IJBPAS, October, 2022, 11(10): 4658-4666

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

EVALUATION OF QUALITY STANDARDS OF *SAMAGUNA BALIJARITA RASASINDURA* (RED SULPHIDE OF MERCURY) BY CONTEMPORARY TECHNIQUES – AN ANALYTICAL STUDY

KUPATI VB[*1] AND JADAR PG[2]

1: Assistant Prof. Department of Rasashastra and Bhaishajyakalpana, KAHER'S Shri B.M Kankanawadi Ayurveda Mahavidyalaya, A Constituent unit of KLE Academy of Higher Education and Research, Belagavi, 590 003, India

2: Prof. Department of Rasashastra and Bhaishajyakalpana, KAHER'S Shri B.M Kankanawadi Ayurveda Mahavidyalaya, A Constituent unit of KLE Academy of Higher Education and Research, Belagavi, 590 003, India

***Corresponding Author: Dr. Veena B Kupati: E Mail: veenakupati@gmail.com**

Received 26th Dec. 2021; Revised 24th Jan. 2022; Accepted 14th Feb. 2022; Available online 1st Oct. 2022

https://doi.org/10.31032/IJBPAS/2022/11.10.6478

ABSTRACT

Rasasindura (Red sulphide of mercury), most commonly used in *Kupipakwa Rasayana* (medicine prepared in bottle) is a sublime product of a mixture of *Shodhita Parada* (purified mercury) and *Shodhita Gandhaka* (purified sulfur), and due to its wide therapeutic utility, it is used in several formulations. Analysis of the products becomes a mandatory to get the quality products. It becomes necessary to adopt modern analytical methodology to determine the important chemical constituents present in the drug qualitatively and quantitatively. **Objectives:** To assess the quality of *Rasasindura*. **Methodology:** The drugs were selected according to *grahya lakshana's* and quantified by modern parameters. The *Parada* and *Gandhaka shodhana,* Preparation of *Kajjali* and *Rasasindura* were done according to the reference of Rasatarangini. Physicochemical and Analytical tests were done before and after preparation. **Results:** XRD report has shown major peaks of HgS, crystals are hexagonal and primitive in lattice. SEM shows particle size of 100nm. ICP-AES has shown concentration of Mercury and Sulphur as 9.822 and 11.336% respectively. **Conclusion:** XRD report has shown major peaks of HgS, crystals are hexagonal and primitive in lattice. SEM image has

shown crystalline nature, *Rasasindura* of particle size 100nm – 1µm. ICP-AES has shown concentration of Mercury and Sulphur as 9.822 and 11.336% respectively. Quality of *Rasasindura* is as per standard guidelines

Keywords- *Rasasindura*, NPST, XRD, ICP-AES, SEM

I. INTRODUCTION

Rasasindura, most commonly used in *Kupipakwa Rasayana* is a sublime product of a mixture of *Shodhita Parada* (processed mercury) and *Shodhita Gandhaka* (purified sulfur), and due to its wide therapeutic utility, it is used in several formulations. In *Ayurveda*, there is an information regarding safety, efficacy of the drugs. These parameters become important steps before administration of the drugs to human beings. Now days *Ayurvedic* products getting globalization and we need scientific base for their uncertainty. To overcome this, in classics the Acharya's mentioned different *siddhi lakshana* to prepared medicaments but these parameters are not enough to mention the chemical composition of the products. So Analysis of the products becomes a mandatory to get the quality products. It becomes necessary to adopt modern analytical methodology to determine the important chemical constituents present in the drug qualitatively and quantitatively. This will help in understanding and interpretation of pharmacological action of any medicament easier and better.

The Analysis of *Rasasindura* is done in the following way which is classified as mentioned below.

1. Physical Analysis
2. Chemical Analysis

1. Physical Analysis

Organoleptic Characters: *Sparsha* (Touch), *Rupa* (Color), *Rasa* (Taste), *Gandha* (Odour),

2. Chemical Analysis

Qualitative: Namburi phase Spot test, X-Ray Diffraction

Quantitative: Inductively coupled Plasma Atomic Emission Spectroscopy, Scanning Electron Microscopy

II. METHODOLOGY

2.1 Physical Analysis

Prepared Rasasindura **[1]** is assessed for physical properties like *Varna, Gandha, Sparsha* and *Rasa*; these tests were as per classical *siddhi lakshana*. *Varna* was *balarka, sparsha* was *slakshna, Rasa* was *nirasa* and *Gandha* was *nirgandha.*

2.2 Solubility

Rasasindura is only soluble in Aquaregia (combination of Conc. HNO_3 and Conc. HCl in the ratio of 1:3).

2.3 Namburi Phased Spot Test: [2]

The 'Namburi Phased Spot Test' is a new technique developed by Dr. Hanumantha Rao after a lot of trial and error. It is an identifying technique for various bhasma and Sindura Kalpanas. In this method, Whatman paper No.1 is invariably impregnated in a suitable reagent and dried. Unlike the conventional method of spot test, in this new technique, the spot is not rejected immediately after treating and noting the chemical reaction. As the reactants continue to react for a long time, the spot is studied at three different time intervals, viz. 1st phase or Immediate Reaction (within five minute after treatment), 2nd Phase or Delayed Reaction (between five and twenty minutes) and 3rd Phase or Late Reaction (8 hours after 1st phase).

2.3.1 Some Special features of this technique:

- Study of the spot at three different time intervals.
- Pattern of colour display of the spot.

2.3.2 Materials:

- Distilled water
- 5 N HO_3
- Potassium iodide paper (Whatman Paper No.1 impregnated 10% Potassium iodide) solution
- Test tubes
- *Rasasindura*
- Petri dishes

2.3.3 Procedure:

Initially 0.25g of *bhasma* was put into test tube containing reagent 0.5 ml of 5N HNO_3 and heated on spirit lamp till the lower end of the test tube becomes red hot. Heating was stopped. The reaction was allowed 72 hours to react, shaking the solution now and then. After 72 hours a drop of supernatant solution was dropped on 10% impregnated potassium Iodide paper and observed at following intervals.
The same procedure was conducted on both sample i.e Sample-G and Sample-P.

2.3.4 Observation at:

1st phase: 0 to 5 min

2nd phase: 05 min to 20 min

3rd phase: 1st phase to8 hours

2.4 X-RAY DIFFRACTION [3]

X-rays are diffracted because crystalline solids are constructed from regularly sample assemblage of components (atom or atomic grouping) repeated in three dimensions and x-ray wavelengths are the same order of magnitude as the spacing of atom centers. From morphological view point, a crystal may be defined as the regular polyhedral form, bounded by smooth surfaces, which is assumed by a chemical compound under the influence of its inter atomic forces when passing from the gaseous or liquid state to solid state.

The geometric shapes of crystals reflect an internal symmetry of atoms and molecules arranged in a regular and repeated pattern in space, which distinguish crystalline solids from amorphous.

An important aspect of solid state is the ability of compounds to crystallize in a variety of symmetrical arrangements of their molecules in space i.e. polymorphic forms which are often quite different from each other in physical characters (habit, melting point solubility) although chemically identical.

The pharmaceutical relevance of polymorphism lies in the fact that the differences in physico-chemical properties between forms by may influence manufacturing processes and importantly, dissolution rate and therefore bio-availability.

The x-ray diffraction methods are necessary to distinguish between difference arrangements.

The condition for diffraction of a beam of x-ray from crystal is given by Bragg's equation.

2d Sin = n t

T = wavelength of x-ray beam

@ = angle between incident x-ray and crystal lattice

d = distance between lattice plane's

The Bragg's equation is fundamental relation underlying all X- ray diffraction measurements.

Only for angles of incidence such that sin @ = nt /2d will x-ray's beam reflected and at all other angles destructive interference occurs.

In powder work irradiated sample consist of small crystallites in random orientation so that Bragg's condition is fulfilled. For every set of lattice planes the reflected rays from each lying along the surface of cone having its apex at specimen, its axis in the direction of the x-ray beam and its semi vertical angles equal to 2@.

In powder camera technique, segment of cones of diffracted x-rays fall in strip of photographic film to give pattern of lines of varying intensity i.e. a power photograph. With the help of powder diffraction, the intensities of diffracted rays are directly measured by radiation detector which moves in a defined are around the specimen. The diffraction for range of Bragg's angles being recorded is peaks on chart, calibrated in values of 2@.

The specimen can be identified by reference libraries. Such as powder Data File or the Joint committee on Powder Diffraction Standards (JCPDS) which contains the diffraction pattern of some tens of thousands of substances in numerical form.

2.5 SCANNING ELECRON MICROSCOPE (SEM): [4]

The SEM is a microscope that uses electrons rather than light to form an image. There are many advantages to using the SEM instead of a light microscope. It is designed for direct studying of the surfaces of solid objects. By scanning with an electron beam that has been generated and focused by the operation of the microscope, an image is formed in much the same way as a television. The SEM allows a greater depth of focus than the optical microscope. For this reason, the SEM can produce an image that is a good representation of the three dimensional sample.

The SEM uses electron instead of light to form an image. A beam of electron is produced at the top of the microscope by heating of a metallic filament. The electron beam follows a vertical path through the column of the microscope. It makes its way through electromagnetic lenses which focus and direct the beam down towards the sample. Once it heats the sample, other electrons are ejected from the sample.

Detectors collect the secondary or backscattered electrons and convert them to a signal that is sent to a viewing screen similar to the one in an ordinary television, producing an image.

2.6 DETERMINATION OF ELEMENTS BY ICP-AES METHOD: [5]

Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP- AES) is a mission spectrophotometric technique, exploiting the fact that excited electrons emit energy at a given wavelength as they return to ground state after excitation by high temperature Argon Plasma. The fundamental characteristic of this process is that each element emits energy at specific wavelengths peculiar to its atomic character. The energy transfers for electrons when they fall back to ground state is unique to each element as it depends upon the electronic configuration of the orbital.

The energy transfer is inversely proportional to the wavelength of electromagnetic radiation, $E = hc/\lambda$ ... (where h is Planck's constant, c the velocity of light and λ is wavelength), and hence the wavelength of light emitted is also unique.

Although each element emits energy at multiple wavelengths, in the ICP-AES technique it is most common to select a single wavelength (or a very few) for a given element. The intensity of the energy emitted at the chosen wavelength is proportional to the amount (concentration) of that element in the sample being analyzed. Thus, by determining which wavelengths are emitted by a sample and by determining their intensities, the analyst can qualitatively and quantitatively find the elements from the given sample relative to a reference standard.

The wavelengths used in AES ranges from the upper part of the vacuum ultraviolet (160 nm) to the limit of visible

light (800 nm). As borosilicate glass absorbs light below 310 nm and oxygen in air absorbs light below 200 nm, optical lenses and prisms are generally fabricated from quartz glass and optical paths are evacuated or filled by a non-absorbing gas such as Argon.

Inductively Coupled Plasma Atomic Emission Spectroscopy is a versatile method for estimation of elements. It finds use in many fields including inorganic chemistry, bio-inorganic chemistry, pharmaceutical industries, biological sciences, geology, oceanography, food industries, polymer industries, pesticide industries, environmental studies and pollution monitoring of water & air and catalyst industries.

2.6.1. Distinctive Applications:

- Precious metal estimation at low level
- Heavy metal estimation at sub ppm level
- Rock, Soil, Fly ash (Complete analysis)
- Environmental sample analysis (Air, Water, Soil, sediments, etc.)
- Biological samples (Urine, tooth, bone, etc.)
- Polymer industries
- Pharmaceutical industries

2.6.2 Procedure:

2.6.2.1 Sample preparation:

0.25 gm of sample was weighed into a Teflon bomb. 8 ml of Milli-Q water, 7 ml of HNO_3 and 1 ml of H_2O_2 was added. The mixture was micro waved for 20 min in microwave digester (Model: Milestone Start D). The samples were then diluted to 50ml with Milli-Q water. Further dilutions may be done if required to attain the working range of the instrument.

3 RESULTS

Table 1: Organoleptic characters of *Rasasindura*

S. No.	Characters	Results
01	Colour	Reddish Brown
02	Odour	Odourless
03	Taste	Tasteless
04	Touch	Fine

Table 2: Solubility test of *Rasasindura*

S. No.	Solvent	*Rasasindura*
01	H_2SO_4	Sparingly soluble
02	HNO_3	Practically insoluble
03	NaOH	Practically insoluble
04	Petroleum ether	Practically insoluble
05	Distilled water	Practically insoluble
06	Aqua regia	Soluble

Table 3: NPST Analysis of *Rasasindura* (Red sulphide of mercury)

S. No.	Phase	Observation
01	1st	Brick red solid spot with dark brown periphery was seen
02	2nd	Dark brown periphery was fades away slowly
03	3rd	Dark brown periphery which was fades away slowly with great extent

Table 4: XRD results of *Rasasindura*

Sample				Standard	
Peak No	**Angle 2 θ**	**d space**	**Intensity**	**d space**	**Intensity**
5	**26.3990**	**3.37623**	**100**	**3.359**	**100**
7	**31. 1351**	**2.87262**	**94.83**	**2.863**	**95**
6	**28.3718**	**3.17769**	**28.51**	**3.165**	**30**
9	**43.4681**	**2.08193**	**24.66**	**2.074**	**25**
8	**51.5348**	**1.77342**	**13.65**	**1.765**	**20**
14	**54.5574**	**1.68210**	**21.78**	**1.679**	**25**
JCPDS PDF No: 6-0256 Name of standard: Cinnabar (HgS) Crystal structure: Hexagonal Lattice: Primitive					

Table 5: SEM results of *Rasasindura*

S. No	Magnification	Particle size
1.	**100,000 X**	**100 nm**
2.	**75,000 X**	**100 nm**
3.	**50,000 X**	**100 nm**
4.	**25,000 X**	**1µm**
5.	**10,000 X**	**1 µm**

Table 6: ICP – AES results of *Rasasindura*

Sample	Hg (%)	S (%)	Fe (%)	Pb (%)	As (%)
Rasasindura	**9.822**	***11.336***	***0.067***	***0.037***	***ND***

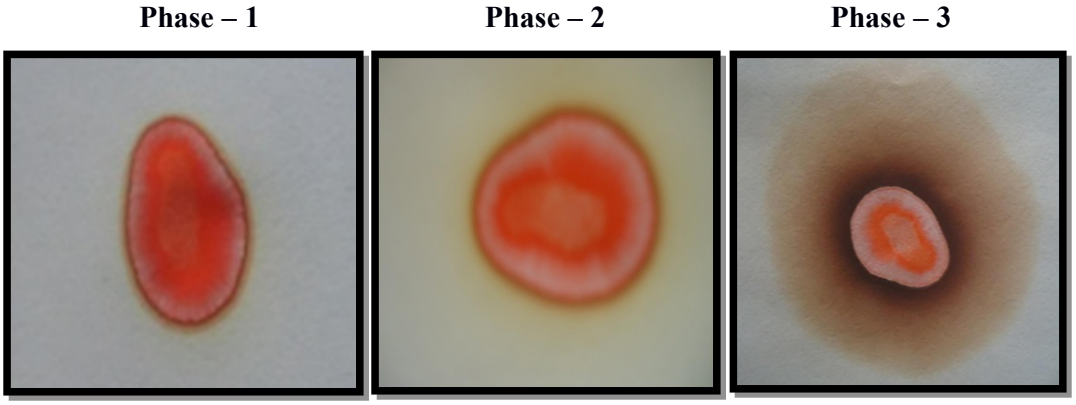

Figure 1: NPST observation

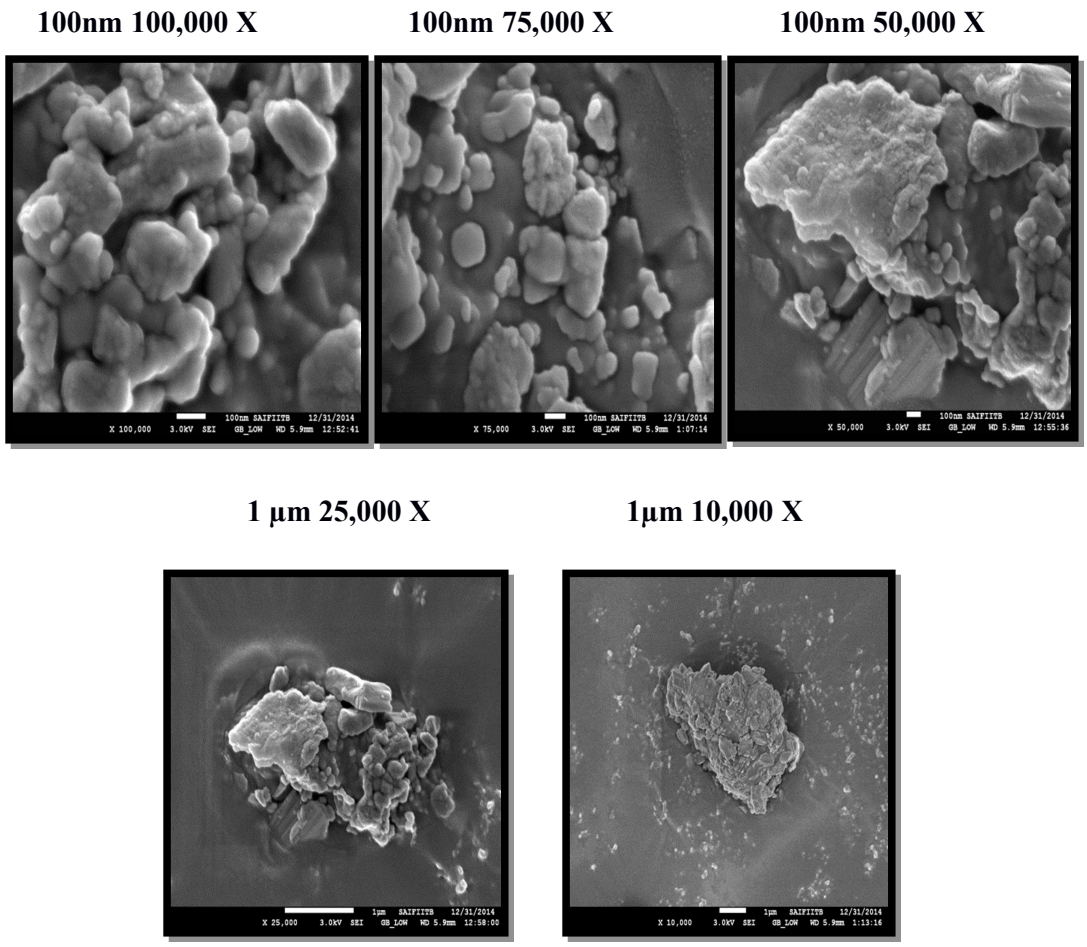

Figure 2: Field Emission Gun Scanning Electron Microscope

4 DISCUSSION

4.1 Solubility of *Rasasindura*

Rasasindura was soluble in Aqua regia and insoluble in Distill water, Sulphuric acid, Nitric acid, Sodium hydroxide and Petroleum Ether.

4.2 NPST Analysis

NPST of *Rasasindura* has shown identical spot compared to standard. The spot was analysed in three phases. In first phase brick red solid spot with dark brown periphery was seen, in second phase dark brown periphery was fades away slowly and in third phase Dark brown periphery which was fades away slowly with great extent. It is comparable to the standard mentioned for *Sindura* group.

4.3 QUANTITATIVE RESULTS

4.3.1 X – ray Diffraction Method (XRD)

The peaks of XRD of *Rasasindura* are identical with the cinnabar i.e. HgS. The particles are hexagonal in structure. In previous study also same parameters were seen.

4.3.2 Scanning Electron Microscopy (SEM)

Crystals of *Rasasindura* have particle size of 100nm in a magnification of

100,000, 75,000 and 50,000 X but size increased to 1µm in 25,000 and 10,000 X. It shows that whenever magnification reduces particle size increases.

4.3.3 Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES)

ICP-AES has shown concentration of Mercury and Sulphur as 9.822 and 11.336% respectively. Arsenic is not detected and other elements are negligible in quantity.

5 CONCLUSION

XRD report has shown major peaks of HgS, crystals are hexagonal and primitive in lattice. SEM image has shown crystalline nature, *Rasasindura* of particle size 100nm – 1µm. ICP-AES has shown concentration of Mercury and Sulphur as 9.822 and 11.336% respectively.

Quality of *Rasasindura* is as per standard guidelines.

6 REFERENCES

[1] Sharma Sadanand, Rasatarangini, Rasavigyana Hindi commentary, 11th Edition, Motilal Banarsidas Publication Delhi, 1979, Reprint 2000, 6/162 – 167, Pp – 135.

[2] Dr Namburi Hanumantha Rao, Manual of Namburi Phased Spot Test, Qualitative Standardisation of Bhasma and Sinduras, Page No.34.

[3] Gribble. C.D, Rutley`s Elements of mineralogy, 27th, Edition, CBS Publishers and distributors Delhi, 1991, Page 26-30.

[4] http://en.wikipedia.org/wiki/Scanning_electron_microscope

[5] Thompson M. and Walsh J.N., Inductively Coupled Plasma Spectrometry, Blackie. (1989).