Determination of Ethanol in Abhayarishta by Gas Chromatography

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A simple, rapid, precise and accurate gas liquid chromatographic method was developed for the determination of ethanol in marketed preparation of *abhayarishta* using flame ionization detector. The separation was performed employing carbowax 20 M stainless steel column using nitrogen as a carrier gas at optimized conditions. The column was maintained at 90°, while injection port and detector were maintained at 110°. The system suitability parameters were studied throughout the study. The method has been applied successfully for the assay of ethanol in *abhayarishta*. The recovery values were found to be in the range of 98.14-104.54% with RSD values less then 0.800%. The proposed method can be used for the determination of generated ethanol content in *abhayarishta* preparation.

Abhayarishta is a common ayurvedic preparation, belonging to *asava* and *arista* categories, generally prepared soaking the drug either in powder form or in the form of decoction (*Kasaya*), in a solution of sugar or jaggery, for a specified period of time, during which it undergoes a process of fermentation generating alcohol, thus facilitating the extraction of active principle contained in the drugs[1].

Gas chromatographic method[2] and internal reflectance spectrophotometeric method[3] are employed in determination of ethanol in toiletries and official drug preparations. Recently GC and mass spectrophotometric methods[4] are reported for the determination of amount of alcohol in OTC products and methanol. In the present communication, we report the optimized gas chromatographic method for the determination of ethanol in *abhayarishta* syrup.

GC analysis was performed on Chemito GC7610 with a dual flame ionization detector (FID) using Carbowax 20 M (stationary phase) packed into a steel column with internal diameter of 2 mm. Nitrogen was used as a carrier gas at a flow rate of 1 kg/cm^2/min. The column temperature was maintained at 90°, while that of injector and detector at 110°. All chemicals used were of Analytical grade. Samples of *abhayarishta* syrup were procured from a local ayurvedic drug store. HPLC grade water was used in the study.

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 1 ml was diluted to 10 ml with HPLC grade water. Then 1 µl of the solution was injected and a chromatogram was recorded. The retention time of ethanol was found to be 1.08. 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).

For the assay, 25 ml of syrup was taken in a 500 ml distillation flask. To this 5 ml of 0.1 N sodium hydroxide solution, 10 mg of phenolphthalein powder and 150 ml of HPLC grade water were added. The resulting mixture was heated to 110° and 100 ml of distillate was collected. Distillate (1 ml) was diluted to 10 ml with HPLC grade water and an aliquot of this solution was analyzed as described earlier and a chromatogram was noted.

Optimum conditions, which are necessary for the 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 such as Porapak Q, 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 kg/cm^2/min. was selected because of its ideal retention time and less time for analysis. The oven temperature was varied from 70-100° and finally it was fixed at 90° due to shorter time of analysis, sharp peak and ideal retention time. The injector and detector temperature were maintained at 110° through out the analysis for sharp peaks and ideal chromatographic behavior.

The calibration curve was plotted and it was found to be linear over the concentration range of 1-5% v/v. The data were analyzed by linear regression least square method. The calibration graph shows negligible intercept and is described by the calibration equation y=a+bx, where y is the peak area, b is the slope, a is the intercept and x is the concentration of the analyte. Linear regression equation y= 0.213x and slope (b) 11320 with correlation of coefficient 'r' values (n=3) was 0.9952.

Chromatographic parameter such as peak asymmetry (tailing factor) and capacity factor (k) were found to be 1.40 and 5.4, respectively. Analytical recovery of ethanol from Ayurvedic formulations was carried out at different

TABLE 1: ANALYSIS OF ETHANOL IN AYURVEDIC FORMULATIONS

Ethanol formulations (% v/v)	Ethanol found* (% v/v)	Relative standard deviation (%)	% Recovery
Syrups			
S-1 [1](NMT 11%)	9.2	0.173	98.14
S-2 [2](No claim)	8.2	0.200	99.14
S-3 [3](NMT11%)	5.6	0.400	104.54
S-4 [4](NMT11%)	10.0	0.800	103.14

*Mean of three determinations, [1,2,3,4] indicate formulations marketed by Baidyanath Bhavan, Dabur India, Sandu and Unza, respectively

concentrations. The supernatant solution was injected and chromatogram was recorded. The results of analysis of *abhayarishta* formulation presented in Table 1 are in good agreement with labeled values.

Analyzing five replicates of fixed amount of ethanol checked precision and accuracy of the proposed method. The precision of the method was calculated in terms of the relative standard deviation. Low values of relative standard deviation (0.800%) indicated high precision and accuracy of the proposed method. In order to study

selectivity of the 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.

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