

# UPLC of Porphyrins

## ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC)

### QUANTITATION OF PORPHYRINS

1. Inject 10 $\mu$ L of supernatant solution of porphyrins in 1.5M HCl into a Waters Acquity UPLC system which includes a binary solvent manager, sample manager, fluorescence detector, column heater and an Acquity UPLC BEH C18, 1.7  $\mu$ M, 2.1 x 100 mm column.
2. Set the fluorescence detector at 404nm excitation and 618nm emission. Keep the sample chamber dark at ambient temperature. Solvent A is 0.2% aqueous formic acid while Solvent B was 0.2% formic acid in methanol.
3. Set the flow rate at 0.40 mL per minute at 60°C for the total run time of 8 min.
4. Use the following successive gradient settings for run time in minutes versus
  - A: 0.0, 60%
  - 2.0, 20%
  - 2.5, 1%
  - 3.5, 1%
  - 3.6, 60%
5. Set the solvent composition gradient from 0.0 to 2.0 min as Waters Gradient 5 (convex with a higher slope at 0.1 min compared to that at 2.0 min).
6. Keep all other gradients are linear.

Use solutions of known concentrations of authentic porphyrins dissolved in 1.5M HCl as standards (Porphyrin Acids, Chromatographic Marker, Product # CMK-1A, Frontier Scientific, Logan, Utah).

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