STANDARDIZATION OF ABHYARISHTA AS PER WHO GUIDELINES


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ABSTRACT

Ayurveda is the oldest surviving complete medical system in the world. Asava and Arishta are unique dosage form discovered by Ayurveda having long shelf life. Abhayarishta is an important ayurvedic formulation used to relieve the problems like piles and constipation, helpful in mild ascites and dropsy, relieve in urinary obstruction, increase digestive power and is helpful in enlargement of liver, spleen and anemia, which is prepared by fermenting the decoction of specified plant materials using fruits of Terminalia chebula. Abhayarishta a marketed ayurvedic formulation containing Gallic acid as one of the active ingredient. The objective of this study was to determine the level of alcohol, and pH in commercially available Abhayarishta to establish a routine procedure for standardization of these Ayurvedic preparations. In present communication, HPLC and UV method was developed for standardization of Abhayarishta by quantitative estimation of major compound, Gallic acid as marker as per WHO guidelines. The developed method was validated with respect to linearity, precision, accuracy, and robustness. Moreover, the alcohol content of the formulation was also determined by Gas chromatography and redox titrimetry. The formulation was also analyzed for microbial load, heavy metal content, pesticide residue, elemental analysis and pharmacological activity.

Keywords: Abhayarishta, Standardization, UV, HPLC.
INTRODUCTION
Formulary of India (AFI) enlists thirty seven asavas and arishtas. Arishtas and Asavas have been used as medicines for more than 3000 years to treat various disorders and are also consumed as appetizers and stimulants. Medicinal value of the preparation, sweet taste and easy availability, people are prone to consume higher doses of these drugs for longer periods. The preparation and sale of 34 varieties of Arishtas and 25 varieties of Asavas has been legalized and listed in the official Ayurveda pharmacopoeia of Sri Lanka. Arishtas are an important group of formulations used in Ayurveda since ancient time. Arishtas and asavas are self generated herbal formulations of traditional Ayurvedic system [1]. They are alcoholic medicament prepared by allowing the herbal juices and their decoctions to undergo fermentation with the addition of the jaggry in it. It is self generated herbal fermentation of traditional ayurvedic system. It is alcoholic medicament prepared by allowing herbal juices or their decoctions to undergo fermentation with addition of sugars. Arishtas are an important group of formulations used in Ayurveda. Abhayarishta is one of the ancient liquid oral formulations prescribed in ayurveda for piles and constipation. The major plant ingredient of this formulation, Terminalia chebula (Combretaceae) is a native plant of India and South East Asia. The concept laid behind the research is to develop a standardization method for Abhayarishta by determining the marker constituents and to study comparative composition of finished formulation with respect to the marker constituent and to correlate these with pharmacological activity. Abhyarishta is recommended for piles and constipation, helpful in mild ascites and dropsy, relieve in urinary obstruction, increase digestive power and is helpful in enlargement of liver, spleen and anaemia. Qualitative analysis gives an indication of the identity of the chemical species in the sample and quantitative analysis determines the amount of one or more of these components [2-9].

MATERIALS AND METHODS
Determination of ethanol content of the formulation by Redox titration
The method uses a redox titration to find the concentration of ethanol in the aqueous solution. The ethanol gets oxidized to ethanoic acid by reacting it with excess of potassium dichromate in concentrated sulphuric acid. [10]

Determination of ethanol content of the formulation by Gas Chromatography
To determine the ethanol content of the marketed formulation, the formulation was subjected to evaporation by rotary vacuum evaporator at controlled temperature of not exceeding 45°C
under vacuum. The evaporation process was carried out until all the liquid content is evaporated. Now, the collected liquid contains mixture of water and ethanol. The condensate was analyzed by Gas Chromatography for the amount of the ethanol in it. Reference standard of ethanol (99.9%) was used to determine retention time of ethanol. The Gas Chromatography method for ethanol determination was validated. The method was found to be accurate, linear and precise. [11]

**Preliminary Phytochemical screening**
Methanolic extract was used for the detection of major class of chemicals like carbohydrates, phenols, flavonoids, tannins, alkaloids, glycosides, saponins, anthraquinones and amino acids. [12]

**Extraction of tannins from the formulation**
Abhayarishta contains tannin complex such as ethyl gallate, methyl gallate, chebulagic acid, chebulinic acid and penta-o-galloyl-β-D-glucose. The formulation was subjected to hydrolysis by refluxing it with 5M sulphuric acid for 10 hours. This caused the complex structure of tannins to break into simpler form of tannins. This simpler form was then passed through the column of silica (column grade, 60-80 mesh) for its purification. The sample was then ready for its HPLC analysis. [13]

**Determination of Gallic acid in the formulation by UV Spectrophotometry and HPLC**
The hydrolyzed marketed Abhayarishta formulation was analyzed by Ultra Violet Spectrometry for amount of gallic acid in it. Standard gallic acid sample was recorded for its UV spectrum and then the hydrolyzed formulation was comparatively examined by UV Spectrophotometry for the amount of gallic acid in it. This method was validated for its linearity, precision, accuracy, robustness and ruggedness.

The reference standard was used for determination of the retention time of gallic acid. The purified compound was then passed through the HPLC column for its analysis. The sample was analyzed by HPLC for gallic acid content. The mobile phase for HPLC analysis composed of acetonitrile: water (30:70) with ortho-phosphoric acid used for maintaining pH of about 3. The method was validated for its linearity, precision and accuracy. [14-16]

**Pesticide residue determination**
Detection of organochloro, organophosphate and carbamate pesticides was done for the
marketed formulation as per WHO guideline. [17-21]

Elemental Analysis
This test was performed to determine the presence of elements like nitrogen, sulphur or any halogen in the formulation. The elements present in the organic sample, carbon and hydrogen are the main constituents. There is no special test for oxygen. Sulphur, nitrogen and halogens are detected by Lessaigne’s test. [21-22]

Heavy metal estimation
As per WHO guidelines, the amount of heavy metals present in the formulation was tested by using Atomic Absorption Spectroscopy.

Microbial load estimation
Microbial load determination was done as per WHO guidelines. For herbal formulations of internal use, the microbial load should not exceed the stated permissible limits. Total viable aerobic count and test for specific organisms such as Enterobacteria, Escherichia coli, Salmonella sp and Shigella sp was performed.

Pharmacological investigation of the formulation
The laxative activity of the formulation was tested on the isolated tissue of rat ileum. The rat tissue was isolated and assembly of organ bath was set to record its peristaltic movement. The peristaltic contraction and relaxation of tissue before drug administration was noted on a kymograph. The formulation was then administered and the change in response of the peristaltic activity was noted.

RESULT AND DISCUSSION
Ethanol content by redox titration method was found to be 11.6 %V/V with standard deviation 0.346. In Ethanol content by GC method (Figure 1) area of each peak of standard was plotted against concentration of ethanol to obtain calibration graph. Calibration curve was found to be linear over concentration range1-3%v/v (R=0.995). Peak area of sample were plotted against concentration using calibration graph and concentration of ethanol was found to be 10.43%v/v. Ethanol content from both methods has comparable values and those are within the labeled claim. Mean pH of formulation was found to be 3.83. It indicates weak acid properties of Abhayarishta. Specific Gravity of formulation was found to be 1.242 g/mL. Preliminary phytochemical tests showed the presence of Anthraquinone glycoside and
reducing sugar, sugars like Ketoses & fructoses, sucrose, glycogen and tannins in the formulation. Quantitation of gallic acid from formulation by UV spectrophotometry was done at 258 nm (Figure 2). The Lambert- Beer’s law was obeyed at a concentration range of 2-12 µg/ml with regression co-efficient of 0.998. The unknown sample formulation concentration corresponds to 1.95 mg/ml of gallic acid of the formulation. The method is precise with standard deviation 0.0036 and % RSD 0.843. The recovery studies were performed at low, medium and high level with recovery in the range of 98.37 to 103.25%. The Standard Error between the absorbance of the two solvent systems and two instruments was found to be 0.002, 0.0018 respectively. LOD and LOQ was found to be 0.225 and 0.681 µg/ml respectively. Quantitation of gallic acid from formulation by HPLC was done at 272 nm. The HPLC method showed a linear relationship between peak areas and concentrations over 2-8 µg/ml with regression co-efficient of 0.996. The unknown sample formulation concentration corresponds to 1.7 mg/ml of gallic acid of the formulation. A signal three times higher than noise was regarded as the detection limit. The LOD and LOQ values was found to be 0.099 and 0.3 µg/ml respectively. The quantitative repeatability of the injection was determined by analyzing the quantity of gallic acid in the formulation. A high repeatability was observed with % RSD values 0.785. Accuracy (expressed as recovery) of the method was determined by analyzing the percentage recovery of marker constituents. The high recovery values (90.87-106.93 %) obtained indicated satisfactory accuracy. Finally, the robustness of the method was studied by changing the mobile phase; minor changes in mobile phase showed no effect on peak resolution (Figure 3) and summarized results were as shown in Table 1. The colour test of the formulation for the pesticide residue determination was done. The formulation was not found to contain any of the pesticide residues such as organochloro, organophosphate and carbamate. The formulation was not found to contain nitrogen, sulphur and any of the halogens (chlorine, bromine, iodine). Heavy metal estimation of the formulation showed presence of lead within permissible limit and cadmium arsenic and mercury was not detected and results were as shown in Table 2. WHO guidelines has stated maximum tolerance limit for the presence of some pathogenic micro organisms and results were as shown in Table 3. The pharmacology activity of the formulation was tested on isolated rat ileum. The contraction of the tissue was observed in correspondence to the peristaltic movement of the isolated tissue. After administration of the formulation, the muscle tone of the tissue was found to decrease and the contractions have been increased. As laxative agents increase peristalsis, the formulation was said to possess laxative activity.
Figure 1: Gas Chromatograph of standard ethanol (2% v/v)

Figure 2. UV spectrum of Gallic acid

Figure 3: HPLC chromatogram of standard Gallic acid
Table 1. Summary of Parameters of UV and HPLC

<table>
<thead>
<tr>
<th>Parameter</th>
<th>UV</th>
<th>HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity</td>
<td>2-12 µg/ml</td>
<td>2-8 µg/ml</td>
</tr>
<tr>
<td>Slope</td>
<td>0.044</td>
<td>185.1</td>
</tr>
<tr>
<td>Intercept</td>
<td>-0.0155</td>
<td>-15.86</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.998</td>
<td>0.997</td>
</tr>
<tr>
<td>LOD</td>
<td>0.225 µg/ml</td>
<td>0.099 µg/ml</td>
</tr>
<tr>
<td>LOQ</td>
<td>0.681 µg/ml</td>
<td>0.3 µg/ml</td>
</tr>
</tbody>
</table>

Table 2. Results for heavy metal contamination of the formulation

<table>
<thead>
<tr>
<th>Elements</th>
<th>Observation</th>
<th>Permissible limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead</td>
<td>0.9 ppm</td>
<td>10 µg</td>
</tr>
<tr>
<td>Cadmium</td>
<td>Not detected</td>
<td>0.3 µg</td>
</tr>
<tr>
<td>Arsenic</td>
<td>Not detected</td>
<td>5 µg</td>
</tr>
<tr>
<td>Mercury</td>
<td>Not detected</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3. Observation table along with permissible limits for different microorganisms

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Observed value</th>
<th>Permissible limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic viable bacteria</td>
<td>150 cfu/ml</td>
<td>10^7 / g</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Absent</td>
<td>100 / g</td>
</tr>
<tr>
<td>Salmonellae sp.</td>
<td>Absent</td>
<td>absent / g</td>
</tr>
<tr>
<td>Shigella sp.</td>
<td>Absent</td>
<td>absent / g</td>
</tr>
</tbody>
</table>

CONCLUSION

The marketed Abhyarishta sample was analyzed as per WHO guidelines. The alcohol content of the sample was within the official limit. The proposed HPLC and UV methods can be used for routine analysis of gallic acid from the marketed ayurvedic formulation to estimate the amount of gallic acid in the formulation. The HPLC method is more sensitive, accurate and selective than UV method. The formulation also passes the test for pesticide residue, heavy metal contaminants and microbial load. The pharmacological activity (laxative activity) was also found to be present during the study.
REFERENCES


