ANALYTICAL CHEMISTRY PROJECT IDEAS
Srch Broadly first: **Google, Google Scholar, Websites (*)**
Srch Broadly Chem. specific databases: **ACS J. Chemical Education (1); other Journals (2)**
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GROUP PROJECT FOCUS: Quantification of Sports Supplements

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Project Idea: Quantify levels of creatine in supplements (NSF approved or not)

Keywords used: creatine, quantification, chromatography,

Measurement of Creatine Monohydrate in Sports Supplements using TLC

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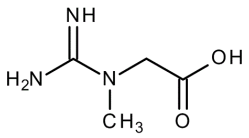
Executive Summary:

Creatine is a common supplement taken by athletes to help gain an edge on competitors. Creatine has been proven to increase stamina and muscle growth in users of the product. Some negative effects are that creatine is a performance enhancing drug thus raising ethical issues on use, and much is unknown about the long term effects of creatine. Because creatine is a dietary supplement there is little regulation in the product, Additionally, some products claim to be certified by the NCAA but the NCAA does not endorse any supplemental product. Our question is, Do these “certified” products contain a more accurate creatine content than those that are not “certified”? Because there are some known health risks, it is important that people know the true amount of creatine they are ingesting.[1] To measure the Creatine content in various samples, thin layer chromatography will be used. Stock samples will be used to create a concentration curve using qTLC, a web app for quantitative analysis of TLC. [2]

Analyte of Interest:

Our designated analyte of interest is Creatine. Creatine is a common supplement that athletes use to gain an advantage on their opponents. It’s primary role in the body is to help regenerate ATP, which ultimately allows athletes to compete at higher levels for longer periods of time. It’s chemical formula is $(H_2N)(HN)CN(CH_3)CH_2CO_2H$. It’s structure consists of an acid on one end and an amine ground of the opposite end as shown by the picture below. Creatine’s solubility in water is 13.3 g/L and melting point is 255 degrees Celsius. Creatine’s molecular mass is 131.135 g/mol. The major species is creatine monohydrate and it is typically in a powdered form prior to consumption. 3



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Experimental Method

Anhydrous creatine standard solution was prepared at a concentration of 1.00 mg/mL in deionized water. Products containing creatine monohydrate were purchased from a health-food store or pharmacy, and appropriate weights (0.100-1.50 g/100 mL) were dissolved in water to produce sample solutions of 1.00 mg/mL, based on label values. All products were powders. Solutions of samples were magnetically stirred for 10 min to ensure that creatine monohydrate was completely dissolved, and the undissolved material was allowed to settle for 15 min prior to analysis.For use in validation of the method, a standard of creatine monohydrate (Fischer, Creatine Monohydrate, Catalog # Ac22690-250, >99% purity) was purchased and dissolved in water at a concentration of 1.00 mg/mL.The standard and all samples were prepared in 100 mL volumetric flasks. Analyses were performed on 20 x 10 high performance silica gel plates with fluorescent indicator, preadsorbent zone, and multiple channels channels. The plates were pre-cleaned by development to the top with dichloromethane-methanol (1:1) and dried before use. Standard and sample solutions were applied to the pre-adsorbent areas of adjacent channels using a 10 µL micropipette.The volumes applied for each analysis were 1.00 µL, duplicate 2.00 µL, and 4.00 µL of the standard (1.00-4.00 µg creatine) and duplicate 2.00 µL of the sample solutions (ca. 2.00 µg theoretical content). The initial zones were dried with a hair dryer on high heat setting for ca. 3 min before development. Plates were developed to a distance 6 cm beyond the origin (pre-adsorbent silica gel interface) with the mobile phase consisting of acetonitrile-deionized water (7:3) in a beaker. After development, the mobile phase was evaporated from the plate by drying in a fume hood for 10 min with a hair dryer, and it was then heated for 5 min at 160°C on an electronic hot plate. The resultant fluorescence-quenched zones of creatine standards and samples were measured by linear scanning using a TLC scanner. The software controlling the densitometer produced a calibration curve by linear regression of the weights and areas of the standard zone scans and interpolated the weights of the sample zones from their scan areas. For each analysis, the percentage creatine was calculated by multiplying the average weight of creatine in the 2.00 µL sample aliquots times the factor (100 x 103 µL/2.00 µL), dividing by the µg of sample weighed into the 100 mL volumetric flask, and multiplying the quotient by 100. Accuracy of the new method was validated by analysis of the certified creatine standard. Precision was validated by spotting four 2.00 µL aliquots of several samples and calculating the relative standard deviations of the scan areas. Further evaluation of reproducibility was obtained by calculating the percent difference between the scan areas of the duplicate sample and standard zones spotted in each analysis.[1]

References

1. Wagner, S. D.; Kaufer, S. W.; Sherma, J. Quantification Of Creatine In Nutrition Supplements By Thin Layer Chromatography-Densitometry With Thermochemical Activation Of Fluorescence Quenching. *Journal of Liquid Chromatography & Related Technologies* **2001**, *24* (16), 2525–2530.
2. **A Toolkit to Quantify Target Compounds in Thin-Layer-Chromatography Experiments**
   Niamh Mac Fhionnlaoich, Stuart Ibsen, Luis A. Serrano, Alaric Taylor, Runzhang Qi, and Stefan Guldin
   Journal of Chemical Education 2018 *95* (12), 2191-2196
   DOI: 10.1021/acs.jchemed.8b00144
3. National Center for Biotechnology Information. PubChem Database. Creatine, CID=586, https://pubchem.ncbi.nlm.nih.gov/compound/Creatine (accessed on April 28, 2020)

(A) 3-4 Sentences Project Goal:

What do you plan on doing?

- Testing NSF regulated vs non-regulated creatine to see the difference in substance between the two

Do you have the context (water, air, soil, etc)?

- In the supplement itself, so chemical makeup and additives

Do you have a question in mind?

- What exactly is in the creatine supplements we are measuring? Do they contain the active ingredient? Is what we find comparable to what it says on the nutrition label?

Do you have a prediction?

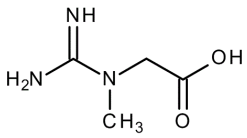
- The non NSF creatine will contain chemicals that are not listed on the nutrition label while the NSF approved creatine will contain everything listed on the nutrition facts label

(B) Background information:

About the context?

- Creatine is a common supplement taken by athletes to help gain an edge on competitors. It helps athletes gain muscle and increase stamina to allow for longer competition.

About the chemical(s)?



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About Health Effects?

- Creatine has been proven to increase stamina and muscle growth in users of the product. Some negative effects are that creatine is a drug and the body can become dependent on it, ethical issues on use, and that it can lead to many kidney and liver issues later in life.

About sources?

- Sources are gained from studies on creatine use over “Short periods” (ranging from a few weeks to several weeks) and “long periods” (up to five years).

About acceptable standards/levels?

- The recommended daily amount of creatine consumption is 5g/day, although some studies have done up to 30g/day with no ill effects.

(C) Methods of Analysis

Do you know what you need to measure?

Need to measure the content of the active ingredient (creatine monohydrate) as well as any potential impurities

Do you know which methods are used to detect?

Possibly HPLC

Thin Layer Chromatography

Do you know if you will have access to this method?

Yes, have done TLC before in previous classes

(D) References (Citation in ACS style and copy of abstract)

[Instructions and details:

1.You should have at least 8-10 relevant articles, 1 ebook, and 2-3 good websites.

2-In this section just write down the correct citation for all the articles you find (ACS style) followed by a copy of the abstract. For example,

Melamed, D., 2005. Monitoring arsenic in the environment: a review of science and technologies with the potential for field measurements. Analytica Chimica Acta, 532(1), pp.1-13.

Abstract: This review examines available field assays and other technologies with the potential to measure and monitor arsenic in the environment. The strengths and weaknesses of the various assays are discussed with respect to their sensitivity, ability to detect the chemical states of arsenic, performance in various media, potential interferences, and ease of operation. The state of the science and development efforts of selected technologies is presented.

3. FOLLOW ACS guidelines for all citations.]

Article#1

4 references-2 methods, 2 related/recent

Kreider, R.B., Kalman, D.S., Antonio, J. *et al.* International Society of Sports Nutrition position stand: safety and efficacy of creatine supplementation in exercise, sport, and medicine. *J Int Soc Sports Nutr* **14,** 18 (2017). https://doi.org/10.1186/s12970-017-0173-z

Creatine is one of the most popular nutritional ergogenic aids for athletes. Studies have consistently shown that creatine supplementation increases intramuscular creatine concentrations which may help explain the observed improvements in high intensity exercise performance leading to greater training adaptations. In addition to athletic and exercise improvement, research has shown that creatine supplementation may enhance post-exercise recovery, injury prevention, thermoregulation, rehabilitation, and concussion and/or spinal cord neuroprotection. Additionally, a number of clinical applications of creatine supplementation have been studied involving neurodegenerative diseases (e.g., muscular dystrophy, Parkinson's, Huntington's disease), diabetes, osteoarthritis, fibromyalgia, aging, brain and heart ischemia, adolescent depression, and pregnancy. These studies provide a large body of evidence that creatine can not only improve exercise performance, but can play a role in preventing and/or reducing the severity of injury, enhancing rehabilitation from injuries, and helping athletes tolerate heavy training loads. Additionally, researchers have identified a number of potentially beneficial clinical uses of creatine supplementation. These studies show that short and long-term supplementation (up to 30 g/day for 5 years) is safe and well-tolerated in healthy individuals and in a number of patient populations ranging from infants to the elderly. Moreover, significant health benefits may be provided by ensuring habitual low dietary creatine ingestion (e.g., 3 g/day) throughout the lifespan. The purpose of this review is to provide an update to the current literature regarding the role and safety of creatine supplementation in exercise, sport, and medicine and to update the position stand of International Society of Sports Nutrition (ISSN).

Jagim AR, Stecker RA, Harty PS, Erickson JL and Kerksick CM (2018) Safety of Creatine Supplementation in Active Adolescents and Youth: A Brief Review. *Front. Nutr*. 5:115. doi: 10.3389/fnut.2018.00115

Creatine has been extensively researched and is well-supported as one of the most effective dietary supplements available. There is overwhelming support within the literature regarding the ability of creatine to augment performance following short term (5–7 days) and long-duration supplementation periods. There is also strong support for creatine regarding its safety profile and minimal risk for adverse events or any negative influence on markers of clinical health and safety. Recent research has also highlighted the ability of creatine to confer several health-related benefits in select clinical populations in addition to offering cognitive benefits. Creatine is also a popular supplement of choice for adolescent athletes; however, research in this area is extremely limited, particularly when examining the safety and efficacy of creatine supplementation in this population. Therefore, the purpose of this review was to highlight the limited number of studies available in adolescent populations and systematically discuss the topic of safety of creatine supplementation in a younger population.

Wagner, S.; Kaufer, S.; Sherma, J. Quantification of Creatine in Nutritional Supplements by Thin Layer Chromatography-Densitometry with Thermochemical Activation of Fluorescence Quenching. *J. Liq. Chromatogr. Relat. Technol*. 2001, *24*, 2525–2530.

A quantitative method using silica gel HPTLC plates with fluorescent indicator; thermochemical, reagent-free reaction to produce fluorescence quenched zones; and automated UV absorption densitometry has been developed for the determination of creatine in nutrition supplements. Eight products containing different amounts of creatine monohydrate

and additional ingredients were analyzed. Accuracy was validated by analysis of a certified reference sample and precision by performing replicated analyses.

Accuracy was found to be within 0.2% of the certified value, and precision was 3–4% relative standard deviation.

Jäger R, Purpura M, Shao A, Inoue T, Kreider RB. Analysis of the efficacy, safety, and regulatory status of novel forms of creatine. *Amino Acids*. 2011;40(5):1369–1383. doi:10.1007/s00726-011-0874-6

Creatine has become one of the most popular dietary supplements in the sports nutrition market. The form of creatine that has been most extensively studied and commonly used in dietary supplements is creatine monohydrate (CM). Studies have consistently indicated that CM supplementation increases muscle creatine and phosphocreatine concentrations by approximately 15–40%, enhances anaerobic exercise capacity, and increases training volume leading to greater gains in strength, power, and muscle mass. A number of potential therapeutic benefits have also been suggested in various clinical populations. Studies have indicated that CM is not degraded during normal digestion and that nearly 99% of orally ingested CM is either taken up by muscle or excreted in urine. Further, no medically significant side effects have been reported in literature. Nevertheless, supplement manufacturers have continually introduced newer forms of creatine into the marketplace. These newer forms have been purported to have better physical and chemical properties, bioavailability, efficacy, and/or safety profiles than CM. However, there is little to no evidence that any of the newer forms of creatine are more effective and/or safer than CM whether ingested alone and/or in combination with other nutrients. In addition, whereas the safety, efficacy, and regulatory status of CM is clearly defined in almost all global markets; the safety, efficacy, and regulatory status of other forms of creatine present in today's marketplace as a dietary or food supplement is less clear.

Joseph Sherma, Fred Rabel. (2019) Advances in the thin layer chromatographic analysis of counterfeit pharmaceutical products: 2008–2019. *Journal of Liquid Chromatography & Related Technologies* 42:11-12, pages 367-379.

Publications reporting thin layer chromatography (TLC) screening and high performance TLC (HPTLC)-densitometry quantification analyses of counterfeit pharmaceutical products are reviewed for the 2008–2019 period. Screening using TLC methods published in the Global Pharma Health Fund (GPHF) Minilab Manual and U.S. Food and Drug Administration (FDA) Compendium, as well as in other sources, are covered. Also included are publications on TLC analysis hyphenated with Raman and mass spectrometry; analyses of counterfeit traditional herbal medicines; earlier published reviews; transfer of screening methods for counterfeit pharmaceutical products in the Minilab Manual and FDA Compendium to HPTLC-densitometry using a model process; development of HPTLC-densitometry methods for pharmaceutical products not included in the Minilab Manual or FDA Compendium using the model process followed by development of corresponding Supplemental FDA Compendium TLC screening methods; and modified Minilab methods with simplified detection based on heating of silica gel F layers to produce fluorescence quenching zones (thermochemical activation) rather than detection using spray, dip, or vapor phase derivatization reagents. Some thoughts on future prospects for the field are also offered.

Measurement of creatine and phosphocreatine in muscle tissue using HPLC

- By Haruki, Katsuo; Itoh, Masanori; Kuroda, Masahiro; Hatakeyama, Shuichi; Hirata, Masayosi; Muramoto, Hiroaki; Koni, Ichirou; Tofuku, Yohei; Takeda, Ryoyu
- From Rinsho Byori (1989), 37(4), 447-9. | Language: Japanese, Database: CAPLUS
- **Creatine** extd. from a lyophilized specimen into $NaHCO_3$ soln. (pH 8-9) was sepd. on a Guanidinopack column, reacted with ninhydrin, and detd. by UV spectrometry. A part of the ext. was acidified to hydrolyze phosphocreatine, and then total **creatine** was detd. Inverse transformation between them was negligible in both alk. and acidic solns.

Yoonsun Mo, David Dobberpuhl, Alekha K Dash,

A simple HPLC method with pulsed EC detection for the analysis of creatine,
Journal of Pharmaceutical and Biomedical Analysis,
Volume 32, Issue 1,
2003,
Pages 125-132,
ISSN 0731-7085,
https://doi.org/10.1016/S0731-7085(03)00028-1.
(http://www.sciencedirect.com/science/article/pii/S0731708503000281)
Abstract: The objective of this study was to develop a simple and sensitive LC method for the determination of creatine in aqueous solutions as well as in rat plasma using electrochemical detection. The chromatographic system consisted of a GP50 gradient pump, an ED40 pulsed electrochemical detector, and an AI-450 chromatography automation system (Dionex). The mobile phase consisted of a mixture of water, acetonitrile, 0.01 M sodium acetate, and 1.0 M sodium hydroxide (2.5:2.5:90:5, V/V/V/V) at a flow rate of 1.0 ml/min. The chromatographic separation was achieved at 45°C on a column with a polyhydroxylated glucose and sulfonated stationary phase. The retention times of creatine and creatinine was 3.50 and 4.73 min, respectively, with creatine fully resolved from its major degradation product, creatinine. The standard curves were linear over the concentration range of 0–20 μg/ml. Within-day and day-to-day relative standard deviations (R.S.D.) were less than 10%. This method was used to study dissolution characteristics of various creatine salts in water.

https://www.healthline.com/nutrition/creatine-safety-and-side-effects#dehydration-and-cramps

Research Project Title and Authors Title – descriptive and concise, with enough detail to engage the readers. **Authors** – Radloff, Garris. Belec, Andrew. Hemmerich, Nathaniel. Shondel, Robert.

Executive Summary (≤ 500 words) Your executive summary, much like an abstract, should highlight the important components with pertinent background information. You should think of this as an argument or proposal for why a funding agency might give you money to do this project.

a) What is your question and what will this study provide to the public.
b) What is the relevant background information for the problem being studied?

- What is the substance being studied (formula, structure, species?) and why is it present in the samples?
- Why is it useful/interesting to obtain quantitative data? (Is it hazardous? toxic? essential for human health, etc.?)
- What have other scientists found? What are normal expected ranges of concentrations or amounts? **c)** What are you studying and how will you compare the data? **d)** Literature citations should be included.

Analyte of Interest (≤ 200 words) In a very brief paragraph, give a description of the analyte of interest,

including the chemical formula, structure, major species in this environment, and important chemical properties (solubility, toxicity, boiling or melting point, etc.)

Experimental Method (no limit, but make sure it is complete) Present the method that you would have performed in two major sections using a written paragraph form and following our discussion of quality assurance and calibration methods.