Original Article

Journal of Pharmaceutical and Medicinal Chemistry
Volume 5 Number 2, July - December 2019
DOI: http://dx.doi.org/10.21088/jpmc.2395.6615.5219.3

Determination of Alcoholic Content in Various Alcoholic Beverages by Using Gas Chromatography Refering Internal Standard

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How to cite this article:
Chattani MA, Ansari YN, Vikas V Patil. Determination of Alcoholic Content in Various Alcoholic Beverages by Using Gas Chromatography Refering Internal Standard. J Pharmaceut Med Chem. 2019;5(2):81-85.

Abstract

Alcohol is a highly prevalent substance that has multiple forms and trends across cultures. Alcoholic beverages vary considerably in ethyl alcohol content and their primary ingredient. With the determination of alcohol content in various beverages, both in fermented and distilled the potency of beverages can be established. For this, many different methods of measuring alcohol content have arisen. A novel method for alcohol quantitation is direct injection Gas Chromatography (GC-FID) using megapore pack column (carbowax 10%, 2 m x 0.12 mm) referring internal standard Acetone. The method effectively and efficiently determines the alcoholic concentration (% v/v) with little preparations in a wide variety of matrices with prominent outcomes in lesser time.

Keywords: Direct injection Gas Chromatography; Alcoholic Beverages; Pure Ethyl alcohol; Quantitative analysis.

Introduction

For centuries, ethyl alcohol has been ubiquitous amongst cultures. Various properties of alcoholic beverages have contributed to their long lasting existence, which contributes to evoke the psychoactive properties. The quantity of alcohol in a particular beverage is an important feature in production, distribution and consumption of an alcoholic product. A Psychoactive drug, alcohol has a depressant effect on consumers. It acts as a CNS depressant leading to impaired sensory and motor functions, delayed cognitive effects, and decreased blood flow to brain, unconsciousness and possible death. As per United State standards the maximum alcohol contents limits for 18 ml. Thus to avoid over consumption of alcohol many regulations have been enacted to control the availability an accepted level of alcohol consumption. For further decreasing the health hazards with alcohol consumption, the manufacturers should accurately quantify the amount of alcohol in their products.[3]

On the other hand, ethanol content serves as the quality index and taxation factor for alcoholic beverages. After notifying the judiciary aspects it has been claimed that many of the countries in the world follows the taxation criteria for alcoholic beverages according to the systems in United States. Mainly it seems that higher the ethanol content in alcoholic beverage more is the tax. Thus, for this simple and accurate, quantitative analysis method for determining ethanol content is needed as a part of quality estimation for beverage manufacturers and as a guide for government related sanitary agencies.[1,6]

There are various methods for determination of ethanol content in alcoholic beverages: (1) boiling point depression of ethanol solution relative to water, (2) densimetric analysis, (3) refractive index

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Received on 06.07.2019, **Accepted on** 14.12.2019

method, (4) oxidation of distillate, (5) dichromate oxidation spectrophotometry, (6) enzymatic method, (7) biosensor method, (8) potentiometry method, (9) gas chromatography, (10) capillary electrophoresis, (11) High Performance liquid chromatography, (12) modular raman spectrometry (13) near infrared spectroscopy (NIR), (14) beer analyzer, (15) flow injection analysis, etc. Due to complicated pretreatment procedures and large sample volume required, the two methods like pycnometry and densimetric analysis are not applicable for samples with small amount. For oxidation of distillate and dichromate oxidation spectrophotometry, more than 5 ml of sample volume is required. Also the reagents used for this method are highly toxic and distinguishable. For enzymatic method, biosensor and potentiometry it shows low stability, low reproducibility and low accuracy. Raman spectrometry and capillary electrophoresis are quite expensive methods. Also sample distillation and accurate weighing process are still required as pretreatment procedures and only spectrophotometry is used for analysis; HPLC method obtains comparatively low sensitivity. NIR spectroscopy and beer analyzer are time consuming with low accuracy, as they can be infrared by other alcohols in alcoholic beverages.[1]

The conclusion esteems that Gas chromatography method is the most appropriate and rapid method for determination of ethanol content in alcoholic beverages with small amount of sample.

The official GC method adapts different sample preparation methods when dealing with different sorts of samples and these samples requires dilution as a part of pretreatment. The retention time and reproducibility of packed column are relatively moderate. Thus we plan to develop a simple, rapid and accurate method in determining ethanol content in alcoholic beverages and assessing in recovery and standard deviation of inter and intraday analysis in order to evaluate the accuracy.[5]

Materials and Method

Materials

Two alcoholic beverages non-distilled spirits (Local Brand wine and Beer) were collected from supermarkets of local place. LC grade (purity> 99.5%) ethanol, Acetone were obtained from Chemox enterprises and Hexon laboratories. Distilled water was obtained from college water distillation unit.[1]

Instrumentation

For the present study Gas Chromatography instrument (model-FL9790-II) was used with dimensions (Length:2 meter, Diameter: 1/8″), Mobile Phase: N2 Gas with 3 kg/cm^2 Pressure, Stationary Phase: 10% Carbowax, Detector Gases: Hydrogen and Air, Detector: FID with 100C.

Solvents

Pure Ethanol and Acetone (Internal Standard) were selected as solvent for developing spectral characteristics of wine samples. The selection was made after assessing the compatibility of solvents with wine sample and beer.[4]

Preparation of standard solution and Linearity calculations

Ten ml of Ethanol and Acetone were dispensed into 1000 ml volumetric flask and then the volume was covered till 1000 ml with the help of distilled water. This made the 1% (v/v) of ethanol and internal standard (Acetone) standard solution.[1,8]

Relative Response Factor (RRF) of ethanol to Acetone

Pure ethanol was mixed with 1% (v/v) Acetone in various ratios (Ethanol: Acetone= 5:5, 10:5, 15:5, 20:5, 25:5). A linear regression line was generated with the GC peak area under curve (AUC) ratio of ethanol to Acetone (Y-axis) against the concentration ratio of ethanol to Acetone (X-axis). RRF is the slope of the regression line as in the equation- RRF= $(A_S/W_S) \div (A_{IS}/W_{IS})$; in which A_S= ethanol AUC, A_{IS}=Acetone AUC, W_S= weight of ethanol (ml), A_{IS}= weight of Acetone.[1,3,7]

Recovery

5 and 10 ml of 1% (v/v) of ethanol was added into 0.5 ml of wine and beer sample individually; then 5 ml of 1% (v/v) internal standard solution was added. After gentle mixing 0.1 µl of sample solution was injected to a GC with the syringe for determination of ethanol content. Each analysis was carried out for thrice. Simultaneously the blank sample was analyzed.[1]

Application of proposed method for determining the percentile amount of alcoholic content in preferred wine sample

- Direct GC-FID method for determining alcoholic content:

Two wine samples named Beer and Local Brand wines were collected. On the basis of linearity responses standard solutions of wine sample and beer were prepared using internal standard and pure Ethanol. Acetone was selected as an internal standard as it was observed to be having its compatibility with ethanol whereas various other volatile solvents like benzene, toluene was found to be having precipitating when mixed with ethanol. Thus constant of 0.5 ml of sample was mixed with internal standard and pure ethanol and was preceded for responses using GC. The curves were monitored and area under curve for each respective curve was noted for finding graphical outputs. Furthermore, the calculations were done for final percentile alcoholic content.[2,3]

Following formula was used for calculations:

$$\%V/V = \frac{\text{(Peak AUC ratio EtOH: Acetone) X 100}}{\text{(EtOH: Acetone dilution) X RRF}} \quad \text{equation 1)}$$

RRF= Relative response factor

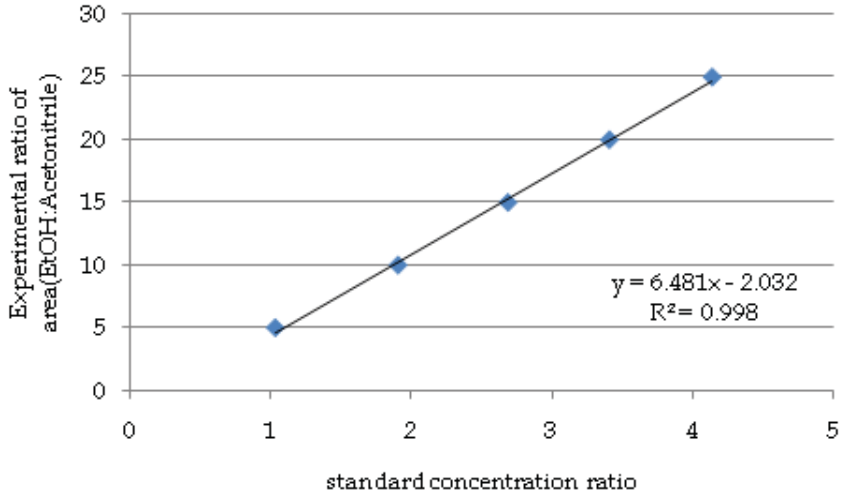

Fig. 1: RRF determination using Ethanol and Acetone

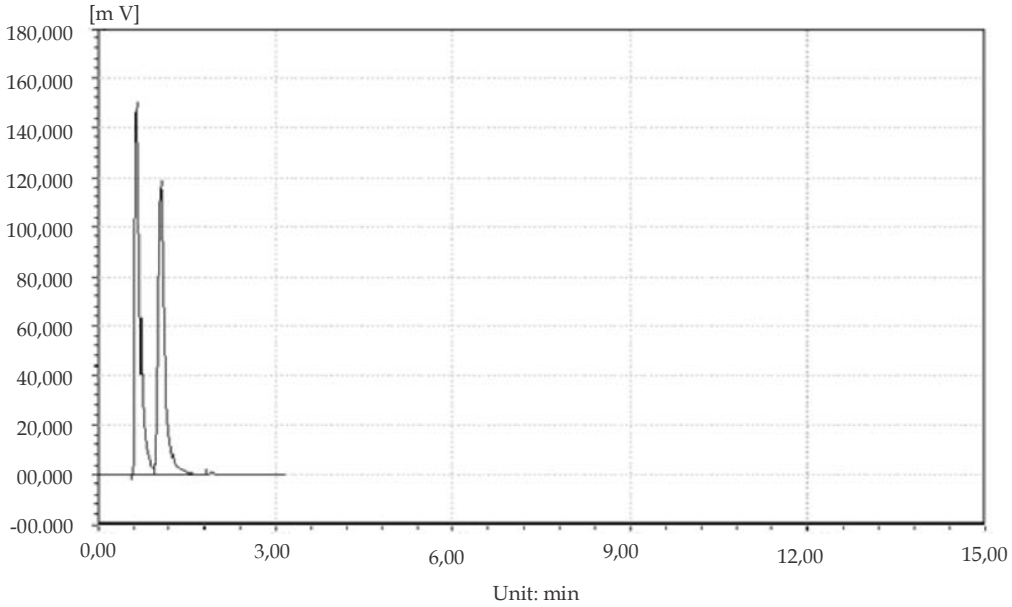

Fig. 2: Gas Chromatogram of 5% ethanol & 5% Acetone as an authentic compound.

Peak 1 = Ethanol; Peak 2 = Acetone

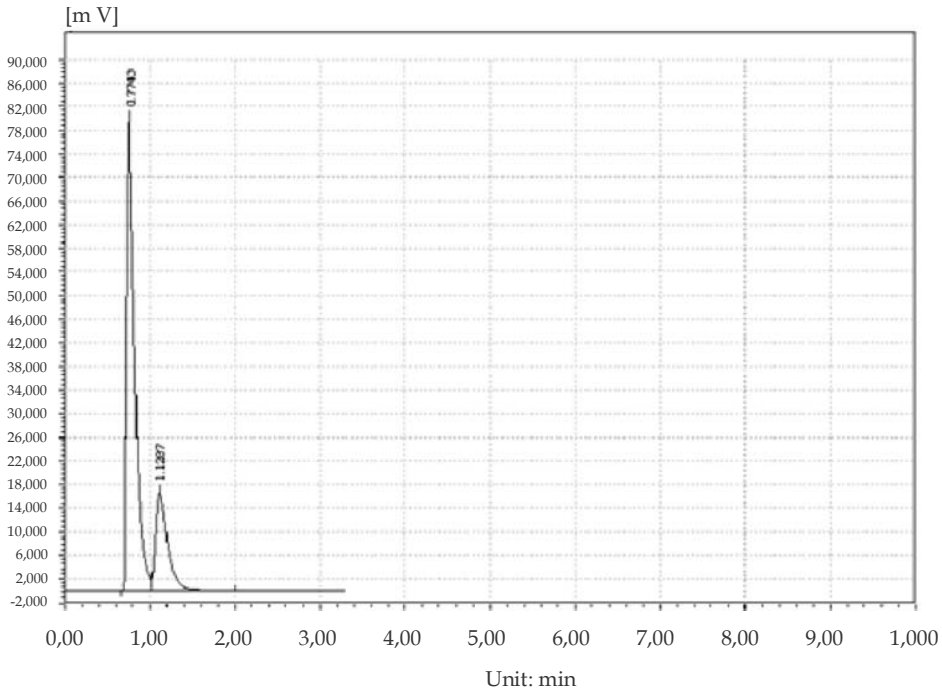

Fig. 3: Gas Chromatogram of Beer Sample

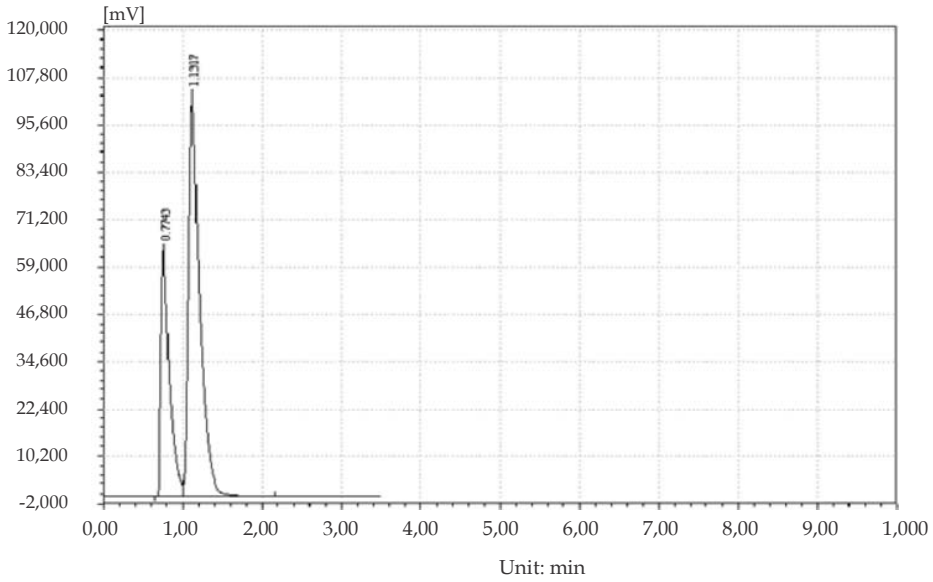

Fig. 4: Gas Chromatogram of Local Brand wine

With the distinct linear trend, the RRF was used for analysis of both the beverage samples. 1:1 (EtOH: Acetone) was reasoned most appropriate and was used for both beer and local brand wine. The furthermore extended dilutions (2:1; 3:1, 4:1, 5:1) of EtOH: Acetone was preceded for more linear curvatures and determination of respective error ranges.[1]

Equation 1 was used to determine experimental alcohol percentage and % Response is the deviation of this data from the manufacturers.

Table 1: Pack column GC results for beverage samples diluted in 1:5 in 1% acetone standard solution

Beverage	Alcohol % (v/v) STD Dev.	% RSD	Expected Alcohol %*	% Response
Beer	0.02015	1.359987	8	9.8314
Local Brand Wine	0.027932	0.272238	42.8%	55.83

* Values given by manufacturer

Results and Discussion

Pack column Gas Chromatography was considered to be an effective and efficient method for determination of alcohol content in alcoholic beverages because there was no need for sample pretreatment procedures required, as the sample was injected directly into GC for analysis. Acetone was selected as an internal standard as it was compatible with ethanol and it showed no overlapping with ethanol when GC peaks were observed. The GC peaks of ethanol and acetone were closer than the other trialed solvents choices for internal standard. Due to the possibility of its existence in some sort of alcoholic beverages, 1-propanol, *t*-butanol, toluene etc are not appropriate as an internal standard for determination of alcoholic content in alcoholic beverages.

For selection of GC conditions, initially a temp of about 60^0 for 2 min was selected which was then increased to about 90^0 and then to 120^0 for 5 min. Ethanol and Acetone were eluted at 90^0 and 100^0 respectively. Through this process, sample components were eluted rapidly almost within 7-8 min to complete the sample analysis.

In order to obtain an accurate quantification, the RRF value of ethanol against Acetone needed to be specified, and then the ethanol components were calculated according to the equation 1. When the AUC ratio of ethanol to Acetone (Y-axis) was plotted against the concentration ratio of ethanol to acetone (X-axis), a linear regression equation was generated ($R^2 \geq 0.998$). The slope of linear regression line is the RRF of ethanol to internal standard. The linearity of the adjusted regression line is in the range of 0-500 mg/ml.

Ethanol content in commercial alcoholic beverages vary a lot, even for some sort of beverages e.g.- 3-6% for beers, 7-21% for fermented wines, 20-50% for distilled spirits. Thus it needs different pretreatment procedures for using other official methods. In this study, a rapid Pack column GC method was used which requires a smaller sample amount and it can be adapted in different sort of alcoholic beverages with ethanol contents between 0-500 mg/ml. The alcoholic content in experimental beverages was found to be in an increased proportion than that of the labeled ones.

Conclusion

In this study, it takes only 8~9 min to complete a sample analysis for the determination of ethanol content in a beverage sample. A sample solution (0.5 ml) is mixed with adequate amount (0.5 ml) of 1% (v/v) internal standard solution (acetone), and injected into a pack column GC. The study method we developed can be applied to alcoholic beverages with different alcoholic contents, and with the advantages of simple sample pretreatment procedures, rapidity and accuracy, and may be used as a routine analysis method in substitution of current official methods used for determining the alcoholic content in beverages.

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