MSU MSMC Protocol 3b

Phytohormone analysis – UPLC/MS/MS method

(last edited 5/11/20)

Samples prepared using protocol *MSU_MSMC_003: Extraction of phytohormones*

- Extracts will be in 80% methanol containing 0.1% formic acid and are ready to analyze by LC/MS/MS
    - For best chromatography of salicylate glycosides, dilute samples with water containing 0.1% formic acid to reduce methanol concentration. Dilution amount will depend on concentration of SAG in the samples but a good starting point would be 1:2 dilution.

A template Masslynx project (Plant_phytohormones.PRO) is located on the Quattro Premier and TQD data systems and can be used to create new user projects.

Tune page file	ESI_Phyto.ipr
MS method file[1]	Phytohormone_MRM_channels.exp Phytohormone_MRM_no standards.exp
Inlet method file[2]	5min_A1B2_column2_phytohormones

[*Note 1: MS methods listed here simply contain a list of MRM channels optimized for the instrument. Each user should create their own personalized MS method based on the compounds they want to measure. MRM channels for compounds without standards available are not optimized, but will work for detection of these known metabolites.*

Note 2: Inlet method file may be different between the QP and TQD due to use of different column positions and solvent lines.]

HPLC column: Ascentis Express C18 2.1x50 mm (2.7 μm particle size) [Sigma cat# 53822-U]. Use with 0.5 μm precolumn filter [IDEX Health and Science A-318 (filter holder), A-102 (frit), U-288 (male-to-male coupler). *This column has a pressure limit of 6000 psi (413 bar)*.

Mobile phase solvents: A) Water + 0.1% formic acid; B) Methanol

LC gradient:

Time (min)	Flow rate (ml/min)	% A	% B
0	0.400	98	2
0.5	0.400	98	2
3.0	0.400	30	70
3.5	0.400	0	100
4.0	0.400	0	100
4.01	0.400	98	2
5.0	0.400	98	2

Column Temp: 40°C

Injection volume: 10 μL

Tune Page parameters: (For the Quattro Premier or TQD)

- Capillary voltage: 1.00 kV (Negative ion mode); 3.00 kV (Positive ion mode)
- Source Temp: 120°C
- Desolvation Temp: 350°C
- Desolvation Gas flow: 800 L/hr
- Cone Gas flow: 50 L/hr

MS/MS parameters

Notes: Concentration of phytohormones can vary significantly across different tissues, treatments, or plant species. Some phytohormones present at very low levels may require additional sample prep procedures not outlined in the standard MSU_MSMC_003 protocol and may require use of more sensitive instrumentation for detection and quantification. The following MS/MS parameters list will include compounds for which standards are available as well as known compounds that are present in plants but for which standards cannot be purchased. Absolute quantification is possible when standards are available but only relative quantification can be performed for compounds without available standards. The following parameters were optimized for a Waters Quattro Premier XE but can be used on other Waters triple quadrupole instruments with the caveat that the CV and CE parameters may not give the optimum sensitivity on the other instruments.

Dwell times: The dwell times listed here are not universal. They will need to be adjusted based on the number of compounds in your MS/MS method and based on how abundant the compounds are in the samples being analyzed. Dwell times should also be set such that there are enough scans (~10-12) across each chromatographic peak to allow for accurate peak integration. It may be necessary to create time-resolved functions in the MS method editor window, where each function monitors a subset of the desired compounds based on their retention times, to get a sufficient number of scans for each compound.

List of MRM channels for compounds having available standards:

Negative ion mode					
Compound	**Precursor ion (*m/z*)**	**Product ion (*m/z*)**	**Dwell time (secs)**	**Cone voltage**	**Collision energy**
Salicylic acid (SA)	137	93	0.05	28	16
[$^{13}C_6$]-SA (*internal standard*)	143	99	0.05	28	16
Abscisic acid (ABA)	263.1	153.1	0.05	22	10
ABA-d_6 (*internal standard*)	269.1	159.1	0.05	22	11
SA glucoside	299.1	137	0.05	28	16
Jasmonic acid (JA)	209.1	59	0.05	28	16
[$^{2}H_5$]-Jasmonic acid (*internal standard*)	214.1	61	0.05	28	16

Jasmonoyl isoleucine (JA Ile)	322.2	130.1	0.05	34	22
Dihydro-JA (*internal standard*)	211.1	59	0.05	34	16
12-Oxophytodienoic acid (OPDA)	291.2	165.1	0.05	46	22
DinorOPDA (dnOPDA)	263.2	165.1	0.05	46	22
[$^{2}H_{5}$]-dnOPDA (*internal standard*)	268.2	170.1	0.05	46	22
Linolenic acid	277.2	277.2	0.05	46	0
Coronatine	318.2	163.1	0.05	34	22
Indole-3-acetic acid (IAA)	174.1	129.9	0.05	21	11

Positive ion mode					
Compound	**Precursor ion (*m/z*)**	**Product ion(*m/z*)**	**Dwell time (secs)**	**Cone voltage**	**Collision voltage**
IAA	176.1	130.1	0.05	20	16
Methyl jasmonate (MeJA)	225.1	151.1	0.05	22	16
Zeatin	220.1	136.1	0.05	20	16

List of predicted MRM channels compounds where standards are unavailable:

Compound	**Precursor ion (*m/z*)**	**Product ion (*m/z*)**	**Dwell time (secs)**	**Cone voltage**	**Collision voltage**
ABA-Glucose ester	425	263	0.05	22	10
12-Hydroxyjasmonoyl isoleucine (12OHJA-Ile)	338.2	130.1	0.05	46	22
OPC:8	293.2	293.2	0.05	28	0
OPC:6	265.2	265.2	0.05	34	0
OPC:4	237.2	237.2	0.05	34	0
Jasmonoyl-Valine	308.2	116.1	0.05	34	22
Jasmonoyl-Threonine	310.2	266.2	0.05	34	16
Jasmonoyl-Glutamine	337.2	145	0.05	34	22
OPC:4-malate	353.2	237.1	0.05	34	15
Jasmonoyl-Alanine	280.2	88.1	0.05	28	22
Jasmonoyl-ACC	292.2	100	0.05	28	16
Jasmonoyl-Methionine	340.2	148.1	0.05	40	22
Jasmonoyl-Phenylalanine	356.2	164.2	0.05	34	22
Jasmonoyl-Tryptophan	395.2	203.1	0.05	28	22
[$^{13}C_{6}$]-JA-Ile (TRC, backordered)	328.2	136.1	0.05	34	22

12-Hydroxyjasmonic acid (12OH-JA)	225.2	59	0.05	28	16

Gibberellins:

The levels of GA's tend to be very low in plant tissues and typically will require more extensive sample prep using larger amounts of extracted tissue and analyte enrichment using either solid phase extraction or HPLC. There are also many different types of GA's in plants, some of which are active while others are not. Users should carefully think about which GA's to measure. In addition, standards are not as easy to purchase or are very expensive. For labeled standards, OlChemIm (www.olchemim.cz) has many deuterated GA's available but they are quite expensive for a very small amount of compound, and many do not have the recommended 3-4 heavy isotopes needed to minimize overlap of signals from the labeled and unlabeled forms. Currently the MSMC does not have any labeled GA standards and only a few GA's have optimized MRM channels on our instruments. For any other GA's the user will need to work with MSMC staff to optimize MS parameters using standards.

Compound	**Precursor ion (*m/z*)**	**Product ion (*m/z*)**	**Dwell time (secs)**	**Cone voltage**	**Collision voltage**
GA4	331.2	213.1	0.1	36	34
GA12	331.2	269	0.1	40	34
GA20	331.2	287.1	0.1	42	21
GA12-d_2	333.2	271	0.1	40	34
GA3	345.16	143.16	0.1	30	28
GA24	345.2	301.2	0.1	40	22
GA1	347.2	213.1	0.1	42	28
GA24-d_2	347.2	303.2	0.1	40	22
GA1-d_2	349	275	0.1	36	30