Metabolite: Isolation from xylem

Supplies and Reagents:

Methanol

Water

SIL stock

Important Notes:

SIL stock are separated in two kinds: non-CoA and CoA cocktail

Protocol:

Non-CoA compounds extraction

- 1. Weight out 50 mg xylem into a prechilled 2 ml microcentrifuge tube.
- 2. Add water (1 ml) and the synthesized SIL (50 ul).
- 3. Vigorously vortexed for 3 min.
- 4. Heat at 60 C for 10 min
- 5. Centrifuge at 10,000 g for 10 min at room temperature
- 6. Separate the supernatural (1) from the tube
- 7. Freeze dry the supernatural
- 8. Add 0.5 ml methanol to the microcentrifuge tube
- 9. Vigorously vortexed for 3 min.
- 10. Heat at 60 C for 10 min
- 11. Centrifuge at 10,000 g for 10 min at room temperature
- 12. Separate the methanol extracts from the tube
- 13. Evaporate the methanol

CoA compounds extraction

- 1. Weight out 50 mg xylem into a prechilled 2 ml microcentrifuge tube.
- 2. Add water (1 ml) and the synthesized SIL (50 ul).
- 3. Vigorously vortexed for 3 min.
- 4. Keep the tube in the room temperature for 10 min
- 5. Centrifuge at 10,000 g for 10 min at room temperature
- 6. Separate the supernatural (1) from the tube
- 7. Freeze dry the supernatural