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RESEARCH ARTICLE

ANALYTICAL CHEMISTRY

CHEMICAL EVALUATION OF TAMRA BHASMA

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ABSTRACT

Bhasmas are traditional Indian medicinal preparations in which metals undergo thermal treatment process to bring about thermal decomposition, phase transition, purification, detoxification and their conversion into a digestible oxide or sulfide form. Tamra Bhasma is copper bhasma.

The present experiment was done to estimate the amount of copper present in Tamra Bhasma of different batches, different manufacturers and formulations containing Tamra Bhasma by Atomic Absorption Spectroscopy. Spectrophotometric and X- Ray Diffraction analysis were also performed on the samples.

It was seen that samples from different batches and different manufacturers contained varying amounts of Tamra Bhasma. Spectrophotometric analysis showed that copper was present predominantly in its cupric state. X- Ray Diffraction analysis showed that the copper in the bhasma was present in its sulphide form. Analysis of formulations showed variations in the copper content from the label claim.

The results from the study stress the need for use of modern analytical tools for standardization and evaluation of traditional medicines.

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KEY WORDS

Copper, Bhasma, Atomic Absorption Spectrophotometry, X- Ray Diffraction, UV Visible Spectroscopy, Standardization, Label Claim, Tamra Bhasma, Chemical evaluation.

INTRODUCTION

Rasa Sastra is the methodology of preparation of Ayurvedic medicine and it includes the extraction of metals from their minerals, their purification and conversion into digestible metallic bhasma. The process of manufacturing metallic bhasma consists of *satvapatana* (metal exrtraction) and *bhasmikarana* (conversion to non toxic form). The processing of metals can be classified into *sodhana* (purification), *marana* (conversion to non toxic fine powder), *mardan* (preparation of intermediate mixture), *putapak* (reactions at high temperature).At the end of processing this microfine medicinal product has easy digestive power and quick reaction with the bile juices.[1]

WHO has concluded that a daily consumption of about 3.5 to 4.5 mg of copper is essential for the normal functioning of vital organs like heart, lungs etc[1]. Tamra Bhasma is copper in its oxide form and used therapeutically as a source of copper. The label claim states that Tamra Bhasma is used as astringent, antispasmodic, antiseptic. It is used to treat painful dyspepsia and anemia.

In the recent past, physicochemical evaluation of Ayurvedic preparations of metallic iron namely, Lauha and Mandura Bhasma has been done[2, 3]. These prompted a study on Tamra Bhasma and its formulations.

Formulations of different batches and different manufacturers were analyzed to estimate the copper content and correlated with their label claim. Quantitation was done by AAS and UV Visible Spectroscopy. The quality of the bhasma was assessed by XRD.

MATERIALS AND METHODS

Tamra Bhasma of two different manufacturers and different batches and formulations containing Tamra Bhasma like Chandrakala Rasa, Sootashekhar Rasa, Nityanand Rasa and Arogyavardhani were procured from an Ayurvedic shop in Mumbai.

The bhasma samples and the formulations were analyzed by routine physicochemical analysis like loss in drying, ash value as well as techniques like UV Visible Spectroscopy, Atomic Absorption Spectroscopy and X Ray Diffraction studies.

Moisture content

0.1g of Bhasma sample and formulation was weighed and kept in the oven at 110°C for 3 hrs. They were weighed again and the difference was determined to find the moisture content.[4]

Organic Content

0.1g of Bhasma sample and formulation was weighed and kept in the muffle furnace at 450°C for 3 days until constant weight was obtained. The difference was determined to find the organic content.[4]

Sample Preparation

0.1 g of bhasma sample was weighed in a crucible and heated in a muffle furnace till a constant weight was obtained. The sample was then treated with 10 ml conc. HNO_3. The mixture was allowed to stand overnight and then heated to dryness. 2 ml perchloric acid was added and heated to half the volume and sample was diluted to 100ml using deionised water in a standard flask[5]. Formulations were treated in similar manner.

Determination of Copper Content and Oxidation state by UV- Visible Spectrophotometer

Copper content in the sample made by two different manufacturers and different batches was determined by Neopcuproin method[6]. 10mL of sample solution was taken in a separating funnel; 5mL of 10% hydroxylammonium chloride solution was added.10mL of 30% sodium citrate was added. Ammonia solution was added until pH was about 4 followed by 10mL of 0.1% solution of Neocuproin in absolute ethanol.10mL chloroform was added and layers were separated. Absorbance was measured at 457 nm on UV Visible Spectrophotometer (Model; Shimadzu UV 1700).

Determination of Copper content by AAS analysis

The Method Validation for Copper by Atomic Absorption Spectrophotometer was performed[7, 5] (Model; Perkin Elmer AAnalyst 700).1000 ppm Cu Standard (CRM) from Merck was used as standard and the standard working range was from 0.25- 4.0 ppm. Method validation was done and linearity, recovery and specificity were checked. The prepared samples were diluted to 10 ppm and assayed.

Determination of form of copper (oxide/ sulphide) by X- Ray Diffraction method

1.0 gm of sample of two different manufacturers was given for X- Ray Diffraction Analysis to Shimadzu Analytical India. Pvt. Ltd.

RESULTS AND DISCUSSION

Sample ID	Sample name
Tam B (A) (I)	Tamra Bhasma of manufacturer A Batch I
Tam B (A) (II)	Tamra Bhasma of manufacturer A Batch II
Tam B (B)	Tamra Bhasma of manufacturer B
Chan R (A)	Chandrakala Rasa of manufacturer A
Chan R (B)	Chandrakala Rasa of manufacturer B
Soot (A)	Sootashekhar Rasa of manufacturer A
Ary (A)	Arogyavardhani of manufacturer A
Nity (B)	Nityanand Rasa of manufacturer B

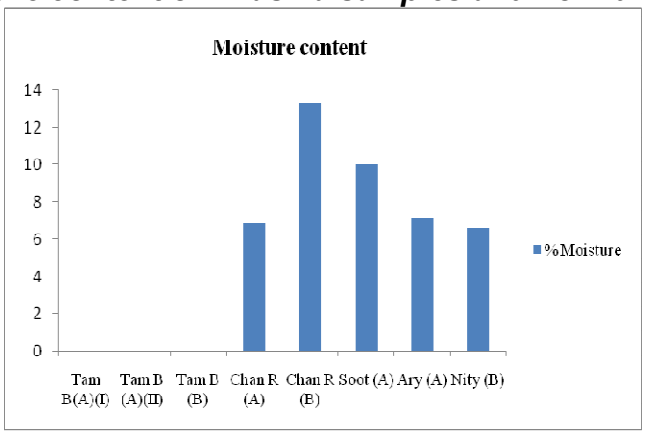
There was negligible moisture content in the bhasma samples and considerable moisture in the formulations (refer table 1, figure 1).

Table 1
Loss on Drying (Moisture content)

Sample ID	Initial weight(g)	Weight loss(g)	% Moisture
Tam B(A)(I)	0.1	0	-
Tam B (A)(II)	0.1	0	-
Tam B (B)	0.1	0	-
Chan R (A)	0.29	0.02	6.9
Chan R (B)	0.15	0.02	13.33
Soot (A)	0.30	0.03	10.00
Ary (A)	0.28	0.02	7.14
Nity (B)	0.30	0.02	6.66

**Conditions: Oven, 3 hours at 110°C.*

Figure 1
Moisture content of Bhasma samples and Formulations



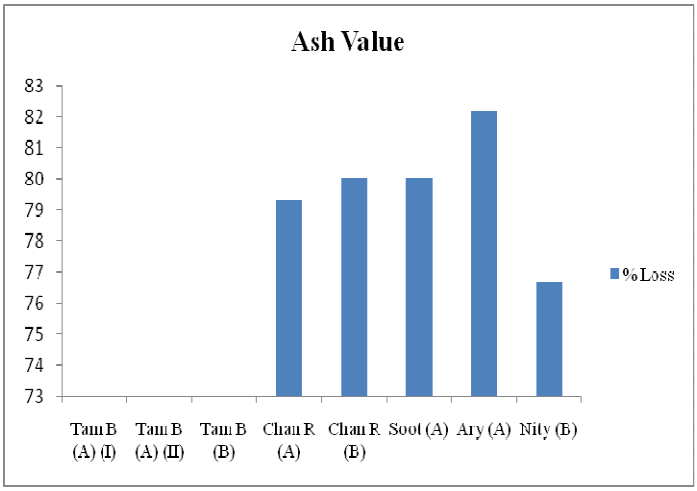
Bhasma samples had high inorganic content while formulations had more organic content. (refer table 2, figure 2).

Table 2
Ash Value (Loss of organic content)

Sample ID	Initial weight(g)	Weight loss(g)	% Loss
Tam B (A) (I)	0.1	0	-
Tam B (A) (II)	0.1	0	-
Tam B (B)	0.1	0	-
Chan R (A)	0.29	0.23	79.31
Chan R (B)	0.15	0.12	80.00
Soot (A)	0.30	0.24	80.00
Ary (A)	0.28	0.23	82.14
Nity (B)	0.30	0.23	76.66

**Conditions: Muffle Furnace, 3 days at 450°C*

Figure 2
Ash Value of Bhasma samples and Formulations



The method for copper estimation by Atomic Absorption Spectroscopy was validated. (refer tables 3, 4, 5, figure 3).

Table 3
AAS Method Validation; Linearity

Cu. std (ppm)	Reading 1	Reading 2	Reading 3	Mean	S.D	% RSD
0.25	0.018	0.018	0.018	0.018	0.0001	0.39
0.5	0.033	0.033	0.034	0.034	0.0002	0.46
1.0	0.065	0.064	0.064	0.064	0.0001	0.17
2.0	0.124	0.124	0.124	0.124	0.0002	0.17
4.0	0.241	0.241	0.241	0.241	0.0002	0.10

**correlation co efficient: 0.99, slope: 0.08019*

Table 4
AAS Method Validation; Recovery Experiment

Standard conc. (ppm)	Sample conc. (ppm)	Effective conc. (ppm)	Calculated conc. (Set I) (ppm)	Calculated conc. (Set II) (ppm)	Recovery % (Set I)	Recovery % (Set II)
1	0.5	1.5	1.5468	1.5468	104.68	104.68
2	0.5	2.5	2.4531	2.4531	97.65	97.65
3	0.5	3.5	3.3438	3.375	94.79	95.83

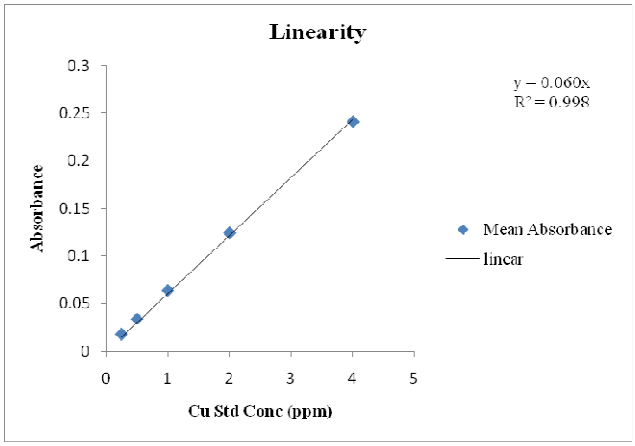
**Percent Recovery:* $\frac{\mu g\ Cu/ml \text{ in strengthened sample} - \mu g\ Cu/ml \text{ in actual sample}}{\mu g\ Cu/ml \text{ added}}$

Table 5
AAS Method Validation; Specificity

Cu. Std (ppm)	Reading 1	Reading 2	Reading 3	Mean	S.D	% RSD
1.0	0.065	0.064	0.064	0.064	0.0001	0.17
1.0 + 5.0* (Zn, As, Pb, Ni)	0.066	0.066	0.066	0.066	0.0002	0.29

**1 ppm Cu standard in presence of 5 ppm each of Zn, As, Pb, Ni.*

Figure 3
AAS Method Validation; Linearity Curve



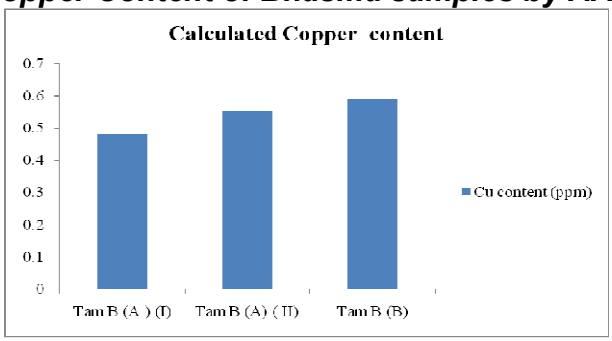
Manufacturers A and B and Batch I and II of Manufacturer A showed variation in copper content in the bhasma sample (refer table 6, figure 4).

Table 6
Cu content of Bhasmas by AAS

Sample ID	Reading 1	Reading 2	Reading 3	Mean	S.D	% RSD*	Cu content (ppm)#	Cu content (%)
Tam B (A) (I)	0.031	0.031	0.031	0.031	0.001	0.34	0.48	48
Tam B (A) (II)	0.034	0.035	0.036	0.035	0.001	3.13	0.55	55
Tam B (B)	0.038	0.037	0.037	0.038	0.002	0.46	0.59	59

#Considering Cu Std 1 ppm = 0.064
**% relative standard deviation (RSD) = (SD/Mean) x 100*

Figure 4
Copper Content of Bhasma samples by AAS



The percentage of copper in the Bhasma samples of Manufacturer A-Batch I, Batch II and Manufacturer B was found to be 48%, 55% and 59% respectively. The amount of copper content in the Bhasma sample must be in the range of 60-65% [8] which was not met by either of the manufacturers. Lead content was checked and found to be absent in all the samples.

Standardization requires that the composition of every formulation be a part of the label claim. Formulations containing Copper Bhasma mention the amount of Copper Bhasma per tablet. However the amount of copper in it is not mentioned. Formulations were analyzed for the amount of copper per tablet. On calculation and correlation with the label claim it was found that formulations contain less copper than the label claim (refer table 7).

Table 7
Cu content of bhasma containing formulations

Sample ID	Mean	S.D	% RSD	Cu conc * (ppm)/tablet	Label claim# mg bhasma / tablet	Expected Cu (ppm)/tablet
Chan R (B)	0.199	0.0006	0.31	3.109	no label claim	-
Soot (A)	0.448	0.0007	0.15	7.0	15.625	8.59
Arv (A)	0.232	0.0003	0.12	3.625	6.944	3.82
Nitv (B)	0.298	0.0007	0.23	4.656	no label claim	-

**Considering Cu Std 1 ppm = 0.064*
#Considering Copper content of tamra bhasma =55%

It can be inferred that either the amount of copper bhasma added to the formulation was less or the percentage of copper in the bhasma raw material used in the formulation was less.

Spectrophotometer analysis showed that copper was present predominantly in its Cupric (II) state (refer table 8, 9, figure 5).

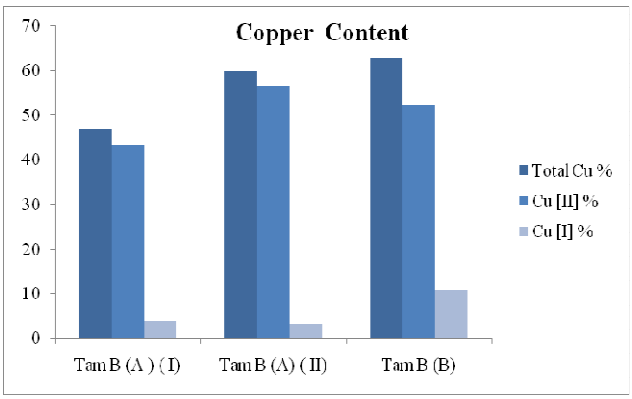
Table 8
Spectrophotometric evaluation of Cu [I] and Cu [II]

Sample ID	Total Cu %	Cu [I] %	Cu [II] %
Tam B (A) (I)	47	3.8	43.2
Tam B (A) (II)	60	3.3	56.7
Tam B (B)	63	10.6	52.4

Table 9
Percentage of Cu [I] and Cu [II] in Bhasma Sample

Sample ID	Cu [I] %	Cu [II] %
Tam B (A) (I)	8.09	91.91
Tam B (A) (II)	5.5	94.5
Tam B (B)	16.83	83.17

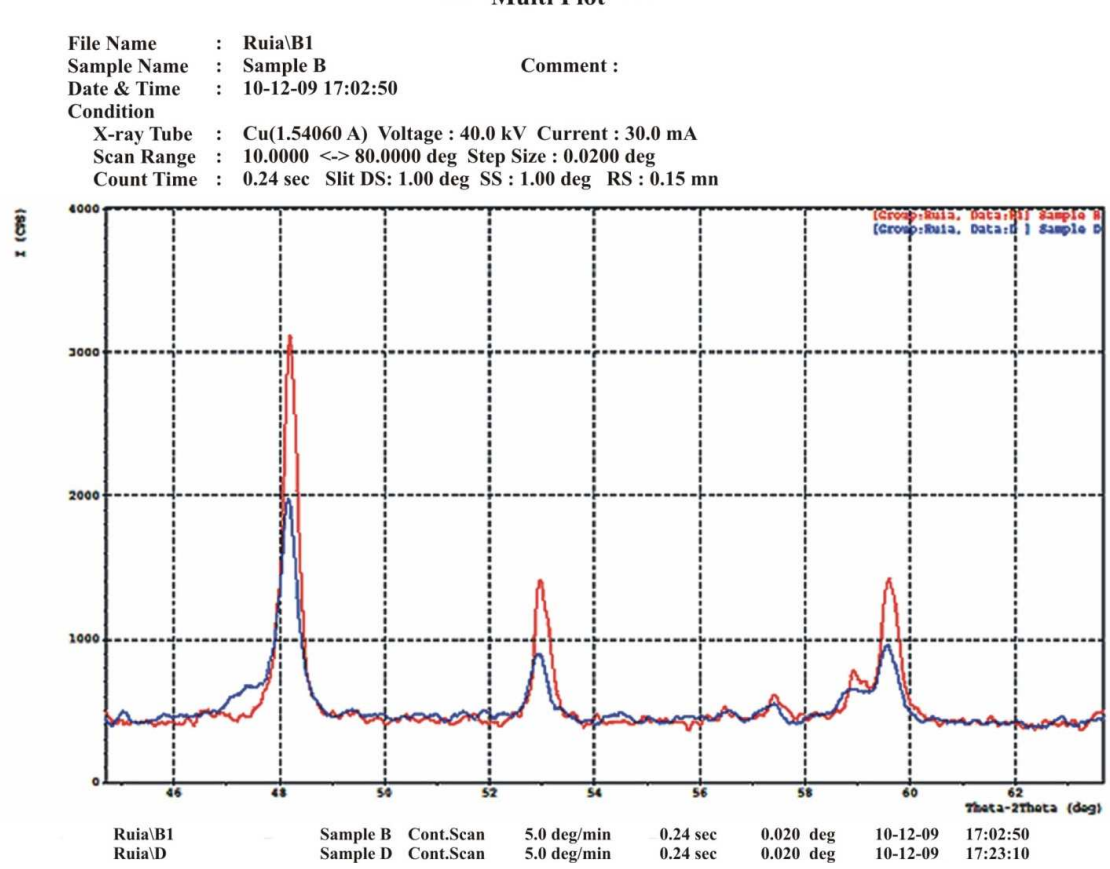
Figure 5
Spectrophotometric Evaluation of Copper [I] and Copper [II]



There was variation in the assay of copper content by the two methods AAS and UV Visible Spectroscopy.

During the process of puta in Bhasma preparation copper sulphide is completely converted to copper oxide. X- Ray Diffraction revealed predominant presence of Copper Sulphide (refer figure 6). [9,10,11]

Figure 6
X Ray Diffraction Spectra of Bhasma samples



Samples in their sulphide form suggest incomplete bhasma preparation .[12]

The data of the present study suggests that the quality of bhasma used in the formulation was not of the standard quality. Inclusion of analytical techniques becomes a necessary prerequisite to evaluate the quality of Bhasma preparations and formulations. Analytical techniques must be used to help set a comprehensive label claim. Further work needs to be done using various animal models to evaluate the extent of absorption and their elemental effect at tissue level of these Bhasmas and their formulations .[13]

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