Application Note

The Determination of Acrylamide Using the Waters Micromass Quattro Premier LC-MS/MS System

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Abstract

In this application note, an instrumental method is described that could be used successfully for the determination of acrylamide in routine analysis.

Benefits

The LOQ achieved from this analysis is significantly lower than those previously reported on the Quattro Micro API using the FDA method

Introduction

Following the publication of two reports[1,2] indicating the presence of high concentrations of the neurotoxic, potentially carcinogenic analyte acrylamide in fried foods, chemists have begun to monitor for acrylamide in a variety of food matrices. Acrylamide is thought to be formed by the reaction of two naturally occurring food ingredients (asparagine and dextrose) when fried at high temperatures. Due to the small molecular weight of acrylamide (71 g/mol), GC-MS has historically been used for its determination. However, this requires the time consuming step of derivatization with bromine.

Scientists at the US FDA Center for Food Safety and Applied Nutrition (CFSAN) have developed an LC-MS/MS method that is widely being used as the basis for other acrylamide methods. The method and sample preparation can be found at the following website address: http://www.cfsan.fda.gov/~dms/acrylami.html.[3] The Limit of Quantification (LOQ) for the most abundant transition is stated as 10 µg/kg or 20 pg on column (S/N=10:1) using the Waters Micromass Quattro micro API Mass Spectrometer.[4]

In food products, the US FDA has not yet established a Maximum Residue Limit (MRL) for acrylamide, but the European Union has initiated a MRL of 10 µg/kg for the migration of acrylamide into food from food contact plastic. This does not apply to acrylamide that forms during cooking. The described method only just achieves this MRL. The ability to achieve lower LOQs would be significantly advantageous for the routine monitoring of acrylamide.

In this application note, an instrumental method is described that could be used successfully for the determination of acrylamide in routine analysis. This paper should be used in conjunction with other Waters Application Notes[5] that describe the extraction method in detail.

Experimental

LC Conditions

LC system:	Alliance 2795 HPLC
Isocratic mobile phase:	Water + 0.5% methanol + 0.1% acetic acid
Column:	Waters Atlantis dC_{18}, 2.1 x 150 mm, 3µm at 35 ˚C

Flow rate: 0.25 mL/min

Injection volume: 20 µL

MS Conditions

MS system : Waters Micromass Quattro Premier Mass Spectrometer

Electrospray mode with positive polarity

Capillary voltage: 3.5 kV

Cone voltage: 20 V

Extractor: 5 V

RF lens: 0 V

Source temp.: 120 °C

Desolvation temp.: 450 °C

Cone gas flow: 50 L/hr

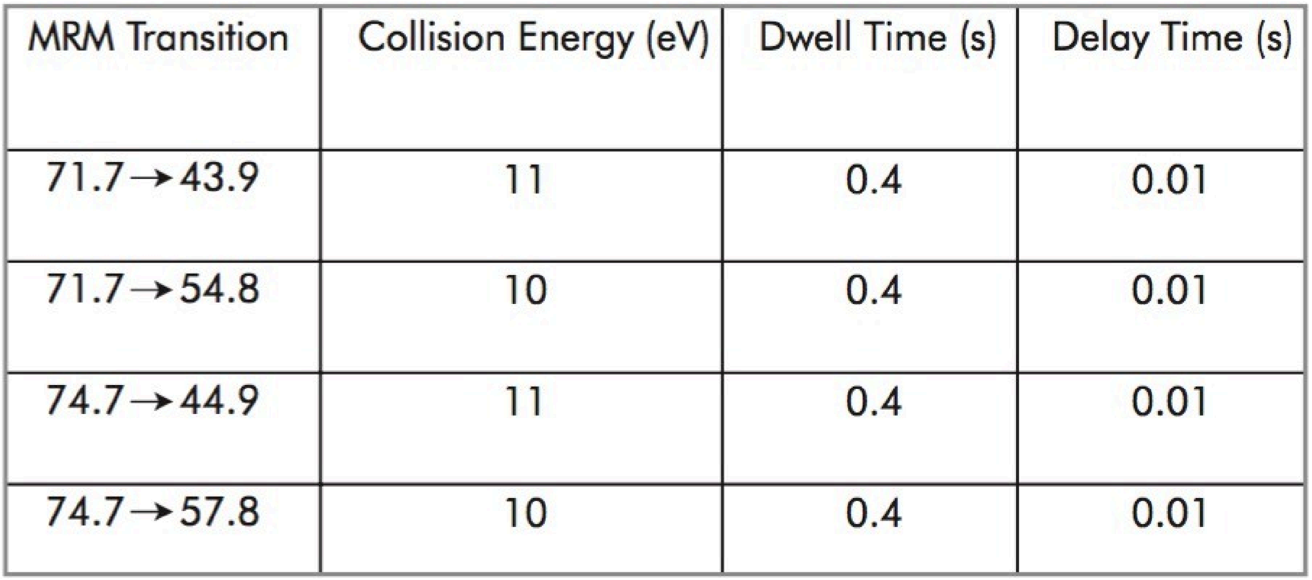
Desolvation gas flow: 850 L/hr

Collision gas pressure: Argon at $1.1e^{-2}$ mBar

Multiplier: 650 V

Acrylamide was tuned so that the precursor and product ions were resolved with a half height peak width of <0.7Da. The MRM transitions, along with the collision energies and dwell times for acrylamide and $^{13}C_3$-

acrylamide, are listed in Table 1. Four MRM transitions were monitored, a quantification and a confirmation transition for both analytes. 50 pg/µL $^{13}C_3$-acrylamide was used as the internal standard.



MRM Transition	Collision Energy (eV)	Dwell Time (s)	Delay Time (s)
71.7→43.9	11	0.4	0.01
71.7→54.8	10	0.4	0.01
74.7→44.9	11	0.4	0.01
74.7→57.8	10	0.4	0.01

Table 1. MRM Method Parameters.

Results and Discussion

This application uses a newer generation of reverse-phase C_{18} columns designed for all aqueous mobile phases. Other reverse-phase columns will show "hydrophobic collapse" and cause a significant decrease in retention times. The Waters Atlantis Column uses a lower ligand density allowing improved access of water polar analytes to the silica surface. Although this is a fully end-capped column, it does exhibit a secondary absorption mechanism necessary for polar molecule retention for analytes such as acrylamide. An example of the retention and the peak shape of acrylamide on an Atlantis Column are illustrated in Figure 1.

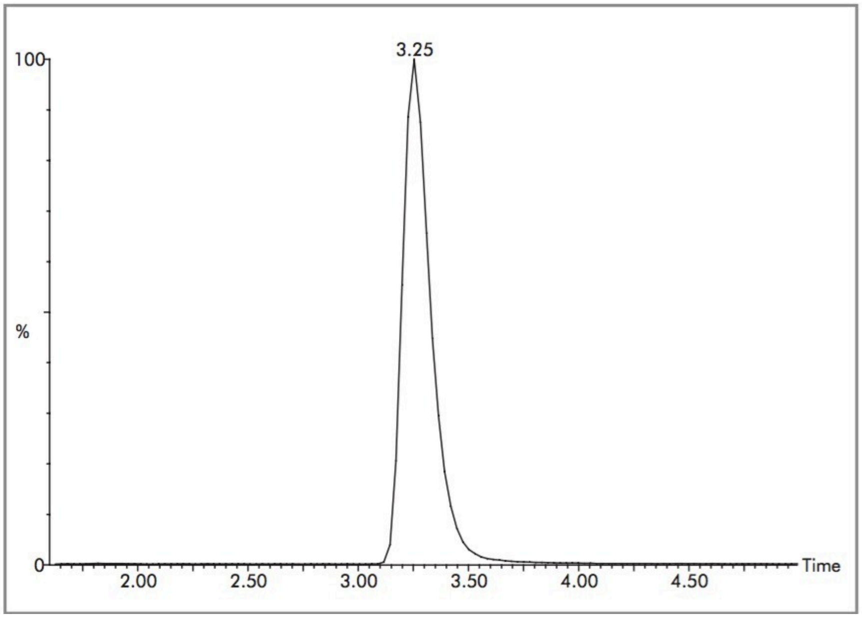


Figure 1. Acrylamide retention and peak shape on the Atlantis Column.

Calibration curves for acrylamide were constructed in the concentration range 0.1 to 250 pg/µL. A representative curve is illustrated in Figure 2 with two injections per vial. The 71.7 → 54.8 transition was used for quantification. The curve gave a coefficient of determination of $r^2 = 0.9999$ using 1/X weighting and internal standardization with $^{13}C_3$-acrylamide.

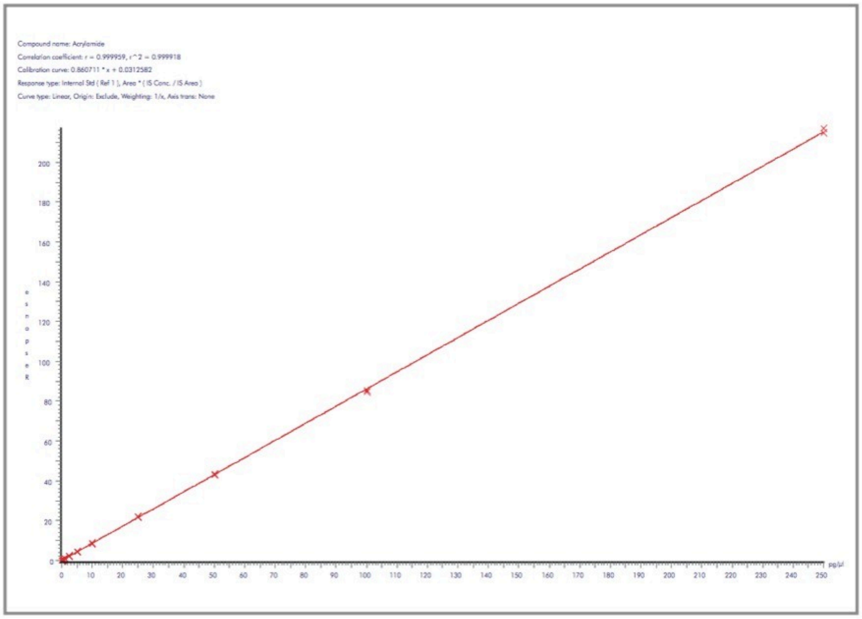


Figure 2. Calibration curve for acrylamide, 0.1–250 pg/µL (ppb).

The 71.7 → 54.8 transition of acrylamide at the 0.1 pg/µL (2 pg on column) concentration level is illustrated in Figure 3. The LOQ in solvent standards (S/N ratio = 10:1) for acrylamide on the Quattro Premier was determined to be 0.1 pg/µL.

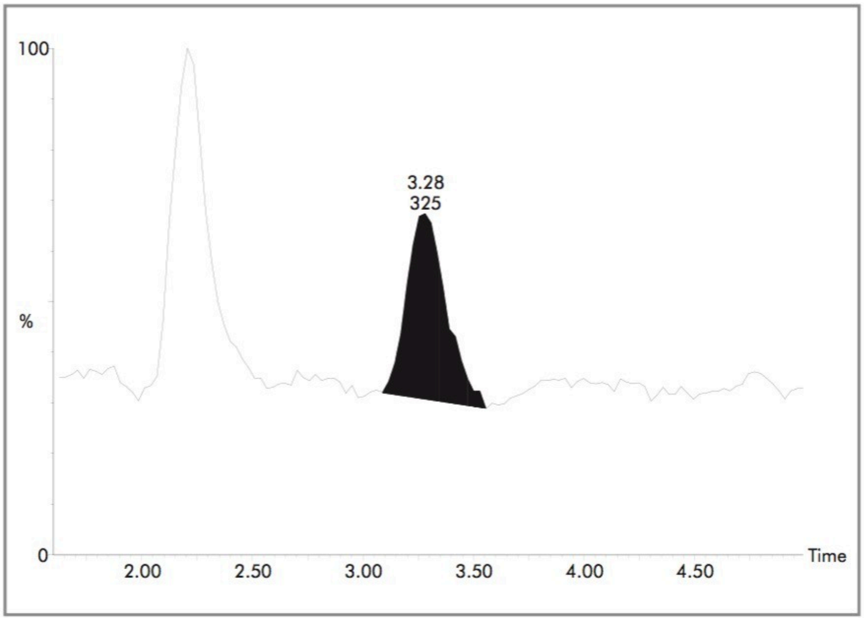


Figure 3. Acrylamide, 0.1 pg/µL, 71.7 → 54.8.

The FDA method recommends acrylamide confirmation using the ion ratio of 72, 55, and 27 for acrylamide with 75, 58, and 29 for the internal standard. Other methods have used the 72 → 44 transition for confirmation rather than the 72 → 27. The sensitivity of each transition will be system dependant. In this experiment, the Quattro Premier gave a better response for the 72 → 44 transition, allowing a confirmation LOQ of between 1 and 2.5 pg/µL (20 and 50 pg on column). The 2.5 pg/µL concentration level is illustrated in Figure 4. This data is significantly lower than that reported on the Quattro micro API.[4]

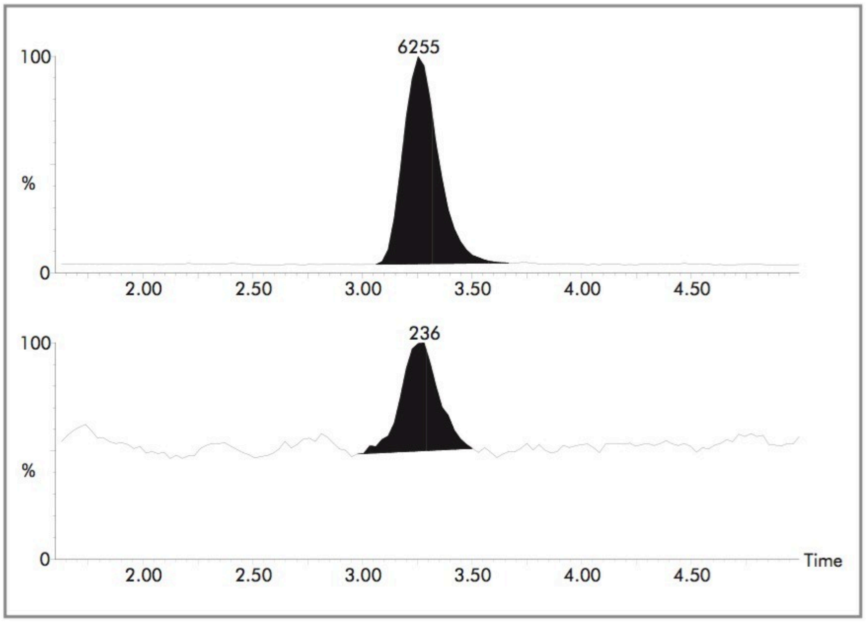
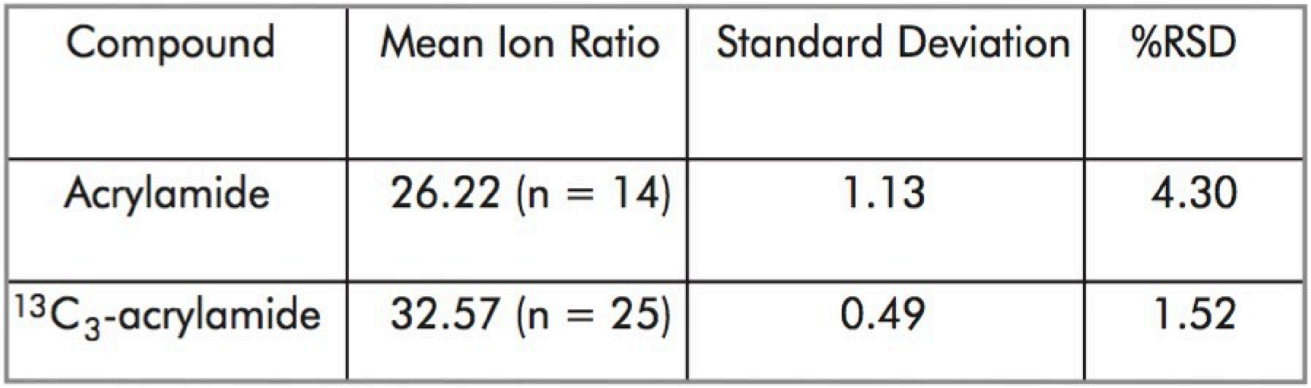


Figure 4. LOQ of acrylamide, 2.5 pg/µL, 71.7 → 54.8 and 71.7 → 43.9.

For confirmation criteria, transition ratio statistics were calculated by dividing the peak area of the quantification transition (72 → 55 or 75 → 58) by the peak area of the confirmation transition (72 → 44 or 75 → 45). The statistics quoted in Table 2 are for all injections greater than the LOQ.



Compound	Mean Ion Ratio	Standard Deviation	%RSD
Acrylamide	26.22 (n = 14)	1.13	4.30
$^{13}C_3$-acrylamide	32.57 (n = 25)	0.49	1.52

Table 2. Transition ratio statistics.

This method should not be attempted using food samples without suitable sample preparation. The high molecular weight carbohydrates and lipids are strongly retained and adversely effect acrylamide retention and performance.

Refer to FDA method[3], or Waters application notes[5] for sample preparation protocols using Waters Oasis HLB and Oasis MCX.

Conclusion

An instrumental method using Waters Micromass Quattro Premier LC-MS/MS System has been developed for the determination of acrylamide. The LOQ achieved from this analysis is significantly lower than those previously reported on the Quattro Micro API using the FDA method.

References

1. Tareke E., ***et al., Chem. Res. Toxicol.,*** 13 (2000) 517–522.
2. Tareke E., ***et al., J. Agric. Food Chem***., 50 (2002) 4998–5006.
3. Detection and Quantitation of Acrylamide in Foods, Feb 2002, http://www.cfsan.fda.gov/~dms/acrylami.html.
4. Roach J., ***et al., J. Agric. Food Chem.***, 51 (2003) 7547–7554.
5. Solid-Phase Extraction and Cleanup Procedure for the LC-MS Determination of Acrylamide in Fried Potato Products, June 2003, WA20799 <https://www.waters.com/webassets/cms/library/docs/wa20799.pdf> .

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