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Research Article

Development and Ratification of a Precise Method (GF-AAS) Used for the Determination of Poisonous Metal Lead (Pb) in Dairy Cow Milk Sample Commonly Available in the Market of Bangladesh

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Abstract

Milk is considered as an ideal food item, and it can be contaminated with the toxic metal (Pb) by various ways. The metal lead (Pb) exposes toxicity greatly when it accumulates gradually inside the body cavity of human or animal. The study is aimed at the detection of poisonous mineral lead in cow's milk sample by Graphite Furnace Atomic Absorption Spectrometry (GF-AAS) method.Besides, public health concern or issues in the food chain of the consumer world is also taken into consideration in this study. Though the method is a bit troublesome, but an attempt was made herein this study to make it easy access for the determination and method validation for the analysis of mineral content in milk sample by complying with the Council Directive 333/2007/E. For the ratification of this method, a pretty good number of criteria including linear range, limits of detection and quantifications, accuracy (%), measuring uncertainty, repeatability and reproducibility or precision checks etc., were assessed for the affirmation of the method. GF-AAS (Model: AA-7000 Shimadzu, Japan) technique was used for the analysis of poisonous element lead in dairy cow milk samples. Lastly, detection of Pb in milk samples was done with ultraviolet/visible detection set at 283.0 nm wave length. The method was confirmed complying with the international guidelines and acceptance values for system suitability, precision, linearity, uncertainty and accuracy or recovery % were met in all aspects. The Relative Standard Deviation (RSD) or Coefficient of Variation (CV%) for system suitability and precision was <10% for the metal (Pb) measured in the milk sample. The linearity of the calibration curves was excellent (r2>0.999) at various concentrations for the lead. The instrumental Limits of Detection (LoD) value in milk were 0.397 and the limits of quantification (LoQ) value in milk samples was 1.32µg//L, respectively, for Pb, And the method of LoD and LoQ for Pb being 0.993 and 3.30 µg/Kg, respectively. The overall recovery (%) found 98.98 for the metal (Pb). The overall RSD or CV% of reproducibility and repeatability percentages being 6.85 and 7.65%, respectively. The value for measurement uncertainty (%) was 7.0 for Pb. The developed validated parameters denote that it is an easy and economical method that can be applied greatly for the regular laboratory analysis of trace mineral element in cow milk samples. After all, the potential health of the consumer health or public health concern should not be ignored at all for the continual ingestion of toxic element contained in the milk, even though the toxic mineral content found in the supplied milk samples were within the acceptable range.

Keywords: Cow Milk; GF-AAS; Method Development; Toxic Mineral Element

Introduction

Milk is the best food item available in the nature, and regarded as a complete or ideal food [1,2]. It is needless to say that new-born babies just live on milk only up to six months of age without any additional nutrient support. The milk is derived from different sort of domestic animals (e.g., cow, buffalo, camel, goat, sheep), which is generally used for the proper nourishment of the consumer world. Milk is a rich source of protein with all sorts of other vital nutrients say lactose, fat, vitamins and minerals. So, increasing trend of milk production by dairy farming could play a pivotal role on the human health for the reduction of the huge protein gap of the country like Bangladesh. In this regard, both commercial and backyard dairy farming are increasing day by day to meet the increased demand of milk of the ever-growing population of Bangladesh. It is reported that the per capita milk requirement is 250 ml/day and milk availability is 39.2 ml/day in Bangladesh [3]. Though milk consumption rate is very low in Bangladesh, but it has no doubt to say that milk production would be increased double in future as private and household dairy

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sectors are increasing rapidly. The increased demand of dairy food products along with the trend of higher production is a burning issue in a developing country like Bangladesh. Because heavy metals and metalloid namely Pb, Cu, Cd, As and Cr etc., might contaminate the food products through consumption of milk and milk products by the consumers. It is reported that the amount of Pb and Cd is found to be higher in cow's milk samples than the permissible range [4-6]. The ingestion of the contaminated dairy food products (milk) works as a potential source for the exposure of heavy metals in the food chain [7]. This contamination of food chain with toxic element like Pb might cause the environmental pollution and in turn, aggravate the situation of consumer world posing a great threat on the health of country people.

The milk contamination is considered as an emerging issue during the last couple of years in Bangladesh. It is obvious that multiple factors are responsible for the contamination of milk. Toxic mineral elements along with other factors say cattle feed, feed composition, water, soil, drug, processing method, lactation period, season, combustion, bushfires, chemicals, fertilizers, industrial wastes, gas extraction, plants etc., are directly or indirectly involved for the contamination of milk and milk products [8-12]. Apart from these, food products can be polluted by accidents, mistakes and frauds that deal with a wide range of chemicals and compromise its safety [13]. People might face severe life-threatening problems if they live in the contaminated areas, contaminated food chain and polluted air or other organisms found in the surroundings [14]. It is reported that about thirty-eight micro and trace minerals are found in the raw milk from different region of the world [15]. All of these mineral elements exist in the cow milk as inorganic ion (e.g., carbohydrate, protein, peptides, fat and other molecules). When the concentration level of heavy metals goes beyond the range (40 to 200-fold) from the acceptable level causes toxicity. As a result, the consumer world might face a significant health hazard such as carcinogenic risk in the near future by the increased consumption of contaminated dairy food materials. Because the non-biodegradable nature of toxic elements could accumulate in the food chain by various ways say bio-transformation, bio-accumulation and bio-magnifications. Besides, the chemical contaminants in milk cannot be eradicated and eliminated readily due to the lipophilic nature of fat molecule found in the milk and milk products [16].

The contamination of milk or dairy food products with metals and other toxic materials is one of the most vulnerable factors now-a-days in the developing countries. Now milk quality, food safety and food security have become a salient factor of the people concern including governments, food industry and manufacturers [17]. It is reported that milk contamination is being increased due to the acceleration of environmental pollution affecting milk quality [18]. We do know that consumer preference and health depend exclusively on the safe, sound, healthy and quality dairy food production. After all, the potential health risk and safety margin of the consumer world would be taken into account as a priority basis, because people will consume cow milk with toxic elements continuously as a good food source. The exploration focusing on the milk contamination with heavy meal (Pb) was not much studied before in a developing country like Bangladesh. In addition, the data are scarce or not available in adequate level regarding the milk contamination with toxic elements, even though few studies have been done earlier to detect the heavy metals in cow's milk and other feedstuffs using validated (GF-AAS) method [19,20]. Besides, some other studies regarding health issues were also done on the different food materials across the globe, which include the detection of arsenic (As) in rice, trace element and aflatoxin in cassavas, and toxic metals in mushroom including others, respectively [21-23].

Though many methods exist for the quantification of heavy metals in various foodstuffs [24-27], but it could warrant further analytical procedures for assuring products quality and official laboratory analyses. For this why, global demand is rising to develop new modern and sophisticated techniques for providing accurate data or reliable information with micro-level of concentration [19]. Apart from these, the method developed herein this study sounds more facile to conduct, cost effective, reliable than others, and it can be applied globally for the routine analyses of milk samples in the laboratory. Furthermore, if the method is validated and implemented then many stakeholders, dairy industry, food and beverage companies, farmers and other personnel involved in the food processing plant might be benefitted from this sort of study.

However, it is noted that method validation and establishing a suitable protocol for conducting particular assay is mostly ignored by the scientists or given less emphasis in developing countries like Bangladesh. But it is reported that the development of new studies or any innovative approach could give a new dimension in research which incur reduced costs, faster test, and less time involvement or analyses [28]. Moreover, the study could play a significant role in detecting toxic elements of milk sample assuring consumer health and food safety of Bangladesh. Considering the above motioned issues, the present study was undertaken to explore the selected toxic metal (Pb) contaminating cow milks in Bangladesh available in the market.

Materials and Methods

Ethical study

The study has been conducted with inert material, so no need any ethical study, as here was no dealing with any live animals for conducting experiment.

Study area and experimental period: The study was conducted in the Quality Control Laboratory (QC Lab, DLS, Savar, Dhaka) from the December 2020 to March 2021. All sort of analyses were done in this laboratory.

Tools, chemicals and reagents: A sophisticated laboratory furnished with all necessary scientific tools and equipment where the present study was conducted. Tools which are used for this study are anatomic absorption spectrophotometer (Model: AA-7000, Shimadzu, Japan) equipped with Graphite Furnace (GFA 7000) and an Auto Sampler (ASC 7000). A cathode lamp was used for the detection of Pb (283.0nm, 10mA and slit 0.7nm). Chemicals namely Nitric Acid (HNO_3, 69%), Hydrogen Peroxide (H_2O_2, 30%) and standard of Pb, were used for sample preparation, Digestion process is done with microwave acid digestion system (Ethos Easy Milestone). The bulk standard solutions were made daily by dilution of metal stock standard solutions, using 1% (w/w) (HNO_3, 69%). Some modifier chemicals (H_3PO_4; $NH_4H_2PO_4$; Merck, Darmstadt,

Germany) were used for the determination of Pb, Deionized water (18 MΩ/cm) produced using an E-pure system (Thermo Scientific, USA) was used to prepare all the solution as well as to clean and wash all containers and glassware prior to use.

Sample preparation & analytical procedure: Milk sample was collected from the market by purchasing method. After that, the samples were stored in the deep freeze before undergoing lab analysis. For the digestion of samples, approximately 4.0ml of milk sample was weighed and digested with 3.5ml of HNO_3 (69%) and 1.5ml of H_2O_2 (30%) in acid pre-washed Teflon vessels. After digestion, it was diluted 10 ml final volume with deionized water. Blank was also prepared with each batch of digestion set. Sample preparation is done triplicate. For atomic absorption spectrophotometer (AAS Shimadzu AA-7000), both diluted samples and the standard solution were kept separately into fresh tubes. Finale heavy metal (Pb) was measured at 283.0 nm wavelength.

Preparation of spiking solution for lead: About 4.0ml (4000uL) sample was taken in digestion vessel and added 3.5mL nitric acid (69%), and 1.5mL hydrogen peroxide (30%), and later mixed 0.04mL (40uL) from each 1000 ppb of Pb solution and allowed to digest this solution. Finale, the amount was made with water up to 10mL.

Criteria for the validation of proposed method: In this study, usually linear range, limits for detection and quantification, accuracy percentage, precision checks and degree of uncertainty measurement etc., were taken into account for the validation of the proposed method [29-31]. The above criteria were appraised by the instructions or guidelines of International Conference on Harmonization (ICH) [25,32] and the US (FDA) [33].

Linearity: Standard mixture of Pb was prepared and a linear equation was established for the metals by plotting the absorbance's versus the concentrations to measure linearity. The calibration curve was obtained on consecutive day with a specified standard concentration of the metal. Linearity was calculated by running aqueous standard solution of the metal at final concentrations of 2, 5, 10, 15, 20, and 40 µg/mL for Pb. From the linear regression and correlation method, the slope, intercept and r2 values were calculated.

Recovery percentage: Three set of spike samples (10, 20, and 30 ug/kg) were prepared and each set replicated seven times. Sample reading was taken by measuring two times. For the estimation of recovery, an accurate amount of metal Pb (10, 20, 30 ug/kg) was added to approximately 1.0g of blank matrix powder, and then the powder was extracted and analyzed for recovery using the formula: recovery (%) = (amount obtained/amount spiked) × 100.

Limit of Detection (LOD), Limit of Quantification (LOQ), Instrument Quantification Limit (IQL), and Instrument Detection Limit (IDL): The lowest qualitative and quantitative concentrations for the tested linearity range were calculated for each metal according to the guidelines of ICH2000. Both LOD and LOQ were calculated using the expression: k x S.D/b, where k = 3.3 for the LOD and 10 for the LOQ, S.D = The standard deviation of the intercept, and b = Slope of the calibration curve tested for linearity. Instrument Detection Limit (IDL) and Instrument quantification limit (IQL) are calculated using following formula: Instrument Detection Limit (IDL) = 3s and Instrument quantification limit (IQL) = 10s; where, s= standards deviation

Repeatability and reproducibility precision check: The precision of the method was evaluated based on repeatability and intermediate precision. The repeatability was calculated on the results obtained from the same day for seven independent mixer solutions of the variable concentration and the intermediate precision was evaluated by calculating the repeatability of the similar concentration by two analysts on different days. The percentage of RSD was calculated for estimating the precision of this study. For reproducibility precision check, three set of samples have been prepared again after 15 days by the same analyst and after 18 days with different analyst spiking at 0.5, 1.0 and 1.5 level and analyzed as before. The mean concentration, standard deviation (RSD) and the coefficient of variation (%) of each level of fortified samples have been calculated.

Statistical analysis

All the data were statistically analyzed using the statistical software [34]. The means and standard deviations of the metal concentrations in samples were calculated. Finally, one-way ANOVA was used to compare the level of heavy metal residues in milk.

Results and Discussion

Linearity

The result of linear regression of the selected metal (Pb) measured in milk sample was shown in Table 1. It is clear that the coefficient correlation (r2) obtained in this study was 0.9996 for the toxic element (Pb). From the result it is obvious that our calculated value has meet the maximum permissible range (r^2=0.995). Our result denotes that it is very nice and perfect linear match as the r^2 value is higher than the acceptance level (0.995) for the selected metal measured in our study [35].

Accuracy of Pb in cow milk sample

Table 2 demonstrated the mean recovery or accuracy% of the selected metal Pb in cow milk sample. The data indicate that the overall recovery % of Pb measured in milk sample was 98.98 %, Our result has meet the criteria of maximum permissible level (mass fractions ≥ 10µg/kg) as required for the assessing of accuracy of this metal measured in the laboratory. The recovery indicates how much analyzed data were obtained from studies during the period of samples analysis in the lab. This is rendered actually to check any lacks and gap or losses of analyte levels occurred due to hindrance at the analyzing point [35]. Generally, recovery implies that how efficiently and accurately analysis is completed from an analytical procedure while sample processing and extraction steps [33][20]. However, we note that there are no justified reference materials for milk sample, thus the recovery % was done to justify the accuracy of

Table 1: Linear regression data of Pb in milk sample.

Metal	Linear Range (µg/L)	Calibration with aqueous standard solution		
		Slope ± SD(µg/L)	Intercept ± SD	Correlation coefficient (r^2)
Pb	2 --40	0.0101 ± 0.0006	0.0136 ± 0.0015	0.9996

Table 2: The recovery (%) of studied data for the analyses of Pb in milk sample.

Metal	Spiked Analyte Concentration (mg/L)	Calculated Analyte Concentration (n=7) (mg/Kg)	Recovery (%) (n=7)	Overall Recovery % (n=21)
Pb	0.5	9.65	96.51	98.98
	1	19.43	97.15	
	1.5	30.99	103.3	

Table 3: Instrument and method Limit of Detection (LoD and Limit of Quantification (LoQs) for the determination of Pb in milk sample.

Parameters	Metal
	Lead (Pb)
Instrumental LoD in solution(µg/l)	0.397
Instrumental LoQs in solution(µg/l)	1.32
Method LoD for milk (µg/kg)	0.993
Method LoQ for milk (µg/kg)	3.31

current method as stated by the previous investigator [36].

Determination of i Limit of Detection (LoD) and Limit of Quantification (LoQ)

The result of instrumental LoD for the analyses of selected metal (Pb) in the milk sample was 0.397µg/l, and the value for LoQ being 1.32µg/l. The result for the method LoD and LoQ was 0.993 and 3.31µg/Kg in the metal (Pb), respectively, as shown in the Table 3. The equipment efficacy or the performance of analytical method basically mean LoD and LoQ. The LoD is the degree of identification or the minimum amount of a sample that can be detected easily and fairly differentiated from nil or zero figure, but not actually determined, while LoQ is termed as the lowest amount of a determinants that can be measured numerically with an accurate level of concentration [37]. However, our result for the methods (LoDs and LoQs) in milk samples was found lower for the Pb than the highest value and well matched according to the Regulations (EC) No. 1275/ [38], and the Regulation 333/ 2007/EC [39], respectively.

Precision checks for the determination of heavy metal in cow's milk samples

The repeatability and reproducibility are the two components for the study of precision check. The precision is usually appraised by Relative Standard Deviation (RSD) or Co-Efficient of Variation (CV%). In this study, the overall CV% of the repeatability and reproducibility data was recorded7.65%,6.85%, respectively, for the selected metal (Pb) measured from the milk sample, as is shown in Tables (4,5). The data revealed that the CV% of either parameter is observed below 10 %, and have meet successfully the acceptable value (10%) [40]. Therefore, we can assume from the values obtained in this study that the developed method showed a good repeatability and reproducibility values. The precision denotes the proximity of compromising data for a series of analysis retrieved from numerous homogeneous samples under the prescribed conditions, and it was followed as per the regulation of /EC [41,42].

Measurement of uncertainty (%) of heavy metal in the cow milk

The value of Measuring Uncertainty (MU) for Lead (Pb) measured from the milk sample is recorded 7.0 in this study. Various data such as method development, validation, reproducibility and repeatability etc., can be used for assessing MU [43,44]. For each and every measurement, it is important for the calculation of MU as the suggestions given by ISO/ IEC 17025, because it checks errors and omission occurred while conducting any assay.

However, milk is a very nutritious, delicious and popular food item to the consumer. Many milk products say yoghurt, Dahi, cream, butter, ghee, cheese, ice cream, powder, sweetmeat, chhana etc., are manufactured from the milk derived from the varieties of domestic animals. People are consuming these milk and milk products continuously, so potential health issue, food hygiene, food contamination and food safety of the consumer world should be considered with great importance. The method development for the detection of contaminating agents in food materials is of great importance. Despite few errors and limitations, we affirm that, the method we formulated here for the detection of toxic metal element in milk sample is very faster, accurate, reliable and cost effective. Thus, the proposed method can be applied greatly for the investigation and detection of selected metal which can keep important role for the laboratory examination of cow milk samples across the globe [45]. However, we also agree with earlier researchers with respect to methods validation, development, detection and assessment of toxic minerals in many foodstuffs [46-49].

Conclusion

It can be concluded that from the results of this study that all the

Table 4: Repeatability precision studies data for the determination of Pb in milk samples.

Name of metal	Days	Spike concentration (µg/Kg)	Overall mean concentration (n=21) (µg/Kg)	SD	CV%	Overall CV %
Pb	1 to 3	0.5	9.96	0.9	8.98	7.65
	1 to 3	1	20.37	1.5	7.18	
	1 to 3	1.5	30.41	2.1	6.78	

Table 5: Reproducibility precision studies data for the determination of Pb milk sample.

Name of metal	Days	Spike concentration (µg/Kg)	Overall mean Concentration (n=21) (µg/Kg)	SD	CV%	Overall CV%
Pb	1 to 3	0.5	9.26	0.7	6.99	6.85
	1 to 3	1	19.43	1.3	6.44	
	1 to 3	1.5	29.36	2.1	7.1	

parameters for method validation and development have fulfilled the required criteria or acceptable range. So, it can be implied from the results that the method can be implemented and approved for the analyzing of toxic minerals in cow milk samples at micro level concentration. The findings of this study might be useful for the food manufacturing companies and other stakeholders across the globe. After all, consumer world would be benefitted from this sort of study to be aware of their health hazard, food contamination and food safety.

Competing Interests

The authors declare that the study has no conflicting matter or interests.

Publisher's Note

The journal will remain neutral with subject to any sort of plaints in published organizational matter.

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