Analysis of blood alcohol concentration with an Agilent Intuvo 9000 GC system

Technology advantage: Modular GC flow path with robust HS sampling

Introduction

Determination of blood alcohol concentration (BAC) requires rigorous control. Accurate calibration and high precision is critical for reducing errors. Most flame ionization detection (FID)-based methods specify two columns: the first for initial identification and quantitation, and the second for confirmation. Depending on the laboratory, this may involve two separate analytical systems or it may take advantage of capillary flow technology (CFT) devices to accomplish this on a single gas chromatographic system.

The Agilent Intuvo 9000 gas chromatograph easily enables dual column analyses through the Intuvo inlet splitter flow chip. The inlet splitter flow chip manages the flow from the inlet through two columns going to two FID (or other atmospheric) detectors. It eliminates the need for users to calculate, measure, and cut restrictors. It also provides a 1:1 split to both columns, provided the column dimensions are matched, for example, if both columns are 30 m × 320 µm. It allows the sample to be analyzed on both columns in a single analytical run.

Agilent Intuvo 9000 GC and Agilent 7697A headspace sampler.

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Experimental

An Intuvo GC system was equipped with an Agilent 7697A headspace sampler. Dual alcohol columns, DB-BAC1 Ultra Inert (UI) and DB-BAC2 Ultra Inert (UI), were configured between a single split/splitless inlet and dual FIDs, and held isothermal (Tables 1 and 2). Ethanol standards ranging from 10 to 800 mg/dL were made in water with methanol, acetone, and isopropanol included at half the concentration, 5 to 400 mg/dL. Ethanol controls were used to evaluated calibration accuracy, and a blood alcohol checkout mix (Table 3) was used to demonstrate the enhanced resolution obtained by the new DB-BAC UI column pair. Headspace vials were prepared by aliquoting 50 µL of the calibration or control into 450 µL of 0.03 % (v/v) *n*-propanol.

Table 1. Instrument conditions are given for the Agilent Intuvo 9000 GC.

Agilent Intuvo 9000 GC	Setpoint
Oven	40 °C (6.5 minutes)
Split/splitless inlet	Split 10:1, 110 °C
DB-BAC1 Ultra Inert (123-9334UI-INT) 30 m × 320 µm, 1.8 µm	Helium constant pressure 21 psi
DB-BAC2 Ultra Inert (123-9434UI-INT) 30 m × 320 µm, 1.2 µm	Controlled by column 1
FID (front and back)	250 °C
H_2	30 mL/min
Air	400 mL/min
N_2 (makeup)	25 mL/min
Jumper chip	110 °C (inlet temperature)
Bus	Default (on 200 °C)
Front/back signal	20 Hz

Table 2. Instrument conditions are given for the Agilent 7697A headspace sampler.

Agilent 7697A headspace sampler	Setpoint
Oven	70 °C
Loop	70 °C
Transfer line	90 °C
Vial equilibration time	7 minutes
Injection duration	0.5 minutes
Vial size	20 mL
Vial shaking	Off
Vial fill mode	Default (50 mL/min to 15 psi, 0.1 minutes)
Vial fill pressure	15 psi
Loop ramp rate	30 psi/min
Loop final pressure	1.5 psi
Loop equilibration time	0.05 minutes

Results and discussion

To determine calibration curves for both the DB-BAC1 UI and DB-BAC2 UI columns (Figure 1), the calibration standards were run in triplicate. The calibration curves were found to be linear for the four analytes included in the calibration standards. A coefficient of determination (R^2) of 0.9995 or better was achieved for ethanol on both columns. The slope difference for ethanol on the two column/detector pairs was only 6.3 %, demonstrating accurate post-inlet 1:1 splitting and detection.

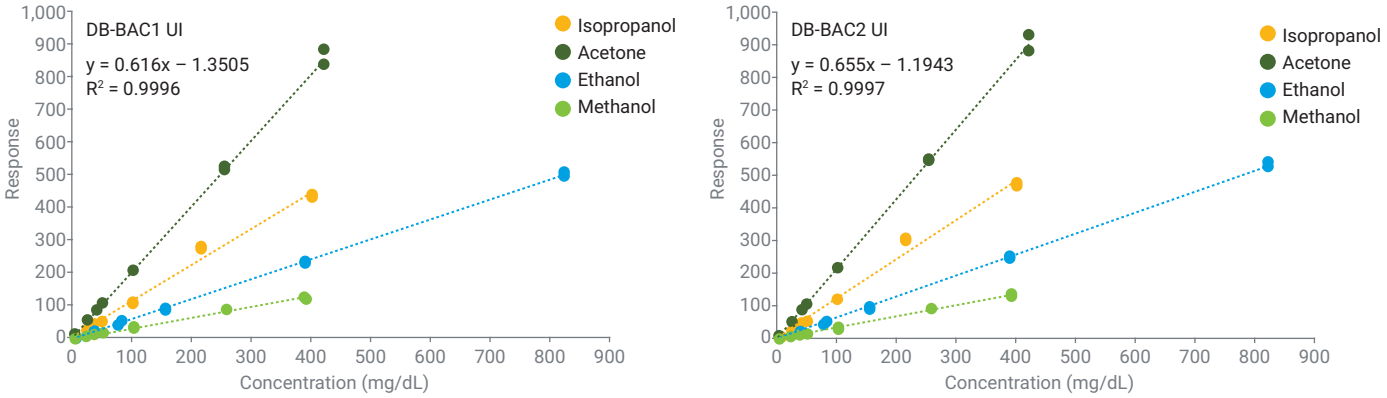

Figure 1. Calibration curves for ethanol, methanol, acetone, and isopropanol for the Agilent DB-BAC1 UI and DB-BAC2 UI columns.

After calibration, five HS samples were made with an 80 mg/dL ethanol control and the blood alcohol checkout mix (50 mg/dL). Area repeatability, measured by the relative standard deviation (RSD) for both channels was found to be 4.1 % or better (Table 4). Retention time precision was also calculated and found to be 0.1 % or better (Table 5). Calibration accuracy was verified with ethanol controls (Table 6).

Table 3. Part numbers for ethanol controls and blood alcohol checkout mix.

Standard	Part number
20 mg/dL	5190-9756
50 mg/dL	5190-9757
80 mg/dL	5190-9758
100 mg/dL	5190-9759
150 mg/dL	5190-9760
200 mg/dL	5190-9761
300 mg/dL	5190-9762
400 mg/dL	5190-9763
Alcohol checkout mix	5190-9765

Table 4. Area precision (RSD) for the 80 mg/dL ethanol standard and the analytes included in the blood alcohol checkout mix for both columns.

Analyte	Agilent DB-BAC1 UI	Agilent DB-BAC2 UI
Ethanol 80 mg/dL standard	3.70%	2.80%
Methanol (50 mg/dL)	4.10%	1.40%
Acetaldehyde (50 mg/dL)	2.80%	3.00%
Ethanol (50 mg/dL)	2.30%	1.10%
Isopropanol (50 mg/dL)	3.30%	1.90%
t-Butanol (50 mg/dL)	2.80%	2.70%
Propanal (50 mg/dL)	3.40%	3.00%
n-Propanol (50 mg/dL)	3.10%	2.10%
Acetone (50 mg/dL)	3.40%	2.90%
Acetonitrile (50 mg/dL)	2.30%	2.80%
2-Butanol (50 mg/dL)	2.00%	3.00%
Ethyl acetate (50 mg/dL)	3.20%	3.10%
2-Butanone (50 mg/dL)	3.10%	3.00%

Table 5. Retention time precision (RSD) for the 80 mg/dL ethanol standard and the analytes included in the 50 mg/dL blood alcohol checkout mix on both columns.

Analyte	Agilent DB-BAC1 UI	Agilent DB-BAC2 UI
Ethanol 80 mg/dL standard	0.04 %	0.10 %
Methanol	0.01 %	0.02 %
Acetaldehyde	0.01 %	0.02 %
Ethanol	0.02 %	0.05 %
Isopropanol	0.02 %	0.04 %
t-Butanol	0.03 %	0.04 %
Propanal	0.01 %	0.02 %
n-Propanol	0.03 %	0.04 %
Acetone	0.02 %	0.03 %
Acetonitrile	0.02 %	0.03 %
2-Butanol	0.04 %	0.04 %
Ethyl acetate	0.02 %	0.03 %
2-Butanone	0.02 %	0.03 %

All controls were found to be within an acceptable tolerance of error (±6 %). The chromatograms in Figure 2 demonstrate the improvement in resolution achieved for the various analytes included in the blood alcohol checkout mix. Common internal standards, *t*-butanol and *n*-propanol, are well resolved from any of the analytes of interest.

Conclusion

The Agilent Intuvo 9000 GC system, equipped with an Agilent 7697A headspace sampler, with an inlet splitter allows identification, quantitation, and confirmation of blood alcohol analytes in a single run. Linearity on both columns and detectors is excellent, as is the precision (area and retention time) and accuracy of concentration determination. With a modular flowpath simplifying the dual column/dual detector configuration, the Intuvo 9000 delivers premium performance. To improve the chromatography experience, the Intuvo 9000 has added benefits such as a smaller footprint, a bright color touchscreen, and click-and-run connections.

Table 6. Concentrations were calculated for a set of ethanol standards evaluated with the collected calibration curves. All concentrations were within 6 % of the expected concentration.

Ethanol standard	Calculated concentration Agilent DB-BAC1 UI	Pass/Fail	Calculated concentration Agilent DB-BAC2 UI	Pass/Fail
20 mg/dL	19.8 mg/dL	Pass	19.3 mg/dL	Pass
50 mg/dL	50.0 mg/dL	Pass	47.1 mg/dL	Pass
80 mg/dL	79.3 mg/dL	Pass	76.8 mg/dL	Pass
100 mg/dL	96.7 mg/dL	Pass	94.4 mg/dL	Pass
150 mg/dL	152 mg/dL	Pass	149 mg/dL	Pass
200 mg/dL	197 mg/dL	Pass	193 mg/dL	Pass
300 mg/dL	302 mg/dL	Pass	302 mg/dL	Pass
400 mg/dL	384 mg/dL	Pass	386 mg/dL	Pass

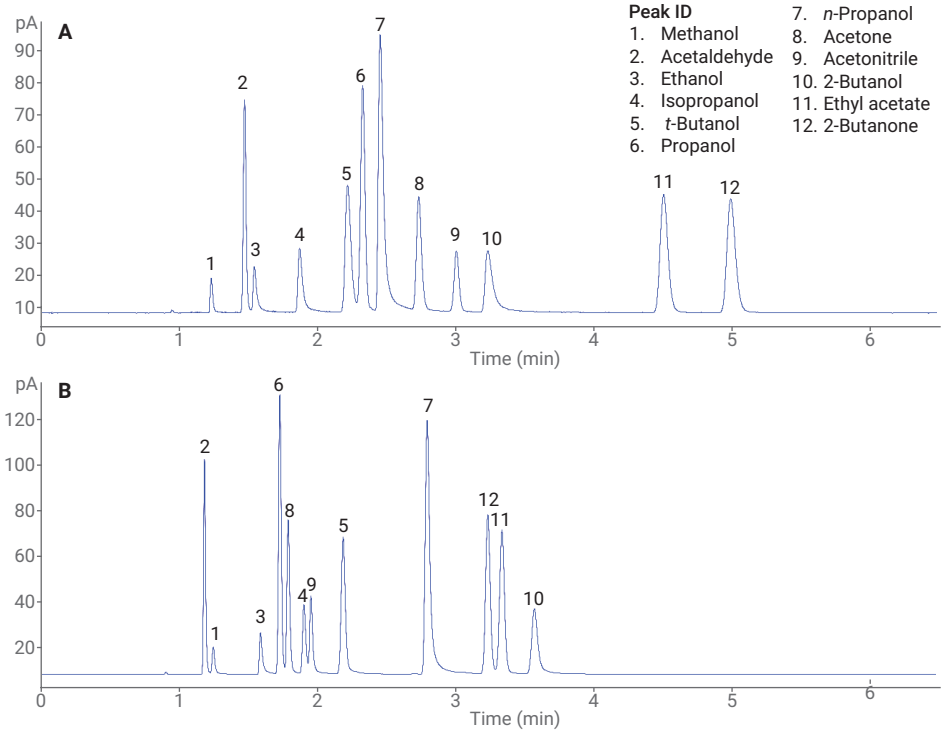

Figure 2. Chromatograms for the blood alcohol checkout mix demonstrate the resolution of *t*-butanol (5) and *n*-propanol (7) from other analytes of interest on an Agilent DB-BAC1 UI column (A). The elution order changes on the complimenting Agilent DB-BAC 2 UI column (B).

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DE.4455439815

Published in the USA, April 2, 2020
5991-8999EN

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