APPLICATION

Acrylamide from Coffee using Novum™ Simplified Liquid Extraction (SLE) Tubes and a Synergi™ Hydro-RP HPLC Column

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Supported Liquid Extraction (or Simplified Liquid Extraction) is very popular in the clinical research industry however the technique is gaining popularity in other industries as a faster, easier, and more reliable alternative to liquid-liquid extraction. This technical note will investigate an application with implications for the food safety industry using Novum SLE to clean up and extract acrylamide from both instant and brewed coffee.

Introduction

According to the American Cancer society, cooking at high temperatures causes a chemical reaction between certain sugars and asparagine which causes acrylamide to form. Acrylamide is commonly found in foods that are made from plants such as potato products, grain products, and coffee whose preparation often requires longer cooking times and higher temperatures.

In this technical note we explore how to use Novum SLE tubes to clean up a coffee matrix in order to quantitate known acrylamide levels, demonstrating that the SLE technique can be applied to a variety of compounds and sample matrices outside of the clinical research industry.

Experimental Conditions

Extraction Procedure

Sample Pre-treatment

Coffee was prepared the same way that it would normally be consumed. Prepared control coffee was left on the bench to reach room temperature before further pre-treatment.

- Ground Coffee Control (40 mg/mL)
  - 60 g of ground coffee was percolated with 1500 mL of boiling water
- Instant Coffee Control (8 mg/mL)
  - 2 g of instant coffee was dissolved in 250 mL of boiling water

Acrylamide standard was spiked into control coffee to reach 100 ng/mL (ground coffee) and 200 ng/mL (instant coffee) by adding 20 µL Acrylamide-$^{13}C_3$ (4 µg/mL in water) to 800 µL of the prepared coffee.

Sample Loading

- Add 150 µL 2 % Ammonium hydroxide in water to the spiked samples (from Pre-treatment step), vortex for 30 seconds.
- Load the sample onto the Novum SLE 6 cc tubes and apply a short and gentle pulse of vacuum (~5-10 seconds at 5" or less of Hg) until the sample has completely entered the media.
- Wait for 5-6 minutes.

Note: Inadequate or excessive wait periods can lead to variable recoveries and poor precision.

Elution

- Dispense 2x 2.5 mL of Ethyl acetate/Tetrahydrofuran (1:1) onto the Novum SLE sorbent and collect the solvent under gravity into a collection tube that contains 10 µL Ethylene glycol.
- Apply vacuum at 5" of Hg (or lower) for 20-30 seconds to complete the extraction.

Note: To reduce analyte loss due to dry down, ethylene glycol was added to the collection tube to prevent the sample from drying completely during the dry down step.

Dry Down

- Evaporate extracted samples to complete dryness under a slow stream of N_2 at 45° C.
- Reconstitute sample in 300 µL of water.

HPLC Conditions

Column: Synergi 4 µm Hydro-RP
Dimensions: 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
Flow Rate: 300 µL/min
Gradient:

Time (min)	% B
0.00	0
0.60	0
0.85	100
3.00	100
3.01	0
5.00	0

Temperature: Ambient
Detection: MS/MS, API 5000™ (AB SCIEX), ESI+
Injection: 5 µL

Table 1. MRM Transitions

Analyte	Q1 Mass (Da)	Q3 Mass (Da)	Dwell (msec)	CE
Acrylamide_1	72	54.9	250	16
Acrylamide_2	72	43.9	250	18
Acrylamide-$^{13}C_3$	75	58.2	250	16

Table 2. Recovery of Acrylamide

Sample ID	Ground coffee (100 ng/mL)	Instant coffee (200 ng/mL)
Mean of area ratio	1.89	3.75
STDV	0.01	0.06
CV (%)	0.78	1.61
Absolute Recovery (%)	94.9	92.8
n=	6	6

Figure 1. Acrylamide from coffee (100 ng/mL)

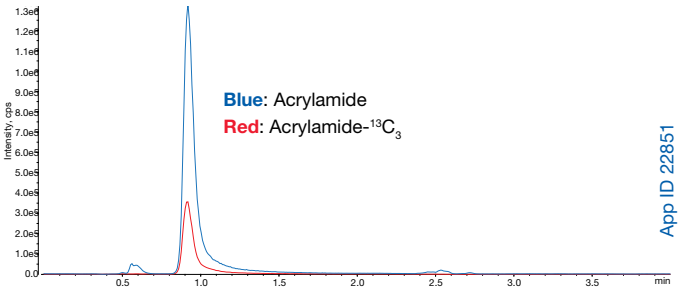


Figure 2. Sample prior to cleanup with Novum™ SLE

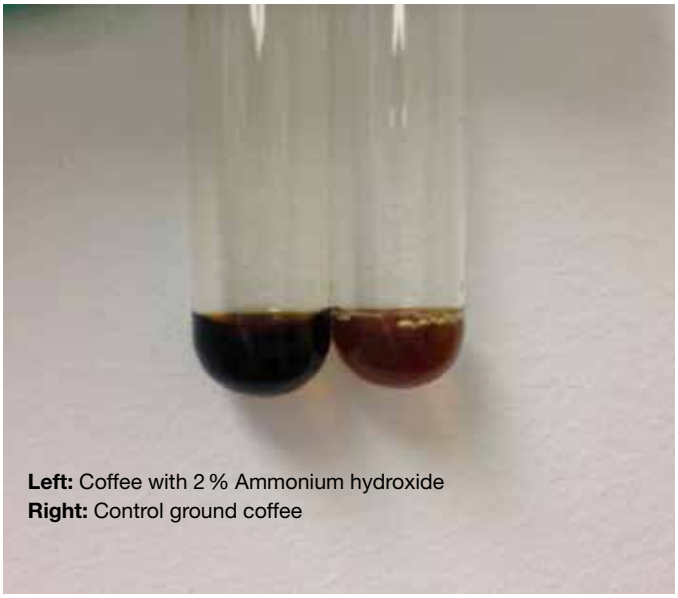
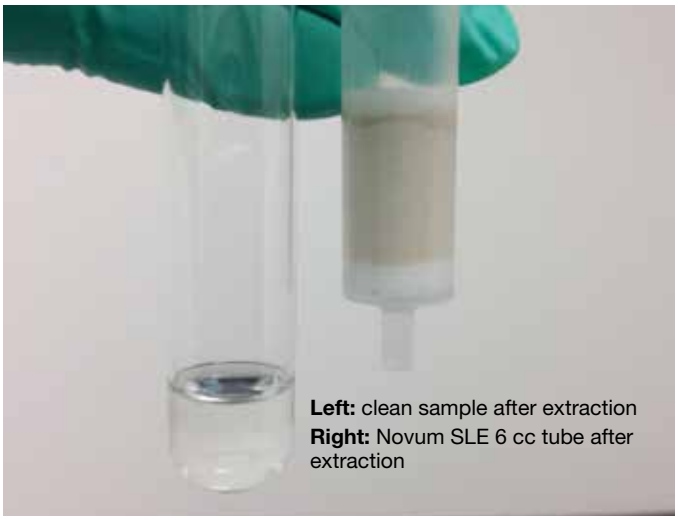


Figure 3. Sample after cleanup with Novum SLE



Results and Discussion

The SLE technique requires that analytes be neutral in charge prior to loading onto the SLE sorbent. This step is important because neutral analytes will partition into the organic elution more efficiently than a charged species. While acrylamide only exhibits charged behavior at extremely low pH, the pre-treated sample was diluted with 2 % Ammonium hydroxide as a way of ensuring that all of the acrylamide was neutralized. This step increases the LogD and facilitates a highly efficient partition to the organic solvent in order to maximize recovery.

After the sample was loaded onto the sorbent, the sample was allowed to soak into the sorbent for 5 minutes. This step allows the sample to disperse amongst the sorbent, creating a higher surface area for interaction with the organic elution solvent. After 5 minutes, a mixture of ethyl acetate/tetrahydrofuran (1:1) was applied to the sorbent in 2 aliquots and the eluent was collected in a collection tube that contained 10 µL of ethylene glycol. Ethylene glycol was added to the collection tube to help prevent analyte loss during the subsequent dry down step. The sample was then blown down under a stream of nitrogen and reconstituted in water.

The SLE procedure resulted in a very clean sample which is depicted in before and after pictures. **Figure 2** shows the ground coffee diluted with 2 % Ammonium hydroxide versus the undiluted ground coffee. Note the darker appearance of the coffee that was diluted with 2 % Ammonium hydroxide. **Figure 3** shows the sample after cleanup, which produced a clear sample that is visually cleaner than the original sample.

Recoveries of acrylamide were studied at 100 ng/mL (ground coffee) and 200 ng/mL (instant coffee) which are values that are in line with typical reported concentration levels in an attempt to mimic a real world sample. **Table 2** shows the absolute recoveries for acrylamide in both the instant and ground coffee which were 92.8 and 94.9, respectively. The resulting HPLC separation of internal standard and acrylamide are depicted in **Figure 1**.

Conclusion

In conclusion, the ethyl acetate/tetrahydrofuran (1:1) elution solvent proved to be an effective solvent choice in terms of both cleanup and recovery. The recovery is especially significant when you consider the polar nature of the acrylamide (LogP -0.27). In addition to high recoveries (>90 %), the low values for the standard deviation and %CV suggest that this method is reproducible. This demonstrates that Novum SLE is capable of being used for non-clinical based samples and effectively functions as a more automatable and easier to use replacement for any liquid-liquid extraction method.

Ordering Information

Novum™ Simplified Liquid Extraction (SLE) Tubes

Part No.	Description	Unit
8B-S138-FAK	Novum SLE 1 cc tubes	100/box
8B-S138-5BJ	Novum SLE 3 cc tubes	50/box
8B-S138-JCH	Novum SLE 6 cc tubes	30/box
8B-S138-KDG	Novum SLE 12 cc tubes	20/box

Vacuum Manifolds

Part No.	Description	Unit
AH0-6023	12-Position Vacuum Manifold Set	ea
AH0-6024	24-Position Vacuum Manifold Set	ea

Synergi™ Hydro-RP HPLC Columns

2.5 µm High Speed Technology (HST) Columns (mm)

Phases	30 x 2.0	50 x 2.0	100 x 2.0	50 x 3.0	100 x 3.0	50 x 4.6
Hydro-RP	00A-4387-B0	00B-4387-B0	00D-4387-B0	00B-4387-Y0	00D-4387-Y0	00D-4387-E0

2.5 µm MercuryMS™ LC/MS Cartridges (mm) / **Guard Columns (mm)**

Phases	10 x 2.0	10 x 4.0	20 x 2.0	20 x 2.0
Hydro-RP	00N-4387-B0-CE	00N-4387-D0-CE	00M-4387-B0-CE	00M-4387-B0

4 µm Capillary Columns (mm) / **Guard Columns (mm)**

Phases	50 x 0.30	150 x 0.30	150 x 0.50	250 x 0.50	20 x 0.30
Hydro-RP	00B-4375-AC	00F-4375-AC	00F-4375-AF	00G-4375-AF	03M-4375-AC

4 µm Microbore and Minibore Columns (mm) / **SecurityGuard™ Cartridges (mm)**

Phases	50 x 1.0	150 x 1.0	250 x 1.0	30 x 2.0	50 x 2.0	75 x 2.0	150 x 2.0	250 x 2.0	4 x 2.0*
									/10pk
Hydro-RP	00B-4375-A0	00F-4375-A0	00G-4375-A0	00A-4375-B0	00B-4375-B0	00C-4375-B0	00F-4375-B0	00G-4375-B0	AJ0-7510
									for ID: 2.0-3.0 mm

4 µm MidBore™ Columns (mm) / **SecurityGuard™ Cartridges (mm)**

Phases	30 x 3.0	50 x 3.0	150 x 3.0	250 x 3.0	4 x 2.0*
					/10pk
Hydro-RP	00A-4375-Y0	00B-4375-Y0	00F-4375-Y0	00G-4375-Y0	AJ0-7510
					for ID: 2.0-3.0 mm

4 µm Analytical Columns (mm) / **SecurityGuard™ Cartridges (mm)**

Phases	30 x 4.6	50 x 4.6	75 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0*
						/10pk
Hydro-RP	00A-4375-E0	00B-4375-E0	00C-4375-E0	00F-4375-E0	00G-4375-E0	AJ0-7511
						for ID: 3.2-8.0 mm

*SecurityGuard™ Analytical Cartridges require holder, Part No.: KJ0-4282

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APPLICATION

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