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# Validation study on spectrophotometric measurement of ethanol from beer and non-alcoholic beer samples distilled by micro steam distillation method

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## Abstract

In this study, a new spectrophotometric method based on the oxidation of sodium dichromate was proposed for the determination of ethanol percentage (v/v) in beer and non-alcoholic beer after distillation with the micro water vapor method, and the method was validated with various parameters. The Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis were used to validate the analytical method we provided. The following aspects of the method were assessed: precision, recovery, linearity, measuring range, limit of detection (LOD), limit of quantification (LOQ), method detection limit (MDL), and measurement uncertainty. The LOD, LOQ, and MDL values obtained for ethanol were 0.04%, 0.05%, and 0.15%, respectively. The relative standard deviation (RSD) values were less than 2.36% and 4.12% for both repeatability and within-laboratory reproducibility, respectively. Recovery percentages of analytes added to the sample at certain levels were determined to be quantitative between 97% and 102%. These findings fulfill the minimal performance standards outlined in AOAC Official Methods of Analysis Appendix F: Guidelines for Standard Method Performance Requirements. In conclusion, the method validated with various parameters in this study has proven to be effectively usable for the routine analysis of ethanol in beer and non-alcoholic beer. This developed analysis method stands out as an innovative approach in terms of collecting ethanol from beers with the help of micro-distillation water vapor and measuring the color resulting from oxidation.

**Keywords:** Beer, ethanol, method validation, micro water vapor distillation, non-alcoholic beer

## 1. Introduction

One of the first known alcoholic beverages, beer, is made from cereals fermented by yeast [1]. Low- or non-alcoholic beer is produced by reducing the alcohol content or eliminating the ethanol in alcoholic beer using a variety of techniques involving physical and biological procedures. Thermal and membrane techniques are examples of physical procedures. The varying volatilities of ethanol and water serve as the basis for the thermal processes of distillation, falling film evaporator, and spinning cone columns. The four types of membrane-based processes include pervaporation, osmotic distillation, dialysis, and reverse osmosis. The idea behind the biological method is to use particular yeasts or limit the amount of ethanol produced by partial fermentation [2].

Due to regulatory restrictions on drivers' alcohol consumption, sports, diet (calorie intake), and health concerns, there has been a surge in interest in non-alcoholic and low-alcoholic beer in recent years [3]. As a result, the brewing industry's market for producing non-alcoholic beer is growing [4]. Beer is a widely consumed alcoholic beverage that has different legal definitions in different nations. The legal restrictions on the alcohol by volume (ABV) of non-alcoholic and low-alcoholic beer vary across nations. Beers with low alcohol content are divided into two categories in the majority of EU countries: non-alcoholic beer with an ABV of less than or equal to 0.5% and low-alcoholic beer with an ABV of no more than 1.2% [5]. According to Turkish law, the ABV of low-alcoholic beer and non-alcoholic beer is less than 0.5%, and 0.5–3.0%, respectively.

Numerous beers with varying alcohol percentages are available on the market. In the brewing sector, it's critical to precisely and properly determine the alcohol concentration for several reasons, including compliance with laws, label verification, quality control, and production consistency [6]. Various techniques, including physical and biological procedures, have been

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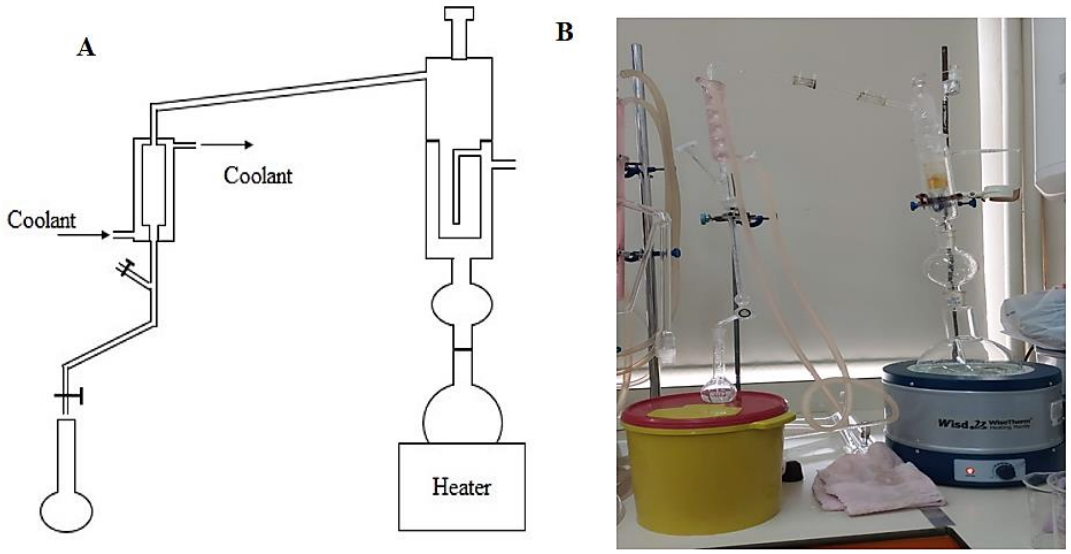


**Figure 1.** Micro water vapor distillation apparatus (A) diagram and (B) picture

proposed in the literature to reduce or eliminate the alcohol content in alcoholic beer to produce low-alcohol or non-alcoholic beer. [7–10].

There are different methods for determining the alcohol content of beer. Beer's ethanol concentration can be found by enzymatic analysis [11], distillation, refractometry [12], gas chromatography [13], catalytic combustion and near-infrared spectroscopy [14]. Volumetric and gravimetric measurements of the distillate's specific gravity serve as the foundation for the distillation process. The distillate is measured both gravimetrically and volumetrically using the labor-intensive conventional distillation method. The spectrophotometric approach uses color to quantify absorbance in the materials. Measuring mistakes can therefore be decreased, in contrast to volumetric and gravimetric approaches.

As a result, the innovative approach of the research is that it can determine the ethanol concentration in a very small sample fraction with an effective model design. Therefore, the study aims to determine the ethanol content in traditional fermented alcoholic beverages spectrophotometrically based on sodium dichromate oxidation after extraction from beer by micro water vapor distillation and to validate the method.

## 2. Materials and methods

The alcohol in beer was rapidly distilled with the micro water vapor distillation method in this study. Instead of volumetric and gravimetric measurements of distillate, it aimed to determine the alcohol content of beer samples with the standard curve by measuring absorbance with a spectrophotometer. The method was based on a reaction between ethanol and sodium dichromate forming green-colored chromate ions in the presence of sulfuric acid and acetate buffer.

### 2.1. Sample

Yeast (*Saccharomyces cerevisiae*) and malt extract (Pilsner, Muntons Plc, Cedar Maltings Stowmarket, Suffolk, UK) were used in this study. After adjusting the malt extract/water ratio recommended by the manufacturer, the yeast was fermented at 20°C A custom-made, airtight fermentation tank (SAIER Verpackungstechnik GmbH & Co. KG, Alpirsbach, Germany) equipped with temperature monitoring capabilities was used for this purpose. Once fermentation was complete, the samples were bottled and kept at +4 °C until needed. Beer and non-alcoholic beer samples were collected from markets in Trabzon and Gümüşhane. All samples were stored at +4 °C until analysis

### 2.2. Chemicals and devices

HPLC-grade solvents and analytical chemicals were supplied by Merck company (Darmstadt, Germany). The standard ethanol (99.99%) was provided by Sigma-Aldrich (St. Louis, Missouri, United States). A UV-Vis 1800 spectrophotometer device was used (Shimadzu, Kyoto, Japan) for the quantitative analysis.

### 2.3. Determination of ethanol

A spectrophotometric approach based on the oxidation of sodium dichromate was used to analyze ethanol. After adding a 5 mL aliquot of beer to the sample cell, micro water vapor distillation was carried out until 25 mL of distillate was collected (Fig. 1).

Following a 2-hour incubation period at room temperature, 500 µL of sodium dichromate (40 mg/L), 500 µL of sodium acetate buffer (pH 4.60) solution, and 2.5 mL of 1 N $H_2SO_4$ were added to 500 µL of the distillate sample. The samples' absorbance was then

measured in the UV-Vis spectrophotometer at 578 nm. The standard curve was created using ethyl alcohol standards at various concentrations (0.10, 0.25, 0.50, 1.00, and 2.00% (v/v)) [15].

### 2.4. Method validation

Validating methods for ethanol detection in beer samples is crucial for accurate and reliable results.

A method's selectivity defines its capacity for detection. Sensitivity means the smallest analyte concentration that the method could reliably measure and also indicates the method's ability to detect low levels of ethanol in the samples. Linearity evaluates the relationship between the concentration of ethanol in the sample and the response of the detection method. It confirms that the method produces results directly proportional to the current ethanol concentration. Linearity evaluates the relationship between the concentration of ethanol in the sample and the response of the detection method. It confirms that the method produces results that are directly proportional to the current ethanol concentration. Ethanol was used in the study at concentrations of 0.10%, 0.25%, 0.50%, 1.00%, and 2.00%.

The limit of detection, or LOD for short, is the lowest analyte concentration that any technique can detect, and also LOD aids in figuring out the method's realistic detection limit. LOD can be calculated by taking 3 times the standard deviation (SD) [16]. Experiments were conducted using a sample that contained 0.15% ethanol to validate the methodology suggested in this study. Ten times the SD can be used to derive the limit of quantification, or LOQ for short, which is the lowest concentration that can be quantitatively measured at a certain confidence level [16]. A sample containing 0.1% ethanol was prepared and examined to determine the LOQ value. The SD value of the results was multiplied 10 times and the LOQ was calculated. Additionally, the SD value was multiplied by the *t*-value to calculate the method detection limit (MDL) at a certain confidence level.

Precision measures the variability of data acquired under various situations (e.g., different analysts, instruments, or days) to assess the repeatability and intermediate precision of the procedure. The term SDR, or Standard Deviation of Reproducibility, describes the standard deviation that is linked to a measurement method's reproducibility. It also measures the variation in results that arises when various operators, tools, or labs carry out the same measurement in identical circumstances.

Relative standard deviation of reproducibility (RSDR%), a concept related to reproducibility, is calculated by dividing the SDR (standard deviation of repeatability) measurements by the mean value and multiplying the result by 100. %RSDR is useful for SDr, a standard deviation related to the repeatability of a measurement method. SDr quantifies the variability in results that occurs when the same operator, using the same instrument, performs repeated measurements under the same conditions. Relative standard deviation of repeatability (RSDr%), like RSDR%, is the relative standard deviation associated with repeatability. It's calculated by dividing the SDr by the mean value of the measurements and multiplying by 100 to express it as a percentage [16].

The method was applied to ethanol solutions at different concentrations by different analysts on different days, both intraday and interday, and %RSDR intraday and %RSDr interday values were calculated from the results obtained. By calculating the proportion of ethanol that can be recovered from spiked samples in comparison to the expected concentration, recovery assesses the method's accuracy [17,18]. The Horwitz equation estimates the relative standard deviation (RSD) expected when replicating measurements within a laboratory or among different laboratories. This is particularly useful for assessing the precision of analytical methods [18]. Predicted relative standard deviation (PRSDR and PRSDr) is a statistical measure used to estimate the precision or variability of a future set of measurements based on existing data. It is calculated using regression analysis or other statistical methods to predict the variability of future measurements under similar conditions [18].

RSDR% and RSDr% values can be calculated from the equations given in Formula 1 and Formula 2 below.

$$PRSDr\% = 2^{1-0.5 \times log\, C} \times 0.66 \quad (1)$$

$$PRSDR\% = 2^{1-0.5 \times log\, C} \quad (2)$$

Where C is the concentration of the measured analyte.

Both formulas should provide an estimate of the repeatability, and reproducibility relative standard deviation as a percentage relative to the concentration of the analyte being measured. For this, three distinct concentrations of recovery, repeatability, and reproducibility tests were carried out by two analysts. Measurement uncertainty accounts for all possible influences on the measurement process, including instrument limits and interference from other substances in samples.

The expanded measurement uncertainty (U) was determined by multiplying the combined uncertainty, which encompasses RSDr%, RSDR%, and mean recovery%, by a coverage factor (k) of 2 [19].

**Table 1.** Analytical parameters applied in the linearity studies

| | Calibration concentration, % | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| | 0.10 | 0.25 | 0.50 | 1.00 | 2.00 |
| **Calibration range, %** | **0.10 – 2.00** | | | | |
| **a** | **0.0629** | | | | |
| **b** | **0.0012** | | | | |
| **$R^2$** | **0.9997** | | | | |
| **$y = ax + b$** | **$y = 0.0629x – 0.0012$** | | | | |
| **Measurement Range, %** | **0.10 – 10.00** | | | | |

### 2.5. Statistical analysis

XLSTAT software (Addinsoft (2024), XLSTAT statistical and data analysis solution, New York, USA, https://www.xlstat.com), in conjunction with the Microsoft Excel application. Using the results of the Cochran and Grubbs tests, outliers were examined and eliminated. The least-squares method was used to complete the linear regression model.

## 3. Results and discussion

A single laboratory validation was completed by the Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis. Selectivity, linearity, LOD, LOQ, recovery, precision, and measurement uncertainty metrics were employed as the method's performance characteristics [20].

### 3.1. Selectivity

Selectivity is a crucial aspect of any analytical method, including spectrophotometry. Selectivity refers to the method's ability to distinguish between the analyte of interest and other substances present in the sample matrix. In other words, it determines how specifically the method can detect the target analyte amidst potentially interfering compounds. Ethanol undergoes oxidation by sodium dichromate ($Na_2Cr_2O_7$) in an acidic medium to form acetic acid and chromium ions. The excess dichromate ions present after the reaction were determined by their absorption at 578 wavelengths using the UV-Vis spectrophotometer. Before initiating the validation phase, the selectivity of the method was evaluated against compounds that occur naturally. The analysis involved comparing the absorbance of representative blank samples ($n = 10$) with that of spiked samples ($n = 10$). The results indicated that at 578 nm, the blank samples exhibited no interference [20].

### 3.2. Linearity and measurement range

Plotting the peak areas of the standard solutions, which were the three series of five distinct concentrations, produced a calibration curve. The formula for the calibration curve is $y = ax + b$, where $x$ is the standard solution's concentration in percentage and $y$ is the standard solution's peak area measured in absorbance. In the studied range, good linearity was found, with an $R^2$ value greater than 0.999 (Table 1 and Fig. 2) [21,22].

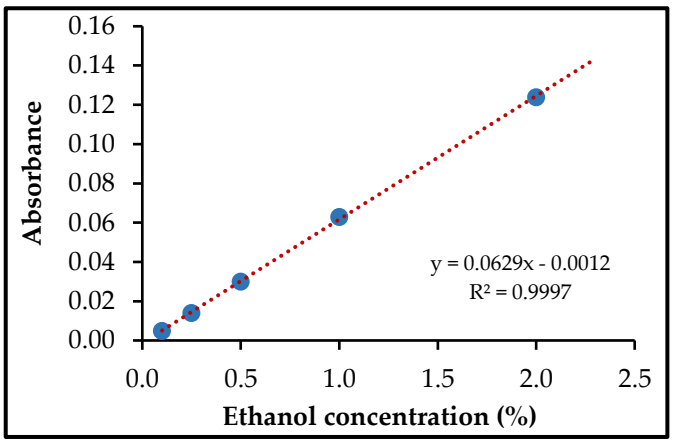


**Figure 2.** Calibration curve for ethanol analysis

Utilizing mass or volume concentration and other analytical parameters is fundamental in establishing a reliable spectrophotometric analysis method. The mass or volume concentration provides crucial information about the amount of the substance present in the solution, allowing for accurate quantification.

Table 1 displays the standard calibration plot for the suggested procedure. The method yielded a standard calibration plot demonstrating robust linearity within the 0.10–2.00% ethanol range. The standard calibration plot for the recommended method is depicted in Fig. 2.

According to Eq. (1), the colorimetric approach is based on the reaction of ethanol and potassium dichromate in acidic solutions, which produces $Cr^{3+}$ and acetic acid [23].

$$16H^+ + 2Cr_2O_7^{2-} + 3CH_3CH_2OH \rightleftharpoons 4Cr^{3+} + 3CH_3COOH + 11H_2O \quad (3)$$

The compound's absorbance was measured to make this approximation. Higher ethanol percentages result in a greater blue color ($Cr^{3+}$); for example, if a molecule appears blue in solution, it presumably absorbs red light, according to Eq. (1) (Fig. 3).

It was discovered that the measurement range operating chart's regression coefficient was higher than 0.995. The F test was used to examine regression at a 95% confidence level. In beer samples, it was discovered to be linear at ethanol concentrations of 0.10–10.00%.

### 3.3. Limit of detection and limit of quantification

The exact LOD and LOQ values are determined by several variables, such as the analyte's unique properties, sample matrix, instrument sensitivity, and analytical technique employed.

**Table 2.** Analytical parameters LOD, LOQ and MDL

| | Spike Concentration = 0.15% | | | |
|---|---|---|---|---|
| | **Analyst 1** | **Analyst 2** | **Ethanol (%)** | **Recovery (%)** |
| Sample 1 | 0.15 | 0.17 | 0.16 | 107.67 |
| Sample 2 | 0.13 | 0.20 | 0.17 | 111.03 |
| Sample 3 | 0.10 | 0.18 | 0.14 | 93.33 |
| Sample 4 | 0.18 | 0.10 | 0.14 | 92.33 |
| Sample 5 | 0.19 | 0.14 | 0.16 | 109.83 |
| Sample 6 | 0.12 | 0.15 | 0.13 | 88.50 |
| Sample 7 | 0.14 | 0.16 | 0.15 | 100.47 |
| Sample 8 | 0.14 | 0.17 | 0.15 | 102.50 |
| Sample 9 | 0.10 | 0.15 | 0.13 | 84.70 |
| Sample 10 | 0.15 | 0.19 | 0.17 | 113.33 |
| **Mean** | **0.15** | | | |
| **Std Dev.** | **0.02** | | | |
| **MDL %** | **0.04** | | | |
| **LOD %** | **0.05** | | | |
| **LOQ %** | **0.15** | | | |
| Spike Level (10xMDL>Spike): | | 0.43 >0.15 | OK, Meets Criteria | |
| Spike Level (MDL<Spike) | | 0.04 <0.15 | OK, Meets Criteria | |
| S/N Estimate (ave./sd): | | 9.88 <10 | OK, Meets Criteria | |
| Ave. % Recovery (98–102%) | | 100.37 | Acceptable | |

Higher sensitivity is indicated by lower LOD and LOQ values, which is advantageous in many analytical applications, especially in environmental sectors. The lowest concentration of an analyte that can be accurately identified but not always measured is known as the limit of detection, or LOD. The smallest concentration of analytes yields a signal that stands out from the noise. The concentration corresponding to a signal-to-noise ratio (S/N) 3:1 is typically used to calculate LOD.

As stated above, the lowest concentration of an analyte at which a measurement can be made with sufficient accuracy and precision is known as the limit of quantification, or LOQ. Typically, the concentration that corresponds to a 10:1 or 3:1 signal-to-noise ratio is used to calculate it. The LOD and LOQ values of the technique were determined by analyzing ten blank samples that had been fortified with 0.15% ethanol. In compliance with the Analytical Detection Limit Guidance [24], the LOD and LOQ values were established.

The SD of the response (s) values was computed by three points three times the response SD to estimate the LOD. Additionally, the projected LOD values were confirmed in compliance with the guidelines [24]. Ten times the response SD was used to calculate the LOQ values. The LOD values for ethanol were identified at 0.05%. The MDL values were established at 0.04%, and the LOQ values were determined to be 0.15% (Table 2). The LOD and LOQ values in a study on the analysis of ethanol in beers were determined to be 0.09% and 0.27%, respectively [23].

### 3.4. Precision

In the precision study, the concentrations were determined according to the maximum and minimum alcohol contents of beers listed in TGK 2006/33 (Low-alcohol beer, non-alcoholic beer, lager, and high-alcohol beer) [20]. Six blank samples treated with ethanol at 0.5%, 3.0%, and 6.0% levels were examined to measure precision. For the repeatability test, samples were prepared in six repetitions, and the same operators finished the analyses in a single day. To determine the within-laboratory repeatability, ten replicate samples were assessed over three days by two different operators over a month.

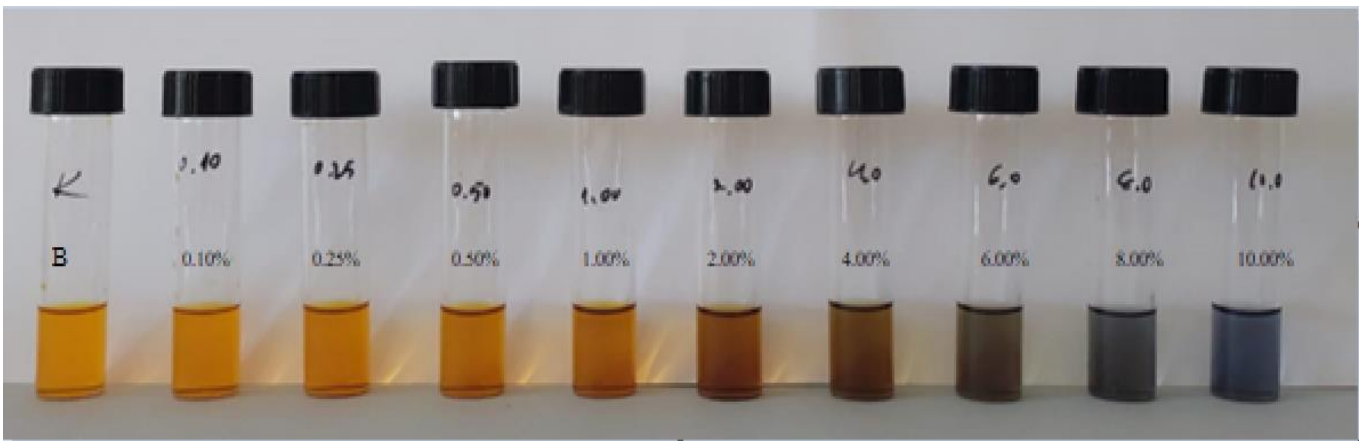


**Figure 3**. Color pictures with an alcohol percentage of 0.10–10.00%

**Table 3.** Analytical parameters $RSD_r$% and $RSD_R$%

| Intra-day (n=6) | | | | | | Inter-day (n=6) | | | |
|---|---|---|---|---|---|---|---|---|---|
| Analyte | Fortification level (%) | Determination level (%) | $SD_r$ (%) | Precision $RSD_r$ (%) | 0.66×Horwitz value $PRSD_r$ (%) | Determination level (%) | $SD_R$ (%) | Precision $RSD_R$ (%) | Horwitz value $PRSD_R$ (%) |
| Ethanol | 0.5[a] | 0.49 | 0.01 | 2.36 | 2.94 | 0.50 | 0.020 | 4.12 | 4.45 |
| | 3.0[b] | 2.94 | 0.05 | 1.85 | 2.24 | 2.95 | 0.032 | 3.19 | 3.40 |
| | 6.0[c] | 5.96 | 0.10 | 1.65 | 2.02 | 6.03 | 0.026 | 2.65 | 3.05 |

**a:** % Alcohol by volume (20 ºC): Low alcohol beer > 0.50,<3.0 and Non-alcoholic beer < 0.50 %, **b:** % Alcohol by volume (20 ºC): Beer > 3.00, <6.0% c: % Alcohol by volume (20 ºC): High alcohol beer > 6.00, <10.0% (TGK 2006/33, 2006)

Table 3 displays the results for the within-laboratory reproducibility represented with $SD_R$ (reproducibility SD), $RSD_R$% (relative SD), the repeatability stated with the SDr (Repeatability SD), and the relative SD (RSDr%). $PRSD_r$% and $PRSD_R$% values predicted by the Horwitz equation cannot be exceeded by reproducibility ($RSD_r$%) and repeatability ($RSD_R$%) values acquired from experimental tests.

$RSD_r$% and $RSD_R$% values of experimental studies were found to be lower than the reference values (Table 3) obtained by applying the Horwitz equation at each of the three concentration levels. These results demonstrated that the proposed method meets the defined minimal performance threshold for Horwitz values $PRSD_r$% and $PRSD_R$%. Therefore, the precision of the approach is sufficient.

$$\text{Reproducibility limit} = 2.8 \times SD_R \quad (4)$$

The repeatability limit of the analysts was determined using the calculation in Formula 3, and it was discovered to be appropriate for the investigated 0.5%, 3.0%, and 6.0% concentrations. Furthermore, it was discovered that the general repeatability control was appropriate for the identical concentrations under investigation.

The $RSD_R$% value was determined to be less than 5% in the interday repeatability investigation on ethanol analysis in beers [23]. Repeatability results in another study ranged from 0.47 to 0.62% [26].

### 3.5. Recovery

Table 4 provides analytical parameters for recovery percentages. To conduct the recovery investigation, samples that had been spiked with ethanol at three distinct concentrations (0.5%, 3.0%, and 6.0% low alcohol beer, non-alcoholic beer, beer, and high alcohol beer) were generated. The suggested procedure for determining the amount of ethanol in beer was applied to the six replicates of the spiked samples. The recovery values obtained (Table 4) fell between 97% and 103% of the values advised by the Association of Analytical Societies (AOAC) in 2016. Thus, it can be concluded that the suggested method for determining the amount of ethanol in beer and nonalcoholic beer samples produced satisfactory findings [18].

In the recovery investigation, samples were treated with varying quantities of ethanol. The amount of ethanol in the beers was then measured through analysis, and a percentage of recovery was computed. Between 97% and 103% of the values advised by the AOAC (2016) were found to be recovery values from this procedure [18]. This demonstrates that the approach yields acceptable outcomes. A recovery number of 97% to 103% shows that the procedure is accurate because the spiked samples recover almost the expected amount of ethanol. This is a good result since it demonstrates that the method can accurately detect the quantity of ethanol in beers that fall within the designated concentration levels. Achieving recovery percentage values near 100% is typically preferred in analytical chemistry since it shows that the technique analyzes the target analyte precisely and without experiencing appreciable loss or interference.

Consequently, outcomes falling within this range show that the method is reliable and appropriate for figuring out how much ethanol is in beer.

### 3.6. Measurement uncertainty

According to ISO 17025/2017 [27], laboratories that are accredited must evaluate the uncertainty of their analytical results. Various methods have been offered for calculating this uncertainty, including the Eurachem/Citac Guide CG 4 [28], and NMKL 4 [29].

**Table 4.** Analytical parameters of recovery %

| Recovery, % ( *n*= 6) | | | | | |
|---|---|---|---|---|---|
| Analyte | Fortification level, % | Low-alcohol beer Recovery, % | Non-alcoholic beer Recovery, % | Beer Recovery, % | High Alcohol Beer Recovery, % |
| Ethanol | 0.5 | 102.00 ± 1.91 | 98.40 ± 1.48 | 99.40 ± 1.43 | 97.65 ± 1.67 |
| | 3.0 | 98.85 ± 1.21 | 98.84 ± 1.33 | 98.76 ± 1.54 | 98.41 ± 1.54 |
| | 6.0 | 99.53 ± 1.11 | 98.53 ± 1.13 | 98.12 ± 1.66 | 98.54 ± 1.53 |

**Table 5.** Analytical parameters of measurement uncertainty

| Measurement Uncertainty | | | |
|---|---|---|---|
| Parameter | Value (X) | u(X) | u(X)/X |
| Trueness (bias) | 100.00 | 0.34 | 0.003 |
| Repeatability | 100.00 | 2.36 | 0.024 |
| Reproducibility | 100.00 | 4..12 | 0.041 |
| Relative combined uncertainty = | | | 0.048 |
| * Expanded measurement uncertainty) = | | | 0.095 |

*95% confidence level, $k = 2$

We employed the analytical validation parameters that were derived at each stage of the procedure in this investigation. Table 5 provides analytical parameters for measurement uncertainty. Measurement uncertainty estimation was performed using data from method performance and validation.

As a result, the likelihood of incorporating every uncertainty component has peaked. Six sources of uncertainty have been considered in order to determine the relative uncertainty (u): (a) volume; (b) mass; (c) calibration curve; (d) technique reproducibility and repeatability; (e) preparation of standards, and (f) precision. Using a coverage factor of 2, which roughly corresponds to a 95% confidence level (Eurachem/Citac Guide CG 4 2000), a relative expanded measurement uncertainty was computed, yielding values of 9.5% (0.095) in beers [28].

### 3.7. Analysis results of beers purchased from the market

Table 6 provides information on the ABV of samples of non-alcoholic beer and beer that is purchased commercially. The ABV values of the non-alcoholic beer samples are significantly lower, ranging from roughly 0.47% to 0.50%, compared to the beer samples, which have ABV values between 4.96% and 5.05%.

The alcohol content is the main distinction between market-bought beer and non-alcoholic beer. The ABV of regular beers usually ranges from 0.5% to 6.0% [25,30]. By contrast, non-alcoholic beers have an ABV of less than 0.5%, about the same as orange juice's alcohol concentration. According to research on the alcohol content of Turkish beers, the ethanol percentage was found to be between 4.2% and 5.2% [31].

## 4. Conclusions

A spectrophotometric technique based on the distillation of ethanol by micro water vapor distillation followed by sodium dichromate oxidation was developed to quantify the quantity of ethanol present in beer matrices. It was discovered that the method's performance characteristics met the minimal requirements set forth by the Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis. The verified approach offers quick and affordable procedures along with precise and accurate results.

**Table 6.** Alcohol analysis results of commercially purchased non-alcoholic beer and beer samples ($n = 3$)

| Sample | Alcohol ABV (%) |
|---|---|
| Sample 1 beer | 5.04 ± 0.03 |
| Sample 2 beer | 5.02 ± 0.06 |
| Sample 3 beer | 4.99 ± 0.03 |
| Sample 4 beer | 5.05 ± 0.02 |
| Sample 5 beer | 4.96 ± 0.01 |
| Sample 6 beer | 4.98 ± 0.03 |
| Sample 7 non-alcoholic beer | 0.48 ± 0.03 |
| Sample 8 non-alcoholic beer | 0.50 ± 0.02 |
| Sample 9 non-alcoholic beer | 0.47 ± 0.02 |
| Sample 10 non-alcoholic beer | 0.50 ± 0.02 |

With sample preparation and detection processes, the suggested method is ideally suited to meet the needs for sensitive and accurate ethanol beer detection. The method demonstrates high sensitivity, allowing for the detection of ethanol in beer matrices even at low concentrations. This sensitivity is important for meeting regulatory requirements and ensuring product compliance. Overall, the described spectrophotometric technique offers a robust and efficient approach for quantifying ethanol in beer matrices, meeting the requirements for method validation and providing reliable results for quality control purposes.

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## Declarations

Approval from an Ethical Perspective: There are no research involving humans or animals in this article.

This study was produced from the doctoral thesis of the Arda Akdoğan.

Permission to Publicate: To publish this work, the writers consented.

Conflicting Interests: There are no pertinent financial or non-financial interests that the authors need to disclose.

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