



**STANDARD OPERATING PROCEDURE**

<b>Department:</b> Quality Control	<b>SOP No.:</b>
<b>Title:</b> Operation & Calibration of HPLC Shimadzu (LC2030) with UV Detector	<b>Effective Date:</b>
<b>Supersedes:</b> Nil	<b>Review Date:</b>
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- 1.0 OBJECTIVE:**  
To describe a procedure for operation and calibration of HPLC Shimadzu (LC-2030) with Lab solution software.
- 2.0 SCOPE:**  
This SOP is applicable for operation and calibration of HPLC Shimadzu (LC-2030) with Lab solution software in Quality Control Department.
- 3.0 RESPONSIBILITY:**  
Officer, Sr. Officer – Responsible to follow this SOP.  
Section In-charge – To compliance of laid down procedure.  
Head QC – Accountable for implementation and compliance of laid down procedure.
- 4.0 PROCEDURE:**
- 4.1 Operation:**
- 4.1.1 Preliminary Check:**
- 4.1.1.1 Ensure that the instrument and the surrounding are clean and dust free. Wipe the Instrument with a soft duster.
- 4.1.1.2 Ensure that the system is connected to the power supply and the supply is switched on.
- 4.1.1.3 Ensure that all solvent tubing is dipped in the solvent bottles. Ensure that the waste bottle is not full.
- 4.1.1.4 Ensure that the instrument is calibrated.
- 4.1.1.5 Press the On/Off button on the LC-2030 Liquid Chromatograph. Wait for the system to initialize.
- 4.1.1.6 Enter five times zero as “Log in Pass-ID No.”. Press select “OK”. On the LC-2030 Liquid Chromatograph for the method screen display.
- 4.1.2 Startup procedure of Lab solution Software:**
- 4.1.2.1 Switch on the computer. Wait for the computer to initialize.



# PHARMA DEVILS

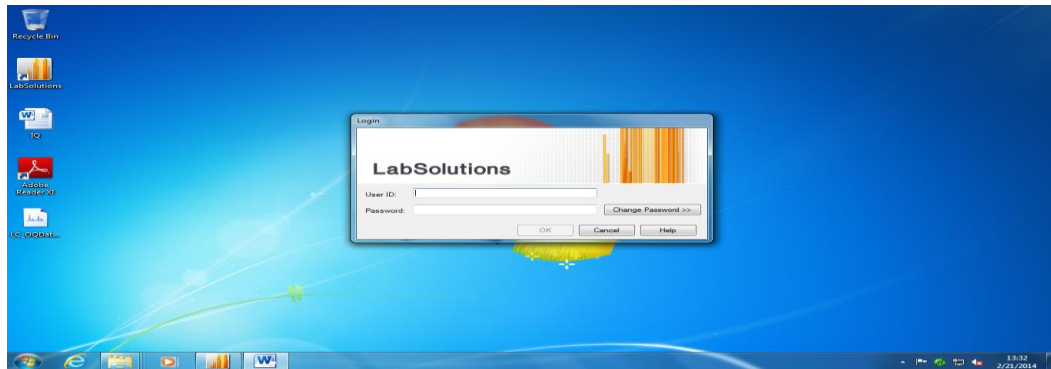
QUALITY CONTROL DEPARTMENT

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4.1.2.2 Double Click on '**Lab Solution**' icon which available on left side of the screen, Screen will display as follows.



4.1.2.3 Enter the user ID and password, Click on '**OK**' Below screen is displayed and next select required project then click on Instruments.



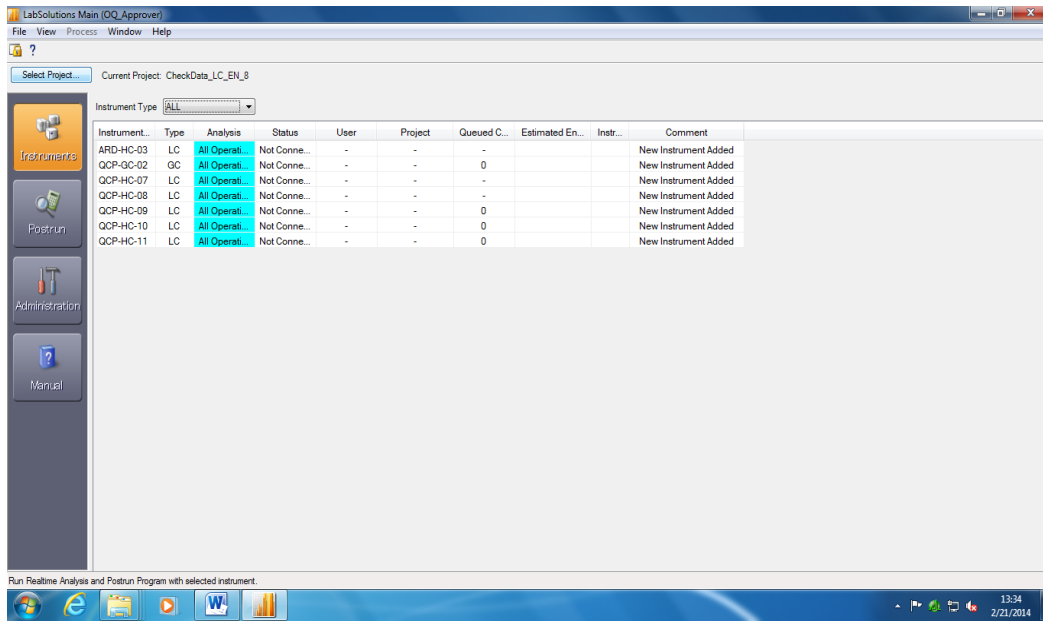
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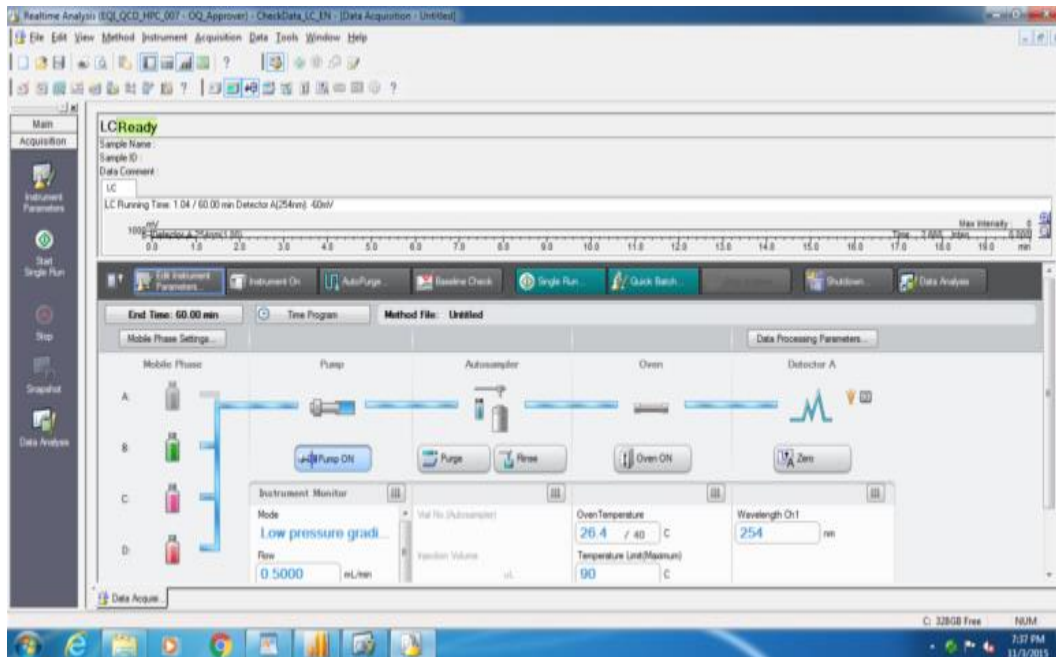
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### 4.1.2.4



### 4.1.2.5

Double click on the instrument ID which is required, following screen ('Real Time Analysis') will be displayed with beep sound.



### 4.1.3

#### Screen Description:

There are three types of windows present in the software:

LC Real Time Analysis –Data acquisition window



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LC Post run Analysis – Data Processing window

LC Analysis Editor- Method editing and Data processing window

**4.1.3.1 LC Real Time Analysis window**

4.1.3.1.1 In “LC Real Time Analysis- Data Acquisition” window, Title bar is present at the top of the screen. It displays the information such as the current application name, Instrument ID, Method file name and Data file name.

4.1.3.1.2 Main menu is present below the title bar which consists of various menus such as File, Edit, View, Method, Instrument, Acquisition, Data, Tools, Window, Help. Menu to be used will vary depending on the user’s current requirement.

4.1.3.1.3 Below Main menu bar, Tool bar icons are present, which provides the frequently used functions, to directly control the instrument. Various icons present in the Tool bar in fragments are as follows:

4.1.3.1.3.1 New -To create a new method file.

4.1.3.1.3.2 Open –To open existing method file.

4.1.3.1.3.3 Save – To save the contents of the method file.

4.1.3.1.3.4 Print- To print method/ Batch/Chromatograms.

4.1.3.1.3.5 Print Preview- To display the output image for printing.

4.1.3.1.3.6 Toggle Data Explorer- To show or hide of “Data Explorer” window.

4.1.3.1.3.7 File Search- To search for an existing file.

4.1.3.1.3.8 Toggle Assistant Bar- To show or hide “Assistant Bar” window.

4.1.3.1.3.9 Toggle Output Window- To show or hide “Output Window”.

4.1.3.1.3.10 Toggle Tab Control- To show or hide Tab control.

4.1.3.1.3.11 Toggle Instrument Monitor- To show or hide “Instrument Monitor” window.

4.1.3.1.3.12 Help- To display the help menu.

4.1.3.1.3.13 Data Acquisition Parameters- To show or hide “Instrument Parameters View” window.

4.1.3.1.3.14 Start- To start the single analysis.

4.1.3.1.3.15 Stop- To stop the data acquisition.

4.1.3.1.3.16 Change Analysis Time- Displays “Change Analysis Time” window to modify the analysis time for data acquisition.



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4.1.3.1.4 Instrument Control tool bar is present below tool bar in fragment. Various icons in the Instrument Control tool bar are as follows.

4.1.3.1.4.1 System Check- To Check the status of consumable items, maintenance parts and performs various other instrument checks.

4.1.3.1.4.2 Startup- To denote the function of Solvent delivery for the time specified at the beginning of the batch processing for purpose such as stabilizing baseline.

4.1.3.1.4.3 Auto Purge- to do the purging of the system.

4.1.3.1.4.4 Baseline Check- To check the baseline drift.

4.1.3.1.4.5 Shutdown- To shutdown the instrument.

4.1.3.1.4.6 Mobile Phase Setup- To display the residual quantity of the mobile phase.

4.1.3.1.4.7 Manual Prime- To open or close the drain valve.

4.1.3.1.4.8 CMD Information- Output the column information log.

4.1.3.1.4.9 Instrument On/Off- To turn on or off pump.

4.1.3.1.4.10 Controller On/Off- To Turn On or Off ready terminals of the system Controller.

4.1.3.1.4.11 Controller Lock/unlock- To lock or unlock controller keys.

4.1.3.1.4.12 Pump On/Off- To turn on or off the pump.






4.1.3.1.4.13 Rinse sampler- To rinse the Auto Sampler

4.1.3.1.4.14 Oven On/Off- To turn the oven On or Off.

4.1.3.1.4.15 Zeros Detector A- To auto zero the detector.

**NOTE: To display any button function move the cursor over a button (don't click).**

4.1.3.1.5 On the extreme left hand side of the screen, "Assistant bar" is displayed provided "Toggle Assistant Bar" icon is activated on the tool bar. Various application icons such as System Configuration, System Check, Data Acquisition, Batch Processing, Report Format and Postrun Analysis are present here to perform important operations.

4.1.3.1.6 "Data Explorer" window is displayed besides Assistant bar, provided "Toggle Data Explorer" icon on the tool bar is activated. Special tabs of application file icons such as  (Data),  (Method),  (Report),  (Batch) and  (All Files) are provided for easy handling of files. Easy file management can be done by switching between folders and dragging and dropping files to the display area.



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4.1.3.1.7 Below Data Explorer “Output Window” is displayed at the bottom of the screen, provided “Output Window” icon on the Tool bar is activated. This window displays activity log, log-in/log-out histories. If an error occurs, this window pops up automatically and displays the error message.

4.1.3.1.8 On the right hand side of the screen is “Chromatographic window”.

4.1.3.1.9 Below “Chromatographic window”, “Instrument Parameters View” pane is displayed.

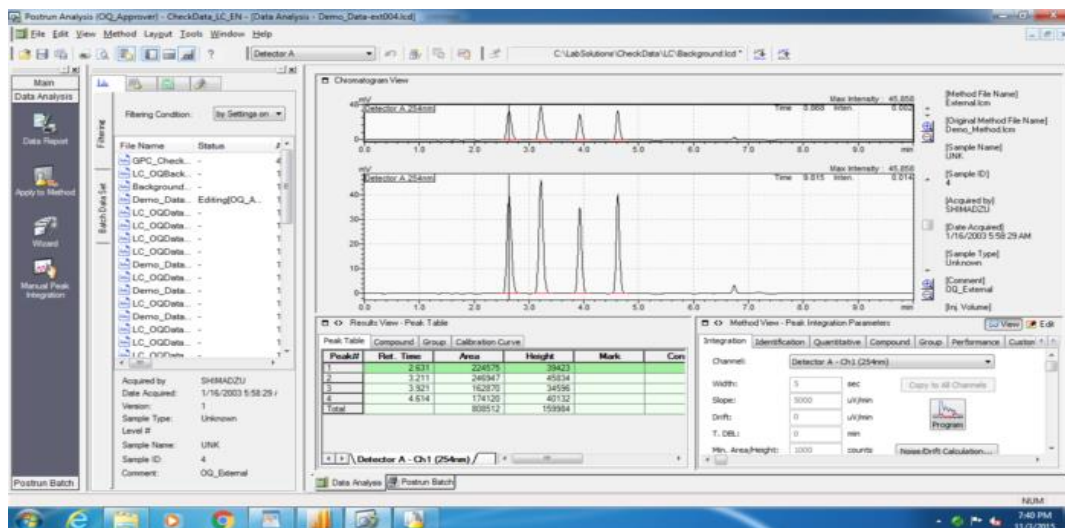
4.1.3.1.10 At the bottom of the screen status of the open windows is displayed.

### 4.1.3.2 LC Post run Analysis

4.1.3.2.1 Click on “Lab solution” tab at the bottom of the screen.

4.1.3.2.2 Click on “Post run” icon. “LC Post run Analysis” window will be displayed on the screen.

4.1.3.2.3 In “LC Post run Analysis” window, Title bar is present at the top of the screen. It displays information about the selected data file.



4.1.3.2.4 Below the Title bar, Main menu is present, which consist of various menus such as File, Table Edit, View, Method, Layout, Tools, Window and Help.

4.1.3.2.5 Below Main menu, various Tool bar icons present in fragments are as follows:

4.1.3.2.5.1 Open- To open Method file, Data file, Batch file or Report file.

4.1.3.2.5.2 Save- To Save Method file, Data file, Batch file or Report file.

4.1.3.2.5.3 Print- To print method, Sequence or report.

4.1.3.2.5.4 Print preview- To view the report before taking printout.





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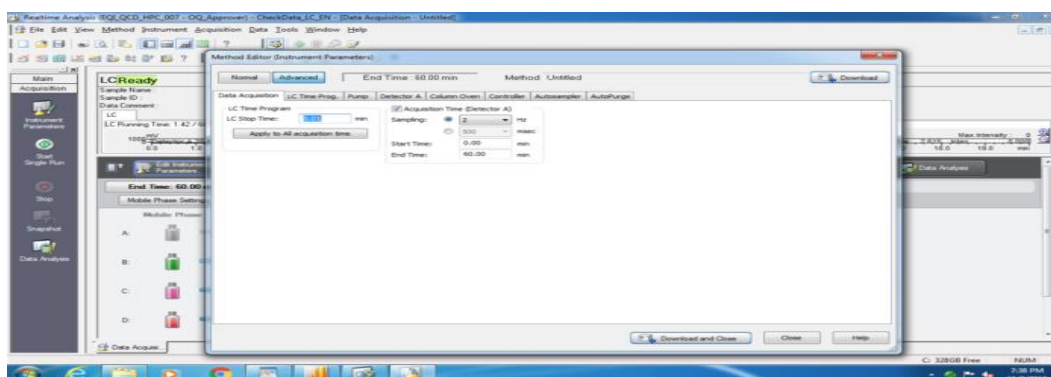
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- 4.1.3.2.5.5 Toggle Data Explorer- To show or hide Data explorer window.
- 4.1.3.2.5.6 Search Files- To search for an existing file.
- 4.1.3.2.5.7 Toggle Assistant Bar-To show or hide Assistant bar.
- 4.1.3.2.5.8 Toggle Output window- To show or hide Output window.
- 4.1.3.2.5.9 Toggle Tab Control- To show or hide Instrument control tabs.
- 4.1.3.2.5.10 Help-To acquire help menu.
- 4.1.3.2.5.11 Channel ID- To display channel (wavelength) under use.
- 4.1.3.2.5.12 Cancel Edit-To cancel the previous action in Edit mode.
- 4.1.3.2.5.13 Compound Table Wizard- To name the peak and set integration parameters.
- 4.1.3.2.5.14 Update- To update the snapshot.
- 4.1.3.2.5.15 Data Analysis Parameters- To set integration parameters.
- 4.1.3.2.5.16 Manual Integration- To perform the manual integration.
- 4.1.3.2.5.17 Pin Background Data File- To display the selected background file to be subtracted.
- 4.1.3.2.5.18 Select Background Data file- To select background file.
- 4.1.3.2.5.19 Cancel Background Subtraction- To cancel the background file.
- 4.1.3.2.6 On extreme left hand side of the screen, “Assistant bar” window is present, provided “Toggle Assistant Bar” icon on the tool bar is activated. Various application icons such as LC Data Analysis, Calibration, Batch Processing Data comparison, Report Format, Library Editor are present here to perform important operations.
- 4.1.3.2.7 “Data Explorer window”, “Output Window”, “Chromatographic View Window” of “LC Post run Analysis” is same as that of “LC Real Time Analysis” window.
- 4.1.4 Operating Method creation:**
  - 4.1.4.1 Click on the “LC Real Time” tab at the bottom of the screen.
  - 4.1.4.2 Ensure “Real Time” application in “Assistant bar” OR click on “Top” icon in Assistant bar to access “Real Time” option on the Assistant bar.
  - 4.1.4.3 Click on “Data Acquisition” icon in Assistant bar.
  - 4.1.4.4 Click on the “File” on main menu and select “New method file” option.



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- 4.1.4.5 "Instrument Parameter View" pane will be activated. Click on the "Advanced" button in this pane.
- 4.1.4.6 "Data Acquisition" option will be displayed on the screen by default.
- 4.1.4.7 Enter run time in "LC Stop Time" box in "LC Time Program" table.
- 4.1.4.8 Ensure "Acquisition Time (Detector A)" option is marked.
- 4.1.4.9 Click on "Apply to All acquisition time" tab to execute the run time in "End Time" box, present in the "Acquisition Time (Detector A)" box.
- 4.1.4.10 Ensure "2" in sampling box and "Start Time" "0.00".
- 4.1.4.11 Click on "Pump" tab. Select "Mode" as "Low pressure gradient" or "Isocratic flow" as desired.
- 4.1.4.12 Enter Flow in case of "Isocratic flow" and flow as well as initial % composition in case of "Low Pressure gradient".
- 4.1.4.13 In pressure Limits box, input maximum pressure as "380" kgf/cm<sup>2</sup>.
- 4.1.4.14 Click on "LC Time Prog". Time Program window will be displayed on the screen.
- 4.1.4.15 Select "Pump" in "Module" column.
- 4.1.4.16 Enter gradient time in "Time" column.
- 4.1.4.17 In "Action" column, select desired % of Solvent.
- 4.1.4.18 Enter "%Composition" of selected channel in "Value" column.
- 4.1.4.19 At the end of the Time program, in last step, select "Controller" in "Module" column and select "Stop" in "Action" column.
- 4.1.4.20 Click on "Detector A". Select Lamp as "D2"; Polarity as "+". Response as "1.0".
- 4.1.4.21 Select cell Temperature "Low". In case of column oven temperature above 35°C, keep cell





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Temperature “High”. Other option available is “OFF”.

- 4.1.4.22 Enter desired wavelength in “Wavelength Ch 1”.
- 4.1.4.23 Click on “Column Oven”. Mark “Enable oven” if column oven temperature is required. Enter desired temperature in “Oven Temperature”.
- 4.1.4.24 Click on “Controller”; mark “Degasser” option.
- 4.1.4.25 Input Rinsing Volume “500 µL”.
- 4.1.4.26 Input Needle stroke “22 mm”.
- 4.1.4.27 Enter sampling speed “15” µL/sec.
- 4.1.4.28 Keep Rinse Mode “Before and after aspiration”. Other options available are: No rinsing, Before aspiration and After aspiration.
- 4.1.4.29 Input “Rinse Dip Time” as 3 Sec.
- 4.1.4.30 Click on “Auto Purge”. “Auto Purge” table will be displayed.
- 4.1.4.31 Select desired line of the solvent to be purged i.e. A, B,C and D in 1<sup>st</sup>, 2<sup>nd</sup>,3<sup>rd</sup> or 4<sup>th</sup> Purge order. Enter purge time as 5 min in the respective column.
- 4.1.4.32 Mark “Autosampler” option and enter total time of the purging against the option.
- 4.1.4.33 In “Warm up” box, ensure wait time “0” and “Total pump A flow” as “0”.
- 4.1.4.34 Click on “Normal” tab. “Simple Settings” option will be displayed on the screen.
- 4.1.4.35 All entered chromatographic parametrs will be displayed in Normal tab. Check to ensure all parameters are correctly entered.
- 4.1.4.36 Click on “LC Time Prong” and “Auto Purge” and check desired parameters.
- 4.1.4.37 Click on “File” on main menu followed by “Save Method File As”. Save file method in the following path.
- 4.1.4.38 The respective method file will be saved with an extension (.lcm).

**4.1.5 Instrument Operation:**

- 4.1.5.1 Click on “LC Analysis-Real Time” tab at the bottom of the screen.
- 4.1.5.2 In “Assistant bar” ensure “Real Time” option is displayed on the screen.
- 4.1.5.3 Click on “Open” icon on tool bar OR click on “File” on main menu and select “Open Method File” option. “Open Method File” window will be displayed on the screen.
- 4.1.5.4 Select desired method from file stored.



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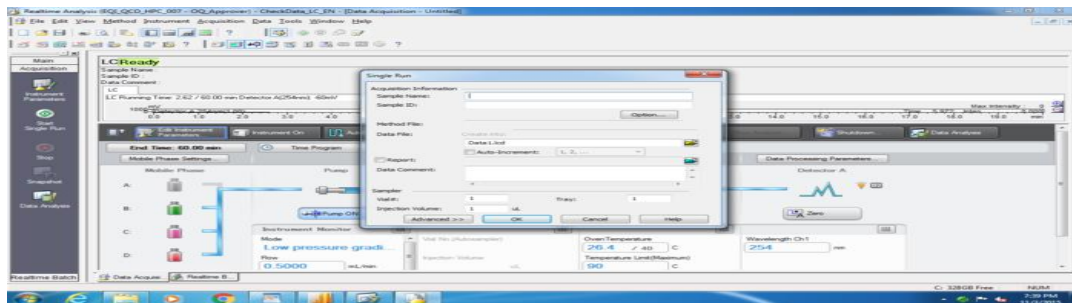
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- 4.1.5.5 To make a change in instrument operating conditions click on respective parameter in “Advanced” tab. Follow any change by “Save”.
- 4.1.5.6 Ensure appropriate solvents in the solvent bottle.
- 4.1.5.7 Purge the system as follows. Fill the pump seal rinse vial with fresh distilled water. Open the purge valve. Click on (Auto purge) icon on Tool bar. “Auto Purge” window will be displayed on the screen.
- 4.1.5.8 Enter purge time of 5 minutes for desired solvent lines. Simultaneously give the Auto sampler rinsing for 10 minutes.
- 4.1.5.9 Fill the Auto sampler reservoir with mobile phase minus buffers or acids. Click on “Purge”. Ensure that no air bubbles are observed in the solvent line. Close the purge valve after the purging is over.
- 4.1.5.10 Connect the column in the direction of flow.
- 4.1.5.11 Click on “Download” tab in “Instrument Parameters View” pane to download the method.
- 4.1.5.12 Click on “Instrument On/Off” icon to start the instrument.

**4.1.6 Data acquisition:**

**4.1.6.1 For Single run:**

- 4.1.6.1.1 Click on “LC solution Real Time” tab at the bottom of the screen.



- 4.1.6.1.2 Click on “Acquisition” on main menu followed by “Single run” OR click on  icon on tool bar to select “Single Start”.
- 4.1.6.1.3 “Single Run” window will be displayed on the screen. Enter sample name in “Sample Name” box.
- 4.1.6.1.4 Enter sample details in “Sample ID”.
- 4.1.6.1.5 Ensure desired method is open in “Method file”.



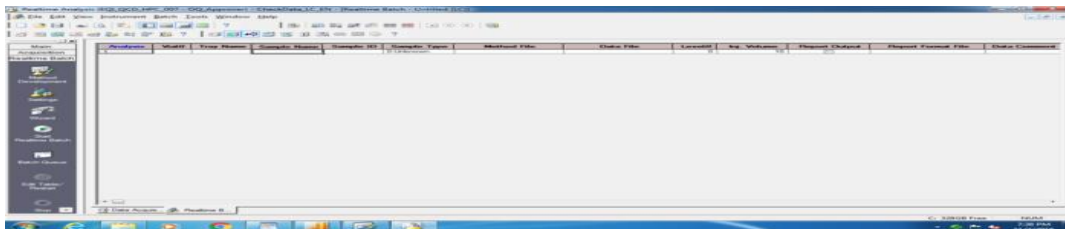
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- 4.1.6.1.6 To create a destination folder for Data file; click on yellow colored open tab against Data file.
- 4.1.6.1.7 Mark "Auto Increment" option to assign next number to file name.
- 4.1.6.1.8 Enter detailed sample information if required in "Data Description".
- 4.1.6.1.9 Enter vial number in "Via #" option.
- 4.1.6.1.10 In "Tray" option enter "1" for left hand side tray and "2" for right hand side tray.
- 4.1.6.1.11 Enter injection volume in terms of  $\mu\text{L}$  in "Injection Volume" box.
- 4.1.6.1.12 Click on "OK" tab to start the run.

**4.1.6.2 For creating and running a Batch file:**

- 4.1.6.2.1 Click on "LC Real Time" tab at the bottom of the screen.
- 4.1.6.2.2 Ensure "Real Time" option in "Assistant bar" window
- 4.1.6.2.3 Click on "Batch Processing" icon in "Assistant bar" window.
- 4.1.6.2.4 Click on "File" on main menu; select "New batch file". "Batch Table-Untitled" will be displayed on the screen.
- 4.1.6.2.5 On extreme left hand side of the table will display "Analysis". This is to ensure, that the batch is created in the "Real Time" window.




- 4.1.6.2.6 Click on the cell below "Vial#" tab. Enter vial number. Move the cursor to the next field using arrow key on the keyboard.
- 4.1.6.2.7 In "Tray Name" column, input "1" for left hand side tray and "2" for right hand side tray. Move the cursor to the next field using arrow key on the keyboard.
- 4.1.6.2.8 Enter sample name in "Sample Name" column. Move the cursor to the next field using arrow key on the keyboard.
- 4.1.6.2.9 Enter test details in "Sample ID" column.





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- 4.1.6.2.10 Click on the cell below “Method File”; Click on tab. “Select Method File” window will be displayed on the screen. Select the desired method file, Click on “Open” tab.
- 4.1.6.2.11 Click on the cell below “Data file”. Click on tab. “Select Data File” window will be displayed on the screen. Select the data path.
- 4.1.6.2.12 Click on the Data file to position the cursor in the cell. Right click on tab Select “Fill Down” option. “Data File” window will be displayed on the screen.
- 4.1.6.2.13 Ensure “1” in “Row #” and enter number of rows to be prepared in the box provided after “-”.
- 4.1.6.2.14 Mark “Auto increment” option and “1” in “Repetitions” option. Click on “OK” tab.
- 4.1.6.2.15 Desired data file will get allotted to the Batch table.
- 4.1.6.2.16 Enter sample details for other samples in “Sample ID” column of the batch table.
- 4.1.6.2.17 Click on the cell below “Inj Volume”. Enter injection volume in micro liters.
- 4.1.6.2.18 Click on “File” on main menu; Select “Save Batch File As”. Enter Batch file name under the path
- 4.1.6.2.19 Click on “Batch” on main menu and select “Settings”. “Settings” window will be displayed on the screen.
- 4.1.6.2.20 Click on “Start Row” tab.
- 4.1.6.2.21 Mark “Start from” option. Ensure 1 in “Start From”. Click on “OK” tab.
- 4.1.6.2.22 Click on green coloured icon  (Batch Start) to run the Batch Table.

**4.1.6.3 To stop the run**

- 4.1.6.3.1 If “Data Acquisition” window is activated, click on red coloured  icon to stop the run.
- 4.1.6.3.2 If “Batch Window” is activated, click on black coloured  icon to stop the run.
- 4.1.6.3.3 “Stop Process” window will be displayed on the screen with two options. Both the options are marked.
- Unmark “Data acquisition under execution” option. Click on “OK” tab. The current run will stop after the run time is over and entire batch will stop for acquisition.
  - Unmark “Batch Processing” option. Click on “OK” tab. The current run will get aborted and will take the next injection.




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iii If both “Data acquisition under execution” and “Batch Processing” options are kept marked and “OK” tab selected, the current run as well as Batch stops immediately.

**4.1.6.4 To Extend run:**

4.1.6.4.1 Click on pink coloured  icon to select “Change Analysis Time”. “Change Analysis Time” window will be displayed on the screen. Enter number of minutes by which the run is to be extended in “Acquisition Time” and click on “OK” tab.

**4.1.6.5 To overlay the reference chromatogram during the data acquisition**

4.1.6.5.1 Click on “LC Real Time” tab at the bottom of the screen.

4.1.6.5.2 Click on “File” on main menu.

4.1.6.5.3 Select “Open Reference Data File” option.

4.1.6.5.4 “Open Data File” window will be displayed on the screen.

4.1.6.5.5 Select desired data file and click on “Open” tab. “Select Channel” window will be displayed on the screen.

4.1.6.5.6 Mark on “Detector A Ch1”.

4.1.6.5.7 Click on “OK” tab to obtain the overlay of the chromatogram.

4.1.6.5.8 To close the reference chromatogram, click on “File” on main menu and select “Close Reference Data File” option. All overlaid chromatograms will be closed.

**4.1.7 To set the Screen Display**

4.1.7.1 Click on “LC Real Time Analysis” tab at the bottom of the screen.

4.1.7.2 Right click on the plot area.

4.1.7.3 Ensure “LC Chromatogram” option is marked to display the chromatogram on the screen.

4.1.7.4 “Initialize zoom” option is selected to un zoom the peak and bring to full view.

4.1.7.5 “Normalize” option is selected to adjust the scale to the peak height.

4.1.7.6 Click on “Display Settings...” option. “Display Settings” window will be displayed on the screen.

4.1.7.7 “General” tab will be displayed by default. Ensure “LC” option is marked in “Show Chromatogram View” to display the chromatogram.

4.1.7.8 In “Show Sample Information” box, mark “Sample Name”, “Sample ID” and “Data



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Description” to display the respective information on the chromatogram.

- 4.1.7.9 In “Time Range” box, the run time will be displayed. Modify the run time if required; click on “OK”.
- 4.1.7.10 To modify Y-axis, click on “LC” tab. In “Chromatogram” box, ensure “Detector A Ch1” is marked.
- 4.1.7.11 In “Intensity Range” box, enter desired lower range and upper range in mV OR click on “Normalize” tab to normalize the y-axis with respect to peak height.
- 4.1.7.12 Right click on Plot area and select “Properties” option. “Properties LC Chromatogram” window will be displayed on the screen. “General”, “Color” and “Font” tabs are available to edit color, font and appearance of the chromatogram.

### 4.1.8 Chromatographic Data Processing:

#### 4.1.8.1 To Select Data File:

- 4.1.8.1.1 Click on “LC Postrun” tab at the bottom of the screen.
- 4.1.8.1.2 Ensure “Data Explorer” window is displayed besides “Assistance Bar” otherwise click on “Toggle Data Explorer” icon on Tool bar to display “Data Explorer” window.
- 4.1.8.1.3 Click on  (Data) icon in “Data explorer” window.
- 4.1.8.1.4 Click on “Select browse folder” icon. ”Project (Folder) selection” window will be displayed on the screen.
- 4.1.8.1.5 Using dropdown key select desired Product name. Click on “+” sign of the desired Product folder. Click on desired date folder followed by “Close” tab. Respective data files will be displayed in the data explorer window.
- 4.1.8.1.6 Double click on the desired data file in data explorer window. Respective chromatogram will open in “Chromatogram View” window.
- 4.1.8.1.7 Alternatively, click on “LC Real Time” tab at the bottom of the screen and select the desired data file by following step no. 4.1.8.1.3 to 4.1.8.1.5
- 4.1.8.1.8 Double click on the desired data file the respective chromatogram will open in new “LC Post run Analysis” window. (If another data file is opened, by following the same way, another new Post run window will open.)





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#### 4.1.8.2 To Set the integration parameters and name the peak

4.1.8.2.1 In the “Post Run Analysis” window, ensure, “LC Data” option in the Assistant bar window.

4.1.8.2.2 Click on “Wizard” icon under “LC Data” option in Assistant bar window.

4.1.8.2.3 “Compound Table Wizard 1/5” window will be displayed on the screen.

4.1.8.2.4 Click on  icon. “Integration Time Program” window will be displayed on the screen.

4.1.8.2.5 Click on the cell below “Command” column. Select desired parameters such as width, slope, Drift, Integration on/off using dropdown key. Enter respective value and Time (min) for the desired parameters.

4.1.8.2.6 Click on “Simulate” tab.

4.1.8.2.7 The chromatogram will get integrated with respect to set parameters. Modify integration parameters if required.

4.1.8.2.8 Click on “OK” tab.

4.1.8.2.9 Click on “Next” tab. “Compound Table Wizard 2/5” will be displayed on the screen.

4.1.8.2.10 Mark in the cell below “Select Column” against R.T. of peak of interest to mark.

4.1.8.2.11 Click on “Next” tab.

4.1.8.2.12 “Compound Table Wizard 3/5” will be displayed on the screen. In “Calculated by:” box ensures “Area” is activated.

4.1.8.2.13 Click on “Next” tab.

4.1.8.2.14 “Compound Table Wizard 4/5” will be displayed on the screen. Ensure window % as “5”.

4.1.8.2.15 Mark “Average” in “Retention Time Update” option. Other options available are “Replace” and “None”. Selecting “Average” option consider the average retention time of all previous injections. Selecting “Replace” option will replace the retention time value with respect to last run. Selecting “None” option will consider only retention time value.

4.1.8.2.16 Click on “Next” tab.

4.1.8.2.17 “Compound Table Wizard 5/5” will be executed on the screen.

4.1.8.2.18 Click on the cell under “Name” column and input the name of peak for respective retention time.

4.1.8.2.19 Click on “Finish” tab.



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- 4.1.8.2.20 Click on “Apply to Method” icon in “Assistant bar” menu under “LC Data” option.
- 4.1.8.2.21 “LC Postrun Analysis” window will be displayed on the screen. Click on “Yes” tab. “Save Method As” window will be displayed on the screen.
- 4.1.8.2.22 Save the processing method as method under respective method file.
- 4.1.8.2.23 Click on “Save” tab.
- 4.1.8.2.24 “Select Method Parameters” window will be displayed on the screen. Ensure “Current Settings” option is marked.
- 4.1.8.2.25 In “LC Data Processing” box ensure “Data Analysis parameters” option is marked. Click on “OK” tab.
- 4.1.8.2.26 Right click on the Chromatogram in “Chromatogram View” window. Select “Data Analysis Parameters” option.
- 4.1.8.2.27 Click on “Performance” tab. In “Calc Method” box, select “USP” from dropdown list.
- 4.1.8.2.28 In “Unretained Peak Time” mark “1<sup>st</sup> Peak Time”.
- 4.1.8.2.29 Enter column Length in mm.
- 4.1.8.2.30 Unmark “Calculate identified peaks only”.
- 4.1.8.2.31 Click on “Apply” tab followed by “OK” tab. Click on “Save” icon on the tool bar.
- 4.1.8.3 To integrate the chromatogram:**
- 4.1.8.3.1 Click on “LC Post run” tab at the bottom of the screen.
- 4.1.8.3.2 Click on “File” on main menu and select “Load Method Parameters” option.
- 4.1.8.3.3 “Select Method Parameters” window will be displayed on the screen. Select respective processing method. Click on “OK” tab.
- 4.1.8.3.4 The integrated chromatogram along with the name and selected parameters will be displayed on the “Chromatogram view” window and results will be displayed in “Compound Table View”.
- 4.1.8.3.5 Similarly load each data file from Post run and follow the steps of 5.8.3.1 to 5.8.3.3 to integrate the peak.
- 4.1.8.3.6 After integrating each chromatogram, click on “Save” icon on tool bar to save the integrated chromatogram.
- 4.1.8.4 To Set System Suitability parameters Display**



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4.1.8.4.1 In the “Chromatographic view” window, in “Compound Table View” pane, click on “Results” tab at the bottom of the pane.

4.1.8.4.2 Right click on the peak table pane and select “Table Style” option.

4.1.8.4.3 “Table Style” window will be displayed on the screen.

4.1.8.4.4 “Column Order” option will be executed by default.

4.1.8.4.5 Select desired parameter such as Name, Retention Time, Area, Tailing F., T. Plate #, Resolution or Peak #, from “Hide Items” box, followed by clicking on “Add” tab to display the desired parameter in the “Display Items” box present on the right hand side of the table.

4.1.8.4.6 To delete the desired parameter select the parameter from “Display Items” followed by “Delete” tab.

4.1.8.4.7 Click on “OK” tab.

**4.1.9 System Suitability:**

4.1.9.1 Click on “LC Postrun Analysis” tab at the bottom of the screen, to open “LC Postrun Analysis” window.

4.1.9.2 Click on  (Report Format) icon in “Data Explorer” window.


4.1.9.3 Click on “Select browse folder” icon at the extreme right hand side of the Data Explorer window.

4.1.9.4 Open the following path:

Local Disk [D:] \ Template

4.1.9.5 Double click on “Template” and click on “Close” tab.

4.1.9.6 Double click on “.....-RSD” file name in Data explorer pane.

4.1.9.7 Click on  (Data) icon in Data explorer window.

4.1.9.8 Click on “Select browse folder” icon

4.1.9.9 Using dropdown key select desired Product name. Click on “+” sign of the desired Product folder. Click on desired date folder followed by “Close” tab. Respective data files will be displayed in the data explorer window.

4.1.9.10 Select the desired data files.

4.1.9.11 Drag the selected files on Report format in the right hand side of the screen.



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4.1.9.12 Overlaid chromatograms along with chromatographic property table will be displayed with Average, % RSD, Maximum, Minimum, and Standard Deviation results.

4.1.9.13 Alternatively, after selecting “..... RSD” file name in Data explore; right click on right hand side window. Select “Properties” option. ”LC/PDA Summary (Compound) Properties” window will be displayed on the screen.

4.1.9.14 Click on “File” tab followed by “Add” tab.

4.1.9.15 “Open” window will be displayed on the screen. Ensure data path.

4.1.9.16 Select all the desired files and click on “Open” tab; Click on “Apply” tab followed by “OK” tab.

4.1.9.17 Overlaid chromatogram along with chromatographic property table with details such as Avg, Min, Max, and RSD will be displayed on the screen.

4.1.9.18 Click on “Print” icon on the tool bar to get a printout.

**4.1.10 Reporting of the Data**

4.1.10.1 Click on “LC Postrun Analysis” tab at the bottom of the screen, to open “LC Postrun Analysis” window.


4.1.10.2 Click on  (Report Format) icon in “Data Explorer” window.

4.1.10.3 Click on “Select browse folder” icon at the extreme right hand side of the Data Explorer window.

4.1.10.4 Open the following path:  
Local Disk [D:]\Lab Solutions\_Data

4.1.10.5 Double click on “Template” and click on “Close” tab.

4.1.10.6 Double click on “.....” file name to open ..... report template.

4.1.10.7 Click on  (Data) icon in Data Explorer window. Open the chromatogram to be integrated from “Select Browse File” or from “Project in” dropdown key in data explorer.

4.1.10.8 Drag and drop the selected file on the report format.

4.1.10.9 Alternatively, after opening the “.....” report template, right click on the template on right hand side of the screen.

4.1.10.10 Select “Properties” option. “Sample information properties” window will be displayed on



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the screen.

4.1.10.11 Click on “Ref” tab.

4.1.10.12 “Open” window will be displayed on the screen.

4.1.10.13 Select individual data file and click on “Open” tab followed by “OK” tab.

4.1.10.14 Click on “Save” icon on tool bar.

**4.1.11 To take the Print out**

**4.1.11.1 Print of individual Chromatogram:**


4.1.11.1.1 Repeat the step no. 4.1.10.1 to 4.1.10.8


4.1.11.1.2 To get the printout of the individual report, after dragging and dropping the integrated chromatogram to the desired report format. Click on “Print” icon on main menu. “Print” window will be displayed on the screen. Select desired printer and click on “OK” tab to get the printout.

**4.1.11.2 To get a continuous print:**

4.1.11.2.1 To get a continuous print of a batch, click on “Batch” icon in “Data explorer” window; click on “Select browse folder” icon. Select desired batch file.

4.1.11.2.2 If the same batch file is open in “Real Time Analysis”, then save it as .off file.


4.1.11.2.3 Click on the cell below “Method File”. Open desired offline method file by clicking on tab. 

4.1.11.2.4 Click on the cell below “Report Format File”. Open desired report template by clicking on tab. 

4.1.11.2.5 Mark the cell on the “Report Output” column of the desired file of which print is to be taken.

4.1.11.2.6 Click on “Save’ icon on tool bar to save the batch file

4.1.11.2.7 Select the set of marked files.

4.1.11.2.8 Click on green coloured  to select “Start”. “Select Batch Execution Range” window will be displayed on the screen.

4.1.11.2.9 Ensure number of files in “Selection Line (s)”.

4.1.11.2.10 Click on “Start” tab.

4.1.11.2.11 The individual chromatogram will be integrated and continuous printout of the chromatogram will be obtained.



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**4.1.11.3 To take Method Printout:**

- 4.1.11.3.1 Click on “LC Real Time” tab at the bottom of the screen to open “LC Real Time Analysis” window on the screen.
- 4.1.11.3.2 In “Assistant Bar” window ensure “Real Time” mode, otherwise, click on “TOP” icon in “Assistant Bar” to execute “Real Time” mode.
- 4.1.11.3.3 Click on “Data Analysis” icon in “Assistant bar” window. “Instrument Parameters View” will be displayed on the right hand side of the screen.
- 4.1.11.3.4 Click on the left corner of the “Instrument Parameter View” to select “Full Size” icon.
- 4.1.11.3.5 Click on “File” on main menu and select “Print Method file” option followed by “Preview” option to view the “Method File Information”.
- 4.1.11.3.6 Click on “Print” tab to get a printout.

**4.1.11.4 To take Sequence Printout:**

- 4.1.11.4.1 Repeat step no. 4.1.11.3.1 to 4.1.11.3.2.
- 4.1.11.4.2 Click on “Batch Processing” icon in “Assistant bar” window.
- 4.1.11.4.3 Respective Batch table will be displayed on the right side of the screen.
- 4.1.11.4.4 Click on “File” on main menu and select “Print Batch Table” option followed by “Preview” option to view “Batch Table”.
- 4.1.11.4.5 Click on “Print” tab to get a printout.

**4.1.12 Procedure to Shutdown the Instrument:**

- 4.1.12.1 Before shutdown flush the system with water: Acetonitrile/Methanol (90:10) followed by Water: Acetonitrile/Methanol (10:90).
- 4.1.12.2 Switch-off the system and the computer.

**4.1.13 Maintenance of the Instrument.**

- 4.1.13.1 In case of malfunction of the instrument inform the Section in-charge for corrective action to be initiated.

- 4.1.14 Make the entries in instrument log book.

**4.2 Calibration :**

**4.2.1 Flow Calibration:**

**Frequency: Quarterly**





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- 4.2.1.1 Purge all solvent lines A, B, C, and D with Purified water.
- 4.2.1.2 Connect a union to the chromatographic system.
- 4.2.1.3 Set the flow 0.5 ml /min. and start the pump with solvent channel A allow equilibrating for 10 minutes.
- 4.2.1.4 Take a clean and dry 10 ml volumetric flask and calibrated stopwatch.
- 4.2.1.5 Start the stopwatch at the same time when collection starts and note down the time required to collect 10.0 ml of water. Note down the time displayed on the stopwatch.
- 4.2.1.6 Calculate the flow rate by using following formula.  
Flow rate =  $\frac{\text{Standard time (sec) to collect 10 ml} \times \text{Set flow rate}}{\text{Observed time to collect 10 ml}}$
- 4.2.1.7 Repeat the procedure 4.2.1.3 to 4.2.1.5 two times. Calculate Mean of Flow rate.
- 4.2.1.8 Repeat the procedure 4.2.1.3 to 4.2.1.5 with the flow rate 1.0 ml /min 2.0 ml/min and 5.0 ml /min.
- 4.2.1.9 Repeat the procedure 4.2.1.3 to 4.2.1.5 for other solvent channel B, C, D.
- 4.2.1.10 Acceptance Criteria:**  
Flow rate accuracy: + 5.0% of set flow rate.
- 4.2.2 Auto Validation:**
- 4.2.2.1 Before starting the Auto validation exit from LC Real Time Analysis and LC Post Run.
- 4.2.2.2 Prepare the 10 mg/ L Caffeine aqueous solution.
- 4.2.2.3 Fill solvent reservoir A with 10 mg/ L Caffeine aqueous solution. Fill reservoir B, C, D with Purified water.
- 4.2.2.4 Connect a union to the LC system.
- 4.2.2.5 Press the key {Maintenance} in {Menu} Screen and select {Auto Validation} in the {Validation} Tab.
- 4.2.2.6 As a solvent Delivery Mode, Select {GRAD} to check gradient concentration, select {ISO} not to Check it. And press {Next}.
- 4.2.2.7 Open the “Preparation” screen and select the following items as instructed on the screen.
- 4.2.2.8 Automatic Validation will inspect the following items.



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Item	Test	Acceptance Criteria
Wavelength Accuracy	Measure the wavelength accuracy using the D2 and Hg Lamp filaments at 656.1nm and 240.6 nm	+ 1.00 nm
Lamp Energy	Measure the reference energy after stabilizing at 220.0nm	NLT 400 mV
Flow rate pulsation	Measure Pressure Pulsation at flow rate 1.0 ml/min. for 5 min.	NMT 0.20 MPa
Temperature Control Precision	Measure temperature change at 40°C for 5 min.	+ 0.20°C
Absorbance Accuracy	Absorbance is analyzed with internal filter of known absorbance inserted in the path of light at 245nm	+ 3.0 % of known absorbance
Noise/ Drift Test	Measure the baseline noise and drift at 250 nm with solvent delivered. Noise for 5 min. and drift for 30 min.	Noise: NMT 0.020 mAU Drift: NMT 0.600
Pressure Limiter	Measure Pressure at flow rate 1.0ml/min, 1.5 ml/min and 0.5 ml/ min.	+ 1 MPa
Gradient	Measure absorbance and concentrations at, 10%, 50%, 90% in gradient from 0 to 100%	+ 2.00% for each A/B, A/C, A/D combination

4.2.2.9 Once the Auto validation is completed, Press {Logged} to save the result. After saving the result print out the system check report.

4.2.2.10 Open LC Real Time Analysis window. Click on “Instrument” on main menu. Select “System Check” option. “System Check” window will be displayed.

4.2.2.11 Click on “Run” tab. Wait for the 100% scanning of the report.

4.2.2.12 Click on “View Result” tab.

4.2.2.13 Click on “Print” tab to get the printout of the System Check report.

### 4.2.3 Injection volume accuracy:

#### Frequency: Quarterly

4.2.3.1 Fill a vial with 1 mL of water and weigh ( $W_1$ ) g.

4.2.3.2 Place the vial in the sample tray.

4.2.3.3 Program the injector to inject 10  $\mu$ L of water from the vial for 50 times.

4.2.3.4 Remove the vial after replicate injections from the sample tray and reweigh ( $W_2$ ) g.



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- 4.2.3.5 Calculate the injected volume in  $\mu\text{L}$  by the formula  
$$\frac{(W_1 - W_2) \times 1000}{d \times \text{No. of Inj.}}$$
  
Where, d = Density of water 0.997044
- 4.2.3.6 Repeat the procedure from 4.2.3.1 to 4.2.3.5 for 20  $\mu\text{l}$ , 50  $\mu\text{l}$  and 100  $\mu\text{l}$  by changing the number of injections to 25, 10 and 5 respectively.
- 4.2.3.7 **Acceptance criteria:**  $\pm 1\%$  of set volume.
- 4.2.4 Repeatability, Auto sampler Linearity, Carry Over and Linearity of detector:**  
**Frequency: Quarterly**
- 4.2.4.1 Chromatographic conditions are as follows:  
Column : C18, 150 x 4.6 mm, 5 $\mu$  (Inertsil)  
Mobile Phase : Acetonitrile: Water (15:85)  
Wavelength : 273 nm  
Column temperature: 25° C  
Flow rate : 1.0 mL/min
- 4.2.4.2 Prepare the Chromatographic system as described above.
- 4.2.4.3 Accurately weigh and transfer 25 mg of caffeine in 100 mL volumetric flask. Add 60 mL Purified water sonicate to dissolve and dilute upto the mark with water. Dilute 5 mL of the solution to 50 mL with mobile phase. (25 $\mu\text{g/ml}$ )
- 4.2.4.4 Inject six replicates of 20 $\mu\text{L}$  and perform system suitability.
- 4.2.4.5 Inject three replicates of each of 2 $\mu\text{L}$ , 10 $\mu\text{L}$ , 20 $\mu\text{L}$ , 50 $\mu\text{L}$  and 100 $\mu\text{L}$  injection volume of above solution. Inject blank solution before changing the injection volume.
- 4.2.4.6 Theoretical plates of Caffeine peak should not be less than 2000 and tailing factor should not be more than 2.0.
- 4.2.4.7 Calculate the % RSD of the peak areas of Caffeine from the 3 replicate injections at each level.
- 4.2.4.8 Calculate the % RSD of the retention times of caffeine at each level.
- 4.2.4.9 Plot a linearity graph of injection volume (X-axis) against average area of caffeine at each level (Y-axis).



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4.2.4.10 Calculate Auto sampler carry over by considering area due to caffeine in the blank injection after 100  $\mu$ L injection using following formula:

$$\frac{\text{Peak area due to caffeine in blank injection after 100 } \mu\text{L injection}}{\text{Mean peak area of caffeine in 100 } \mu\text{L injection}} \times 100$$

4.2.4.11 **Acceptance Criteria:**

- i) The % RSD of retention time of Caffeine from the 3 replicate injections at each level should be less than 1.0 %.
- ii) The %RSD of peak areas of Caffeine from the 3 replicate injections at each level should be less than 1.0 %.
- iii) The Correlation coefficient ' $r^2$ ' obtained from the linearity graph of Caffeine for different levels should not be less than 0.999.
- iv) Carry over of Caffeine: NMT 0.1% of mean area of 100 $\mu$ L.

**4.2.5 Column Thermostat Calibration**

4.2.5.1 Place the temperature probe of the calibrated thermometer in the column seat of the column thermostat and close the compartment.

4.2.5.2 Set the column temperature to 20°C. Allow the system to equilibrate.

4.2.5.3 After the reading is stabilized, note down the value displayed on the calibrated thermometer.

4.2.5.4 Repeat the steps 4.2.5.1 to 4.2.5.3 for 20°C, 40°C, 60°C and 80°C.

**4.2.6 Sampler Cooler thermostat Calibration**

4.2.6.1 Place the temperature probe of the calibrated thermometer in the sample compartment and close the compartment.

4.2.6.2 Set the sample compartment temperature to 4°C. Allow the system to equilibrate.

4.2.6.3 After the reading is stabilized, note down the value displayed on the calibrated thermometer.

4.2.6.4 Repeated the procedure 4.2.6.1 to 4.2.6.3 for 10 °C, 15°C and 25 °C.

4.2.6.5 After the calibration is completed, fill the Calibration format as per Annexure-I and Annexure-II respectively.

4.2.7 After calibration is over enter the status of calibration in the Instrument Calibration tag.



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4.2.8 If the instrument is found out of calibration put “Out of Calibration” tag on the instrument and follow SOP.

**5.0 ANNEXURE (S):**

Annexure-I: Calibration record of flow rate, injection volume accuracy & thermostat.

Annexure-II: Calibration record of auto validation, repeatability, auto sampler, detector linearity and carry over.

**6.0 REFERENCE (S):**

SOP: Handling of out of calibration instrument.

SOP: Preparation, Approval, Distribution control, revision and destruction of Standard operating Procedure (SOP).

**7.0 ABBREVIATION (S)/DEFINITION (S):**

QAD : Quality Assurance Department

RSD : Relative Standard Deviation

HPLC: High Performance Liquid Chromatography

ID : Identification

ml : Millimeter

µL : Micro liter

D2 : Deuterium

Hg : Mercury

NLT : Not less than

NMT : Not more than

nm : Nanometer

° C : Degree Centigrade

**REVISION CARD**



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S. No.	REVISION No.	REVISION DATE	DETAILS OF REVISION	REASON (S) FOR REVISION	REFERENCE CHANGE CONTROL No.
1	00	-	-	New Sop	-





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**ANNEXURE I**  
**CALIBRATION RECORD OF FLOW RATE, INJECTION VOLUME ACCURACY & THERMOSTAT**

CALIBRATION RECORD OF FLOW RATE, INJECTION VOLUME ACCURACY & THERMOSTAT			
			Reference SOP No.
<b>Location</b>		<b>Page No.:</b>	1 of 4
<b>Manufactured By</b>	Shimadzu	<b>Model No.</b>	
<b>Frequency</b>	Quarterly.	<b>Identification No.</b>	
<b>Date of Calibration</b>		<b>Next Calibration Due on</b>	

**Calibration Summary Sheet**

Channel	Observed Flow Rate	Flow Rate 0.5 (ml)	Flow Rate 1.0 (ml)	Flow Rate 2.0 (ml)	Flow Rate 5.0 (ml)	Remark
A	Obs. Flow (ml)					Complies / Does not Comply
B	Obs. Flow (ml)					Complies / Does not Comply
C	Obs. Flow (ml)					Complies / Does not Comply
D	Obs. Flow (ml)					Complies / Does not Comply

**Acceptance Criteria : ( $\pm 5\%$  of Set Flow Rate)**

For 0.5 ml flow should be between(0.475 to 0.525) ml/min

For 1.0 ml flow should be between (0.95 to 1.05) ml/min

For 2.0 ml flow should be between (1.90 to 2.10) ml/min

For 5.0 ml flow should be between (4.75 to 5.25) ml/min

Test Name	Observation	Acceptance Criteria	Remark
Injection volume accuracy	10 $\mu$ l: _____ $\mu$ l , 20 $\mu$ l : _____ $\mu$ l 50 $\mu$ l: _____ $\mu$ l , 100 $\mu$ l: _____ $\mu$ l	$\pm 1\%$	Complies / Does not Comply



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Column Thermostat Calibration	20°C=_____, 40°C=_____, 60°C=_____, 80°C=_____	± 2.0°C of set Temperature	Complies / Does not Comply
Sampler Cooler thermostat Calibration	4°C=_____, 10°C=_____, 15°C=_____, 25°C=_____	± 2.0°C of set Temperature	Complies / Does not Comply
<b>Performed By :</b>		<b>Checked By :</b>	<b>Approved By :</b>

### 1.0) Flow rate calibration by stop watch:

Digital Stop watch ID No.: \_\_\_\_\_ Validity: \_\_\_\_\_

CHANNEL A								
Set flow rate (ml/min)	Standard time in Sec. Required to collect 10 ml	Observed flow rate in ml/ min						Mean Flow rate(ml/min)
		I		II		III		
		Time	Flow	Time	Flow	Time	Flow	
0.5	1200							
1.0	600							
2.0	300							
5.0	120							
CHANNEL B								
Set flow rate (ml/min)	Standard time in Sec. Required to collect 10 ml	Observed flow rate in ml/ min						Mean Flow rate(ml/min)
		I		II		III		
		Time	Flow	Time	Flow	Time	Flow	
0.5	1200							
1.0	600							
2.0	300							
5.0	120							
CHANNEL C								
Set flow rate (ml/min)	Standard time in Sec. Required to collect 10 ml	Observed flow rate in ml/ min						Mean Flow rate(ml/min)
		I		II		III		
		Time	Flow	Time	Flow	Time	Flow	
0.5	1200							
1.0	600							



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2.0	300						
5.0	120						

CHANNEL D								
Set flow rate (ml/min)	Standard time in Sec. Required to collect 10 ml	Observed flow rate in ml/ min						Mean Flow rate(ml/min)
		I		II		III		
		Time	Flow	Time	Flow	Time	Flow	
0.5	1200							
1.0	600							
2.0	300							
5.0	120							

**Acceptance Criteria:** ( $\pm 5\%$  of Set Flow Rate)

For 0.5 ml flow should be between (0.475 to 0.525) ml/min

For 1.0 ml flow should be between (0.95 to 1.05) ml/min

For 2.0 ml flow should be between (1.90 to 2.10) ml/min

For 5.0 ml flow should be between (4.75 to 5.25) ml/min

**2.0 Injection volume accuracy:**

**Balance ID No.:** \_\_\_\_\_

Set Inj. Vol. ( $\mu$ l)	No. of Inj.	Weight of filled vial with water ( $W_1$ ) g	Weight of vial after replicate injections ( $W_2$ ) g	Density of water (d)	Calculation formula	Obs. Injection Volume ( $\mu$ l)	Acceptance criteria ( $\pm 1\%$ ) ( $\mu$ l)
10	50			0.997044	$(W_1 - W_2) \times 1000$ d x No. of Inj.		9.9 to 10.1
20	25			0.997044			19.8 to 20.2
50	10			0.997044			49.5 to 50.5
100	5			0.997044			99.0 to 101.0

Complies /Does not comply.



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**3.0 Column Thermostat Calibration:**

Digital Thermometer ID No. : \_\_\_\_\_

Temperature (°C)	Observed Temperature (°C)	Acceptance Criteria ( $\pm 2.0^\circ\text{C}$ )
20		18 - 22
40		38 - 42
60		58 - 62
80		78 - 82
Complies /Does not comply		

**4.0 Sampler Cooler thermostat Calibration**

Temperature (°C)	Observed Temperature (°C)	Acceptance Criteria ( $\pm 2.0^\circ\text{C}$ )
4		2 - 6
10		8 - 12
15		13 - 17
25		23 - 27
Complies /Does not comply		
<b>Performed By:</b>	<b>Checked By:</b>	



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**ANNEXURE II**  
**CALIBRATION RECORD OF AUTO VALIDATION, REPEATABILITY, AUTO SAMPLER, DETECTOR LINEARITY AND CARRY OVER**

<b>CALIBRATION RECORD OF AUTO VALIDATION, REPEATABILITY, AUTO SAMPLER, DETECTOR LINEARITY AND CARRY OVER</b>			
			Reference SOP
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<b>Manufactured By</b>	Shimadzu	<b>Model No.</b>	
<b>Frequency</b>	Quarterly.	<b>Identification No.</b>	
<b>Date of Calibration</b>		<b>Next Calibration Due on</b>	

**Calibration Summary Sheet**

Test Name	Observation	Acceptance Criteria	Remark
<b>Auto Validation</b>	Wavelength accuracy: at 240.6 nm = _____	± 1.00 nm	Complies / Does not Comply
	at 656.1 nm = _____	± 1.00 nm	
	Lamp energy at 220 nm mV) = _____	NLT400 mV	
	Flow rate pulsation (MPa) = _____	NMT0.20MPa	
	Temperature control precision (°C) = _____	±0.20°C	
	Absorbance accuracy (%) = _____	± 3.0 %	
	Drift (mAU/ h) = _____	NMT 0.50mAU/h	
	Noise (mAU ) = _____	NMT0.020 mAU	
	Pressure Limiter _____	Pass/Fail	
	Gradient A % & B%(10)=_____, (50)=_____, (90)= _____	± 2.00 %	
Gradient A% & C%(10)=_____, (50)=_____, (90)= _____	± 2.00 %		
Gradient A% & D%(10)=_____, (50)=_____, (90)= _____	± 2.00 %		



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<b>Auto sampler Linearity, Repeatability, Carry Over and Linearity of detector (UV)</b>	System Suitability : Theoretical Plates : _____ Tailing Factor : _____	NLT 2000 NMT 2.0	Complies / Does not Comply
---	--	---------------------	----------------------------

	Inj. Vol RSD of RT	Inj. Vol RSD of Area	Acceptance Criteria	Remark
<b>Auto sampler Linearity, Repeatability, Carry Over and Linearity of detector (UV)</b>	2 µl _____ 10µl _____ 20µl _____ 50 µl _____ 100µl _____	2 µl _____ 10µl _____ 20µl _____ 50 µl _____ 100µl _____	RSD of RT NMT 1.0%  RSD of Area NMT 1.0 %	Complies / Does not Comply
	Correlation coefficient 'r <sup>2</sup> ' (2µL to 100µL) =		NLT 0.999	
	Carryover=_____		NMT 0.01%	
<b>Performed By :</b>		<b>Checked By :</b>	<b>Approved By :</b>	

**1.0 Auto Validation:**

Parameters	Observation	Result
Flow rate pulsation (MPa) (Acceptance : NMT 0.20 MPa)		Complies / Does not comply
Pressure Limiter (Acceptance : Pass/Fail)		Complies / Does not comply
Gradient Accuracy B (Acceptance: ± 2%)	B (10%) _____ B (50%) _____ B (90%) _____	Complies / Does not comply



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Gradient Accuracy C (Acceptance: $\pm 2\%$ )	C (10%) _____ C (50%) _____ C (90%) _____	Complies / Does not comply
Gradient Accuracy D (Acceptance: $\pm 2\%$ )	D (10%) _____ D (50%) _____ D (90%) _____	Complies / Does not comply
Temperature control precision ( $^{\circ}\text{C}$ ) (Acceptance : $\pm 0.2^{\circ}\text{C}$ )		Complies / Does not comply
Lamp energy (mV) (Acceptance : NLT 400 mV)		Complies / Does not comply
Wavelength accuracy (nm) (Acceptance Criteria: $\pm 1$ nm)	240.6 nm = _____ 656.1 nm = _____	Complies / Does not comply
Noise (mAU ) (Acceptance : NMT 0.020 mAU)		Complies / Does not comply
Drift (mAU/ h) (Acceptance : NMT 0.600 mAU/Hr.)		Complies / Does not comply
Absorbance accuracy (%) (Acceptance: $\pm 3.0\%$ )		Complies / Does not comply

**Performed By :**

**Checked By :**

### 2.0 Repeatability, Auto sampler Linearity, Carry Over and Linearity of Detector:

#### 2.1 Repeatability

**Balance ID :**

**Batch No. of Caffeine**

#### **Solution preparation :**

Accurately weigh and transfer \_\_\_\_\_ mg (25 mg) of caffeine in \_\_\_\_\_ ml (100 ml) volumetric flask. Add 60 ml water, sonicate to dissolve and dilute up to the mark with water. Dilute \_\_\_\_\_ ml (5 ml) of the solution to \_\_\_\_\_ ml (50 ml) with mobile phase (25 $\mu\text{g/ml}$ ).





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Mobile phase preparation :  
Prepare a mixture of \_\_\_\_\_ ml (15 vol.) of Acetonitrile and \_\_\_\_\_ ml (85 vol.) of water to produce \_\_\_\_\_ ml (100 vol.).

Chromatographic conditions :  
Column : C18, 150 x 4.6mm, 5 $\mu$  (Inertsil)  
Wavelength : 273 nm  
Flow rate : 1.0 ml/min

**System Suitability:**  
Theoretical Plates: \_\_\_\_\_ (NLT 2000)  
Tailing Factor : \_\_\_\_\_ (NMT 2.0)

Injection No.	RT (minutes)	Area
1		
2		
3		
4		
5		
6		
Mean		
RSD (NMT 1%)		

**Complies /Does not comply**

**Performed By:** \_\_\_\_\_ **Checked By:** \_\_\_\_\_

**2.2 Auto sampler Linearity and Linearity of Detector:**

Inj No.	Injection Volume									
	2 $\mu$ l		10 $\mu$ l		20 $\mu$ l		50 $\mu$ l		100 $\mu$ l	
	RT (min)	Area	RT (min)	Area	RT (min)	Area	RT (min)	Area	RT (min)	Area
1										
2										
3										
Mean										
R.S.D. (NMT 1%)										

Correlation coefficient 'r<sup>2</sup>' = \_\_\_\_\_ (Not less than 0.999) (Refer linearity graph attached)

**Complies /Does not comply**



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**2.3 Auto Sampler Carry over:**

Peak area in Blank	Mean Peak area of caffeine in 100 $\mu$ L	Calculation formula	Carry over	Acceptance Criteria
		Peak area in Blank x 100 Mean Peak area of caffeine in 100 $\mu$ L		NMT 0.1 %

**Complies /Does not comply**

<b>Performed By:</b>	<b>Checked By:</b>
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