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ANALYTICAL STANDARDIZATION OF SHATPALA GANDHAKA CHURNA

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ABSTRACT

Rasa Shastra is a specialized branch of Ayurveda which mainly deals with the pharmaceutics of unique and potent preparations. Shatpala Gandhaka churna is an important Rasa Oushadi mentioned in Vaidya Chintamani- Kushtha roga prakaranam The chief ingredients of the formulation are Shuddha Gandhaka, Shuddha Bhallataka, Shuddha Chitrakamula twak, Triphala, Vidanga, Trikatu, Trijataka, Chanaka, and Jeeraka. The main Pharmaceutical procedures adopted for preparation of Shatpala Gandhaka Churna are Shodhana and Churna nirmana. Analytical study plays an important role in the standardization of the drugs. To assess the toxicity, safety and to understand the structural and chemical composition, it was tested through various modern analytical parameters like X-ray diffraction (XRD), Scanning electron microscopy (SEM), Particle size analysis (PSA), Zeta Potential (ZP), UV-Spectroscopy, Fourier transform Infra-Red spectroscopy (FTIR) and Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES). XRD of Shatpala Gandhaka churna shows major peaks of Sulphur(S_8). SEM micrograph showed distribution of particles in Shatpala Gandhaka Churna as clusters of Spherical and Elliptical shaped particles; Particle size was found to be 10.9 nm and Zeta Potential is -40.9 mV. UV- Spectrum of Shatpala Gandhaka churna showed maximum absorption at 226 nm and 270 nm**.** FT-IR analysis showed 10 peaks between the wavelengths 3415.99-406.35cm^{-1} and ICP–OES analysis revealed Sulphur as main constituent in 14376.50 ppm.

KEYWORDS: Shatpala Gandhaka Churna, Modern Analytical parameters, Safety, Toxicity, Standardization.

INTRODUCTION

Analytical study is the key part of any scientific research. It tells us about the correlation between pre-determined hypothetical values and actual results obtained. It gives us valuable information about safety, efficacy, stability etc. of any formulation. Ayurveda, the ancient system of medicine is gaining recognition throughout the world and many herbal, metal and mineral drugs are now clinically tested and accepted. However, one of the impediments in the acceptance of the ancient systems of medical preparation is the lack of standard quality control profiles. The quality of the drugs, that is, the profile of the constituents in the final product has implication in efficacy and safety. Hence highly sensitive modern parameters are employed for gaining information about identity, form, particle size, and structure of contents of the formulation. Considering this, an effort has been made to analyze classically prepared Shatpala Gandhaka Churna through X-ray diffraction, Scanning electron microscopy, Zeta potential, UV- spectroscopy, FT-IR, ICP–OES analysis etc.

MATERIALS AND METHODS

Gandhaka was obtained from Vijayawada. Triphala, Trikatu were obtained from TTD's Sri Srinivasa Ayurvedic Pharmacy, Tirupati. Vidanga, Bhallataka, Chitraka mula twak were obtained from Chennai. Trijataka, Chanaka, Jeeraka were obtained from the local market, Tirupati. Entire preparation of Shatpala Gandhaka Churna was carried out in Depatment of Rasa Shastra and Bhaishajya Kalpana, TTD's S.V. Ayurvedic College and Sri Srinivasa Ayurveda Pharmacy, TTD, Tirupati. Requirement for XRD: Model- Powder XRay Diffractometer D8 advance, ManufacturerBruker Germany. SEM: Model- EVO MA 15, Manufacturer- Carl Zeiss - Germany; PSA and ZP: Model- Horiba scientific Partical Size and Zeta Potential Analyzer, Manufacturer- Horiba instruments, Irvine, CA 92618 USA; UV-Spectroscopy: Model- Nano drop 8000 Spectrophotometer, Manufacturer- Thermo Scientific, India; ICP-OES: Model- Agilent 725, Manufacturer- Agilent technologies, USA.

The process was carried out in two steps

1. Pharmaceutical Study
2. Analytical Study

Pharmaceutical study

The pharmaceutical procedures adopted in this study are Shodhana, Churna nirmana and preparation of capsules of Shatpala Gandhaka Churna. Gandhaka Shodhana was done according to the method that was mentioned in Rasa Ratna Samucchaya.[1] which includes melting of Gandhaka in ghee and pouring into a vessel filled with milk through fine cloth. Final cleaning with hot water removes greasy remnants of milk and ghee. Chitrakamula twak Shodhana, was done according to the method that was mentioned in Rasatarangini[2], by soaking it in Churnodaka for one day and dried in sunlight. Bhallataka Shodhana was done according to the reference Rasa tarangini[3] by rubbing it with Istika churna in a jute bag. Shuddha Bhallataka, Shuddha Chitrakamulatwak, Triphala, Vidanga, Trikatu, Trijataka, Chanaka, Jeeraka were made into fine powder, according to the reference mentioned in Sharangadhara Samhita Madhyama Khanda.[4] Gandhaka obtained after shodhana and the fine powders of herbal drugs were mixed in the ratio as mentioned in the chief reference sloka[5] to obtain the homogenous mixture of Shatpala Gandhaka Churna. Capsules of uniform size were taken. 500 mg of Shatpala Gandhaka Churna was filled in each capsule and weighed. Capsules were preserved in absolute sterile and moisture free glass containers.

Analytical Study

Analysis of Shatpala Gandhaka Churna using modern parameters.

X-Ray Diffraction (XRD)

Shatpala Gandhaka Churna was subjected to XRD at Department of Physics, Yogi Vemana University, Kadapa, Andhra Pradesh.

Procedure

Sample was powdered in agate mortar to very fine powder and it was mounted in sample tray of machine. X-Ray beam bearing a wavelength of 1.5418740 A° from copper source is passed on the sample. Detector was set to identify diffracted beams between 10 -70 degrees of 2 range. Obtained soft files of XRD consisting values of 2θ and intensity are plotted on a graph (2θ on X-Axis and Intensity of Y-Axis) using "Origin Pro 8.5 SR2" Data Analysis Software. Various compounds consisting similar diffraction pattern were identified by matching their peaks with corresponding JCPDS Crystallographic cards. For even better accuracy and precision, XRD soft files were also analyzed for corresponding phase/entry matching with Crystallographic Open Data base (COD - 20120320) – USA, after plotting values in PANalytical X'pert high score plus software 3.0.0.123, UK.

Scanning Electron Microscopy

The practical was performed at Department of Physics, S.V University, Tirupati.

Procedure of SEM

Specimen of the sample to be analyzed was directly kept on the specimen holder for visualization. As the sample employed has non-conductive nature, the sample surface was coated by carbon using melting technique. Then the dried powder was observed under the microscope at 1,000X to 10,000KX and the micrographs were taken with the inbuilt camera.

Particle Size Analysis and Zeta Potential

The practical was conducted at Department of science and Technology, PURSE, S.V. University, Tirupati.

Procedure of PSA

The sample was mixed in water and sonicated for 10 minutes. Then it was poured into the sample chamber, where it passes through the laser beam as homogeneous stream of particles. The scattering of light occurs over a wide range of angles upon interacting with the particles in the suspension which are moving by Brownian motion. Based on this scattering pattern of sample, particle size distributions are calculated comparing with appropriate optical model.

Procedure of ZP

A 1% concentration of Shatpala Gandhaka Churna was prepared in distilled water. The particles were well dispersed before analysis and the sample was taken in a 1ml syringe and injected slowly into the capillary cell (cuvette) through the sample port. Care was taken to see that air bubbles are not formed during this process. As the sample comes out from the second port of the capillary cell, the injection process is stopped. This indicates complete filling of the sample into the capillary cell. The sample ports are then covered with lids and the capillary cell was then placed into the sample holder of the zeta sizer instrument for analysis.

UV- Spectroscopy

Practical was performed at Department of science and Technology, PURSE, S.V. University, Tirupati.

Procedure

5g of Shatpala Gandhaka Churna was macerated with 100 ml of solvent in a closed flask for twenty four hours, shaking frequently during six hours and allowed to stand for eighteen

hours. It was filtered, taking for UV spectroscopic study. The Spectra was taken at 200-800 nm from the peak obtained, the λ-max value was calculated.

Fourier Transform Infrared Spectroscopy (FTIR)

This practical was conducted at Padmavathi Mahila University, Tirupati.

Procedure

Sample was placed in the Potassium bromide plate of FTIR spectrometer and the interference pattern was detected by the infrared detector as variations in the infrared energy level, and the obtained spectral information was calculated.

Inductively Coupled Plasma – optical Emission Spectrometry

This practical was performed at Centre for material for electronics technology (C-MET), Department of Electronics and Information technology, Hyderabad.

Procedure

0.2 g of Shatpala Gandhaka Churna was taken in Teflon tubes and added to 6.0 ml of Nitric acid and 2.0 ml of Hydrogen peroxide and allowed for 10 minutes in outside for reaction. Then samples were dissolved using Microwave Digestion System (Anton PaarMultiwave 3000). Then the Shatpala Gandhaka Churna solutions were made to 25.0 ml and filtered. These solutions were used for Elemental analysis using ICP-OES instrument.

RESULTS

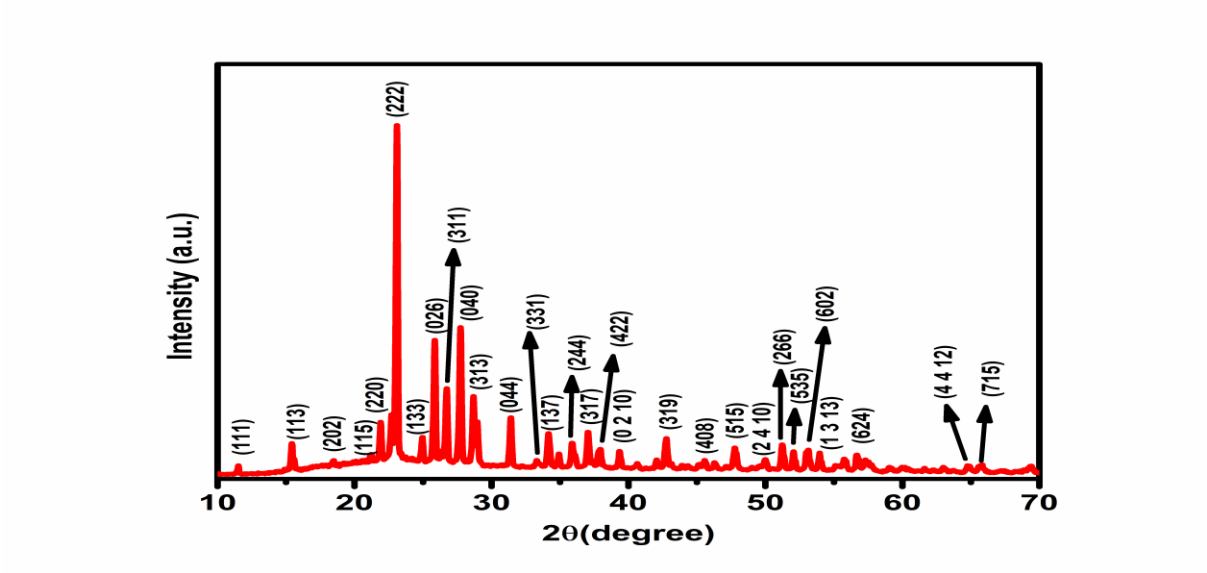
X-Ray Diffraction Studies (XRD)

Table No. 1: Showing the details of matching peaks of XRD data for Shatpala Gandhaka Churna.

S.No	Element/Molecule	JCPDS Ref.No	2θ	Intensity	Height (cts)	FWHM	h	K	L
1.	Sulphur(S_8)	00-008-0247	23.100 27.742 25.859 26.736 28.685	100.0 60.0 40.0 25.0 25.0	7422.20 3050.79 2780.14 1741.38 1567.91	0.1440 0.1680 0.1440 0.1680 0.1440	2 0 0 3 3	2 4 2 1 1	2 0 6 1 3
2.	Sodium Magnesium Sulfate (Vanthoffite) $Na_6 Mg (SO_4)_4$	00-029-1240	55.736	2.0	253.75	0.336	-5	1	4

Table No. 2: Showing Crystal details of JCPDS entries.

Name	**Sulphur(S_8)**
Space group	Fddd
Crystal System	0rthorhombic
Cell Parameters	a = 10.4500 A˚, b= 12.8400 A˚, c = 24.4690 A˚
Z	16.00
Name	**Sodium Magnesium Sulfate (Vanthoffite) $Na_6 Mg (SO_4)_4$**
Space group	P21/c
Crystal System	Monoclinic
Cell Parameters	a = 9.7810A˚ b= 9.1960A˚ c = 8.1970A˚
Z	2.00



Graph No. 1: Showing XRD graph of Shatpala Gandhaka Churna.

- XRD of Shatpala Gandhaka Churna shows major peaks of **Sulphur** with Orthorhombic structure. XRD also showed the existence of Sodium Magnesium Sulfate (Vanthoffite)- **$Na_6 Mg (SO_4)_4$** compound with Monoclinic structure. The Sulphur peaks were detected at a diffraction angle of 23.100, 27.742, 25.859, 26.736, 28.685.

Scanning Electron Microscopy

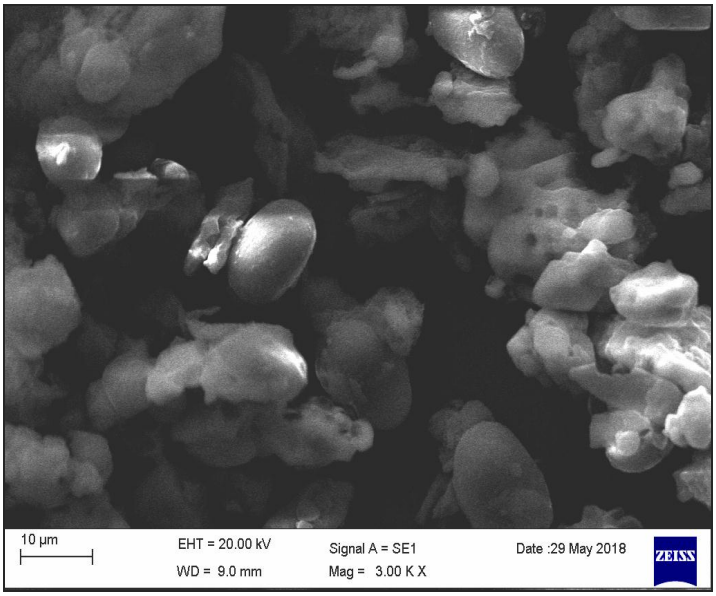


Figure No. 1: Showing SEM result of Shatpala Gandhaka Churna (Mag. 3KX).

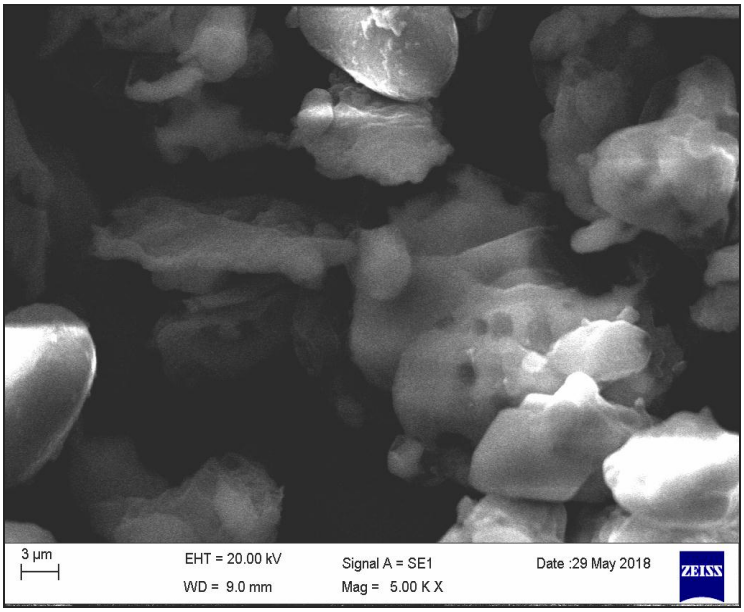
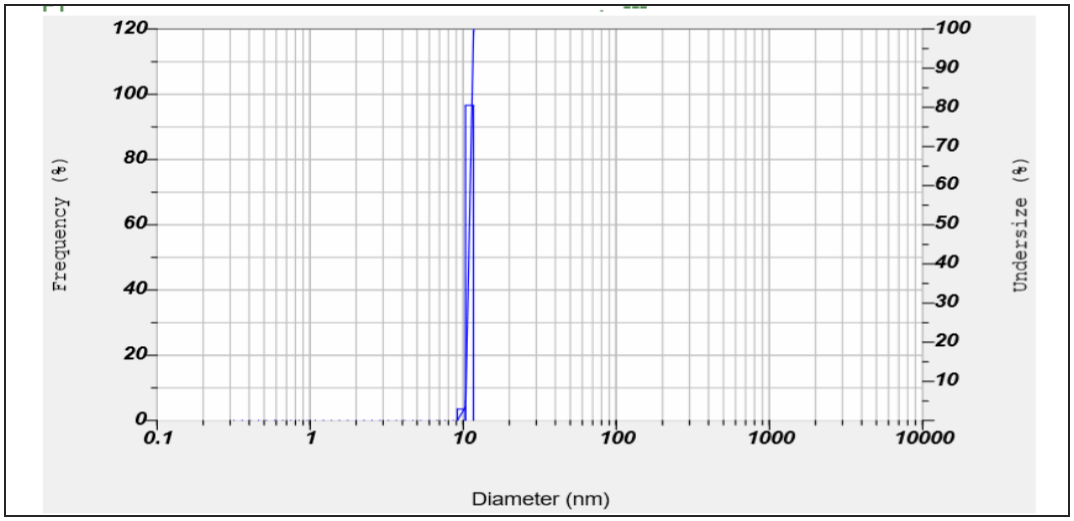


Figure No. 2: Showing SEM result of *Shatpala Gandhaka Churna* (Mag. 5KX).

SEM micrograph showed distribution of particles in Shatpala Gandhaka Churna as clusters of Spherical and Elliptical shaped particals at 3KX and 5KX magnifications.

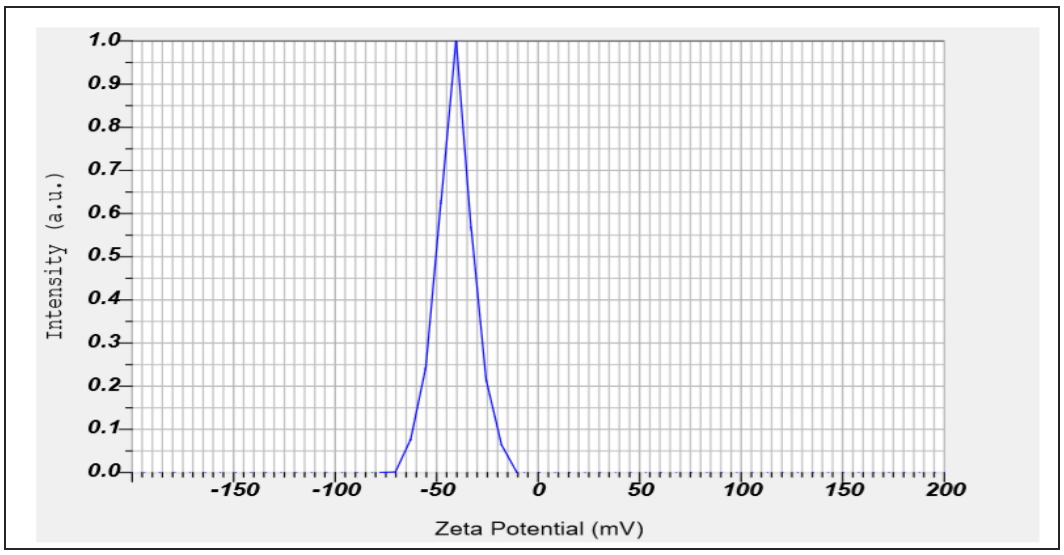
Particle Size Analysis



Graph No. 2: Showing the result of Particle size analysis of Shatpala Gandhaka Churna.

The mean particle size of Shatpala Gandhaka Churna is **10.9 nm.**

Zeta Potential



Graph No. 3: Showing the result of Zeta potential analysis of Shatpala Gandhaka Churna.

- **Shatpala Gandhaka Churna** sample showed a Zeta potential value of **-40.9 mV** which indicates High colloidal stability.

UV- Spectroscopy

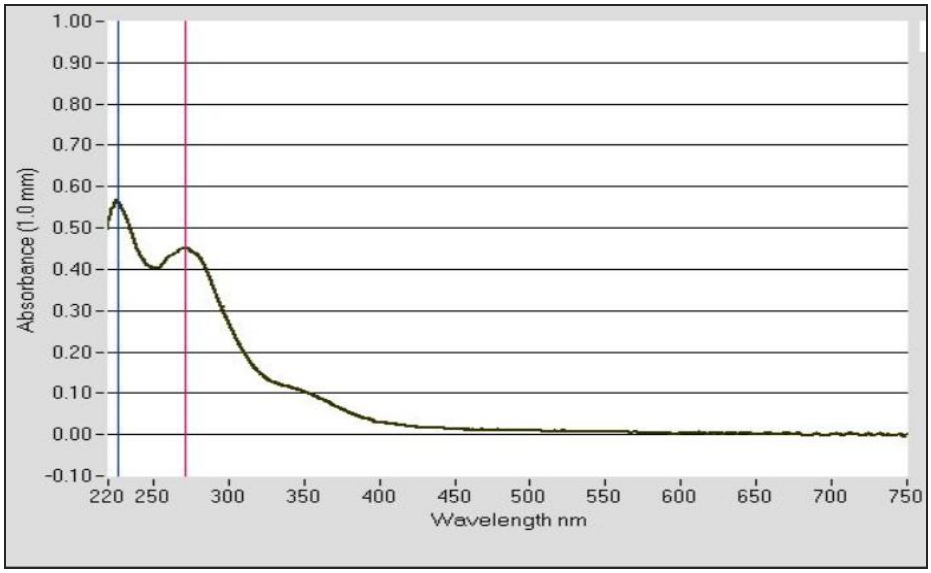


Figure No. 3: Showing UV-Spectrum of Shatpala Gandhaka Churna.

UV-Spectrum of Shatpala Gandhaka Churna showed maximum absorption at **226 nm** and **270 nm.**

Fourier Transform Infrared Spectroscopy (FT-IR)

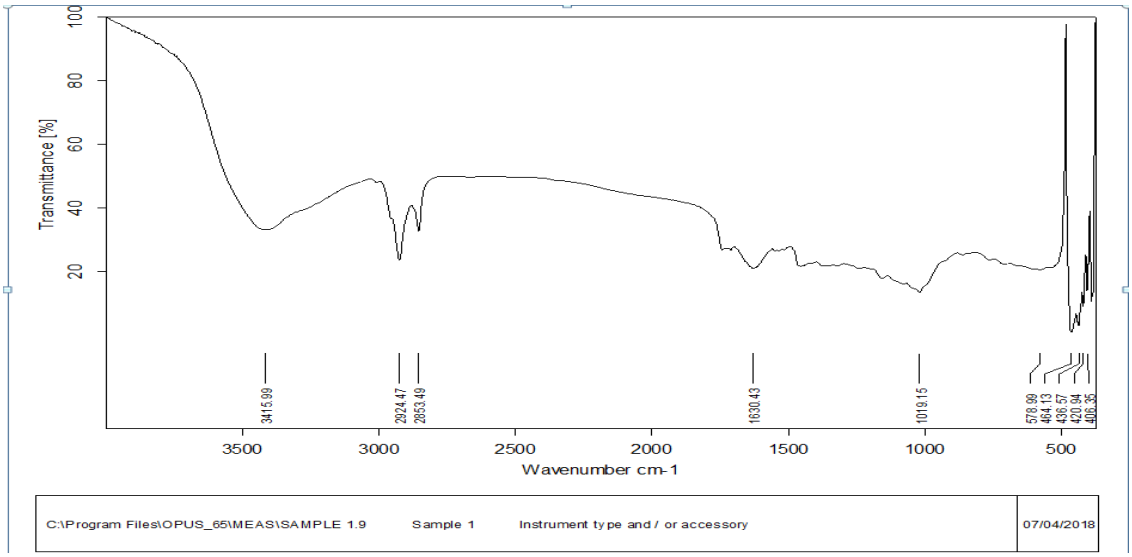


Figure No. 4: Showing various peaks obtained in FTIR analysis of Shatpala Gandhaka Churna.

Table No. 3: Showing details of Peaks obtained in FTIR analysis of Shatpala Gandhaka Churna.

Sample Name	No. of Peaks	Wavelength
Shatpala Gandhaka Churna	10	3415.99, 2924.47, 2853.49, 1630.43, 1019.15, 578.99, 464.13, 436.57, 420.94, 406.35

Table No. 4: Various peaks obtained in FTIR analysis of Shatpala Gandhaka Churna and their correlation with compounds.

S.No.	Peak	Actual peak	Bond	Type of bond	Appearance
1.	3400-3700 cm^{-1}	3415.99	C – H	Alkane	Strong, Broad
2.	2850-2975 cm^{-1}	2924.47 2853.49	O – H	Alcohol	Medium to Strong
3.	1620-1680 cm^{-1}	1630.43	C = C	Alkene	Weak to Medium
4.	1000-1400 cm^{-1}	1019.15	C – F	Alkyl Halide	Strong

Inductively Coupled Plasma – optical Emission Spectrometry

Table No. 5: Showing the result of ICP-OES analysis of Shatpala Gandhaka Churna.

S.No.	Name of the elements analyzed	Tests results in ppm
1.	Sulphur	73460.11
2.	Silver	2.03
3.	Arsenic	Not Detected
4.	Aluminium	1254.61
5.	Barium	23.20
6.	Berylium	Not Detected
7.	Bismuth	21.77
8.	Calcium	2558.79
9.	Cadmium	Not Detected
10.	Cobalt	Not Detected
11.	Chromium	18.02
12.	Copper	7.85
13.	Iron	638.95
14.	Potassium	6239.74
15.	Magnesium	811.01
16.	Manganese	55.94
17.	Sodium	111.27
18.	Nickel	3.81
19.	Lead	Not Detected
20.	Strontium	16.25
21.	Selenium	Not Detected
22.	Vanadium	1.96
23.	Zinc	17.57

DISCUSSION

Analytical study is a process which helps in identification of quantitative and qualitative data of a substance, the components of a solution or mixture, or the determination of the structures of chemical compounds. It is an essential part of any research work. It gives us the knowledge about identity, size, structure of chemical constituents and physical properties. It hints us about toxic properties of drugs, if any.

X-Ray diffraction has been in use in two main areas, for the finger print characterization of crystalline materials and the determination of their structure. Each crystalline solid has its unique characteristic X-Ray powder pattern, which may be used as a "fingerprint" for its identification. Once the material has been identified, X- Ray crystallography may be used to determine its structure, i.e. how the atoms pack together in the crystalline state and what the inter-atomic distance and angle etc. X-Ray diffraction is one of the most important characterization tools used in solid state chemistry and material science. Size and the shape of the unit cell for any compound can be detected most easily using the diffraction of X-rays. XRD of Shatpala Gandhaka Churna shows major peaks of Sulphur with Orthorhombic structure, indicating the highly crystalline nature of Sulphur and the presence of higher amounts of Sulphur in Shatpala Gandhaka Churna. XRD also showed the existence of Sodium Magnesium Sulfate (Vanthoffite)- Na_6 Mg $(SO_4)_4$ compound with Monoclinic structure.

Scanning electron microscopy (SEM) is an analytical technique to know the surface morphology of the drug. It uses electron beam rather than light to form a Figure. It is capable of producing high resolution figures of a sample surface, which means that closely spaced features can be examined at a high magnification. Due to the manner in which the Figure is created, SEM Figures have a characteristic three dimensional appearance and are useful for determining the surface structure of the sample i.e. topography. It can magnify objects to extreme levels where even structure of nano particles could be clearly visible. Agglomerations of Spherical and Elliptical sharp particles were seen in micrographs of Shatpala Gandhaka Churna. This agglomeration in the surface morphology of Shatpala Gandhaka Churna may be due to the presence of natural tannins, resins in the component drugs with binding nature, which may be sticking to the surface of Sulphur.

The size of the particles in the drug plays major role in its therapeutic action and efficacy. Particle size and surface area of solid drug are inversely related to each other. The mean

particle size of Shatpala Gandhaka Churna is 10.9 nm. The nano size of drug is indicative of its quick absorption and faster dispersion into body resulting in better therapeutic efficacy.

Zeta potential is a measure of the magnitude of the electrostatic or charge repulsion or attraction between particles, and is one of the fundamental parameters known to affect stability. The Zeta Potential (mean) value of Shatpala Gandhaka Churna found to be -40.9 mV which indicates High colloidal stability. High zeta potential indicates easy dispersion, whereas less zeta potential indicates strong aggregation of particles in suspension.

UV-Spectroscopy refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. Different molecules absorb radiation of different wavelengths. An absorption spectrum will show a number of absorption bands corresponding to structural groups with the molecule. Electromagnetic spectrum of U.V region is from 190 to 400 nm whereas for visible region it is 400-800 nm. UV-Spectrum of Shatpala Gandhaka Churna showed maximum absorption at 226 nm and 270 nm. which shows its absorbency in UV region.

FTIR was performed to detect the presence of functional groups or organic legends in Shatpala Gandhaka Churna**.** Infrared spectroscopy deals with the infrared region of the electromagnetic spectrum that is light with a longer wavelength and lower frequency than visible light. When infrared light or radiation hits a molecule, the bonds in the molecule absorb the energy of the infrared and respond by vibrating. Shatpala Gandhaka Churna showed 10 peaks between the wavelengths 3415.99-406.35cm^{-1}. Strong intensity of C-H stretching vibrations resulted in one peak at 3415.99cm^{-1} represents Alkanes. Medium intensity of O-H stretching vibrations resulted in two peaks at 2924.47 and 2853.49cm^{-1} which were assigned to Alcohols. A peak at 1630.43cm^{-1} raised due to C=C stretching vibrations represents Alkenes. Strong peak obtained near 1019.15cm^{-1} represents C = F stretching vibrations indicates the presence of Alkyl Halide. This indicates that there are no complex structures in Shatpala Gandhaka Churna.

Inductively Coupled Plasma – Optical Emission Spectrometry is one of the most common techniques for elemental analysis. It is very useful for standardization as well as to develop analytical profile. ICP–OES analysis of Shatpala Gandhaka Churna showed Sulphur as main constituent in 73460.11 ppm. This may be due to the presence of Gandhaka in higher amounts. Potassium is present in 6239.74 ppm. This may be due to the presence of potassium

in Sunthi, Maricha, Pippali, Amalaki[6], Haritaki, Vibhitaki, Twak, Ela, Jeeraka[7] and Bhallataka. Calcium is present in 2558.79 ppm. This may be due to the presence of calcium in Trikatu, Amalaki[8], Haritaki, Sunthi, Maricha[9], Pippali, Twak, Ela and Jeeraka.[10] Aluminium is present in 1254.61 ppm. This may be due to the presence of Aluminium in Bhallataka.[11] Magnesium is present in 811.01 ppm. This may be due to the presence of Magnesium in Amalaki[12], Haritaki, Sunthi, Maricha[13], Pippali, Twak, Ela and Bhallataka. Iron is present in 811.01 ppm. This may be due to the presence of Iron in Jeeraka[14], Amalaki[15], Haritaki, Vibhitaki, Sunthi[16], Maricha, Pippali, Twak and Bhallataka**.** Sodium is present in 111.27. This may be due the presence of Sodium in Amalaki[17], Jeeraka[18], Sunthi and Bhallataka**.** Manganese, Barium and Bismuth were present in 55.94, 23.20 and 21.77 ppm respectively. These minerals are available in Amalaki[19], Haritaki, Vibhitaki, Sunthi, Twak, Ela, Jeeraka and Bhallataka.[20] Chromium, Zinc, Strontium, copper, Nickel, Silver, vanadium were present in trace amounts. These minerals are present in Amalaki[21], Vibhitaki, Sunthi, Maricha, Pippali, Chanaka, Jeeraka and Bhallataka.[22] Toxic elements like Arsenic, Berylium, Cadmium, Cobalt, Lead and Selenium were not detected.

CONCLUSION

From the present study, it can be confirmed that Shatpala Gandhaka Churna is a herbo mineral compound with the presence of Sulphur as the major component. Presence of other micro elements may be due to the other herbal ingredients present in the formulation. Nano particle of the yoga indicates its quick absorption, dissolution to the target site and high therapeutic efficacy; Toxic elements were not identified; this indicates the safety of the formulation. The analytical study depicts the vision of the ancient seers regarding the pharmaceutical procedures adopted in the preparation of Shatpala Gandhaka Churna in making them completely safe for therapeutic usage. This was reflected in all the sophisticated analytical tests employed in this study.

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