HPLC Column Protection

Column Cleaning Procedure

General Information

- Flow rates should be 1/5 - 1/2 of the typical flow rate
- To estimate the column volume, use the following equation:

$V=\pi r^2 L$

V = column volume in mL
r = column radius in cm
L = column length in cm

UNBONDED SILICA COLUMNS (Si)

Rinse with 10 column volumes each of:

- Hexane
- Methylene Chloride
- Isopropanol
- Methylene Chloride
- Mobile Phase

Water Removal: Flush column with 30 mL 2.5 % 2,2-dimethoxy propane and 2.5 % glacial acetic acid in hexane.

REVERSED PHASE COLUMNS (C18, C12, C8, C5, C4, C2, C1, PHENYL, PFP, CN, NH2)

- Rinse with 10 column volumes each of:
- 95 % Water/5 % Acetonitrile (for buffer removal)
- THF
- 95 % Acetonitrile/5 % Water
- Mobile Phase

REVERSED PHASE PROTEIN/ PEPTIDE COLUMNS (C18, C12, C8, C5, C4, Phenyl)

Rinse with 20 column volumes of mobile phase with buffer removed.
Run gradient (2x):
(A) 0.1 % aqueous TFA in water
(B) 0.1 % TFA in Acetonitrile/Isopropanol (1:2)
25 % B to 100 % B for 30 minutes
Equilibrate with 10 column volumes of mobile phase. Do not store column in TFA

GFC/SEC COLUMNS FOR PROTEINS (Yarra SEC, BIOSEP-SEC-S)

Rinse with 5 column volumes of:

- 0.1 M Phosphate buffer pH 3.0.
- For strongly retained proteins:
- Run 100 % Water to 100 % Acetonitrile to 100 % Water over 60 minutes OR wash with 5 column volumes of SDS or 6 M Guanidine Thiocyanate or 10 % DMSO.

Do not backflush columns!

BONDED NORMAL PHASE COLUMNS (CN, NH2, DIOL, PAC)

Rinse with 10 column volumes each of:

- Chloroform
- Isopropanol
- Methylene Chloride
- Mobile Phase

Exception: Recommended for cleaning ***Luna Amino*** when used in *reversed phase mode*:

- Wash with at least 30 column volumes of Sodium Hydroxide pH 11.0
- Flush with at least 30 column volumes of water (HPLC grade)
- Re-equilibrate to mobile phase conditions.

ION-EXCHANGE COLUMNS (SAX, SCX, NH2, WAX, WCX)

Rinse with 10 column volumes each of:

- 500 mM Phosphate Buffer pH 7
- 10 % Acetic Acid (Aq)
- 5 column volumes of Water
- 10 column volumes of Phosphate Buffer pH 7
- 5 column volumes of Water
- 10 column volumes of Methanol
- 10 column volumes of Water

For protein removal, follow the above procedure with this exception: Substitute 10 column volumes of Methanol with 10 column volumes of 5M Urea or 5 M Guanidine Thiocyanate.

HILIC

Rinse with 10 column volumes each of:

- 95 % Water/5 % Acetonitrile (for buffer removal)
- 95 % 100 mM Ammonium Acetate, pH 5.8 / 5 % Acetonitrile
- 95 % Water/5 % Acetonitrile
- Mobile Phase

HPLC columns running water-free, flammable organic solvents (e.g., normal phase, chiral, GPC) can generate static electricity and should be properly grounded to avoid a potentially dangerous electrical discharge.

Download the FREE HPLC Trouble-Shooting Guide at:

www.phenomenex.com/hplcts

If you have any other questions, please contact your local Phenomenex representative below:

Phenomenex products are available worldwide

www.Phenomenex.com
international@phenomenex.com

Authorized distributor in Indonesia:

sbn
PT. SURYA BUANA NUSANTARA

Jl. Budi Mulia No. 5 11/5 Gunung Sahari – Ancol
Jakarta 14420 – Indonesia
P.O. Box 4548 / JKP10045
Tel: 62-21-91214227 ; 62-21-640 6225 ; 68082179
Fax: 62-21-6458467
Email: sales@suryabuana.com

HPLC Column Protection (Continued)

Storing The Column

Column storage conditions affect column lifetime
Never store columns containing buffers or ion-pairing reagents
Flush with at least five column volumes of mobile phase without buffer to remove any buffers or salts

Storage Conditions for Silica-Based HPLC Columns

Column Type	Storage Solvent
Reversed Phase (RP) C18, C12, C8, C4, C2, C1, Phenyl, PFP	65% Acetonitrile/ 35% Water
Normal Phase (NP) Silica, CN, NH_2, PAC, Diol, Alumina	Isopropanol or Hexane
Ion-Exchange SAX, SCX, WAX, WCX	Methanol*
Size Exclusion Diol	0.05% NaN_3 in Water or 10% Methanol
HILIC Luna HILIC	80% Acetonitrile/ 20% Water

*Flush column with 50 mL HPLC grade water prior to storage solvent

Mobile Phase Considerations

- Use only HPLC grade solvents
- Use only highest purity chemicals and reagents
- Degas and filter all mobile phases prior to use
- Make sure solvents are miscible (see Table p.389 from 2014 Catalog www.phenomenex.com/catalog)
- Always check sample solubility
- If possible, use the mobile phase as the diluent (sample solvent)

Stationary Phase Considerations

- Maintain pH between 2.0 and 8.0**
- Use guard columns
- Avoid aldehydes and ketones with amino columns

***Consult Phenomenex for columns that have extended pH ranges.*

Backpressure and Flow Rates

Keep backpressures below 3500 psi (245 bar), unless otherwise specified
Avoid any sudden pressure and flow rate changes
If high backpressure is observed reverse flush the column (Check column care guide before proceeding)
Use a backpressure regulator if you are experiencing out-gassing problems in the detector cell Columns can be operated at any flow rate that is consistent with the backpressure limitations described below. Flow rates should be optimized to provide the highest efficiency for your sample.

Typical Column Flow Rates & Backpressures *(RP)*

*column length

Particle Size (µm)	Internal Diameter(mm)	Typical Flow Rate (mL/min)	Typical Pressure (psi) 150 mm*	250 mm*
1.7	2.1	0.3	6700	NA
2.6	2.1	0.2	6400	NA
2.6	3.0	0.8	5500	NA
2.6	4.6	1.85	5000	NA
3	2.0	0.2	1500	2400
3	3.0	0.3	1500	2400
3	4.6	0.75	1500	2300
5	2.0	0.2	650	1000
5	3.0	0.5	900	1400
5	4.6	1.0	850	1200
10	10.0	5.0	900	1000
Axia Luna	21.2	20.0	350	500

Sample Loading

Amounts of Sample That Can Be Separated

Column Type	ID (mm)	Approx. Dead Volume (mL)*	Typical Flow Rate (mL)	Typical and (Max.) Injection Masses (mg)	Typical and (Max.) Injection Volumes (µL)**
Capillary (Fused Silica)	0.32	0.0075	0.001 - 0.02	0.001 (0.01)	1 (10)
Microbore	1.0	0.07	0.02 - 0.1	0.01 (0.1)	5 (25)
Analytical	4.6	1.5	0.5 - 2.0	0.1 (2.5)	10 (200)
Semi-Prep	10.0	7.3	5.0 - 20	1.0 (25)	50 (1000)
Preparative	20.0	29.2	10 - 200	5.0 (500)	200 (5000)

*The column Dead Volume (Vo) may be estimated from:
Column Dead Volume (mL) = $Vo = 0.487 \times d^2 \times L$
Where: L = column length (cm); 15 cm (150 mm) used for calculation.
d = column ID (cm, not mm)

**The maximum allowable Sample Injection Volume (Vi) can be estimated as follows: Maximum Injection Volume $= Vi = \frac{Vr}{2\sqrt{N}}$

Where: Vr = the retention volume of the first peak (mL)
N = number of theoretical plates per column

Access the HPLC/UHPLC Column Care Guide

www.phenomenex.com/Account/Dashboard/Guides

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www.Phenomenex.com
international@phenomenex.com

Authorized distributor in Indonesia:

PT. SURYA BUANA NUSANTARA
Jl. Budi Mulia No. 5 11/5 Gunung Sahari – Ancol
Jakarta 14420 – Indonesia
P.O. Box 4548 / JKP10045
Tel: 62-21-91214227 ; 62-21-640 6225 ; 68082179
Fax: 62-21-6458467
Email: sales@suryabuana.com