Development Of Fingerprinting Method For Traditional Ayurvedic Formulation Arjunaristha By Gas Liquid Chromatography

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ABSTRACT

Arjunaristha is a well known Ayurvedic formulation described in classical Ayurvedic text Charak-Samhita, Bhaishajyaratnavali and ayurvedic formulary of India. It is a potent ayurvedic medicine containing self-generated alcohol used for heart and lung diseases. In present research study Gas liquid Chromatographic fingerprinting method has been developed that is simple, rapid, precise and accurate for fingerprinting of laboratory and marketed preparation of Arjunaristha using flame ionization detector. The separation was performed employing Carbowax 20 M×4mm stainless steel packed column using nitrogen as a carrier gas at optimized conditions. The column was maintained at 90°, while injection port and detector were maintained at 170°. The system suitability parameters were studied throughout the study. The developed fingerprinting method has been applied successfully for marketed formulations also, found reproducible and repeatable. It also serves as a tool for assay of self-generated alcohol content in Arjunaristha. The recovery values were found to be 99.72% with RSD values less than 0.353%. The proposed fingerprinting method can be used for routine quality control method for laboratory as well as for marketed Ayurvedic formulation Arjunaristha.

KEYWORDS

Fingerprints; Arjunaristha; Gas liquid Chromatography; Fingerprinting; Ayurvedic formulation; Quality control parameter.

INTRODUCTION

Aristhas are classical Ayurvedic formulations made by soaking the drugs(powder or decoction) in a solution of sugar or jaggery for a specified period of time during which it undergoes a fermentation process generating alcohol, thus facilitating the extraction of active principles contained in the drug(s). The alcohol generated, also serves as a preservative1. It is one of the most widely used formulations indicated as general tonic for heart disease, lung disease, in loss of strength, as an immunomodulator and in
azoospermia\textsuperscript{[1,2,3]}. Classical Ayurvedic formulations are time tested but for establishing their modern instrumental evidence from the last two decades, efforts have been made to develop parameters of quality control for traditional formulations (Ayurvedic formulations) by modern instruments and available technology\textsuperscript{[4,5,6]}. The world Health Organization (WHO) Assembly in its resolution WHA 31.33(1978), WHA 40.33(1987) and WHA 42.43(1989) has emphasized the need to ensure the quality of the medicinal plants products by using modern controlled techniques and applying suitable standards\textsuperscript{[7]}. In this contest efforts have been made to develop methods for fingerprinting and determination of alcohol content via Gas liquid chromatographic analysis of the Ayurvedic formulation Arjunaristha.

Gas chromatographic method\textsuperscript{[8]} and internal reflectance spectrophotometric method\textsuperscript{[9]} are employed in determination of ethanol in toiletries and official drug preparations. In the present communication, we report a fingerprinting method that also serves as optimized gas chromatographic method for the determination of alcohol in laboratory and marketed formulations.

**MATERIAL AND METHOD**

**Chemicals**

All the solvents purchased from E.Merck and S.D.Fine Chemicals, Mumbai. All solvents used for experiment were HPLC grade.

**Crude drugs**

Crude drugs for formulation were procured from Crude drug supplier and identified on the basis of microscopic and macroscopic parameters and compared with standard pharmacopoeial monographs\textsuperscript{[10, 11]}.  

**Preparation of the formulation**

Three batches of Arjunaristha were prepared in the laboratory (AJR-I, II, III) according to method given in Ayurvedic formulary of India\textsuperscript{1} and three samples of Arjunaristha (AJR-A, B, C) form different manufacturers were procured from a local Ayurvedic drug store.

**Chromatography condition\textsuperscript{[12]}**

GC analysis was performed on Shimadzu GC14 B with a dual flame ionization detector (FID) using CHROMOPACK. Estimation of alcohol content was carried from different batches (three marketed and three laboratory batches) of Arjunaristha with following chromatographic conditions.

**Column**

CHROMOPACK (Packed Carbowax 20 M packed into a steel column with internal diameter of 4 mm).

**Carrier gas** : Nitrogen  
**Detection** : Flame ionization detector (FID)  
**Injection volume** : 1μl  
**Flow rate** : 1.8kg/cm\(^2\)/min  
**Column temperature** : 90°C-130°C  
**Program rate** : 5°C/min  
**Injector temperature** : 170°C  
**Detector temperature** : 170°C

**Development of fingerprints and estimation of alcohol in arjunaristha**

For the fingerprinting and assay, 25ml of syrup form different batches of laboratory and marketed formulation were taken in a 500ml distillation flask. To this 5ml of 0.1N sodium hydroxide solutions, 10mg of phenolphthalein powder and 150ml of HPLC grade water were added. The resulting mixtures were heated to 110\(^0\) and 100ml of distillate was collected. Distillate(1ml) was diluted to 10ml with HPLC grade water and an aliquot of this solution was analyzed in the developed chromatographic conditions and a chromatogram was noted.

**Preparation of standard solution and standard curve**

A range of standard solutions of ethanol were prepared containing 1, 2, 3, 4 and 5% v/v of ethanol using ethanol(99.98%) and HPLC grade water. From the standard solution 1ml was diluted to 10ml with HPLC grade water. Then 1μl of the solution was injected and a chromatogram was recorded. The retention time of ethanol was found to be 1.6. The area was plotted against the concentration of ethanol to obtain a calibration graph. Throughout the study, the suitability of the chromatographic system was maintained by calculating the capacity factor(k).
and the peak asymmetry (T).

Method validation

The method was validated for linearity, accuracy, limit of detection, limit of quantification, inter-day and intra-day assay precision, tailing factor and capacity factor.

Recovery studies

The recovery studies performed at three levels by adding known volume of ethanol to the formulation, of which the alcohol content has been estimated previously. The data were obtained and recovery was calculated.

RESULT AND DISCUSSION

The classical Ayurvedic formulation Arjunaristha were prepared in laboratory (AJR-I, II, III) and three different marketed samples (AJR-A, B, C) of the formulation were evaluated in the optimized Gas liquid chromatographic conditions for fingerprinting and their self generated alcohol content. Optimum conditions, which are necessary for the reproducible fingerprints and quantitative determination of the ethanol with maximum selectivity, were established by a number of preliminary experiments. Optimum conditions were fixed by varying one parameter at a time fixing other parameters constant and observing its effect on the peak resolution. After evaluating the stationary phase, the stationary phase Carbowax 20M was found to be ideal column for efficient separation of the component with good peak shape. The effect of nitrogen flow rates were examined for recording chromatogram. The nitrogen flow rate of 1.8 kg/cm$^2$/min was selected because of its ideal retention time and less time for analysis. The oven temperature was varied from 90-130°C and finally it was fixed at 90°C due to shorter time of analysis, sharp peak and ideal retention time. The injector and detector temperature were maintained at 170°C throughout the analysis for sharp peaks and ideal chromatographic behavior. The Gas liquid chromatographic fingerprint of alcohol under experimental condition showed a single peak of the at 1.683±0.04 min (Figure 1).

The peak areas of each standard were obtained from the system, and a calibration graph was plotted with concentration vs. peak area. A good linear relationship was obtained over a concentration range of 1-5% v/v of alcohol. The correlation coefficient ($r^2$) was calculated where the $r^2$ valu 0.9979 indicates the good linearity between the concentration and peak area.

The limit of detection for alcohol estimation was found to be 0.086 and the limit of quantification was found to be 0.263. These values are considered to be good enough for a reasonable accuracy in most of the laboratories worldwide.

Intra-day assay precision was found by analysis of alcohol at three times on the same day. Inter-day assay precision was carried out using the standard alcohol at three different days, and % relative standard deviation (RSD) was calculated. The RSD was found to be less than 2 for both inter-day and intra-day assay precision. The low values indicate robustness of the method.

Chromatographic parameter such as peak asymmetry (tailing factor) and capacity factor (k) were found to be 1.10 and 4.7, respectively.

The Gas liquid chromatographic fingerprint and estimation of ethanol from Ayurvedic formulations was carried out at different concentrations. The supernatant solution was injected and chromatogram was recorded. The results of analysis of different laboratory and marketed formulation were found reproducible and repeatable presented in (TABLE 1) are in good agreement with labeled values.

Recovery studies were carried out for the accuracy parameter. The study was carried out at three levels. To the formulation, the standard alcohol was added at 50% 100% and 150% levels; dilutions were made, and analyzed by the method. The mean of % recovery, % RSD and standard error (SE) of three levels were calculated, and found to be within the limit, as listed in TABLE 2. This shows significant
TABLE 1: Analysis alcohol content in arjunaristha

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Formulation (%V/V)</th>
<th>Ethanol content*(%V/V)</th>
<th>Confidence level (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>AJR-I</td>
<td>10.6%\±0.43</td>
<td>0.212</td>
</tr>
<tr>
<td>02</td>
<td>AJR-II</td>
<td>10.5%\±0.62</td>
<td>0.321</td>
</tr>
<tr>
<td>03</td>
<td>AJR-III</td>
<td>10.8%\±0.49</td>
<td>0.272</td>
</tr>
<tr>
<td>04</td>
<td>AJR-A</td>
<td>8.5%\±0.71</td>
<td>0.647</td>
</tr>
<tr>
<td>05</td>
<td>AJR-B</td>
<td>12.6%\±1.72</td>
<td>1.202</td>
</tr>
<tr>
<td>06</td>
<td>AJR-C</td>
<td>9.2%\±1.02</td>
<td>0.928</td>
</tr>
</tbody>
</table>

AJR-A : Marketed formulation I, AJR-B : Marketed formulation II AJR-C : Marketed formulation III, AJR-I, AJR-II, AJR-III : Laboratory formulation

TABLE 2 : Validation parameters

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Retention Time</td>
<td>1.683\±0.04</td>
</tr>
<tr>
<td>2</td>
<td>Linearity (%)</td>
<td>1-5%</td>
</tr>
<tr>
<td>3</td>
<td>Correlation coefficients(r²)</td>
<td>0.9978</td>
</tr>
<tr>
<td>4</td>
<td>LOD</td>
<td>0.086</td>
</tr>
<tr>
<td>5</td>
<td>LOQ</td>
<td>0.263</td>
</tr>
<tr>
<td>6</td>
<td>Precision (%RSD)</td>
<td>a) Inter Day</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) Intraday</td>
</tr>
<tr>
<td>7</td>
<td>Recovery Studies</td>
<td>a)Accuracy (%RSD)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) SE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c) Recovery%</td>
</tr>
<tr>
<td>8</td>
<td>Tailing factor</td>
<td>1.10</td>
</tr>
<tr>
<td>9</td>
<td>Capacity factor</td>
<td>4.7</td>
</tr>
</tbody>
</table>

RSD : Relative standard deviation, LOD : Limit of detection, LOQ : Limit of quantification, SE : Standard error

Precision of methods with 95% confidence level. In order to study selectivity of the fingerprinting method, the interference of commonly associated excipients in the determination of commonly associated excipients in the determination of ethanol was carried out. It was observed that none of the excipients interfered in the determination as evident from the similar retention time of ethanol.

CONCLUSION

The developed Gas liquid chromatographic fingerprint method also serves as an analytical tool for determination of self generated alcohol content (ethanol content) in classical Ayurvedic formulation Arjunaristha.

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