



Full Length Research

## Method Validation for the Determination of the Concentration of Metals in Teff Samples

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**Abstract:** The validity of the method was assured by spiking the samples with a standard solution of known concentration of the target analytes. The calibration curves were prepared from standards of known concentration. Five working standards were used for the calibration curves corresponding to 0, 5, 10, 15 and 20 mg/L for Ca, Cu, Fe, Pb, Mn, Cd and Zn; 0, 1, 2, 3 and 4 mg/L for Al concentration levels. All the working standards of metals solution used for calibration curve exhibited very good linearity with squared regression coefficients ( $R^2$ ) values ranged from 0.9990 to 0.9999. The regression equations for each of the metals were plotted and were best fit equations. In all the cases, the regression coefficients ( $R^2$ ) were found to be in the acceptable linear range. The method detection limits were low enough ( $\leq 0.56$  mg/kg) to detect the concentrations of the metals in the samples at a trace levels using microwave plasma atomic emission spectroscopy. From the stock solution of 1000 ppm, 119.5  $\mu$ L of Fe, 43.5  $\mu$ L of Ca and 15.3  $\mu$ L of Zn were added to 0.5 g of teff sample. For Cu an intermediate standard solution of 100 ppm was prepared and 30  $\mu$ L of Cu was added to 0.5 g of teff sample. For Pb and Cd intermediate standard solutions of 10 ppm were prepared and 63  $\mu$ L of Pb and 45  $\mu$ L of Cd were added to 0.5 g of teff sample. The spiked and non-spiked samples were digested and analyzed in similar condition using the optimized procedure. Six metals were analyzed in triplicate standard metal solutions to evaluate the efficiency of the method and the percentage recoveries lies within the range from 92 to 104%, and were in the acceptable range of  $100 \pm 10\%$ .

**Keywords:** Detection Limits: Metals: Method Validation: Recovery: Spiking: Ethiopia.

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## **1.0 Introduction of the Study**

Recovery is one of the most commonly used techniques utilized for validation of the analytical results and evaluating how far the method is acceptable for its intended purpose. Spike recoveries is one of the most important quality assessment tools of known addition of analyte to a method blank, a field blank or a sample. To determine the a spike recovery, the blank or sample is split into two portions and a known amount of a standard solution of analyte is added to one portion. The concentration of the analyte is determined for both the spiked and unspiked portions, and the percent recovery (%R) is calculated. The limit of detection (LOD) is a measure of how sensitive the analytical method is and is the lowest concentration or weight of analyte that can be measured at a specific confidence level. For the determination of the limit of detection of the analytical method (LOD), replicate blanks must be prepared in parallel to the metal of the interest. In order to determine the method detection limit, the standard deviations (SD) of replicate blanks is calculated and multiplied by three ( $LOD = 3SD_b$ ). The limit of quantification (LOQ) is the smallest quantity of analyte that can be measured with acceptable accuracy and precision and is described as ten times of the standard deviation ( $LOQ = 10SD_b$ ).

Teff is the smallest cereal grain with an average length of around 1 mm (Adebowale et al., 2011). The minuteness of teff grains has nutritional and technological implications. For instance, as teff grains are difficult to decorticate, the cereal is consumed as a wholegrain, improving nutrient intake for consumers. Recently there is a growing interest in teff grain utilization because of nutritional merits and free of the protein gluten that make teff an increasingly important dietary component for individuals who suffer from gluten intolerance or celiac disease (Boka et al., 2013). With respect to soil contamination, teff is extremely exposed and complete removal of the extrinsic Fe from teff is not possible (Baye et al., 2013). Minerals are present in foods at low but variable concentrations and in multiple chemical forms. The role of minerals in food is to provide a reliable source of essential nutrients in a balanced and bio-available form. In cases where concentration and bio-availabilities in food supply are low, fortification has been popular. The mineral contamination of teff is probably due to its small size and suggests increased contact with soil over a larger area (Baye et al., 2014). The contamination of cereal grains in Ethiopia particularly in teff has often been associated with traditional methods of threshing grain under the hooves of cattle. Traditional threshing led to 30-38% increase in Fe content mainly due to soil contamination.

Iron is necessary for red blood cells formation and required for oxygen transport throughout the body. Calcium is essential for developing and maintaining healthy bones and teeth, assists in blood clotting, muscle contraction, nerve transmission and oxygen transport. Zinc is essential part of more than 200 enzymes included in the digestion, metabolism and reproduction and wound healing. It plays critical role in immune response and is an important antioxidant. Copper is both an essential nutrient and a drinking-water contaminant. It has many commercial uses. Copper does not appear to be a cumulative toxic hazard for man, except for individuals suffering from Wilson's disease. Copper is not considered to be mutagenic, carcinogenic or affect reproduction. Teratogenicity/embryo toxicity is observed in some animal studies (FAO/WHO, 2011). Copper is essential to normal red blood cells formation and connective tissue formation. Manganese is a key component of enzymes systems, support brain function and is required for blood sugar regulation. Manganese is naturally occurring in many surface water and groundwater sources, particularly in anaerobic or low oxidation conditions and this is the most important source for drinking-water. The greatest exposure to manganese is usually from food (WHO, 2003).

## 2.0 Analytical Procedures

The validity of the digestion procedures were assured by spiking the samples with a standard solution of known concentration of the target analytes. From the stock solution of 1000 ppm, 119.5  $\mu\text{L}$  of Fe, 43.5  $\mu\text{L}$  of Ca and 15.3  $\mu\text{L}$  of Zn were added to 0.5 g of teff sample. For Cu an intermediate standard solution of 100 ppm was prepared and 30  $\mu\text{L}$  of Cu was added to 0.5 g of teff sample. For Pb and Cd an intermediate standard solutions of 10 ppm were prepared and 63  $\mu\text{L}$  of Pb and 45  $\mu\text{L}$  of Cd were added to 0.5 g of teff sample. The spiked and non-spiked samples were digested and analyzed in similar condition using the optimized procedure. For the determination of limit of detection of the analytical method (LOD), triplicate eight blanks were prepared in parallel and analyzed for their metal contents. The standard deviation (SD) of the eight blanks was calculated and multiplied by three ( $\text{LOD} = 3\text{SD}_b$ ) to determine the method detection limit. The limit of quantification (LOQ) for each blanks were described as ten times of the standard deviation ( $\text{LOQ} = 10\text{SD}_b$ ).

## 3.0 Results and discussion

As shown in Table 1, calibration curves were prepared and equations were displayed for each elements using an appropriate standards at a series of concentrations. The calibration curves were prepared from the standards of known concentration covering the concentration range expected in the sample. The calibration curves were established at five concentration levels corresponding to 0, 5, 10, 15 and 20 mg/L for Ca, Cu, Fe, Pb, Mn, Cd and Zn; 0, 1, 2, 3 and 4 mg/L for Al. All the working standards of metals solution used for calibration curve exhibited very good linearity with squared regression coefficients ( $R^2$ ) values ranged from 0.9990 to 0.9999. The regression equations for each of the metals were plotted and the best fit of the equation was checked using correlation coefficient ( $R^2$ ). In all the cases, the regression coefficients ( $R^2$ ) were found to be above the acceptable linear range value of 0.999. Both the limit of detection and limit of quantification were smaller than the concentrations of the metals obtained. The method detection limits were low enough ( $\leq 0.56$  mg/kg) to detect the concentrations of the metals in the samples at a trace levels.

Table 1: The wavelength, method detection and quantification limit, correlation coefficient and calibration curve equations.

Metal	Wavelength (nm)	<sup>1</sup> MDL (mg/kg)	<sup>2</sup> MQL (mg/kg)	Correlation coefficient	Calibration curve equation*
Al	396.12	0.165	0.50	0.9990	$I = 17002C - 25.00$
Ca	422.673	0.556	1.90	0.9992	$I = 68218C + 8746.00$
Cd	228.802	0.022	0.10	0.9996	$I = 7863C + 733.90$
Cu	324.754	0.399	1.30	0.9998	$I = 10654C + 9283.00$
Fe	371.993	0.043	0.10	0.9999	$I = 5032C + 81.40$
Pb	405.781	0.057	0.20	0.9997	$I = 2889C + 11.22$
Mn	403.076	0.149	0.50	0.9999	$I = 27752C - 255.50$
Zn	213.857	0.221	0.70	0.9990	$I = 2448C - 198.70$

\*I is the intensity and C is the concentration. <sup>1</sup>Method detection limit, <sup>2</sup>Method quantification limit.

As shown in Table 2 six metals were analyzed in triplicate standard metal solutions to evaluate the efficiency of the procedure and the percentage recoveries lies within the range from 92 to 104%, and was calculated using the following formula.

$$\% \text{ Recovery} = \frac{\text{spiked sample} - \text{unspiked sample}}{\text{amount added}} \times 100$$

Table 2: Recovery Tests

Metal	Concentration in sample (mg/kg)	% Spiking	Amount added (mg/kg)	Spiked sample (mg/kg)	Recovery (%)
Ca	348 ± 0.5	25	87	428 ± 2.0	92 ± 3.0
Cd	1.8 ± 0.1	50	0.9	2.73 ± 0.1	103 ± 1.0
Cu	15 ± 0.4	40	6	20.7 ± 0.3	95 ± 0.5
Fe	1195 ± 1.0	20	239	1417 ± 0.8	93 ± 4.0
Pb	2.8 ± 0.1	45	1.26	4.11 ± 0.5	104 ± 2.0
Zn	102 ± 2.0	30	30.6	132 ± 1.0	98 ± 0.6

#### 4.0 Conclusions of the Study

The validity of the method was assured by spiking the samples with a standard solution of known concentration of the target analytes. The calibration curves were prepared from standards of known concentration covering the concentration range expected in the sample. Then, the curves were established at five concentration levels corresponding to 0, 5, 10, 15 and 20 mg/L for Ca, Cu, Fe, Pb, Mn, Cd and Zn; 0, 1, 2, 3 and 4 mg/L for Al. All the working standards of metals solution used for calibration curve exhibited very good linearity with squared regression coefficients ( $R^2$ ) values ranged from 0.9990 to 0.9999. The regression equations for each of the metals were plotted and were best fit equations. In all the cases, the regression coefficients ( $R^2$ ) were found to be in the acceptable linear range. The method detection limits were low enough ( $\leq 0.56$  mg/kg) to detect the concentrations of the metals in the samples at a trace levels. Six metals were analyzed in triplicate standard metal solutions to evaluate the efficiency of the method and the percentage recoveries lies within the range from 92 to 104%, and were in the acceptable range of  $100 \pm 10\%$ .

#### 5.0 References of the Study

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