SHIMADZU

# 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, $H_2$ = 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

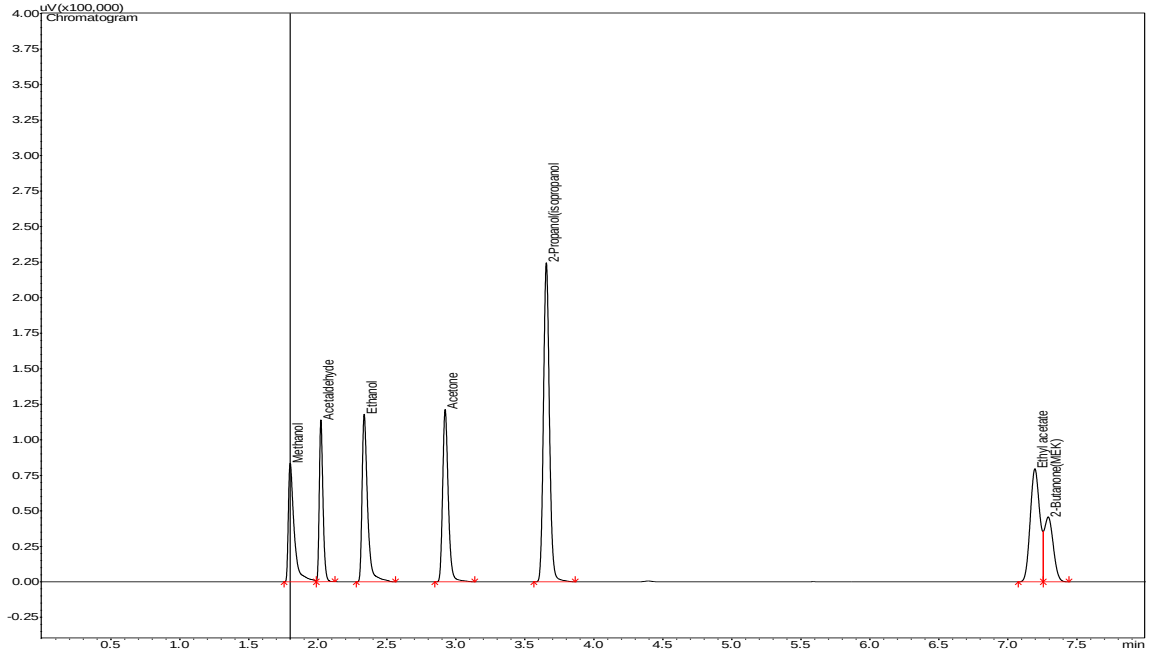



**Figure 1**: Blood alcohol mix resolution control standard, Restek #36256

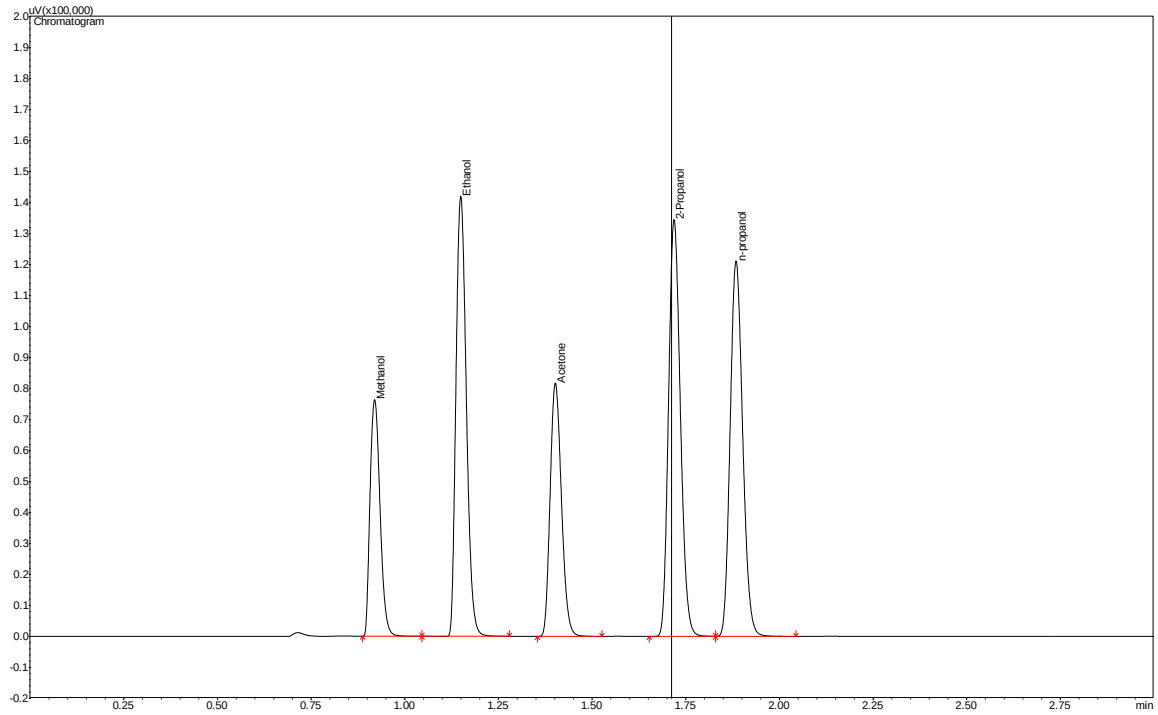



**Figure 2**: Chromatogram of Blood alcohol standard

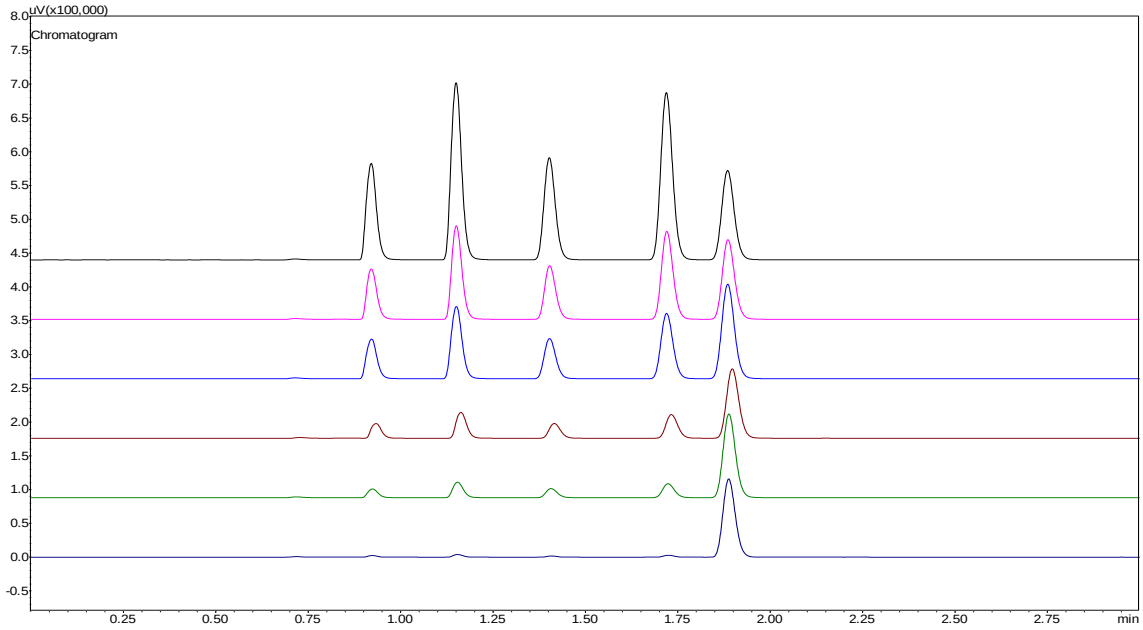



**Figure 3**: Chromatograms of Blood alcohol standard 1 to 6

## 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 |

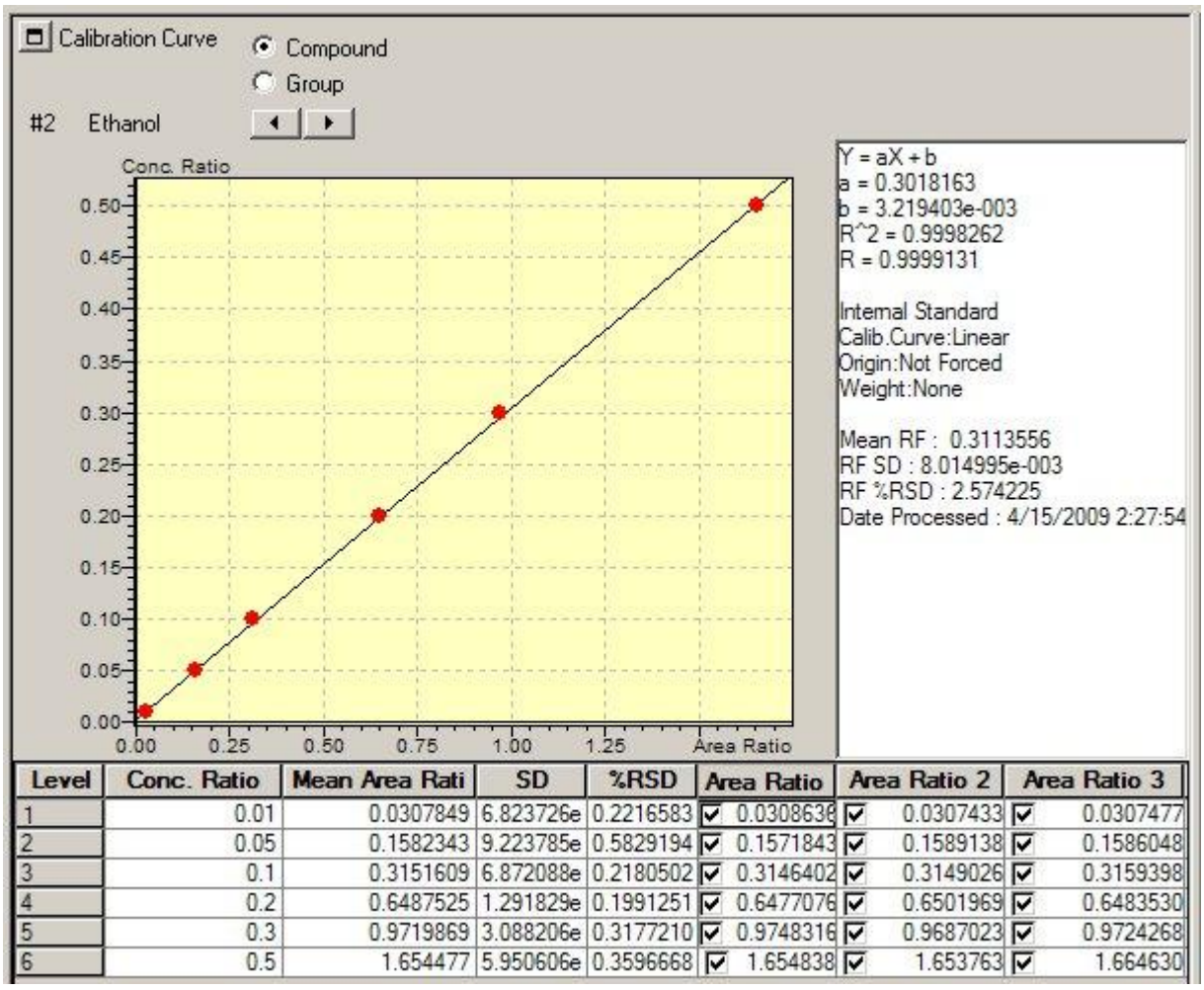
**Table 2**: Retention times in minutes

| 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.




| Level | Conc. Ratio | Mean Area Rati | SD | %RSD | Area Ratio | Area Ratio 2 | Area Ratio 3 |
|---|---|---|---|---|---|---|---|
| 1 | 0.01 | 0.0307849 | 6.823726e | 0.2216583 | 0.0308636 | 0.0307433 | 0.0307477 |
| 2 | 0.05 | 0.1582343 | 9.223785e | 0.5829194 | 0.1571843 | 0.1589138 | 0.1586048 |
| 3 | 0.1 | 0.3151609 | 6.872088e | 0.2180502 | 0.3146402 | 0.3149026 | 0.3159398 |
| 4 | 0.2 | 0.6487525 | 1.291829e | 0.1991251 | 0.6477076 | 0.6501969 | 0.6483530 |
| 5 | 0.3 | 0.9719869 | 3.088206e | 0.3177210 | 0.9748316 | 0.9687023 | 0.9724268 |
| 6 | 0.5 | 1.654477 | 5.950606e | 0.3596668 | 1.654838 | 1.653763 | 1.664630 |

**Figure 4**: Calibration curve of ethanol

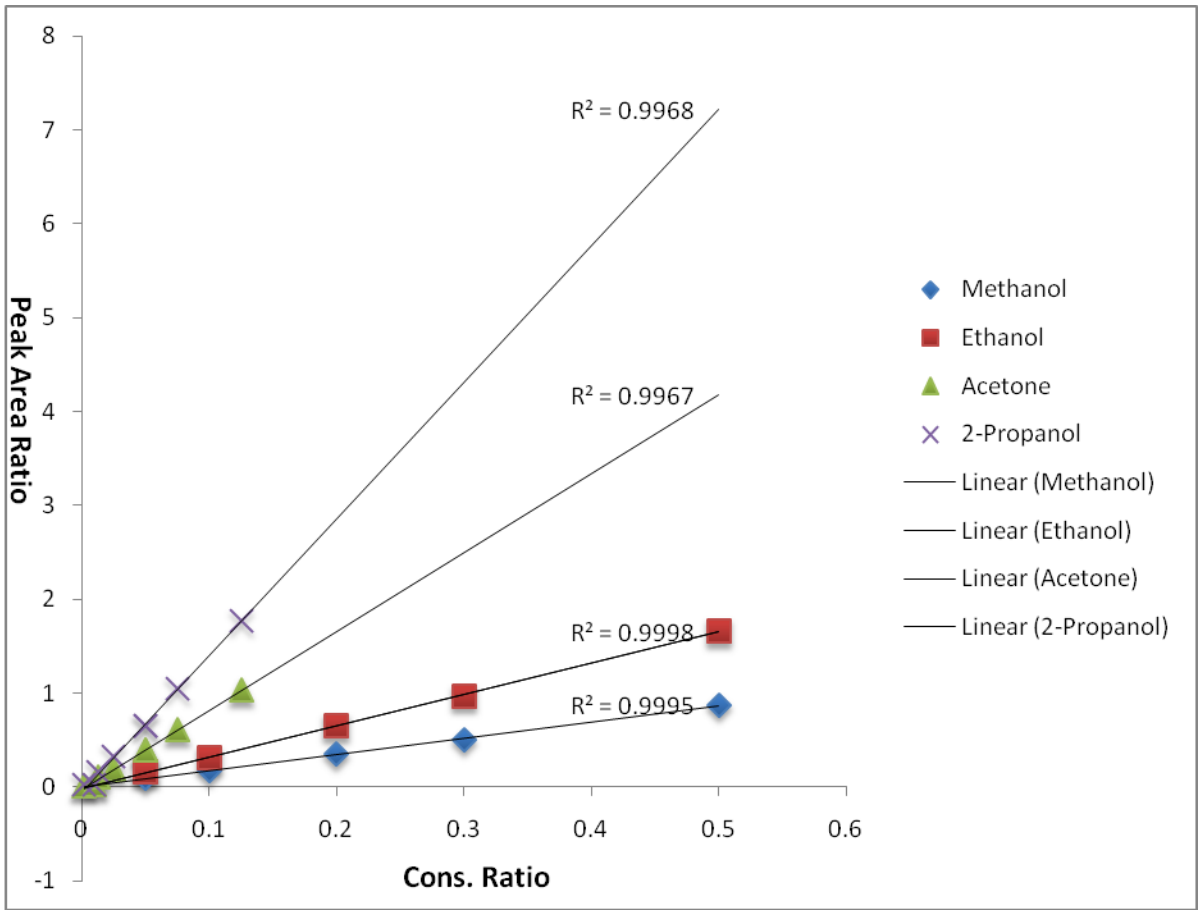



**Figure 5**: Calibration curves of acetone, 2-propanol, ethanol, and methanol

## 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.

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