APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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# Acrylamide Analysis in Waste Water by UHPLC-MS

## Introduction

Specific to waste water, there are currently 31 hazardous compounds under regulation in Korea. In particular, the allowable maximum limit for acrylamide (Figure 1) in Korean waste water was recently set to 15 ppb.[1] Using the HPLC/UV procedure prescribed by EPA method 8316, it is difficult to attain this new sensitivity requirement.

Considering the above, the UHPLC-MS method defined herein was developed to allow for the quantitation of acrylamide in waste water down to low ppb levels, accommodating the current regulation requirements laid down in Korea. Method conditions and performance data, including linearity and repeatability, are presented.

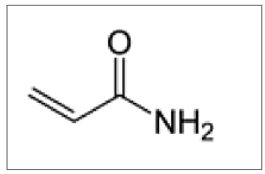


*Figure 1.* Structure of acrylamide ($C_3H_5NO$; MW=71.08).

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## Experimental

### Hardware/Software

A PerkinElmer Altus™ UPLC® system was used, which included the A-30 Sampling and Solvent Delivery Modules (quaternary pump) and Column Heater, as well as the Altus SQ MS detector (PerkinElmer, Shelton, CT, USA). All instrument control, analysis and data processing was performed using the Waters® Empower® 3 Chromatography Data System (CDS) platform.

### Method Parameters

The UHPLC and MS method parameters are shown in Tables 1 and 2, respectively.

*Table 1.* UHPLC Method Parameters.

| | |
|---|---|
| Column: | PerkinElmer Altus UPLC® BEH C18; 1.7 µm, 2.1 x 50-mm (Part # N2972000) |
| Column Temp: | 35 °C |
| Mobile Phase: | Isocratic: 1% methanol and 0.1% formic acid in water |
| Analysis Time: | 2.0 min. |
| Flow Rate: | 0.2 mL/min. |
| Injection Volume: | 5 µL |

*Table 2.* MS Method Parameters.

| | |
|---|---|
| Ionization Mode: | Electrospray (+) |
| Capillary Voltage: | 3.50 kV |
| Source Temp.: | 150 °C |
| Cone Voltage: | 22 V |
| Desolvation Temp.: | 350 °C |
| Desolvation Gas: | 650 L/hr. |
| SIRs (single ion recordings): | m/z 72 (for analysis); m/z 55 (for confirmation) |

### Solvents, Standards and Samples

All solvents and mobile phase additives were LC-MS grade, including water, which was used for all dilutions.

The acrylamide standard (Part # M-8032) was acquired from AccuStandard Inc. A 100-ppb stock standard solution was prepared via dilution with water. Calibration levels of 50, 10 and 5 ppb were prepared via serial dilution of the stock standard.

The waste water sample was obtained from the Seoul Institute of Health & Environment. 2 mL of sample supernatant was filtered through a 0.22-µm nylon filter. 900 µL of the filtered sample was then spiked with 100 µL of the 100-ppb acrylamide stock standard and shaken vigorously, providing a spiked sample concentration of 10 ppb acrylamide. The remaining filtered unspiked sample was used as a sample "blank".

5 µL was injected on column for all analyses.

## Results and Discussion

Figure 2 shows the 4-replicate chromatographic overlay of the 10-ppb acrylamide standard, eluting at just over 1 minute. The initial "peak" represents the column void volume ($t_0$), which is well removed from the analyte. As observed via the overlay and shown in Table 3, the retention time reproducibility is very good.

*Table 3.* Retention time repeatability results for four replicates of the 10-ppb acrylamide standard

| Injection # | Retention Time (min) |
|---|---|
| 1 | 1.038 |
| 2 | 1.038 |
| 3 | 1.039 |
| 4 | 1.038 |
| RSD% | 0.05 |

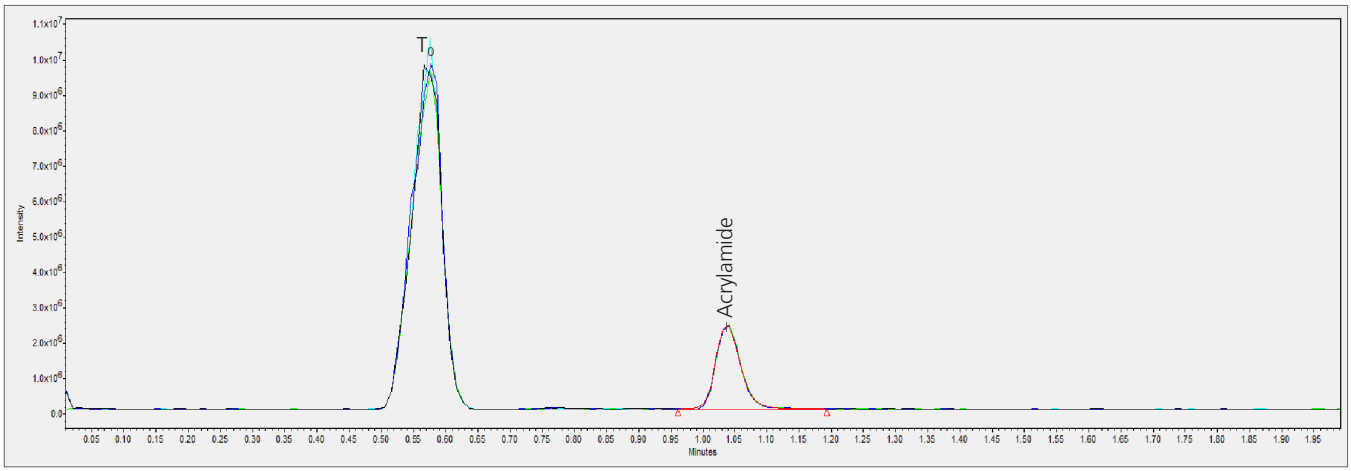


*Figure 2.* Chromatographic overlay of four replicates of the 10-ppb acrylamide standard; m/z 72.

The 3-level calibration plot for acrylamide (Figure 3) shows a very linear response, with an $R^2 > 0.999$.

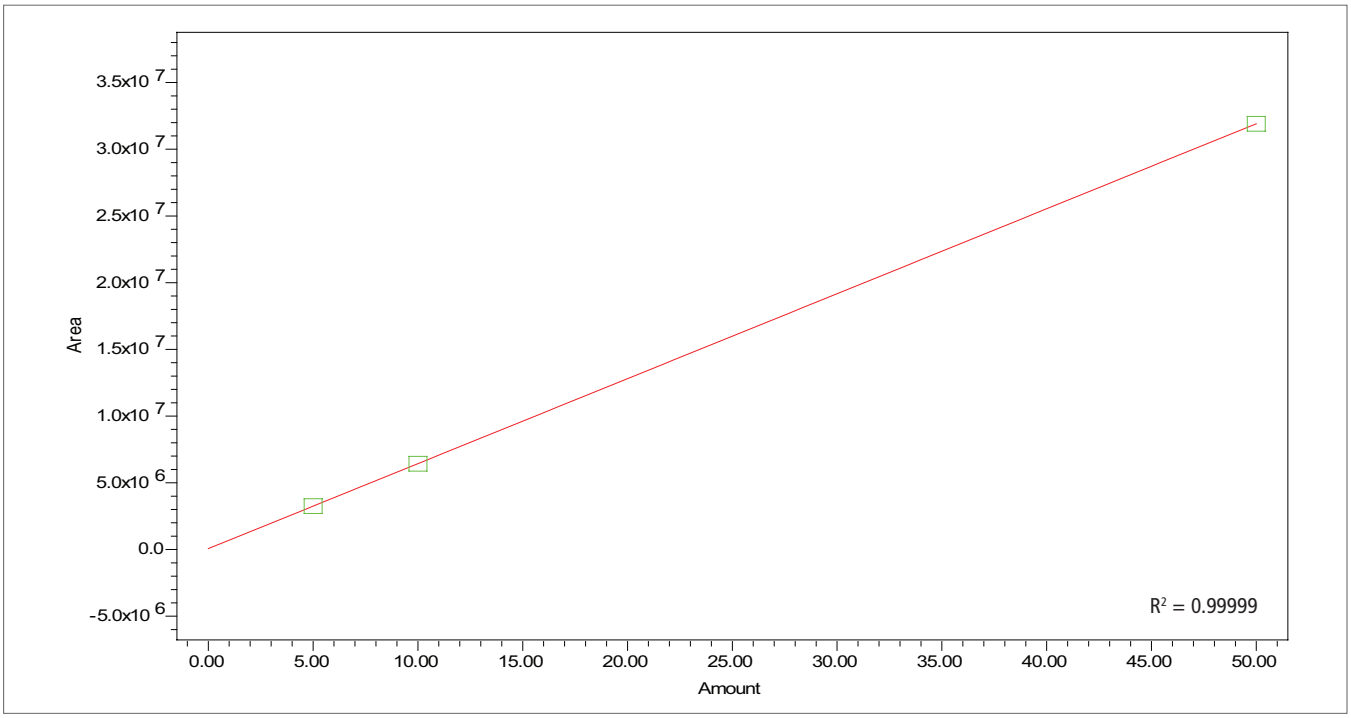


*Figure 3.* 3-level linear calibration results.

The chromatogram and corresponding results of the 5-ppm acrylamide standard are shown in Figure 4 and Table 4. Considering the S/N ratio of 33.48, the calculated LOD and LOQ values were 0.45 and 1.5 ppb, respectively. This was based upon an S/N ratio requirement of ≥ 3 for LOD and ≥ 10 for LOQ.

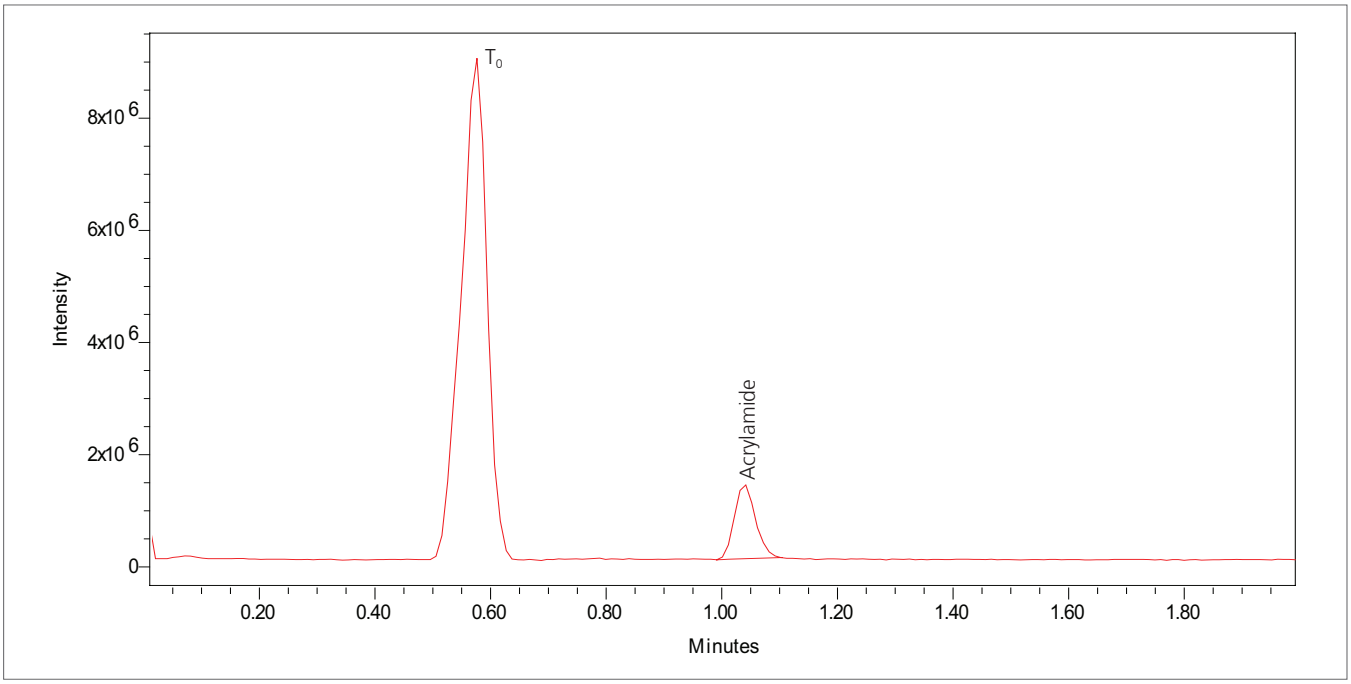


*Figure 4.* Chromatogram of the 5-ppb acrylamide standard; m/z 72.

*Table 4.* Chromatographic results for the 5-ppb acrylamide standard.

| Peak Results | | | | | |
|---|---|---|---|---|---|
| **Component** | **Ret. Time** | **Area** | **Height** | **Units** | **S/N** |
| Acrylamide | 1.039 | 3353834 | 1331570 | Ppb | 33.480 |

As shown in Figure 5, the unspiked waste water sample showed no detectable amount of acrylamide.

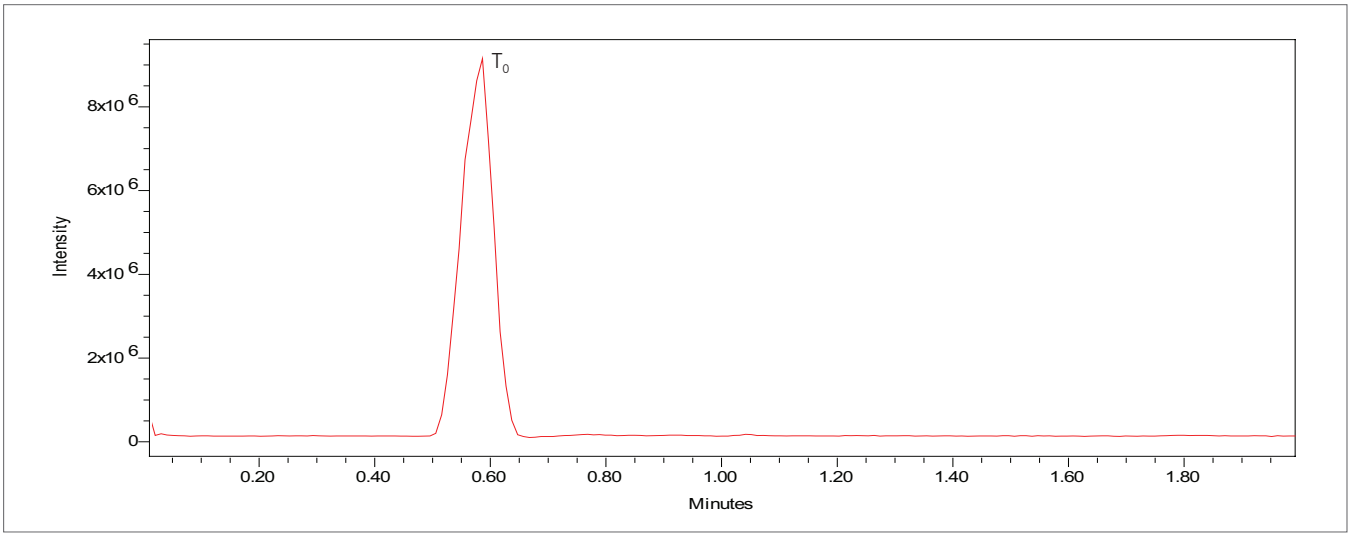


*Figure 5.* Chromatogram of unspiked sample; m/z 72.

Figure 6 shows two chromatograms of the waste water sample spiked with 10-ppb acrylamide, one captured at m/z 72 and the other at m/z 55. The chromatogram captured at m/z 55 (Figure 6b) was used to verify analyte identification, as it is well known that protonated acrylamide can lose ammonia ($NH_3$) during ionization, resulting in the $[CH_2=CH-C=O]^+$ fragmentation ion.[2]

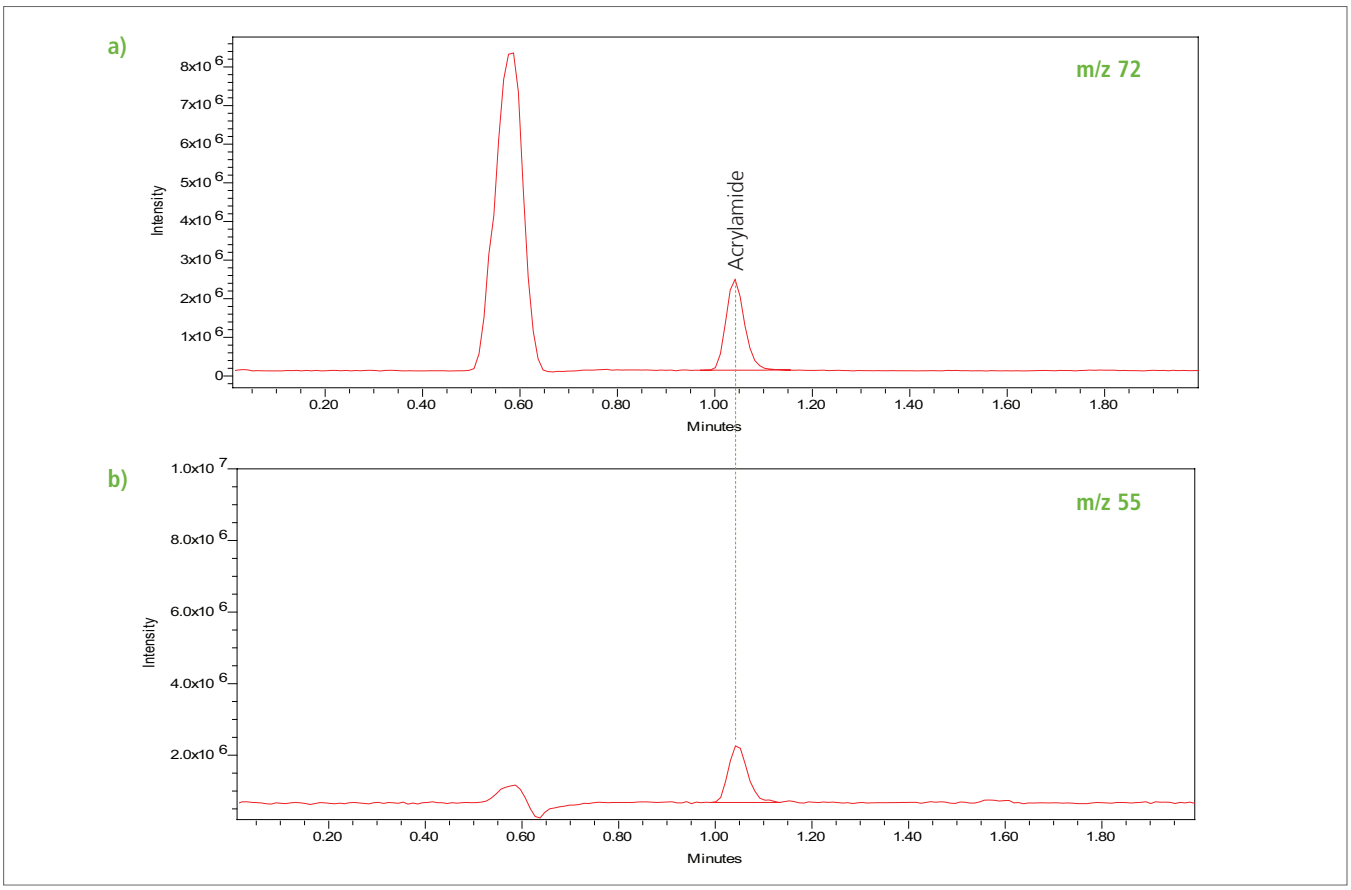


*Figure 6.* Chromatograms of waste water sample spiked with 10-ppb acrylamide; a: m/z 72; b: m/z 55.

Table 5 shows the comparative results for the 10-ppb acrylamide standard and the waste water sample spiked with 10-ppb acrylamide. Based on the area responses, the acrylamide recovery for the spiked waste water sample was determined to be 98%.

*Table 5.* Results for the 10-ppb acrylamide standard compared to the spiked waste water sample.

| | Ret. Time | Area |
|---|---|---|
| 10-ppb Acrylamide Standard | 1.039 | 6424478 |
| Waste water sample spiked with 10-ppb acrylamide | 1.040 | 6207554 |

## Conclusion

Based on the above results, this application presents a fast and effective LC/MS approach for the quantitative determination of acrylamide in waste water samples, down to low ppb levels. The method was shown to provide both very good linearity and chromatographic reproducibility, and offers fast and easy sample preparation.

## References


1. Korean Ministry of Environment (MOE), "Water Quality and Ecosystem Conservation Act", Enforcement Rule No. 2015-238.
2. http://www.epa.gov/sites/production/files/2013-12/documents/liquid-chromatography-acrylamide.pdf.



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