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Alternative determination of BAC by means of ^1H – NMR

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Abstract

Blood alcohol determinations play a very important role in the forensic science. By now, blood alcohol is quantified by the headspace – gas chromatography (GC) and the enzymatic alcohol dehydrogenase (ADH). A new alternative method, the ^1H – NMR spectroscopy, is established. The accuracy and precision are comparable with those of GC headspace and of ADH. The simple sample preparation takes 60 seconds, the analysis time only 180 seconds. For the analysis, 20 µL of capillary blood is sufficient. Comparisons of the BAC values, measured by a medical jurisprudence, with those of the ^1H – NMR, show that only 12% of the deviations are not forensically accepted. The differences could be due to various circumstances. A drinking test demonstrates the identical BAC values and composition of capillary and intravenous blood. A forensic usability with the specified limits can be met.

1. Introduction

Since the 1950s the blood alcohol concentration (BAC) is measured with the headspace – gas chromatography (GC) and the enzymatic alcohol dehydrogenase (ADH) [1,2]. In the *ADH technique* the ethanol reacts with the enzyme ADH and the coenzyme nicotinamide adenine dinucleotide (NAD^+) to acetalaldehyde and the reduced nicotinamide adenine dinucleotide (NADH).

$$C_2H_5OH + NAD^+ \overset{ADH}{\rightleftharpoons} CH_3CHO + NADH + H^+$$

$$CH_3CHO + NAD^+ + H_2O \overset{Al\text{-}DH}{\rightharpoonup} CH_3CHO + NADH + H^+$$

The extinction value of the NADH is measured and recalculated into the ethanol concentration [3]. In the *GC – headspace* the intravenous blood is homogenized, mixed with tertiary butanol and heated up to 60°C for 20 minutes. The gaseous ethanol is qualified and quantified by the gas chromatography [4].

The disadvantages of both methods are the long sample preparation time, the need of a high sample volume, no possibility of gauging and the destruction of the sample during the analysis. With the alternative method of ^1H–NMR spectroscopy all disadvantages of the old methods should be eliminated. Here, a capillary blood drop of 20 µL is adequate for a BAC analysis. The aims of this method are the keeping of the demands of the *Guidelines to determine the BAC for forensic purposes* and the introduction of a fast, precise and non – destructive BAC analysis with a minimum volume of capillary full blood.

2. Materials and Methods

Instruments: NMR – spectrometer, 600 MHz, 5mm CPGNP, BRUKER; Microtubes, 1.2 mL; Na–heparinised capillary pipettes, 20 µL; Materials: Deuteriumoxide (D_2O, 99.90%), Trimethylsilyl propanoic acid (TMSP), Dimethylsulfone (DMS, 99.65%), c = 0.5 g/L.

Sample preparation: A solvent of 1 mL D_2O spiked with TMSP is mixed for 60 seconds with an internal standard, a 20 µL capillary filled with DMS and a 20 µL capillary filled with capillary blood. The sample solution is transferred into a NMR tube. The measurement without water suppression occurs with the NMR spectroscopy (500 or 600 MHz) with fixed parameters: NS = 32, D1 = 1.00 sec, LB = 0.3 Hz, TD = 64k.

3. Results and Discussion

The volume of 20 µL capillary full blood is adequate for the determination of the BAC with the same accuracy like the *"old"* methods. The calibration curve with DMS as internal standard shows, that the actual concentration of whole blood is 100.38% of the nominal concentration with a coefficient of determination of 0.9984.

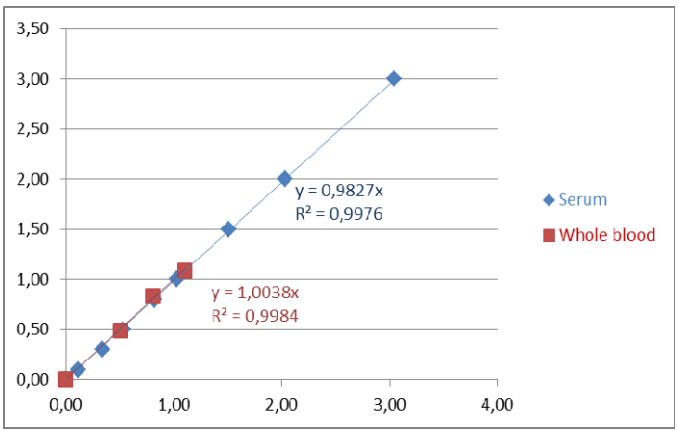

Fig. 1. Calibration curve of serum and whole blood.

The measurement proceeds without water suppression. Experiments show that water suppression lowers the accuracy to 91%. The stability at different storage places, the repeatability, reproducibility, linearity and accuracy are analyzed by preparing calibration series with reference whole blood. A storage of the samples in NMR tubes at room temperature and at 4°C for 14 days shows a concentration changing of ± 2.07%. An influence of light onto the samples in the NMR tubes can be excluded. Repeatability and reproducibility are confirmed. After a seven – months – storage at room temperature the samples show 90% of their former BAC. Factors like personnel, chemical batches and air humidity do not show any effects on the BAC results.

A comparison of the BAC measured with NMR, with GC – headspace and ADH show that 12.6% of the values have a higher deviation than ± 10%. These values are not forensically acceptable. The BAC differences could be due to various circumstances like matrix changing, incorrect storage or adsorption to the sample flask.

A scientific drinking test proves the differences in the BAC and the composition of intravenous and capillary blood. Table 1 shows the deviations of the BAC of intravenous and capillary blood. The values are forensically acceptable.

Tab. 1. BAC with minimum and maximum deviations.

Concentration [g/L]	Min. deviation	Max. deviation
≤ 1.000	0.01 ‰	0.07 ‰
> 1.000	2.31 %	9.00 %

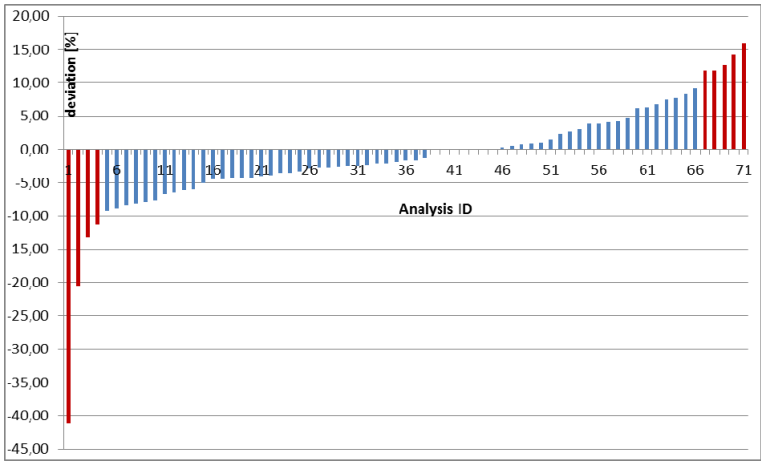

Fig. 2. Deviation [%] of the mean BAC measured with ADH and GC headspace and the BAC measured with ^{1}H – NMR.

4. Conclusion

All experiments show that the ^{1}H – NMR is a good determination method of BAC with the advantages of a simple 60 – seconds – long sample preparation, the possibility of gauging, a small sample volume of 20 µL and a non – destructive short analysis of 180 seconds.

The drinking test proves that a single *capillary* blood drop is sufficient for a BAC determination. A comparison of the BAC results of the forensic jurisprudence, measured by GC headspace and ADH, and of the alternative ^{1}H – NMR – method demonstrates the accuracy of this method with real blood samples. A forensic usability with the specified limits can be met.

5. References

[1] Bücher T, Redetzki H. Eine photometrische Bestimmung von Äthylalkohol auf fermentativem Wege. J Molecular Med 1951;29:35-36.
[2] Machata G. Über die gaschromatographische Blutalkoholbestimmung. Analyse der Dampfphase. Microchimica Acta 1964;52:2-4.
[3] Schwedt G. Analytische Chemie: Grundlagen, Methoden und Praxis. Wiley – VCH. 2008.
[4] Honck M, Siegel J. Fundamentals of forensic science. Academic Press. 2012.