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Title:	Operation, Calibration, and Maintenance of the HPLC System	**Version:**	1.0
Company:	NovaThera Pharmaceuticals Pvt. Ltd.	**Effective Date:**	2025-01-01
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Operation, Calibration, and Maintenance of the HPLC System

Category: Quality Control Laboratory

Standard Operating Procedure (SOP)

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1.0 PURPOSE

This procedure outlines the standard operating procedure for the operation, calibration, and maintenance of the High-Performance Liquid Chromatography (HPLC) system within the Quality Control Laboratory at NovaThera Pharmaceuticals Pvt. Ltd., Pune, India. This SOP ensures the reliable and accurate analysis of pharmaceutical materials through consistent and controlled operation, calibration, and preventative maintenance of the HPLC system to meet GMP requirements for pharmaceutical manufacturing.

2.0 SCOPE

This SOP applies to all Quality Control personnel involved in the operation, calibration, and maintenance of the HPLC system (Model: Agilent 1260 Infinity, System Code: HPLC-001) used for the analysis of raw materials, in-process samples, and finished products within NovaThera Pharmaceuticals Pvt. Ltd. This procedure covers the initial setup, operation, data acquisition, routine calibration, performance verification, troubleshooting, and preventative maintenance activities. The procedure is applicable to all products and batches analyzed using the HPLC system.

3.0 RESPONSIBILITY

QC Inspector: Is responsible for performing the HPLC analysis according to approved test methods, initiating calibration, performing daily performance verification, and reporting any deviations or malfunctions to the QC Supervisor. The QC Inspector is also responsible for maintaining the instrument logbook and performing routine maintenance tasks.

QC Supervisor: Is responsible for overseeing the HPLC operations, ensuring that the equipment is properly calibrated and maintained, and reviewing the data generated by the HPLC system. The QC Supervisor is also responsible for investigating any deviations or malfunctions and for implementing corrective and preventive actions (CAPA) as necessary.

QA Manager: Is responsible for reviewing and approving the calibration and maintenance records, ensuring that the SOP is followed, and that all activities are performed in accordance with GMP requirements. The QA Manager is also responsible for conducting periodic audits to ensure compliance.

Head of QA: Is responsible for the final approval of this SOP and any revisions, ensuring compliance with regulatory requirements, and providing overall oversight of the Quality Control activities. The Head of QA is also responsible for ensuring that adequate resources are available for the proper operation, calibration, and maintenance of the HPLC system.

4.0 MATERIALS & EQUIPMENT

PPE: Safety glasses, laboratory coats, nitrile gloves.

Equipment:

- HPLC System (Agilent 1260 Infinity, System Code: HPLC-001) including:
- Pump (Quaternary Pump G1311C)
- Autosampler (ALS G1329B)
- Column Oven (TCC G1316A)
- Detector (Variable Wavelength Detector VWD G1314B and/or Diode Array Detector DAD G1315C)
- Data Acquisition System (ChemStation Software, Version X.XX)
- Analytical Balance (MET-003)
- Volumetric Flasks (various sizes)
- Pipettes (various sizes)
- HPLC grade solvents (Acetonitrile, Water, Methanol)
- Reference Standards (Certified Reference Material - CRM)
- HPLC Columns (various types as per method requirements)
- Mobile Phase Filters (0.45 µm)
- Sonicator (SON-001)

- Vacuum Filtration Unit
- pH Meter (PHM-002)
- Conductivity Meter (CDM-001)

Documentation:

- HPLC System Logbook (FRM-QC-005-01)
- HPLC Calibration Record (FRM-QC-005-02)
- HPLC Maintenance Record (FRM-QC-005-03)
- Analytical Test Method (ATM-XXX)
- Deviation Report Form (FRM-QA-001-01)
- Corrective and Preventive Action (CAPA) Form (FRM-QA-001-02)

5.0 PROCEDURE

5.1 Initial System Setup and Preparation

5.1.1 The QC Inspector reviews the Analytical Test Method (ATM-XXX) for the specific product to be analyzed.

5.1.2 The QC Inspector verifies that the HPLC system is clean and ready for use, reviewing the HPLC System Logbook (FRM-QC-005-01) for any previous issues or maintenance activities.

5.1.3 The QC Inspector turns on the HPLC system components (pump, autosampler, column oven, detector, and data acquisition system).

5.1.4 The QC Inspector prepares the mobile phase according to the Analytical Test Method (ATM-XXX), using HPLC grade solvents and deionized water.

5.1.5 The QC Inspector filters the mobile phase through a 0.45 µm filter using a vacuum filtration unit to remove particulate matter.

5.1.6 The QC Inspector degasses the mobile phase by sonication for at least 15 minutes to remove dissolved gases.

5.1.7 The QC Inspector installs the appropriate HPLC column as specified in the Analytical Test Method (ATM-XXX).

5.1.8 The QC Inspector sets the column oven temperature as specified in the Analytical Test Method (ATM-XXX).

5.1.9 The QC Inspector programs the HPLC system with the method parameters as specified in the Analytical Test Method (ATM-XXX), including flow rate, gradient program, injection volume, and detection wavelength(s).

5.1.10 The QC Inspector purges the HPLC system with the mobile phase to remove any air bubbles and to equilibrate the column. The system is purged for at least 30 minutes or until a stable baseline is achieved.

5.1.11 The QC Inspector monitors the system pressure and ensures it is within the acceptable range as specified in the instrument manual and Analytical Test Method (ATM-XXX).

5.1.12 The QC Inspector documents all activities in the HPLC System Logbook (FRM-QC-005-01).

5.2 System Suitability Testing (SST)

5.2.1 The QC Inspector prepares the system suitability solution as specified in the Analytical Test Method (ATM-XXX), using the appropriate reference standards.

5.2.2 The QC Inspector injects the system suitability solution multiple times (e.g., 5-6 injections) as specified in the Analytical Test Method (ATM-XXX).

5.2.3 The QC Inspector analyzes the data obtained from the system suitability injections using the data acquisition system (ChemStation).

5.2.4 The QC Inspector evaluates the system suitability parameters, including resolution, tailing factor, plate count, and %RSD of peak area or retention time, against the acceptance criteria defined in the Analytical Test Method (ATM-XXX).

5.2.5 If the system suitability parameters meet the acceptance criteria, the QC Inspector proceeds with the analysis of the samples.

5.2.6 If the system suitability parameters do not meet the acceptance criteria, the QC Inspector troubleshoots the system to identify and correct the problem, such as checking the mobile phase composition, column condition, and system connections. The QC Inspector informs the QC Supervisor of the failure.

5.2.7 After correcting the problem, the QC Inspector repeats the system suitability testing until the acceptance criteria are met.

5.2.8 The QC Inspector documents the system suitability results and any corrective actions taken in the HPLC System Logbook (FRM-QC-005-01).

5.3 Sample Analysis

5.3.1 The QC Inspector prepares the samples as specified in the Analytical Test Method (ATM-XXX).

5.3.2 The QC Inspector injects the samples into the HPLC system according to the injection sequence specified in the Analytical Test Method (ATM-XXX), alternating with standard injections as required.

5.3.3 The QC Inspector monitors the chromatograms during the analysis to ensure proper peak shape, retention time, and resolution.

5.3.4 The QC Inspector records any unusual observations or deviations from the expected results in the HPLC System Logbook (FRM-QC-005-01).

5.3.5 The QC Inspector processes the data obtained from the sample analysis using the data acquisition system (ChemStation).

5.3.6 The QC Inspector integrates the peaks, calculates the results, and compares them to the acceptance criteria defined in the Analytical Test Method (ATM-XXX).

5.3.7 The QC Inspector reviews the data for accuracy and completeness.

5.3.8 The QC Inspector reports the results to the QC Supervisor for review.

5.4 Routine Calibration

5.4.1 The QC Inspector performs a performance verification check daily before use, injecting a standard solution and checking peak area and retention time against pre-established limits.

5.4.2 The QC Inspector performs routine calibration of the HPLC system at least once every six months, or more frequently if required by the Analytical Test Method (ATM-XXX) or if there are any indications of system malfunction.

5.4.3 The QC Inspector uses certified reference standards to calibrate the detector response and retention time.

5.4.4 The QC Inspector prepares a series of standard solutions at different concentrations covering the expected range of the samples.

5.4.5 The QC Inspector injects the standard solutions and analyzes the data to generate a calibration curve.

5.4.6 The QC Inspector evaluates the linearity, accuracy, and precision of the calibration curve.

5.4.7 The QC Inspector ensures that the calibration curve meets the acceptance criteria defined in the SOP-QC-006 (SOP for Calibration of Analytical Instruments) and the Analytical Test Method (ATM-XXX).

5.4.8 The QC Inspector documents the calibration results in the HPLC Calibration Record (FRM-QC-005-02).

5.4.9 If the calibration does not meet the acceptance criteria, the QC Inspector troubleshoots the system and repeats the calibration until the acceptance criteria are met.

5.4.10 The QC Supervisor reviews and approves the calibration record.

5.5 Preventative Maintenance

5.5.1 The QC Inspector performs routine preventative maintenance on the HPLC system according to the manufacturer's recommendations and the schedule outlined in SOP-QC-007 (SOP for Preventative Maintenance of Laboratory Equipment).

5.5.2 Daily Maintenance:

- The QC Inspector flushes the system with appropriate solvents at the end of each day.
- The QC Inspector checks and refills the mobile phase reservoirs as needed.

• The QC Inspector cleans the exterior of the HPLC system components.

5.5.3 Weekly Maintenance:

• The QC Inspector replaces the mobile phase filters.
• The QC Inspector checks the autosampler syringe and replaces it if necessary.
• The QC Inspector checks the column connections for leaks.

5.5.4 Monthly Maintenance:

• The QC Inspector cleans the detector flow cell according to the manufacturer's instructions.
• The QC Inspector replaces the pump seals.

5.5.5 Annually Maintenance:

• The QC Inspector performs a complete system check and calibration by a qualified service engineer.

5.5.6 The QC Inspector documents all maintenance activities in the HPLC Maintenance Record (FRM-QC-005-03).

5.5.7 If any major repairs or maintenance are required, the QC Inspector contacts a qualified service engineer.

5.5.8 The QC Supervisor reviews and approves the maintenance record.

5.6 Troubleshooting

5.6.1 In case of any system malfunction or deviation from the expected performance, the QC Inspector troubleshoots the system to identify the root cause.

5.6.2 Common troubleshooting steps include:

• Checking the mobile phase composition and preparation.
• Checking the column condition and performance.
• Checking the system connections for leaks.

- Checking the detector settings and performance.
- Checking the pump performance.
- Reviewing the HPLC System Logbook (FRM-QC-005-01) for any previous issues.

5.6.3 If the QC Inspector is unable to resolve the problem, the QC Inspector contacts the QC Supervisor or a qualified service engineer for assistance.

5.6.4 The QC Inspector documents all troubleshooting steps and findings in the HPLC System Logbook (FRM-QC-005-01).

5.6.5 Any deviations from the SOP or Analytical Test Method (ATM-XXX) are documented in a Deviation Report Form (FRM-QA-001-01).

5.6.6 The QC Supervisor investigates the deviation and implements corrective and preventive actions (CAPA) as necessary, documenting them on a CAPA Form (FRM-QA-001-02).

6.0 POST-ANALYSIS ACTIVITIES

6.0.1 The QC Inspector flushes the HPLC system with a suitable solvent (e.g., acetonitrile or methanol) to remove any residual sample or mobile phase.

6.0.2 The QC Inspector turns off the HPLC system components (pump, autosampler, column oven, detector, and data acquisition system).

6.0.3 The QC Inspector removes the HPLC column and stores it properly according to the manufacturer's instructions.

6.0.4 The QC Inspector disposes of the waste solvents according to the established waste disposal procedures outlined in SOP-EHS-001 (SOP for Waste Management).

6.0.5 The QC Inspector cleans the work area.

6.0.6 The QC Inspector reviews the data and ensures that all results are properly documented and reported.

6.0.7 The QC Inspector archives the data according to the data retention policy.

6.0.8 The QC Inspector documents all post-analysis activities in the HPLC System Logbook (FRM-QC-005-01).

6.0.9 The QC Supervisor reviews the data and the HPLC System Logbook.

7.0 SAFETY PRECAUTIONS

7.0.1 All personnel operating the HPLC system must be trained on the proper use of the equipment and the hazards associated with the chemicals used.

7.0.2 Wear appropriate personal protective equipment (PPE), including safety glasses, laboratory coats, and nitrile gloves, at all times when operating the HPLC system.

7.0.3 Handle HPLC solvents with care. Avoid inhalation and contact with skin and eyes. Refer to the Material Safety Data Sheets (MSDS) for specific safety information on each solvent.

7.0.4 Use a fume hood when handling volatile solvents or preparing mobile phases.

7.0.5 Dispose of waste solvents properly according to the established waste disposal procedures outlined in SOP-EHS-001 (SOP for Waste Management).

7.0.6 Do not operate the HPLC system if it is not functioning properly or if there are any signs of leaks or damage. Report any problems to the QC Supervisor immediately.

7.0.7 Ensure the HPLC system is properly grounded to prevent electrical shock.

7.0.8 In case of a chemical spill, follow the established spill control procedures outlined in SOP-EHS-002 (SOP for Spill Control).

7.0.9 Familiarize yourself with the location of the emergency eyewash station and safety shower.

7.0.10 Never bypass safety features or interlocks on the HPLC system.

8.0 APPROVALS

Prepared By: QC Inspector

Reviewed By: QA Manager

Approved By: Head of QA

Date:

Document Approval

Role	Name	Signature	Date
Prepared by:	_____	_____	_____
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