

	<b>Standard Operating Procedure</b> <b>HPLC Column Tracking and Maintenance</b>		<b>SOP Number</b> <b>D-814</b>	<b>Revision</b> <b>7</b>
			<b>Effective Date</b> 10/14/22	<b>Page</b> <b>Page 1 of 4</b>
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## 1.0 Purpose

The purpose of this procedure is to describe the process of maintaining, washing, storing, and tracking the lifecycle of the column to help ensure quality and compliance of HPLC methods.

## 2.0 Scope

This procedure is limited to the maintenance, storage, washing, and tracking the lifecycle of HPLC columns in the QC Laboratory.

## 3.0 Responsibility

3.1 It is the responsibility of QC Laboratory Management to align this SOP with current practices and compliance requirements and to ensure that the procedure is followed.

3.2 It is the responsibility of QC Laboratory personnel to follow this procedure.

## 4.0 Definitions

4.1 **HPLC** – High Performance Liquid Chromatography

4.2 **QC** – Quality Control

4.3 **CofA** – Certificate of Analysis

## 5.0 References

None

## 6.0 Procedure

6.1 Any specific column care instructions given in an analyte specific test method supersede any instructions given in this procedure.

6.2 Receipt of New Column

6.2.1 The HPLC Column will be labeled with the following format: CLM-D-XXX-YYY. Where “XXX” will be the method number and “YYY” will be the

sequential number of the column for that method starting with 101. Example: CLM-D-728-101 is the first tracked column for method D-728.

- 6.2.2 The label may also contain the compound being analyzed by that method as well as the date the column was received.
- 6.2.3 The column will then be placed into the dedicated column storage unit in numerical order.
- 6.2.4 If the column is to be used for a USP monograph that does not have an in-house method number, it will be labeled with the following format: CLM-USP-(compound name)-YYY. Example: CLM-USP-Valerian Root-101.
- 6.2.5 A column inventory spreadsheet will document column information including column number, manufacturer, type, dimensions, method(s) the column is used for, received date, and removed from service date.

### 6.3 Cleaning and Storage of Reverse Phase Columns

- 6.3.1 Column performance will degrade over time for a variety of reasons including injection of “dirty” samples, low or high mobile phase pH, high column temperature, and accumulation of particulates from samples or mobile phase. In some cases, the column performance may be restored using a washing procedure.
- 6.3.2 For methods using an isocratic mobile phase program, especially reverse phase methods where the organic content of the mobile phase is low, a column wash after each testing sequence is appropriate.
- 6.3.3 For methods using a gradient elution mobile phase program with a wash step, the column can often be used for multiple runs without extra column washing.
- 6.3.4 Washing procedure for moderately contaminated reverse phase columns
  - 6.3.4.1 For washing, use an appropriate flow rate for the column. This will typically be 1.0 mL/min for a 4.6 mm i.d. column or 0.25 mL/min for a 2.1 mm i.d. column. If in doubt, consult the test method used in the previous sequence.

- 6.3.4.2 For purging, open the purge valve and purge each channel at 5 mL/min for 5 min.
- 6.3.4.3 Purge mobile phase channels A and B with H<sub>2</sub>O/methanol (90/10).
- 6.3.4.4 Wash the column with H<sub>2</sub>O/methanol (90/10) for at least 15 minutes.
- 6.3.4.5 Purge mobile phase channel B with 100% acetonitrile.
- 6.3.4.6 Wash the column with acetonitrile for at least 30 minutes.
- 6.3.4.7 Purge mobile phase channel B with H<sub>2</sub>O/acetonitrile (50/50).
- 6.3.4.8 Wash the column with H<sub>2</sub>O/acetonitrile (50/50) for at least 15 minutes.
- 6.3.5 Washing procedure for severely contaminated columns. The following is a procedure of last resort that may restore column performance, but can also cause damage.
  - 6.3.5.1 As discussed above, use appropriate flow rates for washing and purging steps.
  - 6.3.5.2 The procedure involves flushing the column in the reverse direction (back-flushing). When performing the steps, care should be taken to slowly increase or decrease the flow rate to the desired set point. When back-flushing a column, sudden changes in flow/pressure could cause the column packing to shift resulting in channeling and irreversible damage.
  - 6.3.5.3 Install the column in the reverse direction.
  - 6.3.5.4 Purge mobile phase channels A and B with H<sub>2</sub>O/methanol (90/10).
  - 6.3.5.5 Wash the column with H<sub>2</sub>O/methanol (90/10) for at least 15 minutes.
  - 6.3.5.6 Next, purge mobile phase channel B with 100% isopropanol.
  - 6.3.5.7 Wash the column with isopropanol for at least 60 minutes (use one half the normal flow rate because isopropanol has high viscosity).

- 6.3.5.8 Finally, purge mobile phase channel B with H<sub>2</sub>O/acetonitrile (50/50).
- 6.3.5.9 Wash the column with H<sub>2</sub>O/acetonitrile (50/50).
- 6.3.6 Column storage for reverse phase columns
  - 6.3.6.1 Store reverse phase columns with H<sub>2</sub>O/acetonitrile (50/50).
- 6.3.7 Column care for other column types (normal phase, HILIC, etc.)
  - 6.3.7.1 Consult the analyte specific test method for instruction.
- 6.3.8 Retirement
  - 6.3.8.1 Columns that fail to meet the system suitability requirements may be washed or backwashed using conditions safe for the column matrix to remove contaminants bound to matrix to improve performance.
  - 6.3.8.2 Columns that repeatedly fail system suitability requirements, even after being washed or back-washed are disposed and labelled as out of service in the column inventory spreadsheet.

## 7.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	05/06/10	New	-	-
1	01/24/12	Updated SOP format	-	-
2	02/26/13	Added maintenance and cleaning requirements; eliminated daily use of log; changed SOP title.	13-125	B. Johns
3	12/02/14	Updated SOP format; created a lifecycle log in an electronic format; clarified cleaning and storage procedures.	14-0945	B. Johns
4	02/24/17	Biennial review: updated SOP format; removed physical log.	16-1137	J. Maignan
5	08/23/21	Triennial review; minor SOP updates.	CC-21-0097	M. Hamilton
6	09/07/22	Edit column wash and storage instructions for clarity, add comment about column inventory spreadsheet.	CC-22-0367	S. Sassman