RESEARCH ARTICLE

Particle size estimation and Elemental analysis of *Yashada Bhasma*

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Abstract

Background: Analytical standardization may help to assure adoption of recommended pharmaceutical process, safety, quality, and ultimately, most probably efficacy of medicinal formulation, and thus, it is mandatory for the evaluation of incinerated metallic-mineral preparations, like *Yashada Bhasma* (incinerated Zinc), a widely used medicine in Ayurvedic therapeutics. The present attempt is to revalidate the pharmaceutical process of preparation of *Yashada Bhasma* using two different media. **Materials and Methods:** In this study, analytical standardization of two types of *Yashada Bhasma*, *Vanaspati Jarita Marita Yashada Bhasma* (JMY) and *Parada Marita Yashada Bhasma* (PMY), were initially carried out by means of *Bhasma Pariksha* (classical methods for evaluating *Bhasma*). Afterward, this was evaluated by qualitative and quantitative analytical methods such as ash value, dynamic light scattering (DLS), zeta potential, X-ray diffraction (XRD), scanning electron microscopy (SEM), inductively coupled plasma-atomic emission spectrometry (ICPAES), carbon, hydrogen, nitrogen, and sulfur (CHNS), thin layer chromatography (TLC) and Fourier-transform infrared spectroscopy (FTIR). **Observation and Discussion:** Classical analytical test revealed that *Bhasma* prepared fulfilled all criteria. Modern analytical techniques like XRD identified the PMY and JMY as ZnO and ZnS, respectively. Both JMY and PMY samples were in nanorange as per DLS study. As per SEM study, JMY and PMY sample shows 70. 21% and 44.80% of Zn by weight. In inductively coupled plasma-atomic emission spectrometer analysis, micronutrients such as Cu, Fe, Mg, P, Al, Pb, and Zn were present in ≤1% and Ti, Co, Mn, Ni, Cr, and Cd were detected in ppm level in both JMY and PMY samples. **Conclusion:** *Parada Marita Yashada Bhasma* was detected as ZnS and *Vansapti Jarita Marita Yashada Bhasma* as ZnO. As they correspond to different compounds, it cannot be compared analytically and further clinical evaluation is necessary for the same.

Key words: Analysis, standardization, yashada, zinc

INTRODUCTION

Analytical standardization assures that the products are true beyond any doubt in respect of quality, efficacy, performance, and safety[1] which is inevitable in incinerated metallic-mineral preparations, like *Yashada Bhasma* (incinerated zinc), a widely used medicine in diabetes, Parkinson's disease, eye diseases, night sweating, menorrhagia, and respiratory disorders in Ayurvedic therapeutics. "*Bhasma"* unique Ayurvedic herbo-mineralmetallic compounds of nanodimensions are the products of "Rasa Shastra," classical Indian Alchemy.[2] Physiochemical changes which take place during processing methods and final product characteristics can be standardized by means of various analytical techniques. It also helps to know the probable role of media during the pharmaceutical processing. This is possible by establishing certain qualitative and quantitative parameters of medicines. A major advantage in case of mineral preparations is that it can be analyzed through both classical and modern qualitative and quantitative parameters. The present attempt is to revalidate the ayurvedic *Bhasma* concept, as organometallic ethnonanomedicine through *Yashada*

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Bhasma prepared using two different media, through it's analysis by ancient and modern parameters.

MATERIALS AND METHODS

Method of Preparation of Test Drugs

Parada Marita Yashada Bhasma (PMY) and *Vanaspati Jarita Marita Yashada Bhasma* (JMY) were prepared as per classical Ayurveda guidelines.[3] PMY sample was prepared by means of *Pishti* preparation with *Parada* (Mercury) and *Gandhaka* (sulfur) and JMY sample prepared with prior *Jarana* (roasting) with *Apamarga Panchanga* (whole plant of *Achyranthes aspera* Linn.).[4] Both were subjected to (puta) incineration in electric muffle furnace (EMF).

Analysis

Organoleptic characteristics

Organoleptic characters of PMY and JMY samples satisfying *Bhasma Pariksha* (Classical methods for evaluating *Bhasma*), signify the genuinity of the sample. These characters correspond to the *Panchagyanedriya Pariksha* of *Ayurveda*. Here colour, odour, taste and touch of the sample were examined.

General qualitative analysis

Ash value, Moisture content and Acid insoluble ash value of both samples were analyzed. Total ash of a mineral, is a mixture of inorganic and organic residue remaining, after ignition in a silica dish at a temperature of 600-650º c. In case of minerals, this helps to detect the percent of organic matter remained after incineration.[5]

Specific quantitative and qualitative analysis for Minerals

Techniques like DLS, XRD, SEM, ICPAES, CHNS, TLC and FTIR were used to analyse the samples of *Bhasma*. Details of methodology of techniques used are mentioned below:

Dynamic Light scattering method (DLS)

Characterization of particles was made using DLS method. To determine the mean particle size, the *Yashada Bhasma* was analyzed using zeta sizer. Water was used as a dispersant media and the study was carried out at a temperature of 250°C. Measurement position was 5.50 mm and the count rate was 213.4 kcps. Clear disposable zeta cell was used to carry out the study. Zeta potential was analyzed to measure the stability and charge repulsion/attraction between the nanoparticles.

X-ray diffration (XRD)

X-ray powder diffractometry analyzes the material structure at the atomic level. Bruker AXS, D8 advance model was the instrument used. The sample is smeared over sample holder and fixed on the sample stage in goniometer. The instrument is set with B-B geometry. The current and voltage are set to 40 mV and 35 mA, and data have been collected. The graph representing XRD analysis is called diffractogram. The peak represents a "phase" (compound) present in a specimen. Experimentally observed pattern can be compared to a standard database called powder diffraction file and each "phase" can be identified. Crystallite size was estimated using DS formula.

Scanning Electron Microscope (SEM)/Energy Dispersive X-ray Spectroscopy (EDS)

SEM images a sample by scanning it with a high-energy beam of electrons in a raster scan pattern.[6] It shows simple contrasts between organic-based and metallic-based materials and provides exact chemical nature, size, and morphology of particles. The JEOL JSM-7600F FEG-SEM model was used at a resolution of 1.0 nm (15 kv), 1.5 nm (1 kv) in GB mode.

Inductively Coupled Plasma-atomic Emission Spectrometry (ICPAES)

ICPAES is an instrument useful for measuring higher concentrations of individual ingredients in a compound formulation. The wavelength at which emission occurs identifies the element, while the intensity of the emitted radiation quantifies its concentration.[7] Thermo electron iris intrepid II XSP DUO is the model used. Samples were analyzed by ICPAES for the presence of microelements. The given samples are digested in HNO_3, made up to 50 ml, filtered, and analyzed with ICP-AES system.

Analysis of Carbon (C), Hydrogen (H), Nitrogen (N), and Sulfur (CHNS)

The percentage of elements C, H, N, and S in an organic compound can be individually or simultaneously analyzed over a wide range of sample matrices and concentrations with this instrument.[8] Elementar Vario EL III was the model used.

Fourier-transform Infrared Spectroscopy (FTIR)

FTIR is a technique which is used to obtain an infrared spectrum of a compound. It is the superposition of absorption bands of specific functional groups.[9] Thermo Nicolet Avatar 370 was the model used. Both samples were subjected to FTIR study.

Thin layer chromatography (TLC)

0.5 g sample was mixed with 3mL methanol, 15mint Sonicate, further filtered and injected to TLC plate.

Table 1: *Bhasma Pariksha* during consecutive *puta*

Number of puta and temperature	*Bhasma Pariksha* of PMY sample	Number of puta and temperature (JMY)	*Bhasma Pariksha* of JMY sample
1st–450°C	Didn't satisfy *Varitaratwa* test	1st–450°C	Did not satisfied *Varitaratwa* test. (Floats in water)
2nd–500°C	Didn't satisfy *varitaratwa* test	2nd–500°C	*Bhasma* did not satisfied *Varitaratwa* test
3rd–500°C	100% of *Bhasma* satisfied *Varitaratwa* test	3rd–500°C	100% of *Bhasma* satisfied *Varitaratwa* test. *Bhasma* was not soft when biten with teeth. Furthermore, *Nirutha* test and *Apunarbava* test were negetive
4th–550°C	Didn't pass *Nirutha* and *Apunarbava Pariksha*	4th–500°C	*Bhasma passed Rekha purnatha* test (*Bhasma* could enter the furrows of finger tip) and it was tasteless too
5th–600°C	Do	5th–550°C	*Bhasma cleared Nirutha* test and *Apunarbava* test (Metallic particles were not regained). *Bhasma* was soft when biten with teeth
6th–650°C	Do		
7th–700°C	Satisfied all *Bhasma Pariksha*		

Observation

Bhasma Pariksha

It took 7 *puta* for PMY sample and 5 *putas* for JMY sample to pass relevant *Bhasma Pariksha*. Observations after each consecutive puta are enlisted in Table 1.

Organoleptic characters of samples satisfying *Bhasma Pariksha* were assessed and enlisted in Table 2.

Table 2: Organoleptic characters of PMY and JMY

Parameters	Observations	
	PMY	JMY
Color	Grayish-black	Cream
Odor	Indistinct	Indistinct
Taste	Tasteless	Tasteless
Form and texture	Crystalline	Crystalline

Ash Value

Ash value of PMY sample shows the presence of 11% organic matter. However, there was no organic matter left in JMY sample. Both samples were 98–99% soluble in HCl [Table 3].

Table 3: Ash value and moisture percent in PMY and JMY

Batch code	Ash value % w/w	Moisture content (%)	Acid insoluble ash (%)
PMY	89.360	2.966	2.020
JMY	100.094	1.969	1.612

DLS

Particle size of both samples reduced significantly, which facilitate absorption and assimilation of the drug into the body. JMY particles were distributed with in the range of ≤1–2300 nm. However, a maximum number of particles were in 1726 ± 297.3 nm range. "Z average," the average particle size of JMY samples was 2300 (d. nm) [Figure 1]. A maximum number of particles have zeta potential with in the range of −22.4–+7.50. Moreover, average zeta potential of the particles is −21.1. Zeta deviation (SD) is 9.69 mV. Conductivity is 0.0526 mS/cm [Figure 2].

PMY particles were distributed with in the range of ≤1–10,000 nm, but a maximum number of particles were in the range of 288 ± 15.53 nm. "Z average" is 2647 nm [Figure 3]. A maximum number of particles have zeta potential with in the range of −14.4–+7.50, and average zeta potential of the particles is −9.20, which shows the particles are instable. Zeta deviation (SD) is 33.7 mV. Conductivity is 0.0254 mS/cm [Figure 4].

XRD

XRD phase identified 5 phases of free Zn and 5 phases of ZnO in *Yashada* [YJ] sample (without *Marana*). In JMY,

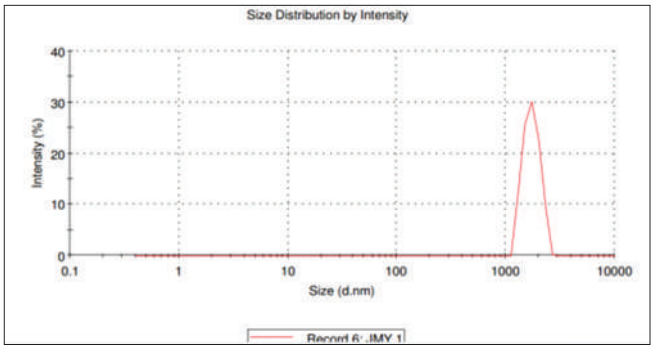

Figure 1: Particle size analysis of JMY sample

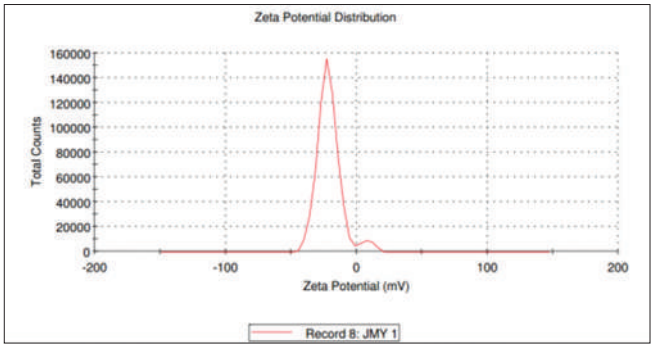

Figure 2: Zeta potential of JMY sample

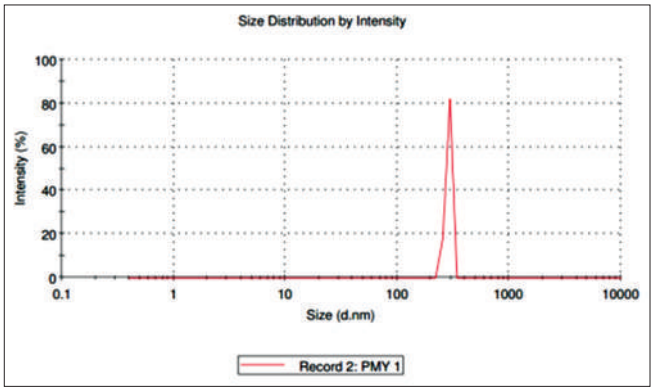

Figure 3: Particle size analysis of PMY sample

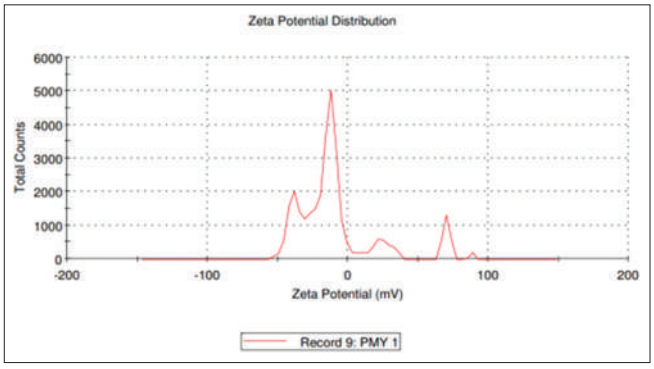

Figure 4: Zeta potential of PMY sample

all phases represent the presence of zinc as zinc oxide and in PMY, all phases of zinc were obtained as zinc sulfide. PMY

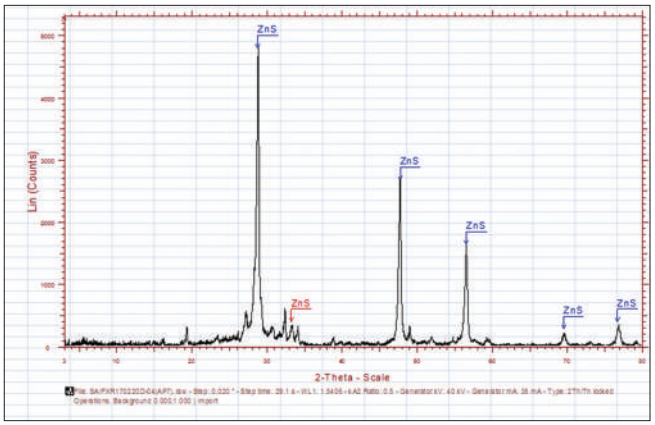

Figure 5: XRD of PMY sample obtained after *Apunarbhava Pariksha*

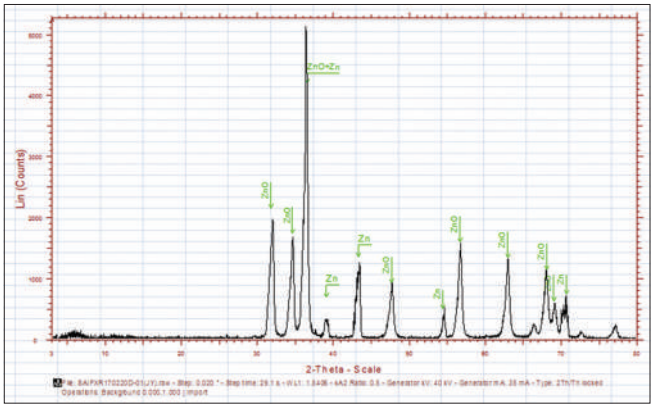

Figure 6: XRD of JY sample

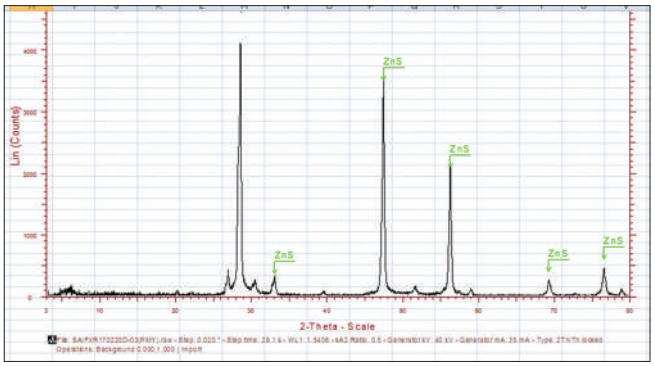

Figure 7: XRD of PMY sample

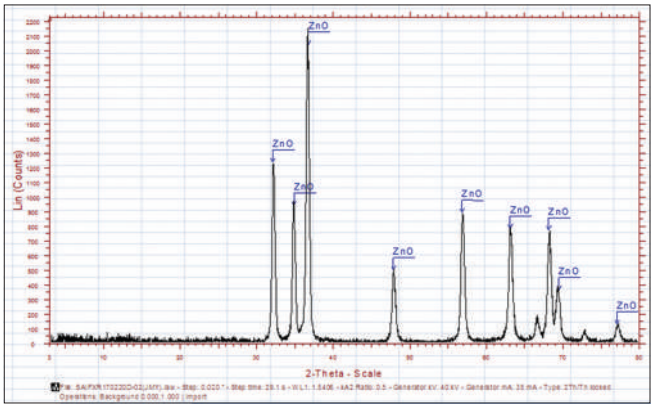

Figure 8: XRD of JMY sample

(7th *puta*) obtained after *Apunarbava* test showed the same chemical constitution as that of PMY in XRD [Figures 5-8].

SEM/EDS: PMY sample shows 44.80% and 23.98% of Zn and $CaCo_3$ by weight. Minor percentage of As, Cu, Fe, FeS2, wollastonite, and KCl was also detected. The graph obtained is the spectrum of the EDS analysis [Table 4 and Figures 9 and 10].

JMY sample showed 70.21% and 20.47% of Zn and silicon dioxide by weight. Elements such as $CaCo_3$, Cu, Fe, and KCl were also detected. Image reveals the presence of nanoparticles [Table 5 and Figures 11 and 12]. EDS was done on a particular area, and the image obtained in both samples reveals the presence of nanoparticles of 10 nm.

ICPAES: Elements such as Cu, Fe, Mg, Ph, Al, Pb, and Zn were present in ≤1% and elements such as Ti, Co, Mn, Ni, Cr, and Cd were detected in ppm level in both JMY and PMY samples, but 0.309% of arsenic and 31.1 ppm mercury were detected in PMY sample only [Table 6].

Analysis of CHNS: Percentage of carbon, nitrogen, and sulfur present in PMY sample was comparatively more than that of JMY sample. Element hydrogen was absent in both samples [Table 7].

FTIR: Based on the peak assignment obtained in FTIR of PMY sample, the presence of functional groups such as alkanes, hydrazine, and cyclopropanes can be assumed. In JMY sample, peaks corresponding to alkanes and dialkyl sulfones were obtained. Metal oxide vibration in FTIR showed the possibility of zinc oxide [Tables 8 and 9 and Figures 13 and 14].

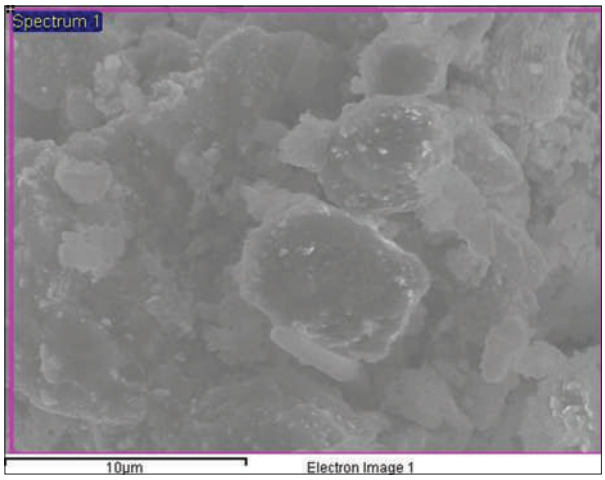

Figure 9: Scanning electron microscopy image of PMY

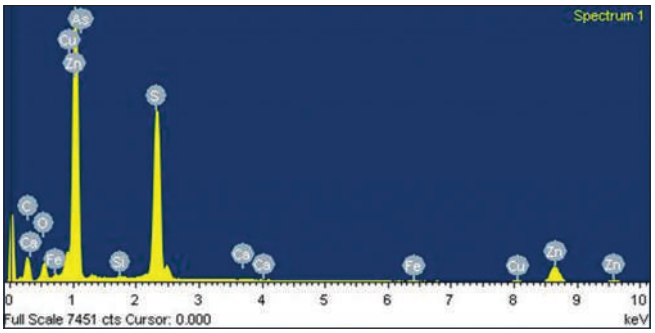

Figure 10: Spectrum of energy dispersive X-ray spectroscopy (PMY)

Table 4: SEM analysis of PMY sample

Element	Weight%	Atomic%
C K	23.98	53.07
O K	5.22	8.67
Si K	0.17	0.16
S K	22.05	18.28
Ca K	0.19	0.12
Fe K	0.64	0.30
Cu L	1.98	0.83
Zn L	44.80	18.22
As L	0.98	0.35
Total	100.00	

Table 5: SEM analysis of JMY sample

Element	Weight%	Atomic%
C K	5.38	15.52
O K	20.47	44.33
Si K	0.67	0.83
Cl K	0.30	0.29
Ca K	0.43	0.37
Fe K	0.84	0.52
Cu L	1.70	0.93
Zn L	70.21	37.21
Total	100.00	

SEM: Scanning electron microscopy

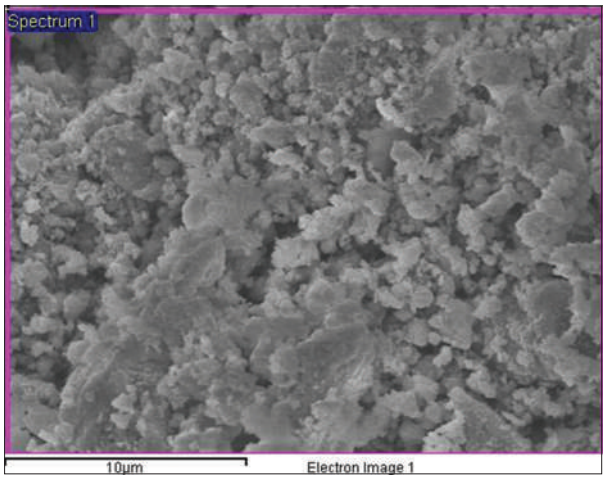

Figure 11: Scanning electron microscopy image of JMY *Bhasma*

TLC

Three major bands were detected in JMY and two major bands in PMY.The colour and corresponding refractive index are given below.

JMY-Blue(Rf-0.68),Violet(Rf-0.79),Blue(Rf-0.90)

PMY-Major bands are Blue (Rf-0.88), Brown (Rf-0.95) [Figure 15 and 16]

DISCUSSION

The pharmaceutical process converts the metal from its zerovalent state to some compound state such as oxides and sulfides in which they function best.[1] The process eliminates the toxic nature of metal along with rendering a higher medicinal value.[10] Hence, evaluation of *Bhasma* is obligatory to make assure of its form.

Bhasma Pariksha done at different stages of *Puta* (incineration setup) revealed that PMY fulfilled classical parameters of *Bhasma* after 7th *Puta*, whereas *JMY* required 5 *Puta* for the same.[3]

Ash value analysis showed that both samples have good solubility in HCl. Variations in the solid content and pH value of the media after *Shodhana* of *Yashada* depicts the transfer of certain impurities from the metal to the Shodhana media. Based on analytical parameters,

Table 6: ICPAES analysis of PMY and JMY

Elements	JMY	PMY	Unit	Detection limit in ppm
Cu3273	0.391	0.113	%	0.10
Fe2599	1.349	0.613	%	0.10
Mg2852	0.234	0.158	%	0.10
Ph1859	0.064	0.011	%	0.10
Al3961	0.081	0.040	%	0.01
Pb2203	0.047	0.012	%	0.05
Zn2062	6.169	4.414	%	0.01
Ti3349	12.79	28.67	ppm	0.05
Co2388	10.66	6.05	ppm	0.01
Mn2576	112.55	22.98	ppm	0.01
Ni3414	30.93	3.29	ppm	0.01
Cr3578	75.15	5.92	ppm	0.01
Cd2144	5.77	3.05	ppm	0.01
As1937		0.309	%	0.10
Hg1849		31.1	ppm	0.10

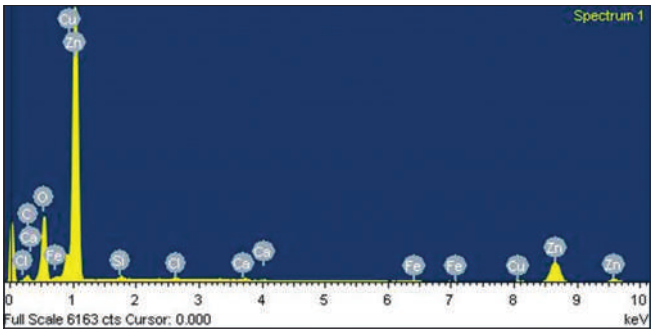

Figure 12: Spectrum of energy dispersive X-ray spectroscopy (JMY)

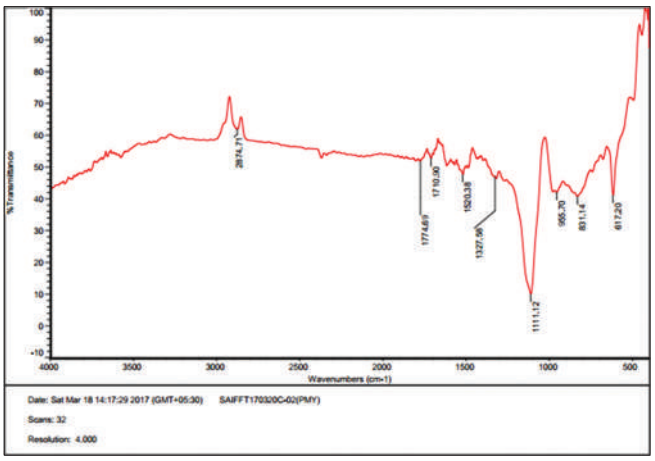

Figure 13: Fourier-transform infrared of PMY sample

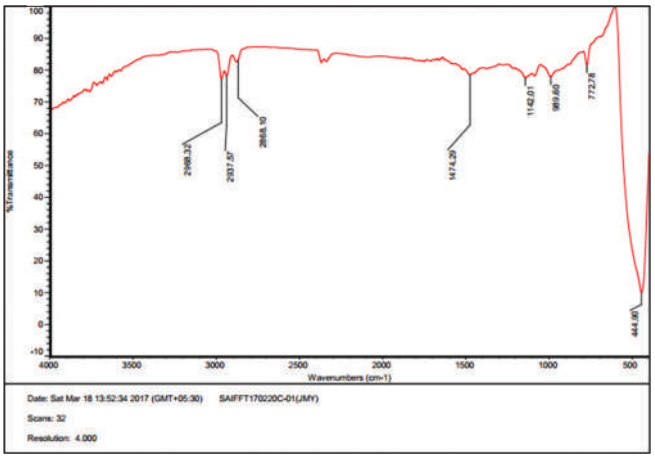

Figure 14: Fourier-transform infrared of JMY sample

Table 7: CHNS-analysis

Sample No	Sample name	N%	C%	S%	H%	Sample weight, mg
1	JMY	0.07	0.22	0.13	ND	7.36
2	PMY	0.09	3.95	31.34	ND	5.19

compound of JMY sample differs from PMY sample and they vary in many factors such as particle size, zeta potential, zinc percentage, and level of trace element. Although both the samples are found to be in nanorange, PMY samples showed comparatively less particle size. Nanoparticles have a natural tendency to agglomerate on storage, so the stability of the nanoparticles plays an important role in preservation of pharmacological properties for long term.

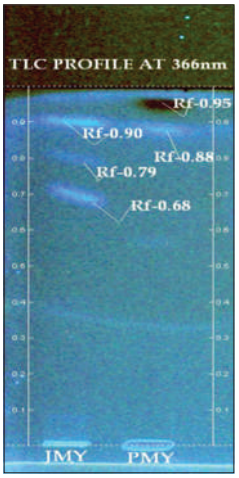

Figure 15: TLC profile at 366nm

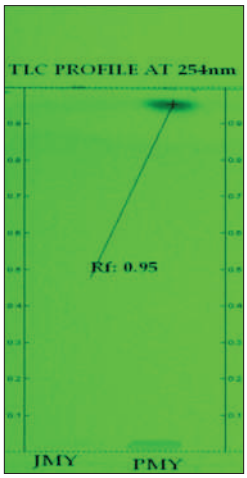

Figure 16: TLC profile at 254nm

Zeta potential is the measurement of stability of nanoparticles. The negative zeta potential value implied that the surface charge of *Bhasma* is negative and a stable suspension was formed in aqueous medium. Surface charge definitely affects cellular uptake, toxicity, and biodistribution of nanoparticles. JMY sample showed a high negative zeta potential value of <−20 mV compared to PMY sample. As per previous studies, zeta potential should be higher than +25 mV or lower than −25 mV to attain a high degree of stability.[11]

The free zinc phase detected in XRD of sample (JY) was not detected in sample subjected to incineration (JMY). Hence, it is clear that mere *Jarana* is not sufficient for the proper conversion of the metal zinc. Although ZnO phases are present in both JY and JMY samples, the intensity of highest peak of ZnO in JY sample is 5150 counts, whereas that in JMY sample is approximately 2150 counts. Simultaneously the intensity of lowest detectable ZnO phase has 500 counts and 150 counts in JY and JMY sample respectively, indicating the difference in the molecular structure of ZnO in two samples. The presence of free as well as oxide form of Zn in *Jarita Yashada* is due to simultaneous carbothermic reaction and

Table 8: FTIR analysis of PMY sample

Sample: PMY		
Frequency	**Peak assignment**	**Possibility**
2874	CH3/CH2 stretching symmetric	N alkanes
1526	Ring stretching	
1327	Ring vibration	Cyclopropanes
1111	NN stretching	Hydrazine
955	Ring breathing	
831	Ring breathing	
617	Ring deformation/CCl stretch	Chloroalkanes

Table 9: FTIR analysis of JMY sample

Sample: JMY		
Frequency	**Peak assignment**	**Possibility**
2968	CH3 stretching- asymmetric	N-alkanes
2937	CH2 stretching- asymmetric	N-alkanes
2868	CH2 stretching- symmetric	N-alkanes
1474	CH3 deformations	N-alkanes
1142	Ring breathing/symmetric SO_2 stretching	Dialkyl sulfones
986	Ring breathing	
772	Ring vibration	Alkyl cyclohexanes/Tertiary alcohols
444	Metal oxide vibration	ZnO

FTIR: Fourier-transform infrared

oxidation, respectively. In a carbothermic reaction, heating with carbon converts the oxide into zinc vapor at a much lower temperature around 950°C. During *Jarana,* some Zn may get vaporize to form ligands with Charred products of *Apamarga churna.*

In SEM study, JMY and PMY samples showed 70. 21 wt % and 44.80 wt % of Zn, respectively. Mass percent of Zn in ZnO[12] (80.340%) matches the SEM result of 70.21 wt % of zinc in JMY sample and mass percent of Zn in ZnS, i.e., 67.094% matches with 56% zinc in PMY sample, after excluding carbon percent. As SEM result corresponds with the compounds detected in XRD to an extent, it can be stated that polycrystalline samples containing ZnS and ZnO as major phase are observed in PMY and JMY samples, respectively, in SEM spectra. The nanoparticles of ZnS can change their crystallographic form easier than particles in the macroscale. The zinc sulfide nanocrystals can transform from zinc the blende (cubic, more stable) to wurtzite (hexagonal) at about 400°C[13] which is evident with polycrystalline nature of ZnS in PMY.

About 10–15 microelements were detected in both samples of *Yashada Bhasma* in ICPAES study. As *Apamarga* is one of the main ingredients in JMY sample, screening of it's elemental composition is also essential to assess the curative effect of the compound. Elemental profile of *Apamarga panchanga* (*A. aspera* Linn.) was determined in a previous study by ICPAES method. Ten elements were detected (Ca, Cu, Ni, Fe, Cr, Na, Mn, Zn, Pb, Cd), in ppm levels at varying concentration, below WHO permissible limits.[14] Although all the above elements were present in both samples of *Yashada Bhasma*, the presence of these trace elements was comparatively more in JMY sample, attributable to the presence of *Apamarga*. Metals such as manganese, chromium, and nickel are the part of basic composition of cast iron.[15] Significantly higher concentration of these metals in JMY may be due to the use of iron vessel for *Jarana*.

Taking into consideration of heavy metal concentration in both the samples, PMY sample possesses As, Hg, Cu, and Pb in permissible limit, while only Cu and Pb were detected in JMY sample. Percentage of these heavy metals in JMY samples was in higher range as compared to PMY sample as per ICPAES analysis. The presence of Hg and As in PMY can be attributed to the use of Hg as an ingredient.

A higher percentage of sulfur detected in PMY in CHNS analysis, and the identification of this sample as ZnS compound is due to the sulfur media used for processing.

Functional groups detected in either sample of *Yashada Bhasma* in FTIR shows the presence of organometallic ligands. This denotes that organic matter is embedded in *Bhasma* even after incineration at a very high temperature of 700°C in EMF. Functional groups signify the structure of the compound.

CONCLUSION

Analytical evaluation helped in assessing the structural and chemical transformation of *Yashada Bhasma* due to pharmaceutical processing. *Bhasma Pariksha* is more qualitative in nature, and modern analytical parameters help to overcome the lacunae in such classical methods to an extent. The study shows that *Bhasma Pariksha* coincides with the analytical studies. Different methods used for the preparation of *Yashada Bhasma* resulted in compounds having different chemical composition and particle size. *Parada Marita Yashada Bhasma* was detected as ZnS and *Vanaspati marita Yashada Bhasma* as ZnO. Their therapeutic efficacy also varies because every element has its individual impact in the structural and functional integrity of living cells. Although the *Bhasma* prepared using *Parada* (mercury) is believed to be best,[16] as the final products correspond to different compounds, it cannot be compared analytically and further clinical evaluation is necessary for the same. The study validated the organometallic ethno nano property of *Yashada Bhasma* and standardized the *Yashada Bhasma* at quality control level, in terms of elemental analysis and particle size. This would definitely help to ensure safety, efficacy, and batch-to-batch uniformity.

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REFERENCES

1. Rajput D, Gokarn R. Analytical standardization of (incinerated iron oxide) Mandura Bhasm. J Indian Syst Med 2016;4. Available from: https://www.researchgate.net/publication/310124131. [Last accessed on 2017 Jul 07].
2. Sharma R, Prajapati PK. Nanotechnology in medicine: Leads fromAyurveda. J Pharm Bioallied Sci 2016;8. Available from: https://www.researchgate.net/publication/286877419. [Last accessed on 2017 Jul 05].
3. Chandran S, Patgiri BJ, Bedarkar P, Nechiyil S, Galib R. Pharmaceutical standardization of "Parada marita and Vanaspati jarita marita yashada Bhasma"-A comparative study. World J Pharm Res 2017;6:2437-50.
4. Sharma SS. Rasatharangini. Reprint Edition., Ch. 19/112-115. Varanasi: Mothilal Banarasi Das Publishers;

2009. p. 576.
5. Joshi D, Joshi G. Quality Control & Standardisazation of Ayurvedic Medicines. 1st ed. Varanasi: Choukamba Orientalia Publications; 2011.
6. Available from: http://www.wikipedia.com. [last accesed on 2017 Feb 25].
7. Sathyanarayana B. Power Point Presentation "Incorporation of Modern Scientific Methods of Analytical Techniques wsr to Rasaushadhi". Jamnagar: TOT, in Dept of RS & BK, IPGT & RA, GAU; 2011.
8. Available from: http://www.rsc.org/images/CHNS-elemental-analysers-technical-brief-29_tcm18-214833.pdf. [Last accessed on 2015 Feb 25].
9. Available from: http://www.en.wikipedia.org/wiki/transform infrared_spectroscopy#cite_note_Ggiffiths. [Last accessed on 2015 Feb 25].
10. Wadekar MP, Rode CV, Bendale YN, Patil KR, Prabhune AA. Preparation and characterization of a copper based Indian traditional drug: Tamra Bhasma. J Pharm Biomed Anal 2005;39:951-5.
11. Huang RF, Wei YJ, Inbaraj BS, Chen BH. Inhibition of colon cancer cell growth by nanoemulsion carrying gold nanoparticles and lycopene. Int J Nanomed 2015;10:2823-46.
12. Available from: https://www.convertunits.com/molarmass/ZnS. [Last acessed on 2017 Oct 06].
13. Fang X, Zhai T, Gautam UK, Li L, Wu L, Bando Y, *et al*. ZnS nanostructures: From synthesis to applications. Prog Mater Sci 2011;56:175-287.
14. Saraf A, Samant A. Evaluation of some minerals and trace elements in *Achyranthes aspera* Linn. Int J Earth Sci 2013;3:229-33.
15. Saxena S. Elements of Metallurgy. Meerut, India: Rastogy Publications, Castirons; 1984. p. 142-3.
16. Vagbhata R, Misra S, editor. Rasaratnasamuchaya, Sidhipradha Hindi Commentary. 1st ed., Ch. 5/12. Varanasi: Choukamba Orientalia Publications; 2011. p. 143.

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