

# A Rapid and Precise Titration Method for Analysis of Ethanol: Comparison among Serum-Whole Blood Alcohol Concentration



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## Abstract

In the present study, we describe a simple and rapid method for determination of ethyl alcohol concentration in blood as well as serum samples. This study was carried out to compare the ratio of ethyl alcohol concentration in whole blood to serum. The result shows that the ratio of serum to blood alcohol concentration is in the range of 0.86: 1 to 1.49:1.

**Keywords:** Serum; Blood; Alcohol concentration; Oxidation diffusion method

## Introduction

Ethanol or ethyl alcohol is a legal drug that is readily available and widely used as intoxicating substance in our society. Ethanol is a small, water soluble molecule that gets easily absorbed in to mucous lining of digestive tract and is distributed by blood throughout all of the water containing components of the body. Amount of absorbed alcohol depends upon the surface area, thickness of the lining of stomach and the blood supply. Unlike most of the other ingested substances, ethyl alcohol is not digested or absorbed directly, only about 20-25% of ingested alcohol is absorbed because stomach has relatively small surface and limited blood supply.

The most important factor affecting absorption of blood is the presence of food in the stomach concurrent with alcohol. High concentration of alcohol is generally attained within 30-60 minutes in cessation of drinking. Ethyl alcohol is eliminated from the body through metabolism, excretion and evaporation. Metabolism accounts for approximately 95% of ethyl alcohol elimination [1,2]. In human body, alcohol is readily detected in body fluids that contains water as well as in breath. Most common of them are blood, breath and urine.

Serum is the liquid that remains when blood is collected without an anti-coagulant and allowed to clot. Serum is usually obtained for medical as well as forensic purposes. Concentration of ethanol in whole blood is not same as in serum. The ratio of serum alcohol concentration to whole blood alcohol concentration is in the range of 1.04:1 to 1.26:1. The approximate

ratio of serum alcohol concentration to whole blood alcohol concentration is 1.14:1 [3-7].

In the present study, we analyzed about eighteen samples to compare the ratio of ethyl alcohol concentration in serum to whole blood.

## Materials and Methods

### Materials

Potassium dichromate and potassium iodide were procured from Sisco Reasearch Laboratory, Mumbai, India. Sodium thiosulphate and starch were purchased from Thomas Baker, India. All chemicals were used without purification and distillation.

### Method

**Extraction of Serum:** Blood sample were collected in a glass tube and left for one hour at room temperature to allow clotting. The glass Pasteur was used to loosen the clot from the side of the tube. It is important not to lyse the red cell as serum cannot then be separated from the blood. The blood was centrifuged at 4000 rpm for twenty minutes at 4 °C. The clear upper layer was removed serum from the clot by gentle pipetting off in another clean tube using a glass pipette.

**Oxidation of ethyl alcohol:** 5ml of 0.05N potassium dichromate was taken in round bottom flask. To this 5ml of concentrated sulfuric acid was slowly added and cooled it to room temperature (i.e. oxidizing reaction mixture). Blood

sample/serum sample (0.5ml) was pipette out in another glass bulb containing 1gm of sodium carbonate (to absorb the ketone bodies and volatile acids from blood). A vacuum was applied to the round bottom flask using three way knob. To which one end contains oxidizing reaction mixture. The glass bulb was attached to other end of the three way knob was immersed the bulb in boiling water bath to vaporize ethyl alcohol. The three way knob was turned to bring the entire vapor in contact with oxidizing reaction mixture. The flask is then kept for 3-4 minutes .20ml of distilled water was then added to the flask

5ml of 10% potassium iodide solution was added to the flask to react unreacted dichromate solution and liberate free iodine. Liberated free iodine was titrated with 0.05 N sodium thiosulfate solutions by using starch as an indicator. End point was faint blue to colorless solution.

### Result and Discussion

Analysis of ethyl alcohol from biological specimens is the most commonly performed forensic science procedure and has occupied that position for many decades. Number of analytical principles and methodological modifications have been developed and published during the past several years for analysis of alcohol in blood and other biological liquids. Currently most of forensic laboratories determine blood alcohol concentration (BAC) by various methods which includes biological, chemical and instrumentation (head space GC) methods [8-12]. The biological and instrumentation methods are comparatively time consuming and costly. Now a day chemical method is widely used because it is simple, easy to work and takes fraction of minutes instead of hours. Chemical method is based on oxidation of ethanol to acetaldehyde by using chromic acid as an oxidizing agent.

The analysis of ethyl alcohol in blood samples as well as in serum or plasma sample plays an important role in an acute phase of forensic alcohol toxicology. The concentration of ethyl alcohol level in plasma, serum and whole blood are not same. The whole blood ethanol concentration is 11% less than that of either in plasma and serum samples from the same person. This variation is due to; serum and plasma contain more water compared to whole blood.

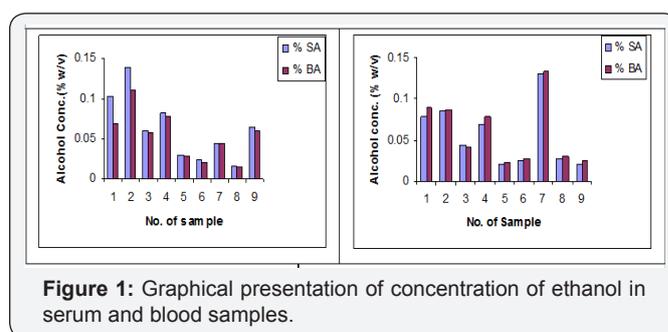
**Table 1:** Ratio of serum alcohol to whole blood alcohol.

S.No.	% SA w/v	% BA w/v	Ratio
1	0.103	0.69	1.49:1
2	0.14	0.11	1.27:1
3	0.060	0.057	1.05:1
4	0.083	0.078	1.06:1
5	0.030	0.028	1.07:1
6	0.023	0.021	1.09:1
7	0.044	0.044	1:1
8	0.016	0.014	1.14:1
9	0.064	0.060	1.06:1

The results obtained in this study are shown in Table 1 & 2. Graphical representation of results is shown in Figure 1. In the literature, the ratio of serum alcohol to blood alcohol has been reported to be in the range of 1.04:1 to 1.26:1. However, in our study it varies from 0.84:1 to 1.49:1. There are many reasons for these variations. For examples, storage as well as processing conditions, preservatives or anticoagulants added and time of collection of blood. Penetar et al. [11] demonstrated that there is no significant effect on types of collecting tubes on ethanol levels in plasma, serum or whole blood [11]. Also they describe no systematic differences in ethanol levels with or without preservatives and/or anticoagulants added. The results obtained in our study are in good agreement with those reported over the last twenty years. The ratio of serum to whole blood ethanol levels is in the ranging from 1.12 to 1.18 has been reported by Barnhill co-workers [3]. The serum alcohol levels observed in the present study is close agreement with Barnhill et al. [3] and Penetar et al. [11].

**Table 2:** Ratio of serum alcohol to whole blood alcohol.

S.No.	% SA w/v	% BA w/v	Ratio
1	0.078	0.090	0.86:1
2	0.085	0.087	0.97:1
3	0.044	0.041	0.93:1
4	0.069	0.079	0.92:1
5	0.021	0.023	0.91:1
6	0.025	0.028	0.89:1
7	0.131	0.133	0.98:1
8	0.028	0.030	0.90:1
9	0.021	0.025	0.84:1



**Figure 1:** Graphical presentation of concentration of ethanol in serum and blood samples.

### Conclusion

In conclusion, oxidation-diffusion method is a simple and rapid method for determination of alcohol in blood as well as serum samples. This study illustrates that the ratio of ethanol concentration in the serum to whole blood is in the range of 0.84:1 to 1.49:1.

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