STANDARDIZATION OF SHUDHA HINGUL W.S.R TO ITS PURIFICATION METHOD

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ABSTRACT

In Ayurveda, minerals and their many Poly formulations are explained but in present scenario standardization of minerals after their purification are most important. Hingul is widely used entity in Herbomineral formulation, hence there is need to standardize it, after purification and before using in formulation by using different analytical tests. Kshalan is one of the unique procedures done after Shodhan of Hingul with Nimbu swaras. This study carried out with the focus on the necessity of the Kshalan process, whether it makes the Shodhit Hingul more potent for internal use or not. In this study we did ICP MS, XRD and HPTLC. In ICP MS we found percentage of Mercury was increased, In XRD no any structural changes were found after purification of Hingul. In HPTLC, white band were seen which shows no physical impurities in purified Hingul.

Key words: Hingul, Kshalan, Shodhan, ICP-MS, XRD, HPTLC.

INTRODUCTION

Rasashastra is one of the important branch of Ayurveda, more inclined towards pharmaceutical and pharmacological angles of different methods of preparations, processing and therapeutical utilization of Mercury, mercurial compounds, metals, minerals, herbo-mineral and metallo-mineral compounds. Improper Shodhan of Rasa aushadhi have delirious toxic effects on the human body. It is essential to Validate, Standardize in details the various aspects of pharmaceutics with the structural changes taking place before, during and after the Shodhan procedure mentioned in our Rasashastra texts. Hingul is one of the most widely used entities, used in preparation of various formulations. Just as it is highly efficacious similarly it is toxic as well. Hence there is a need to standardize the Hingul

Material and Method:
Process of *Hingul shodhan* was carried out in two steps namely trituration of *Ashudha Hingul* (impurified cinnabar) with *nimbusk swaras* (lemon juice) and repeated *kshalan* (washing) of triturated *Hingul*. The *Hingul* was procured from genuine dealer and Lemon for lemon juice was obtained from market.  

**Equipment:** Mortar and pestle, Measuring Cylinder, glass jar, stirrer, Deionized water,  

**Procedure:** At first the *Hingul* was powdered in a mortar with a pestle. Then this Hingul powder was triturated with Lemon juice seven times. After completion of the seventh times triturated *Hingul* turned crystallized to powder form its pH was highly acidic. Then do seven times *kshalan* with DI water of this triturated *Hingul*. After *kshalan Hingul* was done become very smooth dark red in color, lusterless and pH came near neutral. The process was completed in 14 days and the final product called *shudha Hingul* was obtained.  

The raw *Hingul*, seven times triturated *Hingul*, *kshalan* liquid and *shudha Hingul* obtained from the above process were taken for different analysis.  

Modern parameters for analysis  

ICP MS was performed in Texan laboratory at Rabale Thane, for percentage of mercury in *Hingul* samples. XRD was performed in IIT Bombay for the phase identification. HPTLC was performed in Anchrom laboratory at Thane.  

A) ICP MS:  

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is an analytical technique used for elemental determinations. Thermo Scientific ELEMENT XR, a high performance, double focusing magnetic sector field ICP-MS.[2]  

- Three samples for this test were.  
  1) First sample: Raw *Hingul* (H)  
  2) Second sample: Seven times triturated sample. (HB7)  
  3) Third sample: Seven times washed *Hingul* powder sample. (G)  

1) First Sample: Raw *Hingul* (H)  

- **Method:**  
  Test for the detection of total mercury content. Sample preparation as per EPA 3050b using microwave digestion detection and quantification by ICP-MS.[2]  

- **Procedure :**  
  1. 0.2 gm sample was taken in to the microwave container. Then 10 ml of concentrated nitric acid and 1ml of 50% hydrogen peroxide were added.  
  2. The tube was placed in the microwave oven at 220ºc for 1 hour.  
  3. The container was cooled and slowly the pressure was released and the content was transferred into a conical flask.  
  4. If the solution is not clear filter in to the container and again transfer filtered solution quantitatively 50 ml standard volumetric flasks.  
  5. The container was rinsed with ICP grade water.  
  6. Simultaneously prepare blank solution by taking 10 ml nitric acid and 1ml hydrogen peroxide and was treated as above.
7. Both the solutions were subject to ICP-MS.
8. The same procedure was followed for all samples.

- Result:

<table>
<thead>
<tr>
<th>Sr.no</th>
<th>Name of Heavy metal</th>
<th>Concentration (mg/kg)</th>
<th>Detection limit (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Mercury</td>
<td>2.9%</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Note: 1% = 10000 mg/kg (ppm)

2) Second sample: Seven times triturated sample of Hingul (HB7)

- Result:

<table>
<thead>
<tr>
<th>Sr.no</th>
<th>Name of heavy metal</th>
<th>Concentration (mg/kg)</th>
<th>Detection limit (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Mercury</td>
<td>754.0</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Note: 1% = 10000 mg/kg (ppm)

3) Third sample: Seven times washed Hingul powder sample. (G)

- Result:

<table>
<thead>
<tr>
<th>Sr.no</th>
<th>Name of heavy metal</th>
<th>Concentration (mg/kg)</th>
<th>Detection limit (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Mercury</td>
<td>3.0</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Note: 1% = 10000 mg/kg (ppm)

Observation table of ICP-MS result:

<table>
<thead>
<tr>
<th>Sr.no</th>
<th>Name of sample</th>
<th>Name of heavy metal</th>
<th>Concentration (mg/kg)</th>
<th>Detection limit (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>AshudhaHingul (H)</td>
<td>Mercury</td>
<td>2.9 %</td>
<td>0.02</td>
</tr>
<tr>
<td>2.</td>
<td>Seven times triturated Hingul (HB7)</td>
<td>Mercury</td>
<td>745.0</td>
<td>0.02</td>
</tr>
<tr>
<td>3.</td>
<td>After seven times kshalan (G)</td>
<td>Mercury</td>
<td>3.0 %</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Note: 1% = 10000 mg/kg (ppm)

B) XRD:

X-ray powder diffraction analysis (XRD) is perhaps the most widely used X-
ray based analytical techniques for characterizing materials. Phase identification is important because the material properties are highly dependent on structure. XRD analysis has a wide range of applications.

- Material and Method:
Three samples were run for XRD (powder)[3]:
1. First sample: Raw Hingul (H)
2. Second sample: Seven times triturated sample. (HB7)
3. Third sample: seven times washed Hingul powder sample. (G)

1) First sample: Raw Hingul (H)[3]
File name: D:\XRD Data\Year 2014\POWDER DIFFRACTION DATA
Diffractometer system=EMPYREAN
Measurement program = C:\PANalytical\Data Collector\Programs\ROCK POWDER GENERAL METHOD-ADS.xrdmp,
Operator: Indian Institute
Raw Data Origin: XRD measurement (*.XRDML)

2) Second sample: Seven times triturated sample. (HB7)
File name: D:\XRD Data\Year 2014\POWDER DIFFRACTION DATA
Diffractometer system=EMPYREAN
Measurement program = C:\PANalytical\Data Collector\Programs\ROCK POWDER GENERAL METHOD-ADS.xrdmp
Operator: Indian Institute
3) Third sample: Seven times washed Hingul powder sample. (G)  
File name: D:\XRD Data\Year 2014\POWDER DIFFRACTION DATA  
Diffractometer system=EMPYREAN
Measurement program=C:\PANalytical\Data Collector\Programs\ROCK POWDER GENERAL METHOD-ADS.xrdmp.  
Operator:  Indian Institute

This graph shows the intensity of peak of Ashudha Hingul (H), Triturated Hingul (HB7) and Seven times washed Hingul

Result:
1) XRD report shows, the structure of cinnabar is not changed in raw, triturated and seven times washed Hingul sample.  
2) No D’ spacing changes occurs in Raw, Triturated and Seven times washed Hingul sample.  
3) Intensity of peak of cinnabar was increased after completion of kshalan of Hingul.  
4) Atomic spacing or structure is not affected in Raw, Triturated and Seven times washed Hingul sample.

C) HPTLC:  
In the current work HPTLC technique was adopted for deriving the finger print patterns of the samples. Procedure:  
The dried materials were powdered and sieved through ASTM sieve (85/BS sieve) separately and were kept in separate air-tight containers. Samples for HPTLC fingerprinting were prepared by using optimized conditions for extraction.[4]

1) Preparation of raw Hingul: (H)
Step 1: Accurately weighed 500 mg of raw Hingul powder was transferred to 20 cm³ test tube and 5.0 cm³ of methanol was added to it. The test tube was kept on test tube shaker for 90 minutes. The contents were filtered through Whatmann filter paper no. 41.

2) Preparation of seven times triturated Hingul in Nimbu kswaras: (HB7)
Step 2: Accurately weighed 500 mg of seven times triturated Hingul powder was transferred to 20 cm³ test tube and 5.0 cm³ of methanol was added to it. The test tube was kept on test tube shaker for 90 minutes. The contents were filtered through Whatmann filter paper no. 41.

3) Preparation of Hingul powder after seven kshalan: (G)
Step 3: Accurately weighed 500 mg of Hingul powder after seven kshalan was transferred to 20 cm³ test tube and 5.0 cm³ of methanol was added to it. The test tube was kept on test tube shaker for 90 minutes. The contents were filtered through Whatmann filter paper no. 41.

- Chromatography:
High Performance Thin Layer Chromatography was used to establish the fingerprint of the sample extracts. In TLC (>90%) silica is used mainly for the separation of pharmaceuticals and drugs. Normal phase mode of separation was employed. The stationary phase used was Silica gel 60 F₂₅₄. A mobile phase was developed in such a way that the maximum separation was achieved on a TLC plate useful for phytochemical analysis.

- Result:
The proposed HPTLC method can be used to develop a fingerprint for the identification of samples.
1) In this study we found the difference between three samples in the fluorescence we got chromatographic separation of our organic and inorganic substances in the sample of H, HB7 and G.
2) In sample of raw Hingul we saw the color of spectra in fluorescence was yellow and in next sample of seven times triturated Hingul in nimbu swaras the color of spectra in fluorescence was blue with multiple bands and in last sample of Hingul powder after kshalan the color of spectra in fluorescence was white.
Hence according to the result found in these three samples we conclude that may
be the impurities are removed after the completion of shodhan process, mean may be the impurities are removed in the sample of Hingul powder after doing seven times kshalan. HPTLC Fingerprints are suitable for monitoring the identity and purity of crude drugs or processed drug for detecting adulterations. With the help of fingerprints, we can identify the active components of selected disease which is causing it. As the technique is simple and rapid it is a highly used technique. It has benefits of being highly portable methods and can be practiced at every place without any difficulty. The analysis is quick and the fingerprints can easily evaluate to give the analyst an overview of the research. 

The proposed HPTLC methods demonstrated in this study are simple, economical and reliable. The fingerprints which are established are representatives of a given species. These methods are suitable for monitoring the identity and purity of crude and processed drugs and for detecting adulteration. These methods can also be used to check the batch to batch consistency of marketed herbal and Herbomineral formulation.

**DISCUSSION**

ICP MS: In this test three samples were examined i.e. H, HB7 and G. We have analyzed crude cinnabar for mercury content. Where the analysis was about 2.9% means 29,000 mg/kg (parts per million) of mercury in the crude sample. The third sample was treated for purification by applying with trituration with lemon juice and later removing the material applied for purification by kshalan process. In the process other impurities also got removed (wash out). And crude cinnabar got purified.

Where in mercury content level went up in the purified sample, and this was substantiated by analyzing purified sample for mercury content and found to be more than 3% means 30,000 mg/kg (parts per million).

We have also analyzed the seven times triturated sample and observed that the proportion of mercury in the triturated sample obviously got reduced due to addition of this material of trituration in practice it was observed 754 only.

The above observation indicates that pure form of mercury is on higher parameter in sample of Hingul powder after kshalan compared to the other two samples.

XRD: XRD report shows, the structure of cinnabar were not changed in raw, triturated and seven times washed Hingul sample. Intensity of peak of cinnabar was increased after completion of kshalan of Hingul. Atomic spacing or structure is not affected in Raw, Triturated and Seven times washed Hingul sample.

HPTLC: In this study we found the difference between three samples. In the fluorescence we got chromatographic separation of our organic and inorganic substances in the sample of H, HB7 and G. In sample of raw Hingul we saw the color of spectra in fluorescence was yellow and in next sample of seven times triturated Hingul in nimbuk swaras the color of spectra in fluorescence was blue with multiple bands and in last sample of Hingul powder after kshalan the color of spectra in fluorescence was white. It indicates that the last sample which
shows white band i.e. Hingul powder after kshalan indicates the absence of any organic traces i.e nimbuk swarasa is absent.

CONCLUSION
After tituration of Hingul with a Nimbuk swaras for seven times, the acidic nature increases. Later when repeated kshalan of same sample was done with DI water the acidic nature was reduced and the pH became neutral.

The removal of acidic nature of triturated Hingul by the process of kshalan, supports the concept that substances with neutral nature are less gastric irritant which supports the aim of increasing the therapeutic efficacy of the drug after shodhan.

In HPTLC study, the final sample i.e. (G) Hingul after seven kshalan shows white band. Hence we can conclude that the purified Hingul is devoid of any organic impurities as is evident from band color.

To this, supportive result is of XRD and ICP MS tests, which indicates high intensity of cinnabar and high percentage of mercury, which supports the standards that mercury is present 86.2% and 13.8% sulphur in Hingul, according to result of XRD study. Shows that the intensity of peak was increased but atomic structure was not changed which supports the sidhant of gu-nantaradhan.

Kshalan process helps to remove unwanted substances, which is the primary aim of Shodhan i.e Dravyadoshanivaram. Hence it can be concluded that the study or the process of kshalan of shodhit Hingul supports the fundamentals of Shodhan i.e. purification of substance, eradication of impurities and enhancement in their qualities for potent result in therapeutic use.

REFERENCES
2. ICP MS (heavy metal): Sample preparation as per EPA3050b using microwave digestion detection and quantification by ICP/MS.
3. Powder XRD were done by using Diffractometer system-EMPYREAN C:\PANalytical\Data Collector\Programs\ROCK POWDER GENERAL METHOD-ADS.xrdmp
4. Instrument CAMAG HPTLC, Stationary Phase Silica gel 60 F254 HPTLC pre-coated plates, Sample Applicator CAMAG LINOMAT V, Measurements Mode Absorbance & Florescence & visible, Photo Documentation CAMAG TLC Visualizer.

Source of Support: Nil
Conflict Of Interest: None Declared