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APPLICATION OF INDUCTIVELY COUPLED PLASMA–MASS SPECTROMETRY (ICP-MS) IN THE ANALYSIS OF RASA SHASTRA FORMULATIONS

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ABSTRACT

Inductively Coupled Plasma–Mass Spectrometry (ICP-MS) has emerged as a highly sensitive analytical tool for evaluating Ayurvedic herbo-mineral formulations, particularly Rasa Shastra preparations. This review highlights its application in detecting and quantifying trace and toxic metals such as mercury, arsenic, cadmium, and lead at parts-per-trillion levels, ensuring safety and authenticity of Bhasma and Kupipakwa formulations. ICP-MS enables multi-elemental profiling, supporting both standardization and validation of classical purification processes like Shodhana and Marana. Comparative studies demonstrate its superiority over conventional techniques such as AAS and ICP-OES in sensitivity and detection limits. While ICP-MS provides elemental fingerprints crucial for authentication and regulatory acceptance, limitations include lack of speciation data, matrix interferences, and high operational costs. Overall, ICP-MS bridges traditional Rasashastra principles with modern analytical science, reinforcing evidence-based Ayurveda and enhancing global credibility of Ayurvedic pharmaceutics. **Introduction:** Ayurveda employs its own well-defined qualitative and quantitative parameters for the evaluation of drugs and formulations, which are primarily based on classical principles such as *Grahyatva*, *Guna*, *Karma*, and *Pariksha*. Among these, metallic and mineral-based Ayurvedic preparations, collectively known as *Rasa Bhasma*, require special attention due to their complex composition and potent therapeutic nature. Before clinical administration, it is essential to establish their authenticity, safety, and quality through systematic assessment of their physical and chemical characteristics. Traditional *Bhasma Pariksha* methods described in classical texts provide valuable insights into the completion of the processing and transformation of raw materials. However, to achieve a deeper and more objective understanding of the physicochemical changes occurring during various pharmaceutical procedures such as *Shodhana* and *Marana*, the integration of modern analytical techniques has become indispensable. The combined application of ancient and contemporary methods allows for scientific validation of Ayurvedic formulations while preserving their classical foundation.[1] ICP-MS mostly uses a quadrupole mass spectrometer, which can quickly and continuously measure the mass of different elements. Currently, it can be used to analyse more than 70 elements. The detection limit of ICP-MS for more than 70 elements in the solution is one trillion or less, and the linear dynamic range can reach nine orders of magnitude. ICP-MS is an inorganic multi-element analysis technology with inductively coupled plasma as an ion source. and mass spectrometry in the field of analytical chemistry in the early 1980s. In 1980, published the first article on the feasibility of ICP-MS was published, and the first commercial instrument came out 3years later. So far, there are about 20 types of ICP-MS instruments commercialised worldwide. In the field of drug element analysis and safety monitoring, ICP-MS and its combined technology are also increasingly widely used, which seems to have become a common and mature analysis and detection means.[2] ICP-MS is a powerful technique used to measure trace elements in a sample with high precision. It is particularly useful in detecting and quantifying metals such as lead, mercury, arsenic, and other heavy metals, which are commonly found in raw materials before they undergo purification processes.

KEYWORDS: icp-ms, herbo-mineral, ayurveda, standardization etc.

MATERIAL AND METHODS

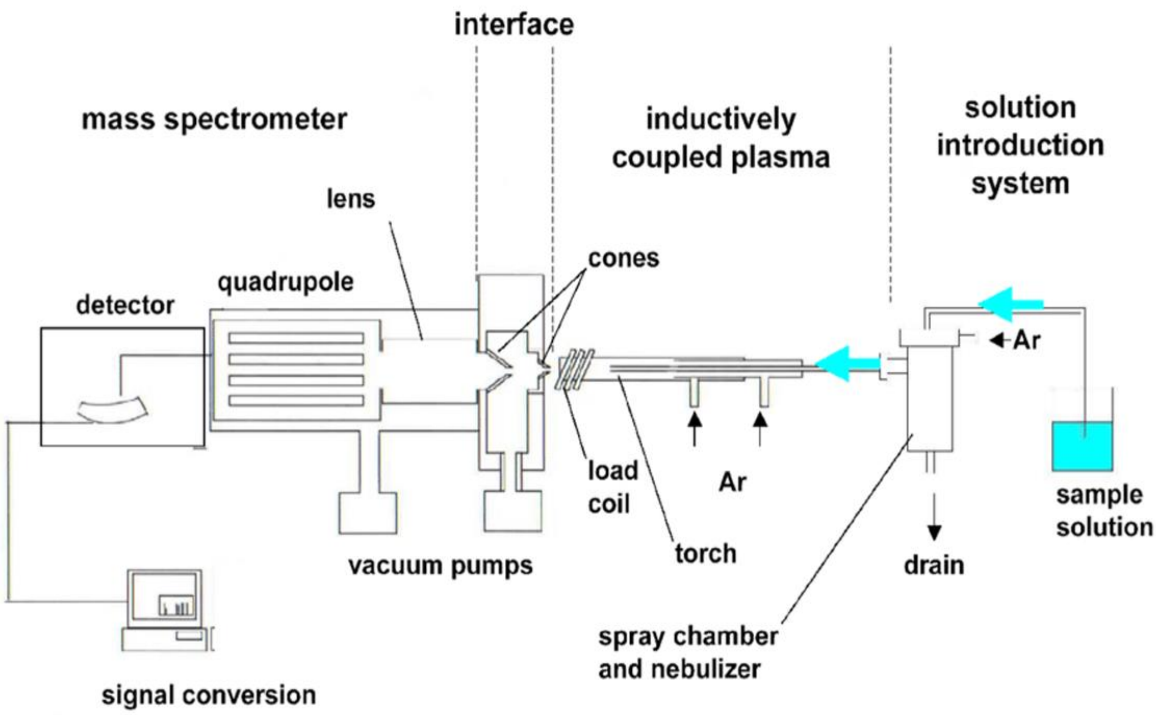
The present study is a narrative review based on published research articles describing the Applications of Inductively Coupled Plasma–Mass Spectrometry (ICP-MS) in Ayurvedic drug analysis. Relevant literature was sourced from peer-reviewed journals such as world journal of pharmaceutical and medical research, International Journal of Ayurvedic Medicine,journal of ayurveda and integrated medical sciences, Elsevier, Scholars International Journal of Traditional and Complementary Medicine,international ayurvedic medical journal, journalofmass spectrometry,African journal of biomedical research, International Journal of Research in AYUSH, Journal of ayurveda, International Journal of Research in AYUSH and Pharmaceutical Sciences (IJRAPS) etc.ICP-MS studies on single herbal drugs, polyherbal formulations, herbo-mineral preparations, and Bhasma were included, with interpretation based on established ICP-MS principles.

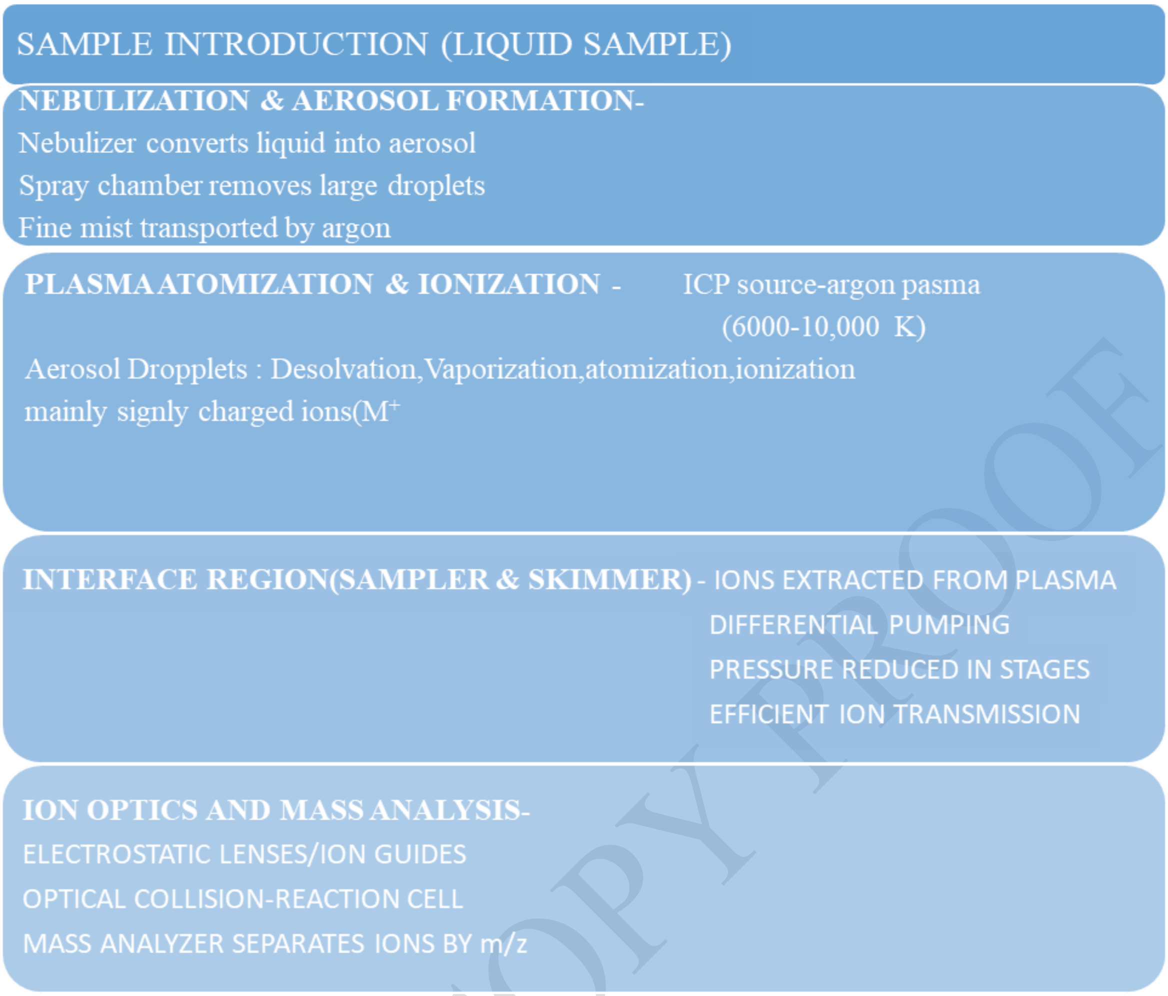
Principle and working of the technique

ICP-MS provides elemental concentrations and isotope ratios with parts-per-trillion (ppt) level detection limits for many elements. The technique's power derives from separating to the sample's conversion into ions (via a high-temperature plasma) and then measuring those ions by mass spectrometry, giving elemental specificity and high sensitivity.[3]

Working Mechanism

1. **Sample introduction and aerosol formation-** Liquid samples are most commonly introduced through a nebuliser that generates an aerosol; a spray chamber removes large droplets, producing a fine mist carried to the plasma.
2. **Plasma atomization and ionisation (the ICP source)-** An argon plasma (~6000–10,000 K in the outer regions) is sustained by an RF coil around a quartz torch. When aerosol droplets enter the plasma, they are rapidly desolvated, atomised, and ionised. Ionisation occurs primarily by electron impact and charge transfer in the hot plasma; most elements form singly charged ions (M^+), which are then sampled into the mass spectrometer. Argon is used for its inertness and favourable ionisation characteristics (high first ionisation energy), which help maintain the sample ions once formed.
3. **Interface: sampling cone(s) and differential pumping-** Ions (and some neutral gas) exit the plasma through a sampler cone into a region of reduced pressure, then pass through a skimmer cone into the high-vacuum region. This interface performs gas-phase sampling and reduces pressure in stages so that a mass analyzer operating at high vacuum can accept the ion beam. The shape, alignment, and cleanliness of cones strongly influence transmission efficiency and background.
4. **Ion optics and mass analysis-** After the skimmer, the extracted ions are focused by ion optics (electrostatic lenses, ion guides) and may pass through collision/reaction cells (see below) before entering a mass analyser.(3)
5. **Detection and quantitation-**Ions are detected typically by electron multipliers or Faraday cups. Measured ion counts are converted to elemental concentrations via calibration with standards.





ADVANTAGES

ICP-MS offers ultra-trace elemental detection at parts-per-billion to parts-per-trillion levels, enabling the accurate quantification of toxic metals such as mercury, arsenic, lead, and cadmium. This high sensitivity is crucial for the safety assessment of Rasa Aushadhi, where metallic constituents must remain within permissible limits to ensure patient safety.

The technique allows simultaneous multi-element analysis in a single analytical run, facilitating the comprehensive evaluation of more than seventy elements. This capability is particularly valuable for the standardisation of Bhasma preparations, as it enables the identification of both major elements (such as iron, calcium, and zinc) and trace elements that may contribute to therapeutic efficacy.

Compared to conventional techniques like Atomic Absorption Spectroscopy (AAS) and Inductively Coupled Plasma–Optical Emission Spectroscopy (ICP-OES), ICP-MS demonstrates significantly superior sensitivity and lower detection limits, making it especially suitable for trace and ultra-trace elemental analysis in complex Ayurvedic formulations.

ICP-MS also supports the modern scientific validation of classical Ras Shastra concepts by correlating traditional qualitative descriptions—such as *nirdhūma* and *niruttha*—with quantitative elemental purity and safety data. This integration strengthens evidence-based Ayurveda and enhances regulatory acceptance at national and international levels.

Another advantage of ICP-MS is its minimal sample requirement, often requiring only milligram quantities of material, which is particularly beneficial when analyzing rare or precious formulations such as Swarna Bhasma.

Furthermore, ICP-MS is widely recognized and accepted by regulatory authorities, including the World Health Organization (WHO), the Ministry of AYUSH, the US Food and Drug Administration (USFDA), and the European Pharmacopoeia, for elemental impurity profiling and quality control.

DISADVANTAGES

ICP-MS measures only the total concentration of elements and does not provide information about their chemical form or biological availability. As a result, it cannot distinguish between elemental mercury and mercury sulfide (HgS) present in formulations such as

Kajjali or Rasa Sindūra, which is a critical consideration in Ras Shastra.

The technique requires acid digestion of the sample prior to analysis, which destroys the original physicochemical structure of the formulation. Consequently, traditional Ayurvedic processes such as Bhasmīkaraṇa and Saṃskāra cannot be directly assessed or interpreted using ICP-MS data alone.

ICP-MS analysis may also be affected by spectral and matrix interferences, particularly due to the formation of polyatomic ions. The complex herbal–mineral composition of Rasāuṣadhi can therefore interfere with accurate elemental quantification if not carefully controlled.

Additionally, ICP-MS is an expensive analytical technique that requires sophisticated instrumentation and trained technical personnel. This limits its availability and routine use in many traditional Ayurveda teaching and research institutions.

Compilation of Studies– Inductively Coupled Plasma Mass Spectrometry (ICP-MS) Purpose: ICP-MS is a highly sensitive and precise analytical technique used for the quantitative estimation of metals and trace elements.

It is especially effective for detecting toxic heavy metals such as **arsenic, cadmium, lead, and mercury** at very low concentrations, ensuring the safety and elemental purity of Ayurvedic herbo-mineral formulations.[5]

Elemental Analysis of Loha after Shodhana

Samanya Shodita Loha

The elemental analysis revealed **iron as the major constituent (99.28%)**, confirming the metallic purity of the sample.

Trace amounts of **carbon (0.57%), silica (0.12%), and manganese (0.03%)** were detected, while **sulphur was not detected**. This indicates that Samanya Shodhana introduces only minimal elemental impurities.

Vishesha Shodita Loha

The analysis showed **iron content of 99.25%**, with trace levels of **carbon (0.61%), silica (0.12%), and manganese (0.02%)**. Sulphur was not detected. The results demonstrate that Vishesha Shodhana maintains elemental purity while slightly modifying trace element composition.[6]

Mercury-Based Formulation

ICP analysis indicated that mercury content was **98.3% (mg/kg)** in the tested sample, reflecting a high degree of purification.[1]

Chemical analysis revealed the presence of **49.50% mercury** and **45.50% sulphur**, confirming the formation of mercury sulphide through proper trituration.[7]

Mritasanjivan rasa

Two formulations (MRC and MRM) were analyzed.

- **MRC** contained **59.23% mercury and 11.43% sulphur**, with trace levels of **lead (4.13 ppm), cadmium (0.1 ppm), arsenic (9.42 ppm)**, and **boron (9366 ppm)**.
- **MRM** showed **52.05% mercury and 8.98% sulphur**, with reduced levels of **lead (1.88 ppm), arsenic (8.50 ppm)**, and **boron (7350 ppm)**.

These values indicate controlled heavy-metal presence within trace limits.[8]

Udayabhaskara Rasa

- ICP-MS analysis showed **copper as the major element**, with **iron present in trace amounts**. Elemental profiling revealed significant concentrations of **mercury (9.177%), arsenic (8.732%), and copper (11.596%)**, confirming the herbo-mineral nature of the formulation.[9]

Shwaskuthara Rasa

- ICP-MS detected **mercury (0.94 ppm)** and **arsenic (8.78 ppm)** in trace quantities. Major elemental composition included **carbon (31.24%), nitrogen (12.40%), oxygen (42.63%)**, along with minor amounts of **calcium (1.62%) and sodium (2.37%)**, suggesting effective detoxification of heavy metals.[10]

Samaguna Gandhaka Jarita Rasa Sindura

- ICP-MS and gravimetric analysis revealed **free mercury content of 95.31%** and **sulphur content of 12.96%**, indicating efficient sulphurization and stabilization of mercury.[11]

Sankhodara Rasa (ICP-MS Analysis)

- Analysis of four samples showed **high levels of calcium and sulphur**, while **mercury was below the detectable limit (BDL)** in all samples, confirming the absence of free mercury:[12]

S.No	SAMPLE IDENTIFICATION	SULPHUR MG/KG	CALCIUM (Ca) MG/KG	MERCURY(Hg) MG/KG
1	S1	32850.00	364000.00	BDL (Below detectable Level)
2	S2	172730.00	410000.00	BDL (Below detectable Level)
3	S3	35360.00	380000.00	BDL (Below detectable Level)
4	S4	122990.00	380000.00	BDL (Below detectable Level)

Comparative Evaluation-

1. Consistent High Sensitivity of ICP-MS for Toxic Metals

Across all studies, ICP-MS demonstrated high sensitivity and accuracy in detecting metals and trace elements, particularly toxic heavy metals such as Hg, As, Cd, and Pb, even at very low concentrations (ppm or below detectable limits). This confirms its suitability for evaluating the safety and elemental integrity of Kupipakwa and herbo-mineral formulations.

2. Effect of Shodhana on Loha (Iron)

Both Samanya Shodita Loha and Vishesha Shodita Loha showed a very high iron content (~99%), indicating that the core metallic identity of Loha remains intact after purification.

Only trace amounts of carbon, silica, and manganese were detected.
Sulphur was not detected in both stages.

Vishesha Shodhana showed a slight reduction in manganese, suggesting improved refinement.Progressive Shodhana procedures enhance **elemental purity without introducing toxic impurities**, supporting classical claims of detoxification.

3. Mercury Stabilization in Kupipakwa and Rasa Preparations

Mercury-based formulations showed **high mercury content**, but in **stabilised or bound forms** rather than free toxic mercury.

Very high mercury percentages in formulations such as **Rasa Sindura (95.31%)** and Kupipakwa preparations indicate **effective fixation of mercury**.

Kajjali showed nearly equal proportions of mercury and sulphur, confirming **Hg–S bonding**.

Mercury is not eliminated but **chemically transformed into stable, therapeutically acceptable forms**.

4. Controlled Presence of Toxic Elements in Mritasanjivana Rasa

Both MRC and MRM variants showed:

- Moderate mercury and sulphur content
- **Lead, cadmium, and arsenic present only in trace (ppm) levels**
- Reduction of heavy metals in MRM compared to MRC

Suggests formulation-specific processing leads to **reduction and regulation of toxic elements**, rather than complete elimination.

5. Elemental Diversity in Udayabhaskara Rasa

ICP-MS revealed:

- **Copper as a major element**
- Mercury and arsenic present in measurable but controlled proportions
- Iron present only in traces

6. Minimal Heavy Metal Burden in Shwaskuthara Rasa

This formulation showed:

- **Mercury and arsenic in very low (ppm) concentrations**
- Predominance of **organic elements (C, N, O)**

Indicates effective detoxification and dominance of organic constituents, enhancing **biocompatibility**.

7. Complete Absence of Detectable Mercury in Sankhodara Rasa

All four samples of Sankhodara Rasa showed:

- **Mercury below detectable limits**
- High levels of **calcium and sulphur**

Significance and difference between AAS,ICP-OES,NMR

	BASIC PRINCIPLES	**SIGNIFICANCE**
ICP-OES(13)	High-temperature argon plasma excites atoms and ions, which emit characteristic wavelengths of light. Emission intensity is proportional to element concentration.	Simultaneous multi-element analysis with good accuracy for major, minor, and trace elements. Commonly used in pharmaceutical, environmental, and metallurgical laboratories.
AAS(14)	Free ground-state atoms absorb light of a specific wavelength. The amount of absorbed light is proportional to the concentration of the element.	Quantitative determination of individual metals such as Fe, Zn, Cu, Pb, Cd, and Hg. Widely used for routine elemental analysis.
NMR(15)	Nuclei absorb radiofrequency energy in a strong magnetic field. The resulting resonance provides information about molecular structure.	Used for structural elucidation, identification of compounds, purity assessment, stereochemistry, and metabolomics.

DISCUSSION

Rasashastra uses metals and minerals like mercury (Parada), gold (Swarna), silver (Rajata), arsenic, lead, etc. ICP-MS helps to quantify toxic and non-toxic metals, ensure metals are within permissible Ayurvedic limits, and confirm proper Shodhana and Marana processes. ICP-MS helps to detect unauthorised metals added intentionally or accidentally.

Identify excess toxic elements due to improper processing. Distinguish between genuine Bhasma and fake or poorly prepared products. For example, excess lead in Naga Bhasma indicates improper Marana.Presence of cadmium → possible industrial contamination.

ICP-MS provides an elemental profile (fingerprint) of a formulation. This fingerprint helps in: Authentication of Rasashastra drugs, Comparing classical vs marketed samples, Supporting pharmacopoeial monographs.

Limitation: ICP-MS gives elemental data, not a complete chemical structure, and there is a strong need for: Standard operating protocols (SOPs) for ICP-MS analysis, Certified reference standards for Bhasmas and Rasoushadhis, Integration of classical Rasashastra principles with modern analytics, Collaboration with: API (Ayurvedic Pharmacopoeia of India), CCRAS, Ministry of AYUSH, Academic and research laboratories.

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