**Desalting using Sep-Pak C18 Cartridge**

1. Resuspend sample in 500 ul Velos buffer A (95% H20, 5% ACN, 0.1% formic). Add an additional 20 ul formic acid to ensure sample is acidic (can check using pH paper). Water bath sonicate for 5 minutes.
2. Condition cartridge by adding 1 mL 100% ACN (x3)
3. Equilibrate by adding 1 mL Velos buffer A (95% H20, 5% ACN, 0.1% formic) (x3)
4. Load sample slowly, 1 drop/sec, take flow through and re-load sample (slowly) again.
5. Desalt by passing 1 mL 95% H20, 5% ACN, 0.1% formic (x3)
6. Elute by adding 1 mL 80% acetonitrile/0 1% FA. Blow cartridge dry
7. Speed vac the elution
8. Resuspend sample in 500ul 95% H20, 5% ACN, 0.1% formic to load on HPLC for offline high pH fractionation.

**HPLC fractionator -- Use TMT\_wash method**

1. Resuspend sample in 500 ul Velos buffer A (95% H20, 5% ACN, 0.1% formic). Water bath sonicate for 5 minutes and spin down at top speed in a centrifuge for 1 minute. Inspect to ensure no particulate matter is present.
2. Wash the column in 80% Buffer B, and then equilibrate in 100% Buffer A
   * 1. Important make sure the pressure responds to the buffer changes. 100% A should be ~116 bar, whereas 80% B should be ~66 bar.
3. Prepare 96 well 1mL deep plates by adding 20uL of 20% formic acid.
4. Switch the injector valve to the load position for the loop (up position).
5. Wash the syringe with Velos Buffer A (95% water, 5% ACN, 0.1% FA) at least twice.
6. Wash the loop with Velos Buffer A (95% water, 5% ACN, 0.1% FA) at least twice.
7. Reset fraction volume on the fraction collector.
8. Reset the fraction collector.
9. Load sample into 500uL loop.
10. Submit sample, and switch to inject position when prompted.
11. Speed vac plate (with balanced plate) and resuspend in Buffer B (80% ACN, 20% water 0.1% FA), combining each column into a single sample.
12. Speed vac concatenated samples and now ready for resuspension and injection onmass spec.