Small Molecule Orbitrap Customer Applications

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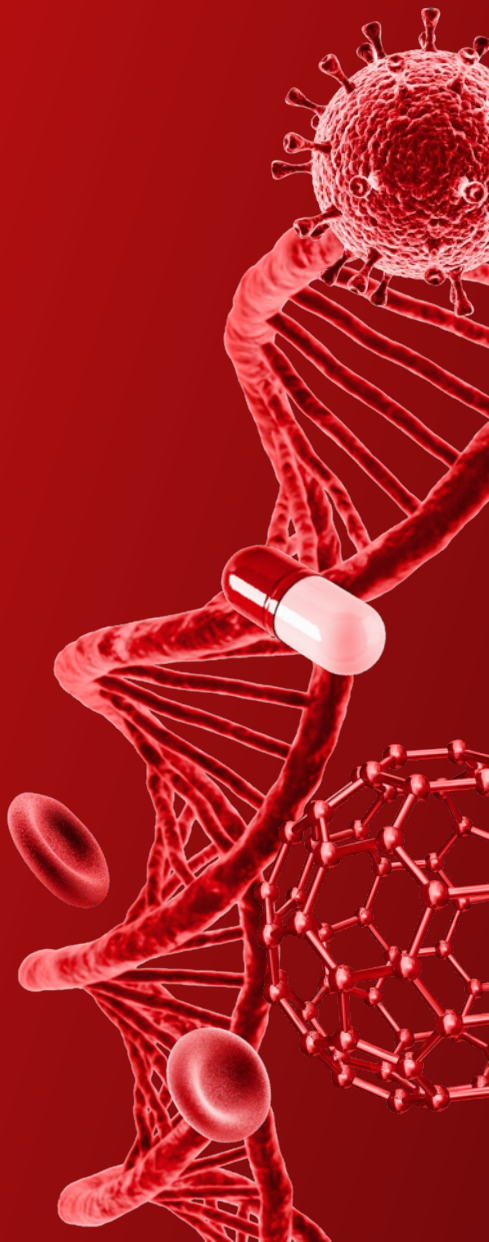


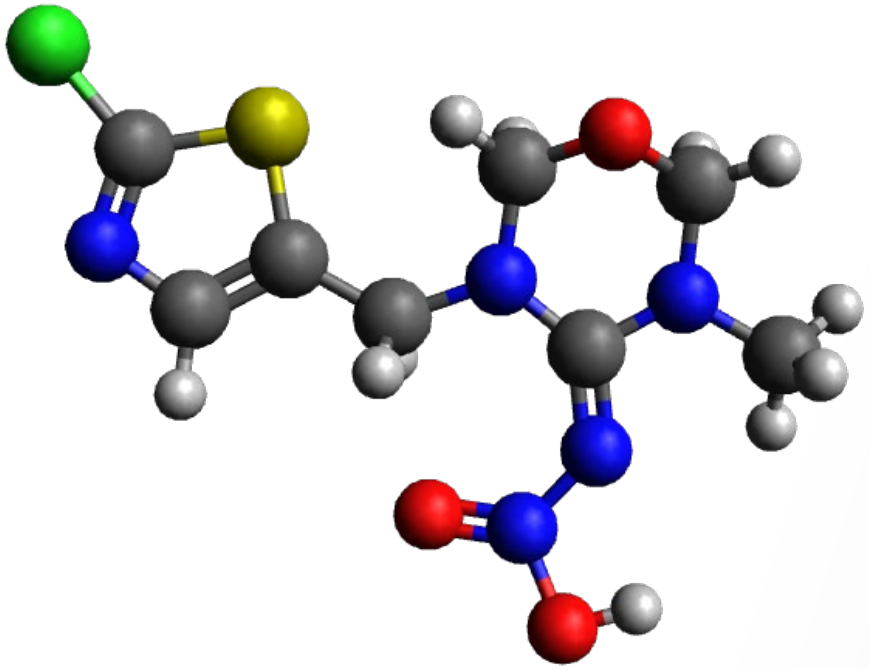
Analysis in Conjunction with Other Labs

- Real world type applications in conjunction with analysis
  - Spray studies with deposition vs. drift compositions
    - Does the Herbicide go where it is put?
    - What part of it moves

- Green house testing
  - Where does the active go under growth conditions?
  - Time resolved experiments

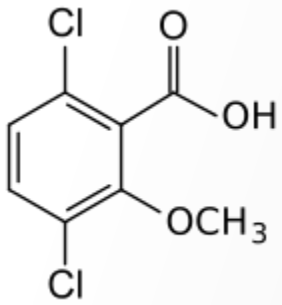
Primary Use for Q-Exactive:

- Looking for Trace amounts of Herbicides in Complex Matrices
- Time resolved residues on/in leaf surfaces
- Herbicide Degradation studies



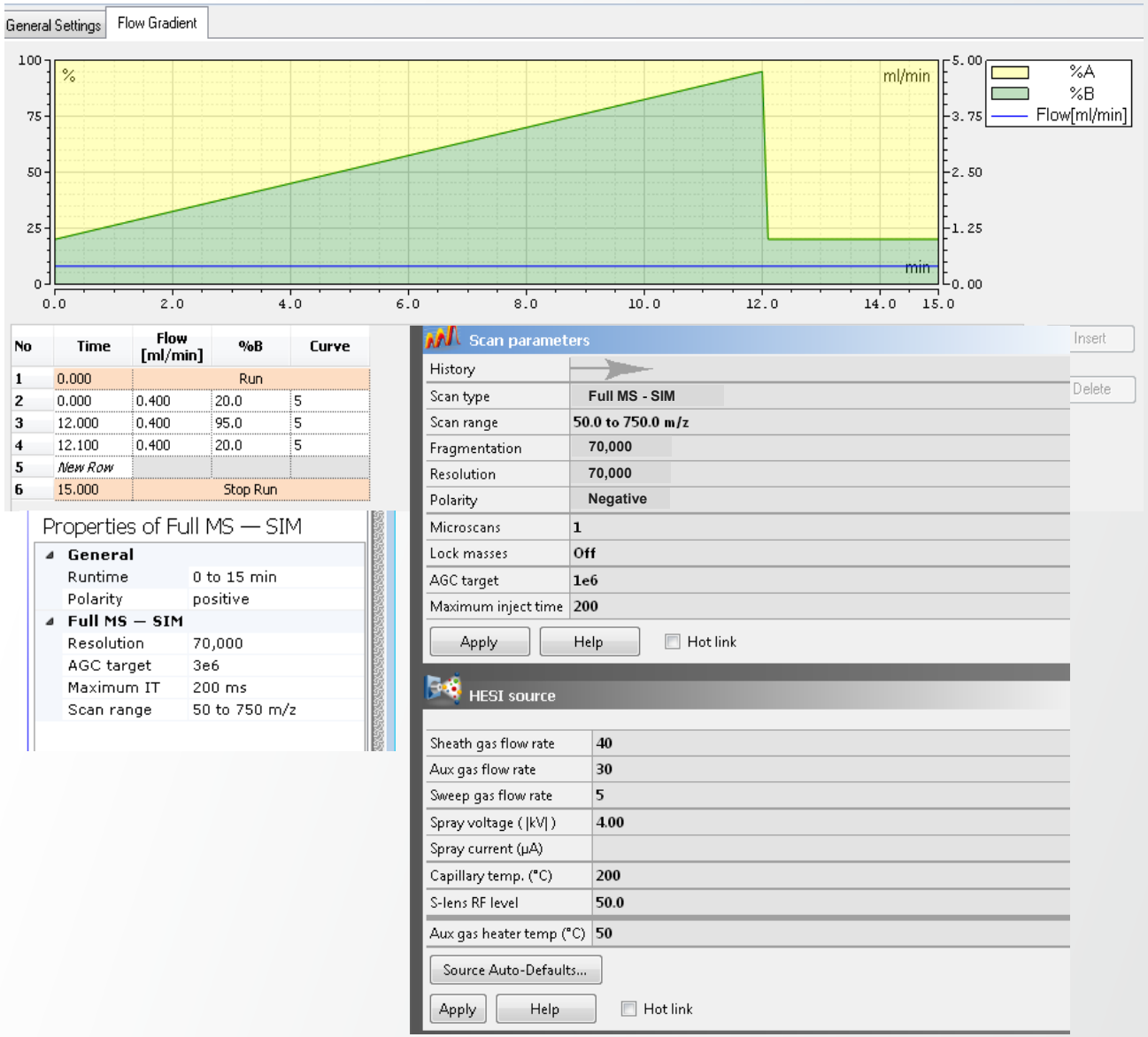
Dicamba Analysis

- A Major problem in the Agriculture industry currently is the translocation of the herbicide Dicamba beyond the desired application site.
- Experimental set up included standard filter papers attached to discrete particle size classifiers at various distances in a field that was sprayed with an Agricultural Mix including Dicamba.
- This experiment was set up in triplicate with four distinct size bins across three distances.
- The filter papers were extracted with MeOH and run on UHPLC-Orbitrap-HRMS to measure the amount of Dicamba captured at various distances from the spray event.



Analysis Conditions

- UHPLC
  - C18 Column
    - 50mm x 2.1mm, 3um Particle
    - 40C
  - 15min run
  - Mobile Phase A – 0.1% Formic Acid
  - Mobile Phase B - Acetonitrile
- HRMS
  - Full MS-SIM
  - (-) mode
  - 70,000 Resolution

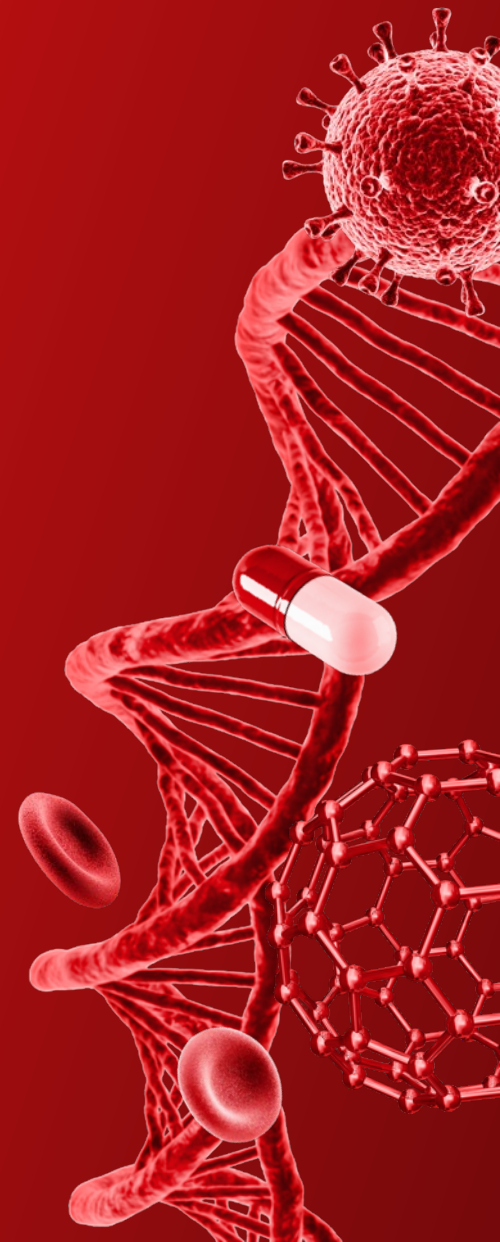


Conclusions

- Ion Extracted Chromatograms were generated from the SIM's.
- Enough data was collected to prove proof of concept, as Dicamba was detected in the nearest collectors.
- Unfortunately, the signals were within the LOD for the prep, they were below LOQ for this method.
- Follow up experiments are underway to concentrate the samples prior to analysis. This should bring the results into an interpretable range.
- Very few studies of this nature have even detected the Dicamba in the environment, so this is a strong step towards quantitation.

Treated Seed Pesticide Analysis by UHPLC-HRMS

Lianna Bestvater
Canadian Grain Commission
Grain Research Laboratory – Grain Safety group
Trace Organics and Trace Elements



Treated Seed Analysis by UHPLC-HRMS

- Treated seed is grain that has been treated with a pesticide product (typically fungicides) prior to planting
- In Canada, treated seed must be dyed to make seeds distinguishable from other grain
- These seeds may be inadvertently present in bulk grain if grain handling equipment is not cleaned well, and may lead to points of pesticide residues in bulk grain
- The Grain Research Laboratory routinely monitors grain using UHPLC-HRMS, and has the capacity to analyze samples for the presence of the 42 seed treatment pesticides that are active ingredients in products registered for use in Canada on grains

Extraction Procedure

Dyed Treated Seed

↓

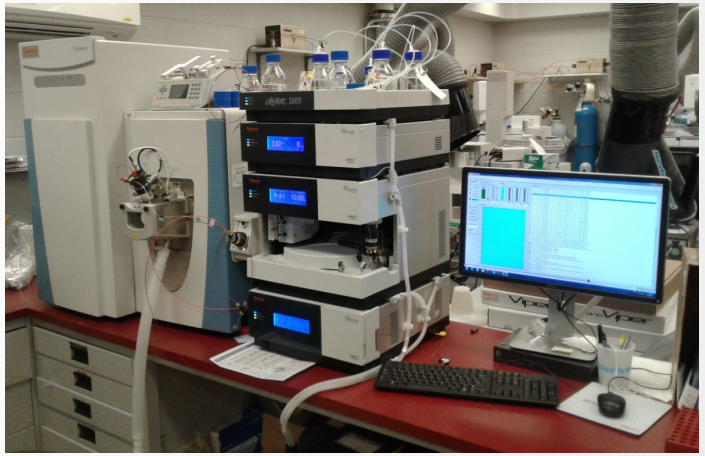
Add Acetonitrile

↓ Vortex, sonicate

Sample Dilutions

↓ Add Internal Standard Syringe Filter

LC-HRMS



UHPLC Conditions

- Column: Waters Acquity UPLC BEH C18, 1.7µm, 2.1x 100mm
- Column flow: 0.4 mL/min
- Column temperature: 40˚C
- Injection volume: 10 µL
- Autosampler tray temperature: 10°C
- Gradient:

Time (min)	%A	%B
Initial	90	10
0.25	90	10
7.75	0	100
8.50	0	100
8.51	90	10
12.00	90	10

HRMS Parameters

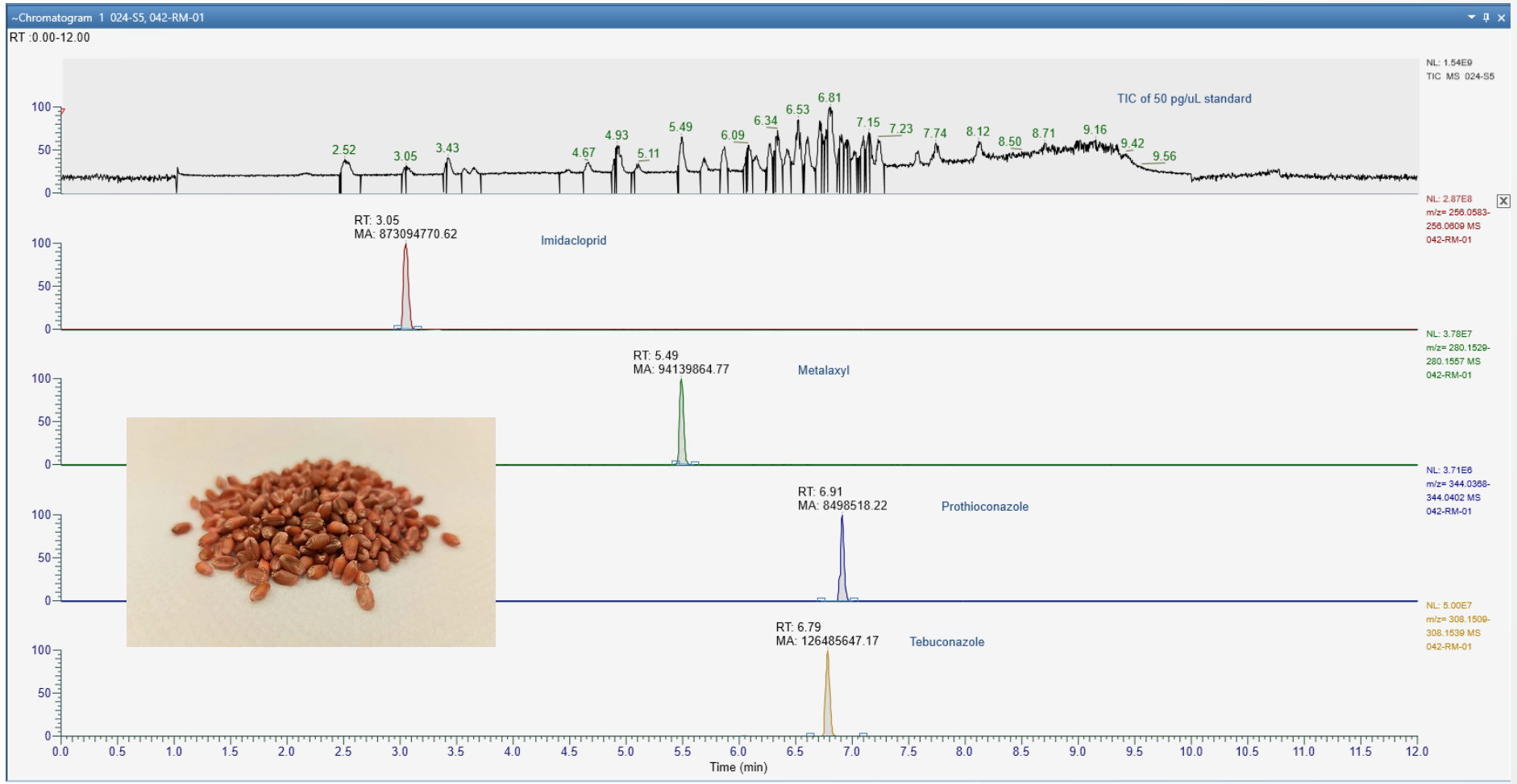
HESI source parameters	
Sheath gas flow rate	50
Auxiliary gas flow rate	13
Capillary temperature	300˚C
Sweep gas flow rate	3
Spray voltage	3.5 kV
S-lens RF level	55
Aux gas heater temperature	425˚C

Experiment type	Full scan/ddMS^2
Ionization mode	ESI +
Scan Range	70 to 680 m/z
Full MS	
Resolution	70,000
AGC target	1e6
Maximum IT	100 ms
ddMS2	
Resolution	17,500
AGC target	1e5
Maximum IT	50 ms
NCE stepped	10, 20, 40

42 Pesticides

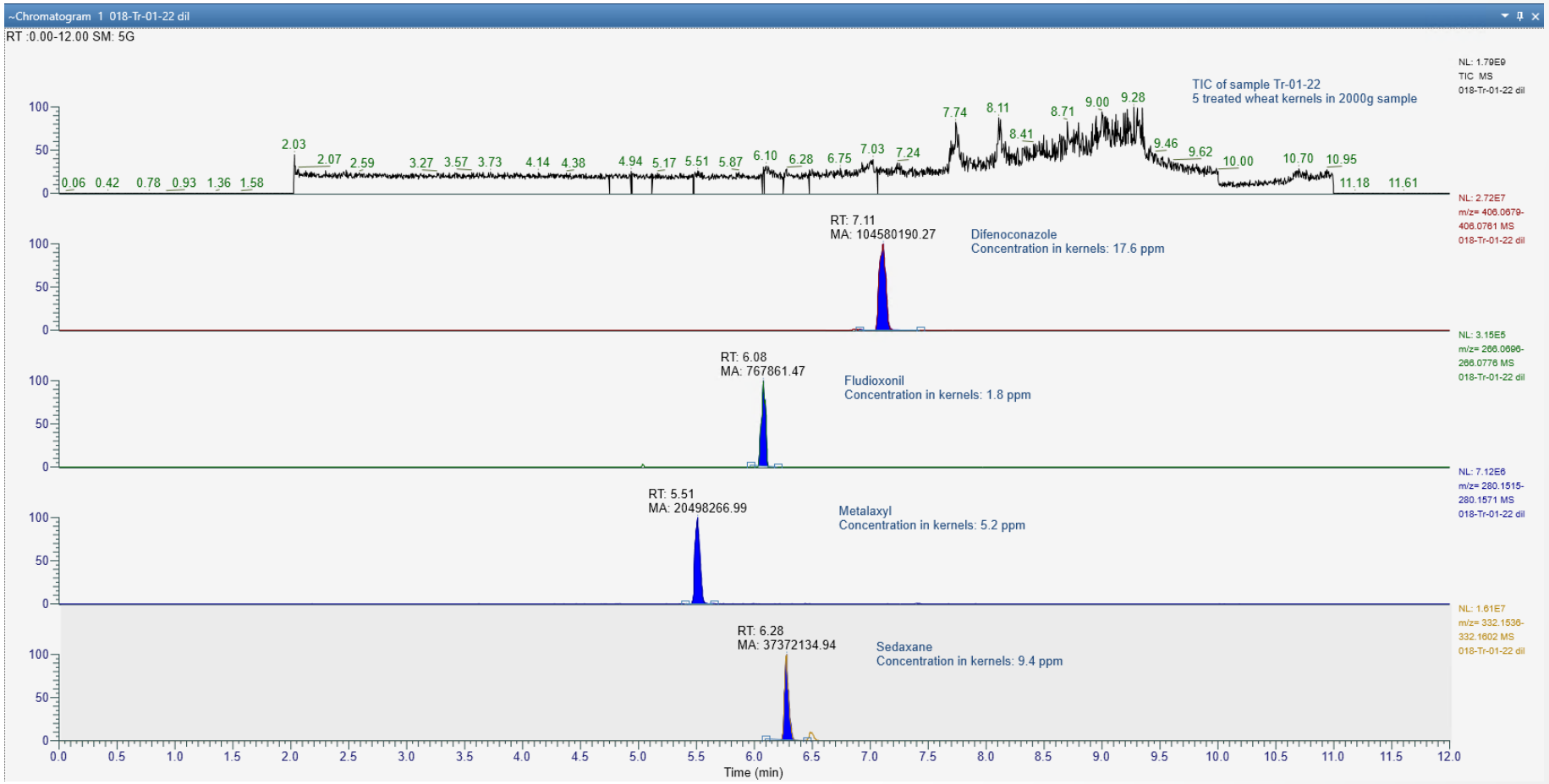
Pesticide	m/z	RT (min)	Pesticide	m/z	RT (min)
Acetamiprid	223.0745	3.40	Metalaxyl	280.1543	5.50
Azoxystrobin	404.1241	5.90	Metconazole	320.1524	7.00
Boscalid	343.0399	6.13	Oxathiapiprolin	540.1487	6.08
Broflanilide	663.0136	7.08	Penflufen	318.1976	6.70
Captan	321.9234	5.74	Penthiopyrad	360.1352	6.76
Carboxin	236.0740	5.00	Picarbutrazox	410.1935	6.61
Chlorantraniliprole	481.9781	5.70	Picoxystrobin	368.1104	6.60
Clothianidin	250.0160	3.10	Prothioconazole	344.0386	6.90
Cyantraniliprole	473.0123	5.15	Pydiflumetofen	426.0349	7.08
Diazinon	305.1083	6.81	Pyraclostrobin	388.1059	6.87
Difenoconazole	406.0720	7.20	Sedaxane	332.1569	6.30
Ethaboxam	321.0838	5.05	Sulfoxaflor	278.0569	3.50
Fludioxonil	266.0736	6.07	Tebuconazole	308.1524	4.79
Fluopyram	397.0537	6.32	Thiabendazole	202.0433	2.54
Fluoxastrobin	459.0866	6.40	Thiamethoxam	292.0266	2.60
Flupyradifurone	289.0550	3.44	Thiophanate methyl	343.0529	4.66
Fluxapyroxad	382.0973	6.19	Thiram	240.9956	4.50
Imidacloprid	256.0596	3.10	Tioxazafen	229.0430	7.00
Inpyrfluxam	334.1726	6.52	Triadimenol	294.1004	6.33
Ipconazole	334.1681	7.30	Trifloxystrobin	409.1370	7.20
Iprodione	330.0407	6.60	Triticonazole	318.1368	6.50

In-house reference material



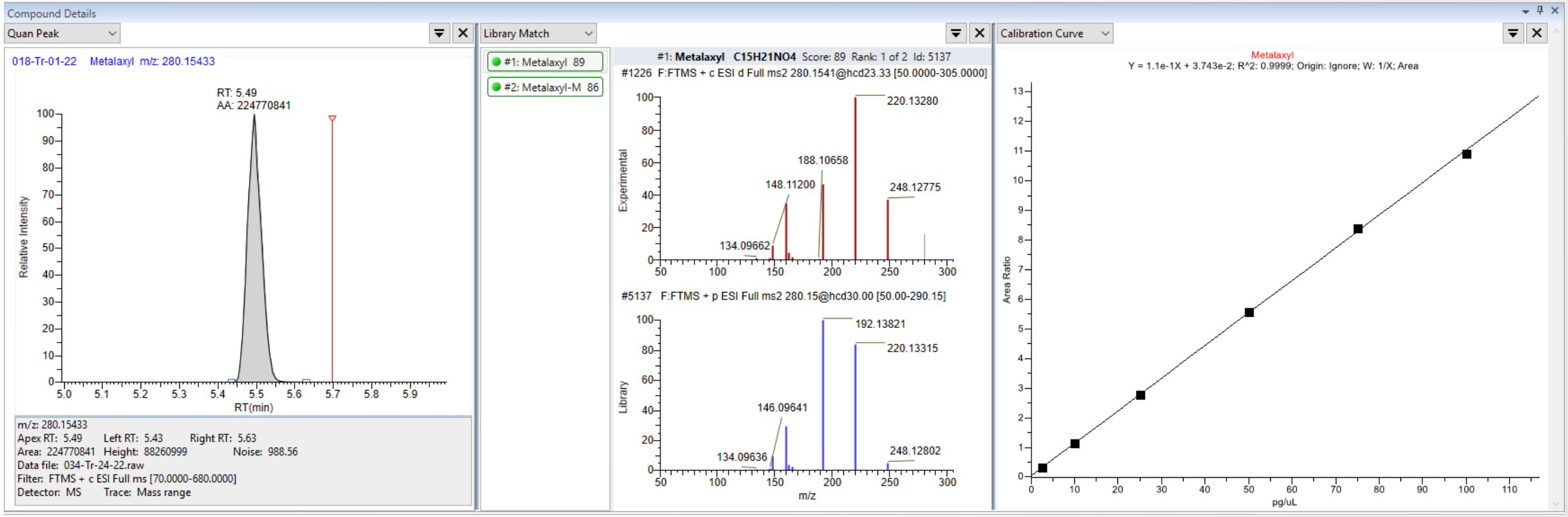
Raxil-Pro Shield seed treatment contains 4 pesticides: imidacloprid, metalaxyl, prothioconazole, tebuconazole

Sample Tr-01-22



5 treated wheat kernels in 2000g sample

Metalaxyl



Quantitation peak

Library match

Calibration curve

Analytical Report

Canadian Grain Commission | Commission canadienne des grains

Grain Research Laboratory
1404-303 Main Street
Winnipeg, Manitoba
Canada R3C 3G8

2022-01-21

SUSPECTED TREATED SEED ANALYTICAL REPORT

Trace Organics Analysis Sample Number: Tr-01-22
Date submitted to Trace Organics Analysis: 2022-01-20

Sample Information:
Submitted by:
CGC IS file number:
Sample identification: [redacted]
Bin:
Tonnes:
Shipper:
Type of Grain: Wheat
Sample size: 2000 g
Suspect condition: 5 seed(s) from 2000 g sample. Seed(s) had a pink discoloration.

Tests performed: Trace Organic Analysis method GS-59

Results: The overall concentration of these chemicals in the sample does not exceed Canadian maximum residue limits. Consequently, the lot is not considered to be contaminated based on the sample provided.

More details provided on the following page.

Recommended action: No action required on food safety grounds.

Sheryl Tittlemier
Program Manager, Trace Organics and Trace Elements

Canadian Grain Commission | Commission canadienne des grains

Results:

Pesticide	Concentration in Stained Seed(s) (ppm)	Concentration in Total Sample, 2000 g (ppm)	Canadian Maximum Residue Limit (ppm)*
Acetamiprid	-	-	0.1
Azoxystobin	-	-	0.03
Boscalid	-	-	0.2
Broflanilide	-	-	0.01
Captan	-	-	0.1
Carboxin	-	-	0.2
Chlorantraniliprole	-	-	6
Clothianidin	-	-	0.01
Cyantraniliprole	-	-	0.1
Diazinon	-	-	0.1
Difenoconazole	17.6	0.002	0.1
Ethaboxam	-	-	0.01
Fludioxonil	1.8	0.0002	0.02
Fluopyram	-	-	1.5
Fluoxastrobin	-	-	0.1
Flupyradifurone	-	-	3
Fluxapyroxad	-	-	0.3
Imidacloprid	-	-	0.05
Inpyrfluxam	-	-	0.01
Ipconazole	-	-	0.01
Iprodione	-	-	0.07
Metalaxyl	5.2	0.0005	0.2
Metconazole	-	-	0.15
Oxathiapiprolin			0.1
Penflufen	-	-	0.01
Penthiopyrad	-	-	0.15
Picoxystrobin	-	-	0.04
Prothioconazole	-	-	0.35
Pydiflumetofen	-	-	0.1
Pyraclostrobin	-	-	0.2
Sedaxane	9.4	0.00008	0.01
Sulfoxaflor	-	-	0.08
Tebuconazole	-	-	0.15
Thiabendazole	-	-	0.1
Thiamethoxam	-	-	0.02
Thiophanate-methyl	-	-	0.1
Thiram	-	-	0.1
Tioxazafen	-	-	0.1
Triadimenol	-	-	0.05
Trifloxystrobin	-	-	0.05
Triticonazole	-	-	0.01

* http://www.hc-sc.gc.ca/cps-spc/pest/part/protect-proteger/food-nourriture/mrl-lmr-eng.php
- Analyte was not detected above limit of quantitation, LOQ (LOQ: the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy

Canada

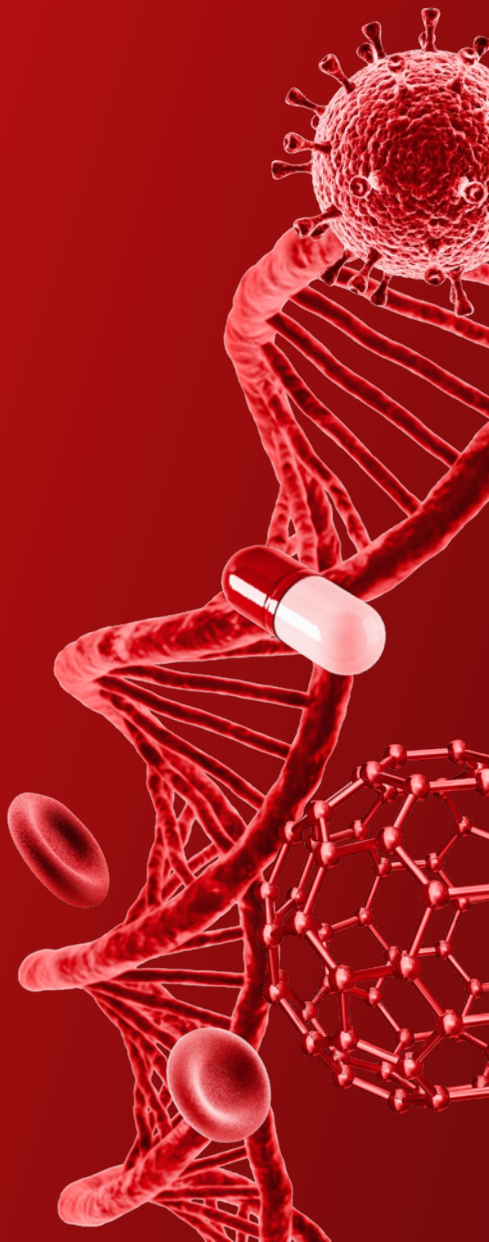
ThermoFisher
SCIENTIFIC

Comprehensive Drug Screening of Whole Blood by LC-HR-MS/MS in a Forensic Laboratory

Jon Benjamin Stephenson

Toxicology Technical Leader

Georgia Bureau of Investigation Division of Forensic Sciences

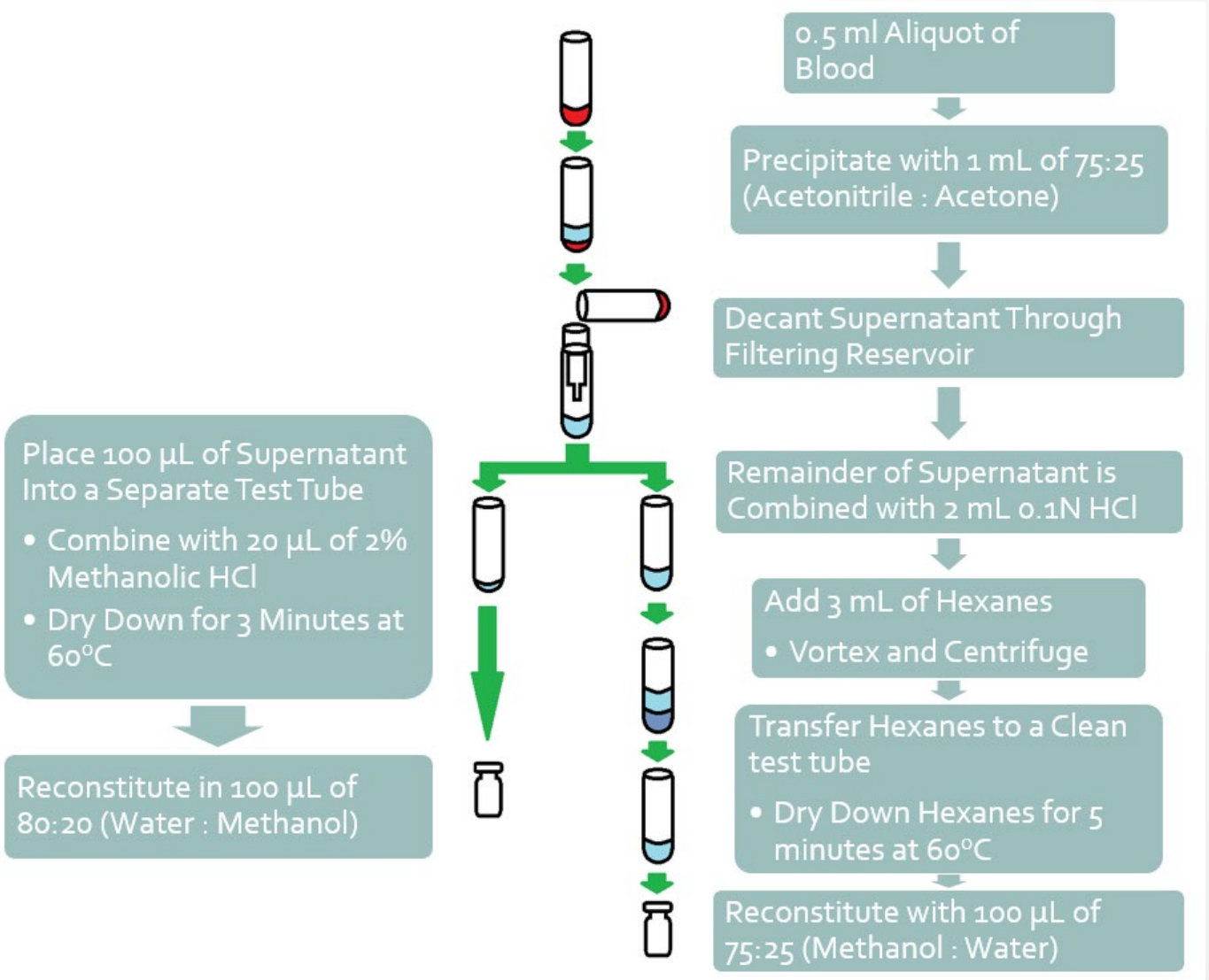


Grant Funding

- This project was supported by Award No. 2016-DN-BX-K005, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice.

  The opinions, findings, and conclusions or recommendations expressed in this program are those of the author and do not necessarily reflect those of the Department of Justice.

Sample Preparation



Instrument Method: LC Setup

Mobile Phase A

- ~15 millimolar Ammonium Formate
- 0.1% Formic Acid
- Optima Grade Water

Mobile Phase B

- ~15 millimolar Ammonium Formate
- 0.1% Formic Acid
- 50 Acetonitrile : 50 Methanol

Syringe Wash

- 50 Methanol : 50 Water

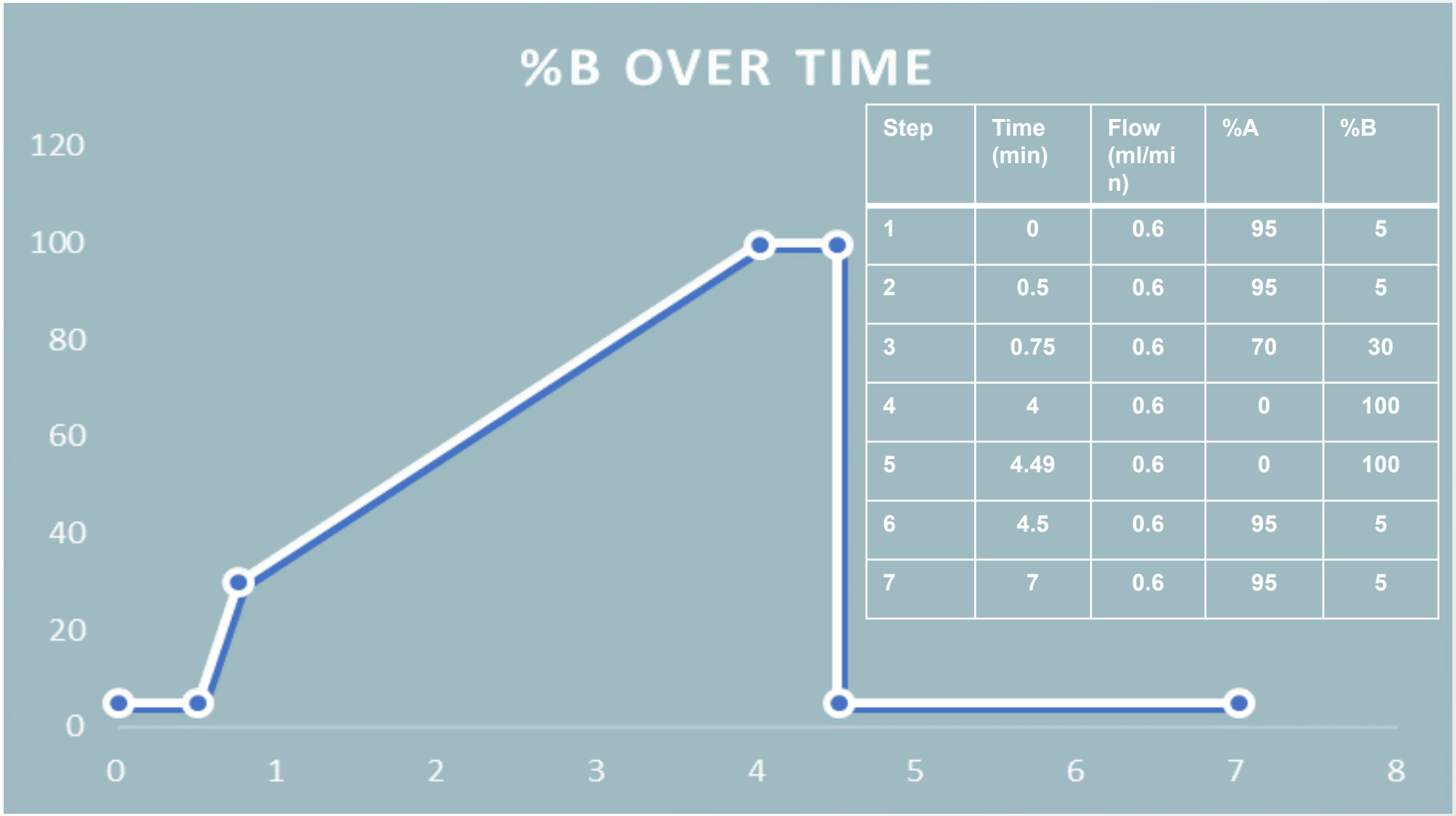
Rear Seal Wash

- 75 % Isopropyl Alcohol
- 25 % Water
- 0.1 % Formic Acid

Agilent Poroshell 120 C18

- 2.1x100 mm
- 2.7 micron packing material

Instrument Method: HPLC Gradient – Positive Ion

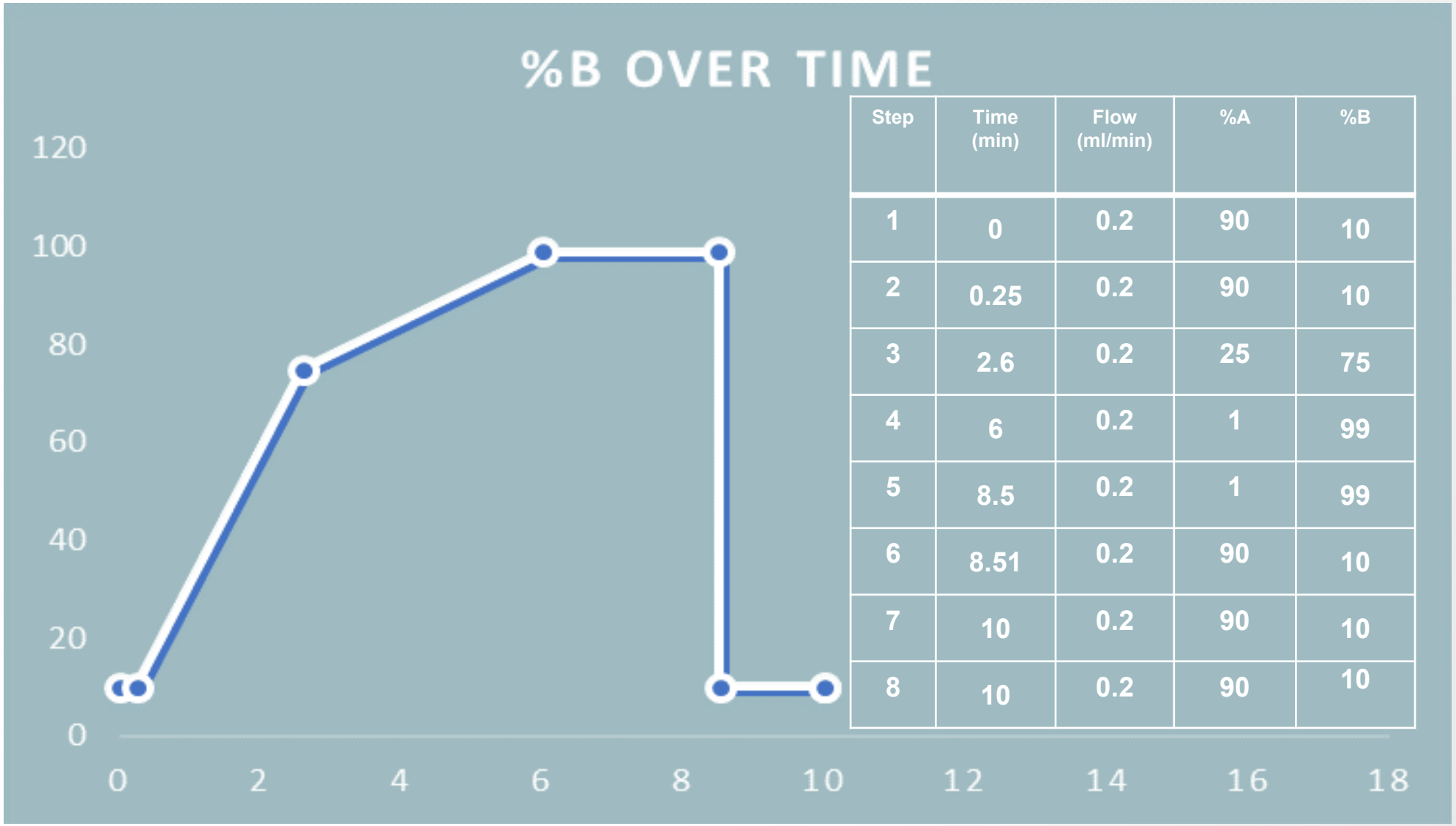


Step	Time (min)	Flow (ml/min)	%A	%B
1	0	0.6	95	5
2	0.5	0.6	95	5
3	0.75	0.6	70	30
4	4	0.6	0	100
5	4.49	0.6	0	100
6	4.5	0.6	95	5
7	7	0.6	95	5

Instrument Method: Orbitrap Positive Mode

- Inclusion list of compounds
  - Accurate mass and name of each compound
  - 0.4 min retention time windows
  - Polarity
  - Exclude
    - Formula
    - Species
    - Charge State
- MS1
  - 35,000 Resolution
  - Scan Range 50 to 750 m/z
- MS2
    - 17,500 Resolution
  - Stepped Collision Energy
    - 10, 25, 50
  - Minimum AGC target
    - Positive Mode 3e3
  - Dynamic Exclusions 6 s

Instrument Method: HPLC Gradient – Pos/Neg Ion



Step	Time (min)	Flow (ml/min)	%A	%B
1	0	0.2	90	10
2	0.25	0.2	90	10
3	2.6	0.2	25	75
4	6	0.2	1	99
5	8.5	0.2	1	99
6	8.51	0.2	90	10
7	10	0.2	90	10
8	10	0.2	90	10

Instrument Method: Orbitrap Pos/Neg Mode

- Inclusion list of compounds
  - Accurate mass and name of each compound
  - 0.4 min retention time windows
  - Polarity
  - Exclude
    - Formula
    - Species
    - Charge State
- MS1
  - 35,000 Resolution
  - Scan Range 50 to 750 m/z
- MS2
    - 17,500 Resolution
  - Collision Energy
    - NCE 10 for Barbituates
    - NCE 30 for THC and Related compounds
  - Minimum AGC target
    - Positive Mode 1e3
  - Dynamic Exclusions (Auto)

Mass Calibration and QC Checks

- Performed weekly
  - Standard Mass Calibration for Positive and Negative modes
  - Custom Calibration done for Positive mode to include lower mass ion 74.09643

- Daily Test mixture
  - Checks
    - Chromatography
    - Mass assignments
    - Sensitivity
  - Masses across the scan range within 5ppm
    - Amphetamine 136.1121
    - Diphenhydramine 256.1696
    - Fentanyl 337.2274
    - Buprenorphine 468.3108
    - Butalbital 223.1088
    - Butabarbital 211.1088

Data Processing

- Generally desire more drugs to pass through processing filters than accidentally exclude something of importance.

- Mass Spectrum Matching
  - Threshold 50
  - Passing value 50
- Isotopic pattern
  - 50% fit threshold (positive mode)
  - 60 ppm mass deviation allowed
  - 100% intensity deviation
  - No requirement in negative mode
- Mass Accuracy
  - 5-10 ppm accuracy
- Retention time
  - 30 second window

- Reviewing scientist evaluates the data and determines what analytes are and are not present based upon the software's pared back results

Validation: LOIs

- Generally the Positive Mode LOI for most drugs is at least 2 µg/L
  - Exceptions
    - MDA, Meprobamate, Phentermine, Zolpidem, and Zopiclone
      - 20 µg/L
    - Phenylephrine, Methylone, and Levetiracetam
      - 10 µg/L
    - Duloxetine, Carisoprodol, and Bromazepam
      - 5 µg/L
    - Fentalogs
      - Furanylfentanyl, carfentanil, and parafluorofentanyl down to 1 µg/L

Validation: Matrix Effects

- Matrix effects were determined by fortifying three separate lots of negative blood post extraction and comparing the average area to the area of an un-extracted non-matrix containing sample.

- Ion suppression or enhancement was universally below 20% with
  - Some analytes such as morphine, nalorphine, perphenazine, norbuprenorphine, mitragynine, nifedipine, acetaminophen, and ranitidine showing ion enhancement from 500% up to more than 1000%
  - Due to the method being a qualitative screen this has no real negative effect on the results as all the reported LOIs are within an acceptable range of detection.

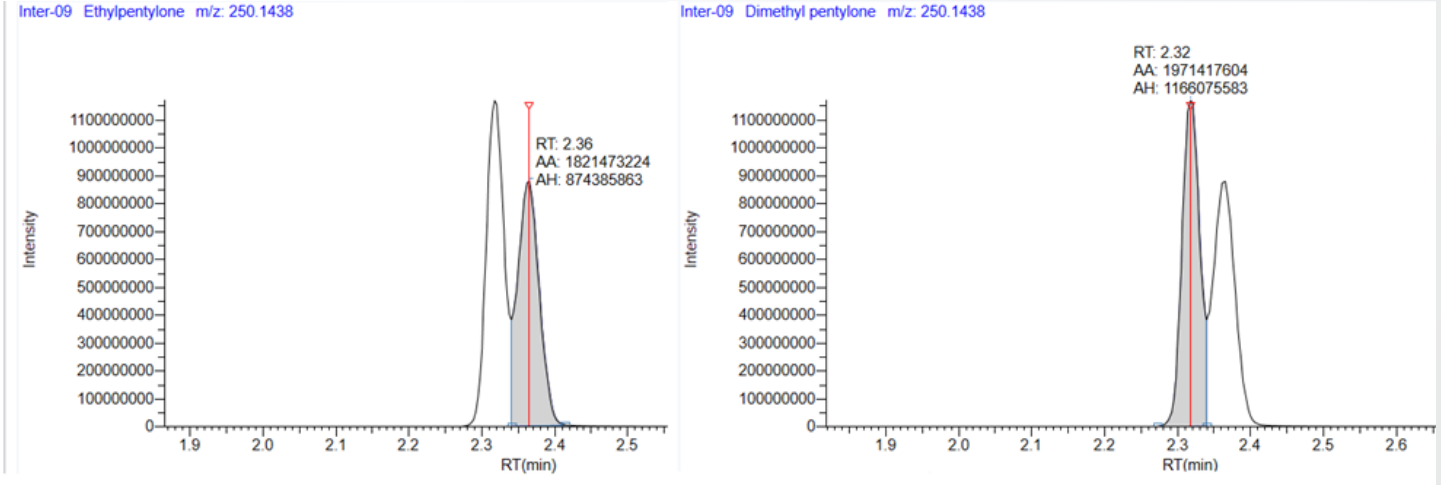
Validation: % Recovery

- Percent recovery was determined by averaging the area of three separate lots of negative blood that was fortified, extracted, and compared to the area of an un-extracted non-matrix containing sample.

- Percent recovery was typically around 100% or greater (mostly due to ion enhancement)
  - Some analytes showed recovery levels below 50% such as meclizine, flubromazolam, lurasidone, ziprasidone, and sertraline
  - Even with some lowered % recovery the LOIs for the analytes remained in an acceptable range.

Validation: Interferences

- Using resolution equation the resolution between all known isomers was determined
- R= 2 x [(tB - tA) / (WB+WA)]
- Resolution (R) equation, tB is the retention time of the later eluting analyte, tA is the retention time of the earlier eluting analyte, WB is the peak width of the later eluting analyte and WA is the peak width of the earlier eluting analyte.
- Resolution of 1.5 indicates two peaks are fully resolved.
- Due to this method being qualititaive only full resolution is not required for identification.
- Example: Ethylpentylone and dimethylpentylone have a resolution of 0.43 and identification is still possible
- Amitriptyline and maprotiline with a resolution of 0.35 are not sufficiently resolved to identify each compound in a single extract



Validation: Interferences

Analyte	Mass	RT	Peak Width (min)	Analytes Used to Calculate Resolution	Resolution
Methamphetamine	150.1277	1.99	0.055	methmphetmine/phentermine	1.8
Phentermine	150.1277	2.08	0.045		
bk-MDDMA	222.1125	1.9	0.09	bk-MDDMA/ethylone	0.666666667
Butylone	222.1125	2.03	0.07	bk-MDDMA/butylone	1.625
Ethylone	222.1125	1.96	0.09	butylone/ethylone	0.875
Metaxalone	222.1125	3.22	0.075	metaxalone/butylone	16.4137931
Methylphenidate	234.1489	2.29	0.07	methylphenidate/normeperidine	2
Normeperidine	234.1489	2.43	0.07		
bk-DMBDB (Dibutylone)	236.1281	2.06	0.08	bk-DMDB/eutylone	0.75
Eutylone	236.1281	2.12	0.08	pentylone/eutylone	2
Pentylone	236.1281	2.27	0.07		
Diphenhydramine	256.1696	2.83	0.085	diphenhydramine/atomoxetine	2.875
Atomoxetine	256.1696	3.06	0.075		
Propranolol	260.1645	2.75	0.085	propranolol/ramelteon	7.5
Ramelteon	260.1645	3.35	0.075		
EMDP	264.1747	3.58	0.085	EMDP/notriptyline	2.818181818
Nortriptyline	264.1747	3.27	0.135		
Norvenlafaxine	264.1958	2.16	0.07	norvenlafaxine/tramadol	1.62962963
Tramadol	264.1958	2.27	0.065		
EDDP	278.1903	2.93	0.095	EDDP/maprotiline	3.428571429
Amitriptyline	278.1903	3.26	0.09	maprotiline/amitriptyline	0.352941176
Maprotiline	278.1903	3.23	0.08		
Imipramine	281.2012	3.19	0.085	imipramine/4-ANPP	5.529411765
4-ANPP	281.2012	2.72	0.085		
Hydromorphone	286.1438	1.69	0.095	morphine/hydromorphone	1.828571429
morphine	286.1438	1.53	0.08	hydromorphone/norcodeine	1.485714286
Norcodeine	286.1438	1.82	0.08		
Hydrocodone	300.1594	1.92	0.09	hydrocodone/codeine	1.142857143
Codeine	300.1594	1.82	0.085		
Temazepam	301.0738	3.48	0.07	temazepam/clobazam	0.533333333
clobazam	301.0738	3.44	0.08		
Noroxycodone	302.1387	1.88	0.07	noroxycodone/oxymorphone	3.294117647
Oxymorphone	302.1387	1.6	0.1		
Scopolamine	304.1543	1.91	0.065	scopolamine/cocaine	9
Cocaine	304.1543	2.54	0.075		
Norpropoxyphene*	308.2009	3.11	0.065	norpropoxyphene/benztropine	1.866666667
Benztropine	308.2009	3.25	0.085		
AH-7921	329.1182	2.91	0.085	AH-7921/U-47700	1.222222222
U-47700	329.1182	2.8	0.095		
Crotonyl fentanyl	349.2274	2.83		crotonyl fentanyl/cyclopropylfentanyl	unresolved
Cyclopropylfentanyl	349.2274	2.83			
FIBF	369.2337	2.97		FIBF/parafluorobuytyrlfentanyl	unresolved
Parafluorobuytyrlfentanyl	369.2337	3			
Dimethylpentylone	250.1438	2.3	0.095	dimethylpentylone/ethylpentylone	0.432432432
ethylpentylone	250.1438	2.34	0.09		
Oxcarbazepine**	253.0972	2.8	0.095	oxcarbazepine/phenytoin	2.171428571
Phenytoin**	253.0972	2.99	0.08		
Oxcarbazepine ***	253.0972	4.98	0.21	oxcarbazepine/phenytoin	1.58974359
Phenytoin***	253.0972	5.29	0.18		

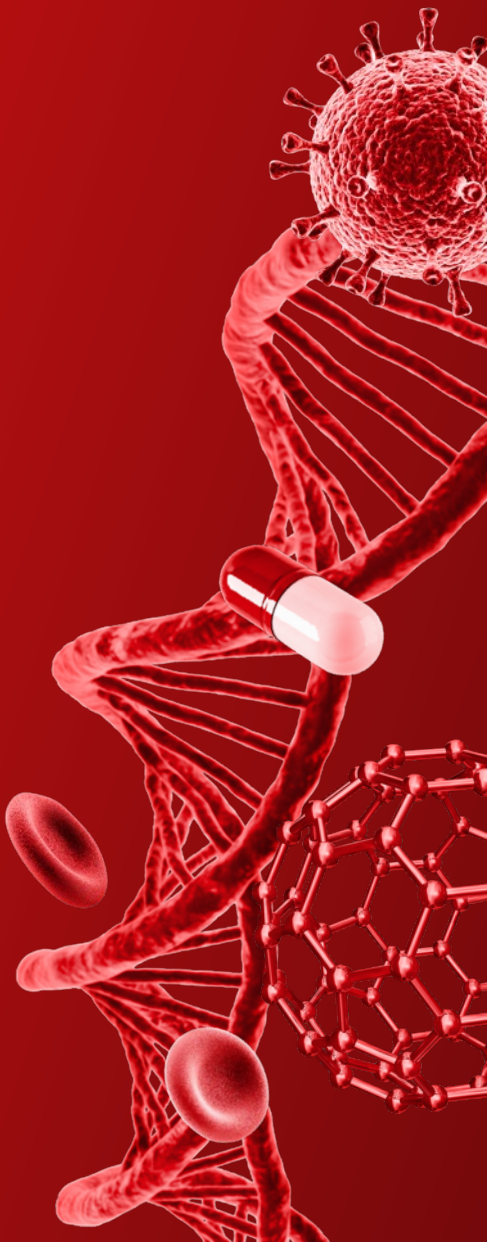
* Mass after in-source loss of water.

** positive method

*** positive/negative switching method

Orbitrap-based Veterinary Toxicology Screening

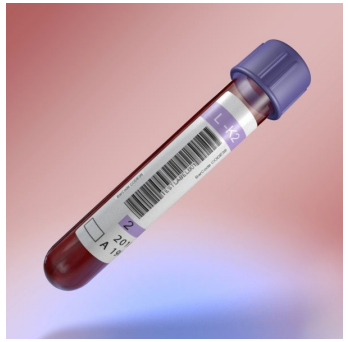
David J. Borts, PhD
Clinical Professor
Analytical Chemistry Services
Veterinary Diagnostic Laboratory
Department of Veterinary Diagnostic and Production Animal Medicine
College of Veterinary Medicine
Iowa State University



Orbitrap-based Veterinary Toxicology Screening

- Case history
  - Midwest US cow-calf farm
  - Calves – sudden ataxia, tremors, and death
  - Cows – same observations ~ 96 hours later
  - Cow rumen (stomach) contents submitted initially
  - Cow milk and calf rumen contents subsequently analyzed
  - Orbitrap-based LC/MS general unknown toxicology screening performed

General Unknown Tox Screening – Orbitrap-based Workflow

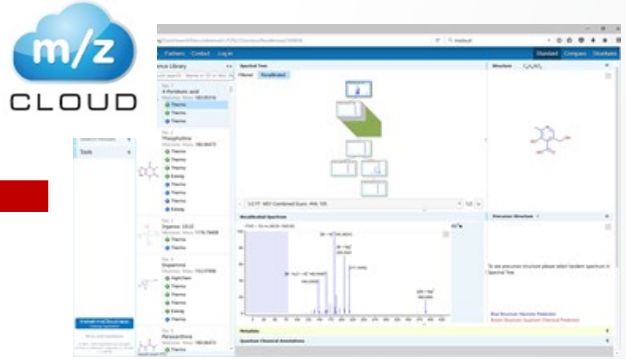


Sample

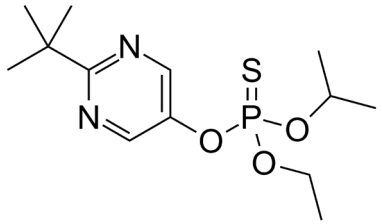
QuEChERS or other 'generic' extraction

Vanquish + Exploris 120 ddMS^2 analysis

Compound Discoverer data processing

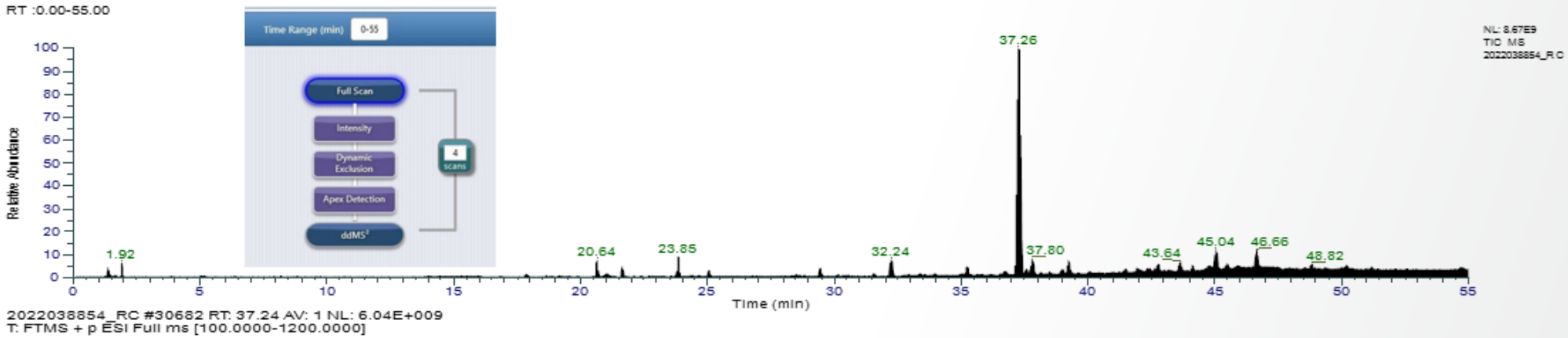


mzCloud database searching

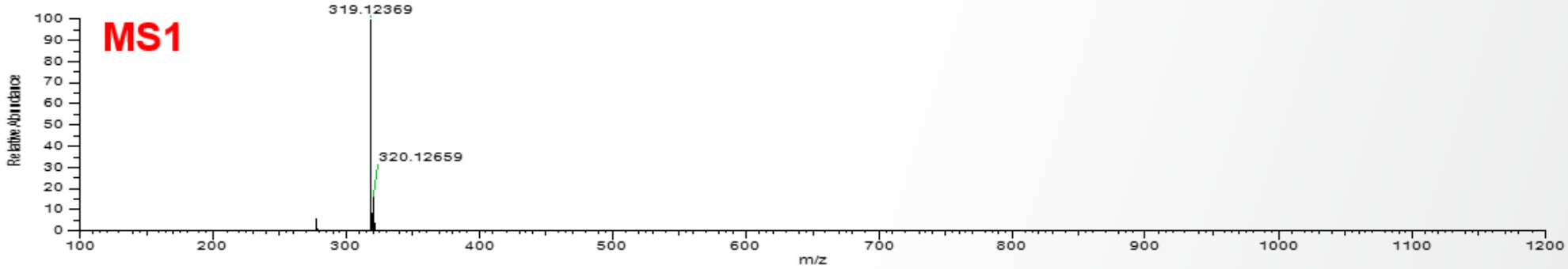


Toxic compound ID

Rumen contents extract



2022038854_RC #30682 RT: 37.24 AV: 1 NL: 6.04E+009
T: FTMS + p ESI Full ms [100.0000-1200.0000]



2022038854_RC #30683 RT: 37.24 AV: 1 NL: 9.30E+008
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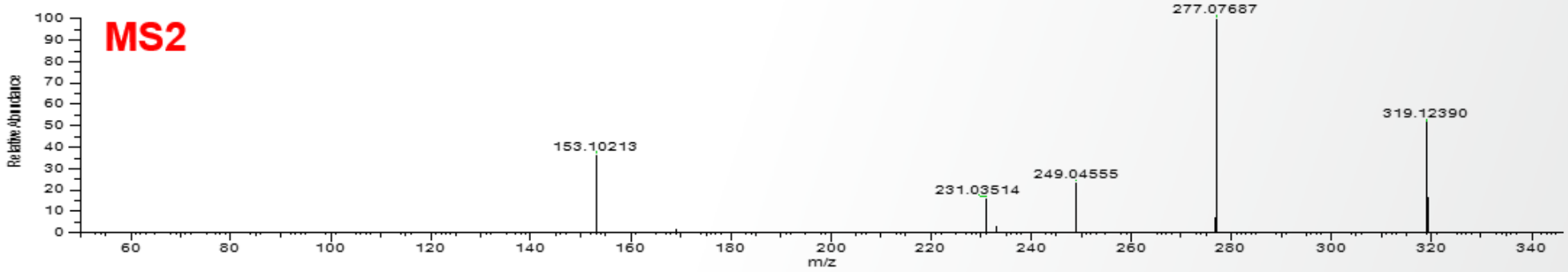
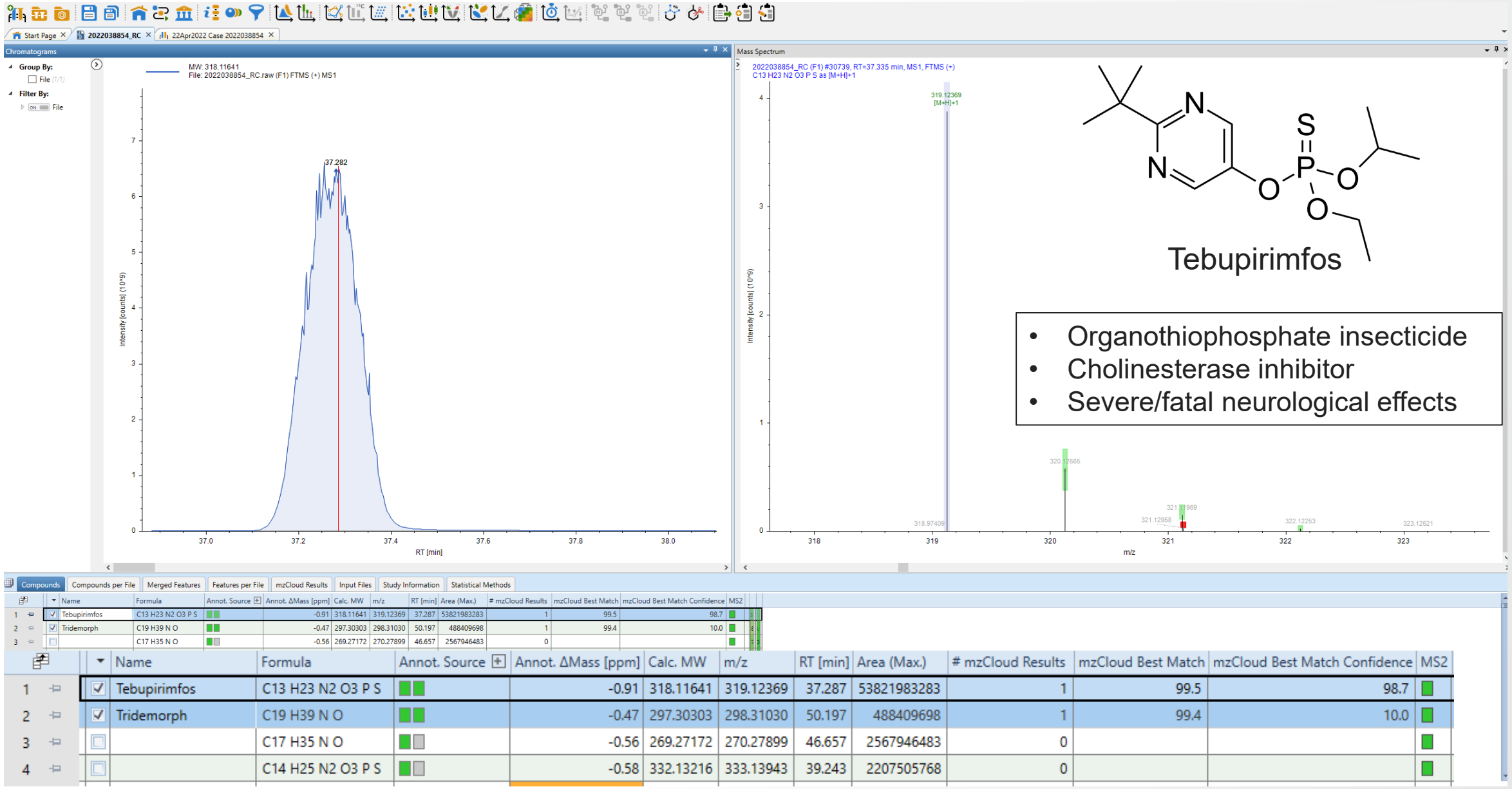
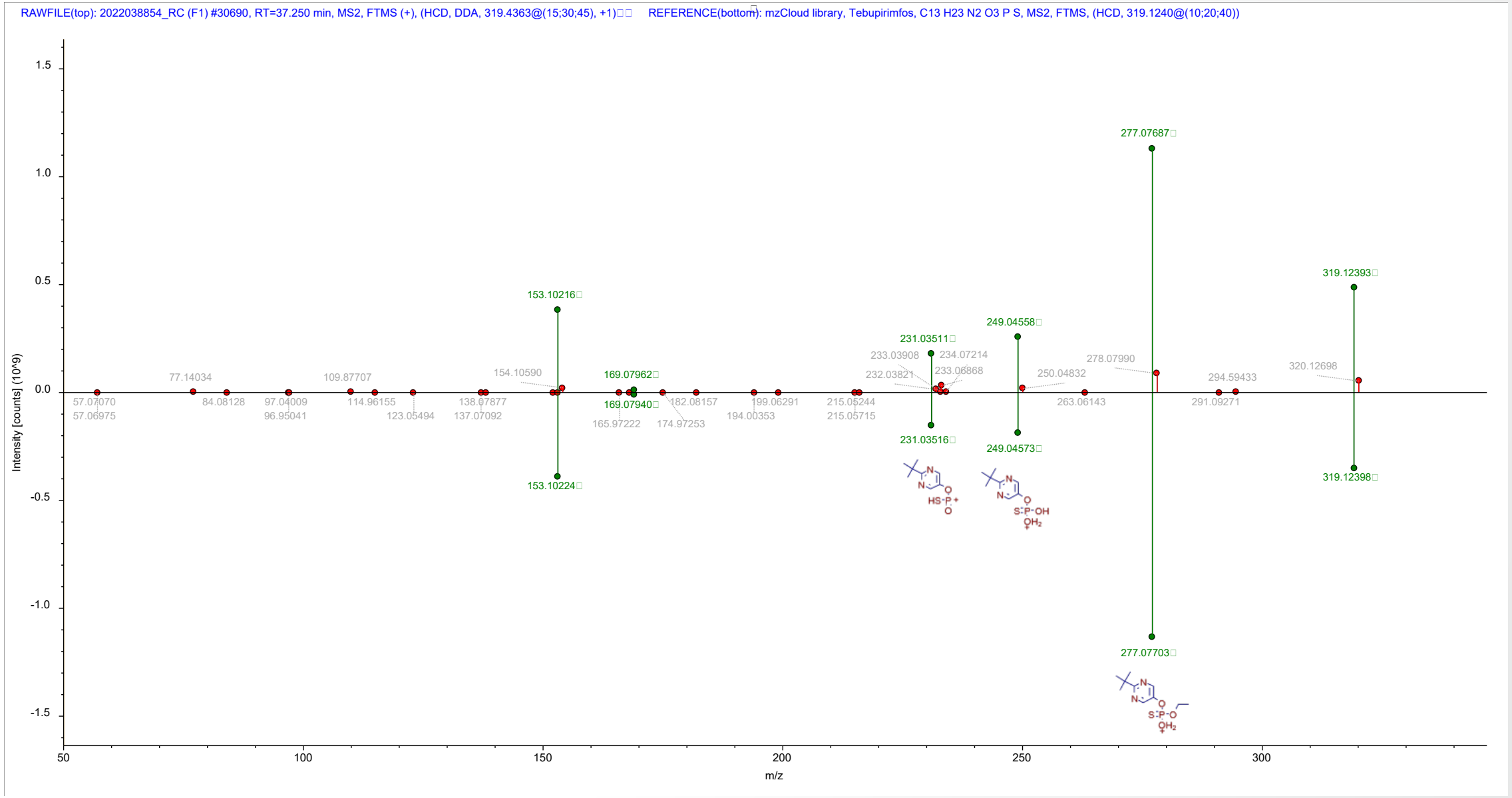


Compound Discoverer



	Name	Formula	Annot. Source	Annot. ΔMass [ppm]	Calc. MW	m/z	RT [min]	Area (Max.)	# mzCloud Results	mzCloud Best Match	mzCloud Best Match Confidence	MS2
1	Tebupirimfos	C13 H23 N2 O3 P S		-0.91	318.11641	319.12369	37.287	53821983283	1	99.5	98.7	
2	Tridemorph	C19 H39 N O		-0.47	297.30303	298.31030	50.197	488409698	1	99.4	10.0	
3		C17 H35 N O		-0.56	269.27172	270.27899	46.657	2567946483	0			
4		C14 H25 N2 O3 P S		-0.58	332.13216	333.13943	39.243	2207505768	0			

Tebupirimfos mzCloud Library Match Score = 99.5



Orbitrap-based Veterinary Toxicology Screening

- Case conclusions
  - Tebupirimfos identified in cow and calf rumen and cow milk
  - On-farm investigation revealed farmhand had inadvertently mixed a tebupirimfos-containing insecticide into cow feed (had similar appearance to a commonly used mineral supplement)
  - Only cows had access to contaminated feed
  - Nursing calves received toxic dose of tebupirimfos through cow milk
  - Feeding equipment cleaned and contaminated feed replaced
  - No further symptoms or fatalities were observed in herd

Acknowledgements

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  - Analytical Chemistry Services Section

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Clinical Assistant Professor

Laura Burns, MS
Lead LC/MS
Chemist

Summary

- Orbitraps are very cool and powerful devices
- Orbitraps are very versatile and can be used in qualitative or quantitative environments across a wide range of industries.

Thank you!