

# Effect of Mobile Phase Preparation on Chromatography

Application  
Technical  
Robert Ricker

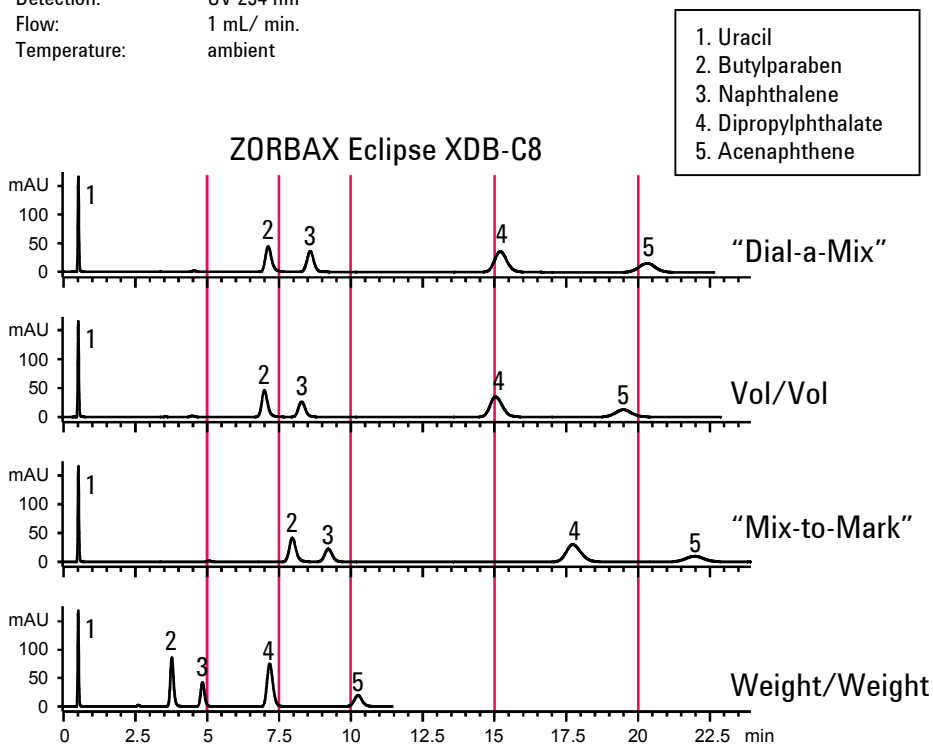
The contraction of volume when mixing methanol and water is well known. Note differences in retention, resulting from how a "50:50" MeOH: water mobile phase is prepared and pumped through an Agilent LC system. Uracil is unretained and is not affected by mobile-phase composition; it serves to indicate proper flow rate.

## Operating Conditions:

HPLC System: Agilent 1100 with quaternary pump  
Column: ZORBAX Eclipse XDB-C8 Rapid-Resolution (3.5 $\mu$ m), 4.6 x 50 mm  
Agilent Part No. 935967-906  
Mobile Phases: Dial-a-Mix= A: water B: MeOH, pump 50% B  
Vol/Vol=250 mL water + 250 mL MeOH, pump 100%  
Mix-to-Mark = 250 mL MeOH, fill to 500 mL with water, pump 100%  
Premixed (w/w) = 200 g MeOH + 200 g water, pump 100%  
Detection: UV 254 nm  
Flow: 1 mL/ min.  
Temperature: ambient

## Highlights

- Significant changes in elution pattern result from the method used for mobile-phase preparation.
- Volume and temperature should be carefully examined when considering reproducibility of mobile-phase preparation.
- When mixing mobile phase, w/w is more accurate than v/v because weight is independent of temperature.



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