Sample Preparation Using Strata-X-C SPE for the Determination of Acrylamide in French Fries by LC/MS/MS

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Abstract

A simple, specific and reliable method based on SPE using Strata-X-C for sample preparation and HPLC/MS/MS detection has been developed for the analysis of acrylamide in food samples. After homogenizing french fries with water and centrifugation, the supernatant was loaded onto Strata-X-C 100mg SPE cartridges, and acrylamide was selectively eluted with water. Synergi Hydro-RP® and Synergi Polar-RP® reversed-phase columns were used for LC/MS/MS analysis. APCI MS ion source was used in positive ion mode. MRM m/z 72→55 & 75→58 transitions were considered for quantitation. The absolute recoveries for acrylamide were between 75-105%, with a limit of quantitation of 50µg/kg food. The precision was 15% RSD at 50ng/mL (n=6), and 3% RSD at 500ng/mL (n=6). The correlation coefficients (r) were > 0.999 for the range 10-1000ng/mL (n=6), and 100-2000ng/mL (n=5), respectively. This sample extraction method based on Strata-X-C SPE is also well suited for the LC-UV analysis of acrylamide at high concentrations in food.

Introduction

Acrylamide - a very small and polar molecular compound - has been classified as a probable carcinogen in humans[1]. The discovery of high levels of acrylamide in a wide range of food products prepared at high temperatures has drawn worldwide attention[2,3]. The increasing need for testing acrylamide content in food requires the development of fast and reliable analytical methods.

Recently published or proposed methods for the determination of acrylamide[4,5,6,7] in food involve either one or two successive SPE steps for sample preparation before LC/MS detection.

Introduction (cont.)

Unfortunately, the one step SPE method does not ensure an efficient sample clean-up. As a consequence, intensive column maintenance is needed after each batch of samples. For two-step SPE methods, typically a hydrophilic/lipophilic cartridge is applied first for removing most matrix components, followed by a mixed bed cartridge used for further clean up. These methods provide cleaner extracts for analysis but are time consuming and costly.

In this poster we present a reliable, specific and simple method which uses one SPE cartridge for the extraction of acrylamide from a food matrix (french fries) using Strata-X-C, a new mixed-mode polymeric cation exchange resin from Phenomenex Inc.

Experimental Conditions

Instrumentation

HPLC: HP 1100 series (www.agilent.com/chem)

MS: Bruker Esquire 2000 (www.bruker.com) Ion Trap MS analyzer, ion source APCI in positive ion mode

LC Conditions

Column: Synergi Hydro-RP® 4μm 80Å 250 x 3.0mm (LC/MS)
Synergi Polar-RP® 4μm 80Å 150 x 3.0mm (HPLC/UV)
or Synergi Polar-RP® 4μm 250 x 4.6mm (HPLC/UV)

Flow rate: 0.5mL/min (LC/MS)

Mobile phase: 94:6 Water:Methanol (both with 0.1% formic acid)
or 96:4 Water:Acetonitrile (both with 0.1% formic acid)

Injection: 25 μL

SPE cartridge Strata-X-C, 100mg, 1 mL

Experimental Conditions (Cont.)

MS/MS Settings

Expert Parameter (Tuning)				**Trap**	
Skim1	15V	Skim2	6.0V	Target	1000
Cap Exit off Set	50V	Cap Exit	65.0V	Max Accu Time	150µs
Octopole Δ	1.79V	Oct RF	50.0VPP	Scan (m/z)	40-150
Octopole	2.4V	Lens1	-5.0V	Average	8 Spectra
Trap Drive	27.6V	Lens2	-60.0V		
Source				**MRM**	
Capillary	-4000V	Dry Gas	10 L/min	Acrylamide $(M+1)^+$	72->55
End Plate off Set	-500V	Dry Temp.	350°C		
Corona (nA)	6000	APCI Temp	400°C	Acrylamide-d3 $(M+1)^+$	75->58
Nebulizer (psi)	50				

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Sample Preparation

- Add 50mL water to 10g pulverized frozen french fries
- Mix or homogenize for 20 minutes
- Centrifuge the decanted solution at 10000rpm for 15 minutes
- Spike IS acrylamide-d3 (prepared in water) in 1mL sample (french fries supernatant)
- Simultaneously, two 1mL samples (unspiked supernatant) are used as blanks for standard calibration

SPE procedure

SPE conditioning

Apply 2 x 1mL methanol, followed by 2 x 1mL water to a Strata-X-C cartridge at a flow rate of about 2mL/min.

Sample loading

Apply supernatant prepared as described above at a flow rate of less than 0.5mL/min and then dry SPE cartridge under full vacuum (10-12 in. Hg) for 0.5 min.

Elution

Elute with 1mL water at a flow rate less than 0.5mL/min; collect eluate in a sample vial; draw any residual water from the sorbent by applying full vacuum. This eluate is used for LC-MS analysis.

Table I. Method Validation Strata-X-C SPE Extraction and LC/MS/MS Detection*[1]

Conc. (ng/mL) (Spiked)	Absolute Recovery, % (n=6) (Daily intervals)	RSD, % (n=6) (Daily intervals)
50	75-105	<15.0
500	≥84	<3.0
2000	>90	<1.0
	Linear Regression (R^2)	
10-1000 (6 pts)	≥0.999	
100-2000 (5 pts)	>0.999	
100-2000*[2](5 pts)	>0.999	
*1-sample pretreatment before SPE not included; *2 -external standard		

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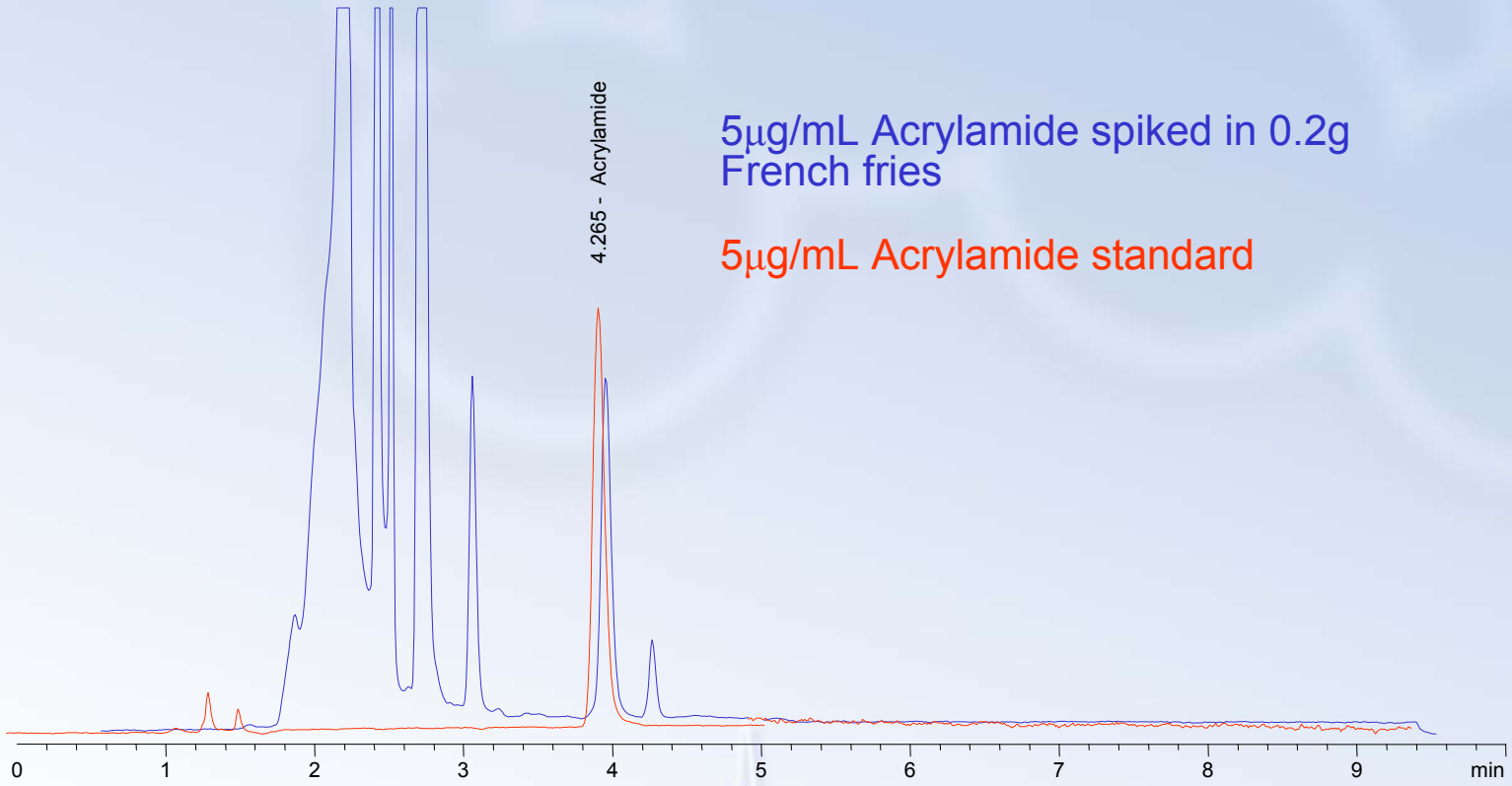
Figure 1 LC/UV chromatogram of Acrylamide in French Fries Extract (1)



LC column: Synergi Polar- RP® 4µm 80Å 250 x 4.6mm; Mobile phase: 96:4/Water:Acetonitrile at 1.0mL/min; UV detection: 210nm; Injection volume: 20µL.

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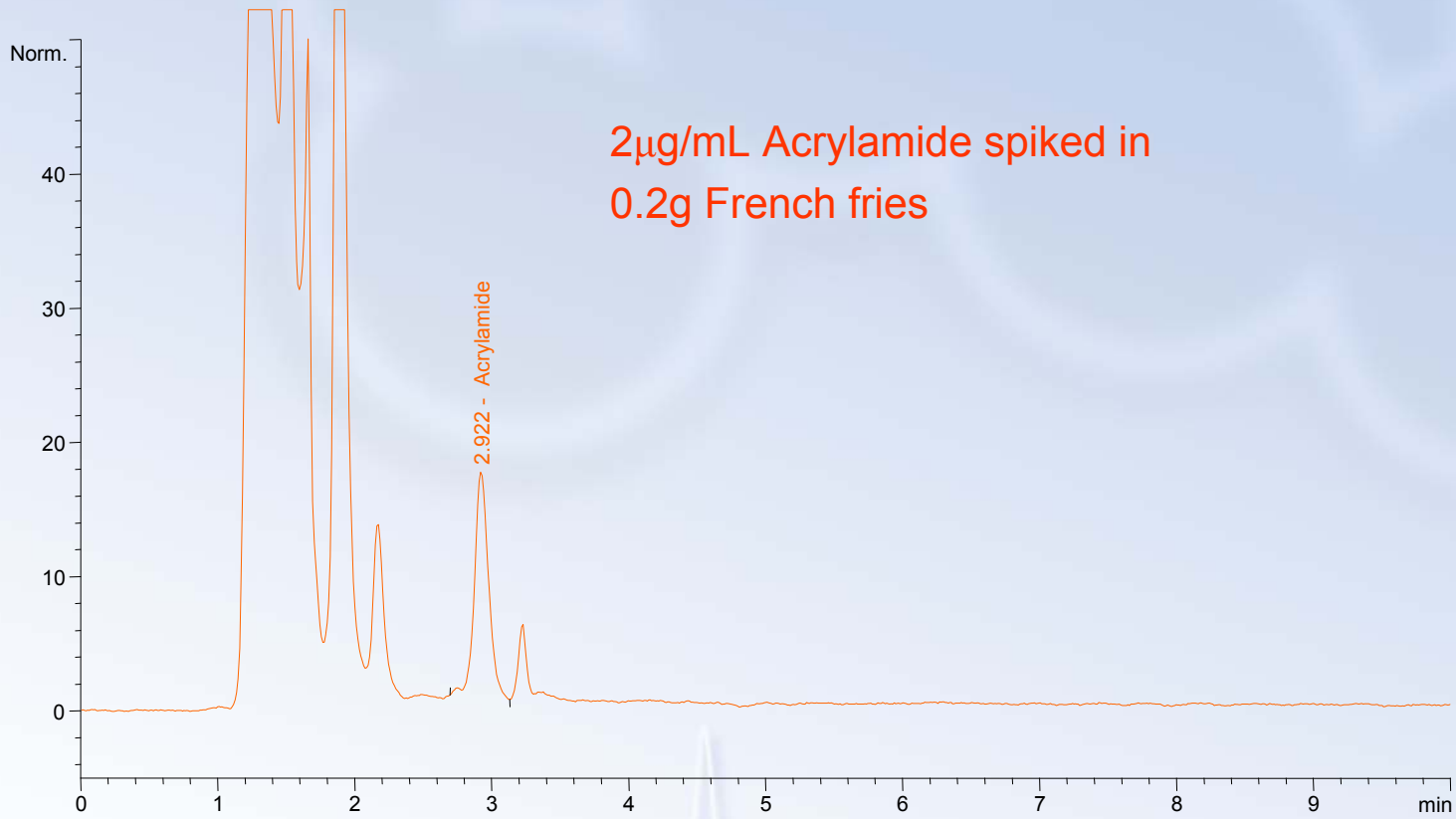
Figure 2 LC/UV chromatogram of Acrylamide in French Fries Extract (2)



LC column: Synergi Polar-RP 4μm 80Å 150 x 3.0mm; Mobile phase: 96:4/Water:Acetonitrile at 0.4mL/min; UV detection: 210nm; Injection volume: 10μL.

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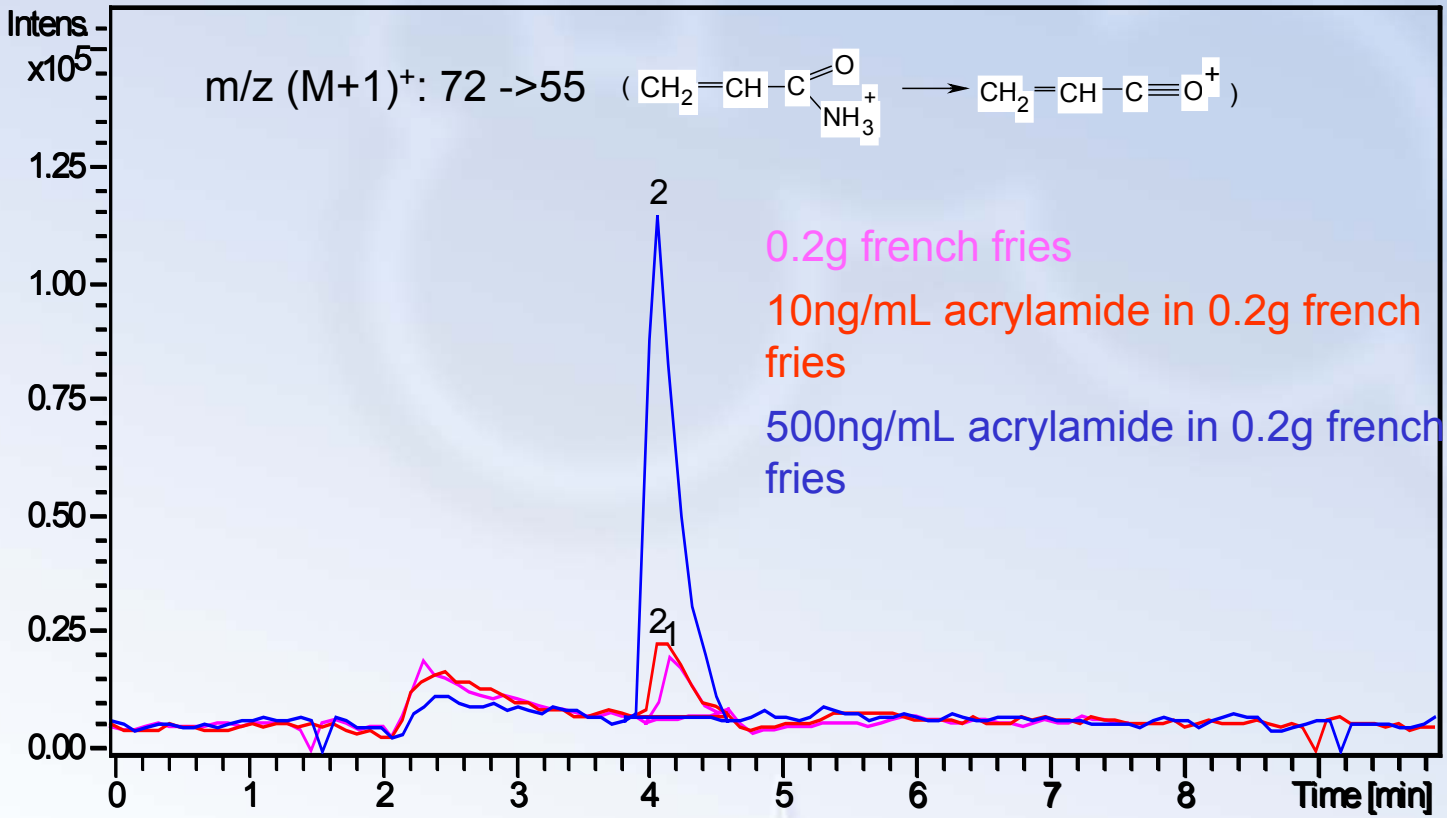
Figure 3 LC/MS/MS Extracted Ion Chromatograms



LC column: Synergi Hydro- RP® 4μm 80Å 250 x 3.0mm, Mobile phase: 94:6/Water:Methanol (both contain 0.1% formic acid) at 0.5 mL/min, Injection volume: 25μL

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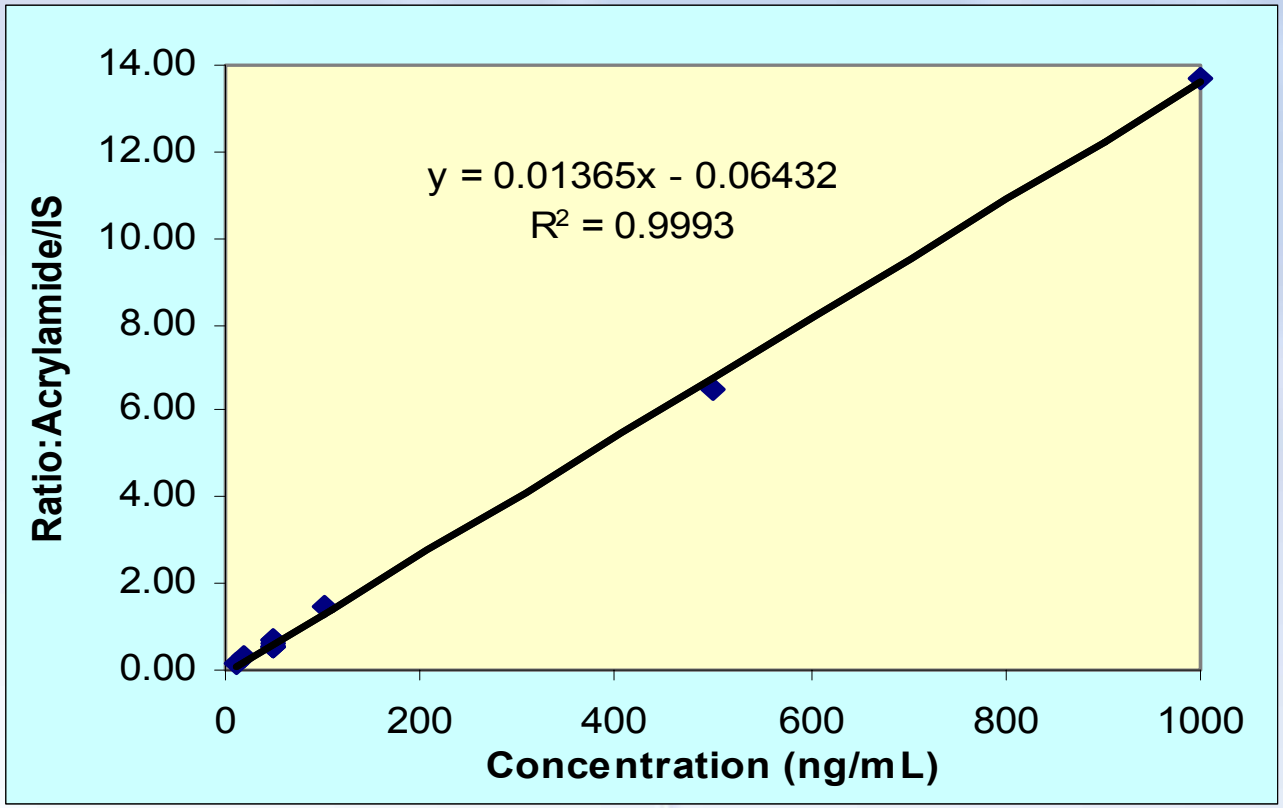
Figure 4 Linearity of Strata-X-C for Acrylamide



The standard calibration curve of acrylamide at concentrations of 10, 20, 50(n=6), 100, 500, 1000 ng/mL, and IS acrylamide-d3 100ng/mLspiked into 1mL of 0.2g/mL french fries extract. Detection by LC/MS/MS.

Assay Application

Table 2. Survey of Acrylamide Levels in French Fries from Fast Food Restaurants	
French Fries	Acrylamide (μg/kg)
Sample1 (n=4)	796 ± 80
Sample2 (n=2)	402 ±32
Sample3 (n=2)	328 ± 32

Discussion of Results

- Stratra-X-C exhibits a binary nature of polar interaction and cation exchange interaction
- Acrylamide may be protonated by the strong acid sorbent surface of strata-X-C and retained by both polar and ion-exchange interactions
- As its high polarity confers acrylamide high affinity for water, elution with water will disrupt its interaction with the sorbent surface. Therefore, the selective extraction of acrylamide from food matrix can be accomplished.
- Other matrix constituents like hydrophobic compounds and cations present in the food will remain on the sorbent by reversed-phase or strong cation ion exchange interactions.

Discussion of Results (cont.)

- The extractions using Strata-X-C give chromatograms with clean background as detected at 210nm and a well separated acrylamide peak from other matrix components as shown in Figure 1 and Figure 2
- The reproducibility of extraction demonstrates excellent precision at three different concentration levels (Table I).
- The absolute recoveries were determined by comparing peak areas of same level standards and spiked acrylamide extracts subtracted for blank. The recoveries are 75-105% for different concentration levels.
- The linearity was studied in two different concentration ranges: 10-1000 ng/mL acrylamide (with IS at 100ng/mL), and 100-2000ng/mL (with IS at 500ng/mL). The linearity based on external standard was also evaluated. Results demonstrate good linearity in all cases with values of R^2>0.998 (Table I).

Conclusion

The extraction of acrylamide from french fries by SPE using Strata-X-C improves the selectivity of extraction and provides clean samples which give chromatograms with relatively little background interference. It also eliminates the necessity of column flushing, as matrix components are not present to build up under weak mobile phase conditions (high ratios of water).

The method of Strata-X-C sample preparation for the determination of acrylamide in french fries by LC/MS/MS analysis is simple, specific and reliable. It provides an attractive alternative to currently applied or proposed methods for the determination of acrylamide in food matrixes. This sample preparation method is also well suited for the HPLC/UV analysis of high concentrations (>500µg/kg) of acrylamide present in food.

References

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