PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE

Title:				
SOP No.:		**Department:**	Microbiology	
		Effective Date:		
Revision No.:	00	**Revision Date:**		
Supersede Revision No.:	Nil	**Page No.:**	1 of 7	

1. **Purpose:**

The purpose of this SOP is to lay down the procedure for Calibration of High Performance Liquid Chromatograph (HPLC).

2. **Scope:**

This SOP is applicable to Calibration of High Performance Liquid Chromatograph (HPLC) being used in Quality control Laboratory.

3. **Responsibility**: Quality Control Executive.

4. **Accountability:** Head of Quality Control

5. **Material and Equipments:**

HPLC, Column, Caffeine .

6. **Procedure:**

6.1. Operate the instrument as per the respective Operation SOP of HPLC.

6.2. Perform the following tests for every 6 months.

6.2.1. Calibration of pump. (Low pressure or High pressure gradient)

6.2.2. Calibration of Gradient proportionating valve (GPV).

6.2.3. High Pressure Gradient Accuracy

6.2.4. Calibration of Auto injector or Rheodyne injector.

6.2.5. Calibration of Detector.

6.2.6. Temperature calibration for Column oven and Sample cooler.

6.2.7. Auto Sampler Carry over.

6.3. Calibration of Pump:

A) Flow Accuracy

6.3.1. Take HPLC grade water and degas it by filtering through 0.45µ membrane. Insert the pump inlet suction filter into it.

6.3.2. Switch on the pump. Connect a 4 mts length and 0.1 mm ID resistance tube to the out let of the pump. Set the flow rate to 1.0 ml/minute and start the pump. In case of HPLC with Quartinary pump configuration, set equal flow rate all pumps.

6.3.3. Check all the connections for any visual leakage. If leakage is observed arrest it by tighting the nuts properly. Allow the flow to stabilize.

6.3.4. Observe the pressure fluctuation. The pressure fluctuation should be below + 2.0 % of the mean value.

6.3.5. Keep a dry, clean and pre-weighed 10ml volumetric flask at the outlet of the restrictor tube. Collect water for 5 minutes.

6.3.6. Weigh the volumetric flask with water. The difference in weight gives the weight of water collected for 5 minutes.

6.3.7. Repeat the collection and weighing for 5 more times as explained above.

6.3.8. Calculate flow rate by the formula $\frac{W}{5 \times 0.99602}$ Where 0.99602 is Density of water at 25°C

6.3.9. Convert all the six observations from weight to volume and calculate ml/min for all the six observations.

6.3.10. Criteria for flow should be with in + 2 % of the set value.

6.3.11. Repeat the same manner for 2.0ml/min flow rate also.

6.3.12. Calculate the Relative standard deviation for each of the above six observation Should not be more than 1.0 %.

B) Flow precision:

6.3.13. Flow precision is confirmed by checking the relative standard deviation for the retention time of the analyte peak for six replicate injections and it should not be more than 1.0 % (Refer Rheodyne injector- Volume precision).

6.4. Calibration of Gradient Proportioning Valve (GPV):

6.4.1. Take mobile phase-I as HPLC grade water and prepare mobile phase-II as 0.05 %v/v aqueous Acetone solution. Degas them separately.

6.4.2. Keep the mobile phase-I as reservoir for A port and mobile phase-II as reservoir for B port.

6.4.3. Connect one end of a resistance tube of 4mts length and 0.1 mm ID to the outlet of the pump and the other end to the inlet of the detector.

6.4.4. Set the instrument parameters as flow rate 2ml/minute, wave length 254 nm and stop time 18 minutes. Set the lowest possible injection volume.

6.4.5. Check that there is no leak in the connections. Set the gradient condition as per the table-1 given below and equilibrate the system.

6.4.6. Calculate the percentage of height ratio for the second (A/B/C) and third peaks (A/B/D) with respect to first peak (C/D). The values thus obtained should be between 9.5 % and 10.5 %.

Table-1

Time	Flow rate (ml/min)	%A	%B	Gradient
Intial	2.000	50	0	Step
2.00	2.000	0	50	Step
6.00	2.000	50	0	Step
10.00	2.000	45	10	Step
12.00	2.000	50	0	Step
14.00	2.000	45	0	Step
16.00	2.000	50	0	Step
18.00	2.000	50	0	Step

6.5. High Pressure Gradient Accuracy:

6.5.1. Take mobile phase-I as HPLC grade water and Prepare mobile phase-II as 0.05 %v/v aqueous Acetone solution. Degas them separately.

6.5.2. Keep pump A suction line in mobile phase-I and pump B suction line in mobile phase-II.

6.5.3. Connect one end of a resistance tube of 4mts length and 0.1 mm ID to the outlet of the pump and the other end to the inlet of the detector.

6.5.4. Set the instrument parameters as flow rate 2.0 ml/minute, wave length 254nm and stop time 14 min. Set the lowest possible injection volume.

6.5.5. Check that there is no leak in the connections. Set the step gradient condition as per the table-2 given below and equilibrate the system.

6.5.6. Calculate the percentage of height ratio for the second peak (A/B) with respect to first peak (B). The value thus obtained should be between 49.0 % and 51.0 %.

Table-2

Time	Flow rate (ml/minute)	%A	%B
Initial	2.00	100	0
2.00	2.00	0	100
4.00	2.00	50	50
8.00	2.00	100	0
10.00	2.00	100	0

6.6. Calibration of Injector:

6.6.1. Volume Accuracy:

6.6.1.1. Take HPLC grade water in one of the vial and stoppered with septa.

6.6.1.2. Weigh the vial with water.

6.6.1.3. Place the vial in the sample compartment. Set the system to inject 10 injections with 20µl- injections volume.

6.6.1.4. Take the vial and reweigh it.

6.6.1.5. Difference in weight gives the weight of water drawn by the auto injector.

6.6.1.6. Calculate the quantity with drawn by the auto injector per injection.

6.6.1.7. Calculate the volume with drawn by the auto injector per injection by the formulae w/10 x 0.99602, Where 0.99602 is density of water at 25°C

6.6.1.8. The volume of water drawn by the auto injector per injection should be within 2.0 % of the set value.

6.6.2. Volume Linearity

6.6.2.1. Take HPLC grade Methanol and degas it by filtering through 0.45µm membrane filter.

6.6.2.2. Connect 250 mm x 4.6 mm, 5µ, C18 (or Equivalent) and keep the mobile phase in the reservoir.

6.6.2.3. Set the instrument parameters as flow rate 1ml/minute, wavelength 272 nm, stop time 5 minutes.

6.6.2.4. Check that there is no leak in the connections. Equilibrate the system.

6.6.2.5. Prepare 5mg/100ml of Caffeine solution in methanol (solution A).

6.6.2.6. Make one injection of solution A at each injection volume of 5µl, 10µl, 15µl, 20µl and 25µl.

6.6.2.7. Record the chromatogram of each injection and draw a graph with injection volume on x-axis and area response on y-axis.

6.6.2.8. Curve should be linear and correlation co-efficient should be > 0.999.

6.7. Calibration of Detector

6.7.1. Wave length accuracy

6.7.1.1. Set the instrument conditions except for wavelength as explained in volume linearity (point 5.6.11,5.6.12 &5.6.13) with injection volume as 20µl. set the appropriate wavelength as explained below. Check that there is no leak in the connections and equilibrate the system.

6.7.1.2. Make one injection of solution A at each wavelength from 203 to 209 nm, from 241 to 247nm and 269 to 275 nm.

6.7.1.3. Record the area response of major peak in each injection.

6.7.1.4. Area response should be maximum at 206+2nm for 203 to 209nm.

6.7.1.5. Area response should be minimum at 244+2nm for 241 to 247nm.

6.7.1.6. Area response should be maximum at 272+2nm for 269 to 275nm.

6.7.2. Detector Linearity

Auto injector:

6.7.2.1. Infer the results obtained from volume linearity (Refer page 8).

6.7.2.2. Curve should be linear and correlation co-efficient should be > 0.999

ii) Rheodyne Injector:

6.7.2.3. Set the instrument conditions as explained in volume linearity (point 5.6.11,5.6.12 & 5.6.13) with injection volume as 20µl. check that there is no leak in the connections and equilibrate the system.

6.7.2.4. Prepare 25mg/100ml of caffeine stock solution in methanol.

6.7.2.5. Prepare the working solutions by pipetting out 5ml, 10ml, 15ml, 20ml and 25ml from the above stock solution in five different 100ml volumetric flasks. Make upto the volume with methanol.

6.7.2.6. Inject all the above working solutions once.

6.7.2.7. Record the chromatogram of each injection and draw a graph with concentration on x-axis and area response on y-axis.

6.7.2.8. Curve should be linearity and correlation co-efficient should be > 0.999.

6.8. If the calibration is not satisfactory then check the HPLC connections and recalibrate.

6.9. If the instrument is out of calibration, tag the instrument with label. Inform to the service engineer for rectification. Calibrate the instrument after rectification.

UNDER MAINTENANCE

7. Distribution and control:

7.1. *Master copy*:

Master copy should keep in lock and key in QA department

7.2. Controlled copy:

Controlled copy should keep with HOD of Production department

7.3. Reference copy:

Reference copy should be display at the SOP stand near the respective area.