



Research Article

PREPARATION AND ANALYTICAL STUDY OF *VATA GAJENDRA SINGH RASA*

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ABSTRACT

Rasaushadhi's (Herbomineral preparations) are unique, acts in low dose, highly potent, don't have annoying taste and gives quick results, hence have edge over the herbal medicines. In the present trend of commercialization, there are many doubts raised regarding authenticity of *Ayurvedic* products. Hence analysis and assessment of raw material, different processes of drug preparation and final product is necessary to maintain authenticity, quality and purity of drug. At present time *Amavata* and *Vatavyadhi* are most common problem due to lifestyle change, changed food habits, lack of exercise etc. *Vata Gajendra Singh Rasa* is a *Kharaliya Rasayana* mentioned in *Bhaishajyaratnawali* under *Amavata Rogadhikara*. The main ingredients of *Vata Gajendra Singh Rasa* are *Abhraka Bhasma*, *Loha Bhasma*, *Tamra Bhasma*, *Naga Bhasma*, *Kajjali* and *Vatsanabha*. In this study *Vata Gajendra Singh Rasa* is prepared as per text of *Bhaishajyaratnawali* and assessment of drug done as per *Ayurvedic* and modern criteria. Chemical analytical studies carried out at government approved quality control laboratory. Analysis of main ingredients like *Abhraka*, *Loha*, *Tamra*, *Naga*, *Vatsanabha*, *Parada* and *Gandhaka* done by methods mentioned Ayurvedic Pharmacopoeia of India. In process analytical studies like analysis of the *Bhasma's* carried out as per methods of Ayurvedic Pharmacopoeia of India and evaluated with the standards mentioned in Pharmacopoeial Standards for Ayurvedic Formulations of Central Council for Research in Ayurveda and Siddha. Analytical tests of the final product (*Vata Gajendra Singh Rasa pills*) carried out as per reference of Ayurvedic Pharmacopoeia of India and Indian pharmacopoeia. Results of the raw material study justifies authenticity of raw material, results of in process study of the *Bhasma* are compared with standards, fulfills assessment criteria. Results of the final product discussed and conclusions are drawn, explained in full paper. Further studies are required to develop standards for the formulation.

KEYWORDS: *Vata Gajendra Singh Rasa*, *Rasaushadhi*, Preparation, Analytical Study.

INTRODUCTION

The great alchemist *Nagarjuna* first time processed *Maharasa*, *Dhatu* etc. (metals and minerals) with various plant juices, *Kwathas* (*decoction*) e.g. *Dashamoola Kwatha* etc. by different mechanisms like *Shodhana*, *Jarana*, *Marana*, *Satvapatana* (standard procedures for *Bhasma* preparation) etc. and converted these once highly toxic inorganic substances into qualified lifesaving medicines.^[1] These medicines are now known as *Rasaushadhi* (Herbo-mineral preparations) and science is known as *Rasashastra* i.e. Alchemy of Indian Medicine. This is said to be the ancient medicinal alchemy. *Rasaushadhi* (Herbo-mineral compounds) acts as a weapon for *Ayurvedic* practitioners as they are highly potent even in low dose, don't have annoying taste, can give quick results and can be preserved for longer period.^[2]

Now a day there is a huge debate all over regarding the toxicity of *Rasaushadhi's*. There is no doubt that the basic principles established by Rasacharya have concrete scientific background, the questions are raised because of faulty methods of preparation and assessment.

Hence development of SOP's, analysis of different processes of drug preparation with the help of well-developed modern science and technology is important. Department of AYUSH (*Ayurveda*, *Yoga*, *Unani*, *Siddha*, and Homeopathy) and C.C.R.A.S. (Central Council of Research in *Ayurveda* and *Siddha*) developed standards for some *Ayurvedic* drugs and formulations in "Ayurvedic Pharmacopoeia of India". Here attempts are made for the preparation and analysis of *Vata Gajendra Singh Rasa* in terms of *Ayurvedic* as well as modern science and technology.

There are several formulations available in the text of *Ayurveda* for the treatment of *Vatavyadhi*. *Mahavatavidhwansa Rasa* is one of the famous formulations for *Vatavyadhi*^[3], but every formulation has its own features and limitations, like this *Mahavatavidhwansa Rasa* cannot be used in *Pitta Prakruti*, and patient with heart diseases because it contains '*Vatsanabha*' in more quantity. The main ingredients of *Mahavatavidhwansa Rasa* and *Vata Gajendra Singh Rasa*

are nearly same but reduction in the quantity of *Vatsanabha* and few other changes makes the formulation so beautiful that it can be used in all types of *Jirna/Kaphanubandhi Vatavyadhi* and *Amavata*.^[4]

MATERIAL AND METHODS

Parada Shodhana

Equal amount of *Sudha* (slaked lime) was mixed with *Parada* and the mixture is triturated for 3 days. It was then strained through twofold cloth and again taken into the *Khalva*. This time mixed with *Lasuna Kalka* (peeled garlic cloves) ½ part and ¼ the part *Saindhava* (Rock salt). It was triturated for such a time, till the mixture become black in colour then washed with hot water to get *Parada* back.^[5]

Gandhaka Shodhana

Ghrita with equal quantity of *Gandhaka* added to pan and subjected to heat. As soon as *Gandhaka* was liquefied the mixture as whole is poured in another pot containing cold *Godugdha*, through a permeable muslin cloth. Then *Gandhaka* was drawn out and washed with hot water. This procedure was repeated for 3 times.^[6]

Preparation of Kajjali

Samaguna Kajjali prepared by triturating equal quantity of *Parada* and *Gandhaka* in *Khalvayantra* till fulfill *Siddhilakshana*.

Abhraka Bhasma Nirmana

A) Shodhana of Abhraka: First blocks of *Krishna Abhraka* subjected to heating on gas stove. Then *Abhraka* quenched (*Nirvapa*) into *Triphalakwatha* when it turned red hot. Procedure repeated for 7 times.^[8]

B) Dhanyabhraka Nirmana: *Shuddha Abhraka* and *Dhana* in proportion 4: 1 tied in jute cloth then it was dipped into *Kanji* for 72 hrs. Thereafter it was rubbed by sole to get coarse powder of *Abhraka*.^[9]

C) Abhraka Marana: for *Nischandikarana* of *Abhraka Bhasma Kalami Sora* and *Guda* (Jaggery) used for first 2 *Putas*. *Arkapatra Swarasa*, *Dashamoola Swarasa* and *Ghritakumari Swarasa* were used as *Bhavana Dravya*, 40 *Gajaputas* given.^[10]

Loha Bhasma Nirmana

A. Samanya Shodhana of Loha: Red hot *Loha* immersed seven times each into *Taila*, *Takra*, *Gomutra*, *Kanji* and *Kulattha Kwatha* respectively.^[11]

B. Vishesa Shodhana of Loha: red hot *Loha* crusts immersed into *Triphala Kwatha* and same procedure repeated seven times.^[12]

C. Marana of Loha: *Bhanupaka* - first *Shuddha Loha* along with *Triphala Kwatha* kept in sunlight till dehydration. Same procedure repeated 7 times. *Sthalipaka*- then *Loha Choorna* was taken in iron pan and *Triphala Kwatha* added, intense heat given till it became dry. *Putapaka* - *Shuddha Loha* and 1/12th part *Hingula Choorna* triturated with *Triphala Kwath*, then *Chakrika* prepared, then it was packed in *Sharava Samputa* and 16 *Gajaputa* given. *Hingula* added upto 4th *puta*.^[13]

Tamra Bhasma Nirman

A) Samanya Shodhana of Tamra –same as *Loha Samanya Shodhana*.

B) Vishesa Shodhana of Tamra - *Swedana* of *Tamra* done with *Gomutra* for 3 hrs by *Dolayantra* method.^[14]

C) Tamra Marana: *Shudhha Tamra* 1 part and *Samaguna Kajjali* 1/4th part triturated in *Khalvayantra* with *Jambir Swarasa* and circular cakes of coin shaped prepared, dried cakes kept into *Sharavasamputa* and subjected to *Putra*, after 19 *Putas Tamra Bhasma* fulfilled *Bhasma Pariksha*. 1/4th part *Kajjali* added upto 4th *Putra*.^[15]

Naga Bhasma Nirmana

A) Shodhana of Naga: *Samanya Shodhana* of *Naga* done as per reference of *Rasaratnasamuchhaya* 5/13.

B) Vishesh Shodhana of Naga: Rods of *Naga* subjected to heat in iron pan on gas stove. Then melted *Naga* poured into *Choorndaka* by using *Pithara Yantra*. Same procedure repeated 7 times.^[16]

C) Naga Marana: *Shuddha Naga* 1 part and *Shuddha Manahshila* 1/12th part triturated in *Khalvayantra* with *Vasa Swarasa* and circular cakes of coin shaped prepared, dried cakes kept into *Sharavasamputa* and subjected to *Kapot Putra*, the procedure repeated upto 40 *Putra*. *Manahshila* added upto 10 *Putra*.^[17]

Vatsanabha Shodhana

Chanaka shaped pieces of *Vatsanabha* were kept immersed in *Gomutra* (cow's urine) placed in earthen pot for three days. *Gomutra* was changed every day. Later on pieces were taken out washed with water, external layer was separated and dried.^[18]

Tankana Shodhana

Tankana is taken in an iron pot and started heating as soon as it melts, it was stirred by spoon. When it was completely free from water it was taken out. This is the *Shuddha Tankana* which can be used for medicinal purpose.^[19]

Preparation of Vata Gajendra Singh Rasa

Ingredients

1. *Abhraka Bhasma* - 1 part - 10 gm
2. *Loha Bhasma* - 1 part - 10 gm
3. *Tamra Bhasma* - 1 part - 10 gm
4. *Naga Bhasma* - 1 part - 10 gm
5. *Shuddha Parada* - 1 part - 10 gm
6. *Shuddha Gandhaka* - 1 part - 10 gm
7. *Shuddha Tankana* - 1 part - 10 gm
8. *Sh. Vatsanabha* - 1 part - 10 gm
9. *Saindhava* - 1 part - 10 gm
10. *Lavanga* - 1 part - 10 gm
11. *Shuddha Hingu* - 1 part - 10 gm
12. *Jatiphala* - 1 part - 10 gm
13. *Twaka* - ½ part - 5 gm
14. *Tejpatra* - ½ part - 5 gm
15. *Sookshma Ela* - ½ part - 5 gm
16. *Haritaki* - ½ part - 5 gm
17. *Bibhitaki* - ½ part - 5 gm
18. *Amalaki* - ½ part - 5 gm

19. *Jiraka* - ½ part - 5 gm20. *Kumari Swarasa* - q.s. (75 ml.)**Procedure**

First all the ingredients measured accurately and sieved through the cloth. Then *Kumari Sara* was crushed in a mixture grinder and juice was kept in a measuring cylinder. Then *Kajjali* was triturated for 3 hour then *Abhraka*, *Tamra*, *Loha*, *Naga Bhasma* were added and triturated for 1 hour. Thereafter the other ingredients were added and *Kumari Swarasa* was added gradually, till the mixture became wet. The mixture was triturated till it became thick and able to form *Vati*. Then *Vati* each of weight 3 *Ratti* (360 mg average) was formed dried and packed in air tight container.^[20]

Analytical Study (According to Modern Science)

The Analytical tests were carried out at FDA and Government authorized, Agmark certified Chemical Laboratory. The Analytical tests are divided in 3 steps - 1) Raw material study 2) In process study and 3) Final product study. 1) Raw material study -It includes analysis of *Ashuddha Parada*, *Ashuddha Gandhaka*, *Ashuddha Abhraka*, *Ashuddha Loha Churna*, *Ashuddha Copper wire*, *Ashuddha Naga* and *Ashuddha Vatsanabha*. 2) In process study -It includes Physio-chemical analysis of *Abhraka Bhasma*, *Loha Bhasma*, *Tamra Bhasma*, *Naga Bhasma* and *Shuddha Vatsanabha*. 3) Final product study - It includes physio-chemical analysis of *Vata Gajendra Singh Rasa*. The following tests were carried out at Laboratory.

1) Raw material study

A) Determination of mercury (Assay for Hg) -(By complexometric Titration method).

0.2 gm sample was taken in volumetric flask and dissolved in HNO₃. 50 ml of distilled water was added. PH was maintained at 6 by adding excess of Hexamine. Mixture in volumetric flask was then titrated with 0.05 N EDTA, by using xylinon orange as indicator. Each ml of EDTA = 0.0104 gm of Mercury.^[21]

B) Determination of sulphur (Assay for S) - (Gravimetric assay)

Solutions: 1) Carbon tetrachloride saturated with Bromine, 2) Barium Chloride - 10% solution in purified water.

Procedure: 1 gm of sample was taken in 250 ml beaker. Then 10 ml carbon tetra chloride saturated with bromine added and kept in fume chamber overnight and 15 ml nitric acid added, digested on water bath then 10 ml hydrochloric acid added, digested it to expel NO₂ fumes till syrupy mass was obtained. Allowed to cool and extracted with hydrochloric acid, made the volume up to 100 ml, boiled and filtered through whatman 40 No. filter paper, then residue washed with hot purified water. Filtrate treated with ammonia solution for R₂O₃ precipitations (R stands for Fe and Al). Filtered through whatman 41 No. filter paper in 500 ml beaker, filtrate acidified with hydrochloric acid and 20 ml of 10% barium chloride solution added, digested on burner. Then precipitate allowed to settle for overnight. Then filtered through whatman 42 No. filter paper and washed with purified water, precipitate kept in muffle furnace in pre weighed platinum crucible upto 850^o. Weighed after cooling.

Calculation: % of Sulphur = Weight of the precipitate x 0.13734 x 100/ Weight of sample. ^[22]

D) Determination of Iron (Assay for Fe) -(By Dichromate method)

Solutions: 1) Stannous chloride solutions - 5 mg of SnCl₂ dissolved in 25 ml hydrochloric acid and diluted to 100 ml (5% solution), 2) Mercuric chloride - saturated solution in purified water. 3) Sulphuric acid + orthophosphoric acid mixture - 60 ml purified water taken, 15 ml sulphuric acid and 15 ml phosphoric acid added, then diluted to 100 ml. 4) Diphenyleamine barium sulphonate - 0.25 g dissolved in 100 ml water. 5) 0.1N standard potassium dichromate solution.

Procedure: An aliquot from the stock solution was taken into 250 ml conical flask and diluted to 100 ml with purified water then 2 drops of methyl red indicator followed by 2 gm of ammonium chloride added, dilute ammonia solution added till brown precipitate appears and solution with precipitate was boiled for 5 minutes the content cooled and filtered through whatman no. 41 filter paper. Residue washed with hot purified water. Then dissolved in dilute hydrochloric acid in 250 ml beaker and made the volume upto 100 ml. Then solution boiled on burner to reduce the Fe³⁺ to Fe²⁺ by adding stannous chloride solution drop wise till solution became colorless. Then 2 drops of stannous chloride, 15 ml 10% solution of mercuric chloride, 25 ml acid mixture and 3 drops of biphenyl amine barium sulphonate indicator added. Thereafter purified water added, titration done against potassium dichromate solution; appearance of violet colour shows end point.

Calculation: 1 ml 1N K₂Cr₂O₇ = 0.05585 g Fe / = 0.7985 Fe₂O₃

% Fe = 0.05585 X normality of K₂Cr₂O₇ X aliquot X ml K₂Cr₂O₇ X 100/Weight of sample X total volume.^[23]

E) Determination of copper (from copper wire) - (By Iodometric titration)

Solutions: 1) Standard 0.1 sodium thiosulphate solution, 2) Potassium Iodide 3) Starch 1% solution- 1 gm dissolved in purified water and boiled to make up to 100 ml.

Procedure: An aliquot of sample from the stock solution was taken in a beaker and 1 gm sodium fluoride added. Then ammonia solution added till precipitation occurs and acetic acid added to dissolve the precipitate, boiled and cooled in water bath. Then potassium 1 gm added titrated the liberated iodine against 0.1N sodium thiosulphate solutions by adding starch solution as indicator in iodine flask. The colour changes from blackish brown to white indicated end point. Calculation of copper value done against 1 ml sodium thiosulphate solution titrating against standard 1000 ppm copper solution.

Calculations: 1 ml N Na₂S₂O₃ = 0.06354 g of Cu

%Cu = 0.06354 X Normality of Na₂S₂O₃ X ml of Na₂S₂O₃ X Aliquot X 100/Weight of sample X Total volume.^[24]

E) Determination of Aluminum in Mica

Preparation of solution: A weighed quantity of well mixed sample was transferred to long necked flask of 250 ml capacity. 10 ml of saturated solution of bromine was added in carbon tetrachloride. Flask was covered and

allowed to stand for about 30 minutes, stirring several times. Then 15 ml of conc. Nitric Acid was added to flask was covered and was allowed to stand for 30 minutes, stirring several times. Flask was given heat over a low flame. Small quantity of conc. Nitric Acid was added from time to time until the solution was clear and didn't darken on standing. Solution was transferred quantitatively to 250 ml beaker with the aid of water evaporated on hot plate to about 5 ml. 20 ml of sample solution was pipetted out and 20 % excess of oxine solution (1 ml will precipitate 0.001g of Aluminium was added. When the complex Al (CaH₆ ON) was formed precipitation was completed by addition of solution 4g of ammonium acetate in the minimum quantity of water mixture was stirred and allowed to cool. Granular precipitate was filtered through a sintered glass crucible of porosity No. 4 and was washed with warm water. The complex was dissolved in warm conc.HCL acid. Solution was collected in 250 ml reagent bottle. Few drops of indicator (0.1% methyl orange solution) were added and 0.5 to 1 gm of pure potassium bromide was also added until colour become pure yellow. To determine exact end point it was better to add a slight excess of standard bromide solution, dilute solution considerably with hydrochloric Acid then 10 ml of 10% potassium iodide solution was added. The liberated iodine was titrated with standard 0.1 N sodium thiosulphate using starch as indicator 0.1 ml of NKBrO₃ = 0.002249 g Al.^[25]

F) Determination of Magnesium in Mica - This was done by Complexometric Titration Method.^[26]

G) Determination of Silica in Mica - Determination of silica as SiO₂ was done by Hydrofluorisation.^[27]

H) Determination of K₂O content in Mica - This was done by flame photometer.^[28]

I) Determination of Fe in Mica - was done by Dichromate method as explained before in Determination of Fe.^[29]

J) Determination of Lead (Assay for Pb) -(By Complexometric Titration Method)

Solutions: 1) Acetic acid – ammonium acetate buffer 5.5 – 6.0 pH, 200 g ammonium acetate taken, 30 ml glacial acetic acid added and made up to 1000 ml. 2) EDTA solution 0.05 M – 18-6120 g of sodium salt of ethylene diamine tetraacetic acid (EDTA) in purified water and made up to 1000 ml. 3) Xylenol orange indicator – 0.2 g indicator dissolved in 100 ml purified water and 2 drops of acetic acid added. 4) Thio urea 5) Ascorbic acid, 6) Urea and 7) Sodium fluoride.

Procedure: 1 gm of powdered sample taken in a beaker and 30 ml aquaregia and digested it on hotplate. After 15 minutes 15 ml sulphuric acid added and solution evaporated to dryness. The residue along with filter paper taken in a beaker and 50 ml acetic acid-ammonium acetate buffer added. Boiled and filtered through whatman no. 40 filter paper, residue washed with hot purified water. A pinch of thiourea and ascorbic acid and 3 drops of xylenol orange indicator added. Solution titrated against 0.05 M ethylene diamine tetraacetic acid (EDTA) solution. Purple content from the ethylene diamine tetraacetic acid used up in titration. Lead value calculated against 1 ml of ethylene diamine tetraacetic acid solution against standard 1000 ppm lead solution.

Calculation: 1ml 0.05 N EDTA = 10.3605 mg of Pb.

%Lead= 10.3605 X Normality X ml of EDTA X ml of EDTA X Aliquot X100/Weight of the sample X Total volume.^[30]

k) Total alkaloids from impure Vatsanabha - The sample was extracted repeatedly using (3 x 50ml) 0.1 N H₂SO₄ in an ultrasonic bath. The solution was filtered the mixed acid solution was washed with 4 successive quantities of 25 ml chloroform. The chloroform washing was then rejected acid solution basified with dilute ammonia solution and extracted with (20 ml x 5) diethyl ether. The combined diethyl ether extracts were then washed with 5 ml distilled water and other evaporated to dryness in a weighed beaker on a water bath. Residue dried to constant weight at 105°C and the total alkaloid content was estimated.^[31]

2) In process study

a) Loss on Drying: About 2 gm sample was taken in a tarred china dish, dried at 105 °C, cooled it in desiccator and weighed. Again the procedure of heating, cooling and weighing was repeated until the constant weight was obtained. The loss on drying was calculated.^[32]

b) Loss on Ignition: Loss on ignition test is used to determine the relative percentage by weight organic portion to inorganic portion of formulate. 1 gm of *Bhasma* sample was taken in porcelain crucible and dried at 110°C. Then after heated to 950°C allowed volatile, substance to escape and organic matter to destroy until its mass ceases to change, mass was redetermined and process was repeated until it shows that mass change was complete. Loss on ignition =100 x (loss in weight)/Wt. of sample taken for test.^[33]

c) Acid Insoluble Ash: 1 gm sample was taken in silica dish. Incinerated at a temperature not exceeding 450°C until become free carbon, cooled in desiccator and weighed samples were heated, cooled and weighed till constant weight was obtained. Thus obtained ash of sample was treated with dil. HCL The insoluble matter was collected on an ash less filter paper and washed with hot water until the filtrate becomes neutral. Then this filter containing insoluble matter was transferred to original crucible dried on hot plate and ignited at 900 °C to constant weight. Acid Insoluble Ash =100x wt. of acid insoluble ash/Wt. of sample taken.^[34]

d) Determination of Iron (in Loha and Abhakra Bhasma): (By Dichromate method) – by same method as Mentioned in raw *Loha* analysis.^[35]

e) Determination of Copper from Tamra Bhasma - (By Ideometric titration) –as mentioned in raw Copper analysis.^[36]

f) Determination of lead from Naga Bhasma - By Complexometric Titration Method as described in analysis of raw lead.

g) Total alkaloids from Purified Vatsanabha -The method was same as mentioned for total alkaloids from impure *Vatsanabha* in Raw material study.

3) Final product study

a) Disintegration time: Pills of sample were placed the tube of disintegration apparatus, the tube was raised and lowered in such a manner that the complete up and down

movement repeated 30 times per minute. The time required to disintegrate the pills was calculated.^[37]

b) Loss on Drying -Method same as given for *Bhasma* in 'In process study'.

c) Total Ash: Method as per mentioned for *Bhasma*, in 'In process study'.

d) Acid Insoluble Ash: Method as per mentioned for *Bhasma*, in 'In process study'.

e) Water Soluble Ash: Ash boiled with 25ml of water for 5 minutes; insoluble matter collected in Gooch crucible, washed with hot water, and ignited for 15 minutes at the temperature not exceeding 450°C. The weight of the insoluble matter subtracted from the weight of the ash; the difference in the weight represents the water soluble ash. The percentage of water soluble ash with reference to the air dried drug calculated.^[38]

f) Alcohol Soluble Extractive: 5 g of the air dried drug coarsely powdered, with 100 ml of alcohol the specified strength kept in closed flask for 24 hours, rapidly filtered by taking precautions against loss of solvent. Evaporated 25ml of the filtrate to dryness in a tarred flat bottomed shallow dish and dried at 105°C, to constant weight and weighed. The percentage of alcohol soluble extractive calculated with reference to the air dried drug.^[39]

g) Water Soluble Extractive: Proceeded as directed for the alcohol soluble extractive, used chloroform water instead of ethanol.^[40]

h) Assay for Pb: Method - By Atomic Absorption Spectrophotometer.

Solutions: 1) 1000 ppm lead standard solution prepared from Pb. 2) 1 ppm, 5 ppm and 10 ppm standard Pb solution prepared. 3) Wavelength 213.9 nm.

Procedure: 0.10 g powdered sample taken in 250 ml beaker and 25 ml aquaregia added, digested on hot plate till syrupy mass was obtained. Filtered through whatman 40 no. filter paper in 100 ml volumetric flask. 1% acidity maintained with nitric acid and washed with hot water made volume to 100 ml. Observation taken on Atomic Absorption Spectrophotometry on appropriate wavelength.

Calculations: ppm (metal) = Reading (Conc.) X original volume/Weight of sample. ^[41]

i) Assay for Fe: Method was same as mentioned for *Bhasma* in 'In process study'.

j) Determination of mercury (Assay for Hg): (By cold vapour Atomic Absorption Spectrophotometer method).

Reagents and Standards: 1) Stannous chloride solution (20% w/v), 2) Potassium dichromate Solution (1% w/v), 3) Potassium permanganate solution (5% w/v), 4) Sulphuric acid (1%), 5) Nitric acid (10% w/v), 6) Sodium hydroxide (20% w/v), 7) Sulphuric acid (1:1 H₂SO₄). 8) Standard Mercury solution – 0.1354 g of mercuric chloride dissolved in 25 ml of 5% nitric acid. 1 ml potassium dichromate solution added and made up to 100 ml with 5% nitric acid. 1 ml of this solution = 1.0 mg Hg/ml = 1000 ug Hg/1 ml = 1000 ppm Hg.

Procedure: 5 g dry fine powder of sample weighed in a conical flask, 15 nitric acid and 5 ml sulphuric acid added and left flask in ice bath for 90 minutes. Heated on purified

water bath for 30 minutes and 150 g potassium permanganate and 3 ml mercury free hydrochloric acid added. Boiled gently for 5 minutes, cooled and transferred into a 50ml plastic volumetric conical tube and made up to volume. After centrifugation clear solution used for determination of mercury content. 10 ml of aliquot pipette out into reaction vessel R2 of mercury analyzer. Procedure followed for reading mercury content as described in operation manual of the instrument.

Calculation: Mercury content (ng) = Mercury content in aliquot/Weight of the sample (g) X Total volume of the aliquot (50ml)/ Volume of aliquot for measurement (10ml).^[42]

k) Assay for Cu- by same method as mentioned for *Bhasma* in 'Raw material study'.

l) Total Sulphur as S- Method – by same method as mentioned in 'Raw material study'.

OBSERVATIONS AND RESULTS

Observations

Abhraka Bhasma Nirmana

During *Marana* procedure, the 85-90% of *Nishchandravta* was achieved after third *Putra*. After first *Putra Abhraka* was swollen and cake like in appearance. After 40th *Putra Abhraka* was *Sookshma*, *Shlakshna*, and *Ishtikabha* and fulfilled all the *Bhasma Pariksha*.

Loha Bhasma

After *Vishesha Shodhana*, it became more brittle, black in appearance and *Triphala Gandhi*. After *Bhanupaka*, it became so brittle that it was easily converted into powder form. The *Sookshmatva* of *Loha Bhasma* was depend on the *Mardana* of *Bhasma* after every *Putra*. *Loha Bhasma* was triturated and filtered through cloth and subjected to next *Putra*. After 16th *Putra Jambuphala Varna*, *Sookshma*, *Shlakshna Nishchandra*, *Varitara Bhasma* was formed.

Tamra Bhasma

Vishesha Shodhana of *Tamra* was done in *Gomutra* there after it became more brittle, brown in colour with blackish coarse particles of *Gomutra Kshara*, which removed after *Tamra Prakshalana* in hot water. The colour of *Tamra* was dark black up to adding the *Kajjali* (till 4th *Putra*) then it became lighter. After 19th *Putra Sookshma*, *Shlakshna*, *Nishchandra* and *Varitara Bhasma* of *Tamra* was formed.

Naga Bhasma

During *Putra* procedure, the *Chakrika* of *Naga* was converted into uniform thick layer which was stuck to *Sharava* after first *Putra*, and then gradually the *Agnipramana* was reduced. It is observed that *Naga* is very sensitive to *Agni* even 300 gms of increase in cow dung cakes leads to increase in hardness of *Chakrika*. After 40 *Putra*, *Niruttha*, blackish, *Sookshma*, *Shlakshna Nishchandra* and *Varitara Naga Bhasma* was formed.

Results**A) ANALYTICAL STUDY (According To Ayurveda)****Table 1: Physical Analysis of Bhasma**

Parameter	Abhraka Bhasma	Loha Bhasma	Tamra Bhasma	Naga Bhasma
Shabda	Dantagrekachakacha bhava	Dantagrekacha kachabhava	Dantagrekacha Kachabhava	Dantagrekacha kachabhava
Sparsha	Shlakshna, Mrudu, Laghu, Sookshma	Shlakshna, Mrudu, Laghu, Sookshma	Shlakshna, Mrudu, Laghu, Sookshma	Shlakshna, Mrudu, Laghu, Sookshma
Rupa	Ishtikavarna, Nishchandra, Rekhapurnatva, Varitara, Unama, Nirdhuma	Jambuphalavarna, Nishchandra, Rekhapurnatva, Varitara, Unama, Nirdhuma	Krishna Varna, Nishchandra, Rekhapurnatva, Varitara, Unama, Nirdhuma	Brownish black, Nishchandra, Rekhapurnatva, Varitara, Unama, Nirdhuma
Rasa	Nhiswadu (tasteless)	Nhiswadu (tasteless)	Nhiswadu (tasteless)	Nhiswadu (tasteless)
Gandha	Nirgandha (odorless)	Nirgandha (odorless)	Nirgandha (odorless)	Nirgandha (odorless)

II) Chemical Analysis

A) Apunarbhava Pariksha - Bhasmas retained their original form even after Apunarbhava Pariksha confirming their stability. They didn't regain their metallic state, it means test is positive.

B) Niruttha Parikshana - Consistency in the weight of silver leaf is the rule of passing the test. Results indicate that the Bhasmas passed through the Niruttha Pariksha successfully.

F) Dadhi Pariksha- It was done for Tamra Bhasma. The test was positive i.e. there was no change in colour of Dadhi after 72hrs.

G) Amala Pariksha - After sprinkling Loha Bhasma over cut surface of fresh fruit of Amalaki. There was no change in colour of Loha Bhasma. This means test is positive.

II) Analytical Study (According to Modern Science)**Results of Raw material study****Table 2: Results of Raw Material Study (As per analytical study)**

Sample	Test	Value
Mercury	Assay as Hg	99.56 w/w%
Sulphur	Carbon disulphide insoluble matter	0.88% w/w
	Assay as Sulphur	99.02 w/w
Mica	Aluminum as Al ₂ O ₃	8.62% w/w
	Iron as Fe ₂ O ₃	11.71% w/w
	Silica as SiO ₂	50.54% w/w
	Magnesium as Mg	2.43% w/w
	K ₂ O content	5.26% w/w
Iron	Assay as Fe	99.28% w/w
Copper	Assay as Cu	99.18% w/w
Lead	Assay for Pb	99.98% w/w
Impure Vatsanabha	Total alkaloids	0.36% w/w

Results of In Process Study**Table 3: Results of Analysis of Bhasma (as per analytical study of Bhasma)**

S. No.	Test	Value of Abhraka Bhasma	Value of Loha Bhasma	Value of Tamra Bhasma	Value of Naga Bhasma
1	Loss on Drying(% w/w)	0.32	0.35	1.06	0.08
2	Loss on Ignition (% w/w)	0.47	0.71	3.10	0.08
3	Acid Insoluble Ash (% w/w)	51.25	0.68	1.05	82.37
4	Iron as Fe (% w/w)	15.29	61.61	-	-
5	Copper as Cu (% w/w)	-	-	63.47	-
6	Lead as Pb (% w/w)	-	-	-	64.57

Vishadravya**Table 4: Results of Analysis of Vatsanabha (Shodhita)**

Sr. No.	Test	Value
1	Total Alkaloids	0.08%

Results of Final Product study**Table 5: Results of Analysis Vata Gajendra Singh Rasa (as per analytical study of final product)**

Sr. No.	Test	Value
1	Disintegration Time (min.)	65
2	Loss on drying (% w/w)	19.14
3	Total Ash (% w/w)	33.51
4	Acid Insoluble Ash (% w/w)	1.25
5	Acid Soluble Ash (% w/w)	32.26
6	Water Insoluble Ash (% w/w)	18.26
7	Water Soluble Extractive (% w/w)	23.45
8	Alcohol soluble Extractive (% w/w)	6.39
9	Assay for Pb (% w/w)	3.02
10	Assay for Fe (% w/w)	4.26
11	Assay for Hg (ppm)	6.82
12	Assay for Cu (% w/w)	3.12
13	Total Sulphur as S	1.27

DISCUSSION**Discussion about Preparation of Vata Gajendra Singh Rasa****Abhraka Bhasma**

For the *Abhraka Bhasma* preparation *Krishnavajrabhraka* was selected which didn't lose its properties on *Agnipariksha*. The cow dung cakes & coal was used as fuel for the *Putra* and its proportion was taken according to weight of *Abhraka*. For the *Nishchandrikarana* of *Abhraka* first two *Putas* given with *Guda* and *Kalamisora* (*Anubhuta*) and *Chandrika* reduced to almost 80-90% after 4th*Putra*. The temperature was increased slowly according to *Agnisahatva* of *Abhraka*. For the *Bhavana* procedure *Arkapatra*, *Kumari Swarasa* and *Dashamoola Kwatha* were used to achieve *Vataghna* property. The *Ishtika Varna* of *Abhraka Bhasma* is because Iron Oxide is a chief constituent of *Abhraka Bhasma* which believed to be causative factor of that typical brick red colour.

Loha Bhasma

During *Shodhana* when *Loha* heated up to red hot stage then some of iron was converted into ferroso-ferric oxide by reacting with atmospheric oxygen which form black layer on the surface which was removed after quenching. *Vishesha Shodhana* of *Loha* was done in *Triphala Kwatha*. Six liquid Medias used during *Shodhana* of *Loha*. The alternate heating and quenching in the acidic and basic liquid media may leads to the corrosive changes and removal of acid and alkali soluble impurities.

Loha Marana

Hingula is said to be best *Maraka Dravya* of *Loha* hence 1/12 quantity of *Hingula* was added up to 4th*Putra*. Cow dung cakes and coal was used as fuel in *Putapaka* according to weight of *Loha*. The colour of *Loha Bhasma* was purple (*Pakva Jambuphala Varna*). *Loha Bhasma* may be considered as mixture of ferrous oxide, ferric oxide, ferrous sulphate and trace elements. Out of this ferrous sulphate is black and ferric oxide is red in colour. Hence combination of all these compounds makes *Loha Bhasma* purple in colour.

Tamra Bhasma

For the *Tamra Bhasma Nirmana* the copper wire of diameter 0.36 mm selected as raw material. As it is said to be the pure form of *Tamra* expect the insulator Vernix

which was removed by heating and dipping into water. It is said that *Rasabhasma Marita Bhasmas* posses good quality. But there are doubts about *Rasabhasma* hence *Kajjali* is selected as *Maraka Dravya* for *Tamra Bhasma*. *Tamra* is soft metal as compared to *Loha* therefore it requires less heat than *Loha* hence only cow dung cakes are selected as fuel. *Tamra Bhasma Nirmana* require specific temperature pattern. The no. of cow dung cakes for first *Putra* was 25 (each of wt. average 280gms) which were gradually reduced to 5 for the 19th *Putra* for 1340 gm of *Tamra*. The black colour of *Tamra Bhasma* may because of cupric oxide (black to brown colour) and copper sulphide (blue-black colour).

Naga Bhasma

Raw *Naga* was purchased from local market as lead-triangular straight rod. As *Naga* is highly sensitive to temperature initially 15 cow Dung cakes (each of weight 280gm) were used as fuel for 1763 gm of mixture, but after first *Putra* the *Chakrika* were converted into the thick layer was stuck to the *Sharava*, it means that proportion of *Agni* was more than the required quantity and orange colour turns to black. Then the no. of cow dung cakes gradually reduced to 12,6,4,3. For 8, 9, 10th *Putra* 3 cow dung cakes (average wt. 900 gms.) was used as fuel. After 10th *Putra* the *Chakrika* were not so hard and could be breakable. Therefore in case of *Putilohas* initially the proportion of *Agni* should be less as possible and should be increase gradually according to *Agnisahatva*.

Vatsanabha Shodhana

According to modern science the toxic content of *Vatsanabha* is Alkaloids it varies from 0.63 - 4.7%. The total Alkaloid in *Ashuddha Vatsanabha* was 0.36% w/w and after *Shodhana* it was reduced to 0.085% w/w which is 4½ times less than *Ashuddha Vatsanabha*, it means that although *Shodhana* of *Vatsanabha* is looks simple process but the results were significant. It justifies the *Vishagna Prabhava* of *Gomutra*.

Discussion about Analytical Study**i) Discussion about Raw drug study**

The percentage of Mercury in *Parada* was 99.56% w/w, Sulphur in *Gandhaka* was 99.28% w/w, Copper in *Tamra* was 99.18% w/w. and Lead in *Naga* was 99.98%

w/w. It means that the selected raw material was of good quality. In the Analysis of Raw *Abhraka* - Aluminum was 8.62%w/w, Iron -11.71% w/w, Silica -50.54% w/w, Magnesium -2.43% w/w and potassium -5.26% w/w was found. These contents are naturally present in *Abhraka*; the analysis was carried for justification of *Abhraka*. The total alkaloids in *Ashuddha Vatsanabha* were found 0.36% w/w. usually it are present in the range of 0.63-04.7%. The

analytical test was carried out to check authentication of *Vatsanabha*. Above results shows that raw material selected was authentic.

ii) Discussion about 'In process study'

A) *Bhasma*: The results of Analytical study of *Bhasma* are compared with the standards of *Bhasma* mentioned in the "Pharmacopoeial standards for Ayurvedic formulations".

Table 6:Assessment of *Bhasmas*

<i>Abhraka Bhasma</i> ^[43]			<i>Loha Bhasma</i> ^[44]		
Test	Standard Value in % w/w	Observed Value in % w/w	Test	Standard Value in % w/w	Observed Value in % w/w
Loss on Drying	< 0.5	0.32	Loss on Drying	< 0.5	0.35
Loss on Ignition	<1	0.47	Loss on Ignition	<1	0.71
Acid Insoluble Ash	40-65	51.25	Acid Insoluble Ash	<1	0.68
Iron as Fe	14-16	15.29	Iron as Fe	65-70	61.61

<i>Tamra Bhasma</i> ^[45]			<i>Naga Bhasma</i> ^[46]		
Test	Standard Value in % w/w	Observed Value in % w/w	Test	Standard Value in % w/w	Observed Value in % w/w
Loss on Drying	< 2	1.06	Loss on Drying	< 0.1	0.08
Loss on Ignition	<5	3.10	Loss on Ignition	<99.9	0.08
Acid Insoluble Ash	<1.5	1.05	Acid Insoluble Ash	<95.0	82.37
Copper as Cu	60-65	63.47	Lead as Pb	60-65	64.57

The value indicates that results of Analytical study of *Bhasma* were nearly matched with the standard parameters means *Bhasma* prepared are of good quality.

iii) Discussion about 'End Product'

The Disintegrate time of *Vata Gajendra Singh Rasa* was 65 minute which is quite high. It may because starch content of *Bhavana Dravya Kumari*. Assay for lead - 3.02% w/w, Assay for Iron - 4.26% w/w, Assay for Mercury - 6.82% w/w, Assay for Copper - 3.12% w/w, Total Sulphur - 1.2% w/w. Presence of these values in final product is applicable for authentication and standardization *Vata Gajendra Singh Rasa*. To develop standards further studies are required, because the standards for *Vata Gajendra Singh Rasa* are not mentioned in the "Pharmacopoeial standards for the Ayurvedic formulations" of C.C.R.A.S.

CONCLUSION

Conclusion regarding Preparation of drug

For the *Nishchandrikarana* of *Abhraka* first 2 *Putas* given with *Guda* and *Kalamisora* after this process there was marked reduction in *Chandrika*. In *Trividha Lauhapaka Loha* became so brittle that its powder was formed easily after *Bhanupaka* and *Sthalipaka* it concludes *Trividha Lauhapaka* is essential for *Marana* of *Loha*. It is said that salts of *Naga* (powder form) are stable for *Putapaka*. Even if, initially the *Agnipramana* should be minimum and it should be increase gradually according to *Agnisatva* of *Naga*. The *Mardana* of *Dhatu Bhasma* enhances the *Bhasma* formation. If *Dhatu Bhasma* triturated properly after each *Putas* the *Bhasma* will be formed early and easily.

Conclusions regarding Analytical Study

Analysis of *Bhasma* fulfills the criteria of *Bhasma Pariksha*. *Dantagrekachakabhava* of all the *Bhasma* (*Abhraka Loha Tamra*, and *Naga*) indicates that there was absence of free metal particles in *Bhasma*. *Sookshmatva*,

Rekhapurnatva, *Varitara*, *Unama Pariksha* of *Bhasma* indicates its micro fineness. *Nirgandha* and *Nirdhuma* indicate that there were no free *Gandhaka* present in the *Bhasma*. Chemical analysis as per *Ayurveda* i.e. *Apunarbhava Pariksha*, *Niruttha Pariksha* was carried out to check the stability of *Dhatu Bhasma*. *Naga*, *Loha*, *Tamra*. *Bhasma* passed through these test, it concludes that *Bhasma* formed were stable i.e. *Marana* of *Dhatu* was achieved. *Dadhi Pariksha* of *Tamra Bhasma* is authentic to check its toxicity. *Amla Pariksha* of *Loha Bhasma* is easy method to check free Iron content in the *Bhasma*.

Analysis as per Modern Science

The total alkaloids in *Shuddha Vatsanabha* was 0.085% which is 4½ times less than found in *Ashuddha Vatsanabha* (0.36% w/w) it concludes that the principle of *Vishadravya shodhana* is to reduce its poisonous properties up to extent, where it does not show any unwanted effect therapeutically. Conclusion drawn about the final product i.e. *Vata Gajendra Singh Rasa* is- The facts observed in the analysis of *Vata Gajendra Singh Rasa* are as follow. The Disintegration time of drug was 65 min. it was quite high it may happen because *Kumari Swarasa* was used for *Bhavana* contain starch leads to tight bonding of ingredients. Acid insoluble Ash in *Vata Gajendra Singh Rasa* was found 1.25% w/w it conclude that 98.75% of the drug is available in the body for absorption and action.

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