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Original Article

# Elemental Impurities for Hypertension Drug Product Injection by Inductively Coupled Plasma-Mass Spectrometry Using Validation and Development

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## Abstract

This study aimed to develop and validate a robust, sensitive, and accurate Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) method for the quantitative determination of elemental impurities in both the drug product and packaging materials of Hydralazine Hydrochloride for Injection, in compliance with ICH Q3D and USP <232>/<233> guidelines. The analysis was performed on a Thermo iCAP RQ ICP-MS system. The method optimized critical instrumental parameters, including dwell time (0.05 seconds), uptake time (60 seconds), wash time (20 seconds), and number of sweeps (30). An online internal standard mode was employed to correct for matrix effects and instrumental drift. The method was validated for system suitability, specificity, linearity, accuracy, precision, and sensitivity in accordance with international regulatory standards. The method demonstrated excellent linearity for all target elemental impurities, with correlation coefficients (r) of 1.000 across the established concentration ranges. Recovery studies yielded mean recoveries of 96% to 107%, confirming the method's accuracy. The precision, expressed as %RSD, was below 20% (n=9), well within the acceptance criteria set by regulatory guidelines. All validation parameters met the predefined acceptance criteria, confirming the method's reliability for its intended use. A comprehensive, fully validated ICP-MS method was successfully established for the routine quality control of elemental impurities in Hydralazine Hydrochloride for Injection. This study is the first to report a validated methodology specifically tailored to this parenteral formulation, addressing critical patient safety requirements and the increasing regulatory demands of the upcoming 2025 pharmacopoeial standards. The method's high sensitivity and multi-element detection capabilities make it a robust tool for ensuring the safety of pharmaceutical products.

***Keywords:*** *Elemental Impurities, ICP-MS, Hydralazine Hydrochloride, Method validation Injection and Internal standard.*

## Introduction

Elemental impurities are trace metals and metalloids that may inadvertently contaminate pharmaceutical products throughout their lifecycle (Norwood *et al.,* 2008). These impurities can arise during various phases, including the manufacturing process, the sourcing of raw materials, or through interactions with packaging components. Understanding the sources and routes of contamination is essential for implementing effective control strategies. Raw materials, including active pharmaceutical ingredients (APIs) and excipients, can contain elemental impurities based on their natural occurrence or methods of synthesis (Ditter *et al.,* 2018; PQRI, 2006). Contamination may result from agricultural practices, catalytic residues, or chemical processes used during manufacturing. Additionally, equipment and machinery used in drug production can contribute to impurities through metal wear or contamination from the production environment, introducing trace metals into drug formulations (ICH Q3B(R2), 2006).

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The impact of packaging on elemental impurities represents another critical source of contamination. Packaging materials such as plastics, glass, and elastomers can interact with drug products, especially under elevated temperatures or prolonged storage. These interactions may result in leachables, which are substances that migrate from packaging into the drug product, with the risk of leaching increasing over time and under varying storage conditions. Extractables, conversely, are substances that can be extracted from materials under specific laboratory conditions and are often assessed during the development phase to predict potential leachables. Regulatory authorities, including the FDA and EMA, have established comprehensive guidelines to assess and control elemental impurities in pharmaceutical products. The ICH Q3D guidelines specifically establish permitted daily exposure (PDE) limits for elemental impurities based on their potential risk to human health, classifying elements into classes based on their toxicity and likelihood of occurrence (ICH Q3D, 2022; USP ⟨232⟩, 2023; USP ⟨233⟩, 2024). Manufacturers must implement appropriate controls to ensure that impurities remain within these limits throughout the product lifecycle, including careful material selection, rigorous testing, and adherence to regulatory guidelines (USP ⟨1381⟩, 2024).

Various analytical techniques are employed for the detection and quantification of elemental impurities, with Inductively Coupled Plasma Mass Spectrometry (ICP-MS) emerging as a highly sensitive technique often preferred for detecting trace metals at parts-per-billion (ppb) and parts-per-trillion (ppt) levels (Fordham *et al.,* 1995). Extractables and leachables (E&L) studies play a crucial role in identifying which packaging materials in contact with drug products may contribute to elemental impurities (Kakuk *et al.,* 2025). This connection is vital for determining potential contamination sources during product stability and storage. Elemental impurities can have significant toxicological effects, posing risks to patient safety (Van Hoecke *et al.,* 2013; Krzydlewska *et al.,* 2004; Fordham *et al.,* 1995). Therefore, understanding how E&L contributes to these impurities is crucial in risk assessment and management to ensure patient safety and therapeutic efficacy.

This study focuses on investigating elemental impurities in glass vials and rubber stoppers intended for hypertension drug product injections, specifically Hydralazine Hydrochloride for Injection. Employing ICP-MS as the primary analytical technique, we aim to develop a robust, sensitive, and validated method for the detection and quantification of elemental impurities. This technique offers high sensitivity and selectivity, making it suitable for the nuanced analysis required in pharmaceutical research (Hladik *et al.,* 2019; Luo *et al.,* 2021). In the subsequent sections, we present the materials and methods employed for the analysis, report our findings, and engage in a detailed discussion of the implications of elemental impurities in the context of hypertension drug product injections.

Based on the literature survey and review of elemental impurities in Hydralazine Hydrochloride for Injection, it was found that limited information exists on analytical methods specifically tailored for this drug product. The author could identify only limited sources of information regarding leachables analytical methods for Hydralazine Hydrochloride for Injection in USP General Chapters (USP ⟨232⟩, 2025; USP ⟨233⟩, 2025; USP ⟨1381⟩, 2024), publications (Van Hoecke *et al.,* 2013), journals (Krzydlewska *et al.,* 2004; Fordham *et al.,* 1995; Jenke, 2015; Ball *et al.,* 2018), and ICH Guidelines (ICH Q3B(R2), 2006; ICH Q3D, 2022). Most available literature pertains to packaging materials and the detection of elemental impurities in non-food packaging applications (Krzydlewska *et al.,* 2004). Using this foundational information, a new method for the determination of elemental impurities in Hydralazine Hydrochloride Injection by ICP-MS was developed. As no specific analytical methods were available for this particular drug product and its packaging system, there was a clear need for this study.

Elemental impurities may remain undetected due to their low concentrations in the ppm and ppt range during routine analysis (Stults *et al.,* 2015; Voss *et al.,* 2017; Udovic*.,* 2025). Hence, there is a critical need for a high-sensitivity method, and its validation using ICP-MS is an essential prerequisite for injectable hypertension products. The developed ICP-MS method can effectively detect elemental impurities present in the glass vials and rubber stoppers of Hydralazine Hydrochloride Injection. High blood pressure, or hypertension, increases the workload of the heart and arteries, and if it persists for a prolonged period, the heart and arteries may not function properly (USP ⟨1381⟩, 2024). Hydralazine hydrochloride is typically administered intravenously for the management of hypertension. Controlling elemental impurities in drug products and their packaging materials is therefore critical for ensuring patient safety and maintaining regulatory compliance (Jenke *et al.,* 2015). This study not only addresses current gaps in the literature but also provides valuable insights for pharmaceutical researchers, regulatory bodies, and manufacturers committed to ensuring the highest standards of pharmaceutical quality and safety.

## Materials and Methods

*Chemicals and reagents:*

Cadmium (Cd), Lead (Pb), Arsenic (As), Mercury (Hg), Cobalt (Co), Vanadium (V), Nickel (Ni), Thallium (Tl), Gold (Au), Palladium (Pd), Iridium (Ir), Osmium (Os), Rhodium (Rh), Ruthenium (Ru), Selenium (Se), Silver (Ag), Platinum (Pt), Lithium (Li), Antimony (Sb), Barium (Ba), Molybdenum (Mo), Copper (Cu), Tin (Sn), Chromium (Cr), Iron (Fe), Zinc (Zn), Boron (B), Aluminum (Al), Silicon （Si） Scandium (Sc), Germanium (Ge), Yttrium (Y), Rhenium (Re) and Bismuth (Bi). 1000µg/mL 34 individual elements standard solutions were provided as gift samples by Kindos Pharma Ltd and Hydralazine Hydrochloride Injection 20mg/mL for this study; 65% Nitric acid, Hydrochloric acid (36%-38%) were purchased from Merck.

*ICP-MS Apparatus and Conditions*:

ICP-MS was achieved on a Thermo iCAP RQ (Thermo Technologies). 100-1000 µL Pipette with RAININ L-1000XLS, 10-100 µL Pipette with Thermo 10-100 µL.

*ICP-MS Conditions*

***Table 1***: ICP-MS Instrument Conditions

| Elements | Mass | Channel | Dwell Time (s) | Spacing (u) | Measurement Mode | Internal Standard Element |
|---|---|---|---|---|---|---|
| Li | 7 | 1 | 0.05 | 0.1 | KED | $Sc^{45}$ |
| B | 11 | 1 | 0.05 | 0.1 | KED | $Sc^{45}$ |
| Al | 27 | 1 | 0.05 | 0.1 | KED | $Sc^{45}$ |
| Si | 28 | 1 | 0.05 | 0.1 | KED | $Sc^{45}$ |
| V | 51 | 1 | 0.05 | 0.1 | KED | $Sc^{45}$ |
| Cr | 52 | 1 | 0.05 | 0.1 | KED | $Sc^{45}$ |
| Fe | 56 | 1 | 0.05 | 0.1 | KED | $Sc^{45}$ |
| Co | 59 | 1 | 0.05 | 0.1 | KED | $Sc^{45}$ |
| Ni | 60 | 1 | 0.05 | 0.1 | KED | $Sc^{45}$ |
| Cu | 63 | 1 | 0.05 | 0.1 | KED | $Sc^{45}$ |
| Zn | 66 | 1 | 0.05 | 0.1 | KED | $Sc^{45}$ |
| As | 75 | 1 | 0.05 | 0.1 | KED | $Ge^{73}$ |
| Se | 78 | 1 | 0.05 | 0.1 | KED | $Ge^{73}$ |
| Mo | 95 | 1 | 0.05 | 0.1 | KED | $Y^{89}$ |
| Ru | 101 | 1 | 0.05 | 0.1 | KED | $Y^{89}$ |
| Rh | 103 | 1 | 0.05 | 0.1 | KED | $Y^{89}$ |
| Pd | 105 | 1 | 0.05 | 0.1 | KED | $Y^{89}$ |
| Ag | 107 | 1 | 0.05 | 0.1 | KED | $Y^{89}$ |
| Cd | 111 | 1 | 0.05 | 0.1 | KED | $Y^{89}$ |
| Sn | 118 | 1 | 0.05 | 0.1 | KED | $Y^{89}$ |
| Sb | 121 | 1 | 0.05 | 0.1 | KED | $Y^{89}$ |
| Ba | 137 | 1 | 0.05 | 0.1 | KED | $Y^{89}$ |
| Os | 189 | 1 | 0.05 | 0.1 | KED | $Re^{187}$ |
| Ir | 193 | 1 | 0.05 | 0.1 | KED | $Re^{187}$ |
| Pt | 195 | 1 | 0.05 | 0.1 | KED | $Re^{187}$ |
| Au | 197 | 1 | 0.05 | 0.1 | KED | $Re^{187}$ |
| Hg | 202 | 1 | 0.05 | 0.1 | KED | $Bi^{209}$ |
| Tl | 205 | 1 | 0.05 | 0.1 | KED | $Bi^{209}$ |
| Pb | 208 | 1 | 0.05 | 0.1 | KED | $Bi^{209}$ |

*Internal Standard stock solution:*

Transfer accurately 0.1 mL of 1000 µg/mL Scanium (Sc) standard solution, 0.1 mL of 1000 µg/mL Germanium (Ge) standard solution, 0.1 mL of 1000 µg/mL Yttrium (Y) standard solution, 0.1 mL of 1000 µg/mL Rhenium (Re) standard solution, 0.1 mL of 1000 µg/mL Bismuth (Bi) standard solution into a 100-mL volumetric flask, then dilute and make up to volume with dilute nitric acid mix well.

*Internal Standard solution*:

Transfer accurately 5.0 mL of internal standard stock solution into a 250-mL volumetric flask, then dilute and make up to volume with dilute nitric acid, mix well.

*Standard and Sample Preparations:*

Different solutions are prepared dilute nitric acid, diluent, 14 Elements mix standard stock solution (Cd, Hg, Pb, Co, Tl, V, Pd, Ir, Rh, Ru, Ag, Pt, As, Ni), 27 elements mix standard stock solution-1, 27 elements mix standard stock solution-2, Linearity solutions, Sample solution, Internal Standard stock solution, Internal Standard solution, and LOQ solution.

***Table 2:*** Preparation of Linearity Solutions

| Items | | Linearity solution-1 (20%) (LOQ) | Linearity solution-2 (50%) | Linearity solution-3 (80%) | Linearity solution-4 (100%) | Linearity solution-5 (120%) | Linearity solution-6 (150%) |
|---|---|---|---|---|---|---|---|
| 27 elements mix standard stock solution-2 (mL) as µg/L | | 0.2 | 0.5 | 0.8 | 1.0 | 1.2 | 1.5 |
| Li | µg/L | 25.000 | 62.500 | 100.000 | 125.000 | 150.000 | 187.500 |
| B | µg/L | 40.000 | 100.000 | 160.000 | 200.000 | 240.000 | 300.000 |
| Al | µg/L | 40.000 | 100.000 | 160.000 | 200.000 | 240.000 | 300.000 |
| Si | µg/L | 60.000 | 150.000 | 240.000 | 300.000 | 360.000 | 450.000 |
| V | µg/L | 1.000 | 2.500 | 4.000 | 5.000 | 6.000 | 7.500 |
| Cr | µg/L | 110.000 | 275.000 | 440.000 | 550.000 | 660.000 | 825.000 |
| Fe | µg/L | 130.000 | 325.000 | 520.000 | 650.000 | 780.000 | 975.000 |
| Co | µg/L | 0.500 | 1.250 | 2.000 | 2.500 | 3.000 | 3.750 |
| Ni | µg/L | 2.000 | 5.000 | 8.000 | 10.000 | 12.000 | 15.000 |
| Cu | µg/L | 30.000 | 75.000 | 120.000 | 150.000 | 180.000 | 225.000 |
| Zn | µg/L | 130.000 | 325.000 | 520.000 | 650.000 | 780.000 | 975.000 |
| As | µg/L | 1.500 | 3.750 | 6.000 | 7.500 | 9.000 | 11.250 |
| Se | µg/L | 8.000 | 20.000 | 32.000 | 40.000 | 48.000 | 60.000 |
| Mo | µg/L | 150.000 | 375.000 | 600.000 | 750.000 | 900.000 | 1125.000 |
| Ru | µg/L | 1.000 | 2.500 | 4.000 | 5.000 | 6.000 | 7.500 |
| Rh | µg/L | 1.000 | 2.500 | 4.000 | 5.000 | 6.000 | 7.500 |
| Pd | µg/L | 1.000 | 2.500 | 4.000 | 5.000 | 6.000 | 7.500 |
| Ag | µg/L | 1.500 | 3.750 | 6.000 | 7.500 | 9.000 | 11.250 |
| Cd | µg/L | 0.200 | 0.500 | 0.800 | 1.000 | 1.200 | 1.500 |
| Sn | µg/L | 60.000 | 150.000 | 240.000 | 300.000 | 360.000 | 450.000 |
| Sb | µg/L | 9.000 | 22.500 | 36.000 | 45.000 | 54.000 | 67.500 |
| Ba | µg/L | 70.000 | 175.000 | 280.000 | 350.000 | 420.000 | 525.000 |
| Ir | µg/L | 1.000 | 2.500 | 4.000 | 5.000 | 6.000 | 7.500 |
| Pt | µg/L | 1.000 | 2.500 | 4.000 | 5.000 | 6.000 | 7.500 |
| Hg | µg/L | 0.300 | 0.750 | 1.200 | 1.500 | 1.800 | 2.250 |
| Tl | µg/L | 0.800 | 2.000 | 3.200 | 4.000 | 4.800 | 6.000 |
| Pb | µg/L | 0.500 | 1.250 | 2.000 | 2.500 | 3.000 | 3.750 |

## Results

Validation of the method was done according to the International Conference on Harmonization guidelines (*ICH, Q2(R2), 2023)*. The method was validated for System suitability, specificity, accuracy and precision, linearity& range, and limit of quantification (LOQ).

*System suitability*

This parameter is evaluated by injecting diluent and linearity solutions and checking the standard solution. The check standard solution was analyzed after not more than ten injections and at the end of the sequence. The drift of concentration results of each element obtained from the check standard solution before and after the analysis of the sample solution were calculated. System suitability results are listed in Table 3. The concentration drift of each element in each post-check standard solution should be NMT ± 20%. The determination coefficient ($R^2$) of each element in this range should be NLT 0.990. The system suitability requirement was met for all experiments. The test results are summarized in Table 3.

***Table 3***: System Suitability

| Concentration Result of Check Solution（μg/L） | | | | Drift of Check Solution (%) | | Determination Coefficient($R^2$) |
|---|---|---|---|---|---|---|
| Name | Initial | 1 | 2 | 1 | 2 | |
| Li | 124.681 | 125.055 | 124.022 | 0 | -1 | 1.000 |
| B | 199.983 | 199.818 | 196.654 | 0 | -2 | 1.000 |
| Al | 200.035 | 200.806 | 198.497 | 0 | -1 | 1.000 |
| Si | 296.430 | 305.306 | 304.264 | 3 | 3 | 1.000 |
| V | 5.003 | 5.034 | 4.936 | 1 | -1 | 1.000 |
| Cr | 554.071 | 558.746 | 547.809 | 1 | -1 | 1.000 |
| Fe | 654.113 | 656.906 | 648.613 | 0 | -1 | 1.000 |
| Co | 2.497 | 2.499 | 2.464 | 0 | -1 | 1.000 |
| Ni | 10.024 | 10.060 | 9.880 | 0 | -1 | 1.000 |
| Cu | 149.872 | 150.543 | 147.951 | 0 | -1 | 1.000 |
| Zn | 649.582 | 654.765 | 642.098 | 1 | -1 | 1.000 |
| As | 7.527 | 7.554 | 7.501 | 0 | 0 | 1.000 |
| Se | 39.906 | 40.157 | 39.947 | 1 | 0 | 1.000 |
| Mo | 752.927 | 765.561 | 755.217 | 2 | 0 | 1.000 |
| Ru | 5.004 | 5.034 | 4.956 | 1 | -1 | 1.000 |
| Rh | 5.008 | 4.996 | 4.946 | 0 | -1 | 1.000 |
| Pd | 5.003 | 4.692 | 4.449 | -6 | -11 | 1.000 |
| Ag | 7.611 | 8.066 | 7.951 | 6 | 4 | 0.998 |
| Cd | 1.004 | 1.005 | 1.001 | 0 | 0 | 1.000 |
| Sn | 299.390 | 302.903 | 298.681 | 1 | 0 | 1.000 |
| Sb | 44.790 | 45.272 | 45.076 | 1 | 1 | 1.000 |
| Ba | 351.715 | 352.836 | 349.472 | 0 | -1 | 0.999 |
| Ir | 4.973 | 5.035 | 4.949 | 1 | 0 | 1.000 |
| Pt | 4.950 | 5.224 | 5.078 | 6 | 3 | 1.000 |
| Hg | 1.492 | 1.513 | 1.487 | 1 | 0 | 1.000 |
| Tl | 4.011 | 4.034 | 3.975 | 1 | -1 | 1.000 |
| Pb | 2.510 | 2.538 | 2.505 | 1 | 0 | 1.000 |

*Specificity*

To assess this specificity parameter, a blank solution and an LOQ (Limit of Quantitation) solution were analyzed. The measured concentration of each element in the diluent did not exceed the LOQ solution, confirming compliance with the specificity requirements. Specificity results are summarized in Table 4.

***Table 4:*** Specificity Results

| Elements | The Measured Concentration in LOQ (µg/L) | The Measured Concentration in Diluent (µg/L) |
|---|---|---|
| Li | 27.016 | 1.082 |
| B | 42.765 | 1.268 |
| Al | 41.241 | -0.814 |
| Si | 60.350 | -2.020 |
| V | 1.026 | 0.001 |
| Cr | 116.007 | 2.704 |
| Fe | 136.669 | 2.182 |
| Co | 0.509 | -0.010 |
| Ni | 2.045 | -0.036 |
| Cu | 32.968 | -0.810 |
| Zn | 136.702 | -1.241 |
| As | 1.572 | -0.015 |
| Se | 8.405 | -0.045 |
| Mo | 157.964 | 3.165 |
| Ru | 1.034 | -0.009 |
| Rh | 1.034 | -0.007 |
| Pd | 1.021 | -0.009 |
| Ag | 1.547 | -0.137 |
| Cd | 0.208 | 0.001 |
| Sn | 65.827 | 1.544 |
| Sb | 9.542 | 0.347 |
| Ba | 76.233 | 2.856 |
| Ir | 1.039 | 0.015 |
| Pt | 1.076 | 0.000 |
| Hg | 0.311 | 0.003 |
| Tl | 0.843 | -0.003 |
| Pb | 0.520 | 0.001 |

### *Linearity and Range*

The calibration curve was generated using ICP-MS software, with linearity confirmed by a determination coefficient ($R^2$). The validated range was assessed for the following elements: Lithium (Li), Boron (B), Aluminum (Al), Silicon (Si), Vanadium (V), Chromium (Cr), Iron (Fe), Cobalt (Co), Nickel (Ni), Copper (Cu), Zinc (Zn), Arsenic (As), Selenium (Se), Molybdenum (Mo), Ruthenium (Ru), Rhodium (Rh), Palladium (Pd), Silver (Ag), Cadmium (Cd), Tin (Sn), Antimony (Sb), Barium (Ba), Iridium (Ir), Platinum (Pt), Mercury (Hg), Thallium (Tl), Lead (Pb). Linearity & Range results are represented in table 5.

***Table 5***: Linearity & Range

| Name | Linear Curve Equation | Determination Coefficient $R^2$ | Slope | Y-axis intercept | Linear Range (µg/L) |
|---|---|---|---|---|---|
| Li | f(x)=463.3383*x-462.7094 | 1.000 | 463.3383 | -462.7094 | 25.000-187.500 |
| B | f(x)=218.1080*x+405.3750 | 1.000 | 218.1080 | 405.3750 | 40.000-300.000 |
| Al | f(x)=823.4965*x+1232.7956 | 1.000 | 823.4965 | 1232.7956 | 40.000-300.000 |
| Si | f(x)=381.8401*x+34863.9377 | 1.000 | 381.8401 | 34863.9377 | 60.000-450.000 |
| V | f(x)=22903.1047*x+2379.5513 | 1.000 | 22903.1047 | 2379.5513 | 1.000-7.500 |
| Cr | f(x)=28979.7308*x-62012.8769 | 1.000 | 28979.7308 | -62012.8769 | 110.000-825.000 |
| Fe | f(x)=31455.0810*x-25506.3780 | 1.000 | 31455.0810 | -25506.3780 | 130.000-975.000 |
| Co | f(x)=56755.5580*x+901.1890 | 1.000 | 56755.5580 | 901.1890 | 0.500-3.750 |
| Ni | f(x)=14602.7747*x+4466.7579 | 1.000 | 14602.7747 | 4466.7579 | 2.000-15.000 |
| Cu | f(x)=35191.1956*x+33416.0667 | 1.000 | 35191.1956 | 33416.0667 | 30.000-225.000 |
| Zn | f(x)=7653.4005*x+19459.8871 | 1.000 | 7653.4005 | 19459.8871 | 130.000-975.000 |
| As | f(x)=4288.6273*x+192.7212 | 1.000 | 4288.6273 | 192.7212 | 1.500-11.250 |
| Se | f(x)=524.3086*x+57.9286 | 1.000 | 524.3086 | 57.9286 | 8.000-60.000 |
| Mo | f(x)=21759.6253*x-67497.0296 | 1.000 | 21759.6253 | -67497.0296 | 150.000-1125.000 |
| Ru | f(x)=30073.8087*x+318.8283 | 1.000 | 30073.8087 | 318.8283 | 1.000-7.500 |
| Rh | f(x)=171340.9255*x+1342.0968 | 1.000 | 171340.9255 | 1342.0968 | 1.000-7.500 |

| | | | | | |
|---|---|---|---|---|---|
| Pd | f(x)=34160.1676*x+1752.6465 | 1.000 | 34160.1676 | 1752.6465 | 1.000-7.500 |
| Ag | f(x)=95482.6854* x+35261.9991 | 0.998 | 95482.6854 | 35261.9991 | 1.500-11.250 |
| Cd | f(x)=17630.0555*x-17.4653 | 1.000 | 17630.0555 | -17.4653 | 0.200-1.500 |
| Sn | f(x)=30408.2484*x-44940.8905 | 1.000 | 30408.2484 | -44940.8905 | 60.000-450.000 |
| Sb | f(x)=33612.4788*x-11516.4867 | 1.000 | 33612.4788 | -11516.4867 | 9.000-67.500 |
| Ba | f(x)=14351.8864*x-40161.2839 | 0.999 | 14351.8864 | -40161.2839 | 70.000-525.000 |
| Ir | f(x)=134051.6297*x+1050.6358 | 1.000 | 134051.6297 | 1050.6358 | 1.000-7.500 |
| Pt | f(x)=40400.6779*x+210.1772 | 1.000 | 40400.6779 | 210.1772 | 1.000-7.500 |
| Hg | f(x)=17581.7234*x+67.5509 | 1.000 | 17581.7234 | 67.5509 | 0.300-2.250 |
| Tl | f(x)=172893.4603*x+1042.1701 | 1.000 | 172893.4603 | 1042.1701 | 0.800-6.000 |
| Pb | f(x)=130232.4674*x+1894.1872 | 1.000 | 130232.4674 | 1894.1872 | 0.500-3.750 |

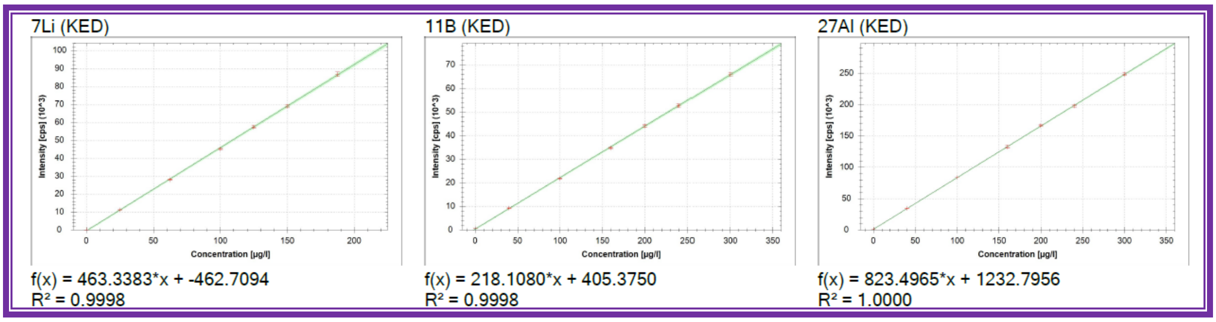

***Figure 1:** Linearity Curves of Li, B, and Al*

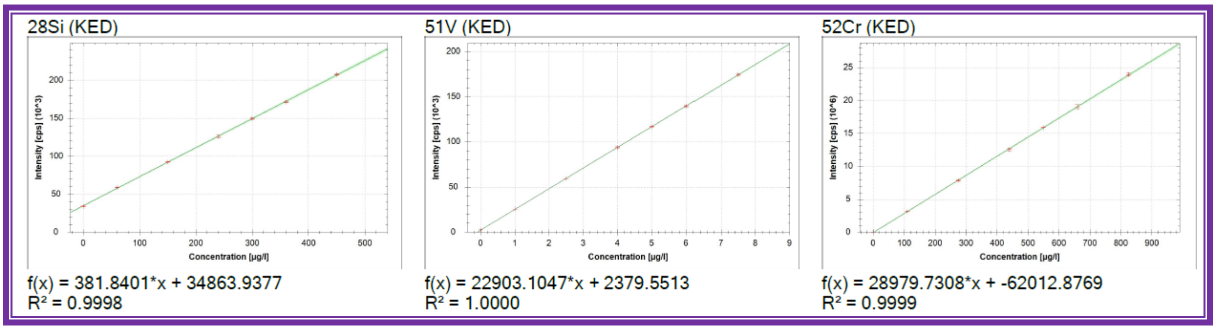

***Figure 2**: Linearity Curves of Si, V, and Cr*

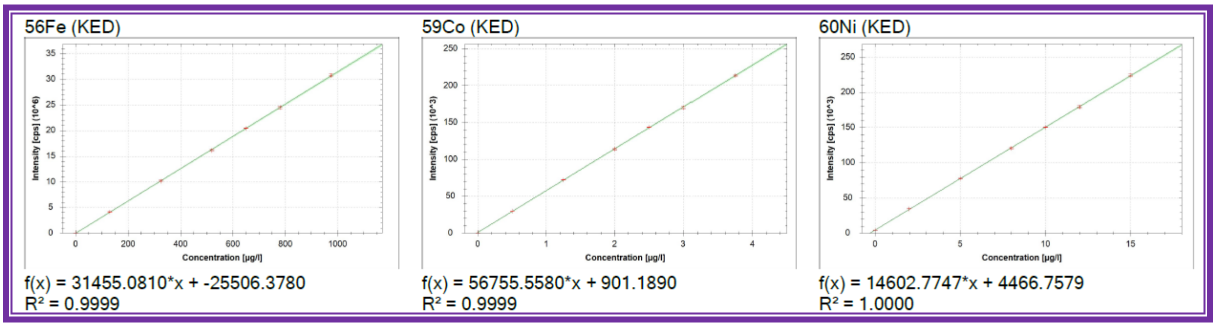

***Figure 3:** Linearity Curves of Fe, Co, and Ni*

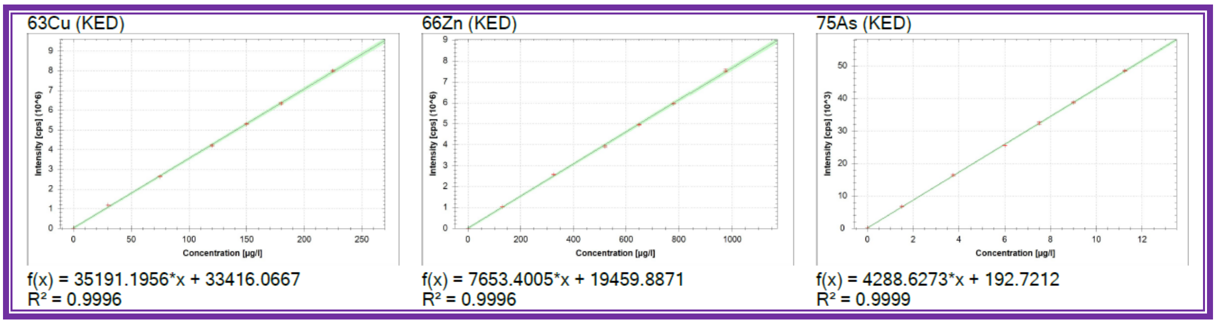

***Figure 4:** Linearity Curves of Cu, Zn, and As*

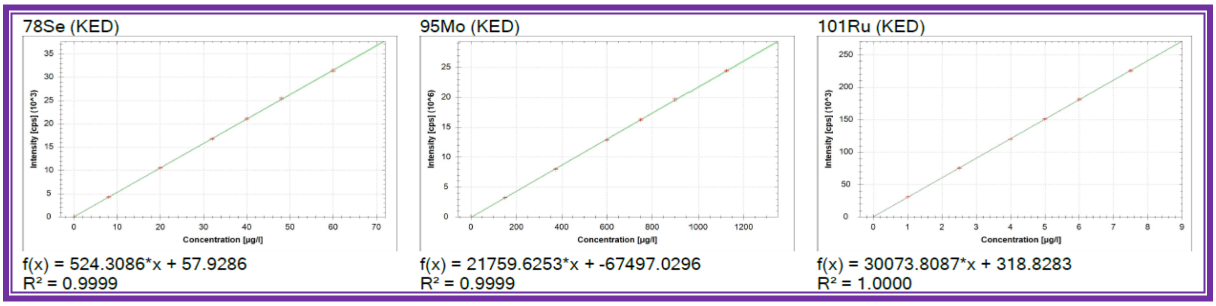

**Figure 5:** *Linearity Curves of Se, Mo, and Ru*

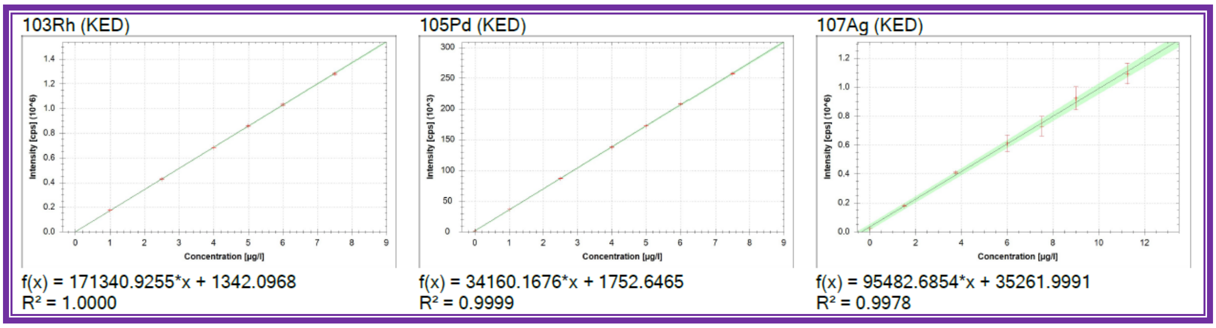

**Figure 6:** *Linearity Curves of Rh, Pd, and Ag*

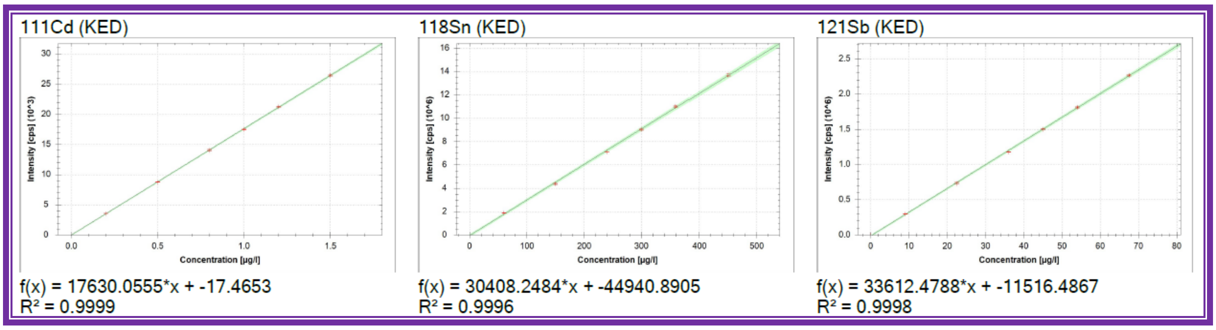

**Figure 7:** *Linearity Curves of Cd, Sn, and Sb*

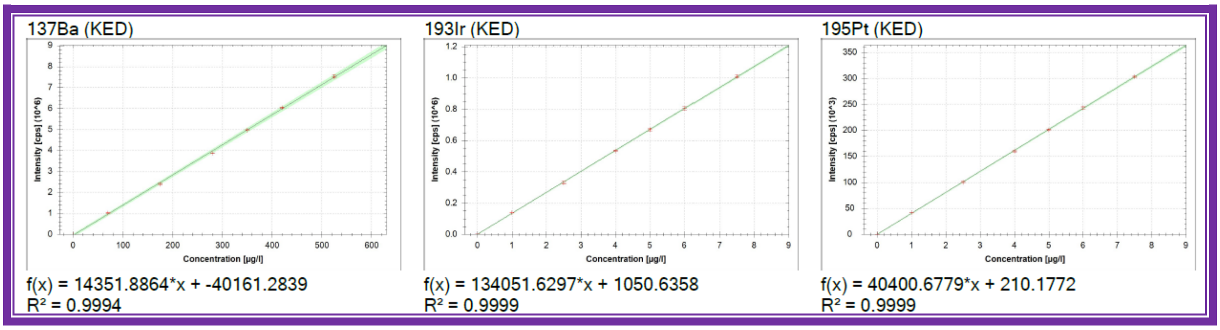

**Figure 8:** *Linearity Curves of Ba, Ir, and Pt*

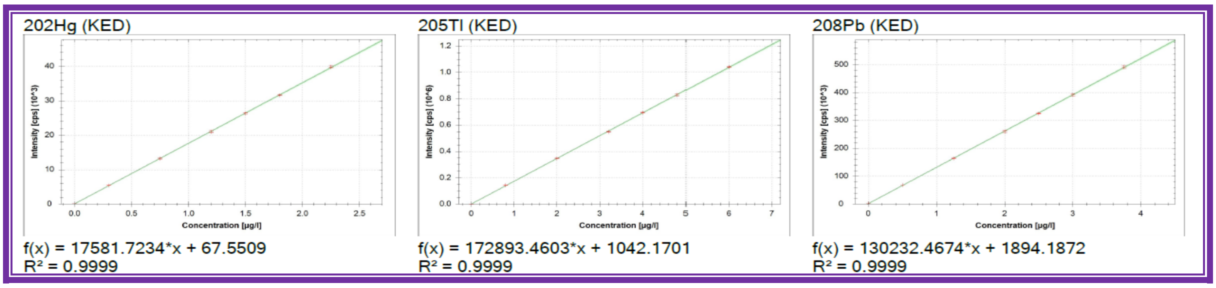

**Figure 9:** *Linearity Curves of Hg, Ti, and Pb*

### *Accuracy*

Three levels of accuracy solutions were analyzed (each level 3 times) to evaluate the accuracy of the method. The spike %recovery of each level for each element was calculated. The mean and relative standard deviation were calculated. Accuracy results, see Table 6.

***Table 6:*** Accuracy Results

| S. No | %Accuracy-1 | | | %Accuracy-2 | | | %Accuracy-3 | | | Mean | %RSD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Elements | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | | |
| Li | 98 | 97 | 99 | 98 | 98 | 99 | 100 | 100 | 100 | 99 | 1 |
| B | 98 | 97 | 98 | 98 | 99 | 98 | 98 | 99 | 99 | 98 | 1 |
| Al | 100 | 100 | 100 | 100 | 99 | 100 | 100 | 101 | 101 | 100 | 1 |
| Si | 99 | 99 | 102 | 99 | 99 | 100 | 101 | 101 | 101 | 100 | 1 |
| V | 100 | 100 | 100 | 101 | 100 | 100 | 100 | 101 | 101 | 100 | 1 |
| Cr | 100 | 100 | 101 | 100 | 100 | 100 | 100 | 102 | 100 | 100 | 1 |
| Fe | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 101 | 1 |
| Co | 100 | 100 | 101 | 99 | 100 | 100 | 100 | 101 | 101 | 100 | 1 |
| Ni | 99 | 99 | 90 | 94 | 94 | 94 | 96 | 97 | 96 | 96 | 3 |
| Cu | 100 | 99 | 101 | 101 | 101 | 101 | 101 | 102 | 102 | 101 | 1 |
| Zn | 103 | 103 | 99 | 97 | 97 | 98 | 99 | 100 | 100 | 104 | 1 |
| As | 104 | 104 | 103 | 103 | 102 | 103 | 104 | 104 | 106 | 104 | 1 |
| Se | 106 | 106 | 106 | 108 | 106 | 106 | 106 | 106 | 108 | 107 | 1 |
| Mo | 100 | 100 | 101 | 101 | 100 | 101 | 101 | 102 | 103 | 101 | 1 |
| Ru | 101 | 100 | 101 | 101 | 100 | 101 | 100 | 101 | 102 | 101 | 1 |
| Rh | 101 | 100 | 101 | 100 | 100 | 101 | 100 | 100 | 101 | 101 | 1 |
| Pd | 98 | 97 | 97 | 96 | 96 | 96 | 96 | 97 | 98 | 97 | 1 |
| Ag | 98 | 106 | 108 | 101 | 91 | 97 | 98 | 99 | 108 | 101 | 5 |
| Cd | 100 | 100 | 100 | 99 | 99 | 100 | 100 | 99 | 102 | 100 | 1 |
| Sn | 99 | 98 | 100 | 99 | 99 | 99 | 100 | 100 | 102 | 100 | 1 |
| Sb | 98 | 98 | 99 | 98 | 98 | 99 | 99 | 100 | 102 | 99 | 1 |
| Ba | 102 | 102 | 102 | 99 | 99 | 100 | 100 | 101 | 103 | 101 | 1 |
| Ir | 99 | 98 | 99 | 98 | 98 | 99 | 98 | 99 | 101 | 99 | 1 |
| Pt | 92 | 92 | 93 | 91 | 90 | 92 | 87 | 89 | 84 | 90 | 3 |
| Hg | 99 | 98 | 100 | 99 | 99 | 100 | 99 | 101 | 102 | 100 | 1 |
| Tl | 99 | 100 | 101 | 101 | 100 | 101 | 100 | 102 | 104 | 101 | 1 |
| Pb | 96 | 97 | 90 | 94 | 94 | 95 | 96 | 97 | 100 | 95 | 3 |

*Preparation of accuracy solutions*:

Accuracy solution I (In triplicate): Accurately transfer 0.1mL Hydralazine Hydrochloride Injection, 20mg/mL, and 0.5 mL 27 elements mix standard stock solution-2 into a 10-mL volumetric flask, then dilute and make up to volume with diluent and mix well.

Accuracy solution II (In triplicate): Accurately transfer 0.1mL Hydralazine Hydrochloride Injection, 20mg/mL, and 1.0 mL 27 elements mix standard stock solution-2 into a 10-mL volumetric flask, then dilute and make up to volume with diluent and mix well.

Accuracy solution III (In triplicate): Accurately transfer 0.1mL Hydralazine Hydrochloride Injection, 20mg/mL, and 1.5 mL 27 elements mix standard stock solution-2 into a 10-mL volumetric flask, then dilute and make up to volume with diluent and mix well.

The recovery of each spike element in each accuracy solution should be 70%-150%, and the %RSD of the nine recovery results of each spike element should be no more than 20%.

### *Repeatability*

Repeatability was evaluated by analyzing six preparations of repeatability solutions. Calculate the percentage recovery of each element in six repeatability solutions and calculate the Mean and relative standard deviations of the % recovery of six results for each element.

Repeatability solution: Accurately transfer 0.1mL Hydralazine Hydrochloride Injection, 20mg/mL, and 1.0 mL 27 elements mix standard stock solution-2 into a 10-mL volumetric flask, then dilute and make up to volume with diluent and mix well. For the repeatability results, see Table 7.

The recovery of the spike element in each repeatability solution should be 70%-150%, and the %RSD of the six recovery results of each spike element should be no more than 20%.

***Table 7***: Repeatability Results

| Elements | % Recovery for Six Samples | | | | | | Mean | %RSD |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | | |
| Li | 99 | 99 | 99 | 98 | 99 | 98 | 99 | 0 |
| B | 98 | 98 | 98 | 97 | 98 | 97 | 98 | 1 |
| Al | 100 | 99 | 100 | 99 | 101 | 99 | 100 | 1 |
| Si | 102 | 102 | 102 | 100 | 103 | 102 | 102 | 1 |
| V | 100 | 99 | 100 | 99 | 101 | 99 | 100 | 1 |
| Cr | 100 | 100 | 100 | 99 | 101 | 100 | 100 | 1 |
| Fe | 100 | 99 | 100 | 98 | 101 | 99 | 100 | 1 |
| Co | 100 | 99 | 100 | 99 | 101 | 99 | 99 | 1 |
| Ni | 95 | 94 | 95 | 95 | 99 | 95 | 95 | 2 |
| Cu | 100 | 100 | 101 | 100 | 101 | 100 | 100 | 0 |
| Zn | 98 | 97 | 98 | 96 | 98 | 98 | 97 | 1 |
| As | 105 | 103 | 103 | 103 | 104 | 103 | 104 | 1 |
| Se | 106 | 106 | 106 | 105 | 108 | 106 | 106 | 1 |
| Mo | 101 | 101 | 101 | 101 | 101 | 100 | 101 | 1 |
| Ru | 101 | 100 | 101 | 100 | 100 | 99 | 100 | 1 |
| Rh | 101 | 100 | 100 | 100 | 100 | 99 | 100 | 1 |
| Pd | 98 | 96 | 96 | 95 | 95 | 94 | 96 | 1 |
| Ag | 107 | 95 | 102 | 106 | 102 | 106 | 103 | 4 |
| Cd | 100 | 100 | 101 | 99 | 100 | 99 | 100 | 1 |
| Sn | 100 | 99 | 100 | 99 | 100 | 99 | 99 | 1 |
| Sb | 99 | 99 | 99 | 98 | 99 | 98 | 99 | 0 |
| Ba | 99 | 99 | 100 | 99 | 100 | 98 | 99 | 1 |
| Ir | 98 | 98 | 98 | 98 | 99 | 98 | 98 | 1 |
| Pt | 93 | 92 | 91 | 92 | 93 | 93 | 93 | 1 |
| Hg | 100 | 99 | 99 | 98 | 100 | 98 | 99 | 1 |
| Tl | 101 | 99 | 100 | 99 | 101 | 99 | 100 | 1 |
| Pb | 95 | 94 | 96 | 94 | 95 | 96 | 95 | 1 |

## Discussion

The present study was undertaken to develop and validate a robust analytical method for the quantification of elemental impurities in Hydralazine Hydrochloride for Injection, a critical prerequisite for ensuring patient safety and adhering to stringent international regulatory standards, including *ICH Q3D (2022)* and *USP ⟨233⟩ (2025).* While regulatory guidelines provide the framework for validation, a comprehensive, fully validated ICP-MS methodology specifically tailored for this parenteral antihypertensive drug has not been previously reported. The validation results confirmed that the proposed ICP-MS method is reliable, reproducible, and suitable for its intended purpose in routine quality control, thereby addressing this notable gap in the pharmaceutical literature.

The validation parameters confirmed the stability and selectivity of the analytical system. System suitability data demonstrated excellent instrumental stability, with concentration drifts remaining well within the acceptable ±20% limit, thereby ensuring data integrity throughout the analytical runs. Specificity testing further substantiated the method's reliability by confirming the absence of interfering signals from the diluent or sample matrix at the mass-to-charge ratios of the target analytes. This high degree of selectivity is particularly crucial for pharmaceutical injectables, where complex excipients and formulation components can potentially lead to matrix-induced interferences or signal suppression if not properly evaluated *(*Hladik *et al., 2019).* The method subsequently exhibited exceptional linearity across the calibration range, with correlation coefficients (R²) consistently exceeding 0.998 for all elements under investigation. This wide linear dynamic range, spanning concentrations from trace (ppb) levels to higher levels, is essential for the accurate quantification of elemental impurities relative to the stringent Permitted Daily Exposure (PDE) limits established for parenteral products.

The accuracy and precision of the method were comprehensively evaluated through recovery studies and repeatability testing. The mean recoveries for most elements fell within the acceptable range of 90–110%, aligning with regulatory guidelines. However, the observed recoveries for elements such as Nickel (Ni), Silver (Ag), and Platinum (Pt) were marginally lower (90–96%), though still within the acceptable limit of 70–150% at the specified concentration levels. This phenomenon is consistent with the existing literature, which has documented the analytical challenges associated with quantifying noble metals such as Pt and Pd. These challenges are often attributed to their complex solution chemistry, including the formation of stable complexes or memory effects within the ICP-MS sample introduction system *(*Van Hoecke *et al., 2013;* Korbi *et al., 2025;* Harrington *et al., 2025;* Kakuk *et al., 2025).* Furthermore, the method demonstrated high precision, with relative standard deviations (%RSD) for repeatability below 4% for all elements. The slightly elevated variability observed for Ag, while still acceptable, suggests that its quantification may benefit from further optimization, such as the inclusion of a specific stabilizing agent or a dedicated rinse protocol to mitigate adsorption or memory effects.

### *Limitations and Future Scope*

Despite the successful validation of this method, certain limitations must be acknowledged. The primary limitation is the method's current specificity to Hydralazine Hydrochloride for Injection; its applicability to other drug matrices, particularly those with different excipient profiles or dosage forms, would require re-validation to account for potential matrix effects. Additionally, while ICP-MS offers unparalleled sensitivity, it is susceptible to specific polyatomic interferences (e.g., from chloride in the formulation), which were mitigated but not entirely eliminated in this study. Furthermore, the high instrumentation costs and specialized technical expertise requirements may limit the widespread adoption of this technique across all quality control environments. Consequently, future work should focus on expanding the validated method to a broader range of high-risk parenteral drug products to establish a platform approach for elemental impurities testing. Given the increasing regulatory emphasis on packaging-derived contaminants, as highlighted by the draft ICH Q3E guideline for extractables and leachables, subsequent studies should also adapt this ICP-MS method to investigate the potential migration of elemental leachables from the primary container system (e.g., glass vials and rubber stoppers) over the product's shelf-life. Finally, further optimization of the analytical conditions for inherently challenging elements, particularly Ag and Pt, remains a worthwhile pursuit to enhance method robustness.

## Conclusion

The present study successfully developed and validated a robust, sensitive, and reliable Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) method for the quantitative determination of elemental impurities in Hydralazine Hydrochloride for Injection, in full compliance with ICH Q3D and USP <232>/<233> guidelines. The method demonstrated excellent system suitability, specificity, linearity and precision, with recovery values consistently within the acceptable range. The high sensitivity of ICP-MS enabled detection of trace metals at ppb/ppt levels, which is essential for ensuring the safety of parenteral formulations and meeting stringent regulatory requirements.

While the method performed robustly for most target elements, the relatively lower recoveries observed for Nickel (Ni), Silver (Ag), and Platinum (Pt) indicate a need for further optimization, potentially through modified digestion protocols or the use of specialized stabilizing agents. Nevertheless, the validation data confirms that the method is suitable for its intended purpose, including routine quality control and batch release testing.

This work holds significant practical value for pharmaceutical quality control laboratories, offering a high-throughput, multi-element analytical solution that addresses the critical safety requirements specific to injectable antihypertensive therapies. The novelty of this study lies in its systematic optimization and validation tailored to overcome the unique matrix challenges of Hydralazine Hydrochloride for Injection, establishing a benchmark for elemental impurities testing similar parenteral formulations.

Future research should focus on extending the applicability of this method to other high-risk drug products and investigating the migration of elemental leachates from primary packaging materials over the product shelf-life, in alignment with emerging regulatory guidance such as the draft ICH Q3E.

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### Conflict of interest

The authors declare that they have no conflicts of interest among the authors.

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