

Inductively coupled plasma-mass spectrometry versus flame atomic absorption spectrophotometry for the analysis of Fe, Cu, Zn, Mn and Cr in medicinal plants: a comparison study

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Abstract:

Analysis of essential and toxic elements is a vital analytical process that employs different analytical instruments depending on the procedures. This paper presents the results of a comparative study of inductively coupled plasma-mass spectrometry (ICP-MS) and flame atomic absorption spectrophotometry (FAAS) as quantification methods for analyzing the essential elements in the medicinal plants. The concentrations of iron (Fe), copper (Cu), chromium (Cr), manganese (Mn) and zinc (Zn) were quantified by using the ICP-MS and FAAS in nineteen medicinal plants from Nyamira County, Kenya and the results revealed that the two methods were statistically not different ($p < 0.05$). The detection limits (LODs) and quantification limits (LOQS) were between 0.0003 and 7.716 mg/kg for ICP-MS and 0.0011 and 25.7202 mg/kg for FAAS with the recovery of the standard reference material of 93.07 ± 4 to $97.04 \pm 4\%$ and 86.30 ± 7 and $90.37 \pm 4\%$ respectively the relative standard deviations (RSDs) were lower than 10%. The correlation coefficient values for the results of the elements were close to unit indicating very strong positive relationship between the element concentrations determined by the two methods. Though ICP-MS method has superior analytical characteristics in terms of sensitivity, bias, detection limit, precision and selectivity, it can be complimented by use of FAAS for quantification of the mineral elements in plants when dealing with few elements and fewer samples of plants, environmental and soils.

Keywords: Medicinal plant, Regression, Correlation analysis, Essential elements

1. Introduction

Different methods have been used in the analysis of essential and toxic elements in plant and soil samples. Most of the methods used in the analysis are very expensive to acquire and maintain for most researchers in the developing countries. There is need to assess the results obtained with different methods in order to elucidate their suitabilities in terms of cost, maintenance and the accuracy of the results. The criteria that can be used to decide whether a given

instrumental method is suitable for a given analysis include precision, bias, sensitivity detection limits and selectivity of the methods [1]. The precision of a given method is measured by the degree of agreement of the data that has been obtained from a given sample in the same way [2, 3]. It provides a measure of the random or indeterminate error of analysis and the numerical terms which express these characteristics are absolute standard deviation, relative standard deviation, standard deviation of the mean, coefficient of variation and variance [4, 5]. Mogwasi [6] reported that bias of a given

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method is determined by elucidating the mean of one or more reference materials whose analyte concentration and subsequently obtaining their differences. Sensitivity of a method has been defined as the ability to discriminate between small differences in the analyte concentration and can be determined from the slope of the calibration curve and the reproducibility or the precision of the method [1]. Therefore, the characteristics of the given method should be assessed in order to select a method to be used in a particular analysis.

Ji et al. using ICP-MS/MS method determined accurately 49 elements with the instrumental detection limits (LODs) of 0.0003 and 7.716mg/kg and quantification limits (LOQs) of 0.0011 and 25.7202mg/kg [4]. The method recovery was in the range of 85.82% to 104.98% while the relative standard deviations (RSDs) were lower than 10% and they revealed that the content of multi-element in the discriminant and cultivation models of *P. notoginseng* were accurately determined by the neural networks (prediction accuracy was 0.9259 and area under ROC curve was 0.9750) and the support vector machine algorithm. Alqadami et al. used microwave digestion and inductively coupled plasma atomic emission spectrometry method to determine cadmium (Cd), bismuth (Bi), mercury (Hg), titanium (Ti), lead (Pb) and metalloid arsenic (As) in skin-whitening cosmetics revealing the excellent quality parameters of detection limits, As (4.6 ppb), Bi (7.9 ppb), Cd (0.45 ppb), Hg (3.3 ppb), Pb (3.8 ppb), Ti (4.3 ppb), linearity ($r^2 > 0.999$) and run-to-run and day-to-day precisions with relative standard deviations of <3% and recovery rates for standard reference materials of between 90 and 105% [2]. Khan et al. determined manganese (Mn), cobalt (Co), nickel (Ni), zinc (Zn), arsenic (As), cadmium (Cd) and lead (Pb) in acrylic color paints commonly used by the school children using microwave digestion and iCAPQ inductively coupled plasma mass spectrometry (ICP-MS) system and revealed that their concentrations ranged from 0.05 to 372.59 $\mu\text{g/g}$ [5]. Azam et al. further used AAS to effectively determine the levels of Cu (II) adsorbed by the adsorbent made from ajwa date pits [7]. Azam et al. determined trace levels of Pb (II) in various industrial wastewater effluents by optimized SPE and iCAP Q ICP/MS method which offered excellent validation conditions in terms of coefficient of determination (CoD, $R^2 > 0.999$), detection limit (DL, 0.011mg/L), quantification limit (QL, 0.034mg/L) and day-to-day precision (RSD <6%) [8]. Liu et al. reported the Pb²⁺ monitoring by N-methyl mesoporphyrin IX, which is highly specific to G-quadruplex [9]. The utilization of G-quadruplex specific dye and terminal-labeled fluorophore allowed a ratiometric signal outputs towards Pb²⁺, dramatically increasing the lead detection in a sample. DNAzyme assay has been reported to precisely detect Pb²⁺ in tap water, milk and fish to as low as 0.12 nM and ended a dynamic range from 0.1 nM to 30 nM [10].

Different methods have been used to determine the concentration of mineral elements in medicinal plants.

Mtunzi et al. reported the levels of Fe (iron), Cu and Zn in medicinal plant products in South African market to be 3.47+0.1 $\mu\text{g/g}$, 0.81+0.33 $\mu\text{g/g}$ and 2.71+0.30 $\mu\text{g/g}$ respectively by using AAS [11]. Lukose et al. used infrared technique to establish the bonding nature of ligands for complexes of Fe (III), Co (III) and Mn (III) which were bioactive against the *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Klebsiella pneumonia* [12]. Moradkhani et al. reported the cation chelating affinity with salvigenin to Fe (III) > Cu (II) > Zn (II) using UV-Visible spectroscopy [13]. Konieczynski et al. used FAAS and UV-visible spectrophotometer to determine the total iron and bioavailable Fe (II) levels in the medicinal plants to the human body while Konieczynski & Wesolowski used FAAS to report the levels of zinc and iron in several botanical species of medicinal plants of Poland [14,15]. Korfali et al. used EDXRF and AAS to report the concentrations of K (potassium), Ca (Calcium), Fe, Zn, Cu, Mn, Pb(lead), As (arsenic), Cd (cadmium) and Cr in medicinal herbs and infusion of Lebanon to be within the ranges recommended permissible tolerable levels [16]. Manzoor & Mahmood reported that some Pakistan herbal plants to have excess essential elements while others had deficiency using ICP-MS [17]. Rathore and Upadhyay used atomic absorption spectrophotometer to report zinc concentrations in *Azadirachta indica*, *Moringa oleifera* and *Osmium sanctum* to be 32.6 $\mu\text{g/g}$, 21.3 $\mu\text{g/g}$ and 48.5 $\mu\text{g/g}$ respectively [18]. Sadia et al. reported the levels of essential elements to be higher as compared to the toxic elements in 24 common medicinal plants using atomic absorption spectroscopy [19]. Majolagbe et al. further used FAAS to determine the total concentration and bioavailability of iron in some selected blood-building medicinal plants of Nigeria and classified the medicinal plants as high iron source going by the WHO standards [20]. Mogwasi et al. (2019) reported on the comparison of aqueous and enzymatic extraction combination with sequential filtration for the profiling of selected trace elements in twelve medicinal plants from Kenya using ICP-MS while Mogwasi (2023) reported on the bio accessibility of Fe and Cu from seven Kenyan anti-anemia plants using FAAS [21, 22]. These implied that a number of methods are available for the determination of elements in plants and the characteristics of different methods should be elucidated to enable the most suitable one to be selected under the prevailing circumstances.

2. Materials and methods

2.1 Collection and storage of samples

The twelve anti-diabetic medicinal plants (*Plectranthus barbatus*, *Magnifera indica*, *Bidens Pilosa*, *Senna didymobotrya*, *Acacia abyssinica*, *Erythrina abyssinica*, *Vernonia auriculifera*, *Ultrica dioica*, *Solanum mauense*, *Tabernaemontana stapfiana*, *Acacia hockii* and *Solanum indicum*) [22] and seven anti-anemia medicinal plants

(Aloe Vera, *Carissa edulis*, *Croton macrostachyus*, *Clerodendrum myricoides*, *Melia azedrach*, *Toddalia asiatica* and *Warburgia ugandensis*) [22] were collected from forests in Borabu sub county of Kenya.

The plant samples collected were botanically identified, washed with deionized water to remove soil and other dust particles, placed in polythene paper, sealed, air-dried under shade and ground using a pestle and wooden mortar (for all the samples) to avoid contamination. The ground medicinal plants were placed in polythene paper, sealed and stored. This was done with ultimate care to ensure that there was no contamination during collection or storage, whereby each plant was collected and stored separately. Each sample was placed in a transparent polythene bag, labeled and stored in a cool dry area until it was analyzed.

2.2 Reagents, chemicals and apparatus

All the reagents used in the study were of analytical grade of merck, Suprapur®. Standard stock solutions of iron, chromium, manganese, copper and zinc were prepared from merck, titrisol solutions. They were diluted with ultra-pure deionized water (high purity deionized water) supplied by a Millipore-milli Q water to obtain the working solutions. The blank values for heavy metals in the ultra-pure water were 0.05µg/L Cu, 0.04µg/L Zn, 0.004µg/L Fe, 0.01µg/L Cr and 0.001µg/L Mn. The glassware was decontaminated by soaking overnight in 5% nitric acid and rinsing thoroughly with ultra-pure deionized (high purity deionized water) water several times after each use and dried overnight in an oven at 80°C.

2.3 Preparation of samples for essential element analysis by FAAS

1.0g of powdered each plant sample was weighed into a 125ml of Erlenmeyer flask which had been previously washed with 0.1 M HNO₃ and rinsed with distilled water. 4ml concentrated HClO₄, 25ml concentrated HNO₃ and 2ml concentrated H₂SO₄ (all chemicals of analytical grade) were added. The content was mixed and heated, initially at low temperature of 60°C and then on a hot plate under an acid fume-hood, the heating was continued until dense white fumes appeared. The mixture was strongly heated for half a minute afterward. The content was allowed to cool after heating. 50ml of distilled water was added after cooling and boiled further for half a minute on the hot plate. The solution was allowed to cool again and filtered using a Whatman No. 42 filter paper. The solution was made up to the 100ml mark with distilled water and analyzed for Fe, Zn, Mn, Cu and Cr using 210 VGP FAAS. Each sample was extracted in triplicate and analyzed to test for reproducibility. General laboratory quality assurance measures were observed to prevent sample contamination and instrumental errors.

2.4 Elemental analysis by ICP-MS

Determination of elemental contents in medicinal plant part (0.3g) was conducted in triplicate (by digesting the plant in triplicates) by adopting Mogwasi et al. [21] and Nischwitz et al. [24] techniques. Samples digested using closed vessel microwave system with nitric acid and hydrogen peroxide were supplied to ICP-MS multi element analyzer for analysis. Analysis was performed by ICP-MS using external calibration method with matrix-adapted standards of varying concentration for each metal. For selected samples the determined concentrations of the trace elements were compared with the results from standard addition to check for matrix effects. In addition, certified reference material was analyzed for quality control.

2.5 Data analysis

The binomial t-test was to compare the concentration of the elements determined by the two methods to establish the relationship of concentrations. Pearson correlation analysis was further used to determine whether the concentrations of the elements in the medicinal plants were statistically different. One-way Anova was used to determine the correlation between the levels of copper and iron within the plants of a given study area and between the three study areas. Statistical packages for social scientists (SPSS), version 23.0 (IBM-SPSS Inc., Chicago, IL, USA) was used in the analysis. An- α value of 0.05 was adopted as the critical level for all statistical testing giving a 95% confidence level.

3. Results and discussion

Iron, copper, zinc, manganese and chromium standard solutions were used to prepare graphs to compare the levels of the elements determined by ICP-MS versus FAAS (Figures 1-9). The absorbance measurements for the standards used were taken in triplicates. The regression parameters for the calibration curves were summarized in Table 1. The results obtained were validated using regression analysis. The intercepts were very close to zero, indicating that the lines passed near the origin and thus minimum matrix interference. Spearson's correlation factors r^2 were close to 0.999 and the points were on straight lines (Figures 1-5). The results indicated linearity within the concentrations used and therefore the regression equations can be used for quantification of the elements in the plant samples. Twenty standard solutions of Fe, Cu, Zn, Mn and Cr consisting of 10mg/kg were prepared and their absorbencies measured and used to determine the co efficiency of variance for each element. The values of co efficiency of variances were 0.035%, 0.105%, 0.403%, 0.653%, 0.425% and 0.0825% respectively indicating high reproducibility for the elements.

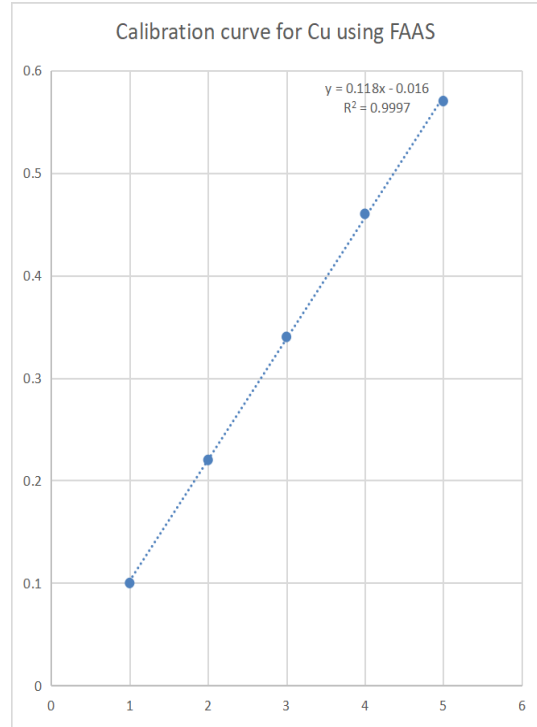
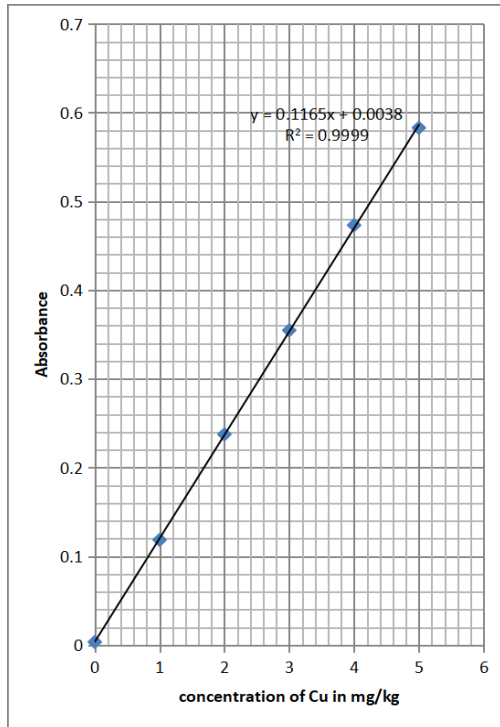


Figure 1. Calibration curve for Cu using ICP-MS

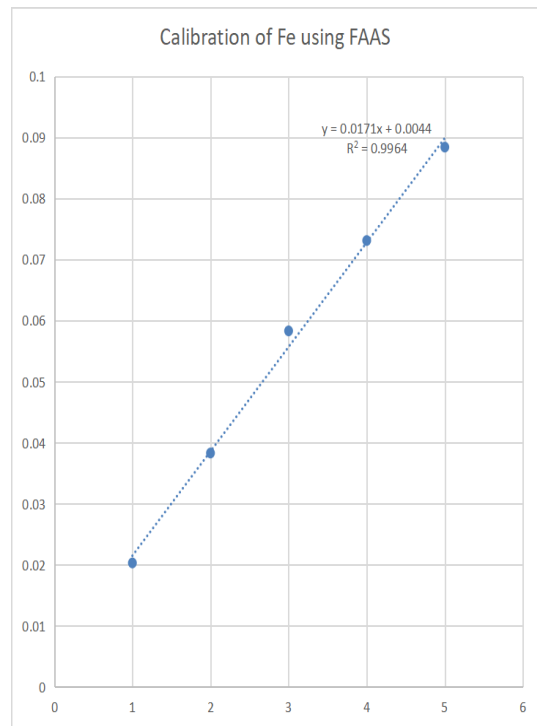
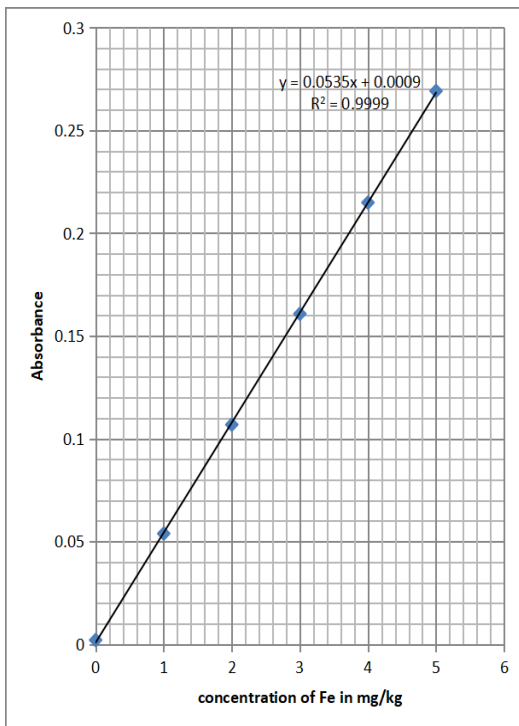


Figure 2. Calibration curve of Fe using ICP-MS

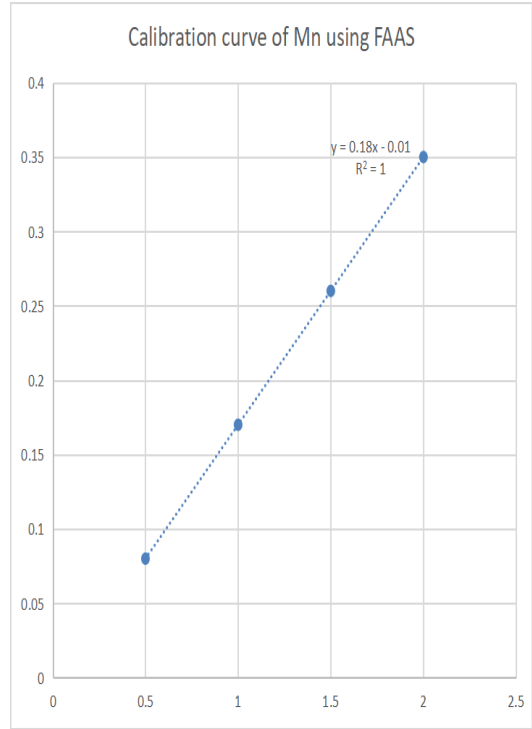
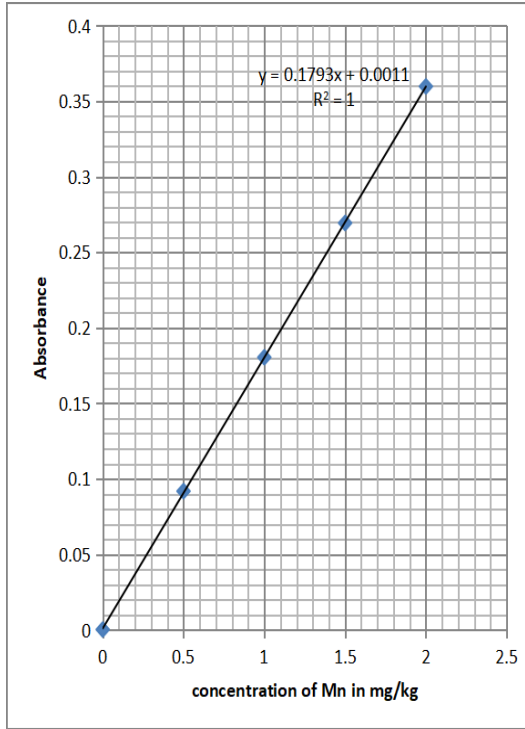


Figure 3. Calibration curve of Manganese using FAAS

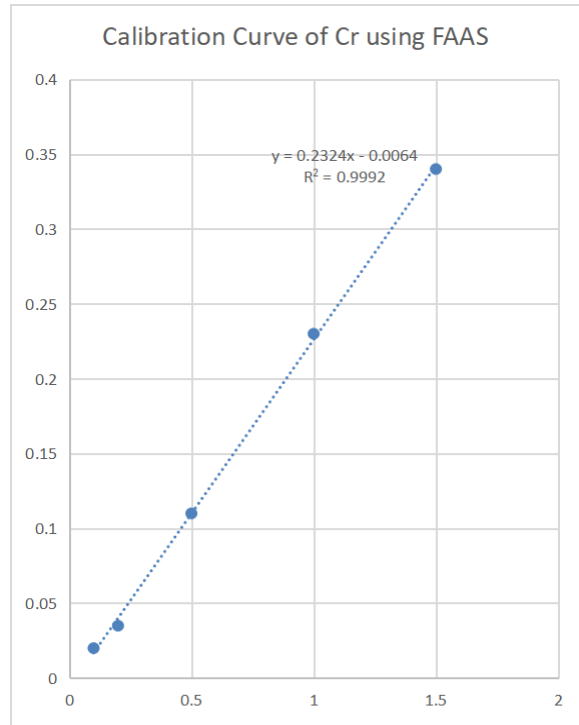
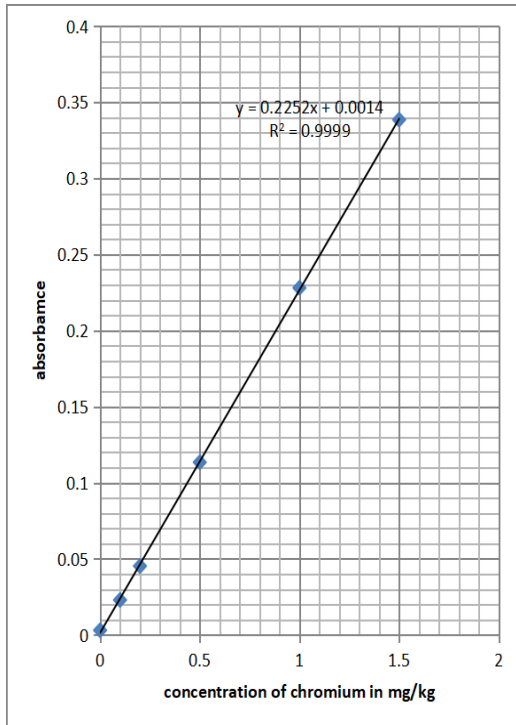


Figure 4. Calibration curve of Cr using ICP-MS

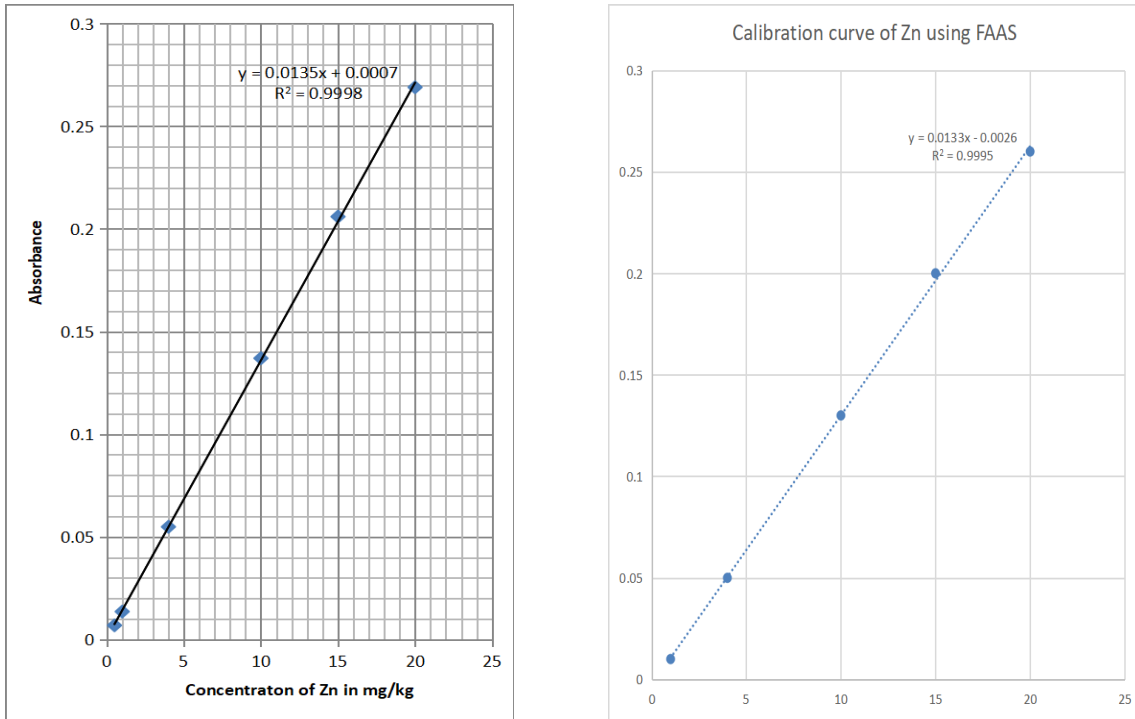


Figure 5. Calibration curve of Zn using ICP-MS

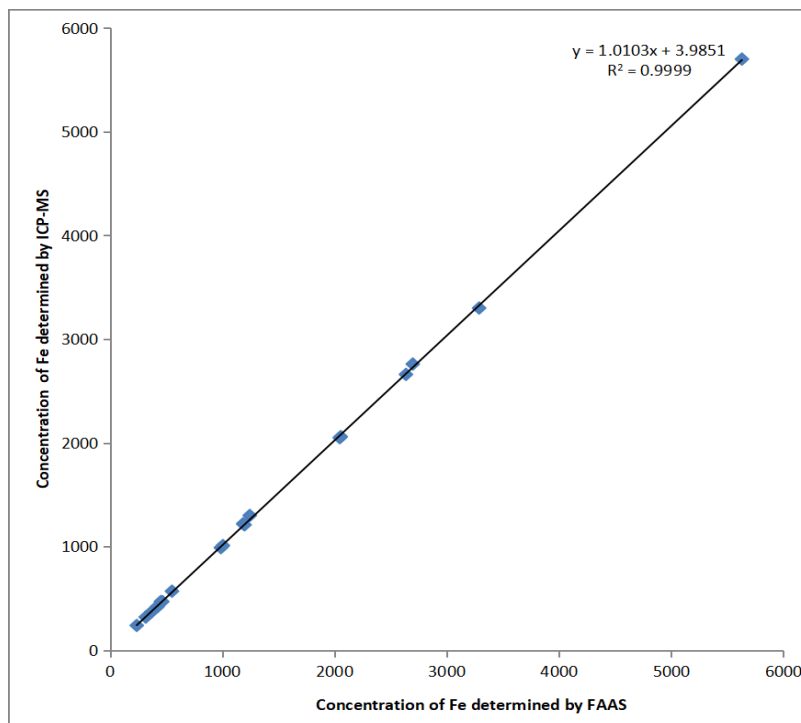


Figure 6. Concentration of Fe in Borabu medicinal plants determined by ICP-MS vs FAAS

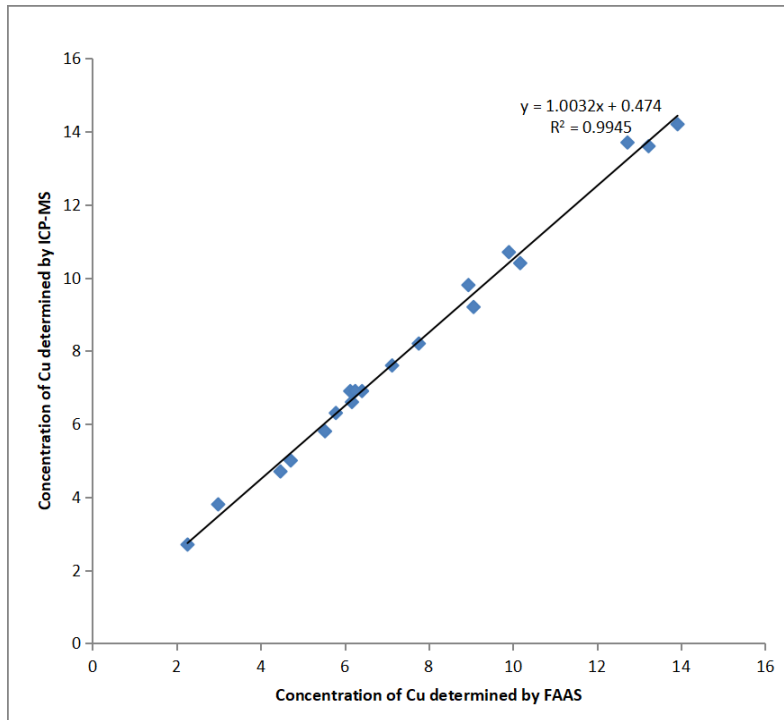


Figure 7. Concentration of Cu in Borabu medicinal plants determined by ICP-MS vs FAAS

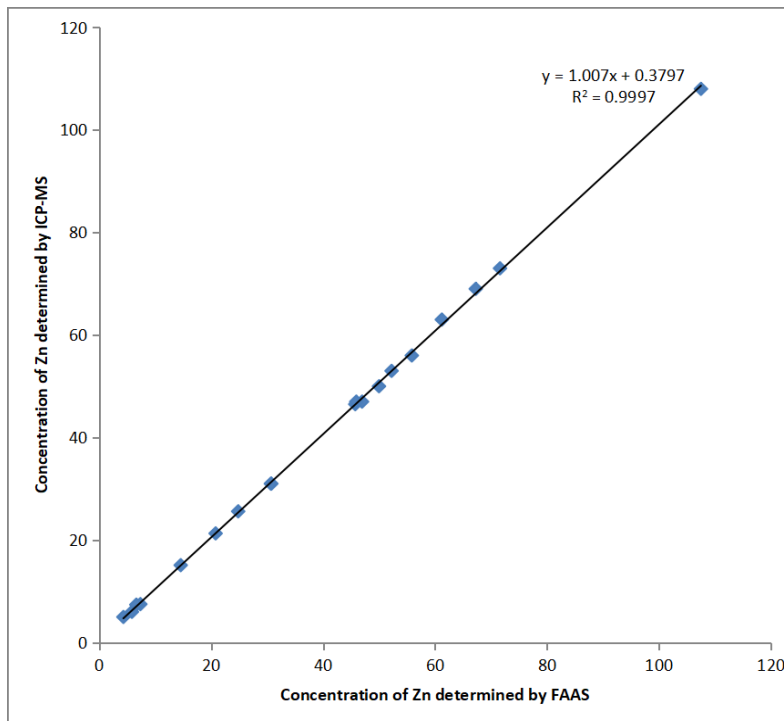


Figure 8. Zn concentration in Borabu medicinal plants determined by ICP-MS vs FAAS

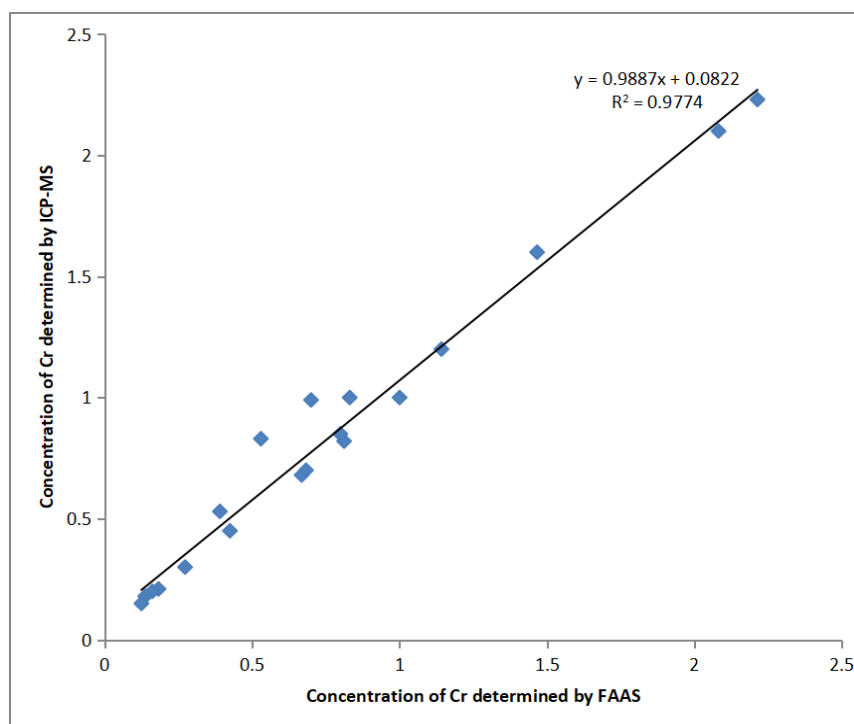


Figure 9. Cr concentration in Borabu medicinal plants determined by ICP-MS vs FAAS

Table 1. Summary of regression parameters for comparison iron, copper, zinc, manganese and chromium

Element	Concentration, mg/kg	ICP-MS		FAAS	
		Line of regression	R ²	Line of regression	R ²
Iron	1-5	$y = 0.0535x + 0.0009$	0.9999	$y = 0.0171x + 0.0044$	0.9964
Copper	1-5	$y = 0.1165x + 0.0038$	0.9999	$y = 0.118x - 0.016$	0.9997
Zinc	1-20	$y = 0.0135x + 0.0007$	0.9998	$y = 0.0133x - 0.0026$	0.9995
Chromium	0.1-1.5	$y = 0.2252x + 0.0014$	0.9999	$y = 0.2324 - 0.0064$	0.9992
Manganese	0.5-2	$y = 0.1793x + 0.0011$	1	$y = 0.18x - 0.01$	1

y= absorbance; x = concentration

The mean recoveries of the reference material (NIST 1547 -peach leaves with 6.4% moisture) for the concentration of the elements determined by ICP-MS were 97.04±4% for Cr, 94.96±1% for Mn, 95.47±2% for Cu, 95.46.3±5 for Fe and 94.21±3% for Zn while that for FAAS were 89.58±2% for Mn, 87.73± 5% for Cu, 89.63±7% for Cr, 86.3±4% for Fe and 88.03±3% for Zn (Table 2). Mogwasi et al. [21] reported NIST 1640a reference material to have recoveries with ICP-MS for Co, Cu, Ni, V, Mo, Mn, Zn and Cr to range of 96% to

104% while Nischwitz et al. [24] reported recoveries in the range from 96.4% to 107.7% (22 elements with certified values) in addition plant reference materials were analyzed and moisture corrected recoveries for NIST 1515 and NIST 1547 in a range from 82% to 116% for B, Mg, Al, K, Ca, V, Mn, Cu, Zn, Sr, Mo, Ba and Pb (mean 98% with standard deviation 8%) using ICP-MS which were comparable to those reported in the present study. This shows that ICP-MS method had higher precision and bias than FAAS.

Table 2. Concentration of Fe, Cu, Cr, Mn and Zn (mg·kg⁻¹) in certified reference material (NIST1547) and percentage recovery of ICP-MS and FAAS

Element	Certified concentration	Concentration determined by ICP-MS (n=20)	% Recovery	Concentration determined by FAAS (n=20)	% Recovery
Zn	17.97 ± 0.53	16.93±0.41	94.21±3	15.83±0.43	88.09±3
Cu	3.75 ± 0.38	3.58±0.33	95.47±2	3.29±2.33	87.73±5
Cr	1.35± 0.13	1.31±0.83	97.04±4	1.21±0.71	89.63±7
Mn	97.8 ± 1.8	92.87±1.3	94.96±1	87.61±1.1	89.58±2
Fe	219.8±6.8	209.83±4.7	95.46± 5	189.69±3.7	86.30±4

The slope of the calibration curves for the elements were Cr (0.2252), Mn (0.1793), Cu (0.1165), Fe (0.0535) and Zn (0.0135) for ICP-MS while that for FAAS were 0.2324, 0.18, 0.118, 0.0171 and 0.0133 (Figures 1-5). The R² value for the results obtained by ICP-MS were higher than those for FAAS for the five elements. This means that ICP-MS was more sensitive than FAAS. The calibration sensitivity suffers from the fact that it fails to take into account the precision of the technique. Precision needs to be included in a meaningful mathematical statement of sensitivity to give the following definition of analytical sensitivity Y,

$$Y = \frac{M}{Ss}$$

where M is the slope of the calibration curve and Ss is the standard deviation of the signal. Analytical sensitivity is relatively insensitive to amplification factors, for example increasing the gain of the method by a factor of eight will produce an eight-fold increase in M, which will be accompanied by a corresponding increase in Ss, hence leaving the analytical sensitivity constant and it's also independent of the measurement units of S. The detection limits (LODs) and quantification limits (LOQS) were between 0.0003 and 7.716mg/kg for ICP-MS and between 0.0011 and 25.7202mg/kg for FAAS, which further indicated that ICP-MS method was more sensitive than FAAS. Our results are similar to those reported by Alqadami et al. [2] of detection limits, As (4.6 ppb), Bi (7.9 ppb), Cd (0.45 ppb), Hg (3.3 ppb), Pb (3.8 ppb), Ti (4.3 ppb), linearity (r² > 0.999) and run-to-run and day-to-day precisions with relative standard deviations < 3% with NIST SRM 1570a Spinach leaves reference material using ICP-AES method.

3.1 Comparison of results of essential elements in plants quantified by FAAS and ICP-MS

The levels of the essential elements in the plants from two study areas were determined by FAAS and ICP-MS techniques. The FAAS analysis was carried out at the Chemistry Department Laboratories Kisii University of Kenya, while the ICP-MS was done at the central institute for chemical analysis (ZEA-3), Research Centre Julich, Germany. The triplicate levels of the elements in the medicinal plants determined by ICP-MS and FAAS in Borabu were compared by t-test, correlation coefficient, R² and the regression equations. The results of ICP-MS, FAAS and the table of comparison are given in Table 4.

The mean levels of the elements in the plant species determined by ICP-MS and FAAS had a positive correlation for the five elements. Regression curves for the elements in the plants in the study areas were determined by plotting the concentration of the elements determined by ICP-MS against those determined by FAAS (Table 4). The regression curves for the ICP-MS against FAAS gave R² values ranging from between 0.977 to 1, indicating strong positive correlation between the two techniques used in the determination of the elements (Figures 6-10 and Table 3). The regression equations gave slopes which were very close to unit and the lines which passed close to the origin for each element. This indicated that these two techniques gave the similar results. The t-test values obtained for the elements was less than 2.101 (df = 18), indicating that there were no significant differences for the results obtained by the two methods used in the analysis at p ≥ 0.05 though the results were statistically significant for the five elements at p ≥ 0.01 (Table 6).

The comparison of concentration of Fe, Cr, Cu, Zn and Mn determined by ICP-MS and FAAS in medicinal plants from Borabu were given in Figures 6-10 and Tables 2-6. The concentration of Fe determined by ICP-MS ranged from 230±8 to 5700±99, Cu from 2.7±0.03 to 14.2±1.98, Zn from 5±0.37 to 108±10.33, Cr from 0.12±0.003 to 2.43±0.23 and Mn from 57±6.1 while that determined by FAAS was from 216.08±5.6 to 5630.08±93, 1.87±0.2 to

13.12±1.23, 3.320±0.92 to 101.49±10.43, 0.18±0.01 to 2.23±0.91 and 54.01±4.7 to 873.36±13.3 mg/kg for Fe, Cu, Zn, Cr and Mn respectively (Table 3). The ranges of Zn, Cr and Mn are comparable to those we reported by Nischwitz et al. [24] of 5.48 to 105, 0.05 to 2.28 and 58 to 1661 in Borabu anti-diabetic medicinal plants. The said anti-diabetic plants were reported by Mogwasi et al. [22] to have said element in low molecular size species (<3 kDa) and hence the plants can be used in the management of diabetes. Mogwasi et al. [23] using FAAS method reported the Fe and Cu levels in the anti-anemia medicinal form Borabu to range from 464.00±2.37 to 2635.48±17.33 and 3.2±0.63 to 8.94±1.5 mg/kg which were comparable

to those in our study. They further reported the plants to avail substantial amounts of bio accessible concentration of Fe and Cu and hence they can be used in the management of anemia. The paired sample t-test (Table 5) presents the difference in concentration of the elements in the plants determined by ICP-MS compared to that determined by FAAS. The concentrations of Zn, Cr Mn and Cu determined by the two methods were statistically significant. Fe reported a p-value more significant than a 5% level of significance (Fe-ICP-MS - Fe-FAAS t (56) = 0.737, p = 0.435) (Table 5). Therefore, the Fe in the plants determined by FAAS and ICP-MS had no significant difference for the nineteen medicinal plants.

Table 3. Mean concentration (±) standard deviation mg/kg and % ratio element determined by FAAS/ICP-MS of Fe, Cu, Zn, Mn and Cr in medicinal plants

Fe		Cu		Zn		Cr		Mn		% Elemental content determined by FAAS/ICPMS				
ICP-MS	FAAS	ICP-MS	FAAS	ICP-MS	FAAS	ICP-MS	FAAS	ICP-MS	FAAS	Fe	Cu	Zn	Cr	Mn
420±8	394±7	5±0.9	3.72±0.12	7.5±0.74	6.35±0.87	0.12±0.03	0.18±0.01	170±23	168.01±17.2	89.2	94.4	89	98.1	98.8
2030±70	2015.15±74	6.9±0.93	5.13±0.35	25.6±2.074	21.8±1.87	0.82±0.06	0.76±0.04	130±17	127.88±14.2	99.8	88.8	97	99	94
990±50	985.76±45	5.8±0.83	4.53±0.36	21.3±1.074	20.8±1.23	0.45±0.02	0.35±0.03	104±18	102.9±8.3	99.6	95.3	97.6	97.8	98.9
420±10	418.06±7.8	6.6±0.67	6.17±0.48	6±0.44	5.24±0.87	0.18±0.01	0.16±0.02	121±21	120.9±8.7	99.5	93.5	97.3	94.4	99.7
420±12	411.57±8.4	6.9±0.57	6.41±0.67	31± 4.03	28.74±2.33	0.15±0.02	0.13±0.01	147±29	144.3±9.2	100.4	92.9	99.2	100	98.2
370±20	367.26±18	14.2±1.93	13.12±1.23	46.5±3.31	42.74±3.97	0.24±0.03	0.2±0.01	212±32	209.75±12.3	99.3	98.03	98.4	100	98.9
2000±64	1954.55±79	10.7±1.33	9.82±0.93	47± 4.17	43.95±4.87	1.23±0.08	1.2±0.075	400±36	397.82±17.3	97.7	92.5	99.9	100	99.5
3230±78	3187.83±73	6.3±0.45	5.73±0.33	47± 5.12	44.92±3.98	1.65±0.07	1.54±0.81	290±28	285.9±13.4	99.8	95.5	97.7	97.5	98.6
1300±12	1184.37±7	8.2±0.87	7.26±0.37	31±1.87	29.67±3.22	0.68±0.04	0.65±0.04	1650±56	1637.49±65	91.1	94.6	98.9	94.1	99.2
5700±98	5630.08±93	13.7±1.77	12.32±0.97	73±8.07	67.56±5.79	2.15±0.07	1.87±0.12	580±29	578.71±18.7	98.8	92.8	98	99.05	99.8
920±12	874.3±7.5	9.8±0.87	8.44±0.67	53±4.03	50.23±7.33	1±0.09	0.89±0.04	540±34	532.8±11.3	98.5	91.2	98.5	100	98.7
2600±65	2496.5±71	9.2±0.87	9.06±0.47	108±10.33	101.49±10.34	2.43±0.23	2.23±0.91	330±8	327.76±10.4	99.9	98.5	99.5	99.6	99.3
230±8	216.08±5.6	10.4±1.23	8.17±0.59	15.1±1.67	12.56±1.23	0.3±0.01	0.23±0.05	880±17	873.36±13.3	98.8	97.8	96.4	100	99.2
320±7	316.1±6.4	4.7±0.08	3.47±0.01	5±0.37	3.32±0.92	1.21±0.12	0.81±0.06	81±7	81.53±6.2	98.8	95.1	86.4	100	100.7
560±14	550.1±13.3	2.7±0.03	1.87±0.02	7.4±0.47	5.63±0.46	0.53±0.04	0.38±0.03	57±6.1	54.01±4.7	98.2	83.7	89.6	96.2	94.8
1150±17	1197.02±14	6.9±0.78	3.97±0.33	50±6.43	43.97±3.97	0.83±0.06	0.53±0.04	279±13.3	228.63±8.9	104.1	90.6	99.9	96.4	99.9
2600±87	2635.48±93	7.6±0.67	7.13±0.83	63±5.69	58.7±5.97	0.7±0.04	0.56±0.03	269±12.1	264.86±11.8	98.0	93.8	97.1	104.3	98.5
450±7	451.01±7.3	13.6±1.21	11.23±0.93	69±7.31	63.27±6.77	0.99±0.09	0.73±0.04	610±15.6	608.68±13.6	98.3	97.3	97.5	97.98	99.8
1300±23	1233.22±27	3.8 ±0.07	2.99±0.06	56±4.77	52.84±5.33	0.85±0.07	0.63±0.03	260±7.3	254.49±5.9	95.6	78.7	99.7	101.1	97.9

1. Warburgia ugandensis 2. Solanum indicum 3. Toddalia asiatica 4. Erythrina abyssinica 5. Senna didymobotrya 6. Vernonia auriculifera 7. Plectranthus barbatus 8. Ultrica dioica 9. Croton macrostachyus 10. Bidens pilosa 11. Melia azedarach 12. Solanum mauense 13. Magnifera indica 14. Acacia hockii 15. Acacia abyssinica 16. Clerodendrum myricoides 17. Carissa edulis 18. Tabernaemontana stapfiana 19. Aloe vera "±"mg/kg standard deviation

Table 4. The t-test, r, R2 and the regression of Fe, Cu, Zn, Mn and Cr in Borabu

Study area		Borabu			
Element	T	R	R ²	Regression	
Iron	0.9670	0.9999	0.999	Y=1.01x+3.99	
Copper	0.6460	0.9972	0.994	Y=1.003x+.47	
Zinc	0.9416	0.9998	0.999	Y=1.007x+.38	
Manganese	0.9793	1	1	Y=1.006x+.78	
Chromium	0.7103	0.9886	0.977	Y=0.988x+.08	

Table 5. T-test for the levels of Fe, Cu, Zn, Cr and Mn in medicinal plants

		T	Df	P
Pair 1	Fe-ICP-MS - Fe-FAAS	.787	56	.435
Pair 2	Cu-ICP-MS - Cu-FAAS	13.278	56	.000
Pair 3	Zn-ICP-MS - Zn-FAAS	13.352	56	.000
Pair 4	Cr-ICP-MS - Cr-FAAS	10.531	56	.000
Pair 5	Mn-ICP-MS - Mn-FAAS	5.328	56	.000

Table 6. Comparison of the concentration of Fe, Cu, Zn, Cr and Mn

		Fe		Cu		Zn		Cr		Mn	
		ICP-MS	FAAS	ICP-MS	FAAS	ICP-MS	FAAS	ICP-MS	FAAS	ICP-MS	FAAS
Fe	ICMS	1.00									
	FAAS	.912**	1.00								
Cu	ICP-MS	.099		1.00							
	FAAS		.090	.871**	1.00						
Zn	ICP-MS	.421**		.391**		1.00					
	FAAS		.440**		.403**	.937**	1.00				
Cr	ICP-MS	.454**		.145		.429**	0.254**	1.00			
	FAAS		.484**		.181*		.425**	.916**	1.00		
Mn	ICP-MS	.204*		.506**		.442**		0.246**		1.00	
	FAAS		.169		.465**		.481**		0.275**	.940**	1.00

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

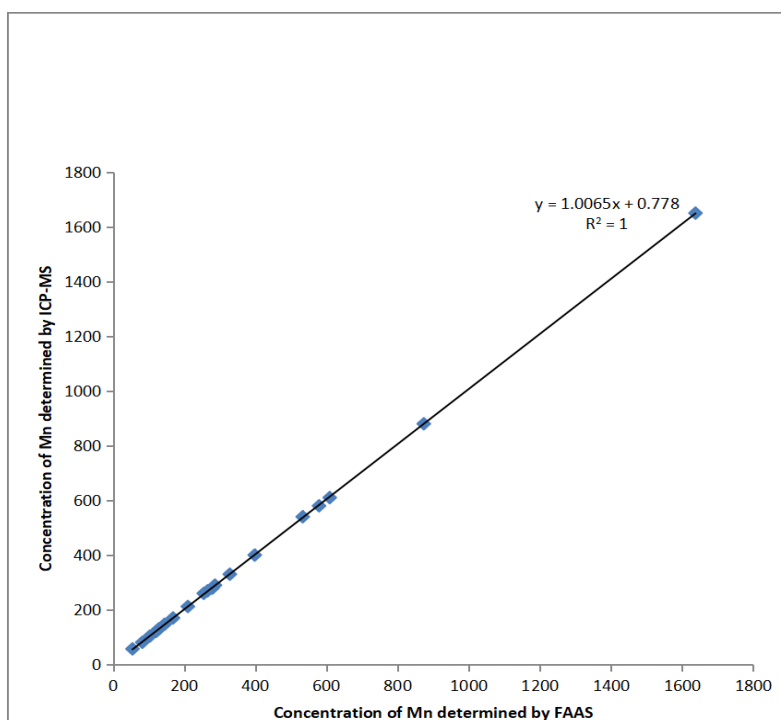


Figure 10. Mn concentration in Borabu medicinal plants determined by ICP-MS vs FAAS

The correlation between the concentration of the elements determined by ICP-MS and FAAS in the medicinal was significantly positive at ($P > 0.01$), when the individual elements were compared. Conversely, the correlation between the concentration of the different elements in the plants determined by a given method was statistically significant ($P \geq 0.01$) except for Cu/Cr for FAAS, Cu/Cr, Fe/Mn for ICP-MS which were positively significant at ($P \geq 0.05$) and Fe/Cu for FAAS and ICP-MS, Fe/Mn for FAAS and Cu/Cu for ICP-MS whose concentrations were not statistically different (Table 6). The percentage of Cr in the plants determined using FAAS to ICP-MS ranged from 94.1 (*C. macrostachyus*) to 104.3 (*C. eduklis*) with a mean of 97.97%, Mn from 94 (*A. abyssinica*) to 100.7 (*A. hockii*) with a mean of 98.88%, Cu from 78.7 (*A. vera*) to 98.5 (*S. mauense*) with a mean of 92.71%, Zn from 86.4 (*A. hockii*) to 99.9 (*C. myricoides* & *P. barbatus*) with a mean of 97.19% and Fe from 89.2 (*W. ugandensis*) to 104.1 (*C. edulis*) with a mean 95.2%. The mean ratio of the elements determined by the two methods (FAAS/ICP-MS) for Cr, Zn, Cu and Mn were above 95% while that of Cu was 91%. Azam et al. reported that the optimized SPE and iCAP Q ICP/MS method offered excellent validation conditions in terms of coefficient of determination (CoD, $R^2 > 0.999$), detection limit (DL, 0.011 mg/L), quantification limit (QL, 0.034 mg/L), and run-to-run and day-to-day precision (RSD < 6%) similar to those we reported in the present study [26]. Khan et al. used microwave digestion (using reagents

nitric acid (HNO_3 , 65%, 5 mL) and hydrofluoric acid (HF, 40%, 2 mL)) and iCAPQ inductively coupled plasma mass spectrometry (ICP-MS) system to assay seven potential heavy metals manganese (Mn), cobalt (Co), nickel (Ni), zinc (Zn), arsenic (As), cadmium (Cd) and lead (Pb) in Acrylic paints of different colors reported recovery values ranging between 99.33% and 105.67% [6].

The recoveries of ICP-MS were better than those of FAAS for the elements however, the recoveries for the two methods can be improved by controlling adjusting or altering analytical conditions. This will eliminate or minimize interferences from the matrix to enhance accuracy of the determined concentration of the elements. The use of HCl , HClO_4 , H_3PO_4 and H_2SO_4 in sample preparation in ICP-MS may cause considerable spectral problems [10] and should be avoided in most ICP-MS analyses and if this is not possible, sample separation using chromatography should be done before introduction into the plasma to remove unwanted species and preconcentrate the analyte [5, 8, 10]. Other methods used to overcome the problems are electro-thermal vaporization (ETV), mixed gases and the use of high-resolution magnetic sector ICP-MS to resolve masses less than 0.1 Dalton apart, hence eliminating most spectral interferences [10]. Therefore, solutions for ICP-MS analysis should be carefully prepared in nitric acid. Polyatomic interferences are caused by Cl^+ , P^+ , S^+ ions in conjunction with other matrix elements like Ar^+ , O^+ , H^+ such as $^{35}\text{Cl}^{40}\text{Ar}$ on ^{75}As and $^{35}\text{Cl}^{16}\text{O}$ on ^{51}V . Doubly charged ions also causes

spectral interference at half the m/z of the singly charged ions, e.g., $^{138}\text{Ba}^{++}$ on $^{69}\text{Ga}^+$ or $^{208}\text{Pb}^{++}$ on $^{104}\text{Ru}^+$. These interferences are minimized or eliminated by optimizing the system before the analysis.

Ionization interference in ICP-MS for samples containing high concentrations of Group I and II elements has been reported by Nischwitz et al. [24] and can overcome by matrix matching, sample dilution, standard addition, isotope dilution, extraction or separation by chromatography in which space charge effects occur behind the skimmer cone, where the net charge density becomes significantly different from zero [25]. The high ion density leads to interaction between ions present in the ion beam causing preferential loss of the light ions in the presence of the heavy ones such as Pb^+ on Li^+ . Matrix matching, or careful choice of internal standards across the mass range of the analytes, help to compensate for these effects, although this may prove difficult in practice and therefore isotope dilution will be effective though expensive [2], although it can also be overcome most effectively by diluting the sample [4].

As a result of the low temperature of the air/acetylene flame (2,200°C) used in FAAS there are many chemical interferences such as PO_4 on Ca and the effect of metals on others [3]. The use of "releasing agents" can overcome these interferences such as Lanthanum Chloride for the Ca in phosphate solutions and Uranium Oxide or Lanthanum Oxide for precious metals. As there are many metals which interfere with each other much work should be involved in designing ways to overcome the interference before the analysis [3, 9, 25]. Most of these interferences are well documented, but it is useful to work with reference materials to ascertain the accuracy.

Transport effects in ICP-MS such as the spray chamber effects and differences in viscosity between sample solutions and calibration standards will change the efficiency of aerosol production from one solution to another [2]. This calls for matrix matching, although internal standardization can be used to overcome this effect and rapid scanning speed of ICP-MS does give superior results when using an internal standard [4, 9, 24, 25].

FAAS, like ICP-MS uses a nebulizer and spray, so it has similar interferences such as viscosity differences between samples and calibration standards [26]. Matrix matching is required, due to direct aspiration of the sample and the method of standard additions is used because the use of an internal standard is not possible on FAAS. Spray chamber "adaptation" effects are less in FAAS probably due to the large droplet size and volume of aerosol in the spray chamber.

The short-term precision of ICP-MS is 1-3% which can be improved routinely by use of multiple internal standards [24]. The longer-term precision (over a period of hours) is still $<0.5\% \text{RSD}$ [9]. The use of isotope dilution can improve the precision and accuracy of the results, though the high cost of the standard is prohibitive

for most routine analysis [26] while the precision of FAAS is excellent 0.1-1%, however, long term precision is poor especially if nitrous oxide/acetylene flame is used which can be improved with constant manual "de-coking" of the burner by the operator [1].

ICP-MS has a LDR (linear dynamic range) in excess of 10^5 and this can be extended up to 10^7 by de-sensitizing one of the ion lenses, use of detector analog mode, or use of a separate faraday cup as a second detector [1, 4, 9] however, high matrix component concentrations may cause problems best solved by dilution. Samples with high dissolved solids, ICP-MS method should be used for trace/ultra-trace analysis and with isotope dilution method, very good results are obtained with high concentrations. FAAS has a LDR of approximately 10^3 and constant dilutions of the samples are required to give reliable results [1, 6, 9]. The two methods used in the analysis of the nineteen medicinal plants for borabu used in the management of diabetes and anemia exhibited that the methods can be used to complement each other in the determination of the mineral elements involved in the management of the two conditions. Mn, Cr, Zn, V and Mg have been shown to be hypoglycemic [21, 24] while Fe and Cu have the potential to manage anemia. These indicate that the applied digestion and quantification methods provided valid and reliable results and can be used for the quantification of Zn, Cu, Cr, Mn and Fe in plants, soil and other environmental samples.

4. Conclusions

The concentrations of Zn, Mn, Cr, Fe and Cu in the anti-diabetic and anti-anemia medicinal plants determined by ICP-MS and FAAS are similar, though the concentrations of the elements determined by ICP-MS were higher and had high sensitivity, bias, selectivity and precision. The two methods can complement each other in the analysis of concentration of Zn, Mn, Cr and other hypoglycemic elements and Fe and Cu, in anti-diabetic and anti-anemia medicinal plants. This is because FAAS has low accuracy, cheap to acquire, maintain and low running costs and can be used to analyze few samples while ICP-MS can be used when more samples are to be analyzed.

Disclosure

The authors have no competing interests to declare that are relevant to the content of this article.

Authors' contributions

Mogwasi Richard designed the project, carried out the

project and wrote the manuscript; Onyancha M Evans carried out project and edited the manuscript; Mobegi Eric carried out project and edited the manuscript; Nyabaro Obed carried out project and edited the manuscript.

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