Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Supplementary Information

Rapid Plant Uptake of Isothiazolinone Biocides and Formation of Metabolites by Hydroponic *Arabidopsis*

Claire P. Muerdter,[†,‡] Megan M. Powers,[†,‡] Sraboni Chowdhury,[†,‡] Alyssa L. Mianecki,[†,‡] and Gregory H. LeFevre[,†,‡]*

[†] Department of Civil and Environmental Engineering, University of Iowa, 4105 Seamans Center, Iowa City, Iowa, 52242, United States

[‡] IIHR—Hydroscience and Engineering, University of Iowa, 100 C. Maxwell Stanley Hydraulics Laboratory, Iowa City, Iowa, 52242, United States

[*] **Corresponding Author**: gregory-lefevre@uiowa.edu; Phone: 319 335 5655, Department of Civil and Environmental Engineering, 4105 Seamans Center for Engineering, University of Iowa, Iowa City IA, 52242, United States

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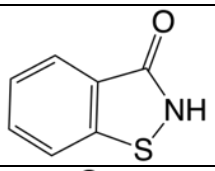
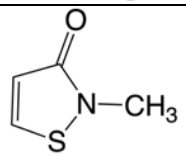
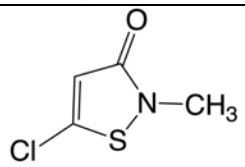
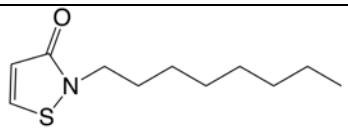
Table S1: Isothiazolinone biocides used in this work

Name	CAS Number	Chemical Structure	Molecular Weight ($g\ mol^{-1}$)	Log K_{OW}
Benzisothiazolinone (BIT)	2634-33-5		151.2	0.64[1–3]
Methylisothiazolinone (MIT)	2682-20-4		115.1	-0.49[1]
Chloromethylisothiazolinone (CMI)	26172-55-4		149.6	0.40[1]
Octylisothiazolinone (OIT)	26530-20-1		213.3	2.61[2,3]

Additional Methods Details

Seed Sterilization Procedure

A previously published seed sterilization procedure was used with Arabidopsis Columbia ecotype "0" (Col-0) seeds,[4] with the following minor modifications:

- Instead of conducting the procedures over a flame, seed sterilization was conducted in a biosafety cabinet.
- Rather than 50 µL of seed, between 10 and 50 µL of seed were used.

Arabidopsis Growth Procedure

A previously published Arabidopsis growth procedure[4] was used to grow up the sterilized seeds, before exposure to isothiazolinones, with the following modifications:

- 30 ± 2 seeds per box were used
- Growth chamber temperatures were 23°C during the light period and 21°C during the dark period
- Plants were grown for 10–11 days before exposure to isothiazolinones

Plant Isothiazolinone Exposure Experiment Details

The exposure experiments were modeled on previous work.[4,5] After a 10–11 day period of growth in unspiked sterile hydroponic medium, the boxes were taken from the growth chamber into a biological safety cabinet and the following procedures conducted using sterile technique.

A master mix of medium was spiked with the isothiazolinone(s) of interest and any other compounds used for the experiment, e.g., inhibitors. 3–4 samples were taken from the master mix, typically at 1 mL each, and filtered with nylon filters (0.2 µm, 13 mm diameter, mdi SY13NN) into LC vials. These medium samples were frozen at -20°C at the end of each timepoint and kept frozen until analysis.

After master mix sampling for the t=0 timepoint, the microporous tape was removed from each box and the box tilted to allow for the medium to leave the box while the plant tissue remained in the box. The box lid was then removed and freshly isothiazolinone-spiked plant growth medium was added to each box, at 25 mL per box except for the experiment comparing BIT, CMI, and MIT uptake, which was conducted at 15 mL per box. The box lid and microporous tape were then replaced.

Additionally, an abiotic control was created at t=0 for each treatment. Each control replicate consisted of the same amount of master mix of medium as the plant boxes, pipetted into a washed and autoclaved Magenta box. The lid was replaced and microporous tape applied in the same manner as the plant boxes. Each treatment and control was conducted at n=3–4.

Except for sampling, boxes were kept in the Percival growth chamber alternating between 16 hours light at 23° C and 8 hours dark at 21° C. Relative humidity was maintained at 50%. All sampling was conducted using sterile technique in the biosafety cabinet. Samples ranged from 0.6 to 1.0 mL and were collected as described above, with filtering by nylon filters and freezing of samples after collection.

Plant Tissue Harvest Details

The microporous tape was removed from each box and the box tilted with the lid still on to allow for the medium to drain out while the plants were retained in the box. The box was then inverted onto a clean paper towel, and the box removed. The plant tissue was gently patted with the paper towel to remove any remaining medium. Clean tweezers were then used to move the tissue into 1.5 or 2 mL microcentrifuge tubes with locking lids, with the tweezers cleaned with ethanol between each box. Plant tissue was then frozen at -20 °C until overnight freeze drying (to determine dry weight, for select samples) and extraction (only for metabolomics samples).

Plant Extraction for Metabolomics

The following procedure from LeFevre et al. 2015[4] was used:

A single stainless steel homogenization bead (5 mm) and 1.0 mL of 1:1 methanol/water solution were added to freeze-dried plant tissues in a microcentrifuge tube. The tubes were frozen at −80 °C for 30 min. Samples were thawed and placed on a Retsch mixer mill for 5 min at 30 Hz. The samples were then sonicated for 10 min, vortexed for 1 min, and centrifuged at

10 000 × g for 10 min. Following centrifugation the supernatant was removed with a 22G x 1 ½ BD precision glide needles needle syringe and filtered through a 0.2 µm, 13 mm diameter PFTE filter (mdi) into an empty autosampler vial. The extraction procedure was repeated sequentially two additional times by adding only 0.5 mL (rather than 1.0 mL) of the methanol:water solution for each subsequent extraction and otherwise exactly repeating the extraction procedure (*i.e.*, homogenization, sonication, vortex, centrifugation, filtration). All three fractions were combined in a single autosampler vial for analysis.

Plant Health Experiments

Plant health experiments were conducted per the plant isothiazolinone exposure experiments above, but with no negative control. For all inhibitor experiments, including plant health experiments, a nominal BIT C_0 of 150 µg/L was used. This concentration was selected because of the relatively high BIT plant uptake rate at 112 µg/L (Figure 2). Thus, any changes in uptake created by inhibitor presence were expected to be especially noticeable at this concentration. A BIT-spiked plant control was run in parallel with one or more inhibitor treatment. Inhibitor treatments were the same as the BIT plant control but with the addition of an inhibitor chemical at the desired concentration. No medium samples were taken. Plant health was visually inspected at t = 0, 2, 24, and 48 hours. If plant health was not visually distinguishable between the treatment and control, a full inhibitor experiment was conducted at the tested inhibitor concentration. If plant health was distinguishable between the two, then the plant health experiment was repeated at a lower inhibitor concentration, until no difference in plant health was observed between the treatment and control. That inhibitor concentration was used for the inhibitor experiment. Inhibitor concentrations used in the final experiments were:

- DEPC (Diethylpyrocarbonate): 100 µM

- Glycerol: 1 mM
- Quinidine: 100 µM
- 9-AC (Anthracene-9-carboxylic acid): 100 µM
- 2,4-DNP (2,4-dinitrophenol): 25 µM
- 1-ABT (1-aminobenzotriazole): 0.15 µM

Analytical Methods

Samples were analyzed via high performance liquid chromatography (Agilent 1260) coupled to a triple quadrupole mass spectrometer (LC-MS/MS; Agilent 6460 Triple Quadrupole MS with MassHunter, version B.07.00) operating in multiple reaction monitoring (MRM) positive ionization mode. LC-MS/MS method details are given on the next page. The sample tray was kept at 4 °C. All compounds quantified to a concentration basis (rather than C/C_0 based on peak area) were run with at least a five-point (not counting the blank) standard curve, which was isotopically labeled (d4) imidacloprid-normalized to account for matrix effects during ionization. Peak area was quantified from chromatograms using Agilent MassHunter Qualitative Analysis software. For the standard curve, the log of the quotient of target compound peak area over the internal standard peak area was plotted versus the log of the target compound concentration on the x-axis, as recommended and used in the literature for calibration curves with large concentration ranges and/or low concentrations.[6–8] A linear regression of this line was used to calculate target compound concentration in samples.

BIT Transporter vs Transpiration-Driven Uptake Rate Calculation

Based on LeFevre et al. 2015[4] Supporting Information "4. BT Uptake Ratio Calculation," with some modifications.

Calculation Summary (see Notes for further definition of terms):

$$(7)\ Expected\ mass\ of\ BIT\ removed\ from\ medium\ through\ transpiration\ driven\ uptake =$$

$$\frac{(1)\ Volume\ of\ medium\ in\ plant\ at\ experiment\ end + (2)\ Volume\ of\ medium\ transpired\ out\ of\ plant\ during\ experiment}{(5)\ Initial\ volume\ of\ medium\ in\ box}$$

$$\times\ (6)\ Initial\ total\ mass\ of\ BIT$$

Fold change of observed plant BIT removal vs transpiration-driven BIT uptake =

$$\frac{(8)\ Observed\ mass\ of\ BIT\ removed\ from\ medium}{(7)\ Expected\ mass\ of\ BIT\ removed\ from\ medium\ through\ passive\ uptake}$$

(1) Volume of medium in plant at experiment end = (Wet plant biomass at experiment end) – (Dry plant biomass at experiment end)
(2) Volume of medium transpired out of the plant during experiment = (3) Volume of medium evapotranspired during experiment – (4) Volume of medium evaporated during experiment
(3) Volume of medium evapotranspired during experiment = Total BIT plant treatment box weight at experiment start – Total BIT plant treatment box weight at experiment end – 2 mL (for C1 and C2 timepoint sampling)
(4) Volume of medium evaporated during experiment = Total abiotic control box weight at experiment start – Total abiotic control box weight at experiment end – 2 mL (for C1 and C2 timepoint sampling)
(5) Initial volume of medium in box at experiment start
(6) Initial total mass of BIT = (5) Volume of medium in box at experiment start * Concentration of BIT in plant medium at experiment start
(7) Expected mass of BIT removed from the medium through transpiration-related uptake (*i.e.*, both the BIT in the plant at the end of the experiment and the BIT removed from the plant via transpiration during the experiment): see equation above
(8) Observed mass of BIT removed from medium = (6) Initial total mass of BIT – [(Concentration of BIT in plant medium at experiment end)*(Volume of medium at experiment end = (5) – (3))]

Notes:

- These ratios are approximations determined gravimetrically (hydroponic plant media specific gravity assumed = 1). They are meant only to approximate the fold change between the observed removal of BIT from the plant medium and the expected BIT removal from the system due to transpiration. Exact measurements of transpiration were outside the scope of this work.

- The observed removal encompasses both sorption to plant tissue and plant uptake. Per Figure S2, sorption is expected to be a part of BIT fate but plant uptake is expected to be the primary mechanism for BIT removal from the medium.
- "Experiment start" was at t=0, at the time of BIT spiking into the plant medium
- "Experiment end" was after 48 hours of BIT exposure
- At 48 hours the plants were 93–95% water. Therefore, the change in dry biomass due to plant growth between t=0 and t=48 hours is assumed to be negligible.
- The experiment was not sacrificial. Sampling volume (2 samples of 1 mL between experiment start box weights and experiment end box weights) is accounted for in the calculations below.
- All calculations below were performed with n=4 and the average of n=4 was used for each final value below

Results:

	For a C_0 of 49 µg BIT/L	**For a C_0 of 357 µg BIT/L**
(1) Volume of medium in plant at experiment end	0.5293 mL	0.6773 mL
(2) Volume of medium transpired out of the plant during experiment	0.3493 mL	0.4887 mL
(3) Volume of medium evapotranspired during experiment	0.9075 mL	1.131 mL
(4) Volume of medium evaporated during experiment	0.5582 mL	0.6422 mL
(5) Initial volume of medium in box at experiment start	25.0 mL	25.0 mL
(6) Initial total mass of BIT	1.225 µg	8.925 µg
(7) Expected mass of BIT removed from the medium through transpiration-related uptake	0.04305 µg	0.4162 µg
(8) Observed mass of BIT removed from medium	1.221 µg	8.914 µg
Fold change of observed plant BIT removal vs passive (transpired) BIT uptake	**28**	**21**

Table S2: LC-MS/MS and MRM Transition Details

Target Compound(s)	Chromatography	Method Parameters	Target Compound	Qualitative or Quantitative Transition	Q1 *m/z*	Q3 *m/z*	Dwell time (ms)	Frag-mentor voltage (V)	Collision energy (V)	Cell Accelerator Voltage (V)
BIT	0.2 mL min^{-1} method for 24 min. Mobile phase A = Fisher Optima LC/MS Water with 0.1% Optima LC/MS grade formic acid Mobile phase B = Fisher Optima LC/MS acetonitrile with 0.1% Optima LC/MS grade formic acid Gradient: • 0 min: 100% A, 0% B • 5.0 min: 50% A, 50% B • 15.0 min: 20% A, 80% B • 15.5 min: 0% A, 100% B • 20 min: 100% A, 0% B Approximate retention time (minutes): • BIT: 21.0 min Column used: Agilent Eclipse Plus C18 (5 µm, 4.6 x 150 mm) Sample tray temperature: 4 deg C	Injection volume: 10 µL Column temperature: 25 °C Gas temperature: 300 °C Gas flow: 5 L min-1 Nebulizer pressure: 45 PSI Sheath gas temperature: 250 °C Sheath gas flow: 11 L min-1 Positive and negative capillary voltage: each 3,500 V Positive and negative nozzle voltage: each 500 V Polarity for all transitions: positive	BIT	Quantitative	152.02	109.0	200	120	20	4
			BIT	Qualitative	152.02	134.0	200	120	24	4
			Imidacloprid d4	Quantitative	260.09	213.0	200	59	12	4
			Imidacloprid d4	Qualitative	260.09	179.1	200	59	16	4

Target Compound(s)	Chromatography	Method Parameters	Target Compound	Qualitative or Quantitative Transition	Q1 *m/z*	Q3 *m/z*	Dwell time (ms)	Frag-mentor voltage (V)	Collision energy (V)	Cell Accelerator Voltage (V)
CMI and MIT	0.6 mL min^{-1} isocratic method for 4 min: • 20% Fisher Optima LC/MS Water with 0.1% Optima LC/MS grade formic acid • 80% Fisher Optima LC/MS acetonitrile with 0.1% Optima LC/MS grade formic acid Approximate retention times (minutes): • CMI: 2.8 min • MIT: 2.6 min Column used: Agilent Eclipse Plus C18 (5 µm, 4.6 x 150 mm) Sample tray temperature: 4 deg C	Injection volume: 10 µL Column temperature: 25 °C Gas temperature: 300 °C Gas flow: 5 L min-1 Nebulizer pressure: 45 PSI Sheath gas temperature: 250 °C Sheath gas flow: 11 L min-1 Positive and negative capillary voltage: each 3,500 V Positive and negative nozzle voltage: each 500 V Polarity for all transitions: positive	CMI	Quantitative	149.98	115.0	200	107	24	4
			CMI	Qualitative	149.98	135.0	200	107	24	4
			MIT	Quantitative	116.0	101.0	200	107	16	4
			MIT	Qualitative	116.0	98.0	200	107	16	4
OIT Some method details were sourced from [14]	0.3 mL min^{-1} method for 26 min. Mobile phase A = Fisher Optima LC/MS Water with 0.1% Optima LC/MS grade formic acid Mobile phase B = Fisher Optima LC/MS acetonitrile	Injection volume: 10 µL Column temperature: 30 °C Gas temperature: 300 °C Gas flow: 5 L min-1 Nebulizer pressure: 45 PSI Sheath gas temperature: 250 °C Sheath gas flow: 11 L min-1 Positive capillary voltage: 3,800 V	OIT	Quantitative	214.1	84.0	200	118	42	4
			OIT	Qualitative	214.1	102.0	200	120	14	4

Target Compound(s)	Chromatography	Method Parameters	Target Compound	Qualitative or Quantitative Transition	Q1 *m/z*	Q3 *m/z*	Dwell time (ms)	Frag-mentor voltage (V)	Collision energy (V)	Cell Accelerator Voltage (V)
	with 0.1% Optima LC/MS grade formic acid Gradient: • 0 min: 90% A, 10% B • 8.0 min: 50% A, 50% B • 18.0 min: 40% A,60% B • 21.0 min: 10% A, 90% B • 23.1 min: 90% A, 10% B Approximate retention time (minutes): • OIT: 14.6 min Column used: Agilent XDB-C18 ZORBAX, 3.5 µm, 2.1 x 50 mm Sample tray temperature: 4 deg C	Negative capillary voltage: 3,500 V Positive and negative nozzle voltage: each 500 V Polarity for all transitions: positive	Imidacloprid d4	Quantitative	260.09	213.0	200	59	12	4
			Imidacloprid d4	Qualitative	260.09	179.1	200	59	16	4

Table S3: Q-Exactive Settings for Full MS Scan

The column, mobile phases, injection volume, flow rate and gradient, column temperature, source parameters, and sample tray temperature from the BIT method in Table S2 above were used, along with the following:

Polarity	Positive
Resolution	70,000
AGC Target	1,000,000
Max IT	200 ms
Scan Range	70-1,000 m/z

Table S4: Q-Exactive Settings for the Positive and Negative ddMS2 Scans (the same settings were used for both)

The column, mobile phases, injection volume, flow rate and gradient, column temperature, source parameters, and sample tray temperature from the BIT method in Table S2 above were used, along with the following:

Resolution	17,500
AGC target	50,000
Max IT	50 ms
Loop Count	5
Isolation Window	1.5 m/z
Stepped NCE	20, 40, 60
Min AGC target	8,000
Apex trigger	Not used
Exclude Isotopes	On
Dynamic Exclusion	5.0 s

Compound Discoverer Analysis

Compound Discoverer (Thermo Scientific) analysis was run January 18, 2021 (Compound Discoverer version used not noted) using the .RAW files produced by the Q-Exactive. Run as samples were the nine BIT-exposed plant tissue extract sample MS scan files, nine unexposed plant tissue extract sample MS scan files, one composite C0 medium sample MS scan file, and one composite t=24 hours medium sample MS scan file. Polarity switching was used for the MS scan (i.e., both positive and negative modes were run in the same sample run). Run as Identification only were both the negative and positive ddMS2 files of the composite medium at 24 hours, the composited BIT-exposed plant tissue extracts, and the composited unexposed plant tissue extracts. ddMS2 samples were run in positive and negative modes separately. Additionally, five blanks from throughout the run were input to Compound Discoverer as blanks. The established workflow within Compound Discoverer, "Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases and mzLogic" was used. An image of the workflow tree is shown in Figure S3. All workflow settings are listed below. mzCloud, Metabolika, and ChemSpider searches were based on online databases and were completed using the versions available on January 18, 2021.

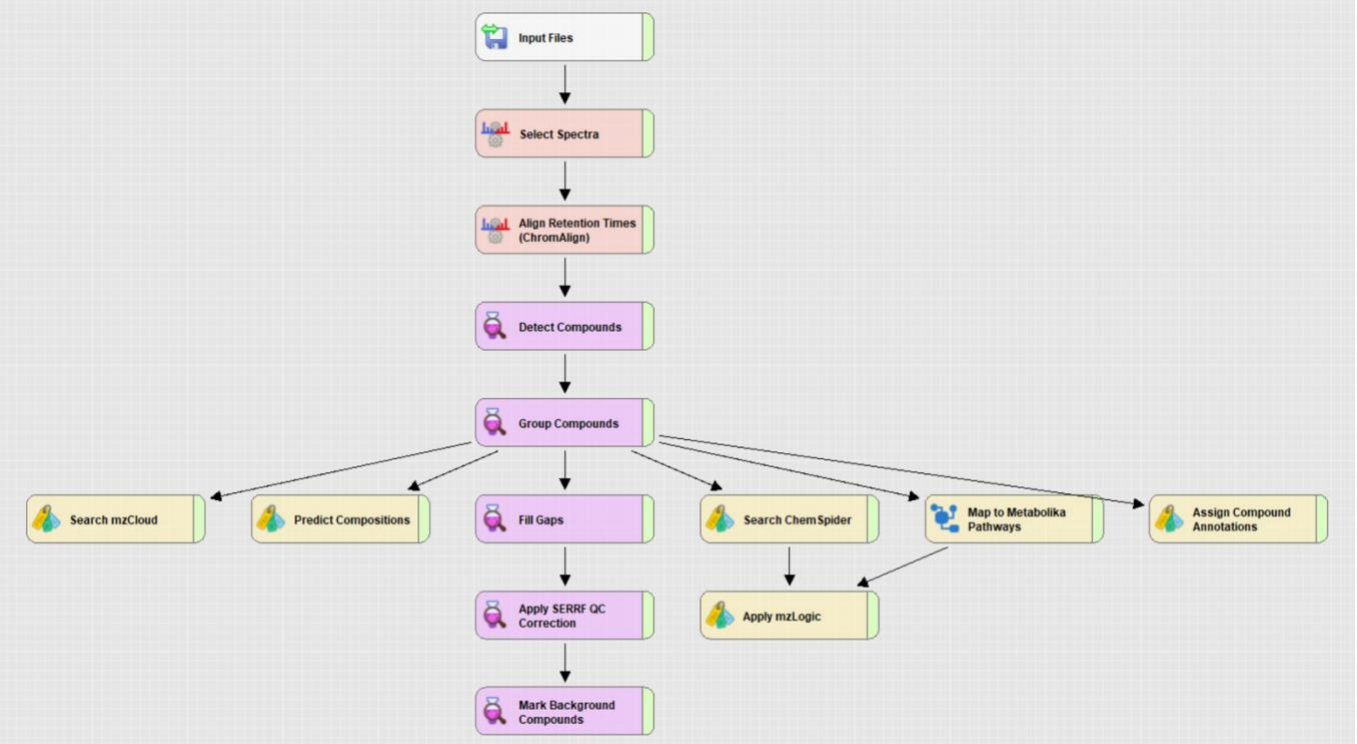


Figure S1: Workflow tree showing the components of the Compound Discoverer automated analysis (screenshot).

- Workflow nodes:
    - Select Spectra
        - Precursor selection MS(n-1)
        - Use Isotope pattern in precursor evaluation = True
        - Precursor mass range 0-5,000 Da
        - Intensity threshold = 0
        - Minimum peak count = 1
        - S/N Threshold = 1.5
    - Align Retention times (ChromAlign to first BIT-exposed plant tissue sample)
    - Detect Compounds
        - Mass tolerance = 5 ppm

- Min. peak intensity = 10,000
- Min. # Scans per Peak = 5
- S/N Threshold = 1.5
- Use Most Intense Isotope Only: True
- Ions: [2M+ACN+H]+1; [2M+ACN+Na]+1; [2M+FA-H]-1; [2M+H]+1; [2M+K]+1; [2M+Na]+1; [2M+NH4]+1; [2M-H]-1; [2M-H+HAc]-1; [M+2H]+2; [M+3H]+3; [M+ACN+2H]+2; [M+ACN+H]+1; [M+ACN+Na]+1; [M+Cl]-1; [M+DMSO+H]+1; [M+FA-H]-1; [M+H]+1; [M+H+K]+2; [M+H+MeOH]+1; [M+H+Na]+2; [M+H+NH4]+2; [M+H-H2O]+1; [M+H-NH3]+1; [M+K]+1; [M+Na]+1; [M+NH4]+1; [M-2H]-2; [M-2H+K]-1; [M-H]-1; [M-H+HAc]-1; [M-H+TFA]-1; [M-H-H2O]-1
- Base Ions: [M+H]+1; [M-H]-1
- Remove singlets = True
- Filter out Features with Bad Peaks Only = true
- Max peak width = 1.0 min

- Group Compounds
  - Mass tolerance = 5 ppm
  - RT tolerance = 0.2 min
  - Preferred Ions: [M+H]+1; [M-H]-1
- Search mzCloud
  - Compound classes: All
  - Precursor Mass Tolerance: 10 ppm
  - FT Fragment Mass Tolerance = 10 ppm

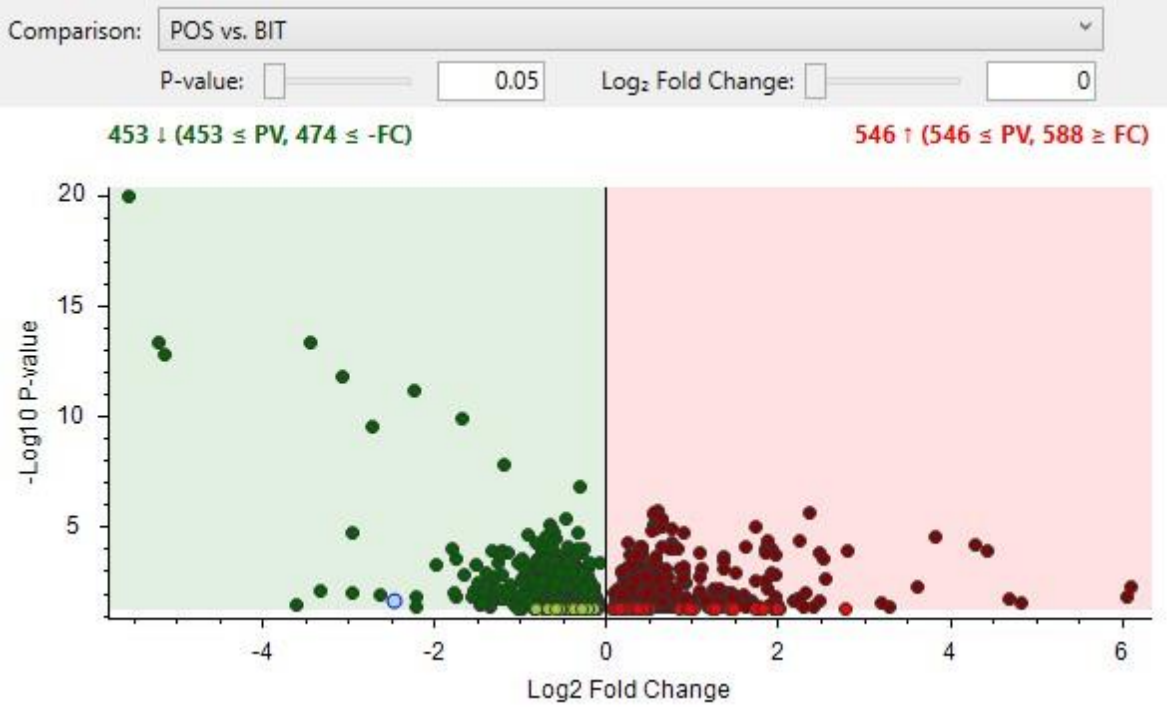
        - IT Fragment Mass Tolerance = 0.4 Da
        - Library: Autoprocessed; Reference
        - Post Processing: Recalibrated
        - Max # Results = 10
        - Annotate Matching Fragments = True
        - Search MSn Tree = True
        - Identity search = Cosine
        - Match Activation Type = True
        - Match Activation Energy = Match with Tolerance
        - Activation Energy Tolerance = 20
        - Apply Intensity Threshold = True
        - Similarity Search = Confidence Forward
        - Match Factor Threshold = 50
        - Use DIA scans for search = False
        - Max isolation width = 500 Da
        - Match activation type = False
        - Match activation energy = Any
        - Activation Energy Tolerance = 100
        - Apply Intensity Threshold = False
        - Match Factor Threshold = 20
    - Predict Compositions
        - Mass Tolerance = 5 ppm
        - Min Element Counts: C H

        - Max Element Counts: C90, H190, Br3, Cl4, N10, O18, P3, S5
        - Min RDBE = 0
        - Max RDBE = 40
        - Min H/C = 0.1
        - Max H/C = 3.5
        - Max # Candidates = 10
        - Max # Internal Candidates = 200
        - Intensity Tolerance = 30%
        - Intensity Threshold = 0.1%
        - S/N Threshold = 3
        - Min. Spectral Fit = 30%
        - Min. Pattern Coverage = 90%
        - Use Dynamic Recalibration = True
        - Use Fragment Matching = True
        - Mass Tolerance = 5 ppm
        - S/N Threshold = 3
    - Fill Gaps
        - Mass tolerance = 5 ppm
        - S/N threshold = 1.5
        - Use real peak = true
    - Search ChemSpider
        - Databases: BioCyc; Human Metabolome Database; KEGG
        - Search Mode: By formula or mass

        - Mass tolerance 5 ppm
        - Max # of results per compound = 100
        - Result Order = Order by Reference count (DESC)
        - Max # of predicted compositions to be searched per compound = 3
        - Check all predicted compositions = True
    - Map to Metabolika Pathways
        - Metabolika Pathways = (all are checked)
        - Search Mode = By Formula or Mass
        - Mass Tolerance = 5 ppm
        - Max. # of Predicted Compositions to be searched per Compound = 3
        - Mas. # Pathways in 'Pathways' column = 20
    - Apply mzLogic
        - FT Fragment Mass Tolerance = 10 ppm
        - IT Fragment Mass Tolerance = 0.4 Da
        - Max. # Compounds = 0
        - Max. # mzCloud Similarity Results to consider per Compound = 10
        - Match Factor Threshold = 30
    - Assign Compound Annotations
        - Mass Tolerance = 5 ppm
        - Data source #1 = mzCloud Search
        - Data source #2 = Predicted Compositions
        - Data source #3 = MassList Search
        - Data source #4 = ChemSpider Search

        - Data source #5 = Metabolika Search
        - Use mzLogic = True
        - Use Spectral Distance = True
        - SFit Threshold = 20
        - SFit Range = 20
        - Clear Names = False
    - Apply SERRF QC Correction
        - Min. QC Coverage [%]: 50
        - Max. QC Area RSD [%]: 30
        - Max. Corrected QC Area RSD [%]: 25
        - Max. # Files Between QC Files: 15
        - # Batches: 2
        - Interpolate Gap-filled QC Areas: True
        - Correct Blank Files: False
        - # Trees: 200
    - Mark Background Compounds
        - Max Sample/Blank = 5
        - Max. Blank/Sample = 0
        - Hide Background = True

(a)


Comparison: POS vs. BIT
P-value: 0.05
Log₂ Fold Change: 0
453 ↓ (453 ≤ PV, 474 ≤ -FC)
546 ↑ (546 ≤ PV, 588 ≥ FC)
-Log10 P-value
Log2 Fold Change

(b)

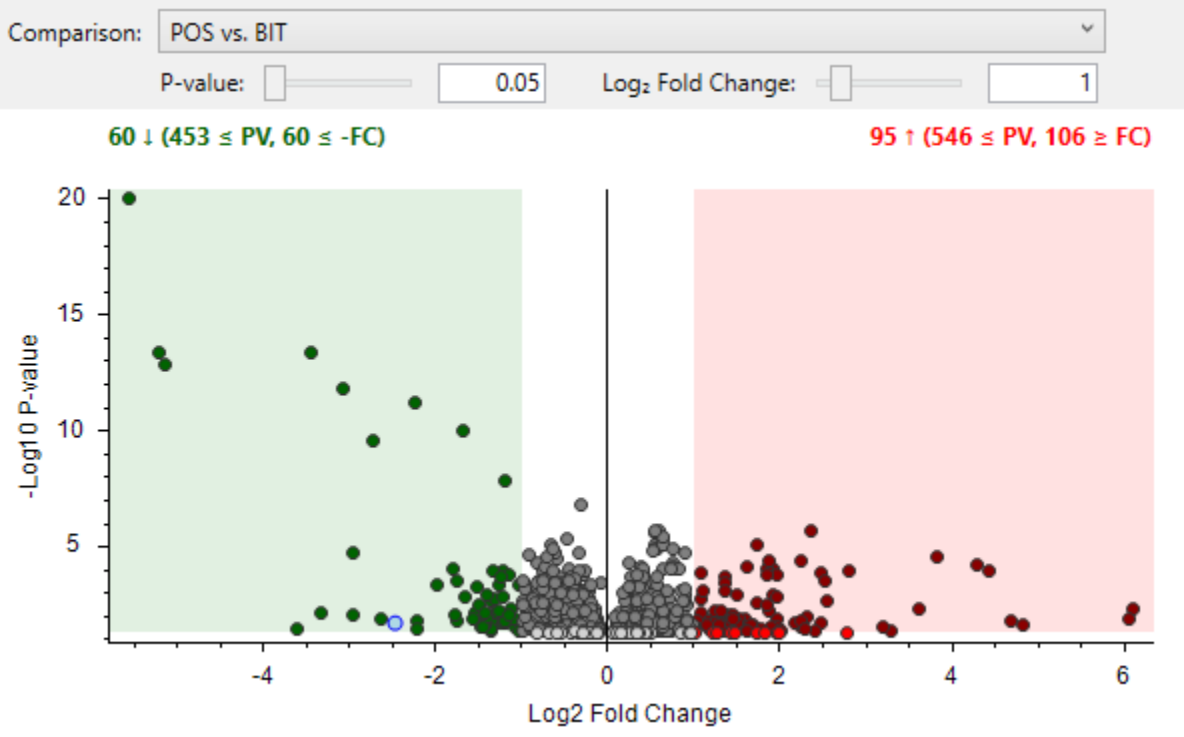

Comparison: POS vs. BIT
P-value: 0.05
Log₂ Fold Change: 1
60 ↓ (453 ≤ PV, 60 ≤ -FC)
95 ↑ (546 ≤ PV, 106 ≥ FC)
-Log10 P-value
Log2 Fold Change

Figure S2: To illustrate the number of features changed in plant tissue by BIT exposure: the plant extract metabolites with a p-value of ≤0.05 in the fold-change ratio between exposed plants and unexposed plants and (a) a log2 fold change >0, indicating a different amount of compound in the BIT-unexposed ("POS") and BIT-exposed ("BIT") plant tissue extracts and (b) a log2 fold change > |1|, indicating a two-fold or greater change between the treatments. (a) is meant to illustrate the number of features overall affected by BIT exposure—453 upregulated, 546 downregulated, and (b) illustrates the number of features changed at least two-fold by BIT exposure—60 upregulated, 95 downregulated. A more restrictive five-fold or greater change cutoff along with p-value of ≤0.05 was used for metabolite analysis in this paper. Note that the ratio was (unexposed plants)/(exposed plants), so negative x-axis values indicate compounds upregulated in the exposed plants and positive x-axis values are compounds downregulated in the exposed plants. This is the opposite of what is indicated by the arrows in the figure. Features decreased in peak area in BIT-exposed vs. unexposed plants were not examined further in this work, but HRMS data for these features is available upon request. Medium samples were run as composite samples so p-values are not calculated and thus are not pictured.

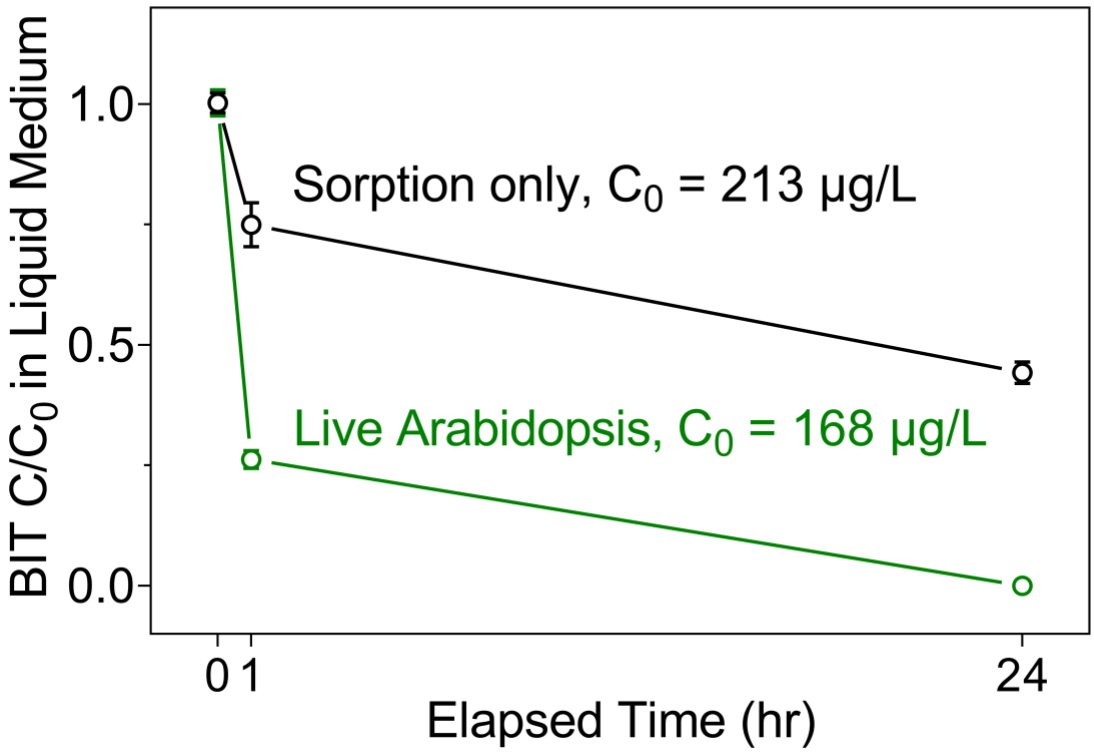
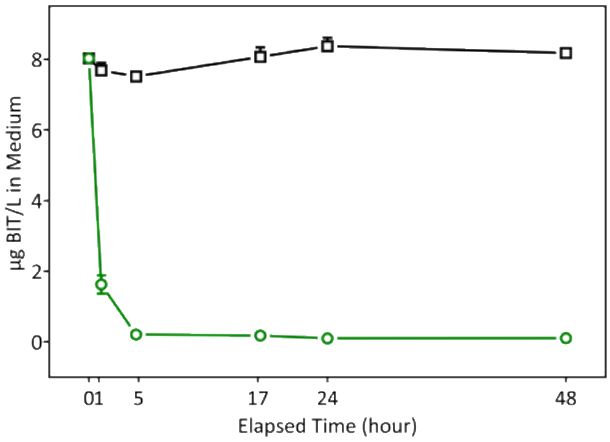
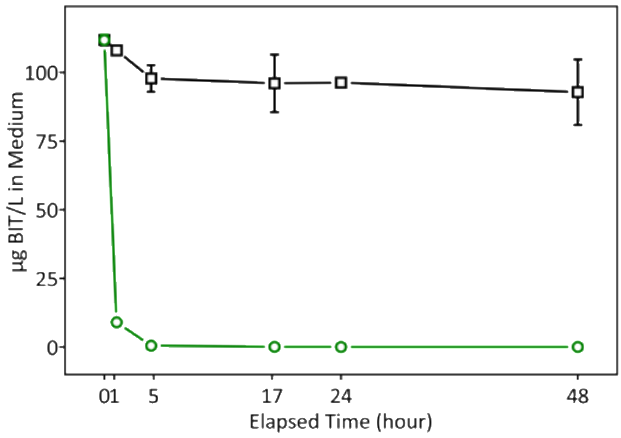


Figure S3: Sorption test with dead (freeze-dried) Arabidopsis (C_0 of 213 µg BIT/L, n=4) compared with BIT removal from the medium with live Arabidopsis (C_0 of 168 µg BIT/L, not conducted in parallel with sorption experiment, n=4). Error bars are +/- standard error and are small enough to not be visible for many timepoints. At a starting concentration of 213 µg BIT/L, in the sorption test 25% of the starting concentration of BIT was removed from the medium after one hour and 56% after 24 hours. At a similar nominal starting concentration of 168 µg BIT/L with live plants, 75% of BIT was removed after one hour and >99% at 24 hours. Thus, as an approximation, sorption accounts for ~33% (25%/75%) of the BIT removal at one hour.

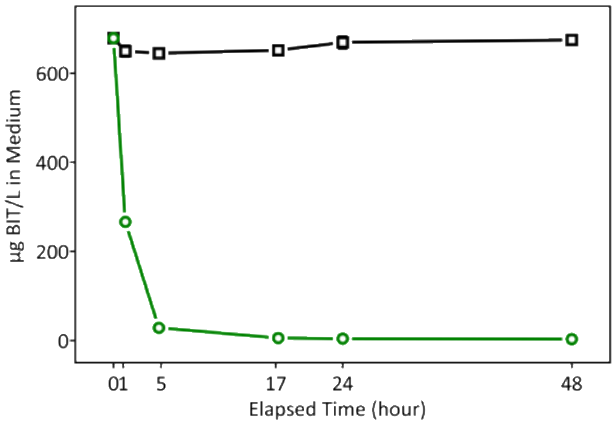
(a) 8 µg BIT/L



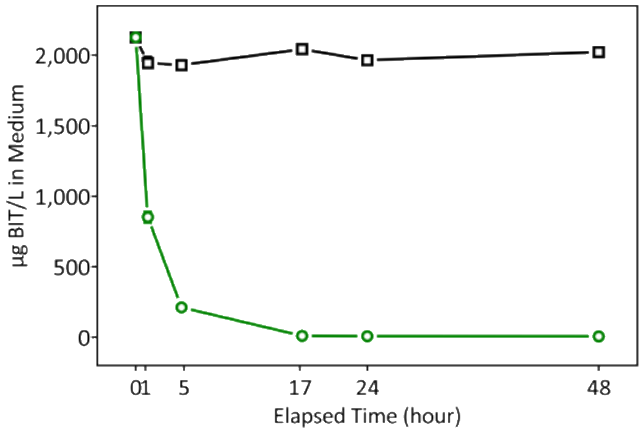
(b) 112 µg BIT/L



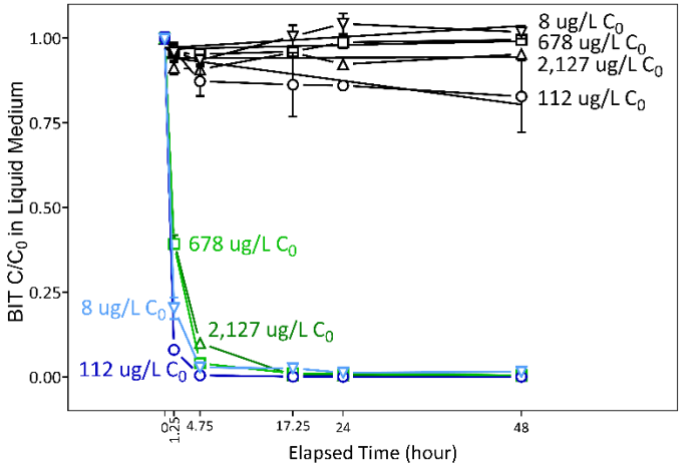
(c) 678 µg BIT/L



(d) 2,127 µg BIT/L

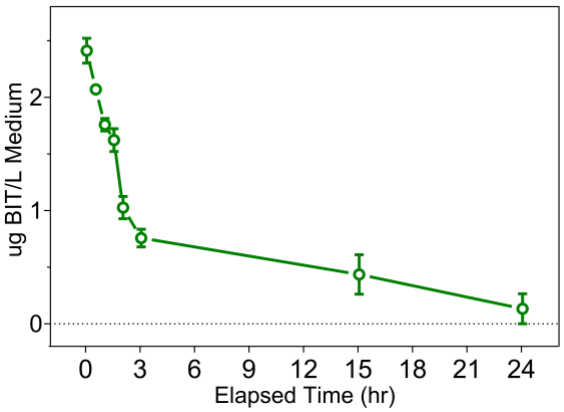


(e)

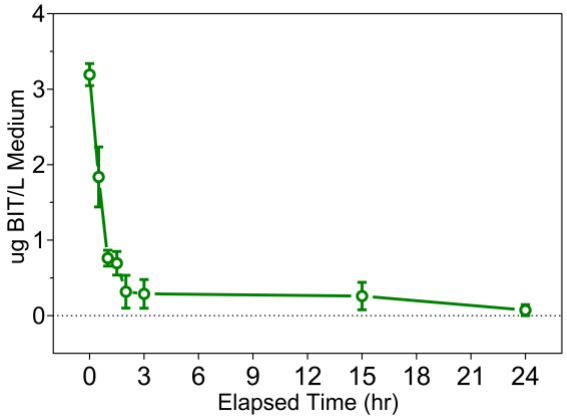


(f) 2.4 µg BIT/L

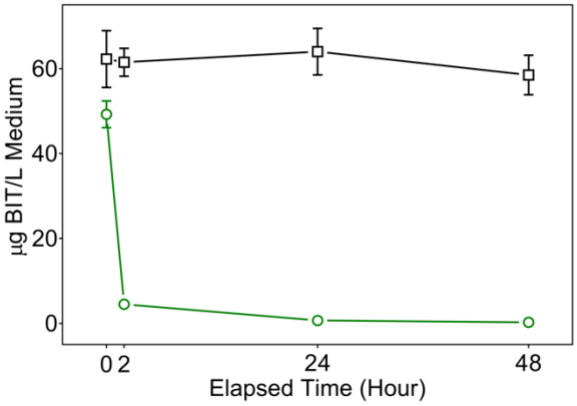
(g) 3.2 µg BIT/L



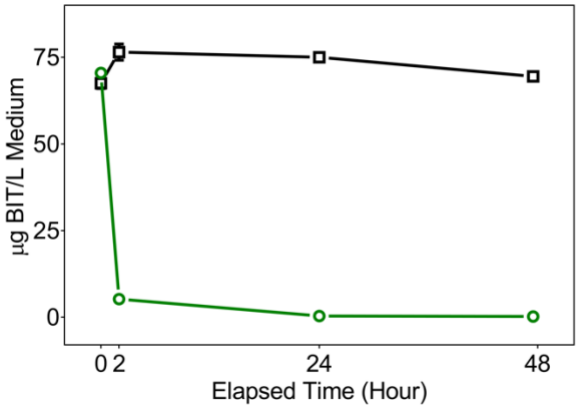
(h) 49 µg BIT/L



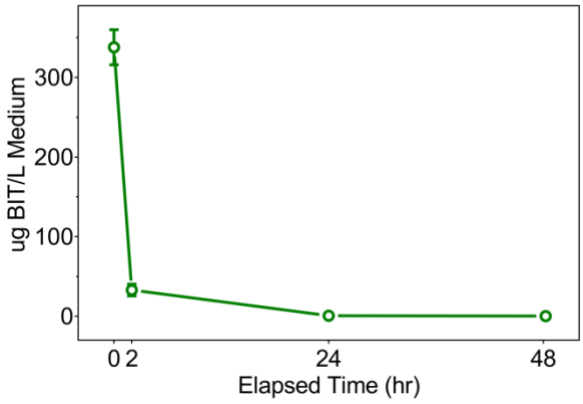
(i) 71 µg BIT/L

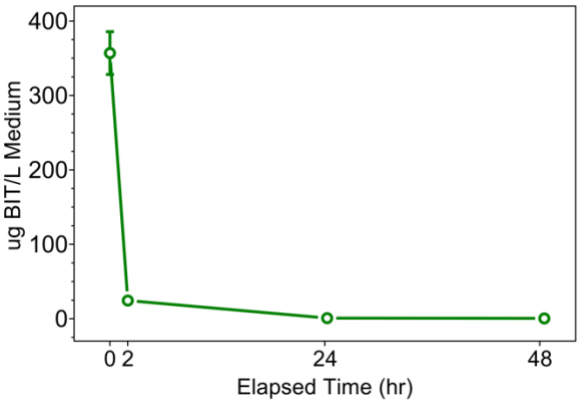


(j) 338 µg BIT/L



(k) 357 µg BIT/L





(l) 1,050 µg BIT/L

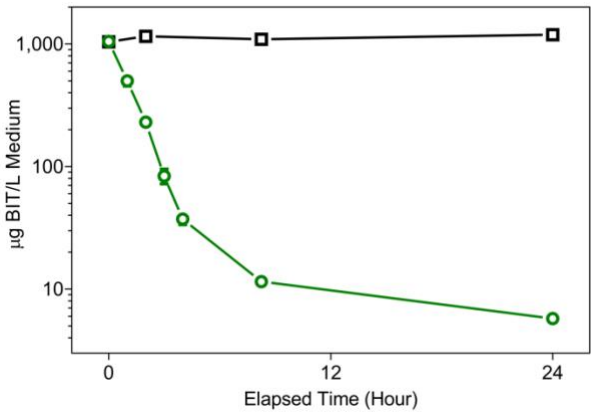


Figure S4: BIT removal from liquid medium by Arabidopsis plants grown from surface-sterilized seeds in sterile medium at four starting concentrations of BIT in the liquid medium, conducted in parallel: (a) 8 µg BIT/L, (b) 112 µg BIT/L, (c) 678 µg BIT/L, (d) 2,127 µg BIT/L, and (e) All four initial concentrations as C/C_0, to allow for comparison between different concentrations. Additionally, all data represented in Figure 2 is represented in (a) through (l) above (representing BIT depletion kinetics experiments conducted asynchronously from experiments f–l). The removal rate calculated from each graph above is represented by one data point in Figure 2, for a total of 11 data points in Fig 2. For all graphs above, black lines and symbols are no-plant controls (not included in all experiments); blue and green lines and symbols are plant treatments. Non-sacrificial sampling, n=4 per timepoint. Error bars are +/- standard error and are small enough to not be visible for many timepoints.

Table S5: r^2 values for curve fitting each data point in Figure 2.

C_0 (µg BIT/L)	**k from µg/L data**	**Zero order r^2**	**First order r^2**	**Second order r^2**
2.4	0.2074	0.6271	0.8891	0.9062
3.2	0.7310	0.2333	0.8670	0.8575
8	0.4209	0.2637	0.9939	0.9940
49	0.1003	0.3622	0.9819	0.9822
71	0.08842	0.3585	0.9986	0.9986
112	0.08841	0.2131	0.9987	0.9983
338	0.0138	0.3678	0.9802	0.9801
357	0.01913	0.3431	0.9734	0.9734
678	0.002225	0.3521	0.8054	0.9868
1,050	0.001653	0.2653	0.9891	0.9528
2,127	0.0006343	0.3861	0.7829	0.9930
	Average ->	**0.3259**	**0.9213**	***0.9510***

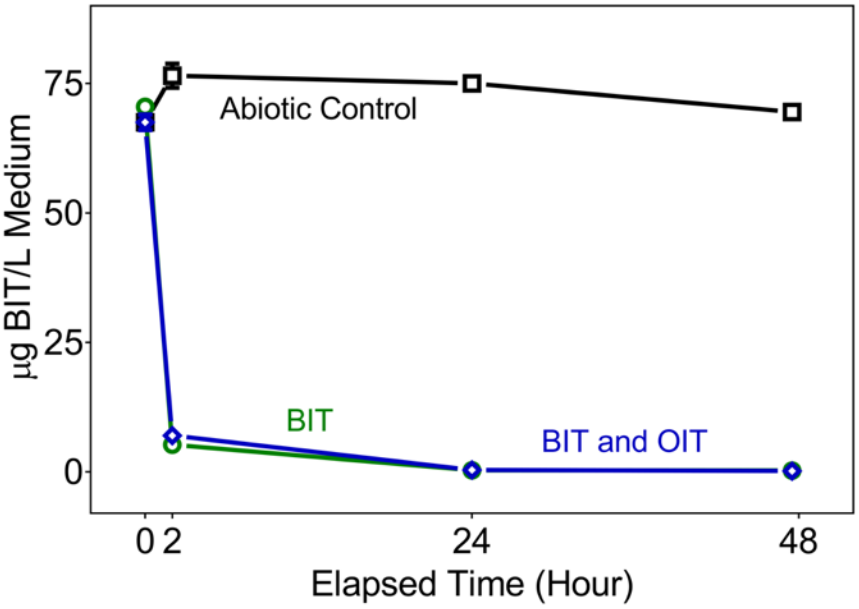


Figure S5: BIT and OIT competitive uptake experiment. Nominal C_0s were 50 µg BIT/L and 70 µg/L (an equimolar concentration to 50 µg BIT/L). BIT C_0 was measured as 71 µg BIT/L. OIT C_0 was not measured. BIT treatment was spiked with BIT only. BIT and OIT treatment and the abiotic control were spiked with both BIT and OIT. The BIT treatment and BIT and OIT treatment were found to not be significantly different (p=0.77).

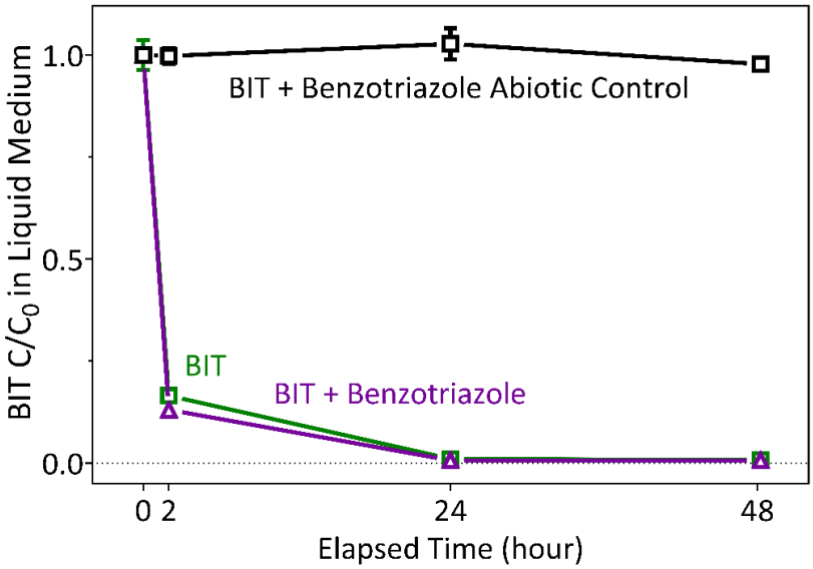


Figure S6: BIT relative concentration in the plant medium with a nominal C_0 mixture of 50 µg BIT/L and 50 µg benzotriazole/L and live Arabidopsis plants. The BIT treatment was not found to be significantly different than the BIT and benzotriazole mixed treatment (p=0.34), indicating no exhibited competitive inhibition.

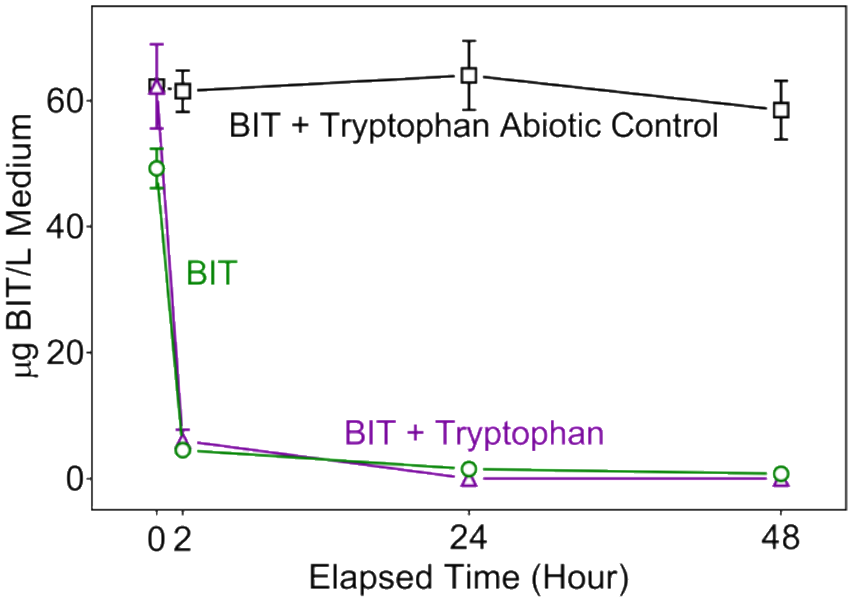


Figure S7: Results of the BIT and tryptophan competitive plant uptake experiment. BIT and BIT + Tryptophan treatments are not significantly different (p=0.43), indicating that tryptophan does not create significant competitive inhibition for plant uptake of BIT. The abiotic control does not demonstrate significant removal (p=0.65), indicating that biotic processes (*i.e.*, plant uptake and sorption to plant tissue) are dominant.

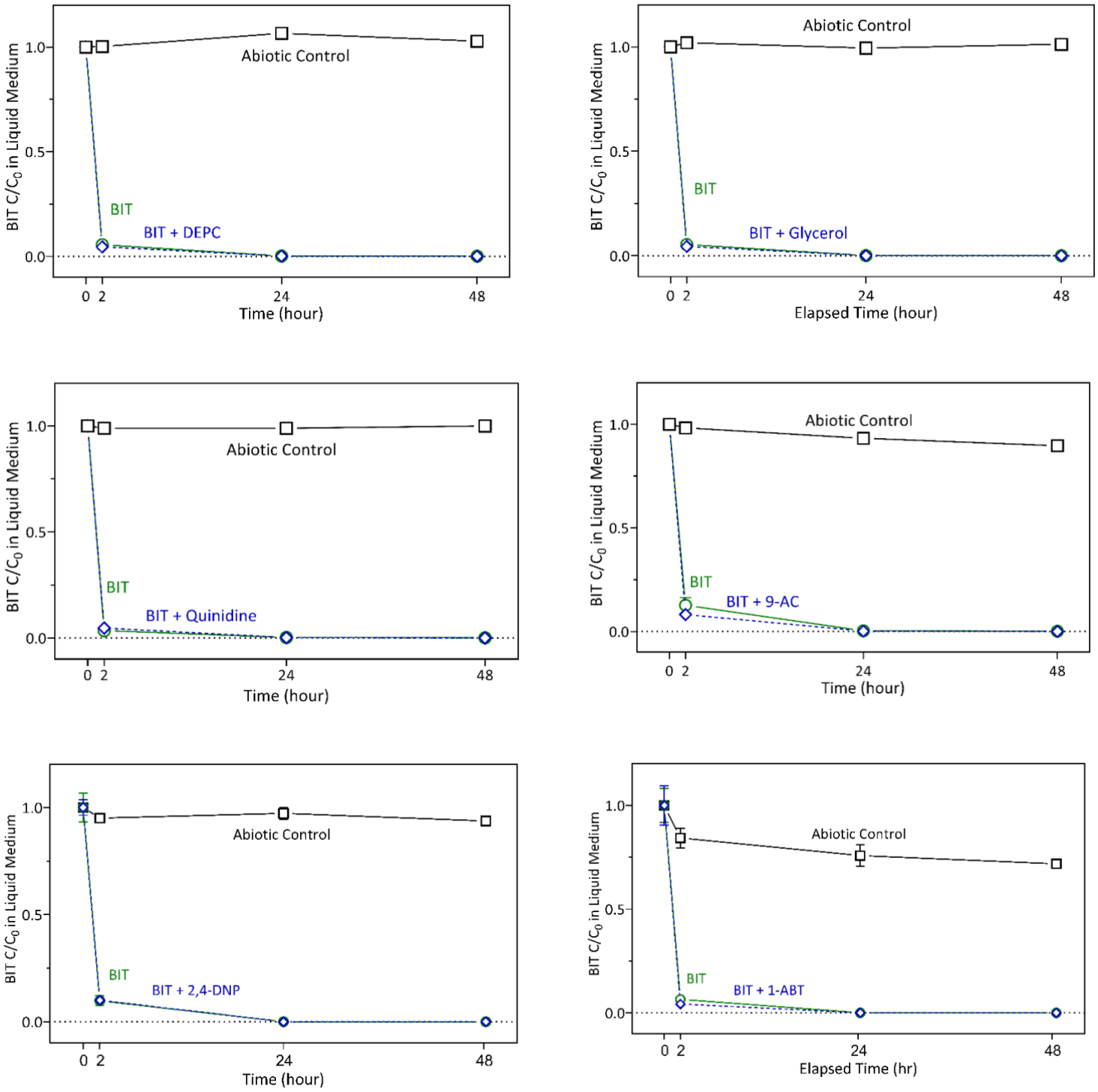
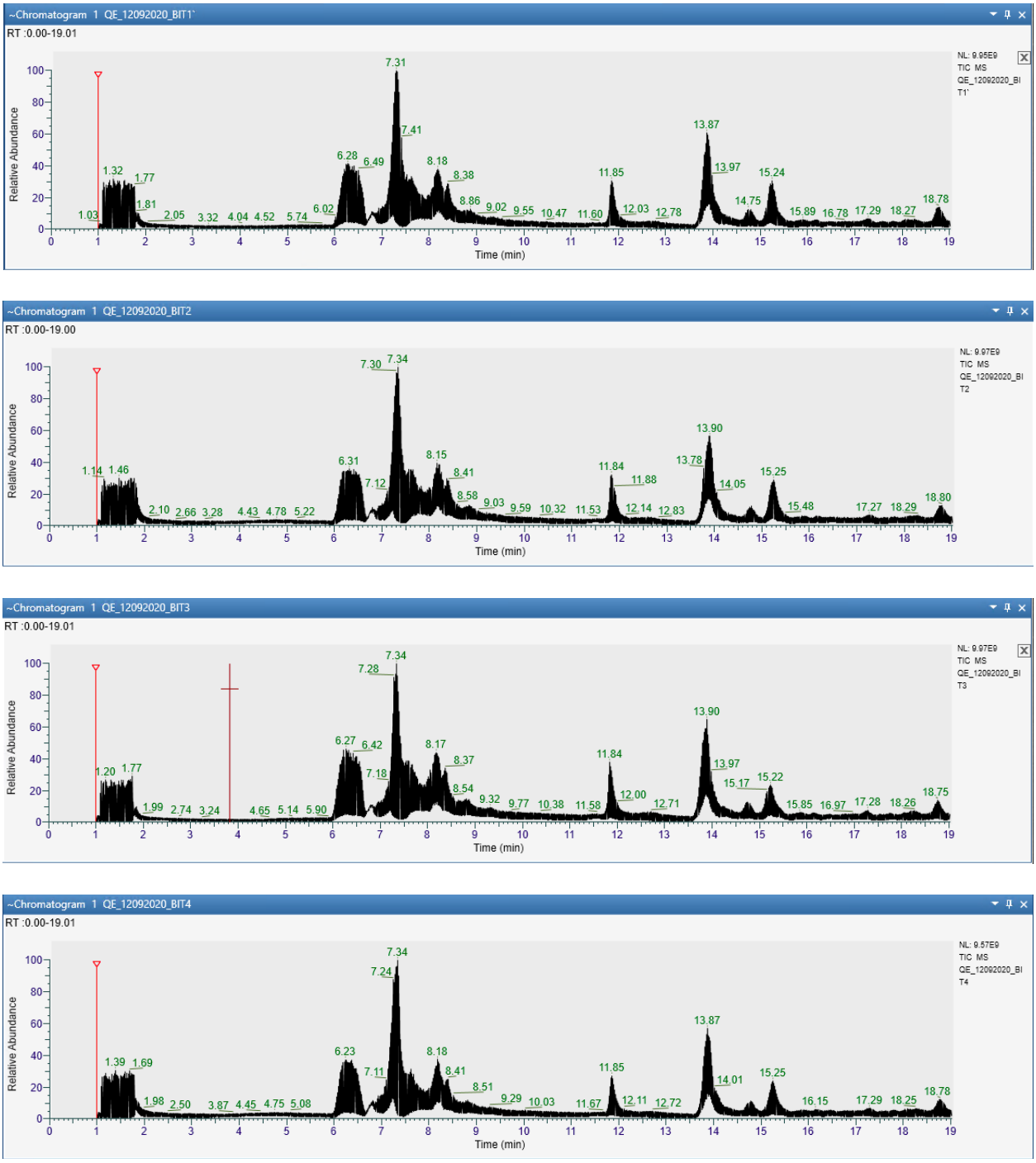
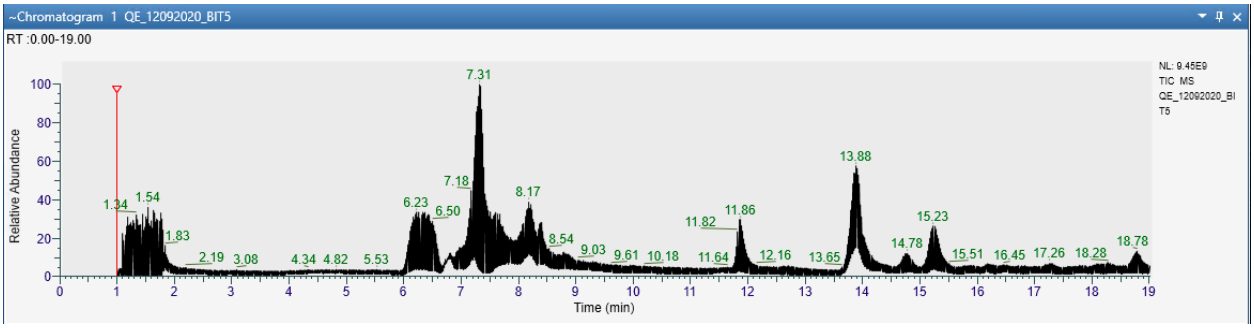
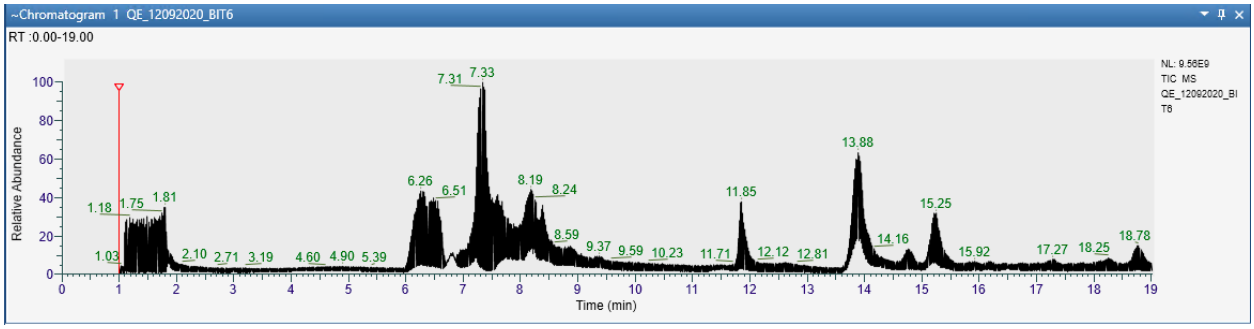


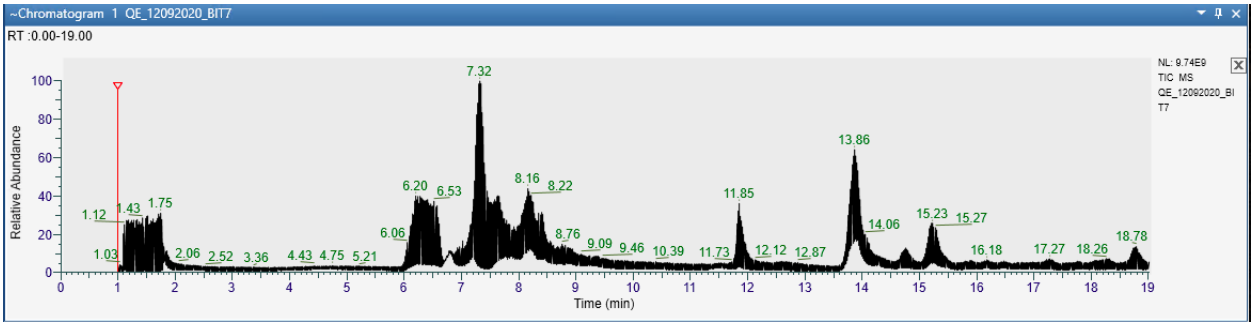
Figure S8: Full inhibitor results of BIT removal from the hydroponic medium. Nominal C_0 BIT concentration for all experiments was 150 µg BIT/L. None of the BIT and BIT + inhibitor treatment pairs were significantly different (top to bottom, p=0.34 for DEPC, p= 0.38 for glycerol, p=0.43 for quinidine, p=0.36 for 9-AC, p=0.39 for 2,4-DNP, p=0.39 for 1-ABT), indicating no significant inhibition of BIT uptake. Two outlier samples in the abiotic control of

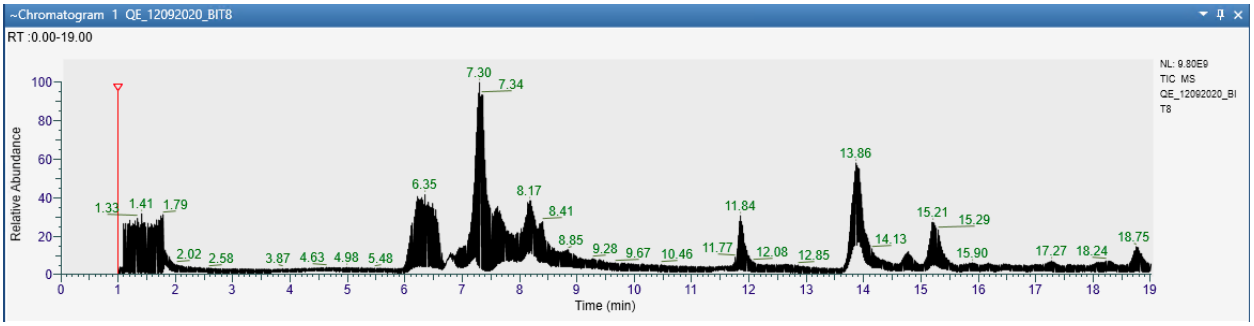
the 2,4-DNP inhibitor experiment and one outlier sample in the abiotic control of the 9-AC experiment were removed using the Prism ROUT method with Q = 1%. Including outlier removal, n=3–4 for each timepoint. Error bars represent standard error and are small enough to be obscured by the symbols in some cases.




~Chromatogram 1 QE_12092020_BIT5
RT :0.00-19.00
NL: 9.45E9
TIC MS
QE_12092020_BI
T5
Relative Abundance
Time (min)


~Chromatogram 1 QE_12092020_BIT6
RT :0.00-19.00
NL: 9.56E9
TIC MS
QE_12092020_BI
T6
Relative Abundance
Time (min)


~Chromatogram 1 QE_12092020_BIT7
RT :0.00-19.00
NL: 9.74E9
TIC MS
QE_12092020_BI
T7
Relative Abundance
Time (min)


~Chromatogram 1 QE_12092020_BIT8
RT :0.00-19.00
NL: 9.80E9
TIC MS
QE_12092020_BI
T8
Relative Abundance
Time (min)

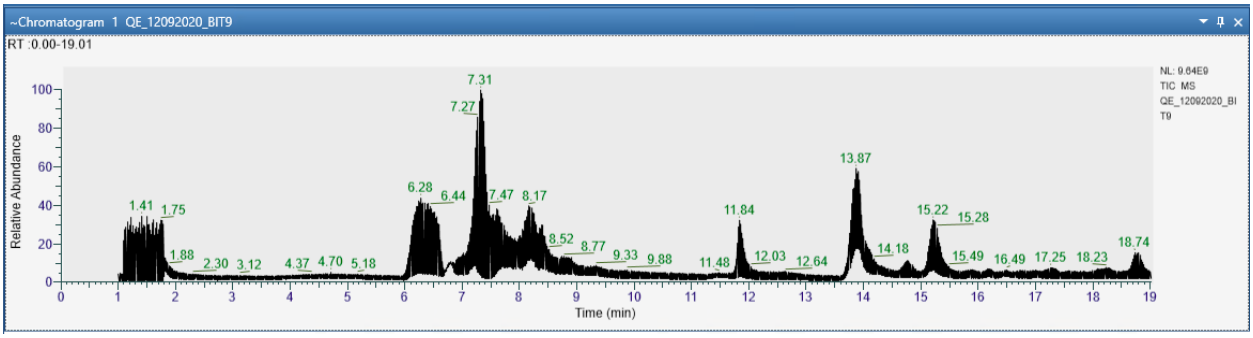
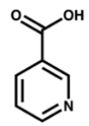
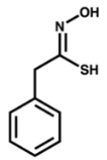
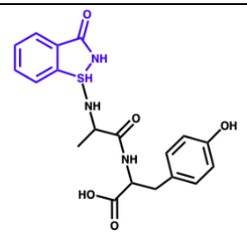


Figure S9: Chromatograms for the nine BIT-exposed plant tissue extracts. Samples were extracted from the nine plants exposed to BIT for 24 hours. The retention time for the earliest compound where peak area was increased at least five-fold in plant tissue exposed to BIT vs unexposed was 1.917 min.

Table S6: Summary of Compounds Increased in BIT-Exposed Arabidopsis Plant Tissue

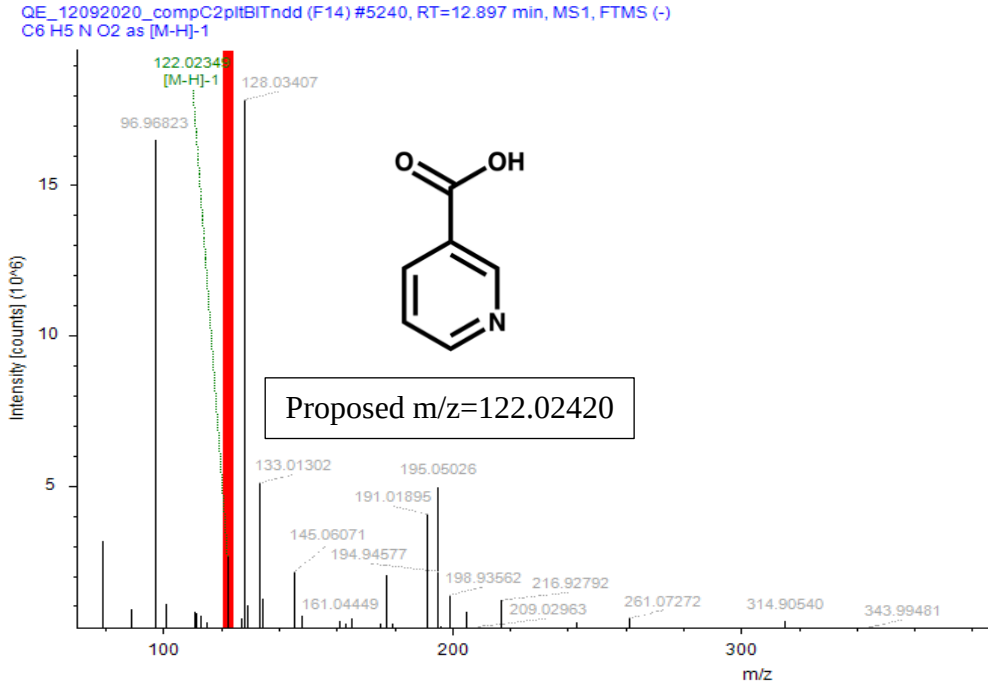
Proposed Metabolite									
Compound Name	**Proposed Metabolite Structure**	**Proposed Metabolite Formula**	**Confidence Level**[9]	**Retention Time**	**Measured m/z, Positive/ Negative Ion**	**Exact Mass of Proposed Ionized Formula**	**Accurate Mass Deviation (ppm)**	**Fragment Measured m/z**	**Proposed Fragment Molecular Formula**
Nicotinic Acid		$C_6H_5NO_2$	Level 1: Confirmed with Reference Material (and Library Spectrum Match MS^2)	12.912 min	122.02349 $[M-H]^-$	122.02475	10	78.03349	C_5H_5N
Phenylthioaceto-hydroximic acid		C_8H_9NOS	Level 3a:[67] Tentative Candidate (based on MS, fragments, exp. data, MS^2)	14.769 min	168.04785 $[M+H]^+$	168.04831	3	151.02130 151.02127 151.02145	C_8H_8NS (same for all three ions)
BIT-Alanine-Tyrosine Conjugate		$C_{19}H_{21}N_3O_5S$	Level 3b:[67] Tentative Candidate (based on MS, fragments, exp. data)	13.891 min	403.11679 $[M+H]^+$	403.12019	8	166.08640	$C_9H_9O_3^-$
TP 470 [Unknown Accurate Mass of Interest Significant Upregulated]	N/A	N/A	Level 5: Accurate Mass of Interest	1.917 min	470.15134 $[M-H]^-$	N/A	N/A	N/A	N/A
TP 410 [Unknown Accurate Mass of Interest Significant Upregulated]	N/A	N/A	Level 5: Accurate Mass of Interest	6.726 min	410.86249 $[M-H]^-$	N/A	N/A	N/A	N/A

Mass Spectra and Proposed Fragments for Proposed Metabolites

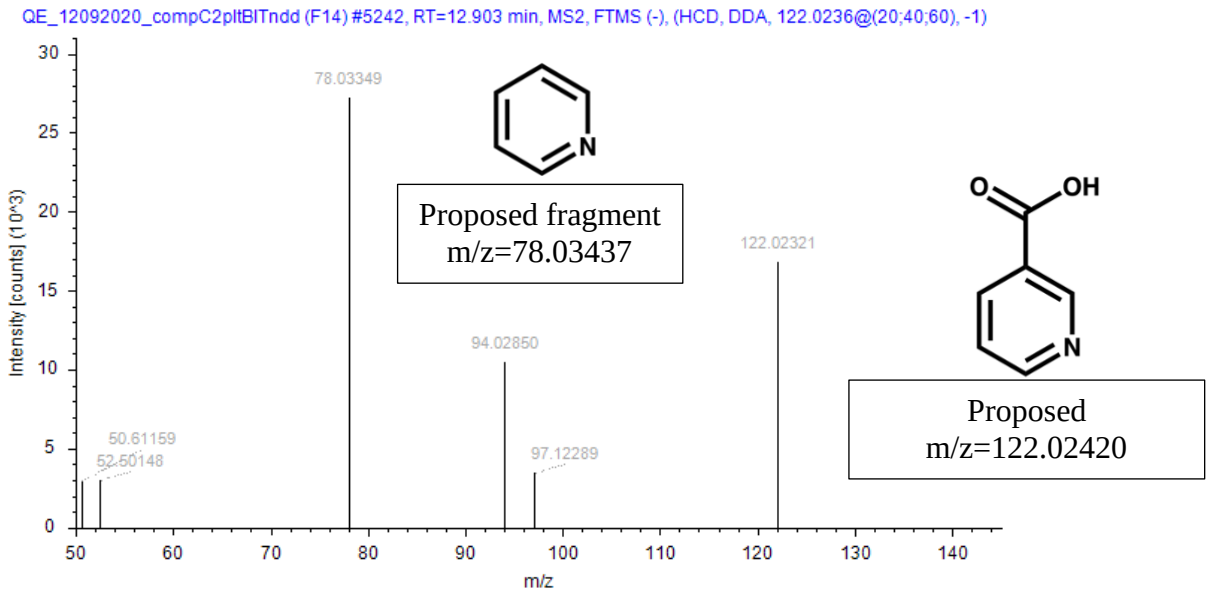
(NOTE: all proposed metabolites and fragment structures are drawn unionized, with proposed m/z ($[M+H]^+$ or $[M-H]^-$) values representing ionized versions of the drawn structures to correspond to the ionization state presented in the spectra).

Nicotinic Acid

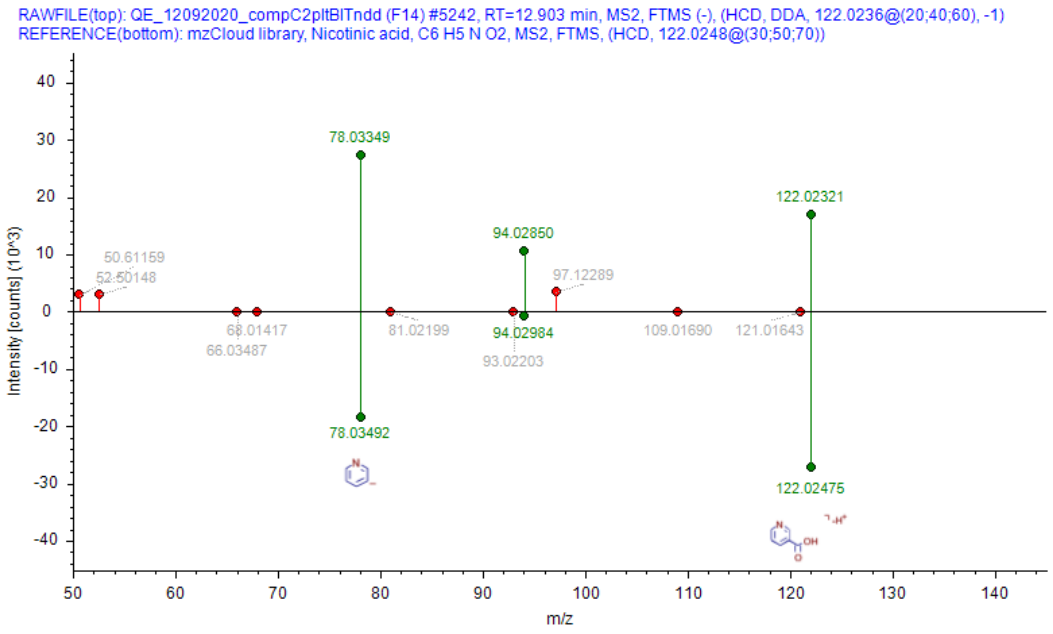
MS:



MS2:



mzCloud match with MS2 experimental data ***(mzCloud Best Match score=86.9)***:



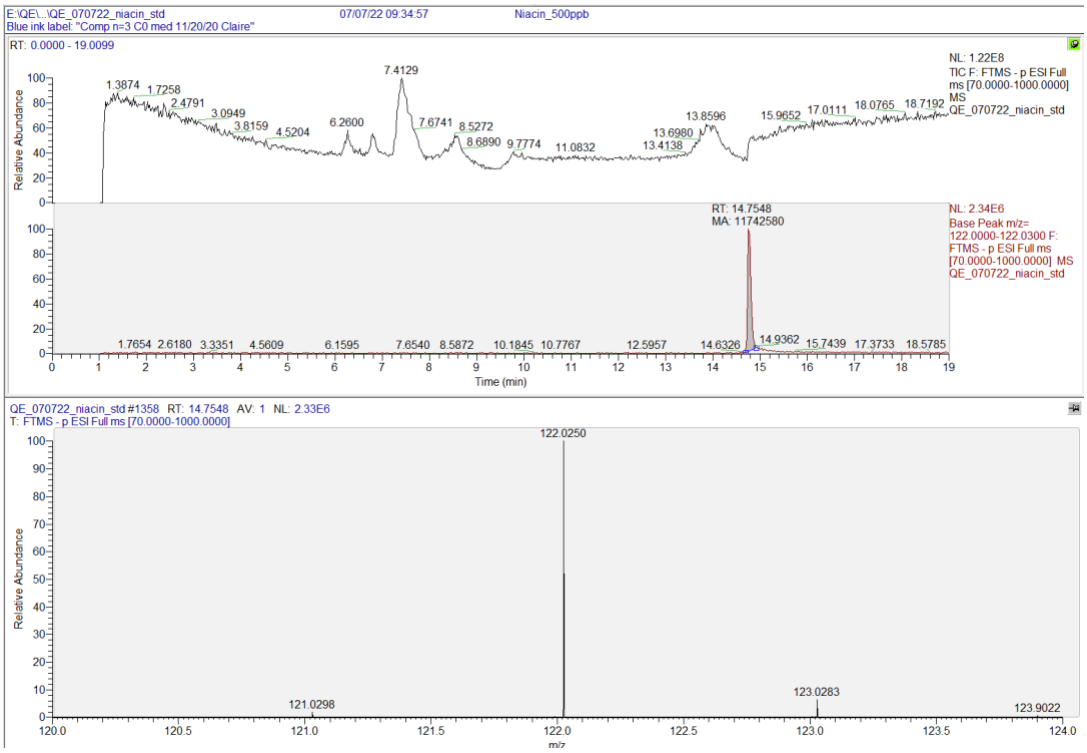
RESULTS of Niacin Standard Addition test proving niacin presence to Level 1 Confidence.

To prove that the plant extract samples contained nicotinic acid to a Level 1 Confidence, we ordered a commercial standard of nicotinic acid (niacin; Supelco Sigma-Aldrich Certified Reference Material; CAS 59-67-6, product number: PHR1276) and diluted it to 500 ppb in 1:1 MeOH:H_2O to match the solvent of the plant extract. We then spiked the niacin commercial reference standard in the plant extract. We ran the samples on the LC-HRMS and quantified the extracted ion counts for the niacin peak for: (a) the niacin reference standard, (b) niacin in the plant extract [as an endogenous compound], and (c) the niacin standard addition into the plant extract at a 1:1 volume ratio [a blank showing no background was run for quality assurance purposes].

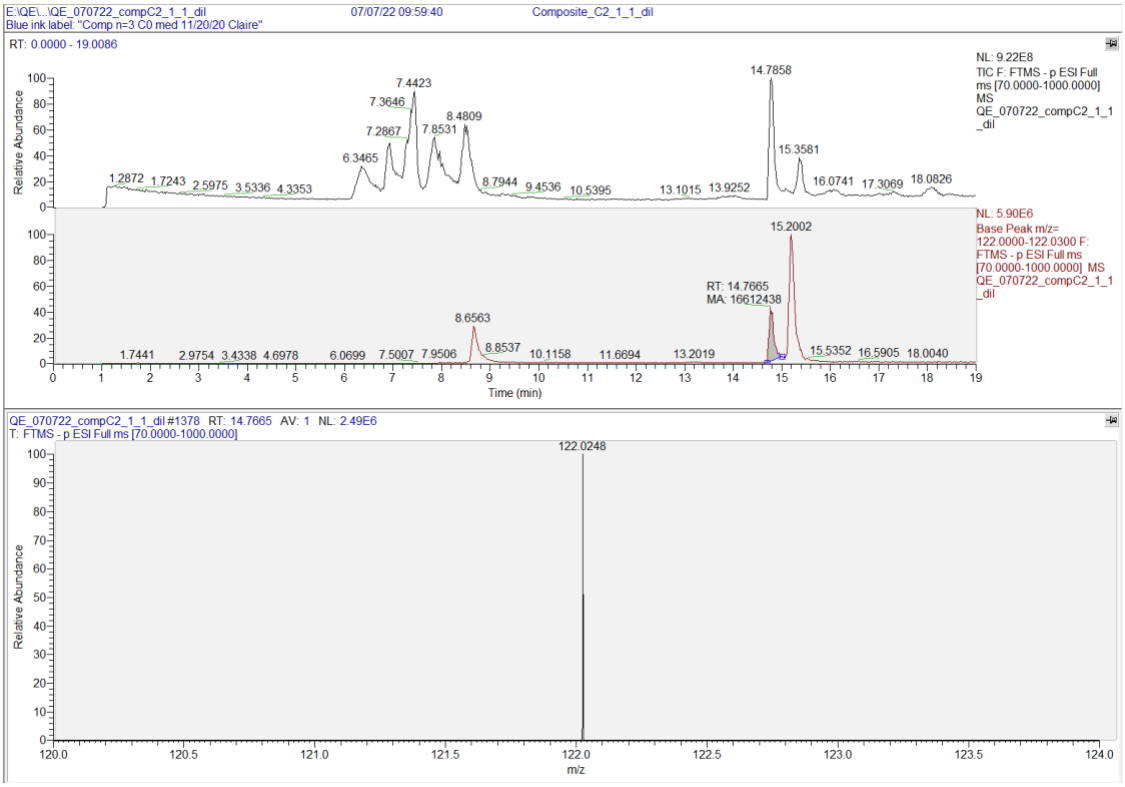
The retention times and accurate masses all match; see below (note: the RT is different from the original metabolomics-based sample run because the standard addition was conducted at a later date). The niacin peak area significantly increases following standard addition; indeed, the peak area standard addition spike was ~double that of the commercial standard or plant extract when added at a 1:1 volume ratio, demonstrating responsiveness at the same peak. ***We thus demonstrate that the compound we identified as upregulated endogenous niacin in the plant extract via HRMS/MS is confirmed with to a Level 1 Confidence with a reference material.***

Sample Run	**m/z (ESI-)**	**RT (min)**	**Peak Area**
Niacin Commercial Reference Material Standard	122.025	14.76	11742580
Plant Extract ('composite' Sample)	122.0248	14.77	16612438
Niacin Standard Addition to plant extract sample	122.0248	14.76	25840662

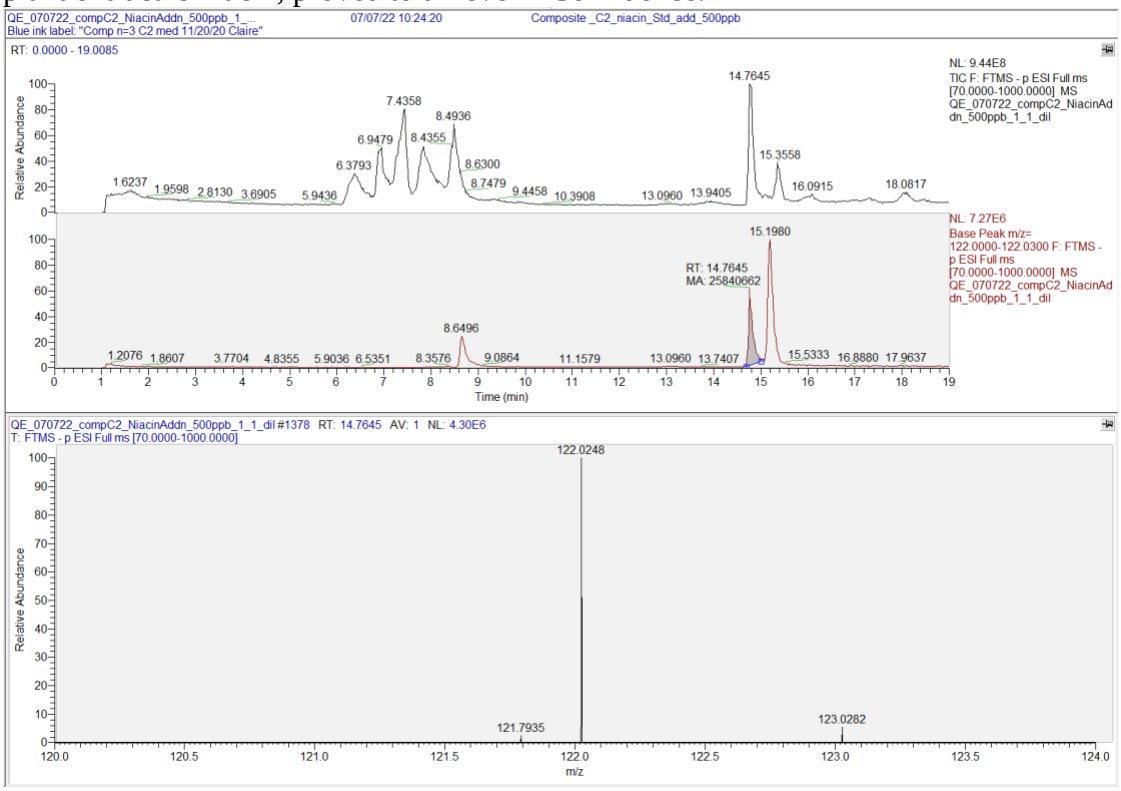
Niacin Commercial Standard spike:



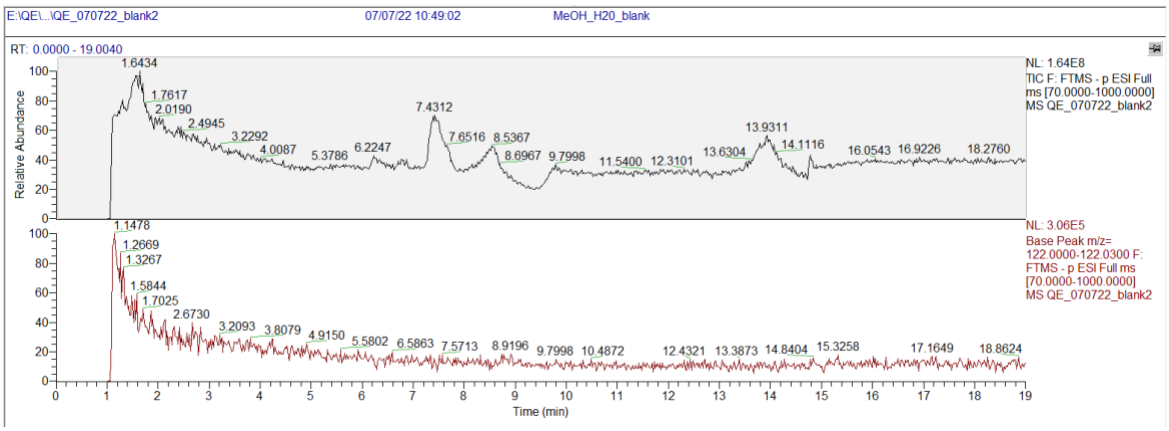
Composited Plant Extract:



1:1 volume Standard Addition of niacin standard into the plant extract: the significantly increased peak area of the singular niacin peak demonstrates that the compound identified in the plant extract is niacin, proved to a Level 1 Confidence.

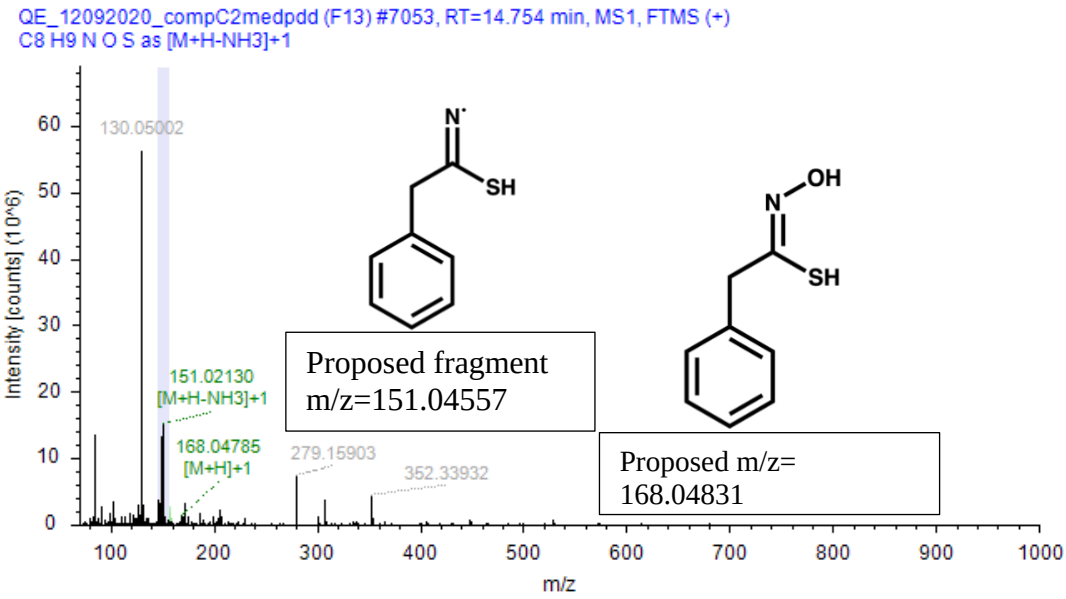


Quality Control Blank indicates no background niacin (total or extracted ion count):

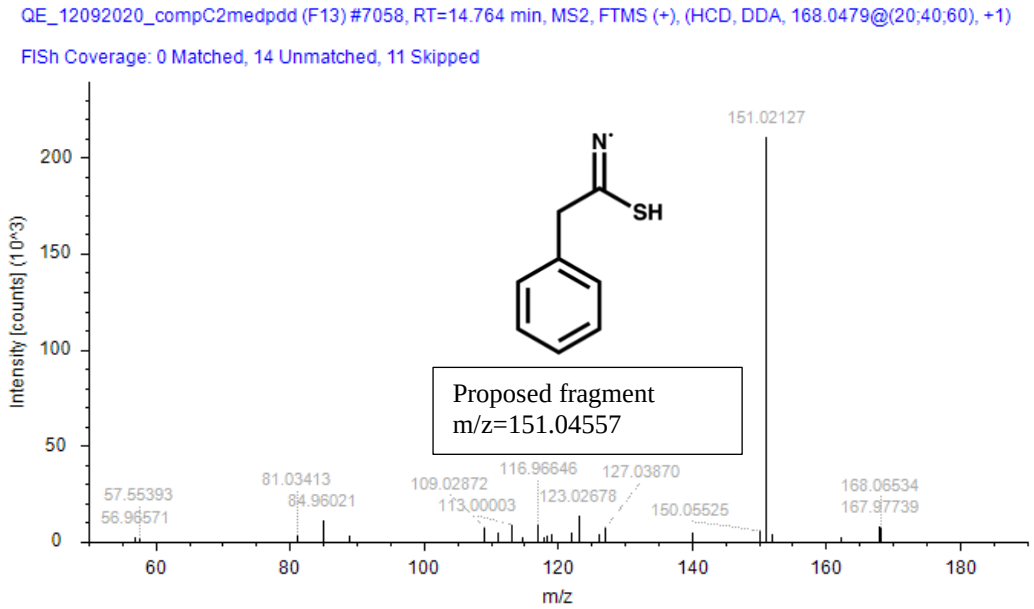


Phenylthioacetohydroximic Acid

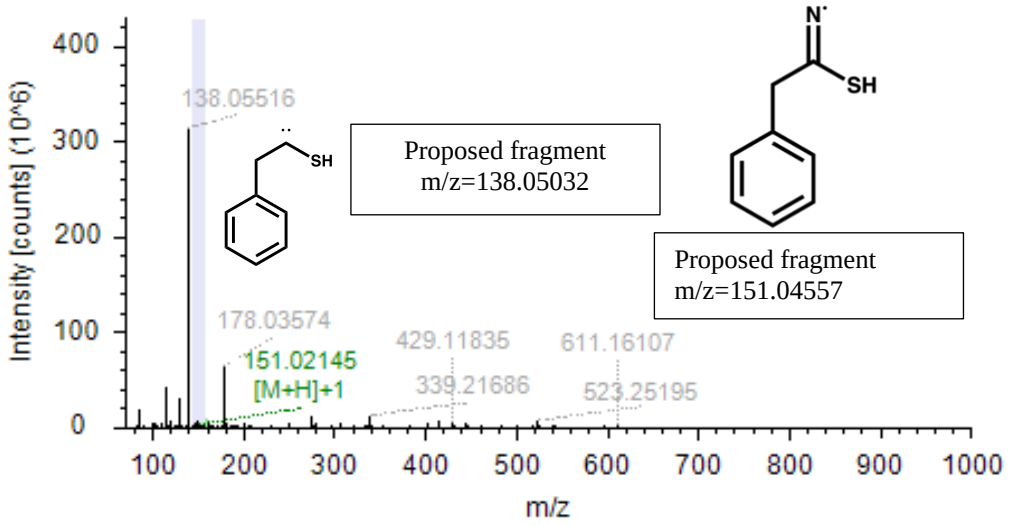
MS:



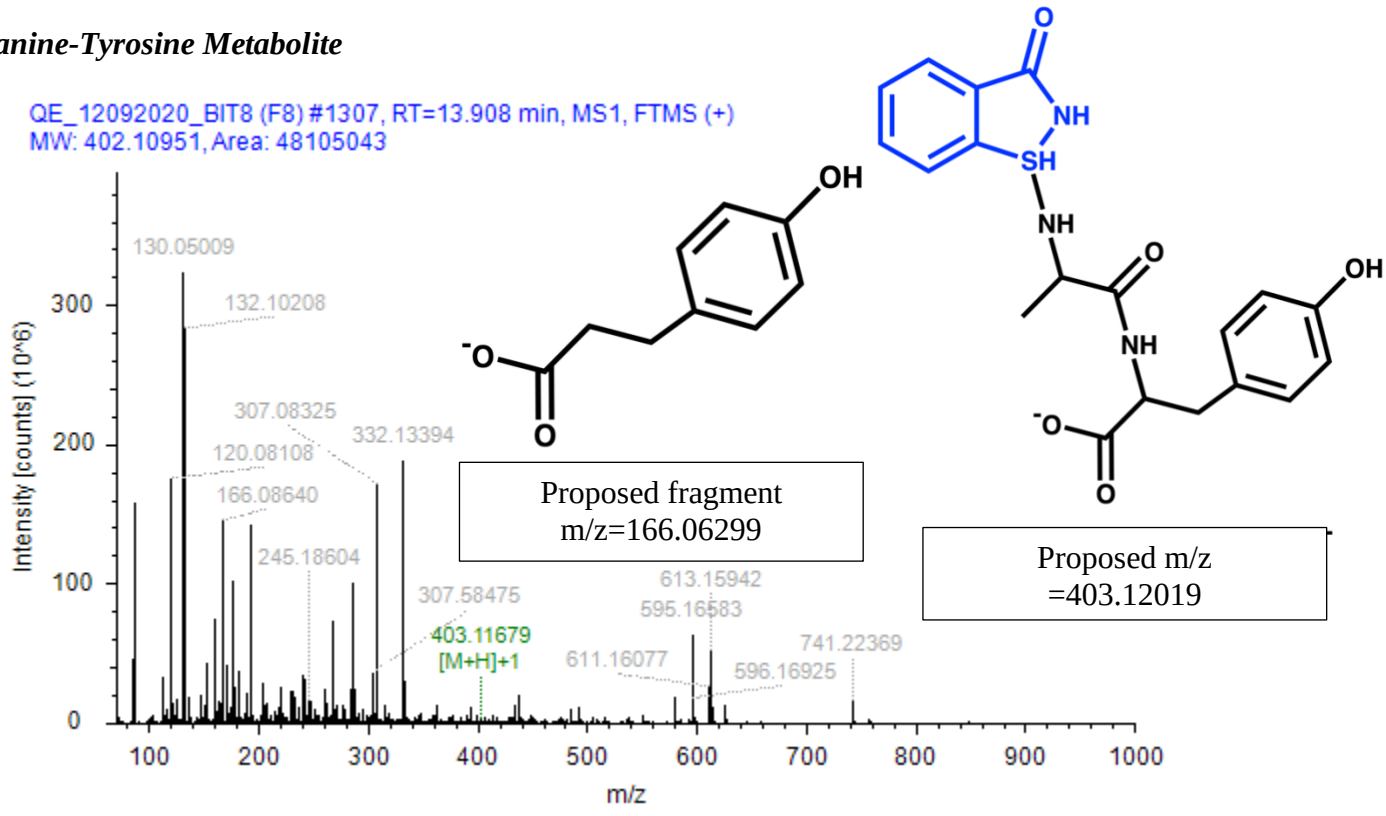
MS2 with proposed phenylthioacetohydroximic acid fragment:



Additional peak proposed phenylthioacetohydroximic acid fragment:



Alanine-Tyrosine Metabolite

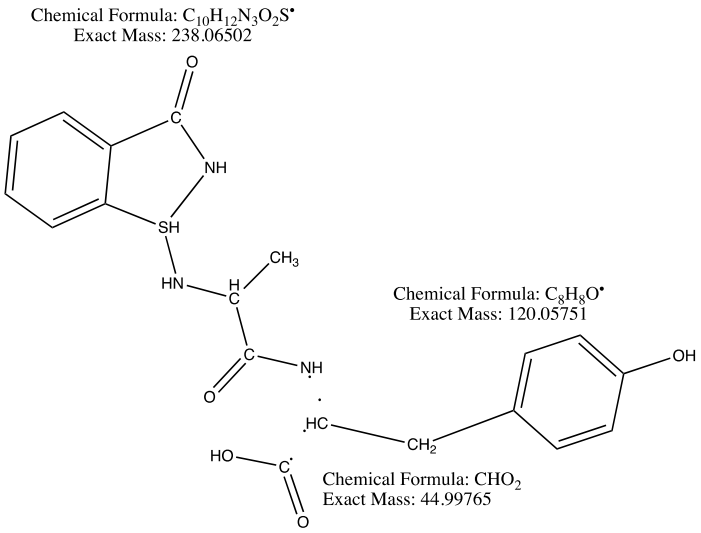


Note: We would expect (based on the accurate mass measured) that the compound is likely deprotonated at ambient condition based on the pKa of tyrosine (pKa= 4.0;) however, tyrosine is known to ionize in either ESI+ or ESI- modes. Citation:

Liigand, P.; Kaupmees, K.; Haav, K.; Liigand, J.; Leito, I.; Girod, M.; Antoine, R.; Kruve, A. Think Negative: Finding the Best Electrospray Ionization/MS Mode for Your Analyte. *Anal. Chem.* **2017**, *89* (11), 5665–5668. https://doi.org/10.1021/acs.analchem.7b00096.

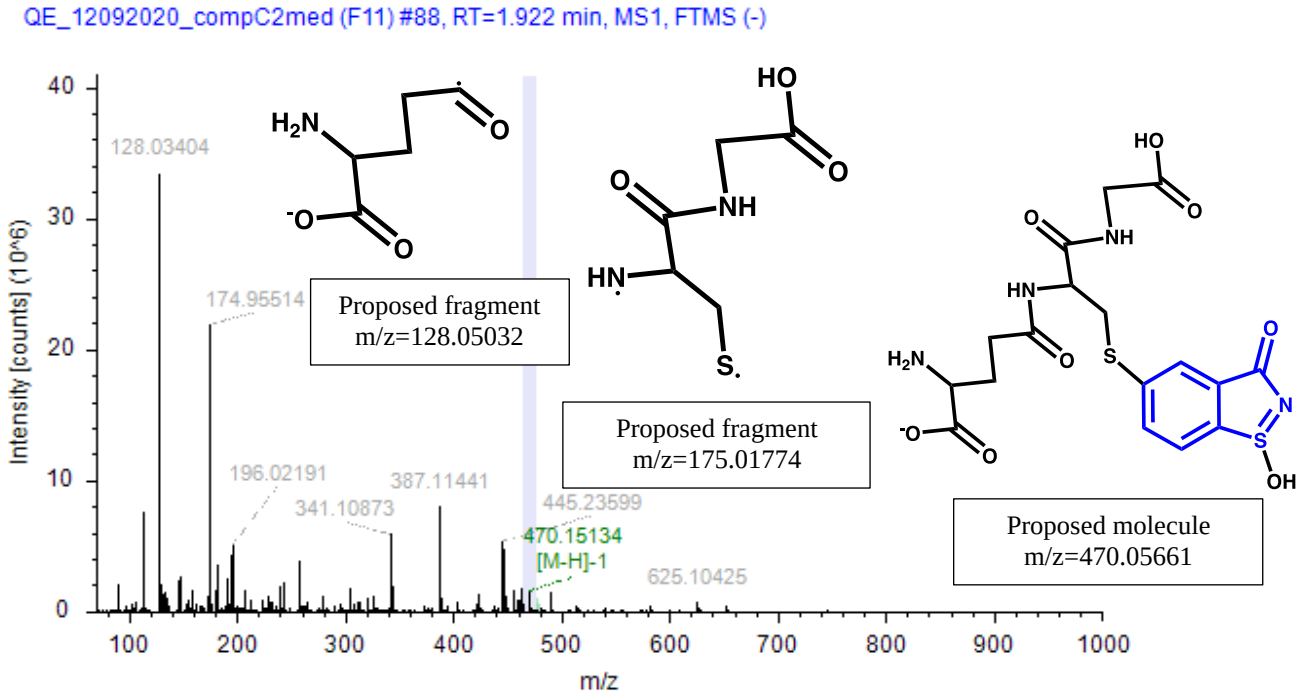
(Note: No MS2 Available for BIT-Alanine-Tyrosine conjugate)

Proposed possible m/z 120.08108 fragmentation:



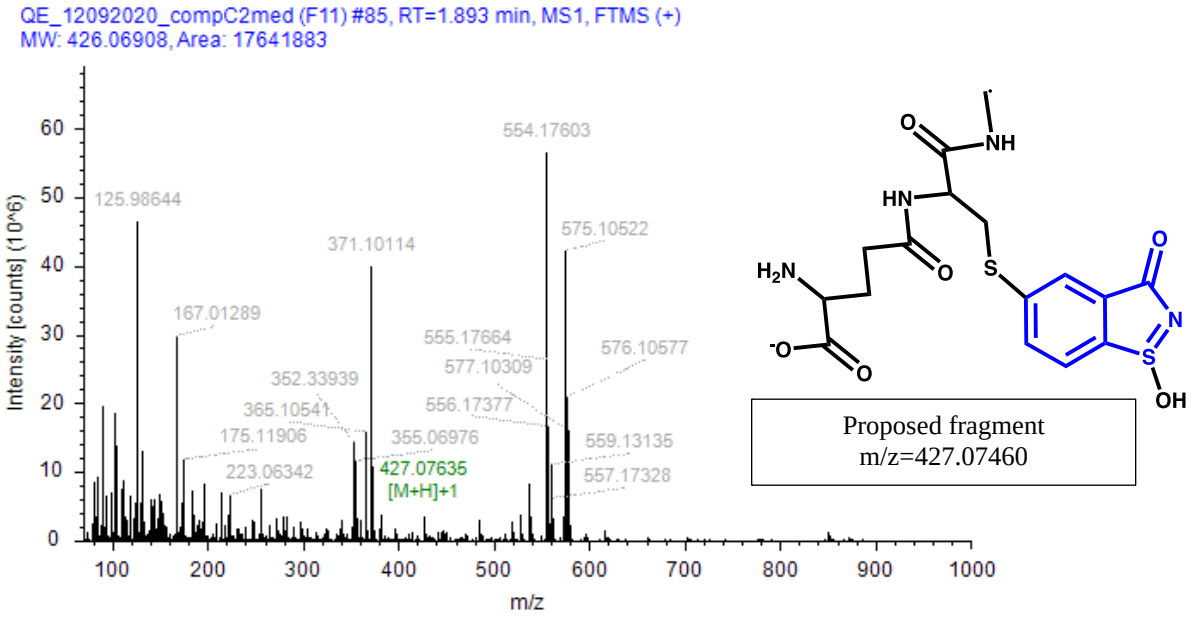
TP470 Metabolite: NOTE, due to the high mass deviations (>100 ppm) of the possible structures below we are presenting the structures for context only, and we formally report the metabolite only as a level 5 accurate mass of interest.

MS:

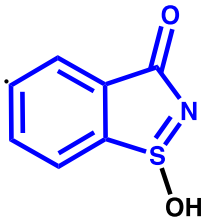


(No MS2 available)

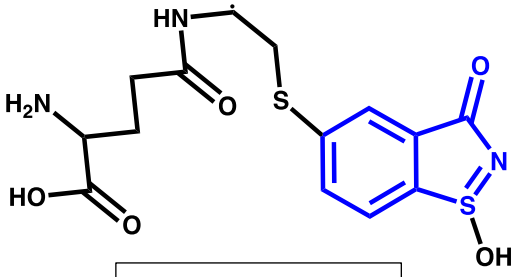
Additional feature $[M+H]^{+1}$: 427.07635



Proposed fragment
m/z=427.07460



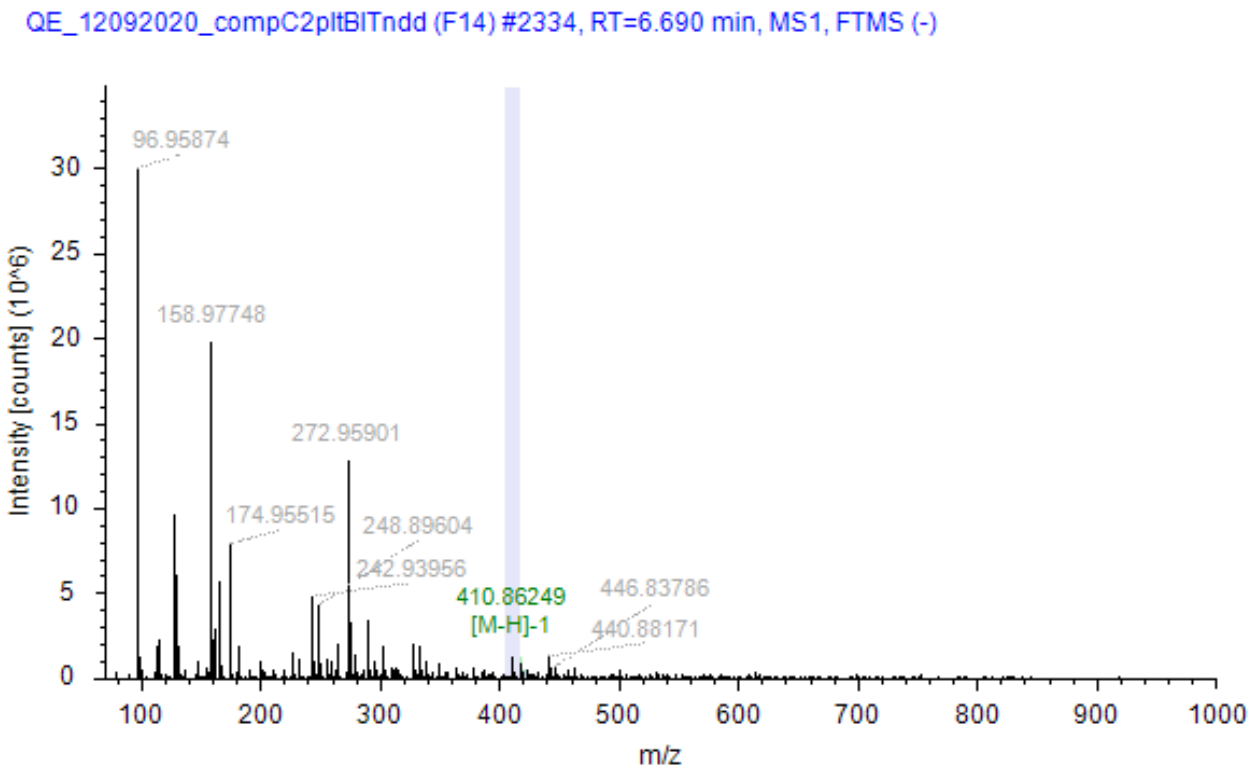
Proposed fragment
m/z=167.00410



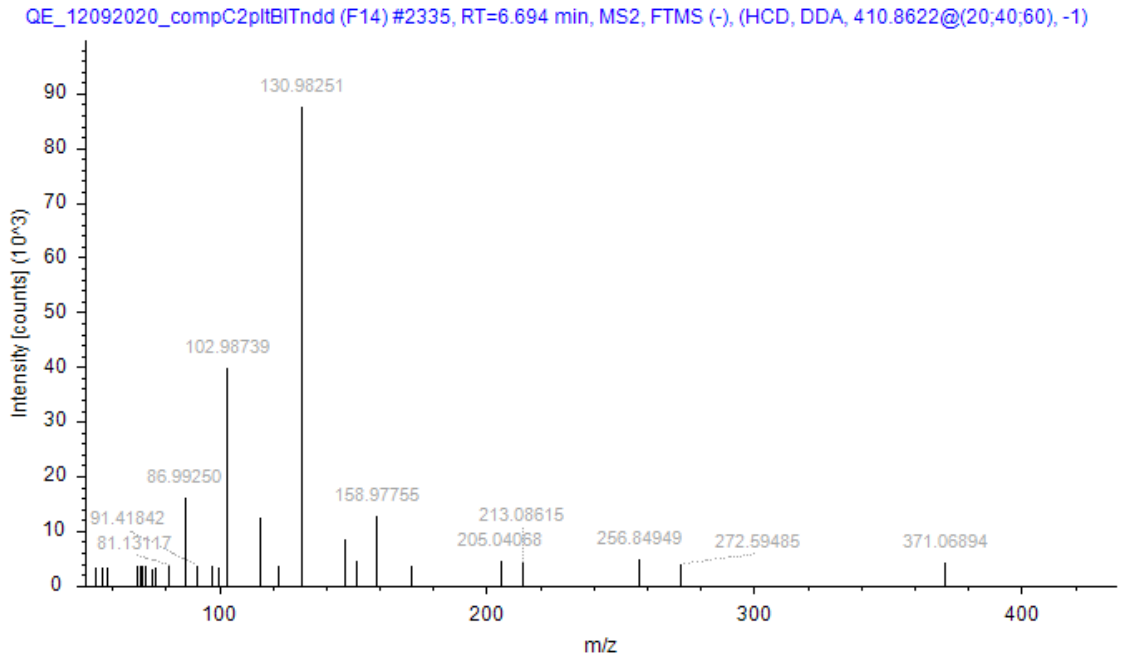
Proposed fragment
m/z=371.06096

TP 410 Accurate Mass of Interest (Unknown 6.76 min Metabolite)

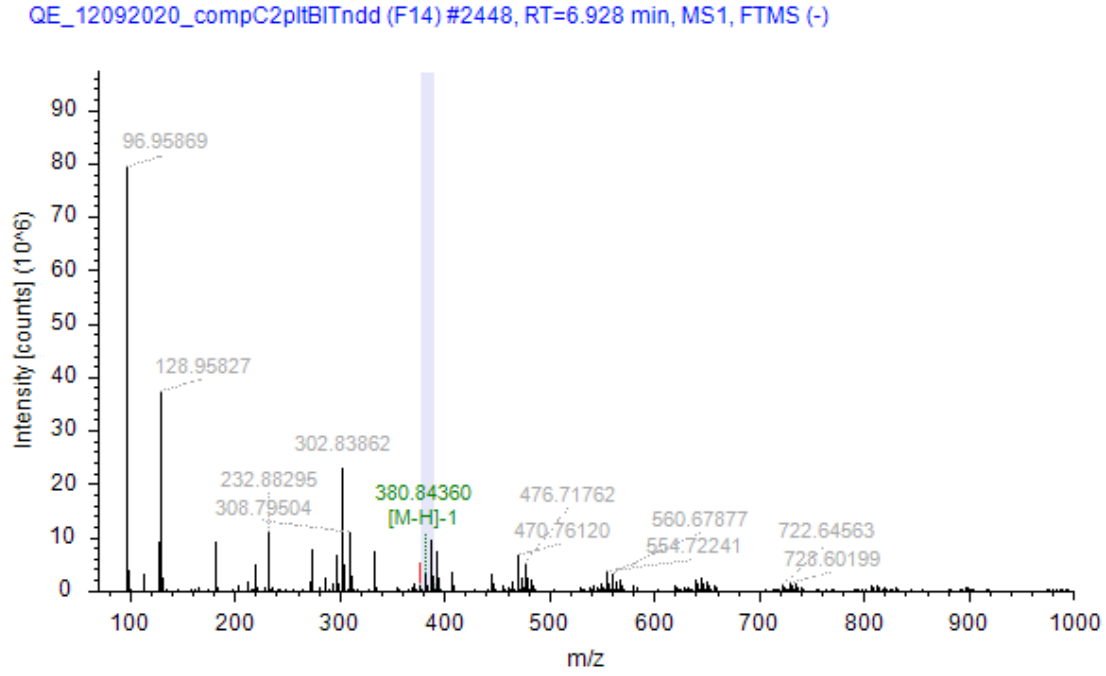
MS:



MS2:



Additional feature at $[M-H]^{-1}$ = 380.84360:



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