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SOLID-PHASE EXTRACTION AND CLEANUP PROCEDURES FOR THE LC/MS DETERMINATION OF ACRYLAMIDE IN FRIED POTATO PRODUCTS

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INTRODUCTION –

Acrylamide ($H_2C=CHCONH_2$) is a highly polar, highly water soluble, highly useful industrial chemical. Its chemical structure includes the amide functionality that imparts the high water solubility and the vinyl functionality that makes the molecule amenable to polymerization. The most common industrial use for acrylamide monomer is for production of polyacrylamide resins useful in chromatography, water purification, and in production of textiles and other materials. There have been recent reports of acrylamide levels in excess of 1000 µg/kg in fried potato products. The acrylamide is presumed to be formed from the high temperature reaction of asparagine with certain carbohydrate moities naturally occuring in the foodstuffs. Acrylamide has traditionally been determined using GC or GC/MS after derivatization with bromine. However, since the recent discovery of acrylamide in foods, there has been an interest in the development of less cumbersome methods of analysis.

The purpose of this research was to develop an improved SPE protocol for preparation of fried potato samples for LC/MS analysis. The analyte is extracted from the matrix using 2 M NaCl and an aliquot of the initial extract is loaded onto a reversed-phase cartridge. After the analyte is eluted from the cartridge, the eluent is cleaned up by passing through a mixed-mode cation-exchange cartridge. The eluent is then evaporated and the residue is reconstituted in mobile phase prior to LC/MS analysis. Recoveries compared to an added internal standard ranged from 96 to 101 % with RSDs from 5 % to 11 %. Linear response was observed in the concentration range from 100 - 2000 µg/kg with a coefficient of determination (R^2) of 0.992 (n = 25). An interday study showed good accuracy and precision of the method over a three day period with recovery of 98 % and RSD of 9.5 % (n = 15). The analysis of six incurred potato chip samples showed concentrations of acrylamide ranging from 250 to 1500 µg/kg.

PREPARATION OF POTATO CHIP SAMPLES-

- 1 gram crushed potato product was weighed into a centrifuge tube
- 15 mL of 2M NaCl and 10 µL of ISTD (acrylamide-d_3) solution was added to the tube and the contents were vigorously shaken for a period of 30 minutes
- The tube was then centrifuged at 10,000 x *g* for 12 minutes
- A 1.5 mL aliquot of the supernatant was taken from the centrifuge tube for SPE extraction and cleanup

HPLC CONDITIONS-

Instrument: Alliance® 2695 Separations Module
Column: Atlantis™ dC_{18}, 2.1 x 150 mm, 5 µm
Part Number: 186001301
Flow Rate: 0.20 mL/min
Mobile Phase: 0.1% Formic acid in water
Injection Volume: 20.0 µL
Column Temp: 30 °C

MS CONDITIONS-

Instrument: Waters® ZMD Mass Detector
Interface: Positive Electrospray (ESI+)
Multiple Selected-Ion Recording (SIR)
Dwell Time - 0.2 Seconds
Interchannel Delay Time - 0.02 Seconds
Optics: Capillary - 2.9 kV
Extractor - 4 V
RF Lens - 0.1 V
Source Block - 150 °C
Desolvation - 350 °C
SIR Parameters:

Compound:	Mass	Cone Voltage
Acrylamide	72	20 V
	55	40 V
Acrylamide-d_3	75	20 V
	58	40 V

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SPE PROTOCOL FOR POTATO CHIP SAMPLES

Part A *Oasis® HLB* *6 cc, 200 mg* Acrylamide Retention

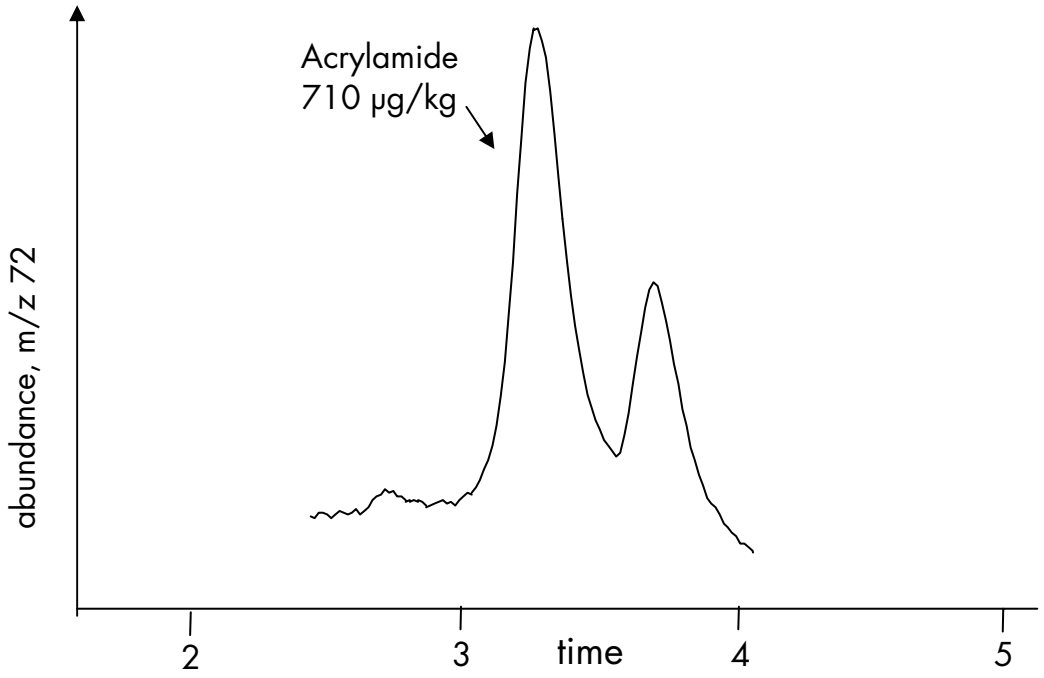
- Condition/Equilibrate
  2mL Methanol/ 2mL 2M NaCl
- Load
  1.5mL Potato Extract
- Wash
  0.8mL Water
- Elute
  3mL Methanol (1% Formic acid)

Proceed To Part B

Part B *Oasis® MCX* *3 cc, 60 mg* Pass-Through Cleanup

- Condition
  2 ml Methanol
- Pass Eluent from Part A
  Collect in total
- Rinse
  0.5 mL Meoh, combine with passed eluent
- Evaporate and Reconstitute
  0.4mL Water

LC/MS DETERMINATION OF ACRYLAMIDE IN POTATO CHIP A



METHOD PERFORMANCE-

Initial Validation

Because no potato chip samples were found with an incurred level of acrylamide below 200 µg/kg, initial validation studies were accomplished using commercial dehydrated mashed potato flakes with 10 % by weight added soybean oil; the acrylamide level in these flakes was well below 100 µg/kg. Quantitation was by internal standard calibration with five replicate samples analyzed per level.

Fortification Level (µg/kg)	Amount Found (µg/kg)	% RSD
100	96	12
200	211	8.7
500	488	5.8
1000	1010	8.0
2000	2000	6.5

Interday Study With Incurred Samples:

Replicate samples from a single batch of potato chips (potato chip A) were analyzed over a three day period. Five replicate 1 g samples were prepared and analyzed on successive days (n = 15).

Results:

Day 1; 770 µg/kg ± 7.0 % RSD
Day 2; 660 µg/kg ± 7.1 % RSD
Day 3; 700 µg/kg ± 8.7 % RSD

Interday Study With Fortified Samples:

Replicate samples from the same batch of potato chips was used for an interday analysis of fortified samples. Five replicate 1 g samples were spiked with 2000 µg/kg of acrylamide and were then analyzed on successive days over a three day period (n = 15).

Results:

Day 1; 2660 µg/kg ± 4.3 % RSD
Day 2; 2710 µg/kg ± 7.7 % RSD
Day 3; 2600 µg/kg ± 2.7 % RSD

The analysis indicates that 1950 µg/kg of acrylamide was recovered from each sample (98 % recovery).

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ANALYSIS OF COMMERCIAL POTATO CHIPS

Potato Chip	Acrylamide (µg/kg)	Chip Type
A	710	Regular
B	640	Baked
C	460	Onion/sour cream
D	780	Regular
E	1470	Regular
F	260	Kettle

CONCLUSIONS-

The SPE protocol described in this paper provides sample enrichment and cleanup acceptable for the routine determination of acrylamide in potato chip samples using single quadrupole electrospray mass-spectrometry. Response was linear in the sample range from 100-2000 µg/kg. Results obtained from fortified potato samples indicate that the limit of quantitation (LOQ) was below 100 µg/kg. Results obtained from a study conducted on actual potato chip samples show that the method is reproducible over several days. Analysis of 6 potato chip samples obtained from commercial markets showed levels of acrylamide in all products was well above the quantitation limit of the method. The acrylamide levels found in commercial potato chips ranged from 250 to 1400 µg/kg.

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