



### PHYSICO-CHEMICAL CHARACTERIZATION OF VANGA BHASMA

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#### ABSTRACT

Bhasma is very effective when prepared by appropriate method and used in accurate therapeutic dose. Vanga Bhasma [VB] is being used in genitor-urinary disorders since long in Ayurveda. The pharmaceutical processing of VB was performed by four steps i.e. Samanya Shodhana, Vishesh Shodhana, Jarana & Marana. To assure the quality of bhasma, rasa shastra quality control tests like rekhapurna, varitara, Niruttha, etc., were used. After the bhasma complied with these tests, the bhasma was analyzed [Physicochemical characterization] using ICP-AES, X-ray Diffraction (XRD) revealed that Vanga bhasma contains major compound SnO<sub>2</sub> and Thermo Gravimetric analysis (TGA) with DTA showed no weight loss and no physical or chemical changes so it can be an alternative and supportive to Niruttha Pariksha. It may be concluded that raw Vanga is a Simple compound which gets converted into a mixture of complex compounds after the particular process of marana.

**KEY WORDS:** Vanga bhasma, X-ray diffraction, Thermo Gravimetric analysis (TGA), ICP-AES

#### INTRODUCTION

Various metals are described in texts of Rasashastra viz. Suvarna (Gold), Rajat (Silver), Tamra (Copper), Naag (Lead), Vanga (Tin), Loha (Iron), etc. Among these; Vanga i.e. Stannum is a well known metal to Indians since the Vedic period. It is included in the category of 'Puti-Loha'. Vanga is used as an important ingredient in several Metallic preparations<sup>1</sup>. Preparations of VB are frequently used due to its proficient therapeutic effectiveness in genitorurinary disorders<sup>2</sup>. Toxicity of Vanga is also described by ayurvedic authors<sup>3</sup>, but also acknowledged its efficacy in alleviation of diseases and its role in growth and development of human body. Hence, Rasashastra scholars developed systematic procedures to process the metal for removing its toxicity and potentizing its therapeutic effect.

Mostly the Bhasma are chemically mixed oxides of one or more metals, from this point of view it is essential to carry out their structural characterization and also to study the role of various steps involved in the preparation of Bhasmas, so this work was towards, describing the importance of exact process and to attempt for the decision of the character of prepared Bhasma.

Physico-chemical parameters being indirect indicators of therapeutic properties, they are selected as criteria for analysis of the sample obtained during and at the end of the process were taken as a task of present study.

#### MATERIAL AND METHODS

##### Procurement of Raw Materials

Raw Vanga (Sn), Tila Tail (Sesame oil), Takra (buttermilk), Gomutra (cow urine), Kanji, Kulattha seeds, Nirgundileaves (*Vitex nigundo* Linn.), Churnodaka (Slake lime water) were used as raw materials. Stannum was considered as Vanga for their similar characteristics and was collected from local market of Nanded, Maharashtra and authenticated as per classical texts mentioned.

##### Pharmaceutical Processing

##### Samanya Shodhana

Preparation of accessory drugs: Takra, Kanji and Kulattha kwatha were prepared as per classics for the process of

Samanya Shodhana.

##### Process of Shodhana

##### Ingredients

**Main Drug** – Vanga, 1200 g.

**Accessory Drugs** – Tila taila 9L; Takra 9L; Gomutra 9L; Kanji 9 L; Kulattha kwatha 9L.

**Procedure** – Vanga was heated in an iron laddle and after melting, it was poured in each liquid media for 7 times. Each time fresh liquid media was used<sup>4</sup>.

##### Vishesh Shodhana

**Main Drug** – Vanga obtained after Samanya Shodana, 1145 g.

**Ingredients** – Churnodaka 21L.

**Procedure** – For this purpose Lead was heated in an iron laddle and after melting, it was poured in Churnodaka for 7 times<sup>5</sup>.

##### Jarana of Shuddha Vanga

Shuddha (purified) Vanga was heated in an iron pan and after melting, Ashwattha-twak-churna (ATC) was added (apprx. 10-15 g) and triturated with iron ladle. After completion of its burning (5 min.), same amount was again added and the procedure was repeated till Vanga got converted into powder form. Then jarita Vanga powder was collected in centre, covered with earthen saucer and strong heat was applied up till red hot stage (12hrs). On next day, Jarita Vanga powder was collected and sieved through 120 no. mesh. The product was washed thrice with distilled water to remove excessive salts<sup>6</sup>.

##### Preparation of Vanga Bhasma by Putapaka Method

The preparation of Vanga Bhasma by Putapaka method was done as per the reference of Rasamitra<sup>7</sup>.

**Procedure** – Juice of Aloe Vera q.s. was added to the Jarita Vanga and triturated for 6 hrs. After triturating, small pellets of uniform size and thickness were prepared and dried in sunlight. Pellets were kept inside a sharava (shallow earthen disc) and another sharava was inverted over it. The junction was sealed by mud smeared cloth and allowed for complete drying. Then it was subjected for Laghuputa (25 cow-dung cakes). On the next day the material was collected and

ground. The process was repeated 13 times to obtain final product of Vanga bhasama & was subjected to analysis.

**Table 1: Weight of the material aftersamanyashodhana, vishesha shodhana, Jarana & putapaka**

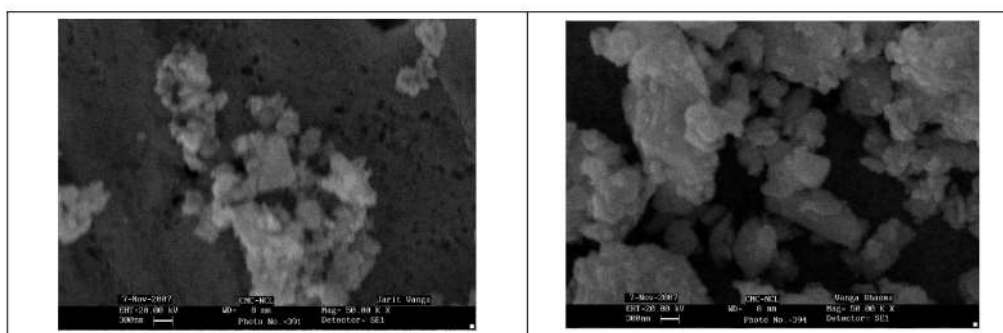
Pharmaceutical procedure	Media	Initial weight (in gm)	Final weight (in gm)
Samanyashodhana	Kanji, Takra, Kulattha kwatha, Gomutra, Tilataila	1200	1145
Vishesha shodhana	Churnaodaka	1145	1032
Jarana	Ashvattha churna	1032	1048
Marana	Aloe Vera Juice	200	198

**Table 2: Ash Value of Raw Vanga, Jarit Vanga & Vanga Bhasma**

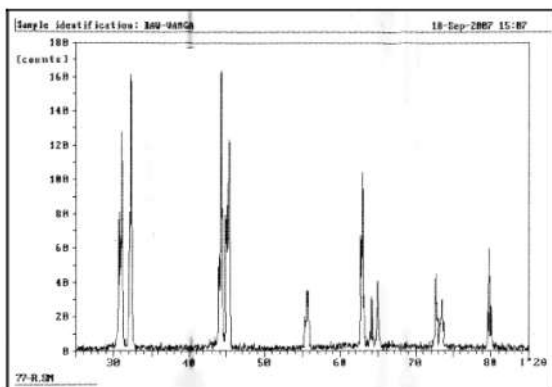
Parameter	Raw Vanga	Jarit Vanga	Vanga Bhasma
Total Ash %	99.14	99.5	99.09
Acid Insoluble Ash %	92.39	90.04	83.09
Water Soluble Ash %	0.97	2.82	4.56

**Table 3: Elemental assay of Raw Vanga, Jarit Vanga & vanga Bhasma using acid digestion method, ICP-AES technique**

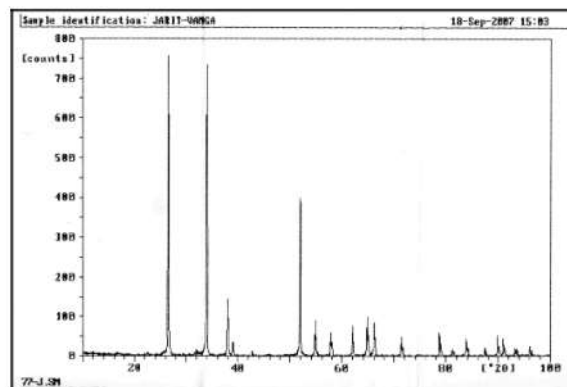
Sr. No.	Element	Raw Vanga (%)	Jarit Vanga (%)	Vanga Bhasma (%)
1	Tin (Sn)	99.9	74.4	76.5
2	Lead (Pb)	<0.01	<0.01	<0.01
3	Calcium (Ca)	0.01	1.4	1.5
4	Arsenic (As)	<0.01	<0.01	<0.01
5	Zinc (Zn)	<0.01	0.02	0.02
6	Cadmium (Cd)	<0.01	<0.01	<0.01
7	Nickel (Ni)	<0.01	<0.01	<0.01
8	Iron (Fe)	<0.01	0.06	0.54
9	Silica (Si)	<0.01	<0.01	0.48
10	Magnese (Mn)	<0.01	<0.01	0.01
11	Magnesium (Mg)	0.01	0.10	0.27
12	Aluminium (Al)	0.58	0.06	0.45
13	Pottassium (K)	0.02	0.16	0.24



**Figure 1: Scanning electron microscopy feature of Jarit Vanga & Vanga Bhasma**



**Figure 2: XRD Analysis of Raw Vanga (Sn)**



**Figure 3: XRD Analysis of Jarit Vanga**

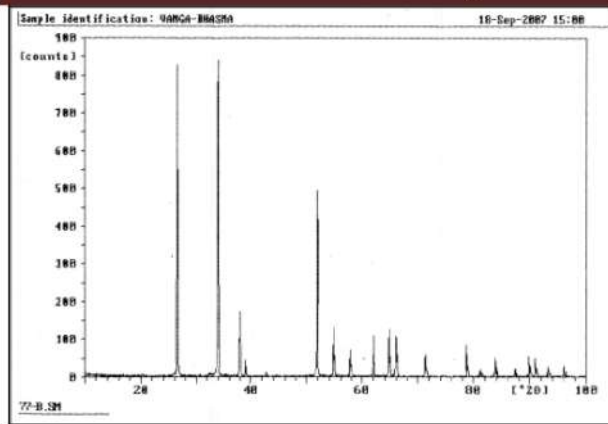


Figure 4: XRD Analysis of Vanga Bhasma

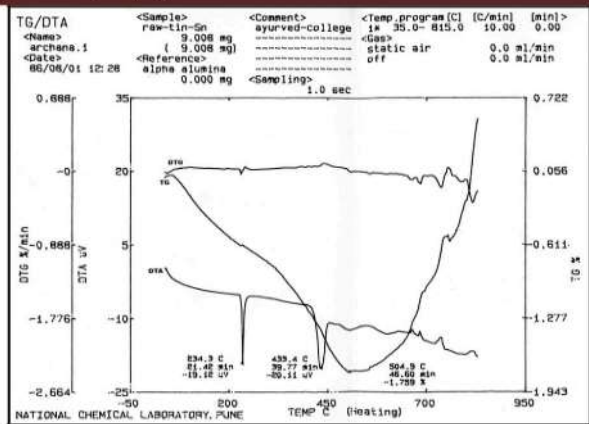


Figure 5: TG/DTA Analysis of Raw Vanga

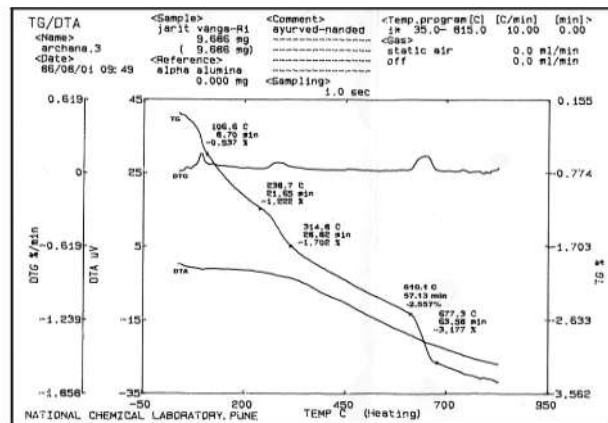


Figure 6: TG/DTA Analysis of Jarit Vanga

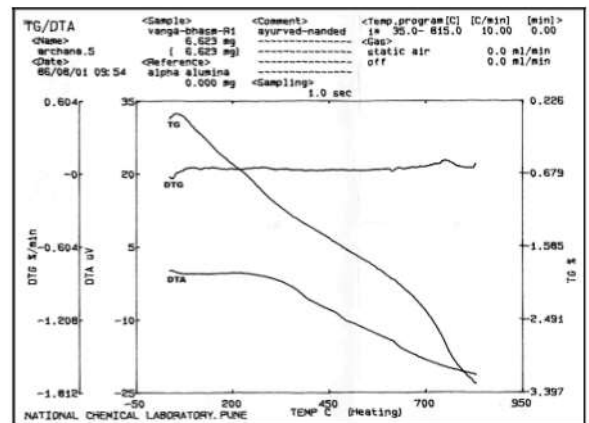


Figure 7: TG/DTA Analysis of Vanga Bhasma

## OBSERVATION & RESULTS

### Analysis Using Parameters Described In Ayurveda Texts

The final product (VB) was analyzed on quality control measures described in Ayurvedic texts as follows and found appropriate<sup>8</sup>.

#### Nischandratva

The bhasma was taken in a Petri dish and observed for any luster in daylight through magnifying glass. No luster was observed in the bhasma.

#### Rekhapurnatvam

A pinch of bhasma was taken in between the thumb and index finger and rubbed. It was observed that the bhasma entered into the lines of the finger, and was not easily washed out from the cleavage of the lines.

#### Varitaratavam

A small amount of the prepared bhasma was sprinkled over the still water in a beaker. It was found that the bhasma particles floated over the surface of the water.

#### Nisvadutvam

The prepared bhasma was found to be tasteless when a small amount was kept on the tongue.

#### Avami

Ingestion of 5-10 mg of the bhasma did not produce any nausea / vomiting.

#### Uttam

When a grain was placed on the varitara film of Bhasma, it was float like swan on water; such Bhasma is termed as Uttam.

#### Niruttha

Bhasma is heated at high temperature in a koshthi along with measured quantity of silver. At the end of the process, the quantity of silver should not increase.

### Apunarbhava

Bhasma when mixed with mitrapanchaka and heated at high temperature should not undergo any change in its physical properties. The bhasma should not regain its original state.

### Analysis Using Modern Parameters

#### Physico-chemical Analysis

Physical characteristics of the material were noted down before and after each puta. The Tables 1-2 show observations and variation in weight of the material before and after each pharmaceutical procedure.

The Bhasma was further analyzed using the following techniques

- Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES) for the elemental assay
- Scanning electron microscope (SEM)
- X-Ray diffraction (XRD) studies
- Thermal analysis using Thermo Gravimetric analysis (TGA).

#### Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP - AES)

This test was carried out at Bhabha Atomic Research Center, Hyderabad.

#### Technique –

Line spectra are a type of emission spectra. A line spectrum consists of discrete irregularly spaced line. This type of spectrum is obtained when the light emitting substance is in the atomic state. The origin of the line spectrum can be explained on the basis of Bohr's theory. If an atom in ground state, its electrons are present in the lowest permitted energy levels. When atom gets excited by electric or thermal methods, its electrons move from inner orbital to outer

orbital. The excited electrons rapidly emit a photon of energy and occupy the orbital with the lowest energy or ground state. The emitted radiation from the excited atoms in the form discrete spectral lines forms the basis of emission spectroscopy. Results are summarized in **Table 3**.

#### Scanning Electron Microscope (SEM) Studies

**Instrument:** Electron probe analyzer of model JXA-840 A Scanning Electron Microscopy (SEM) was performed at 'National Chemical Laboratory', Pune. The mounted sample was placed inside the microscope's vacuum column through an airtight door, and then the air was pumped out. After the air was pumped out of the column, a beam of electrons was emitted by an electron gun from the top. This beam travels downward through a series of magnetic lenses designed to focus the electrons to a very fine spot. Near the bottom, a set of scanning coils made the focused beam to move back and forth across the mounted sample, row by row. As the electron beam hits each spot on the sample, secondary electrons are backscattered from its surface. A detector counts these electrons and sends the signals to an amplifier. The final image was built up from the number of electrons emitted from each spot on the sample.

The particles of Vanga Bhasma were viewed under successively increasing magnification up to 50,000X. The smaller particles are found adhered to larger particles. The particles of Bhasma were of irregular shapes in both Jarit and Vanga Bhasma. The numbers of smaller size particles are less in Jarit than Vanga Bhasma. 35% of particles of Jarit were found < 1  $\mu\text{m}$  size and 50% of particles of Vanga Bhasma were found < 1  $\mu\text{m}$  size. The smaller particles of Bhasma were observed as agglomerates i.e. they were found as collected mass. The agglomerates formation was found more in Vanga Bhasma than Jarit Vanga. Some particles having 3  $\mu\text{m}$  size also found in Vanga Bhasma. At 50,000X magnification the nanoparticles about 300 nm were observed in Jarit and Vanga Bhasma. But the percentage of nanoparticles observed more in Vanga Bhasma than Jarit Vanga. **[Figure 1]**

#### X-Ray Diffraction Study

**Instrument:** Philips Holland XRD system PW 1710 using cu - Tube anode

X-ray diffraction studies were performed at 'Sophisticated Analytical Instrument Facility', Nagpur. Vanga Bhasma coded as 77-B, Raw Vanga - 77 - R, Jarit Vanga 77 - J, were subjected to study. The powdered sample was spread onto a double-side tape with a spatula, which was then placed on an aluminum sample holder; it was covered & sealed with glass plate. It was then exposed to x-ray beam of intensity 35KV and 20MA. All the peaks were recorded on the chart, and the corresponding  $2\theta$  (theta) values were calculated. Results are shown in **Figure 2-4**.

The high and sharp peaks were observed in raw Vanga which indicates the presence of crystal structure. But such crystals are infrequent in Jarit and Vanga Bhasma which becomes clear that sharp crystals are very less in number in Vanga Bhasma and Jarit Vanga.

#### Thermo Gravimetric Analysis

**Instrument:** Thermal analyzer, Type-TA 4000, Model -TG 50, Make - Mettler, Switzerland.

Thermogravimetry study was performed at National Chemical Laboratory, Pune, India. The instrument for thermogravimetry is a precision balance programmed for a linear rise of temperature. Vanga Bhasma sample was placed in sample holder and heating was preceded. The temperature

was gradually increased from 35°C to 815°C the rate of temperature increases was maintained at 10°C minute. Weight changes during this period were recorded. A plot of weight change versus temperature of time represents results from the programmed operation of thermo balance. This plot is referred to as the 'Thermogravimetric curve' - TG curve. TG curve gives information that how much weight lost, by heating a sample to a given temperature. The result was presented in the form of graph of weight loss plotted against temperature. Change in weight per unit change in temperature was derived and it's another graph against the temperature i.e. derivative of mass (dm) change with respect to time i.e.  $dm / dt$  was recorded as a function of temperature or time. Another curve i.e. DTA (Differential Thermal Analysis) was obtained it is often considered an adjunct to TG is, in fact more versatile and yields data of a considerably more fundamental nature. This technique is simple as it involves the technique of recording the difference in temperature between a substance and a reference material against either time or temperature as the two specimens are subjected to identical temperature regimes in an environment heated or cooled at the controlled rate. Thus the differential thermogram consists of a record temperature (differential temp;  $\Delta T$ ) plotted as a function of time t, sample temperature ( $T_s$ ), reference temperature ( $T_r$ ) or furnace temperature ( $T_f$ ). DTA allows the detection of every physical and chemical change whether or not it is accompanied by a change in weight.

The resultant curves were obtained as -

- Thermo Gravimetry (TG) curve.
- Derivative Thermo Gravimetry (DTG) curve.
- Differential Thermal Analysis (DTA) curve.

#### Thermogravimetric Analysis of Raw Vanga (R)

**Wt. of the sample** - 9.008 mg

DTA of Raw Vanga shows some endothermic peaks out of these one is sharp endothermic peak which give ideas of change in crystallinity where as another one is somewhat broad endothermic peak which signify the dehydration reaction. The first endothermic peaks observed at 234.3°C and second endothermic peaks observed at 433.4°C. **[Figure 5]**

#### Thermogravimetric Analysis of Jarit Vanga (J)

**Weight of the sample** - 9.686 mg

The thermo-gram of Jarit Vanga shows % weight loss (-3.177) in the temp of (35°C - 815°C which is not a very much significant. DTA of Jarit Vanga shows no any endothermic or exothermic peaks, means there is no any physical and chemical change observed in Jarit Vanga. **[Figure 6]**

#### Thermogravimetric Analysis of Vanga Bhasma

**Weight of the sample** - 6.623 mg

TG - Curve of Vanga Bhasma shows there is no weight gain or loss observed in the temperature range 35°C - 815°C and DTA shows no any endothermic or exothermic peaks means no any physical and chemical changes observed again same as like Jarit, nature of the sample have change. **[Figure 7]**

#### DISCUSSION

Vanga Marana was prepared using Aloe Vera juice through Mardana (trituration) and Puta procedure. According to text Rasa Tarangini, Jarit powder of Vanga without Puta procedure was called as 'Bhasma' so both products were analysed on physicochemical parameters. In present study observed that the Bhasma Parikshas like Varitara, Rekhapurna was not completely passed in Jarit Bhasma but Vanga Bhasma prepared by puta procedure was passed all the

Parikshas. Marana results in formation of complex compound form of Vanga due to collective impact of all the materials and procedures. It has not been possible yet, to describe the exact nature of Bhasma. However following hypotheses get supporting clues from the analytical tests performed.

The loss in the weight at 105°C shows the Moisture Content of the material. The excess Moisture Content in the prepared Bhasma can lead to growth of microbes, fungi and it is not a good sign for Bhasma, because it causes deterioration of Bhasma. If Bhasma contains more moisture then it does not pass the Varitara Pariksha. Hence it is necessary to develop range of standard Moisture Content value the Bhasma.

On analysis of raw Vanga (R), Jarita Vanga (J) and Vanga Bhasma (B) by ICP-AES technique [acid digestion method] it was observed that the percentage of Sn decreased in the Jarita and Vanga Bhasma due to the Heating process as compared to their percentage in raw sample. The percentage of tin in raw sample is 99.9% it was decreased 74.4% in Jarit and 76.5% in Vanga Bhasma, while the percentage of elements Fe, Mg, Ca, K, Al etc. increased in Vanga Bhasma than Jarit Vanga. These additional elements may have origin from the herbs and other additives used during the preparation. These additional elements do not found in Ayurvedic literature but it seems to be used in maintaining fluid balance in the human body.

On XRD Analysis In present study both the Bhasma (Jarit and Vanga Bhasma) showed similar structure i.e. Tin oxide (SnO<sub>2</sub>) (Tetragonal Futile Phase). The particle size to be around 200-300 nm are found in SEM studies and from XRD it was found 28 nm called as 'nano particles'. The considerable numbers of nano particles have been reported during SEM studies. But reported percentage is more in Vanga Bhasma than Jarit Bhasma. This fact suggests that the Bhasma possibly works on the principle of nanotechnology. In the prepared Vanga Bhasma the particles were found to be nanosized which might be working into blood circulation or reach upto any molecule in any tissue of the body. And some particles having size less than 1 µm or 3 µm might be working as catalyst. For this pharmacological action of Bhasma in order to know the actual action of particles of Bhasma is needed. The thermal analysis of Bhasma was also done. These give information about any change in constitution of material is certainly reflected in the nature of curve. The graph is in the form of upward and downwards line, these lines of graph indicates the increase and decrease in weight. The weight goes on changing due to different reactions occurring in the material due to heating. The weight loss suggests escape of volatile material or escape of some gaseous materials resulting due to breakdown reactions. In present study the some weight loss was observed in Jarit Vanga. Which is not very much significant, but in Vanga

Bhasma there was not any weight loss means weight remains constant, due to complete formation or conversion of SnO<sub>2</sub>.

DTA of raw Vanga shows some peaks and the direction of these peaks was downwards. This direction shows some endothermic reaction was carried out in the sample. On this DTA peak we can say that there was some physical changes or reaction was carried out. In the sample of raw Vanga two downwards peaks was observed. From which one was sharp and another was somewhat broad than first. The sharp peak observed at the temperature 234.3°C and the melting point of Vangs is also that. That means melting point can be easily determined by DTA and this can be a direct check on the purity of the compound as a quality control.

On above observation we can say that above technique can be used as a confirmative or supportive test for any Bhasma or Mritaloha. In our Rasgrantha various Bhasma Pariksha was described by Rasaacharyas. Among these Niruttha Pariksha is the one of them. In which the sample of Bhasma is strongly heated along with silver and the main criterion for assessment of Bhasma is related to the weight of silver after Pariksha. In this Pariksha Bhasma is heated along with silver. In this context, this technique can be alternative and supportive to Niruttha Pariksha. And the Advantages over Niruttha Pariksha as it requires minimum quantity of sample i.e. up to few mg, specific controlled mode of heating can be maintained, automated mechanism increased perfection of the results. This technique can be established as the quality control test of Bhasma in reaction of Niruttha Pariksha.

From above observation we can say that Puta procedure plays an important role in Bhasma preparation. After Jarana procedure particle size of Vanga definitely gets reduced but not whole element get converted in to Bhasma which can be removed by Puta & more of nano particles formed. Hence importance of puta remains untouched and it enhances pharmacoclinical action of bhasma.

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