THOMPSON RIVERS UNIVERSITY

UREAP WINTER 2023 REPORT

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An Analytical Method for Quantifying Tetrahydrozoline Found in Eye Drops Using Capillary Electrophoresis

July 2023

Table of Contents

Abstract

Eye drops are most often saline solutions with medications in them to treat various eye diseases. They are used as artificial tears to treat dry eyes or simple irritation such as itching or redness. One of the main components of eye drops is tetrahydrozoline, a decongestant used to relieve redness in the eyes caused by minor eye irritations (ex: smog, swimming, dust, smoke). Unfortunately, recently the oral consumption of eye drops has risen causing poisoning due to the tetrahydrozoline in the eye drops. Capillary electrophoresis, an analytical technique that separates ions based on their electrophoretic mobility by using an applied voltage, has yet to be used to isolate tetrahydrozoline from eye drops. Capillary electrophoresis provides several advantages, including high separation efficiency, low cost, short analysis times, easy use, and low waste generation. Therefore, the objective of my study was to develop and optimize an analytical method using capillary electrophoresis (CE) to isolate and determine tetrahydrozoline in commercially available eye drops. The results of this research have provided insight into the successful detection of tetrahydrozoline from commercially available eye drops to identify which brands of eye drops are potentially the safest. It may also provide companies with a better means to ensure that their products contain the correct amount of tetrahydrozoline. Furthermore, the method was validated to evaluate its precision and accuracy.

Introduction

In recent years, the number of poisonings caused by the ingestion of eye drops has been rising. Eye drops designed to treat redness are not intended to be consumed orally. If swallowed, the medication can cause illness and even death. This is caused by tetrahydrozoline, the main component of eye drops.

Tetrahydrozoline (TH) is a derivative of imidazoline that serves to reduce the redness of the eye caused by minor ocular irritants. Tetrahydrozoline works by temporarily narrowing the blood vessels in the eye. Alongside constricting blood vessels, tetrahydrozoline can cause difficulty breathing and a slowed heartbeat (1). However, when consumed orally, tetrahydrozoline passes quickly through the gastrointestinal tract, rapidly reaching the blood and the central nervous system, causing toxic blood levels and the possibility of slipping into a coma when consumed in large quantities (1,2).

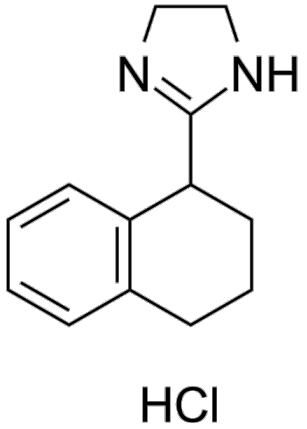

Figure 1. The chemical arrangement of tetrahydrozoline hydrochloride.

Previously, analytical instruments such as liquid chromatography-mass spectrometry (LC-MS) and high-performance liquid chromatography (HPLC) have been used to detect toxins in blood and urine samples of patients suffering from poisoning from eye drop ingestion (3). The isolation and identification of tetrahydrozoline from ophthalmic solutions have also been done using these methods (4). Interestingly, such studies have not been done using capillary electrophoresis (CE). CE as an alternative method will alleviate the number of disadvantages associated with LC-MS and HPLC methods.

HPLC is a type of column chromatography that relies on different polarities of compounds in a solution to separate them. HPLC uses pressure to force the solution through the column more quickly (5). However, the cost and complexity of the HPLC are often a deterrent to its usage. LC-MS is used to isolate the compounds of a sample mixture with the power of mass spectrometry as a detector. The main disadvantages of LC-MS are its cost, complexity, and that it only works with volatile buffers. The advantages of CE include better sensitivity, low cost, short analysis times, and easy use in comparison to other analytical techniques such as HPLC and LC-MS.

In this study, we proposed to isolate and identify tetrahydrozoline from commercially available eye drops through a novel capillary electrophoresis method. Furthermore, the method will be validated to evaluate its precision, accuracy, peak area, and migration time. The proposed investigation will establish CE as a means of a low-cost and efficient method of identifying tetrahydrozoline in patients suffering from eye drop ingestion.

Materials and Methods

Chemicals

Tetrahydrozoline hydrochloride (1000 ppm) powder was obtained by Millipore Sigma, Oakville, Ontario. Methanol (MeOH), sodium phosphate monobasic monohydrate (NaH_2PO_4) powder, and water (18 MΩ) were obtained to prepare the buffer solution. The four samples of eye drops (Life Original Eye Drops, Life Advanced Relief Eye Drops, Visine Original Red Eye, and Visine Red Eye Triple Action Advance Plus) were obtained from the Kamloops Superstore.

Preparation of Buffer Solutions

To prepare 20 mM phosphate buffer, 0.27598 g of NaH_2PO_4 powder was dissolved in 100 mL of water. The pH was adjusted to 7.00 with 1M NaOH using a pH meter (Meter Toledo). The buffer solution was filtered using a 0.45 μm Nylon syringe filter and was kept at room temperature. To prepare the standard solutions, 0.1 g of tetrahydrozoline hydrochloride (1000 ppm) powder was measured using an analytical balance and was dissolved in 100 mL MeOH. To create a 100 ppm tetrahydrozoline hydrochloride (TH) stock solution, 10 mL of the 1000 ppm TH was further added to 100 mL of MeOH. Standard TH solutions of 5, 10, 15, 20, and 25 ppm were prepared using the 100 ppm TH stock solution. All the standard solutions were run on the CE. Peak areas and migration times were recorded. Calibration curve was then generated.

Sample Preparation

The eye drop samples were prepared by mixing 1 mL of the sample in a 5-mL volumetric flask and topping it up with MeOH. The samples were further filtered using a 0.45 μm Nylon syringe filter and was kept at room temperature. The four samples were then run on the CE. Peak areas and migration times were recorded.

CE Methodology

Tetrahydrozoline in commercially available eye drops was analyzed by CE. CE is an analytical technique that separates ions based on their electrophoretic mobility with the use of an applied voltage (6). The electrophoresis mobility depends on both the particle properties (surface charge, density, and size), and solution properties (ionic strength, electric permittivity, and pH) (7). The size and charge of the ion will determine the rate of separation, which is also directly proportional to the applied electric field. Neutral species are not affected; only ions move with the electric field. If two ions are of the same size, the one with a greater charge will move faster. For ions of the same charge, the smaller ion will have less friction and an overall faster migration rate (7).

The ions were detected by the UV detector, and the results were processed with the 32 Karat software, which operates the CE instrument. The wavelength of the UV detector, pH and concentration of the buffer, and the voltage and injection time conditions on the CE were optimized to obtain high resolution and baseline peaks for the analytes. Prepared eye drops solutions were run on the CE with the help of my primary supervisor, Dr. Kingsley Donkor. The linear relationship between the concentration of the tetrahydrozoline and their corresponding peak area on the electropherogram was determined and used to determine the amounts of tetrahydrozoline present in the eye drops. This method was validated for reproducibility of the peak area and migration times of the analytes, accuracy, limits of detection and quantification, percent recovery, and intraday and interday precision of the analysis.

Results and Discussion

After running the 5, 10, 15, 20, and 25 ppm TH standards on the CE, a calibration curve was created on Excel (Figure 2.). The curve observed was linear and had a R^2 value of 0.9824. Essentially, an R^2 value of 0.9824 indicates that 98.24% of the variance of the dependent variable being studied is explained by the variance of the independent variable. Additionally, as the concentration (ppm) of the standards increased, the peak areas recorded from the CE increased as well, showing that there was a steady increase in TH.

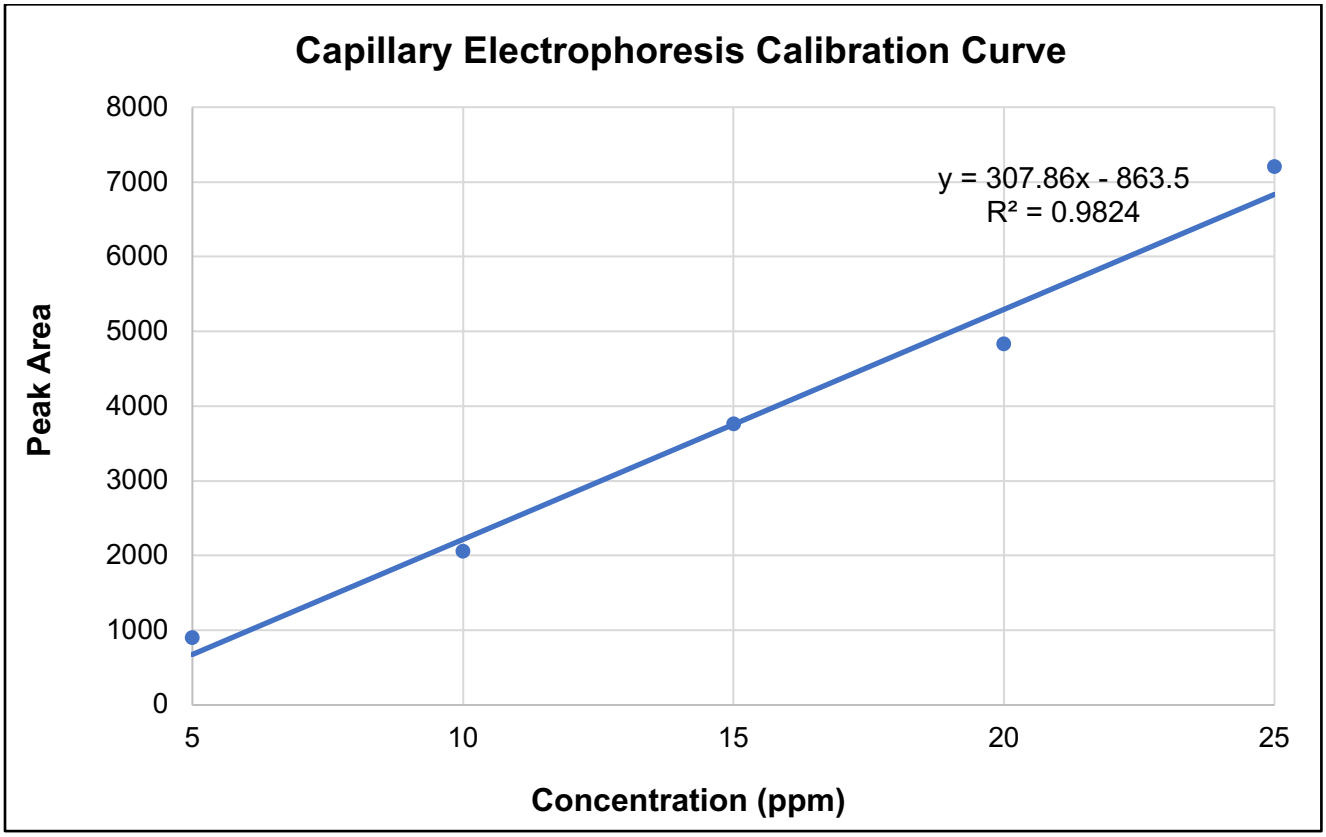

Figure 2. Calibration curve indicating the peak areas of the tetrahydrozoline standards made at 5, 10, 15, 20, and 25 ppm on CE.

The equation of the line from Figure 2 was then used to determine the concentration of tetrahydrozoline hydrochloride in the standard solutions. An electropherogram of one of the 5 standards is shown in Figure 3. The peak seen at the 2.2 – 2.4-min mark was used to determine the concentration of TH, specifically investigating the peak areas and migration times.

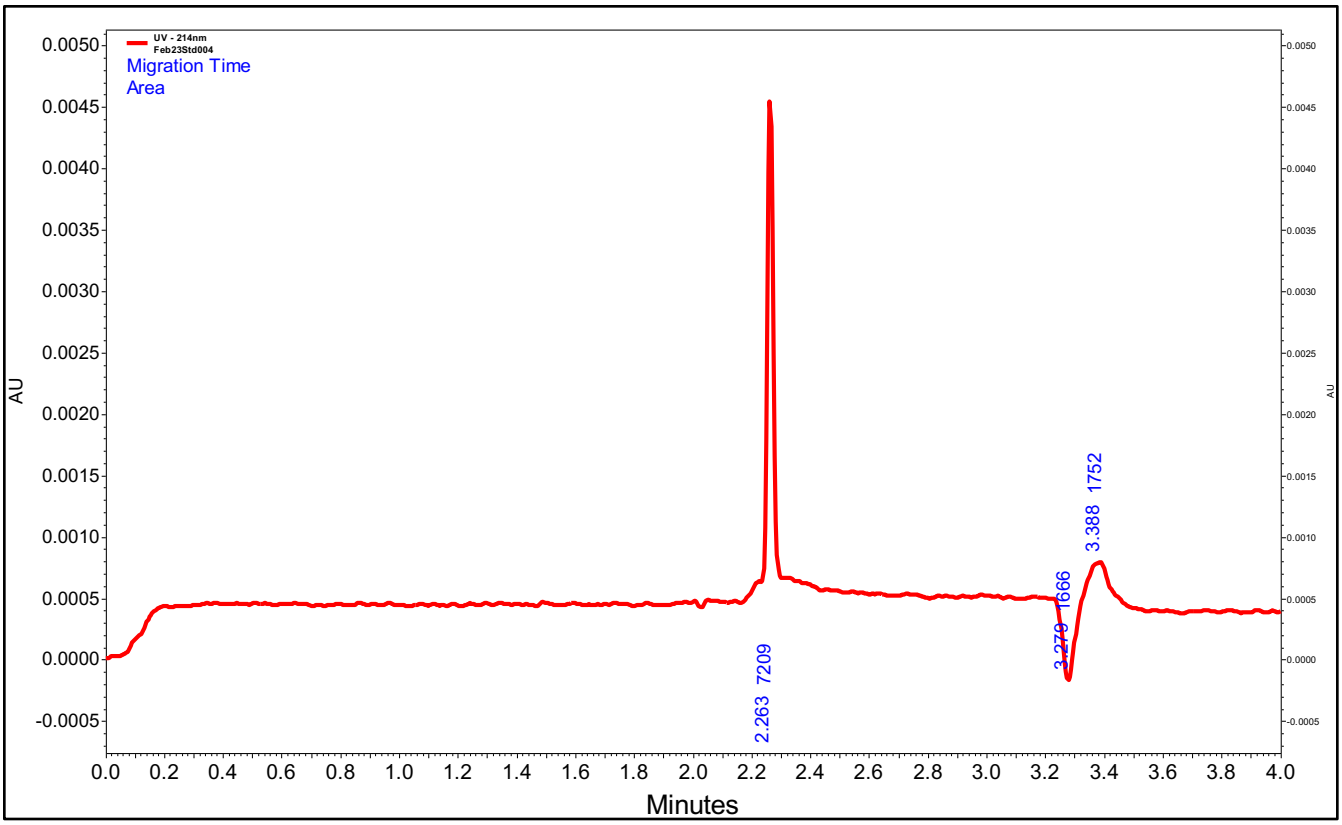

Figure 3. Electropherogram indicating the peaks of the 20 ppm tetrahydrozoline standard produced by the CE.

In Figure 3, a sharp peak can be seen at 2.263 min, indicating the presence of TH, with a peak area of 7209.

Similarly, to the TH standard solutions, the four eye drop samples were run on the CE and electropherograms were generated. From the equation of the line of Figure 2, the migration time and peak areas of each sample were used to generate the TH concentration of each eye drop sample. The concentrations were then compared and displayed as a graph in Figure 4.
Out of the four samples, the highest concentration of TH was seen in Life Advanced Relief Eye Drops (52 ppm) meaning it can potentially be quite dangerous if consumed in comparison to Visine Original Red Eye Drops (24 ppm) which has the lowest tetrahydrozoline concentration.

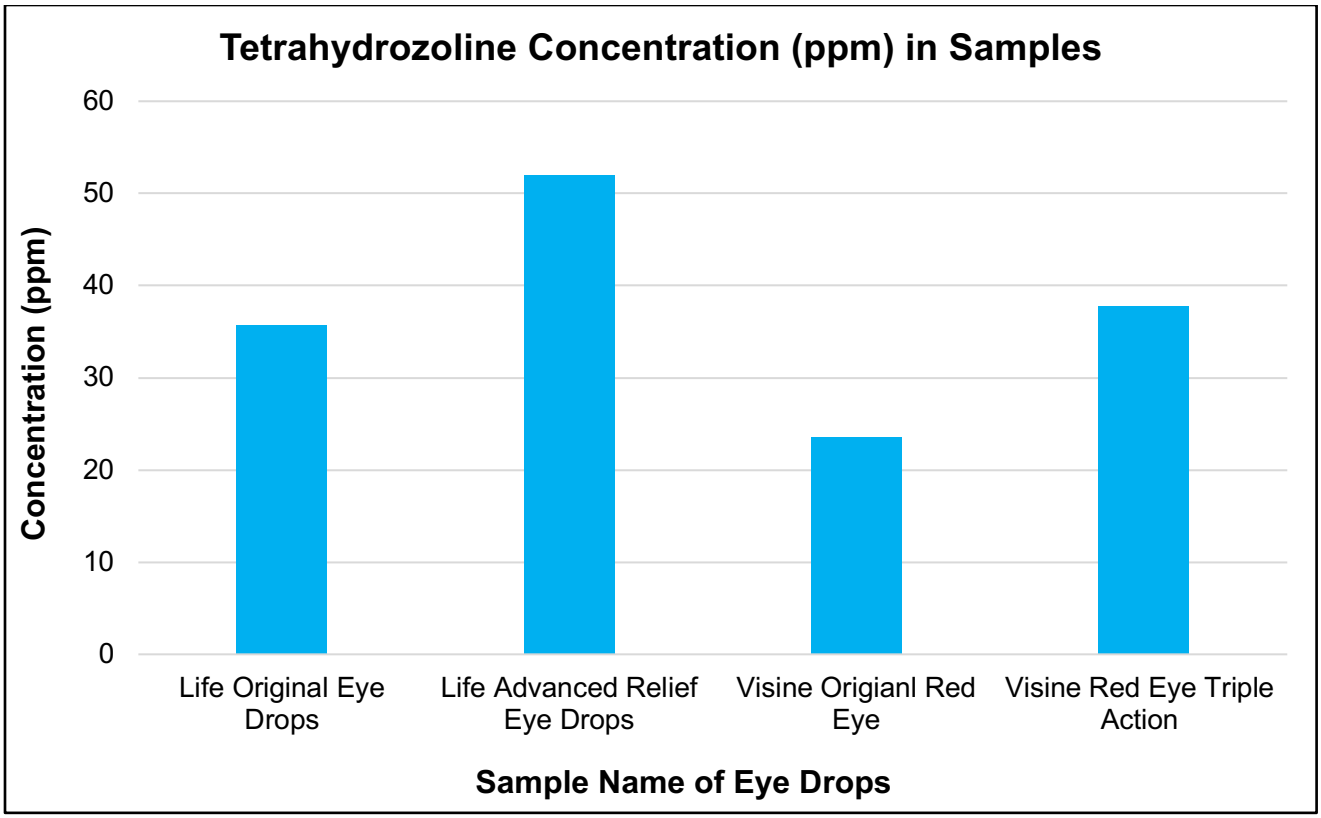

Figure 4. Comparison between the experimentally determined concentrations of the four eye drop samples determined by CE.

To determine the precision and accuracy of the CE methodology, a percent recovery was conducted by spiking each sample with 15 ppm TH stock. The resulting concentrations were recorded and compared to the unspiked concentrations in Figure 4. The percent of TH recovered was then indicated in Figure 5. The percent recovery showed a successful recovery since almost each eye drop garnered a 80-120% percent recovery. Having a percent recovery within this range indicates that the accuracy of the developed method is good.

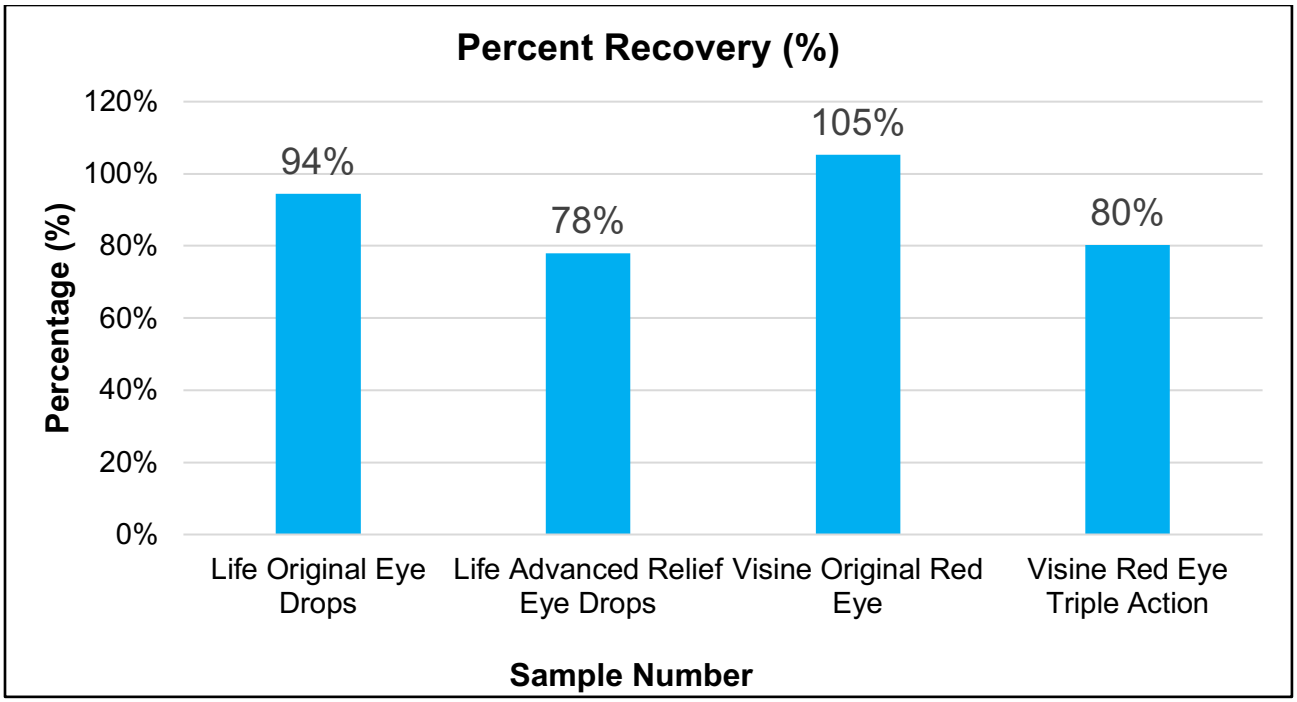

Figure 5. The four commercially available eye drops that were used in this study to determine tetrahydrozoline concentrations from.

Following percent recovery, percent of relative standard deviation of peak area and migration time was calculated to identify the accuracy and precision of collected data. Interday and intraday precisions of the standards at 5, 15, and 25 ppm were also conducted. From this, percent of relative standard deviation of peak area and migration time was calculated and shown in Tables 1 and 2. The values of the interday precision are reasonably low indicating that the results obtained on different days are tightly clustered around the mean, implying greater consistency and reproducibility. Therefore, it indicates that the methodology can be repeated on different days with consistent outcomes. This increases the reliability of the data and reduces the chances of obtaining erroneous results. Similarly to interday, the values of intraday were reasonably low indicating that the results obtained on the same day but at separate times are tightly clustered around the mean, implying greater consistency and reproducibility of the method. It implies that the experimental procedure is highly reliable and stable over time.

Standard Concentration (ppm)	% RSD Peak Area	% RSD Migration Time
5	25.73 %	3.22 %
15	23.61 %	6.42 %
25	21.58 %	1.31 %

Table 1. Interday precision of standards at 5, 15, and 25 ppm (n = 9).

Standard Concentration (ppm)	% RSD Peak Area	% RSD Migration Time
5	16.34 %	0.90 %
15	16.23 %	1.34 %
25	14.64 %	1.18 %

Table 2. Intraday precision of standards at 5, 15, and 25 ppm (n = 9).

Future Work

Although this experiment validates the accuracy and precision of using CE to detect TH in eye drop solutions, to further validate the reproducibility of the CE methodology developed, this method can be carried out on other components of eye drops. Additionally, to confidently assess the advantages of using CE over other analytical instruments, we would want to conduct this experiment on LC-MS and HPLC-DAD to compare the results.

Conclusion

Through this study, it can be concluded that the CE is an accurate and efficient analytical technique that can be used to detect tetrahydrozoline concentrations in eye drops.
From the electropherograms, we can conclude that CE provides high separation efficiency, fast analysis times, and excellent sensitivity thus proving to be advantageous over other analytical techniques for this type of study. From the results, the tetrahydrozoline concentrations range from 24 ppm to 52 ppm, with the Visine Original Red Eye brand containing the lowest tetrahydrozoline concentration and the Life Advanced Relief brand containing the highest tetrahydrozoline concentration. The interday and intraday precision and percent recovery results confirm the methodology's validity, reproducibility, and accuracy. Therefore, these results provide insight into the successful detection of tetrahydrozoline from commercially available eye drops to identify which brands of eye drops are potentially the most unhealthy.

Acknowledgements

I am grateful to Dr. Kingsley Donkor for providing me with the opportunity to gain research experience and conduct this study. I would like to thank the TRU Chemistry Department for their resources and the TRU Undergraduate Research Experience Award Program (UREAP) for funding this project. Financial support from NSERC is gratefully acknowledged.

References

(1) *Tetrahydrozoline poisoning*. Mount Sinai Health System. (n.d.). Retrieved October 10, 2022, from https://www.mountsinai.org/health-library/poison/tetrahydrozoline-poisoning

(2) Churchill, J., & Soglin, A. (2021, February 22). *Red-eye drops: Poisonous to drink, safe if used correctly*. American Academy of Ophthalmology. Retrieved October 10, 2022, from https://www.aao.org/eye-health/treatments/red-eye-drops-safe-if-used-correctly

(3) O'Leary, M. A., Schneider, J. J., & Isbister, G. K. (2004). Use of high performance liquid chromatography to measure tetrodotoxin in serum and urine of poisoned patients. *Toxicon : official journal of the International Society on Toxinology*, *44*(5), 549–553. https://doi.org/10.1016/j.toxicon.2004.07.008

(4) Al-Rimawi, F., Zareer, W., Rabie, S., & Quod, M. (2011). Development and validation of a reversed-phase HPLC method for analysis of tetrahydrozoline hydrochloride in eye drop formulations. *Journal of Pharmaceutical Analysis*, *2*(1), 67–70. https://doi.org/10.1016/j.jpha.2011.11.001

(5) Burdick, R. (2019, March 2). *What are the disadvantages of HPLC?* Sciencing. Retrieved October 10, 2022, from https://sciencing.com/list-7237518-disadvantages-hplc-.html

(6) Mao, R., Bayrak-Toydemir, P., & Lyon, E. (2012). Capillary electrophoresis for the detection of fragile X expanded alleles. *Methods in Molecular Biology*, 275–285. https://doi.org/10.1007/978-1-62703-029-8_24

(7) Thormann, W. (2018). Theoretical principles of capillary electromigration methods. *Capillary Electromigration Separation Methods*, 21–44. https://doi.org/10.1016/b978-0-12-809375-7.00002-2

(8) Thomas, D. L. (2018, September 4). *Health risks and benefits of Eye Drops*. News. Retrieved October 10, 2022, from https://www.news-medical.net/health/Health-Risks-and-Benefits-of-Eye-Drops.aspx

(9) Hauser, C. (2020, January 17). *Woman used eye drops to kill husband, Court says*. The New York Times. Retrieved October 10, 2022, from https://www.nytimes.com/2020/01/17/us/visine-eye-drop-poisoning.html