RESEARCH PAPER

Spectroscopic and Instrumental Characterization of Abhrasindoora: A Sublimated Mercurial Preparation

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ABSTRACT

Introduction

Abhrasindoora (AS) is a Kupipakwa Rasayana (Sublimated mercurial formulation) which is one of the dosage forms of Chaturvidha Rasayana and it deals by Rasashastra (Iatrochemistry). Therapeutic applications of AS are well-documented, yet there remains a need for systematic scientific validation. Standardization through modern analytical tools is essential to ensure the safety, efficacy, and reproducibility of this formulation.

Objectives

This study aims to bridge classical Ayurvedic parameters with contemporary analytical methods to establish comprehensive quality benchmarks for Abhrasindoora.

Methods

Multiple analytical tools were employed, including particle size analysis, X-ray Diffraction (XRD), X-ray Fluorescence (XRF), Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to determine the fineness and uniformity, crucial parameters that directly influence the bioavailability and therapeutic potential of the formulation.

Results

Particle size analysis revealed a distribution with a mean particle size of 3.2 µm, indicating fine and uniform particle characteristics. XRD analysis exhibited multiple crystalline phases with varying peak intensities, signifying the complex nature of the formulation. The most prominent peak at 12.34° 2θ corresponded to Hematite (Fe_2O_3), confirming the presence of iron oxide as a major constituent. Additional crystalline phases identified included Fe_3O_4, $CaCO_3$, SiO_2, FeS, ZnO, PbO, MnO_2, and CuO, representing the compound's diverse mineral composition. XRF analysis demonstrated the presence of Sulphur, Bromine, Mercury, and other trace elements providing its therapeutic and structural characteristics.

Conclusion

The study establishes a strong scientific rationale for the classical preparation method of Abhrasindoora and supports the formulation's standardization through advanced analytical characterization. These findings contribute significantly to developing quality standards for Rasoushadhis, ensuring safety, efficacy, and consistency in Ayurvedic pharmaceutics.

KEYWORDS: Analytical Tools, Ayurvedic Pharmaceutics, Kupipakwa Rasayana, XRD, Rasashastra.

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Introduction:

Kupipakwa Rasayana (KR) (a Sublimated herbo-metalo-mineral preparations), one of the commonly prescribed dosage forms of *Rasashastra* (a branch of Ayurveda where medicines are prepared by using herbs- metal-minerals) is prepared in a specially designed glass bottle by gradual heating immersion in sand bath (*Valuka Yantra*)/vertical electrical muffle furnace[1]. Out of many examples of KR, *Abhrasindoora* (AS) is one, which derived its name because of one of the ingredients used is Black mica (*Abhra*) and color of the final product resembles like vermilion (*Sindura*). AS is prescribed therapeutically for the management of Bronchial Asthma (*Shwasa*) and Fever (*Tridoshaj Jwara*)[2]. The method of preparation of this drug involves complex process like purification (*Shodhana*), trituration (*Mardana*), levigation (*Bhavana*) and chronological heating pattern/incineration (*Jarana*)[3]. Because of these processes the final product obtained is palatable, fast acting and effective in small dosages with long shelf life without losing potency and thus believed to be superior to herbal preparation[4]. Despite its longstanding use, because of variations in preparation and lack of standardization often lead to inconsistencies in quality

and efficacy and sometime may lead to exhibition of toxic effects[5]. That's why standardization and chemical characterization, using modern analytical tools are crucial which also ensure the safety, efficacy, and reproducibility of the formulation. Thus, the study was planned with an aim to establish a comprehensive quality benchmark for AS by bridging the classical Ayurvedic parameters with contemporary analytical methods.

The objective of this research was to develop a comprehensive standardization protocol for AS by preparing it according to authoritative *Rasashastra* references and evaluating the formulation through physicochemical, spectroscopic, and advanced instrumental analytical techniques to establish validated quality parameters.

1. Materials and Methods:

Pharmaceutical procedure of AS

Collected raw materials i.e, Mercury, sulphur, and mica, *Calotropis procera Linn*. (*Arka Kṣeera*) and *Ficus benghalensis Linn*. (*Vaṭasunga*) were authenticated and subjected to procedures like *shodhana, dhanyabhrkikarana, murcchana, bhavana* and *jarana* to obtain the red colored crystalline solid which was collected after shelf cooling and triturated to obtain AS.

Shodhana (Purification)[6]: This method is frequently employed to eliminate chemical and physical contaminants and to ease the subsequent procedure involved. The Black mica was refined by the heating and quenching (*Nirvapa*) in Decoction of *Triphala*[7], which eliminated physical impurities like sand particles and made the mineral brittle. Sulphur was refined by melting in ghee and pouring in milk (*Dhalana*), which eliminates the bad odour and incorporated therapeutic properties of milk and *ghee*[8]. Mercury was subjected to intense trituration (*Mardana*) with lime stone powder, garlic pastes, and half part rock salt for seven days and later was rinsed with hot water which helped in removing certain heavy metals found in impure mercury itself[9]. (Fig: 1-6)

Preparation of *Dhanyabhraka*: This method involves maceration of purified black mica with paddy (*Dhanyaka*) to remove any physical impurities if remained and to reduce the size of mica particles to be utilized directly to manufacture black sulphide of mercury (*Kajjali*) for subsequent process[5]. (Fig. 6)

Murchana[10]: In this process purified Mercury is triturated with *Dhanyabhraka* and Sulphur until it becomes fine black powder known as *Kajjali.* Trituration not only helps mercury to become stable and therapeutically potent but also made it free from any free metallic particle, which can be examined by its non-shininess (*Nischandrata*). (Fig. 7) The prepared *kajjali* is subjected to levigation (*Bhavana*) with the latex of *Calotropis procera* and the juice of *Ficus Benghalensis* in a mortar until the product becomes smooth black and dried. This method is specifically utilized to eliminate contaminants, and increase the therapeutic effect by incorporating the phytochemicals and trace elements of those herbs use apart from reducing the particle size[11]. (Fig. 8-12) The levigated black powder is further subjected to gradual heating (*Jarana*) which is performed in a long-necked glass bottle. During the procedure the black *kajjali* turns red and crystalline; eventually sublimates` and settled at the neck of the bottle[12]. (Fig. 14-18) This final product obtained is known as AS, which is considered potent because of cooking of sulphur with mercury in slow gradual heat and thus used to treat conditions like fever and Bronchial asthama[13]. (Fig. 18)

2. Result:

Pharmaceutical Procedure of AS shown in figure 1-24.

Figures 1-24: Pharmaceutical Procedure of AS

13. Kajjali (Black compound of Hg+S+Mica) 14. Glass bottle (Mud cloth wrapped) 15. Valuka Yantra (Sand Bath)

16. Sublimated AS 17. Abhrasindoora (AS) 18. Triturated final compound

Analytical Study of *Abhrasindoora*:

Table 1: Showing Analytical parameters for *Abhrasindoora*

Sr. No.	Particulars
Physicochemical analysis	
1	pH (1% w/v solution)
2	Total ash
3	Acid-insoluble ash
4	Water-soluble extractive
5	Loss on drying at 105°C
6	Alcohol soluble extractives
7	Total mercury
8	Free mercury
9	Total Sulphur
Spectroscopic & Instrumental Analysis	
7	Helos Particle Size Analysis: To analyse particle size AS
8	XRD: To identify crystalline phases and particle size of AS
9	XRF: To determine the elemental composition of AS.
10	ICP-OES: To determine the heavy metals present in AS

The physicochemical tests of *Abhrasindoora* results are mentioned in Table 2, and all parameters were found to be within the specified limits.

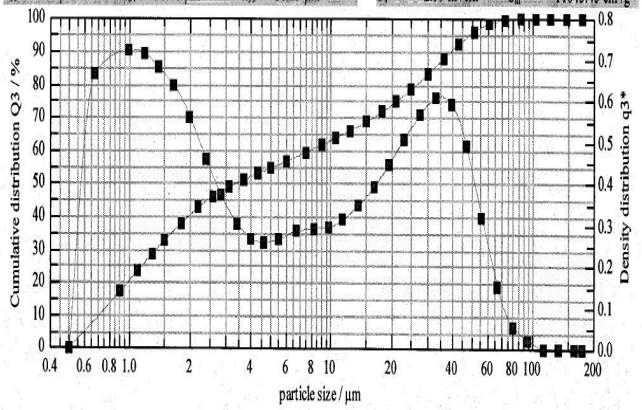
Graph 1: Particle size analysis of *Abhrasindoora*

HELOS Particle Size Analysis
WINDOX 5

SOPHISTICATED INSTRUMENTATION CENTRE FOR APPLIED RESEARCH AND TESTING
Vallabh Vidyanagar - 388120

HELOS (H1004) & SUCELL, R3: 0.5/0.9...175µm
Abhrasindoora 2023-02-07, 10:39:49,890

x_{10} = 0.74 µm x_{50} = 3.45 µm x_{90} = 39.58 µm SMD = 2.00 µm VMD = 13.08 µm
x_{16} = 0.88 µm x_{84} = 31.40 µm x_{99} = 68.29 µm S_V = 2.99 m²/cm³ S_m = 11043.45 cm²/g



comment:

user parameters:
Company Name: Dr. Rakesh, Parul university.
Sample Name: Abhrasindoora
Batch Name:

cumulative distribution

x_0/µm	Q_3/%	x_0/µm	Q_3/%	x_0/µm	Q_3/%	x_0/µm	Q_3/%
0.90	16.97	3.70	50.95	15.00	68.51	61.00	98.30
1.10	23.23	4.30	52.68	18.00	71.62	73.00	99.45
1.30	28.39	5.00	54.35	21.00	74.59	87.00	99.85
1.50	32.61	6.00	56.43	25.00	78.41	103.00	100.00
1.80	37.64	7.50	59.17	30.00	82.88	123.00	100.00
2.20	42.51	9.00	61.47	36.00	87.67	147.00	100.00
2.60	45.82	10.50	63.43	43.00	92.22	175.00	100.00
3.10	48.63	12.50	65.79	51.00	95.83		

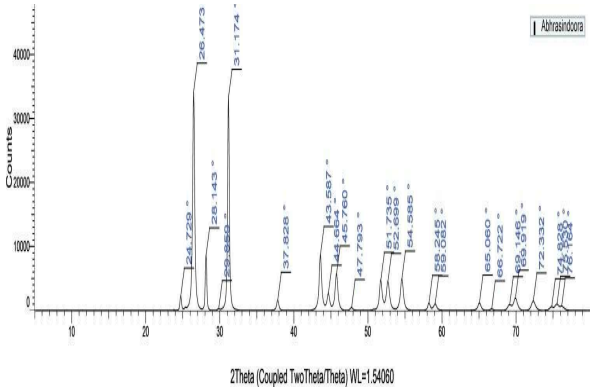
density distribution (log.)

x_m/µm	q_3/lg	x_m/µm	q_3/lg	x_m/µm	q_3/lg	x_m/µm	q_3/lg
0.67	0.66	3.39	0.30	13.69	0.34	55.78	0.32
0.99	0.72	3.99	0.26	16.43	0.39	66.73	0.15
1.20	0.71	4.64	0.26	19.44	0.44	79.69	0.05
1.40	0.68	5.48	0.26	22.91	0.50	94.66	0.02
1.64	0.64	6.71	0.28	27.39	0.57	112.56	0.00
1.99	0.56	8.22	0.29	32.86	0.60	134.47	0.00
2.39	0.46	9.72	0.29	39.34	0.59	160.39	0.00
2.84	0.37	11.46	0.31	46.83	0.49		

evaluation: WINDOX 5.5.1.0, *HRLD*
revalidation:
reference measurement: 02-07 10:38:16
contamination: 0.00 %
trigger condition: Parul Institute
start: c.opt >= 0.1%
valid: always
stop: 2s c.opt <= 0.1% or 10s real time
time base: 100.0 ms

product: Abhrasindoora
density: 2.7100 g/cm³
shape factor: 1.000
C_{opt}: 17.24 %
dispersing method: Parul University.
dispersant: DI Water + 0.1% Tw..
sonication duration: 30 s
sonication pause: 5 s
stirrer speed: 60

Graph 2: XRD analysis report of AS



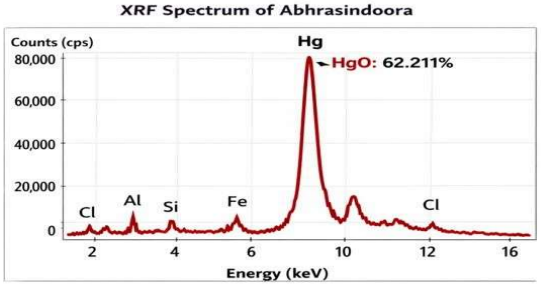


Table 2: Physicochemical tests of *Abhrasindoora*

SN	Test Name	AS
1	Ph (1% w/v solution)	6.04
2	Ash Value	0.90%
3	Water soluble ash	0.53%
4	Alcohol soluble extractives	0.28%
5	Loss on drying	0.02%
6	Total mercury	83.15%
7	Free mercury	Nil
8	Total Sulphur	11.06%

Table 3: Showing XRD values of *Abhrasindoora*

Index	Angle	d Value	FWHM	Rel. Intensity	Intensity
1	24.729 °	3.59741 Å	0.239	6.6%	2253.070
2	26.473 °	3.36420 Å	0.292	100.0%	34266.010
3	28.143 °	3.16828 Å	0.230	24.9%	8515.788
4	29.859 °	2.98992 Å	0.160	0.8%	269.514
5	31.174 °	2.86678 Å	0.256	97.2%	33294.490
6	37.828 °	2.37641 Å	0.318	4.6%	1582.731
7	43.587 °	2.07482 Å	0.345	25.4%	8707.375
8	44.664 °	2.02725 Å	0.406	7.8%	2669.558
9	45.760 °	1.98120 Å	0.391	16.7%	5708.645
10	47.793 °	1.90156 Å	0.362	1.3%	454.967
11	51.735 °	1.76556 Å	0.378	13.7%	4697.958
12	52.699 °	1.73550 Å	0.451	13.2%	4529.520
13	54.585 °	1.67991 Å	0.410	14.3%	4883.834
14	58.245 °	1.58276 Å	0.358	3.2%	1097.318
15	59.082 °	1.56233 Å	0.436	2.9%	1007.031
16	65.060 °	1.43248 Å	0.404	3.4%	1148.130
17	66.722 °	1.40078 Å	0.354	0.7%	224.832
18	69.146 °	1.35747 Å	1.277	2.6%	878.801
19	69.919 °	1.34434 Å	0.507	5.6%	1924.061
20	72.332 °	1.30531 Å	0.485	4.3%	1461.062
21	74.828 °	1.26783 Å	0.160	1.7%	571.734
22	75.530 °	1.25779 Å	1.186	2.8%	963.790
23	76.164 °	1.24889 Å	1.221	2.0%	672.208

Table 4: The results of XRF analysis of AS

SN	Test Parameters	Concentration in %
1	Al2O3	0.077
2	SiO2	0.218
3	SO3	37.396
4	Fe2O3	0.040
5	HgO	62.211
6	Cl	0.057

Table 5: Showing the result of ICP-OES analysis of AS

SN	Particular	Result value % in ppm
1	Hg	2.41
2	As	74.587
3	Cd	1.997
4	Pb	Not detected

Discussion:

Authentication of the raw materials is a vital step in

creating a herbo-metallo-mineral formulation prior to the start of the pharmaceutical process. The essential characteristics of raw materials with variation have been explicitly addressed in *Rasashastra* to address the authenticity. In the era of evidence-based medicine and advancement of modern machineries, sometime these parameters may not be reliable; hence the physiochemical and instrumental testing using the advanced techniques also are equally important. Accordingly, the raw materials were authenticated. Raw Mercury obtained was bright shiny, silvery liquid[14] (Figure 1) and Raw Sulphur was greenish yellow, unctuous, smooth touch and hard appearance with rotten eggs odour confirming the criteria laid down for authenticity[15] (Fig. 3). Raw mica was shiny, black, hard, heavy, platy and unctuous with presence of Silica, iron, aluminium and other trace elements aligning the standards of Ayurvedic Pharmacopoeia[15] (Fig. 3). The analytical results obtained after different procedures was nothing but substantial reflection of transformations as described in the authoritative texts. The reduction in particle size and change in crystalline structure enhances bioavailability and therapeutic efficacy, aligning with the concept of *Sukshmatva*. The XRD analysis shows a range of peaks with varying intensities, indicating the presence of multiple crystalline phases in AS. The most prominent phase identified was Hematite (Fe2O3) at 12.34° 2θ, indicating the presence of iron oxide. Other significant phases include Magnetite (Fe3O4), Calcite (CaCO3), Quartz (SiO2), Iron Sulfide (FeS), Zinc Oxide (ZnO), Lead Oxide (PbO), Manganese Oxide (MnO2), and Copper Oxide (CuO). (Graph 2, Table 3). These prominent peaks suggest that the corresponding crystalline phases are major components of the formulation[16].

The process involves purification, preparation of *dhanyabhraka*, (Fig. 6) trituration (wet and dry), and heating process utilizing a specially designed glass bottle placed in a sand bath over a heating device (*Koshti*). (Fig. 15) This sequential process permits the reduction of Metals/Minerals particles to a finer state, ultimately yielding a compound with a bright red color. (Fig. 17 & 18) This demonstrates the pragmatism and significance of the techniques employed in *Rasashastra* for achieving nanoparticles[17]. The particle size analysis of AS using the HELLOS particle analyzer, revealed a distribution of particle sizes with a mean particle size of 3.2 µm. The particle size distribution shows a broad polydisperse profile with a median particle diameter (D50) of 3.45 µm. The fin fraction is significant, with D10=0.74 µm, indicating the presence of ultrafine particles. The coarse tail extends to D90 = 39.58 µm, confirming a wide distribution range. The Sauter mean diameter (SMD) of 2.00 µm suggests a high specific surface area, consistent with the measured surface value of 2.93 m2/cm3. The volume mean diameter (VMD) of13.08 µm indicates that larger particles contribute substantially to the volume fraction despite the dominance of fine particles in number. The calculated span value of 0.8 reflects moderate dispersion uniformity, suggesting controlled milling or processing condition. (Graph 1) The detailed particle size distribution helps in understanding the fineness and uniformity of the sample, which can influence its therapeutic efficacy and bioavailability[17]. The WD-XRF analysis shows major and minor elemental oxides i.e., HgO (Mercuric oxide), 62.21%, SO_3 (Sulfur trioxide), 37.39%, and SiO_2 (0.218%), Al_2O_3 (0.077%), Fe_2O_3 (0.04%), Cl (0.057%) respectively. (Graph 3)

The analysis indicates that AS mainly consists of mercuric oxide (HgO) and sulphur compounds (SO_3), suggesting the presence of mercury sulfide (HgS)-related phases, a typical character of properly prepared KR having the colour of vermilion (Sindoor); confirming the successful formation of AS (HgS compound) as per traditional standards. The minor presence of silica, alumina, and iron oxide implies trace mineral impurities. Al_2O_3 in trace amounts suggests it does not contribute meaningfully to the formulation's therapeutic efficacy or adverse effects. Silicon dioxide is present in low concentration. (Table 4) This level is typical for formulations and suggests that silica, if present, does not dominate the composition of the herbo-mineral preparation. The high concentration of Sulphur trioxide is significant. This suggests that Sulphur containing compounds are a major component of the formulation, which could be attributed to the use of certain mineral or herbal ingredients known for their sulphur content. The iron oxide content is very low, reflecting minimal iron presence in the preparation. This aligns with typical expectations for this type of formulation where iron is not a major ingredient. The results obtained by using modern analytical tools validate ancient methods of *Shodhana, Murcchna* and *Jarana*, highlighting the scientific basis of traditional *Rasashastra* practices[18]. (Table 5)

Conclusion:

The present study establishes a connection between traditional principles and modern analytical parameters for standardization of AS. The combined approach ensures safety, quality, and reproducibility of this formulation. The unique methods like *Shodhana, Marana, Murchhana* and *Jarana* incorporated to prepare herbo-metalo- mineral compound help in converting inorganic substances to form a compound fine powder. AS possesses hexagonal crystal structures with presence of Hg and S as major elements and Magnetite, Calcite, Quartz, Iron Sulfide, Zinc Oxide, Lead Oxide, Manganese Oxide, and Copper Oxide as minor elements. Such evidence- based standardization can serve as a model for other herbo-metalo-mineral formulations, strengthening the scientific foundation of Ayurvedic Pharmaceutics.

Conflict of Interest:

The authors have no conflicts of interest regarding this investigation**.**

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