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The Advantages of Automated Blood Alcohol Determination by Head Space Analysis

Gottfried Machata

Institute of Forensic Medicine, Chemistry Department, Vienna (Austria)

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Summary. Precision, specifity and interpretation of the results are reported. Using only few different columns all volatile substances of importance are detected specifically. With new developed stationary phases, graphite as support, the analysis could be done with a sufficient resolution in 1 min. The absolute peak height of the internal standard or the amount of acetaldehyde determines the status of the sample and enables a very precise analysis of alcohol in blood.

Zusammenfassung. Die Genauigkeit, Spezifität und Interpretation der gaschromatographischen Blutalkoholbestimmung wird eingehend besprochen. Zur Abkürzung der Analysenzeiten sind neuentwickelte Säulenfüllungen mit Graphit als Trägermaterial geeignet. Bei gleicher Trennleistung wie mit Standardsäulen kann die Analysenzeit auf 1 min verkürzt werden. Auf die Berechnung des Blutalkoholgehaltes bei verschiedenen Proben (Sera, hämolytische Blute, Leichenblute, Fluoridblute) wird eingehend hingewiesen, wobei zur Kennzeichnung der unterschiedlichen Proben der Wassergehalt bzw. die Acetaldehydmenge herangezogen werden kann.

Key words: Alkohol, Gaschromatographie — Blutalkohol, Gaschromatographie — Gaschromatographie, Blutalkoholbestimmung.

The gas chromatographic determination of blood alcohol is the only routine procedure that enables ethanol to be specifically determined in biological materials and at the same time permits the determination of other volatile components. In routine operation the direct manual injection of the samples, even when the blood samples have been diluted, is not easy to carry out continously. For this reason, mechanized procedures are to be preferred, the head space technique in particular. The introduction of the sample in gas form brings sharp peaks, high separation performance and a stable base line for the chromatogram.

The specifity of a single GC determination is naturally limited. If, however, the volatile components which come into question are examined more closely, there are only a few that must be differentiated. Among such substances are acetaldehyde, acetone, iso-propanol, methanol, ethanol and n-propanol. Furthermore, the internal standard must appear separately. By means of diagrams of the relative retention times (RRT) of four different phases—polyethylene glycol 1500 (K), Hallcomid M 18 (H), Porapak Q (Q) and Porapak R (R)—it can be seen that the phases H and Q are the most suitable for the solution of this problem (Figs. 1 and 2). In practice, however, the phase K is the most widely used because of the shorter total duration of the analysis, even though methanol is not separated from the internal standard.

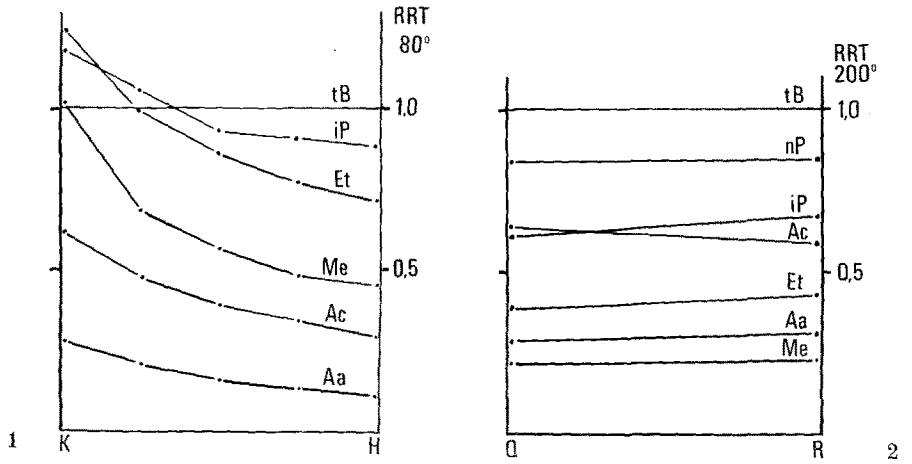

Fig. 1. Relative retention time (*RRT*) of stationary phase *K* (Polyethyleneglycole 1500) and *H* (Hallcomid M 18) of five substances in relation to t-Butanol (*tB*). *Aa* Acetaldehyde, *Ac* Acetone, *Me* Methanol, *Et* Ethanol, *iP* iso-Propanol

Fig. 2. Relative retention time (*RRT*) of phase *Q* (Porapak Q) and *R* (Porapak R)

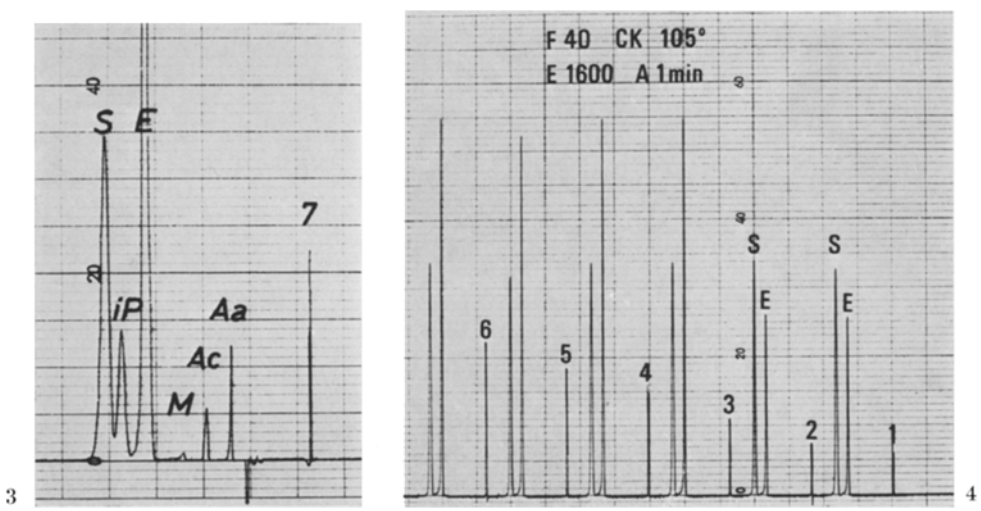

Fig. 3. Analysis of blood after intoxication with technical alcohol (S reference substance: t-Butanol) on Hallcomid with F 40

Fig. 4. Routine analysis of blood alcohol; F 40, Column CK (Polyethyleneglycole 0.4% on Graphite 60/80 mesh), analysis time 1 min

In Europe, methanol is practically without importance in forensic analyses concerned with the road traffic regulations. Furthermore, methanol intoxication is relatively easy to recognize due to the circumstances of the case. If such a case is suspected, the analysis of the blood or urine can be carried out without the

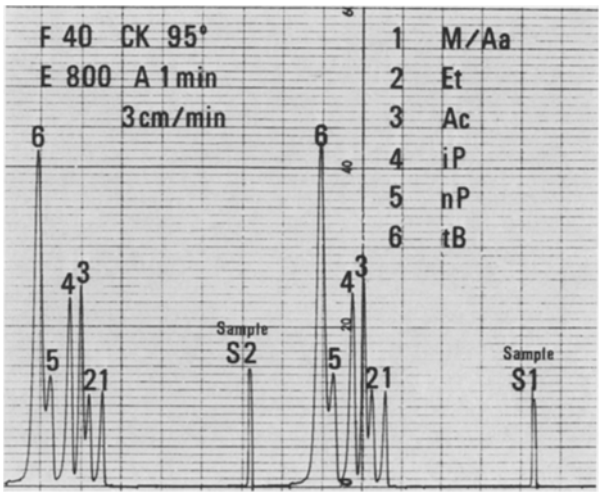

Fig. 5. Analysis of a mixture of different volatile substances; F 40, Column CK; chart speed 3 cm/min, analysis time 1 min

addition of an internal standard, or another stationary phase can be employed. The chromatogram shows such an analysis on a case of technical solvent poisoning (Fig. 3). In principle, SCOT- or WCOT-columns could be employed to obtain a higher separating performance. These capillary columns require a flow-splitter or a special adaptor in the gas chromatograph. A long-term trial of these columns in routine operation has not yet taken place.

A further improvement is the introduction of column fillings having carbon as the support material. Coating the carbon with polyethylene glycol 1500 brings about an excellent separating performance and enables the analysis time to be reduced. A reduction of 1 min in the analysis time gives a time saving for 1000 analyses (in each case double determinations) of 33 hrs, or approximately 4 working days.

In practice, an analysis time of only 1 min is sufficient and the same separating performance is obtained as for a normal carbowax filling (Fig. 4).

By selecting a relatively low column temperature, all substances of interest can be separated in a relatively short time (Fig. 5).

For quantitative determinations, an internal standard is absolutely necessary to maintain the high accuracy and to carry out a continuous check on the functioning of the instrument. Tertiary-butanol has been chosen for this purpose because its vapour pressure curve is parallel to that of ethanol and a change of temperature does not alter the ratio of the vapour quantities to any appreciable extent (Fig. 6) [1, 2]. An internal standard which meets all the requirements is hardly to be found since it must not have a too long retention time (total analysis time) and must not be present in the sample. In particular, the requirements of forensic medicine must be taken into account (putrifying alcohol, solvents).

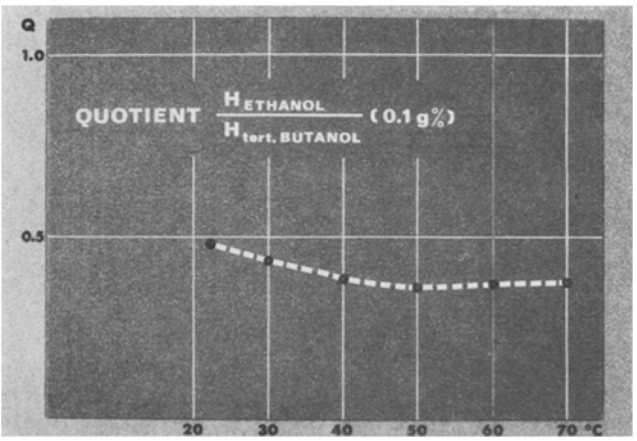

Fig. 6. Relation of the quotient of the vapour pressure from Ethanol and t-Butanol versus temperature

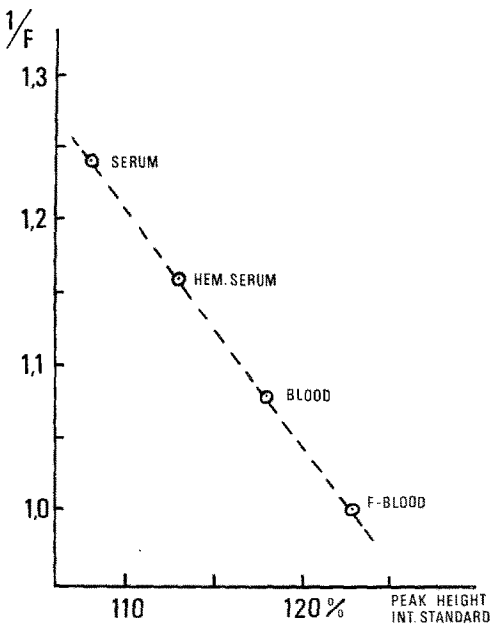

Fig. 7. "Floating factor" for correct calculation of blood alcohol in samples with different water content

The quantitative determination is carried out by determining the ratio of alcohol to internal standard. At the same time, the internal standard can be used for the quantitation of other volatile substances.

For the manual evaluation and in particular with the automatic procedure conversion, factors for the calculation of the blood alcohol concentration must

be taken into account to allow for the various types of sample, i.e. serum or blood-containing serum [3]. The condition of the blood (water content) must also be taken into account so that the original alcohol content—referred to a normal water content—can be determined [4]. For this two procedures are recommended:

1. When alcohol is present, blood also contains intermediate acetaldehyde. This acetaldehyde is bound to the erythrocytes of the blood and is only released by heating to 60°C when the labile thermobands are broken [5]. The quantity is in the first case dependent upon the number of erythrocytes and is thereby proportional to the quantity of blood. The acetaldehyde content is independent of the quantity of alcohol present and is proportional to the Widmark degradation factor β. The peak height of the acetaldehyde therefore gives a good indication of the condition of the blood and can be employed in the evaluation.

2. The alteration of the vapour pressures of volatile components at a given temperature is dependent upon the water content of the sample. The relative alteration of the peak height of the internal standard is proportional to the condition of the sample and is a direct measure for the water content. With manual evaluation, the correction factor can be taken in discreet steps from a table [6]. With EDH (electronic data handling) evaluation, a "floating factor" has been in use for several years which allows a conversion for every vapour pressure (Fig. 7).

This is particularly important in routine determinations in forensic analyses where fresh serum, haemolyzed blood samples, fluoride blood and congealed corpse blood must all be analyzed.

With this procedure, automatic sample recognition is given and the blood alcohol concentration corresponding to normal blood exactly calculated.

Statistical comparisons on fresh, random samples, in each case collectively on 10000 single determinations, gave in comparison to other procedures—Widmark (W) and ADH—a mean value difference of maximum 5% (Table 1). This difference can be noticeably reduced if the second method is automated[1].

With the automated head space procedure [7, 8], the relative standard deviation for a single sample employing an internal standard is $\pm 0.5\%$, while for alcohol alone a value of $\pm 0.7\%$ can be obtained. The deviation of the single

Table 1. Mean differences between gaschromatographic-, Widmark- and TDH-method from several institutes of forensic medicine in GFR

Differences of the average values			
method	N	remarks	mean differences
GC/W (Göttingen)	400	< 100 mg%	4.7 mg%
		> 100 mg%	3.2 mg%
GC/ADH (Aachen)	500	mean 148 mg%	3.6 mg%
GC/ADH (Duisburg)	500	mean 166 mg%	3.2 mg%
GC/ADH (Münster)	600	mean 170 mg%	3.1 mg%

[1] The author thanks the mentioned Institutes of Forensic Medicine for supporting valuable data.

Table 2. Precision datas on blood alcohol analysis by GC with the F 40

Accuracy, repeatability, deviation			
N	remarks	*s*	types
120	100 mg%	± 0.7%	Ethanol, peak height, Aqu.
120	100 mg%	± 0.5%	Q (Ethanol/Standard), Aqu.
1000	50 mg%	± 2.0%	Serum (Q)
270	110—140 mg%	± 1.6%	Serum (Q)

values, calculated from double determinations on 2000 random samples, is ± 4.3% [9] (Table 2).

According to the legal requirements in the majority of European countries, the blood alcohol concentration is the determining forensic factor. A respiratory alcohol determination must be converted to the blood alcohol content. The exact chemical determination of the respiratory alcohol is analytically possible, but the conversion to the exact blood alcohol value is not easy due to the physiological properties. The varying proportion of the alveolar air, the random influence of the respiratory volume and the ratio of respiratory alcohol to respiratory air must all be taken into consideration.

At the present stage of development of instrumental analysis, the GC head space technique is the optimum method for the determination of the blood alcohol concentration. Also, no other analytical method will be available in the foreseeable future which will be better or can replace this technique.

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Professor Dr. G. Machata
Institute of Forensic Medicine
Chemistry Department
A-1090 Wien, Sensengasse 2