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BAC Analysis Utilizing GCMS and FID Combined with Fully Automated Sample Prep Performed by Robotic Sampler

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Introduction

Blood Alcohol Content (BAC) analysis by gas chromatography (GC) and gas chromatography/mass spectrometry (GCMS) is a standard analytical technique that has been around for years. However, the standard and sample prep is laborious and tedious. Using a custom script and robotic autosampler with syringe swap capability, a protocol was developed to automatically prepare calibration and check standards, perform internal standard addition, and perform headspace (HS) injections into a GCMS paired with FID establishing a turn key system for BAC analysis.

In this study, the quantitative accuracy and reproducibility of four analytes were tested with a Shimadzu GCMS/FID with AOC-6000 multifunction robotic autosampler.

Experimental Methods

The four analytes tested were: Methanol, Ethanol, Isopropanol, and Acetone. n-Propanol was used as an internal standard. These four analytes and internal standard are specified in the Procedure for HS GC for Blood Alcohol Analysis of the North Carolina Department of Justice (NCDOJ).[1] A stock solution containing the 4 analytes and a stock solution of the internal standard were prepared. The autosampler was programmed to prepare 5 diluted standards in triplicate. Quality control check standards were prepared in triplicate identical in concentration to the calibration standards. Standards were injected on GCMS/FID with detector splitter.

Table 1. Concentrations (g/dL) of Calibration and Check Standards

Analyte	Std1	Std2	Std3	Std4	Std5	Check Std
Methanol	0.0101	0.0403	0.100	0.201	0.504	0.0201
Ethanol	0.0100	0.0402	0.100	0.201	0.502	0.0201
Isopropanol	0.0100	0.0400	0.100	0.200	0.500	0.0200
Acetone	0.0100	0.0400	0.100	0.200	0.500	0.0200

Internal standard n-propanol concentration: 0.0482 g/dL
The autosampler was used to prepare each vial with 100 µL of standard and 1 mL of internal standard.

Standard Preparation

Standards were prepared by the AOC-6000 autosampler. 10, 25, and 1000 uL syringes were used. Large volume wash stations and 20mL vials were used to store stock standard solutions, water rinse solutions, and, and water for dilution. A 2.5 mL headspace syringe was used for injecting standards.

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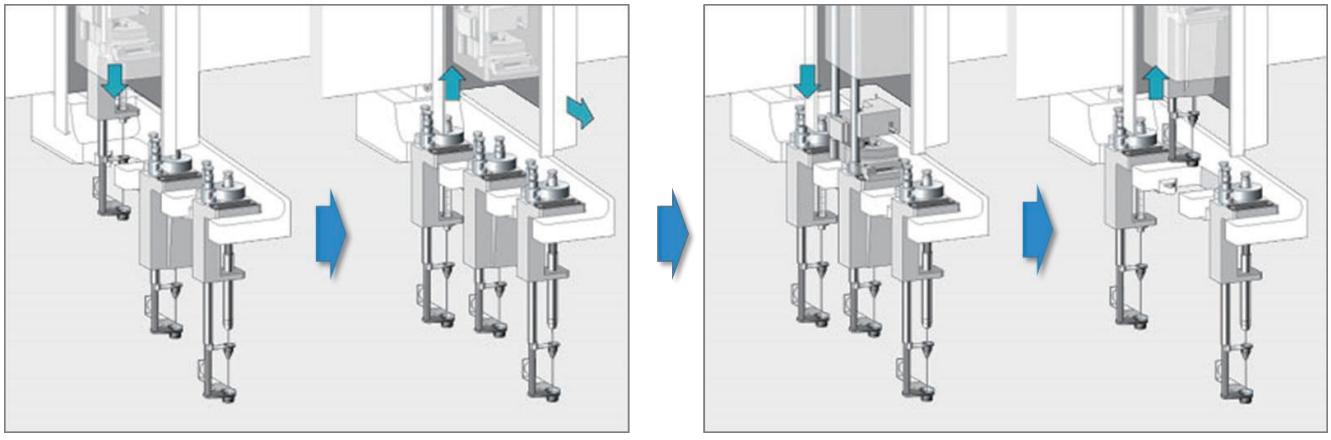

Figure 1. Robotic Tool Changer allows for switching between three liquid syringes (10, 250, 1000 µL) and HS syringe (2.5 mL)

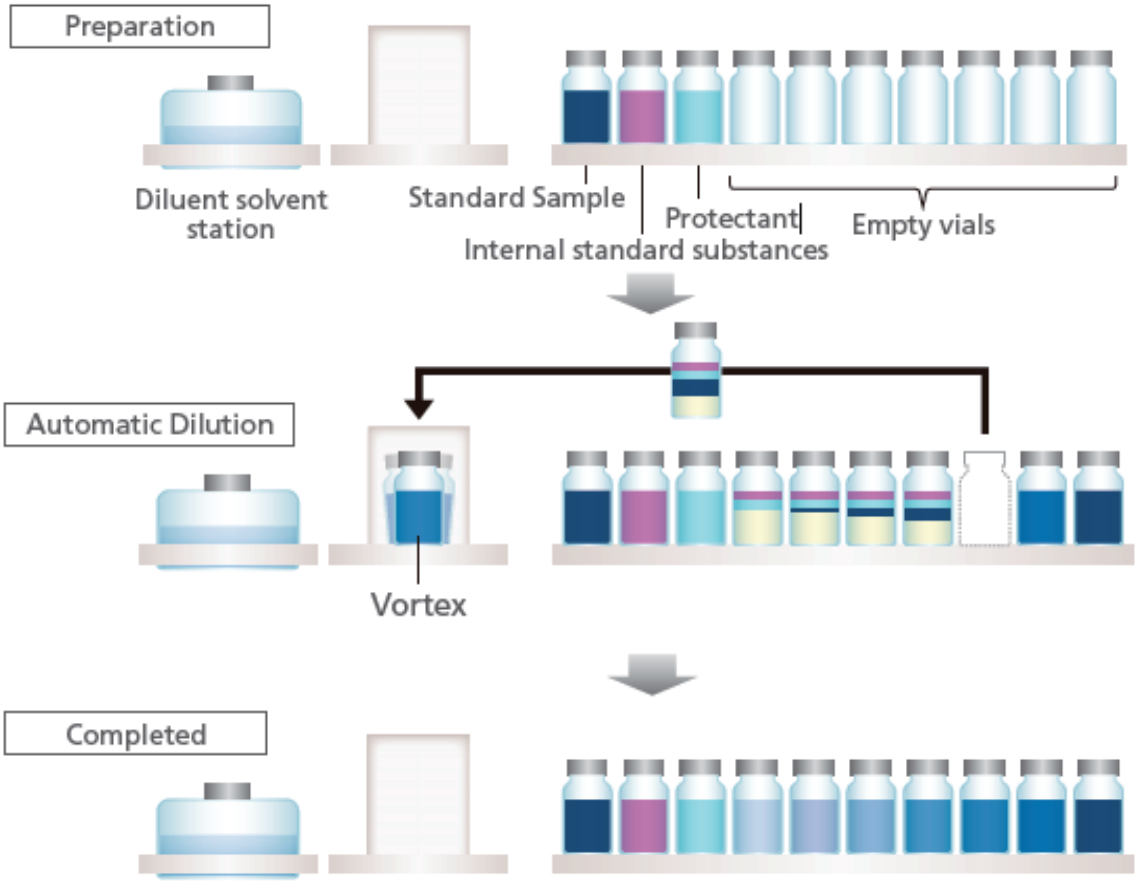

Figure 2. Sample schematic of Automatic Dilution

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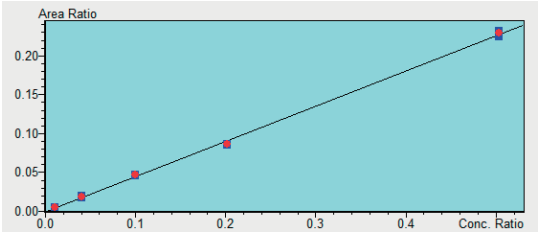
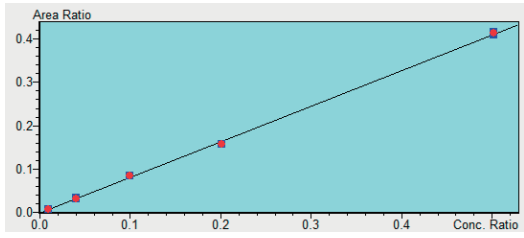
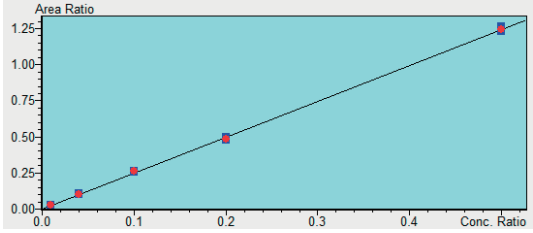
Results



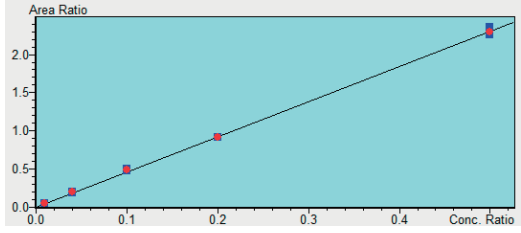
Methanol: r^2 = 9996 (m/z 31)



Ethanol: r^2 = 9994 (m/z 31)

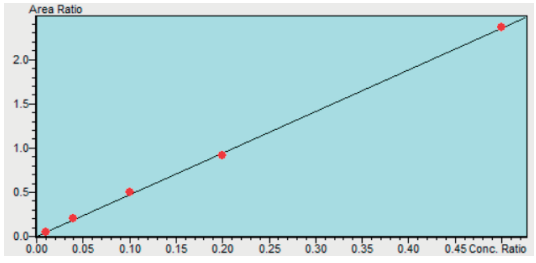


Isopropanol: r^2 = 9998 (m/z 45)

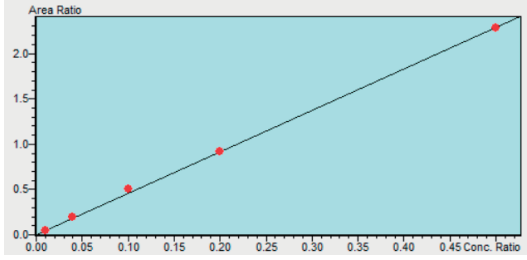


Acetone: r^2 = 9999 (m/z 43)

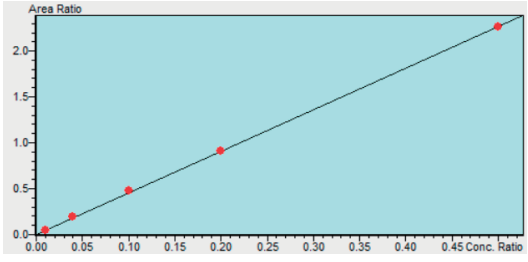
Figure 3. Calibration Curves run on MS
NCDOJ requires r^2 of 0.995 or greater[1]



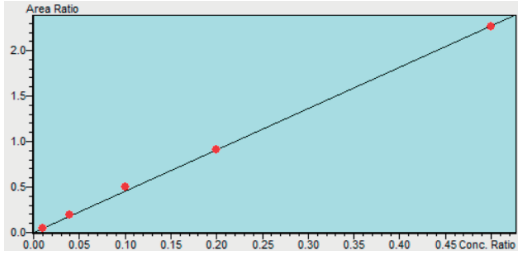
Methanol: r^2 = 9999 (m/z 31)



Ethanol: r^2 = 9999 (m/z 31)



Isopropanol: r^2 = 9999 (m/z 45)



Acetone: r^2 = 9998 (m/z 43)

Figure 4. Calibration Curves run on FID
NCDOJ requires r^2 of 0.995 or greater[1]

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Table 2. FID Error in %Difference of Check Standards
NCDOJ requirement: All Ethanol concentrations ±5% of Target
All others ±10% of Target[1]

MeOH			EtOH			IPA			Acetone		
Meas Conc	Actual Conc	%Diff	Meas Conc	Actual Conc	%Diff	Meas Conc	Actual Conc	%Diff	Meas Conc	Actual Conc	%Diff
0.0103	0.0101	1.96	0.0101	0.01	1.00	0.0101	0.01	1.00	0.0101	0.01	1.00
0.0102	0.0101	0.99	0.0102	0.01	1.98	0.0102	0.01	1.98	0.0098	0.01	2.02
0.0104	0.0101	2.93	0.0101	0.01	1.00	0.0103	0.01	2.96	0.0096	0.01	4.08
0.02	0.0202	1.00	0.0199	0.0201	1.00	0.0201	0.02	0.50	0.0211	0.02	5.35
0.0201	0.0202	0.50	0.0199	0.0201	1.00	0.0201	0.02	0.50	0.0203	0.02	1.49
0.0203	0.0202	0.49	0.0198	0.0201	1.50	0.0207	0.02	3.44	0.0209	0.02	4.40
0.0401	0.0403	0.50	0.0408	0.0402	1.48	0.0402	0.04	0.50	0.0409	0.04	2.22
0.0404	0.0403	0.25	0.0405	0.0402	0.74	0.0398	0.04	0.50	0.0403	0.04	0.75
0.0418	0.0403	3.65	0.0403	0.0402	0.25	0.0393	0.04	1.77	0.0395	0.04	1.26
0.101	0.1	1.00	0.101	0.1	1.00	0.102	0.1	1.98	0.0994	0.1	0.60
0.098	0.1	2.02	0.099	0.1	1.01	0.101	0.1	1.00	0.101	0.1	1.00
0.101	0.1	1.00	0.098	0.1	2.02	0.102	0.1	1.98	0.102	0.1	1.98
0.198	0.201	1.50	0.2	0.201	0.50	0.201	0.2	0.50	0.19	0.2	5.13
0.199	0.201	1.00	0.199	0.201	1.00	0.198	0.2	1.01	0.19	0.2	5.13
0.198	0.201	1.50	0.202	0.201	0.50	0.197	0.2	1.51	0.19	0.2	5.13
0.506	0.504	0.40	0.503	0.502	0.20	0.494	0.5	1.21	0.485	0.5	3.05
0.51	0.504	1.18	0.507	0.502	0.99	0.495	0.5	1.01	0.485	0.5	3.05
0.508	0.504	0.79	0.508	0.502	1.19	0.504	0.5	0.80	0.491	0.5	1.82

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Table 3. Mass Spec Error in %Difference of Check Standards
NCDOJ requirement: All Ethanol concentrations ±5% of Target
All others ±10% of Target[1]

MeOH			EtOH			IPA			Acetone		
Meas Conc	Actual Conc	%Diff	Meas Conc	Actual Conc	%Diff	Meas Conc	Actual Conc	%Diff	Meas Conc	Actual Conc	%Diff
0.0105	0.0101	3.86	0.0101	0.01	0.7	0.009	0.01	9.6	0.0101	0.01	1
0.0108	0.0101	6.73	0.0102	0.01	1.5	0.0091	0.01	9.2	0.0098	0.01	1.9
0.0108	0.0101	6.53	0.0101	0.01	1	0.009	0.01	9.6	0.0096	0.01	3.6
0.0213	0.0202	5.54	0.0197	0.0201	2.04	0.019	0.02	5.25	0.0211	0.02	5.5
0.0205	0.0202	1.58	0.0199	0.0201	1	0.0193	0.02	3.75	0.0203	0.02	1.65
0.0209	0.0202	3.66	0.0199	0.0201	0.85	0.0192	0.02	4.25	0.0209	0.02	4.5
0.0421	0.0403	4.49	0.0419	0.0402	4.18	0.0402	0.04	0.53	0.0409	0.04	2.33
0.0431	0.0403	6.95	0.0414	0.0402	3.03	0.0398	0.04	0.4	0.0403	0.04	0.65
0.0418	0.0403	3.67	0.0403	0.0402	0.32	0.0393	0.04	1.85	0.0395	0.04	1.15
0.109	0.1	8.63	0.104	0.1	3.99	0.102	0.1	1.65	0.0994	0.1	0.54
0.107	0.1	6.51	0.103	0.1	2.46	0.101	0.1	1.4	0.1	0.1	0.04
0.109	0.1	8.74	0.103	0.1	2.99	0.102	0.1	1.77	0.102	0.1	2.45
0.196	0.201	2.5	0.194	0.201	3.28	0.19	0.2	4.81	0.19	0.2	4.69
0.197	0.201	2.31	0.194	0.201	3.38	0.191	0.2	4.48	0.19	0.2	5.05
0.198	0.201	1.9	0.192	0.201	4.17	0.192	0.2	3.98	0.19	0.2	4.89
0.506	0.504	0.56	0.502	0.502	0.09	0.494	0.5	1.24	0.485	0.5	3
0.515	0.504	2.33	0.507	0.502	1.01	0.495	0.5	0.97	0.485	0.5	2.96
0.519	0.504	3.16	0.508	0.502	1.12	0.504	0.5	0.76	0.491	0.5	1.81

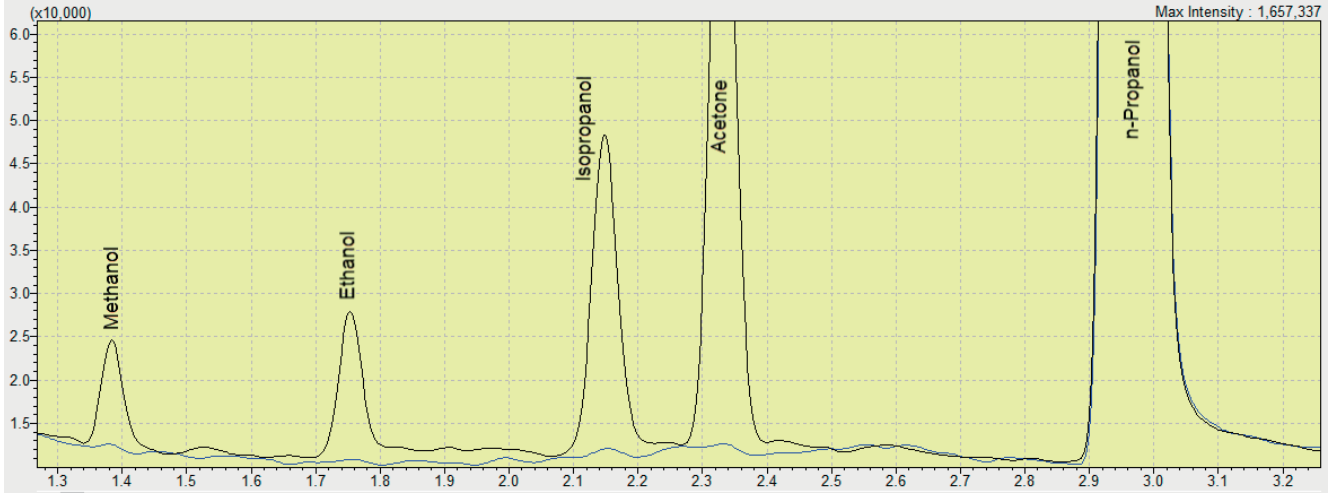

Figure 6. Sample Chromatogram
0.01 g/dL standard overlaid with negative control

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Conclusion

Using the AOC-6000 Autosampler on a GCMS/FID is an effective method of standard preparation, while using both FID and MS for confirmatory analysis. Only two standards were prepared by the user – the stock calibration standard and internal standard. All other standards were prepared by the autosampler and all samples were spiked with internal standard by the autosampler. All prep and analyses were run automatically in a continuous run. The resulting calibration curves had excellent linearities and the check standards had reproducibility that exceeded stringent state requirements.

References

North Carolina Department of Justice Crime Lab ISO Procedures.
https://www.ncdoj.gov/About-DOJ/Crime-Lab/ISO-Procedures/Headspace-Gas-Chromatography-to-Quantitate.aspx
(accessed Jan 24, 2019)

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