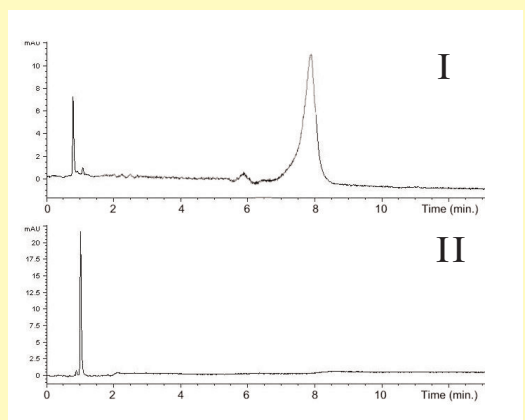
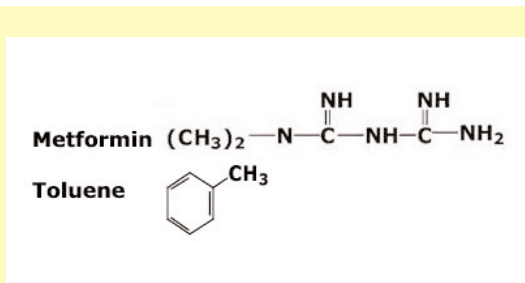


Using an Inverse Gradient Technique to Determine Best HPLC Mode.

Analysis of Metformin



Note: Due to the gradient conditions you might observe fronting of the peak for the basic polar compound as in Figure I. This is expected. When using an MS as a detector a higher percentage of formic acid can be used than with UV. For UV detection a higher percent of acid will cause the distortion of the baseline due to the difference in refraction indices between solvent A and solvent B in gradient analysis.

Method Conditions

Column: Cogent Bidentate C18™, 4µm, 100A
Catalog No.: 40018-75P
Dimensions: 4.6 x 75 mm
Mobile phase: A= DI water + 0.1% formic acid
 B= Acetonitrile

Inverse Gradient

Time (min.)	%A	%B
0.00	0.00	100.00
0.00 – 15.00	50.00	50.00
15.00 – 20.00	50.00	50.00
20.00 – 20.01	0.00	100.00
20.01 – 25.00	0.00	100.00

Flow rate: 1.0 mL/min.
Injection Volume: 1 µL
Sample: I. Metformin 1mg/mL in DI water
 II. Toluene 1 µL/mL in acetonitrile
Detection: UV 254 nm

Discussion

A simple Inverse (ANP) gradient was used to Determine the most useful HPLC Mode for:

- I – Basic, polar compound - Metformin
- II – Neutral compound – Toluene

Gradients can be used to quickly determine if a compound will be retained in the Aqueous Normal Phase (ANP) mode. From the gradient profile, one can predict what the retention for a basic polar compound will be in an isocratic mode on the same column. For example, with Metformin, using 80% acetonitrile and 0.1% formic acid, the retention time is 2.5 minutes ($t_0 = 0.7$ minutes) when using the same column and flow rate as in the ANP Gradient. This compound is well retained in ANP. When the gradient is used for neutral compounds they remain in the dead volume; unretained in ANP (see Figure II).

For more information visit www.MTC-USA.com

Cat. No.	Description
40018-75P	Cogent Bidentate C18 HPLC Column, 100Å, 4µm, 4.6 x 75 mm, Standard End Fittings.