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Department	Analytical Research Development	Effective Date	12/02/25
Superseded No.	Nil	Review Date	11/02/27

1.0 OBJECTIVE

- 1.1 To lay down the procedure for Operation and Calibration of High-Performance Liquid Chromatography (Shimadzu P Series With PDA)

2.0 SCOPE

- 2.1 This procedure is applicable for Operation and Calibration of High-Performance Liquid Chromatography (Shimadzu P Series With PDA) in ARD Department at Pritam International Pvt. Limited.

3.0 RESPONSIBILITY

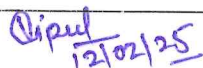
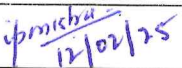
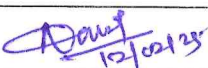
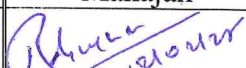
- 3.1 Chemist/Executive/Designee-ARD/Person shall follow the cleaning, operation & calibration of HPLC.
- 3.2 ARD Section Person shall be responsible for calibration of HPLC.
- 3.3 Manager-Designee shall ensure efficacy of the procedure of cleaning, operation & calibration of HPLC.

4.0 ACCOUNTABILITY

- 4.1 ARD Designee
- 4.2 ARD & QA Manager

5.0 PROCEDURE**5.1 OPERATION PROCEDURE**

- 5.1.1 Switch ON the Instrument (Pump, Injector, Detector, Column Oven and Computer loaded with Lab Solution Software for the Data computation)
- 5.1.2 Start the Lab Solution Software by double-clicking the Lab Solution icon located on desktop.
- 5.1.3 Each time start an instrument application (by double clicking the instrument icon (HPLC written), an **Instrument Wizard** shall appear. This wizard is designed to direct to the basic functions of the instrument window.
- 5.1.4 Then go to the **File** option on the menu bar and then click on **Method**, then click on **new** or **open** to create or to follow the existing method respectively.
- 5.1.5 For each sample enter the run parameters required for the sample under test.
- 5.1.6 Data acquisition and instrument control parameters: –

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- (a) **Instrument Setup:** Select each tab and enter the correct parameters for running the sample.
- (b) **Integration events:** Additional integration events are not necessary for a test sample.
- (c) **Calibration of Peaks/Groups:** Calibration parameters are not necessary for a test sample.
- (d) **Advanced method options:** Advanced method options are not necessary for a test sample.
- (e) **Reports:** From the **File** menu, select **Report Template** and then click **Open**. From the list of templates, select **Area%. rep** to generate an Area %report when run the sample
- 5.1.7 Create a sequence:** This button starts the **Sequence Wizard** that steps you through creation of an acquisition reprocessing sequence. Or for creating sequence enter file menu which display Sequence, from Sequence enter new method.
- 5.1.8 Single sample run:** This button opens a dialog, from this select a previously saved method to run a single sample.
- 5.1.9** In the **Single Run Acquisition** dialog box, enter a number to be used as a **Sample ID**.
- 5.1.10** Click the method open button  and then select the method file that was provided or the method you saved in the previous step.
- 5.1.11** Enter a **Data file** name you wish to use, or click the adjacent button  to select a parameter that will be used to create the data file name for you.
- 5.1.12** Enter the **Vial number** and **Injection Volume** to be used.
- 5.1.13 Run sequence of samples:** This button opens the Run Sequence dialog where you can start data acquisition using a stored sequence.
- 5.1.14 Save the method.** When you have completed setting up the method parameters, from the **File** menu, followed by **Method** and click **Save As**. Type a name to be used for your method and then click **OK**.
- 5.1.15** Report any discrepancy observed during operation and calibration of instrument to Section In charge or his representative for corrective and preventive action.
- 5.1.16** Section In charge or his representative shall take the necessary action and report the same to Quality Manager. Affix 'Under Maintenance' label on HPLC

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5.2 CALIBRATION PROCEDURE

5.2.1 CALIBRATION FREQUENCY: HALF YEARLY $\pm$ 20 days.

5.2.2 PRECAUTIONS

5.2.2.1 For calibrating the Column oven, use only calibrated thermometer.

5.2.2.2 For calibration of pump flow rate use only calibrated stopwatch & Analytical Balance.

5.2.3 CALIBRATION PARAMETERS

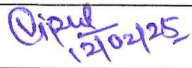
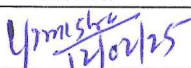
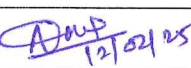
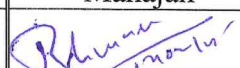
The following components of system shall be calibrated.

- | | |
|----------------------------|--|
| A) Pump Calibration | i) Flow rate accuracy. |
| | ii) Gradient composition accuracy. |
| B) Injector calibration: | i) Linearity & Carry over test. |
| | ii) Injector Precision / Injector Reproducibility & Repeatability |
| | iii) Injector Volume Accuracy. |
| | iv) Vial Positioning. |
| C) Column Oven Temperature | i) Temperature Calibration |
| D) Detector Calibration | i) Linearity of Detector Response |
| | ii) Wavelength accuracy |
| | iii) Noise and drift |
| E) Lamp Intensity | i) Lamp intensity to be verified and maintain the record on monthly basis. |

A) PUMP CALIBRATION

(i) FLOW RATE ACCURACY:

- Use Milli Q water (Degassed & filter) as a mobile phase for calibration of pump.
- Before starting the calibration purge the system two or three times to remove air bubbles.
- Before starting calibration set pump at constant flow and discard the volume for five minutes.
- Take a previously dried 10 ml Volumetric Flask and weigh it, note the weight of the Volumetric Flask (W1) and install a union in column compartment simultaneously start the required flow and stop watch for 5 minutes and collect it. After five minutes stop the flow and weight the collected volume in Volumetric Flask (W2) calculate the actual volume collected in Volumetric Flask (W2-

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W1=W3). Calculate the corresponding flow rate by using formula = (weight of collected volume / Time Taken x 0.997).

- e. Select a flow rate of 0.5ml/min, 1.0 ml/min, 1.5ml/min, and 2.0 ml/min and perform the same exercise. Repeat the step same as above for calibration of pump Chancel B C D (if applicable).

Acceptance Criteria= The flow rate should be within $\pm 2\%$ of the set value.

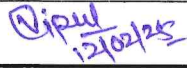
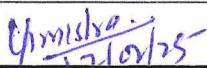
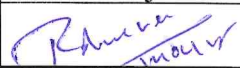
(ii) GRADIENT COMPOSITION ACCURACY

Set the following chromatographic conditions

Column : Restriction capillary
Solvent for Pump A : Filtered and degassed purified water
Solvent for Pump B,C& D : 10 ppm caffeine in purified water
Flow rate : 2.0 ml/min
Wavelength : 272 nm
Injection volume : 0.00 μ l
Run time : 30 min

Gradient programming:

Step	Time in minute	Flow Rate (ml/min)	% Solvent A (Water)	%Solvent B (10 ppm Caffeine)	%Solvent C (10 ppm Caffeine)	%Solvent D (10 ppm Caffeine)
1	5.00	2.0	100.0	0.0	0.0	0.0
2	5.01	2.0	90.0	10.0	10.0	10.0
3	10.00	2.0	90.0	10.0	10.0	10.0
4	10.01	2.0	50.0	50.0	50.0	50.0
5	15.00	2.0	50.0	50.0	50.0	50.0
6	15.01	2.0	10.0	90.0	90.0	90.0
7	20.00	2.0	10.0	90.0	90.0	90.0
8	20.01	2.0	0.0	100.0	100.0	100.0
9	25.00	2.0	0.0	100.0	100.0	100.0
10	25.01	2.0	100.0	0.0	0.0	0.0
11	30.00	2.0	Stop			

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5.2.4 Procedure of Pump Calibration:

* Purge all pump including rinse at required time.

Set the flow rate at 2.0 ml per minute. Keep the system in this flow rate for some time to equilibrate. Start the gradient programming. After completion of Gradient programming calculate actual concentration by using formula below

S. No.	Set Nominal Height (%)	Observed Height $\frac{A \times 100}{B}$			Tolerance Limit 2%
			=		
1	0		=		0.00
2	10		=		9.8 – 10.2
3	50		=		49.0 – 51.0
4	90		=		88.2 – 91.8
5	100		=		98.0 – 102.0

A = Actual Height

B = Maximum height at 100 %

Acceptance criteria: At each absorbance level $\pm 2\%$ of set concentration

5.2.4.1 PREPARATION OF CALIBRATION STANDARD SOLUTION:

(A) Requirements:

- Standard caffeine
- Acetonitrile and water HPLC Grade.
- Diluent (70:30) Acetonitrile water.
- 100 ml, 50 ml and 20 ml Volumetric Flask.
- 1 ml, 2 ml, 5 ml, 10 ml Bulb pipette.

(B) Preparation of caffeine stock solution

Weigh accurately about 50.0 mg of caffeine and transfer to a 100.0 ml volumetric flask, add 50 to 75 ml of mobile phase, sonicate for 5 minutes for dissolve and makeup the volume up to 100.0 ml with mobile phase. This solution contents about 0.5 mg/ml of caffeine.

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(C) Working Solution of caffeine:

- Dilute 1 ml of above stock solution to 100.0 ml with diluent (5 ppm)
- Dilute 2 ml of above stock solution to 100.0 ml with diluent (10 ppm)
- Dilute 2 ml of above stock solution to 50.0 ml with diluent (20 ppm)
- Dilute 5 ml of above stock solution to 50.0 ml with diluent (50 ppm)
- Dilute 10 ml of above stock solution to 50.0 ml with diluent (100 ppm)
- Dilute 10 ml of above stock solution to 20.0 ml with diluent (250 ppm)

(D) LINEARITY OF DETECTOR RESPONSE:

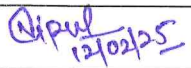
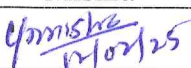
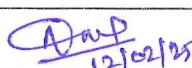
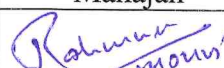
For Photo Diode Array Detector (PDA)

Chromatographic parameter

Flow rate : 1.0 ml/min.
Wave length : 272 nm (200-400)nm
Column : ODS, C18, 250mm X 4.6 mm, 5 μ or any suitable column
Mobile Phase : 70:30(Acetonitrile: Water)

Procedure:

- Set the detector at λ max.
- Place the vials containing mobile phase i.e. blank and caffeine solutions having conc. of 5 ppm, 10 ppm, 20 ppm, 50 ppm & 100 ppm in the given sequence in the auto sampler. Program the system to successively inject 20 μ l each of the mobile phase (MP) to serve as blank, therefore- mentioned five caffeine solutions and the MP again (second blank) at the end and record their chromatograms.
- Read the areas of principal peaks in each chromatogram.
- Enter the peak areas corresponding to each conc. And also, area of any caffeine peak in the chromatogram of the second blank.
- A plot of the solution concentrations vs. the areas should be a straight line with its correlation coefficient **greater than 0.999**.

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(E) CARRY OVER

Inject 20 µl solution as per Sequence Blank → Standard solution 250 ppm → Blank, calculate area if any peak at the same RT of standard. Operate the Instrument as per given parameters. Acceptance criteria is **NMT 0.01%**.

Calculate the carryover % & as shown below.

$$\text{Carryover \%} = \frac{\text{Peak area in Blank} \times 100}{\text{Peak area in Sample}}$$

(F) INJECTOR PRECISION (Reproducibility & repeatability):

Inject 5 injection of Standard caffeine 20.0 ppm solution prepared above
And calculate the RSD of area and RT

Acceptance Criteria: NMT 1.0% for area and NMT 0.5% for RT

(G) WAVE LENGTH ACCURACY:**For Photo Diode Array Detectors (PDA)****Chromatographic parameter**

Flow rate : 1.0 ml/min.

Wave length : (190-800)nm

Column : ODS, C18, 250mm X 4.6 mm, 5µ or any suitable column

Mobile Phase : 70:30(Acetonitrile: Water)

Set detector wavelength at 190 nm to 800 nm.

Inject 20 µl of 0.01 mg/ml solution of caffeine.

Record spectrum and report maxima and minima.

Acceptance Criteria

Wavelength maxima found should be between 273±2 nm.

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Wavelength maxima found should be between 205±2 nm.

Wavelength minima found should be between 245±2 nm.

(H) INJECTOR CALIBRATION:

a. Injector Volume Accuracy:

- Fill a HPLC vial with Degassed HPLC grade water.
- Seal the vial with septum and cap.
- Zero the analytical balance, and then carefully weigh the vial, Record the weight W1.
- Place the weighed vial in position 1 of the sample tray in the sample compartment.
- Fill the reservoir A with degassed 100% HPLC- grade water, then prime the solvent delivery system
- Prepare programmed in software as injection volume inject 15 injections of 20.0 µl.
- Remove the vial and reweigh the vial (W2). Repeat the process again as per above.
- To calculate the average volume of water injected per injection.
- Repeat it with 15 injections of 10 µl, 15 injections of 50 µl & 10 injections of 100 µl.

$$[(W1-W2) \times 1000] / \{ \text{Number of injection} \times \text{Density of Water} \} = \mu\text{L per injection}$$

Where density of water at 25°C is 0.997g/ml

Acceptance criteria: ±2.0 %

b. Injector Linearity:

- Inject separately 5, 10, 20, 50 and 100µl injection in duplicate of 20ppm solution.

c. Test of Vial Positioning:

- Detector Linearity solution shall be use for the vial positioning test.
- Keep the Linearity solution vials at random position across the injector tray.
- All injector trays should be used with random position.

Acceptance Criteria:

- All Vials to be picked/punctured as per program.

(I) Column Oven Calibration (Temperature Calibration)

- Switch on the column oven and set Temperature at 40°C.

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- Remove the lift and right-side plastic opening center clip and insert the standard Calibrated thermometer to the middle of column compartment.
 - Properly packs the side space of thermometer with cotton for proper insulation.
 - Now wait up to ready position lamp (up to set temperature is achieved).
 - Note down the Temperature in thermometer and repeat the exercise with temperature at 20°C & 60°C.
- Acceptance Criteria:** The observed reading (current) and standard thermometer reading do not differ by more than $\pm 2^\circ\text{C}$ from the set temperature.

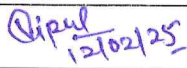
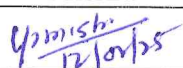
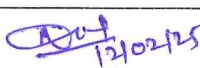
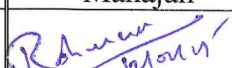
Note: Calibration of Column Oven Temperature from External Lab once in a year.

(J) Noise and Drift

This procedure based on ASTM standard E 685-93 determines the noise & drift.

Conditions:

Sequence	: PQ_PDA_NOISE_DRIFT
Program	: PDA_DET_NOISE_AND_DRIFT
Flow	: 1 mL/minute
Stop Time	: 21.0 minutes
Solvent A	: Milli Q water
Temperature	: 40.0°C
Wavelength	: 254

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Procedure: The checks for noise, drift, and lamp intensity are included in the PDA Noise Drift sequence. For those checks, water is pumped through the cell at a flow rate of 1 ml/min. The PDA signal is recorded at 254 nm.

To calculate drift and noise, the measuring signal is split into 20 intervals of 1 minute each. For each interval, Chromeleon calculates a regression based on measured values, using the method of least squares. The slope of the curve indicates the drift of the measured signal; the absolute value of the slope indicates the absolute value of the drift. The noise is the distance between two parallel lines through the measured minimum and maximum values and the regression line. The calculated values are averaged for all 20 intervals to establish the final value.

Acceptance criteria: Noise: $\pm 0.2\text{mAU}$

Drift: $\pm 2.0\text{mAU/h}$ (for VWD)

$\pm 5.0\text{mAU/h}$ (for DAD)

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Sign / Date	<i>Vipul</i> 12/02/25	<i>Chandra</i> 12/02/25	<i>Amol</i> 12/02/25	<i>Rahul</i> 12/02/25

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	STANDARD OPERATING PROCEDURE		11 of 12
TITLE	Operation and Calibration of High-Performance Liquid Chromatography (SHIMADZU P Series with PDA)		Issue No.: 01
SOP No.	PIPL/ARD/001-00	Issue Date	12/02/25
Department	Analytical Research Development	Effective Date	12/02/25
Superseded No.	Nil	Review Date	11/02/27

6.0 REFERENCES

Indian Pharmacopoeia Commission, In-House

7.0 ABBREVIATION (S)

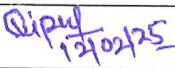
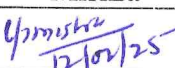
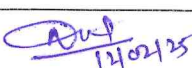
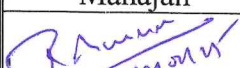
S. No	Abbreviation	Full Description
1	SOP	Standard Operating Procedure
2	PIPL	Pritam International Pvt. Ltd.
3	No.	Number
4	ARD	Analytical Research Development
5	RSD	Relative Standard Deviation
6	HPLC	High performance Liquid Chromatography
7	OQ	Operation Qualification
8	PDA	Photo Diode Array Detector

8.0 ANNEXURE (S)

Annexure No.	Details/Title of Annexure	Format No.
PIPL/ARD/001-A01	Format for Calibration Index for HPLC	PIPL/ARD/001/F01-00
PIPL/ARD/001-A02	Format for Calibration Data Sheet	PIPL/ARD/001/F02-00
PIPL/ARD/001-A03	Format for HPLC Calibration Summary Sheet	PIPL/ARD/001/F03-00
PIPL/ARD/001-A04	Format for standard preparation sheet	PIPL/ARD/001s/F04-00

9.0 CHANGE HISTORY

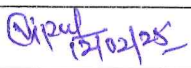
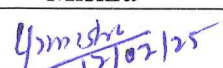
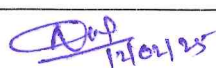
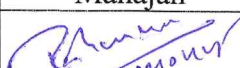
S. No.	Supersedes	Changes made	Effective Date
01	Not Applicable	New Sop	12/02/25

	Prepared by	Reviewed by	Approved by	Authorised by
Designation	Asst. Chemist ARD	Dy. Manager ARD	QA Manager	Quality Head
Name	Vipul Rajput	Chandra Prakash Mishra	Amol Thorve	Dr. Rahul Mahajan
Sign / Date	 12/02/25	 12/02/25	 14/02/25	 14/02/25

	PRITAM INTERNATIONAL PVT. LIMITED		Page No.
	STANDARD OPERATING PROCEDURE		12 of 12
TITLE	Operation and Calibration of High-Performance Liquid Chromatography (SHIMADZU P Series with PDA)		Issue No.: 01
SOP No.	PIPL/ARD/001-00	Issue Date	12/02/25
Department	Analytical Research Development	Effective Date	12/02/25
Superseded No.	Nil	Review Date	11/02/27

10.0 DISTRIBUTION/ISSUANCE

S. No.	Department	Issuance	S. No.	Department	Issuance
1.	Quality Assurance	Yes ☒ No ☐	10.	Planning & Purchase	Yes ☐ No ☒
2.	Quality Control	Yes ☒ No ☐	11.	Accounts	Yes ☐ No ☒
3.	Microbiology Lab	Yes ☐ No ☒	12.	F&D	Yes ☐ No ☒
4.	Production (Mfg.)	Yes ☐ No ☒	13.	NPD	Yes ☐ No ☒
5.	Production (Packing)	Yes ☐ No ☒	14.	Human Resources	Yes ☐ No ☒
6.	RM Store	Yes ☐ No ☒	15.	Maintenance	Yes ☐ No ☒
7.	PM Store	Yes ☐ No ☒	16.	Information Technology	Yes ☒ No ☐
8.	FG Store	Yes ☐ No ☒	17.	House Keeping	Yes ☐ No ☒
9.	Warehouse	Yes ☐ No ☒	18.	ARD	Yes ☒ No ☐

	Prepared by	Reviewed by	Approved by	Authorised by
Designation	Asst. Chemist ARD	Dy. Manager ARD	QA Manager	Quality Head
Name	Vipul Rajput	Chandra Prakash Mishra	Amol Thorve	Dr. Rahul Mahajan
Sign / Date	 12/02/25	 12/02/25	 12/02/25	 12/02/25

**PRITAM INTERNATIONAL PVT. LIMITED****ANNEXURE - PIPL/ARD/001-A01**

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Sign./Date: *Sonam* 12/02/2025

PIPL ROORKEE

Format for Calibration of HPLC

Location		Instrument ID	
Make		Model No.	
Calibration Date		Calibration due date	
Reference SOP No		Frequency	6 months $\pm$ 20 days

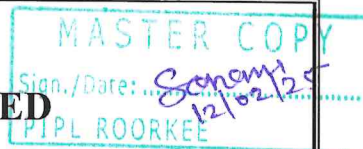
Sr. No	Calibration parameter	Page No
1	Index	
2	Flow Rate Accuracy	
3	Gradient Composition Accuracy	
4	Standard Preparation Sheet	
5	Injection Precision/ Injector Reproducibility & Repeatability	
6	Injector Calibration	
7	Vial Positioning	
8	Carry Over Test	
9	Linearity of Detector Response	
10	Wavelength Accuracy	
11	Noise and Drift	

Remarks: The Instrument Calibration & Performance is Complies / Not Complies

Calibrated By	Checked By	Approved By
Date:	Date:	Date:

**PRITAM INTERNATIONAL PVT. LIMITED**

ANNEXURE - PIPL/ARD/001-A02

**HPLC Calibration****CALIBRATION DATA SHEET****Calibration Parameter – 1: Flow Rate Accuracy**

Instrument ID	:	
Current Location	:	ARD Instrument Room-01
Calibration Frequency	:	HALF YEARLY ± 20 Days
Date of Calibration	:	
		Next Due Date of Calibration :

Name of Equipment	ID No. of Equipment	Validity of Calibration
Analytical Balance		
Stop Watch		

Density of Water at 25°C = D (0.997)

Calibration of Pump -A

Sr. No.	Set Flow rate (ml/min)	Total Time (min) = T	Wt. of beaker (g) = W1	Wt. of beaker + water (g) = W2	Weight of water W2-W1 = W	Volume of water (ml) W/D = V	Observed Flow rate (ml/min) = V/T	Tolerance Limit (ml/min)
1.	0.5							0.49-0.51
2.	1.0							0.98-1.02
3.	1.5							1.47-1.53
4.	2.0							1.96-2.04

Remarks: Calibration of Pump for Flow rate accuracy Complies/ does not complies with the Tolerance limits.

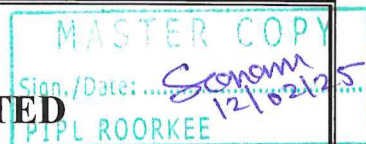
Calibration of Pump -B

1.	0.5							0.49-0.51
2.	1.0							0.98-1.02
3.	1.5							1.47-1.53
4.	2.0							1.96-2.04

Remarks: Calibration of Pump for Flow rate accuracy Complies/ does not complies with the

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ANNEXURE - PIPL/ARD/001-A02

**HPLC Calibration****Tolerance limits.****Calibration of Pump -C**

Sr. No.	Set Flow rate (ml/min)	Total Time (min) =T	Wt. of beaker (g) =W1	Wt. of beaker + water (g) =W2	Weight of water W2-W1= W	Volume of water (ml) W/D = V	Observed Flow rate (ml/min) =V/T	Tolerance Limit (ml/min)
1.	0.5							0.49-0.51
2.	1.0							0.98-1.02
3.	1.5							1.47-1.53
4.	2.0							1.96-2.04

Remarks: Calibration of Pump for Flow rate accuracy Complies/ does not complies with the Tolerance limits.

Calibration of Pump -D

1.	0.5							0.49-0.51
2.	1.0							0.98-1.02
3.	1.5							1.47-1.53
4.	2.0							1.96-2.04

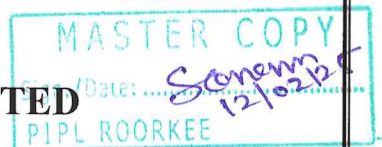
Remarks: Calibration of Pump for Flow rate accuracy Complies/ does not complies with the Tolerance limits.

Calibration By:
Sign. /Date:

Checked By:
Sign. /Date:



PRITAM INTERNATIONAL PVT. LIMITED
ANNEXURE - PIPL/ARD/001-A02



HPLC Calibration

CALIBRATION DATA SHEET
Calibration Parameter – 2: Gradient Composition Accuracy

Chromatographic Conditions:

Parameter	Details	Parameter	Details
Run Time (Min.)		Wavelength (nm)	272 nm
Solvent for Pump		Mobile Phase	10 ppm Caffeine

Gradient Composition

Sr. No.	Set Nominal Height (%)	Observed Height			Tolerance Limit (%)
		$\frac{A \times 100}{B}$	=	(%)	
1.	0		=		0.00
2.	10		=		9.8 – 10.2
3.	50		=		49.0 – 51.0
4.	90		=		88.2 – 91.8
5.	100		=		98.0 – 102.0

A= Actual Height

B = Maximum height at 100 %

Acceptance criteria: $\pm 2\%$ of set concentration

Remarks: Complies / does not comply

Calibration By:
Sign. /Date:

Checked By:
Sign. /Date:

**PRITAM INTERNATIONAL PVT. LIMITED****ANNEXURE - PIPL/ARD/001-A02**

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/Date: *12/02/2015*
PIPL ROORKEE**HPLC Calibration****Chromatographic Conditions:**

Parameter	Details	Parameter	Details
Run Time (Min).		Wavelength (nm)	272 nm
Solvent for Pump		Mobile Phase	10 ppm Caffeine

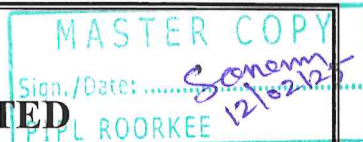
Gradient Composition

Sr. No.	Set Nominal Height (%)	Observed Height			Tolerance Limit (%)
		$\frac{A \times 100}{C}$	=	(%)	
1.	0		=		0.00
2.	10		=		9.8 – 10.2
3.	50		=		49.0 – 51.0
4.	90		=		88.2 – 91.8
5.	100		=		98.0 – 102.0

A= Actual Height

B = Maximum height at 100 %

Acceptance criteria: $\pm 2\%$ of set concentration**Remarks: Complies / does not comply****Calibration By:**
Sign. /Date:**Checked By:**
Sign. /Date:

**PRITAM INTERNATIONAL PVT. LIMITED****ANNEXURE - PIPL/ARD/001-A02****HPLC Calibration****Chromatographic Conditions:**

Parameter	Details	Parameter	Details
Run Time (Min).		Wavelength (nm)	272 nm
Solvent for Pump		Mobile Phase	10 ppm Caffeine

Gradient Composition

Sr. No.	Set Nominal Height (%)	Observed Height			Tolerance Limit (%)
		$\frac{A \times 100}{D}$	=	(%)	
1.	0		=		0.00
2.	10		=		9.8 – 10.2
3.	50		=		49.0 – 51.0
4.	90		=		88.2 – 91.8
5.	100		=		98.0 – 102.0

A= Actual Height

B = Maximum height at 100 %

Acceptance criteria: $\pm 2\%$ of set concentration**Remarks: Complies / does not comply****Calibration By:****Sign. /Date:****Checked By:****Sign. /Date:**

**PRITAM INTERNATIONAL PVT. LIMITED****ANNEXURE - PIPL/ARD/001-A02****HPLC Calibration**

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Sign./Date:
12/10/22

CALIBRATION DATA SHEET**Calibration Parameter – 3: Injection Precision (Reproducibility & repeatability)****Use 20 ppm Standard Caffeine Solution****Solvent/Diluent****:****Water: Acetonitrile (30:70)**

Injection No.	Retention Time	Area
1		
2		
3		
4		
5		
Mean		
SD		
RSD (%)		

Acceptance Criteria

- 1. Retention Time = RSD should not be more than 0.5 %**
- 2. Area = RSD should not be more than 1.0 %**

Remarks: Complies / does not comply.**Calibration By:**
Sign. /Date:**Checked By:**
Sign. /Date:



PRITAM INTERNATIONAL PVT. LIMITED

ANNEXURE - PIPL/ARD/001-A02



HPLC Calibration

CALIBRATION DATA SHEET

Calibration Parameter – 4: Injector Calibration

Chromatographic conditions:

Mobile phase/Diluent	:	Water (100%)
Injection volume (µl)	:	10 µl
Run Time (Min.)	:	

A. Calibration of Injection Volume Accuracy: -

Injection Volume	10µl from Vial.
Number of injections	15
Weight of vial with water (g) [W1]	
Weight of vial after injection (g) [W2]	
W1-W2=W (in g)	
Density of water at 25°C	0.997
Observed Volume [W x1000/0.997x15]	
Acceptance criteria (µl) (±2% of Injection volume)	9.8 to 10.2

Calibration By:
Sign. /Date:

Checked By:
Sign. /Date:

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ANNEXURE - PIPL/ARD/001-A02

**HPLC Calibration****Chromatographic conditions:**

Mobile phase/Diluent	:	Water (100%)
Injection volume (μ l)	:	20 μ l
Run Time (Min.)	:	

B. Calibration of Injection Volume Accuracy: -

Injection Volume	20 μ l from Vial.
Number of injections	15
Weight of vial with water (g) [W1]	
Weight of vial after injection (g) [W2]	
W1-W2=W (in g.)	
Density of water at 25°C	0.997
Observed Volume [W x1000/0.997x15]	
Acceptance criteria (μ l) ($\pm$ 2% of Injection volume)	19.6 to 20.4

Calibration By:
Sign. /Date:Checked By:
Sign. /Date:

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ANNEXURE - PIPL/ARD/001-A02

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PIPL ROORKEE 12/02/25

HPLC Calibration**Chromatographic conditions:**

Mobile phase/Diluent	:	Water (100%)
Injection volume (μl)	:	50 μl
Run Time (Min).	:	

C. Calibration of Injection Volume Accuracy: -

Injection Volume	50μl from Vial.
Number of injections	15
Weight of vial with water (g) [W1]	
Weight of vial after injection (g) [W2]	
W1-W2=W (in g)	
Density of water at 25°C	0.997
Observed Volume [W x1000/0.997x15]	
Acceptance criteria (μl) (±2% of Injection volume)	49.0 to 51.0

Calibration By:
Sign. /Date:Checked By:
Sign. /Date:

**PRITAM INTERNATIONAL PVT. LIMITED**

ANNEXURE - PIPL/ARD/001-A02

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Sign./Date:
PIPL ROORKEE**HPLC Calibration****Chromatographic conditions:**

Mobile phase/Diluent	:	Water (100%)
Injection volume (μl)	:	100 μl
Run Time (Min.)	:	

D. Calibration of Injection Volume Accuracy: -

Injection Volume	100μl from Vial.
Number of injections	10
Weight of vial with water (g) [W1]	
Weight of vial after injection (g) [W2]	
W1-W2=W (in g)	
Density of water at 25°C	0.997
Observed Volume [W x1000/0.997x15]	
Acceptance criteria (μl) (±2% of Injection volume)	98.0 to 102.0

Calibration By:
Sign. /Date:Checked By:
Sign. /Date:

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12/02/22

PIPL ROORKEE

HPLC Calibration**E. Injector Linearity:****Chromatographic conditions:**

Mobile phase/Diluent	Water: Acetonitrile (30:70)
Injection volume (μL)	20 μl
Column ID	
Flow rate (ml/min)	1.0 ml/min
Detector Wavelength (nm)	272 nm
Column Oven temperature ($^{\circ}\text{C}$)	30 $^{\circ}\text{C}$

Inject separately 5 μl , 10 μl , 20 μl , 50 μl & 100 μl injection in duplicate of 20 ppm Caffeine Standard Solution

Injection Volume (μl)	Mean Area
5 μl	
10 μl	
20 μl	
50 μl	
100 μl	

Acceptance Criteria = Area vs. Injection volume graph should be linear and Correction coefficient should be greater than 0.999**Result:**

1. Area vs. Injection volume graph is linear /not linear.
2. Correction coefficient is greater/not greater than 0.999. (Correction coefficient).

Calibration By:**Sign. /Date:****Checked By:****Sign. /Date:**

**PRITAM INTERNATIONAL PVT. LIMITED****ANNEXURE - PIPL/ARD/001-A02****HPLC Calibration****F. Vial Positioning:****Procedure: Inject Blank and single injection of standard solution of each dilution.****Note: Use the all concentration solution of Detector Linearity test dilution.**

Sr. No.	Vial Program	Tray Name	Concentration	Vial Analysed	Remark
1			5 ppm		
2			10 ppm		
3			20 ppm		
4			50 ppm		
5			100 ppm		
Acceptance Criteria: All Vials to be picked/punctured as per program.					

Calibration By:
Sign. /Date:**Checked By:**
Sign. /Date:



PRITAM INTERNATIONAL PVT. LIMITED

ANNEXURE - PIPL/ARD/001-A02

HPLC Calibration

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PIPL ROORKEE 12/02/25

Calibration Parameter – 5: Carry Over

Chromatographic conditions:

Mobile phase/Diluent	:	Water: Acetonitrile (30:70)
Injection volume (µL)	:	20µl
Column ID	:	
Detector Wavelength (nm)	:	272 nm

Use 250 ppm Caffeine standard solution:

Calculation for Carry over (%)

Area observed of any peak due to Caffeine in post blank (A) :

Area of Principal peak in the chromatogram of the Caffeine solution (B) :

Carry over (%) =
(Ax100/B)

Acceptance Criteria: NMT 0.01%

Remarks : Complies / does not comply.

Calibration By:
Sign. /Date:

Checked By:
Sign. /Date:





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ANNEXURE - PIPL/ARD/001-A02

HPLC Calibration

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ROORKEE 12/02/20

CALIBRATION DATA SHEET
Calibration Parameter – 7: Wavelength Accuracy

Chromatographic conditions:

Mobile phase/Diluent	Water: Acetonitrile (30:70)
Injection volume (μL)	20 μL
Column ID	
Flow rate (ml/min)	1.0 ml/min
Detector Wavelength (nm)	268 nm to 276 nm
Column Oven temperature (°C)	30 °C
Use Caffeine standard	20 ppm

Wavelength(nm)	Area of Caffeine solution of 20 ppm
269	
270	
271	
272	
273	
274	
275	
276	

Observation: λ max obtained at:nm.

ACCEPTANCE CRITERIA = Area should be maximum at wavelength 272 ± 2 nm.



PRITAM INTERNATIONAL PVT. LIMITED

ANNEXURE - PIPL/ARD/001-A02

HPLC Calibration

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Chromatographic conditions:

Mobile phase/Diluent	Water: Acetonitrile (30:70)
Injection volume (μL)	20 μL
Column ID	
Flow rate (ml/min)	1.0 ml/min
Detector Wavelength (nm)	241 nm to 248 nm
Column Oven temperature ($^{\circ}\text{C}$)	30 $^{\circ}\text{C}$
Use Caffeine standard	20 ppm

Wavelength(nm)	Area of Caffeine solution of 20 ppm
241	
242	
243	
244	
245	
246	
247	
248	

Observation: λ min obtained at:nm.

ACCEPTANCE CRITERIA = Area should be minimum at wavelength 244 ± 2 nm.

**PRITAM INTERNATIONAL PVT. LIMITED**

ANNEXURE - PIPL/ARD/001-A02

HPLC CalibrationMASTER COPY
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Roorkee 12/02/25**Chromatographic conditions:**

Mobile phase/Diluent	Water: Acetonitrile (30:70)	
Injection volume (μL)	20 μL	
Column ID		
Flow rate (ml/min)	1.0 ml/min	
Detector Wavelength (nm)	202 nm to 209 nm	
Column Oven temperature (°C)	30 °C	
Use Caffeine standard	20 ppm	

Wavelength(nm)	Area of Caffeine solution of 20 ppm
202	
203	
204	
205	
206	
207	
208	
209	

Observation: λ max obtained at:nm.**ACCEPTANCE CRITERIA =** Area should be maximum at wavelength 205 ± 2 nm**Remarks:** Complies/does not complies



PRITAM INTERNATIONAL PVT. LIMITED

ANNEXURE - PIPL/ARD/001-A02

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ROORKEE 12/02/25

HPLC Calibration

Calibration Parameter – 9: Noise and Drift

Observation:

Remarks: Complies/does not complies

Note: Calibration of Column Oven Temperature from External Lab once in a year.

Calibration By:
Sign. /Date:

Checked By:
Sign. /Date:



PRITAM INTERNATIONAL PVT. LIMITED
ANNEXURE - PIPL/ARD/001-A03

Format for HPLC Calibration Summary Sheet

INSTRUMENT ID :		Calibration Date :					
Calibrated By :		Calibration Due Date :					
Test Name	Flow Rate (ml/min)	Observation				Acceptance Criteria ($\pm 2\%$ of set flow rate)	Status
		A	B	C	D		
Calibration of the pump flow rate	0.5					0.49-0.51	Complies / Does not Complies
	1.0					0.98-1.02	
	1.5					1.47-1.53	
	2.0					1.96-2.04	
Gradient Composition Accuracy	0 %	10 %	50 %	90 %	100 %	At each absorbance level $\pm 2\%$ of set concentration	Complies / Does not Complies
Limit $\longrightarrow$	0.00	9.8 to 10.2	49.0 to 51.0	88.2 to 91.8	98.0 to 102.0		
Column Oven calibration / External Lab	25°C		40°C		60°C	$\pm 2.0^\circ\text{C}$ of set value	Complies / Does not Complies
Injector Precision (Reproducibility & repeatability) {Using 20 ppm Caffeine}	RSD of Retention Time					RSD of RT NMT 0.5%	Complies / Does not Complies
	RSD of Area					RSD of area NMT 1.0 %	



PRITAM INTERNATIONAL PVT. LIMITED
ANNEXURE - PIPL/ARD/001-A03

Format for HPLC Calibration Summary Sheet

MASTER COPY

Sign./Date:
PIPL ROORKEE

Injector Linearity	Correlation coefficient 'r' =	NLT 0.999	Complies / Does not Complies
Injector volume Accuracy	15 injection of 10 µL	9.8-10.2	Complies/ Does not complies
Injector volume Accuracy	15 injection of 20 µL	19.6-20.4	Complies/ Does not complies
Injector volume Accuracy	15 injection of 50 µL	49.0-51.0	Complies/ Does not complies
Injector volume Accuracy	10 injection of 100 µL	98.0-102.0	Complies/ Does not complies
Vial Position	Refer to Detector Linearity Vials	All Vials to be picked/punctured	Complies/ Does not complies
Wavelength accuracy	Wavelength = ----- nm (Using Caffeine 20 ppm)	272.0 nm ± 2.0 nm (Maxima)	Complies / Does not Complies
Wavelength accuracy	Wavelength = ----- nm (Using Caffeine 20 ppm)	244.0 nm ± 2.0 nm (Minima)	Complies / Does not Complies
Wavelength accuracy	Wavelength = ----- nm (Using Caffeine 20 ppm)	205.0 nm ± 2.0 nm (Maxima)	Complies / Does not Complies



PRITAM INTERNATIONAL PVT. LIMITED
ANNEXURE - PIPL/ARD/001-A03

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Sign./Date:
PIPL ROORKEE 12/12/25

Format for HPLC Calibration Summary Sheet

Linearity of Detector Response	Correlation coefficient 'r' = -----	NLT 0.999	Complies / Does not Complies
Carryover Test	Carryover = ----- %	Carryover of Caffeine NMT 0.05% of area of 20 µl injection of 250 ppm Standard Solution.	Complies / Does not Complies
Noise & Drift (VWD)		Noise: ±0.2 mAU Drift: ±2.0 mAU	Complies / Does not Complies

Remarks: All the parameters for the calibration as per predefined acceptance criteria are under limit hence the instrument is suitable for routine analysis

Calibrated By:
Sign./Date:

Checked By
Sign./Date



PRITAM INTERNATIONAL PVT. LIMITED

ANNEXURE - PIPL/ARD/001-A04

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Date: ...
PIPL ROORKEE 12/02/25

Standard Preparation Sheet

Mobile Phase: 70 Volume of ACN & 30 Volume of water

Column Name: Inertsil ODS, C18, 250 mm x 4.6 mm, 5 μ

Column ID No:

Injection Volume: As Per Required Parameters

Use Std.: Caffeine

Detector: As Per Required Parameters

Flow Rate: 1.0 ml / min

Name of Standard: _____

Batch No.: _____

Valid up to: _____

Stock Solution:

Weight aboutgm of Caffeine in 100 ml Volumetric Flask add 50-75 ml of mobile phase, Sonicate 5 min for dissolve, make volume up to mark with mobile phase.

5 ppm: Take 1 ml of this solution into 100 ml of mobile phase.

10 ppm: Take 2 ml of this solution into 100 ml of mobile phase

20 ppm: Take 2 ml of this solution into 50 ml of mobile phase

50 ppm: Take 5 ml of this solution into 50 ml of mobile phase

100 ppm: Take 10 ml of this solution into 50 ml of mobile phase

250 ppm: Take 10 ml of this solution into 20 ml of mobile phase

Blank: Mobile phase

Remarks: The Prepared Solution is OK/Not OK.

Prepared By :

Checked By:

Date :

Date: