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Estimation of Ethanol in Marketed Mouthwashes by Gas Chromatography

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The present study was oriented towards gas chromatographic estimation of alcohol, as a quality control process. The determination of ethanol in alcoholic mouthwash is very essential to maintain the quality and efficacy. In this study simple and rapid gas chromatographic method was developed for determination of ethanol in mouthwash using packed column (15 % FFAP, 2 m × 0.125 inch) with detection by flame ionization detector (FID). The retention time of alcohol was found to be less than 6 min. The estimate of the standard deviation of the chromatography for duplicate injections is ± 0.02 %. This gas chromatography method determines ethanol separately from the other components of mouthwash that interfere in detection method and without distillation or chemical reaction. When large numbers of samples are analyzed, it reduces analysis time per sample and with adequate accuracy of estimation. The developed method was validated with various validation parameters *viz.* accuracy, precision, ruggedness, robustness and could be one of the rapid methods for quality control analysis of alcohol containing mouthwashes.

Keywords: Gas Chromatography, Mouth wash, ethanol.

INTRODUCTION

Gas chromatography has become increasingly preferred for accurate quantitative as well as qualitative analyses of many substances especially of volatile nature. The quantitative and qualitative determination of ethanol are perhaps the most important analysis when it is present in formulations like mouthwash. In the context of substitute alcohol consumption, the possibility of ingestion of mouthwashes (*i.e.* cosmetic or medicinal products intended for oral rinsing) was regularly pointed out in the past [1]. The products preferred as substitute for alcohol contain typically between 10 and 30 % volume of alcohol with the most common products being between 20 and 27 % volume, which is higher than the strength of wine [2-10]. The toxicity of mouthwash exposure appears to be rather low and only few cases of intoxications due to intentional ingestion of very large amounts in adults were reported [11,12]. The estimation of alcohol in mouthwash is needed to control the level of alcohol in formulation for state and federal government tax and regulatory purposes. To achieve this alcoholic beverage industry and various regulatory agencies have devoted much effort in recent years to develop a faster, specific, more accurate and automated method [13]. Older ethanol analysis methods, involving distillation and/or mass determinations, are known to include small inaccuracies due to the presence of interfering volatile or other components in samples [14]. Gas-liquid chromatography is one of the modern analytical techniques, dating from 1952. Recently with the utilization of sophistication in equipment, gas chromatography has become increasingly preferred for accurate quantitative & qualitative analyses of alcohol. Alcohol content in pharmaceuticals depends on formulation and varies in the wide range from fraction to tens of percent [15-17]. The highest ethanol concentrations are characteristic for liquid formulations, including solutions, syrups, suspensions and emulsions. On the other hand, these preparations are most convenient for pediatric patients who are very often unable to swallow the solid preparations like capsules or tablets [18]. Scientists have reported that nearly 80 % of pediatric medicines are produced as liquids and ethanol content in these products is in the range from 2.3 to 20 % [19]. In consequence, a simple and accurate methods for the determination of ethanol are needed, but there is limited information in the literature about determining of ethanol contents in dental formulations. Literature reports that for the quantitative analysis of ethanol in liquid herbal drugs [20], Ayurvedic formulations [21] and cough syrup [22] gas chromatography method is used. The most popular method is based on the density measurements of distillates by pycnometer or aerometer [23-25]. This method is also recommended by the European Pharmacopoeia [26].

Mouthwash is used for cleansing the mouth and teeth also known as collutorium and its traditional methods of analysis, involving distillation and/or mass determinations, are known to include small inaccuracies due to the presence of interfering complex volatile or other components. With an appropriately chosen column packing, gas chromatography is inherently specific, separating volatile compounds on the basis of compound specific partitioning properties between a gas phase and a liquid phase. In this investigation, determination of ethanol content in mouthwash involves use of diluted samples without previous distillation.

EXPERIMENTAL

The analyses were performed using a gas chromatography (Perkin Elmer, Clarus-500) with Turbochrom Workstation data handling system (Perkin Elmer/Pe Nelson). The detector employed was FID detectors (Perkin-Elmer, Norwalk, USA) with an insert liner of 0.6 mm I.D. A dedicated gas chromatography packed column with length 2 m, internal diameter 0.125 inch, packed with liquid 15 % Free fatty acid phase (FFAP) on 80/100 mesh Chromosorb WHP (Temperature 225 °C) was used for the separation of alcohol using nitrogen gas as a carrier. The weighing was performed on electronic weighing balance, (Shimadzu, AUX 220, Kyoto, Japan), Injector syringe of 10 µL capacity was procured from SGE Analytical Science Pvt. Ltd., Australia and Millipore water system (Millipore India Pvt. Ltd.) was used for deionized water.

Ethanol of standard analytical grade (95.9 % GC) was procured from Merck India Ltd. Three typical mouthwash samples containing alcohol were purchased from market for GC analysis.

Solutions preparation

Standard solution of ethanol: Five ethanol standards were prepared by diluting HPLC grade ethanol with HPLC grade water to cover the range 2 to 10 % (v/v) ethanol. Pure ethanol and distilled water were chosen to minimize the possibility of non-ethanol components interfering in determination of the ethanol content of the standards.

Test sample preparation: 10 mL of sample was pipette out from each marketed mouthwash preparation and were used for injection in to GC system with sample size 0.2 µL.

Chromatographic method: The analysis was performed using gas chromatographic system with flame ionization detector. The separation was achieved with packed column with liquid 15 % free fatty acid phase on 80/100 mesh and nitrogen gas was employed as a carrier gas.

Following optimized chromatographic conditions were applied: Oven temperature: 200 °C; Run time: 6.0 min; Injector temperature: 200 °C; FID temperature: 180 °C; Carrier gas (N2) flow rate: 10mL/min; Sample size injected: 0.2 µL.

With these chromatographic conditions the peak response was obtained and peak areas were integrated for the calculation of results.

Application of proposed method for detection of alcohol: 0.2 µL portion of sample solution and the standard solution was injected and chromatograms were recorded. Retention time (R_t) was recorded as the analytical parameter (Figs. 1 and 2).

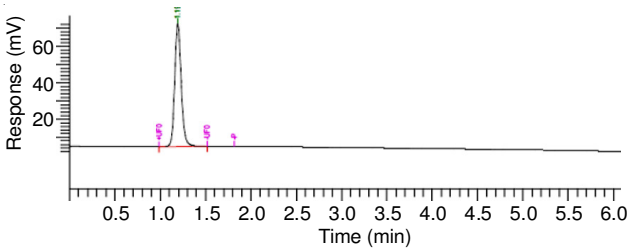

Fig. 1. A typical chromatogram of standard ethanol sample

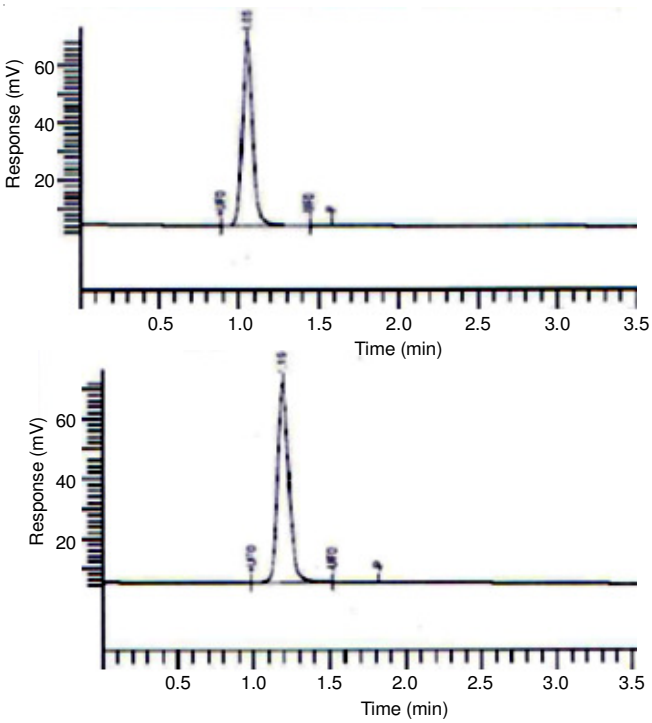

Fig. 2. A typical chromatogram of test samples

Analysis of standard ethanol: A range of standard was prepared as discussed earlier containing five different concentration of ethanol. 0.2 µL portion of each was injected and chromatograms were obtained. The graph of concentration of ethanol *versus* peak area was found to be linear with, slope value 42953, Y intercept 37348 and R^2 = 0.9977 (Fig. 3).

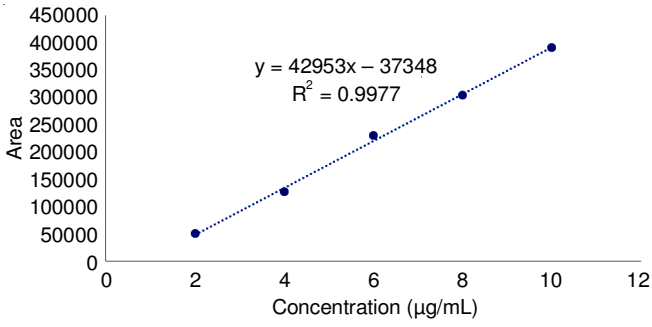

Fig. 3. Linearity of ethanol (% v/v) *vs.* peak area of chromatogram

Analysis of marketed sample by proposed method using gas chromatography: 0.2 µL portion of each sample solution was injected and chromatograms were obtained. The concentration was determined from standard working curve. The results of all the marketed samples (Brand 1 to 3) are given in Table-1.

TABLE-1
ETHANOL CONTENTS OF DIFFERENT MARKETED MOUTHWASH SAMPLES

Sample	Label claim	% Ethanol estimation v/v ± SD	Coefficient of variation*
Brand-1	7.2	7.10 ± 1.85	2.01
Brand-2	7.2	7.22 ± 1.53	2.42
Brand-3	7.2	7.11 ± 1.50	1.96

*n = 3, Each reading is a average of three determinations

Method validation: The methods are validated according to International Conference on Harmonization (ICH) guidelines [27,28] for validation of analytical procedures in order to determine the linearity, precision and recovery, limit of detection, limit of quantification.

Accuracy and precision: Recovery tests were performed for evaluation of applied methods accuracy. Mouthwash (Brand-1) used for recovery tests contained 2, 4, 5 and 7 % of ethanol. Ethanol contents of selected brand was determined according to previously described methods. The analyses were replicated three times for each concentration level. The values obtained for intra- and inter-day precision (% RSD) at different concentration levels was found to be within 2 % (Table-2).

Recovery studies: The accuracy of the method was determined by sample with known amounts of the alcohol as standard to achieve three different levels 50, 100 and 150 % levels. The percentage of estimation of mean recoveries for sample was found to be 100.02 ± 1.7 % (Table-2).

Specificity (Selectivity): The selectivity of the chromatographic method depends on the resolution of the targeted compounds and on the absence of interference. The ethanol-containing liquid formulation can also include the other alcohols like methanol. European Pharmacopoeia recommends test of these alcohols content in liquid drug preparations. The specificity of the gas chromatography method was checked by analysis of a blank sample and a sample containing alcohol standard.

Linearity and range: Linearity of methods was studied by analyses of standard solutions at different concentration levels, with triplicate determination at each level. Standard solutions were prepared and analyzed according to the above described methods. The calibration curves were constructed by plotting detector responses against corresponding concentrations. As the detector response, ethanol peak areas were used and concentrations expressed ethanol content in mouthwash. The calibration curves values of slope 'm' (42953), 'C' intercepts (37348) and correlation coefficients 'R^2' (0.9977) were obtained. The concentrations of calibration standards correspond to the range from 7 to 27 % v/v of ethanol concentration in commercial mouthwash samples.

Limit of detection (LOD) and limit of quantitation (LOQ): The blank samples were prepared and ethanol contents were determined. It was near the expected value of LOD (1 % v/v). Standard deviations (SD) of results were calculated and the limits of detection were determined.

$$LOD = 3.3\ \delta/S$$

The limits of quantitation were calculated by triplicate multiplying of LOD values. The limit of detection and quantitation for this method was 0.24 % v/v. The concentration range for which the method was validated was above the values of LOQ and the calculated limit of quantitation using formula:

$$LOQ = 10\ \sigma/S$$

It was below 1 % v/v *i.e.* 0.7 % v/v, therefore, the developed method is suitable to control the ethanol content in mouthwash sample.

RESULTS AND DISCUSSION

The samples of mouthwash and standard ethanol solution were analyzed in triplicate. The ethanol content was estimated direct from linear equation of proper calibration curve. Results calculated from calibration curves were used to obtain the ethanol content in the selected preparations. All results are summarized in Table-1. It was found that the ethanol concentration in the analysed samples was in the range of 7 to 8 % v/v. Differences between standard deviations of determined ethanol content were significant. The chromatographic conditions were optimized in order to provide a simple, accurate and economical analytical method, which can be employed for routine quality control of ethanol in mouthwash. The injection port and detector temperature were set to 200 and 180 °C respectively and oven temperature program was set at 200 °C. The solvent, column and acquisition parameters were chosen to be a starting point for the method development. However, the separation produced using these parameters were excellent. The retention time of methanol was approximately 1.15 ± 0.2 min (Fig. 1) with good peak shape and tailing factor was approximately 1.0. A five point calibration curve was constructed with working standards of ethanol and was found linear (R^2 = 0.9977) (Fig. 3). The developed GC method with FID detector was accurate, precise, reproducible and sensitive. All the validation parameters of the method were shown to be satisfactory. Accuracy and precision were determined by elaboration of standard calibration curve *i.e.* (intra-day and inter-day). The intra-day and inter-day precision (% RSD) at different concentration levels was found to be within ± 2 %. The ethanol showed 98-102 % recoveries from the sample with standard ethanol at 50, 100 and 150 % levels.

TABLE-2
RESULTS OF RECOVERY STUDY

Sample	Recovery (%)* Level of concentration			Mean recovery ± SD**	Coefficient of variation
	80 %	100 %	120 %		
Brand-1	97.76	100.43	101.87	100.02 ± 1.700	1.702
Brand-2	97.16	99.44	101.59	99.39 ± 1.800	1.820
Brand-3	97.01	101.14	98.09	98.74 ± 1.749	1.771

*Average of three determinates, **Average value for different levels

The calculated LOQ and LOD concentrations confirmed that the method was sufficiently sensitive. The methods was specific as none of the excipients interfered with the analytes of interest. Hence, the method was suitably employed for assay of ethanol in commercial mouthwash formulations.

Conclusion

The developed method of gas chromatography was used in quality control analysis of marketed mouthwashes containing alcohol . The aim of the present study was to show the need of quality control of alcohol as ingredient of mouthwash. The gas chromatography with FID detector system was used to trace down potential quantization and confirm the presence of alcohol in mouthwash as per labelled claim. The GC applying right column set-up SGE polar column (15 % FFAP, 2 m 0.125 inches) enhances separation of alcohol from other ingredients. So the identification of level of alcohol, thanks to sufficient separation, may be also done only based on retention times without mass spectral information. The connection of gas chromatography to other more specific detector such as electron capture detector would provide additional sensitivity. This study takes only 6-7 min to complete a sample analysis for the determination of ethanol content in a mouthwash samples. A sample solution (0.2 µL) is injected into a packed gas chromatography column. The study method we developed with the advantages of simple sample pretreatment procedures, with rapidity and accuracy.

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