Development and validation of a method for congener analysis in serum and application to a pilot experiment addressing endogenic 1-propanol

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Introduction

Congener alcohol analysis is a useful tool for testing the plausibility of a claim of drinking alcohol after a criminal act but before blood sampling ("Nachtrunk"). The major components for the assessment are methanol as well as 1-propanol and 2-methyl-1-propanol (isobutanol) and also their concentration ratio. As a result it may be essential in some cases to consider endogenic 1-propanol production in the presence of elevated blood ethanol concentrations.

Materials and methods

Method development was performed using a Perkin Elmer gas chromatograph (GC) equipped with a capillary column (Restek RTX®-502.2, 60 m, 0.53 mm ID, 3 µm film thickness), an FID detector and an automatic Headspace sampler. The following analytes were included into the method: 1-butanol, 1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, acetone, isobutanol, 2-propanol (isopropyl alcohol), 2-butanone (methyl ethyl ketone) and methanol. For sample work-up 0.5 mL serum was used and t-butanol served as an internal standard. To increase vapor pressure, 1 g of sodium sulfate was added to each vial. The method was validated according to the guidelines of GTFCh for congener analysis [GTFCh 2010].

Drinking experiment

A male person (42 years, 75 kg) drank 400 mL vodka (Grasovka, 40 vol%), mixed with Bitter Lemon within 3 h. Serum samples were obtained every 1.5 hours over a 10 h period. Furthermore, both vodka and bitter lemon were analyzed for congener alcohols.

Results and discussion

Method validation demonstrated sufficient selectivity and specificity of the method. A linear relationship between the detector response and the concentration was confirmed by Mandel test (99% significance). Weighting was not necessary (homoscedasticity of variance was given for all analytes). The intra- and interday precision were in the range of ±15%. Accuracy was in the range of ± 30% (± 40% near the LOD) and fulfilled the requirements. The limits of detection (LOD), the lower limits of quantification (LLOQ) and the upper limits of quantification (ULOQ) are listed in table 1.

Table 1: LOD, LLOQ and ULOQ

	1-butanol	1-propanol	2-butanol	2-methyl-1-butanol	3-methyl-1-butanol
LOD [mg/L]	0.02	0.01	0.01	0.01	0.02
LLOQ [mg/L]	0.05	0.04	0.03	0.05	0.05
ULOQ [mg/L]	2.0	2.0	2.0	2.0	2.0
	acetone	isobutanol	isopropyl alcohol	methyl ethyl ketone	methanol
LOD [mg/L]	0.07	0.01	0.02	0.02	0.06
LLOQ [mg/L]	0.24	0.05	0.05	0.05	0.25
ULOQ [mg/L]	10	2.0	2.0	2.0	20

Both bitter lemon and vodka did not contain detectable amounts of 1-propanol or isobutanol. The maximum BAC (blood alcohol concentration) reached in the experiment was 1.63‰ (4.5 h after start of drinking). The maximum 1-propanol serum concentration during the experiment was measured about seven hours after start of drinking and reached 0.58 mg/L (BAC at this time 1.26‰). In the phase of ethanol resorption, the rate of 1-propanol formation increased very fast and went through a maximum shortly before the BAC-maximum (see Fig. 1).

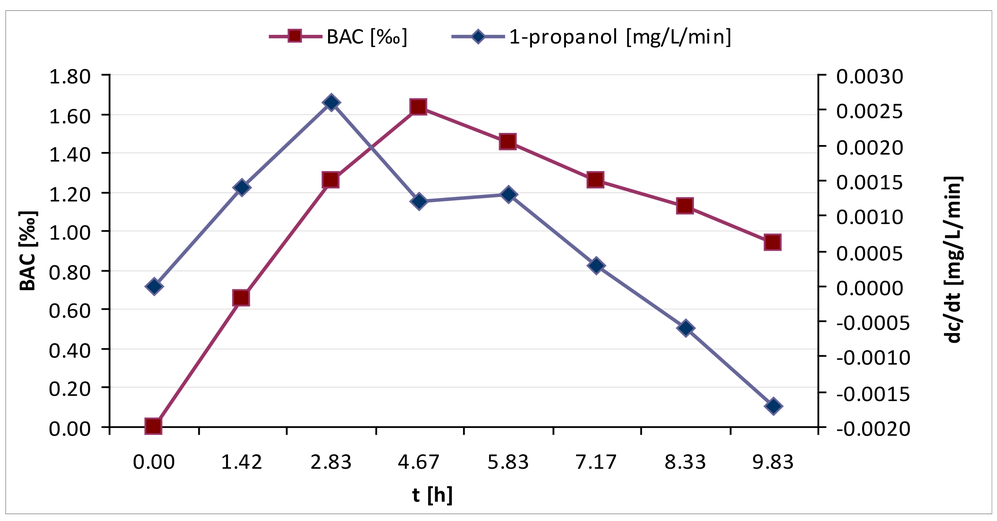

Fig. 1: BAC and production velocity (dc/dt) of 1-propanol over time (start of drinking at t = 0)

Conclusion

A method for analyzing alcohol congeners in serum was successfully validated and applied to a drinking experiment. Significant formation of endogenic 1-propanol was shown in the presence of ethanol although such formation was not stated so far in the scientific literature. As the concentration of endogenic 1-propanol appears to depend on the BAC and may vary interindividually, more research needs to be done. However, in forensic cases experts should be aware of such potential interference, particularly when high BAC's before the incidence can not be excluded.

References

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Acknowledgement

The authors would like to thank the laboratory stuff of the Institute of Forensic Medicine, Freiburg (Germany) for assistance.

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