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TITLE: OPERATION AND CALIBRATION OF HPLC (SHIMADZI)			

1.0 Objective:

To describe the written standard procedure for Calibration & Operation of HPLC LC- 2050C

2.0 Scope:

This SOP is applicable for Calibration & Operation of HPLC LC-2050C

3.0 REFERENCE

In-House, Instrument Manual

- 4.0 Responsibility:
 - 4.1 Officers and Executive F&D
 - **4.1.1** Proper Operation of HPLC, LC- 2050C
 - **4.1.2** Calibration as per frequency & method.
 - **4.1.3** To give training as per written approved procedure by time to time.
 - **4.2** Engineering Department
 - **4.2.1** Maintenance of HPLC as per intimation from Analytical Development Department.
- 5.0 Accountability:
 - **5.1** Manager- AD
- 6.0 Procedure:
 - **6.1** Instrument Details:

HPLC Auto sampler - Instrument ID-XXXX & AD/LC/XXX Model-LC-2050C & LC2050 Make-Shimadzu.

- **6.2** General About Instrument :-
 - **6.2.1** Gradients pump.
 - **6.2.2** Thermostat column compartment.
 - **6.2.3** Auto sampler & Injector.
 - **6.2.4** Detector with wave length.
 - **6.2.5** Computer with window 10 (Pro) bases for LC-2050C with Lab Solution Software.
 - **6.2.6** Solvents reservoir Bottle A, B, C, D. Rinse and seal wash.
 - **6.2.7** Store the instrument clean. Always keep the instrument in AC room.
- 6.3 Basic Start UP Procedure For HPLC Systems:-

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- **6.3.1** Ensure that the system is connected to stabilize with UPS Power supply.
- **6.3.2** Switch ON the main for power supply.
- **6.3.3** Switch ON the main for instruments screen show.
- **6.3.4** wait for Ok & asking user password,
- **6.3.5** Switch ON the pump & oven if require.
- **6.3.6** Switch ON Detector.
- **6.3.7** Computer ON opens with Lab solution Software.

6.4 Lab Solution Software Operation:

- **6.4.1** Cursor put on **Lab** solution icon & Double Click.
- **6.4.2** Enter the user ID and password, Click on 'OK'
- **6.4.3** Double click on the instrument ID which is required.
- **6.4.4** Now Lab solution software open for operation & come beep sound 2 times.
- **6.4.5** Open new method file & generate new method for new product
- **6.4.6** Close method file last open, before open new method file (existing)
- **6.4.7 Post rum File:** for running, existing data graphs observation & printing.

6.5 Data Acquisition Parameters:-

- **6.5.1 Data Acquisition:** LC time process (sample run time)
- **6.5.2 Gradient time Process:** Sample gradient analysis time setting.
- **6.5.3** Flow Rate: 0.2 to 2.0 ml, & Down Load.
- **6.5.4** M.P. Lines A. B, C, D tubes Volume set in %.
- **6.5.5** Maximum Pressure set (as require Max: 440)
- **6.5.6** Set Lamp ON/OFF & Down Load
 - [a] Temp: L/H.
 - [b] Wave Length selection Like 254 nm.
 - [c] Volt : OK
 - [d] Range: 1.0 %
- **6.5.7 Oven:** Column Temperature selection 20°C to 70°C as require & in degases.

6.5.8 Auto Sampler:-

6.5.8.1 Rack 1, 2, 3 or 4 : Select vials 1.5 ml x 54, when D2 on

6.5.8.2 Rinsing : 200 μl

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6.5.8.3 Needle : 52 mm

6.5.8.4 SMP Speed : 15 ul / sec.

6.5.8.5 Rinse Needle. : Before & after

6.5.9 Auto Purging:-

6.5.9.1 Purging Order: A, B, C, D and rinse port.

6.5.9.2 Mobile Phase : A, B,C, D.

6.5.9.3 Purging Time : 1....to....10 min. **6.5.9.4** Purging Pump : LC-2030C & Plus

6.5.10 (Window open for new method & new product file (Unknown file Single & Multiple Injection):-

6.5.10.1 For Window: - Change in wizard

6.5.10.2 New Batch file Sequences

6.5.10.2.1 Show window - \rightarrow Batch file \rightarrow Wizard \rightarrow Method show (XYZ) \rightarrow Tray No \rightarrow injection volume \rightarrow No of samples \rightarrow (New) unknown only \rightarrow sample name \rightarrow sample ID \rightarrow data file \rightarrow finish.

6.6 Calibration Procedure of HPLC:-

- 6.6.1 A] Calibration of Pump:-
 - **6.6.1.1** Calibration of Pump is to be done with the help of distilled water at $25^{\circ}\text{C}\pm2^{\circ}\text{C}$.
 - **6.6.1.2** Set the flow of the pump at 1ml/min. keep the system at this flow rate for about 10 minutes to equilibrate the system without the Column.
 - **6.6.1.3** Now dry to A grade measuring cylinder & weight the empty A grade measuring cylinder before starting the procedure.
 - **6.6.1.4** Start the stopwatch & simultaneously start collecting the eluent from the pump outlet in a class A grade measuring cylinder (**10ml capacity**).
 - **6.6.1.5** Measure the time it takes for water to fill up to the graduation marked and calculate the flow accuracy and flow rate.
 - 6.6.1.6 Calculate the Flow rate accuracy by following formula

 Flow rate accuracy (%) = (actual Flow rate/Set Flow rate-1)x100

 Set time 0.5 ml/min 1200(s), 1.0 ml/min 600(s), 2.0 ml/min 300(s),
 - 6.6.1.7 Calculate the Actual Flow rate by following formula
 Actual Flow rate = Set flow rate X set time (s)/actual measurement time
 Set time 0.5 ml/min 1200(s), 1.0 ml/min 600(s), 2.0 ml/min 300(s),

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6.6.1.8 Set the flow 0.5, 1.0, & 2.0 ml & repeat the above experiment, allowing the system to equilibrate at least for 10 minutes at each flow set.

6.6.1.9 Acceptance Criteria:

For: 0.5 ml/min \pm 0.010 ml/min [0.490-0.510 ml/min] For: 1.0 ml/min \pm 0.020 ml/min [0.980-1.020 ml/min] For: 2.0 ml/min \pm 0.040 ml/min [1.960-2.040 ml/min]

6.6.1.10 Same way Calibrate A, B, C, D pump individually as above procedure.

6.6.1.11 B) Noise & Drift Calibration For UV;(Limit : Noise: 20 x10⁻³ AU/h **& Drift :** 500 x10⁻³ AU/h

For PDA: Limit: Noise: 50 x10⁻³ AU/h & Drift: 500 x10⁻³ AU/h

Pre-treatment procedure: for System Before Calibration.

- Sonicate suction filter for 10 minutes in 10% v/v Nitric acid and then 5 minutes in 100 % methanol.
- ➤ Set the each 4 port with 25% ratio and run HPLC grade IPA 100% for 30 min with flow rate 5ml / min.
- ➤ Deep all ports in HPLC grade Hot water & purge all lines.
- ➤ Connect the tubes with union & Replace the Column.
- > Set the each 4 port with 25% ratio and run HPLC grade hot water for 30 min with flow rate 5ml/min.

Condition for drift and Noise:

Mobile phase : HPLC grade water (Dip all port in water)

Flow rate : 1.0 ml/min. Wave Length : 250 nm, Column Oven : 25°C

Column: connect with union or capillary column.

Saturation time: 60 minutes Run Time: 15 minutes

6.6.1.12 C) Gradient Pump Calibration (Limit $\pm 2.0\%$)

Mobile Phase	A Line & C Line	Water
	B Line & D Line	10 ppm Caffeine in water
Chromatographic condition	Flow	2 ml/min
	Wavelength	272 nm
	Run time	26 Min
	Vial	System Blank

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Min	A	В	С	D	
1	50	0	50	0	
1.01	40	10	40	10	
4	40	10	40	10	
4.01	30	20	30	20	
7	30	20	30	20	
7.01	20	30	20	30	
10	20	30	20	30	
10.01	10	40	10	40	
13	10	40	10	40	
13.01	0	50	0	50	
16	0	50	0	50	
21	50	0	50	0	
26	50	0	50	0	STOP

- **6.6.1.12** Determine Lamp Energy: NLT 400 mv by Lab solution software.
- **6.6.2 Vial Position Test**: Use linearity solution to perform this test, for different position of sample injection in tray as below and calculate the linearity.

Vial Position at tray		
1-1	2 22	3-54
2-9	3-33	4-46

- 6.6.3 Calibration of Detector:-
 - **6.6.3.1** Repeatability (Precision)
 - **6.6.3.2** Absorbance linearity
 - 6.6.3.3 System Repeatability of Peak area and Retention time:-

System Parameters:

Column : C18 250 X 4.6 mm, 5 μ Mobile phase : Water: Methanol = 60:40

Flow rate : 1.00ml/min

Wavelength : 272 nm

Column oven Temp : 40°C

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Volume : 10 μl Analysis Time : 3-8Min

Caffeine Solution : 20mcg/ml in MP: Separately 6 replicates Injections.

6.6.3.4 Linearity of Detector absorbance:

System Parameters: same as above system & condition: Caffeine solution : 10/15/20/25/30 mcg/ml in MP.

Caffeine anhydrous Stock solution: Caffeine 20mg------→100ml

Sr. No.	Diluted Solution	Conc. mcg/ml	Area	R square NLT 0.99
01	1ml20	10		
02	1.5 ml20	15		
03	2 ml20	20		
04	2.5ml20	25		
05	3ml20	30		

6.6.4 Calibration of Injector (Loop):-

6.6.4.1 Chromatographic Condition:-

Column : C18 250 X 4.6 mm, 5 μ

Mobile phase : Methanol : Water (40 : 60)

Flow Rate : 1.0 ml/min Wavelength : 272nm

6.6.4.2 Preparation of Standard Solution:-

- **6.5.4.2.1** Weigh accurately 20 mg of caffeine into a 100ml volumetric flask, dissolve & dilute to volume with water. Further dilute 10 ml. of this solution to 100 ml with water. (20 mcg/ml.)
- **6.5.4.2.2** Inject 10μl, 20μl, 50μl of the standard solutions five replicates & record the chromatograms.
- **6.5.4.2.3** Calculate the %RSD of the peak area of caffeine from the 5 replicate injections at each level.
- **6.5.4.2.4** Calculate the average peak area of caffeine from the 5 replicate injections at each level.

6.5.4.2.5 Acceptance Criteria:-

% RSD limit for 5 replicate injection samples should NMT 2.0%.

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6.6.5 Calibration of Thermostat for column oven:

- **6.6.5.1** Purge the system with purified water to remove any solvents & previous buffer salts.
- **6.6.5.2** Keep the thermometer in the right side of the column oven.
- **6.6.5.3** Set the temperature at 20°C. Wait till the set temperature is attained & the temperature display on the instrument is stable.
- **6.6.5.4** Record the temperature displayed by the instrument & the thermometer.
- **6.6.5.5** Repeat the steps for temperature setting at 30°C, 40°C, 50°C, 70°C.
- **6.6.5.6** Record the observation.
- **6.6.5.7** Record the temperature displayed by the instrument & the thermometer.
- **6.6.5.8 Acceptance Criteria:** The difference between the set temperature & the displayed temperature on the thermometer should not be more than $\pm 1^{\circ}$ C.

6.6.6 Wavelength Accuracy Calibration :- (Auto sampler HPLC)

6.6.6.1 Verify the wavelength accuracy use **20 mcg/ml** dilution of caffeine in water.

Caffeine solution- mg \longrightarrow 100 ml \longrightarrow 10 ml \longrightarrow 50 ml

6.6.6.2 Mobile Phase - Methanol: Water (40: 60).

Column - C18 250 X 4.6 mm, 5 μ

6.6.6.3 Flow Rate - 1 ml/min

Primary Std. No.

6.6.6.4 Inject the solution & enter the value of wave length 269 nm to 275 nm.

Note down the areas observed. And other wavelength 202 nm to 208 nm

Note down the areas observed. And other wavelength 241 nm to 247 nm

- **6.6.6.5** For PDA detector used 1st injection of Repeatability test and scan with spectra.
- **6.6.6.6 Acceptance Criteria** Maxima 273± 2 nm

Acceptance Criteria - Minima 243 ± 2 nm

Acceptance Criteria - Maxima 204± 2 nm

6.6.7 Carry over Test (NMT 0.01%)

6.6.7.1 For carry over test first inject water as blank followed by three injections of 200 mcg/ml dilution of caffeine in mobile phase & then blank inj.

Calculation:

% Carry Over = (Area of caffeine in blank 2 - Area of caffeine in blank 1) \times 100 Mean area of caffeine in sample solution

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6.6.8 Frequency of Calibration: -

6.6.8.1 Calibration shall be carried out in every six months or if any major break down observed in specific parts (hardware),the calibration shall be carried for same related part before use the same instrument as below mention table:-

Sr.	Name of HPLC Parts	Related test for calibration after breakdown
No	under breakdown	
1	Pump	Pump calibration
2	D2 Lamp	Wavelength accuracy
3	Solenoid valve	Calibration of GPV
4	Detector	Detector calibration point & test Repeatability
		test
5	Injector Loop	Injection Volume Precision
		(Calibration of injector)

- **6.6.8.2** Breakdown shall be reported through laboratory incident ref. to Respective SOP.
- **6.6.8.3** After calibration record all the chromatograms and results filled to Annexure I. If all result are within the SOP specification limit than use the same HPLC system for routine analysis.

6.6.9 Calibration of Sample Cooler:-

- **6.6.9.1** Purge the system with purified water to remove any solvents & previous buffer salts.
- **6.6.9.2** Keep the thermometer in the right side of the column oven.
- **6.6.9.3** Set the temperature at 5°C. Wait till the set temperature is attained & the temperature display on the instrument is stable.
- **6.6.9.4** Record the temperature displayed by the instrument & the thermometer.
- **6.6.9.5** Repeat the steps for temperature setting at 10°C, 15°C, 20°C, 25°C.
- **6.6.9.6** Record the observation.
- **6.6.9.7** Record the temperature displayed by the instrument & the thermometer.
- **6.6.9.8 Acceptance Criteria:** The difference between the set temperature & the displayed temperature on the thermometer should not be more than $\pm 1^{\circ}$ C.

7.0 Abbreviations:

7.1 SOP : Standard Operating Procedure7.2 QA : Quality Assurance

7.3 HPLC: High Performance Liquid Chromatography

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8.0 Annexure (s):

Format No.: F/AD/044/A/00

Annexure I – Calibration of HPLC Auto Sampler

F/AD/044/B/00

Annexure II –HPLC User Log Book

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SUMMARY OF HPLC CALIBRATION

Annexure - I Format No.: F/AD/044/A/00

Instrument Name:	HPLC AUTO SAMPLER	Model Number:	LC2050C
Serial number:		Instrument ID number:	AD/LC/
Date of Calibration		Next Calibration due date:	

Sr. No.	Test Performed	l	Acceptance criteria		Observation		
1	A] Flow accuracy of pump (ml/min)		Tolerance (mL/min)	A	В	С	D
	0.5		0.490 to 0.510				
	1.0		0.980 to 1.020				
	2.0		1.960 to 2.040				
		For UV	Noise 20 x10 ⁻³ AU/h	Noise : Drift :			
	B) Calibration of Noise &		Drift 500 x10 ⁻³ AU/h				
	Drift	For PDA	Noise 50 x10 ⁻³ AU/h) Noise:				
			Drift 500 x10 ⁻³ AU/h	Drift:			
	C] Calibration of GPV						
	0.0 %		NA				
	20 %		18 % - 22 %				
	40 %		38 % - 42 %				
	60 %		58 % - 62 %				
	80 %		78 % - 82 %				
	100 %		98 % - 102 %				
	UV Lamp Energy		NLT 400 mv				
2	DETECTOR CALIBRATION TEST		Area -NMT 2 .0 %				
	A) Repeatability test.		R-time-NMT 1.0 %				
	B) Absorbance Linearity Test.		R ² – NLT: 0.997 %				
	C) Vial Position Test.		Complies				
3	Injection Volume Precision			Inj.Vol.	% RSD-Are	a	% RSD-RT
	(Calibration of injector)	(Calibration of injector)		10 μl			
			% RSD= NMT 2.0	20 μ1			
				50 μl			
4	Thermostat Oven		NMT ± 1° C				
5	Carry over Test		0.01 %				
6	Wavelength Accuracy		Maxima 273 ± 2 nm				
			Maxima 204 ± 2 nm				
			Minima 243 ± 2 nm				
7	Sample Oven		NMT ± 1° C				

CALIBRATION STATUS: The calibration of the instrument is within limit/ out of limit.

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TITI F. OPERATION AND CALIBRATION OF HPLC (SHIMADZIL)					

Calibrated By Sign/Date:

Checked By Sign/Date:

CALIBRATION OF PUMP: (Dist water Temp at 25°C - 27°C)

Set the flow 0.5, 1.0 & 2.0 ml & repeat the above experiment, allowing the system to equilibrate at least for 10 minutes at each flow set.

STOP WATCH ID No: Calibration due date:

Acceptance criteria: $\pm 2.0 \%$ in ml

Pump. A

Set Flow rate (ml/min)	Set Time (s)	Actual Measurement time	Flow Rate Accuracy	Actual Flow rate
0.5 ml	1200			
1.0 ml	600			
2.0 ml	300			

Pump. B

Set Flow rate	Set Time (s)	Actual Measurement time	Flow Rate Accuracy	Actual Flow rate
0.5 ml	1200			
1.0 ml	600			
2.0 ml	300			

Pump. C

Set Flow rate	Set Time (s)	Actual Measurement time	Flow Rate Accuracy	Actual Flow rate
0.5 ml	1200			
1.0 ml	600			
2.0 ml	300			

Pump. D

Set Flow rate	Set Time (s)	Actual Measurement time	Flow Rate Accuracy	Actual Flow rate
0.5 ml	1200			
1.0 ml	600			
2.0 ml	300			

Calibrated by (Sign/Date):

Checked by (Sign/Date):

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Calibration of Noise and Drift:

Detector	Noise Limit	Drift:	Observed Result	
			Noise	Drift
PDA	Noise 50 x10 ⁻³ AU/h	500 x10 ⁻³ AU/h		
UV	Noise 20 x10 ⁻³ AU/h	500 x10 ⁻³ AU/h		

Calibrated by (Sign/Date):

Checked by (Sign/Date):

Gradient Pump Calibration (Limit +/- 2.0%)

Quaternary gradient system	Limit	Observation (result)
0 %	NA	
20 %	18 % - 22 %	
40 %	38 % - 42 %	
60 %	58 % - 62 %	
80 %	78 % - 82 %	
100 %	98 % - 102 %	

k	lesu	lts:	comp	lies /	not	comp	lies
---	------	------	------	--------	-----	------	------

UV Lamp Energy test: NLT 400 MV :.....

Calibrated by (Sign/Date):

Checked by (Sign/Date):

2] DETECTOR CALIBRATION POINT & TEST

(a) REPEATABILITY TEST

(b) ABSORBANCE LINEARITY TEST

a] System repeatability of peak area and retention time (Limit -RSD NMT 1.0 %)

System Parameters: 1st wash with Methanol.

Column ID :..... (C18 250 X 4.6 mm, 5µ)

Mobile phase : Water: Methanol = 60:40

Flow rate : 1.00ml/min
Wavelength : 272 nm
Column oven Temp : 40°C

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Volume : $10 \mu l$ Analysis time : 3-8Min

Caffeine solution : 20mcg/ml in MP: Separately 6 replicates injections.

<u>Fest Preparation</u> : (Caffeine	mgml-→
--	--------

Primary Std Batch No - Opened date: Exp. Date:

OR W.S No._____ Vial No.____

Sr. No.	Conc.	Area	%RSD	Retention Time	%RSD
01	20 mcg/ ml				
02	20 mcg/ ml				
03	20 mcg/ ml				
04	20 mcg/ ml				
05	20 mcg/ ml				
06	20 mcg/ ml				
]	Mean				

Calibra	ted	hv	(Sion.	/Date)	١:
Cambra	uu	W 1	(DIZII	Date	

Checked by (Sign/Date):

b] LINEARITY OF DETECTOR RESPONSE (Absorbance linearity Test)

System Parameters: Same as above system & condition:

Caffeine solution : 10/15/20/25/30 mcg/ml in water

<u>Caffeine Stock Solution</u>: (Caffeinemg------→ml)

OR W.S No._____ Vial No.:____

Sr. No.	Diluted	Conc.	Area	R square
	Solution	mcg/ml		NLT 0.997
01	1ml20	10		
02	1.5ml20	15		
03	2ml20	20		
04	2.5ml20	25		
05	3ml20	30		

Calibrated by (Sign/Date):
FORMAT NO.: F/DQA/XXX/A/XX

Checked by (Sign/Date):

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3] CALIBRATION OF INJECTOR (loop):

Chromatographic cond	dition:					
Column ID (C18 250 X 4.6 mm, 5µ)						
Mobile phase	: Water: Methanol = 60:40					
Flow rate	: 1.00 ml/min					
Wavelength	: 272 nm					
Column oven Temp	: 40°C					
Caffeine standard so	lution: 20 mcg/ml in water					
Primary Std: Batch N	o					
OR W.S No	OR W.S No Vial No					
Inject 10μl, 20μl, 50μl, of the standard solutions five replicates & record the chromatograms.						
Calculate the %RSD of the peak area of caffeine from the 5 replicate injections at each level.						
Calculate the average	peak area of caffeine from the 5 replicate injections at each level.					
Acceptance criteria (% RSD: NMT 2 0)						

Sr. No.	10µl area	20µl area	50µl area	0	% RSD	
				Inj. Volume	Area	RT
01				10.1		
02				10 µl		
03				201		
04				20 μl		
05				501		
Average				50 μl		

Calibrated by (Sign/Date):

Checked by (Sign/Date):

4. CALIBRATION OF THERMOSTAT COLUMN OVEN:

Thermometer Id No:Range: Calibration Due date:..........

Set the temperature at 20° C. Wait till the set temperature is attained & the temperature display on the instrument is stable. Record the temperature displayed by the instrument & the thermometer. Repeat the steps for temperature setting at 20° C, 30° C, 40° C, 50° C & 70° C. Record the observation.

Acceptance criteria: (NMT +/- 1° C)

Sr. No.	20°C	30°C	40°C	50°C	70°C
01					
02					
Mean					

Calibrated by (Sign/Date):

Checked by (Sign/Date):

RAVENBHEL			EL HEALTHCARE CO, KARTHOLI, J		
TANDAR	D OPERATING PI	ROCEDUR	E	PAGE NO.: 15 o	of 17
DEPARTMENT: ANALYTICAL		SOP. NO.	: RHPL/AD/044/00	SUPERSEDES I PROCEDURE I	
EVELOP	MENT	EFFECTI	VE DATE:	REVIEW DATE	Ε:
ITLE: OP	PERATION AND C	ALIBRATI	ON OF HPLC (SH	IMADZU)	
5. <u>CARR</u>	Y OVER TEST:				
Primary S	Std: Batch No		Opened date:	Exp. Date:	
OR W.S	No	Vial	No		
Sample P	Preparation: - Prepare	e 200 mcg/ml	of caffeine in water.		
Caffeine s	solution mg	> m	l>ml>	≻ ml	
Sr. No.	Mean Area of cal		Area of caffeine in blank injection	% Carry over	Limit
			Blank 1		
			Blank 2		0.01%
			Diank 2		
Calculati Calibrate	ion: ed by (Sign/Date):	Mean are	ea of caffeine in sample	e solution Checked by (Sign/Date):	
6. WAVE	ELENGTH ACCURA	ACY CALIB	RATION: (For UV de	etector)	
	the wavelength accurates the wavelength accurates the wavelength accurates the wavelength accurate the wavelength accurates the wavelength accurate the wavelength accurates the wavelength accurate the w		cg/ml dilution of caffei →	ine in water.	
Mobile Pl	hase - 40 Methanol: 60) water			
Column I	D		(C18 250 X 4.6 mm, 5	ίμ)	
Flow rate	- 1 ml/min				
Primary S	Std: Batch No		Opened date:	Exp. Date:	
OR W.S	No	Vial	No		

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STANDARD OPERATING PR	PAGE NO.: 16 of 17			
DEPARTMENT: ANALYTICAL	SOP. NO.: RHPL/AD/044/00	SUPERSEDES EARLIER PROCEDURE DATE: NA		
DEVELOPMENT	EFFECTIVE DATE:	REVIEW DATE:		
TITLE: OPERATION AND CALIBRATION OF HPLC (SHIMADZU)				

Inject the solution & enter the value of wave length 269 nm-275 nm.

Note down the areas observed.

1st Maxima Acceptance Criteria - Maxima 273± 2 nm

Sr. No.	Wave length	Area	Maxima
01	269 nm		
02	270 nm		
03	271 nm		
04	272 nm		
05	273 nm		
06	274 nm		
07	275 nm		

2nd Maxima Acceptance Criteria - Maxima 204± 2 nm

Inject the solution & enter the value of wave length 202 nm-208 nm. Note down the areas observed

Sr. No.	Wave length	Area	Maxima
01	202 nm		
02	203 nm		
03	204 nm		
04	205 nm		
05	206 nm		
06	207 nm		
07	208 nm		

^{3&}lt;sup>rd</sup> Minima Acceptance Criteria - Minima $243 \pm 2 \text{ nm}$

Inject the solution & enter the value of wave length 241 nm-248 nm. Note down the areas observed

Sr. No.	Wave length	Area	Minima
01	241 nm		
02	242 nm		
03	243 nm		
04	244 nm		
05	245 nm		
06	247 nm		
07	248 nm		

Calibrated by (Sign/Date):

Checked by (Sign/Date):

WAVELENGTH ACCURACY CALIBRATION: (For PDA detector)

Take 1st injection of repeatability and scan with spectra

STANDARD OPERATING PR	PAGE NO.: 17 of 17				
DEPARTMENT: ANALYTICAL	SOP. NO.: RHPL/AD/044/00	SUPERSEDES EARLIER PROCEDURE DATE: NA			
DEVELOPMENT	REVIEW DATE:				
TITLE: OPERATION AND CALIBRATION OF HPLC (SHIMADZU)					

Sr. No.	Wave length	Acceptance Criteria	Result
01	241 nm-248 nm	Minima 244 ± 2 nm	
02	202 nm-208 nm	Maxima 205± 2 nm	
03	269 nm-275 nm	Maxima 272± 2 nm	

Calibrated by (Sign/Date):			Checked by (Sign/Date):				
7. <u>CALIBRA</u>	ATION OF SAMP	LE OVEN:					
Thermomete	r ID No.:	Raı					
Acceptance of	eriteria: (NMT +/-	1° C)					
The difference than $\pm 1^{\circ}$ C.	e between the set te	emperature & the dis	splayed temperature	on the thermo-meter	should not be more		
Sr. No.	5°C	10°C	15°C	20°C	25°C		
01							
02							
Mean							
Calibrated by (Sign/Date): Affix the Calibration Label on Instruments:				Checked by (Sign/Date):			
AIIIX the Cal	ibration Labei on	instruments:					
Name of Inst	rument : Shimadz	u HPLC, LC-2050C					
Instrument ID	: AD/LC/.						
Date of Calib	oration :						
Due Date (After Six month/ as when required if any breakdown):							
Calibrate by : Sign/Date :				Checked by : Sign/Date :			

Approved By Manager (Sign/Date):

END OF DOCUMENTS