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Determination of Ethanol in Blood: Analytical Aspects, Quality Control, and Theoretical Calculations for Forensic Applications

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Abstract: According to current Swiss traffic law, a person with 0.8 g/kg or more of alcohol in the blood is not permitted to drive. Given special circumstances, driving under the influence of alcohol can be diagnosed even with less than 0.8 g/kg. The Federal Department of Justice and Police defines how the analysis of blood samples taken from road traffic participants has to take place. The Swiss Federal Roads Authority is responsible for the implementation of these regulations. Other measurements of blood alcohol with a forensic background are done in the same way. Each blood sample must be tested fourfold, twice with one method, twice with another. The enzymatic method (ADH) and gas chromatographic methods (GC) are currently certified for this. It is possible to adopt new methods once they receive scientific recognition. Procedures using GC columns with a clearly distinct separation behavior and distinct internal standards are considered as separate methods. As an injection procedure, the headspace technique has gained general acceptance, while the flame ionization detector (FID) is used for detection. With respect to the homogeneity of the four measurements there are statistical specifications to be complied with.
Furthermore there are concrete specifications on the calibration of the test systems and internal quality checks to be implemented. As far as external quality checks are concerned, each laboratory has to participate in four interlaboratory tests each year. These are organized by the Centre Suisse de Contrôle de Qualité (CSCQ) on behalf of the Swiss Federal Roads Authority. The laboratories which perform alcohol testing of blood samples from road traffic participants have to be accredited by the Swiss Federal Roads Authority. At present there are ten accredited laboratories in Switzerland.

Keywords: Analytical chemistry · Ethanol in blood · Forensics

Introduction

Every year in Switzerland more than 15000 driving licenses are confiscated as the result of driving under the influence of alcohol. Our institute, which is in charge of the alcohol testing of blood samples in the area of the cantons St. Gallen, Thurgau, the Grisons, Appenzell Innerrhoden and Appenzell Ausserrhoden, analyzed over 3000 blood samples in 2001. Fig. 1 shows the results of the tests for 2000. The graph evidences, first, a high proportion of high and very high alcohol values and, second, a high proportion of drivers involved in accidents. In the field of clinical-toxicological questions the aim is to rapidly and, with respect to the patient, accurately reach a valid test result. In the case of forensic questions, however, the aim is not only precision and correctness but also a test result that allows the legal-medical specialist to deliver an expert opinion. The Federal Government laid down these requirements in the 'Regulations on the definition of driving under the influence of alcohol' [1]. These regulations specify in detail the staffing and equipment configuration of test laboratories. On the basis of the law on road traffic [2] the following is regulated: the taking of blood samples, the medical examination of the subject, the testing and subsequent storage of blood samples, the storage of documents as well as the interpretative basis for the test results. The correct implementation of the regulations is furthermore monitored by the so-called FABA commission (Kommission für Fragen der Fahrfähigkeit wegen Alkohol-, Betäubungsmittel- oder Arzneimittelkonsum) which represents the Swiss Federal Roads Authority, the Federal Office of Metrology and Accreditation, as well as experts from the laboratories and from the medical field. This commission monitors the implementation of the regulations for the whole of Switzerland.

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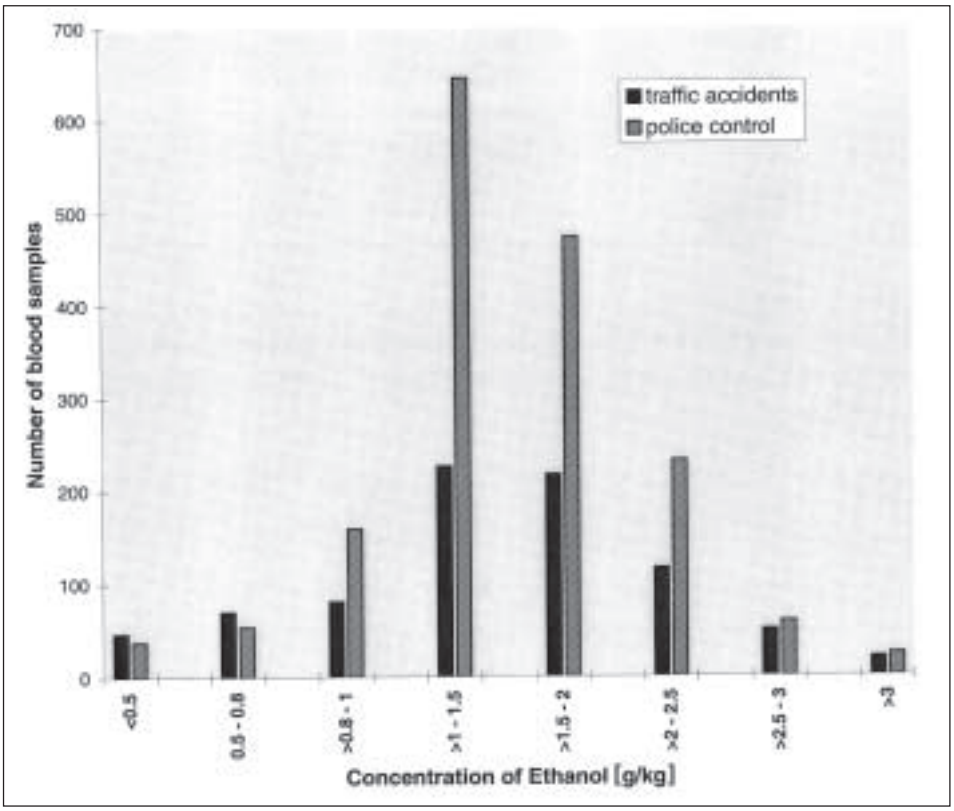

Fig. 1. Distribution of blood alcohol concentrations of samples from road traffic, which were tested in the year 2000 by the IRM SG

2. Measurement

Each blood sample must be tested fourfold, twice with one method and twice with another. The following methods are mentioned in the regulations:

- enzymatic method (ADH)
- gas chromatography methods (GC)

Some laboratories use the enzymatic method together with a gas chromatographic method, the majority of accredited test laboratories, however, use two different gas chromatographic methods.

2.1. *Determination of Alcohol Concentration*

2.1.1. *Enzymatic Method (ADH)*

The enzymatic method is based on the catalytic oxidation of alcohol to acetaldehyde by means of alcohol dehydrogenase with the concurrent reduction of NAD to NADH. A wide variety of reagents and laboratory instruments is offered for these tests. Enzymatic alcohol measurements can be done with the analytical systems common for clinical chemistry. Hence the method is used in laboratories with a main field of activity in clinical chemistry.

2.1.2. *Gas Chromatographic Methods*

The determination of ethanol in blood samples by gas chromatography uses with preference the headspace technique. The principle of this procedure is the heating up of the sample in a closed system and the analysis of the developing vapor. The concentration of the substance in question in the vapor is proportional to its concentration in the solution. The big advantage of this method is the low level of contamination of injector and column with non-volatile particles of the blood. The conditions for equilibrium do not differ from those of a liquid sample if dilution is sufficient and if the conditions of equilibration are suitable. It is not necessary to add salt. Both packed and capillary columns can be used for the chromatographic separation.

The flame ionization detector (FID) is the only detector with sufficient sensitivity. As mentioned above, in the case of the use of two gas chromatographic methods, the columns in question need to show a distinct separation behavior. Furthermore the internal standards need to be distinct. Tert-butanol and dioxan have proven to work well as internal standards. Important volatile substances such as methanol, acetaldehyde, acetone, i-propanol, n-propanol and n-butanol have to be clearly separated. The equipment is to be calibrated for each test series with at least four standards of different concentrations. Liquid ethanol standard solutions are offered in good quality and in various concentrations by several companies. In the context of internal quality control at least one control serum or one control blood has to be included in each series of measurements. Table 1 describes a GC method. Fig. 2 shows the chromatogram of a blood sample tested with the method described in Table 1. Validating data as well as the results of control measurements, finally, are given in Table 2.

Table 1. Equipment and GC conditions

Sample preparation
In a 10 ml headspace tube 2 ml of internal standard (0.4 ml tert-butanol in 1000 ml water) are added to 200 mg of sample blood. After that the bottles are tightly closed.

Equipment and GC conditions

Headspace:		CombiPal (CTC Analytics)
	Incubat Temp.	50 °C
	Incubat Time	10 min
	Agi Speed	500 rpm (on 5 sec, off 45 sec)
	Sample Volume	0.5 ml
	Needle Temp	55 °C
Gas chromatography		Carlo Erba HRGC 5300 Mega
	Column	DB-ALC1 (J&W Scientific)
		30 m × 0.32 mm i.d., 1.8 μm film thickness
	Oven Temp.	120 °C
	Inlet Temp.	200 °C
	Detector Temp. (FID)	250 °C (Carlo Erba EL 480)
	Carrier Gas	N_2
	Split	20 : 1

2.2. *Determination of Blood Alcohol Content*

The data communicated to the investigation authorities consists of the mean of the four measurements and the confidence interval. The width of the confidence interval was determined by the legislator on the basis of a multitude of statistically analyzed test data.

The confidence interval for samples with a mean of up to 1.0 g/kg is set at ±0.05 g/kg whereas for samples with a mean higher than 1.0 g/kg it is set at ±5% of the mean. For example: A measured mean of 0.80 g/kg results in a confidence interval of 0.75 g/kg to 0.85 g/kg. To determine whether someone has been driving under the influence of alcohol or not the lower value (0.75 g/kg) is regarded as relevant.

2.3. *Internal Quality Control*

The spread of the four measurements has to be located within the confidence interval with a probability P1 of at least 95%. Another requirement of the regulations states that the global reproducibility, which is represented by the standard deviation of the samples of a test series, is smaller or equal to a defined standard deviation, and this with a probability of at least 95%. The corresponding calculations are stated in detail in the report of the working group of Perrochet [3]. The group has developed an Excel program, which allows a comfortable calculation of the necessary data. The report as well as the program can be downloaded from the Internet in both German and French (*www.cscq.ch/al/Med_leg_al/med_leg1_al.htm*).

The examination of the reproducibility and the correctness of the test series has to be guaranteed by co-analyzing reference serums and reference blood samples with each series (Table 3). The report exemplifies this with a system of control charts.

2.4. *External Quality Control*

All accredited test laboratories have to participate in four interlaboratory tests each year. The tests are organized by the CSCQ. Each interlaboratory test consists of two samples. The results of the interlaboratory tests are supervised by the FABA commission. In order to check the correct application of the regulations, the commission furthermore regularly arranges laboratory inspections by external specialists.

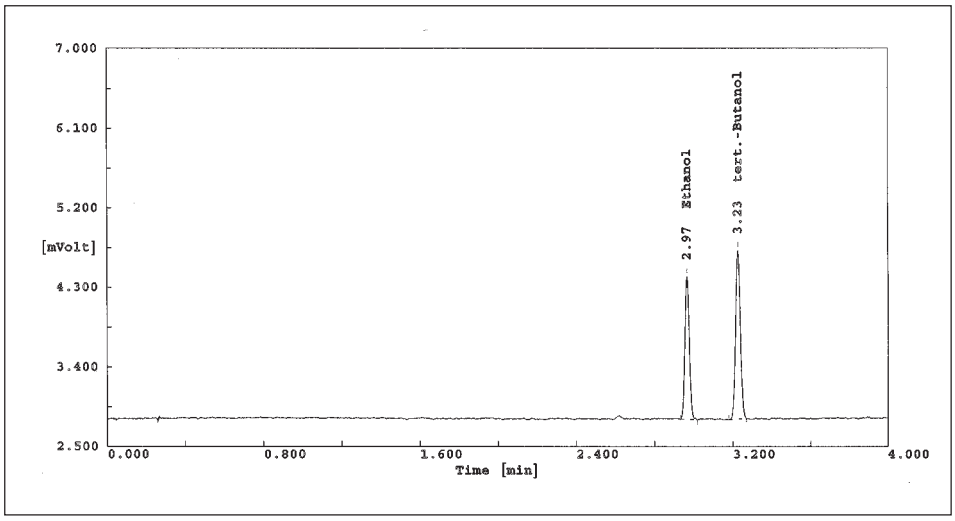

Fig. 2. Chromatogram of a blood sample (1.219 g/kg)

Table 2. Validation and internal quality control

Calibration curve	Calibration points at 0.1, 0.5, 1.0, 2.0, and 3 mg/g Correlation coefficients were typically greater than 0.999	
Limit of detection (LOD)	0.0202 mg/g (DIN 32645)	
Limit of quantitation (LOQ)	0.0442 mg/g (DIN 32645)	
Accuracy and precision	Whole blood control	Serum control
Example of month with 15 measuring series	Medidrug®	Medidrug®
	Assigned value: 0.739 g/kg	Assigned Value: 1.95 g/kg
	N = 15	N = 15
	Mean: 0.733	Mean: 1.940
	SD: 0.010	SD: 0.014
	% CV: 1.4	% CV: 0.74

Table 3: Theoretical blood alcohol calculation after Widmark

$$c(t) = \frac{A}{p \times r} - beta_{60} \times t_{AE}$$

c (t) = presumed blood alcohol concentration at time t
A = quantity of pure alcohol consumed in grams
p = body weight in kg
r = distribution factor
$beta_{60}$ = hourly elimination rate
t_{AE} = time in hours from beginning of drinking to critical incident

Example
A 70 kg man has drunk 1 liter of beer (4.8 vol.%) within 1 h. What is the blood alcohol concentration 2 h after the end of consumption?

A: 1000 ml × 0.048 × 0.8g/ml = 38.4 g of pure alcohol
r : 0.7
$beta_{60}$: 0.15 g/kg

$$c = \frac{38\ g}{70\ kg \times 0.7} - 3h \times 0.15\ g/kg \times h = 0.3\ g/kg,$$ respectively o/oo.

3. Point of Time of Incident Calculation

The resorption of alcohol can basically take place *via* all mucous membranes, the skin and by inhalation. The main place of resorption is the upper part of the small intestine. The resorption of alcohol from the gastro-intestinal tract continues after consumption. The main variable to determine the total duration of resorption is the extent to which the stomach is filled. Due to its physical characteristics the distribution of ethanol in the body is as follows: roughly 96% is found in the body fluid and 3–4% in the fatty tissue. Biotransformation in the liver is by far the most important way of elimination of ethanol. Elimination starts relatively soon after the beginning of drinking. Fig. 3 shows schematically the blood ethanol concentration after consumption of alcohol. In the ascending section of the curve, *i.e.* the drinking phase, resorption outweighs elimination, in the top section the two processes are roughly equal. After the end of resorption the curve reflects the linear reduction.

In some cases it is necessary to calculate the alcohol content of a blood sample back to the point of time when an incident occurred (*e.g.* hit-and-run road accident). Thanks to numerous investigations it is known that the termination of resorption is hardly less than 20 min and hardly more than 120 min after the end of consumption. Once resorption has ended and the distribution in the body has reached a balance, the blood ethanol concentration decreases – largely independently of its initial value – by an average of 0.15 g/kg per hour (beta60 value). As a rule the calculation back in time may only be done within the linear section of the curve, *i.e.* once resorption has terminated. In the case of transgression against traffic law and with the aim of a uniform investigation one assumes a maximum resorption time of 120 min and a minimum hourly reduction of 0.1 g/kg.

4. Approximate Calculation of the Blood Ethanol Concentration

A chemically determined blood alcohol count is most indicative as a proof. In the absence of a chemically determined value the judge has to orient himself on a purely calculated value. The calculation usually takes place with the formula after Widmark (Table 3). The alcohol concentration is calculated on the basis of the consumed alcohol quantity on the one hand and of the body weight of the person on the other. With the help of the distribution factor 'r' individual differences are taken account of. Persons with more fat are granted a relatively low r-value of 0.55–0.6, whereas persons with little fat have r-values of up to 0.80. Elimination of alcohol has to be taken into account already during the drinking phase. For someone with an average constitution the calculation with a distribution factor 'r' of 0.70 and an elimination rate 'beta60' of 0.15 g/kg yields results which usually can be reproduced experimentally quite well.

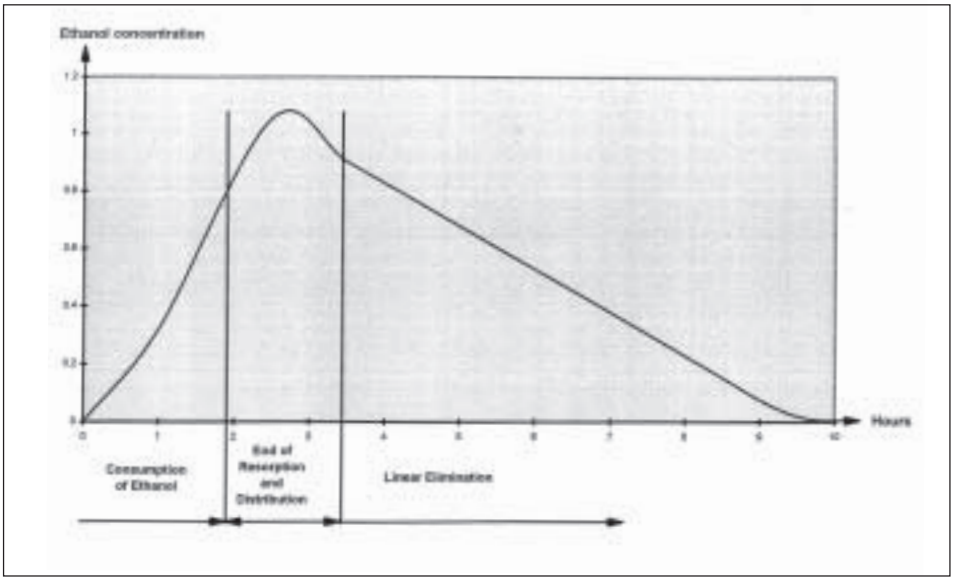

Fig. 3. Blood alcohol concentration as a function of time. The drinking phase, the end of resorption and distribution, and the phase of linear elimination are shown.

5. Conclusions

Blood alcohol testing is still the most frequent testing done in a forensic laboratory. The regulations issued by the Federal Government are meant to ensure a correct measurement of the alcohol concentrations and a uniform interpretation thereof. Test laboratories need to be accredited by the Swiss Federal Roads Authority.

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[1] Weisungen betreffend die Feststellung der Angetrunkenheit, Verordnung über die Zulassung von Personen und Fahrzeugen zum Strassenverkehr (VZV), Art.150, Abs. 6.
[2] Strassenverkehrsgesetz (SVG), 19.12.1985 (under revision).
[3] J.F. Perrochet, 'Statistische Verfahren zur internen Qualitätskontrolle für die Analyse des Blutalkoholwertes', Version 2000.10, Kommission für Fragen der Fahrfähigkeit wegen Alkohol-, Betäubungsmittel- oder Arzneimittelkonsum (FABA), Bundesamt für Strassen. Legally binding since 1.1.2002.