

Blood Alcohol Determination with Teledyne Tekmar HT3™ Automated Static/Dynamic Headspace Analyzer

Application Note

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Introduction

Analysis of biological fluids for volatile components by headspace analysis is routinely used by forensic laboratories around the world. One of the more common of these headspace analyses is the determination of blood alcohol in cases involving suspected DUI or OVI. This determination is typically accomplished with an injection of the sample onto two columns, one for quantitation and the other for confirmation of the volatile compounds, detected with separate flame ionization detectors (FID).

This paper documents one set of Teledyne Tekmar's HT3™ Automated Static/Dynamic headspace system's instrument parameters useful for the analysis of blood alcohol levels. The volatile compounds presented in this paper include acetaldehyde and acetone, metabolized components of ethanol in the blood, methanol, a potential contaminate of ethanol, and isopropanol, absorbed or inhaled from rubbing alcohol vapors. The compounds are quantitated using the internal standard method, with n-propanol as the internal standard.



Figure 1: HT3™ Automated Static/Dynamic Headspace Analyzer

Instrument Parameters:

The HT3™ Automated Static/Dynamic Headspace Analyzer was connected to an Agilent 6890 GC with dual FID's. The Static (Loop) instrument conditions for the HT3™ are presented in Table 1. Parameters for the GC, columns and FID detectors are presented in Table 2.

Variable	Value
Constant Heat Time	On
GC Cycle Time	3 min
Valve Oven Temp	200°C
Transfer Line Temp	200°C
Standby Flow Rate	200mL/min
Platen/Sample Temp	70°C
Platen Temp Equil. Time	1.00 min
Sample Equil. Time	10.00 min
Mixer	Off

Variable (Cont'd)	Value
Mixing Time	0.0 min
Mixing Level	Level 5
Mixer Stabilize Time	0.50 min
Pressurize	10 psig
Pressurize Time	2.0 min
Pressure Equil. Time	0.20 min
Loop Fill Pressure	5 psig
Loop Fill Time	0.20 min
Inject Time	0.50 min

Table 1: Static HT3™ Parameters (Loop)

Quantitation Column	Phenomenex Zebron™ ZB-BAC-1, 30m x 0.53, 3.00 micrometer film, column flow of 12mL/minute (P/N: 7HK-G021-36)
Confirmation Column	Phenomenex Zebron™ ZB-BAC-2, 30m x 0.53, 2.00 micrometer film, column flow of 12mL/minute (P/N: 7HK-G022-32)
Inlet	Split ratio 10:1, inlet temperature of 200°C, 1 mm IP-deact liner, columns connected to inlet with a Restek Universal “Y” Press-Tight® connector
Oven	40°C constant
FID	250°C, Hydrogen flow - 35mL/min, Air flow - 300 mL/min, Constant column and make up flow - 35mL/min

Table 2: Agilent 6890 Conditions

Standard Preparation:

Blood alcohol analysis by headspace typically utilizes an internal standard with a buffering agent mixed with the blood sample. An internal standard solution of 0.015% v/v n-propanol in 0.5M ammonium sulfate in deionized water was used to prepare 0.40, 0.020, 0.010, 0.05, 0.025, 0.010, 0.0025% v/v ethanol standards along with methanol, acetaldehyde, isopropanol and acetone. These concentrations correspond to blood ethanol concentrations of 0.32, 0.16, 0.08, 0.04, 0.02, 0.008 and 0.002 mg/dL. The units expressed in this paper correspond to the DUI % calculations used by various states. The concentration of the compounds in each standard expressed in DUI blood alcohol mg/dL is listed in Table 3.

The standards were prepared by pipeting a known amount of the neat solvents into 100mL volumetric flasks containing the 0.015% n-propanol internal standard solution. A refrigerated syringe was used to pipet the acetaldehyde, to reduce the error due to boiling of the standard at close to room temperature.

1mL of these standard solutions was then pipeted into Teledyne Tekmar standard 22mL sample vials with a 5mL Class A graduated pipet. The vials were then capped with Teflon lined silicon septa and headspace crimp caps and properly crimped. These standards were used to determine correlation coefficients of the analysis. Seven samples of the 0.008 mg/dL ethanol level was used to determine the precision of the assay. The pipet used to prepare the headspace sample vials had a precision of 2.0%.

Compounds	Standard Concentration mg/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

Table 3: Concentrations of Methanol, Acetaldehyde, Ethanol, Acetone and Isopropanol in the mixed standards were used for this paper.

Data:

The chromatography on the quantitation Phenomenex Zebtron™ column ZB-BAC-1 and the confirmation column ZB-BAC-2 exhibited excellent peak shape and response. Figure 1 shows the chromatography on the quantitation column, top, and the confirmation column, bottom of a 0.008 mg/dL standard. The precision of the 0.008 mg/dL standard is presented in Table 4 for the quantitation column and Table 5 for the confirmation column. The correlation coefficients of the seven point calibration curves for each of the analytes are presented in Table 5.

Sample Chromatograms:

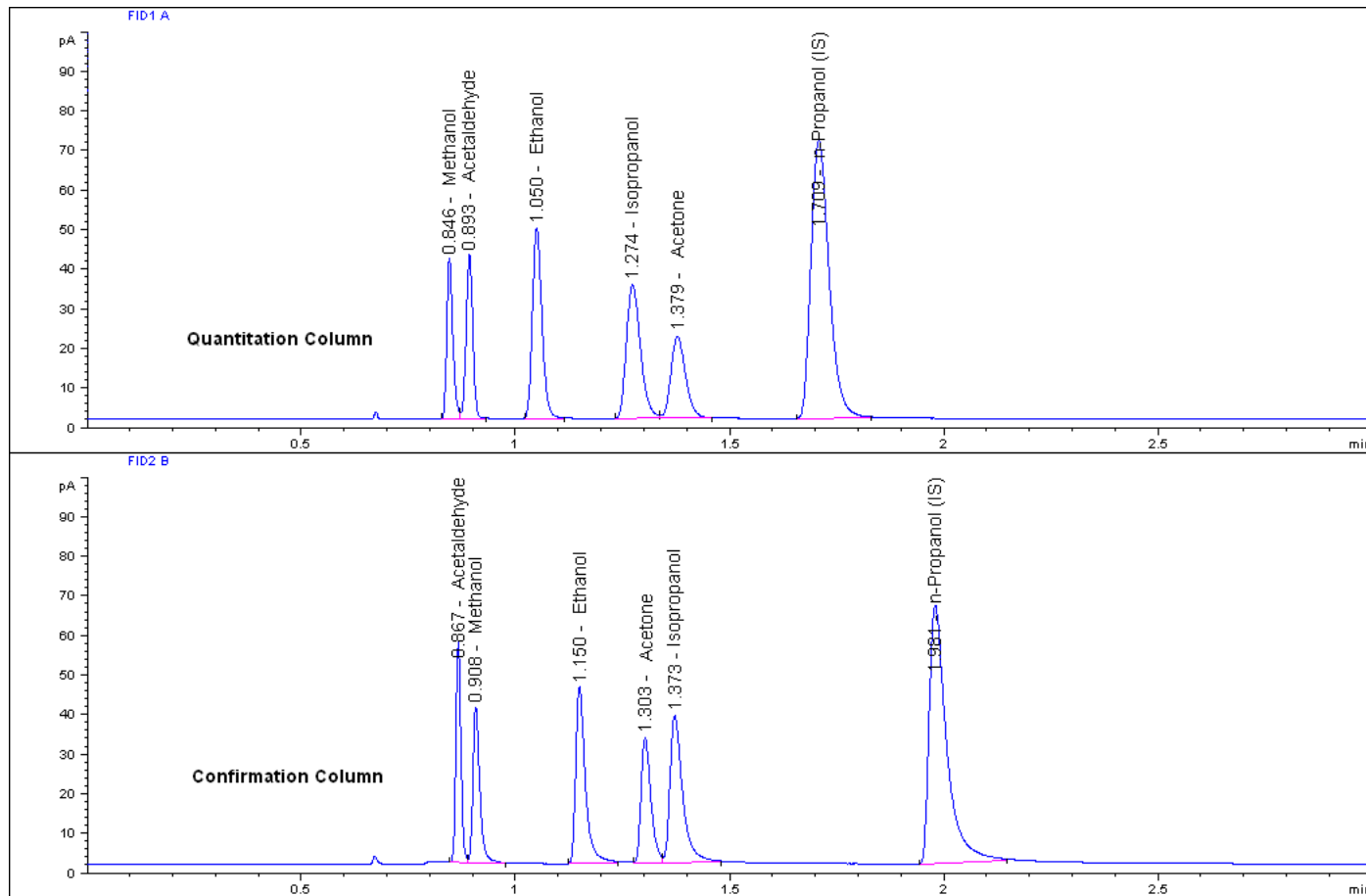


Figure 1: Top - ZB-BAC-1 Quantitation Column Chromatogram

Bottom- ZB-BAC-2 Confirmation Column Chromatogram of a 0.008 mg/dL Ethanol Standard

Sample	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

Table 4: Precision of Methanol, Acetaldehyde, Ethanol, Isopropanol, Acetone and n-Propanol for 7 samples of a 0.008 mg/dL Ethanol standard on the quantitation column.

Sample	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

Table 5: Precision of Acetaldehyde, Methanol, Ethanol, Acetone, Isopropanol and n-Propanol for 7 samples of a 0.008 mg/dL Ethanol standard on the confirmation column.

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	0.0005 to 0.08
Isopropanol	0.9995	0.00125 to 0.20

Table 6: Correlation Coefficients for a seven point calibration curve from standards prepared at 0.32%, 0.16%, 0.08%, 0.04%, 0.02%, 0.008% and 0.002% Ethanol standards. The concentrations of the other compounds are listed in Table 3.

Conclusion:

The determination of blood alcohol levels using headspace GC with dual FID's can be easily accomplished with Teledyne Tekmar's HT3™ Automated Static/Dynamic Headspace Analyzer.

The set of parameters followed in this paper provides a correlation coefficient of 1.0000 for a seven point calibration curve ranging from 0.002 mg/dL to 0.32mg/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 and its effect when preparing standards from the neat solution.

The internal standard method precision was 0.8% for the 0.008 mg/dL ethanol standard from 7 samples prepared using a graduated pipet with 2% RSD.

The HT3™ Automated Static/Dynamic Headspace Analyzer combined with ZB-BAC-1 and ZB-BAC-2 capillary GC columns produced blood alcohol data with excellent linearity and precision for ethanol concentrations ranging from 0.32 mg/dL to 0.002 mg/dL. The columns also allowed the quantitation and confirmation of blood alcohol in less than 3 minutes using n-propanol as an internal standard.

Acknowledgments

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