PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Quality Control	**SOP No.:**
Title: Operation, Cleaning and Calibration of HPLC	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

1.0 OBJECTIVE:

To lay down procedure for operation, cleaning and calibration of HPLC.

2.0 SCOPE:

This SOP is applicable for operation, cleaning and calibration of HPLC (Make: WATERS, Model: 2695 ALLIANCE).

3.0 RESPONSIBILITY – Execution- Executive QC
Checking -Assistant Manager QC

4.0 ACCOUNTABILITY - Manager Quality Control

5.0 PROCEDURE:

5.1 (OPERATION PROCEDURE):

MOBILE PHASE PREPARATION

5.1.1 Prepare a Pre labeled mobile phase bottle having details of product / mobile phase for analysis, date of preparation and signatures of analyst

5.1.2 Prepare the mobile as per STP. Filter through 0.45µ filter membrane and put in above labeled container.

5.2 INSTRUMENT START UP

5.2.1 Waters Alliance.

Power up the system and detector.

5.3 COLUMN INSTALLATION

5.3.1 Select appropriate column as per STP. Check physical condition of column.

5.3.2 Fit the column in right direction between detector and auto injector and ensure the tightness of ferrules at both the ends, using spanners

5.4 MILLENNIUM/EMPOWER START UP

5.4.1 Click START at the bottom of desktop. Point to PROGRAMME Select MILLENNIUM 32/EMPOWER programme folder.

5.4.2 Select MILLENNIUM/EMPOWER LOGIN from cascade menu. The login screen appears. Click LOGIN.

5.4.3 Enter USERNAME and PASSWORD. And click OK.

5.4.4 Double Click on BROWSE PROJECT. List of projects will appear. Click on the desired project and click OK.

5.4.5 Selected project opens up.

5.4.6 Click on TOOLS. Select RUN SAMPLES.

5.4.7 The list of instruments configured with the software will appear.

5.4.8 Select the system on which work has to be done and click OK.

5.4.9 RUN SAMPLE screen will appear.

5.4.10 Select the INSTRUMENT METHOD from screen appearing on right hand bottom of desktop. Click SETUP.

5.4.11 Equilibrate the column with the mobile phase for at least half an hour and monitor the base line on the screen by clicking MONITOR on the bottom of RUN SAMPLE screen.

5.4.12 After stabilisation of base line, place vial in the autosampler. Feed the following information in the SAMPLE TABLE on RUN SAMPLE screen. Vial no. / sample name /sample type /Injection volume / method set/ No. of injections / Run Time/ column ID, STD lot no. , etc. and injection delay in case of gradient.

5.4.13 Click on MODE drop down list. Select the RUN AND REPORT MODE and click on INJECT and then RUN SAMPLE SET

5.4.14 Give the sample set name and click OK.

5.4.15 After completion of the run, chromatogram will be automatically printed on giving specific auto print command.

5.4.16 Do the calculations as per STP.

5.4.17 In case of any problem / clarification contact LAB INCHARGE.

5.5 CALLIBRATION PROCEDURE

Frequency: Once in a 6 month.

5.5.1 General Maintenance

5.5.1.1 Remove the column from the system and replace with a Dead volume connector.

5.5.1.2 Flush the system with hot water (50-70 deg C) for about half an hour, using all channels at a flow rate of 2ml/min, using following composition :

[Channel A(25%), B(25%), C(25%) and D(25%)]

NOTE: Allow at least half an hour for the UV lamp to warm up and the system to equilibrate.

5.5.2 CHROMATOGRAPHIC CONDITIONS:

Column	:	Nova pack C18, 4u, (3.9 x 150 mm)
Mobile phase	:	
Channel A	:	30 % (Methanol)
Channel B	:	30 % (Methanol)

Channel C	:	20 % (Water)
Channel D	:	20 % (Water)
Flow rate	:	1 ml /min
Injection volume	:	20 uL
Detection	:	UV at 254 nm.
Run time	:	10 min.
Retention time	:	~ 6 min

5.5.3 CALIBRATION OF PUMP:

Calibration of the pump shall be done to check the performance of the following:

i) Flow rate accuracy

ii) Flow rate consistency

iii)Compositional accuracy (gradient profile)

iv) Delay volume of the system

5.5.4 FLOW RATE ACCURACY

5.5.4.1 Remove the column and put all the channels inlets in reservoirs of HPLC grade water. Set the flow rate at 1 ml/min., using the following composition:

[Channel A(25 %), B(25 %), C(25 %) and D(25%)]

5.5.4.2 Collect the mobile phase, at column inlet, in a dry 10 ml volumetric flask and note down the time taken to fill the volumetric flask till the mark using calibrated stopwatch. Perform the exercise in duplicate.

5.5.4.3 Calculate the corresponding flow rate.

5.5.4.4 Select a flow rate of 2.0 ml/min and 3.0 mL/min. Perform the same exercise in duplicate.

5.5.4.5 Acceptance Criteria

The flow rate should be within ± 2% of the set value.

5.5.5 FLOW RATE CONSISTENCY

5.5.5.1 Calculate the RSD for the retention times obtained in the chromatograms with injection volume 10 ul and 20 µL

(Auto Injector - Injection Linearity and Precision).

5.5.5.2 Acceptance Criteria

RSD should not be more than 1%.

5.5.6 COMPOSITIONAL ACCURACY (Gradient Profile)

5.5.6.1 Remove the column from the system and replace with Dead Volume connector.

5.5.6.2 Create an instrument method in empower using the parameters listed as under

Flow rate : 1 mL/min

Detection : UV at 254 nm

5.5.6.3 Check the compositional accuracy of the HPLC system with the conditions given below:

Flow rate : 1 mL/min
Detection : UV at 254 nm

Time (min)	% HPLC grade Water [Channel A,B]	0.25% Acetone In Water [Channel C,D]
0	100	0
4	100	0
6	80	20
10	80	20
12	60	40
16	60	40
18	20	80
22	20	80
24	0	100
28	0	100
30	100	0

Injection delay : 15 min

5.5.6.4 Run the gradient using Channel combination A,C and B,D.

5.5.6.5 Inject 0 µl or minimum volume of HPLC grade water and record the gradient profile.

5.5.6.6 Acquire the data till 28 minutes.

5.5.6.7 Print the overlay plot of gradient profile of A,C and B,D (the difference of not more than 0.01 AU in absorbance and the difference of not more than 20 sec. in time is acceptable).

Note : If the number of channels are less than 4 then the compositional accuracy should be done on the combination of the available channels.

5.5.6.8 Accetpance Criteria

The gradient profile of A,C and B,D should overlay with each other.

5.5.7 AUTO INJECTOR CALIBRATION

5.5.7.1 INJECTION ACCURACY

5.5.7.1.1Purge auto injector with HPLC grade water.

5.5.7.1.2 Fill a standard vial with HPLC grade water and seal with a cap.Weigh this vial and record weight(W1) in gms.

5.5.7.1.3Program HPLC system for a flow rate of 1.0 ml/min of water and run time of 1 min.

5.5.7.1.4 Inject 20ul from vial and repeat it for 18 times.After completion of 18 injections, remove

the vial and weigh again(W2) in gms.

5.5.7.1.5 Calculate the average volume (in ul) injected per injection using formula

[(W1-W2)/18]x1000 = mg of water/injection = ul/injection

5.5.7.1.6Acceptance Criteria

Average volume of injection (ul/injection) shall be 20ul +/- 0.4 ul.

NOTE : Water is used in Auto injector injection accuracy test because its density, 0.9982 g/ml at 20°C and 0.9970 g/ml at 25°C, introduces less than 0.3% error when volume is assumed equal to weight.

5.5.8 AUTO INJECTOR LINEARITY AND PRECISION

5.5.8.1 Flush the HPLC system with HPLC grade water for about half an hour.

5.5.8.2 CHROMATOGRAPHIC CONDITIONS:

Column	:	Novapack C18, 4u, (3.9 x 150 mm)
Mobile phase	:	Methanol:Water(60:40)
Flow rate	:	1 ml/min
Detection	:	UV at 254 nm
Run time	:	10 min
Retention time	:	~ 6 min

5.5.8.3 Accurately weigh about 100 mg of Benzophenone in a 100 ml volumetric flask. Dissolve in 10 ml of methanol and make up the volume with mobile phase. Further dilute accordingly with mobile phase to get solution having concentrations of about 25 ppm.

5.5.8.4 Inject 5 µL of 25ppm Benzophenone standard into chromatograph and record the chromatograph. The tailing factor of Benzophenone peak should not be more than 1.5.

5.5.8.5 Inject Benzophenone six times with injection volume 5ul.calculate %RSD.

5.5.8.6 Repeat step with varying injection volumes (10ul, 20ul and 50 ul) and calculate %RSD.

5.5.8.7 Plot regression curve with injection volume on the x-axis and mean response of six injections on y-axis. Calculate Correlation Cofficient.

5.5.8.8 Accetpance Criteria

RSD = Not more than 1%

Correlation Cofficient = Not less than 0.9990

5.5.9 CARRYOVER

5.5.9.1 After sixth injection of Benzophenone with injection volume 50 uL , inject 50 uL of the mobile phase.

Carryover(%) = (Blank peak area / Standard peak are) x 100

Acceptance criteria = Carryover <= 0.010%.

5.5.10 DETECTOR PERFORMANCE

Calibration of the Detector shall be done to check the performance of the following:

i) Detector Linearity

ii) Detector Noise

iii)Wavelength Accuracy

5.5.11 DETECTOR NOISE

5.5.11.1 Inject the mobile phase (Blank) six times.

5.5.11.2 Calculate the Baseline Noise or the Standard deviation of the Noise.

5.5.11.3 Acceptance criteria

The Baseline Noise or three times the Standard deviation of the Noise should not be more than 100 microvolts and the RSD of Baseline Noise should not be more than 33%.

5.5.12 DETECTOR LINEARITY

5.5.12.1 Set up the HPLC system using chromatographic conditions

5.5.12.2 Accurately weigh about 100 mg of Benzophenone in a 100 ml volumetric flask. Dissolve in 10 ml of Methanol and make up the volume with mobile phase. Further dilute accordingly with mobile phase to get solutions having concentrations of about 0.001, 0.01 and 0.10 mg/ml.

5.5.12.3 Inject 0.001, 0.01 and 0.10 mg/ml solutions of Benzophenone, prepared above, in duplicate.

5.5.12.4 From the data obtained, plot a graph of Mean area counts in y-axis versus concentration (ug/ml) in x-axis and calculate the value of Correlation Cofficient.

5.5.12.5 Acceptance criteria:

Correlation Coefficient should not be less than 0.9990.

5.5.13 WAVELENGTH ACCURACY

For Non-diode array detectors

5.5.13.1 Flush detector with HPLC grade water.

5.5.13.2 Prepare a diluent comprising of 95% water and 5% Acetonitrile.Set the detecting wavelength at 270 nm.

5.5.13.3 Flush the flow cell of detector with the diluent and auto-zero the detector. Cancel and disable auto-zero function of the detector.

Inject 0.01 mg/mL solution of Caffeine in the flow cell of detector (there must be no trapped air bubbles in the cell).

5.5.13.4Set detector wavelength at 268 nm and record absorbance displayed on the front panel of detector.

5.5.13.5 Repeat step 5.5.13.3 for wavelengths from 269 to 278 nm with 1 nm increment.

5.5.13.6 Repeat step 5.5.13.3 for wavelengths from 200 to 210 nm with 1 nm increment.

5.5.13.7 Repeat step 5.5.13.3 for wavelengths from 239 to 249 nm with 1 nm increment.

5.5.13.8 Plot a graph of absorbance(y-axis) versus wavelength(x-axis) and calculate the wavelength maxima and minima.

5.5.13.9 Acceptance criteria

For step 5.5.13.5 wavelength maxima found should be between 273 ± 2 nm.

For step 5.5.13.6 wavelength maxima found should be between 205 ± 2 nm.

For step 5.5.13.7 wavelength minima found should be between 245 ± 2 nm.

5.5.14 COLUMN OVEN TEMPERATURE

5.5.14.1 Set the Column Oven Temperature at 30 deg.C After about 10 minutes record the observed temperature using a calibrated digital temperature indicator or thermometer.

5.5.14.2 Set column temperature at 50 deg.C

5.5.14.3 Acceptance Criteria

The observed temperature should be not more than ± 3 deg.C of the set temperature.

5.5.15 TEMPERATURE ACCURACY OF SAMPLE COOLER

5.5.15.1 Use a calibration thermometer to measure the actual temperature in the sample cooler and compare it to the set value.Use a set value of 4 deg C.

5.5.15.2 About 90 minutes are required for adequate cooling.

Acceptance Criteria

Temperature Accuracy should be ± 3.0°C of the set temperature

5.6 The records of the calibration shall be maintained as per Annexure -I

5.7 CLEANING PROCEDURE

Frequency: Daily or after each use.

5.7.1 Open the front part of the instrument .

5.7.2 Wipe out any material in the sample holder assembly by means of tissue paper.

5.7.3 Clean all the sampling accessories with tissue paper after analysis and keep them in proper place.

5.7.4 Clean the outer surface of the instrument with dry cotton cloth.

5.7.5 Place the 'self indicating' coarse silica gel bag in the sample holding assembly after analysis.

5.7.6 If the colour changes of the silica bag shown by the instrument (changes from blue to pink), regenerate the silica by keeping it in oven at 70º C for some time till it gets blue color again.

5.7.7 Record the details of cleaning in log card.

6.0 SAFETY & PRECAUTIONS:
Not Applicable

7.0 REVISION HISTORY:

Revision No.	Reason for Revision	Superseded from & date

8.0 DISTRIBUTION:

Copy No.	Issuance Record				Withdrawal Record		Destruction Record	
	Date	Dept. issued	Name / Signature of receiver	Issued By Name / Signature	By	Sign/ Date	By	Sign/ Date

9.0 REFERENCES:
Not Applicable

10.0 ABBREVIATIONS & ANNEXURES:

SOP : Standard Operating Procedure

No. : Number

QC : Quality Control

º : Degree Celsius

Annexure I- X : ANALYTICAL RAW DATA SHEET (HPLC CALIBRATION)

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ANNEXURE I
ANALYTICAL RAW DATA SHEET (HPLC CALIBRATION)

Instrument No . :_____ Issued to :_____
Make :_____ Issued by :_____
SOP No. :_____ Issued on :_____
Calibration on : _____ Next due on:_____

S.No.	TEST	ACCEPRANCE CRITERIA	OBSERVED VALUE	RESULT
PUMP				
1	FLOW RATE	± 2% OF SET VALUE		
	1 ml/ min	9.8 – 10.2		
	2 ml/ min	4.9 – 5.1		
	3 ml/ min	3.26 – 3.39		
2	FLOW RATE CONSISTENCY	RSD NMT 1.0%		
3	COMPOSITION ACCURACY	DIFFERENCE IN ABSORBANCE NMT 0.01 AU		
		DIFFERENCE IN TIME NMT 20 sec.		
4	DELAY VOLUME	NMT 1.5 ml (for 1 ml delay volume system)		
AUTOINJECTOR				
1	INJECTOR VOLUME ACCURACY	AVERAGE VOLUME FOR 18 INJECTIONS = 19.6 – 20.4 µl		
2	PRECISION	RSD NMT 1.0%		
3	INJECTION VOLUME LINEARITY	Correlation Coefficient NLT = 0.9990		
4	CARRYOVER	Carryover ≤ 0.010%		
DETECTOR				
1	LINEARITY	Correlation Coefficient NLT = 0.9990		
2	NOISE	RSD of baseline noise NMT 33%		
3	WAVELENGTH ACCURACY	MAXIMA 270 – 276 nm		
		MAXIMA 202 – 208 nm		
		MINIMA 241 – 249 nm		
COLUMN OVEN AND SAMPLE COOLER				
1	TEMPERATURE ACCURACY (Column Heater)	± 3°C of set temperature		
2	TEMPERATURE ACCURACY (Sample Cooler)	± 3°C of set temperature		
SOFTWARE VERIFICATION				

Remarks:_____

DONE BY:_____CHECKED BY:_____

ANNEXURE II
ANALYTICAL RAW DATA SHEET(HPLC CALIBRATION)

i) Auto injector Accuracy:

Weight of vial with water before injection (W1) :_____gm

Weight of vial with water after injection (W2) : _____gm

ii) Auto injector Linearity and Precision:

Weight of Benzophenone :

Gross Weight :_____gm

Tare Weight : _____gm

Net Weight : _____gm

Actual Concentration Calculation :

iii) Detector Linearity :

Weight of Benzophenone :

Gross Weight :_____gm

Tare Weight : _____gm

Net Weight : _____gm

Actual Concentration Calculation :

iv) Wavelength Accuracy:

Weight of Caffeine:

Gross Weight :_____gm

Tare Weight : _____gm

Net Weight : _____gm

Actual Concentration Calculation :

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ANNEXURE III

ANALYTICAL RAW DATA SHEET (HPLC CALIBRATION)

Instrument No . :_____ Issued to :_____
Make :_____ Issued by :_____
SOP No. :_____ Issued on :_____
Calibration on : _____ Next due on:_____

FLOW RATE

FLOW RATE	VOLUMETRIC COLLECTED	TIME	MEAN
1ml /min	10 ml		
2 ml/min	10 ml		
3 ml/min	10 ml		

Volumetric flask No.:_____

Remarks:_____

DONE BY:_____ **CHECKED BY:**_____

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ANNEXURE IV
ANALYTICAL RAW DATA SHEET (HPLC CALIBRATION)

Instrument No .:_____ Issued to :_____
Make :_____ Issued by :_____
SOP No. :_____ Issued on :_____
Calibration on : _____ Next due on:_____

FLOW RATE CONSISTENCY

S.No.	RETENTION TIME
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	
MEAN	
% RSD	

Remarks:_____

DONE BY:_____ CHECKED BY:_____

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ANNEXURE V

ANALYTICAL RAW DATA SHEET (HPLC CALIBRATION)

Instrument No : _____
Make :_____
SOP No. :_____
Calibration on : _____

Issued to :_____
Issued by :_____
Issued on :_____
Next due on:_____

AUTO INJECTOR PRECISION AND LINEARTY

S.No.	VOLUME	OBSERVED AREA	MEAN AND RSD
1	5µL		
2			
3			
4			
5			
6			
1	10µL		
2			
3			
4			
5			
6			
1	20µL		
2			
3			
4			
5			
6			
1	50µL		
2			
3			
4			
5			
6			
	Correlation Coefficient	NLT 0.9990	

Remarks:_____

DONE BY:_____ **CHECKED BY:**_____

ANNEXURE VI

ANALYTICAL RAW DATA SHEET (HPLC CALIBRATION)

Instrument No .:_____ Issued to :_____
Make :_____ Issued by :_____
SOP No. :_____ Issued on :_____
Calibration on : _____ Next due on:_____

INJECTOR VOLUME ACCURACY

INJECTOR VOLUME ACCURACY = (W1-W2) ---------- X1000 18 **Where W1(Weight of vial with water before injection)=** **Where W1(Weight of vial with water after 18 injections)=**	**Calculation**

CARRYOVER (AUTO INJECTOR)

BLANK PEAK AREA	STANDARD PEAK AREA	CARRYOVER(%)

Formula: Carryover = (Blank peak area)/ Standard peak area) X 100

Remarks:_____

DONE BY:_____ CHECKED BY:_____

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ANNEXURE VII

ANALYTICAL RAW DATA SHEET (HPLC CALIBRATION)

Instrument No .:_____ Issued to :_____
Make :_____ Issued by :_____
SOP No. :_____ Issued on :_____
Calibration on : _____ Next due on:_____

DETECTOR NOISE

INJECTION NUMBER	BASELINE NOISE
1	
2	
3	
4	
5	
6	
MEAN	
% RSD	

Remarks:_____

DONE BY:_____ CHECKED BY:_____

ANNEXURE VIII

ANALYTICAL RAW DATA SHEET (HPLC CALIBRATION)

Instrument No .:_____ Issued to :_____
Make :_____ Issued by :_____
SOP No. :_____ Issued on :_____
Calibration on : _____ Next due on:_____

DETECTOR LINEARTY

S.No.	CONCENTRATION	AREA	MEAN
1	0.001 mg/ml		
2			
1	0.01 mg/ml		
2			
1	0.1 mg/ml		
2			
	Correlation Coefficient	NLT 0.9990	

Remarks:_____

DONE BY:_____ **CHECKED BY:_____**

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ANNEXURE IX
ANALYTICAL RAW DATA SHEET (HPLC CALIBRATION)

Instrument No .:_____ Issued to :_____
Make :_____ Issued by :_____
SOP No. :_____ Issued on :_____
Calibration on : _____ Next due on:_____

WAVE LENGTH ACCUEACY

W AVE LENGTH (nm)	ABSORBANCE	WAVE LENGTH (nm)	ABSORBANCE	WAVE LENGTH (nm)	ABSORBANCE
269		200		239	
270		201		240	
271		202		241	
272		203		242	
273		204		243	
274		205		244	
275		206		245	
276		207		246	
277		208		247	
278		209		248	
		210		249	

Remarks:_____

DONE BY:_____ CHECKED BY:_____

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ANNEXURE X
ANALYTICAL RAW DATA SHEET (HPLC CALIBRATION)

Instrument No .:_____
Make :_____
SOP No. :_____
Calibration on : _____

Issued to :_____
Issued by :_____
Issued on :_____
Next due on :_____

COLUMN OVEN CALIBRATION

SET POINT	OBSERVED VALUE	INSTRUMENT DISPLAY
30 °C		
50°C		

SAMPLE COOLER CALIBRATION

SET POINT	OBSERVED VALUE	INSTRUMENT DISPLAY
30 °C		

Remarks:_____

DONE BY:_____ **CHECKED BY:**_____