

HPLC

Materials

1. HPLC
2. Pipets
3. Samples
4. Buffers
5. Sonicator
6. Column
 - a. XTerra MS C18 by Waters Inc.
7. ..

Protocol

1. Make stock solution of compounds being tested (IMI + NADPH)
 - a. 2mM NADPH + 5mL H₂O
 - b. 2mM IMI + 5mL H₂O
 - c. Filter through 0.45 um paper
2. Make resuspension buffer
 - a. 50mM Tris
 - b. 150mM NaCl
 - c. 0.5mM DTT
3. Pellet cells + resuspend in resuspension buffer
4. Add 10uL protease inhibitors
5. Sonicate cells at 1W for 10 sec/sample, four times each sample
6. Make mixtures (IMI, IMI+NADPH, cell lysate alone, etc..)
7. Incubate at 37degree for 1:00 hour
8. Centrifuge samples at 21,000g for 25 min
9. Filter 0.6mL sample through 0.1um paper (with syringe)
10. Inject 90uL and run HPLC at 0.7mL/min
 - a. Detect IMI and IMI metabolites at 270nm and 265nm with UV