Detection and Quantitation of Acrylamide in Foods

Researchers in Sweden, the U.K., and Norway have reported finding the chemical acrylamide in certain foods. The acrylamide appears to form as a byproduct of certain cooking processes, such as frying. FDA scientists have developed a method to measure acrylamide levels in foods. The FDA is posting this method on its website to provide other researchers the opportunity to review and use the method.

Purpose

To provide an analytical method for the quantitative determination of acrylamide in foods.

Update Information

This section will identify differences between the present method and the last method posted on the web site. This is the second revision of the method.

Changes made in the first revision (23 July 2002) were as follows:

The methanol elution step was removed because higher recoveries were achieved with a water wash of the Oasis column instead of the methanol wash. An additional solid phase extraction (SPE) step was added to the method using a sorbent bed of C8, strong anion and cation exchange media. This step improved signal to noise ratios in samples with high levels of coextracted materials when used in conjunction with the Oasis SPE step. Post-column addition of 2-propanol to the liquid chromatography (LC) eluent eliminated background interference with the acrylamide parent mass.

Changes in the second (current) revision are as follows:
Cleanup - Samples are centrifuged in groups of 6, with 12 at a time cleaned up by SPE. Extraction time on the rotating shaker was increased to 20 min to permit completion of the centrifugation steps for one group of 6 prior to centrifugation of the second group of 6 needed to make a set of 12 test portions available for SPE cleanup. Centrifugal phase separation time was reduced from 30 min to 15 min. Centrifugal filtration time was reduced from 4 min to 2-4 min because most portions were completely filtered within 2 min. The volumes of methanol and water used to condition the SPE cartridges in steps 7 and 9 were reduced to save time and solvent. Less test portion is now loaded and collected in step 8 to achieve better cleanup. These changes allow one person to prepare 12 portions for analysis in about 1.5 hours.

Liquid chromatography/tandem mass spectrometry (LC/MS/MS) Determination - The optimal collision energy was determined for each of the transitions recorded by multiple reaction monitoring (MRM). Our best collision energy settings, desolvation gas flow and cone gas flow are reported. Optimal voltage and gas flows vary between instruments. These parameters should be determined for each instrument used to analyze for acrylamide. A formula for calculation of acrylamide level in parts per billion is provided.

Cautionary statement

Acrylamide monomer has been implicated as a genetic and reproductive toxicant. It has also been reported to be a neurotoxicant. Appropriate laboratory safety precautions should be used when working with this chemical. It is stable in acid, decomposes in base, and is sensitive to light. The method described in this report has not been fully validated, and is still in the development process. Comments, in the form of method notes (MN), are indicated in parentheses, and note important steps or items that continue to undergo development.

Chemical Stability

Appropriate caution should be exercised in working with acrylamide. Use of red glass volumetric flasks to prepare standard solutions is suggested. Place extracts and standards in amber autosampler vials for analysis. Stock solutions of acrylamide are stable for up to 6 months at room temperature in red glass containers. Working standard solutions of 40 ppb and less in 0.1% formic acid held in clear glass containers will degrade substantially in 1 week. Verify the linearity of the standard curve for each data set and replace standards as warranted. Extracts are analyzed overnight and
discarded after their data have been processed and examined. Long-term extract stability is not known.

**Reagents and Consumables**

- Acrylamide (Sigma Chemical Company, St. Louis, MO)
- $^{13}$C$_3$-labeled acrylamide (Cambridge Isotope Laboratory, Andover, MA)
- HPLC grade acetonitrile (Omnisolv, EM Science, Gibbstown, NJ)
- HPLC grade methanol (Omnisolv, EM Science, Gibbstown, NJ)
- HPLC grade 2- propanol (Omnisolv, EM Science, Gibbstown, NJ)
- HPLC grade water (Omnisolv, EM Science, Gibbstown, NJ)
- Formic acid 99% (Sigma Chemical Company, St. Louis, MO)
- Glacial acetic acid 99% (Sigma Chemical Company, St. Louis, MO)
- Maxi- Spin filter tube, 0.45 μm PVDF (Alltech Associates, Deerfield, IL)
- 50 mL polypropylene conical tube with cap (Becton Dickinson)
- Hydro- RP 80A HPLC column (2 x 250 mm), 4 micron packing (Phenomenex, Torrance, CA). Wash column a minimum of 20 min with 50:50 methanol:acetonitrile after 48 samples or at end of daily operations. Mobile phase re-equilibration for analyses will require 1.5 hr.
- OASIS HLB 6 mL solid phase extraction cartridge, 200 milligram packing (Waters Corporation, Milford, MA).
- Bond Elut - Accucat (mixed mode, C8, SAX and SCX) 3 mL solid phase extraction cartridge, 200 milligram packing (Varian Inc., Harbor City, CA).

**Instrumentation**

- Agilent (Palo Alto, CA) Model 1100 autosampler, binary HPLC pump and column heater
• Micromass Inc. (Manchester, UK) Quattro micro triple quadrupole mass spectrometer

Sample Preparation

1. Crush and homogenize a portion of sample equal to the manufacturer's recommended serving size with a food processor or equivalent device.

2. Weigh a one-gram portion of crushed sample into a 50 mL polypropylene graduated conical tube with cap.

3. Add 1 mL of internal standard solution (\(^{13}\)C\(_3\)-labeled acrylamide in 0.1% formic acid, 200 ng/mL), followed by 9 mL of water to the test portion. Shake by hand or vortex briefly to disperse test portion in water prior to step 4.

4. Mix for 20 minutes on a rotating shaker. (MN: Do not heat or sonicate, as this may generate an extract that will clog the SPE column.)

5. Centrifuge at 9000 rpm for 15 min. Promptly remove 5 mL portion of clarified aqueous phase for spin filtration and SPE. Avoid top oil layer and bottom solids layer when removing portion of aqueous phase.

6. Place 5 mL portion in Maxi-Spin filter tube, 0.45 \(\mu\)m PVDF (Alltech #2534). Centrifuge at 9000 rpm for 2-4 min. If filter clogs, insert new filter into tube, pour unfiltered liquid onto new filter and continue centrifugation until most of the liquid has passed through filter.

7. Condition OASIS SPE cartridge with 3.5 mL methanol, followed by 3.5 mL of water. Discard methanol and water portions used to prepare cartridge. A number of SPE cartridges were tested during development of this method, and all of them had different analyte retention and elution characteristics. Do not substitute another SPE sorbent in this step without testing.

8. Load OASIS SPE cartridge with 1.5 mL of the 5 mL test portion extract. Allow extract to pass completely through the sorbent material. Elute column with 0.5 mL water and discard. Elute column with additional 1.5 mL water and collect for Varian SPE cartridge cleanup. Do not use a vacuum to speed up the elution process in any of the SPE steps.
9. Place mark on outside of Varian SPE cartridge at height of 1 mL liquid above sorbent bed. Condition Varian SPE cartridge with 2.5 mL methanol, followed by 2.5 mL of water. Discard methanol and water portions used to prepare cartridge. Load 1.5 mL portion collected in step 8 and elute to 1 mL mark before collecting remainder of eluted portion. Transfer to 2 mL amber auto-sampler vial for LC/MS/MS analysis. This step removes a number of early eluting co-extractives, resulting in better precision for sub-50 ppb measurements. Do not load more than 1.5 mL of extract onto Varian SPE cartridge.

Liquid Chromatography/Mass Spectrometry

1. Mobile phase composition: Aqueous 0.1% acetic acid, 0.5 % methanol
2. Column flow rate: 200 μL/min
3. Post-column makeup flow rate: 50 μL/min 1% acetic acid in 2-propanol
4. Injection volume: 20 μl
5. Column temperature: 26°C
6. Acrylamide elution time: approximately 7.1 minutes
7. Ionization Mode: Positive ion electrospray
8. Probe temperature: 240°C
9. Source temperature: 120°C
10. Desolvation gas flow: 710 L/hr nitrogen
11. Cone Gas flow: 153 L/hr nitrogen
12. Collision gas pressure: 1 Torr argon
13. MRM ions: Acrylamide (m/z 72, 55, 27), Internal Standard (75, 58, 29). Collision energy of transitions for MRM: 72 > 72 and 75 > 75, 5 volts; 72 > 55 and 75 > 58, 10 volts; 72 > 27 and 75 > 29, 19 volts. Dwell time 0.3 sec each with 0.02 sec inter-channel and inter-scan delay.
14. Quantitation: Parts per billion acrylamide = (200 ng internal standard)(area of
m/z 55)/(area of m/z 58)(g of portion analyzed)(response factor). The response factor is the average response factor obtained from a concurrently run standard curve encompassing the range of apparent acrylamide levels in the test portions. Limit of quantitation is defined as the level at which a 10:1 signal/noise ratio is observed for the analyte quantitation ion (m/z 55).