Protocol for ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC) QUANTITATION OF INTERMEDIATE PORPHYRIN ISOMERS

Hector Bergonia 03-26-20

Inject 10µL 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 µM, 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 1M formic acid adjusted to pH 5.16 with glacial acetic acid, while Solvent B was 0.2% formic acid in methanol. Set the flow rate at 0.4 mL per minute at 60˚C for the total run time of 12 min. Use the following successive gradient settings for run time in minutes versus %A: 0.0, 80; 5.0, 40; 5.5, 5; 7.5, 5; 8.0, 80. Set the solvent composition gradient from 0.0 to 5.0 min as Waters Gradient 7 (concave, with increasing slope of %B from 0.0 to 5.0 min). Set the solvent composition gradient from 5.0 to 5.5 min as Waters Gradient 5 (convex, with decreasing slope of %B from 5.5 to 5.5 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).