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Application News

No. GCMS-2005

Gas Chromatography Mass Spectrometry

Simultaneous GC-MS and FID Analysis of Blood Alcohol Content utilizing Robotic Autosampler for Automated Sample Preparation

■ Abstract

Blood alcohol (BAC) analysis has been a staple application in the Forensic field for many years, typically performed using routine GC with dual FID and a dedicated closed loop headspace autosampler. However, more and more labs are transitioning to a combined GC-FID and GC-MS method for analysis. In this application, a GC-MS was used with FID for simultaneous BAC data acquisition. The purpose of this study was to determine if the AOC-6000 could perform repeatable sample preparation from start to finish, ending with sample injection. A total of six sets of calibration standards were prepped and analyzed by the system resulting in linearities of r^2 = 0.999 and greater. The standard deviation of ethanol concentration was also reported at less than 0.003 when performing a repeatability study of n=30 injections of 0.08 g/dL BAC standard solution.

■ Introduction

With many forensic toxicology labs transitioning from FID to a combination of GC-MS with FID data collection, it is imperative to produce a solution that allows for simultaneous analysis. This application also tackles one of the more fundamental problems faced during BAC sample analysis: sample preparation. Depending on state regulations, both calibration and check standards need to be prepared in multiple replicates, which can be time consuming for the analyst. Unknowns must then also be prepared for analysis, which ultimately leads to a large amount of time spent by the analyst just prepping samples for analysis. The goal of this application was to eliminate the time spent prepping samples allowing the analyst to utilize time needed for other projects or data analysis. An additional benefit of the automated sample prep is the removal of the human element from the preparation of samples eliminating human error from the equation. Using the robotic sampler, an AOC-6000 RTC 120cm autosampler, all calibration standards and unknown samples were prepped and subsequently analyzed with a high level of repeatability and accuracy for BAC.

■ Samples and Analytical Conditions/Experimental

Instrument Configuration

A Shimadzu GCMS-QP2020 NX was utilized in unison with an FID-2030 to acquire simultaneous BAC data output. The advanced flow technologies (AFT) were configured for detector splitting to ensure sample was actively split between both detectors keeping flow to the mass spec at low levels without sacrificing sensitivity and typical BAC analysis run time. The instrument was also outfitted with an AOC-6000 RTC 120cm rail as the sample introduction device. Not only was the headspace injection used, but the liquid capabilities were used for sample preparation. Large wash stations were installed to accommodate the large volumes of standards and diluents necessary for sample preparation. The AOC-6000 was also equipped with two Tool Parking stations in order to automatically switch between the three liquid syringes and 2.5mL HS syringe.

Sample Preparation

The AOC-6000 was used to perform sample preparation of the BAC standards from start to finish, which includes the headspace injection of the before mentioned standards. A 1.0 g/dL BAC stock solution consisting of methanol, ethanol, isopropanol, and acetone was placed in a 20mL HS vial in the first position of the first 60 vial headspace rack. Large wash station module one was reserved for deionized water and it was used for standard dilution and syringe pre/post wash cycles. Large wash station module two contained 100mL of a stock 0.03g/dL internal standard n-propanol standard in the first vial position. The second vial position was filled with deionized water for syringe pre/post wash cycles. The second headspace vial rack was filled with empty 20mL screw cap HS vials in positions 1-34.

Using the AOC-6000, five calibration standards with a final volume of 100uL were created resulting in the following concentrations: 0.01, 0.04, 0.1, 0.2, 0.5g/dL.

The standards were prepared in replicates of three, skipping a vial after each calibration prep. Vials 1-5, 7-11, and 13-17 contained 100uL of the BAC standards while vials 6 and 12 remained empty. The previously mentioned process was repeated to create calibration check standards to ensure the instrument is functioning accordingly. Vials 18-22, 24-28, and 30-34 contained 100uL of the BAC standards while vials 23 and 29 remained empty.

Internal standard was then added by the AOC-6000 in 1mL aliquots to vials 1-34. After internal standard was added, each vial was transported to the vortex module to ensure homogeneity. The blank vials at positions 6, 12, 23 and 29 also received 1mL aliquots of internal standard and were used as control samples by confirming zero carryover after each calibration analysis. Following the addition of internal standard, each was sampled via HS injection using the HS syringe. For method conditions regarding the headspace injection, see Table 1 below.

Table 1: GCMS with FID Method Parameters

AOC-6000	**HS Method Parameters**
Sample	1mL Sample Injection Volume
	20mL HS Vial
Equilibration	15 minutes at 60°C
	Syringe Temp 70°C
	Agitator 500 RPM
GC	**GC-2030**
Injection	Split 30:1
	Column Flow 4mL/min
Column	SH-Rtx-BAC1, 30.0m x 0.32mm ID x 1.80um
	Helium Carrier Gas
	Constant Pressure 86.3 kPa
	Linear Velocity 72.1 cm/s
Oven Program	Isothermal 40°C
	Total GC Run Time 5.25 min
	Total cycle time 10 min
APC	25 kPa for Detector Splitter
Detector 1	**MS**
Operating Mode	Scan Mode 29m/z - 200m/z 2.00min - 5.25min
Ion Source	200°C
MS Interface	250°C
Detector 2	**FID**
FID Temperature	250°C
FID Flow Rates	H_2 = 32.0 mL/min
	Air = 200 mL/min
	Makeup = 24.0 mL/min

■ Results and Discussion

Chromatography

The headspace sample was actively split between GC-MS and FID using the AFT Detector splitting kit using a constant APC pressure of 25kPa at the splitter. The result showed nearly the exact same retention times for all peaks on both detector's respective chromatograms shown in Figure 1.

All five components were resolved within the 5-minute run time with the method set to a linear velocity of 72 cm/s.

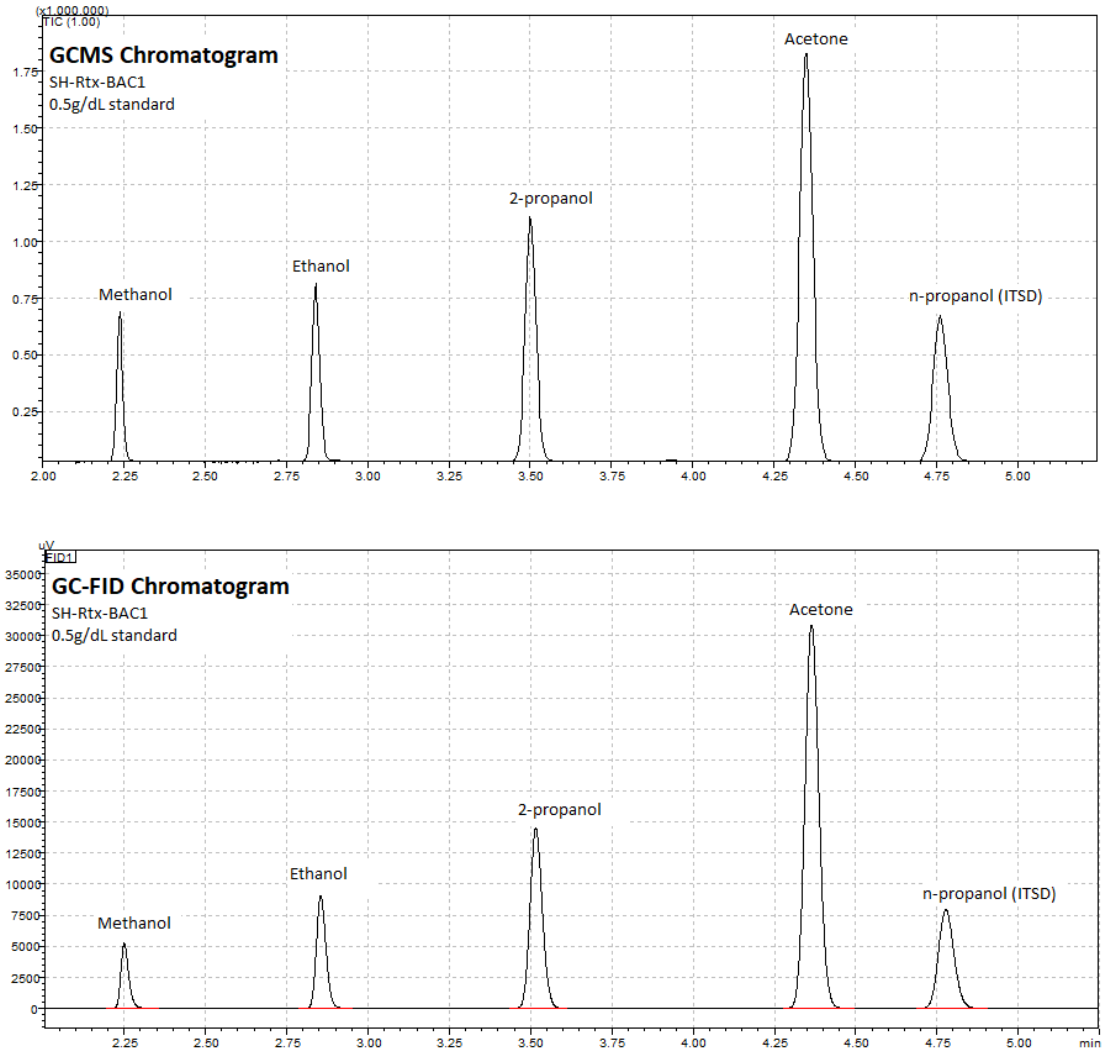

Figure 1: GCMS Chromatogram (top) of the 0.5g/dL BAC standard and the GC-FID Chromatogram (bottom) of the same 0.5g/dL.

Mass Spec Confirmation

Using the MS for mass spectral confirmation is advantageous when performing BAC analysis. Confirming the spectra in the chromatogram removes the need for dual-column analysis, which previously used to confirm elution order on GC-FID.

The mass spectral information for all peaks were cross referenced with the latest Wiley/NIST library for the most accurate results. Even at the lowest level of 0.01 g/dL, the similarity index was calculated at 96 (shown in Figure 2).

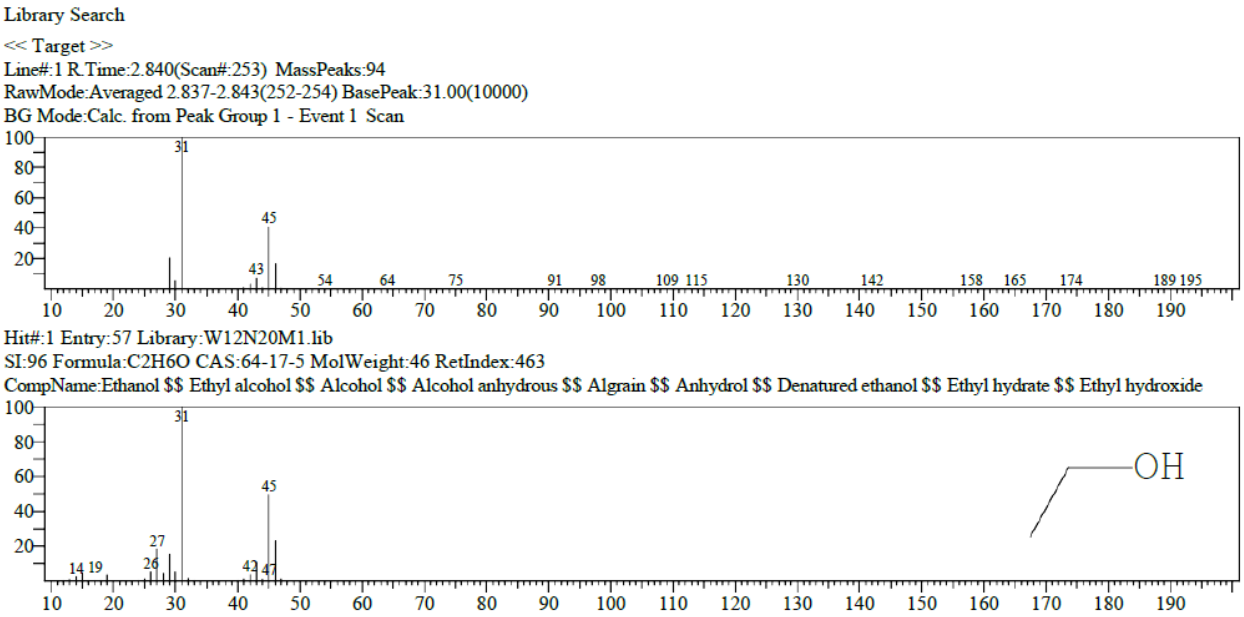

Figure 2: Library search results of ethanol at 0.01 g/dL using Wiley/NIST library

Calibration

Five calibration standards (0.01, 0.04, 0.1, 0.2, 0.5g/dL) were prepared from start to finish by the AOC-6000; this included the internal standard addition.

Each set of standards was prepped in triplicates followed by a blank containing only internal standard, which was used to ensure there was no carryover (Figure 3).

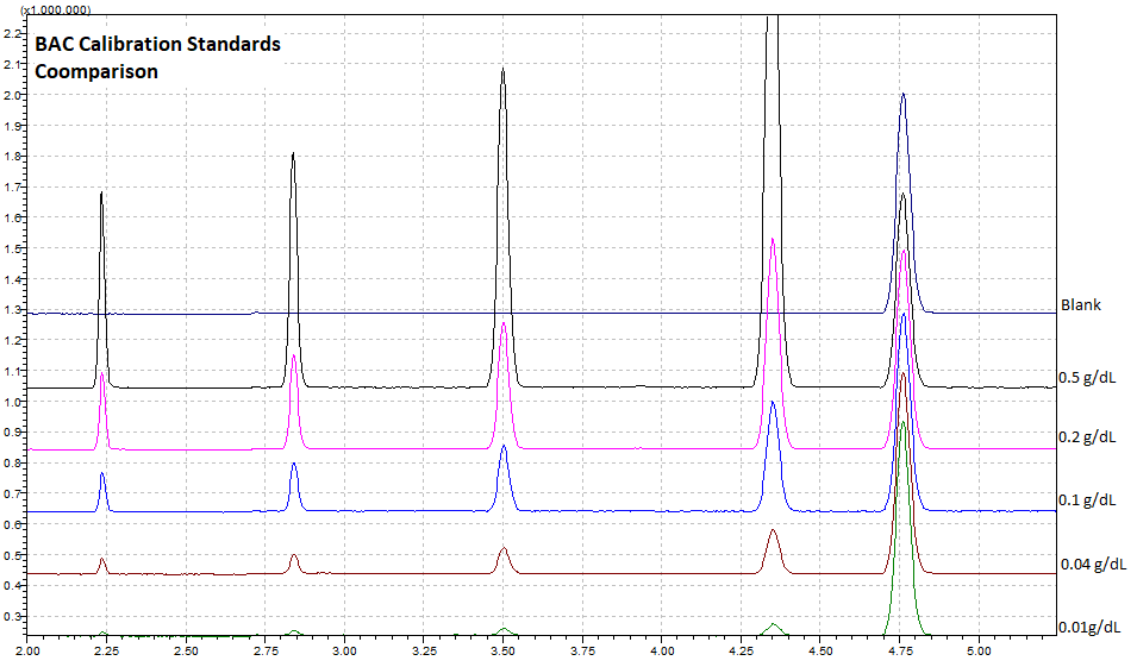

Figure 3: Data comparison of the BAC calibration standards prepped and sampled by the AOC-6000. The blank is the top chromatogram with only the internal standard peak present.

The calibration curves for both GC-MS and GC-FID were built using a total of six replicates for each level: three repetitions of calibration standards and three repetitions of check standards. Linearity of a minimum r^2 = 0.999 were obtained for all analytes on both FID and MS detector.

The calibration curve information for ethanol on both detectors is shown in Figure 4. The linearity values for the additional three compounds can be found in Table 2.

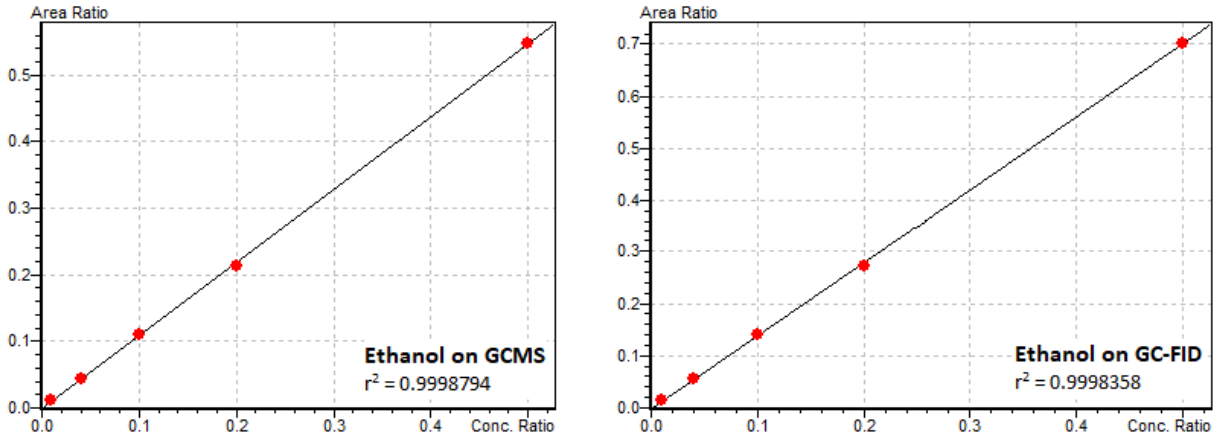

Figure 4: Comparison of calibration curves for ethanol on both GC-MS and GC-FID with their respective linearities.

Table 2. Linearity values for 4 target compounds on GC-FID and GCMS

	GCMS	FID
Methanol	0.9998657	0.9998063
Ethanol	0.9998794	0.9998358
2-propanol	0.9999022	0.9998546
Acetone	0.9998253	0.9997563

Reproducibility

After calibration samples were analyzed, 30 samples at 0.08 g/dL were run with internal standard and quantified for ethanol concentration. As shown in Table 3, the average concentration of ethanol samples were 0.0779 g/dL and 0.0768 g/dL for GCMS and GC-FID respectively.

In addition to the more than acceptable RSD values, the standard deviation of ethanol concentration remained below .005.

Table 3. Repeatability values for ethanol (n=30 samples)

	GCMS	FID
Average RT (min)	2.838	2.853
% RSD for RT	0.033	0.017
Average EtOH conc. (g/dL)	0.0779	0.0768
Standard Deviation of EtOH conc.	0.0022	0.0021

■ Conclusion

Using a GC-MS paired with FID for simultaneous data collection for BAC analysis provides highly repeatable compound identification and ethanol quantification. Using the robotic sampler for sample preparation eliminates the need for analyst sample creation while maintaining a high level of accuracy and repeatability across a broad linear range.

With the addition of mass spectral confirmation, ethanol along with the other BAC components can be accurately identified even at lower concentration levels. The instrument solution matched with the above method parameters proved not only efficient but reliable for automated BAC analysis.

■ Consumables

REST-18003	Rtx-BAC1 Cap. Column 30m, 0.32mm ID, 1.80um
225-19744-03	SYRINGE, 10UL GT 0.47 [AOC-6000]
225-19744-08	SYRINGE, 250UL GT 0.47 [AOC-6000]
225-19744-10	SYRINGE, 1mL GT 0.47 [AOC-6000]
225-19744-11	SYRINGE, 2.5mL GT 0.47 [AOC-6000] HS
220-97331-16	Vials, 20ml Headspace vial w/ 18mm Magn Screw Cap & Silicone/PTFE Septa, Kit, round bottom, 100/pk

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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