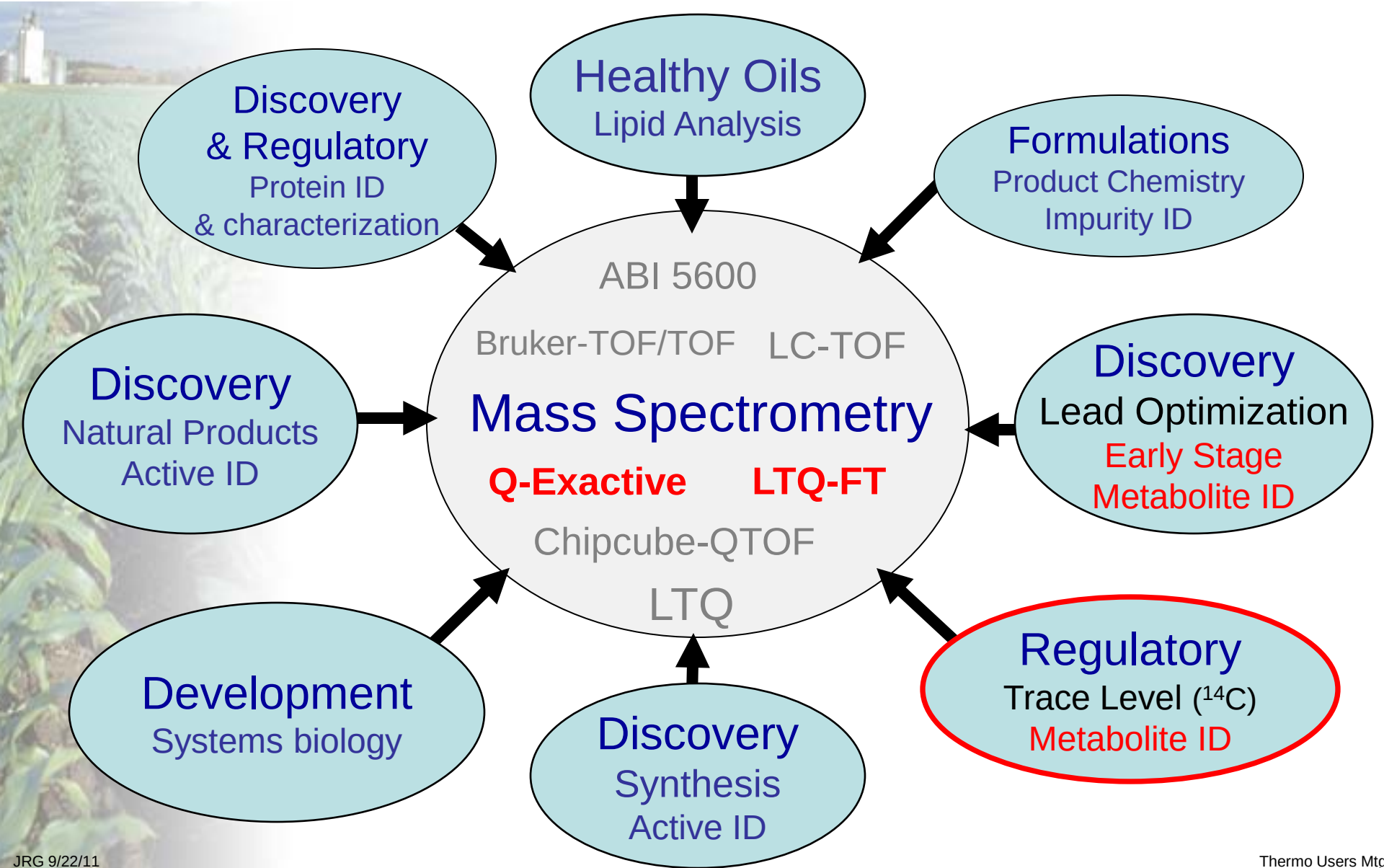
Trace Level Metabolite Identification Using the Latest Generation of LIT-FTMS and Q-orbitrap Instruments

Jeffrey R. Gilbert*[1], Jesse L. Balcer[1], Yelena Adelfinskaya[1], Kate Comstock[2], Yingying Huang[2],Suresh Annangudi[1], Ayanna Jackson[1], Mike J. Hastings[1], Brian M. Wendelburg[1], Mingming Ma[1], and David G. McCaskill[1]

1. Dow AgroSciences, 9330 Zionsville Road, Indianapolis, IN 46268
2. Thermo Fisher Scientific, 355 River Oaks Pkwy, San Jose, CA 95127

Mass Spectrometry at Dow AgroSciences

Our Mission: To solve complex **identification problems** encountered in the discovery, development, registration, and defense of Dow AgroSciences products using state of the art expertise and techniques in mass spectrometry.



Regulatory Metabolite Identification - Systems Studied

Metabolism:

- Animal Metabolism - ruminants (goat/cow and poultry)
- Plant Metabolism - groupings (root vegetables, leafy crops, fruits, cereals)

Environmental Fate:

- Aerobic Soil/Sediment
- Anaerobic Soil/Sediment
- Hydrolysis
- Photolysis

(^{14}C) Radiolabeled Parent is Available!!

Challenges

- Complex matrices – plant and soil much dirtier than urine/plasma/feces
- Low dose levels/application levels – low soil dose rates ~ 0.020 ppm
  must identify 5% of applied = **0.001 ppm**
- Slow metabolite formation – soil metabolites can take **weeks/months** to form
  - Residue method development must incorporate required metabolites
- Typical customer supplies < 200ul of an extract containing several metabolites
  - We must inject 30-50 ul for on-line radiochemical detection

How can we maximize the information obtained from each injection?

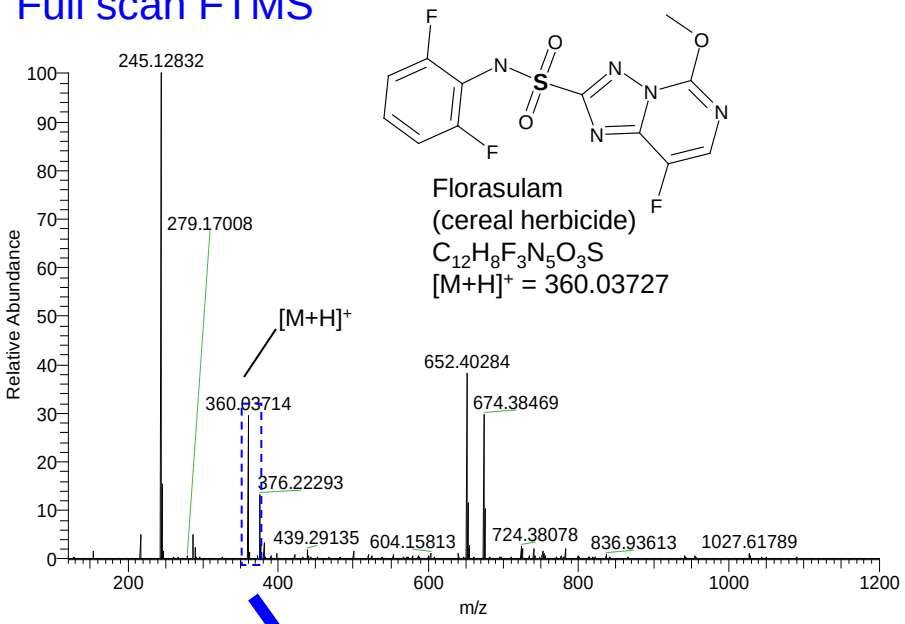
MS Tools to Aid in Metabolite Identification

- Thermo LTQ-FT Ultra
  - Sensitivity
  - Pos/Neg operation
  - High Mass Accuracy (typically < 1ppm)
  - High Resolution (up to 1,000,000)
  - MS^n
- IRMPD (Infrared Multi Photon Dissociation)
  - Fragmentation and Detection in the FT
    - No 1/3 cut-off for fragment ions
    - No time-of-flight effect in product ion spectra
- Advion Nanomate
  - Collect fractionated sample (after RAM)
  - Chip-based infusion enables thorough interrogation of samples
  - Extended infusion allows use of slower scan functions (very high res)
- Controlled Isotopic Labeling (^{13}C, ^{15}N, ^{2}H) – ‘easily’ recognize the metabolites
- Thermo Q-Exactive
  - Improved Sensitivity (~10-50x over LTQ-FT)
  - High Acquisition Speed
  - Pos/neg switching
  - High Mass Accuracy

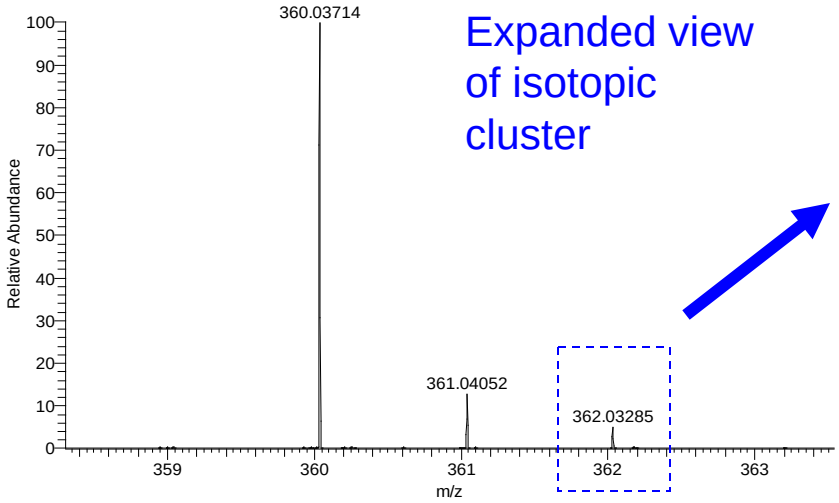
Benefits of high resolution – M+2 Sulfur counting

Florasulam in Wheat Cell Extract

Full scan FTMS

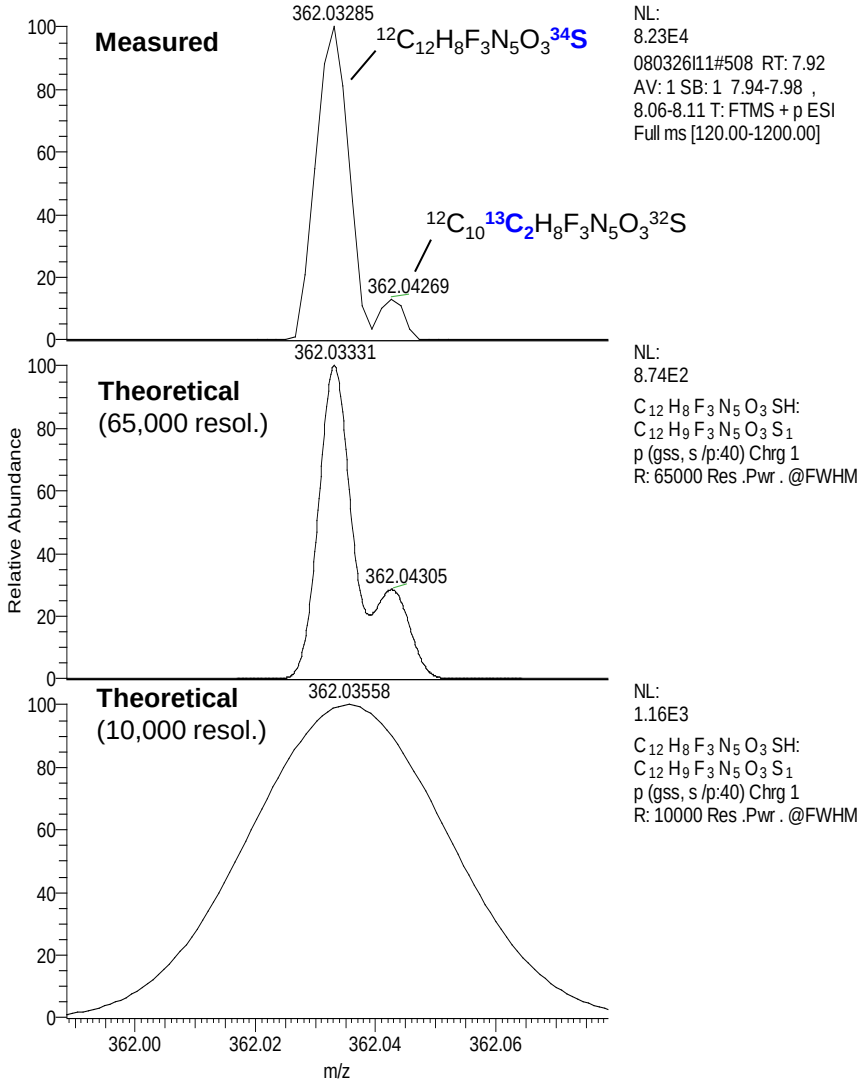


Florasulam
(cereal herbicide)
$C_{12}H_8F_3N_5O_3S$
$[M+H]^+ = 360.03727$

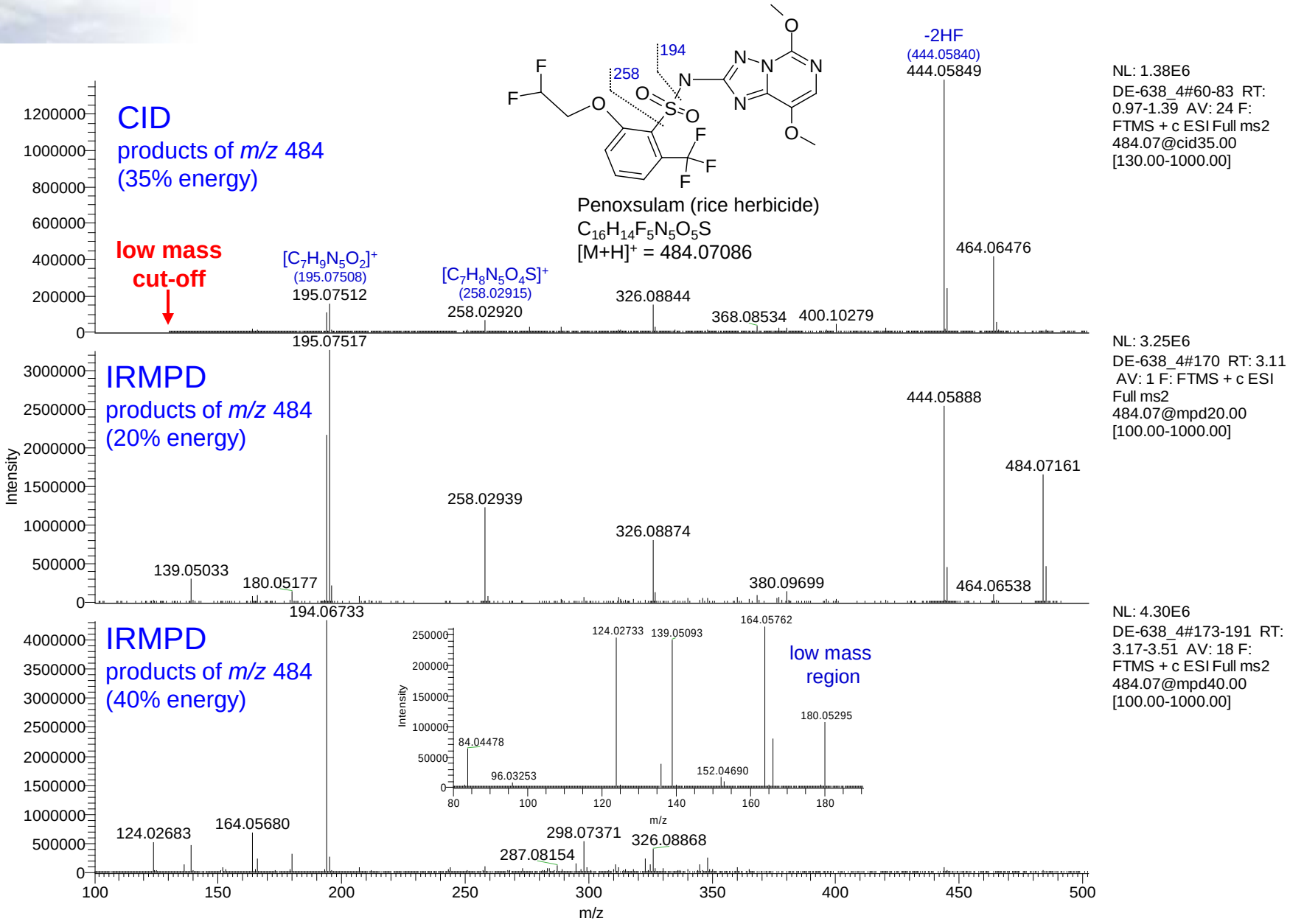


Expanded view of isotopic cluster

M+2 isotope

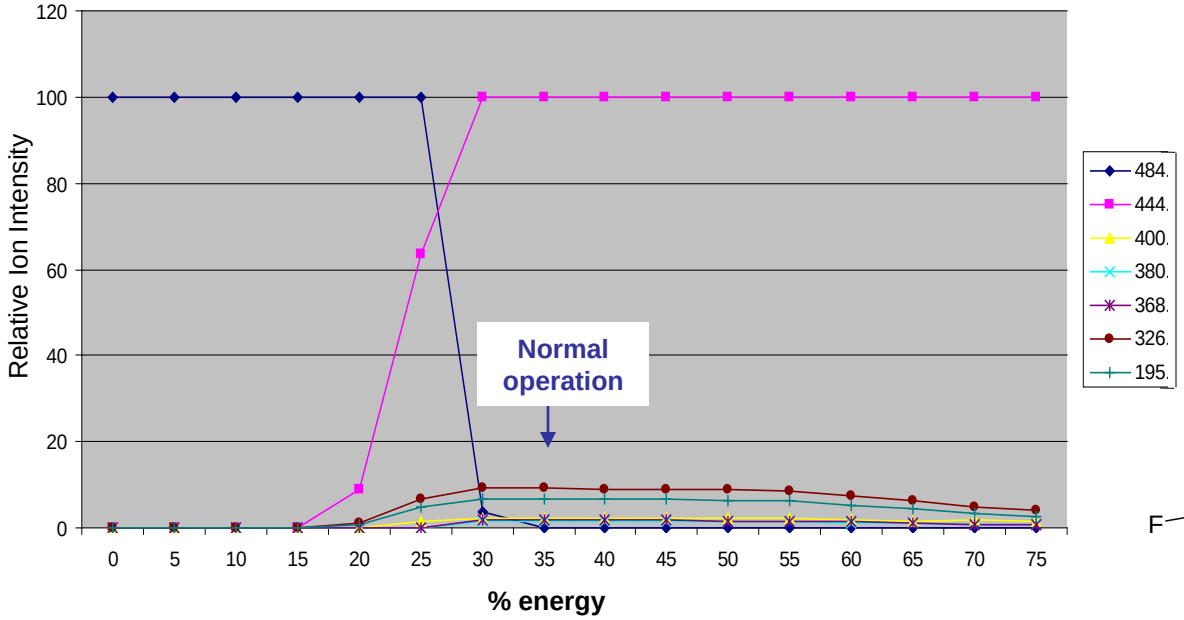


CID vs. IRMPD – Low Mass Ion Detection

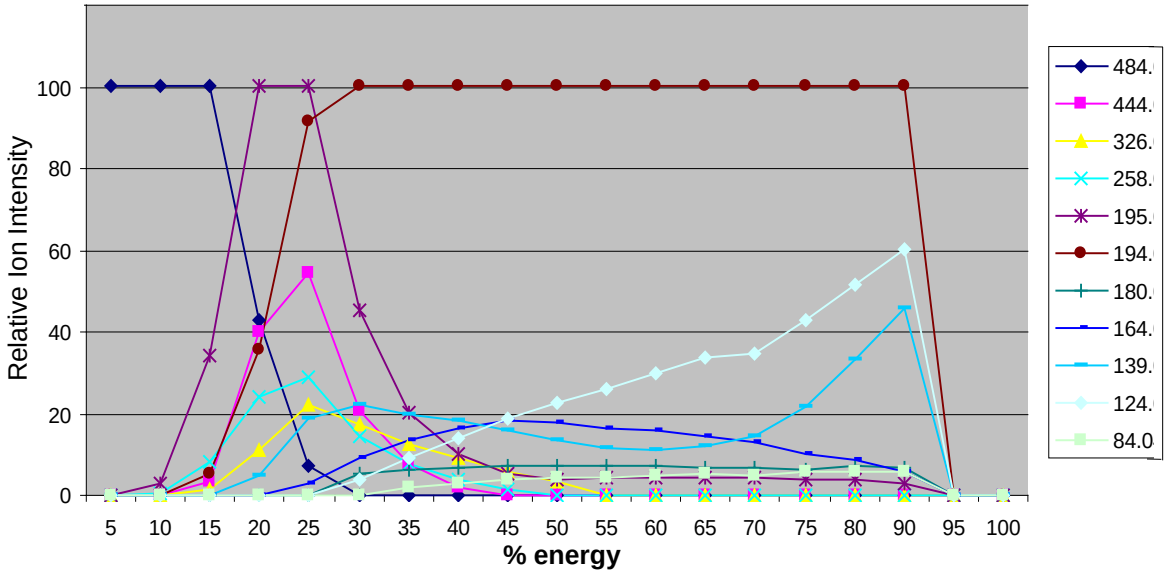


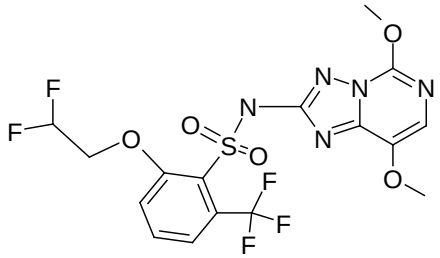
CID vs. IRMPD – Energy Dependence of Fragmentation

CID



IRMPD

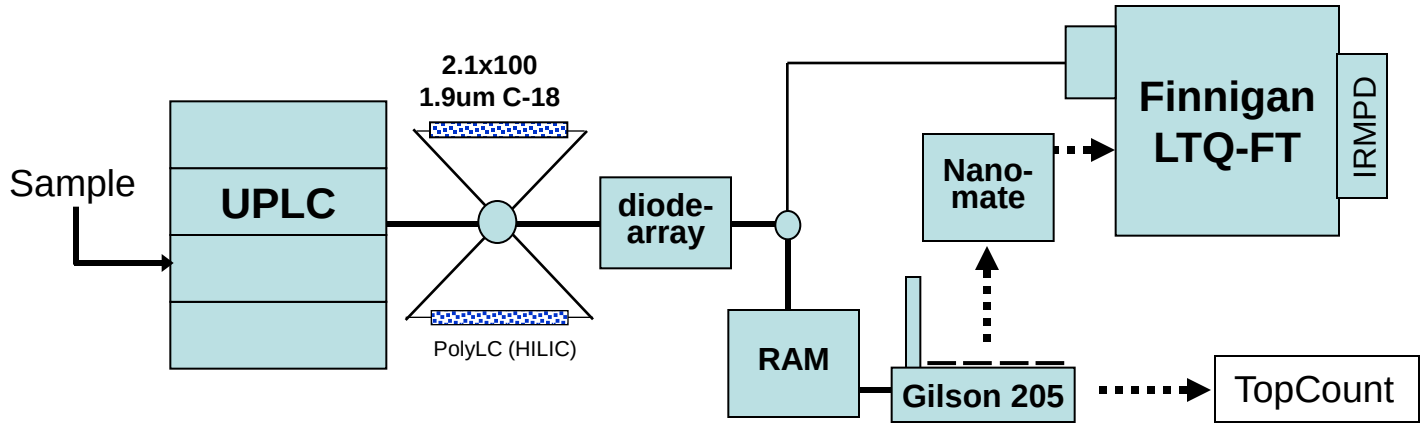




Penoxsulam (rice herbicide)
$C_{16}H_{14}F_5N_5O_5S$
$[M+H]^+$ = 484.07086

More optimization required for IRMPD

Metabolite Identification on the LTQ-FT

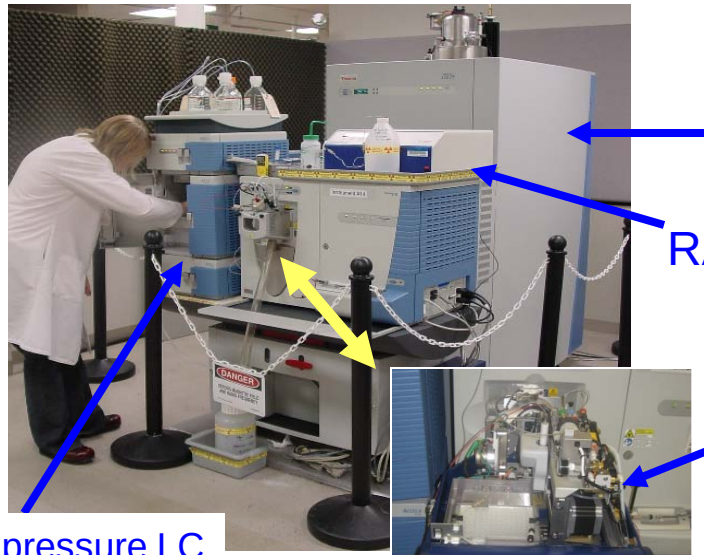


General Workflow:

1) LC/MS analyses (Sample + Stds.)
   - post column split (ESI:RAM)
   - +/- ESI-FTMS
   - DD-MS^n
   - IRMPD (1-2 energies)
   - fractionation (collect 96-well plates)

2) Retention time and mass spectral match with standards?

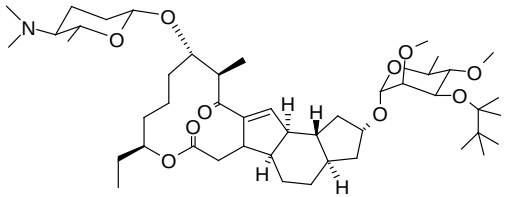
3) Nanomate infusion of fractionated sample from step 1.
   - Detailed IRMPD
   - High resolution MS



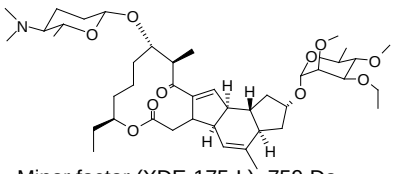
Spinetoram – lettuce leaf metabolism

Spinetoram

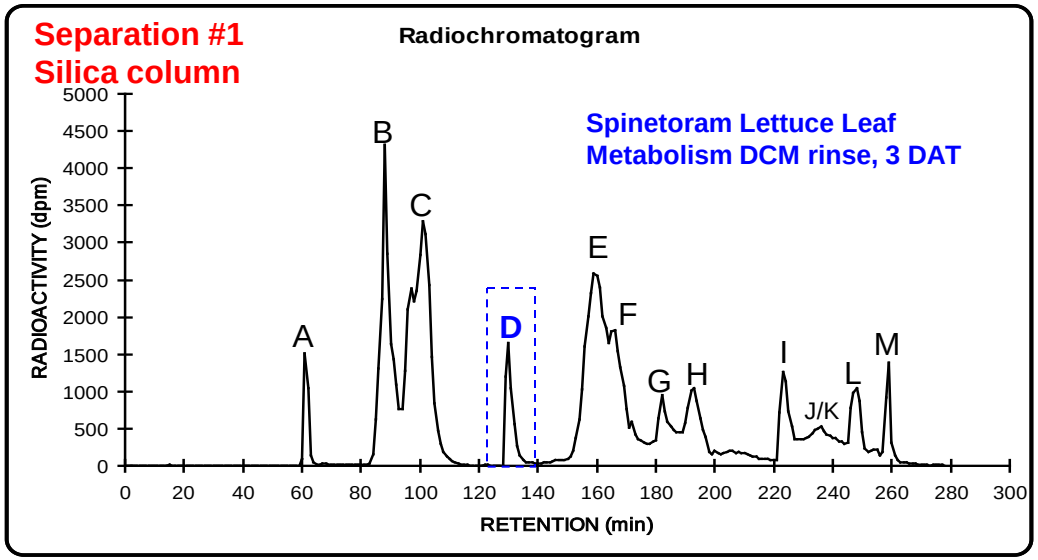
- New natural product-derived insect control agent
- Broad spectrum of insect activity
- Novel mode of action (resistance management)
- Low persistence in the environment
- Consists of two primary factors

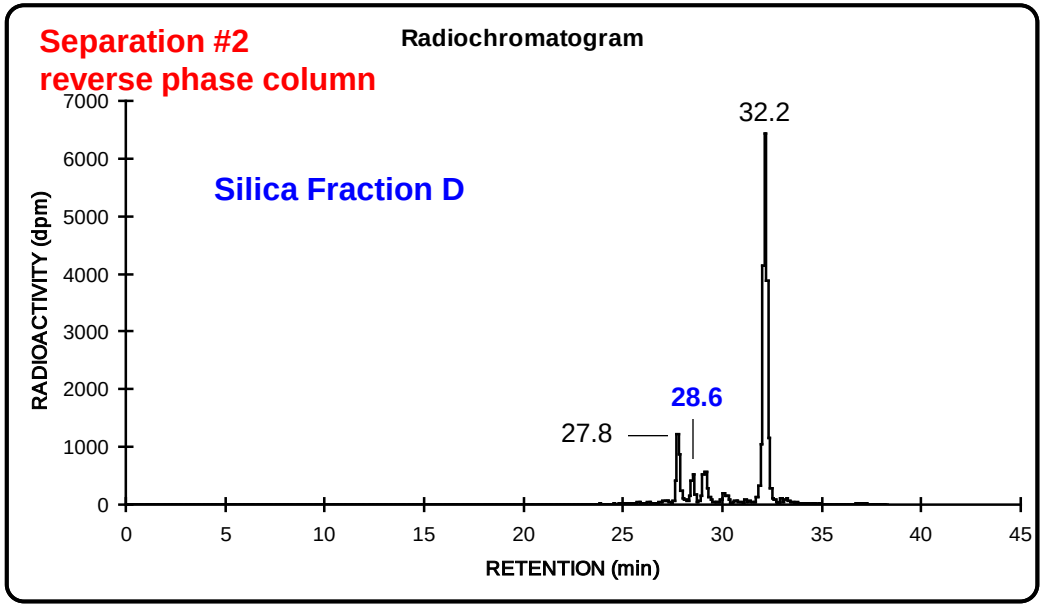


Spinetoram Major Factor (XDE-175-J)
$C_{42}H_{69}NO_{10}$
$[M+H]^+$ = 748.4994

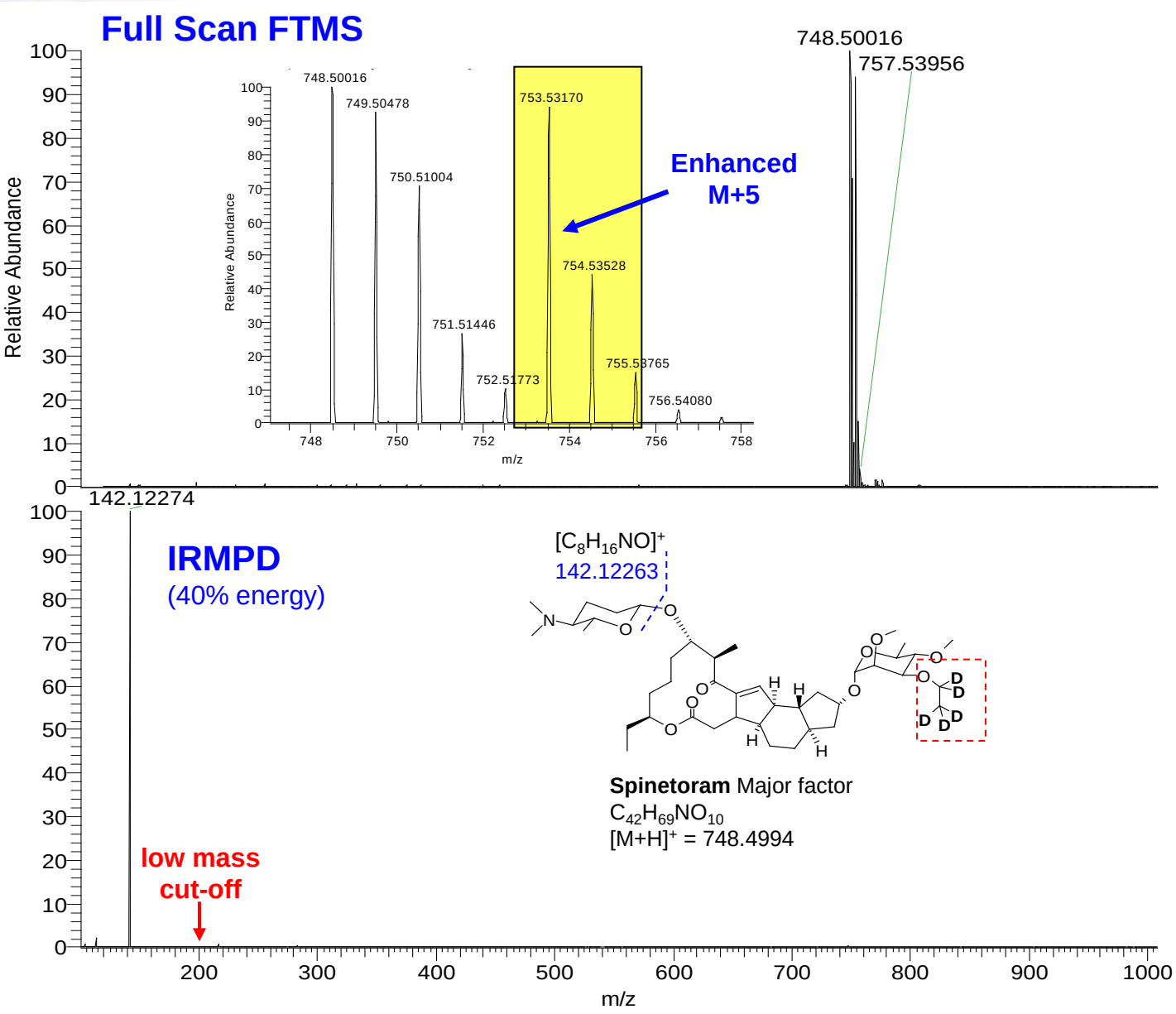


Minor factor (XDE-175-L), 759 Da



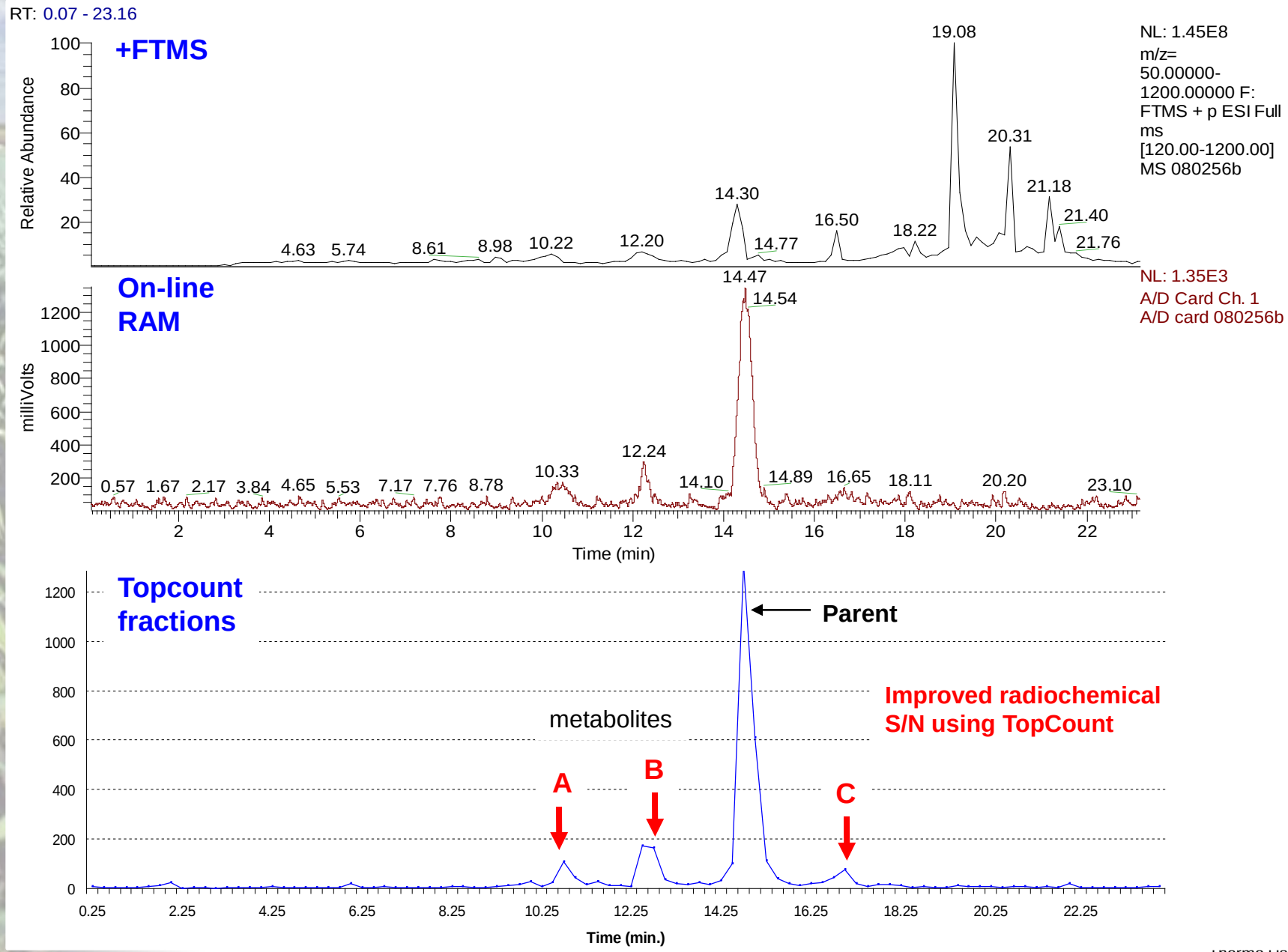


Spinetoram labeling scheme aids in metabolite detection



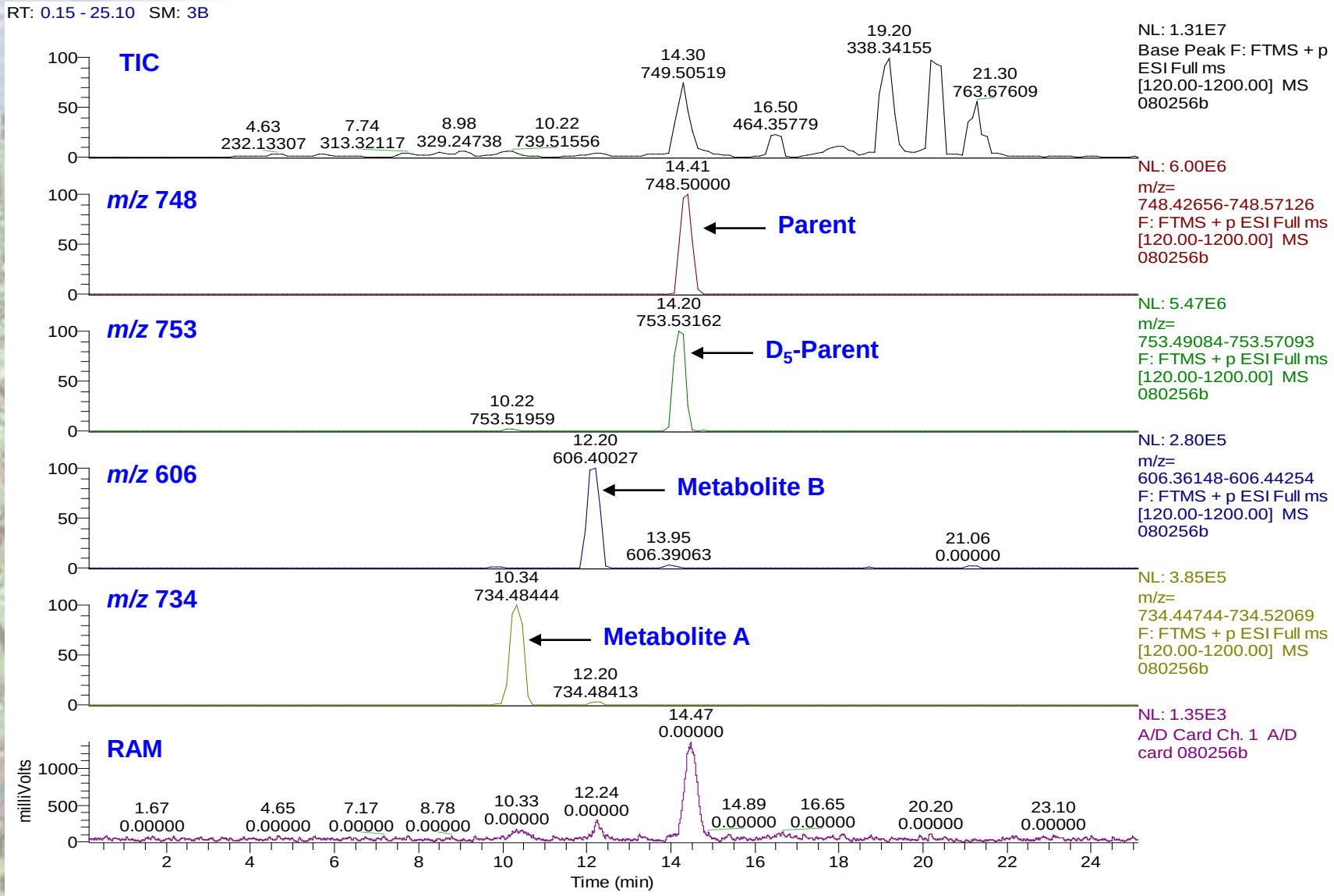
Spinetoram – Turnip Leaf Extract

FTMS TIC and Radiochemical Traces

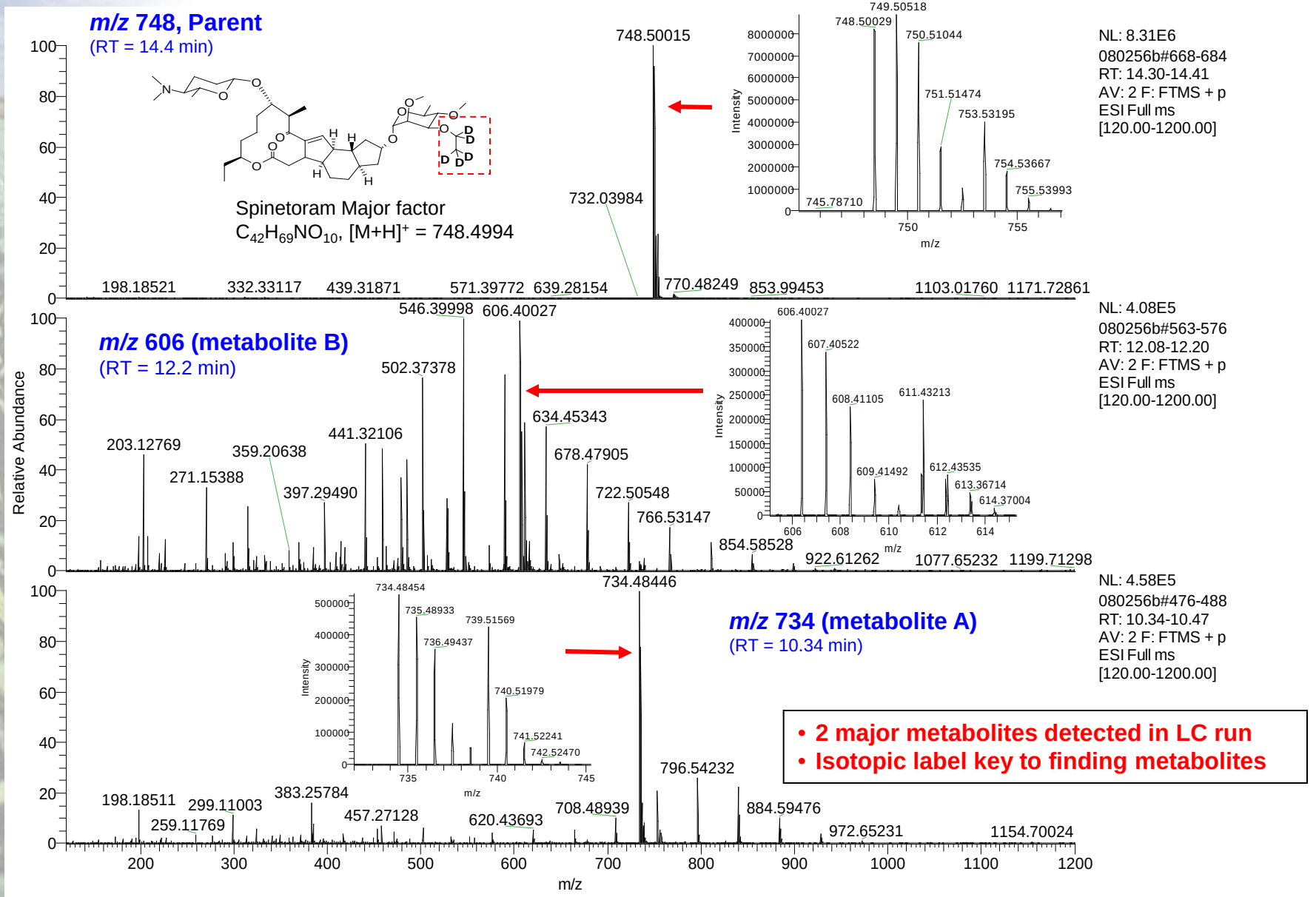


Spinetoram – Turnip Leaf Extract

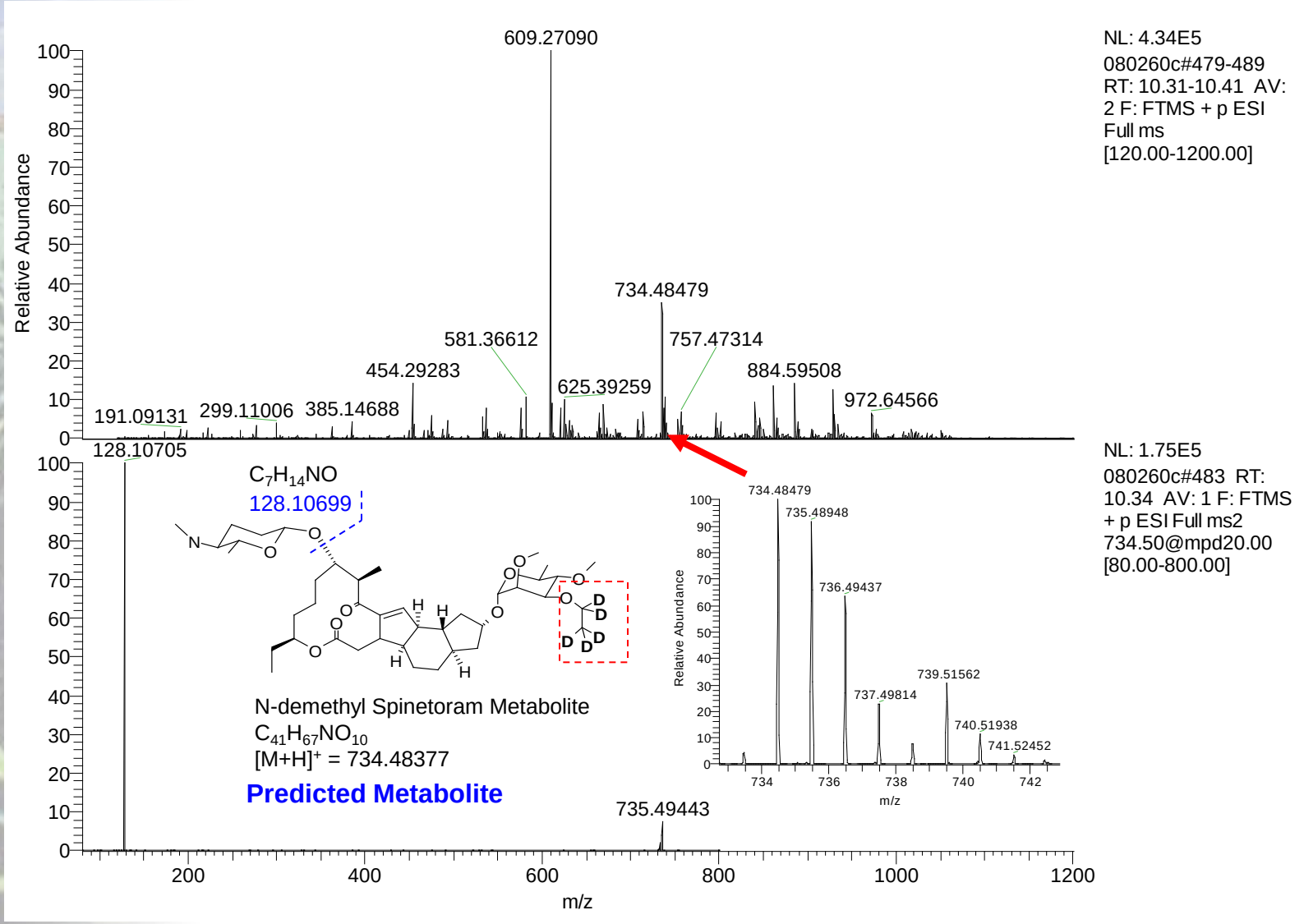
FTMS TIC, selected ion, and RAM traces



Spinetoram – Turnip Leaf Extract
full scan FTMS spectra from LC run

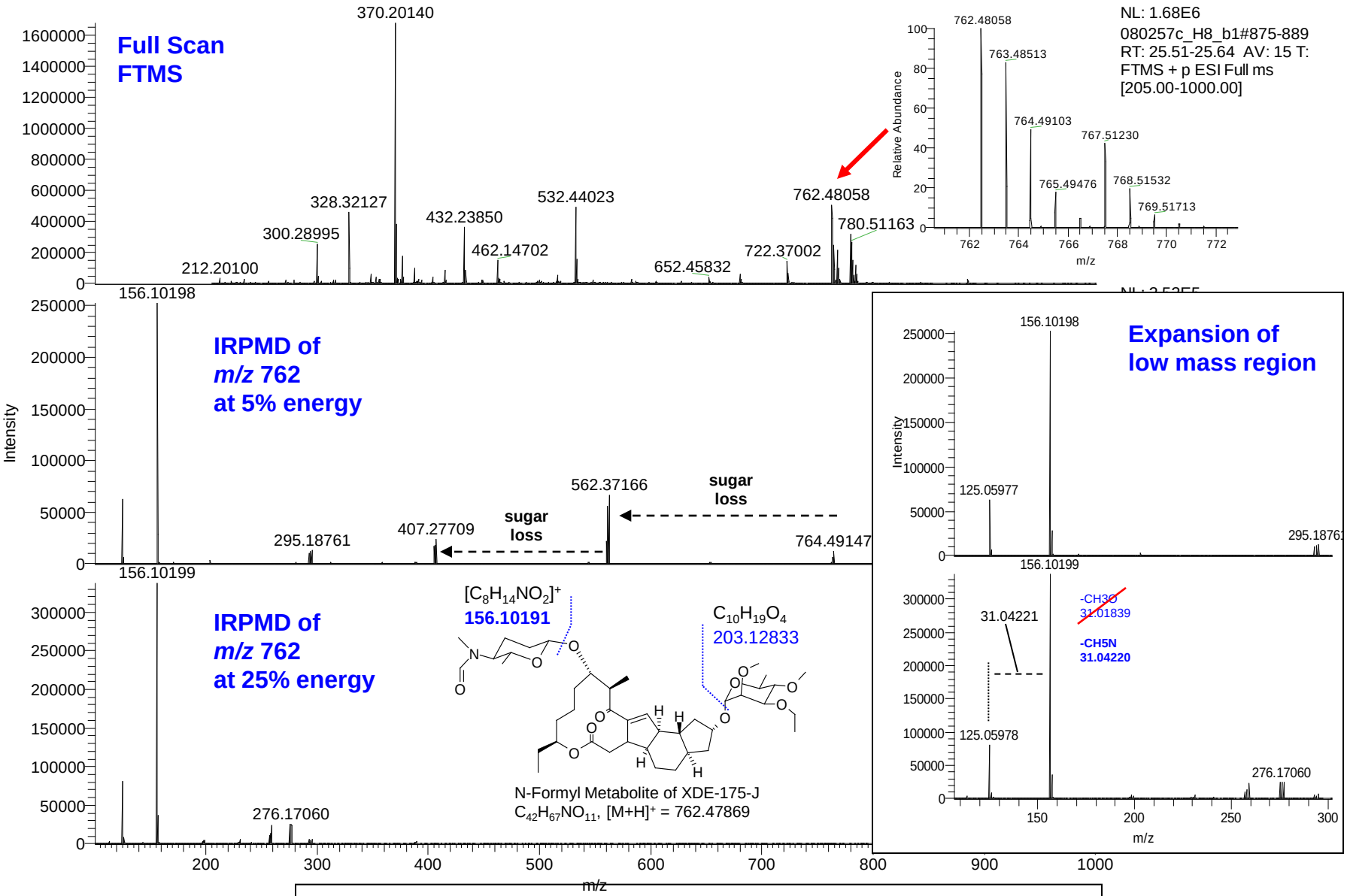


IRMPD of Spinetoram Metabolite A at 10.3 min



Nanomate infusion of Spinetoram Turnip Leaf Fraction

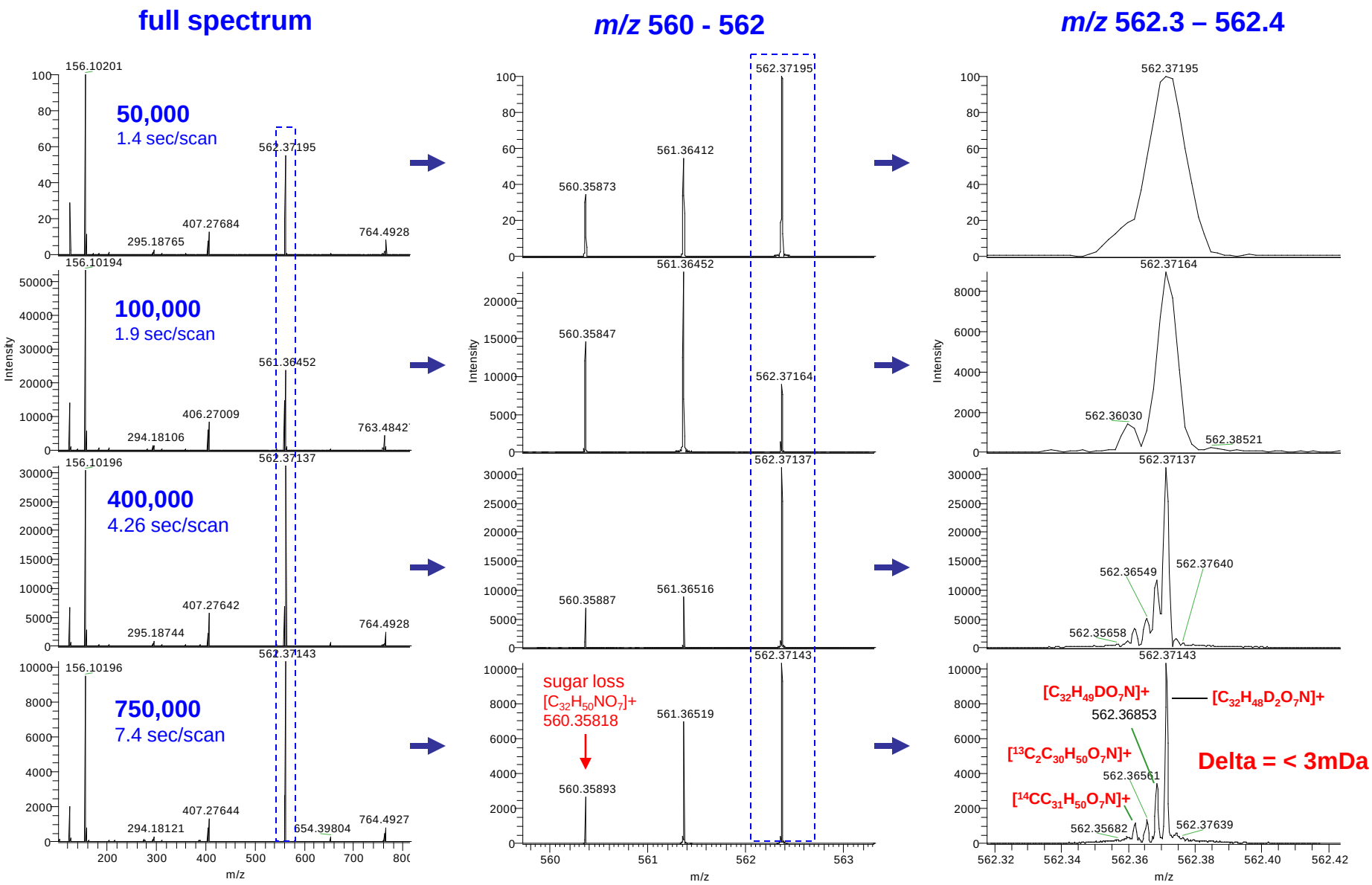
Fragmentation of *m/z* 762 (**metabolite C**) at varying IRMPD energies



N-formyl Spinetoram J represented a novel metabolite.
Use of Nanomate allowed extended data acquisition from initial sample injection

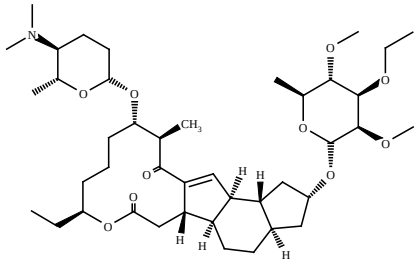
Nanomate infusion of spinetoram fraction

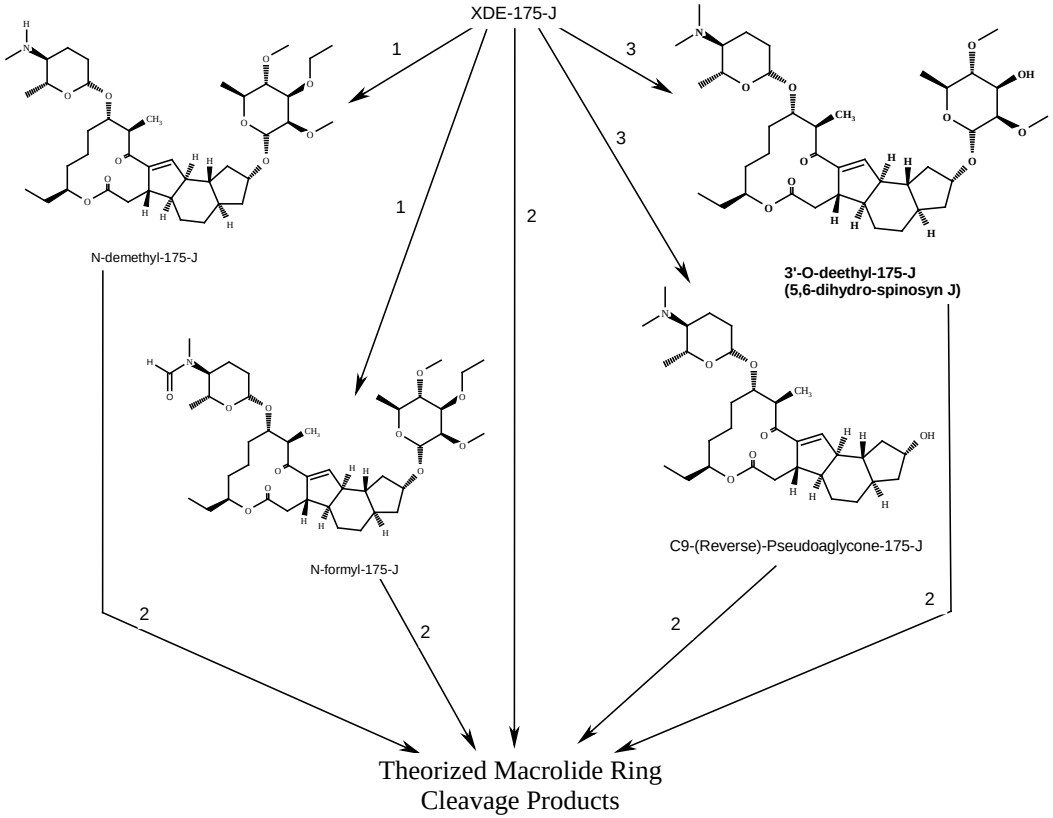
IRPMD product of *m/z* 762 at increasing resolution



Spinetoram (XDE-175) Plant Metabolic Pathway

Spinetoram Plant Metabolic Pathway





Systems studied as **required for registration**:

Soil

- biodegradation - aerobic conditions
- biodegradation - anaerobic conditions
- abiotic degradation
- aerobic photodegradation

Sediment

- biodegradation - aerobic conditions
- biodegradation - anaerobic conditions

Water

- Hydrolysis
- photodegradation in buffered water
- photodegradation in natural water

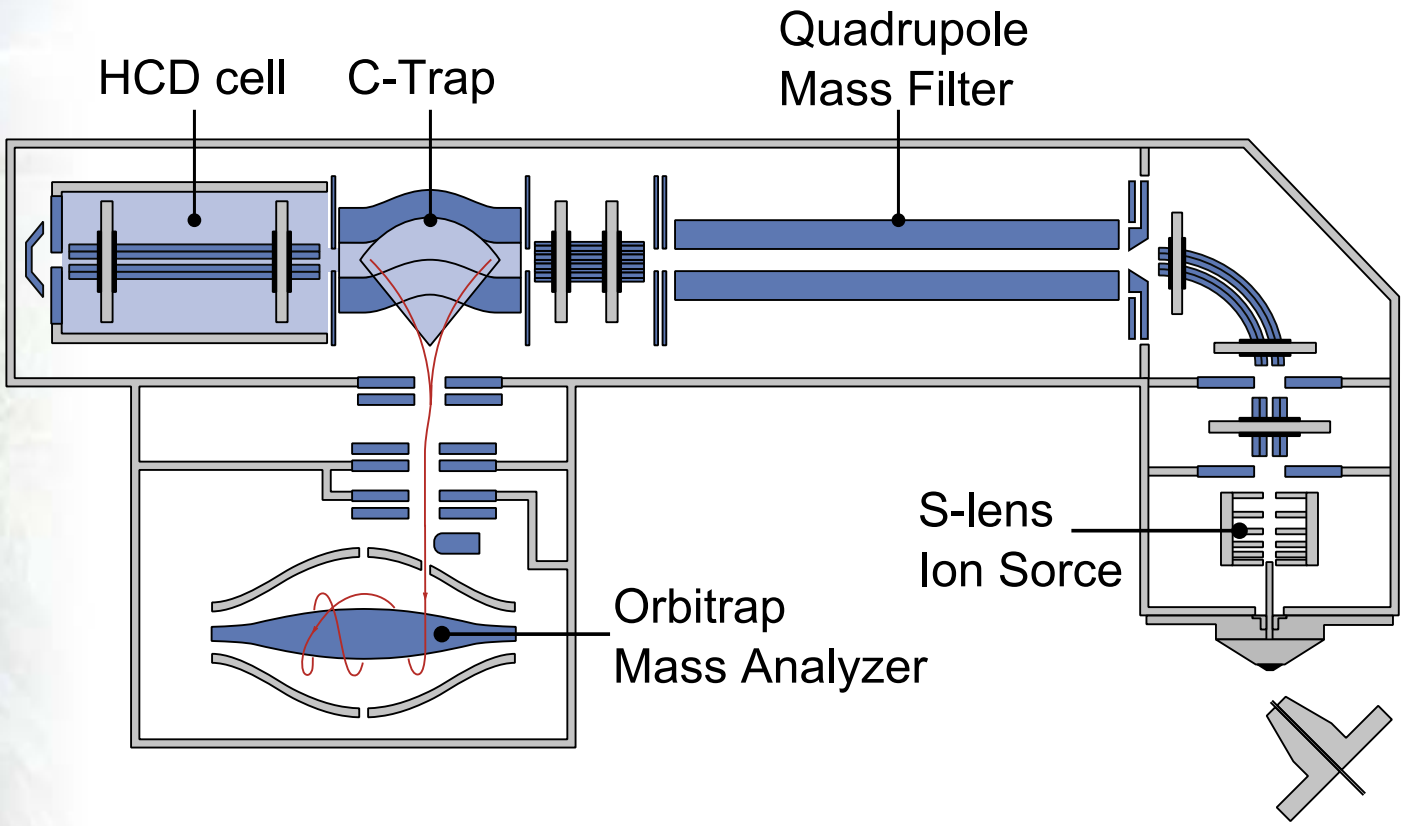
Metabolism

- Lettuce (foliar applications)
- Turnips (foliar applications)
- Apples (foliar applications)
- Rice (after treatment of seedling box soil)

Metabolism

- Poultry
- Lactating goats.
- Rats

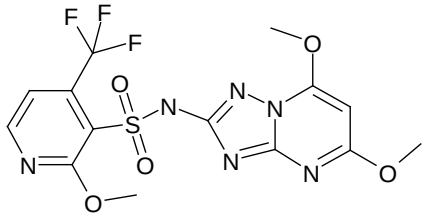
Schematic of the Q Exactive



Capabilities that may aid in metabolite ID:

- Sensitivity
- Mass accuracy < 5ppm (ext. cal.), <1ppm (int. cal.)
- Pos/Neg switching
- Fast MS, and MS/MS acquisition rates (up to 12Hz)
- High resolution up to 140,000 (FWHM @ *m/z* 200)

General Q Exactive Experiments Used to Study the Soil Metabolism of pyroxsulam (herbicide)



Pyroxsulam

$C_{14}H_{13}F_3N_6O_5S$

M = 434.0620

[M+H]+ = 435.0693

[M-H]- = 433.05475

Experiments:

1) FTMS - p ESI Full ms [100.00-1000.00] @ 70K resolution
FTMS - p ESI Full ms2 top-5@HCD35
FTMS + p ESI Full ms [100.00-1000.00] @ 70K resolution
FTMS + p ESI Full ms2 top-5@HCD35

Goal: **General screen** for metabolites at medium resolution using **pos/neg switching**, acquire MS/MS spectra for anticipated metabolites

2) FTMS + p ESI Full ms [100.00-1000.00] @ 140K resolution
FTMS + p ESI Full ms2 [100.00-1000.00] @ 140K resolution
FTMS + p ESI mSIM top-5@HCD35 @ 140K resolution

Goal: **Target metabolites** detected in experiment #1 **(+ESI) for high resolution +ESI MS, MS/MS, and mSIM** spectra – optimal mass measurement and characterization of isotopic cluster

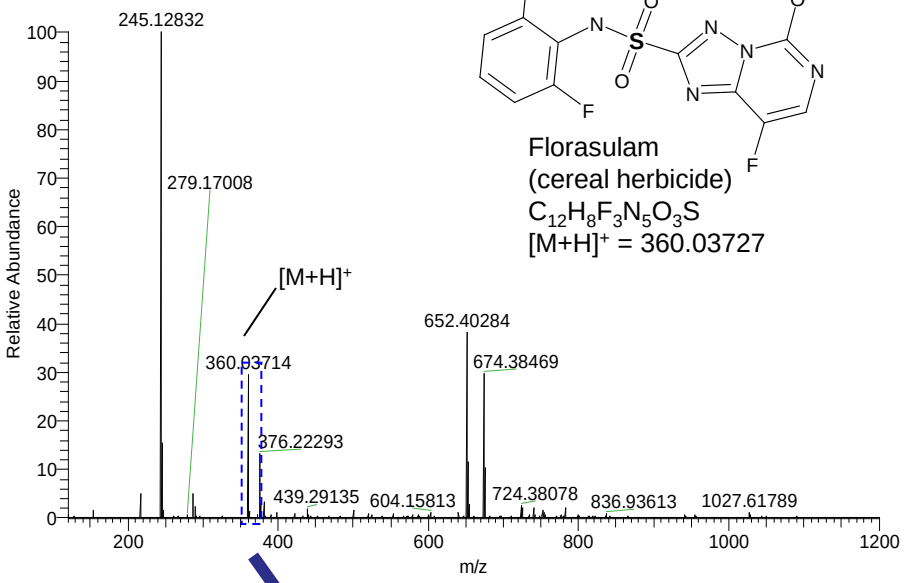
3) FTMS - p ESI Full ms [100.00-1000.00] @ 140K resolution
FTMS - p ESI Full ms2 [100.00-1000.00] @ 140K resolution
FTMS - p ESI mSIM top-5@HCD35 @ 140K resolution

Goal: **Target metabolites** detected in experiment #1 **(-ESI)** for **high resolution -ESI MS, MS/MS, and mSIM** spectra – optimal mass measurement and characterization of isotopic cluster

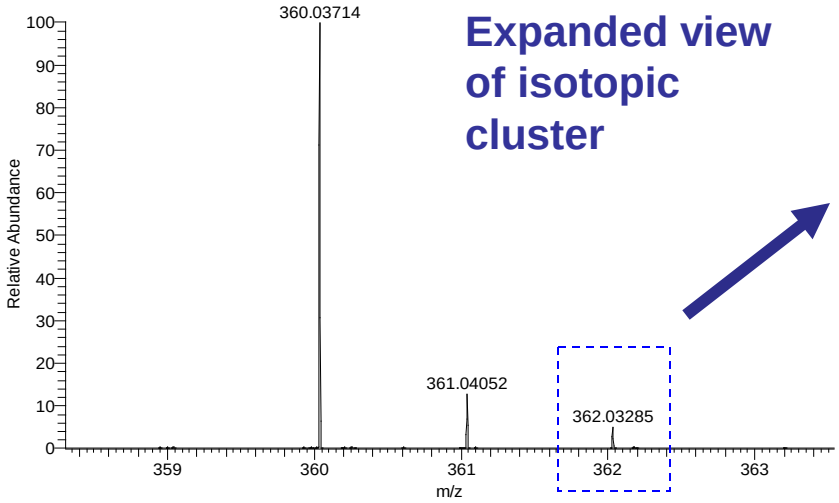
Benefits of High Resolution – M+2 Sulfur Counting

Florasulam in Wheat Cell Extract

Full scan FTMS

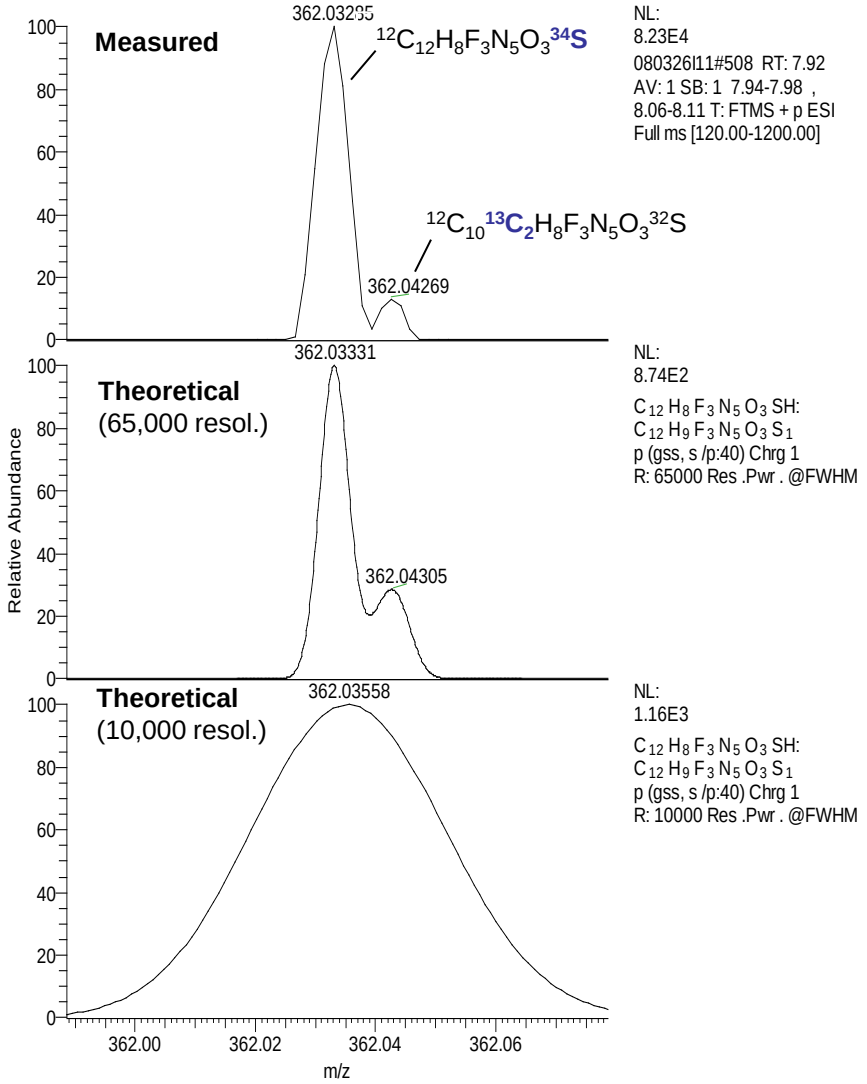


Florasulam
(cereal herbicide)
$C_{12}H_8F_3N_5O_3S$
$[M+H]^+ = 360.03727$



Expanded view of isotopic cluster

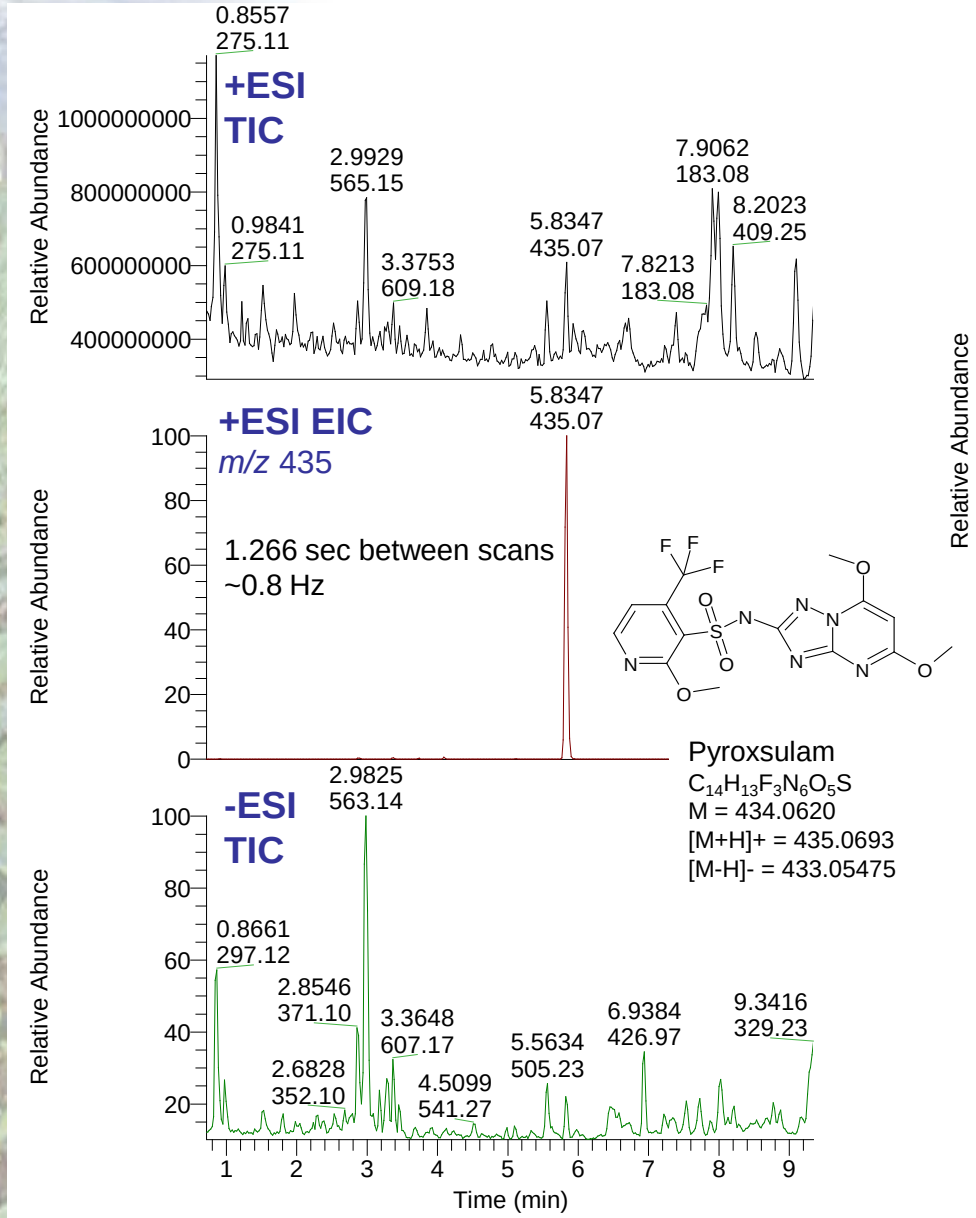
M+2 isotope

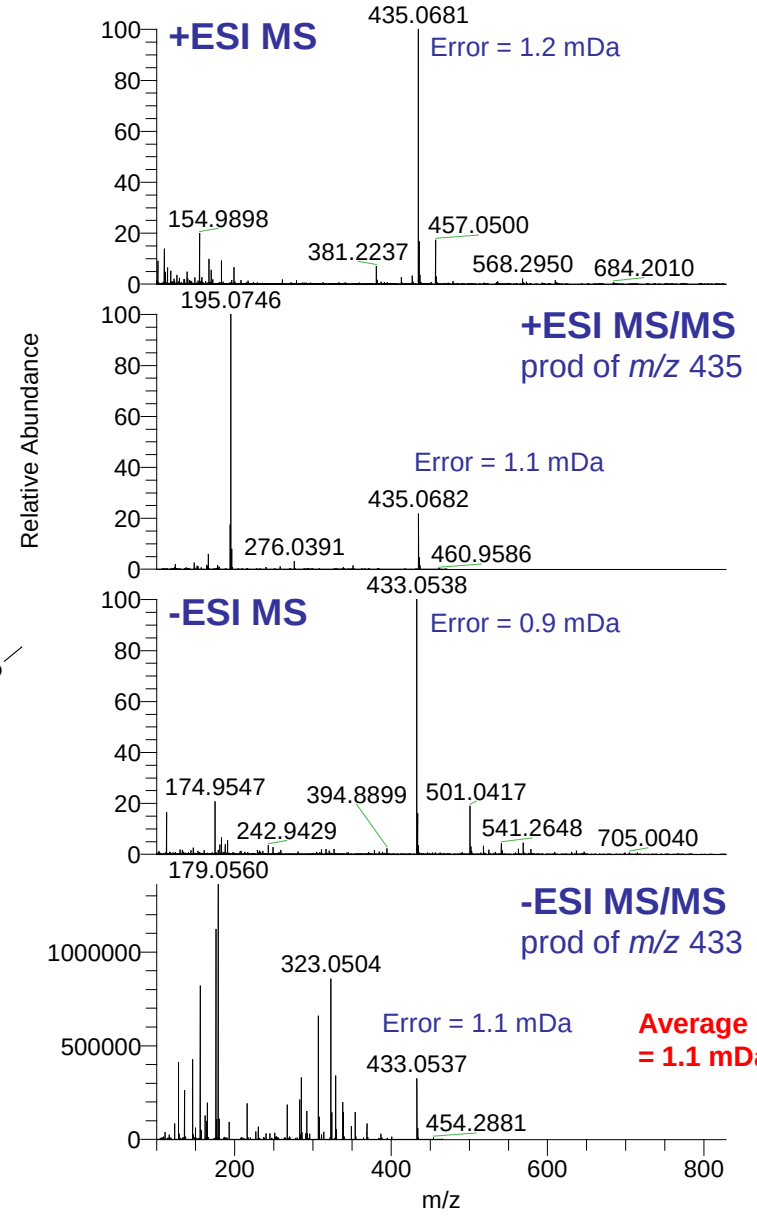


Typically requires fairly concentrated sample because of low M+2 intensity

Soil Extract Containing Pyroxsulam Metabolites

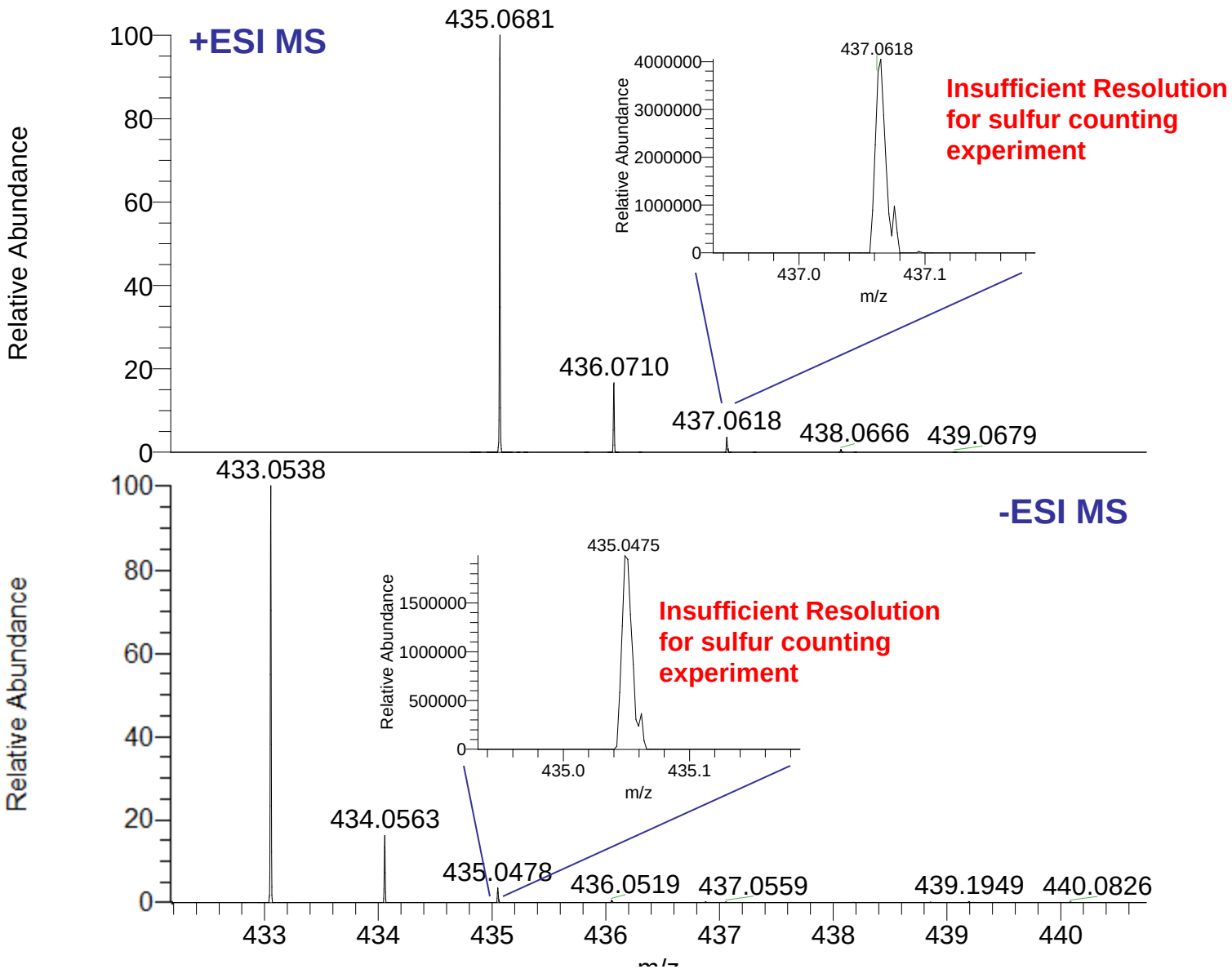
(0.5 ppm, 0.5 day), Q Exactive Experiment #1 (70K, +/-, top-5 DD MS/MS)





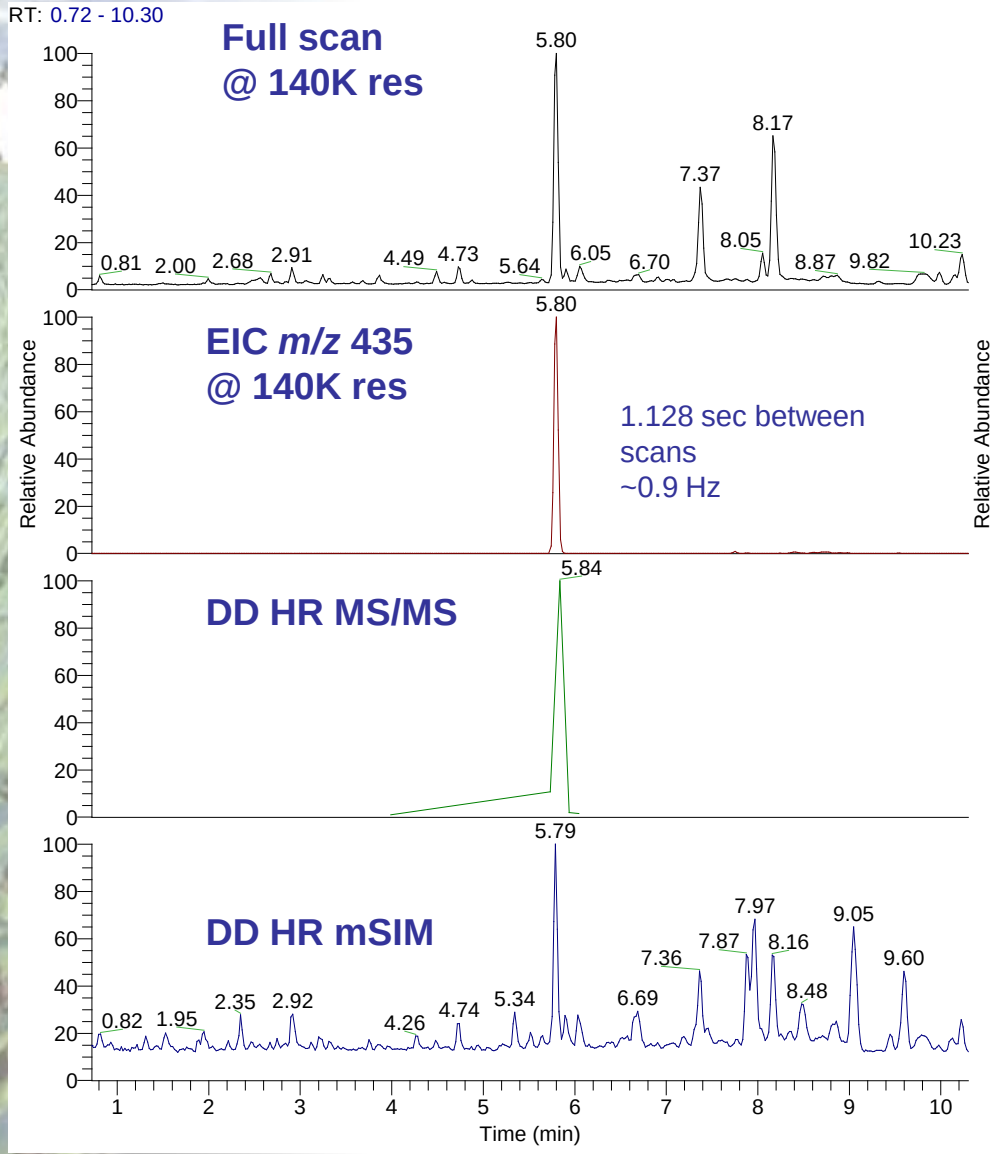
Soil Extract Containing Pyroxsulam Metabolites

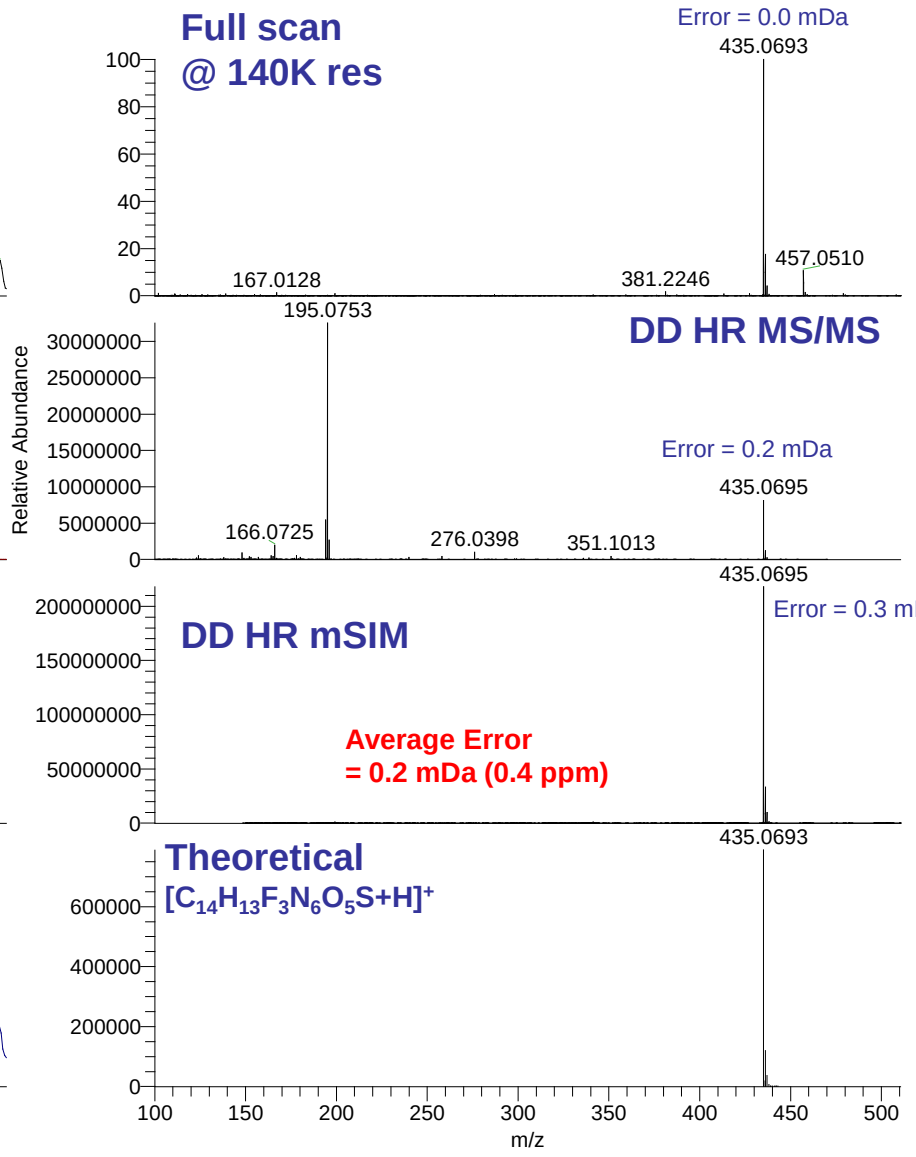
(0.5 ppm, 0.5 day), Q Exactive Experiment #1 (70K, +/-, top-5 DD MS/MS)



Soil Extract Containing Pyroxsulam Metabolites

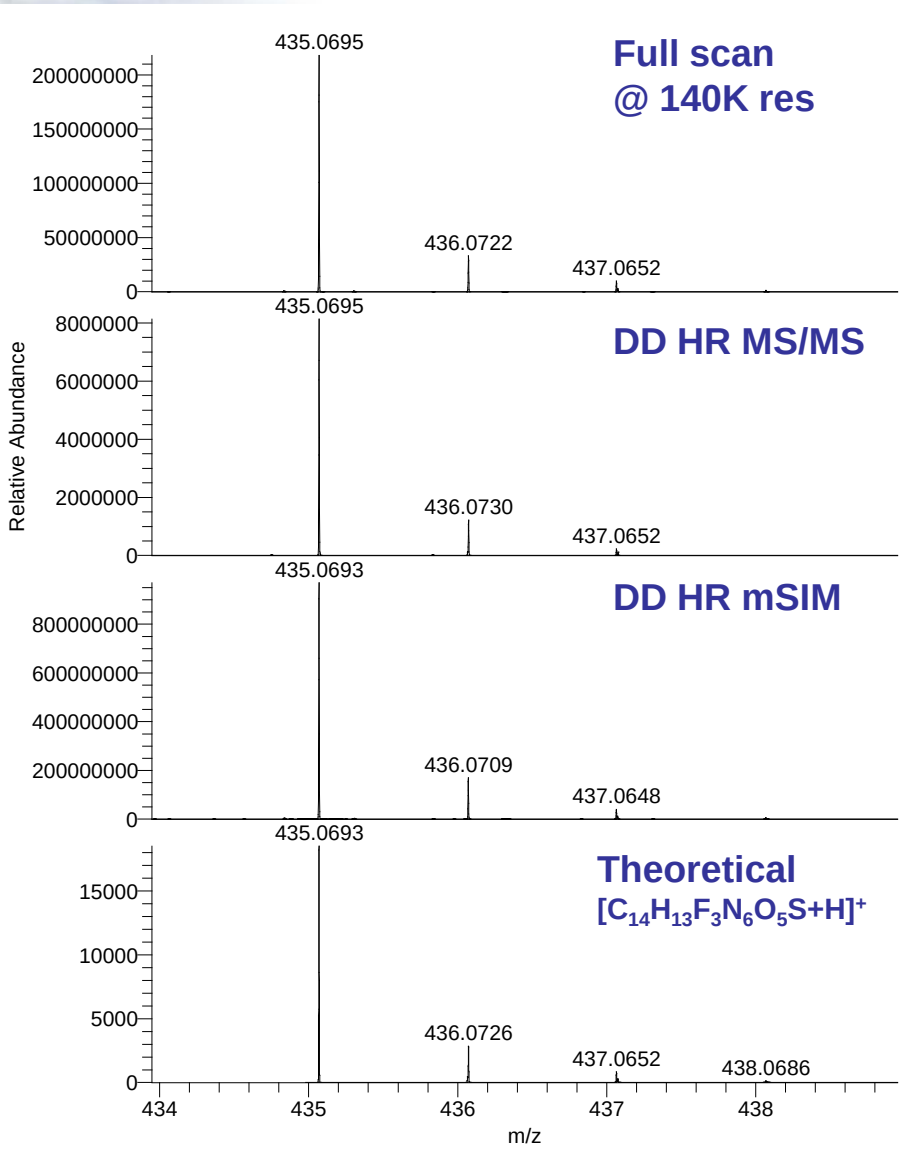
Q Exactive **Experiment #2** (+ESI 140K MS, 140K MS/MS and 140k mSIM)

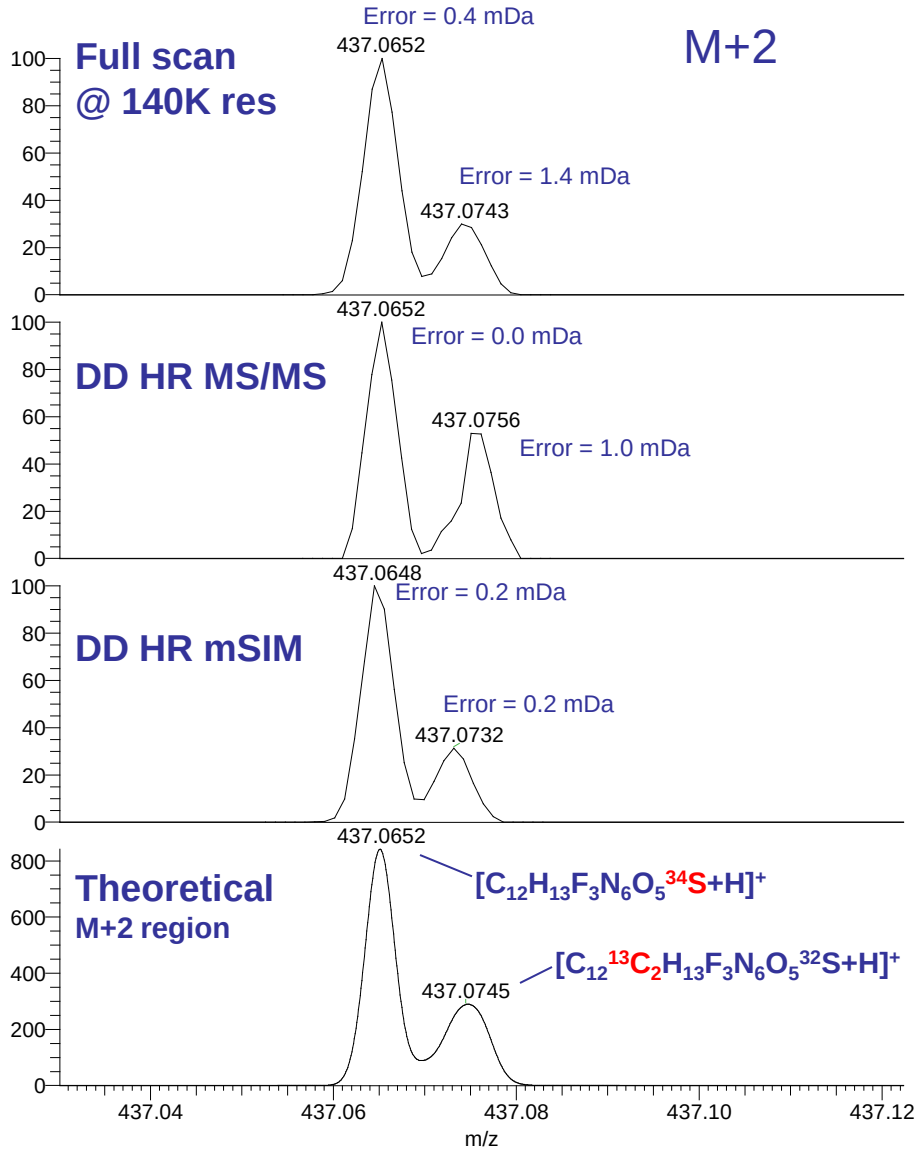




Soil Extract Containing Pyroxsulam Metabolites

Q Exactive **Experiment #2** (+ESI 140K MS, 140K MS/MS and 140k mSIM) – expanded view



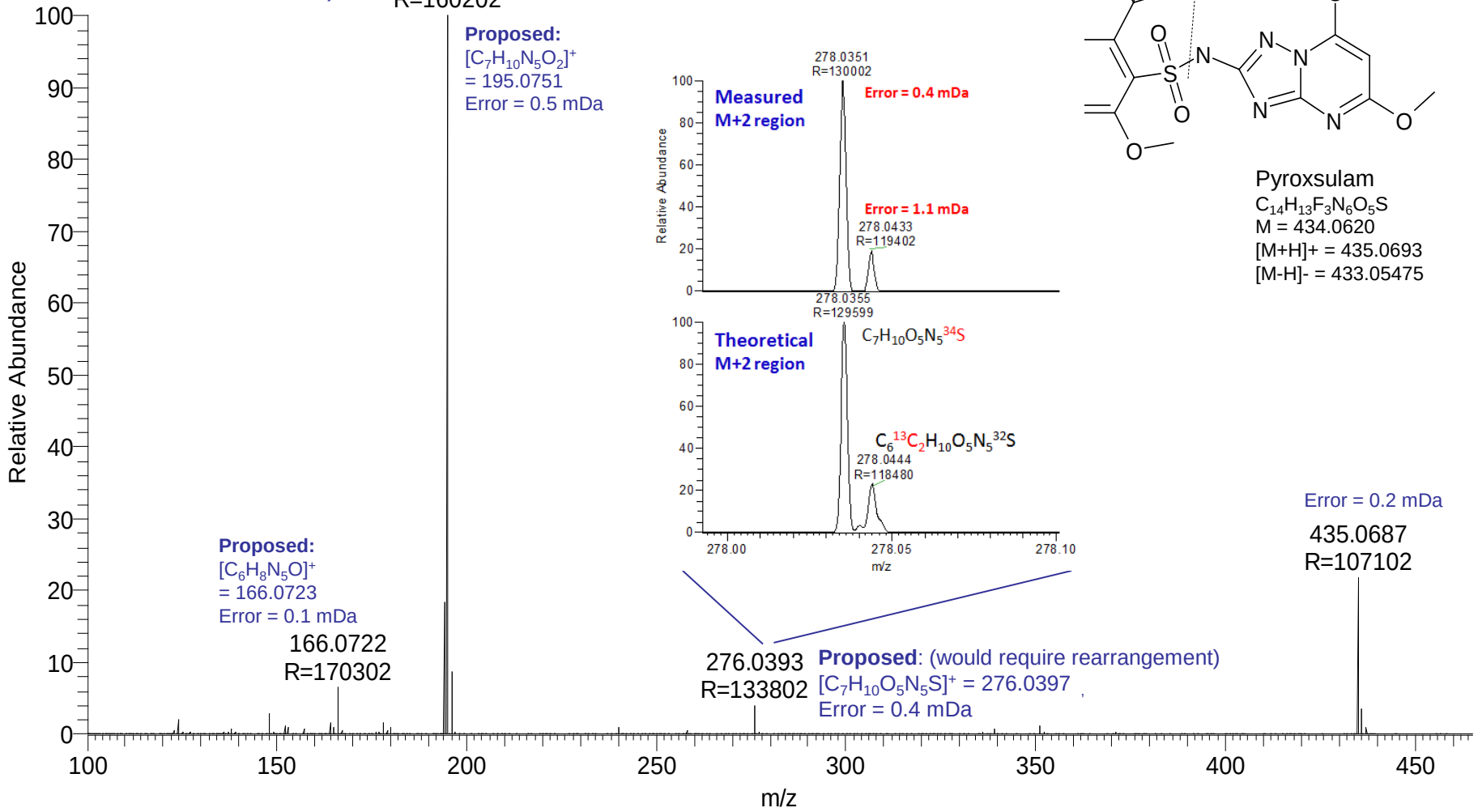


Soil Extract Containing Pyroxsulam Metabolites

Q Exactive **MS/MS Spectrum** from Experiment #2 (140K MS/MS)

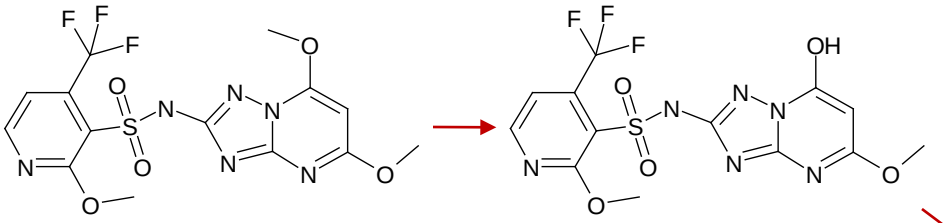
MS/MS

(products of m/z 435
at ~ 130,000 resolution)



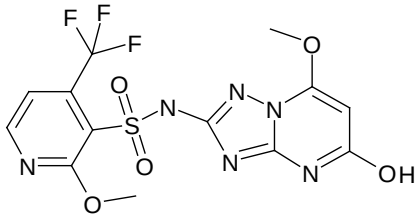
Pyroxsulam
$C_{14}H_{13}F_3N_6O_5S$
M = 434.0620
[M+H]+ = 435.0693
[M-H]- = 433.05475

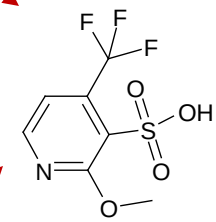
Pyroxsulam Soil Metabolism – Q Exactive Results



Pyroxsulam
$C_{14}H_{13}F_3N_6O_5S$
M = 434.0620
$[M+H]^+$ = 435.0693
$[M-H]^-$ = 433.05475

O-demethylation
$C_{13}H_{11}F_3N_6O_5S$
M = 420.0464
$[M+H]^+$ = 421.0536
$[M-H]^-$ = 419.0391





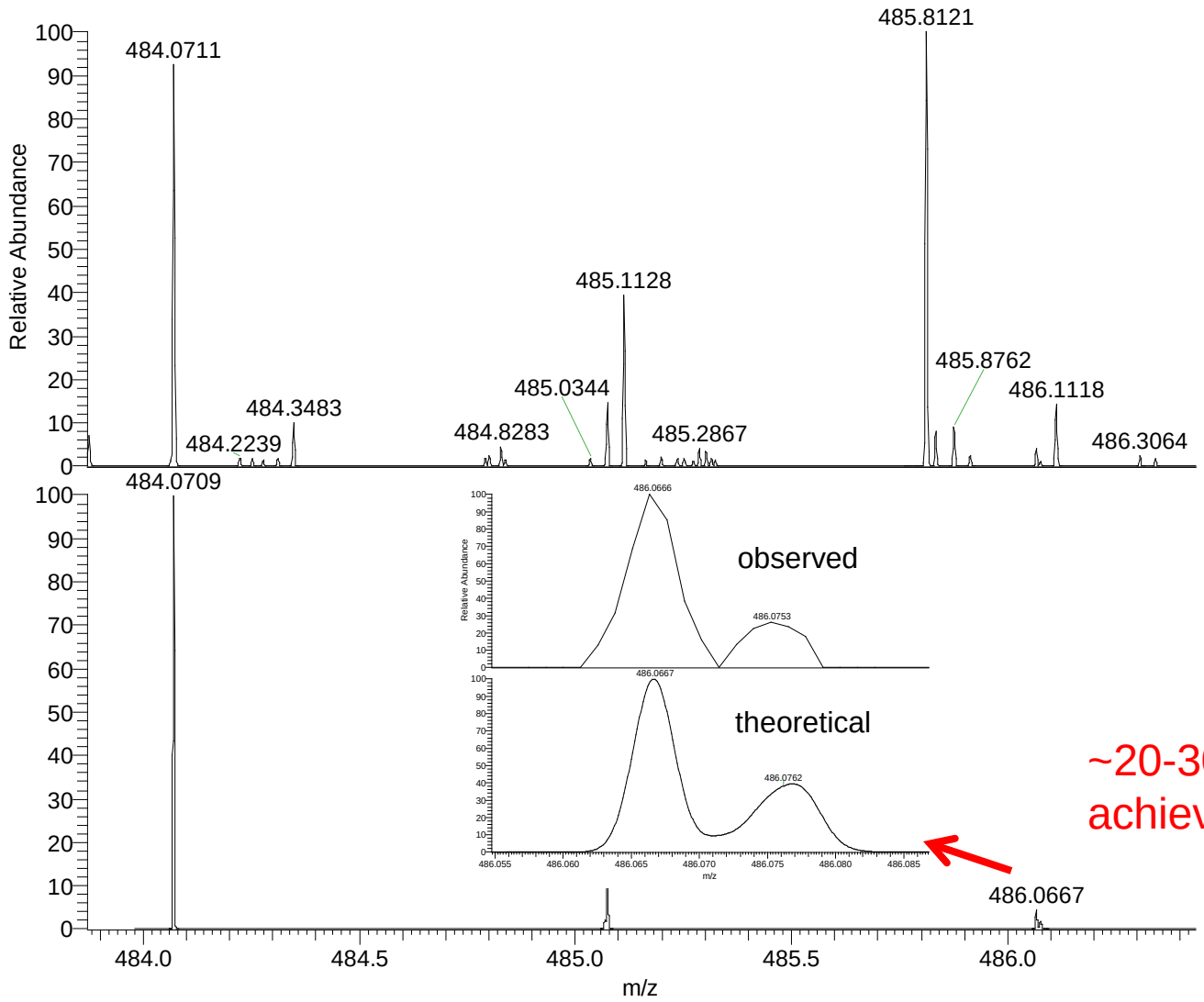
Sulfonic Acid
$C_7H_6F_3NO_4S$
M = 256.9970
~~$[M+H]^+$ = 258.0042~~
$[M-H]^-$ = 255.9897

Only detected In -ESI

	Experiment 1			Experiment 2			Experiment 3	
pyroxsulam	pos	neg			pos			neg
theo	435.0693	433.0548			435.0693			433.0548
+/- MS	435.0681	433.0538		140k MS	435.0693		140k MS	433.0550
MS/MS	435.0682	433.0537		140k MS/MS	435.0695		140k MS/MS	433.0551
Ave =	435.0682	433.0538		mSIM	435.0696		mSIM	433.0552
ave. error (Da) =	0.0011	0.001		Ave =	435.0695		Ave =	433.0551
error (ppm) =	2.53	2.31		StDev =	0.0002		StDev =	0.0001
				ave. error (Da) =	0.0002		ave. error (Da) =	0.0004
ave error (ppm) =		2.42			0.23			0.69
Overall for all metabolites Detected								
ave error (ppm) =		2.47			0.15			1.18

All with external calibration

At 10 pg on column in mSIM mode - A+2 splitting is still observed



NL:
1.28E4
DOW_STD_140K_mSIM_Top1_#A_10pg_OC#450 RT: 7.37 AV: 1 T: FTMS + ESI SIM msx ms [465.56-474.56, 479.57-488.57, 727.97-736.97, 741.98-750.98, 804.00-813.00]

NL:
1.82E4
$C_{16}H_{14}O_5N_5S_1F_5$ +H:
$C_{16}H_{15}O_5N_5S_1F_5$
p (gss, s /p:40) Chrg 1
R: 140000 Res .Pwr . @FWHM

~20-30x lower than we can achieve on our LTQ-FT Ultra

Conclusions

- Metabolite identification remains a challenging component of agrochemical discovery and development.
  - Constantly lowering application rates = lower levels of resulting metabolites.
  - Sample complexity is high, but timelines often require ID with limited sample clean-up.

- New instruments, including the LTQ-FT, have significantly improved our ability to meet these challenges by providing:
  - Improved Sensitivity
  - High Mass Accuracy (typically better than 1ppm)
  - High Resolution (up to 1,000,000)
  - MS^n

- Additional methods to interrogate an unknown, including IRMPD and high resolution MS combined chip-based nanospray infusion allows the acquisition of a wide array of data with minimal sample consumption.

- Addition of the Thermo Q Exactive, a new benchtop quadrupole-orbitrap, may further expand our capabilities by providing:
  - Improved MS and MS/MS sensitivity
  - Pos/Neg switching
  - Maintaining Fast MS, and MS/MS acquisition rates
  - High resolution (~140,000 FWHM) – allowing high resolution experiments, including sulfur counting experiments at levels 20-30x better than our LTQ-FT Ultra.

Acknowledgements

Dow AgroSciences

Gerry Deboer
Jeff Godbey
Debbie Schwedler

Thermo

Mary Blackburn
Tim Stratton
David Peake
Bjoern Rose
Markus Kellmann
Esther Lewis
Butler John