Application Note 10317

Rapid, Automated, and Accurate Determination of Volatiles in Human Blood by Headspace Gas Chromatography

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Key Words
Blood alcohol content, headspace analysis, valve-and-loop, ethanol, methanol, isopropanol

Goal

The purpose of this application note is to describe the determination of blood alcohol levels using the TRACE 1310 GC, the new TriPlus 300 Headspace autosampler, and Chromeleon CDS.

Introduction

Blood alcohol content analysis is one of the most common tests in forensic science. It is generally performed via headspace gas chromatography (GC) with a flame ionization detector (FID). A chromatographic system composed of a Thermo Scientific™ TriPlus™ 300 Headspace autosampler and a Thermo Scientific™ TRACE™ 1310 GC controlled by the Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software can be employed to perform the analysis with a dual column configuration in a rapid, automated, and reproducible manner.

For purposes of law enforcement, blood alcohol content is used to define the level of intoxication. Blood alcohol level can also provide a rough measure of impairment. Most countries forbid operation of motor vehicles and heavy machinery by anyone who has levels of blood alcohol content above a legal limit. The content of alcohol in blood is most commonly expressed as grams per deciliter (g/dL).

Experimental

A TRACE 1310 GC and the TriPlus 300 Headspace autosampler are used. The data are collected and processed with Chromeleon 7.1 CDS. The system is equipped with two Instant Connect FIDs and one Instant Connect Split/Splitless (SSL) injector with a dedicated headspace liner (P/N 453A1335).

Two capillary columns are connected to the SSL injector via a Y-shaped press tight connector and a 5 m × 0.32 mm i.d. deactivated precolumn (P/N 516454). The columns used are a Thermo Scientific™ TraceGOLD™ TG-ALC 1 30 m × 0.32 mm × 1.8 µm (P/N 26074-3390) and TraceGOLD TG-ALC 2 30 m × 0.32 mm × 1.2 µm (P/N 26073-2260).

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First, the analytical conditions are optimized to obtain proper peak resolutions. Add 1 mL of Restek™ blood alcohol mix resolution standard (P/N 36256) into a headspace vial together with 120 µL (0.1 g/dL) of n-propanol used as an internal standard, apply the cap, and analyze it according to the conditions below. The chromatograms shown in Figures 1 and 2 illustrate the resolution obtained respectively on the two columns.

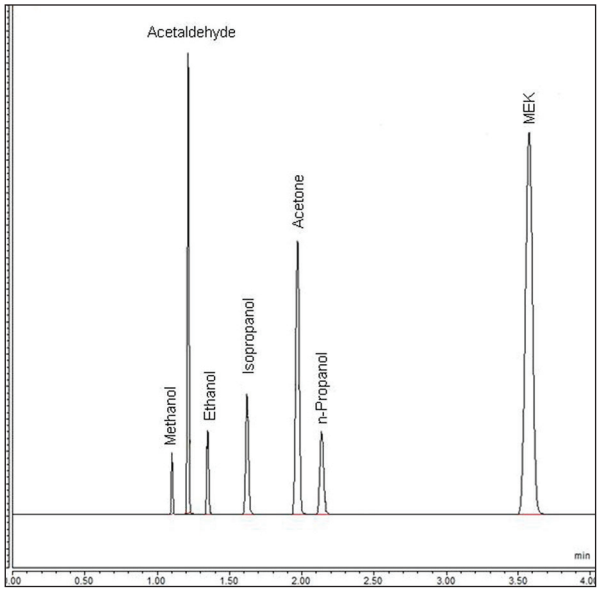

Figure 1. Resolution mix plus internal standard (*n*-Propanol) 0.1 g/dL on column 1 (TraceGOLD TG-ALC 1).

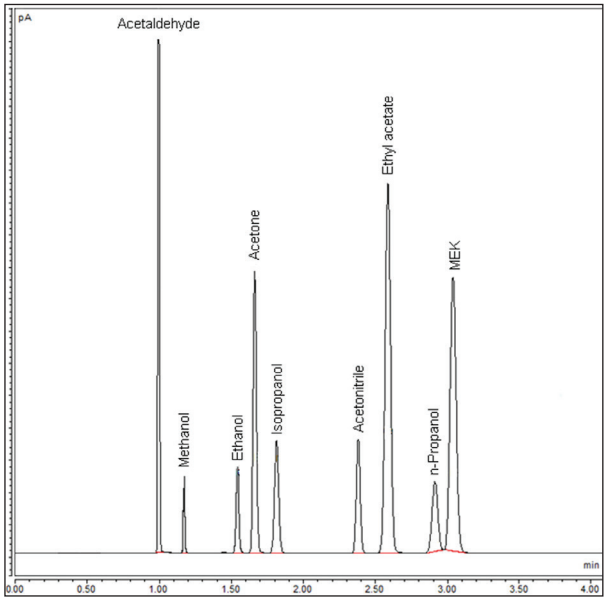

Figure 2. Resolution mix plus internal standard (*n*-Propanol) 0.1 g/dL on column 2 (TraceGOLD TG-ALC 2).

Next, calibration curves are prepared for methanol, ethanol, isopropanol, and acetone (the most commonly investigated compounds in blood alcohol analysis) with and without the use of an internal standard. For each analyte, the calibrant concentrations are: 0.01, 0.02, 0.04, 0.1, 0.2, 0.4 g/dL each, and the total volume into the headspace vial is 1 mL.When working in internal standard mode, 120 µL of n-propanol is also added to give a concentration of 0.1 g/dL into the headspace vial. To analyze real samples, transfer 1 mL of a biological specimen into a 20 mL headspace vial, add 120 µL (0.1 g/dL) of the *n*-propanol internal standard, apply the vial cap, and mix.

Analysis

The TriPlus 300 Headspace sampling system parameters are configured as follows:

- Equilibration time of the sample is 15 min with vial shaking set to high
- Oven temperature set to 70 °C
- Manifold temperature set to 70 °C
- Transfer line temperature set to 105 °C
- Pressurization mode is set to ***Pressure***; pressure is set to 1 bar and the pressure equilibration time to 0.2 min
- Loop filling mode is set to ***Pressure***; pressure is set to 1 bar with an equilibration time of 0.1 min
- Loop size is 1 mL
- Injection mode is ***Standard***, and injection time is set to 0.5 min
- Sample line is purged after injection for 1 min at 80 mL/min
- Vial venting is set to ***On***, and the loop is purged for 1.0 min at 100 mL/min

The TRACE 1310 GC parameters are configured as follows:

- Hold at 40 °C isothermal for 4 minutes
- SSL inlet temperature is set to 150 °C, split mode with split flow 200 mL/min with a split ratio of 20:1
- Carrier used is helium, with a constant flow of 10 mL/min
- FID temperature is set to 300 °C

Results and Discussion

Chromatograms for methanol, ethanol, and the internal standard used (*n*-propanol) on both the analytical columns are reported in Figures 3 and 4.

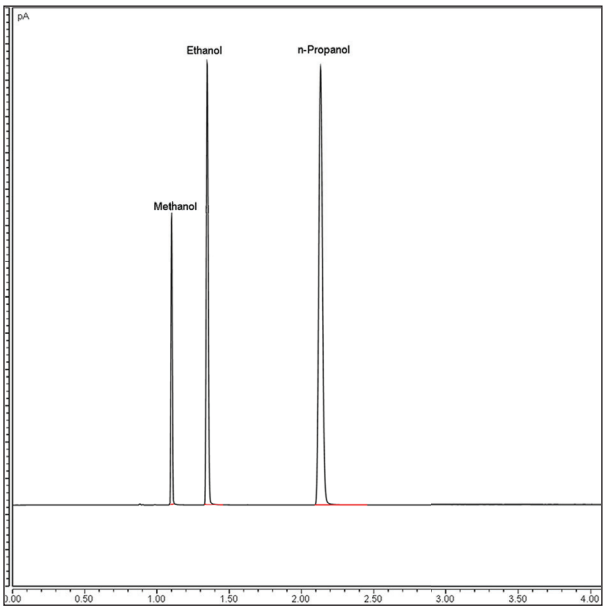

Figure 3. Sample chromatogram on Column 1.

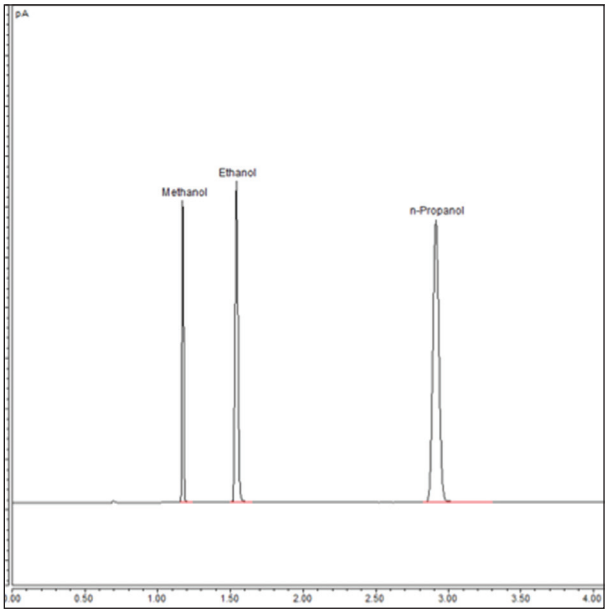

Figure 4. Sample chromatogram on Column 2.

Method repeatability was tested for methanol, ethanol, and *n*-propanol on 20 consecutive injections of the same sample. The area RSD% calculated for each alcohol is shown in Table 1.

Table 1. Area RSD% for alcohols in 20 consecutive injections of sample.

Alcohol	RSD%
Methanol	0.78
Ethanol	0.75
n-Propanol	0.85

Next, excellent linearity was obtained both with and without using an internal standard (*n*-propanol). The calibration curves for methanol, ethanol, isopropanol, and acetone without an internal standard are displayed in Figures 5a–5d. The calibration curves for methanol, ethanol, isopropanol, and acetone with *n*-propanol used as an internal standard are shown in Figures 6a–6d respectively.

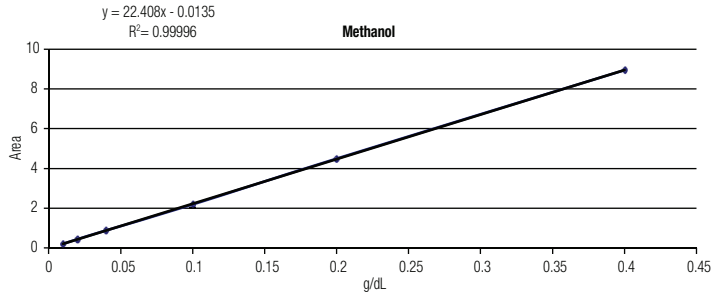

Figure 5a. Calibration curve for methanol with no internal standard

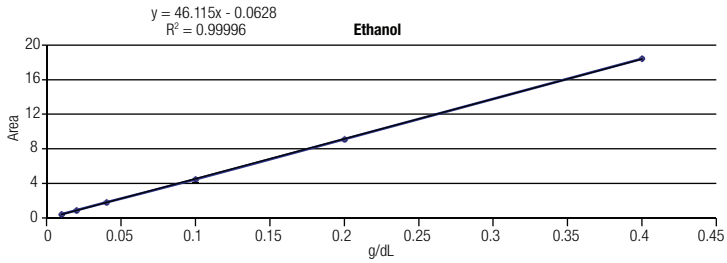

Figure 5b Calibration curve for ethanol with no internal standard.

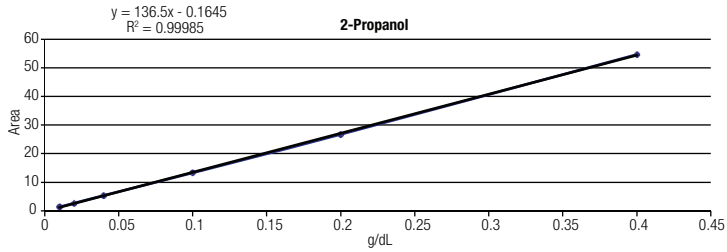

Figure 5c. Calibration curve for isopropanol with no internal standard.

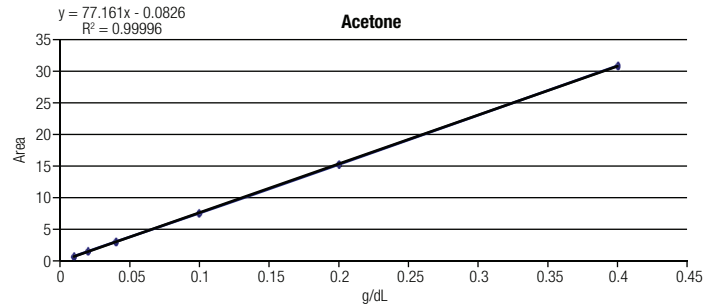

Figure 5d. Calibration curve for acetone with no internal standard.

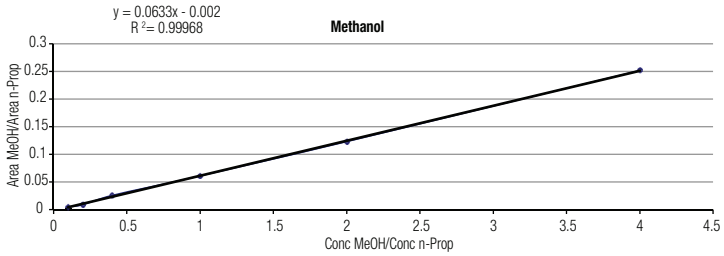

Figure 6a. Calibration curve for methanol with an *n*-propanol internal standard.

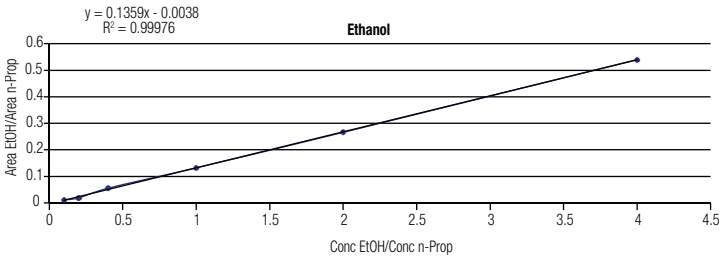

Figure 6b. Calibration curve for ethanol with an *n*-propanol internal standard.

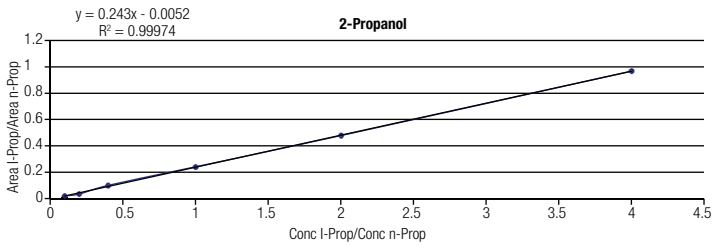

Figure 6c. Calibration curve for isopropanol with an *n*-propanol internal standard.

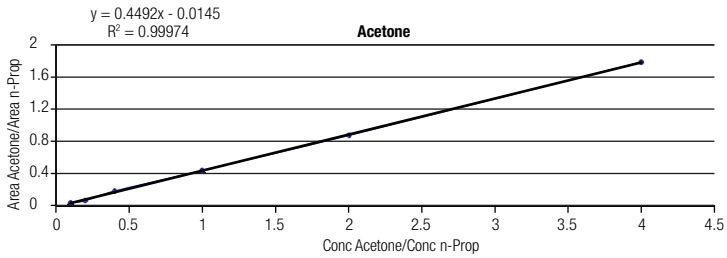

Figure 6d. Calibration curve for acetone with an *n*-propanol internal standard.

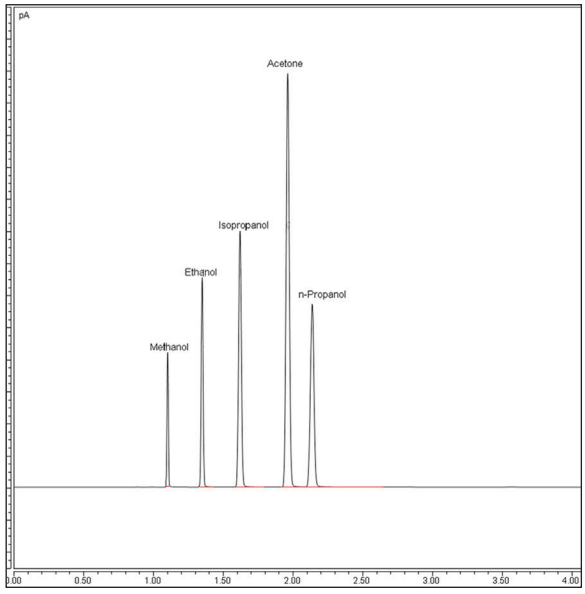

Figure 7. Example of the second Calibration point on Column 1.

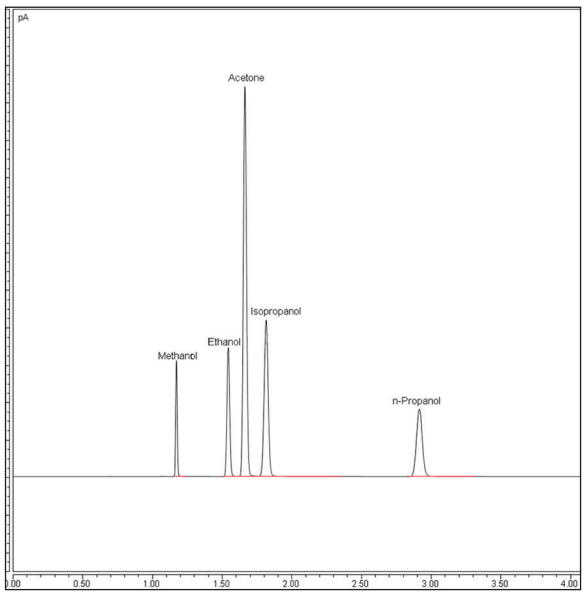

Figure 8. Example of the second calibration point on Column 2.

Figures 7 and 8 display the chromatograms of the second calibration point acquired on the two columns.

Conclusion

As the results confirm, the system comprised of the TriPlus 300 Headspace autosampler, the TRACE 1310 GC, and Chromeleon CDS is a reliable and automated solution for blood alcohol analysis. With its 120-vial sample tray and the large 18-vial incubation oven overlap capacity it guarantees excellent throughput. The inertness of the entire sample path and high temperature capability eliminate any carryover effect, ensuring the highest sample integrity and result consistency. The optional barcode capabilities of the autosampler and the accurate auditing capabilities of Chromeleon CDS also offer the possibility of operating this system in a regulated environment ensuring the necessary data quality and traceability.