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Validation of Wavelength-Dispersive X-Ray Fluorescence Technique for Rapid Screening Iron and Zinc Concentration in Rice Seed

Piyanuch Orpong[1,*], Kanokporn Boonsirichai[1], Roppon Picha[1], Ratchai Funklin[2], Supalak Kongsri[1], Chunyapuk Kukusamude[1] and Sasiwimon Naksuriyawong[2]

[1]Nuclear Technology Research and Development Center, Thailand Institute of Nuclear Technology (Public Organization), Nakhon Nayok 26120, Thailand
[2]Nuclear Technology Service Center, Thailand Institute of Nuclear Technology (Public Organization), Nakhon Nayok 26120, Thailand

([*]Corresponding author's e-mail: piyanuch@tint.or.th)

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Abstract

This study focuses on the validation of a wavelength dispersive X-ray fluorescence (WD-XRF) spectrometer for the rapid determination of iron (Fe) and zinc (Zn) concentrations in certified reference materials (CRMs). Five CRMs were used to establish calibration curves. The results were analyzed in terms of accuracy, precision, repeatability and linearity to assess the performance of the WD-XRF method. Accuracy was evaluated by comparing measured values to certified values, with relative accuracy calculated for each sample. Precision was determined through repeatability, where samples were measured in at least 7 replicates across different days, and relative standard deviation (RSD) was calculated. Linearity was assessed by plotting measured intensities and concentrations against certified concentrations, with the correlation coefficient (R^2) values close to 1, indicating a strong linear relationship. Additionally, the WD-XRF method demonstrated high repeatability, with low RSD values across multiple measurements. The study concluded that WD-XRF is a reliable method for the rapid analysis of elemental composition, providing strong accuracy, precision and linearity for Fe and Zn determination in CRMs and can be applied as a valuable method for rapid and non-destructive analysis in various agricultural and food-related applications.

Keywords: Iron, Zinc, Method validation, WD-XRF, Rice, Rapid screening, Calibration

Introduction

There are various analytical techniques available to determine the concentration of elements in plant samples. Widely known techniques include: (1) Atomic absorption spectrophotometry (AAS), used in coffee [1,2] and *Salvia* species [3]; (2) inductively coupled plasma spectrometry (ICP), applied to medicinal plants and herbal medicines such as globe artichoke (*Cynara scolymus* L.), devil's claw (*Harpagophytum procumbens* D.C.) and espinheira-santa (*Maytenus ilifolia* (Mart) ex Reiss.) [4], coffee [2,5], peppers [6] and rice [7,8]; (3) neutron activation analysis (NAA), used in brook-mint, oregano, St. John's wort (*Hypericum perforatum*), thyme and fireweed [9] and rice [10,11]; and (4) X-ray fluorescence (XRF), applied to wheat [12] and thyme [13]. Each technique has its own advantages and disadvantages, and the selection of an appropriate method depends on the type and amount of measured element, the facility availability and the purpose of the analysis.

XRF is a technique that relies on the excitation of samples with high-energy X-rays. Subsequently, secondary X-rays, emitted following de-excitation of the electrons, are collected by a detector, and the signals are processed. These X-rays have characteristic energies which can be used to identify the elements in the sample, while their intensities correlate with elemental concentrations. There are 2 types of XRF systems: Energy Dispersive (ED) and Wavelength Dispersive (WD). Both types are based on the same principle. However, in a WD-XRF system, a crystal is utilized to diffract X-rays based on their energies. Therefore, WD-XRF detects 1 element at a time and often has a better resolution. ED-XRF is generally more cost-effective, compact, and can often be used as a handheld unit [14]. XRF can be used with minimal sample preparation (no need for chemical digestion), making it useful in applications where rapid analysis and sample retention post testing are necessary.

Iron (Fe) and zinc (Zn) are trace elements crucial for both plant growth and human health. They are involved in many processes such as enzyme activity, nucleic acid synthesis, protein synthesis and oxygen transport [15]. In rice seed, iron and zinc are more concentrated in the aleurone layer or outer layer than endosperm or inner layer [16,17]. In unpolished rice seeds, iron concentrations vary from 6.9 to 22.3 mg/kg, while zinc concentrations range from 14.5 to 35.3 mg/kg. However, more than 50 % of these elements are lost during the polishing process [18-20]. Because the iron and zinc concentrations in rice can vary significantly, a reliable analytical method is necessary to accurately determine their levels. Method validation ensures the reliability of the analytical method by verifying that it is appropriate and can provide accurate results. The aim of this study is to validate WD-XRF as a rapid method for determining iron and zinc contents for use in rapid sample screening.

Materials and methods

Sample preparation

Five certified reference materials (CRMs) were used to determine the concentration calibration curve for the measurements. These CRMs included Wheat flour (1567b) and Trace Elements in Pine Needles (Pinus taeda) (1575a), both produced by the National Institute of Standards and Technology (NIST), as well as Trace Elements in White Rice Flour (7501-a), Trace Elements in White Rice Flour (7502-a) and Trace Elements in Soybean (7511-a), produced by the National Metrology Institute of Japan (NMIJ). Two known concentrations of samples, NIST® Rice Flour (1568b) and NMIJ® Arsenic Compounds and Trace Elements in Brown Rice Flour (7533-a), were used to validate the method. For each of the standard samples, 3 g were weighed, placed into 34-mm-diameter sample cups sealed at the bottom with a 6-μm Mylar thin film and covered with a cap to prevent contamination.

Instrumentation

The elemental composition in all standard samples were determined using a Bruker S8 Tiger WD-XRF spectrometer, a stationary lab-scale unit. The device operates with a maximum X-ray tube energy of 50 keV. The measurement conditions were configured in Organic Compounds mode. The measurement time was approximately 7 min per sample.

Calibration procedure

Calibration curves were generated by plotting the certified concentration values against the measured values (intensity and concentration) from the 5 CRMs. The linear equations and the linear regression coefficients of these calibration curves were evaluated based on the results. This helped verify that the device provided a linear response to different concentrations of elements in the samples.

Analytical performance test

All standard samples were measured in least 3 replicates. For each sample, the measured concentration and intensity of the element from WD-XRF were used to calculate the mean, standard deviation (SD), relative standard deviation (RSD) and relative accuracy.

Accuracy

Accuracy refers to how close a measured value is to the true value [21]. The equation used for the calculation of the relative accuracy is as follows:

$$\text{Relative accuracy} = (\text{Analyzed value}/\text{True value})\times 100 \quad (1)$$

Precision

The precision of the method was evaluated based on the repeatability of the measurements. All standard samples were measured at least 7 times on different days. RSD, which indicates the consistency of the measurements, reflecting the analysis precision, is calculated as follows:

$$\text{RSD} = (\text{SD}/\text{Mean})\times 100 \quad (2)$$

Repeatability

Repeatability was assessed by performing multiple measurements on different days for each standard sample under the same operating conditions.

Linearity

The intensity of each element measured by the WD-XRF device was plotted against the certified concentration from the standard samples. A linear regression analysis was performed to determine the correlation between intensity and concentration for each element. The correlation equation and the coefficient of determination or r-squared (R^2) were calculated for each element.

Statistical analysis

The statistical analysis was performed using R Software (Version 4.4.1).

Results and discussion

Analytical performance testing is essential step in ensuring the reliability, accuracy and precision of an analytical method or instrument. This process involves systematically evaluating how well the method or instrument performs under various conditions, ensuring that it consistently produces the reliable results. By conducting these tests, researchers can identify any potential issues, confirm that the methodology meets established standards and ultimately enhance the overall quality and robustness of their analytical processes. For this study, the elemental composition of 5 CRMs was analyzed using WD-XRF. **Table 1** presents the iron and zinc concentrations of the CRMs as determined by both the certified values and the measurements, as well as the measured intensities, obtained from the WD-XRF device.

In WD-XRF analysis, peak and background positions are predetermined, and only intensities at these positions are recorded, whereas in ED-XRF, the entire spectrum is captured, and the peak area is commonly used to measure peak intensity. The output from the device includes both the concentration and net intensity of each element, which correlates with the concentration of each element in the sample. In XRF analysis, primary X-rays are directed at the sample, causing it to emit characteristic X-ray lines, such as Kα, Kβ and Lα. These characteristic X-ray lines are measured for intensity. The intensity depends on the atomic number of the chemical elements interacting with the X-ray. Therefore, this intensity can be used to determine the chemical element composition of the sample.

Table 1 Iron and zinc concentrations (ppm) and intensities (cps) of the CRMs. The values are shown as mean ± sd.

Standard materials	Fe			Zn		
	Certified conc. (ppm)	WD-XRF conc. (ppm)	WD-XRF intensity (cps)	Certified conc. (ppm)	WD-XRF conc. (ppm)	WD-XRF intensity (cps)
1567b	14.11 ± 0.33	10.20 ± 1.33	1.20 ± 0.15	11.61 ± 0.26	6.50 ± 0.67	2.42 ± 0.24
1575a	46.00 ± 2.00	27.00 ± 1.59	2.76 ± 0.17	38.00 ± 2.00	19.70 ± 1.15	6.32 ± 0.32
7501-a	4.04 ± 0.24	5.60 ± 0.66	0.67 ± 0.07	20.10 ± 0.70	10.47 ± 0.96	3.99 ± 0.31
7502-a	4.48 ± 0.20	5.43 ± 0.50	0.67 ± 0.04	26.00 ± 0.90	13.13 ± 0.72	4.92 ± 0.28
7511a	69.10 ± 2.40	38.15 ± 2.40	3.17 ± 0.20	57.80 ± 1.70	30.33 ± 1.19	7.87 ± 0.30

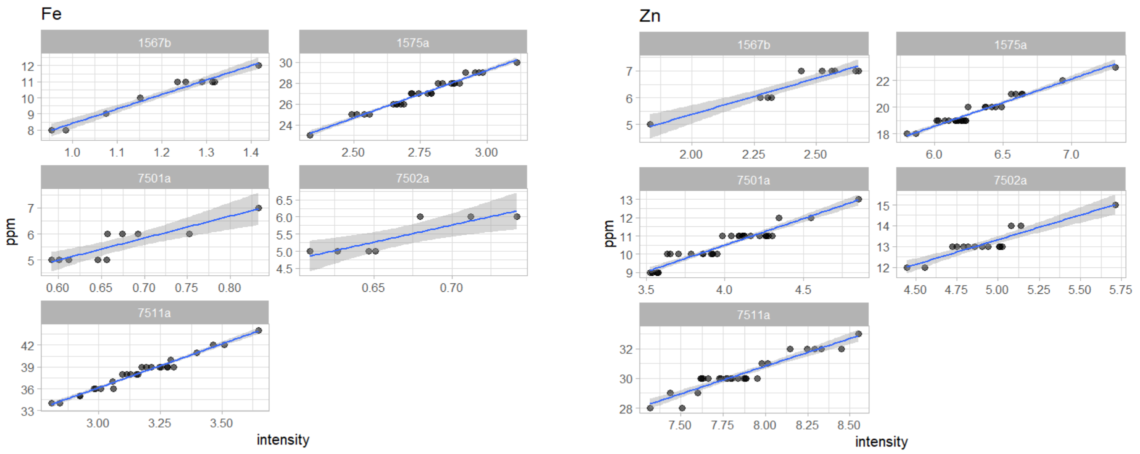

Figure 1 Scatter plot of measured concentration values (ppm) vs intensity (cps).

The WD-XRF results showed that the intensity values recorded by the machine were reported with greater precision, as decimal numbers, while the concentration values in parts per million (ppm) were expressed as integers. This can be seen in **Figure 1**, where multiple distinct intensity values (x-axis) correspond to the same concentration value (y-axis). This indicates that the WD-XRF instrument provides higher resolution in intensity measurement compared to the concentration data, leading to finer intensity values even when concentration values remain unchanged.

The linearity of the analytical method was assessed from the relationship between the measured and certified values analyzed by linear regression. The strength of the linear relationship between 2 variable quantities is expressed as the correlation coefficient which varies from −1 to +1. The closer value is to −1 or +1 indicates the strong of correlation [22]. A very strong correlation coefficient should be close to 1 and not be less than 0.90 [23]. **Figure 2** shows the calibration curve of the measured concentration versus the certified concentration for iron and zinc. The correlation coefficients (R^2) for iron and zinc were 0.9996 and 0.9988, respectively. These values indicated a strong linear relationship between the measured value and certified concentrations. Additionally, the certified concentration value and measured intensity of iron and zinc were also analyzed using a linear regression, as shown in **Figure 3**. The coefficient of determination for this relationship were 0.9718 and 0.9634, respectively. Consequently, the analysis results suggest that the WD-XRF method can be reliably used for determining the elemental composition of the CRMs, as indicated by the strong linearity and correlation with the certified values, as evidenced by the correlation coefficients and the calibration curves.

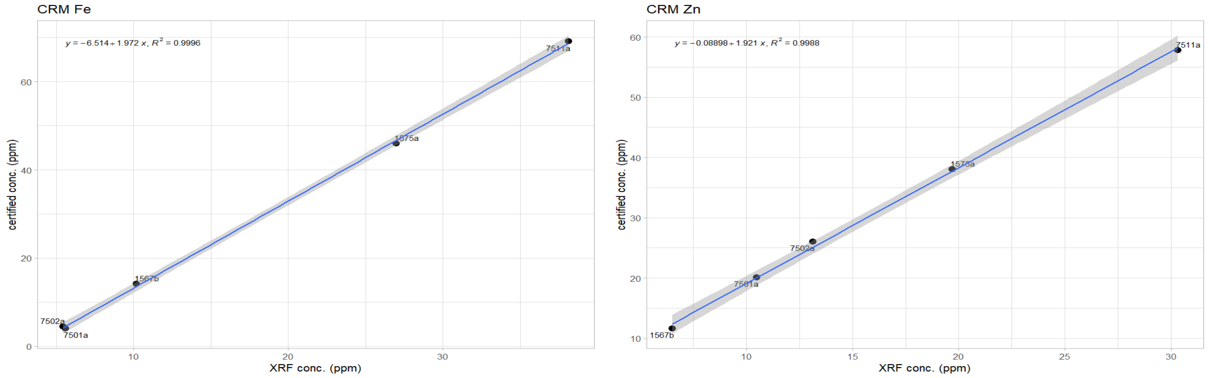

Figure 2 Calibration curves from linear regression of scatter plots between measured concentration and certified concentration.

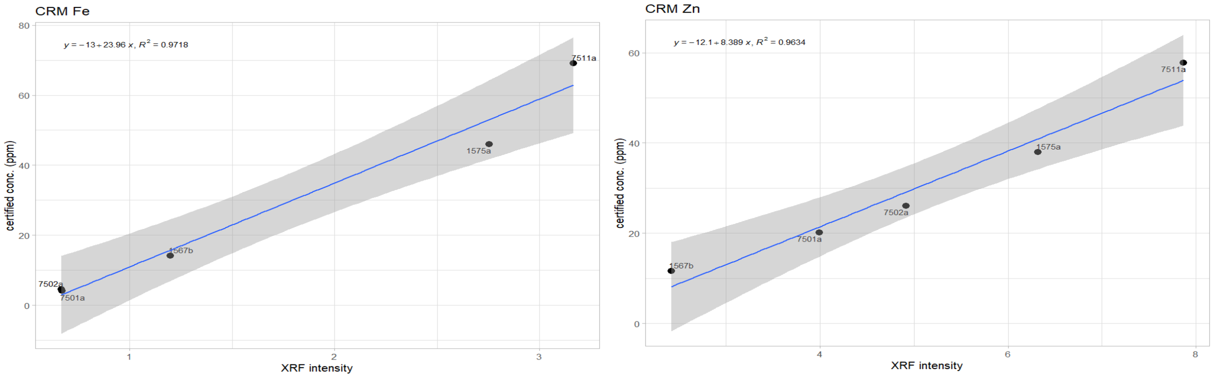

Figure 3 Calibration curves from linear regression of scatter plots between measured intensity and certified concentration.

To calculate iron and zinc concentrations of the 2 known samples, the calibration curves can be used. The iron concentration was calculated from the equation of calibration curve; y = 23.961x – 12.997, while the zinc concentration was calculated from the equation of calibration curve; y = 8.389x – 12.102. The concentration of iron and zinc in the first known sample (rice flour CRM (1568b)) were 7.65 and 21.30 ppm, respectively. In the second known sample, the Arsenic Compounds and Trace Elements in Brown Rice Flour (7533-a), the concentration of iron and zinc are 13.43 and 31.56 ppm, respectively. The obtained values were compared with the certified value and were calculated the average accuracy percentage. The acceptable average threshold of accuracy is recommended to be 80 - 120 %. The accuracy of first known sample iron and zinc concentration are 103.09 and 109.66 %, respectively. In the second known sample, the accuracy of iron and zinc concentrations were 98.75 and 108.09 %, respectively. These results were in the acceptable average threshold of accuracy. Therefore, WD-XRF is an efficient method for determining iron and zinc contents in rice seed sample for use in rapid sample screening.

For the analytical performance test, the accuracy of the analytical method can be evaluated by comparing the measured results to known reference values or certified values can be calculated by percent recovery, bias or error. On the other hand, precision denotes the closeness of repeated measurements to each other when taken under the same conditions. The precision of the analytical method can be evaluated by determining the repeatability with the calculated precision metrics such as relative standard deviation (RSD) or coefficient of variation (CV). While accuracy focuses on the proximity of a measurement to the true value, precision assesses consistency and reproducibility. These factors, although independent, are both vital considerations in measurement processes as they reflect the reliability of the obtained results. The RSD of the measured concentrations and intensities, as well as those of the certified values for comparison, are summarized in **Table 2**. The RSD values of the measured quantities are mostly under 10 %, indicating good precision of the method. The high RSD values (above 10 %) in some measurements may be due to sample heterogeneity and the low concentrations of Fe and Zn in the samples. At low concentrations, the signal-to-noise ratio decreases which is leading to increased variability in measurements [24]. Typical detection limits (LODs) of Fe and Zn using the WD-XRF technique are around 5 ppm [25]. Additional repeated measurements are expected to help reduce the variability.

Table 2 Relative standard deviations (RSD) of iron and zinc concentrations (ppm) and intensities (cps) of the CRMs.

Standard materials	Fe RSD (%)			Zn RSD (%)		
	Certified concentration	Measured concentration	Measured intensity	Certified concentration	Measured concentration	Measured intensity
1567b	2.34	13.04	12.50	2.24	10.31	9.92
1575a	4.35	5.89	6.16	5.26	5.84	5.06
7501-a	5.94	11.79	10.45	3.48	9.17	7.77
7502-a	4.46	9.21	5.97	3.46	5.48	5.69
7511a	3.47	6.29	6.31	2.94	3.92	3.81

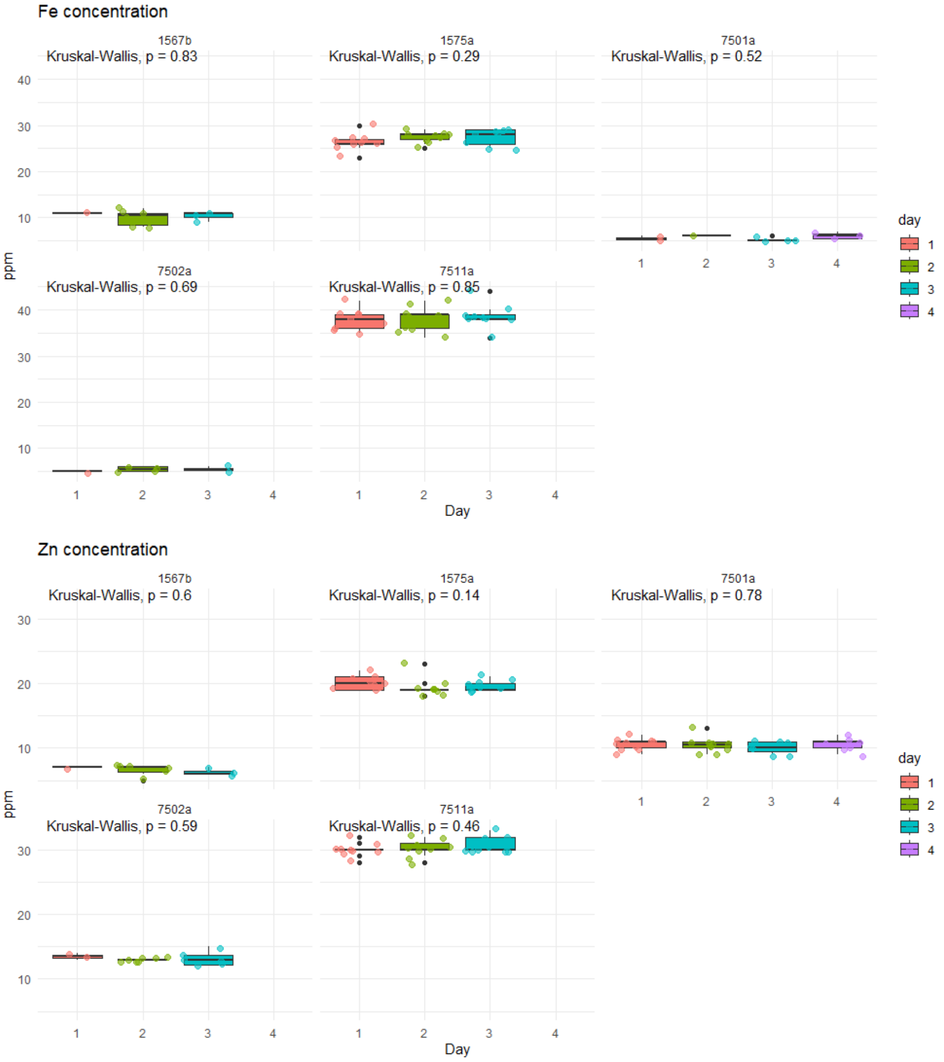

Figure 4 Distribution plots of measured concentration, grouped by CRM and measurement day.

Repeatability refers to the ability of the method to produce consistent results under the same operating conditions over a short time period. A low RSD value would indicate high repeatability, demonstrating that the WD-XRF method provides consistent and reliable results when used under similar conditions. Repeatability is closely related to precision, but the former term focuses more on the repeated measurements under identical conditions, whereas the latter also considers slightly different conditions. For the repeatability assessment, the distributions of the measured values grouped by each day are plotted as shown in **Figure 4**. The Kruskal-Wallis Test was used to compare the 5 CRMs. The results showed that the *p*-value for all samples were greater than 0.05, indicating that there were no statistically significant differences between the measured concentration from different days, for each CRM. A visual inspection of the values indicated that the measurements taken on different days carried out under the same condition remained consistent, suggesting good repeatability.

Conclusions

This study evaluated the effectiveness of wavelength dispersive X-ray fluorescence (WD-XRF) as a reliable method for determining the elemental composition, specifically iron (Fe) and zinc (Zn), in plant-based certified reference materials (CRMs). Calibration curves were established for Fe and Zn, demonstrating a strong linear relationship between measured values (both intensity and concentration) and certified concentrations. The precision and accuracy of the method were validated through repeat measurements, with relative standard deviation (RSD) values reflecting consistent results across multiple trials. The WD-XRF method exhibited high accuracy, with recovery rates for Fe and Zn in 2 known samples (1568b and 7533-a) falling within the acceptable accuracy range. The correlation coefficients (R^2) for Fe and Zn were very close to 1, indicating excellent linearity in the calibration curves. Furthermore, the strong alignment between measured and certified values for both elements highlight the reliability of WD-XRF for elemental analysis. Future studies may involve additional CRMs to further validate these findings and expand the method's applicability. Overall, WD-XRF proves to be a precise, accurate and efficient analytical tool for determining the elemental composition of plant-based CRMs, making it a valuable method for rapid and non-destructive analysis in various agricultural and food-related applications.

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