Application Note

Forensics

Agilent
Trusted Answers

# Method Translation and Evaluation to Implement Nitrogen Carrier Gas in the Dual-Flame Ionization Detector Configuration for Blood Alcohol Analysis

## Using the Agilent 8697 Headspace Sampler/8890 GC System

**Author**

Abbey Fausett
Agilent Technologies, Inc.

## Abstract

A dual-column headspace GC/flame ionization detector (FID) method for blood alcohol analysis converted from helium to nitrogen carrier gas using the Agilent Method Translator tool was evaluated. The objective in this translation effort was to achieve matching retention times for all target peaks in the original helium carrier method. Sufficient chromatographic resolution for all peaks was maintained under the nitrogen carrier gas conditions. Statistically, the modified method produced calibration and repeatability data with equivalent performance when compared with the original helium carrier method.

## Introduction

Fluctuations in helium supply can create productivity interruptions for many analytical laboratories. Conversion of methods away from helium carrier is a potential solution that insulates the lab from supply uncertainties, reduces operational expenses, and frees up helium resources for other less adaptive methods without compromising system robustness or performance. While some methods are not easily migrated to alternative carrier gases, the blood alcohol analysis conditions only require an adjustment to the inlet pressure setpoint for nitrogen carrier gas when translated from traditional helium methods.

Work previously done with this configuration[1] used high-purity helium as the carrier gas, and high-purity nitrogen as both the make-up gas on the detectors and as the pressurization gas for the headspace. Converting the carrier gas to nitrogen creates a completely helium-free system.

Three features available on the Agilent 8890 GC/8697 headspace sampler system make this translation simple to execute and authenticate. The first is the Method Translator tool, which is available through the GC method editor within the Agilent GC driver for the data system, or via download from the Agilent website.[2] The second feature is the integrated 8697 headspace control from within the data system or browser interface access. This convenience provides a singular pathway to manage both the sampling and acquisition parameters in the same interface. The third feature is the Gas Identity diagnostic. Configuring the proper gases on a GC is critical to a successful translation. However, sometimes tracing plumbing lines back to the gas source is cumbersome, if the possibility exists at all. The 8890 GC Intelligence offers an algorithm to check the configured gas properties against the gas supplied to the inlet electronic pneumatics control to verify a correct assignment. This test is located under the Diagnostics menu of the GC touch screen or browser interface.[3]

## Experimental

An Agilent 8890 GC with a single split/splitless inlet and dual-flame ionization detectors was configured with an Agilent 8697 headspace sampler. An unpurged splitter was used to split the injection onto two complementary column chemistries commonly used in blood alcohol analysis (Figure 1). Instrumental parameters for both helium and nitrogen acquisition methods are shown in Tables 1 and 2. Consumables and standards used in the evaluation are provided in Table 3.

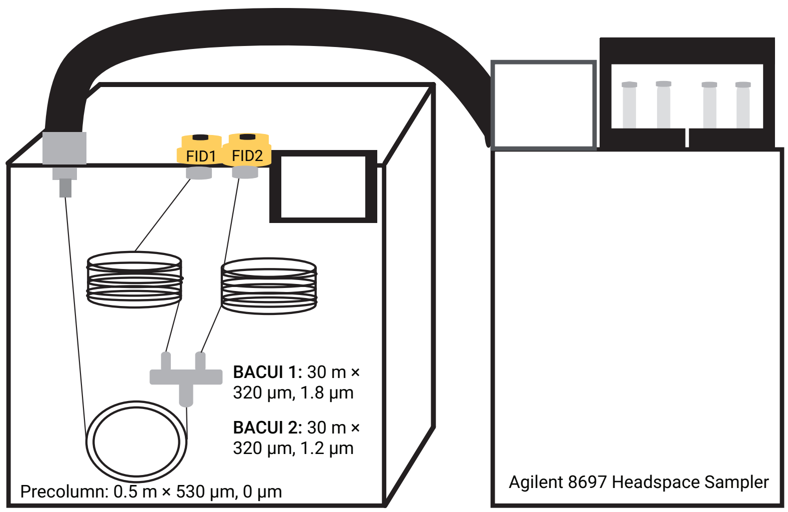


**Figure 1.** System configuration for blood alcohol content analysis.

**Table 1.** GC method parameters for both helium and nitrogen carrier methods.

| Agilent 8890 GC Conditions | | | | |
|---|---|---|---|---|
| Inlet | Split/Splitless (Helium) | Split/Splitless (Nitrogen) | Detectors | Flame Ionization Detector (Front and Back) |
| Temperature | 150 °C | 150 °C | Temperature | 250 °C |
| Split Ratio | 10:1 | 10:1 | Air flow | 400 mL/min |
| Mode | Constant pressure, 21 psi | Constant pressure, 18 psi | Hydrogen flow | 30 mL/min |
| | | | Make-up (nitrogen) | 25 mL/min |
| Oven | 40 °C, isothermal for 5 min | 40 °C, isothermal for 5 min | | |

**Table 2.** Headspace method parameters for both helium and nitrogen carrier methods.

| 8697 Headspace Sampler Conditions (Both) | |
|---|---|
| Oven Temperature | 70 °C |
| Loop Temperature | 80 °C |
| Transfer Line Temperature | 90 °C |
| Vial Equilibration | 7 min |
| Injection Time | 1 min |
| Vial Size | 20 mL |
| Vial Fill Mode | Default |
| Fill Pressure | 15 psi |
| Pressurization Gas | Nitrogen |
| Loop Fill Mode | Custom |
| Final Loop Pressure | 1.5 psi |
| Loop Equilibration | 0.05 |
| Loop Volume | 1 mL |

Using the parameters for helium carrier gas and the Method Translator tool (available either as a standalone download or accessed from the Agilent GC data systems under the GC Calculators menu shown in Figure 2), nitrogen carrier gas parameters are quickly generated from the helium method.

For this method translation, a speed gain of 1 was applied, which conserves the column holdup time. This approach is an ideal choice when the intent is to keep retention time and run time close to the original method.

**Table 3.** Consumables and chemical standards used in this evaluation.

| Consumables | Part Number | Standards | Part Number | Vendor |
|---|---|---|---|---|
| 20 mL Vials and Crimp Caps | 5190-2286 | BAC Resolution Mix | 5190-9765 | Agilent |
| Inert Liner, Ultra Inert, 2 mm id | 5190-6168 | Ethanol Calibration | G3440-85036 | Agilent |
| Transfer Line (Fused Silica) | 160-2535-5 | *t*-Butanol, >99% | 24127 | Millipore/Sigma |
| Precolumn: 0.5 m × 0.53 mm, 0 µm | 160-2535-10 | Custom Solvent Mix | Custom | Restek |
| Column 1: BAC1 UI (30 m × 0.32 mm, 1.8 µm) | 123-9334UI | Dispensed MilliQ Water | N/A | Millipore/Sigma |
| Column 2: BAC2 UI (30 m × 0.32 mm, 1.2 µm) | 123-9434UI | | | |

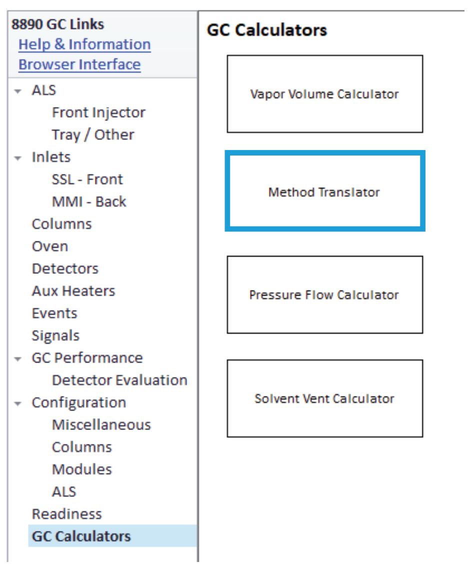


**Figure 2.** Screen capture of the Agilent Method Translator tool location inside Agilent data systems.

As shown in Figure 3, when changing the carrier gas while maintaining all other parameters, the inlet pressure decreased from 21 psi (helium) to 19 psi (nitrogen). As this method is not a single-column analysis but rather a confirmational configuration with a precolumn split into two columns, the optimal inlet pressure was determined to be slightly lower than calculated: 18 psi. This value provided slightly improved resolution without significant broadening of the peaks and negligible retention time adjustments within the calibration table. The example in Figure 3 uses column details consistent with the DB-BAC1 UI column dimensions. Entering the dimensions of the DB-BAC2 UI column results in the same calculated inlet pressure, although the flow and average linear velocities are slightly lower, resulting in a marginally higher holdup time of 0.705 minutes.

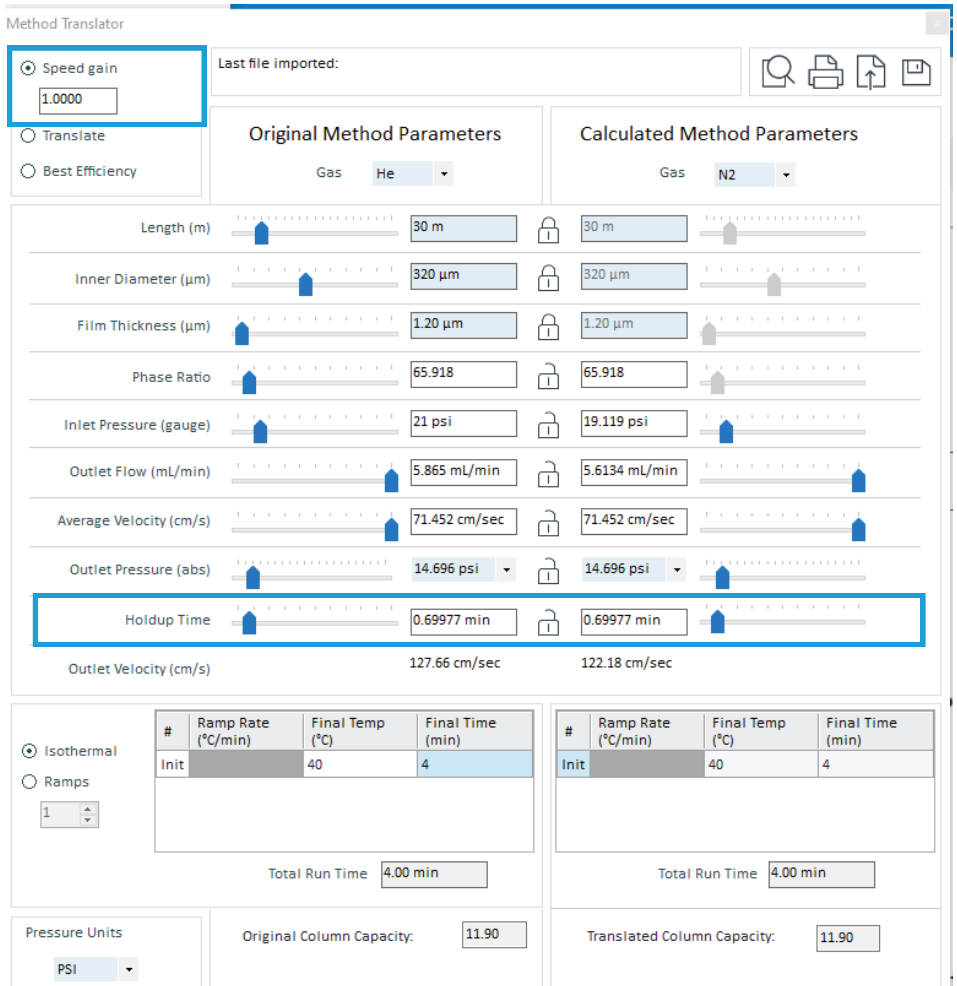


**Figure 3.** Screen capture from the Agilent Method Translator tool, provided within the GC data system. Translation between helium and nitrogen carrier gas is demonstrated using the conditions for the DB-BAC1 UI column in the parameter fields.

## Results and discussion

Ethanol linearity, vial-to-vial reproducibility, and compound resolution assessments were performed on the system to compare performance with helium carrier.

### Ethanol linearity

Linearity of ethanol was evaluated by preparing a six-point calibration curve between 20 and 400 mg/dL. Calibration samples were prepared by adding 50 µL of ethanol standard to 450 µL of water containing 100 mg/dL *t*-butanol as the internal standard. Calibration curves are shown in Figure 4 and indicate no loss of linearity or sensitivity using nitrogen carrier gas. Figure 5 contains calibration curves on both FIDs under the helium carrier method, and Table 4 details the comparison of the linear curve for both configurations.

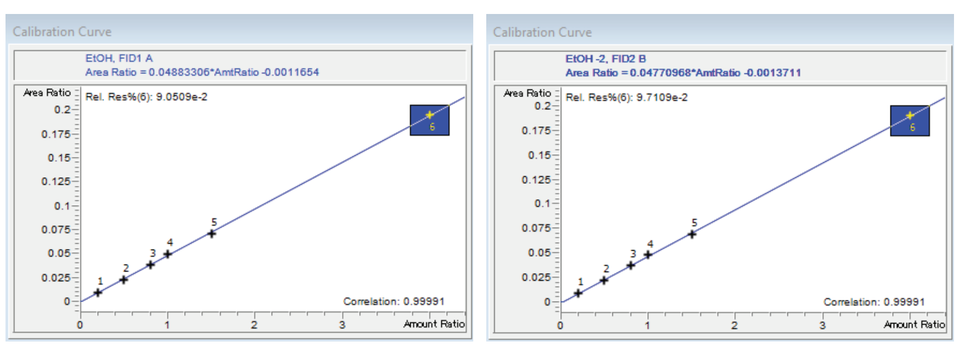


**Figure 4.** Calibration curves for ethanol on both Agilent J&W DB-BAC1 UI and DB-BAC2 UI columns using helium carrier gas.

## Repeatability

Repeatability statistics (Table 5) were calculated on 10 sequential injections of a quality control (QC) mix, a custom blend prepared by Restek. Vials were prepared with 450 µL water containing 100 mg/dL *t*-butanol as an internal standard, and 50 µL of the QC mix to make a solution concentration of 50 mg/dL in each vial.

As shown in Table 5, the configuration using nitrogen carrier gas provided equivalent or superior data when compared to the helium configuration on most analytes. In headspace applications, repeatability performance is highly dependent on sample preparation and capping technique, in addition to running the analysis on a leak-free, properly maintained system.

## Resolution

A theoretical loss of chromatographic resolution is one of the common reasons that nitrogen is often considered to be a nonviable substitution for helium carrier gas in many GC methods. Both the helium and nitrogen runs are shown in Figure 6 to show that under these conditions, the retention times are largely unaffected across the run under translated conditions. As the retention times of the analytes are a critical part of the resolution calculation, detailed data comparing retention times under both carrier gas conditions are provided.

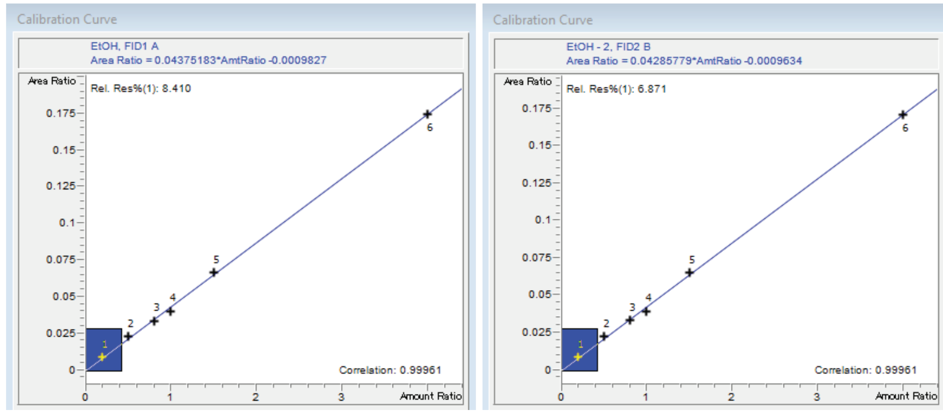


**Figure 5.** Calibration curves for ethanol on both the Agilent J&W DB-BAC1 UI and DB-BAC2 UI columns using nitrogen carrier gas.

**Table 4.** Comparison of ethanol calibration curve constants between helium and nitrogen carrier gas.

| | Helium Carrier Gas | | Nitrogen Carrier Gas | |
|---|---|---|---|---|
| | DB-BAC1 UI | DB-BAC2 UI | DB-BAC1 UI | DB-BAC2 UI |
| Slope | 0.0488 | 0.0477 | 0.0438 | 0.0429 |
| Intercept | −0.0011 | −0.0014 | −0.0010 | −0.0010 |
| Correlation | 0.9999 | 0.9999 | 0.9995 | 0.9995 |

**Table 5.** Retention time (RT) and relative response factor (RRF) statistical data for 12 sequential injections of a 50 mg/dL QC mix.

| | Helium Carrier | | | | Nitrogen Carrier | | | |
|---|---|---|---|---|---|---|---|---|
| | DB-BAC1 UI | | DB-BAC2 UI | | DB-BAC1 UI | | DB-BAC2 UI | |
| Compound | RT | RRF | RT | RRF | RT | RRF | RT | RRF |
| Methanol | 0.03% | 2.06% | 0.03% | 1.72% | 0.03% | 2.64% | 0.04% | 2.19% |
| Acetaldehyde | 0.04% | 2.09% | 0.00% | 2.11% | 0.01% | 0.76% | 0.01% | 0.83% |
| Ethanol | 0.00% | 2.16% | 0.02% | 1.69% | 0.03% | 2.27% | 0.02% | 2.16% |
| Isopropanol | 0.02% | 1.49% | 0.03% | 1.34% | 0.03% | 1.79% | 0.01% | 1.83% |
| Acetone | 0.02% | 0.74% | 0.00% | 1.01% | 0.02% | 1.09% | 0.01% | 0.72% |
| 2-Butanone | 0.04% | 1.90% | 0.02% | 1.61% | 0.03% | 0.83% | 0.03% | 0.99% |

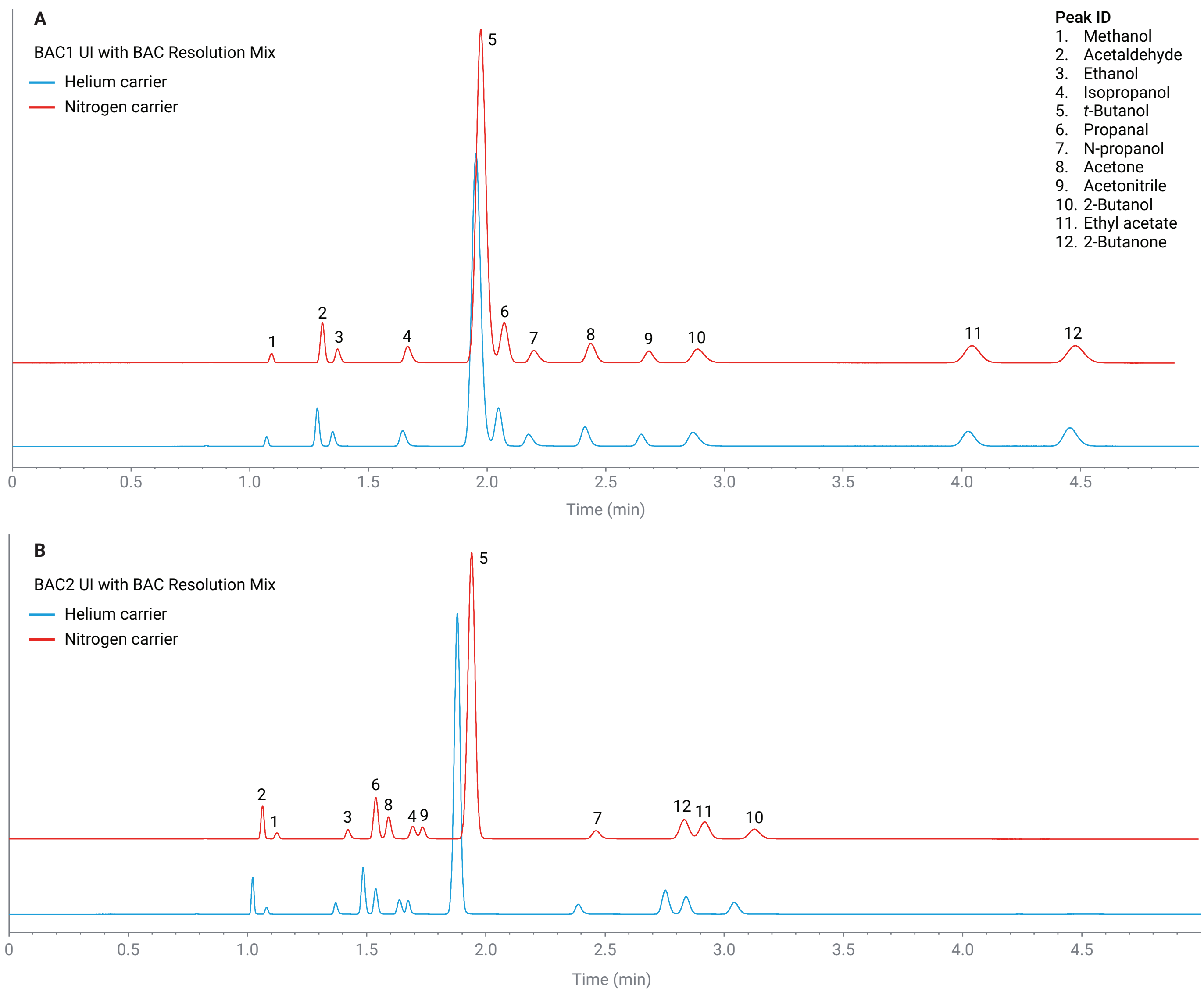


**Figure 6.** Chromatograms of a 50 mg/dL injection of the Agilent Blood Alcohol checkout mix using nitrogen carrier gas.

Figure 7 provides the retention time values used in the resolution calculations for both columns under both carrier gas options.

Resolution was calculated using the USP formula available in most current data systems, and a comparison for helium and nitrogen results on both the DB-BAC1 UI and DB-BAC2 UI column chemistries is shown in Figure 8. While the resolution values from the nitrogen carrier method are slightly lower on critical separations, the values are adequate to meet the needs of most laboratory QC requirements.

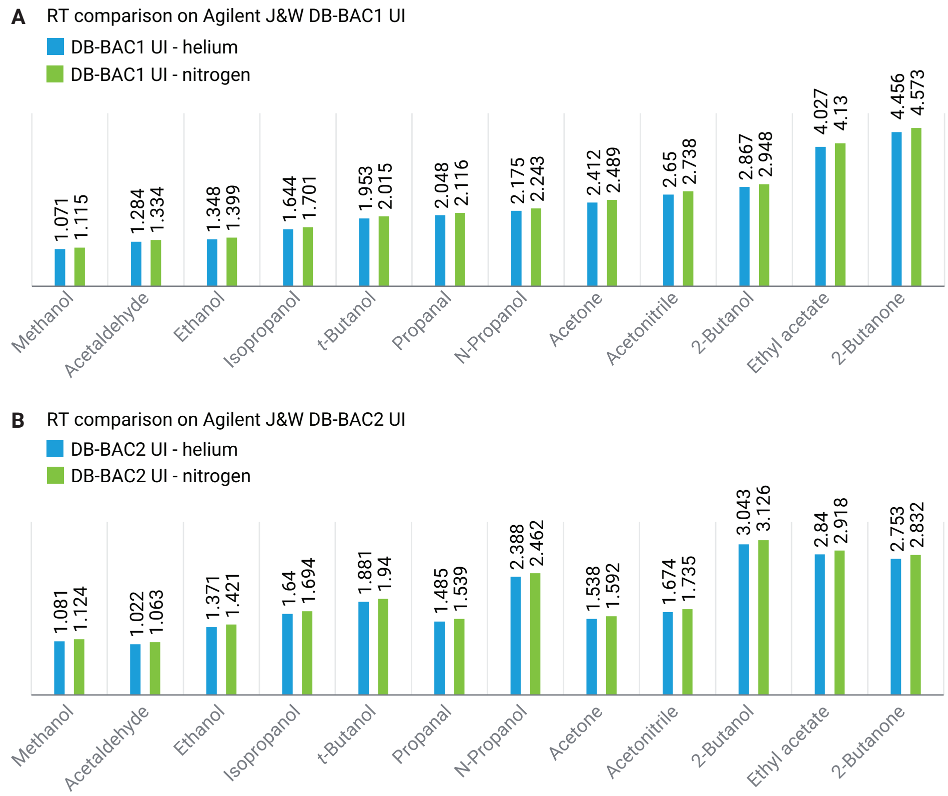


**Figure 7.** Retention time (RT) comparison between helium and nitrogen carrier gas on both Agilent J&W DB-BAC1 UI (A) and DB-BAC2 UI (B) columns.

## Feature focus: Gas Identification Test

The Gas Identification Test, built into the 8890, 8860, and Intuvo 9000 GC memory, provides a noninvasive diagnostic test to confirm the gas plumbed to the inlet module matches the gas configured by the system. This diagnostic test is helpful when the GC configuration is being evaluated under different carrier gas options. Properties of the configured gas are used to calculate the pressure required to deliver a flow, for example. If the configured gas does not match the actual gas, errors or incorrect results can occur. This diagnostic takes less than one minute, and eliminates error when a user is changing carrier gas settings on their gas chromatograph. For best results, an inlet pressure of 10 psig or higher is recommended. Figure 9 displays results for a failing carrier gas result with an option to correct as well as a passing diagnostic test.

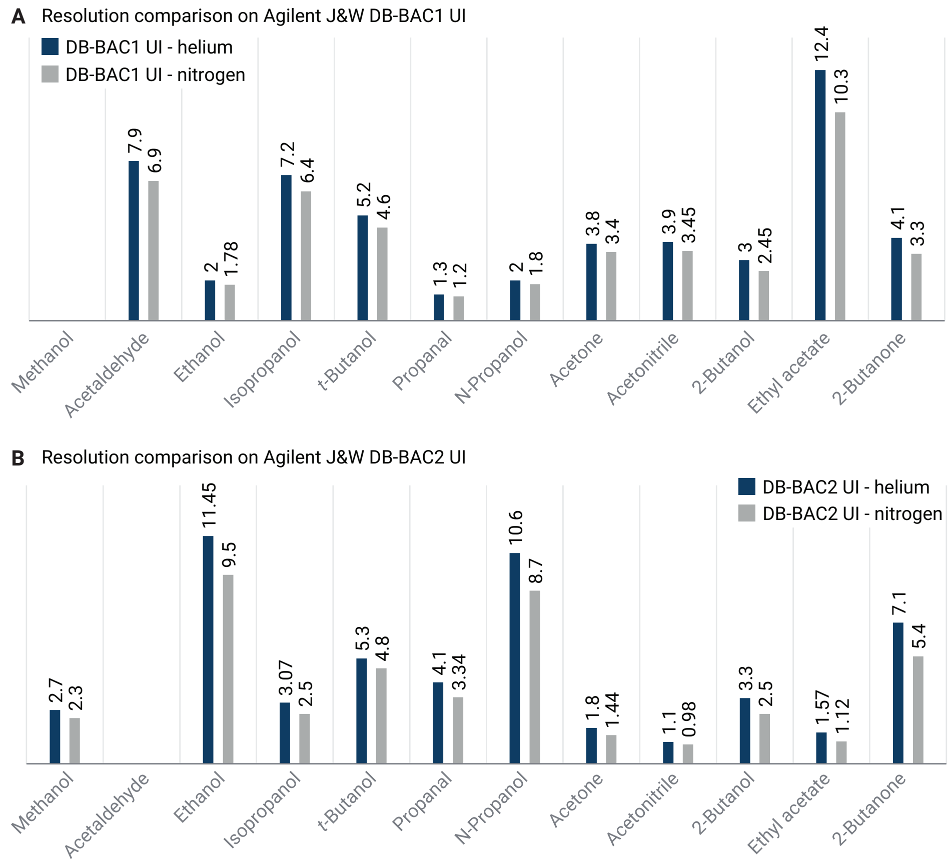


**Figure 8.** USP resolution (Rs) comparison between helium and nitrogen carrier gas on both Agilent J&W DB-BAC1 UI (A) and DB-BAC2 UI (B) columns. Resolution values are unavailable for methanol (DB-BAC1 UI) and acetaldehyde (DB-BAC2 UI) because they are the initial peaks in the run.

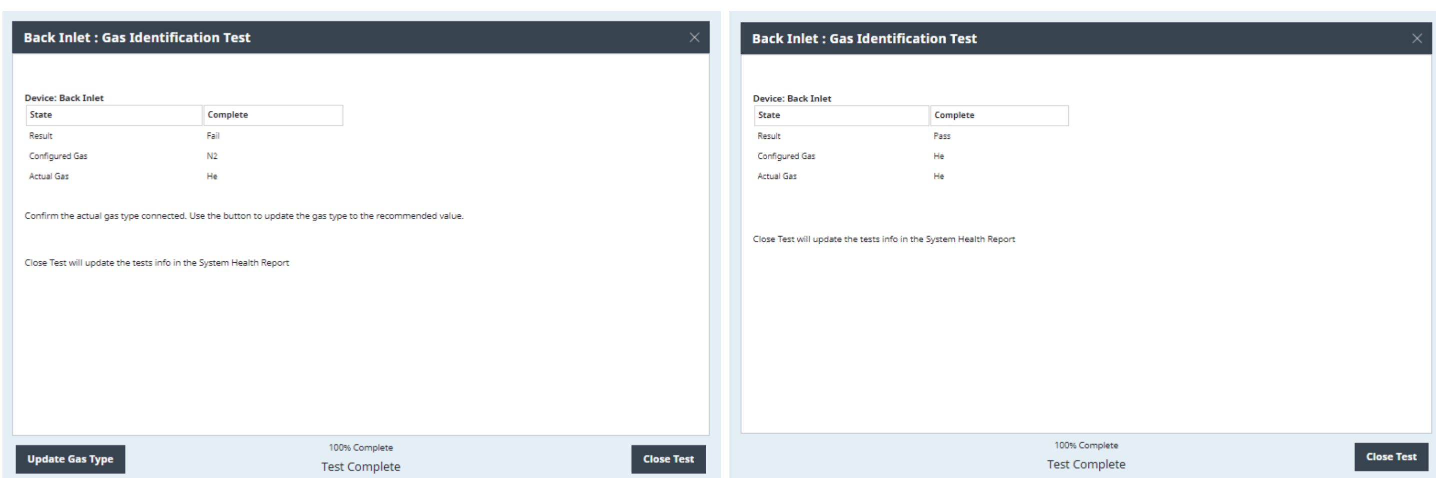


**Figure 9.** Screen captures of the Gas Identification Test. In the first box, a failing result is returned when the configured gas does not match the actual gas type. By verifying the gas type at the source, the setting can be corrected and the diagnostic initiated, in this case, correcting the gas configuration and returning a passing result where the configured gas matches the actual gas.

## Conclusion

Helium is a critical resource to many labs, yet a recurrent pattern is emerging in its availability. These results show that nitrogen—a more sustainable and economical carrier gas—can be used in the analysis of ethanol in headspace, without sacrificing data integrity. Method performance including linearity, repeatability, and chromatographic resolution were compared against the original helium method, resulting in near equivalent performance while circumventing this period of helium shortages. Legacy features such as the Agilent Method Translator tool, combined with new GC intelligence offerings, ease the burden of converting methods, effectively shifting the focus to more efficient operations with fewer concerns and interruptions due to critical resource availability.

## References


1. Fausett, A. Blood Alcohol Analysis with the Integrated 8697 Headspace Sampler on 8890 GC-Dual FID System, *Agilent Technologies application note*, publication number 5994-3126EN, **2021**.
2. GC Calculators and Method Translation Software. https://www.agilent.com/en/support/gas-chromatography/gccalculators (accessed **2022**-11-07).
3. Agilent 8890 Browser Interface Diagnostics. Agilent Technologies. YouTube, Apr 14, **2022**. https://www.youtube.com/watch?v=vRe8j78Bp8s&list=PLThrdl2ragom_g5O55hddaMc-hcwr2_1d&index=114 (accessed **2022**-11-07).



**www.agilent.com**

For Forensic Use.

RA45124.4069907407

This information is subject to change without notice.





Printed in the USA, July 18, 2023
5994-6508EN

Agilent
Trusted Answers