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

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Abstract

Fertilizer samples are analyzed to ascertain their nutritional content; however, the results differ according on the technique employed. The study's main objective was to develop and evaluate an approach for measuring Copper using an atomic absorption spectrophotometer. Copper content in fertilizer samples was measured using the Atomic Absorption Spectrophotometer Technique, which was verified by Dera Ghazi Khan's Soil & Water Testing Laboratories. As part of the validation process approach, tests were conducted on selectivity, bias, linearity, recovery, detection limit, repeatability, linearity, and selectivity. The findings showed that the sample's LOD and LOQ values for Copper, as well as its repeatability (relative standard deviation of 4.98 and T-value 0.063 being less than the calculated T value of 2.0262), recovery of 97%. The Copper concentration's linear curve, which displayed linearity R2 = 0.998 %, was obtained between 0.5 mg Kg^{-1} and 2.0 mg Kg^{-1} (Cu). The laboratory took part in the Magruder Fertiliser Sample Check Programme, an American proficiency testing programme. The Z-score values of -0.39 of the Copper samples examined using this approach are within an acceptable range (i.e., -2 to +2). The method's uncertainty was ±0.026%. Therefore, Copper analysis using the recommended method in accordance with standard may be carried out at the Dera Ghazi Khan Soil and Water Testing Laboratory. This association leads us to the conclusion that the plan was effective.

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Introduction

Analytical techniques are essential in a wide range of fields, such as the research of medicines, medical difficulties, environmental variables, and food goods. It is essential to utilise the most straightforward analytic method available to produce the most accurate, consistent, and dependable data (Ahmad et al., 2015; Gumustas et al., 2013; Kurbanoglu et al., 2014). Validation, which is predicated on outcomes verification, keeps an eye on the procedure's dependability.

A method's validity is determined by a number of factors, such as repeatability, sensitivity, detection and quantification limits, accuracy, and precision. Validated approaches are essential to provide high-quality outcomes (Aboul-Enein, 2012; Arkaban et al., 2021; Striegel, 2021). Any analytical technique must be validated to make sure it satisfies the required criteria, according to the ISO/IEC 17025 standard. The purpose of this study was to confirm that Copper levels in fertiliser samples could be measured or examined using the Atomic Absorption Spectrophotometry method.

Objective

1. To create and verify an Atomic Absorption Spectrophotometry method for analysing Copper content in fertiliser sample.
2. Determining the uncertainty budget in our current laboratory setting and measuring the uncertainty of the verified approach.
3.

Materials and Methods

The Certified Reference Material (Cu 1000 ppm) was used to create the working standards for the solutions using the Bano et al. formula. Where C_2 is the required Cu concentration (ppm), C_1 is the concentration of the stock solution (ppm), and V_1 is the quantity extracted from the stock solution, $C_1V_1 = C_2V_2$ (ml).

Quantification of Copper (Cu):

Water-Based Extraction: A 100 mL glass beaker was filled with a 1.0g sample of fertilizer, added around 75 millilitres of distilled water heated water for thirty minutes. Filtered in a volumetric flask of one litre and made volume with distilled water. Rediluted, if necessary. To analyze the amount of Copper in solution (mg/L) calibration curve was used.

Repeatability:

The relative standard deviation, sometimes known as "repeatability," is used to measure the degree of agreement between independent results that were obtained using the same analytical method on the same test material, under the same conditions (same user, equipment, and laboratory) and after a short period of time.

Accuracy:

Generally speaking, measures of accuracy such the relative standard deviation (Cu%) were evaluated based on repeatability and reproducibility. The repeatability test for Copper (Cu) was carried out in a laboratory environment using comparable tools, personnel, and short time intervals. With the relative standard deviation, the repeatability measurement was computed. The relative standard deviation was used to compute the repeatability measurement.

Limits of Detection (LOD) and Quantification:

The sensitivity of the Atomic Absorption Spectrophotometer was evaluated using LOD and LOQ computations. The minimal concentration of any substance that is only always observable and easily differentiated from zero, but not always measurable, is known as the lowest detectable dose (LOD). Gonzalez et al. (2010) and Renger et al. (2011) state that the lowest quantity of any substance that can be evaluated with a reasonable degree of precision and accuracy is referred to as the limit of quantification (LOQ).

Recovery:

The accuracy of the technique under investigation was verified using Cu recovery computations. In order to assess the accuracy of the approach, recoveries experiments were carried out to confirm Cu loss due to contamination during sample preparation and matrix interference during analysis. For analyte concentrations of 1g/mL, Taverniers et al. (2004) state that the desirable range of the recovery is 95% to 105%.

The Eurachem Guide was used to measure the uncertainty. The operator, the analytical technique, the accommodations and surroundings, the reagents and instruments, and other factors might all contribute to this uncertainty in the results. The combined effects of all the previously listed components result in combined uncertainty. The budget for uncertainty included all components of uncertainty (Cortez, 1995; Örnemark, 2004). This estimation of uncertainty was performed using a 68% confidence level. The testing laboratories must describe their uncertainties as extended uncertainty and provide a clear confidence level in accordance with ISO/IEC 17025 standard (Aslam et al., 2021; Nazir et al., 2020; Van der Veen and Cox, 2021).

Expanded uncertainty = Combined uncertainty × confidence level

Standard Testing Method, Estimation of Copper in fertilizer sample by AAS

Principle

Atomic absorption spectroscopy involves generating a gaseous population of free atoms by heating a sample in a flame and then passing narrow bandwidth light at a certain wavelength through the atoms in the flame. These conditions result in absorption of radiation that is selective for a particular element. Absorbance is measured and Beer's Law, which defines the simple linear relationship between absorbance and concentration, is applied to enable quantitative analysis of the sample for the particular element under analysis.

Method:

Placed 1.00g test portion into 100 mL glass beaker. Added 75mL D.I water and boiled for 30 minutes. Filtered in 1-liter volumetric flask, washed and filter with D.I water. Made the volume up-to the mark with D.I water. Re-diluted if necessary. Determine concentration of element in solution (mg/L) from calibration curve or digital concentration readout following the standard operating parameters.

Calculation:

Concentration % of Copper = AAS Reading x dilution factor/10000.

1. Certified Reference Material (CRM)

The following CRM used for preparation of standard

Table.1 Certified Reference Material (CRM)

Concentration	Company	Product ID	Lot No.
1000ppm Cu	VWR	BDH82025-974	S2-CU708105

Sample	Product Name	Company Guaranteed Contents
Multimicronutrient	Crop Grow	Cu = 1%

Results:

Precision:

Reproducibility and repeatability were often used to evaluate accuracy using metrics like the relative standard deviation (Cu%). The Copper (Cu) repeatability test was carried out in a lab environment using comparable tools, personnel, and short time intervals. Relative standard deviation was used to determine the repeatability measurement, yielding a repeatability standard deviation of 0.048 percent Table-1.

Table-2 Repeatability for analysis results of Copper Fertilizer

Analyst-1		
Sr. No.	**Repeat**	**Cu=1%**
1	1	1.0
2	2	1.0
3	3	1.0
4	4	0.9
5	5	1.0
6	6	1.0
7	7	1.0
8	8	1.0
9	9	0.9
10	10	0.9
	Average%	0.97
	Stdev%	0.048
	RSD%	**4.98**

Table 2 contains the repeatability data. While investigating Cu reproducibility, it was ascertained if Cu standards' results from the Atomic Absorption Spectrophotometer were reliably reliable (consistent for different parameters). This was not considered for mistakes pertaining to sample handling or preparation; rather, it was solely considered for errors linked to the system (Eka et al., 2012; Horwitz and Latimer, 2005; Pointner et al., 2014; Ullah et al., 2017). Two independent analysts performed Cu studies on Atomic Absorption at different times, and the data reproducibility of their results was determined using the T-test. The estimated T value (0.063) was found to be smaller than the T-tabulated value (2.262). Hence the method is able to furnish reproducible results while duplicating analyses with standard deviation of ±0.048and ±0.0526 %, respectively (Table-3).

t- test= =((0.97-0.961)/SQRT((0.048)*2/10)+(0.0526)*2/10)= 0.063
t- Tabulated =2.262 at 95% confidence level

Table-3 Table-3 Reproducibility of Copper test result

S/N	Analyst-1	Analyst-2
1	1	1
2	1	0.9
3	1	1.01
4	0.9	1
5	1	0.9
6	1	0.9
7	1	1
8	1	1
9	0.9	0.9
10	0.9	1
Average	0.97	0.961
SD	0.048	0.0526

However, the two analysts independently performed a duplicate determination at different times with relative standard deviations of 0.048% and 0.0526, respectively.

The parameter is deemed successful in its reproducibility and is awarded a passing grade. The findings of reproducibility are shown in Table 2. For the analyte concentration of 1g/L, the maximum standard deviation (relative RSD) values permitted are almost 16%. Consequently, the process can yield repeated outcomes. Given that replication is regarded as successful, the parameter receives a pass grade (González et al., 2010; González and Herrador, 2007; Uno, 2016). Based on the data from 10 repetitions (Table 2), the Copper technique is predicted to be repeatable with a relative standard deviation (RSD) of 4.98%, which is much lower than 15% and indicates data homogeneity. parameter is therefore, classified as pass.

Reproducibility

Table 3 presents the data that elucidates the similarity between Copper findings obtained separately using a comparable approach on the same test sample, but in contrasting situations (different user, different climatic conditions, and different times). The t-test was used in this investigation. According to the t-test, the calculated t-value (0.063) is less than the tabulated t-value (2.262). This means that the results are not statistically significant. The method can produce repeatable results when duplicate analyses are performed by two separate analysts working independently at different times, with standard deviations of 0.048% and 0.0526, respectively. Reproducibility is consequently graded as passable as it is seen as successful.

The method detection limit (LOD)

The minimal concentration of a substance that can be detected and reported with a 95% confidence level that the analyte concentration is greater than zero is known as the method detection limit (LOD). This value is obtained by examination of a sample in a specific matrix that contains the analyte. By increasing the technique factor, the LOD for this investigation is determined to be 0.145% Copper in a fertiliser sample. To calculate LOD, ten samples' worth of data were analyzed.

LOD = blank value + $k.s_r$ = $0 + 3 \times 0.048 = 0.145\%$

The Method Limit of Quantification (LOQ):

LOQ is the lowest concentration of analyte that can be examined with a satisfactory degree of accuracy. Most of the time, LOQ is calculated by multiplying the concentration of any analyte that corresponds to the standard deviation measured at a very low level by a factor called kq, which is typically set to 10. By increasing the technique factor, the LOQ for Copper in the fertiliser sample was determined to be as 0.48 % Copper in this investigation. As shown below, the LOQ in this instance is determined by multiplying the repeatability standard deviation by ten times the blank value:

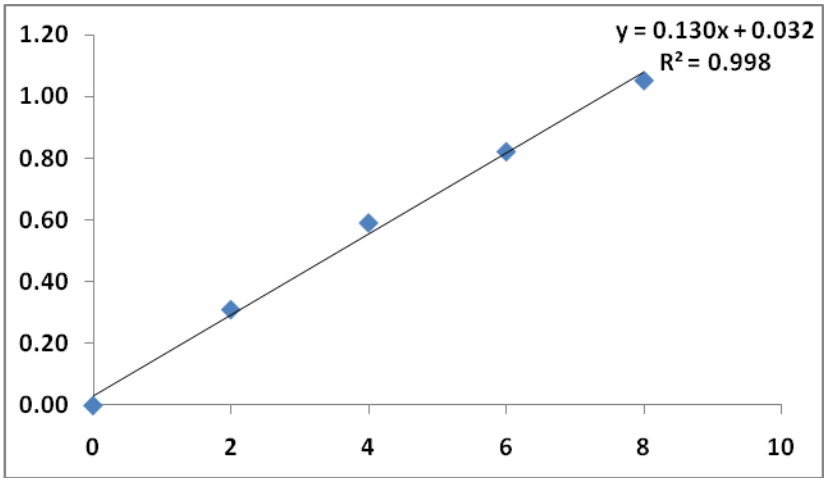
LOQ = Blank + k .s_r = $0 + 10 \times 0.048 = 0.48\ \%$

Table 4 Standard curve for Copper determination

S. No	Copper	Reading
	0	0.00
	2	0.31
	4	0.59
	6	0.82
	8	1.05

Calibration Curve:

By measuring the concentration that matches the instrument reading, correcting for the blank, and multiplying by the dilution factor, one may determine the concentration of a sample from the calibration curve (at point 5). A calibration curve should be able to use the linear part of a "curvy linear" relationship to calculate the sample concentration. With an R^2 value of 0.998 and a score larger than the threshold limit of 0.95, the 5-point Cu calibration curve duly displayed in Figure 1 indicates that the parameter is passable and exhibits excellent behavior and predictability.



Linearity:

When a dependent variable can be computed as the linear function of an independent variable (Cu) because it has a relationship with one or more independent variables, it is said to be linear. The study's straight line in Figure 1 exhibits good behavior and predictability, with R2=0.998 above the necessary value of 0.95 as per the criterion.

Recovery:

The measured recovery (97 %) for Cu samples that lie closer to the midpoint of the calibration curve and the highest calibrator is within the allowable range of the criterion, that is, within ± 10% of the recovery range (Table 5). The approach in issue is thus given a pass rating.

Table 5: Evaluation of Copper Recovery

S. No.	Standard sample	Sample detail	Copper% Expected	Copper% Observed	Recovery (%) Obs/exp*100	Verification range (± 10 % of 100% Recovery)	Remarks
1	Cu Quality sample	1% Cu	1	0.97	97	90-110 %	Verified

Bias:

There is bias in the discrepancy between the expected test result and a recognised reference value. The test results fall within an acceptable range (Table 6) of Z score; as a result, the parameter is considered passed. Table 6 demonstrated that the Proficiency Testing (PT) results are within the permissible range (that is, the Z-Score values for the Copper sample during 2023 is less than 2, which is -0.39).

Table-6.Magruder PT Results for Copper

Analyte	SWTL, D.G.Khan	Robust Mean	Z score (SWTL, D.G.Khan)	Number of Labs participated	Remarks
Copper (Sample # 231111), Magruder USA, 31-12-2023)	10.00	10.147	-0.39	62	Pass

Uncertainty

The state of being uncertain or the doubt about Copper measurement is expressed at K=2 (95% probability) and is calculated as ±0.026% per unit of Copper content. 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). It can be regarded as good for this method at Soil and Water Testing Laboratory SWTLDGK environment. Detail is given in Table-6 expressing uncertainty sources, expanded uncertainty per unit of the product.

Uncertainty Budget for Copper								
S/N	Sources of Uncertainty	Uncertainty	Type A/B	K Factor	Uncertainty Contribution	Average or Value	Relative Uncertainty	Combining Uncertainty
1	Analyst	1.26211	A	1	1.26211	0.961	1.313329865	1.724835
2	Vol. Flask 100 ml	1	B	2	0.510204082	99.77	0.005113803	2.6151E-05
3	Cylinder 50 ml	0.5	B	2	0.255102041	45.56	0.005599255	3.13517E-05
4	Pipett 01 ml	0.1	B	2	0.051020408	0.992	0.051431863	0.0026452
5	Equipment, AAS	0.01	B	2	0.005102041	2	0.00255102	6.50771E-06
6	Environment	0.005	A	1	0.005	25.93	0.000192827	3.71822E-08
7	Analytical Balance	0.001	B	2	0.000510204	0.5	0.001020408	1.04123E-06

8	Equipment, Hot Plate	5.2	B	2	2.653061224	280.2	0.009468455	8.96516E-05

Combined Uncertainty (Uc)	1.3144	**@**	**95 % CL**
CL (K)	2	2	**2**
Expanded Uncertainty (Ue)	2.6288	**@**	**2**
Expanded Uncertainty per unit	**0.026**	**%**	

Table. 7. Summary for Method Validation of Cu in Fertilizer

Sr. No	Validation Parameter	Limit/Range/Action	Result	Remarks
1	CRM	Cu Containing Material, 1%	0.97%	Pass
2	Repeatability	RSD<10 %	RSD=4.98%	Pass
3	Reproducibility	T values< 2.262	T Value=0.063	Pass
4	LOD	Should be calculated	Yes, Cu=0.145%	Pass
5	LOQ	Should be calculated	Yes, Cu=0.48%	Pass
6	Calibration Curve	R^2>0.95	R^2=0.998	Pass
7	Linearity	R^2>0.95	R^2=0.998	Pass
8	Uncertainty	Should be calculated	Uncertainty per unit=±0.026%	Pass
9	Recovery	90 - 110 %	97	Pass
10	Bias	Z score range of acceptance -2.0 to +2.0	-0.39	Pass

Discussion:

The Type-A and Type-B uncertainties are within the permissible range, according to the uncertainty budget. Additionally, the Expanded and Combined uncertainty meet the required standards. The proficiency testing (PT) results fall within the acceptable range; for example, the Copper sample Z-scores for 2023 are -0.39. The technique under research is capable of delivering repeatable findings, as seen by the validation parameters results, which indicated that all the parameters—repeatability, reproducibility, LOD, LOQ, recovery, and uncertainty—are within an acceptable range. Our results are consistent with earlier research by Ullah et al. (2022), which found that the Atomic Absorption Spectrophotometry method for analysing copper in fertiliser showed similar trends in terms of PT, LOQ, LOD, Recovery, Linearity, and uncertainty. Consequently, the process can yield repeated outcomes. Since repeatability is seen as successful, the parameter receives a passing grade (González et al., 2010; González and Herrador, 2007; Uno, 2016).

Conclusion:

After validation study, it is evident that the Soil and Water Testing Laboratory, Dera Ghazi Khan is capable of performing the Copper analysis by the said method according to standards.

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