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Blood Alcohol Determination using Static Headspace Analysis with Optimized Sample Throughput

Application Note - Environmental

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Abstract

There are several ways to determine the amount of alcohol that is in a person's system. The most common methods are breath analysis in the field and blood analysis in the lab. Blood alcohol determination in the laboratory is used predominantly when a person refuses a breath test. In order to determine blood alcohol content, a person's blood has to be withdrawn as soon as possible after the occurrence. Furthermore, the blood needs to be collected in duplicate in order to confirm the test results. This application note will examine static headspace sampling of alcohol standards using Gas Chromatography (GC) for separation and Flame Ionization Detection (FID) for analysis. The linearity of the compounds of interest will be examined and compared using a secondary column for confirmation. Additionally, as many forensic labs have an excess of samples to examine, the use of software innovations will aid in optimizing sample throughput.

Introduction:

Throughout the United States, it is illegal to drive when your blood alcohol level is above a certain point, most commonly 0.08%. If a driver is found to be above this level, he/she will be arrested for driving under the influence (DUI). In the field, there are assorted tests to determine a driver's level of intoxication. Most commonly, police officers use a field sobriety test and/or a breathalyzer. However, when an offender refuses to take a breathalyzer test, police are required to bring the suspect in for a blood alcohol test.

Due to the complex matrix of blood, static headspace sampling is the sampling method used for blood alcohol analysis; while GC/FID is employed for analyte separation and analysis. Since the results of this sampling and analysis have the potential to be disputed in court, the testing has to be both accurate and reproducible so as to be able to stand up to scrutiny. Forensic labs that do this kind of testing not only have to be able to meet the requirements of this analysis but also are expected to optimize sample throughput in order to meet laboratory demands.

The focus of this paper will be on optimizing sample throughput while maintaining both the precision and the accuracy of the analysis. Six point curves, and precision and accuracy studies will be performed on both the primary blood alcohol column and the confirmation column. Furthermore, using innovative software, a method was developed to optimize headspace sampling while preserving the GC/FID four minute cycle time.

Experimental:

The sampling system used for this analysis was the EST Analytical FLEX autosampler fitted with a 2.5ml headspace syringe, while an Agilent 7890 GC/FID was used for separation and analysis. Blood alcohol analysis requires testing using two columns, one for initial testing and one for confirmation. The columns used for this study were the Restek Rtx®-BAC Plus 1 and Rtx®-BAC Plus 2. After the sampling and analysis systems were set up, the experimental parameters were optimized in order to shorten analysis cycle times. Refer to Tables 1 and 2 for the sampling and analysis parameters.

Autosampler	FLEX
General	
Method Type	Headspace
GC Ready	Continue
GC Cycle Time	4.1min
Constant Heat Mode	Yes
Timing Conflict	Continue
Sample Incubate Agitate	
Incubation Temp.	60°C
Incubation Time	10.1min
Agitation Speed	100%
Agitation Delay	0.1min
Agitation Duration	10.0min
Wait	
Wait on Input	Yes
Wait Input	GC Ready
Sample Fill	
Syringe Temperature	70°C
Syringe Needle Depth	80%
Sample Depth Speed	20%
Sample Volume	40% (1000μl)
Sample Fill Rate	10%
Sample Fill Delay	1.0sec
Injection	
Needle Depth Speed	30%
Needle Depth	90%
Injection Rate	10%
Injection Volume	40% (1000μl)
Pre-Injection Delay	Off
Post-Injection Delay	Off
Injection Start Input	Start
Sweep Needle	
Needle Temperature	70°C
Syringe Pumps	5
Syringe Pump Volume	80% (2000μl)
Syringe Pump Speed	50%

Table 1: FLEX Autosampler Experimental Parameters

GC/FID	Agilent 7890
Inlet	Split/Splitless
Inlet Temp.	220°C
Inlet Head Pressure	16.105 psi
Split	80:1
Liner	Restek SKY Liner Splitless, 2mm x 6.5 x 78.5
Column	Rtx-BAC Plus 1 and Rtx-BAC Plus 2 30m x 0.32mm x 1.8um
Oven Temp. Program	40°C hold for 4.0 min
Column Flow Rate	4.0ml/min.
Gas	Helium
FID Temp.	250°C

Table 2: GC/MS Experimental Parameters

Blood alcohol reagents were purchased from Sigma Aldrich. The reagents were all of ≥99.5% purity. Dilutions were performed on the reagents in order to prepare a 0.01 to 0.40 g/dL curve using n-propanol as the internal standard at a 0.20 g/dL concentration. Secondary ethanol standards were purchased from Sigma Aldrich in order to confirm the calibration. Furthermore, a six component blood alcohol resolution standard was purchased from Restek in order to verify analyte resolution. Six point calibration curves, seven replicate precision and accuracy standards, secondary standard confirmation and analyte resolution studies were performed on both blood alcohol columns. Curve results are listed in Table 3, Precision and Accuracy results are listed in Tables 4 and 5, K Factor Results are presented in Table 6, Secondary Standard Recoveries are displayed in Table 7 and Table 8 shows the carryover after a 0.40g/dL standard. Finally, Figures 1 and 2 display chromatograms of compound resolution using both columns.

Compound	BAC1		BAC2	
	Curve %RSD	Curve R^2	Curve %RSD	Curve R^2
methanol	1.05	.0000	3.06	.0000
acetaldehyde	2.87	0.9999	1.59	0.9994
ethanol	0.89	.0000	2.61	.0000
isopropanol	1.79	1.0000	1.26	1.0000
acetone	1.68	1.0000	1.23	0.9997
t-butanol	2.93	1.0000	1.51	0.9999

Table 3: Curve Results

0.20g/dL Precision BAC1							
Filename	n-propanol (IS)	methanol	acetaldehyde	ethanol	isopropanol	acetone	t-butanol
BAC0512045.D	27831027.53	6586516.53	69348428.09	14120432.00	8476891.88	7119747.50	70623443.00
BAC0512046.D	27627995.71	6585795.85	70569173.16	14159777.66	28714439.41	68257257.50	71371253.82
BAC0512047.D	26977764.36	6512436.00	68220946.25	13910238.50	7869167.37	5948946.25	68885075.00
BAC0512048.D	27452510.86	6537740.75	70499826.60	14052526.66	28537478.13	67986082.00	71075418.75
BAC0512049.D	27959800.47	6852999.35	69347186.40	14531570.63	8883395.63	7333571.00	71025974.25
BAC0512050.D	28102019.76	6676375.27	71561616.48	14389831.71	29189818.25	69263677.63	72557142.20
BAC0512051.D	27416544.50	6568745.85	70458310.63	14139074.53	8681662.25	8130409.38	71414756.09
Ave.	27623951.88	6617229.94	70000783.94	14186207.38	8621836.13	7719955.89	70993294.73
Std. Dev.	353393.35	107349.77	1016873.28	193469.98	377549.80	967846.18	1025064.09
%RSD	1.28	1.62	1.45	1.36	.32	.43	1.44

Table 4: Precision and Accuracy BAC1

0.20g/dL Precision BAC2							
Filename	n-propanol	methanol	acetaldehyde	ethanol	isopropanol	acetone	t-butanol
BAC0509037.D	27215350.75	6607779.37	62884065.50	14025338.50	28026103.50	3619360.25	8942593.00
BAC0509038.D	26486052.38	6254403.62	62675587.14	13424178.48	27257890.00	63064290.63	67779193.50
BAC0509039.D	26813439.90	6305377.96	63032373.70	13528439.57	27474348.38	3430626.50	8353013.25
BAC0509040.D	27954990.97	6828283.74	63832186.11	14467786.90	28758979.50	64922989.25	70589107.00
BAC0509041.D	26709978.56	6439660.46	62229633.55	13795353.02	27837305.13	3371421.13	8880939.50
BAC0509042.D	26322912.11	6245267.29	62263699.99	13457283.03	27407194.50	63023459.00	68154608.88
BAC0509043.D	26837722.46	6365348.68	62472010.36	13654414.19	27603286.75	3221588.50	8444765.50
Ave.	26905778.16	6435160.16	62769936.62	13764684.81	27766443.97	3521962.18	8734888.66
Std. Dev.	502007.50	198138.87	514939.31	346749.24	471653.41	603745.58	843688.82
%RSD	1.87	3.08	0.82	2.52	1.70	.95	.23

Table 5: Precision and Accuracy BAC2

Run #	BAC1			BAC2		
	n-propanol	ethanol	K Factor BAC1	n-propanol	ethanol	K Factor BAC2
1	7831028	14120432	0.394	27215351	14025339	0.388
2	27627996	14159778	0.390	26486052	13424178	0.395
3	6977764	13910239	0.388	26813440	13528440	0.396
4	27452511	14052527	0.391	27954991	14467787	0.386
5	7959800	14531571	0.385	26709979	13795353	0.387
6	28102020	14389832	0.391	26322912	13457283	0.391
7	7416545	14139075	0.388	26837722	13654414	0.393
Ave.	27623952	14186207	0.389	26905778	13764685	0.391
Std Dev.	353393	193470	0.003	502007	346749	0.004
%RSD	1.28	1.36	0.704	1.87	2.52	0.704

Table 6: K Factor Results

Test	BAC1 % Recovery	BAC2 % Recovery
1	00	96
2	98	98
3	98	96
4	99	98
5	99	99
Ave.	99	97

Table 7: Secondary Standard Recoveries

Compound	BAC1 %Carryover	BAC2 %Carryover
methanol	.170	0.069
acetaldehyde	0.047	0.022
ethanol	.150	0.069
isopropanol	0.032	0.029
acetone	0.04	0.028
t-butanol	0.018	0.015

Table 8: %Carryover after a 0.40g/dL standard

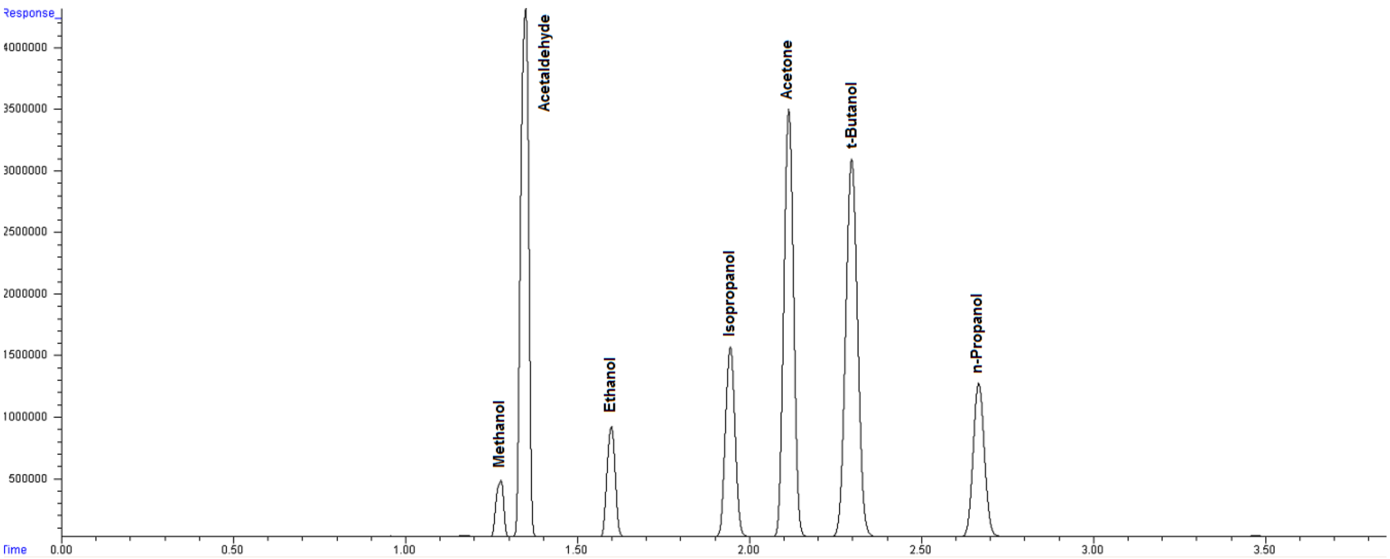

Figure 1: BAC1 Resolution Standard at 0.20g/dL Concentration

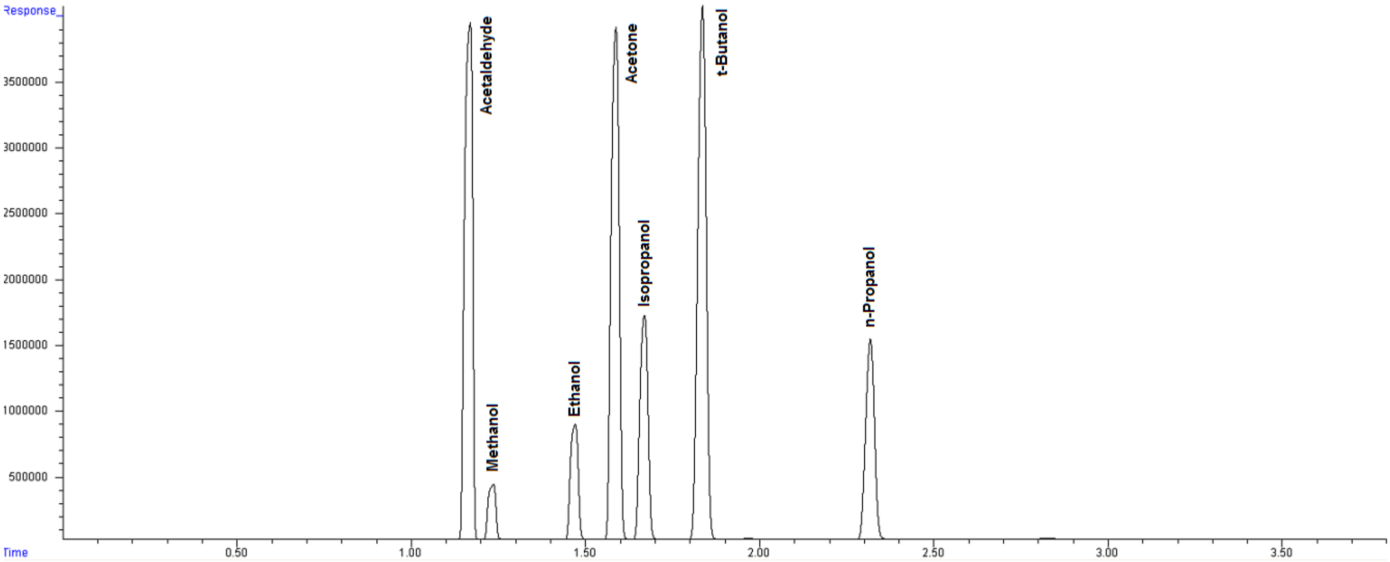

Figure 2: BAC2 Resolution Standard at 0.20g/dL Concentration

Conclusions:

Static headspace sampling coupled with GC/FID analysis of blood alcohol samples is an effective tool for the detection of alcohol in blood. Using the innovative software of the FLEX autosampler, samples can be incubated for ten minutes and still have a four minute cycle time. The analytes of interest displayed excellent linearity, and precision and accuracy while recoveries of secondary standards were also very accurate. For the busy forensic lab, more sample trays can be added to the autosampler in order to increase efficiency of sample throughput making the FLEX autosampler an excellent addition to your lab.

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