Rapid and Reproducible Extraction of Acrylamide in French Fries using a Single SPE Sorbent - Strata™-X-C

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Acrylamide (MW 71, m.p. 84.5°C, logP 0.67) belongs to the enamide class of organic compounds and is an extremely important industrial chemical. The polyacrylamide family of polymers and copolymers derived from this monomer find applicability in widely differing areas such as clarification of drinking water, flocculants for waste water treatment, oil recovery, soil conditioning, agriculture and biomedical purifications. However, the neurotoxicity of acrylamide poses a serious health risk and leads to a reluctance by the public at large to accept the polyacrylamides as safe materials.[1] To add to these concerns, the presence of acryamide has recently been detected in food products prepared at high temperatures.[2,3] Earlier methods for the detection of acrylamide involved liquid-liquid extraction followed by derivatization (bromination) and GC/ECD or GC/MS analysis.[4,5] The high detection limits associated with such methods limit their applicability for the determination of low levels of acrylamide in food matrices, in addition to being tedious and laborious.

Recently published methods for the determination of acrylamide[6,7,8] utilize either one or two successive solid phase extraction (SPE) sorbents for sample preparation before LC/MS detection. The method that uses a single reversed-phase sorbent is based on nonretentive SPE, removing only the hydrophobic components of the sample while the acrylamide passes, unretained, through the sorbent. A more extensive clean up procedure has been proposed that requires two SPE sorbents. In this method, a hydrophilic/lipophilic sorbent is applied first for the removal of most sample components. The sample is then passed through a second tube that contains a mixed mode sorbent that refines the extract of the target analyte - acrylamide. These methods provide cleaner extractions for analysis, but are time consuming and costly.

In this application note, we present a simple and efficient method which uses a single SPE sorbent **- strata-X-C -** for the extraction of acrylamide from a food sample (French fries). strata-X-C is a revolutionary, patent pending polymeric resin that has been functionalized with polar and strong cation exchange groups. As a result, strata-X-C exhibits numerous retention mechanisms including hydrophobic, hydrophilic, π-π and strong cation exchange, making it ideal for the extraction of acrylamide from food samples. All of these retention mechanisms will come into play for the effective cleanup of the acrylamide sample.

Instrumentation

HPLC:	Agilent 1100 series (Palo Alto, CA USA)
MS:	Bruker Esquire 2000 Ion-Trap MS analyzer, (Billerica, MA USA) Ion source APCI in positive ion mode MRM (acrylamide m/z:72→55, acrylamide-d3 m/z:75→58).
LC columns:	Synergi Hydro-RP 4µ 250 x 3.0mm (LC/MS) Synergi Polar-RP 4µ 150 x 4.6mm (HPLC/UV)

Experimental Conditions

Specimen 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 sample with internal standard - acrylamide-d3. Apply supernatant to a conditioned strata-X-C tube as described below.

SPE method

1. **condition:** apply 2 x 1mL methanol, followed by 2 x 1mL water to strata-X-C tube at a flow rate of 2mL/min.
2. **sample load:** apply supernatant (prepared as described above) at a flow rate of less than 0.5mL/min.
3. **dry:** 30 seconds under vacuum (10-12 inches Hg).
4. **elution:** 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 ready for LC/MS analysis.

Important! The recommended volumes are for 100mg of sorbent bed mass. The volumes will have to be adjusted for smaller or larger sorbent masses.

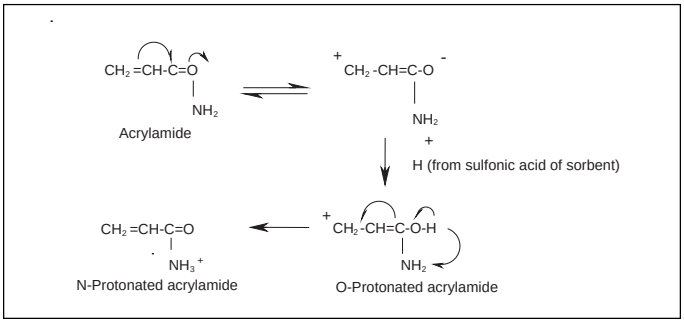
Analysis

A calibration curve was generated by spiking acrylamide and acrylamide-d3 (both prepared in water) at different concentrations in 1mL of French fries supernatant. Two unspiked samples of supernatant served as blanks.

Results and Discussion

For retaining a polar and highly water-soluble (215.5g/100mL water) analyte such as acrylamide on an SPE sorbent, one has to harness every possible mode of polar interaction. It is well known that enamides can be protonated with Bronsted acids to form an N-acyliminium cationic species in solution.[9,10] Such behavior can be favorably utilized to retain acrylamide on a cation exchange sorbent

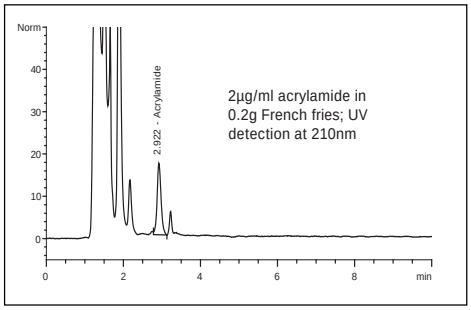
Scheme 1. Protonation of acrylamide by strata-X-C.



such as strata-X-C, which can also undergo other polar interactions as well. The strongly acidic sulfonic acid moieties on strata-X-C can furnish the hydronium ion for protonating acrylamide, as shown in **Scheme 1**. There is the choice of using either silica- or polymer-based cation exchange sorbent for extracting acrylamide. However, there are several advantages in using the polymeric sorbent. First, strata-X-C has larger surface area (800m²/g vs. 500m²/g) and hence greater retentivity than silica based materials. Second, there is a greater concentration of ion exchange moieties on a polymeric

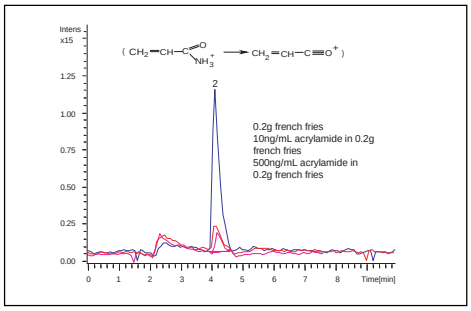
surface than a silica surface. Furthermore, a polymeric sorbent does not carry any silanol groups as the silica surface does. This is significant as silanol groups interact with sulfonic acid groups through more than one mechanism and hence render some of the sulfonic acid groups unavailable for interactions with acrylamide. A comparative evaluation of Strata SCX and strata-X-C sorbents reveals the silica-based SCX material yields around 50% recovery of acrylamide, while the recovery from the polymeric strata-X-C sorbent is near quantitative. Lastly, the polymeric sorbent has more retention modes for interaction with sample interferences. For extracting acrylamide from French fries, one has to use water not only due to the high solubility of acrylamide in this solvent, but also to minimize dissolution of hydrophobic molecules in this food product. Furthermore, in developing an SPE protocol for acrylamide, the wash step has to be excluded in view of its solubility in water. Elution with water also has the advantage of eliminating the desorption of all hydrophobic impurities which remain adsorbed on the sorbent.

Figure 1. LC/UV chromatogram of acrylamide in a French fries extract using strata-X-C.



LC conditions: Synergi Polar-RP 4µ 150 x 3.0mm, Mobile phase: 94:6 (v:v) water: CH_3CN at 0.4mL/min. Injection volume: 10µL.

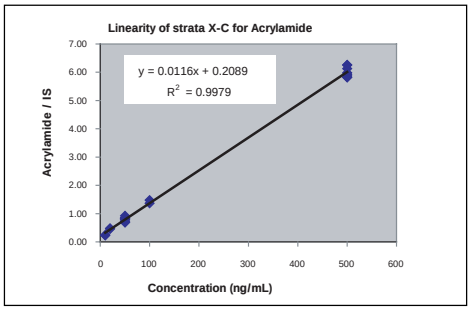
Figure 2. LC/MS/MS extracted ion chromatograms for m/z: 72 ->55.



LC conditions: Synergi Hydro-RP 4µ 250 x 3.0mm, Mobile phase: 94:6 (v:v) water: methanol (both contain 0.1% formic acid) at 0.5mL/min. Injection volume: 25µL

As shown in **Figure 1**, HPLC(UV) chromatograms recorded at 210nm demonstrate that extraction using strata-X-C gives clean extracts with low background and a well separated acrylamide peak from all matrix components. **Figure 2** shows the ion chromatogram for the transition of the protonated molecular ion of acrylamide (m/z 72) to the acryloyl cation (m/z 55) through the elimination of NH_3 (ammonia). The mobile phase for LC/MS comprised of 96:4 water/methanol containing 0.1% formic acid. The data in this figure shows the high sensitivity of the MS mode of detection, even for trace levels of acrylamide. The reproducibility of extraction demonstrates excellent precision at three different concentration levels (**Table 1**). The absolute recoveries were determined by comparing peak areas for same level standards and for spiked acrylamide extracts subtracted for blank. The linearity was studied in two different concentration ranges: 10-500 ng/mL acrylamide (with IS at 100ng/mL), (see **Figure 3**) and 100-2000ng/mL (with IS at 500ng/mL) as shown in Table I. The linearity based on external standard was also evaluated. Results demonstrate good linearity in all cases with values of R^2>0.998.

Figure 3. Linearity of extraction of acrylamide using the strata-X-C method.



Calibration curve prepared by spiking 10, 20, 50(n=6), 100, 500 (n=6) ng/mL acrylamide, respectively, and 100ng/mL IS acrylamide-d3 into 1mL of 0.2g/mL French fries extract. Detection: LC/MS/MS.

Conclusions

The novel selectivity of strata-X-C allows for the rapid extraction of acrylamide from French fries using a simple and reproducible method. Since the method uses a single SPE sorbent it is an attractive alternative to currently applied or proposed methods for the extraction of acrylamide in food samples. The versatility of this clean up method allows for the analysis of acrylamide present in food at ppb or ppm concentration levels, giving chromatograms with relatively little background interference. It also eliminates the necessity of column flushing, as sample components are not present to build up under weak mobile phase conditions (high percentage of water).

Table 1. Results of SPE & LC/MS/MS method validation

Concentration (ng/mL)	RSD, % (n=6)	Recovery, % (n=6)
50	9.55	>78
500	2.20	≥84
2000	0.82	>91
	R^2	
10-500 (5 pts)	>0.999	
100-2000 (5 pts)	>0.999	
100-2000*(5 pts)	>0.999	
* external standard		

References
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Order Information

Order Number	Description
8B-S029-TAK	strata-X-C 30mg/1mL Tubes (100/Box)
8B-S029-UBJ	strata-X-C 60mg/3mL Tubes (50/Box)
8B-S029-ECH	strata-X-C 100mg/6mL Tubes (30/Box)
8B-S029-FCH	strata-X-C 200mg/6mL Tubes (30/Box)
8B-S029-HCH	strata-X-C 500mg/6mL Tubes (30/Box)
8E-S029-AGB	strata-X-C 96-Well Plate 10mg/well (2/Box)
8E-S029-TGB	strata-X-C 96-Well Plate 30mg/well (2/Box)
00G-4375-Y0	Synergi Hydro-RP 4µ 250 x 3.0mm
00F-4336-E0	Synergi Polar-RP 4µ 150 x 4.6mm