SOP: SP026
3/21/17

Operation of Waters 2535 HPLC System

Materials and Reagents: (note 1)

1. Endotoxin-free water
2. 20% ethanol (made from absolute ethanol)
3. HPLC column
4. Appropriate buffers for column being used
5. 10 ml syringe
6. 8. Injection needle
7. 9. Compressed helium cylinder
8. 10. Waters 2535 HPLC
9. Computer running Empower software
10. 11. Detector (either UV or RI)
11. 12. Fraction collector

Protocol: (note 2)

Setup and Priming the Lines

1._____ Turn on the HPLC. Turn on the Helium source at the knob on the tank.

2._____ Open the Empower program on the computer (note 3). Username: Doboslab (no password).

3._____ Select "Run Samples" and open the folder that is appropriate for the sample being analyzed. HPLC control panel is located on the lower left corner of the screen.

4._____ Right click on the control panel and chose "Set Sparge." Click on the "Enable Sparge" button (note 4).

5._____ Rinse each line in a beaker with filtered endotoxin free water and then place in a bottle containing filtered endotoxin free water (note 5).

6._____ Ensure that the waste container is not full.

7._____ Right click on the control panel, set flow scale to small, then choose "Prime Solvents." Prime the lines to be used with filtered endotoxin free water. Then run "Prime Seal Wash."

8._____ Click on the "Flow Rate" and set between 2-5 ml/min to wash the sample loop and all HPLC lines into water.

Running

1._____ Attach the column(s) (note 6).

2._____ Wash the column in water to remove the storage buffer as per protocol.

3._____ Prime lines in their appropriate buffers.

4._____ Equilibrate column in the start buffer as per protocol.

5._____ Select the appropriate method set, injection volume, and run time for your column (note 7).

6._____ Draw your sample into a 10 ml syringe and attach the injection needle. Be sure to expel any air bubbles from the syringe and needle (note 8).

7._____ Click on the inject icon.

8._____ On the HPLC, flip the injection lever to load.

9._____ Insert the needle completely, and inject your sample into the HPLC, then flip the lever to inject and hit start on the fraction collector (note 9).

10._____ When the run is complete, prime all lines in water and wash the column in water.

11._____ Prime the lines and wash the column in 20% ethanol for storage (unless otherwise stated in the column instructions, note 10).

12._____ Remove the column and prime the lines in 10% methanol. Leave the lines for storage.

13._____ Turn off the HPLC pump, detector, and helium cylinder.

Notes:

1. All buffers used for the HPLC must be filtered through a 0.45µm filter.
2. This SOP is to be used as a reference tool only. You MUST be trained by lab personnel before use of this machine.
3. In addition to being trained on the use of the HPLC, you must also receive training on the use of the Empower program.
4. The sparge default is 20 minutes at 100%, followed by 20% (intermittent) sparge. This will degas buffers, as well as ensuring that no residual buffer is trapped in the lines before transferring from one buffer to another.
5. You only need to proceed with the lines being used. All others can be left in their storage buffer.
6. It is important to avoid introducing air into the columns. Keep the HPLC running at 0.25 ml/min and introduce one drop at a time before connecting. If the column was stored with the spring syringe, keep this attached until the column is connected to the HPLC.
7. The instrument method will determine the length of the run, however setting the run time for a sample will determine how long the computer collects data.
8. All samples should be filtered through a 0.2 µm filter before injection on the HPLC.
9. If more than 10 ml of sample are required for one HPLC run, multiple injections will be necessary. In this case, begin your injections while the HPLC is still under manual control. Allow enough time between injections for the 10 ml injection loop to clear. Proceed to step 5 for the last injection.
10. If using a C18 or C4, flush column with filtered water for 2 column volumes to ensure salt removal and store column in 50% methanol.