

LSOP Title	Phytohormone extraction
LSOP No.	LSOP06
Version	1.1
Location	UQ Node/Centre-wide
Policy/Procedure Link	UQ- Equipment OGTR
Risk Assessments	
Approved by	Lindsay Shaw
Date Approved	13/10/2021
Date Effective	02/06/2021
Next Review Date	02/06/2022
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1.0 Scope

This procedure covers the extraction of phytohormones for four plant hormone classes: cytokinin, auxin, abscisic acid and gibberellin from plant tissues.

2.0 Definitions



AcOH – Acetic Acid

ISTD – lipid internal standards



MeOH – Methanol



MeCN - Acetonitrile

LC-MS - Liquid chromatography–mass spectrometry

C18 Sep Pak Cartridge - Adsorbs analytes

Speed Vac – Speed vacuum

3.0 Materials and Equipment

1. Eppendorf Tubes



2. Steel beads

3. Liquid Nitrogen



4. Scalpel or Scissors

5. Extraction Solution



6. Methanol (MeOH)

7. Acetic Acid (AcOH)

8. 2010 Geno/Grinder

9. Weigh balance

10. Centrifuge

11. Speed Vacuum

12. C18 sep pak cartridge

13. LC-MS

14. Vertex pipette tips without filters

4.0 Prescribed Actions

First Day

1. Weigh 1.5 ml Eppendorf tubes with steel bead inside.



2. Harvest samples and immediately put them in liquid nitrogen

3. Measure the weight and calculate the weight of plant tissues for extraction.

4. Homogenize frozen plant tissues with the 2010 Geno/Grinder (SPEX SamplePrep, USA) under 4 °C



5. Add 1 mL extraction solvent (80% MeCN with 1% acetic acid (AcOH) in Milli-Q water) containing 5 µL ISTD working solution. Refer to Cao et al. 2020 for details on how to prepare ISTD working solution. Prepare fresh solvent before grinding samples and store in 4 °C until ready to use.

6. Vortex

7. Leave at -20°C for 5 min (only necessary if extracting RNA) and then centrifuge at 15,900 rcf for 10 min under 4°C

8. Transfer supernatant to new Eppendorf tubes and extract RNA from the pellet
9. Evaporate supernatants using speed vac at room temperature (~3 hours)

Second Day



10. Add 1 mL 1% AcOH to samples and quickly vortex and spin down.
11. Using a vacuum manifold in the fume hood, wash C18 sep pak cartridge (Waters, USA) with 1 ml 100% MeOH ensuring that vacuum doesn't pull liquid through too rapidly. Always leave a small volume of liquid at bottom of cartridge preventing it from drying out for all steps except elution of sample.



12. Activate C18 cartridge by 1 ml 1% AcOH/Water.

13. Load samples on column



14. Wash column with 1 mL 1% AcOH/water



15. Change collection tubes and elute with 2 mL 80% MeCN with 1% AcOH

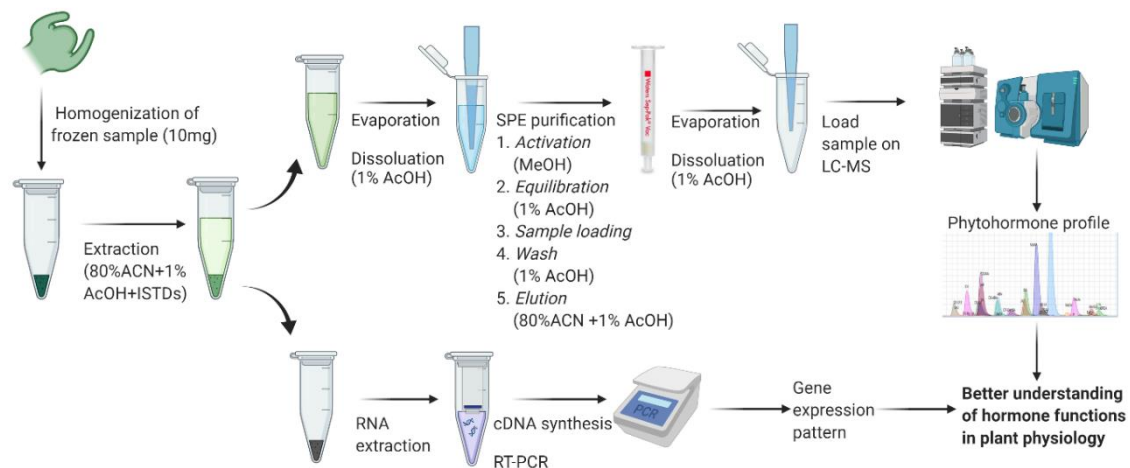
16. Evaporate in speed vac at room temperature (~4 hours)



17. Dissolve with 20 µL or 50 µL 1% AcOH/water

18. Store sample in -80°C before loading on LC-MS

5.0 Appendix



NB: Image has ACN as acetonitrile abbreviation – which is more commonly known as MeCN as referenced in the protocol