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APPLICATION NOTE 10713

Rapid, automated, and accurate determination of blood alcohol concentration (BAC) by headspace coupled to gas chromatography and flame ionization detection

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Keywords

Blood alcohol concentration, BAC, headspace analysis, valve and loop, ethanol, human blood, headspace gas chromatography, HS-GC, flame ionization detector, FID, TriPlus 500 HS, robustness

Goal

The aim of this work is to demonstrate the performance of the new Thermo Scientific™ TriPlus 500™ headspace (HS) autosampler coupled to a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph, using the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) for fast, accurate, and routine determination of blood alcohol concentration (BAC).

Introduction

Blood alcohol concentration analysis is one of the most common tests performed in forensic science. For the purposes of law enforcement, BAC is used to define the level of intoxication and can also provide a rough measure of impairment. Many countries forbid operation of motor vehicles or heavy machinery by anyone with alcohol concentration above a legal limit, usually expressed in grams per deciliter (g/dL). BAC legal limits vary in different countries: 0.08 g/dL in the majority of USA states, England, and Wales; 0.05 g/dL in Italy and Scotland; 0.02 g/dL in Sweden and Norway. In some cases, zero tolerance BAC laws are enforced, either for all (e.g. Brazil, Hungary, Kuwait), for specific age groups (e.g. under 20 years old in Japan), for a specific time after gaining a driving license (e.g. drivers in their first two years after gaining a license in Italy), or for those in some jobs (e.g. military).

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BAC analysis is routinely carried out using the headspace sampling technique coupled to gas chromatography (GC) with flame ionization detection (FID) or mass spectrometry (MS) detection, as this is a simple and fast analytical technique allowing for high sample throughput. The main challenges related to BAC determination that can lead to inaccurate results are carryover from previous injections, resulting in elevated and in some cases false positive results and non-linear ethanol calibration, due to poor instrument performance or inadequate method optimization.[1]

Forenisc toxicology laboratories require accurate, reliable results, that are obtained timely and robustly 24/7. Reduced/limited sample preparation, minimizing preparation errors, and increasing sample throughput is also preferred. Lack of analytical robustness can result in biased results and delayed turnaround times with increased analysis costs.

Experimental

Sample preparation

Blood alcohol mix resolution control standards, 0.1 g/dL (P/N 36256) containing eight target components in water (acetaldehyde, acetone, acetonitrile, ethanol, ethyl acetate, 2-propanol (isopropanol), methanol and methyl ethyl ketone), were acquired from Restek (Bellefonte, PA, USA).

Whole blood certified control samples containing 0.02, 0.05, 0.08, and 0.3 g/dL ethanol (P/Ns WH02-030, WH05-030, WH08-030, and WH30-030) and whole blood blank check samples (P/N 11WH025) were acquired from ACQ Science (Rottenburg, Germany).

For targeted blood alcohol quantitative analysis, methanol, ethanol, acetone, isopropanol, acetonitrile, ethyl acetate, and 1-propanol (internal standard) individual stock standards at 10 g/dL in water (LC/MS grade) were prepared. Diluting from the individual stock standards, mixed calibration working standards over 5 levels (ranging from 0.01 to 0.2 g/dL) were prepared in water. Diluting from the 1-propanol (internal standard) stock standard, a 0.2 g/dL working internal standard was prepared in water.

Standards/blood samples or water blanks (500 µL) were transferred to a 10 mL crimp headspace vial (P/N 10-CV) containing 60 µL 1-propanol (0.2 g/dL, internal standard). The vial was sealed (caps P/N 20-MCBC-ST3) and mixed prior to analysis.

Instrument and method setup

A Thermo Scientific™ TriPlus 500™ HS autosampler coupled to a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph with a Thermo Scientific™ Instant-Connect Split/Splitless Injector (SSL) and two Thermo Scientific™ Instant-Connect Flame Ionization Detectors (FID) were used for all experiments (Figure 1).

Figure 1. TriPlus 500 HS autosampler (240 vial configuration) connected to the Trace 1310 GC, offering the highest sample throughput in the most compact design

Chromatographic separation of the target analytes was achieved using two capillary GC columns, a Thermo Scientific™ TraceGOLD™ TG-ALC1, 30 m × 0.32 mm i.d. × 1.8 µm, film capillary column (P/N 26074-3390) and Thermo Scientific™ TraceGOLD™ TG-ALC2, 30 m × 0.32 mm i.d. × 1.2 µm, film capillary column (P/N 26073-2260).

A dual-column, dual-FID configuration was implemented (Figure 2) through a Thermo Scientific™ Microfluidic 3-port column connector (P/N 60201-398), connected to the flow from the HS via a guard column, Thermo Scientific™ GuardGOLD™, 5 m × 0.32 mm i.d. (P/N 26050-0532). This configuration, using columns with different chemistries and selectivities, results in different

target compound elution order and retention times, which enables compound identification and confirmation for BAC analysis.

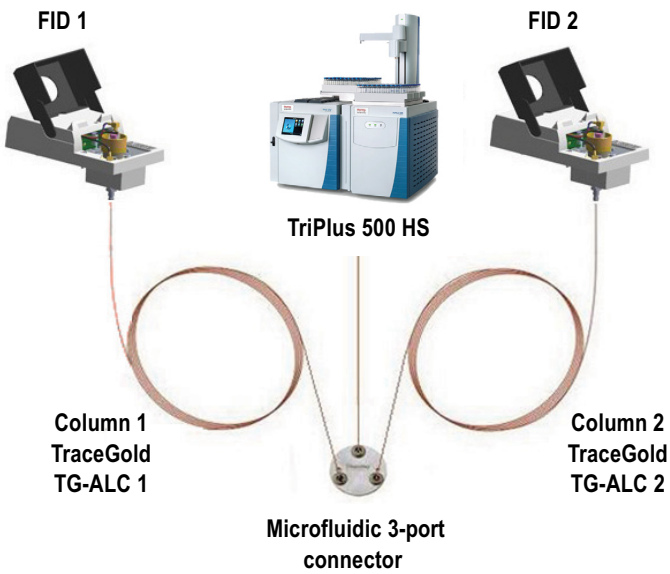

Figure 2. Dual-FID configuration implemented through a microfluidic 3-port connector, connected to the TriPlus 500 HS autosampler via a guard column

Additional HS-GC-FID parameters are detailed in Tables 1 and 2. Conditions were optimized to reduce analysis time, improve sample throughput, and maintain robust analytical performance, with a total GC runtime of 5 min (last peak eluting at 2.6 min). The TriPlus 500 HS autosampler sample overlapping capability and the automatic cycle time optimization are essential for long, unattended sample sequences, supported by the vial capacity expandable up to 240 vials.

Data processing

Data were acquired, processed, and reported using Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS), version 7.2. Chromeleon CDS allows the analyst to setup acquisition, processing, and reporting methods with easy data reviewing and flexible data reporting.

Table 1. GC-FID conditions

TRACE 1310 GC system parameters	
Injection Mode:	Split
Split Ratio:	20:1
Carrier Gas, Carrier Mode, Flow (mL/min):	He, constant flow, 15
Column 1:	TraceGOLD TG-ALC1, 30 m × 0.32 mm i.d. × 1.8 µm, film capillary column (P/N 26074-3390)
Column 2:	TraceGOLD TG-ALC2, 30 m × 0.32 mm i.d. × 1.2 µm, film capillary column (P/N 26073-2260)
Guard Column (to connect HS to the microfluidic device)	GuardGOLD, 5 m x 0.32 mm i.d. (P/N 26050-0532)
Oven Temperature Program	
Temperature 1 (°C):	50
Hold Time (min):	5
FID	
Temperature (°C):	300
Air Flow (mL/min):	350
H_2 Flow (mL/min):	35
N_2 Flow (mL/min):	40
Acquisition Rate (Hz):	25

Table 2. Headspace conditions

TriPlus 500 HS autosampler parameters	
Incubation Temperature (°C)	70
Incubation Time (min)	15
Vial Shaking	Fast
Vial Pressurization Mode	Pressure
Vial Pressure (kPa) (Auxiliary Gas Nitrogen)	100
Vial Pressure Equilibration Time (min)	0.20
Loop Size (mL)	1
Loop/Sample Path Temperature (°C)	70
Loop Filling Pressure (kPa)	50
Loop Equilibration Time (min)	0.10
Needle Purge Flow Level	4
Injection Mode	Standard
Injection Time (min)	0.5

Results and discussion

BAC analysis requires analytical methods that are fast, reliable, and cost effective. Analytical performance was tested for the detailed HS-GC-FID configuration, including chromatographic separation of target analytes, compound linearity, peak area repeatability, recovery, carryover, and quantitation of BAC in blood samples.

Chromatography

Chromatographic resolution and chromatographic peak shape are vital in determining peak area, and in turn, the precise concentration of the target analytes.[1] The chromatographic separation of the components in the blood alcohol mix resolution control standard at 0.1 g/dL in water, applying the HS-GC-FID conditions described in Tables 1 and 2, on both analytical columns, is illustrated in Figure 3.

Chromatographic separations on both analytical columns for a mixed alcohol working calibration standard at 0.1 g/dL in water, containing methanol, ethanol, acetone, isopropanol, acetonitrile, ethyl acetate, 1-propanol (as internal standard) are shown in Figures 4 and 5.

Chromatographic separations on both analytical columns for a whole blood control standard at 0.08 g/dL ethanol (1-propanol as internal standard) are shown in Figures 6 and 7.

As the two dedicated GC capillary columns have different stationary phases with different polarity, different retention times and elution order are achieved (e.g., acetone and acetonitrile co-elutes on the TraceGOLD TG-ALC1 column but they are well separated on the TraceGOLD TG-ALC2 column), enabling confident compound identification and confirmation. Excellent peak shape is achieved, with peak asymmetry values between 0.94 and 1.04 for all target compounds in a 0.1 g/dL mixed alcohol calibration working standard, as illustrated for ethanol in Figures 4b and 5b. Excellent peak shapes are also observed for ethanol in blood samples with peak asymmetry values of 1.01 and 1.03, as illustrated in Figures 6B and 7B for the 0.08 g/dL whole blood control sample.

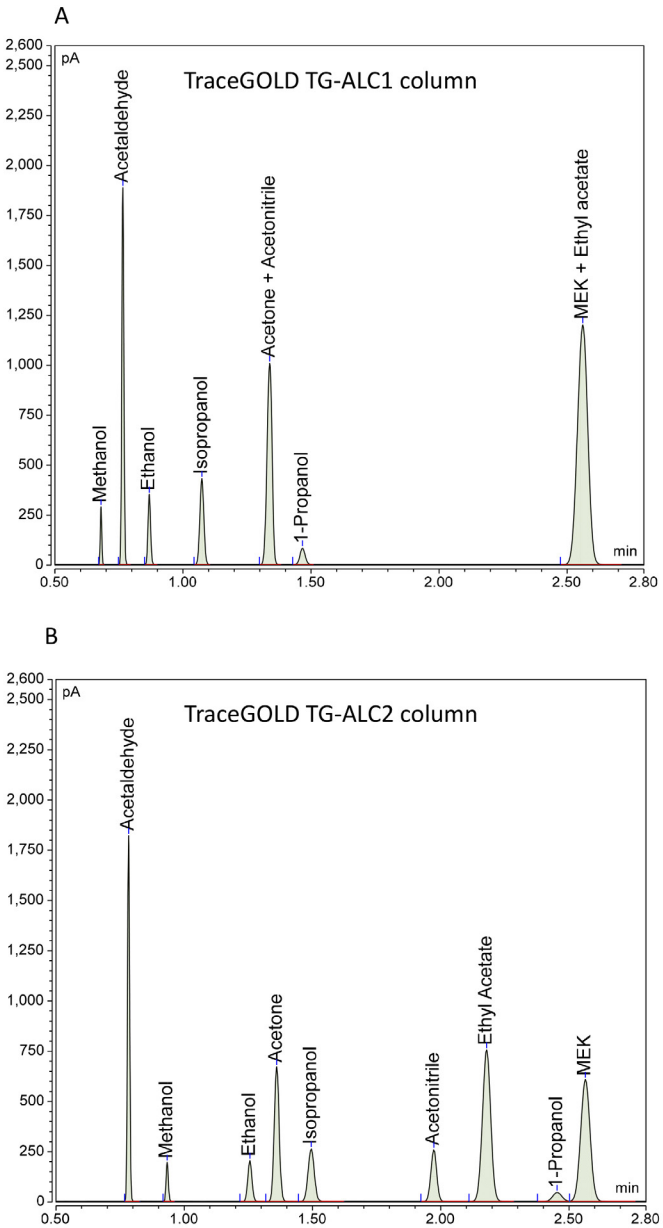

Figure 3. Chromatographic separation of target components in a blood alcohol mix resolution control standard at 0.1 g/dL in water (1-propanol as internal standard) on the TraceGOLD TG-ALC1 (A) and TraceGOLD TG-ALC2 (B) capillary columns

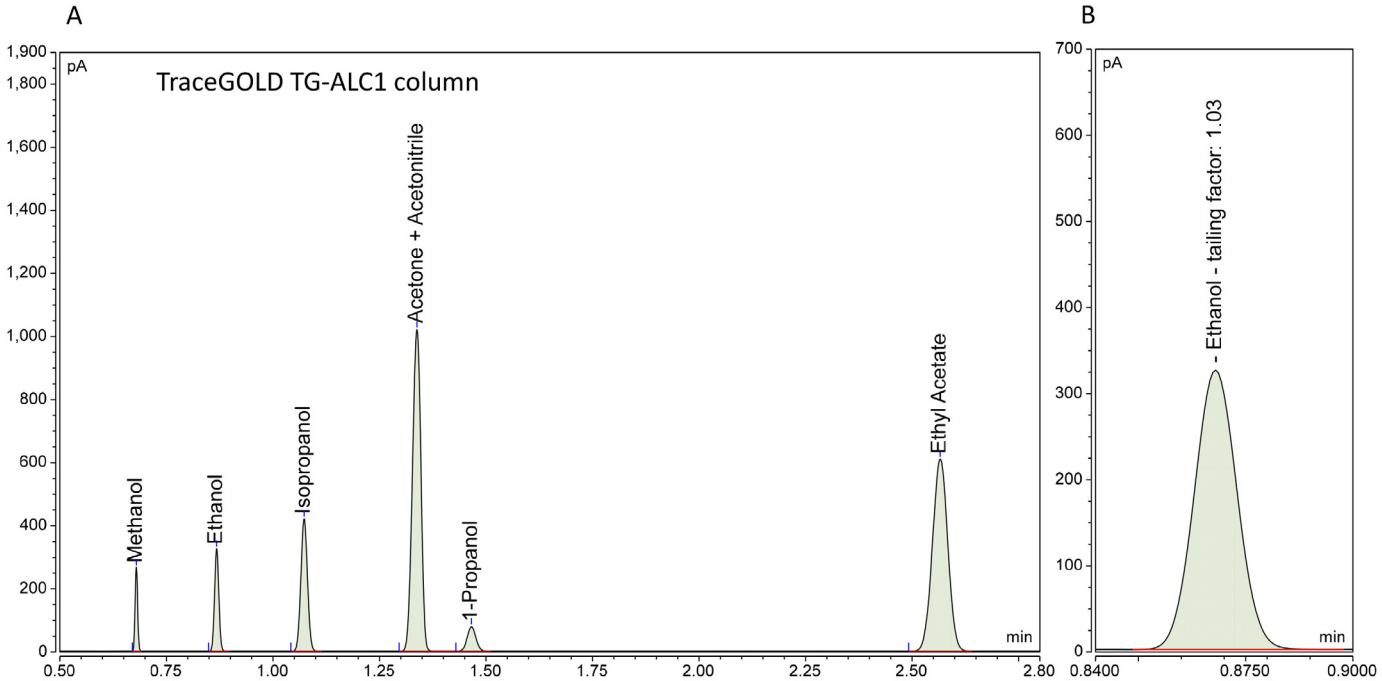

Figure 4. Chromatographic separation of target compounds in a mixed alcohol calibration working standard at 0.1 g/dL in water, plus internal standard (1-propanol) on the TraceGOLD TG-ALC1 capillary column (A), peak asymmetry for ethanol, with a tailing factor (Tf) of 1.03 indicating an almost perfect Gaussian peak (B)

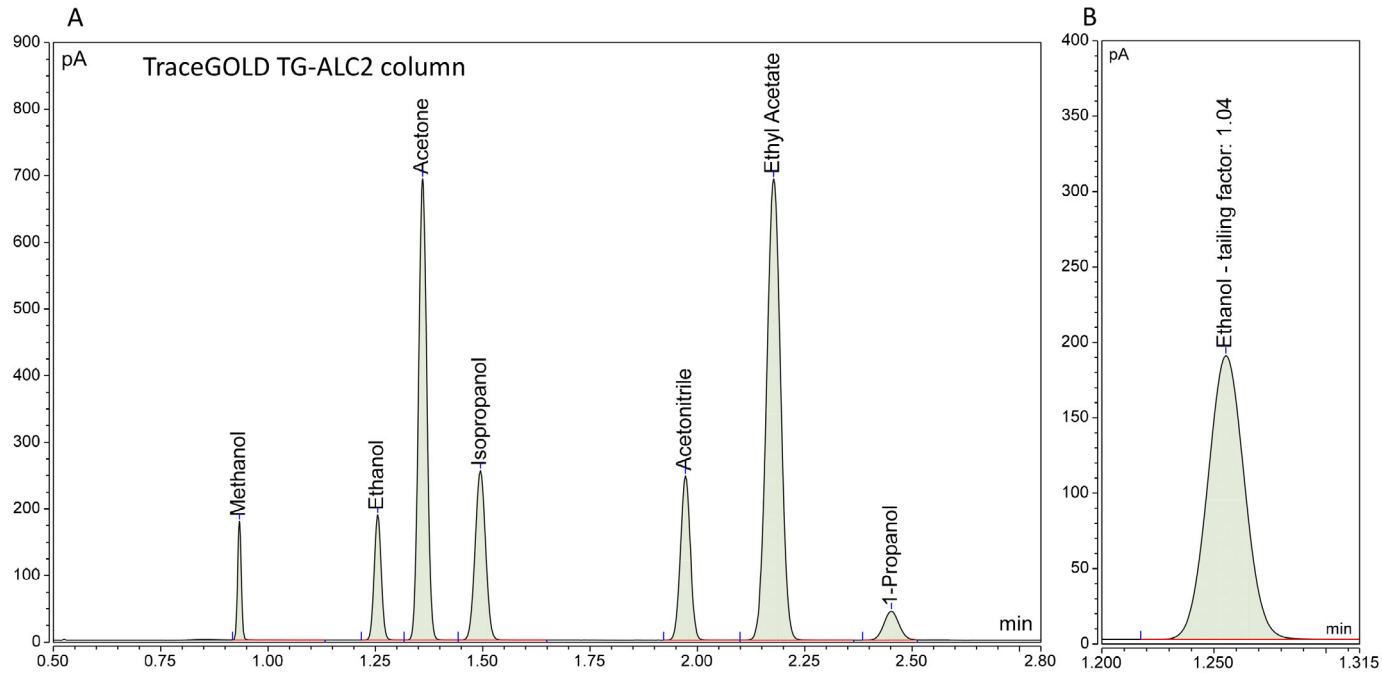

Figure 5. Chromatographic separation of target compounds in a mixed alcohol calibration working standard at 0.1 g/dL in water, plus internal standard (1-propanol) on the TraceGOLD TG-ALC2 capillary column (A), peak asymmetry for ethanol, with a tailing factor (Tf) of 1.04 indicating an almost perfect Gaussian peak (B)

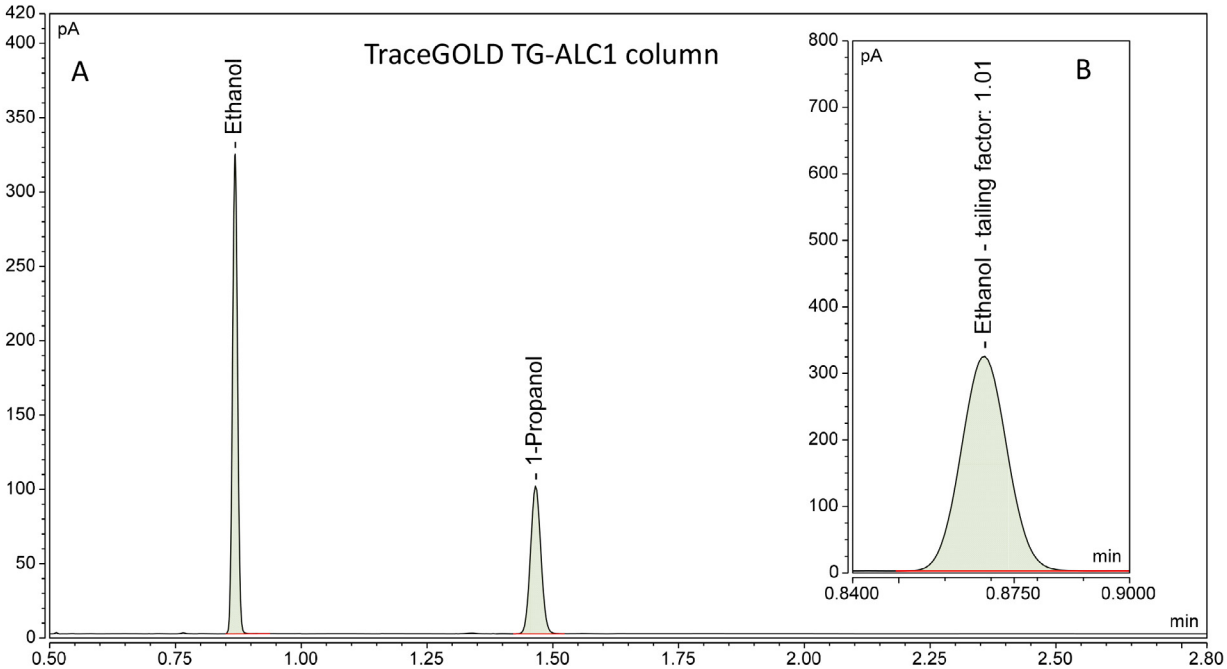

Figure 6. Chromatographic separation of ethanol and 1-propanol (internal standard) in a whole blood control sample, 0.08 g/dL ethanol, on the TraceGOLD TG-ALC1 capillary column (A), peak asymmetry for ethanol, with a tailing factor (T_f) of 1.01 indicating an almost perfect Gaussian peak (B)

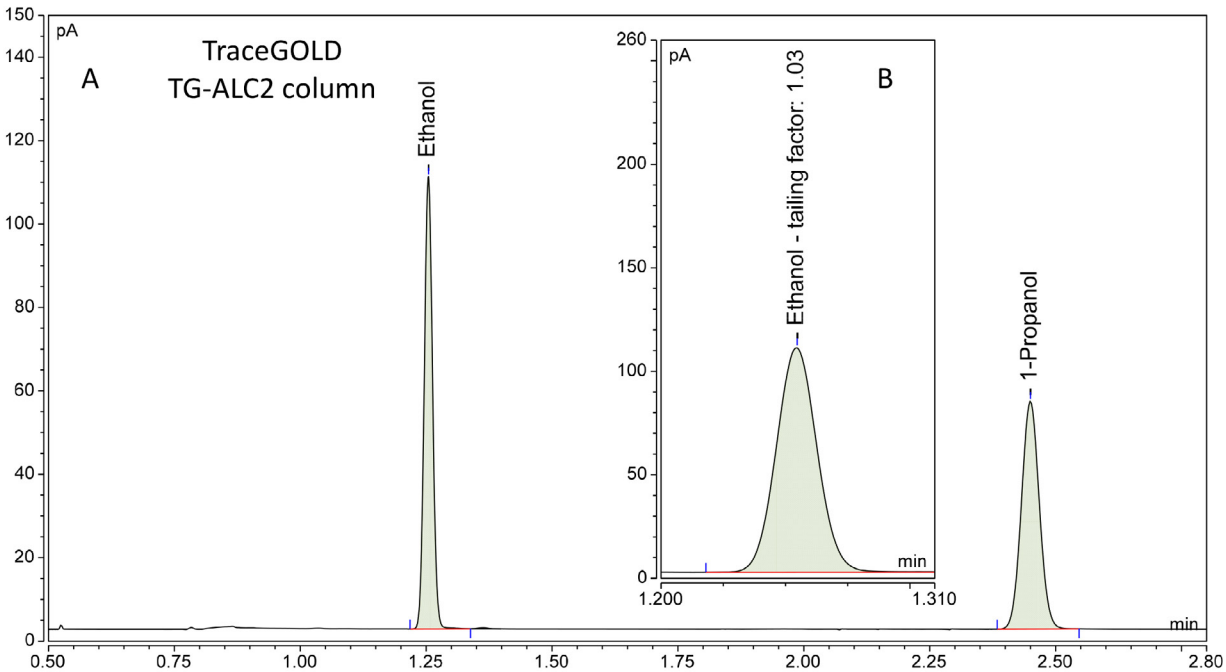

Figure 7. Chromatographic separation of ethanol and 1-propanol (internal standard) in a whole blood control sample, 0.08 g/dL ethanol, on the TraceGOLD TG-ALC2 capillary column (A), peak asymmetry for ethanol, with a tailing factor (T_f) of 1.03 indicating an almost perfect Gaussian peak (B)

Linearity of response

Obtaining linear response is critical for accurate and precise compound quantification. To assess compound linearity, a five-level calibration curve (with concentrations ranging from 0.01 to 0.2 g/dL) was used with internal standard (1-propanol) correction of the responses.

Excellent linearity was obtained for all target compounds with coefficient of determinations R^2 >0.998, and average calibration factors %RSD ≤6. A detailed report of linearity assessment for all target compounds is given in Tables 3A and 3B. The calibration curve for ethanol is shown in Figure 8, using the TraceGOLD TG-ALC2 capillary column. The peak area repeatability (as %RSD) of the internal standard (1-propanol) for n=5 injections was <2.7 on both columns, indicating outstanding precision of the method.

Additionally, linearity for ethanol was tested using whole blood control samples over five levels ranging from 0.02 to 0.3 g/dL, with demonstrated coefficient of determination R^2 of 0.9993, and average calibration factor %RSD of 3.1 (Figure 9).

Peak area repeatability

Confident quantification of BAC in routine testing relies on obtaining stable analyte response in solvent standards and ultimately in blood samples. Repeatability of absolute peak area responses was tested in solvent standards as well as in blood samples. Repeatability in mixed alcohol standards was assessed by carrying out n=15 consecutive analysis of mixed alcohol standards at 0.04 and 0.1 g/dL in water. Additionally, ethanol peak area repeatability was evaluated from n=7 injections of whole blood certified control samples at 0.3 g/dL.

Table 3A. Calibration linearity assessment, showing retention time, average calibration factor (ACF) %RSD, and coefficient of determination (R^2) for the target compounds using the TraceGOLD TG-ALC1 capillary column

Compound	TraceGOLD TG-ALC1		
	RT (min)	ACF %RSD	R^2
Methanol	0.68	3.3	0.9995
Ethanol	0.87	3.4	0.9996
Acetone + Acetonitrile	1.34	5.8	0.9986
Isopropanol	1.07	2.3	0.9998
Ethyl acetate	2.60	3.6	0.9995

Table 3B. Calibration linearity assessment, showing retention time, average calibration factor (ACF) %RSD, and coefficient of determination (R^2) for the target compounds using the TraceGOLD TG-ALC2 capillary column

Compound	TraceGOLD TG-ALC2		
	RT (min)	ACF %RSD	R^2
Methanol	0.93	3.2	0.9983
Ethanol	1.26	2.5	0.9997
Acetone	1.36	5.2	0.9987
Isopropanol	1.50	3.9	0.9991
Acetonitrile	1.97	6.0	0.9984
Ethyl acetate	2.18	3.7	0.9992

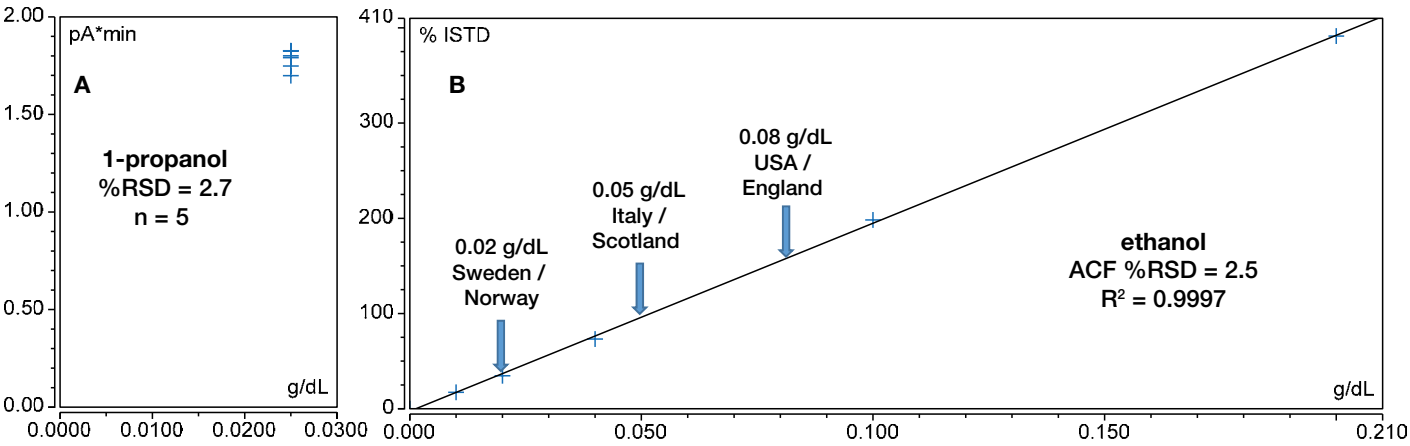

Figure 8. Average peak area for 1-propanol (internal standard), annotated with the peak area repeatability (as %RSD) (A), the linearity response for ethanol over a concentration range of 0.01 to 0.2 g/dL on the TraceGOLD TG-ALC2 capillary column, annotated with the coefficient of determination (R^2) and the average calibration factor (ACF) (as %RSD). The blue arrows indicate blood alcohol legal limits for vehicle operation in different countries (B).

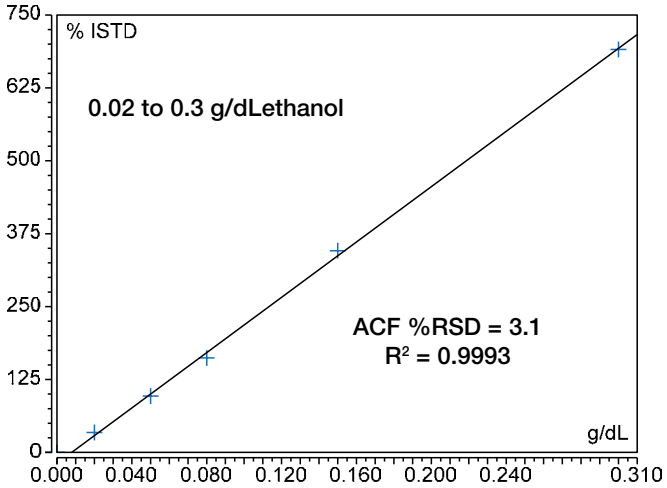

Figure 9. Linearity response of ethanol in whole blood control samples over five levels (ranging from 0.02 to 0.3 g/dL), on the TraceGOLD TG-ALC2 capillary column, annotated with the coefficient of determination (R^2) and the average calibration factor (ACF) (as %RSD).

The results of these experiments demonstrate excellent precision, with peak area %RSD between 0.7 and 3.2 (Table 4), assisted by the advanced features of the TriPlus 500 HS autosampler such as the optimized pneumatic control, the sample path inertness, and the direct connection between the sampling loop and the chromatographic column.

Quantification of BAC in real blood samples

To test the method performance, whole blood control samples over five levels ranging from 0.02 to 0.3 g/dL were analyzed and quantified against the mixed alcohol working standards, with internal standard correction of the responses.

Accurate recovery values are critical in BAC analysis to clearly define the level of intoxication, provide a rough measure of impairment, and accurately determine compliance against legal levels. Excellent recovery of ethanol from whole blood certified control samples (between 93% and 107%) are shown in Table 5 and Figure 10.

Carryover assessment

Compound carryover (contamination from either previously injected standards or blood samples) is one of the known issues when performing HS-GC analysis of BAC and can cause erroneous, false positive results.

Table 4. Assessment of precision (as repeatability of peak area responses) in mixed alcohol standards (n=15) at 0.1 and 0.04 g/dL in water, and whole blood control samples (n=7) at 0.3 g/dL ethanol

Compound	TraceGOLD TG-ALC2			TraceGOLD TG-ALC1
	0.1 g/dL (water) n=15 %RSD	0.04 g/dL (water) n=15 %RSD	0.3 g/dL (blood) n=7 %RSD	0.3 g/dL (blood) n=7 %RSD
Methanol	0.9	1.5	-	-
Ethanol	0.8	1.5	1.5	1.5
Acetone	0.7	2.2	-	-
Isopropanol	0.8	1.0	-	-
Acetonitrile	0.8	1.7	-	-
Ethyl acetate	1.1	3.2	-	-

Table 5. Calculated ethanol concentration in whole blood certified control samples, versus the specified ethanol concentrations, and the associated recovery values

Specified concentration (g/dL)	TraceGOLD TG-ALC2		TraceGOLD TG-ALC1	
	Concentration (g/dL)	Recovery (%)	Concentration (g/dL)	Recovery (%)
0.30	0.303	101	0.321	107
0.15	0.154	102	0.154	103
0.08	0.076	94	0.076	95
0.05	0.047	94	0.047	93
0.02	0.020	101	0.020	100

To assess potential carryover from solvent standards, injections of a mixed alcohol standard (n=15) at 0.1 g/dL in water, using the TraceGOLD TG-ALC2 capillary column, were carried out followed by a blank (water) injection. No detectable carryover was achieved as shown in Figure 11A for ethanol.

To assess potential carryover from blood samples, injections of whole blood control sample (n=7) at 0.3 g/dL ethanol, using the TraceGOLD TG-ALC1 and TraceGOLD TG-ALC2 capillary columns were carried out followed by blank (n=3) water injections. No detectable carryover for ethanol was achieved as shown in Figure 11B and 11C, respectively.

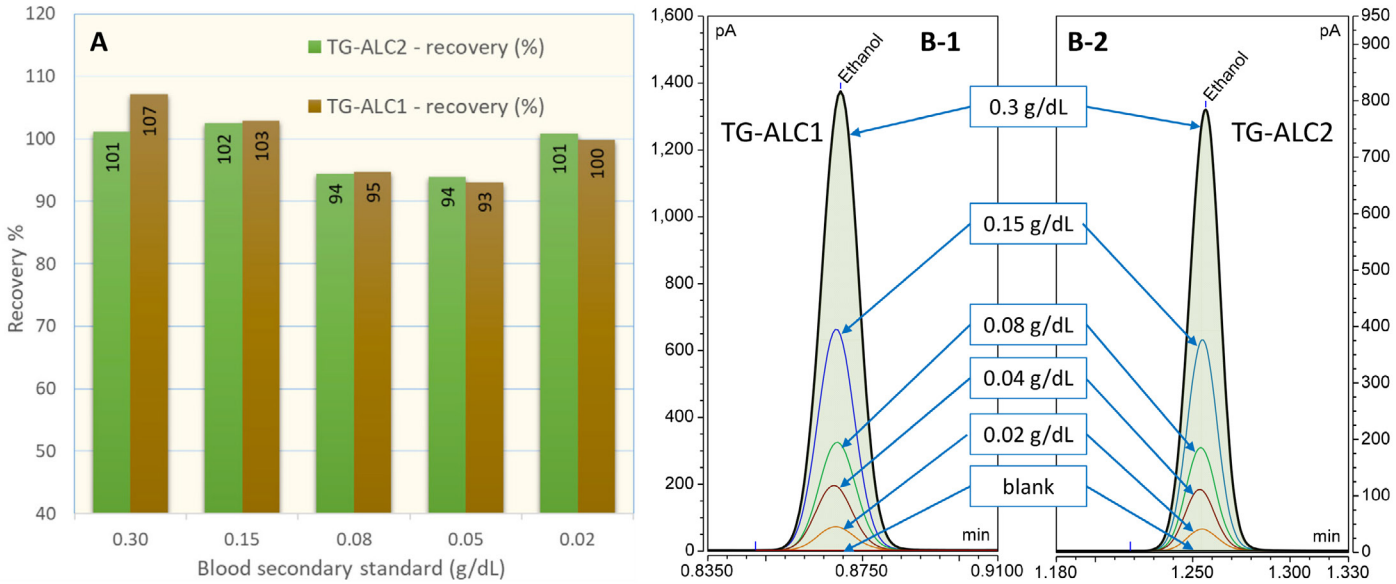

Figure 10. For whole blood certified control samples: ethanol recovery using the TraceGOLD TG-ALC1 (brown) and the TraceGOLD TG-ALC2 (green) capillary columns (A); overlaid chromatograms using the TraceGOLD TG-ALC1 (B-1), and the TraceGOLD TG-ALC2 (B-2) capillary columns for whole blood certified control samples

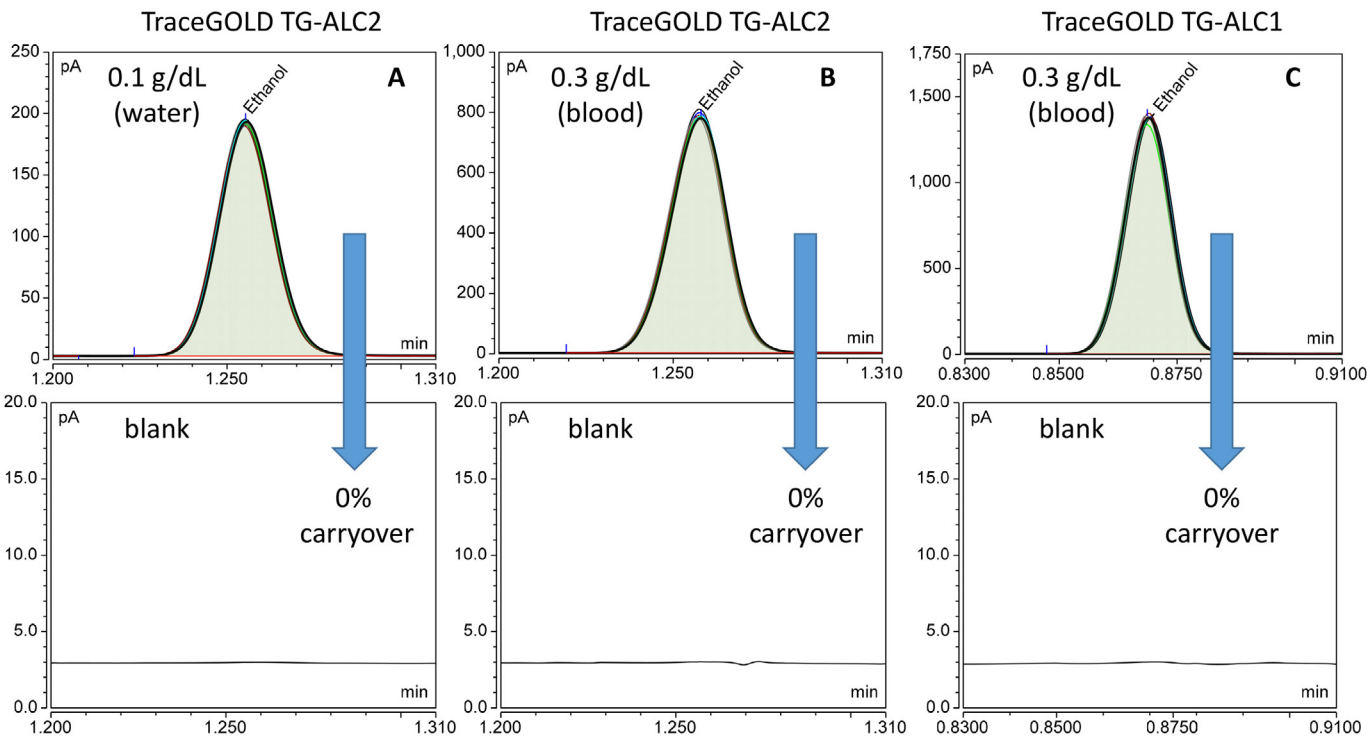

Figure 11. Ethanol carryover (%) was assessed from solvent standards with injections of a mixed alcohol standard (n=15, 0.1 g/dL in water) using the TraceGOLD TG-ALC2 capillary column (A), followed by a blank (water) injection; from blood samples with injections of a whole blood control sample (n=7, 0.3 g/dL ethanol) using the TraceGold TG-ALC2 (B) and TG-ALC1 (C) capillary columns, followed by blank (n=3) water injections.

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Conclusions

- The data demonstrated that the TriPlus 500 HS autosampler can provide high level performance for reliable quantitation of blood alcohol content and fulfills the needs of forensics laboratories for fast, accurate, and high-throughput routine analysis.
- Using a dual column / FID configuration compound separation in <5 min was achieved, with excellent peak shapes (peak asymmetry values between 0.94 and 1.04). Such a short run time allows for high-throughput analysis, aided by automatically optimized overlapped headspace incubation cycles and unattended analysis of up to 240 samples.
- Compound linearity obtained for methanol, ethanol, acetone, isopropanol, acetonitrile, and ethyl acetate in aqueous standards over a calibration range of 0.01 to 0.2 g/dL resulted in coefficients of determination R^2 >0.998 and average calibration factor %RSD of ≤6, indicating an excellent linear response for all the target analytes.
- Excellent precision was achieved for the analysis of 0.04 g/dL standards (n=15) with peak area %RSD for all compounds between 0.7 and 3.2, assisted by the advanced pneumatic control and high sample path inertness of the TriPlus 500 HS autosampler.
- Ethanol recoveries of 94% and 107% were achieved for the analysis of whole blood control samples.
- Aided by the efficient pneumatic purging of the TriPlus 500 HS autosampler and the short inert sample path, no detectable carryover was achieved for the target compounds in the blank samples analyzed after the injection of a mixed alcohol standard (at 0.1 g/dL concentration in water) and a whole blood control sample (at 0.3 g/dL ethanol).
- Chromeleon CDS software offers an ideal solution for the targeted analysis of BAC with user-friendly data processing for high-throughput analysis, with easy data reviewing and flexible data reporting.

References

1. Boswell, H.A and Frank, F.L. Uncertainty of Blood Alcohol Concentration (BAC) results as Related to Instrumental Conditions: Optimization and Robustness of BAC analysis headspace parameters. *Chromatography,* **2015,** 2, 691–708.
2. Calculate MDL using Environmental Protection Agency (EPA) Method for Detection of MDL, 40 CFR part 136. Appendix B, Revision 1.11.

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