Current Standard Operating Protocols (SOP), NCBS-CCAMP MS-Facility Metabolomics –

Quantification of Plant Hormones

Purpose: To provide general guidelines for conducting the quantification of plant hormones using tandem triple quadrupole mass spectrometry.

Reagents: All solvents and reagents used are of LC-MS quality.

Protocol:

A. Preparation of Standards:

- Prepare the individual stock solutions (STDs) of each plant hormone (~1mg/mL) in methanol (Stock A). Prepare 10µg/mL stocks in methanol of BAP and Z, by taking the required amounts (~10µl) from Stock A.

- Prepare mixed stock of all seven STDs (Stock B): 5µg/mL (IAA, IPA, IBA, GA and ABA) and 0.5µg/mL (BAP and Z) in 100% methanol by taking ~5µl from Stock A of IAA,IPA,IBA,GA and ABA and ~50µl from the 10µg/ml stocks of BAP and Z.

- Prepare individual stock solution of ISTD, Tryptamine-D4 (~1mg/mL) in 50%MeOH (0.1%FA) (Stock C) and 10µg/mL stock in 50%MeOH (0.1%FA), by taking ~10µl from Stock C.

- Prepare 2.5µg/mL stock (Stock D) of ISTD in 50% methanol (0.1%FA), by taking ~250µl from 10µg/ml stock.

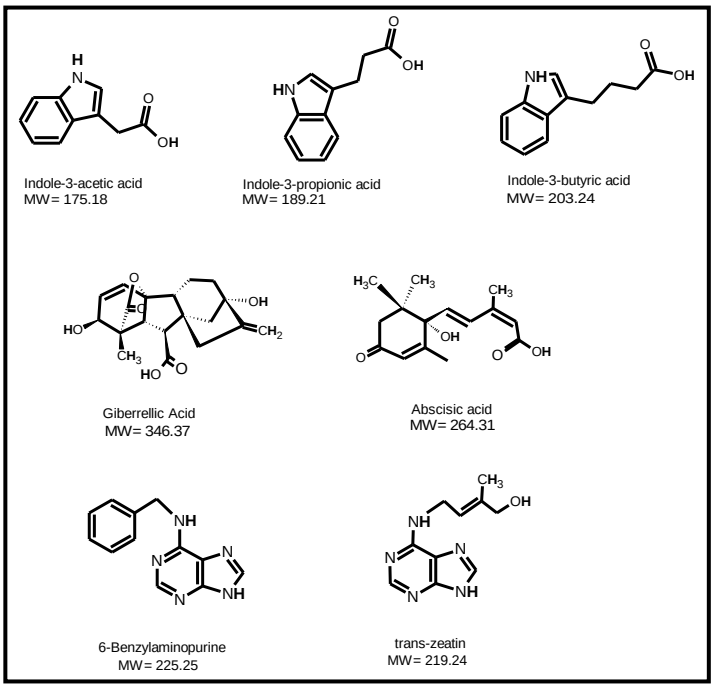

Figure 1: List of seven plant hormones

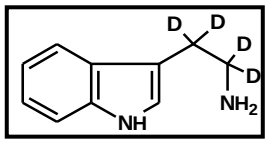

Figure 2: Internal standard- Tryptamine-D4.

- Take 10µL each from Stock B and Stock D and dilute it to 50µL of 5% MeOH. Transfer this into an HPLC vial and place it in the auto sampler for analysis.

B. LC-SRM Analysis:

- Equilibrate the Shim-pack ODSIII column (Phenomenex 2µ, 2 X 150 mm) with 5%acetonitrile.

- Use the mobile phase solvents A: water (10 mM ammonium acetate, 0.1 % FA), B: Acetonitrile (0.1% FA), and a flow rate of 200 µL/min for analysis.

- Set the following gradient (0 to 2 min-5 % B, 2 to 13 min- 5 to 90% B, 13 to 15 min-90% B, 15 to 15.1 min- 90 to 5% B, and 15.1 to 20 min-5% B) in the LC system.

- In the MS set the source parameters like spray voltage, (+ve): 3500 V and (-ve): 2500V; source temperature, 30º C; ion transfer capillary temperature, 270 °C; collision gas argon, S-lens voltage- as per table 1; sheath gas-20 and auxiliary gas-10; and scan time-50 milli sec for each transition. Set the following injector settings: 0-3 min: waste, 3-15 min: load, 15-20 min: waste.

- Select the most intense product ion and the corresponding collision energy and S-lens voltage of each transition for the LC-SRM analysis as shown in table 1.

Table 1: SRM Table for plant hormones analyzed in the method

S. No.	Plant Hormone	Polarity	Precursor ion (m/z)	Product ion (m/z)	Collision Energy	S-lens Voltage
1	Indole-3-acetic acid	+ve	176.1	130.08	16	60
2	Indole-3-propionic acid	+ve	190.32	130.07	18	63
3	Indole-3-butyric acid	+ve	204.2	186.07	13	68
4	Zeatin	+ve	220.12	136.06	18	82
5	6-Benzyl aminopurine	+ve	226.2	91.07	27	89
6	Abscisic acid	+ve	247.2	229.14	10	77
7	Gibberellic acid	-ve	345.2	143.13	40	93
8	Tryptamine-D4*	+ve	165.2	148.13	12	36

*Tryptamine-D4 is the Internal Standard but not a plant hormone

- Inject 10 µL of the sample (1-10ng on column) for the actual analysis.

- The expected result is shown in the following figure 3.

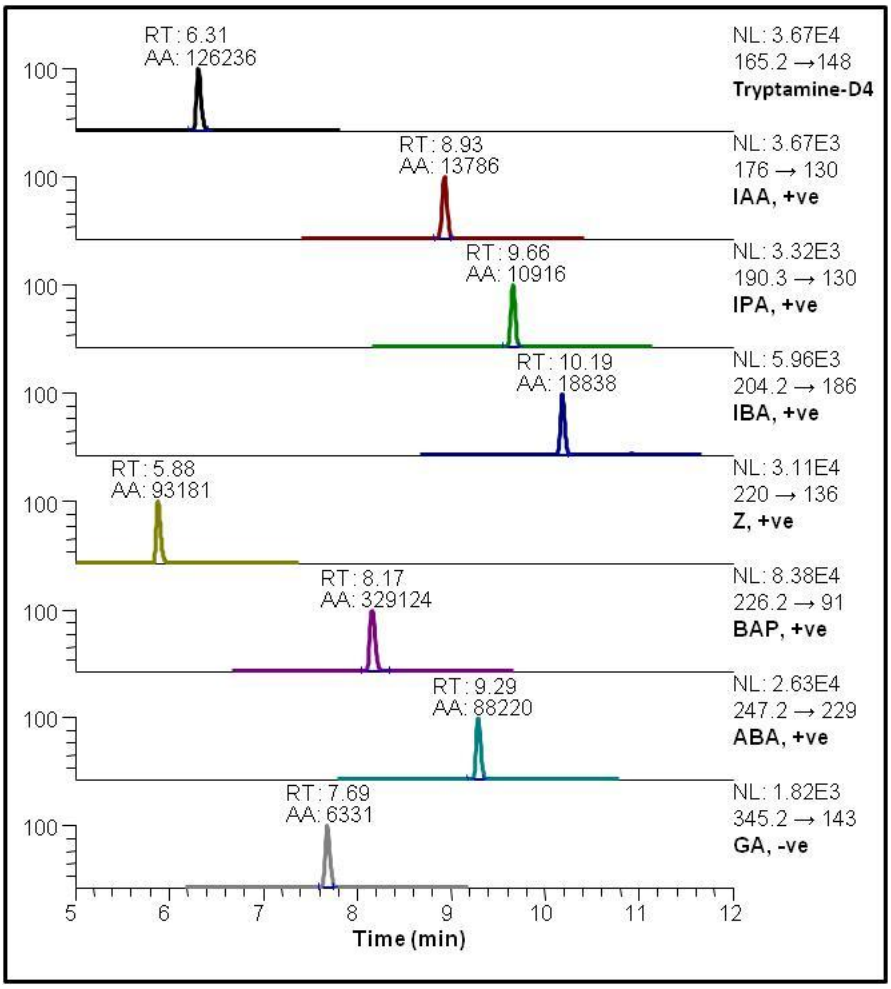

Figure 3: LC-MS/SRM chromatogram of plant growth hormones.

C. Preparation of samples for analysis:

- Obtain the extract as a lyophilized powder. Solubilize the extract in 5%MeOH (approximately 7ml for 2g of extract).

- Vortex the sample for 5mins and then sonicate (bath) for 3mins.

- Centrifuge at 4000rpm for 10mins. Separate the supernatant and spike with ISTD, 10µl from Stock D.

- Perform clean-up of the spiked supernatant by loading it onto a Strata-X 33u Polymeric Reverse phase cartridge (30mg/1ml).Before loading, condition and equilibrate the cartridge with methanol and 0.1%FA in water. Load the sample (7x1ml). After loading, wash twice with 0.1%FA in water and once with 5%MeOH.

- Finally, elute with 1ml 0.1%FA in acetonitrile and dry in a speed vac.

- Reconstitute the residue in 50µl of 5%MeOH and transfer to an HPLC vial and place it in the auto sampler for analysis.

- Inject 10µl for actual analysis.