Old vs New: A Novel Approach to Blood Alcohol Analysis In the Modern Day Forensic Laboratory

Timothy Fassette
Thermo Fisher Scientific, San Jose, CA, USA

Abstract

Purpose: The objective of this work is the development of an instrument setup that allows for the analysis of blood alcohol headspace samples along with solvent extracted whole blood samples on a single instrument. This instrument set up will utilize a single autosampler for both the headspace samples and liquid extracted samples as well as using a blood alcohol column with temperature tolerances up to 320 °C , which allows the toxicologist to also run high temperature analyses for their blood drug tests on the same instrument. The work on this project addresses the limitations faced by forensic toxicologists when it comes to the versatility of the instruments that can be used for blood alcohol headspace testing.

Introduction

GC/FID analysis for the measurement of blood alcohol in forensic samples utilizing the dual column, dual FID set up has been the gold standard in the forensic toxicology laboratory for many years. A few laboratories have switched over to splitting their blood alcohol sample on an FID detector and a mass spectrometer to attain both retention time and mass spectral identification of the sample. Whether labs use the dual FID method or the FID/MS method, the main limitation for headspace analysis of blood alcohol samples on almost every instrument is that the analyst always had to use an instrument whose sole purpose in the toxicology lab was for blood alcohol analysis. This limited the variety of testing that could be performed on that instrument. The main limitation for not being able to use these instruments for other toxicology testing purposes are the low temperature tolerances of the traditional blood alcohol GC columns (approximately 220 °C – 230 °C) and the limited sampling capabilities of a traditional headspace autosampler. This work seeks out an alternate testing method using a GC column not traditionally used in blood alcohol analysis and an autosampler that can perform the headspace testing for blood alcohol analysis and liquid sampling analysis used in blood-drug toxicology testing. To determine feasibility of this new method, we investigated the linearity, accuracy, and reproducibility differences between this new testing approach vs the traditional dual column, dual FID set up and found that the results were in line with what we saw with the standard dual column, dual FID analysis.

Materials and methods

Sample Preparation

Certified mixed volatile standards made up of ethanol, methanol, acetone and isopropanol from Cerilliant were prepared in five levels spanning from 0.01 g/dL to 0.40 g/dL. 100 uL of each sample was added to 1000 uL of a 1-propanol internal standard in water and sealed in a 20 mL headspace vial. Control samples were prepared at 0.05 g/dL to assess the accuracy of the calibration curve created by the prepared calibration standards.

Chromatography

Analytes were separated with the Thermo Scientific TRACE 1610 GC system with a TG-624 Sil MS column (30m x 0.25mm ID x 1.4um).The Triplus RSH autosampler placed the headspace vials into an incubator/agitator @ 70 °C for 6 minutes and then a 700ul headspace sample was injected for analysis. The sample was spilt post column using a Restek MXT Y-connector to send the sample to the mass spectrometer and the Flame Ionization Detector for analysis. For the post column split, a Restek GC guard column (5m x 0.10mm ID x 0.363 mm OD) was used for transfer of the sample to the mass spectrometer and a Restek GC guard column (5m x 0.15mm ID x 0.363 mm OD) was used to transfer of the sample to the flame ionization detector.

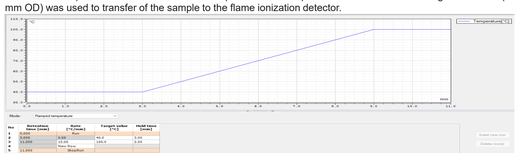

Figure 1. Temperature ramp table for the method.

Mass Spectrometry and FID Detector

The TRACE 1610 GC was coupled to a Thermo Scientific TSQ 9610 mass spectrometer for the analysis. The retention time, quantitation peak and confirming peaks for each analyte is listed in the table 1. The flame ionization detector used for the method was a Thermo Scientific TRACE 1600 FID module.

Table 1. Mass Spectral information for the method.

Detection | MS Detection | MS Component Table | Calibration | MS Settings | MS Library Screening | SST/IRC | Advanced Settings | Peptide Table | Composite Scoring

Component Table

Group Area — Drag a column header here to group by that column. Run Component Table Wizard... Show Properties...

#	Name	Ret. Time	Window	MS Quantitation Peak	MS Confirming Peak 1	MS Confirming Peak 2	Display Window	Display Time	Cal Type	Conc. Unit	Ref. M
1	Methanol	3.475	0.129 AN	31.0	32.0	29.0	RW	RT	Lin,		
2	Ethanol	4.415	0.073 AN	31.0	45.0	46.0	RW	RT	Lin,		
3	Acetone	4.924	0.121 AN	43.0	58.0	42.0	RW	RT	Lin,		
4	Isopropanol	5.148	0.101 AN	45.0	43.0	59.0	RW	RT	Lin,		
5	1-Propanol	6.555	0.088 AN	31.0	29.0	42.0	RW	RT	Lin,		
*									Click here to add a new component		

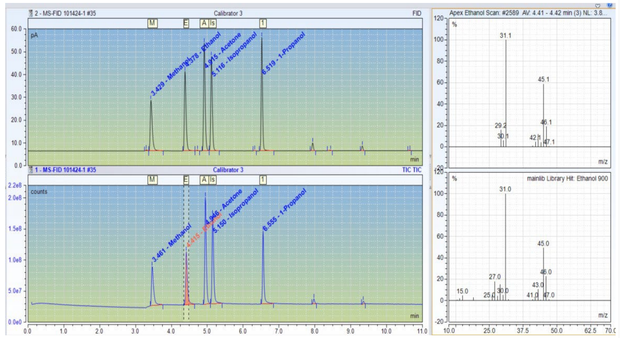

Figure 2. Mass spectrometer and flame ionization detector chromatograms with corresponding library match

Data Analysis

Data was acquired and processed with Thermo Scientific Chromeleon ™ software, version 7.3.1 which stores information including molecular formula, retention time and fragment ions for all compounds of interest. Only those compounds which fall within the retention time range and the mass ion ratio parameters will be reported.

Results

All analytes of interest are depicted in the chromatogram in Figure 2. The limit of quantitation (LOQ), Coefficient of Determination (R^2) and calibration range were determined from the 5-point calibration range. The standard deviation and relative standard deviation values were determined from the repeatability study of the control sample. The 5-point calibration curves and corresponding mass spectrometer ions for each analyte are shown in figure 4. The retention times for both the FID data and the Mass spectrometer data were well within acceptable limits of each other as seen in figure 2.

A control sample that falls within the calibration range was run to determine the standard deviation and relative standard deviation for each analyte. One set of samples was run on day 1 and a second set was run on day 2. In between the runs, a blood drug screen was performed that required the GC to ramp up to 280 °C for 20 samples to test the robustness of the column after exposure to high temperatures routinely seen in normal toxicology screening and confirmation work. The analytes all have relative standard deviations less than 10% and those values can be found in table 2.

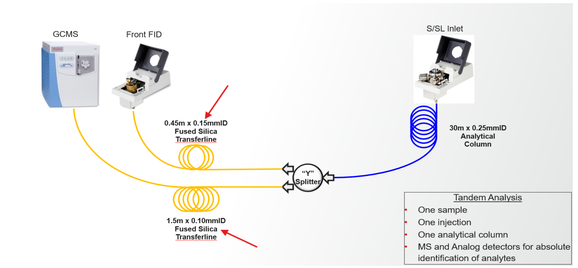

Figure 3. Post Column split diagram: Important attention needs to be paid to the length and ID of the transfer lines to the mass spectrometer and the flame ionization detector. Using the correct length and ID will assure tight retention times for the post column split.

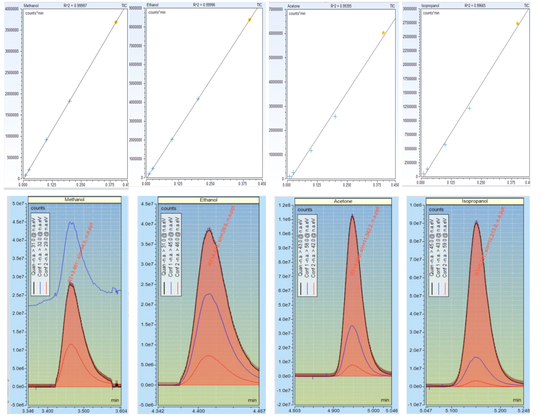

Figure 4. Calibration curves and corresponding Mass Spectrometer ions: All R-squared values are above 0.990 and retain linearity through the calibration range.

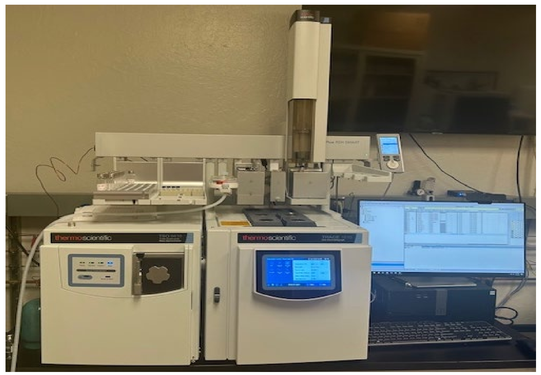

Figure 5. Instrument setup for the method.

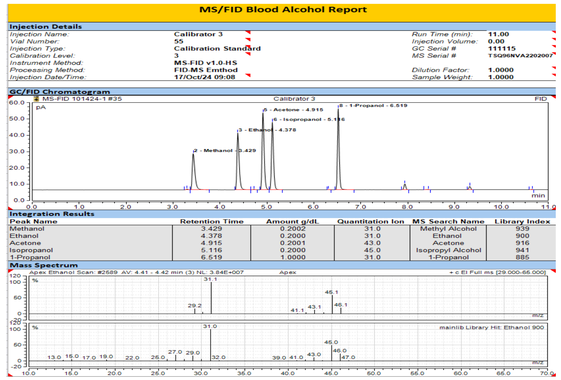

Figure 6. Sample Report

Pre-run RSD Values | **Post-run RSD Values**

Inj No	Injecton Name	Amount				Inj No	Injecton Name	Amount			
		Methanol	Ethanol	Acetone	Isopropanol			Methanol	Ethanol	Acetone	Isopropanol
26	0.05 Control sample - 1	0.0513	0.0523	0.0512	0.0558	38	0.05 Control sample - 1	0.0583	0.0508	0.0539	0.0497
27	0.05 Control sample - 2	0.0518	0.0533	0.0567	0.0574	39	0.05 Control sample - 2	0.0532	0.0543	0.052	0.0531
28	0.05 Control sample - 3	0.0527	0.0547	0.0542	0.0522	40	0.05 Control sample - 3	0.0555	0.0545	0.0586	0.0529
29	0.05 Control sample - 4	0.0571	0.0517	0.0539	0.0546	41	0.05 Control sample - 4	0.0574	0.0544	0.0531	0.0539
30	0.05 Control sample - 5	0.0538	0.0557	0.0564	0.0588	42	0.05 Control sample - 5	0.0601	0.0532	0.0589	0.0565
Maximum		0.0571	0.0557	0.0567	0.0588	Maximum		0.0601	0.0545	0.0589	0.0565
Average		0.0533	0.0535	0.0545	0.0558	Average		0.0569	0.0534	0.0553	0.0532
Minimum		0.0513	0.0517	0.0512	0.0522	Minimum		0.0532	0.0508	0.0520	0.0497
Standard Deviation		0.0023	0.0017	0.0022	0.0025	Standard Deviation		0.0027	0.0016	0.0032	0.0024
Relative Standard Deviation		4.33%	3.10%	4.08%	4.57%	Relative Standard Deviation		4.66%	2.95%	5.83%	4.58%

Table 2. Control sample values pre and post toxicology drug screen

Conclusions

The goal of this study, which was to implement a single instrument setup that could be used for the analysis of headspace blood alcohol samples along with running other toxicology related tests was achieved. Linearity was achieved from LOQs as low as 0.01 g/dL to ULOQs of 0.40 g/dL which exemplifies the sensitivity and dynamic range of the instrument setup used in this study. The results of the study also showed that a method could be developed that is linear and precise in its analysis of the four main analytes of blood alcohol analysis. This method is fast and highly flexible for toxicology and clinical research labs. There may no longer be a need to keep an instrument around just for blood alcohol analysis, and perhaps more labs can move towards implementing instrument set ups in their lab that allow for more robust testing and not just a single use. The importance of the exact measurements for the post column split shown in figure 3 can not be stressed enough. Deviation from those dimensions and lengths can lead to unacceptable differences in the retention times of the analytes of your analysis on the two different detectors.

The next step is to modify some of the method parameters to achieve faster analysis times to shorten the run times to approximately 4 minutes which is standard for most forensic laboratories using the dual column, dual FID method.

References

1. Principles of Forensic Toxicology, Fifth Edition. 2020

Trademarks/licensing