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Precision, Accuracy and Relevance of Breath Alcohol Measurements[1]

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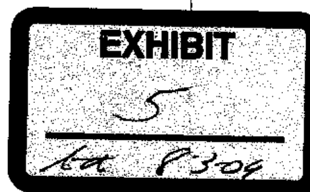

A physiological relationship exists between the concentration of alcohol in end expired alveolar air and in the blood. This is defined by the *in vivo* blood/breath partition ratio which is generally considered to be 2,100 to 1 at an expired breath temperature of 34 °C (2). As a biological parameter the blood/breath partition ratio is subject to interindividual and intraindividual variations (9). Nevertheless, during the post-absorption phase of ethanol metabolism the variability is sufficiently low for breath alcohol analysis to be used as an estimator of the blood alcohol concentration (BAC).

A wide range of methods are available for breath alcohol analysis and depending on the analytical principles involved either a qualitative or a quantitative measure of the underlying blood alcohol concentration is possible. The current interest in breath alcohol analysis as a routine procedure in clinical and medicolegal work has stimulated detailed studies into reliability and usefulness of breath-testing equipment for this purpose.

For many years elaborate programs have been used in our laboratory for evaluating breath-testing instruments, comprising the following experimental stages. (1) *In vitro* studies using breath simulator techniques under various conditions with air-alcohol mixtures of known concentration. (2) *In vivo* experiments with subjects given known doses of alcohol in a laboratory environment. (3) Field studies under double-blind conditions with laboratory control over blood sampling and analysis. (4) Roadside testing of motorists.

This communication concerns recent findings with regard to the physiological background and the experimental and statistical techniques suitable for evaluating the reliability of a wide range of new breath-testing devices. Detailed reports of each individual instrument will be published elsewhere.

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Table I. Breath alcohol instruments evaluated

Instrument or testing device	Operating principle
Mark II gas chromatograph intoximeter	gas-liquid chromatography using a flame ionisation detector
Alcohol screening device (ASD)	fuel cell detector
Breathalyzer model 900	photometric measurement of potassium dichromate reduction
Alcolmeter pocket instrument	fuel cell detector
Alco-Limiter	electrochemical oxidation
Intoxilyzer	infra-red absorption
Alcotest 0.5 and 0.8 ‰ ampoules	visible colour change after potassium dichromate reduction
Alcolyser 0.5 and 0.8 ‰ ampoules	visible colour change after potassium dichromate reduction

Methods

Instruments for Breath Alcohol Analysis

The breath-testing instruments evaluated within this program are shown in table I, as well as the analytical principles used for the detection and analysis of ethanol in breath.

Alcohol Vapour Standards

The errors inherent in the analytical methods employed in each breath instrument have been investigated using air-alcohol vapour standards. Such standards are also used for instrument calibration before testing subjects. Comparisons have been made between a dynamic breath simulator method operating at 34 °C and a new static method based on sampling the equilibrium head space vapour above a known strength aqueous ethanol solution (8).

In vivo *Studies*

Healthy male subjects (n = 55) have been used in the present series of experiments. In general, whisky 36 % w/v was used thoughout the present series and given in a standard dose of 2 ml/kg body weight equal to 0.72 g alcohol/kg. The calculated dose was required to be consumed neat over a period of 20 min. In order to 'standardise' alcohol absorption kinetics the subjects were required to be in a fasted condition.

Blood sampling. A blood sample was taken immediately before drinking to check alcohol-free status and thereafter capillary blood samples were taken in triplicate at regular intervals throughout the whole blood alcohol course. The sample volume (10 μl) was measured using disposable blood pipettes and transferred into Autoanalyser cups containing 1 ml of 0.05 *M* sodium fluoride.

Breath sampling. Breath samples were taken in accordance with the requirements for each specific instrument with emphasis on obtaining end expired alveolar air. The standard procedure was to take one breath sample before and one after the blood sampling, the time difference between the two breath samples being only 2 min. Samples were taken before drinking, at 30-min intervals for the first 2 h after drinking, and thereafter at 1-hour intervals until the subjects were alcohol-free, usually for a total of 7–9 h. This procedure enables each phase of alcohol metabolism to be carefully monitored, and in particular to monitor

the instrument performance when the subjects were around the critical legal limit, 0.5, 0.8 or 1 mg/ml (‰), respectively, and when they were approaching zero blood alcohol.

Blood alcohol analysis. The alcohol concentrations from blood samples were measured using an automated enzymatic micro-technique (4), recently modified to include a distillation step for increased sensitivity (3). This technique has the capacity to analyse 50–60 samples per hour enabling 400–500 independent blood samples to be run each day. The analytical precision for triplicate samples of blood has been shown to be 0.01 mg/ml at a mean concentration of 0.5 mg/ml (3) – 0.5 mg/ml (‰) is equivalent to 50.0 mg/100 ml.

Results

In vitro *Studies*

In vitro experiments are the logical first step in evaluating a breath alcohol instrument since biological variation is eliminated. The merits of the analytical principle and the precision of the sampling mechanism can be investigated under these circumstances. Precision can be estimated from multiple analysis at fixed concentration levels and linearity determined over relevant concentration ranges. To illustrate an example from this stage of testing, some results with the Gas Chromatograph Intoximeter are reported. Figure 1 shows the instrument response measured in peak area units in relation to the blood alcohol equivalent concentration of the two alcohol vapour standards. Ten measurements at each concentration level for both the simulator and head-space standards were made.

The relationship between detector response and concentration for both the simulator and head-space methods of preparing air-alcohol standards was first determined by fitting individual least squares regression lines for each standard. As shown by covariance analysis the slopes as well as the elevations of these two lines were not statistically different, $p > 0.05$ in each case. This implies that they are samples from the same parent population enabling a pooling of the results and the fitting of a single rectilinear regression line. The regression equation was $y = 0.433 + 35.8\,x$ and the y-intercept 0.433 was not significantly different from zero, $t = 0.522$ (d.f. = 6, $p > 0.05$).

A measure of the *in vitro* precision of the gas chromatograph intoximeter is furnished by the variability of the points around the least squares fitted line. In statistical terms this is given by s_{yx}, the error estimate, being ± 0.977 peak area units or 3.70 % of the mean. Dividing s_{yx} by the regression coefficient b gives the variability of the corresponding x-variate (1) being ± 0.027 mg/ml or 3.72 % of the mean blood alcohol equivalent concentration.

In vivo *Studies*

Precision of breath alcohol analysis. As an example of how the *in vivo* precision of breath alcohol instruments has been determined, some results from experiments using the alcohol screening device (ASD) will be presented.

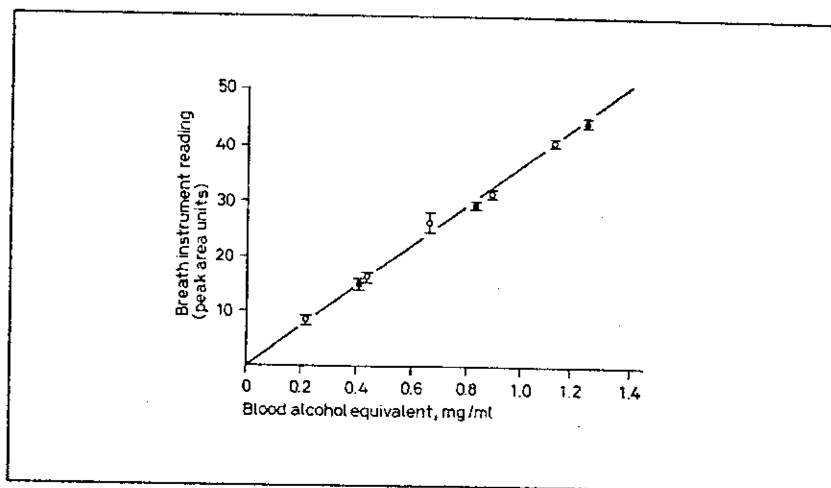

Fig. 1. The *in vitro* relationship between peak area and alcohol vapour concentration using a Gas Chromatograph Intoximeter and air-alcohol standards prepared by two independent methods. The mean and range of 10 determinations at each concentration level are plotted. • = Simulator; o = head space.

The short interval of 2 min between breath sampling means that each pair of readings may be considered as duplicates, the differences between these duplicates were used to estimate the precision and experimental errors of the method. A total of 147 duplicate breath samples were analysed, ranging from 0.04 to 1.30 mg/ml (mean 0.50 mg/ml). The variability (SD) of a single determination was calculated from the SD of the differences between duplicate results according to the following formula:

$$s_x = \frac{\sqrt{\frac{S(d - \bar{d})^2}{n - 1}}}{\sqrt{2}}, = \frac{Sd}{\sqrt{2}}$$

where d = difference between duplicate determinations; $\bar{d}$ = mean of the differences; n = number of differences; s_d = SD (variability) of the differences; s_x = SD (variability) of a single determination.

This procedure requires that the individual differences are randomly distributed with a mean not significantly different from zero. This is proved statistically using a Student's t test, the mean difference was -0.0031 ± 0.0064 mg/ml; $t = -0.4830$ (d.f. = 146, $p > 0.05$) and confirms the random nature of these differences. The SD of the differences was found to be $\pm$ 0.0753 mg/ml and the precision of a single determination $\pm$ 0.0532 mg/ml.

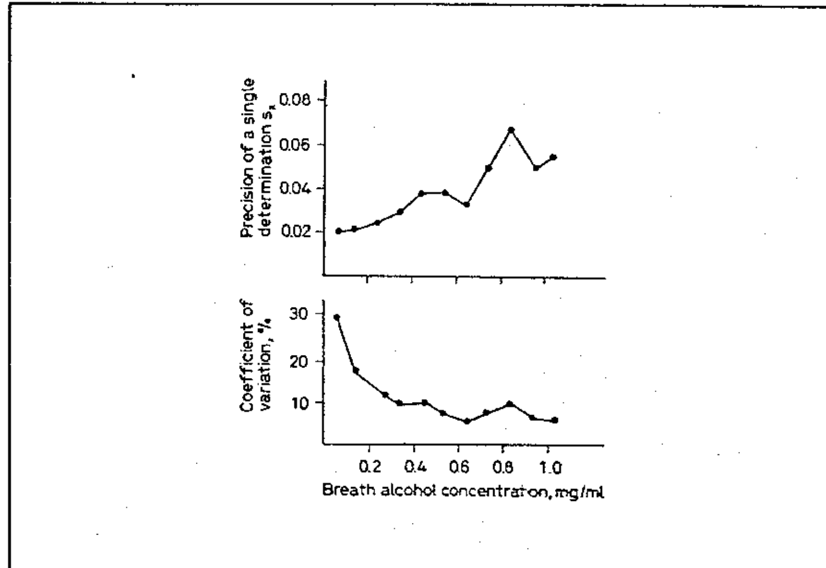

Fig. 2. The precision of breath alcohol analysis as a function of concentration using the ASD. Results based on duplicate determinations.

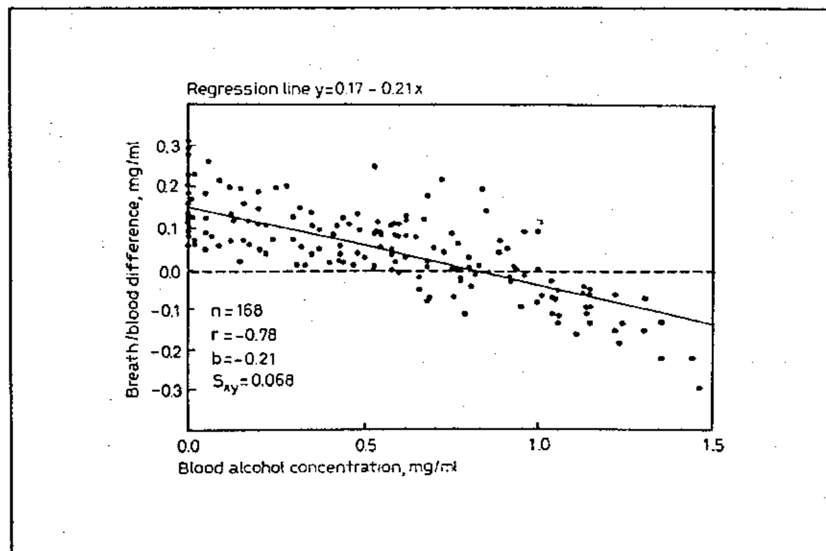

Fig. 3. The breath/blood alcohol concentration difference in relation to the blood alcohol concentration using the ASD for breath analysis.

Since in this work all analyses were based on duplicate determinations, the experimental error is defined as the SD of a double determination, and is calculated as:

$$\frac{0.0532}{\sqrt{2}} = \pm\ 0.038 \text{ mg/ml.}$$

In order to see whether the precision of breath analysis is related to the breath alcohol concentration, precision was related to breath alcohol over a range of concentrations. Figure 2 shows the variability of a single determination in relation to concentration in both absolute (upper part) and in relative terms (lower part).

The precision varies essentially inversely with the variability. The precision in relative terms, i.e. the coefficient of variation, of a single determination was found to be 10.6 % at a mean concentration of 0.5 mg/ml (fig. 2, lower part).

Accuracy of breath alcohol analysis. As a measure of the accuracy of an analytical technique, it is necessary to consider the nature of the differences between the concentration observed and the true values, whether systematic or random. In these experiments, if the results of blood analysis are taken as the standard, the differences between the blood and breath results serve as a measure of the accuracy of the breath instrument. From a statistical point of view, an accurate technique will be characterised by a random distribution of the differences around a mean of zero.

This statistical technique has been used to analyse the results from a field study using the ASD. The breath/blood differences have been plotted against blood alcohol concentration and are shown in figure 3.

The regression of breath/blood difference on BAC is defined by the equation $y = 0.166 - 0.208\,x$ indicating a negative regression with a highly significant correlation $r = -0.776$ ($p < 0.001$). This particular ASD instrument reads too high at BACs below 0.5 mg/ml and too low above 1.0 mg/ml.

The mean breath/blood difference was found to be + 0.051 mg/ml but the individual differences show a *trend* when one considers them in relation to BAC. For example, at zero BAC, the ASD gave readings significantly greater than zero. The maximum ASD difference was found to be + 0.32 mg/ml when the true blood alcohol concentration was zero. The mean zero reading, i.e. the regression equation y-intercept, was 0.166 ± 0.009 mg/ml, $t = 18.44$ ($p < 0.001$).

Blood/breath correlation. The precision and accuracy of breath alcohol instruments may be analysed further using a blood/breath scatter diagram. The results of extensive experiments using the Breathalyzer (5) are shown in figure 4.

The appropriate technique for a mathematical analysis of these data is that of regression analysis, using the BAC as the independent variable. In figure 4, the regression equation was found to be $y = 0.012 + 1.02\,x$ and breath and blood

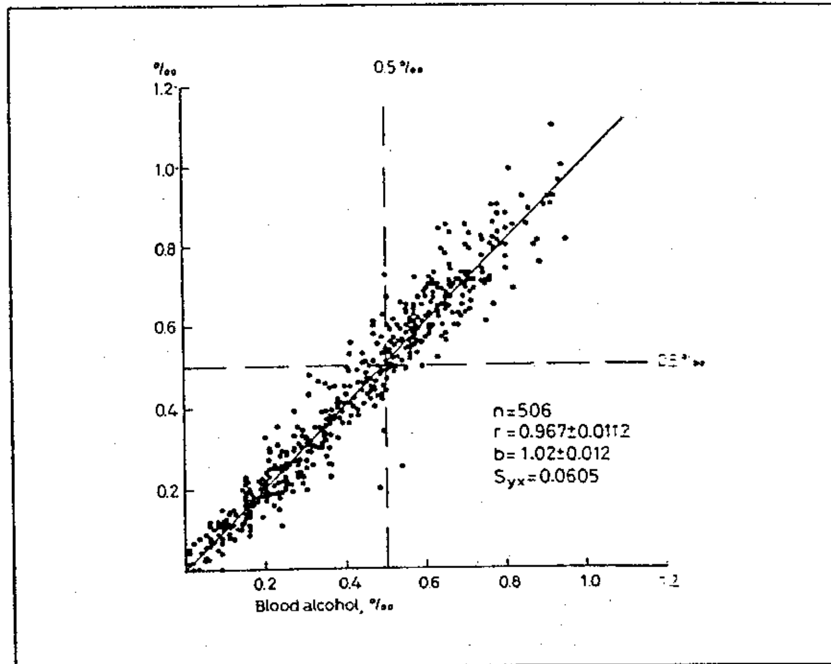

Fig. 4. The regression relationship between simultaneous blood and breath analysis using a Breathalyzer 900.

were highly correlated r = 0.967 ± 0.011 ($p < 0.001$). The regression coefficient 1.021 ± 0.012 ($p < 0.001$) was not significantly different from unity and the y-intercept 0.012 not significantly different from zero, i.e. a perfect 1:1 relationship in this study, there being no systematic differences between the breath and blood alcohol concentrations within the range of BAC levels used. The variability of the individual points around the least squares regression line s_{yx} was low, ± 0.0605 mg/ml, and is a measure of the high accuracy of this instrument.

From this figure of ± 0.0605 the 95 % confidence limits for unbiased estimates of the blood alcohol concentration using this instrument may be shown to be ± 0.13 mg/ml at a mean breath alcohol result of 0.50 mg/ml. This means that if a random breath sample is taken from another subject, there is only a 1 in 20 chance that it will differ by more than ± 0.13 mg/ml from the true blood concentration at the 0.5 mg/ml level.

The blood/breath ratio in relationship to the phase of alcohol metabolism. The design of the present experiments involved serial blood and breath alcohol measurements for the entire time alcohol was in the body, i.e. during the absorp-

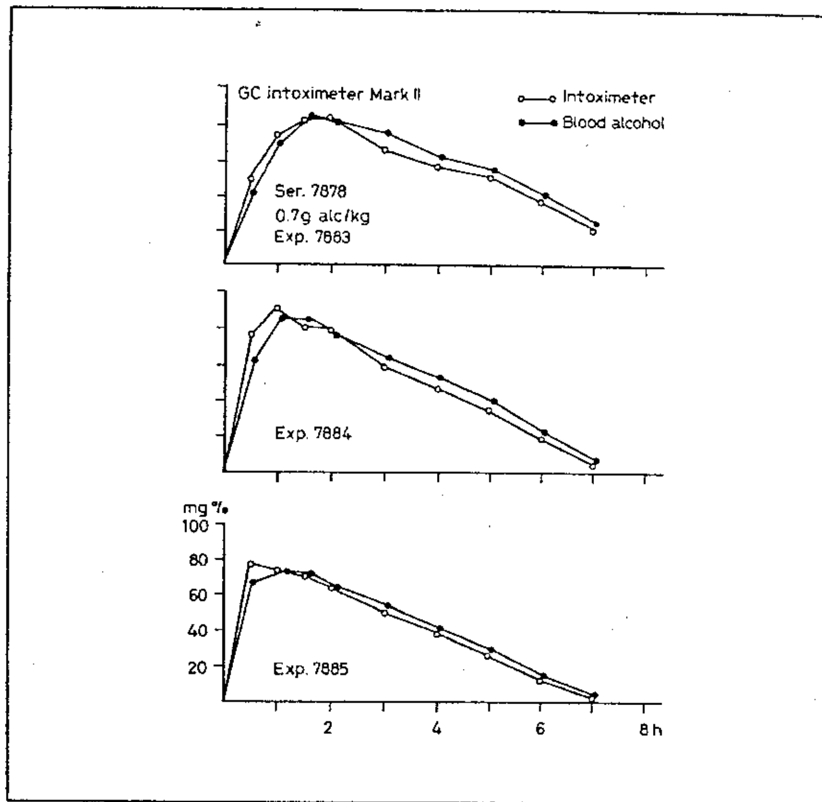

Fig. 5. The alcohol concentration time-course for both breath and blood samples during the absorption, distribution and elimination phase of metabolism in three subjects. Tests were made on the same day and with the same instrument calibrated using a 2,100 to 1 blood/breath ratio.

tion, distribution and elimination phases of metabolism. This enables a study of the variability of the *in vivo* blood/breath relationship. Some typical curves for three different subjects are shown in figure 5. The Gas Chromatograph Intoximeter was used for breath analysis and calibrated using a 2,100 to 1 blood/breath partition ratio.

During active *absorption* of alcohol, in these experiments occurring between 30 and 90 min from the start of drinking, the BAC predicted by breath analysis was somewhat *higher* than that found by capillary blood analysis. The breath alcohol concentration reflects pulmonary arterial blood concentration and during conditions of rapid absorption there is an arterio-venous alcohol difference. *Harger et al.* (6) showed that during the absorption phase, the alcohol concentration in samples of blood from different parts of the vascular system

were in the order arterial > capillary > venous blood concentration; breath alcohol concentration was nearest to the arterial blood concentration in this study.

During the *distribution* phase, between 90 and 120 min, the breath alcohol and capillary blood concentrations show *no* systematic differences, breath analysis being a reliable estimator of BAC during this stage of metabolism. A rectilinear *elimination* of alcohol occurs after the distribution equilibrium has been reached. During this stage, the breath alcohol results *underestimate* the true capillary blood concentration (fig. 5). For most instruments calibrated using a 2,100 to 1 partition ratio systematic *low* readings are obtained; recent studies have shown that the blood/breath ratio *in vivo* at 34 °C is theoretically nearer 2,300 to 1 (8).

Discussion

In experiments to investigate the properties of breath-testing instruments, where the actual BAC is the standard for comparison, it is essential that the method used for blood alcohol analysis has a low experimental error, otherwise part of the differences found between the blood and breath results will be due to the errors in the BAC values.

The experimental error of the present method of blood analysis of ± 0.01 mg/ml is so low that its contribution to the overall variability of the blood/breath relationship has been shown to be negligible.

If at a mean blood alcohol concentration of 0.5 mg/ml the precision in relative terms is taken as ± 2 %, i.e. precision as a percentage of the mean, then from the blood/breath correlation diagram (fig. 4) with an error estimate of ± 0.065 mg/ml or 13 % of the mean concentration, the variability unexplained and resulting from biological sources is given by:

$$\sqrt{13.0^2 - 2.0^2} = \pm\ 12.8\ \%,$$

i.e., the error in blood analysis has an insignificant contribution.

The results from *in vitro* experiments will reflect the maximum limits of precision and accuracy possible for each particular instrument since there is no human variables to be considered. The reliability of the methods of preparing alcohol vapour standards has been confirmed in these experiments by using two independent physical principles, i.e. static and dynamic equilibration operating at two different temperatures, i.e. the vapour pressure-temperature relationship for alcohol reported in the literature may be considered correct (7, 8).

The technique of simultaneous blood and breath analysis and serial sampling from individual subjects enables an exact mathematical expression for the relationship between these two variables to be computed. In addition, the blood/breath relationship can be analysed during each phase of alcohol metabolism and the intra- and inter-subject variability can be investigated.

Our observations imply that the *in vivo* blood/breath partition ratio varies in a regular and systematic way depending on the phase of alcohol kinetics. Theoretically, for a precise estimate of the underlying capillary blood alcohol concentration a different ratio would apply according to the phase of metabolism. If a partition ratio higher than 2,100 to 1 is used, e.g. 2,300 to 1, then a reliable instrument would show no differences during the elimination phase but accentuate the differences during the absorption phase. Conversely, an instrument which happens to show no differences during the absorption phase will show large differences during the elimination phase.

The results found with the ASD are typical of an instrument having a high precision but a low accuracy. The difference between these two terms when applied to an analytical method or breath alcohol instrument need to be clearly defined and differentiated. The precision was ± 0.038 mg/ml for duplicate samples being high considering the many variable factors involved, e.g. breath temperature, breathing pattern of subjects, etc. The accuracy was low, the instrument giving positive readings when there was no alcohol in the blood, and the magnitude and direction of the breath/blood differences changing with the blood alcohol concentration.

The findings from comprehensive evaluation programs, such as that outlined here, enable specifications to be set regarding each particular instrument, covering both *in vitro* and *in vivo* reliability and the practical usefulness of the method. Although there is an inherent biological variability to be considered in any method of breath analysis, the fact that many samples can be taken over a short period of time to give an on-the-spot objective diagnosis of intoxication make a method acceptable for medico-legal work provided that both precision and accuracy are documented and are within the limits of the specifications laid down for each type of breath instrument.

Summary

The methods employed for evaluating the performance of some currently available breath alcohol devices are described. A program consisting of *in vitro* experiments. extensive *in vivo* laboratory studies, controlled field studies, and finally roadside random testing has been used. The importance of considering the underlying physiological mechanisms particularly with regard to the blood/breath partition ratio and its variability is emphasized. An important finding is

that the relationship varies with the phase of alcohol metabolism in a regular and systematic way. Some statistical techniques are outlined for calculating the precision and accuracy of breath-testing instruments, which are important properties of a method to be used for medico-legal purposes.

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