

HPLC protocol for supernatant samples – juni 2019

- Take a supernatant sample of 200 μ l
- Add 20 μ l ice-cold 35% PCA
- Vortex
- Put on ice for 10'
(Samples can be instantly frozen (N₂ (l)) after this step if needed and then put in the -80°C freezer; before next step thaw samples on ice)
- Add 21 μ l of ice-cold 5M KOH in 0.2M MOPS
- Vortex
- Put on ice for 10'
- Spin down at maximum speed, 4°C, 10'
- Filter supernatant through a 0.2 μ m filter and put in a HPLC vial
- Make sure that there are no bubbles in the HPLC vial

We used Shimadzu LC20-AT with a UV + RI detector and a Rezex (Phenomenex) column at 55 °C. The flow of the eluent (5 mM H₂SO₄ in milli-Q) was 0.5 mL/min.