

**Protocol for ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY
(UPLC) QUANTITATION OF PORPHYRINS
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Inject 10 μ 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 μ M, 2.1 x 100 mm column. 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. Set the flow rate at 0.4 mL per minute at 60 $^{\circ}$ C for the total run time of 6 min. 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. Set the solvent composition gradient from 0.0 to 2.0 min as Waters Gradient 5 (convex, with decreasing slope of %B from 0.0 to 2.0 min). 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).