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Phytohormone profiling: obtaining highest sensitivity and throughput

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Overview

Several approaches were evaluated to discern the best selectivity and sensitivity for the analysis of plant hormones. Target hormones were derivatised and analysed using UFMS capable single quadrupole and triple quadrupole gas chromatography-mass spectrometry platforms (Figure 1).
Electron ionisation (EI) and chemical ionisation (CI), with methane, isobutane and ammonia as reagent gases, were assessed for selectivity and sensitivity.

Figure 1: The information content that may be generated from a single sample is enhanced by the use of simultaneous scheduled-MRM for targeted screening and a scan event for untargeted screening. The full scan data may also be used for profiling of sample populations and characterisation of the matrix. Simultaneous acquisition is enabled by UFMS capable GCMS and GCMSMS systems.

Introduction

Phytohormones regulate a plant's cellular processes including normal growth and development and their defensive responses to biotic and abiotic stresses.
While phytoprofiling can be extremely complex, the classes of phytohormones can be considered to include auxins (e.g indole-3-acetic acid), cytokinins (e.g zeatin), gibberellins (e.g Gibberellin A1), abscisic acid and ethylene. Each class of compound can act in an inhibitory fashion or engender multiple positive effects. In agricultural monitoring and research, the detection and measurement of all members of the family is important.
We required a rapid, broad spectrum method to analyse the large sample sets that were generated during a series of plant-pathogen interaction studies. In the studies, 11 phytohormones were chosen for targeted analysis. To achieve biologically relevant levels of quantification with reasonable sample sizes and sample preparation techniques that were compatible with the scale of the study, required the use of methods using either selective ion monitoring (SIM) or multiple reaction monitoring (MRM).
A simultaneous full scan/MRM method was also evaluated for untargeted analysis. We found that this approach offerd advantages in information content without significant compromise of the method (Figure 2 and Table 1).
An extraction and derivatisation protocol was developed that could be used to efficiently target small organic acids and amino acids from wet plant material.

Methods

Extraction

100mg of ground fresh plant material was suspended in a basic aqueous/methanol solution and methyl chloroformate was used to derivatise compounds to their methyl ester form. The methyl ester derivatives were extracted using chloroform.

Analytical method

Standards were purchased from Sigma-Aldrich (Missouri, USA).
Shimadzu GCMS-TQ8030 triple quadrupole mass spectrometer in both EI and CI mode (Shimadzu Corporation, Japan) was configured with a split/splitless inlet for a 3uL injection with 40psi pressure pulse at 250°C and 1mL/min flow. The column was a VF-5ms 30m × 0.25mm × 0.25µm + 10m EZ-Guard (Agilent Technologies, Netherlands). The oven was programmed from 40°C held for 1 min then ramped at 20°C/min to 320°C and held for 2 minutes.
For EI: Ion source @ 200°C; Full scan: 40-400amu; SIM: Ions chosen for sensitivity and selectivity scan; MRM: transitions optimised for best response/exclusiveness
For CI: Ion source @200°C; Reagent gases: methane, iso-butane, ammonia; Full scan: 40-400amu; SIM and MRM transitions optimised for sensitivity and selectivity.

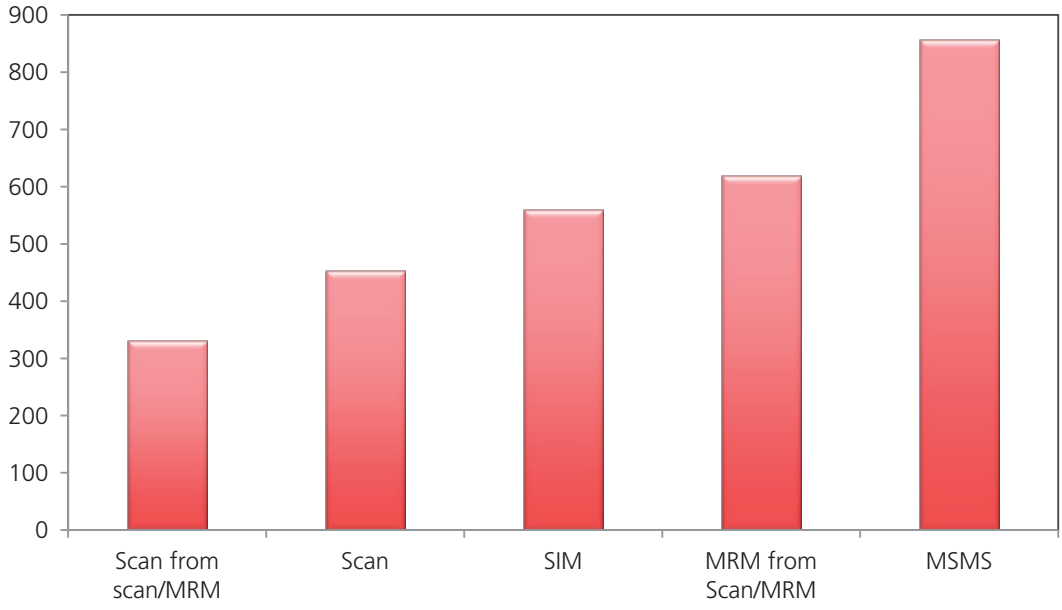

Figure 2. Signal to noise (S/N) of 1-aminocyclppropanoic 1-carboxylic acid methyl ester at 10ng/mL using full scan, SIM, MRM and full scan/MRM in EI mode.

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Table 1. Detection limit (ng/mL) – as determined by a S/N > 50 and a %RSD <10% - of the methylated products of benzoic acid (MeBA), 1-aminocyclopropanoic 1-carboxylic acid (Me-ACC carbamate), cinnamic acid (MeCA), Salicylic acid (MeSA-carbamate, MeMeSA), Azelaic acid (MeAz), Jasmonic acid (MeJA), 1-indole acetic acid (MeIAA), Linoleic acid, Linolenic acid, Abscisic acid (MeABA), 13-epi-12-oxophytodienoic acid (MeOPDA) using EI and CI in full scan, SIM and MRM modes.

	EI			Methane			Iso-butane			Ammonia		
	Scan	SIM	MRM	Scan	SIM	MRM	Scan	SIM	MRM	Scan	SIM	MRM
MeBA	2	1	<0.2	500	2	0.5	<2	<2	<0.2	>5000	5000	5000
MeACC -carbamate	2	2	<0.2	200	20	<0.2	50	5	<0.2	20	2	1
MeCA	2	2	<0.2	50	5	<0.2	10	2	<0.2	100	50	<0.2
MeSA –carbonate	2	2	<0.2	100	20	<0.2	50	5	<0.2	10	<2	<0.2
MeAz	2	2	<0.2	100	20	<0.2	50	2	<0.2	5	<2	<0.2
MeJA	5	1	1	500	200	<0.2	100	20	<0.2	20	2	<0.2
MeIAA	2	2	<0.2	200	50	<0.2	100	5	<0.2	50	2	<0.2
Linoleic Acid, Me Ester	5	2	<0.2	500	200	50	200	20	<0.2	50	5	2
Linolenic Acid, Me Ester	5	2	1	1000	200	50	200	20	<0.2	50	2	2
MeABA (cis)	2	2	<0.2	1000	200	100	500	200	100	100	20	1
MeOPDA	10	5	1	1000	500	10	500	100	20	500	20	10

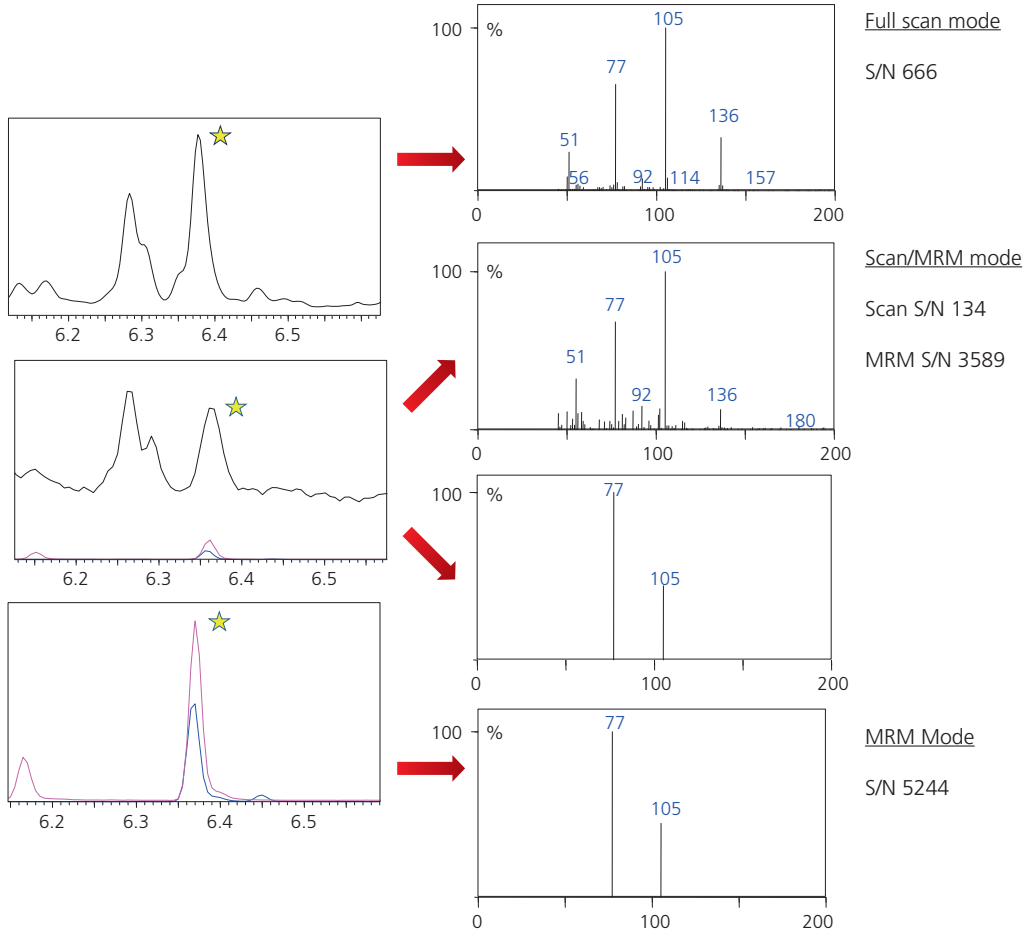

Figure 3. a) full scan, b) full scan/MRM and c) MRM data of methyl benzoate in a real plant sample (Medicago truncatula) and their corresponding mass spectra with the associated S/N.

Results

SIM ions and MRM transitions were optimised for sensitivity and selectivity for EI & CI (same transitions used for all reagent gases). EI data was collected in full scan, SIM, MRM and simultaneous full scan/MRM modes. Detection limit criteria: S/N > 50 and RSD < 10% based on 10 replicates. CI mode allowed cis- and transisomers of abscisic acid to be resolved.
Recoveries and detection limits were calculated based upon a comparison of spiked plant material versus unspiked materials (Tables 1 and 2) .

Table 2. Recovery of analytes spiked directly into fresh ground plant material, then extracted per the protocol. Fresh ground material also extracted to calculate baseline.

	%Recovery	%RSD
$MeCA_{-d6}$ (IS)	99.0	1.6
MeBA	96.0	6.6
MeSA	132.8	5.8
MeACC carbamate	79.3	3.8
MeCA	97.7	4.4
MeAz	84.5	5.2
MeSA carbonate	67.4	5.8
MeJA	91.9	3.3
MeIAA	99.1	4.0
Linoleic acid, Me Ester	55.7	4.9
Linolenic acid, Me Ester	86.1	10.5
MeABA (cis)	102.1	3.9
MeABA (trans)	109.8	3.6
MeOPDA (isomer 1)	55.6	15.7
MeOPDA (isomer 2)	59.4	14.8

Conclusion

Using UFMS triple quadrupole technologies enabled a rapid and robust method to be developed to detect a selected range of phytohormones. No single technique achieved the best detection limit across all 11 quantified phytohormones. MRM detection limits in EI mode were superior to currently published protocols, which are optimised for a smaller ranges of chemistries.
Plant samples showed variable basal levels across the target analytes. Calibrations were linear over 5 orders of magnitude and detection limits were biologically relevant and linear across the large concentration range expected in plant samples.
In addition to a highly sensitive targeted MRM method, full scan data can still monitor other small organic acids and amino acids (eg. Figure 3). Future extraction protocols will be extended to cover a wider range of plant types and materials.

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