COMPARISON OF AN ENZYMATIC ALCOHOL DEHYDROGENASE ASSAY AND ALCOHOL HEADSPACE GC-FID METHOD USING STATISTICAL ANALYSIS ON REAL FORENSIC BLOOD AND URINE SAMPLES

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ABSTRACT: Two independent measurement techniques to determine alcohol, Syva-Emit® and headspace GC-FID were compared using a paired sample t-test, regression analysis, and a graphical procedure. For the real forensic blood samples examined, a deviation between both methods was found in the higher but not in the lower concentration range.

KEY WORDS: EMIT; Gas chromatography (GC); Ethanol.

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INTRODUCTION

The determination of ethanol in biological fluids is probably the most commonly performed forensic test in existence. At our laboratory, two independent measurement techniques are used to determine alcohol levels in forensic samples. Primarily, a screening is performed using the Syva-Emit, enzymatic alcohol dehydrogenase assay (EM). Alcohol dehydrogenase catalyzes the oxidation of ethyl alcohol to acetaldehyde, and during this reaction NAD^+ is reduced to NADH. The increase in absorption at 340 nm is proportional to the concentration of the alcohol present in the sample. Subsequently, headspace sampling (HS) with gas chromatography and flame ionization detection (GC-FID) is used to confirm the concentrations of ethyl alcohol and to search for the presence of other volatiles in the samples. Over the last five years, many samples were analyzed with both methods, enabling a statistically based comparison of both methods' performance for blood (BL) and urine (UR).

ORIGIN OF THE SAMPLES

All selected blood and urine samples were from forensic sources. When using real samples of which the concentration is not known a priori, one tends to analyze a higher

number of samples in the medium concentration range and only few at the lowest and the highest concentration levels. To avoid this, the samples were randomly selected out of the whole sample, but pool equally divided over the range of the headspace calibration curve (0.5–3 g ethanol/l). The distribution of the selected samples is visualized with proper histograms for the examined sample populations (Figure 1). Table I summarizes the descriptive statistics of the sample populations.

TABLE I. DESCRIPTIVE STATISTICS

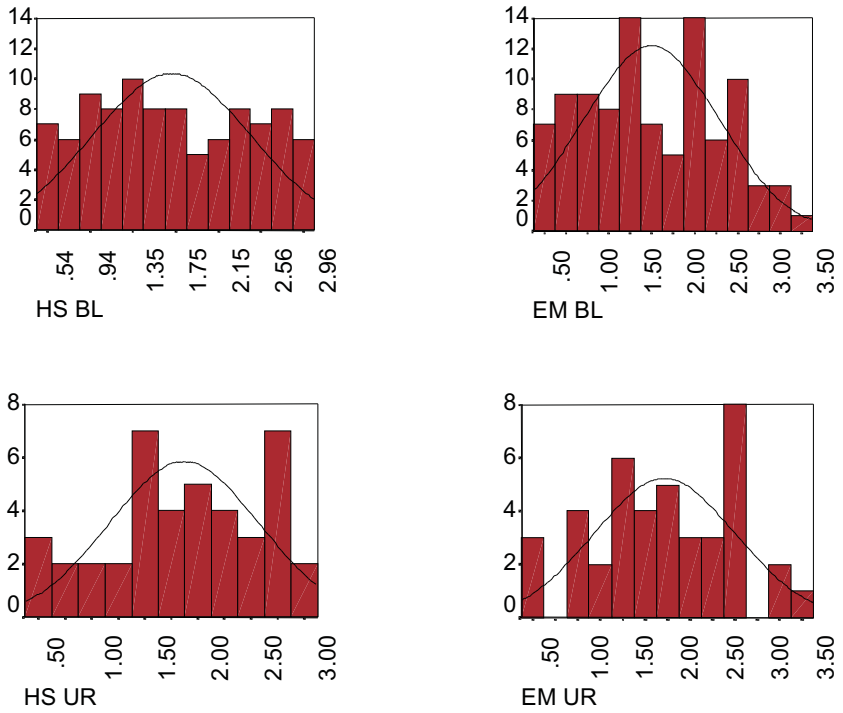

Fig. 1. Histograms of the selected headspace blood samples (HS BL), emit blood samples (EM BL), headspace urine samples (HS UR), and emit urine samples (EM UR).

Method/ material	N	Min	Max	Mean	SD
HS BL	96	.50	2.99	1.71	.74
EM BL	96	.50	3.54	1.74	.78
HS UR	41	.50	2.95	1.86	.69
EM UR	41	.52	3.41	1.96	.78

PAIRED SAMPLE T-TEST

Assumptions

The paired sample t-test is based on the assumptions that the variances of the compared sample populations are equal and that the data of the sample populations follow a normal distribution. The Levene test statistic to check for homogeneity of variances is presented in Table II.

TABLE II. TEST FOR HOMOGENEITY OF VARIANCES

Material	Levene statistic	Df1	Df2	Sig.
Blood	.271	1	190	.603
Urine	.597	1	80	.442

The assumption of normal distribution was checked as well graphically (Figure 2) as with the Kolmogorov-Smirnov test of normality (Table III).

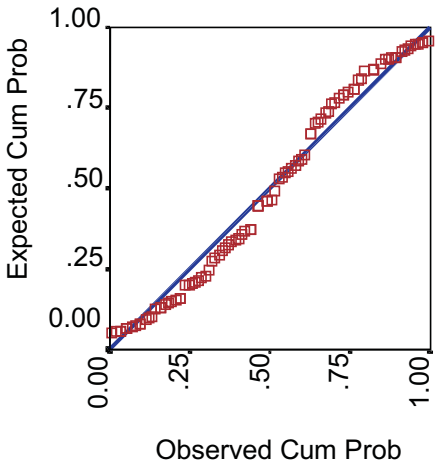

Fig. 2. Normal p-p plot of the selected headspace samples (HS BL).

TABLE III. KOLMOGOROV-SMIRNOV TEST OF NORMALITY

Method/ material	Test statistic	Df	Sig.
HS BL	0.075	96	0.200
EM BL	0.083	96	0.103
HS UR	0.088	41	0.200

EM UR	0.071	41	0.200

These facts and figures demonstrate that their are no violations of both the assumptions.

Paired samples t-test

Table IV summarizes the results of the actual paired sample t-test, performed on blood and urine.

TABLE IV. PAIRED SAMPLE t-TEST

Comparison of methods	ð	SD	SEM	95% CI	
				Lower	Upper
EM BL/HS BL	$3.53 \cdot 10^{-2}$	0.158	$1.61 \cdot 10^{-2}$	$3.23 \cdot 10^{-3}$	$6.73 \cdot 10^{-2}$
EM UR/HS UR	$9.68 \cdot 10^{-2}$	0.181	$2.82 \cdot 10^{-2}$	$3.98 \cdot 10^{-3}$	0.1539
	t	Df		Sig. (2 tail.)	
EM BL/HS BL	2.185	95		0.031	
EM UR/HS UR	3.431	40		0.001	

At the chosen significance level ($\alpha = 0.05$), a bias was detected in one or both of the methods, and this for the two matrices analyzed.

REGRESSION ANALYSIS

Alternatively, the methods were compared by regression analysis (Table V). Both x, and y values were experimental results and subject to random error. In this case one should use a regression analysis with the residuals orthogonal to the regression line instead of ordinary least square regression. However, since the spread of the headspace values was large compared to the measurement error on a single headspace value, classical regression analysis could be used, without making significant errors [2].

TABLE V. REGRESSION ANALYSIS

Blood		SD	95% CI	
			Lower	Upper
Intercept	$-1.61 \cdot 10^{-2}$	0.040	-0.096	0.064
Slope	1.030	0.022	1.012	1.073
Urine		SD	95% CI	
			Lower	Upper
Intercept	$7.69 \cdot 10^{-2}$	0.077	–0.233	0.079
Slope	1.093	0.039	1.015	1.171

Regression analysis gives more information than the paired sample t-test. Seeing that, for both matrices analyzed, the slopes differ from one, a proportional discrepancy was detected between both methods.

BLAND AND ALTMAN PLOT

Of great diagnostic value was the plot of the difference between the methods (d) against their mean, as proposed by Bland and Altman in 1986 [1], (Figure 3).

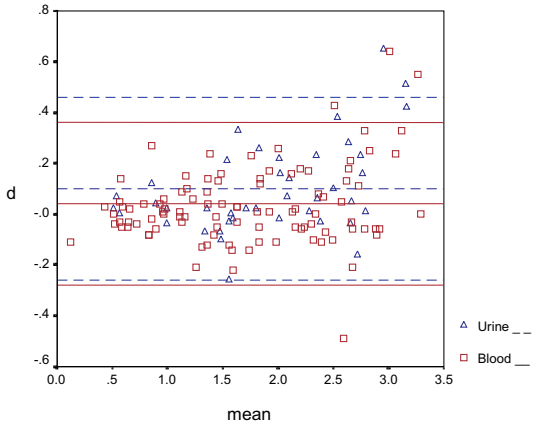

Fig. 3. Bland and Altman plot for blood and urine.

A considerable lack of agreement between the emit and the HS method was displayed in the higher concentration range (> 2.5 g/l).

PAIRED SAMPLE T-TEST ON SUB-POPULATIONS

Subsequently, the selected samples were divided into two groups following the emit values (emit value > 2.5 g/l). The paired sample t-test was performed on the sub-populations (Table VI).

TABLE VI. PAIRED SAMPLE t-TEST ON SUB-POPULATIONS

Concentration of ethanol	t	Df	Sig. (2-tailed)
Blood < 2.5 g/l	0.574	75	0.568
Blood ≥ 2.5 g/l	2.680	19	0.015
Urine < 2.5 g/l	2.081	28	0.052

Urine ≥ 2.5 g/l	3.097	11	0.010

A statistically significant difference between emit and the HS method was now detected, only for the higher concentrations (sig. < 0.05).

CONCLUSION

The two independent measurement techniques Syva-Emit® and headspace GC-FID to determine alcohol levels in forensic samples were equivalent in the lower but not in the higher concentration range. This may be due to a calibration error in the emit method where a one point calibration was performed at the 1 g/l level. An additional cause of the higher emit values for some of the blood samples might be partially/completely attributed to an earlier published interference of LDH in the Syva-Emit® assay [3]. This can be the cause of the deviation found in some of higher concentrated real forensic samples analyzed in our study.

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