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Application News

No. GC-2104A

Gas Chromatography

Analysis of Blood Alcohol by Headspace Gas Chromatography with Dual Flame Ionization Detection and AOC-6000 Plus Autosampler

■ Introduction

Blood alcohol content (BAC) analysis by gas chromatography with dual flame ionization detection (GC-FID) utilizing a headspace autosampler is the typical instrument configuration for this toxicology lab standard analysis. A single injection is split between two different columns, each leading to an FID. The peak areas obtained from the two separate chromatograms are used to calculate the reported BAC concentrations. This technique could be performed using a dedicated headspace autosampler or a multi-injection rail system (AOC-6000 Plus) with headspace capabilities. This application describes the latter.

■ Standard and Sample Preparation

Two separate stock solutions were prepared. The first solution was made by adding 4 mL of acetone to 100 mL of deionized (DI) water in a 250 mL volumetric flask and then filling to the final volume with DI water. The second added 4 mL methanol, ethanol, and isopropanol each to 10 mL of DI water in a 25 mL volumetric flask and then filled to the final volume with DI water. These two stock solutions were combined to create one intermediate stock solution containing all four components by adding 20 mL of each stock solution to 100 mL of DI water in a 250 mL volumetric flask and then filled to the final volume with DI water.

This intermediate stock solution was used to make the six aqueous calibrator solutions shown below in Table 1, each made in 100 mL volumetric flasks. Once prepared, ~1 mL of each calibrator stock solution was aliquoted into individual 3 mL polypropylene tubes and stored between 2-8°C. Using a diluter pipette, 100 µL of each calibrator and 1 mL of the n-propanol internal standard (ISTD) were added to a labeled 20 mL headspace vial and then crimped.

An ISTD intermediate stock solution was prepared by accurately weighing out 1.50 g of n-propanol into a 50 mL beaker containing 25 mL water, transferred to a 1 L volumetric flask, and then filled to the final volume with DI water. The final ISTD stock solution was created by adding 50 mL of the intermediate stock solution to a 500 mL volumetric flask containing 250 mL of DI water, and then filled to the final volume with DI water.

Table 1. Concentrations for each of the aqueous calibrator solutions.

CAL Point	Intermediate Stock (mL)	Prepared Concentrations (g/dL)			
		Methanol	Ethanol	Isopropanol	Acetone
6	50	0.506	0.505	0.502	0.051
5	35	0.355	0.354	0.352	0.035
4	20	0.203	0.202	0.201	0.020
3	10	0.101	0.101	0.100	0.010
2	5	0.051	0.051	0.050	0.005
1	2	0.020	0.020	0.020	0.002

Low and high concentration positive controls were prepared in whole blood using a secondary preparation of the two calibration stock solutions as described previously. The high control intermediate solution was made by adding 20 mL of each of the two calibration stock solutions to a 50 mL volumetric flask and then filled to the final volume with DI water. The low control intermediate solution was prepared by adding 10 mL of the high control intermediate solution to a 100 mL volumetric flask and then filled to the final volume with DI water. The high concentration whole blood control was prepared by adding 20 mL of the high control stock solution to a 250 mL flask with 100 mL of negative whole blood then filled to the final volume with negative whole blood. The low concentration whole blood control was prepared by adding 25 mL of the low control stock solution to a 250 mL flask with 100 mL of negative whole blood then filled to the final volume with negative whole blood.

See Table 2 below for the concentrations of each whole blood positive control. Once prepared, ~1 mL of each whole blood positive control was aliquoted into individual 3 mL polypropylene tubes and stored frozen at -80°C. Using a diluter pipette, 100 µL of each whole blood positive control and 1 mL of the n-propanol ISTD were added to a labeled 20 mL headspace vial and then crimped.

For unknown samples, 100 µL were added to a labeled 20 mL headspace vial with 1 mL of the ISTD using a diluter pipette and then crimped. If a sample dilution was needed, 50 µL of sample with 1 mL of the ISTD may be used for a 1:1 (x2) dilution.

Table 2: Concentrations for each of the whole blood positive controls.

Whole Blood Positive Control	Prepared Concentrations (g/dL)			
	Methanol	Ethanol	Isopropanol	Acetone
Low Control	0.051	0.051	0.050	0.005
High Control	0.405	0.404	0.402	0.040

■ Instrument Configuration

The AOC-6000 Plus was used for the headspace autosampler paired with a Shimadzu Nexis GC-2030 and dual FID-2030 for detection. The columns used for analysis were SH-Rtx-BAC Plus 1 (30 m x 0.32 mm i.d. x 1.80 µm df) and SH-Rtx-BAC Plus 2 (30 m x 0.32 mm i.d. x 0.60 µm df). Table 3 describes the analytical instrument settings for the autosampler and GC system.

Table 3: Instrument Settings

AOC-6000 Plus	
Incubation Temperature	60°C
Agitator Speed	300 rpm
Pre-Purge Time	5 seconds
Post-Purge Time	15 seconds
Analysis Time	3.5 minutes
Fillings Strokes Count	0
Filling Strokes Volume	2 mL
Filling Strokes Aspirate Flow Rate	12 mL/min
Sample Post-Aspirate Delay	1 second
Sample Vial Penetration Speed	50 mm/sec
Injection Penetration Speed	50 mm/sec
Pre-Injection Dwell Time	0.5 seconds
Post-Injection Dwell Time	0.5 seconds
Agitator On Time	5 seconds
Agitator Off Time	2 seconds
Syringe Heater (Max, Min, Stby)	150°C, 30°C, 65°C
Agitator (Max, Min, Stby)	200°C, 30°C, 60°C
Needle Flush N2 Pressure	1.0 bar

GC-2030	
Inlet Temperature	220°C
Injection Mode	Split
Split Ratio	9
Oven Temperature (isothermal)	35°C
Carrier gas	Helium
Column Flow	7 mL/min
Purge Flow	3 mL/min
Total Flow	73 mL/min
Stop Time	3 minutes
FID-2030	
Temperature	250°C
Sampling Rate	40 msec
Makeup Gas	Nitrogen
Make-up Flow	8 mL/min
Hydrogen Flow	32 mL/min
Air Flow	300 mL/min

■ Results and Discussion

Calibration curves each had an r^2 ≥0.999 or better with a quadratic fit as shown in Figure 1 below. Figures 2 and 3 are example chromatograms of a high positive control sample from FIDs 1 and 2, respectively.

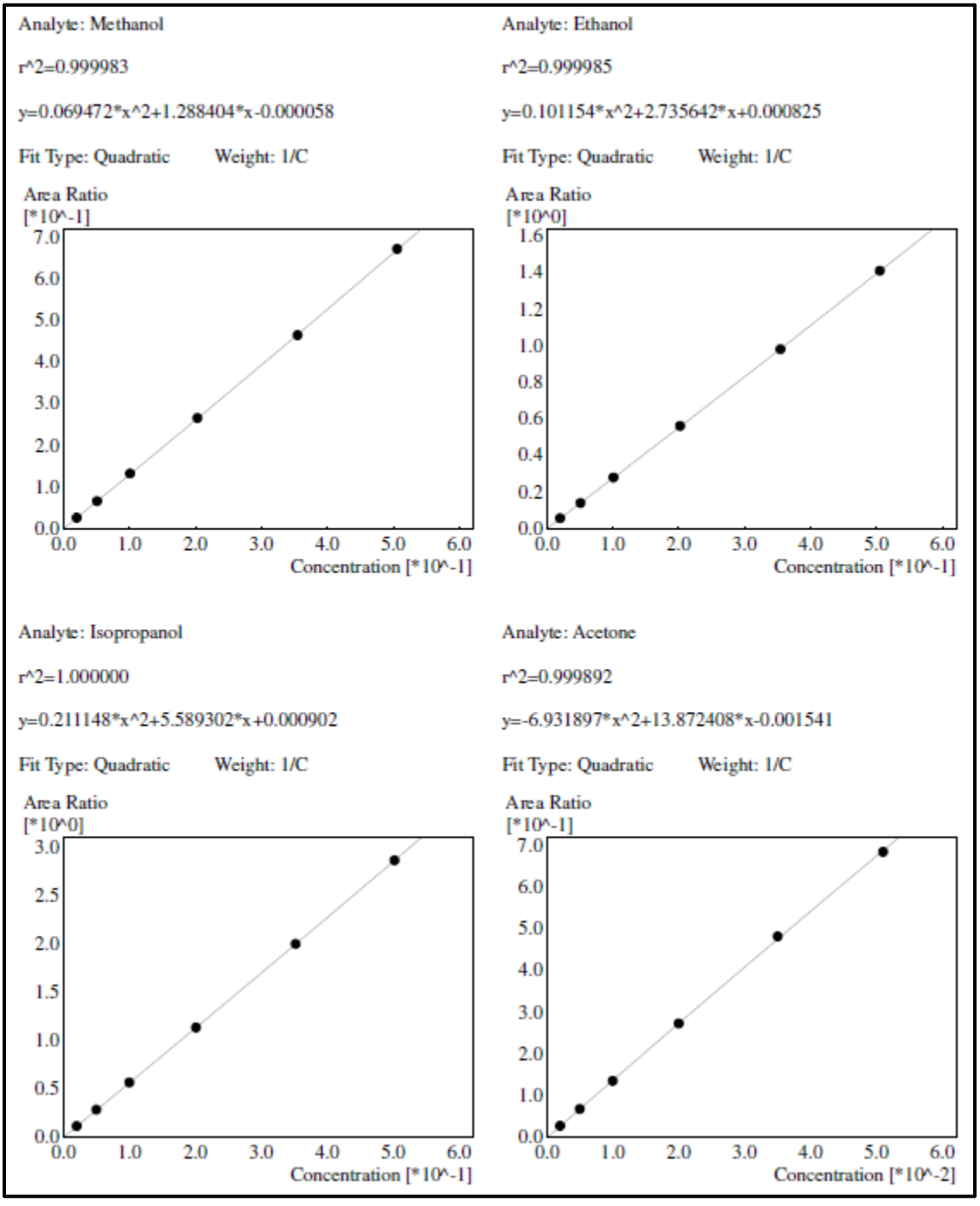
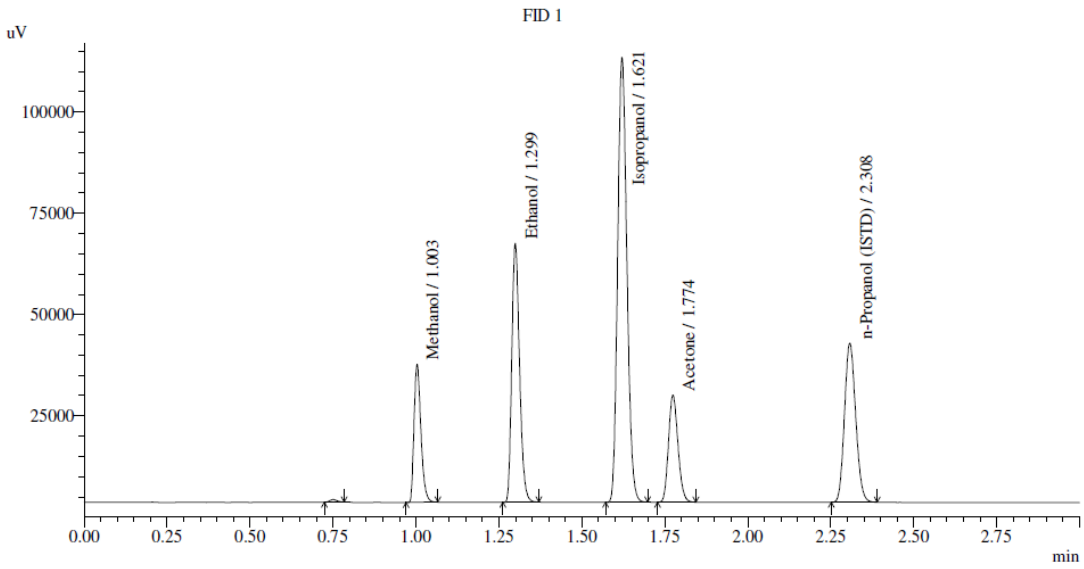


Figure 1: Calibration Curves

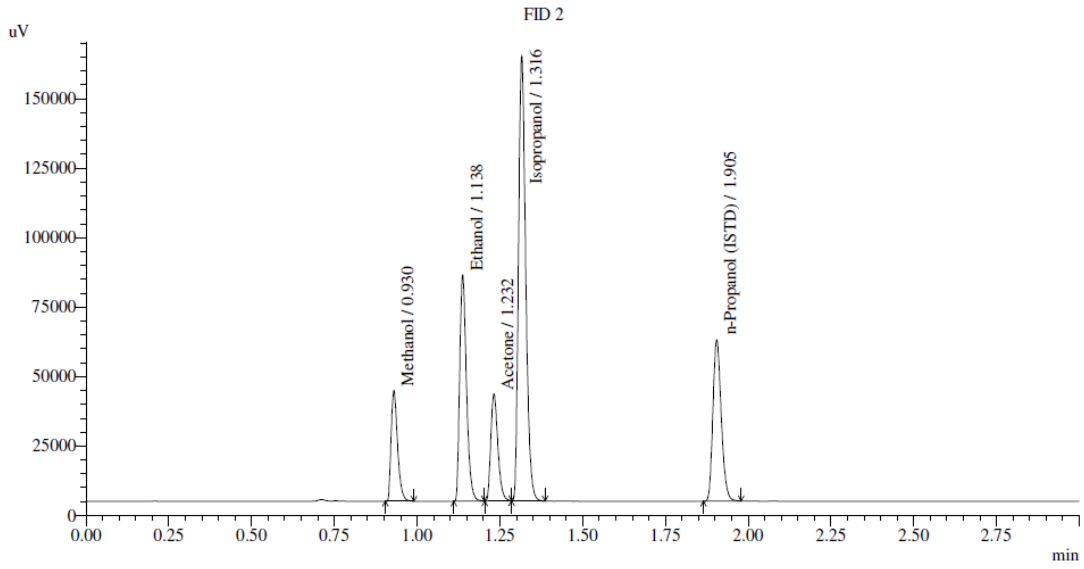


Quantitative Results

FID 1

Name	Ret. Time	Area	Conc.	Unit
Methanol	1.003	52113	0.404	%
Ethanol	1.299	110181	0.405	%
Isopropanol	1.621	226304	0.407	%
Acetone	1.774	55737	0.042	%
n-Propanol (ISTD)	2.308	97956	0.000	%

Figure 2: High Control FID 1.



Quantitative Results

FID 2

Name	Ret. Time	Area	Conc.	Unit
Methanol	0.930	57422	0.404	%
Ethanol	1.138	123098	0.405	%
Acetone	1.232	60720	0.042	%
Isopropanol	1.316	256150	0.407	%
n-Propanol (ISTD)	1.905	108121	0.000	%

Figure 3: High Control FID 2.

■ Conclusion

The instrument parameters provided here prove to be a reliable and robust method with baseline separation of all analytes and repeatable concentrations even between columns of different film thickness.

■ Consumables

Item	Part Number
SH-Rtx-BAC PLUS 1 (30 m x 0.32 mm i.d. x 1.80 µm df)	227-36260-01
SH-Rtx-BAC PLUS 2 (30 m x 0.32 mm i.d. x 0.60 µm df)	227-36263-01

First Edition: November 2021

SHIMADZU Corporation
www.shimadzu.com/an/

SHIMADZU SCIENTIFIC INSTRUMENTS
7102 Riverwood Drive, Columbia, MD 21046, USA
Phone: 800-477-1227/410-381-1227, Fax: 410-381-1222
URL: www.ssi.shimadzu.com