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AN ANALYTICAL STUDY OF *SWARNAMAKSHIKA BHASMA*

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ABSTRACT

Rasashastra is Pharmaceutical science of *Ayurveda* known as *Ayurvedic* Pharmaceutics an integral part of *Ayurveda* which deals with metals, minerals and techniques of conversion of the drugs. Metals and minerals are said to be very effective when used in the form of *Bhasma* and not harmful to body. The preparation of *Bhasma* includes various processing like *Shodhana, Marana, Satvapatana, Amritikarana* etc. *Swarnamakshika* (Chalcopyrite) is one of the main ore of Copper. It is also known as Copper Pyrite. *Swarnamakshika Bhasma* is one such a drug, which is being used since ancient days. Analysis of the *Bhasma* preparation is a very necessary for global acceptence therefore an attempt will be carried out in this project to compare the different samples of *Swarnamakshika Bhasma* on standard *ayurvedic* and modern parameters. The detailed analytical study and results were documented.

KEYWORDS: *Rasashastra, Shodhana, Swarnamakshika Bhasma, Marana.*

INTRODUCTION

Increasing demand of *ayurvedic* products as well as the exponential growth of *ayurveda* pharmaceutical industry in the global market, has at the same time lead to increasing

concerns regarding the safety and efficacy of *ayurvedic* formulations especially *bhasma* preparation. The use of metals and minerals in therapeutics in the form of *Rasyoga* has been started from the period of classical texts, however their use has been flourished only after the development of *Rasashastra*.

These *Rasaushadhis* are supposed to be superior to other herbal drugs on account of their effectiveness in low dosage, easy palatability and quick action without causing any untoward effects. All these properties made *Rasaushadhis* more popular. Thus from centuries we are using metallic and mineral preparations for therapeutic purposes. Though Metals are essential for the growth and development of body, are not suitable for consumption in their natural state. So our Acharyas have found specific methods for their detoxification and *Bhasma* preparation, which make them suitable for clinical use in therapeutic doses. Among these *Bhasma* is specialized form of medicinal formulations. The metallic preparations are mainly termed as '*Bhasma'*.

Swrarnamakshika Bhasma is widely used as compared to others. Before internal administration, these metals and minerals should be subjected to *Shodhana* and *Marana* (Calcination) processes. Quality of a drug depends upon its formulation, processing, and applications. It is essential to fix some standards for manufacture of drugs so that the genuineness of the drug is not compromised. *Ayurveda* texts have described several methods for quality control of finished product such as.

AIM AND OBJECTIVES

(1.) To analyze with objective to characterize the *bhasma* preparation.

(2.) To analyze all samples of *swarnamakshika bhasma* as per classical and modern parameters.

MATERIALS AND METHODS

Preparation of Sample

The all samples of *Swarnamakshika bhasma*[1] were prepared in pharmaceutical lab of department of *Ras Shastra and Bhaishajya Kalpana* and further subjected to analytical studies for various parameters.

All Parameters was taken according to "Protocol of testing of ASU medicines[2]" & Ayurvedic Pharmacopoeia of Indian Medicines, published by Govt. of India, Dept. of Ayush.

Most of tests were conducted at departmental analytical lab and remaining tests were conducted at S.R.LABS, Pratap Nagar, Jaipur.

Procedures

Ancient Parameters

***Varitara* Test**

Take two glass of water and allowed for stagnation. Then very small amount of *bhasma* was sprinkled from a short distance on the surface of stagnant water in glass and observed.

***Rekhapurnata* Test**

Take small amount of *bhasma* between index finger and thumb, then Rubbed it and observed whether the *bhasmas* fill the furrows of the finger tips or not.

Nishchandrata test

Firstly pellets of swarnamakshik samples after *Marana* was observed in sunlight, after their little amount of the *Bhasma* was taken on palm and, observed in sunlight for presence of any luster particles.

Niruttha Test

1 g of *swarna makshik Bhasma* was taken in a crucible and added to an accurately weighted solid piece of silver. It was heated to the similar grade of heat(600ºC) Retain at that temperature for about 5 to 10 seconds before allowing to cool. After complete self cooling, piece of silver was collected and weighed.

***Apunarbhavata* test**

20 g of *swarnamakshik Bhasma* was mixed with equal quantity of each of the ingredients of *Mitra Panchaka* (*Guda*, *Gunja*, *Ghrita*, *Madhu* and *Tankana*) and ground, pellets were prepared and kept in *Sarava Samputa*. After there it was subjected to the similar grade of heat used for the preparation of *Bhasma* (750°C as highest temperature for a duration of 1 hour at highest temperature) and after self cooling, pellets were collected and observed for any lustred particles or accumulated masses.

Nisvadutvam

Small amount of prepared bhasma was kept on tongue found to be testless.

Amla Pariksha

Sour curd was taken into three separate petri dishes. Little bit amount of swarnamakshik bhasma sample A after 10 puta, *swarnamakshika bhasma* sample B after 8 puta, *Swarnamakshika bhasma* sample C after 5 puta were sprinkled over this curd separately. Then mixture is examined next day.

Organoleptic Characters

"Organoleptic evaluation" of drugs refers to the evaluation of a drug by Colour, Odour, Size, Shape, Taste and special features including touch, texture etc. with the help of *Jnanendriya*. The obtained results were shown in table no. 2.

Physico-chemical analysis[3]

pH Determination

Immerse the Digital pH meter in the solution under examination & measure the pH at the same temperature which was used for standard solutions. At the end of the set of measurements, record the pH of the solution used to standardize the meter. If the difference between this reading & the original value is greater than 0.05, the set of measurements must be repeated.

Note the value of pH. The same procedure is applied for 3 sample each time. The obtained results were shown in table no. 3.

L.O.D.

About 10 g of drug is accurately weighed and kept in atarred petri dish. After this petri dish is kept in oven and dried at 105^0C for 5 hours and weighed. Continuous drying and weighing should be done at one hour interval until constant weight is reached in 2 consecutive reading. (2 Consecutive weighing should not show difference of more than 0.01gm). Weight loss (loss on drying) is calculated and expressed as % w/w. The obtained results were shown in table no. 3.

Ash Value

Incinerate 2 gm of drug accurately weighed and ground in a tarred silica dish. Keeps the crucible in a muffle-furnace at a temperature 550^0C until free from carbon (white ash). Then cool the content and weigh. Calculate the percentage of ash with reference to the air-dried drug. The obtained results were shown in table no. 3.

Acid-Insoluble Ash

Transfer the ash obtained from Total Ash test in a 250 ml beaker without loss of ash and adds 100 ml of diluted hydrochloric acid. Wash the crucible with 10 ml of acid and transfer the washing to the beaker. Heat the beaker till the liquid boils. Then filter the solution and collect the insoluble matter on an ash less filter paper (Whatman no.41). Wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible. Dry on a hot plate and ignite at 600^0C in a muffle furnace (until it becomes white ash). Allow the residue to cool in suitable desiccators for 30 minutes and weigh without delay. Repeat the process until constant weigh is obtained. Calculate the Acid-insoluble ash with reference to the air dried drug. The obtained results were shown in table no. 3.

Water Soluble Ash

Water Soluble Ash shows quantity of water soluble inorganic Substance. Calculate the Water - insoluble ash with reference to the air dried drug. The obtained results were shown in table no. 3.

Sulphated Ash

Heat a silica crucible to red for 10 minutes. Allow to cool in desiccators and weight (W_2).

Put accurately weighed 1 to 2 gm (W_1) of the powdered substance into the crucible. Ignite gently at first until the substance is thoroughly charred. Cool, moisten the residue with 1 ml of concentrated sulphuric acid. Heat gently until white fumes no longer evolved. Ignite at 800˚C ± 25˚C until all black particles have disappeared. Conduct the ignition in a place protected from air currents. Allow the crucible to cool, add a few drops of sulphuric acid and heat. Ignite as before; allow cooling and weighing (W_3). Repeat the operation until two successive weighing do not differ by more than 0.5 mg. The experiment should be conducted in a fume cub-board. The obtained results were shown in table no. 3.

Mineral Determination by AAS

Significance

1. Estimation of heavy metals which is a criterion to judge the quality or purity of Sample.
2. Tool for standardization of sample.

Applicability: Raw material & finished product (except bhasmas & rasaushadi).

Equipments & Glasswares: Weighing Analytical balance capable of determining, 0.1 mg, Micropipette, 10100
ìL & 1001000
ìL, Volumetric flasks – Class A, 10 ml, 50ml and 100 ml, Whatman no. 1 filter paper or equivalent, Filter funnel, or equivalent , AAS (Atomic Absorption Spectrophotometer), Porcelain Crucible, 100 ml Glass Beaker.

Reagents & Chemicals: Conc nitric acid (suprapure), Conc HCl (suprapure), polypropylene, polyethylene, distilled water.

Procedure

Wet Digestion

Accurately weigh 0.5 to 2 g test portion of well mixed material and transfer to 250 mL beaker and add 210 mL HNO3 cover beaker with watch glass, heat to 95°C on hot plate, and the digestion continued till no brown fumes evolved and solution becomes clear and colour less. Cool the beaker to room temperature. Transfer the solution into a volumetric flask and dilute the mark with doubled distilled water. Dilute sample if necessary.

Preparation of Standards for AAS, Fe and Cu

(i) Preparation of Iron Standard:

Prepare 0.5, 1.0, 1.5 and 2.0 ppm standard for calibration. Plot a linearity curve by using Mentioned standard solutions. Correlation coefficient should not be less than 0.990.

(ii) Preparation of Copper Standard

Prepare 0.25, 0.5, 1.0 and 2.0 ppm standard for calibration. Plot a linearity curve by using Mentioned standard solutions. Correlation coefficient should not be less than 0.990.

Measure the sample corresponding to the linearity plotted by standards.

Sulphur Estimation

Take 0.5 to 1 g of sample in 250 ml beaker. Add 10 ml of CCl_4 saturated with bromine and keep in cold condition for overnight. Add 10-15 ml of HNO_3 digest on waterbath, then add 10 ml of HCl again digest it to expel the NO_2 fumes till syrupy mass is obtained. Cool and extract with HCl, make up volume up to 100 ml, boil and filter through Whatman filter paper no. 40 and wash the residue with hot purified water. Treat the filterate with ammonia solution for R_2O_3 precipitations, here R stands for Fe and Al. Filter through Whatman no. 41 filter

paper in 500 ml beaker, acidify the filterate with HCl and 20 ml of 10% barium chloride solution. Stir the solution and digest on burner. Allow to precipitate to settle for overnight. Filter the precipitate with Whatman no. 42 filter paper and wash with purified water. Ignite the precipitate in muffle furnace in pre weighed platinum crucible upto 850^0C. Allow to cool and weigh. Calculate the weight of sulphur by multiplying the weight of precipitate with 0.13734. The obtained results were shown in table no. 3.

X-Ray Diffraction

Principle

The distance between each set of atomic planes (i.e. inter atomic space'd') is determined with the help of wave length (λ) of x-ray beam and angle of diffraction (θ). by applying Bragg's Law (n λ= 2d sin θ).

No two substances have absolutely identical diffraction patterns. The'd' spacing of the ten most intense reflecting planes of atoms are calculated and results are compared with the data of x-ray powder data file and identification of the sample is done.

Purpose and Scope

This document outlines the procedures for sample analysis using x-ray diffraction (XRD). The x-ray diffractograms will be used to identify minerals from rock pile materials, alteration scars, and rock samples. The types of products generated will include x-ray spectra and pattern matching tables including identification of minerals present. All pattern matching will be based on standard powder diffraction files.

Procedure

At the start of the day, check the calibration of the XRD machine with a silicon (Si) standard. Mount the silicon standard in the XRD. Go to Controller in DataScan. Under Controller, go to Scan Line. Make sure the silicon standard peak will be scanned (make sure the scan range is set so the Si peak will be covered). After the machine has stopped, it will display a single peak with a vertical line. The vertical line is the position of the Si (111) peak where it appears according to the computer. This should match the peak the XRD found. If the two don't match, you will have to move the vertical line to calibrate the XRD to the Si standard. The computer will ask if you want to change the set angle to the new value. If the new value is okay, press the YES button with the cursor, if not, press the NO button and move the vertical line until it matches the peak.

Fill in the File ID and Scan ID. File ID is the name of the computer file the data will be stored in and Scan ID is the sample number or name.

Scan from 2.0 degrees to 35.0 degrees 2θ, with an s stepsize of 0.03 degrees and a dwell time of 0.5 seconds. Always scan with the axes coupled C. INSTRUCTION FOR USING JADE 1) Open powder pattern(s) saved from the Datascan analysis 2) Select background correction or background edit to remove or modify background pattern 3) Select scan match, set to Minerals, to match pattern to known powder patterns.

Select Peak ID extended to print table of peak match data.

Select Phase ID report to print graphic file of scan and superimposed match spectra.

Save manipulation files in appropriate directory. The obtained results were shown in table no. 4, 5 and 6.

Particle size analysis

Laser Diffraction Method

Laser diffraction has become one of the most commonly used particle sizing methods, especially for particles in the range of 0.5 to 1000 microns. It works on the principle that when a beam of light (a laser) is scattered by a group of particles, the angle of light scattering is inversely proportional to particle size (ie. the smaller the particle size, the larger the angle of light scattering). Laser diffraction has become very popular because it can be applied to many different sample types, including dry powders, suspensions, emulsions and even aerosols. It is also a very fast, reliable and reproducible technique and can measure over a very wide size range. The obtained results were shown in table no. 7.

Other Methods

There are many other methods for analysing particle size, other than laser diffraction. Sieving is one of the oldest particle sizing methods and is still widely used for relatively large particles (ie. > 1mm). When measuring very small particles (ie. < 0.5um), Dynamic Light Scattering is by far the easiest methods to use. And if you need to measure morphological properties of particles, (ie. shape as well as size), then image analysis methods are the only way to gain the extra information.

RESULTS

1. Ancient Parameters

Table no.1: showing Analysis of All samples of *Swarnamakshika Bhasmas* on the basis of Ancient parameters.

Parameters	*Sample (A)*	*Sample (B)*	*Sample (C)*
Varitaratva	Positive	Positive	Positive
Rekhapurnatva	Positive	Positive	Positive
Nischandrata	Positive	Positive	Positive
Niruttha	Positive	Positive	Positive
Apunarbhavata	Positive	Positive	Positive
Nisvadutvam	Positive	Positive	Positive
Amla Pariksha	Positive	Positive	Positive

2. Organoleptic Characters

Table no. 2: showing the Physical description of all Samples of *Swarnamakshika Bhasma* Sample A, sample B, Sample C.

Sr. no.	Tests	Sample (A)	Sample (B)	Sample(C)
1.	Appearance	Very Fine powder	Very Fine powder	Very Fine powder
2.	Colour	Dark Reddish	Black and Reddish	Black
3.	Odour	Characteristic	Characteristic	Characteristic
4.	Taste	Tasteless	Tasteless	Tasteless

3. Physico-Chemical Parameters

Table no. 3: showing the Physico-chemical description of all Samples *Swarnamakshik Bhasma* Sample *A*, Sample *B* and Sample C *Bhasma.*

Parameters	*Swarnamakshik Bhasm* (A)	*Swarnamakshik Bhasma* (B)	*Swarnamakshik Bhasma*(C)
pH value(1%v/w)	6.18	4.14	3.73
Loss on drying(by oven)	.98% w/w	1.04% w/w	.87% w/w
Total Ash	99.42% w/w	99.17% w/w	95.02% w/w
Acid insoluble Ash	92.27% w/w	85.54% w/w	80.91% w/w
Water soluble Ash	1.34% w/w	3.96% w/w	17.56% w/w
Sulphated Ash	99.54% w/w	99.07% w/w	81.62% w/w
Assay of Element **Iron(Fe)** **Copper(Cu)** **Sulpher(S)**	 9.43% w/w 2.77% w/w .52% w/w	 9.59% w/w 3.32% w/w 2.04% w/w	 11.34% w/w 3.88% w/w 10.05% w/w

Table No. 4: Showing The main compound diffraction peaks for *Swarnamakshika Bhasma* Sample A with Intensity.

S. no.	Compound name	2^0 Value	Intensity
1.	**CuS**	30.2557	3170
2.	**Fe_3O_4**	35.6101	5302
3.	**$FeSO_4$**	43.3386	3027
4.	**Fe_3O_4**	57.2297	3398
5.	**Cu_2S**	62.9376	3705

Table No. 5: Showing The main compound diffraction peaks for *Swarnamakshika Bhasma* Sample B with Intensity.

S. no.	Compound name	2^0 Value	Intensity
1.	CuS	29.8011	3098
2.	Fe_3O_4	35.1555	5214
3.	$FeSO_4$	43.0355	3079
4.	Fe_3O_4	56.8761	3418
5.	Cu_2S	62.584	3602

Table No. 6: The main compound diffraction peaks for *Swarnamakshika Bhasma* Sample C with Intensity.

S. no.	Compound name	2^0 Value	Intensity
1.	CuS	29.5485	2001
2.	Fe_3O_4	32.7309	2533
3.	Fe_3O_4	55.9163	2452

Table No. 7: Showing Partical size analysis of all Samples of *Swarnamakshika Bhasma* (Analysis in triplicate)

S. no.	Sample name	Average Particle Size (µm)	Particle Size distribution 50% (µm)	% intensity
1.	SMB A I	1.707	0.631	100
2.	SMB A II	2.027	0.892	100
3.	SMB A III	2.639	1.185	100
4.	Average-	2.124	0.902	
5.	SMB B I	3.39	0.903	100
6.	SMB B II	2.825	1.133	100
7.	SMB B III	3.327	1.260	100
8.	Average-	3.18	1.098	
9.	SMB C I	3.336	0.561	100
10.	SMB C II	2.24	0.439	100
11.	SMB C III	1.814	0.578	100
12.	Average-	2.463	0.526	

DISCUSSION

Analytical study deals with the analysis of the values of some physical constants and chemical values of the prepared formulation. In present research work all samples of *Swarnamakshika bhasma* were tested on various preliminary parameters as well as on some of the sophisticated analytical tests.

Classical orgenoleptic examination shows changes in physical properties of the *bhasma*, on the basis of *Shabda, Sparsha, Rupa, Rasa* and *Gandha*. Test of *Dantagrakacakacabhava* shows almost negligible sound was present in the *bhasma* of all samples. Final *bhasma* of all samples was very smooth. In all samples blackish brown powder was converted into reddish brown colour *bhasma* in sample A, black and red colour *bhasma* in sample B and black brown colour *bhasma* in sample C. All samples of finished product were tasteless and without any peculiar odour.

Classical test for *bhasma* –All samples of finished products (Bhasma after 10^{th},8^{th} and 5th *varahapuţa*) show complete positive *rekhapurnatva*. *Varitara* test is positive for all finished product. In *Amla pariksha* no any bluish tinge was appeared in all finished products. So it can say that final prepared *bhasma* of all samples has become free from toxic effects of the copper. *Bhasma* obtained after 10^{th}, 8^{th},and 5^{th} *Varahapuţa* has the quality of *Shlaksṇatva* (smoothness), *Mŗidutva* (softness) and *Nishchandŗatva* (free from lustre). So all the classical organoleptic and *bhasma* examination have been successfully accomplished. All samples of finished product has shown satisfactory results. Though all these classical tests are based on only physical properties of the *bhasma*, but the significance of these tests is that, *bhasma* can be obtained in such a form that it could be easily acceptable for body system without any toxic effects.

Physico chemical tests of sample A, sample B and sample C *Swanamakshika bhasma reveal* that all the sample of *Bhasma* contain very high ash value of 99.42%, 99.17% and 95.02%. Ash value indicate presence of inorganic contents of *Bhasma*. Very high total ash value of three *bhasma* the *Bhasma* is indicative of presence of very high inorganic content. loss on drying value of the three *bhasma* was very low having values. 98%, 1.04%, 87%. Very minute loss on drying of the *Bhasmas* are indicative of presence of very little amount of moisture. Test for acid insoluble ash was carried out to evaluate the percentage of insoluble inorganic content of the *Bhasma* in dilute acid. Since a drug must first pass into solution before it can be absorbed, so the acid insoluble ash test of *Bhasma* is therapeutically very

important. It is intended to provide a step towards the evaluation of the physiological availability of the *bhasma* sample A *Bhasma* contains comparatively more acid insoluble ash (92.27%) than that of sample B *Bhasma* 85.54% and sample C *bhasma* 69.81%.The pH of the sample A,sample B and sample C were having 6.73%,4.14%,3.73% respectively. water soluble ash is less in sample A 1.34%, than sample B 3.96% and sample C 17.56%. The sulphated ash of the sample A, sample B and sample C were having 6.73%,4.14%,3.73% respectively.

Table No. 8: Showing results of Assay of Element of all samples.

Assay of Element	Sample A	Sample B	Sample C
Iron(Fe)	9.43% w/w	9.59% w/w	11.34% w/w
Copper(Cu)	2.77% w/w	3.32% w/w	3.88% w/w
Sulpher(S)	.52% w/w	2.04% w/w	10.05% w/w

% of Fe, Cu are comparatively same in all samples of *Swarnamakshika bhasma* but % of S is comparatively more in Sample C may be due to *Ghandhaka* use as a marak Dravya in sample c method.

X-Ray diffraction

This technique was used for the characterization of compound through the crystalline phase identification. Sample identification was done by matching with intensity with the standard JCPDS database. Analysis of all samples of *Swarnamakshika bhasma* were done by powder crystal method, as shown in the results of XRD graphs. XRD study was showing various peaks crystallographic structure, chemical composition and physical properties of materials.In sample A Sharp single peaks shows that major compounds of Iron, Copper, Sulphur.In Sample B Sharp single peaks shows that major compounds of Iron, Copper, Sulphur.In Sample c Sharp single peaks shows that major compounds of Iron, Copper.

Table No. 9: Showing results of XRD pattern of All *Swarnamakshika Samples.*

Samples	Compounds
Sample A	CuS +Fe_3O_4+Fe_3So_4+Cu_2S
Sample B	CuS +Fe_3O_4+Fe_3So_4+Cu_2S
Sample C	CuS +Fe_3O_4

Particle size

Particle size is one among the several factors which affect the distribution and dissolution of drug. Particle size and area of solid drug are inversely proportional to each other, smaller the particle size greater the area for chemical reaction and thus greater the activity of drug. As

seen from the table 4.7 among the different samples the least particle size was observed in Sample C ie. 526µm followed by Sample A having .902µm and Sample B having a particle size of 1.098 µm. Thus among the three preparations Sample *C* had lowest particle size which may have in turn lead to its greater bio availability.

Table No. 10: Showing Partical size analysis of all Samples of *Swarnamakshika Bhasma.*

Samples	Average Particle Size (µm)	Particle Size distribution 50% (µm)
Sample A	2.124	.902
Sample B	3.18	1.098
Sample C	2.463	.526

CONCLUSION

Analytical test results of all samples of *Swarnamakshika bhasma* were within normal limits. In XRD Study of sample A and Sample B the peaks shows that major compounds of Iron, Copper, Sulphur in oxide form but in Sample C peaks shows that major compounds of Iron in oxide form and Copper in sulphide form. On the basis of Particle size analysis data Sample C found to be best in Particle size as it possess lowest size. These quality control parameters can be considered as tool for preparation , safety and efficacy of formulations. Hence, further clinical study can be helpful for evaluating the results.

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