

Phytohormone Quantification

Method

Sample extraction

- Approximately 100 mg tissues were weighed and transferred into a 2-ml lysing tube containing ceramic beads (MP Biomedical, Australia)
- 70% methanol supplied with 500 ng/ml of internal standards was added to each sample tube – 800 µl for leaf and 400 µl for spike tissues.
- Samples were homogenized using Precellys 24 tissue homogenizer (Bertin Technologies, France) for 3 cycles of 30 s at 6000 rpm with 90 s pause between the cycles. The extracts were centrifuged at 13000 rpm for 15 min.
- Supernatants were transferred into a LC-MS glass vial and subjected to LC-MS analysis.

LC-MS analysis

- 5 µl of samples was injected into the LC-MS machine under the following condition.
 - LC machine: 1200 series, Agilent Technologies
 - LC column: Zorbax Eclipse EDB-C18, 2.1 × 100 mm, 1.8 micron, Agilent Technologies
 - Column temperature: 45°C
 - Mobile phase A: water + 0.1% formic acid
 - Mobile phase B: acetonitrile + 0.1% formic acid
 - LC gradient program

At	%A	%B
0 min	80%	20%
2 min	80%	20%
3 min	50%	50%
12 min	5%	95%
16 min	5%	95%
17 min	80%	20%
23 min	80%	20%

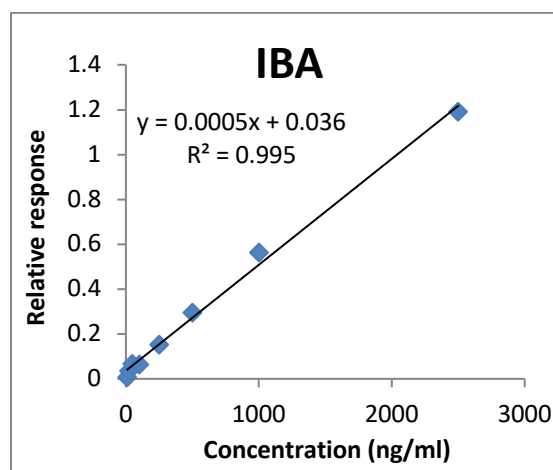
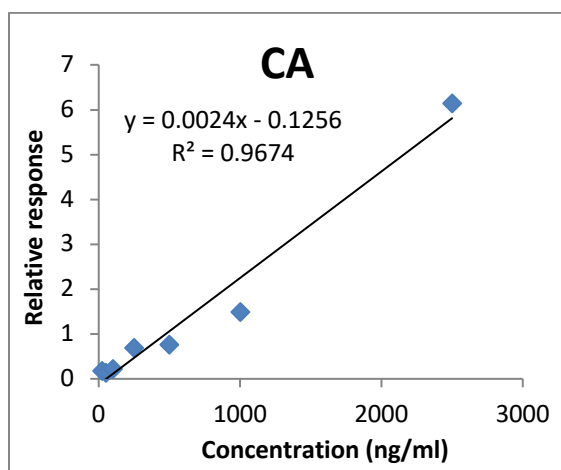
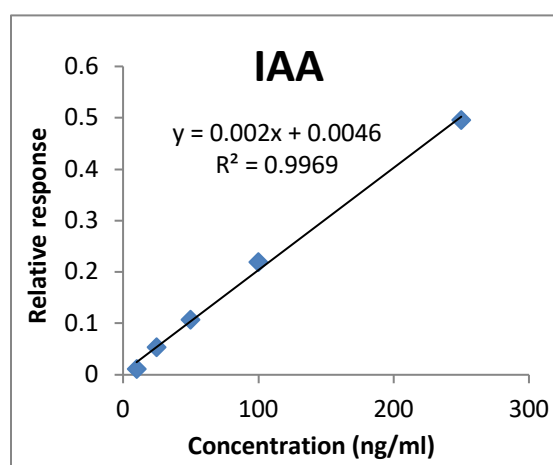
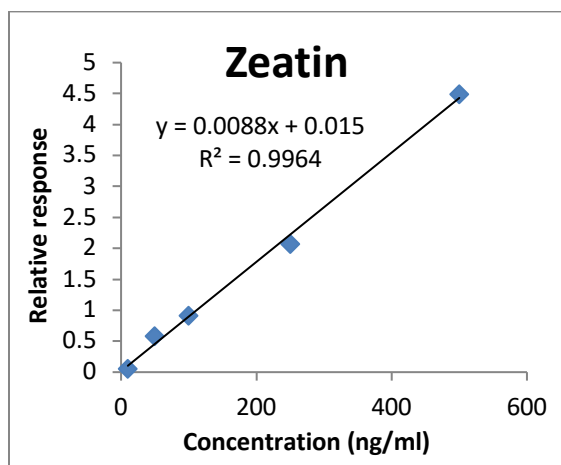
- MS machine: Triple-Quad 6410, Agilent Technologies
- Gas temperature: 250°C
- Gas flow: 13 l/min
- Nebulizer: 55 psi
- Capillary voltage: 5500 V (positive mode) and 4500 V (negative mode)
- MRM transitions

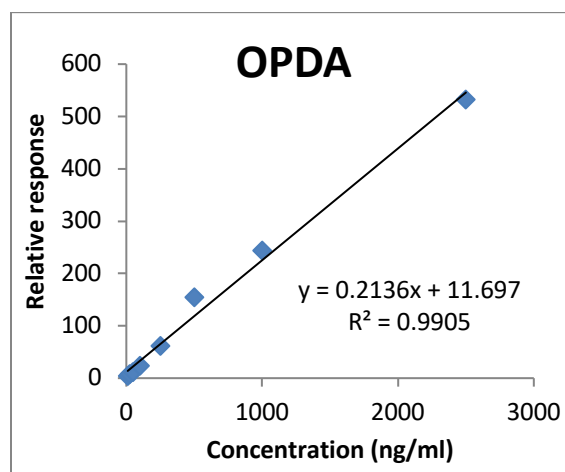
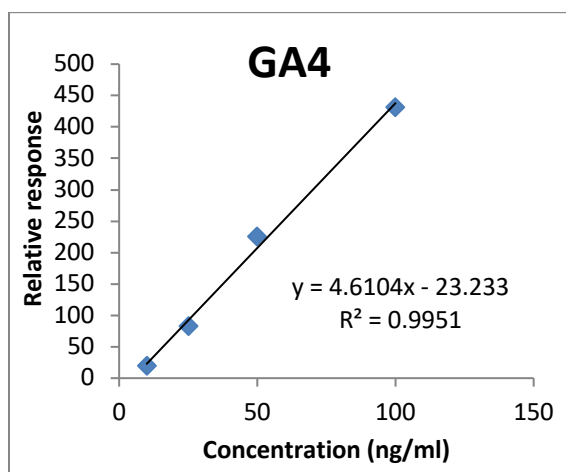
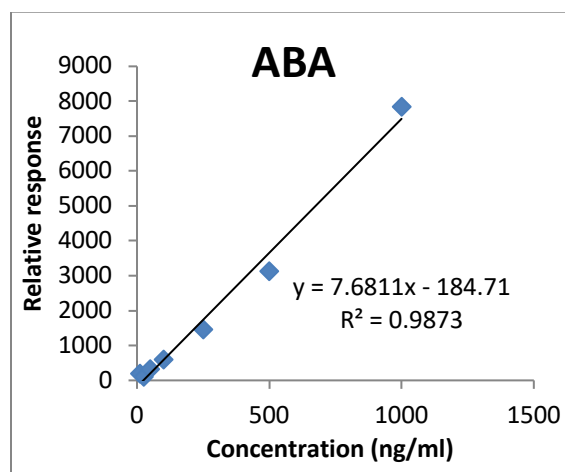
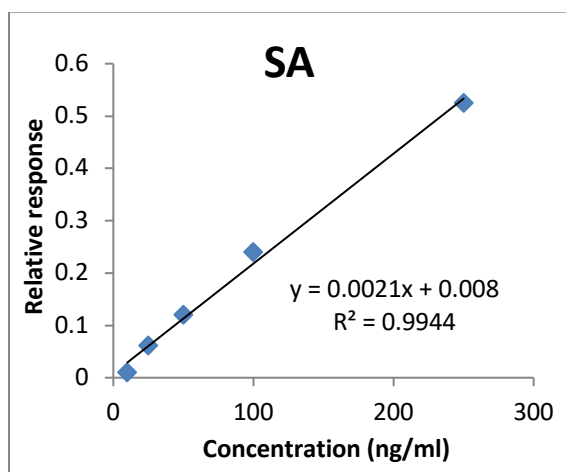
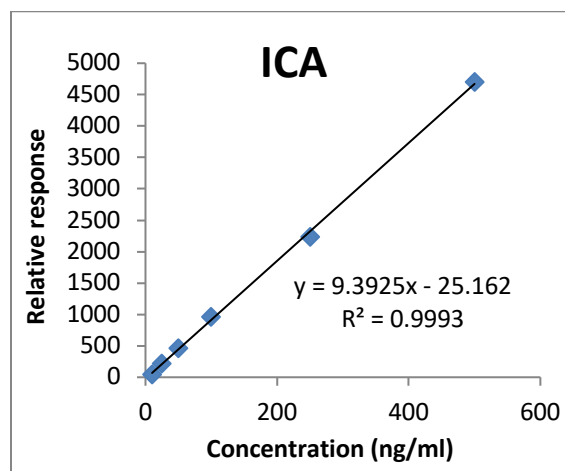
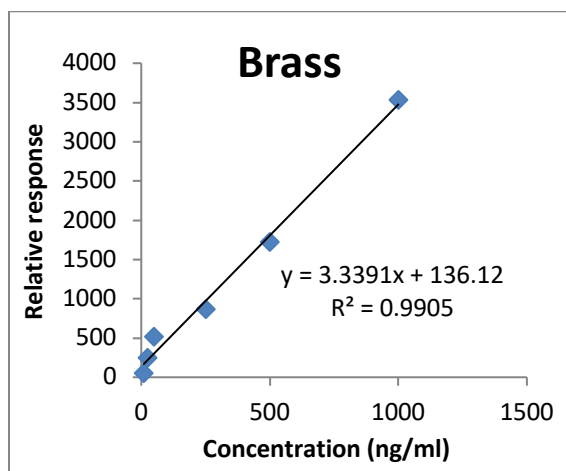
Compound	Precursor ion (m/z)	Production (m/z)	Retention time (min)	Dwell time (ms)	Collision energy (eV)	Polarity
Standards						
Zeatin	220.1	136.1	0.93	200	14	Positive
IAA	176.1	130.1	3.83	200	10	Positive
CA	149.1	103.1	5.19	200	20	Positive
IBA	204.1	186.1	5.48	200	10	Positive
MeIAA	190.1	130.0	5.79	200	16	Positive
Brass	481.5	315.3	6.18	200	10	Positive
MeJA	225.0	151.0	7.14	200	10	Positive
GA3	345.1	143.1	2.03	200	24	Negative
ICA	160.1	116.1	2.86	200	10	Negative
SA	137.0	93.0	3.97	200	16	Negative
ABA	263.1	153.1	4.92	200	8	Negative
JA	209.1	59.0	5.55	200	8	Negative
GA4	331.2	213.0	5.93	200	30	Negative
JA-Ile	322.1	129.9	6.19	200	24	Negative
OPDA	291.0	164.9	8.10	200	20	Negative
Internal standards						
d ₅ -Zeatin	225.2	137.1	0.92	200	20	Positive
d ₂ -IAA	178.1	132.0	3.79	200	12	Positive
d ₇ -CA	156.1	109.0	5.16	200	22	Positive
d ₄ -SA	141.0	97.1	3.88	200	16	Negative
d ₆ -ABA	269.1	159.1	4.92	200	8	Negative
H ₂ JA	211.1	59.0	5.99	200	12	Negative

- Injection was performed in 3 technical replicates for each sample.
- Calibration curves were created from 4-8 dilutions of commercial standard. The curve is varied in a range of 10-5,000 ng/ml based on concentration of each phytohormone in the samples.
- Concentrations were measured by comparing relative peak area against the standard curve.

Result

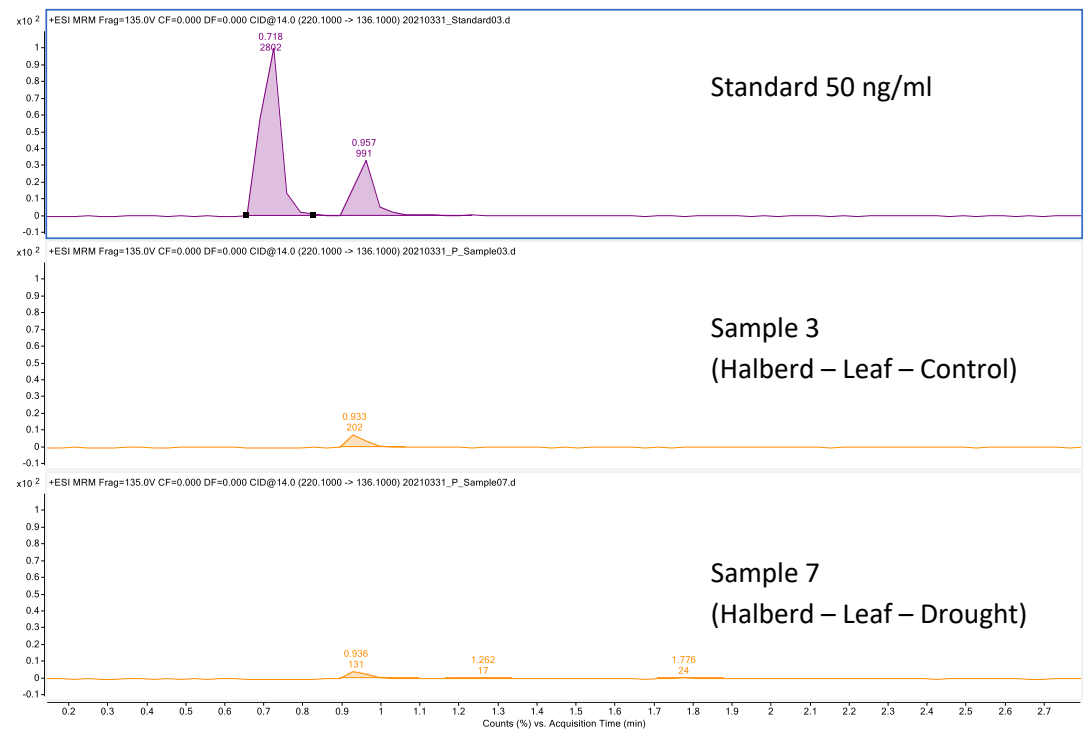
Calibration curves



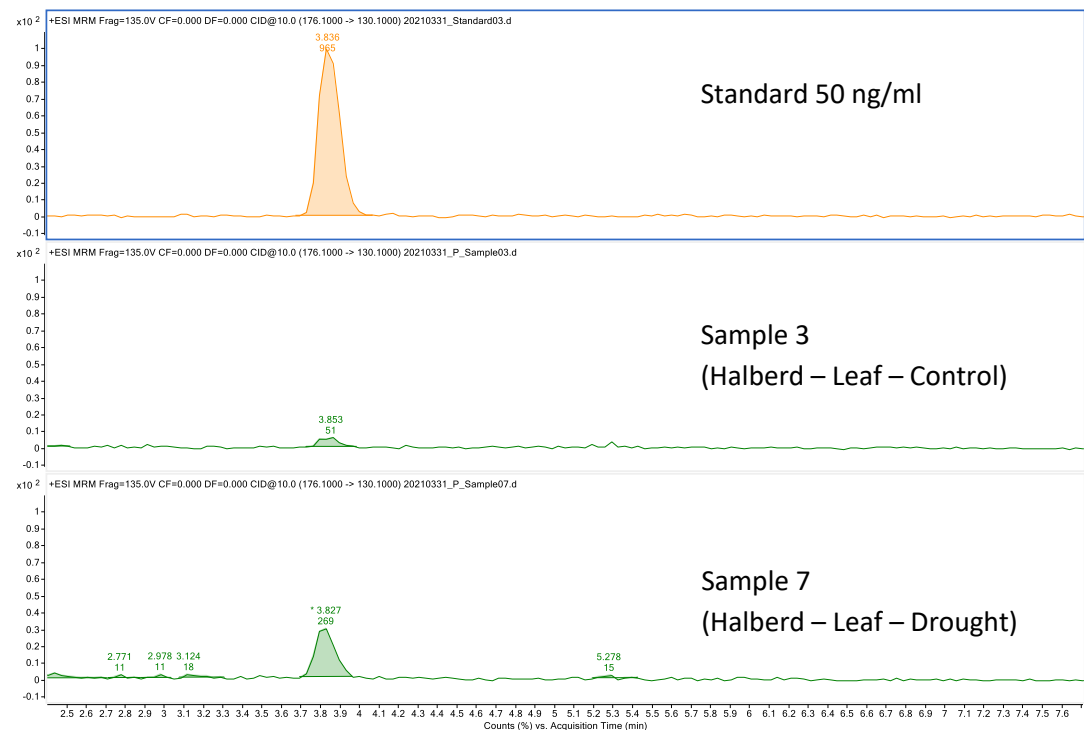


Example chromatograms

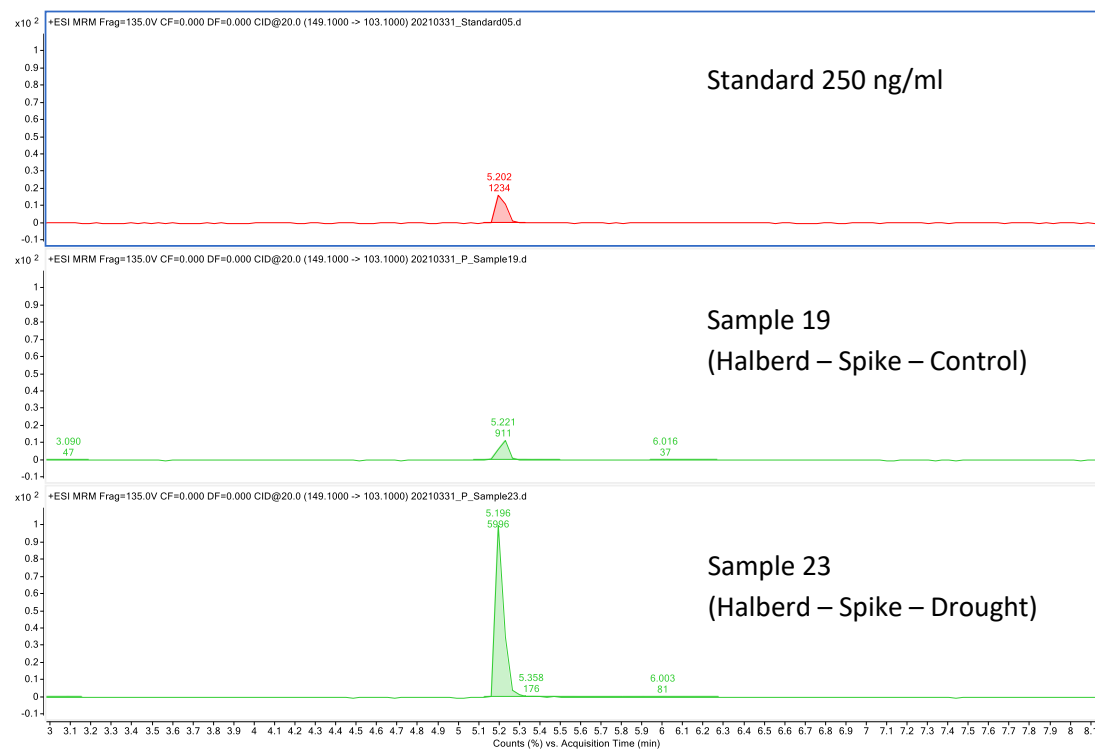
Zeatin



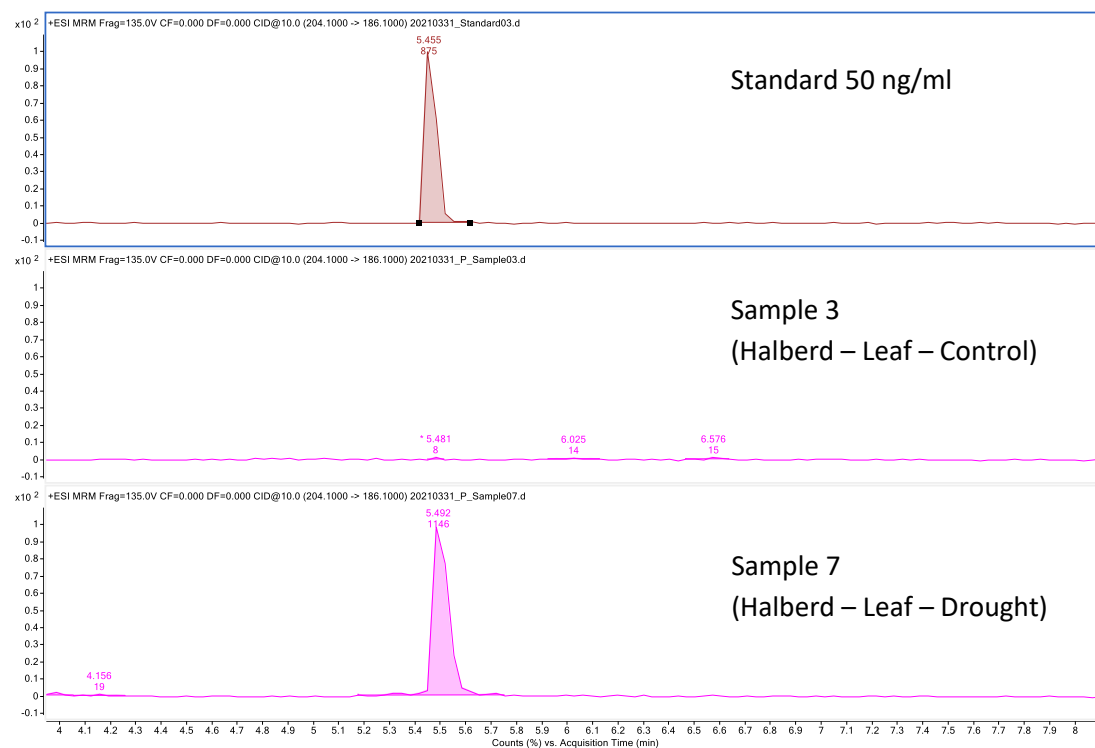
IAA



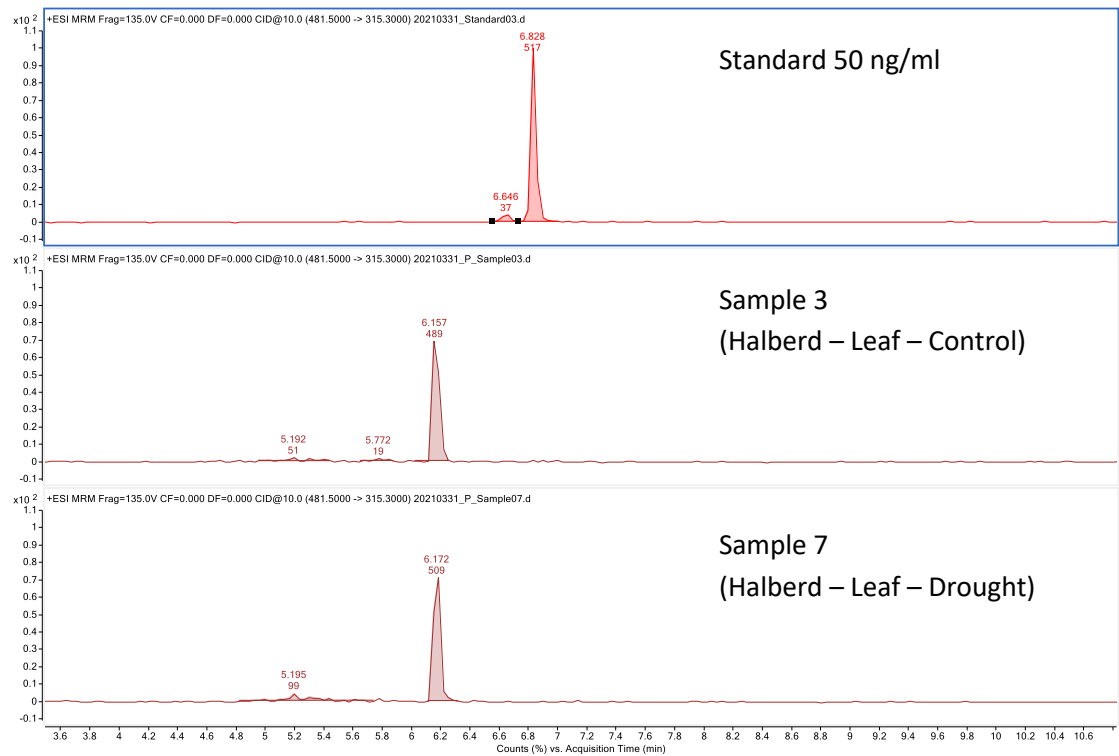
CA



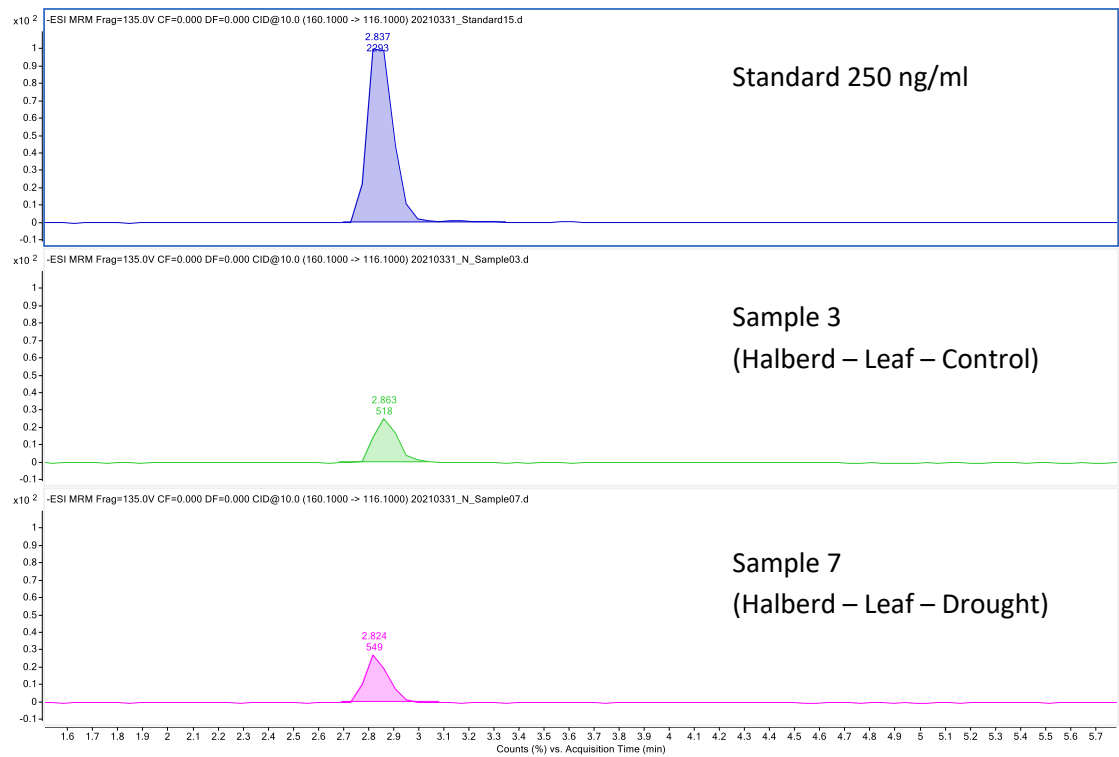
IBA



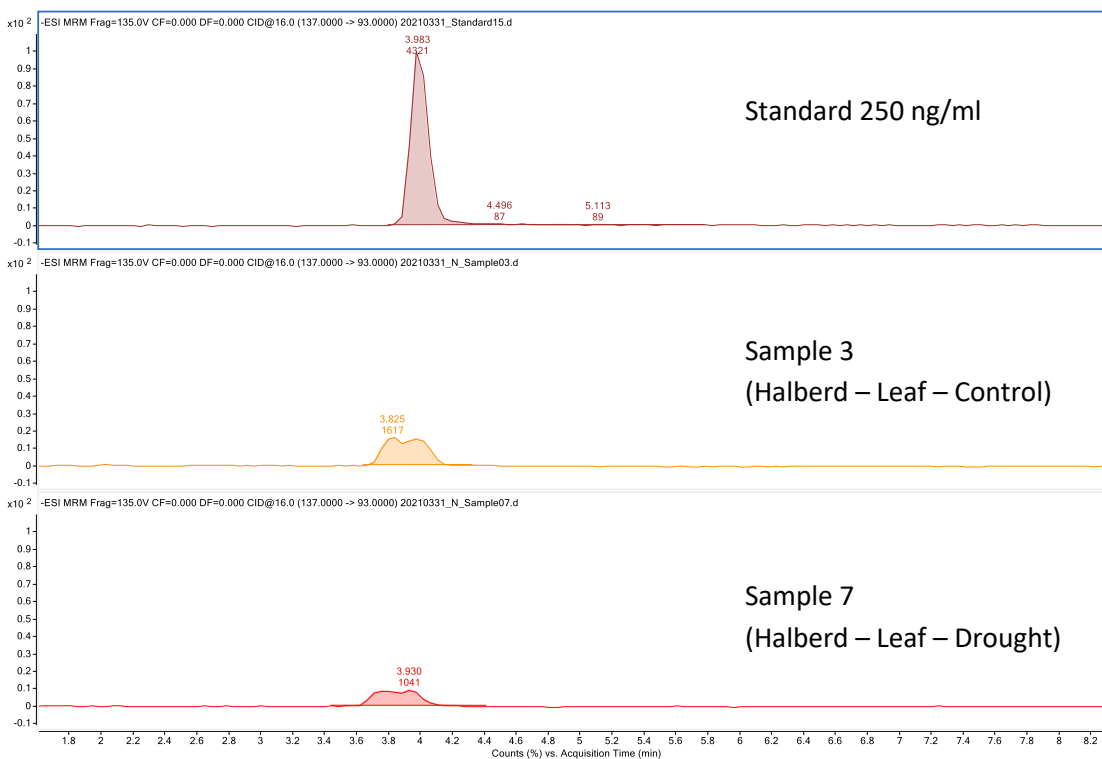
Brass



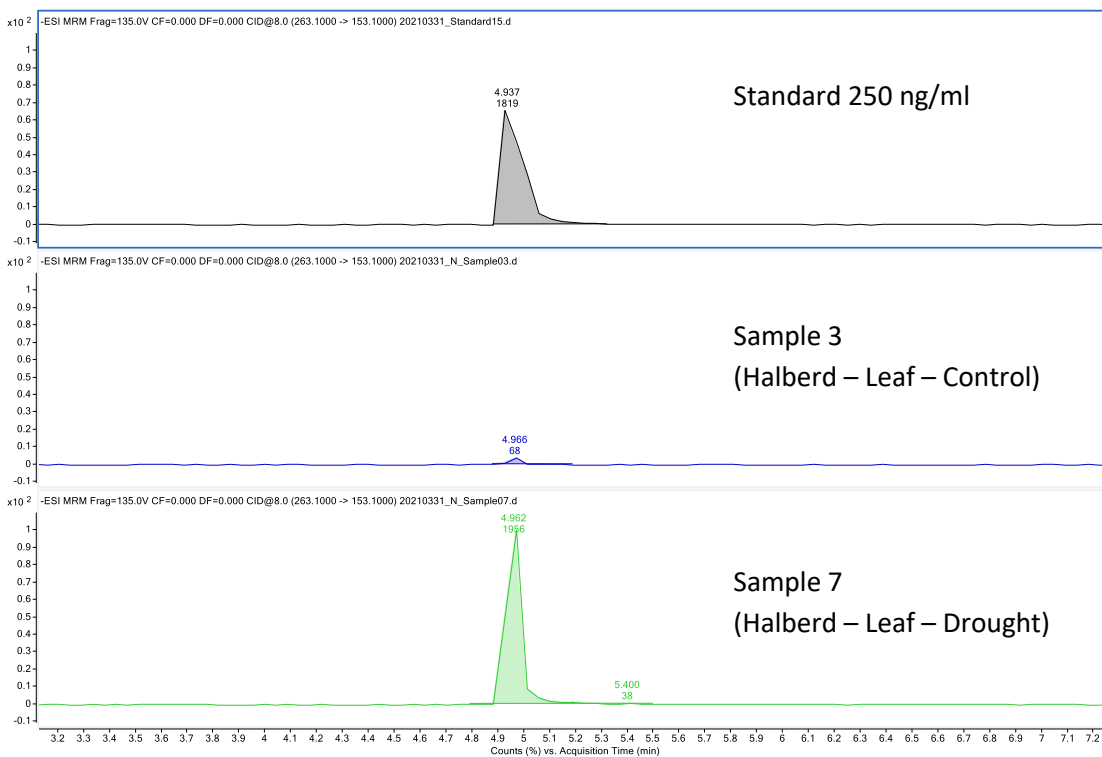
ICA



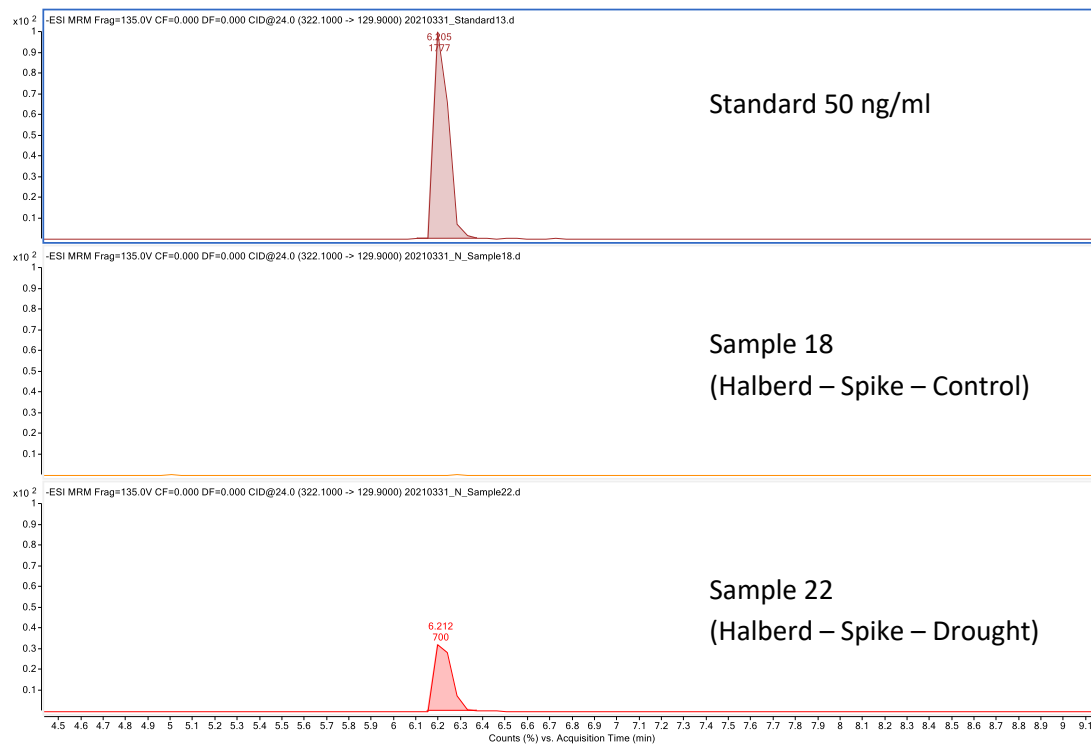
SA



ABA



JA-Ile



OPDA

