EVALUATION OF HYDROXY ETHANE AND HYDROXY METHANE CONTENT IN NON-ALCOHOLIC BEVERAGES BY GC-FID

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ABSTRACT

Based on the use of gas chromatography with flame ionisation detector (GC-FID), a simple, sensitive, accurate, direct, and cost-effective approach for detecting if non-alcoholic beverages could result in positive "alcohol results" has been created. The results of validation parameters such as linearity, specificity, accuracy, LOD, and LOQ were satisfactory and within the limitations. Unlike other traditional methods that require sample preparation, this method allows us to analyse samples directly without the need for sample preparation techniques such as SLE or LLE, and it also decreases cost and analysis time. Low amounts of ethanol and methanol were also discovered and measured. A total of 30 alcohol-free samples were acquired from local markets in India and analysed for alcohol level. Ethanol concentrations ranged from 0.002 g/L to 0.36 g/L. Methanol concentrations ranged from 0.03 g/L to 0.13 g/L. The concentrations of ethanol and methanol in all of the samples are within the acceptable ranges.

Key Words: Non-Alcoholic beverages, gas chromatography, flame ionisation detector.

1. INTRODUCTION

Drinking too much alcohol can lead to a variety of health issues. These issues are divided into two categories: "short-term health hazards" and "long-term health risks." Automobile accidents, falls, drownings, and burns are all short-term health concerns. Homicide, suicide, and sexual assault are all examples of violence. Risky sexual behaviours, such as having several sexual relationships, can result in the transfer of diseases like HIV and AIDS, as well as "foetal alcohol spectrum disorders (FASDs)." Long-term health hazards include hypertension, heart attack, liver disease, and digestive issues. Alcohol also causes cancers of the breast, mouth, throat, oesophagus, voice box, liver, colon, and rectum when consumed. Long-term alcohol consumption weakens the immune system, increasing the risk of becoming ill as well as mental health issues such as depression and anxiety [1]. As a result, the popularity of "alcohol-free" beverages has grown in many nations where drinking alcoholic beverages is prohibited. As an alternative to alcoholic beverages, the popularity of "alcohol-free" beverages, energy drinks, and fruit juices has grown on global markets. Consumers of

non-alcoholic beverages believe that this product is alcohol-free because it is marked as such on the package [2]. Most non-alcoholic drinks have 0.5 percent ABV since it is more profitable than distilling it to 0.05 percent ABV, which is typically found in products offered by non-alcoholic beverage firms [3]. According to THE ALCOHOL ACT, chapter 1, section 3 (4.1.2001/1) paragraph 3, a non-alcoholic beverage is one that contains no more than 2.8 percent ethyl alcohol by volume [4]. Alcohol-free beers have a low alcohol level, according to literature assessments on manufacturing techniques. Alcohol-free beer is made using either fermentation-free brewing malt and a dilution technique, followed by dealcoholisation to remove the alcohol [5]. Energy drinks, on the other hand, are beverages that, in addition to calories, contain caffeine and other energy-boosting ingredients like taurine, herbal extracts, and B vitamins [6, 7]. There is an old approach called semi-quantitative ebulliometry that is based on identifying boiling beverages for a long time and determining density [8]. In some methods they require sample preparation techniques like solid phase extraction SLE, LLE [9, 10]. These procedures are time-consuming to implement. Although the specific gravity method is more accurate, it is a time-consuming approach that necessitates sample extraction for laboratory analysis [11, 12]. There are numerous methods for determining ethanol and methanol in non-alcoholic beverages, energy drinks, and fruit juices that have been authorised [13, 14, 15]. Some methods necessitate a significant amount of time for sample preparation. Extraction processes, which are time-consuming operations, may be used in the sample preparation approaches [16]. Helium was utilised as a carrier gas in some of the procedures. When compared to alternative carrier gases, helium gas is more expensive, and certain procedures require a gradient temperature programme and longer run times [17,18]. We devised a simple, affordable, efficient, and less time-consuming approach for evaluation of hydroxyl ethane and hydroxyl methane content in non-alcoholic beverages using GC-FID to tackle the aforementioned disadvantages. The approach that was created was tested and found to be effective.

2. MATERIALS AND METHODS

2.1 Chemicals and samples

SHIMADZU provided certified standard solutions of HPLC grade ethanol (99.8%) and Methanol (99.8%) and water for gas chromatography flame ionisation detector (GC-FID). Local supermarkets, grocery stores, and bakeries provided the samples for analysis. The collected samples were kept in a refrigerator at a temperature of 4.0 to 5.0 °C.

2.2 General Procedure

2.2.1 Ethanol standard preparation

Using a clean and dry pipette, pipette 1ml of HPLC grade ethanol into a clean, dry 100ml volumetric flask and make up with HPLC grade water to the mark.

2.2.2 Methanol standard solution

Using a clean and dry pipette, pipette 1ml of HPLC grade methanol into a clean, dry 100ml volumetric flask and make up with HPLC grade water to the mark.

2.2.3Sample preparation

With the use of a SHIMADZU syringe, all of the obtained samples were manually injected into the GC-FID apparatus.

2.3 Detection Method (GC-FID Analysis)

Using a SHIMADZU gas chromatography model number GC-2010 PLUS with Lab solutions software and a Flame Ionisation Detector and manual sample injector, ethanol and methanol concentrations were calculated. The separation of the standards and samples was done using a DB-WAX (fused silica) capillary column with dimensions of 30m x 0.53mm ID x 1 µm film thickness. The temperature in the column oven is set to 45°C. The injection mode is split. Manual injection of 17 samples into a sample injection port with a split ratio of 1:20 was performed. The injection port temperature was set to 200°C. The carrier gas is hydrogen, with a flow rate of 40 ml/min at 83 Kpa pressure and a linear velocity of 20.36 ml/min. The air flow rate was set at 400 millilitres per minute. The flame ionisation detector (FID) is a device that detects the presence of flames at a temperature of 250°C. All the samples were analysed for ethanol and methanol content and the results were satisfactory and all the results are within the limits.

2.4 Method validation

The developed method was validated. The validation parameters include specificity, linearity, and precision, limit of detection (LOD) and limit of quantification (LOQ).

3. RESULTS AND DISCUSSION

3.1 Specificity

Specificity was carried out to determine whether is there any interference of blank or impurities on the retention time of ethanol and methanol. Specificity was carried out by injecting blank in to gas chromatographic system. There is no interference of blank on the retention time of ethanol and methanol.

3.2 Linearity

The goal of determining the linearity of an analytical method is to find test findings that are proportionate to the analyte concentration. The linearity ranges for ethanol and methanol are 79-474 g/ml and 79-474 g/ml, respectively. Concentration was plotted on the x-axis and peak area on the y-axis to create a linearity curve. For ethanol and methanol, the correlation coefficient value (R2) was found to be 0.9927 and 0.9964, respectively. For ethanol and methanol, the regression line was found to be Y= 3065.4X-35408, Y=2289.4X-17946, respectively. Figures 4 and 5 show the linearity curves for ethanol and methanol, respectively. Tables 2 and 3 show the linearity data for ethanol and methanol.

3.3 Precision

When a technique is repeated on many samples, the degree of agreement between individual test findings is called precision. Precision is determined by analysing a set of samples taken from several homogeneous slot samplings. The standard deviation (SD), mean values, and precision as percent relative standard deviation (%RSD) is determined from the measured data. The percent RSD number should be less than 2. There are two types of precision. Intraday precision and interday precision.

I. Intraday precision

It expresses precision over a short period of time on the same day and under the same operational conditions. Intraday precision %RSD findings for ethanol and methanol are 0.10 and 0.17 respectively.

II. Interday precision

It expresses the precision under laboratory changes such as different days, analysts, and equipment, among others. Interday precision %RSD results for ethanol and methanol are 0.12 and 0.17 respectively.

3.4 Limit of detection (LOD)

Under the provided experimental conditions, the smallest amount of analyte in a sample that can be detected but not necessarily measured.

LOD= 3.3 σ/ slope

LOD results for ethanol and methanol is 0.83 and 1.41 respectively.

3.5 Limit of quantification (LOQ)

Lowest amount of analyte in a sample which can be quantitatively determined with suitable precision.

LOQ= 10 σ/ slope

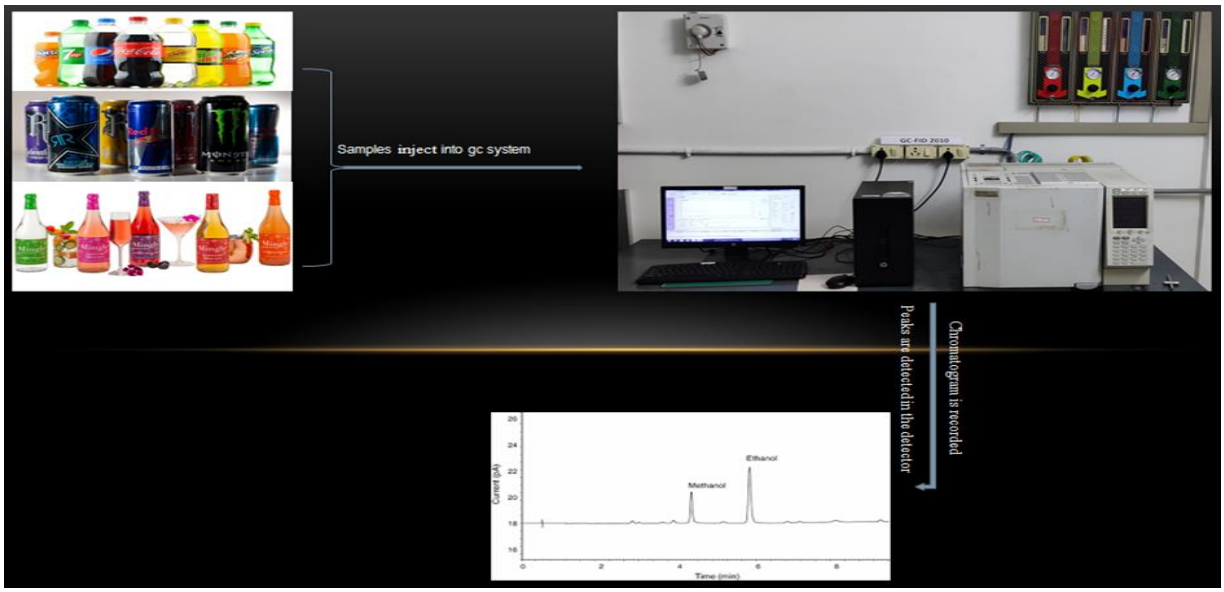
LOQ results for ethanol and methanol is 2.53 and 4.2 respectively.

4. CONCLUSION

The developed GC-FID method for determining ethanol and methanol in non-alcoholic beverages is simple, cost-effective, and time-efficient. The method has been validated, and all of the parameters such as specificity, linearity, precision, LOD, and LOQ have yielded satisfactory and within-limits results. For ethanol and methanol, the correlation coefficients are 0.9927 and 0.9964, respectively, and the %RSD is less than 2. Sample preparation techniques such as SLE, LLE, and centrifugation are used in other methodologies. Helium is employed as a carrier gas in various ways; however it is more expensive than other carrier gases. My method involves injecting the sample straight without any sample preparation, using hydrogen as the carrier gas, and running for 5 minutes. So, in comparison to other approaches, my method is easy, cost-effective, efficient, exact, and less time-consuming. As a result, the suggested approach is utilized to determine the concentrations of ethanol and methanol in non-alcoholic beverages.

5. ART WORK

5.1 Graphical Abstract



5.2 Figures

Figure1. Blank chromatogram **Figure2. Ethanol chromatogram**

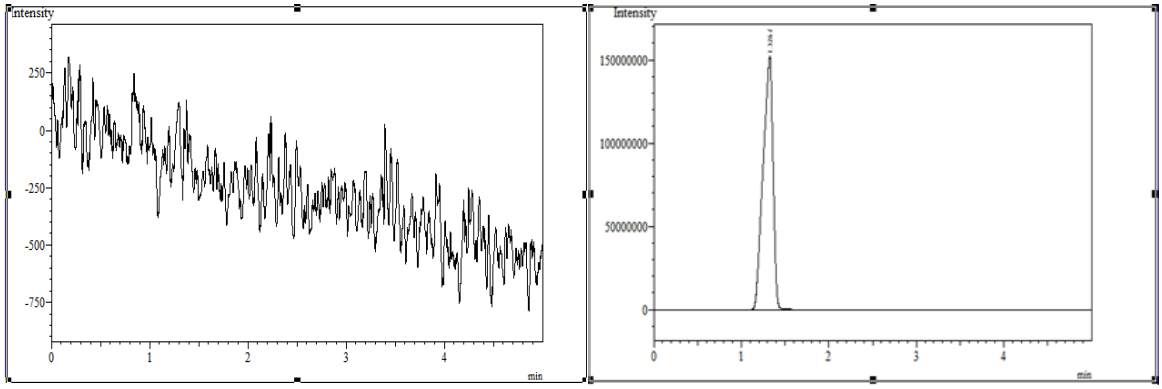


Figure3. Methanol chromatogram

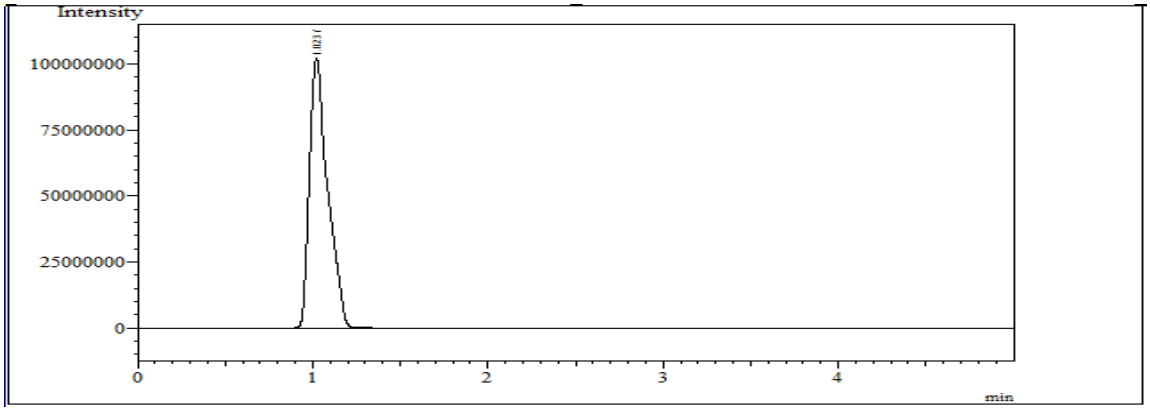
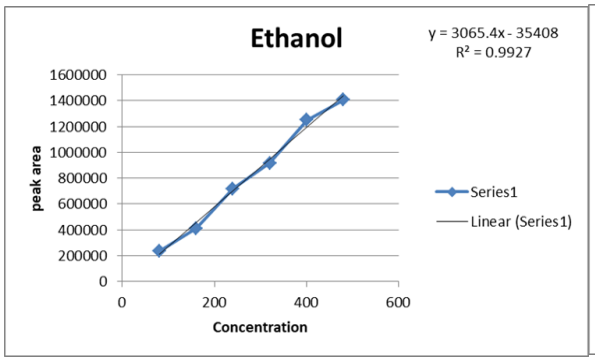
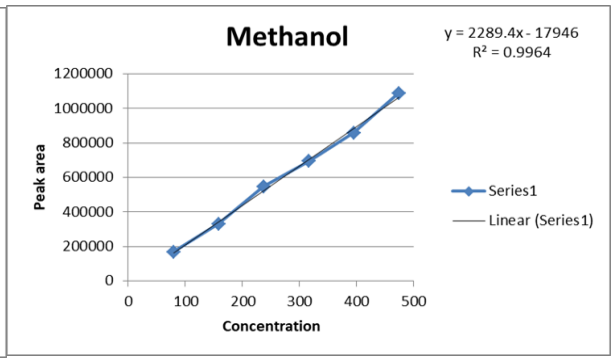


Figure4. Linearity curve for ethanol **Figure5. Linearity curve for methanol**





5.3 Tables

Table1. Information about presence of absence of ethanol and methanol in the samples

S.NO	Name of the sample	Ethanol	Methanol
01	Brand 1	x	x
02	Brand 2	x	x
03	Brand 3	x	✓
04	Brand 4	✓	x
05	Brand 5	x	x
06	Brand 6	x	x
07	Brand 7	✓	x
08	Brand 8	x	✓
09	Brand 9	x	x
10	Brand 10	✓	x
11	Brand 11	x	x
12	Brand 12	✓	x
13	Brand 13	x	x
14	Brand 14	✓	x
15	Brand 15	x	x
16	Brand 16	x	✓
17	Brand 17	✓	x

Note: x mark represents the absence of ethanol and methanol; ✓ mark represents the presence of ethanol and methanol.

Table2. Linearity data for ethanol

Concentration µg/ml	Peak area	Statistical data	
80	233702	Slope	3065.4
160	412205	y-intercept	35408
240	716784		
320	917404	Correlation coefficient	0.9927
400	1249595		
480	1407785		

Table3. Linearity data for methanol

Concentration µg/ml	Peak area	Statistical data	
79	168982		
158	332076	Slope	2289.4
237	546569		
316	694886	y-intercept	17946
395	858182		
474	1089667	Correlation coefficient	0.9964

Table4: Ethanol and Methanol Intraday Precision

	Injection	Ethanol peak area	Methanol peak area
	1	715468	549236
	2	713958	546458
	3	710358	543165
	4	714569	548219
	5	713466	548625
	6	713724	547121
Statistical parameters	**Mean**	713595	547137
	Standard Deviation	777.69	980.38
	%RSD	0.10	0.17

Table5: Ethanol and Methanol Interaday Precision

	Injection	Ethanol peak area	Methanol peak area
	1	715328	536283
	2	717462	538123
	3	709786	538469
	4	716843	536218
	5	713592	534219
	6	715921	537125
Statistical parameters	**Mean**	714822	536739
	Standard Deviation	1255.26	689.55
	%RSD	0.17	0.12

Table6. LOD and LOQ data for ethanol and methanol

LOD		LOQ	
Ethanol	**Methanol**	**Ethanol**	**Methanol**
LOD= 3.3 σ/ slope	LOD= 3.3 σ/ slope	LOQ= 10 σ/ slope	LOQ= 10 σ/ slope
=3.3×777.69/ 3065.4	=3.3×980.38/ 2289.4	=10×777.69/ 3065.4	=10×980.38/ 2289.4
=0.83	=1.41	=2.53	=4.2

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