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.

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"

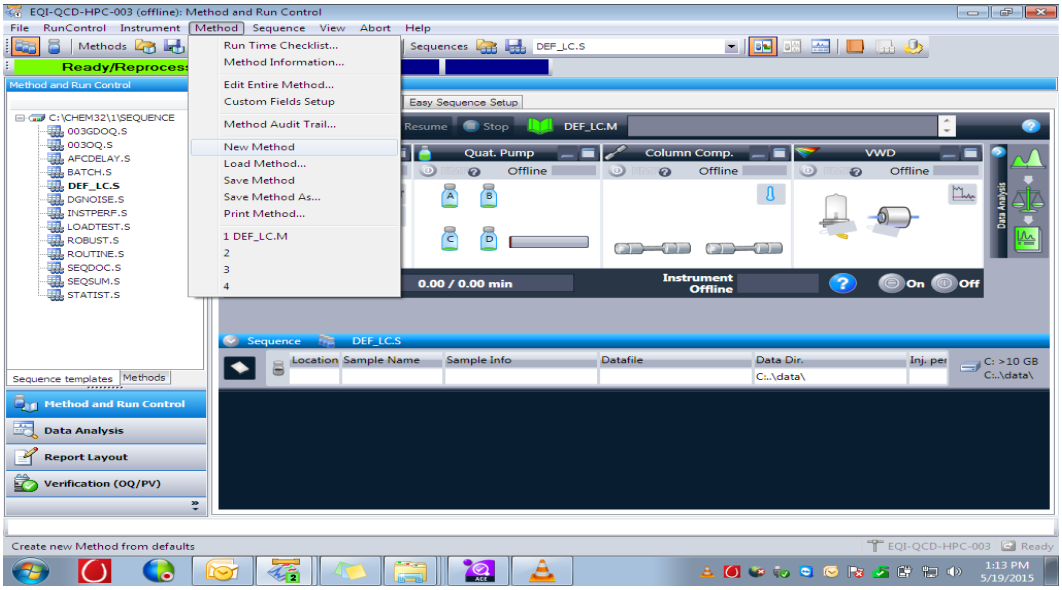
4.2.7.1 Click on sampler and select "Method" Injection Volume.

4.2.7.2 Entre the desired injection volume and click on "OK"

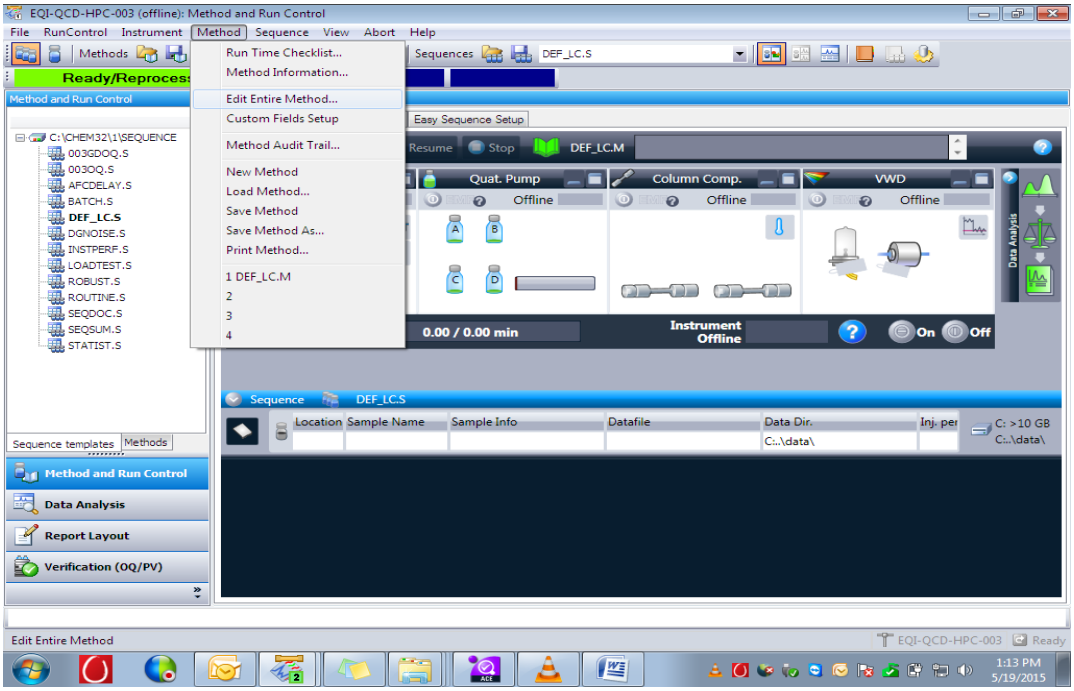
4.2.8 To create a New method

4.2.8.1 Click the "Method" icon followed by click the "New Method"

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.:**

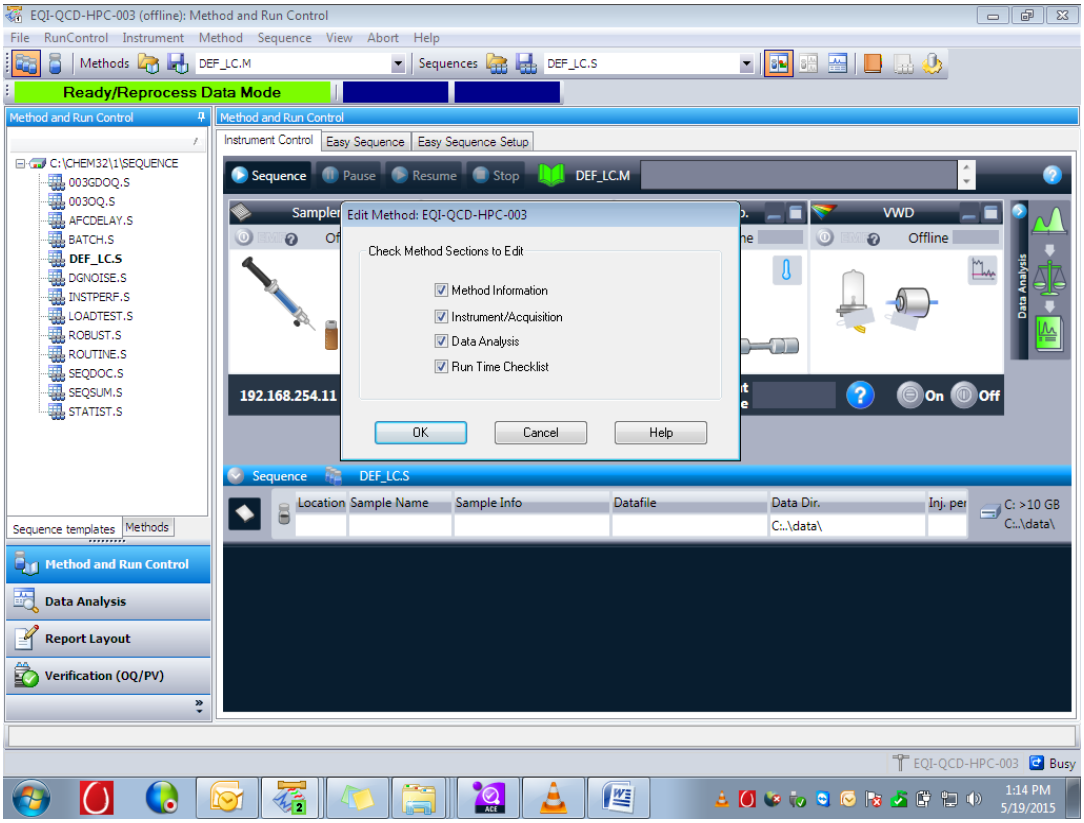


4.2.8.2 Again click method icon and following by edit entire method.



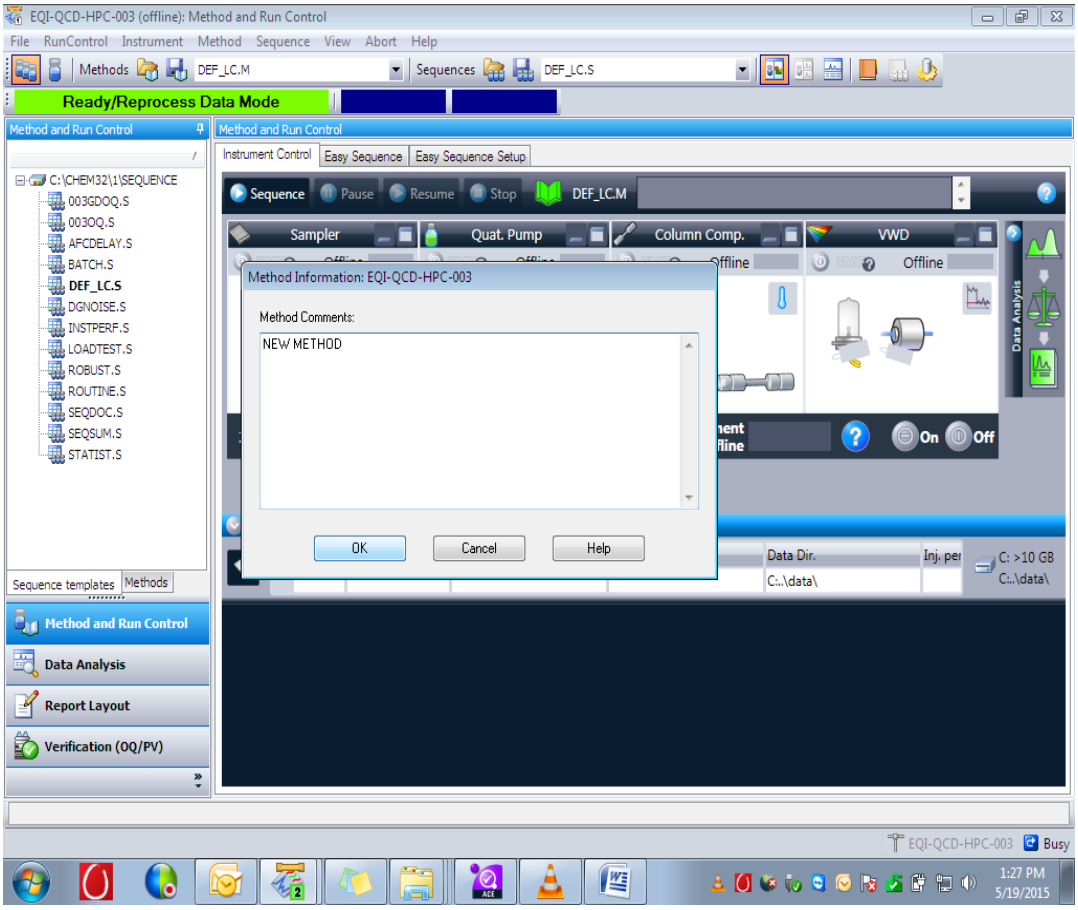
4.2.8.3 Click method section to edit OK.

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.:**



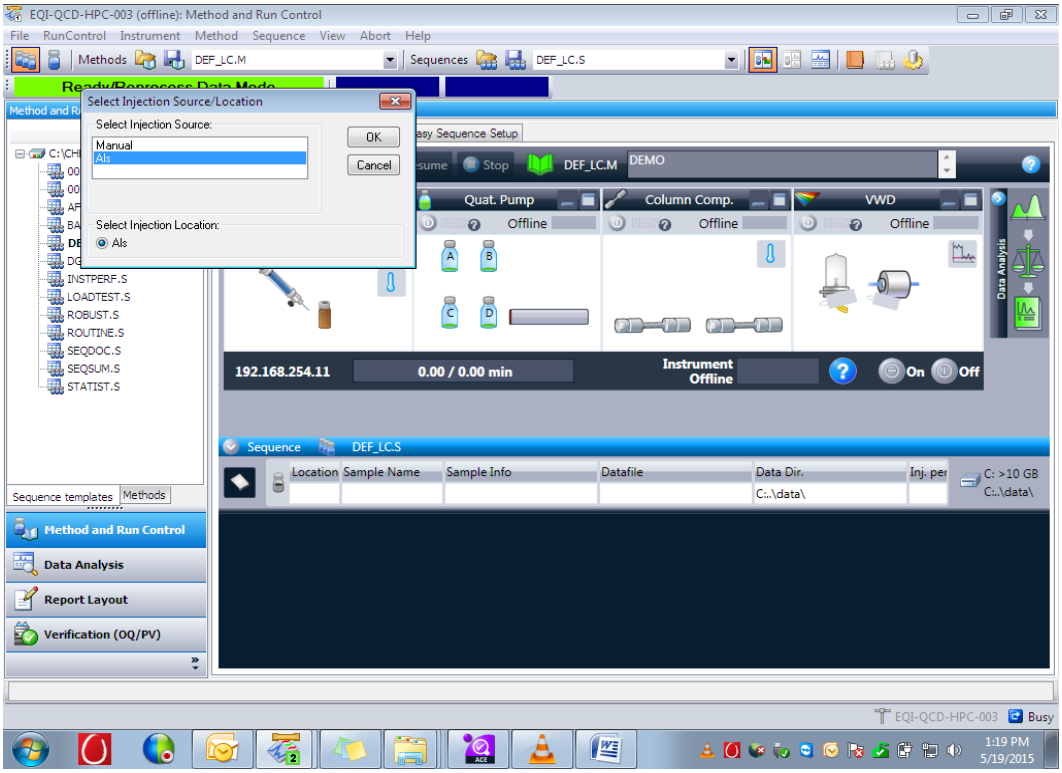
4.2.8.4 Entre method comments and clicks OK.

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

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.

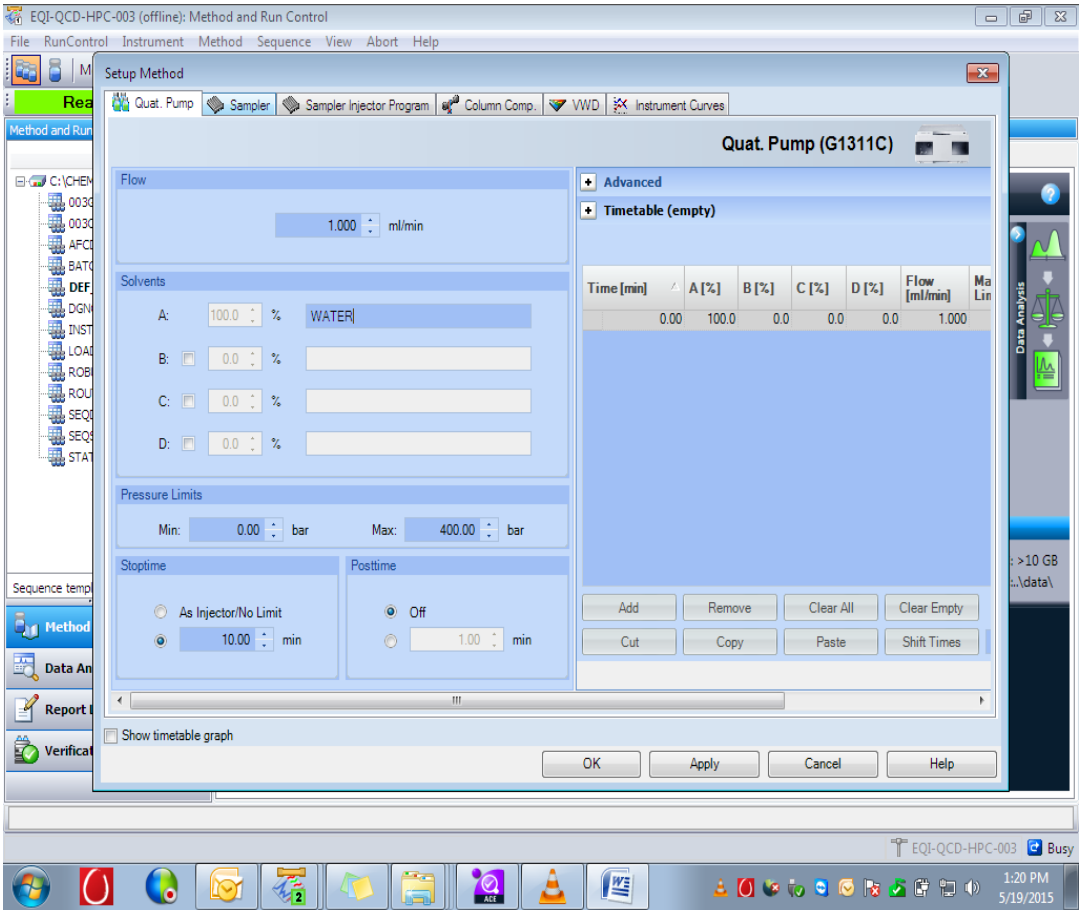
4.2.8.7 Select edit integration events and click OK.

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.:**



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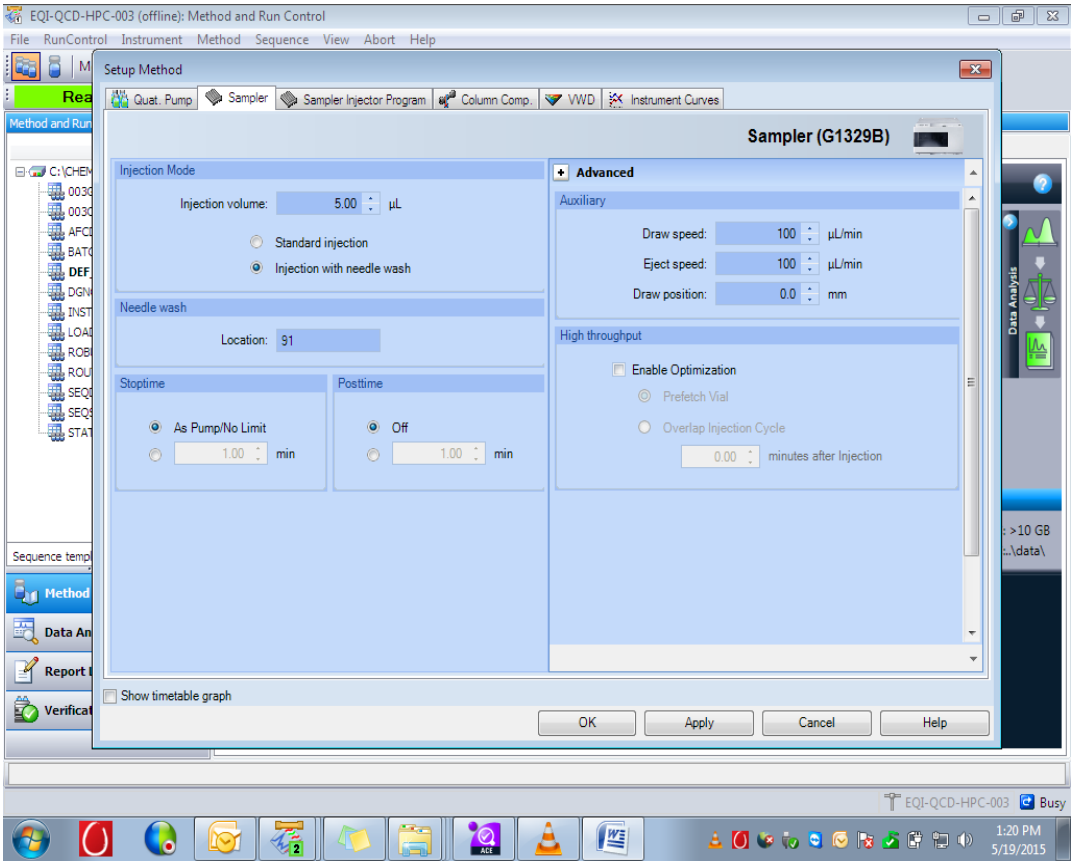
QUALITY CONTROL DEPARTMENT

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.:**



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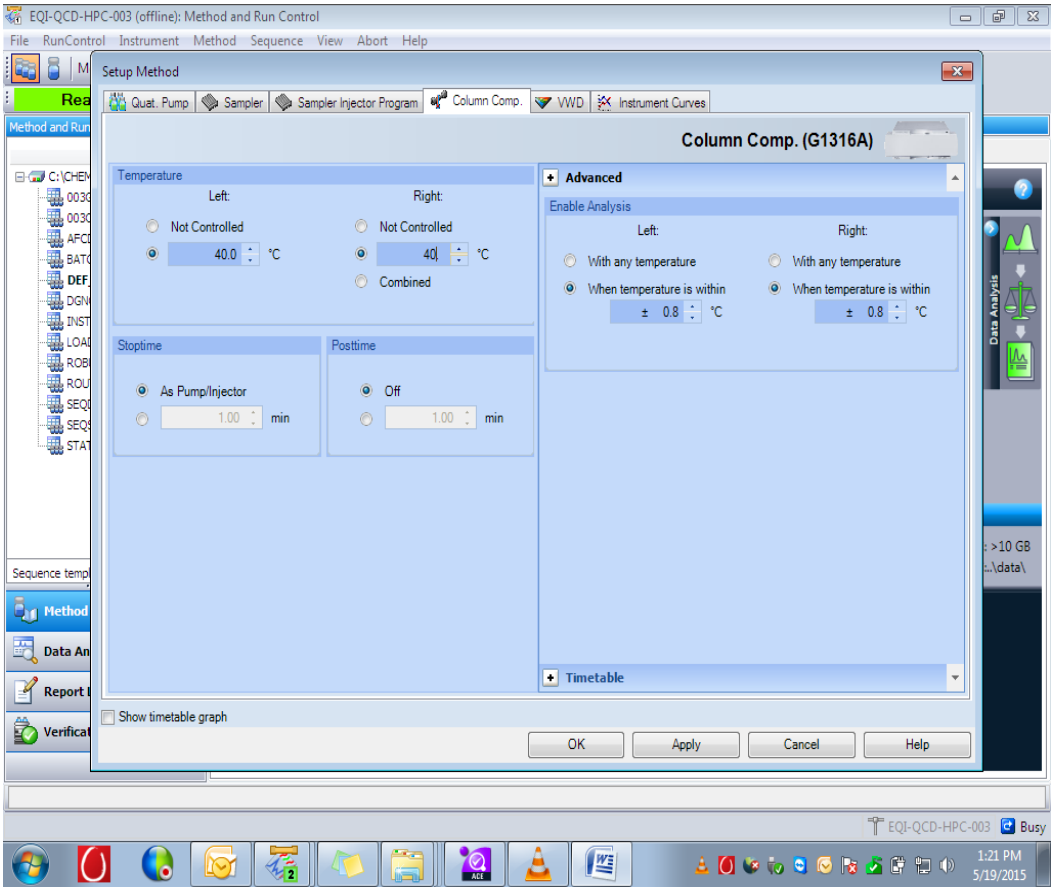
QUALITY CONTROL DEPARTMENT

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.:**



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QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Quality Control	**SOP No.:**
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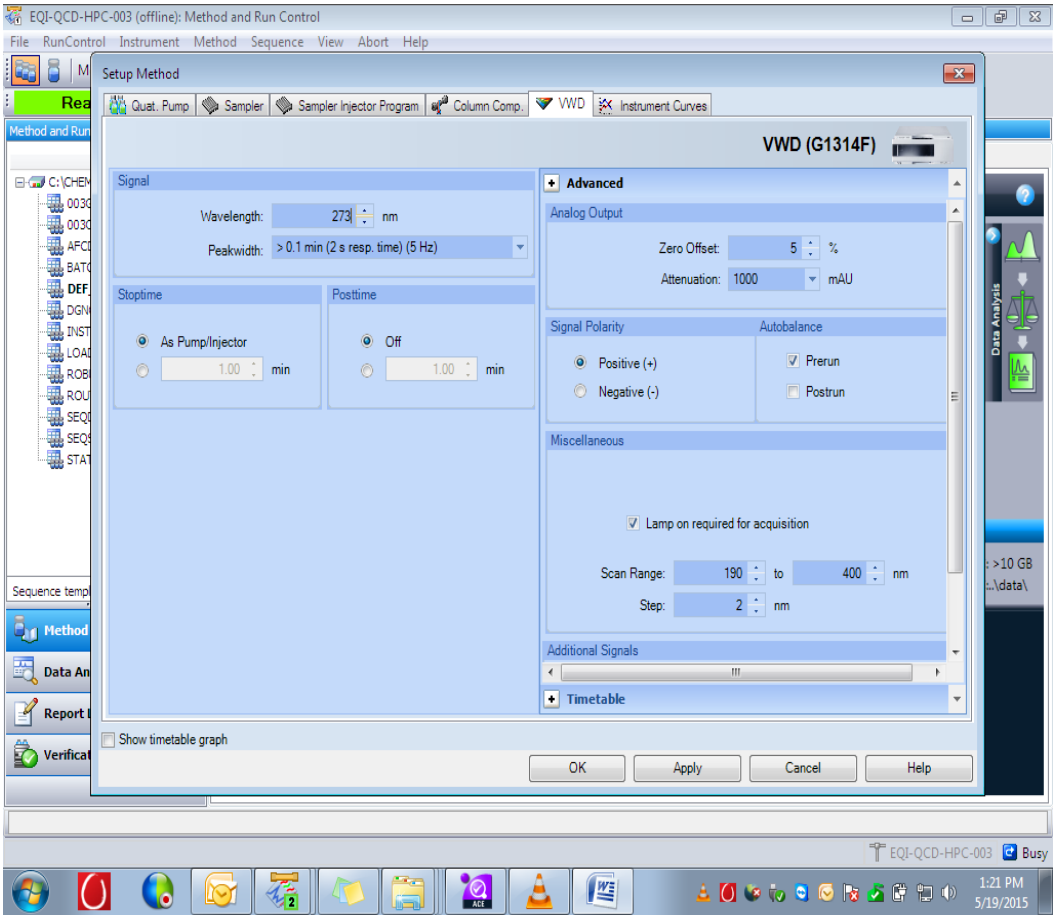

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QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE	
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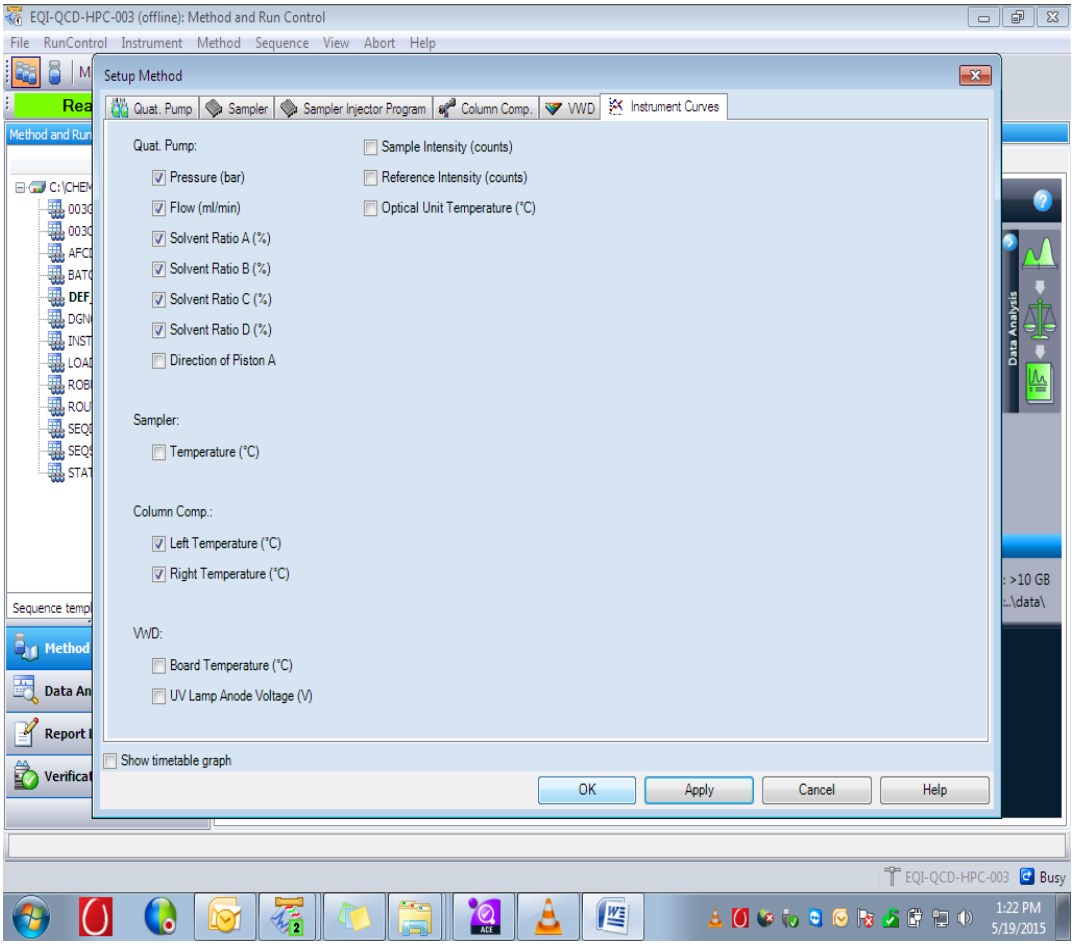

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STANDARD OPERATING PROCEDURE	
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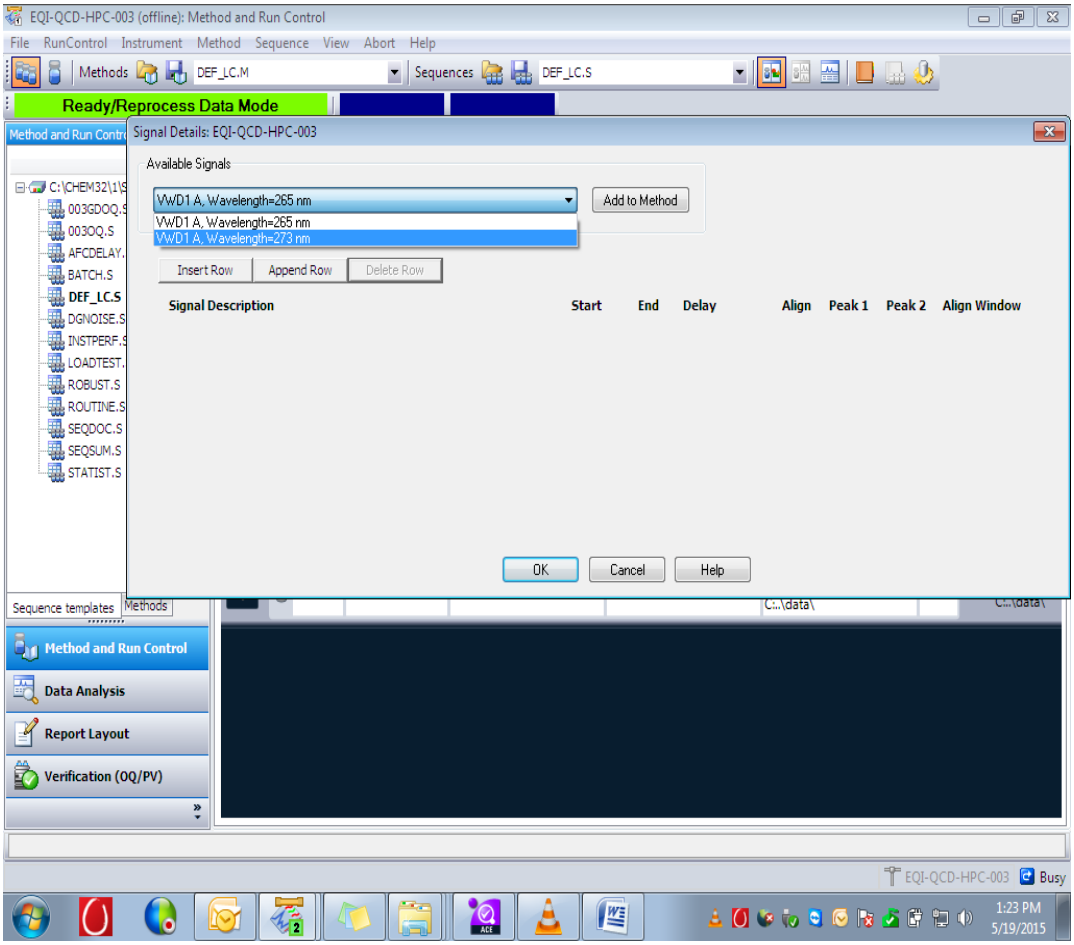
Issue Date:	**Page No.:**



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QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Quality Control	**SOP No.:**
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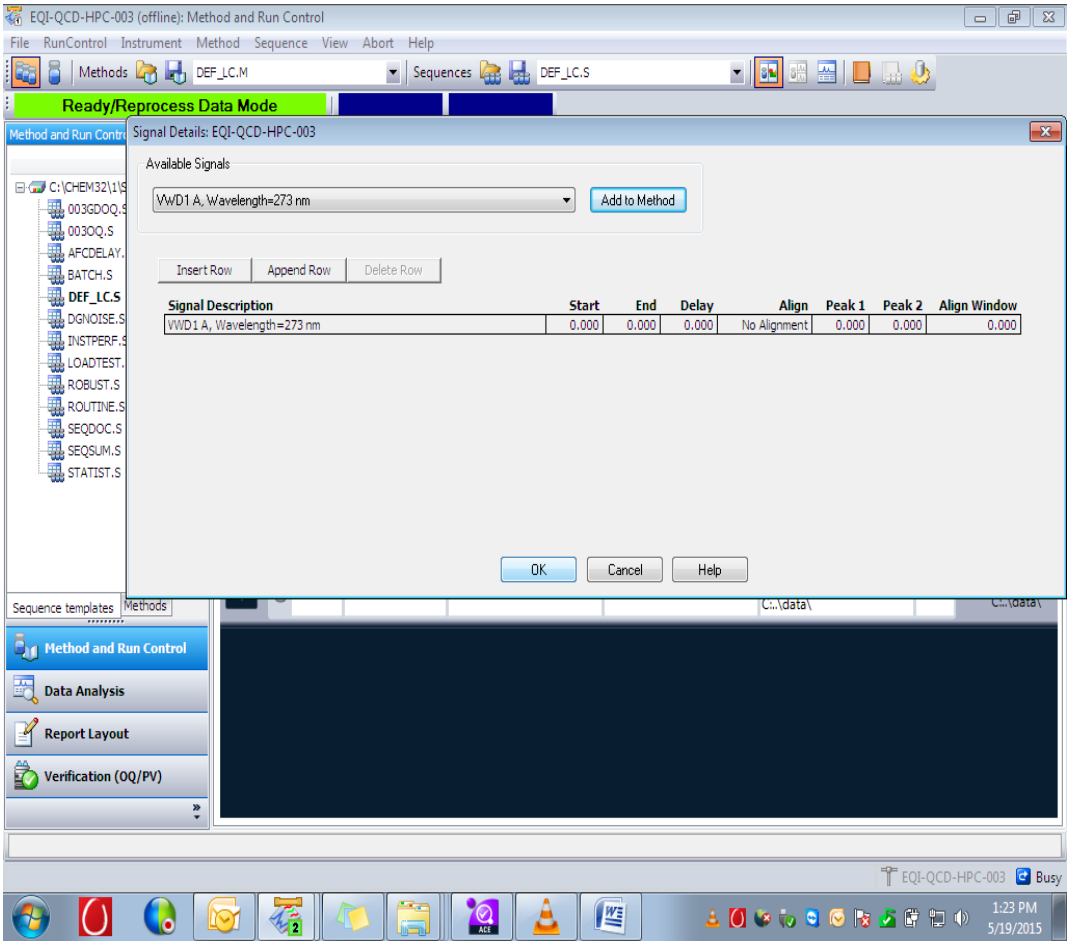

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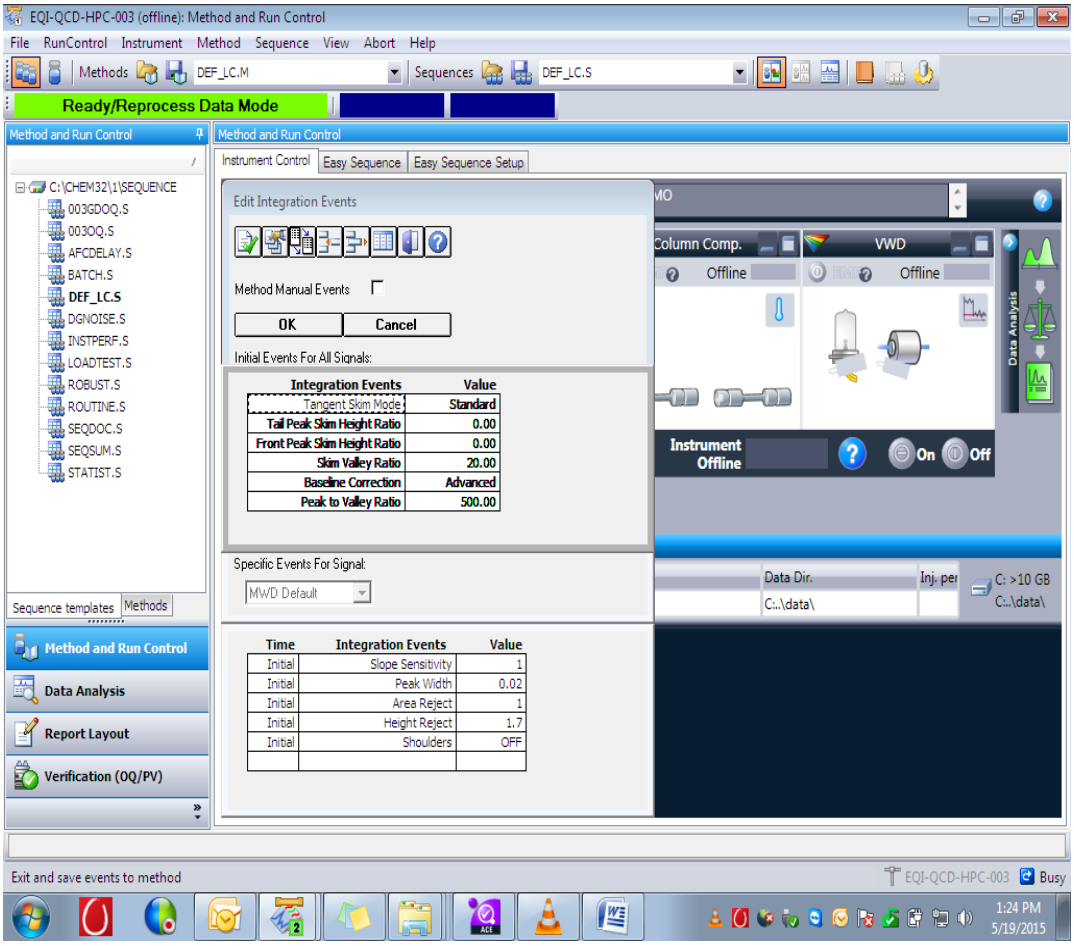
QUALITY CONTROL DEPARTMENT

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.:**



PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Quality Control	**SOP No.:**
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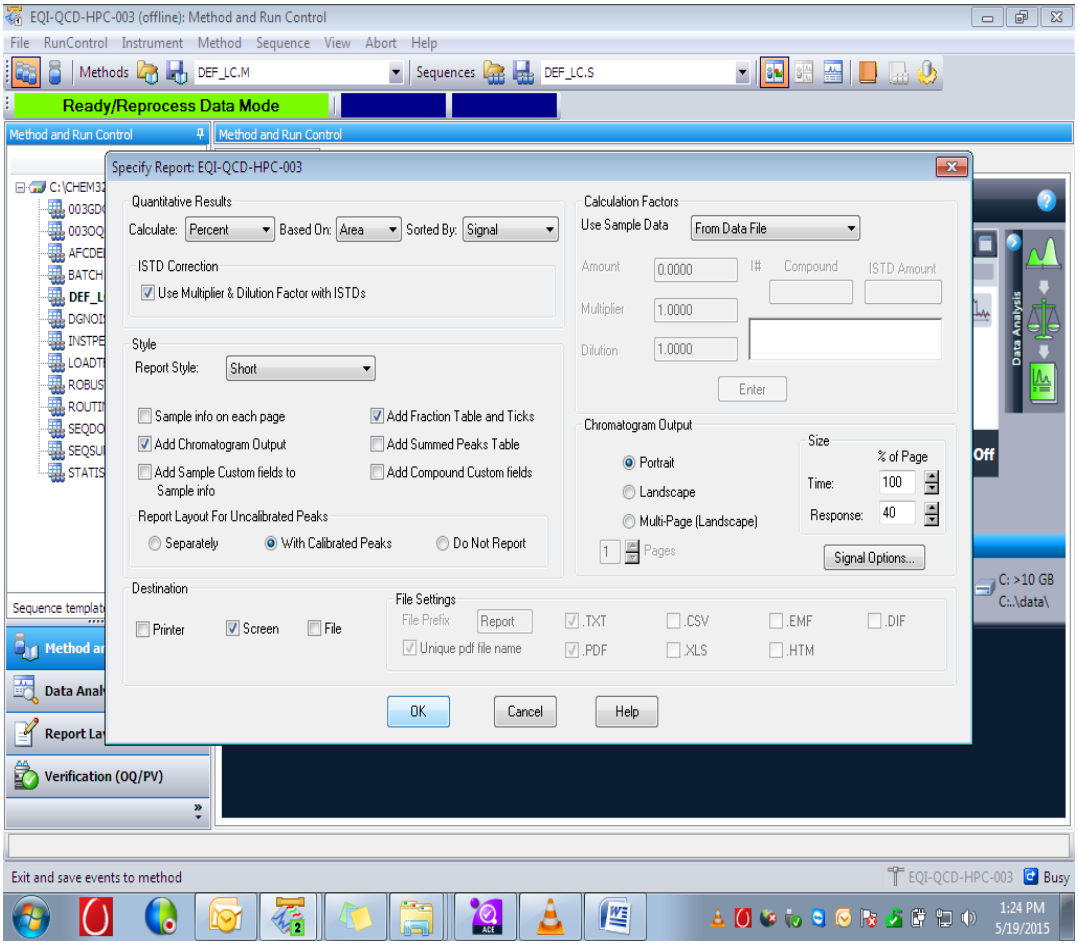
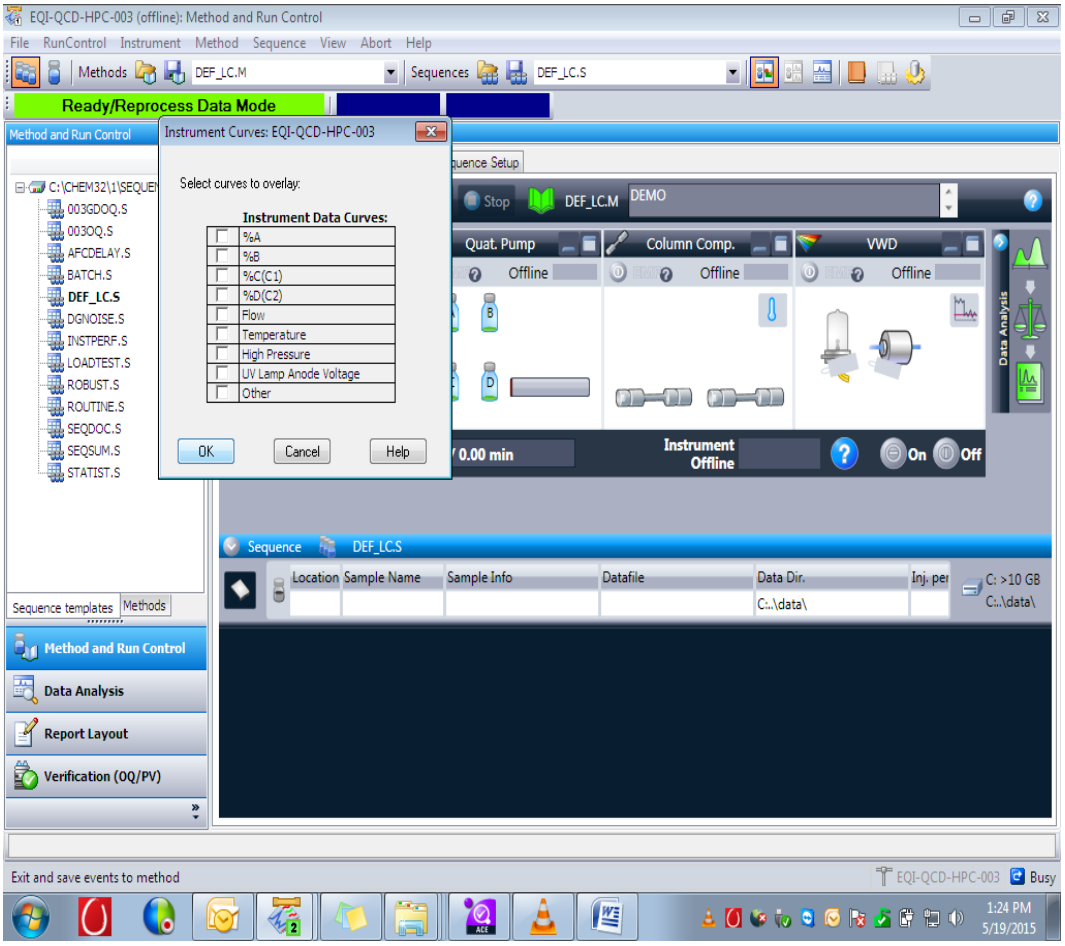
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**



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.:**



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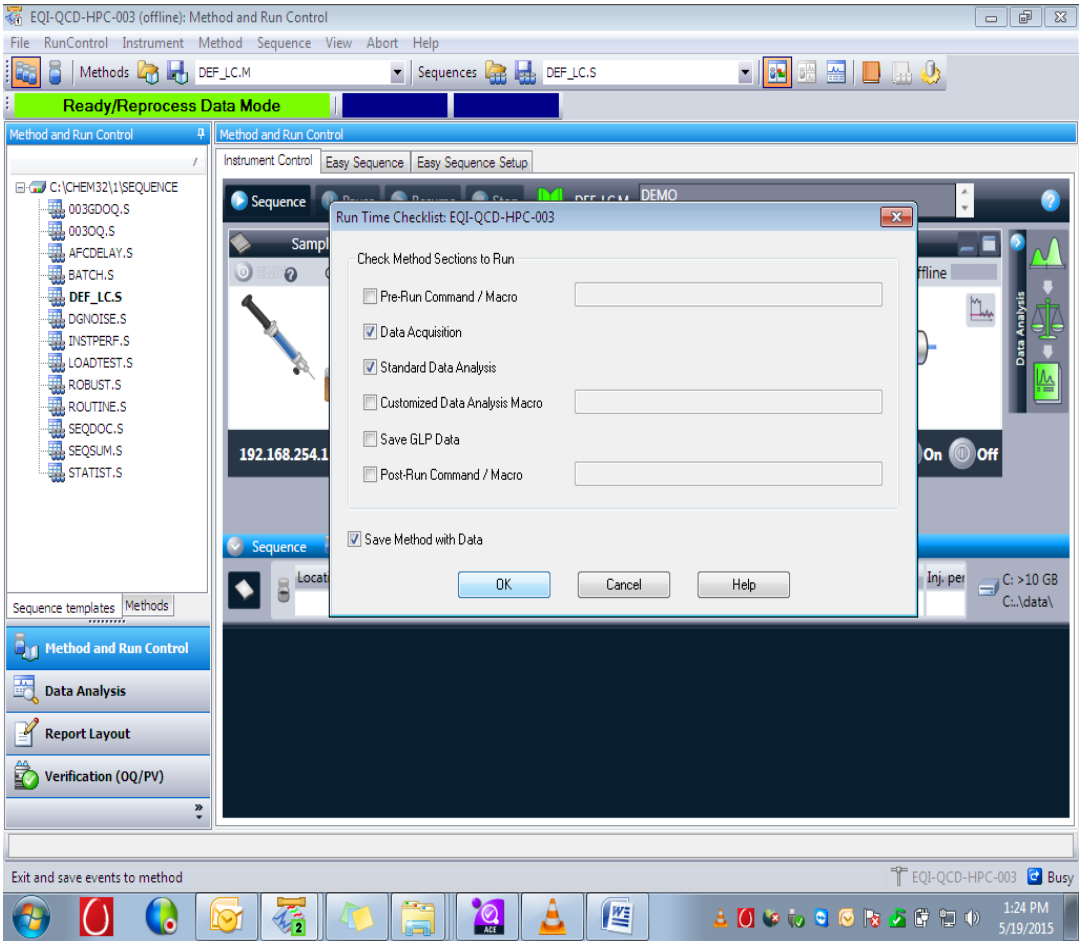
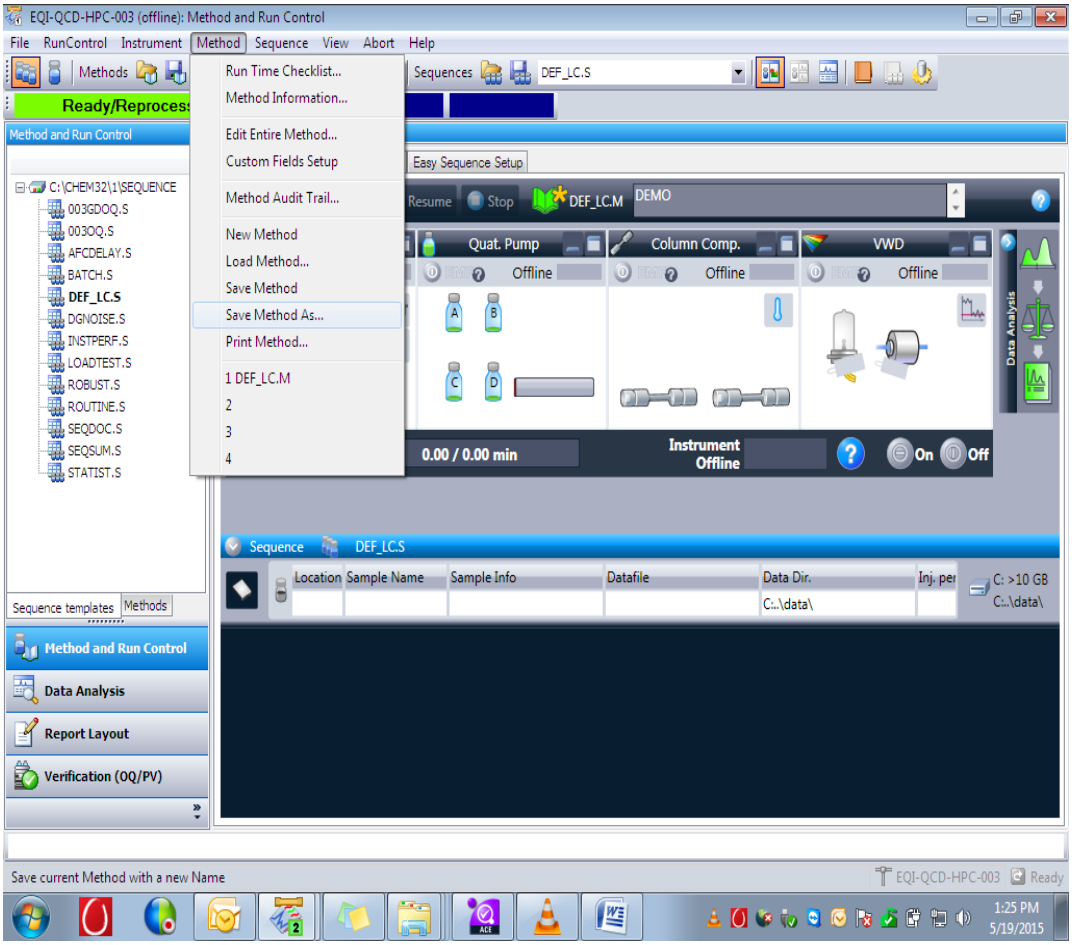
Issue Date:	**Page No.:**



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

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.:**

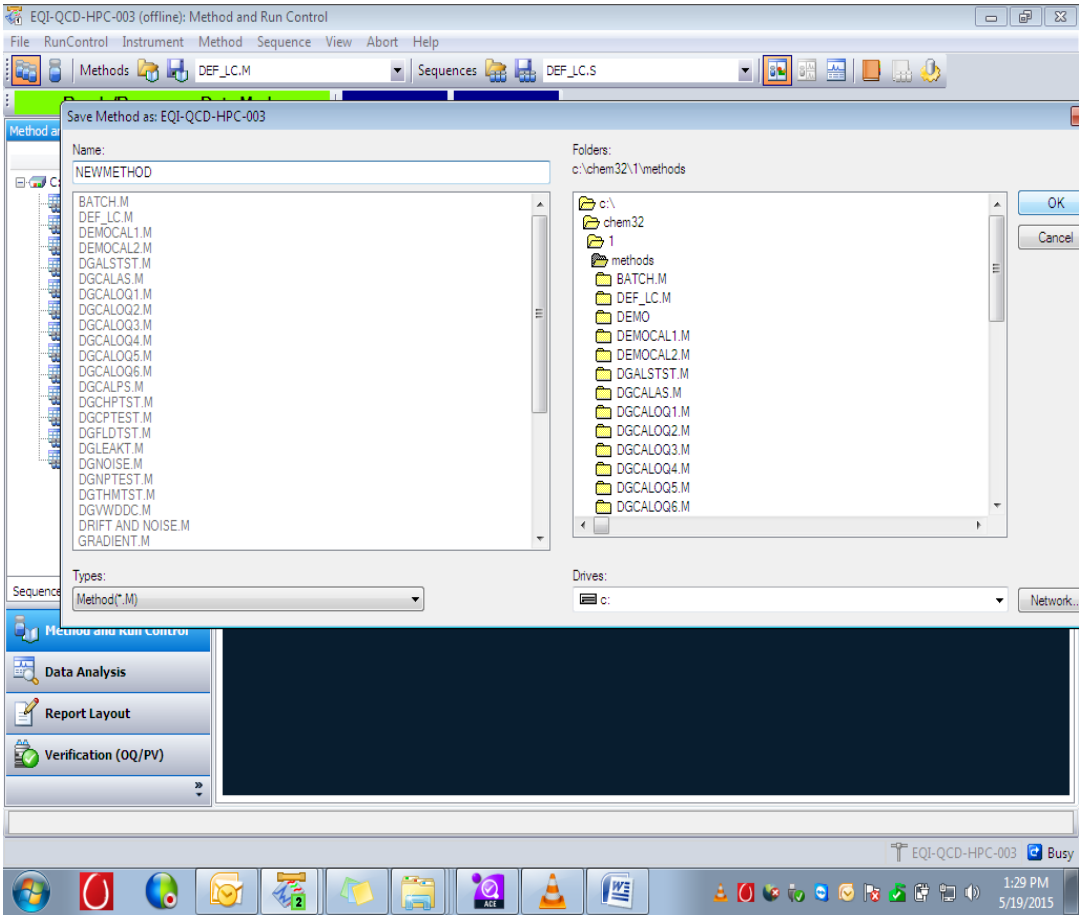


4.2.8.10 To create new method click in the following order method/new Method save it in following path:

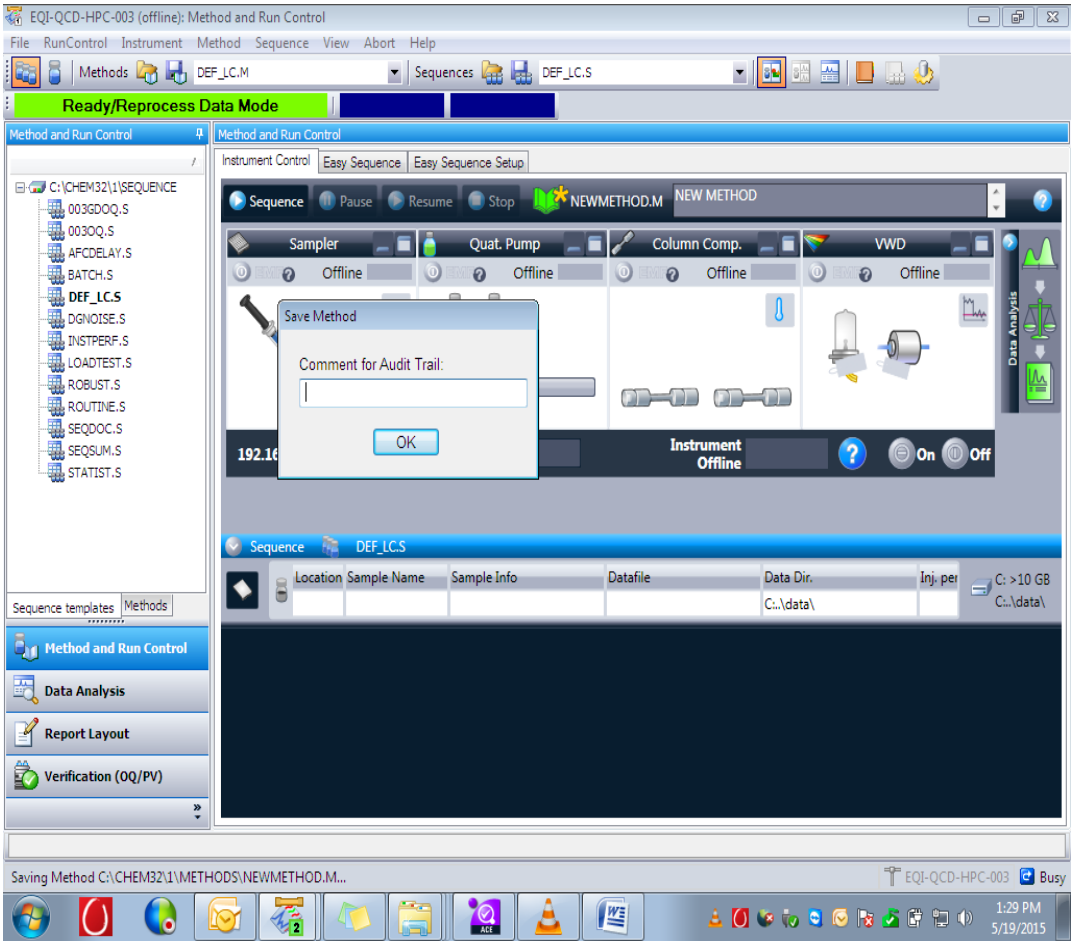
PHARMA DEVILS

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.:**



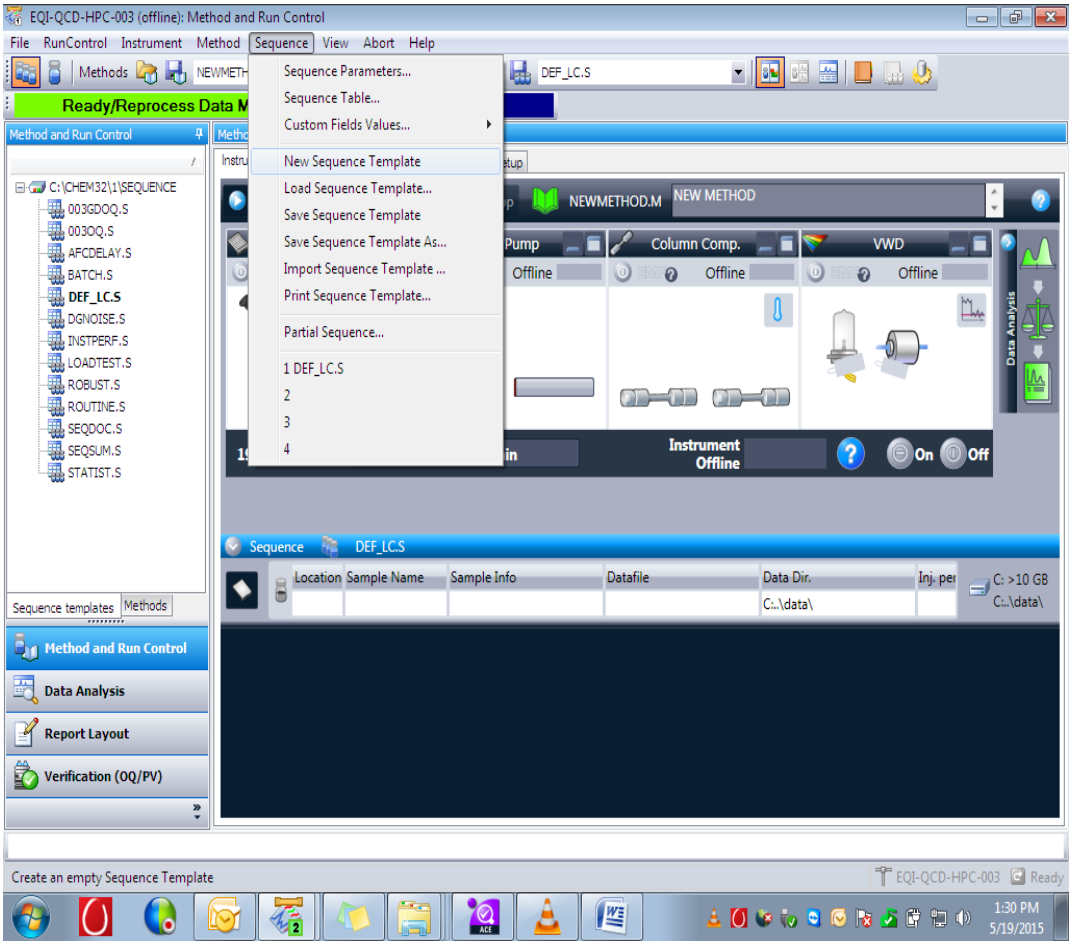
4.2.8.11



4.2.9 Creating Sequence in Chemstation Software

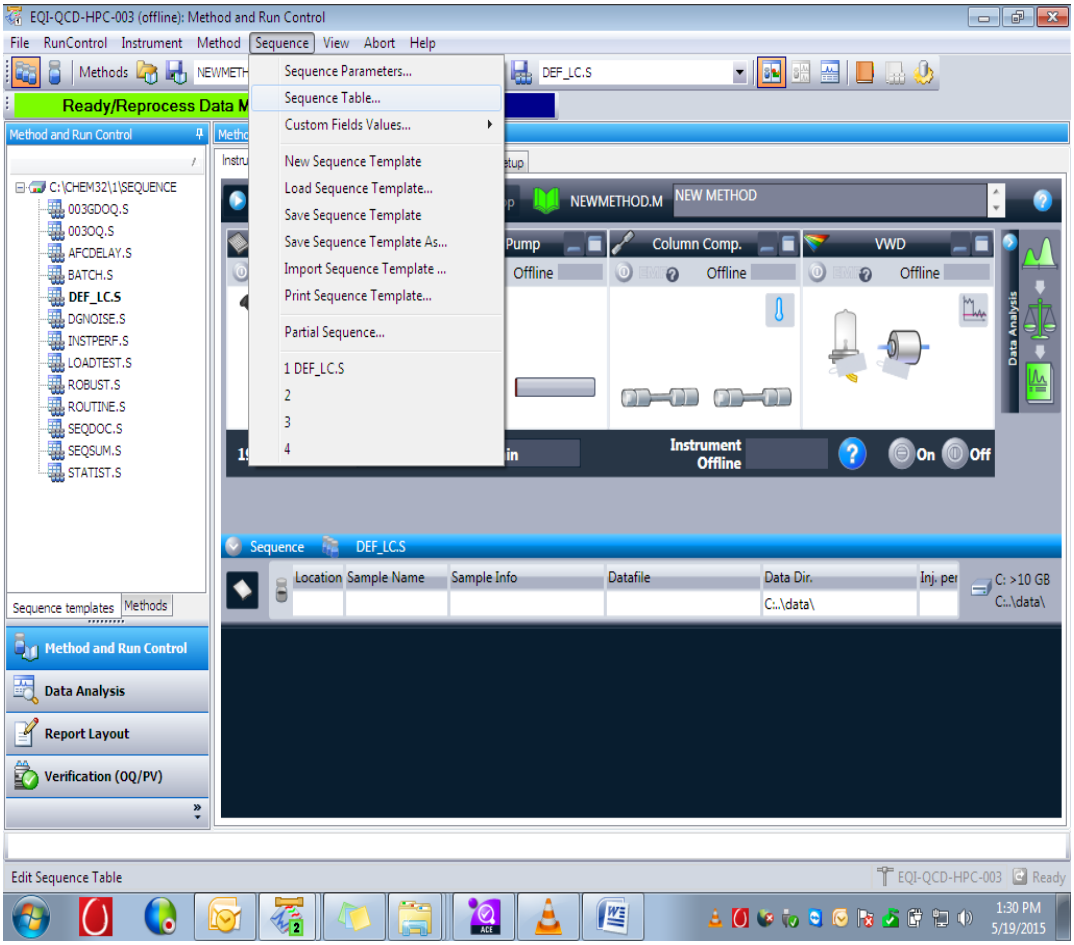
4.2.9.1 Click the “Sequence”icon followed click the “New Sequence”

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.:**

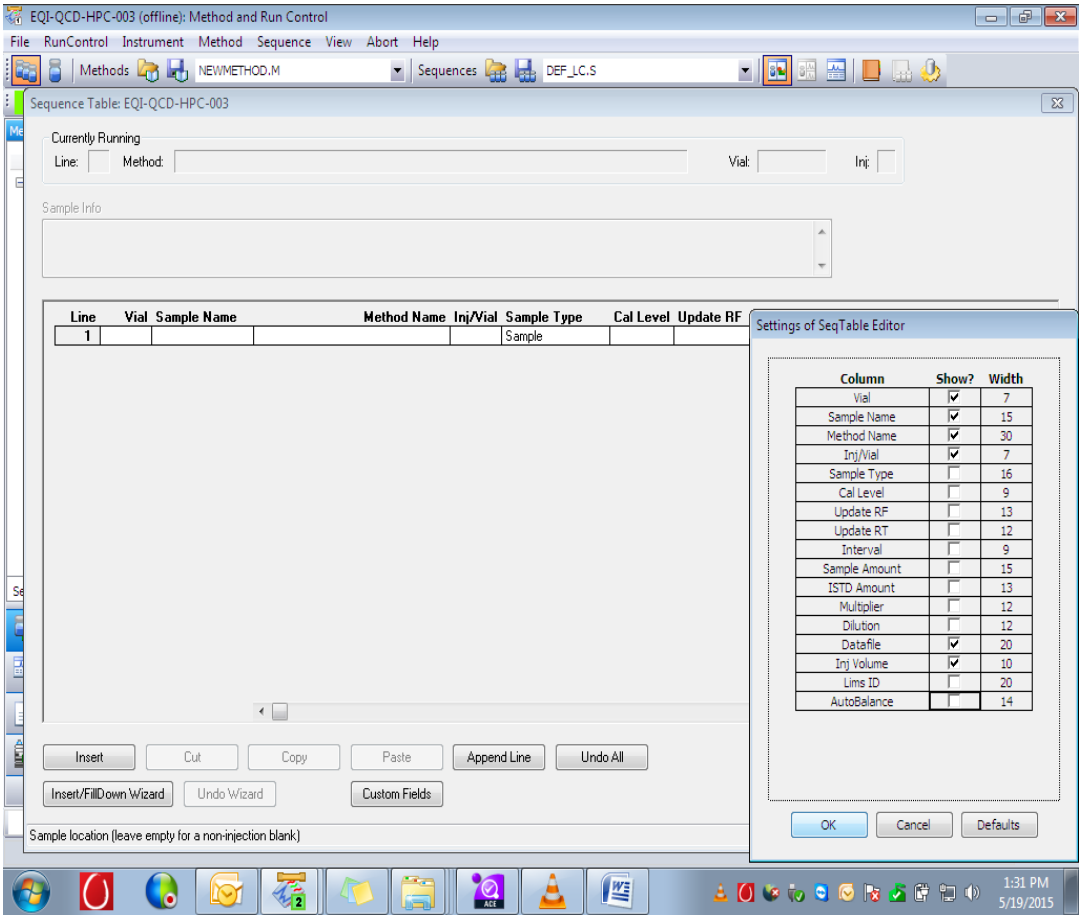


4.2.9.3 The sequence table by choosing the Injector source and completed the following entriese. Location,sample name,Method Name,Injection/ Location and sample type,injection Volume,vail Number.

PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

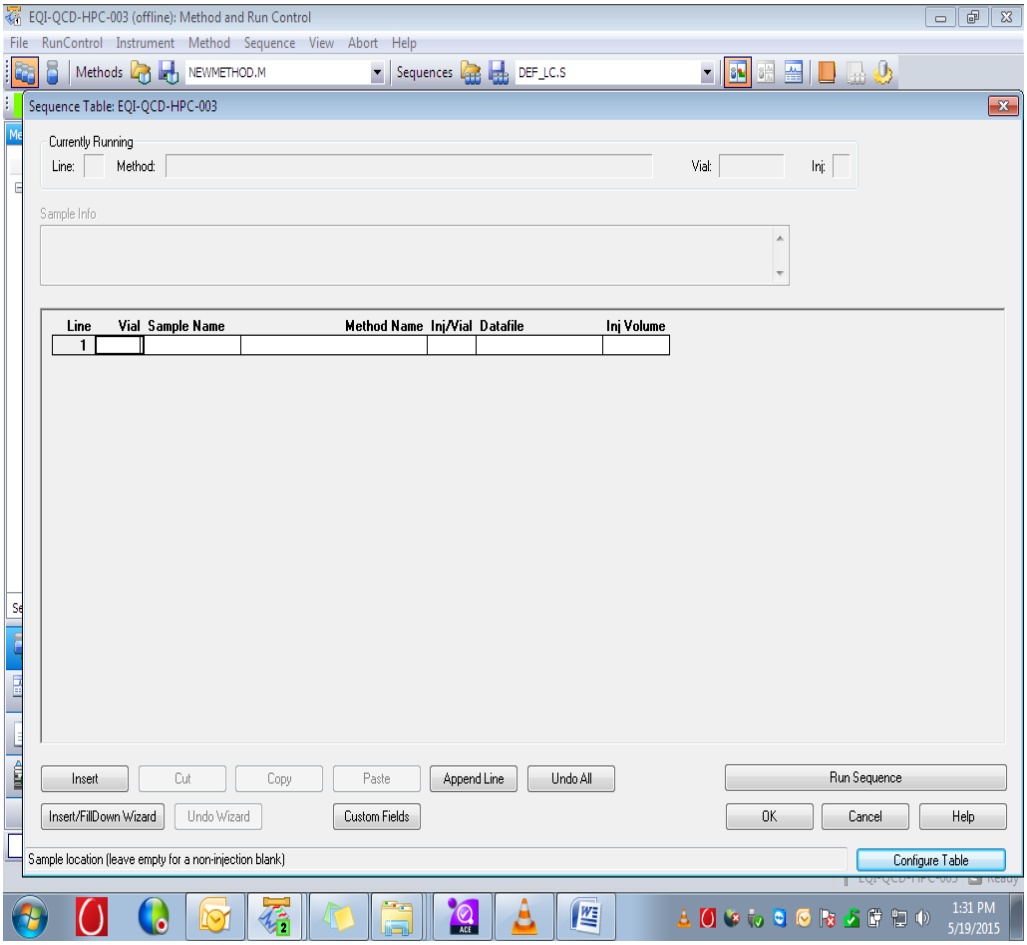
STANDARD OPERATING PROCEDURE	
Department: Quality Control	**SOP No.:**
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**



PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

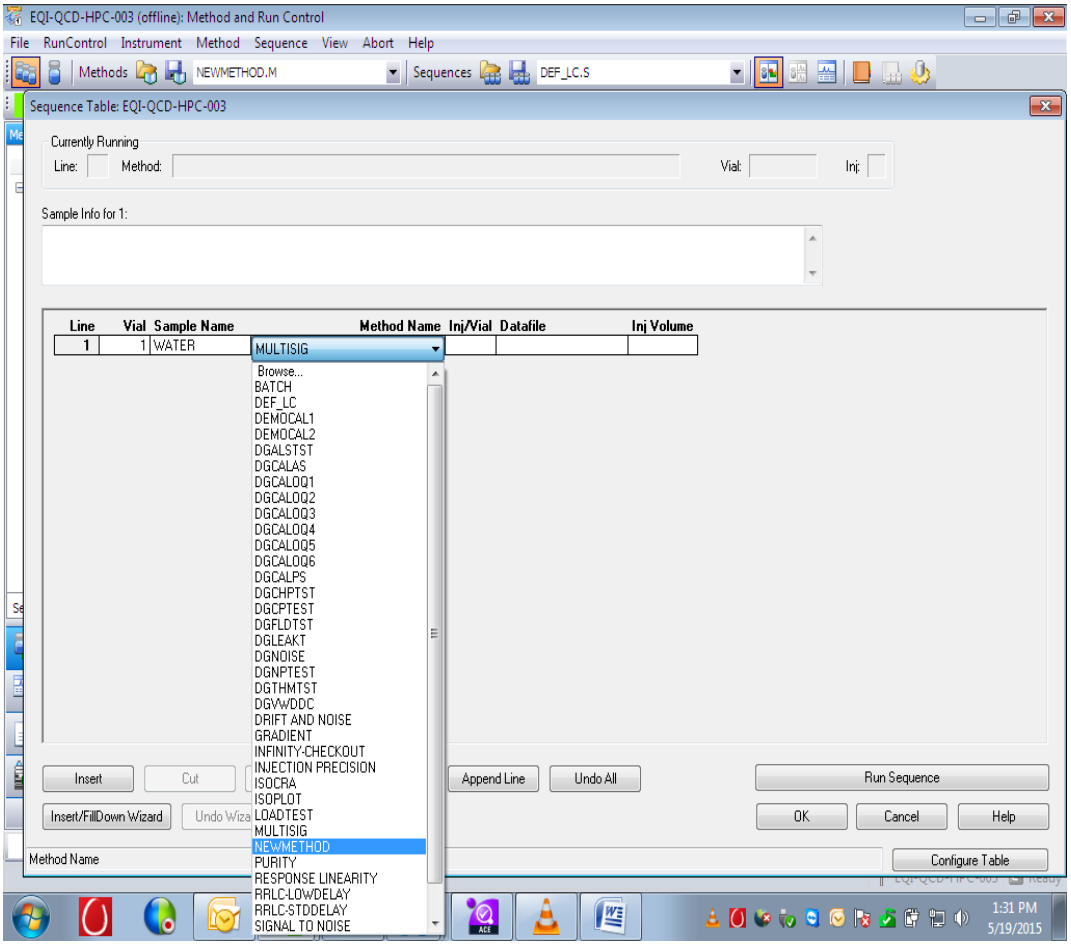
STANDARD OPERATING PROCEDURE	
Department: Quality Control	**SOP No.:**
Title: Operation and Calibration of HPLC Agilent (1260 Infinity series) with UV Detector	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**



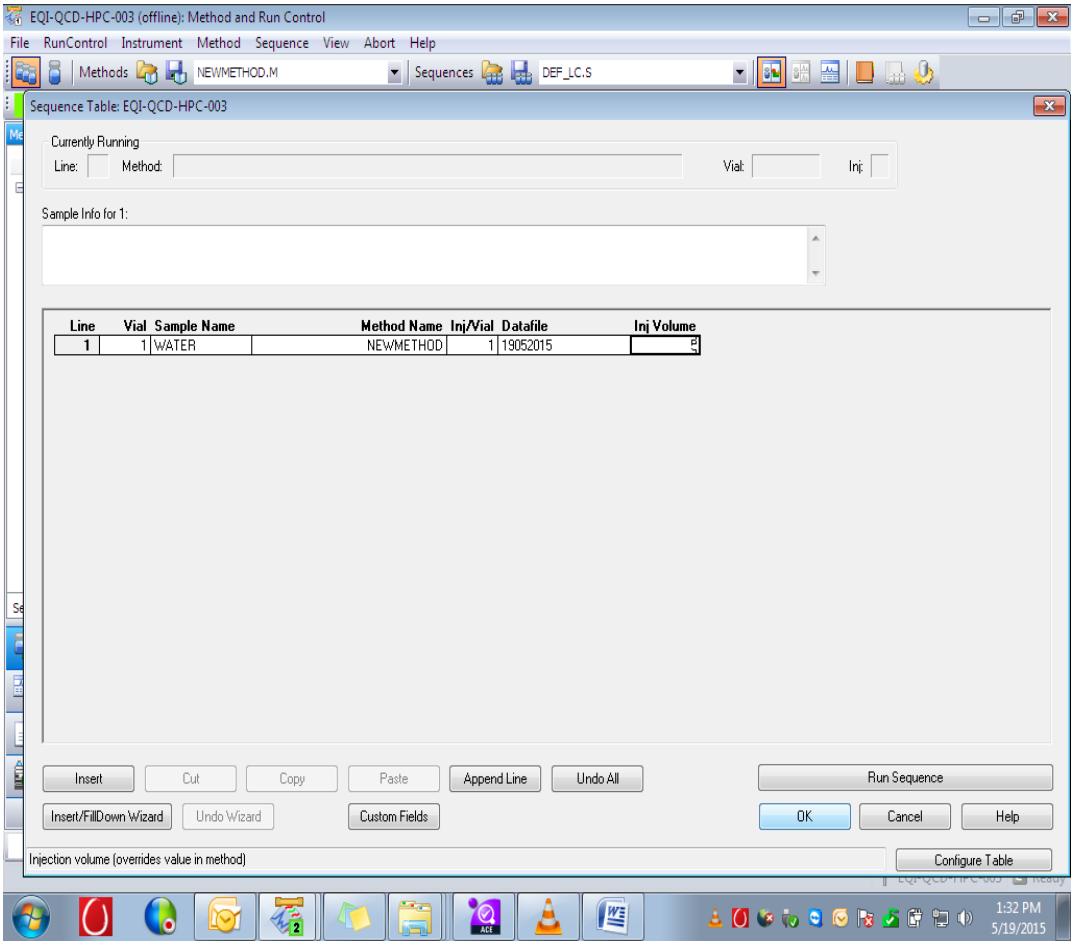
PHARMA DEVILS

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.:**



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.:**

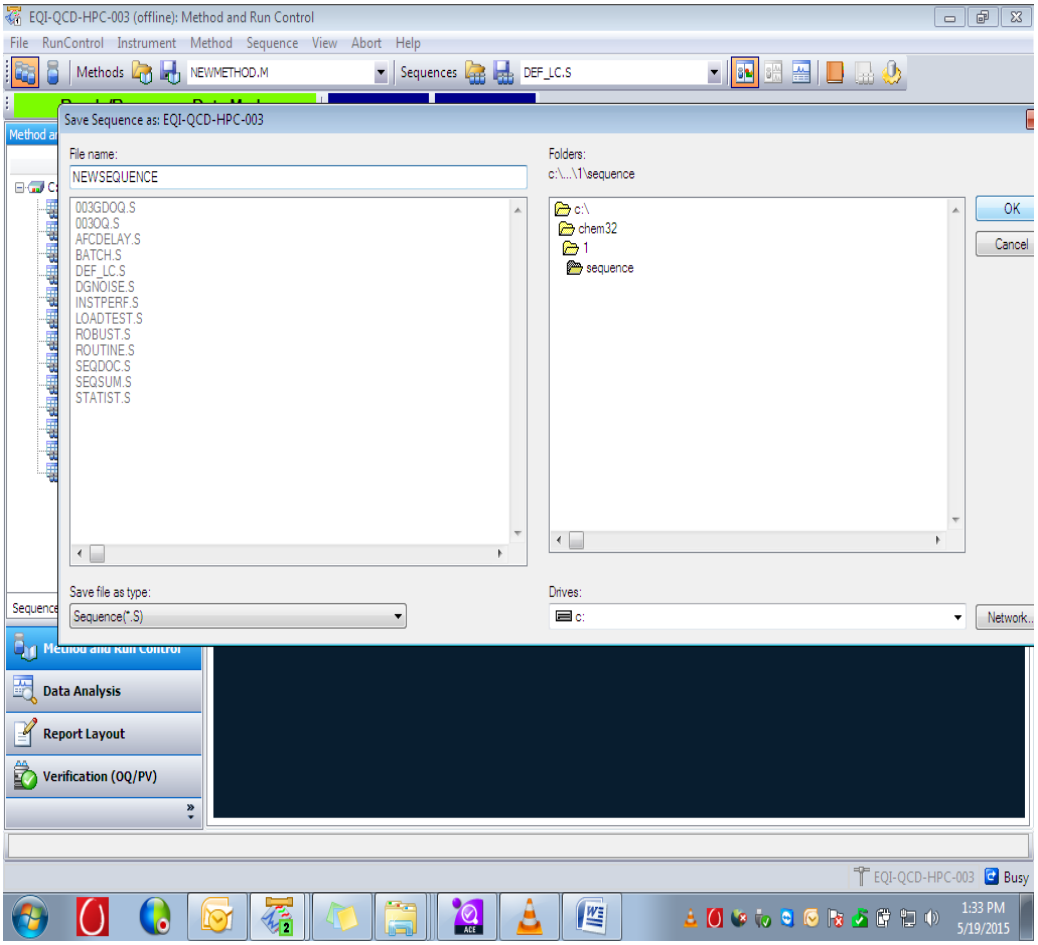


4.2.9.4 To select the data file couter and storage location click in the following order sequence/sequence parametres and choose the path.

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.:**
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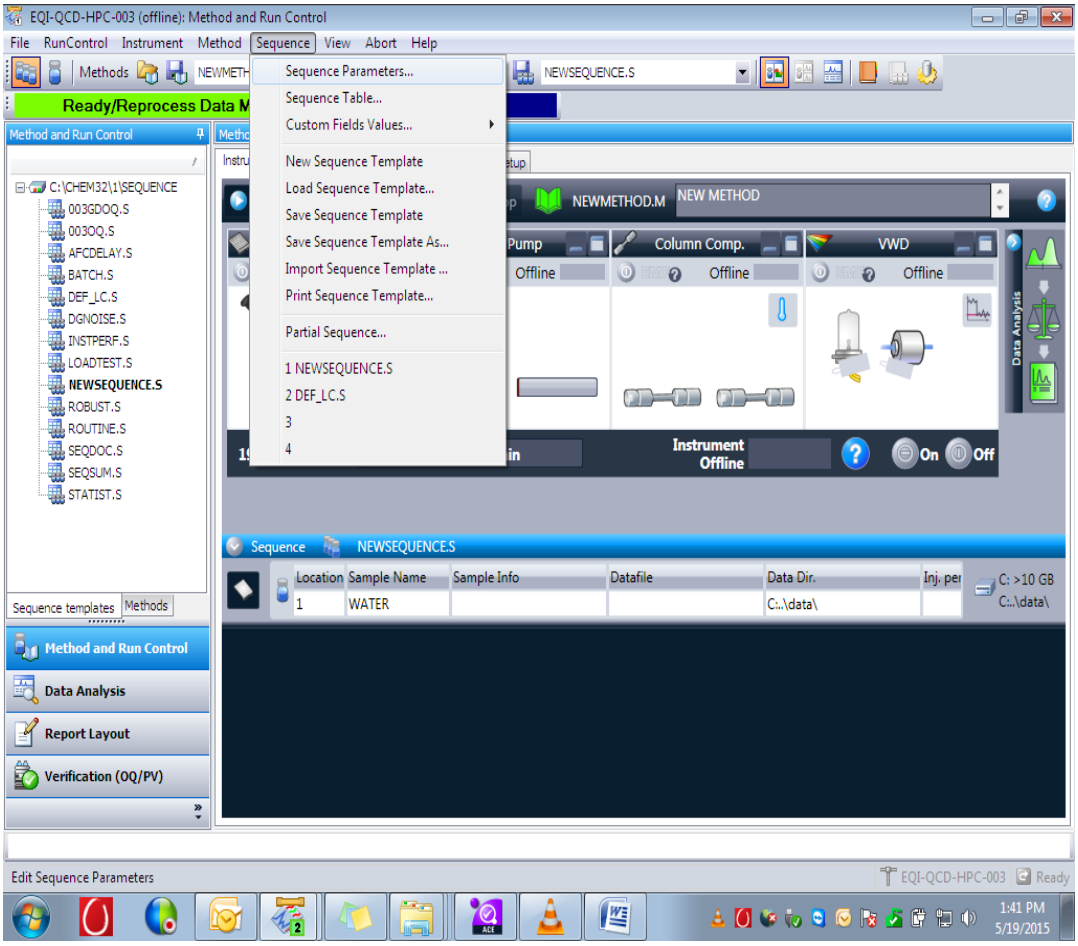
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**



PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Quality Control	**SOP No.:**
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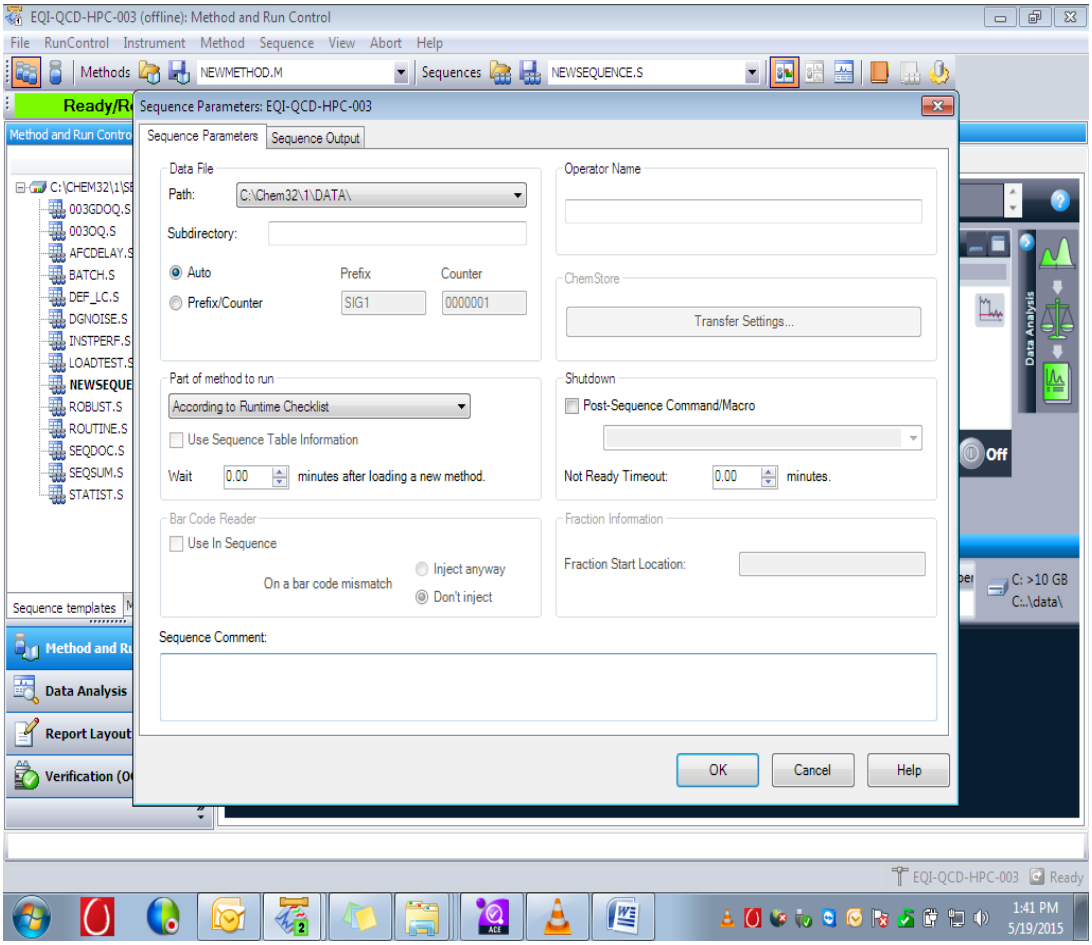
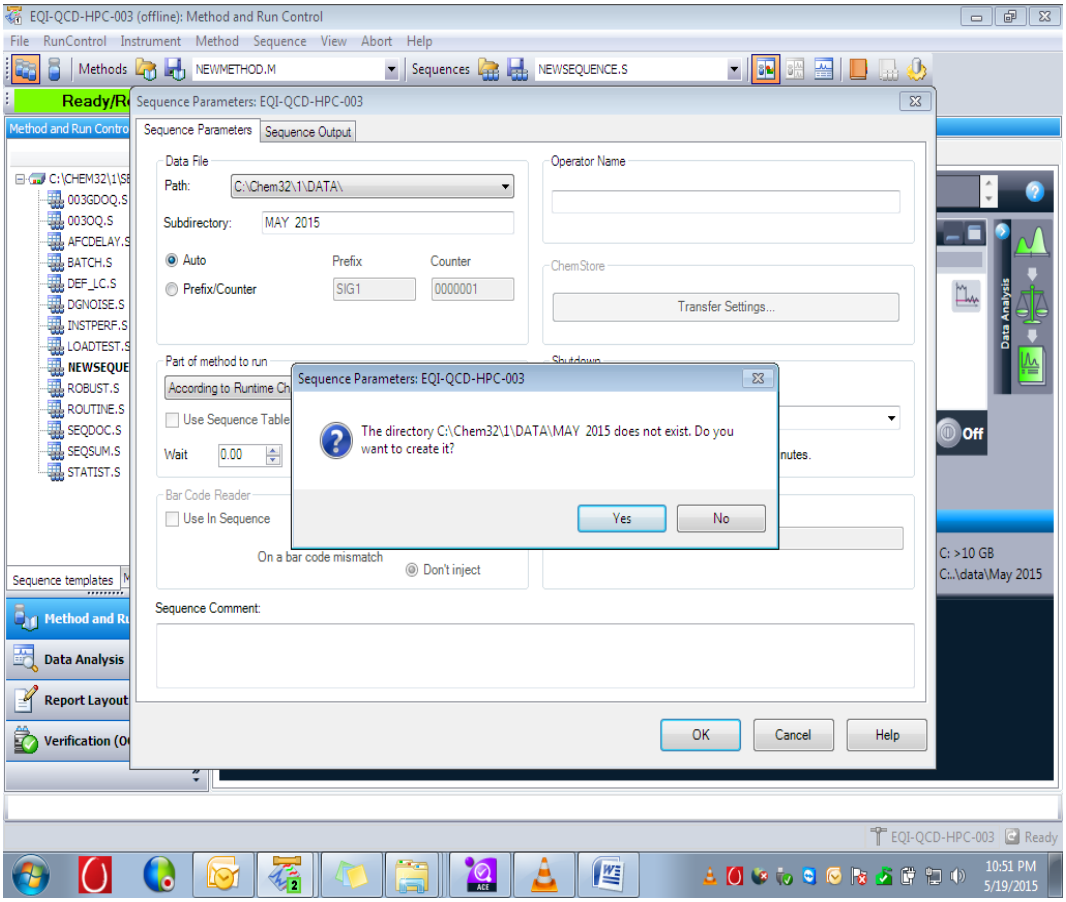
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**



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.:**



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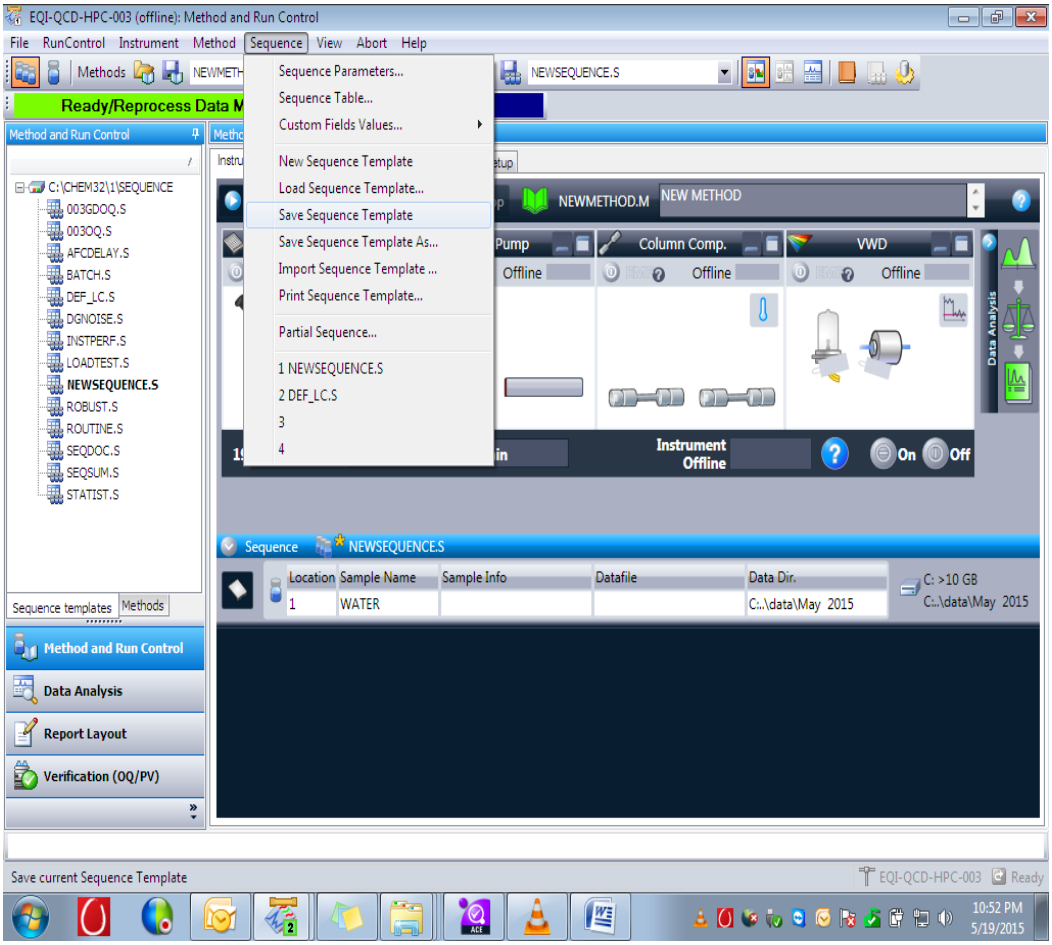

4.2.9.5 Click on “Run Control” and select Run sequence or “START” dispayed above the sequence .

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.

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.

Flow rate = (W2-W1) / (5 x 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 (W_1) g.

4.4.2.2 Place the vial in the sample tray.

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 (W_2) 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),

Minimum at 245 nm ± 2 nm (Between 242 nm to 248 nm),
Maximum at 273 ± 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 5µl,10µl,20µl,50µl and 100µl 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 100µL. Calculate the carry over using following formula:

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

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 '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 0.2% of mean area of 100µL.

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.

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.

4.3.8.8 Inject 0µl 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).

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

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.:**

REVISION CARD

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

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: _____

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 ($W_2 - W_1$g	Observed flow rate in ml/min $\frac{(W2 - W1)}{5\times 0.997044}$
CHANNEL A				
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.

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				$\frac{(W_1-W_2) \times 1000}{0.997044 \times \text{No of Inj.}}$		
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 (± 2.0°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)

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 :**	

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 'r^2'=_____(NLT=0.999)					

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 % = $\frac{\text{Area of Caffeine in Blank} \times 100}{\text{Mean Area of 100 µl}}$

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 'r^2' 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.

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 :**	

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 :**

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 (± 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.

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 (± 1%)	REMARK
10 µl			Complies / Does not Comply
20µl			Complies / Does not Comply
50µl			Complies / Does not Comply
100µl			Complies / Does not Comply

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:**

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 : _____ Minimum between 242 nm to 248 nm :_____ Maximum between 270 nm to 276 nm : _____	205 ± 2 nm 245 ± 2 nm 273 ± 2 nm	Complies / Does not Comply

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:**

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 :**

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 III
HPLC USER LOG BOOK

Instrument Name :

Instrument ID :

Date	Time		Used for			Column ID No.	Number of Injections	Total run in hours	Used by	Remarks
	From	To	Material / Product	Test	A.R.No.					