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

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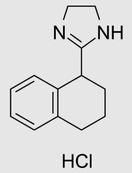
Introduction

- 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 is a derivative of imidazoline that serves to reduce the redness of the eye caused by minor ocular irritants.
- Alongside constricting blood vessels in the eye, tetrahydrozoline can cause difficulty breathing and a slowed heartbeat.



- Previously, analytical instruments such as liquid chromatography-mass spectrometry (LC-MS) and high-performance liquid chromatography (HPLC) have been used to detect tetrahydrozoline from ophthalmic solutions.
- Interestingly, such studies have not been done using capillary electrophoresis (CE); an alternative method that can alleviate the number of disadvantages associated with LC-MS and HPLC methods.
- The advantages of CE include better sensitivity, small samples, low cost, short analysis times, and easy use in comparison to HPLC and LC-MS.
- The objective of my study is to develop and optimize an analytical method using CE to identify and determine the concentrations of tetrahydrozoline in commercially available eye drops.

Experimental

- CE is an analytical technique that separates ions based on their electrophoretic mobility with the use of an applied voltage.
- The capillary is filled with a buffer of a fixed pH.
- The application of the voltage generates the flow of the buffer through the capillary known as electroosmotic flow.
- The electrophoresis mobility depends on the ion properties (charge, density, and size), and solution properties (ionic strength, electric permittivity, and pH).
- The size and charge of the ion determine the rate of separation, which is also directly proportional to the applied electric field.

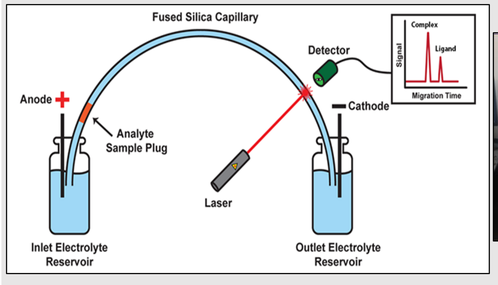


Figure 1. Schematic of CE (left) and CE instrument at TRU used for this study.

Experimental

- The ions were detected by the UV detector and the results were processed with the 32 Karat software, which operates the CE.
- 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.
- A calibration curve was created using the peak areas of the electropherograms obtained from running 5, 10, 15, 20, and 25 ppm tetrahydrozoline standards on the CE.
- Peak areas and migration times levels of the four eye drops were also obtained.
- The linear relationship between the tetrahydrozoline concentrations and their corresponding peak area on the electropherogram was determined and used to determine the amounts of tetrahydrozoline present in each eye drops solution.
- This method was validated for reproducibility of the peak area and migration times of the analytes, accuracy, percent recovery, and intraday/interday precision of the analysis.

Results

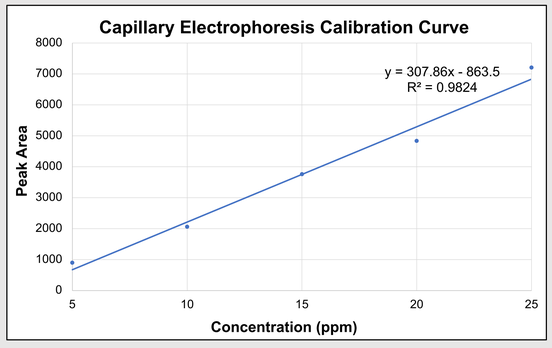


Figure 2. Calibration curve indicating the peak areas of the tetrahydrozoline standards made at 5, 10, 15, 20, and 25 ppm on CE. The equation was further used to quantify tetrahydrozoline concentrations in eye drops.

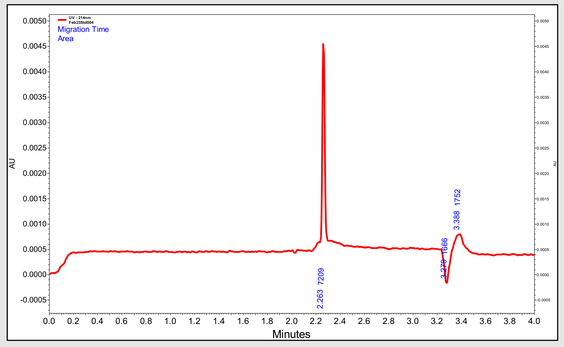


Figure 3. Electropherogram indicating the peaks of the 20 ppm tetrahydrozoline standard produced by the CE. A peak indicating tetrahydrozoline was observed at 2.263 minutes with a peak area of 7209.

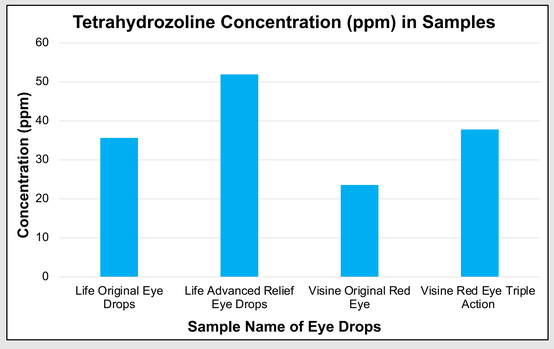


Figure 4. Comparison between the experimentally determined concentrations of the four eye drop samples determined by CE.

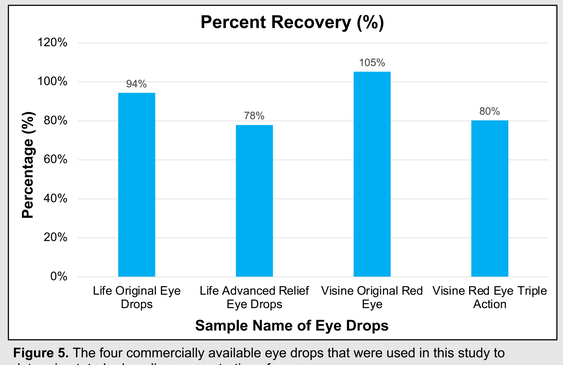


Figure 5. The four commercially available eye drops that were used in this study to determine tetrahydrozoline concentrations from.

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. Percent of Relative Standard Deviation of peak area and migration time was calculated to identify the accuracy and precision of collected data (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. Percent of Relative Standard Deviation of peak area and migration time was calculated to identify the accuracy and precision of collected data (n = 9).

Discussion

- 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.
- The 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 healthy.
- 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.
- 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.

Future Work

- To further validate the CE method developed by comparison with another analytical method such as LC-MS.

Acknowledgements

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

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