

Blood Alcohol Determination with the Teledyne Tekmar HT3 and Young Lin YL6500 Gas Chromatograph

Application Note

Introduction

Police and forensic laboratories around the world routinely use headspace-gas chromatography for volatile components in biological fluids. One of the more common headspace analyses is the determination of blood alcohol in cases involving suspected DUI or OVI. The blood alcohol limit for some countries, like Korea and South Africa, is as low as 0.05%.

This study evaluates Teledyne Tekmar's HT3 automated headspace vial sampler for the analysis of blood alcohol using the new YL6500 Gas Chromatograph from Young Lin Instrument Co., Ltd.

The volatile compounds presented in this paper include acetaldehyde and acetone, metabolized components of ethanol in the blood, methanol, a potential contaminant of ethanol, and isopropanol, which can be absorbed or inhaled from rubbing alcohol vapors. The compounds are quantified using external and internal standard methods. Either n-propanol or tert-butanol was used as internal standards.



Instrument Parameters

For this study, the HT3 was connected to a YL6500 GC with an FID. Table 1 lists the instrument conditions for the HT3. Table 2 lists the GC parameters, column, and FID detectors settings.

Variable	Value	Variable	Value
Constant Heat Time	On	Mixing Time	5.00 min
GC Cycle Time	22.50 min	Mixing Level	Level 5
Valve Oven Temp	105° C	Mixer Stabilize Time	0.50 min
Transfer Line Temp	110° C	Pressurize	10 psig
Standby Flow Rate	50 mL/min	Pressurize Time	2.00 min
Platen/Sample Temp	80° C	Pressure Equil. Time	0.20 min
Platen Temp Equil. Time	1.00 min	Loop Fill Pressure	7 psig
Sample Equil. Time	15.00 min	Loop Fill Time	2.00 min
Mixer	Off	Inject Time	2.00 min

Table 1: HT3 Parameters

YL6500 GC/FID Parameters	
Column	ZB-Waxplus, 30 m x 0.32 mm ID, 0.25 µm film, column flow of 2.0 mL/min, helium
Inlet	Split ratio 30:1, Inlet temperature - 150° C
Oven	35° C for 6 min, then 25.0° C/min to 150° C, final hold of 5.0 min
FID	230°C, Hydrogen flow – 35.0 mL/min, Air flow – 300.0 mL/min, Make up flow – 35.0 mL/min

Table 2: YL6500 GC/FID Parameters

Standard Preparation

Blood alcohol analysis by headspace typically utilizes an internal standard mixed with the blood sample. A 0.05% w/v internal standard solution was prepared by adding 65 µL of t-butanol and 62 µL of n-propanol to 100 mL of laboratory water.

The 0.2% (w/v) stock/working standard consists of acetaldehyde, acetone, methanol, ethanol, and isopropanol. This was prepared by adding the appropriate volume (in µL), equivalent to 200 mg, to 100 mL of laboratory water. This stock/working standard was diluted to prepare 0.10%, 0.05% (Legal Limit), 0.01% and 0.002% working standards.

The sample preparation consists of placing 200 µL of the working standard, 200 µL of a saturated sodium chloride solution (36g in 100 mL of laboratory water), and 100 µL of the internal standard solution into a 22 mL headspace vial. These were capped and sealed with Teflon lined septa and aluminum crimp seals.

The 0.002%, 0.05% and the 0.10% standards were used to determine the reproducibility (by %RSD) at each level by analyzing seven replicate samples of each standard.

Results

The correlation coefficients (r^2) of the seven-point calibration curves for all analytes were calculated by external standard and internal standard method using both internal standards for the calculation. Table 3 presents these correlation coefficients.

The percent relative standard deviation (%RSD) of each compound was calculated by external and internal standard methods, using both internal standards for the calculation. The %RSD of three standard concentrations representing a low-level blood alcohol, 0.002% (w/v), the Korean and South African blood alcohol limit, 0.05% (w/v) and twice the blood alcohol limit, 0.10% (w/v) are presented in Table 4.

A chromatography of a 0.05% (w/v) standard is presented in Figure 1.

Compound	Correlation Coefficient (r^2)		
	Ext	IS nPro	IS tBut
Acetaldehyde	0.9988	0.9999	0.9993
Acetone	0.9992	0.9999	0.9996
Methanol	0.9993	1.0000	0.9993
Isopropanol	0.9992	1.0000	0.9993
Ethanol	0.9989	1.0000	0.9991

Table 3: Correlation Coefficients (r^2) using External (Ext), and Internal Standard (IS) Calculation versus n-Propanol (nPro) and t-Butanol (tBut)

Compound	0.002% (w/v)			0.05% (w/v)			0.10% (w/v)		
	Ext	IS nPro	IS tBut	Ext	IS nPro	IS tBut	Ext	IS nPro	IS tBut
Acetaldehyde	1.51	3.01	2.60	0.79	1.10	0.96	1.51	1.65	0.87
Acetone	2.10	2.79	2.22	1.09	1.40	1.16	2.16	1.87	0.70
Methanol	3.48	1.30	2.98	2.21	2.01	2.87	3.27	1.72	2.91
t-Butanol	2.56	2.27		1.34	1.30		1.81	1.27	
Isopropanol	3.08	1.05	2.27	1.46	0.61	1.60	1.71	0.86	0.85
Ethanol	5.80	4.25	4.87	1.53	1.02	2.12	2.61	1.36	2.20
n-Propanol	3.33		2.29	1.34		1.31	1.95		1.26

Table 4: %RSD of for Seven Samples at Three Critical Levels using External (Ext), and Internal Standard (IS) Calculation versus n-Propanol (nPro) and t-Butanol (tBut).

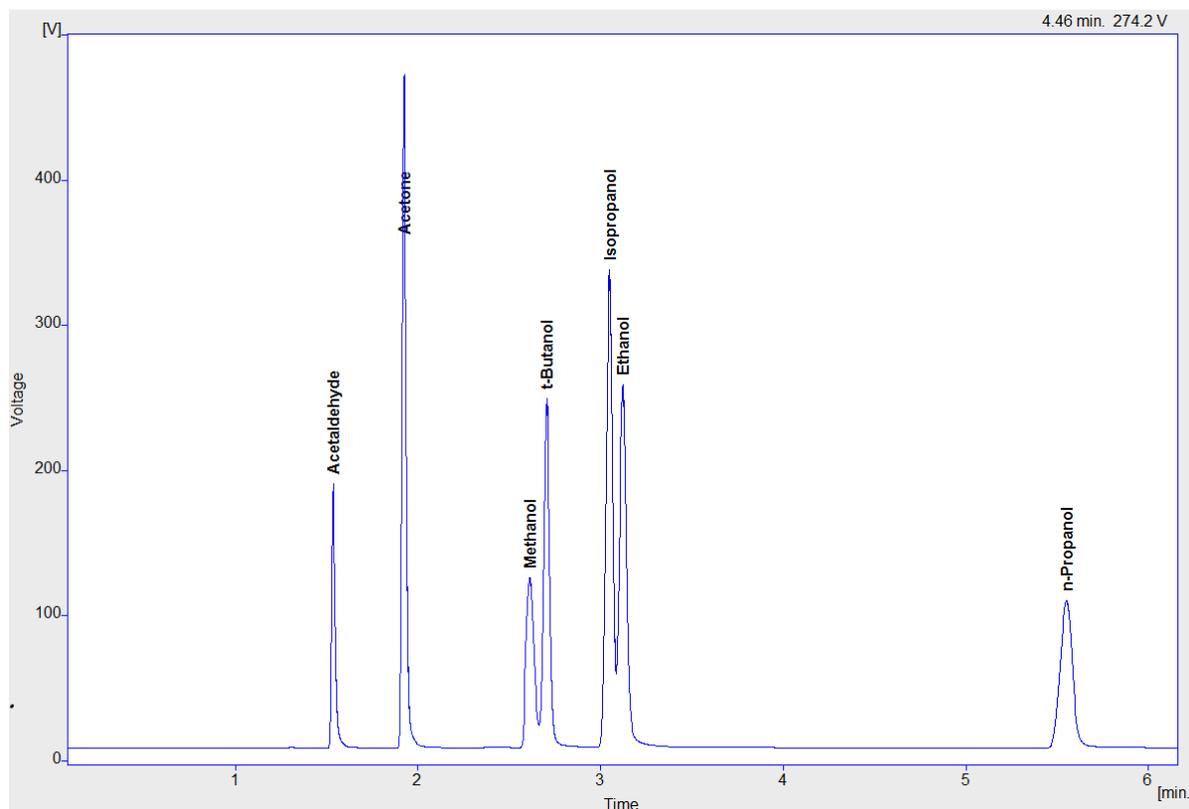


Figure 1: Chromatogram of a 0.05% (w/v) Standard Solution of Acetaldehyde, Acetone, Methanol, t-Butanol (IS), Isopropanol, Ethanol, and n-Propanol (IS)

Conclusion

Blood alcohol levels are easily determined with Teledyne Tekmar's HT3 automated headspace vial sampler and Young Lin YL6500 GC/FID. The 0.05% (w/v) blood alcohol limit exhibits excellent percent relative standard deviation and linearity for acetaldehyde, acetone, methanol, isopropanol, and ethanol by external or internal standard calculations with either n-propanol or t-butanol as the internal standard.

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