

# Tracing the Fate of Chromium Nutritional Supplements in the Body

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*Chromium has been proposed to be an essential trace element useful in the treatment of diabetes, and several chromium(III) compounds have been used as nutritional supplements. However, the fate of the chromium from nutritional supplements has been investigated solely by techniques, such as radio-labeling, which allow only the chromium atom to be traced, not its chemical form. This investigation examined the ability of liquid chromatography-mass spectrometry (LCMS) to follow these supplements and to elucidate the chemical composition of their degradation products in the body. Rats were utilized to examine chemical composition of a chromium supplement provided to the rats by gavage administration. Results indicate that LCMS is not a viable analytical technique for elucidating the speciation and quantification of chromium nutritional supplements at even pharmacological doses.*

## Introduction

The element chromium (Cr) in the +3 oxidation state has been proposed to be an essential trace element for mammals [14]. The National Academy of Sciences of the United States has set the adequate intake (AI) of chromium at 35  $\mu\text{g}$  daily for men and 25  $\mu\text{g}$  daily for women [13]. Even though the typical American diet provides an adequate amount of chromium, chromium supplements are currently on the market and include chromium chloride, chromium picolinate, and chromium nicotinate. However, a consensus exists among researchers that chromium supplementation of healthy rats does not provide any therapeutic effects except for the compound Cr3 [10, 8, 15, 1]. Several reasons may explain this difference among Cr chloride, Cr nicotinate, Cr picolinate and the cation Cr3.

Olin et al. [9] and Anderson et al. [2] have shown that Cr chloride, Cr nicotinate, and Cr picolinate are absorbed with efficiencies between 0.5-1.3 % of the gavaged dose after 24 hours. However, for Cr3, 40% of the Cr at a pharmacological dose and 60% of the Cr at a nutritionally relevant dose are absorbed over a 24 hour period. (The nutritional dose was 3  $\mu\text{g}$  Cr/kg body mass while the pharmacological dose was 3 mg Cr/kg body mass). The better absorption efficiency of Cr3 may explain its better effectiveness as a therapeutic agent.

Cr chloride used in nutritional supplements is not actually the simple salt  $\text{CrCl}_3$  with waters of hydration but is actually the compound *trans*- $[\text{CrCl}_2(\text{H}_2\text{O})_4]\text{Cl}$ .

Oligomerization, the chemical process of forming a degree of polymerization from a monomer, commonly occurs, especially at basic pHs, and results in the formation of multinuclear hydroxo-bridged chromic species. This diminishes the therapeutic value of the  $\text{CrCl}_3$  since the solubility of the oligomerized and polymerized species is limited, especially while passing through the basic pH levels of the intestines [4]. Chromium chloride is commonly used as a model for dietary chromium.

The chemical structure of chromium nicotinate has not yet been fully characterized, but the compound is known to have only limited solubility in water (i.e., it is essentially insoluble in water). The ligands of the chromium nicotinate tend to allow creation of other Cr forms that can bind to various biomolecules and undergo olation due to their relative lability. Thus the nicotinate species degenerate in the gastrointestinal tract of the rats [7].

Chromium picolinate has poor solubility in water [6] and other common solvents and also lacks strong degree of lipophilicity [3]. Its solubility actually decreases as pH is lowered until the hydrogen ion concentration is so high that the compound decomposes. This pH is approximately that of the stomach lining. However, Cr3 is extremely soluble in water and stable in dilute mineral acid and is thus better able to maintain its chemical structure under physiological conditions, such as conditions in the stomach [11]. Cr3 has been proposed to act as a biomimetic of chromodulin, a naturally present

oligopeptide that stimulates tyrosine kinase activity of the insulin receptor. Thus, Cr3 has been shown in healthy and type 2 diabetic model rats to increase insulin sensitivity while also decreasing plasma triglycerides and total cholesterol and low-density lipoprotein (LDL) cholesterol concentrations [4]. After a period of three months, the daily intravenous administration of Cr3 at a concentration of 20 µg Cr/kg body mass lowered plasma triglycerides and total and LDL cholesterol levels in healthy rats [12]. Another study also demonstrated that six months of intravenous administration of Cr3 at the pharmacological doses of 5-20 µg Cr/kg body mass resulted in a reduction in plasma triglycerides, total and LDL cholesterol, insulin concentrations and an increase in insulin sensitivity for both healthy and type 2 diabetic model rats.

Since the fate of these nutritional supplements in the body has not been well established, the use of liquid chromatography mass spectrometry (LCMS) could allow the fate of chromium from these chromium supplements to be followed better inside the body than with previously utilized techniques. Prior studies primarily relied on the use of radioactive Cr-51, which yielded liquid chromatography column elution profiles which provided approximate molecular weights of the radiolabeled chromium-containing species, not the identity of compounds.

The purpose of this project is to elucidate the speciation of chromium from chromium supplements provided by gavage administration within the body tissues of rats as a function of time. The four chromium species that were utilized were CrCl<sub>3</sub>, Cr picolinate, Cr nicotinate, and triaqua-µ<sub>3</sub>-oxo-µ-hexapropionatotrichromium(III) cation, also known as Cr3. Only non-radioactive chromium was utilized to gavage the rats. Blood samples and body tissues were harvested for investigation and include liver, spleen, heart, kidney, testes, fat, pancreas, skeletal muscle, and femur. The significance of this research is that it may be possible to determine the fate of Cr from the supplements in body organs of the rat. This was not previously possible with other techniques; now, however, the fate of the chromium might be followed better throughout the body. This knowledge could be utilized to better understand the mode of action by which chromium can potentially be used to treat type 2 diabetes.

## Materials and Methods

<sup>51</sup>CrCl<sub>3</sub> in 1.0 M HCl was obtained from ICN. [Cr<sub>3</sub>O(O<sub>2</sub>CCH<sub>2</sub>CH<sub>3</sub>)<sub>6</sub>(H<sub>2</sub>O)<sub>3</sub>]NO<sub>3</sub> was made by the method of Earnshaw et al. [5]. Cr picolinate and Cr nicotinate were available from previous work in the Vincent laboratory. All procedures were performed using doubly-deionized water unless otherwise noted. A liquid chromatography mass spectrometer was used. The LC portion is an Agilent 1200 Capillary LC, and the MS portion is a Bruker Esquire HCT Ultra Ion Trap. The column utilized for the LC portion of the LCMS experiment was an Agilent Technologies Zorbax SB-C18, 150 x 0.5 mm with a 5 µm diameter. An UV/visible spectrophotometer (Hewlett-Packard 845X UV Visible Chemstation) was utilized to collect all ultraviolet/visible spectra. Calf liver was obtained from a local vendor and used as a representative tissue to test the feasibility of detecting chromium-containing species extracted from tissues by LCMS. A TissueMiser tissue homogenizer was used to homogenize the bovine liver. High Performance Liquid Chromatography (HPLC) grade acetonitrile (ACN) and H<sub>2</sub>O were utilized for all LCMS and MS experiments. When necessary, phenolphthalein (PHT) was utilized as an internal standard for LCMS experiments.

### *Testing solubility of chromium compounds*

Three nutritional supplements were utilized: chromium propionate, chromium nicotinate, and Cr3. These three Cr nutritional supplements were tested for solubility in common LCMS solvents: acetonitrile, dimethyl sulfoxide (DMSO), and methanol (MeOH). One milliliter of each of the three organic solvents was added to 100 mg samples of Cr3, chromium picolinate, and chromium nicotinate. The samples were allowed to sit for 24 hours.

### *Testing ability to extract Cr3 from tissue homogenates*

Since Cr3 was soluble in all three organic solvents, its ability to be extracted from tissue homogenates was tested with calf liver and each of the three organic solvents. Three samples were made, each containing 0.670 g cow liver, 0.7 mL H<sub>2</sub>O, and 50 mg Cr3. The samples were homogenized using a TissueMiser tissue homogenizer and then freeze-dried for 18 hours. Each sample was extracted with 2 mL of the respective solvent, and this solution was clarified by centrifugation. The extraction procedure was

repeated a second time. An UV/visible spectrum was obtained for each solution. ACN was chosen as the extraction solvent.

#### LCMS studies on Cr3

Before the presence of Cr3 in ACN extractions of liver homogenates could be tested, the behavior of Cr3 in the LCMS had to be examined. The molecular weight of the parent cation of Cr3 is 664.4 g/mol. Previous electrospray ionization (ESI) MS studies on Cr3 had indicated that Cr3 lost water during the experiment; thus, additional signals could be expected from Cr3 [Table 1].

**Table 1:** Signals from Cr3 expected in ESI MS experiments

m/z	Species
664	$[\text{Cr}_3\text{O}(\text{O}_2\text{CCH}_2\text{CH}_3)_6(\text{H}_2\text{O})_3]^+$
646	$[\text{Cr}_3\text{O}(\text{O}_2\text{CCH}_2\text{CH}_3)_6(\text{H}_2\text{O})_2]^+$
628	$[\text{Cr}_3\text{O}(\text{O}_2\text{CCH}_2\text{CH}_3)_6(\text{H}_2\text{O})]^+$

#### LCMS experiments on tissue extracts

Cr3 (50 mg) was added to 1 g calf liver and 1 mL H<sub>2</sub>O. The sample was homogenized with the TissueMiser tissue homogenizer, and the product was freeze-dried overnight. The Cr3 was extracted from the sample with 5 mL ACN, and the product was clarified by centrifugation. The ACN was removed under vacuum, and the product was dissolved in 1 mL H<sub>2</sub>O. A 1 μL aliquot was used in the LCMS experiment. After establishing that Cr3 could be detected by LCMS, the LCMS detection limit for Cr3 in a tissue sample had to be determined. Samples were prepared by adding 25 mg, 10 mg, 5 mg, 2.5 mg, 1 mg, or 0.5 mg Cr3 to 1 g bovine liver and 1 mL LCMS grade H<sub>2</sub>O. These samples were freeze-dried and then extracted with 1 mL LCMS grade ACN. These solutions were clarified by centrifugation, and the ACN was removed by vacuum. A mixture of 50:50 ACN: H<sub>2</sub>O was added to the dissolve the samples. Finally, the solutions were filtered through a 22 μm Fisher filter to remove any particulates. This improves the resolution of the peaks in the LCMS experiment while cutting down on MS background noise. One μL aliquots were used in the LCMS experiments. Since Cr3 was successfully identified in some of the experiments, an internal standard was needed in order to quantify the amount of Cr3. Phenolphthalein (PHT) was tested as an internal standard; 1 mg PHT

and 1 mg Cr3 were added to 1 mL ACN. Ten μL of this solution was diluted with 990 μL of 50:50 ACN: H<sub>2</sub>O, and the resulting solution was used to obtain mass spectra.

#### LCMS experiments on rat liver

Two male Sprague-Dawley rats (227.1 g and 226.5 g) were obtained from Charles River Laboratories; rats were allowed to acclimate for at least one week after their arrival and before use. Rats were maintained on a 12 hour light/dark cycle. The rats were gavaged with an aqueous solution of Cr3 at the pharmacological dose (3 mg Cr/kg body mass) of 2.89 mg Cr3 in 500 μL H<sub>2</sub>O at 9:00 am. After administration, the rats were allowed to feed on a commercial rat chow and drink ad libitum. After six hours, the rats were sacrificed by carbon dioxide asphyxiation, and tissue samples (heart, liver, testes, spleen, kidneys, epididymal fat, pancreas, and muscle [musculus triceps surae] from the hind legs) were harvested, weighed and frozen at -78°C. Ten grams of liver was procured from one of the rats, divided into five samples of two grams each and freeze dried for 12 hours. After this period, the freeze dried liver samples were pulverized to a dust. 2 mL HPLC ACN was added to each of the five samples and allowed to sit for 24 hours to extract Cr3 from the liver. The ACN extractions of the liver were then added together and the ACN removed by vacuum. One mL HPLC H<sub>2</sub>O was added and this solution was filtered through a 22 μm Fischer filter to remove any particulates. A 1 μL aliquot was used in the LCMS experiment. The University of Alabama Institutional Animal Use and Care Committee approved all procedures involving the use of rats.

#### Results

The solubility of Cr picolinate, Cr nicotinate, and Cr propionate was tested in ACN, DMSO, and MeOH. Chromium nicotinate did not dissolve in any of the three organic solvents, chromium picolinate dissolved appreciably only in DMSO, and chromium propionate readily dissolved in all three solvents. The solubility of the Cr picolinate in DMSO was too low to make further studies feasible. Thus, only Cr3 was chosen for further study.

The extraction test with 50 mg Cr3 added to bovine liver homogenates followed by extraction with the respective organic solvents revealed that only the

ACN solution could ambiguously be shown to contain Cr3 [Figure S1]; consequently, ACN was chosen as the solvent for subsequent extractions.

Initially in the LCMS experiments on the tissue extracts, the extraction method did not include filtration with a 22 µm Fisher filter, and Cr3 could not be detected. With this technique, Cr3 was first detected by LCMS in the sample derived from 50 mg Cr3 homogenized with 1 mL H<sub>2</sub>O and 1 g liver. MS signals for Cr3, along with its degradation products, showed up at their characteristic masses. Additionally a feature was observed at m/z of 702, corresponding to the ACN adduct of Cr3. This verified the feasibility of detecting Cr3 from a tissue sample by analysis with LCMS [Figure S2]. Once the feasibility was established, a detection limit for the amount of Cr3 that could be detected in a tissue sample needed to be established. The mass spectra of the liver homogenates containing masses of Cr3 from 0.5 mg to 25 mg failed to contain the characteristic signals from Cr3 and its degradation products but rather contained much background noise. A 22 µm Fisher filter was then added to the extraction technique in order to remove any particulates, help decrease background noise, and obtain better MS data. With the filtration step, Cr3 added to tissues at a level as low as 0.5 mg Cr3 per g of liver tissue could be detected. As levels at which Cr3 could be detected in tissue homogenates had been established, the LCMS experiments were performed in a time dependent fashion (i.e., the intensity of the 664 m/z signal was measured as a function of time as the samples passed through the LC column and were detected by the MS) [Figure S3 & S4 and Table 1 & 2]. The Cr3 eluted from the column from 29 minutes to 32 minutes under the operation conditions. The gradient conditions utilized are given in Table 3.

To characterize the concentration of Cr3 in the samples, an internal standard is needed. The proposed internal standard, PHT, was tested by mass spectrometry along with the Cr3 in the 50:50 ACN: H<sub>2</sub>O solution [Figure S5]. The Cr3 was detected as well as PHT adducts with H<sup>+</sup>, Na<sup>+</sup>, and K<sup>+</sup>. Thus, PHT appears to be an excellent candidate for use as an internal standard.

**Table 2:** Assignment of LCMS data for 2.5 mg Cr3 in homogenate of 5 mL H<sub>2</sub>O and 5 g liver from Figure S3.

m/z	Species
628	[Cr <sub>3</sub> O(O <sub>2</sub> CCH <sub>2</sub> CH <sub>3</sub> ) <sub>6</sub> (H <sub>2</sub> O)] <sup>+</sup>
646	[Cr <sub>3</sub> O(O <sub>2</sub> CCH <sub>2</sub> CH <sub>3</sub> ) <sub>6</sub> (H <sub>2</sub> O) <sub>2</sub> ] <sup>+</sup>
663.9	[Cr <sub>3</sub> O(O <sub>2</sub> CCH <sub>2</sub> CH <sub>3</sub> ) <sub>6</sub> (H <sub>2</sub> O) <sub>3</sub> ] <sup>+</sup>
702.7	[Cr <sub>3</sub> O(O <sub>2</sub> CCH <sub>2</sub> CH <sub>3</sub> ) <sub>6</sub> (H <sub>2</sub> O) <sub>3</sub> ] <sup>+</sup> · ACN

**Table 3:** LCMS Gradient Data for 2.5 mg Cr3 in homogenate of 5 mL H<sub>2</sub>O and 5 g liver from Figure S3 & S4.

Time	Function	H <sub>2</sub> O Solvent A%	ACN Solvent B%
0	Solvent Mix	100	0
1	Solvent Mix	98	2
3	Solvent Mix	98	2
8	Solvent Mix	88	12
34	Solvent Mix	75	25
55	Solvent Mix	20	80
60	Solvent Mix	20	80
75	Solvent Mix	98	2

The LCMS experiment with the rat liver failed to characterize Cr3 at the concentration of 3 mg Cr/kg body mass. Since the rats were sacrificed at six hours after ingestion of Cr3, the time with maximum chromium absorption as indicated in the prior literature [4] and the experiment utilized the liver, which contains the highest concentration of chromium among body tissues [4], no further LCMS experiments were necessary on the other harvested rat tissues. Thus, LCMS is not a viable analytical tool for the characterization of Cr3 at the concentration representative of a pharmacological dose of chromium in humans.

## Discussion

Chromium propionate has been implicated as a possible treatment for type 2 diabetes due to its high absorption rates in the body at both nutritional and pharmacological doses. Cr3 has been shown to reduce plasma triglycerides, low density lipoprotein and total cholesterol concentrations and to increase insulin sensitivity. Therefore, establishing a method to determine the chromium speciation in the body and also to quantify the levels of the chromium species in various body tissues and fluids, as a function of time, could help elucidate the mode of action of Cr3.

Liquid chromatography mass spectrometry (LCMS) was hypothesized to potentially be a more effective method of determining the speciation and

concentration of Cr<sup>3+</sup> and its degradation products in tissues and body fluids than previous methods because specific molecular weights can be assigned to species by MS after components of the mixtures are separated by LC. Progress was hampered by occasional instrumental problems with the MS. In part, these were solved by adding a thorough flushing of the MS with 100% ACN, 50:50 ACN: H<sub>2</sub>O, and 100% H<sub>2</sub>O for half an hour or longer. The lowest amount of Cr<sup>3+</sup> per gram of liver detectable by the LCMS was 0.5 mg/g. This amount is still too high to detect Cr<sup>3+</sup> in samples of tissue from rats given a pharmacological dose of 3 mg Cr/ 1 kg body weight. The amount of Cr needed to gavage the rats to be detectable by LCMS would be 117 mg Cr/ 1 kg body weight.

The choice of phenolphthalein as an internal standard appears promising as it was readily detected along with Cr<sup>3+</sup> and its degradation products in the mass spectrum. The PHT bonded with H<sup>+</sup> at 319, Na<sup>+</sup> at 342, and K<sup>+</sup> at 357. The utilization of PHT as an internal standard would allow the concentration of the Cr<sup>3+</sup> to be determined by finding the area under the curves of both PHT and Cr<sup>3+</sup> on the chromatogram from the LCMS.

### Conclusion

The solubility test showed Cr<sup>3+</sup> is the most soluble compound among itself, chromium picolinate, and chromium propionate in acetonitrile, methanol, and dimethyl sulfoxide. The low solubility of the other compounds prevented their use in further studies. Cr<sup>3+</sup> was utilized in the extraction ability test to determine which of the three organic solvents was best at extracting the Cr<sup>3+</sup> from the bovine liver. ACN was determined to be the best organic solvent for extracting Cr<sup>3+</sup> from tissue. Liquid chromatography mass spectrometry (LCMS) was shown as a viable option for characterizing Cr<sup>3+</sup> (or its decomposition products) in the body at a concentration of 0.5 mg Cr<sup>3+</sup> per 1 g tissue. This research also showed PHT at a concentration of 10 µg/mL is a good internal standard for LCMS. However, this research determined that LCMS is not a viable analytical option for characterizing Cr<sup>3+</sup> in the body when administered at a pharmacological dose.

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### References

- [1] Althuis MD, Jordan NE, Ludington EA, et al. (2002). Glucose and insulin responses to dietary chromium supplements: A meta-analysis. *Am. J. Clin. Nutr.*, 76: 148-155.
- [2] Anderson RA, Bryden NA, Polansky MM, et al. (1996). Lack of toxicity of chromium chloride and chromium picolinate in rats. *J. Trace Elements Exp. Med.*, 9: 11-25.
- [3] Chakov NE, Collins RA, Vincent JB. (1999). A re-investigation of the electronic spectra of chromium(III) picolinate complexes and high yield synthesis and characterization of Cr<sub>2</sub>(µ-OH)<sub>2</sub>(pic)<sub>4</sub>·5H<sub>2</sub>O (Hpic=picolinic acid). *Polyhedron*, 18: 2891-2897.
- [4] Clodfelder BJ, Chang C, Vincent JB. (2004). Absorption of the Biomimetic Chromium Cation Triqua-µ<sub>3</sub>-oxo-µ<sub>6</sub>-hexapropionatotrichromium(III) in Rats. *Biol. Trace Elem. Res.*, 98: 159-169.
- [5] Earnshaw A, Figgis BN, Lewis J. (1966). Chemistry of polynuclear compounds. Part VI. Magnetic properties of trimer chromium and iron carboxylates. *J. Chem. Soc.*, A: 1656-1663.
- [6] Evans GW, Pouchnik DJ. (1993). Composition and biological activity of chromium-pyridine carboxylate complexes. *J. Inorg. Biochem.*, 49: 177-187.
- [7] Kingry KF, Royer AC, Vincent JB. (1998). Nuclear magnetic resonance studies of chromium(III) pyridinecarboxylate complexes. *J. Inorg. Biochem.*, 72: 79-88.
- [8] Nissen SL, Sharp RL. (2003). Effect of dietary supplements on lean mass and strength gains with resistance exercise: a meta-analysis. *J. Appl. Physiol.*, 94: 651-659.

- [9] Olin KL, Stearns DM, Armstrong WH, et al. (1994). Comparative retention/absorption of  $^{51}\text{Cr}$  chromium ( $^{51}\text{Cr}$ ) from  $^{51}\text{Cr}$  Chloride,  $^{51}\text{Cr}$  nicotinate and  $^{51}\text{Cr}$  picolinate in a rat model. Trace Elements Electrolytes, 11: 182-186.
- [10] Pittler MH, Stevinson C, Ernst E. (2003). Chromium picolinate for reducing body weight: meta-analysis of randomized trials. Int. J. Obes., 27: 522-529.
- [11] Shute AA, Vincent JB. (2002). The fate of the biomimetic cation triaqua- $\mu$ -oxohexapropionatotri-chromium(III) in rats. J. Inorg. Biochem., 89: 272-278.
- [12] Sun Y, Mallya K, Ramirez J, et al. (1999). The biomimetic  $[\text{Cr}_3\text{O}(\text{O}_2\text{CCH}_2\text{CH}_3)_6(\text{H}_2\text{O})_3]^+$  decreases plasma insulin, cholesterol and triglycerides in healthy and type II diabetic rats but not type I diabetic rats. J. Biol. Inorg. Chem., 4: 838-845.
- [13] Trumbo P, Yates AA, Schlicker S, et al. (2001). Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. J. Am. Diet. Assoc., 101: 294-301.
- [14] Vincent JB. (2001). The bioinorganic chemistry of chromium(III). Polyhedron, 20: 1-26.
- [15] Vincent JB. (2003). The potential value and potential toxicity of chromium picolinate as a nutritional supplement, weight loss agent, and muscle development agent. Sports Med., 33: 213-230.

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