PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Quality Control	**SOP No.:**
Title: Procedure for management of HPLC columns	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

1.0 OBJECTIVE:

To lay down procedure for management of HPLC column.

2.0 SCOPE:

This SOP is applicable to instrument lab.

3.0 RESPONSIBILITY – Execution- Executive QC
Checking -Assistant Manager QC

4.0 ACCOUNTABILITY - Manager Quality Control

5.0 PROCEDURE:

5.1 Receipt of new Column:

5.1.1 On receipt of the column, analyst shall verify the details like batch no./column serial no. in certificate and on column.

5.1.2 Analyst shall allocate the column no. as per following procedure :
As HCXXX
where:
H = HPLC
C = Column
XXX = Serial no. starting from 001.
For example : The first column received in the QC lab shall be numbered as HC001

5.1.3 Analyst shall write the allocated column no. on the certificate and put initial and date . File the certificate in respective file.

5.1.4 After the allocation of column no. analyst shall make entry of column details for "Column No"., "Column Type" (C8, C18, Silica etc….), "Make"(Hypersil,waters, Merck etc….), "Column Serial No."(Available on column or in Certificate), "date of Purchase", "Reference Page No. of column usage log" and "Dedicated for"(Name of product) in "Column Inventory /Index".(Annexure-I)

5.1.5 Analyst shall write the required details "Column type ", "Particle size", "Dimension," "Column No." and "For " (Name of the product for which column is dedicated)on the column label and affix it on the column.(Annexure- II)

5.1.6 Analyst shall make entry of column details like "Column No." "Column Type" , "Make" and "Dedicated for" in "Column Usage Log" first page and "Column No." on remaining pages (Annexure-III)

5.2 Usage of the Columns:

5.2.1 After the satisfactory of column performance check, Executive QC or designee shall issue a dedicated column for analysis . Enter the details like,"Date in use"into "column inventory

/index" (Annexure-I)

5.2.2 In case a different column (i.e. Other than dedicated make) is required to use , take authorization (In remarks column of "Column Inventory / Index") of head QC.

5.2.3 Before starting analysis , wash the column (other than the silica column) with water for 30 mins. And saturate for 30 mins (Or saturate more if required) with mobile phase at a required flow rate.

5.2.4 Column shall first wash with the required solvent (Recommended by manufacturer) at low to high flow rate. (Example : 0.2 ml/min for 15 mins., 0.5ml/min for 15 mins and 1.0ml/min for 30 mins.

5.2.5 Make necessary entries on every usage of the column in respective Column usage log for "Date", "Product/ Sample", "B.No. /A.R. No., and "No of Inj." "Cumulative Injections", "Usage Time" (Start time)and End time of column usage), "Washing Time" (Start time and End Time of column washing), :washed with" (Name of solvent/reagent), "Sign" (Analyst initial) and " Remarks" (If column performance is found as per the requirement than use "Satisfactory". If column performance is not found as per the requirement mention Specific reason (Like: Theoretical plates or Tailing factor or resolution are not compling) (Annexure-III)

5.2.6 After completion of analysis wash the column with water: ACN (Acetonitrial) (80:20) (Filtered through 0.45µ /0.2µ nylon filter for about 1 $^{1/2}$ hrs. or more depends on the concentration of buffer used in the mobile phase . (Column may be stored in different solvent if recommended by manufacturer)

5.2.7 For Silica column, before starting analysis wash the column with Isopropyl alcohal (HPLC grade)for about 30mins and saturate for about 30mins with mobile phase at a required flow rate .

5.2.8 After completion of analysis wash the Silica column with Isopropyl alcohal-HPLC for about 1 $^{1/2}$ hrs. and finally with methanol for about 30mins.

5.2.9 After completion of analysis and washing disconnect the column , fix column ends tightly and keep the column at designated place.

5.3 Column Efficiency Check and column regeneration

5.3.1 Any new column first Analyst shall check the column performance using respective "HPLCCOLUMN EFFICIENCY CHECK LOG"(Annexure-IV,V) prior to use.

5.3.2 For any specific make column where in –house method of column efficiency does not give satisfactory results or do not have in- house method, consider manufacturer's column efficiency report or use manufacturer methodology for checking of efficiency and take the Authorisation of Head-QC for the usage of the same .Check the system suitability parameters

by analyzing the product, for which column is dedicated . If system suitability parameters found satisfactory , dedicate the column for that product .If system suitability parameters are not found satisfactory ,reject the column for that particular product. Attach the system suitability raw data of product along with the manufacturer's column efficiency report.

5.3.3 After the completion of column performance check analyst shall give "HPLC COLUMN EFFICIENCY CHECK LOG" along with the raw data for checking to designated person.

5.3.4 If column performance found satisfactory then column shall be issued for the analysis by Executive QC or designee and put initial in column of "Date in use" in "Column Inventory /Index". In case of column performance not found satisfactory then follow step 5.3.2 for the analysis.

5.3.5 After every 600±100 cumulative injection .Analyst shall check column efficiency and check the system suitability parameters against the criteria. In case above no. of Injection are exceed during the analysis , then perform efficiency check after the completion of analysis

5.3.6 Analyst shall regenerate the column using "HPLC COLUMN REGENERATION LOG" Column regeneration shall be done with manufacturer method or instruction for specific column.

5.3.7 If the system suitability criteria of column for that dedicate product found unsatisfactory , then do the column regeneration and after regeneration check System suitability again for that dedicate product. System suitability criteria found to be satisfactory continue the usage for analysis . If , system suitability criteria are found again unsatisfactory then discontinue the column usage and discard the column after authorization of Head -QC or designee.

5.4 Discarding of the column:

5.4.1 Discard column , in case of non-compliance of system suitability parameters against the criteria for the dedicate product.

5.4.2 Head QC or designees shall make entry of "DISCARDED ON", "NO. OF CUMULATIVE INJECTIONS" AND "REASON FOR DISCARD" IN "COLUMN inventory / Index" also in "HPLC Column Usage Log" for "Remarks" column .

5.4.3 Discard the column in case a loss of packing material is found in the column and take the authorization of Head QC or designee.

5.4.4 Discard the column if it gives high backpressure even after cleaning the frits and regeneration; take the authorization of Head QC or designee.

6.0 SAFETY & PRECAUTIONS:

Not Applicable

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7.0 REVISION HISTORY:

Revision No.	Reason for Revision	Superseded from & Date

8.0 DISTRIBUTION:

Copy No.	Issuance Record				Withdrawal Record		Destruction Record	
	Date	Dept. issued	Name / Signature of receiver	Issued By Name / Signature	By	Sign/ Date	By	Sign/ Date

9.0 REFERENCES:

Not Applicable

10.0 ABBREVIATIONS & ANNEXURES:

SOP : Standard Operating Procedure

QC : Quality Control

No. : Number

HPLC : High Performance Liquid Chromatography.

Annexure –I : Column Inventory /Index

Annexure –II : Column Utilization Register

Annexure –III : Column Identification Tag/Label

Annexure –IV: Column Inventory /Index

Annexure –V: HPLC Column Regeneration Protocol

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ANNEXURE –I

COLUMN INVENTORY /INDEX

Column No.	Column Type	Make	Column Serial No.	Date of Purchase	Ref. Page No. of Column Usage Log	Dedicated For	Date in Use	Discarded on

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ANNEXURE –II
COLUMN UTILIZATION REGISTER

S.No.	Date	Product name	B.No.	Test Performed	Running Time	No. of injections	Cumulative No. of injections	Analyzed by	Theoretical plates

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ANNEXURE –III
COLUMN IDENTIFICATION TAG / LABEL

Type: _____

Particle Size: _____µm

Dimension: _____

Column No.: _____

For: _____

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ANNEXURE –IV
HPLC-COLUMN EFFICIENCY CHECK PROTOCOL

HPLC-Column Efficiency Check Protocol

Column Type:		**Column No.:**	
Column Make:		**Column Dimension:**	
No. of Cumulative Injections:			
Reason for Efficiency Check:			
Mobile phase preparation:			
Test mixture preparation:			
Procedure:			
		Test Condition	**Applied Condition**
1.0	**HPLC Set up**		
1.1	Flow Rate		
1.2	Wave Length		
1.3	Injection Volume		
2.0	**Integration Parameter**		
2.1	Width		
2.2	Threshold		
2.3	Other (If any)		
3.0	**System Suitability for Toluene Peak**		
3.1	**Theoretical Plates**		
3.2	**Tailing Factor**		
Conclusion: The Column is Satisfactory / Not Satisfactory for the Analytical use.			
Remarks:			
Analyst: **Date:**		**Checked by:** **Date:**	**Approved by:** **Date:**

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ANNEXURE –V
HPLC COLUMN REGENERATION PROTOCOL

Report No.:

Column Type: _____(MOS / C18 / C8 / BDS CPS (Cyano) / NH2 / PHENYL)

Column No.	Make	Dimensions

Reason for Regeneration: (Ref.: Column Efficiency Check Report No.)

Remark: Where frits are removable Remove the frits of the column. Rinse the frits with water and sonicate for 15 min. in 6M Nitric Acid. Discard the Nitric acid and rinse the frits with water. Assemble the frits to the column.

Procedure:

Attach the column to be regenerate to the HPLC pump. Keep outlet of the column directed towards the waste. Set the pump to the desired flow rate. Keep the inlet in the hot water at about 55°C through suction filter, start the pump and wash the column with hot water for specified time and while washing inject 4 x 100 µl of DMSO (Dimethyl sulphoxide). On completion of water washing. Change the mobile phase solvent as specified in the following table and wash the column with the solvent only in the sequence given in the table. Start the pump and wash the column with the specified solvents for specified time. Note the time of each washing. On completion of the regeneration procedure, perform Column efficiency check.

Solvent		Flow Rate		Time		Remark
Test Condition	Applied Condition	Test Condition	Applied Condition	Test Condition	Applied Condition	
Water at 55°C		1.0 ml		50 min.		With DMSO Injection
Methanol		1.0 ml		50 min.		
Chloroform		1.0 ml		50 min.		
Methanol		1.0 ml		50 min.		

Remark:

Analyst: **Checked By:** **Approved By:**
Date: **Date:** **Date:**

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ANNEXURE –V
HPLC COLUMN REGENERATION PROTOCOL

Column Type: SILICA

Column No.	Make	Dimensions

Reason for Regeneration: (Ref.: Column Efficiency Check Report No.)

Remark: Where frits are removable, remove the frits of the column. Rinse with water and sonicate for 15 min. in 6M Nitric Acid. Discard the Nitric acid and rinse the frits with water. Assemble the frits to the column.

Procedure:

Attach the column to be regenerated to the HPLC pump. Keep outlet of the column directed towards the waste. Set the pump to the desired flow rate. Keep the inlet in the Hexane through suction filter, start the pump and wash the column. Change the mobile phase solvent as specified in the following table and wash the column with the solvent only in the sequence given in the table. Start the pump and wash the column with the specified solvents for specified time. Note the time of each washing. On completion of the regeneration procedure, perform Column efficiency check.

Solvent		Flow Rate		Time	
Test Condition	**Applied Condition**	**Test Condition**	**Applied Condition**	**Test Condition**	**Applied Condition**
Hexane		1.0 ml		50 min.	
Methylene Chloride		1.0 ml		40 min.	
Isopropyl Alcohol		1.0 ml		40 min.	
Methyl Chloride		1.0 ml		30 min.	
Hexane		1.0 ml		30 min.	

Remark:

Analyst: **Checked By:** **Approved By:**
Date: **Date:** **Date:**