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CHEM 3170 Laboratory

Determination of Creatine in Workout Supplement by Capillary Electrophoresis

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Unknown Sample: Tango Creatine Recovery Formula

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Abstract

The concentration of creatine was in a sample of Tango Creatine Recovery Formula was determined using capillary electrophoresis (CE). CE is a rapid, high-resolution method for determining organic and inorganic ions. This analytical technique separates ions based on their relative ionic mobility upon the introduction to an electric filed in a silica capillary. The CE system includes two electrodes, a pump, a silica capillary, a UV detector and a selectable UV filter. A set of five standard solutions were prepared by dissolving creatine monohydrate in water. A calibration curve that displayed peak area versus concentration of creatine was created using the five standards. The concentration of creatine in the Tango Creatine Recovery Formula was determined by interpolating the concentration from the equation of the least squares line from the calibration curve. The equation of the least squares line was determined to be $y = 492.93x + 7803.7$. The sample was determined to have a concentration of 13830 ppm ± 40.37 ppm with a percent relative standard deviation of 7.20%.

Introduction

Capillary electrophoresis (CE) is an analytical technique that is used for the separation and identification of analytes in solution. The separation is achieved by passing a solution through a silica capillary in the presence of an electric field. The electric filed is generated by applying a voltage buffer to solutions containing creatine. This works because different ions have different properties including size and charge meaning they will interact differently in the electric field and will migrate at different rates through the capillary. Migration time is the time taken for an analyte to move through the capillary and to the detector. In CE, the solution is passed from the positively charge electrode to the negatively charged electrode. In general, small cations are eluted first followed by large cations, neutral analytes, large negative ions and finally small anions. The term electrophoretic mobility is reference to the ability of an analyte to move through an electric field. Electroosmotic flow is reference to the displacement of buffer solution in electric field. In UV detection, light is passed through the sample and the absorbance at a specific wavelength is recorded. The detector response is shown on an electropherogram where analytes can be identified based on migration time.

The analyte in this experiment was creatine. Creatine is an amino acid located mostly in the body's muscles as well as in the brain. Creatine is commonly found in red meats and seafood and can also be produced in small amounts by the liver, pancreas, and kidneys. Creatine supplements are becoming more common for athletes who want to build muscles. Even though these compounds are found commonly throughout natural products, they can be potentially harmful in high concentrations which can result in kidney damage. For this reason, it is crucial to determine the amount of creatine in samples of workout supplements. The determination of creatine in supplement samples by CE appears to be a suitable analytical method as it pairs the separation capabilities of gas chromatography with the ability of mass spectrometry to identify chemical species. Standard solutions were prepared to provide a reference of migration time in order to identify the analyte peak on the electropherogram.

Experimental

Standard and Sample Preparation – Procedure

Standard solutions were prepared in clean and dry 10 mL volumetric flasks. The solutions were then transferred to GC glass sample vials using a glass Pasteur pipette. First, 0.4636g of creatine monohydrate was dissolved in 500 mL of water in a 1 L beaker. The solution was mixed for 30 min to allow the powder to dissolved. Then, an Eppendorf micropipette was used to transfer the desired volumes of 927.2 ppm creatine standard solution into the 10 mL volumetric flasks. The volumetric flasks were then topped up to the 10 mL line with water. The solution was then filtered with using a 0.45 µm membrane syringe filter attached to a syringe and added to the sample vials. The vials were then capped and mixed using a vortex.

The sample was prepared by weighing 10.92 g of supplement powder into 330 mL of water. The solution was mixed for 30 min to allow the powder to dissolved. Next a 100-fold dilution was completed by pipetting 1.0 mL of solution into a 100 mL volumetric flask. The volumetric flasks were then topped up to the 100 mL line with 18 MOhm water. The solution was then filtered with using a 0.45 µm membrane syringe filter attached to a syringe and added to the sample vials. The vials were then capped and mixed using a vortex.

Chemicals and Solvents:

Creatine Monohydrate

Sodium Hydroxide (0.1 M)

Deionized Water (MOhm)

20 mM Borate Buffer pH 9

Sample Information:

Tango Creatine Recovery Formula

GC/MS Instrument Information :

Beckman P/ACE System MDQ capillary electrophoresis system.

Table 1. Instrumental parameters for the Beckman P/ACE System MDQ capillary electrophoresis system.

Capillary:	Fused silica, 50-µm I.D. x 375-µm O.D. x 50-cm total length (40 cm to detector)
Operating Temperature:	25°C
Run Time:	20 minutes
Detection:	UV, 214 nm (Direct absorbance)
Rinse Pressure (0.1 M NaOH):	20 psi for 5.0 min
Rinse Pressure (Water):	20 psi for 1.0 min
Rinse Pressure (Rinse Buffer):	20 psi for 3.0 min
Injection:	Pressure, 1 psi for 5.0 s
Separation Voltage:	20kV

Table 2. Creatine standards information.

Standard (#)	**Desired Concentration (mg/L)**	**Volume of 927.2 ppm Creatine Solution (µL)**	**Water (µL)**	**Final Volume (µL)**
1	100.0	50.0	450.0	500.0
2	200.0	100.0	400.0	500.0
3	300.0	150.0	350.0	500.0
4	400.0	200.0	300.0	500.0
5	500.0	250.0	250.0	500.0

Data and Results

Table 3. Peak area results obtained for the analysis of creatine in the standards and sample on the Beckman P/ACE System MDQ capillary electrophoresis system.

Standard	**Concentration (ppm)**	**Peak Area**	**Migration Time**
S1	92.79	3.862	60498
S2	185.58	3.875	103721
S3	278.37	3.888	112626
S4	371.16	3.908	188635
S5	463.95	3.913	235029
Sample 1	-----	3.933	64537
Sample 2	-----	3.933	73218
Sample 3	-----	3.954	90173

Table 4. Calibration curve data and uncertainties for creatine obtained from Figure 1 and the uncertainty table in Appendix I.

Slope (m)	492.93
Uncertainty in Slope (S_m)	17.47
Y-intercept	7803.7
Uncertainty in y-intercept (S_b)	4917.99
R^2	0.9962
Equation of the line	y = 492.93x + 7803.7

Table 5. Calculated concentration for the samples from the peak area given by the Beckman P/ACE System MDQ capillary electrophoresis system.

Sample Number	**Diluted Concentration (ppm)**	**Original Concentration (ppm)**
Sample 1	115.094	11509
Sample 2	132.705	13270
Sample 3	167.1014	16710
Average	----	13830

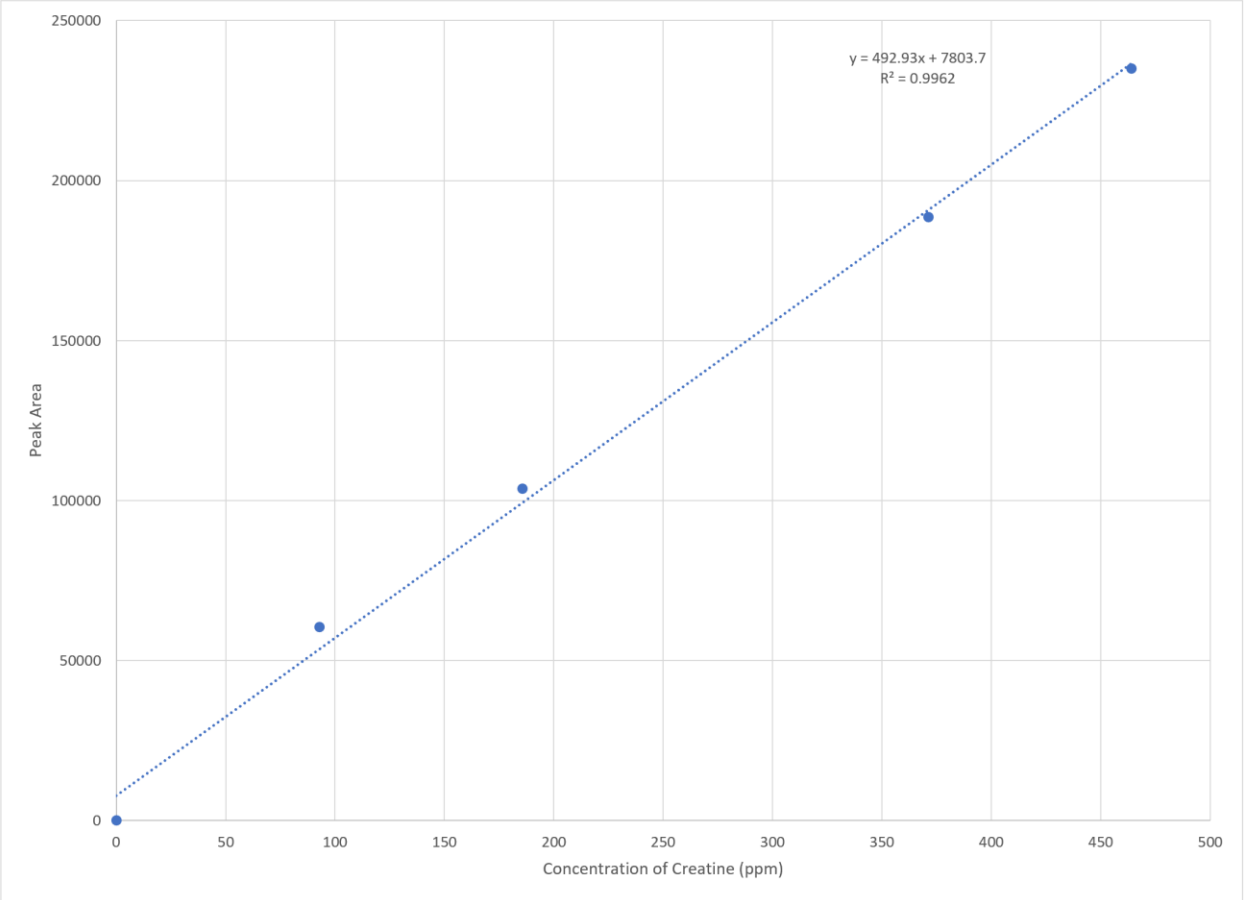

Figure 1. Calibration curve for creatine from the analysis of the standards on the Beckman P/ACE System MDQ capillary electrophoresis system (n=5).

Calculations for creatine

1. Concentration of creatine in standard solutions for standard 2 from Table 2.

C_1 = Initial stock concentration = 927.9 ppm

V_1 = Initial volume of stock creatine = 50.00 µL

V_2 = Final volume = 10.00 mL

C_2 = Final creatine concentration = ?

$C_1V_1 = C_2\ V_2$

$$C2 = \frac{C_1V_1}{V_2} = \frac{(927.9ppm)(0.050mL)}{0.50mL} = 92.79\ ppm$$

2. Concentration of creatine in the diluted mouthwash sample.
   Equation of calibration curve trendline: y = 492.93x + 7803.7 (table 4)
   Creatine Peak Area: = 64573 = y (Table 3)

$$x = \frac{64573 - 7803.7}{492.93} = 115.094\ ppm$$

3. Average concentration of Creatine in sample.

$$(115.09\ + 132.71 + 167.10)/3 = \ 138.30\ ppm$$

4. Concentration of creatine in the undiluted mouthwash sample 0.5 mL aliquot.

$$Concentration\ of\ creatine\ = \ 138.30\ ppm$$

$$Concentration\ of\ creatine\ in\ sample = \left(138.30\ \frac{\mu g}{mL}\right)\left(\frac{10.00\ mL}{0.100\ mL}\right) = 13830\ ppm$$

5. Uncertainty in Slope (S_m) from Appendix I Uncertainty Table.

$$S_m = \frac{\sqrt{(1181620.8^2)(5)}}{740458} = 17.47$$

6. Uncertainty in y-intercept (S_b) from Appendix I Uncertainty Table.

$$S_b = \frac{\sqrt{(1181620.8^2)(34375)}}{740458} = 4917.99$$

7. Uncertainty in unknown result (S_x)

$$S_x = \frac{S_y}{|m|}\sqrt{\left(\frac{1}{k}\right) + \left(\frac{1}{n}\right) + \frac{(y - \bar{y})^2}{(m^2 \sum (x_i - \bar{x})^2}}$$

$$S_x = \frac{6724.47}{|492.93|}\sqrt{\left(\frac{1}{3}\right) + \left(\frac{1}{5}\right) + \frac{(117576 - 75976)^2}{(492.93^2)(10937)}} = 9.962 \text{ ppm}$$

8. Propagation of uncertainty for creatine.

$$(\frac{S_{c2}}{C_2})^2 = (\frac{S_{vol1}}{Vol_1})^2 + (\frac{S_{vol2}}{Vol_2})^2 + (\frac{S_{c1}}{C_1})^2$$

$$(\frac{S_{c2}}{13830\ ppm}) = \sqrt{(\frac{0.02\ mL}{10.00\ mL})^2 + (\frac{0.02\ mL}{10.000\ mL})^2 + (\frac{9.962}{13830\ ppm})^2}$$

$$S_{c2} = 40.37\ ppm$$

9. %RSD

$$\frac{9.962}{138.30} * 100\% = 7.20\%$$

Discussion

Capillary electrophoresis (CE) with UV detection was used to determine the concentration creatine, in a workout supplement sample. Overall, the experiment and analysis were successful as the concentration of creatine was determined to be 13830 ppm ± 40.37 ppm with a percent relative standard deviation of 7.20%. In this experiment, five standards and three samples were run on the Beckman P/ACE System MDQ capillary electrophoresis system. Using the supplied CE Software, the peak areas and migration times were determined. The analyte was identified on the electropherograms of the sample using the retention times provided by the standards. As shown in Table 3, the peak areas for each standard increased with an increasing concentration of methylparaben. On the electropherograms, this relationship should be shown by an increase in peak size for each standard of increasing concentration. The electropherograms were also expected to have high resolution and separation for each peak. According to the van Deemter equation, the band broadening due to the multiple path and mass transfer contributions could be eliminated because no stationary phase was used. This should have resulted in a decreased plate height and an increased number of theoretical plates and resolution compared to another analytical method such as HPLC.

The external standards calibration method was used to determine the concentration of creatine in the sample. Five standards were analyzed (table 2) and a calibration curve was generated relating the peak area of the standardized creatine to its known concentration. Each standard was analyzed using Beckman P/ACE System MDQ capillary electrophoresis system. The calibration curves were generated by plotting the peak areas versus the known concentration of creatine (Figure 1). The square of the correlation coefficient (R^2) was found to be 0.9962 for creatine which is greater than the desired level of 0.995.

The concentration of creatine in the sample were determined by solving the equation of the least squares line. The peak area for the analytes were given using the Beckman P/ACE System MDQ capillary electrophoresis system. The equation of the least squares line was determined to be y = 492.93x + 7803.7 as generated by excel. The workout supplement sample was determined to have a concentration of 13830 ppm ± 40.37 ppm.

The degree of uncertainty observed can be attributed to many sources of error. A source of error in this experiment is error in the preparation of the standards. This includes error in micro pipetting technique and the sample preparation. This source of error can be minimized by having the person performing the experiment take extra care with their technique and preparation of samples. In addition, more replicates of the standard solutions containing known concentrations of the analyte could be used to decrease the degree of uncertainty. The more data points in the calibration curve the more accurate the equation of the least square's lines would be. This could also increase the accuracy of the final value that was calculated. The accuracy of this experiment was relatively high. The literature value for creatine on the sample bottle showed a concentration of 14066ppm. These values are close to the experimental values of the calculated concentration of 13830 ppm.

Conclusion

The Tango Creatine Recovery Formula was determined to have a concentration of 13830 ppm ± 40.37 ppm with a percent relative standard deviation of 7.20%.

References

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2. McNair H. M., Miller J. M. Basic Gas Chromatography, 2nd ed., John Wiley & Sons, Inc., New Jersey, 2009.
3. Harris, D. C. Quantitative Chemical Analysis, 8th ed., W. H. Freeman & Co.: New York, 2010, Chapter 25.

Appendix I - Uncertainty Tables

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1	Xi	Yi	XiYi	Xi^2	di	di^2	$Y_{unknown}$		∑ Xi	∑Yi	∑XiYi	$\sum Xi^2$	$\sum di^2$		Number of points		5		**Results:**	S_y	6724.475
2	0	0	0.000	0	-7803.698	60897697	64537		1113.48	587883	2.04E+08	396059.3	1.36E+08		Slope (m)		492.9271398			S_b	4917.995
3	92.79	60498	5613609.420	8609.984	6955.593	48380274	73218								Intercept (b)		7803.697674			S_m	17.47403
4	185.58	103721	19248543.180	34439.94	4439.884	19712567	90173								D		740458.6326			S_x	9.962653
5	371.16	188635	70013766.600	137759.7	-2123.535	4509400									k		3			DEVSQ	148091.7
6	463.95	235029	109041704.550	215249.6	-1468.244	2155741														R^2	0.996244
7															Xi average		222.696			Derived x	138.301
8															Yi Average		117576.600				
9															$Y_{unknown}$ Average		75976			RSD	7.20%

Figure 1. Uncertainty table for creatine.