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The stability of ethanol in unstoppered tubes

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ABSTRACT

Objectives: The exact time frame within which ethanol can be reliably measured in unstoppered tubes is not known. The aim of this study was to investigate the stability of alcohol concentration in unstoppered tubes.

Design and methods: 44 samples with alcohol concentration >2.7 mmol/L were included in the study. Measurements were done on Vitros 250 analyzer with original Vitros reagents. After the initial alcohol measurement, each sample was aliquoted into two separate clean tubes (1 mL). One of the aliquoted tubes was stoppered immediately after aliquoting and remained stoppered during the experiment; while the other two tubes (original sample tube and the second aliquoted tube) remained open. During the experiment all three tubes were kept at room temperature. Alcohol concentration was measured at 30 minutes, 1, 2 and 3 hours after the initial measurement in all 3 tubes. The differences between the time intervals for each test tubes were examined using repeated measures Anova or Friedman test. The deviation from the initial concentration was calculated for all three test tubes for each time interval. The calculated deviations were compared with desirable imprecision specifications (DI) according to the RiliBÄK (DI < 9%).

Results: We found a statistically significant difference between the initial concentration and the concentration in unstoppered tubes for all the investigated time intervals; however, the DI was exceeded only in the original tube and in the tube B, 3 hours after the initial measurement (−9.2% and −12.6%, respectively).

Conclusions: Alcohol concentration can be accurately measured in the unstoppered samples within two hours upon decapping the tube, when stored at room temperature. Longer storage time (>2 hours) in the unstoppered samples introduces significant bias in alcohol concentration.

Introduction

Alcohol concentration measurement is a frequently requested laboratory analysis for diagnostics, therapeutic monitoring or other purposes [1]. In order to assure an accurate total testing process, besides having a standardized analytical procedure, it is important to know the pre-analytical variables that might influence the results, such as sample type or storage conditions.

So far several studies have investigated the stability of alcohol concentration in samples from living human subjects within days from blood collection. In 1983 Winek and Paul [2] analyzed the stability of alcohol in whole blood samples stored in sealed tubes at room temperature (22–29 °C) or in a refrigerator (0–3 °C) for 14 days and found that the stability of ethanol did not change significantly during their experiment. In addition, Penetar and colleagues [3] also investigated the stability of ethanol concentrations. They used plasma samples (EDTA and potassium oxalate/sodium fluoride), serum samples (plain serum and serum with sodium fluoride as additive) and whole blood samples (EDTA and potassium oxalate/sodium fluoride) that were aliquoted into polystyrene tubes and stored for 10 days at room temperature (25 °C) or in a refrigerator (4 °C). Similar to the results of Winek and Paul, their findings have shown that alcohol concentration does not change significantly regardless of sample type or storage conditions. A different study explored the stability of alcohol concentration within 35 days from the collection and showed that alcohol concentration may decrease in whole blood if stored in a closed tube on 26.7, 32.2 or 37.8 °C, but not in serum or plasma samples when kept in the same conditions [4].

Alcohol concentration decreases in unstoppered samples within the first few hours from the collection and such samples are not suitable for alcohol analysis. Although, to the best of our knowledge, the evidence for the evaporation rate of alcohol from uncapped samples has not yet been provided, it is recommended to keep the tubes stoppered to avoid possible evaporation of ethanol [5]. Therefore, the aim of this study was to investigate the stability of alcohol concentration in unstoppered tubes during the first three hours after measuring the ethanol concentration and removing the stopper from the tube.

Materials and methods

The study was carried out in the Clinical Institute of Chemistry, University Hospital Center "SESTRE MILOSRDNICE" during February

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Table 1
The results of repeated measures Anova analysis.

Tube	30 minutes	1 hour	2 hours	3 hours
Original bias; *P*	−1.06 ± 2.17; 0.0275	−1.52 ± 1.95; <0.001	−2.60 ± 3.04; <0.001	−2.82 ± 3.04; <0.001
A bias; *P*	−0.81 ± 1.94; 0.155	−0.02 ± 1.95; 1.000	−0.53 ± 2.39; 1.000	−0.43 ± 1.95; 1.000

and March 2013. Samples with alcohol concentration >2.17 mmol/L (>0.1 g/L) were included in the study ($N = 44$). Original sample tubes used in the experiment were Vacuette sample tubes (Greiner Bio-One, Kremsmünster, Austria) either with serum clot activator or lithium heparin, as additives. All alcohol measurements were carried out on Vitros 250 analyzer (Ortho Clinical Diagnostics, Johnson & Johnson Medical S.p.A., Milano, Italy) with original Vitros reagents. According to the manufacturer's instructions ethanol concentration remains stable if the tubes are kept tightly closed at room temperature for 3 hours, at 2–8 °C for one week or frozen for 6 months [6].

After the initial alcohol measurement, each sample was aliquoted into two separate clean tubes (1 mL). One of the aliquoted tubes (tube A) was stoppered immediately after aliquoting and remained stoppered during the experiment; while the other two tubes (original sample tube (tube O) and the second aliquoted tube (tube B)) remained open. Tubes made of polystyrene (PS) used for sample storage during the experiment were manufactured by Starstedt (Nümbrecht, Germany). During the experiment all three tubes were kept at room temperature. The average room temperature in our laboratory is 23.5 °C (min 22.1 °C–max 25.1 °C). The average ambient humidity is 55%. Alcohol concentration was measured at 30 minutes, 1, 2 and 3 hours after the initial measurement in all 3 tubes.

Statistical analysis

All data sets have been tested for normality using Kolmogorov–Smirnov test. The differences between the time intervals for each test tubes were examined using repeated measures Anova if the data were normally distributed, or using Friedman test in case of non-Gaussian distributions.

Additionally, the deviation from the initial concentration was calculated for all three test tubes for each time interval according to the formula $[(Cx - Ci)/Ci)] \times 100$; where Ci represents the mean value for the initial measurement and Cx the mean value of each tube and each time interval.

The calculated deviations were compared with the desirable imprecision (DI) according to the RiliBÄK (desirable imprecision < 9%) [7].

In order to investigate whether the stability shows concentration dependence we examined the relationship between the alcohol concentrations in each tube for each time interval and the deviation from the initial concentration for each time interval and each tube. The relationship was examined using Pearson's correlation coefficient if the data were normally distributed, or using Spearman's correlation coefficient in case of non-Gaussian distributions.

The statistical analysis was done using the MedCalc® programme, version 11.5.1.0 (F. Schoonjans, Belgium). *P* values < 0.05 were considered significant. The deviation from the initial concentration for all test tubes for each time interval was calculated using Microsoft Excel (Microsoft office 2003, Microsoft, Redmond, Washington, USA).

Results

The mean initial alcohol concentration was 32.47 mmol/L (range: 20.60–84.20 mmol/L), which is equal to 1.49 g/L (range: 0.95–3.88 g/L).

The results of repeated measures Anova test are presented in Table 1. There was no statistically significant difference between the initial concentration and the concentrations in the closed tube A for the investigated time intervals. For the unstoppered tubes, a statistically significant difference was observed between the initial concentration and the measured concentrations during the entire experiment.

Deviations from the initial concentration exceeded the desirable imprecision in the original tube and in the tube B, 3 hours after the initial measurement. The observed biases were −9.2% and −12.6% (biases found for the original tube and the tube B, respectively). Mean alcohol concentrations in the original tube and in the tube B 3 hours after the initial measurement were 28.93 and 27.63 mmol/L (1.33 and 1.27 g/L), respectively. The deviation of alcohol concentration was within the acceptable criteria for the closed tubes during the entire experiment. The decrease of alcohol concentration with time is presented in Fig. 1.

The results of the correlation analysis are presented in Table 2. Overall, the stability showed poor or no concentration dependency.

Discussion

Current recommendations state that samples for alcohol measurements should be tightly closed in order to prevent evaporation into the atmosphere [5]. Despite the fact that there was a statistically significant difference between the initial concentration and the concentration in unstoppered tubes for all the investigated time intervals, the desirable bias was not exceeded during the first two hours of the experiment.

A few studies have so far investigated the stability of alcohol in samples from living humans and found that alcohol concentration is stable within days if properly stored. For example, back in 1983 Winek and Paul [2] examined the stability of ethanol in whole blood samples that were taken from participants in uncontrolled conditions (variable additives and anticoagulants in sample tubes) and in controlled conditions (additives: sodium heparin or potassium oxalate with sodium fluoride). The samples were stoppered and stored at room temperature (22–29 °C; number of samples in the three studied groups taken under uncontrolled and controlled conditions with

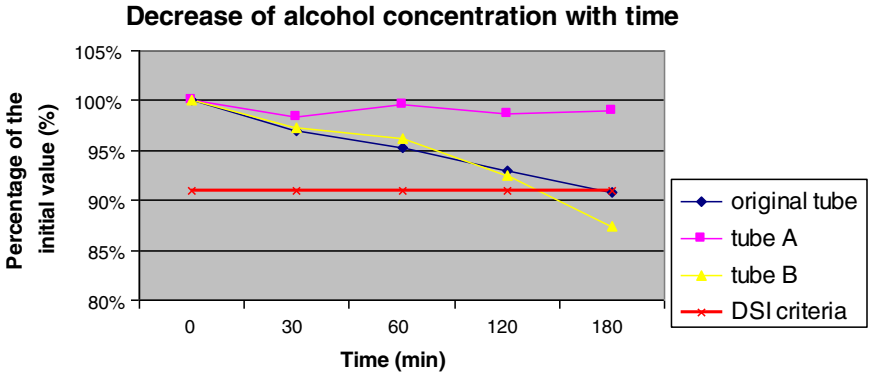

Fig. 1. Decrease of alcohol concentration with time. DSI criteria — desirable specification for imprecision according to the RiliBÄK (desirable imprecision < 9%).