DETERMINATION OF URIC ACID IN HUMAN SALIVA USING CAPILLARY ELECTROPHORESIS

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Table of Contents

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Abstract

Uric acid is a waste product found in all humans. It is created from the breakdown of purines and dissolves in the blood. Uric acid can crystallize on joints, causing arthritis, particularly gout, or on kidneys, forming kidney stones. Therefore, there is a need for a rapid and simple method to determine uric acid concentrations for fast diagnosis of gout. An analytical method has been developed to determine the amount of uric acid in human saliva samples using capillary electrophoresis. This method utilizes small sample volumes and quick analysis times to provide accurate, sensitive, and precise results. The developed method was validated using intraday and interday precision, percent recovery, limits of detection and quantitation, and linearity of the calibration curve. This study is useful in quantifying uric acid for diagnostic purposes in medical research using capillary electrophoresis.

Introduction

Background

Uric acid is an organic compound found in human bodies and is the final product of purine metabolism. This molecule (see Figure 1 below) is a waste product that is removed by the kidneys and excreted primarily through urine. The average salivary concentrations found in humans are 199 ± 27 µmol/L or 33.4 ± 4.5 ppm (Vernerová et al.).

While there are many other inorganic and organic compounds found in saliva, uric acid is particularly important because it is linked to many diseases. When uric acid is present in high concentrations in humans, this condition is called hyperuricemia. Hyperuricemia can arise from a diet high in purines or the inability to excrete this waste effectively. It is correlated with hypertension, strokes, metabolic syndrome, Lesch-Nyhan syndrome, and, most importantly, gout.

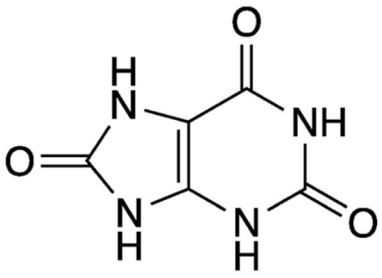

Figure 1. Uric acid molecule.

Figure 2. Monosodium urate crystals.

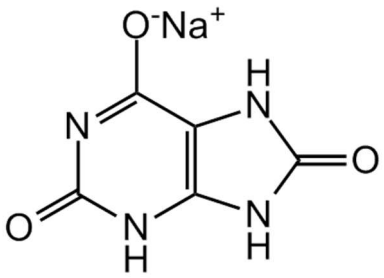

Figure 3. Monosodium urate molecule.

Gout is a form of arthritis that is caused by the deposit of monosodium urate crystals on joints, resulting in joint inflammation (CDC). Monosodium urate crystals (shown in Figures 2 and 3 above) are formed from the acid dissociation of uric acid and ionic bonding with one molecule of sodium. These crystals have a needle-like structure that rubs against parts of the joint, causing the characteristic symptoms of inflammation.

As the concentration of uric acid increases, the risk of uric acid crystallizing on the joints of humans and causing gout also increases. Patients with hyperuricemia have higher levels of uric acid, which can lead to increased crystallization and inflammation.

Theory

Determining the concentration of uric acid in humans is crucial for diagnosing conditions based on the levels and symptoms demonstrated. To achieve accurate and precise measurements of uric acid concentration in small saliva samples, a capable instrument must be used. The capillary electrophoresis system allows for the fast analysis of very small samples. This instrument utilizes a capillary with a diameter of 50 microns that is placed under an electric potential, and a small sample volume is passed through it. The absorbance is then measured by an ultraviolet detector to determine the amount of analyte. With this, the concentration of each analyte can be determined and used for diagnosis.

Significance of Project

With a novel, rapid method to analyze the concentration of uric acid in human saliva, doctors can now have an inexpensive and reliable way of diagnosing patients. Compared to blood analysis, extracting patient blood can be troublesome as it requires the presence of medical personnel, and if the patient is anemic, removing blood could have negative effects on their health. For urine samples, the patient must physically move themselves to and from a bathroom to collect a sample, which can be troublesome when the patient's condition is not adequate. However, with a saliva sample, the collection time is relatively quick and easy. The patient can remain in bed and even collect the sample themselves if they are in good condition. This method provides an overall ease in the detection of hyperuricemia and the associated diseases rapidly and effectively.

Experimental

Instrumentation

For this procedure, a Beckman Coulter ProteomeLab PA800 Capillary Electrophoresis system (see Figure 4 below) was used. This system used a capillary of 50-micron diameter and effective length of 50 cm. Across the capillary, a voltage of 20kV was applied when analysing samples at normal polarity. The separation of the analyte and rinsing of capillary was all done at 25.0°C. The analytes were injected into the capillary at a pressure of 1.0psi. In order to quantitatively determine the concentration of uric acid, the absorbance of the analyte was determined by using an ultraviolet detector set at 214nm. The software used to run the samples and analyse the data was the 32 Karat software.

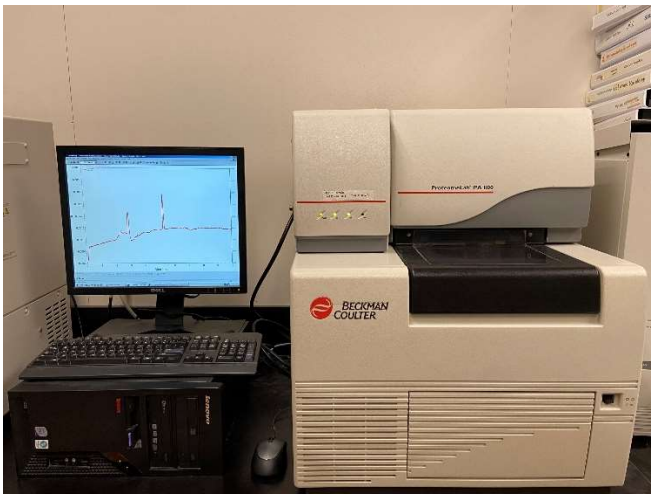

Figure 4. Beckman Coulter ProteomeLab PA800 CE with desktop showing the 32Karat Software.

Reagents

Pure uric acid was used to prepare the standard solutions. This product was purchased from SIGMA Life Sciences as well as the sodium tetraborate decahydrate used to prepare the borate buffer used to rinse and separate the analyte. Only 18 MΩ water was used to prepare standards and buffer solutions.

Standard Preparation

The seven standard solutions ranging from 0.99ppm to 6.93ppm were prepared by diluting the pure uric acid in a volumetric flask. The stock solution was prepared to 99ppm and the pH was raised dropwise by the addition of 1M NaOH to fully dissolve the uric acid. The solution was then filtered and stored at an average fridge temperature of 4°C to be used for preparing standard solutions.

Sample Preparation

The samples were collected by swabbing patients from Royal Inland Hospital. The swabs were then placed in test tubes with water and allowed to diffuse out. After 16 hours, the swabs were removed, and the uric acid-containing solutions were filtered through a 0.22nm filter. The filtered samples were stored at an average fridge temperature of 4°C.

Methodology

All of the standard and sample solutions, having been filtered, were transferred directly into capillary electrophoresis sample vials. The solutions were then run through the instrument using the parameters mentioned in the Instrumentation section, using sequence runs. The standard solutions were run in duplicate, while the samples were run only once. The standard solutions were used to create a calibration curve of concentration against peak area, which was gathered from the ultraviolet absorbance. The calibration curve was then used to determine the sample concentrations based on their peak areas.

Results and Discussion

A calibration curve (see Figure 5 below) was created with an excellent regression value of 0.9888. The curve also has high sensitivity due to the high slope.

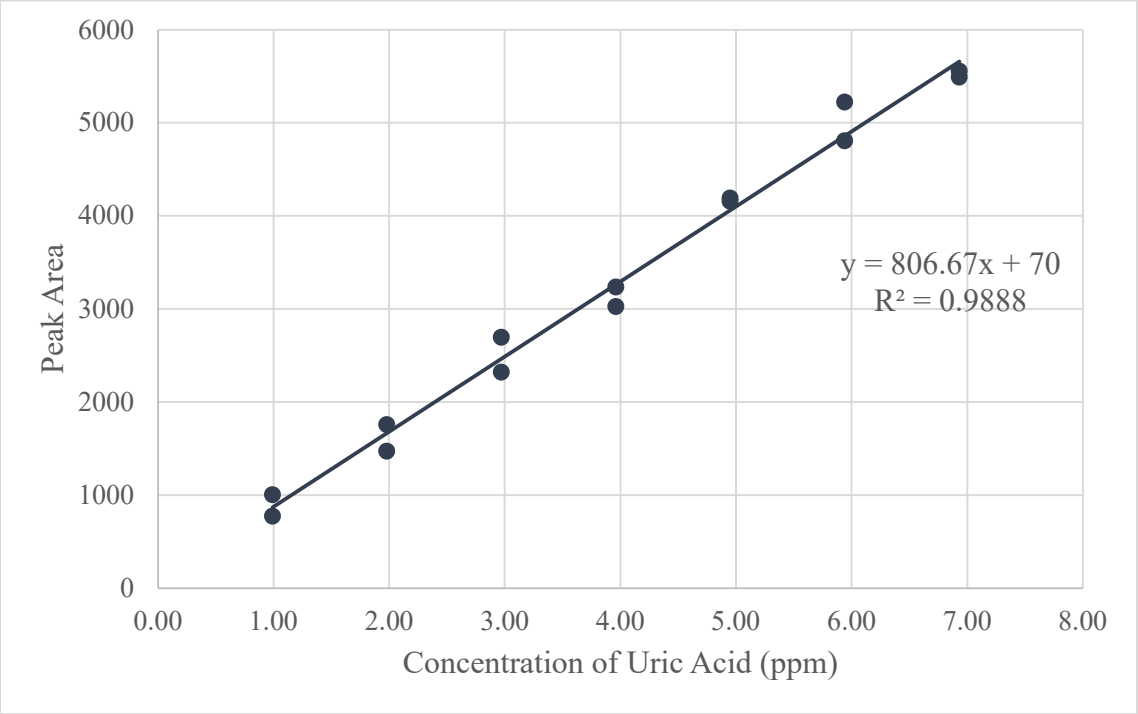

Figure 5. Calibration curve of peak area of uric acid peaks of the standards solutions made at varying parts

Table 1. Uric acid standard solution concentrations and their experimental peak areas.

Standard	Concentration (ppm)	Peak Area
S1	0.99	1004
S1	0.99	774
S2	1.98	1758
S2	1.98	1471
S3	2.97	2697
S3	2.97	2321
S4	3.96	3236
S4	3.96	3025
S5	4.95	4190
S5	4.95	4155
S6	5.94	4805
S6	5.94	5221
S7	6.93	5555
S7	6.93	5490

The values of the concentrations and peak areas of each standard are shown in Table 1 above. Below are two electropherograms (see figures 6 and 7), one from a standard solution and one from the entire sample solution, with the uric acid peak highlighted. The peak had a greater migration time in the sample, which can be explained by the increased viscosity of the matrix.

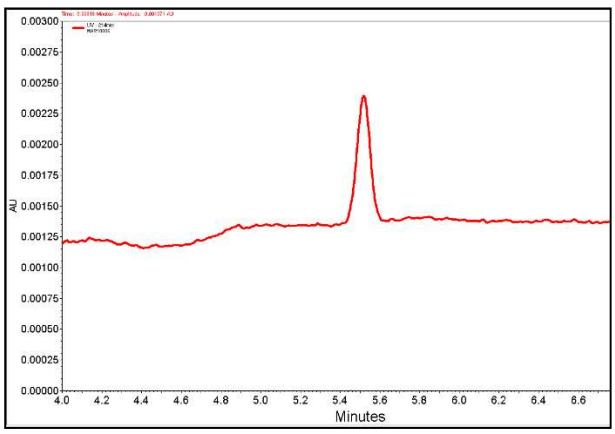

Figure 6. Electropherogram of uric acid standard 6 run 1 showing the uric acid peak.

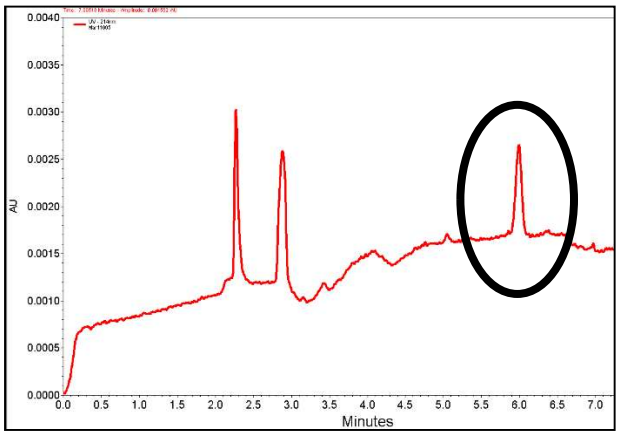

Figure 7. Electropherogram of human saliva sample 5 with the circled uric acid peak.

Using the equation of the calibration curve, the concentrations of the sample solutions can be calculated from their peak areas. The calculated concentrations are presented as a bar graph in Figure 8 below. However, upon further consideration, the concentrations in the figure are not representative of the original saliva on the swab, but rather the concentration of the solution in which the swab was placed. Since the original volume or weight of the swab was not measured, no dilution calculations can be made.

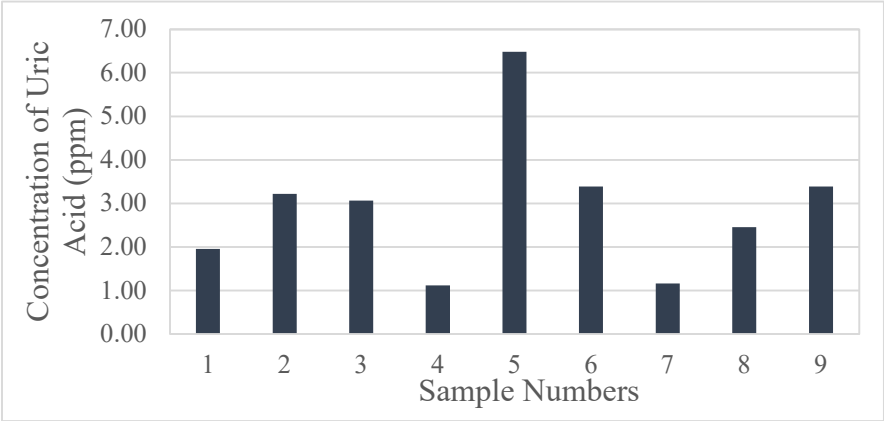

Figure 8. Bar graph showing concentration of uric acid determined in each saliva sample.

Conclusions

The concentration of uric acid in a solution can be determined by using a capillary electrophoresis system with an ultraviolet detector. The calibration curve created was of high sensitivity and regression. There was also a successful detection and quantization of the uric acid peaks at a migration time just under six minutes. Overall, the run takes less than twenty minutes to complete with all the rinsing steps included making it a quick and effective method. With a bit of work in the sample collection, this method will be able to serve as a valuable resource in the diagnosis of gout and other hyperuricemia-related diseases.

Future Work

For future studies, there are multiple considerations and improvements that can be made to this research. Firstly, the sample collection could be redone by using spit samples instead of swab samples. By doing so, the saliva could be filtered, stored, and run easily without having to do any dilutions. If swab samples are used, the mass of the swab must be measured before and after collection to allow for a determination of the quantity of saliva present in the swab. When allowing the saliva to diffuse from the swab, a high pH must be used to ensure uric acid's solubility in water. Finally, the method should undergo precision studies such as interday and intraday studies to ensure its precision. Recovery studies of spiked samples as well as limit of quantitation and detection will determine the accuracy of the method. By conducting these studies, the method can be validated and demonstrated to be reliable and capable.

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