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A NEW ICP-MS BASED APPROACH FOR THE ANALYSIS OF HEAVY METALS IN FEEDER INSECT *TENEBRIO MOLITOR*

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ABSTRACT

Heavy metal accumulation in feeder insects is becoming a major food safety concern as it is a threat to human health through the transmission of toxic compounds into the human food chain. For the determination of heavy metals in *Tenebrio molitor* feeder insect by ICP-MS, an accurate and sensitive approach has been devised and validated in this study. The criteria for new methods, such as linearity and range, accuracy, precision, selectivity, limit of detection, and limit of quantitation are used to verify this method. The current technique exhibits linearity with correlation coefficient values not less than 0.99. The developed method shows accuracy ranging from 91.13 to 104.93%. When the RSD of the responses of replicates of the metals at specification levels is less than 20%, the approach is also precise. This method enables the detection and quantification of certain metals at low concentrations due to their low limit of detection and limit of quantification.

Key words: *Tenebrio molitor* cadmium, lead, arsenic, mercury, impurities, inductively coupled plasma-mass spectrometry, method development, validation, toxicity, microwave digester, agriculture waste

Insects are used as cattle food because of their high protein, mineral, and bioactive chemical content. Heavy metal accumulation in feed is becoming a major food safety concern as it offers a threat to human health through the transmission of toxic compounds into the human food chain and poses a substantial challenge to animal health (Ayman et al., 2023). Feeder insects reared on agricultural waste have been found to carry traces of heavy metals and pesticides. Feeder insects first appeared as a protein supplement in avian diets decades ago. Feeder Insects have been highlighted as a distinctive protein resource for both animals and humans, with the potential to increase feed and food supplies (Mabelebele et al., 2022). Insects can partially replace extensively imported feed proteins from other nations in animal feeds. Feeder insects are used as feed additives or supplements because of their high protein content, vitamins, and minerals. Although insects are regarded as an alternative protein source for animals, there are significant hazards associated with their use, especially when generated under regulated conditions (Kohelr et al., 2019). Although trace elements are required for an organism's proper functioning, they become poisonous when their amounts surpass suggested levels. Heavy metals are metal elements that have a density five times that of water and are known to harm both animals and plants (Wu et al., 2021). Because of its hazardous effect on living creatures, this group of elements is classified as an environmental contaminant.

Ingestion of polluted substrates, such as plant by products with fertiliser or residues of pesticides and manure from animals with veterinary medicine debris, causes metal element bioaccumulation in insect tissue (Morshedul Haque et al., 2021). This is still a serious issue in some underdeveloped nations, where grasshoppers and locusts are collected from around farms and fed to chickens following insecticide treatment. Cadmium, lead, arsenic and mercury are frequently found in feeder insects such as yellow meal worms, grasshoppers, super worms, termites, locusts. These metal elements are highly poisonous and easily accessible trace elements; they are also not metabolized in other intermediate compounds, making them difficult to break down in the body (Abdullahi et al., 2022). Heavy metal accumulation in the environment is becoming a serious food safety concern, as these metals endanger human health when they enter the food chain. As a result, feeder insects must be tested and verified safe for use as a supplement feed for cattle (Amir et al., 2019). Attempts have been undertaken to identify and summarise the primary heavy metals found in feeder insects and then to compare these amounts with dangerous and safe levels found in animal feed. It is becoming increasingly critical to find heavy metal concentrations simultaneously, swiftly, reliably, precisely, and at low concentrations. ICP-MS, also known as inductively coupled plasma mass spectrometry, has emerged as one of the most fascinating detection techniques and is widely used in forensic, food, earth, and environmental sciences, as well as the materials, chemical, and nuclear sectors. The ICP-MS is a potent technology for ultra-trace-level element detection due to its improved sensitivity. It is a multi-element approach with significantly lower detection limits than other multi-element methods capable of analysing liquid samples with high selectivity and sensitivity (Scott et al., 2019). This study evaluates a method for detecting several trace metals in water using ICP-MS, which are commonly detected in contaminated water. The method is validated in accordance with the innovative method standards, which include linearity, precision, accuracy, detection limit, and quantitation limit.

MATERIALS AND METHODS

The study was conducted at the the yellow meal worm P&N Pharma and Neutraceuticals in Chennai. The yellow meal warm *Tenebrio molitor* samples were collected from farmers field in Trichy, Tamil Nadu. Experiments were conducted using the Agilent ICP-MS and the Anton Paar microwave digester. Through an inorganic venture, cadmium, lead, arsenic and mercury standards were purchased Nitric acid and hydrochloric acid without any elemental impurities were purchased from Merck India. Used ultrapure water, all standards and sample solutions were created. *Tenebrio molitor* samples were softly frozen in a -20^{o}C freezer before being blanched and oven-dried for one hour at 120^{o}C. Insect samples were placed into 50 ml PTFE vessels, added nitric acid and hydrochloric acid in a ratio of (3:2). In a digester temperature ramped from 50^{o}C to 150^{o}C holded for 15 minutes. After completion of digestion the samples were centrifuged at 6000 rpm for 10 minutes. Furthermore blank and control samples were performed. Diluted to volume 50 ml with water. Five point calibration curve standards were prepared according to the WHO recommended (Coline et al., 2021) heavy metals specifications, from 25 to 200%. For all heavy metal the linearity curve's intercept and slope were calculated. A correlation coefficient of 0.99 or more is preferred. A known amount of standard solutions was spiked with a feeder insects sample at four concentrations ranging from 25 to 150%. Insect samples were weighed into a PTFE vessels, added with 3 ml of nitric acid, 2 ml of hydrochloric acid. PTFE vessels introduced into a digester. The syngistix software version 2.3, which is integrated with ICP-MS, was used to calculate statisticals.

RESULTS AND DISCUSSION

Following the successful validation of an analytical method developed for the simultaneous determination of the four heavy metals, it was utilised to analyse the presence of heavy metals in *T. molitor*. This analysis followed standard procedures based on the available literature (David Thomas et al., 2019). Sample preparation procedure utilised was after Mabelebele et al. (2022). These results collectively indicate that samples (*T. molitor)* taken from agricultural regions treated with pesticides have cadmium, lead, mercury, and arsenic (Table 1). According to numerous studies, feeding agricultural waste and pesticides treatment to insects are the main causes of the buildup of heavy metals in insects. Insects raised on animal manure and other agricultural waste contain heavy metals as cadmium, lead, arsenic and mercury. These are harmful to humans and other animals when consumed in excess of recommended doses (Abdullahi et al., 2022). Therefore, it is necessary to identify their concentration levels using suitable analytical method. The proposed analytical method has been validated in accordance with ICH and WHO recommendations (Coline et al., 2021). Several solvent ratios have been used in the technique development process to get positive results. It was determined that a solvent ratio of 3 ml of nitric acid and 2 ml of hydrochloric acid produced outcomes that were acceptable by regulatory standards. The proposed ICP-MS technique has been verified in terms of recovery, precision, linearity, limit of detection, limit of quantification, and intermediate precision. The elemental impurity's linearity curve was discovered to be linear and displays a correlation coefficient >0.99. To evaluate the accuracy of the established approach, samples were spiked with standards at various concentrations. Elemental impurities % recovery was

Table 1. Heavy metal contents in *T. molitor*

S. No.	Heavy metals	Amount found (ppm)	Standard limit as per WHO/ FAO (ppm)
1.	Cadmium	1.402	0.05 - 2
2.	Lead	2.109	0.01 - 3
3.	Arsenic	0.103	0.1- 0.2
4.	Mercury	0.504	0.5- 1

Table 2. Heavy metal contents-method validation in *T. molitor*

S.No.	Parameters	Standard limit	Cadmium	Arsenic	Lead	Mercury
1.	Method precision	% RSD NMT 20	2.59	5.09	1.01	1.93
2.	Linearity	NLT 0.99	0.9992	0.9993	0.9934	0.9988
3.	Accuracy-25%	70–150 %	97.26	93.18	101.44	97.85
4.	Accuracy -50%	70–150 %	96.81	93.36	104.44	99.34
5.	Accuracy-100%	70–150 %	97.22	92.49	102.38	97.66
6.	Accuracy-150%	70–150 %	97.66	91.47	102.68	98.10
7.	Ruggedness	% RSD NMT 25	2.47	5.08	1.55	1.47
9.	LOQ	No limit	1.990	0.199	1.004	3.017
10.	LOD	No limit	0.116	0.017	0.083	0.249
11.	LOQ Precision	% RSD NMT 20	2.79	1.15	0.33	1.38

found within the range of 70 to 150% for each stage. Recovery studies' results show that the new approach is more accurate. Lesser standard deviation readings for method precision demonstrate the developed technique's repeatability and reproducibility. The lowest concentrations of an analyte that can be quantified precisely and the lowest concentration of an analyte that can be detected, respectively, are shown by the limit of quantification value and limit of detection value (Table 2). It shows how long-lasting is the method is for the simultaneous determination of heavy metals in *T. molitor*.

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AUTHOR CONTRIBUTION STATEMENT

Ravishankar M: Consceptulization, methodology; Alexandar S: Supervision revewing and analyzed the results; Senthil Kumar R: Original draft preparation; Kumar M: Editing and reviewing; Venkateswarlu B S: Draft correction.

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CONFLICT OF INTEREST

No conflict of interest.

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