Determination of Manganese in Tri Calcium Phosphate (TCP) by Atomic Absorption Spectrometry

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Abstract

Tricalcium phosphate Anhydrous Powder typically contains less than 10 ppm (mg/Kg) (w/w) manganese. This level can be determined utilizing Flame Atomic Absorption Spectrometer (AAS) and standard based on known standards. A number of analytical methods are presently used for the analysis of metals in the biochemical. The instrumental techniques available are Inductively Coupled Plasma (ICP/MS), X-Ray Fluorescence, UV-VIS Spectrophotometry, and Atomic Absorption Spectrometry. Flame AA has gained widespread acceptance as an analytical technique and is used for many applications. In this study, we have determined the amount of manganese metal present in Tricalcium Phosphate (TCP) using Flame Atomic Absorption Spectrophotometer. The method has high precision and accuracy. The percent recovery was found to be 99.8% for spiked sample. The results meet the requirement.

1. Introduction

Manganese (Mn) is associated with iron in many minerals. It is a common ingredient of alloy as well as iron and steel. It is present in small amounts in many biological samples [1]. Eating a small amount of manganese each day is important in maintaining your health. Too much manganese, however, can cause se-

rious illness. Studies in animals have shown that very high levels of manganese in food or water can cause changes in the brain [2].

Tri-calcium phosphate (TCP) is a calcium salt of phosphoric acid with the chemical formula $Ca_3(PO_4)_2$. It is known as tri-basic calcium phosphate, calcium orthophosphate, tert-calcium phosphate and bone phosphate of lime. Tri-calcium phosphate salt occurs naturally in several forms, including as a rock, in milk, in skeletons and teeth of animals and found in many nutritional supplements. Calcium phosphate rocks have a content of 30% to 40% P_2O_5 in weight. The human body needs phosphorus as well as calcium, and tri-calcium phosphate supplies both [2]. However, tricalcium phosphate is a concentrated source of calcium and taking too much can cause high calcium levels of *hypercalcemia*. High levels of calcium can cause; constipation, nausea, vomiting, stomach pain, muscle pain, weakness, excessive urination. It may also cause loss of appetite, thirst, dehydration, fatigue, and mental confusion. Tricalcium is commonly used in dental powders, household items, including baby powder, toothpaste, and medically as an antacid or calcium supplement.

2. Materials and Method

2.1. Reagents

- Certified 1000 mg/L in 5% HCl, Atomic Absorption Standard Manganese Solution, Alfa Aesar.
- Stock Standard Solution for Quality Control (QC): Manganese, 1000 mg/L. Dissolve 1.000 g of manganese metal in a minimum volume of (1 + 1) HNO_3. Dilute to 1 liter with 1% (v/v) HCl.
- Deionized doubly distilled water.
- Analytical grade concentrated hydrochloric acid (HCl), and tri-calcium phosphate (TCP) were used.
- Tricalcium Phosphate (TCP) was obtained from Morre-Tec Industries, Analytical Grade.

2.2. Equipment/Instruments

- Perkin Elmer 5100 PC Atomic Absorption Spectrometer with Manganese lamp and 2 inches Nitrous Oxide/Acetylene burner head.
- Lamps: Mn element hallow cathode lamp.
- Glassware: All glassware is washed in the following sequence: Alconox detergent solution, tap water, 1:1 nitric acid, tap water, 1:1 hydrochloric acid, tap water, and final rince with deionized water.
- Pipettes: 5, 10, 20, 50, 100, 200, 500, and 1000 microliters (μL) pipets with disposable tips are used.

Instrument/Operating Parameters

Blank: 100 mL of D.I. water is taken through the digestion process.

Duplicate: A separate aliquot of random sample is digested or analysis (approximately (10%).

Spike: Using the formula $C_1V_1 = C_2V_2$ an appropriate volume and concentration of standards is brought through spiked into a sample and the digestion procedure.

Instruments: Perkin-Elmer 5100 PC Atomic Absorption Spectrophotometer

Start program: Perkin-Elmer 5100 PC Atomic Absorption Spectrum

Wavelength: 403.1 nm-UV

Slit width: 0.2 nm

Relative sensitivity: 1.0 μg/mL

Lamp energy: 67.0

Lamp current: 20 mA

Light source: Hallow Cathode Lamp

Burner Head: Nitrous oxide/Acetylene

Type of Flame: Nitrous oxide/Acetylene flame

Integration Time: 1.5 seconds (for optimizing only)

Average Readings: 3

2.3. Solutons

1) Standard Preparation: 1000 mg/L manganese stock solution.

The first consideration is preparing a set of standard is to stay within the linear range of the instrument, if a linear calibration is to be used. Refer to table, column labeled "Linear Range". That is the highest standard to be used for s linear range. Do not make standard lower than the column sensitivity. Prepare three to five standards covering the range. Pipet 25 mL of 1000 ppm (mg/L) Certified Manganese Standard Solution into a 250 mL volumetric flask. Add 10 mL of concentrated HCl. Dilute to volume with deionized water and mix well [3]. Another consideration is preparing standards is added components.

Prepare AA standards for Manganese of 0, 0.2, 0.5, 1.0, 2.0, 5.0 mg/L with the matrix solution 1 mL 6N HCl per 100 mL solution.

2) Stock and Serial Manganese Standards for Quality Control (QC): Manganese, 1000 mg/L. Dissolve 1.000 g of manganese metal in a minimum volume of $(1 + 1)$ HNO_3. Dilute to 1 liter with 1% (v/v) HCl

3) Calcium Matrix Solution, 6000 mg/Kg For the Mn test, 1.0 g sample is used in 100 mL, and standard is prepared with nearly 6000 mg/L Ca. The sample concentration here is higher by a factor of about ten times. Accurately weigh 15 g of Calcium Carbonate and transfer to a 1000 mL volumetric flask. Add approximately 100 mL deionized water and swirl to form slurry. Add concentrated. HCl dropwise to affect dissolution. Allow the CO_2 escape before subsequent additions of HCl to prevent the solution from bubbling out of the flask. When the calcium carbonate completely dissolves and the solution clears, dilute to volume with deionized water and mix well [4].

4) Sample Preparation/Acid Digestion:

Analytically weigh 1.0 g grams of tri-calcium phosphate and transfer to a 100 mL volumetric flask. Add approximately 25 mL of DI water and swirl to form a slurry. Then add 10 mL of concentrated HCl and swirl to dissolve. Adding acid

to dry product can cause coagulation of the product and impede dissolution. Dilute to volume with DI water and mix well. The sample prep for determination of Mn by AA is based on the USP monograph for tricalcium phosphate, sample prep for heavy metals test [5].

3. Procedure

1) In a 50 mL beaker with glass bead weigh 2.5 g sample to the nearest 0.1 mg. add 18 mL 3N HCl and heat to boiling. Cool to room temperature, quantitatively transfer to 25 mL volumetric flask, and dilute to mark with water.

2) Prepare AA standards for Manganese of 0, 0.2, 0.5, 1.0, 2.0, 5.0 mg/L with the matrix solution 10.72 g calcium chloride and 1 mL 6N HCl per 100 mL solution.

3) Run AA for Manganese as follows. Blank the instrument with water. Run water then a standard. Run water then a sample. Continue until all standards and samples have been measured.

4) Subtract average water signal from each standard reading. Construct a calibration curve and report slope, intercept, and correlation coefficient.

5) Subtract average water signal from each sample reading. Calculate concentration using calibration slope and intercept.

6) Calculate mg/kg using the concentration in the mass taken, and the volume of solution.

4. Experimental

4.1. Contamination Control

All reagent were of a high purity grade. Eppendorf pipettes with plastic tips were used for all pipetting. All sample handling operations were conduct under a laminar flow hood to reduce atmospheric contamination.

4.2. Calibration Standards

The aqueous calibration standards were prepared in pre-cleaned 100-mL volumetric flasks using glass pipettes and fixed-volume Effendorf pipette for volume transfers (Table 1 and Table 2). These standards represent the stock calibration standards were prepared.

Table 1. Serial of Mn standards from 1000 mg/L Mn stock solution.

Working Std. Sol (mL)	Dilute to (mL)	Concentration of Mn Standards mg/L
0.00	100	0.00
0.02	100	0.02
0.05	100	0.50
0,10	100	1.00
0.50	100	5.00

Table 2. Absorbance of Mn standards.

Standard Concentration (mg/L)	DF	Absorbance	Blank-corrected Absorbance
Air		0.0000	
Water	1	0.0002	
Blank	1	0.0006	
0.20	1	0.0107	0.0092
0.50	1	0.0263	0.0244
1.00	1	0.0505	0.0481
5.00	1	0.2314	0.2274

4.3. Background

The technique of atomic absorption spectrometry (AAS) depends on generating a cloud of free atoms in the ground or unexcited state. The first AA spectrometers used a flame to generate atoms of the element to be determined [5]. The most common method of background correction in atomic absorption (AA) spectrophotometry uses a continuum source such as a deuterium lamp to measure the background. Background is electronically subtracted from total absorbance to give background corrected atomic absorbance [6].

4.4. Calibration and Operation

Before operation the calibration of the instrument is necessary. Rinse aspirator tube with D.I. water. Aspirate sample, ensure within range (between blank and highest standard). Dilute sample if necessary, and repeat for each sample.

Protocol:

Blank (purpose of this second blank measurement is to "zero" the reading)

Water

Standard

Water

Sample

Water

Sample dup

Water

Matrix Spiked

Water

Matrix Spiked Duplicate

Water

5. Result and Discussion

Historically, we have matched the standard matrix to the sample's matrix. When

determining Mn in tricalcium phosphate, our Mn standards have calcium at concentrations calculated to equal those in the prepared sample solution. This may require some investigation for this reason: 1) if the reagent used for modifying the standard matrix contains, as a contaminant, traces of the element to be determined, this will artificially lower the result for the sample. 2) Therefore, use reagent of utmost purity for matrix matching standards to samples. Spike and recovery can be used to confirm that Mn can be determined accurately despite the fact that tricalcium phosphate sample contain PO_4^{3-}.

Table 2 Raw data, absorbance of Manganese standard solutions, and the blank-corrected absorbance calculated using Equation (1).

Table 3 Data used to plot the calibration curve graph, [Mn] concentration and blank-corrected absorbance; slope, intercept, and R^2 values for the line of best fit.

Table 4 Data of absorbance of sample solutions and spiked sample solutions, each analyzed in duplicate; concentration of Mn calculated using Equation (2) and (3).

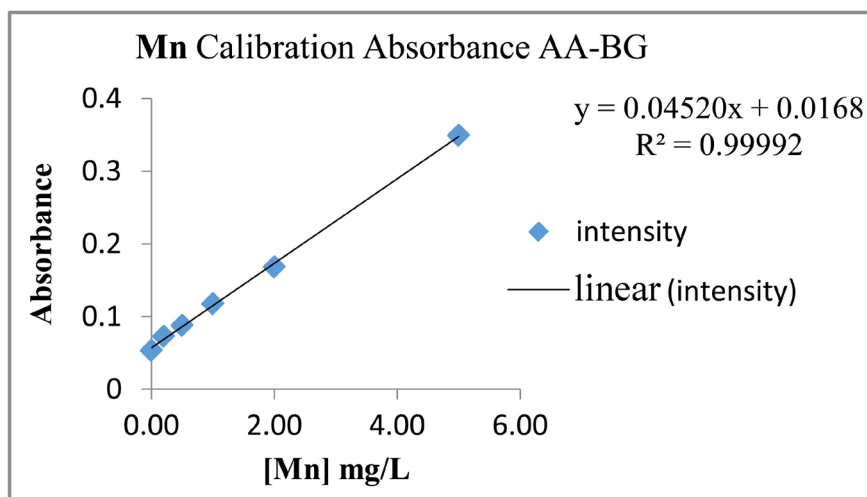Equation (1): Blank-corrected absorbance = Observed absorbance − Blank absorbance

Equation (2): [Mn] (mg/L) = (Blank-corrected Absorbance − 0.0059)/0.0005

Equation (3): [Mn] (mg/kg) = [Mn] (mg/L) * Final volume (mL)/Sample weight (g)

6. Conclusions

Perkin-Elmer 5100PC Atomic Absorption Spectrophotometer was used with a short part burner and nitrous oxide/acetylene flame optimized with hollow cathode lamp and a single element was measured. The background correction was selectable on an element by element basis. Water had been run between each reading; AA measurements were thus performed [7] [8].

The results of a single method for the detection of manganese in the tricalcium phosphate were obtained and are shown in Figure 1 and Tables 2-4.

Figure 1. Calibration curve of manganese linear plot of [Mn] concentration against blank-corrected absorbance at 403.1 nm.

Table 3. Mn calibration curve data.

[Mn] (mg/L)	Abs	m	0.04520
0.00	0.0002	b	0.00168
0.20	0.0092	R^2	0.99992
0.50	0.0244		
1.00	0.0481		
5.00	0.2274		

Table 4. Absorbance of sample and QA/QC study.

Sample	DF	Absorbance	Blank-corr Ab	[Mn] mg/L	W (g)	V (mL)	[Mn] mg/L
Water		0.0006					
Blank	1	0.0010	0.0004				
Water		0.0015					
0.2 mg/L	1	0.0107	0.0092				0.18
Water		0.0019					
0.5 mg/L	1	0.0263	0.0244				0.51
Water		0.0024					
1.0 mg/L	1	0.0505	0.0481				1.03
Water		0.0032					
2.0 mg/L	1	0.0967	0.0935				2.03
Water		0.0040					
5.0 mg/L	1	0.2314	0.2274				5.00
Water		0.0046					
Water		0.0046					
Sample	1	0.0257	0.0211	0.4297	1.0002	10	4.30
Water		0.0046					
Water		0.0046					
Sample-duplicate	1	0.0258	0.0211	0.4320	1.0002	10	4.32
Water		0.0047					
Spike	1	0.0484	0.0437	0.9298	1.0002	10	9.30
Water		0.0047					
Spike-duplicate	1	0.0484	0.0437	0.09298	1.0002	10	9.30
Water		0.0050					

Atomic Absorption Spectrophotometric determination of manganese in tricalcium phosphate (TCP) was found to be adequately sensitive in terms of linearity, repeatability, and accuracy. The determination of correlation coefficient (R^2) was found to be 0.99992. Concentration of spiking solution: 100 mg/L,

spiking solution: 0.5 mL, sample volume: 10 mL, spiked amount: 5.0 mg/L. Average percent matrix spike recovery (%R) was 99.8%, and relative percentage difference (%RPD) was 0.00%. The percent recovery was found to be 99.8% for spiked sample, and 99.8% for the duplicate spiked sample. The results were within the specification of 10 mg/Kg maximum, with the average concentration of Mn in sample found to be 4.31 mg/Kg. The results meet the requirement.

Acknowledgements

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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