Analysis of Ethanol in Blood using LLE-^{1}H-NMR method with Spinsolve 60 Ultra SPA 1880

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Abstract

The Spinsolve 60 Ultra SPA 1880 NMR is an economic instrument configuration for measuring ethanol in blood. The established method for liquid-liquid extraction of ethanol from blood to organic solvent ensures good repeatability and simple-to-use operation, ideal for a forensic lab.

According to the national standard, driving under the influence of alcohol is established when the blood ethanol concentration is 0.2 g/L or higher. To meet this quantitative requirement, the calibration curve for the method was generated over a wide range from 0.1 g/L to 5.0 g/L. This way, ethanol could be determined in every possible case.

Introduction

Ethanol is a common psychoactive substance that has been widely consumed in several parts of the world. Gas chromatography (GC) coupled with a flame ionization detector (FID) has often been used to determine blood alcohol concentration. However, the gas chromatograph is an expensive instrument that requires an experienced analyst to use this instrument.

This application note provides a simple, reliable method for determining the ethanol content in blood using a simple bench-top NMR instrument.

Strengths: No deuterated solvent is required

Experimental

Typical NMR conditions

Software: Spinsolve 1.17.7
NMR Mode: 1H PROTON+
Scans: 32
Acquisition time: 6.4 s
Repetition time: 15 s
Pulse Angle: 90
Measurement time: 8 min

Sample preparation

Introduce 200 µL of **Cocktail A** to Eppendorf and add 500 µL of blood. Vortex mixture for about 10 min, centrifuge and collect 450 µL of supernatant.

Introduce 450 µL of collected supernatant to Eppendorf filled with **Cocktail B**. Vortex for 15 min and centrifuge. Transfer 450 µL of solvent from the upper layer to new Eppendorf, add hexamethylcyclotrisiloxane (HMCS) serving as an internal standard, vortex for 10 s and transfer to NMR probe.

Results

NMR spectrum

Figure 1 shows the overlapped H-NMR spectra of blank blood samples (A), and ethanol spiked blood samples (B). The ethanol spiked blood samples are prepared by adding ethanol-water solution into a 2.85 mL blank blood sample. They are submitted to the extraction procedure described in the sample preparation section.

Signal integration

Only the central triplet signal of the -CH_3 group is integrated as it is not interfered with matrix components.

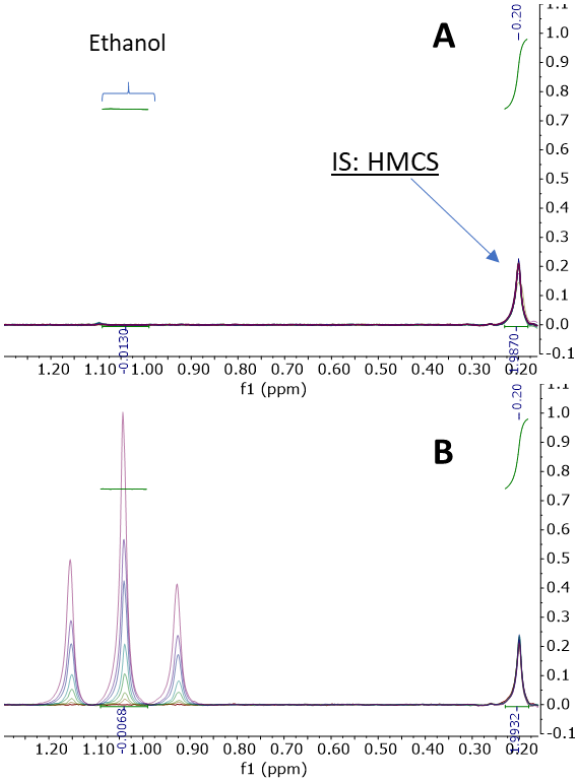

Figure 1. ^{1}H-NMR spectra of blank blood samples (A) and ethanol spiked blood samples (B) with indicated signals for internal standard (HMCS) and ethanol integration zone.

Linearity

Two levels of ethanol-water solution are prepared in 10 mL flasks with concentrations of 10 g/L and 100 g/L. Then appropriate ethanol-water solution and water are added into blank blood sample in 5 mL probe and vortexed. The final concentrations of ethanol standard in blood samples were: 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, 5.0 g/L. Five hundred microliter of each blood standard solution was subjected to liquid-liquid extraction. In the last step, an internal standard was added. Figure 2 shows the calibration curve for seven levels.

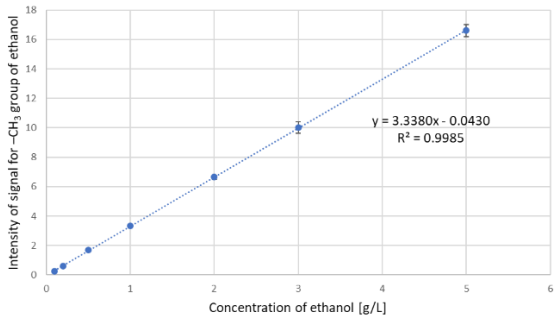

Figure 2. Calibration curve of ethanol standards in a blood sample.

Repeatability

Five spiked blood samples were analyzed with a concentration of 0.2 g/L. The repeatability results are shown in Table 1.

Table 1. RSD (%) Ethanol concentrations in five spiked blood samples established by H NMR

Number of ethanol spiked blood samples	5
The established average concentration of ethanol in blood, g/L	0.2
Standard deviation, g/L	0.01
Relative standard deviation, %	3.0

Accuracy

Certified Reference Materials were investigated (blood samples containing six levels of ethanol) by the established ^{1}H-NMR method. Table 2 shows that differences between concentrations based on both approaches ($|C_{NMR}-C_{CRM}|$) do not exceed 0.05 and combined uncertainty range of both methods.

Table 2. Accuracy of the method for determination of ethanol content in blood concerning certified values

Ethanol in CRM, g/L	Ethanol by NMR, g/L	Rec. %	$\lvert C_{NMR}-C_{CRM}\rvert$
0.189 ± 0.018	0.234 ± 0.045	129	0.045
0.303 ± 0.020	0.305 ± 0.031	93	0.002
0.500 ± 0.060	0.505 ± 0.020	99	0.005
1.102 ± 0.044	1.144 ± 0.029	99	0.042
2.004 ± 0.066	1.960 ± 0.039	97	0.044
3.032 ± 0.140	3.005 ± 0.127	97	0.027

Conclusions

LLE-H-NMR method provides a fast and economical solution for the analysis of ethanol in blood. Liquid-liquid extraction of ethanol allows its determination without the use of deuterated solvents and can be easily automated. NMR provides excellent repeatability for both signal intensity and shift.

Funding

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