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# Protocol development and its Efficacy testing for extraction of Nickel from Milk

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## Abstract

*Milk, recognized as an essential source of nutrition, is increasingly subjected to examination concerning heavy metal contamination, notably Nickel (Ni), because of its deleterious toxicological effects. The objective of this research was to establish an environmentally sustainable extraction methodology for the detection of Nickel in milk utilizing Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Milk specimens fortified with specified Nickel concentrations (1–9 ppm) underwent treatment with glacial acetic acid and sodium lauryl sulphate, followed by centrifugation, filtration, and subsequent analysis. The proposed method exhibited effective recovery rates and reliable detection capabilities, particularly at elevated concentrations. This protocol presents a sustainable, economically viable alternative for the routine monitoring of food safety and the safeguarding of public health.*

**Keywords:** Nickel detection; milk contamination; heavy metals; ICP-MS; environmentally friendly extraction; food safety; analytical chemistry; sustainable methodologies.

## Introduction

Milk is the secreted fluid of the mammary glands of female mammals. It contains nearly all the nutrients necessary to sustain life. Milk is an opaque liquid that appears white or yellowish-white in colour. Milk has a mildly sweet taste with a faint odour and flavour under normal conditions. Components of milk include carbohydrates that are solubilized in milk serum, proteins that exist as both solubilized whey and suspended casein micelles, and fats that are present as emulsified globules[1]. Milk is often regarded as the "perfect food" due to its vital role in nourishment, hydration, and immune system development. While most mammals consume milk only in infancy, humans uniquely continue milk consumption into adulthood, sourcing it from various species. Cow's milk dominates global production (83%), followed by buffalo (13%), goat (2%), sheep (1%), and camel (0.4%). The domestication of dairy animals has significantly influenced agriculture, societal development, and human evolution over the past 10,000 years[2]. Even today, milk remains one of the most widely consumed beverages in India, with 239.30 million tonnes produced during 2023-24[3].

However, the rising demand for milk is accompanied by growing concerns over its safety and quality. One of the major concerns is the presence of heavy metals in milk. Heavy metal contamination in milk primarily occurs through multiple sources.

Untreated sewage water and industrial effluents used for crop irrigation are the main sources of heavy metal contamination in milk, which also results in contaminated animal feed. Furthermore, near industrial areas, contaminants in the soil, water, and feed may be consumed by milk-producing animals. Additionally, processing equipment and transport apparatus can cause contamination; processed milk frequently contains higher levels of metals than raw milk. Additional environmental factors that contribute to elevated levels of heavy metals in milk include being close to busy roads and industrial regions[4].

These heavy metals, once introduced into the food chain, pose significant health risks to both animals and humans. Among them, Nickel (Ni) is particularly concerning due to its toxic effects even at low concentrations.

Numerous health hazards, including respiratory conditions such chronic lung inflammation and fibrosis, have been connected to the use of Nickel compounds. Reduced immune responses and altered lymphoid structure are two consequences of immune function impairment. Prolonged exposure can have developmental impacts including fetal loss and reproductive damage like decreased sperm motility and count. Nickel compounds are classified as carcinogenic to humans by the IARC and the U.S. Department of Health and Human Services[5].

The growing worries about food safety and the possible health consequences associated with heavy metal contamination have led to a demand for better methods for detecting Nickel in milk. Conventional techniques might not provide the environmental safety, sensitivity, or accuracy needed for current testing standards. By avoiding the use of strong acids like nitric acid, providing a more ecologically friendly method, and simplifying and making the procedure easier for labs to utilize, this methodology solves these problems. This protocol shows great extraction capacity, allowing for detection by instrumental analysis as well as chemical testing. Positive results were obtained from this study's utilization of Inductively Coupled Plasma Mass Spectrometry (ICP-MS), guaranteeing accurate and dependable Nickel identification in milk. This development helps to better protect the public's health by improving the monitoring of heavy metal contamination in food products.

## Methodology

A nickel chloride stock solution of 1000 ppm was made by adding 0.03 g of nickel chloride to 30 mL of milk. From the 1000 ppm stock solution, an intermediate 100 ppm was made by taking 1 mL of the 1000 ppm stock solution and diluting it with 9 mL of milk. Then a 10 ppm saline solution was made using the same method as the intermediate solution, by taking 1 mL of the 100 ppm solution and diluting it with 9 mL of milk. All working standards were made from the 10 ppm dilution using milk as diluent.

The procedures for creating the working standard levels used to produce the metal standards were as follows: 1 ppm standard was made by combining 0.5 mL of the 10 ppm working stock with 4.5 mL of milk; 5 ppm standard was made by combining 2.5 mL of the 10 ppm stock solution with 2.5 mL of milk; and 9 ppm standard was made by combining 4.5 mL of the 10 ppm working stock with 0.5 mL of milk.

To prepare the samples for analysis, 2 mL of glacial acetic acid was added to each prepared sample and allowed to stand for 5 minutes. Subsequently, 5 mL of sodium lauryl sulphate (SLS) solution (prepared by dissolving 0.3 g sodium lauryl sulphate in 50 mL of distilled water) was added to all prepared samples. All prepared samples were vortexed for 1 minute to ensure uniform dispersion and then centrifuged for 5 minutes at 3000 RPM. Only the supernatant was collected from all prepared samples and filtered through filter paper. The resultant filtrate was then analyzed for the determination of metal concentration using Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

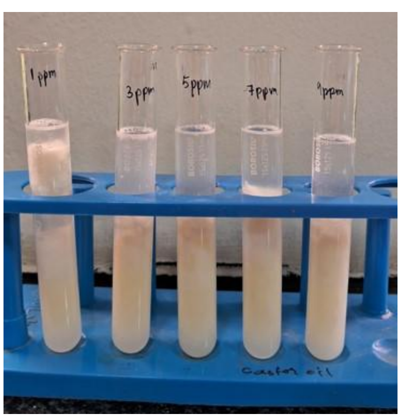


**Figure-1:** Milk with nickel chloride (1 ppm, 3 ppm, 5 ppm, 7 ppm, 9 ppm) with curdling agent and demulsifier.


**Figure-2:** Vortexing of the mixture for 1 minute.


**Figure-3:** Centrifugation of the mixture for 5 minutes at 3000 rpm.

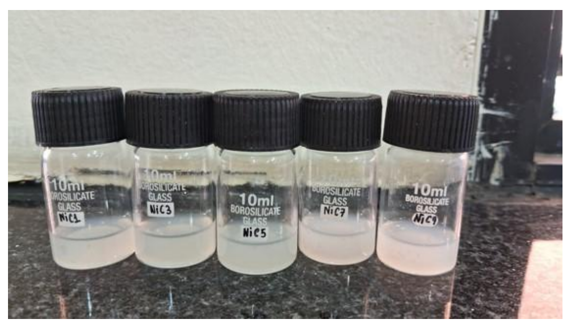


**Figure-4:** Samples filtered and collected in vials.

## Results and Discussion

The experimental protocol was primarily centered on the extraction of Nickel from milk samples that were prepared at defined concentrations, which were analyzed utilizing Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). The milk samples were augmented with nickel chloride at three concentration tiers, spanning from 1 ppm to 9 ppm, with the resultant recovered concentrations documented in table 1. In the analyzed Nickel samples, the recovery values exhibited an increase in response to rising concentrations and displayed a more consistent and incremental trend. Nickel was detected with a strong signal at m/z 60, indicating its significant presence in the sample.

**Table-1:** concentration of Nickel recovered from milk samples.

| Concentration (in ppm) | Recovered concentration (in ppm) |
|---|---|
| 1 | 0.33 |
| 5 | 0.54 |
| 9 | 0.89 |

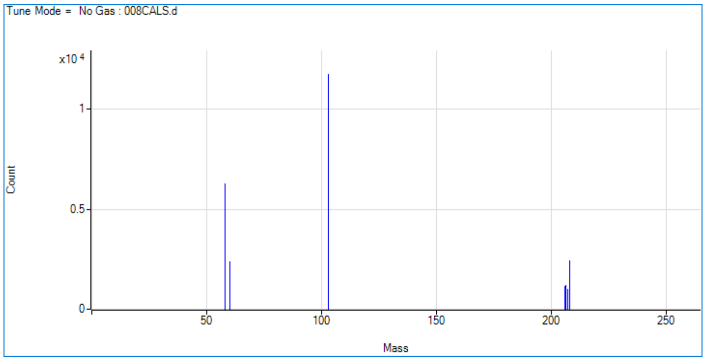

**Figure-5:** ICP-MS spectrum for 1 ppm sample.

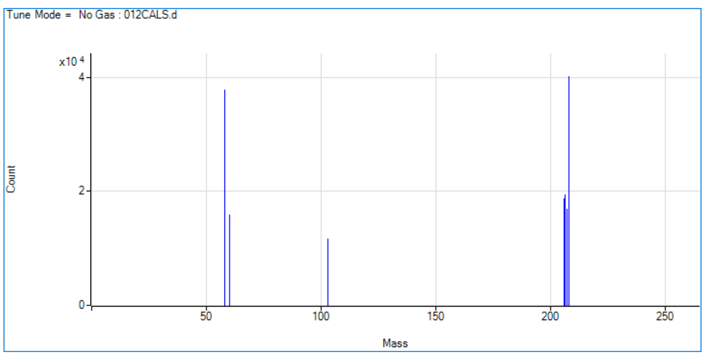

**Figure-6:** ICP-MS spectrum for 5 ppm sample.

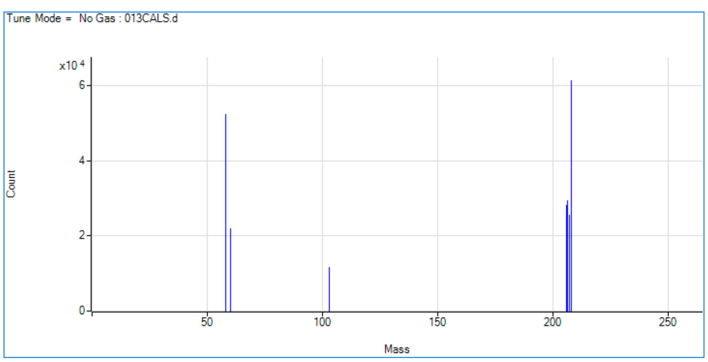

**Figure-7:** ICP-MS spectrum for 9 ppm sample.

**Discussion:** This investigation sought to formulate and validate an innovative extraction methodology for the identification of heavy metals—specifically Nickel (Ni)—in dairy products, employing Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). The results indicate that the methodology effectively extracted and facilitated the identification from milk samples with known concentrations of Nickel, thereby suggesting the method's promising efficacy in assessing heavy metal contamination within intricate food matrices.

While the notion of metal extraction from milk is not entirely novel, the present study contributes to the field by proposing a more streamlined and environmentally-friendly methodology that adapts established principles for improved practicality. Consequently, this investigation aids in addressing a methodological void by presenting a more accessible technique specifically designed for the analysis of heavy metals in milk.

Contextualizing these findings within the broader spectrum of existing literature, the results of this investigation exhibit a strong correlation with previously documented data regarding heavy metal concentrations in milk, particularly from studies employing ICP-MS. In a study conducted on commercially available milk in Istanbul, Turkey by Saribal[6], the mean concentration of Nickel was reported to be 38.476 µg/L (0.0385 ppm). Although the present study utilized controlled concentration levels, this correlation underscores that the developed protocol possesses the capacity to detect metals encountered in actual milk samples. The consistency of the recovered concentrations in this study further substantiates the method's potential application in contexts necessitating the monitoring of heightened heavy metal levels. Additionally, the uniform application of ICP-MS across various studies accentuates the robustness and precision of this analytical methodology, thereby reinforcing confidence in the usability of the newly established extraction protocol for the surveillance of heavy metals in milk.

A noteworthy observation is that the recovery values at lower concentrations were relatively modest (e.g., 0.33 ppm for Ni at a concentration of 1 ppm), which may be ascribed to the matrix effects inherent in milk. The composition of milk encompasses proteins, fats, and minerals that may bind to metals or disrupt extraction efficiency. While this phenomenon was anticipated due to the complex nature of milk, the consistent enhancement in recovery across the tested concentration range suggests that the extraction methodology is adaptable to varying levels and could be further optimized to improve performance at lower concentrations.

The ICP-MS spectrum reveals a prominent peak at m/z 60, which is indicative of the most abundant isotope of Nickel. The intensity of the signal at this specific mass indicates a substantial concentration of Nickel within the sample. The minor peaks detected at alternative mass-to-charge ratios are likely attributable to other elemental background signals; however, these do not interfere with the detection of Nickel isotopes. The lack of significant spectral noise at m/z 60 further substantiates the selectivity of this analytical method for the detection of Nickel.

In summary, these findings contribute substantially to the advancement of more sustainable and accessible methodologies for the detection of toxic contaminants in milk, particularly Nickel. This study exemplifies the potential of a practical, environmentally conscious, and cost-effective extraction technique for detecting Nickel in milk through the utilization of ICP-MS. Beyond routine surveillance, such methodologies hold significant value for probing instances of intentional adulteration or contamination.

## Conclusion

This study aimed to develop and validate an environmentally friendly extraction protocol for the detection of Nickel in milk using ICP-MS. The method proved effective in detecting both metals within the complex milk matrix, contributing to ongoing advancements in trace metal analysis in food while presenting a greener alternative to conventional techniques.

The method's potential for food safety applications is particularly relevant for protecting sensitive populations, such as infants, who are more vulnerable to heavy metal exposure. By offering a rapid, cost-effective, and sustainable approach, the protocol supports routine monitoring and facilitates investigations into intentional adulteration or contamination from environmental sources. Furthermore, the connection between milk contamination and industrial or environmental pollutants underscores the broader public health and ecological implications of the findings.

Nonetheless, certain limitations were noted. The matrix complexity of milk likely affected metal recovery, indicating a need for further optimization to enhance extraction efficiency. Future studies should focus on refining the method, extending its scope to other contaminants, and validating its performance across diverse milk types and real-world samples.

In summary, this research advances the development of accessible and sustainable analytical tools for food quality assessment. It underscores the critical role of eco-conscious methodologies in improving food safety practices and promoting environmental stewardship in analytical laboratories. With continued refinement, this protocol holds strong potential for integration into public health monitoring frameworks.

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