METHOD DEVELOPMENT FOR ESTIMATION OF ALCOHOL IN AYURVEDIC FORMULATIONS USING GAS CHROMATOGRAPHY

Dhara K. Vora, Saurabh K. Banerjee* and Gurmeet S. Chhabra

Department of Pharmaceutical Chemistry, School of Pharmacy and Technology Management, SVKM's NMiMS, Shirpur Campus, Babulde, Shirpur, Maharashtra

*E-mail: saurabhbannerjee15@gmail.com
Tel.: +91-9765362007

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MARKETED HERBAL PREPARATIONS CONTAINING SELF GENERATED ALCOHOL (HERBAL ORIGIN AND ALLOPATHIC) MAY AFFECT THE HEALTH OF PATIENTS ON FAULTY DOSING. THE METHOD WAS SET UP FOR THE QUANTITATIVE ESTIMATION OF ALCOHOL BY GAS CHROMATOGRAPHY. THE METHOD WAS DEVELOPED BY CHANGING VARIOUS PARAMETERS OF GAS CHROMATOGRAPHY. FURTHERMORE, THE METHOD WAS VALIDATED IN ORDER TO CONFIRM ITS RELIABILITY AND POTENTIALS TO USE AS QUALITY CONTROL TOOL IN QC LABORATORIES. IN AYURVEDIC PREPARATION ARISHTHA, THE ESTIMATED ALCOHOL (AS SELF GENERATED ALCOHOL) WAS FOUND TO BE 8.1% (KHADIRARISHTA), 10.2% (DRAKSHAKUMARI), 7.8% (SARASWATARISHTA) AND OBEYED THE LABEL CLAIM. THE PRESENT WORK CAN BE USED AS THE METHOD FOR ROUTINE QUALITY AND SAFETY STANDARDIZATION.

KEY WORDS: HERBAL FORMULATION, ALCOHOL, GAS CHROMATOGRAPHY, DRAKSHAKUMARI, KHADIRARISHTA, SARASWATARISHTA

INTRODUCTION

Alcohol is the diluent in more than hundred proprietary products and is found in concentrations up to 70%. This causes significant trouble in patient management. e.g. patient under disulfiram therapy, in the patients with active peptic ulcer disease and in the patient under treatment with central nervous system depressants or other substances that interact with alcohol. The alcohol contents can cause various difficulties with abstinence programs and adolescent alcohol abuse (Dukes et al. 1997). Pharmaceutical products containing alcohol like arishtas (containing self generated alcohol) has tremendous medicinal value (Kroes et al. 1993), sweet taste and easy availability, due to which people are prone to abuse it by consuming higher doses of these drugs for longer periods. Hence standardization and development of reliable quality protocols for the formulations containing alcohol using sophisticated techniques of analysis is extremely important (Santosh et al. 2003; Dash and Hashyap, 1987).

The Medicines Control Council is concerned about the influence of the simultaneous ingestion of alcohol and medicines on certain psychomotor functions and the consequent inability of the consumer to perform tasks which require mental clarity. Health care personnel should be aware of the alcohol content of medications when counseling patient's receiving drugs against alcoholism or patients who should avoid alcohol (Dangor and Veltman, 1985). The content of ethanol, methanol, isopropyl alcohol in herbal extracts is routinely measured by gas chromatography. Routine GC applications include analysis of herbal extracts to comply to good laboratory and good manufacturing practices, several GC methods to monitor residual solvents have been reported in the literature (Puranik et al. 2007; 2009; Mukherjee, 2002). In continuation of our work on method development for bulk drug and dosage forms...
(Patil et al 2011; Prasanthi et al 2011; Shah et al 2011; Suresh et al 2011), the present work was undertaken to estimate alcohol content in ayurvedic formulations.

**EXPERIMENTAL**

**Instruments and materials**
The GC system (Perkin Elmer 500) was used in the present study. The detection was performed by means of FID. Separation was achieved using a packed column with the dimensions of 2 m length and 3.170 mm diameter. Stationary material was made up of WHP having 0.14 mm in mesh size range. The column used was packed, carrier gas was nitrogen, detector used was FID and the data were acquired through Total Chrome Navigator software (version 6.3.10504). This developmental work involved the use of various simple laboratory chemicals of HPLC grade including ethanol, DMSO, benzene, toluene (Merck) and water (Double distilled). Samples of Khadirarishta, Drakshkumari and Saraswatarishta were purchased from Ayurvedic stores.

**Method development**

*Preparation of standard*
Toluene was selected as standard and sample diluent based on its ability to dissolve wide variety of substances and high boiling point that does not interfere with more volatile solvents tested by GC for method involving analysis of high boiling point solvents. Standard stock of ethanol was prepared by diluting with toluene in 10 ml volumetric flask.

*Preparation of sample*
Accurately weighed 1.2 ml of Khadirarishta, Drakshkumari and Saraswatarishta sample was transferred in 10 ml volumetric flask and volume was made up with toluene. The supernatant obtained contained alcohol in extracted form and was injected in the chromatographic system.

**Gas chromatographic conditions**
The experimental conditions used were: 0.01 µl volume of either standard or sample solutions was injected in GC injection port maintained at 150°C. Nitrogen was used as a carrier gas at the flow rate of 25 ml/min. Temperature of detector was set at 200°C with temperature gradient maintained initially at 85°C for 10 min. Further, trails were carried out to optimize the final method for estimation of alcohol content.

**Method validation**
The analytical method validation was carried out according to ICH method validation guidelines. The validation parameters addressed were specificity, linearity, precision, limit of detection, limit of quantization, ruggedness and system suitability.

**RESULTS AND DISCUSSION**
The optimized parameters were considered on the basis of symmetry obtained. For this, the injector and detector temperature (200°C) and oven temperature was maintained. Final temperature gradient was maintained at 70°C for 3 min and then, increased at the rate of 10°C to 150°C for 3 min. The flow rate of 15 ml/min and injection volume 0.01 µl were fixed. The retention time of ethanol was found to be 6.11 min. The presence of ethanol in different herbal formulations were presented in Table 1 and Figures 1-4. The specificity of analytical method was determined by injecting a blank solution and pure toluene solution under the sameexperimental conditions. No peak was observed from the chromatogram obtained by injecting 0.01 µl of toluene as blank. The plot of peak area versus concentration was linear over the concentration range. The regression line equation calculated was $y = 473256x + 30880$ with a correlation coefficient of 0.9974. The areas obtained were directly proportional to the concentration of analyte (Figure 5). The precision of method is extent to which the individual test results of multiple injections of standard agree. Precision of the analytical method usually expressed in standard deviation (as coefficient of variance) and % RSD. Method precision was expressed through relative standard deviation of six replicates of samples.

**Table 1.** % Label claim of ethanol in herbal formulations

<table>
<thead>
<tr>
<th>Test (Herbal formulation)</th>
<th>% Label claim observed</th>
<th>% Label claim given</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saraswatarishta</td>
<td>7.8</td>
<td>9.5</td>
</tr>
<tr>
<td>Khadirarishta</td>
<td>8.1</td>
<td>9.5</td>
</tr>
<tr>
<td>Drakshkumari</td>
<td>10.2</td>
<td>12</td>
</tr>
</tbody>
</table>
Fig. 1. Chromatogram of standard solution

Fig. 2. Chromatogram for Drakshkumari

Fig. 3. Chromatogram for Saraswatarishta

Fig. 4. Chromatogram of Khadirarishta
Relative standard deviation in the precision study for the ethanol was less than 1.0 % and confirmed that the method was highly precise. Intermediate precision is to determine method precision in different experiments using different analysts. The six replicates of standard solution were injected by the two analysts. The % RSD for ethanol in this study was less than 1%, thus, it confirmed that the method developed was highly precise.

Limit of detection (LOD) was found to be 0.075 µg/ml of ethanol and the limit of quantification (LOQ) was found to be 0.125 µg/ml. The ruggedness was established by determining ethanol using the same chromatographic system and the same column by two analysts on a different day. The results indicated good separation of the peaks and the % alcohol obtained was precise which suggested that the method was rugged.

CONCLUSION
The study represented a simple and validated gas chromatographic method for estimation of alcohol content in herbal formulations. Toluene was selected amongst various solvents as it showed comparatively good resolution and no interference with sample and standard peaks. By trying various set of temperature programming for column oven temperature, the best suited parameters for desired analysis were sorted and optimized. The developed method was found to be specific, accurate, precise and rugged. The amounts of alcohol estimated in the herbal formulations were found to be well within the acceptable label claim.

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REFERENCES

Fig. 5. Calibration curve of analyte