Development and validation of a method for congener analysis in serum and application to a pilot experiment addressing endogenous 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 1-propanol and isobutanol and also their concentration ratio. As a result it can be essential in some cases to know if endogenous 1-propanol production occurs in the presence of elevated blood ethanol concentrations.

Materials and methods

Method development was done with a Perkin Elmer GC equipped with a capillary column (Restek RTX[®]-502.2, 60 m, 0.53 mm ID, 3 μm film thickness) and an FID detector. For sample work-up 0.5 mL serum was used and *t*-butanol served as an internal standard. To increase vapor pressure, 1 g sodium sulfate was added to each vial. The method was validated according the guidelines of GTFCh for congener analysis.

Pilot test

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

Results and discussion

Method validation demonstrated sufficient selectivity and specificity of the method. A linear relationship between the 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 $\pm 15\%$. Accuracy was in the range of $\pm 30\%$ ($\pm 40\%$ near the LOD) and fulfilled the requirements. The limits of detection and the lower limits of quantification are listed in Table 1.

Table 1: LOD and LLOQ

| | 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 |
| | acetone | isobutanol | isopropyl alcohol | methyl- ethylketone | 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 |

Both bitter lemon and vodka did not contain detectable amounts of 1-propanol or isobutanol. The maximum BAC 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

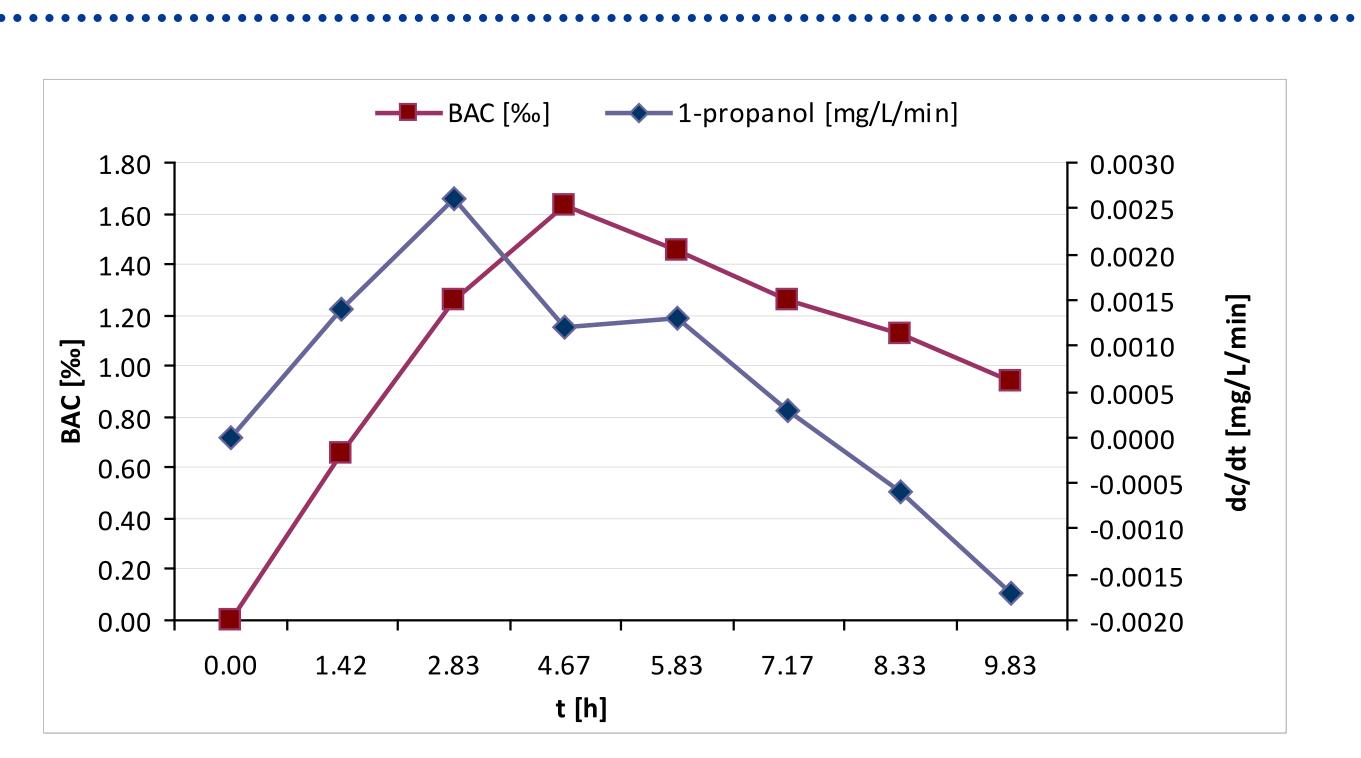


Fig. 1: BAC and production velocity (dc/dt) of 1-propanol over time (start of drinking at t = 0)

Conclusion

A method for analysing alcohol congeners in serum was successfully validated and applied to a pilot experiment. Significant formation of endogenous 1-propanol was shown in the presence of ethanol. As the concentration of endogenous 1-propanol appears to depend on the BAC

