

## Applications of Ultra Fast HPLC

### Abstract

This application note focuses on the high-speed analysis of xanthine derivatives and triarylmethane ink dyes with Ultra Fast HPLC.

### Introduction

The Shim-pack XR-ODS columns, which have 2.2 $\mu$ m particles and can reduce column flow resistance, result in considerably shorter analytical cycle times with systems comprised of conventional hardware, achieving performance similar to commercial sub-2 $\mu$ m columns.

As an example of Ultra-Fast LC, high-speed analyses of xanthine derivatives and triarylmethane dyes are introduced here using ultra fast HPLC with a Shim-pack XR-ODS column.

### Experimental Conditions

#### Xanthine derivatives

Instruments: Shimadzu Prominence UFLC  
Column: Shim-pack XR-ODS (3.0mm I.D. x 50 mmL, 2.2 $\mu$ m)  
Mobile Phase: A: 20mM (Sodium) phosphate buffer <pH=2.6>  
B: 20mM (Sodium) phosphate buffer <pH=2.6>/ Acetonitrile =70/30  
B concentration 10% (initial)  $\rightarrow$  85% (1.20min)  
Flow rate: 1.2 mL/min  
Column temp: 40°C  
Injection vol: 5  $\mu$ L  
Detection: 273nm

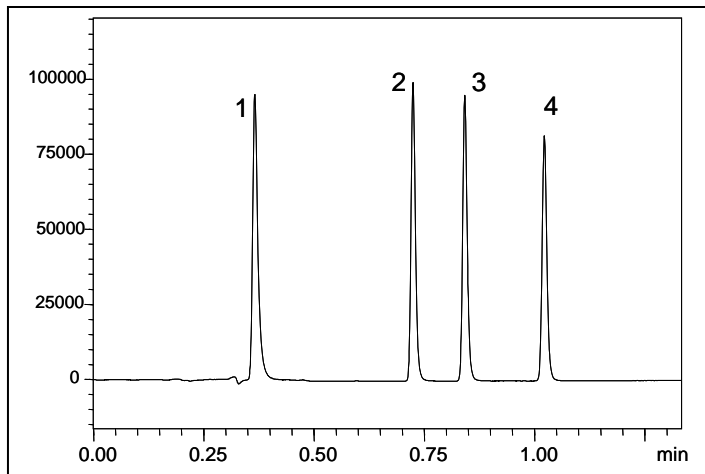
#### Triarylmethane dyes

Instruments: Shimadzu Prominence UFLC  
Column: Shim-pack XR-ODS (3.0mm I.D. x 50 mmL, 2.2 $\mu$ m)  
Mobile Phase: A: 20mM (Sodium) phosphate buffer <pH=2.6>  
B: Acetonitrile  
B concentration 30% (initial)  $\rightarrow$  85% (0.80min)  
Flow rate: 1.75 mL/min  
Column temp: 50°C  
Injection vol: 10  $\mu$ L  
Detection: UV 500-700nm (PDA detector)

### Results

Figures 1, 2 show chromatograms of xanthine derivatives and triarylmethane dyes, respectively. The analyses were performed in approximately 1 minute by using ultra-fast LC combined with a Shim-pack XR-ODS column. The retention time and peak area reproducibility were excellent as shown in Tables 1 and 2, even when using steep gradient conditions.

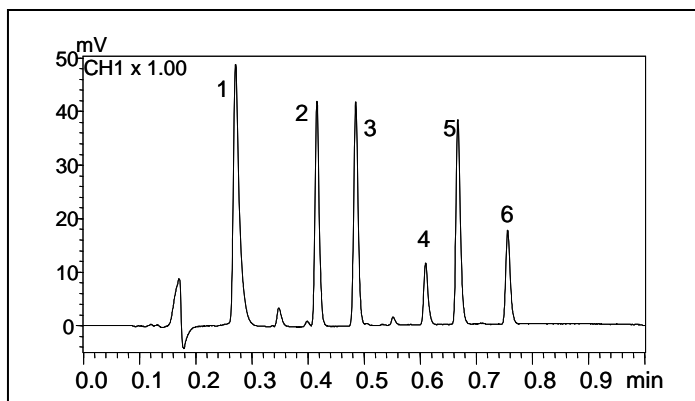




**Figure 1:** UFLC Chromatogram of Xanthine Derivatives (10ug/mL).  
 1.Xanthine, 2.Theobromine, 3.Theophylline, 4.Caffeine.

Compound	RT % RSD	Peak Area % RSD
Xanthine	0.200%	0.147%
Theobromine	0.106%	0.140%
Theophylline	0.094%	0.102%
Caffeine	0.078%	0.154%

**Table 1:** Retention Time and Peak Area Reproducibility of Xanthine Derivatives (n=10).



**Figure 2:** UFLC Chromatogram of Triarylmethane Dyes (1ug/mL).  
 1.Pararosaniline, 2.Patent Blue VF, 3.Patent Blue V, 4.Methyl Violet, 5.Crystal Violet, 6.Victoria Blue B.  
 \*Methyl Violet is an impurity of Crystal Violet.

Compound	RT % RSD	Peak Area % RSD	Wavelength
Pararosaniline	0.132%	0.157%	540nm
Patent Blue VF	0.106%	0.150%	635nm
Patent Blue V	0.113%	0.268%	635nm
Crystal Violet	0.087%	0.290%	590nm
Victoria Blue B	0.074%	0.178%	616nm

**Table 2:** Retention Time and Peak Area Reproducibility of Triarylmethane Dyes (n=6)

## Conclusions

Xanthine derivatives and triarylmethane dyes were successfully analyzed in approximately 1 minute by ultra-fast LC using a Shim-pack XR-ODS column without extremely high pressure, while maintaining high separation efficiency and system reproducibility performance.

