

# Fast Gradient Analysis and Fast Re-Equilibration Using LC/MS Columns

Application  
Technical  
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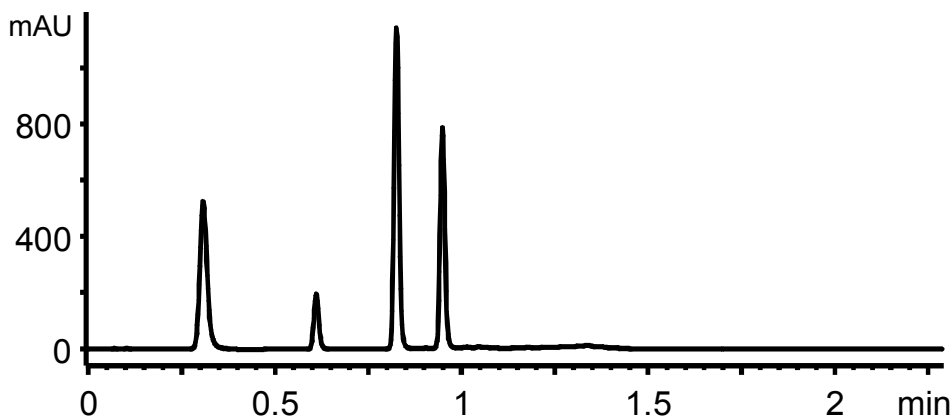
Using shorter columns with smaller particles (3.5 vs. 5  $\mu\text{m}$ ) is a popular way to reduce analysis time since it does not change relative retention ( $k'$ ) and selectivity ( $\alpha$ ) of peaks. Gradients are another popular way to speed up analyses. Gradient methods work with low-volume columns, offering good chromatography and fast equilibration time. Below, four compounds are resolved in less than one minute, using a 0 to 100% gradient. Because the column volume is small and the flow rate is high, re-equilibration takes only 45 seconds:

$$(0.3 \text{ ml column volume}) \times (10 \text{ column-volumes flush of starting eluent}) / (4 \text{ ml/min.}) = .75 \text{ min or 45 seconds.}$$

Thanks to state-of-the-art LC/MS cartridge columns, this separation can be repeated in less than two minutes.

## Highlights

- Fast gradient methods utilizing ZORBAX 3.5  $\mu\text{m}$  Rapid-Resolution LC/MS columns are ideal for clinical screening, combinatorial chemistry, LC/MS, and LC/MS/MS.
- Low-volume cartridge columns are as rugged and reproducible as traditional size columns.
- Narrow peak widths of only 0.02 to 0.05 min. are common when using ZORBAX 3.5  $\mu\text{m}$  Rapid-Resolution LC/MS columns in gradient mode.



Conditions: LC: Agilent 1100  
Column: Eclipse XDB-C18, 4.6 x 30mm (3.5 $\mu\text{m}$ ), Agilent P/N: 933975-902  
Gradient: 0 - 100% B / 1 min.  
A= 50 mL H<sub>2</sub>O + 450 mL MeOH + 2 mL H<sub>3</sub>PO<sub>4</sub>  
B= 450 mL H<sub>2</sub>O + 50 mL MeOH + 2 mL H<sub>3</sub>PO<sub>4</sub>  
Sample: ~3 mg/mL each compound x 5  $\mu\text{L}$  = 15  $\mu\text{g}$   
UV: 268 nm; Flow: 4.0 mL / min.; 23°C



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