

BrMT Protocol

Materials

-X grams of derivative BrMt (take spectra) from spectra determine appropriate concentration to load unto column. See vial...

- C18 BioBasics Column
- Shimadzu HPLC
- Nanodrop Spec.
- ~200uL 5% Acetonitrile, .1% TFA solution

Method

- 1.) Blank the Spectrophotometer using water and loading 2 uL of solution. Record and measure blank after performing "blank" command.
- 2.) Load BrMT sample, ~ with a concentration greater than 20ug/ mL. (***Jon should we attempt this in several steps? 20, 50, 100 ug/ mL?***)
Monitor the absorbance at the following wavelengths: 240nm and 280nm.
- 3.) After determining the **max absorbance**, and determining the optimal loading concentration for the C18 ~ greater than 20ug /mL (consider UV/ Spec limitations on HPLC).
- 4.) Prepare 100uL solution of 5% ACN, .1% TFA, with 1uL (X conc.) in 100uL of the BrMT derivative; take into account this is a 1:100 dilution when determining optimum absorbance parameters on the detector for the HPLC.
- 5.) Program the HPLC to perform the following functions,

After running the HPLC pumps at 5% Solvent B for 15mins, while in "inject" mode on the sample loop, and allowing the UV Deuterium lamp to warm up for 30 mins. You are now ready to perform the run. Ensure that the fraction collect is sampling @ every minute, 1mL fractions. Vials should be appropriately programmed as fit on collector rack. Time-based collection, for preliminary runs.

Run for 2 mins @ 5 % Solvent B.

Up the gradient to 40% in 2 mins.

Increase the gradient further to 70% Solvent B over 30 mins.

Increase it further to 95% in 2 mins, and then hold at 95% for 2 mins.

Total Run Time: 36 mins

Analysis

Using the injection volume and sample's concentration be sure to determine the mole input ratio to mole output ratio as collected in the fraction collector.

Optimize loading amount of BrMT on the column.

Also determine the percentage of solvent B, where BrMT comes off and its appropriate collection vial from the collector

Next, optimize protocol to maximize resolution peaks for BrMT after preliminary runs.