



Research Article

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QUALITY STANDARDIZATION OF A TRADITIONAL AYURVEDIC FORMULATION PANCHAMRUT LOHA GUGGUL TABLET

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Received on: 25/06/17 Accepted on: 28/08/17

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DOI: 10.7897/2277-4343.084213

ABSTRACT

Panchamrut Loha Guggul is herbo-mineral ayurvedic preparation indicated in treatment of cervical spondylitis, neuromuscular conditions, Gridhrasi (Sciatica), pain in waist and knees, and other Vata-vyadhi (diseases caused by aggravated Vata dosha). The present research work was conducted to standardize Panchamrut Loha Guggul Tablets on the basis of Physico-chemical screening, Elemental analysis, Chromatographic and IR spectroscopic study. Physico-chemical screening was done by evaluating Ash, Acid insoluble ash (AIA), Loss on Drying (LOD), Water Soluble Extractive (WSE) and Alcohol Soluble Extractive (ASE). The Atomic Absorption Spectrophotometric method (AAS) was applied to determine the Iron (Fe), Silver (Ag) and Copper (Cu) content. Chromatographic analysis was performed to estimate Guggulsterone (E & Z) content using High Performance Thin Layer Chromatography (HPTLC). The chemical fingerprint was taken by using Fourier transform infra-red spectroscopy (FT-IR). The Physico-chemical studies showed Ash content less than 50 %, AIA less than 25 %, LOD less than 6 %, WSE more than 25 % and ASE more than 15 %. FT-IR reveals unique transmittance spectra in the range of 4000 - 600 cm⁻¹. Iron (Fe), Silver (Ag) and Copper (Cu) content found to be more than 20 mg/tab, 10 mg/tab and 1.5 mg/tab respectively. The HPTLC analysis showed the Guggulsterone (E & Z) content more than 0.50 mg/tab. This study will help to develop quality control profile for Panchamrut Loha Guggul Tablet for future reference in determining the quality of Panchamrut Loha Guggul Tablets.

Keyword: Standardization, Panchamrut Loha Guggul, High Performance Thin Layer Chromatography (HPTLC), Fourier transform infra-red spectroscopy (FT-IR), Guggulsterone (E & Z)

INTRODUCTION

Ayurveda is a science that deals not only with treatment of some diseases but is a complete way of life¹. It is known to be a complete medical system and a science of life with a holistic approach to health and personalized medicine². Ayurveda has ability to treat many chronic diseases such as arthritis, cancer, asthma, diabetes etc³.

Panchamrut Loha Guggul is one of the most important Guggulkalpa containing herbo-mineral ingredients like Abhrak Bhasma, Roupya Bhasma, Suvarnamakshik Bhasma, Loha Bhasma, Shuddha Parad, Shudha Gandhak and Shuddha Guggul⁴. Guggulkalpa means medicine that contains equal or more amount of Guggul as compared to other ingredients⁵⁻⁶.

Ayurveda describes Guggul as an antiseptic, anti-bacterial, astringent, anti-spasmodic⁷. Various experimental studies confirm anti-inflammatory and hypolipidaemic actions of guggul⁸⁻¹⁰. Guggul contains Guggulsterone (E & Z) as its main Phyto-constituents which is a potent anti-inflammatory agent¹¹. Abhrak Bhasma which is one of the ingredients of Panchamrut Loha Guggul is known for treatment of various chronic diseases, has found to be safe in acute toxicity study at a dose of 5000 mg/kg BW¹². Roupya bhasma known for its Vatashamak property acting on Kidney, Brain and Nerves has analgesic activity and used to treat many disease conditions like pain, neuralgias, inflammation, anxiety, convulsions, memory loss etc¹³⁻¹⁴. Suvarnamakshik Bhasma is indicated in the effective management of Mandagni (Poor digestion), Anidra (Insomnia), Apsmara (Convulsions), Pandu (Anemia) etc.¹⁵⁻¹⁶. Loha

bhasma is very useful in treatment of various diseases like Pandu (Anaemia), Shotha (Oedema), Sthaulya (Obesity)¹⁷⁻¹⁹. A toxicity study on albino rats has shown that Loha Bhasma is safe at the therapeutic dose and also at five times the therapeutic dose levels²⁰.

Panchamrut Loha Guggul has analgesic and anti-inflammatory properties. It improves blood circulation to the brain and acts on the brain, nerves, blood vessels, heart, muscles, bones, and joints. It is commonly used in the treatment of mental disorders, symptoms associated with nervine weakness or weakness of the brain, Neuralgia, sciatica, Neuritis, lumbar pain (a low backache), osteoarthritis, frozen shoulder (adhesive capsulitis) and rheumatoid arthritis²¹.

Ayurvedic medicines were developed at time of limited access to technologically variable norms of standardization. Hence, Standardization of Ayurvedic formulation is essential and need broader considerations²². Standardization of drug means confirmation of its identity and determination of its quality and purity. It is an important aspect for the establishment of a consistent chemical profile or a quality assurance program for the manufacturing of an Ayurvedic drug²³. Since, Quality specifications of Panchamrut Loha Guggul Tablet are not yet published in Pharmacopoeia's, we have made an effort to establish quality norms of this Ayurvedic formulation on the basis of Physico-chemical screening, Elemental analysis, Chromatographic and IR spectroscopic study.

MATERIALS AND METHODS

Three Batches of Panchamrut Loha Guggul were prepared as per Baishajya Ratnavali (Mastishkaroga) 101/14-17 at manufacturing unit of Shree Dhootapapeshwar Ltd. Batch codes designated were as Sample-1, Sample-2 and Sample-3. These batches were analysed for Physico-chemical parameters, Elemental assay, Chromatographic and IR spectroscopic evaluations to standardize Panchamrut Loha Guggul tablets. All chemicals and reagents (Toluene, Acetone, Methanol, HNO₃, HCl, etc.) used in analysis were of analytical reagent grade of Merck. Certified reference materials of Silver (1000 PPM), Iron (1000 PPM) from Merck and Copper (1000 PPM) from REGICON were used for Elemental analysis. Reference standards (RS) Guggulsterone E & Guggulsterone Z of purity 99.60 % (CAS No, 39025-24-6) & 97.60 % (CAS No, 39025-23-5) respectively were used for the Chromatographic study.

Organoleptic Evaluation

Various parameters such as colour, taste and texture of the samples of Panchamrut Loha Guggul tablets were observed and recorded.

Physico-chemical screening

In Physico-chemical screening, the samples of Panchamrut Loha Guggul tablet were analysed for various Physico-chemical Parameters such as Hardness, Friability, Disintegration, weight variation Loss on Drying (LOD), Ash, Acid Insoluble ash (AIA), Water soluble extractive (WSE) and Alcohol soluble extractive (ASE) as per The Ayurvedic Pharmacopoeia of India (API)²⁴.

Elemental analysis by Atomic Absorption Spectroscopy (AAS)

The samples of Panchamrut Loha Guggul tablet were digested in a MARS 6 microwave digestion system (CEM corp., USA) equipped with Teflon closed vessels (Easy Prep Plus vessel) for safe operation under 800 psi. The instrumental conditions used for digestion of samples are given in Table 1. After completion of digestions elemental content (Fe, Ag & Cu) were determined by Atomic Absorption Spectrometry using a Perkin Elmer model - Analyst AA 400 Atomic Absorption Spectrometry. The instrumental conditions of AAS used for Elemental analysis are given in Table 2.

Chromatographic analysis

HPTLC Instrument Camag with Linomat 5, TLC Scanner 4 and Wincat Software was used for chromatographic analysis of Methanolic extract of Panchamrut Loha Guggul tablets. Twin trough chamber was used for development of HPTLC plate. Photo documentation cabinet fitted with High Resolution camera was used for capturing images at different wavelengths. Densitometer TLC Scanner 4 equipped with D2 lamp was used to obtain spectra for quantitative determination of compound.

The solvent system Toluene: Acetone (9 : 1 v/v) was selected for estimation of Guggulsterone (E & Z), which gave good resolution. Clear chromatogram was attained with R_f value 0.32 ± 0.04 for Guggulsterone E and 0.38 ± 0.04 for Guggulsterone Z. The wavelength of 252 nm was used for quantification of sample.

IR spectroscopic evaluation

The samples of Panchamrut Loha Guggul tablet were analysed in range of 4000 cm⁻¹ to 600 cm⁻¹ using ALPHA FTIR, Bruker with ATR sampling mode and OPUS software.

RESULTS AND DISCUSSION

Panchamrut Loha Guggul tablets characterized as Brown to blackish brown in colour, bitter in taste and round coated biconvex in shape. (Table 3)

The Physico-chemical studies showed Friability less than 1 %, Hardness greater than 1.5 kg/cm², Disintegration time less than 60 min., Ash content less than 50 %, Acid insoluble ash (AIA) less than 25 %, Loss on drying (LOD) less than 6 %, Water soluble Extractive (WSE) more than 25 % and Alcohol soluble Extractive (ASE) more than 15 %. (Table 3) All the samples were found to comply the weight variation test as per API.²⁴ Weight variation is an important factor that is affected by tooling of the compression machine, head pressure, machine speed and flow properties of the powder, powder or granulate density and particle size. The friability test helps to determine the physical strength of the tablets which is attributed to the tablet breaking force. The disintegration test is a measure of the time required under specified conditions for the tablets to disintegrate into particles²⁵. LOD is used to measure the amount of water and volatile matters in a sample when the sample is dried under specified conditions. This is the major factor responsible for the deterioration of the drugs and formulations. Low moisture content is always useful for higher stability of drugs²⁶.

The ingredients such as Abhrak Bhasma, Suvarnamakshik Bhasma and Loha Bhasma contributed to the content of Iron (Fe) in Panchamrut Loha Guggul tablet. As well as Suvarnamakshik Bhasma and Roupya Bhasma contributed to Copper (Cu) and Silver (Ag) content respectively. The contents of Iron (Fe), Silver (Ag) and Copper (Cu) in samples were determined by AAS and results were tabulated in Table 4. Iron (Fe), Silver (Ag) and Copper (Cu) content in Panchamrut Loha Guggul tablet found to be more than 20 mg/tab, 10 mg/tab and 1.5 mg/tab respectively.

The HPTLC analysis confirms the presence of Guggulsterone E and Guggulsterone Z at R_f value 0.32 + 0.04 and 0.38 + 0.04 respectively. (Figure 1) The total Guggulsterone (E & Z) content in Sample-1, Sample-2, and Sample-3 of Panchamrut Loha Guggul tablet found to be 0.83 mg/tab, 0.76 mg/tab and 0.61 mg/tab respectively.

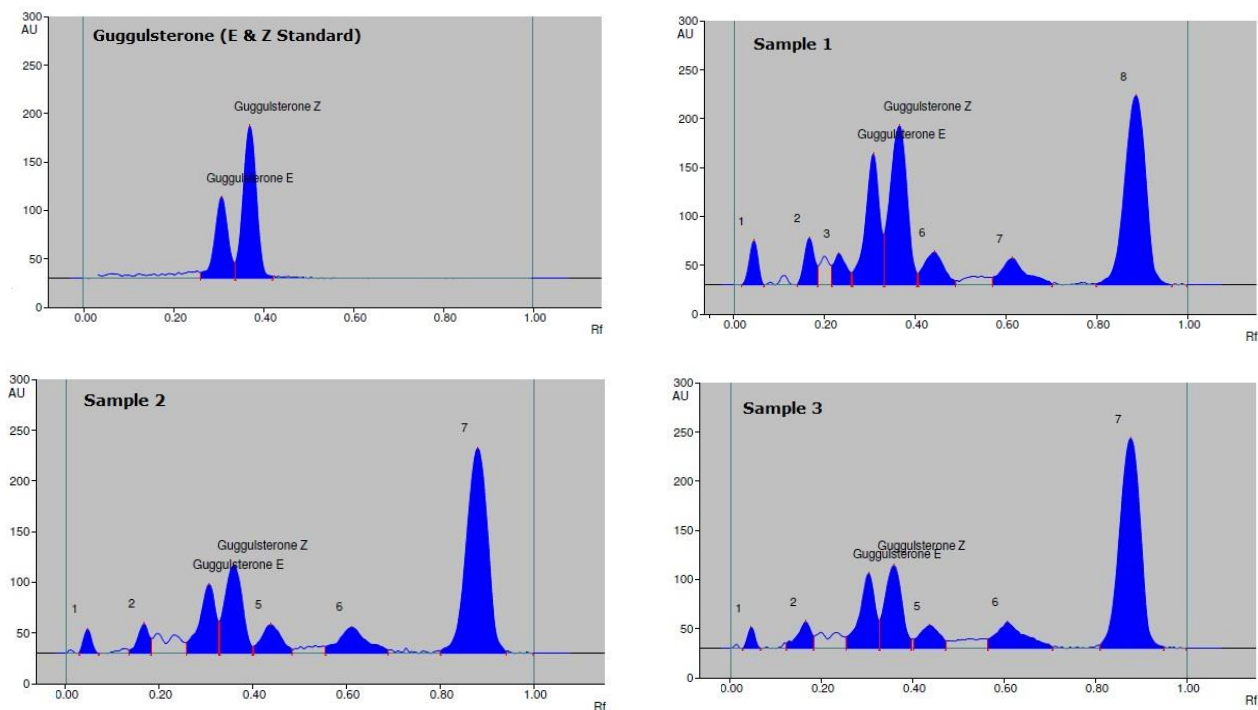


Figure 1: Peak Display of Guggulsterone (E & Z) standard and Panchamrut Loha Guggul tablet (Sample-1, Sample-2 & Sample-3)

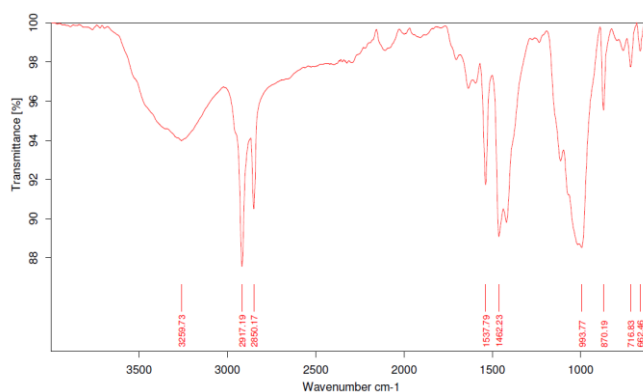


Figure 2: FT-IR Spectrum of Panchamrut Loha Guggul tablet

Table 1: CEM MARS 6 microwave digestion system operating parameters

Instrumental Parameters	Silver (Ag)	Iron (Fe)	Copper (Cu)
Acid used for Digestion	HNO ₃	HCl	Aquaregia (HCl : HNO ₃ :: 3 : 1)
Method	Ag	Fe	Cu
Temperature	170°C	170°C	190°C
Pressure	650 psi	650 psi	650 psi
Ramp Time	15 min.	20 min.	20 min.
Hold Time	1 min.	10 min.	15 min.
Cooling Time	15 min.	15 min.	15 min.

Table 2: AAS operating parameters

Instrumental Parameters	Silver (Ag)	Iron (Fe)	Copper (Cu)
Lamp	Ag	Fe	Cu
Lamp Current	10 mA	30 mA	15 mA
Wavelength	328.07 nm	248.33 nm	324.75 nm
Slit	0.8 nm	1.35 nm	0.8 nm
Burner position	Normal	Normal	Normal
Flame	Air-Acetylene	Air-Acetylene	Air-Acetylene

Table 3: Results of organoleptic and physico-chemical parameters

Batch code	Sample-1	Sample-2	Sample-3
Colour	Brown	Dark Brown	Brown
Taste	Bitter	Bitter	Bitter
Shape	Coated round biconvex tablet, SDL mark on one side		
Friability (%)	0.03	0.19	0.09
Disintegration Time (min.)	25	15	20
Hardness (kg/cm ²)	4.5	3.5	5.5
Weight variation	Pass	Pass	Pass
LOD (%)	3.34	2.68	2.35
Ash (%)	34.81	38.23	43.26
AIA (%)	14.26	16.38	19.23
WSE (%)	38.57	35.83	41.35
ASE (%)	17.68	16.29	18.16

Table 4: Elemental content in Panchamrut loha guggul tablet

Sample	Batch code	Silver (Ag) (mg/tab)	Iron (Fe) (mg/tab)	Copper (Cu) (mg/tab)
Panchamrut Loha Guggul Tablet	Sample-1	10.43	26.59	3.21
	Sample-2	10.45	25.42	2.39
	Sample-3	10.66	27.28	2.52

Table 5: FT-IR fingerprint of Panchamrut loha guggul tablet²⁷

Wave number (cm ⁻¹)	Vibrational modes in IR region	Functional groups
3259	O - H (H-bonded)	Phenol
2917	C - H (Stretch)	Alkanes
2850	C - H (Stretch)	Aldehyde
1537	C = C (Stretch)	Aromatic
1462	- CH ₂ - (Bend)	Alkanes
993	S = O (Stretch)	Sulfoxide
870	C - H (Stretch)	Aromatic
716	C - H (Stretch)	Alkenes
662	C - H (Stretch)	Alkenes

The FT-IR spectrum of Panchamrut Loha Guggul tablet reveals the presence of O-H stretching at 3259 cm⁻¹, C-H stretching at 2917 cm⁻¹, 2850 cm⁻¹, 870 cm⁻¹, 716 cm⁻¹ and 662 cm⁻¹, C=C stretching at 1537 cm⁻¹, -CH₂- bending at 1462 cm⁻¹ and S=O stretching at 993 cm⁻¹. This is shown in Table 5 and Figure 2. It confirms the presence of Phenols, Alkanes, Aldehydes, Sulfoxides, Alkenes and Aromatic groups in Panchamrut Loha Guggul²⁷.

CONCLUSION

In this study, Panchamrut Loha Guggul tablet was prepared and analyzed according to the standard pharmacopoeia procedures for its Physico-chemical parameters, Elemental assay, Chromatographic and IR spectroscopic evaluations. This study will help to develop quality control profile for future reference in determining the quality of Panchamrut Loha Guggul Tablets.

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Cite this article as:

K S Thakur et al. Quality standardization of a traditional ayurvedic formulation Panchamrut loha guggul tablet. *Int. J. Res. Ayurveda Pharm.* 2017;8(4):42-46 <http://dx.doi.org/10.7897/2277-4343.084213>

Source of support: Nil, Conflict of interest: None Declared

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