

Original Article

Homogeneity Test of Elemental Content in Agricultural Soil Reference Material Candidate Using ICP-MS

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Abstract:

The aim of any analytical measurement is to obtain accurate, reliable, and consistent data. Certified Reference Materials (CRMs) are vital in obtaining reliable results in various analytical fields such as environmental and medical studies. This paper discusses the production of an agricultural soil CRM candidate from the Al-Ahsa region, Saudi Arabia, using an ICP-MS instrument. The CRM is intended to be applied for quality assurance of toxicological and environmental analyses of soil matrices for some elements at macro, micro, and trace levels. This article demonstrates homogeneity tests as an essential parameter for CRMs. A one-way Analysis of Variance (ANOVA) was used to assess the between-bottle homogeneity at a confidence interval of 95%. The p-values of all elements in this CRM candidate are > 0.05 , indicating no significant variation in elemental concentration.

Keywords:

CRM, between bottle homogeneity, IC P-MS, accuracy, quality control, quality assurance.

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Introduction:

Reference materials (RMs) are very important in analytical chemistry. They are cornerstones to trace the result of an individual laboratory with an SI unit or another suitable reference measurement system. The International vocabulary of metrology (ISO/IEC Guide 99:2007 2007) has defined the term “Reference material” as “Material, sufficiently homogeneous and stable with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties,” while has defined CRMs as “Reference material, accompanied by documentation issued by an authoritative body and providing one or more specified property values with associated uncertainties and traceabilities, using valid procedures.”

Institutions, which are concerning with the production of RMs, distribute in many countries in the world. However, RMs have neither been produced in the Middle East nor in Arabic Countries (Idris al, 2015).

This paper is an attempt to produce Saudi Certified Reference Materials (SCRMs) with a high quality and a

metrological value. SCRMs includes agricultural soil of date palm which collected from Al-Ahssa farms, which the largest area of date palm farming in the eastern region in Saudi Arabia.

The soil elements content along with other properties are important for determination of soil quality and are used to assess soil health and suitability for cultivation of crops (Małgorzata Rutkowska, 2018). Soil is composed of minerals, organic matter, living organisms, air, and water, and it regulates the natural cycles of these components [3-4]. Heavy metals occur naturally in soils, which are formed by geological processes, such as alteration and erosion of the geological underground materials [5, 6]. Environmental contamination by heavy metals has become a world-wide problem in recent years due to the fact that heavy metals unlike some other pollutants are not biodegradable [7].

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and medical studies. This paper discusses the production of an agricultural soil CRM candidate from the Al-Ahsa region, Saudi Arabia, using an ICP-MS instrument. The CRM is intended to be applied for quality assurance of toxicological and environmental analyses of soil matrices for some elements at macro, micro, and trace levels. This article demonstrates homogeneity tests as an essential parameter for CRMs. A one-way Analysis of Variance (ANOVA) was used to assess the between-bottle homogeneity at a confidence interval of 95%. The p-values of all elements in this CRM candidate are > 0.05 , indicating no significant variation in elemental concentration.

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Materials and Methods.

Sample Collection and Preparation.

Soil CRM candidate has been proposed with the code KACST401 from agricultural area in Alahssa farms, Eastern Region- to be certified for V, Cr, Mn, Co, Ni, Cu, Ga, As, Rb, Sr, Cd, In, Tl and U. About 20 kg of KACST401 were collected. Extraneous particles such as stones, roots, waste, etc. were manually eliminated. The soil was ground and sieved to obtain particle-size ≤ 1000 μm . Two homogenization steps, before and after the irradiation step, were carried out in a 50 L mixer for 12 h for each step. The container and the paddles of the mixer were made of stainless steel and coated with polyethylene sheets. The irradiation processes was carried out using Co^{60} facility, Nordion Gamma Cell 220

(Nordion, Canada Ltd., Laval, Canada). The dose rate for the sample was 4.1 kGy/ h at 25 kGy of absorbed dose ($1 \text{ Gy} = 100 \text{ rad} = 1 \text{ J.kg}^{-1}$). The irradiation treatment was performed in vessels of Pyrex-glass at room temperature. The irradiation dose is suitable for the preservation of CRMs. After the second phase of homogenization, the soil CRM was packed in light protected bottles (Figure 2).

Treatment of CRM samples for analysis

About 200 mg was exactly weighed from seven different bottles. Soil matrices were placed into Teflon digestion vials. Materials of soil matrices underwent hydrofluoric acid digestion to guarantee total content determination. To appropriately weighed samples of soil, 4.5 mL of hydrochloric acid 36-37% supplied by Scharlab S.L, Galo Perez 33-P.I. Mas d'En Cisa 08181 Sentmental Spain, 1.5 mL of nitric acid 69% supplied by SIGMA-ALDRICH Lot #SZBB1660V and 1.0 mL of hydrofluoric acid 40% supplied by BDH laboratory, Poole, BH 15 1TD, were added. A two steps microwave program was employed. The digestion solutions were filtered

and filled up to 25 mL by deionized water. These solutions were diluted at ratio 1:10 for measurement, depending

on the element concentration, to keep measurement concentrations in a reasonable range of calibration curve.

Instrumentation

The instrument used in this study was ICP-MS supplied by thermo-Fisher scientific.

Quality Control.

The limits of detection (LODs) and quantification (LOQs) were calculated by 10 measurements with 1% HNO₃. The calculations were as follows:

LOD = 3 × SD of the blank and

LOQ = 10 × SD of the blank.

A continuing calibration verification (CCV) was used as a quality control (QC) test for each run. This was performed by testing 50.0 µg/L of a mixed standard containing all measured elements after each set of twenty-six runs. A spiked sample with a specific concentration (50 µg/L) of each measured element in a mixture was analyzed.

Calibration.

For quality assurance a standard solution, Inorganic ventures IV-STOCK-21 includes 29 elements, was measured. The calibration curves were

obtained by plotting signals i.e. count per second (cps) versus corresponding concentrations. The linearity of curves was assessed by means of correlation

coefficient, r and determination coefficient R^2 .

Recovery.

The recovery of elements in soil samples was obtained from the first sample, 1-1-1. The concentration of the CRM (stock solution) is 10 ppm or 10,000 ppb. 0.125 ml of the stock solution were taken in 25 ml volumetric flask and completed to 25 ml by HNO₃ 1% to prepare 50 ppb solution which was referred to *spiked 50*.

2.5 ml of the sample 1-1-1 were taken in 25 ml volumetric flask and 0.125 ml of stock standard solution were added to it and completed by de-ionized water to the mark to prepare spiked sample.

2.5 ml of the sample 1-1-1 were taken in 25 ml volumetric flask and completed by deionized water to the mark to prepare *non-spiked sample*.

$$\% \text{Recovery} = \frac{(\text{conc of spiked sample} - \text{conc of non-spiked sample})}{\text{conc of spiked 50}} \times 100$$

Elemental Measurements Using ICP-MS.

In total, 14 elements were measured in all collected samples. Concentrations of fourteen trace elements (V, Cr, Mn, Co, Ni, Cu, Ga, As, Rb, Sr, Cd, In, Tl and U) were measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (iCAP Q,ermo Fisher Scientific, Waltham, MA, USA) in all 14 samples. Samples were analyzed in triplicate ($n = 3$).

Results and Discussion:

For assessment of the quality of measurements of agricultural soil samples by ICP-MS, the elements concentrations showed good repeatability during measurements except samples 1-2-2, 1-4-2 and 1-6-1 for all elements and samples 1-1-1, 1-2-2, 1-3-2, 1-4-2, 1-6-1 and 1-7-2 for Indium showed poor repeatability. Concentrations were determined within reasonable relative standard deviations (RSDs) regarding total concentrations and element specific limit of quantifications (LOQs). RSDs ranged from the lowest value, 0.0% for Indium in sample No 1-7-1 to the highest value, 35.5% for Indium in sample No 1-4-2.

Calibration.

The 14 elements proposed in this paper were calibrated using standard ready

prepared by Inorganic ventures IV-STOCK-21. The ICP-MS was programmed to produce calibration curves with the following concentrations: 5, 10, 20, 50 and 100 $\mu\text{g/L}$, each concentration level was measured three times i.e. triplicates. The results obtained from the calibrations of elements were compiled in table 5. The linearity of the calibration process was investigated by means of the correlation coefficient (r) and determination coefficient, (R^2).

Values of r and R^2 of V, Cr, Mn, Co, Ni, Ga, As, Rb, Sr, Cd, In, Tl and U are greater than 0.99 indicating that their linearity is good. Cu element showed poor linearity i.e. its values of r and R^2 were lower than 0.99.

Quality Control.

The accuracy of the proposed method was evaluated by determining the recoveries of the analytes by spiking experiments. The recoveries, LODs and LOQs of all 14 elements were calculated and listed in table 1 below. Good accuracy in the range of 76.98–96.58% was recorded. More sensitive detection was obtained by ICP-MS. It recorded limits of detection (LODs) in the range of 0.003–3.0 $\mu\text{g/L}$.

Table (1): Recovery % of 14 metals

Element	Recovery %	LOD	LOQ
V	88.19	0.015	0.050
Cr	87.84	0.015	0.050
Mn	85.44	0.216	0.720
Co	86.58	0.021	0.070
Ni	82.54	1.524	5.080
Cu	76.98	0.243	0.810
Ga	88.89	3.300	11.00
As	86.67	0.147	0.490
Rb	88.51	0.015	0.050
Sr	86.78	0.051	0.170
Cd	82.63	0.141	0.470
In	92.11	0.117	0.390
Tl	83.89	0.258	0.860
U	96.58	0.003	0.010

Elemental Analysis by ICP-MS.

Mean concentrations ($\mu\text{g/g}$) of the measured elements in wet weight are shown in Tables below. Concentration in $\mu\text{g/g}$ of the 14 elements were measured in 14 samples of agricultural soil ($n = 3$)

Table (2): Mean concentrations and RDS of V, Cr, Mn, Co, Ni elements in agricultural soil CRM candidate

No	V		Cr		Mn		Co		Ni	
	Conc	RSD%	Conc	RSD%	Conc	RSD%	Conc	RSD%	Conc	RSD%
1-1-1	34.19	7.1	148.83	7.1	161.69	7.5	4.52	9.6	51.52	8.0
1-1-2	29.73	4.2	137.75	4.7	143.38	4.5	4.0	6.1	45.68	5.6
1-2-1	28.75	6.2	157.44	7.6	143.9	7.9	3.95	5.5	48.57	5.2
1-2-2	26.17	<u>15.6</u>	118.4	<u>13.9</u>	129.31	<u>14.2</u>	3.54	<u>14.6</u>	43.78	<u>14.1</u>
1-3-1	27.06	5.7	109.46	5.5	129.67	5.5	3.62	6.1	42.56	4.7
1-3-2	26.39	5.0	117.35	4.5	127.29	4.0	3.47	6.7	40.64	5.4
1-4-1	26.39	3.5	105.98	3.9	129.63	2.4	3.91	0.96	42.43	2.7
1-4-2	27.98	<u>23.5</u>	111.76	<u>24.1</u>	134.15	<u>24.3</u>	3.81	<u>26.7</u>	45.02	<u>26.6</u>
1-5-1	27.5	3.4	113.83	4.0	136.27	4.1	3.65	5.2	42.84	5.4
1-5-2	28.06	6.8	110.32	4.1	132.35	2.9	3.72	4.4	42.42	3.8
1-6-1	25.93	<u>21.0</u>	113.12	<u>21.1</u>	128.76	<u>20.2</u>	3.61	<u>20.3</u>	41.0	<u>19.7</u>
1-6-2	26.73	4.6	111.22	2.6	128.5	3.0	3.45	3.1	43.22	4.2
1-7-1	26.99	5.4	119.08	6.1	130.69	6.2	3.48	5.6	42.06	5.1
1-7-2	31.42	4.9	129.04	5.1	152.56	4.4	3.92	3.8	46.65	4.5

Table (3): Mean concentrations and RDS of Cu, Ga, As, Rb and Sr elements in agricultural soil CRM candidate

No	Cu		Ga		As		Rb		Sr	
	Conc	RSD%	Conc	RSD%	Conc	RSD%	Conc	RSD%	Conc	RSD%
1-1-1	14.59	8.4	7.44	5.3	5.14	4.0	39.78	7.0	322.30	7.9
1-1-2	10.85	5.2	5.57	5.3	3.67	8.9	34.40	6.7	278.76	6.9
1-2-1	18.35	5.6	5.58	6.2	3.10	10.7	34.13	6.9	273.87	7.4
1-2-2	9.87	<u>15.8</u>	5.04	<u>13.8</u>	3.25	<u>22.5</u>	31.45	<u>11.0</u>	248.43	<u>12.6</u>
1-3-1	9.62	4.6	5.18	4.6	3.24	<u>13.3</u>	32.00	3.4	256.77	2.9
1-3-2	9.54	4.0	5.16	4.9	3.20	7.5	31.50	7.2	255.62	7.8
1-4-1	11.88	4.1	5.46	8.0	3.25	2.4	31.57	6.5	263.24	7.0
1-4-2	9.93	<u>27.7</u>	5.47	<u>24.4</u>	3.38	<u>25.3</u>	32.44	<u>22.5</u>	271.90	<u>26.4</u>
1-5-1	9.83	4.4	5.18	5.9	3.20	7.5	32.37	4.3	231.68	4.7
1-5-2	9.45	4.1	5.4	5.2	3.27	7.3	33.13	8.1	263.80	7.2
1-6-1	9.45	<u>20.8</u>	4.84	<u>15.9</u>	2.82	<u>21.5</u>	30.61	<u>15.8</u>	101.52	<u>16.8</u>
1-6-2	10.55	3.6	5.34	3.3	3.16	5.7	30.79	5.0	222.02	3.0
1-7-1	10.10	4.7	5.28	3.5	3.11	4.2	31.64	4.7	250.95	4.5
1-7-2	9.98	3.7	5.81	4.8	3.50	9.2	36.91	4.5	257.18	5.6

Table (4): Mean concentrations and RDS of Cd, In, Cs, Ba, Tl and U elements in agricultural soil CRM candidate

No	Cd		In		Tl		U	
	Conc	RSD%	Conc	RSD%	Conc	RSD%	Conc	RSD%
1-1-1	2.71	8.8	0.50	<u>11.4</u>	0.33	9.96	1.66	9.1
1-1-2	2.51	6.9	0.22	5.7	0.27	4.7	1.15	5.6
1-2-1	2.18	3.0	0.38	3.3	0.26	4.8	1.11	6.0
1-2-2	1.09	<u>20.7</u>	0.16	<u>13.5</u>	0.21	<u>15.2</u>	1.01	<u>17</u>
1-3-1	1.45	6.2	0.10	7.5	0.21	5.9	0.95	4.7
1-3-2	1.78	4.4	0.04	<u>17.5</u>	0.20	6.3	1.00	4.5
1-4-1	10.6	4.6	0.69	7.3	0.46	5.7	1.01	4.9
1-4-2	0.70	<u>24.8</u>	0.05	<u>35.5</u>	0.20	<u>18.8</u>	1.00	<u>25.2</u>
1-5-1	1.19	3.16	0.07	10.2	0.20	3.6	0.94	5.3
1-5-2	2.24	8.2	0.53	6.3	0.24	5.3	0.97	6.9
1-6-1	3.70	<u>21.3</u>	0.23	<u>22.1</u>	0.26	<u>18.3</u>	0.85	<u>19.5</u>
1-6-2	0.92	10.7	0.35	3.6	0.19	9.99	0.92	6.7
1-7-1	0.41	3.1	0.08	0.0	0.18	4.1	1.00	3.3
1-7-2	0.56	4.4	0.04	<u>17.5</u>	0.21	5.99	1.02	5.7

Tables 2, 3 and 4 show the mean concentrations and RSD% of elements in each sample of CRM candidate of agricultural soil matrices (KACST401) obtained from triplicate analysis.

Statistical Analysis.

Statistical analysis carried out for all analytes was calculated normally, a one way analysis of variance (ANOVA) approach using SPSS version 20 was used to assess the between bottle and within bottle homogeneity. Table 6 showed the ANOVA factors sum of squares, degrees of freedom, mean of squares,

F values, critical F value and p -values or significance.

Where SS provides the sums of squares, df the associated degrees of freedom, MS the mean squares, and the F -test indicated that the result of the homogeneity is insignificant ($F < F_{crit}$, the critical value of F for $\alpha = 5\%$). The P -value gives the level for which the observed F equals F_{crit} . All P -values (sig) are > 0.05 indicating that no significant difference between concentrations measurements which mean that the soil samples are homogeneous.

Table (6): Analysis of variance (ANOVA) for elements

Element	Source of variation	SS	df	MS	F	P -value	F_{crit}
V	Between Groups	44.986	6	7.498	2.095	0.178	3.87
	Within Groups	25.052	7	3.579			
	Total	70.038	13				
Cr	Between Groups	2303.324	6	383.887	2.893	0.095	
	Within Groups	928.840	7	132.691			
	Total	3232.164	13				
Mn	Between Groups	870.827	6	145.138	1.903	0.210	
	Within Groups	533.975	7	76.282			
	Total	1404.802	13				
Co	Between Groups	0.737	6	0.123	2.474	0.131	
	Within Groups	0.348	7	0.050			
	Total	1.085	13				
Ni	Between Groups	74.195	6	12.366	1.849	0.220	
	Within Groups	46.809	7	6.687			
	Total	121.003	13				
Cu	Between Groups	36.854	6	6.142	1.462	0.314	
	Within Groups	45.538	7	6.505			
	Total	82.392	13				
Ga	Between Groups	2.736	6	0.456	1.462	0.314	
	Within Groups	2.184	7	0.312			
	Total	4.921	13				
As	Between Groups	2.602	6	0.434			

	Within Groups Total	1.237 3.840	7 13	0.177	2.454	0.133
Rb	Between Groups Within Groups Total	52.569 32.758 85.327	6 7 13	8.761 4.680	1.872	0.215
Sr	Between Groups Within Groups Total	21661.428 9105.000 30766.428	6 7 13	3610.238 1300.714	2.776	0.104
Cd	Between Groups Within Groups Total	31.731 54.100 85.832	6 7 13	5.289 7.729	0.684	0.670
In	Between Groups Within Groups Total	0.199 0.384 0.583	6 7 13	0.033 0.055	0.604	0.722
Tl	Between Groups Within Groups Total	0.031 0.041 0.072	6 7 13	0.005 0.006	0.889	0.549
U	Between Groups Within Groups Total	0.342 0.139 0.482	6 7 13	0.057 0.020	2.864	0.097

Conclusion:

ICP-MS technique was used to quantify some heavy metals V, Cr, Mn, Co, Ni, Cu, Ga, As, Rb, Sr, Cd, In, Tl

and U in agricultural soil CRM candidate. The main objective of the measurements is to verify initial within-bottles and between-bottles homogeneity test and to compare the level of heavy metals in the agricultural soil from Alahsa region, Saudi Arabia with risk levels associated with different concentrations of heavy metal in soil according to the standards set in the Finnish legislation for contaminated soil (Ministry of the Environment - MEF, Finland, 2007).

CRM candidate of agricultural soil recorded within-bottle RSDs (CV_{within}) range from 0.0 to 35.5. the CRM samples show acceptable repeatability except samples No 1-2-2, 1-4-2 and 1-6-1 for all elements and samples 1-1-1, 1-2-2, 1-3-2, 1-4-2, 1-6-1 and 1-7-2 for in showed poor repeatability due to random errors and storing long period after digestion and before measurement.

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