METHOD VALIDATION OF TRACE ELEMENTS IN WATER BY ATOMIC ABSORPTION SPECTROMETER DETERMINATION

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ABSTRACT

An analytical method for determination of the dissolved trace elements; copper (Cu), iron (Fe), Zinc (Zn), manganese (Mn), Cadmium (Cd), Lead (Pb), Arsenic (As), Mercury (Hg), Cobalt (Co), Chromium (Cr), and Nickel (Ni) in the water samples (primarily drinking water); had been validated and introduced into the scope of the Central Laboratory of Residue analysis of Pesticides and Heavy Metals in Food . The acidified water samples were analyzed by direct aspiration by atomic absorption spectroscopy (flame or electro thermal vaporization) for total metals determination without digestion. The analytical parameters of the method such as limit of quantification and linearity have been investigated. The limits of quantitation were found to be 0.1, 0.05. 0.1, 0.005, 0.3, 2, 1, 1, 0.05, 0.1, and 0.05 mg/L for Cu, Fe, Mn, Zn, Cd, Pb, As, Hg, Co, Cr and Ni, respectively. The method showed to be linear from the LOQ levels up to 4, 5, 2, 1, 0.004, 0.03, 0.04, 0.04, 6, 5, and 5 mg/L levels for Cu, Fe, Mn , Zn, Cd, Pb, As, Hg, Co, Cr and Ni, respectively. The average recoveries of Cu, Fe, Mn, Zn, Cd, Pb, As, Hg, Co, Cr and Ni at different levels varied between 70 and 120%. The reproducibility expressed as relative standard deviation of all tested elements was less than 20 %. The measurement uncertainty expressed as expanded uncertainty in terms of relative standard deviation at 95% confidence level for all elements is within the range 15.9%, 31.4%, 16.1%, 29.6% , 26.8%, 26%, 32.6%, 30%, 5.1%, 3.1%, 8.2% for Cu, Fe, Mn, Zn, Cd, Pb, As, Hg, Co, Cr and Ni,   respectively.
Keywords: Method validation, trace elements, water, atomic absorption spectroscopy, determination

INTRODUCTION

Heavy metals pollution in the environment is one of the most important problems for human (Deming 2005; Mikkelsen *et al.*, 2005 and Yaman 2005). With the development of mining, smelting and other industrial activities, heavy metals are increasingly being found in freshwater sources which can pose severe threats to human and environmental health. Pollution by heavy metals (such as Cd, Pb, etc.) not only affects the productivity of crops, but also the quality of the atmosphere as well as water bodies and threatens the health and life of animals and human beings by way of the food chain. The environmental pollution caused by these heavy metals is a long-term and irreversible process. Such metals are not required for routine functioning of the human body and can be toxic even at low concentration. Drinking water from a tap, such as a private well or public water system, is a source of potential exposure to environmental contaminants. Natural contamination of heavy metals usually originates from weathering of

minerals, rocks and aquatic environments which result in the entry of heavy metals into water bodies. Disposal of industrial effluents, wastes (domestic and industrial), such as sewage sludge and mining effluents, are other causes of contamination. Many of the metals are retained relatively strongly in the surface water and soil and do not readily leach out – causing accumulation that may ultimately pose a threat to humans, animals, plants and microbes, and its concentration has increased in the air, soil and waters, especially in big cities and industrial areas. Health effects of contaminants found are cardiovascular of blood toxicity, cancer, developmental toxicity, endocrine toxicity, immune-toxicity, kidney toxicity, gastrointestinal or liver toxicity, musculoskeletal toxicity, neurotoxicity, reproductive toxicity, respiratory toxicity and skin sensitivity. By heavy metals we shall understand the most toxic elements, primarily lead, cadmium, mercury, arsenic, selenium, tellurium, as well as copper, nickel, cobalt, zinc, chromium, silver, and some other metals. Extensive literature, including reviews (Robinson, 1982), Mizuike, 1983), and (Zolotov and Kuzmin, (1982) is devoted to concentration and determination of heavy metals in natural water.

Validity is an essential component of the measures that a laboratory should implement to allow it to produce reliable analytical data (ISO17025, 2005). Validation of analytical method is recognized as a potentially weak link in the quality chain of laboratories. The validation procedure needs to be considered the context of the fitness for the purpose and cost benefit criteria. Validation of analytical methods is the measurement of performance characteristics such as accuracy, precision, specificity, linearity and range, limits of detection and quantitation, intra-laboratory variations (robustness), and inter-laboratory variations (ruggedness).

The aims of this validation method study were to confirm that the analytical procedure employed for a specific test is suitable for its intended use validate the analytical method for water analysis and results from the method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.

MATERIAL AND METHODS

Chemicals and reagents:
All reagents must be pro Analyze quality.
- Deionized water, from water purification system (Gen Pure and Pacific Up. TKA / Germany).
- Nitric acid (HNO_3) (supra pure, 65%), (Merck-reagent grade).
- Nitric acid (2 mol/L). (130 ml of HNO_3 is diluted to 1L with distilled water) used for cleaning the digestion flasks.
- Nitric acid (0.3 % HNO_3) (5 ml conc. acid is diluted to 1L with distilled water).
- Reagents used as matrix modifier: A mixture of 10 gm of Ammonium-di-hydrogen phosphate ($NH_4H_2PO_4$) and 0.87 gm of Magnesium nitrate (Mg $(NO_3)_2$. 6 H_2O).

- Pb, Cd, Cu, Hg, Zn, Fe, Mn, As, Co, Cr and Ni stock standards, (1000 mg/L)(Merck) or similar quality).
- Intermediate and working solutions of Pb, Cd, and Cu prepared from stock solution with different concentrations in 0.3 % HNO_3.
- Hydrochloric acid, (37%), (Merck) or similar quality.
- Hydrochloric acid, (10%), 270 ml conc. Hydrochloric acid, (37%) is diluted to 1L with deionised water.

Preparation of reference standard solutions:

- **Metal stock standard solutions (1000 μg/ml) (Merck) or similar quality** Pb, Cd, Cu, Hg, Zn, Fe, Mn, As, Co, Cr and Ni metals.
- **Intermediate standard solutions:-**

I. (100 μg/ml) intermediate standard solutions of Pb, Cd, Cu, Hg, Fe, Zn, As, Mn, Co, Cr and Ni were prepared by diluting of 10 ml of standard solution (1000 mg/L) of Pb, Cd, Cu, Zn, Fe, Mn, As, Co, Cr and Ni to 100 mL with 0.3% HNO_3, while in the case of Hg with 10 % HCL .

II. (10 μg/ml) intermediate standard solution of Pb , Cd , As, Hg, Fe , Cu, Zn , Mn, Co, Cr and Ni were prepared by diluting of 10 ml of standard solution (6.5.2.1) to 100 ml with 0.3% HNO3 in case of Pb, Cd, As, Zn, Fe, Mn, Co, Cr and Ni, and with 10 % HCL in case of Hg.

III. (1 ug/ml) intermediate standard solution (1 μg/ml) for Pb, Cd , As, Zn , Co and Hg, were prepared by diluting of 10 ml of standard solution (10 μg/ml) to 100 ml with 0.3% HNO_3 in case of Pb , Cd , As , Zn, and Co, whereas with 10 % HCL in case of Hg.

Working standard solutions:-

- (0.1 μg/ml) working standard solutions for Cd, As and Hg, prepared by diluting of 10 ml of standard solution (1 μg/ml) to 100 ml with 0.3% HNO3 and with 10 % HCL in case of Hg.
- (20 μg/ml) working standard solutions (20 μg/ml) for Cu and Fe prepared by diluting of 20 ml standard solution (100 μg/ml) to 100 ml with 0.3% HNO3.

Spiking mixture standard solutions:-

I. Spiking mixture standard solutions for Cd, Pb, As, Cu, Mn, Fe, Zn, Cr, Ni, and Co, were prepared by diluting of 2 ml of stock standard solution (1000 mg/L) for Mn, 5 ml of standard solution (10 μg/ml) of each of Pb, As, and Zn, and 1.5 ml of standard solution (10 μg/ml) of Cd, 5, 5, 2.5, 2.5, and 3 ml of standard solution (100 μg/ml) of Fe ,Cu, Cr, Ni, and Co to 100 ml volumetric flask and then complete the volume with 0.3% HNO_3.

II. Spiking standard solution for Hg is prepared by diluting of 5 ml of standard solution (1 μg/ml) for Hg up to 100 ml with 10 % HCL.

Calibration standard solutions:-

- Calibration standard solutions for Pb is prepared by diluting of 0.5, 1, 2, and 3 ml of standard solution (1 μg/ml) up to 100 ml with 0.3% HNO_3 to get conc. 5, 10, 20 and 30 ug/L, respectively.
- Calibration standard solutions for Cd is prepared by diluting of 0.5, 1, 2, and 3 ml of standard solution (0.1 μg/ml) up to 100 ml with 0.3% HNO_3 to get conc. 0.5, 1, 2 and 3 (μg /L), respectively.

- Calibration standard solutions for Cu is prepared by diluting of 0.5, 5, 10, and 20 ml of standard solution (20 µg/ml) up to 100 ml with 0.3% HNO3 to get conc. 0.1, 1, 2, and 4 mg/L, respectively.
- Calibration standard solutions for Hg is prepared by diluting of 1 and 2 ml of standard solution (0.1 µg/ml) and   0.5, 1, 2, and 4 ml of standard solution (1 µg/ml) up to 100 ml with 10 % HCL to get conc.1,2, 5, 10, 20, and 40 µg /L, respectively for Hg.
- Calibration standard solutions for Fe is prepared by diluting of 0.1, 0.5, 1, 3, and 5 ml of standard solution (100 µg/ml) to 100 ml with 0.3% HNO_3 to get conc. 0.1, 0.5, 1, 3, and 5 mg/L, respectively.
- Calibration standard solutions for Zn is prepared by diluting of 1, 5 and 10 ml of standard solution (100 µg/ml) to get conc. 0.1, 0.5 and 1 mg/L, respectively, and 1, 5 ml of standard solution (1 µg/ml) to 100 ml with 0.3% HNO_3 to get conc.0.01, 0.05 (mg/L), respectively.
- Calibration standard solutions for Mn is prepared by diluting of 1 ml of standard solution (10 µg/ml) to get conc. 0.1 (mg/L), and 0.5, 1, and 2 ml of standard solution (100 µg/ml) to 100 ml with 0.3% HNO_3 to get conc. 0.5, 1, and 2 (mg/L), respectively.
- Calibration standard solutions for As is prepared by diluting of 2 ml of standard solution (0.1 µg/ml) of As up to 100 ml with 0.3% HNO_3 to get conc (2 µg /L), and dilute 0.5, 1, 2, and 4 ml of standard solution (1 µg/ml) up to 100 ml with 0.3% HNO_3 to get conc. 5, 10, 20 and 40 ug/L, respectively.
- Calibration standard solutions for Co is prepared by diluting of 5 ml and 10 ml of standard solution (1 µg/ml) of Co up to 100 ml with 0.3% HNO_3 to get conc. (0.05 and 0.1 mg/L), and dilute 0.5 ml of standard solution (100 µg/ml) up to 100 ml with 0.3% HNO_3 to get conc. 0.5 mg/L, respectively. Dilute 1 ml of standard solution (6.5.2.1) up to 100 ml with 0.3% HNO_3 to get conc. 1 mg/L. Dilute 2,4, and 6 ml of standard solution (100 µg/ml) up to 100 ml with 0.3% HNO_3 to get conc. 2,4, and 6 mg/L, respectively.
- Calibration standard solutions for Cr is prepared by diluting of 1 ml and 3 ml of standard solution (10 µg/ml) of Cr up to 100 ml with 0.3% HNO_3 to get conc. (0.1 and 0.3 mg/L), and dilute 0.5, 1, 2, 3, and 5 ml of standard solution (100 µg/ml) up to 100 ml with 0.3% HNO_3 to get conc. 0.5, 1, 2, 3, and 5 mg/L, respectively.
- Calibration standard solutions for Ni is prepared by diluting of 5 ml and 10 ml of standard solution (1 µg/ml) of Ni up to 100 ml with 0.3% HNO_3 to get conc. (0.05 and 0.1 mg/L), and dilute 0.5, 3, and 5 ml of standard solution (100 µg/ml) up to 100 ml with 0.3% HNO_3 to get conc. 0.5, 3, and 5 mg/L, respectively, and dilute 10 ml of standard solution (10 µg/ml) up to 100 ml with 0.3% HNO_3 to get conc. 1 mg/L.

Apparatus and Equipment:
- Atomic Absorption Spectrometer (Thermoscientific M6 series AA Spectrometer with   Zeeman and deuterium - background corrections).
- Graphite furnace GF95Z with auto sampler (FS95).
- Flame (air/acetylene burned through 10 cm premix burner) and (air/acetylene/nitrous oxide burned through 5 cm premix burner).

- Background correction, deuterium Lamp, and Zeeman
- Hollow cathode lamps (HCL) specific for each element (Pb, Cd, Cu , Zn, Fe, and Mn).
- Gas supply for argon, acetylene, and nitrous oxide of high purity.
- Computer with software of SOLLAR AAS SYSTEM.
- Atomic Absorption Spectrometer (Thermoscientific S4 series AA Spectrometer with Continuous flow vapor (VP100), with autosampler (CETAC 520).
- Background correction, deuterium Lamp and Zeeman.
- Hollow cathode lamps (HCL) specific for each element .
- Polypropylene tubes 50 ml.
- Volumetric flask 50 ml.

All glass ware should be carefully soaked overnight in nitric acid 2 mol HNO_3 then washed and rinsed well three times by deionised water, then kept dry until use*.*

Procedure:

Sample preparation:

The samples were prepared according to Standard Methods for the Examination of water and wastewater, 20th Edition, (2000). The required sample preparation depends on the metal form being measured (dissolved, suspended, total, or acid- extractable).

Samples containing particulates or organic material generally require pretreatment before spectroscopic analysis. The colorless, transparent samples (primarily drinking water), no odor, and single phase may be analyzed directly by atomic absorption spectroscopy (flame or electro thermal vaporization) for total metals determination without digestion. For further verification or if changes in existing matrices are encountered, compare digested and the undigested samples to ensure comparable results. On collection, acidify such samples to pH < 2 with conc. nitric acid (**65%)** (1.5 ml HNO_3/L is usually adequate for drinking water) and analyze it directly by direct aspiration by atomic absorption spectroscopy (flame or electro thermal vaporization) for total metals determination without digestion. However, digest all other samples before determining total metals. To analyze for dissolved metals, filter sample, acidify filtrate, and store until analyses can be performed.

Atomic Absorption measurement:

The instrument is started, calibrated by measuring of CM (Characteristic Mass) and programmed according to the instructions of the manufacture. Tables from (1 to 4) described the instrumental parameters of Thermo M6-AAS and S4 Cold Vapour (VP100) - AAS and used in the determination of the tested elements.

Table (1): Instrumental Parameters of Flame Atomic Absorption Spectrometer (AAS):-

Parameter	Cu	Fe	Zn	Mn	Co	Ni	Cr
Technique	Flame	Flame	Flame	Flame	Flame	Flame	Flame
Wave length (nm)	324.8	246.3	213.9	279.5	240.7	232	357.9
Slit band pass (nm)	0.5	Full 0.2	0.5	0.5	0.2	0.1	0.5nm
Lamp current %	75% - 80%	75% - 100%	75% - 100%	75% - 80%	75% - 100%	75% - 100%	100%
Signal type.	Continues	Continues	Continues	Continues	Continues	Continues	Continues
Background correction.	On	On	On	On	On	On	On
Heating source.	Air – acetylene With flow rate (1.1 l/min)	Air – acetylene with flow rate (0.9 l/min	Air – acetylene With flow rate (1.2 l/min	Air – acetylene With flow rate (1.5 L/min)	Air – acetylene With flow rate (1.3 L/min)	Air – acetylene With flow rate (1.2 L/min)	Nitrous oxide-acetylene With flow rate (2.4 L/min)

Table (2): Instrumental Parameters of Graphite Furnace Atomic Absorption Spectrometer (AAS):-:

Parameters	Pb	Cd	As
Technique	Graphite	Graphite	Graphite
Wave length (nm)	217.0	228.8	
Slit band pass (nm)	0.5	0.5	0.5
Lamp current %	75% - 100%	75% - 100%	75% - 100%
Signal type.	Transient	Transient	Transient
Background correction	On	On	On
Inert gas	Argon	Argon	Argon
Heating source	Electro thermal	Electro thermal	Electro thermal

Table (3): Graphite Furnace Program for Cd, Pb and As:

Element	Phase	Temp. (^oC)	Time (sec)	Ramp ^oC/Sec	Gas type	Gas flow (ml/min)	Command	Matrix modifier	Inj. volume
Cd & Pb	Drying	130	35	30	3	2		$NH_4H_2PO_4$ +Mg $(NO_3)_2$x $6H_2O$	20 ul sample + 10 ul matrix modifier
	Ashing	800	20	50	3	2			
	Atomization	1800	3	0	3	0	Read, Temp. control		
	Cleaning	2500	3	0	3	2	TC		
	Cooling	20	5	0	3	2			
As	Drying	130	45	10	3	2		Pd $(NO3)$+Mg $(NO_3)_2$x $2H_2O$	20 ul sample + 5 -10 ul matrix modifier
	Ashing	1100	20	15	3	2			
	Atomization	2400	3	0	3	0	Read, Temp. control		
	Cleaning	2500	3	0	3	2	TC		

Table (4): Instrumental Parameters of Cold Vapor (VP100) AAS for Hg:

Parameters	*Hg*
Technique	Cold vapor
Wave length (nm)	235.7
Slit band pass (nm)	0.5
Lamp current %	75% - 100%
Signal type.	Continues
Background correction	On
Inert gas	argon
Heating source	No heating
Flow rate	150 ml/min
The pump speed	60
Reductant	Acidified borohydride soln. 1 % (w/v)

Calibration:

The calibration curves of Pb, Cd, Cu, Hg, Zn, Fe, As, Mn, Co, Cr, and Ni (Absorbance as Y axis and conc. as X axis) must be constructed with every set of samples.

Normal segmented curve is used in the case of GAAS, while in the case of the FAAS normal linear curve must be used. The concentration of the metal in the sample solution (the instrument is started, calibrated by measuring of CM (Characteristic Mass) and programmed, according to the instructions of the manufacture.

Calculation: **For determination of metal concentration by direct aspiration by AAS:** Read the metal value from the calibration curve or directly from the read-out system of the instrument.

mg metal / L= C.F   Where:

C= metal concentration as read directly from the instrument or from the calibration curve, mg/L, and F= dilution factor.

RESULTS AND DISCUSSION

The method validation:

The selected parameters for the verification were mainly taken from Eurachem guidelines (1998).

Limit of quantitation (LOQ):

The limit of quantitation is the minimum concentration of analyte in the test sample that can be determined with acceptable precision (repeatability) and accuracy under the stated conditions of the test. The lowest practical limits of quantitation of each element were estimated by repeated the analysis of spiked water samples on about the expected lowest levels. The LOQ's of the elements were found to be in range from 0.001 to 1 mg/L. The average recovery (%), and the relative standard deviation (CV %) for each element are shown in Table (5).

Table (5): Recovery tests at different concentration levels of trace elements in the water samples:

Elements	Level 1 (LOQ)				Level 2 (spiking level)				Level 3			
	Expected (mg/L)	No. of replicates	Mean recovery %	CV%	Expected (mg/L)	No. of replicates	Mean recovery %	CV%	Expected (mg/L)	No. of replicates	Mean recovery %	CV%
Cu	0.1	7	101	8	1	7	100	5	2	7	99	4
Fe	0.05	7	91	14	0.1	7	102	16	0.2	7	109	13
Zn	0.005	7	116	3	0.01	7	98	15	0.02	7	112	5
Mn	0.1	7	97	5	0.4	7	94	8	0.8	7	96	6
Cd	0.3	7	107	5	3	7	95	2	6	7	107	5
As	1	7	76	3	10	7	99	5	20	7	97	6
Hg	1	7	79	9	1	7	79	9	2	7	89	5.8
Co	0.05	7	108	14.9	3	7	104	2.4	6	7	99	1.1
Cr	0.1	7	94	5.5	2.5	7	100.4	1.3	5	7	99	1
Ni	0.05	7	110	9.7	2.5	7	104	3.9	5	7	98	1.1
Pb	2	7	98	13	10	7	108	5	20	7	95	2

Recovery tests:

The method recoveries for all 11 elements were tested by performing repeated spiked water samples at different concentration levels. The average recovery was determined and relative standard deviation on each level was calculated according to the following equation. RSD% = (S/X. 100), where S is Standard deviation, x is Mean of the found concentration in (n) samples. The results of recovery tests are shown in the table (5).

Linearity:

Linearity was tested by performing recovery tests at concentrations of used trace elements in the water samples. The correlation between the concentration and response of each metal was studies. The method showed to be linear from the LOQ levels up to 4, 5, 2, 1, 0.004, 0.03, 0.04, 0.04, 6, 5, and 5 mg/L levels for Cu, Fe, Mn, Zn, Cd, Pb, As, Hg, Co, Cr and Ni, respectively. The results of recovery tests are shown in the table (5).

Repeatability:

The closeness of agreement between the results of successive measurements obtained with the same method on identical test sample, under the same conditions (same operator, same apparatus, same laboratory and short intervals of time was performed with seven replicates of real contaminated water samples. Repeatability relative standard deviations were found to be 5.04 %, 14.8 %, 5.9 % , 14 %, 6.8%, 1.3%, 1.79%, 1.59%, 1.27%, 1.92 and 1.53% for Cu, Zn, Mn , Fe, As, Cd, Hg, Pb, Co, Cr, and Ni, respectively. The results of recovery tests are shown in the table (6).

Table (6): The repeatability experiments are shown in the following table:

Replicate Number	Cu (mg/L)	Zn (mg/L)	Mn (mg/L)	Fe (mg/L)	As (mg/L)	Cd (mg/L)	Hg (mg/L)	Pb (mg/L)	Co (mg/L)	Cr (mg/L)	Ni (mg/L)
1	1.102	0.0094	0.780	0.050	0.87	1	0.736	0.77	3.95	3.23	1.14
2	1.101	0.0106	0.790	0.048	0.9	0.99	0.768	0.75	3.88	3.11	1.12
3	1.014	0.0097	0.800	0.047	0.95	1.01	0.729	0.74	3.9	3.14	1.1
4	1.014	0.0104	0.790	0.053	1.04	1	0.738	0.76	3.86	3.09	1.13
5	1.014	0.0101	0.680	0.037	0.89	1.02	0.73	0.75	3.97	3.22	1.13
6	1.028	0.0117	0.790	0.047	0.88	0.98	0.74	0.753	3.82	3.11	1.16
7	1.139	0.0070	0.78	0.036	0.96	0.99	0.735	0.774	3.86	3.12	1.13
Mean	1.06	0.01	0.77	0.05	0.93	0.998	0.74	0.76	3.89	3.14	1.13
Sd	0.05	0.0015	0.05	0.01	0.064	0.0135	0.0132	0.012	0.0493	0.0602	0.0173
RSD$_r$%	5.04	14.78	5.88	13.96	6.83	1.35	1.79	1.59	1.27	1.92	1.53

Reproducibility

The closeness of test result obtained under conditions, with the same method on identical test items in different laboratories with different operators using different equipments. In this study, both of intra-laboratory reproducibility and inter– laboratory reproducibility were considered. The intra-laboratory reproducibility was performed by repeating the analysis of fortified water sample at certain level of each element 7 times by different operators at different times. The reproducibility relative standard deviation (CV %) ≤ 16 of each element is shown in Table (7).

Table (7): Reproducibility experiments are shown in the following table:

Replicate Number	Cu (mg/L)	Zn (mg/L)	Mn (mg/L)	Fe (mg/L)	As (mg/L)	Cd (mg/L)	Hg (mg/L)	Pb (mg/L)	Co (mg/L)	Cr (mg/L)	Ni (mg/L)
1	0.106	0.0094	0.410	0.109	10.0700	2.84	1.71	11.6	3.12	2.46	2.54
2	0.093	0.0106	0.330	0.107	9.1400	2.88	1.8	11	3.04	2.53	2.57
3	0.103	0.0097	0.400	0.111	9.1800	2.83	1.95	10.42	3.10	2.47	2.55
4	0.106	0.0104	0.390	0.104	9.8200	2.82	1.8	10.6	3.04	2.56	2.54
5	0.112	0.0101	0.380	0.095	10.51000	2.77	1.8	10.72	3.08	2.51	2.62
6	0.089	0.0117	0.340	0.120	10.2000	2.94	1.7	10.07	3.21	2.53	2.53
7	0.101	0.0070	0.380	0.070	9.7300	2.91	1.6	10.71	3.07	2.51	2.84
Mean	0.101	0.0098	0.3757	0.102	9.881	2.841	1.770	10.815	3.113	2.5	2.598
Sd	0.008	0.00145	0.0299	0.0161	0.52	0.06749	0.10240	0.50285	0.0750	0.033	0.1025
RSD$_R$ %	7.9%	14.8%	8%	15.7%	5.2%	2.4%	5.8%	5%	2.41%	1.3%	3.9%

The interlaboratory reproducibility was considered by performing inter-laboratory comparison with different laboratories by participation in Laboratory Environmental Analysis Proficiency Testing (LEAP). The proficiency test samples were analyzed by using the developed method. The

Z- Score of each test sample were accepted (-2 ≤ Z ≥2). The results of the proficiency tests are shown in table (8).

Table (8): Inter-Laboratory comparison between QCAP and Leap proficiency tests:

Elements	R08014			R08015			R08016			R08018			R08019		
	Found (mg/L)	Assign	Z-score	Found (mg/L)	Assign	Z-score	Found (mg/L)	Assign	Z-score	Found (mg/L)	Assign	Z-score	Found (mg/L)	Assign	Z-score
Cd	1.01	1.04	-0.3	0.48	0.511	-0.6	2.03	2.1	-0.3	2.07	2.07	0	3.22	3.03	0.6
Cr	2.59	2.5	0.4	0.95	0.97	-0.2	3	3.06	-0.2	3.02	2.99	0.1	0.98	0.997	-0.2
Ni	1.55	1.51	0.3	2.48	2.54	-0.2	1.05	1.04	0.1	4.05	4.12	-0.2	2.08	2.1	-0.1
Pb	0.75	0.728	0.3	1.01	0.987	0.2	2.02	2.05	-0.1	2.04	2	0.1	4.02	3.96	0.1
Cu	3.1	3.07	0.1	3.9	3.98	-0.2	1.1	1.03	0.7	5.3	5.11	0.4	1	0.996	0
Zn	2.33	2.09	1.2	3.4	3.04	1.2	4.3	4.17	0.3	2.17	2.07	0.5	5.2	5.01	0.4
As	1.18	0.984	2	-	-	-	2.05	2.01	0.2	2.04	2.03	0	1.13	1.04	0.9
Hg	0.768	0.996	0.6	-	-	-	-	-	-	-	-	-	0.7	0.748	-0.6

Measurement Uncertainty

Accumulated data from different quality control procedures was used for estimation of measurement uncertainty for the method. Eurachem guidelines (2000) were followed in estimation of measurement uncertainty.

Relative Standard Uncertainty:

The random effects were estimated as relative standard deviation of repeated spikes samples at different concentration levels. The bias of the analytical procedure was investigated from recovery data using spiked samples. The lowest recovery (89%) was observed for Cu with standard deviation S = 8 and n= 7, the lowest recovery (70%) for Zn with standard deviation S = 14.6 and n= 7. The lowest recovery (70%) was observed for Fe with standard deviation S = 16.1 and n= 6 and the lowest recovery (83%) for Mn with standard deviation S = 7.5 and n= 7. The lowest recovery (95 %) was observed for Cd with standard deviation S = 12.5 and n= 20, the lowest recovery (99%) for as with standard deviation S = 15.1 and n= 7. The lowest recovery (98 %) was observed for Pb with standard deviation S = 12.2 and n= 8, the lowest recovery (89%) for Hg with standard deviation S = 12 and n= 12. The lowest recovery (100.4%) was observed for Cr with standard deviation S = 1.3 and n= 8, the lowest recovery (104%) for Co with standard deviation S = 2.5 and n= 8, and the lowest recovery (104%) for Ni with standard deviation S = 4.1 and n= 8.

The Standard uncertainty was calculated as the standard deviation of the mean ($\frac{s}{\sqrt{n}}$):

Element	standard uncertainty	Relative Standard Uncertainty (U_{Bias})
Zn	5.499%	5.612%
Fe	6.066 %	5.947 %
Cu	3.016%	2.986%
Mn	2.827%	3%
Cd	2.795%	2.986 %
As	5.707 %	6.17 %
Pb	4.313%	4.401
Hg	3.47 %	4.332 %
Co	0.884%	0.85 %
Cr	0.46 %	0.458 %
Ni	1.45%	1.394 %

A significance test (t-test) was applied to test if the recovery is significantly different from 100 %. In the case of Cu for 6 degrees of freedom t_{tab} was 2.45 and t_{calc} was 0.33, in the case of Zn, for 6 degrees of freedom t_{tab} was 2.45 and t_{calc} was 0.36, in the case of Fe, for 6 degrees of freedom t_{tab} = 2.45 and t_{calc} = 0.33 and in the case of Mn, for 6 degrees of freedom t_{tab} = 2.45 and t_{calc} = 2.12. In the case of As, for 6 degrees of freedom t_{tab} = 2.45 and tcalc= 1.31, in the case of Pb, for 7 degrees of freedom t_{tab} = 2.36 and t_{calc} = 0.46 In the case of Cr, for 7 degrees of freedom t_{tab} = 2.36 and t_{calc}= 0.87

$$t_{calc} = \frac{\left|1 - \overline{\text{Re}c}\right|}{StndardUncerta\,\text{int}\,y}$$, Where: $\overline{\text{Rec.}}$ is mean recovery

In this case Co and Ni for 7 degrees of freedom for each, the t_{tab} = 2.36 and t_{calc}= 4.53 for Co and the t_{tab} = 2.36 and t_{calc} =2.76 for Ni, and In this case Cd and Hg for 20, and 12 degrees of freedom, respectively, the t_{tab} = 2.09 and t_{calc} = 2.29 for Cd and the t_{tab} = 2.2 and t_{calc} = 5.74 for Hg, (since t $_{calc}$ is greater than t tab). However, if the t $_{calc}$ is greater than ttab, the recovery is statistically significantly different from 100 %, but in the normal application of the method no correction is applied. The uncertainty must be increased to take account of the fact that the recovery has not been corrected.

Other sources: All the important volumetric measuring devices are under regular control. Precision and recovery studies take into account the influence of the calibration of the different volumetric measuring devices because during the investigation various volumetric flasks and pipettes have been used. The uncertainty due to reference standard preparation was estimated by accounting for reference standard purity tolerance, volumetric flask and pipettes. The uncertainty component due to reference standard preparation for all tested elements was found to be 0.8 %, for each.

Combined Uncertainty (UC)

Combined uncertainty, is the positive square root of the sum of the squares of different uncertainty components. In case precision is calculated from recovery tests. Combined uncertainty was found to be 7.9%, 14.8%, 8%, 15.7%, 13.4%, 16.3%, 13%, 15%, 2.7%, 1.5%, and 4.3% for Cu, Zn, Fe, Mn, Cd, As, Pb, Hg, Co, Cr, and Ni. The following equation is used for combined uncertainty calculations;

$$U_C = \sqrt{(U_{precision})^2 + (U_{sp}) + (U_{Rec})^2 + + U_{Ref}}$$

Expanded Uncertainty

Expanded uncertainty is obtained by multiplying the combined uncertainty by a coverage factor k, for confidence level of 95% k is 2. The expanded uncertainty (at 95 % confidence level) was found to be 15.9%, 29.6%, 31.4%, 16.1% , 26.8%, 32.6%, 26% , 30%, 2.7%, 1.5%, and 4.3% for Cu, Zn, Fe, Mn, Cd, As, Pb, Hg, Co, Cr, and Ni, respectively. Table (9) summarizes the uncertainty calculations.

Table (9): Summary of the uncertainty estimation of the tested metals:

Uncertainty components	Relative Standard Uncertainty											Remarks
	Cu	**Zn**	**Fe**	**Mn**	**Cd**	**As**	**Pb**	**Hg**	**Co**	**Cr**	**Ni**	
1.Precision	7.9%	14.8%	15.7%	8 %	13.4 %	16.3%	13%	15%	2.4%	1.3%	4%	From recovery tests
2. Bias	2.99%	5.61%	5.95%	3%	2.99%	6.17%	4.3%	3.47%	0.85%	0.46%	1.39%	Spiked Samples
3. Others (Standards preparation)	0.8%	0.8%	0.8%	0.8%	0.8%	0.8%	0.8%	0.8%	0.8%	0.8%	0.8%	Reference standard
Combined Uncertainty (Uc)	7.9%	14.8%	15.7%	8 %	13.4 %	16.3%	13%	15%	2.7%	1.5%	4.3%	-
Expanded Uncertainty (2xUc)	15.9%	29.6%	31.4%	16.1%	26.8%	32.6%	26%	30%	5.4%	3.1%	8.6%	-

Conclusions:

The established method was found to be precise. Satisfactory recoveries and repeatability were observed and satisfactory Z score results of LEAP's proficiency tests

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تطوير طريقة تقدير العناصر الثقيلة فى المياة بواسطة جهاز الامتصاص الذرى

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المعمل المركزى لتحليل متبقيات المبيدات والعناصر الثقيلة فى الأغذية – الدقى- الجيزة

تم استخدام طريقة تقدير بعض العناصر الثقيلة الذائبة فى عينات المياة بعد إجراء خطوات إثبات كفاءة الطريقة وذلك ضمن متطلبات المعمل المركزى لتحليل متبقيات المبيدات والعناصر الثقيلة فى الأغذية وتشمل العناصر الاتية النحاس ــ الحديد ــ الزنك- المنجنيز- الكادميوم- الرصاص- الزرنيخ ــ الزئبق ــ الكوبلت ــ الكروم ــ والنيكل. ويتم تقدير تلك العناصر فى عينات المياة المعاملة بالحامض بواسطة الحقن المباشر لجهاز الامتصاص الذرى بدون الحاجة لهضم العينات. وتم اختبار كفاءة الطريقة عن طريق اختبار العناصر المختلفة للطريقة منها حدود التقدير الكمى للعناصر الثقيلة المختبرة وتوكيد خطية الطريقة المستخدمة. وكانت حدود التقدير الكمى للعناصر الاتية النحاس- الحديد ــ المنجنيز- الزنك- الكادميوم- الرصاص- الزرنيخ ــ الزئبق ــ الكوبلت ــ الكروم ــ والنيكل هى ٠.١ ــ ٠.٠٥ ــ ٠.١ ــ ٠.٠٠٥ ــ ٠.٣ ــ ٠.٠٠٥ ــ ٢ ــ ٠.١-١-٠.٠٥ ــ ٠.١ ــ ٠.٠٥ مجم / لتر؛على التوالى. كما اثبتت ان الطريقة خطية عند التركيزات المختلفة من حدود التقدير الكمى حتى ٤- ٥- ٢- ١- ٠.٠٠٤ -٠.٠٣ -٠.٠٤ -٠.٠٤ -٦- ٥- ٥ مجم / لتر للنحاس و الحديد و المنجنيزو الزنك و الكادميوم و الرصاص و الزرنيخ و الزئبق و الكوبلت و الكروم والنيكل ؛على التوالى. كما تراوح متوسط معدل الاسترجاع للطريقة مابين ٧٠ الى ١٢٠%. كما كان الانحراف المعيارى للعناصر المختبرة أقل من ٢٠%. وكذلك كانت نسبة اللايقين (عند مستوى يقين ٩٥%) للعناصر مجال الدراسة أقل من ٣٣%.

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