High Data Quality, 24/7 Productivity and Result Defensibility for Blood Alcohol (BAC) determination

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ABSTRACT

Purpose: A newly designed static headspace autosampler connected to a dual GC-FID system was evaluated to offer forensic and clinical toxicology laboratories a fast, easy to use and reliable solution for routine blood alcohol analysis, combining high sample throughput to highly precise, accurate and defensible results.

Method: A newly designed valve and loop headspace autosampler was connected to a dual-column/dual-FID GC configuration. The GC was run in isothermal conditions to achieve maximum throughput along with ID confirmation. The overall conditions were optimized to shorten the cycle time, while delivering the best area counts RSD%.

Results:

- GC separation time < 3 min
- Ethanol linearity R^2 > 0.999
- Ethanol area counts repeatability 0.8% (15 replicates, 0.1 g/dL std)
- No detectable ethanol carryover after high concentrated samples

INTRODUCTION

When dealing with GC analyses of volatiles in biological samples, highly automated and reliable sampling solutions are a must to maximize sample integrity and data accuracy while assuring timely response capability for laboratories serving the toxy/forensic market. This work aims to demonstrate how new technological advances can offer extended unattended workflows for high quality and defendable data supporting laboratories in keeping up high demand for services.

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).

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.

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.

MATERIALS AND METHODS

Instrument Setup

The Thermo Scientific™ TriPlus™ 500 Headspace Autosampler was connected to a dual-column/dual-FID configuration of the Thermo Scientific™ TRACE™ 1310 GC via a Thermo Scientific™ microfluidic 3-port connector.

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 phase thickness (P/N 26074-3390) and Thermo Scientific™ TraceGOLD™ TG-ALC2, 30 m × 0.32 mm i.d. × 1.2 µm phase thickness (P/N 26073-2260).

A Thermo Scientific™ GuardGOLD, 5 m x 0.32 mm i.d. capillary deactivated fused silica (P/N 26050-0532) was used to connect the headspace autosampler to the 3-port microfluidic device. The hardware set up is represented in **Figure 1.**

Sample Preparation

Blood alcohol mix resolution control standard, 0.1 g/dL (Restek, Bellefonte, PA, USA) contains eight target components in water (acetaldehyde, acetone, acetonitrile, ethanol, ethyl acetate, 2-propanol, methanol and methyl ethyl ketone).

Whole blood certified control samples containing 0.02, 0.05, 0.08, and 0.3 g/dL ethanol and whole blood blank check samples 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).

Data Analysis

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. Instrumental conditions

TriPlus 500 HS Autosampler parameters	
Incubation Temp	70° C
Incubation Time	15 min
Vial Shaking	Fast
Vial Pressurization Mode	Pressure
Vial Pressure	100 kPa (Nitrogen)
Loop Volume	1 mL
Lopp/Sample Path Temp	70° C
Loop Filling Pressure	50 kPa
Loop Equilibration Time	0.1 min
Purge Flow Level	4
Injection Mode	Standard
Injection Time	0.5 min

TRACE 1310 GC parameters	
Carrier Gas	Helium
Carrier Gas Flow	CF @ 15 mL/min
Injection Mode	Split
Split Ratio	20:1
GC Oven	
Temp Program	50° C (5 min)
FID	
Temperature	300° C
Air Flow	350 mL/min
H2 Flow	35 mL/min
N2 Flow	40 mL/min
Acquisition Rate	25 Hz

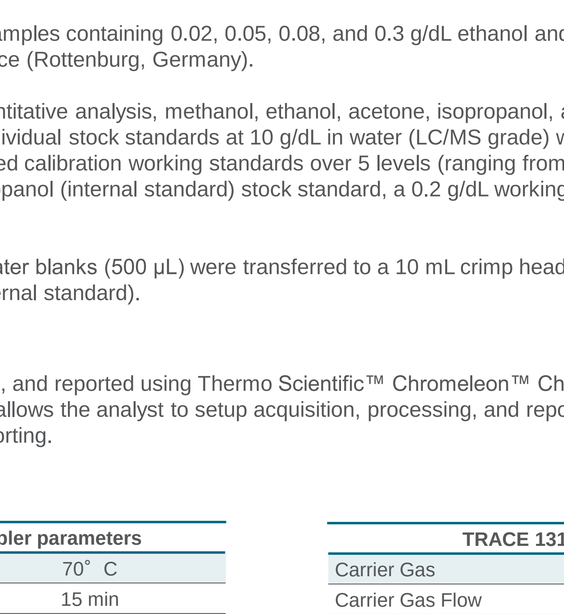


Figure 1. Dual-column/dual-FID GC configuration implemented through a microfluidic 3-port connector, connected to the TriPlus 500 HS autosampler via a guard column

RESULTS

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. Chromatographic separation 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 Figure 2.

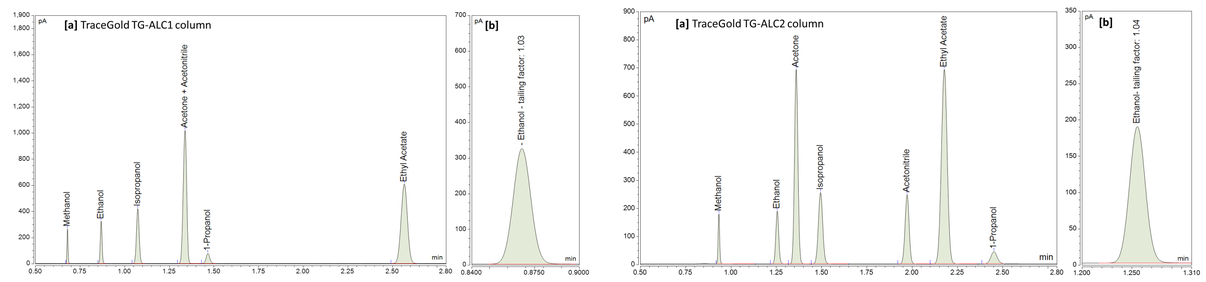


Figure 2. Chromatographic separation of target compounds in a mixed alcohol calibration working standard at 0.1 g/dL in water, plus internal standard (1-propanol) [a], peak asymmetry for ethanol, with a tailing factor (Tf) indicating an almost perfect Gaussian peak [b].

Linearity

Excellent linearity was obtained for all target compounds with coefficient of determination R^2 > 0.998 and calibration response factors %RSD of ≤ 6.0%. A detailed report of linearity assessment for all target compounds is given in **Table 2,** while 5-level calibration curves for ethanol, using the TG-ALC2 capillary column, are shown in **Figure 3.**

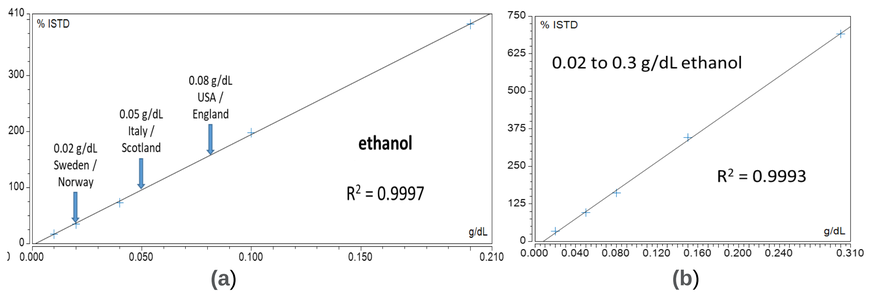


Figure 3. a) Calibration working standard solution: linearity response for ethanol. Blue arrows indicate blood alcohol legal limits for vehicle operation in different countries. b) Linearity response for ethanol in a whole blood control sample

Compounds	TG-ALC1 R^2	TG-ALC2 R^2
Methanol	0.9995	0.9983
Ethanol	0.9996	0.9997
Acetone	0.9986	0.9987
Acetonitrile	-	0.9984
Isopropanol	0.9998	0.9991
Ethyl acetate	**0.9995**	**0.9992**
1-propanol (IS)	-	-

Table 2. Linearity assessment of target compounds

Repeatability

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. The results of these experiments demonstrate excellent precision, with %RSD between 0.7 and 3.2 % (**Table 3**), assisted by the pneumatic control and the sample path inertness featured by the TriPlus 500 HS.

Table 3. Peaks area repeatability

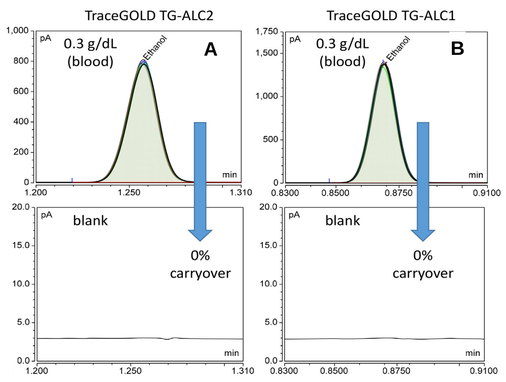
Compound	TG-ALC2		TG-ALC1	
	0.1 g/dL (water)	0.04 g/dL (water)	0.3 g/dL (blood)	0.3 g/dL (blood)
	%RSD (n=15)	%RSD (n=15)	%RSD (n=7)	%RSD (n=7)
Methanol	0.9	1.5	-	-
Ethanol	0.8	1.5	1.5	1.5
Acetone	0.7	2.2	-	-
Isopropanol	0.8	1.0	-	-
Acetonitrile	0.8	1.7	-	-
Ethyl acetate	1.1	3.2	-	-

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, in order 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 4**.

Table 4. Calculated ethanol concentration in whole blood certified control samples, versus the nominal concentrations, and the associated recovery values.

	TG-ALC2		TG-ALC1	
Nominal concentration	Calculated concentration	Recovery	Calculated concentration	Recovery
g/dL	g/dL	%	g/dL	%
0.3	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	100	0.020	100



Ethanol carryover (%) was assessed from blood samples with injections of a whole blood control sample (n=7, 0.3 g/dL ethanol) using the TG-ALC2 (A) and TG-ALC1 (B) capillary columns, followed by blank water injection.

CONCLUSIONS

- The data demonstrated that the TriPlus 500 HS autosampler provides high level performance for reliable quantitation of blood alcohol content and it is fully compliant with the needs of forensics laboratory for fast, accurate, and high throughput 24/7 routine analysis.
- Using a dual-column/dual-FID configuration, compounds separation is achieved in <5 min with excellent peak shape (tailing factors between 0.94 - 1.04). Such 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 all targets, in aqueous standards over a calibration range of 0.01 to 0.2 g/dL, and in whole blood control samples in the range 0.02-0.3 g/dL, resulted in coefficient of determination R^2 > 0.998 and calibration response factors %RSD of < 6.0 %.
- Excellent precision was achieved for the analysis of 0.04 g/dL standards (n=15) achieving area counts %RSD for all compounds between 0.7 and 3.2%, assisted by the pneumatic control and sample path inertness of the TriPlus 500 HS.
- Ethanol recoveries of 94 and 107% were achieved for the analysis of whole blood control samples, aided by the direct column connection to the valve manifold used in the TriPlus 500 HS.
- Aided by the efficient pneumatic purging of the TriPlus 500 HS autosampler, no detectable carryover was achieved for the target compounds in the blank samples analysed after the injection of a mixed alcohol standard (n=15, 0.1 g/dL in water), and a whole blood control sample (n=7, 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.

TRADEMARKS/LICENSING