

# Create Your Own Column Chemistry

## Chromolith® WP 300 Epoxy 2 mm I.D. HPLC Columns

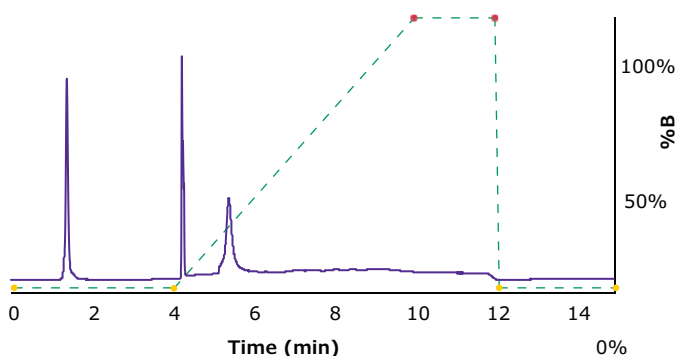
Chromolith® WP 300 Epoxy columns are specially designed for the user-specific immobilization of ligands and their later application in HPLC. The unique, bimodal pore structure of silica monoliths allows efficient coupling independent of molecule size. The wider mesopores also enable the use of proteins and antibodies as both ligand immobilized on the column, and later analyte separated by an immobilized column. Potential applications: attach trypsin to obtain HPLC column-protein digestion reactor; attach protein and measure other protein interactions with the attached one; attach any chiral selector to obtain a chiral column, attach any affinity ligand to obtain custom made affinity column, etc. With the 2 mm I.D. column geometry, improved efficiency, sensitivity, and MS-compatibility can be realized.

### Immobilization of iminodiacetic acid

- According to Epoxy method
- Chromolith® WP 300 Epoxy 100-4.6 mm column
- 1 g iminodiacetic acid dissolved in 25 mL 50 mM Disodium hydrogen phosphate + 1.9 M Ammonium sulfate pH 8.0
- Immobilization for 72 hours at 0.2 mL/min
- No quenching of remaining epoxide functions
- Column was flushed with Copper sulfate solution before separation

### Immobilization of iminodiacetic acid – Affinity chromatography

<b>Eluent A</b>	20 mM sodium phosphate + 100 mM sodium chloride pH 7.0
<b>Eluent B</b>	Eluent A + 200 mM imidazole
<b>Flow rate</b>	1.0 mL/min
<b>Detection</b>	280 nm
<b>Temperature</b>	25 °C
<b>Injection volume</b>	20 µL



### Key Benefits:

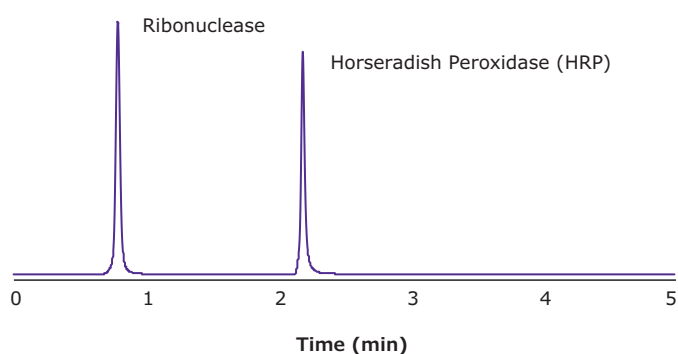
- Easy to perform immobilization of ligand opens up limitless customization options
- Monolithic format allows for high flow rates at low backpressure, enabling high throughput
- Narrow column I.D. enables improved performance and improved MS-compatibility

### Immobilization of concanavalin A

- According to Epoxy method
- Chromolith® WP 300 Epoxy 100-2 mm column
- 50 mg concanavalin A from Jack bean dissolved in 25 mL 50 mM Disodium hydrogen phosphate, 1 mM + 1.9 M Ammonium sulfate pH 8.0
- Immobilization for 4 hours at 0.2 mL/min
- Quenching of remaining epoxide functions with glycine

### Immobilization of concanavalin A – Affinity chromatography

<b>Eluent A</b>	50 mM sodium acetate, 200 mM sodium chloride, 1 mM calcium chloride pH 5.3		
<b>Eluent B</b>	Eluent A + 100 mM Methyl- $\alpha$ -D-mannopyranoside		
<b>Flow rate</b>	2.0 mL/min		
<b>Detection</b>	214 nm		
<b>Temperature</b>	25 °C		
<b>Injection volume</b>	5 µL		
<b>Gradient</b>	<b>Time</b>	<b>%A</b>	<b>%B</b>
	0	100	0
	1	100	0
	1.25	0	100
	3.5	0	100
	3.6	100	0
	5	100	0

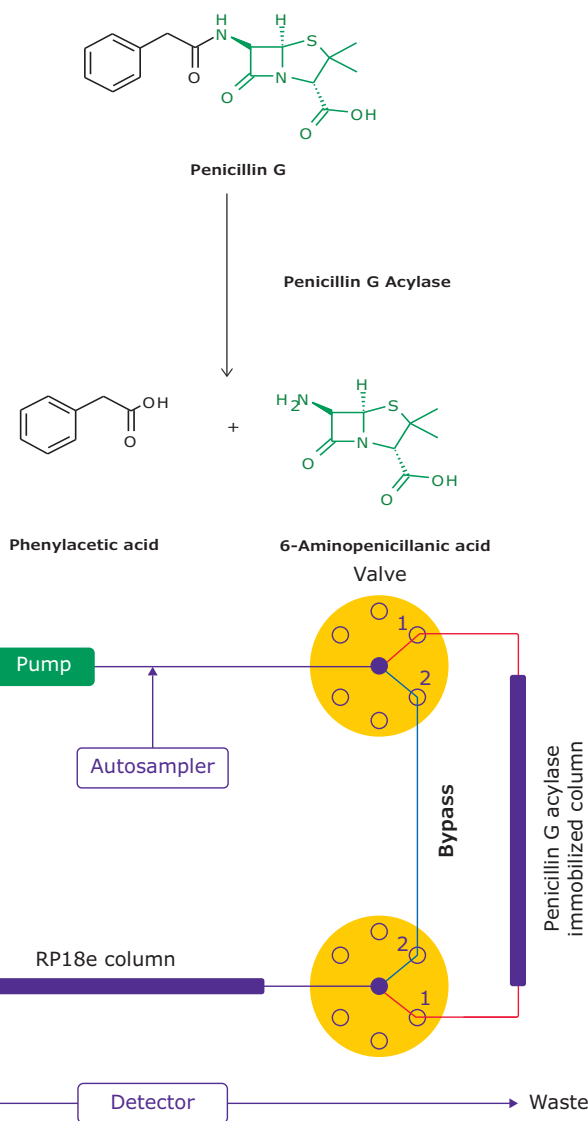
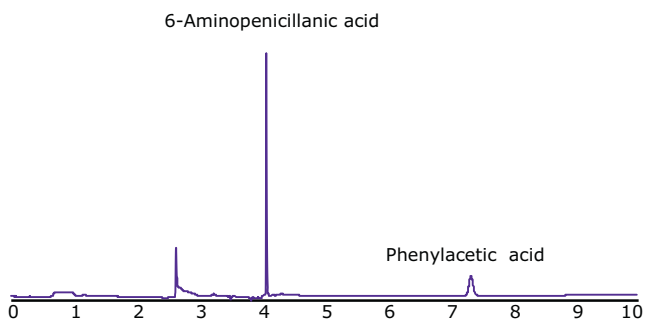


## Immobilization of penicillin acylase

- According to Epoxy method
- Chromolith® WP 300 Epoxy 100-2 mm column
- 80 mg penicillin acylase dissolved in 25 mL 50 mM sodium phosphate + 1.9 M ammonium sulfate, pH 8.0
- Immobilization for 24 hours at 0.2 mL/min
- Quenching of remaining epoxide groups with glycine

### Immobilization of penicillin acylase – Enzymatic bioreactor

<b>Eluent A</b>	10 mM sodium phosphate pH 7.0
<b>Eluent B</b>	10 mM sodium phosphate pH 3.0
<b>Eluent C</b>	Acetonitrile
<b>Flow rate</b>	1.0 mL/min
<b>Temperature</b>	23 °C
<b>Detection</b>	UV 225 nm
<b>Sample</b>	1.0 µL Penicillin G (3.5 mg/mL)
<b>Gradient</b>	
	<b>Time</b> <b>Valve</b> <b>A</b> <b>B</b> <b>C</b>
	0            1            100    0        0
	2            1            100    0        0
	2            2            0        80      20
	4            2            0        80      20
	9            2            0        50      50
	9.5        2            0        50      50
	9.6        2            0        80      20
	15         2            0        80      20



## Ordering Information

Part Number	Description	Length (mm)	I.D. (mm)
1.52350.0001	Chromolith® WP 300 Epoxy Column	100	2.0
1.52351.0001	Chromolith® WP 300 Epoxy Column	50	2.0
1.52352.0001	Chromolith® WP 300 Epoxy Column	25	2.0
1.52353.0001	Chromolith® WP 300 Epoxy Guard Column (3 units)	5	2.0

## To place an order or receive technical assistance

Order/Customer Service: [SigmaAldrich.com/order](https://www.sigmaaldrich.com/order)  
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