Variability of BAC/BrAC, BAC/SAC and SAC/BrAC Ratios During Absorption and Elimination of Alcohol

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Abstract
A group of 38 volunteers participated in 74 experiments with multiple collection of blood and saliva samples and simultaneous breath testing. BAC/BrAC, BAC/SAC and SAC/BrAC ratios were calculated for every sampling time for each examined person in each experiment. It gave 975 values of the distribution ratio of ethanol for each system. The mean values of BAC/BrAC ratio calculated for absorption and elimination phases differed significantly (1822.1±315.9 and 2330.9±384.7, respectively, t=19.0, p<0.001). BAC/BrAC ratio slightly grows with increase of ethanol concentration in absorption phase (BAC/BrAC = 1583±60 + 466±110 x BAC, r=0.26, p<0.001) and with fall of ethanol concentration during elimination (BAC/BrAC = 2758±40 – 836±75 x BAC, r=-0.39, p<0.001). The difference in BAC/SAC ratio in both phases was also statistically significant (832±0.152 and 1.037±0.157, t=18.1, p<0.001). SAC/BrAC ratio did not show any dependency on the phase (2217.6 ± 384.6 and 2264.3 ± 357.4, t=1.81, p=0.05). Both in the absorption and elimination phase the SAC/BrAC ratio value felt with alcohol concentration (absorption: SAC/BrAC = 2572±66 – 595±104 x BrAC, r=-0.34, p<0.001; elimination SAC/BrAC = 2514 ± 33 – 517±64 x BrAC, r=-0.29, p<0.001).

Introduction
Reliable, valid and non-invasive methods of the sobriety testing are important in forensic, work place, medical and research settings (1). To date mainly breath testing is used for this purpose. In the eighties evidential breath alcohol instruments were approved for law enforcement purposes and threshold limits of breath alcohol concentration (BrAC) were introduced alongside the existing statutory blood alcohol concentration (BAC) limits (2). Nevertheless measurements of breath alcohol may not be accurate in some cases, i.e. in febrile or hypothermic patients, unconscious victims, or patients with bronchopulmonary disease (3). At the present time there is increasing emphasis on saliva as a biological specimen for analysing drugs of abuse, therapeutic agents, ethanol, environmental chemicals and many endogenous substances (4). The ratio of blood flow to tissue mass of the salivary gland is so large, the concentration of alcohol entering saliva reflects the concentration in arterial blood (5). It is very favourable because acute alcohol effects on the brain function are correlated with the concentrations in this type of blood. It is a reason the saliva /blood ratio of ethanol is not constant during pharmacokinetic phases because saliva primarily reflects the arterial and not the venous branch of blood circulation system (6). The saliva specimens serve as substitutes for blood, and the analytical finding is usually translated into the presumably equivalent blood alcohol concentration. The aim of this study was to estimate the variability of breath/blood, saliva/blood and saliva/breath ethanol distribution coefficients and to assess the possibility of saliva analysis as a direct method for sobriety testing.
Material and Methods

A group of 38 volunteers (26 men and 12 women, aging 23 – 60 years at the beginning of the study) participated in 74 experiments. The volunteers were social drinkers with no history of alcohol abuse. The subjects in each experiment consumed alcohol in the form of 40 % v/v vodka, within 15 minutes, two hours after last meal. They received a dose of ethanol: men – 0.7 g and women – 0.6 g of ethanol/kg of body weight. After inserting a catheter into a large cubical vein, blood samples were taken at 15 minutes intervals. Simultaneously the mixed saliva secretion samples were collected and breath alcohol concentration was measured using Alcometr A 2.0. Blood and saliva alcohol concentrations were determined by means of headspace gas chromatography using Perkin Elmer Autosystem apparatus equipped with autosampler HS 40. Separation was achieved on a 0.2% Carbowax 1500 / Graphpack-GC column under isothermal conditions (100 °C). The temperature of the flame ionization detector (FID) was 200 °C. A 0.2 ml blood or saliva was mixed with 1.8 ml of 0.002 g/l 2-methyl-2-propanol (tert-butyl alcohol) used as internal standard (IS). The samples were incubated in the autosampler for 22 minutes at 60 °C. Chromatograms were received and calculations were done using Turbochrom computer program. The statistical analysis were performed with use of STATISTICA software (Statsoft Inc., Tulsa, OK, USA).

Results

The study covered 74 experiments with multiple collection of blood and saliva samples and simultaneous breath testing. The samples were collected up to time when BrAC felt below 0.1 mg/l. Moreover the results with BAC and SAC lower than 0.2 g/l were rejected. This operation was done because of the great uncertainty in estimation of partition coefficients below these values. It gives 975 results of alcohol concentration in each specimen. 256 of them was classified as absorption phase while the rest as elimination one. The maximum ethanol concentration in blood was the criterion for division of the data.

In Figure 1 the mean values of BAC/BrAC, BAC/SAC and SAC/BrAC ratios calculated for absorption and elimination phases were compared.

Fig.1. Comparison of mean values of BAC/BrAC, BAC/SAC and SAC/BrAC ratios in absorption and elimination phase.
The mean values of BAC/BrAC ratio calculated for absorption and elimination phases amounted to 1822.1 ± 315.9 and 2330.9 ± 384.7, respectively, and the difference was statistically significant (t=19.0, p<0.001). The differences in BAC/SAC ratio in both phases were also significant (0.832 ± 0.152 and 1.037 ± 0.157, t=18.1, p<0.001). SAC/BrAC ratio did not show any dependency on the phase of alcohol changes (2217.6 ± 384.6 and 2264.3 ± 357.4, t=1.81, p>0.05).

In Figure 2 the changes in alcohol concentration and partition coefficients during the experiments were shown. Each point represents different sampling time.

![Figure 2](image.png)

**Fig. 2.** The changes in alcohol concentration and partition coefficients during experiments.

BAC/BrAC ratio rose from 1658 for 15 minutes timed from finishing of consumption to 2719 for 270 minutes after this time. The great interindividual variability in the partition coefficient caused high values of standard deviation (SD). In order to compare means the *post-hoc* Scheffe and Tukey tests were applied. At the applied significance level (α=0.05) the Scheffe test did not detect the differences between means for all points lying after 120 min, and Tukey test – after 135 min. BAC/SAC ratio started from 0.78 and finished reaching value of 1.15. But in this case the homogeneity of the means was reached much earlier. The Scheffe test did not detect differences between means for sampling times greater than 60 min, and Tukey test – greater than 75 min. SAC/BrAC partition coefficient is stable during processes of alcohol absorption and elimination. Both Scheffe and Tukey test did not detect differences between means for the individual sampling times at α=0.05 significance level.

The relationships between values of partition coefficients and actual alcohol concentration were estimated. The regression lines, \( y = b_0 + b_1 \cdot x \), calculated by means of least squares method were fitted to experimental data. The results were shown in Table 1.
Table 1. The regression and correlation coefficients.

<table>
<thead>
<tr>
<th>Function</th>
<th>Absorption</th>
<th>Elimination</th>
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<tbody>
<tr>
<td></td>
<td>b₁</td>
<td>b₀</td>
</tr>
<tr>
<td>BAC/BrAC=f(BAC)</td>
<td>466±110</td>
<td>1583±60</td>
</tr>
<tr>
<td>BAC/SAC=f(BAC)</td>
<td>0.341±0.050</td>
<td>0.658±0.027</td>
</tr>
<tr>
<td>SAC/BrAC=f(BrAC)</td>
<td>-595±104</td>
<td>2572±66</td>
</tr>
</tbody>
</table>

As one can see in Table 1, BAC/BrAC and BAC/SAC ratios slightly grew with increase in ethanol concentration during absorption phase and felt during elimination. In contrast to both ratios, SAC/BrAC ratio felt both during absorption and elimination.

Discussion
The partition coefficients of ethanol between blood, saliva and breath were calculated separately for absorption and elimination phases. When different materials have to be used interchangeably the partition coefficients between the specimens should be constant during whole course of ethanol changes in the body. As one can see in Figure 1 the BAC/BrAC and BAC/SAC ratio values for absorption phase are lower than those obtained during elimination one. It follows that results of breath testing and saliva analysis in the absorption phase are overestimated in comparison to the results of blood alcohol analysis and underestimated during elimination. SAC/BrAC ratio does not depend on phase of ethanol metabolism. This phenomenon can be explained by the fact that the concentration of alcohol in saliva should run closer to the concentration in arterial blood compared with the venous blood; this might account for the somewhat different time profiles, namely arteriovenous differences in the pharmacokinetics of ethanol. Concentrations of ethanol in breath are also closer to concentrations in arterial blood therefore the obtained time profiles for BrAC and SAC are practically identical.

The course of BAC/BrAC and BAC/SAC changes shown in Fig. 2 indicates that for the same alcohol concentration in absorption and elimination phase the different values of the ratios are obtained.

In the study the parallel changes of BAC/BrAC and BAC/SAC ratios, and the stability of SAC/BrAC ratio, were observed. It indicates the similar nature of changes in alcohol concentration in saliva and breath. Therefore, in our opinion analysis of saliva for ethanol content seems to be a good supplement, or even alternative, to breath testing.

References