

# AccuTOF™

## Analysis of Acrylamide in Food by LC/Time-of-Flight Mass Spectrometry

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### Introduction

Acrylamide has been shown to cause cancer in animal studies. It has also been shown to cause nerve damage in people who have been exposed to very high levels at work. In April 2002 the Swedish National Food Authority reported the presence of elevated levels of acrylamide in certain types of food processed at high temperatures. Since then, acrylamide has been found in a range of cooked and heat-processed foods in many countries including the United States<sup>[1]</sup>. In order to assess acrylamide risk to humans, food levels need to be measured accurately. This prompts the need for development of analytical methods for acrylamide analysis. The US Food and Drug Administration has published an LC/MS/MS method for acrylamide<sup>[2]</sup>. Due to the low molecular weight of acrylamide, GC/MS has also been used for its analysis<sup>[3,4]</sup>. However, GC/MS requires time-consuming derivatization. Here, we describe a simple and rapid LC/Time-of-flight MS method with accurate mass measurement and “in-source CID” fragmentation for acrylamide analysis.

### Experimental

#### Chemicals and preparation of standard solutions

All solvents used were of HPLC grade. The acrylamide stock solution was prepared by dissolving acrylamide (Sigma-Aldrich) in water with a concentration of 1 mg/mL. A series of working solutions was prepared by diluting the stock solution in water with concentrations from 10 ng/mL to 10 µg/mL. D<sub>3</sub>-Acrylamide was used as internal standard for quantitation and prepared in water with the concentration of 1 µg/mL.

#### Sample preparation

The solid-phase extraction (SPE) of acrylamide from food samples followed the FDA method<sup>[2]</sup> with some modifications. Briefly, a portion of sample equal to the manufacturer’s recommended serving size was crushed and homogenized. Approximately 200 mg of crushed sample was weighed and 1.8 mL of water and 200 µL of internal standard were added. After mixing for 20 min on a rotating shaker, 500 µL of the above solution was transferred to a micro-centrifuge vial and centrifuged at 2,700 g for 15 min. 300 µL of the supernatant extract was loaded onto a preconditioned Oasis HLB 1cc SPE cartridge (Waters). The cartridge was then washed with 100 µL of water and eluted with 300 µL of water. The eluent was then injected into the LC/TOF-MS system.

#### LC/TOF-MS analysis

An Agilent 1100 HPLC system was used. The HPLC separation was carried out on a 2 x 100mm, 3µm particle, Luna C<sub>18</sub> column (Phenomenex) with the mobile phase of methanol/0.1% formic acid (10/90, v/v). The flow rate was set at 0.2 mL/min. The injection volume was 20 µL.

The mass spectrometer system consisted of a JEOL AccuTOF™ time-of-flight mass spectrometer with standard electrospray ion source (ESI) and a JEOL MassCenter™ workstation. The system was tuned to achieve a resolution of 6,000 (FWHM). The ESI source was set at positive mode. The needle voltage and the MCP voltage were set at 2,000 and 2,600 V, respectively. The temperatures for the desolvating chamber and orifice 1 were set to 250 °C and 80 °C, respectively. The orifice 1 voltages were switched between 35 and 120 V to produce both protonated molecular ions and fragment ions. The methanol dimer ion was used as “lock mass” for accurate mass measurement. High-resolution mass chromatograms were generated for the measurement of peak areas.

## Results and discussion

With current HPLC conditions, the measurement can be completed in 3 minutes. Without using the secondary SPE cartridge in the FDA extraction method, the sample preparation time was significantly reduced. But this can only be achieved by a high resolution mass spectrometer. Figure 1A shows the mass chromatogram of acrylamide extracted from a French fry sample with 6,000 (FWHM) resolution, while Figure 1B shows the mass chromatogram with unit resolution. A comparison of two chromatograms shows that the abundance of an early-eluting interference peak is significantly reduced in the high-resolution mass chromatogram. Therefore, accurate peak areas for quantitation can be obtained.

The orifice 1 voltages were switched between 35 and 120 V automatically in one run to obtain both  $[M+H]^+$  and the fragment ion for acrylamide identification. The mass spectrums are shown in Figure 2. With accurate mass measurement, the fragment ion  $m/z$  55 was confirmed as  $[M+H-NH_3]^+$ . The mass errors for both protonated and fragment ions are less than 2 mmu, indicating excellent mass accuracy and positive identification.

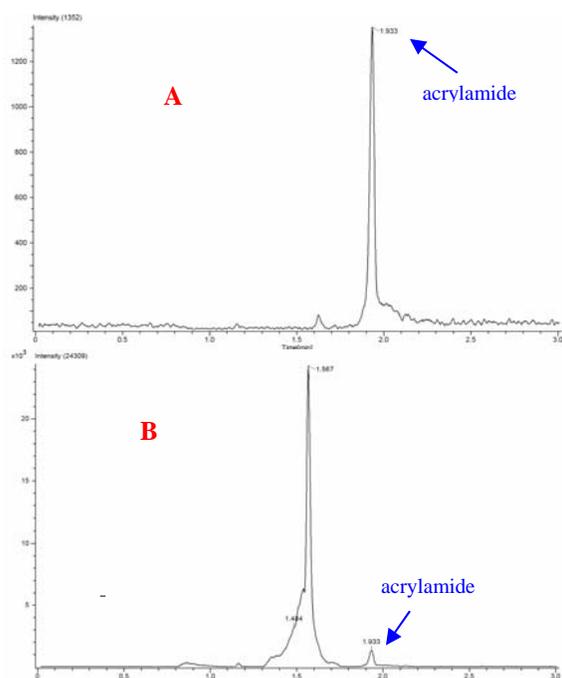


Fig.1 Mass chromatograms of acrylamide extracted from French fry sample. (A) high resolution, (B) low resolution

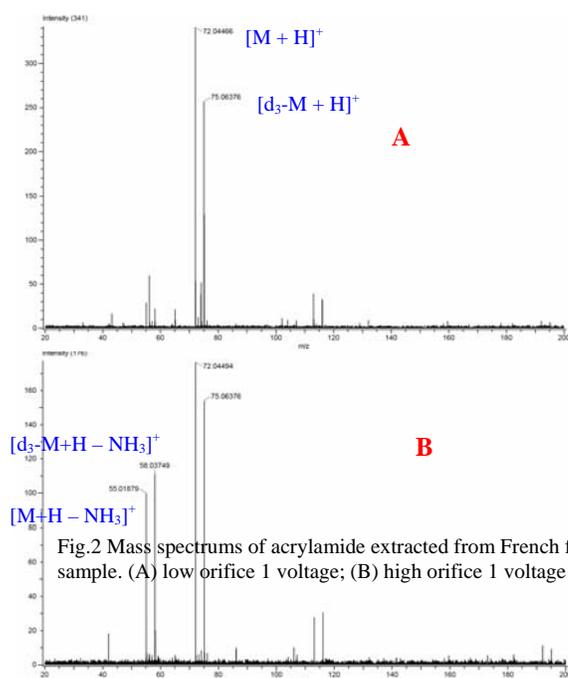


Fig.2 Mass spectrums of acrylamide extracted from French fry sample. (A) low orifice 1 voltage; (B) high orifice 1 voltage

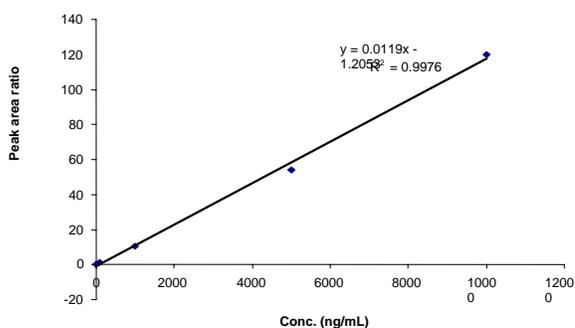


Fig. 3 Calibration curve for acrylamide

Sample	Conc. ( $\mu\text{g/g}$ )	C.V. (%) (n=5)
French fry 1	0.43	2.3
French fry 2	0.36	2.7
French fry 3	0.21	3.2
Potato chip 1	0.92	1.5
Potato chip 2	0.76	1.7
Potato chip 3	0.52	2.0

Table 1. Acrylamide levels in some French fry and potato chip samples

A signal-to-noise ratio of 62 was obtained when 12 ng/mL acrylamide solution was injected, indicating good sensitivity. D<sub>3</sub>-acrylamide was used as an internal standard for quantitation. The quantitation standard curve is shown in Figure 3. The R<sup>2</sup> for the curve is 0.9976, indicating good linearity. The acrylamide level in some French fry and potato chip samples are listed in Table 1.

## Summary

An LC/TOF-MS method has been developed for analyzing acrylamide in food samples. The method is accurate, simple, and rapidly completed.

## References:

- [1] [http://www.who.int/foodsafety/publications/chem/acrylamide\\_faqs/en/index.html](http://www.who.int/foodsafety/publications/chem/acrylamide_faqs/en/index.html)
- [2] <http://www.cfscan.fda.gov/~dms/acrylami.html>
- [3] <http://www.chem.agilent.com/cag/prod/CA/b0467.pdf>
- [4] LC/GC Europe, Application Book, Sept. 2003