PO-CON1345E

SHIMADZU
Excellence in Science

Use of CI and EI for enhanced selectivity and sensitivity for the analysis of phytohormones

ASMS 2013 WP-304

Catherine Rawlinson[1], Lars Kamphuis[2], Paul Wynne[4,5], Karam Singh[2], Riki Kitano[3], Robert Trengove[1], Bruce Fraser[4,5]
Shimadzu Australasia, Rydalmere, New South Wales, Rowville, Victoria, AAUSTRALIA and Palmerston North NEW ZEALAND

Use of CI and EI for enhanced selectivity and sensitivity for the analysis of phytohormones

1. Overview

Several approaches were evaluated to discern the best selectivity and sensitivity for the analysis of plant hormones. Twelve target hormones were derivatised and analysed using single quadrupole and triple quadrupole gas chromatographic technologies. Electron ionisation (EI) and chemical ionisation (CI) – using methane, iso-butane and ammonia as reagent gases - were also assessed for their selectivity.

2. Introduction

- Phytohormones regulate many cellular processes including normal growth and development of the plant and defensive responses to biotic and abiotic stresses.
- Current analytical methods have limited capacity for diverse plant hormone analysis due to intensive extraction protocols required for biologically relevant detection limits.
- Volatility of several phytohormones has led to many LC based protocols, where removal of water is not of primary concern.
- Methyl chloroformate is a derivatisation agent especially suited to the esterification of small organic and amino acids in the presence of water.
- Here we describe the analysis of a range of plant hormones using several GC based techniques.

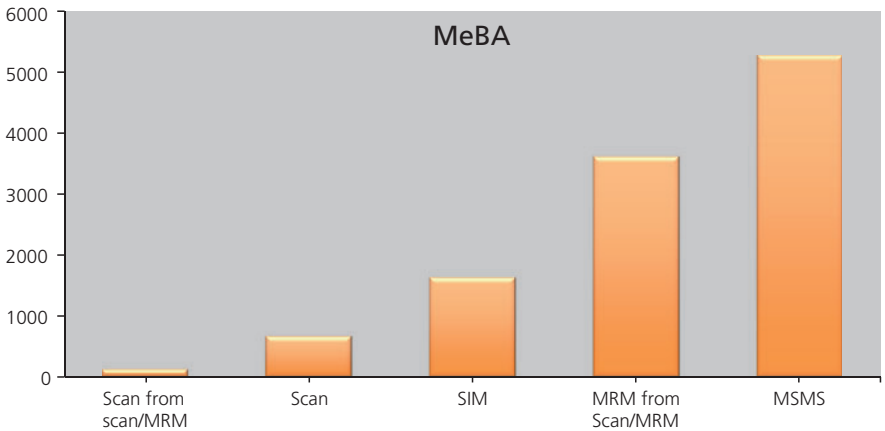

Fig. 1 Signal to noise (S/N) of Methyl Benzoate at 10 ng/mL using full scan, SIM, MRM and full scan/MRM

Use of CI and EI for enhanced selectivity and sensitivity for the analysis of phytohormones

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, cinnamic acid (MeCA), Salicylic acid (MeSA, 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	MSMS	Scan	SIM	MSMS	Scan	SIM	MSMS	Scan	SIM	MSMS
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

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

3. Methods - Extraction

- Ground fresh plant material was suspended in a basic aqueous/methanol solution and derivatised to their methyl ester form using methyl chloroformate. Methyl esters were extracted using chloroform.

Analytical method

- All data obtained using the TQ 8030 triple quadrupole mass spectrometer in both EI and CI mode (Shimadzu Scientific, Japan)
- Column – VF - 5 ms 30 m × 0.25 mm × 0.25 µm + 10 m EZ-Guard (Agilent Technologies, Netherlands)
- Ramp - 40 °C (1 min hold) ↑ @ 20 °C/min to 320 °C (2 min hold)
- Inlet - S/SL, 3 uL injection with 40 psi pressure pulse. 250 °C inlet temperature with 1 mL/min flow.
- Mass Spectrometer – EI: Ion source @ 200 °C. Full scan: 40 - 400 amu. SIM: Ions chosen using pure standards (Sigma-Aldrich, Missouri), see table x. MSMS: transitions optimised for best response/uniqueness, see table x. CI: Ion source @ 200 °C. Reagent gases: methane, iso-butane, ammonia. Full scan: 40 - 400 amu. SIM: Ions chosen using pure standards. MSMS: transitions optimised for best response/uniqueness.

4. Results

- SIM ions and MRM transitions optimised for best sensitivity and selectivity for electron and chemical ionisation.
- Detection limits determined by achieving and %RSD of less than 10% as a signal to noise greater than 50. %RSD was calculated using 10 replicates.
- CI mode could discern between the cis and trans isomers of Abscisic acid.
- Recoveries were calculated based on comparing spiked plant material vs unspiked material. Baseline was calculated on 10 unspiked samples.
- Good linearity for plant hormones exists over 5 orders of magnitude (data not shown).

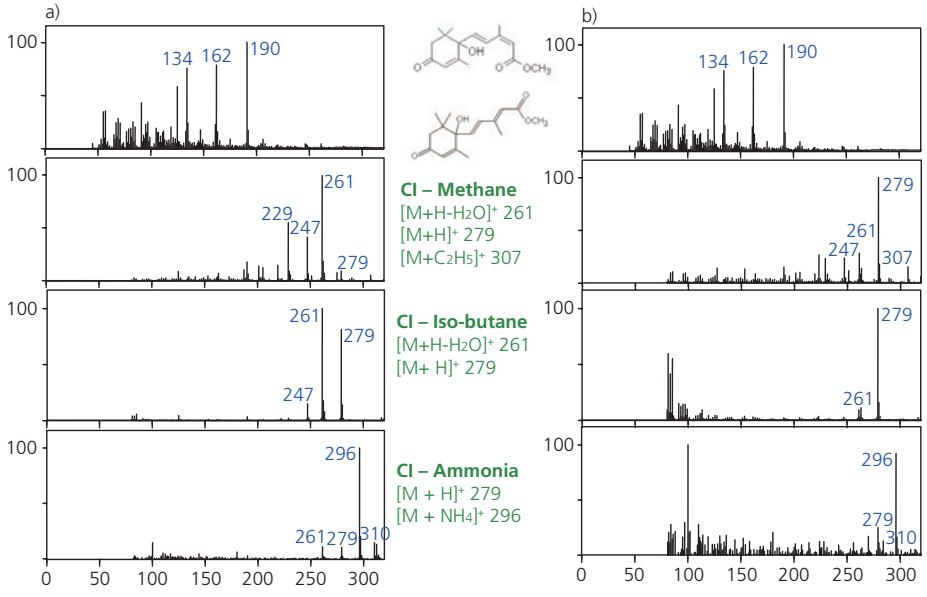

Fig. 2 Mass spectra obtained for the analysis of cis (a) and trans (b) Abscisic acid using EI and CI with methane, iso-butane and ammonia as reagent gases

5. Conclusions

- A quick and easy extraction protocol was developed for the analysis of volatile and semi-volatile plant hormones.
- Detection limits were achieved that were biologically relevant as well as linearity across a large concentration range expected in plant samples.
- Future extraction protocols will be developed to validate recoveries across a wide range of plant types and materials.

6. Acknowledgements

Murdoch University would like to acknowledge and thank Shimadzu Scientific for the opportunity to validate and use the TQ 8030 GC-QQQ-MS.

First Edition: June, 2013

SHIMADZU

Shimadzu Corporation

www.shimadzu.com/an/