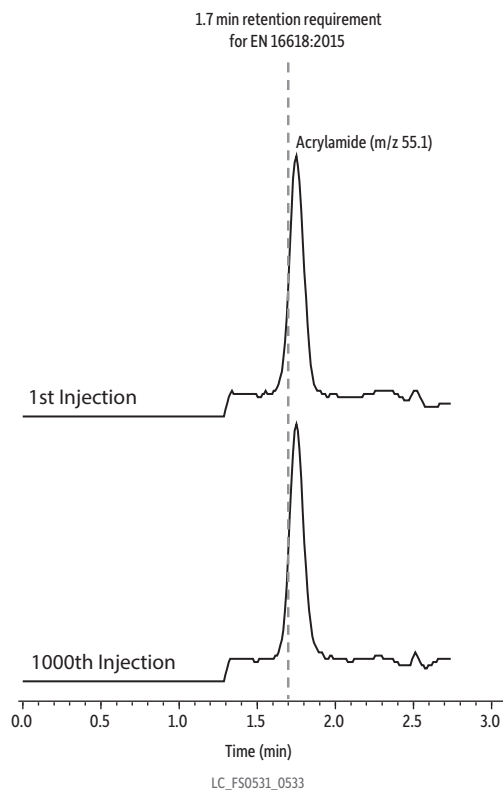


Lifetime Test: EN16618:2015 Coffee Extract on Allure Acrylamide



Peaks	Conc. (ng/mL)	Precursor Ion	Product Ion
1. Acrylamide-d3 (IS)	200	75.1	58.1
2. Acrylamide	Endogenous	72.1	55.1

Column Allure Acrylamide (cat.# 9167552)
Dimensions: 50 mm x 2.1 mm ID
Particle Size: 5 µm
Pore Size: 60 Å
Guard Column: Allure Acrylamide 10 mm, 2.1 mm ID, 5 µm (cat.# 916750212)
Temp.: 22 °C

Standard/Sample

Diluent: Water
Inj. Vol.: 10 µL

Mobile Phase

A: 0.001% Formic acid in water
B: 0.001% Formic acid in acetonitrile

Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	100	0
1.00	0.4	100	0
2.00	0.4	10	90
2.01	0.4	100	0
5.50	0.4	100	0

Detector MS/MS

Ion Mode: ESI+
Mode: MRM

Instrument HPLC

Sample Preparation Extracted per EN 16618:2015

Weighed 2.0 g of ground coffee into a 50 mL centrifuge tube. Added 5 mL *n*-hexane and 40 mL water followed by the addition of internal standard. Shook by hand for 30 sec, by vortexer for 15 sec, and then on a mechanical shaker for 60 min set to maximum sample extraction agitation. Centrifuged in a refrigerated centrifuge at 10 °C, 3600 x g for 20 min. Removed the aqueous layer after centrifugation, taking care to avoid the top, hexane layer, or the solids at the bottom of the tube. Placed the aqueous extract in an appropriate container.

For cleanup, the first SPE cartridge (multimode SPE column with nonpolar, SAX and SCX properties, 1000 mg/6 mL) was conditioned with 3 mL methanol and x2, 6 mL aliquots of water. Passed 10 mL of the aqueous extract through the column and collected eluate. For the next cleanup step, the second SPE cartridge (crosslinked polystyrene/poly-DVB SPE column, 500 mg/6 mL) was conditioned with 5 mL methanol and 5 mL water. Passed the eluate from the previous step entirely through the column. Rinsed the loaded cartridge once with 4 mL water and discarded the rinsing solvent. Eluted the acrylamide with 2 mL of 60% methanol in water. Collected the sample and transferred into an evaporation tube. Placed the tube in an evaporator at a temperature no higher than 40 °C to remove the methanol. Evaporated until the final volume was 0.5–0.8 mL using a gentle flow of nitrogen. Transferred the final sample into an autosampler vial and analyzed by LC-MS/MS.