

Comparative Forensic Characterization of Blood Components by Increased Alcoholic Concentrations

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Forensic toxicological research is concerned with the collection and storage of blood samples. In the experimental technique, the samples of four people have collected for 4 hrs. Before consumption of alcohol and sequential analysis was done for 6 hrs. After consumption at regular intervals. After subsequent analysis blood was transferred and collected in different sets. Whole blood was collected and Disodium ethylenediaminetetraacetate was added as an anticoagulant. Plasma samples containing EDTA and sodium fluoride samples after centrifugation were stored. Sample analysis was done after 24 h and 8 days at room temperature and after refrigeration. Gas chromatography was used to determine the concentration of alcohol before and after consuming it. The alcohol levels are found to be different in plasma and whole blood. Whole blood showed less concentration of ethanol than plasma. This indicates that there is no effect on the concentration of ethanol by adopting various preservation techniques.

Introduction

BAC accurate and precise levels are important for forensic studies [1]. The sample collection and various methods adopted for determining blood alcohol concentrations is an important tool in forensic science and toxicological studies [2]. The differences in the concentrations of plasma and whole blood are discussed in this report. It has been reported earlier that whole blood has less water content as compared to plasma [3]. The reported ratio of the two is 1.0 - 1.1 [4]. The objective is to maintain the blood samples and analyze them against alcohol concentrations.

In the vials used for collecting samples, preservatives and anticoagulants are added and then stored at different temperatures. In the *in - vivo* techniques [5], various difficulties are there. EDTA and Sodium fluoride are common preservatives for blood samples [6,7]. This report summarizes the role and effects of coagulant on BAC at concentrations of 30, 50, and 100 mg/dL from 2 mL ethanol, and *n*-propyl alcohol was taken as the internal standard. An aqueous solution of sodium tungstate (10% w/v) was used to precipitate the proteins. Plasma samples were centrifuged for 8 min at 3200 rpm at room temperature. Samples were extracted using 300 μ L each of internal standard, adding 10% sodium tungstate. Extractions were centrifuged at 3500 rpm for 5 min at 35°C and vials were set for instrumentation.

Material and methods

Blood samples of physically fit four adults (2 men and 2 women) were collected at the Paliwal Diagnostics (P) Ltd., Kanpur. Samples were collected of the adults who regularly consumed 10-20 drinks per week. These four adults were tested for fasting and abstaining from alcohol for at least 24h. After the consumption of alcohol, the first sampling was done, by HS-GC-MS, at CAF, Trivandrum, (Model, Clarus 580, Perkin Elmer, HS-40), then after, a gap was maintained for 1h. Post breakfast samples were taken four times in a gap of two hours.

Experimental

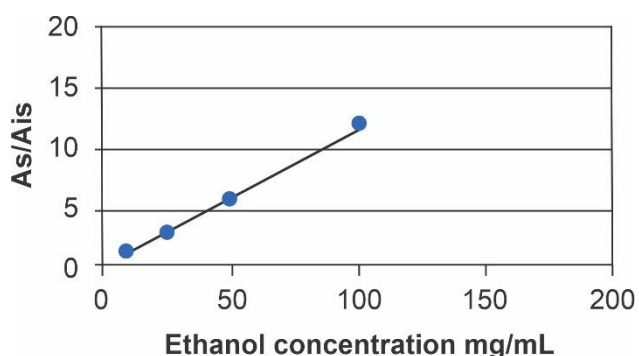
Blood was transferred and collected in yellow capped tubes with EDTA as an anticoagulant. Red-capped tubes with sodium fluoride and green-capped vials with sodium fluoride and potassium oxalate as anticoagulants. Plasma samples containing EDTA and sodium fluoride, after centrifugation for 10 min. at 3200 rpm, were analyzed and remaining kept for refrigeration [8]. Whole blood was also tested and stored as such, adding both the chemicals. Analysis was also carried out after 8 days. Both at room temperature and after refrigerating. Ethanol standardized solutions were prepared.

Alcohol concentrations were subjected to HS-GC. Temperature [9] was set at 30°C for 4.0 min and then ramped at 20°C/min to a final temperature of 100°C. The

purge valve was on for a split injection. The injector was at 237°C and the detector was set at 300°C. Standardization of the analytes was observed and a regression curve was obtained for linearity. CVs were 5.8 % and 6.6 %, and target values were 150 and 200 mg/ml.

Ethanol(mg/mL)	(AS/AIS)	REM (%)	RSD (%)
20	2.5	2.4	4.6
50	5.7	3.5	4.4
150	15.0	1.8	3.9
200	21.0	3.8	2.8

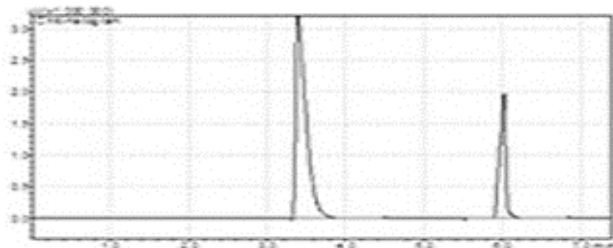
Table 1. Linear Regression of Calibration curve of ethanol concentration



Results and discussion

This report is consistent with the earlier reports, showing plasma and whole blood ratio of 1.11 [4,7]. Hodgson and Shajani [4] reported an averaged plasma to whole blood ratio of 1.11 for two-time points after drinking.

Thus, it has been observed for different procedures and sets of collection of vial samples, the ratio of plasma and whole blood remains nearly equal [10-12]. The results are a good match, as far as alcohol concentrations are concerned. Numeral reports [13-17] state that sustainable levels of alcohol can be obtained from whole blood samples at room temperature, i.e., 25-30°C for 1-2 days and those refrigerated.



Time of retention using n-propanol as internal standard.

The present study indicates a small depreciation in alcohol levels when the blood is stored in tubes for one week. Samples processed within 24 h were, on average, 10% higher in ethanol concentration than samples processed after a week. This clearly shows the loss of

alcohol. This was observed regardless of whether the samples were refrigerated or kept at room temperature.

The addition of the type of preservative had nearly no effect on the BAC, indicating that within a week, irrespective of preservation technique, ethanol level remains the same in the sample. The levels of ethanol observed in our samples following drinking were not significantly changed by the method adopted to collect the sample or by the technique adopted for preservation. [6,7]. The present study is the extension of the previous reports the obtained values of plasma and whole blood were nearly identical in all respects.

Keywords: Ethanol, Forensic toxicological research, gas chromatography

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