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Dr. Vidyaa G Honde
Assistant Professor, Department of Rasashastra and Bhaishajya Kalpana, CSMSS Ayurved Mahavidyalaya, Chhatrapati Sambhajinagar, Maharashtra, India

Dr. Piyush K Gandhi
Associate Professor, Department of Rasashastra and Bhaishajya Kalpana, Government Ayurvedic College, Nanded, Maharashtra, India

Dr. Rajesh K Ingole
Professor & Head of Department, Department of Rasashastra and Bhaishajya Kalpana, Government Ayurvedic College, Nanded, Maharashtra, India

Corresponding Author:
Dr. Vidyaa G Honde
Assistant Professor, Department of Rasashastra and Bhaishajya Kalpana, CSMSS Ayurved Mahavidyalaya, Chhatrapati Sambhajinagar, Maharashtra, India

An ancient and modern analytical examination of dhatu bhasma - a review article

Vidyaa G Honde, Piyush K Gandhi and Rajesh K Ingole

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Abstract
There are different dhatu in Ayurveda, like Swarna, rajata, etc,
Aim: is to compile such available research work done on analytical examination of dhatu bhasmas and the Ancient bhasma pariksha mentioned in different samhitas to avoid the searching time.
Materials and Methods: The bhasmas need to fulfil the siddilakshanas like rekhapurnatva, varitartva, nischandrtva, nirthatva, apunarbhav etc, similarly obtain the panchavidh parikshan of shabd,sparsha, ras, rupa, gandha, and the modern analytical parameters like XRD, XRF, SEM-EDAX, TEM, etc, also the phisico-chemical parameters like ph value, lod, total ash, acid insoluble ash, water soluble ash, specific gravity etc.
Conclusion: The Bhasmas which passes both the ancient and the modern analytical parameters i.e. physical, physico-chemical and the elemental analysis, metals and the other trace elements present in Bhasmas are within limit of API standards. Which are therapeutically very effective and safe to use, doesn't having any side effects.

Keywords: Antimalarial drug, antiplasmodial, cell lines, malaria, neurotoxicity

Introduction
The Indian system of medicine is the first amongst all traditional medicine systems. Ayurveda is a science with rich heritage and antiquity (knowledge of metal and mineral) [1] Over a period of time, this got systemised to professionalism for the prevention, promotion and care of disease condition Rasashastra being a tribute of Ayurveda contribute to promote and maintain health. The use of metal and mineral therapeutic was the speciality of this science. Bhasmas are metallic preparation obtain by repeated incineration of metal.
Rasaushadhis (herbo- mineral preparation) especially metallic bhasma (incinerated metal) are known to improve immunity and stability in the body. As these bhasma acts as rasayana (rejuvenation) and yogvahi (targeted drug delivery) Use of bhasma reduced the quantity of the dose administered and increased the palatability, tissue availability of drugs [1].
In the mediaeval period, the golden era of rasashatra, bhasmas were used widely of the metallic elements, which are involved significantly in the anatomy and physiology of the body, now in present era, we remain wondering the depth of these elements in the body, being highly efficacious in small doses and having long shelf life, metallic preparation become very popular among ayurvedic physicians within very short period of time.
But unfortunately, now a days, these herbo-metalo-mineral formulations are suffering a lot thus struggling to build their crumbling image, serious doubts rose on safety and efficacy of these formulations in last decade, to overcome this the properly prepared and standardised (analytically tested in both ancient and modern method) bhasmas are very important. Properly prepared bhasmas have been proved to work wonders in clinical practices.
Quality of drugs depends upon its formulations processing, applications, it is essential to fix some standards for manufacture of drugs so that the genuineness of drug is not compromised.
There have been concerns regarding the safety and efficacy of ayurvedic drugs mainly the bhasmas, Ayurvedic texts have described several methods for quality control of finished products like varitartva, rekhapurnatva, nischandratva, niruttha, apunarbhav, unam, dantagre-kachkachabhav etc. and modern analytical examinations are physico-chemical tests i.e., ph value, total ash, acid insoluble ash, water soluble ash, loss on drying, qualitative & quantitative test like, XRD (phase identification of diffractogram using x ray diffraction), XRF (x ray fluorescence), SEM (scanning electron microscopy) E-DAX, (energy dispersive x ray spectroscopy) FT-IR (Fourier transform infrared spectrometry) PSA (particle size distribution) Bhamas are prepared mainly from these dravyas -DHATU- swarn, rajat, tamra, loha, nag,

vang, yashad, chapal, parade. etc MISHRADHATU-kansya, pital, trivang,etc UPDHATU-abhrak, makshik, mandur, vimal, hingul, mruddarshrung, hingul etc SUDHAVARGA-shankh, shukti, kapardik, mouktik, godanti, praval, hastidant, ajasthi, mrugshrung, etc MALLA- hartal, somal etc SIKATA VARGA- ratna- gomeda, nil, manikya, pushkraj, vaidurya, hiraka, paanch, etc upratna - vaikrant etc mani- Chandrakant, piroja, rajavarta, sphatika, Suryakant [2] etc

Here we are taking important dhatu which are mainly used in clinical practices that are Suvarna, rajat, tamra, loha, nag, vanga, yashad for this review article.

Aims

To review the different ancient and modern analytical parameters for bhasma standardization.

Objectives

To study the literature regarding the bhasmas from various samhitas and the research articles.

Materials and Methods

Ancient analytical examination

Table 1: Organoleptic character of bhasma

Parameter	Swarna[3]	Rajat [8]	Tamra [24]	Loha [19]	Naga[30]	Vang [35]	Yashad [32]
Shabda	No sound	No sound	No sound	No sound	No sound	No sound	No sound
Sparsha	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Rupa	Brick red in colour/ champaka	Black in color	Black in colour, greyish brown, dark brown[26]	Pakvajambu phal varna / ishtika varna[22]	black /red/ kapota	Milky white/light pink/ chandrama	Creamish colour/ Peeta
Rasa	Tasteless (niswadu)	Tasteless	Tasteless	No taste	No taste	No taste	No taste
Gandha	No specific	No Specific	No specific	No odour	No odour	No odour	No odour

Table 2: Bhasma pariksha maintioned in classics

Parameter	Swarna	Rajata	Tamra	Loha	Naag	Vang	Yashad
Rekhapoornatva	+	+	+	+	+	+	+
Varitaratva	+	+	+	+	+	+	+
Nischandratva	+	+	+	+	+	+	+
Unnama		+	+	+	+	+	+
Apunarbhav	+	+	+	+	+	+	+
Niruttha	+	+	+	+	+	+	+
Nirdhuma		+	+	+	+		+
Dantagre kachkachabhav	+		+	+	+		+
Avami			+	+	+		+
Sukshma	+	+	+	+	+	+	+
Amla paiksha greenish tincture in acidic solution (dadhi pariksha)			+				
On sprinckling amala doesn't turn black				+			
Shine in sunrays /peacock green colour tinch,			+				
Fine powder after grinding							

Modern Analytical Examination

Table 3: Physico-chemical analysis

Pariksha	Swarna	Rajata	Tamra	Loha	Naga	Vang	Yashad
Ph value	6.48[3]	5.43 [10], 4.81[11]	3.61% w/w [27]	4.41 [22]	9.8 [30]	8.75[35]	11.2.
Total ash value	97.60 % [3]	99.50 [10], 100[11]	98.10 [24]	98.24%[19] 99.38 [22]	96.27%[30]	99.75%[35]	99.76% [32]
Acid insoluble ash	21.80% [3]	11.50 [10], 87.599[11]	3.32 [24]	80.88 [22]	23.21% [30]	93.15% [35]	46.10% [32]
Water soluble ash	0.12% [3]	Nil [10], 47.756[11]	0.78 [24]		11.28% [30]		1.66% [32]
Loss on drying at 110° 105°	0.27% [3]	0.00%[10] 0.3205[11]	0.15 [24]	0.39 gm%	0.46% [30]	0.08%	0.01% [32]
Water soluble extractive		4.28[11]	0.78 [24]	20%		0.37% [35]	
Alcohol soluble extractive		4.888[11]	1.33 [24]	17%		0.86% [35]	
Specific gravity API-	15.6-18.3 [36]	1.0224[11] 10.1-11.1% [36]	8-9 % [24]	6-6% [36]			

Table 4: Elemental analysis

Parameter	Swarna	Rajat	Tamra	Loha	Naga	Vang	Yashad
XRD	Peaks of Au and HgS 5.606 to1.2331[3]	Strongest peak of Ag_2S [10] crystalline with Ag_3as, S_3, Fe_3o_4	CuS [24], Cu 67.50%[25] CuO & $CuMn_2O_4$ in	Irregular in shape [22]	Peaks at angle 2Ø=25.96, [28] high (2θ) of 10-60° at	Peaks match with the tin oxide, other major peaks at 2Ø=	Showed four peaks, at 31.9, 36.4, 47.7, 56.5 presence of

		[11]	minor phase		the rate of 0.01°/sec [29] crystalline nature, also the carbon & oxygen peaks	22.56, 34.20, 44.98, 52.04 [34]	hexagonal zinc sulphide. [31], peak of ZnO Observed [32].
SEM	P.S-ranging between 21.85nm-28.80 nm [3]	P.S-105nm[11], 54.6nm[10], 10-60 nm[9]	Volumetric mean diameter 28.70 µm[24]	P.S -11 micron [22]		P.S smaller than 100nm [34].	P.S- 200-500 nm. [31]
EDX	93.96 Au &other trace elements, O,Mg,K,Ca,Fu,& Au	Ag- 74.67, C,O,Ng,Mg, Si,S,Cl,K,Ca, Cu, no Hg&Pb [10]	Cu-58.56, S-22.48, Fe-0.31, As,pb,hg, mn, zn: traces, cd,se; not detected[24]	Iron-64.01% [22]	Presence of mg ca & fe [28] % of lead -37.93[30]	O, Sn, C, Zn, Mg, Si, P,Fe, Al, S, Cu, with 24.33% of tin may be due to its conversion in tin oxide[34]	29.8% zinc content in the sample [31]
ICP-OES	Au-52.33% As-o.316, Hg-o.054, Cd&Pb-BDL [7, 11]	Ag-80.12% S-9.4261, Al-0.363, As- 1.892, Hg 0.000076, Cu-0.866, Fe-0.682, Pb-0.202, Cd-0.0016% [11]	As-28.26 Cd-3.81 Hg- ND, Na-205.69 P-281.21 Pb-27.7 Sn-32.3 F-186 Cl-414 [21] Cu -56.42 % S - 45.35[25]	SiO_2-9.07 Fe_2O_2-89.48, CaO-0.11, MgO-0.88, Na_2O-1.01, K_2O-0.17%Cl-0.05%, SO_4-0.02%[19]	Pb-40.26%, Fe-7.69, Ca- 1.45, K-7.14, O-10.89, Cl- 0.80, Sn-1.77.	Na- 0.29%, Mg-1.40, Al-0.73, Si-3.51, S-2.54, Fe-1.42, Sn-90.11 [35]	Oxygen- 34.3%, sodium -15.2%, sulfur-13.3, pottasium-7.5%. Toxic metals like-hg, ng, arsenic etc are not detected [31]
TEM		Particles are spherical in range of 5-50 nm9				Particles are poly crysstelline in nature with size 20 nm. [34]	more or less spericle. Arranged in hexagonal pattern [31]
UV visible spectrum		Absorption at around 423nm [9]					
Namburi phased spot test				Central deep blue spot in phase 1, expanded blue spot in phase 2, no other changes in phase 3[19, 21]	1 st phase - A solid yellow spot was formed which turned immediately into deep yellow central solid spot with a light red spot surrounding the yellow spot. 2 nd phase - It continued to be the same in the 2nd phase. 3 rd phase - The yellow colour around the central spot faded partially. [36]	1st phase - A solid yellow spot was formed which turned immediately into deep yellow central solid spot with a light red spot surrounding the yellow spot.2nd phase - same. rd phase - The yellow colour around the central spot faded partially	In the 1st phase (0-5 min), a wet central spot spread outside with Immediate formation of bright white glittering surface over the spot. In 2nd phase (5-20 min) spreading of the drop stopped. Thin reddish outer ring around the white spot was seen. The white spot was very bright in this stage. 3rd phase (20 min24 hours) the brightness of the white spot was maintained. There was a clear yellowish periphery around the centre spot. [34]
AAS (atomic absorption spectroscopy				Mg-285.2 nm, Al-309.3 nm K-766.5 Ca- 422.7 Cr-357.9 Mn-279.5 Fe- 248.3 Cu-324.8 Zn-213.9 nm [21]	lead -98.98% PbO-94.21, CaO-2.79, K_2O-1.08, MgO-0.43, P_2O_5 - 0.25, Fe_2O_3 - 0.26, SiO_2 - 0.15% [2]		
FTIR-Permissible limit as per API	Lead-10 ppm Mercury- 1ppm Cadmium-0.3ppm Arsenic-3ppm		API- should not contain more than- ar-1ppm, cd-5ppm [38] Pb-5 ppm, May contain-zn--25ppm, ag-10ppm, au-135 ppm	API- Zn-95 ppm, mn-500 ppm, ag-5 ppm [38]	between 4000 and 400/cm in FTIR spectrometer [29]	Functional group like -NH_2, -OH, CH_3,O-CH_3, C=O, C=C,C-H are present, Sn-O bonding & Sn-C bonding present [35]	
XRF					34 mm diamet er pellets of moderate thickness[29]		

API Parameters[38]	Hg, pb, ar, cd should be absent.	Cu-1.40%, S-traces, au-o.oo1%		60%iron, should not be greater than this Ar-2ppm, cd-7 ppm			

Discussion

Ayurveda cannot remain confined to the use of conventional, conservative normas of medication. It has to accept new challenges, and be prepared to answer the queries of the modern man, to meet this new trust of inquisitiveness, standardization of the drugs of Indian system of medicine is mandatory. Use of rasdravyas increased after development of Rasashastra, which is an integral part of Ayurveda. Bhasma of metals or minerals are one of the suitable pharmaceutical forms in Rasashastra. Role of shodhana, bhavana and marana is important in bhasma preparation, shodhana and marana are considered highly necessary for converting the metels into suitable form in which they could be administered.

Physico chemical analysis provides the objective parameters to fix up the standards for quality of raw drugs as well as finished products. Analytical study of a drug also helps to interpret the pharmacokinetics and pharmacodynamics of the same. In this phase of study, the physico chemical analysis of bhasma evaluated.

Organoleptic characteristics

- Shabda sound produced when the bhasma is chewed which should be ideally absent (dantanaam kachkachabhav), sound is quality of metel, absent of sound indicates the finess of bhasma, and conversion of metal into specific compound.
- Sparsha bhasma is taken between thumb and forefinger and rubbed, no grittiness is to be present, and should be smooth and soft to touch, that for the fineness of bhasma [39].
- Rupa colour and form of the bhasmas, A specific colour is mentioned for each Bhasma. Alteration in specific colour suggests that Bhasma is not prepared properly. Because a particular metallic compound is formed during Bhasma preparation and every chemical compound possesses specific colour[04]

Colour of compounds

- **Swarna:** Gold oxide (Au_2O_3) -red brown.
- **Rajat:** Silver oxide (Ag_2O) - fine black/dark brown. Silver sulphide (Ag_2S) - dense black solid.
- **Tamra:** Copper sulphide, chalcosite (Cu_2S) - dark grey/black. Copper sulphide, covellite (CuS) - black. Copper oxide (CuO) - black.
- **Loha:** Iron oxide (FeO) - black. Ferric oxide (Fe_2O_3) - red brown.
- **Naga:** lead oxide (PbO) - Letharge - red, massicot-yellow. Vanga - tin oxide (SnO) - black colour. SnO_2 - white colour.
- **Yashad:** Zinc sulphide (ZnS) - originally white in colour, usually black due to impurities, it can be transparent.

The colour of bhasma is given in text of Swarna bhasma is brick red or champaka & colour of compound gold oxide is red brown that means the acharyas are expected the gold oxide is the final product of Swarna bhasma.

The rajat bhasma colour in text is black, silver oxide and silver suphide both are black in colour that suggests rajat bhasma can be its oxide or sulphide form.

Colour of tamra bhasma is black, greyish brown or dark brown & the colour of compounds is copper sulphide dark grey or black copper oxide black, that means tamra bhasma can be it's both forms.

Colour of loha bhasma is pakvajambuphalvat or ishtika varna & the colour of ferric oxide is red brown that means the loha bhasma must be in ferric oxide form.

Colour of naga bhasma is black or kapota varna that means, naga bhasma must be in letharge form.

Colour of vanga bhasma is chandramasankash that suggests the final product of vanga is SnO2.

The colour of yashad bhasma is peeta or creamish that means the final product contain zinc sulphide without any impurities.

Rasa - taste of bhasma, devoid of taste of bhasma resembles conversion of metal into specific compound [39].

Gandha - there is no specific odour of bhasma, suggect metal converts into a specific stable compound.

Bhasma pariksha mentioned in classics-

- Varitara water taken in a plastic jar and allows for stagnation. Then small amount of Bhasma was taken in between index fin ger and thumb and pressed to form a small flat mass and that is slowly kept on the surface of stagnant water in jar from a short distance and observed, applied to study lightness and fineness of Bhasma [40].
- **Unnama Test:** If grains are put over the floating sample of the bhasma it should continue its floating. Applied to test the lightness of bhasma.
- Rekhapoorna test very little amount of bhasma is taken in between thumb and index finger and rubbed and observed whether the bhasma fills the furrows of the finger tips or not. This test is applied to study fineness of Bhasma. Bhasma particles should be of minimum size for easy absorption and assimilation in the body [40].
- Nischandra test bhasma observed in sunlight thereafter little amount of Bhasma is taken on palm and observed in sunlight for presence of any lustre particles. Chandratva (luster) is a character of metal. After proper incineration, luster of metal should not remain. For this test, Bhasma is observed under bright sun light, whether luster is present or not; if luster is still present, it indicates further incineration [40].
- **Apunarbhava Test:** 2grams of Bhasma was mixed with equal quantity of each of the ingredients of the Mitra Panchaka (Guda, Ghruta, Madhu, Tankana, Gunja) and mardana is done, chakrikas are done and then made samputa. There after it was subjected to the similar grade of heat used for the preparation of Bhasma and after self-cooling, chakrikas were collected and observed for any lustered particle or any accumulated masses. Lustrous particles in it show presence of free metal, which is indicative of improper incineration [40].
- **Niruttha**: Niruttha is to test inability to regain metallic form of metallic Bhasmas. In this test, Bhasma is mixed with a fixed weight of silver leaf, kept in earthen pots and similar grade of heat is applied and after self-cooling, weight of silver is taken. Increase in weight of silver leaf indicates improperly prepared Bhasma [40].
- **Anjana sadrisha**: It should be black in colour like collyrium. Anjana (collyrium) is smooth in character and

it does not create any irritation whenever applied. Properly incinerated Bhasma should be smooth and should not create any irritation to mucous membrane of gastrointestinal tract [40].

- **Nirdhuma:** No fumes should be produced when it is heated. Which suggests no free unwanted materials likes, as etc [39].
- **Slakshnatvam:** It is tactile sensation produced by Bhasma by simple touch with finger tips. Properly incinerated Bhasma attains this quality. Slakshna Bhasma can be absorbed and assimilated in the body without producing any irritation to mucous membrane of gastrointestinal tract [40].

Physico-chemical analytical test -

- **Ash value:** Ash value is useful in determining authenticity and purity of sample and also these values are important qualitative standards, it is measure of amount of the amount of inorganic matter or minerals present in the sample. Ash value of the bhasmas is around 97% that showed organic matter present in all bhasma are negligible.
- **Water soluble ash:** The part of the total ash dissolved by water. The water-soluble ash values of Swarna bhasma- 0.12%, rajat bhasma - nil, tamra- 0.78, naga - 11.28, yashad - 1.66, the decreased water-soluble ash values showed that the inorganic matter present in the samples was insoluble in water.
- **Acid insoluble ash:** Insoluble residue remaining when the ash treated with hydrochloric acid. The acid insoluble ash values of Swarna bhasma - 21.80%, rajat -11.50, tamra-3.32, loha - 80.80, naga- 23.21, vang- 93.15, yashad- 46.10%. Increased acid insoluble ash value of bhasma of loha & vang may be due to presence of components like silica, carbonates, which are insoluble in hcl.
- **Loss on drying:** Loss of any volatile matter from the sample. Loss in drying does not usually refer to molecularly bound water or water of crystallisation. The LOD seen to be very minimal in all bhasmas, that suggests there is no any volatile matter or the moisture left in the bhasma.
- **Extractive values:** Extractive values by different solvents are used to assess quality, purity and to detect adulteration due to exhausted and incorrectly processed drugs. All the bhasmas contain very less extractive value that means the bhasma has good quality.
- **Specific gravity**: The specific gravity is the ratio between the density of an object, and a reference substance. The specific gravity can tell us, based on its value, if the object will sink or float in water which always has a density of 1 gram per milliliter or 1 gram per cubic centimeter. All the bhasma has specific gravity more than 1 gm per cubic centimeter, that why bhasma float on surface of water(varitartva)
- **Ph value:** ph value give us idea about the acidic or basic nature of the material tested, ph value of bhasmas are- swarna bhasma- 6.48, rajata - 5.43, tamra- 3.61, loha - 4.41, naga - 9.8, vanga- 8.75, yashad - 11.2, that suggests sample of tamra loha & rajat are acidic in nature, Swarna bhasma is around neutral, & naga vang yashad are basic in nature.

Instrumental methods of analysis

XRD (X-RAY Diffraction): This test was carried out for the crystalline phase identification of the compounds present in the sample also provides vital information regarding the arrangement of sample, all the bhasma has highest peak is above the 60% of that metal, that indicates bhasma is prepared from pure metal.
2Ø values of all bhasma corresponds to Swarna bhasma- auric oxide, rajat bhasma- silver sulfide ag2s, tamra copper sulfide,iron- auric oxide, naga- lead oxide, vang - tin oxide, yashad- zinc oxide. In case of metal, sulfides have lower melting point than oxide.

SEM (scanning electron microscope): Is used for finding out the particle size, also applied to the surface related physical properties such as topography, depth profiling studies. Particle size of bhasmas is in nano size in the range of 1-100 nm, due to this bhasmas are easily absorbed in the body.

EDS, EDX, or EDAX (Energy Dispersive X-RAY Spectroscopy): Is an analysis tool used to determine the elemental composition of a sample for elemental analysis. Other trace elements in the final product of bhasma, are comes from the drugs used in various processes like shodhana marana etc, that trace elements increase the quality of bhasma.

ICP-OES: inductively coupled plasma atomic/optical emission spectroscopy: Inductively coupled plasma optical emission spectrometry is an analytical technique for the detection of trace metals, and the % of toxic elements if any present, all the bhasmas showed 0% of toxic elements like arsenic, mercury, cadmium etc or within the permissible limit acc to API standard.

FT-IR: Fourier Transform Infrared: The preferred method of infrared spectroscopy. Which measures intensity over a narrow range of wavelengths, use in the identification and structural analysis of organic compound, natural polymer. Functional group in a given organic compound can be identified.

TEM: Transmission Electron Microscopy: TEMs are capable of imaging at a significantly higher resolution than light microscopes,used to study the local structures, morphology,cross sections and crystallization of metallic alloys and microstructure of composite material. Indicates the shape of particles present in bhasma.

Namburi Phased Spot Test: This test was performed for standardization of bhasma. This is the method developed by Dr. Namburi Hanumanth a Rao in 1970 which was accepted and propagated by C.C.R.A.S.

The Bhasmas prepared with Parada or Parada Bhasma as media are considered superior to others. In this study, it is observed that, bhasma prepared by using Kajjali (black sulphide of mercury - HgS) having negligible % of mercury or the kajjali in the final product, this may be due to kajjali acts as catalyst during the process and later it is evaporated, other trace of micronutrients found which are different in different bhasma, these are as follows.

Trace elements in Sapta Dhatu Bhasma [40].

- Swarna Bhasma -As, Nb,

- Rajat Bhasma -Fe, Cu, Pb, Cd, S,
- Tamra Bhasma -Cu, Fe, Al, S, As, Hg,
- Louha Bhasma- K, Cu, Zn, Mn, Mg, Ca, Na, Cl,
- Vanga Bhasma- Ca, As, Fe, Si, P, Al, Cl,
- Naga Bhasma- Ca, Sn, Mo, K, Mn, Fe, Mg,
- Yashad Bhasma- Sn, Pb, Fe, Ca, Mg, Cu, Co.

Conclusion

From above mentioned ancient literature and the mentioned research paper we can conclude that the Bhasmas which passes both the ancient and the modern analytical parameters i.e physical, physico-chemical and the elemental analysis, metals and the other trace elements present in Bhasmas are within limit of API standards. Which are therapeutically very effective and safe to use, doesn't having any side effects.

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