High-Throughput Detection of Acrylamide in Food with a Rapid, Sensitive LC/MS/MS Method

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Introduction

Acrylamide is a monomer used in the synthesis of polyacrylamides; polymers that are used as flocculants in the treatment of municipal water supplies and in paper and pulp processing. The monomer is a white, crystalline solid at room temperature that is freely soluble in methanol, ethanol, water, and acetone. Spontaneous (and potentially dangerous) polymerization occurs once acrylamide reaches it's melting point or when exposed to oxidizing agents. Industrial applications generally control the initiation of polymerization by the use of UV light. In addition to it's use in water treatment, polyacrylamides have found wide usage as additives in cosmetics, soil-conditioning agents, and in gel electrophoresis of biological samples (PAGE). Acrylamide itself is also reactive from a biological perspective and has been found to cause tumors in rats after long-term exposure (1). Results from these and other experiments, in vivo and in vitro, has shown acrylamide to be genotoxic, a carcinogen (2) and may have several detrimental effects on the male reproductive system in animals. It has also been shown to cause nerve damage in humans, evident as peripheral neuropathy (3).

It has been suggested that acrylamide in cooked food arises by the reaction of glucose with asparagine (4). After consumption of food containing acrylamide the body primarily metabolizes it into glycidamide which has been shown to react with DNA and is presumed to be the mutagenic or cancer-causing agent.

Due to these carcinogenic and mutagenic effects, the World Health Organization (WHO) and the EPA guidelines suggest a limit for acrylamide in drinking water of 0.5 mg/L corresponding to 1 mg/day acrylamide intake. In 2003, the European Union adopted a 0.1 mg/L limit and the UK independent Committee on Carcinogenicity of Chemicals in Food advises that exposure to genotoxic carcinogens such as acrylamide be as low as reasonably possible.

Presented here is a high throughput method for accurately detecting acrylamide in food by use of a Varian 1200L LC/MS/MS system in combination with a CombiPAL autosampler. This method can analyze any combination of 96 samples (standards or unknowns) in a little more than 5 hours, including the sample extraction time. The exact example given here is 28 samples analyzed in triplicate with a single calibration curve of 10 points (triplicate runs). Total time for this analysis was approximately 6 hours as the calibration curve was run in triplicate bringing the total number of samples analyzed to 120.

Instrumentation

- Varian ProStar™ 210 Solvent Delivery Modules (2)
- Varian 1200L LC/MS/MS equipped with an ESI source
- CTC Analytics HTS PAL AutoSampler

Materials and Reagents

All chemicals were reagent or HPLC grade from Sigma-Aldrich Corporation (St. Louis, MO) with the exception of Acrylamide-1,2,3-$^{13}C_3$ internal standard solution (1 mg/mL in methanol) from Cambridge Isotope Laboratories, Inc. (Andover, MA).

Sample Preparation

Stock solutions were prepared in water at 1 mg/mL. All diluted samples were also prepared in water (HPLC grade).

Twenty-eight (28) samples of dried foodstuffs (for human and animal consumption) were obtained from various sources. Samples, if not already in powder form, were ground in a food mill and stored in glass vials, at 4° C in dried/powdered form. Samples were weighed out in triplicate and placed (one well per sample) into 96-well 2 mL deep-well polypropylene plates (84 unique samples). Approximately 50 mg of each sample was weighed and recorded. To these dried samples was added 1 mL HPLC grade water containing 500 ppb of the internal standard (IS). The first row of samples (Row A) of the plate was left empty so that it could be used for a calibration curve, allowing it to be run first before the samples. The plate was sealed with a silicon mat and gently shaken for 2 hours at room temperature. After 2 hours the samples were removed, with the aid of a multichannel pipette, to a Varian Captiva® 96-well filter kit plate with a PVDF membrane. Samples were allowed to pass thru the membrane (with the aid of a vacuum) into a second 96-well 2 mL polypropylene plate. Wells A11 and A12 were filled with 1 mL of water (blanks) and wells A01 thru A10 were filled with acrylamide solutions to form a standard curve (from low to high concentration). The injected samples were analyzed by the Varian 1200L with the following MS/MS conditions.

Mass Spectrometry Conditions

Ionization Mode:	ESI positive
Collision Gas:	2.0 mTorr Argon
API Drying Gas:	33 psi at 260° C
API Nebulizing Gas:	50 psi
Scan Time:	1 sec
SIM Width:	0.7 amu
Needle:	4000V
Capillary:	30V
Shield:	600V
Detector:	2000V

Samples were injected by the CTC Analytics HTS PAL Autosampler according to the following method.

CPAL Method

Injection Mode:	LC
Read Bar Code:	Never
Required Syringe:	100 µL Liquid
Pre-Inj Washes Solvent 1:	0
Pre-Inj Washes Solvent 2:	2
Pre-Inj Sample Flushes:	0
Sample Vial Penetration Depth Pct:	90%
Plunger Fill Speed:	5.0 µl/sec
Fill Strokes:	0
Viscosity Delay:	0.3 sec
Air Volume Below Sample:	0 µL
Injector:	LC Vlv1
Pre-Injection Delay:	0.5 sec
Plunger Inject Speed:	5 µl/sec
Post-Injection Delay:	0.5 sec
Post-Inj Washes Solvent 1:	0
Post-Inj Washes Solvent 2:	3
Post-Inj Valve Washes Solvent 1:	0
Post-Inj Valve Washes Solvent 2:	0
LC Cycle Time (Prep Ahead):	OFF

LC Conditions

Column:	Varian Pursuit® MS 5 mm 100 x 2 mm
Solvent A:	1 mM Ammonium Acetate in Water
Solvent B:	0.1% formic acid in Methanol
LC Program:	Isocratic (95% A / 5% B) – 4 min
Flow rate:	0.2 mL per min
Injection Volume:	10 µL

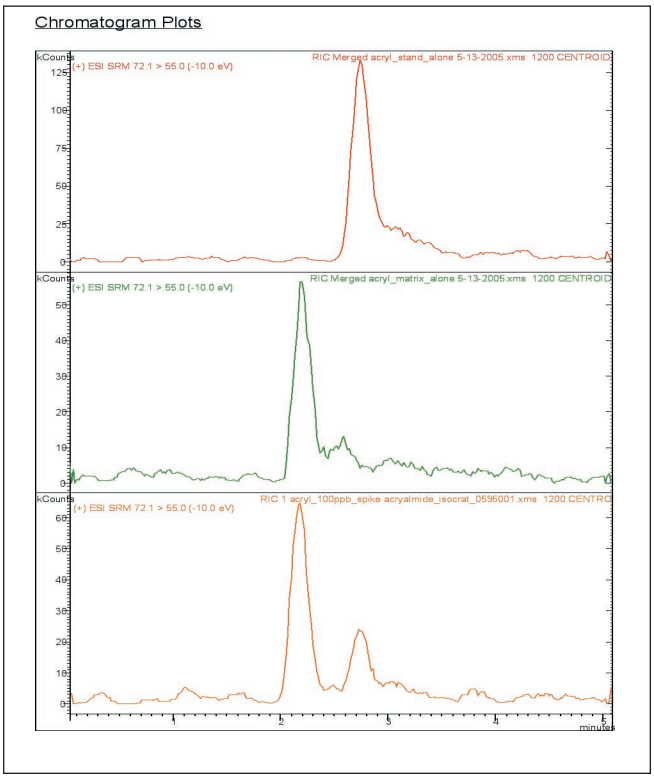

Figure 2. Chromatograms of A) acrylamide standard, B) matrix alone and C) 100 ppb acrylamide spiked into matrix.

MS/MS Scan Parameters

Analyte	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (V)
Acrylamide	72.1	55	-10
13C-Acrylamide (IS)	75.1	NA	NA

Results and Discussion

In this method, good separation of the acrylamide is obtained, in less than 5 minutes, on a Varian Pursuit MS column with 1 mM Ammonium Acetate (Solvent A) and 0.1% formic acid in Methanol (Solvent B) and an isocratic method (see LC Method, above). The Pursuit MS column has a unique fluorinated phase, allowing for separation of polar compounds without tailing or phase collapse sometimes seen in standard C18 columns. This unique phase allows for a separation that is up to 25% faster than competing methods at the same detection limits. More importantly, the method does not require the sample to undergo lengthy extraction processes or derivitization as in some GC/MS methods. Linear calibration curves were obtained in the range of 10,000 to 20 ppb (n=3, r=0.994) (Figure 3) and limits of detection and quantification are 8 and 20 ppb, respectively. These limits are comparable or superior to what exists in other methods available, in particular a currently available kit where the calibration curve does not extend below 50 ppb. Moreover, the competitors system only tests 12 samples per kit whereas one Captiva kit contains 5 filter plates, each with 96-wells (480 total samples/ standards).

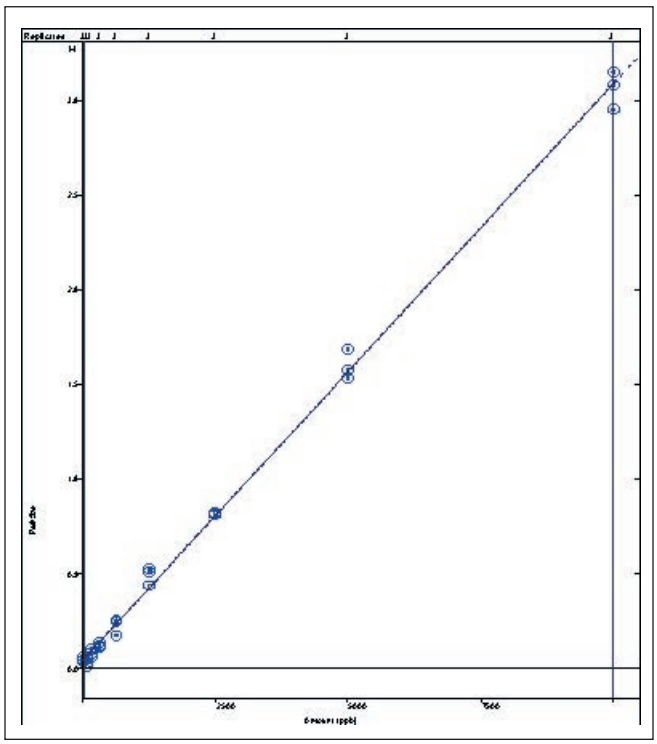

Figure 3. Calibration curve of acrylamide.

Sample	Triplicates Average (pg/µl)	Amount of Material (mg)	Results (mg/kg)
S1	133.6	53.92	2.48
S2	93.6	51.95	1.80
S3	70.7	56.4	1.25
S4	46.5	51.4	0.91
S5	10.2	56.1	0.18
S6	37.9	54	0.70
S7	131.8	51	2.58
S8	41.5	52.3	0.79
S9	50.2	56.6	0.89
S10	163.2	55.95	2.92
S11	78.9	56.13	1.41
S12	15.1	51.9	0.29
S13	4.74	50.8	0.09
S14	18.1	51	0.36
Chip1	28	53.4	0.52
Chip2	21.25	52.8	0.40
Chip3	34	56.5	0.60
cereal1	ND	56.7	ND
cereal2	ND	52.6	ND
cereal3	ND	51.2	ND
MM1	82.085	51.7	1.59
MM2	83.89	58.5	1.43
MM3	78.2	53.7	1.46
CA1	297.4	49.6	6.00
CA2	235	57.9	4.06
CA3	267.3	56.2	4.76
CAT1	ND	53.6	ND
CAT2	ND	52.3	ND

Table 1 Results of twenty-eight unique samples of various foodstuffs.

Utilizing the high-throughput nature of the Captiva plates, twenty-eight unique samples were weighed out in triplicate, extracted, and analyzed with the aid of a CombiPAL HTS autosampler and the LC/MS/MS system mentioned. Results for all samples are shown in Table 1. Samples "cereal" and "CAT" (denoting a breakfast cereal and dried cat food) were known to be baked, not fried, therefore it is not surprising that they did not contain detectable levels of acrylamide.

Conclusion

A high-throughput LC/MS/MS method for the detection of acrylamide in food is presented. This method allows for the direct analysis of acrylamide without the lengthy derivatization process required for the GC/MS analysis. Calibration curves are linear in the range used and allows for better detection (lower LODs) than competing kits with a simpler and less time-consuming method.

References

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4. C and EN, Pg. 6, Dec. 2, 2002.