Development and validation of a GF-AAS method and its application for the trace level determination of Pb, Cd, and Cr in fish feed samples commonly used in the hatcheries of Bangladesh

A. K. M. Atique Ullah[*], M. A. Maksud, S. R. Khan, L. N. Lutfa and Shamshad B. Quraishi[*]

Abstract

Background: A sensitive, accurate and precise method was developed and validated in regard to Council Directive 333/2007/EC, Commission Decision 657/2002/EC, ISO/IEC 17025:2005 and EURACHEM 1998 for the trace level determination of Lead (Pb), Cadmium (Cd) and Chromium (Cr) in fish feed samples using Graphite Furnace Atomic Absorption Spectrometry (GF-AAS).

Methods: For method validation, selectivity, working and linear ranges, limit of detection (LoD), limit of quantification (LoQ), trueness (recovery) and precision (repeatability and reproducibility) have been assessed as performance criteria.

Results: The method LoDs for Pb, Cd and Cr were found to be 0.078, 0.010 and 0.022 μg/g respectively, whereas, the method LoQs were obtained at 0.156, 0.021 and 0.044 μg/g respectively. Moreover, the recovery of the analytes was 90 – 104% and the RSD values for precision were 4.90 – 9.60% respectively.

Conclusions: The method validation performance criteria indicate the method's suitability for trace level quantification of Pb, Cd, and Cr in fish feed samples.

Keywords: Validation, Heavy metals, Trace level, Fish feed, GF-AAS

Background

Fish is the most important single source of high quality protein providing about 17% of the annual protein consumed by world's population (Food and Agricultural Organization 2016). Moreover, in Bangladesh, fish made up 55% of animal protein intake (Food and Agricultural Organization 2014) and contributes 49.5 g/capita/day (Bangladesh Bureau of Statistics 2015). Fish can accumulate contaminants in their tissues that they ingest with food. Moreover, fish can concentrate the contaminants from water through diffusion across the gills and skin (Gobas et al. 1999). Heavy metals are one of the most potential environmental contaminants even though they are present at low concentrations (Jakimska et al. 2011). Several researchers found that feeds contain significant concentrations of heavy metals that can bioaccumulate in fish (Indrajit et al. 2011; Maule et al. 2007). On the contrary, growing concern for public health has stimulated the European Commission to set maximum permissible limits for major toxic ingredients in feed.

There are several techniques available for the quantification of heavy metals at very low level, but it is essential to ensure the quality of analytical results generated by the laboratories approved for official control. For this reason, there is a growing global interest to develop and establish

* Correspondence: atique.chem@gmail.com; mumu3222@yahoo.com
Analytical Chemistry Laboratory, Chemistry Division, Atomic Energy Centre, Bangladesh Atomic Energy Commission, Dhaka 1000, Bangladesh

Ullah *et al. Journal of Analytical Science and Technology* (2017) 8:15

Page 2 of 7

analytical methods which can provide accurate and precise measurements with low detection limit. This should be achieved by practicing proper quality assurance systems and more specifically by applying methods validated following the international guidelines and standards (European Commission 2002a, 2002b, 2006, 2007, 2008). ISO/IEC 17025 (2005) specifies the general requirements for the competence in order to perform chemical testing including sampling. It covers testing performed using standard methods, non-standard methods, and laboratory-developed methods.

Few studies have been carried out on metal contamination in different fish feed samples in earlier (Shamshad et al. 2009; Anhwange et al. 2012; Psoma et al. 2014). Studies have shown that fish feed contains measurable levels of some contaminants, e.g., Pb, Cd, Cr, As, and Hg. Moreover, according to FAO, feed quality is one of the key constraints to the sustainable aquaculture development in Bangladesh (Food and Agricultural Organization 2014). For this reason, attempt has taken to determine the heavy metal contents particularly Pb, Cd, and Cr in fish feed samples commonly used in the hatcheries of Bangladeshi using a validated method. Several analytical techniques such as Flame Atomic Absorption Spectrometry (FAAS), Graphite Furnace-Atomic Absorption Spectrometry (GF-AAS), Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES), Differential Pulse Anodic Stripping Voltammetry (DPASV), Neutron Activation Analysis (NAA), Energy Dispersive X-ray Fluorescence (EDXRF) have been employed for the determination of trace metals in different environmental samples (Akter et al. 2014; Ullah et al. 2014, 2015; Aydin and Soylak 2010; Panahi et al. 2009; Shubina and Kolesov 2002; Hwang et al. 2016). In the present study, GF-AAS was used for studying trace metal contents in fish feed samples as it is highly accurate, sensitive, selective, and cost effective (Zhong et al. 2016). Moreover, to the best of our knowledge, this is the first attempt to quantify the toxic metals, Pb, Cd, and Cr, in fish feed samples widely used in the hatcheries of Bangladesh using a validated analytical method.

Methods
Instruments and reagents
A Varian AA280Z atomic absorption spectrometer with Zeeman background correction system equipped with graphite furnace (GTA 120) and an auto sampler (PSD 120) was used. The purity of argon gas was 99.999%. Hollow cathode lamps were used for Pb (283.3 nm and slit 0.5 nm), Cd (228.8 nm and slit 0.5 nm), and Cr (357.9 nm and slit 0.2 nm) and operated according to the conditions recommended by the manufacturer. Atomic signals were measured in peak area mood. The digestions were performed using a microwave oven (MARS'5, CEM Corporation, USA).

The bulk standard solutions 75 $\mu g_{Pb}L^{-1}$, 6 $\mu g_{cd}L^{-1}$, and 20 $\mu g_{cr}L^{-1}$ were daily prepared by appropriate dilution of 1000 μgL^{-1} Pb, Cd, and Cr stock standard solutions respectively using 1% (w/w) Suprapur grade nitric acid (Merck, Darmstadt, Germany). The working standard solutions were prepared automatically by the autosampler from bulk standard solution. Phosphoric acid (H_3PO_4; Merck, Darmstadt, Germany) and ammonium phosphate monobasic ($NH_4H_2PO_4$; Merck, Darmstadt, Germany) were used as chemical modifiers for the determination of Pb and Cd, respectively. The reagents were all of analytical grade, and all solutions were prepared using deionized water (18 MΩ/cm) produced using an E-pure system (Thermo Scientific, USA). All containers and glassware were cleaned by soaking into 20% (w/w) nitric acid for at least 24 h and rinsed three times with deionized water prior to use.

Sample preparation
One gram of the homogenized fish feed samples was weighted into the polytetrafluoroethylene digestion vessel, and 6 mL of Suprapur nitric acid were added. Subsequently, the samples were digested with the microwave oven using a two-step temperature program. During the first step, the temperature was linearly increased to 180 °C for 10 min, the maximum power of the rotating magnetron was 1600 W. During the second step, the temperature was maintained at 180 °C for 15 min. After digestion and cooling, each solution was diluted to a final volume of 10 mL with deionized water.

Quality control programs
Internal quality control charts (IQCs) were also constructed in order to monitor whether results are reliable enough to be released. The objective of IQCs is the elongation of method validation: continuously checking the accuracy of analytical data obtained from day to day in the laboratory. The analytical system is under control if no more than 5% of the measured values exceed the warning limits and none of them the action or control limits (Taverniers et al. 2004). The external quality assurance program was maintained through the participation in proficiency programs organized by Asia Pacific Metrology Program-Asia Pacific Laboratory Accreditation Cooperation (APMP-APLAC) and LGC, UK. The results of proficiency tests were within ±2 Z scores.

Ullah *et al. Journal of Analytical Science and Technology* (2017) 8:15

Page 3 of 7

Results and discussion

Method validation

The validation of the analytical procedure for quantitative determination of Pb, Cd, and Cr in fish feed was performed by evaluating linearity and selectivity, limit of detection (LoD), limit of quantification (LoQ), accuracy (recovery), and precision (repeatability and reproducibility) (European Commission 2007; ISO/IEC 17025 2005; EURACHEM 1998).

Linearity and selectivity

R^2 value greater than 0.995 is required for accurate quantification as analytical response is linear over certain concentration ranges, or the peak height has a similar trend to that of relevant peak areas (EURACHEM 1998; Jeevanaraj et al. 2015). The linearity for Pb, Cd, and Cr was demonstrated by analyzing a number of different concentrations of the studied elements. The calibration curves were prepared using aqueous standard solutions at 15, 30, 45, 60, and 75 µg/L for Pb; 1.0, 1.5, 3.0, 4.5, and 6.0 µg/L for Cd; and 4, 8, 12, 16, and 20 µg/L for Cr. The coefficient correlation (r) values obtained corresponding to linear regression equation were 0.998 (Pb), 0.997 (Cd), and 0.998 (Cr). Table 1 complies the linearity and range parameters for analysis of Pb, Cd, and Cr. The R^2 value greater than 0.995 for all three elements in our study indicates an excellent and precise liner relationship between concentration and the corresponding peak area.

Selectivity is the ability of a method to accurately quantify the analyte in the presence of interferences, under the stated conditions of the assay for the sample matrix being studied (EURACHEM 1998; Senila et al. 2014). The selectivity in the case of AAS method is related to possible interferences of the absorption spectrum at specific wavelength. To verify the selectivity of the method, matrix matching (MM) calibration curves were also prepared for Pb, Cd, and Cr within the linearity range obtained for aqueous standard solutions (Soares and Nascentes 2013). The MM curves were prepared from a feed that presented no analytical signal for Pb, Cd, and Cr during GF-AAS analysis. The slope of the calibration curve from aqueous standards is almost the same as the slope of the curve plotted by feed standard addition indicating no matrix interferences in the matrix (Catarino et al. 2002) (Table 1). Comparison of the slopes of the curves by means of Student's t test revealed no significant differences at the 95% confidence level. Therefore, aqueous calibration curves were used for calibration in all experiments. Moreover, modifiers used during GFAAS stabilize the elements during the graphite furnace cycle and permits increase in the charring and atomization temperature allowing a better separation of the elements from interferences (Blake and Bourqui 1998).

Determination of LoD and LoQ

The performance of an instrument or an analytical method is assessed by determining LoD and LoQ. LoD is the lowest concentration of an analyte that can be detected and reliably distinguished from zero, but not necessarily quantified, while LoQ is defined as the lowest concentration of an analyte that can be determined quantitatively with an acceptable level of precision and accuracy (Gonzaler and Herrador 2007). Based on guideline by Commission Regulation (EC) No. 333/2007 of 28 March 2007, instrument LoD was calculated as the three times the standard deviation of the mean of 21 blank determinations, while instrument LoQ was measured as six times of the standard deviation of the mean of 21 blank determinations (European Commission 2007). The LoDs and LoQs for fish feed samples were calculated by multiplying each instrument LoD and LoQ with a factor of 100 for Pb, Cd, and Cr (1 g sample and a final volume of 100 mL) (Perring et al. 2001). LoDs and LoQs for instrument and method are given in Table 2

The calculated method LoDs and LoQs were found to be 64 and 32 times lower for Pb and 100 and 200 times lower for Cd than the maximum permissible limits as presented in Regulations (EC) No. 1275/2013 (European Commission 2013), and well met the criteria as defined in Commission Regulation 333/2007/EC (European Commission 2007) that LoD and LoQ should be one fifth and one tenth of the maximum limit indicating that developed method is very sensitive and can quantify toxic metals at trace level for the assessment of fish feed.

Table 1 Linear regression and selectivity data of Pb, Cd, and Cr for aqueous and matrix matching calibration curves

Analyte	Linear range (µg/L)	Calibration with aqueous standard solutions			Calibration with matrix matched standard solutions		
		Slope ± SD (µg/L)$^{-1}$	Intercept ± SD	Correlation Coefficient	Slope ± SD (µg/kg)$^{-1}$	Intercept ± SD	Correlation coefficient
Pb	15–75	0.00084 ± 0.00002	0.00053 ± 0.00025	0.998	0.00086 ± 0.00002	0.00521 ± 0.00164	0.998
Cd	1–6	0.01941 ± 0.00019	0.01430 ± 0.00374	0.997	0.01984 ± 0.00065	0.03423 ± 0.00319	0.997
Cr	4–20	0.00607 ± 0.00002	0.02070 ± 0.00479	0.998	0.00615 ± 0.00003	0.00463 ± 0.002811	0.998

Ullah *et al. Journal of Analytical Science and Technology* (2017) 8:15

Page 4 of 7

Table 2 Instrument and method LoDs and LoQs for determination of Pb, Cd, and Cr in fish feed

Parameter	Pb	Cd	Cr
Instrument LOD in solution (µg/L)	0.780	0.103	0.221
Instrument LOQ in solution (µg/L)	1.560	0.206	0.442
Method LOD for feed (µg/g)	0.078	0.010	0.022
Method LOQ for feed (µg/g)	0.156	0.021	0.044

Accuracy

The performance of an analytical method is demonstrated by acceptable accuracy, the closeness of agreement between the values which is acceptable either as a conventional true value or an accepted reference value and the value found (European Medicine Agency 2006). Certified reference materials similar to fish feed were not available; thus, the recovery studies were performed in order to check the accuracy of the proposed method (European Commission 2002a; Eka et al. 2012; Rawar and Rohman 2016; Rohman and Wijayanti 2015). These recovery studies were done to confirm the lack of analyte levels due to the losses or contamination during sample preparation, and matrix interferences during the measurement step (Ertasa and Tezel 2005). The recovery determination was carried out by spiking technique, i.e., the known concentration of Pb, Cd, and Cr solutions were added to fish feed samples before digestion and the resulting spiked samples were measured, calculated and compared to the known value of Pb, Cd, and Cr solutions added. The samples were spiked at three different levels of maximum level as per the recommendation of Commission Regulation 657/2002/EC (European Commission 2002a). The recovery values for accuracy studies of the fish feed samples spiked with different levels of Pb, Cd, and Cr are shown in Table 3. For the analyte level of approximately 1 µg/g, the acceptable recovery range is 80–110% (Taverniers et al. 2004; Huber 1998). In the present study, the recovery was found to be 90–104%

Table 3 Accuracy studied data for analysis of Pb, Cd, and Cr

Analyte	Spiked analyte concentration (µg/g)	Calculated analyte concentration (µg/g)	Recovery (%)
Pb	2.50	2.26	90.400
	5.00	4.60	92.000
	7.50	7.33	97.733
Cd	0.50	0.49	98.000
	1.00	1.04	104.000
	1.50	1.53	102.000
Cr	2.50	2.32	92.800
	5.00	4.62	92.400
	7.50	6.94	92.533

for Pb, Cd, and Cr suggesting the developed method was accurate for the quantification of Pb, Cd, and Cr in fish feed samples.

Precision

The precision demonstrates the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions and is usually measured as relative standard deviation (RSD) (Jeevanaraj et al. 2015). The analytical precision was determined by assessing the repeatability and reproducibility of instrument response to analyte according to the commission regulation (2002/657/EC) (European Commission 2002a). In order to assess the repeatability, measurements were also done for three different concentrations levels (0.5, 1, and 1.5 times the permitted limit), and in each level, the analysis was performed with six replicates. The steps were repeated on two other occasions. The mean concentration, relative standard deviation, overall mean concentration and overall relative standard deviation are summarized in Table 4.

The precision was also assessed in terms of reproducibility of the analytes. In order to assess the reproducibility, measurements were also done for three different concentrations levels (0.5, 1, and 1.5 times the permitted limit), and in each level, the analysis was performed with six replicates. The steps were repeated on two other occasions with different operators and different environmental conditions. The mean concentration, relative standard deviation, overall mean concentration and overall relative standard deviation are summarized in Table 5.

According to RSD Horwitch function (Gonzaler and Herrador 2007), the maximum RSD values acceptable for the level of ≥1000 µg/mL is 10% (European Commission 2002). Therefore, it can be stated that the developed method exhibited a good reproducibility precision based on RSD values obtained.

Measurements uncertainty

Measurement uncertainty (MU) is a parameter associated with the results of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measurand. Thus, according to ISO/IEC 17025, an estimation of measurement uncertainty is required for every measurement. Method development and validation data can be used for estimating measurement uncertainty (EURACHEM/CITAC 2012). The most common practice is using reproducibility data for the estimation of measurements uncertainty (Nordtest Report TR 537 2004). The measurement uncertainty can be calculated at the 95% confidence level using the equation below (Ora Laboratory Procedure 2005).

Ullah *et al. Journal of Analytical Science and Technology* (2017) 8:15

Page 5 of 7

Table 4 Repeatability precision studies data for determination of Pb, Cd and Cr

Analyte	Day	Spiked concentration (µg/g)	Mean concentration (n = 6) (µg/g)	RSD (%)	Overall mean concentration (µg/g)	Overall RSD (%)
Pb	1	2.50	2.26	7.40	2.26	4.90
	2	2.50	2.27	3.21		
	3	2.50	2.26	3.82		
	1	5.00	4.60	9.73	4.59	7.46
	2	5.00	4.59	7.06		
	3	5.00	4.59	4.50		
	1	7.50	7.10	6.46	7.20	5.89
	2	7.50	7.16	8.24		
	3	7.50	7.33	1.50		
Cd	1	0.50	0.52	7.51	0.51	9.12
	2	0.50	0.53	9.41		
	3	0.50	0.49	9.06		
	1	1.00	1.04	5.61	1.06	6.03
	2	1.00	1.06	6.75		
	3	1.00	1.09	5.66		
	1	1.50	1.55	8.51	1.54	5.98
	2	1.50	1.55	6.24		
	3	1.50	1.53	4.41		
Cr	1	2.50	2.28	9.63	2.28	9.60
	2	2.50	2.32	9.01		
	3	2.50	2.25	8.13		
	1	5.00	4.25	6.18	4.48	7.18
	2	5.00	4.62	7.91		
	3	5.00	4.57	4.79		
	1	7.50	6.35	2.86	6.55	8.16
	2	7.50	6.36	1.98		
	3	7.50	6.94	9.49		

$$U = k \times \text{RSD} \qquad (1)$$

where U = Uncertainty and k = coverage factor (for 95% and 18 points, k = 1.740).

It is reported that the uncertainty tends to become extreme at very low concentrations (Nordtest Report TR 537 2004). In the present study, the calculated measurement uncertainty (%) was found to be 15.8, 12.6, and 11.8 for Pb, Cd, and Cr, respectively.

Determination of Pb, Cd, and Cr in fish feed samples

Eleven fish feed samples commonly used in the hatcheries of Bangladesh were collected from different local markets. Samples were milled and homogenized to obtain a representative material. Each sample was digested and measured three times. Contents were measured on a wet weight basis. Procedural blanks were always run. As shown in Table 6, the contents of Pb, Cd, and Cr determined in fish feed samples using the validated method ranged from 0.29 to 1.82, 0.05 to 0.22 and 0.05 to 46.5 mg kg^{-1}, respectively. The contents of Pb and Cr in fish feed samples were lower than the maximum permissible limits (ML), as described by Directive 2013/1275/EC concerning undesirable substances in animal feed. However, ML for Cr is not included in the Directive 2013/1275/EC (European Commission 2013).

Conclusions

The current work describes development and validation of a method for the trace level analysis of Pb, Cd, and Cr in fish feed in regard to Council Directive 333/2007 EC, ISO/IEC 17025:2005, and EURACHEM 1998 using Graphite Furnace Atomic Absorption Spectrometry (GF-AAS). The validation results are presented and organized in tables in order to provide an easy

Ullah *et al. Journal of Analytical Science and Technology* (2017) 8:15

Page 6 of 7

Table 5 Reproducibility precision studies data for determination of Pb, Cd and Cr

Analyte	Day	Spiked concentration (µg/g)	Mean concentration (µg/g)	RSD (%)	Overall mean concentration (µg/g)	Overall RSD (%)
Pb	1	2.50	2.26	7.40	2.46	9.11
	2	2.50	2.55	7.35		
	3	2.50	2.59	6.78		
	1	5.00	4.88	9.65	5.10	8.47
	2	5.00	5.16	3.23		
	3	5.00	5.27	1.26		
	1	7.50	6.99	7.73	7.26	8.84
	2	7.50	6.83	3.03		
	3	7.50	8.11	9.05		
Cd	1	0.50	0.52	7.51	0.51	7.25
	2	0.50	0.51	9.23		
	3	0.50	0.51	5.51		
	1	1.00	1.04	9.31	1.02	7.76
	2	1.00	0.97	6.12		
	3	1.00	1.06	3.05		
	1	1.50	1.57	8.23	1.54	6.69
	2	1.50	1.60	2.72		
	3	1.50	1.46	6.34		
Cr	1	2.50	2.28	9.63	2.32	6.79
	2	2.50	2.35	4.71		
	3	2.50	2.34	3.94		
	1	5.00	4.25	6.18	4.69	8.13
	2	5.00	4.84	4.64		
	3	5.00	4.99	1.97		
	1	7.50	6.07	5.18	6.99	9.04
	2	7.50	7.52	0.75		
	3	7.50	7.39	1.99		

Table 6 Mean (*n* = 3) and measurement uncertainty values for Pb, Cd, and Cr in fish feed

Sample	Concentration (mean ± U), mg/kg		
	Pb	Cd	Cr
1	1.72 ± 0.27	0.20 ± 0.03	46.50 ± 5.49
2	0.71 ± 0.11	0.16 ± 0.02	1.17 ± 0.14
3	1.04 ± 0.16	0.11 ± 0.01	0.67 ± 0.08
4	0.32 ± 0.05	0.11 ± 0.01	0.25 ± 0.03
5	1.81 ± 0.29	0.05 ± 0.01	4.55 ± 0.54
6	0.50 ± 0.08	0.15 ± 0.02	0.24 ± 0.03
7	1.08 ± 0.17	0.16 ± 0.02	0.68 ± 0.08
8	0.47 ± 0.07	0.05 ± 0.01	0.50 ± 0.06
9	0.78 ± 0.12	0.22 ± 0.03	0.59 ± 0.07
10	0.29 ± 0.05	0.17 ± 0.02	0.28 ± 0.03
11	0.52 ± 0.08	0.13 ± 0.02	0.05 ± 0.01
EU	5	1	–

overview of the method's performance. The validation data show that the present developed method has met all acceptance criteria defined in 657/2002/EC and 333/2007/EC for LOD, LOQ, Accuracy, and precision, and fit for the analysis of Pb, Cd, and Cr in fish feed at very low level (µg/g). The method was implemented in routine analysis in order to investigate the fish feed quality commonly used in the hatcheries of Bangladesh. The results indicated that the elemental contents were lower than the legislative values, as described by the Council Directive 2002/32/EC concerning undesirable substances in animal feed.

Authors' contributions
AKMAU and SBQ designed the experiment. AKMAU, MAM, SRK, and LNL carried out the sample preparation and analysis. AKMAU wrote the manuscript, and SBQ revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Ullah *et al. Journal of Analytical Science and Technology* (2017) 8:15

Page 7 of 7

Competing interests
The authors declare that they have no competing interests.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

Akter M, Sikder T, Ullah AKMA. Water quality assessment of an industrial zone polluted aquatic body in Dhaka, Bangladesh. Am J Environ Protect. 2014;3:232–7.

Anhwange BA, Asemave K, Kim BC, Nyiaatagher DT. Heavy metals contents of some synthetic fish feeds found within Makurdi Metropolis. Int J Food Nutr Saf. 2012;2:55–61.

Aydin FA, Soylak M. Separation, preconcentration and inductively coupled plasma-mass spectrometric (ICP-MS) determination of thorium(IV), titanium(IV), iron(III), lead(II) and chromium(III) on 2-nitroso-1-naphthol impregnated MCI GEL CHP20P resin. J Hazard Mater. 2010;173:669–74.

Bangladesh Bureau of Statistics. Statistical Pocket Book of Bangladesh 2015. Government of the People's Republic of Bangladesh. Dhaka: Bangladesh Bureau of Statistics, Ministry of Planning; 2015.

Blake C, Bourqui B. Determination of lead and cadmium in food products by graphite furnace atomic absorption spectroscopy. Atomic Spectra. 1998;19:207–13.

Catarino S, Curvelo-Garciaa AS, Sousa RB. Determination of aluminum in wine by graphite furnace AAS: validation of analytical method. Atomic Spectra. 2002;23:161–5.

Eka E, Astuti, Retno S, Rohman A. Validation and quantitative analysis of cadmium and lead in snake fruit by flame atomic absorption spectrophotometry. Int Food Res J. 2012;19:937–40.

Ertasa OS, Tezel H. A validated cold vapor AAS method for determining cadmium in human red blood cells. J Pharm Biomed Anal. 2005;36:893–7.

EURACHEM. The fitness for purpose of analytical methods. Teddington: LGC; 1998.

EURACHEM/CITAC. Quantifying uncertainty in analytical measurement. UK: LGC; 2012.

European Commission. Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Off J Eur Communities. 2002a;657/EC: L 221/8–L 221/36.

European Commission. Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. 2002b. p. L 140/10–L140-21.

European Commission. Regulation (EC) No. 1275/2013 of 6 December 2013 amending Regulation (EC) No. 2002/32/EC on undesirable substance in animal feed. 2013. p. L328/86–L328/92.

European Commission. Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. 2006. p. L 364/5–L 364/24.

European Commission. Regulation (EC) No. 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs. 2007. p. L 88/29–L 88/38.

European Commission. Regulation (EC) No. 629/2008 of 2 July 2008 amending Regulation (EC) No. 1881/2006 setting maximum levels for certain contaminants in foodstuffs. 2008. p. L 173/6–L173/9.

European Medicine Agency. Validation of analytical procedures: Text and methodology. 2006.

Food and Agricultural Organization. The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all. 2016.

Food and Agricultural Organization. Fisheries and Aquaculture Department, Fishery and Aquaculture Country Profiles, The People's Republic Of Bangladesh; 2014.

Gobas FAPC, Wilcockson JB, Russell RW, Haffner GD. Mechanism of biomagnification in fish under laboratory and field conditions. Environ Sci Technol. 1999;33:133–41.

Gonzaler AG, Herrador MA. A practical guide to analytical method validation, including measurement uncertainty and accuracy profiles. Trends Anal Chem. 2007;26:227–38.

Huber L. Validation and quantification in analytical laboratories. East Englewood: Interpharm Press; 1998.

Hwang BG, Lee SJ, Gil K. In-vivo analysis of the uptake process of heavy metals through maize roots by using synchrotron X-ray fluorescence spectroscopy. J Korean Phys Soc. 2016;69:1824–9.

Indrajit S, Ajay S, Shrivastava VS. Study for determination of heavy metals in fish species of the River Yamuna (Delhi) by inductively coupled plasma-optical emission spectroscopy (ICP-OES). Adv Appl Sci Res. 2011;2:161–6.

ISO/IEC 17025:2005. General requirements for the competence of testing and calibration laboratories. 2005.

Jakimska A, Konieczka P, Skora K, Namiesnik J. Bioaccumulation of metals in tissues of marine animals, Part 1: the role and impact of heavy metals on organisms. Pol J Environ Stud. 2011;20:1117–25.

Jeevanaraj P, Hashim Z, Elias SM, Aris AZ. Total mercury (THg), lead (Pb), cadmium (Cd) and arsenic (As) in hair samples: method validation and quantification among women at reproductive age in Selangor. Int J Sci: Basic Appl Res. 2015;24:332–47.

Maule AG, Gannam AL, Davis JW. Chemical contaminants in fish feed used in federal salmonid hatcheries in the USA. Chemosphere. 2007;67:1308–15.

Nordtest Report TR 537. Handbook for calculation of measurement uncertainty in environmental laboratories. 2ª ed.; 2004.

Ora Laboratory Procedure. Estimation of uncertainty of measurement. Food and Drug Administration; 2005.

Panahi HA, Kalal HS, Moniri E, Nezhati MN, Menderjani MT, Kelahrodi SR, Mahmoudi F. Amberlite XAD-4 functionalized with m-phenylendiamine: synthesis, characterization and applications as extractant for preconcentration and determination of rhodium (III) in water samples by Inductive Couple Plasma Atomic Emission Spectroscopy (ICP-AES). Microchem J. 2009;93:49–54.

Perring L, Alonso MI, Andrey D, Bourqui B, Zbinden P. An evaluation of analytical techniques for determination of lead, cadmium, chromium, and mercury in food-packaging materials. Fresenius J Anal Chem. 2001;370:76–81.

Psoma AK, Pasias IN, Rousis NI, Barkonikos KA, Thomaidis NS. Development, validation and accreditation of a method for the determination of Pb, Cd, Cu and As in seafood and fish feed samples. Food Chem. 2014;151:72–8.

Rawar EA, Rohman A. Validation of atomic absorption spectrophotometry and mercury analyzer for analysis of cadmium, lead and mercury in red chili pepper. Asian J Agric Res. 2016;10:65–71.

Rohman A, Wijayanti E. Development and validation of atomic absorption spectrometry for the determination of zinc and mercury analyzer for determination of mercury in cream cosmetics. J Food Pharm Sci. 2015;3:23–6.

Senila M, Drolc A, Pintar A, Senila L, Levei E. Validation and measurement uncertainty evaluation of the ICP-OES method for the multi-elemental determination of essential and nonessential elements from medicinal plants and their aqueous extracts. Anal Sci Technol. 2014;5:37.

Shamshad BQ, Shahidur RK, Tasrena RC. Studies on toxic elements accumulation in shrimp from fish feed used in Bangladesh. As J Food Ag-Ind. 2009;2:440–4.

Shubina NA, Kolesov GM. Determination of heavy metals as environmental pollutants: use of instrumental neutron activation analysis. J Anal Chem. 2002;57:912–9.

Soares AR, Nascentes CC. Development of a simple method for the determination of lead in lipstick using alkaline solubilization and graphite furnace atomic absorption spectrometry. Talanta. 2013;105:272–7.

Taverniers I, Loose MD, Bockstale EV. Trends in quality in the analytical laboratory: analytical method, validation and quality assurance. Trends Anal Chem. 2004;23:535–52.

Ullah AKMA, Akter M, Anam MR, Firoz MSH. Preconcentration of copper, cadmium and lead on Amberlite XAD-4 resin functionalized with 2-hydroxynaphthalene-1-carbanaldehydethiosemicarbazone and their determination in green tea leaves by GF-AAS. Am J Chem Eng. 2014;2:35–41.

Ullah AKMA, Khalil MI, Kibria AKMF. Determination of uranium in stream water, beach sand and rocks by stripping voltammetry following adsorptive accumulation of the U(VI)-chloranilic acid complex. Int Res J Eng Technol. 2015;2(8):54–63.

Zhong W-S, Ren T, Zhao L-J. Determination of Pb (lead), Cd (cadmium), Cr (chromium), Cu (copper), and Ni (nickel) in Chinese tea with high-resolution continuum source graphite furnace atomic absorption spectrometry. J Food Drug Anal. 2016;24:46–55.