Analysis of Ethanol in Blood using Master SHS Static Headspace Sampler and Master GC Gas Chromatograph

APPLICATION NOTE - AN167

Abstract

Blood Alcohol Content (BAC) is analyzed by gas chromatography (GC). Headspace (HS) extraction is the most common method of extraction and sample introduction into the GC. Accuracy ,precision and data defendibility are essential in analysis involving legal matters. Speed of analysis, sample throughput and cost efficiency are additional concerns with high throughput laboratories performing forensic analysis.

Introduction

Driving an automobile under the influence of alcohol is a major contributor to motor vehicle accidents, injuries and death on motorways throughout the world. To increase public safety from impaired driving, the analysis of blood alcohol content (BAC) is the most common analysis requested in the forensic science laboratory. In legal matters, reliability of analysis is paramount. But the sheer volume of impaired driving cases also requires rapid analysis and high throughput to handle heavy case volume.

Gas chromatography is the analytical instrumentation utilized for the analysis of volatile organic components such as ethanol. Direct injection of raw blood or urine will work for a few injections, but contamination of the injection port liner and column will result from the non-volatile red cells in blood and salts in the urine. Headspace extraction of the volatile ethanol into the vapor phase eliminates the contamination from the biological matrix, producing consistent system performance and extended column lifetime. The combined instrumentation of headspace-gas chromatograph (HS-GC) is the configuration of choice for the analysis of biological fluids for ethanol in the forensic analysis of blood alcohol content (BAC).

Experimental

Instrumentation Configuration as tested:
Dani Master SHS, Static Headspace Sampler, with 120 vial capacity Vial Handler,
Dani Master GC, Gas Chromatograph, capillary injector, 2 FIDs to handle many unattended samples.

Master SHS Conditions	
Oven temperature	70° C
Manifold temperature	90° C
Transfer Line temperature	90° C
Loop	Fill Mode - Pressure
Loop Pressure	0.5 bar
Loop equil time	0.2 min
Vial equil time	15 min
Shaking	High
Pressurize mode	Pressure
Aux pressure	1.0 bar
Pressure equil time	0.2 min
Injection Time	1.0 min
Injection mode	Standard
Vial Venting	No
Purge time	0.2 min
Purge flow	30ml/min
Total purge time	1.2 min
GC time	2.0 min
Vial size	20 ml
HS vials bar coded for positive identification to the report	

Table 1: Master SHS Analysis Conditions

Master GC	
Oven	45°C isothermal for two minutes
Injector	SL/IN at 150°C
Flow	8ml/min Helium
Split flow	40 ml/min
FID	250°C
H2	40mL/min
Air	280ml/min
MU gas N2	25mL/min
Columns	
Column1	30m, 0.32mm, 1.8um DB-Alc1
Column2	30m, 0.32mm, 1.2umDB-Alc2

Table 2: Master GC Analysis Conditions

Press tight wye splitter, 1 injector to 2 columns and 2 detectors.
Dual column analysis producing different elution order improves qualitative identification of ethanol.

Sample

100ul sample + 1ml internal standard solution (0.2% 1-propanol aqueous and 0.5M ammonium sulfate) with electronic pipettor/diluter.
20ml screw cap vial, magnetic caps, PTFE lined septa
Dilution of sample with 10- fold water dilutes viscous blood to normalize viscosity.
Whole blood is collected in vials with anti-coagulation additives:
sodium fluoride (100mg/10ml) and potassium oxalate (20mg/10ml).

Choice of Internal Standard

n-propanol is the most common internal standard of choice. Acetonitrile has been used as internal standard but co-elutes with acetone on some columns. T-butanol has gained popularity as internal standard for faster throughput with some columns.

Calibration Standards : ethanol in aqueous dilutions at: 0.005, 0.04, 0.08, 0.15, 0.25, 0.35, 0.50%
7-point calibration curve is shown for display purposes. Most laboratories use 5-point curve.
Three repetitions for each calibration level were acquired for reproducibility calculations.
Standards are checked against NIST certified reference materials within 5% or 0.005 of target.
One control sample of known concentration is acquired every 10 injections.
Aqueous blank control must prove blank with no peak detected at ethanol retention time.
Calibration meets a linear correlation of 0.999 or better from 0.005% to 0.50% ethanol.
Resolution > 1.0 for methanol, acetaldehyde, ethanol, iso-propanol, acetone and n-propanol.

Concentration units used in blood alcohol legislation vary by jurisdiction around the world.

Concentration equivalents: 0.08% = 0.08g/100ml = 800ppm = 80mg/dl

RESULTS

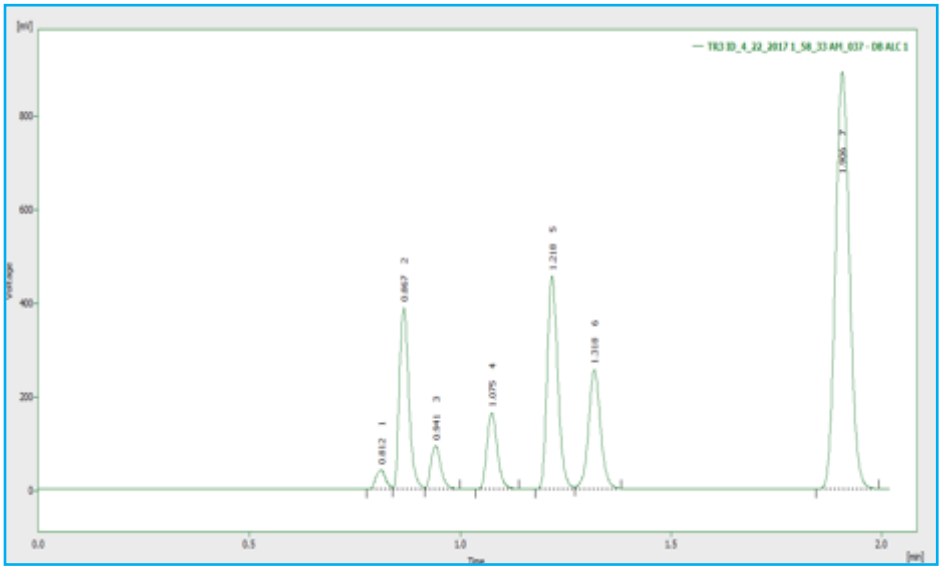

Figure 1 : Resolution mix on DB-Alc1

	DB-Alc1	DB-Alc2
	RT	RT
Acetaldehyde	0.879	0.847
Methanol	0.823	0.873
Ethanol	0.953	1.009
iso-propanol	1.087	1.123
acetone	1.230	1.107
n-propanol	1.331	1.463
t-butanol	After 2.0	1.220
acetonitrile	1.230	1.270

Table 3: ELution for DN-Alc columns

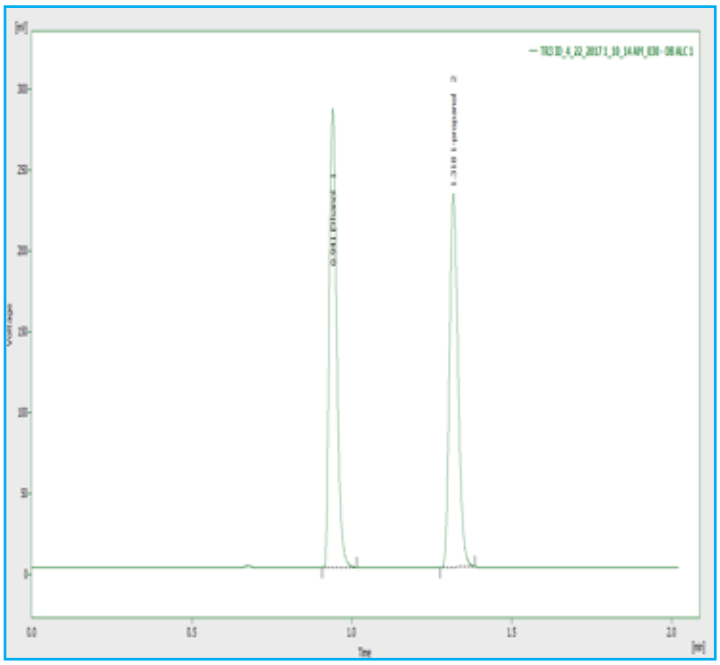

Figure 2 : Typical BAC chromatogram of ethanol and n-propanol ISTD

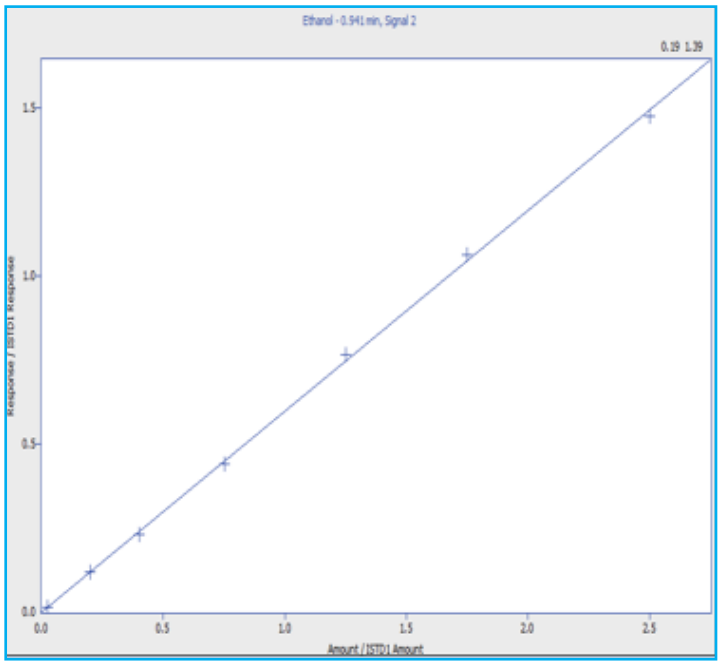

Figure 3 : ethanol 7- point calibration curve 0.005% - 0.5% ethanol on DN-Alc1, He

Using the paired columns of DN-Alc1 and DN-Alc2, the factor limiting throughput is the elution of the internal standard component. Using n-propanol as the internal standard, the final peak elutes around 1.5 minutes on DB-Alc2 with 1.2u stationary film thickness.

Headspace extraction allows for minimal sample preparation, a high degree of automation, highly reproducible results that are essential in high case load laboratories.
Fast separation in less than 1.0 - 1.5 minutes allows for high throughput.
The Dani Master SHS Static Headspace Sampler offers large 120 vial autosampler tray capacity for high throughput of 900 samples per day.
Bar code reading of vials insures sample integrity and chain of custody from vial to report.

CONCLUSION

The Dani Master SHS Static Headspace Sampler coupled with the Dani Master GC Gas Chromatograph permits BAC analysis of ethanol and internal standard in less than 90 seconds with He carrier.
The system offers a fast, reliable solution to the analysis of ethanol in bodily fluids.

REFERENCES

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2. Bruno Kolb and Leslie S. Ettre, Static Headspace-Gas Chromatography : Theory and Practice - 2nd Edition, 2006, John Wiley & Sons, Inc.

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