APPLICATION NOTE AN097

Blood Alcohol Determination By Versa Headspace Sampling

INTRODUCTION

Blood alcohol analysis is one of a variety of headspace methods performed in forensic toxicology laboratories. The Versa was evaluated to determine its suitability to perform an occasional blood alcohol test. Methanol, acetone, ethanol, isopropanol and acetaldehyde along with n-propanol, a typical internal standard, were tested with the Versa headspace instrument. These volatile organic compounds which must be accurately identified and quantified, especially in legal medical cases in which the compounds are contributing factors to death or criminal investigations.

Figure 1. SCION Instruments Versa Headspace Sampler

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EXPERIMENTAL

The Versa headspace instrument was connected to GC with FID for this application note. A ZB-BAC-1 column, 30m x 0.53mm x 3.0µm was used to perform the quantitation portion of the analysis.

Table 1 displays the Versa parameters for the blood alcohol analysis. Table 2 displays the GC/FID parameters used for the separation of the six blood alcohol components.

Table 1. Versa Loop Parameters

Variable	Value	Variable	Value
GC Cycle Time	11 min	Pressurize	10 psig
Valve Oven Temp	70°C	Pressurize Time	2.00 min
Transfer Line Temp	75°C	Pressurize Equil Time	0.25 min
Platen/Sample Temp	65°C	Loop Fill Pressure	7 psig
Platen Temp Equil Time	0.50 min	Loop Fill Time	2.00 min
Sample Equil Time	10 min	Inject Time	0.50 min
Mixer	off		

Table 2. GC/FID Method parameters

Parameter	Setting
Column	ZB-BAC-1, 30m, 0.53mm ID, 3.0µm; Linear Velocity 38 cm/sec
Inlet	Split Ratio 5:1, Temperature 140°C, Helium Carrier Gas, Purge Flow 0.5 mL/min
Oven	40.0°C, isothermal, 10 minutes
FID	250°C, Hydrogen Flow: 35.0 mL/min, Air Flow: 400 mL/min, Column and Makeup Flow: 30 mL/min, Sampling Rate

An internal standard solution was prepared by adding approximately 620µL of n-propanol into a 1L flask containing 20g of sodium chloride dissolved in deionized water.

An accuracy test mix was prepared by adding approximately 90µL of acetaldehyde, 175µL of methanol, 70µL of acetone, 110µL of isopropanol and 105µL of ethanol into a 100mL volumetric flask containing deionized water. A set of 7 accuracy standards were prepared by adding 1mL of the internal standard and 0.5mL of the test mix into 22mL headspace vials. These were capped with Teflon lined septa and sealed. Blanks were prepared by placing 1mL of internal standard and 0.5mL of deionized water into 22mL headspace vials.

A linearity test mix was prepared by adding approximately 370µL of acetaldehyde, 690µL of methanol, 270µL of acetone, 430µL of isopropanol, and 420µL of ethanol into a 100mL volumetric flask containing deionized water. A linear standard series was prepared by pipetting 3µL, 10µL, 30µL, 60µL, 125µL, 250µL, and 500µL into separate 22mL headspace vials containing 1mL of internal standard solution. Water was added to the vials so that the total volume of test mix and water was equal to 1.5mL. These were sealed with Teflon-lined septa and sealed. Table 3 lists the concentrations of the accuracy and linearity standards in g/dL.

Table 3. Accuracy and Linearity Approximate Concentrations in g/dL for the 0.5mL Samples Added to 1mL of Internal Standard Solution

Compound	Accuracy	500µL	250µL	125µL	60µL	30µL	10µL	3µL
Methanol	0.138	0.546	0.273	0.136	0.065	0.033	0.011	0.003
Acetaldehyde	0.071	0.290	0.145	0.073	0.035	0.017	0.006	0.002
Ethanol	0.083	0.331	0.166	0.083	0.040	0.020	0.007	0.002
Isopropanol	0.086	0.338	0.169	0.084	0.041	0.020	0.007	0.002
Acetone	0.055	0.214	0.107	0.053	0.026	0.013	0.004	0.001

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Results

The CDS software was used to measure the peak areas of all solvents from both the accuracy and the linearity study. The peak area data was entered into a Microsoft Excel spreadsheet. The average and the percent relative standard deviations (%RSD) were calculated from the accuracy samples by both external and internal standard methods. N-Propanol was used as the internal standard. This data is presented in Table 4.

The correlation coefficient (r^2) was calculated from the linearity standards by both external and internal standard methods. This data is presented in Table 5.

The chromatogram of the standard is presented in Figure 2.

Table 4: Average and %RSD of Methanol, Acetaldehyde, Ethanol, Isopropanol, Acetone and n-Propanol of the 7 Accuracy Standards Calculated by External (Area) and Internal Standard (IS) Methods

	Methanol		**Acetaldehyde**		**Ethanol**		**Isopropanol**		**Acetone**		**n-Propanol**
Sample	Area	IS	Area	IS	Area	IS	Area	IS	Area	IS	Int Std
1	675725	0.31518	3004372	1.40135	941593	0.43919	2071477	0.96621	2434915	1.13573	2143918
2	670092	0.32373	2942336	1.42150	924437	0.44661	2029247	0.98037	2379069	1.14938	2069880
3	705702	0.32471	3130144	1.44025	979136	0.45052	2154548	0.99136	2530903	1.16453	2173334
4	683987	0.33399	3029362	1.47922	945340	0.46161	2074703	1.01307	2450154	1.19640	2047939
5	692344	0.33999	3116792	1.53056	961454	0.47214	2110877	1.03659	2500489	1.22792	2036367
6	657449	0.32840	2953801	1.47545	922583	0.46084	2028341	1.01318	2380615	1.18914	2001965
7	681411	0.32661	2990310	1.43330	951117	0.45588	2085893	0.99980	2424213	1.16196	2086318
Average	680959	0.32752	3023874	1.45452	946523	0.45526	2079298	1.00008	2442908	1.17501	2079960
%RSD	2.28	2.41	2.45	3.00	2.11	2.39	2.14	2.34	2.33	2.68	2.91

Table 5: Correlation Coefficient (r^2) of the Seven Point Calibration Curve of Methanol, Acetaldehyde, Ethanol, Isopropanol, Acetone Calculated by External (Area) and Internal Standard (IS) Methods. The average[A] and the %RSD[B] are calculated for the internal standard, n-propanol.

	Methanol		**Acetaldehyde**		**Ethanol**		**Isopropanol**		**Acetone**		**n-Propanol**
µL Added	Area	IS	Area	IS	Area	IS	Area	IS	Area	IS	Int Std
3	13453	0.00650	51969	0.02512	17944	0.00867	39115	0.01891	53472	0.02585	2068761
10	47334	0.02212	183765	0.08588	63123	0.02950	138389	0.06468	183762	0.08588	2139681
30	139587	0.06675	567645	0.27145	192549	0.09208	422092	0.20185	559034	0.26733	2091150
60	292393	0.13485	1164017	0.53686	398063	0.18359	870323	0.40140	1150372	0.53056	2168207
125	638367	0.29501	2501647	1.15609	864788	0.39965	1882933	0.87017	2485936	1.14883	2163878
250	1367549	0.63057	5185309	2.39093	1833353	0.84535	3956936	1.82453	5195643	2.39569	2168742
500	2582989	1.27666	9583915	4.73691	3426346	1.69349	7293127	3.60467	9582685	4.73630	2023243
r^2	0.9990	0.9996	0.9983	0.9999	0.9987	0.9998	0.9982	0.9998	0.9982	0.9999	2117666[A]
											2.71[B]

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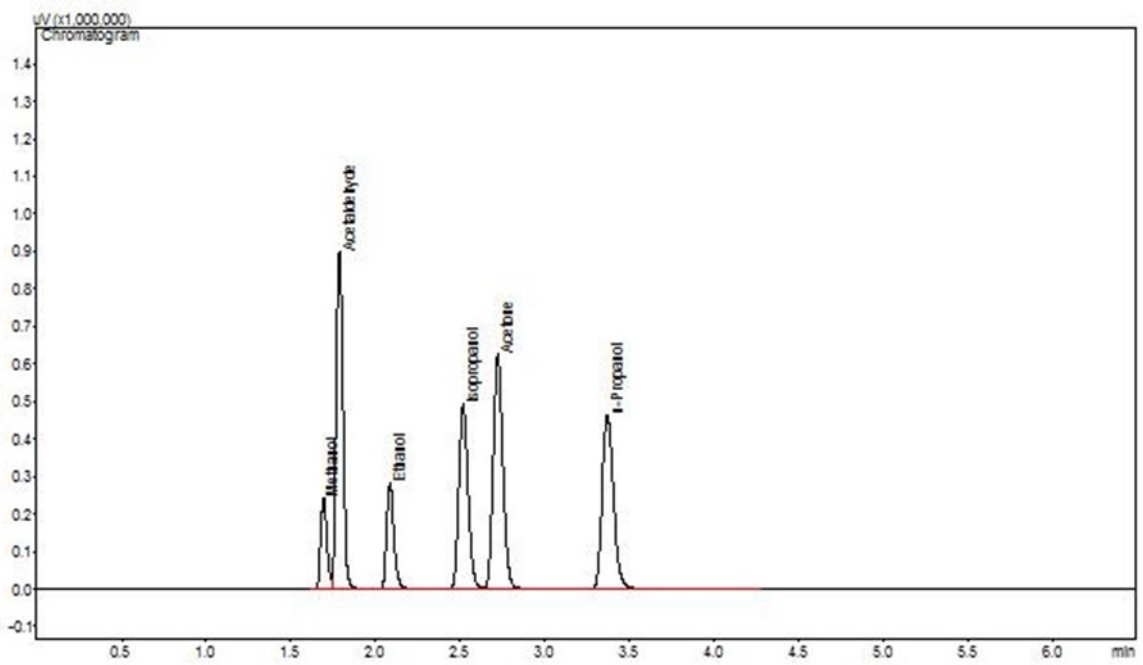

Figure 2. Chromatogram of the Blood Alcohol Standards

CONCLUSION

The Versa was tested with a typical blood alcohol method to determine the percent relative standard deviation (%RSD) of a set of 7 standards. The %RSD was less than 3% for the six typical blood alcohol solvents calculated by either the external or internal standard method.

A linearity study from 0.002g/dL to 0.33g/dL ethanol provided a correlation coefficient (r^2) greater than 0.999 by either the external or internal standard calculation method. The other solvents had a correlation coefficient (r^2) greater than 0.998 by either the external standard or internal standard calculation method for a similar concentration range.

SCION Instruments

US
11840 W Market Place, Suite K-L
Fulton, MD 20759
Phone: +1 844 547 0022
sales-usa@scioninstruments.com

The Netherlands
Amundsenweg 22-24
4462 GP Goes
The Netherlands
Phone: +31 (0) 113 287 600
sales-eu@scioninstruments.com