

Adjusting Acid Digestion Procedure to Determine the Concentration Levels of Essential Metals in Teff Samples

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Abstract: An efficient and cost effective method was developed and optimized for the digestion of Fe, Ca, Cu, Zn, Mn, Al, Cd and Pb in teff samples by using MP-AES. The effect of the digestion media, digestion time and digestion temperature on the efficiency of extraction of metals from the teff samples was examined. The levels of the metals in the Teff samples were determined for the samples collected from three selected areas (Bure, Debre Markos and Bahir Dar) of Ethiopia. Concentrated HNO₃ and HClO₄ were determined to be the most efficient digestion media for all the metals studied. After proper sample pretreatment, 5 mL HNO₃: 1 mL HClO₄, 240°C and 2:30 h were the optimized digestion conditions. Then using the optimized conditions sample preparation was made and levels of metals were determined by micro-plasma atomic absorption spectroscopy (MP-AES). The optimized conditions in this study were economical in terms of time and reagent volumes used compared to other authentic reports. The accuracy of the optimized procedure was evaluated by analyzing the digest of the spiked samples with standard solution and the percentage recoveries varied from 92% to 104%, which is good and was in the acceptable range of 90% ± 10.

Keywords: Acid Digestion, Determination, Metals, Optimization, Teff Sample

1. Introduction

Wet acid digestion is one of the methods that are involved to get free metal ions in dissolved form complex organic matrix based on changing different digestion parameters like volume ratio of reagents added, digestion temperature and duration of time [13]. One of the wet acid digestions can be carried out by Kjeldahl apparatus in which organic components are assumed to decompose in the form of different gaseous forms and other metallic elements are left in the solution except those easily volatile metals like Hg [14]. Moreover it is assumed that digestion is assumed to be complete if the solution is clear and colorless [5].

MP-AES consists of microwave induced plasma interfaced to an atomic emission spectrophotometer (AES). It is used for simultaneous multi-analyte determination of major and minor elements. MP-AES employs microwave energy to produce a plasma discharge using nitrogen supplied from a gas cylinder or extracted from ambient air, which eliminates the need for sourcing gases in remote locations or foreign countries [2, 15]. Samples are typically nebulized prior to

interaction with the plasma in MP-AES measurements. The atomized sample passes through the plasma and electrons are promoted to the excited state. The light emitted electrons return to the ground state light is separated into a spectrum and the intensity of each emission line measured at the detector. Most commonly determined elements can be measured with a working range of low part per million (ppm) to weight percent. MP-AES is a technique comparable to traditional AA and AES but with several potential advantages including lower cost of operation and elimination of the requirement for flammable gasses. MP-AES instruments are bench top instruments. While the technique is mature, there are a limited number of manufacturers supplying commercial MP-AES instruments [2].

2. Research Methodologies

2.1. Chemical Reagents and Materials

All measurements were performed using an Agilent 4200 MP-AES (USA), with nitrogen supplied from an Agilent 4107

nitrogen generator. The sample introduction system consisted of a micro mist nebulizer and double-pass glass cyclonic spray chamber. An external gas control module (EGCM) accessory and auto sampler were used. The MP-AES was controlled using the intuitive MP Expert software, which recommends wavelengths for the selected elements and automatically sets the nebulizer flow rate and EGCM settings. Auto background

correction was used to resolve the element emission line from the organic matrix. The instrument operating conditions are shown in Table 1. The sample introduction system consisted of PVC peristaltic pump tubing, a single-pass glass cyclonic spray chamber and the One Neb nebulizer. The Agilent MP Expert software was used to automatically subtract the background signal from the analytical signal.

Table 1. Agilent 4200 MP-AES operating conditions.

Parameters	Fe	Ca	Cu	Zn	Mn	Al	Cd	Pb
Wavelength (nm)	372.0	422.7	324.8	213.9	403.1	396.1	228.8	405.8
Background correction	Auto	Auto	Auto	Auto	Auto	Auto	Auto	Auto
EGCM setting	High	High	High	High	High	High	High	High
Replicates	3	3	3	3	3	3	3	3
Pump speed (rpm)	15	15	15	15	15	15	15	15
Blank subtraction	On	On	On	On	On	On	On	On
Stabilization time (s)	20	15	15	15	15	15	16	20
Sample uptake time (s)	30	27	25	25	25	30	30	30
Sample uptake fast pump	On	On	On	On	On	On	On	On
Rinse time (s)	10	10	10	10	10	10	10	10
Read time (s)	3	3	3	3	3	3	3	3
Nebulizer flow (L/min)	0.65	0.6	0.7	0.95	0.9	0.95	0.5	0.75

All chemicals used in this study were of analytical grade reagents. Perchloric acid (70%) and nitric acid (69-72%) were used (Sigma Aldrich Steinleim, Germany). The reference standards of the metals under study were the products of Perkin Elmer. The stock standard solutions 1000 mg L⁻¹ were prepared from the nitrate salts of the metals. The working standard solutions of the selected metals were prepared freshly from the intermediated standard solutions (100 mg L⁻¹) which was obtained by diluting stock standard solutions.

The materials used in the laboratory were sample preparation utilized PVC flasks, polyethylene conical flasks, filter paper, 50 mL volumetric beakers for sample and solution preparation, round bottom flask, ceramic mortar and pestle (USA) for grinding and homogenizing the samples, digital analytical balance (four digit) and Kjeldahl technique (England) for the purpose of digestion. All the glassware used were first kept overnight in a 10% HCl solution and then repeatedly washed with distilled water and dried in an oven for 24 h before use.

2.2. Optimization of Digestion Procedure

Different digestion procedures were carried out for the teff samples using HNO₃ and HClO₄ acid mixtures by varying volume of the acid mixture, digestion time and digestion temperature [1]. Optimized procedures were selected based on the usage of lesser reagent volume, shorter digestion time and reasonable mild temperature for obtaining clear and colorless solutions of the resulting digests [13]. Based on this fact the optimized digestion conditions for the teff samples in this study were (5 mL HNO₃: 1 mL HClO₄) volume ratio of reagents, 240°C digestion temperature and 2:30 h digestion time. Compared to [6], the optimum conditions used in this digestion process were better both in digestion time and temperature. According to [6] 3:00 h and 300°C were the optimum conditions. The optimized condition in this study is also better than other different optimized conditions in terms of the reagents used. In this experiment only two acids

(HNO₃ and HClO₄) were used for digestion of the teff samples, but others used more. Therefore the optimized conditions in this study were economical in terms of time and reagent volumes used. The optimum conditions for digestion of the teff samples in this study are shown in Table 3.

2.3. Samples for Digestion

Applying the optimized conditions, 0.5 g of powdered teff samples were transferred into a 100 mL round bottom flask. Then 6 mL of a mixture of HNO₃ (69-72%) and HClO₄ (70%) with a volume ratio of 5:1 (v/v) was added and the mixture was digested on a Kjeldahl digestion apparatus fitted with a reflux condenser by setting the parameters temperature and time. The digest was allowed to cool to room temperature for 10 min without dismantling the condenser and for 10 min after removing the condenser. To the cooled solution 10 mL of distilled water was added to dissolve the precipitate formed on cooling and to minimize dissolution of filter paper by the digest residue while filtering with filter paper (Whatman 125 mm diameter, Germany) into 50 mL volumetric flask. The round bottom flask was rinsed subsequently with around 5 mL distilled water until the total volume reached around 40 mL. Then finally the solution was filled to the mark (50 mL) using distilled water. The digestion was carried out in triplicate for each sample. Digestion of the blank was also performed in parallel with the teff samples keeping all digestion parameters the same. Then the metal concentrations in the digested sample solutions were determined by using MP-AES [7].

2.4. Calibration Instruments

Calibration standard solutions were prepared for each of the metals which were prepared from the MP-AES standard stock solutions that contained 1000 mg L⁻¹. These intermediate standards were diluted with distilled water to obtain five

working standards for each metal of interest. Then Fe, Ca, Cu, Zn, Mn, Al, Cd and Pb were analyzed with MP-AES. Three replicate determinations were carried out on each sample. All the above listed metals were determined by emission/concentration mode and the instrument readout was recorded for each solution. The same analytical procedure was employed for the determination of elements in the digested blank solutions. The calibrated instrumental operating conditions

including its determined method detection limits are shown in Table 2. Five points calibration curve were established by running series of the prepared working standard solutions for each metal. Correlations coefficients obtained for the calibration curves were > 0.999 . After calibration the sample solutions were aspirated into the MP-AES instrument and readings of the elemental concentrations was recorded. Three replicate determinations were carried out on each sample.

Table 2. The wavelength, method detection and quantification limit, correlation coefficient and calibration curve equations.

Metals	Wavelength (nm)	IDL (mg/kg)	MDL ¹ (mg/kg)	MQL ² (mg/kg)	Correlation coefficient	Calibration curve equation*
Fe	372.0	0.10	0.40	1.30	0.9999	$I = 5032C + 81.40$
Ca	422.7	0.10	0.56	1.90	0.9992	$I = 68218C + 8746.00$
Cu	324.8	0.10	0.40	1.30	0.9998	$I = 10654C + 9283.00$
Zn	213.9	0.10	0.22	0.70	0.9990	$I = 2448C - 198.70$
Mn	403.1	0.10	0.15	0.50	0.9999	$I = 27752C - 255.50$
Al	396.1	0.10	0.17	0.50	0.9990	$I = 17002C - 25.00$
Cd	228.8	0.01	0.02	0.10	0.9996	$I = 7863C + 733.90$
Pb	405.8	0.01	0.06	0.20	0.9997	$I = 2889C + 11.22$

* I is the emission intensity and C is the concentration. IDL is the instrument detection limit, ¹Method detection limit, ²Method quantification limit.

2.5. Calibration Curves of Standards for Each Metal

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 Fe, Ca, Cu, Zn, Mn, Cd and Pb and 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.

2.6. Method of Detection and Quantification Limits

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 [8]. 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 ($LOD = 3SD_b$) to determine the method detection limit [9, 1]. The limit of quantification (LOQ) is the smallest quantity of analyte that can be measured with acceptable accuracy and precision and it is described as ten times of the standard deviation [10]. In this study the limit of detections for all of the eight elements in the teff samples were smaller than all the results obtained and are given in Table 2.

3. Results and Discussion

Calibration curve for each element was constructed using an appropriate standard at a series of concentrations. Regression equation for each metal was constructed and the best fit of the equation was checked using correlation coefficient (R^2). In all the cases, the regression coefficient (R^2) was found to be above the accepted linear range value of 0.999. As can be seen from the Table 2, the method detection limits are low (≤ 0.56 mg/kg) enough to determine the metals in the samples at trace levels. The calibration curves were with good correlation coefficients.

Table 3. Reagent ratios and volumes, temperature and time attempted during optimization of digestion of 0.5 g of teff sample.

Trials	Reagent volume (mL)			Temperature (°C)	Time (h)	Results
	HNO ₃	HClO ₄	Total			
1	1	1	2	240	2:30	Yellow with suspension
2	2	1	3	240	2:30	Cloudy yellow
3	3	1	4	240	2:30	Nearly colorless
4	4	1	5	240	2:30	Slightly colorless
5	5	1	6	240	2:30	Clear colorless*
6	6	1	7	240	2:30	Clear colorless
7	3	2	5	240	2:30	Slightly colorless
8	4	2	6	240	2:30	Nearly colorless
9	4	1	5	240	2:30	Nearly colorless
10	5	2	7	240	2:30	Clear colorless
11	5	1	6	240	0:30	Yellow with suspension
12	5	1	6	240	1:00	Yellow with suspension
13	5	1	6	240	1:30	Cloudy light yellow
14	5	1	6	240	2:00	Light yellow
15	5	1	6	240	2:30	Clear colorless*

Trials	Reagent volume (mL)			Temperature (°C)	Time (h)	Results
	HNO ₃	HClO ₄	Total			
16	5	1	6	240	3:00	Clear colorless
17	5	1	6	150	2:30	Cloudy yellow with suspension
18	5	1	6	180	2:30	Cloudy yellow with suspension
19	5	1	6	210	2:30	Slightly yellow
20	5	1	6	240	2:30	Clear colorless*
21	5	1	6	270	2:30	Clear colorless
22	5	1	6	300	2:30	Clear colorless

*The optimized conditions for the three parameters (reagents volume ratio, time and temperature).

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 [11]. Due to the absence of certified reference material (CRM) for the teff samples in the laboratory, 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 mg/L, 120 µL of Fe, 44 µL of Ca and 15 µL of Zn were added to 0.5 g of teff sample. For Cu intermediate standard solution of 100 mg/L was prepared and 30 µL of Cu was added to 0.5 g of teff sample. For Cd and Pb intermediate standard solutions of 10 mg/L were prepared and 45 µL of Cd and 63 µL of Pb 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. As shown in Table 4 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 were calculated using the following formula.

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

The suitable optimized conditions for each variable (digestion media, temperature and time) were selected for each

metal being analyzed. Due to the large number of teff samples to be analyzed the optimized conditions that gave the best metal recoveries and could be applied to the widest range of metals was desirable. Such a procedure would reduce analysis time as well as the quantity of chemicals to be used, which lowers risks and ensures cost effectiveness. The correlation coefficient in Table 2 for each metals shows that the change in emission with concentration is in good correlation. The experiment was conducted using two acid combinations while varying the digestion temperatures and times. The optimized digestion conditions for the teff samples in this study were (5 mL HNO₃: 1 mL HClO₄) volume ratio of reagents, 240°C digestion temperature and 2:30 h digestion time. The digested samples were clear and colorless which indicated there was complete digestion of the sample and therefore release of the bound heavy metals from the sample matrix. This finding was confirmed by the analysis of spiked recovery. A spiked recovery analysis was performed as a quality control to calculate the recovery of the analyte spike added to the sample prior to sample preparation [12] (Table 4). It was used to determine any matrix effects and sample preparation losses [3]. The spiked recoveries indicated that there were minimal losses through volatilization using nitric acid since the recoveries varied from a low of 92% to a high of 104%.

Table 4. Analytical results for recovery test of Teff sample.

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

4. Conclusions

From the recoveries of the spikes, (5 mL HNO₃: 1 mL HClO₄) volume ratio of reagents, 240°C digestion temperature and 2:30 h digestion time were the most efficient digestion protocol for the evaluation of Fe, Ca, Cu, Zn, Mn, Al, Cd and Pb. The optimum conditions used in this digestion process were better both in digestion time and temperature. The optimized condition in this study is also better than other different optimized conditions in terms of the reagents used. In this experiment only two acids (HNO₃ and HClO₄) were used for digestion of the teff samples, but others used more.

Therefore the optimized conditions in this study were economical in terms of time and reagent volumes used. This study proves that efficient digestion of teff sample can be achieved with minimal losses using simple controlled open acid digestions.

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