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Development of a statistical model for predicting the ethanol content of blood from measurements on saliva or breath samples

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Abstract: Blood, saliva and breath samples from a population of males and females subjected to the intake of preselected amounts of ethanol, whilst in different physical conditions (at rest, after physical exertion, on an empty stomach and after eating), were analysed by automatic methods employing immobilized (blood) or dissolved (saliva) enzymes and a breathanalyser. Treatment of the results obtained enabled the development of a statistical model for prediction of the ethanol concentration in blood at a given time from the ethanol concentration in saliva or breath obtained at a later time.

Keywords: *Ethanol; breath or saliva assay; statistical model; prediction of blood alcohol concentrations.*

Introduction

Despite the numerous studies performed on the determination of ethanol in biological fluids [1–9], little is known of the relationship between the ethanol content in different human fluids (blood, breath, saliva) and its rate of change with time [10].

This problem is considered herein with two basic purposes. On the one hand, the necessity to know whether or not the ethanol concentration in saliva and breath is directly related to that of blood in the same individual after a given time. Whereas the former is readily available, the assay of the alcohol content of blood requires the prior drawing of samples, that must be performed by skilled workers according to legal regulations.

On the other hand, it is necessary to appreciate that the measurement of the degree of alcoholemia in individuals involved in traffic accidents may be performed up to one or more hours after the incident by the breathanalyser test. This gives an estimate of the concentration in blood at that moment, but not at the time of the accident. Therefore, it is necessary to develop a statistical model allowing the reliable prediction of the degree of alcoholemia in blood at the time of the traffic accident by extrapolation.

A thorough study of the changes of ethanol concentrations in different human fluids with time has been performed in order to develop a statistical model that enables the

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ethanol concentration in blood to be determined from that in saliva or breath. The comparative study of the ethanol content in these fluids allows one to determine whether such a content bears a constant relationship in different individuals, taking into account factors potentially modifying the rate of biotransformation of this drug within the organism.

Experimental

Reagents

The characteristics of the solutions used to develop the analytical methods are as described previously for blood [11] and saliva [12], respectively.

Apparatus

A Perkin–Elmer LS-1 fluorimeter equipped with a flow-cell of 4 μl internal volume, a Pye-Unicam SP6-500 spectrophotometer equipped with a Hellma 178.12QS flow-cell (inner volume 18 μl) and an Alcotest Dräger 7310 breath autoanalyser were used. A Gilson Minipuls-2 peristaltic pump, a home-made dual injection valve furnished with two Rheodyne 5041 variable-volume injection valves, and Tecator "chemifolds" type I and II were also used. A General Data MV 4000 computer and a Hewlett–Packard HP-85 microcomputer were also employed.

Sampling and sample treatment

Samples (50 μl) of fluid (saliva or blood — from a finger tip) diluted with pyrophosphate buffer were tested. Breath was sampled directly into the autoanalyser.

Methods of analysis

The determination of ethanol in blood was based on a fluorimetric flow-injection analysis (FIA) procedure [11], involving the use of immobilized enzymes.

Ethanol in saliva was determined by an FIA photometric method by using dissolved enzymes [12].

The ethanol content in breath was determined on the above-described commercial instrument.

The technical data and operational details of the methods are given in Table 1. Fifty microlitre volumes of sample were diluted with pyrophosphate buffer solution (pH 9.0) to give a volume of 5 ml. The solution was aspirated to fill the loop of the injection valve and introduced into the carrier stream, the enzymatic reaction taking place in the open (saliva) or in the packed reactor (blood), the products being monitored on passage through the flow-cell (measurement of the peak height or signal increment for blood and saliva, respectively).

Results and Discussion

Features of the sampling

A population of individuals of both sexes, aged around 24 years and with an average weight of 50 kg (females) and 70 kg (males) were given a preset amount (100 ml to females and 150 ml to males) of whisky containing 33.36% (m/v) of ethanol, over a period of 20 min. The simultaneous sampling of the three body fluids was started 10 min after the whisky was consumed, and was repeated at 15-min intervals, six times for each

Table 1
Features of the methods employed for the determination of ethanol in blood, saliva and breath

(A) *Optimum values of the variables*

Sample	Reactor length (cm)	Flow rate (ml min^{-1})	Injection volume (µl)	|ADH| (U ml^{-1})	|NAD^+| (mg 100 ml^{-1})	Temperature (°C)
Blood	70	2.35	130	*	45	25
Saliva	20	1.80	100	90	45	25

(B) *Features of the determinations*

Sample	Detector	Range (µg ml^{-1})	r^2	RSD (%)	Sampling frequency (h^{-1})	Determination limit (µg ml^{-1})
Blood	Fluorimetric	2.0–24.0	0.9926	0.64	50	0.5
Saliva	Photometric	2.5–15.0	0.9998	0.68	40	0.5
Breath	Conductimetric	0–3†	—	0.1	60	0.1†

*Immobilized on controlled pore glass.
†Expressed as per cent of alcohol in blood.

Table 2
Types of experiments performed

Experiment	Population	Condition	Weight (kg)
I	24 individuals (12 M and 12 F)	At rest and on an empty stomach	47–83
II	12 individuals (6 M and 6 F)	On an empty stomach and after physical exertion	47–83
III	12 individuals (6 M and 6 F)	At rest and after eating	47–83

individual. The experiments performed were grouped into three types as shown in Table 2. "Physical exertion" involved two periods of 10 min jogging before the first and second samplings and "after eating" the ingestion of a copious meal.

Influence of mouth rinsing before the first saliva sampling

To obtain a representative sample it was necessary to free the mouth from residual ethanol from the intake, so that the measurement was representative of the analyte in equilibrium with blood [10]. To estimate the error arising from the sampling conditions a series of experiments involving two successive samplings with and without rinsing the mouth with water 10 min after finishing the intakes (the interval between both samplings was negligible) was performed. The results of these determinations (Table 3) show that concentrations of ethanol up to 90% higher may be obtained when sampling is performed before rinsing the mouth; confirming the need to perform the rinsing prior to sampling. As the results obtained in the second sampling were consistent with their counterparts in other fluids, the first sampling of saliva was performed after rinsing the mouth in subsequent experiments.

Qualitative aspects of the study

The average values for the concentrations of ethanol in breath, saliva and blood at

Table 3
Influence of mouth rinsing on the sampling of saliva 10 min after intake

Experiment	Ethanol concentration $\|SAL\|^*$ before R	$\|SAL\|^*$ after R	Difference	% Increment
1	8.31	7.55	0.76	10.06
2	22.70	19.00	3.70	19.47
3	10.98	9.46	1.52	16.07
4	11.75	7.74	4.01	51.81
5	10.70	7.74	2.96	38.24
6	9.27	6.21	3.06	49.28
7	12.32	10.22	2.10	20.55
8	13.66	10.40	3.26	31.35
9	10.22	5.64	4.58	81.21
10	11.56	6.02	5.54	92.03
11	10.41	8.50	1.91	22.47
12	9.26	6.40	2.86	44.69

*Concentration in $\mu g\ ml^{-1}$; R, rinsing.

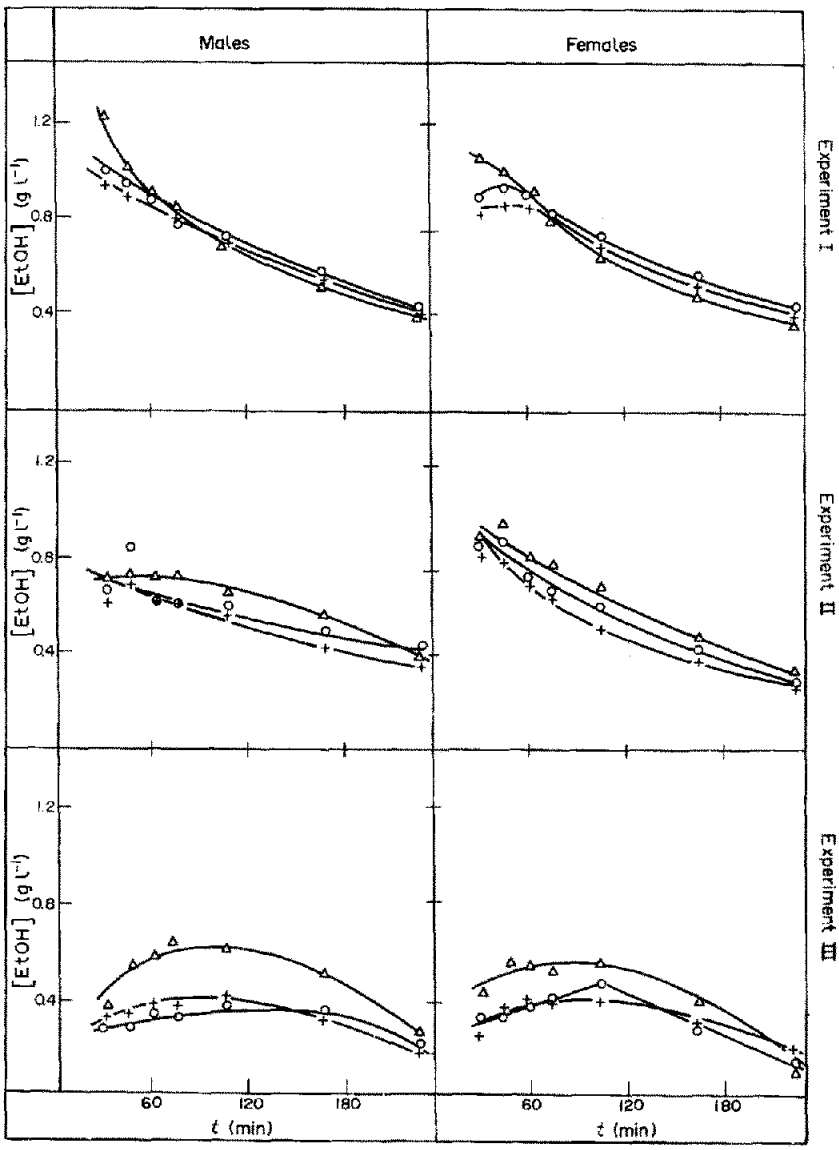

Figure 1
Evolution of the ethanol content in breath (○), saliva (△), and blood (+) for the three types of experiments.

various times in the three experiments are shown (Fig. 1). The distinction between sexes reveals a slightly different behaviour, namely:

(i) the maximum of the ethanol concentration in all three fluids shifted with time (30 and 45 min for males and females, respectively) after finishing the intake in experiment I;
(ii) physical exertion had no influence on the shape of the biotransformation curves but shifted them in males, 75 min after finishing the intake;
(iii) the change in the shape of the curve in experiment III was similar for both sexes.

The maximum concentration was obtained 105 min after finishing the intake in all three fluids.

Establishment of the model

Owing to the shape of the curves, the data corresponding to the first sampling were deleted.

Definition of variables. The development of the model required the prior definition of the variables involved, namely: condition (E), sex (S), weight (W) and time; of these, the first two are qualitative and require the formulation of a representative mathematical function by the artificial variable technique (the variable, sex, has values -1 and $+1$ representing male and female, respectively; the variable E must be represented by two artificial variables, $E_1 = 1$, $E_2 = 0$ for condition 1; $E_1 = 0$, $E_2 = 1$ for condition 2, and $E_1 = -1$, $E_2 = -1$ for condition 3. It was also necessary to consider, in principle, the interactions between variables (condition–time, condition–weight, condition–sex, time–weight, time–sex and weight–sex, which proved to be insignificant for the model.

Study of the individual relationship between the data

By using the general analysis of variance/covariance BMDP-3V [13] program for the variance analysis of the ethanol concentrations in breath, saliva and blood were treated in pairs. In Table 4 are listed the average values of the individual determination coefficients (r^2) in the three groups of experiments, as well as their relative standard deviations (RSDs). From these data one may conclude that there is a better relationship between the ethanol concentration in saliva and blood, which is logical taking into account that these biological liquids are less markedly influenced by external factors than breath. Thus, in experiment III, the breath–saliva relationship had a determination coefficient of 0.729, with an RSD of 43.18%, which indicates a wide variation in the results for individuals ingesting food. Similar though less significant, was the behaviour of the breath–blood relationship. Physical exertion resulted in no increase in dispersion with respect to individuals at rest.

Study of the joint linear relation of the data

The program BMDP-3V [13] was used to treat the ethanol concentrations obtained from breath, saliva and blood for the entire population in pairs at each time (see Table 5).

Table 4
Comparative study of the linear regression analysis of the individual data

Experiment	Cases	Determination coefficient (average)	RSD (%)	Related methods
		0.8825	13.80	Saliva–blood
I	24	0.8652	12.85	Breath–blood
		0.8647	11.89	Breath–saliva
		0.9105	11.36	Saliva–blood
II	12	0.9008	9.64	Breath–blood
		0.8537	14.67	Breath–saliva
		0.8600	16.96	Saliva–blood
III	12	0.7544	32.68	Breath–blood
		0.7290	43.18	Breath–saliva

Table 5
Comparative study of the joint linear regression data

Experiment	Cases	Correlation coefficient (r) Saliva–blood	Blood–breath	Breath–saliva
I	24	0.8068	0.8501	0.8846
II	12	0.9592	0.9616	0.9476
III	12	0.9288	0.6856	0.7836
Overall	48	0.8875	0.9178	0.9044

The overall experiment included all 48 cases, including each of the three sample types. Discrimination between sexes enabled the determination of the influence of this variable on the linear relationship. The most significant influence was obtained in experiment II on the saliva–blood relationship (slopes of 0.582 and 1.287 for females and males, respectively).

The overall correlation coefficients were rather variable. They were satisfactory for experiment II, which indicates that the agreement between the ethanol concentration in all three fluids was better for empty stomachs and after physical exertion. Conversely, the concentration of ethanol in blood found after eating was very different from that in breath, the correlation coefficient in this case being 0.4347. The overall experiment involving all four cases in the different conditions yielded correlation coefficients of between 0.7877–0.8424, the average of those given above.

Linear models of covariance analysis

In order to develop a model accounting for the changes in a variable (ethanol concentration in this case), denoted by Y, an experiment taking into account various factors causing such changes was designed. These factors can be quantitative or covariables (time elapsed from the intake, t; weight–dose, W; breath measurements, BR; saliva measurements, SAL), and qualitative or treatment variables (sex and physical condition), as well as the possible interactions of combined effects of two or more factors. In each model the endogenous variable, Y, was divided into two parts; the estimated value, $\hat{Y}$, accounted for causal factors and random disturbance ϵ, which represents the variability of Y not accounted for by causal factors; thus

$$Y = \hat{Y} + \epsilon = f(t, W, BR, SAL, S, E) + \epsilon, \tag{1}$$

which must be specified by determining the functional form and selecting the significantly explicative variables; estimated by using the least-squares method with artificial variables for the categorical factors; and contrasted with the correct specification of the model. Then, it can be used for predictions (e.g. back extrapolation for estimation of alcoholemia at a time prior to measurement).

The detection of causal models was performed by the corresponding statistical T and F-tests of hypotheses; yet, as it was necessary to perform tests of hypothesis of type (1) estimated by least-squares methods with artificial variables. From general formulation, numerous alternative models were tested by residual analysis and T and F-tests of the model parameters.

$$Y_t = b_0 + b_1 W + b_2 t + b_3 SAL + e_1 E_1 + e_2 E_2 + s_1 S_1 + b_4 E_1 t + b_5 E_2 t + \epsilon_t,$$

where

$$S = e_1E_1 + e_2E_2;\ S = s_1S_1.$$

The extrapolation with the estimated regression models allows performing predictions by point and by interval. For a measurement performed at time t, the model selected in each case allows estimation of the value of Y at time $t - \Delta t$, for which it is necessary to assign values to the explicative variables used in each model. Such variables are represented by the vector:

$$\mathbf{x}_i = \begin{pmatrix} 1 \\ \text{Weight} \\ t - \Delta t \\ |BR| \\ |SAL| \\ \vdots \end{pmatrix}$$

for the individual i, on which the prediction is made. Thus, the estimation by point of the variable Y for individual i is obtained from the expression:

$$\hat{Y}_i = b_0 + b_i\text{Weight} + b_2(t - \Delta t) +, \ldots, = \mathbf{bx},$$

with a $1 - \alpha$ confidence interval for Y of

$$I_{(1-\alpha)}\ (\hat{Y}_i) = (\hat{Y}_i - \eta;\ \hat{Y}_i + \eta),$$

the amplitude interval being

$$\eta = t_{\alpha/2(288-k-1)} \cdot \bar{s}_e \cdot \sqrt{1 + \mathbf{x}_i'\ (X'X)^{-1}\mathbf{x}_i},$$

with $\mathbf{b}$ = vector of the estimated model coefficients, $\bar{s}_e$ = quasi-residual standard deviation, $t_{\alpha/2(288-k-1)}$ = the quantyl $1 - \alpha/2$ of the Student's t-distribution, with $288 - k - 1$ d.f., k = number of explicative variables, and $n = 288$ the number of data (48 individuals and six samples per individual).

Selected models

Three models were selected, namely those covering to the greatest extent the variance Y accounted for the causal factors, i.e. those corresponding to the estimation of the ethanol concentration in blood from that found in saliva, breath and both.

The models were developed from the series of multiple regression programs in the statistical ADDE [14] package and that or PIR from the regression series of the BMDP package.

Blood–breath model

The most suitable model for prediction of the ethanol concentration in blood from that in breath is illustrated in Table 6, which indicates that the 80.67% of the variance of Y is

Table 6
General models

Relation	Equation	r^2	s_e	$F_{8.279}$
Blood–breath	$Y_t = 0.6684 - 0.004587W - 0.001028t + 0.45019\|BR\|_t$ (−4.05) (−7.19) (10.07)			
	$+ 0.1304E_1 + 0.1537E_2 - 0.0485S_1 - 0.000327E_1t$ (6.08) (5.88) (−3.76) (−2.29)	0.8067	0.1117	145.52
	$- 0.000577E_2t + \epsilon_t$ (−3.39)			
Blood–saliva	$Y_t = 0.7828 - 0.006804W - 0.000871t + 0.4139\|SAL\|_t$ (−6.11) (−5.21) (8.80)			
	$+ 0.1685E_1 + 0.2218E_2 - 0.06536S_1 - 0.000331E_1t$ (8.15) (9.28) (−5.05) (−2.23)	0.7937	0.1150	134.15
	$- 0.000778E_2t + \epsilon_t$ (−4.54)			
Blood–breath–saliva	$Y_t = 0.5571 - 0.00469W - 0.000662t + 0.3263\|BR\|_t$ (−4.29) (−4.15) (6.39)			
	$+ 0.237\|SAL\|_t + 0.1167E_1 + 0.1461E_2 - 0.047S_1$ (4.56) (5.57) (5.77) (−3.77)	0.8201	0.1079	140.85
	$+ 0.000226E_1t - 0.000536E_2t + \epsilon_t$ (−1.62) (−3.25)			

r^2 = Determination coefficient.
s_e = Quasi-residual typical deviation.
$F_{8.279}$ = Snedecor's statistic.
Values in parentheses correspond to T from the Student's test.

accounted for by causal factors. The variance analysis is clearly significant, showing the predictive capacity of the explicative variables. All the coefficients of the model significantly different from zero show a similar behaviour.

The Durbin–Watson statistic is DW = 0.78872, which indicates a slight autocorrelation in the residual; possibly a first-order autoregressive transformation would be desirable.

The estimated value–residue diagrams showed no trends in the variance average, so that the above-described model was considered acceptable and was characterized for the different conditions and sexes (Table 7).

Blood–saliva model

The most suitable model found in this case for predicting the ethanol concentration in blood from that in saliva is also illustrated in Table 6. The model accounted for 79.37% of the covariance of Y explained by causal factors. As with the above-described model, the variance analysis was very significant; hence its clear predictive capacity. The Durbin–Watson statistic indicated a slight autocorrelation (DW = 0.7695) in the residual. If these were clearly autocorrelated, it would affect the back-forecasted Y values.

Table 7
Models characterized by condition and sex

(Condition, sex)	E_1	E_2	S_1	
				Blood–breath model
(1.1)	1	0	−1	$Y_t = 0.8473 - 0.004587W - 0.001355t + 0.45019BR_t + \epsilon_t$
(1.2)	1	0	1	$Y_t = 0.7503 - 0.004587W - 0.001355t + 0.45019BR_t + \epsilon_t$
(2.1)	−1	1	−1	$Y_t = 0.7402 - 0.004587W - 0.001278t + 0.45019BR_t + \epsilon_t$
(2.2)	−1	1	1	$Y_t = 0.6432 - 0.004587W - 0.001278t + 0.45019BR_t + \epsilon_t$
(3.1)	0	−1	−1	$Y_t = 0.5632 - 0.004587W - 0.000451t + 0.45019BR_t + \epsilon_t$
(3.2)	0	−1	1	$Y_t = 0.4662 - 0.004587W - 0.000451t + 0.45019BR_t + \epsilon_t$
				Blood–saliva model
(1.1)	1	0	−1	$Y_t = 1.0167 - 0.006804W - 0.001202t + 0.4139SAL_t + \epsilon_t$
(1.2)	1	0	1	$Y_t = 0.8859 - 0.006804W - 0.001202t + 0.4139SAL_t + \epsilon_t$
(2.1)	−1	1	−1	$Y_t = 0.9015 - 0.006804W - 0.001318t + 0.4139SAL_t + \epsilon_t$
(2.2)	−1	1	1	$Y_t = 0.7707 - 0.006804W - 0.001318t + 0.4139SAL_t + \epsilon_t$
(3.1)	0	−1	−1	$Y_t = 0.6264 - 0.006804W - 0.000093t + 0.4139SAL_t + \epsilon_t$
(3.2)	0	−1	1	$Y_t = 0.4956 - 0.006804W - 0.000093t + 0.4139SAL_t + \epsilon_t$
				Blood–breath–saliva model
(1.1)	1	0	−1	$Y_t = 0.7208 - 0.0047W - 0.000928t + 0.3263BR_t + 0.237SAL_t + \epsilon_t$
(1.2)	1	0	1	$Y_t = 0.6248 - 0.0047W - 0.000928t + 0.3263BR_t + 0.237SAL_t + \epsilon_t$
(2.1)	−1	1	−1	$Y_t = 0.6335 - 0.0047W - 0.000932t + 0.3263BR_t + 0.237SAL_t + \epsilon_t$
(2.2)	−1	1	1	$Y_t = 0.5395 - 0.0047W - 0.000932t + 0.3263BR_t + 0.237SAL_t + \epsilon_t$
(3.1)	0	−1	−1	$Y_t = 0.458 - 0.0047W - 0.000126t + 0.3264BR_t + 0.237SAL_t + \epsilon_t$
(3.2)	0	−1	1	$Y_t = 0.364 - 0.0047W - 0.000126t + 0.3263BR_t + 0.237SAL_t + \epsilon_t$

The estimated value–residue plots show a trend towards positive residues; nevertheless, it can be considered acceptable and can be characterized for different conditions and sexes.

Blood–breath–saliva model

The two methods proposed above can be improved if the ethanol concentrations in saliva and breath are used as joint variables in a single prediction model (as shown in Table 6), in which 82.01% of the variance of Y is explained by causal factors; DW = 0.7698.

The estimated value–residue diagram does not show in this case the variance trend of the above-described model. In Table 7 the model characterized for the different conditions and sexes is illustrated.

Comparison of the models

The same sequence in the influence of the variables can be observed in all three models (Table 7), but the joint model shows a greater contribution to the breath variable than does the saliva variable.

A smaller determination coefficient is obtained when the main variables (saliva, breath) are considered separately. This indicates that the independent variable (ethanol concentration in blood Y) is accounted for better by the causal factors when the two dependent variables are used jointly. On the other hand, the residual typical deviation decreases in the third model, making it more reliable than the other two.

Application of the proposed models to real cases

From the study of the variation of the ethanol concentration in saliva and breath with time, it can be inferred that the curves conform to the exponential regression equation:

$$|\mathrm{EtOH}|_{BR,SAL} = A_0\, e^{-bt}.$$

The features of these curves for each fluid are summarized in Table 8. These models have been estimated by least-squares after a logarithmic transformation.

Table 8
Features of the exponential curves

Sample	A_0	b	r^2
Saliva	1.247	5.425×10^{-3}	0.9865
Breath	1.146	4.288×10^{-3}	0.9982

If the expressions for each sex are considered separately, a great similarity between their coefficients (slight differences in the third decimal place) is found. Thus, the average was considered and a generic equation was used for both sexes.

In this manner, a first approximation can be made:

$$|BR|_t = A_0\, e^{-bt},$$

where A_0 considered the influence of the individual and b is the relative measure of constant decrease of the exponential curve.

By taking natural logarithms,

$$\ln |BR|_t = \ln A_0 - bt$$

and

$$\ln |BR|_{t-\Delta t} = \ln A_0 - b(t - \Delta t).$$

Subtracting and rearranging gives the expression:

$$\ln |BR|_{t-\Delta t} = \ln |BR|_t + b\Delta t,$$

which enables the estimation of the ethanol concentration at any time prior to the sampling event.

By using a similar procedure for samplings performed at different known times, several estimated ethanol concentrations in breath or saliva at a time prior to the sampling can be obtained. These, in turn, enable the calculation of the ethanol concentration in blood at the same time prior to the sampling in either fluid by applying the blood–breath or blood–saliva model.

To establish the reliability of prediction with the proposed models, experiments were carried out on 12 individuals of both sexes in the three different conditions (blood, saliva and breath) at different time intervals and analysed by the methods described above.

This study is an approximation to a real case of a traffic accident after which the individual is taken to a suitable centre (hospital or surgery), where two saliva or breath samples are taken at a known interval after the actual time of the accident. Thus, considering samples of both fluids (saliva and breath) the following parameters are known:

$\Delta t_1 = t_1 - t_0$ = time elapsed between the accident and the first sampling;
$\Delta t_2 = t_2 - t_0$ = time elapsed between the accident and the second sampling;
$[BR]_1$, $[SAL]_1$ = concentration in breath and saliva found at t_1;
$[BR]_2$, $[SAL]_2$ = concentration in breath and saliva found at t_2;

which, substituted into the above expressions, enables the measurement of the ethanol concentration in blood at the time of the accident from two samples of breath or saliva taken at later known times. The results obtained in these experiments are summarized in

Table 9
Results obtained by the application of the model to real cases. Breath–blood

Experiment	Sex	Δt	$\|BR\|_t$	$\|BR\|_{t-\Delta t}$	$\|BR\|^*_{t-\Delta t}$	$\|BL\|^*_t$	$\|BL\|^*_{t-\Delta t}$	$\|BL\|_t$	$\|BL\|_{t-\Delta t}$
I	F	165	0.3	0.6	1.008	0.356	0.896	0.330	0.996
		105	0.5		0.950	0.528	0.873	0.562	
II	F	120	0.4	0.8	0.915	0.437	0.831	0.454	0.892
		60	0.5		0.757	0.563	0.760	0.608	
I	F	105	0.5	0.6	0.950	0.482	0.827	0.550	0.885
		45	0.6		0.793	0.608	0.756	0.704	
I	F	180	0.7	0.7	1.471	0.555	1.146	0.700	1.027
		30	1.1		1.228	0.938	1.037	1.023	
I	M	60	0.6	0.7	0.857	0.644	0.841	0.708	0.981
		30	0.7		0.828	0.730	0.829	0.869	
I	M	165	0.4	0.8	1.107	0.391	0.934	0.435	0.915
		105	0.4		0.850	0.473	0.818	0.592	
I	M	120	0.6	1.1	1.114	0.567	0.962	0.504	1.027
		30	0.8		0.929	0.779	0.878	0.992	
I	M	60	0.9	1.0	0.557	0.765	0.692	0.582	0.829
		15	0.9		0.964	0.826	0.876	0.992	
II	F	105	0.3	0.6	0.750	0.297	0.634	0.316	0.631
		45	0.4		0.593	0.419	0.564	0.472	
II	M	165	0.1	0.7	0.807	0.167	0.696	0.260	0.627
		105	0.3		0.750	0.334	0.670	0.296	
III	F	180	0.2	0.2	0.971	0.258	0.687	0.150	0.575
		120	0.3		0.815	0.330	0.616	0.212	
III	M	105	0.2	0.5	0.650	0.234	0.484	0.263	0.506
		45	0.4		0.593	0.351	0.458	0.437	

*Values estimated by the model.

Table 10
Results obtained by the application of the model to real cases. Saliva–blood

Experiment	Sex	Δt	$\|SAL\|_t$	$\|SAL\|_{t-\Delta t}$	$\|SAL\|^*_{t-\Delta t}$	$\|BL\|^*_t$	$\|BL\|^*_{t-\Delta t}$	$\|BL\|_t$	$\|BL\|_{t-\Delta t}$
I	F	165	0.239	0.812	1.134	0.369	0.938	0.330	0.996
		105	0.344		0.904	0.484	0.842	0.562	
II	F	120	0.277	0.831	0.928	0.389	0.802	0.454	0.892
		60	0.564		0.889	0.580	0.786	0.608	
I	F	105	0.439	0.831	1.009	0.456	0.818	0.550	0.885
		45	0.554		0.879	0.575	0.764	0.704	
I	F	180	0.516	1.023	1.411	0.511	1.098	0.700	1.027
		30	0.869		1.032	0.847	0.877	1.023	
I	M	60	0.659	1.118	0.985	0.686	0.893	0.708	0.981
		30	0.812		0.975	0.985	0.888	0.869	
I	M	165	0.334	0.735	1.229	0.407	0.976	0.435	0.915
		105	0.229		0.799	0.435	0.798	0.592	
I	M	120	0.592	1.194	1.243	0.592	1.006	0.504	1.027
		30	0.888		1.051	0.823	0.827	0.992	
I	M	60	0.745	1.091	1.070	0.701	0.907	0.582	0.829
		15	1.054		1.135	0.883	0.934	0.760	
II	F	105	0.450	0.675	0.975	0.326	0.682	0.316	0.631
		45	0.563		0.807	0.452	0.611	0.472	
II	M	165	0.203	0.776	1.098	0.211	0.799	0.260	0.627
		105	0.367		0.37	0.358	0.733	0.296	
III	F	180	0.140	0.915	1.116	0.228	0.649	0.150	0.575
		120	0.360		1.011	0.325	0.605	0.212	
III	M	105	0.366	0.822	0.936	0.252	0.510	0.263	0.506
		45	0.602		0.846	0.368	0.472	0.437	

*Values estimated by the model.

Tables 9 and 10 for breath–blood and saliva–blood models, respectively. In column Δt, the first datum corresponds to Δt_1 and the second to Δt_2; $[BR]_t$, $[SAL]_t$ and $[BL]_t$ are concentrations in samples taken at t_1 and t_2; $[BR]_{t-\Delta t}$, $[SAL]_{t-\Delta t}$ and $[BL]_{t-\Delta t}$ are concentrations estimated by the exponential model; and $[BR]^*_{t-\Delta t}$, $[SAL]^*_{t-\Delta t}$ and $[BL]^*_{t-\Delta t}$ are concentrations estimated by the proposed model. As can be seen by comparing columns 8 and 9 in Tables 9 and 10, the values estimated by the proposed method and the real values of the ethanol concentration in blood are consistent. The errors are always smaller than 20%; thus, the proposed model is suitable for the prediction in the conditions established by the features of the experiments on which the model is based. The errors provided by the exponential model are very high in some cases.

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