



VALIDATION OF AAS ANALYSIS PROTOCOLS FOR TUNA FISH HOMOGENATE: IAEA-436

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Tuna fish flesh homogenate, IAEA-436, a certified reference material received from IAEA under the Analytical Quality Control Services intercomparison programme for trace metal determination, was analyzed for Cu, Fe, K, Mg, Mn and Zn by using atomic absorption spectrophotometric (AAS) technique employing the optimized instrumental parameters. The reliability of the methodology adopted was checked by analyzing IAEA-CRM (Copepod Homogenate MA-A-1) under identical conditions and comparing the results obtained with the certified values which are in quite good agreement with each other. High precision was observed for Fe, K, Mg and Zn, whereas Cu and Mn showed relatively poor precision in their determination.

Keywords: Trace metals, Tuna fish homogenate, Atomic absorption spectrophotometry

1. Introduction

During the last few decades the human environment has been deteriorated to a significant extent due to urbanization, industrialization, agricultural and other human activities. Currently the international trade is facing difficulties due to legislation enforcement by the WTO especially for the quality of edible items. To meet such challenges accredited laboratories are running under the guidelines of international bodies such as EURACHEM, IUPAC and ISO [1-3]. To obtain the quality data and reliable chemical analysis the use of reference materials (RMs) is one of the integral parts of the routine procedures of a certified laboratory [3]. Such practices enable to identify analytical difficulties in laboratories and to initiate remedial actions or to make appropriate modifications to laboratory analytical procedures to improve the quality of data. This is also useful to characterize trends of analytical performance in the determination of elements at trace levels in specific matrices over the years [4-5].

Among the various available analytical techniques for trace metal determination, atomic absorption spectrophotometry (AAS) is one of the preferred techniques over other due to its rapidness, specificity and lower detection limits [6]. Our laboratory has participated in the AQCS inter-

comparison exercise for the determination of trace metals in the proposed reference material i.e., Tuna Fish Flash Homogenate (IAEA-436). According to the IAEA rating our laboratory was categorized in group 2, i.e., laboratories with Z-score < 3 for 75% to < 90% of the data set.

The present paper deals with the validation of methodology adopted for quantification of metals at trace levels in the proposed reference material i.e., Tuna Fish Flash Homogenate (IAEA-436) in the intercomparison programme of IAEA and to discuss the problems associated with the determination of certain metals with the adopted mode of quantification. The participation in such exercise will help to establish the potentials of the techniques for their uses in biological matrix and to highlight the problems associated with the quantification of certain elements in such matrices. It will also help to establish the baseline levels of trace metals in marine biological material under the specified environment.

2. Experimental

2.1. Instrumentation

All the atomic absorption measurements were performed with Hitachi model 180/80 polarized Zeeman atomic absorption spectrophotometer, which was coupled with a microprocessor-based

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Table 1. Instrumental parameters used for the determination of trace metals by AAS.

Parameters	Metals					
	Cu	Fe	K	Mg	Mn	Zn
Lamp current (mA)	7.5	10.0	10.0	7.5	7.5	10.0
Resonance absorbance line (nm)	324.8	248.3	766.5	285.2	279.5	213.8
Slit width (nm)	1.3	0.2	1.3	2.6	0.4	1.3
Burner type	Standard*					
Burner height	7.5	7.5	7.5	7.5	7.5	7.5
Fuel (C ₂ H ₂) pressure (kg/cm ²)	0.3	0.35	0.3	0.25	0.3	0.25
Oxidant (air) pressure (kg/cm ²)	1.6	1.6	1.6	1.6	1.6	1.6

* See experimental section.

data-handling facility. A water-cooled premix, fishtail type burner having a $10 \times 0.05 \text{ cm}^2$ slot was used for the air-acetylene flame. Concentration mode of FAAS was used for all measurements. Single element hollow cathode lamps of the desired metals were used as radiation sources.

2.2. Reagents

Stock solutions (1000 mg L^{-1}) of metals of interest were prepared by dissolving appropriate amount of metals or their oxides (Johnson & Matthey Chemicals Limited) separately in minimum amount of nitric acid and the volume was made up to mark with water. Standard solutions for the construction of calibration curves were prepared by appropriate dilution of these stock solutions. Fresh working standards were prepared immediately before use. Analytical grade perchloric acid (70%) and nitric acid (65%) were used for digestion of samples. Glassware was cleaned by overnight soaking in nitric (1+1) followed by multiple rinsing with water. Distilled and deionized water was used throughout this work.

2.3. Sampling and sample preparation

The IAEA reference material tuna fish flesh homogenate was prepared by Marine Environmental Studies Laboratory (MESL) of International Atomic Energy Agency. A large quantity of the tuna fish filet was collected from the Mediterranean Sea and purchased from a local supplier. The material was freeze-dried, ground and sieved then it was homogenized by mixing in a stainless steel rotating drum for two weeks. Aliquots of about 20 g were packed into glass bottles with teflon lined screw caps and sealed in plastic bags. The material homogeneity for trace elements was tested using a standard protocol and

found to be satisfactory for the purpose of intercomparison exercise (for 200 mg sub-sample). Since the material has the tendency to absorb moisture easily during storage, therefore, the sample was oven dried at 105°C for 24 hours as recommended by the supplier.

2.4. Procedure

About 0.5 g of dried sample was taken in six replicates in 100 mL flask fitted with a 30 cm air condenser and 5 mL of nitric acid was added to the sample. The mixture was heated at 60°C for 45 minutes. After cooling the mixture, 2 mL of perchloric acid was added and then heated at 200°C with occasional shaking till white fumes evolved. The clear solution obtained was transferred into a 10 mL volumetric flask and the volume was made up with water. A blank was prepared similarly. The signals of standard blank, standards and samples were measured by aspirating the solutions into the atomizer using the optimized instrumental parameters given in Table 1. A minimum of three readings were taken for each solution and the mean value of the absorption signal was used for subsequent calculations. The absorption signals were evaluated by subtracting the value of blank from the signal of the sample.

3. Results and Discussion

The concentration of six metals (Cu, Fe, K, Mg, Mn and Zn) at trace levels has been determined in the certified reference material "Tuna fish flesh homogenate (IAEA-436)", using atomic absorption spectrometry by employing the optimized instrumental parameters (Table 1). The criterion of the optimization was the selection of parameters,

Table 2. Determined metal concentration ($\mu\text{g g}^{-1}$) in IAEA – CRM

Element	Copepod homogenate (MA-A-1)					Average	SD	Certified values	$C_{\text{Det}}/C_{\text{Cer}}$
	1	2	3	4	5				
Cu	8.12	7.83	7.02	8.54	7.76	7.85	0.56	7.6 ± 0.2	1.03
Fe	67.58	55.53	55.56	62.89	59.29	60.17	5.14	60.0 ± 2.0	1.06
K	8456	6698	5922	6953	–	7007	1061	–	–
Mg	6973	5779	6273	5283	5877	6037	631	–	–
Mn	2.48	2.22	2.46	2.46	2.32	2.39	0.12	2.9 ± 0.2	0.82
Zn	138.23	143.16	159.24	139.90	160.74	148.25	10.87	158.0 ± 2.0	0.94

which could produce maximum and reproducible absorption signal with minimum background.

For digestion of the sample, different proportions of the nitric acid and perchloric acid were tested to find out the best composition. A mixture of 5.0 mL of nitric acid and 2.0 mL of perchloric acid proved to be the most satisfactory composition for the sample weight used in the present study. The initial heating at about 60°C was found to be essential in order to decompose maximum of organic matter. Perchloric acid was then added to complete the destruction of remaining organic matter at higher temperature to get clear and homogeneous resultant solution.

The detection limit defined as the smallest amount of an analyte which could produce a signal equal to three times the value of the standard deviation of at least ten determinations of blank solution or near blank level [2], were found to be 0.02, 0.02, 0.03, 0.03, 0.02 and $0.01\mu\text{g g}^{-1}$ of Cu, Fe, K, Mg, Mn and Zn respectively. It was observed that the detection limits of all the elements studied were much below the certified amounts of these elements in the sample. The above mentioned procedure using a mixture of nitric acid and perchloric acid was, therefore, found suitable for the digestion of the biological material for trace metal determination.

Quality assurance of the results obtained was checked by analyzing Certified Reference Material i.e., IAEA-Copepod Homogenate (MA-A-1) for the metals of interest employing identical experimental conditions and the results are reproduced in Table 2 along with the certified values. The determined concentrations of metals in the IAEA-CRM are in quite good agreement with the certified values indicating the reliability of the procedure adopted.

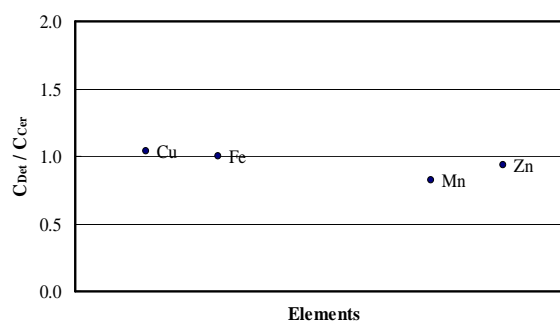


Figure 1 Ratio of the determined and certified concentration in IAEA – CRM Copepod Homogenate (MA-A-1).

The accuracy of the results is well explained through the ratio of determined metal concentration (C_{Det}) and the certified concentration (C_{Cer}) in the Certified Reference Material [7] or by calculating the Z-scores and comparing with the analytical criteria [5]. In the present study the first criteria has been adopted and the results have been shown in Fig. 1 which is a plot between $(C_{\text{Det}})/(C_{\text{Cer}})$ ratio and metals. The plot depicts that the $(C_{\text{Det}})/(C_{\text{Cer}})$ ratio of most of the metals lie close to unity (0.82–1.06) indicating good agreement of our determined values with the certified values. The ratio of $(C_{\text{Det}})/(C_{\text{Cer}})$ for K and Mg could not be plotted due to non-availability of the certified values for these metals.

The determined metal concentrations in tuna fish flesh homogenate (IAEA-436) from Mediterranean Sea have been reported in Table 3 along with the certified values [8]. All calculations were made on dry weight basis. The concentration of each determined metal was reported as six independent determinations, average value and standard deviation. The last column of Table 3 shows the $C_{\text{Det}}/C_{\text{Cer}}$ ratio of the determined elements and is depicted in Fig. 2. Our calculated

Table 3. Determined metal concentration ($\mu\text{g g}^{-1}$) in IAEA – CRM Tuna fish flesh homogenate (IAEA – 436)

Element	Determined concentration						Average	SD	Certified values	$C_{\text{Det}}/C_{\text{Cer}}$
	1	2	3	4	5	6				
Cu	1.34	1.37	1.09	1.08	1.27	0.88	1.17	0.18	1.73 ± 0.19	0.68
Fe	89.17	86.86	89.40	86.53	89.15	91.83	88.82	1.93	89.3 ± 4.2	0.99
K	12508	12520	11696	12615	13315	12531	12531	514	12300 ± 615	1.01
Mg	845	766	784	862	839	–	819	41.7	1070 ± 44	0.76
Mn	0.33	0.30	0.26	–	0.40	0.30	0.32	0.05	0.24 ± 0.04	1.34
Zn	16.37	15.08	14.25	14.15	13.88	14.19	14.65	0.93	19.0 ± 1.3	0.77

$C_{\text{Det}}/C_{\text{Cer}}$ ratios for the determined metals were comparable to the results reported by Siddique et al. [7].

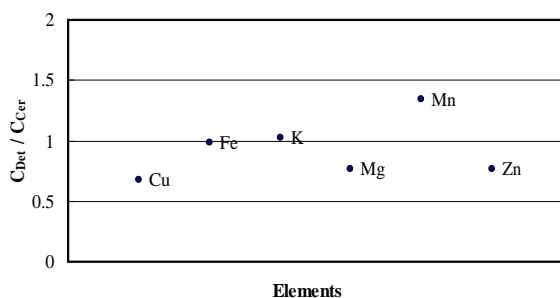


Figure 2. Ratio of the determined and certified concentration in Tuna fish flesh homogenate (IAEA-436).

Among the determined metals K and Mg were found as major elements whereas Cu, Fe, Mn and Zn were at trace levels. The determined precision was found to be up to 6.4% for Fe, K, Mg and Zn. Relatively higher relative standard deviation of about 16% were observed for Cu and Mn, which could probably be due to low concentration of these metals in the sample. Comparatively higher determined value for Mn is in accordance with the IAEA report [8], indicating low sensitivity of FAAS technique specially operating for low concentration of this metal. It is, therefore, suggested to select the graphite furnace AAS technique for the determination of low concentration of Mn in such matrices.

4. Conclusion

IAEA Certified reference material of marine biological origin i. e., tuna fish flash homogenate (IAEA-436) has been characterized for trace metals by employing atomic absorption spectrophotometry. The elements were determined with good precision except for Cu and Mn which were present at very low concentrations for determination with flame AAS and are reported

with low degree of confidence. The proposed procedure has proved to be simple, rapid and economical for routine analysis.

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