

Application News

Gas Chromatography

Blood Alcohol Analysis using the Shimadzu GC-2010 and AOC-5000 Headspace Autosampler

Introduction

Driving Under the Influence (DUI) is a serious criminal offense. To prove a suspect guilty often calls for solid scientific evidence. The generally accepted method is to accurately measure the Blood Alcohol Content (BAC). This application presents a static headspace method using Shimadzu GC-2010 and AOC-5000 headspace autosampler, which offers reliable qualitative analysis, reproducibility and short run times allowing for high throughput.

Instrumentation

Instrument configuration includes a Shimadzu GC-2010, a split/splitless (SPL) injector, Flame Ionization Detector, an Rtx-BAC1 column, and an AOC-5000 autosampler. An additional analytical line with a second BAC column can be added in to confirm the testing results.

Standards

Blood alcohol mix resolution control standard was purchased from Restek, #36256. Calibration standards were prepared by serial dilution, as shown in Table 1.



Analyte (in g/dL)	Methanol	Ethanol	Acetone	2-Propanol	n- Propanol (IS)
Standard 6	0.5	0.5	0.125	0.125	0.141
Standard 5	0.3	0.3	0.075	0.075	0.141
Standard 4	0.2	0.2	0.05	0.05	0.141
Standard 3	0.1	0.1	0.025	0.025	0.141
Standard 2	0.05	0.05	0.0125	0.0125	0.141
Standard 1	0.01	0.01	0.002	0.002	0.141

Table 1: Calibration standards

Analytical Conditions

GC-2010

- Injector Temp = 150 °C
- Column Temp = 40 °C
- FID Temp = 300 °C, H₂ = 40 mL/min, Air = 400 mL/min, Makeup = 30 mL/min.
- Carrier Gas: He
- Column: Rtx-BAC1 0.32mmX30mX1.8µm
- Flow Control Mode: Linear Velocity @ 80 cm/s, Column Flow = 7 mL/min, Purge = 3 mL/min, Split Ratio = 5:1
- Injection Volume: 1mL Headspace
- GC run time: 3 min

AOC-5000

- Syringe: 2.5 mL – HS
- Incubation Temp = 65 °C
- Incubation Time = 15 min
- Syringe Temp = 70 °C
- Agitator Speed: 500 rpm
- Fill Speed: 500 µL/s
- Pullup Delay: 500 ms
- Injection Speed: 1000 µL/s
- Pre Inject Delay: 0 ms
- Post Inject Delay: 3000 ms
- Flush Time: 20 s

Chromatograms

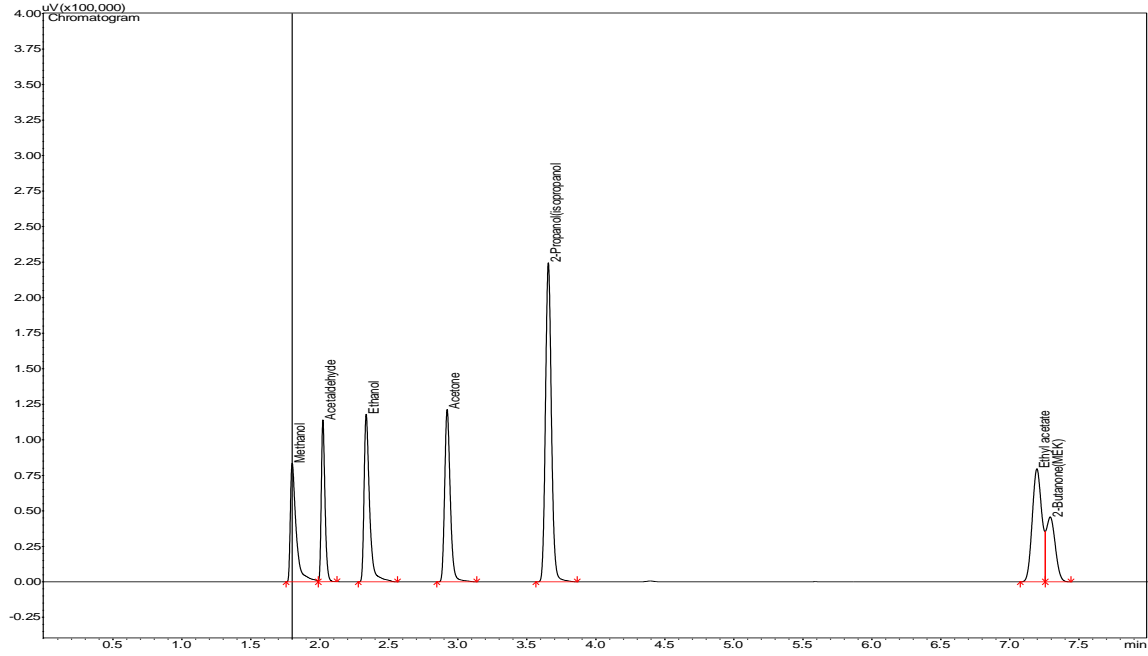


Figure 1: Blood alcohol mix resolution control standard, Restek #36256

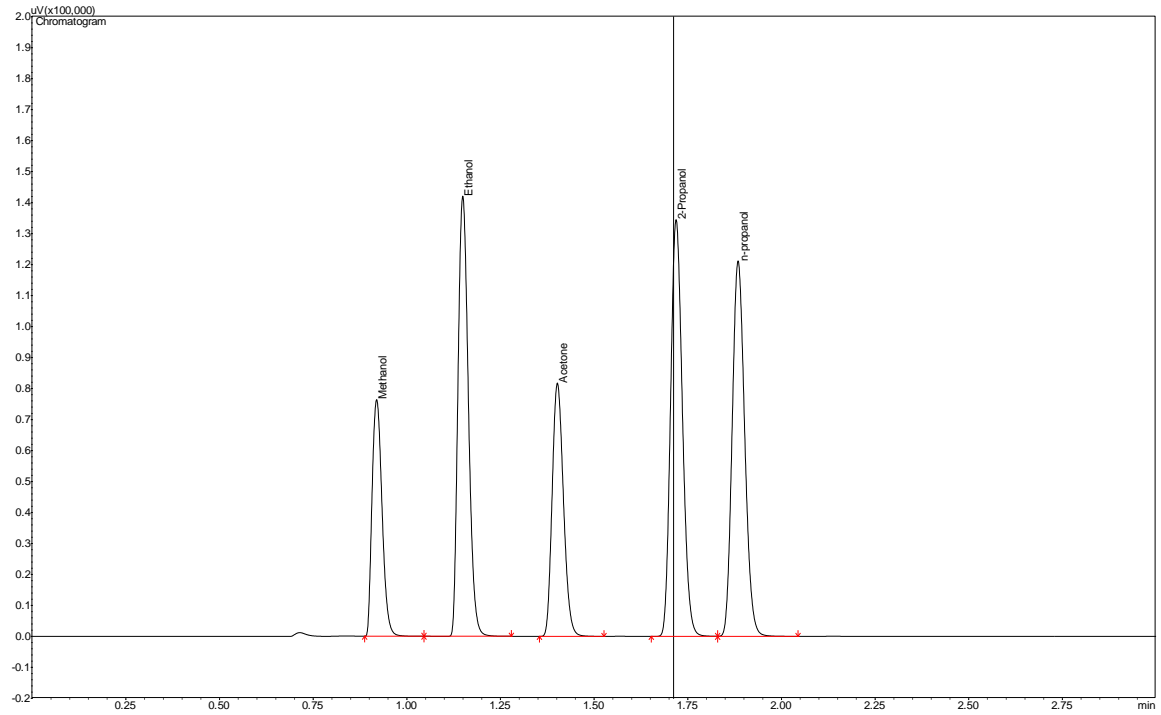


Figure 2: Chromatogram of Blood alcohol standard

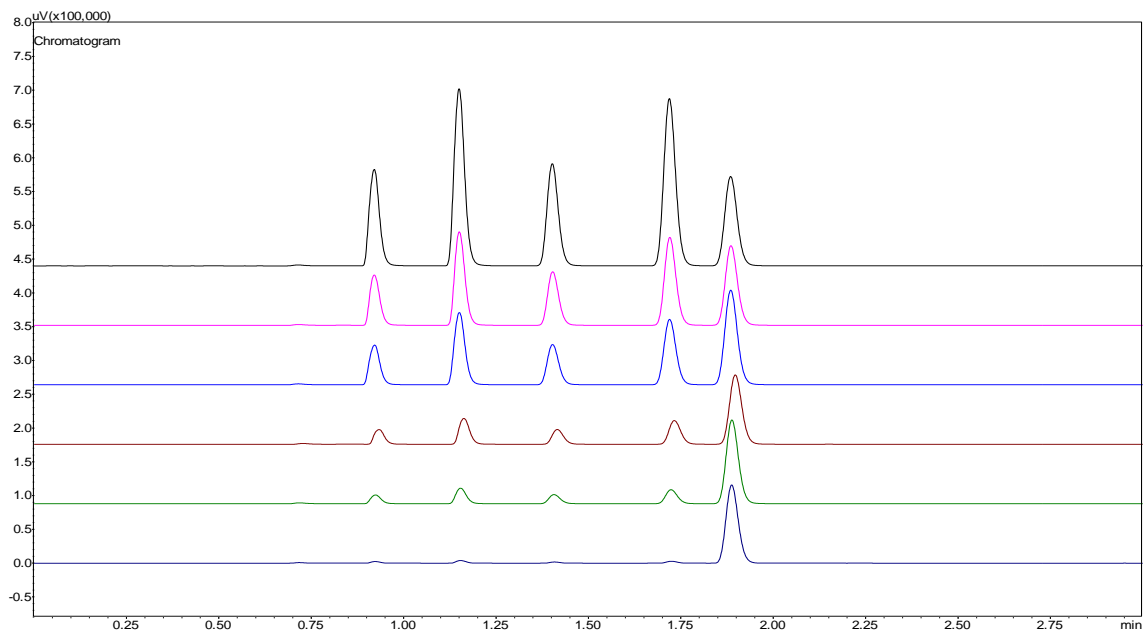


Figure 3: Chromatograms of Blood alcohol standard 1 to 6

Qualitative Analysis

For most GC measurements, peak identification is generally performed by comparing retention times of unknown sample to that of the standards. However, retention time varies due to changing parameters such as temperature, pressure, flow rate, and sample matrix, etc. To enforce the integrity of forensic testing results, a Relative Retention Time (RRT) method is used to accurately identify the peaks and therefore avoid any false positive or false negative results. RRT is defined by using retention time of unknown peak divided by that of the internal standard. Table 2 and 3 list the retention times and corresponding RRTs, from 6 consecutive runs. The relative standard deviations of RRT indicate excellent reproducibility.

Analyte	Run1	Run2	Run3	Run4	Run5	Run6
Methanol	0.923	0.92	0.923	0.934	0.93	0.935
Ethanol	1.153	1.15	1.153	1.163	1.159	1.164
Acetone	1.407	1.404	1.407	1.416	1.412	1.417
2-Propanol	1.723	1.72	1.723	1.732	1.729	1.734
n-Propanol	1.886	1.883	1.886	1.897	1.894	1.898

Table 2: Retention times in minutes

Analyte	Run1	Run2	Run3	Run4	Run5	Run6	%RSD
Methanol	0.4894	0.4886	0.4894	0.4924	0.4910	0.4926	0.17
Ethanol	0.6113	0.6107	0.6113	0.6131	0.6119	0.6133	0.10
Acetone	0.7460	0.7456	0.7460	0.7464	0.7455	0.7466	0.04
2-Propanol	0.9136	0.9134	0.9136	0.9130	0.9129	0.9136	0.03

Table 3: Relative retention times (RRT) and standard deviation

Calibrations

Internal standard calibration was employed to compensate sample injection variations and minimize matrix effect. Equal amount of the internal standard, n-propanol, was added to all calibration standards and unknown samples. A calibration curve was generated by plotting peak area ratio of unknown: internal standard as a function of the concentration ratio of unknown: internal standard, shown in Figure 4 and 5. The area ratio of unknown: internal standard was used to determine the unknown ethanol concentration.

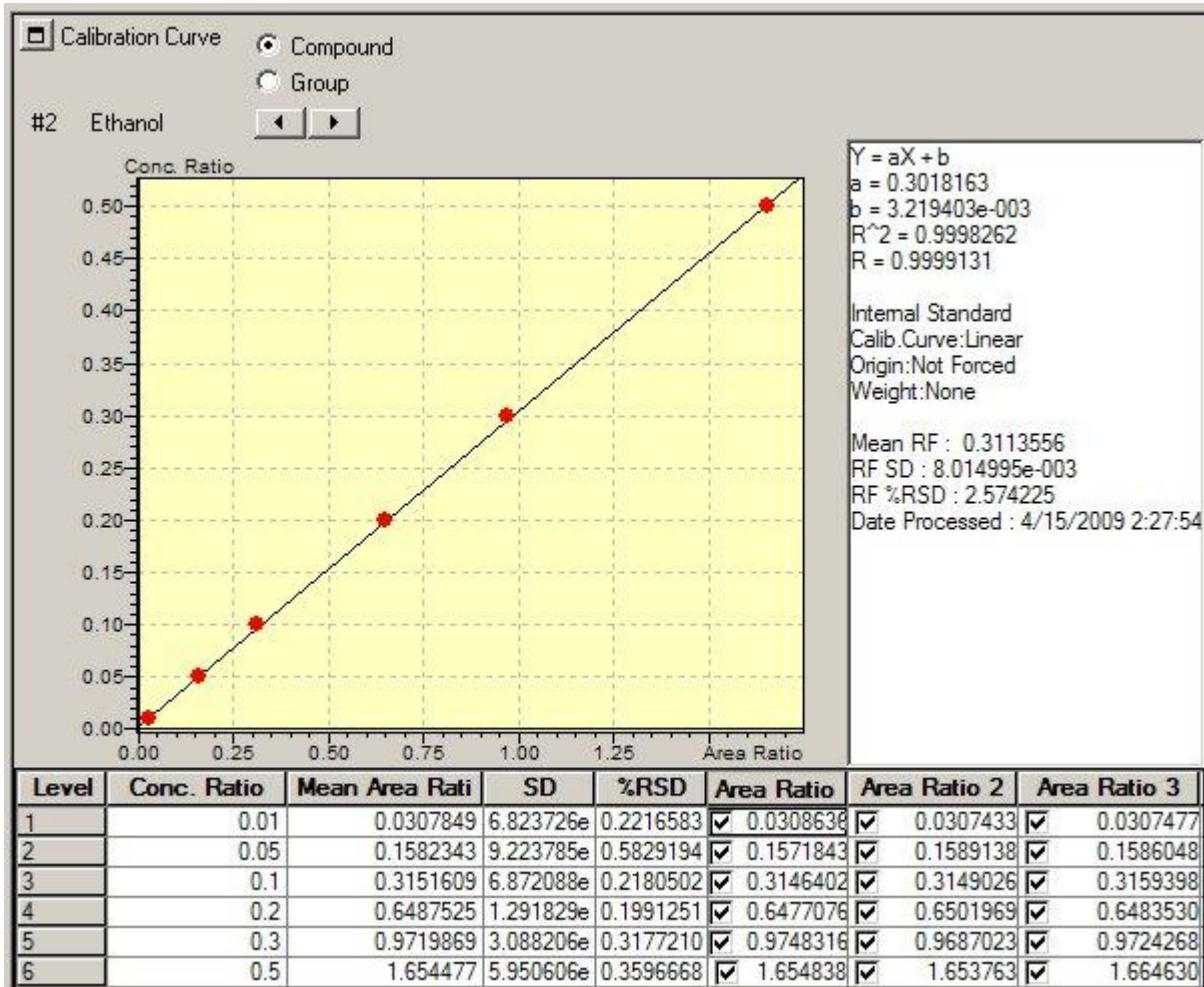


Figure 4: Calibration curve of ethanol

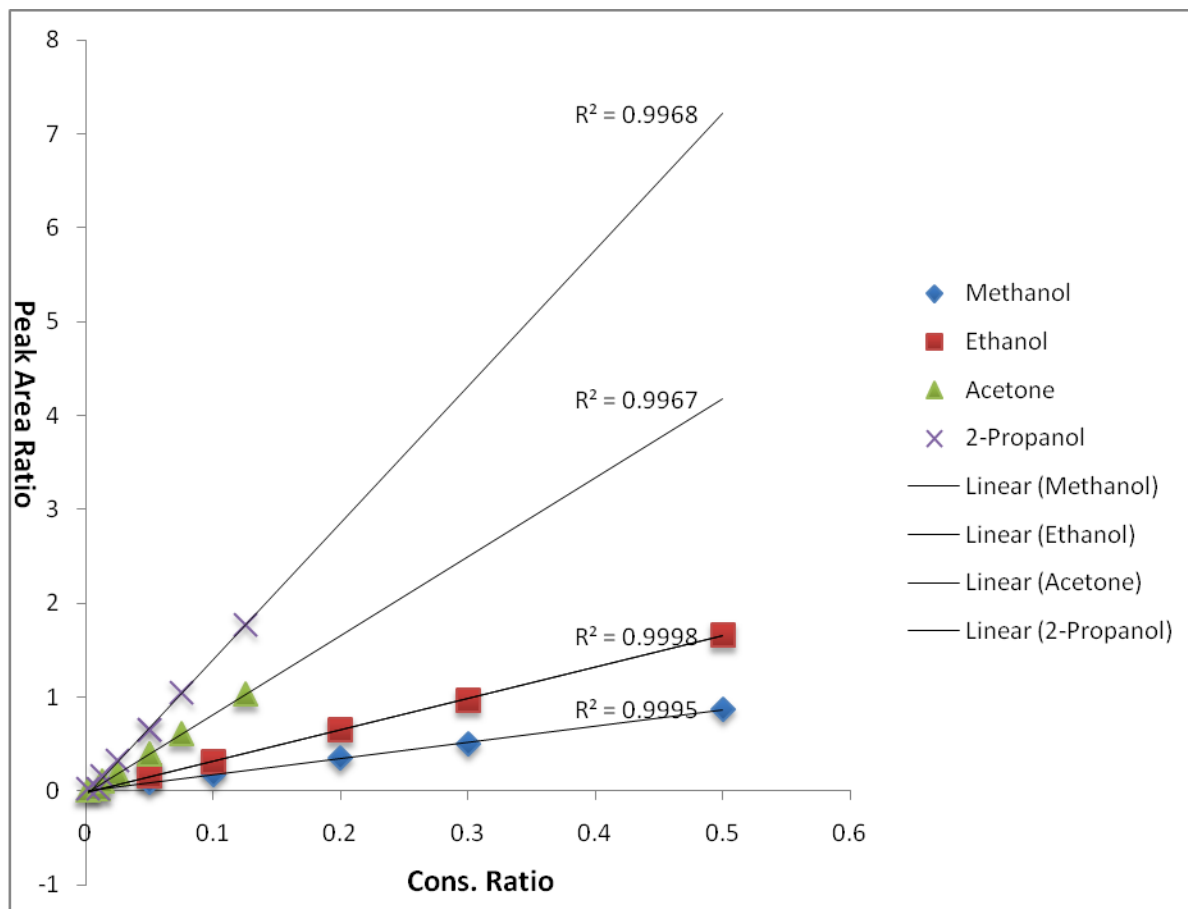


Figure 5: Calibration curves of acetone, 2-propanol, ethanol, and methanol

Measurement of Spiked BSA

To investigate matrix effect, Bovine Serum Albumin (BSA) was chosen to mimic blood content. 1% BSA was spiked with certain amount of methanol, ethanol, acetone, and 2-propanol. Recovery was calculated by subtracting the result of BSA blank runs from that of the spike runs, as shown in Table 4. Reproducibility was summarized in Table 5.

		Methanol	Ethanol	Acetone	2-propanol
BSA (g/dL)	Conc.1	0.0040	0.0318	0.0000	0.0000
	Conc.2	0.0000	0.0297	0.0000	0.0000
	Conc.3	0.0045	0.0296	0.0000	0.0000
Average		0.0028	0.0303	0.0000	0.0000
Dilution factor		0.8400	0.8400	0.8400	0.8400
Corrected Conc. (g/dL)		0.0024	0.0255	0.0000	0.0000
Spiked_BSA (g/dL)	Conc.1	0.1082	0.1218	0.0288	0.0259
	Conc.2	0.1044	0.1201	0.0288	0.0259
	Conc.3	0.1042	0.1193	0.0287	0.0255
	Conc.4	0.1053	0.1172	0.0288	0.0257
	Conc.5	0.1014	0.1151	0.0289	0.0262
	Conc.6	0.1039	0.1171	0.0287	0.0256
	Conc.7	0.1060	0.1204	0.0287	0.0256
	Conc.8	0.1022	0.1159	0.0288	0.0261
	Conc.9	0.1026	0.1162	0.0288	0.0260
Average		0.1042	0.1181	0.0288	0.0258
Spiked Amount (g/dL)		0.1000	0.1000	0.0250	0.0250
Recovered Con (g/dL)		0.1019	0.0926	0.0288	0.0258
Recovery (%)		101.8645	92.6177	115.0444	103.3289

Table 4: Recovery of spiked BSA

ID#2 Compound Name: Ethanol

Title	Ret. Time	Area %	Conc.	Units
BSA_041409_01.gcd	1.156	18.163	0.12179	g/dL
BSA_041409_02.gcd	1.161	18.026	0.12007	g/dL
BSA_041409_03.gcd	1.151	17.995	0.11925	g/dL
BSA_041409_04.gcd	1.163	17.677	0.11719	g/dL
BSA_041409_05.gcd	1.149	17.450	0.11510	g/dL
BSA_041409_06.gcd	1.151	17.720	0.11713	g/dL
BSA_041409_07.gcd	1.157	18.055	0.12036	g/dL
BSA_041409_08.gcd	1.155	17.515	0.11587	g/dL
BSA_041409_09.gcd	1.153	17.564	0.11622	g/dL
Average	1.155	17.796	0.11811	
%RSD	0.408	1.495	1.969	
Standard Deviation	0.005	0.266	0.00233	

Table 5: Reproducibility of spiked BSA

Conclusions

This study describes a straightforward configuration of the Shimadzu GC-2010 and AOC-5000 autosampler for blood alcohol static headspace analysis. Relative retention times were used for peak identification and proved to be a viable qualitative analysis method. The investigation of matrix effect demonstrated good reproducibility and excellent recoveries from spiked samples.