Quantitative Estimation of Class 1 and Class 2A Elemental Impurities in Anti-Tussive and Cold Syrup Formulations Using ICP-MS

Mehar Raghavendra Yeggina [1]*, Krishnamanjari Pawar Amgoth[1]

[1]Department of Pharmaceutical Analysis, A.U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530003, Andhra Pradesh, India.

Corresponding Author:
Mehar Raghavendra Yeggina
***Address:** Department of Pharmaceutical Analysis, A.U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530003, Andhra Pradesh, India.*
Email: meharraghavendra.yeggina@gmail.com
Tel: +91-9963851449

Abstract
Elemental impurities such as arsenic, cadmium, mercury, and lead can pose significant toxicological risks in pharmaceutical products. This study investigates the presence of the seven major elemental impurities, vanadium (V), cobalt (Co), nickel (Ni), arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) in five commercially available antitussive and cold syrup formulations using Inductively Coupled Plasma–Mass Spectrometry (ICP-MS). Sample digestion was performed using closed-vessel microwave digestion, followed by trace analysis as per ICH Q3D and USP <233> guidelines. The method demonstrated excellent recovery (70–150%) and reproducibility across all matrices, with measured concentrations significantly below permissible daily exposure (PDE) limits. The validated method is robust and suitable for routine quality control of liquid oral formulations.

Keywords: *Elemental Impurities, ICP-MS, Anti-tussive Syrup, ICH Q3D, Permitted daily exposure, Pharmaceutical Quality Control*

1. Introduction

The common cold is a widespread illness affecting millions of individuals globally, contributing to considerable morbidity and economic burden [1]. To manage symptoms such as cough, nasal congestion, and sore throat, anti-tussive and cold formulations are extensively used [2]. Despite the widespread use of antitussive and over-the-counter cold medications, nonspecific cough suppressant therapies have demonstrated limited efficacy in managing persistent cough. Optimal treatment requires a precise diagnosis and a comprehensive understanding of the underlying pathophysiology [3]. However, like all pharmaceutical products, anti-tussive and cold formulations are susceptible to contamination by elemental impurities, which may pose significant health risks to consumers [4].

Elemental impurities, particularly heavy metals can enter pharmaceutical products through raw materials, manufacturing processes, or packaging systems [5]. Among these,

Class 1 elements, including arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb), are of particular concern due to their high toxicity and lack of therapeutic benefit. Similarly, Class 2A elements, such as vanadium (V), cobalt (Co), and nickel (Ni), though less toxic than Class 1 elements, are also monitored closely as they are more likely to be present naturally in pharmaceutical ingredients [6, 7]. The presence of these impurities is concerning due to their potential to cause adverse health effects, including organ toxicity, genotoxicity, and carcinogenicity. Several studies have identified these elements in anti-tussive and cold formulations using advanced analytical techniques such as Inductively Coupled Plasma Mass Spectrometry (ICP-MS) [8]. Furthermore, considerable variation in the levels of these contaminants across different commercial brands has been reported [9, 10].

Comparative studies evaluating ICP-MS, Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), and Atomic Absorption Spectroscopy (AAS) have consistently demonstrated that ICP-MS offers superior sensitivity and accuracy for trace-level detection of heavy metals in pharmaceutical matrices [11] and also in bio-monitoring of trace elements [12]. In recognition of these concerns, regulatory bodies such as the International Council for Harmonisation (ICH) have issued guidelines, ICH Q3D to control elemental impurities in drug products [13]. Given its analytical advantages, ICP-MS is widely used for routine multielemental trace and ultratrace analysis in diverse liquid matrices and has become the preferred technique for regulatory compliance and quality control in pharmaceutical testing [14].

This study aims to assess the levels of class 1 and class 2A elemental impurities in various anti-tussive and cold formulations using ICP-MS, with the objective of evaluating product safety and ensuring adherence to established regulatory standards.

2. Materials and Methods

2.1. Reagents and Materials

All reagents and materials used in this study were identical to those employed in our previously developed methodology for elemental impurity analysis, which is currently under review (Mehar et al.). Concentrated nitric acid (trace metal grade) was obtained from Fisher Scientific, while hydrochloric acid was sourced from Sigma-Aldrich and distilled-deionized water was produced using a Millipore purification system. Elemental standard solutions of vanadium (V), cobalt (Co), nickel (Ni), arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb), yttrium (Y), bismuth (Bi), and gold (Au) were acquired from Inorganic Ventures. A tuning solution for instrument optimization was obtained from Agilent Technologies.

2.1.1. Sample Materials

The present study focused on the analysis of elemental impurities in five commercially available over-the-counter pharmaceutical formulations. These were designated as Formulation A – a combination of an antihistamine and a decongestant, Formulation B – a cough suppressant and expectorant syrup, Formulation C – an antihistamine-based cough syrup, Formulation D – a pediatric cold and allergy suspension, and Formulation E – a sedative antihistamine formulation. All products were procured from licensed retail pharmacies in their original, unopened packaging and were used as received, without any modification. Each formulation was handled in accordance with standard laboratory practices to ensure sample integrity during preparation and analysis.

2.2. Methodology

2.2.1. Instrumentation: Elemental impurity analysis was conducted using an Inductively Coupled Plasma–Mass Spectrometer (ICP-MS, Agilent 7800) equipped with MassHunter software (Agilent Technologies). The instrument operating conditions were consistent with those described in our previously developed methodology (Mehar et al., manuscript under communication) and are summarized in Table 1.

Table 1. Instrument Conditions

S.no	Condition	Value
1	Gas mode	Helium
2	RF power	1550 W
3	Spray Chamber temperature	2 °C
4	Carrier gas (Argon)	1.01 L/min
4	Helium gas flow	4.3 mL/min
5	Acquisition mode	Spectrum
6	Rinsing port	Rinsing solution (Diluent)
	Pre Run	
7	Sample uptake time	45 sec
8	Stabilization	40 sec
9	Uptake speed	0.5 rps
	Post run	
10	Probe rinse	25 sec
11	Nebulizer pump speed	0.5 rps

2.2.1 Evaluation of Maximum Allowable Daily Intake and Establishment of Specification Limits: Method development commenced with the evaluation of the maximum allowable daily intake (MDI) for each of the five pharmaceutical formulations under study. The MDI was calculated based on the maximum daily dose (MDD) of the individual active pharmaceutical ingredients (APIs) present in each formulation. Each formulation's MDI was expressed in grams (g) and derived either from direct label claims or from dose-volume relationships, considering the highest possible therapeutic regimen per day.

The specification limits for elemental impurities were established by applying the concentration of an elemental impurity in the drug product, expressed in micrograms per

gram (µg/g), is calculated by dividing the permitted daily exposure (PDE, µg/day) by the maximum daily intake of the drug product (g/day).
Each formulation is described below in terms of composition, calculated MDI (Table 2) and derived elemental impurity specification limits (Table 3).

Table 2. Composition and Maximum Daily Intake of Syrup Formulations

Formulation	Composition, Each 5 mL contains	Maximum Daily Dose (mg)	Daily Intake (mL)	Weight per mL (g/mL)	Maximum Daily intake (g) (Daily Intake X Wt per mL)
A	Phenylephrine Hydrochloride 5mg,	60 [15]	60	1.271	76.26
	Chlorpheniramine Maleate 2mg,	24 [16]	60		
	Flavoured syrupy base, Excipients .....q.s, Colour: Sunset Yellow	-	-		
B	Dextromethorphan Hydrobromide 10mg,	120 [17]	60	1.190	71.40
	Phenylephrine hydrochloride 5mg,	60	60		
	Chlorpheniramine Maleate 2mg,	24	60		
	Flavoured base q.s, Colour: Ponceau	-	-		
C	Dextromethorphan Hydrobromide 15 mg,	120	40	1.156	46.24
	Colour: Quinoline Yellow, in a pleasantly flavoured mentholated syrup base	-	-		
D	Paracetamol 125 mg,	4000 [18]	160	1.240	74.40
	Phenylephrine Hydrochloride 5 mg,	60	60		
	Chlorpheniramine Maleate 1 mg,	24	20		
	Excipients q.s, Colour: Sunset Yellow, in Flavoured syrup base	-	-		
E	Promethazine Hydrochloride 5 mg	25 [19]	100	1.188	29.7

The maximum daily dose of the API was based on the formulation's highest therapeutic regimen.

Table 3. PDE and Specification Limits for Elemental Impurities

Element	PDE Limits (µg/day)	Specification Limits (µg/g) for formulation				
		A	B	C	D	E
V	Not more than 100	1.31	1.40	2.16	1.34	3.37
Co	Not more than 50	0.66	0.70	1.08	0.67	1.68
Ni	Not more than 200	2.62	2.80	4.33	2.69	6.73
As	Not more than 15	0.20	0.21	0.32	0.20	0.51
Cd	Not more than 5	0.07	0.07	0.11	0.07	0.17
Hg	Not more than 30	0.39	0.42	0.65	0.40	1.01
Pb	Not more than 5	0.07	0.07	0.11	0.07	0.17

Each of the above specification limits represents the maximum allowable concentration of elemental impurities in the respective formulation, ensuring safety in line with toxicological thresholds set by ICH Q3D guidelines. These limits guided the subsequent analytical method development, which ensured that sensitivity, accuracy, and reliability were sufficient to meet regulatory expectations.

2.3. Preparation of Solutions

The solution preparation followed an established procedure previously optimized for another pharmaceutical dosage (Mehar et al.). The same protocol was applied here without alteration and is outlined below.

2.3.1. Diluent Preparation*:* To prepare the diluent, 50 mL of concentrated nitric acid was combined with 10 mL of concentrated hydrochloric acid in a 1000 mL volumetric flask containing 500 mL of high-purity distilled-deionized water. The mixture was thoroughly agitated to ensure uniformity, and the final volume was brought up to the 1000 mL mark using the same water.

2.3.2. Internal Standard and Gold Stabilizer Solution: A combined internal standard and stabilizer solution was formulated by transferring 0.25 mL each of yttrium and bismuth standard solutions, along with 0.5 mL of a gold standard solution (each at 1000 µg/mL concentration), into a 50 mL volumetric flask already containing 5 mL of the prepared diluent. The mixture was well homogenized and subsequently diluted to volume using the same diluent.

2.3.3. Calibration Blank Preparation: To prepare the calibration blank, 2.5 mL of nitric acid and 0.5 mL of hydrochloric acid were transferred into a 50 mL volumetric flask. This was followed by the addition of 1 mL distilled-deionized water and 0.5 mL of the previously prepared internal standard/gold solution. The

mixture was then diluted to the final volume with distilled-deionized water and mixed thoroughly.

2.3.4. Standard Stock Solutions: Stock Solution A was prepared by separately adding 0.5 mL each of Vanadium, Cobalt, and Nickel standard solutions, and 0.1 mL each of Arsenic, Cadmium, Mercury, and Lead standard solutions (all certified at 1000 µg/mL) into individual 50 mL volumetric flasks. Each solution was then brought to volume with the prepared diluent and mixed well.
To prepare **Stock Solution B** (a mixed standard), 0.4 mL of vanadium, 0.2 mL of cobalt, 0.8 mL of nickel, 0.3 mL of arsenic, 0.1 mL of cadmium, 0.6 mL of mercury, and 0.1 mL of lead were combined from their respective individual standard solutions into a 20 mL volumetric flask. The contents were then diluted to the mark with the prepared diluent and thoroughly mixed.

2.3.5. Selection of diluent for samples preparation: Samples were treated with a 5:1 ratio of nitric acid to hydrochloric acid (5 mL HNO_3 and 1 mL HCl), followed by a controlled digestion process to break down the organic matrix. After digestion, the samples were diluted to a final volume of 25 mL with distilled deionized water. This acid mixture was chosen for its proven effectiveness in solubilizing organic excipients and retaining trace-level elemental impurities in the sample solution. Nitric acid acts as a strong oxidizing agent, facilitating the decomposition of organic components during sample digestion, while hydrochloric acid improves the solubility and stability of certain metal ions particularly, Class 1 elements such as mercury and lead.
The combination of these acids ensured complete dissolution of the syrup matrix and minimized potential matrix effects during ICP-MS analysis. Diluent was prepared using distilled deionised water to eliminate background contamination and ensure analytical accuracy. This diluent system was also utilized for rinsing protocols throughout the analytical run to maintain cleanliness of the sample introduction system and prevent carryover between samples [20].

2.3.6. Sample Digestion Procedure: Sample digestion was performed using a closed-vessel microwave digestion system optimized for the complete breakdown of the organic matrix in the five formulations. The digestion procedure used in this study was adapted from an in-house method currently under communication (Mehar et al.). Approximately 0.2 g of each syrup sample was accurately weighed into microwave digestion vessels. To each vessel, 5 mL of concentrated nitric acid (HNO_3), 1 mL of concentrated hydrochloric acid (HCl) and 2 mL of water were added. The vessels were sealed and subjected to a two-stage microwave digestion program designed to ensure complete oxidation of excipients and effective release of elemental impurities.

The microwave digestion program consisted of the following steps:
• Ramp to 180 °C over 10 minutes, followed by a 20-minute hold,
• Ramp to 190 °C over 5 minutes, followed by a 15-minute hold.

After digestion, the vessels were allowed to cool to room temperature. The digested samples were quantitatively transferred and diluted to a final volume of 25 mL using ultrapure Milli-Q water. Reagent blanks and spiked samples were processed in parallel to monitor background contamination and evaluate recovery performance. The resulting digests were clear and particle-free, indicating the efficiency of the digestion process and confirming their suitability for ICP-MS analysis.

3. Results and Discussion

3.1 Method Adaptability and Performance Evaluation

The analytical method, initially developed and validated for a different pharmaceutical dosage form, was successfully applied to five commercially available anti-tussive and cold syrup formulations without modification. The goal was to evaluate the adaptability of the method under ICH Q3D (R2) guidelines and USP <233> requirements, focusing on its accuracy and precision at the limit of quantification (LOQ) and 100% spike levels.

3.2 Accuracy and Recovery at LOQ and 100% Levels

Spike recovery studies were performed on three preparations of each of the five formulations for each analyte (V, Co, Ni, As, Cd, Hg, and Pb) at concentrations corresponding to the limit of quantification (LOQ) and 100% of the specification limits derived from the maximum daily intake of another pharmaceutical dosage form, based on our previously developed and validated method (Mehar et al., manuscript under review). The method showed acceptable recovery for all elements across all five cough syrup matrices. Recoveries were within the acceptable range of 70–150% (Table 4 and Figure 1) and RSD values remained below 20%**,** in compliance with USP <233> criteria [21].

Table 4. Mean Recovery of Formulations at LOQ and 100% Level.

Level	Element	A		B		C		D		E	
		Mean	RSD	Mean	RSD	Mean	RSD	Mean	RSD	Mean	RSD
Accuracy at LOQ	V	86.7	1.3	95.6	1.2	87.8	1.3	85.9	0.7	95.6	3.1
	Co	84.4	5.3	103.7	1.2	97.8	4.5	96.3	7.0	91.1	2.4
	Ni	97.4	1.7	104.6	3.8	99.4	1.5	98.7	1.7	95.2	0.7
	As	92.9	7.7	95.2	11.5	100.0	7.1	92.9	7.7	107.1	6.7
	Cd	100.0	0.0	116.7	12.4	108.3	13.3	91.7	15.7	108.3	13.3
	Hg	100.0	3.7	95.1	2.3	101.2	5.6	103.7	3.6	102.5	5.5
	Pb	108.3	13.3	91.7	15.7	108.3	13.3	100.0	0.0	100.0	0.0
Accuracy at 100%	V	100.4	0.5	100.2	0.6	99.6	0.1	99.2	0.4	97.0	0.4
	Co	102.9	0.5	101.3	1.4	98.5	0.6	99.1	0.8	95.6	0.7
	Ni	101.9	0.8	100.2	0.2	99.9	0.2	100.8	0.4	102.1	0.3
	As	105.6	1.7	99.7	0.5	101.5	1.3	104.7	2.1	103.8	1.8
	Cd	105.4	2.6	100.9	4.1	105.4	2.6	99.1	4.2	100.0	2.7
	Hg	100.1	0.7	101.2	0.3	101.8	0.7	101.6	0.7	99.3	3.6
	Pb	103.6	6.6	101.8	4.1	104.5	1.5	100.0	2.7	100.9	4.1

Accuracy expressed as mean recovery (%); %RSD calculated from three replicate spike recoveries at each level.

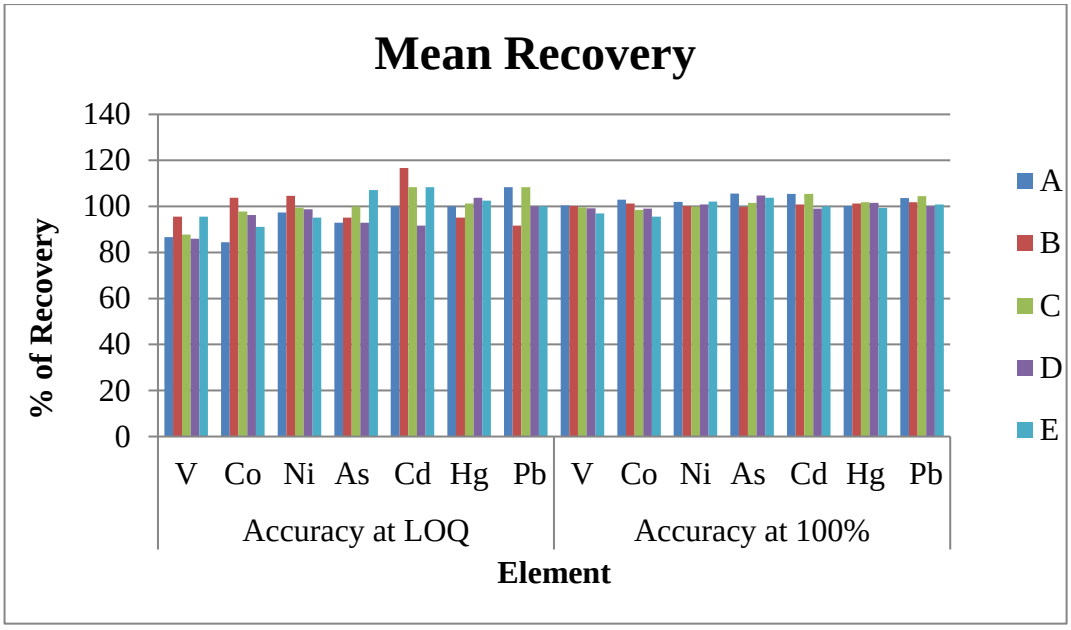

Figure 1. Mean Recovery of Cough Syrup Formulations for Class 2A (As, Cd, Hg and Pb) and Class 1 (V, Co and Ni) Elements at LOQ and 100% Level.

This consistent performance across different formulations confirms the robustness and adaptability of the method. No significant matrix interference or signal suppression was observed, indicating the effectiveness of the sample preparation procedure and internal standard correction.

3.3 Elemental Impurity Profiles in market Samples

The concentrations of all seven elemental impurities measured in the five cough syrup formulations (A, B, C, D, and E) were found to be well below the permitted daily exposure (PDE) limits established by the ICH Q3D guidelines for oral drug products (Table 5 and Figure 2).

Table 5. Measured Concentrations of Elemental Impurities in the Marketed Formulations

Formulation	Concentration (ppm)						
	V	Co	Ni	As	Cd	Hg	Pb
A	0.008	0.012	0.380	0.010	0.012	0.038	0.017
B	0.008	0.005	0.371	0.013	0.016	0.040	0.025
C	0.013	0.018	0.411	0.015	0.030	0.035	0.008
D	0.012	0.014	0.361	0.012	0.026	0.040	0.017
E	0.007	0.008	0.264	0.012	0.016	0.033	0.017

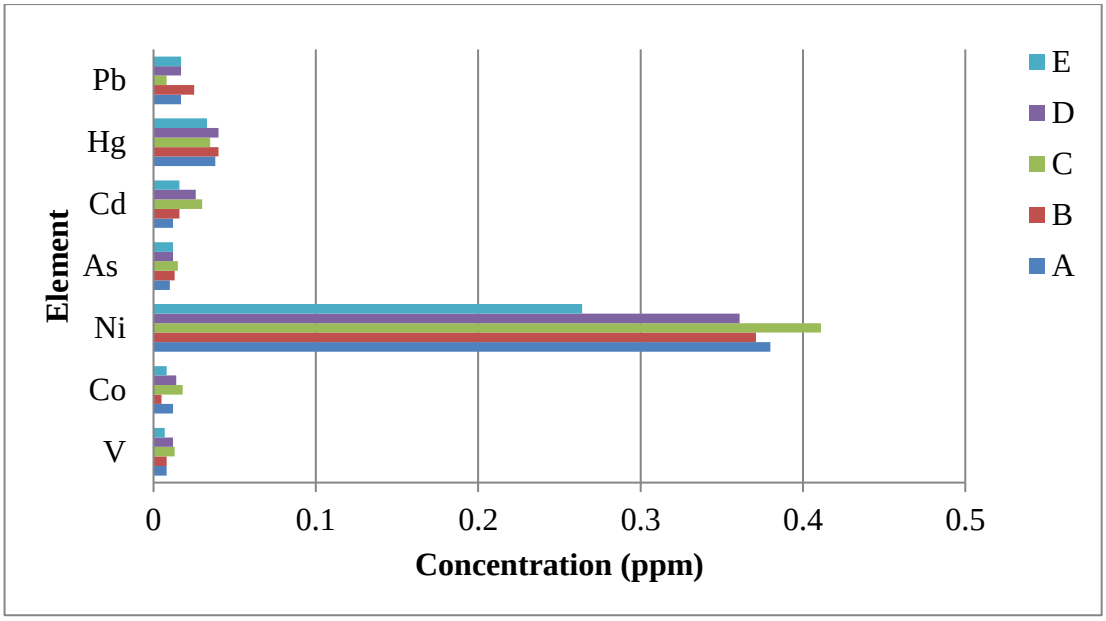

Figure 2. Measured Concentration of Class 2A (As, Cd, Hg and Pb) and Class 1 (V, Co and Ni) Elemental Impurities in the Marketed Cough Syrup Formulations.

3.4 Suitability for Routine Quality Control

The method demonstrated excellent sensitivity, reproducibility, and accuracy for trace metal detection in complex liquid oral matrices. Its ability to meet validation criteria without matrix-specific modifications highlights its suitability for routine QC analysis of oral liquid formulations. Moreover, the use of 5% Nitric acid and 1% Hydrochloric acid as a diluent proved effective in maintaining analyte stability and minimizing matrix effects during ICP-MS analysis.

4. Conclusion

The validated ICP-MS method applied in this study provided accurate, sensitive, and reproducible detection of elemental impurities across five different liquid pharmaceutical formulations. It demonstrated reliable quantification of Class 1 and Class 2A trace metals while effectively minimizing matrix interference. All detected impurity levels complied with ICH Q3D regulatory guidelines, reaffirming the safety of these pharmaceutical products for human use. The method's robustness was evident through its consistent performance across varying sample matrices, underscoring its matrix-independent nature. With its high sensitivity, precision, and adaptability, this ICP-MS method is well-suited for routine quality control in pharmaceutical testing environments, facilitating regulatory compliance and ensuring safety of the product.

Conflicts of Interest

None

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