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Method Development/ Validation and Uncertainty Measurement for Determination of Zinc (Zn) Using Atomic Absorption Spectrophotometry Technique

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Abstract

Samples of fertilizer are analyzed to determine their nutrient content; however, the findings vary depending on the method used. The major goal of the study was to design as well as to test a method for determination of Zinc through Atomic Absorption Spectrophotometer. The Soil & Water Testing Laboratories, Dera Ghazi Khan, validated the use of the Atomic Absorption Spectrophotometer Technique to measure the Zinc concentration in fertilizer samples. Repeatability, reproducibility, detection limit, quantification limit, linearity, recovery, selectivity and bias were all tested as part of the technique of validation process. The results revealed that the repeatability, (Relative standard deviation of 1.01), reproducibility (T-value 0.05 is less that tabulated-T value 2.0262, LOD 0.98 % Zinc in the sample, LOQ 0.9 % Zinc in the sample), recovery 98.9%, Linear curve for the Zinc Conc. was attained from 0.5 mg Kg^{-1} to 2.0 mg Kg^{-1} (Zn) showing Linearity R^2 = 0.999 %. The laboratory participated in Proficiency testing program of USA (Magruder Fertilizer Sample Check Program) Test results of the Zn samples analyzed by this method of Zinc have Z-score values of -0.93 and 1.65 during 2021 and 2022 which fall in acceptable range (i.e., -2 to +2). The uncertainty of the method was 0.0011%. Hence the Soil and water testing Laboratory, Dera Ghazi Khan is capable of performing Zinc analysis by the suggested method as per standard. Based on this relationship, we may conclude that the strategy worked quite well. As a result, the approach can be used to determine Zinc levels in fertilizer samples.

Keywords: Uncertanity; Validation; Atomic Absorption; Zinc

1. Introduction:

Analytical methods are crucial in many different domains, including the study of food products, environmental factors, pharmaceuticals, and medicinal issues, among others. It is necessary to use the simplest analysis technique possible to obtain the most reliable, reproducible, and accurate data (Ahmad *et al*., 2015; Gumustas *et al*., 2013; Kurbanoglu *et al*., 2014). The reliability of the procedure is monitored by validation, which is based on the verification of the results. The criteria for judging a method's validity

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includes (i.e., accuracy, precision, detection and quantification limit, sensitivity as well as repeatability. For the purpose of achieving high quality results, validated techniques are inevitable (Aboul-Enein, 2012; Arkaban *et al*., 2021; Striegel, 2021). According to ISO (2005) standard, any analytical method must be validated to ensure that it meets the necessary standards. This study's goal was to validate the use of the Atomic Absorption Spectrophotometry technique to measure or analyze zinc in samples of fertilizer.

1.1. Objectives:

- To develop and validate Atomic Absorption Spectrophotometry technique for Zinc analysis in fertilizer samples.
- Measurement of uncertainty of the validated method and to calculate the uncertainty budget in our existing Laboratory environment.

2. Materials and Methods:

2.1. The Preparation of Standard Solutions:

Using the Bano *et al*. formula, the Certified Reference Material (i.e Zn 1000 ppm) was utilized to prepare the working standards for the solutions. Where V1- is the quantity taken from the stock solution, C2 is the needed Zn concentration (ppm), and C1 is the stock solution's concentration (ppm), C1V1 = C2V2 (ml).

2.2. Measurement of Zinc (Zn):

2.2.1. Aqueous Extraction:

1.00 g sample of fertilizer was taken in 100 mL glass beaker. Added about 75mL D.I water and boiled for 30 minute. Filtered in 1 litre volumetric flask. Made the volume with D.I water. Rediluted if needed. Analysed the quantity of Zn in solution (mg/L) from calibration curve or digital concentration readout following the standard operating parameters.

Calculation:

Zn% = Reading x dilution factor/10000

Table 1: Certified Reference Material (CRM)

Concentration	**Company**	**Product ID**	**Lot No.**
1000ppm Zn	Agilent Technologies	5190-8326	0100792248

Fertilizer Sample Detail

Sample Code	**Product Name**	**Fertilizer Manufacturer**	**Guaranteed Contents**
AB-13	Zinkron Plus	Suncrop Pesticides	Zn: 10%

2.3. Repeatability:

The nearness of agreement between the independent results was obtained with the same analytical method on the same test material, under the identical conditions (same user, equipment and same laboratory and after short time interval) the measurement of repeatability is considered as the relative standard deviation which is termed as 'repeatability'.

2.4. Precision:

In general, repeatability and reproducibility were used to assess precision such as the relative standard deviation (Zinc %). The Zinc (Zn) repeatability test was conducted in a laboratory setting with similar operator, equipment, and small-time intervals. The repeatability measurement was calculated using the relative standard deviation.

2.5. Limits of Detection (LOD) and Quantification:

LOD and LOQ calculations were used to assess the Atomic Absorption Spectrophotometer sensitivity. The lowest detectable dose (LOD) is the minimum concentration of any chemical that can only be necessarily detected and clearly distinguished from zero, but not always quantifiable. According to Gonzalez *et al*. (2010) and Renger *et al*. (2011), the LOQ is considered as the lowest amount of any drug that can be assessed with satisfactory level of precisions and accuracy.

2.6. Recovery:

The calculations of Zn recovery were used to test the correctness of the method under study. The recoveries studies were performed in order to verify

Zn loss because of contamination during preparation of sample and matrix interference during analysis in order to evaluate the method's accuracy. According to Taverniers *et al.* (2004), the satisfactory range of the recovery is 95% to 105% for analyte concentrations of 1 lg/ mL.

For the measurement of uncertainty, the Eurachem Guide was employed. This uncertainty in the results could be because of several factors including operator, analytical method, accommodation and environment as well as reagents and instruments. Combined uncertainty is due to the combined impacts of all above mentioned factors. The uncertainty budget comprised of all the uncertainty of all the aspects (Cortez, 1995; Örnemark, 2004). This uncertainty calculation was done at 68% confidence level. According to ISO/IEC 17025 standard the testing laboratories have to represent their uncertainties with clear confidence level which is narrated as expanded uncertainty (Aslam *et al*., 2021; Nazir *et al.*, 2020; van der Veen and Cox, 2021)

Expanded uncertainty = Combined uncertainty × confidence level.

3. Results:

3.1. Precision:

In general, repeatability and reproducibility were used to assess precision such as the relative standard deviation (Zinc %). The Zinc (Zn) repeatability test was conducted in a laboratory setting with the similar operator, equipment, and small-time intervals. The repeatability measurement was calculated using the relative standard deviation, which resulted in a repeatability standard deviation of 1.01 %. The repeatability data are provided in Table 2.

Table 2: Repeatability for analysis results of Zinc fertilizer

S. No.	Repeat	Zn=10%
1	1	9.9
2	2	9.8
3	3	9.9
4	4	10
5	5	9.9
6	6	10
7	7	9.8
8	8	9.7
9	9	9.9
10	10	10
	Average%	9.89
	Stdev %	0.099
	RSD%	**1.01**

Whilst examining the reproducibility of Zn, it was determined if the Atomic Absorption Spectrophotometer readings of Zn standards were consistently accurate (same for various parameters). This was only taken into account for system-related errors and not for errors related to sample handling or preparation (Eka *et al*., 2012; Horwitz and Latimer, 2005; Pointner *et al*., 2014; Ullah *et al*., 2017). The T-test was used to calculate the data reproducibility of two different analysts who performed Zn analyses on Atomic Absorption at various times, and the results exhibited that the calculated T value (0.05) value is less than the T-tabulated value (2.262). As a result, the results were not significantly different from one another, and the procedure can produce repeatable outcomes. On the other hand, the two analysts separately did a duplicate determination having relative standard deviations of 0.099 and 0.1033%, respectively, at various times.

Reproducibility is seen as successful; as a result, the parameter receives a passing grade. Table 2 provides the reproducibility results. The highest standard deviation (relative RSD) values that are allowed for the analyst concentration of 1 lg/L are approximately 16%. As a result, the procedure can produce repeatable results. The parameter is given a pass grade since reproducibility is seen as successful (González *et al*., 2010; González and Herrador, 2007; Uno, 2016).

Suncrop Pesticides, Zinkron Plus of known concentration of Zinc (Zn=10%) was used for repeatability, reproducibility and other studies. The data of 10 repeats (Table-2) predict that the Zinc method is repeatable with relative standard deviation (RSD) of 1.01% which is fairly less than 15% indicating homogeneity of data. Hence parameter is categorized as pass.

3.2. Reproducibility:

The data (Table-3) explain closeness of Zinc results gained independently with the similar methodology on the same test material but under dissimilar environment (different user, different environmental conditions and after different time intervals). T-test was employed in this study.

Table 3: Reproducibility of Zinc test result

S.No.	Analyst 1	Analyst 2
1	9.9	10
2	9.8	9.8
3	9.9	9.7
4	10	9.8
5	9.9	9.9
6	10	9.9
7	9.8	10
8	9.7	10
9	9.9	9.8
10	10	9.9
Average	9.89	9.88
SD	0.099	0.1033

T- Test= ((9.89-9.88)/SQRT ((0.099) *2/10) +(0.1033) *2/10) = 0.05; **T- Tabulated =2.262 at 95% confidence level**

As per t-test, calculated t value (0.05) is as compared to tabulated t value (2.262) therefore these results are non-significant with each other and method is able to give reproducible result while duplicating analyses with standard deviation of ±0.099 and ±0.1033 %, respectively done by the two different analysts working alone at different interval of time. Reproducibility is therefore considered to be successful; therefore, reproducibility is marked as pass.

3.3. Method Limit of Detection (LOD):

The method detection limit (LOD) is defined as the minimum concentration of a substance that can be measured and reported with 95% confidence level that analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte. The LOD in this study is obtained as 0.298%Zinc in a fertilizer sample by multiplying method factor). The data of ten samples was used to determine LOD.

LOD = Value of blank +k. s

Where k= a factor which is multiplied with the standard deviation for calculating the uncertainty. In this context, a factor of 3 is employed, s= standard deviation for a sample with a very little content of the particular analyte or for blank specimen. In present case, no blank value is involved being machine is adjusted to zero for every observation (Blank=0). Consequently, the standard deviation is for the laboratory reproducibility. Now;

LOD = blank value + $k.s_r$ = *0 + 3 x* 0.099= 0.298 %

3.4. Method Limit of Quantification (LOQ):

The LOQ is the minimum level of any analyte that can be analyzed with an acceptable level of performance. Calculation of LOQ is done in most of the cases as the concentration of any analyte corresponding to the standard deviation obtained at very low level multiplied with a factor, kq which is commonly used as 10. Following this practice, the LOQ in this study was obtained as 0.9 % Zinc (in fertilizer sample) after multiplying method factor. The LOQ in this case is calculated as the blank value + 10 times of the standard deviation of the repeatability as detailed below:

LOQ = Blank + k .s_r = 0 + 10 x 0.099= 0.9 %

3.5. Calibration Curve:

The concentration of a sample was obtained from calibration curve (at 5 point) by reading of the concentration that correspond to the instrument reading, corrected for the blank and multiplied with the dilution factor. A calibration curve should have the capability for computing a sample concentration by using a linear portion of a "curvy linear" relationship. The 5-points K calibration curve dully shown in Figure-1 carries the R2value as 0.999 and it is greater than critical limit of 0.95, therefore, parameter is pass and characterized by good behaviour and predictability.

Table 4: Standard curve for Zinc determination

Zinc (ppm)	Reading
0.0	0
0.5	0.23
1.0	0.47
1.5	0.69
2.0	0.92

3.6. Linearity:

Linearity is the condition where dependent variable has a relation with one or more independent variables and hence, can be calculated as the linear function of independent variable (Zn). The linear line in Figure-1 of the above-mentioned study is characterized by good behavior and predictability being R2=0.994which is >0.95 as required by the criteria.

3.7. Recovery:

For Zn samples falling near to middle of calibration curve and also nearer to the highest calibrator, the recorded recovery (100.56 %) is within admissible range of criteria i.e., ± 5 % of range of recovery (Table-5), Thus, the method in question is graded as pass.

Table 5: Evaluation of Zinc Recovery

S. No.	Sample detail	Zn% Expected	Zn% Observed	Recovery (%) Obs/exp*100	Verification range (± 5 %)	Remarks
1	10% Zn	10	9.89	98.9	95 - 105 %	Verified

3.8. Bias:

The difference between the expectation of the test result and an accepted reference value is biased. The test results (Table 6) are within acceptable range of Z score; hence, parameter is regarded as pass. Table-5 highlighted that the results of Proficiency Testing (PT) are within admissible range (i.e., Z-Score values for Zinc samples during 2021 and 2022 are -0.93 and 1.65, respectively which are less than 2)

Table 6: Magruder PT Results for Zinc (Zn)

S. No.	Sample Detail	Z score range of acceptance	SWTL, D.G. Khan Z-Score Results	Remarks
1	Bias Magruder sample No. 21012 (February 2021)	-2.0 to +2.0	Z score= -0.93	Pass
2	Bias Magruder sample No. 220111 (February 2022)	-2.0 to +2.0	Z score= 1.65	Pass

The uncertainty budget indicates that the Type-A and Type-B uncertainties are within admissible range. It is also depicted in Uncertainty tabulated data that the combined and Expanded uncertainties also fall in the acceptable criteria as per Eurachem guide (Table 7).

Table 7: Estimation of Uncertainty of Measurement and Uncertainty Budget

S. #.	Sources of Uncertainty	Uncertainty	Type A/B	K Factor	Uncertainty Contribution	Average or Value	Relative Uncertainty	Combining Uncertainty
1	Analyst	0.103	A	1	0.103	9.88	0.010425101	0.000108683
2	Flask 250 ml	3	B	2	1.530612245	249.5	0.006134718	3.76348E-05
4	Pipett 01 ml	0.1	B	2	0.051020408	0.992	0.051431863	0.002645237
7	Environment	0.084	A	1	0.084	25.14	0.003341289	1.11642E-05
8	Balance	0.001	B	2	0.000510204	2	0.000255102	6.50771E-08
9	Equipment (AAS)	0.01	B	2	0.005102041	1.5	0.003401361	1.15693E-05
10	Equipment (Hot Plate)	5.2	B	2	2.653061224	280.2	0.009468455	8.96516E-05

Combined Uncertainty (Uc)	0.0539	@	**95 % CL**
CL (K)	2	2	**2**
Expanded Uncertainty (Ue)	0.1078	@	**2**
Expanded Uncertainty per unit	0.0011	%	

Table 8 exhibited that the Repeatability, reproducibility, LOD, LOQ, recovery as well as uncertainty are within acceptable limit, therefore method under study is capable of producing reproducible results in our existing Laboratory analytical environment.

Table 8: Summary for Method Verification of Zinc in Fertilizer.

S. No.	Verification Parameter	Limit/Range/Action	Result	Remarks
1	RM	Zn Containing Material 10 %	9.89%	Pass
2	Repeatability	RSD<10 %	RSD=1.01%	Pass
3	Reproducibility	T values< 2.262	T Value=0.05	Pass
4	LOD	Should be calculated	Yes, Zn=0.298%	Pass
5	LOQ	Should be calculated	Yes, Zn=0.9%	Pass
6	Calibration Curve	R^2>0.95	R^2=0.999	Pass
7	Recovery	95 - 105 %	98.9 %	Pass
8	Uncertainty	Should be calculated	Uncertainty per unit= ±0.0011%	Pass
Bias				
1	Bias Magruder sample Sample No. 21012 February 2021)	Z score range of acceptance -2.0 to +2.0	Z score= -0.93	Pass
2	Bias Magruder sample No. 220111 February 2022)	Z score range of acceptance -2.0 to +2.0	Z score= 1.65	Pass

4. Discussion:

The uncertainty budget indicates that the Type-A and Type-B uncertainties are within admissible range. Also, the Combined and Expanded uncertainties fall in the acceptable criteria. The results of Proficiency Testing (PT) are within admissible range (i.e Z-Score values for Zinc samples during 2021 and 2022 are -0.93 and 1.65, respectively) Validation parameters results showed that all the parameters i.e Repeatability, reproducibility, LOD, LOQ, recovery as well as uncertainty are within acceptable limit, therefore method under study is capable of producing reproducible results. Previous findings of (Ullah *et al.,* 2022) are in line with our results as they reported that the Flame photometry method for Potassium Fertilizer analysis exhibited the same trend of PT, LOQ, LOD, Recovery, Linearity and uncertainty. As a result, the procedure can produce repeatable results. The parameter is given a pass grade since reproducibility is seen as successful (González *et al.,* 2010; González and Herrador, 2007; Uno, 2016)?

5. Conclusion:

After validation study, it is concluded that the said laboratory is competent to perform the Zinc analysis by the suggested method as per standards.

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