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Determination of Acrylamide in Water by LC-HRMS Analysis Using a Luna™ 3 µm Polar Pesticides HPLC Column

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Introduction

Acrylamide is a white odorless solid, soluble in water and several organic solvents. Most of the Acrylamide produced is used as a chemical intermediate or as a monomer in the production of polyacrylamide. Both Acrylamide and Polyacrylamide are used mainly in the production of flocculants for the clarification of potable water and in the treatment of municipal and industrial effluents. It has been found to be neurotoxic and carcinogenic, so the level in water and food are strictly regulated. EU Directives 2020/2184 establish the Acrylamide limits in water at 0.10 µg/L, which refers to the residual monomer concentration in the water as calculated according to specifications of the maximum release from the corresponding polymer in contact with the water. In this technical note, a method combining enrichment with carbon SPE and LC-HRMS that is able to reach the legislation limits is shown.

LC Conditions

Column: Luna 3 µm Polar Pesticides
Dimensions: 100 x 2.1 mm
Part No.: [00D-4798-AN](#)
Mobile Phase: A: 0.1 % Formic Acid in Water
 B: 0.1 % Formic Acid in Acetonitrile
 A / B (1:99, v/v)
Flow Rate: 0.2 mL/min (Isocratic)
Injection Volume: 20 µL
Temperature: 35 °C
Instrument: Vanquish™ UHPLC
Detection: SIM
Detector: Q Exactive™ Orbitrap™

Sample Preparation

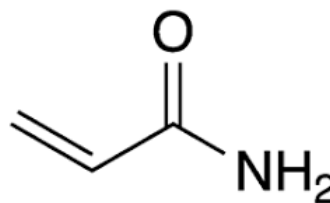
| Step | Description |
|------------------------------|---|
| Sample Pre-treatment: | 5 different aliquots of Tap Water (500 ml) were spiked with 15, 30, 50, 75, and 100 µl of Acrylamide (1 ppm) and 500 µl of D ₃ -Acrylamide (100 ppb) to obtain a final concentration of 30, 60, 100, 150, and 200 ppt of Acrylamide. |
| Condition: | GS-Tek ACA 500 mg/ 6 ml cartridges with 8 mL of Methanol. |
| Equilibrate: | With 8 mL of Water. |
| Load: | 500 mL of pre-treated Tap Water. |
| Dry: | Vacuum at 5-10 in. Hg for 15 minutes. |
| Elute: | With 6 mL of Methanol. |
| Reconstitute: | Dry down and dissolve in 1 mL of Dichloromethane. |

Table 1. Exact Mass of Analytes.

| Analyte | Exact Mass [M-H] ⁺ |
|----------------------------|-------------------------------|
| Acrylamide | 72.04514 |
| D ₃ -Acrylamide | 75.06392 |

MS/MS Conditions

Polarity: Positive
Sheet Gas: 35 L/min
Auxiliary Gas: 10 psi
Auxiliary Temperature: 200 °C
Spray Voltage: 3000 V
Capillary Temperature: 250 °C
Max Injection Time: 50 ms



Acrylamide



Results and Discussion

To generate the calibration curve, 5 different aliquots of tap water (500 ml) containing Acrylamide and D₃-Acrylamide at 30, 60, 100, 150, and 200 ppt were extracted and enriched 500 times, as reported in the sample preparation protocol before LC-HRMS analysis using a Luna™ 3 µm Polar Pesticides HPLC Column. As seen in **Figure 1**, Acrylamide was detectable at the lowest concentration of 30 ppt in tap water. A calibration curve was also drawn (**Figure 2**) and shows that this method is linear within the concentration range of 15 to 100 ppb, with a R² value of 0.998.

Acrylamide and D₃-Acrylamide show a very high stability in term of retention time as reported in the **Figure 3**. Finally, to prove the method recovery, two independent samples were spiked respectively with 60 and 150 ppt of Acrylamide and 100 ppt of D₃-Acrylamide as internal standard. Each sample was extracted using the same carbon SPE protocol to obtain an enrichment of 500 times before the injection into the LC-HRMS system using a Luna 3 µm Polar Pesticides HPLC Column. Each result obtained was normalized over the internal standard response to calculate the Acrylamide in water sample.

Figure 1. Extracted Ion Chromatogram of Acrylamide at 15 ppb Corresponding to 30 ppt in Tap Water.

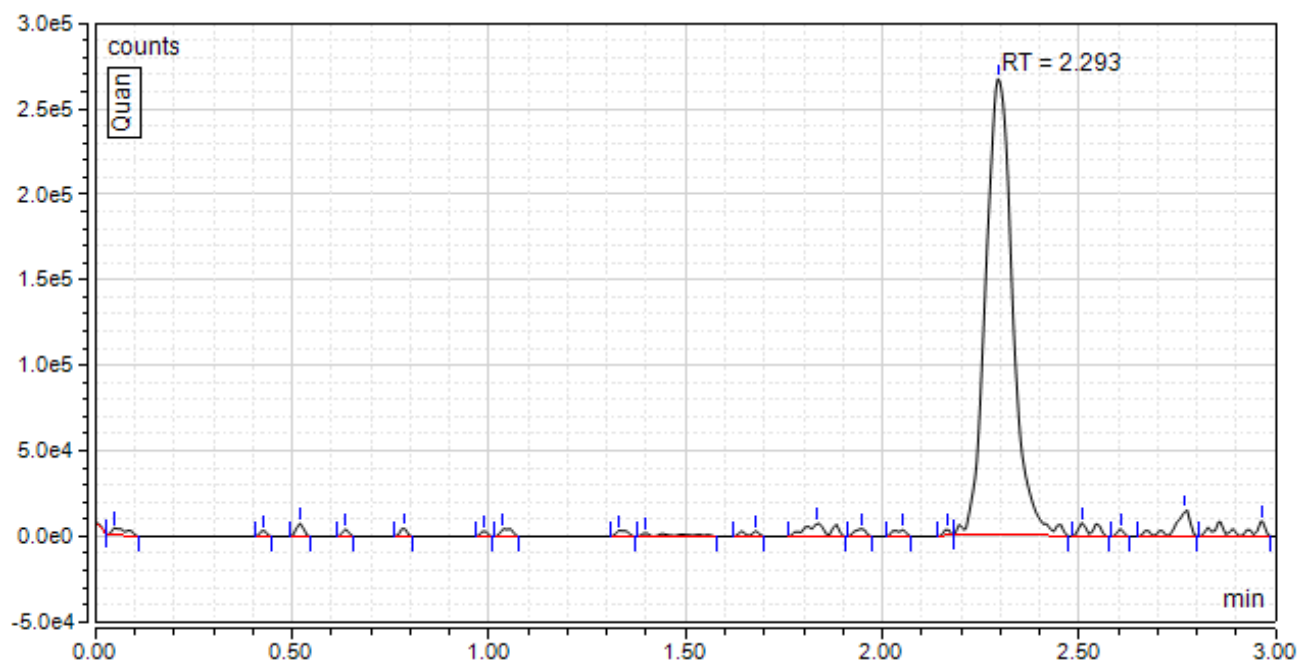


Figure 2. Calibration Curve for Acrylamide.

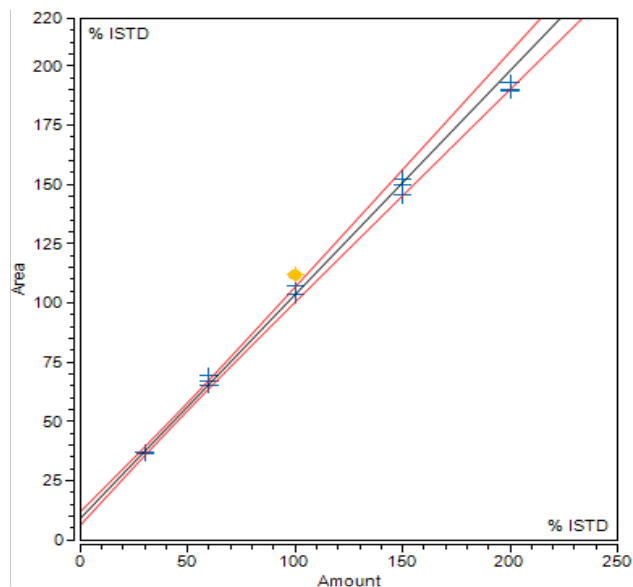
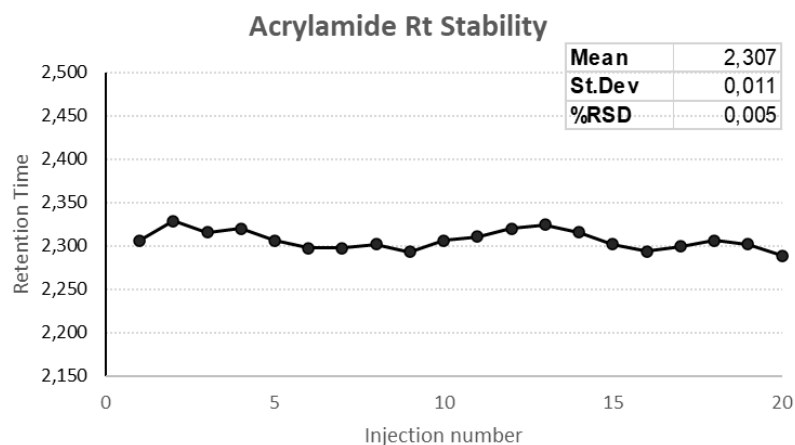


Table 2. Calibration Curve Data for Acrylamide.

| Sample Name | Cal level | Acrylamide Tap Water Level Before Enrichment (ppt) | Area Counts*min | ISTD Area | Area Ratio % |
|--------------------|-----------|--|-----------------|-----------|--------------|
| 15 ppb Acrylamide | 1 | 30 | 5984 | 16311 | 36,7 |
| 15 ppb Acrylamide | 1 | 30 | 5996 | 16097 | 37,3 |
| 15 ppb Acrylamide | 1 | 30 | 5913 | 16050 | 36,8 |
| 30 ppb Acrylamide | 2 | 60 | 8519 | 13027 | 65,4 |
| 30 ppb Acrylamide | 2 | 60 | 8371 | 12529 | 66,8 |
| 30 ppb Acrylamide | 2 | 60 | 8844 | 12726 | 69,5 |
| 50 ppb Acrylamide | 3 | 100 | 21134 | 20446 | 103,4 |
| 50 ppb Acrylamide | 3 | 100 | 23326 | 21799 | 107,0 |
| 50 ppb Acrylamide | 3 | 100 | 21421 | 19152 | 111,8 |
| 75 ppb Acrylamide | 4 | 150 | 19327 | 13290 | 145,4 |
| 75 ppb Acrylamide | 4 | 150 | 19664 | 13110 | 150,0 |
| 75 ppb Acrylamide | 4 | 150 | 19129 | 12545 | 152,5 |
| 100 ppb Acrylamide | 5 | 200 | 25502 | 13427 | 189,9 |
| 100 ppb Acrylamide | 5 | 200 | 25125 | 13250 | 189,6 |
| 100 ppb Acrylamide | 5 | 200 | 26014 | 13462 | 193,2 |

Figure 3. Measured Retention Time Over 20 Injections.



Conclusion

Since the levels of Acrylamide in drinking water sources are strictly regulated, it is critical to be able to quickly and accurately determine Acrylamide levels. The ACA SPE was suitable for the extraction of Acrylamide from water in the ppt concentration range. The Luna™ Polar Pesticides column provided a short run time of less than 4 minutes for high throughput analysis. By coupling ACA SPE and the Luna Polar Pesticides HPLC column, we achieved quality extraction and process efficiency by reducing run time for both sample preparation and determination of Acrylamide in tap water samples.

Order Information

| Luna 3 µm Analytical Columns (mm) | | | | | | SecurityGuard™ ULTRA Cartridges* |
|-----------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|----------------------------------|
| Phase | 30 x 2.1 | 50 x 2.1 | 100 x 2.1 | 150 x 2.1 | 150 x 3.0 | 3/pk |
| Polar Pesticides | 00A-4798-AN | 00B-4798-AN | 00D-4798-AN | 00F-4798-AN | 00F-4798-YO | AJ0-8789 |

For ID: 2.1-4.6 mm

*SecurityGuard ULTRA Cartridges require holder, Part No.: [AJ0-9000](#)



Have questions or want more details on implementing this method? We would love to help! Visit www.phenomenex.com/Chat to get in touch with one of our Technical Specialists

Need a different column size or sample preparation format?

No problem! We have a majority of our available dimensions up on www.phenomenex.com, but if you can't find what you need right away, our super helpful Technical Specialists can guide you to the solution via our online chat portal www.phenomenex.com/Chat.

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