Application Note: AN 9195

Optimizing the Analysis of Acrylamide in Food by Quadrupole GC/MS

Key Words

- Food/Beverage
- Trace DSQ
- PTV
- Carbohydrates
- Carcinogen

Chromatography and Mass Spectrometry
GC/MS Application Note #9195

Trisa Robarge, Eric Phillips, Meredith Conoley
Thermo Electron Corporation, Austin, TX

Overview

Purpose: Several GC/MS methods for the analysis of acrylamide monomer in foods will be described, various ionization modes explored, and future areas for development offered.

Methods: Analytical methods for acrylamide in food using the Finnigan™ TRACE DSQ™ in positive EI and positive and negative chemical ionization (PCI and NCI) modes were developed. Two extraction methods with minimal sample preparation involved both derivatized and underivatized acrylamide. Selected Ion Monitoring (SIM) was chosen to increase sensitivity. A Finnigan TRACE™ GC Ultra with a PTV inlet provided the means for sample introduction and offered flexibility for optimized injections to accommodate sample matrix.

Results: The methods had a limit of detection of 5 ppb in EI+ and PCI SIM and of 2 ppb for NCI SIM, with linearity ranging from 2 to 1000 ppb. PCI SIM using ammonia as reagent gas provided the best blend of sensitivity and selectivity. Productivity was enhanced by using methods that did not include an evaporation step for concentration of the final extract.

Introduction

In April of 2002, Sweden's National Food Administration announced that levels of acrylamide monomer in common food items exceeded published intake limits for acrylamide in drinking water.[1] The Maillard reaction, responsible for characteristic browning, taste, and odors, is implicated in the increased levels of acrylamide, particularly in foods with high carbohydrate content. It also appears that the amino acid asparagine plays an essential role. A potential mechanism for this reaction is summarized in Figure 1.[2,3]

Because acrylamide is deemed a neurotoxin and a genotoxic carcinogen, the United States Environmental Protection Agency (USEPA) established a Minimum Contamination Level Goal of zero for acrylamide in drinking water.[4] The World Heath Organization (WHO) set a limit of 0.5 µg/L (0.5 ppb) for residual acrylamide in drinking water.[5] Limited studies have found acrylamide concentrations in foods of up to 1200 µg/kg (1200 ppb).[6] At these levels, acrylamide in food may present a long-term health risk.

To adequately assess this risk to humans, food levels need to be accurately measured and compiled. This prompts the need for development of analytical methods for extraction and quantitation of acrylamide. The United States Food and Drug Administration (FDA) has published an LC/MS/MS method for acrylamide, and others have also published methods using either GC, GC/MS, or LC/MS.[7,8]

The TRACE DSQ is a quadrupole mass spectrometer that incorporates a curved pre-filter which essentially eliminates non-chemical noise created by excited neutrals striking the detector. This reduction in neutral noise allows the GC/MS operator greater sensitivity and detectivity. Published detection limits for acrylamide in food range from 10-50 ppb depending on instrumentation. Here, the TRACE DSQ is used to analyze acrylamide with linear dynamic ranges extending from 2-1000 ppb, depending on the ionization mode.

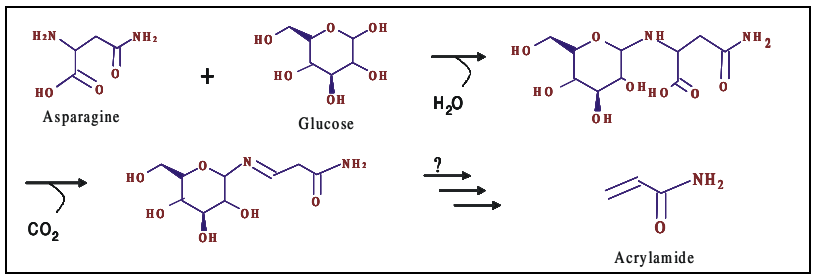

Figure 1: Potential pathway for acrylamide formation via the Maillard Reaction.[2,3]

Methods

Extraction Method A, Underivatized:[9] 7 mL of 0.1% formic acid solution, 100 µL of a 2000 ng/mL solution of ^{13}C3-Acrylamide solution (Cambridge Isotope Laboratories, Inc, Andover, MA), and 1 g of crushed food sample were mixed for 20 minutes using a wrist action shaker. The sample was centrifuged and any oil layer was removed prior to transferring the supernatant to another tube. Unusually turbid samples were filtered using 0.45 µm nylon syringe filters (Restek Corp, Bellefonte, PA) prior to extraction. Standards ranging from 5-500 ppb were prepared using acrylamide (Cambridge Isotope Laboratories, Inc) in 7 mL of 0.1% formic acid, and 100 µL of the $^{13}C_3$-Acrylamide solution were added to each standard. CarboPrep™ 200 cartridges (Restek Corp.) were conditioned with 2 mL methanol followed by 2 mL 0.1% formic acid. Samples were added and allowed to extract using gravity. 500 µL of water were then added to each tube, and tubes were dried under high vacuum for five minutes. Sample elution with 1 mL acetone occurred under gravity. Eluates were transferred to autosampler vials and analyzed without further drying by the TRACE DSQ in EI+ SIM and NCI SIM, using methane as reagent gas.

Extraction Method B, Brominated:[10] 1 gram of unknown sample and 100 µL of $^{13}C_3$-Acrylamide solution (2000 ng/mL) were combined in 10 mL water. Standards were prepared in water as well, with 100 µL internal standard added to each. Samples were mixed for 20 minutes, then centrifuged. The supernatant was filtered using 0.45 µm nylon syringe filters. 200-300 µL of brominating reagent were added to 3 mL of the filtered sample and standards, which were gently mixed, and then allowed to react in an ice bath for 1 hour. One drop of 1.0N sodium thiosulfate was added to each sample to decompose any remaining bromine. Samples were extracted with 2 mL ethyl acetate, then centrifuged for 10 minutes. The organic layer was transferred to autosampler vials for analysis. Bromination of acrylamide according to this method yields 2,3-dibromopropionamide. Analysis of the more stable 2-bromopropenamide, created in situ from the thermal decomposition in the injector, using ammonia as a reagent gas provided stable adduct ions that were used for PCI SIM.

Figure 2: The Finnigan TRACE DSQ and TRACE GC Ultra, shown with vacuum interlock and PTV injection port

	Ionization Mode		
Component	EI+ SIM	NCI SIM	PCI SIM
Acrylamide	71, 55, 44	70	
$^{13}C_3$-acrylamide	74, 58	73	
2-bromo-propenamide			167, 169
2-bromo-$^{13}C_3$-propenamide			170, 172

Table 1: Summary of SIM masses and ionization modes

Instrument Methods: The TRACE DSQ and TRACE GC Ultra (Figure 2) were set up using the PTV injection port in splitless mode, utilizing programmed transfer, evaporation, and cleaning phases and a 0.3 minute splitless duration. The oven program ramped from 40-220 °C at 30 °C/min. A 15 m x 0.25 mm i.d. x 0.25 µ Stabilwax™ Crossbond® Carbowax® column (Restek Corp) was chosen as the analytical column.

TRACE DSQ methods were set up for EI+, NCI, and PCI analyses. The SIM masses selected for EI+, PCI and NCI are summarized in Table 1. Methane and ammonia were both used as reagent gases. The reagent gas flow rate in each method was 2.0 mL/min. The TRACE DSQ was tuned in EI mode, and the pre-filter voltage was reduced as needed to increase low mass sensitivity, since the acrylamide molecule has a low mass-to-charge ratio, *m/z*. TRACE DSQ instrument parameters are detailed in Table 2.

Results

The TRACE DSQ was a valuable tool in the analysis of acrylamide in food due to its flexibility. In EI+ SIM, acrylamide monomer was found to be linear from 5-500 ppb. NCI yielded a linear range of 2-500 ppb when methane was used as the reagent gas. Analysis of 2-bromopropenamide in PCI was linear from 5-1000 ppb.

Mode	Setting
EI SIM	Prefilter: -1.2 V
	Electron Energy: 70 eV
	Emission Current: 150 µA
NCI SIM	Prefilter: -2.0 V
	Electron Energy: 70 eV
	Emission Current: 150 µA
	Electron Lens: 10 V
PCI SIM	Prefilter: -8.0 V
	Electron Energy: 120 eV
	Emission Current: 50 µA
	Electron Lens: 10 V

Table 2: Selected Instrument Parameters for the TRACE DSQ

A simple solid-phase extraction, which eliminates final dry-down steps to concentrate the samples, provides sufficient sample preparation for EI+ SIM. Using 3-ion SIM for confirmation, the correlation coefficient was 0.9992 from 5-500 ppb (Figure 3). Run time was approximately 7 minutes. This method provides sensitivity and productivity for a rapid determination of acrylamide monomer in food. However, due to the possible presence of interfering ions in the ranges used, chemical ionization was also evaluated for applicability to this analysis.

Selection of reagent gas was dependent on availability and affinity. NCI with methane as the reagent gas provided sensitivity down to 2 ppb, with a correlation coefficient of 0.9993 from 2-500 ppb. (Figure 4). NCI SIM eliminated the chemical interferences present in EI SIM, as seen in Figure 5, where the top trace is *m/z* 70 for NCI and the lower trace is *m/z* 55 for EI SIM. The sample in this case is extracted underivatized potato chips. Analysis of brominated samples using ammonia as reagent gas in NCI yielded a spectra consistent with free bromine, and thus this ionization technique was not used for the brominated samples.

However, the use of ammonia in PCI to analyze the 2-bromopropenamide form of acrylamide leads to the formation of two primary adduct ions, at *m/z* 167 and 169 for the unlabeled form and 170 and 172 for the ^{13}C-labeled compound. A method was set up using these masses, and calibration from 5-1000 ppb was performed in PCI, with a correlation coefficient of 0.9998 (Figure 6). Figure 7 displays the overlaid extracted ion chromatograms for *m/z* 167 and 169 for the 5 ppb standard. The DSQ enables users to automatically optimize tune parameters in PCI and NCI modes through use of the appropriate autotunes. For all analyses, the prefilter setting on the DSQ was lowered from the tune result to optimize

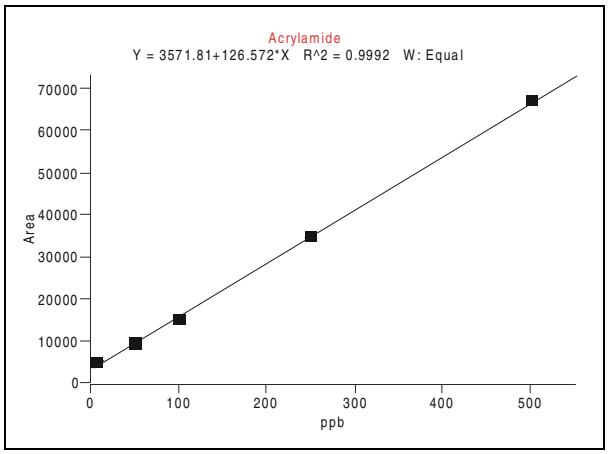

Figure 3: EI+ SIM Calibration Curve, 5-500 ppb, r^2 = 0.9992

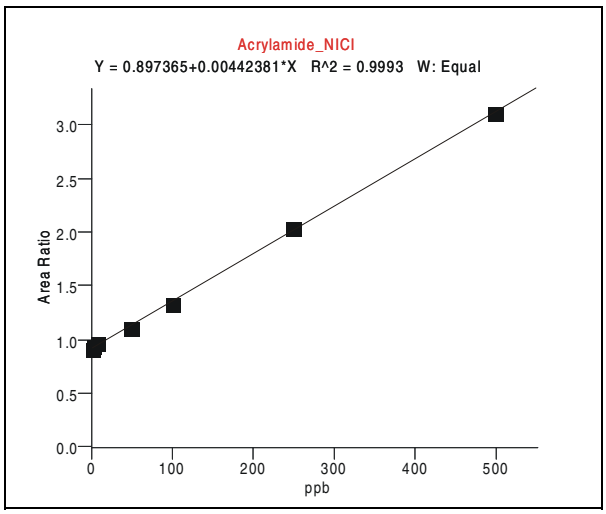

Figure 4: Calibration curve for acrylamide monomer in NCI SIM with Methane, from 2-500 ppb, r^2 = 0.9993 (solvent contamination noted)

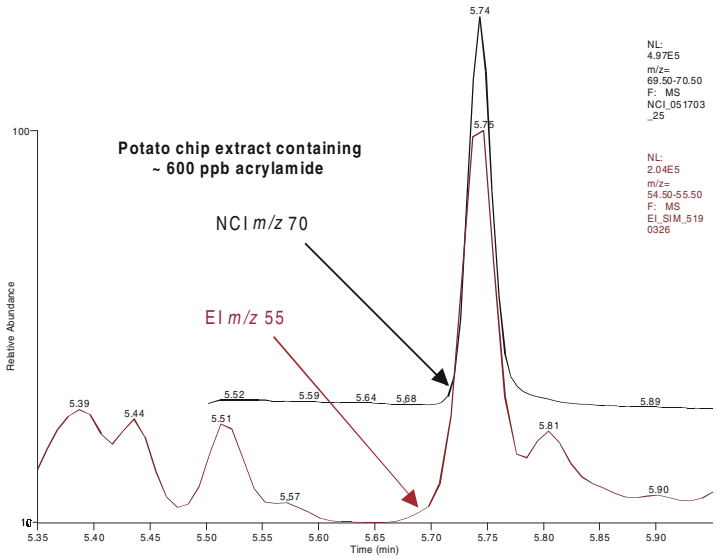

Figure 5: Comparison of EI+ SIM and NCI SIM, extracted potato chip (1.0 gram sample)

low mass sensitivity. The tunes were saved and elected for the appropriate methods. The reagent gas flow rate was set at 2.0 mL/min for both PCI and NCI. Use of a digital flow controller for CI reagent gas ensures accurate flow, quantitative accuracy and day-to-day reproducibility.

Alternating between ionization modes was accomplished through the use of the vacuum interlock. The interlock enabled the changing of ion volumes specific to EI or CI types of analyses without venting the instrument. This minimized instrument downtime and also facilitated ion volume cleaning when necessary. This is an important feature since food extracts are known to rapidly dirty the ion volume.

For optimization of acrylamide analysis in food, care must be taken to avoid acrylamide contamination. Also, the presence of interference needs to be carefully considered when interpreting the chromatographic and mass spectral data. Verification of solvent purity beforehand is recommended, as in this case, the acetone contained acrylamide. Additionally, the autosampler program needs to include sufficient rinse steps in order to eliminate carryover.

Conclusions

The TRACE DSQ is a valuable tool for identification and quantitation of acrylamide in foods. It offers versatility and sensitivity that makes it adaptable to different methodologies for this analysis. Simplified extractions increase sample throughput, and the vacuum interlock reduces instrument downtime by enabling routine maintenance and switching of ion volumes without venting the analyzer. With detection limits ranging from 2 ppb in NCI to 5 ppb in EI and PCI, and linearity to 1000 ppb, it offers the necessary dynamic range for this application. Use of the TRACE GC Ultra with a PTV enables the user to program evaporation, transfer, and cleaning steps that facilitate sample transfer to the analytical column and help eliminate carryover and matrix interference.

Two important aspects of the TRACE DSQ that may also lead to successful performance of acrylamide analysis will be explored in future applications. The use of pulsed positive ion/negative ion chemical ionization (PPINICI) allows sequential acquisition of both positive and negative ions in a single injection. Additionally, a sequential full scan/SIM acquisition in EI+ offers additional specificity by providing full scan data in addition to the enhanced sensitivity that SIM provides.

References

[1] Swedish National Food Administration. Acrylamide is formed during the preparation of food and occurs in many foodstuffs. April 2002.

[2] Yarnell, A. Acrylamide Mystery Solved. Chemical & Engineering News. 2002, 80, 40, 70. ISSN: 0009-2347

[3] Becalski, A; Lau, B P-Y; Lewis, D; Seaman, S. Acrylamide in Foods: Occurrence, Sources, and Modeling. J. Agric. Food Chem. 2003, 51, 802-808.

[4] US EPA Groundwater and Drinking Water Consumer Factsheet on Acrylamide. http://www.epa.gov/safewater/dwh/c-voc/acrylami.html

[5] World Health Organization. Guidelines for drinking-water quality, 2nd ed. http://www.who.int/water_sanitation_health/GDWQ/Chemicals/Acrylamidesum.htm

[6] US FDA CFSAN. Exploratory Data on Acrylamide in Foods. http://www.cfsan.fda.gov/~dms/acrydata.html

[7] US FDA CFSAN. Detection and Quantitation of Acrylamide in Foods (Draft), http://www.cfsan.fda.gov/~dms/acrylami.html

[8] Brandl, F et al. A rapid and convenient procedure for the determination of acrylamide in foodstuffs. EJEAFChe. 2002. ISSN: 1579-4377

[9] Restek Corp Application Note # 59485. Acrylamide Analysis by Gas Chromatography. http://www.restekcorp.com/appnotes/59485.pdf

[10] Analysis of Acrylamide in Processed Foods in Japan, http://aa.iacfc.affrc.go,jp/en.

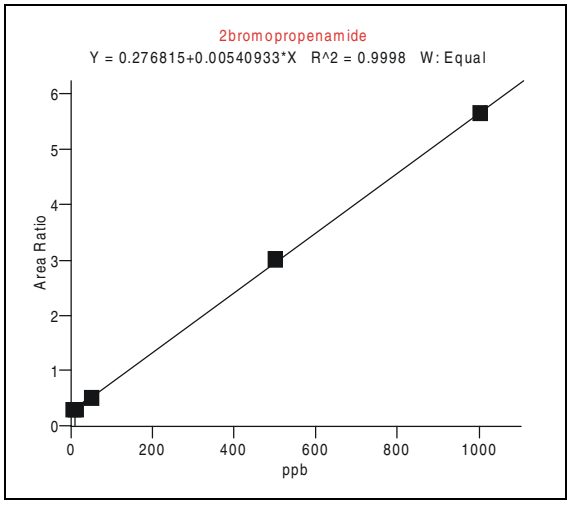

Figure 6: Calibration curve for brominated samples in PCI SIM using ammonia for reagent gas, from 5-1000 ppb, r^2 = 0.9998

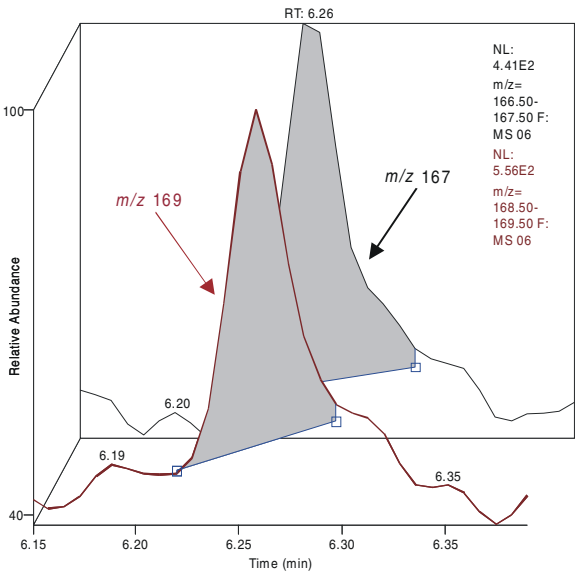

Figure 7: EIC for *m/z* 167 and 169, PCI SIM for 2-bromopropenamide, 5 ppb standard

Thermo
ELECTRON CORPORATION
www.thermo.com/finnigan

Thermo Electron Corporation has direct subsidiary offices in North America, Europe, and Japan. To complement these direct subsidiaries, we maintain a network of representative organization throughout the world. Use this reference list or visit our Web site to locate the representative nearest you.

Australia
Tel. (61) 2 9898 9000

Austria
Tel. (43) 1 333 50340

Belgium
Tel. (32) 010 450025

Canada
Tel. (905) 712 2258

Canada only
Tel. (800) 721 4260

Central and South America
Tel. (01) 512 2511503

France
Tel. (33) 1 69 18 88 10

Germany
Tel. (49) 6103 4080

Italy
Tel. (39) 02 950 591

Japan
Tel. (81) 45 453 9100

The Netherlands
Tel. (31) 765 411 800

People's Republic of China
Tel. (86) 10 6621 0839

Singapore (SEA Head Office)
Tel. (65) 6 464 6302

Spain
Tel. (34) 91 657 4930

Sweden
Tel. (46) 8 556 468 00

Switzerland
Tel. (41) 732 31 31

United Kingdom
Tel. (44) 1 442 233555

United States
Tel. (01) 800 532 4752

AN9195/03.08