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SHORT COMMUNICATION

SALIVA AS AN ALTERNATIVE SPECIMEN FOR ALCOHOL DETERMINATION IN THE HUMAN BODY

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Saliva as an alternative specimen for alcohol determination in the human body. W. GUBAŁA, D. ZUBA. Pol. J. Pharmacol., 2002, 54, 161–165.

Saliva, breath, and blood samples were collected from 49 volunteers and over 700 values of ethanol concentration were obtained. The profiles of time-dependent changes in saliva and expired air-breath ethanol concentration were similar. The average difference between the respective values determined in blood and saliva amounted to –0.031 ± 0.096 g/l, whereas the difference between the results for breath and saliva was –0.034 ± 0.080 g/l. These differences in ethanol concentrations do not exceed those which occur between blood and breath (0.003 ± 0.093 g/l). Introducing a correction value of 1.08, stemming from the varying water content in saliva and blood, results in a good agreement between the results for saliva and breath (0.005 ± 0.077 g/l). The headspace gas chromatographic method applied for ethanol determination in saliva is specific (resolution > 1), shows good accuracy (recovery = 100.7%) and precision (SD = 0.0155 g/l). There is no matrix effect when water solutions are used for calibration instead of saliva.

Key words: *blood, breath, correlation, ethanol, methods, saliva, validation*

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INTRODUCTION

Ethanol has a low molecular weight, is highly soluble in water, and does not bind to plasma proteins, which makes it a suitable compound for analysis in body fluids, i.e. blood, urine, saliva, and sweat [2, 5, 6]. The distribution of ethanol in the body is proportional to water content in the tissue. Therefore, the concentration of ethanol reaching saliva should reflect that found in the water fraction of whole blood. The ratio of blood flow to the tissue mass of the salivary gland is so high that the concentration of ethanol entering saliva should accurately reflect its concentration in blood [1].

At present, the determination of ethanol content in saliva is treated strictly as a screening method, despite observations that ethanol concentration values in this material are largely consistent with those found in simultaneously obtained blood samples. There are companies that manufacture quick tests for determination of ethanol content in saliva, like QED kits, or AlcoScreen [4, 7]. In the authors' opinion, saliva constitutes an analysis specimen adequate for use in the assessment of sobriety for forensic purposes.

METHODS

The alcohol concentration in saliva and blood samples was measured by headspace gas chromatography (GC) using Perkin Elmer AutoSystem XL with HS 40 autosampler. Separation was achieved on a 0.2% Carbowax 1500/Graphpack-GC column under isothermal conditions (at 100°C). The temperature of the flame ionisation detector (FID) was 200°C. A 0.2 ml of saliva or blood sample was mixed with 1.8 ml of 0.02 g/l 2-methyl-2-propanol (tert-butyl alcohol) used as an internal standard (IS). The samples were incubated in the autosampler for 22 min at 60°C.

The saliva, expired air-breath and blood samples were collected from 49 volunteers (37 men and 12 women), ageing 23–60 years at the beginning of the study, participating in the experiments. They had consumed: 0.7 g (men) and 0.6 g (women) of ethanol per kg of body weight in the form of undiluted 40% v/v vodka. The samples were taken in 15-minute intervals. The research performed on the studied group yielded over 700 values of ethanol concentration in saliva, breath and blood.

RESULTS and DISCUSSION

Validation of the method

The applied conditions allow for good separation of ethanol from its metabolite (acetaldehyde) and other volatile compounds which may co-exist in saliva (methanol, acetone, n-propanol and isopropanol). Linearity was verified in the range of 0.1–4.0 g/l of ethanol in aqueous solutions. The limit of determination (LOD) was lower than 0.05 g/l. In order to estimate the matrix effect, two parallel series of calibration solutions were prepared. This was done by spiking a vial filled with water or saliva with the certified reference solutions of ethanol. The calibration curve for ethanol in saliva ($y = 0.122x + 0.001$) did not differ significantly from the one obtained for aqueous solutions ($y = 0.123x$

Table 1. The results of assessment of method accuracy and precision. In the study the certified reference solution purchased from Merck were analyzed

C_{exp} [g/l]	C_{obt} [g/l]	N	SE [%]	R [%]	SD [g/l]	RSD [%]
0.5	0.479	14	– 4.1	95.9	0.037	7.7
1.0	0.992	66	– 0.8	99.2	0.037	3.7
1.5	1.494	23	– 0.4	99.6	0.052	3.5
2.0	1.999	38	– 0.1	99.9	0.075	3.7
2.5	2.529	8	1.2	101.2	0.065	2.6
3.0	3.045	40	1.5	101.5	0.078	2.6
4.0	4.039	9	1.0	101.0	0.112	2.8

C_{exp} – ethanol concentration in the certified reference solutions, C_{obt} – mean ethanol concentration obtained from analyses, N – number of analyses, SE – standard error, R – recovery, SD – standard deviation, RSD – relative standard deviation

+ 0.001). The recovery calculated for all calibration levels amounted to 100.7%. The data show the absence of systematic error when the aqueous solutions were used instead of saliva for calibration. The precision of the method was checked using duplicate determinations of 122 randomly chosen routine samples used for ethanol level evaluation in saliva. The intra-assay precision expressed as standard deviation (SD) amounted to 0.0155 g/l, while reproducibility (r) was 0.045 g/l, and relative standard deviation (RSD) was 3.6%. The variance was homogenous between the persons and the days of analysis.

Correlation between the concentrations of alcohol in saliva and in blood and breath

Ethanol is a depressant of the central nervous system and its effect on the brain functions is of great importance for medicolegal purposes. Ethanol reaches the brain with arterial blood, thus symptoms of its effects are directly proportional to its concentration in arterial blood. Unfortunately, collecting samples of this type of blood is extremely difficult, making the proposition of its use in routine analyses rather senseless. The application of a blood test requires drawing venous blood from the arm. This is a source of error due to the time elapsing (from several to over ten minutes) between the moment when blood has contact with the brain and the moment when it reaches the veins of the arm. It can be said that alcohol concentrations determined through the analysis of venous blood define alcohol's effects on the brain, and thus on the behavior of a given individual, with somewhat a delay. The profiles of time-dependent changes in alcohol concentrations in blood, saliva, and breath (applying a blood/breath ratio of 2100:1), based on the analysis of the alcohol time-concentration curves in 49 people, are shown in Figure 1. The profiles were drawn up to 270 min after the start of alcohol consumption, because after that point in time, alcohol was completely eliminated from the systems of most of the subjects. Hence, in subsequent 15-minute intervals, minimal alcohol concentrations were obtained. Concentrations of alcohol in breath, as compared to those in the blood, are greater during the absorption phase, and lower in the elimination phase. This is borne out by the difference in ethanol content in venous and arterial blood. The saliva and breath time-concentration curves are similar. Introducing a correction value of 1.08, stemming from the varying water content in saliva and blood, results in the almost perfect over-

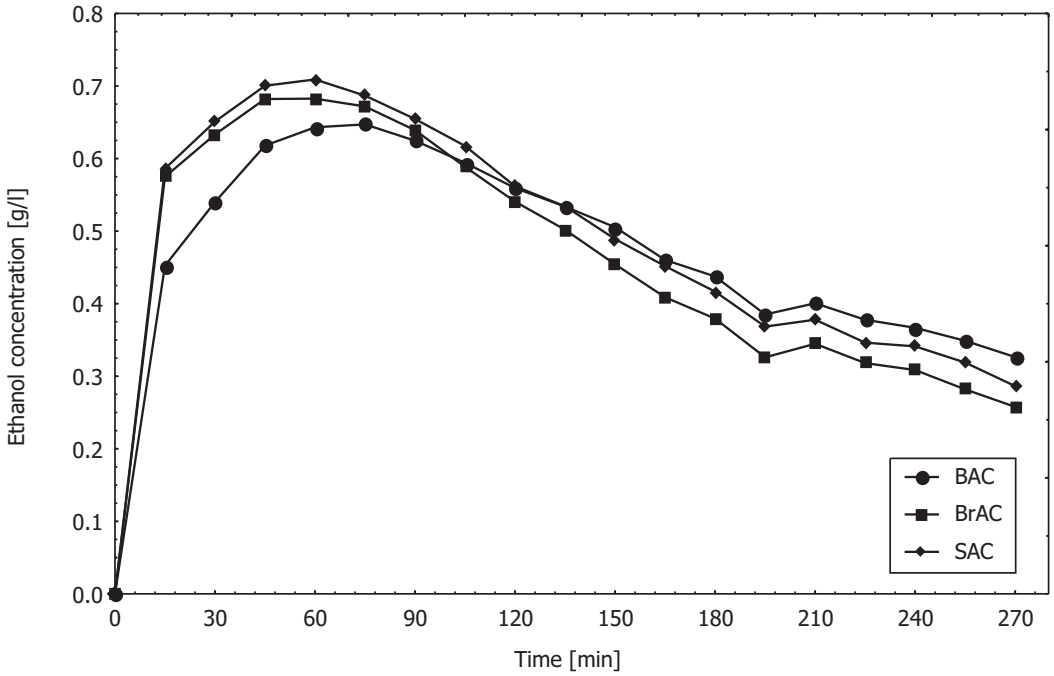

Fig. 1. The profiles of time-dependent changes in ethanol concentration in blood (BAC), breath (BrAC) and saliva (SAC). The results are expressed as means calculated for 49 persons. Samples were collected in 15 min intervals

lapping of these two curves. The maximum concentration in saliva appears simultaneously with that of breath. The differences in alcohol content in saliva, as opposed to blood and breath, are shown as a Bland and Altman plot [3] in Figures 2 and 3. The average difference in blood and saliva alcohol concentration amounted to –0.031 ± 0.096 g/l, whereas the difference between the results for

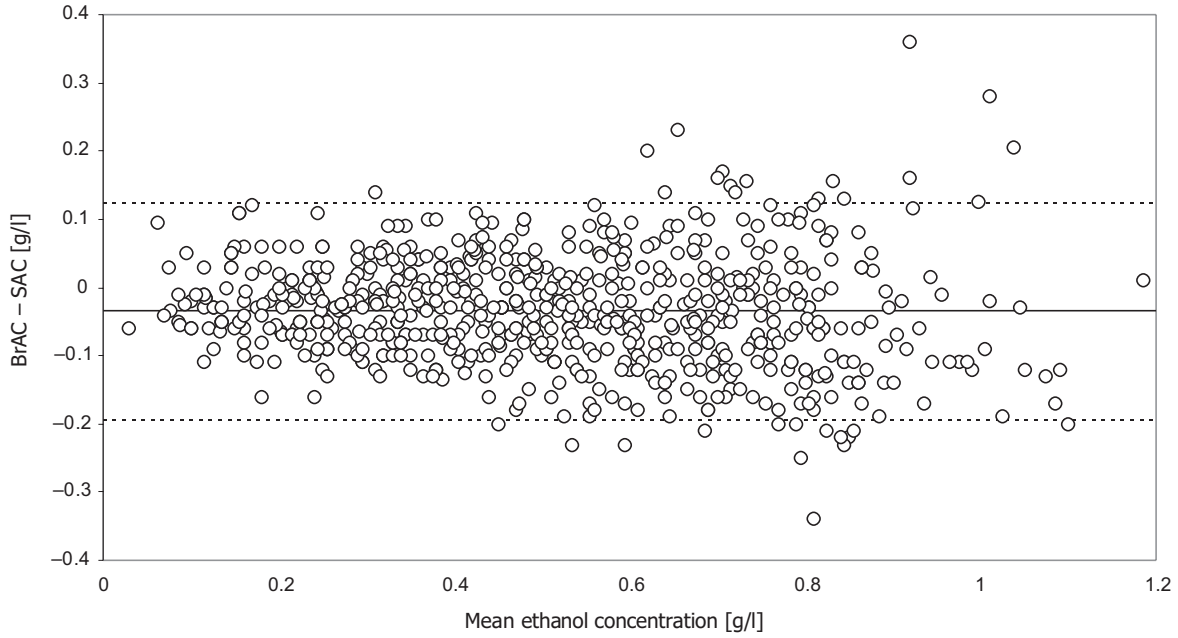

Fig. 2. Bland and Altman plot for differences in ethanol concentration in saliva and breath. Solid line denotes the mean difference between breath and saliva alcohol concentration (–0.034 g/l), the dashed lines indicates confidence limits (mean ± 2 SD, –0.034 ± 0.080 g/l)

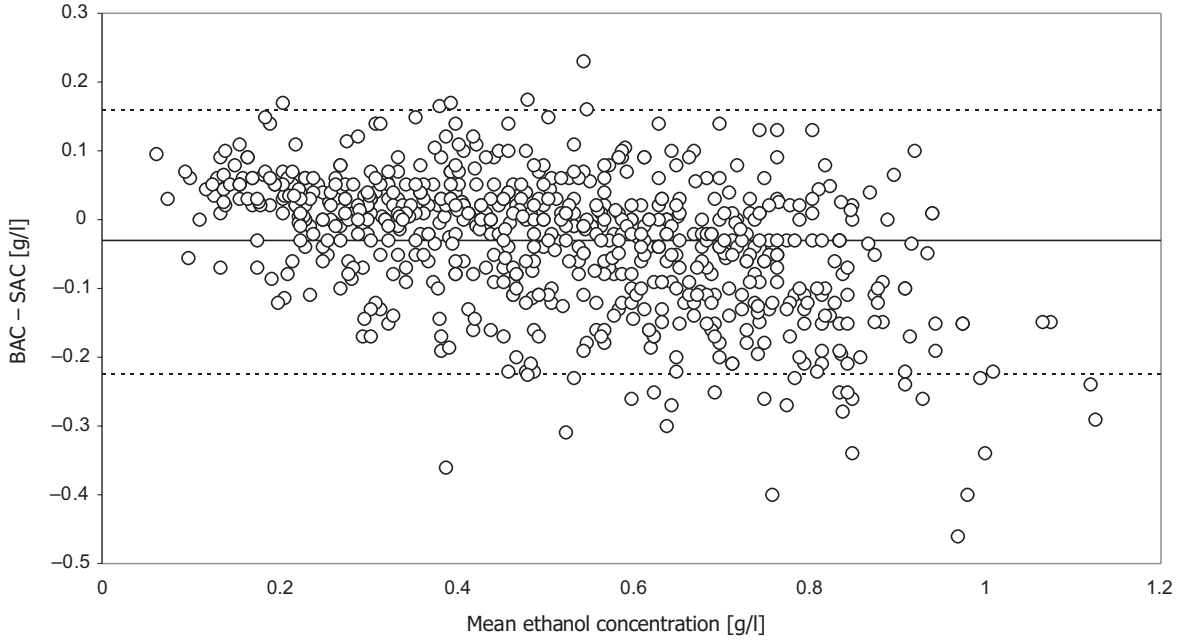

Fig. 3. Bland and Altman plot for differences in ethanol concentration in saliva and blood. Solid line denotes the mean difference between saliva and blood alcohol concentration (–0.031 g/l), the dashed lines indicates confidence limits (mean ± 2 SD, –0.034 ± 0.096 g/l)

breath and saliva was –0.034 ± 0.080 g/l. The obtained results indicate a high level of consistency between concentrations of alcohol in saliva and breath or blood. The values of the differences in ethanol concentration in saliva, blood, and breath do not exceed those which occur between blood and breath (0.003 ± 0.093 g/l).

CONCLUSIONS

The results of validation show that an analytical error committed during routine ethanol determinations in saliva is comparable to that characterizing blood ethanol determinations.

Saliva ethanol concentrations, the same as the levels in breath, are closely correlated with its concentration in the arterial blood. Therefore, it seems that the saliva analysis for assessment of sobriety testing could be treated independently of blood analysis.

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