Quantitation and Confirmation of Blood Ethanol Content using a New GC/FID/MS Blood Alcohol Analyzer

Application Note

Forensics and Toxicology

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Abstract

This application note highlights the development of a method for determining blood alcohol concentration using an Agilent 7890B GC with FID coupled to an Agilent 5977A MSD. The combination of detection by FID and MSD provides precise quantitation of alcohol concentration along with spectral confirmation of alcohol presence within a complex blood sample matrix.

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Introduction

Blood alcohol concentration (BAC) corresponds directly to the level of impairment an intoxicated driver has when operating a vehicle. To address driving while intoxicated cases, law enforcement agencies have established threshold values for BAC. Breathalyzer and field sobriety tests provide subjective indication of impairment. Defensibility in court requires quantitation of ethanol content making BAC the most widely run test in toxicology laboratories.

Due to the number of samples received and their relative short hold times, toxicology laboratories require rapid, accurate, and reliable tests for BAC. Headspace gas chromatography is widely used by law enforcement laboratories [1]. While this technique meets many of these lab's requirements, the possibility of false positives through sample carry-over or co-elution of a contaminant has elevated the demand for mass spectral confirmation of ethanol presence above the routine analysis by gas chromatography coupled with dual flame ionization detection (GC-FID). The use of headspace GC coupled with a flame ionization detector (FID) and a mass spectrometer (MS) provides simultaneous quantitation and spectral confirmation of ethanol presence in blood.

This application tests the combination of an Agilent 7697A Headspace Sampler, 7890B GC with FID and a 5977A GC/MS for the separation, quantitation, and confirmation of alcohol compounds in blood. The purpose of this study was to illustrate that the addition of mass spectrometry validates alcohol identification and adds legal defensibility to the data. Agilent instrumentation was used for accurate and precise results, in rapid, high capacity analyses.

Experimental

This experiment was performed on an Agilent 7890B GC equipped with a split/splitless inlet and coupled to an FID, an Agilent 5977A GC/MSD, and an Agilent 7697A Headspace Sampler. Figure 1 depicts the experimental setup. A capillary flow technology (CFT) two-way splitter was used to split the flow from the GC column to the FID and MS detectors. This arrangement provided accurate and reproducible flow to both detectors (Figure 2).

The software used was Agilent MassHunter GC/MS Aquisition B.07.00.SP2 and MSD ChemStation Enhanced Data Analysis F.01.00.1903.

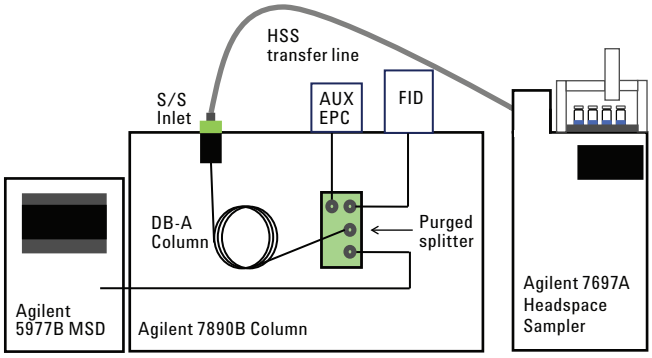

Figure 1. GC/FID/MS configuration for blood alcohol.

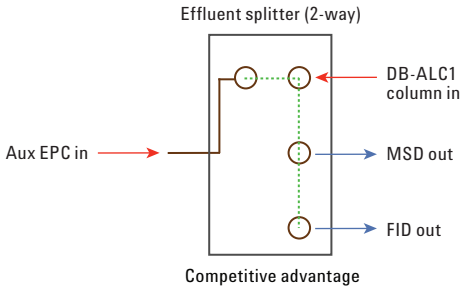

Figure 2. Reproducible flow to both detectors using a CFT two-way purged splitter.

Instrument conditions

Column	DB-ALC-1
Carrier	Helium
Oven	55 °C Isothermal
Inlet	Capillary Split/Splitless with EPC
Inlet liner	Ultra inert (p/n 5190-4047)
GC	Agilent 7890B GC
Detector	FID with EPC
MSD	Agilent 5977A Mass Spectrometer
Sampler	Agilent 7697A Headspace Sampler
Transfer line	Deactivated fused silica, 0.53 mm id
CFT device	2-way purged splitter
GC septum	Bleed and temperature optimized, BTO 11 mm septa (p/n 5183-4757)
Gold seals	Ultra Inert Gold Seals (p/n 5190-6145)
CFT ferrules	Flexible metal ferrules (p/n G3188-27502 for 0.32-id column, p/n G3188-26503 for 0.53 mm id tubing), internal nut (p/n GB2855-20530)
Inlet/FID	85:15 Vespel graphite ferrules (p/n 5062-3514, 10 pk)
MSD flow rate	1.25 mL/min

Sample preparation

Ethanol reference standards were prepared by the addition of 500 µL of each reference standard solution to 4.5 mL distilled water and 5 µL diluted internal standard.

The stock internal standard solution (ISTD) was prepared by performing a 1:10 dilution of *n*-propanol in distilled water to a final working concentration of nominally 0.08 g/dL. Performance of this method was evaluated through the analysis of real world samples.

Ethanol calibrators were prepared in a separate mix.

Results and Discussion

Figure 3 shows the chromatograms from the DB-ALC1 column for separation and elution order of analytes for the multicomponent mix using combined FID and MS signals. Figure 4 depicts the retention time alignment for the multicomponent mix. Under the analytical conditions for baseline separation of ethanol, other possible sample constituents such as methanol, 2-propanol, and acetone, was achieved in less than three (3) minutes.

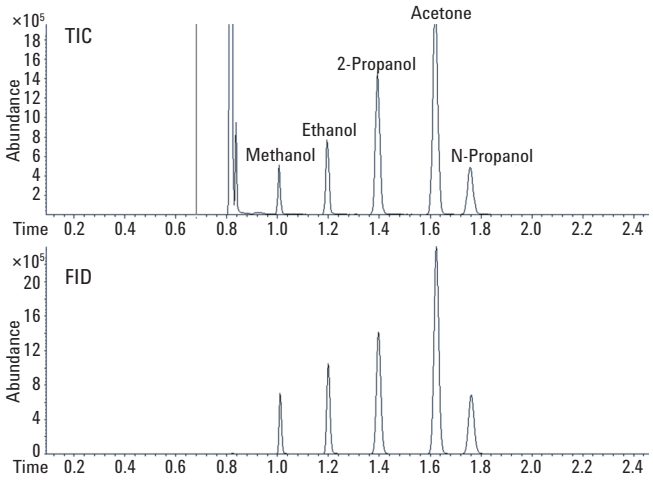

Figure 3. Chromatographic separation of multicomponent mix at 0.4% g/dL.

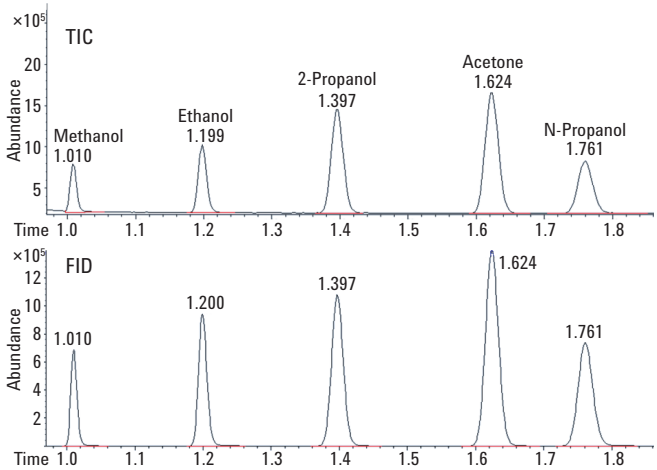

Figure 4. Retention time alignment for FID and MS Chromatograms for multicomponent mix.

To verify recovery, *n*-propanol was added to the calibration standards and samples. System calibration was performed for ethanol from 0.02 to 0.4 g/dL, at seven levels with 10 replicates per level. Figure 5 shows the calibration curves generated for ethanol on both detectors. As illustrated, the calibration curves were linear for both FID and MS, with R^2 of 0.9991 and 0.9989 respectively.

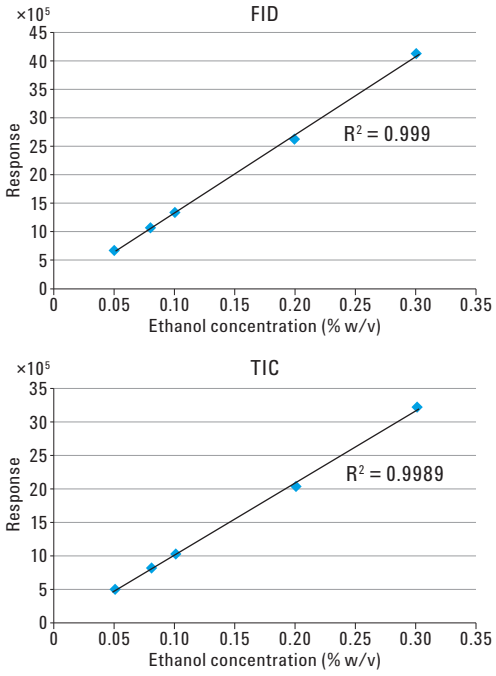

Figure 5. Calibration curves for ethanol detection with FID and MS.

Sample carryover can be of concern with running high concentration BAC samples. This study not only focused on testing carryover from sample to sample, but carryover from possible sample residue remaining in the headspace sampler. The verification was performed by analyzing a multicomponent mix with a high concentration of ethanol (0.4 g/dL), followed by the analysis of an ISTD blank. Figure 6 verifies the absence of ethanol in the ISTD blank, demonstrating that there is no sample carryover with this method.

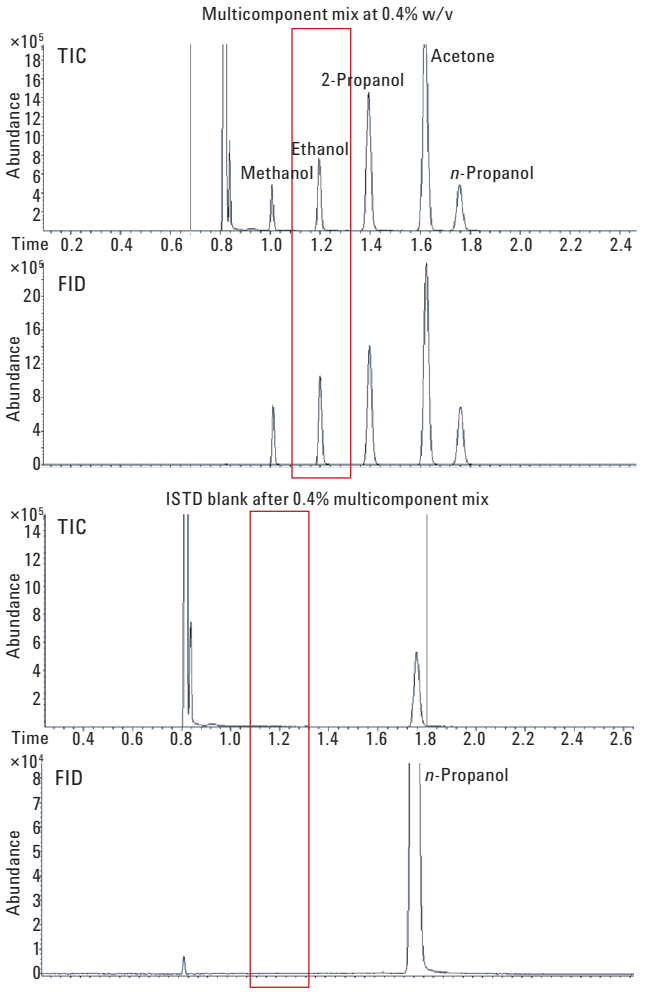

Figure 6. The top chromatograms show the analysis of ethanol in the high concentration standard (4 g/dL). The bottom chromatograms verify the absence of ethanol carry over from the 4 g/dL standard in the ISTD blank

Agilent ChemStation software offers custom report options for data presentation and comparison. The sample report shown in Figure 7 includes quantitation results from a sample analysis, the FID response is used for quantitation, and a comparison of collected MS spectra to NIST spectral data is displayed. This report provides an easy review of concentration data, and visual confirmation of the presence of ethanol in the sample analyzed. The system is designed so that retention times match for each component on the FID and MSD channels.

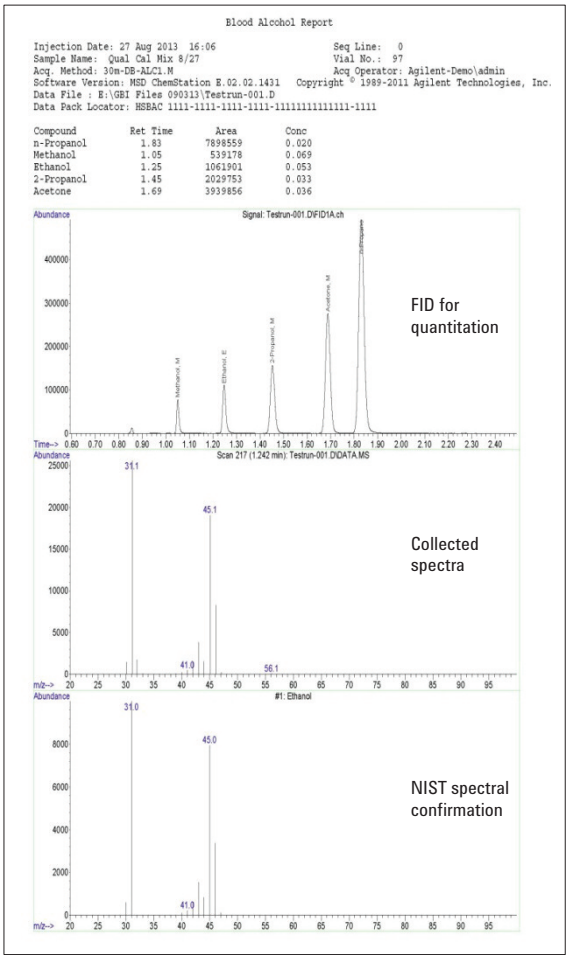

Figure 7. Agilent ChemStation custom report.

Conclusion

This study confirms the rapid, robust, and accurate BAC analysis using the 7890B GC configured with an FID and 5977A MSD. The direct coupling of the 7697A Headspace Sampler transfer line to the split/splitless inlet and EPC controlled vial sampling at pressures above ambient provided reproducible performance across a wide calibration range, and eliminated carryover. EPC controlled CFT provided reproducible split of column flow between the FID and MSD to allow for simultaneous detection and spectral confirmation of ethanol presence in a single injection. The system showed no carryover from sample to sample, even after challenging the system with a high ethanol concentration injection. Additionally, this method provides defensible data with quantitation using FID detection and spectral confirmation by MS confirmation for ethanol and other targets. The flexible custom reporting options also provide concentration information, example chromatography, and a comparison of collected and reference library spectra. Overall, use of the Agilent 7890B GC/FID, coupled to an Agilent 5977A MSD and 7697A Headspace Autosampler, is an excellent tool demonstrating precise, accurate, reproducible, and defensible data in the detection and quantitation of BAC for law enforcement.

References

1. J.L. Westland, F.L. Dorman, *Forensic Sci. Int.*, **231**(2013), pp. 50-56.

2. H. Boswell, F. Dorman "Determine Blood Alcohol with Dual Column/Dual FID for Precision and Reproducibility" *Agilent Technologies publication* 5991-3671EN.

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