

Original Article

PHYSICOCHEMICAL CHARACTERISTICS OF RASAMANIKYA-AN AYURVEDIC ARSENICAL FORMULATION

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Received: 06 May 2017 Revised and Accepted: 29 Mar 2019

ABSTRACT

Objective: The objective of this study was standardization and Chemical characterization of *rasamanikya* prepared as per standard operating procedures (SOP) mentioned in the classical text.

Methods: *Rasamanikya* was prepared by putting *churnodaka shodhita haratala* (Orpiment-As₂S₃) between two *abhraka* (white mica) sheets which are heated for a while to obtain a red colored finished product. The Ayurvedic specifications for the analysis of *rasamanikya* were performed through qualitative and quantitative analysis. Physicochemical analysis, assay of elements by atomic absorption spectrometer (AAS) and inductively coupled plasma-atomic emission spectrometry (ICP-AES) were carried out and some other tests such as x-ray diffraction (XRD), x-ray photoelectron spectroscopy (XPS) and energy dispersive x-ray analyzer (EDAX) were also performed to ensure the quality of the drug.

Results: In the finished drug Arsenic and Sulphur are present in the form of As₄S₄, As₂S₃, As₂S₅. On the basis of XPS survey scans, scanning electron microscopy-energy dispersive x-ray analyzer (SEM-EDAX) and carbon, hydrogen, nitrogen, sulphur (CHNS) analysis the Arsenic to Sulphur (As to S) ratio is thus standardized as 39-47: 53-61. In addition to this powder, XRD shows a major conversion into an amorphous phase.

Conclusion: The results could be used to lay down a new set of pharmacopoeial standards for the preparation of *rasamanikya* for getting optimal efficacy of medicine. Therefore, the information will help the Scientists and Researchers to build comprehensive standards, to screen the compounds responsible for different bioactivities, and to elucidate the molecular mechanism of action.

Keywords: Physico-chemical, *Rasamanikya*, Ayurvedic arsenical

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DOI: <http://dx.doi.org/10.22159/ijpps.2019v11i5.29429>

INTRODUCTION

Rasamanikya is a well known ayurvedic arsenical preparation, which is prepared out of *shuddha haratala*. The formulation has been first described in *Rasendra Chintamani* in the 13th century A. D. as *Rasamanikya prabham* [1]. The same product has been described in *Siddha Bhesaja Manimala* as *kumuda rasa* [2]. *Tala manikya* is one of the synonyms of *rasamani kya*.

It is commonly and effectively used in various *kustha roga* (skin diseases), *shwasa* (Bronchial asthma), *vicharchika* (Eczema), *bhaganadara* (Fistula), *vatarakta* (Gout), *phiranga roga* (Syphilis), *jwara* (Fevers), *kasa* (Cough), and *nadi vrana* (Chronic wounds) with different *anupanas* in various dosage forms [3, 4].

Haratala (As₂S₃), the only ingredient is described as *dhatuvisha* and *phenasma* in *samhitas* [5, 6]. *Rasamanikya* is one among the numerous preparations made out of *haratala*. Being an arsenical preparation and, considering the importance of *rasamanikya* in clinical use; SOP for its preparation and chemical characterization, safety/toxicity studies becomes the mandate for its acceptance. In this current study, an attempt has been made to evaluate preliminary physicochemical characteristics of *rasamanikya*.

MATERIALS AND METHODS

Pharmaceutical processing

Rasamanikya was prepared by following standard methods mentioned in the Ayurvedic Formulary of India [7]. The whole process of preparation is divided into the following steps.

1. Preparation of *churnodaka*
2. *Haratala shodhana* (Processing of *Haratala*)
3. Preparation of *rasamanikya*

Preparation of *churnodaka*

Lime powder (250 mg) and water (60 ml) were mixed thoroughly with the help of a stirrer. The solution thus obtained was kept aside undisturbed for 12 h. Next day morning, the whole contents were filtered through a clean cloth. The filtrate thus obtained was collected and preserved in a clean glass container [8].

Haratala shodhana

Small pieces of *haratala* were bundled in a muslin cloth (*pottali*) immersed in *dolayantra* containing *churnodaka* and subjected to moderate heat for three hours [9]. Utmost care was taken to immerse *pottali* completely in *churnodaka* throughout the procedure. At the end of three hours, *haratala* was taken out from the *pottali*. It was then washed in potable hot water, dried in open air and *shodhita haratala* [10] was collected.

Preparation of *rasamanikya*

Powdered *shodhita haratala* was spread in between two mica sheets and the joints were closed by using 'U' pins. This was held with the help of tongs and heated over the LPG stove. After the melting is completed, it was withdrawn from heating and ruby colored *rasamanikya* was collected [11].

Reagents and standards

All chemicals, reagents and solvents were used analytical grade and obtained from authentic suppliers.

Physicochemical analysis

Physicochemical analysis, viz. Description, estimation of Loss on drying, ash content, acid insoluble ash, water/alcohol soluble extractive, pH, etc., qualitative/quantitative elemental testing, pesticide, microbiological examination and tablet parameters

hardness, friability, average wt., dissolution time etc. were carried out by following standard methods as per ayurvedic pharmacopoeia of India (API) guidelines [12-14]. The quantitative estimation of heavy metals viz. As, Fe, Mg, B, Al, Ca, Pb and Cr were carried out by atomic absorption spectrometer (Perkin Elmer (USA) Analyst 400) and ICP-AES (Thermo Electron Corporation's model IRIS INTREPRID II XDL). However Sulphur, were quantified by using CHNS analyzer as well as conventional methods [12] EDAX, X-ray diffraction, XPS survey scans, and CHNS analysis were performed to evaluate the elemental composition ratio [15-18].

X-ray diffraction

Powder x-ray diffraction (XRD) analysis of *Rasamanikya* was carried out using Rigaku Ultima-IV X-ray diffractometer with CuK α radiation ($\lambda = 1.54\text{\AA}$) operating at 40 kV and 30mA. Pattern was recorded for angle (2θ) ranging from 10-100° at a scanning rate of 1°/second and scan step of 0.1°. Sample identification was done by matching d-spacing with the standard database.

X-ray photoelectron spectroscopy (XPS)

XPS measurements of *rasamanikya* were obtained on a KRATOS AXIS 165 instrument equipped with dual aluminum-magnesium

anodes using Al K α radiation. The x-ray power supply was run at 15 kV and 5 mA. The pressure of the analysis chamber during the scan was 10⁻⁹ Torr. The peak positions were based on calibration with respect to the C 1s peak at 284.6 eV. The obtained XPS spectra were fitted using a nonlinear square method with the convolution of Lorentzian and Gaussian functions after the polynomial background subtraction from the raw data (fig. 3).

RESULTS AND DISCUSSION

The Organoleptic observation shows that the prepared *rasamanikya* is in the form of a yellowish fine powder having no characteristic odour and taste. The qualitative analysis shows the positive test for the presence of arsenic and sulphur. The chemical analysis revealed that it contains 60.0% of Arsenic and 39.5% of Sulphur on average together with minor elements viz. boron, iron, magnesium, chromium, aluminum, lead, and calcium. Moisture content 0.20% was found when the determined loss on drying at 105 °C. Total ash content (approx 2.75%) is left after burning of volatile matter (96.75%). The observations show that water-soluble (1.75%) and alcohol soluble (2.0%) matter are also present in this formulation. (Details are mentioned in table 2). Particles size is about 300 nm, and is homogeneously distributed which showed the presence of the microfine particle.

Table 1: Observations of three batch analysis

S. No.	Parameter tested	Observed results of three batch analysis
1.	Organoleptic Characters	
	Colour	Yellowish
	Taste	Tasteless
	Odor	Odorless
	Appearance	Fine powder
2.	Physico-chemicals parameters	
	Identification	Yields the reaction characteristics of Arsenic and Sulphur
	Loss on drying	0.15-0.25
	Total Ash (%w/w)	2.0-3.5
	Acid Insoluble Ash (%w/w)	1.5-3.0
	Water soluble extractive(%w/w)	1.0-2.5
	Alcohol (90%) soluble extractive(%w/w)	1.5-2.5
	pH of aqueous extract	5.0-6.0
	volatile matter	96.73±0.02
	Specific gravity	0.9976±0.015
	Particle size distribution	
	10%,	21.24-44.69 μm
	50%,	104.31-144.32 μm
	90%	289.95-308.82 μm
3.	Assay of elements (%w/w)	
a.	Arsenic	59.0-61.0
b.	Sulphur	39.0-40.0
	Boron	0.01-0.10
	Iron	0.12-0.17
	Magnesium	0.15-0.80
	Chromium	0.005-0.02
	Aluminium	0.03-1.10
	Lead	0.25-0.65
	Calcium	0.50-2.50

The three *rasamanikya* (3 batches) samples have near identical XRD patterns with two small but prominent peaks at 26.7 and 27.9 degrees respectively; only one sample shows a peak at 67.8 degrees to the samples were compared with earlier reported XRD patterns [19]. This shows the XRD of the '*haratala*' (arsenic trisulphide/Orpiment) used to make these samples by heating *haratala* at high temperatures while holding the samples between mica sheets. The XRD of *rasamanikya* samples show a complete transformation into an amorphous state, barring two samples

showing a peak due to a crystal phase (some fraction of *hartala* might have remained).

The present set of samples too, show a major conversion into an amorphous phase but the peaks seen at 26.7, 27.9 and 67.8 degrees could not be specifically assigned to any crystalline phase but the XRD patterns quite resemble the amorphous patterns of samples as mentioned therein [19]. The XRD pattern is shown in fig. 1 and fig. 2.

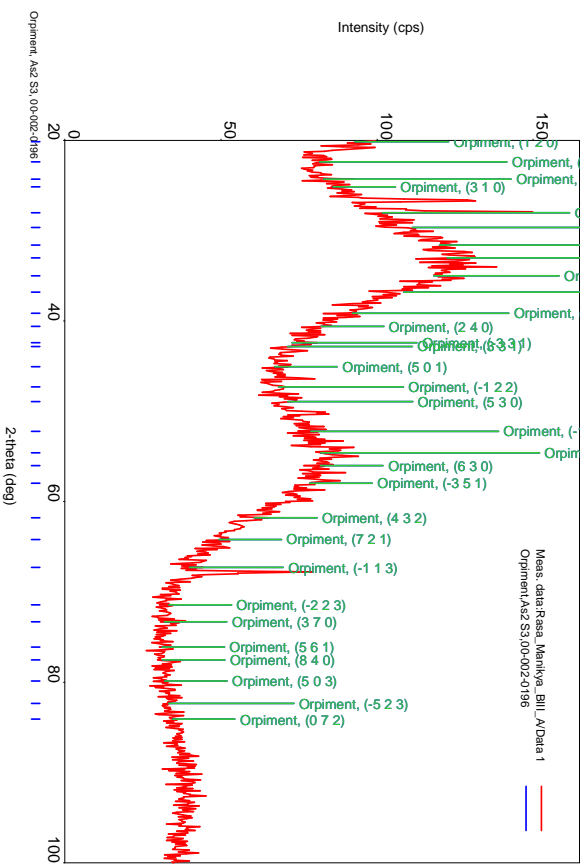
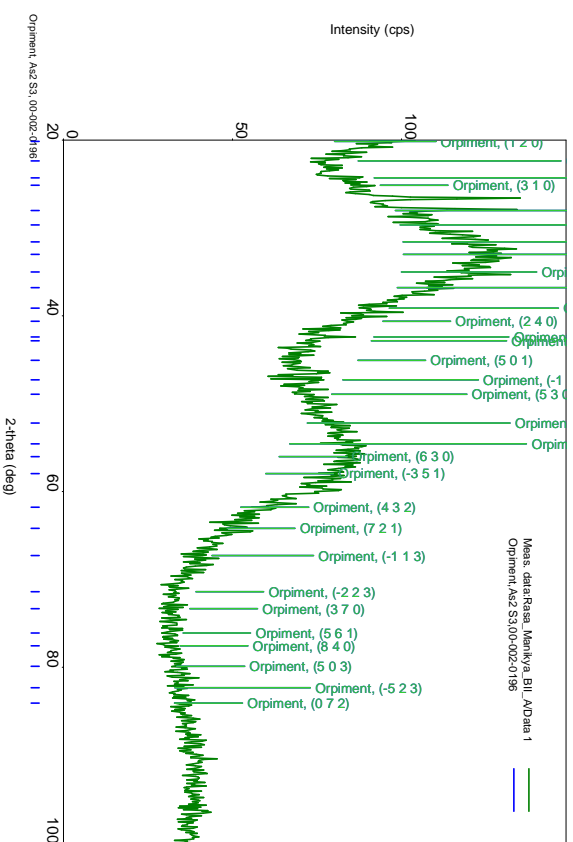
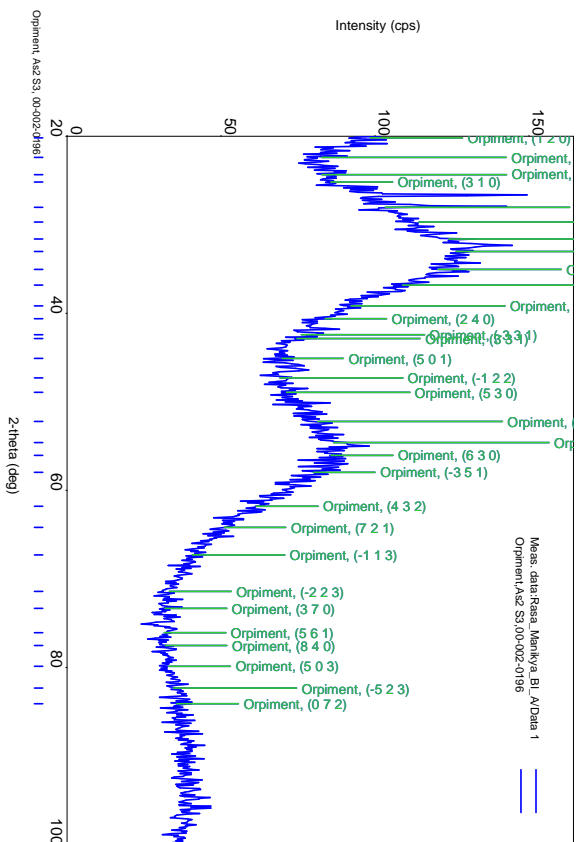


Fig. 1: XRD pattern of Rasamanikyva (Batch-I, II, III)

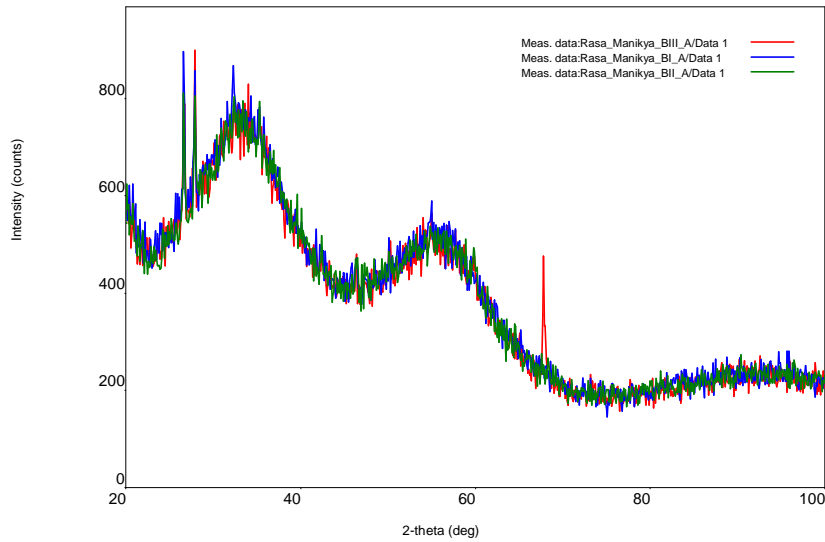
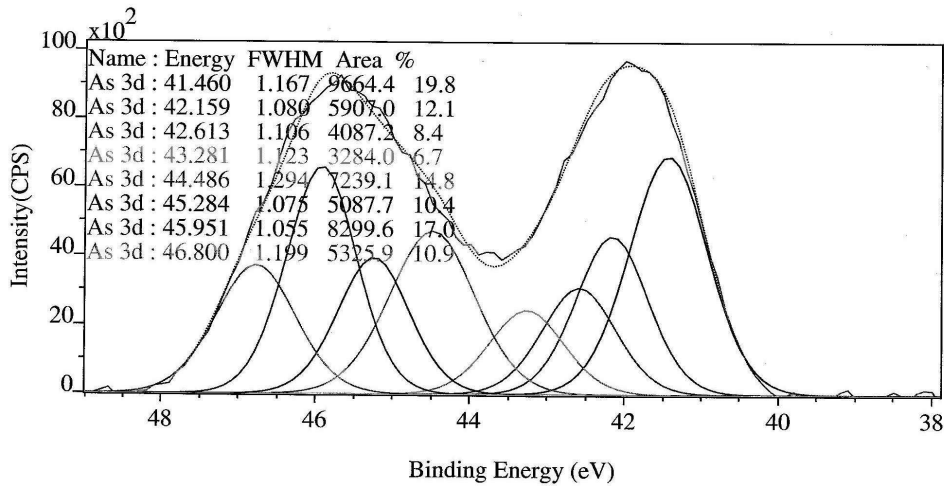
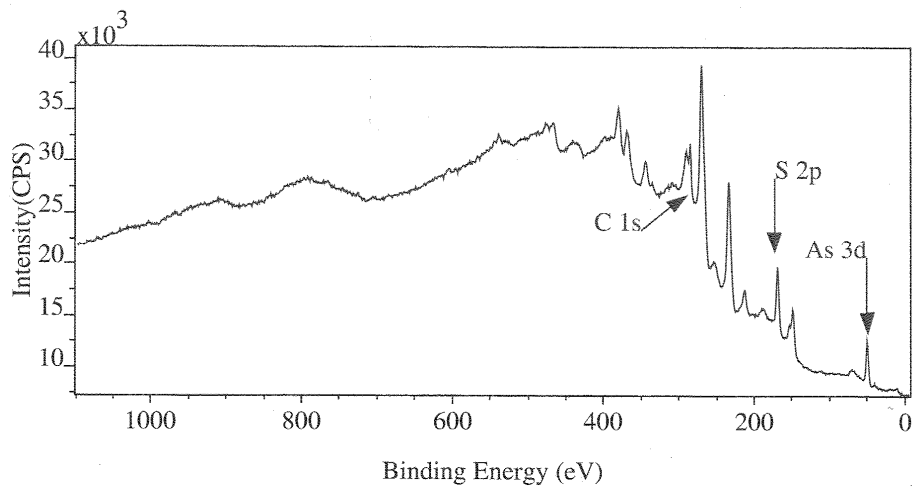


Fig. 2: Overlay of XRD pattern of Rasamanikya (batch-I, II, III)

The observed binding energy peaks for both As 3d and S 2p clearly suggest the coexistence of these two metals in multivalent states. Based on the literature, the various oxidation states of As and S and the corresponding binding energies are given in table 3 and 4. In addition to As in metallic form, it is

present in the form of As₄S₄, As₂S₃, As₂S₅ with characteristic binding energy peaks at ~ 42.6 eV, 44.5 eV and 45.9 eV respectively. From the area under the curves the approximate percentage of the various forms, namely As (0), As₄S₄, As₂S₃, As₂S₅ are 32.1%, 36.2%, 20.7%, 11.0%, respectively.



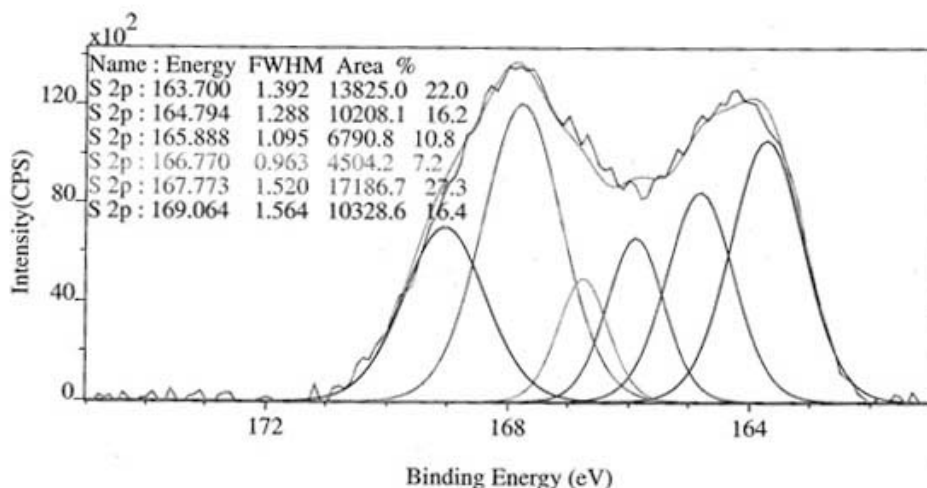


Fig. 3: Typical ESCA (XPS), the observed high-resolution narrow scans for both As 3d and S 2p are illustrated in tables 2 and 3.

Table 2: Observed binding energy peaks for As 3d in *Rasamanikya*

Element's Oxidation state	Binding energy (eV) peak at 3d _{5/2}	Binding energy (eV) peak at 3d _{3/2}
As(0)	41.46	42.16
As(+1)	42.61	43.28
As(+3)	44.49	45.28
As(+5)	45.95	46.80

Table 3: Observed binding energy peaks for S 2p in *Rasamanikya*

Element's Oxidation state	Binding Energy (eV) peak at 2p _{3/2}	Binding Energy (eV) peak at 2p _{1/2}
S	163.70	164.79
S	165.88	166.77
S	167.77	169.06

XPS survey scans do not show any remarkable changes for all the three batches. However, the atomic concentration quantification report for the elements As and S for batch II shows a ratio of 39:61 (As: S) whereas in batches I and III they are in the ratio 47:53 and 43:57 respectively. As: S ratio was also determined by SEM-EDAX for all batches and were found to be 45.6:54.4, 49.9:50.1 and 49.8:50.2, respectively. Sulphur content in batches I, II, and III as determined by elemental analysis by CHNS showed 36.30, 39.05, and 37.73 % respectively. On the basis of XPS survey scans, SEM-EDAX and CHNS analysis the Arsenic to Sulphur (As to S) ratio is thus standardized as 39-47: 53-61.

CONCLUSION

The preliminary profiles of *rasamanikya* evaluated in this attempt could be used to lay down a new set of pharmacopoeial standards. This information will help the scientists and researchers to build comprehensive standards, to screen the compounds responsible for different bioactivities, and to elucidate the molecular mechanism of action.

ACKNOWLEDGMENT

The authors express their heartfelt thanks and would like to acknowledge Dr S. K. Sharma, Former Advisor (Ayurveda), Ministry of AYUSH, Government of India; Dr M. M. Padhi, Former Dy. Director General, CCRAS; and Dr Pramila Pant, Assistant Director (Chemistry) for valuable guidance; and Dr V. K. Singh, M/sMaharishi Ayurveda Pharmacy, Noida, India, for technical inputs. The authors are thankful to Dr J. Arunachalam, Former Director, National Centre for Compositional Characterization of Materials (BARC), Hyderabad, India, for helping in data analysis and interpretation of the results.

Thanks conveyed to Dr. Aarti Sheetal, Bhavana Dwivedi, Dr. Suman Singh, Dr. Divya Mishra, and Yadunandan Dey senior research fellows of CCRAS for technical assistance.

FUNDING

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The study was funded by the Ministry of AYUSH, Government of India.

ETHICAL APPROVAL

Not applicable for this research study

AUTHORS CONTRIBUTIONS

Designing of the study, data analysis and interpretation, preparation of manuscript: Dr. Sarada Ota, Dr. Arjun Singh; Data acquisition and preparation of manuscript: Dr. Galib R; Data acquisition: Dr. Sreedhar Bojja; Revised the article critically for important intellectual content and final approval for the version to be published: Dr. N. Srikanth; Agreement to be accountable for all aspects of the work: Dr. Kartar Singh Dhiman

CONFLICTS OF INTERESTS

We declare that we have no conflict of interest

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