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Colorimetric Method for the Estimation of Ethanol in Alcoholic-Drinks

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Abstract

A method for estimating ethanol in alcoholic-drinks by direct reaction is presented. The method consists of color reaction of ethanol with sodium dichromate. The colorimetric quantification was based on the formation of green colored chromate ions resulting from treatment of ethanol and sodium dichromate as limiting reactant in presence of sulfuric acid and acetate buffer pH 4.3. The absorbance maxima for the ethanol were found to be 578 nm. The influence of acetate buffer pH, reaction time, and Beer's law on color development and sensitivity were investigated and optimal assay conditions established.

The limits of detection and quantification for ethanol were determined to be 0.6 mg/mL and 1.9 mg/mL.

Keywords: colorimetric method; limiting reactant, Ethanol, alcoholic drinks

Introduction

Ethanol is one of a wide variety of structurally dissimilar agents that depress the functioning of central nervous system [1]. With its legal and widely acceptable use, is the enormous personal and societal cost of its abuse. With millions of individual becoming alcohol abusers or alcoholics and proportional toxic effects has led to a growing concern about its purity and quantity of ethanol in beverages and alcoholic-drinks.

The cost of instrument as well as sophistication in handling prevents access to quick and accurate analyses of ethanol. The goal of this study was to establish a simple and rapid colorimetric method for estimating ethanol in alcoholic-drinks. On the basis of color reaction [2] consisting oxidation of ethanol with sodium dichromate as limiting reactant in presence of sulfuric acid and acetate buffer, we established the following design criteria: the method must be simple, sensitive, reproducible, and solution based so a spectrophotometer could be used for quantification, able to extend for beverages and ethanol containing pharmaceutical preparations.

To date, no methods for spectrophotometric determination of ethanol have been reported, whereas Gas Chromatographic method for estimation of ethanol in wine [3] and in concentrated cell suspensions [4] has been reported. None of these methods meet all of the criteria established above. The colorimetric method for the determination of ethanol in beverages using cerium IV reagent [5] do not meet rapid and simple criterion as reaction requires distillation and extraction to remove interfering reducing substances in the sample.

Materials and Methods

All solutions were prepared with purified water obtained from Millipore Milli-Q system. The absolute ethanol, BDH AnalaR Ethanol lot L536001 (Comply with ACS specification) was used for the preparation of stock solution. Three alcoholic-drink brands Romanov, White Mischief, manufactured by United Spirits Limited, Bangalore and Magic Moments Radico Khaitan Ltd. Rampur, Uttar Pradesh, India, were purchased from market. Sodium dichromate AR and sodium acetate AR were purchased from Qualigens. Sulfuric acid AR and Glacial Acetic acid AR grade were purchased from Qualigens.

Preparation of Ethanol Stock Solution

Ethanol as described previously was used to prepare 1.6 mg/mL stock solution in water. The ethanol was poured in volumetric flask containing water to prevent loss due to volatility. The ethanol stock solution was freshly prepared prior to use.

Preparation of Sample Solution

Samples were passed through activated charcoal and procedure as followed for the preparation of stock solution was used for preparing three alcoholic-drinks sample solutions.

Preparation of Sodium Dichromate Reagent

Sodium dichromate about 4 g previously dried at 120^{o}C for 3 hrs was used to prepare 40 mg/mL stock solution in water.

Preparation of Acetate buffer (pH 4.3)

The acetate buffer pH 4.3 was prepared as per US pharmacopoeia specification [6].

Preparation of sulfuric acid 1N

Accurately measured quantity about 54 mL of sulfuric acid dissolved in 1000 mL of water.

Color reaction and development of colorimetric method

To an aliquot of standard stock solution containing 1.6 mg/mL, 5 mL of sodium dichromate solution , 5 mL of acetate buffer pH 4.3 and 25 mL of 1N sulfuric acid was added in 50 mL of volumetric flask. The mixture was shaken gently for 1 min and allowed to stand for 120 min as incubation period at room temperature resulted in formation of green colored reaction product. Following incubation period the absorbance at 578 nm was read on Schimadzu Pharmaspec 1700 spectrophotometer. This procedure was followed for each of the three samples prepared in triplicates. Software supplied with the instrument was used to plot concentration curve for standard and concentration of sample was calculated using equation.

Percentage of ethanol in sample (%) = (Cs/Cu) (Au/As) x 100

where Cs = Concentration of standard, Cu = Concentration of sample as per Labeled Claim, Au = Absorbance of standard, As = Absorbance of sample.
The GC method (3), using Agilent 7890A with flame ionization detector, was taken as validity tool for comparative study of results obtained with those of proposed method.

Determination of Limits of Detection and Quantification

Limits of detection (LOD) and quantification (LOQ) were estimated in terms signal-to-noise ratio by comparing measured signals from samples with known low concentrations of analyte with those of blank samples. To determine the LOD and LOQ, serial dilutions of samples from 0 to 800 ppm were evaluated. LOD was established as ethanol concentration that yielded an absorbance greater than the sum of the mean plus 3 x the standard deviation of the zero ppm standard (n=3), whereas LOQ was reported as the concentration that yielded an absorbance greater than the sum of the mean plus 10 x the standard deviation of the zero ppm standard (n=3).

Spike Recovery Experiments

Spike recovery experiments were carried using BDH AnalaR ethanol (6.4 mg/mL) spiked with marketed alcoholic-drink brands containing 0, 3.2, 6.4, 9.6 mg/mL of ethanol.
Three independent experiments were conducted at each level of spike following color reaction and quantification procedures described above.

Results and discussion

Initiating our studies with the knowledge of color reaction of ethanol with sodium dichromate (2) in presence of sulfuric acid, we have developed a method for estimating the total ethanol in marketed alcoholic-drink brands (Table 1). Absolute ethanol was chosen as standard since it is easily commercially available with this standard in hand, we developed a method that is simple, accurate, less time consuming as it is absolutely free from extraction of colored compound from organic solvent.
Colorimetric quantification is based on the formation of green color resulting from the treatment of standard or sample with sodium dichromate in presence of sulfuric acid. Absorbance maximum for ethanol was found to be 578 nm. (Fig1).
The linearity of absorbance versus concentration of ethanol was observed by plotting calibration curve (Fig.2). Linearity using standard was found in the range of 1.6 mg/mL to 12.8 mg/mL.
The retention time for colored reaction product was enhanced by using acetate buffer solution. Effect of different acetate buffer solutions on stability of colored reaction product was studied using pH 4.1, 4.3, 4.5, 4.7 and 4.9 acetate buffer solutions. The colored product was most stable at pH 4.3 (Fig. 3).
The time required to complete the formation of colored reaction product was studied. Maximum time required for the completion of color reaction at 25^{o}C was observed to be 90 min established as incubation period. (Fig. 4).

Conclusion

A simple and sensitive colorimetric method was developed using 4% solution of sodium dichromate, sulfuric acid and acetate buffer pH 4.3 for determining total ethanol in marketed alcoholic-drink brands. During optimization, the influence of sodium dichromate concentration, reaction time and stability of colored reaction product were investigated. The limit of quantification and linear nature of the method make it a valuable tool for determining ethanol in alcoholic drinks. Although the assay was only demonstrated for alcoholic drinks, it can be readily adapted for the analysis of other alcohol containing pharmaceutical preparations, beverages, herbal formulations.
In addition, the methods described do not require expensive equipments, chemicals and skills thus extending application potential to researchers, alcohol processors and beverage manufacturers.

Acknowledgements

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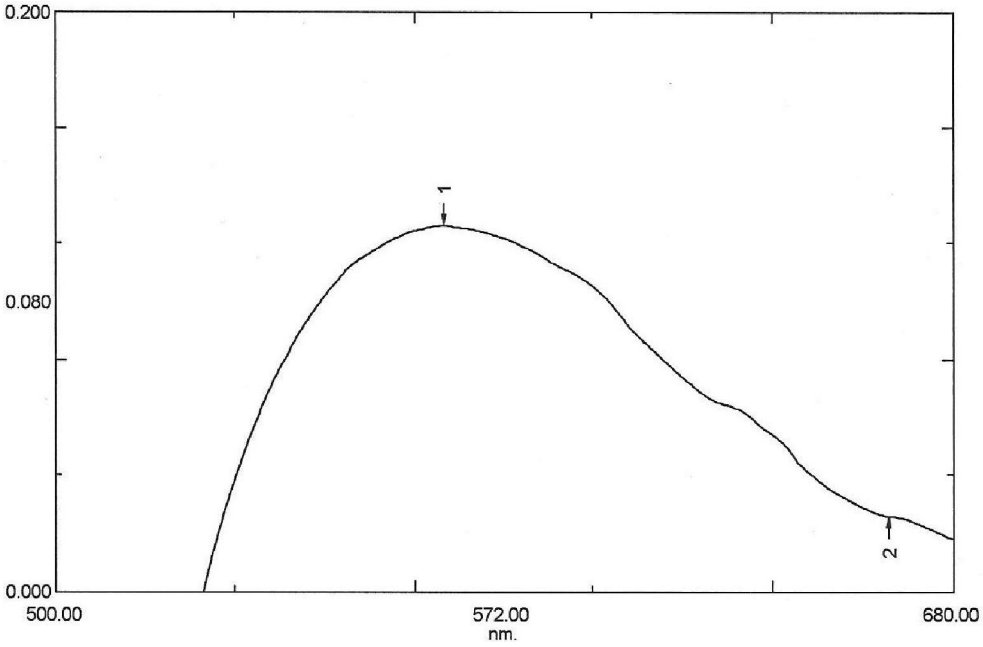

Figure 1. Visible spectra (480-680 nm) of reaction product of ethanol. After 150 min incubation at room temperature, spectrum was obtained using a Schimadzu Pharmaspec 1700 UV spectrophotometer.

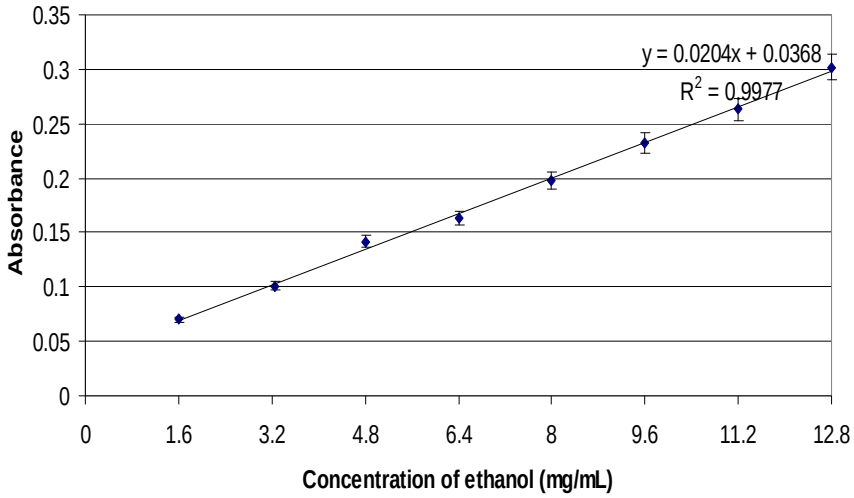

Figure 2. Plot of absorbance vs concentration (mg/mL) for the ethanol stock solution demonstrates reactivity of sodium dichromate reagent with ethanol at rising concentration.

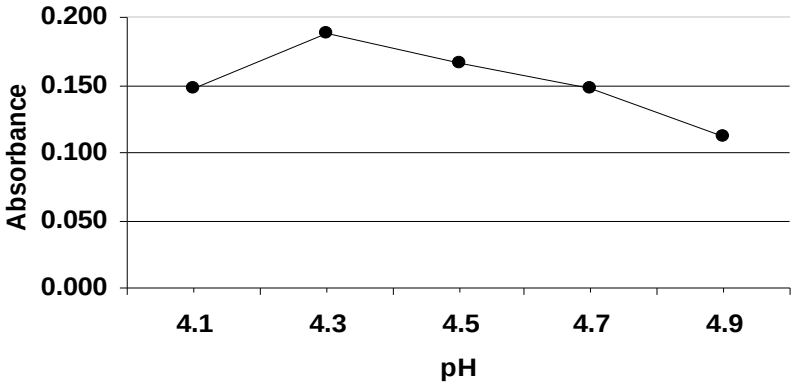

Figure 3. Effect of acetate buffer pH on formation of colored reaction product

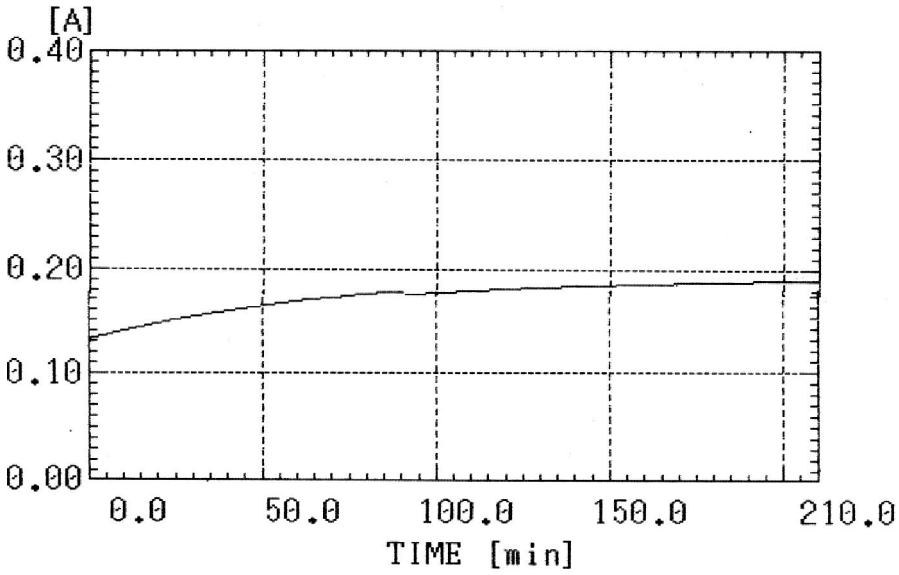

Figure 4. Effect of time on colored reaction product stabilized by acetate buffer pH 4.3

Table 1 Total percentage of ethanol found in commercial alcoholic-drink samples (Mean ± Standard Deviation, n = 3)

Sample	Percentage of Estimated Label Claim		% Recovery
	GC Method	Proposed Method	
Romanov	99.50 ± 0.69	98.92 ± 0.82	97.68 ± 0.91
White Mischief	97.05 ± 1.26	97.21 ± 0.60	97.30 ± 0.76
Magic Moments	94.74 ± 1.46	93.62 ± 0.53	95.20 ± 0.82