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Application News

No. GCMS-1403

Gas Chromatograph Mass Spectrometer

Analysis of Blood Alcohol by Headspace with Simultaneous GC-FID and MS Detection

■ Introduction

Determination of Blood Alcohol Content (BAC) has been a standard analytical method in criminal labs for many years. The typical instrument configuration consists of a static headspace instrument for sample introduction, followed by gas chromatography (GC) with two dissimilar capillary columns for separation, and two Flame Ionization Detectors (FIDs) for detection and quantitation. Two sets of data are obtained simultaneously, and the quantitative results from the two FIDs are compared for confirmation of the reported BAC levels.

With the BAC method, compound identification is done by comparing the retention time (RT) of blood alcohol in the unknown sample to the RT obtained from analysis of an analytical standard. Recently however, additional compound identification provided by matching the ethanol mass spectrum to a library spectrum, in addition to RT, has proven to offer an additional level of confirmation. This application note describes BAC analysis using a GC-FID in parallel with a mass spectrometer (MS) for positive compound identification.

■ Experimental

Instrument Configuration

The Shimadzu HS-20 Loop headspace sampler (Figure 1) was used in the static-loop headspace mode for sample introduction. Effluent from the HS-20 was split 20-to-1, and then divided to two identical columns using a 3-way "T" fitting. The outlet ends of the two columns were connected to the FID and MS detectors. Because the MS detector was under vacuum, RTs for the two columns were different and the exact split ratio between the FID and the MS was not determined. Instrument configuration and operating parameters are outlined in Table 1.

Figure 1: Shimadzu HS-20 Loop Headspace Sampler with GCMS-QP2010 SE

Table 1: Instrument Operating Conditions and Method Parameters

Head Space	**HS-20 Loop Model**
Operation Mode	Static headspace with loop
Sample	1-mL sample volume 10-mL headspace vial
Equilibration	15 minutes at 65 °C Agitation level 3 (of 9 levels)
Sample Loop	1-mL loop Vial pressurization 0.5 min, equilibration 0.1 min Loop load time 0.5 min, equilibration 0.1 min Injection time 0.5 min
Sample Pathway Temperature	150 °C
Transfer Line Temperature	150 °C

Gas Chromatograph	**GC-2010 Plus**
Injection	Split injection from HS-20, with 20:1 split ratio to inlet side of SGE SilFlow pre-column splitter ("T" fitting) Nominal 50:50 division to two capillary columns
Column	Pre-column "T" fitting splitter to two columns Rtx-BAC1, 30 m x 0.32 mm x 1.8 µm film (x2) Helium carrier gas Constant linear velocity, 40 cm/second (each column)
Oven Program	Isothermal at 40 °C Total GC run time 5.0 minutes Total cycle time 6.0 minutes

Detector #1	**GCMS-QP2010 SE**
Operating Mode	Scan mode 30-150 m/z
Ion Source	200 °C, EI mode, 70 eV
Solvent Cut Time	0.9 min
MS Interface	200 °C
Detector #2	**Flame Ionization Detector**
FID Temperature	240 °C
FID Gas Flow Rates	H_2 = 40 mL/min Air = 400 mL/min Makeup (He) = 30 mL/min

Sample Preparation

Forensic ethanol solutions were purchased commercially with concentrations of 0.01, 0.05, 0.2, and 0.4 g/dL. An internal standard (IS) solution of n-propanol was prepared at 0.2 g/dL in TOC-grade water. Finally, a control standard (CS) was prepared by mixing methanol, ethanol, acetone, and isopropanol in TOC-grade water at 0.05 g/dL. Aliquots for analyses were prepared by mixing 1.0 mL of the IS solution with 100 µL of the individual calibration or control standard in a 10-mL headspace vial, and sealing immediately with a crimper prior to analysis.

■ Results and Discussion

Chromatography

The FID was at atmosphere and the MS was under vacuum, so the Retention Times (RT) for the 4 target compounds were different in the two chromatograms. The different RTs are inconsequential, since all compounds were individually calibrated on each of the two detectors, and RTs using the standard procedure (i.e., dissimilar columns and two FIDs) would also have been different. No effort was made to adjust the RTs for this project, but this can be done quite easily by adding a restriction to the outlet of the FID column. The FID and MS chromatograms are shown in Figure 2 with the target compounds and internal standard labeled.

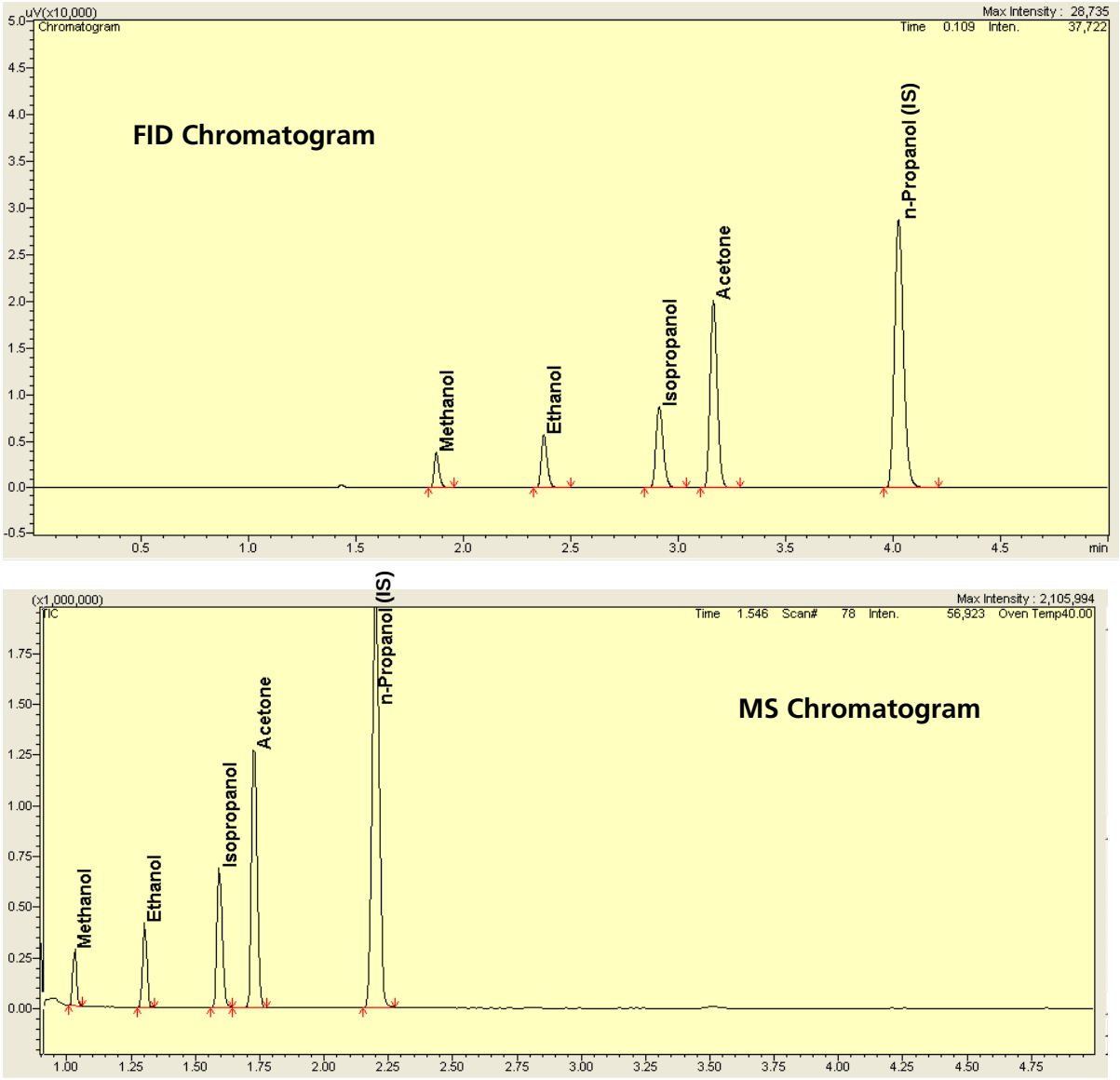

Figure 2: Chromatograms from the FID and MS with Compound Peaks Labeled

Ethanol Confirmation

Identity of the ethanol was confirmed in the MS chromatogram by matching the mass spectrum for the ethanol peak to the standard spectrum in the NIST Library. In all cases the identity of ethanol was confirmed through library matching with a similarity index of 98 or better. Figure 3 illustrates the NIST Library matching and confirmation of ethanol.

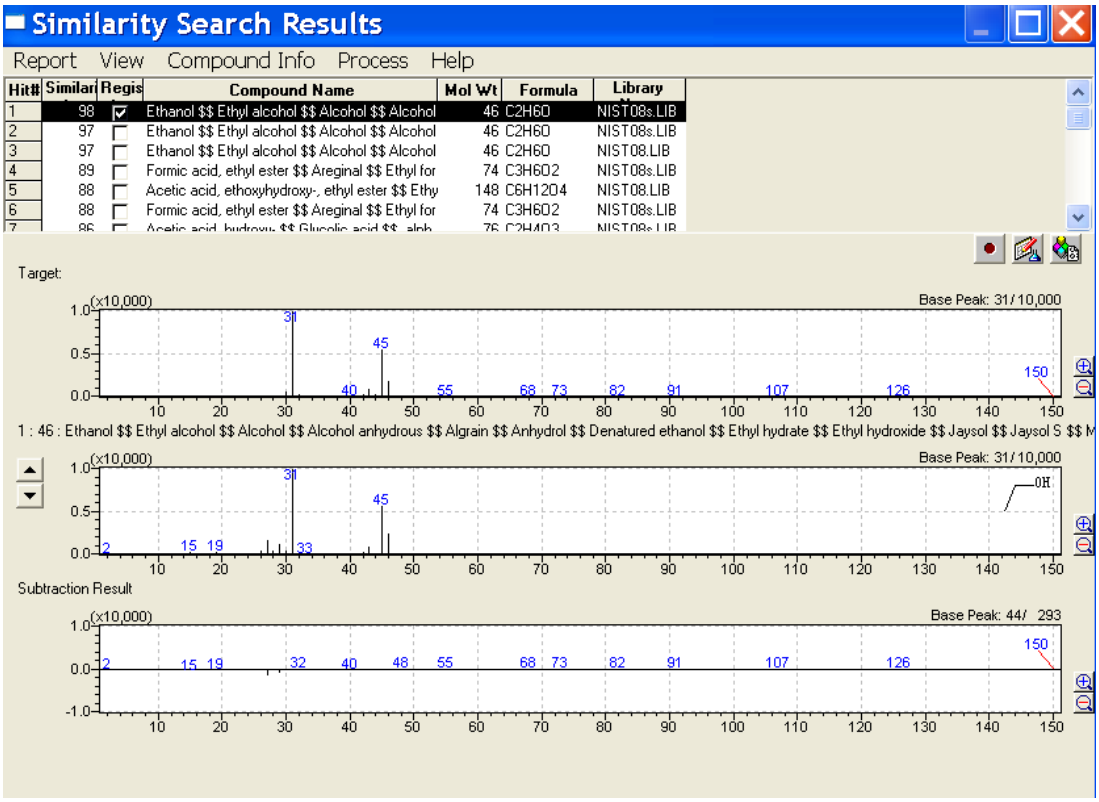

Figure 3: Mass Spectral Library Search Using the NIST11 Library to Confirm the Identity of Ethanol

Calibration

A 4-point calibration curve was generated by analyzing 3 individual aliquots at each calibration level. Data were collected on both the FID and the MS, and individual curves plotted using the internal standard technique. Calibration curves were created using the average of the data collected for the 3 individual standards at each concentration level. Figure 4 is the plotted calibration curves for ethanol on the FID and MS detectors. Table 2 shows the linearity for all 4 compounds in the FID and MS detectors.

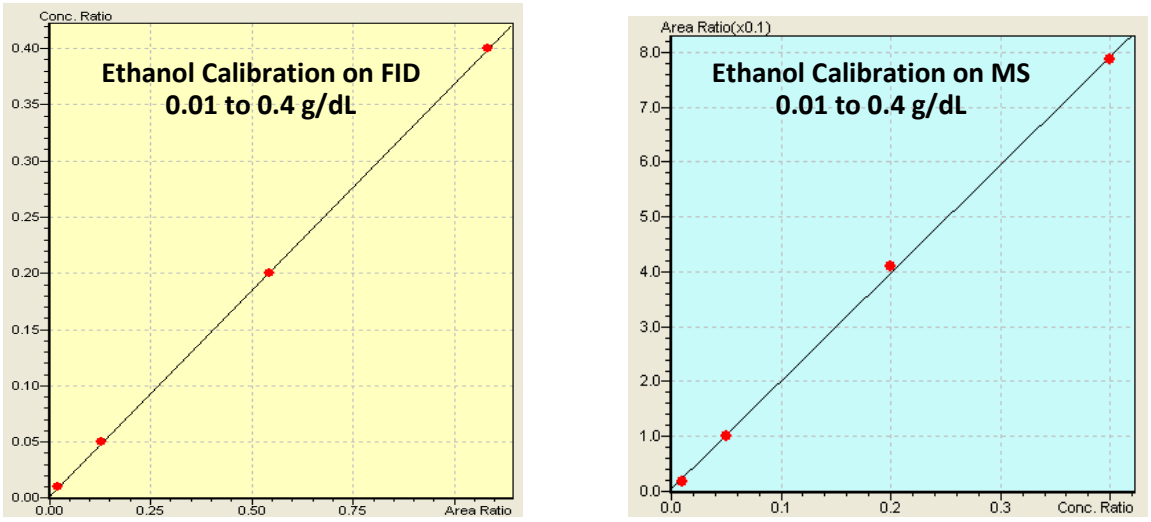

Figure 4: Calibration Curves for Ethanol on the FID and MS Detectors

Table 2: Linearity of Calibration Compounds on the FID and MS Detectors over Range of 0.01 to 0.4 g/dL

Compound	R^2 on FID	R^2 on MS
Methanol	0.9999	0.9995
Ethanol	0.9999	0.9998
Isopropanol	0.9999	0.9991
Acetone	0.9999	0.9992

Precision

Six replicate aliquots of the CS (0.05 g/dL) were prepared and analyzed using the conditions outlined in Table 1 to measure the analytical precision of the system. Overlaid chromatograms from the FID and MS are shown in Figure 5. Table 3 lists the precision results for all 4 target compounds.

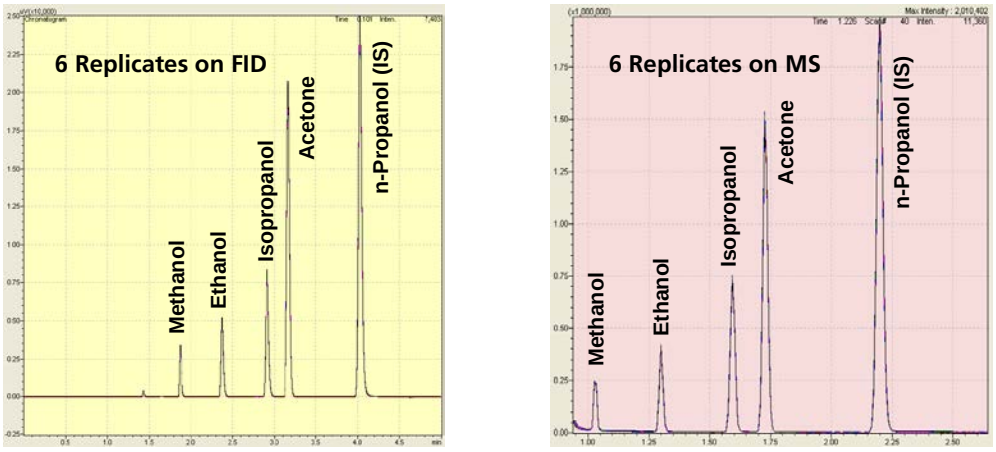

Figure 5: Overlaid Chromatograms from 6 Replicate Analyses of the Control Standard Run on the FID and the MS

Table 3: Precision Results for 6 Replicate Analyses of the Control Standard at 0.05 g/dL

Compound	RSD on FID (n = 6)	RSD on MS (n = 6)
Methanol	1.6%	1.0%
Ethanol	1.4%	0.9%
Isopropanol	1.1%	1.5%
Acetone	0.8%	1.7%

■ Summary and Conclusions

When a mass spectrometer is used in parallel with a GC-FID for analysis of blood alcohol content, the additional compound identification provided by matching the alcohol mass spectrum to an industry-standard library spectrum provides unambiguous, defensible confirmation of the ethanol. Calibration over the target concentration range is linear on both detectors, and precision is demonstrated below 2% for analysis of six replicate standards at the concentration range of interest.

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