Supelco®
Analytical Products

MilliporeSigma

Create Your Own Column Chemistry

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

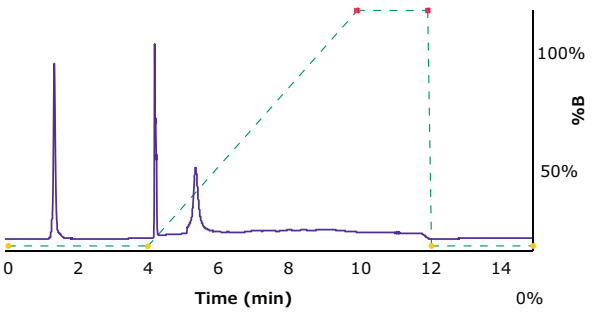
Chromolith® WP 300 Epoxy columns are specially designed for the user-specific immobilization of ligands and their later application in HPLC. The unique, bimodal pore structure of silica monoliths allows efficient coupling independent of molecule size. The wider mesopores also enable the use of proteins 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 imidodiacetic 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 imidodiacetic acid– Affinity chromatography

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

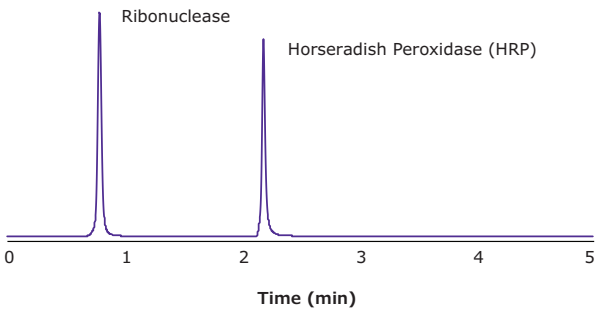
- According to Epoxy method
- Chromolith® WP 300 Epoxy 100-2 mm column
- 50 mg concanavalin A from Jack bean dissolved in 25 mL 50 mM Disodium hydrogen phosphate, 1 mM + 1.9 M Ammonium sulfate pH 8.0
- Immobilization for 4 hours at 0.2 mL/min
- Quenching of remaining epoxide functions with glycine

Immobilization of concanavalin A – Affinity chromatography

Eluent A	50 mM sodium acetate, 200 mM sodium chloride, 1 mM calcium chloride pH 5.3
Eluent B	Eluent A + 100 mM Methyl-α-D-mannopyranoside
Flow rate	2.0 mL/min
Detection	214 nm
Temperature	25 °C
Injection volume	5 µL

Gradient

Time	%A	%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

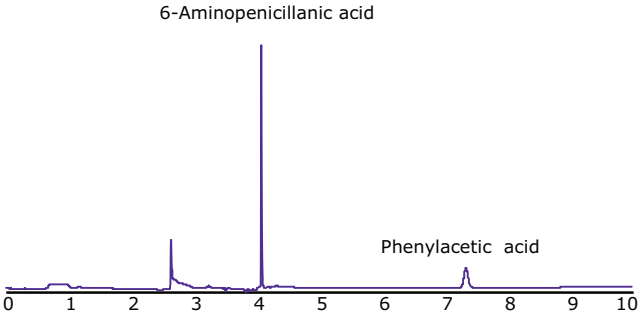
- According to Epoxy method
- Chromolith® WP 300 Epoxy 100-2 mm column
- 80 mg penicillin acylase dissolved in 25 mL 50 mM sodium phosphate + 1.9 M ammonium sulfate, pH 8.0
- Immobilization for 24 hours at 0.2 mL/min
- Quenching of remaining epoxide groups with glycine

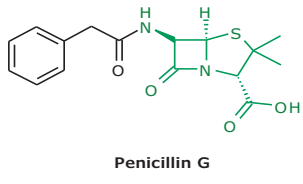
Immobilization of penicillin acylase – Enzymatic bioreactor

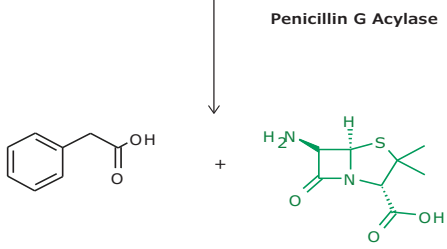
Eluent A	10 mM sodium phosphate pH 7.0
Eluent B	10 mM sodium phosphate pH 3.0
Eluent C	Acetonitrile
Flow rate	1.0 mL/min
Temperature	23 °C
Detection	UV 225 nm
Sample	1.0 µL Penicillin G (3.5 mg/mL)

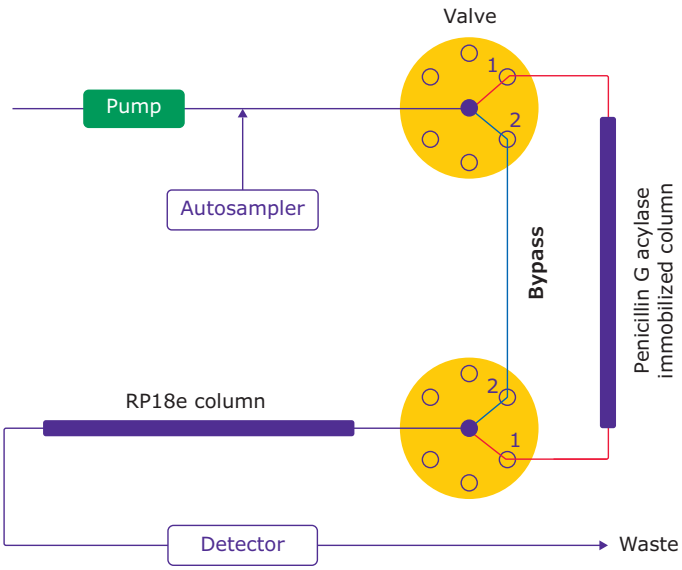
Gradient

Time	Valve	A	B	C
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

Cat. No.	Description	Length (mm)	I.D. (mm)
11-010-272	Chromolith® WP 300 Epoxy Column	100	2.0
11-010-273	Chromolith® WP 300 Epoxy Column	50	2.0
11-010-274	Chromolith® WP 300 Epoxy Column	25	2.0
11-010-275	Chromolith® WP 300 Epoxy Guard Column (3 units)	5	2.0

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