INAA Application for Trace Element Determination in Biological Reference Material

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INAA Application for Trace Element Determination in Biological Reference Material

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Abstract. Trace element determination in biological samples is often used in the study of health and toxicology. Determination change to its essentiality and toxicity of trace element require an accurate determination method, which implies that a good Quality Control (QC) procedure should be performed. In this study, QC for trace element determination in biological samples was applied by analyzing the Standard Reference Material (SRM) Bovine muscle 8414 NIST using Instrumental Neutron Activation Analysis (INAA). Three selected trace element such as Fe, Zn, and Se were determined. Accuracy of the elements showed as %recovery and precision as %coefficient of variance (%CV). The result showed that %recovery of Fe, Zn, and Se were in the range between 99.4-107%, 92.7-103%, and 91.9-112%, respectively, whereas %CV were 2.92, 3.70, and 5.37%, respectively. These results showed that INAA method is precise and accurate for trace element determination in biological matrices.

1. Introduction

Increasing knowledge and advancement in technology demand that researchers improve research method in getting accurate and precise research results. One of the field studies that should have good quality control (QC) is the study of biological samples, because many of them are used in the health sciences and toxicology. Elemental analysis on research related to toxicology and health must describe the results as close as possible to the actual content in the sample studied [1]. This is of particular importance when small differences in concentration values may help better understanding of the role played by the trace elements under investigation [2]. Determination change to its essentiality and toxicity of trace element requires suitable method that can yield accurate results, which implies that a good Quality Control (QC) procedure should be performed. Neutron Activation Analysis (NAA) is a nuclear analytical technique that has high accuracy in determining the elements both qualitatively and quantitatively, and has the ability to measure many elements simultaneously, has high sensitivity and is capable of analyzing the elements of major, minor and traces [3,4]. NAA is based on the measurement of induced radioactivity of the sample, formed in the reaction of atomic nuclei by neutron particles [5]. Some of the elements that have thin boundaries between essentiality and toxicity in the body are Fe, Zn and Se, so they require a set of data analysis that are controlled and selective and should be verified by quality assurance procedures. Therefore, laboratory performing elemental analysis must always perform the quality control of the data analysis results. To ensure the quality of the test results, which is also as one of the requirements in ISO/IEC 17025:2005, method validation activity using reference material is performed. Reference materials are materials or substances that

have homogeneous and stable properties and serve as standards of measurement or in testing a sample [6,7]. Reference materials play a very important role in validating the accuracy of the data; especially for testing laboratory, in this case is the Laboratory of Center for Applied Nuclear Science and Technology (PSTNT) that uses reference materials as validation of results from testing a sample. Reference materials aim to facilitate the accurate testing of the whole system testing during the development or application of an analytical method. There are several types of reference materials, among which are the Reference Material (RM), Certified Reference Material (CRM), and Standard Reference Material (SRM). One of SRM that been used for validating the NAA method in PSTNT laboratory, particularly for biological matrices samples was SRM NIST 8414 Bovine Muscle, that is produced by NIST (National Institute of Standards and Technology, USA) [8]. In this study, method validation of NAA in determining the elements in biological matrices sample was done by measuring the concentration of target elements in SRM NIST 8414, then comparing with the certificate value for its accuracy and precision. QC chart was made for evaluating the performance of analytical method during routine use. The results of these activities were expected to show that the result data in the field of biological research using nuclear analytical techniques, especially generated by our laboratory are accurate.

2. Methodology

2.1. NAA relative method

NAA relative method was applied for trace element determination in SRM NIST 8414 Bovine Muscle. In this method, the standards and the samples were irradiated together in order to obtain the same conditions. Therefore, that the levels of the elements in the sample can be calculated by comparing the activity of the sample (A spl) with the known activity of prepared standard (A std). A level of elements in the sample is calculated by the following equation [9]:

$$Wx = \frac{A\,spl}{A\,std} \times Wstd \quad (1)$$

Wx and *Wstd* are weight of interested elements of sample and standard, respectively.

2.2. Preparation of NAA standards

Preparation of NAA standards was carried out by pipetting 100 µl of ICP multi-elemental standard solution (E-Merck) then dripping into the polyethylene vial. The filled vials were then dried under the infrared lamp and sealed by heating. Masses of elements in the prepared standard are shown in table 1.

Table 1. Elemental mass of prepared standards.

Elements	Mass (µg)
Fe	59.9
Se	10.2
Zn	10

2.3. Preparation of biological reference materials

SRM NIST 8414 Bovine muscles biological reference material samples were weighed as much as 25 mg, placed in the 0.273 mL polyethylene vial, and then sealed by heating the end of the lid. After checking for leakage possibility, the samples were ready for irradiation. Leakage of the vial can affect the reference material and cause contamination of the research reactor irradiation facility.

2.4. Irradiation and counting of biological reference material.
Biological reference material NIST 8414 Bovine muscle was irradiated in a multipurpose research reactor, PRSG G. A. Siwabessy located in Serpong, Banten province with neutron flux of ~10^{13} n.cm^{-2}.s^{-1} for 120 minutes with cooling time until 4 weeks to get the long half-life elements such as Fe, Zn and Se. Counting was performed using Gamma spectrometer equipped with HPGe detector (Canberra) in PSTNT laboratory for 5000 seconds, to form the characteristic energy peak that shows the elements Fe, Zn and Se.

2.5. Quality control and data analysis.
The SRM was used as quality control assessment of data validity. The results of SRM analysis were compared with its certificate value and evaluated its accuracy and precision by %Recovery and %CV calculation. Net areas from Genie 2000 were used to calculate the concentrations of elements in the samples using a comparative method. Further evaluation of validation results was carried out by plotting it on a diagram of control. Data on control charts were evaluated statistically using the average value and standard deviation with the following conditions [10]:

- Upper warning limit = mean + 2SD
- Lower warning limit = mean - 2SD
- Upper control limit = mean + 3SD
- Lower control limit = mean - 3SD

After statistical evaluation, accuracy and precision were then calculated. Accuracy testing was done by comparing the results obtained with the value of the certificate and commonly describes as %Recovery, which is expressed in the following equation:

$$\%Recovery = \frac{Value_{measurement}}{Value_{certificate}} \times 100\% \quad (2)$$

Precision is the ability to produce consistent measured value. Precision is measured as the standard deviation or coefficient of variance (CV). Parameters precision in the validation of methods, expressed in %CV as shown in the following equation:

$$\%CV = \frac{SD}{Mean} \times 100\% \quad (3)$$

Where CV is coefficient of variance, SD is standard deviation and Mean is mean concentration.

The results of accuracy (%Rec) and precision (%CV) were evaluated by observing the acceptability criteria of accuracy results required by the AOAC guidelines for single laboratory validation, and of precision required by Horwitz equation, as shown in the following table 2 and 3.

Table 2. Acceptance criteria for accuracy [11].

Concentration	***Recovery* (%)**
100%	98-101
10%	95-102
1%	92-105
0.1%	90-108
0.01%	85-110
10 ppm	80-115
1 ppm	75-120
10 ppb	70-125

Table 3. Acceptance limits of precision by Horwitz equation [11].

Concentration	Horwitz (%RSD)
100%	2
10%	2.8
1%	4
0.1%	5.7
100 ppm	8
10 ppm	11.3
1 ppm	16
100 ppb	22.6
10 ppb	32
1 ppb	45.3

According to the table 2 and 3, it can be stated that the analytical method observed is valid if the validation data, meet the acceptance values listed in the tables 2 and 3 above. If it does not meet the acceptance criteria then it will be necessary to evaluate the whole measurement activity beginning with the sample preparation to data processing, otherwise it should be considered that the methods used do not correspond to the reference materials used.

3. Results and Discussion

NAA has been applied in PSTNT laboratory for several samples including biological matrices. Data quality assurance was performed in this laboratory. The easiest and really effective way to carry out data QA controls is to analyze using the same procedure, standard reference materials with well-known and established data on the trace elements under investigation. If correct results are obtained, the overall analytical performance meets the precision and accuracy requirements for trace analysis [12]. Accuracy and precision of NAA method for Fe, Se and Zn was tested by analyzing SRM NIST 8414 Bovine muscle. Results are shown in table 4. Table 4 shows that Fe has an average concentration of 72.9±2.13 ppm. The results obtained from measurements of the 5 samples of SRM gives standard deviation of 2.13 ppm. The values of the measurements were still within the range of the certificate value; 71.20±9.20 ppm, with %Recovery were in the range of 99.4 until 107%, and the %CV was 2.92%. The results of subsequent measurements are described in a diagram control as shown in figure 1. Figure 1 shows that the analysis results of SRM NIST 8414 using NAA method from repeated measurements lie within the mean±2SD. With all the results of testing that has been done, it can be ascertained that the measurement of Fe element content contained in NIST SRM 8414 using NAA method is valid. Zn was also analyzed for the parameters of validation, as shown in table 4. Mean concentration of Zn was 140 ppm, with the range values were 132-146 ppm. The measurements values were still within the range of certificate value; 142.0±14.0 ppm, with the %Recovery were in the range of 92.7-102.75%, and the value of %CV was 3.70%. Performance evaluation of NAA method of test results Zn showed by its control diagram in figure 2.

Table 4. The results of the validation using SRM 8414 Bovine muscle.

No	Sample	Fe		Zn		Se	
		Measured (mg/kg)	Accuracy (%Rec)	Measured (mg/kg)	Accuracy (%Rec)	Measured (mg/kg)	Accuracy (%Rec)
1	SRM BM A	73.7±2.92	104	132±2.15	92.7	0.075±0.008	99.1
2	SRM BM 1	70.8±1.48	99.4	141±0.60	99.6	0.075±0.006	98.7
3	SRM BM 2	72.4±7.14	102	146±1.43	103	0.085±0.009	112
4	SRM BM 3	71.5±2.49	100	140±0.44	98.5	0.079±0.007	104

5	SRM BM B	76.2±3.73	107	141±0.65	99.2	0.076±0.010	99.95
	Mean	72.9±9.04	102	140±2.76	98.5	0.078±0.02	103
	Certificate	71.2±9.20		142±14.0		0.076±0.010	
	%CV	12.4		1.97		23	

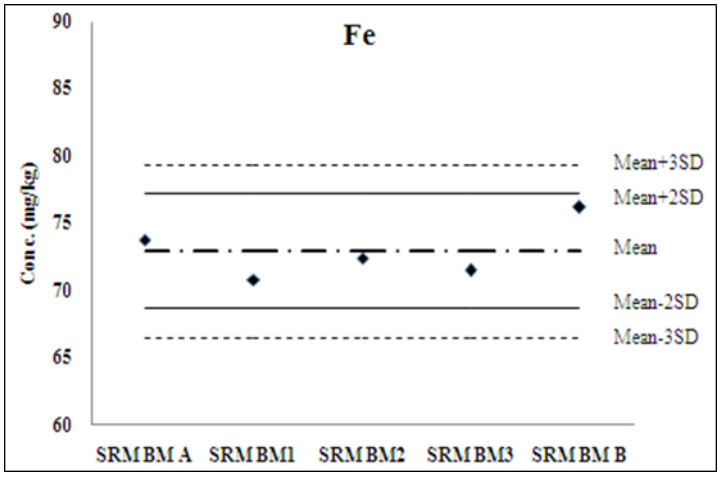

Figure 1. QC chart of Fe in SRM NIST 8414 Bovine muscle.

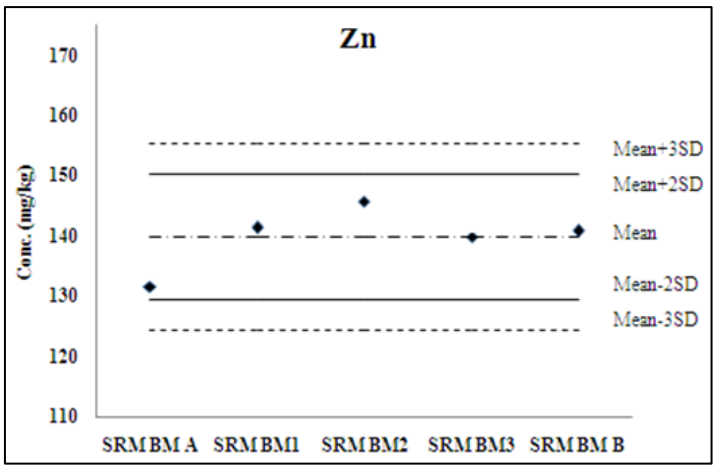

Figure 2. QC chart of Zn in SRM NIST 8414 Bovine muscle.

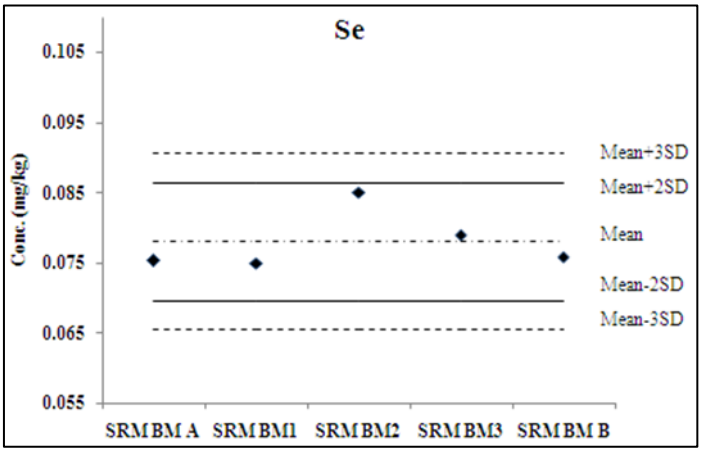

Figure 3. QC chart of Se in SRM NIST 8414 Bovine muscle.

Diagram control for Zn as shown in figure 2, present the range of measurement results for SRM NIST 8414 using NAA between 130 until 150 ppm and still within the range of mean±2SD values. The control diagram and the results of accuracy and precision of Zn measurements contained in SRM NIST 8414 using NAA showed valid results. Validation method of NAA for Selenium was also performed by analyzing SRM NIST 8414. Selenium is an element that usually quite difficult to be analyzed using the NAA. Table 4 shows the element Se has an average concentration of 0.078 ppm, with the results values were in the range of 0.075 until 0.085 ppm, and standard deviation from 5 measurements was 0.004 ppm. This value was still within the range certificate value that is 0.076±0.010 ppm, with the value of %Recovery range between 98.68 until 111.84%, and the %CV was 5.37%. QC chart for Se is shown in figure 3, and the concentration of 5 measurements was still in the mean±2SD, it means that the performance of this method is good. Based on of both accuracy and precision test, Se determination in SRM NIST 8414 using NAA showed good results. The accuracy and precision obtained from this study were affected by several factors such as weighing procedure of the Fe, Se and Zn in the prepared standards for NAA relative method and stability of the neutron flux during irradiation. From these results, it can be concluded that the NAA method in determining of Fe, Zn and Se in SRM NIST 8414 are valid, can thus be applied with accuracy and precision on similar biological matrices samples.

4. Conclusion

The results obtained from testing the accuracy (%Recovery) and precision (%CV) of the Fe, Zn and Se contents in SRM NIST 8414 Bovine muscle are within the range of their acceptance criteria, and are strongly supported by the repeated measurements as described in the control diagram. It can be stated that the NAA method has high accuracy and precision for the determination of elements observed in the SRM Bovine muscle, thus NAA method is valid and can be applied to determine trace elements in similar biological matrices samples.

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