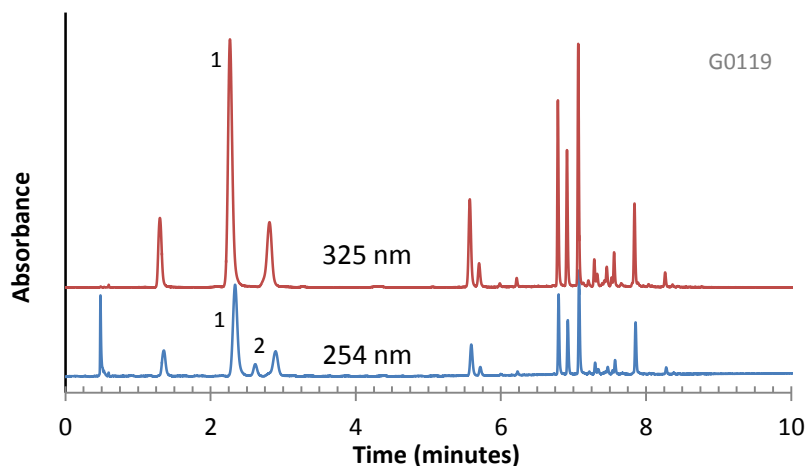


HPLC Analysis of Chlorogenic Acid in Green Coffee Extract on HALO C18, 2.7 µm



PEAK IDENTITIES:

1. Chlorogenic acid
2. Caffeine

TEST CONDITIONS:

Column: HALO C18, 3.0 x 100 mm, 2.7 µm

Part Number: 92813-602

Mobile Phase: A/B

A= Water + 0.1% formic acid

B= Acetonitrile + 0.1% formic acid

Gradient:

Time	%B
0.0	10
4.0	10
9.0	50
11.0	100
13.0	100

Flow Rate: 0.75 mL/min.

Pressure: 250 Bar (initial pressure)

Temperature: 30°C

Detection: UV 254, 325 nm, VWD

Injection Volume: 1.0 µL

Sample Solvent: 50/50 water/acetonitrile

Response Time: 0.02 sec.

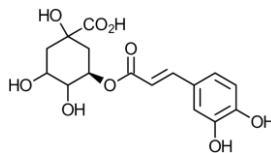
Data rate: 25 Hz

Flow Cell: 2.5 µL semi-micro

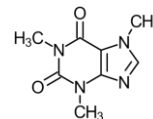
LC System: Shimadzu Prominence UFLC XR

ECV: ~14 µL

STRUCTURES:



Chlorogenic Acid



Caffeine

Green coffee extract is sold as a dietary supplement to aid in weight loss. Chlorogenic acid is the active ingredient. The chlorogenic acid and caffeine can be easily analyzed using HPLC. A bottle of green coffee extract capsules was purchased at a local pharmacy. The powder from one capsule was placed into a vial containing 20 mL of 50/50: acetonitrile/methanol and vortexed to mix. The vial was sonicated for 10 minutes and then allowed to settle. Five mL of the supernate was filtered through a 13 mm, 0.45 µm porosity Nylon syringe filter. A portion of this solution was diluted 1:10 using a 50/50 mixture of water and acetonitrile. The diluted sample was injected into the HPLC. Caffeine was only detectable using the 254 nm wavelength, while chlorogenic acid was detected with increased sensitivity at the 325 nm wavelength. The solvent gradient extends past 10 minutes to remove some minor late eluting peaks.