

2013 Missouri Life Sciences Week Workshop

Practical application of UPLC-MS/MS for targeted metabolite analysis

Abraham J.K. Koo



What is Metabolomics?

“Metabolomics is the scientific **study of chemical processes involving metabolites.** “
-wikipedia.org

“Metabolomics is a newly emerging field of "omics" research concerned with the **comprehensive characterization of the small molecule metabolites** in biological systems.”

-Metabolomics Society

How many metabolites are there?

KEGG (Kyoto Encyclopedia of Genes and Genomes): ~15,000 compounds (from animals, plants and bacteria)

E. coli and yeast: **600 ~ 800** (Forster et al., 2003; Keseler et al., 2005)

Human: **2,900** (Human Metabolome Project)

Plant kingdom: **200,000 ~ 1,000,000** (Dixon and Strack, 2003)

Arabidopsis thaliana: ~ **5,000**

**Structural Diversity
(chemical properties)**

**Wide Concentration
Range (10^6)**

PlantMetabolomics
detect approximate



Water

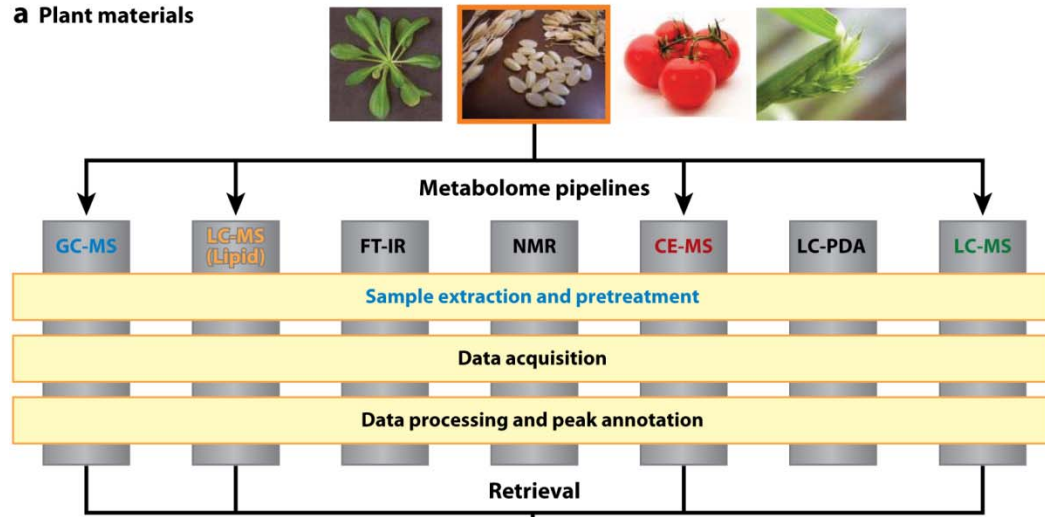
mics.org) :
emically defined.

Human Metabolom

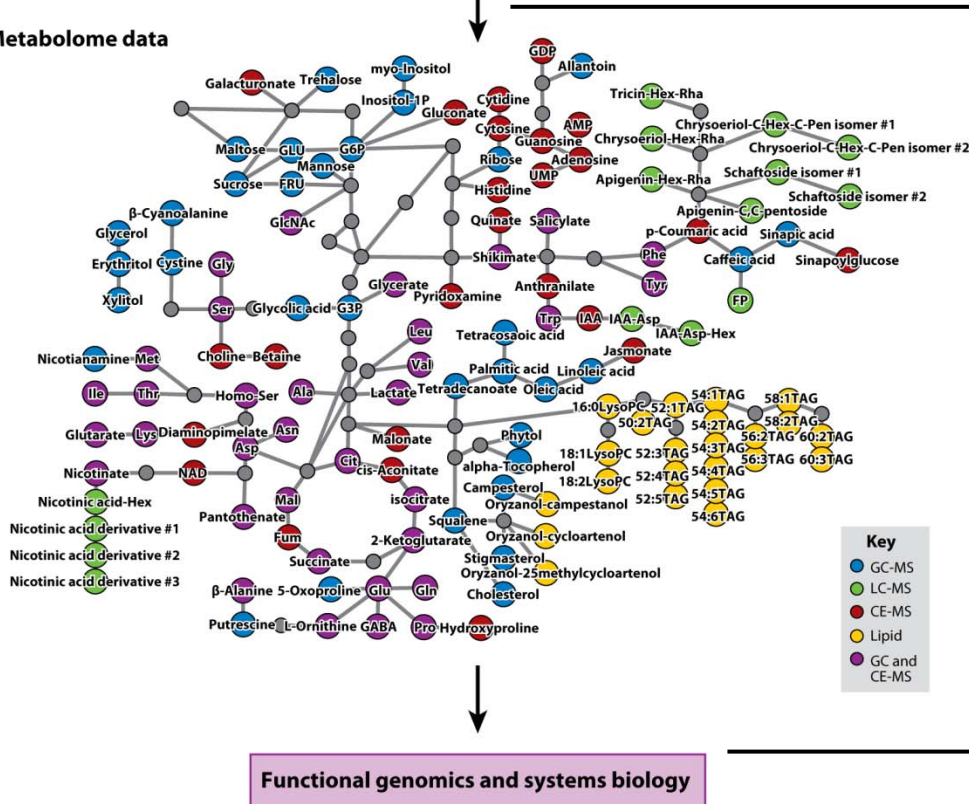
MS/MS data for **800** compounds, experimental ^1H and ^{13}C NMR data for **790** compounds and GC/MS spectral and retention index data for **260** compounds.

[ca/](#)): experimental

a Plant materials



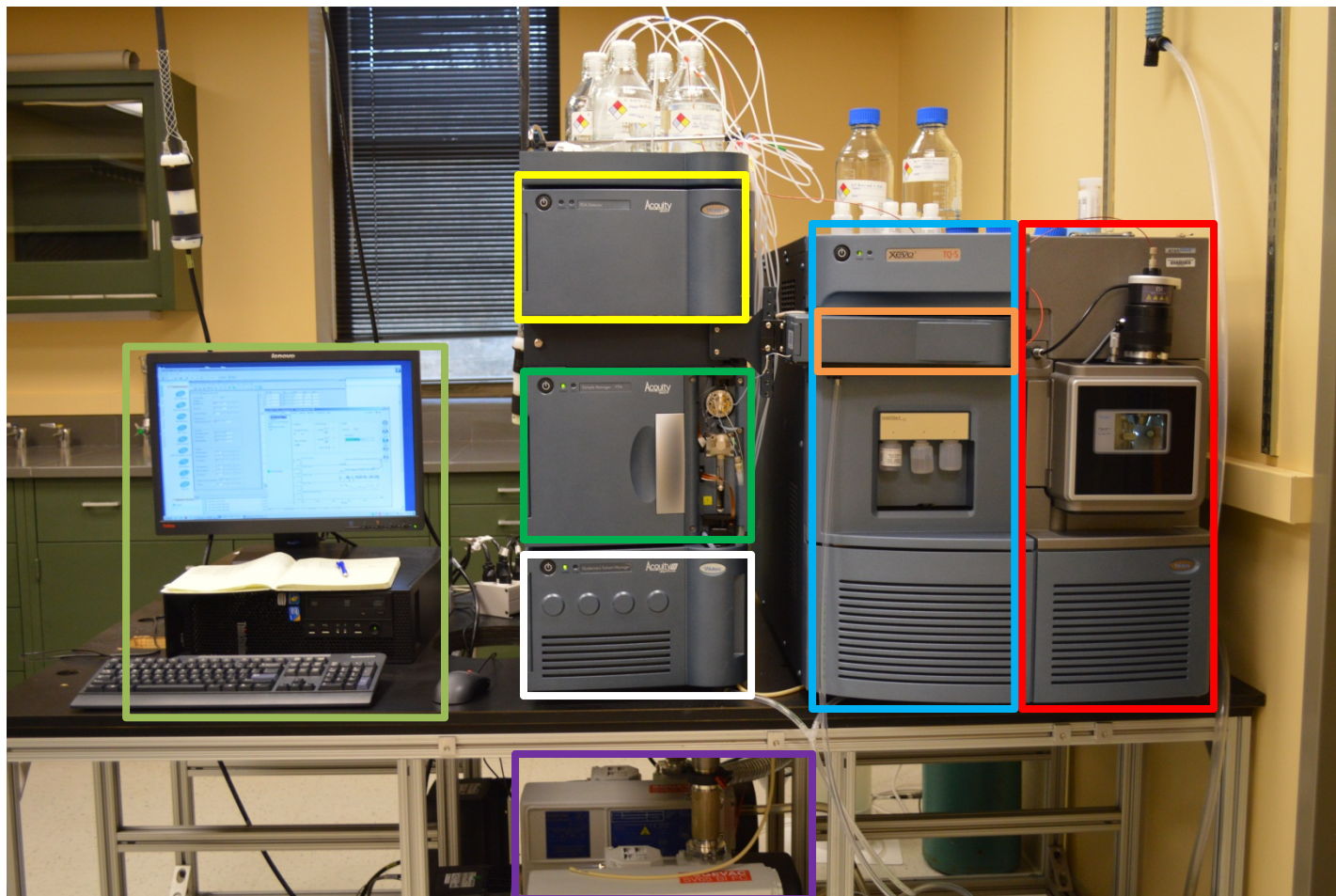
b Metabolome data



Computation /
Bioinformatics

Functional genomics and systems biology

UPLC-MS/MS system at MU (19 Schweitzer Hall)



HPLC unit (ACQUITY UPLC)

Chromatographic Separation of Complex Sample Mixture

Mass Spectrometer (Xevo TQ-S)

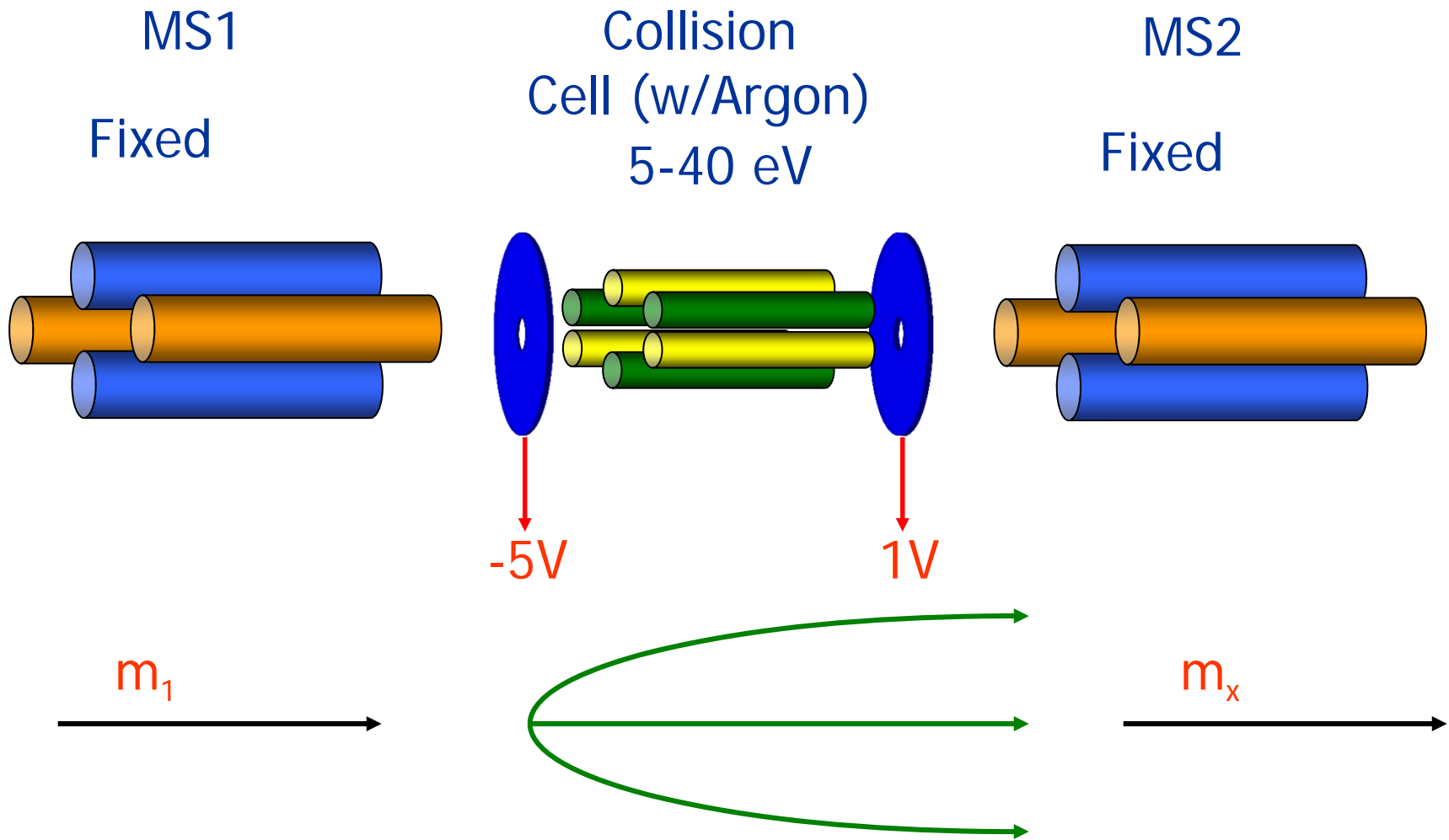
Ionization and detection of ions' **mass-to-charge ratio (m/z)**

Triple quadrupole: MS/MS Tandem Mass Spectroscopy (MRM)

PDA (ACQUITY UPLC PDA detector)

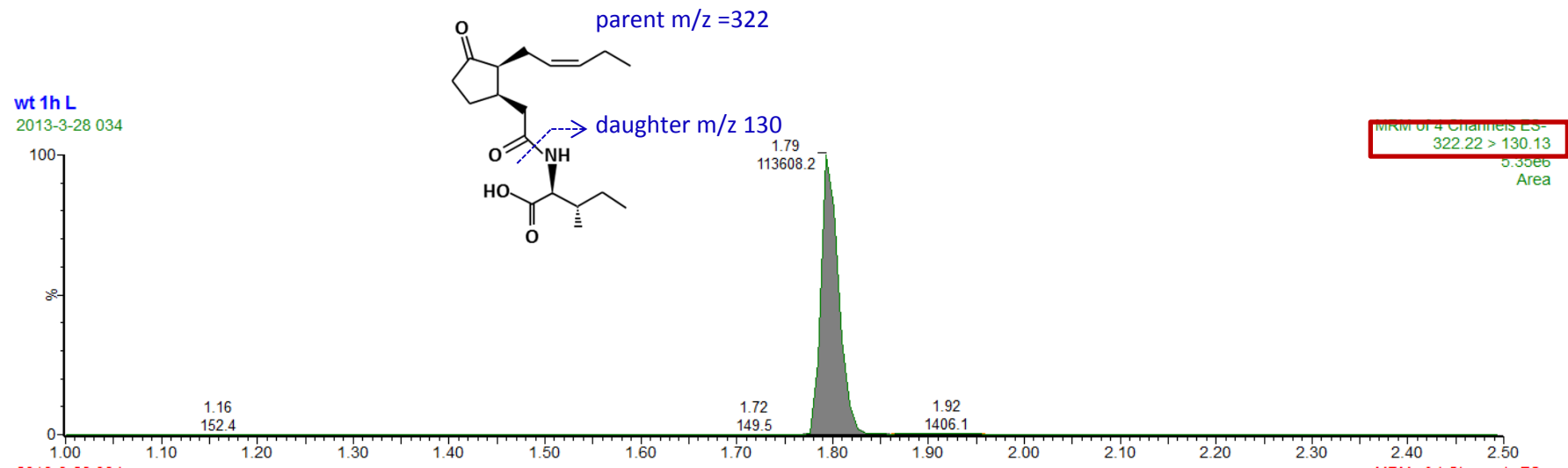
Spectrophotometric detection of **absorbance** at wavelength range 190 nm – 500 nm with 1.2 nm resolution

MRM (Multiple Reaction Monitoring)



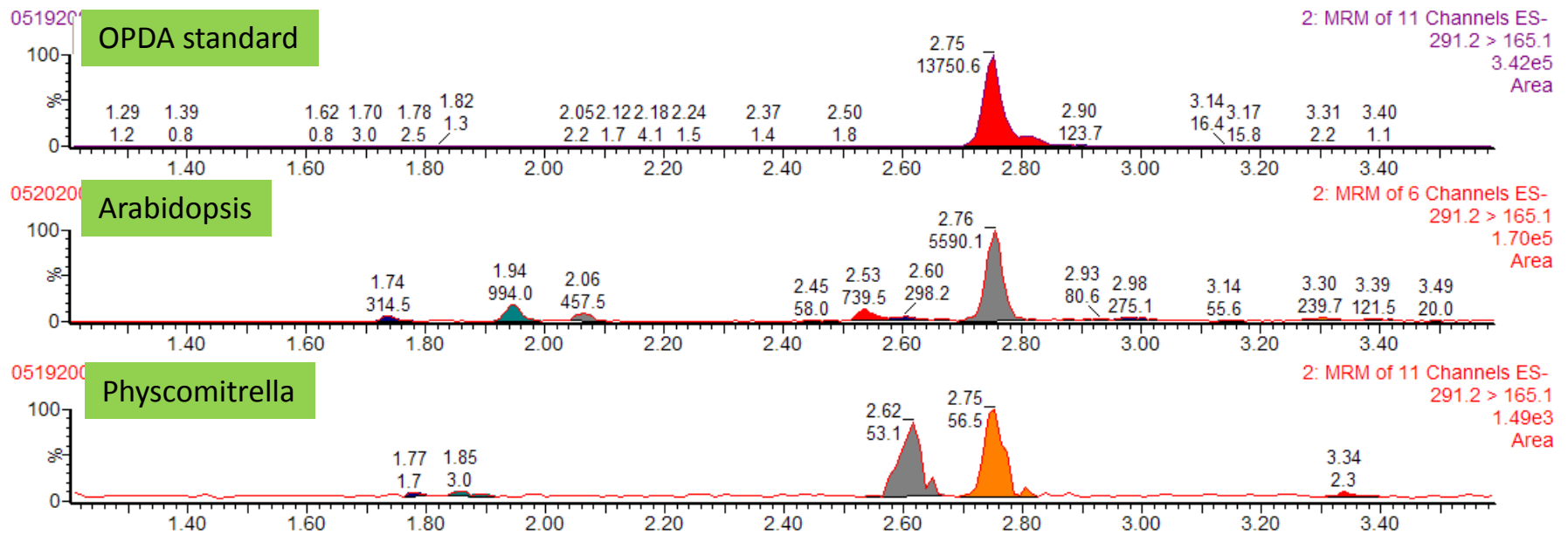
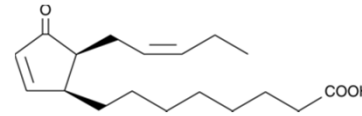
MRM's are used to monitor selected analyte(s) via their daughter ions

Selective detection of a target compound in complex sample matrix

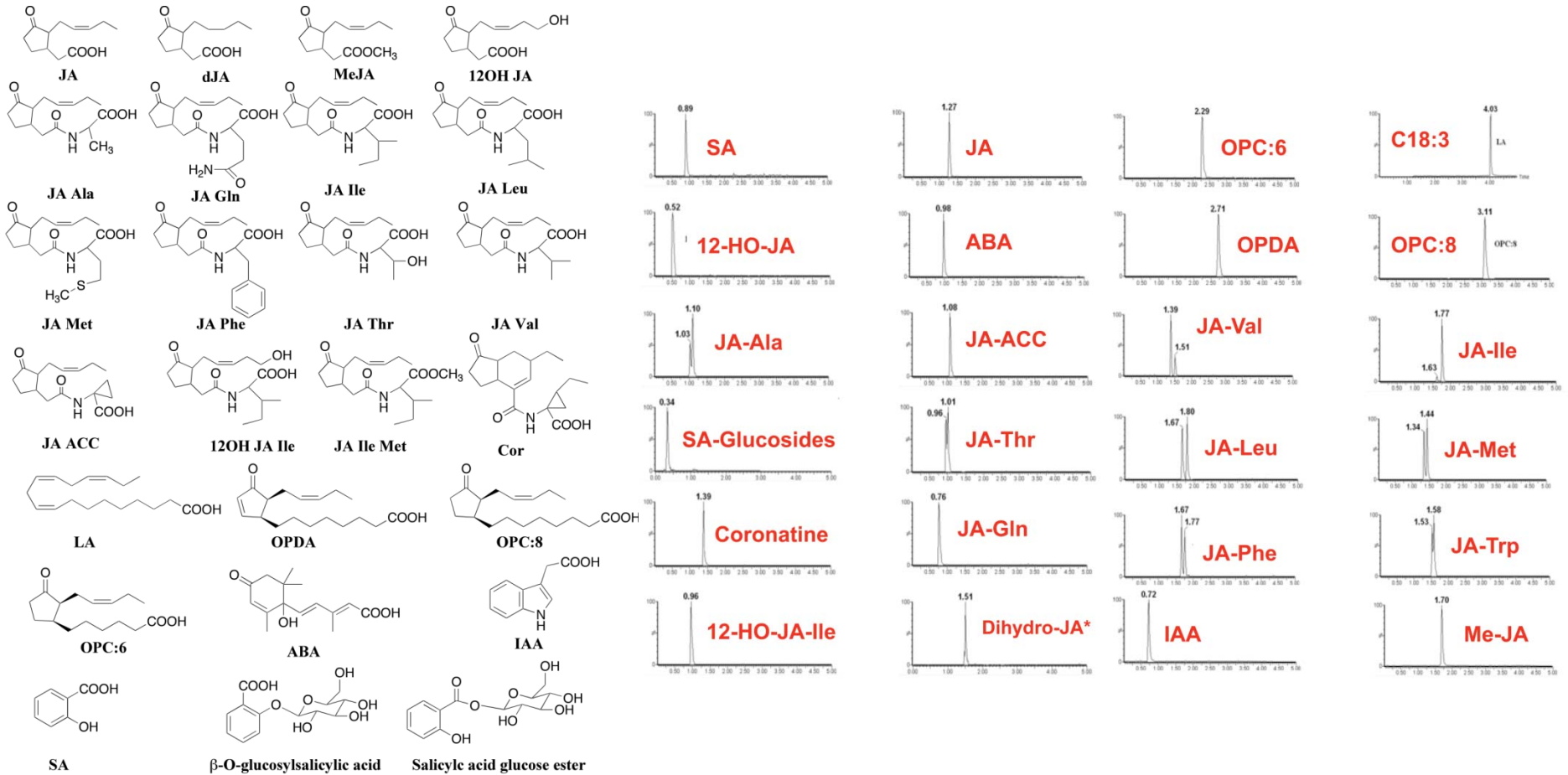


Methanol extract from Arabidopsis leaf

Chromatography improves specificity



MRM allows Simultaneous Detection of Multiple Ions



Metabolite analysis using MRM requires pre-knowledge about the compound (molecular mass) or to have standards

Public metabolomics database (KEGG >15,000 compounds)

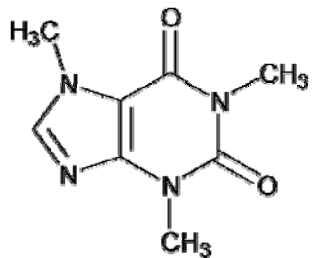
> 212,000 compounds available from Sigma-Aldrich

What kind of molecules can be analyzed?

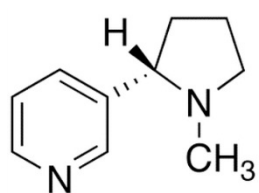
In the fields of pharmacology and biochemistry, a **small molecule is a low molecular weight (<800 Daltons) organic compound** that may serve as an enzyme substrate or regulator of biological processes. The upper molecular weight limit for a small molecule is approximately 800 Daltons which allows for the possibility to rapidly diffuse across cell membranes so that they can reach intracellular sites of action.

Mass range: 2 to 2048 m/z

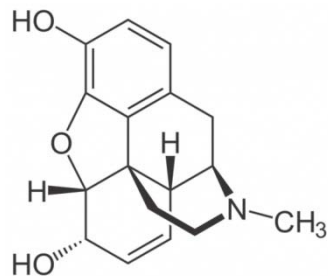
Caffeine



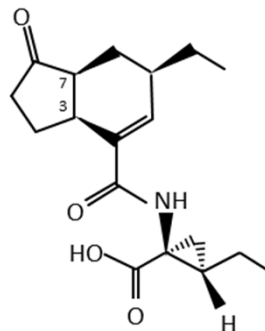
Nicotine



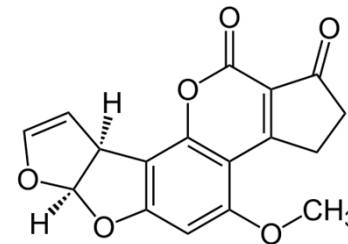
Morphine



Coronatine

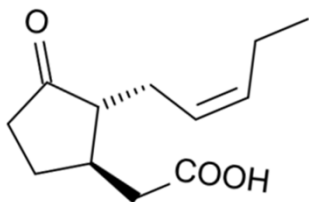


Aflatoxin B1

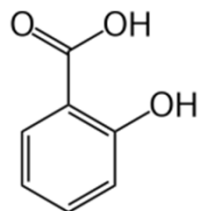


m/z = 137

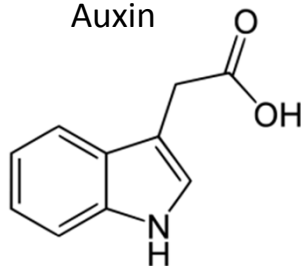
Jasmonic Acid



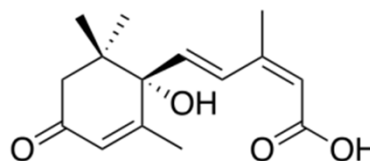
Salicylic Acid



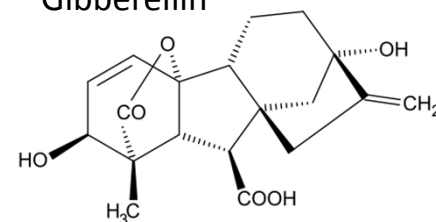
Auxin



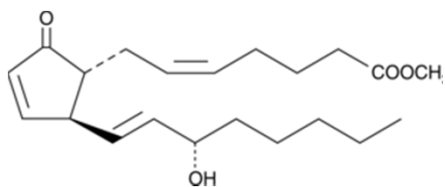
Abscisic Acid



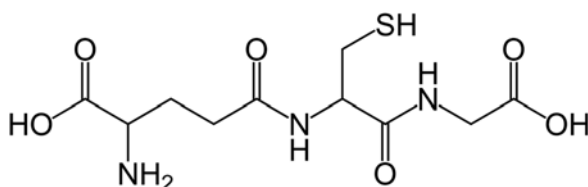
Gibberellin



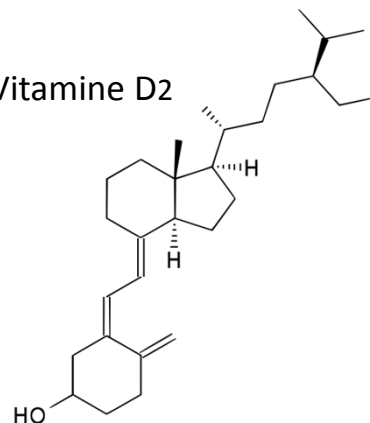
Prostaglandin A2



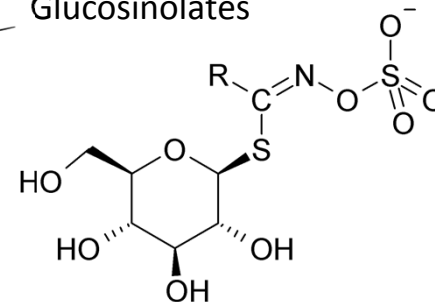
Glutathione (GSH)



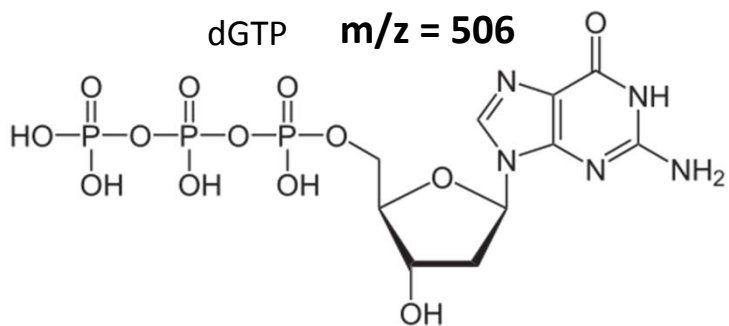
Vitamine D2



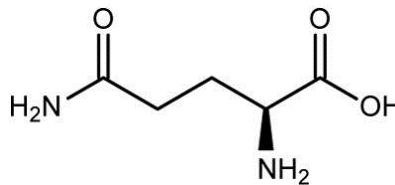
Glucosinolates



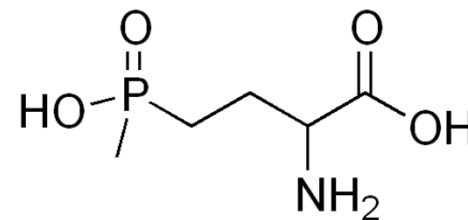
dGTP **m/z = 506**



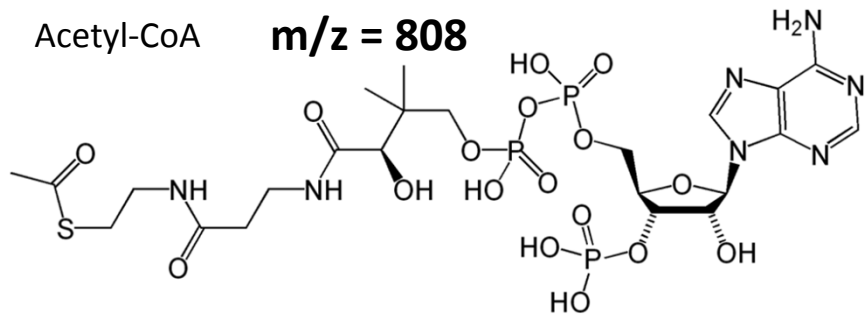
Glutamine



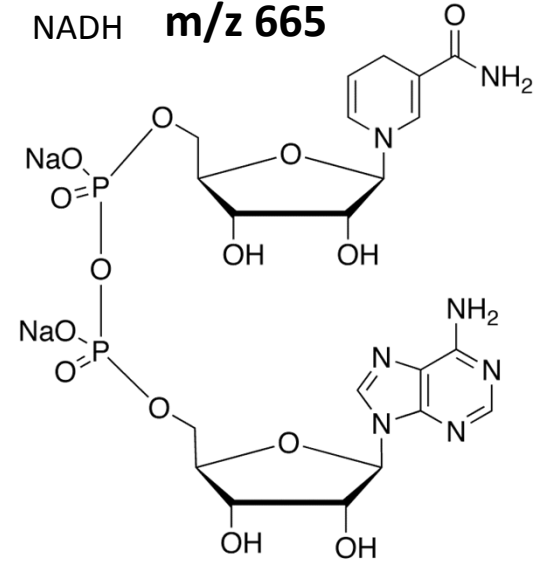
Glycophosphate



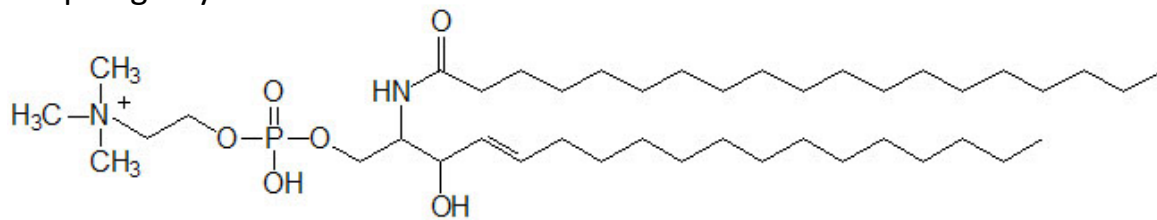
Acetyl-CoA **m/z = 808**



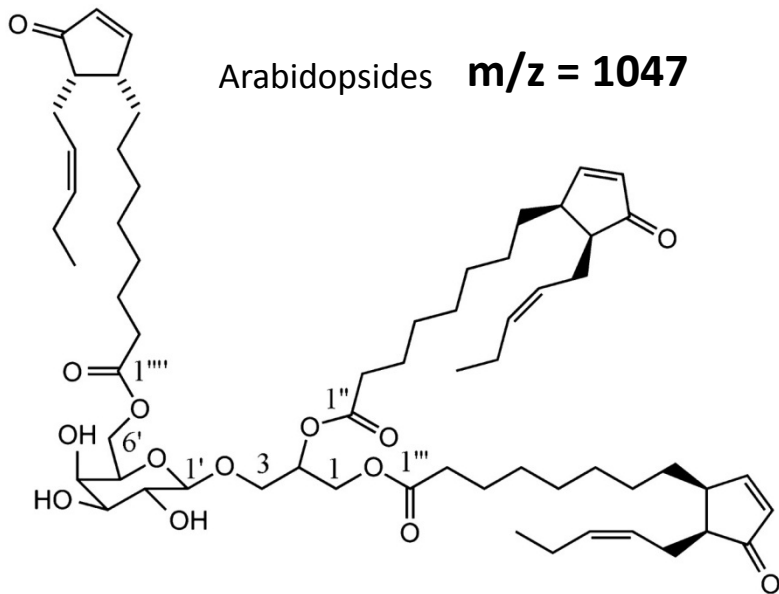
NADH **m/z 665**



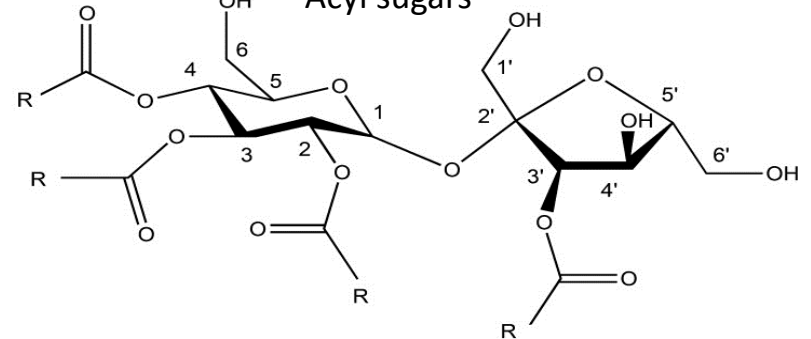
Sphingomyelin



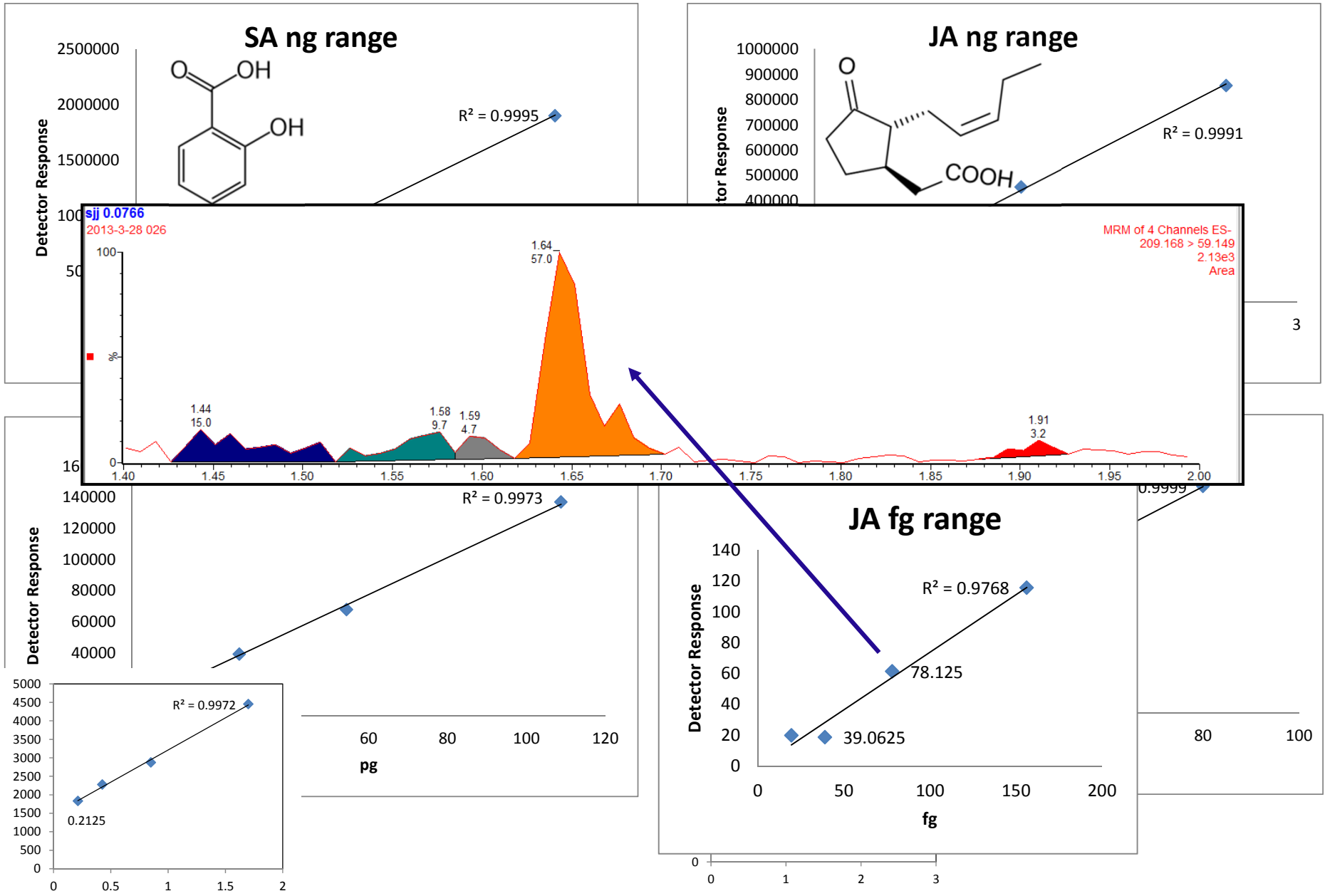
Arabidopsides **m/z = 1047**



Acyl sugars



How Sensitive is it? (detection limit)

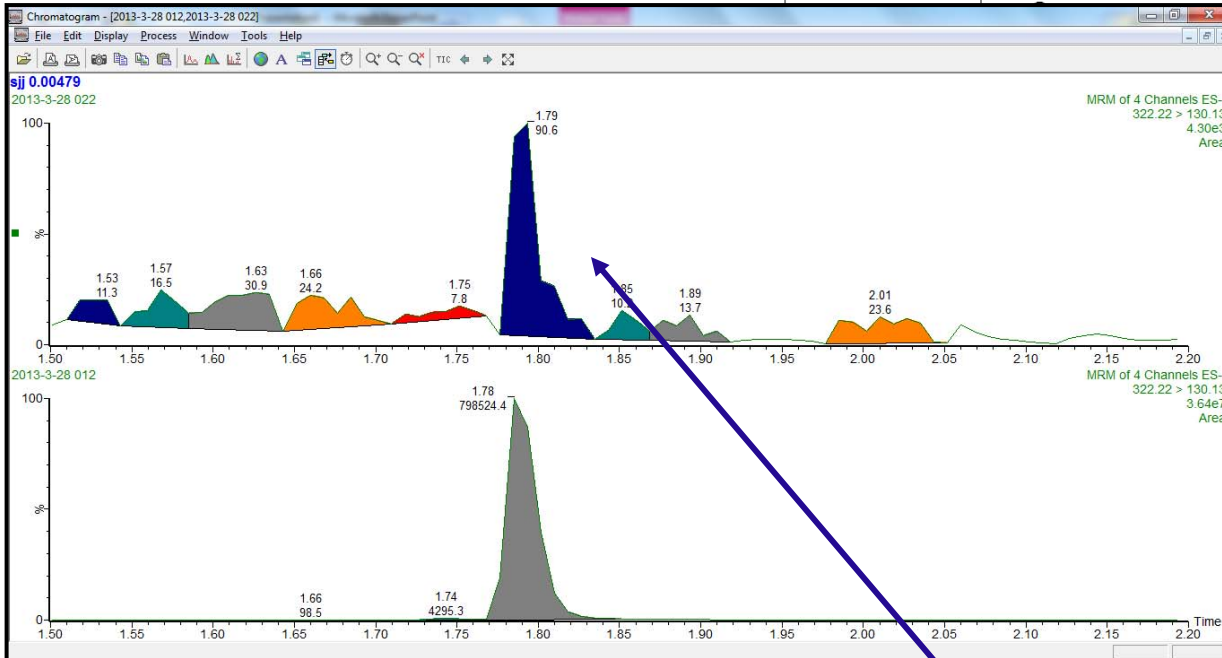


3

80 100

900000

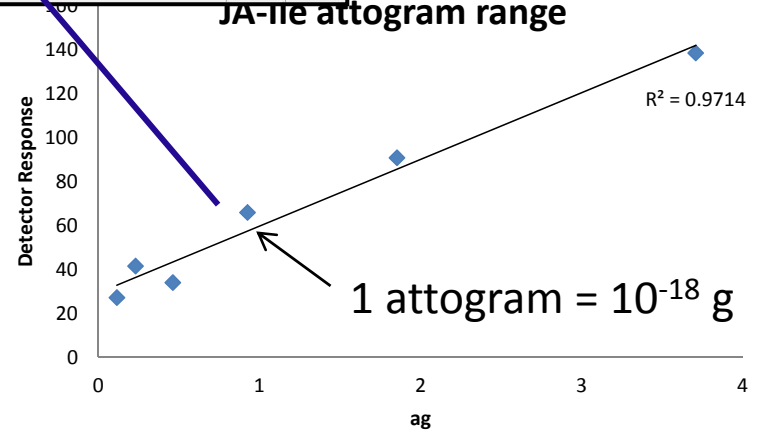
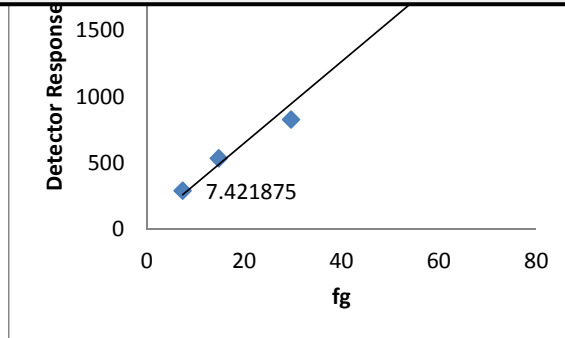
JA-Ile pg range



$R^2 = 0.9999$

60 80 100 120 140
pg

JA-Ile attogram range



What does this mean in real tissue?



Mechanical wounding 1h



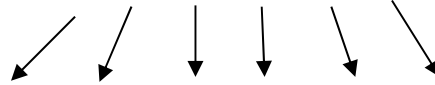
Bulk leaf tissue (~400 mg)



Frozen liquid N₂



Grind into powder



Divide into smaller weights (mg)

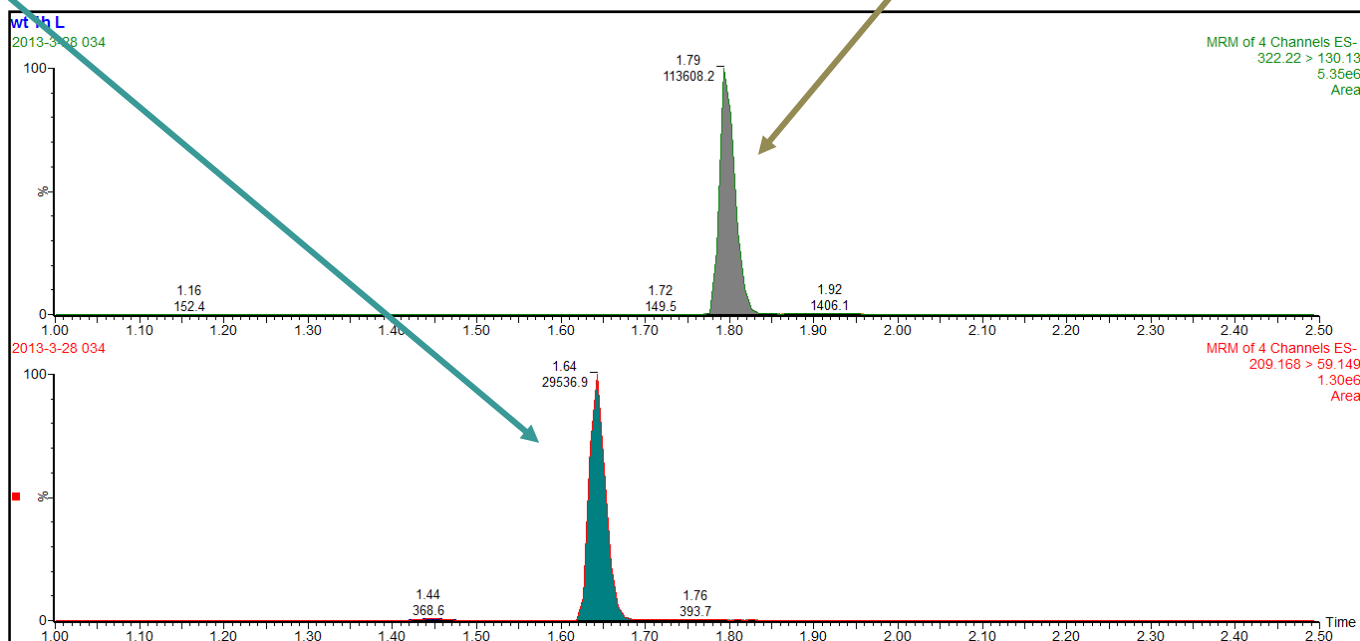
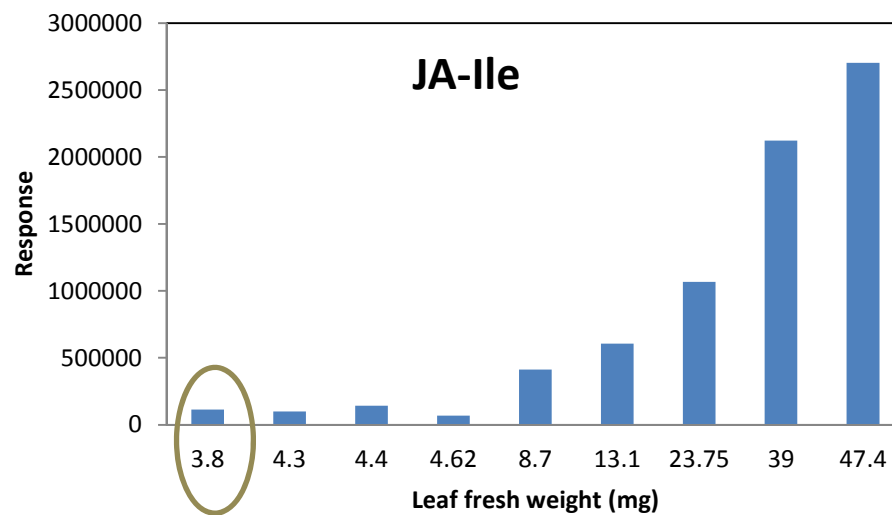
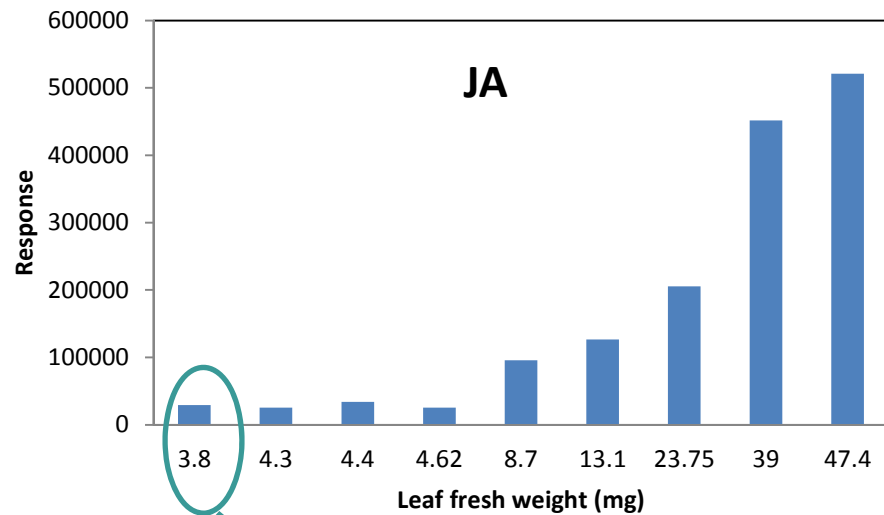
3.8, 4.3, 4.4, 4.62, 8.7, 13.1, 23.75, 39, 47.4, 88.2



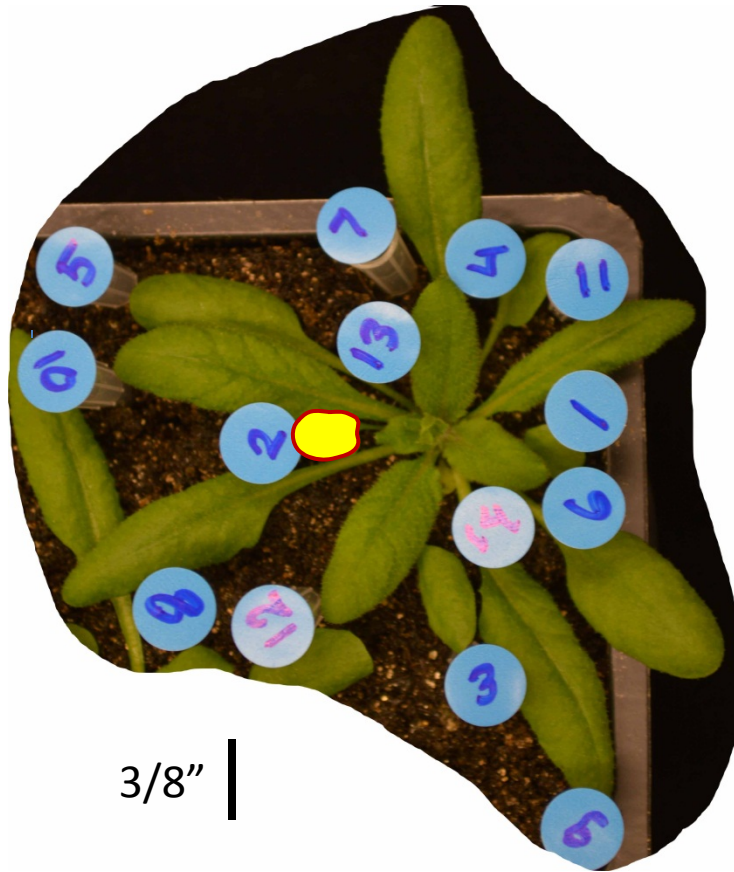
Hormone extraction (100 μ L)



UPLC MS/MS (5 μ L)



How small is 3.8 mg?



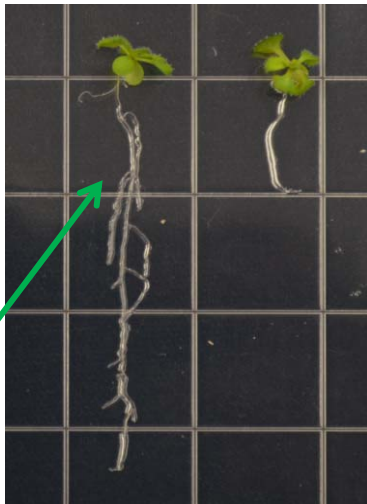
30d old Col-0

Arati Nepal

**Hormone analysis from individual leaves possible
-phenotyping of mutants and transgenics**

leaf no.	mg
1	6.89
2	7.3
3	22.9
4	20.7
5	39.7
6	50.85
7	83.6
8	65.05
9	81.33
10	67.61
11	45.35
12	41.25
13	32.32
14	16.71

Hormone analysis from individual seedlings

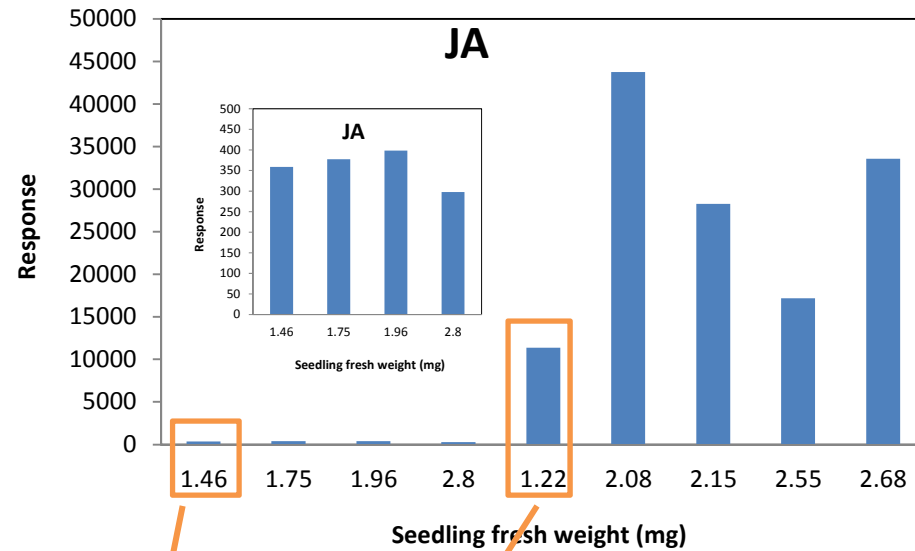


3 %

10 d old plate grown

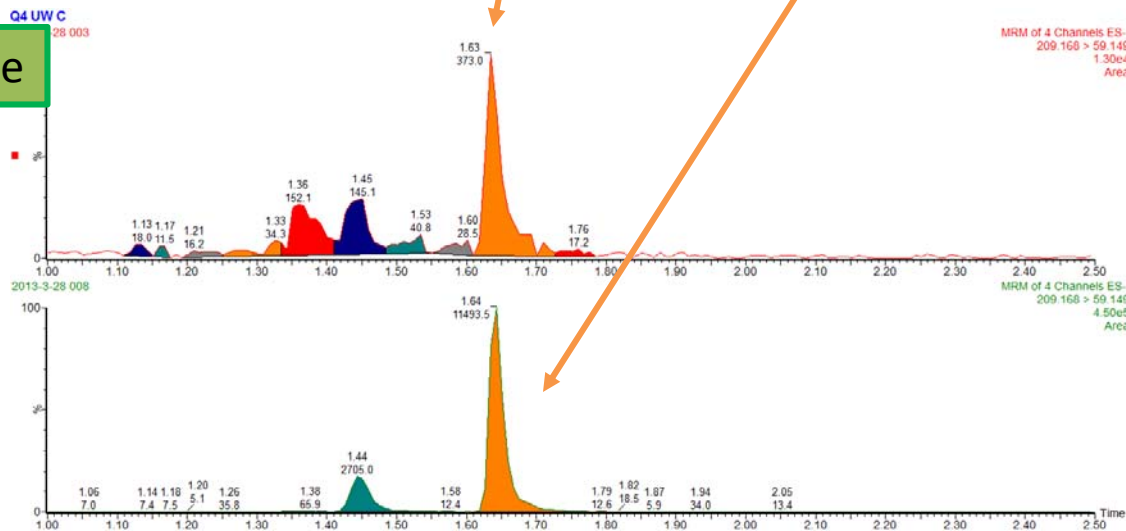
JA-Ile ~40 fold JA anbhag

0.08 % ~ 1 µg tissue

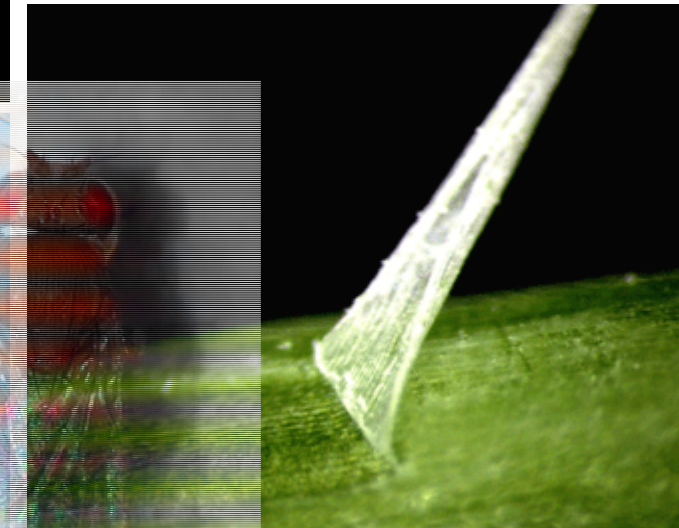
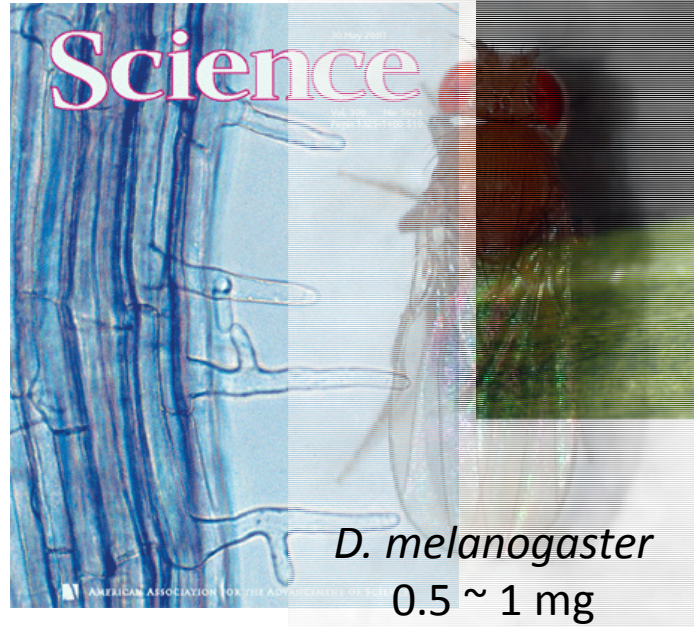
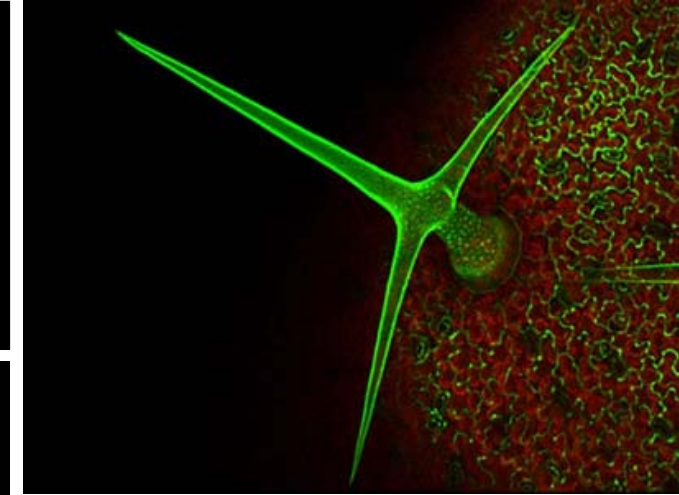


unwounded

Wounded

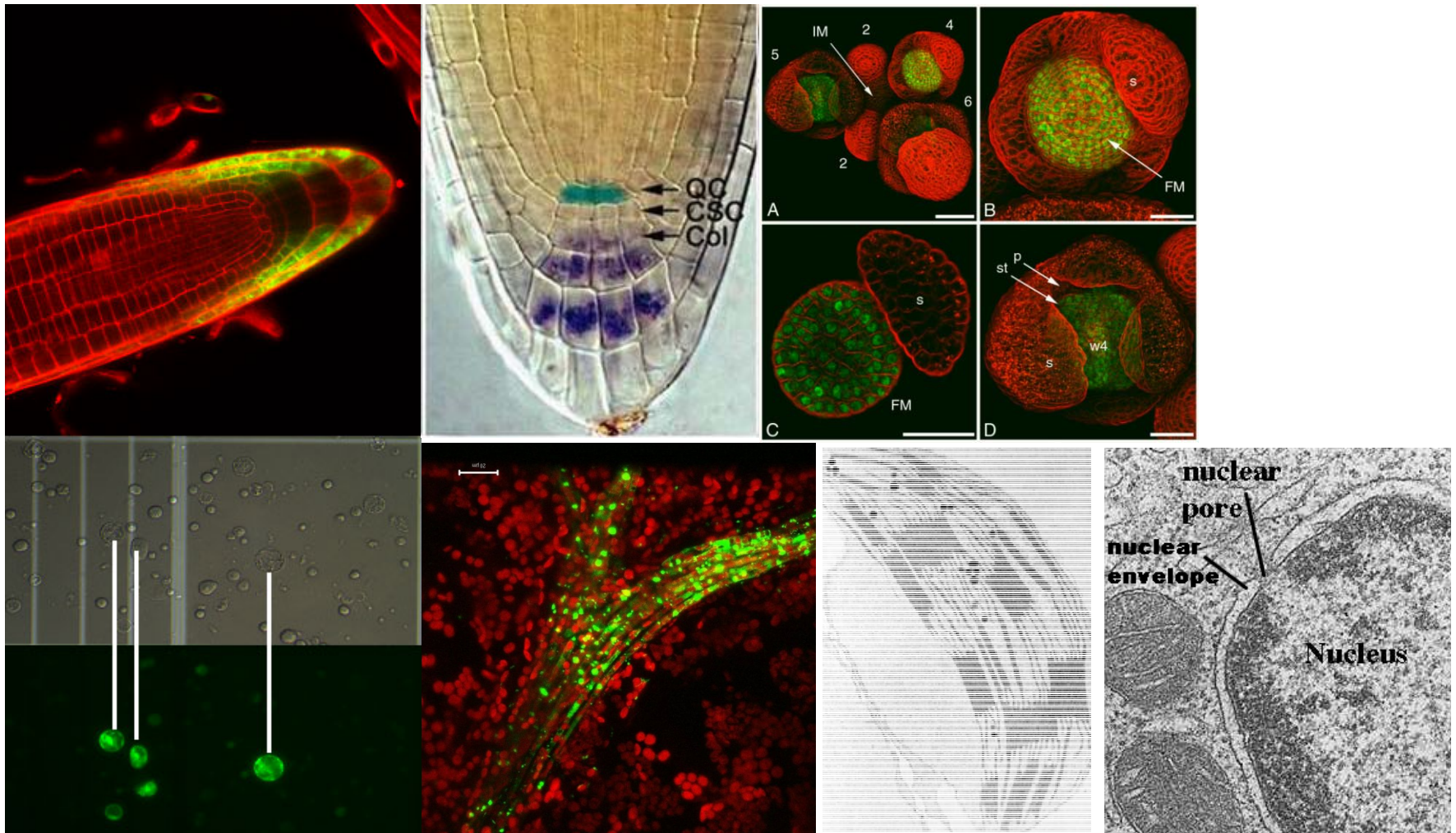


Organ specific metabolite analysis



Canola
Soybean
Corn

Cell type specific metabolite analysis (abundant metabolites)

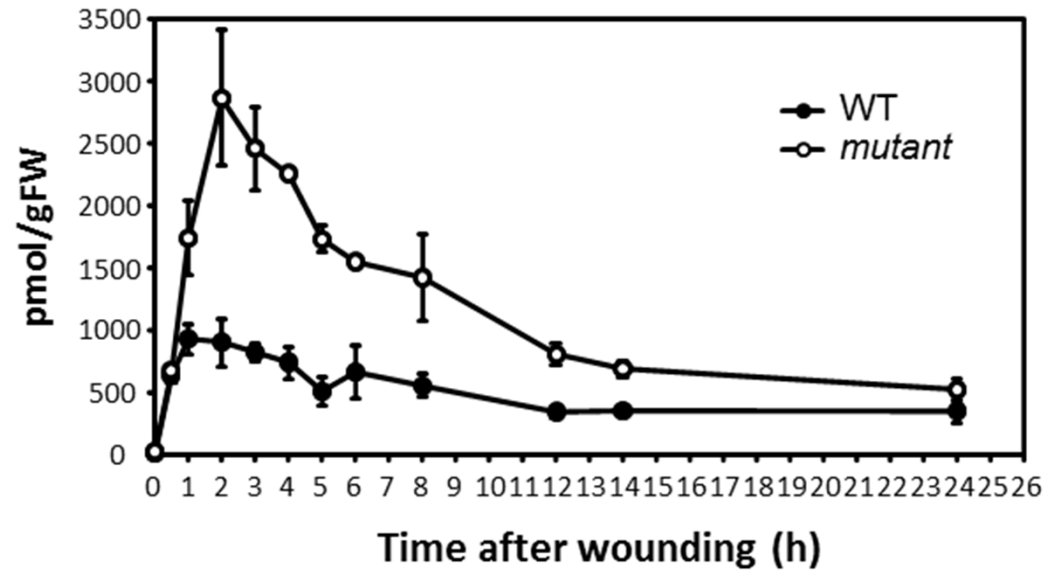


Organelle specific metabolite analysis

Higher Sample Throughput (Shorter Run Time) using UPLC



Conventional chromatography often takes long time!



12 time points X 4 replicates X 2 genotypes = 96 samples

45 minute method X 96 samples = 4320 minutes = 72 hours \$\$\$

Higher Sample Throughput (Shorter Run Time) by UPLC-MS/MS

3 min method X 96 samples = 288 minutes = **4.8** hours

Inlet parameters

Column: ACQUITY UPLC® BEH C18 **1.7 μm**,
2.1 x 50 mm

Mobile phase A: Water, 0.1 % formic acid

Mobile phase B: Methanol

Column Temperature: 40 °C

Flow rate: **0.4 mL/min**

Injection volume: 5 μL

Time (min)	Flow Rate (mL/min)	%A	%B
0.00	0.4	70	30
1.50	0.4	0	100
2.50	0.4	0	100
2.51	0.4	70	30
3.00	0.4	70	30

Higher Sample Throughput (Shorter Run Time) by UPLC-MS/MS

Higher operating pressure
(back pressure)



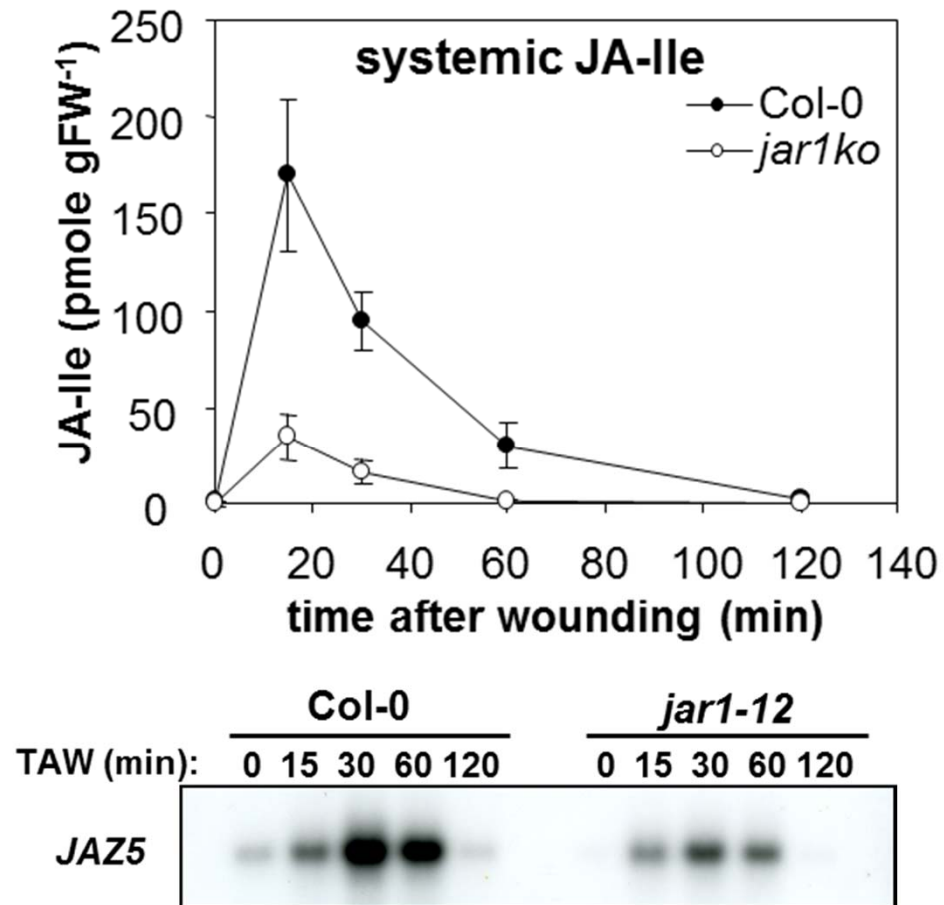
ACQUITY UPLC H-Class
(**15,000 psi** or 1,034 bar)

- Narrower peak width require **faster detector sampling rate**
- Low system and dwell volume (minimal dispersion in the detector cell)
- Injection valves should prevent extreme pressure fluctuations on column
- Fast injection cycle time
- Low volume injections with minimal carryover

Applications that need running many samples

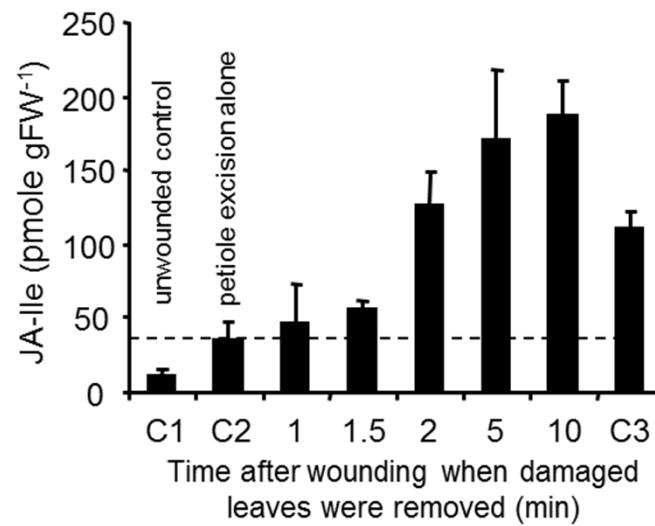
Not just yes/no but How Much?

Triple Quadrupole good for Quantitative Analysis



Not just yes/no but How Much?

Triple Quadrupole good for Quantitative Analysis



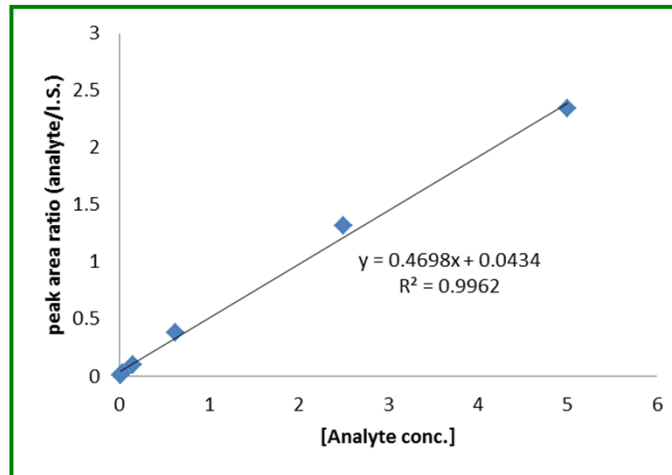
Not just yes/no but How Much?

Triple Quadrupole good for Quantitative Analysis

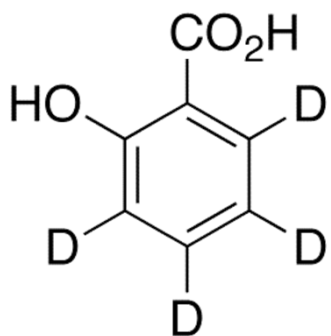
Internal standards (to account for Experimental Drift)

- very similar but not identical to the analyte
- known amount added before extraction or LC injection

Calibration curves



Example of an Internal Standard



d4-SA

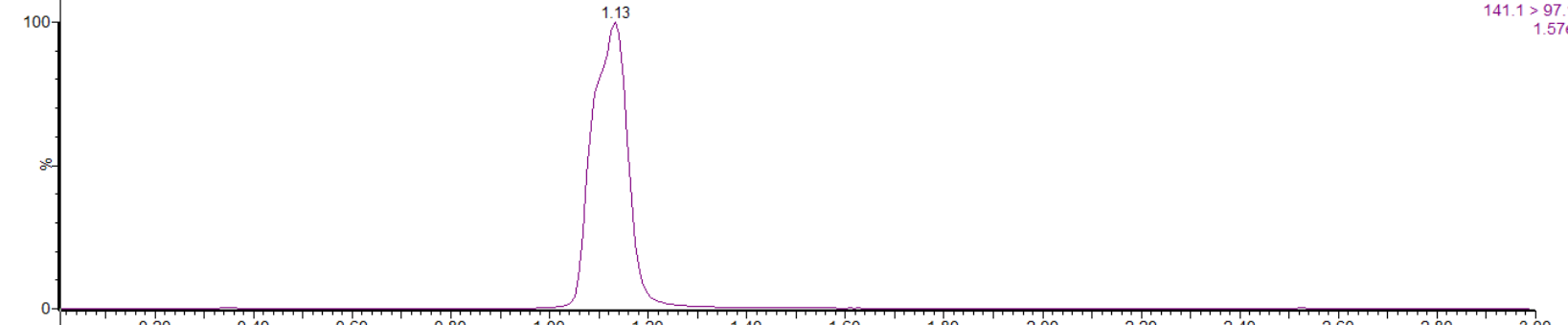
MRM transition [M-H]⁻ (m/z)

141 > 97

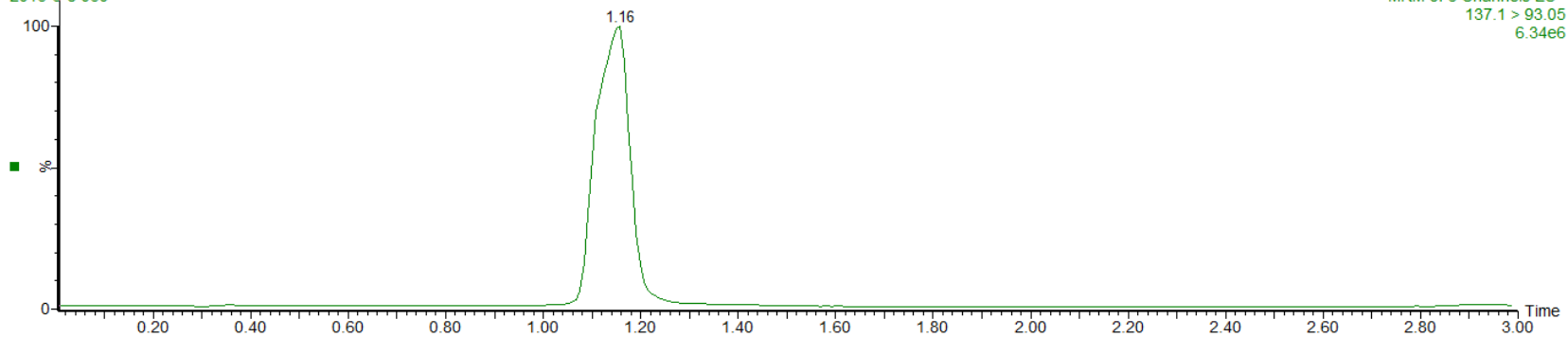
SA

137 > 93

STM910.625
2013-08-030



2013-08-030



Simple and Quick Sample Preparations

- No derivatization necessary

Things to consider

How much tissue sample is needed? (final volume can be adjusted)

Record tissue weight (or other **normalization** factors)

Solubility in extraction solvent / buffer

Extractability (tissue homogenization)

Rapid Quenching (stop chemical reactions, hot isopropanol)

Stability (temp, pH, purge w/ inert gas, anti-oxidants, fresh extract, storage)

Remember to include known amount of **Internal Standard**

Remove insoluble debris (column life, filter / precipitate / centrifuge hard)

Sample Prep Example:

Jasmonate Extraction Protocol

Tissue collect

↓
Weigh

F

T

A

M

M

Tr



Prep with beads)

(Add 0.5% acetic acid)

10000 RPM 30 min



Larger Scale (0.1- 1g)

Tissue collect

↓
Weigh

↓
Freeze in liquid N2 (until use)

↓
Grind

↓
Add I.S. and Ethyl Acetate and Mix

↓
Spin down (10,000 x g)

↓
Transfer organic phase to glass tubes

↓
Adjust pH and re-extract from pellet

↓
Dry down under N2 gas / heating

↓
Reconstitute dried residue in 70% methanol

↓
Keep in -20 °C

↓
Micro-centrifuge 4 °C 13,000 RPM 30 min

↓
Transfer to LC vials

UPLC-MS/MS Method Development

LC Me

MS M

Modify ACQUITY Quaternary Solvent Manager Instrument Method

Acquity Quaternary Solvent Manager

Auto Blend Plus™ Run Time: 3.50 min

General Misc Data

Solvents

A: water+0.01%FA+0.05%
B:
C: Methanol
D: Water Fomic acid 0.15

Pressure Limits ?
Low: 0 psi
High: 15000 psi

Seal Wash Period: 5.00 min

Gradient:

	Time	Flow (mL/min)	%A	%B	%C	%D	Curve
1	Initial	0.400	0.0	0.0	40.0	60.0	Initial
2	1.50	0.400	0.0	0.0	100.0	0.0	6
3	2.50	0.400	0.0	0.0	100.0	0.0	6
4	2.51	0.400	0.0	0.0	40.0	60.0	6

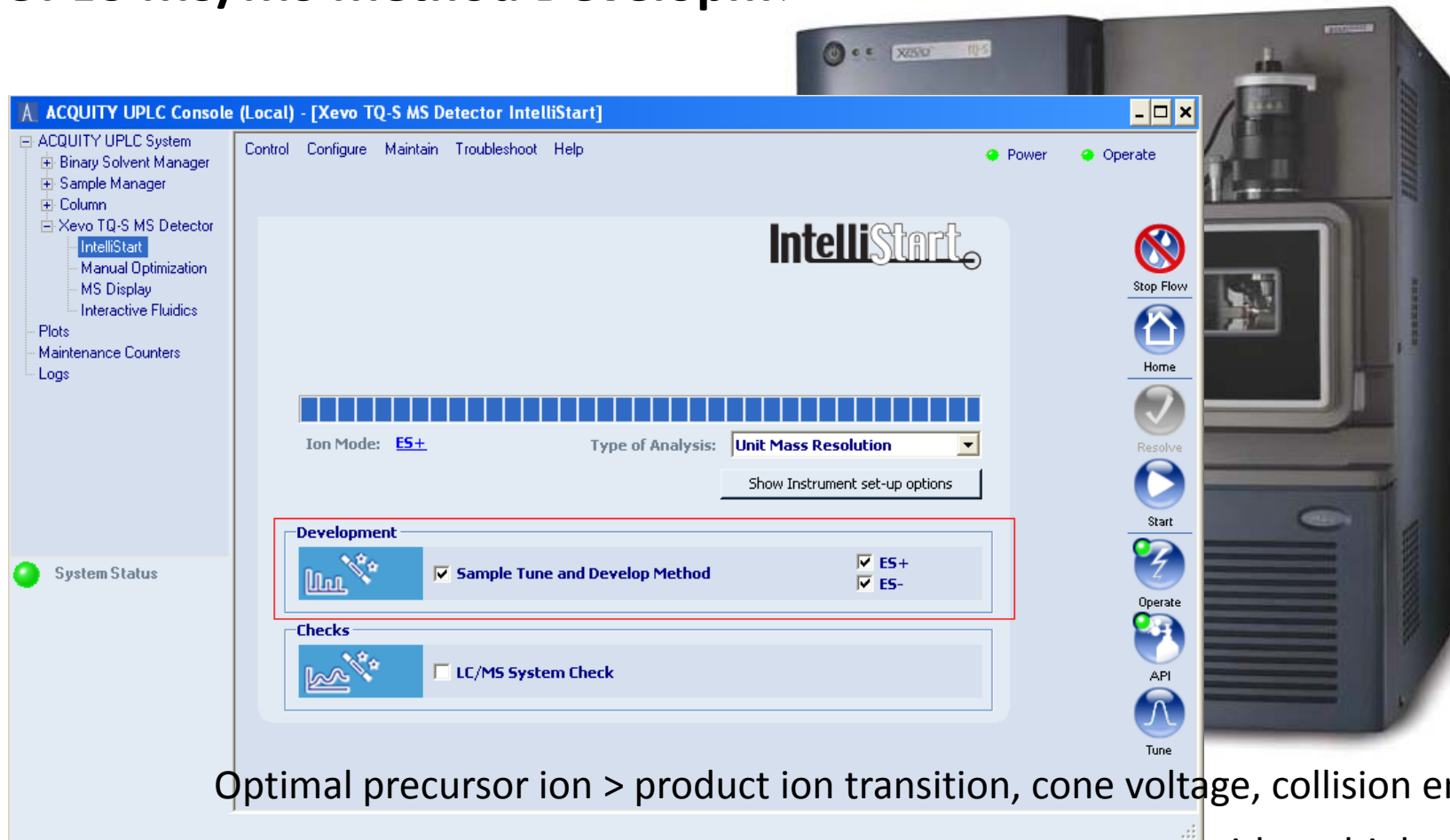
Comment:

OK Cancel

- Inlet Method

A 0.0 %
B 0.0 %
C 100.0 %
D 0.0 %

UPLC-MS/MS Method Development

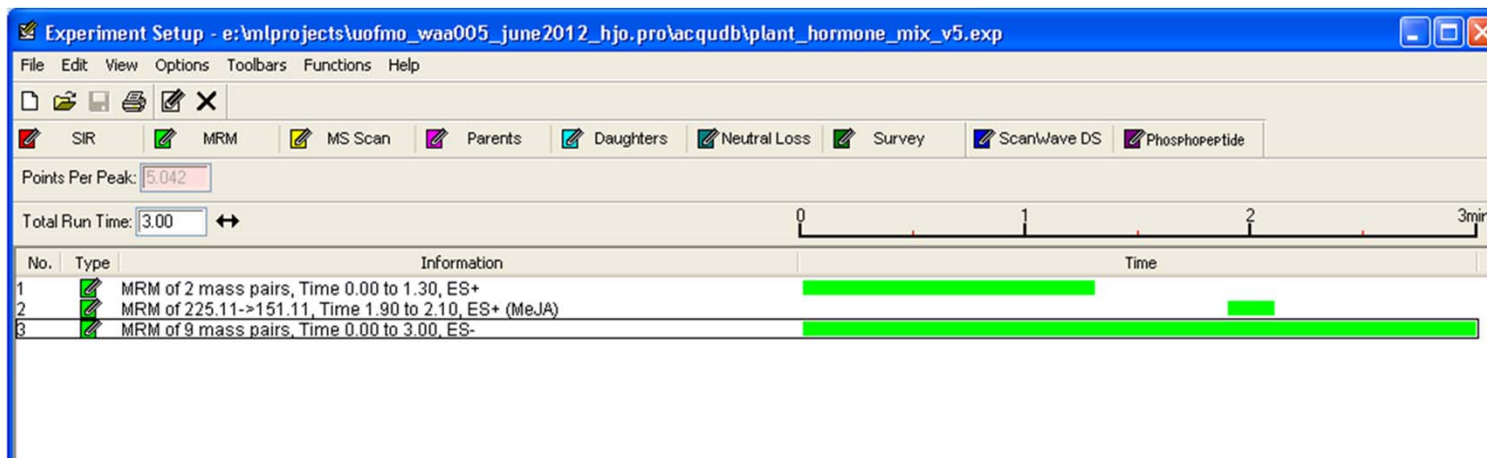


The screenshot displays the ACQUITY UPLC Console software interface. The title bar reads "ACQUITY UPLC Console (Local) - [Xevo TQ-S MS Detector IntelliStart]". The interface includes a navigation tree on the left with categories like "Binary Solvent Manager", "Sample Manager", "Column", and "Xevo TQ-S MS Detector". The main area features the "IntelliStart" logo and a progress bar. Below the progress bar, the "Ion Mode" is set to "ES+" and the "Type of Analysis" is "Unit Mass Resolution". A "Development" section is highlighted with a red box, containing a checked "Sample Tune and Develop Method" option with sub-options for "ES+" and "ES-". Below this is a "Checks" section with an unchecked "LC/MS System Check" option. On the right side, there are several control buttons: "Stop Flow", "Home", "Resolve", "Start", "Operate", "API", and "Tune". The background shows a physical Xevo TQ-S MS detector unit.

Optimal precursor ion > product ion transition, cone voltage, collision energy
- Based on IntelliStart Recommendations develop IMS method with multiple analytes

UPLC-MS/MS Method Development

MS method editor



F1 (ES+)
(0-1.3 min)

	Compound Na	Parent (m/z)	Daughter (m/z)	Aut	Dwell (s)	Cone (V)	Collision (V)
1	MeSA	153.0200	135.0000	<input type="checkbox"/>	0.025	35	8
2	IAA	176.0314	130.0604	<input type="checkbox"/>	0.025	16	16

F2 (ES+)
(1.9-2.1 min)

	Compound Na	Parent (m/z)	Daughter (m/z)	Auto	Dwell (s)	Cone (V)	Collision (V)
1	MeJA	225.1093	151.1060	<input type="checkbox"/>	0.025	2	14

F3 (ES-)
(0-3 min)

	Compound Na	Parent (m/z)	Daughter (m/z)	Auto	Dwell (s)	Cone (V)	Collision (V)
1	SA	136.9998	92.9775	<input type="checkbox"/>	0.025	32	14
2	JA	209.0937	59.0384	<input type="checkbox"/>	0.025	32	12
3	dh-JA	211.1093	59.0350	<input type="checkbox"/>	0.025	6	14
4	12OH-JA	225.0612	59.0352	<input type="checkbox"/>	0.025	28	14
5	Linoleic acid	277.1927	277.5712	<input type="checkbox"/>	0.025	44	18
6	12OPDA	291.1719	165.1438	<input type="checkbox"/>	0.025	2	20
7	JA-Ile	322.2416	130.1169	<input type="checkbox"/>	0.025	34	20
8	GA3	345.1735	239.1464	<input type="checkbox"/>	0.025	36	14
9	Rutin	609.1215	300.1908	<input type="checkbox"/>	0.025	60	36

Running the Sample

MassLynx - K002 - RE Col-0 jh2a ill6-1 4-9-13.SPL

File View Run Help

Queue Is Empty

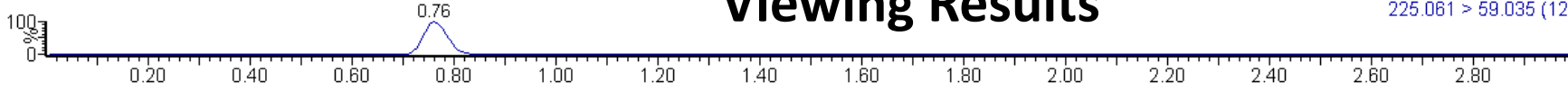
Spectrum Chromatogram Map Edit Samples

	File Name	File Text	Inlet File	MS File	MS Tune File	Vial	Inj Vol
1	2013-4-9 001	INTSTD 1/10 2-8-13	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:A,1	5.0
2	2013-4-9 002	INTSTD 1/3 Apr 2013	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:A,2	5.0
3	2013-4-9 003	Col-0 QA 1/3	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:A,3	5.0
4	2013-4-9 004	jh2a 0A 1/3	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:A,4	5.0
5	2013-4-9 005	ill6-1 0A 1/3	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:A,5	5.0
6	2013-4-9 006	P25 0A 1/3	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:A,6	5.0
7	2013-4-9 007	Col-0 2hA 1/3	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:A,7	5.0
8	2013-4-9 008	jh2a 2hA 1/3	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:A,8	5.0
9	2013-4-9 009	ill6-1 2hA 1/3	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:B,1	5.0
10	2013-4-9 010	P25 2hA 1/3	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:B,2	5.0
11	2013-4-9 011	Col-0 6hA 1/3	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:B,3	5.0
12	2013-4-9 012	jh2a 6hA 1/3	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:B,4	5.0
13	2013-4-9 013	ill6-1 6hA 1/3	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:B,5	5.0
14	2013-4-9 014	P25 6hA 1/3	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:B,6	5.0
15	2013-4-9 015	Col-0 10hA 1/3	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:B,7	5.0
16	2013-4-9 016	jh2a 10hA 1/3	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:B,8	5.0
17	2013-4-9 017	ill6-1 10hA 1/3	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:C,1	5.0
18	2013-4-9 018	P25 10hA 1/3	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:C,2	5.0
19	2013-4-9 019	STM6 II Feb 12 1/3 0.00327	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:C,3	5.0
20	2013-4-9 020	STM6 II Feb 12 1/3 0.013	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:C,4	5.0
21	2013-4-9 021	STM6 II Feb 12 1/3 0.052	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:C,5	5.0
22	2013-4-9 022	STM6 II Feb 12 1/3 0.208	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:C,6	5.0
23	2013-4-9 023	STM6 II Feb 12 1/3 0.833	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:C,7	5.0
24	2013-4-9 024	STM6 II Feb 12 1/3 1.67	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:C,8	5.0
25	2013-4-9 025	STM8 II Feb 12 1/3 0.00327	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:D,1	5.0
26	2013-4-9 026	STM8 II Feb 12 1/3 0.013	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:D,2	5.0
27	2013-4-9 027	STM8 II Feb 12 1/3 0.052	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:D,3	5.0
28	2013-4-9 028	STM8 II Feb 12 1/3 0.208	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:D,4	5.0
29	2013-4-9 029	STM8 II Feb 12 1/3 0.833	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:D,5	5.0
30	2013-4-9 030	STM8 II Feb 12 1/3 1.67	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120HJA 120HJA-Ile 12000H 3pt5min	MRM 2013-2-11	1:D,7	5.0
31	2013-4-9 031	STM10 I 0.00024	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:D,8	5.0
32	2013-4-9 032	STM10 I 0.00098	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:E,1	5.0
33	2013-4-9 033	STM10 I 0.0039	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:E,2	5.0
34	2013-4-9 034	STM10 I 0.0156	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:E,3	5.0
35	2013-4-9 035	STM10 I 0.0625	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:E,4	5.0
36	2013-4-9 036	STM10 I 0.25	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:E,5	5.0
37	2013-4-9 037	STM10 I 1	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:A,1	5.0
38	2013-4-9 038	INTSTD 1/10 2-8-13	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:A,2	5.0
39	2013-4-9 039	INTSTD 1/3 Apr 2013	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:A,6	5.0
40	2013-4-9 040	Col-0 0B 1/3	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:E,6	5.0
41	2013-4-9 041	jh2a 0B 1/3	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:E,7	5.0
42	2013-4-9 042	ill6-1 0B 1/3	UPLC 400uL_3pt5 min II 2-15...	MRM JA dhJA JA-Ile 13C 120...	MRM 2013-2-11	1:E,8	5.0

Ready

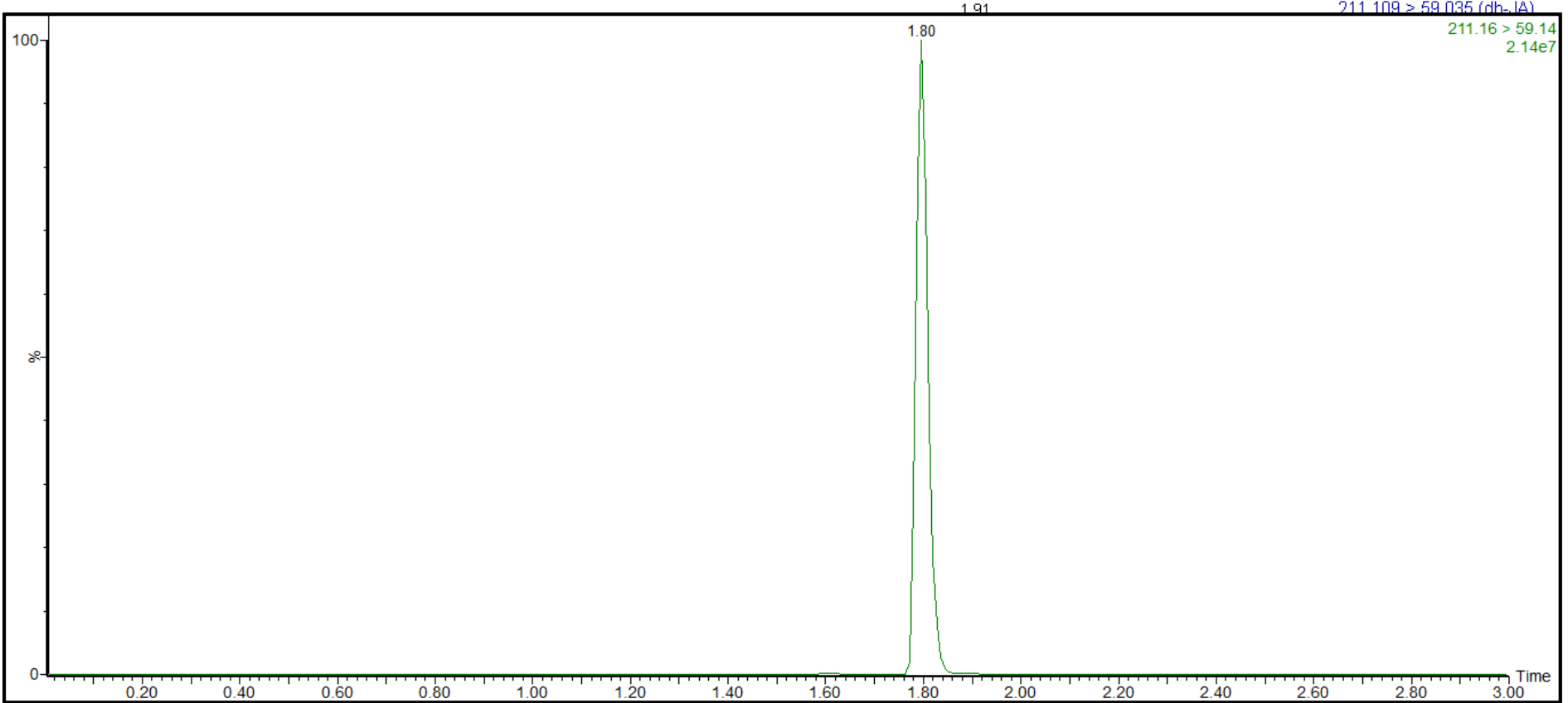
Viewing Results

standard106



3: MRM of 9 Channels ES-
225.061 > 59.035 (12OH-JA)
1.67e6

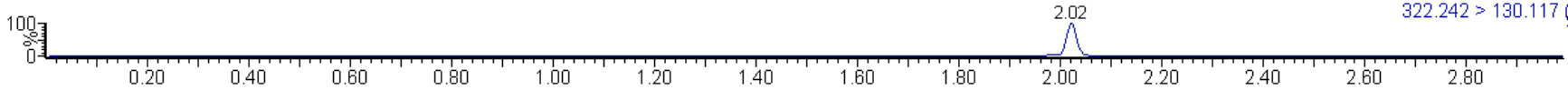
standard106



3: MRM of 9 Channels ES-
211.109 > 59.035 (dh-JA)

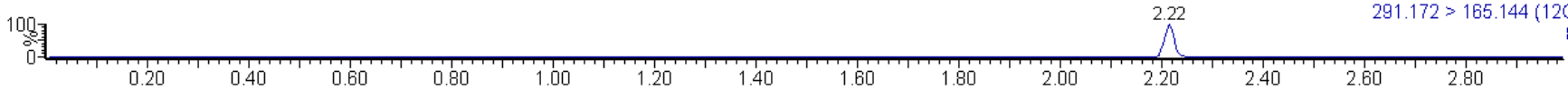
211.16 > 59.14
2.14e7

standard106



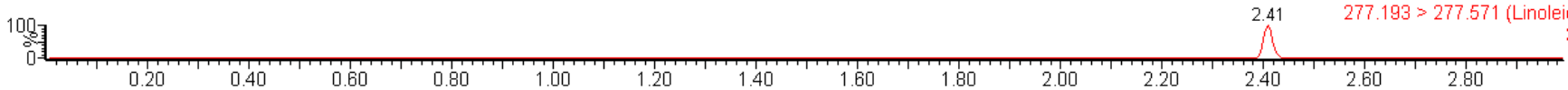
3: MRM of 9 Channels ES-
322.242 > 130.117 (JA-Ile)
7.27e7

standard106



3: MRM of 9 Channels ES-
291.172 > 165.144 (12OPDA)
8.29e6

standard106

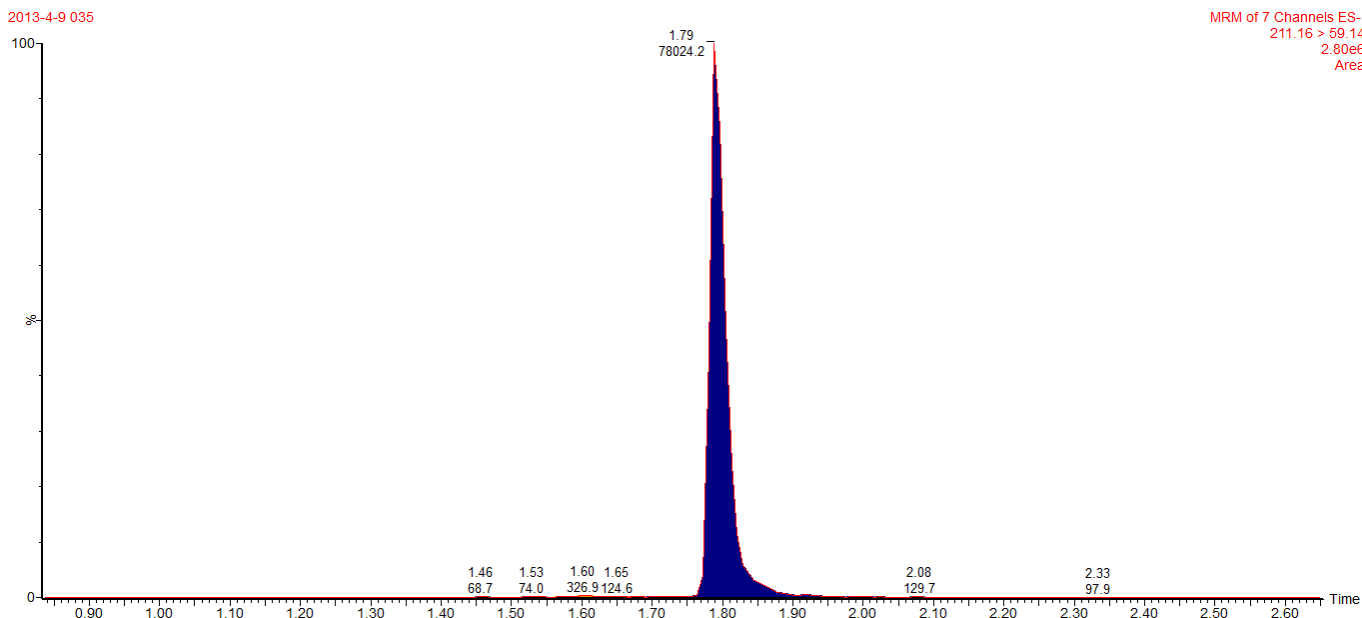


3: MRM of 9 Channels ES-
277.193 > 277.571 (Linoleic acid)
3.26e7

Data Analysis

-manual

-TargetLynx



#	Name	Sample Text	Type	Std. Conc	RT	IS Area	Area	Response	Conc.
1	standard083	Blank, 5 ul inj.	Blank						
2	standard084	Mix, 0.4166 nM, 5 ul inj.	Standard	0.417	1.91	59	58.501	58.501	0.3396
3	standard085	Mix, 4.166 nM, 5 ul inj.	Standard	4.166	1.91	593	593.480	593.480	4.0583
4	standard086	Mix, 41.66 nM, 5 ul inj.	Standard	41.660	1.91	6506	6505.699	6505.699	45.1543
5	standard087	Mix, 416.6 nM, 5 ul inj.	Standard	416.600	1.91	62892	62892.266	62892.266	437.0986
6	standard088	Mix, 833.3 nM, 5 ul inj.	Standard	833.300	1.91	113679	113678.523	113678.523	790.1151
7	standard092	Blank, 5 ul inj.	Blank						
8	standard093	Mix, 0.4166 nM, 5 ul inj.	Standard	0.417	1.91	67	66.805	66.805	0.3974
9	standard094	Mix, 4.166 nM, 5 ul inj.	Standard	4.166	1.91	608	607.897	607.897	4.1585
10	standard095	Mix, 41.66 nM, 5 ul inj.	Standard	41.660	1.91	6702	6701.528	6701.528	46.5155
11	standard096	Mix, 416.6 nM, 5 ul inj.	Standard	416.600	1.91	63870	63869.531	63869.531	443.8916
12	standard097	Mix, 833.3 nM, 5 ul inj.	Standard	833.300	1.91	114581	114580.672	114580.672	796.3860
13	standard101	Blank, 5 ul inj.	Blank						
14	standard102	Mix, 0.4166 nM, 5 ul inj.	Standard	0.417	1.91	58	58.281	58.281	0.3381
15	standard103	Mix, 4.166 nM, 5 ul inj.	Standard	4.166	1.91	613	612.683	612.683	4.1918
16	standard104	Mix, 41.66 nM, 5 ul inj.	Standard	41.660	1.91	6950	6949.885	6949.885	48.2418
17	standard105	Mix, 416.6 nM, 5 ul inj.	Standard	416.600	1.91	65053	65052.539	65052.539	452.1147
18	standard106	Mix, 833.3 nM, 5 ul inj.	Standard	833.300	1.91	117320	117319.914	117319.914	815.4265

Summary

- UPLC-MS/MS for targeted analysis of known metabolites
- Highly sensitive detections
- Short running time, increased throughput
- Quantitative analysis
- Quick and simple sample preparations
- Overview of UPLC-MS/MS steps
- User friendly software interface
- Robust system (low maintenance / large sample number)

What is your compound of interest?

Quantification of a Male Sea Lamprey Pheromone in Tributaries of Laurentian Great Lakes by Liquid Chromatography–Tandem Mass Spectrometry

Xiaodan Xi,[†] Nicholas S. Johnson,^{†,||} Cory O. Brant,[†] Sang-Seon Yun,^{†,⊥} Keali L. Chambers,[†]
A. Daniel Jones,^{†,§} and Weiming Li^{*,†}

