

PIRKLE-TYPE COLUMNS

Care and Use Guide

Column Quality Report

Every Regis column is tested to ensure performance and ships with a quality report. Column-specific information, such as material lot number, column serial number, a test chromatogram with operating conditions, and performance results are included with each report.

Recommended Conditions for Column Use

Columns are shipped containing the storage solvent listed on the column's quality report. Care should be taken to ensure solvent compatibility before switching mobile phase systems. An arrow on the label indicates the recommended direction of flow. Typical flow rates for normal-phase HPLC operation, as a function of column i.d. and particle size, are listed in Table 1.

Column i.d.	Typical Flow Rate					
	1.8 μm	3.5 μm	5 μm	10 μm	16 μm	20 μm
2.1 mm	0.9	0.5	0.3	0.2	N/A	N/A
3 mm	1.8	0.9	N/A	0.3	N/A	N/A
4.6 mm	4.2	2.1	1.5	0.8	0.5	0.4
10 mm	N/A	N/A	7.1	3.5	N/A	N/A
21.1 mm	N/A	N/A	32	16	N/A	N/A
30 mm	N/A	N/A	64	32	N/A	N/A
50 mm	N/A	N/A	177	88	N/A	N/A

Table 1. Typical normal-phase HPLC flow rate as a function of column i.d. and particle size.

Typical flow rate also depends on the mode of operation and mobile phase viscosity. When operating in SFC mode, flow rates can be 2 – 3x higher than those listed in Table 1. In reversed-phase HPLC, flow rates are typically 3x lower than those listed in Table 1.

Column lifetime will vary depending on operating conditions. For example, high pressure operation at high pH (>7.5) and elevated temperature (>60° C) will lead to shorter lifetime. Recommended operating ranges for pH, temperature, pressure, and buffer concentration are described below.

pH Range

The recommended pH range for silica-based pirkle-type columns is 2.5 to 7.5. Low pH (<2) can lead to hydrolysis of the bonded phase, while high pH (>8) can lead to silica dissolution.

Operating Temperature

Chiral selectivity is often enhanced at lower operating temperatures. The recommended range is 20 - 30° C. Do not exceed the upper temperature limit of 60° C.

Pressure

Pirkle-type columns equal to or smaller than 21.1 mm i.d. are stable at pressures up to 6,000 psi. Columns larger than 21.1mm i.d. can tolerate 3,000 psi. Extended use at elevated pressures may shorten column lifetime.

Modifier

When mobile phase modifiers are used, the column should be flushed thoroughly with the same mobile phase, without the modifier present, before flushing the column with storage solvent. When acidic or basic modifiers, such as trifluoroacetic acid (TFA) or N,N-diethylamine (DEA), are used as mobile phase modifiers, it is satisfactory to leave this mobile phase in the column overnight. However, if the column will not be used for several days it is recommended that the system be flushed with mobile phase that does not contain modifiers so that the column is not damaged.

Buffer Concentration

Aqueous buffers are commonly used as a mobile phase component when using columns in the reversed-phase mode. When a buffer solution has been used, it is imperative to flush the column with the identical mobile phase, without the buffering salt present, before converting the column to the recommended storage solvent.

Column Storage

Do not allow water to remain in these or any silica-based columns. Remove any buffers by washing with 20 column volumes of water and then flush the column with at least 20 column volumes of compatible organic solvent before leaving the column overnight.

If used only under normal phase conditions, the column may be stored in that mobile phase. If an acidic or basic modifier is present, flush the column with a pure organic solvent prior to storage.

The column is to be kept wet during storage. Each column is shipped with two removable end plugs to prevent the drying of the column bed. Save these plugs and reinstall them whenever the column is removed from the HPLC system.

Troubleshooting


Increased backpressure, changes in peak shape, and/or shifting retention times may indicate the need to perform column maintenance.

Pirkle-type Column Selection

α -Burke 2
 β -Gem 1
DACH-DNB

Leucine
Phenylglycine
Pirkle 1-J

ULMO
Whelko-O 1
Whelko-O 2



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