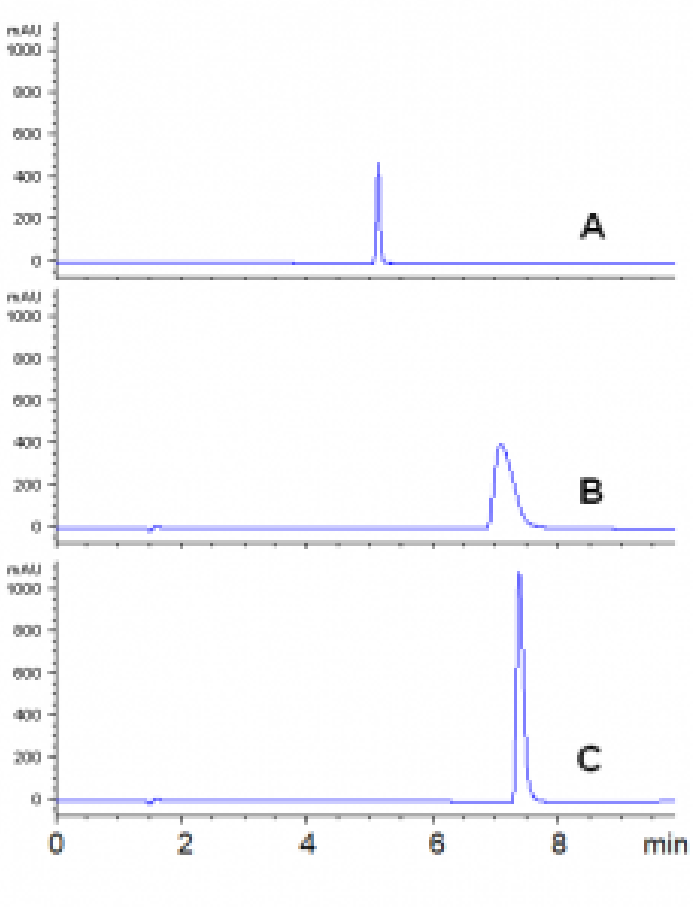
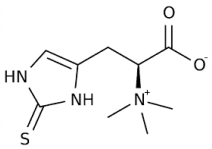
Changing column ID means changing your gradient – Tips & Suggestions

Gradient changes with column ID change.

An example is shown below with ergothioneine being analyzed using 4.6×100 mm column and ANP gradient method using UV detection. When the column dimensions were changed from a 4.6mm x 100mm to 2.1mm x100 mm, it may seem the column ID have made the peak efficiency worse.

Column ID has effects on peak height response, back pressure, and elution time. One must keep these variables in mind during column dimension changes. Below is a demonstration of how an adjustment of gradient conditions were necessary to obtain good peak symmetry for this compound.





Peak:

Ergothioneine

Chromatogram A: column A, gradient 1, flow rate: 1 mL / minute

Chromatogram B: column B, gradient 1, flow rate: 0.2 mL / minute

Chromatogram C: column B, gradient 2, flow rate: 0.2 mL / minute

MicroSolv Technology Corporation

9158 Industrial Blvd. NE, Leland, NC 28451

tel. (732) 380-8900, fax (910) 769-9435

Email: customers@mtc-usa.com

Website: www.mtc-usa.com

Method Conditions

Column: Cogent Diamond Hydride™, 4µm, 100Å

Catalog No.: Column A: 70000-10P Column B: 70000-10P-2

Dimensions: Column A: 4.6×100 mm Column B: 2.1x100mm

Mobile Phase:

A: DI water with 0.1% formic acid (v/v)

B: acetonitrile with 0.1% formic acid (v/v)

Gradient:

Gradient 1		Gradient 2	
time (*minutes*)	%B	time (*minutes*)	%B
0	90	0	90
5	50	4	30
7	50	6	30
8	90	7	90

Flow rate: A: 1.0mL / minute, Column B: 0.2 mL / minute

Detection: UV @ 254nm

Injection vol.: 1µL

Sample Preparation: 0.1 mg / mL in 90% DI water / 10% acetonitrile with 0.1% formic acid

Note: Ergothioneine is a naturally occurring amino acid and is a thiourea derivative of histidine, containing a sulfur atom on the imidazole ring. Ergothioneine is found mainly in mushrooms, as well as red and black beans.

MicroSolv Technology Corporation

9158 Industrial Blvd. NE, Leland, NC 28451

tel. (732) 380-8900, fax (910) 769-9435

Email: customers@mtc-usa.com

Website: www.mtc-usa.com