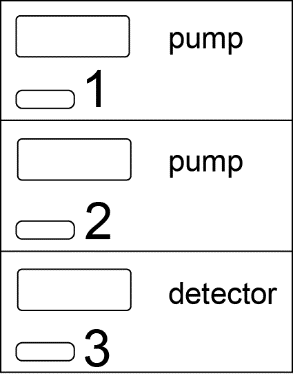
**Shimatsu HPLC Checking List**

* **Date (yyyy.mm.dd) \_\_\_\_.\_\_.\_\_**

**Check the Buffer A and B for last run**

|  |  |  |  |
| --- | --- | --- | --- |
| **Buffer** | **Composition 1** | **Composition 2** | **Filtered?** |
| **A** |  |  |  |
| **B** |  |  |  |

Usually A is water, B is 65% CH3CN.

## Start the Machine

In HPLC, Buffer A is for aqueous/running buffer and B is for storage or elution solution.

* Turn on the machine by following the order
* Turn the program (Unit 1)
* Until the sensor worm up

The System is originally in **B** which is 65% Acetonitrile (CH3CN) for storage the column. To begin with, we need to reduce the organic phase to running phase which is 5% CH3CN.

* Check the line, if there is no bubble, skip the following PURGE step
* **Check here if the PURGE steps are skipped.**
* Go to advance mode and check the pump label
* Set the flow rate to 0
* Set the pressure limit according to the limitation of the column type
* Record the back pressure or generate a notebook later.
* Purge A:



* + Open the purge by rotating the switch counter clockwise
  + Set the percentage of liquid A and B (here B is 0%)
  + Turn on the pump
  + Press “PURGE” on the machine. Then the machine will start to purge for 5min
  + Check everywhere for leaking. If anywhere is found, stop PURGE and doing trouble shooting
* Check the baseline of the machine
* Purge B:
  + Make sure the purge switch is opened by rotating the switch counter clockwise
  + Set B concentration (percentage) to 100%
  + Make sure the pump is on
  + Press “PURGE” on the machine. Then the machine will start to purge for 5 min
  + Check everywhere for leaking. If anywhere is found, stop PURGE and doing trouble shooting.
  + After finishing the PURGE, Set B concentration (percentage) to 0% (total A)
  + Close the Purge swtich by turn it clockwise (slightly tight)
  + Turn on the pump
  + Adjust the flow rate of the pump by following the order and monitor the pressure of the pump.
  + 0 🡪 [1min]🡪0.2🡪[1min]🡪0.5 🡪[1min]🡪0.75🡪[1min]🡪1 (ml/min)

## Replace the buffer to running condition

* **Running condition:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**<5% CH3CN>**

* Make sure the pump is on
* Adjust the percentage of B (CH3CN) to 100% in pump page
* Adjust the flow rate by following order

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Rate(ml/min) | 0.2 | 0.5 | 0.7 | 1 | 1.2 | 1.5 |
| Check |  |  |  |  |  |  |

**Now we want to run the gradient to 10% B which will be ~6.5% CH3CN**

* Reduce the concentration of B by 10% every 2 min until 10% B reached.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 100 | 90 | 80 | 70 | 60 | 50 | 40 | 30 | 20 | 10 |
|  |  |  |  |  |  |  |  |  |  |

* Slow the flow rate to 0

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Rate(ml/min) | 1.5 | 1.2 | 1 | 0.7 | 0.5 | 0.2 | 0 |
| Check |  |  |  |  |  |  |  |

## Purge to the running buffers

* Change buffer A to aqueous buffer
* Change buffer B to organic buffer

|  |  |  |  |
| --- | --- | --- | --- |
| Buffer | Component 1 | Component 2 | Filtered? |
| A | 0.1M NH4OAc | 5% CH3CN |  |
| B | 100% CH3CN |  |  |

* Open the purge switch
* If you have programmed protocol, check this box and Skip the following steps
* Set B to 50%
* Adjust total flow to 2 ml/min

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Rate(ml/min) | 0.2 | 0.5 | 0.7 | 1 | 1.5 | 2 |
| Check |  |  |  |  |  |  |

* Set the flow rate to 0

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Rate(ml/min) | 1.5 | 1 | 0.7 | 0.5 | 0.2 | 0 |
| Check |  |  |  |  |  |  |

* Change the percentage of B to 0%

## Replace the Column to starting condition

* After changing B to 0%, Close the purge switch then start the flow rate from 0 to 1.5 ml/min

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Rate(ml/min) | 0.2 | 0.5 | 0.7 | 1 | 1.2 | 1.5 |
| Check each 2 mins |  |  |  |  |  |  |

* Wait for the baseline stable. Here wash the column by 4 times column volumn (3 ml \* 4)
* Normalized the window when signal is flat. (Usually need 10 min)