



Improved Resolution and Peak Shape Performance for the Determination of Blood Alcohol Concentration

Vanessa Abercrombie

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Abstract

One of the most widely used applications of headspace gas chromatography is the determination of ethanol content in blood. Blood alcohol concentration (BAC) is routinely analyzed in both ante-mortem and post-mortem samples using headspace injection and dual column GC-FID analysis. Column choice plays a major role in data quality and reliability. Analysis is carried out using two columns with different stationary phases. Agilent J&W DB-BAC1 Ultra Inert and Agilent J&W DB-BAC2 Ultra Inert GC columns are the only blood alcohol columns on the market that can separate t-Butanol and n-Propanol from frequently encountered interferences in blood matrix.

Use of an internal standard for quantitative analysis, either n-propanol or t-butanol, assisted in compensating for matrix differences, due to their similarity to ethanol. Using the internal standard method for the calibration of ethanol resulted in lower percent errors when compared to the external standard method.

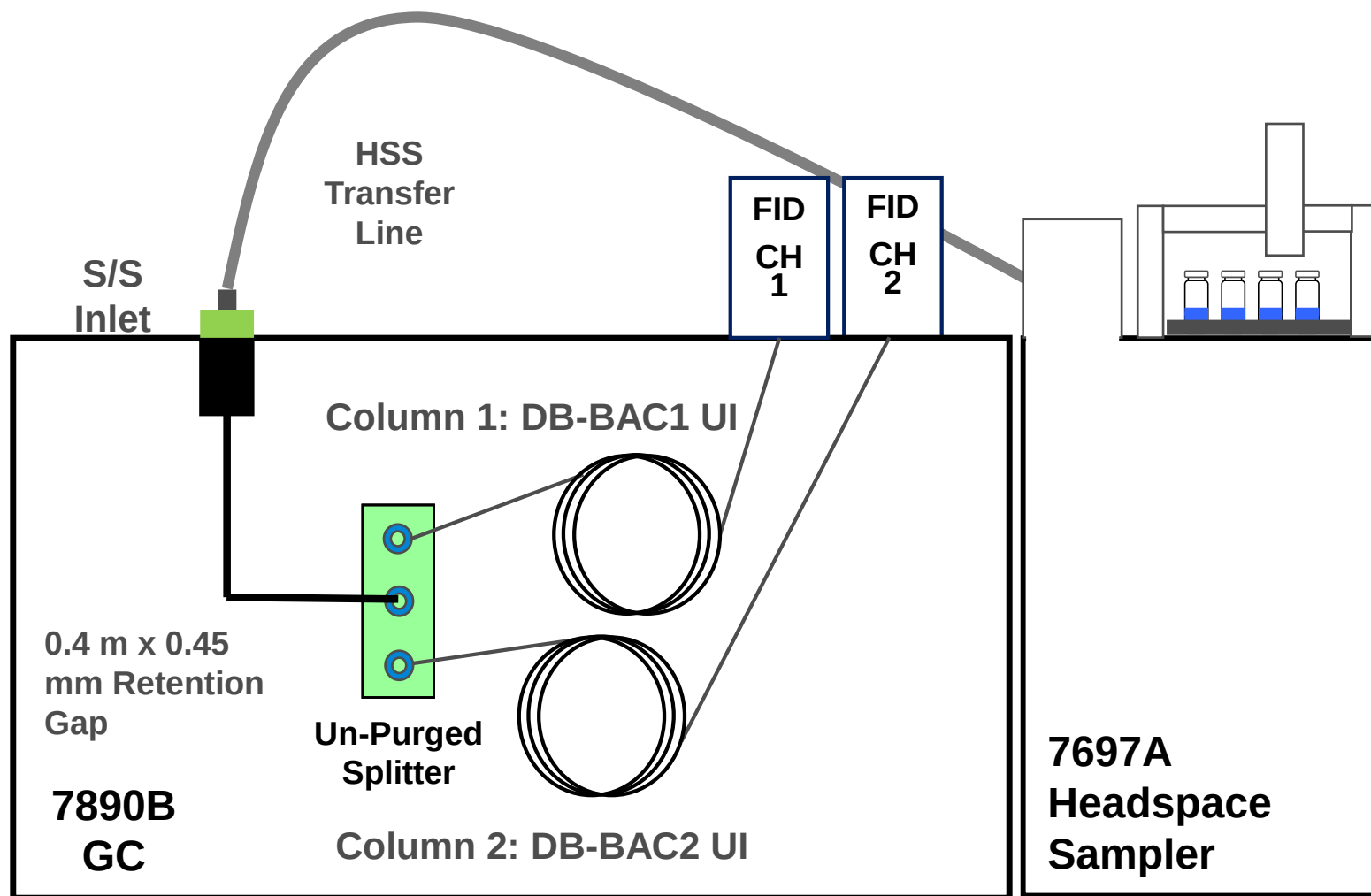
Together with ethanol calibration standards, traceable to both NIST and European Reference Materials (ERM), Agilent now offers a complete solution for the analysis of blood alcohol concentration.

Ante and Post Mortem Blood Alcohol

- Universal threshold 0.08 g/dL
- Dual column analysis by HS/GC/FID
- Ante Mortem
- Post Mortem
 - Interferences can cause resolution issues



Instrument Set Up



Instrument Conditions

Headspace Conditions

Oven: 70°C (15 min equilibration)
Loop: 80°C
Transfer liner: 90°C

GC Conditions

Columns: Agilent J&W DB-BAC1 UI 30 m × 0.32 mm, 1.8 µm (p/n 123-9334UI)
Agilent J&W DB-BAC2 UI, 30 m × 0.32 mm, 1.2 µm (p/n 123-9434UI)
Carrier: Helium, constant flow, 6 mL/min
Oven: 40°C (5.00 min)
Inlet: Split Mode, 210°C, split ratio 20:1
Inlet liner: Ultra Inert straight liner, 75 mm (p/n 5190-4048)
GC/FID: Agilent 7890B GC equipped with dual FIDs
Sampler: Agilent 7697A Headspace Autosampler

FID Conditions

Temperature: 250°C
Hydrogen: 30 mL/min
Air: 350 mL/min
Col + Makeup: 35 mL/min



Blood Alcohol Checkout Mix

- NIST Traceable Standard
- 50 mg/dL each in water
- 1 mL ampules

Simple Sample Prep

- 500 μ L Sample
- 4.5 mL DI Water
- 5.0 mL Internal Standard
 - t-Butanol
 - n-Propanol

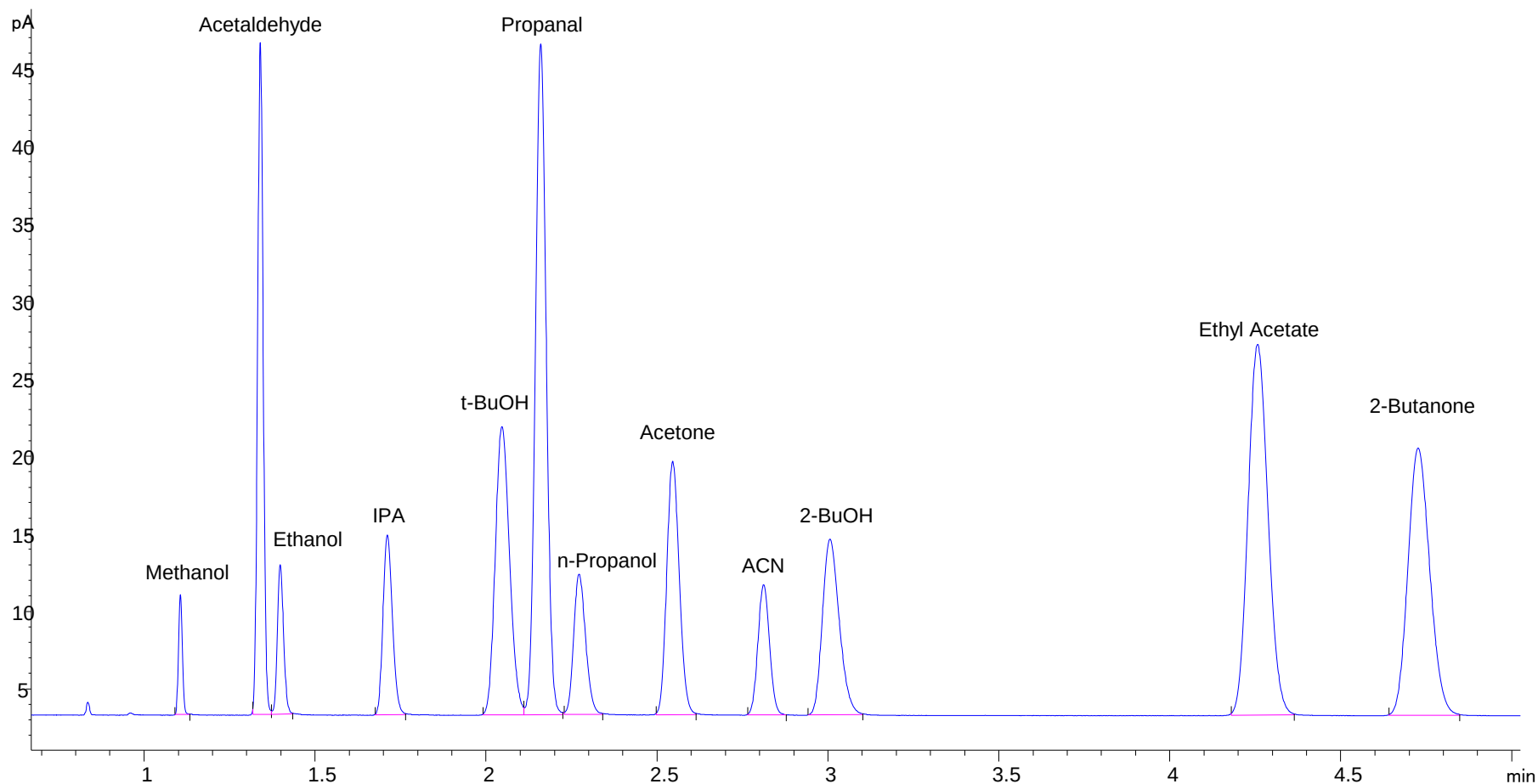


No	Compound
1	Methanol
2	Acetaldehyde
3	Ethanol
4	Isopropanol
5	t-Butanol
6	Propanal
7	n-Propanol
8	Acetone
9	Acetonitrile
10	2-Butanol
11	Ethyl Acetate
12	2-Butanone

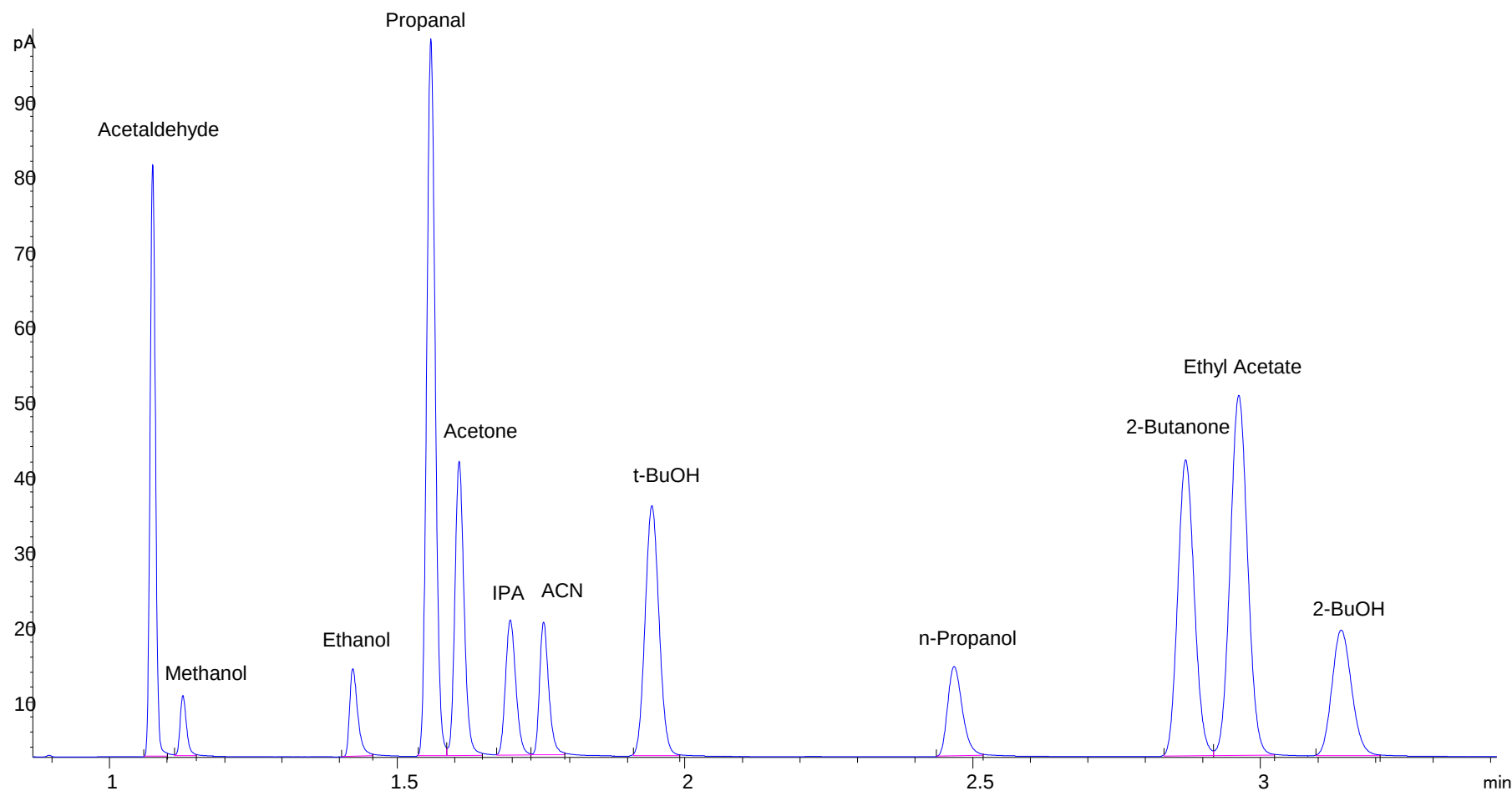
p/n 5190-9765



Complete Resolution of 12 component Mix on DB-BAC1 UI

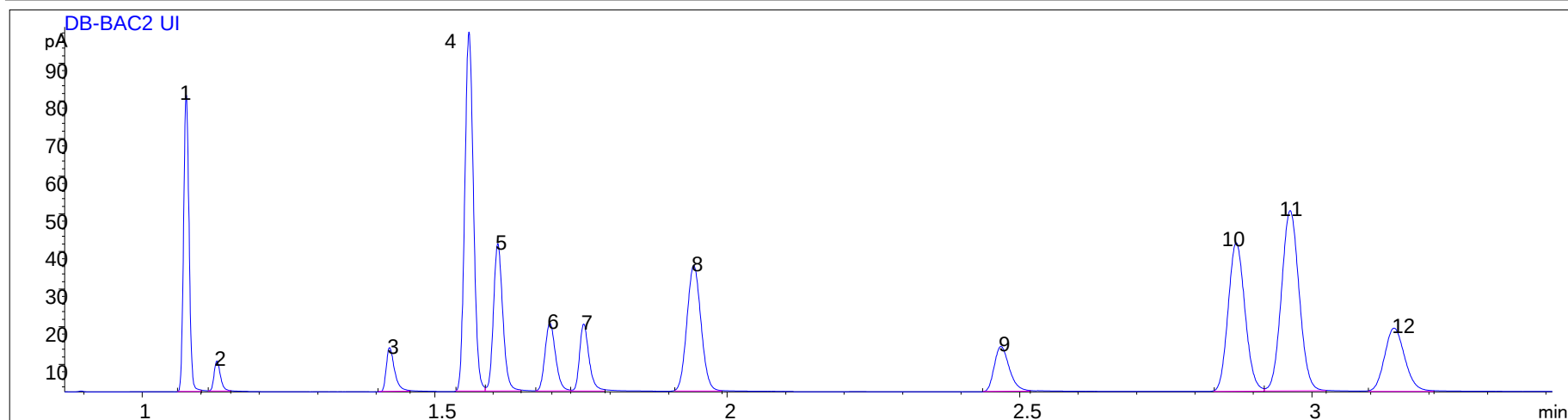
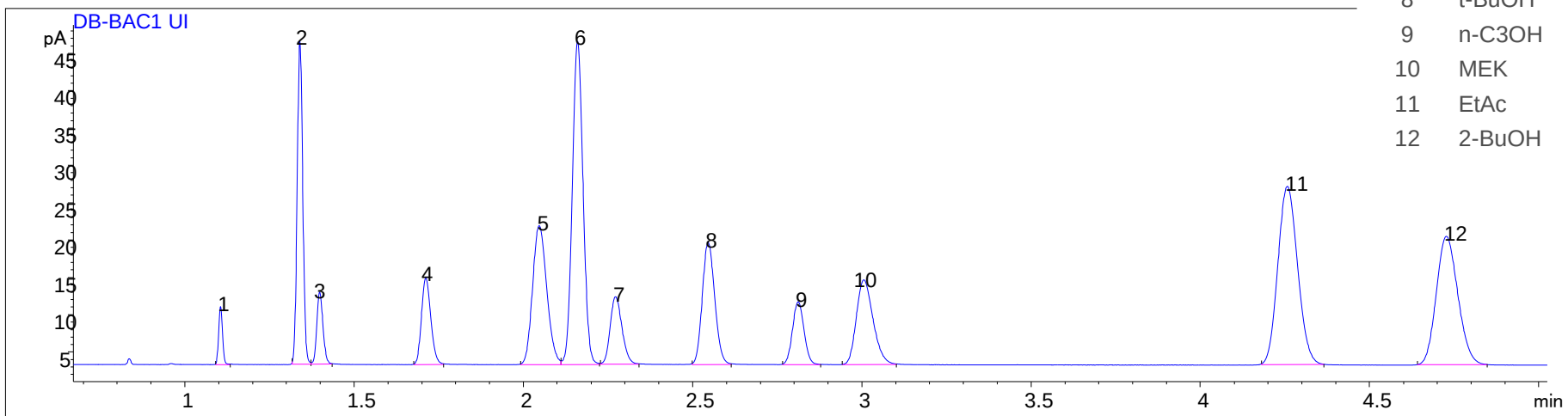


Complete Resolution of 12 component Mix on DB-BAC2 UI



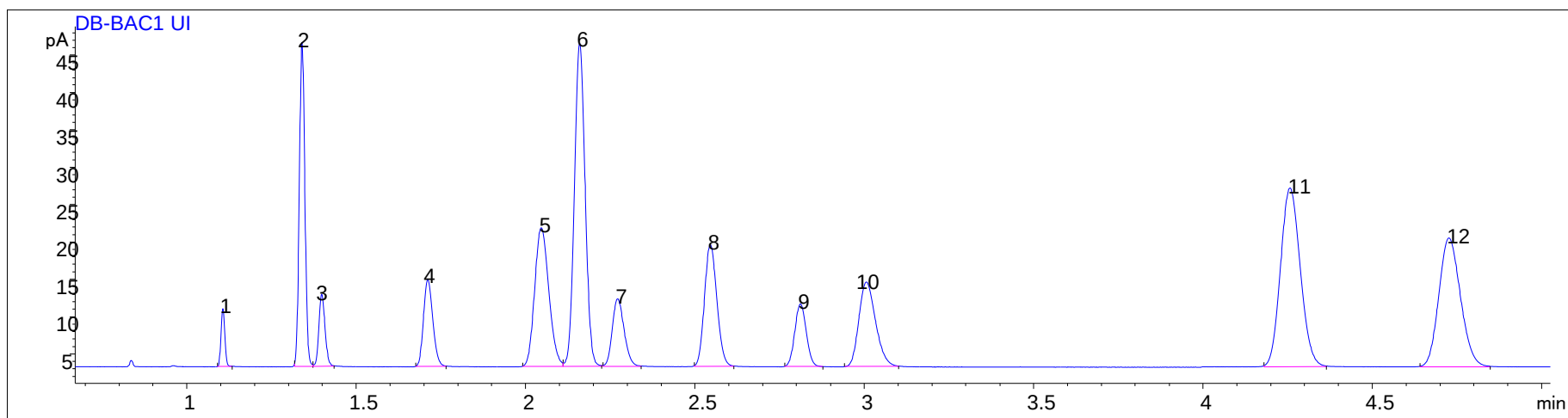
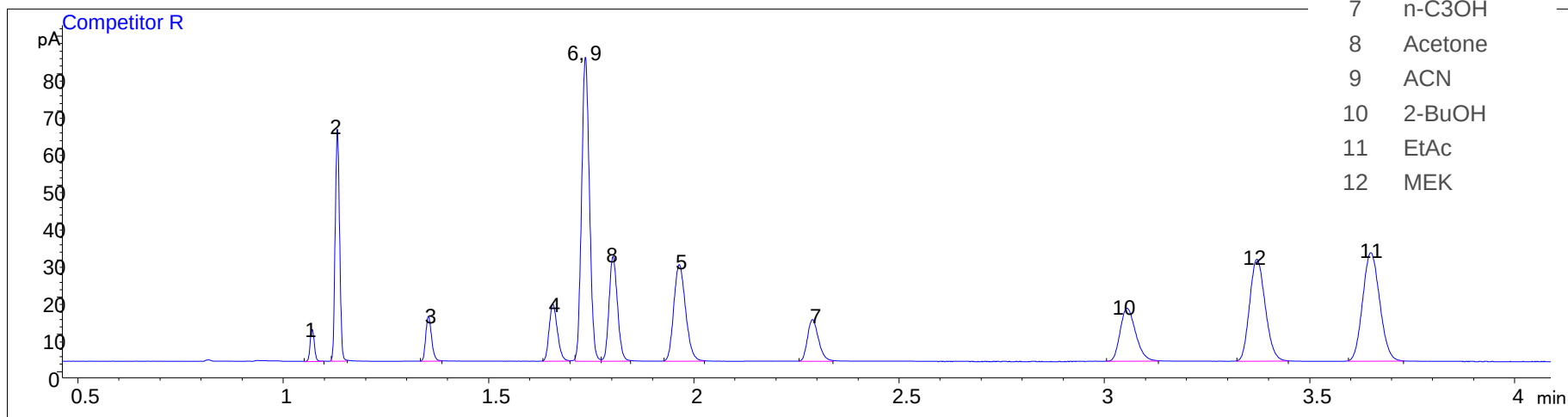
Improved Resolution of 12 Compounds on Both DB-BAC1UI and DB-BAC2 UI

- 1 Acetaldehyde
- 2 Methanol
- 3 EtOH
- 4 Propanal
- 5 Acetone
- 6 IPA
- 7 ACN
- 8 t-BuOH
- 9 n-C3OH
- 10 MEK
- 11 EtAc
- 12 2-BuOH



Comparison to competitor

- 1 Methanol
- 2 Acetaldehyde
- 3 EtOH
- 4 IPA
- 5 t-BuOH
- 6 Propanal
- 7 n-C3OH
- 8 Acetone
- 9 ACN
- 10 2-BuOH
- 11 EtAc
- 12 MEK



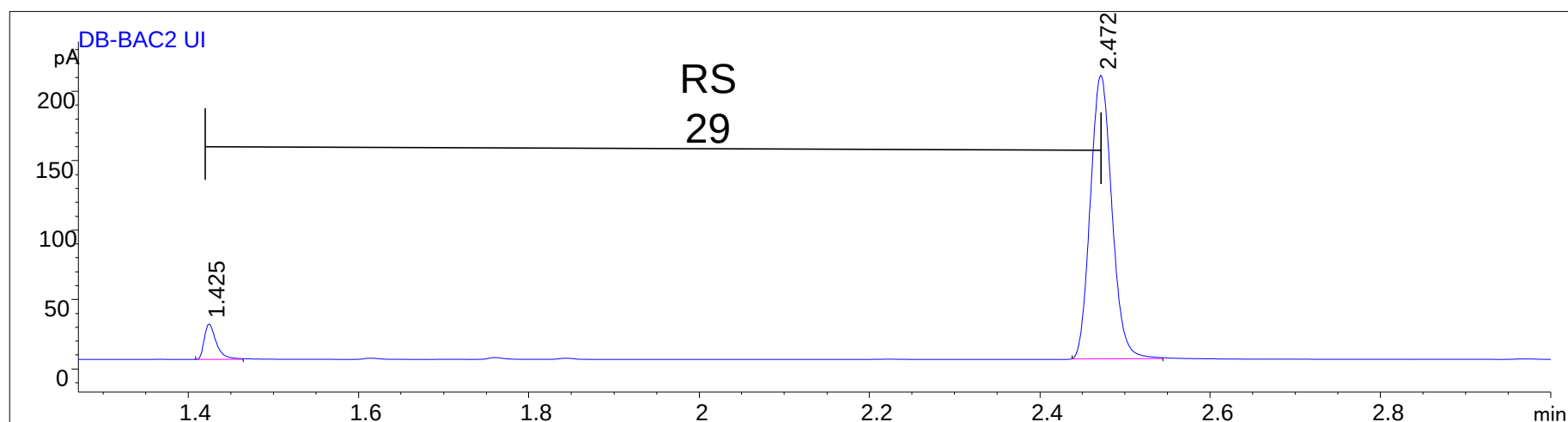
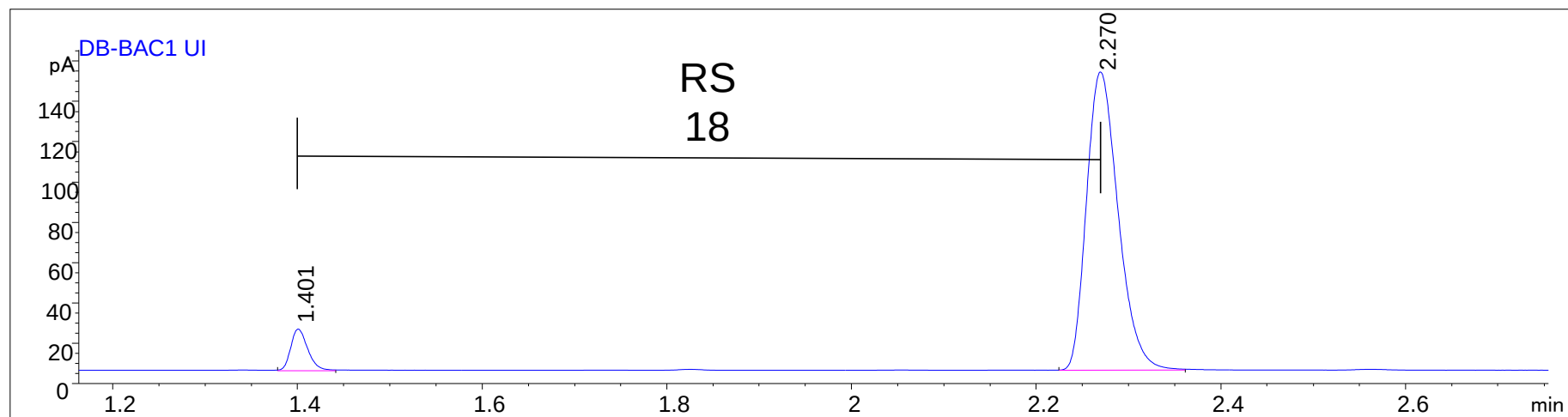
Calibration Standards

NIST and European Reference Materials (ERM) traceable calibration standards



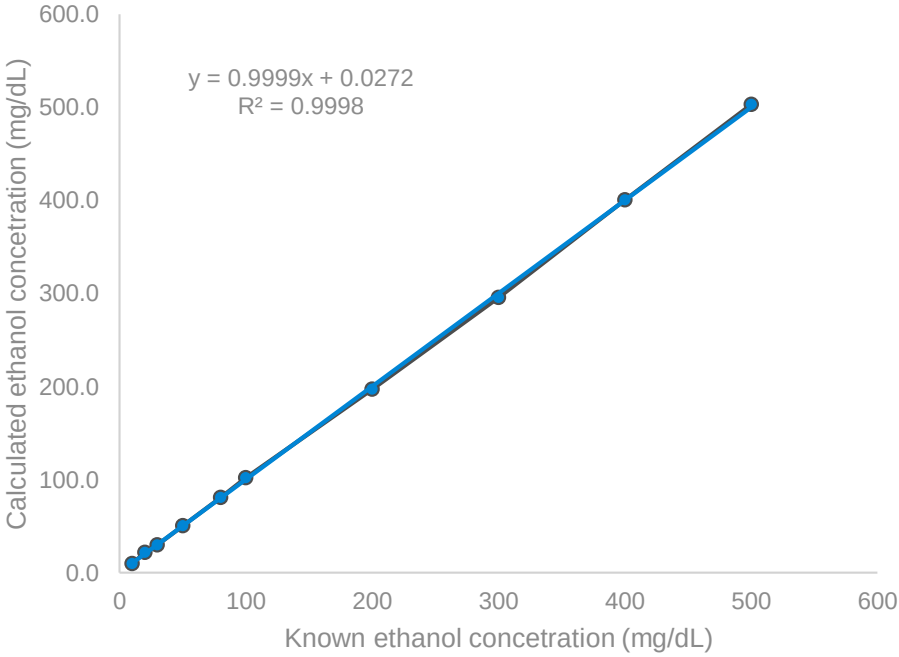
PN	Name	Description
5190-9756	Ethanol 20 mg/dL standard	Ethanol 20 mg/dL or 0.2 g/L, in water, (1mL x 10)
5190-9757	Ethanol 50 mg/dL standard	Ethanol 50 mg/dL or 0.5 g/L, in water, (1mL x 10)
5190-9758	Ethanol 80 mg/dL standard	Ethanol 80 mg/dL or 0.8 g/L, in water, (1mL x 10)
5190-9759	Ethanol 100 mg/dL standard	Ethanol 100 mg/dL or 1.0 g/L, in water, (1mL x 10)
5190-9760	Ethanol 150 mg/dL standard	Ethanol 150 mg/dL or 1.5 g/L, in water, (1mL x 10)
5190-9761	Ethanol 200 mg/dL standard	Ethanol 200 mg/dL or 2.0 g/L, in water, (1mL x 10)
5190-9762	Ethanol 300 mg/dL standard	Ethanol 300 mg/dL or 3.0 g/L, in water, (1mL x 10)
5190-9763	Ethanol 400 mg/dL standard	Ethanol 400 mg/dL or 4.0 g/L, in water, (1mL x 10)

80 mg/dL standard ethanol with n-propanol

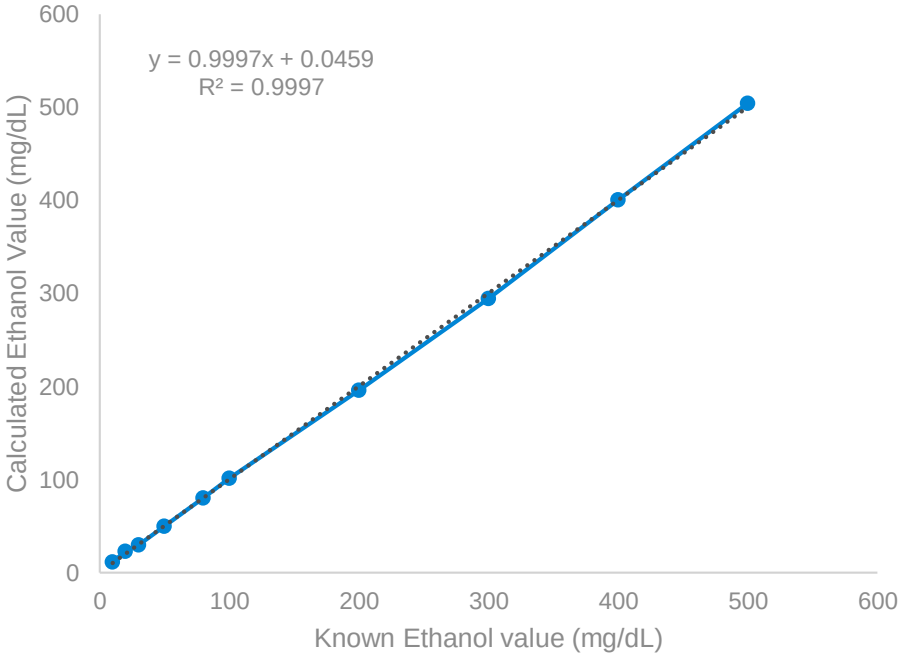


Ethanol Calibration with t-Butanol Internal Standard

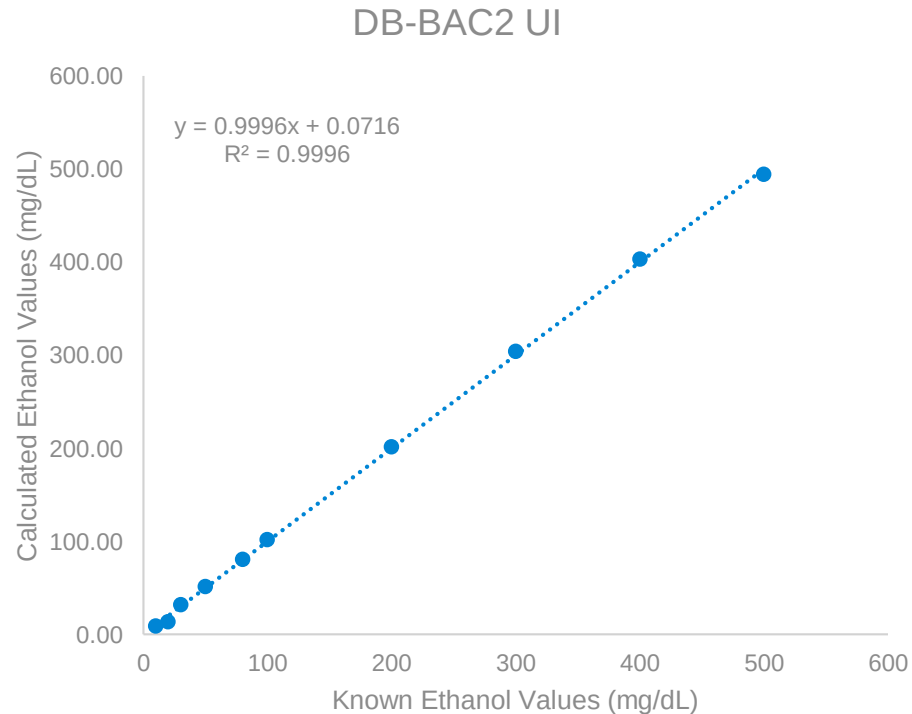
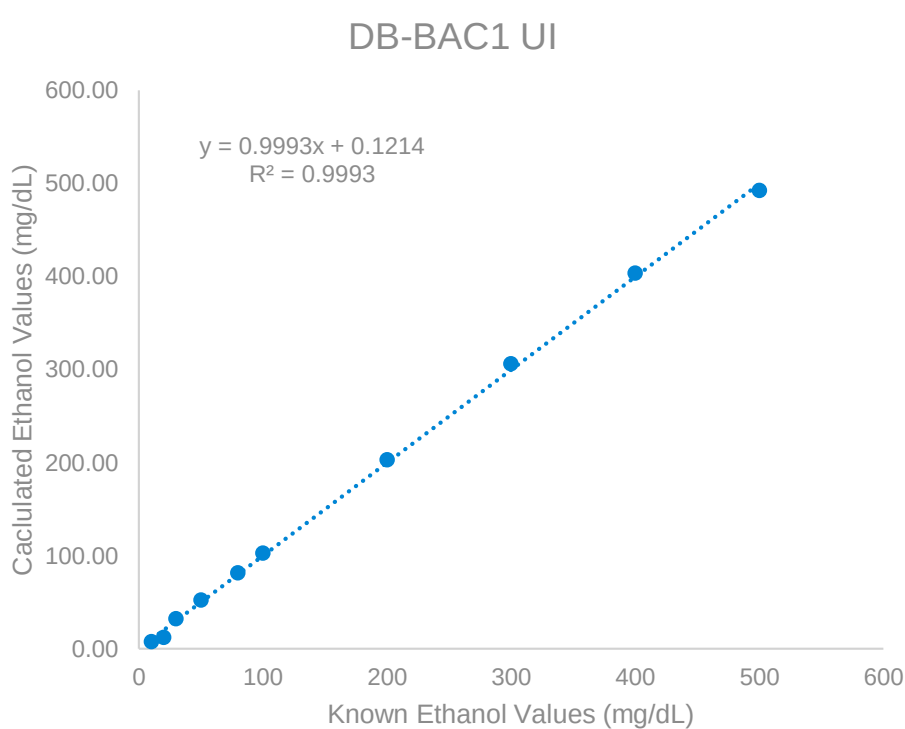
DB-BAC1 UI



DB-BAC2 UI

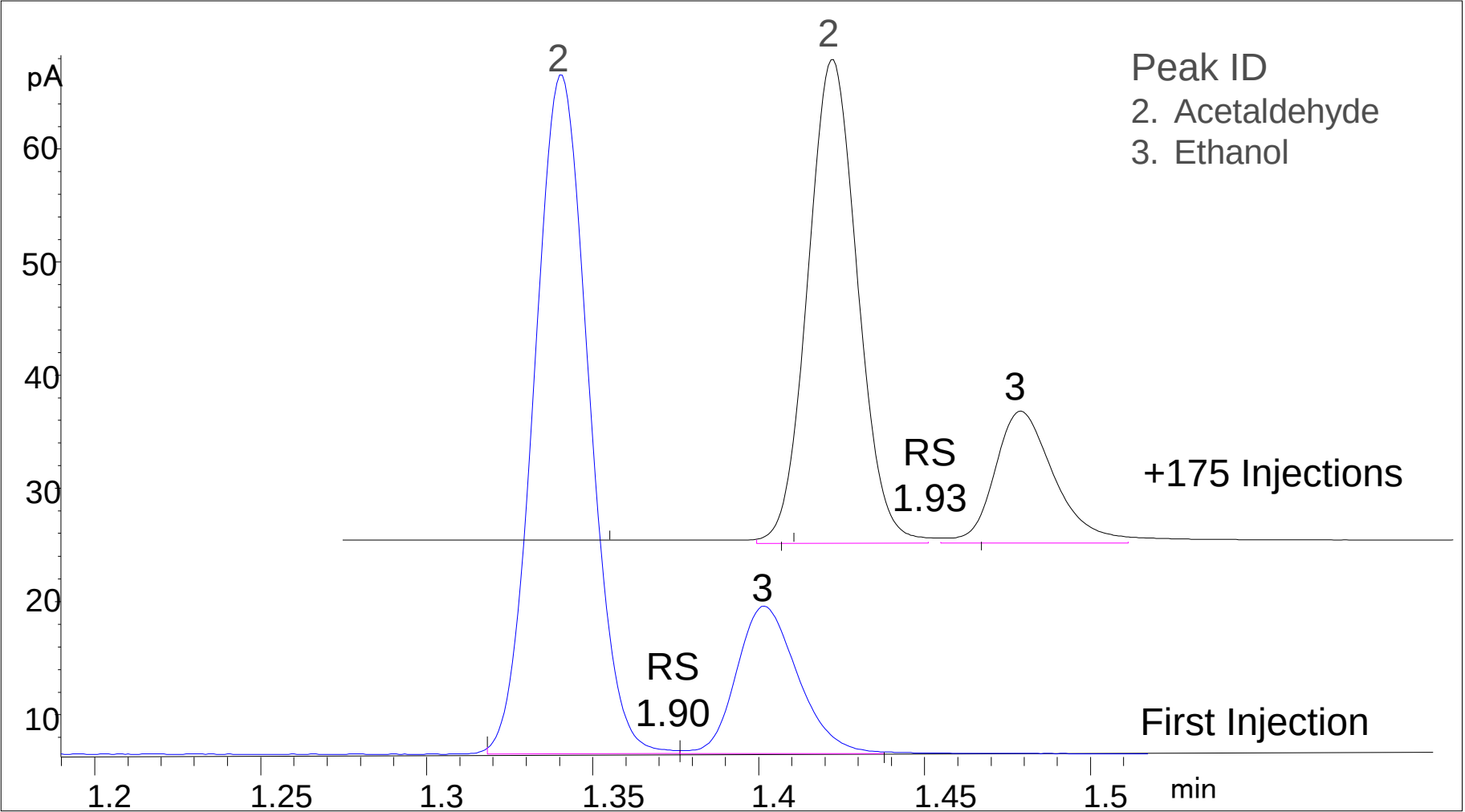


Ethanol Calibration with n-Propanol Internal Standard



Resolution of Acetaldehyde and Ethanol

DB-BAC1 UI



DB-BAC1 UI

10 Replicates

	Replicate Number										Average	%RSD
	1	2	3	4	5	6	7	8	9	10		
Methanol	1.107	1.107	1.107	1.107	1.107	1.107	1.107	1.107	1.107	1.107	1.107	0.000
Acetaldehyde	1.340	1.340	1.341	1.340	1.341	1.341	1.341	1.340	1.341	1.340	1.341	0.039
EtOH	1.402	1.402	1.403	1.402	1.403	1.403	1.403	1.403	1.403	1.403	1.403	0.034
IPA	1.717	1.718	1.718	1.718	1.719	1.719	1.719	1.719	1.719	1.718	1.718	0.041
t-BuOH	2.054	2.055	2.055	2.055	2.056	2.055	2.056	2.055	2.056	2.055	2.055	0.031
Propanal	2.162	2.162	2.162	2.162	2.162	2.162	2.162	2.162	2.162	2.162	2.162	0.000
n-C3OH	2.281	2.282	2.283	2.283	2.284	2.284	2.285	2.284	2.285	2.284	2.284	0.056
Acetone	2.551	2.552	2.552	2.552	2.552	2.552	2.552	2.552	2.552	2.551	2.552	0.017
ACN	2.815	2.815	2.815	2.815	2.815	2.815	2.815	2.815	2.815	2.814	2.815	0.011
2-BuOH	3.019	3.020	3.021	3.021	3.023	3.023	3.024	3.023	3.024	3.023	3.022	0.057
EtAc	4.267	4.267	4.267	4.267	4.268	4.268	4.268	4.268	4.268	4.267	4.268	0.012
2-Butanone	4.739	4.739	4.740	4.740	4.740	4.740	4.740	4.739	4.740	4.739	4.740	0.011



DB-BAC2 UI

10 Replicates

	Replicate Number										Average	%RSD
	1	2	3	4	5	6	7	8	9	10		
Acetaldehyde	1.075	1.075	1.075	1.075	1.075	1.075	1.075	1.075	1.075	1.075	1.075	0.000
Methanol	1.129	1.130	1.130	1.130	1.130	1.130	1.130	1.130	1.130	1.130	1.130	0.028
EtOH	1.426	1.427	1.427	1.426	1.428	1.427	1.428	1.427	1.428	1.426	1.427	0.057
Propanal	1.559	1.559	1.559	1.559	1.559	1.559	1.559	1.558	1.559	1.558	1.559	0.027
Acetone	1.609	1.609	1.609	1.609	1.609	1.609	1.609	1.609	1.609	1.609	1.609	0.000
IPA	1.700	1.701	1.701	1.701	1.702	1.701	1.702	1.701	1.702	1.700	1.701	0.043
ACN	1.755	1.755	1.755	1.755	1.756	1.755	1.756	1.755	1.756	1.755	1.755	0.028
t-BuOH	1.948	1.948	1.948	1.948	1.949	1.948	1.949	1.948	1.949	1.948	1.948	0.025
n-C3OH	2.475	2.475	2.476	2.475	2.476	2.476	2.476	2.475	2.477	2.475	2.476	0.028
MEK	2.873	2.873	2.873	2.873	2.873	2.873	2.873	2.873	2.873	2.872	2.873	0.011
EtAc	2.965	2.965	2.965	2.965	2.965	2.965	2.965	2.964	2.965	2.964	2.965	0.014
2-BuOH	3.150	3.150	3.150	3.150	3.151	3.150	3.151	3.150	3.151	3.149	3.150	0.020

Conclusions

- Improved Resolution
- Calibrate with either n-Propanol or t-Butanol
- Resolution between acetaldehyde and ethanol still greater than 1.5 after over 175 injections
- Retention Times stable over many injections

