Protocol for HPLC Analysis of Peptoids

Purpose: Equipment used to measure the peaks from the sample
Password: Breeze

1) Check solvent levels in ACN and Water with TFA. If levels are near bottom of labels it time to refill.
   To refill: Line A – DI water with TFA = 4L DI H_2O plus 4ml TFA
   Line B – New bottle of Acetonitrile plus 4ml TFA
   Keep float in bottom of bottles under solution level to prevent air from entering column.
   Filter solutions in both bottles after additions.

2) Select correct column. Prep column (to purify) large green ended column. Semi prep column (to purify) smaller chrome column. Attached to the right side of this column is a guard column. Close column that you change out by attaching the screws to the ends. Check the detectors inside the machine. There are 2 you can use. One is for semi-prep and the other is for analytical. Be sure you are using the correct one.

   Dissolve the lyophilized pellet with an 80%/20% solution of DI H_2O and Acetonitrile. Measure into tube containing sample ~2200 µl total with1700µl DI H_2O and 500µl Acetonitrile. Vortex to mix and dissolve pellet. Remove solution from peptoid tube and put solution into a 3ml syringe. Filter the solution into a 2ml vial. Make sure <u>no air is in the bottom of the vial or along the sides of the vial</u>. <u>Must have at least 2ml of solution in vial.</u>

3) Set machine. Put vial in position 1 (behind door at the bottom of the HPLC).
   Starting gradient – Pressure (100% A) water
   "Flow" (move arrow up to 10) for 10ml per minute for prep scale.
   Press "GO"
   Click top button – screen will appear (Specify Single Inject. Parameters)
   Input – Name (of peptoid)
   Method (80/20 prep)
   Inject volume (2000 µl)
   Run time (80 min.)
   Press (inject)

4) Collect samples into glass tubes to ~ 1/3 full. Keep a list of tube numbers used for each peak of sample collection.
   Let the program continue to the end of the 80 minute setting. This will automatically wash out the lines and will stop at the end of the timed setting. Base line is flat when finished.

5) If you manually stop the program. Press the 3rd button down from the top on left side of screen. Press "stop pump
   symbol" . Wash lines.
   Ready for Mass Spec. selection of samples.
   Prepared by: Rosemary Wilson Date: 8/1/05