

**SOP: SP025.3**

**Modified 4/21/2017 by MCL**

**Operation of Waters 600E 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. 20 ml syringe
6. 10 ml syringe
7. Injection needle
8. Compressed helium cylinder
9. Waters 600E HPLC
10. Computer running Empower software (optional)
11. Detector (either UV or RI)
12. Fraction collector

**Protocol:** (note 2)

Priming the Lines

1. \_\_\_\_\_ Set the sparge to 100 ml/min. This will ensure that no residual buffer is trapped in the sparge line.
2. \_\_\_\_\_ Rinse each line with water and then place in the appropriate buffer.
3. \_\_\_\_\_ Turn the sparge down to 20 ml/min.
4. \_\_\_\_\_ Turn the flow switch to the right.
5. \_\_\_\_\_ Set the flow to 2.00 ml/min.
6. \_\_\_\_\_ Attach a 20 ml syringe to the priming port.
7. \_\_\_\_\_ Lift the syringe so that the port is in the “prime” position and draw 20 ml into the syringe (note 3).
8. \_\_\_\_\_ Move the port into the “off” position, remove the syringe, and discard the buffer from the syringe into a waste container.
9. \_\_\_\_\_ Repeat draw twice more (steps 6-8).
10. \_\_\_\_\_ After the third draw, do not close the outlet. Tap the syringe to knock any air bubbles away from the front of the syringe.
11. \_\_\_\_\_ Turn the flow to 10.00 ml/min and inject approximately 10-15 ml back in, being sure not to introduce any air into the system.
12. \_\_\_\_\_ Close the outlet port and discard the remaining buffer.
13. \_\_\_\_\_ Set flow rate back to 2.00 ml/min.
14. \_\_\_\_\_ Check the flow to be sure that it is flowing evenly. If not, it may be necessary to repeat the priming.
15. \_\_\_\_\_ When done priming all lines, turn the flow switch back to the left.

Running

1. \_\_\_\_ Turn on the HPLC and detector. If the detector lamp is off, turn it on.
2. \_\_\_\_ Determine how many buffer lines will be needed to run the column of interest (note 4)
3. \_\_\_\_ On the Setup screen on the HPLC, enable each line to be used.
4. \_\_\_\_ Turn on the helium.
5. \_\_\_\_ Switch the screen over to Direct mode.
6. \_\_\_\_ Rinse and prime all lines in filtered endotoxin free water.
7. \_\_\_\_ Let ~100 ml water to run through the HPLC, periodically flipping the load/inject lever.
8. \_\_\_\_ Attach the column.
9. \_\_\_\_ Wash the column in water to remove the storage buffer.
10. \_\_\_\_ Prime lines in their appropriate buffers.
11. \_\_\_\_ Equilibrate column in the start buffer.
12. \_\_\_\_ On the HPLC computer, start up the Empower program (note 5).
13. \_\_\_\_ Select the appropriate method set, injection volume, and run time for your column (note 6).
14. \_\_\_\_ Click on the Inject icon. This should change the HPLC over to the Operate Program mode.
15. \_\_\_\_ 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 7).
16. \_\_\_\_ On the HPLC, flip the injection lever to load.
17. \_\_\_\_ 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 8).
18. \_\_\_\_ When the run is complete, prime all lines in water and wash the column in water.
19. \_\_\_\_ Prime the lines and wash the column in 20% ethanol for storage (unless otherwise stated in the column instructions, note 9).
20. \_\_\_\_ Remove the column and leave the lines in 20% ethanol.
21. \_\_\_\_ Turn off the HPLC pump, detector, and helium cylinder.

**Notes:**

1. All buffers used for the HPLC must be filtered through a 0.45 $\mu$ m 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. Be sure to keep the syringe even with the priming port. If the syringe is pulled too far in any direction, it will not create a good seal and air will be pulled into the syringe.
4. You only need to proceed with the lines being used. All others can be left in their storage buffer.
5. In addition to being trained on the use of the HPLC, you must also receive training on the use of the Empower program. If the Empower software is not available, gradients can be programed directly through the Waters 600 controller, however no data will be collected.
6. 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.

7. All samples should be filtered through a 0.2  $\mu\text{m}$  filter before injection on the HPLC.
8. 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 in Direct mode (before step 14). Allow enough time between injections for the 10 ml injection loop to clear. Proceed to step 14 for the last injection.
9. 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