

COLUMN OPERATION AND STORAGE

Good column care requires little additional time and will extend the lifetime of your SIELC HPLC columns. The following are general recommendations. More detailed information is available at www.sielc.com.

Mobile Phases for Column Operation and Storage **General Guidelines**

1. **TRY TO AVOID USING ALCOHOLS** with Primesep (A, 100, 200, C, B2, AB and P), Promix (SP and AP), and Obelisc R columns. Alcohols can esterify carboxylic acids attached to the surface of silica, significantly affecting reproducibility and retention time of some analytes.
2. Do not use or store SIELC HPLC columns in pH outside of recommended ranges. SIELC columns require buffered or acidified mobile phases to be present in the column at all times (Table 1).
3. Do not store SIELC HPLC columns in mobile phase containing modifiers that degrade easily, such as triethylamine (TEA) tetrahydrofuran (THF) and trifluoroacetic acid (TFA). TEA and TFA are more likely to become contaminated when stored at ambient temperatures, and should be refrigerated during long storage. Contamination may change the chromatography or change the column. Use HPLC-grade solvents and additives for your chromatography.
4. To achieve reproducible results, please use a column thermostat. Operating temperature for all SIELC columns is 0-50°C.
5. To achieve reproducible results, always equilibrate SIELC column prior to the injection. In some cases replacement of the mobile phase inside the column does not make the column sufficiently equilibrated. SIELC columns' stationary phase retain components of the mobile phase as counter ions. To equilibrate the column in this case requires a replacement of all the ions adsorbed in the column stationary phase. Typically, 50 mM buffer would equilibrate a column in 1 hour. If only the concentration of the buffer changed, then 2-4 volumes of the column are sufficient to reach an equilibration state.
6. It is important to operate columns below the specified maximum pressure limits (5000 psi). If you exceed maximum pressure the column can be destroyed. Leaking column is an indication of excessive pressure usage.
7. Limit the use of tetrahydrofuran to 10% with SIELC columns.
8. An arrow on the column label is used to specify direction of flow through the column. Typically you should operate the column in the direction marked. If column need to be washed in opposite direction use reduced flow rate in order to not create pressure which can disturb packing.
9. Use only your fingers to tighten PEEK fittings to the column connection.

Table 1. Operational pH ranges for SIELC Columns

Column packing	pH Limits/ Recommended Operating Range	Short Term Storage	Long Term Storage	Alcohol in mobile phase
Primesep A, Primesep 100, Primesep 200, Primesep C, Primesep P, Primesep AB, Primesep B2, Obelisc R, Promix SP, Promix AP	1.5-7.0/ 2.0-6.5	Buffered or acidified mobile phases within recommended pH range	ACN/water/formic or acetic acid=75/25/0.1 ACN/water/acetic acid	Not allowed
Primesep B, Primesep D, Primesep B4, Primesep SB, Promix MP, Primesep N, Primesep PB, Primesep S, Obelisc N	1.5-5.0/ 2.0-4.5	Mobile phases within recommended pH range	ACN/water/formic acid or acetic acid=75/25/0.1	Allowed
PEI	1.5-5.0	Mobile phase	Mobile phase	Allowed
SHARC	No water	Mobile phase	Mobile phase	Allowed
Newcrom	1.5-7	Mobile phase	ACN/water/formic acid or acetic acid=75/25/0.1	Allowed

Other Recommendation

Perform the column performance test using conditions described in Certificate of Analysis and compare with the result in the report. Due to the variations in particular LC systems you may observe slightly different results.