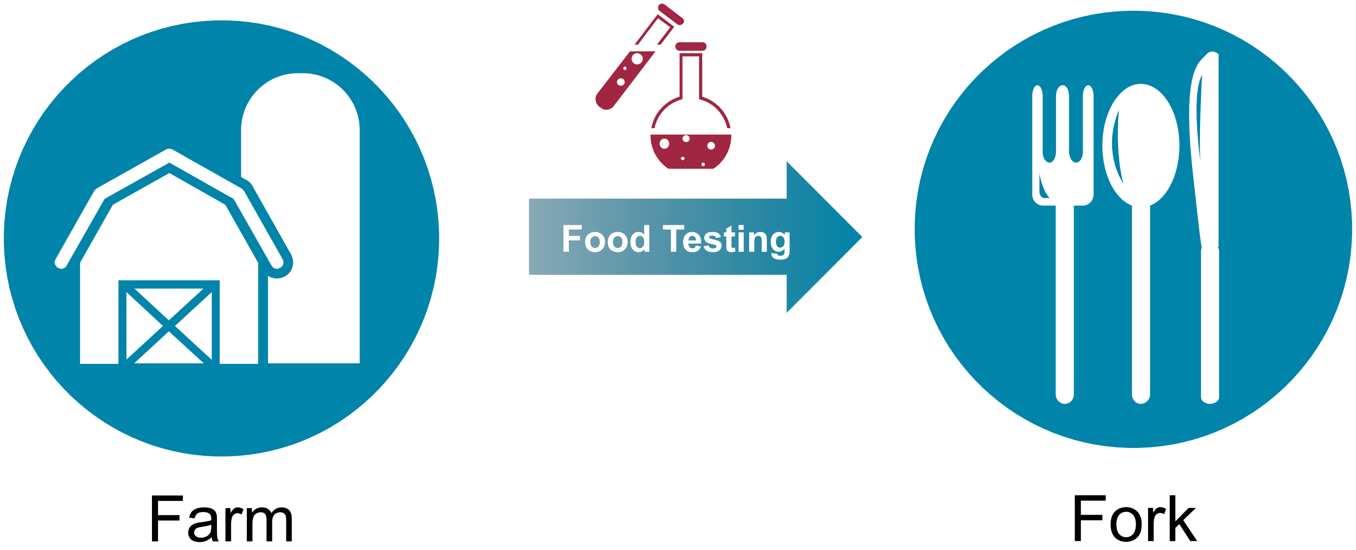
New Tools in Food Analysis for Residue Screening and Authenticity

Jianru Stahl-Zeng, PhD
Global Technical Manager – Market Development
SCIEX

Today's Food Movement

Emphasis on ensuring that food is safe and free of chemical residues and contaminants as it transits from the farm to the consumer.



Farming practices?

Growth climate and conditions?

Adulteration?

Everything occurring between:

Manufacturing, processing, packaging, storage, transportation…

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Target & Non-Target Screening on Food Testing

In 1 Injection……

Extraction → Clean-up → LC separation → MS analysis → Identification → Quantitation

Extraction: Comprehensive enough for wide compound coverage?
(ie. broad polarity, MW, solubility)

Clean-up: Get rid of matrix but retain the contaminants?
(and, what contaminants??)

LC separation: LC conditions for widest compound coverage?

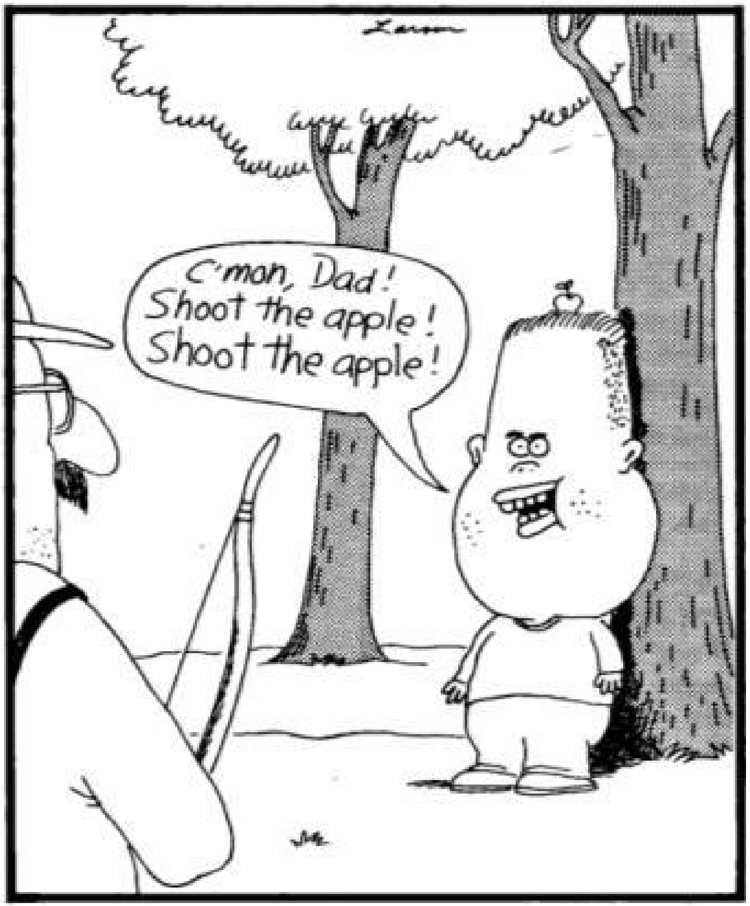
MS analysis: **MS conditions for widest compound coverage?**

Hardware that's powerful enough to catch everything?

Identification: **Data mining – how do you find what you don't know you're looking for?**

Quantitation: A get more for less scenario – Can I quantify something once I find it?

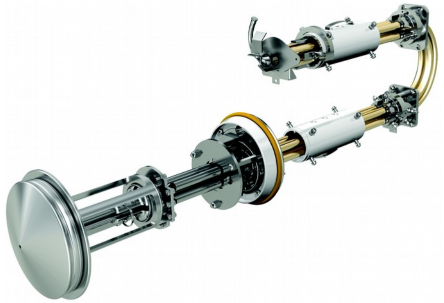
So You Know What You Are Looking For?

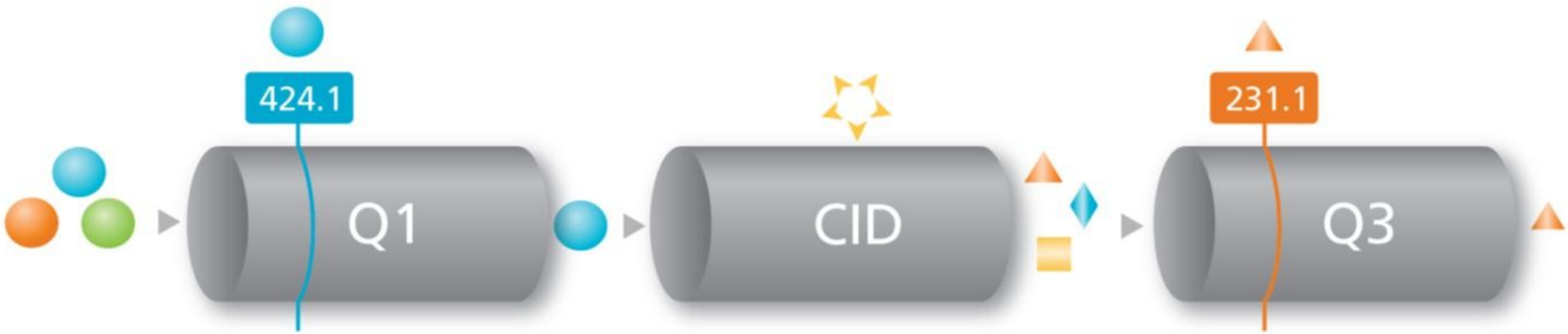


But what could you be missing?

LC-MS/MS with Multiple Reaction Monitoring (MRM)

Selectivity and Sensitivity for Screening and Quantitation





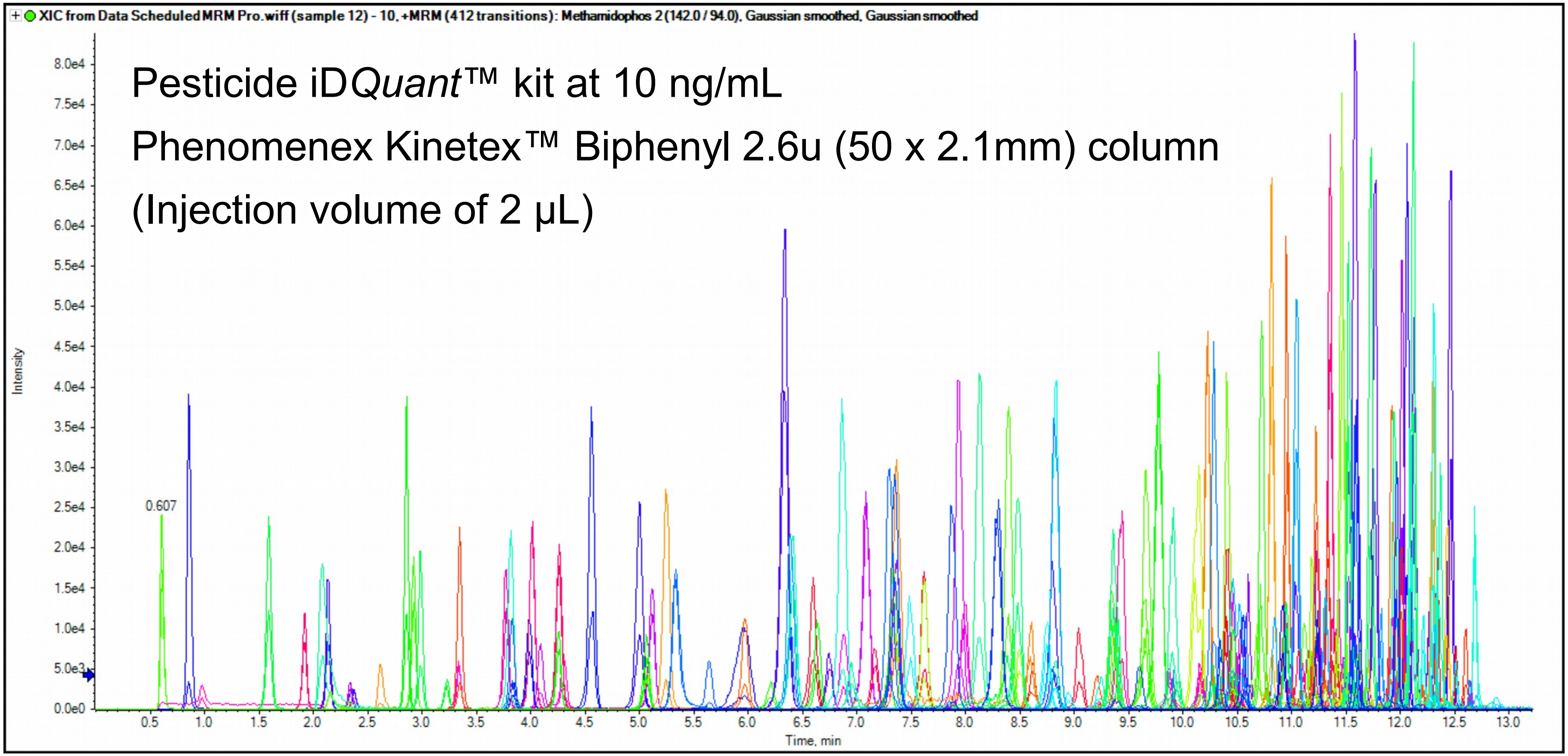
Filter

Fragment

Filter

Triple Quad™ 3500 System – Pesticide Screening

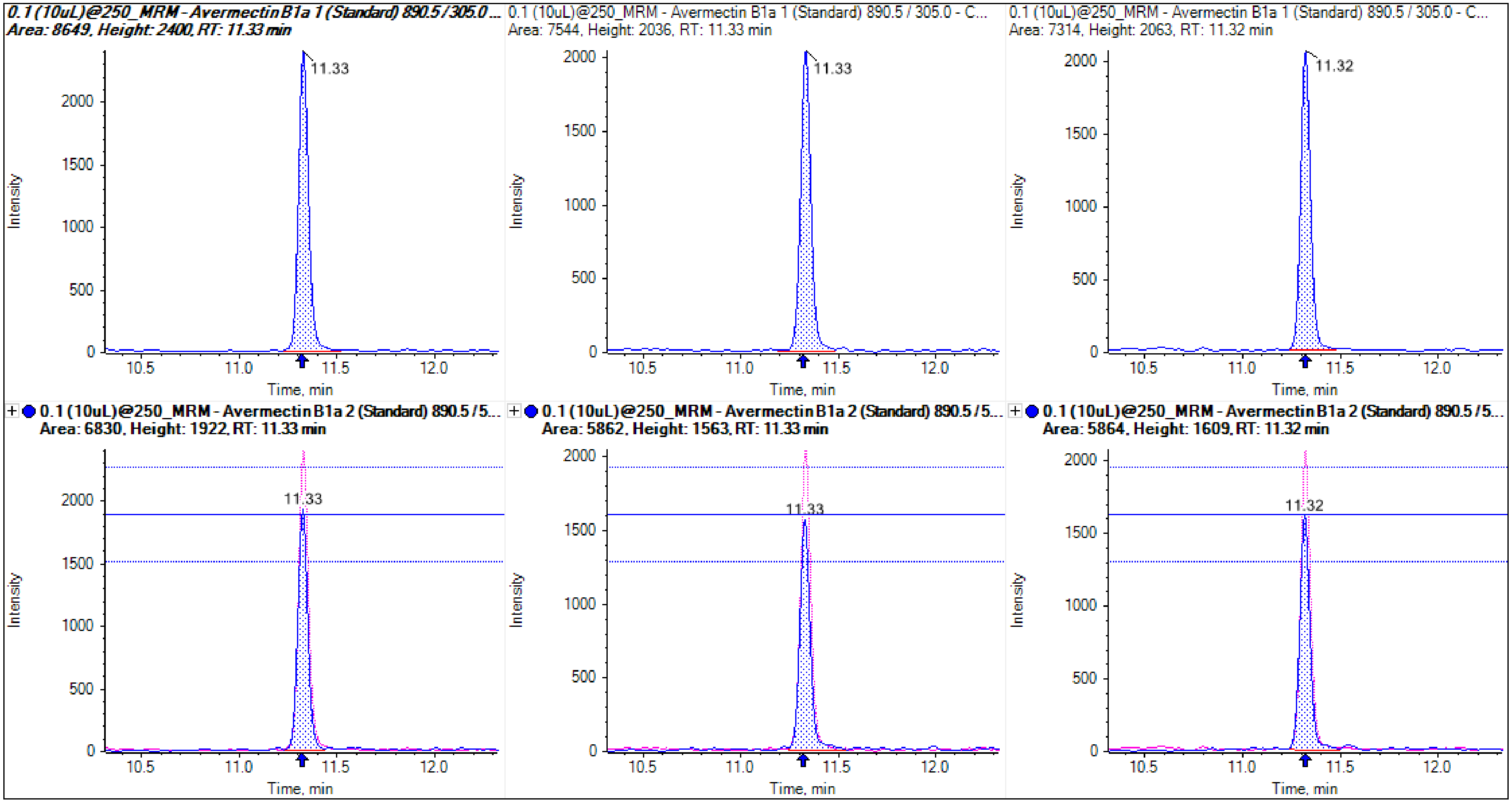
Hundreds of Compounds in a Single Analysis



Over 400 MRM transitions monitored using the *Scheduled* MRM™ Pro algorithm to quantify and identify pesticides in QuEChERS extract of fruit and vegetable samples at established maximum residue limits

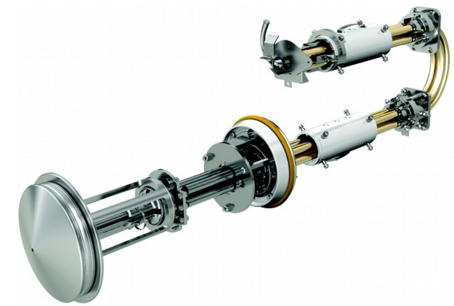
QTRAP® 6500 System – Ion Ratios

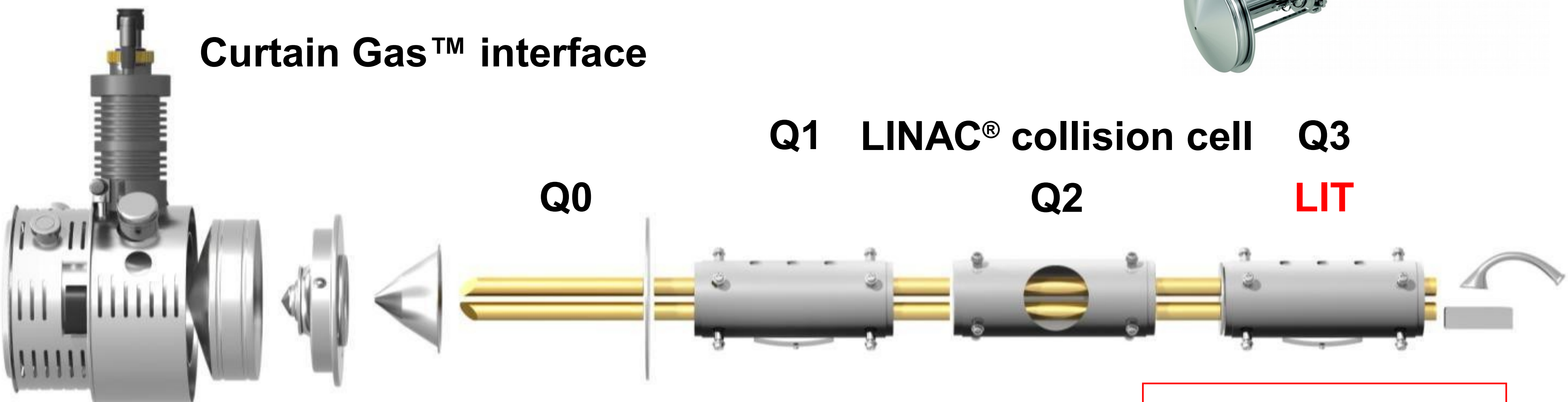
0.1 ng/mL Avermectin B1a (10 µL injected)



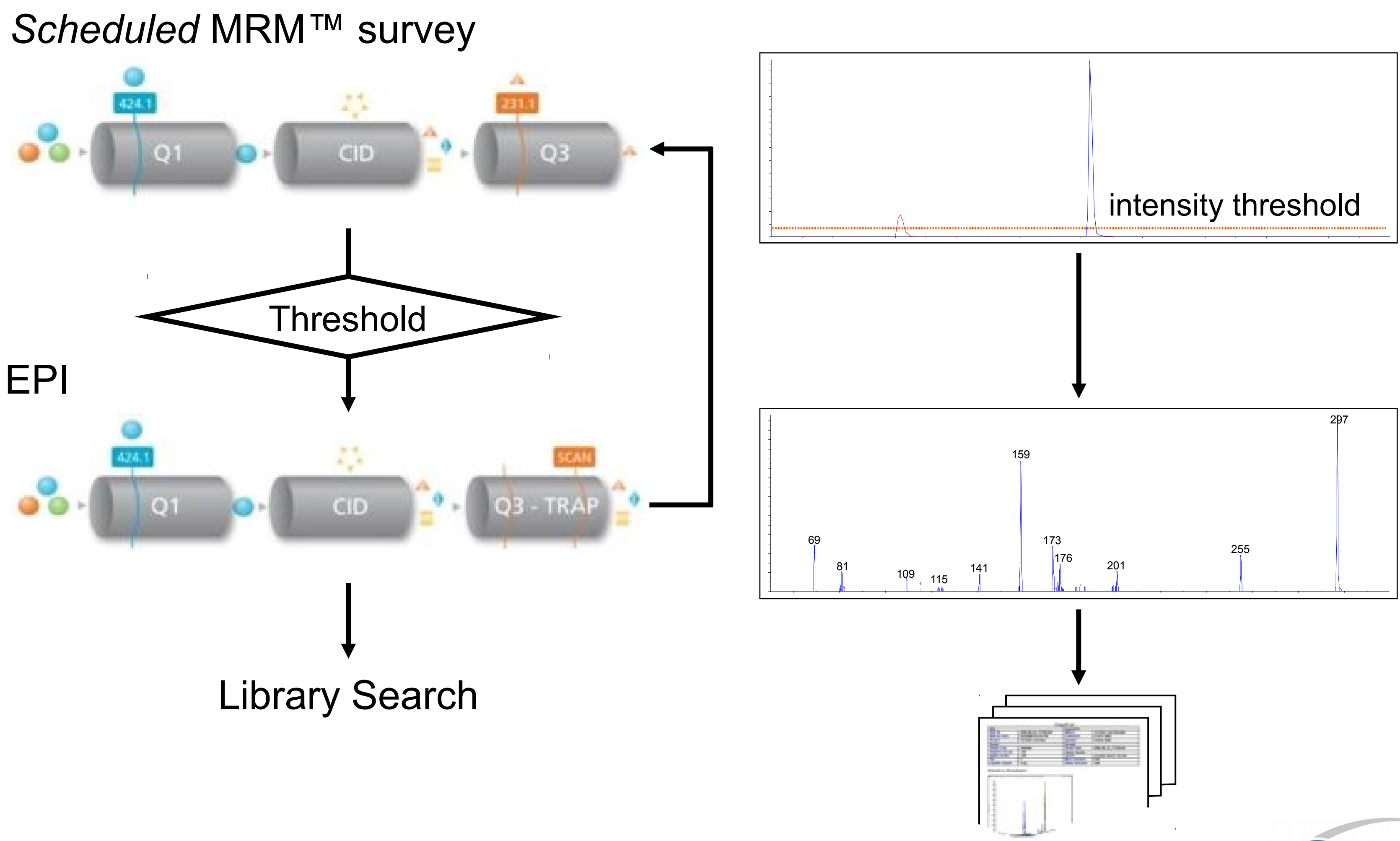
Full Scan MS/MS using QTRAP® Technology

Screening and Quantitation with Increased Confidence in Identification



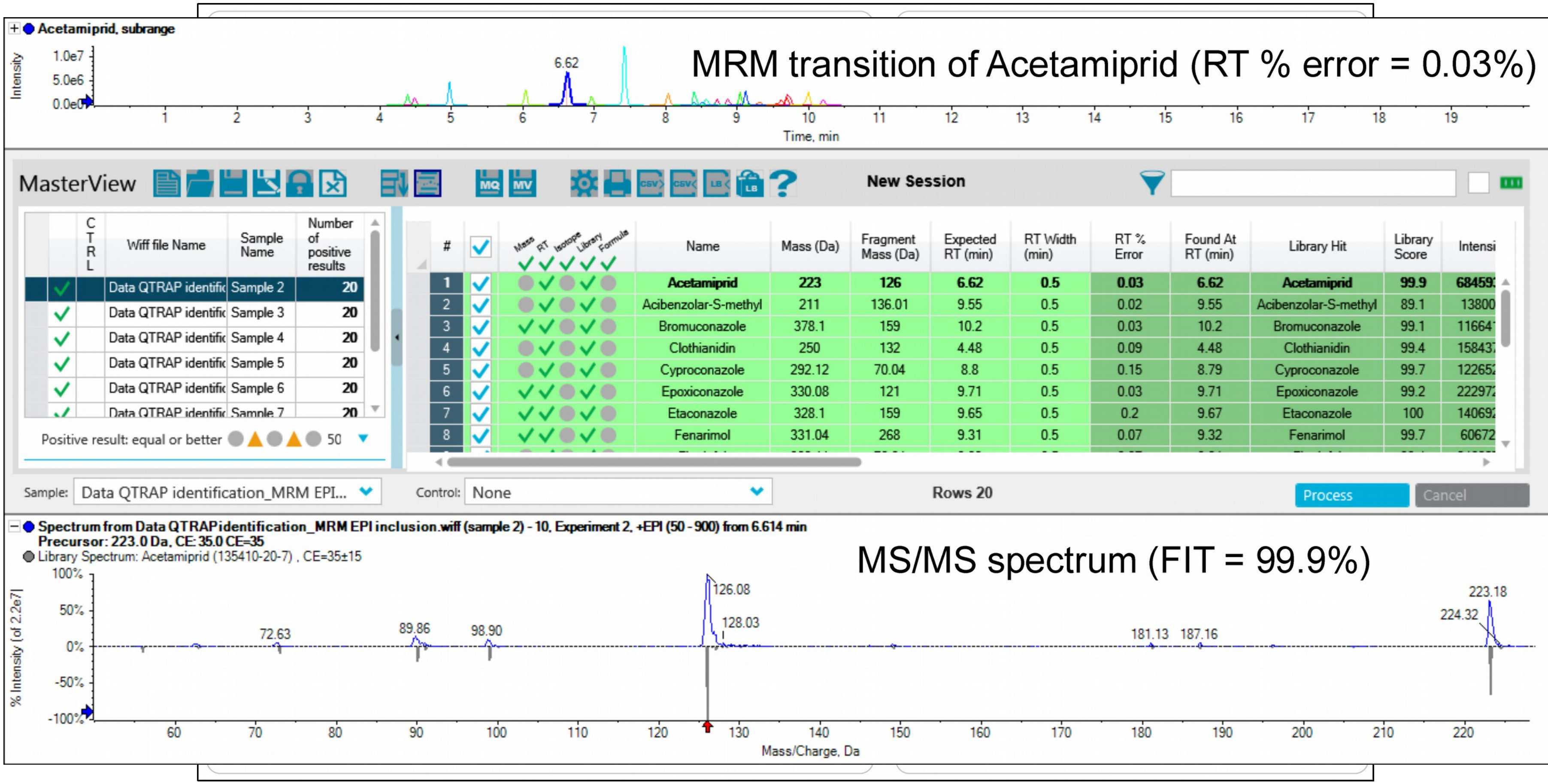


Information Dependent Acquisition (IDA)



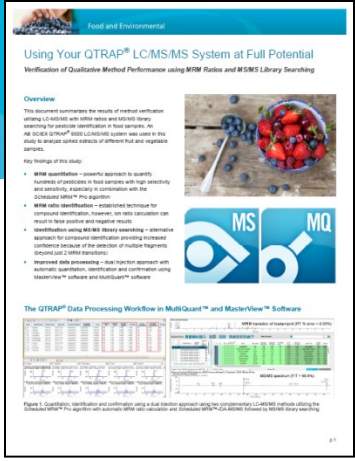
Compound Identification using MS/MS Full Scan

Scheduled MRM-IDA-MS/MS complementary to MRM Ratios



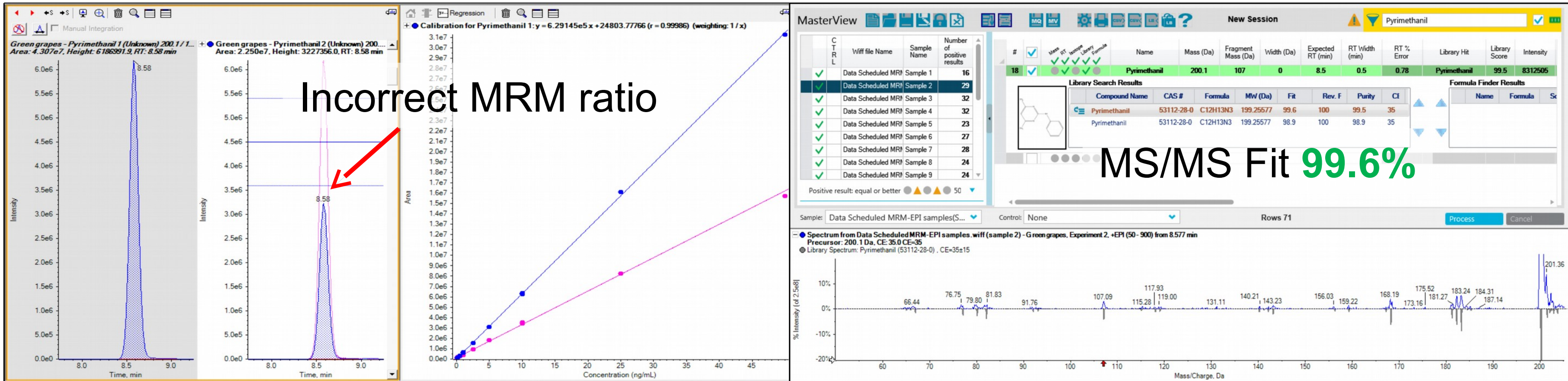
Processing in MasterView™ Software

Why QTRAP® ?
Increase Confidence in Identification

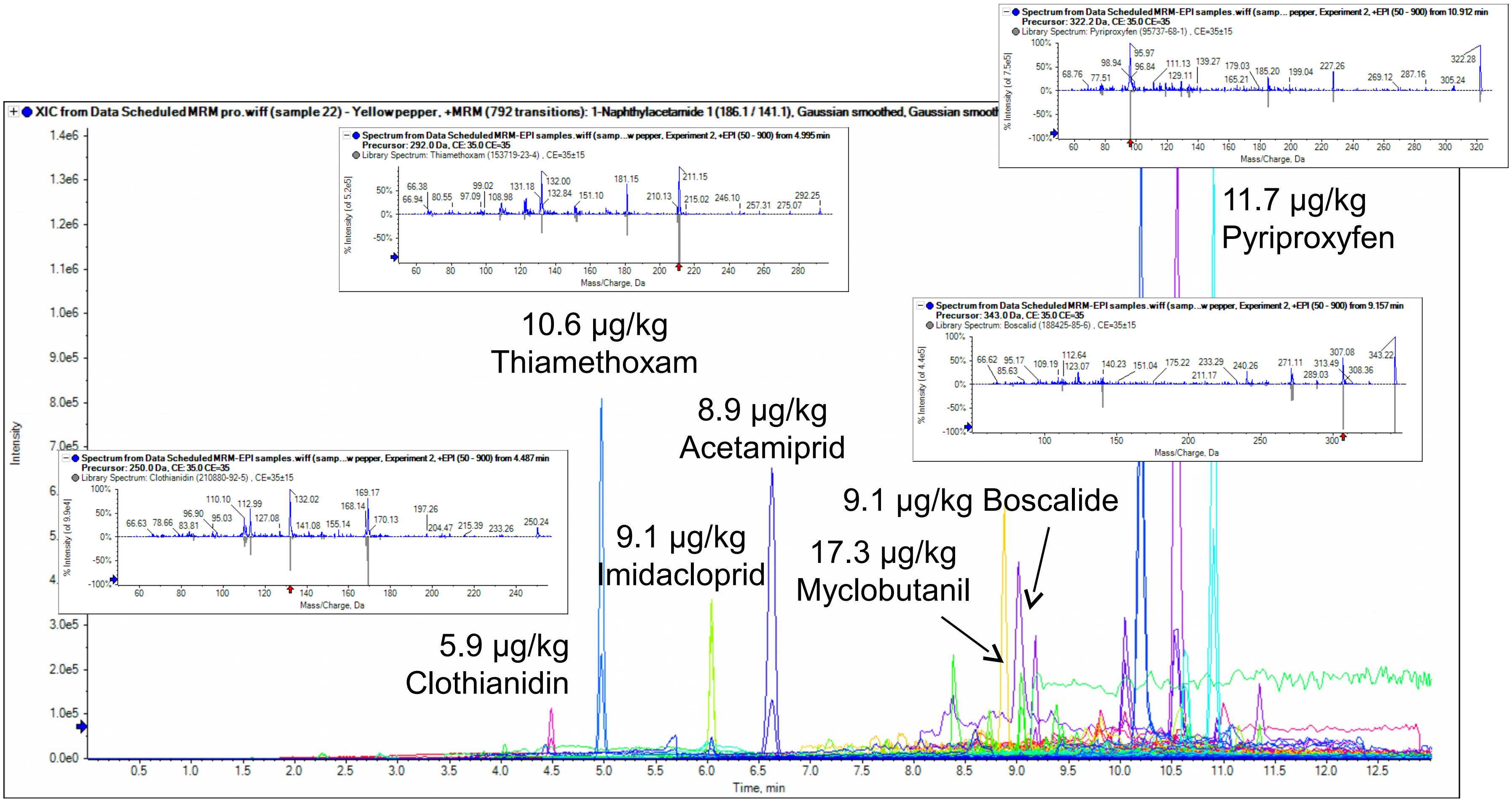


20 pesticides spiked into different fruit samples

- Identification based on MRM ratio
  - **95%** correct identification, **1%** questionable (review), **4%** false negative (outside acceptable MRM tolerance) – all confirmed by MS/MS library searching
- Identification based on MS/MS library searching
  - **99%** correct identification, **1%** questionable (review) – confirmed with ratio

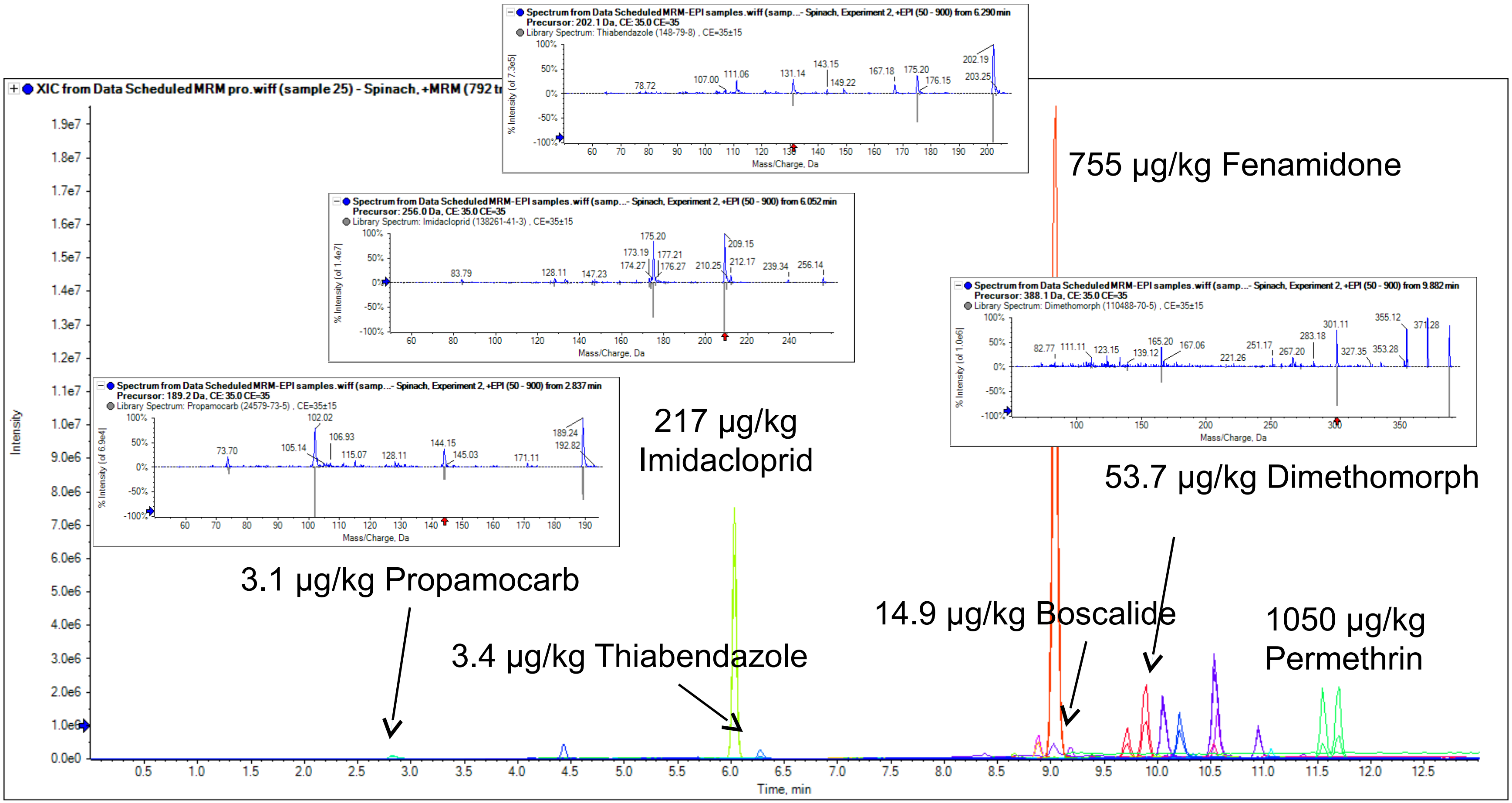


Pesticides in Yellow Pepper



QuEChERS Phenomenex roQ (EN 15562 with PSA dSPE)
Extract of 10 g with 10 mL and 10x dilution of extract with water

Pesticides in Spinach

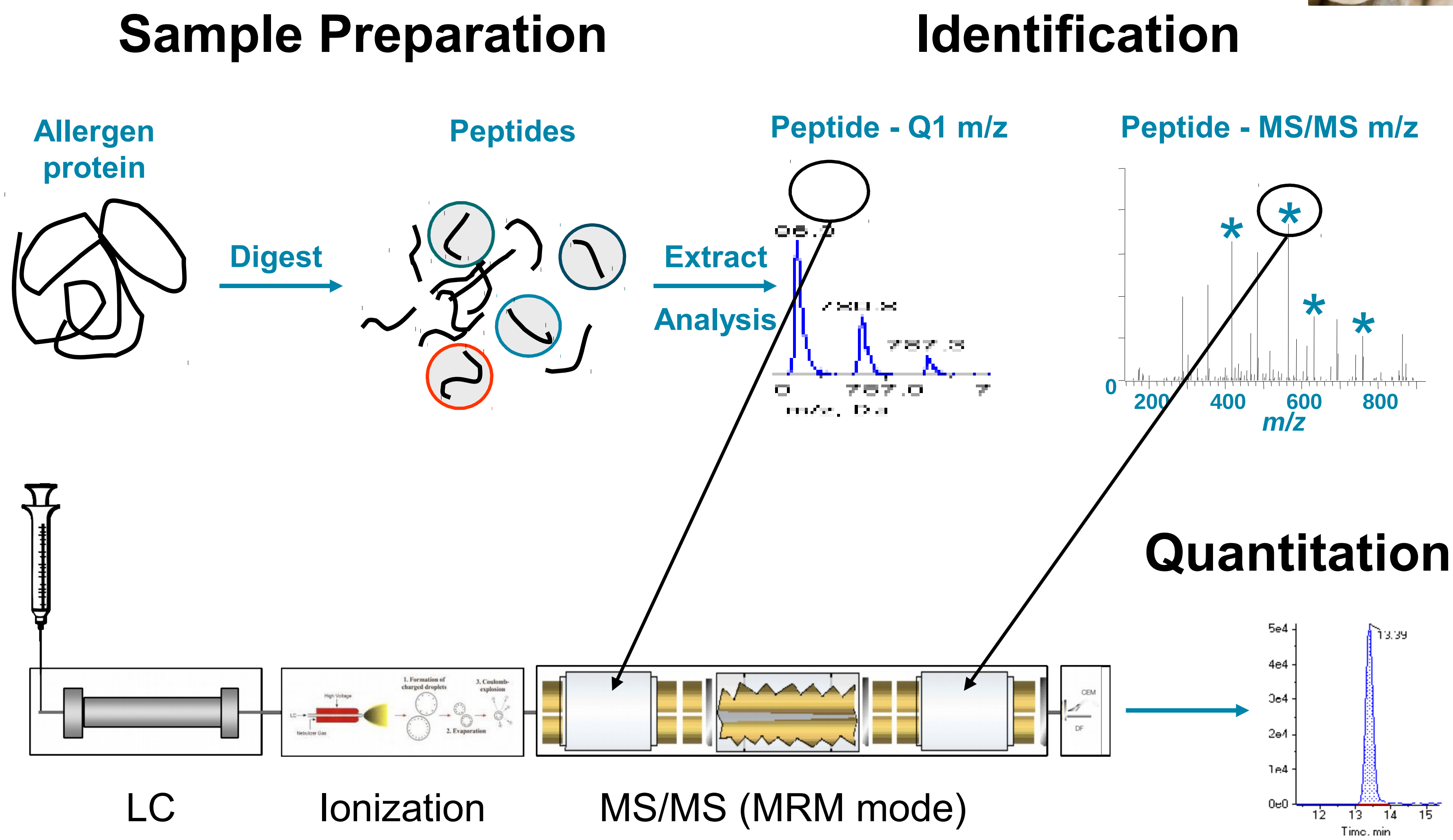


QuEChERS Phenomenex roQ (EN 15562 with PSA/GCB dSPE)
Extract of 10 g with 10 mL and 10x dilution of extract with water

Allergen Analysis by LC-MS/MS

- Over 150 million people worldwide suffer from a food allergy
  - Food allergy statistics in North America:
    - ~8% of children
    - 3.7% of adults
    - … and rising
- What are allergens?
  - Complex mix of chemicals including proteins and sulfites that induce an adverse response in the body
- The cure?
  - There is none! The only solution is to avoid consumption of the allergen.
- As a result, accurate food labeling for allergens is extremely important and requires analysis.

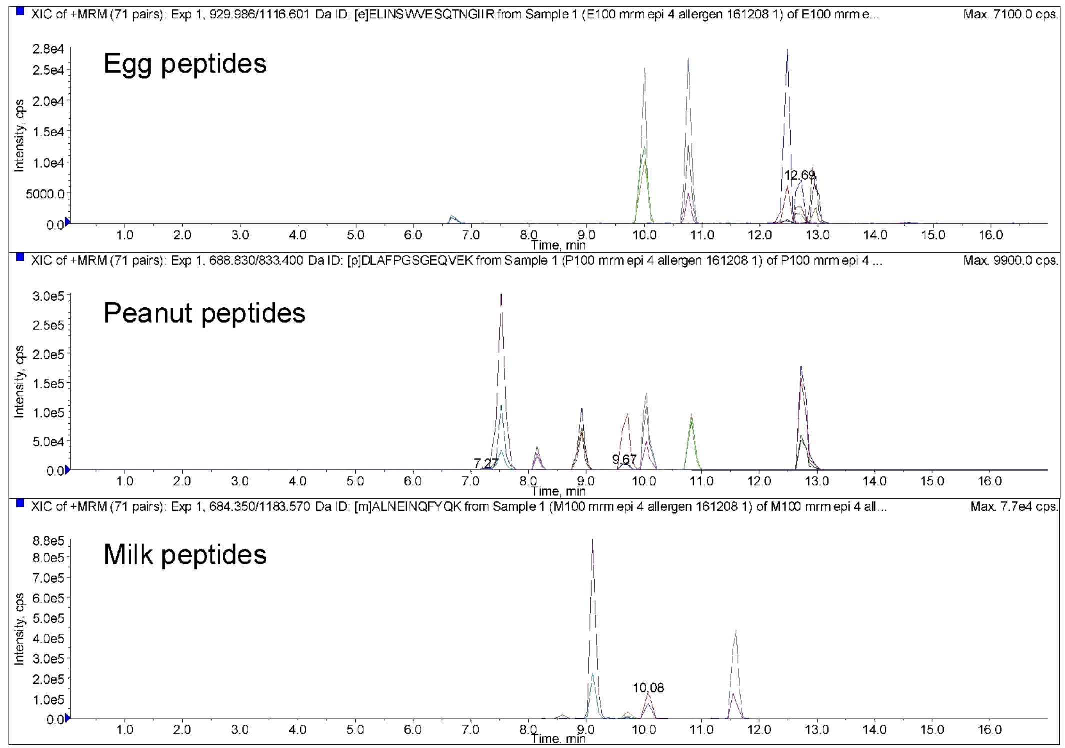
Allergen Analysis by LC-MS/MS



Multi-Allergen Screening

Method built on the foundation of the work of Heick and Pöpping

- Benefits of LC-MS/MS:
  - Multiple allergens in one injection
  - Fewer false positive or false negative results
  - Multiple fragments for each allergen detected
  - Full MS/MS fragmentation enables sequencing for highest level of selectivity
  - Accurate quantitation
  - More Confidence



Multi-allergen analysis

Expending the Allergen-Method (1)

- LC-MS/MS for nut allergen analysis:
  - Very large class
  - Includes tree nuts (i.e. pistachio, brazil, hazelnut, cashew, macadamia, walnut, pecan)
  - Includes peanut
  - Includes sesame

Is what we eat authentic?


Horse meat scandal highlights need for systematic testing

By Joe Whitworth+ 28-Aug-2013 Post a comm

Related tags: Brand protection, Eurofins, Danone, Horsemeat, , Testing, Regulation

Related topics: Financial & Industry, Meat, fish and savoury ingredients

The horse meat scandal in Europe highlights the need for more systematic testing, according to Eurofins.

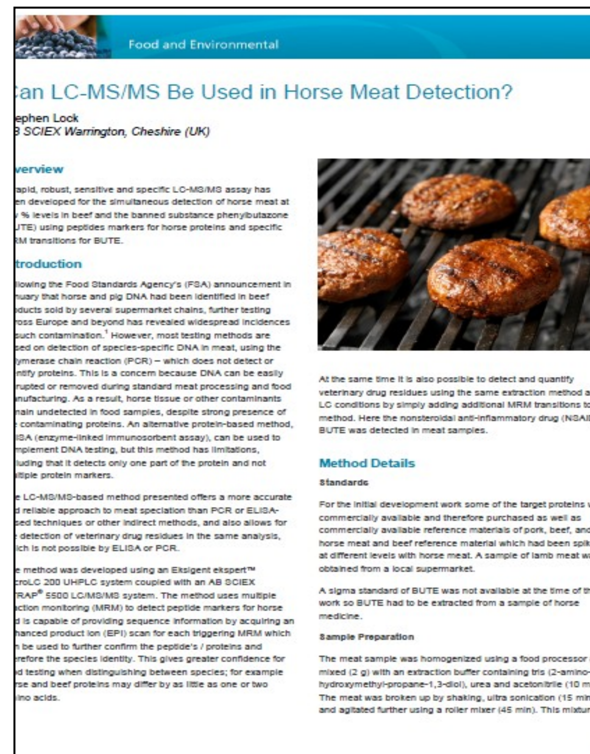
After horsemeat, pork may be next scandal, say farmers

By Fiona Barry , 27-Aug-2013 Post a comm

Picture Credit: (Neeltje van Helvoort/flickr)

Authenticity of meat

Horse, pork, duck, lamb, fox…


Food and Environmental

Can LC-MS/MS Be Used in Horse Meat Detection?

ephen Lock
B SCIEX Warrington, Cheshire (UK)

verview

apid, robust, sensitive and specific LC-MS/MS assay has en developed for the simultaneous detection of horse meat at % levels in beef and the banned substance phenylbutazone UTE) using peptides markers for horse proteins and specific RM transitions for BUTE.

troduction

llowing the Food Standards Agency's (FSA) announcement in nuary that horse and pig DNA had been identified in beef oducts sold by several supermarket chains, further testing ross Europe and beyond has revealed widespread incidences such contamination.[1] However, most testing methods are sed on detection of species-specific DNA in meat, using the lymerase chain reaction (PCR) – which does not detect or ntify proteins. This is a concern because DNA can be easily rupted or removed during standard meat processing and food nufacturing. As a result, horse tissue or other contaminants nain undetected in food samples, despite strong presence of contaminating proteins. An alternative protein-based method, ISA (enzyme-linked immunosorbent assay), can be used to mplement DNA testing, but this method has limitations, luding that it detects only one part of the protein and not ltiple protein markers.

e LC-MS/MS-based method presented offers a more accurate d reliable approach to meat speciation than PCR or ELISA-sed techniques or other indirect methods, and also allows for detection of veterinary drug residues in the same analysis, ich is not possible by ELISA or PCR.

e method was developed using an Eksigent ekspert™ croLC 200 UHPLC system coupled with an AB SCIEX RAP® 5500 LC/MS/MS system. The method uses multiple ction monitoring (MRM) to detect peptide markers for horse d is capable of providing sequence information by acquiring an hanced product ion (EPI) scan for each triggering MRM which n be used to further confirm the peptide's / proteins and erefore the species identity. This gives greater confidence for d testing when distinguishing between species; for example rse and beef proteins may differ by as little as one or two ino acids.

At the same time it is also possible to detect and quantify veterinary drug residues using the same extraction method and LC conditions by simply adding additional MRM transitions to th method. Here the nonsteroidal anti-inflammatory drug (NSAID) BUTE was detected in meat samples.

Method Details

Standards

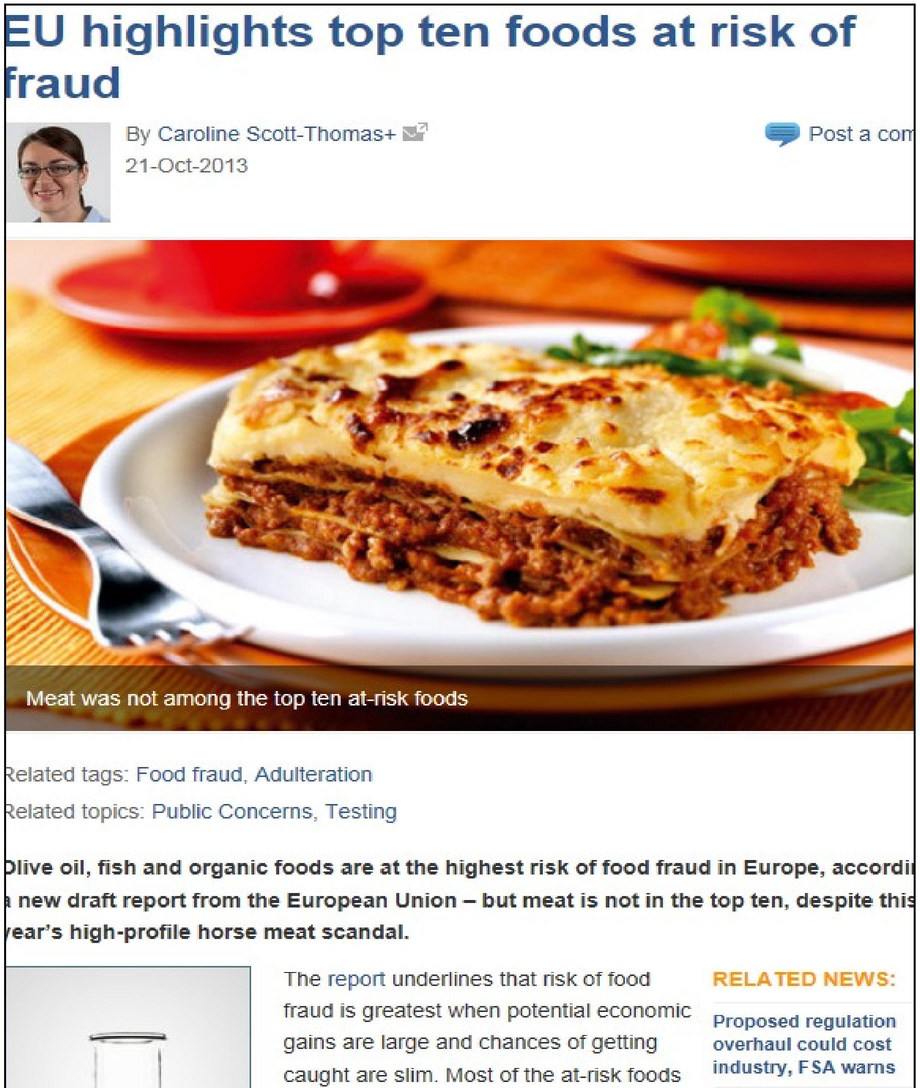
For the initial development work some of the target proteins we commercially available and therefore purchased as well as commercially available reference materials of pork, beef, and horse meat and beef reference material which had been spiked at different levels with horse meat. A sample of lamb meat was obtained from a local supermarket.

A sigma standard of BUTE was not available at the time of this work so BUTE had to be extracted from a sample of horse medicine.

Sample Preparation

The meat sample was homogenized using a food processor an mixed (2 g) with an extraction buffer containing tris (2-amino-2-hydroxymethyl-propane-1,3-diol), urea and acetonitrile (10 mL). The meat was broken up by shaking, ultra sonication (15 min) and agitated further using a roller mixer (45 min). This mixture

Hot Topics and Trends – Top 10 Authenticity



EU highlights top ten foods at risk of fraud

By Caroline Scott-Thomas+

21-Oct-2013

Post a com

Meat was not among the top ten at-risk foods

Related tags: Food fraud, Adulteration

Related topics: Public Concerns, Testing

Olive oil, fish and organic foods are at the highest risk of food fraud in Europe, accordi a new draft report from the European Union – but meat is not in the top ten, despite this year's high-profile horse meat scandal.

The report underlines that risk of food fraud is greatest when potential economic gains are large and chances of getting caught are slim. Most of the at-risk foods

RELATED NEWS:

Proposed regulation overhaul could cost industry, FSA warns

- Olive oil
- Meat and fish
- Organic produce
- Milk
- Grains
- Honey and maple syrup
- Coffee and tea
- Spices (chili, saffron)
- Fruit juices
- Wine

Wine for cats launched in Japan

By Mark Astley+

17-Oct-2013

Post a com

Meat Speciation – Is it Authentic?

- Similar workflow to allergen analysis can be used for meat speciation and authenticity testing
- Extraction, digestion, cleanup, LC-MS/MS

Sample Preparation

Extract proteins from food sample (1 hour in buffer)

↓

Centrifuge and collect extract (30 min)

↓

Reduce, alkylate, and digest overnight with trypsin (14 hours)

↓

Filter digested extract (15 min)

↓

SPE clean-up, dry-down eluent, and reconstitute (2.5 hours)

↓

LC-MS/MS analysis

- Extraction of proteins
- Digestion into peptides
- Off-line SPE extraction
- LC-MS/MS analysis
  - Micro flow LC
  - Halo C18 50 x 0.5 mm
  - Gradient water/acetonitrile + 0.1% formic acid
  - 20 µL/min
  - SCIEX QTRAP® 5500 system
  - MRM-IDA-MS/MS (quantitation and identification)

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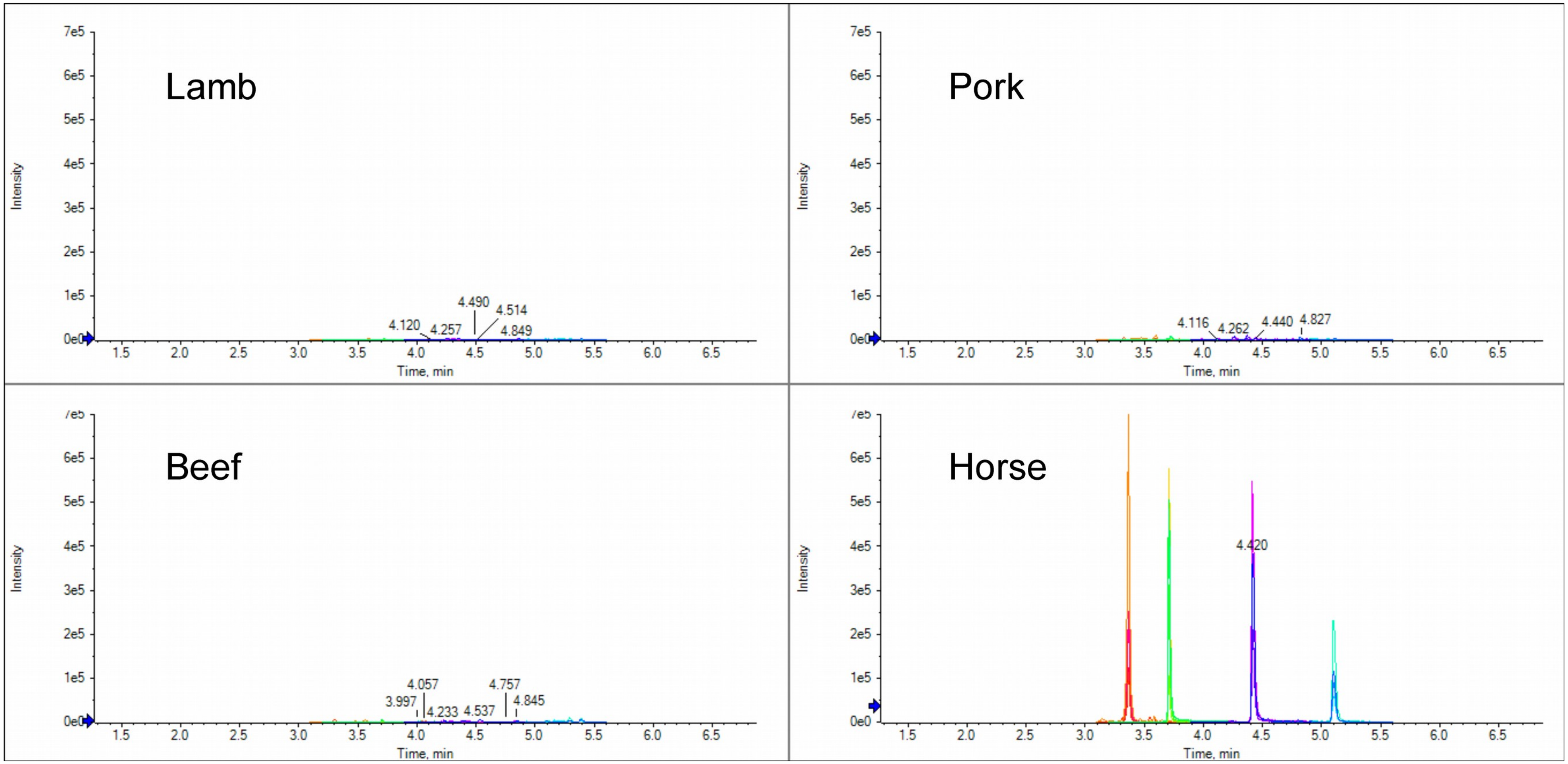
Detection of Lamb, Beef, Horse Pork, and Horse Markers

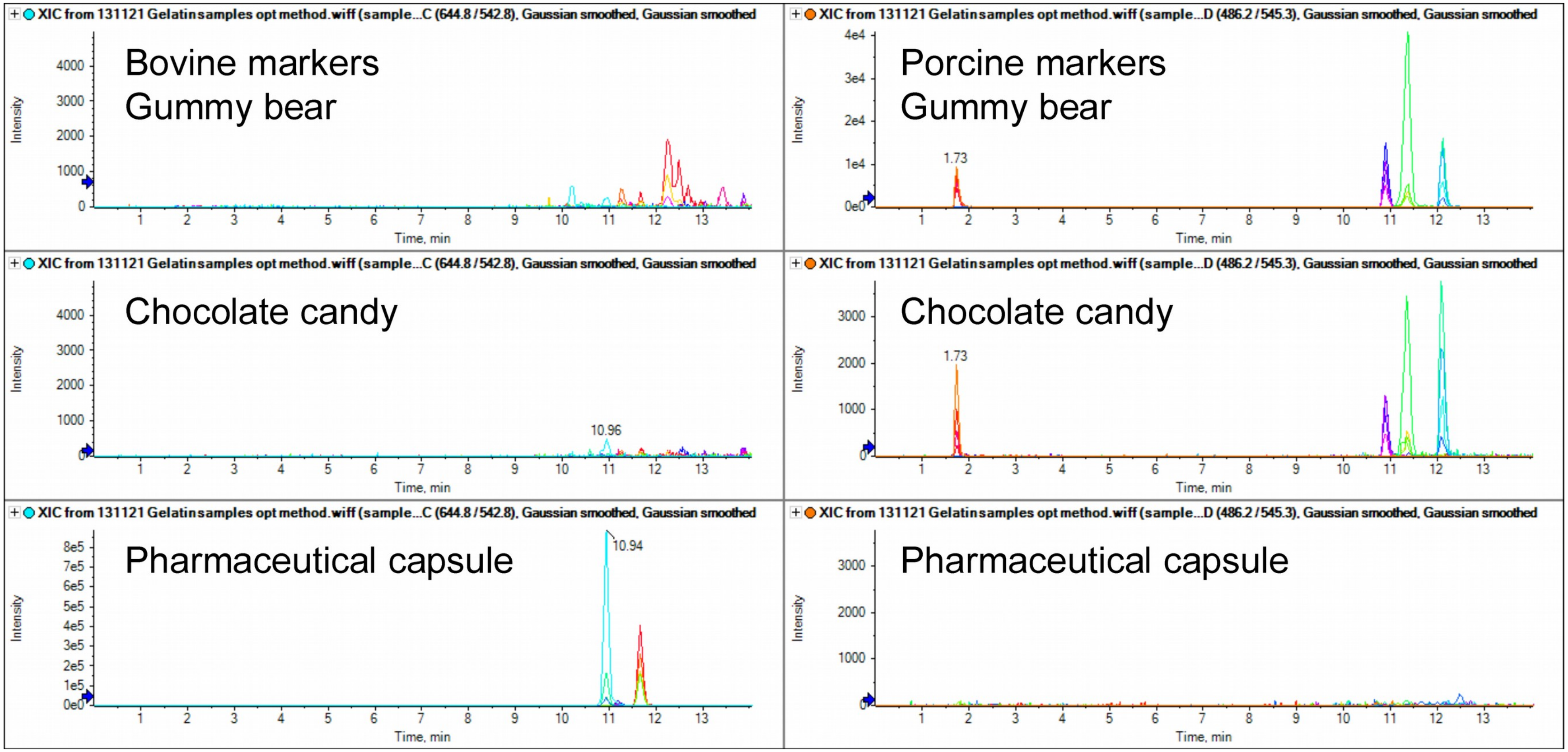
Meat Reference Materials Analyzed by LC-MS/MS



Horse adulteration detectable at 1%

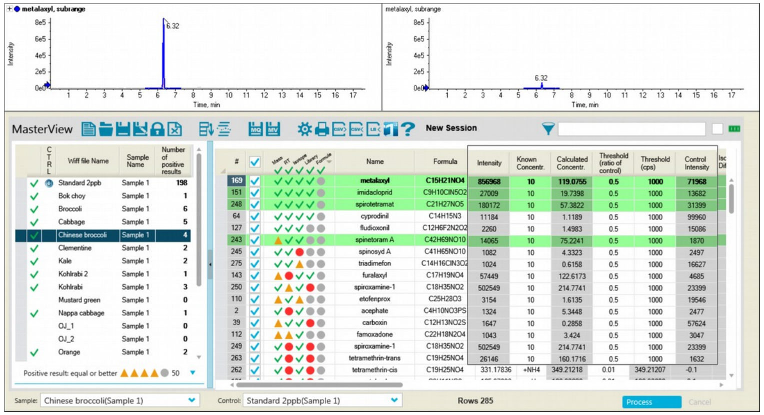
Updated method also used to identify duck, rat, fox… and to quantify veterinary drugs

Detection of Pork Gelatin Markers



Pork and beef gelatin markers detected in gummy bears, candy, and pharmaceutical capsule

Unknown Screening using High Resolution Accurate Mass LC-MS/MS



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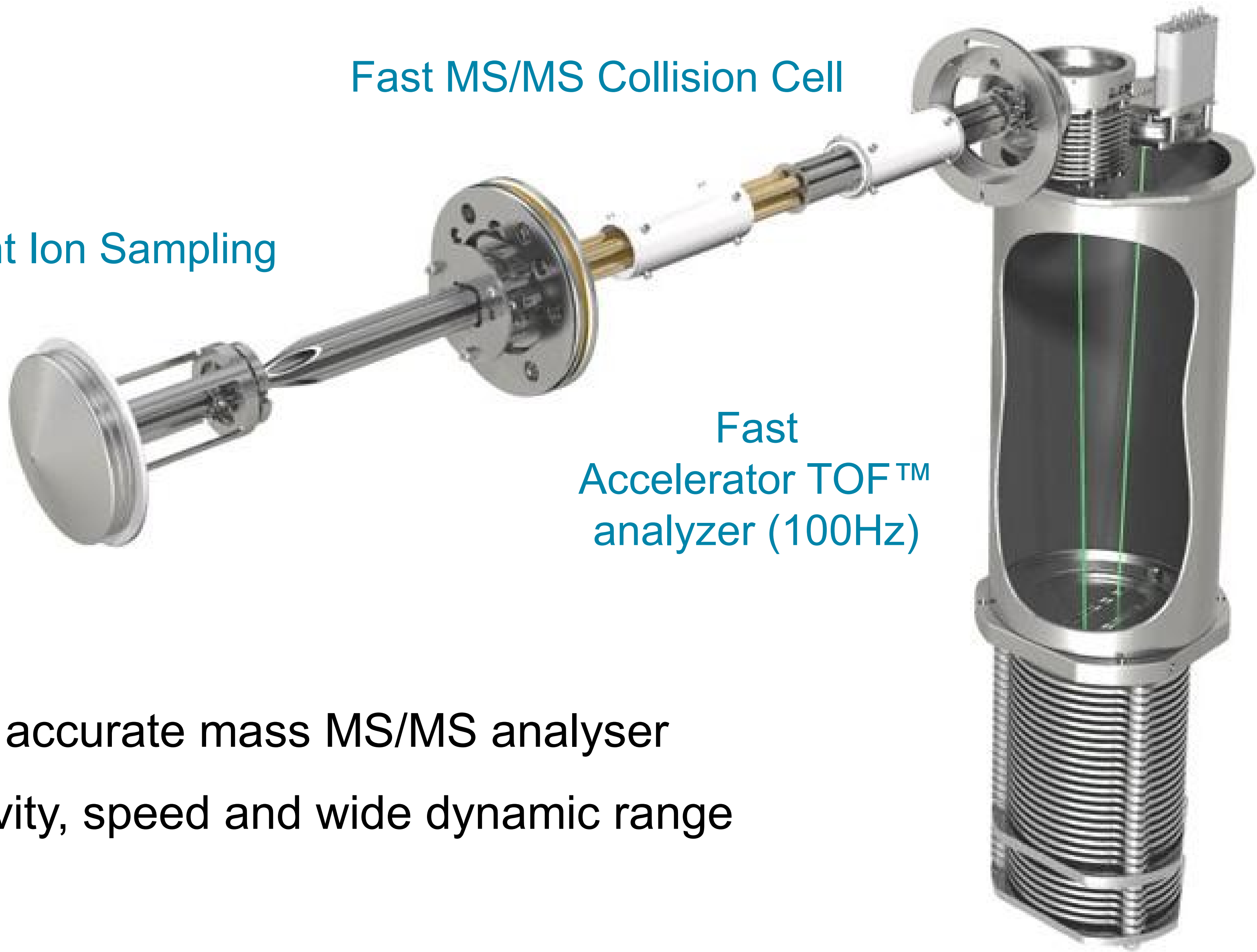
Accurate Mass MS and MS/MS

- Increased specificity through High Resolution acquisition for both MS and MS/MS data
  - Ability to screen for everything that can be ionised
- Calculate and postulate potential elemental formulas
  - Better to use both MS and MSMS information
- Accurate Mass MS/MS data, provides a chemical fingerprint of a molecule
  - Increases confidence in the data through less ambiguous results from MS only data (reduced number of false positive answers)
  - Allows the postulation of chemical structure based on fragmentation

SCIEX TripleTOF® QTOF System



- Hi-Res accurate mass MS/MS analyser
- Sensitivity, speed and wide dynamic range

So, what is in my sample?

Two Possible Workflows

Targeted processing:

Quickly screen against your target list:

- Identification using 4 criteria:
  - Retention time
  - Accurate MS
  - Isotopic patterns
  - Accurate MS/MS

Perform quantitation:

- Ability to quantitate how much is there if a compound is identified

Non-targeted processing:

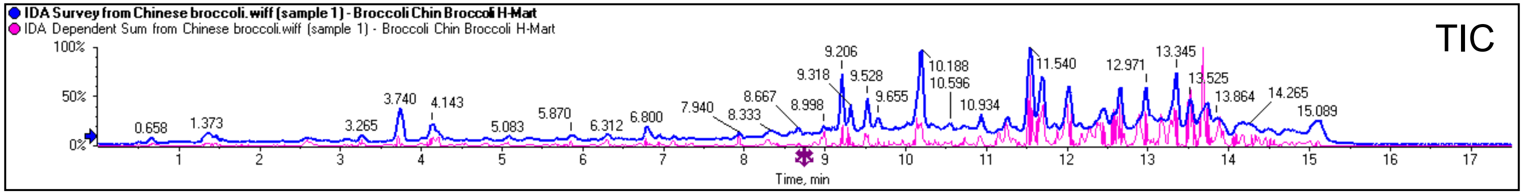
Effectively find unknowns by:

- Sample-control comparisons
- Automatic formula finder
- Library searching
- ChemSpider search for structure elucidation

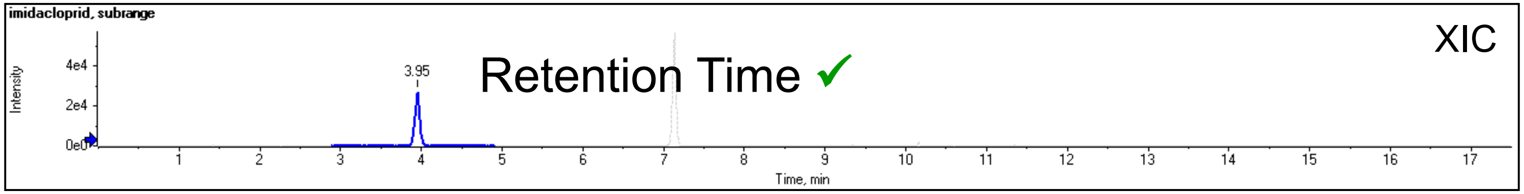
Searching for what you know

Automatic Target Identification and Quantitation

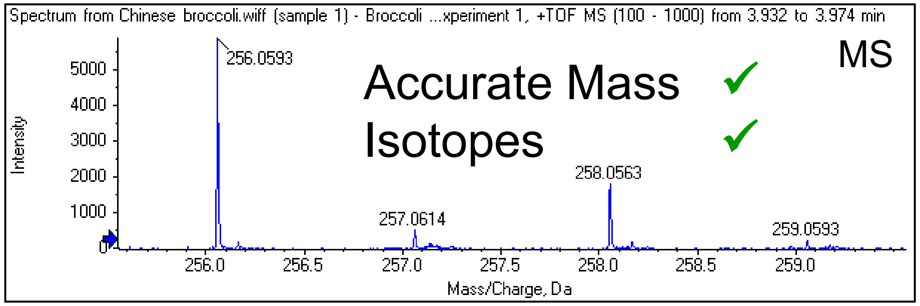
TIC of TOF-MS-IDA-MS/MS



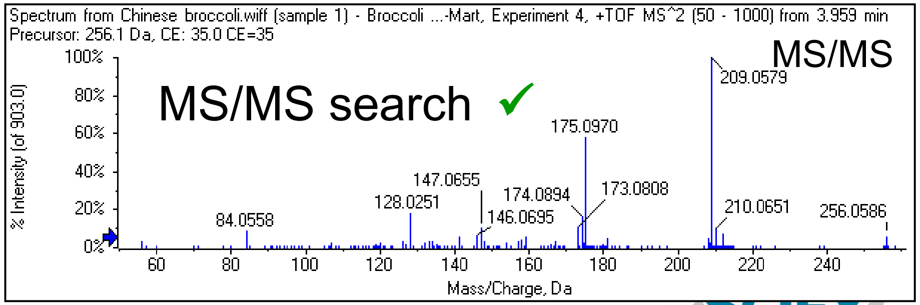
Generation of Extracted Ion Chromatogram (XIC) for each Target



Evaluation of TOF-MS



Evaluation of TOF-MS/MS (Library Search)



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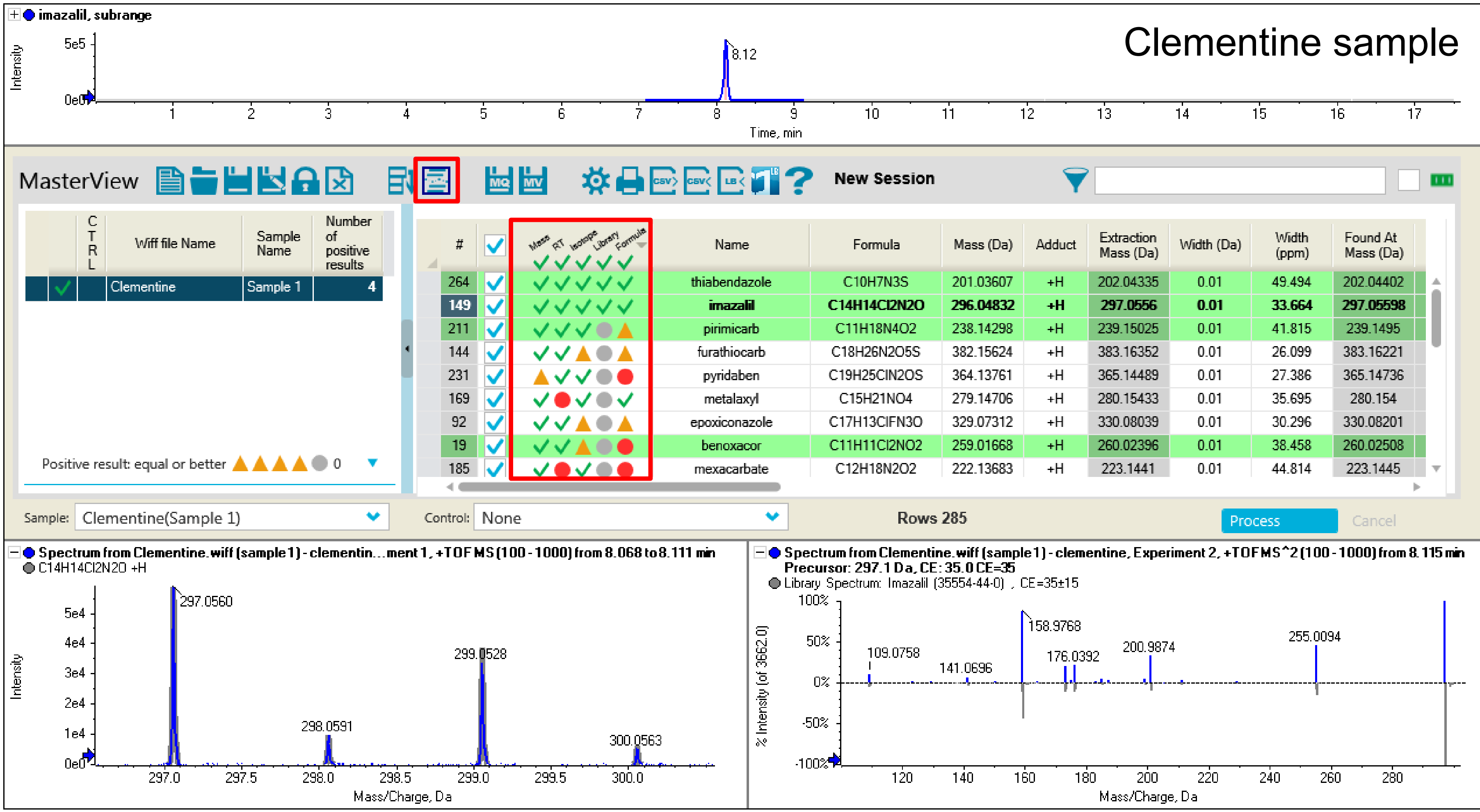
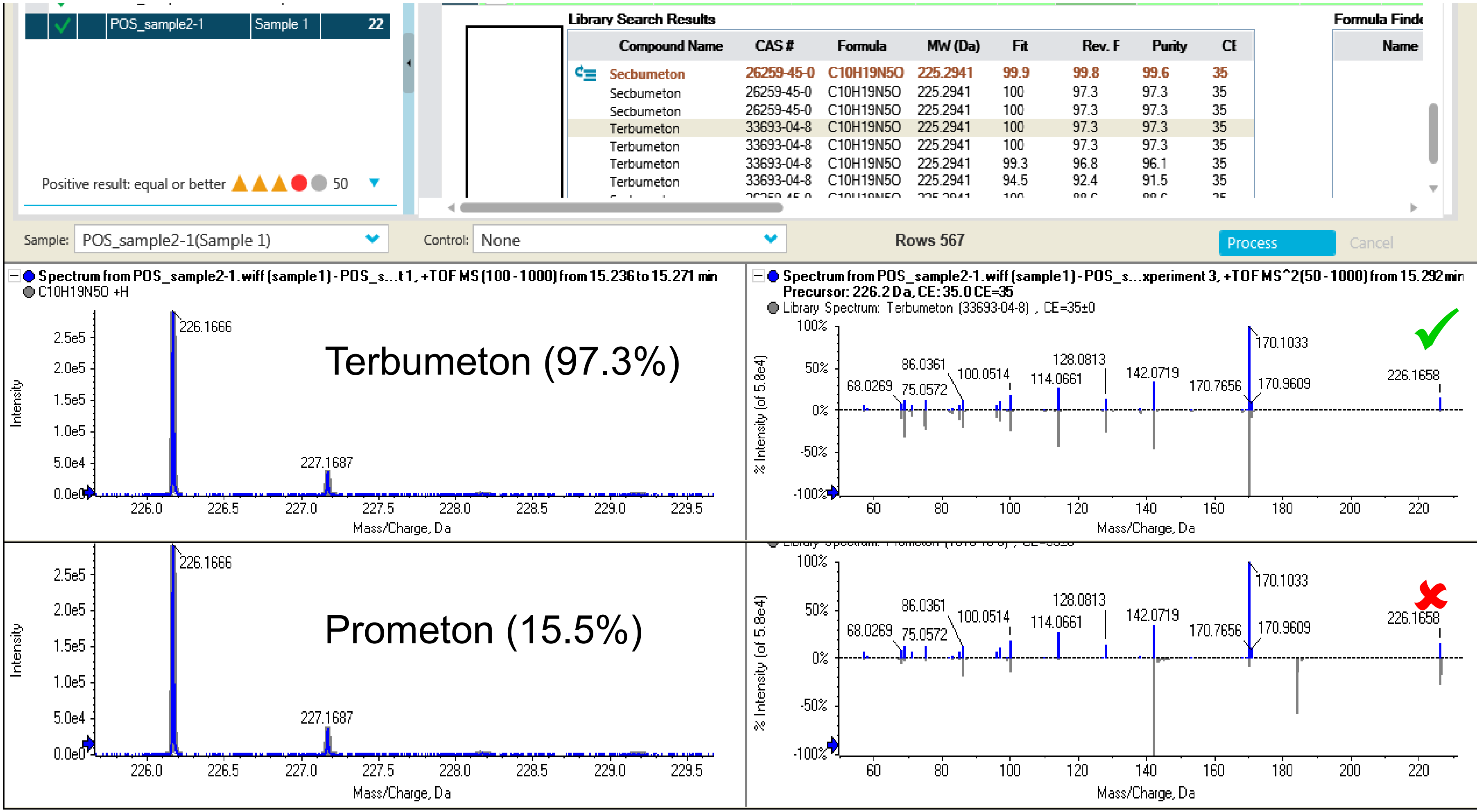
Process Results Based on Targeted List with MS/MS



Data EU Proficiency Test

MS/MS Information is Crucial to Minimize False Positive Results



High probability of false positive for Prometon without MS/MS information

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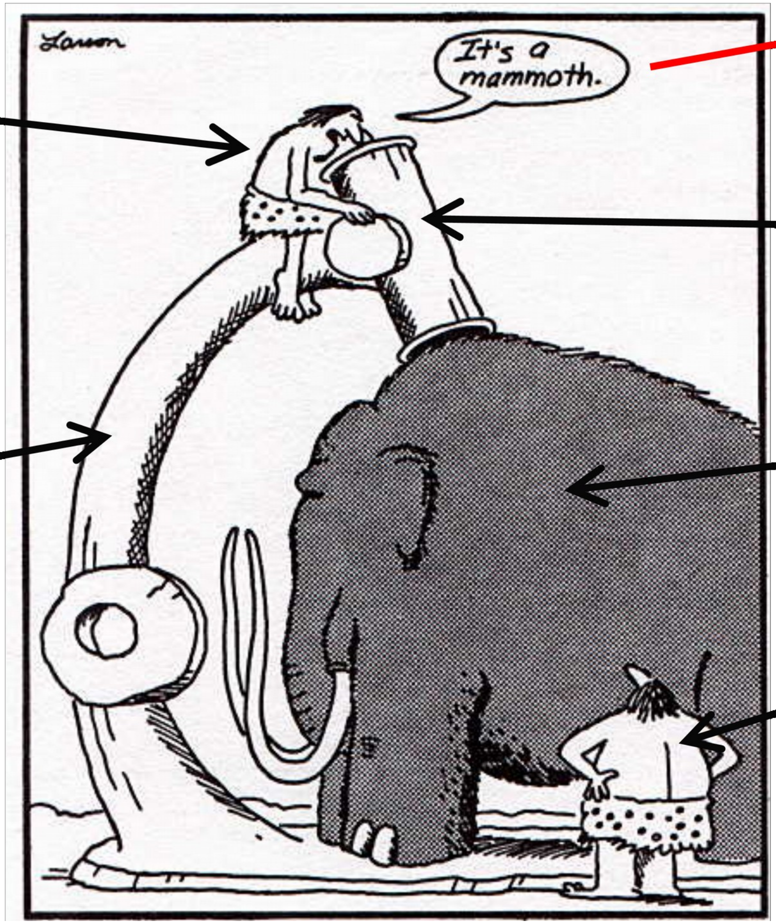
EU Proficiency Test Data - Results

- Sample were analyzed using a SCIEX TripleTOF® 5600 system
- Samples were processed in MasterView™ software
  - XIC table and library of 567 pesticides
  - Data processing < 30 seconds
- 43 out of 44 pesticides in the provided leek and pear sample were correctly identified
  - One false negative result (low QuEChERS recovery for Ethoxyquin)
  - Mass errors between -1.7 and 2.1 ppm (positive polarity) and -0.7 and 2ppm (negative polarity) were well below required 5 ppm and retention time errors were well below required 2.5% (SANCO/12495/2011)
  - No false positive results due to MS/MS library searching

What's Really in my Sample? – Non Target Screening

Skilled operator (extensive knowledge or spectral library)

Hardware



The answer!

Software interface

Sample

QC

So, WHAT really is in my sample?

Two streamlined data processing workflows:

Non-targeted processing:

Effectively find unknowns by:

- Sample-control comparisons
- Automatic formula finder
- Library searching
- ChemSpider search for structure elucidation

Simplifying Data Complexity

Comparative Unknown Screening

What is it and why is this a good approach?

Comparative screening = comparing your unknown sample to a known control sample to <u>more quickly isolate unique features</u>

vs. organic food product

vs. clean water sample

vs. un-treated sample

Comparative screening processing can reduce the list of compounds of interest from **tens of thousands** to **less than a hundred**

- Get to the answer of what is in the sample faster and more accurately

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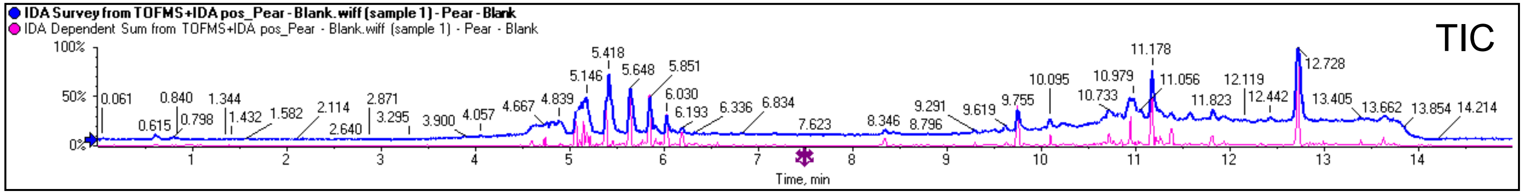
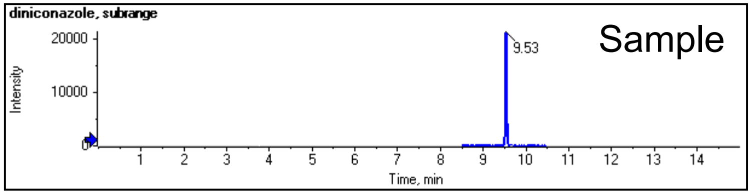
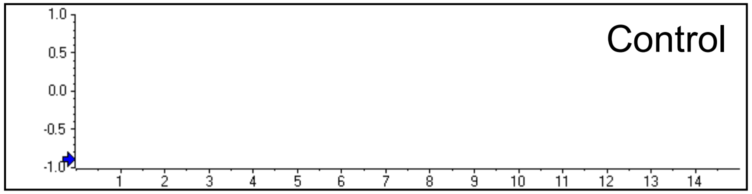
Data processing workflow for non-targeted screening

TIC of Sample and Control

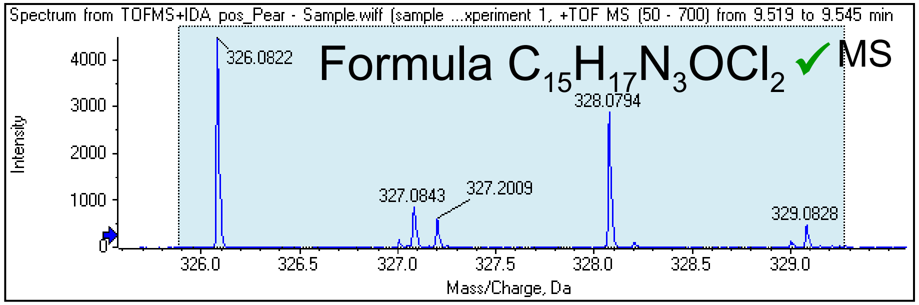


Non-Target XIC Generation and Sample-Control-Comparison

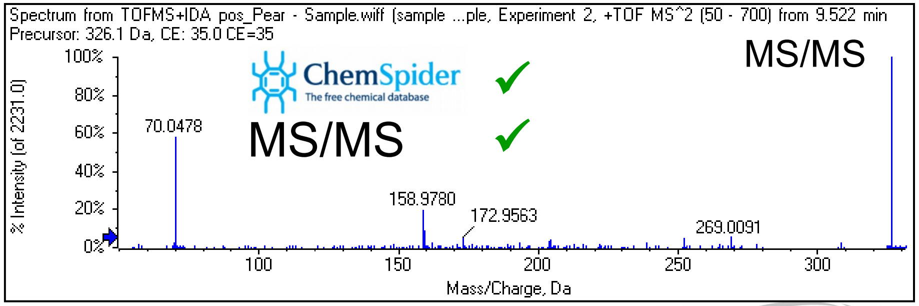




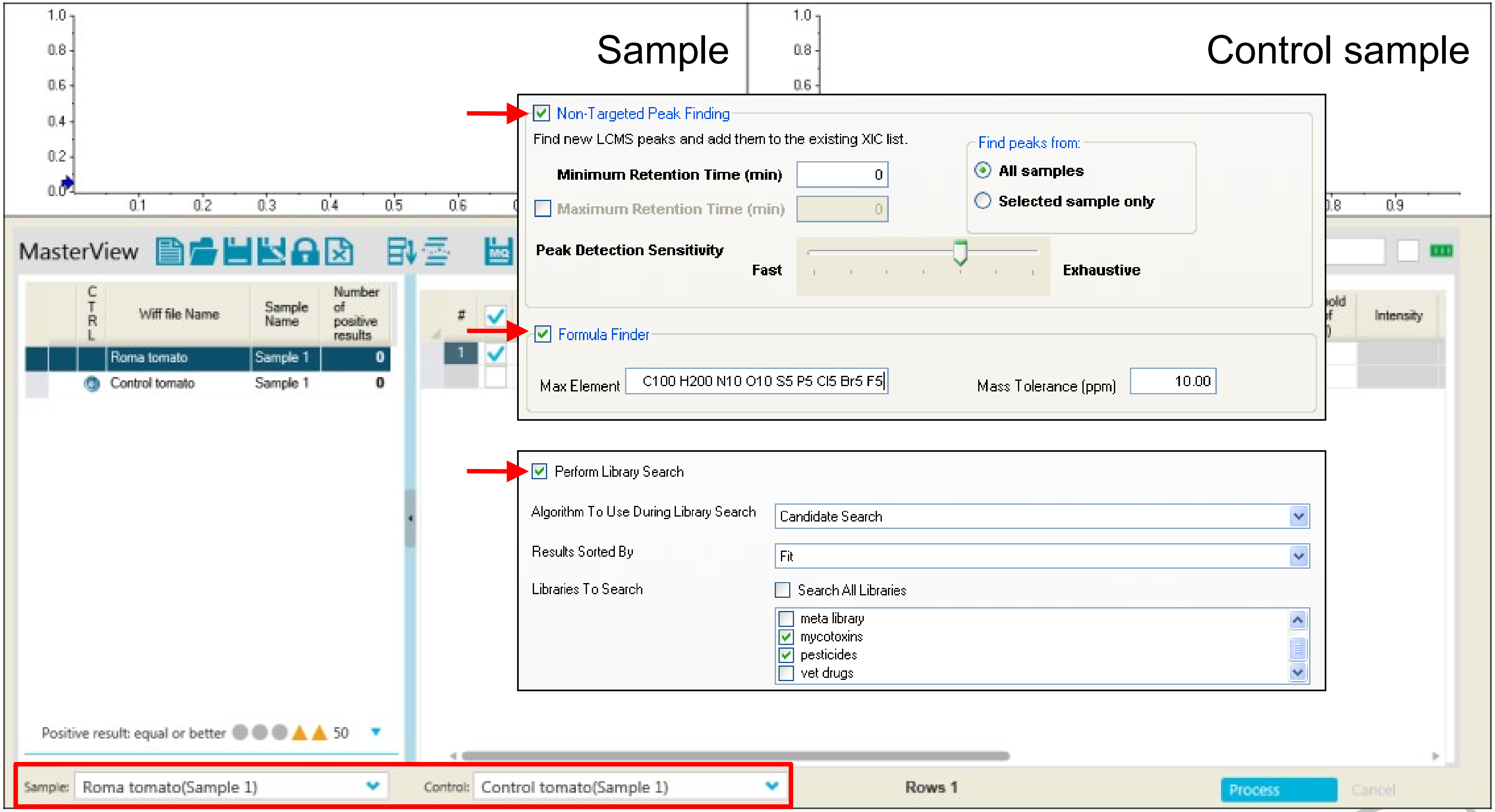
Evaluation of TOF-MS (Formula Finder)



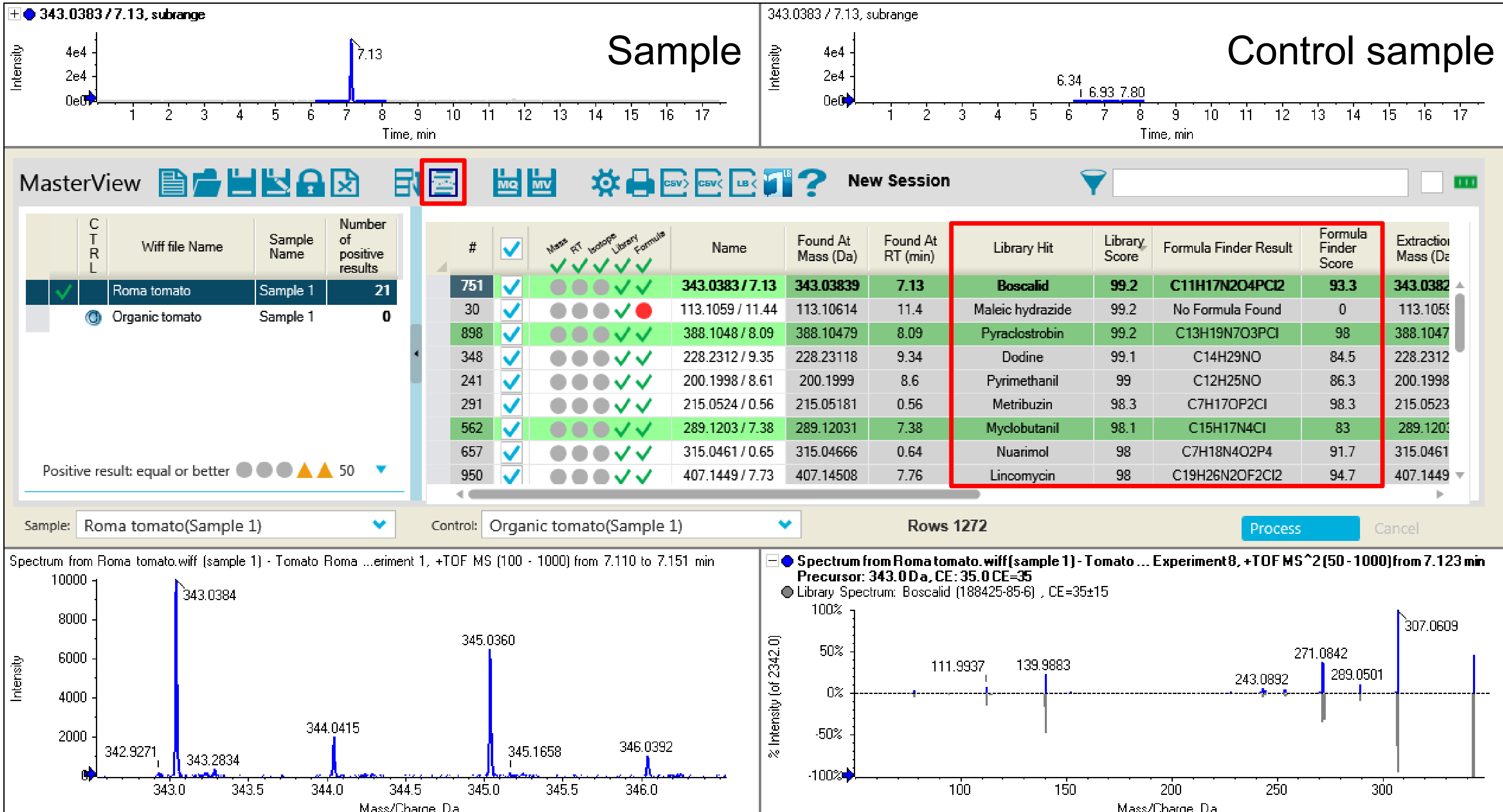
Evaluation of TOF-MS/MS (Search)



Sample to Control Comparison



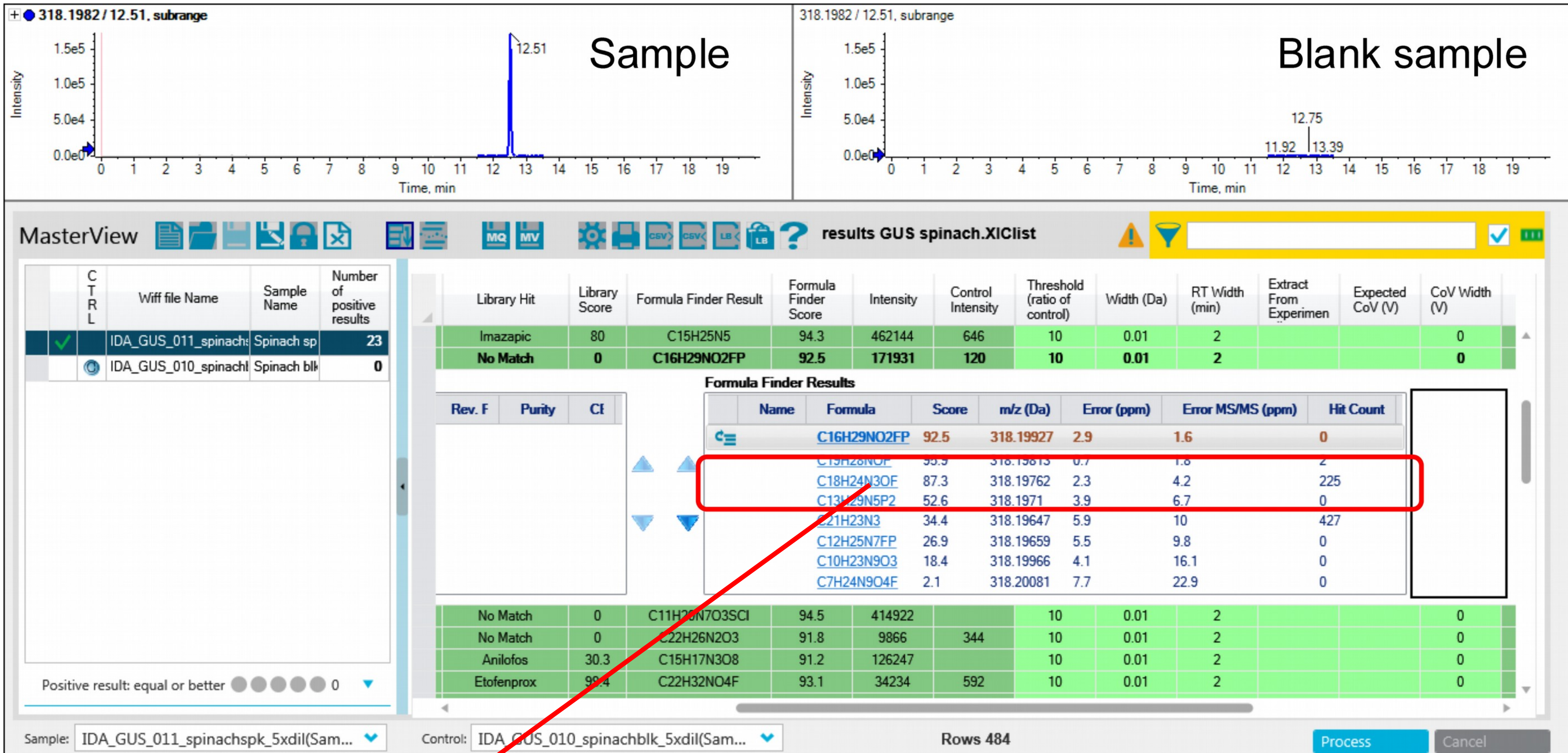
Automatic Formula Finding and MS/MS Search



1272 chromatographic peaks and 21 relevant signals

Non-Target Screening using MasterView™

Review of Formula Finding and Library Searching Results



ChemSpider
The free chemical database

MS and MS/MS match plus ChemSpider hit count to quickly identify most likely formula

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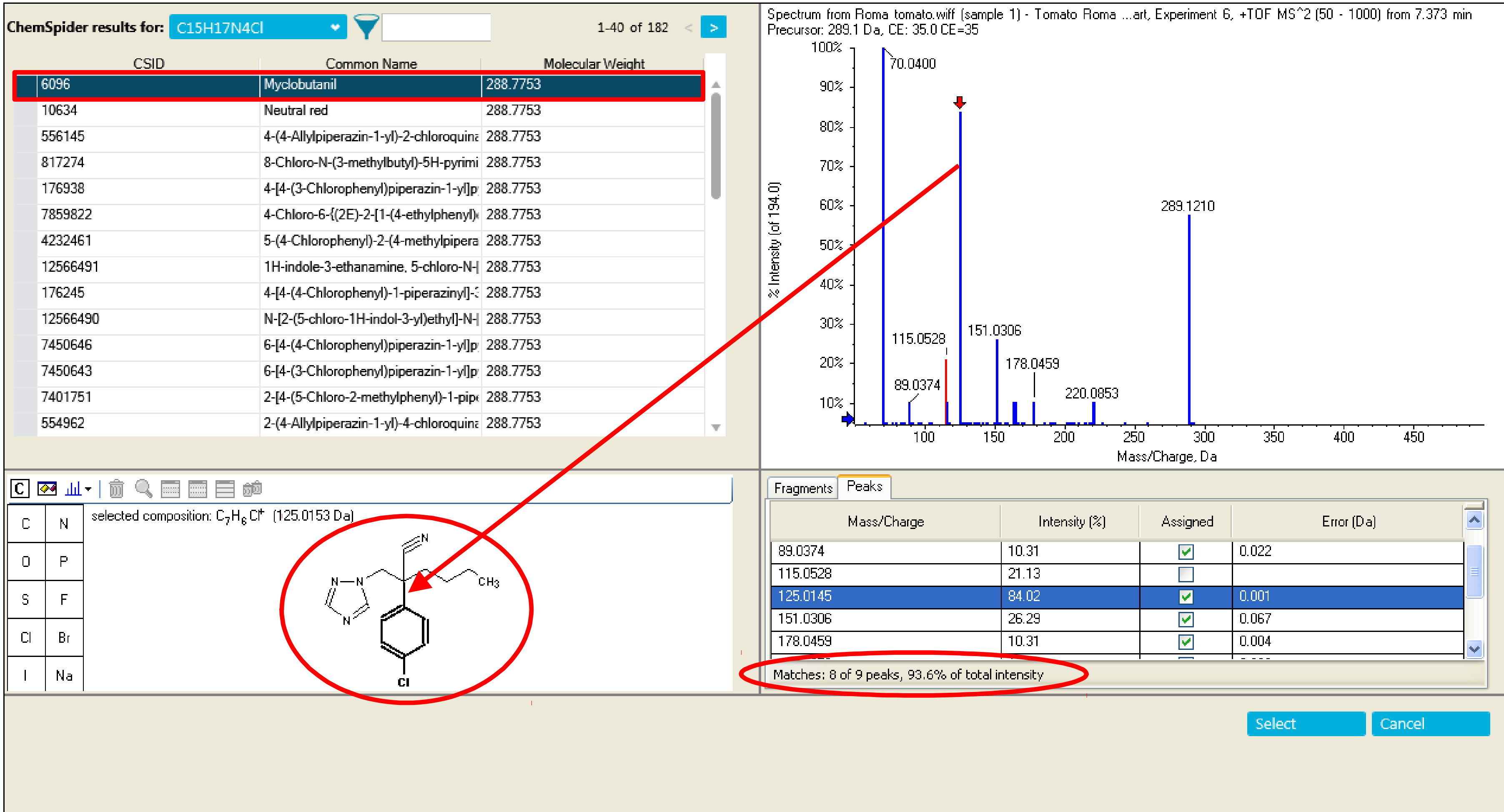
ChemSpider Search & MS/MS Interpretation



ChemSpider hits are automatically compared against MS/MS spectrum.

Hi-Res Accurate Mass MS/MS as a routine tool?

Making the ‘Needle’ in the ‘Haystack’ Easier to find

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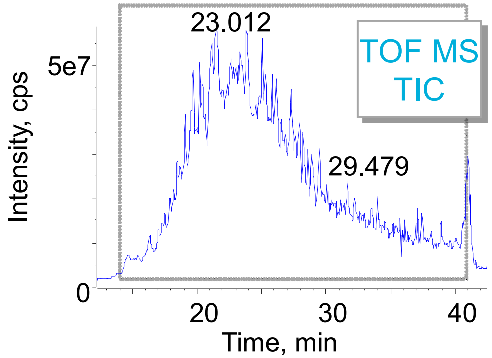
Answers for Science.
Knowledge for Life.™

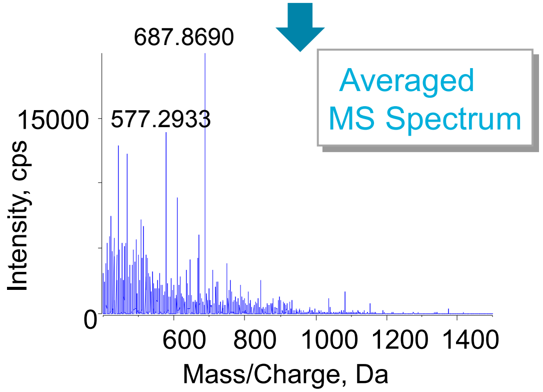
SWATH with Variable Window Acquisition for Small Molecule Analysis

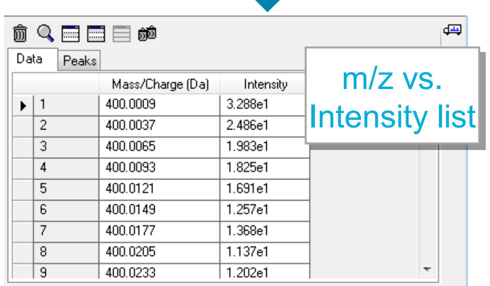
PPCPs in Water

Variable Window Calculator Tool

- Excel Sheet for Designing based on Constant Precursor Density

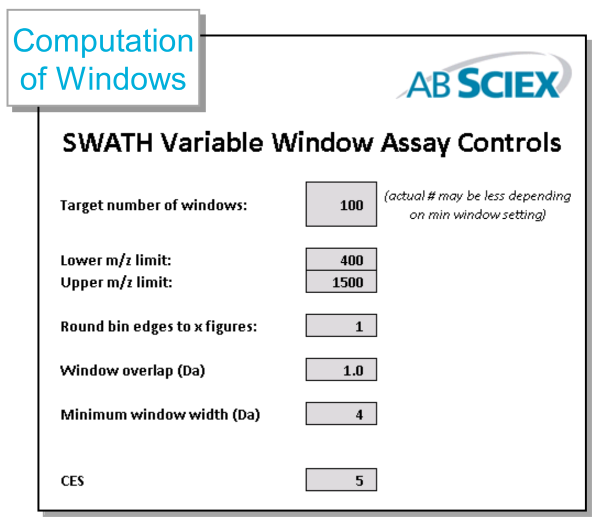






	Mass/Charge (Da)	Intensity
1	400.0009	3.288e1
2	400.0037	2.486e1
3	400.0065	1.983e1
4	400.0093	1.825e1
5	400.0121	1.691e1
6	400.0149	1.257e1
7	400.0177	1.368e1
8	400.0205	1.137e1
9	400.0233	1.202e1

- Open TIC of target Compounds (Pesticides) and extract out a MS spectrum of the whole LC run
- Generate m/z vs intensity list to paste into Excel
- Set assay parameters and build method
- www.absciex.com\VariableWindowsCalculator



399.5	449.5	5
448.5	481.5	5
480.5	505.5	5
504.5	527.5	5
526.5	547.5	5
546.5	565.5	5
564.5	579.5	5
578.5	594.5	5
593.5	608.5	5
607.5	623.5	5
622.5	637.5	5
636.5	651.5	5
650.5	664.5	5
663.5	677.5	5
676.5	691.5	5
690.5	705.5	5
704.5	718.5	5
717.5	732.5	5
731.5	745.5	5
744.5	757.5	5
756.5	772.5	5

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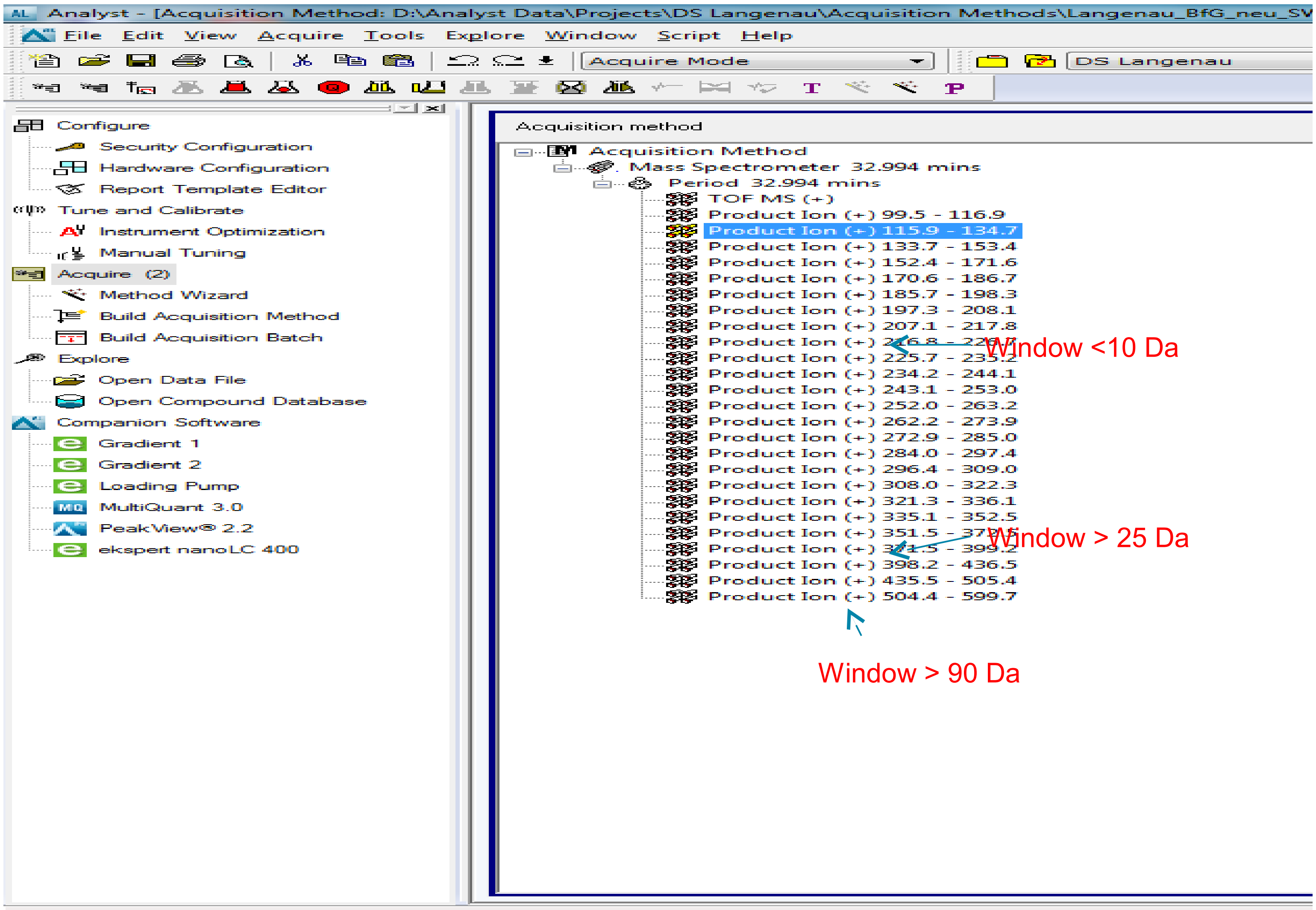
Acquisition Method – Variable Mass Windows

Example of Acquisition Method for a Mixture of 264 PPCP Compounds



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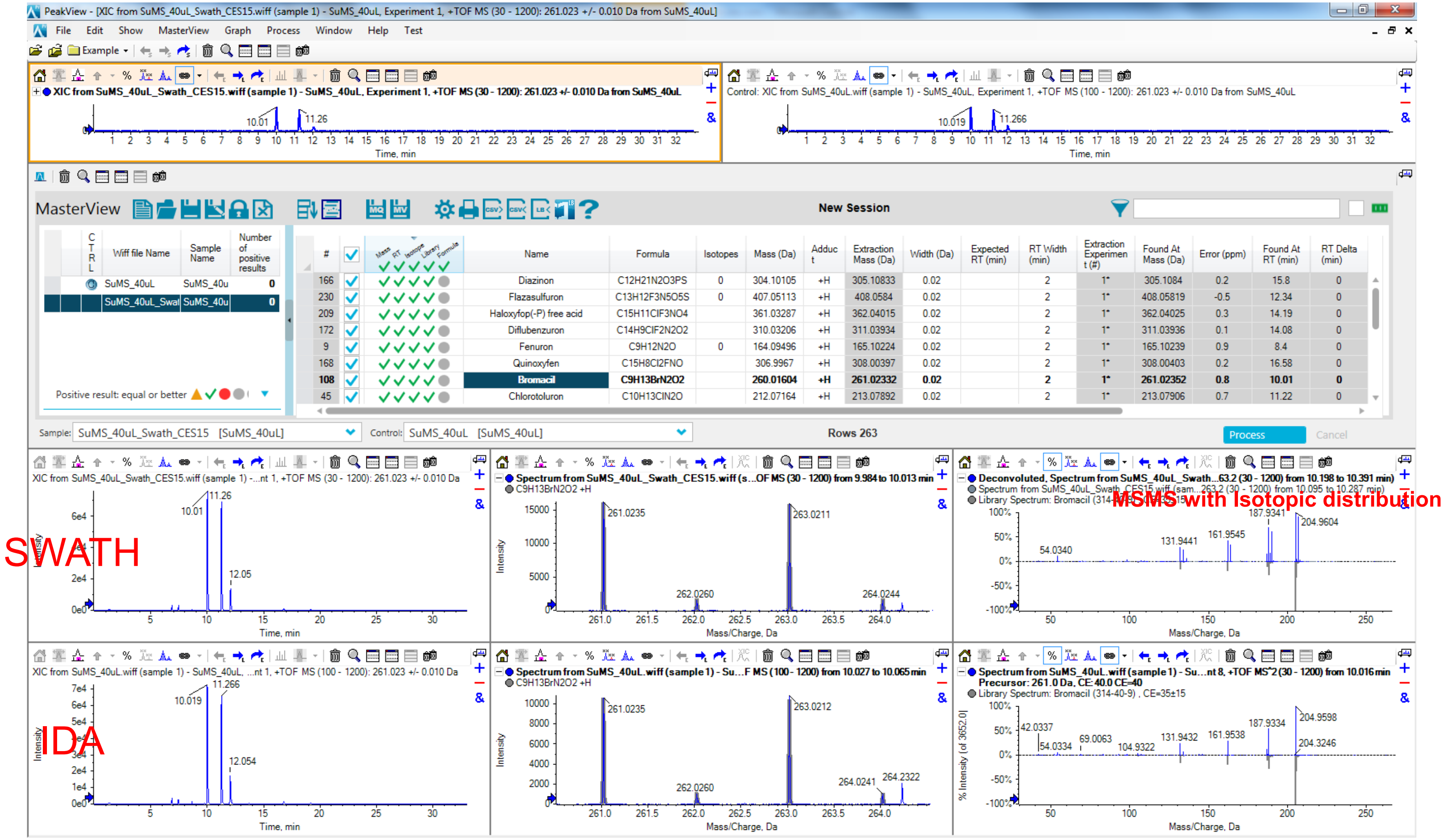
SWATH & IDA Acquisition

Compounds can be identified for both SWATH and IDA data using MasterView

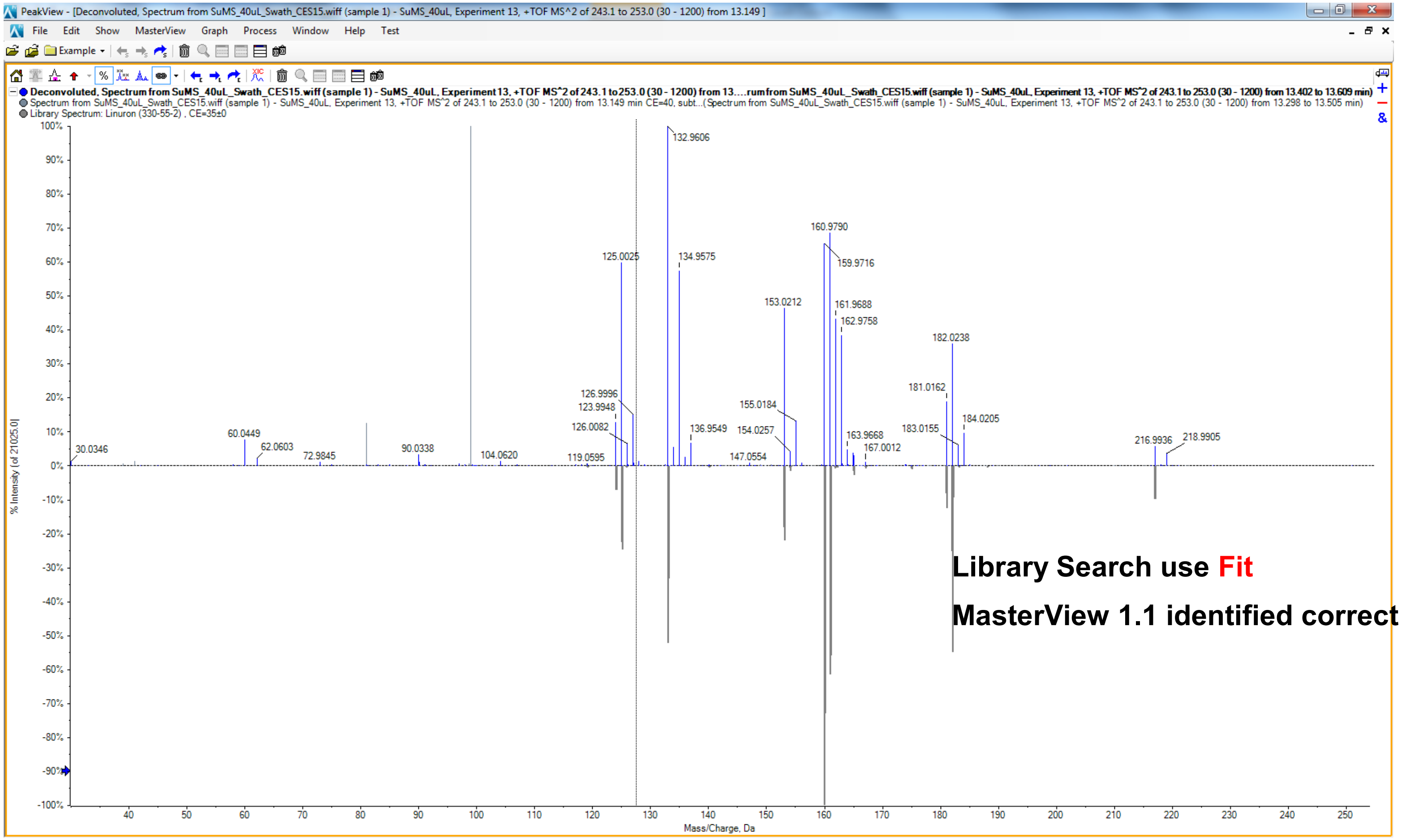


Results SWATH vs IDA

Example Linuron, SWATH Window has more than one precusor

Results SWATH vs IDA

Example Linuron, SWATH Window has more than one precusor



Library Search use Fit

MasterView 1.1 identified correct compound

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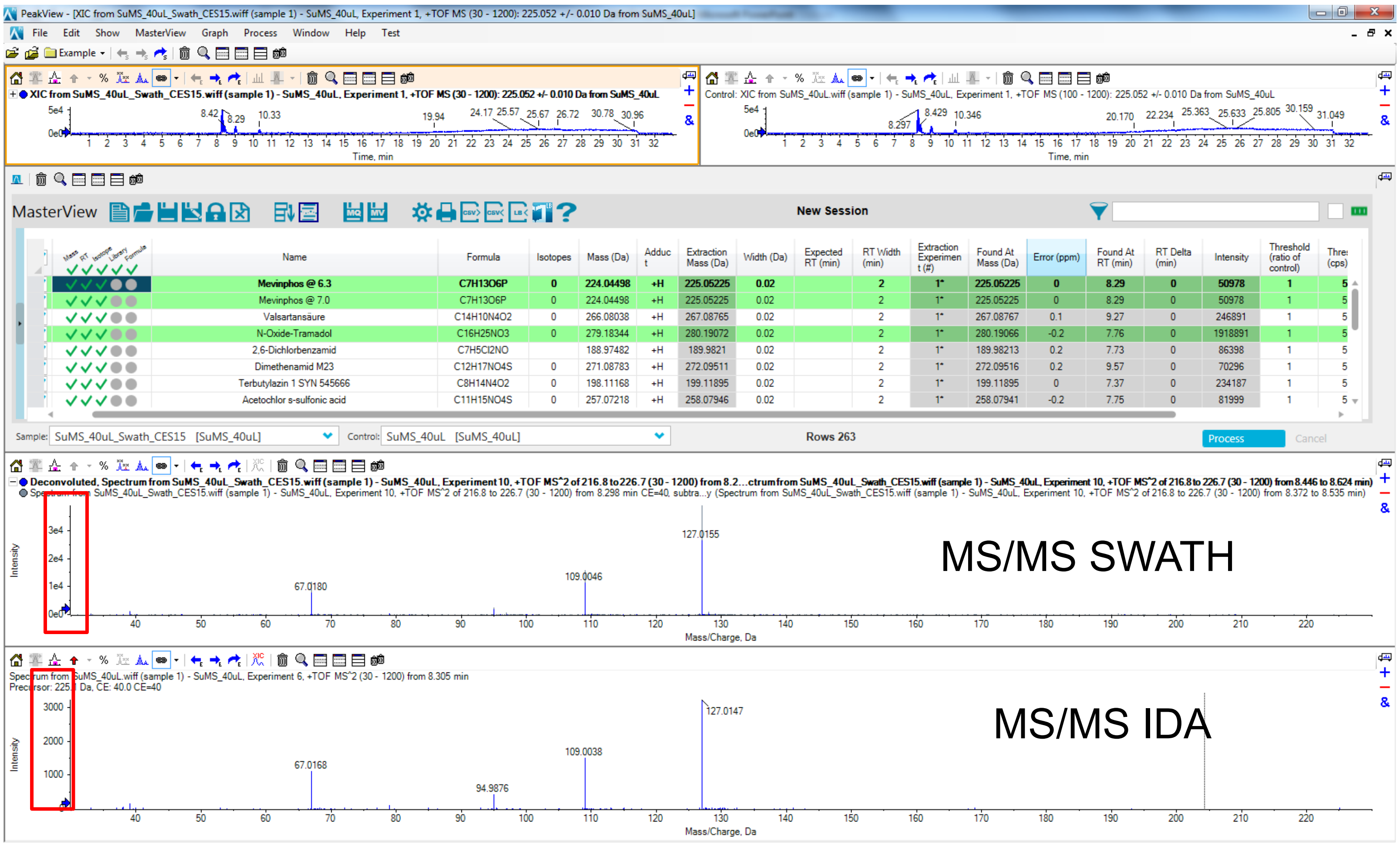
MS/MS SWATH vs IDA

Intensity comparison



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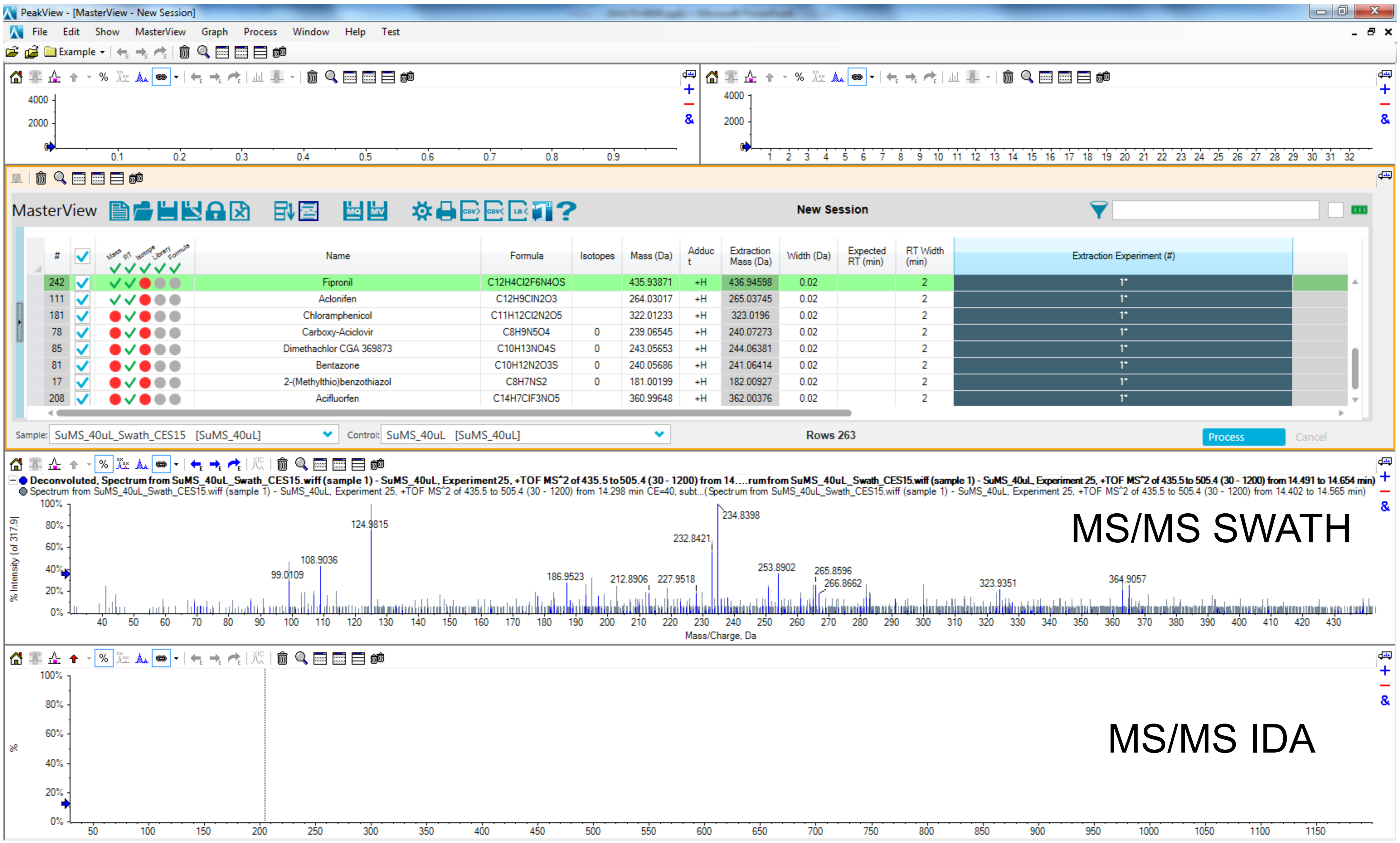
MS/MS SWATH vs IDA

No MS/MS spectrum with IDA



Conclusions & Futures?

- Non-targeted or unknown screening in food safety testing is possible
- Accurate Mass MS (but more importantly accurate mass MS/MS) data reduces matrix problems and increase selectivity for more confident identification
- Accurate mass MS/MS provides the capability of library searchable spectra that can be processed through ChemSpider for intuitive deconvolution
- Fast acquisition systems such as the TripleTOF allow all analytes ionised to be detected in both MS & MS/MS without reduction in data quality
- Software processing plays a big part….

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