# Extraction and Cleanup of Acrylamide in Complex Matrices Using Accelerated Solvent Extraction (ASE®) Followed by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

## INTRODUCTION

Acrylamide is formed during the cooking process of certain plant-based foods which are rich in carbohydrates and low in protein. Specifically, it forms when asparagine reacts with sugars such as glucose at high temperatures. Acrylamide was detected in fried foods by the Swedish National Food Authority in 2002. Since then, many food laboratories have successfully performed determinations for this compound on a variety of different food matrices. Acrylamide is a known carcinogen in animals.

ASE is an excellent technique for extraction of acrylamide from various fried food products; until recently, however, extraction of this compound from matrices such as coffee and chocolate has proven difficult. Traditional extraction techniques are time consuming, and may cause bottlenecks in sample preparation. This Application Note describes a new ASE method that combines the extraction of low-levels of acrylamide from coffee and chocolate with an in-cell, solid-phase cleanup step.



## EQUIPMENT

Dionex ASE 200 with 33-mL stainless steel extraction cells (P/N 048763)

Dionex cellulose filters (P/N 049458)

Dionex collection vials 60 mL (P/N 048784)

Dionex SE 500 Solvent Evaporator (P/N 063221, 120 v) (P/N 063218, 240 v)

Standard laboratory tissue homogenizer

Standard laboratory centrifuge (rated to 10,000 rpm or greater)

Centrifuge tubes (40–50 mL)

## CHEMICALS AND REAGENTS

Acrylamide, purity 99% (Sigma-Aldrich)

*d3*-Acrylamide (2,3,3-*d3*-2-propenamide) (Cambridge, Isotope Laboratories USA)

Florisil, 60–100 mesh (Sigma-Aldrich)

Potassium hexacyanoferrate (II) trihydrate (Carrez I) (Sigma-Aldrich)

Zinc sulfate heptahydrate (Carrez II) (Sigma-Aldrich)

Dionex ASE Prep DE (P/N 062819)

Termamyl® 120 L (Type L thermostable amyloglucosidase enzyme) (Novozymes, Denmark)

Ethyl acetate (Fisher Scientific, HPLC Grade)

Dichloromethane (Fisher Scientific, HPLC Grade)

Methanol (Fisher Scientific, HPLC Grade)

### *FOOD SAMPLES*

The coffee and chocolate samples were purchased from a local grocery store and stored at room temperature.

### *REAGENT SOLUTIONS*

#### 0.68 M potassium hexacyanoferrate (II) trihydrate (Carrez I) solution

Dissolve 28.722 g of $K_4Fe(CN)6\ 3H_2O$ in 100 mL of water.

#### 2 M zinc sulfate heptahydrate (Carrez II) solution

Dissolve 57.512 g of $ZnSO_4\ 7H_2O$ in 100 mL of water.

### *STANDARD SOLUTIONS*

Prepare aqueous stock solutions of acrylamide and *d3*-acrylamide at concentrations of 50 and 5 µg/mL, respectively.

To make 50 µg/mL acrylamide, add 5 mg to 100 mL water.

To make 5 µg/mL *d3*-acrylamide, add 0.5 mg to 100 mL water.

Dilute the acrylamide solutions in water to obtain the following matrix-equivalent levels: 0, 10, 50, 200, 500, and 2500 µg/kg. The matrix-equivalent concentration of *d3*-acrylamide should be 250 µg/kg.

### *SAMPLE PREPARATION*

#### Hydrolysis

Weigh 2.0 g of sample into a centrifuge tube and add 10 mL of water, (heated to 60 °C) then add 50 µL of Termamyl. Place the tube in a water bath at 90°C for 45 min. Homogenize the mixture for 1 min. To precipitate the proteins, add 1 mL of the 0.68 M potassium hexacyanoferrate (II) trihydrate solution and 1 mL of the 2 M zinc sulfate heptahydrate solution to the centrifuge tube, swirling constantly for 1–2 min. Add 5 mL dichloromethane and swirl for an additional minute. Centrifuge for 15 min at 10,000 rpm.

#### Preparing the ASE Cell

Prepare the 33-mL extraction cell by successively inserting: (1) a cellulose filter, (2) 6 g Florisil deactivated with 3% deionized water, (3) a second cellulose filter, and (4) at least 8 g ASE Prep DE so that there is approximately 0.5 cm of empty space at the top of the cell.

Transfer 6 mL of the supernatant from the centrifuge tube and drip onto the ASE Prep DE layer in the prepared extraction cell. Fill the extraction cell to the top with additional ASE Prep DE and cover with a third cellulose filter. Carefully place the extraction cell cap on the cell and tighten by hand.

### *ASE CONDITIONS*

| | |
|---|---|
| Solvent: | Ethyl acetate (100%) |
| Temperature: | Ambient* |
| Pressure: | 1500 psi** |
| Static Time: | 3 min |
| Static Cycles: | 3 |
| Flush: | 100% |
| Purge: | 60 s |

** Although the authors of this Application Note used ambient temperature for the extraction, this is not a typical extraction temperature for ASE. Normal extraction temperatures range from 40–200 °C. If recoveries are lower than expected using ambient temperature, increasing temperature may improve results.*

***Pressure studies show that 1500 psi is the optimum extraction pressure for all ASE systems.*

### *EXTRACTION*

Place the prepared extraction cells onto the ASE carousel. Enter the ASE conditions into the *Method Editor* screen, and save this method with the desired number. Begin the extraction by pushing *Start*. The method can also be set up as a Schedule in the *Schedule Editor* screen. Running the ASE system under *Schedule* control enables the system to track any errors that may occur throughout an extraction run. This is especially helpful if the system is set up to run unattended overnight. Any problems are logged in the *Error Log* for the user to view the next morning.

When the extraction is complete, evaporate the extracts under vacuum (40 °C, 200 mbar) until only a few droplets remain. Evaporate the residual ethyl acetate under a gentle stream of nitrogen, or transfer the vials to the Dionex SE 500 Solvent Evaporation System (P/N 063221~120v; 063218~240v) and evaporate to dryness using standard conditions. Redissolve the extract with 500 µL water.

If necessary, the extract can be filtered through a 0.2-µm cellulose filter. Decant approximately 180 µL of the extract and mix with 90 µL of methanol prior to analysis by LC-MS/MS.

## RESULTS AND DISCUSSION

The ASE method automates the extraction and cleanup steps of extraction of acrylamide from cocoa and coffee. Compared to the manual method, ASE greatly reduces the time and the amount of sample handling required (Table 1). The addition of Florisil to the extraction cell eliminates the need for an additional cleanup step of the extract. Figure 1 shows coffee extracted with various amounts of Florisil, followed by filtration through SPE cartridges. Method optimization determined that 6 g of Florisil were sufficient to obtain a clear extract, however some samples may require additional filtering before analysis.

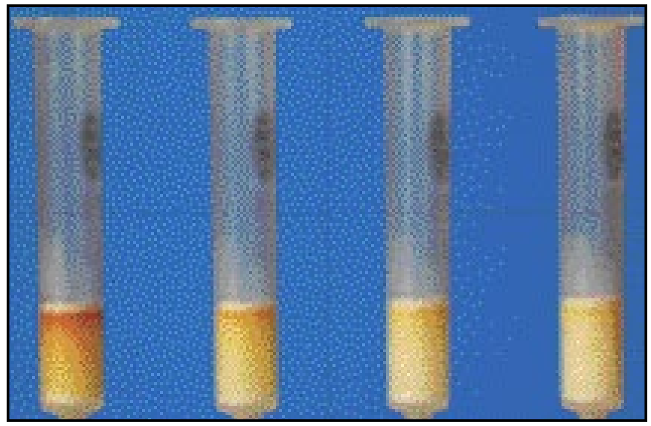

*Figure 1. Residual coextractables from coffee extracts, trapped on an Isolute Multimode SPE cartridge. The four extraction cells contained from 0 to 6 g of Florisil. From left to right: (1) no Florisil, (2) 2 g Florisil, (3) 4 g Florisil, and (4) 6 g Florisil.*

**Table 1. Comparison of the Sample Preparation Steps for the Manual Method and ASE Method for Extraction of Acrylamide from Coffee and Chocolate**

| Manual Method | ASE Method |
|---|---|
| 1. Weigh 2 g of sample, add 100 µL of d3-acrylamide solution (5 µg/mL) and 10 mL of water into a centrifuge tube. Homogenize for 1 min. | 1. Weigh 2 g of sample, add 100 µL of d3-acrylamide solution (5 µg/mL) and 10 mL of water into a centrifuge tube. Homogenize for 1 min. |
| 2. Add 1 mL Carrez I, swirl, add 1 mL Carrez II, swirl. Add 5 mL dichloromethane, shake vigorously for 1 min. | 2. Add 1 mL Carrez I, swirl, add 1 mL Carrez II, swirl. Add 5 mL dichloromethane, shake vigorously for 1 min. |
| 3. Centrifuge at 3–5 °C, 10,000 rpm for 15 min. | 3. Centrifuge at 3–5 °C, 10,000 rpm for 15 min. |
| 4. Transfer 6 mL of the supernatant into a centrifuge tube containing 1.8 g of NaCl, swirl to dissolve. | 4. Prepare ASE cell by successively adding 1 cellulose filter, 6 g of Florisil (deactivated with 3% water), a second cellulose filter and 8 g ASE Prep DE. |
| 5. Add 13 mL ethyl acetate and shake vigorously (1 min). | 5. Transfer 6 mL of the extract on the ASE Prep DE, fill the rest of the column with ASE Prep DE, add a third cellulose filter and close the cell. |
| 6. Centrifuge at 3–5 °C, 13800 g for 15 min. | 6. Perform the ASE extraction step. |
| 7. Transfer the organic phase into an amber vial containing 2 mL water. Shake vigorously for 1 min. | 7. Evaporate the organic fraction under vacuum (40 °C, 200 mbar) to about 500 µL and finish the evaporation with a gentle stream of N2. |
| 8. Evaporate the organic phase with N2 at 40 °C. | 8. Redissolve the extract in 500 µl water. |
| **Repeat the ethyl acetate extraction (2x), steps 5-7.** | **Add 90 µL of methanol to 180 µL of extract. Proceed with LC-MS/MS analysis (60 µL injected).** |
| 9. Condition the SPE cartridge with 3 mL methanol, then twice with 3 mL distilled water. | |
| 10. Load the aqueous extract onto the cartridge, elute and rinse with 1 mL water. Collect both fractions. | |
| 11. Reduce the extract volume to approximately 500 µL (N2, 40 °C). | |
| 12. Add 90 µL of methanol to 180 µL of extract. Proceed with LC-MS/MS analysis (60 µL injected). | |

Table 2 compares the results of manual extraction to ASE extraction of blank samples spiked with acrylamide standard.

**Table 2. Comparison of Manual Extraction versus ASE for Quantification of Acrylamide Spiked Samples in soluble Chocolate Powder (n = 6)**

| | Manual Extraction | | ASE Extraction | |
|---|---|---|---|---|
| Spiking Levels | Recovery % | %RSD | Recovery % | %RSD |
| 12.7 µg/kg | 103.7 | 17.2 | 94.6 | 4.3 |
| 304.7 µg/kg | 106.0 | 6.3 | 102.2 | 7.0 |
| 2504 µg/kg | 104.3 | 5.3 | 101.5 | 2.4 |

Table 3 shows the results of using ASE for the extraction of acrylamide from various difficult matrices.

**Table 3. ASE of Roast Ground Coffee, Soluble Coffee, Coffee Surrogate, and Cocoa**

| | Acrylamide level (µg/kg) | | | |
|---|---|---|---|---|
| | | Spiked at 150 µg/kg | | |
| Materials | Incurred[a] | Expected[b] | Measured[a] | CV% |
| R&G coffee | 136 | 286 | 298 | 3.1 |
| Soluble coffee powder | 299 | 449 | 435 | 2.9 |
| Coffee surrogate | 632 | 782 | 782 | 1.0 |
| Cocoa powder | 192 | 342 | 343 | 1.1 |

[a]Mean of two independent determinations. [b]Mean incurred level + spike level.

## CONCLUSIONS

ASE has consistently proven to be an excellent alternative to the traditional labor-intensive extraction methods used for determination of acrylamide in food. ASE allows extraction and cleanup to be performed simultaneously, eliminating the need for a post-extraction cleanup step. Automation of ASE allows for unattended extraction, and can be set up to run over night to provide the user with filtered extracts that are ready for analysis in the morning. The advantages of speed and decreased sample handling as compared to manual extraction techniques are clear. Acrylamide recoveries obtained with the ASE method were comparable to traditional methods, proving that ASE is an effective tool for the extraction of polar compounds from complex matrices.

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## SUPPLIERS

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