Determination of Blood Alcohol with Dual Column/Dual FID and the Agilent Intuvo 9000 GC

Application Note

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Abstract

This Application Note demonstrates dual column/dual detector separation and detection of blood alcohol components on an Agilent Intuvo 9000 GC equipped with an Agilent 7697A Headspace Sampler. The simplified inert flow path with integrated inlet splitting provides precise and accurate quantification and retention time identification of ethanol across a range of concentrations.

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Introduction

Blood alcohol concentration involves the determination of the ethanol content in blood samples, often by headspace sampling. In fact, this measurement is one of the most common headspace-gas chromatography (HS-GC) applications[1]. Not surprisingly, HS-GC is regularly used by law enforcement laboratories when an individual is charged with driving while intoxicated[2]. Accurately determining the ethanol content in blood samples is imperative because it directly corresponds to a person's intoxication level. Given that, in the United States, the threshold for blood alcohol concentration is 0.08 % (80 mg/dL), there can be significant legal consequences depending on the reported value.

A dual column/dual detector method can be useful in the identification and quantification of ethanol and other compounds of interest. Two columns of different stationary phases will show different retention behavior for the analytes in the sample. By comparing the retention times for both columns, accurate compound identification is achieved. With dual detectors, the concentration of the analyte(s) of interest is determined from two calibration curves, and can serve as quantitative confirmation.

This Application Note describes a dual column/dual detector system with the Agilent Intuvo 9000 GC and an Agilent 7697A Headspace Sampler. The Intuvo 9000 GC features a simplified inlet splitting flow path that allows for precise and accurate sample splitting. Accurate calibration and high precision is shown for the dual column system.

Experimental

Two working standard solutions were made in water. The first was made with ethanol at 800 mg/dL (0.8 % w/v), and methanol, acetone, and isopropanol at 400 mg/dL (0.4 % w/v). The second was made with ethanol at 500 mg/dL (0.5 % w/v), and methanol, acetone, and isopropanol at 250 mg/dL (0.25 % w/v). The working standards were diluted in water to achieve final ethanol concentrations of 200 mg/dL, 100 mg/dL, 80 mg/dL, 50 mg/dL, and 10 mg/dL (0.2 %, 0.1 %, 0.08 %, 0.05 %, and 0.01 % w/v, respectively).

An internal standard solution of *n*-propanol was prepared at 0.3% (v/v) in water. Ethanol controls were obtained from Cerilliant (Round Rock, TX).

Headspace samples were prepared in 20-mL headspace vials by adding 450 µL of internal standard and 50 µL of either calibrator or control.

Instrumentation

An Agilent Intuvo 9000 GC was equipped with a split/splitless inlet and dual FIDs. An Agilent 7697 Headspace Sampler was used for sample introduction. Instrument parameters are given in Table 1.

Table 1. The instrument conditions for the BAC dual FID application are shown.

Agilent Intuvo 9000 GC	
Inert flow path configuration	Inlet splitter
Carrier gas	Helium
Inlet	Split/Splitless inlet in split mode 110°C
Split ratio	10:1
Split flow	20 mL/min
Septum purge flow	3 mL/min
Gas saver	15 mL/min after 3 min
Intuvo Jumper Chip	110°C (p/n G4587-60575)
Column	DB ALC1, 30 m × 0.32 mm, 1.8 µm (p/n 123-9134-INT) DB ALC2, 30 m × 0.32 mm, 1.2 µm (p/n 123-9234-INT)
Constant pressure	21 psi
Column temperature program	40°C (4 minutes)
Detector/Detector tail	250°C
H_2	30 mL/min
Air	400 mL/min
Makeup flow	25 mL/min
Data rate	20 Hz
Agilent 7697A Headspace	
Oven	70°C
Loop	70°C
Transfer line	90°C
Vial equilibration time	7 minutes
Inject time	0.5 minutes
Fill flow	50 mL/min
Fill pressure	15 psi
Equilibration time	0.1 minutes
Loop fill	30 psi/min to 1.5 psi
Loop equilibration time	0.05 minutes Single extraction
Purge flow	200 mL at 3 minutes Vent after extraction

Results and Discussion

Figure 1 shows chromatograms for DB-ALC1 and DB-ALC2 on the Intuvo 9000 GC. Very good peak shape was obtained, and the expected elution order change for acetone and isopropanol was observed. The ethanol and methanol peaks, whose retention time was not expected to change, showed only a 3.6-second difference in retention times, allowing for accurate identification of ethanol.

Calibration curves for the seven levels were run on the HS-GC-FID/FID system. Figure 2 shows the resulting plots for ethanol, methanol, acetone, and isopropanol. The linear regressions and calibration curve coefficients are listed on the plot for ethanol. The calibration curves for all four analytes were found to be extremely linear, with R^2 values greater than 0.9992. The ethanol analytical sensitivity for both column/detector pairs (slope of the calibration curve) was found to be within 5 % of each other, demonstrating accurate post-inlet splitting and consistent detection.

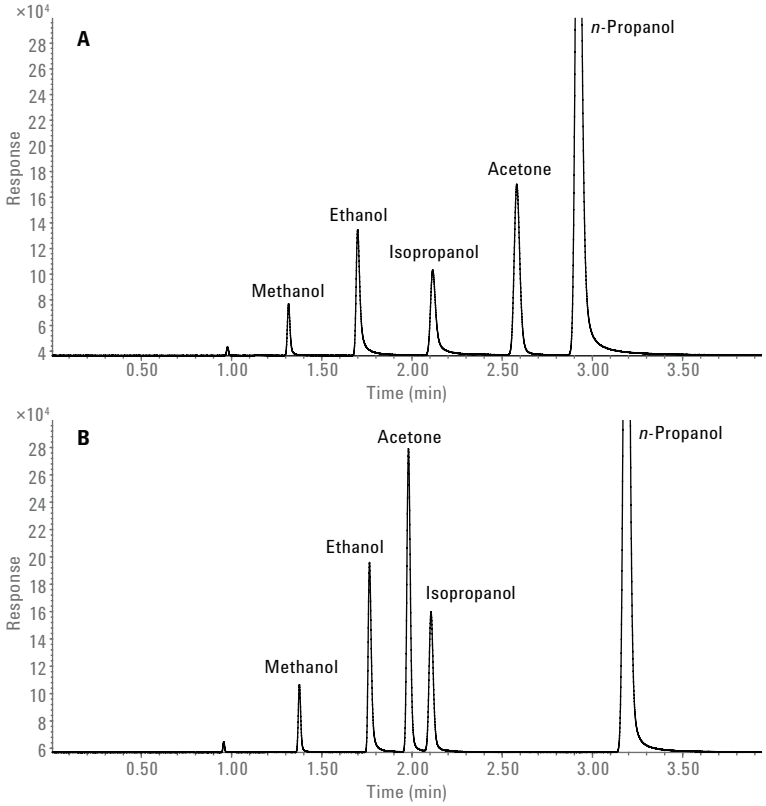

Figure 1. Chromatograms for the DB-ALC1 column (A) and DB-ALC2 column (B) for the 0.05 % calibrator showed very good peak shape and the expected elution order change for acetone and isopropanol.

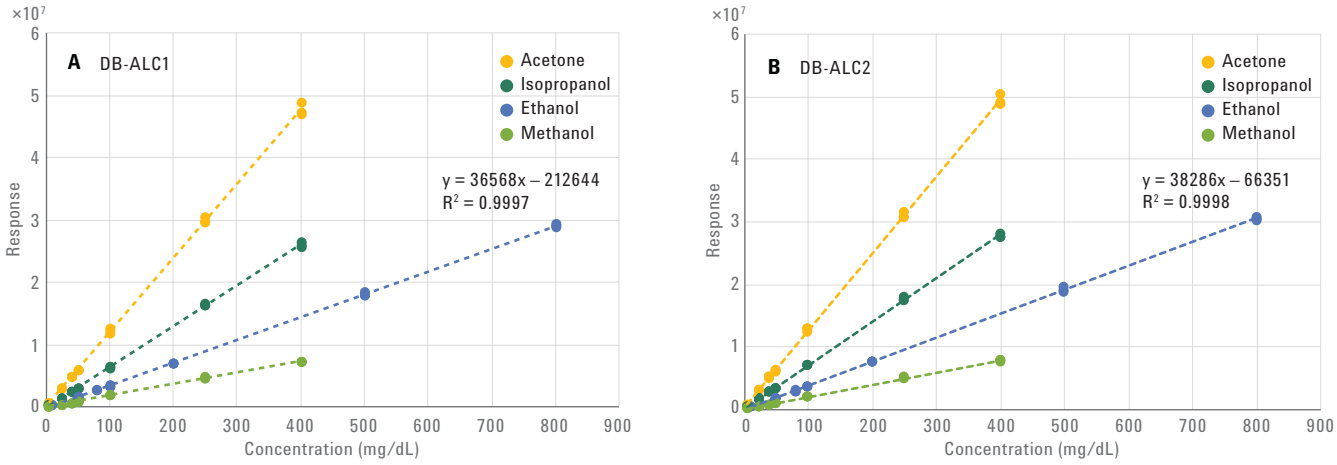

Figure 2. Calibration curves for ethanol, acetone, isopropanol, and methanol show excellent linearity on both column/detector pairs. The ethanol calibration shows good agreement between both channels.

After calibration, ethanol controls were evaluated for accuracy. While peak area calibration is shown in Figure 1, calibration curves based on response ratios were also generated. The ethanol control concentration was calculated from both the area and response ratio calibration (Table 2). Ethanol controls were determined to be within accepted tolerances (±6 % of the target concentration or ±0.004 % w/v)[3]. All ethanol controls were determined be within these specification metrics for both area and response ratio calibration.

Repeatability and reproducibility was also evaluated for multiple vials as well as multiple injections from a single vial. Area repeatability (RSD) across six different vials made with the 0.05 % control was found to be 1.2 %. The calculated concentration was determined to be 0.048 % w/v, which is within the accuracy tolerance of ±0.004 % w/v for this level. Area repeatability for 10 injections made from the same vial is worse, as expected from a headspace vial at 15.9 %. However, when response ratios are used, the repeatability for 10 injections from the same vials decreases three-fold to 5.2 %. When using response ratios to calculate the concentration of the ethanol control used for 10 injections, the control was found to be within specification, at 0.051 % w/v, which is again within the accepted tolerance.

Table 2. Ethanol controls at various levels were found to be within accepted tolerance when determined from either area or response ratio calibration curves.

Control concentration (w/v)	Area		Response ratio	
	DB-ALC1	DB-ALC2	DB-ALC1	DB-ALC2
0.40 %	0.392 %	0.393 %	0.400 %	0.389 %
0.30 %	0.300 %	0.302 %	0.306 %	0.298 %
0.20 %	0.193 %	0.194 %	0.198 %	0.193 %
0.10 %	0.099 %	0.100 %	0.101 %	0.099 %
0.08 %	0.080 %	0.080 %	0.081 %	0.079 %
0.05 %	0.050 %	0.050 %	0.051 %	0.059 %
0.02 %	0.022 %	0.020 %	0.022 %	0.021 %
0.01 %	0.013 %	0.011 %	0.013 %	0.011 %

Conclusions

Calibration curve linearity, accuracy, and repeatability was demonstrated for an Agilent Intuvo 9000 GC equipped with an Agilent 7697A Headspace Sampler. Excellent linearity was achieved for the four analytes evaluated with very good agreement in ethanol calibration curves for both the DB-ALC1 and DB-ALC2 column/detector pairs. Ethanol controls were found to be within tolerance for a range of concentrations, when determined from either area or response ratio calibration curves. Measurement repeatability was also excellent for six replicate vials. When evaluating a large number of injections from a single headspace vial, it is advisable to use response ratios, as it greatly improves the repeatability and measurement confidence. Peak shape and retention times showed very good agreement for ethanol on both columns, allowing for reliable target identification and quantification.

References

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