



Blood alcohol determination by HT3 Automatic Static/Dynamic Headspace Sampler

INTRODUCTION

Forensic and toxicology laboratories around the world are using headspace analysis to analyse biological fluids for volatile components. The determination of blood alcohol components (BAC) is a routinely performed headspace analysis, frequently used in the suspicion of driving under influence (DUI). Using a headspace injection of the sample onto two columns, one for quantitation and the other for confirmation of the volatile compounds, who are finally detected with separate flame ionization detectors. (FID)

This application note presents the results of the determination of blood alcohol using headspace sampling. The HT3 headspace sampler is perfectly capable for the analysis of several volatile compounds in blood (ethanol), including acetaldehyde and acetone (metabolized components of ethanol in blood), Isopropanol (absorbed or inhaled from rubbing alcohol vapours). These compounds are quantitated by using an internal standard n-propanol. Figure 1 shows the SCION Instruments HT3 headspace sampler used throughout this application with the 8X00 GC platform.



Figure 1. SCION Instruments HT3 Headspace Sampler and the SCION Instruments 8X00 GC platform

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INSTRUMENTS PARAMETERS

The HT3 headspace sampler was connected to a SCION Instruments 8X00-GC, equipped with dual FID's. Table 1 is showing the analytical conditions of the GC system. The static loop instruments conditions are presented in table 2.

EXPERIMENTAL

Blood samples are mixed with an internal standard with buffering agent. The internal standard solution of 0,015% v/v% n-propanol is dissolved in 0,5M ammonium sulphate and deionized water.

A set of standards is prepared for the components ethanol, methanol, acetaldehyde, isopropanol and acetone in concentration range of 0.40, 0.20, 0.10, 0.05, 0.025, 0.010, 0.0025% v/v. These concentrations correspond to blood ethanol concentrations of 0.32, 0.16, 0.08, 0.04, 0.02, 0.008 and 0.002g/dL. The concentration of the compounds in each standard expressed in DUI blood alcohol mg/dL is listed in table 3.

Standards were prepared by pipetting a known amount of neat solvents up into 100mL volumetric flasks, containing the 0,015% n-propanol internal standard solution. A refrigerated syringe was used to pipet the acetaldehyde, for reducing the error during sample preparation of the volatile compound. 1mL of these standard solutions is transferred into a HT3 22mL sample vial with a graduated pipet, capped with a Teflon lined silicon septa and headspace crimp caps and properly crimped.

Standards from table 3 are injected to generate correlation coefficients of the analysis.

Table 1: Analytical settings GC

Part	Settings
Injector	200°C
(split splitless)	10:1
Column	1: SCION-WAXMS 2: SCION-WAXMS
Oven Program	40°C
Carrier	Helium
Column flow	12 ml/min FID with ceramic jet, 250°C
Detector	Air: 300 ml/min, Fuel gas (H ₂): 35 ml/min, Make up (N ₂): 23 ml/min
Inj. Volume	1 ml
Software	Compass CDS

Table 2: Static loop instruments conditions HT3

Variable	Value	Variable	Value
Constant Heat Time	On	Mixing time	0.0 min
GC Cycle Time	3 mins	Mixing level	Level 5
Valve Oven Temp	200°C	Mixer stabilize time	0.50 min
Transfer Line Temp	200°C	Pressurise	10 psig
Standby Flow Rate	200 mL/min	Pressurise Time	2.0 min
Platen/Sample Temp	70°C	Pressurise Equil Time	0.20 min
Platen Temp Equil. Time	1.00 min	Loop Fill Pressure	5 psig
Sample Equil. Time	10.00 min	Loop Fill Time	0.20 min
Mixer	Off	Inject Time	0.50 min

Table 3: Concentrations of the standards

Compounds	Standard Concentration (g/dL)						
	1	2	3	4	5	6	7
Methanol	0.32	0.16	0.08	0.04	0.02	0.008	0.002
Acetaldehyde	0.08	0.04	0.02	0.01	0.005	0.004	0.0005
Ethanol	0.32	0.16	0.08	0.04	0.02	0.008	0.002
Acetone	0.08	0.04	0.02	0.01	0.005	0.004	0.0005
Isopropanol	0.20	0.10	0.05	0.025	0.0125	0.005	0.00125

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RESULTS AND DISCUSSION

A sample chromatogram is presented in figure 2, showing the chromatogram of the 0.008g/dL ethanol standard on the quantitation column.

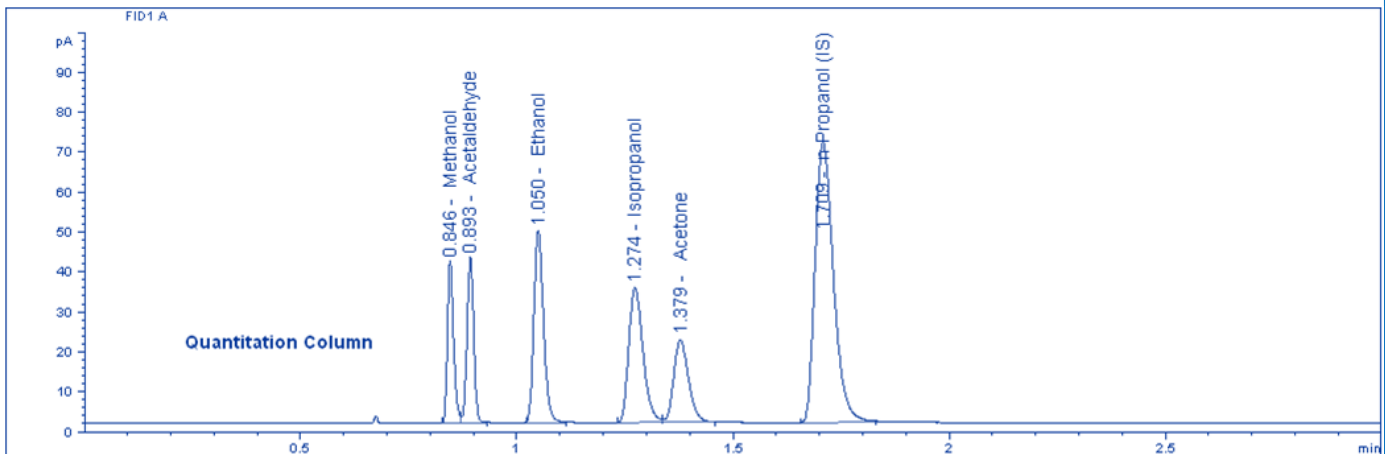


Figure 2: Chromatogram 0.008g/dL ethanol standard on the quantitation column.

Table 4: Precision of the quantitation column.

	Methanol		Acetaldehyde		Ethanol		Isopropanol		Acetone		n-Propanol (IS)
	Area	IS Ratio	Area	IS Ratio	Area	IS Ratio	Area	IS Ratio	Area	IS Ratio	Area
1	37.9879	0.1973	42.1094	0.2187	69.6079	0.3615	72.3644	0.3758	46.8225	0.2431	192.5791
2	40.5376	0.2037	42.6875	0.2145	73.1351	0.3674	74.4834	0.3742	47.3482	0.2379	199.0514
3	39.7432	0.1951	42.6868	0.2095	73.7363	0.3619	76.3297	0.3747	48.0622	0.2359	203.7249
4	40.1865	0.2081	40.7507	0.2111	71.4818	0.3702	71.7019	0.3713	45.2439	0.2343	193.0856
5	40.2395	0.1972	41.9664	0.2056	74.3102	0.3641	76.0220	0.3725	47.3413	0.2320	204.0724
6	38.6157	0.2034	40.9111	0.2154	69.5918	0.3665	70.5772	0.3717	45.1332	0.2377	189.8920
7	37.6379	0.2025	39.1901	0.2109	67.9005	0.3653	69.1451	0.3720	43.4294	0.2337	185.8657
Average	39.2783	0.2010	41.4717	0.2122	71.3948	0.3653	72.9462	0.3732	46.1972	0.2364	195.4673
%RSD	3.0	2.3	3.1	2.0	3.4	0.8	3.8	0.5	3.6	1.6	3.6

Figure 2 is showing a good separation between the components, within 3 minutes analysis time. Precision of the assay is determined by injecting seven times the same sample (0,008 g/dl ethanol standard). The precision for each component in the standard is presented in Table 4 for the quantitation column. A sample chromatogram is presented in figure 3, showing the chromatogram of the 0.008g/dL ethanol standard on the quantitation column. This chromatogram shows a good separation between the components, within 3 minutes analysis time.

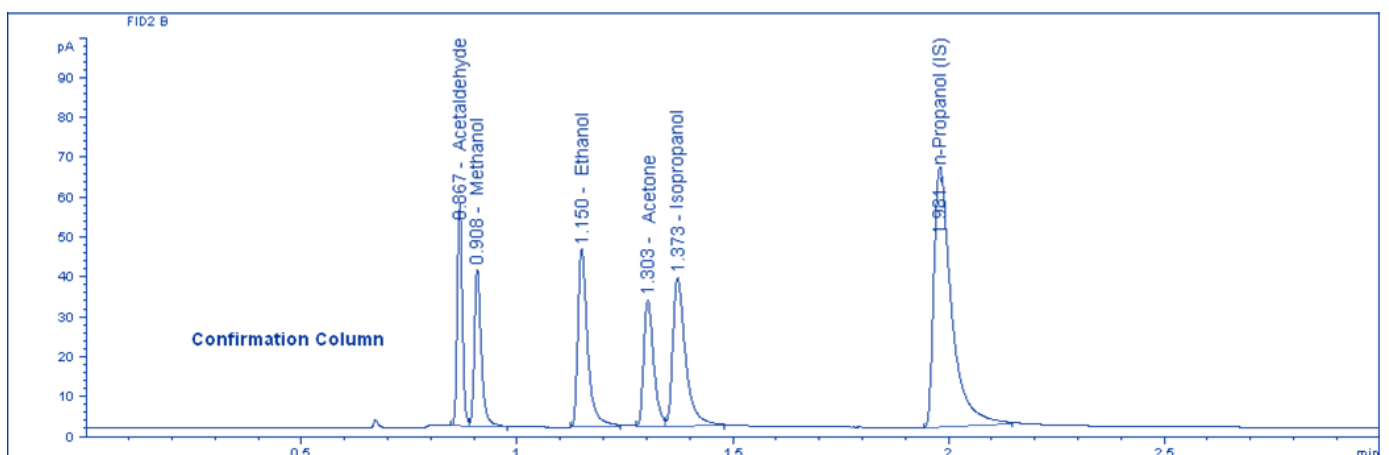


Figure 3: Chromatogram 0.008g/dL ethanol standard on the confirmation column.

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Table 5: Precision of the confirmation column.

	Acetaldehyde		Methanol		Ethanol		Acetone		Isopropanol		n-Propanol (IS)
	Area	IS Ratio	Area	IS Ratio	Area	IS Ratio	Area	IS Ratio	Area	IS Ratio	Area
1	43.6512	0.2628	41.0730	0.2473	63.3810	0.3816	47.4316	0.2856	68.8978	0.4148	166.1035
2	44.2913	0.2567	43.7153	0.2533	66.6057	0.3860	47.8574	0.2773	70.9253	0.4110	172.5634
3	44.2272	0.2506	42.8884	0.2430	67.2499	0.3811	48.6533	0.2757	72.7133	0.4121	176.4668
4	42.4985	0.2548	43.2528	0.2593	64.9051	0.3892	45.7679	0.2744	68.2796	0.4094	166.7748
5	43.6353	0.2451	43.3207	0.2433	72.5385	0.4074	47.9482	0.2693	72.7095	0.4083	178.0594
6	42.6253	0.2600	41.4251	0.2527	62.8839	0.3836	45.5205	0.2777	66.7594	0.4072	163.9421
7	40.8633	0.2560	40.4448	0.2534	61.4196	0.3847	43.8038	0.2744	65.2469	0.4087	159.6378
Average	43.1132	0.2551	42.3029	0.2503	65.5691	0.3876	46.7118	0.2763	69.3617	0.4102	169.0783
%RSD	2.8	2.3	3.1	2.4	5.6	2.4	3.7	1.8	4.2	0.6	4.0

Precision of the assay is determined by injecting seven times the same sample (0,008 g/dL ethanol standard). The precision for each component in the standard is presented in Table 5 for the confirmation column.

Calibration curves

Table 6 presents the correlation coefficient for a seven point calibration curve from standards prepared at 0.32%, 0.16%, 0.08%, 0.04% and 0.02%, 0.008% and 0.002%. set of parameters followed in this paper provides a correlation coefficient of 1.0000 for a seven point calibration curve ranging from 0.002g/dL to 0.32g/dL ethanol concentration. The correlation curves for methanol, acetone and isopropanol were all greater than 0.999 for this method for their corresponding concentration. The correlation coefficient for acetaldehyde was greater than 0.998, and was expected because of the volatility of the compound during sample preparation.

Table 6: Correlation coefficients for a seven point calibration curve.

Compound	Correlation Coefficients	Concentration range (%)
Methanol	0.9995	0.002 to 0.32
Acetaldehyde	0.9983	0.0005 to 0.08
Ethanol	1.0000	0.002 to 0.32
Acetone	0.9999	0.005 to 0.08
Iso Propanol	0.9995	0.00125 to 0.20

CONCLUSION

The determination of blood alcohol, in association with the SCION Instruments 8X00 GC is easy to perform with the HT3 SCION Instruments Headspace Sampler.

The correlations coefficients of the seven point calibration curves are for most components greater than 0.999. Exceptional is acetaldehyde with a correlation coefficient of 0.998, due to the volatility of the compound during standard preparation.

The internal standard method precision was 0.8% for 0.008 mg/dL ethanol standard, measured in septenary, prepared by graduated pipet with 2% RSD.

The HT3 with 2 capillary columns produced blood alcohol data with excellent linearity and precision for ethanol concentrations in the range of 0.32 g/dL to 0.002g/dL, within a analysis time of 3 minutes using n-propanol as internal standard.

Although the 4X6-GC series is not shown in this application note, it is also possible to perform this method on the SCION instruments 4X6 GC series. Ordering information can be found in the table below. For customisation please contact your local sales representative.

Part number	SCION HT3 Headspace sampler
SC149300000	HT3 Headspace Autosampler 110V.
SC149300100	HT3 Headspace Autosampler 230V.
SC149300005	HT3 Dynamic Headspace Autosampler 110V.
SC149300105	HT3 Dynamic Headspace Autosampler 230V.

SCION Instruments

UK
4 Michaelson Square
Livingston
EH54 7DP, Scotland, UK
Phone +44 1506 300 200
sales-eu@scioninstruments.com

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