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Application News

Blood alcohol content (BAC) analysis using a liquid injection approach

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User Benefits

- Blood alcohol analysis using liquid injection is an alternative to the headspace analysis of blood samples for clinical purposes
- It provides reliable results for whole blood and plasma samples without adverse matrix effects

■ Introduction

Blood alcohol content (BAC) is relevant for legal regulations regarding driving limits, but also investigated for forensic and clinical purposes with respect to poisoning. Blood alcohol analysis does not only focus on ethanol, which is the classical drinking alcohol, but also investigates other alcoholic substances like methanol and isopropanol as well as metabolites like acetone. Ethanol is present in alcoholic beverages, but also used for antiseptics, various daily life products, and pharmaceuticals. Being a central nervous system depressant, consumption can cause severe intoxication with toxic levels at above 100 mg/dL blood [1]. Methanol intoxication could originate from the consumption of counterfeit alcohol, with as few as 30 mL pure methanol consumed being potentially lethal [2]. Isopropanol is widely used as solvent or antiseptic in households and sometimes used as cheap substitute for ethanol by alcohol abusers. It is metabolized by alcohol dehydrogenase to acetone, which alike isopropanol is a central nervous depressant [3].

Blood alcohol analysis can be performed using gas chromatography (GC) with flame-ionization detection (FID). Due to the complexity of blood as matrix, headspace analysis is a widely used technique for simple sample transfer onto the GC column, which is especially valuable in forensics, where the matrix states investigated can drastically vary depending on the time the sample is taken. For clinical analysis, fresh blood is used, making the sample more uniform. In this approach, the analysis of BAC using a liquid injection technique as alternative to headspace sampling is presented.

■ Sample Preparation and Calibration

The blood samples are analyzed twice for blood alcohol content: Methanol and ethanol content are determined from the whole blood, whereas isopropanol and acetone are analyzed in the blood plasma. In both cases dilution in serum is used to ensure the blood samples are within the calibration range. This spans concentrations of 100 to 2000 mg/L for methanol, isopropanol, and acetone, and concentrations of 200 to 4100 mg/L for ethanol. n-propanol is used as internal standard and water as matrix. Despite analyzing part of the targets from whole blood and part from plasma, one analytical method and the same data processing for all compounds are used, facilitating data evaluation and routine work.

■ Results

Fig. 1 shows exemplary calibration curves for the four target compounds methanol, ethanol, isopropanol, and acetone proving excellent linearity.

Compound quantification is not affected by the whole blood and plasma matrices, all peaks are reliably detected at the lowest level in both matrices (Fig. 2).

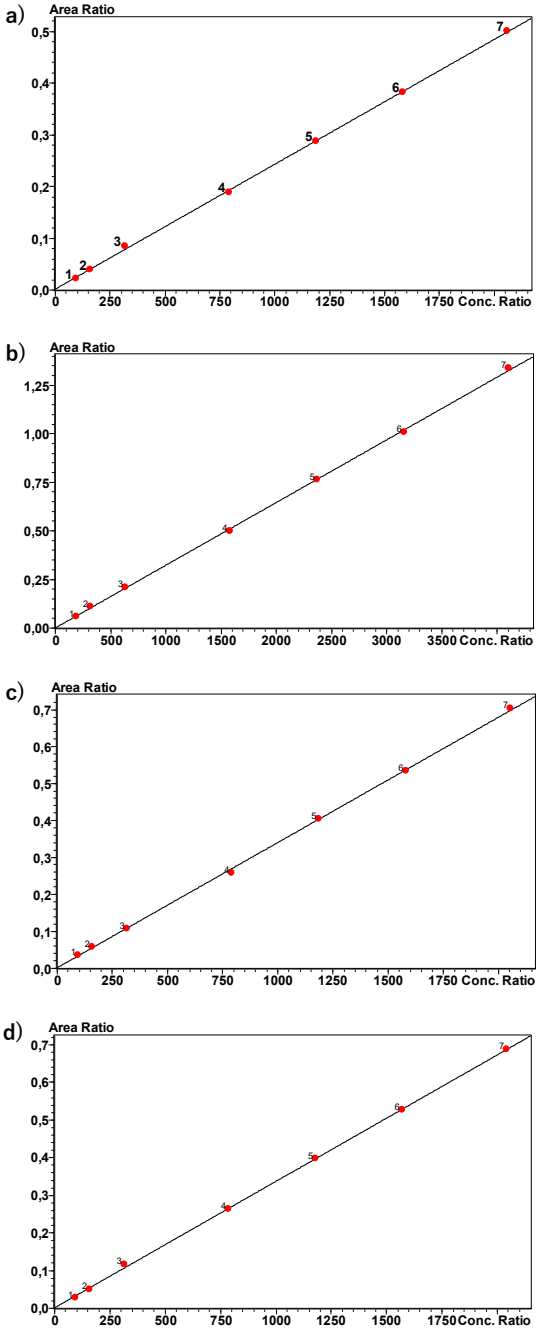

Fig. 1 Exemplary calibration curves for a) methanol, b) ethanol, c) isopropanol, and d) acetone

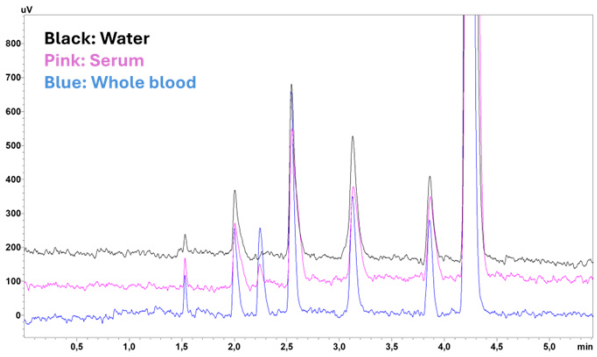

Fig. 2 Compound signals at the lowest level in water (black), serum (pink), and whole blood (blue)

Reproducibility of retention time and area for each compound are summarized in table 1 for compound concentrations of 300 mg/L ethanol / 150 mg/L other compounds and 3200 mg/L ethanol / 1600 mg/L other compounds, determined from 5 consecutive measurement series. The values for ethanol and methanol refer to whole blood as matrix, in which these two compounds are analyzed, whereas the values for isopropanol and acetone refer to plasma as matrix, as quantification of these compounds is performed via blood plasma samples.

Table 1 %RSD retention time and %RSD area (n = 5)

Name	Level 1*		Level 2*	
	% RSD Ret. Time	% RSD Area	% RSD Ret. Time	% RSD Area
Methanol	0.1	4.0	0.2	2.2
Ethanol	0.1	1.9	0.1	2.4
Isopropanol	0.2	3.1	0.2	2.3
Acetone	0.1	3.7	0.1	3.3

* Level1: 400 mg/L Ethanol, 200 mg/L others; Level 2: 4000 mg/L Ethanol, 2000 mg/L others

■ The Package

The recommended analytical hardware and software configuration is listed below.

❑ *Main Unit*

Nexis GC-2030 with FID-2030: Gas chromatograph with flame ionization detector

❑ *Accessory*

AOC-30i autosampler: Liquid autosampler with 30 vials capacity

❑ *Main Consumables*

SH-BAC1 column (30 m x 0.32 mm x 1.8 µm;

P/N 221-76135-30)

❑ *Software*

LabSolutions LCGC

Fig. 3 Nexis GC-2030 equipped with FID-2030 detector and AOC-30i autosampler

■ Conclusion

Liquid sample injection presents a reliable alternative to headspace injection when analyzing blood samples for clinical purposes. Ethanol and methanol can be determined from whole blood, isopropanol and acetone from plasma samples without adverse matrix effects. Both whole blood and plasma analysis can be done using the same analytical method and one data processing approach, facilitating data evaluation and routine work.

■ Acknowledgements

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■ References

[1] D. Perry, D. Klimaszyk, Z. Kolacinski, J, Szajewski, "Ethyl Alcohol (Ethanol)" in *McMaster Textbook of Internal Medicine*. Available: https://empendium.com/mcmtextbook/chapter/B31.II.20.2.1

[2] D. Perry, D. Klimaszyk, Z. Kolacinski, J, Szajewski, "Methyl Alcohol (Methanol)" in *McMaster Textbook of Internal Medicine*. Available: https://empendium.com/mcmtextbook/chapter/B31.II.20.2.2

[3] D. Perry, D. Klimaszyk, Z. Kolacinski, J, Szajewski, "Isopropyl Alcohol" in *McMaster Textbook of Internal Medicine*. Available: https://empendium.com/mcmtextbook/chapter/B31.II.20.2.4.

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