Analysis of Acrylamide in French Fries using Agilent Bond Elut QuEChERS AOAC kit and LC/MS/MS

Food Application

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Abstract

This application note describes the use of a quick, easy, cheap, effective, rugged, and safe (QuEChERS) sample preparation procedure. The QuEChERS method employs an extraction and cleanup protocol for the determination of acrylamide with $^{13}C_3$-acrylamide as the internal standard. The analyte and internal standard were separated on a reversed phase C-18 column (2.1 mm × 150 mm, 3 µm) by isocratic elution and analyzed by a triple quadrupole mass spectrometer operating in focused positive electrospray ionization mode. The acrylamide recoveries ranged from 97% to 116% with a relative standard deviation of less than 5%.

Introduction

In 2002, researchers at the Swedish National Food Administration and Stockholm University reported finding acrylamide at levels up to 3 mg/kg in a wide range of potato and cereal-based products such as potato chips, french fries, roasted and baked potatoes, bread, breakfast cereals, and biscuits. Acrylamide forms in carbohydrate-rich foods that are subjected to high temperatures (>120 °C) such as frying, baking and extrusion (1). It is recognized as a neurotoxin and a carcinogen in animals, and a possible carcinogen in humans by the International Agency for Research on Cancer (IARC) (2). High levels of acrylamide are found primarily in potato products, bakery and cereal products, and coffee. Acrylamide is generated from certain food products during heat treatment as a result of the Maillard reaction between certain amino acids and reducing sugars (1). Most laboratories use liquid chromatography/triple quadrupole mass spectrometry (LC/MS/MS) for the analysis of underivatized acrylamide in food samples. Liquid chromatography methods use water to extract acrylamide. A defatting step with hexane, toluene or cyclohexane is

used before or combined with the extraction step, and the aqueous phase is centrifuged (3). The cleanup steps often involve solid-phase extraction (SPE) such as that developed by the U.S. FDA (4).

The QuEChERS method, which was originally designed for the analysis of pesticides in food was modified by Mastovska and Lehotay for the extraction of acrylamide (5). The method involves two major steps: extraction and dispersive SPE cleanup. Water extracts the sample, hexane defats the sample, and the $MgSO_4$ and NaCl induce separation of the water and acetonitrile layers, forcing the majority of acrylamide into the acetonitrile layer. An aliquot of the organic layer is cleaned up by dispersive solid-phase extraction (dSPE) by using primary secondary amine (PSA) to remove organic acids and anhydrous $MgSO_4$ to reduce any water left in the extract. The final extract is then analyzed by liquid chromatography/ triple quadrupole mass spectrometry operating in positive ion multiple reaction monitoring (MRM) mode.

This application note presents a method for the analysis of acrylamide in french fries with LC/MS/MS. The method includes sample preparation with Agilent Bond Elut QuEChERS Extraction kit for acrylamides (p/n 5982-5850) and Agilent Bond Elut AOAC Dispersive SPE 2 mL kit (p/n 5982-5022).

Experimental

Reagents and Chemicals

All reagents were Optima or LC/MS grade. Acetonitrile, n-hexane, formic acid and water were purchased from Fisher Scientific (Hanover Park, IL, USA). Acrylamide (Figure 1) and $^{13}C_3$-acrylamide (internal standard) were purchased from Cambridge Isotope Laboratories (Andover, MA, USA).

Acrylamide

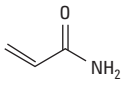

Figure 1. Chemical structure of acrylamide.

Standard Solutions

The acrylamide standard stock solution (1 mg/mL) was prepared by dissolving 100 mg of the acrylamide in 100 mL acetonitrile and stored at 4 °C. The internal standard (methacrylamide) stock solution (100, 000 µg/mL) was made up by pipetting 0.5 mL of the 1 mg/mL standard into 50 mL acetonitrile and stored at 4 °C. All working solutions were prepared daily by serial dilution in acetonitrile.

Equipment and Material

The analysis was performed on an Agilent 1200 Series HPLC system equipped with an Agilent 6460 Triple Quadrupole LC/MS system (Agilent Technologies, Inc., CA, USA). Separation of the chemicals was performed on a reversed phase C18 column (2.1 mm × 150 mm, 3 µm). The data was processed by Agilent MassHunter software.

Extraction and cleanup were achieved with an Agilent Bond Elut QuEChERS Extraction kit for acrylamides (p/n 5982-5850) and an Agilent Bond Elut QuEChERS AOAC Dispersive SPE kit (p/n 5982-5022), Agilent Technologies (CA, USA).

Instrument Conditions:

Table 1. LC/MS/MS Conditions

Column	Reversed C-18 column, 2.1 mm × 150 mm, 3 µm
Column temperature	30 °C
Isocratic mode (%B)	2.5% methanol/97.5% of 0.1% formic acid
Flow rate	0.2 mL/min
Injection volume	10 µL
Run time	7 min
Post run time	3 min
Mass Spectrometer	Positive electrospray ionization mode with jet stream technology
Capillary voltage	4000 Volts
Nozzle voltage	500 V
Sheath gas temperature	325 °C at 5 L/min
Drying gas temperature	350 °C at 11 L/min

Sample preparation

Frozen par-fried potato slices were purchased from a local store.

Extraction

One gram of french fries was weighed into a 50 mL centrifuge tube from the Agilent Bond Elut QuEChERS Extraction kit. The internal standard ($^{13}C_3$-acrylamide) was added at 500 ng/g. Hexane (5 mL) was added and the tube was vortexed. Water (10 mL) and acetonitrile (10 mL) were added followed by the Agilent Bond Elut QuEChERS extraction salt mixture for acrylamides (p/n 5982-5850). The sample tubes were shaken for 1 min vigorously, and centrifuged at 5000 rpm for 5 min.

Dispersive SPE cleanup

The hexane layer was discarded and 1 mL of the acetonitrile extract was transferred to a 2 mL Bond Elut QuEChERS AOAC Dispersive SPE tube (p/n 5982-5022). This tube contained 50 mg of PSA and 150 mg anhydrous $MgSO_4$. The tubes were vortexed for 30 sec and then centrifuged at 5000 rpm for 1 min. The supernatant was then placed in an autosampler vial for LC/MS/MS analysis.

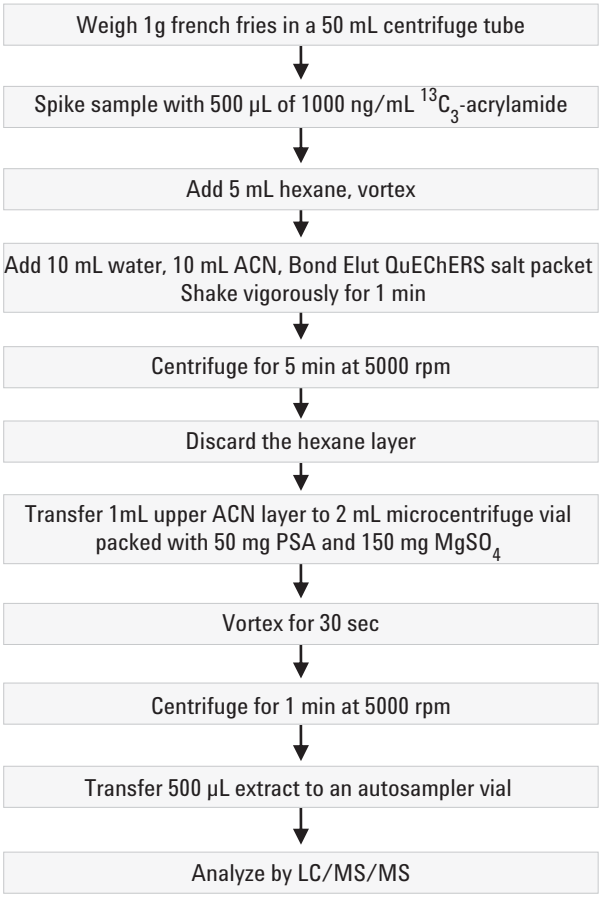

Figure 2. Flow chart for the QuEChERS sample preparation procedure.

Results and Discussion

Chromatographic analysis

The separation of acrylamide and methacrylamide was obtained on a reversed phase C-18 column (2.1 x 150 mm, 3 µm) where an isocratic elution was used with 2.5% methanol/97.5% of 0.1% formic acid. The column temperature was set at 30°C, while the flow rate was maintained at 0.2 mL/min. Figure 3 shows a typical standard chromatogram of acrylamide and methacrylamide.

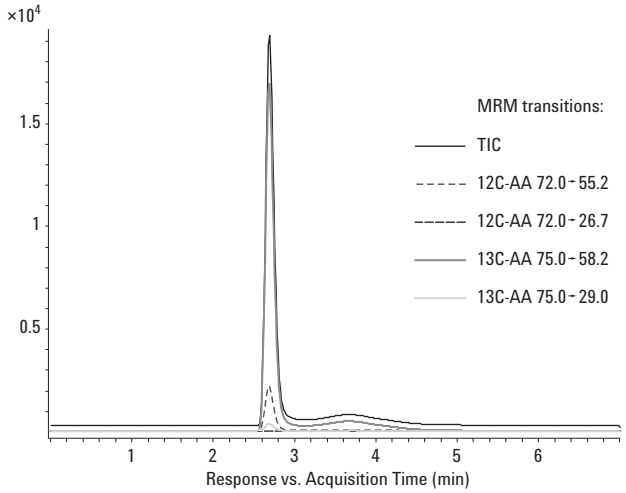

Figure 3. Chromatogram of the 10 ng/mL acrylamide standard and 500 ng/mL internal standard $^{13}C_3$-acrylamide.

QuEChERS method

Hexane was used to defat the extract. Addition of water was necessary to facilitate extraction of acrylamide from french fries. An Agilent Bond Elut QuEChERS salt packet (p/n 5982-5850) containing 4 g $MgSO_4$ and 0.5 g NaCl was used to extract acrylamide from a 1 g sample of french fries. Addition of salt allowed for the separation of the acetonitrile and water phase. Dispersive SPE was employed for sample cleanup (5).

The QuEChERS protocol in this application note is simple. The long and tedious SPE process, which may cause low recoveries of acrylamide, is not necessary.

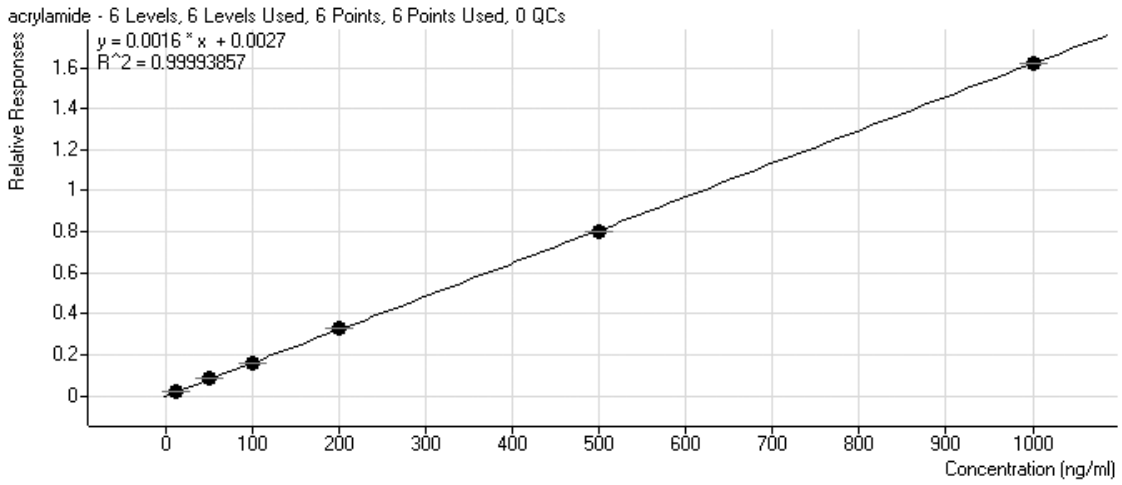

Figure 4. Acrylamide calibration curve.

Linearity

A linear calibration curve was obtained by plotting the relative responses of analyte (peak area of analyte (acrylamide)/peak area of IS (methacrylamide) to the relative concentration of analyte (acrylamide)/concentration of IS ($^{13}C_3$-acrylamide). The curve was generated by spiking acetonitrile with acrylamide at a concentration range of 0 – 1000 ng/mL (Figure 4). Excellent linearity was observed (r^2>0.9999).

Recovery and Reproducibility

Acrylamide was spiked into 1:1 acetonitrile:water at two different fortification levels (50 and 100 ng/mL). The analysis was performed in triplicates at each level (n=3). Frozen par-fried french fry samples containing 33 ng/mL acrylamide were also spiked at two different fortification levels (100 and 200 ng/mL) and blank corrections were made, since it was difficult to find blank french fry samples. A chromatogram of the spiked french fry sample is shown in Figure 5. The analysis was performed in triplicates at each level (n=3). Table 2 shows the recoveries and RSD values for acrylamide.

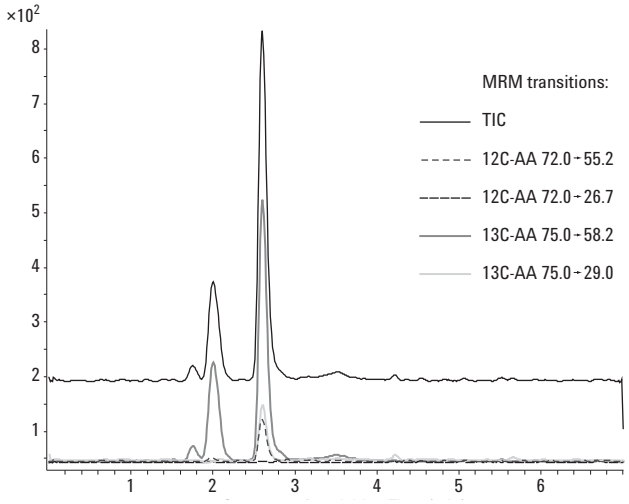

Figure 5. Chromatogram of the spiked french fry extract.

Table 2. Recoveries and RSDs for Acrylamide in Spiked French Fry Samples and 1:1 Water:Acetonitrile (n=3)

Matrix	Concentration of acrylamide spike (ng/mL)	%Recovery (n=3)	%RSD (n=3)
1:1 ACN-water	50	116.6	4.07
1:1 ACN-water	100	114.06	4.85
French fries	100	97.14 (after blank correction)	5.04
French fries	200	97.50 (after blank correction)	2.55

Conclusion

A simple and fast multiresidue method based on Agilent Bond Elut QuEChERS for acrylamide extraction and cleanup with LC/MS/MS analysis has been developed. High extraction yields were obtained with excellent RSD. Therefore, this method can be applied to monitor acrylamide in food samples.

References

1. Al-Taher, F. 2009. Effect of heat-processed foods on acrylamide formation, In : Intentional and Unintentional Contaminants in Food and Feed. F. Al-Taher, L. Jackson, and J.W. DeVries, eds. Oxford University Press, New York, NY, pp.91-113.
2. IARC. Acrylamide. Some Industrial Chemicals: Monographs on the Evaluation of Carcinogenic Risks to Humans; International Agency for Research on Cancer: Lyon, France, 1994.
3. Wenzl, T.; Calle, B. Anklam, E. Analytical methods for the determination of acrylamide in food products: a review. Food Addit. Cont. 2003, 20, 885-902.
4. Roach, J.A.G.; Andrzewjewski, D.; Gay, M.L.; Nortrup, D.; Musser, S.M. Rugged LC/MS/MS survey analysis for acrylamide in foods, J. Agric. Food Chem. 2003. 51, 7547-7554.
5. Mastovska, K. and Lehotay, S.T. Rapid sample preparation method for LC/MS/MS or GC-MS analysis of acrylamide in various food matrices, J. Agric. Food Chem. 2006. 54, 7001-7008.

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