



# **Column Certificate of Analysis**

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 (mL/min)			
	3 µm	5 µm	10 µm	20 µm
2.1 mm	0.9	0.3	0.2	0.1
3 mm	1.8	0.6	0.3	0.2
4.6 mm	4.0	1.5	0.8	0.4
10 mm	20	7.0	3.5	2
21.1 mm	88	32	16	8
30 mm	178	64	32	16
50 mm	490	177	88	45

 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. When using aqueous-based, reversed-phase solvents, flow rates are typically 3x lower than those listed in Table 1.

Column lifetime will vary depending on operating conditions. For example, extended use at the upper range of recommended limits will lead to shorter lifetime. Recommended operating ranges for pH, temperature, and pressure are listed on the back of this card along with compatible solvents (mobile phase and sample diluent) and modifiers.

# pH Range

The recommended pH range for Reflect coated polysaccharide columns is 3.5 – 6.5.

#### **Operating Temperature**

Chiral selectivity is often enhanced at lower operating temperatures. The recommended range is  $20 - 30^{\circ}$  C. Do not exceed the upper temperature limit of  $40^{\circ}$  C.

#### Pressure

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

## Mobile Phases and Sample Solvents

Avoid mobile phases and sample solvents that may dissolve the coated polysaccharide phase, such as acetone, chloroform, dichloromethane, DMF, DMSO, ethyl acetate, THF, etc. Make sure that flow paths are clear of these solvent prior to use.

## Modifiers

When using acidic (e.g. TFA, acetic acid, formic acid, etc.) or basic (e.g. DEA, butylamine, enanolamine, etc.) modifiers, common concentration levels are 0.1%. It is recommended not to exceed 0.5% modifier concentration.

## **Column Storage**

Columns should be cleared of modifier before storage. Flush the column with mobile phase that does not contain modifier, using a higher ratio of strong solvent. For example, if alkane/alcohol solvent is used, a higher ratio of alcohol should be used to flush the column. For longterm storage, the shipping solvent is recommended.

## Troubleshooting

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



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