# High Performance Liquid Chromatography

University of Exeter iGEM 2017

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# Protocol:

1. Make up a 50ml solution of 4% mannose (by mass), and serial dilute with a 50:50 water mannose dilution down to 0.03125%, to make a standard curve.
2. Pipette 100µl of mannose samples from the "Testing the Effectiveness of the Metal Binding Reactor Protocol" and from the calibration curve into a 1.5ml Eppendorf for each sample.
3. Pipette 900µl of 10mM sulphuric acid (H2SO4) into the Eppendorfs.
4. Spin down samples using a centrifuge at 16,100rcf, for 10 minutes at 4°C.
5. Pipette the solution into an HPLC vile, and freeze at -20°C until required.
6. When all the samples are ready, they should be run through the HPLC in the following order:

- 1. Maximum concentration (4%) × 1
- 2. Minimum concentration (0.03125%) × 3
- 3. In order from second lowest (0.0625%) to second highest (2%)
- 4. Test samples from "Testing the Effectiveness of the Metal Binding Reactor Protocol"

Some samples from the standard curve should also be run during the test samples to ensure the standard curve has not changed. Blank water samples should also be run through at regular periods to prevent blockages.