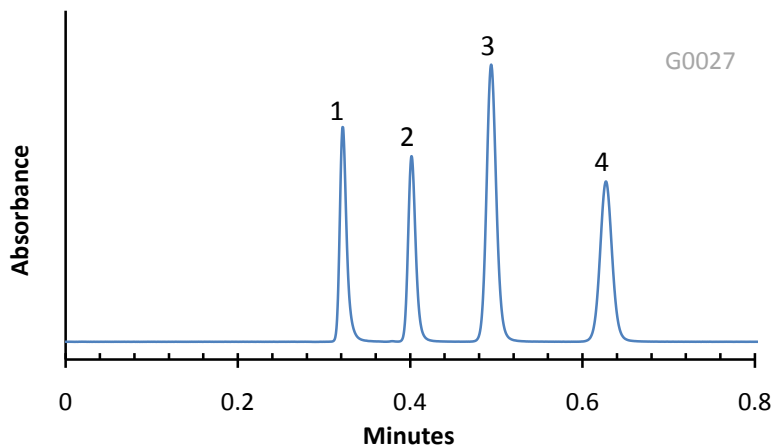


Application Note: 048-XA

## Separation of Xanthines on HALO RP-Amide Phase



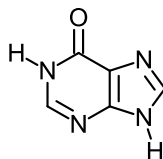
### PEAK IDENTITIES:

1. Hypoxanthine
2. Theobromine
3. Theophylline
4. Caffeine

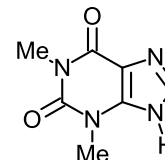
### TEST CONDITIONS:

Column: 4.6 x 50 mm, HALO RP-Amide  
Part Number: 92814-407  
Mobile Phase: 85/15: A/B  
A=0.03 M phosphate buffer, pH=3, in water  
B= Acetonitrile  
Flow Rate: 1.5 mL/min.  
Pressure: 150 Bar  
Temperature: 35° C  
Detection: UV 254 nm, VWD  
Injection Volume: 0.5 µL  
Sample Solvent: 30% Methanol in water  
Response Time: 0.02 sec.  
Flow Cell: 2.5 µL semi-micro  
LC System: Shimadzu Prominence UFLC XR  
ECV: ~14 µL

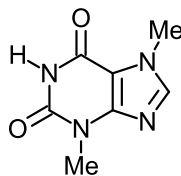
### STRUCTURES:



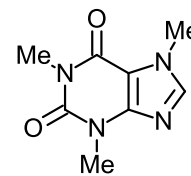
Hypoxanthine



Theophylline



Theobromine



Caffeine

These xanthines can be readily separated on a HALO RP-Amide column in less than one minute.