HEMIN, PROTOPORPHYRIN IX AND ZINC PROTOPORPHYRIN IX EHTYL ACETATE / ACETIC ACID EXTRACTION AND UPLC

Solvent Extraction

Prepare extraction solvent (EA) by mixing four volumes of ethyl acetate to one volume of glacial acetic acid.

Dilute sample homogenates with homogenization buffer to about 10 mg protein/mL or less.

Mix 50µL adjusted sample homogenate with freshly prepared 200µL EA. Vortex vigorously for one minute.

Centrifuge at 16000xg for 0.5 min. Collect the resulting supernatant, which is around 90% of the total volume.

Quantify by UPLC (ultra performance liquid chromatography).

UPLC Quantitation.

Inject 10µL of the supernatant solution above into a Waters Acquity UPLC system which includes a binary solvent manager, sample manager, photodiode array detector (PDA), fluorescence detector (FLR), column heater and an Acquity UPLC BEH C18, 1.7 µM, 2.1 x 100 mm column. Set the PDA to measure hemin absorbance at 398nm and the FLR to measure fluorescence of protoporphyrin IX (PPIX) at 404 nm excitation and 630 nm emission and of Zn protoporphyrin IX (ZnPPIX) at 406 nm excitation and 586 nm emission. Keep the sample chamber dark and 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 0.40 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%; 7.5, 1%; 9.5, 1%; 10.0, 80%. Set the solvent composition gradient from 0.0 to 7.5 min as Waters Gradient 5 (convex with a higher slope at 0.0 min compared to that at 2.0 min). Keep all other gradients are linear.

For standards, extract solutions of known concentrations of authentic hemin, PPIX and ZnPPIX dissolved in 1% aqueous trimethylamine or 0.1M NH_4OH.