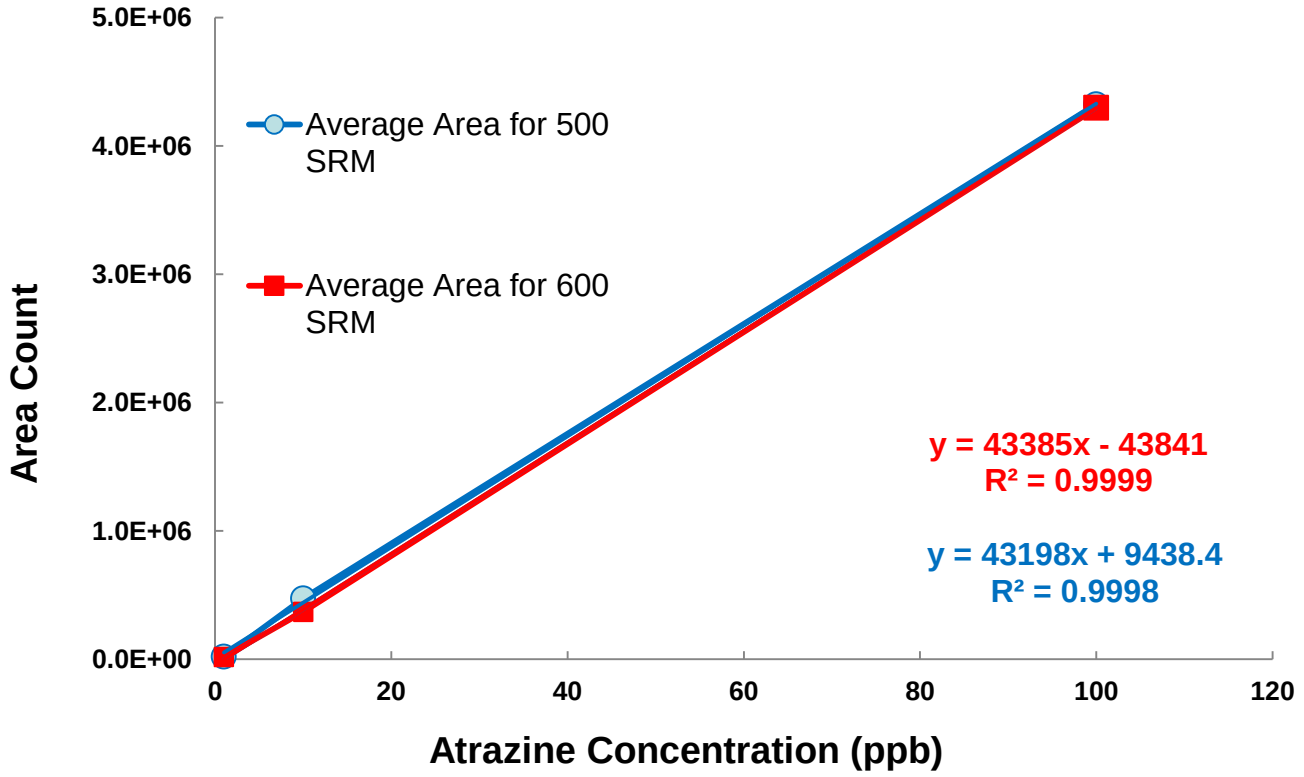
Performance at the extreme – 500 SRMs/sec vs 600 SRMs/sec

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



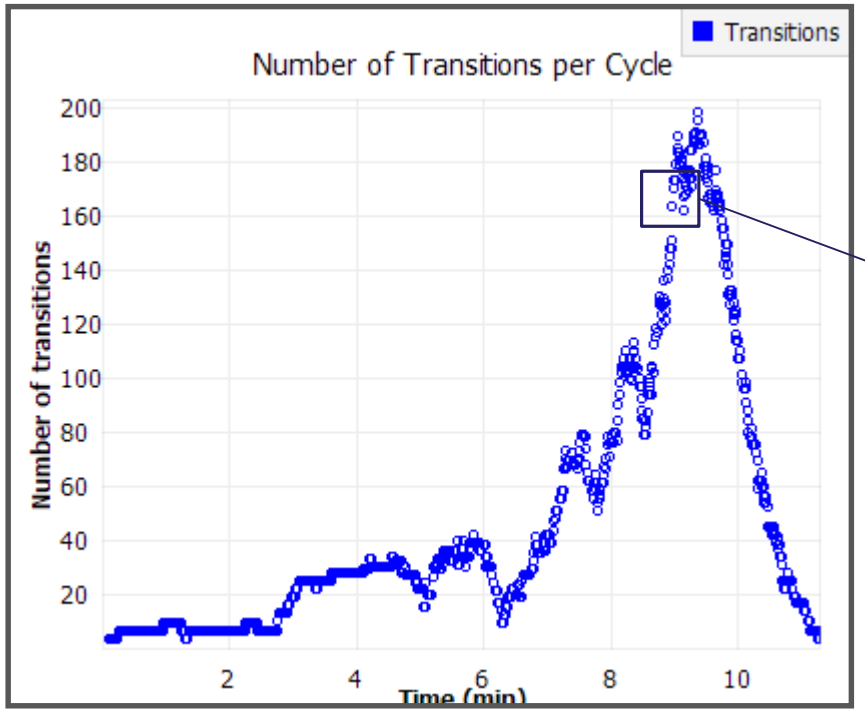
SRMs/Sec	Total Number of Transitions	Dwell Time (mSec)
500	1075	0.769
600	1291	0.437

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

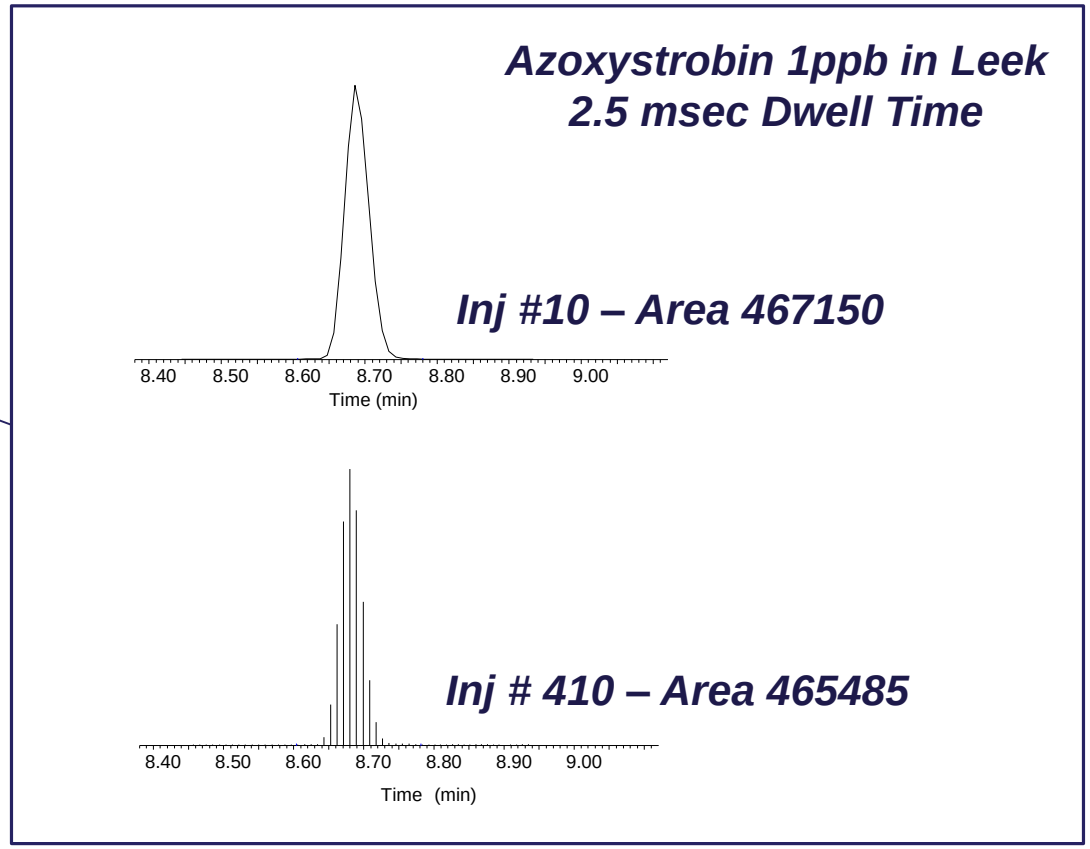
TP 387 - Application of high speed TSQ MS with a prototype RF/DC rod driver to pesticide analysis

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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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

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Compounds Studied and Chemical Classes

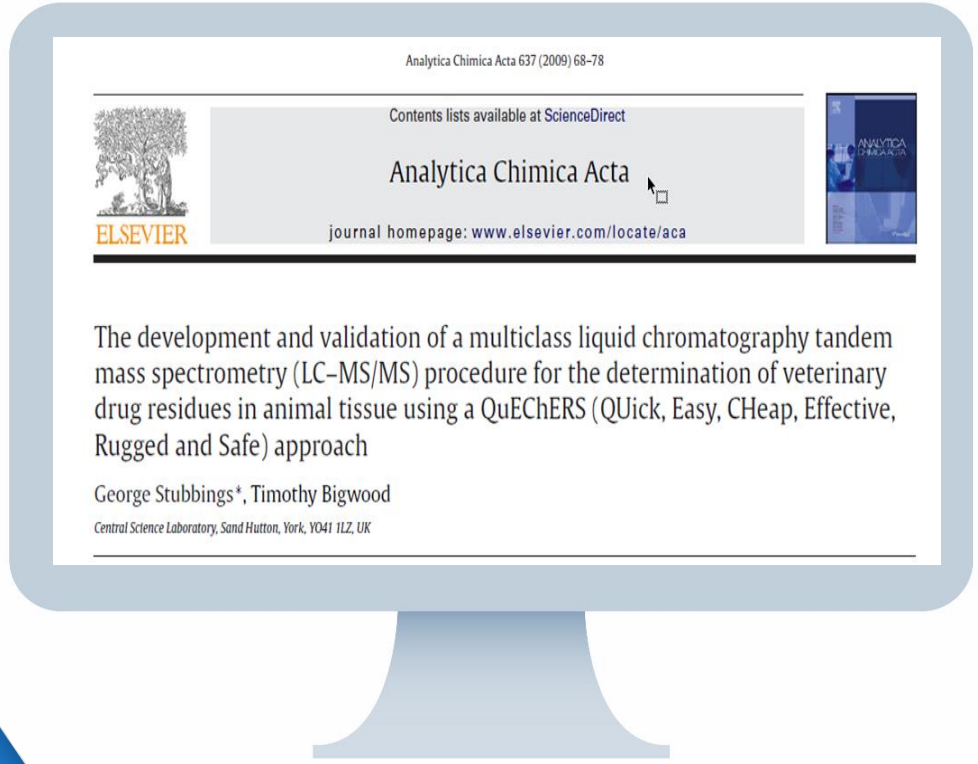
- Antibiotics-68
- β-agonist-11
- Coccidiostat-17
- NSAID-13
- Aquaculture (Dyes and metabolites)-12
- Antihelmintic-23
- Steroids-9
- Other-23

Sample Preparation and LC Conditions

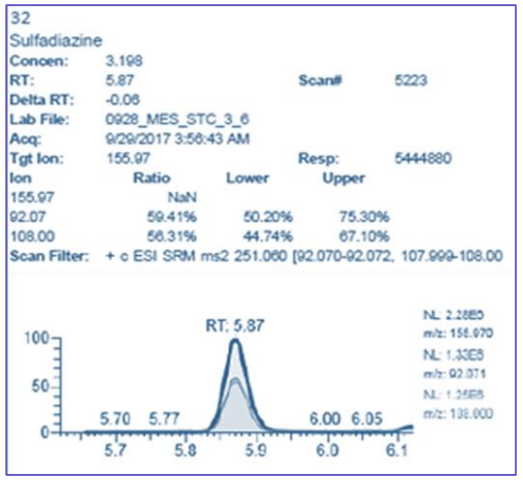
- **QuEChERS based approach**
  - EDTA/NH_4 oxalate solution and acetonitrile
  - Sample homogenised until fully dispersed
  - Sodium sulphate added before centrifugation
  - Dispersive SPE (CEC-C_{18}) clean-up
  - Add 1 mL H2O to 3mL extract, filter, inject

- **LC conditions**
  - Thermo Scientific™ 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

- **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

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

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

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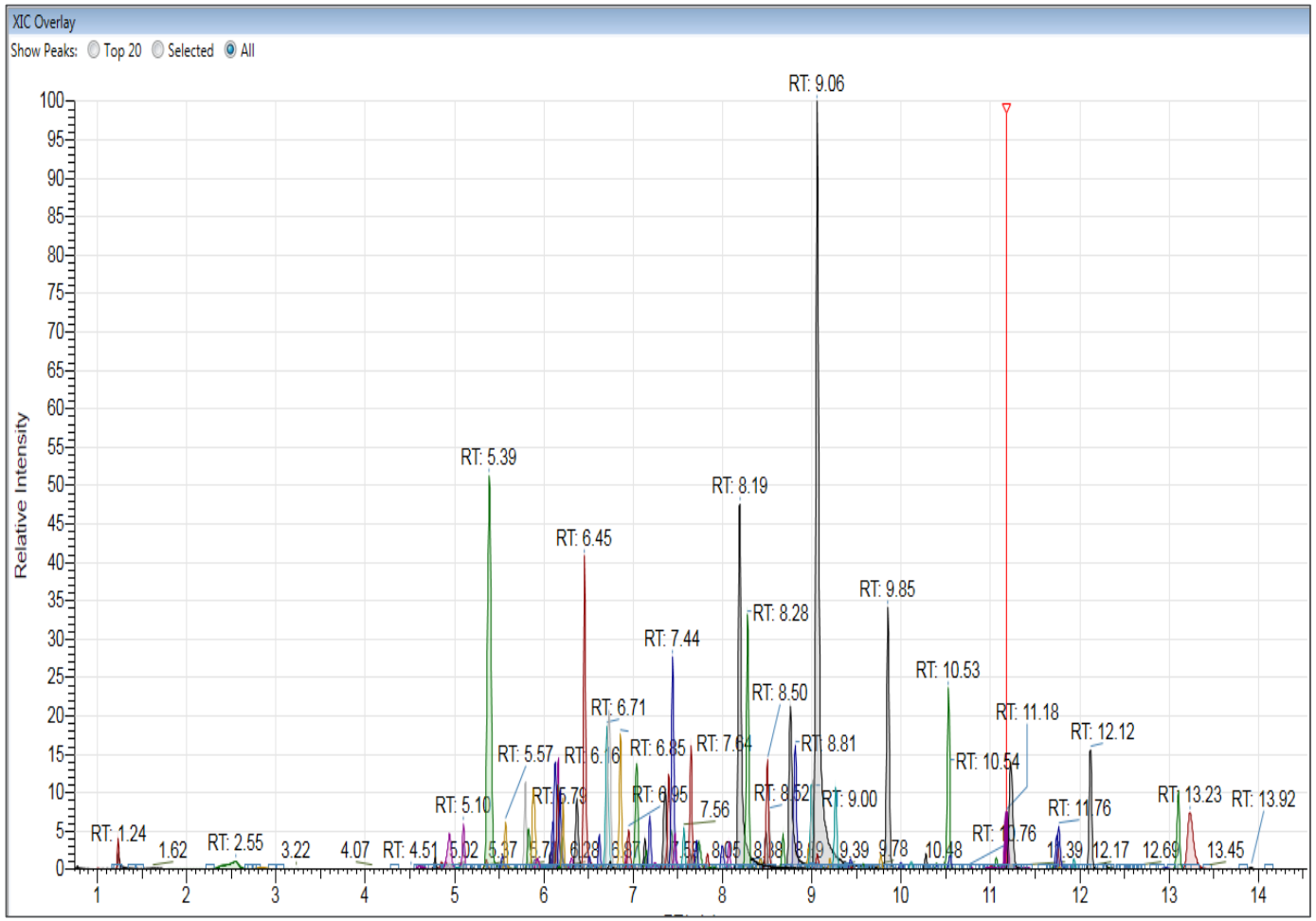
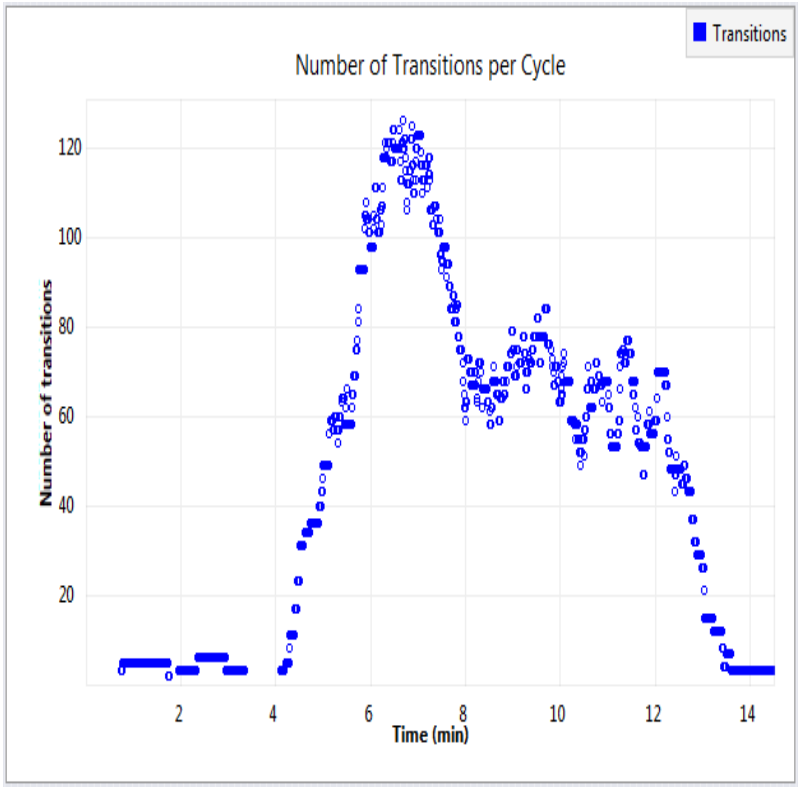
Extracted SRMs for Multi-Class VetDrugs

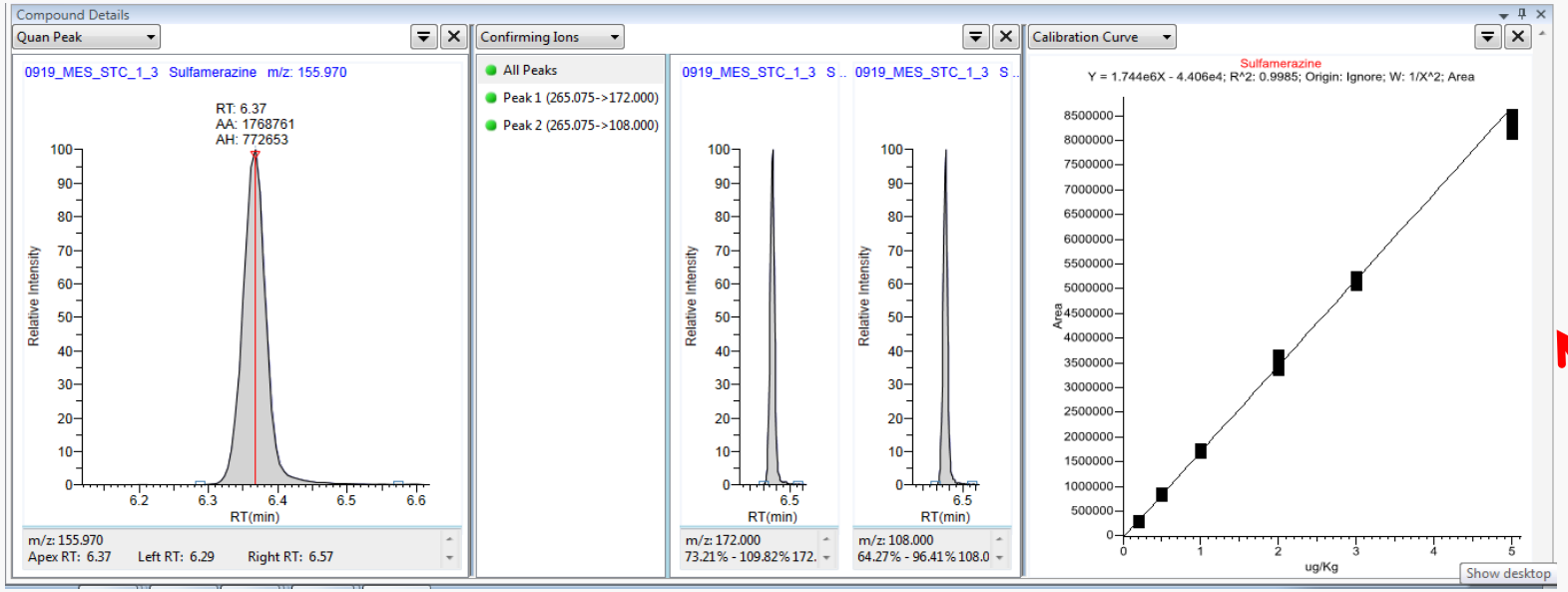


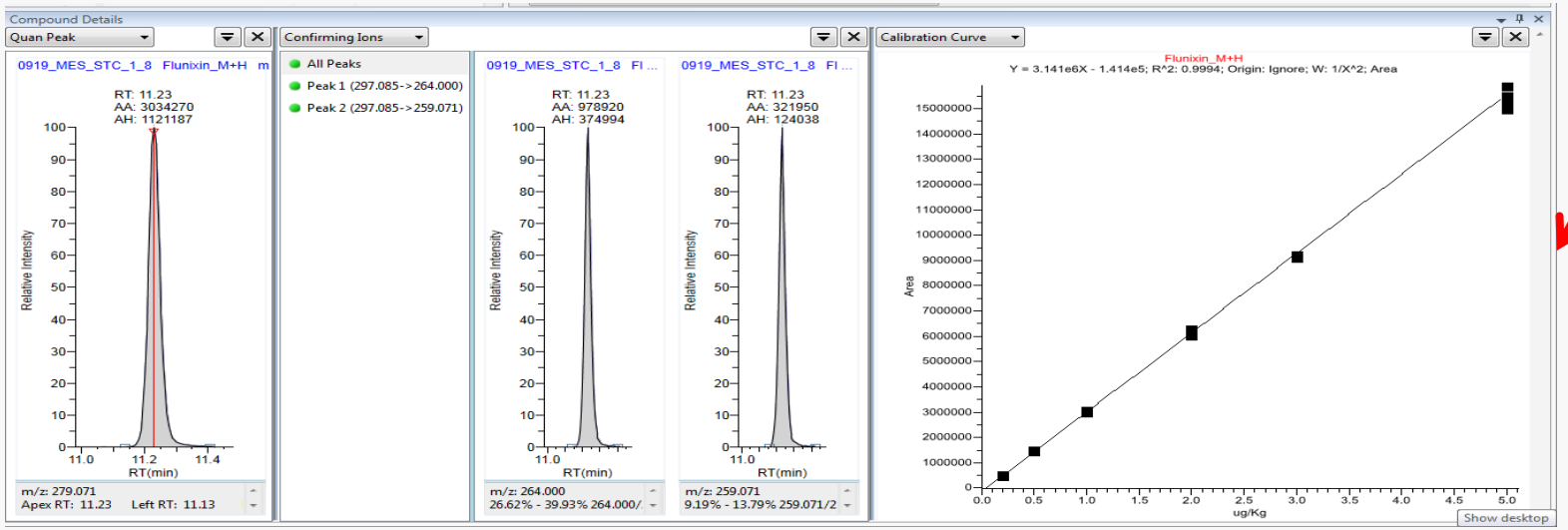
Extracted SRMs at 0.5 x STC in MES



TSQ Altis- total of 525 transitions from analysis at left

Quantitative Results- 0.2 to 5 x STC- Bovine





Sulfa Drugs- Sulfamerazine

8 replicates plotted per each point

NSAIDs- Flunixin

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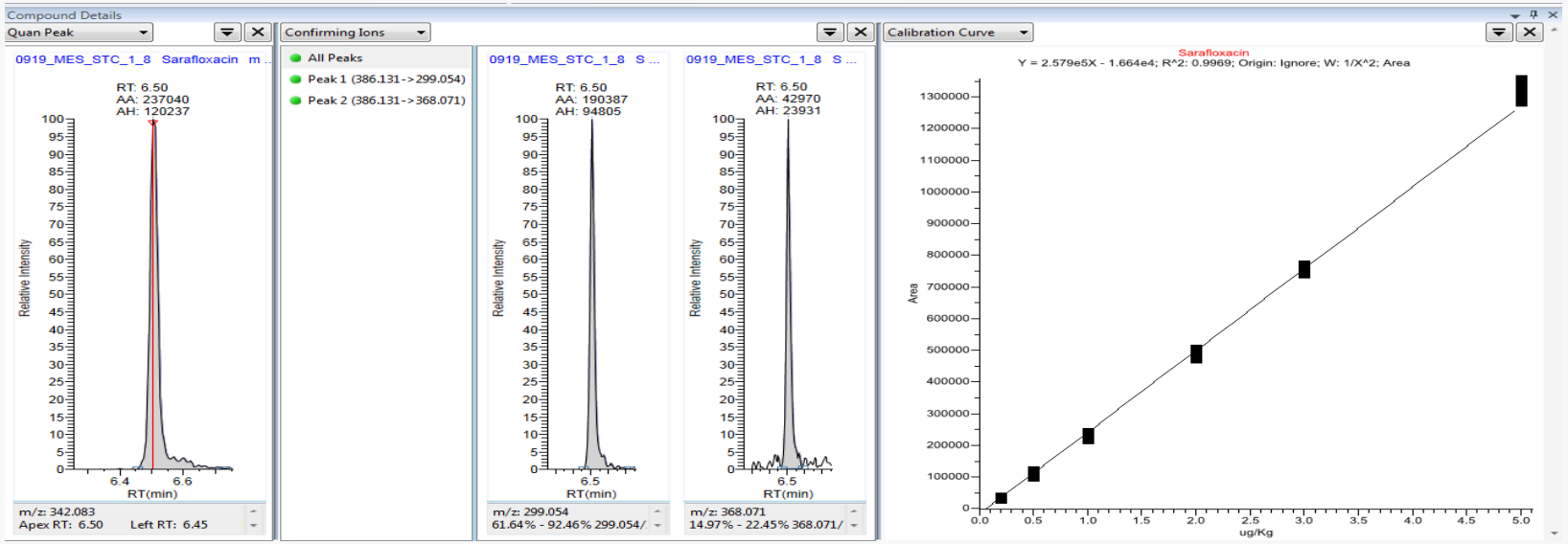
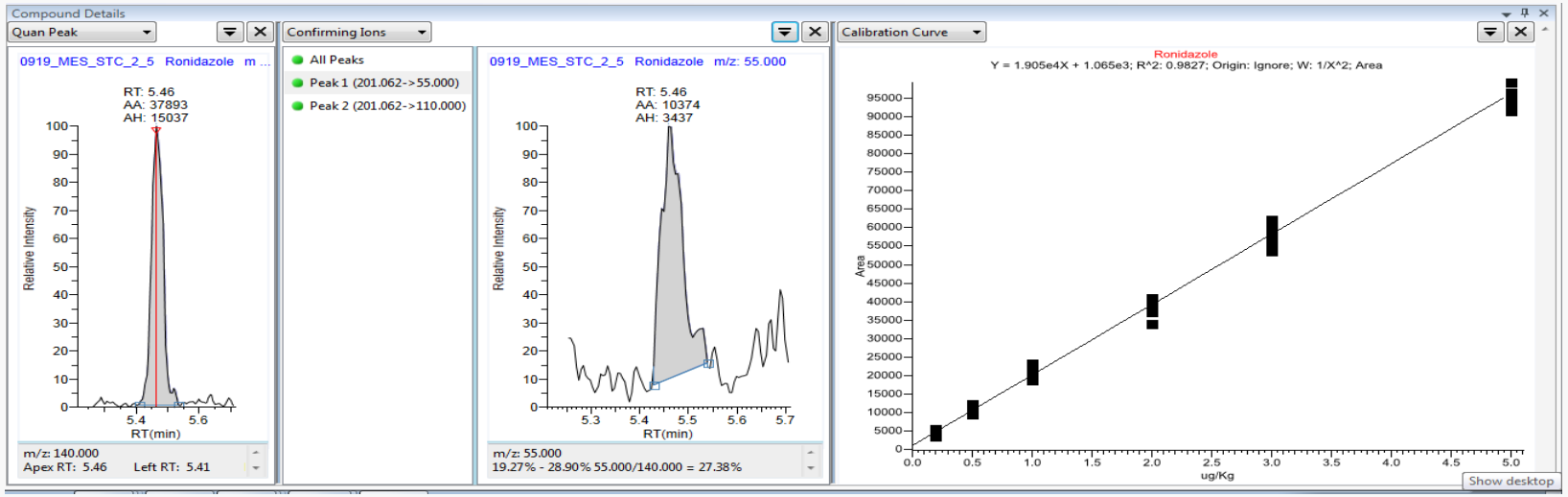
Quantitative Results- 0.2 to 5 x STC- Bovine



Quinolones-Sarafloxacin



Nitroimidazoles - Ronidazole

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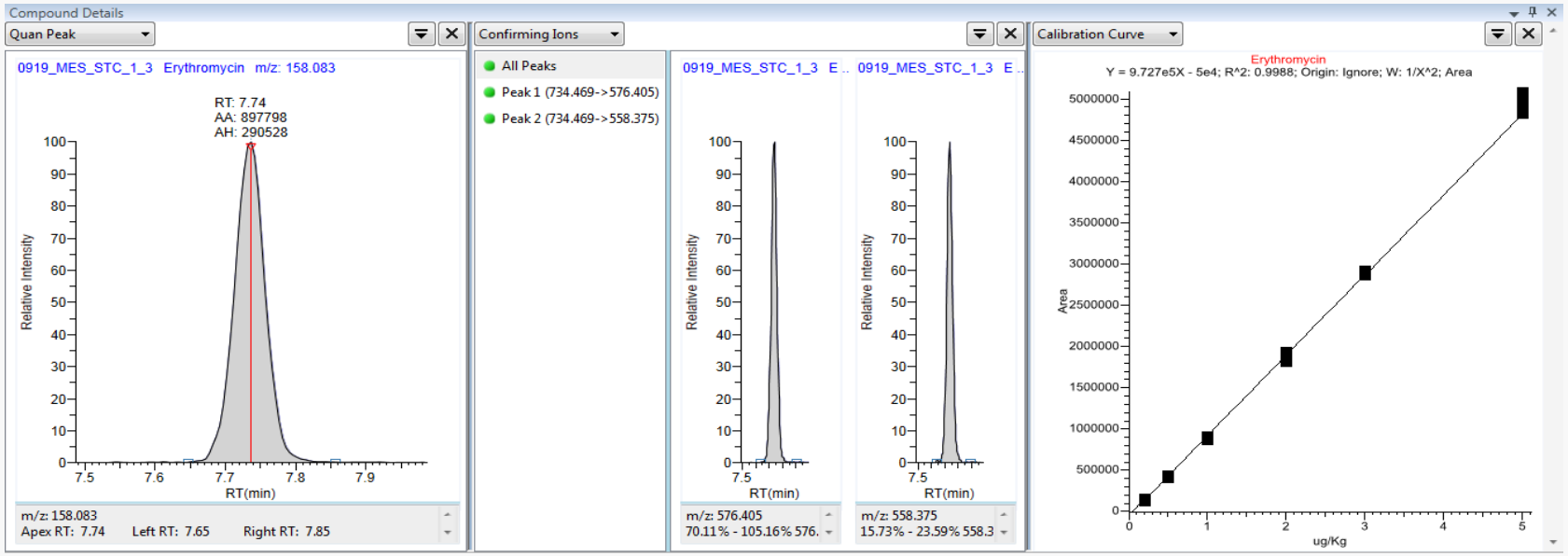
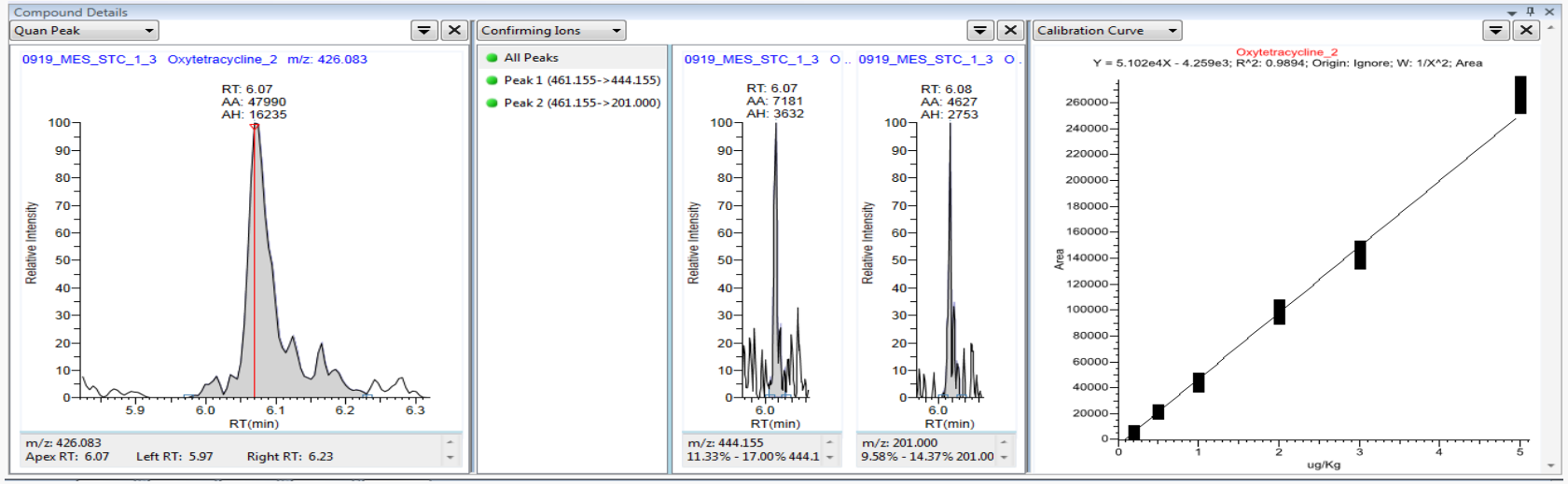
Quantitative Results- 0.2 to 5 x STC-Bovine



Antibiotics-Erythromycin



Antibiotics-Oxytetracycline

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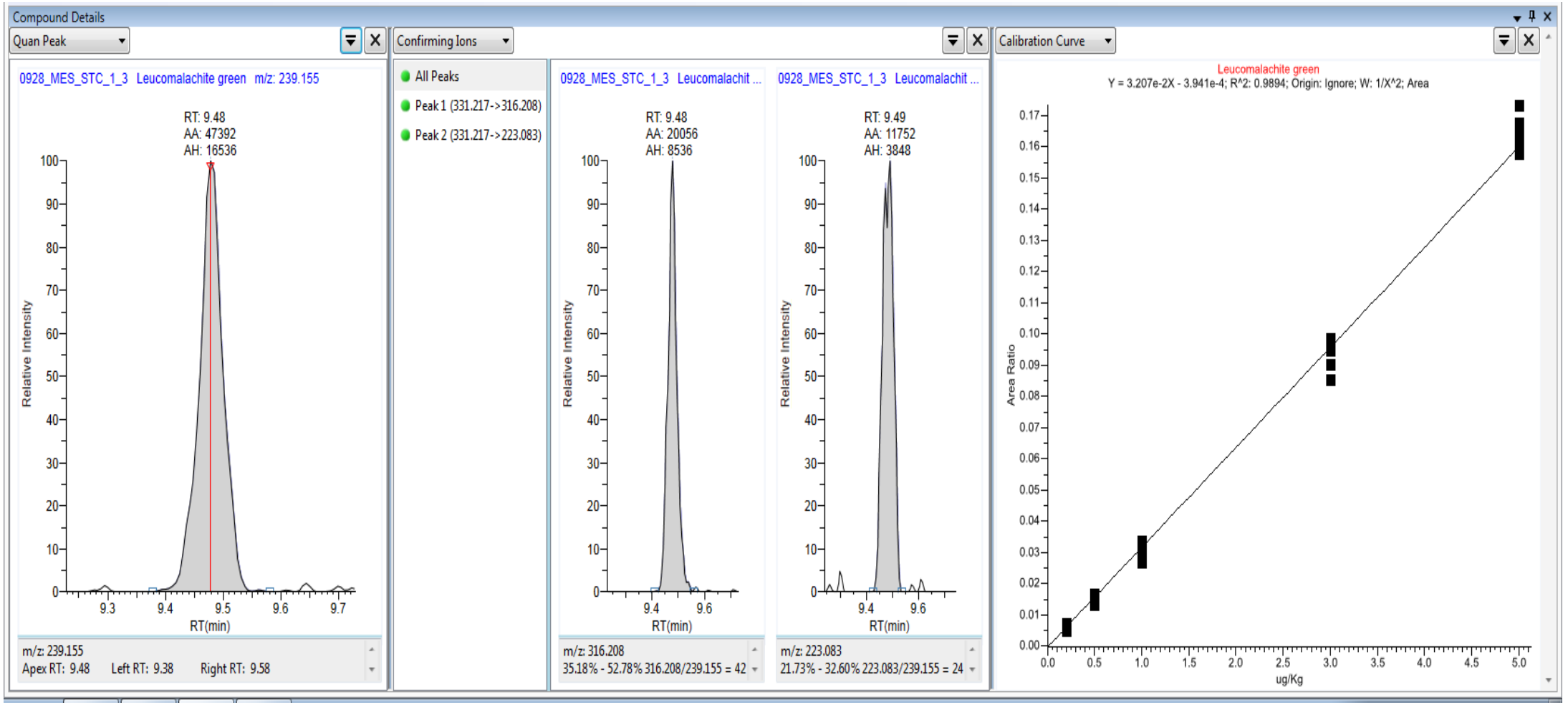
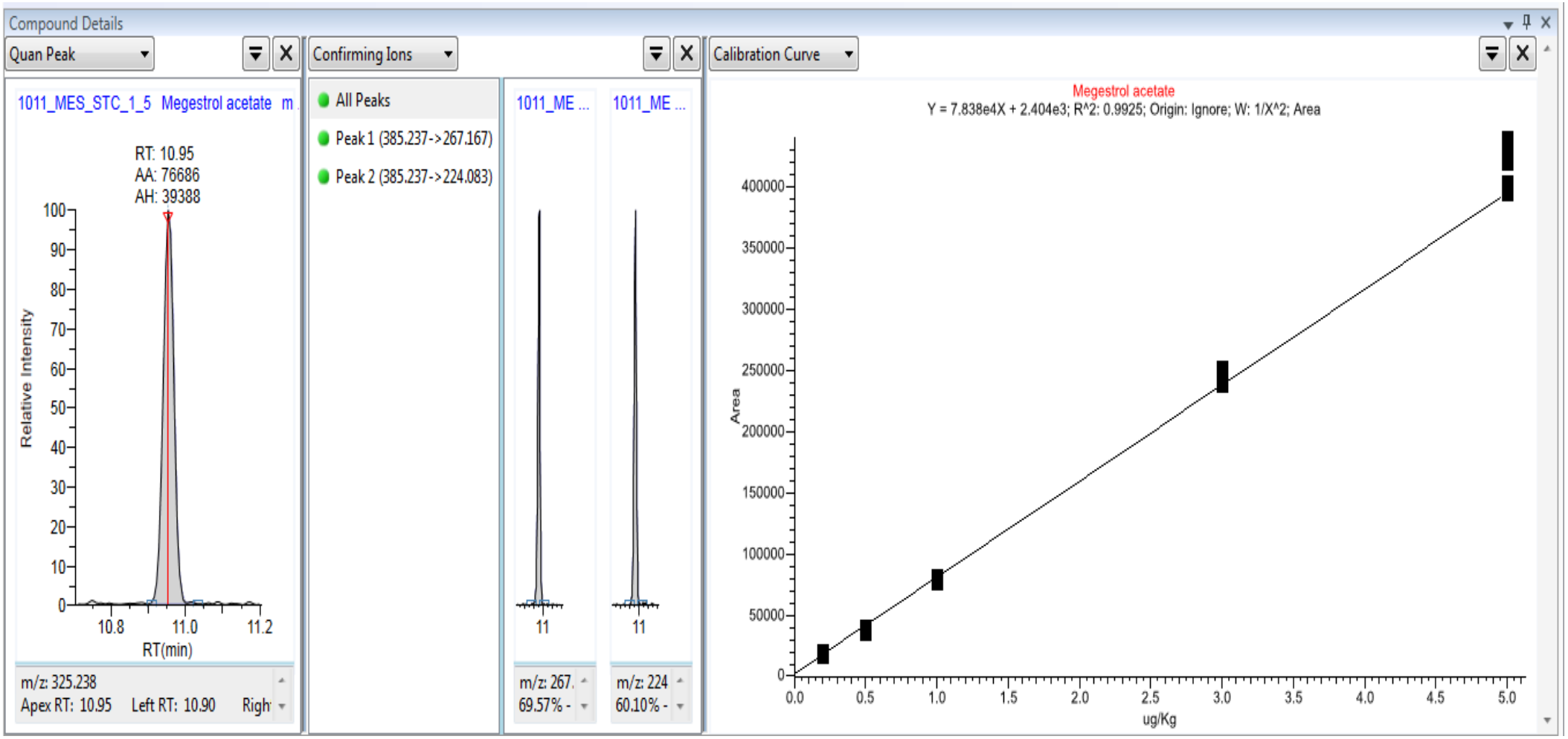
Quantitative Results- 0.2 to 5 x STC-Salmon Fillet



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

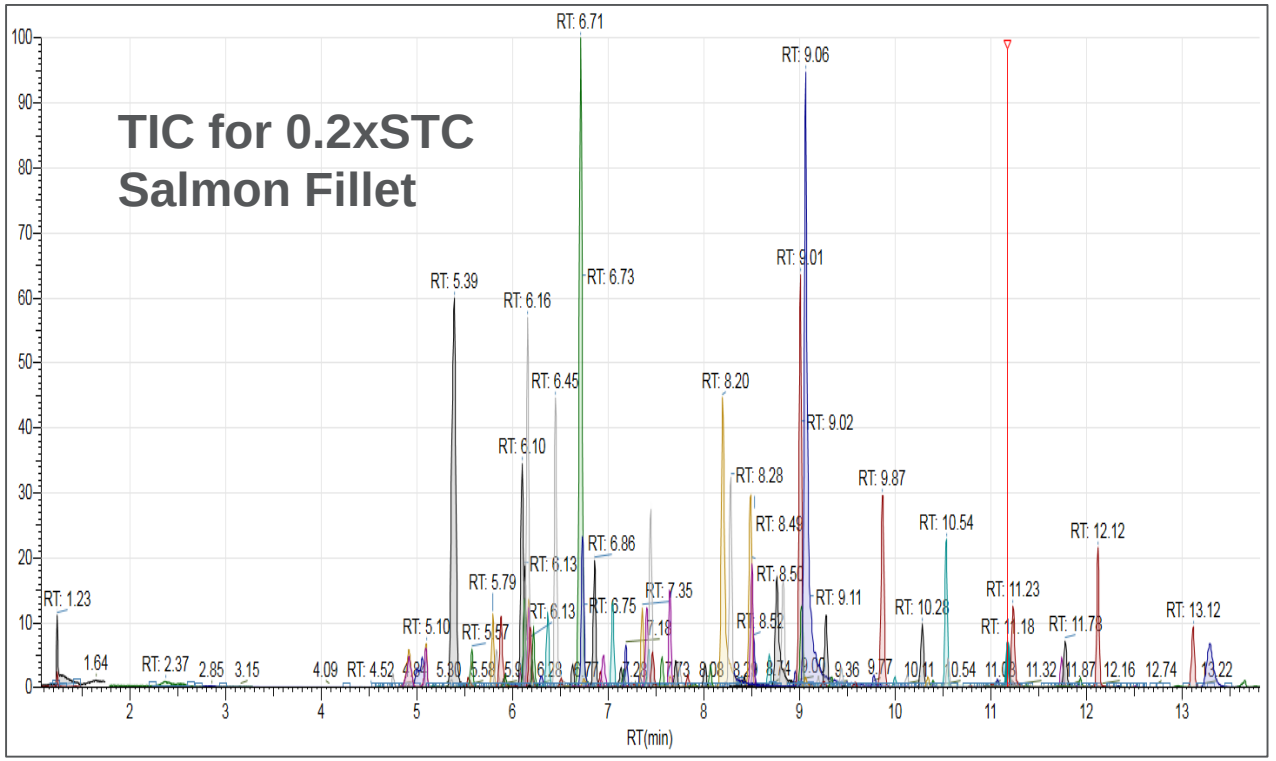
Quantitative Results- 0.2 to 5 x STC-Milk



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

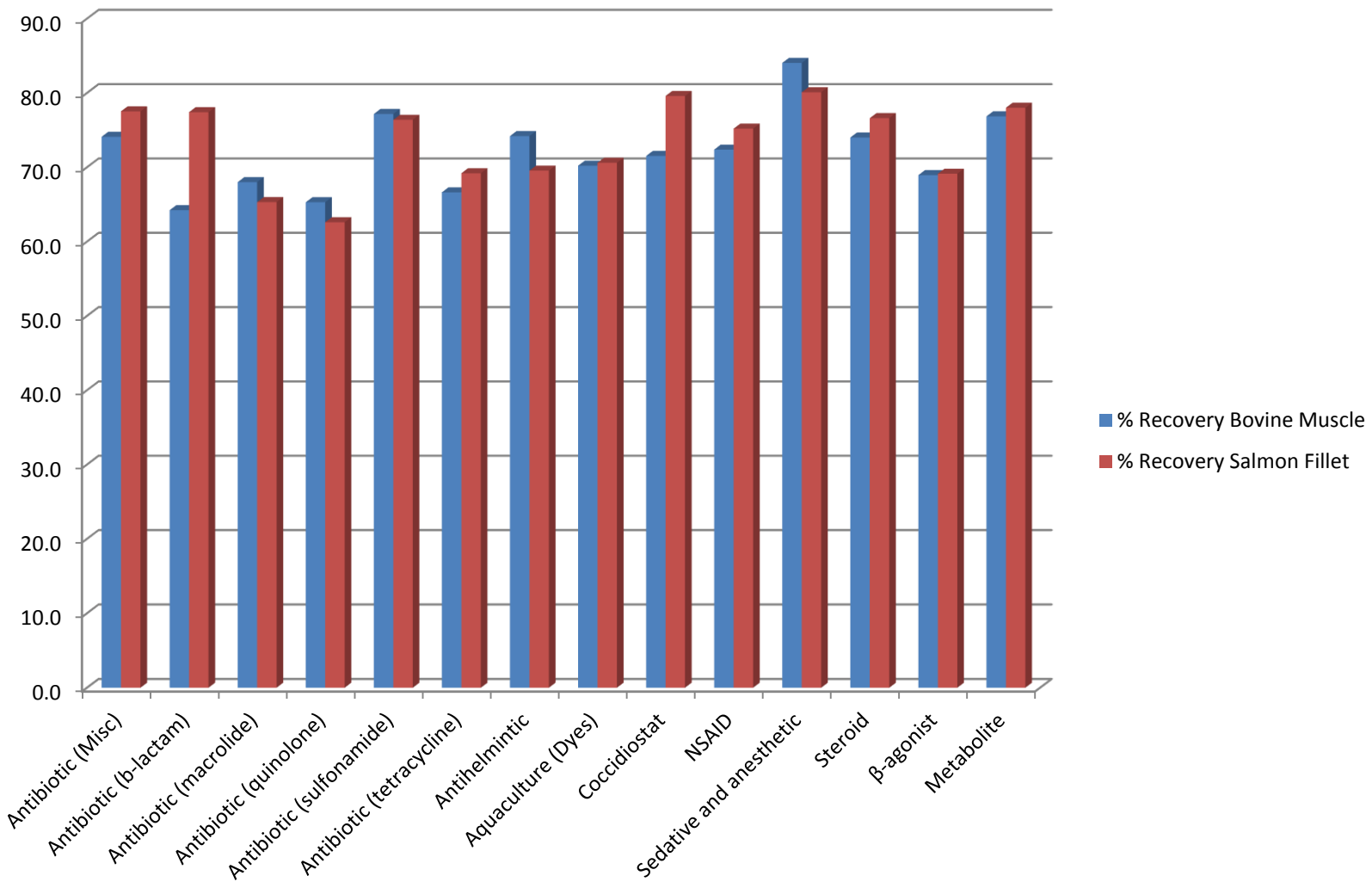
Parameter	Bovine Muscle	Salmon Fillet	Milk*
MDL Average (ng/g)	2.7	3.4	NA
MDL Range (ng/g)	0.01-76	0.01-126	NA
% Recovery-Mean	72.7	73.2	NA
% Recovery Range	39.7-97.5	34.4-101	NA

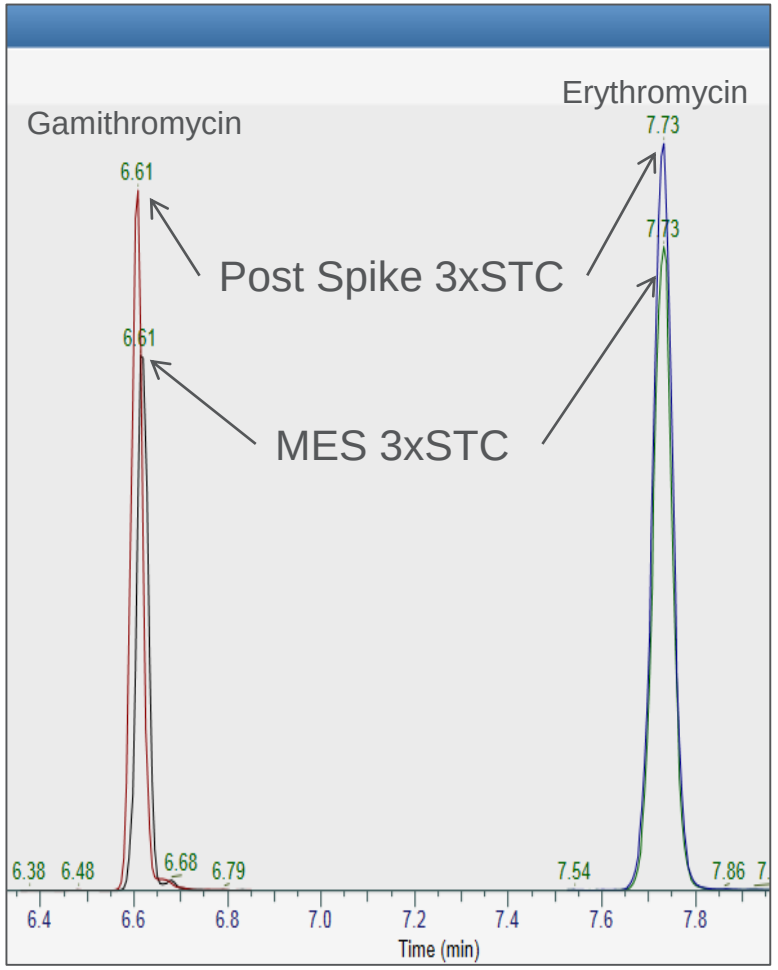


Notes:

- *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

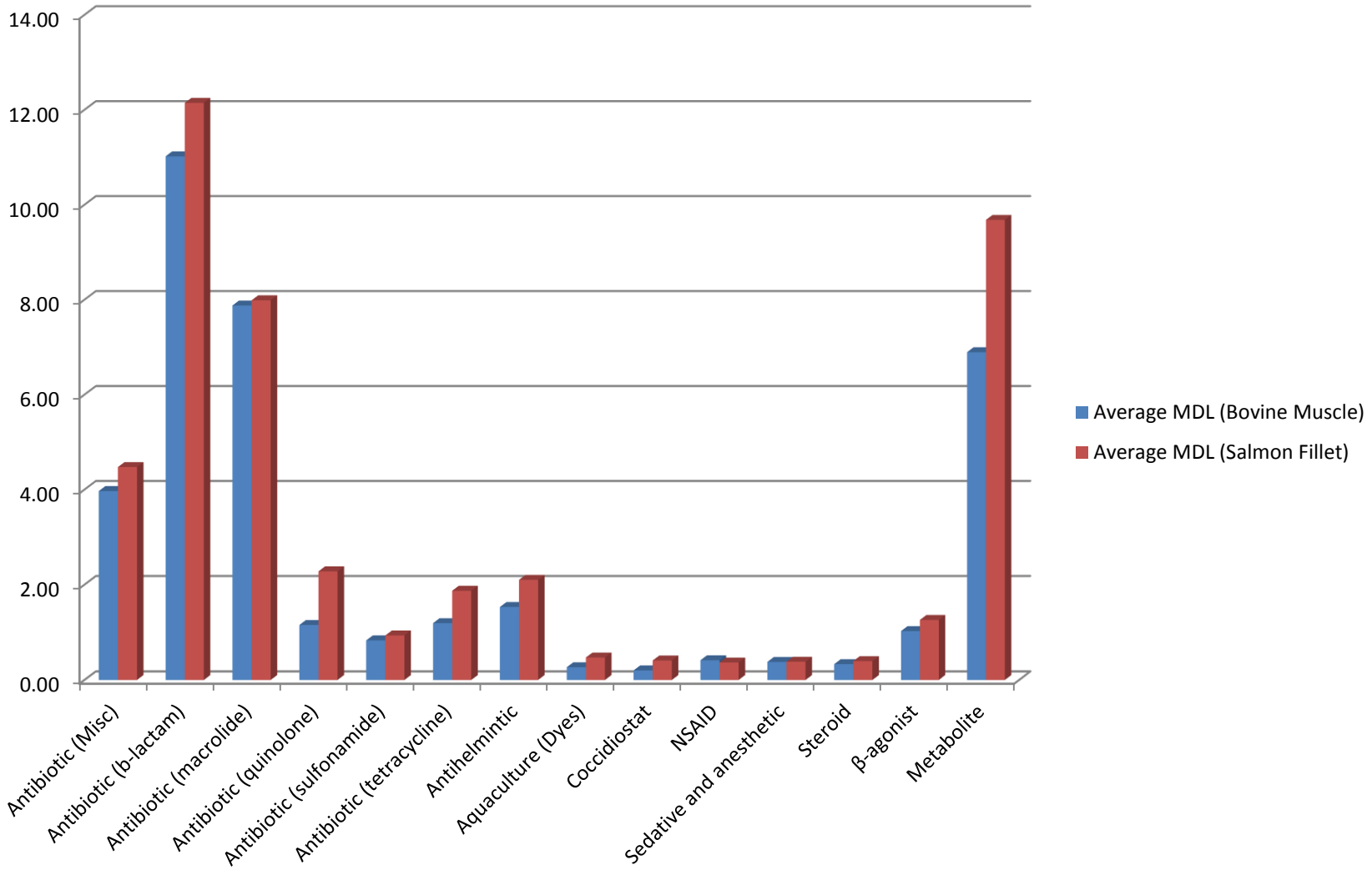
Compound Class- Average % Recovery (Absolute)

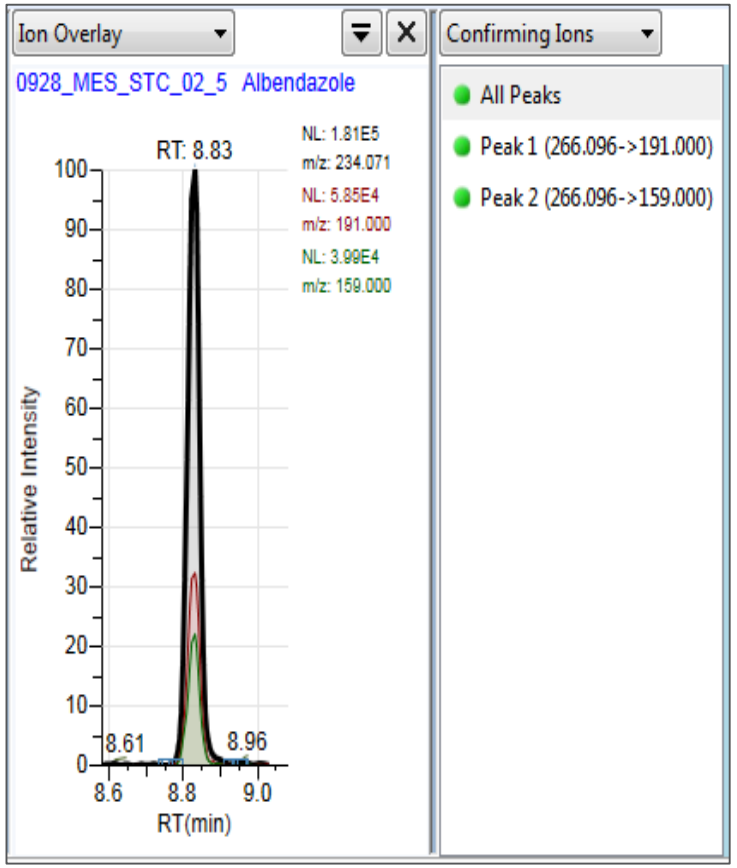




Example comparison of matrix extracted spike vs. post-spike to show absolute recovery from the extraction process

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



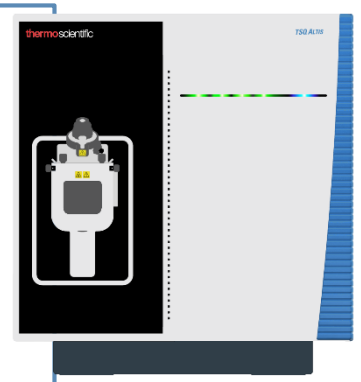


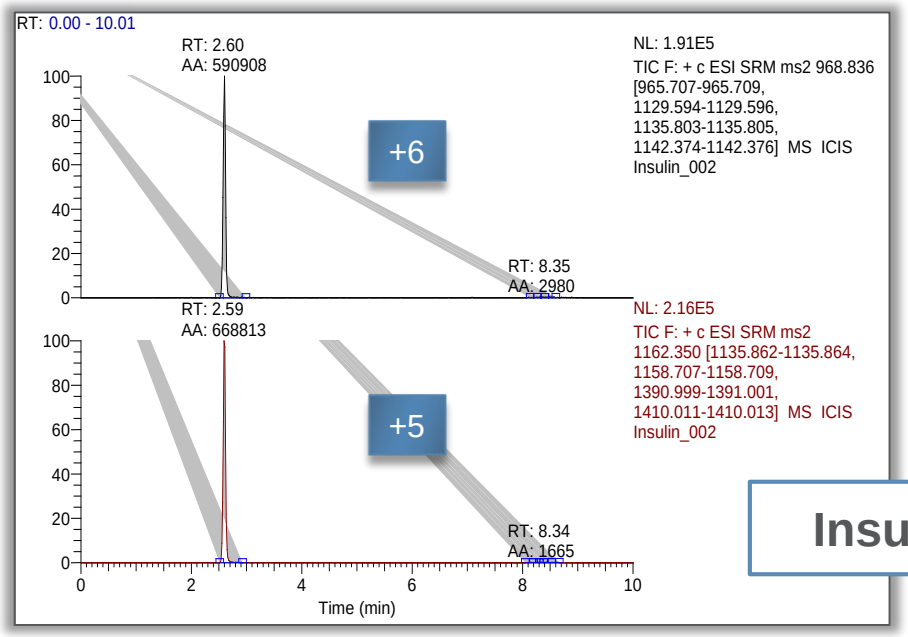
Example Albendazole in Salmon Fillet 0.2 x STC in Thermo Scientific™ TraceFinder™ software

Quantitation of Mixture of Large Molecules

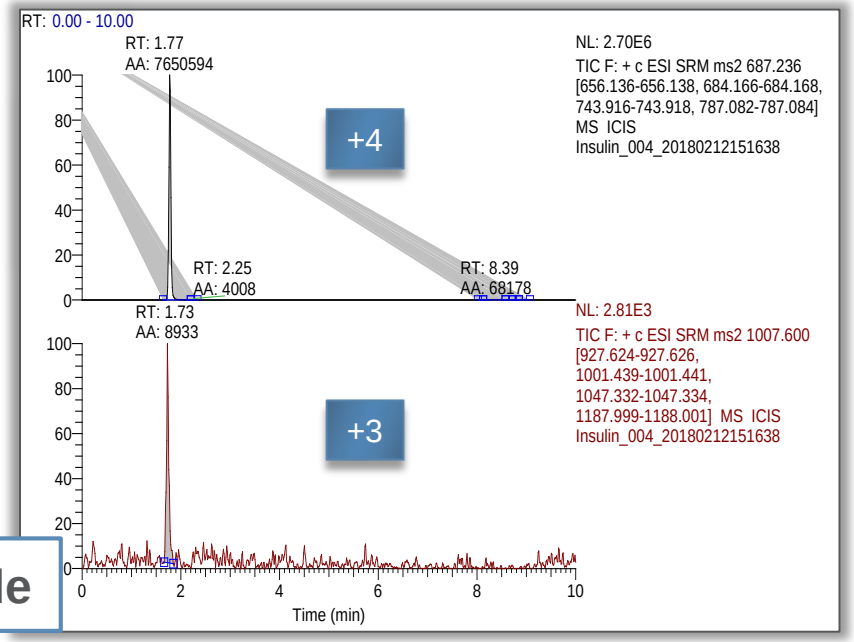
- Column: 2.1 x 50 mm, 1.5 um Accucore Vanquish C18
- Column Temp: 60 C
- Mobile Phase: [A] H_2O + 0.1% Formic Acid; [B] ACN + 0.1% Formic Acid
- Injection Volume: 10 uL
- Sample Temp: 10 C

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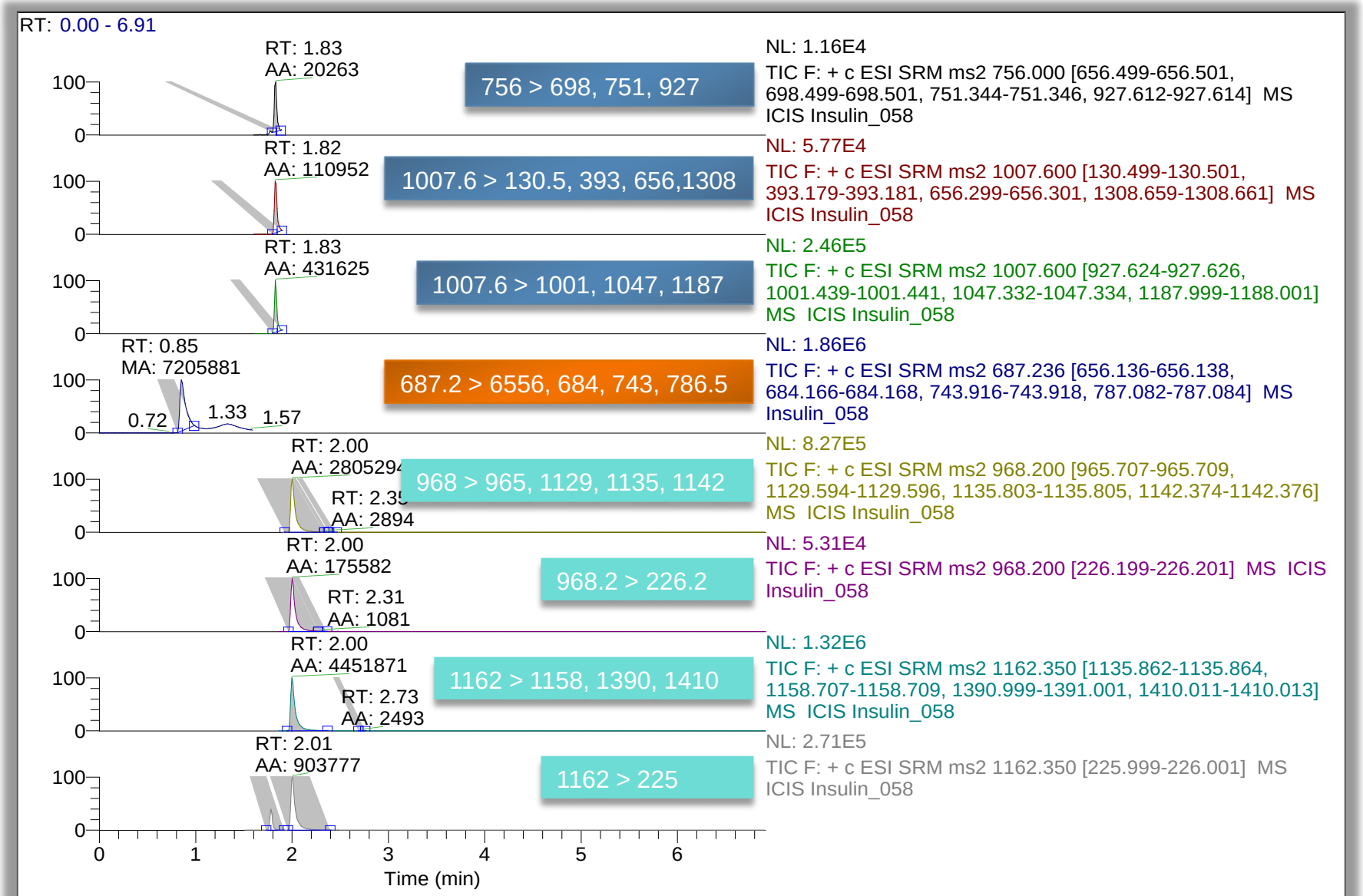
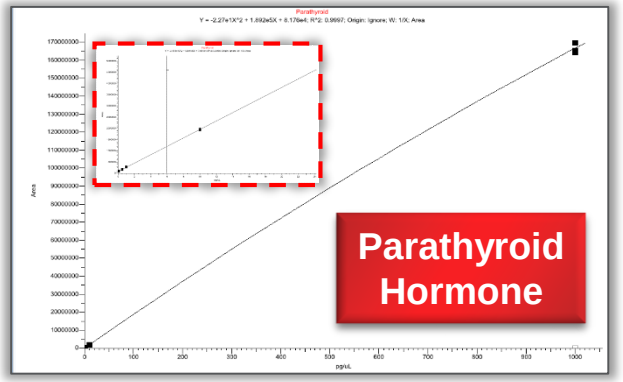
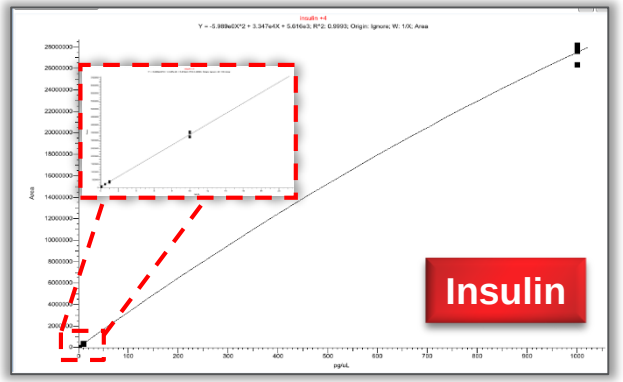
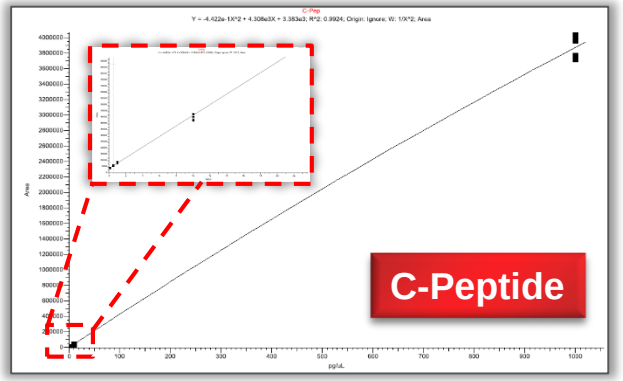
Optimized LC/MS – C-Peptide, Parathyroid Hormone, and Insulin









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Conclusions

- New Thermo Scientific™ TSQ Altis™, Quantis™ and Fortis™ 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

Success with QqQs:

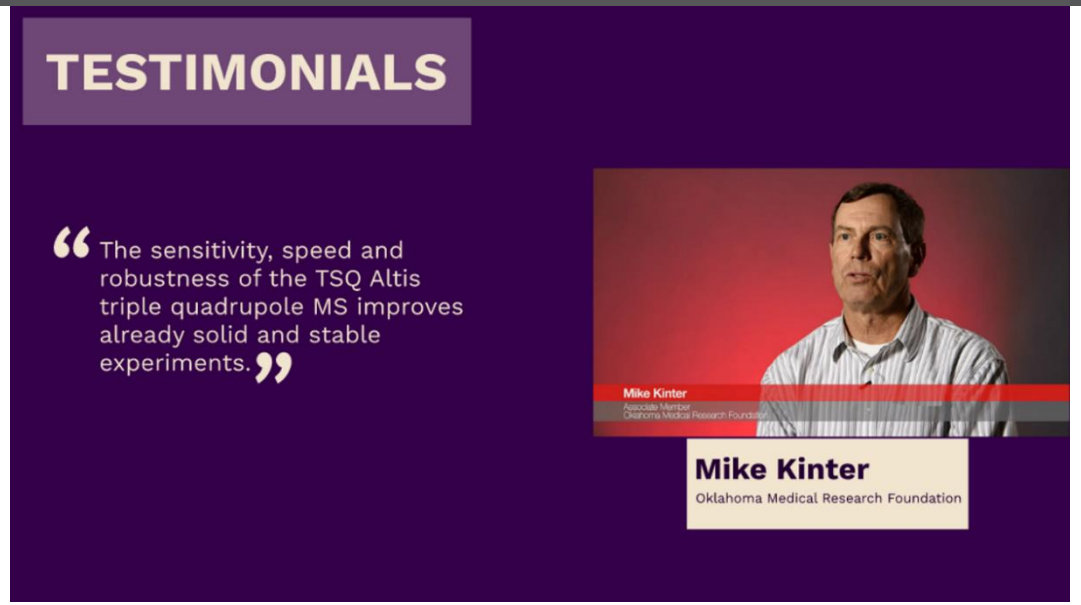

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Table showing high profile food and beverage scandals

Product	Adulteration	Years	Financial & Health Effects
Olive oil	Industrial oil denatured with aniline	1981	600 deaths reported in Spain
Orange juice	Beet sugar syrup, water and malic acid	1980's	Prosecutions of juice suppliers
Wine - Austria	Ethylene glycol	1985	Market recall – huge damage to Austrian wine industry
Chili spices - Asia	Sudan and other illegal dyes in spices, palm oil and processed foods	2005	Largest supermarket recall ever in UK costing £millions
Milk powder - China	Melamine & cyanuric acid	2008-2009	300,000 victims, 6 infant deaths & 54,000 babies hospitalized
Animal feed - Ireland	Adulteration of pig feed with waste oil	2008	PCBs and dioxins in pork – Estimated €200 million financial losses
Animal feed - Germany	Adulteration of feed with contaminated waste cooking oil	2010	PCBs and dioxins in meat – restrictions on 5000 farms
Sports & tea drinks - Taiwan	Phthalates (DEHP) 2-20 ppm added as clouding agent to replace palm oil	2011	Health effects unknown but exposure above TDI for up to 15 years
Meat & meat products - Europe	Horse meat	2013	Large scale food recalls, RASFF alerts and prosecutions of processors
Cumin spice – India, Turkey	Ground peanut & almond shells	2015	Dangerous to allergen suffers but no individuals identified
Eggs contamination -15 EU countries	Fipronil	2017	Millions of eggs have been pulled from the shelves of supermarkets
Outbreak of Cyclospora Illnesses Salad Mix Served at McDonald's, US		**2018**	**Removed existing lettuce blend from 3000 McDonald's**

What is the risk to have a Hamburger?

Meat Substitution

A real scandal !!

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Meat Substitution

- Motivation : $$$
  - **Addition of meat from undeclared species to a specific meat product in order to lower production cost and increase profitability**

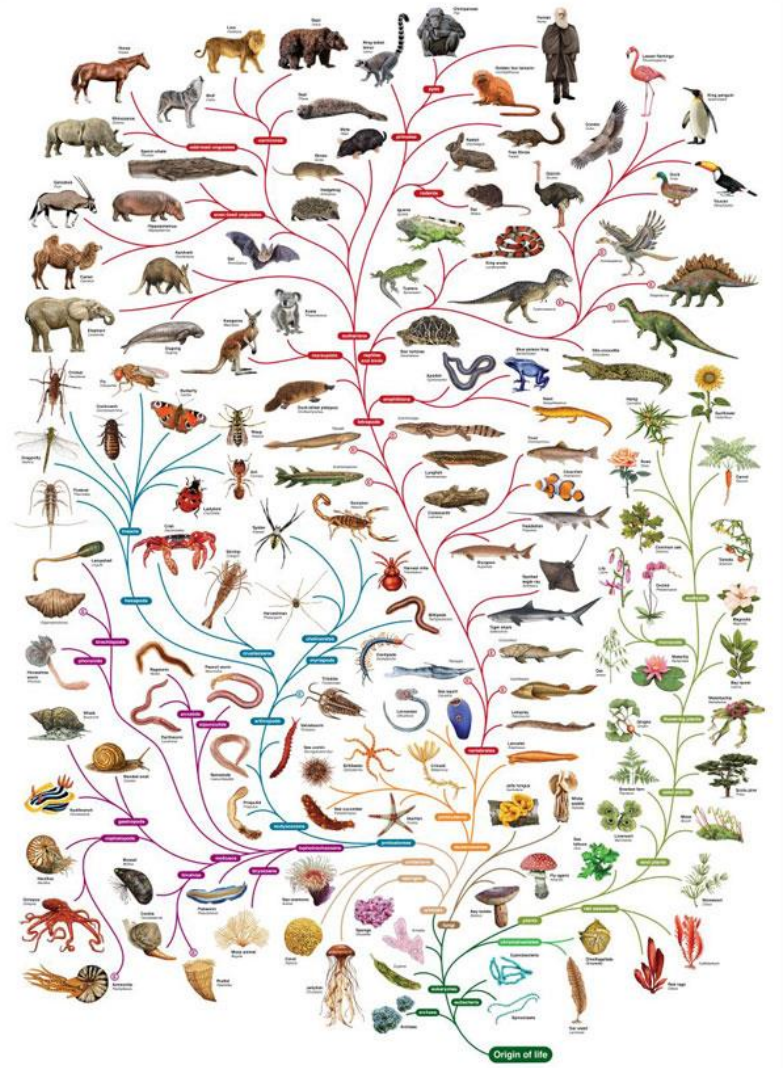
    Cost per kg: Horse meat << Beef meat
- It is an international issue
  - **It is economic fraud**
  - **It represents health issues due to specific dietary restrictions**
  - **It is an ethical problem**
  - **It is also an important cultural and religious issues**

Current methods used in regulated laboratories

Assessment of Meat Authenticity methods:

- Two-dimensional polyacrylamide gel electrophoresis and western-blot analysis
- Qualitative Real-Time PCR
- Enzyme-linked immunosorbent assay (ELISA)
  - **These methods are mostly qualitative**
  - **Molecular information obtained is limited**
  - **Data can’t be revisited post-acquisition for data mining**
  - **They are not generic approaches and need to be heavily customized**

Why bottom-up proteomics workflow is an interesting option to develop an MS based assay



All life forms are related by common ancestry and descent. The construction of phylogenies provides explanations of the diversity seen in the natural world.

Today, phylogenies is constructed using **DNA sequence data.**

Relationships between genes and species is central for meat speciation

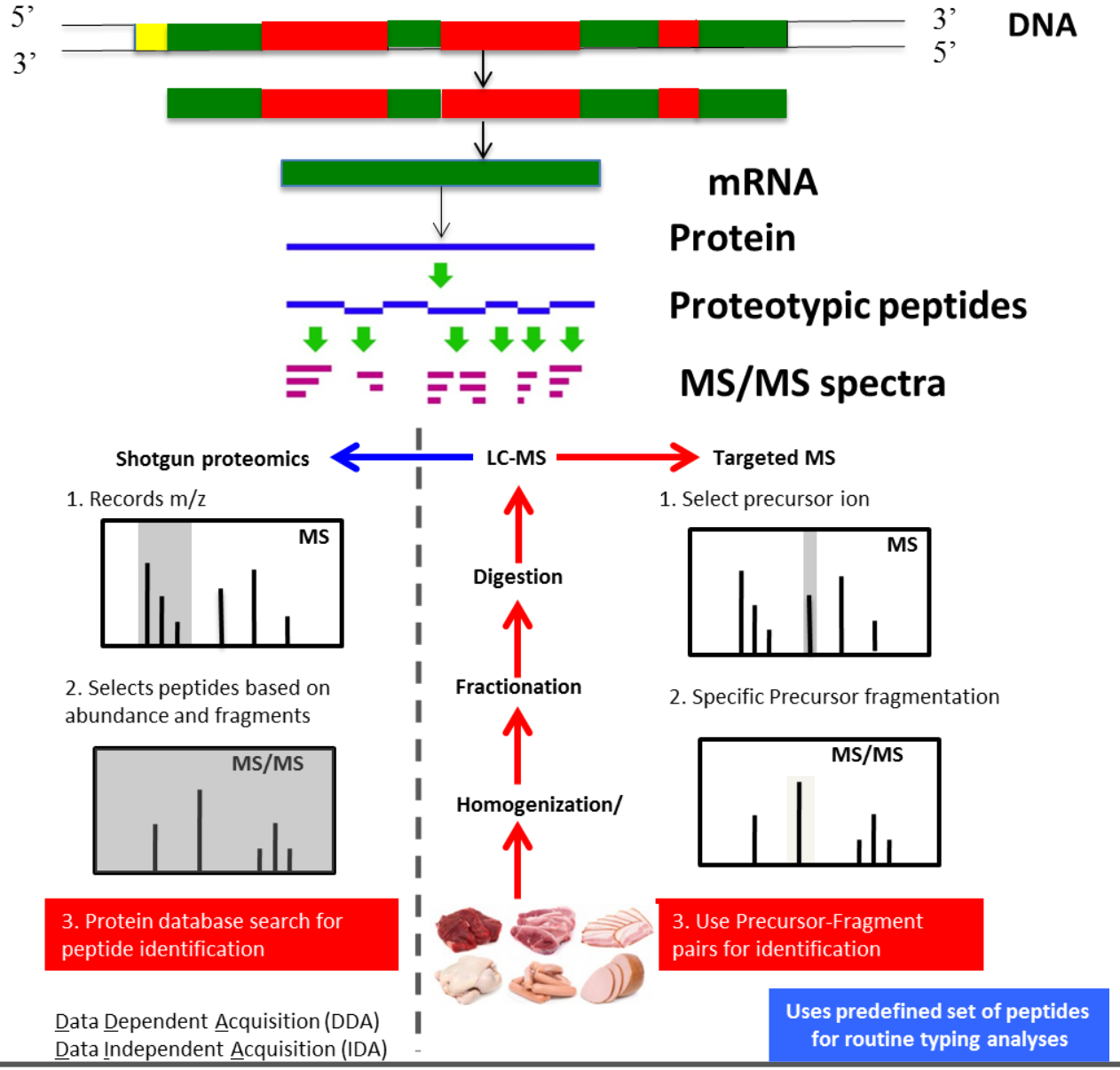
Define Set of Proteins

Select Peptides

Select precursor and product ions

Validate precursor and product ions

Protein Detection and Quantitation



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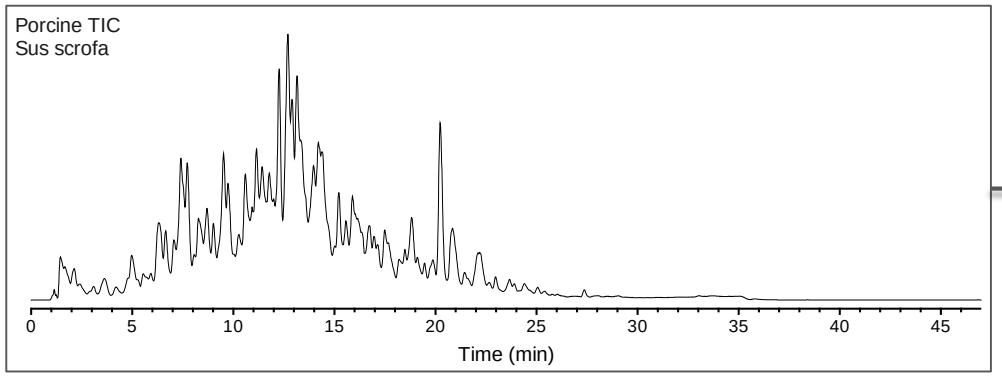
Traditional Peptide Fingerprinting Approach



Generate a mass list (MS and MS/MS data)

Database search (MASCOT)

MS and MS/MS matching

Peptide fingerprinting

Beef

17 specific peptides

Horse

23 specific peptides

Using a relatively high abundance threshold …

7 429 common unique peptides identified

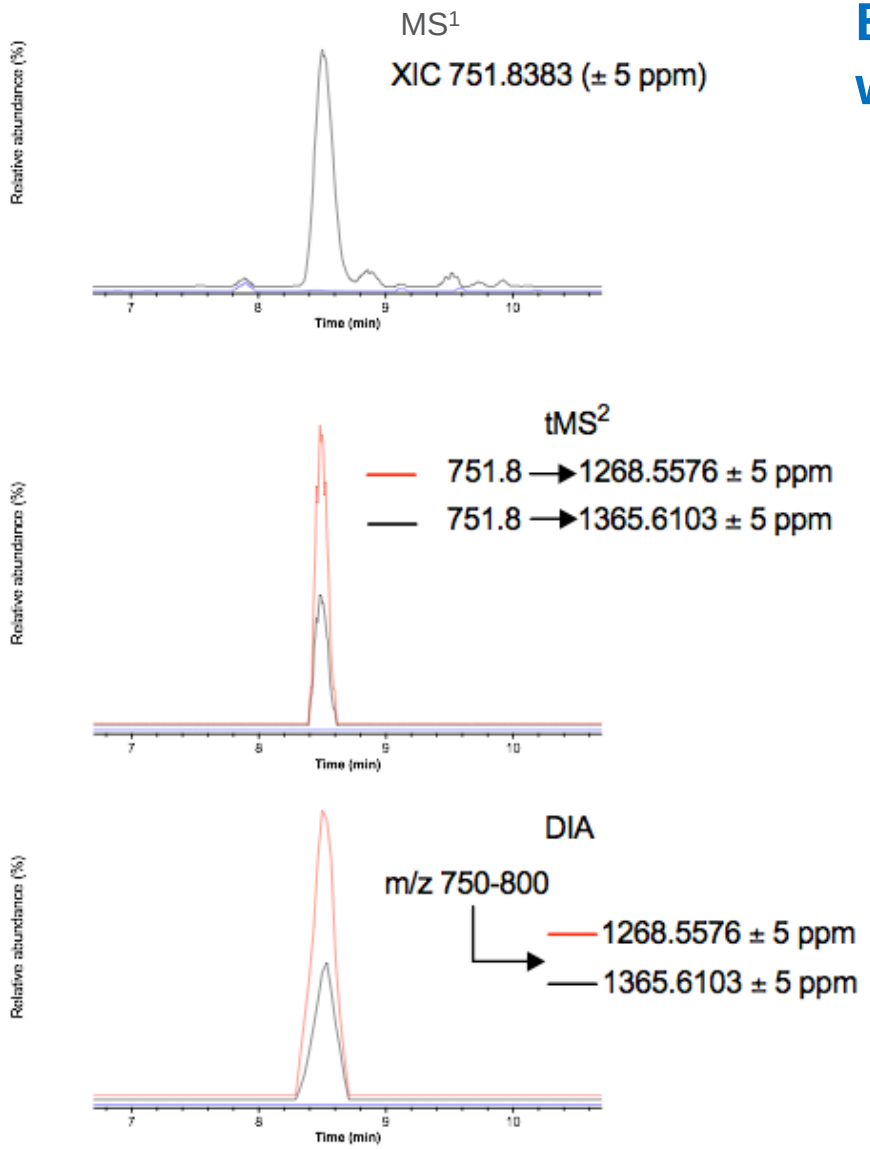
Pork

14 specific peptides

Lamb

9 specific peptides

Beef meat sample fortified with 1% (w/w) with pork meat



We can detect adulteration on beef meat sample with pork meat

Quadrupole - Orbitrap HRAM & Triple Quadrupole for Halal Applications

Meat Authenticity Assessment by Orbitrap MS/MS Analysis

Food Additives & Contaminants: Part A, 2015
http://dx.doi.org/10.1080/19440049.2015.1064173

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Assessment of meat authenticity using bioinformatics, targeted peptide biomarkers and high-resolution mass spectrometry

Alberto Ruiz Orduna[a], Erik Husby[b], Charles T. Yang[b], Dipankar Ghosh[b] and Francis Beaudry[a]*

[a]*Département de biomédecine vétérinaire, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, QC, Canada;*
[b]*Department of Environment & Food Safety, Thermo Fisher Scientific, San Jose, CA, USA*

(*Received 21 May 2015; accepted 16 June 2015*)

In recent years a significant increase of food fraud has been observed, ranging from false label claims to the use of additives and fillers to increase profitability. Recently in 2013 horse and pig DNAs were detected in beef products sold from several retailers. Mass spectrometry (MS) has become the workhorse in protein research, and the detection of marker proteins could serve for both animal species and tissue authentication. Meat species authenticity is performed in this paper using a well-defined proteogenomic annotation, carefully chosen surrogate tryptic peptides and analysis using a hybrid quadrupole-Orbitrap MS. Selected mammalian meat samples were homogenised and proteins were extracted and digested with trypsin. The samples were analysed using a high-resolution MS. Chromatography was achieved using a 30-min linear gradient along with a BioBasic C8 100 × 1 mm column at a flow rate of 75 µl min^{-1}. The MS was operated in full-scan high resolution and accurate mass. MS/MS spectra were collected for selected proteotypic peptides. Muscular proteins were methodically analysed *in silico* in order to generate tryptic peptide mass lists and theoretical MS/MS spectra. Following a comprehensive bottom-up proteomic analysis, we detected and identified a proteotypic myoglobin tryptic peptide (120–134) for each species with observed *m/z* below 1.3 ppm compared with theoretical values. Moreover, proteotypic peptides from myosin-1, myosin-2 and β-haemoglobin were also identified. This targeted method allowed comprehensive meat speciation down to 1% (w/w) of undesired product.

Keywords: high-resolution mass spectrometry; HPLC; proteomics; food; meat; authenticity; biomarkers

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Analytical Workflow for Meat Speciation Determination

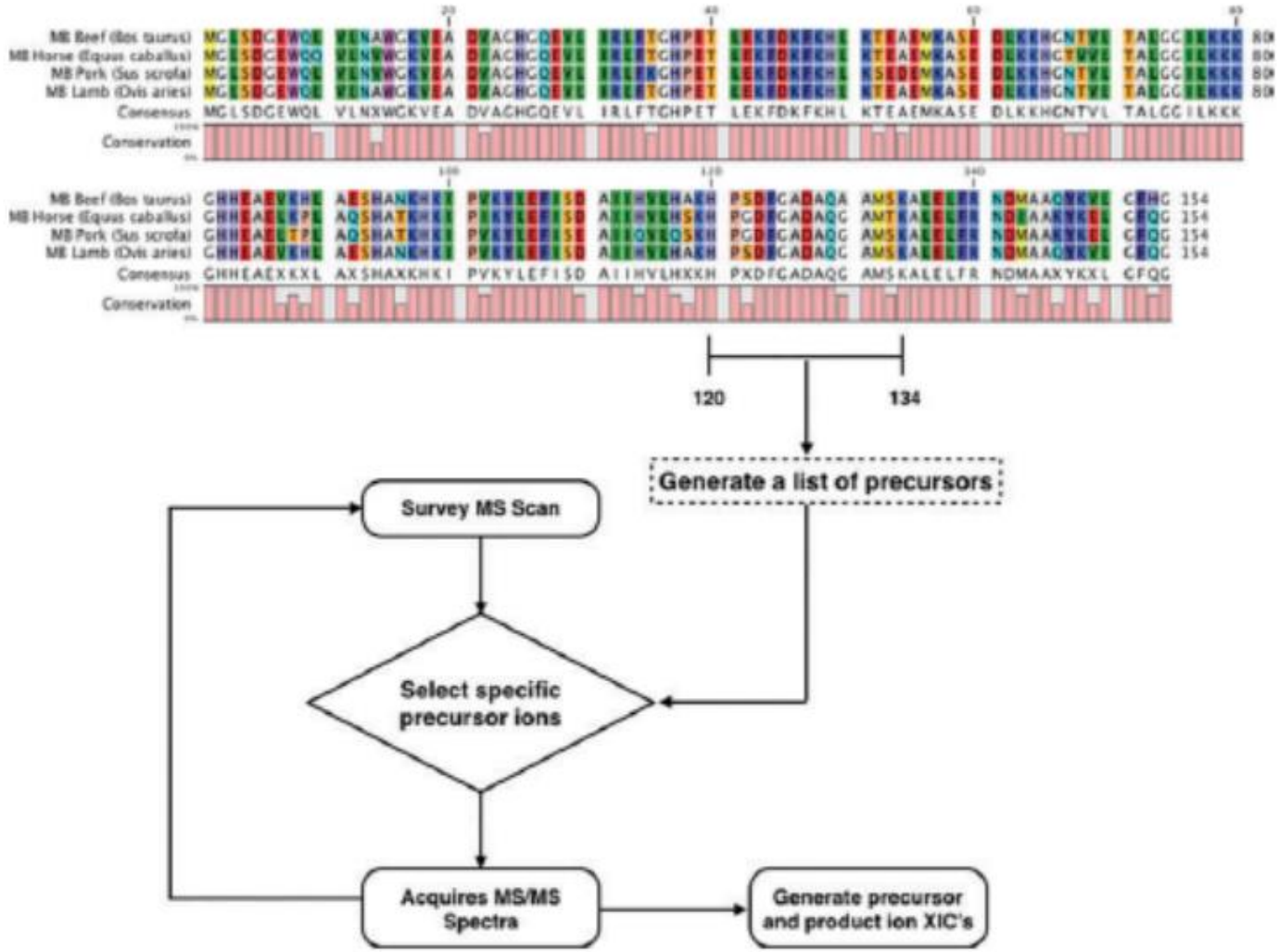

Figure 1. (colour online) Bioinformatic analysis of targeted mammalian muscular proteins. Species-specific myoglobin (MG) sequences were aligned and thoroughly analysed. Proteotypic peptides were identified as being located between amino acid positions 120 and 134. Thus, a specific precursor ion mass list can be generated and MS/MS experiments can be performed on species-specific biomarkers.

HRAM Meat Speciation Determination

Table 3. Other specific proteotypic peptides identified for selected mammalian meat species.

Species	Protein	Uniprot Accession number	Peptide sequence	AA position	Theoretical mass ($z = 2$)	Observed mass ($z = 2$)	Mass accuracy (ppm)	R_t (min)
Beef	Myosin-1	Q9BE40	TLALLFSGPASGEAEGGPK	619–637	901.4702	901.4694	−0.89	16.8
Horse	Myosin-1	Q8MJV0	TLALLFSGPASADAEAGGK	619–637	888.4623	888.4620	−0.34	17.0
Pork	Myosin-1	Q9TV61	TLAFLFTGAAGADAEAGGGK	619–638	912.9600	912.9594	−0.66	17.4
Lamb	Myosin-1	XM_004012706.1 (RefSeq)	TLAFLFSGAASAEAEGGGAK	619–638	927.9652	927.9650	−0.21	17.6
Beef	Myosin-2	Q9BE41	TLAFLFSGTPTGDSEASGGTK	619–639	1022.4971	1022.4968	−0.29	16.4
Horse	Myosin-2	Q8MJV1	TLALLFSGAQTADAEAGGVK	617–636	960.5073	960.5070	−0.31	17.0
Pork	Myosin-2	Q9TV63	TLAFLFSGAQ TGEAEAGGTK	619–638	978.4891	978.4894	−0.31	17.1
Lamb	Myosin-2	XM_004012707.1 (RefSeq)	TLALLFSGTPTAESEGSGTK	617–636	984.0020	984.0022	0.20	16.5
Beef	β-Haemoglobin	P02070	FFESFGDLSTADAVMNNPK	40–58	1045.4804	1045.4796	−0.77	16.9
Horse	β-Haemoglobin	P02062	FFDSFGDLSNPGAVMGNPK	42–60	1000.4646	1000.4637	−0.90	17.2
Pork	β-Haemoglobin	P02067	FFESFGDLSNADAVMGNPK	42–60	1023.4673	1023.4670	−0.29	16.8
Lamb	β-Haemoglobin	P02075	FFEHFGDLSNADAVMNNPK	40–58	1076.9915	1076.9906	−0.84	15.3

Adulteration with Porcine-based Products

- Adulteration of meat species in ground and comminute products have been a widespread problem in some retail markets.
- In Jan 2013, Food Standards Agency (FSA) reported detection of horse and pig DNA in beef products, subsequently many fraudulent and deception cases reported worldwide involving adulteration of haram ingredients in halal food.
- Majority of food manufactures choose to use porcine derivatives because they are cheap and readily available.

Protein Extraction from Raw Meat

1 Slice and blend the meat samples

Slice

Blend

2 Preparation of sample mixtures

Minced pork

+

Minced beef

Spike different amounts of pork in beef

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Protein Extraction from Raw Meat

3 Protein Extraction
using ***Thermo Scientific Dionex ASE 350 Accelerated Solvent Extractor System*** under high pressure and temperature

Protein Extraction

4 Trypsin Digestion

5 Peptide Identification/Confirmation with HRAM MS
using ***Thermo Scientific Q Exactive Plus Hybrid Quadrupole-Orbitrap MS*** with a shotgun proteomic approach

6 Detection of Targeted Peptide with Triple Quadrupole
using ***Thermo Scientific Vanquish UHPLC-TSQ Quantiva Triple Quadrupole MS*** with MRM workflow

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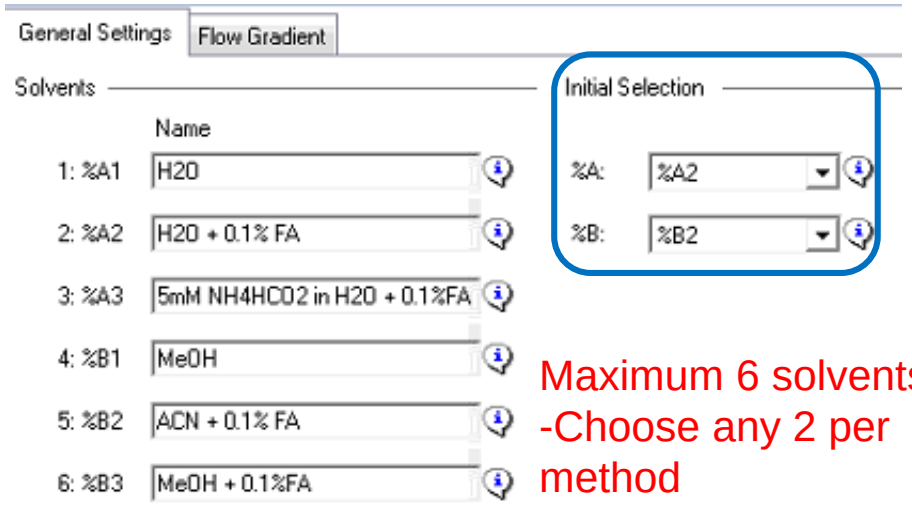
Instrument Method Setup

Vanquish Horizon Binary UHPLC (max pressure 1,500 bar)

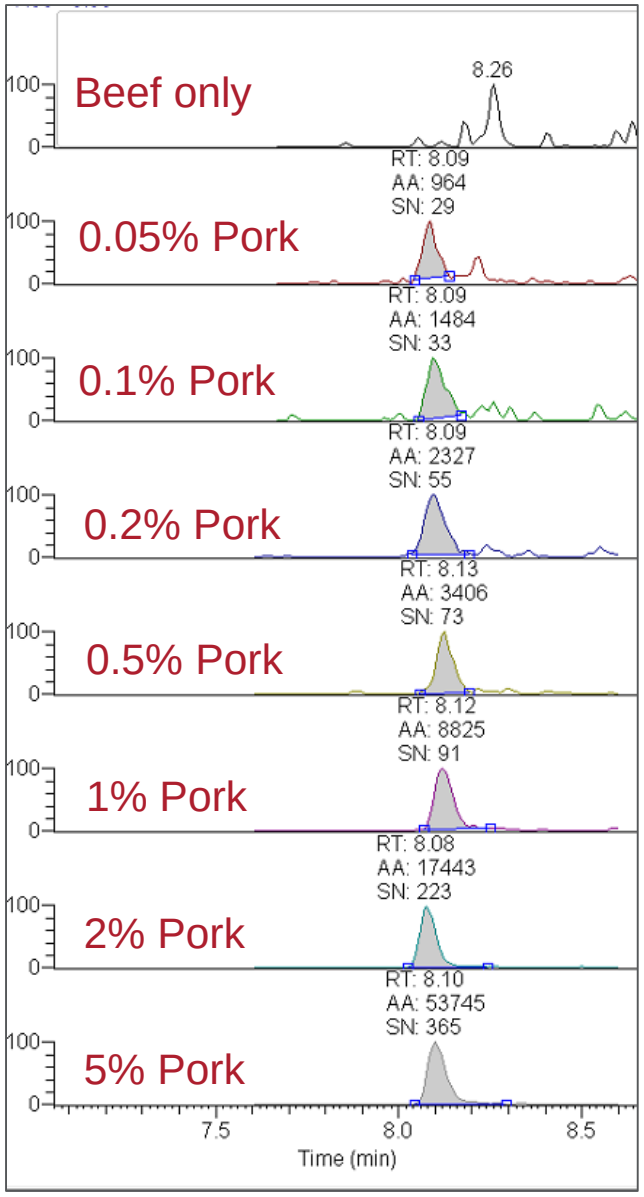
- Thermo Scientific Hypersil Gold 100mm x 1.0 mm ID x 1.9 µm
- Mobile Phase:
  A2: H_2O with 0.01% Formic Acid
  B2: Acetonitrile with 0.01% Formic Acid
- Analytical Flow Rate: 100 µL/min
- Column Temperature: 35 °C
- Injection Volume: 10 µL
- 13 minutes cycle time



Maximum 6 solvents -Choose any 2 per method

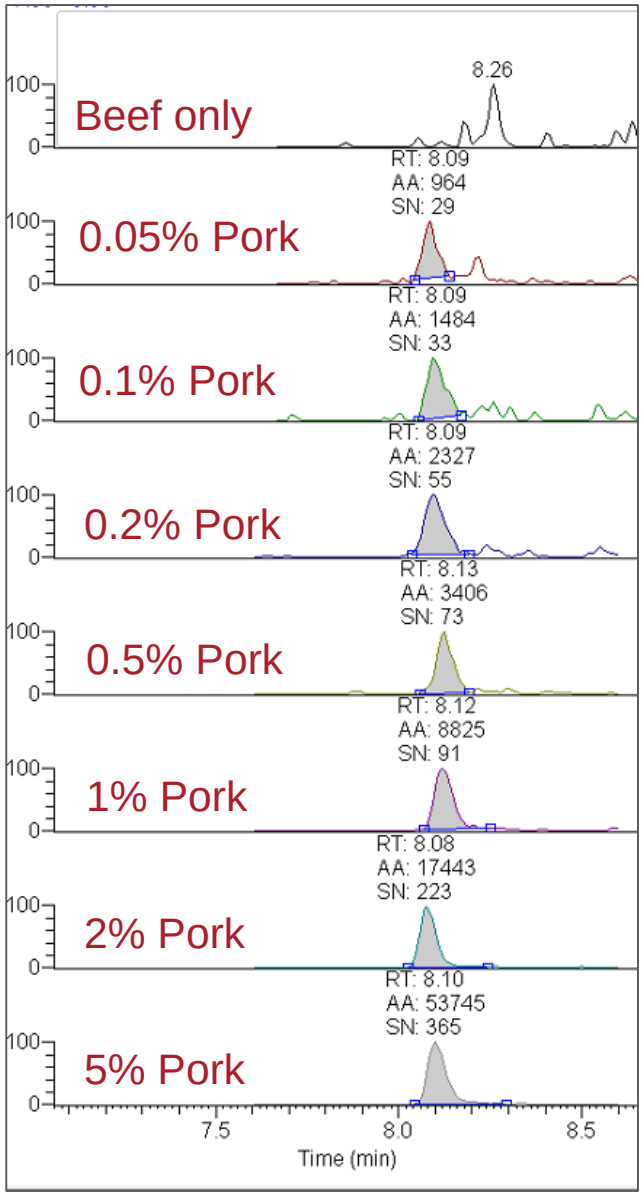
No	Time	Flow [ml/min]	%B	Curve
1	0.000	Equilibration		
2	0.000	0.100	2.0	5
3	*New Row*			
4	0.000	Run		
5	0.000	0.100	2.0	5
6	3.500	0.100	2.0	5
7	8.000	0.100	60.0	5
8	8.100	0.100	95.0	5
9	10.000	0.100	95.0	5
10	10.100	0.100	2.0	5
11	*New Row*			
12	13.000	Stop Run		

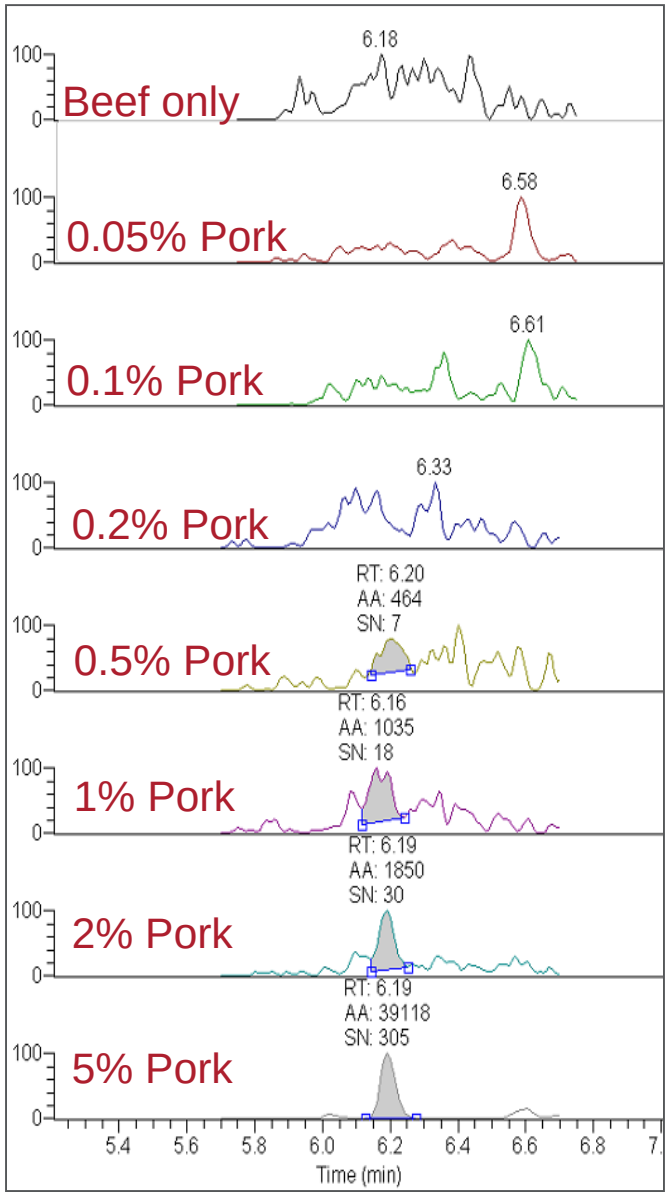
XIC of Peptide Marker in Pork-Beef Mixtures



- The identified peptide is unique for porcine meat and does not observe in beef.
- meat can be detected at 0.05% spike level in cooked pork.
- Consistent retention time (8.10±0.03 min) and narrow peak width were achieved.

Comparison of Different Targeted Peptide Biomarkers





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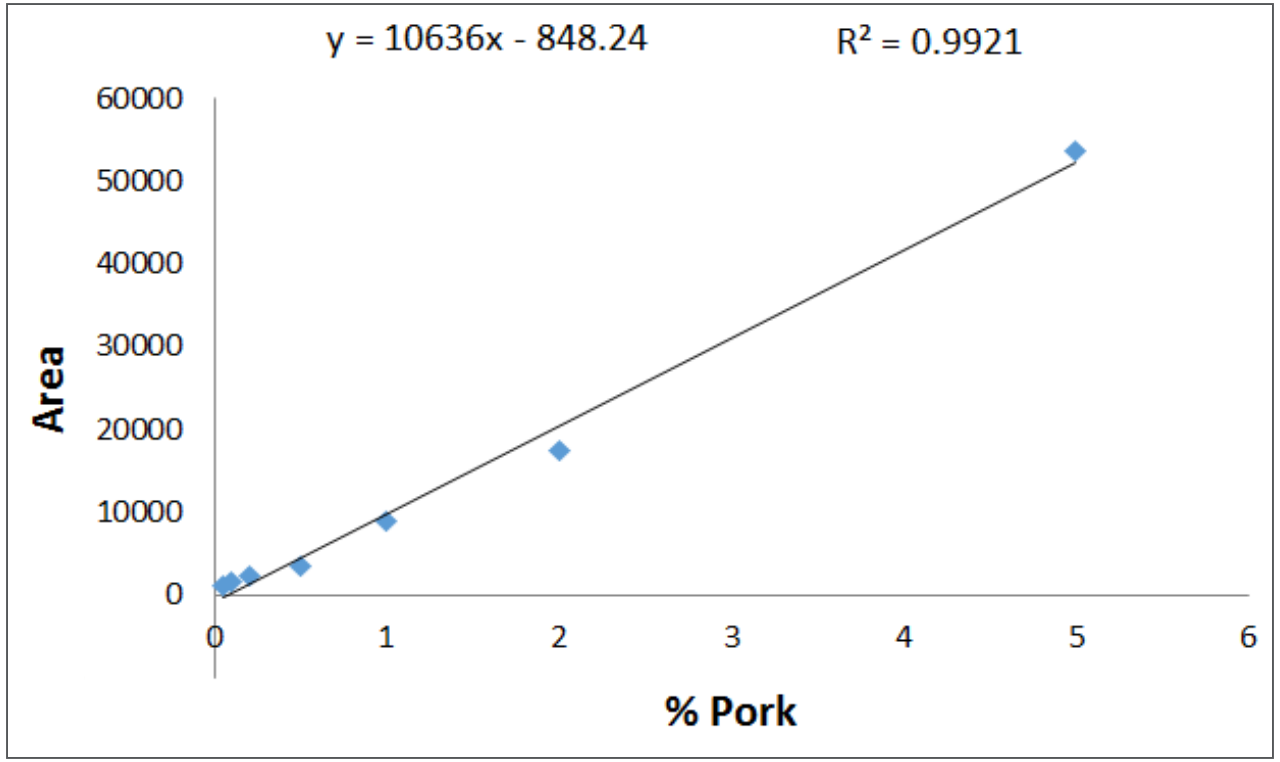
Calibration and results



- 7 points calibration curve was constructed.
- Peak abundances were directly proportional to the quantity of pork meat in the mixture and provided adequate linearity.

The Seafood Industry

Allergen detection

- Food allergy is a growing public health concern.
- 9 million, or 4%, of adults have food allergies
- 6 million or 8% of children have food allergies
- Food Allergies are on the Rise an increased of approximately 50% between 1997 and 2011.
- The economic cost of children's food allergies is nearly $25 billion per year.

Criminal fraud in the food supply chain

•It is estimated that it costs the world economy $49 billion annually and is growing

•The horsemeat scandal

•33% of seafood is mislabeled in the US

•The rapid rise of food fraud is considered a global threat

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THE PROBLEM

Criminal Fraud In The Supply Chain Is Considered A Global Threat

THE COST OF SEAFOOD FRAUD

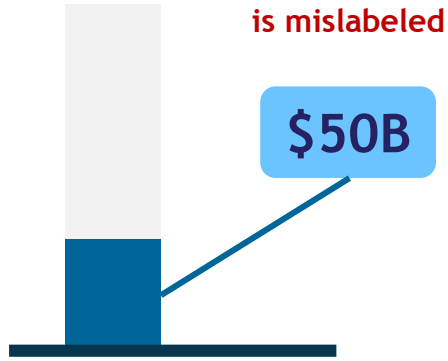
If You Mean To Buy:

But You Get:

	GROCERY	RESTAURANT
	GROUPER	
	$7.00	$27.00
	TILAPIA	
	$2.99	$15.00
	YOU LOSE $4.01	YOU LOSE $12.00

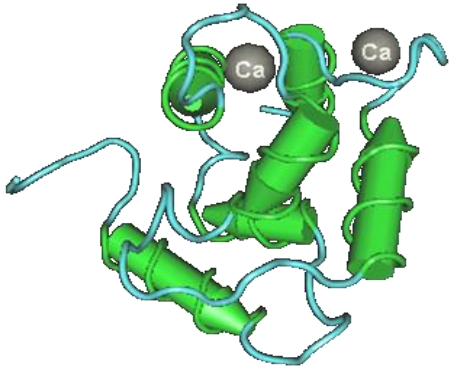
1 in 3

Fish tested in the US is mislabeled



THE SOLUTION

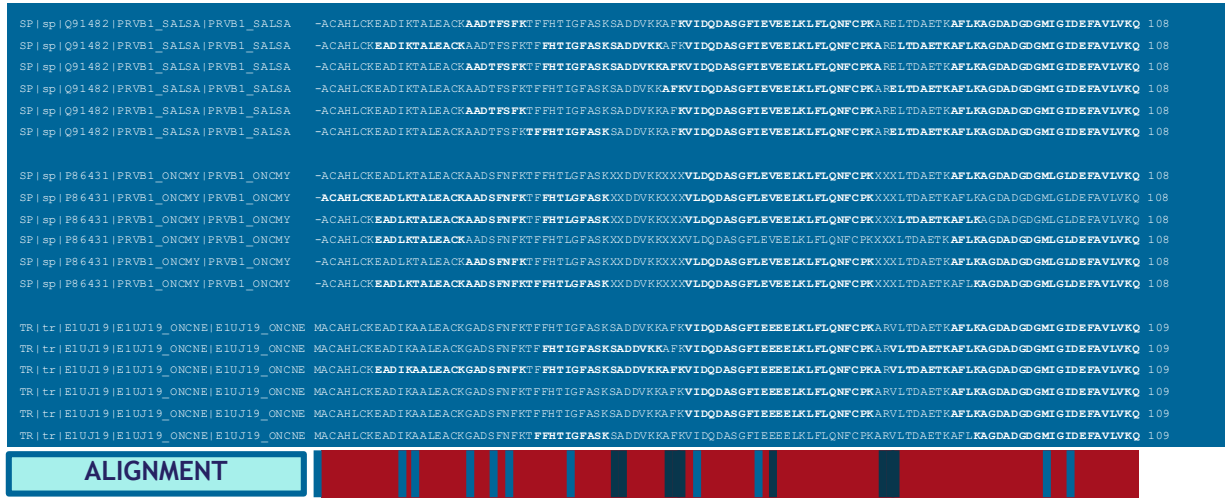
The Primary Allergen In Fish Is Parvalbumin PRVB



Calcium-binding protein
Mr: 10-12 kDa
pI: 3-5
Length :108 aa

Resistant To,
- Heat
- Ph
- Enzyme Digestion

Sequence Alignment Showed Very Conserved Sequence Regions



Allergen Detection

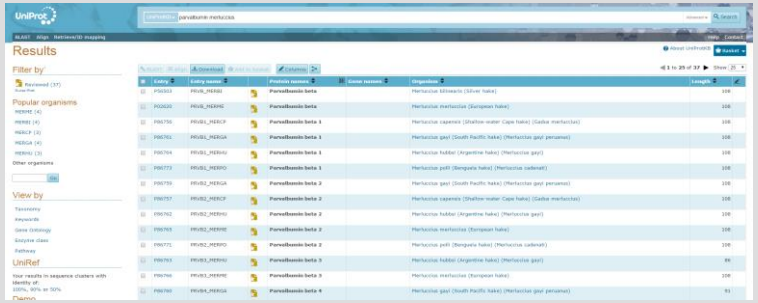
CONSERVED REGIONS

Food Authenticity

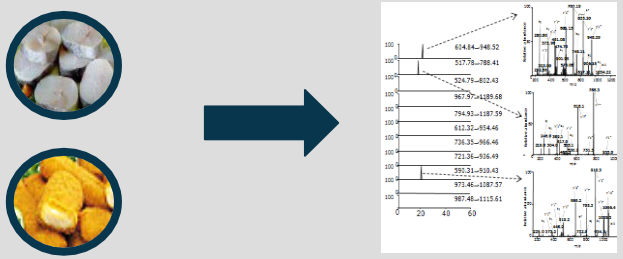
DIFFERENTIAL REGIONS

FIRST ATTEMPTS

38 new complete protein sequence of allergens



Fast monitoring of a kit of 19 peptide biomarkers by selected ms/ms ion monitoring



Allergen Detection <2h

VALIDATION IN
50 COMMERCIAL FOOD PRODUCTS
FROM SPANISH MARKETS (FROZEN, PROCESSED, PRECOOKED)

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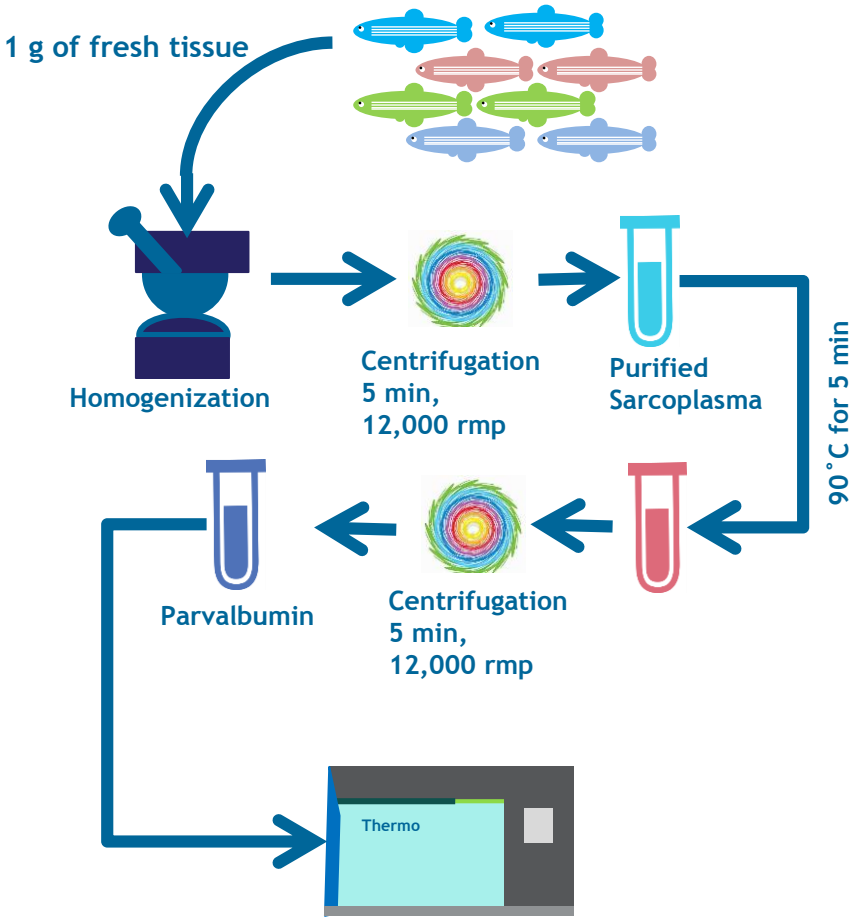
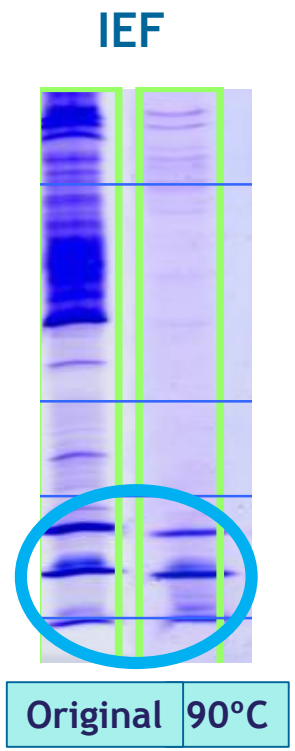
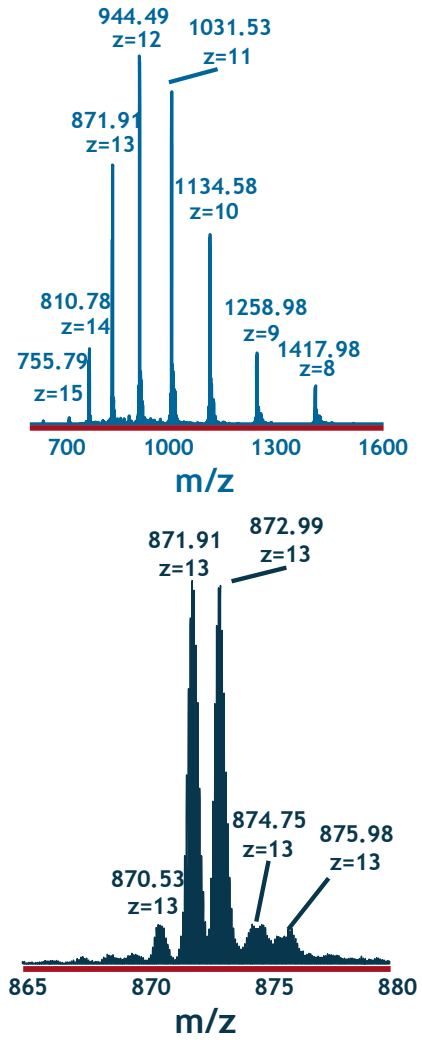
RESULTS

FAST PRVB PURIFICATION







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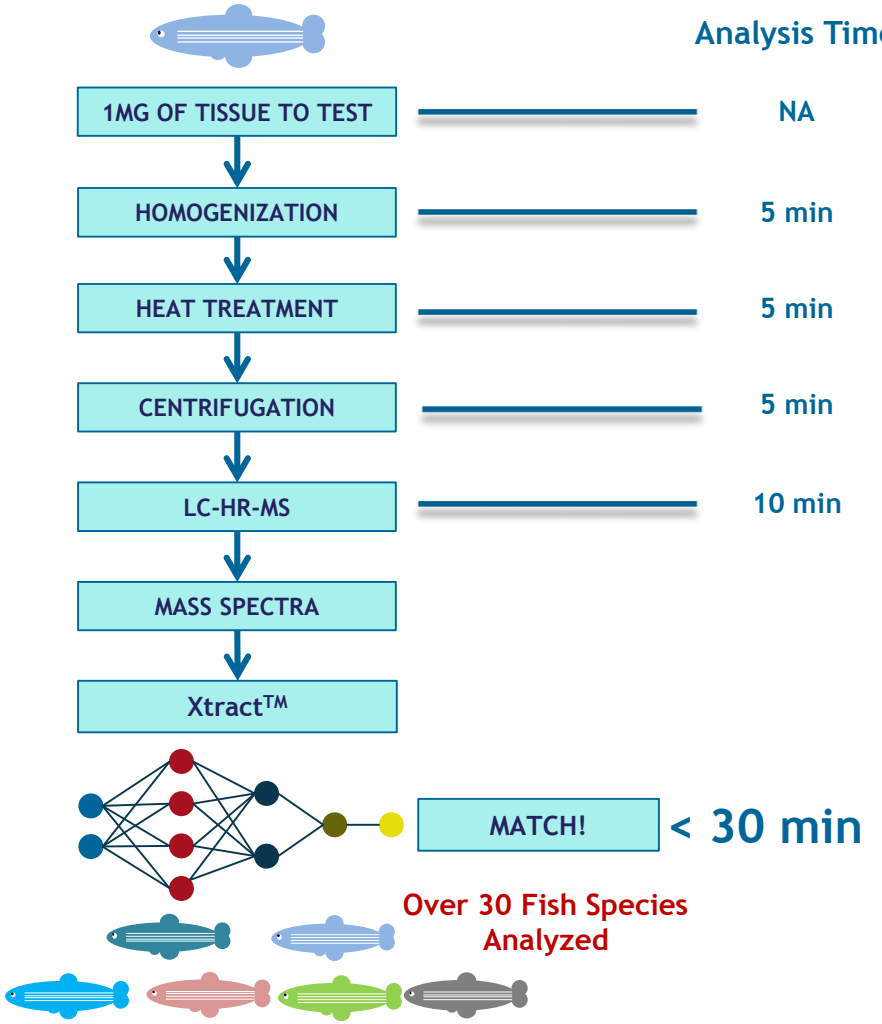
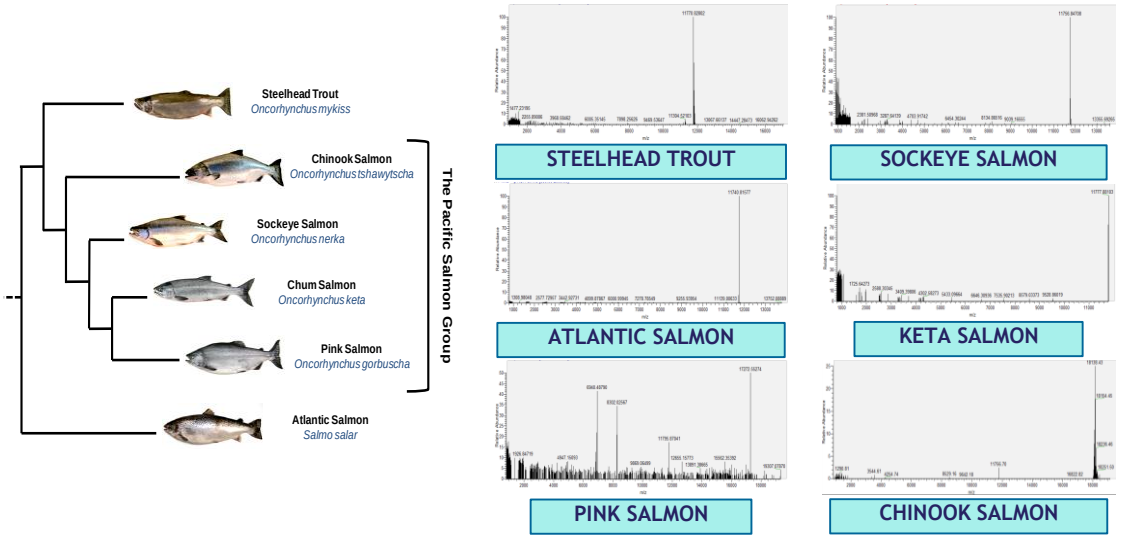
Top-Down, High-Throughput Proteomics for allergen and food

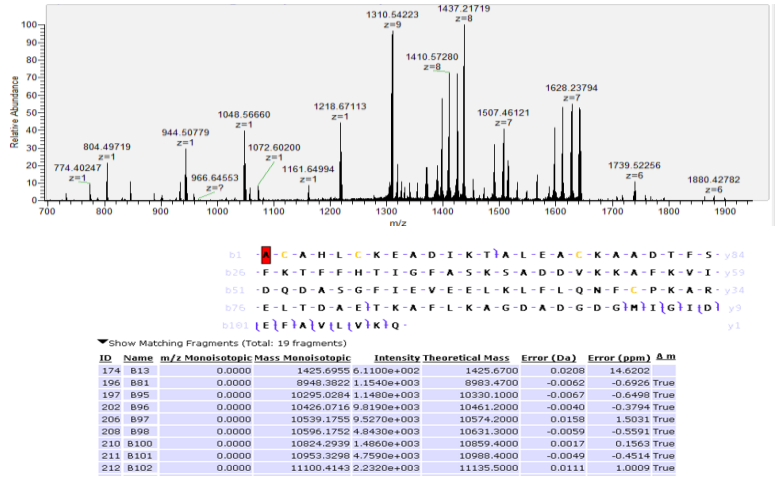
GLOBAL ANALYTICAL STRATEGY



Proof of Principle



Representative After Spectra Deisotoping & Deconvolution Using Xtract™



Show Matching Fragments (Total: 19 fragments)

ID	Name	m/z Monoisotopic	Mass Monoisotopic	Intensity	Theoretical Mass	Error (Da)	Error (ppm)	Δ m
174	B13	0.0000	1425.6955	6.1100e+002	1425.6700	0.0208	14.6202	
196	B81	0.0000	8948.3822	1.1540e+003	8983.4700	-0.0062	-0.6926	True
197	B95	0.0000	10295.0284	1.1480e+003	10330.1000	-0.0067	-0.6498	True
202	B96	0.0000	10426.0716	9.8190e+003	10461.2000	-0.0040	-0.3794	True
206	B97	0.0000	10539.1755	9.5270e+003	10574.2000	0.0158	1.5031	True
208	B98	0.0000	10596.1752	4.8430e+003	10631.3000	-0.0059	-0.5591	True
210	B100	0.0000	10824.2939	1.4860e+003	10859.4000	0.0017	0.1563	True
211	B101	0.0000	10953.3298	4.7590e+003	10988.4000	-0.0049	-0.4514	True
212	B102	0.0000	11100.4143	2.2320e+003	11135.5000	0.0111	1.0009	True

Top Down Proteomics Adds And Extra Dimension Of Knowledge

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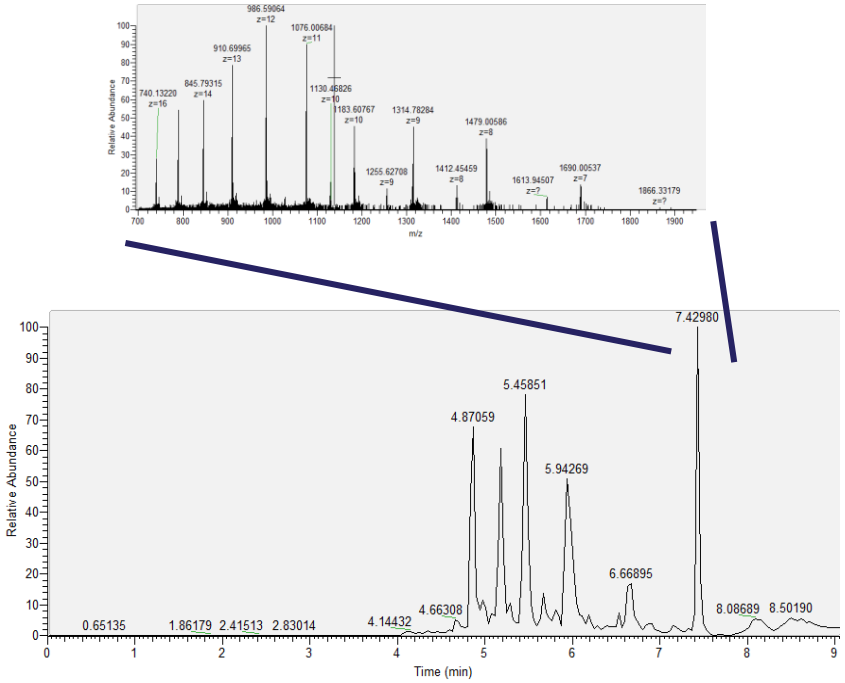
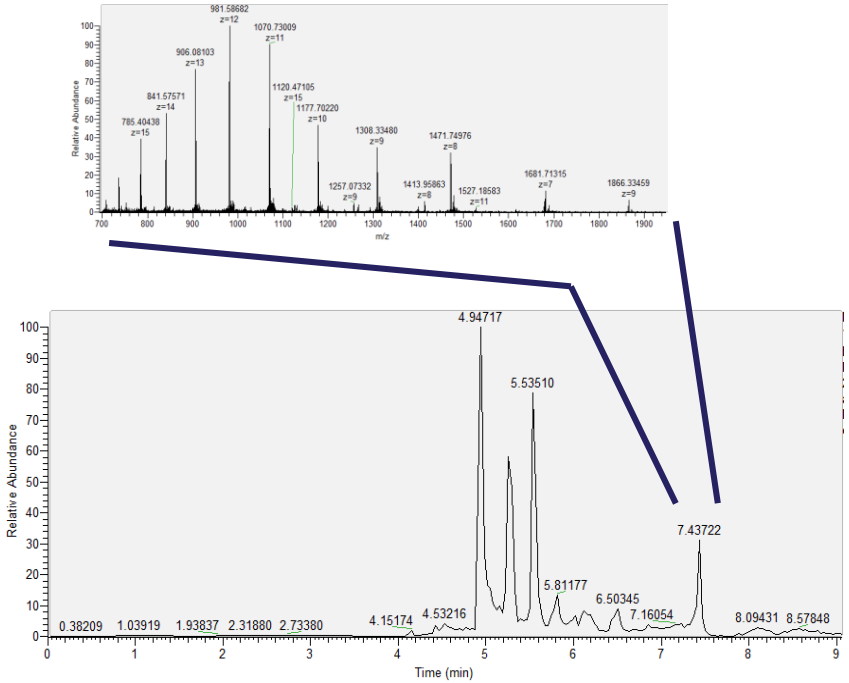
Top-Down, High-Throughput Proteomics for allergen and food authenticity



Sockeye Salmon

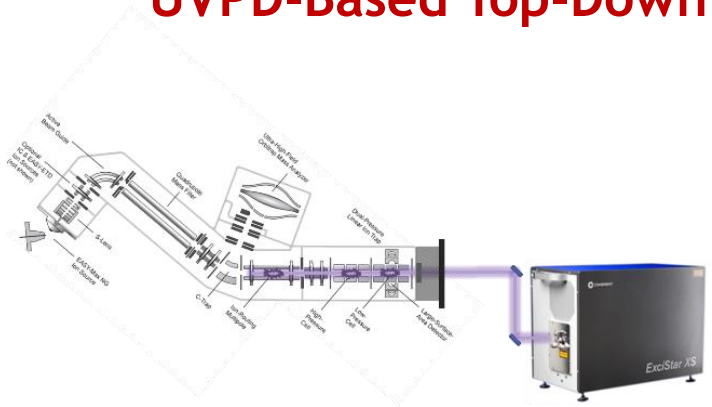


Atlantic Salmon

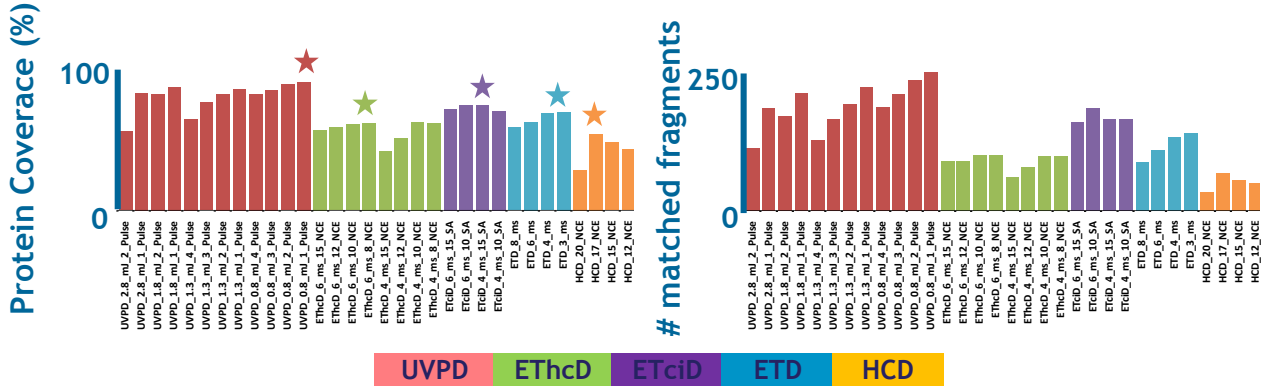
An easy and robust method for salmon speciation has been developed utilizing the high speed and high resolution of the Q Exactive HF. Using parvalbumin proteoforms as a signature for the species identification reveals the following benefits:

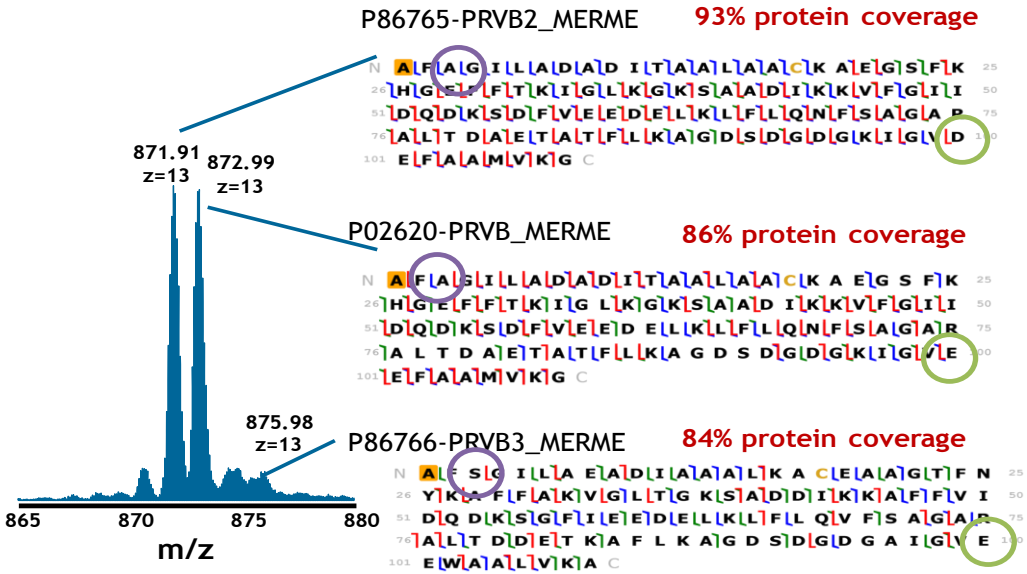
- Minimal sample preparation
- High sensitivity and throughput
- Bypass extensive *de novo* sequencing due to the high homology among the amino acid sequences from the different species.

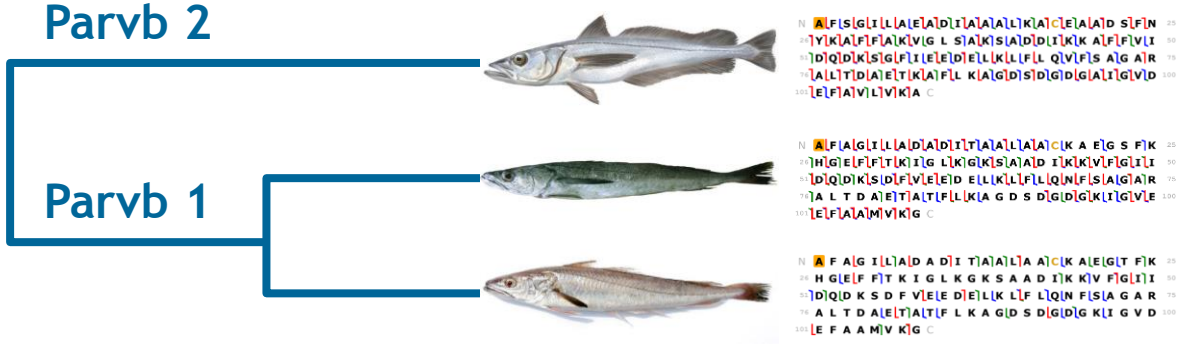
UVPD-Based Top-Down Proteomics For Complete Protein Coverage



Orbitrap Fusion with Integrated UVPD Kit







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CONCLUSIONS

- An Easy And Robust Method For Fish Allergen Detection Has Been Developed.
- The Use Of Protein Ms-barcodes Allows For Rapid Identication Of Commercially Important Species Of Fish
- Using Parvalbumin Proteoforms As A Signature For The Species Identification Reveals The Following Benefits:
  - Minimal Sample Preparation
  - High Sensitivity And Throughput
  - Bypass Extensive *De Novo* Sequencing Due To The High Homology Among The Amino Acid Sequences From The Different Species.

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Success and Beyond

Fish

Poster summary PN 64845

From ocean to table: an integrated mass spectrometry approach to identify the fish on your plate

Edible oils

Application summary AB 30276

EA-IRMS: Detection of squalane from animal and vegetable sources

Beverages

Application summary AB 201

Determination of carbohydrates in coffee using a compact ion chromatography system

Honey

Poster summary PN 30397

Food and beverage fraud prevention using stable isotope fingerprints

Spices

Application summary AB 163

Determination of capsaicinoids in chili pepper using HPLC-ECD

Beverages

Application summary AN 1068

Determination of organic acids in fruit juices and wines by high-pressure IC

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Thank you