PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Quality Control	**SOP No.:**
Title: Handling of HPLC Column	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

1.0 OBJECTIVE:

To lay down a procedure for Receipt, Issuance, Usage, Storage, Performance, Regeneration, and Destruction of HPLC column in the Quality Control laboratory.

2.0 SCOPE:

This procedure is applicable to Receipt, issuance, usage, Storage, Performance, Regeneration and destruction of HPLC column in the Quality Control laboratory.

3.0 RESPONSIBILITY:

Officer, Executive – Quality control

Head – Quality Control

4.0 PROCEDURE:

4.1 Receipt of HPLC Column:

4.1.1 On receipt of new HPLC column, check the pack of column physically as per indent and check the performance report of the manufacturer.

4.1.2 Record the date of receipt, Name of Column, Batch/Sr. No, Cat. No, dimensions, Make/ Suppliers and column destruction date in "Column Inward Record" as per Annexure-I.

4.1.3 The manufacturer's column performance certificate shall be filed by Responsible QC Officer and retained for future reference

4.2 Issuance of HPLC Column

4.2.1 Record the name of column, Column ID. No., Product Name/ Raw material, Test, Date of issuance and Issued by in "Column issuance record" at the time of Column issue as per Annexure-II

4.2.2 Assign HPLC column ID number

LCC/XXX/YY

Where,

LCC : Liquid Chromatography Column

XXX : Serial number

/ : Slash

YY : Year (i.e. 15 for the year 2015)

4.3 Usage of HPLC Column

4.3.1 Record for usage of column in the respective column usage logbook as per Annexure-III.

4.3.2 In case the product analysis requires the same column for two different tests at a time, then additional column shall be issued and used for carrying out the analysis.

4.3.3 For the same products having multiple columns, analyst shall use the column based on the FIFO (First In First Out).

4.3.4 Only If the system suitability of the column is meeting the system suitability requirement for the test performed, then the analysis shall be continued. Otherwise the column regeneration has to follow as per 4.6 or 4.7 depends upon type of chromatography otherwise new column has to issue

4.4 Performance of HPLC Column

4.4.1 At the time of first analysis GLP Person has to ensure to receive the System suitability chromatogram from analyst, fill System suitability criteria on the Annexure-V as per respective STP and attach the chromatogram with manufacturer's column performance certificate.

4.4.2 Accept the column only if all the system suitability parameter are within limits.

4.5 Storage of HPLC column:

4.5.1 **For reverse phase column:** After washing, store the column in the solvent (acetonitrile or methanol as applicable) or as per the manufacturer's instructions.

4.5.2 **For normal phase column:** Hexane or IPA.

If required regenerate the column when system suitability is not achieved.

Note: For column other than those mentioned in the SOP, follow the manufacturer instructions for storage of columns

4.6 Column Regeneration (For reverse phase)

4.6.1 Before starting the regeneration, clean the frits by using sonication in hot water for about 30 minutes.

4.6.2 Rinse the column with about 40 ml of hot distilled water (55°C), simultaneously inject the 4 x 100 µl of dimethyl sulfoxide solution.

4.6.3 Rinse the column with 40 ml methanol followed by 40 ml chloroform simultaneously.

4.6.4 Finally, rinse with 40 ml methanol. After completion of regeneration, check the column system suitability as per 4.6.5.

4.6.5 For Reverse phase column

4.6.5.1 **Chromatographic condition:**

Flow rate : 1.0ml/min.

Wavelength : 254nm

Injection volume : 20 µl

4.6.5.2 **Mobile phase:** Take accurately 700 ml of acetonitrile and 300ml of water HPLC grade mix well and filter through 0.45 µ filter. Degas the mobile phase for 4 minutes.

4.6.5.3 **Test mixture preparation:** Take about 70 ml of mobile phase in a 100ml volumetric flask, add accurately about 1ml of Benzene and 1ml of toluene, mix well and make up the volume with mobile phase. Dilute 5ml of above solution to 50ml with mobile phase.

4.6.5.4 Make single injection of blank and five injections of test mixture preparation and record the response as per given in Annexure-IV.

4.6.5.5 **Acceptance criteria:**

Repeatability of RT and Area: RSD NMT 2.0% of five replicates.

Number of theoretical plates: NLT 2000 for Benzene and toluene.

Peak Asymmetry: NMT 2.0 for Benzene and toluene.

Resolution between two peaks: NLT 2.0

4.6.6 Accept the column only if above parameters are within limits and store the column in the mobile phase recommended by the manufacturer.

4.6.7 If above parameters are not complying, inform to In charge of Quality control and reject the column.

4.6.8 Before starting analysis, wash the column for approximately 30 minutes with mixture of 50: 50 Water and Methanol or Acetonitrile, for reverse phase chromatography and use only methanol for normal phase chromatography.

4.6.9 After analysis, if buffer is used in Mobile phase, wash the column initially with water for about 30 minutes followed by 50 % solution of methanol or Acetonitrile, for about 30 minutes. If no buffer is used in mobile phase, directly wash the column with 50 % solution of methanol or acetonitrile about 30 minutes at flow of 1.0 ml /min.

4.7 Column Regeneration (For Normal Phase)

4.7.1 Before starting the regeneration, clean the frits by using sonication in IPA for about 30 minutes.

4.7.2 Flush the column with 50 ml of Chloroform, 40 ml IPA, 30 ml of methylene chloride and 25 ml of mobile phase.

4.7.3 After completion of regeneration, check the column system suitability as per 4.7.4

4.7.4 For Normal Phase column and for Cyano, Silica etc.

4.7.4.1 **Chromatographic condition:**

Flow rate : 1.0ml/min.

Wavelength : 254nm

Injection volume : 10.0 µl

4.7.4.2 **Mobile phase:**

Take accurately 850 ml of Iso Octane and 150 ml of Ethanol mix well and filter through 0.45 µ filter. Degas the mobile phase.

4.7.4.3 **Test preparation:**

Take 0.1 ml of Nitrobenzene & 1.0 ml of Toluene in a 100 ml volumetric flask and make up the volume with mobile phase. Dilute 5 ml of above solution to 50 ml with mobile phase.

4.7.4.4 **Acceptance criteria:**

Repeatability of RT and Area: RSD NMT 2.0% of five replicates Injections. theoretical plates: NLT 2000 for Nitrobenzene and toluene.

Peak Asymmetry: NMT 2.0 for Nitrobenzene and toluene.

Resolution between two peaks: NLT 2.0

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4.8 Destruction of HPLC Column:

4.8.1 After regeneration if column system suitability is not within the limits, discard the column and enter details in Annexure-I.

4.8.2 Bend the column up to 90° and discard in to scrap area.

5.0 ANNEXURE(S):

Annexure –I : Column Inward Record.

Annexure –II : Column Issuance record.

Annexure –III : Column Usage Record.

Annexure –IV : Column Performance Report (Regeneration).

Annexure –V : Column Performance Report

6.0 REFERENCE (S):

USP <621> / BP Appendix III / Ph. Eur. 2.2.46/IP 2.4.13 & 2.4.14

SOP: Preparation, approval, distribution, control, revision and destruction of Standard Operating Procedure (SOP).

7.0 ABBREVIATION (S)/ DEFINITION (S):

HPLC: High performance liquid chromatography

µl : micro liter

NMT : Not more than

NLT : Not less than

RT : Retention Time

USP – United States Pharmacopoeia

BP – British Pharmacopoeia

Ph. Eur. – European Pharmacopoeia

IP – Indian Pharmacopoeia

STP- Standard Test procedure

° - Degree

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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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ANNEXURE I
COLUMN INWARD RECORD

S.No.	Date of Receipt	Name of Column	Batch No. / Sr. No	Cat. No.	Column Dimension/ Material	Make/ Suppliers	Received by	Destruction date	Destructed by	Remarks

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ANNEXURE II
COLUMN ISSUANCE RECORD

S.No.	Name of column	Column ID. No.	Name of Product/Raw material	Test	Date of Issuance	Issued by Sign

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ANNEXURE III
COLUMN USAGE RECORD

Name of Column:			**Column Id. No.:**		**Dimension:**	
S.No.	**Date Of Analysis**	**Name of product**	**No. of Injection**	**Cumulative Injection**	**Analyst**	**Remarks**

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ANNEXURE IV

Name of Column		**Column ID. No.**	
Name of the Mfg.		**Supplier Name**	
Date of Receipt		**Date of Analysis**	
Use for Product			

1. **Column Efficiency Report:**

   Column Type:

   Chromatographic conditions:

Parameter	Actual
Wave Length	
Flow Rate	
Injection Volume	
Temp.	

Mobile Phase:

Test mixture:

Observation: For

Chromatogram	Standard RT Response	Standard Area Response	Asymmetry Factor	Theoretical Plate
1				
2				
3				
4				
5				
Mean				
% RSD				
Limits				

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Observation: For

Chromatogram	Standard RT Response	Standard Area Response	Asymmetry Factor	Theoretical Plate	Resolution
1					
2					
3					
4					
5					
Mean					
% RSD					
Limits					

1. **Limit For Reverse Phase Column:**
   Repeatability of RT and Area: RSD NMT 2.0% of five replicates

   Number of theoretical plates: NLT 2000 for Benzene and toluene.

   Peak Asymmetry: NMT 2.0 for Benzene and toluene.

   Resolution between two peaks: NLT 2.0

2. **Limit For Normal Phase Column:**
   Repeatability of RT and Area: RSD NMT 2.0% of five replicates

   Number of theoretical plates: NLT 2000 for Nitrobenzene and toluene.

   Peak Asymmetry: NMT 2.0 for Nitrobenzene and toluene.

   Resolution between two peaks: NLT 2.0

3. **For other columns as per Mfg.**

Conclusion: The column is satisfactory/ Not satisfactory for analytical use.

Analysed By:
Date:

Checked By:
Date:

Approved By:
Date:

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ANNEXURE V
COLUMN PERFORMANCE REPORT

Observation: For

System Suitability Criteria	Observations	Limits
Theoretical Plate		
Tailing Factor		
Resolution		
% RSD		

Conclusion: The column is satisfactory/ Not satisfactory for analytical use.

Analyzed By:
Date:

Checked By:
Date:

Approved By:
Date: