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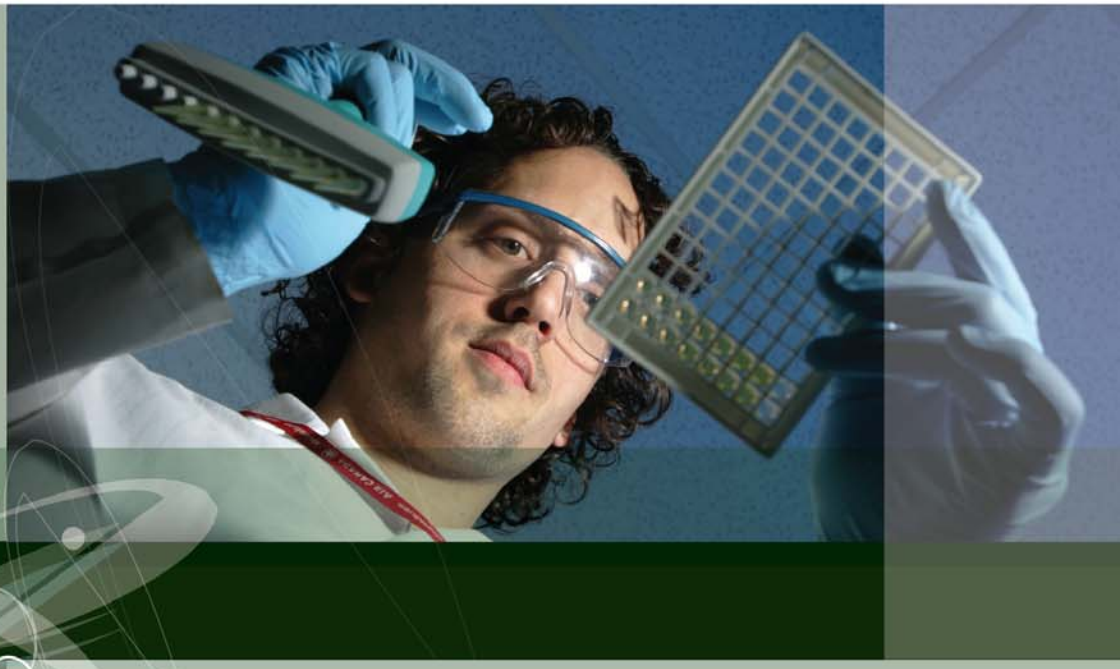
The Determination of Acrylamide in Foods by LC-ESI-MS-MS



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The Determination of Acrylamide in Foods by LC-ESI-MS-MS,
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The Determination of Acrylamide in Foods by LC-ESI-MS-MS

1.0 Purpose:

The sample is homogenized with water, spiked with a known amount of isotopically labeled $^{13}C_3$-acrylamide. The non-polar interferents are extracted with dichloromethane. An aliquot of the sample is centrifuged and the aqueous supernatant passed through a 5,000 Nominal Molecular Weight Limit (NMWL) centrifugal filter. Further cleanup is performed on both, a hydrophilic-lipophilic balanced copolymer Solid Phase Extraction (SPE) cartridge and a mixed mode strong cation / strong anion SPE cartridge. Calibration is performed using the method of external standards with a volumetric internal standard. Quantitation is aided by the use of a surrogate internal standard. Instrumental analysis is performed by reversed phase liquid chromatography with detection by Electrospray Ionization (+ve ion mode)-Mass Spectrometry/Mass Spectrometry.

2.0 Scope:

This method was developed by the Food Research Division, Food Directorate, Health Products and Food Branch. It has been validated for the determination of acrylamide in breakfast cereals. The method may be used for other foods including: French fries, bread, roasted nuts and coffee.

The limit of detection has been reported to be 5 ng acrylamide/g sample (matrix dependent) by Health Canada, Food Research Division.

3.0 Responsibilities:

The analyst performing the method is responsible for the execution of all outlined procedures.

4.0 Definitions:

Volumetric internal standard: a known quantity of a compound added to an extract or standard solution which is used to reduce or remove the dependence of the final analytical result on the injection volume or total solution volume.

Surrogate internal standard: a known quantity of a compound (ideally an isotopically labeled version of the target analyte, (MS Detection only) added at as early a stage as possible in the extraction procedure and used to correct for analyte losses.

5.0 Procedure:

5.1 Equipment/Materials

5.1.1 High-Performance Liquid Chromatograph consisting of:

- Tertiary pump capable of reproducibly delivering 0.175 mL/min. (Waters Alliance Separations Module 2695 or equivalent)
- Injector, capable of reproducible 10 µL injections, (Waters Alliance Separations Module or equivalent)
- SecurityGuard HPLC Guard Cartridge System, (Phenomenex p/n KJO-4282 or equivalent) utilizing a Phenomenex C18 Cartridge, (Phenomenex p/n AJO-4286)
- Thermo-Hypersil Analytical Column, Aquasil C18, 2.1 X 250 mm, d_p 5 µm, (Thermo p/n 77505-252130)
- Column heater capable of maintaining 28.0 ± 1.0°C, (Waters Alliance Separations Module 2695 or equivalent)

5.1.2 Mass Spectrometer

- Triple-stage Quadrupole, (Micromass Quattro Ultima or equivalent)

5.1.3 4/5 place analytical balance, (Mettler AE240 or equivalent)
5.1.4 Homogenizer, (Brinkman Polytron PT-MR 3000 or equivalent)
5.1.5 Shaker, horizontal, (Eberbach or equivalent)
5.1.6 High-speed refrigerated Centrifuge, (DuPont Instruments Sorvall RC-5B or equivalent)
5.1.7 5,000 Nominal Molecular Weight Limit centrifugal filter, (Millipore Centricon Plus-20 or equivalent)
5.1.8 Refrigerated Centrifuge, (Beckman GS-6R Centrifuge or equivalent)
5.1.9 SPE Vacuum Manifold, with 16 mm culture tube rack
5.1.10 Waters Oasis HLB SPE columns, 6 cc, 200 mg, (Waters p/n WAT106202)
5.1.11 Strong cation exchange/strong anion exchange mixed mode SPE cartridge, Bond Elut-Accucat, 3 cc, 200 mg, (Varian p/n 12282003)
5.1.12 50mL Teflon Centrifuge tube, FEP (Fluorinated ethylene propylene) 28.8 X 107.7 mm, (Nalgene p/n 3114-0050 or equivalent)
5.1.13 Variable Volume Pipettors, 10-100 µL & 100-1000 µL, (Eppendorf, or equivalent)
5.1.14 Gas-tight syringes, 10 µL, 50 µL, 100 µL & 250 µL volumes, (Hamilton, or equivalent)
5.1.15 Volumetric Flasks, Class A, 10 mL & 100 mL
5.1.16 5-10 mL Pipettor and tips, (Oxford or equivalent)
5.1.17 16 X 100 mm borosilicate glass disposable culture tubes, (Fisherbrand, Fisher cat. no. 14-961-29, or equivalent)
5.1.18 nylon membrane filters, 0.45 µm pore size, 47 mm diameter, (Whatman, Fisher cat. no. 09-902-10, or equivalent)

5.2 Reagents & Standards

5.2.1 Reagents

5.2.1.1 Methanol, Pesticide Residue Grade, (Burdick & Jackson, or equivalent)

5.2.1.2 Methylene Chloride, Distilled in Glass (DIG), (Caledon, or equivalent)
5.2.1.3 Ammonium Formate, 99.995+% (Aldrich, or equivalent)
5.2.1.4 Acrylamide, 99+%, (Aldrich, or equivalent)
5.2.1.5 Acrylamide-1,2,3-$^{13}C_3$ 1 mg/mL in Methanol, 99%, (Cambridge Isotope Laboratories, Inc. p/n CLM-813-1.2)

5.2.2 Preparation of Standards

Notes: It is highly recommended that the standards be prepared volumetrically using syringes instead of pipettors.

The concentration of the volumetric internal standard (i.e. 1,2,3-$^{13}C_3$-acrylamide) in each of the working standards is 50 ng/mL.

5.2.2.1-Stock 250 µg/mL acrylamide standard; accurately weigh out approximately 12.5 mg of acrylamide into a 100 mL amber sample bottle. Add a calculated mass of Milli-Q water (approximately 50 mL) to produce an acrylamide concentration of 250 µg/mL. Accounting for the temperature and density of the water, calculate the exact acrylamide concentration.
5.2.2.2 Intermediate 10 µg/mL acrylamide standard; dilute 0.4 mL of the stock 250 µg/mL acrylamide standard (5.2.2.1) to 10 mL in a volumetric flask with water. Calculate the exact concentration.
5.2.2.3 Stock 250 µg/mL 1,2,3-$^{13}C_3$-acrylamide standard; accurately dispense 0.5 mL of the 1 mg/mL 1,2,3-$^{13}C_3$-acrylamide into a 2 mL volumetric flask. Dilute to the mark with Milli-Q water.
5.2.2.4 Intermediate 10 µg/mL 1,2,3-$^{13}C_3$-acrylamide standard; dilute 0.4 mL of the stock 250 µg/mL 1,2,3-$^{13}C_3$-acrylamide standard (5.2.2.3) to 10 mL in a volumetric flask with water.
5.2.2.5 Prepare the working standards as outlined in Table I: Preparation of the Working Standards.

Table I: Preparation of the Working Standards

Standard no.	Volume of 10 µg/mL 1,2,3-$^{13}C_3$-Acrylamide (µL)	Volume of 10 µg/mL Acrylamide (µL)	Total volume (mL)	Nominal [Acrylamide] (ng/mL)
1	500	50	100	5
2	50	10	10	10
3	50	25	10	25
4	50	100	10	100
5	50	500	10	500
6	50	1000	10	1000
7	50	2000	10	2000

5.2.3 Preparation of Reagents

5.3.1.1 Stock ammonium formate: Prepare a stock 1 M ammonium formate solution by dissolving 0.63±0.01 g ammonium formate in about 5 mL of Milli-Q water followed by dilution to 10.0 mL with Milli-Q water.

5.3.1.2 1mM ammonium formate: Dilute 1 mL of the stock 1M ammonium formate (5.3.1.1) to 1 L with Milli-Q water

5.3.1.3 Mobile phase: Prepare the mobile phase by diluting 120 mL of methanol to 1 L with the 1 mM ammonium formate (5.3.1.2). Filter (0.45 µm) the mobile phase.

5.3 Process

5.3.1 Sample Preparation and Extraction

5.3.1.1 Reduce the sample particle size using the Retsch mill. Store the sample in the freezer until ready for use.

5.3.1.2 Using the homogenizer, homogenize 16.0 g of sample with 80 mL of Milli-Q water in a 150 mL beaker.

5.3.1.3 Transfer 24 g of the homogenate (equivalent to 4 g of sample) to a 50 mL teflon centrifuge tube. Using a syringe add 16 µL of the 250 µg/mL 1,2,3-$^{13}C_3$-acrylamide standard. Add 10 mL of dichloromethane.

Note: the sample contains 1 µg/g (1000 ng/g) 1,2,3-$^{13}C_3$-acrylamide.

5.3.1.4 Shake the sample solution at high speed on the horizontal mixer for 15 minutes.

5.3.1.5 Centrifuge at 15,000 rpm in the refrigerated centrifuge for 0.5-2 hours at 4°C. Use dichloromethane to balance the tubes. Centrifugation is deemed to be complete when the upper aqueous phase displays clarity (i.e. not cloudy) and there is enough volume to enable the removal of 9 mL of the aqueous phase.

5.3.1.6 Transfer 9 mL of the supernatant to the 5,000 NMWL centrifugal filter and centrifuge at 4000 rpm in the refrigerated centrifuge for 1-4 hours at 4°C. Centrifugation is deemed to be complete when enough volume has been collected to enable the removal of 2 mL of sample solution for sample clean-up.

5.3.2 Sample Clean-up

5.3.2.1 Condition the Waters Oasis HLB SPE cartridge(s) with 5 mL of methanol followed by 2 X 5 mL aliquots of Milli-Q H_2O.

5.3.2.2 Apply 2 mL of the filtrate (5.3.1.6) to the Oasis HLB SPE, followed by rinsing the cartridge with 1 mL of Milli-Q H_2O. Discard all eluates.

5.3.2.3 Elute the Oasis HLB SPE cartridge with 1 mL of Milli-Q H_2O, collecting the eluate in a culture tube.

5.3.2.4 Condition the Varian Accucat SPE cartridge(s) with 3 mL of methanol followed by 2 X 3 mL aliquots of Milli-Q H_2O.

5.3.2.5 Apply the eluant (5.3.2.3) to the conditioned Accucat SPE cartridge, discard the first 0.5 mL (approximately) of eluate followed by collection of the remaining eluate in a culture tube. Elute the cartridge with an additional 1 mL of Milli-Q H_2O. Transfer the sample to an amber vial and/or store in the dark at 4°C until ready for analysis.

5.3.3 Sample Queue

The sample queue shall be set up as follows: startup, blank, calibration standards, intersperse up to 5 samples, calibration standards, intersperse up to 5 samples, etc., shutdown.

5.3.4 Instrumental Conditions

5.3.4.1 LC Conditions

Column:	2.1 X 250 mm Keystone C18 Aquasil with a Phenomenex C18 guard column
Mobile Phase:	12% methanol in 1 mM Ammonium formate (isocratic)
Flowrate:	0.175 mL/min.
Injection volume:	10 µL
Column temp.:	28°C

5.3.4.2 Mass Spectrometer Conditions

Ionization mode:	+ve ESI
Desolvation temp.:	250°C
Source temp.:	120°C
Desolvation gas flow:	595 L/hr
Cone gas flow:	150 L/hr
Collision gas pressure:	2.9 X 10^{-3} mbar
Resolution settings:	13.5/14 for quadrupoles 1/3 respectively
Ion energies:	0.5/1.0 V for quadrupoles 1/3 respectively

Selected Reaction Monitoring (SRM)

mass span	0.1 dalton
inter channel delay	0.1 sec.

Notes: Acrylamide is quantitated using the 72>55 transition for the native form of acrylamide and the 75>58 transition for 1,2,3-$^{13}C_3$-acrylamide.

The 72>54 transition is used as the primary confirmation transition for acrylamide. The remaining transitions may be used for confirmation if the primary confirmation transition suffers from a matrix interferent.

Table II: Selected Reaction Monitoring				
Precursor Ion (m/z)	Product Ion (m/z)	Dwell Time (sec)	Cone Voltage (V)	Collision Energy
72	27	0.3	34	16
72	44	0.3	34	14
72	54	0.3	34	11
72	55	0.3	34	11
75	58	0.3	34	11

5.3.5 Calculations

Input the concentrations of the calibration standards' concentrations (nominal values listed in Table I, Preparation of Working Standards) into the MassLynx sample list table. 1,2,3-$^{13}C_3$-acrylamide is identified as an internal reference for acrylamide in the processing method editor of QuanLynx, (MassLynx 4.0). The 1,2,3-$^{13}C_3$-acrylamide concentration in the standards is 50 ng/mL and 1000 ng/g in the sample, (assuming all were prepared as outlined).

The calibration curve is generated using a linear regression, with the origin excluded and 1/x weighting.

For this method of quantitation the response (defined below) is plotted on the y-axis and acrylamide concentrations on the x-axis.

$$\mathrm{Re}\,sponse = Area_{acrylamide}\ \frac{[1,2,3-{}^{13}C_3-acrylamide]}{Area_{1,2,3-{}^{13}C_3-acrylamide}}$$

The slope of the calibration is the response divided by the acrylamide concentration.

$$Slope = \frac{Area_{Acrylamide}}{[Acrylamide]} \times \frac{[1,2,3-{}^{13}C_3-acrylamide]}{Area_{1,2,3-{}^{13}C_3-acrylamide.}}$$

Rearranging y=mx+b, to solve for x

$$\left(\frac{y}{m}\right) - b = x$$

A dimensional analysis of y/m leaves the [acrylamide] and b is in units of [acrylamide].

5.3.6 Reporting

The results are reported in units of ng of acrylamide/g of sample to 3 significant figures, (the 3rd significant digit having some uncertainty associated with it). Negative results are reported as < 5ng acrylamide/g sample.

5.4 Quality Control/Results Verification

5.4.1 Identification

Acrylamide is identified by its Retention Time (RT) of about 6 minutes in addition to its characteristic transition of 72>55. The RT of acrylamide in the sample(s) should match the standards' RT to within ±5%. The RTs of the labelled and the native acrylamide should be within ±0.05 min of one another.

5.4.2 Control Sample (if available)

Include one positive control sample in the sample set. The positive control shall produce a result that is within ±3 standard deviations of the mean based on historical data and ideally within ±2 standard deviations of the mean.

Pringles (no. 479) has historically produced the following statistics: mean = 734 ng/g; standard deviation = 49; n=7. Therefore, sample results ranging from 587 to 881 ng acrylamide/g of sample would be considered suitable for the acceptance of all sample results in a sample set.

5.4.3 Spike Sample

Notes: Spike one sample with every sample set.

The spike level selected is based on prior knowledge of a commodity's acrylamide concentration. For example if a sample is suspected to contain 1000 ng/g of acrylamide a 10 ng acrylamide/g sample spike would be inappropriate. A sample suspected to contain 1000 ng/g of acrylamide would be more appropriately spiked with 500 ng acrylamide/g of sample.

Spike breakfast cereal samples at 100 ng/g.

Spike potato chip samples at 500 ng/g.

5.4.3.1 Aliquot two 24 g portions of the homogenate (5.3.1.3) for the selected spike sample.

5.4.3.2 Spike one of the two 24 g homogenates (equivalent to 4 g of sample) (5.3.1.3). Spike a breakfast cereal sample homogenate with 40 µL of the 10 µg/mL acrylamide intermediate (5.2.2.2). Spike a potato chip sample homogenate with 200 µL of the 10 µg/mL acrylamide intermediate (5.2.2.2).

5.4.3.3 The spike sample recovery shall produce a recovery which is between ±3 standard deviations of the mean based on historical data and ideally within ±2 standard deviations of the mean. Historical data produced a mean recovery of 92% with a standard deviation of 7 (n=5), therefore the recovery must be within the range of 70-110%.

6.0 Safety & Environmental Information:

Analysts must become familiar with the hazards of all chemicals and reagents used in this procedure by referencing the MSDSs'.

7.0 Associated Documents:

Quantitative Trace Analysis by Combined Chromatography and Mass Spectrometry Using External and Internal Standards; Boyd, R.K.; Rapid Communications in Mass Spectrometry; vol. 7; pp. 257-271.