Application Note

Forensics

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Analysis of Blood Alcohol Content with the 120-Vial Agilent 8697 Headspace Sampler -XL Tray and Dual-FID Agilent 8890 GC System

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Abstract

Blood alcohol content (BAC) testing is an important methodology for forensic laboratories around the world. While the legal ethanol limit for motorists varies by location, the long testing backlog for forensic laboratories is a commonly reported challenge. This application note demonstrates the use of the 120-vial Agilent 8697 Headspace Sampler -XL Tray coupled to the Agilent 8890 GC System with dual-column and dual-flame ionization detector (FID) to analyze BAC. The 8697 -XL Tray integrates the precision and intelligence of the 8697 with an expanded 120-vial-capacity tray. Combining the 8697 -XL Tray with the 8890 GC in a dual-column, dual-FID configuration yields excellent ethanol linearity between 0.025 and 0.3%, vial-to-vial reproducibility of 2.0% RSD at 0.05% concentration, and no ethanol carryover at 0.4% concentration.

Introduction

BAC testing is a well-established, routine testing method for many forensic and diagnostic laboratories. The method parameters can vary by jurisdiction; however, headspace GC and a linear ethanol calibration model that brackets the legal blood-ethanol limit is a common approach. Headspace sampling is popular for BAC analysis because, unlike standard liquid injections, none of the nonvolatile sample matrix is injected into the instrument. The sample is instead sealed in a vial and heated to vaporize the volatile components, including ethanol and its metabolites. This vapor is injected into the GC while the nonvolatile matrix remains in the vial, yielding a simplified chromatogram that is devoid of excess extraneous peaks and baseline disturbances.

This approach to sample introduction greatly prolongs the lifetimes of instrument consumables, such as inlet liners and columns, by protecting them from unnecessary exposure to the salts and proteins present in the nonvolatile matrix. Also, the samples do not require any complicated cleanup steps or special preparation outside of adding an internal standard (ISTD). The resulting fast sample preparation combined with long instrument uptimes greatly increases personnel and laboratory throughput without sacrificing method figures of merit.[1] This application note demonstrates the analysis of BAC using the 8697 -XL Tray coupled to the 8890 GC with dual-column and dual-FID.

Experimental

An Agilent 8890 GC was configured with a split/splitless (SSL) inlet and dual FIDs. Connected to the GC was an 8697 -XL Tray using a heat-traced transfer line directly into the SSL inlet. The inlet was plumbed with a short precolumn to an unpurged splitter, which was connected to both an Agilent J&W DB-BAC1 Ultra Inert (UI) primary column and an Agilent J&W DB-BAC2 UI confirmatory column, enabling dual, simultaneous separations from a single sample injection. Table 1 details the instrument parts and consumables, and Table 2 details the chemical standards used in this method.

A 1 L stock solution of 0.1% (v/v) *n*-propanol in water (Millipore dispensed) was created as an ISTD. Each headspace sample consisted of 450 µL ISTD solution and 50 µL sample for a total volume of 500 µL in each vial. All solutions were stored in a refrigerator at 4 °C. Table 3 shows the method parameters for the GC and headspace sampler.

Table 1. Agilent instrument parts and consumables.

Parts and Consumables	
Description	**Part Number**
Crimp Top Vials, 20 mL	5190-2286
Crimp Top Caps/Septa	5183-4477
Inlet liner, Ultra Inert, 2 mm id	5190-6168
Precolumn, 0.5 m × 0.53 mm (Deactivated Fused Silica)	160-2535-10
Column 1: DB-BAC1 UI	123-9334UI
Column 2: DB-BAC2 UI	123-9434UI
CFT Unpurged Two-Way Splitter	G3181-60500

Table 2. Chemical standards.

Standards		
Description	**Part Number**	**Test**
Agilent Blood Alcohol Checkout Mix	5190-9765	Repeatability/resolution
Agilent Ethanol Calibration Kit	G3440-85035	Ethanol calibration
Agilent Multicomponent Alcohol Calibration Kit	G3440-85036	Carryover check
1-Propanol	34871 (Millipore Sigma)	ISTD

GC cycle time

GC cycle time is an important parameter when optimizing the automation of the headspace sampler for maximum throughput. In simple terms, the GC cycle time is the amount of time from injection of a sample to the next "Ready" state of the system. More specifically, it is the sum of the GC acquisition time, the oven cooldown time (for non-isothermal methods), the temperature and pressure equilibration times for each controlled zone, and computer/software processing time. Variance in equilibration times can occur due to changes in the laboratory environment (such as a door opening causing a pressure change or a nearby GC venting during cooldown) and adding a buffer of approximately 1 minute is generally recommended. This application note uses a 5-minute run time and a 6-minute GC cycle time.

If the GC cycle time is set too short, the system can be programmed to either "Abort" the sequence, "Skip" the sample, or "Wait" for GC readiness. This option can be set in the 8697 -XL Tray method settings under Sequence Actions, as shown in Figure 1. To assist the user in setting the optimal GC cycle time, the activity log in OpenLab CDS will calculate the actual GC cycle time for samples run using the method. Figure 2 shows a screen capture of the reported GC cycle times from the activity log.

Table 3. Agilent 8697 Headspace Sampler -XL Tray and 8890 GC System method parameters.

Agilent 8697 Headspace Sampler -XL Tray Conditions	
Oven Temperature	70 °C
Loop Temperature	80 °C
Transfer Line Temperature	90 °C
Vial Equilibration	7 min
Injection Duration	1 min
GC Cycle Time	6 min
Vial Size	20 mL
Vial Shaking	250 shakes/min (setting 9)
Vial Fill Mode	Pressure
Fill Pressure	15 psi
Pressurization Gas	Nitrogen
Pressure Equilibration Time	0.05 min
Loop Fill Mode	Custom
Loop Ramp Rate	40 psi/min
Final Loop Pressure	1.5 psi
Loop Equilibration	0.05 min
Extraction Mode	Single
Vent Vial Pressure After Extraction	On
Post-Injection Purge	Custom
Purge Flow	200 mL/min
Purge Time	3 min

Agilent 8890 GC System Conditions	
Inlet Temperature	150 °C
Carrier Gas	Helium
Split Ratio	10:1
Control Mode	Constant pressure
Inlet Pressure	21 psi
Septum Purge	3 mL/min
Gas Saver	20 mL/min after 2 min
Oven Program	40 °C isothermal for 5 min
FID A/B Temperature	250 °C
FID A/B Air Flow	400 mL/min
FID A/B Hydrogen Flow	30 mL/min
FID A/B Makeup Flow (N_2)	25 mL/min

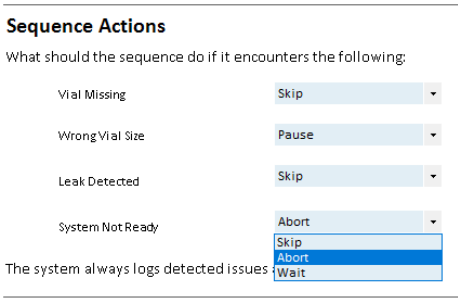

Figure 1. Method parameter settings in Agilent OpenLab CDS for the Agilent 8697 Headspace Sampler -XL Tray to control sequence behavior when the headspace sampler encounters issues.

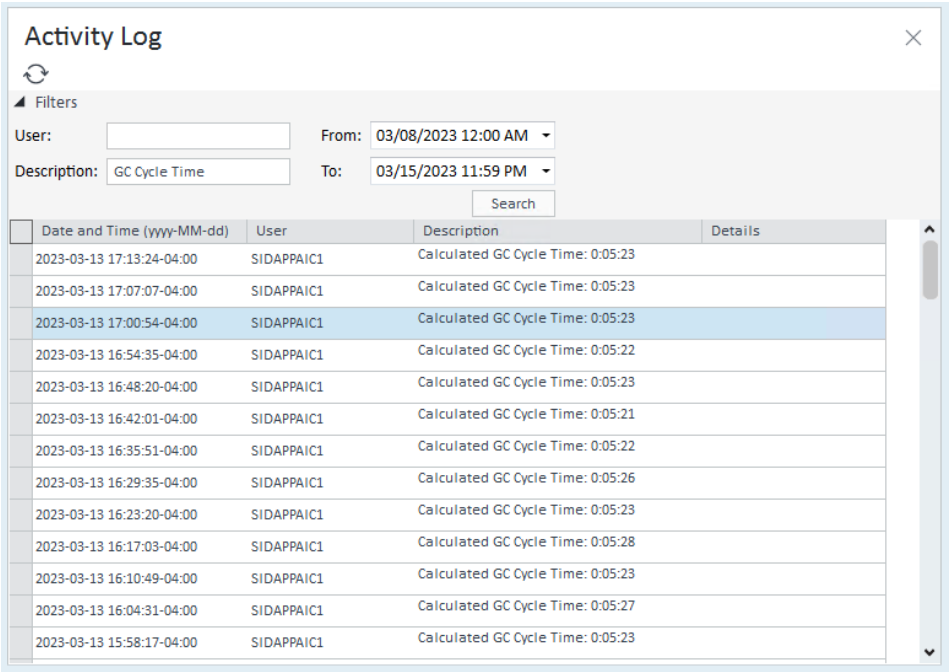

Figure 2. Screen capture of the Agilent OpenLab CDS software activity log showing the calculated GC cycle time for a sequence of BAC samples.

Results and discussion

Calibration

Calibration standards containing 50, 80, 100, 200, and 300 mg/dL of ethanol in water were analyzed in triplicate. Also, a 25 mg/dL standard was created by adding 25 µL of the 50 mg/dL standard and 25 µL water to the headspace vial (to maintain a final volume of 0.5 mL, consistent with the other standards) and this was also analyzed in triplicate.

The relative response of ethanol to *n*-propanol (Y-axis) was plotted against the relative amount of ethanol to *n*-propanol (X-axis) and an unweighted linear curve model was established in OpenLab CDS. The model was set to ignore the origin and to calculate using the average for each calibration level. The R^2 value for FID A was 0.99994 and for FID B was 0.99993. Figures 3 and 4 show the calibration models for FID A and B respectively.

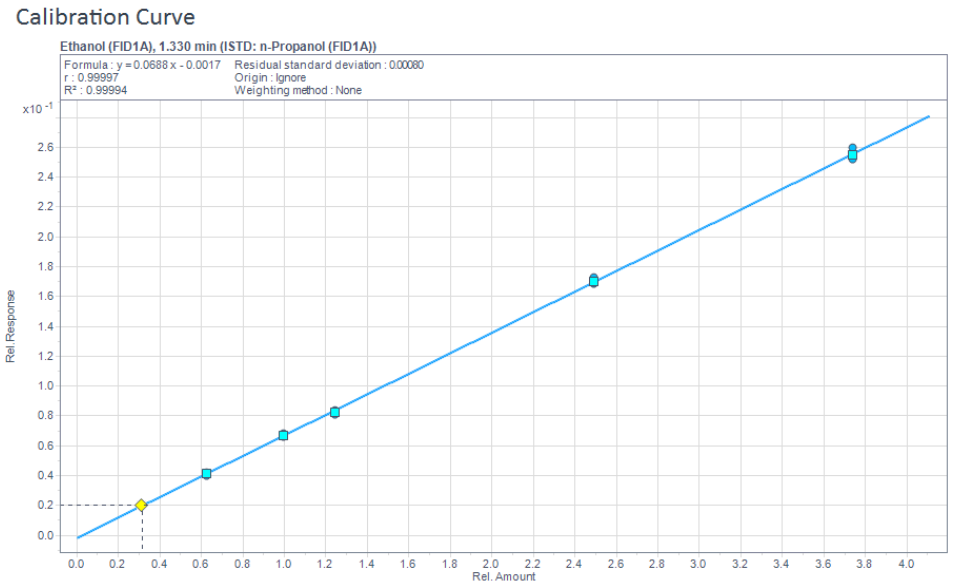

Figure 3. Calibration model for ethanol on FID A (Agilent J&W DB-BAC1 Ultra Inert primary column).

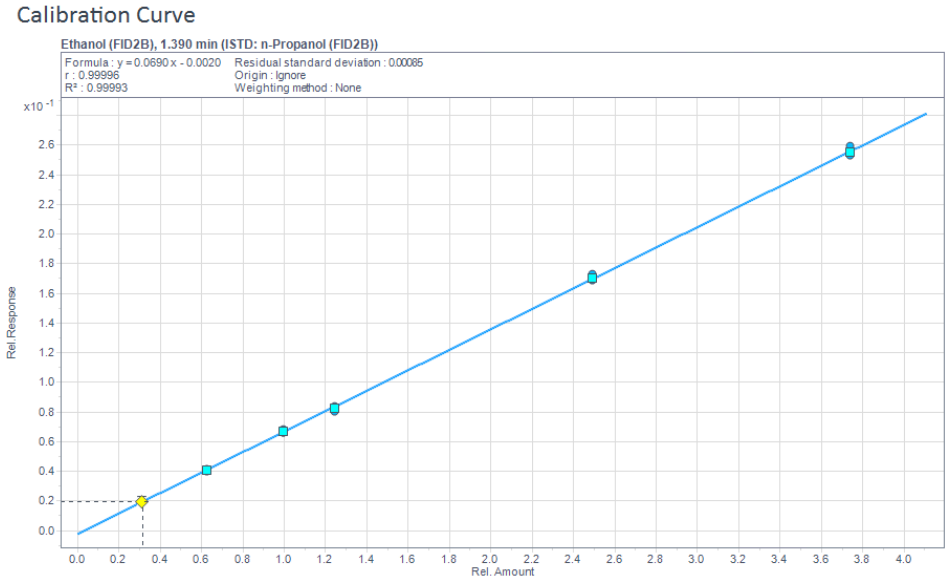

Figure 4. Calibration model for ethanol on FID B (Agilent J&W DB-BAC2 Ultra Inert confirmatory column).

Repeatability

The Agilent Blood Alcohol checkout mix (part number 5190-9765) is a 12-compound mixture that is used to confirm proper chromatographic performance of the system. Each compound is present at 50 mg/dL, which makes the mixture an excellent standard for characterizing method precision. Figures 5 and 6 show the separation of the Blood Alcohol checkout mix on both DB-BAC1 UI and DB-BAC2 UI columns respectively, and Table 4 lists the identification and retention times of the peaks. To demonstrate the repeatability of the method, 12 replicates of the checkout mix were injected consecutively and analyzed, and the results are shown in Table 5.

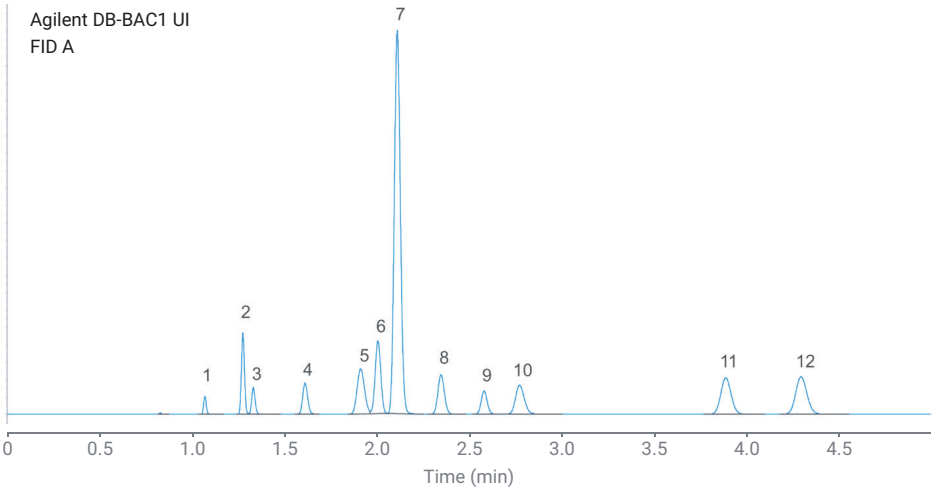

Figure 5. FID A chromatogram of the Agilent Blood Alcohol checkout mix on the Agilent J&W DB-BAC1 Ultra Inert column.

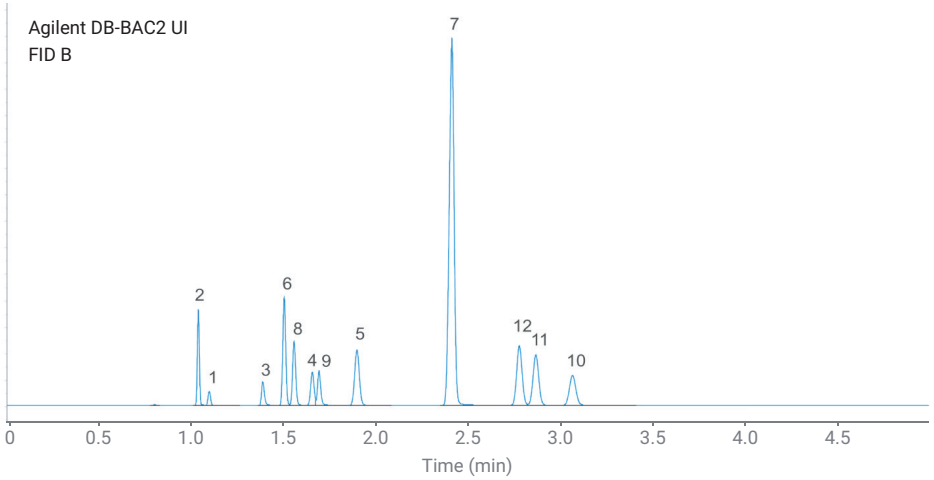

Figure 6. FID B chromatogram of the Agilent Blood Alcohol checkout mix on the Agilent J&W DB-BAC2 Ultra Inert column.

Table 4. Peak identities and retention times (RT) of the Agilent Blood Alcohol checkout mix shown in Figures 5 and 6.

Peak Label	Compound	RT DB-BAC1 UI (min)	RT DB-BAC2 UI (min)
1	Methanol	1.066	1.095
2	Acetaldehyde	1.271	1.036
3	Ethanol	1.327	1.385
4	Isopropanol	1.606	1.653
5	*t*-Butanol	1.906	1.895
6	Propanal	1.999	1.501
7	*n*-Propanol (ISTD)	2.103	2.409
8	Acetone	2.339	1.555
9	Acetonitrile	2.571	1.689
10	2-Butanol	2.762	3.062
11	Ethyl acetate	3.873	2.864
12	2-Butanone	4.279	2.774

Carryover

To demonstrate complete transfer of sample from the headspace oven to the GC, 10 replicates of a 400 mg/dL standard containing methanol, ethanol, isopropanol, acetone, and *n*-propanol (ISTD) were analyzed in series and bracketed with blank runs. Figures 7 and 8 show the overlaid 10 replicate chromatograms and a zoomed-in view of the blank run immediately following the replicates for the DB-BAC1 UI and DB-BAC2 UI columns, respectively. As can be seen, the blank signals for both columns show no measurable carryover for all compounds in the standard.

Table 5. Repeatability (%RSD) of retention time, area response, and peak height for each compound in the Agilent Blood Alcohol checkout mix.

		%RSD		
Name	**Signal Description**	**RT**	**Area**	**Height**
2-Butanol	FID1A	0.022	1.342	1.441
	FID2B	0.010	1.363	1.406
2-Butanone	FID1A	0.012	1.810	1.838
	FID2B	0.000	1.804	1.767
Acetaldehyde	FID1A	0.000	2.036	1.998
	FID2B	0.000	2.048	1.911
Acetone	FID1A	0.017	1.544	1.511
	FID2B	0.026	1.536	1.493
Acetonitrile	FID1A	0.000	1.497	1.444
	FID2B	0.000	1.534	1.362
Ethanol	FID1A	0.000	2.018	2.034
	FID2B	0.034	1.995	2.021

		%RSD		
Name	**Signal Description**	**RT**	**Area**	**Height**
Ethyl Acetate	FID1A	0.008	2.747	2.777
	FID2B	0.016	2.759	2.834
Isopropanol	FID1A	0.025	1.455	1.513
	FID2B	0.028	1.454	1.435
Methanol	FID1A	0.047	2.454	2.443
	FID2B	0.000	2.706	2.224
n-Propanol	FID1A	0.022	1.378	1.455
	FID2B	0.022	1.424	1.530
Propanal	FID1A	0.000	2.442	2.321
	FID2B	0.000	2.258	2.259
t-Butanol	FID1A	0.021	1.310	1.319
	FID2B	0.027	1.341	1.297

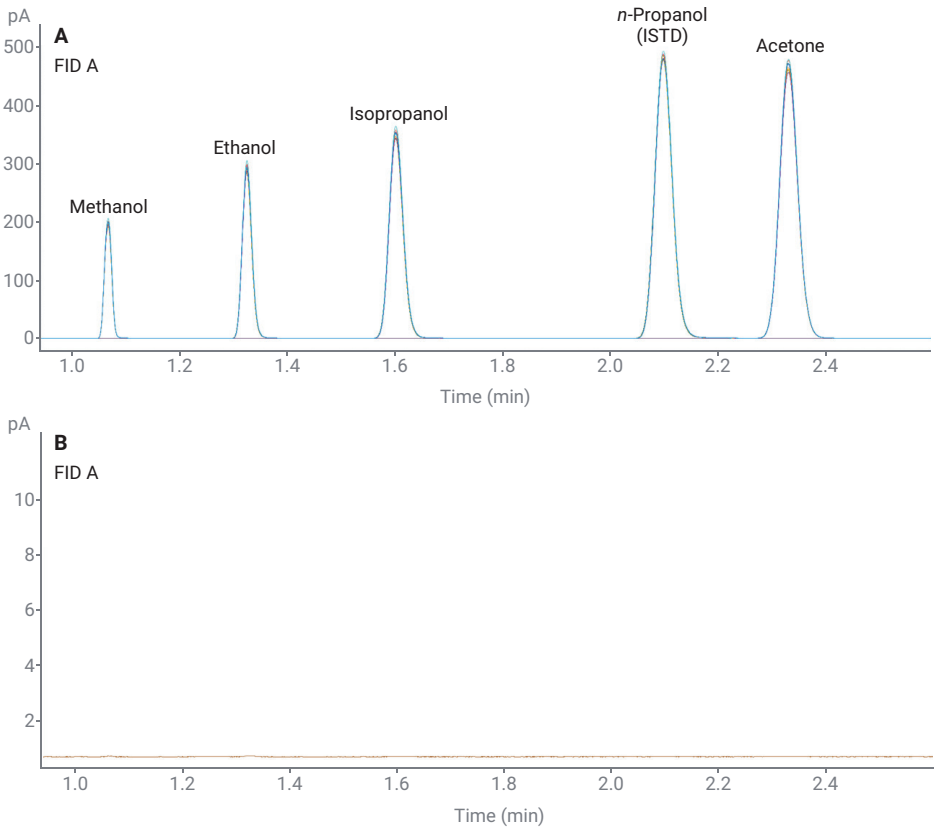

Figure 7. Overlay of 10 replicates of 400 mg/dL mixture (A) and blank run immediately after (B) on the Agilent J&W DB-BAC1 Ultra Inert primary column (FID A).

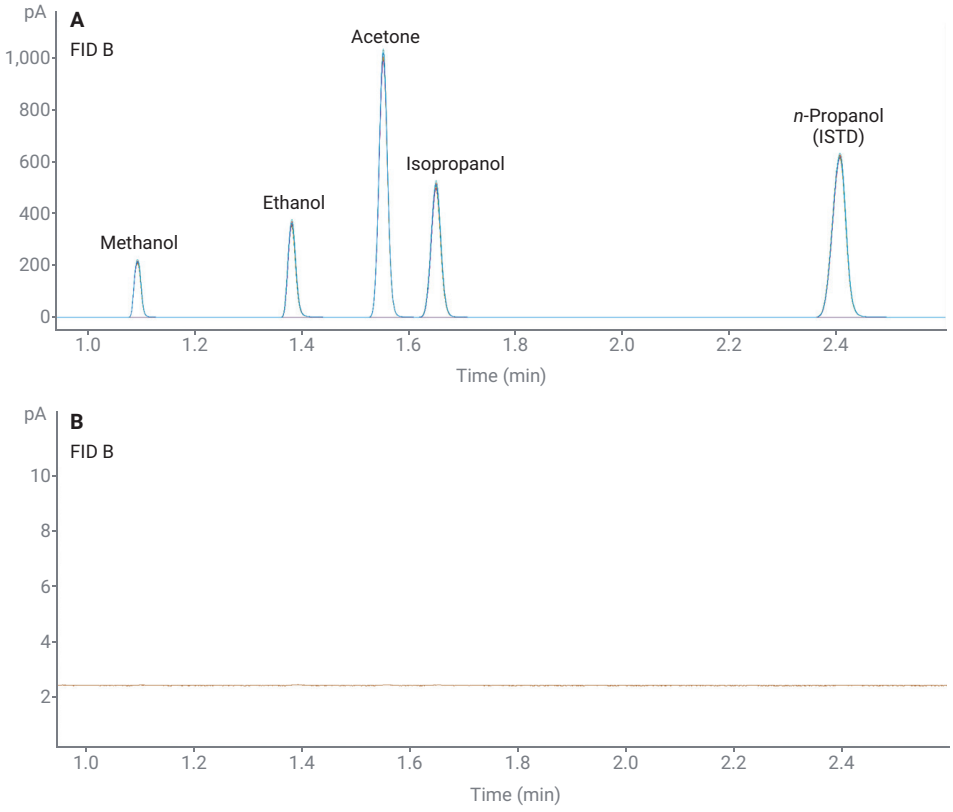

Figure 8. Overlay of 10 replicates of 400 mg/dL mixture (A) and blank run immediately after (B) on the Agilent J&W DB-BAC2 Ultra Inert confirmatory column (FID B).

Conclusion

The 8697 -XL Tray builds upon the precision, robustness, and intelligence previously established by the 8697 -XL Tray by expanding the tray capacity to 120 vials. Combining these features with the Agilent 8890 GC enables the testing of BAC with zero carryover, excellent linearity, and the functionality needed to finally clear the sample backlog.

References

1. Blood Alcohol Analysis with the Integrated Agilent 8697 Headspace Sampler on 8890 GC-Dual FID System. *Agilent Technologies application note*, publication number 5994-3126EN, **2022**.

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