

New Developments in HRMS Screening Method for Veterinary Drugs and Other Chemical Contaminants in Aquacultured Products

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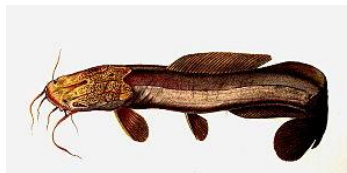
US FDA, Animal Drugs Research Center/Denver Laboratory

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Previous Work



- **Developed HRMS screening method for veterinary drug residues in aquacultured products**
 - Generic sample preparation with acidic acetonitrile extraction, pass-through SPE cleanup
 - Q-Exactive Orbitrap coupled to C18 LC with acetonitrile/0.1% formic acid gradient
 - Different types of data acquisition evaluated
- **Validated method**
 - Tested in 5 different types of aquacultured products with 70 veterinary drugs
 - Fortified at target testing levels and compared to one-point extracted calibration standard
 - Determined number of false positives/negatives (semi-quantitative limits test)
- **Applied method to incurred and imported samples**
 - Monitored for both target analytes (70 compounds initially validated)
 - Also compared to larger database (N > 500) to find new metabolites, unexpected residues
 - Found new metabolite of amoxicillin in dosed fish and ofloxacin in croaker and eel sample



J Ag. Food Chem. (2017)

Analytical Bioanalytical Chem. (2018)

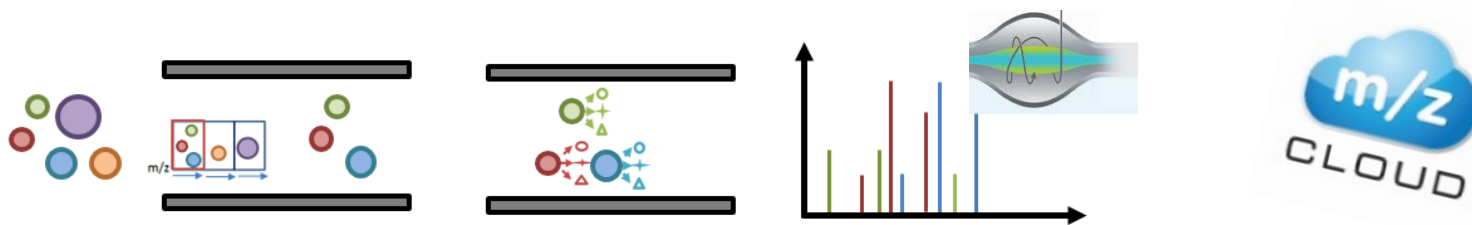
New Developments



1) Expansion of method to mixed contaminants



2) Improvement of method – data acquisition and processing



Expanding method

Validating for addition chemical contaminants



- **Disinfectants/Antimicrobial Soaps**

- Benzalkonium chlorides, triclocarban, triclosan



- **Pesticides**

- Few dozen likely to be found in aquaculture from agricultural run-off
- LC-MS compounds



- **Human Pharmaceuticals/Emerging Contaminants**

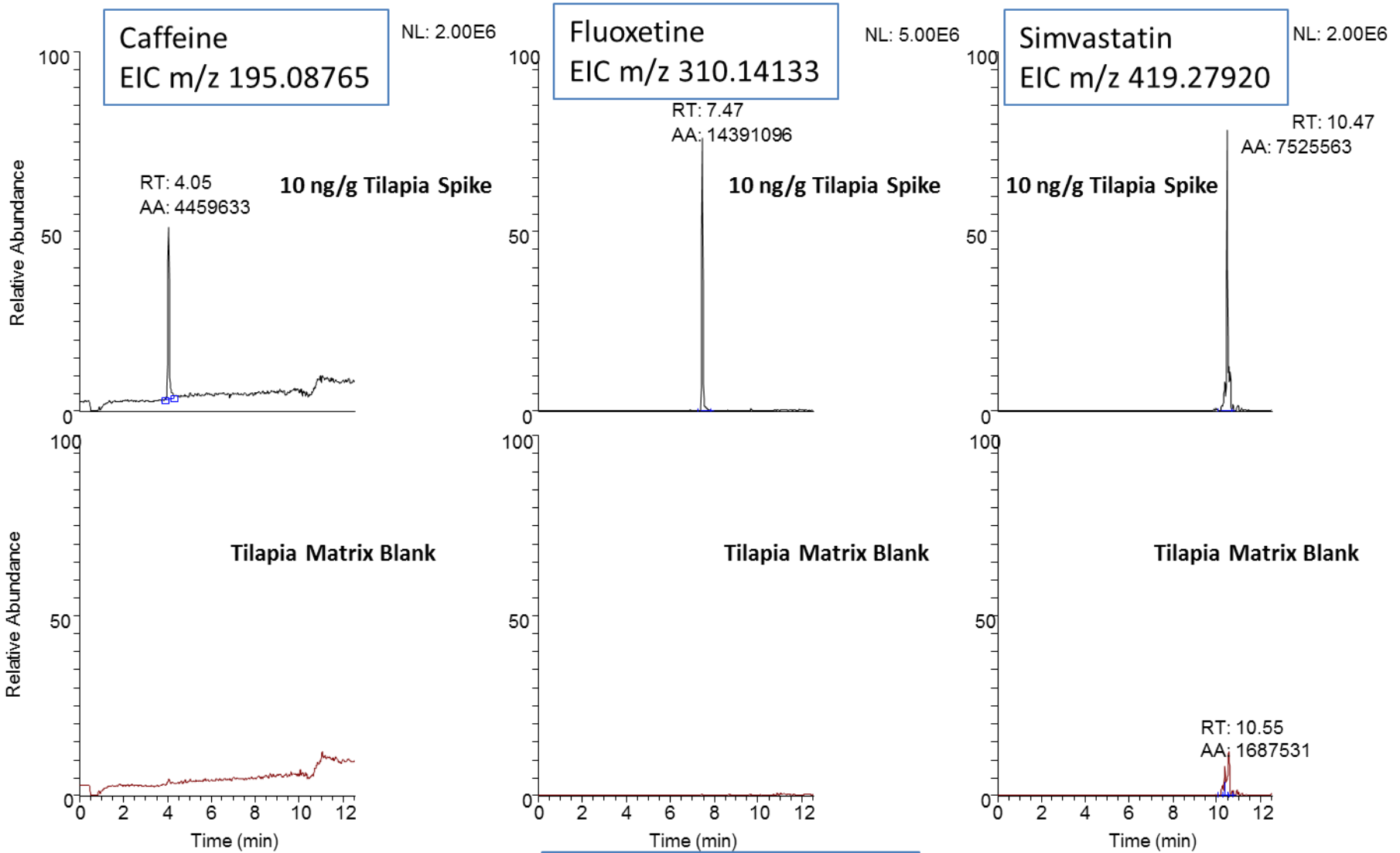
- Those commonly found in surface water
- Includes drugs for depression, hypertension, pain



- **Additional Veterinary Drug Compounds**

- More antibiotics, anti-wormers, etc.

Example: Human drugs in tilapia

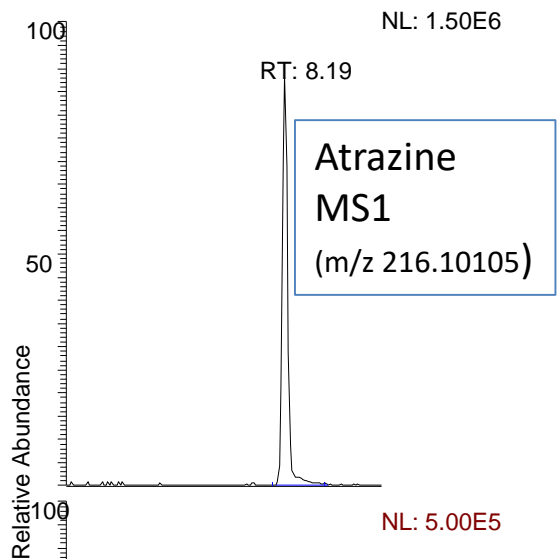


MS¹ (5 ppm window) AIF

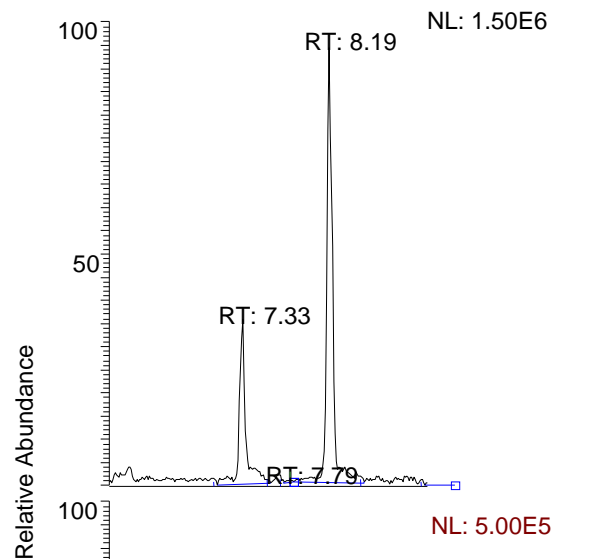
Example: Atrazine in shrimp



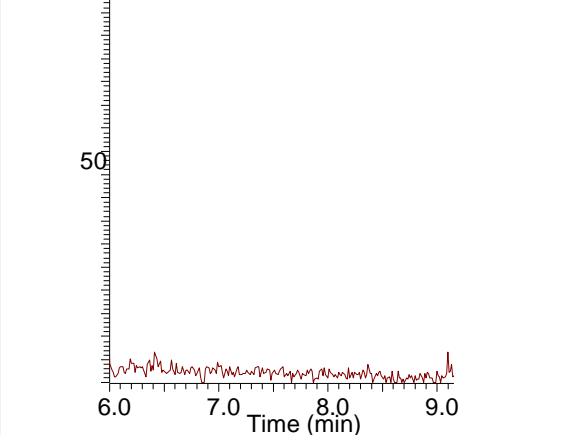
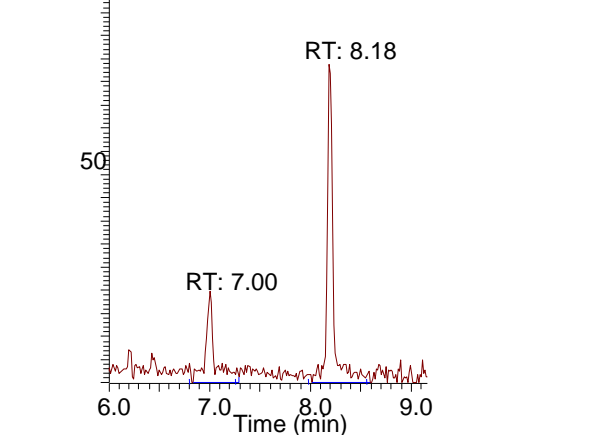
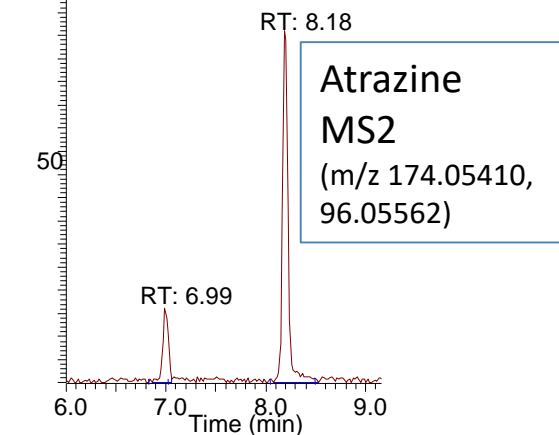
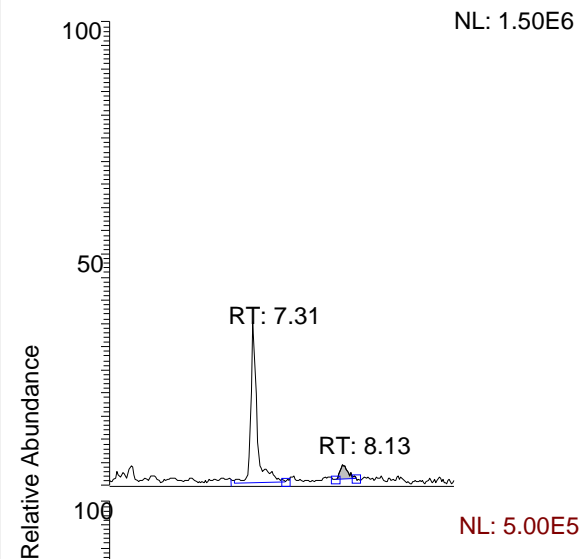
1 ng/g Solvent Standard



1 ng/g Shrimp Spike



Shrimp Blank



(5 ppm window)

Expanding method



Validating for additional chemical contaminants

~~1,3-Dibromo-5,5-dimethylhydantoin~~

~~1,3-Dichloro-5,5-dimethylhydantoin~~

Benzalkonium chlorides

Triclocarban

Triclosan

Amitraz (degradant)

Atrazine

Azadirachtin

Azamethiphos

Benzocaine

Carbaryl

Carbofuran

~~Cypermethrin~~

Dichlorvos

~~Etofenprox~~

Fipronil/Fipronil sulfone

Malathion

Phoxim

Praziquantel

Propazine

Quinalphos

Simazine

Trichlorfon

~~Trichloroisocyanuric acid~~

~~Trifluralin~~

Quinoclamine

Atenolol

Caffeine

Carbamazepine

Clarithromycin

Clofibric acid

Diclofenac

Diltiazem

Diphenhydramine

Fluoxetine

Gemfibrozil

Ibuprofen

Metformin

Naproxen

Propranolol

Ranitidine

Sertraline

Simvastatin

Sotalol

Valsartan

Rifampin

Aldicarb/Aldicarb sulfone/Aldicarb sulfoxide

Methylene blue

Acriflavine/Proflavine

Rotenone

Thiabendazole

Sulfisoxazole

Rifaximin

Roxithromycin

Marbofloxacin

Orbifloxacin

Baqueloprim

Virginiamycin M1

- Initially ~ 60 additional compounds
- The majority **worked well** through the method, some **were not detected**, and others were detected **only at higher levels**
- Tested 4 different fish fortified at 100, 10 and 1 ng/g
- This increased the number of residues validated for our method and expands the scope of the type of contaminants we are monitoring for in aquaculture.

Expanding method

Detection of additional chemical contaminants



Using HRMS screening method, several eel samples were initially presumptive positive for additional chemical contaminants. (HRMS identification criteria were met using non-targeted data acquisition)

- Further analysis (targeted MS² data acquisition, standard addition, analysis on separate QqQ method) **confirmed thiabendazole** (~ 6 ng/g) in one eel sample.
- **Acriflavine was** presumptive positive in many eel samples, but further analysis (targeted MS² data acquisition, standard addition) **ruled out** the presence of this compound.

Expanding method

Confirmed thiabendazole in eel

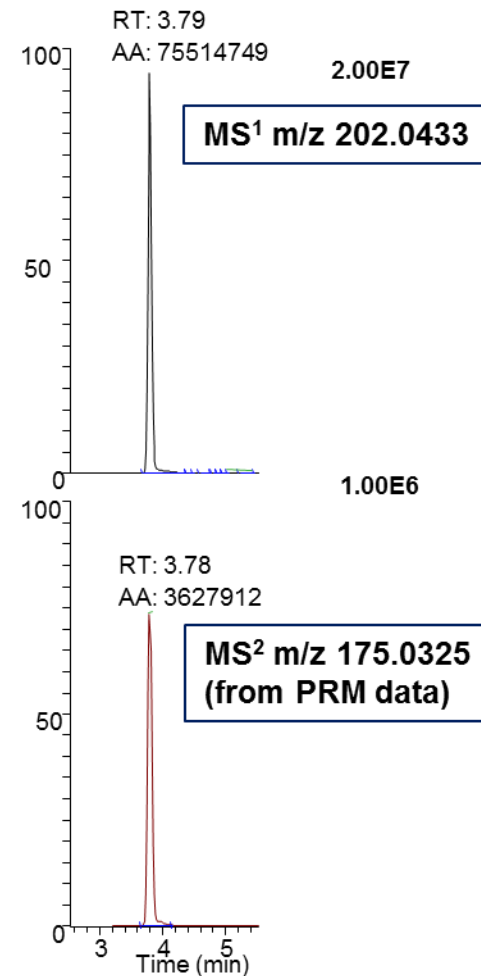
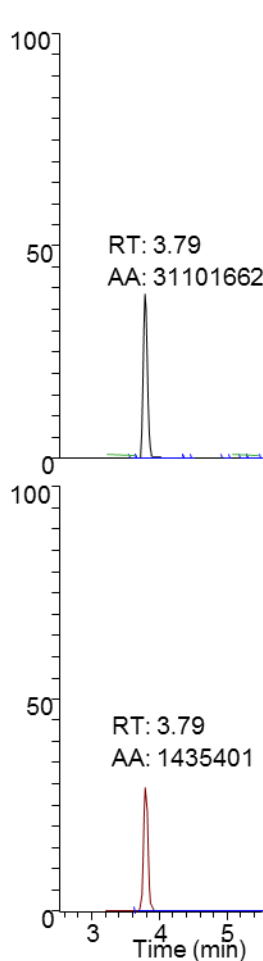
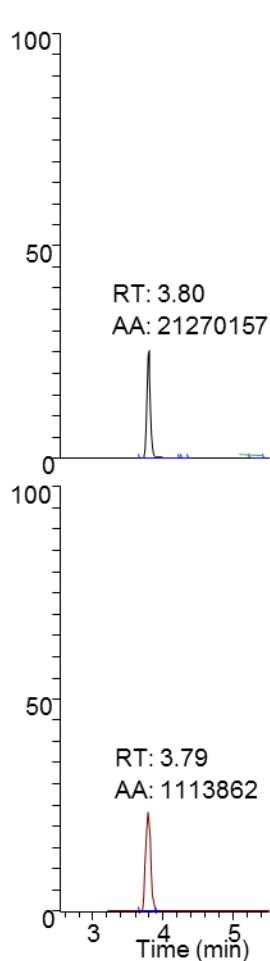
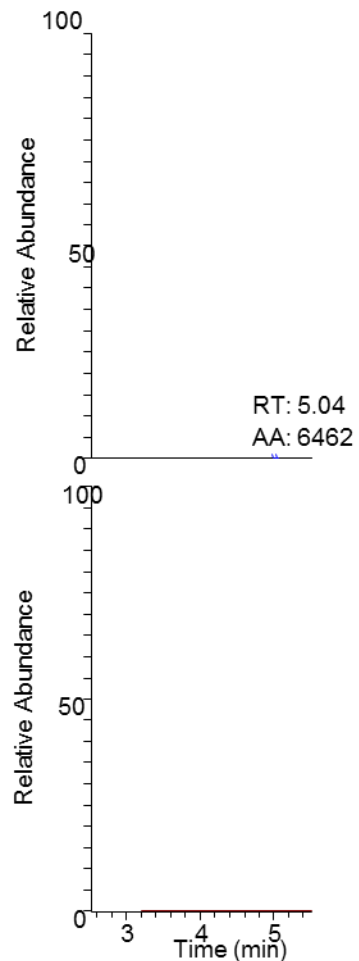


Control eel

Eel sample

Spiked eel control

Spiked eel sample

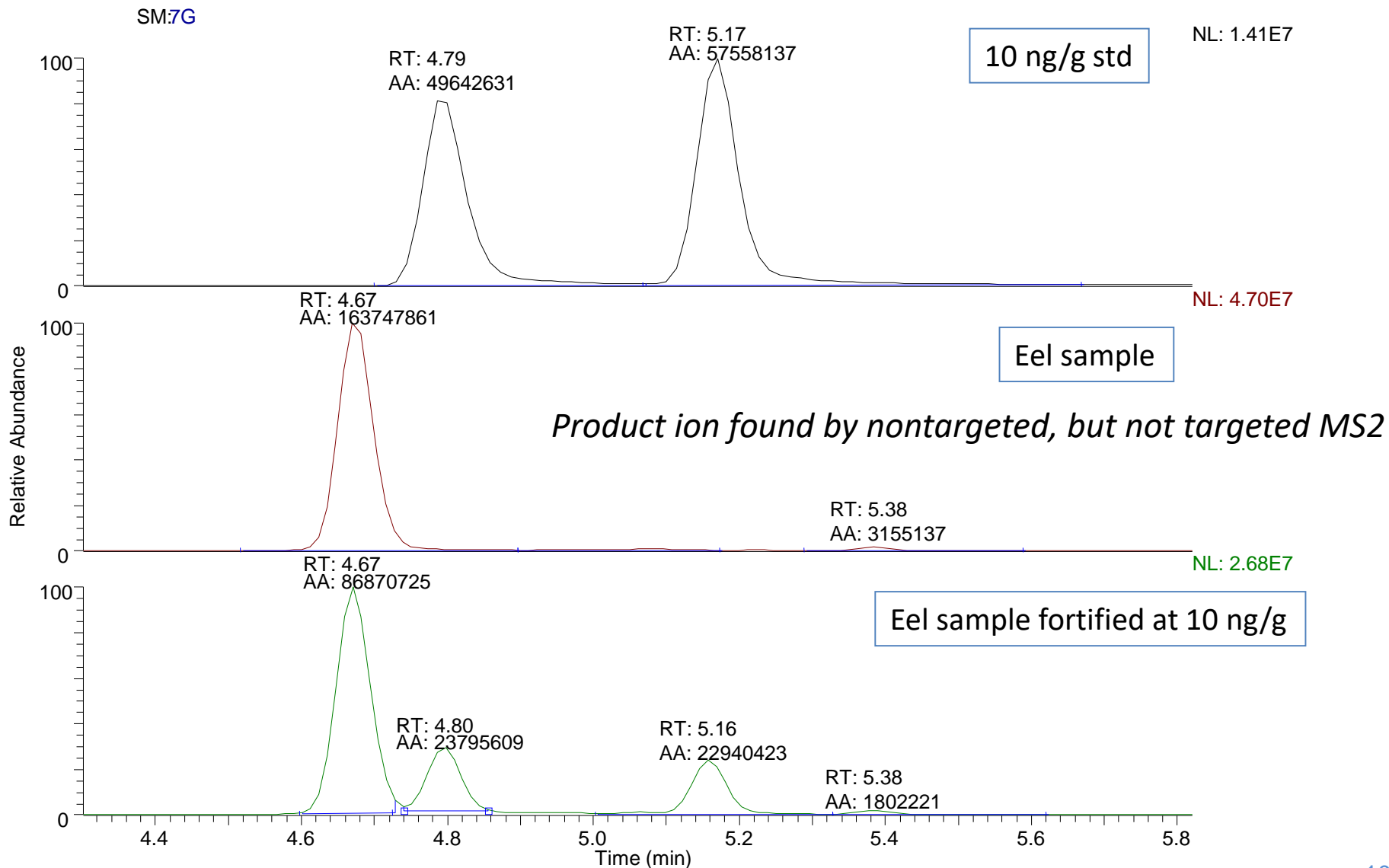


Expanding method



m/z 224.1182

False positive for acriflavine in eel



MS data acquisition

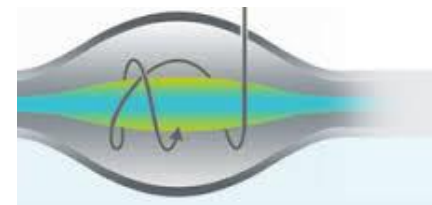
Thermo Q-Exactive Orbitrap High Resolution MS with a heated electrospray source
(using both classic QE and QE-HF)

Full scan MS¹ data always collected (m/z 150-1000)

Two types of MS² data acquisition programs were evaluated:

Nontargeted: collect product ion data for all precursor ions. *All Ion Fragmentation (AIF) or Data Independent Analysis (DIA)*

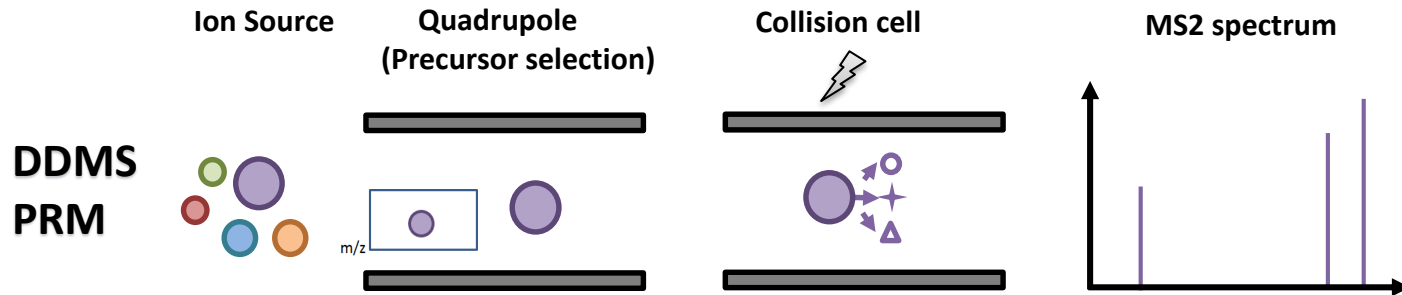
Targeted: collect product ion data of targeted precursor ion on a list. *Data Dependent MS² (DDMS²) or Parallel Reaction Monitoring (PRM) using inclusion lists*



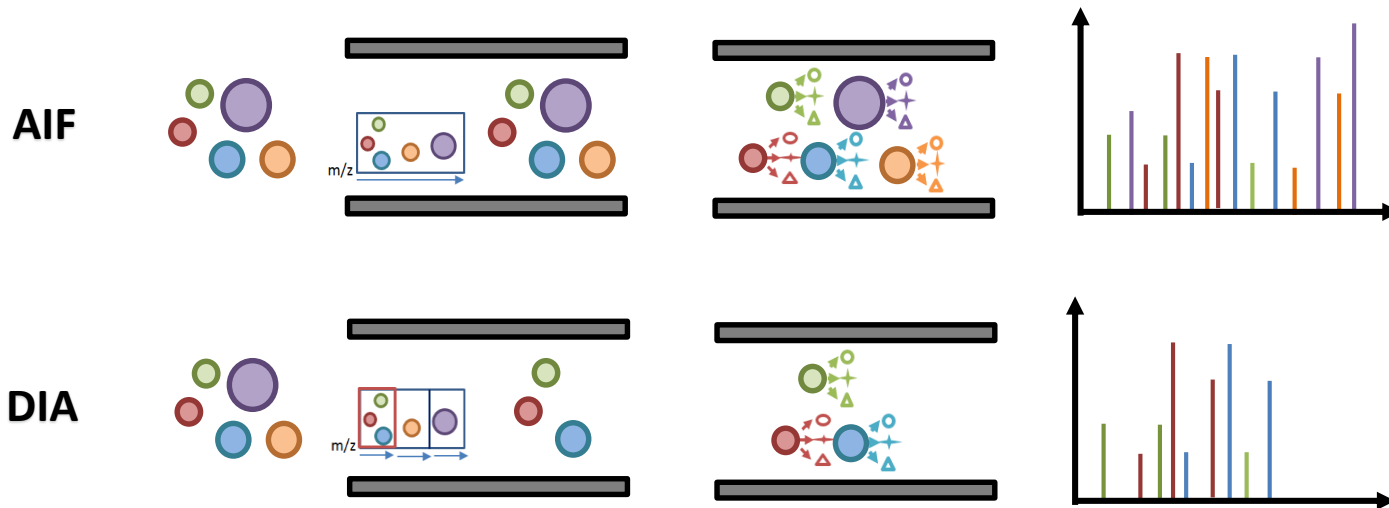
Types of data acquisition



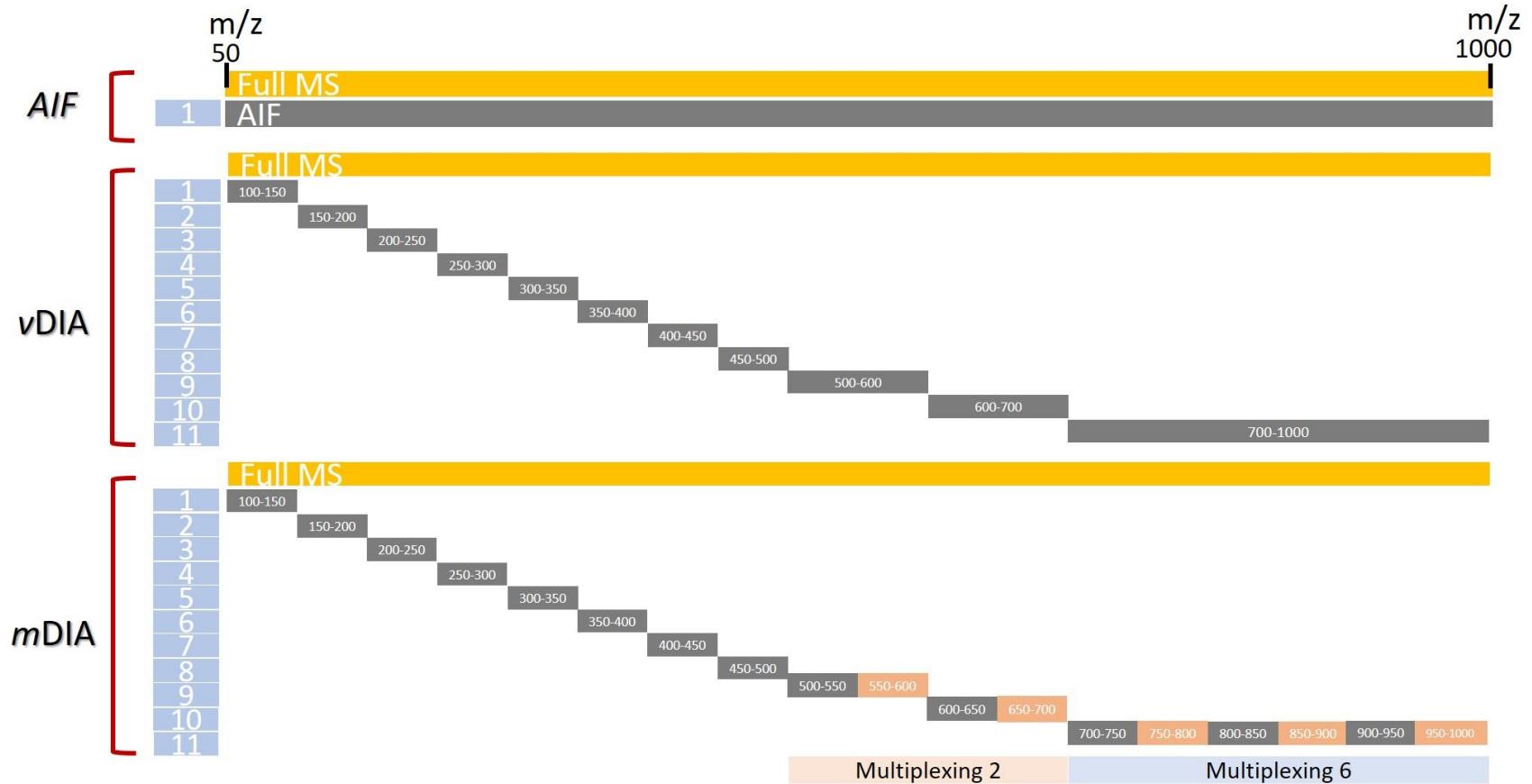
Targeted acquisition



Non-targeted acquisition



Types of nontargeted data acquisition



Comparison of these methods similar to work done previously:

Wang et al *Analyt Bioanalyt Chem* **2018**

Wong et al *J Ag Food Chem* **2018**

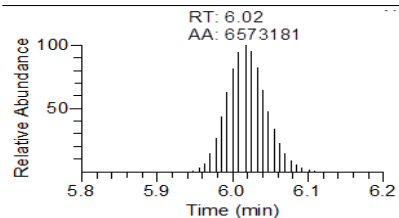
Comparison of scan types



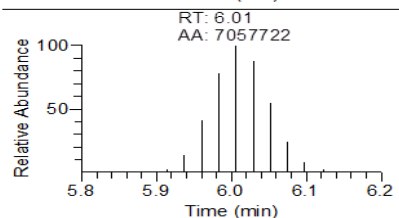
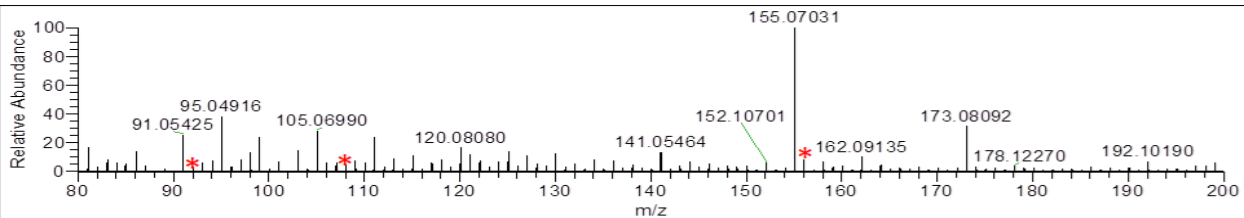
Sulfadoxine 10 ng/g in spiked eel

EIC of MS1 (m/z 311.0809)

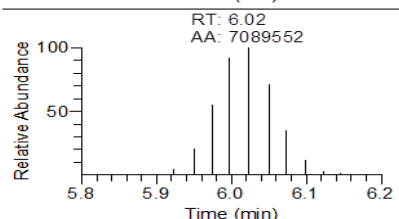
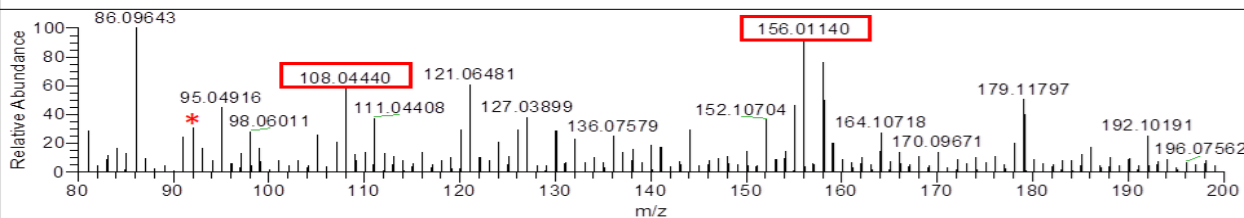
MS2 Spectra



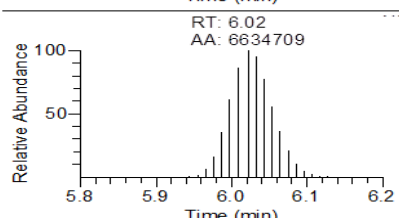
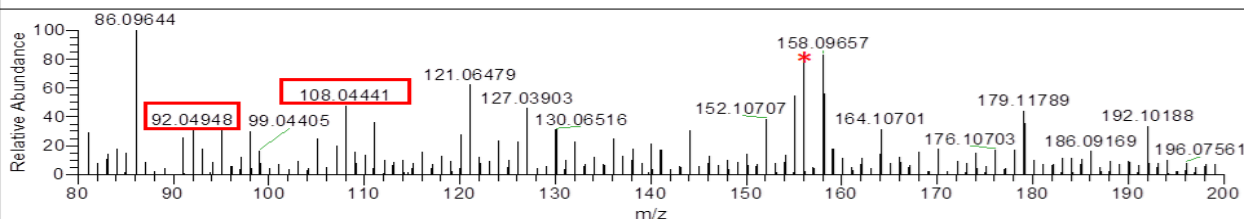
AIF



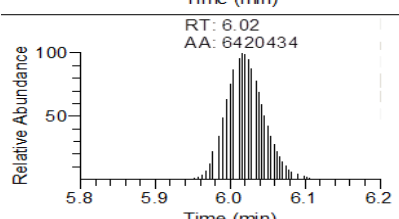
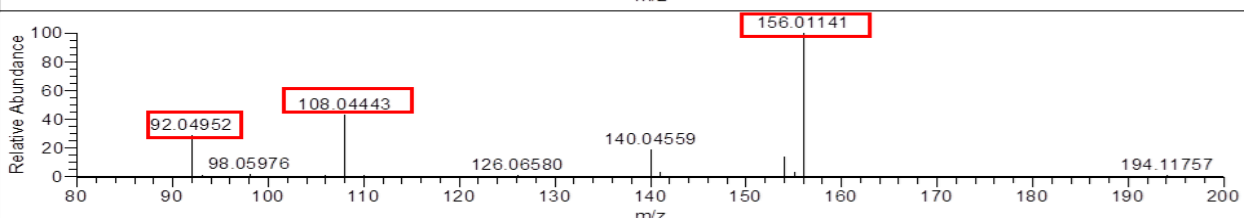
vDIA



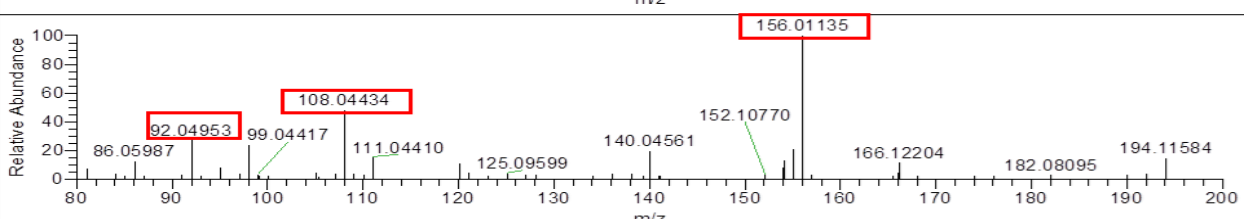
mDIA



PRM



DDMS



Comparison of data acquisition



FDA identification criteria for exact mass data:

Identified = MH^+ (5 ppm), one product ion (10 ppm), RT match

Nontargeted

- > 90% validation compounds detected and identified at 1X with AIF
- Most identified with AIF at much lower levels (0.1-0.5X of target testing level)
- Recently compared different DIA methods to AIF with similar results

Targeted

- ~ 70% of validation compounds identified with DDMS²
- Compounds with low target testing levels (dyes) or low method recovery (β -lactams) don't meet threshold to trigger DDMS²
- Some identified at higher levels
- Recently compared PRM (limited # of compounds) to DDMS² w/ better results (~90% of residues identified)

Comparison of Scan Types



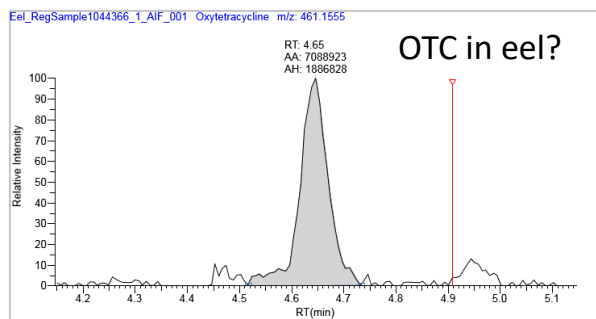
Non-selective AIF data can lead to false positives

- Oxytetracycline (OTC) often “identified” in eel samples with AIF
- Less often detected using DIA; not usually confirmed by PRM, DDMS²

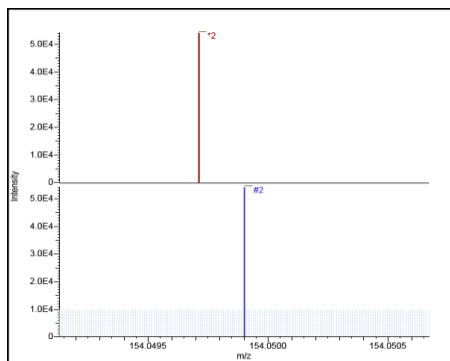
Example:

OTC initially identified in this eel at 37 ng/g – met exact mass criteria by AIF

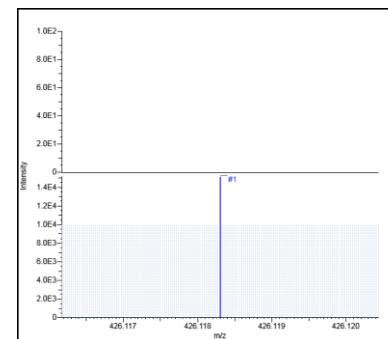
But...only one product ion found, mass accuracy worse than usual, retention off by 0.06 min



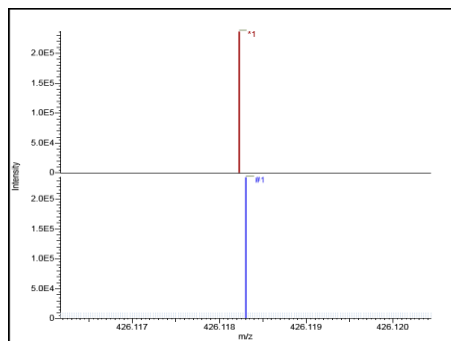
m/z 154.04988 (-1.213 ppm)



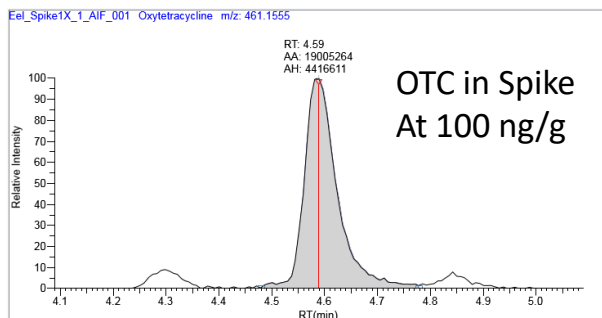
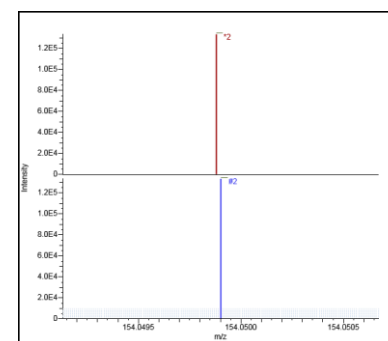
m/z 426.1183 Not Found



m/z 154.04988 (-0.123 ppm)



m/z 426.1183 (-0.176 ppm)

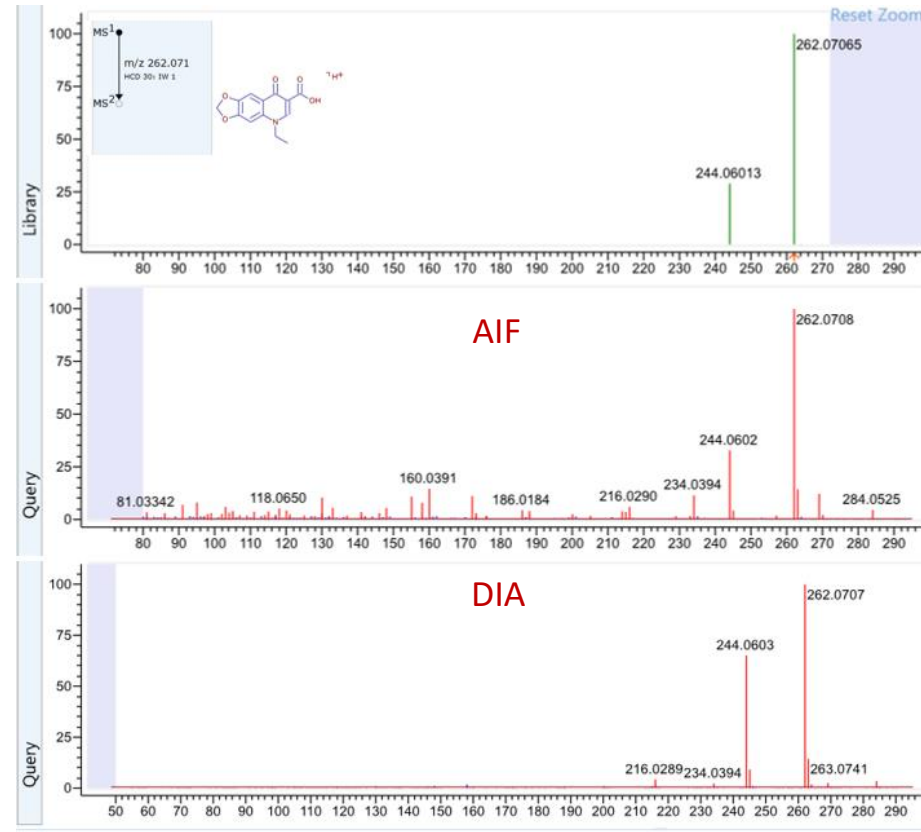
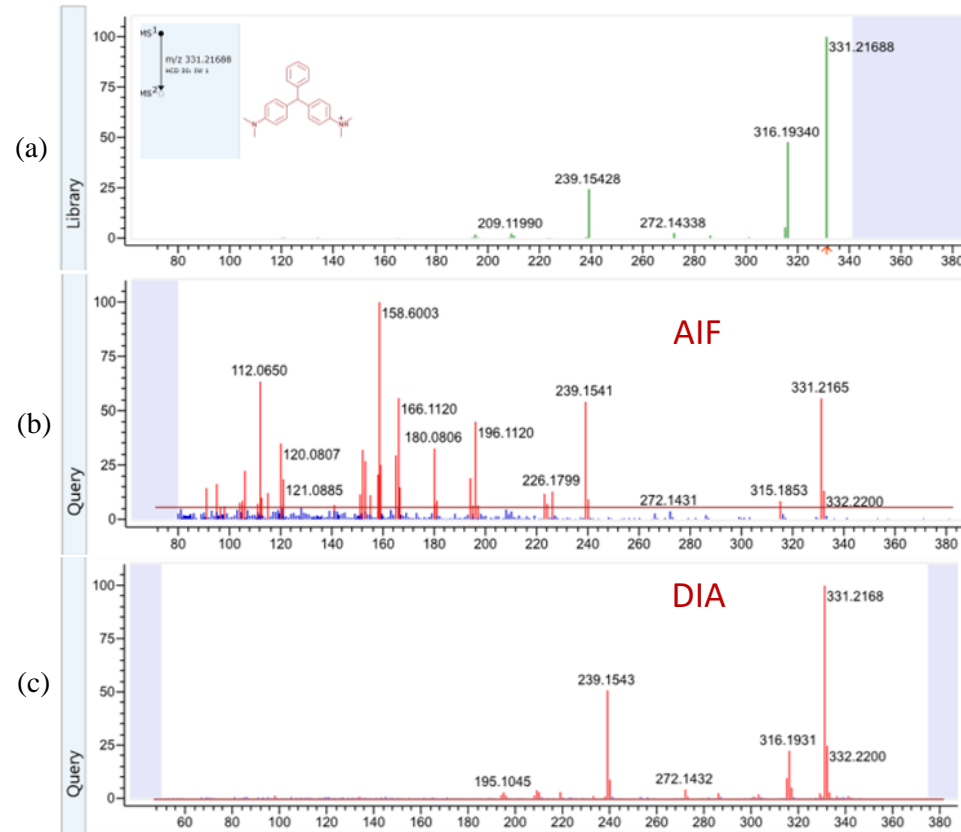


Comparison of Scan Types



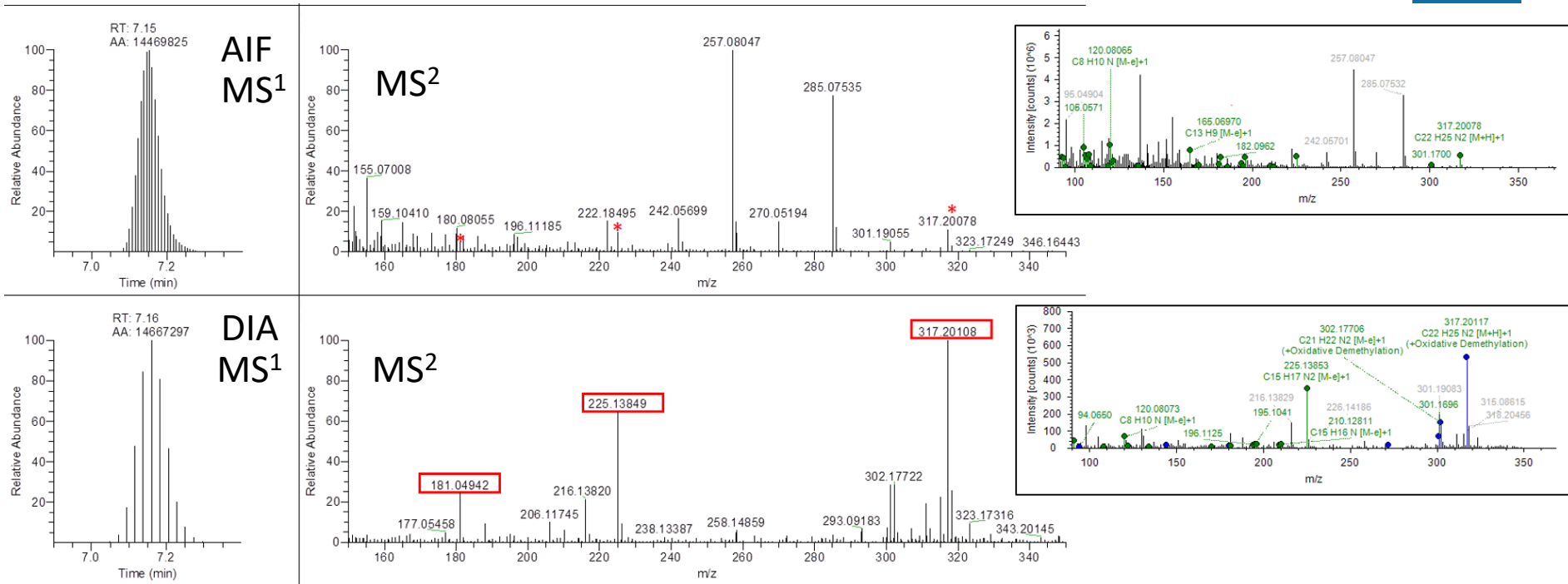
Leucomalachite green (QqQ 214 ng/g)

Oxolinic Acid (QqQ 1950ppb)



- Residues found in imported eel sample
- DIA MS² spectra can be easier to compare to on-line databases such as m/z cloud

Nontarget compound analysis



- Eel sample violative for several analytes including leucomalachite green investigated to look for additional compounds.
- Extracted ion chromatogram and MS² spectra for Des-LMG (C₂₂H₂₅N₂, m/z=317.20123) using AIF and DIA acquisition methods.
- Inserts show the annotated spectrum for the proposed structure using Compound Discoverer software to generate fragments for proposed structure and match the fragments observed in the unknown peak.

Ongoing improvements to HRMS Screen



- Continue to keep evaluating data analysis work flow strategies (more non-targeted with using available software tools)
- Work to transfer method to more routine analysis
- Evaluate alternative chromatography – look at nanoflow interface to increase sensitivity, reduce matrix effects, simplify extraction (similar to Alcántara-Durán et al, *Food Chemistry* 2018)



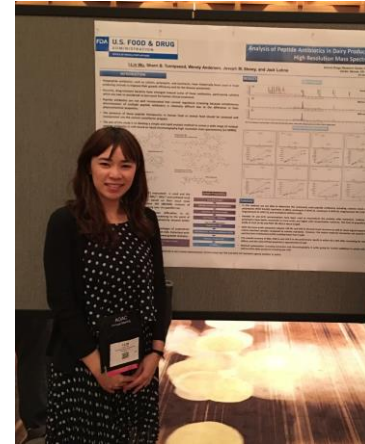
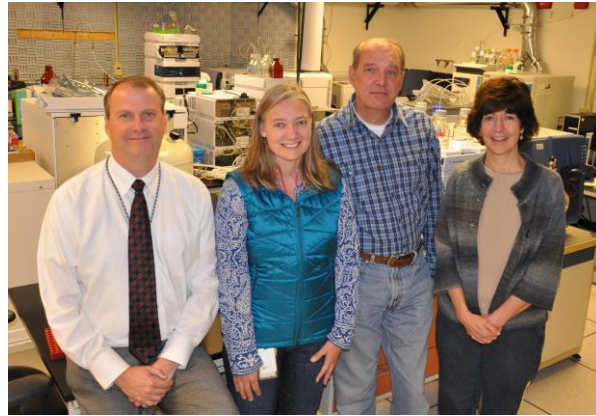
Thermo Scientific EASY-nLC 1200 System



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Thermo Scientific Application Scientists and Engineers



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