

## TN-0149

# Robust Analysis of Acrylamide in Coffee using Strata™ Activated Carbon Extraction

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### Introduction

Acrylamide is a chemical that forms through a natural chemical reaction between sugars and asparagine in plant-based foods during high-temperature cooking. It has been found to be neurotoxic and carcinogenic when consumed in large quantities. Coffee forms one of the principal dietary sources of Acrylamide, where it is normally drunk in large quantities. Extracting Acrylamide from coffee proves to be difficult since it is a neutral polar compound and traditional sample preparation using Solid Phase Extraction (SPE) might not be efficient. Since the analyte is polar, a specialized extraction technique is needed. In addition, there are several matrix components including pigments, alkaloids, amines, and esters that prevent accurate extraction of Acrylamide from this challenging concoction. Activated carbon contains porous carbon with defined pore volume and high surface area that are an excellent choice for polar analytes.

In this technical note we have extracted Acrylamide from ground coffee and instant coffee using Strata Activated Carbon cartridges. In addition, multiple batches of Strata Activated Carbon cartridges were evaluated to prove the consistency of the newly developed porous activated carbon material.

### Sample Preparation

<b>Sample Pretreatment:</b>	<i>Neutral Condition:</i> 100 µL of Acrylamide standard (1.0 µg/mL) and 50 µL Acrylamide- <sup>13</sup> C <sub>3</sub> (1.0 µg/mL) spiked in 850 µL of prepared coffee to reach concentration of 100 ng/ml and 50 ng/ml of Acrylamide and Acrylamide- <sup>13</sup> C <sub>3</sub> , respectively. <i>Basic Condition:</i> Same as <i>Neutral Condition</i> but add 150 µL of 20 % Ammonia.
<b>Condition:</b>	Strata Activated Carbon, 400 mg cartridge (Part No. <a href="#">CS0-9210</a> ) or Waters® Sep-Pak® AC2 Plus Activated Carbon, 400 mg with 2 washes of 3 mL Methylene Chloride followed by 2 washes of 3 mL Methanol
<b>Equilibrate:</b>	Cartridges with 2 washes of 3 mL Water
<b>Load:</b>	1 mL pretreated coffee sample using a low vacuum
<b>Dry:</b>	Cartridges under vacuum for 5 min
<b>Elute:</b>	With 3 mL Methylene Chloride and repeat elution 3 more times (12 mL total volume). Add 10 µL Ethylene Glycol.
<b>Water Removal:</b>	Pass eluent through Sodium Sulfate tubes, 1 g/6 mL (Part No. <a href="#">8B-S124-JCH</a> ) that is pretreated with Methylene Chloride
<b>Evaporate:</b>	Solvent to approximately 0.1 mL under a gentle stream of Nitrogen
<b>Adjust:</b>	Final volume to 0.5 mL with Water

### LC Conditions

**Column:** Synergi™ 4 µm Hydro-RP  
**Dimension:** 50 X 2.0 mm  
**Part No.:** [00B-4375-B0](#)  
**Mobile Phase:** A: 0.1 % Formic Acid in Water  
 B: 0.1 % Formic Acid in Methanol

Gradient:	Time (min)	%B
	0	100
	0.6	100
	0.85	0
	3	0
	3.01	100
	5	100

**Flow Rate:** 0.3 mL/min  
**Injection Volume:** 5 µL  
**Temperature:** 25 °C  
**Detector:** MS/MS  
**System:** Agilent® 1260 Binary UHPLC  
**Detection:** SCIEX® 5500 Triple Quad

### MS/MS Conditions

**Source:** ESI-Positive  
**Source Temperature:** 500 °C  
**GS1:** 50  
**GS2:** 50  
**CUR:** 40  
**IS:** 4500

Table 1. MRM Transitions

Analyte	Q1 (m/z)	Q3 (m/z)
Acrylamide	72	54.9
Acrylamide- <sup>13</sup> C <sub>3</sub>	75	58.2



## Results and Discussion

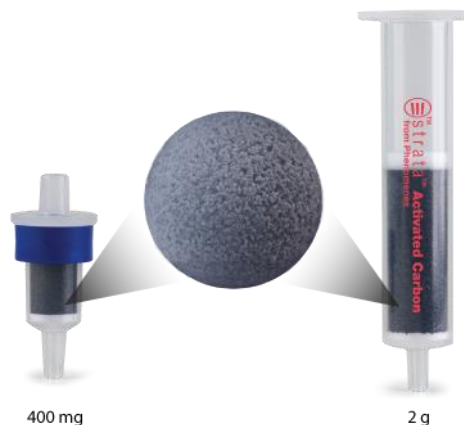
**Figure 1** is a representation of the Strata™ Activated Carbon extraction cartridges. The porous carbon is optimized for reproducible extraction of polar analytes. Although the 400 mg format was employed in this application, there is also a 2 g/6 mL format available for larger sample sizes.

In order to determine the analytical range of the method, calibration standards were made at 25 parts per billion (ppb), 50 ppb, and 100 ppb. **Figure 2** shows the linear progression of the Acrylamide standard. A calibration curve was also drawn (**Figure 3**) and shows that this method is linear within the concentration range of 25 to 100 ppb, with a  $R^2$  value of 0.9999. Together this data shows that under these conditions, Acrylamide can be reliably detected between 25 ppb and 100 ppb. **Figure 4** is a representative chromatogram comparing standards of 100 ppb Acrylamide and 50 ppb Acrylamide- $^{13}\text{C}_3$  through the same extraction process.

Two different coffee extracts were prepared: Instant coffee and coffee prepared from ground coffee beans. 100 ppb Acrylamide and 50 ppb Acrylamide- $^{13}\text{C}_3$  were spiked into the instant coffee preparation and extracted using the Strata Activated Carbon cartridge. **Figure 5** is a representative chromatogram of the results of this extraction. **Figure 6** shows an overlay of the extraction of Acrylamide from instant coffee and instant coffee post-spike. **Figure 7** shows a representative chromatogram of 100 ppb Acrylamide and 50 ppb Acrylamide- $^{13}\text{C}_3$  that were spiked into the ground coffee preparation and extracted using the Strata Activated Carbon cartridge. **Figure 8** shows an overlay of the extraction of Acrylamide from ground coffee and ground coffee post-spike.

Acrylamide recovery and variability in results are presented in **Table 2**. Acrylamide is a neutral compound, but there are several other matrix components, including pigments and caffeine. In order to study the cleanliness and recovery related to matrix components, both a neutral and basic pretreatment condition were explored. We compared the results of six extractions using the Strata Activated Carbon extraction cartridges to the Waters® Sep-Pak® AC2 Plus Activated Carbon extraction cartridges under both sample pretreatment conditions. The % recovery of Acrylamide in the instant coffee sample increased slightly from the neutral pretreatment condition to the basic pretreatment condition after extraction using the Strata Activated Carbon cartridges, with variability of 3 % and 2 %, respectively. The Strata Activated Carbon extraction cartridges provided consistent recovery for the instant coffee sample with neutral and basic pretreatment conditions. For the ground coffee sample, the Waters Sep-Pak AC2 Plus Activated Carbon extraction cartridges gave over 147% recovery with the basic pretreatment condition. This is an indication of matrix components added to the recovery and providing a false positive. With the Strata Activated Carbon extraction cartridges, reasonable recoveries were obtained for both neutral and basic pretreatment conditions extending its usage for both neutral pH and basic pH to optimize accuracy of extraction and to remove matrix interference.

**Figure 1.** Strata Activated Carbon Extraction Cartridges



**Figure 2.** Overlap of Acrylamide Calibration Standards: 1) 100 ppb, 2) 50 ppb, 3) 25 ppb

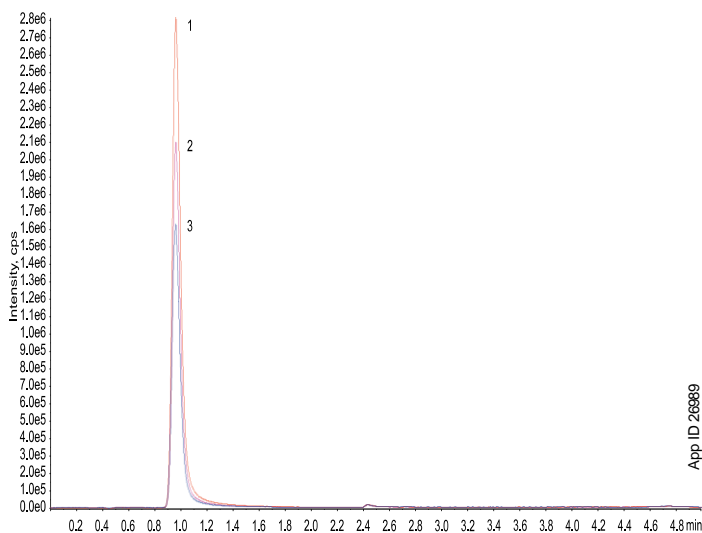


Figure 3. Acrylamide Calibration Curve

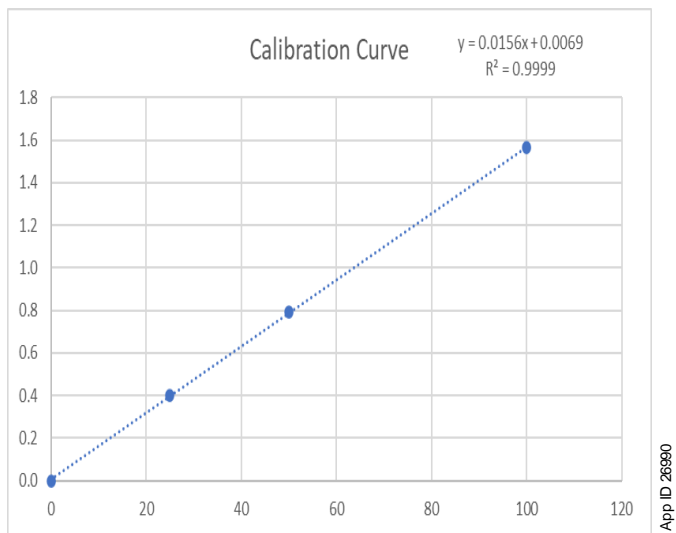


Figure 4. Chromatogram of 1) 100 ppb Acrylamide and 2) 50 ppb Acrylamide-<sup>13</sup>C<sub>3</sub>

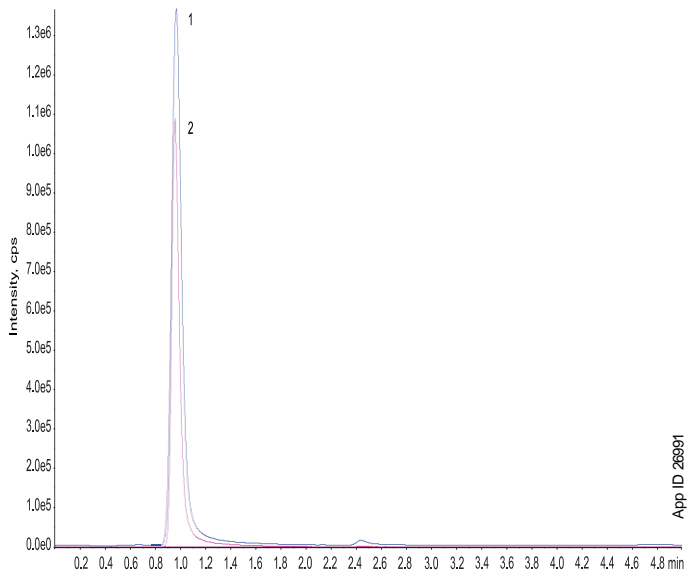


Figure 5. Chromatogram of 1) 100 ppb Acrylamide and 2) 50 ppb Acrylamide-<sup>13</sup>C<sub>3</sub> in an Instant Coffee Sample After Extraction Using the Strata™ Activated Carbon Cartridge

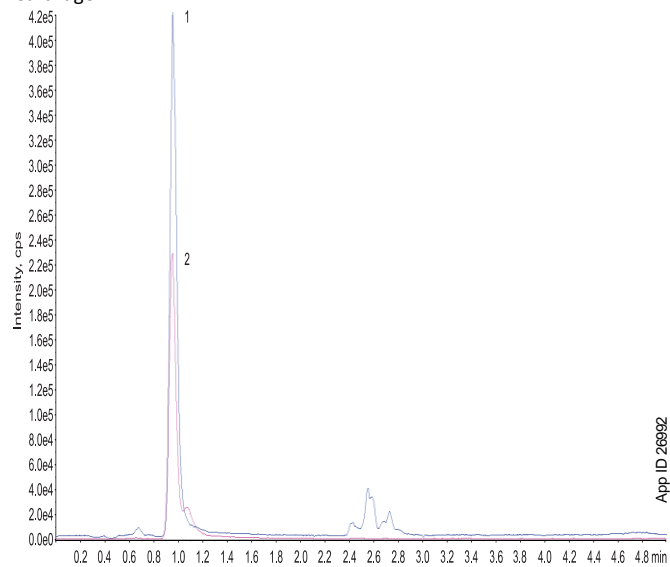
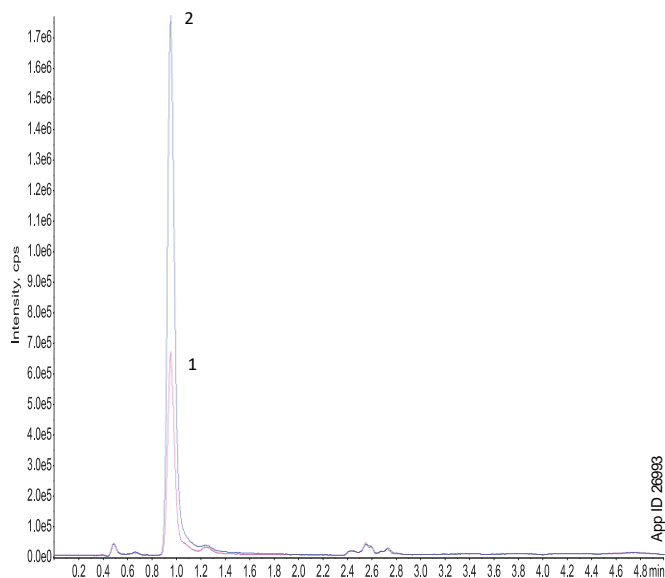
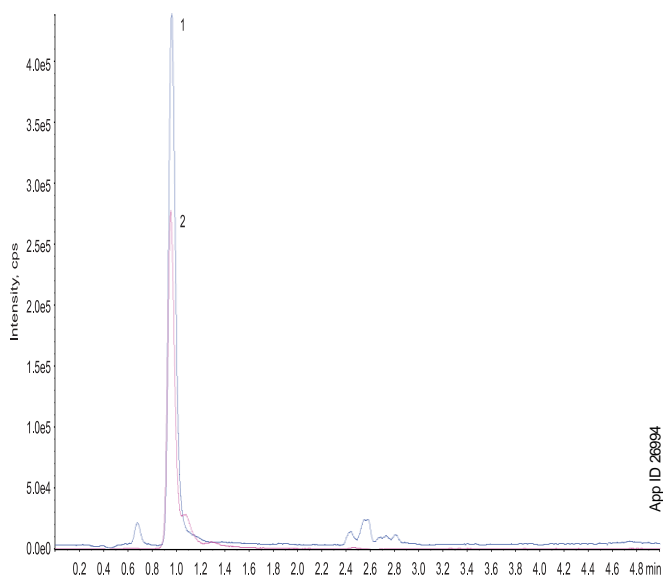


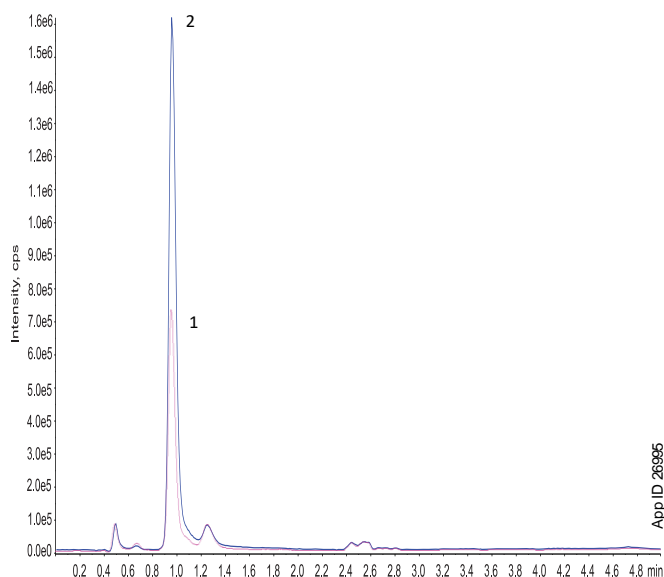
Figure 6. Overlay of 1) Instant Coffee and 2) Instant Coffee spiked of 100 ppb Acrylamide



**Figure 7.** Chromatogram of 100 ppb Acrylamide and 50 ppb Acrylamide-<sup>13</sup>C<sub>3</sub> in a Ground Coffee Sample After Extraction Using the Strata™ Activated Carbon Cartridge



**Figure 8.** Overlay of 1) Ground Coffee and 2) Ground Coffee Spiked of 100 ppb Acrylamide



**Table 2.** Comparison of % Recovery of Acrylamide in Instant Coffee and Ground Coffee Under Two Different Sample Pretreatment Conditions

Extraction	Instant Coffee				Ground Coffee			
	Neutral Pretreatment		Basic Pretreatment		Neutral Pretreatment		Basic Pretreatment	
	Strata Activated Carbon	Waters® Sep-Pak® AC2 Plus Activated Carbon	Strata Activated Carbon	Waters Sep-Pak AC2 Plus Activated Carbon	Strata Activated Carbon	Waters Sep-Pak AC2 Plus Activated Carbon	Strata Activated Carbon	Waters Sep-Pak AC2 Plus Activated Carbon
1	104.40	112.44	112.87	112.29	103.83	98.81	115.17	147
2	105.98	110.79	108.02	109.56	103.78	99.74	116.91	149.46
3	108.41	110.96	110.38	110.88	103.88	102.56	115.29	146.07
4	111.82	110.43	114.15	110.34	103.14	98.69	113.76	152.83
5	111.29	110.12	113.51	109.2	99.91	101.98	111.98	142.38
6	110.58	111.77	112.62	110.44	103.55	100.9	114.34	151.83
<b>Average</b>	<b>108.75</b>	<b>111.09</b>	<b>111.93</b>	<b>110.45</b>	<b>103.02</b>	<b>100.45</b>	<b>114.58</b>	<b>148.26</b>
<b>Standard Deviation</b>	<b>3.03</b>	<b>0.87</b>	<b>2.30</b>	<b>1.09</b>	<b>1.55</b>	<b>1.63</b>	<b>1.66</b>	<b>3.90</b>
<b>%RSD</b>	<b>3%</b>	<b>1%</b>	<b>2%</b>	<b>1%</b>	<b>2%</b>	<b>2%</b>	<b>1%</b>	<b>3%</b>

**Conclusions**

Strata Activated Carbon provided reproducible extraction of Acrylamide from instant coffee and ground coffee in the ppb concentration range and is suitable for both neutral and basic sample pretreatment conditions. Results demonstrated low variability between batches due to a narrow range of pore size and surface area of Strata Activated Carbon.



## Strata™ Activated Carbon Tubes and Cartridges Ordering Information

Strata Activated Carbon				
Part	Sorbent Mass	Volume	Format	Units/pk
<a href="#">CS0-9209</a>	2 g	6 mL	Tube	30
<a href="#">CS0-9210</a>	400 mg	Pass through	Cartridge	50

## Synergi™ Ordering Information

4 µm Microbore and Minibore Columns (mm)									SecurityGuard™ Cartridges (mm) *
Phases	50 x 1.0	150 x 1.0	30 x 2.0	50 x 2.0	75 x 2.0	150 x 2.0	250 x 2.0	50 x 1.0	3/pk
Max-RP	<a href="#">00B-4337-A0</a>	—	<a href="#">00A-4337-B0</a>	<a href="#">00B-4337-B0</a>	<a href="#">00C-4337-B0</a>	<a href="#">00F-4337-B0</a>	—	<a href="#">00B-4337-A0</a>	<a href="#">AJ0-6073</a>
Hydro-RP	<a href="#">00B-4375-A0</a>	<a href="#">00F-4375-A0</a>	<a href="#">00A-4375-B0</a>	<a href="#">00B-4375-B0</a>	<a href="#">00C-4375-B0</a>	<a href="#">00F-4375-B0</a>	<a href="#">00G-4375-B0</a>	<a href="#">00B-4375-A0</a>	<a href="#">AJ0-7510</a>
Polar-RP	—	—	<a href="#">00A-4336-B0</a>	<a href="#">00B-4336-B0</a>	<a href="#">00C-4336-B0</a>	<a href="#">00F-4336-B0</a>	<a href="#">00G-4336-B0</a>	—	<a href="#">AJ0-6075</a>
Fusion-RP	<a href="#">00B-4424-A0</a>	<a href="#">00F-4424-A0</a>	<a href="#">00A-4424-B0</a>	<a href="#">00B-4424-B0</a>	<a href="#">00C-4424-B0</a>	<a href="#">00F-4424-B0</a>	<a href="#">00G-4424-B0</a>	<a href="#">00B-4424-A0</a>	<a href="#">AJ0-7556</a>

for 2.0 – 3.0 mm ID

\*SecurityGuard Analytical Cartridges require holder, Part No.: [KJ0-4282](#)

## Strata Sodium Sulfate Tubes Ordering Information

Strata Sodium Sulphate			
Format	Sorbent Mass	Part Number	Unit
Tube	1 g	<a href="#">8B-S124-JCH</a>	6 mL (30/box)
Giga™ Tube	5 g	<a href="#">8B-S124-LEG</a>	20 mL (20/box)



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