

# DEVELOPMENT OF J&W DB-BAC1 ULTRA INERT AND DB-BAC2 ULTRA INERT GC COLUMNS: IMPROVED RESOLUTION AND PEAK SHAPE PERFORMANCE FOR THE DETERMINATION OF BLOOD ALCOHOLS

Agilent  
**CrossLab**

From Insight to Outcome

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## Blood alcohols

Blood alcohol concentration (BAC) is routinely analyzed in both ante-mortem and post-mortem samples. The main component is Ethanol together with other alcohols. Metabolites and interfering compounds can be present as well so a comprehensive separation is important. Propanal can occur naturally in post-mortem blood samples while acetaldehyde is formed by the metabolism of ethyl alcohol. Acetone is often detected because of Ketoacidosis of untreated type 1 diabetes mellitus or can occur due to prolonged alcoholism [1].

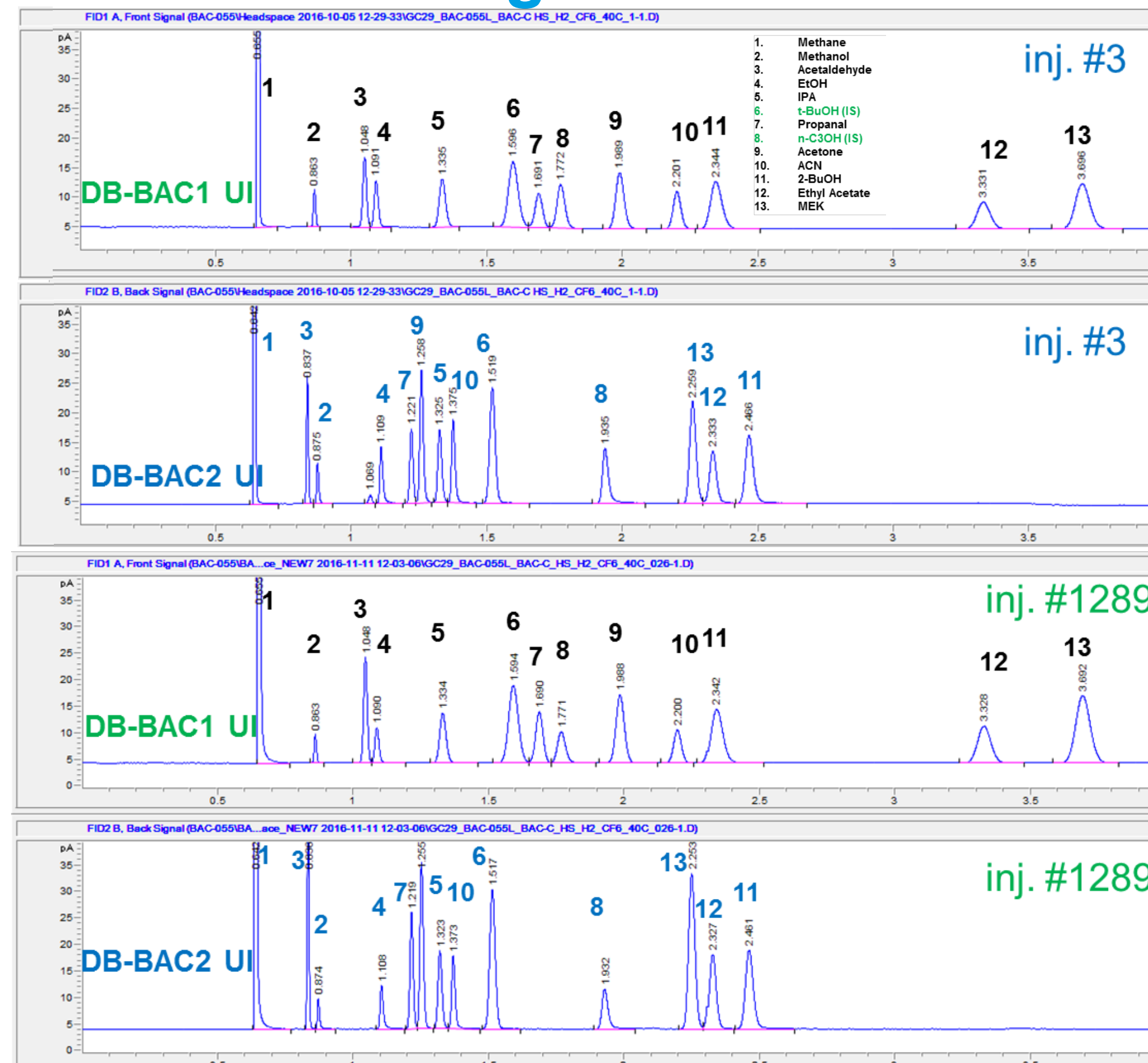
n-Propanol or t-butanol are commonly used as internal standards (IS) to compensate for matrix variations. In cases n-propanol is detected, ACN is often used as an alternative IS. Cross-contamination of this common solvent needs to be prevented [2].

Many HS-GC-FID procedures use dual-column confirmation by splitting the sample after injection to 2 chromatographic columns of sufficient different polarity. This to change retention and elution order of ethanol and other volatiles of interest [3-5]. Alternatively, one column can be used with dual FID/MS detection for quantification and identification. The new DB-BAC UI columns have been developed for use with FID and MS detection, both in ante-mortem and post-mortem samples.

Besides the new BAC columns, Agilent also has introduced ethanol CRM standards and a checkout mix for reliable blood alcohol analysis.



## Lifetime testing



DB-BAC1 UI and DB-BAC2 UI  
30m x 0.32mm

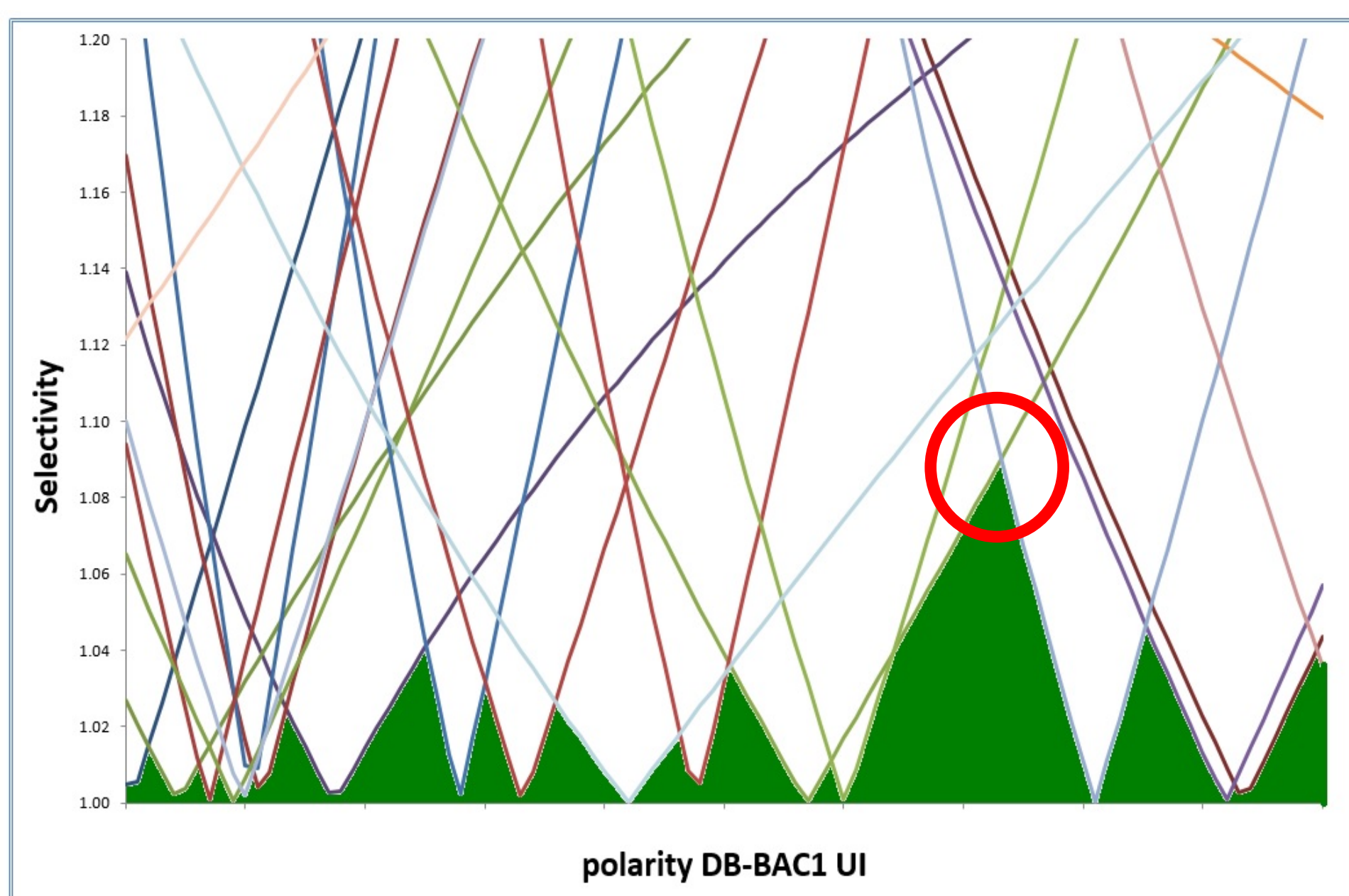
**Repeated Sequence:**  
1. 50 mg/dL (0.05%) 12 components in MQ+CH<sub>4</sub>, check column performance, n=2 inj.  
2. Ethanol and t-Butanol, 20 mg/dL (0.02%), n=99 inj.

**Headspace conditions:**  
oven 70°C (7 min), loop and transfer line 110°C, 1 mL gas injection  
20 mL Headspace vial with 50µL sample+450µL MQ water and IS.

**GC Oven:** 40°C  
**Hydrogen, CF** 6 mL/min (≈ 76 cm/s H<sub>2</sub>)  
**Split** 1:50  
**FID detection**  
**OpenLabs Chemstation**

## GC Column Development & Window Diagramming

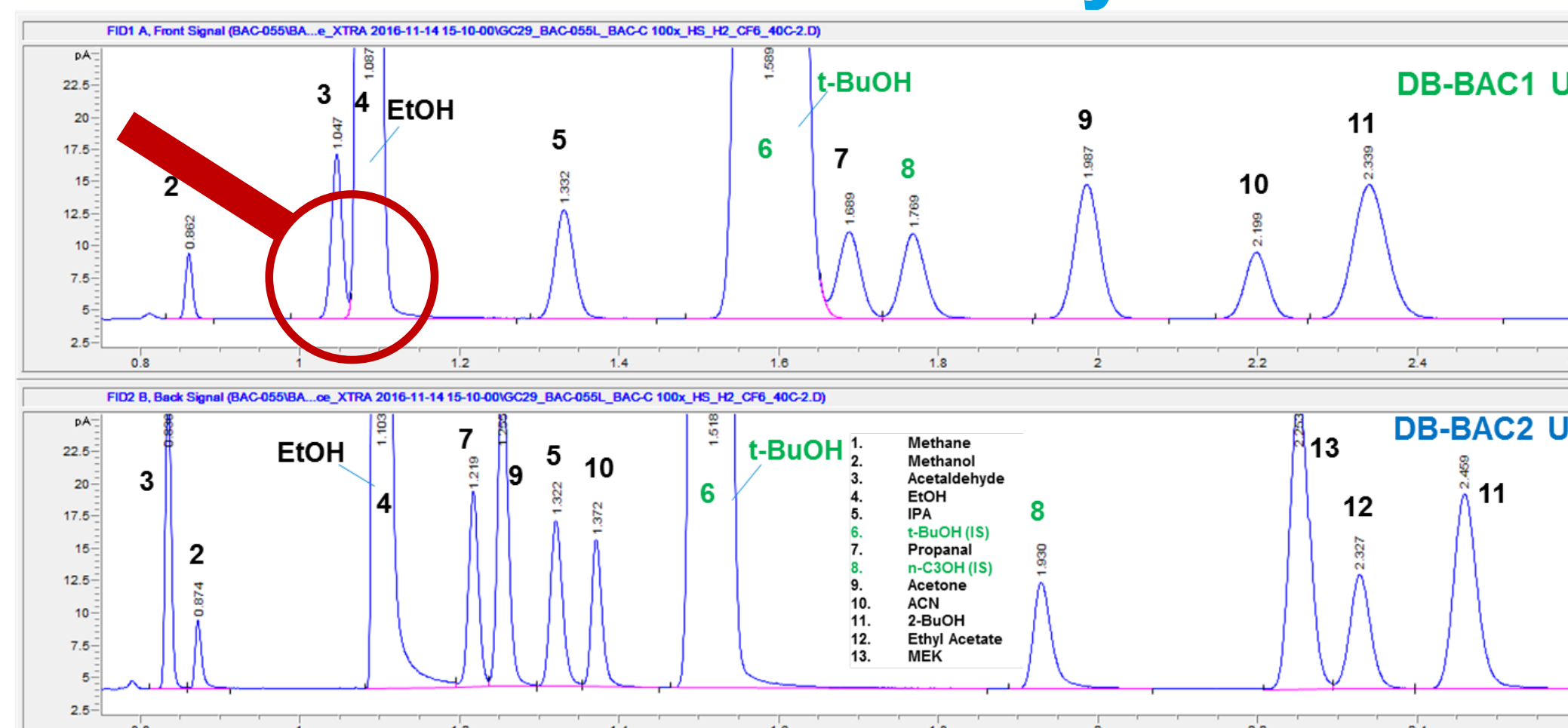
During the development of the DB-BAC UI columns, window diagramming has been used to optimize the polarity and selectivity of the new stationary phase [6]. DB-BAC1 UI is able to deliver a complete resolution of BAC target compounds.



As a next step, the DB-BAC2 UI column was optimized to generate a similar runtime but with maximum alternative selectivity and elution order.

Selectivity plot of 12 critical BAC compounds at 40°C: green marked area is the window with a selectivity > 1 for all peak pairs. The red circle marks the optimum corresponding with the highest α

## Critical look at Acetaldehyde-Ethanol separation

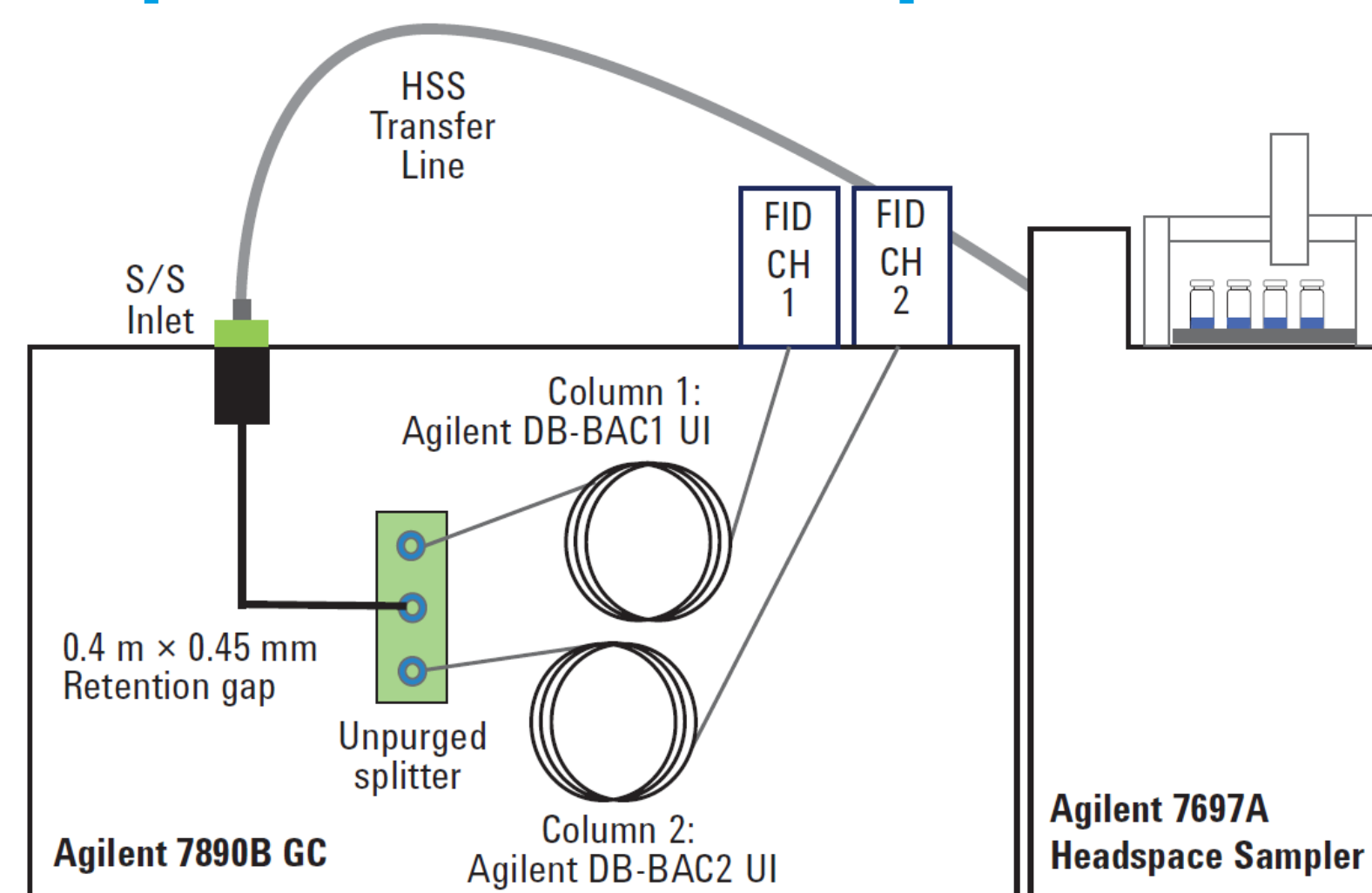


50 mg/dL (0.05%)  
12 components in MQ with a 100x addition of Ethanol and t-BuOH (5%)

**Conditions:**  
see Lifetime test

**Far above optimum gas velocity:**  
≈ 76 cm/s H<sub>2</sub>  
**Fast runtime:**  
2.3 min for compounds 1-10

## Experimental setup



Static headspace GC is widely accepted as method of choice for this type of analysis. Inlet and column maintenance are reduced compared to a direct liquid injection.

Experimental setup using Agilent dual-column/FID for the detection of blood alcohol. Details: Agilent Application note 5991-8206EN

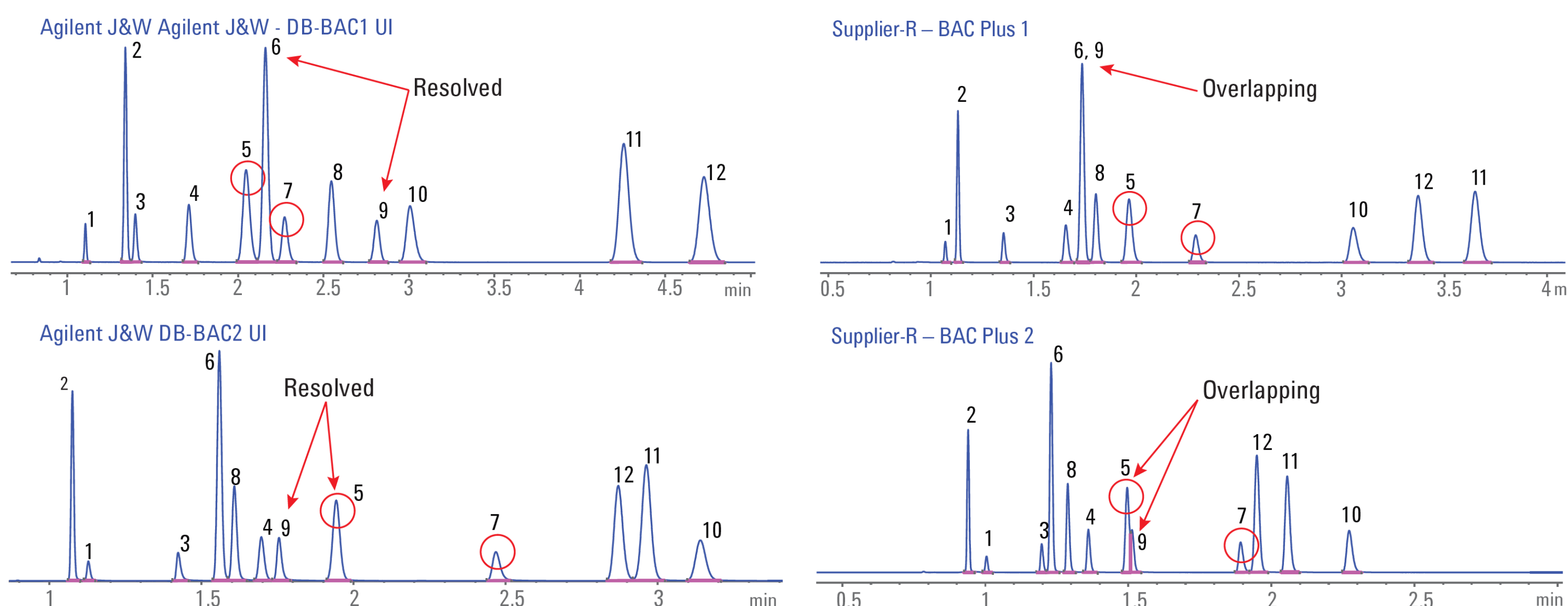
## BAC Headspace Application

The Agilent J&W DB-BAC1 UI and DB-BAC2 UI columns show sharp, highly-resolved, well-separated peaks for the Blood Alcohol Checkout Mix as compared to corresponding columns from Supplier-R under identical conditions.

**GC Method:** 60 cm/s, He, 40°C; Split 1:20; 30m x 0.32mm id GC columns  
**Headspace Method:** 500µL res mix (50 mg/dL); 15 min 70°C, 20 mL vial  
Further details: Brochure 5991-7781EN and Application note 5991-8206EN (including reproducibility and calibration)

Target compounds contained in Agilent Blood Alcohol Checkout mix (p/n 5190-9765)

No	Compound	No	Compound
1	Methanol (MeOH)	7	n-Propanol (n-C <sub>3</sub> H <sub>7</sub> OH)
2	Acetaldehyde	8	Acetone
3	Ethanol (EtOH)	9	Acetonitrile (ACN)
4	Isopropanol (IPA)	10	2-Butanol (2-BuOH)
5	t-Butanol (t-BuOH)	11	Ethyl acetate (EtAc)
6	Propanal	12	2-Butanone



The DB-BAC1 UI Acetaldehyde-Ethanol separation and quantification is compared to DB-BAC2 UI as reference. Results with an extreme high Ethanol peak still show sufficient resolution and similar peak area ratio which proves these conditions can be used for quantitative analysis (using a calibration curve).

## Summary

In the BAC analysis, t-butanol and n-propanol as the internal standards are important. The data shows both compounds are well separated from all other analytes of interest and common interferences. The use of ACN as IS is also without interference problems.

The Agilent J&W DB-BAC Ultra Inert columns show:

- Optimized resolution and baseline separation of critical blood alcohol peaks and all internal standards
- Excellent peak shape and accurate integration of low-concentration compounds
- Accurate identification of challenging polar analytes, even at trace levels
- Accurate, credible quantitation with NIST- and ERM-traceable Agilent ethanol calibration standards

## Available GC column configurations and chemical standards

Part No.	GC Column Description	Chemical Standards																											
123-9334	DB-BAC1 UI 30m, 0.32mm, 1.8u	<table><tr><th>Name</th><th>Description</th><th>Part No.</th></tr><tr><td>Ethanol 20 mg/dL</td><td>Ethanol 20 mg/dL or 0.2 g/L, in water, (1mL x 10)</td><td>5190-9756</td></tr><tr><td>Ethanol 50 mg/dL</td><td>Ethanol 50 mg/dL or 0.5 g/L, in water, (1mL x 10)</td><td>5190-9757</td></tr><tr><td>Ethanol 80 mg/dL</td><td>Ethanol 80 mg/dL or 0.8 g/L, in water, (1mL x 10)</td><td>5190-9758</td></tr><tr><td>Ethanol 100 mg/dL</td><td>Ethanol 100 mg/dL or 1.0 g/L, in water, (1mL x 10)</td><td>5190-9759</td></tr><tr><td>Ethanol 150 mg/dL</td><td>Ethanol 150 mg/dL or 1.5 g/L, in water, (1mL x 10)</td><td>5190-9760</td></tr><tr><td>Ethanol 200 mg/dL</td><td>Ethanol 200 mg/dL or 2.0 g/L, in water, (1mL x 10)</td><td>5190-9761</td></tr><tr><td>Ethanol 300 mg/dL</td><td>Ethanol 300 mg/dL or 3.0 g/L, in water, (1mL x 10)</td><td>5190-9762</td></tr><tr><td>Ethanol 400 mg/dL</td><td>Ethanol 400 mg/dL or 4.0 g/L, in water, (1mL x 10)</td><td>5190-9763</td></tr></table>	Name	Description	Part No.	Ethanol 20 mg/dL	Ethanol 20 mg/dL or 0.2 g/L, in water, (1mL x 10)	5190-9756	Ethanol 50 mg/dL	Ethanol 50 mg/dL or 0.5 g/L, in water, (1mL x 10)	5190-9757	Ethanol 80 mg/dL	Ethanol 80 mg/dL or 0.8 g/L, in water, (1mL x 10)	5190-9758	Ethanol 100 mg/dL	Ethanol 100 mg/dL or 1.0 g/L, in water, (1mL x 10)	5190-9759	Ethanol 150 mg/dL	Ethanol 150 mg/dL or 1.5 g/L, in water, (1mL x 10)	5190-9760	Ethanol 200 mg/dL	Ethanol 200 mg/dL or 2.0 g/L, in water, (1mL x 10)	5190-9761	Ethanol 300 mg/dL	Ethanol 300 mg/dL or 3.0 g/L, in water, (1mL x 10)	5190-9762	Ethanol 400 mg/dL	Ethanol 400 mg/dL or 4.0 g/L, in water, (1mL x 10)	5190-9763
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125-9434	DB-BAC2 UI 30m, 0.53mm, 2.0u	<table><tr><td>Agilent Blood Alcohol Checkout Mix 50 mg/dL (equal.)</td><td>Methanol, Acetaldehyde, Ethanol, Isopropanol, t-Butanol, Propanal, n-Propanol, Acetone, Acetonitrile, 2-Butanol, Ethyl Acetate, 2-Butanone, in water, 1 mL</td><td>5190-9765</td></tr></table>	Agilent Blood Alcohol Checkout Mix 50 mg/dL (equal.)	Methanol, Acetaldehyde, Ethanol, Isopropanol, t-Butanol, Propanal, n-Propanol, Acetone, Acetonitrile, 2-Butanol, Ethyl Acetate, 2-Butanone, in water, 1 mL	5190-9765																								
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Visit our website for more information: [www.agilent.com/chem/db\\_bac\\_ui](http://www.agilent.com/chem/db_bac_ui)

## References

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- V.A. Bumba et al. Patterns of the most abundant volatiles detected in post-mortem blood, Rom J Leg Med, [20] 147-154 [2012]
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- Walt Jennings, E. Mittlehehl, P. Stremple. Analytical Gas Chromatography, 2nd Edition. Academic Press, 1997, p. 274-277.

