RASĀYAN *J. Chem.*
Vol. 15 | No. 4 |2632-2641| October - December | 2022
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

HEAVY METALS IN SEA FOOD: METHOD VALIDATION AND EVOLUTION BY INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY IN ACCORDANCE WITH COMMISSION REGULATION (EC) 333/2007, 582/2016

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ABSTRACT

The goal of this study was to validate the analytical technique for determining the immediate development of lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) in various Indian seafood products. According to Commission Regulation (EC) 333/2007, various marine foods, including crustaceans, cephalopods, and fish species, were employed for the validation of the developed method by ICP-MS. HNO_3 and H_2O_2 were combined to prepare the sample during microwave digestion. Specificity/selectivity, linearity, LOD, LOQ, precision-repeatability and reproducibility, accuracy-recovery, robustness, and fitness studies were used to validate the approach. The maximum RSD value and Horrat value (HorRat) for the within-lab reproducibility for all analytes (Pb, Cd, Hg, and As) in marine food were 5% and 1 respectively. The mean recovery for all analytes examined at three spiking levels (0.5, 1 & 1.5 of the permitted limit) was between 92.67 and 107.33%.Whereas limit of detection (LOD) values for Pb, Cd, Hg and As were 0.018 µg/g, 0.032 µg/g, 0.031 µg/g and 0.034 mg/kg for repeatability 6% and <1 respectively. 0.061 µg/g, 0.127 µg/g, 0.0.103 µg/g and 0.101 µg/g respectively, were the limit of quantitation (LOQ) values. At a 95% confidence level, the method's relative extended measurement uncertainty (k=2) was 9%. In fact, the developed method's precision was examined by taking part in LGC proficiency testing (round 253, sample 742); the outcomes (Z score, i.e. < 1) showed that this analytical method could be used for the routine analysis of these four toxic metals in seafood with acceptable analytical performance in the laboratory.

Keywords: Seafood, ICP-MS, Heavy metal, Method Validation, LOD, LOQ.

RASĀYAN*J. Chem.*, Vol. 15, No. 4, 2022

INTRODUCTION

In both industrial and research-related operations, analytical measures are essential to maintaining the quality of processes and products.[1] Growing customer demand for high-quality goods and services has placed a substantial emphasis on the development of analytical testing to restore the reliability of test findings and guarantee the product's quality. Consuming fish has a number of nutritional advantages, mainly because it contains sufficient amounts of nutrients including vitamins, omega-3 fatty acids, and other nutrients like amino acids[2,3,4], The trade-off between advantages and hazards associated with ingesting chemical pollutants, however, has not been well defined. Consequently, regulatory agencies are more concerned about seafood contamination exceeding the limit.[5] Seafood may accumulate a number of inorganic contaminants, such as the most frequently studied metals Hg, Cd, Pb, and As, which persist in the environment through bio-accumulation and bio-magnification in the food chain. The noxiousness of various kinds of marine food can be determined by looking for bio-accumulations and bio-magnifications[6,7,8], where the main factors that may affect the accumulation of inorganic contaminants in fish tissues and organs include sex, size, and physiological constitution.[9] Even after discharges stop, the presence of these metals in the environment has long-lasting impacts.[10] Some researchers have suggested that fish and other aquatic life could serve as biological indicators of environmental heavy metal pollution.[11] The fact that fish and fisheries products contain traces of inorganic pollutants, commonly known as heavy

http://doi.org/10.31788/RJC.2022.1546653

metals, makes them one of the main sources of pollution for humans. The acceptable limits of certain heavy metals in fish and fishery products for eating have been standardized by regulators like the European Commission and WHO/FAO due to the importance of the issue. Because ICPMS has lower detection thresholds and the ability to instantly identify several components in a little run time, its operation is significant. The preferred method for evaluating heavy metals at lower levels is hence ICP-MS.[12,13] In this work, a simple, reliable, and rational method is created to find heavy metals in seafood in accordance with Commission Regulation.[14,15]

EXPERIMENTAL

Materials and Procedures

Instrumentation**:** Thermo Scientific ICP-MS was used as the inductively coupled plasma mass spectrometer in this study (CAP Qc, Thermo Fisher Scientific, Bremen, Germany). It was integrated with collision and reaction cells, sample cones, cyclonic spray chamber (quartz), nebulizer (FPA), Peltier, injector (quartz, 2.5 mm ID), Chiller (Thermoflex 2500), and Qtegra operational software (version 1.5.1189.1) for data collection. The argon gas used had a spectral purity of 99.9998%. The instrument was tuned for everyday performance using Tune-B solution before each use (Ba, Bi, Ce, Co, In, Li, and U). The operational circumstances and instrumental settings were as listed in Table-1 and Table-2.

Table-1: ICP-MS Operating Conditions for Lead, Cadmium, Mercury, and Arsenic

Spectrometer	ICP-MS
RF power (kW)	Meinhard
Nebulizer	Cyclonic
Spray chamber	1.35
Measurement mode	Mass
Cooling gas flow	14 (L/min)
Auxiliary flow	0.8 L/min
Nebulizer flow	1.0 L/min
Chiller Temperature	20.0°c - 22.0°c
Peristaltic pump	25 rpm
Isotopes	Pb208, Cd111, Hg 202 & As75

Table-2: Summary of Isotopes Mass of Investigated Elements in Seafood

S. No.	Element name	Isotope Mass	First Ionization potential
1	Arsenic	75	9.815 ev
2	Cadmium	111	8.994 ev
3	Lead	208	7.417 ev
4	Mercury	202	10.438 ev

The samples were digested using a microwave reaction system, model Anton Paar Multiwave ECO, that is configurable for time and power between 600 and 1500W and has 16 high-pressure PTFE-TFM vessels (maximum design specification 310 °C/35 bar (508 psi).

Analytical Grade Reagents

ICP multi-element reference standard solutions of 100 mg/L (Merck, KGaA, Frankfurter Sir, 250, 64293, Darmstadt, Germany) NIST Traceable were used for analytical curves of Pb, Cd, and AS, and standard reference solutions of 1000 mg /L (Merck, KGaA, Frankfurter Sir, 250, 64293, Darmstadt, Germany) NIST traceable were used for Hg. Milli Q water (resistivity-18.2 MΩ·cm) was obtained from a Milli-Q A10 water purification system and used for analysis (Millipore, Bedford, MA, USA).

Sea Food Samples

In the month of Jan-Feb 2018, 20 Samples of seafood of different species (crustacean and cephalopods) approximately 350 g of each type were obtained from the fish market in Delhi, India. These samples were immediately preserved in an insulated ice box and transported to the laboratory in -4°C conditions, where they were weighed and kept frozen at -18°C until further analysis.

Sample Preparation and Digestion

Nearly 0.5 gm of sample is retained in the microwave digester for digestion at 190 ^{0}C, with 15-minute ramps to temperature, at a maximum power of 1500 watts, for 30–40 min (Table-3). The digested sample was then put into a 50 mL flask (volumetric) and allowed to cool for approximately 15 minutes before being topped off with Milli Q water.[16] Additionally prepared and subjected to the same microwave digestion procedure was a set of blank samples. Ten subsamples in total were routinely dispersed throughout the microwave digester disc.

Table-3: Microwave Digestion Program

Step	Power	Ramp Time	Hold Time	Fan
1	400 watts	10 min	10 min	1
2	700 watts	15 min	10 min	1
3	1500 watts	15 min	20 min	1
4	0 watt	-	20 min	3

Calibration Procedure

For the quantitative analysis, standard solutions for seven different concentrations were set up after dilution with HNO_3 as expressed in Tables-4, 5, and 6. Finally, the seven-point calibration curves starting from LOD up to 0.050 µg/g were made.

Table-4: Preparation of Stock Standard Solution and Working Standard for Pb, Cd, and As:

Stock STD. conc.	The volume of Stock Std.	Solvent volume	Final volume	Final concentration	Label
100 mg/L	5 mL	45 mL	50 mL	10 mg/L	WS 1
10 mg/L	5 mL	45 mL	50 mL	1000 µg/L	WS 2
1000 µg/L	5 mL	45 mL	50 mL	100 µg/L	WS 3

Table-5: Preparation of Stock Standard Solution and Working Standard for Hg

Stock STD. conc.	The volume of Stock Std.	Solvent volume	Final volume	Final concentration	Label
1000 mg/L	5 mL	45 mL	50 mL	100 mg/L	WS 1
100 mg/L	5 mL	45 mL	50 mL	10 µg/L	WS 2
10 mg/L	5 mL	45 mL	50 mL	1000 µg/L	WS 3
1000 µg/L	5 mL	45 mL	50 mL	100 µg/L	WS 4

Table-6: Preparation of Working Standard for Calibration Curve

S. No.	Master standard Conc. (µg/L)	The volume of Working standard (mL)	Solvent volume (mL)	Final volume (mL)	Final Conc. (µg/L)	Label
1	500.0	5.0	45.00	50	50.0	CC7
2	100.0	10.0	40.00	50	20.0	CC6
3	100.0	5.0	45.00	50	10.0	CC5
4	50.0	5.0	45.00	50	5.00	CC4
5	10.0	5.0	45.00	50	1.00	CC3
6	5	5.0	45.00	50	0.50	CC2
7	1	5.0	45.00	50	0.10	CC1

Additionally, calibration curve standards for Pd, Cd, Hg, and As were created using solvent dilutions ranging from 0.10 to 50.0 g/L to obtain minimum linearity of 7 points for each element. The calibration curves' correlation coefficients (R^2) exceeded 0.995, demonstrating a straight-line link between the calibration curve's concentration ranges. Spiked samples were also analyzed at regular intervals throughout the trial to validate the test results. Furthermore, Milli-Q water as a blank was routinely collected for analysis alongside samples to track any divergence caused by contamination during sample processing.[17]

Validation of Analytical Method

In order to validate the test method for evaluating Pb, Cd, Hg, and As in seafood (Fig.-1) as a regular analysis method, the following parameters, as listed below (Fig.-2), were evaluated as per European regulation for the method validation.[1,14]

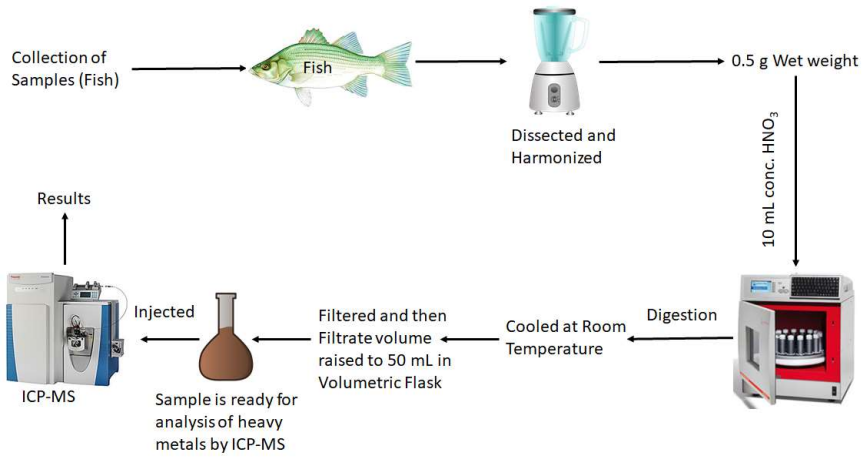

Fig.-1: Schematic Diagram for Sample Preparation for Analysis of Metals in Sea Food

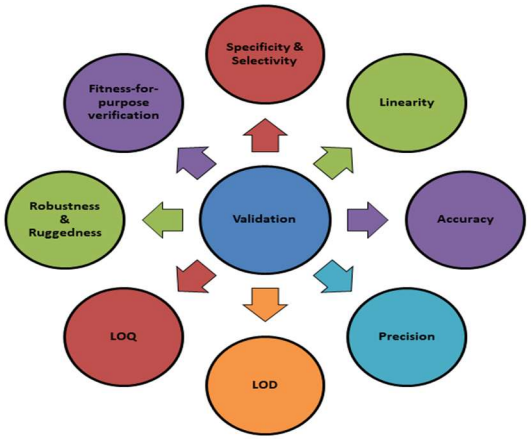

Fig-2: Parameters to Validate the Analytical Methods for Metal Analysis

HorRat Value

The HorRat value is the difference between the anticipated reproducibility relative standard deviation (RSD_R, %) and the reproducibility relative standard deviation ($PRSD_R$%).[18]

$$\text{HorRat} = \frac{\text{RSD}_\text{R}\%}{\text{PRSD}_\text{R}\%}$$

$PRSD_R$, % = $2C^{-0.1505}$

or

$PRSD_R$, % = $2^{(1-0.5*\log C)}$

C=estimated mean concentration articulated as (Table-7).

Table-7: Predicted Relative Standards Deviations

Conc. (C)	Mass Fraction (C)	$PRSD_R$ %	$PRSD_R$ %
100 %	1.0	2	1
1 %	0.01	4	2
0.01 %	0.0001	8	4
1 ppm	0.000001	16	8
10 ppb	0.00000001	32	16
1 ppb	0.000000001	45	22

- $HORRAT_r$ = The difference between the observed and estimated RSDr values obtained using the (modified) Horwitz equation under the premise that r = 0.66 R.

$$HorRat\ (r) = \frac{RSD_r\%}{PRSD_r\%}$$

- $HORRAT_R$ = The ratio of the measured RSDR to the RSDR value calculated using the (modified) Horwitz equation.

$$HorRat\ (R) = \frac{RSD_R\%}{PRSD_R\%}$$

By observing the goodness-of-fit or correlation coefficient, the range of linearity is assessed. (R^2) value within a specified range of linearity, calibration curve values are used to assess test outcomes that are directly proportional to the concentration of analytes in samples.[19] Prepared with points was a calibration curve. $R_2 > 0.995$ denotes that the calibration curve's linearity is well-considered and acceptable.[20,21] The capacity of a method to distinguish between the analytes being assessed and the matrix effect is known as specificity and selectivity. This characteristic can vary depending on the element and matrix but primarily depends on the adequacy of the determining method. A minimum of 7 matrix blank samples are produced according to the technique and run/injected into the instrument to assess the specificity. Conduct the analysis and look for any spectral or matrix interferences (signals, counts, ion traces). The approach is particular and selective if there are no matrix or spectral interferences.[21,22] Accuracy is defined as "the degree to which a test result and the established reference value agree in terms of their relative magnitude" (European Regulation). Recovery was carried out to verify the lack of Analyte concentration, which could be caused by Analyte loss, cross-contamination while preparing samples, and matrix effects while processing samples in an instrument, as well as to check the accuracy of the analytical method. Recovery is defined as the percentage of a substance's true concentration recovered during the analytical procedure. [23]. The six samples were infused with three different concentrations—0.5, 1, and 15 times the legal limit suggested by Commission Regulation.[24] Calculate the % recovery, mean recovery, and % RSD.

$$\%\ Recovery = \frac{S_{spiked} - S_{blank}}{C_{spiked}} X\ 100$$

Where,
S_{spiked} = Result of the spiked sample
S_{blank} = Result of a blank sample
C_{spiked} = Spiking level

$$\%\ RSD = \frac{SD}{\mu} X\ 100$$

Where,
SD = Standard deviation
RSD= Relative standard deviation
μ= Mean

For all substances included in the scope of a technique, acceptable mean recoveries fell within the range of 80-110% with a corresponding repeatability RSD of 20%.[24] Precision stands for the “closeness of agreement between independent test results obtained under predetermined conditions”. According to the commission regulation[25], the measure of precision is concluded by evaluating the repeatability and reproducibility of the analyte during the analysis. In order to achieve precision under repeatability conditions, tests must be conducted independently using the same technique on identical test samples in the same laboratory by the same analyst using the same instrument over a brief period of time. Reproducibility (within a lab) is a form of accuracy to be attained in the same location under predetermined conditions (regarding, for example, test techniques, materials, analyst, and environmental conditions) over an acceptable long period of time. Six samples of each are spiked with the analyte(s) to make concentrations equivalent to 0.5, 1, and 1.5 times the permitted limit for repeatability and reproducibility precision, and the results are compared to the acceptable coefficient of variation (CV)/RSD (i.e., < 20% as per commission regulation[24]) and the HorRAT value (i.e., < 2 as per European commission regulation).[14] When it comes to the method's performance, a value >2 typically denotes unsatisfactory. To evaluate an instrument's or an analytical method's performance, the terms Limit of Detection (LOD) and Limit of Quantification (LOQ) are used. While LOQ is defined as the lowest detection level of an analyte that can be quantitatively attained while meeting precision and accuracy requirements, LOD is the lowest detection level of an analyte that can be achieved and consistently differentiated from zero but is not necessarily quantifiable[26]. According

to the commission's advice in rule[14], LOD was estimated to be three times the standard deviation of the mean of a matrix with more than 20 blank spaces.[14] For the acceptance of LOD and LOQ, the recovery percentage and RSD should be within the range of 80-110% and < 20% respectively. Ruggedness means to verify the ability of the test method to be unchanged by slight alterations in method and additionally offers a sign of its consistency during regular practice. The Ruggedness is studied by spiked sample and analysis is done with Slight changes in the normal method. The recovery is considered within the acceptable criteria of 80-110% for its performance.

Fitness-for-Purpose Verification

To determine whether a test method is appropriate for use in routine analysis, "fitness-for-purpose" could be applied to methods that have undergone internal validation. The results from newly developed methods must have standard measurement uncertainty (Uf) that is less than the commission-recommended maximum (Uf), which is calculated using the equation below.

$$Uf = \sqrt{(LOD/2)^2 + (\alpha\, C)^2}$$

Where:(µg/kg); C=the concentration of interest (µg/kg); α = a numeric factor to be used based on the value of C as given below (Table-8).

Table-8 Relation between concentration of interest and Numeric factor

C (µg/kg)	α
≤ 50	0.2
51 to 500	0.18
501 to 1 000	0,15
1 001 to 10 000	0.12
> 10 000	0.1

Performance Criteria

The European Commission established performance standards (Table-9) for test methods to be used for routine analysis of investigated heavy metals in seafood samples.[1,14]

Table-9: Performance Criteria for Methods of Analysis for Lead, Cadmium, Mercury and Arsenic (EC/333, Commission Regulation (EC) No 333/2007, Laying Down the Methods of Sampling and Analysis for the Official Control of the Levels of Lead, Cadmium, Mercury, Inorganic tin, 3-MC PD and Benzo(a) Pyrene in Foodstuffs, off. J. Eur. Union. (2007)

Parameter	Criterion		
Specificity	Free from matrix/spectral interferences		
Repeatability (RSD_r)	$HorRaT_r$ <2		
Reproducibility (RSD_R)	$HorRaT_R$ <2		
Recovery	80-110% (As per AOAC)		
LOD	3 LOQ/10		
LOQ	Pb	Cd, Hg, As	
	ML≥0.1 µg/g	ML<0.100 µg/g	ML≥0.100 µg/g
	≤ ML/5	≤ ML/5	≤ ML/10

Quality Control

To continuously assure the accuracy of the analytical data generated during laboratory analysis, internal quality checks were also carried out. Using the described approach, Pd, Cd, Hg, and As were analyzed for LGC Proficiency Testing (round 253, sample 742). By bolstering and then evaluating the percent recovery, the quality control (QC) of the test procedures for all analytes was also checked.[25]

RESULTS AND DISCUSSION

By evaluating selectivity/specificity, linearity, LOD, LOQ, accuracy, precision, and ruggedness, the analytical method for quantitative evaluation of Pb, Cd, Hg, and As in seafood samples was validated.[14,19,27]

Method Validation

Selectivity was evaluated by preparing 7 blank matrix samples were prepared as per the method and injecting them into the ICP-MS system. It was found there was no matrix or spectral interference observed.

Heavy metal standard solutions in concentrations between 0.10 and 50 µg/L were used to prepare the linearity. All the analytes exhibited excellent regressions- coefficient of correlation (R^2>0.995) in calibration curve, that were reproducible as well (Table-10). The sensitivity was appraised by LOD and LOQ. According to the guideline by Commission Regulation (Table-10), The LOD was verified as a lower concentration than three-tenths of LOQ in a blank matrix fortified with a mixed standard solution having interesting elements (Table-10). The LOQ was confirmed as the lower concentration ≤ one-fifth of the ML (maximum limit) for Lead and ≤ one-tenth of the ML for cadmium, mercury, and arsenic which came out from a blank matrix fortified with a mixed standard solution having all studied elements (Table-10). The LOD values attained (see Table-10) were 0.018 µg/g for Pb, 0.032 µg/g for Cd, 0.031 µg/g for Hg, and 0.034 µg/g for As, while the LOQs were 0.061 µg/g for Pb, 0.120 µg/g for Cd, 0.103 µg/g for Hg and 0.101 µg/g for As, which was under the maximum permissible limit for heavy metal as per European regulations[4] (0.3 µg/g for Pb, 0.5 µg/g for Cd, 0.5 µg/g for Hg and 1 µg/g for As in seafood for direct human consumption). The accuracy was verified by evaluating the mean recoveries of the heavy metals in the spiked samples. 18 fractions of samples (0.5 g each) were fortified with low (0.15 µg/g), intermediate (0.30 µg/g) and high levels (0.45 µg/g) of heavy metal standards with six replicates at each spiked level. These samples were digested with HNO_3 and H_2O_2 solution and afterward processed according to the method given in detail above.

Table-10: Linearity Range, Equation, R^2 Value, RSD, LOD, and LOQ of Toxic Heavy Metals

Analyte	Linearity range (µg/kg)	Equation	R^2	RSD %	LOD (µg/g)	LOQ (µg/g)	ML (µg/g)
Pb	0.1-50	Y = 171349X+1592.9	0.995	3.168	0.018	0.061	0.3
Cd	0.1-50	Y = 34712X 7014.6	0.996	2.724	0.032	0.120	0.5
Hg	0.1-50	Y = 25883X +5847.9	0.998	2.341	0.031	0.103	0.5
As	0.1-50	Y = 25883X +5847.9	0.995	6.769	0.034	0.101	1

The obtained results summary in Table-11 showed that average recovery at the lower spiking level surpassed 92.67%, whereas the average recoveries of intermediate and high spiking levels were in the range from 97 to 103.67% and from 96.67 to 102.44%, respectively.

The average recovery at the higher spiking level was marginally improved than at the lower spiking level. Similarly, for the repeatability and reproducibility studies, the blank matrix was chosen and fortified with all four heavy metals. Six replicates were performed on the same day for the repeatability study with repetition of these steps on three other occasions, Further tests were carried out every day, nonstop for three days, for the reproducibility study with different analysts and different environmental conditions. RSDs and HorRat Value of the repeatability and reproducibility studies showed the suitability of the developed method (Table-12).

Table-11: Accuracy Studied Data for Pd, Cd, Hg, and As

Analyte	Spiked concentration (µg/g)	Obtained concentration (µg/g)	Recovery %
Pb	0.150	0.139	92.67
	0.300	0.291	97.00
	0.450	0.458	101.78
Cd	0.150	0.161	107.33
	0.300	0.309	103.00
	0.450	0.461	102.44
Hg	0.150	0.145	96.67
	0.300	0.311	103.67
	0.450	0.459	102.00
As	0.150	0.161	107.33
	0.300	0.293	97.67
	0.450	0.435	96.67

Table-12: Repeatability and Reproducibility of Analytes Spiked in Blank Sea Food

Analyte	Spiked concentration (µg/g)	Repeatability RSD_r (n=14)	$HorRat_r$	Reproducibility RSD_R (n=21)	$HorRat_R$
Pb	0.15	2.482	0.117	2.436	0.115
	0.30	5.426	0.283	3.125	0.163
	0.45	1.097	0.061	5.954	0.328
Cd	0.15	2.715	0.128	2.703	0.127
	0.30	2.115	0.110	3.461	0.181
	0.45	1.974	0.109	2.135	0.118
Hg	0.15	2.326	0.109	3.426	0.161
	0.30	5.673	0.296	2.751	0.144
	0.45	2.315	0.128	4.652	0.257
As	0.15	4.577	0.215	4.306	0.203
	0.30	2.157	0.113	3.451	0.180
	0.45	3.521	0.194	5.075	0.280

n=number of replicates.

The technique validation for the current approach carried out in this work, may be regarded as selective, accurate, exact, and robust for the evaluation of heavy metals (Pb, Cd, Hg, and As) in seafood samples when all these factors are taken into account. As per the ruggedness study, the method was found with the capacity to remain unaffected by slight variations. As uncertainty measurements calculated at 100 µg/kg for all analytes come lower than the maximum standard uncertainty (Uf) in Table-13, it described the fitness for the purpose of the developed method. Moreover, verification of the validated method was performed by participating in LGC, UK Proficiency testing in accordance with ISO 17043, where satisfactory z-score were obtained for all analytes (Table-14).

Table-13: Fitness for Purpose Approach for all Analytes at 100 µg/kg

	Lead	Cd	Hg	As
Uf	30.81	30.81	30.81	30.81
Standard Uncertainty	6.25	2.15	6.32	8.36
Fitness for purpose	comply	comply	comply	comply

Table-14: Results Summary of LGC PT (round 253, sample 742) in Fish-Based Sample

Analyte	Result	Z-score	Assigned Value	Ux AV	SDPA	Exp.SDPA	No. of results	Median	Mean	Robust SD	SD
As	2.50	0.00	2.50	0.08	0.250	0.262	19	2.60	2.52	0.326	0.398
Cd	0.14	0.00	0.14	0.00	0.014	N/A	21	0.14	0.14	0.015	0.019
Hg	0.78	-0.46	0.82	0.03	0.082	0.087	12	0.78	0.78	0.119	0.134
Pb	0.83	0.37	0.80	0.02	0.080	N/A	20	0.80	0.80	0.082	0.110

Unit=µg/g

Sample Analysis

Numerous studies have reported on the build-up of metal in various seafood samples.[28-30] The four heavy metals were then detected over time in 20 samples of seafood from Delhi, India's fish market using the newly devised technology. The four heavy metals under examination were not found, with the exception of a small number of samples whose findings fell below the permitted range as per European regulation (Table-15).[4] This demonstrated that the marine foods under investigation are safe for human consumption in terms of heavy metal contamination and are commercially available in Delhi, India.

Table-15: Mean value of Pb, Cd, Hg and As in different Sea Food Samples

	Concentration (µg/g)					
Analyte	*Litopenaeus Vannamei*	*Penaeusindicus*	*Penaeus monodon*	*Uroteuthis Siboge*	*Sepia pharaonis*	*Octopus vulgaris*
Pb	BDL-0.081	BDL-0.051	BDL	BDL	BDL-0.073	BDL

Cd	BDL-0.015	BDL-0.018	BDL	BDL-0.021	BDL	BDL-0.021
Hg	BDL	BDL	BDL	BDL	BDL	BDL
As	BDL -0.17	BDL-0.11	BDL	BDL	BDL-0.10	BDL-0.15

BDL (Below detection limit)

CONCLUSION

The Pb, Cd, Hg, and As the content of samples of seafood may be accurately and precisely analyzed thanks to the validation and optimization of the inductively coupled plasma mass spectrometry technology. Framing tactics to overcome matrix interferences and meet acceptable recovery standards are also important. The mean recovery for all analytes measured at three spiking levels (0.5, 1, and 1.5 of the permissible limit) was between 92.67 to 107.33 %. The maximum RSD value and Horrent value ($HorRaT_R$ & $HorRaT_r$) for the within-lab reproducibility & repeatability for all analytes (Pb, Cd, Hg, and As) in seafood were <6% and <1 respectively. LOD values for Pb, Cd, Hg, and As were 0.018 µg/g, 0.032 µg/g, 0.031 µg/g and 0.034 mg/kg respectively, while LOQ values were 0.061 µg/g, 0.120 µg/g, 0.0.103 µg/g & 0.101 µg/g respectively. The method's 95% confidence level regarding expanded measurement uncertainty (k=2) was 9%. It has been determined that the created method is adequate for the intended purpose after using it to analyze 20 market samples. It is sufficient for ensuring compliance with tolerances and guidelines because of the low detection limit, high accuracy, and high precision.

ACKNOWLEDGEMENT

The Amity University in Haryana provided support during the research, and Intertek Laboratory in Haryana, India provided vital facilities for the analytical work.

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