



STANDARD OPERATING PROCEDURE

Department:	SOP No.:
Title: Validation of Instrument Software	Effective Date:
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1.0 OBJECTIVE:

To lay down the procedure for validation of Instrument Software (HPLC) used in Quality Control Department.

2.0 SCOPE:

This procedure is applicable for validation of Instrument Software (HPLC) used in Quality Control Department at.....

3.0 RESPONSIBILITY:

Officer, Executive – Quality Control Department
Head – Quality Control Department

4.0 DEFINITION(S):

NA

5.0 PROCEDURE:

5.1 All the HPLC software is to be operated using individual instrument operation procedure. Software is to be validated as mentioned below.

5.1.1 Instrument condition :

Column : Inertsil ODS-3 C18,(250 x 4.6 mm), 5 μ or any other equivalent column.

Flow rate : 1.5 ml/min

Wavelength : 254 nm

Injection volume : 20 μ l

Mobile phase: Methanol : Water (70:30)

5.1.2 Preparation of benzene solution (1) :

Pipette out 0.5 ml of benzene and transfer to 50 ml volumetric flask, add 30 ml methanol, sonicate for 5 mins and dilute to 50 ml with methanol. Further diluted 5 ml to 50 ml with methanol (0.1%)



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5.1.3 Preparation of benzene and toluene solution (2):

Pipette out 0.5 ml of benzene and 0.5 ml of toluene and transfer to 50 ml volumetric flask, add 30 ml methanol, sonicate for 5 mins and dilute to 50 ml with methanol. Further diluted 5 ml to 50 ml with methanol (0.1%).

5.1.4 Separately inject 20 µl of solution 1 (5 injections) and solution 2 (1 injection) (0.1%).

5.1.5 Manual calculation as per USP :

5.1.5.1 Tangent (N) = $16 (t/W)^2$ or $N = 5.54 (t/W_{h/2})^2$

The number of theoretical plates, N is the measure of column efficiency. Column efficiency is measure of peak sharpness which is important for the detection of trace compounds. Where 't' is the retention time of the substance and 'W' is the width of the peak at its base, obtained by extrapolating the relatively straight sides of the peak to the baseline. $W_{h/2}$ is the peak width at half height.

5.1.5.2 Tailing factor (T) : = $W_{0.05}/2f$

The tailing factor, T is a measure of peak symmetry is unity for perfectly symmetrical peaks and its value increases as its value becomes more pronounced. As peak symmetry increases, integration and hence precision becomes less reliable.

t = retention time measured from time of injection to time of elution of peak maximum.

W = peak width at 5% of peak height.

f = distance from the peak maximum to the leaving edge of the peak, the distance having measured at a point 5% of the peak at from the baseline.

5.1.5.3 Resolution (R): The separation of two compounds in a mixture of resolution 'R' is determined by the equation.

$$R = 2 (t_2 - t_1) / (W_2 + W_1)$$

In which t_2 and t_1 are the retention times of the two compounds.

W_2 and W_1 are the corresponding widths at the base of the peaks obtained by extrapolating the relatively straight side of the peaks to the baseline.

5.1.5.4 Relative standard deviation in percentage :

$$R = \frac{100}{\bar{X}} \left(\frac{\sum_{i=1}^n (x_i - \bar{X})^2}{N - 1} \right)^{1/2}$$

R = Relative Standard Deviation in %

\bar{X} = Arithmetic mean of all observations.



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x_i = one observation
 N = number of observations.

5.2 System Suitability Parameters Calculation:

- 5.2.1 Select the data or chromatogram from the instrument software, which is to be validated.
- 5.2.2 Obtain the printout of system suitability parameters (Tangent, Tailing factor and Resolution) calculated by the instrument software.
- 5.2.3 Calculate the selected parameters manually.
- 5.2.4 Compare the results calculated by the instrument software with that calculated manually.
- 5.2.5 Acceptance Criteria : Results obtained by software and manual calculations for system suitability parameters should not be more than $\pm 5.0\%$

5.3 % RSD(Relative Standard Deviation) Calculation

- 5.3.1 Take printout of replicate injection
- 5.3.2 Calculate the same % RSD manually.
- 5.3.3 Compare the results obtained by software with manual calculation.
- 5.3.4 Acceptance Criteria: Results obtained through the software and manual calculations should be same.

5.4 Frequency

- 5.4.1 Validate the instrument software once in a year.
- 5.4.2 Revalidate the instrument software after every major breakdown, software up-gradation and in case of software failure.



PHARMA DEVILS
QUALITY CONTROL DEPARTMENT

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6.0 ABBREVIATION(S):

QCD – Quality Control Department

HPLC – High Performance Liquid Chromatography

7.0 REFERENCE(S):

NA

8.0 ANNEXURE(S):

Annexure-1 : Software validation of HPLC

9.0 REVISION CARD:

S.No.	Revision No.	Revision Date	Details of Revision	Reasons of revision



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ANNEXURE I

Software Validation of HPLC

AIM: To validate the instrument to check the system suitability parameters and ascertain whether the instrument gives reproducible results.

DATA PRESENTATION:

Parameters	Observation		% Variation with respect to Instrument value
	Instrument value	Manually calculated value	
Theoretical Plates			
Tailing factor			
Resolution			
Relative standard deviation			

RESULTS: The acceptance criteria for instrument values and manually calculated values is Achieved/Not Achieved.

Initiated By:	Checked By:	Approved By:
Date:	Date:	Date: