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Identification and Quantification of Atrazine in the North River Using SPE and HPLC

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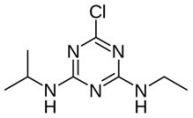
Introduction

The North River, along with many rivers, have low concentrations of pesticides that can be magnified due to human consumption of aquatic life. Atrazine is a commonly used herbicide that prevents growth of broadleaf and grassy weeds. It is not soluble in water and exhibits slow to no biodegradation in surface waters. There are detrimental side effects on humans, such as damage to the nervous system and the liver due to atrazine exposure.



The purpose of this study is to identify and quantitate atrazine in the North River. A sample from the North River was collected near River Road at Bridgewater college and analyzed using solid phase extraction (SPE) and high-performance liquid chromatography (HPLC).

Experimental

Filtration and Solid Phase Extraction (SPE):

- The water sample was filtered through a glass microfibre filter before SPE.
- The octadecyl (C18) SPE column was conditioned with two 5-mL aliquots of 3:1 ethyl acetate/ethanol, 5 mL of ethanol, and three 5-mL aliquots of 10 g/L ascorbic acid.
- The filtered water sample was extracted, and the column left to dry for 5 days.
- Sample was eluted using 13 mL of ethyl acetate/ethanol 3:1 with 0.2% trifluoracetic acid (TFA) in aliquots of 5, 5, and 3.

Sample Preparation:

- Sample was neutralized with 16 µL of 1.5 M sodium hydroxide (NaOH).
- The neutralized sample was concentrated down to 1 mL using nitrogen (N_2) gas.
- Concentrated sample was placed in an autosampler vial and stored at 4°C.

High-Performance Liquid Chromatography (HPLC) Parameters for Atrazine Analysis:

- Column and stationary phase: 5 µ, 50x4.6 mm ODS-2
- Gradient elution: 70/30 MeOH/H2O for 1 min, transition to 80/20 MeOH/H2O in 1 min, hold 80/20 MeOH/H2O for the remainder of the sample run.
- Injection volume and run time: 10 µL, 6 minutes.
- Processing: integrate peak areas at atrazine's retention time and inhibit integration outside that retention time window.

External Standard Calibration and Water Sample Analysis:

- Five calibration standards were made from an atrazine stock solution and analyzed using the atrazine HPLC parameters.
- The standards' integrated peak areas were utilized to generate a calibration curve to be used for the quantitation of atrazine.
- The water sample was injected by HPLC and analyzed using the atrazine parameters.

Results and Discussion

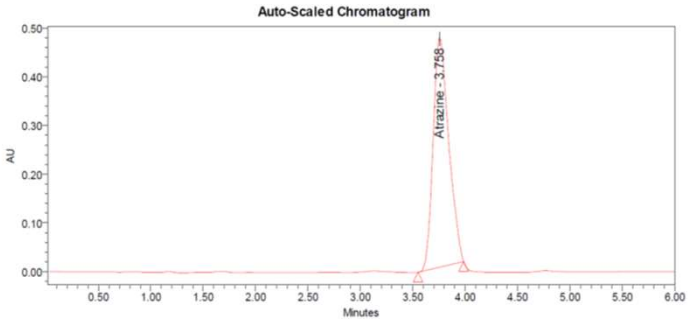


Figure 1. Chromatogram of atrazine calibration standard #1, displaying the retention time of atrazine (3.758 mins). The chromatogram was obtained by injecting atrazine calibration standard #1 (0.0625 mg/mL) and running it with the atrazine HPLC parameters. The data was processed using the atrazine processing method, and the atrazine peak was integrated.

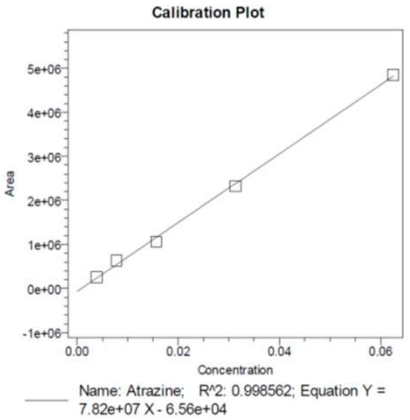


Figure 2. External standard calibration curve of atrazine. Five calibration standards of concentrations 0.0625, 0.0313, 0.0156, 0.00781, and 0.00391 mg/mL were made from an atrazine stock solution. Their chromatogram peaks were integrated, and the peak areas were utilized to generate a calibration curve. The calibration curve can be used to quantitate atrazine in the water sample.

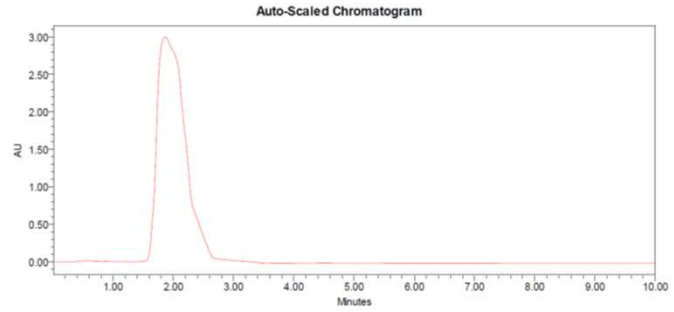


Figure 3. Chromatogram of the North River (Bridgewater, VA) water sample. There were no atrazine peaks observed in the chromatogram obtained at 222 nm, so atrazine cannot be quantitated. A peak at 1.95 minutes was displayed and was suspected to be caused by ethyl acetate, which was used to elute the sample from the SPE column. Ethyl acetate's UV cutoff is around 260 nm; therefore, it would have absorbed at the chosen wavelength of 222 nm.

Figure 4. Waters Alliance 2695 Separations Module was used to generate the atrazine calibration curve and analyze the water sample.

Conclusions

- The water sample was successfully extracted and eluted from the C18 SPE column.
- HPLC instrument and processing methods were created for the analysis of atrazine in the water sample.
- An atrazine calibration curve was successfully generated for the quantitation of atrazine in the water sample.
- No atrazine was found in the water sample, but another peak was observed and was suspected to be caused by ethyl acetate.

Future Work

The HPLC can be used for the separation of many complex mixtures. Future projects can include whole cell sugar analysis of a bacterial cell wall and determination of caffeine concentrations in different kinds of coffee and tea.

Acknowledgements

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References

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2. Werner, S. L.; Burkhardt, M. R.; DeRusseau, S. N. *Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory--Determination of Pesticides in Water by Carbopak-B Solid-Phase Extraction and High-Performance Liquid Chromatography;* Open-File Report; U.S. Geological Survey: Denver, 1996.