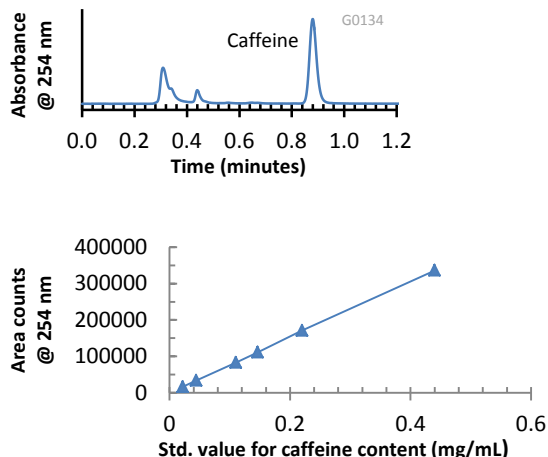


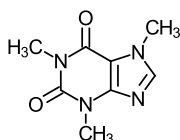
## Determination of Caffeine in Soda Using HALO 5 C18



### TEST CONDITIONS:

Column: HALO 5 C18, 3.0 x 50 mm, 5 µm with HALO 5 guard column  
 Part Number: HALO 5 C18, 95813-402  
 Part Number: Guard column: 95813-102  
 Mobile Phase: 75/25: A/B  
 A= 0.1% formic acid in water  
 B= Methanol  
 Flow Rate: 0.8 mL/min.  
 Pressure: 120 Bar  
 Temperature: 30°C  
 Injection Volume: 1.0 µL  
 Sample Solvent: (caffeine std.) mobile phase  
 Detection: UV 254 nm, VWD  
 Response Time: 0.02 sec.  
 Date rate: 25 Hz  
 Flow Cell: 2.5 µL semi-micro  
 LC System: Shimadzu Prominence UFLC XR  
 ECV: ~14 µL

### STRUCTURE:



Caffeine

### DISCUSSION:

A selection of sodas was purchased along with several energy drinks at a local grocery store. An approximate 10 mL sample from each soda was placed in a separate 20 mL scintillation vial and capped. The vials were shaken several times and then the cap was loosened and the vial sonicated for 10 minutes to remove CO<sub>2</sub>. Then a 1 mL aliquot was placed into a 1.5 mL HPLC sample vial. A one microliter quantity was injected into the HPLC under conditions tabulated elsewhere on this page. A guard column was used to prevent the buildup of a brown material on the analytical column packing. The material was likely caramel coloring.

The chromatogram shown is from a regular cola drink.

A standard curve of peak area vs. caffeine concentration was made over the range of 0.11-0.44 mg/mL. These values were used to calculate the line fit of  $Y=mX + b$ . From the peak response the concentration of (mg of caffeine)/mL was calculated and then multiplied by the number of mL in the beverage can (usually 355 mL). Results are shown below.

Sample	Caffeine tested mg/(355 mL)	Can value mg/(355 mL)
Store brand cola 1	12	N/A
Cola 2	53	54
Cola 3	43	43
Cola 4	36	38
Cola 5	38	38
Store brand diet cola 1	12	N/A
Diet cola 2	45	46
Diet cola 3	34	34
Diet cola 4	36	35
Energy drink 1*	160	160
Energy drink 2**	79	80
Diet Energy drink**	79	80
Non-cola drink 1	53.3	54
Non-cola drink 2	22	22
Diet non-cola drink	43	41
Diet cola 1 non caffeinated	0	N/A
Diet cola 2 non-caffeinated	0	N/A
Diet cola 3 non-caffeinated	0	N/A

355 mL = 12 oz.

\*amount in 16 oz. (473 mL) cans

\*\*amount in 8.4 oz (248 mL) cans