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Validation and Uncertainty Evaluation of an Atomic Absorption Spectrophotometric Method for Iron Determination in Fertiliser Samples

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ABSTRACT

Routine analysis of fertiliser samples is conducted to quantify their nutrient content; however, the measured nutrient concentrations may vary depending on the analytical methodology used. The principal objective of this study was to develop and validate a method for determining Iron (Fe) in fertiliser samples using atomic absorption spectrophotometry (AAS). The method was validated at the Soil and Water Testing Laboratory (SWTL), Dera Ghazi Khan, accredited to ISO/IEC 17025:2017, for repeatability, reproducibility, limit of detection (LOD), limit of quantification (LOQ), linearity, recovery, and bias, while the measurement uncertainty was estimated following the Eurachem Guide. The method produced a repeatability relative standard deviation (RSD) of 0.24%, and reproducibility was confirmed by a calculated t-value of 0.021, well below the tabulated t-value of 2.101 at the 95% confidence level. The LOD and LOQ were 0.014% and 0.05% Fe, respectively. A five-point calibration curve constructed over the range of 0-8 ppm Fe showed excellent linearity (R^2 = 0.99). The recovery of Fe from a certified quality-control sample (2% Fe) was 99.85%, well within the acceptance range of 95-105%. Laboratory performance was

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further confirmed through participation in the Magruder Fertiliser Sample Check Program (USA), with Z-scores of 1.34 and 0.00 recorded for two independent proficiency-testing rounds, both within the acceptable range of -2.0 to +2.0. The expanded measurement uncertainty, calculated at k = 2 (95% confidence level), was ±0.0004% Fe per unit of product. All validation parameters met the acceptance criteria, demonstrating that the Soil and Water Testing Laboratory, Dera Ghazi Khan, is competent to perform routine Iron analysis in fertiliser samples by the proposed AAS method.

Keywords: Iron; Method Validation; Atomic Absorption Spectrophotometry; Measurement Uncertainty; Fertiliser; ISO/IEC 17025

Introduction

Analytical methods occupy a central place in agriculture, environmental monitoring, pharmaceutical analysis, and medical diagnostics. To obtain the most accurate, reliable, and reproducible data, the simplest available analytical technique should be employed and rigorously validated (Ermer & Nethercote, 2025; Haque et al., 2025; Kurbanoglu et al., 2014). Validation ensures the reliability of an analytical procedure by demonstrating that its performance meets predefined acceptance criteria. In accordance with ISO/IEC 17025:2017, analytical methods employed by accredited testing laboratories must undergo validation to verify compliance with established performance criteria before routine use (Sahoo et al., 2018).

Iron (Fe) is an essential micronutrient that plays a fundamental role in plant metabolism despite its relatively low requirement. It functions as a cofactor for enzymes involved in chlorophyll biosynthesis, electron transport during photosynthesis and respiration, and biological nitrogen fixation. Therefore, inadequate Fe availability in soils or fertilisers can lead to interveinal chlorosis, reduced photosynthetic efficiency, impaired growth, and significant yield losses (Sági-Kazár et al., 2022; Zhang et al., 2019). Because micronutrient fertilisers are frequently formulated with guaranteed Fe content, an accurate and validated analytical method is essential to verify label claims, support farmer advisory services, and satisfy regulatory and proficiency-testing requirements.

Atomic Absorption Spectrophotometry (AAS) remains one of the most widely used techniques for trace and minor element determination in agricultural and environmental matrices because of its sensitivity, selectivity, and relatively simple sample preparation requirements (Raposo & Ibelli-Bianco, 2020). The present study extends a series of method validation studies previously carried out at the Soil and Water Testing Laboratory, Dera Ghazi Khan, for Copper (Ullah et al., 2024), Zinc (Ullah et al., 2023), Potassium (Ullah et al., 2022), and Humic Acid (Noor et al., 2025), and applies the same validation and uncertainty-estimation framework to the determination of Iron in fertiliser samples.

Objectives

To develop and validate an Atomic Absorption Spectrophotometry method for the determination of Iron content in fertiliser samples.

To estimate the measurement uncertainty of the validated method and to compute the

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http://amresearchreview.com/index.php/Journal/about

uncertainty budget under the existing laboratory environment, in accordance with the Eurachem Guide (Ellison et al., 2019).

Materials and Methods

The validation study was conducted at the Soil and Water Testing Laboratory for Research, Dera Ghazi Khan, Punjab, Pakistan, which is accredited under ISO/IEC 17025. The analytical method was validated using an internal protocol developed in accordance with the International Conference on Harmonisation (ICH) guideline on the validation of analytical procedures and the Codex Alimentarius/AOAC International guidelines for single-laboratory method validation (Cannavan et al., 2016; Sahoo et al., 2018).

Preparation of Standard Solutions

A Certified Reference Material (CRM) of Iron (1000 mg Fe/L stock solution) was used to prepare the working standards by using the dilution formula of Bano et al. (2021): $C_1V_1 = C_2V_2$, where C_1 is the concentration of the stock solution (ppm), V_1 is the volume taken from the stock solution (mL), C_2 is the required Fe concentration (ppm), and V_2 is the total volume required (mL).

Table 1. Certified Reference Material (CRM) used for the preparation of Iron working standards.

Concentration	Company	Product ID	Lot No.
1000 ppm Fe	AppliChem	82450,0500	22.4132907.50

3.2 Fertiliser Sample

Table 2. Details of the fertiliser sample used for repeatability, reproducibility, recovery, and uncertainty studies.

Sample Code	Product Name	Fertiliser Type	Guaranteed Content
AB-13	Bloco Multi Micronutrient	Boraq Agro Chemicals	Fe: 2%

Instrumentation

Table 3. Instrumental operating parameters for Iron determination by AAS.

Element	Wavelength (nm)	Flame	Range (µg/mL)
Iron	248.3	Air-C_2H_2	0.2-5

Apparatus used included an analytical balance, a hot plate, an Atomic Absorption Spectrophotometer, a 1-litre volumetric flask, a funnel, Whatman No. 1 filter paper, and calibrated pipettes.

Extraction and Determination Procedure

Aqueous extraction was carried out following AOAC Official Method 965.09 (AOAC International, 2017). A 1.00 g test portion was placed in a 100 mL glass beaker, and 75 mL of deionised (DI) water was added. The mixture was boiled for 30 min, filtered into a 1 L volumetric flask, and the residue was washed thoroughly with DI water. The combined filtrate was made up to volume with DI water, and further dilutions

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http://amresearchreview.com/index.php/Journal/about

were performed as required. The Fe concentration (mg L^{-1}) was measured using atomic absorption spectrophotometry (AAS) based on the instrument calibration curve and standard operating parameters.

Concentration (%) of Iron = AAS reading × dilution factor / 10000.

Method Validation Parameters

Method validation was carried out by evaluating repeatability, reproducibility, the limit of detection (LOD), the limit of quantification (LOQ), linearity, recovery, selectivity, and bias, following ICH guidance and the approach previously applied to Cu, Zn, K_2O, lambdacyhalothrin and Humic Acid in this laboratory (Ullah et al., 2022, 2023, 2024; Noor et al., 2025; Aslam et al., 2021).

Repeatability is defined as the closeness of agreement between independent test results obtained by applying the same analytical method to identical test material under repeatability conditions, including the same analyst, equipment, laboratory, and a short time interval. Repeatability is expressed as the relative standard deviation (RSD) of replicate measurements.

Reproducibility is defined as the closeness of agreement between independent test results obtained using the same analytical method on identical test material under reproducibility conditions, including different analysts, laboratories, equipment, and/or time intervals. In this study, reproducibility was calculated statistically using the t-test: $t = (X_1 - X_2) / \sqrt{[(2\times SD_1/n) + (2\times SD_2/n)]}$, where X_1 and X_2 are the mean results and SD_1 and SD_2 the standard deviations obtained by the two analysts, and n is the number of repeats. The calculated t-value was compared against the tabulated t-value (2.101) at the 95% confidence level (n - 1 = 9, df=18, degrees of freedom for each analyst, two-tailed).

The limit of detection (LOD) is the lowest concentration of analyte that can be detected, but not necessarily quantified, with 95% confidence that the analyte concentration is greater than zero (Taleuzzaman, 2018). It was calculated as LOD = blank value + k × s_r, with k = 3 and s_r the repeatability standard deviation, the instrument blank being adjusted to zero for every reading.

The limit of quantification (LOQ) is the lowest concentration of analyte that can be determined with an acceptable level of precision and accuracy, calculated as LOQ = blank value + $k^i \times s_r$, with $k^i = 10$.

Linearity was assessed from a five-point calibration curve (0-8 ppm Fe) by linear regression of instrument reading against standard concentration, with an acceptance criterion of $R^2 > 0.95$.

Recovery was assessed on a certified quality-control sample of known Fe content, calculated as Recovery (%) = (Observed/Expected) × 100, against the acceptable range of 95-105% recommended for analyte concentrations near 1 µg/mL (Raposo & Ibelli-Bianco, 2020).

Bias was assessed from participation in the Magruder Fertiliser Sample Check Program (USA), an international proficiency-testing (PT) scheme, by comparing the Z-score of the laboratory's result against the acceptance range of -2.0 to +2.0.

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http://amresearchreview.com/index.php/Journal/about

Measurement Uncertainty

Measurement uncertainty was performed following the Eurachem Guide (Magnusson & Örnemark, 2014; Ellison et al., 2019). Sources of uncertainty considered included the analyst (Type A), volumetric glassware, the analytical balance, the AAS instrument, the hot plate (Type B), and the laboratory environment (Type A). The combined uncertainty (uc) was calculated as the square root of the sum of squares of the relative uncertainty contributions of all sources:

$$uc = \sqrt{[\ (u_1)^2 + (u_2)^2 + (u_3)^2 + \ldots + (u_n)^2\ ]}$$

The expanded uncertainty (U_e) was obtained by multiplying the combined uncertainty by the coverage factor k = 2, corresponding to a confidence level of approximately 95% (Cortez, 1995; Nazir et al., 2020; Van der Veen & Cox, 2021):

$$U_e = uc \times k$$

Results

Precision: Repeatability

Precision was evaluated under repeatability and reproducibility conditions and reported as the relative standard deviation (RSD, %). The Iron repeatability test was carried out under similar operator, equipment, and short time-interval conditions, using the Bloco Multi Micronutrient fertiliser sample (Fe = 2%, Code AB-13). The data from ten repeats (Figure 1) show that the Fe method is repeatable, with a relative standard deviation (RSD) of 0.24%, which is well below the 10% acceptance threshold and indicates good homogeneity of the data. The method satisfied the repeatability acceptance criterion. As shown in Figure 1, all ten replicate measurements fell within the ±2 SD control limits, and no systematic trend or drift was observed, supporting the low relative standard deviation (RSD) obtained.

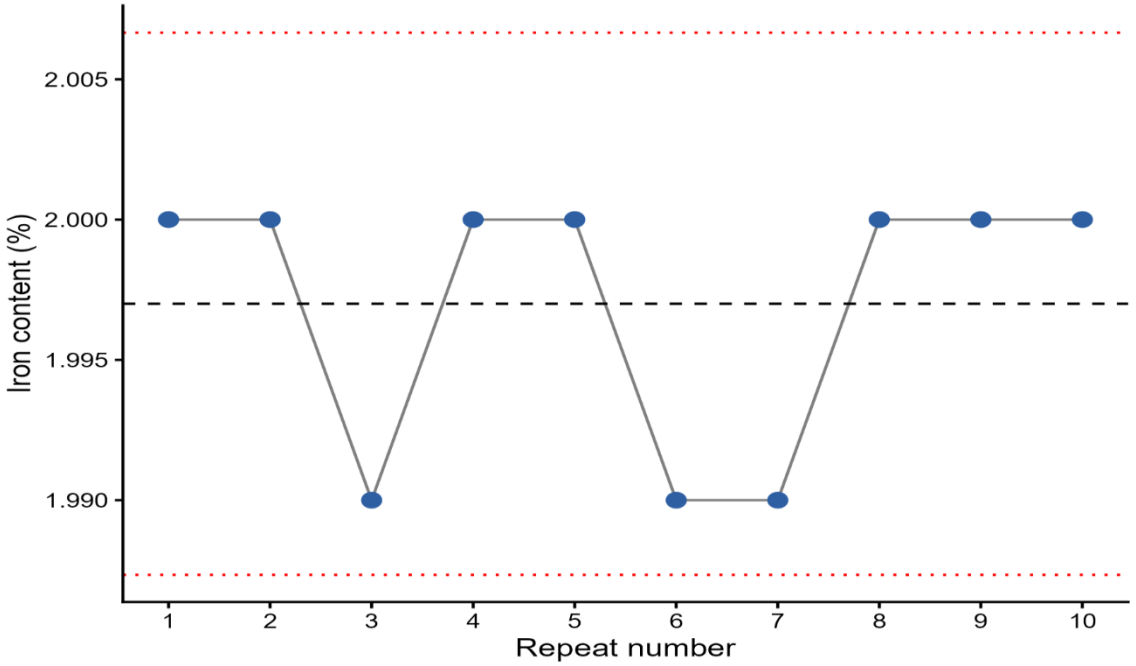

Figure 1. Repeatability run chart for Iron determination in fertiliser samples (10 replicates).

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Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

Reproducibility

Figure 2 presents the closeness of agreement between Iron results obtained independently using the same method on identical test material, but under different conditions (different analyst, different environmental conditions, different time interval). The t-test was applied: $t = (1.997 - 1.996) / \sqrt{[(2\times0.0048/10) + (2\times0.0070/10)]} = 0.021$. As the calculated t-value (0.021) is far smaller than the tabulated t-value (2.101) at the 95% confidence level, the results obtained by the two analysts are not statistically different, confirming that the method furnishes reproducible results, with standard deviations of ±0.0048% and ±0.0070% obtained by Analyst 1 and Analyst 2, respectively. Reproducibility is therefore graded as pass. Figure 2 demonstrates close agreement between the results obtained by the two analysts across all ten replicate measurements, with no evidence of systematic bias, consistent with the non-significant t-test result.

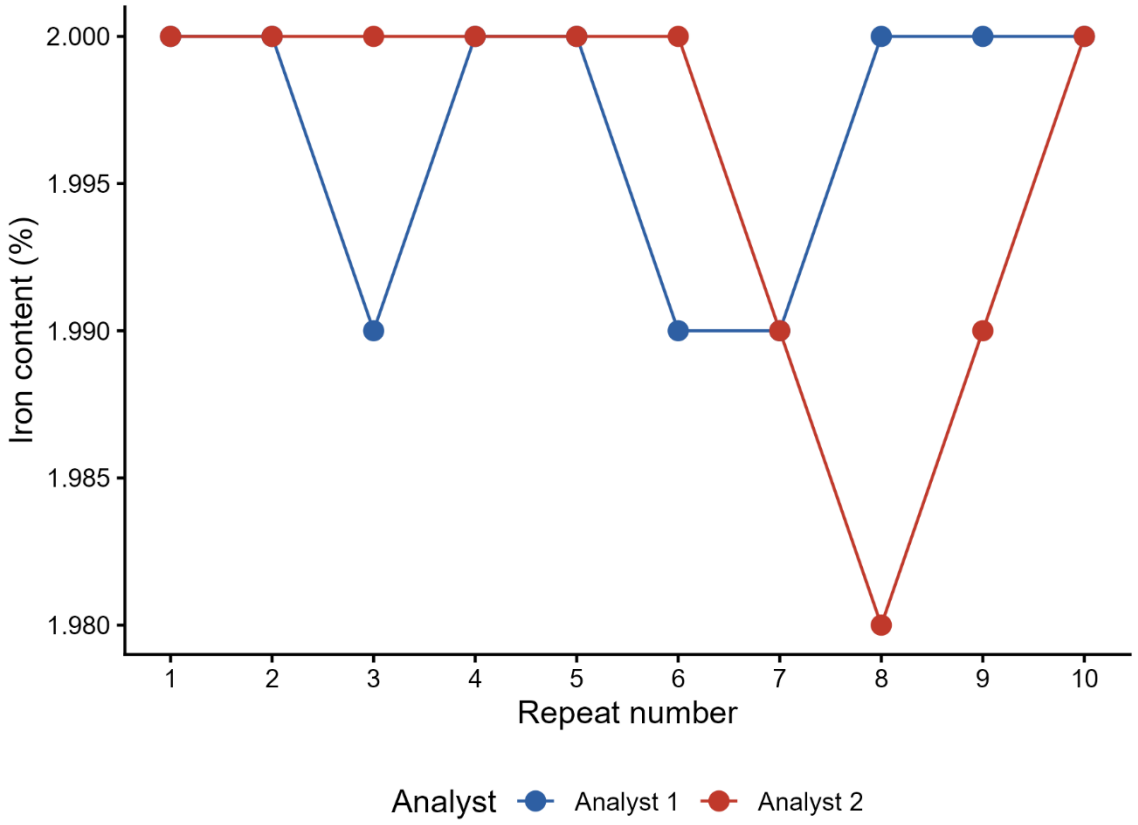

Figure 2. Reproducibility plot comparing results from two independent analysts.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The method detection limit (LOD) is the lowest concentration of Iron that can be measured and reported with 95% confidence that the analyte concentration is greater than zero, determined from analysis of ten samples. Using a factor k = 3 and the repeatability standard deviation (s_r = 0.0048%):

$LOD = blank + k \times s_r = 0 + 3 \times 0.0048 = 0.014\%$ Fe.

The limit of quantification (LOQ), calculated using a factor of 10 times the

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Volume 4, Issue 6 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

repeatability standard deviation, was:

$LOQ = blank + k^i \times s_r = 0 + 10 \times 0.0048 = 0.05\%$ Fe.

Both the LOD (0.014% Fe) and LOQ (0.05% Fe) are well below the guaranteed Fe content (2%) of the fertiliser samples routinely tested at this laboratory, confirming adequate sensitivity of the method for its intended use.

Calibration Curve and Linearity

The concentration of a sample is derived from a calibration curve by reading off the concentration corresponding to the instrument reading, corrected for the blank value and multiplied by the dilution factor. A calibration curve should be capable of computing sample concentration using the linear portion of a curvilinear relationship.

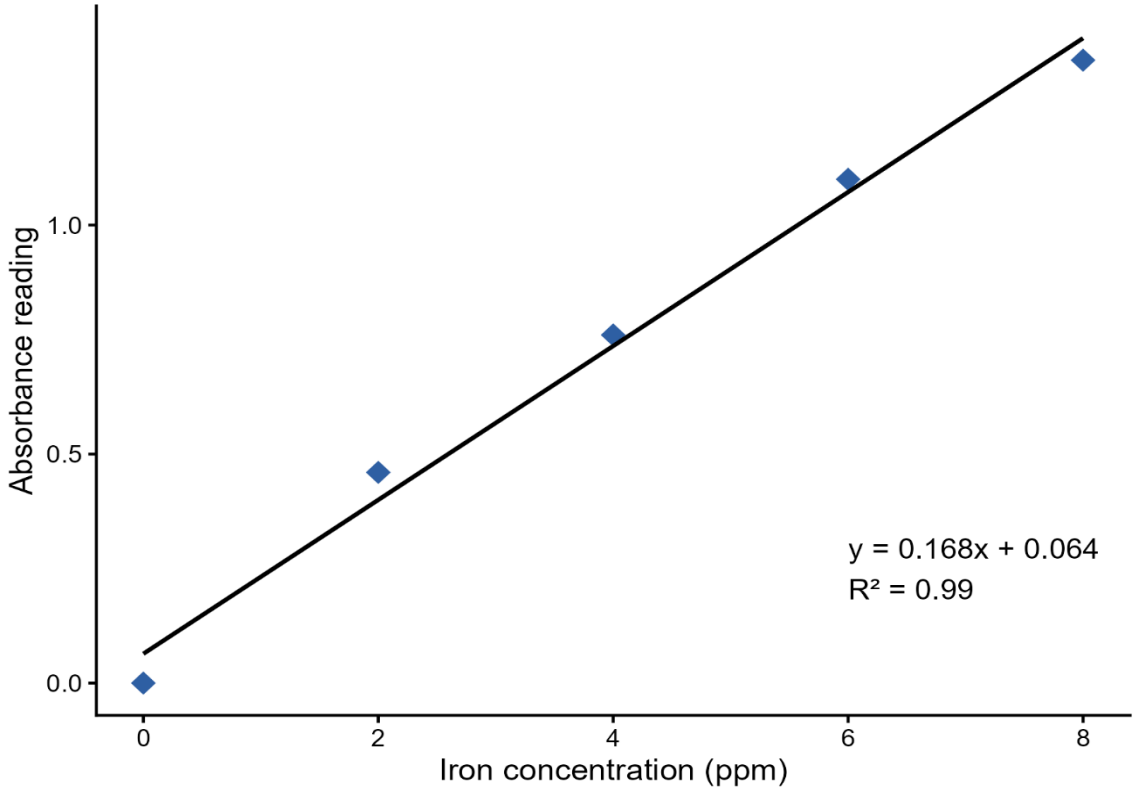

Figure 3. Five-point calibration curve for Iron determination (y = 0.168x + 0.064, R^2 = 0.99).

The five-point Fe calibration curve shown in Figure 3 gave a coefficient of determination (R^2) of 0.99, exceeding the critical limit of 0.95; the parameter is therefore graded as pass, with the curve exhibiting good linearity and predictability across the tested working range (0-8 ppm Fe).

Recovery

The measured recovery of Fe (99.85%) from the quality-control sample is well within the acceptable range of the criterion (95-105%); the method is therefore given a pass rating for accuracy. Figure 4 illustrates that the measured recovery value is within the acceptable range of 95–105%, confirming the accuracy of the method for iron determination.

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Volume 4, Issue 6 (2026)

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http://amresearchreview.com/index.php/Journal/about

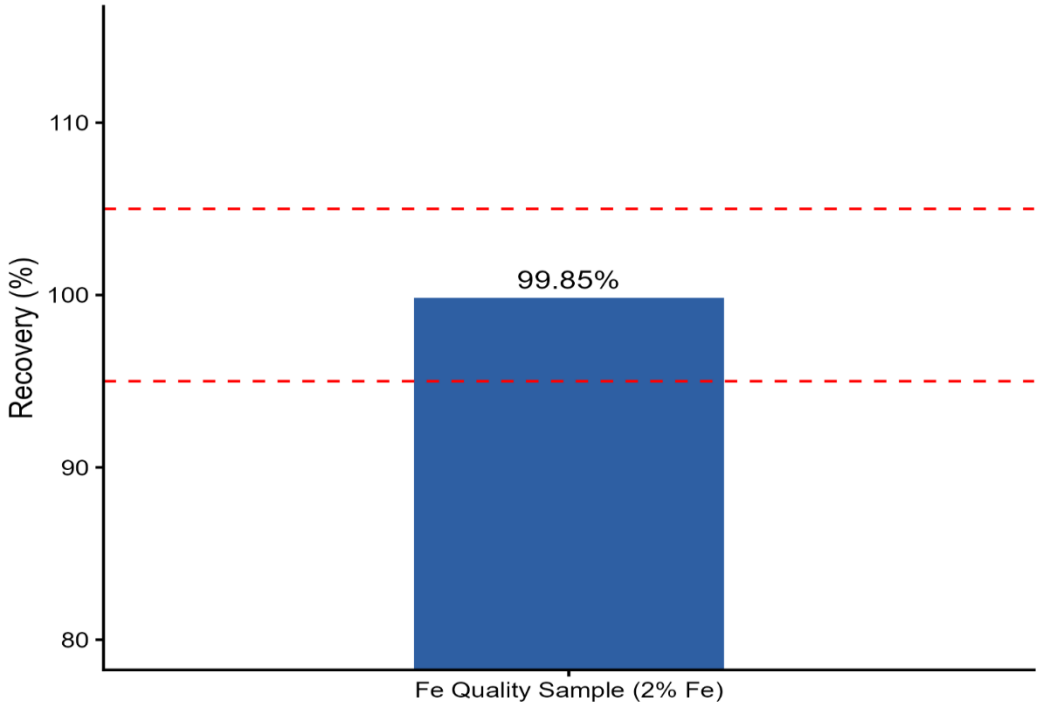

Figure 4. Recovery of Iron from the quality control sample.

Bias / Proficiency Testing

Bias is the difference between the expectation of the test result and an accepted reference value. The laboratory participated in the Magruder Fertiliser Sample Check Program (USA), an international proficiency-testing (PT) scheme. Table 4 demonstrates that the laboratory's Z-scores for Iron during the 2024-2025 testing rounds were within the acceptable range of -2.0 to +2.0; the parameter is therefore considered passed.

Table 4. Magruder Fertiliser Sample Check Program proficiency-testing results for Iron.

Analyte (Magruder Sample)	SWTL, D.G. Khan	All-Labs Mean	Z-score	Remarks
Soluble Fe, Sample #191121, 31-12-2024 (58 labs)	4.150	3.703	1.34	Pass
Soluble Fe, Sample #200213, 31-03-2025 (69 labs)	1.050	1.050	0.00	Pass

Measurement Uncertainty

The uncertainty budget for the estimation of Iron in a fertiliser sample (Fe = 2%, Code AB-13) is presented in Table 5. Type-A uncertainty was estimated from repeated measurements made by the analyst and from variation in the laboratory environment (temperature), while Type-B uncertainty was obtained from calibration certificates and manufacturer specifications of volumetric glassware, the analytical balance, the AAS instrument, and the hot plate.

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Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

Table 5. Uncertainty budget for the estimation of Iron in a fertiliser sample by AAS.

S/N	Source of Uncertainty	Uncertainty	Type A/B	K Factor	Average/Value	Combining Uncertainty
1	Analyst	0.0437	A	1	3.80	0.000132
2	Vol. Flask 100 mL	0.0100	B	1.73	99.96	0.0000000033
3	Cylinder 50 mL	0.8000	B	1.73	49.30	0.0000880
4	Pipette 1 mL	0.0020	B	1.73	0.99	0.0000013
5	Equipment, AAS	0.0058	B	1.73	8.00	0.0000002
6	Environment	0.2000	A	1	25.00	0.0000640
7	Analytical Balance	0.0004	B	1.73	2.00	0.0000000
8	Equipment, Hot Plate	5.2000	B	1.73	280.20	0.0001151

Table 6. Combined and expanded measurement uncertainty for Iron determination.

Quantity	Value	Confidence Level
Combined Uncertainty (uc)	0.0200	-
Coverage factor (k)	2	95%
Expanded Uncertainty (U_e)	0.0400	95%
Expanded Uncertainty per unit	±0.0004 %	95%

The uncertainty budget indicates that both Type-A and Type-B uncertainties fall within the admissible range. The combined and expanded uncertainties also meet the acceptance criteria of the Eurachem Guide (Ellison et al., 2019; Magnusson & Örnemark, 2014), and the expanded measurement uncertainty of ±0.0004% Fe per unit of product is considered acceptable for the existing laboratory environment at SWTL, Dera Ghazi Khan. The relative contribution of each uncertainty source to the combined uncertainty is shown in Figure 5, which indicates that the hot plate and the analyst component together account for the majority of the combined uncertainty, while the volumetric glassware, balance, and AAS instrument contribute comparatively little.

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http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 6 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

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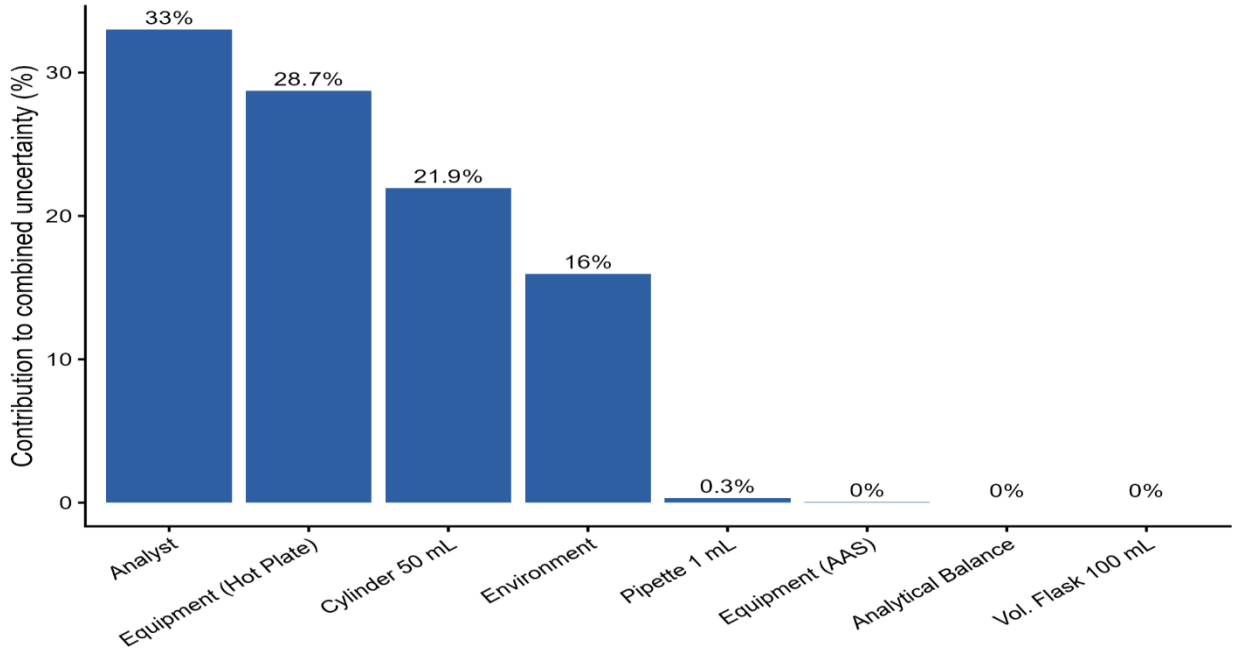

Figure 5. Relative contribution of each source to the combined measurement uncertainty.

Summary of Method Validation

Table 7. Summary of method validation parameters for Iron in fertiliser.

Sr. No.	Validation Parameter	Limit/Range/Action	Result	Remarks
1	Reference Material	Fe Containing Material, 2%	1.997%	Pass
2	Repeatability	RSD < 10%	RSD = 0.24%	Pass
3	Reproducibility	t < 2.101	t = 0.021	Pass
4	LOD	Should be calculated	Fe = 0.014%	Pass
5	LOQ	Should be calculated	Fe = 0.05%	Pass
6	Calibration Curve	$R^2 > 0.95$	$R^2 = 0.99$	Pass
7	Linearity	$R^2 > 0.95$	$R^2 = 0.99$	Pass
8	Recovery	95-105%	99.85%	Pass
9	Bias	Z-score: -2.0 to +2.0	Z = 1.34, 0.00	Pass
10	Uncertainty	Should be calculated	±0.0004% Fe	Pass

Discussion

The validation results demonstrated that the Atomic Absorption Spectrophotometry (AAS) method for iron determination in fertiliser samples is reliable under the operating conditions of the Soil and Water Testing Laboratory, Dera Ghazi Khan. The method exhibited excellent precision, with a repeatability RSD of 0.24% and a reproducibility t-value of 0.021, which was well below the critical value (2.101). These values were lower than those reported for previously validated methods for copper (RSD = 4.98%, t = 0.063; Ullah et al., 2024), zinc (RSD = 1.01%, t = 0.05;

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Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

Ullah et al., 2023), and humic acid (RSD = 0.47%, t = 0.05; Noor et al., 2025), indicating superior method precision and lower susceptibility to operator and time-related variability.

The method demonstrated excellent analytical sensitivity, with limits of detection (LOD) and quantification (LOQ) of 0.014% Fe and 0.05% Fe, respectively. Both values were substantially lower than the Fe concentrations typically encountered in commercial micronutrient fertilisers (1–10% Fe), confirming that the method is sufficiently sensitive for routine quality control and regulatory analysis. The observed sensitivity is consistent with that reported for previously validated analytical methods for Cu, Zn, and K_2O at the same laboratory (Ullah et al., 2022, 2023, 2024).

The five-point calibration curve gave an R^2 of 0.99, comfortably exceeding the 0.95 acceptance threshold recommended by ICH guidance and applied uniformly across the laboratory's validated methods (Rao, 2018). The recovery of 99.85% from a 2% Fe quality-control sample falls within the 95-105% range recommended by Raposo & Ibelli-Bianco, (2020) for analyte concentrations near 1 µg/mL, and is comparable to the recovery values reported for K_2O (98.8%; Ullah et al., 2022) and Zn (98.9%; Ullah et al., 2023) by the same laboratory.

Bias, evaluated through the laboratory's participation in the Magruder Fertiliser Sample Check Program (USA), yielded Z-scores of 1.34 and 0.00 for two independent rounds, both within the internationally accepted range of -2.0 to +2.0 (Cortez, 1995; Magnusson & Örnemark, 2014), confirming that the laboratory's Fe results are unbiased relative to the consensus value obtained from the participating laboratory network.

The expanded measurement uncertainty of ±0.0004% Fe (k = 2, 95% confidence level) reflects a well-controlled measurement process. As in the Cu, Zn, and Humic Acid uncertainty budgets generated by this laboratory, the largest contributions to the combined uncertainty arose from the hot plate (sample digestion temperature control) and the analyst component, while volumetric glassware, the analytical balance, and the AAS instrument itself contributed comparatively little to the overall uncertainty. This pattern is consistent with the Eurachem Guide's emphasis on sample preparation steps, rather than final instrumental measurement, as the dominant source of uncertainty in wet-chemistry/AAS workflows (Magnusson & Örnemark, 2014).

Overall, the validation results are consistent with previous studies conducted at the Soil and Water Testing Laboratory (SWTL) in Dera Ghazi Khan for Cu, Zn, K_2O, and humic acid analyses (Noor et al., 2025; Ullah et al., 2022, 2023, 2024). These studies likewise demonstrated acceptable repeatability, reproducibility, recovery, linearity, proficiency-testing performance, and measurement uncertainty, supporting the reliability of AAS- and wet chemistry-based analytical methods for fertiliser quality assessment.

Conclusion

The validation results confirmed that the proposed Atomic Absorption Spectrophotometry (AAS) method for iron determination in fertiliser samples complies with the performance requirements of ISO/IEC 17025. All validation parameters, including repeatability, reproducibility, linearity, recovery, limit of detection (LOD), limit of quantification (LOQ), bias, and measurement uncertainty,

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satisfied the predefined acceptance criteria. These findings demonstrate that the method is fit for routine iron determination in fertiliser quality control at the Soil and Water Testing Laboratory, Dera Ghazi Khan.

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Conflict of Interest
The authors declare no known competing financial interests or personal relationships that could have influenced the work reported in this study.

References

AOAC International. (2017). Method 965.09, Fertilisers. In Official methods of analysis of AOAC International (18th ed., Chapter 2, Subchapter 6). AOAC International.

Aslam, Z., Avais, M. A., Farooq, M. R., Rafique, M. A., Haq, M. Z. U., Nazarat, A., ... & Khalid, M. A. (2021). Method development, validation and calculation of uncertainty for the determination of lambdacyhalothrin from commercial formulations through reverse-phase liquid chromatographic approach. *Journal of Agricultural Research (JAR).*, *59*(3), 271-278.

Cannavan, A., Kay, J. F., & Jandrić, Z. (2016). Method Validation and Quality Assurance/Quality Control Approaches for Multi-residue Methods. *Chemical Analysis of Non-antimicrobial Veterinary Drug Residues in Food*, 549-574.

Cortez, L. (1995). Future trends in analytical quality assurance—the estimation of uncertainty. Microchimica Acta, 119(3-4), 323-328.

Ellison, S., Magnusson, B., Bremser, W., Brzyski, A., Christie, E., Kaarls, R., ... & Wood, R. (2019). SLR Ellison and A Williams (Eds) Eurachem/CITAC Guide: Metrological Traceability in Analytical measurement (2019). ISBN: 978-0-948926-34-1.

Ermer, J., & Nethercote, P. W. (Eds.). (2025). *Method validation in pharmaceutical analysis: A guide to best practice*. John Wiley & Sons.

Haque, S. M., Kabir, A., Abu-Thabit, N. Y., Kachhawaha, A. S., Prajapati, P., Pa, S., & Siddiqui, M. R. (2025). The Advancement of Analytical Techniques in Drug Development and Validation. *Critical Reviews in Analytical Chemistry*, 1-32.

ISO. (2017). ISO/IEC 17025: General requirements for the competence of testing and calibration laboratories. International Organization for Standardization.

Kurbanoglu, S., M Ashrafi, A., Vytras, K., Uslu, B., & A Ozkan, S. (2014). Determination and validation of the antiviral drugs using reverse phase high performance liquid chromatographic method. *Current Drug Therapy*, *9*(4), 256-260.

Magnusson, B., & Örnemark, U. (Eds.). (2014). Eurachem guide: The fitness for purpose of analytical methods — A laboratory guide to method validation and related topics (2nd ed.). Eurachem. ISBN 978-91-87461-59-0.

Online ISSN 3007-3197
Print ISSN 3007-3189

Nazir, A., Iqbal, J., Iqbal, M., Abbas, M., & Nisar, N. (2020). Method validation for bifenthrin emulsifiable concentrate and uncertainty calculation using gas chromatographic approach. *Future Journal of Pharmaceutical Sciences*, *6*(1), 5.

Noor, S., Ullah, R., Qazi, M. A., Abbas, Z., Habib, F., Bashir, F., ... & Khan, A. G. (2025). Validation and Development of Gravimetric Method for Humic Acid Determination. *International Journal of Agriculture Innovations and Cutting-Edge Research (HEC Recognised)*, *3*(2), 46-53.

R Core Team. (2024). R: A language and environment for statistical computing. R Foundation for Statistical Computing. https://www.R-project.org/

Rao, T. N. (2018). Validation of analytical methods. *Calibration and validation of analytical methods—a sampling of current approaches*, 131-141.

Raposo, F., & Ibelli-Bianco, C. (2020). Performance parameters for analytical method validation: Controversies and discrepancies among numerous guidelines. *TrAC Trends in Analytical Chemistry*, *129*, 115913.

Sági-Kazár, M., Solymosi, K., & Solti, Á. (2022). Iron in leaves: Chemical forms, signalling, and in-cell distribution. *Journal of Experimental Botany*, *73*(6), 1717-1734.

Sahoo, C. K., Sudhakar, M., Ramana, D. V., Satyanarayana, K., & Panda, K. C. (2018). Validation of analytical procedures-A review. *Asian J. Pharm. Anal*, *8*(2), 109-116.

Taleuzzaman, M. (2018). Limit of blank (LOB), limit of detection (LOD), and limit of quantification (LOQ). *Org. Med. Chem. Int. J*, *7*(5), 127-131.

Ullah, R., Abbas, Z., Bilal, M., Habib, F., Iqbal, J., Bashir, F., ... & Saeed-ur-Rehman, H. (2022). Method development and validation for the determination of potassium (K2O) in fertiliser samples by flame photometry technique. *Journal of King Saud University-Science*, *34*(5), 102070.

Ullah, R., Qazi, M. A., Abbas, Z., Bilal, M., Habib, F., Iqbal, J., Bashir, F., Noor, S., Murtaza, G., Khan, A. G., Naz, S., Bashir, S., & Bashir, S. (2023). Method development/validation and uncertainty measurement for determination of zinc (Zn) using atomic absorption spectrophotometry technique. GU Journal of Phytosciences, 3(2), 112-119.

Ullah, R., Qazi, M.A., Abbas, Z., Noor, S., Bhatti, M. A., Bilal, M., Habib, F., Bashir, F., Khan, A. G., Rauf, A., Jamil, S., & Illahi, W. (2024). Method development/validation and uncertainty measurement for determination of copper (Cu) using atomic absorption spectrophotometry technique. Journal of Xi'an Shiyou University, Natural Science Edition, 20(4), 517-530.

Veen, A. M. V. D., & Cox, M. G. (2021). Getting started with uncertainty evaluation using the Monte Carlo method in R. *Accreditation and quality assurance*, *26*(3), 129-141.

Zhang, X., Zhang, D., Sun, W., & Wang, T. (2019). The adaptive mechanism of plants to iron deficiency via iron uptake, transport, and homeostasis. *International journal of molecular sciences*, *20*(10), 2424.