Application Note: 319

Quantitation of Acrylamide in Food Samples on the TSQ Quantum Discovery by LC/APCI-MS/MS

Kevin J. McHale, Witold Winnik and Gary Paul; Thermo Fisher Scientific, Somerset, NJ, USA

Key Words

- TSQ Quantum Discovery™
- Hypercarb™
- Acrylamide
- Quantitation
- Triple Quadrupole MS

Introduction

Acrylamide has been identified as a potential human carcinogen. This is important not only because acrylamide is a common industrial chemical, but acrylamide has been shown to be present at significant levels in food samples,[1] particularly cooked foods high in carbohydrates. This has led many government health agencies around the world to assess the risk of short- and long-term exposure to acrylamide in humans.

This has led to the development of LC-MS/MS methodology for the quantitative analysis of acrylamide in foodstuffs.[1-5] While a GC/MS protocol for the analysis of acrylamide exists, this method requires extensive sample cleanup and chemical derivatization.[6] The advantage of LC-MS/MS is that chemical derivatization is not necessary prior to acrylamide analysis.

To date, most LC-MS/MS methods for the assay of acrylamide have utilized an electrospray ionization (ESI) source for the production of acrylamide ions.[1-4] Yet it is well-known that ESI-MS is problematic when highly aqueous solutions, such as those required for the reversed-phase LC separation of acrylamide, are used.[7] On the other hand, water does not pose a problem for the formation of a stable corona discharge used in APCI. One published report has demonstrated that APCI is a viable ion source for the production of acrylamide ions for LC-MS/MS detection.[5] Furthermore, a study comparing ESI and APCI ion sources for the LC-MS/MS analysis of acrylamide showed that under the same chromatographic conditions, APCI-MS/MS yielded an improved detection limit.[8]

This report presents data acquired on the Thermo Scientific TSQ Quantum Discovery for the analysis of acrylamide. A simple LC-MS/MS method using the APCI source is used to measure acrylamide, via selective reaction monitoring (SRM), over a wide concentration range. A small selection of food samples was analyzed for acrylamide content following extraction with water. To preclude the need for a time-consuming solid-phase extraction procedure, a column-switching method was employed to selectively "fractionate" acrylamide from polar matrix interferences prior to LC-MS/MS detection.

Goals

1. **Development**–A sensitive and rugged LC/APCI-MS/MS assay for the analysis of acrylamide
2. **Application**–An on-line column-switching technique to aqueous food extracts as an alternative to solid-phase extraction (SPE) cleanup
3. **Measurement**–Acrylamide content in selected food samples

Experimental

Chemicals and Reagents: Acrylamide (>99.0%) was purchased from Fluka (Buchs SG, Switzerland). 2,3,3-d3-acrylamide (98%) was obtained from Cambridge Isotope Laboratories (Andover, MA, USA). HPLC grade water was acquired from Burdick and Jackson (Muskegon, MI, USA). All chemicals were used as received without further purification.

Sample Preparation: Standards were prepared by dilution of a stock solution of 1.0 mg/mL acrylamide or 1.0 mg/mL d3-acrylamide in water. The stock solutions were stored at 4 °C for a period of no longer than two weeks.

Two brands of potato chips and two brands of breakfast cereals were purchased and stored at room temperature until processed. After homogenizing approximately 50 grams of a food sample, two grams were weighed into a 35 mL polypropylene centrifuge tube. Aqueous extraction of acrylamide was initiated by the addition of 20 mL water containing 1000 ng d3-acrylamide as the internal standard (final concentration = 50 ng/mL). The sample was vortexed for 30 s then subsequently centrifuged at 18,000 g for 15 minutes. Ten milliliters of the supernatant was decanted into a clean 35 mL centrifuge tube and centrifuged at 18,000 g for 10 minutes. Prior to analysis, 0.49 mL of the aqueous extract was filtered through a 0.45 µm centrifuge filter (Millipore Corp., Bedford, MA, USA) at 9,000 g for 5 minutes.

Sample Analysis: LC experiments were conducted with the Thermo Scientific Surveyor™ HPLC system. A Thermo Scientific Hypercarb 2.1 × 50 mm column was utilized as the analytical LC column. Separations of acrylamide were achieved under isocratic conditions using 100% water as the mobile phase at a flow rate of 0.4 mL/min. The injection volume for all LC experiments was 10 µL.

Thermo SCIENTIFIC

To eliminate the need for solid phase extraction (SPE) purification prior to the analysis of the food sample extracts, a column-switching LC method was employed. Briefly, the sample extract was loaded onto a 2.1 × 50 mm Thermo Scientific Aquasil™ C18 column, which was positioned before a 6-port switching valve. The eluent from the C18 column was diverted to waste except for the period when acrylamide eluted from the C18 column, whereby the valve was switched to the Hypercarb column for MS/MS detection. This column-switching method required a second Thermo Scientific Surveyor MS pump, which also delivered 100% water at 0.4 mL/min. Both Surveyor MS pumps and the 6-port switching valve were controlled using Xcalibur™ version 1.3 software.

The experimental conditions for the TSQ Quantum Discovery were as follows:

Source: APCI
Ion polarity: Positive
Vaporizer Temperature: 375 °C
Discharge Current: 5 µA
Ion Transfer Capillary Temperature: 250 °C
Source CID Offset: 6 V
Scan Mode: Selective Reaction Monitoring
Q2 Pressure: 1.0 mTorr argon
SRM Transitions: *m/z* 72 → 55 for acrylamide;
m/z 75 → 58 for d3-acrylamide
Collision Energy: 13 eV
Scan Width: 1.0 u
Scan Time: 0.3 s (each SRM transition)
Q1, Q3 Resolution: Unit (0.7 u FWHM)

Results and Discussion

Prior to the acquisition of acrylamide standards, it was important to determine if there was any detectable native acrylamide contribution originating from the deuterated internal standard. As shown in Figure 1, there is no acrylamide signal observed for the *m/z* 72 → 55 SRM transition at the same retention time as the 50 ng/mL d3-acrylamide standard.

The limit of quantitation (LOQ) for acrylamide on the TSQ Quantum Discovery was 0.25 ng/mL acrylamide or 2.5 pg on column (Figure 2). This compares favorably to LOQs previously reported by other research groups, including an 8-fold improvement over the mass LOQ by LC/ESI-MS/MS (20 pg)[1] and a 40-fold improvement over the concentration LOQ on the TSQ 7000 (10 ng/mL),[5] which used an LC/APCI-MS/MS method.

The calibration curve for acrylamide from 0.25 ng/mL to 2500 ng/mL is displayed in Figure 3. This calibration curve was generated using the column-switching LC method just prior to the acquisition of the food extracts data. A linear regression fit to these data using 1/x weighting yielded the following equation: $y = 5.5997 \times 10^{-4} + 0.0206125x$. The correlation coefficient for this curve was $r^2 = 0.9999$, indicating excellent linearity across the four orders of magnitude dynamic range. Table 1 summarizes the statistical results for the acrylamide calibration curve. At the LOQ,

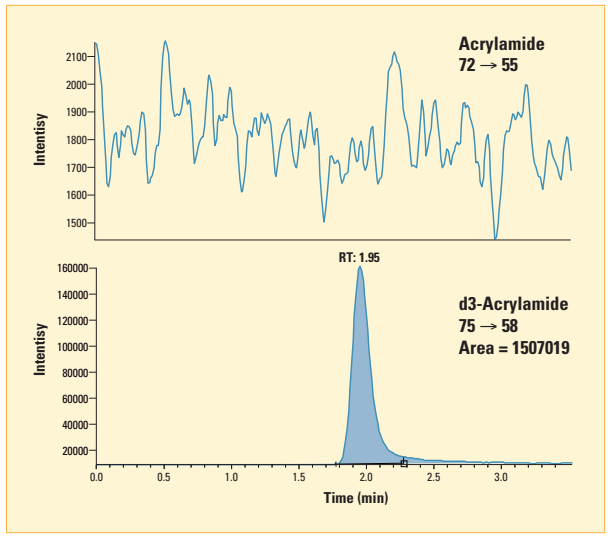

Figure 1: SRM chromatograms for 50 ng/mL d3-acrylamide

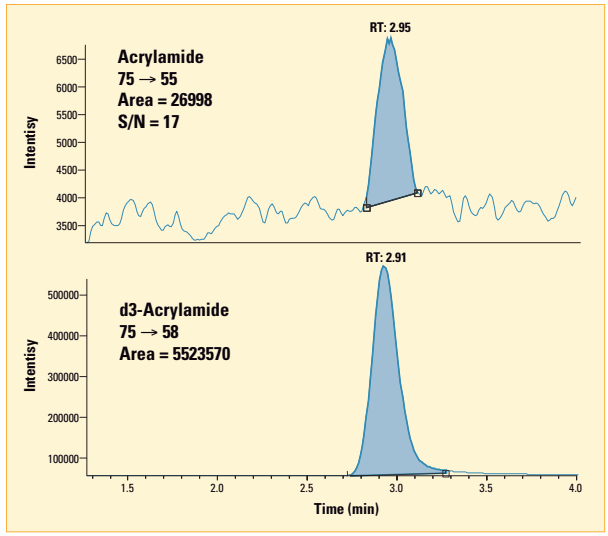

Figure 2: SRM chromatograms for 0.25 ng/mL acrylamide (LOQ) with 50 ng/mL d3-acrylamide

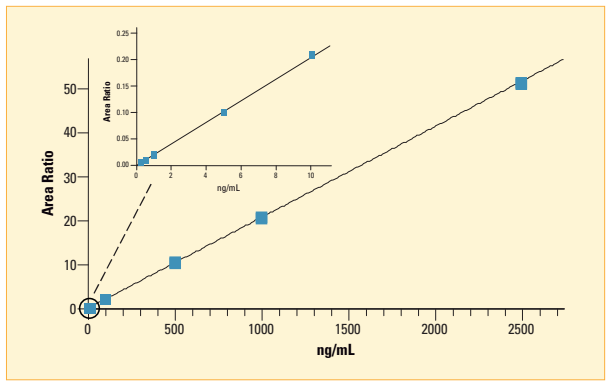

Figure 3: Calibration curve for acrylamide using column-switching LC method with APCI-MS/MS detection

Nominal (ng/mL)	Mean Conc. (ng/mL)	% Rel. Error	% CV	Number of Replicates
0.250	0.253	1.1	12.1	5
0.500	0.485	-2.9	6.7	5
1.00	1.00(4)	0.4	4.6	5
5.00	4.86	-2.7	0.9	5
10.0	10.2	2.1	0.7	5
100	101	0.7	0.5	5
500	512	2.4	0.8	3
1000	1006	0.6	0.6	3
2500	2481	-0.8	0.6	3

Table 1: Statistical data for the calibration curve of acrylamide

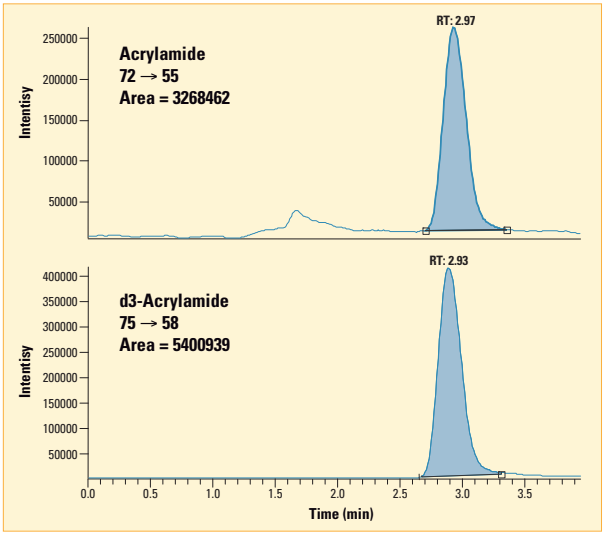

Figure 4: SRM chromatograms of the Potato Chip 2 sample aqueous extract

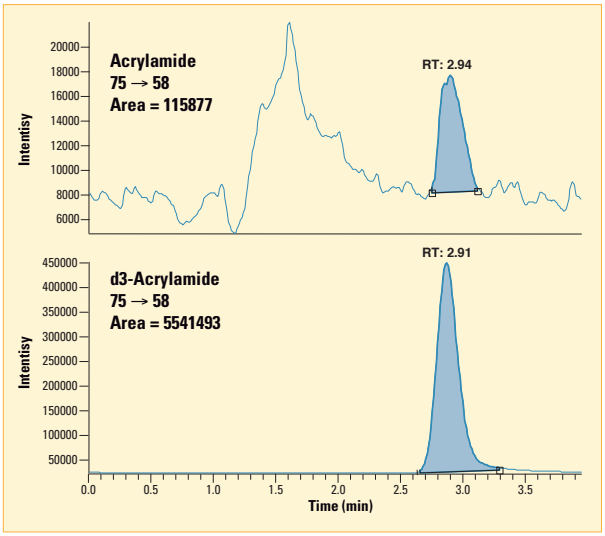

Figure 5: 1 ng/mL acrylamide standard analyzed directly after duplicate injections of the aqueous extract of the Potato Chip 2 sample

the accuracy, as a percent relative error, is 1.1% and the precision, as a percent coefficient of variance (%CV), is 12.1% for five replicate injections. Above the LOQ, the relative error varied from -2.9 to +2.4% and the %CV ranged from 0.5 to 6.7%.

Results obtained from the aqueous extract of Potato Chip 2 are presented in Figure 4. By utilizing a C18 column positioned before a switching valve to selectively elute acrylamide onto the Hypercarb column, background interferences are reduced. Unlike most of the other acrylamide reports where SPE cleanup was used following extraction of the sample with water,[1-4] the column-switching LC method employed here provides an on-line means of acrylamide fractionation. This has the advantage of minimizing sample losses during SPE and greatly reduces sample preparation time.

To monitor the consistency and reproducibility of the column-switching LC-MS/MS method, a 1 ng/mL acrylamide standard was analyzed immediately following each food sample. An example of this quality control standard analyzed after the Potato Chip 2 sample is shown in Figure 5. Although the baseline for the *m/z* 72 → 55 SRM transition is somewhat elevated near the retention time for acrylamide, the calculated concentration for this standard is 0.99 ng/mL, equating to a relative error of -1.0%.

Table 2 reports the results for four different food samples that were assayed for acrylamide using the column-switching LC method and MS/MS detection. The acrylamide concentrations in each food sample were calculated by multiplying the measured solution concentration from duplicate injections by the extraction volume and dividing by the food sample mass that was extracted. The determined acrylamide concentrations correlated well to those reported elsewhere for these classes of food.[1-5]

	Cereal 1	Cereal 2	Potato Chip 1	Potato Chip 2
Injection 1	17.17 ng/mL	55.93 ng/mL	57.11 ng/mL	29.18 ng/mL
Injection 2	17.00 ng/mL	56.18 ng/mL	56.52 ng/mL	29.14 ng/mL
Mean	17.09 ng/mL	56.06 ng/mL	56.82 ng/mL	29.16 ng/mL
Extraction Vol.	20.0 mL	20.0 mL	20.0 mL	20.0 mL
Mass Sample	2.003 g	2.007 g	2.021	1.995
Acrylamide Conc.	171 ng/g	559 ng/g	562 ng/g	292 ng/g

Table 2: Results of acrylamide assay from food samples

Conclusions

An LC-MS/MS method has been developed for the measurement of acrylamide on the TSQ Quantum Discovery. Using APCI for the analysis of acrylamide from 100% water, an LOQ of 0.25 ng/mL acrylamide or 2.5 pg on column was achieved. Incorporation of a column-switching LC method prior to MS/MS detection of acrylamide eliminated the need to purify food sample extracts by SPE. The method was successfully demonstrated for the analysis of four brands of food samples using TSQ Quantum Discovery in conjunction with a column-switching LC method.

References

(1) Tareke, E.; Rydberg, P.; Karlsson, P.; Eriksson, S.; Tornqvist, M. *J. Agric. Food Chem.*, **2002**, *50*, 4998-5006.

(2) Rosen, J.; Hellenas, K. *Analyst*, **2002**, *127*, 880-882.

(3) Musser, S. M. http://www.cfsan.fda.gov/~dms/acrylami.html

(4) Becalski, A.; Lau, B.P.; Lewis, D., Seaman, S.W. *J. Agric. Food Chem.*, **2003**, *51*, 802-808.

(5) Brandl, F.; Demiani, S.; Ewender, J.; Franz, R.; Gmeiner, M.; Gruber, L.; Gruner, A.; Schlummer, M.; Smolic, S.; Stormer, A.; Wolz, G. *Electron. J. Environ. Agric. Food Chem.*, **2002**, *1(3)*, 1-8.

(6) Castle, L.; Campos, M.J.; Gilbert, J. *J. Sci. Food Agric.* **1993**, *54*, 549-555.

(7) Ikonomou, M.G.; Blades, A.T.; Kebarle, P. *J. Am. Soc. Mass Spectrom.*, **1991**, *2*, 497-505.

(8) McHale, K.J.; Winnik, W.; Paul, G. *Proceedings of the 51st ASMS Conference on Mass Spectrometry and Allied Topics*, Montreal, 2003.

View additional Thermo Scientific LC/MS application notes at: **www.thermo.com/appnotes**

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

Africa
+43 1 333 5034 127
Australia
+61 2 8844 9500
Austria
+43 1 333 50340
Belgium
+32 2 482 30 30
Canada
+1 800 530 8447
China
+86 10 5850 3588
Denmark
+45 70 23 62 60
Europe-Other
+43 1 333 5034 127
France
+33 1 60 92 48 00
Germany
+49 6103 408 1014
India
+91 22 6742 9434
Italy
+39 02 950 591
Japan
+81 45 453 9100
Latin America
+1 608 276 5659
Middle East
+43 1 333 5034 127
Netherlands
+31 76 587 98 88
South Africa
+27 11 570 1840
Spain
+34 914 845 965
Sweden/Norway/ Finland
+46 8 556 468 00
Switzerland
+41 61 48784 00
UK
+44 1442 233555
USA
+1 800 532 4752

www.thermo.com

Thermo Fisher Scientific, San Jose, CA USA is ISO Certified.

AN62606_E 01/08S