

Porting the Agilent Blood Alcohol Application Note 5990-9021EN

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Key Words

Blood Alcohol, Headspace Analysis, TRACE 1310 GC, TriPlus 300 HS Autosampler

Goal

To demonstrate the successful portability of a popular Agilent application note on a system comprising the Thermo Scientific™ TRACE™ 1310 gas chromatograph (GC) and the Thermo Scientific™ TriPlus™ 300 HS autosampler

Introduction

The TRACE 1300 Series GC offers the best alternative to an Agilent GC. On top of soundly key performance advantages such as superior injector robustness, detector sensitivity, user-exchangeability of instant connect injectors and detectors, easier maintenance, and faster oven cycles, the TRACE 1300 Series GC provides easy and fast implementation of applications previously run on Agilent GC instruments with a split/splitless injector. This compatibility with Agilent methods can also be extended to workflow involving sampling devices extracting samples prior to the GC analysis. The TriPlus 300HS is a valid example of extracting device, able to automated head space extraction and injection, using valve and loop technology.

To demonstrate this portability the blood alcohol analysis performed according to Agilent Application Note 5990-9021EN¹ with the Agilent 7820A GC and 7697A headspace autosampler has been selected as example.

This is considered a “classic” analysis, and is well known among laboratories worldwide. In this technical note, blood alcohol analysis is performed using the TriPlus 300 HS autosampler with a Thermo Scientific™ TRACE™ 1300 Series gas chromatograph (GC), and parameters such as retention time, peak area reproducibility, and linearity are measured to show comparable results with the original application note.



Method Parameters of Agilent Application Note 5990-9021EN

Sample Size:	0.5 mL + 100 µL internal standard (t-Butanol 200 mg/100 mL)
Column:	Agilent J&W DB-ALC2, 0.32 mm × 30 m × 1.2 µm
GC:	Oven: 40 °C, hold 7min Inlet: 200 °C, split ratio 10:1, constant flow 12 mL/min FID: default values

Headspace Autosampler

Temperatures:	Oven 85 °C Loop 85 °C Transfer line 100 °C
Times:	Equilibration time 15 min
Vial and Loop	
Filling Settings:	Vial fill mode: flow limited pressure Fill flow: 50 mL/min Fill pressure: 15 psi Loop fill mode: custom Loop fill ramp: 20 psi/min Final pressure: 10 psi

Calibrations and Sample Preparation

Following the guidelines of the original application note, prepare five ethanol/water solutions in 100 mL flasks at concentrations of 500, 1000, 2000, 4000, and 8000 mg/100 mL. Add 10 μ L of ethanol/water solution to a 0.49 mL blank blood sample in a 20 mL headspace vial. The final concentrations of ethanol standard blood samples should be: 10, 20, 40, 80, and 160 mg/100 mL. Add 100 μ L of 200 mg/100 mL t-butanol to each vial as an internal standard. Use ethanol at 60 mg/100 mL to assess the repeatability of the system.

Original Method Results

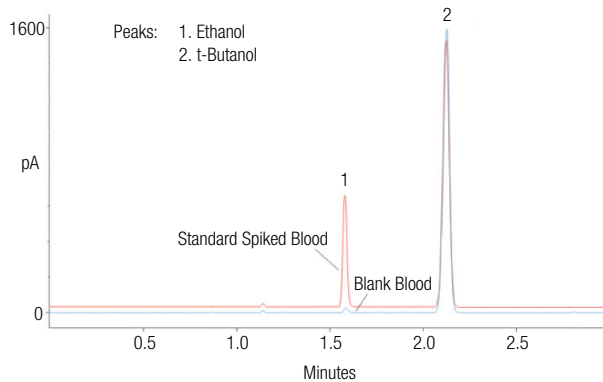


Figure 1. Chromatograms of blank blood sample and ethanol-spiked blood sample (ethanol spiked concentration is 40 mg/100 mL) in the original application note.

Method Porting

The parameters used by the 7697A instrument can be precisely transferred and deployed by the TriPlus 300 HS autosampler. This is made possible by the flexibility of the TriPlus 300 HS autosampler, which can accept up to four different modalities of sample vial pressurization and loop filling and even different unit parameters (psi, kPa, bar). The flow pressurization and custom loop filling modes can reproduce the 7697A operations with precision. On the TriPlus 300 HS autosampler, the analysis parameters are as follows:

Vial pressurization mode: Flow, 50 mL/min, 15 psi
 Loop fill mode: Custom 20 psi/min 10 psi 0.05 min

The GC is run using the same conditions as the Agilent 7820A, the column used is a Thermo Scientific™ TraceGOLD™ TG-ALC II at 30 m \times 0.32 mm \times 1.2 μ m (P/N 26073-2260).

Results

Figure 2 shows a typical chromatogram obtained from this analysis. Proper adjustment of the carrier gas column flow rate on the TRACE 1300 allows perfect matching of retention times with those shown in the original application note.

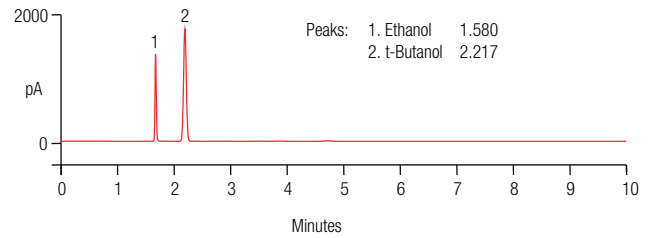


Figure 2. Chromatogram acquired by the TRACE 1310 GC/TriPlus 300 HS system at 40 mg/100 mL.

The linearity of the system has been tested using the same calibration mixture indicated in the original application note.

Table 1. Calibration results.

Conc (mg/dL)	Area Ratio
10	0.05816
20	0.19444
40	0.37810
80	0.75731
160	1.51795

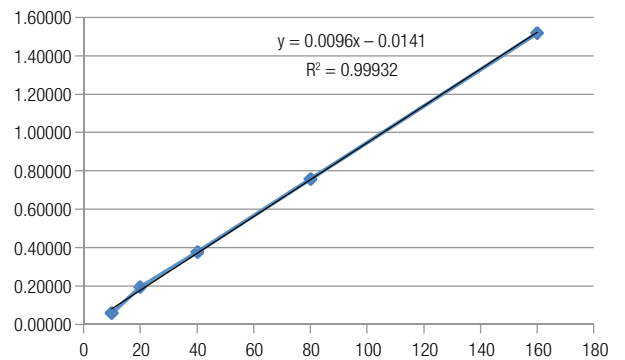


Figure 3. Calibration curve for ethanol.

Repeatability was tested on 10 replicates of ethanol and t-butanol standard at 60 mg/100 mL concentration.

The good linearity and peak area repeatability as indicated by the low level RSD% indicate the capability of this system to provide quality results for this analysis.

Table 2. Ethanol and t-butanol peak area repeatability.

Run #	Ethanol	t-Butanol
1	5996.55	16167.24
2	6108.05	16245.84
3	6077.72	16580.42
4	6033.05	16658.93
5	6047.79	16670.65
6	6038.39	16317.57
7	6092.62	16211.54
8	6034.76	16868.23
9	5974.74	16545.33
10	6179.51	16447.13
Avg	6058.32	16471.29
Std Dev	58.89	232.11
RSD%	0.97	1.41

Conclusion

The performance of the TriPlus 300 headspace autosampler is solid and consistent. In combination with TRACE 1300 Series GC, it enables simple, rapid, and pain-free portability of this and other HS methods.

This comparison demonstrates that an existing, validated method running on an Agilent HS-GC system can be adapted to the TriPlus 300 HS-TRACE 1300 Series GC system without any change in method or time spent on revalidation.

Not only does the method remain unchanged, but the same consumables (HS vials and septa, SSL injector consumables, ferrules, etc.) can be used on the Thermo Scientific systems, further reducing the cost of migrating the method from an older platform to a more modern HS-GC system.

References

1. Application Note 5990-9021EN, *Analysis of Ethanol in Blood with the Agilent 7820A GC and 7697A Headspace Sampler*, Agilent Technologies, Inc., Shanghai, People's Republic of China, 2013. [Online] <http://www.chem.agilent.com/Library/applications/5990-9021EN.pdf> (accessed October 31, 2014).

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