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 In order to determine blood alcohol content, a person's blood has to be withdrawn as soon as possible after the occurrence.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 testing; 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 a Shimadzu GC-2010 Plus GCFID 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	80%
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	5%
Injection Volume	40% (1000μl)
Pre-Injection Delay	Off
Post-Injection Delay	Off
Injection Start Input	Start
Sweep Needle	
Needle Temperature	Ambient
Syringe Pumps	5
Syringe Pump Volume	80% (2000μl)
Syringe Pump Speed	50%

Table 1: FLEX Autosampler Experimental Parameters

GC/FID	Shimadzu GC-2010 Plus
Inlet	Split/Splitless
Inlet Temp.	220ºC
Inlet Head Pressure	78.2 kPa
Split	80:1
Linear Velocity	39.1 cm/sec
Column	Rtx-BAC Plus 1 and Rtx-BAC Plus 2 30m x 0.32mm x 1.8um
Oven Temp. Program	40ºC hold for 4.0 min
Column Flow Rate	2.5 ml/min.
Gas	Helium
FID Temp.	240ºC

Table 2: GCFID 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. Six point calibration curves and seven replicate precision and accuracy standards were performed on both blood alcohol columns. Curve results are shown in Table 3, Precision results are listed in Tables 4 and 5, K Factor Results are presented in Table 6, and Ethanol accuracy results are stated in table 7. Finally, Figures 1 and 2 display 0.20g/dL standard chromatograms using both blood alcohol columns.

Compound	BAC1		BAC2	
	Curve %RSD	Curve R^2	Curve %RSD	Curve R^2
methanol	8.34	0.9999	4.62	0.9999
acetaldehyde	3.61	0.9999	1.96	0.9999
ethanol	2.93	0.9999	1.38	0.9999
isopropanol	1.32	0.9999	3.71	1.0000
acetone	1.77	1.0000	1.70	1.0000
t-butanol	5.83	1.0000	3.60	0.9999

Table 3: Curve Results

0.20g/dL Precision BAC1							
Replicate Number	n-propanol (IS)	methanol	acetaldehyde	ethanol	isopropanol	acetone	t-butanol
1	309250	75340	579557	155260	328324	779956	858999
2	312275	75147	586599	156041	331901	789726	869730
3	311634	77677	585194	159584	333774	787367	868600
4	317232	78140	597712	162123	341107	806485	893388
5	315073	76737	594872	158788	335706	799949	880922
6	315442	75804	590930	157866	334704	796957	880521
7	319838	78267	598423	162587	342401	807179	895474
Ave.	314392.00	76730.29	590469.57	158892.71	335416.71	795374.14	878233.43
Std. Dev.	3334.22	1227.24	6515.92	2589.94	4584.39	9411.05	12389.13
%RSD	1.06	1.60	1.10	1.63	1.37	1.18	1.41

Table 4: Precision BAC1

0.20g/dL Precision BAC2							
Replicate Number	n-propanol (IS)	methanol	acetaldehyde	ethanol	isopropanol	acetone	t-butanol
1	322677	64408	633304	160239	326358	830769	896350
2	314842	66299	657360	156859	334027	853452	912858
3	320322	62685	638682	159971	323701	831012	891563
4	328223	66285	651244	164662	335510	850368	921083
5	327953	65051	643754	163686	349645	857677	910711
6	318758	65518	657074	160283	333066	852500	913575
7	332900	62938	666215	163570	337416	866680	943836
Ave.	323667.86	64740.57	649661.86	161324.29	334246.14	848922.57	912853.71
Std. Dev.	5837.36	1368.00	10781.93	2557.72	7779.40	12408.00	15849.30
%RSD	1.80	2.11	1.66	1.59	2.33	1.46	1.74

Table 5: Precision BAC2

Replicate Number	BAC1				BAC2			
	n-propanol	ethanol	K Factor BAC1	± 1.5%	n-propanol	ethanol	K Factor BAC2	± 1.5%
1	309250	155260	0.398	Pass	322677	160239	0.403	Pass
2	312275	156041	0.400	Pass	314842	156859	0.401	Pass
3	311634	159584	0.391	Pass	320322	159971	0.400	Pass
4	317232	162123	0.391	Pass	328223	164662	0.399	Pass
5	315073	158788	0.397	Pass	327953	163686	0.401	Pass
6	315442	157866	0.400	Pass	318758	160283	0.398	Pass
7	319838	162587	0.393	Pass	332900	163570	0.407	Pass
Ave.	314392	158893	0.396	0.390/0.402	323668	161324	0.401	0.395/0.407

Table 6: K Factor Results

Replicate Number	BAC1 % Recovery	BAC2 % Recovery
1	101	103
2	101	107
3	103	104
4	103	104
5	102	104
6	101	104
7	102	102
Ave.	102	104

Table 7: Ethanol Standard Recoveries

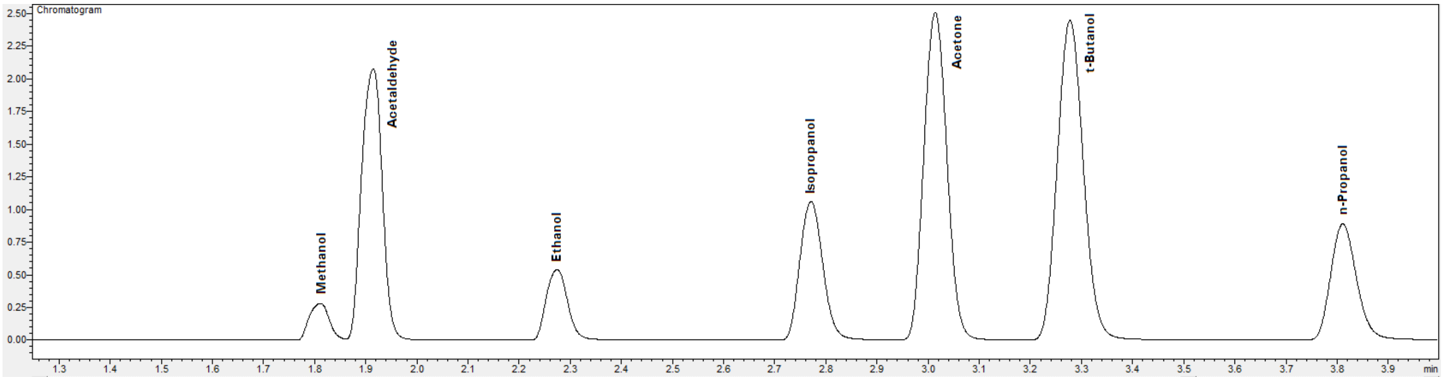

Figure 1: BAC1 Column Chromatogram at 0.20g/dL Concentration

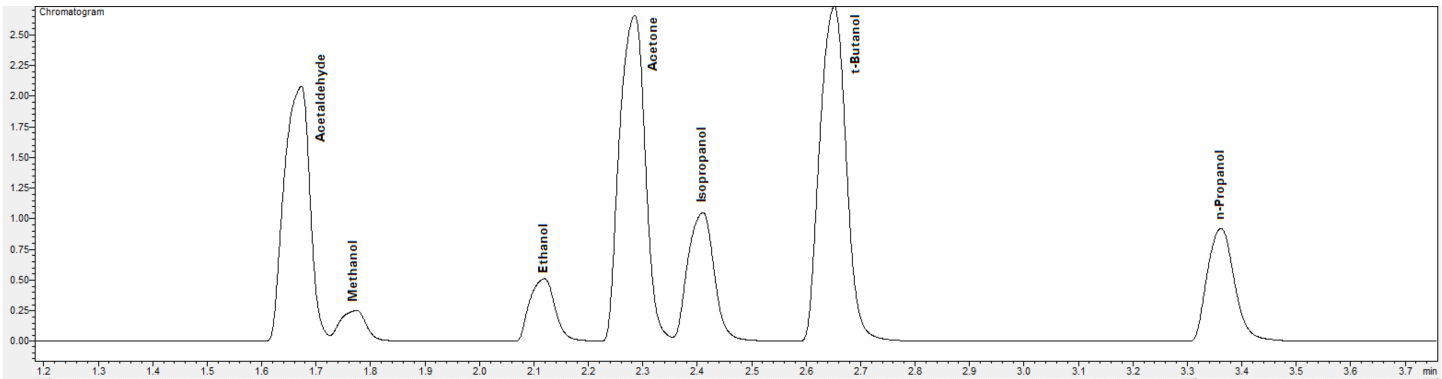

Figure 2: BAC2 Column Chromatogram at 0.20g/dL Concentration

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 sample cycle time. The analytes of interest displayed excellent linearity and precision while the accuracy of the ethanol recoveries was exceptional. Furthermore, the K factor for the replicate samples met the ±1.5% criteria specified for blood alcohol analysis. 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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