Determination of Acrylamide in Food

1. Scope

This method is for the determination of acrylamide in food (coffee is used in this experiment).

2. Solution Preparation

Accurately weigh 5 mg (to an accuracy of 0.02 mg) of the standard into a 50 mL volumetric flask. Dissolve it in methanol and dilute to the mark. This solution contains 0.10 mg of acrylamide per 1.0 mL and serves as the acrylamide standard stock solution with a concentration of 100 μg/mL. Accurately pipette 1 mL of the 100 μg/mL stock solution and dilute to 10 mL with 20% methanol in water, yielding a concentration of 10 μg/mL.

3. Extraction Procedure

Weigh 1 g of the sample into a 15 mL centrifuge tube, add 10 mL of acetonitrile, vortex for 2 minutes, and centrifuge for 1 minute at 4000 r/min. Transfer the supernatant to a 50 mL centrifuge tube. Add another 10 mL of acetonitrile to the 15 mL centrifuge tube and extract again. Combine the two extracts and add 20 mL of n-hexane. Shake vigorously for 5 minutes. Remove the upper hexane layer and repeat with another 20 mL of n-hexane, shaking for 5 minutes and removing the hexane again. Transfer the lower aqueous phase to a flask, add 4 mL of water, mix well, and evaporate under reduced pressure to less than 2 mL for purification.

4. SPE Purification Procedure

SPE Column: Welchrom® C18E

Specification: 500 mg/6 mL

Activation: 5 mL methanol, followed by 5 mL water

Loading: Load the purified sample solution and collect the eluate

Elution: Rinse the flask with 2 mL of 30% methanol in portions, load the solution onto the column, collect the eluate, and dry.

Combine the loading solution and eluate, dilute to 5 mL with 30% methanol in water, filter through a 0.22 μm membrane, and proceed to HPLC for detection.

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5. Notes

In elution process, the eluate was previously water but has been changed to 10%, 20%, 30%, 40%, and 50% methanol in water, respectively. It was found that 30% methanol in water had better elution efficiency than the other eluates.

6. Chromatographic Conditions

Column: Ultisil® XB-C18 4.6×150 mm, 3 µm
Mobile phase: Methanol: water (20:80)
Column temperature: 35°C
Injection volume: 5 µL
Detection wavelength: 210 nm
Flow rate: 0.5 mL/min

7. Chromatograms or Spike Recovery Results

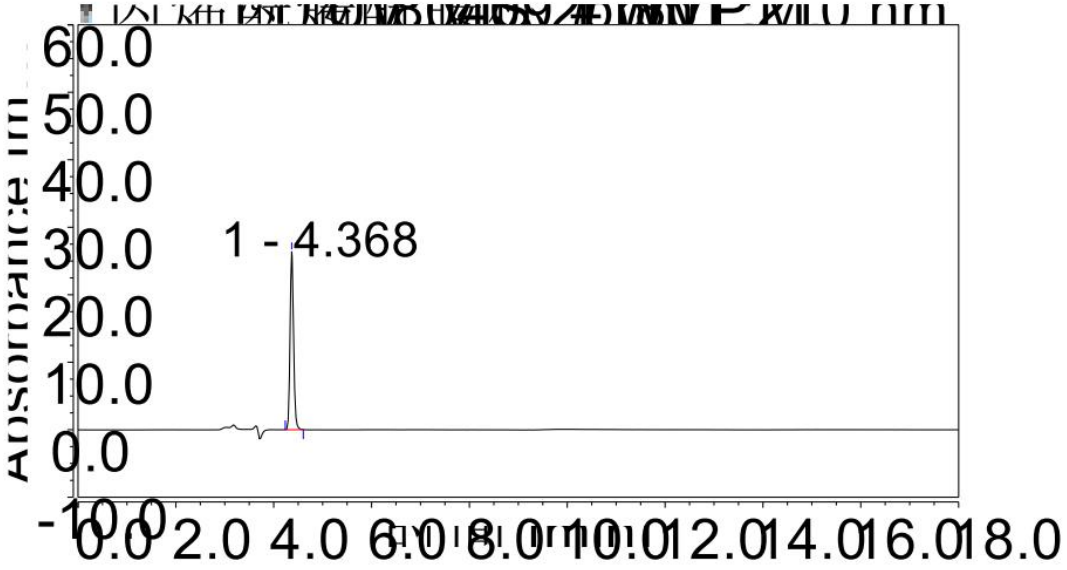


Figure 1: Acrylamide standard at 2 mg/L

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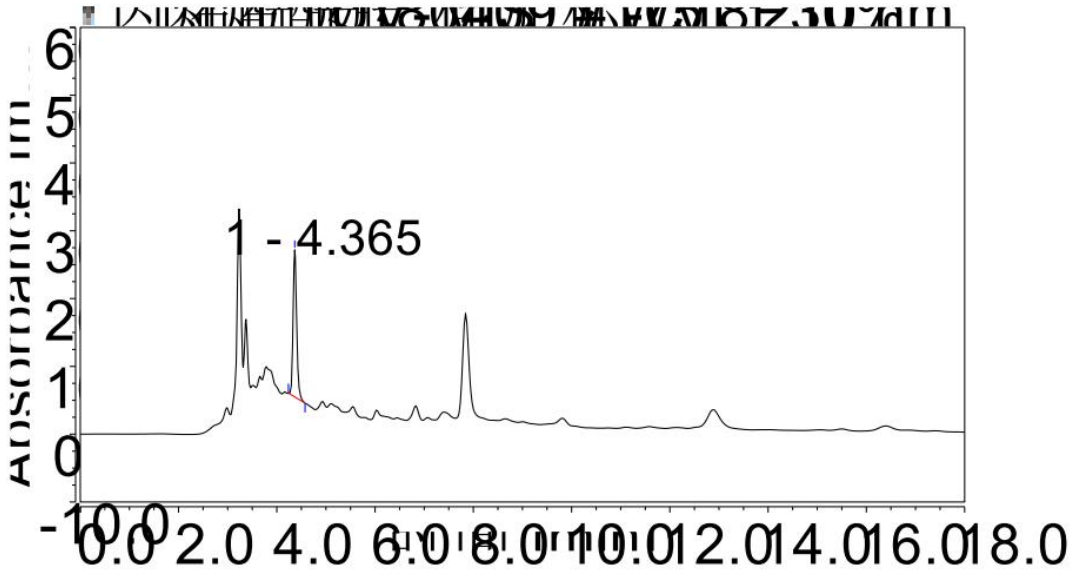


Figure 2: Coffee sample spiked at 10 mg/kg

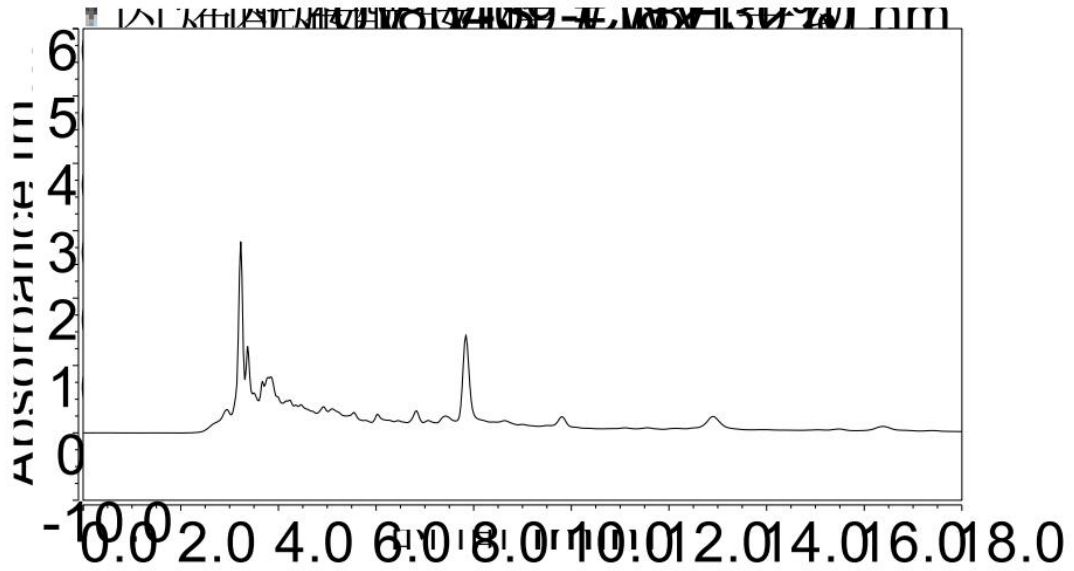


Figure 3: Coffee sample

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Table 1: Spike Recovery Rates

Compound	Spike (mg/kg)	Recovery (%)	Rt (min)	RSD (%)
Acrylamide	10	84	4.36	3.77
	10	84		
	10	84		
	10	86		
	10	83		
	10	82		
	10	87		

8. Related Product Information

Catalog No.	Name	Specification
00559-11006	Welchrom® C18E	500 mg/6 mL, 30 pack
00824-31001	Welch Solid Phase Extraction Device	12-position square cylinder
00802-02201	Nylon Syringe Filter	13×0.22 μm, 100 pack
00824-11101	Disposable Syringe	200 pack/box, 1 mL with needle, rubber tip
00822-00007	Sample Vial Kit	2 mL clear short-thread wide-mouth vials with writable area, pre-slit white PTFE/red silicone septa, 9 mm blue short-thread open caps, 6 mm center hole, 100 pack
00201-21041	Ultisil® XB-C18	4.6×150 mm, 3 μm

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