



STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

1.0 OBJECTIVE:

To describe a procedure for operation and calibration of Agilent (1260 Infinity series) HPLC system with Chemstation software.

2.0 SCOPE:

This procedure is applicable for operation and calibration of Agilent (1260 Infinity series) HPLC system with Chemstation software in Quality Control Department.

3.0 RESPONSIBILITY:

Officer, Executive – 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 Preliminary Check:

4.1.1 Ensure that the instrument and the surrounding are clean and dust free. Wipe the Instrument with a soft duster.

4.1.2 Ensure that the system is connected to the power supply and the supply is switched on.

4.1.3 Ensure that all solvent tubing's are dipped in the solvent bottles. Ensure that the waste bottle is not full.

4.1.4 Ensure that the instrument is calibrated.

4.2 Operation:

4.2.1 The System Consists of

4.2.1.1 Quaternary Gradient Pump(G1311C)

4.2.1.2 Auto Sampler (G1329B)

4.2.1.3 Thermostat Compartment (G1330B)

4.2.1.4 Column Compartment (G1316A)

4.2.1.5 Variable Wavelength Detector(G1314F)

4.2.2 Starting Up Procedure

4.2.2.1 Switch on the LC-Modules in the following manner.



STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

4.2.2.2 Switch on the mains for pumps.

4.2.2.3 Switch on the mains for auto sampler.

4.2.2.4 Switch on the mains for column compartment.

4.2.2.5 Switch on the mains for detector.

4.2.3 Operation Procedure for “Chemstation”

4.2.3.1 Put on the computer Click on “Start”

4.2.3.2 Select the Instrument.

4.2.4 Operating Procedure for “Pump”

4.2.4.1 Open the purge valve by turning it in the anti-clockwise direction for purging the system.

4.2.4.2 Click on pump icon. Click on “Method” and set required flow rate.

4.2.4.3 Click on pump icon and then “Control” to switch On/ Off the pump.

4.2.4.4 Before starting any analysis purge the system with water. Further flush the system with mobile phase at the flow rate of 1.0 ml/ min for about 5Minutes without the Column.

4.2.4.5 Enter the stop-time in “Method” and click on “OK”

4.2.4.6 Click on “Method” and select the required port(A to D), entre the % of solvent in each of the selected and click on OK

4.2.5 Operating Procedure for “Column Compartment”

4.2.5.1 Click on the column compartment icon and select “Method” Entre the desired temperature and click on ok if temperature is not required click on “Not Control” Click on the column thermostat icon and then on “Control” to switch on/off thermostat.

4.2.6 Operating Procedure for “VWD (Detector)”.

4.2.6.1 Click on detector icon and select “Method” VWD-Signal.

4.2.6.2 Entre the desired wavelength and click on “OK”

4.2.6.3 Click on detector icon and then “Control” to put on/ off the detector.

4.2.7 Operating Procedure for “Auto sampler”

4.2.7.1 Click on sampler and select “Method” Injection Volume.

4.2.7.2 Entre the desired injection volume and click on “OK”

4.2.8 To create a New method

4.2.8.1 Click the “Method” icon followed by click the “New Method”

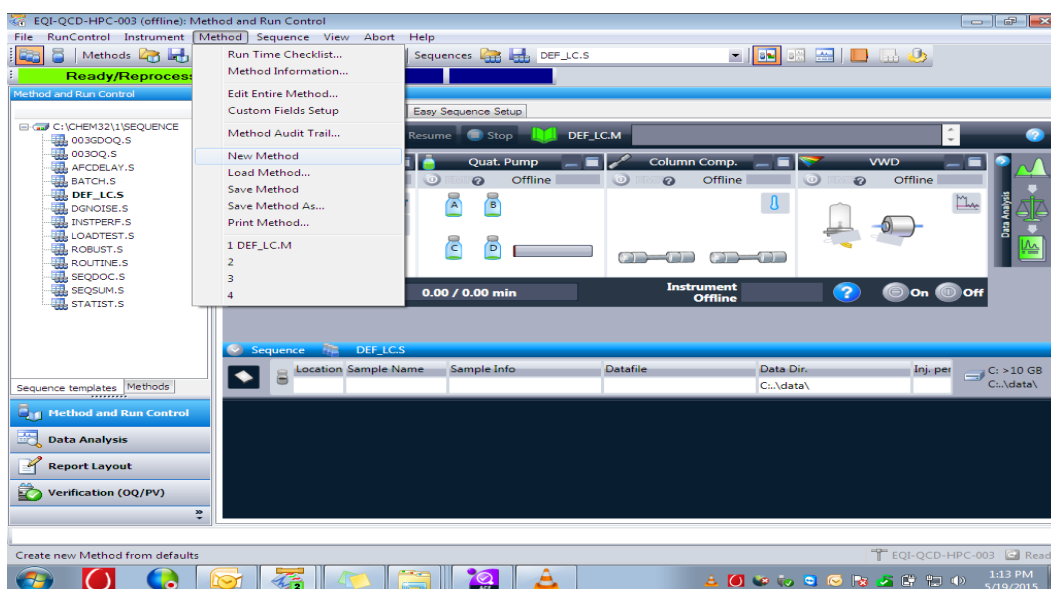


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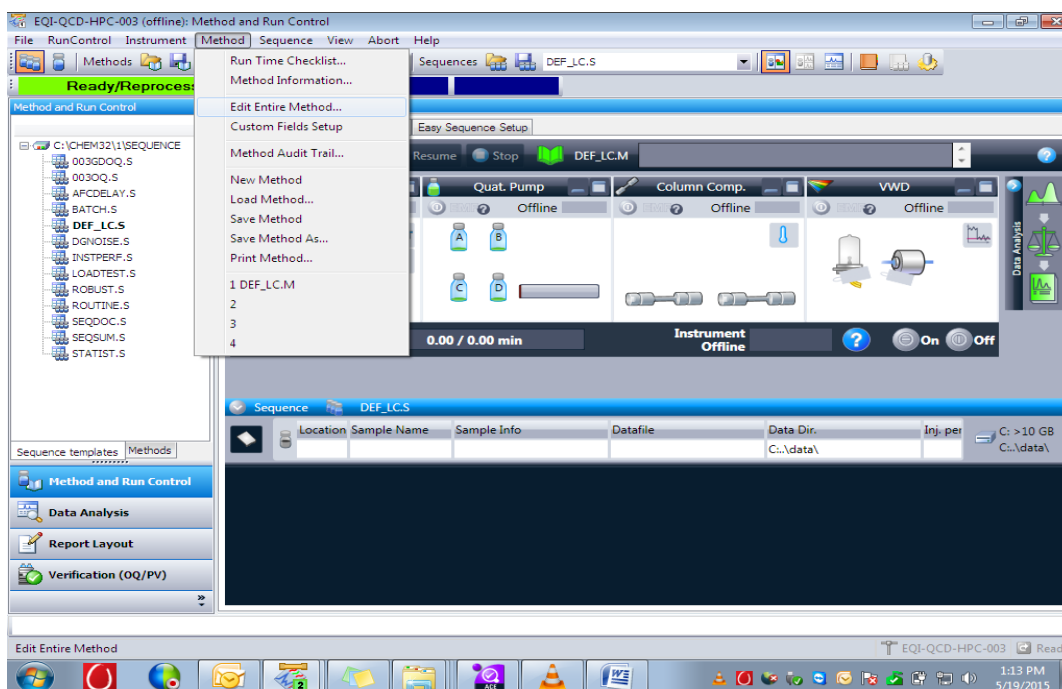
QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:



4.2.8.2 Again click method icon and following by edit entire method.



4.2.8.3 Click method section to edit OK.

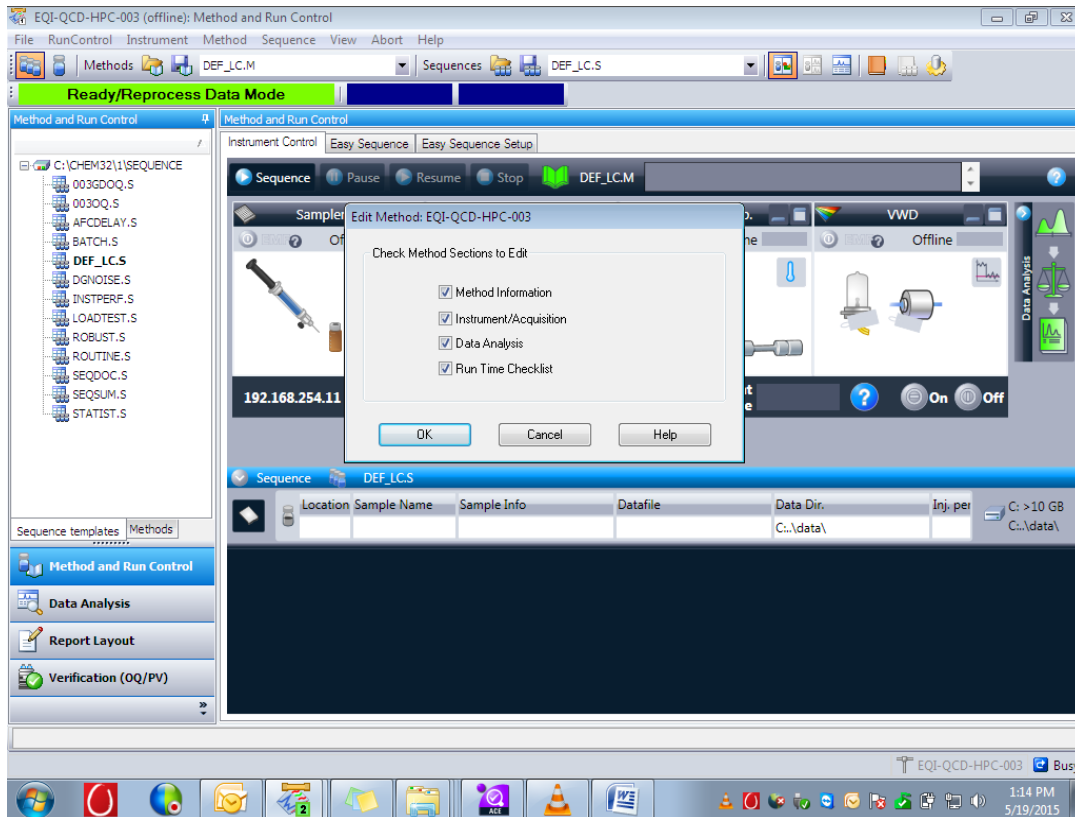


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QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:



4.2.8.4 Entre method comments and clicks OK.



PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control

SOP No.:

Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector

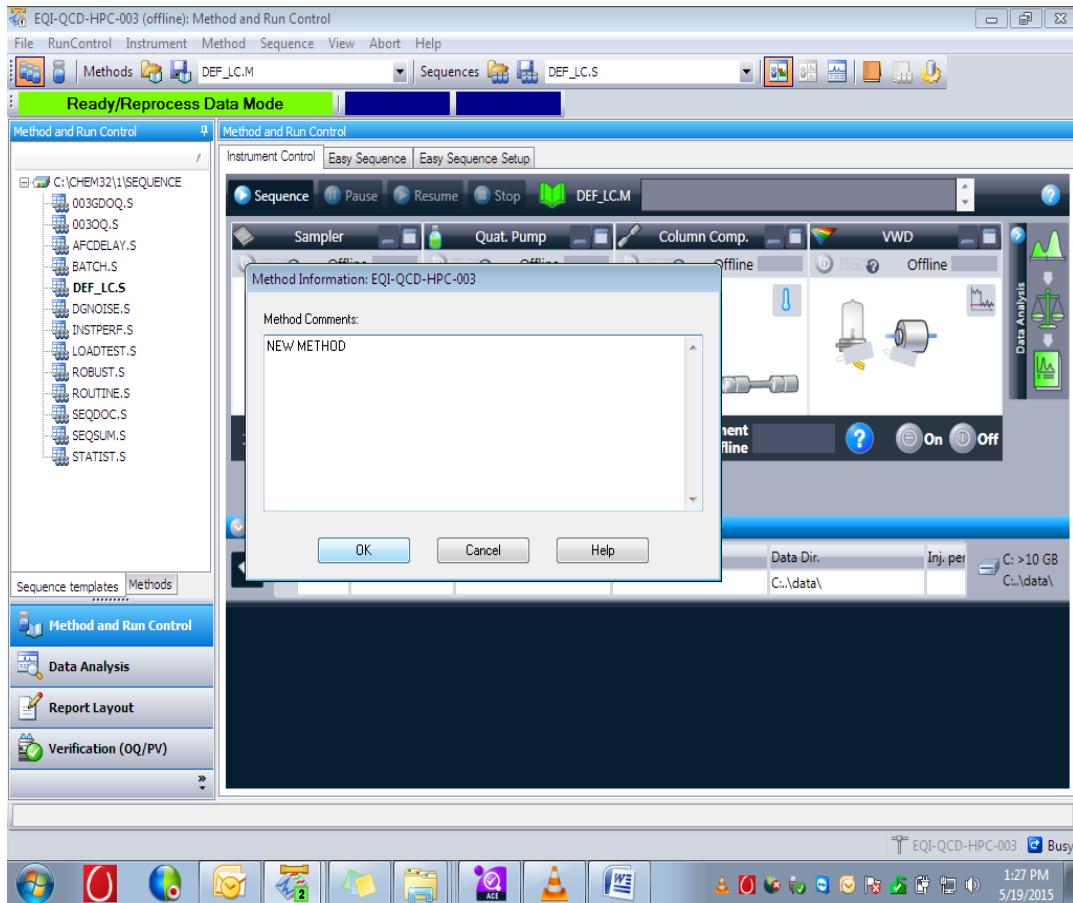
Effective Date:

Supersedes: Nil

Review Date:

Issue Date:

Page No.:



4.2.8.5 Select Injection Source



PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control

SOP No.:

Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector

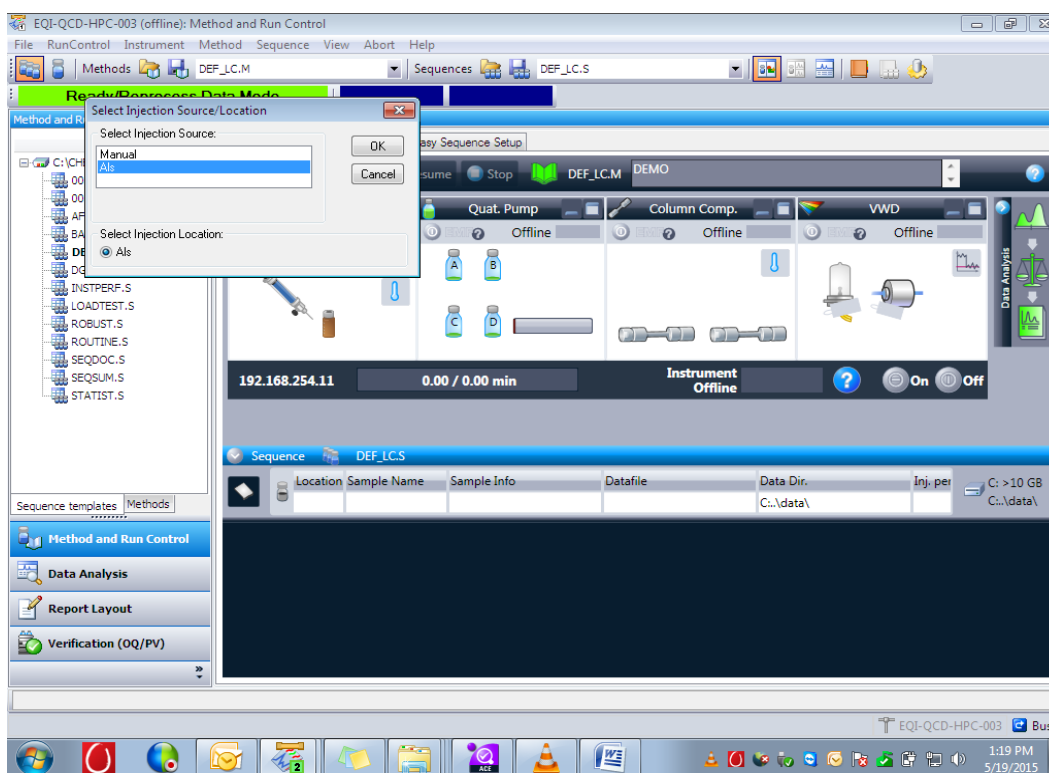
Effective Date:

Supersedes: Nil

Review Date:

Issue Date:

Page No.:



4.2.8.6 Set up pump parameter. Injector, Column compartment VWD single (Detector compartment single details (Wavelengths) and click OK after each events.

4.2.8.7 Select edit integration events and click OK.

4.2.8.8 Set up specify reports, Instrument curve runtime checklist and click "OK" after each events.



PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control

SOP No.:

Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector

Effective Date:

Supersedes: Nil

Review Date:

Issue Date:

Page No.:

The screenshot displays the 'Setup Method' dialog box for a Quat. Pump (G1311C) in the Agilent ChemStation software. The dialog is titled 'Setup Method' and has a close button (X). The main window shows the following settings:

- Flow:** 1.000 ml/min
- Solvents:**
 - A: 100.0 % WATER
 - B: 0.0 %
 - C: 0.0 %
 - D: 0.0 %
- Pressure Limits:** Min: 0.00 bar, Max: 400.00 bar
- Stoptime:** 10.00 min (selected)
- Posttime:** Off (selected)

The 'Advanced' section is expanded, showing a 'Timetable (empty)' table with the following columns: Time [min], A [%], B [%], C [%], D [%], Flow [ml/min], and Ma Lin. The table contains one row: 0.00, 100.0, 0.0, 0.0, 0.0, 1.000, and Ma Lin. Below the table are buttons for 'Add', 'Remove', 'Clear All', 'Clear Empty', 'Cut', 'Copy', 'Paste', and 'Shift Times'. At the bottom of the dialog are 'OK', 'Apply', 'Cancel', and 'Help' buttons. The background shows the main software interface with a sidebar on the right containing icons for 'Data Analysis', '>10 GB', and '..\data\'. The taskbar at the bottom shows the system tray with the time 1:20 PM and date 5/19/2015.



PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control

SOP No.:

Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector

Effective Date:

Supersedes: Nil

Review Date:

Issue Date:

Page No.:

The screenshot displays the 'Setup Method' dialog box for a 'Sampler (G1329B)' in the Agilent ChemStation software. The dialog is titled 'Setup Method' and has a menu bar with 'File', 'RunControl', 'Instrument', 'Method', 'Sequence', 'View', 'Abort', and 'Help'. The main area is divided into several sections:

- Injection Mode:** 'Injection volume' is set to 5.00 µL. There are two radio buttons: 'Standard injection' (unselected) and 'Injection with needle wash' (selected).
- Needle wash:** 'Location' is set to 91.
- Stoptime:** 'As Pump/No Limit' is selected, with a value of 1.00 min.
- Posttime:** 'Off' is selected, with a value of 1.00 min.
- Advanced:** This section is expanded to show 'Auxiliary' settings: 'Draw speed' is 100 µL/min, 'Eject speed' is 100 µL/min, and 'Draw position' is 0.0 mm. Under 'High throughput', 'Enable Optimization' is checked, with 'Prefetch Vial' selected and 'Overlap Injection Cycle' unselected. The 'Overlap Injection Cycle' value is 0.00 minutes after Injection.

At the bottom of the dialog are buttons for 'OK', 'Apply', 'Cancel', and 'Help'. The background shows the main software interface with a file tree on the left and a taskbar at the bottom. The system tray shows the date 5/19/2015 and time 1:20 PM.



PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control

SOP No.:

Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector

Effective Date:

Supersedes: Nil

Review Date:

Issue Date:

Page No.:

EQI-QCD-HPC-003 (offline): Method and Run Control

File RunControl Instrument Method Sequence View Abort Help

Setup Method

Quat. Pump Sampler Sampler Injector Program Column Comp. VWD Instrument Curves

Method and Run

C:\CHEM\0030\0030\AFC0\BAT0\DEF\DGN\INST\LOAD\ROB\ROU\SEQ\SEQ\STAT

Sequence temp

Method

Data An

Report

Verificat

Show timetable graph

OK Apply Cancel Help

EQI-QCD-HPC-003 Busy

1:21 PM 5/19/2015



PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control

SOP No.:

Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector

Effective Date:

Supersedes: Nil

Review Date:

Issue Date:

Page No.:

The screenshot displays the 'Setup Method' dialog box for the VWD (G1314F) instrument. The window is titled 'VWD (G1314F)' and contains several configuration sections:

- Signal:** Wavelength is set to 273 nm. Peakwidth is set to > 0.1 min (2 s resp. time) (5 Hz).
- Stop/Post Time:** Stop time is set to 1.00 min (As Pump/Injector). Post time is set to 1.00 min (Off).
- Advanced:**
 - Analog Output:** Zero Offset is 5%, Attenuation is 1000 mAU.
 - Signal Polarity:** Positive (+) is selected.
 - Autobalance:** Prerun is checked, Postrun is unchecked.
 - Miscellaneous:** Lamp on required for acquisition is checked.
 - Scan Range:** 190 nm to 400 nm, Step is 2 nm.
 - Additional Signals:** A scrollable list with 'm' selected.
 - Timetable:** A scrollable list.

The interface includes a 'Show timetable graph' checkbox and buttons for 'OK', 'Apply', 'Cancel', and 'Help'. The background shows the main software window with a file tree on the left and a taskbar at the bottom.

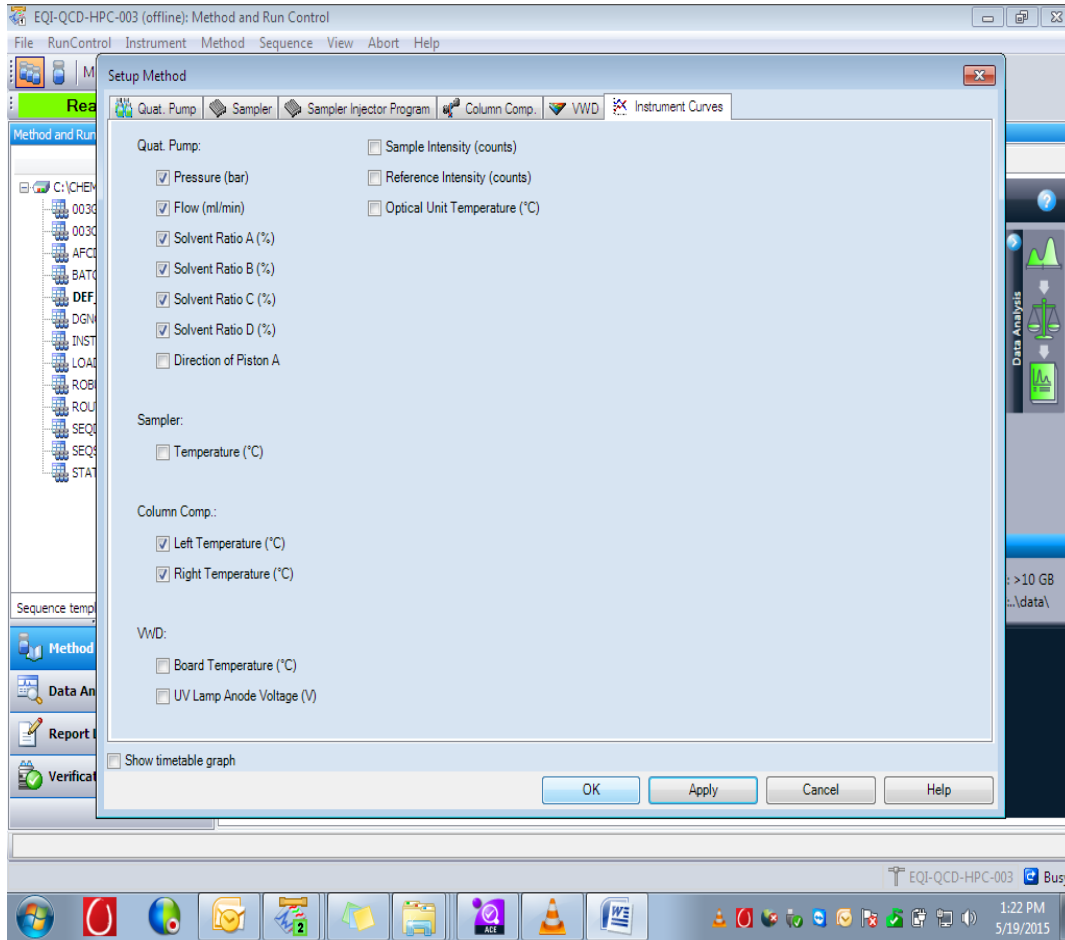


PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:





PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control

SOP No.:

Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector

Effective Date:

Supersedes: Nil

Review Date:

Issue Date:

Page No.:

EQI-QCD-HPC-003 (offline): Method and Run Control

File RunControl Instrument Method Sequence View Abort Help

Methods DEF_LC.M Sequences DEF_LC.S

Ready/Reprocess Data Mode

Method and Run Control Signal Details: EQI-QCD-HPC-003

Available Signals

VWD1 A, Wavelength=265 nm Add to Method

VWD1 A, Wavelength=265 nm

VWD1 A, Wavelength=273 nm

Insert Row Append Row Delete Row

Signal Description	Start	End	Delay	Align	Peak 1	Peak 2	Align Window
--------------------	-------	-----	-------	-------	--------	--------	--------------

OK Cancel Help

Sequence templates Methods

Method and Run Control

Data Analysis

Report Layout

Verification (OQ/PV)

EQI-QCD-HPC-003 Busy

1:23 PM 5/19/2015



PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control

SOP No.:

Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector

Effective Date:

Supersedes: Nil

Review Date:

Issue Date:

Page No.:

EQI-QCD-HPC-003 (offline): Method and Run Control

File RunControl Instrument Method Sequence View Abort Help

Methods DEF_LC.M Sequences DEF_LC.S

Ready/Reprocess Data Mode

Method and Run Control Signal Details: EQI-QCD-HPC-003

Available Signals

VWD1 A, Wavelength=273 nm Add to Method

Insert Row Append Row Delete Row

Signal Description	Start	End	Delay	Align	Peak 1	Peak 2	Align Window
VWD1 A, Wavelength=273 nm	0.000	0.000	0.000	No Alignment	0.000	0.000	0.000

OK Cancel Help

Sequence templates Methods

Method and Run Control

Data Analysis

Report Layout

Verification (OQ/PV)

EQI-QCD-HPC-003 Busy

1:23 PM 5/19/2015



PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control

SOP No.:

Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector

Effective Date:

Supersedes: Nil

Review Date:

Issue Date:

Page No.:

The screenshot displays the Agilent Method and Run Control software interface. The main window is titled 'EQI-QCD-HPC-003 (offline): Method and Run Control'. The 'Edit Integration Events' dialog box is open, showing the following data:

Method Manual Events:

Initial Events For All Signals:

Integration Events	Value
Tangent Skim Mode	Standard
Tail Peak Skim Height Ratio	0.00
Front Peak Skim Height Ratio	0.00
Skim Valley Ratio	20.00
Baseline Correction	Advanced
Peak to Valley Ratio	500.00

Specific Events For Signal: MWD Default

Time	Integration Events	Value
Initial	Slope Sensitivity	1
Initial	Peak Width	0.02
Initial	Area Reject	1
Initial	Height Reject	1.7
Initial	Shoulders	OFF

The background interface shows the 'Instrument Control' section with 'VWD' and 'Instrument Offline' status. The system tray at the bottom indicates the time as 1:24 PM on 5/19/2015.



PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control

SOP No.:

Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector

Effective Date:

Supersedes: Nil

Review Date:

Issue Date:

Page No.:

The screenshot displays the 'Specify Report' dialog box for report EQI-QCD-HPC-003. The dialog is divided into several sections:

- Quantitative Results:** Calculate: Percent, Based On: Area, Sorted By: Signal.
- ISTD Correction:** Use Multiplier & Dilution Factor with ISTDs.
- Style:** Report Style: Short.
- Report Layout For Uncalibrated Peaks:** Separately, With Calibrated Peaks, Do Not Report.
- Destination:** Printer, Screen, File.
- File Settings:** File Prefix: Report, .TXT, .CSV, .EMF, .DIF, Unique pdf file name, .PDF, .XLS, .HTM.
- Calculation Factors:** Use Sample Data: From Data File. Amount: 0.0000, Multiplier: 1.0000, Dilution: 1.0000.
- Chromatogram Output:** Portrait, Landscape, Multi-Page (Landscape). Size: 1 page, Time: 100%, Response: 40%.

The background shows the 'Method and Run Control' interface with a file tree on the left and a 'Data Analysis' panel on the right. The system tray at the bottom indicates the time is 1:24 PM on 5/19/2015.

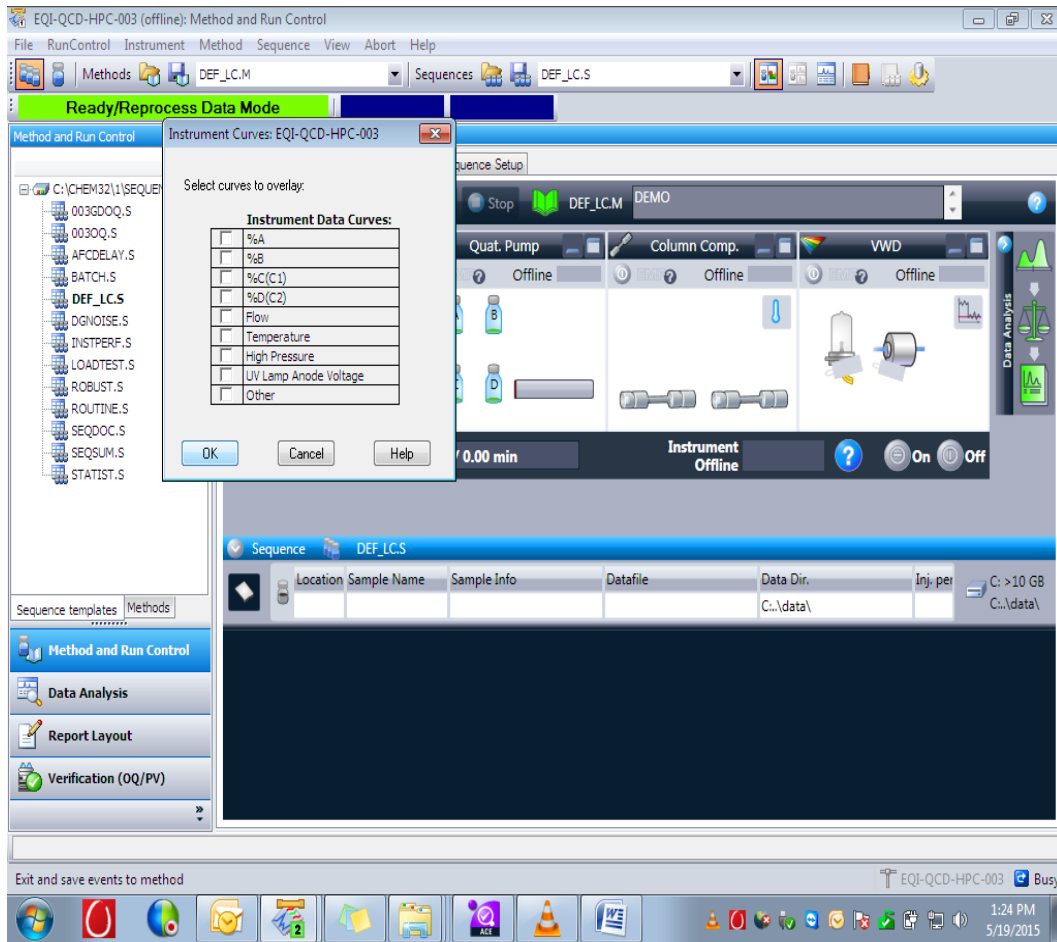


PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:



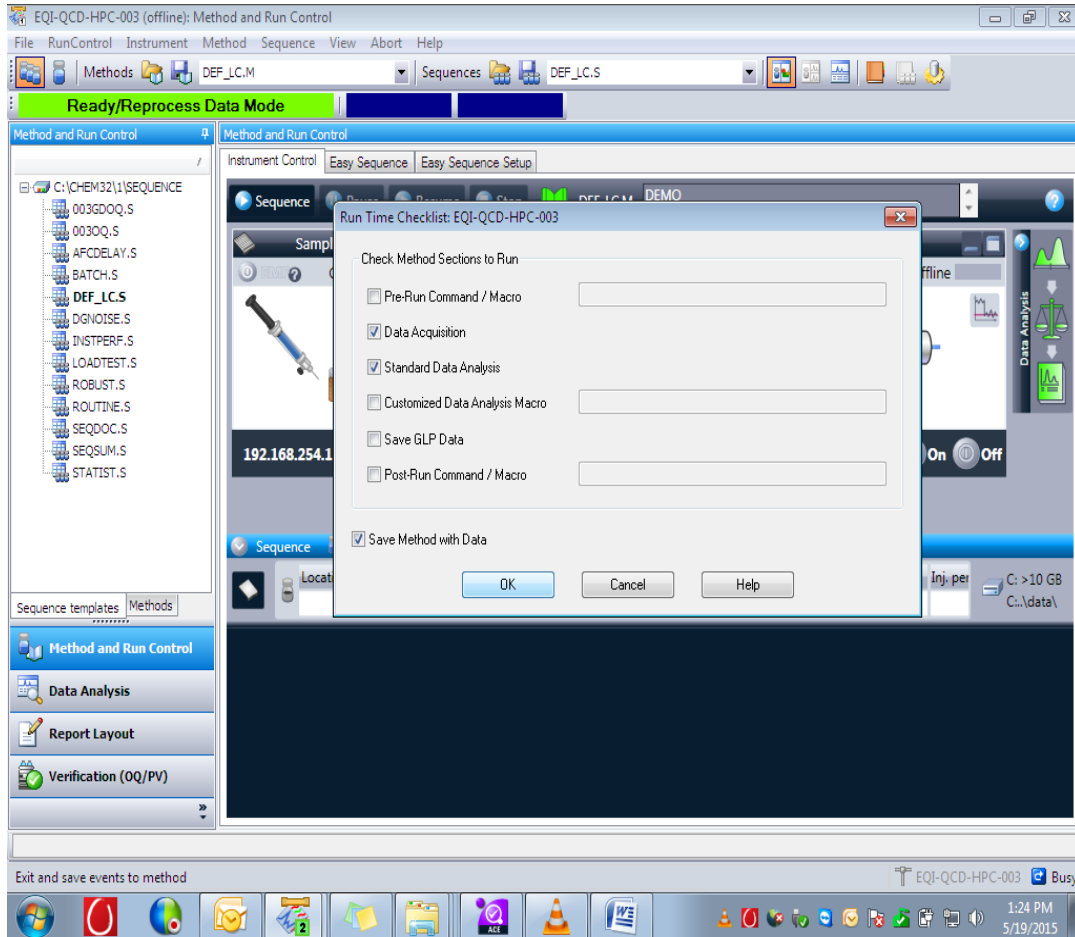


PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:



4.2.8.9 Again click “method” icon and followed by save method as .

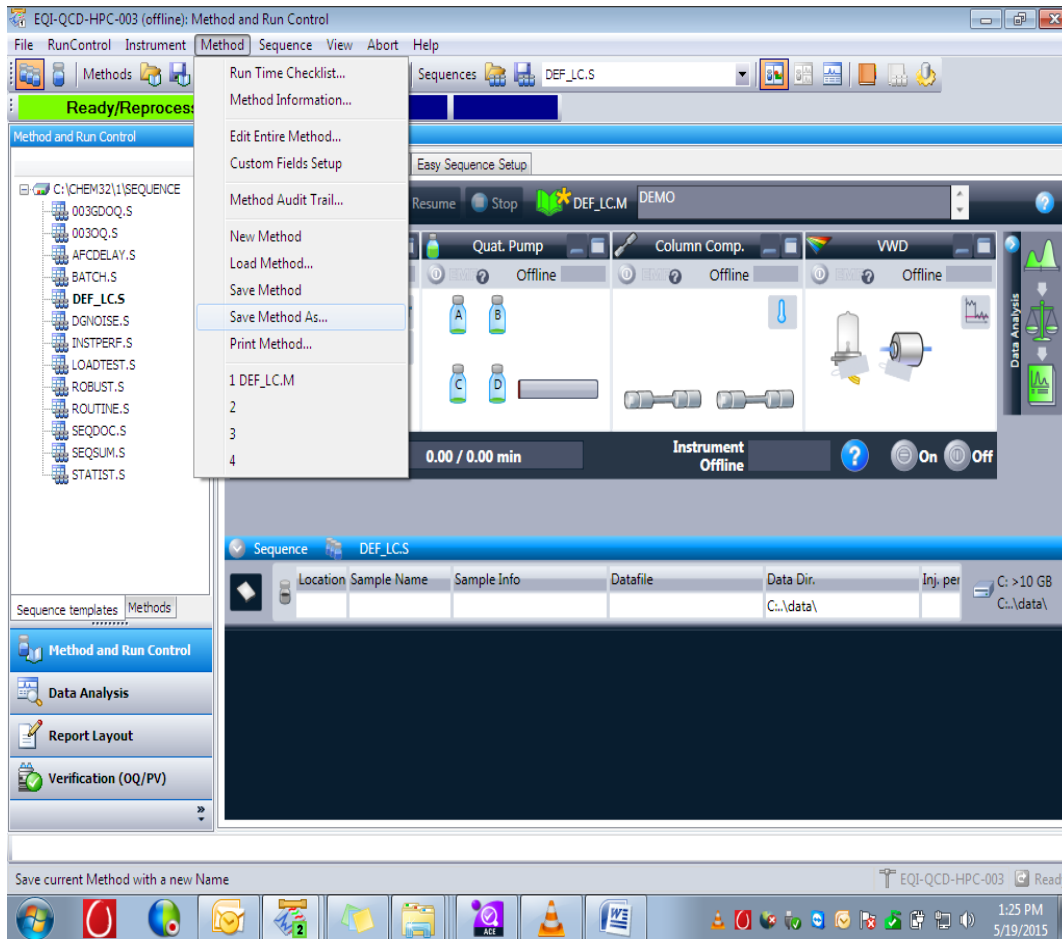


PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:



4.2.8.10 To create new method click in the following order method/new Method save it in following path:



PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control

SOP No.:

Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector

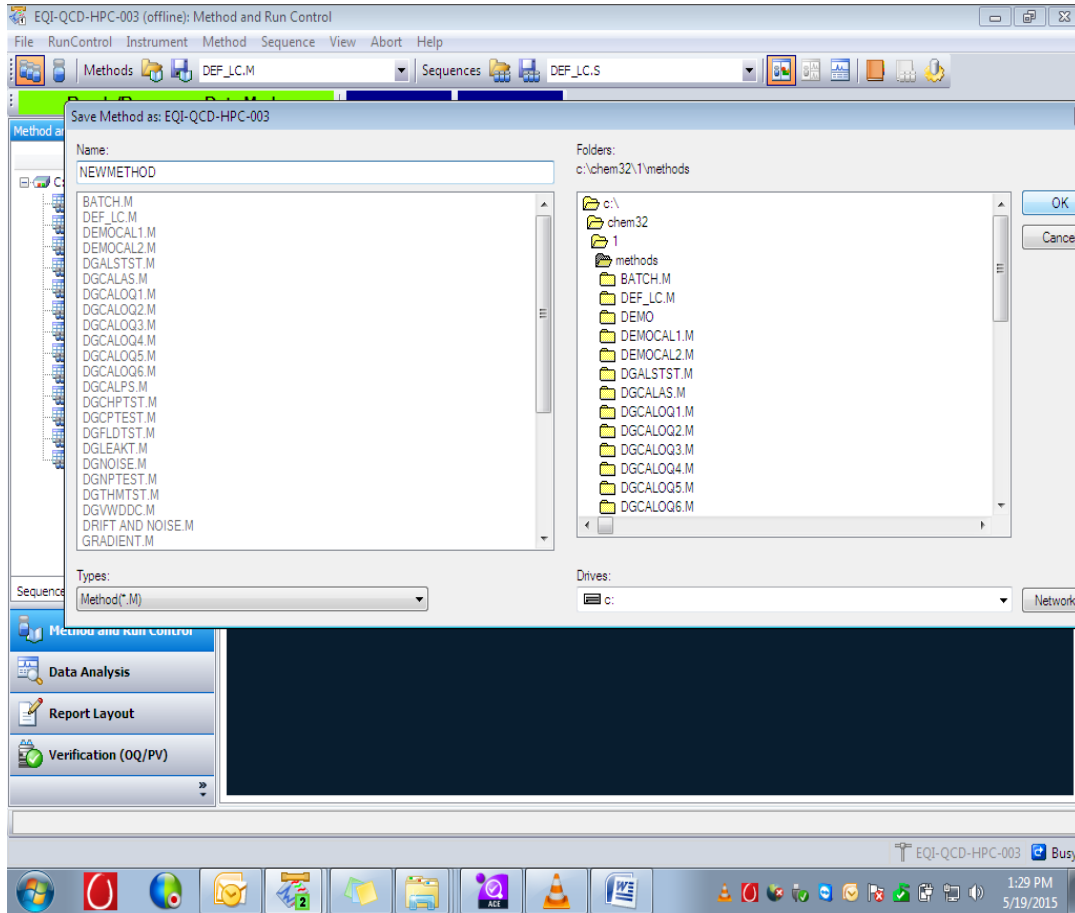
Effective Date:

Supersedes: Nil

Review Date:

Issue Date:

Page No.:





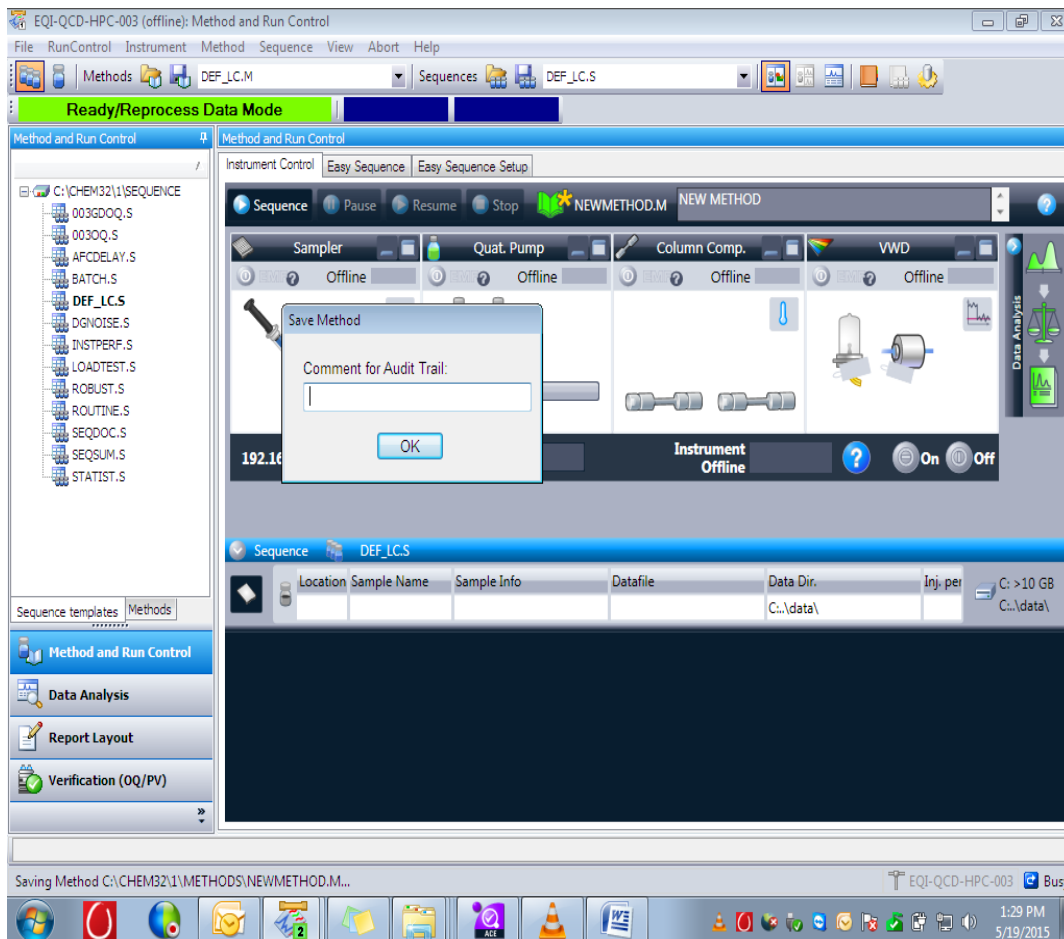
PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

4.2.8.11



4.2.9 Creating Sequence in Chemstation Software

4.2.9.1 Click the “Sequence” icon followed click the “New Sequence”



PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control

SOP No.:

Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector

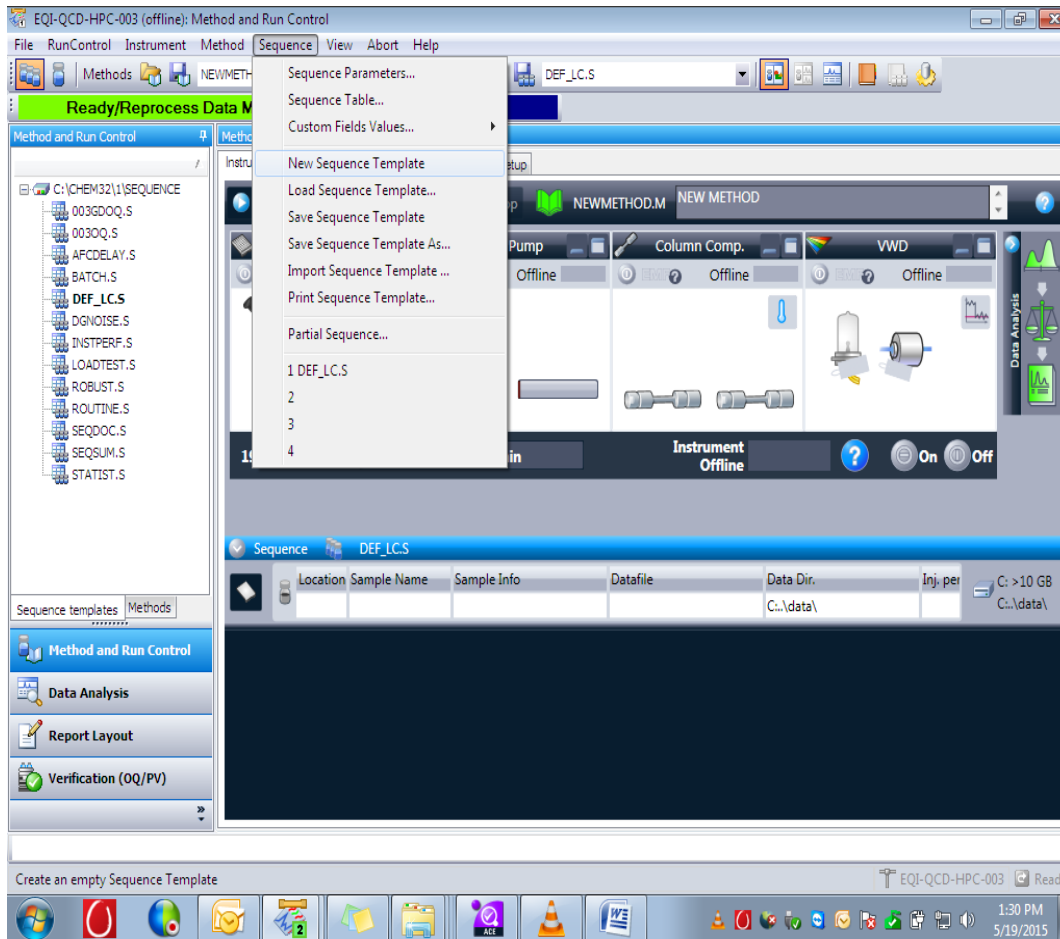
Effective Date:

Supersedes: Nil

Review Date:

Issue Date:

Page No.:

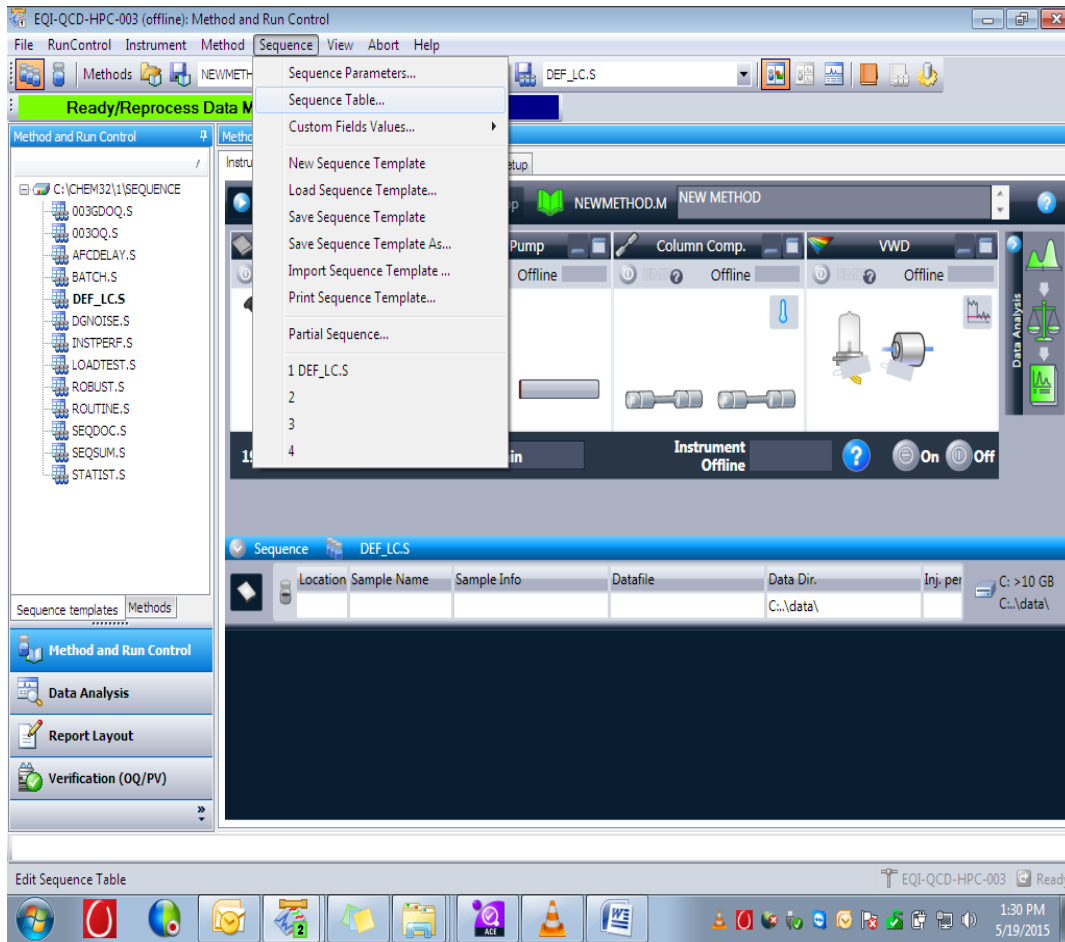


4.2.9.2 Again click “Sequence” icon and following by sequence table.



STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:



4.2.9.3 The sequence table by choosing the Injector source and completed the following entries. Location,sample name,Method Name,Injection/ Location and sample type,injection Volume,vail Number.



PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control

SOP No.:

Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector

Effective Date:

Supersedes: Nil

Review Date:

Issue Date:

Page No.:

EQI-QCD-HPC-003 (offline): Method and Run Control

File RunControl Instrument Method Sequence View Abort Help

Methods NEWMETHOD.M Sequences DEF_LC.S

Sequence Table: EQI-QCD-HPC-003

Currently Running
Line: Method: Vial: Inj:

Sample Info

Line	Vial	Sample Name	Method Name	Inj/Vial	Sample Type	Cal Level	Update RF
1					Sample		

Settings of SeqTable Editor

Column	Show?	Width
Vial	<input checked="" type="checkbox"/>	7
Sample Name	<input checked="" type="checkbox"/>	15
Method Name	<input checked="" type="checkbox"/>	30
Inj/Vial	<input checked="" type="checkbox"/>	7
Sample Type	<input type="checkbox"/>	16
Cal Level	<input type="checkbox"/>	9
Update RF	<input type="checkbox"/>	13
Update RT	<input type="checkbox"/>	12
Interval	<input type="checkbox"/>	9
Sample Amount	<input type="checkbox"/>	15
ISTD Amount	<input type="checkbox"/>	13
Multiplier	<input type="checkbox"/>	12
Dilution	<input type="checkbox"/>	12
Datafile	<input checked="" type="checkbox"/>	20
Inj Volume	<input checked="" type="checkbox"/>	10
Lims ID	<input type="checkbox"/>	20
AutoBalance	<input type="checkbox"/>	14

Insert Cut Copy Paste Append Line Undo All

Insert/FillDown Wizard Undo Wizard Custom Fields

Sample location (leave empty for a non-injection blank)

1:31 PM 5/19/2015



PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control

SOP No.:

Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector

Effective Date:

Supersedes: Nil

Review Date:

Issue Date:

Page No.:

EQI-QCD-HPC-003 (offline): Method and Run Control

File RunControl Instrument Method Sequence View Abort Help

Methods NEWMETHOD.M Sequences DEF_LC.S

Sequence Table: EQI-QCD-HPC-003

Currently Running
Line: Method: Vial: Inj:

Sample Info

Line	Vial	Sample Name	Method Name	Inj/Vial	Datafile	Inj Volume
1						

Insert Cut Copy Paste Append Line Undo All Run Sequence
Insert/FillDown Wizard Undo Wizard Custom Fields OK Cancel Help

Sample location (leave empty for a noninjection blank) Configure Table

1:31 PM 5/19/2015



PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control

SOP No.:

Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector

Effective Date:

Supersedes: Nil

Review Date:

Issue Date:

Page No.:

EQI-QCD-HPC-003 (offline): Method and Run Control

File RunControl Instrument Method Sequence View Abort Help

Methods NEWMETHOD.M Sequences DEF_LC.S

Sequence Table: EQI-QCD-HPC-003

Currently Running
Line: Method: Vial: Inj:

Sample Info for 1:

Line	Vial	Sample Name	Method Name	Inj/Vial	Datafile	Inj Volume
1	1	WATER	MULTISIG			

Insert Cut Append Line Undo All Run Sequence OK Cancel Help

Insert/FillDown Wizard Undo Wiza

Method Name

NEWMETHOD

PURITY
RESPONSE LINEARITY
RRLC-LOWDELAY
RRLC-STDDelay
SIGNAL TO NOISE

1:31 PM
5/19/2015



PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control

SOP No.:

Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector

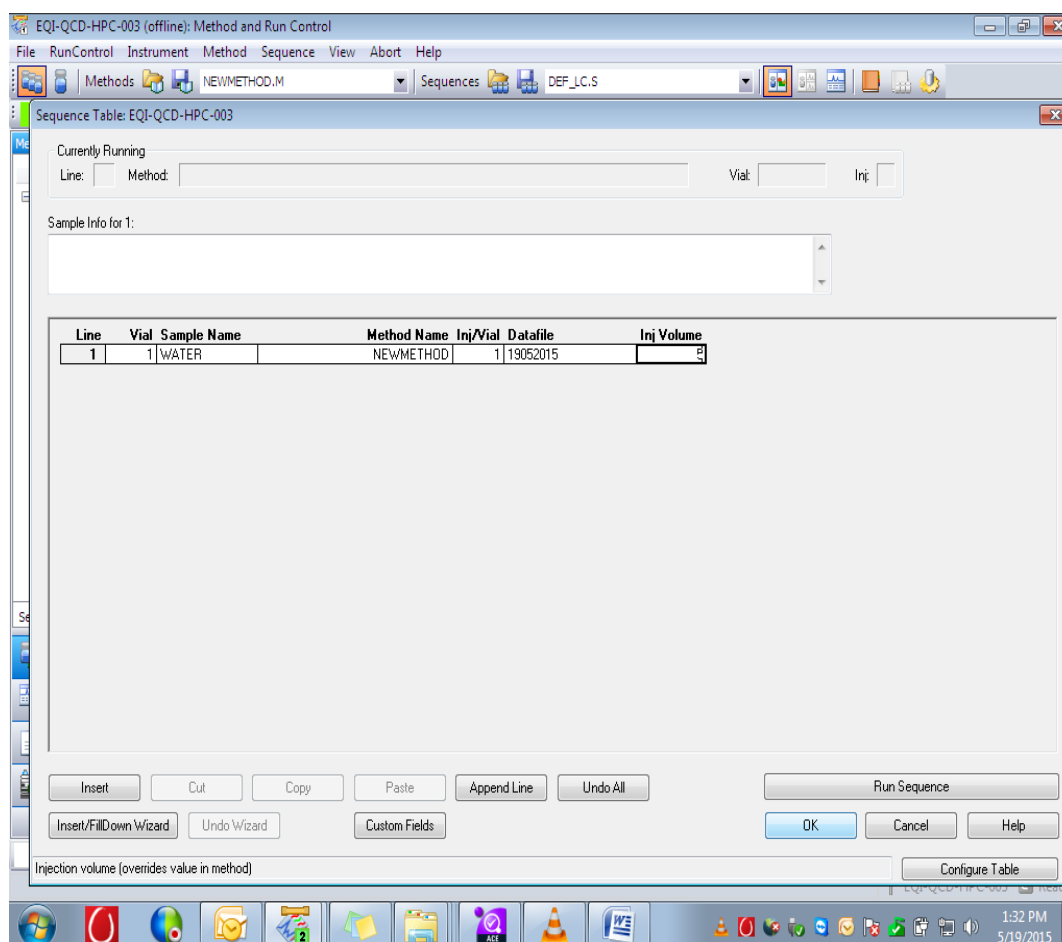
Effective Date:

Supersedes: Nil

Review Date:

Issue Date:

Page No.:



4.2.9.4 To select the data file counter and storage location click in the following order sequence/sequence parametres and choose the path.



PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control

SOP No.:

Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector

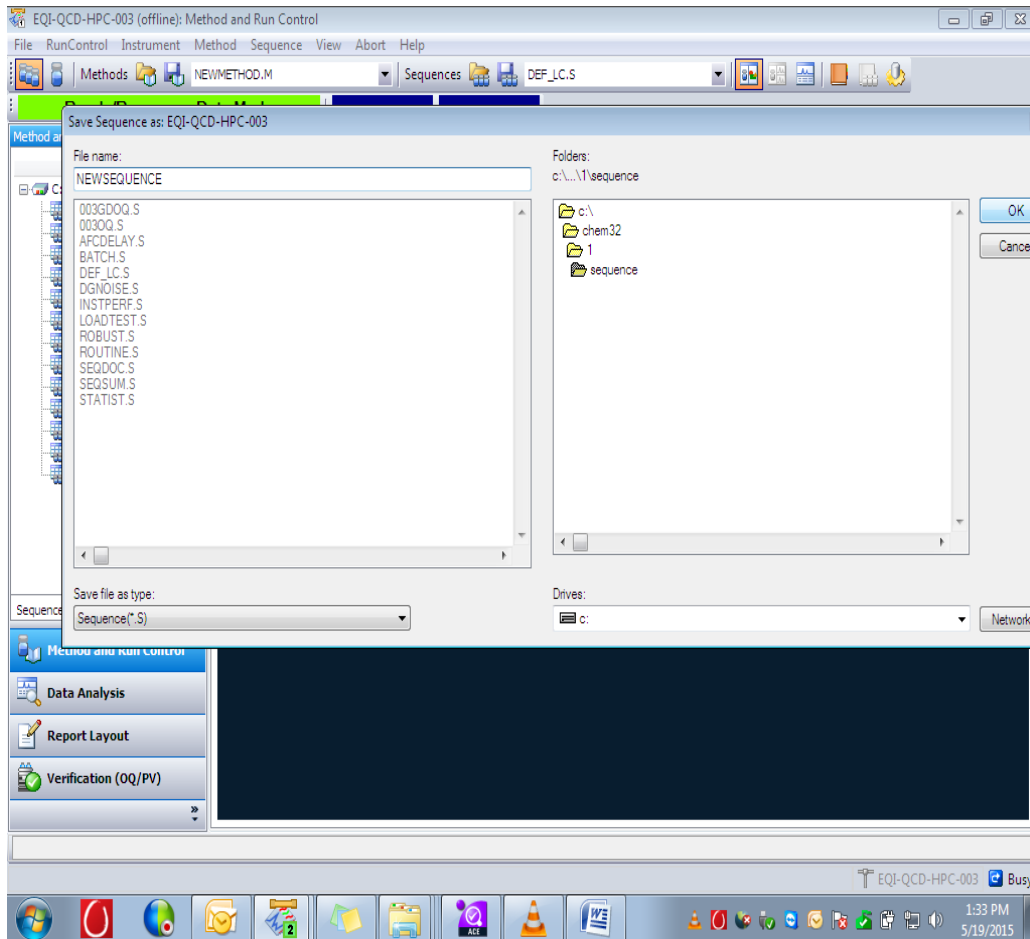
Effective Date:

Supersedes: Nil

Review Date:

Issue Date:

Page No.:





PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control

SOP No.:

Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector

Effective Date:

Supersedes: Nil

Review Date:

Issue Date:

Page No.:

The screenshot shows the Agilent Method and Run Control software interface. The 'Sequence' menu is open, displaying various options for managing sequence templates. The main window shows a sequence table with the following data:

Location	Sample Name	Sample Info	Datafile	Data Dir.	Inj. per
1	WATER			C:\data\	

The interface also shows the instrument status as 'Offline' and the sequence name as 'NEWSEQUENCE.S'. The system tray at the bottom indicates the time as 1:41 PM on 5/19/2015.



PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control

SOP No.:

Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector

Effective Date:

Supersedes: Nil

Review Date:

Issue Date:

Page No.:

EQI-QCD-HPC-003 (offline): Method and Run Control

File RunControl Instrument Method Sequence View Abort Help

Methods NEWMETHOD.M Sequences NEWSEQUENCE.S

Ready/Run

Method and Run Control

Sequence Parameters: EQI-QCD-HPC-003

Sequence Parameters Sequence Output

Data File Path: C:\Chem32\1\DATA\

Subdirectory:

Auto Prefix Counter

Prefix/Counter SIG1 0000001

Operator Name

ChemStore

Transfer Settings...

Part of method to run

According to Runtime Checklist

Use Sequence Table Information

Wait 0.00 minutes after loading a new method.

Shutdown

Post-Sequence Command/Macro

Not Ready Timeout: 0.00 minutes.

Bar Code Reader

Use In Sequence

On a bar code mismatch Inject anyway Don't inject

Fraction Information

Fraction Start Location:

Sequence Comment:

OK Cancel Help

Sequence templates

Method and Run Control

Data Analysis

Report Layout

Verification (O)

EQI-QCD-HPC-003 Ready

1:41 PM 5/19/2015



PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control

SOP No.:

Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector

Effective Date:

Supersedes: Nil

Review Date:

Issue Date:

Page No.:

The screenshot displays the Agilent ChemStation software interface. The main window is titled 'EQI-QCD-HPC-003 (offline): Method and Run Control'. The 'Sequence Parameters' dialog box is open, showing the following fields:

- Data File Path:** C:\Chem32\1\DATA\
- Subdirectory:** MAY 2015
- Operator Name:** (empty)
- Chem Store:** (empty)
- Prefix/Counter:** SIG1 / 0000001
- Part of method to run:** According to Runtime Ch...
- Wait:** 0.00 minutes
- Bar Code Reader:** Use In Sequence (unchecked)
- On a bar code mismatch:** Don't inject (selected)
- Sequence Comment:** (empty)

An error dialog box is overlaid on the main window, displaying a question mark icon and the message: 'The directory C:\Chem32\1\DATA\MAY 2015 does not exist. Do you want to create it?'. The dialog has 'Yes' and 'No' buttons.

The Windows taskbar at the bottom shows the system clock as 10:51 PM on 5/19/2015. The system tray includes icons for network, volume, and power.



STANDARD OPERATING PROCEDURE

Department: Quality Control

SOP No.:

Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector

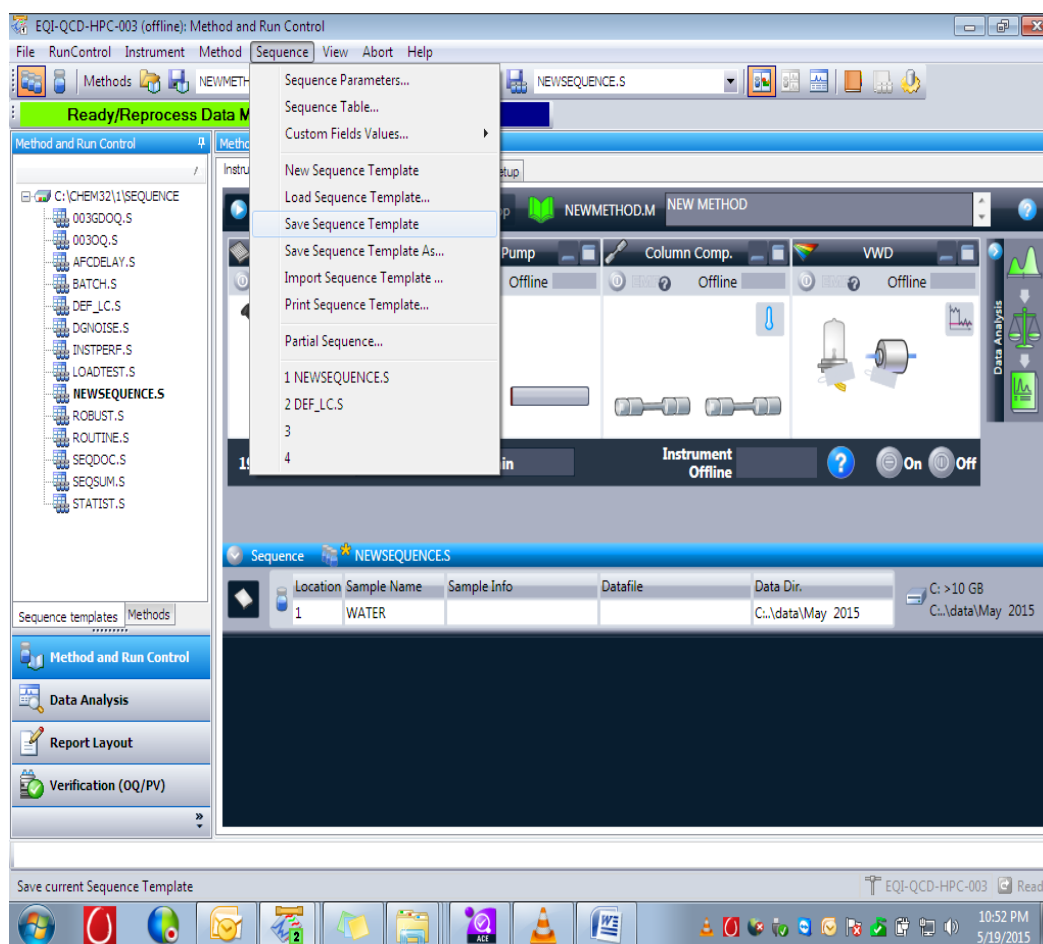
Effective Date:

Supersedes: Nil

Review Date:

Issue Date:

Page No.:



4.2.9.5 Click on “Run Control” and select Run sequence or “START” displayed above the sequence .

4.3 Calibration:

Frequency: Quarterly

4.3.1 Flow Calibration:

4.3.1.1 Purge all solvent lines A, B, C, and D with Purified water.

4.3.1.2 Connect the Union

4.3.1.3 Set the flow 0.5 ml /min and start the pump with solvent channel A allow to equilibrate for 10 minutes.

4.3.1.4 Take a clean and dry 10 ml volumetric flask and calibrated stopwatch.

4.3.1.5 Weight accurately the above volumetric flask with lid(W1g).

4.3.1.6 Keep the outlet of the detector into volumetric flask and start the stop watch immediately. Collect the water for 5 minute and take out the flask. Immediately after 5 minutes.



STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

4.3.1.7 Close the flask with lid and weight (W₂g)

4.3.1.8 Calculate the flow delivered by pump per minute by using the following formula.

$$\text{Flow rate} = (W_2 - W_1) / (5 \times 0.997044)$$

4.3.1.9 Repeat the procedure 4.3.1.3 to 4.3.1.4 with the flow rate 1.0 ml /min 2.0 ml/min and 5.0 ml /min.

4.3.1.10 Repeat the procedure 4.4.1.8 to 4.4.1.12 for other solvent channel B, C, D.

4.3.2 Acceptance Criteria:

4.3.2.1 ± 5% of the set flow rate.

4.3.2.2 1) For Flow 0.5 ml/min: Between (0.475 to 0.525) ml/ min.

4.3.2.3 2) For Flow 1.0 ml/min: Between (0.95 to 1.05) ml/ min.

4.3.2.4 3) For Flow 2.0 ml/min: Between (1.90 to 2.10) ml/ min.

4.3.2.5 4) For Flow 5.0 ml/min: Between (4.75 to 5.25) ml/ min.

4.3.3 Temperature accuracy of Column Oven

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

4.3.3.2 Set the column temperature to 20°C. Allow the system to 10 minute equilibrate.

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

4.3.3.4 Repeat the steps 4.3.3.1 to 4.3.3.3 for, 40°C, 60°C and 80°C.

4.3.3.5 **Acceptance Criteria:** + 2.0°C of set temperature

4.3.4 Sample Thermostat Calibration

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

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

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

4.3.4.4 Repeat the procedure 4.4.4.2 to 4.4.4.3 for 10° C, 15° C and 25° C.

4.3.4.5 **Acceptance Criteria:** + 3.0 °C of set temperature.

4.3.5 Injection volume accuracy:

4.4.2.1 Fill a vial with water and weigh (W₁) g.

4.4.2.2 Place the vial in the sample tray.



STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

4.4.2.3 Program the injector to inject 10 µL of water from the vial for 50 times.

4.4.2.4 Remove the vial after replicate injections from the sample tray and reweigh (W₂) g.

4.4.2.5 Calculate the injected volume in µL by the formula

$$\frac{(W_1 - W_2) \times 1000}{0.99602 \times \text{No. of Inj.}}$$

Where, d = Density of water 0.99602 at 25°C

4.4.2.6 Repeat the procedure from 4.2.3.1 to 4.2.3.5 for 20 µL, 50 µL and 100 µL by changing the number of injections to 25, 10 and 5 respectively.

4.4.2.7 **Acceptance criteria:**

± 1 % of set volume.

4.3.6 Calibration of wavelength accuracy:

4.3.6.1 Chromatographic conditions are as follows:

Column : C18, 150 x 4.6 mm, 5µ (Inertsil)

Mobile Phase : Acetonitrile: Water (15:85)

Wavelength : 273 nm

Column Temperature: 25° C

Flow rate : 1.0 mL/min

Injection volume : 20 µL

Run time : 10 min

4.3.6.2 Prepare the Chromatographic system as described above.

4.3.6.3 Accurately weigh and transfer 25 mg of caffeine in 100 ml volumetric flask, add 60 ml purified water sonicate to dissolve and dilute up to the mark with water. Dilute 5 ml of the solution to 50 ml with mobile phase. (25µg/ml).

4.3.6.4 Inject the above Caffeine solution and record the peak response at each wavelength between 202 nm to 208 nm, 242 nm to 248nm and 270 nm to 276 nm. Inject blank before starting of the each sequence of wavelength accuracy.

4.3.6.5 **Acceptance Criteria:**

The peak response should be wavelength:

Maximum at 205 nm ± 2 nm (Between 202 nm to 208nm),



STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

Minimum at 245 nm \pm 2 nm (Between 242 nm to 248 nm),
Maximum at 273 \pm 2 nm (Between 270 nm to 276 nm).

4.3.7 Precision, Linearity, Carry Over, Auto sampler and Detector:

4.3.7.1 Prepare the Chromatographic system and solution as per procedure 4.3.6.1 and 4.3.6.3.

4.3.7.2 Inject the 5 μ l,10 μ l,20 μ l,50 μ l and 100 μ l of the standard solution in six replicates and record the chromatogram.

4.3.7.3 Calculate the % RSD of the peaks areas of caffeine from the six replicate injections at each level.

4.3.7.4 Calculate the % RSD of the retention times of Caffeine at each level.

4.3.7.5 Plot a linearity graph of injection volume on the X-axis and average area caffeine at each level on the Y-axis.

4.3.7.6 No peaks due to Caffeine should be observed in the chromatograms obtained due to blank injection which is injected after 100 μ L. Calculate the carry over using following formula:

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

4.3.7.7 Acceptance Criteria:

i) The % RSD of retention time of Caffeine from the six replicate injections each level should be less than 1.0 %.

ii) The % RSD of peak areas of Caffeine from the six replicate injections each level should be less than 2.0 %.

iii) The Correlation coefficient 'r²' obtained from the linearity graph of Caffeine for different levels should not be less than 0.999.

iv) Carry over of Caffeine: NMT 0.2% of mean area of 100 μ L.

4.3.8 Checking of Compositional accuracy for Gradient system:

4.3.8.1 Remove the column from the HPLC instrument and connect the union.

4.3.8.2 Flush the HPLC instrument for about 30 min with hot water(40-60°C) by selecting all channels at a flow rate of 5.0 ml/ min ,ith the following composition: Channel: A(25%), B(25%),C(25%) and D(25%)

4.3.8.3 Prepare a solution of 0.25% v/v solution of acetone in water by accurately pipetting 5.0 ml of acetone in a 2000 ml volumetric flask and make up the volume to 2000 ml with water.



STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

4.3.8.4 Place Channel A and Channel B in the solvent bottle having purified water and place Channel C and Channel D in the solvent bottle having 0.25 % v/v solution of acetone in water.

4.3.8.5 Flush the HPLC Instrument for about 20 min at a flow rate of 1.0 ml/ min using the composition as per the following table:

Time	%HPLC grade water (Channel A, B)	0.25% Acetone in water (Channel C, D)
0	25+25	25+25
10	25+25	25+25
12	50+50	0+0
20	50+50	0+0

4.3.8.6 Check the gradient accuracy of the HPLC system with the condition given below Flow rate : 1.0 ml/min

Detection : UV at 254 nm

Set the time program as follows:

Time in minute	%HPLC grade water (Channel A, B)	0.25% Acetone in water (Channel C, D)
0	100	0
4	100	0
6	80	20
10	80	20
12	60	40
16	60	40
18	20	80
22	20	80
24	0	100
28	0	100
30	100	0

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



STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

- 4.3.8.8 Inject 0 μ l or minimum volume of HPLC grade water and record the gradient profile.
- 4.3.8.9 Acquire the data till 30 minutes.
- 4.3.8.10 Print the overlay plot of gradient profile of A,C and B,D (the difference shall be not more than 0. AU for absorbance and the difference shall be not more than 20 sec for time).

4.3.8.11 Acceptance Criteria:

The Gradient Profile A, C and B, D shall be overlay with each other.

The difference in absorbance shall be NMT: 0.01AU.

The difference in time shall be NMT : 20 Sec.

4.3.9 Calibration of Noise & drift of the Detector:

- 4.3.9.1 To carry out above test , set the chromatographic condition as mentioned below chromatographic conditions:

Column : Restriction capillary or equivalent

Mobile phase : Degassed Water

Flow rate : 1.0 m/min

Wavelength : 254 nm for Noise & drift

Run Time : 60 min

Column Temperature: 25° C

4.3.9.2 Acceptance Criteria :

Detector Noise : NMT 0.000125 AU

Detector Drift : NMT 0.005 AU / hr

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

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

- 4.3.9.5 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 : Raw data entry format for calibration of HPLC Agilent (1260 infinity series).

Annexure-II: Calibration Summary Report of Agilent (1260 infinity series).



STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

Annexure-III: HPLC User Log Book.

6.0 REFERENCE (S):

SOP: Handling of out of calibration instrument

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

SOP: Maintenance of Quality Control Instruments Log.

7.0 ABBREVIATION (S)/DEFINITION (S):

QAD: Quality Assurance Department

RSD: Relative Standard Deviation

HPLC: High Performance Liquid Chromatography

ID : Identification

ml : Milli liter

μL : Micro liter

D2 : Deuterium

Hg : Mercury

NLT : Not less than

NMT : Not more than

nm : Nanometer

°C : Degree Centigrade

μAU : Micro absorbance unit

μAU/h : Micro absorbance unit per hour

nm : Nanometer



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STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

REVISION CARD

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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STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

ANNEXURE I

RAW DATA ENTRY FORMAT FOR CALIBRATION OF HPLC AGILENT (1260 INFINITY SERIES)			
Location		Page No.	2 of 52
Manufactured By		Model No.	
Calibration Frequency:		Instrument ID No.	
Date of calibration		Next Calibration Due on	

1.0 Flow rate calibration by stop watch:

Stop watch ID No : _____

Validity: _____

CHANNEL A				
Set flow rate in ml/min	Weight of empty Volumetric flask (W _{1g})	Weight of Volumetric flask after collection of water for 5 minutes (W _{2g})	Weight of Water collected (W ₂ – W _{1g})	Observed flow rate in ml/min $\frac{(W_2 - W_1)}{5 \times 0.997044}$
0.5				
1.0				
2.0				
5.0				
CHANNEL B				
0.5				
1.0				
2.0				
5.0				
CHANNEL C				
0.5				
1.0				
2.0				
5.0				
CHANNEL D				
0.5				
1.0				
2.0				
5.0				

Acceptance criteria (± 5% of the set flow rate)

- 1) For Flow 0.5 ml/min: Between (0.475 to 0.525) ml/ min.
- 2) For Flow 1.0 ml/min: Between (0.95 to 1.05) ml/ min.
- 3) For Flow 2.0 ml/min: Between (1.90 to 2.10) ml/ min.



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STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

4) For Flow 5.0 ml/min: Between (4.75 to 5.25) ml/ min.

Result: Complies / does not comply.

2.0 Injection Volume Accuracy:

Balance ID No.: _____

Set Inj. Vol. (µL)	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 (µL)	Acceptance criteria (± 1%) (µL)
10	50				$(W_1 - W_2) \times 1000$ $0.997044 \times \text{No of Inj.}$		
20	25						
50	10						
100	5						

Complies /Does not comply.

Performed By : _____

Checked By : _____

3.0 COLUMN THERMOSTAT CALIBRATION:

Digital Thermometer ID No. : _____ Valid up to: _____

Temperature (°C)	Observed Temperature (°C)	Acceptance Criteria (± 2.0°C)
20		18 - 22
40		38 - 42
60		58 - 62
80		78 - 82

Complies /Does not comply.

Performed By : _____

Checked By : _____



STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

4.0 SAMPLE THERMOSTAT CALIBRATION:

Digital Thermometer ID No. : _____ Valid up to: _____

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.

Performed By :

Checked By :

5.0 WAVELENGTH ACCURACY, LINEARITY, PRECISION, CARRY OVER OF AUTO SAMPLER AND DETECTOR

Balance ID: EQ/QCD/	B. No. of Caffeine:
----------------------------	----------------------------

Mobile phase preparation:

Prepare a mixture of _____ ml (15 vol.) of Acetonitrile and _____ ml (85 vol.) of water to produce _____ ml (100 vol.).

Solution preparation:

Accurately weigh and transfer _____ mg (25 mg) of caffeine in _____ ml (100 ml) volumetric flask. Add 60 ml of water, sonicate to dissolve and make volume with water. Dilute _____ ml (5 ml) of the solution to _____ ml (50 ml) with mobile phase. (25µg/ml)



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QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

Chromatographic Condition:

Column ID . :

Mobile Phase : Acetonitrile: Water (15:85)

Wavelength : 273 nm

Column Temperature: 25° C

Flow rate : 1.0 ml/min

Injection volume : 20 µL

Run time : 10 min.

OBSERVATIONS: WAVELENGTH ACCURACY

Expected absorbance Maxima and Minima in nm	Observed Absorbance Maxima and Minima in nm	Acceptance Criteria (± 2 nm from expected wavelength)	Results (Pass / Fails)
205 (Maxima)		203 to 207	
245 (Minima)		243 to 247	
273 (Maxima)		271 to 275	

Complies /Does not comply.

Performed By :

Checked By :



PHARMA DEVILS
QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

OBSERVATIONS: LINEARITY, PRECISION, CARRY OVER OF AUTO SAMPLER AND DETECTOR

RT (Caffeine Solution 25 µl/ ml)					
S.No.	Inject(5µl)	Inject(10µl)	Inject(20µl)	Inject(50µl)	Inject(100µl)
1					
2					
3					
4					
5					
6					
Avg. Area					
RSD %					

Area (Caffeine Solution 25 µl/ ml)					
S.No.	Inject(5µl)	Inject(10µl)	Inject(20µl)	Inject(50µl)	Inject(100µl)
1					
2					
3					
4					
5					
6					
Avg. Area					
RSD %					

Correlation coefficient 'r²'=_____ (NLT=0.999)



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QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

CARRY OVER:

	Area of Caffeine	Acceptance Criteria (0.2%)	Results (Pass / Fails)
Standard Caffeine (100 µl mean)			
Blank – 1			

Carry over % = $\frac{\text{Area of Caffeine in Blank} \times 100}{\text{Mean Area of 100 } \mu\text{l}}$

Mean Area of 100 µl

Performed By:

Checked By:

Acceptance Criteria:

- v) The % RSD of retention time of Caffeine from the six replicate injections each level should be less than 1.0 %.
- vi) The % RSD of peak areas of Caffeine from the six replicate injections each level should be less than 2.0 %.
- vii) The Correlation coefficient 'r²' obtained from the linearity graph of Caffeine for different levels should not be less than 0.999.
- viii) Carryover of Caffeine: NMT 0.2% of mean area of 100µL.



PHARMA DEVILS
QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

6.0 Gradient system:

Preparation of 0.25% v/v solution of Acetone:

Take: _____ (5 ml) of Acetone dilute to: _____ (2000 ml) of Purified water.

Flow rate: _____ 1.0 ml/ min

Wavelength: _____ 254 nm

Parameter	Observation	Acceptance Criteria	Results (Pass / Fails)
The difference in absorbance		NMT: 0.01AU	
The difference in time		NMT : 20 Sec.	
Performed By :		Checked By :	



PHARMA DEVILS
QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

7.0 OBSERVATION: - Drift and Noise Test

Column : Restriction capillary or equivalent

Mobile phase : Degassed Water

Flow rate : 1.0 ml/min

Wavelength : 254 nm for Noise & drift

Run Time : 60 min

Column Temperature: 25° C

Observed Detector Noise: _____ AU

Observed Detector Drift: _____ AU / 60 minutes.

Acceptance Criteria:

Detector Noise: NMT 0.000125 AU

Detector Drift: NMT 0.005 AU / hr

Remarks: The instrument is **OK/Does not OK** for routine analysis.

Performed By :

Checked By :

Approved By :



PHARMA DEVILS
QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

ANNEXURE II

CALIBRATION SUMMARY REPORT OF AGILANT (1260 INFINITY SERIES)

Location		Page No.	
Manufactured By		Model No.	
Calibration Frequency:		Instrument ID No.	
Date of calibration		Next Calibration Due on	

1.0) FLOW RATE

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

Acceptance criteria ($\pm 5\%$ of the set flow rate)

- 1) For Flow 0.5 ml/min: Between (0.475 to 0.525) ml/ min.
- 2) For Flow 1.0 ml/min: Between (0.95 to 1.05) ml/ min.
- 3) For Flow 2.0 ml/min: Between (1.90 to 2.10) ml/ min.
- 4) For Flow 5.0 ml/min: Between (4.75 to 5.25) ml/ min.



PHARMA DEVILS
QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

2.0) INJECTION VOLUME ACCURACY

TEST NAME	OBSERVATION	ACCEPTANCE CRITERIA ($\pm 1\%$)	REMARK
10 μ l			Complies / Does not Comply
20 μ l			Complies / Does not Comply
50 μ l			Complies / Does not Comply
100 μ l			Complies / Does not Comply



PHARMA DEVILS
QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

3.0) Column Thermostat Calibration

TEST NAME	OBSERVATION	ACCEPTANCE CRITERIA (+ 2.0°C)	REMARK
20°C			Complies / Does not Comply
40°C			Complies / Does not Comply
60°C			Complies / Does not Comply
80°C			Complies / Does not Comply

4.0) SAMPLE THERMOSTAT CALIBRATION

TEST NAME	OBSERVATION	ACCEPTANCE CRITERIA (+ 2.0°C)	REMARK
4°C			Complies / Does not Comply
10°C			Complies / Does not Comply
15°C			Complies / Does not Comply
25°C			Complies / Does not Comply

Performed By:

Checked By:



PHARMA DEVILS
QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

5.0 WAVELENGTH ACCURACY:

Test Name	Observation	Acceptance Criteria	Remark
Wavelength accuracy	Maximum between 202 nm to 208 nm : _____	205 ± 2 nm	Complies / Does not Comply
	Minimum between 242 nm to 248 nm : _____	245 ± 2 nm	
	Maximum between 270 nm to 276 nm : _____	273 ± 2 nm	

Performed By:

Checked By:

6.0 LINEARITY, PRECISION, CARRY OVER OF AUTO SAMPLER AND DETECTOR

Injection Vol.	Area (µAU*sec)		Retention time (min)		Acceptance Criteria	Remark
	Mean	RSD%	Mean	RSD %		
5µl					% RSD of RT NMT 1.0	Complies / Does not Comply
10µl						
20µl					% RSD of Area NMT 1.0	
50µl						
100µl						
Correlation coefficient 'r ² ' = _____					NLT 0.999	Complies / Does not Comply
Carryover = _____					NMT 0.2%	Complies / Does not Comply

Performed By:

Checked By:



PHARMA DEVILS
QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

7.0 PUMP GRADIENT ACCURACY:

PARAMETRE	OBSERVATION	ACCEPTANCE CRITERIA	Remark
The difference in absorbance		NMT : 0.01 AU	Complies / Does not Comply
The difference in time		NMT : 20 Sec	Complies / Does not Comply

Performed By:

Checked By:

8.0) NOISE AND DRIFT OF DETECTOR:

PARAMETRE	OBSERVATION	ACCEPTANCE CRITERIA	Remark
Noise		NMT 0.000125 AU	Complies / Does not Comply
Drift		0.005 AU / hr	Complies / Does not Comply

Remarks: The instrument is **OK/Does not OK** for routine analysis.

Performed By :	Checked By :	Approved By :
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