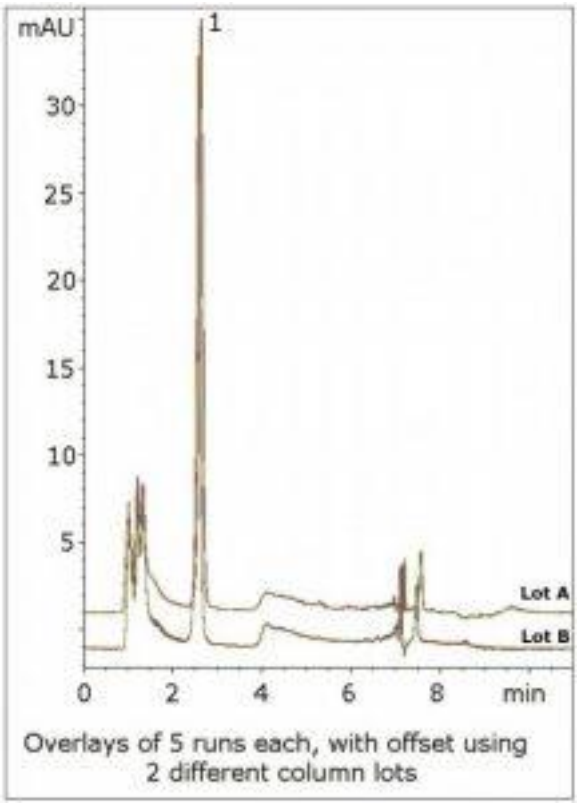
Caffeine in Coffee Analyzed by HPLC - AppNote

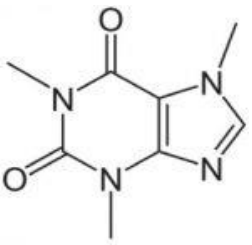
Unique Retention Mode Affords Superior Specificity

Although Caffeine retains well in Reversed Phase, it is found to be difficult to obtain a well-resolved Peak free of interference from other matrix peaks with a complex sample such as coffee. In this Method, most of the matrix peaks elute near the void volume and do not interfere with the Caffeine Peak, which is well-resolved from the others.

Complex matrices can also adversely affect run-to-run repeatability due to compounds that do not elute from the Column and change the chromatography. Here the data shows no sign of contaminant build-up on the Column, as the run-to-run overlays show. The lot-to-lot reproducibility is good as well. Finally, the Method conditions are LCMS compatible.



Overlays of 5 runs each, with offset using 2 different column lots



Peak:

Caffeine

Method Conditions

Column: Cogent Diamond Hydride™, 4µm, 100Å
Catalog No.: 70000-7.5P
Dimensions: 4.6 x 75mm

Mobile Phase:
A: DI Water / 0.1% Formic Acid (v/v)
B: Acetonitrile / 0.1% Formic Acid (v/v)
Gradient:

Time *(minutes)*	%B
0	98
2	98
7	50
8	98

Post Time: 3 minutes
Injection vol.: 1µL
Flow rate: 1.0mL / minute
Detection: UV @ 275nm

Sample Preparation: Commercially available ground coffee was brewed and filtered with a 0.45µm Nylon Syringe Filter (MICROSOLV Tech Corp.). It was then diluted 1:10 with a diluent of 50:50 Solvent A / Solvent B. The Caffeine Peak identity was confirmed with a USP reference standard.
t_0: 0.9 minutes

Note: *Caffeine is a Xanthine Alkaloid found in the coffee plant, the tea bush, the kola nut, and other plants. It is the most commonly consumed psychoactive drug in the world.*

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