Agilent GC-MS:

Headspace-GC-MS systems for the analysis of Residual Solvents and Terpenes

Ronald Honnold, Ph.D.

Robert Kubas

Agilent GC-MS Applications Scientists

Agilent products and solutions are intended to be used for cannabis quality control and safety testing in laboratories where such use is permitted under state/country law.
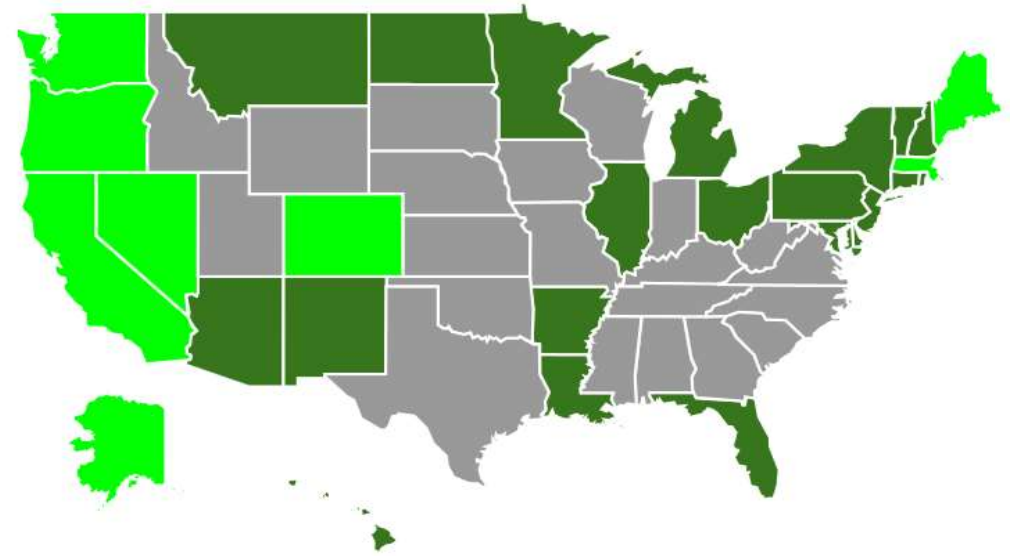
Agilent Technologies

STATES WHERE MARIJUANA IS LEGALIZED

■ Legalized marijuana ■ Legalized medical marijuana ● Legislation passed Nov. 2016
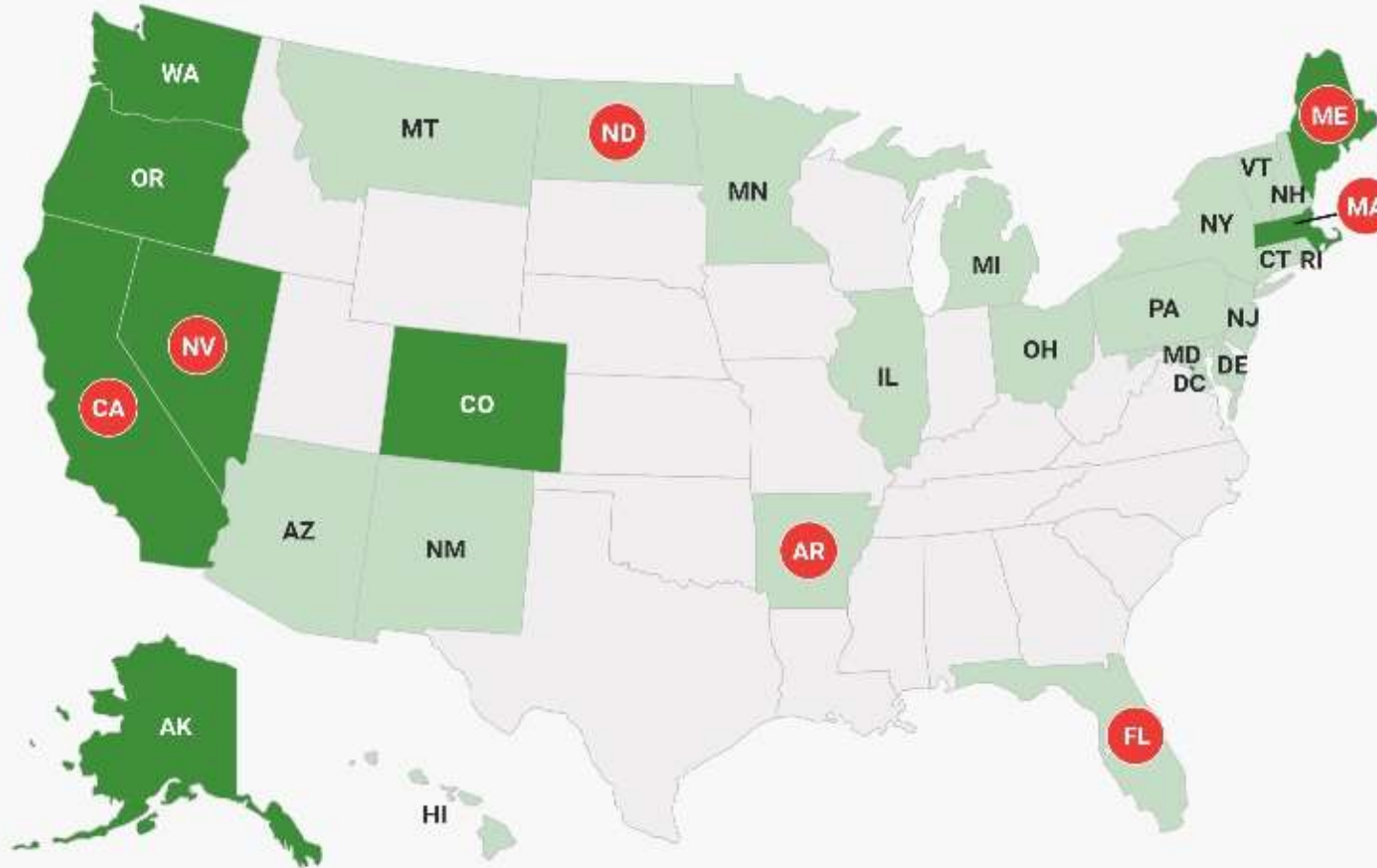
SOURCES: Politico; Reuters

http://www.businessinsider.com/where-is-marijuana-legal-2016-11

Agilent Technologies

Marijuana Legalization Status
- Medical marijuana broadly legalized
- Marijuana legalized for recreational use
- No broad laws legalizing marijuana

Mexico legalized medicinal marijuana in June, 2017
Canada expected to fully legalize in 2017

Agilent Technologies

Testing

- All methods should be consistent with FDA, ELAP, USP, and EPA guidelines.

- Unfortunately, all states are not yet uniform in testing requirements.

- It's not enough to know that the product has been tested; the science behind it must be reliable.

- For analytical testing to be reliable, established methods must be used and available for scientific scrutiny.

- Detailed testing procedures should be available.

Analytical Challenges

Matrix

    Raw plant material

    Extracts

    Food

        Edibles

Analysis needs

- ## Residual Solvents
  - Headspace GC-FID
  - Headspace GC-MS

- ## Terpenes
  - Headspace GC-FID
  - Headspace GC-MS
  - Liquid injection if using a portion of the Potency Test extract

  - While one instrument can be used, current sample loads dictate using an optimized dedicated system for Residual Solvents and one for Terpene analysis.

Residual Solvent and Terpene Analyses

Residual Solvents

- Solvents used in extraction of cannabinoids
- No health-based residual solvent limits have been established for cannabis
- States using various models:
    - USP <467> Residual Solvents
    - ICH Residual Solvents
    - Commission of the European Communities, Scientific Committee on Food

Terpenes

- Provide the unique bouquet or aroma
- Relationship between terpenes and cannabinoids determine strain
- Debate as to effect / importance
- Agilent GC-FID or Agilent GC-MS

Applying the Agilent 5977A MSD to the Analysis of USP<467> Residual Solvents with the 7697A Headspace Sampler and 7890B GC
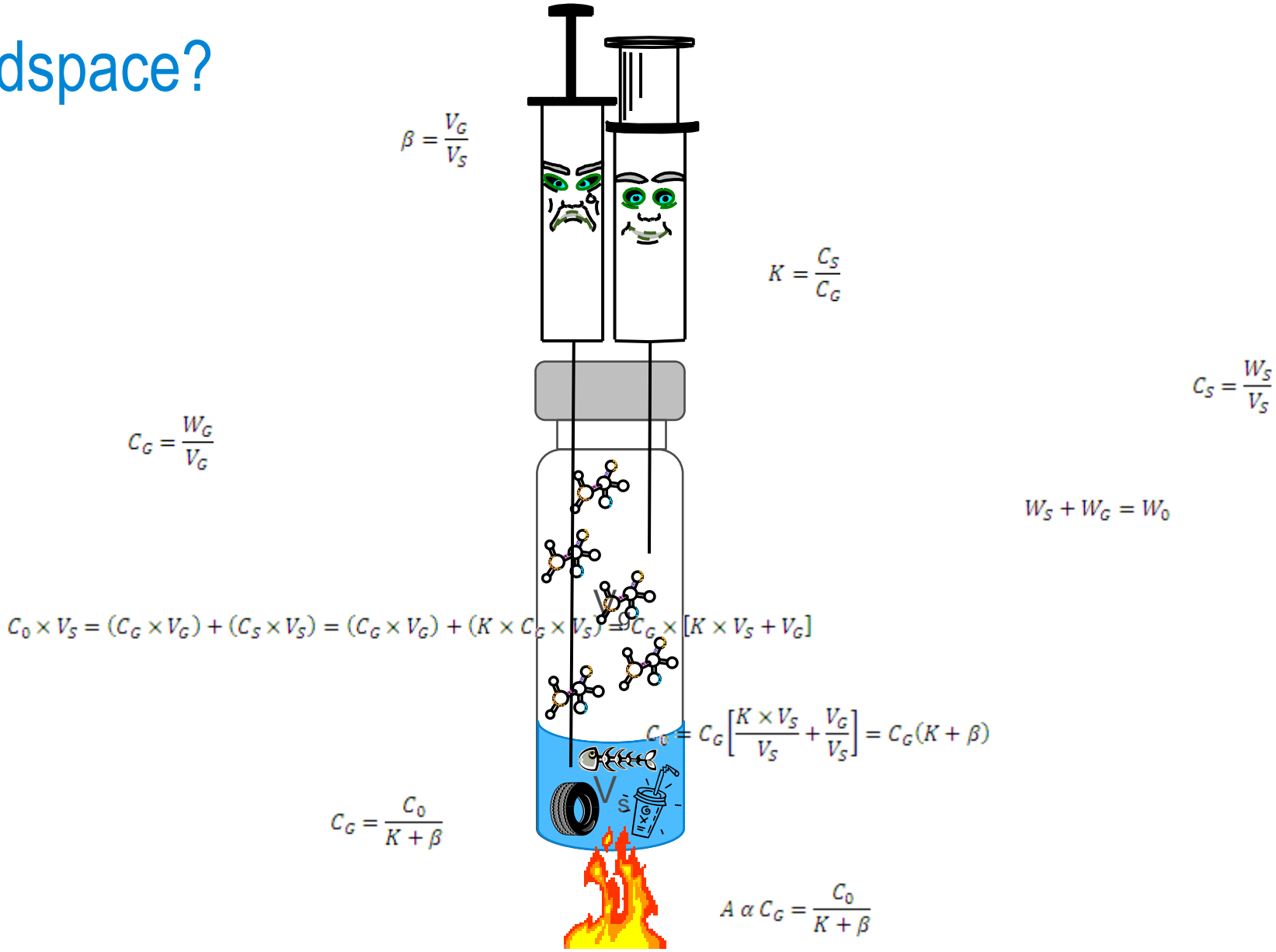
Application Note

Pharmaceuticals

Authors

Roger L. Firor and Mike Szelewski
Agilent Technologies, Inc.
2850 Centerville Rd
Wilmington, DE 19808-1610
USA

Abstract

The Agilent 7697A Headspace Sampler coupled to an Agilent 5977 Series GC/MSD System was used for the analysis of USP <467> residual solvents at their limit concentration in aqueous solution according to procedure A of the method. Scan data give repeatability generally better than 2.5% RSD for Class 1, Class 2A, and Class 2B solvents. The Multi Mode inlet (MMI) with an Ultra Inert 1 mm id liner was used for sample introduction from the fused silica headspace transfer line. Several liners were investigated including Atune and Etune for data acquisition with source and quadrupole temperatures of 250 °C and 200 °C, respectively. MassHunter Quant software was used for data analysis.

Agilent Technologies

What Is Headspace?

$$\beta = \frac{V_G}{V_S}$$

$$K = \frac{C_S}{C_G}$$

$$C_S = \frac{W_S}{V_S}$$

$$C_G = \frac{W_G}{V_G}$$

$$W_S + W_G = W_0$$

$$C_0 \times V_S = (C_G \times V_G) + (C_S \times V_S) = (C_G \times V_G) + (K \times C_G \times V_S) = C_G \times [K \times V_S + V_G]$$

$$C_0 = C_G \left[\frac{K \times V_S}{V_S} + \frac{V_G}{V_S} \right] = C_G (K + \beta)$$

$$C_G = \frac{C_0}{K + \beta}$$

$$A \, \alpha \, C_G = \frac{C_0}{K + \beta}$$

Headspace…How To?

1. **User - Place an accurate amount of sample into the vial and seal it with a crimp cap.**

2. **Instrument - Heat the vial for a fixed time to reach equilibrium.**

3. **Instrument - Deliver a reproducible volume of the headspace gas to the GC.**

4. **User - Assume peak area to be proportional to original concentration.**

7697A – *Productivity* Features

Three racks of 36 vials	*Plus* Three priority positions	12 vial oven	Bar code reader

111 SAMPLE POSITIONS!

Agilent Technologies

7697A – *Performance* Features

Advanced Sampling Design

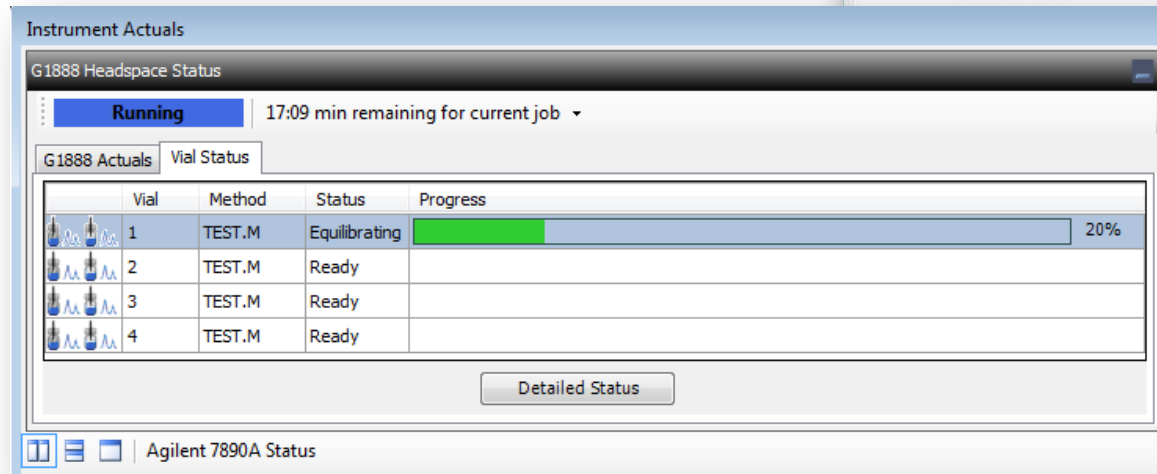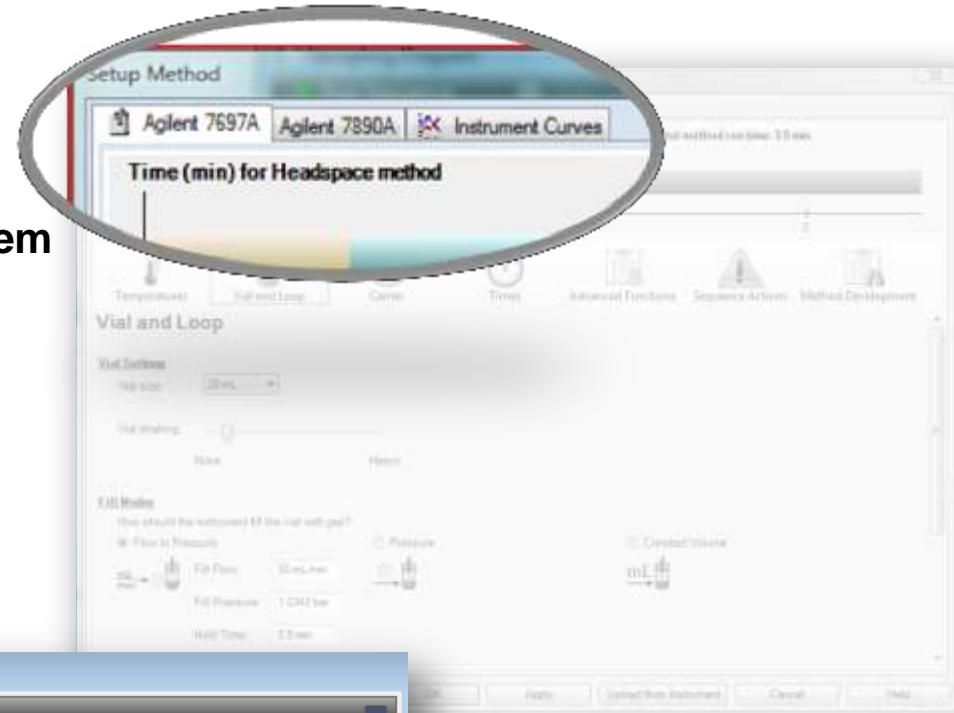**On-board electronic pneumatic control (EPC)**

Vial integrity check (leak test)
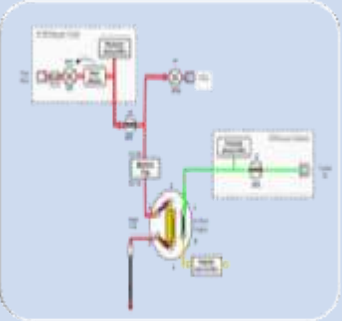
Automatic flow-path cleaning
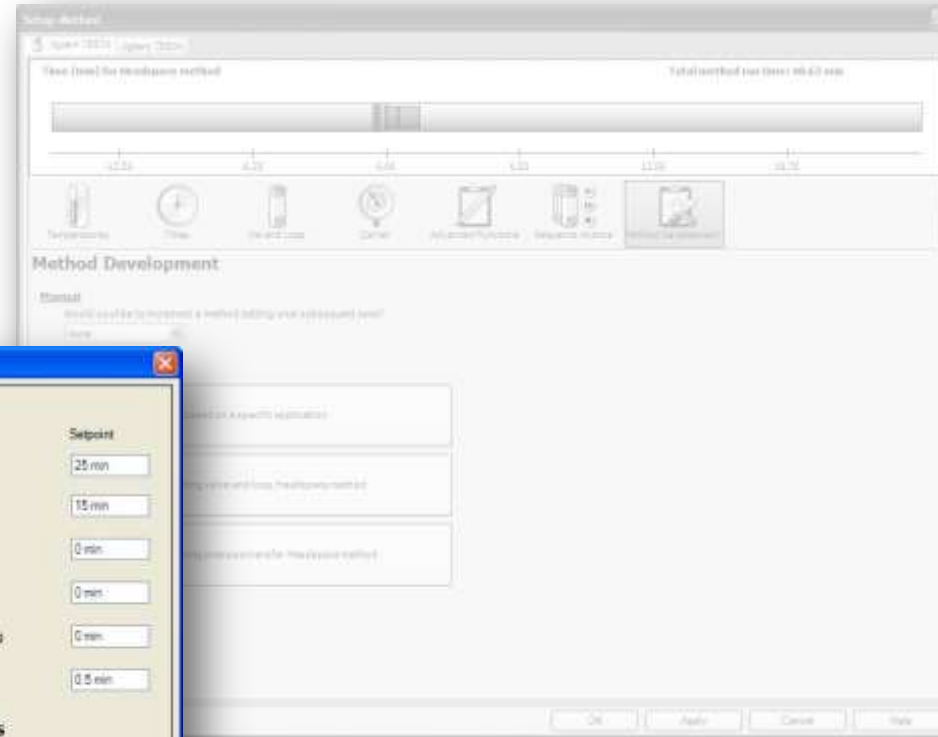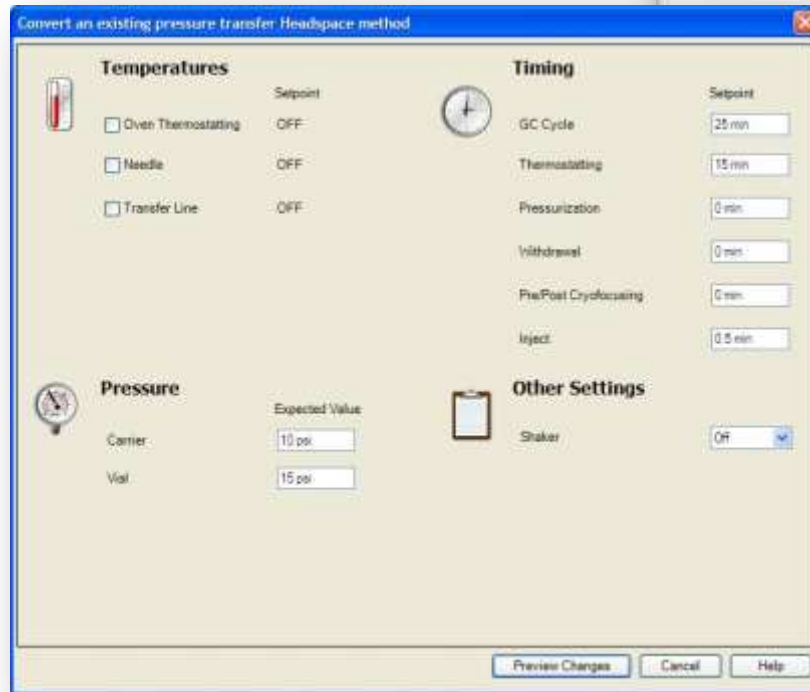
7697A – *New* Control Software

- **Fully integrated software**
  - Just another instrument in the system

- **Operates nearly like a 7693 ALS**
  - Sequences are sequences

- **Status-At-A-Glance**

7697A – *New* Control Software

Method Development Tools
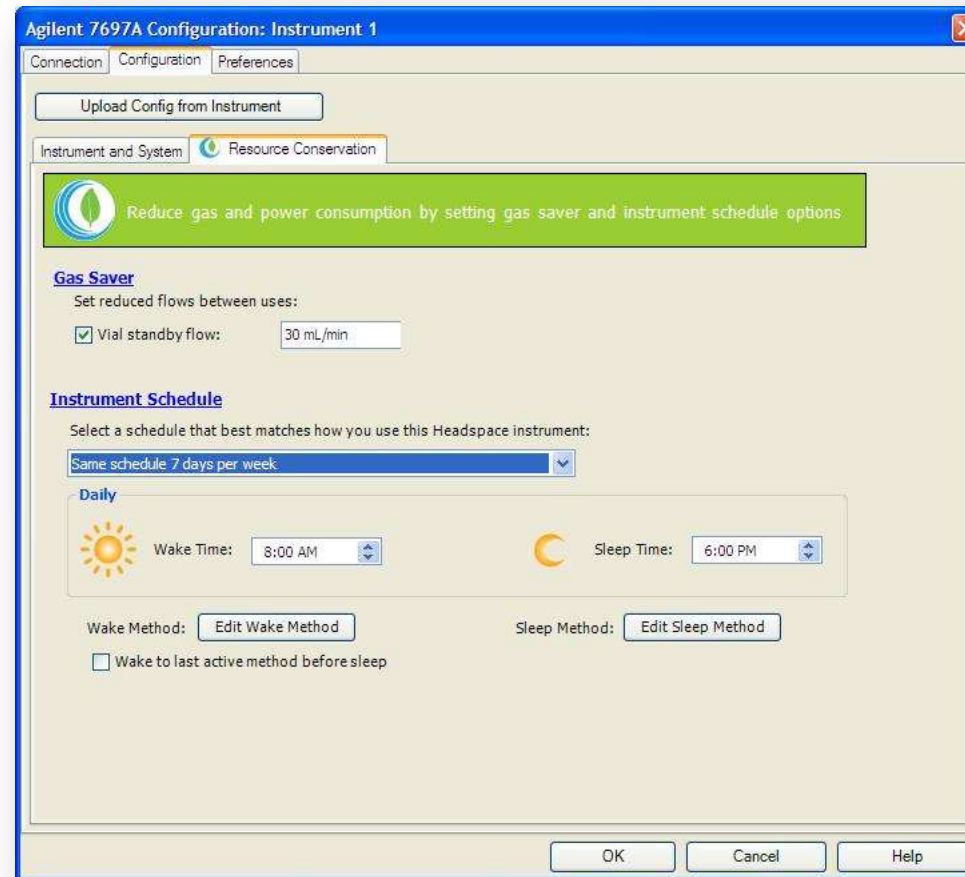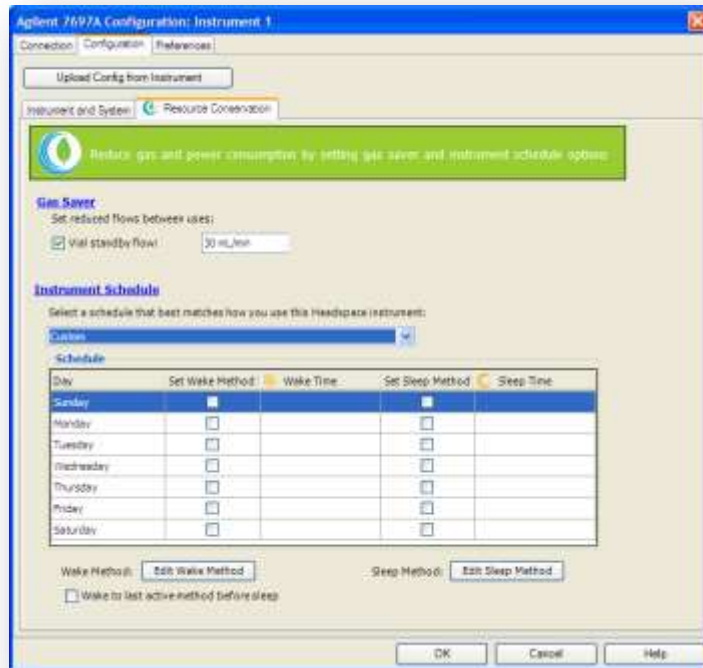
- **Builder**
- **Converter**

7697A – EcoBranding

Resource Conservation

- **Empowers the user to control their "greeness"**

Agilent Technologies

The Agilent 7697A Headspace Sampler

Three 36 position trays with 3 priority slots

Integrated bar code reader

12 Vial oven allows for easy prep ahead

Controlled venting

No O-rings to replace

AGILENT 7693A AUTOMATIC LIQUID SAMPLER

Agilent Technologies

Residual Solvent Configuration

The extraction of cannabis to produce materials for use in oils, edibles, and other products often utilizes solvents such as butane, propane, isopropanol, or acetone.

These solvents are harmful to health, so they should be absent from the final product.

1. Headspace-GC-Split between FID and MS, column of choice is the DB-624
   a) Headspace GC is typically used and detection is performed with MS and FID.
   b) Because solvents are volatile, HS-GC-FID/MS can be used for residual solvent analysis.

Agilent Technologies
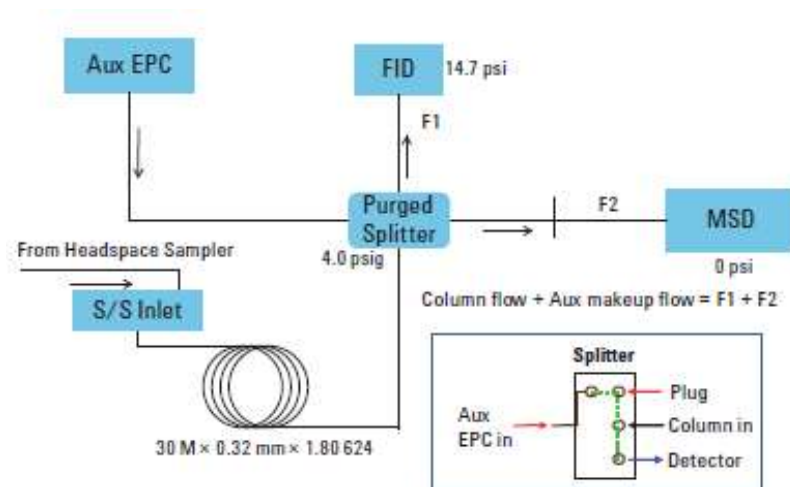
Cannabinoid Extraction Methods

Dry Sieve

This is a natural method of extracting compounds by sifting trim and shake through a fine-weave screen filter. This solvent-free process does not always produce the cleanest or most effective results.

Water

This method uses ice, screens of different micron sizes, and agitation to remove trichomes. While slightly more effective than dry sieve, there is a danger of mold growth if the extract is not dried thoroughly.

Carbon Dioxide

This is a very effective, although expensive, extraction method that does not use solvents and creates a clean and pure product.

Isopropyl Alcohol

Due to the ability to dissolve waxes, Isopropyl alcohol is best used as a quick wash to increase the purity of the cannabis extract.

Ethanol

The two-wash Quick Wash Ethanol, or QWET, process is probably the most common and easily executed extraction process. The first wash can extract 75 to 80% of the oil, the second extracts most of the balance.
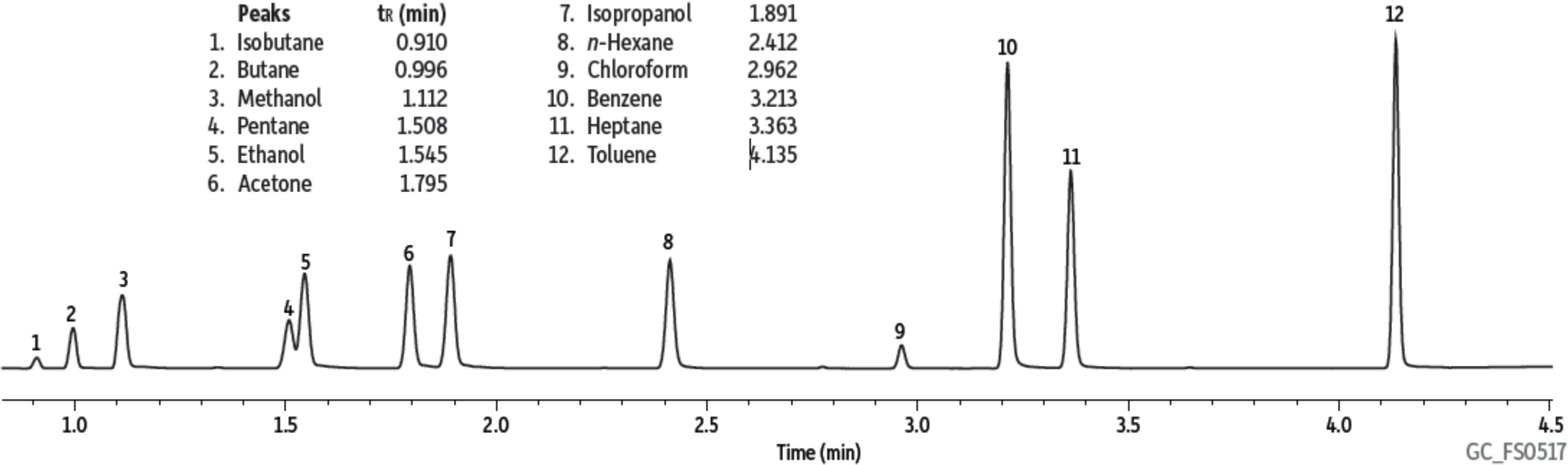
Butane/Propane

When performed safely in a laboratory, butane and propane extractions are inexpensive and create clean, potent products. Either solvent is easily purged.

Hexane

Although an effective solvent, hexane is more toxic than butane, and is insoluble in water, extremely flammable, and potentially explosive.

Agilent Technologies

Residual solvents by Restek using Headspace and Agilent GC-FID

Peaks	t_R (min)			
1. Isobutane	0.910	7. Isopropanol	1.891
2. Butane	0.996	8. *n*-Hexane	2.412
3. Methanol	1.112	9. Chloroform	2.962
4. Pentane	1.508	10. Benzene	3.213
5. Ethanol	1.545	11. Heptane	3.363
6. Acetone	1.795	12. Toluene	4.135

GC_FS0517

Restek.com. GC Columns: Fused Silica Guard/Retention Gap Columns. Retrieved January 23, 2017 from:
http://lab-comp.hu/wp-content/uploads/2015/06/Restek_GC_Fused_Silica_Capillary_Columns.pdf.

Agilent Technologies

Agilent SIM Acquisition setup Class 2A&B Residual Solvents, Scan is used for the Class 1 Residual Solvents

Class 2A

Group	Compound	SIM ions
1	Methanol	31,29
2	Acetonitrile	39,41,84,86,96,98
	Dichloromethane	
	trans-1,2-Dicloroethene	
	cis-1,2-Dichloroethene	
3	Tetrahyrofuran	56,71,72,84,96,98
	Cyclohexene	
	Methylcyclohexane	
4	1,4-Dioxane	58,83,88,98
5	Toluene	91,92
6	Chlorobenzene	91,106,112,114|
	Ethylbenzene	
	m-Xylene, p-Xylene o-Xylene	

Class 2B

Group	Compound	SIM ions
1	Hexane	56,57
2	Nitromethane|	46,61
3	Chloroform	83,85
4	Dimethoxyethane	45,60
5	Trichloroethene	130,132
6	Pyridine, 2-Hexanone	52,58,79,85
7	Tetralin	104,132

Coelutions can occur on the 624 phase when all three classes of solvents are considered. Using the MSD in SIM overcomes this problem when using just the 624 phase.
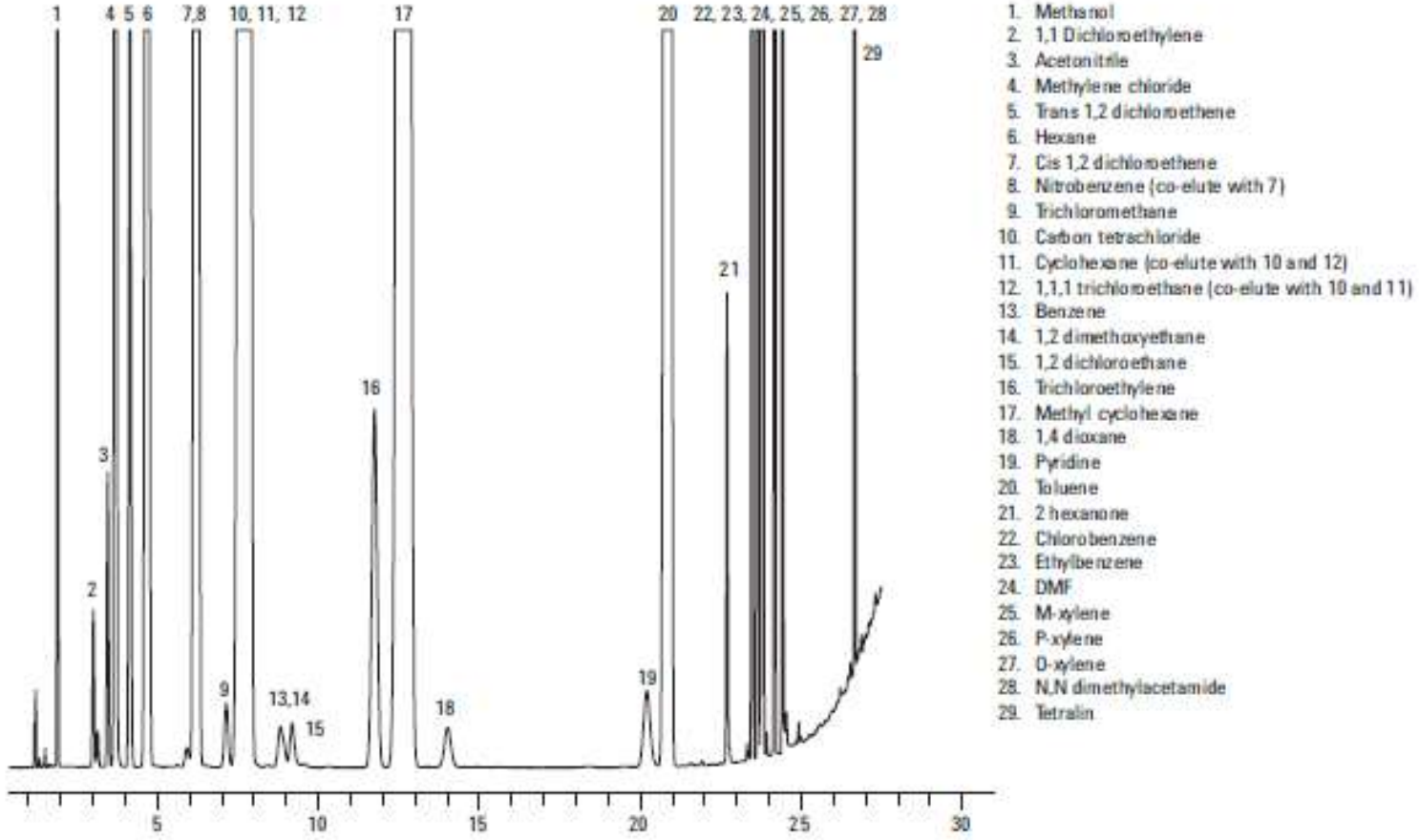
Agilent Technologies

Agilent example of residual solvents analysis

Figure 1. Class 1 and Class 2 residual solvents.

1. Methanol
2. 1,1 Dichloroethylene
3. Acetonitrile
4. Methylene chloride
5. Trans 1,2 dichloroethene
6. Hexane
7. Cis 1,2 dichloroethene
8. Nitrobenzene (co-elute with 7)
9. Trichloromethane
10. Carbon tetrachloride
11. Cyclohexane (co-elute with 10 and 12)
12. 1,1,1 trichloroethane (co-elute with 10 and 11)
13. Benzene
14. 1,2 dimethoxyethane
15. 1,2 dichloroethane
16. Trichloroethylene
17. Methyl cyclohexane
18. 1,4 dioxane
19. Pyridine
20. Toluene
21. 2 hexanone
22. Chlorobenzene
23. Ethylbenzene
24. DMF
25. M-xylene
26. P-xylene
27. O-xylene
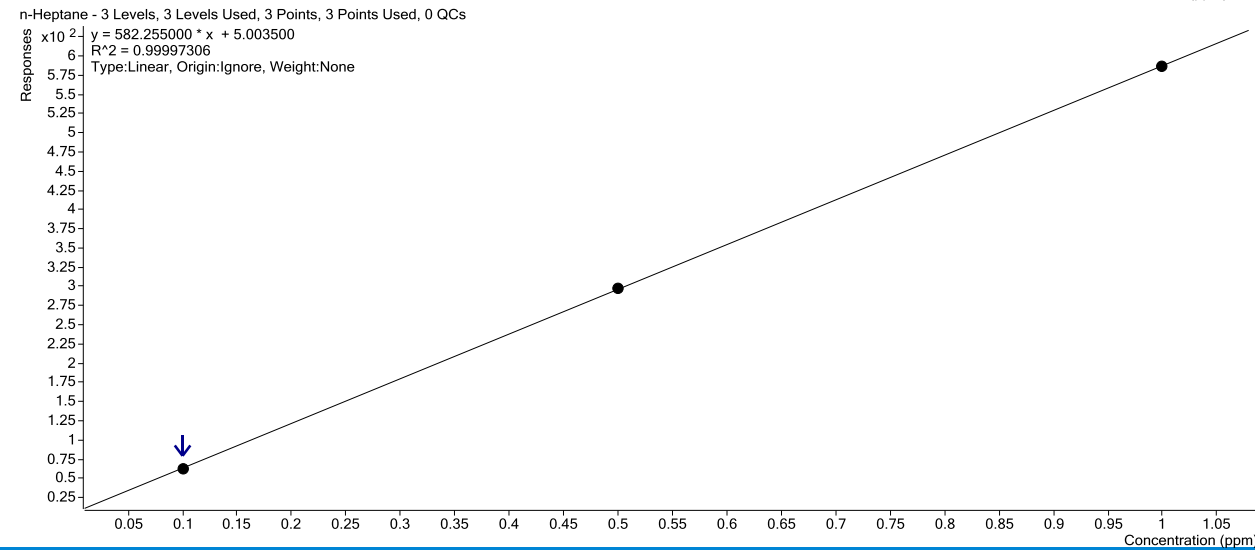28. N,N dimethylacetamide
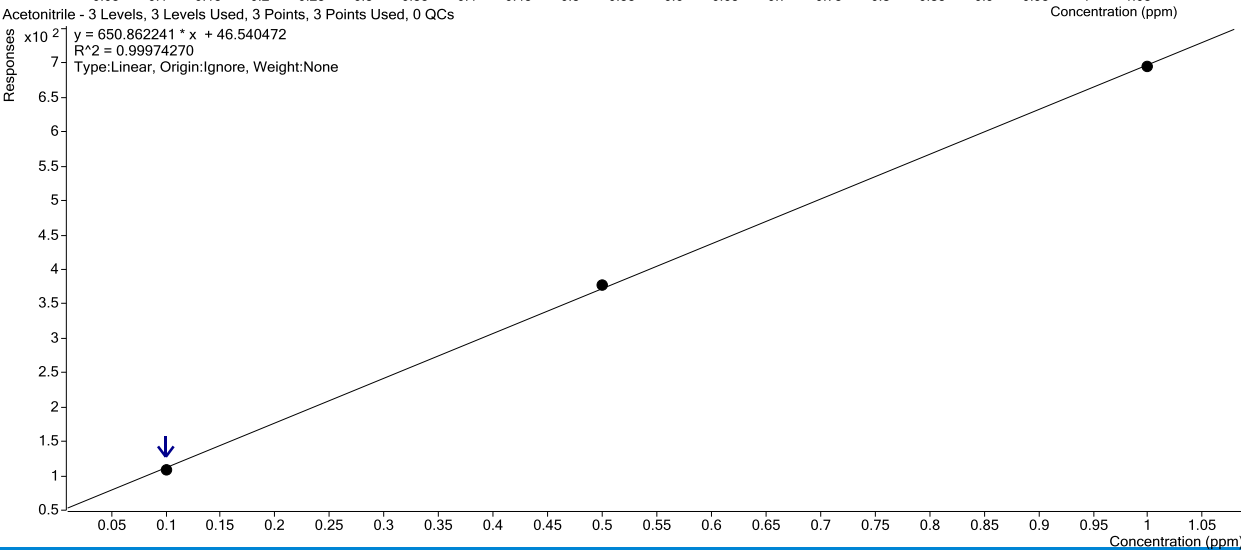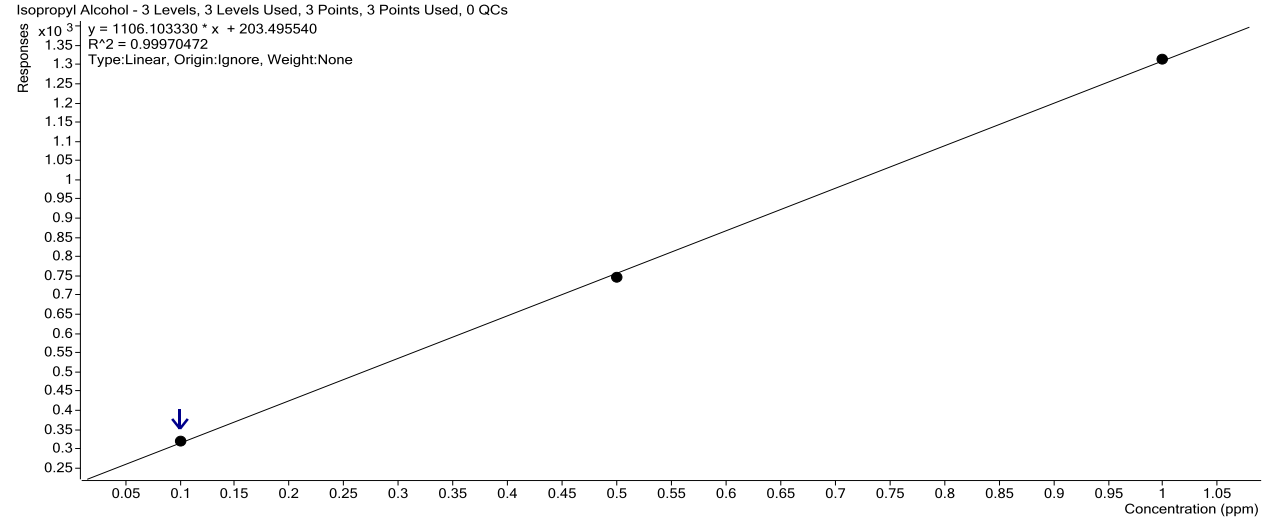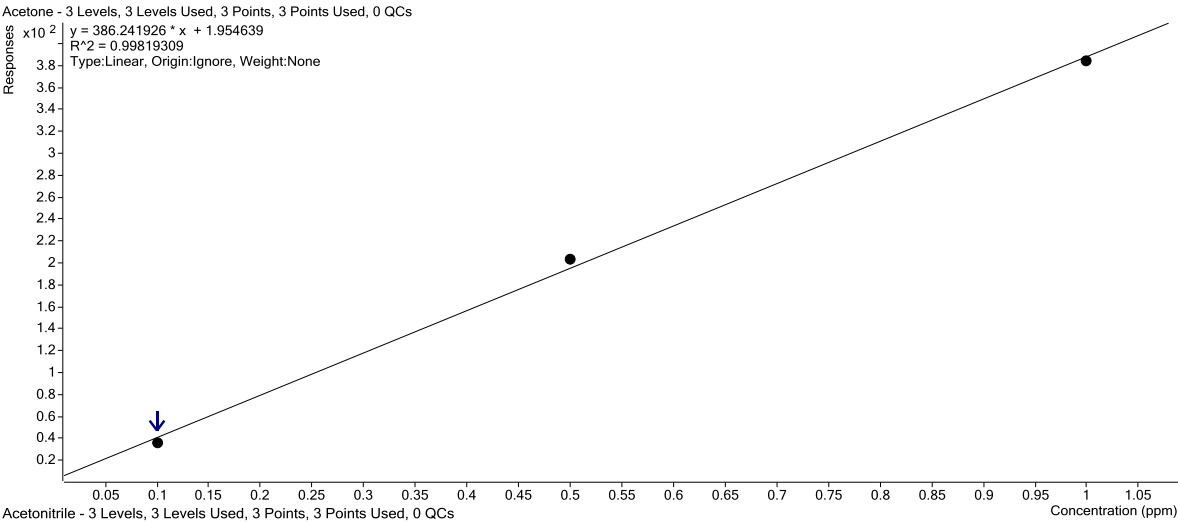29. Tetralin

Agilent Technologies

Some labs prefer the MS as the detector of choice

- Mass spectrometry has the benefit of specificity
  - MS for residual solvents adds confidence to compound identification
  - Somewhat limited in linear dynamic range when going into high percent values

- FID is linear over a wider concentration range but does not have the specificity that MS offers
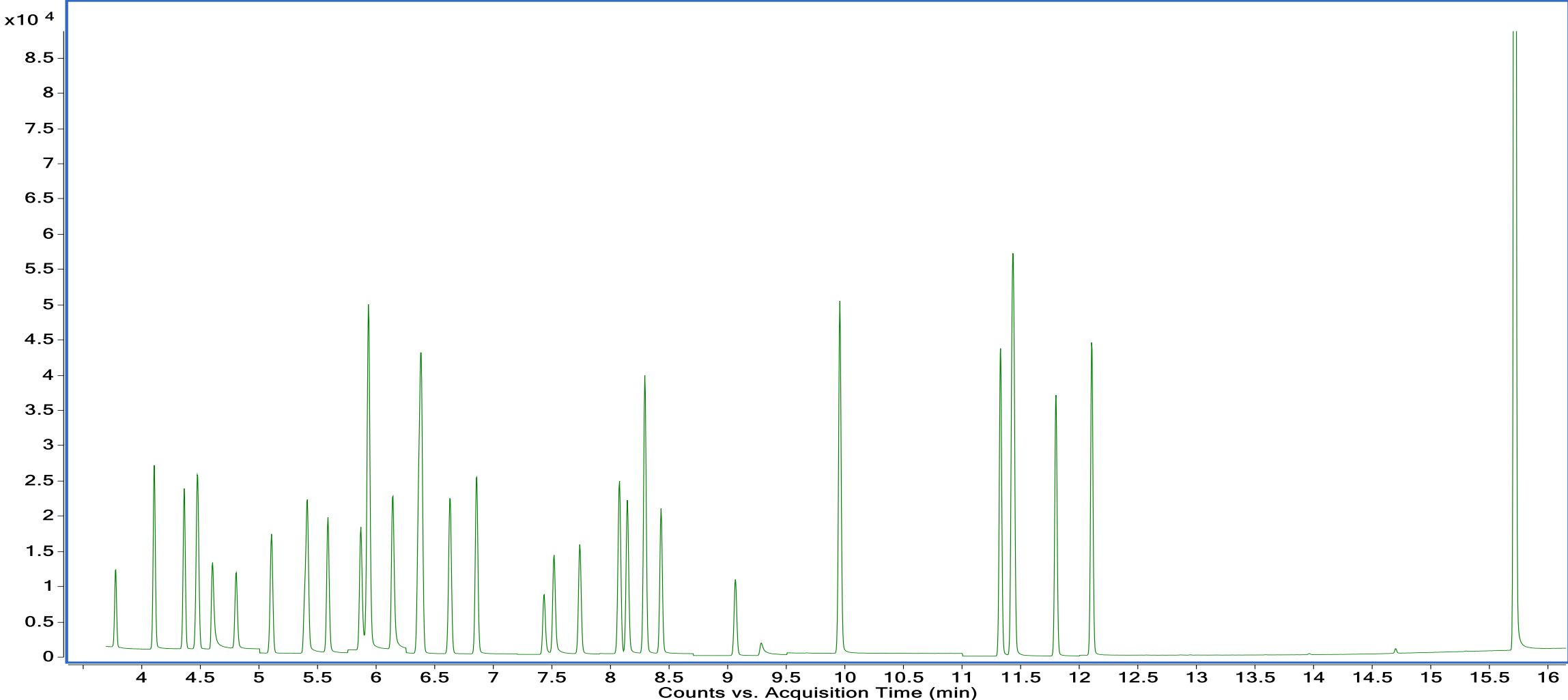- The best choice depends on the analytes and allowable limits

Mid level Calibration of Residual Solvents, scan mode using the 7697 Headspace and an Agilent 5977 MSD
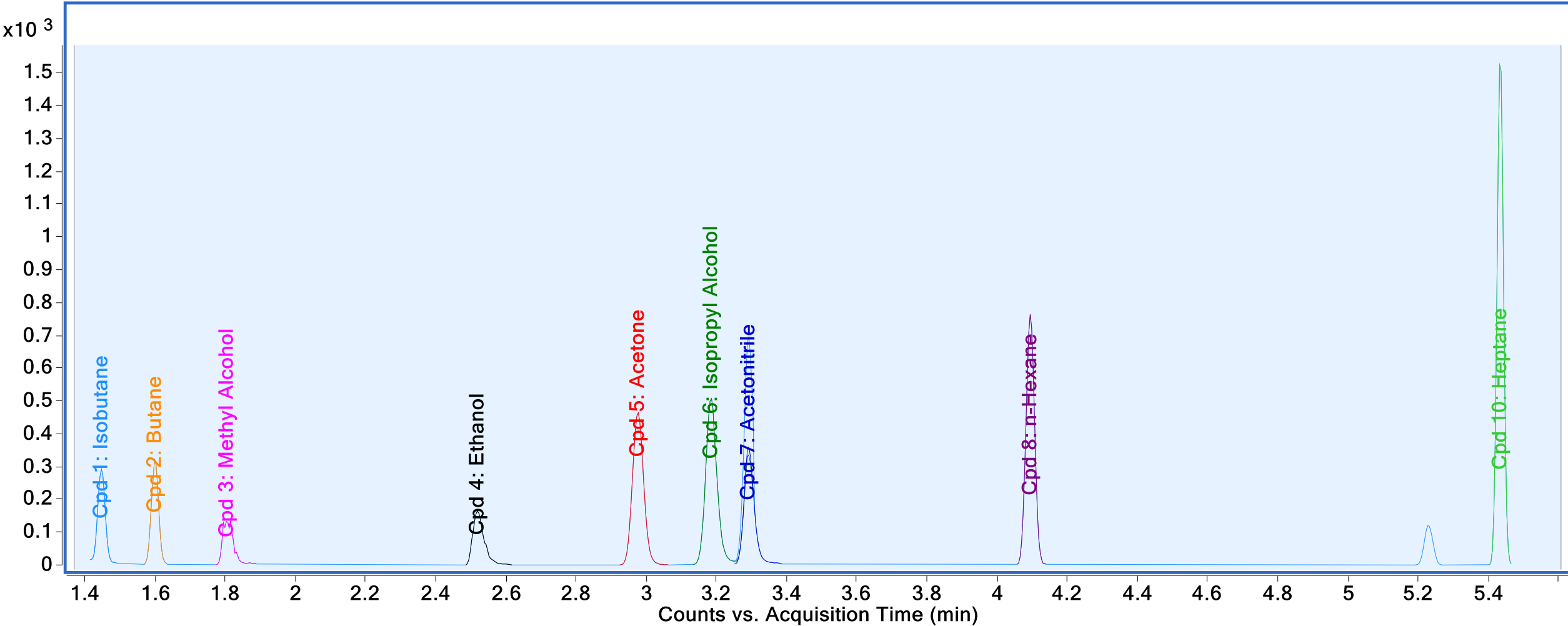
7697 Headspace and Agilent 5977 MSD scan mode, Calibration 0.1 ppm to 1.0 ppm all r2 values over 0.997 using scan data

Acetone - 3 Levels, 3 Levels Used, 3 Points, 3 Points Used, 0 QCs
y = 386.241926 * x + 1.954639
R^2 = 0.99819309
Type:Linear, Origin:Ignore, Weight:None

Isopropyl Alcohol - 3 Levels, 3 Levels Used, 3 Points, 3 Points Used, 0 QCs
y = 1106.103330 * x + 203.495540
R^2 = 0.99970472
Type:Linear, Origin:Ignore, Weight:None

Acetonitrile - 3 Levels, 3 Levels Used, 3 Points, 3 Points Used, 0 QCs
y = 650.862241 * x + 46.540472
R^2 = 0.99974270
Type:Linear, Origin:Ignore, Weight:None

n-Heptane - 3 Levels, 3 Levels Used, 3 Points, 3 Points Used, 0 QCs
y = 582.255000 * x + 5.003500
R^2 = 0.99997306
Type:Linear, Origin:Ignore, Weight:None
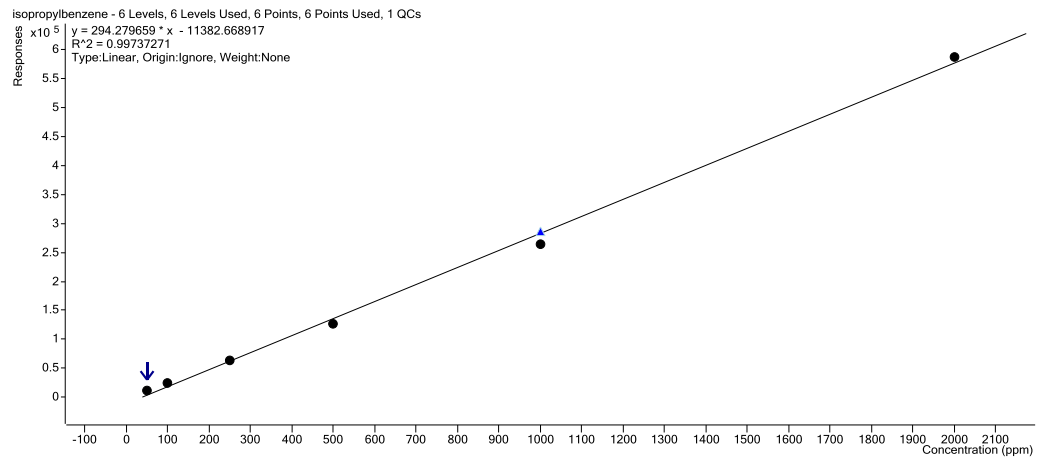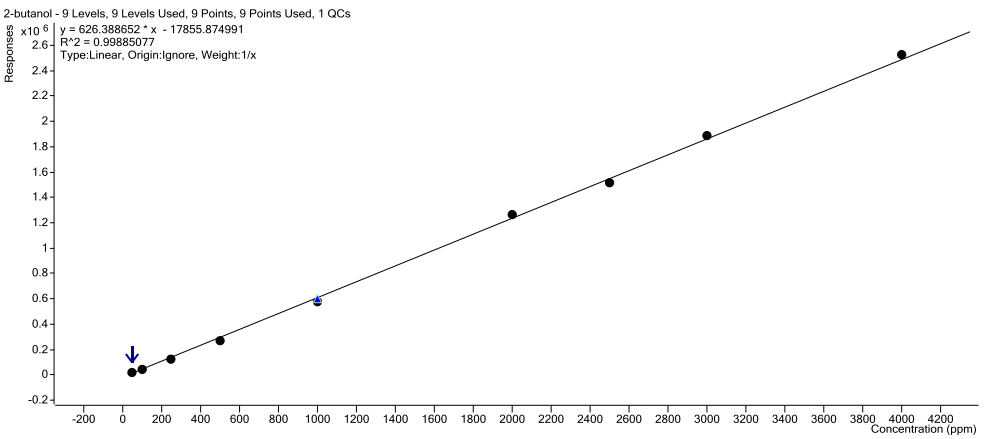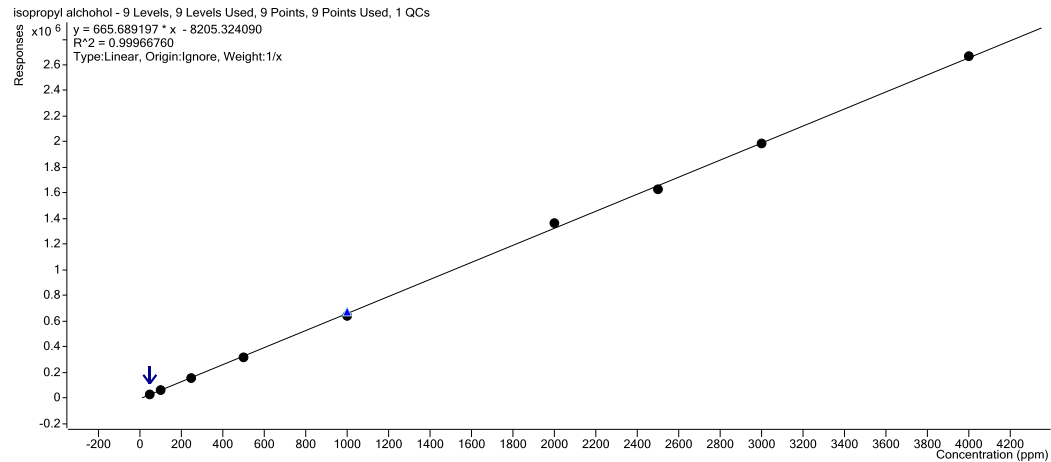
Agilent Technologies

Low level Calibration of Residual Solvents by SIM mode using Headspace and an Agilent 5977 MSD

Agilent Technologies

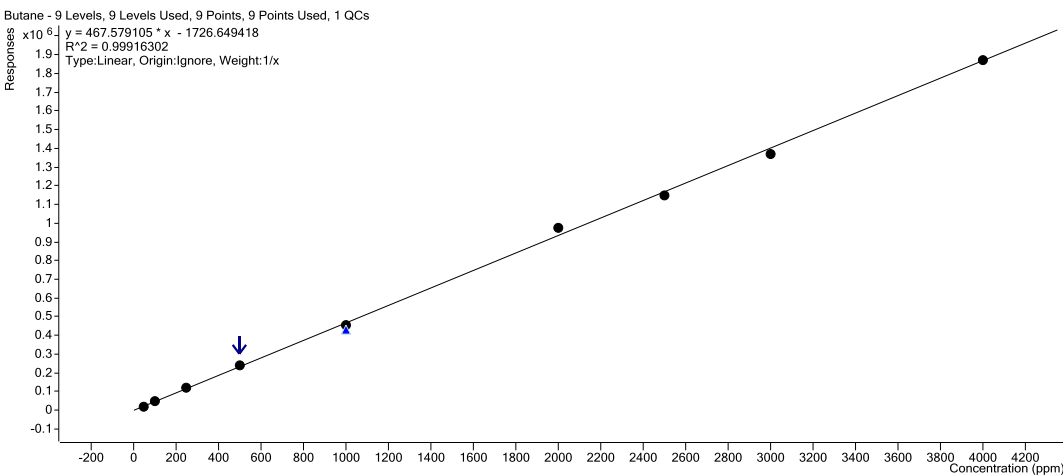# Headspace and Agilent 5977 MSD scan mode, 9 point Calibration 50 ppm to 4000 ppm all r2 values over 0.997 using SIM data

Butane - 9 Levels, 9 Levels Used, 9 Points, 9 Points Used, 1 QCs
y = 467.579105 * x - 1726.649418
R^2 = 0.99916302
Type:Linear, Origin:Ignore, Weight:1/x

isopropyl alchohol - 9 Levels, 9 Levels Used, 9 Points, 9 Points Used, 1 QCs
y = 665.689197 * x - 8205.324090
R^2 = 0.99966760
Type:Linear, Origin:Ignore, Weight:1/x

2-butanol - 9 Levels, 9 Levels Used, 9 Points, 9 Points Used, 1 QCs
y = 626.388652 * x - 17855.874991
R^2 = 0.99885077
Type:Linear, Origin:Ignore, Weight:1/x

isopropylbenzene - 6 Levels, 6 Levels Used, 6 Points, 6 Points Used, 1 QCs
y = 294.279659 * x - 11382.668917
R^2 = 0.99737271
Type:Linear, Origin:Ignore, Weight:None

Agilent Technologies

The Agilent 7890B GC/5977 Series MSD & 7697 Headspace system

Outstanding repeatability for the analysis of residual solvents in cannabis materials.

However, most headspace methods suffer from poor repeatability at low concentrations

- Use of advanced pneumatics, excellent thermal zone control, prep ahead with precise timing translates to better reproducibility and precision for residual solvent analysis.

Agilent Technologies

Why does terpene analysis matter?*

Terpene analysis can be beneficial for patients, providers, and breeders alike.

Understanding terpenes is essential to helping patients identify the right strain for their symptoms.

Terpene analysis allows collectives and budtenders to customize treatment programs for their clients and optimize a strain's pharmacological effects.

Through understanding terpenes, breeders can selectively modulate the terpene ratios of their strains, in order to maximize desired benefits.

Different storage conditions can change terpene results over time and this should be taken into consideration when analyzing cannabis samples as the results show less than expected results.

*For research use only. These statements have not been evaluated by the FDA and are not intended to diagnose, treat or cure any disease.

Terpenes typically done by GC-FID and or GC-MS

Nerolidol	(+)-3-Carene	Camphene
β-Pinene	(-) Caryophyllene Oxide	Sabinene
p-Cymene	α-Pinene	Guaiol
Isopulgeol	Eucalyptol	Phytol
Geranyl acetate	Myrcene	Terpineol
Fenchol	Terpinolene	Camphor
Pulegone	β-Caryophyllene	Isoborneol
Menthol	Limonene	α-Terpinene
α-Humulene	Geraniol	α-Phellandrene
α-Bisabolol	Linalool	α-Cedrene
	(+)Valencene	

Agilent Technologies

Terpene Sample Prep

For cannabis plant material, it is recommended that samples be frozen prior to grinding or that grinding occur under liquid nitrogen. This keeps the samples cold during the grinding process, reducing loss of the more volatile terpenes.

The headspace method utilizes full evaporation technique (FET) sample preparation because cannabis product matrices are extremely varied and plant material will not dissolve in solvent. This involves the use of a very small sample amount (10–50 mg).

A constant incubation temperature and extraction time was used to ensure volatilization of all terpenes and terpenoids in the sample for reproducible, quantitative results.

The prep ahead feature of the 7697 allows an optimum workflow for the short sample analysis time.

Agilent Technologies

Terpene Profiling Configuration

Cannabis has a complex terpene profile, which is theorized to increase its therapeutic effects.

Terpene profiling is used for product quality testing and strain identification.

Terpenes are normally done by GC-FID as the spectra is similar for most compounds.

By resolving the compounds GC-MS is a preferred method.

Headspace-GC-Split between FID and MS

a) Headspace GC is typically used and detection is performed either with MS or FID.

b) Because terpenes are volatile, HS-GC-FID/MS can be used for terpene analysis.
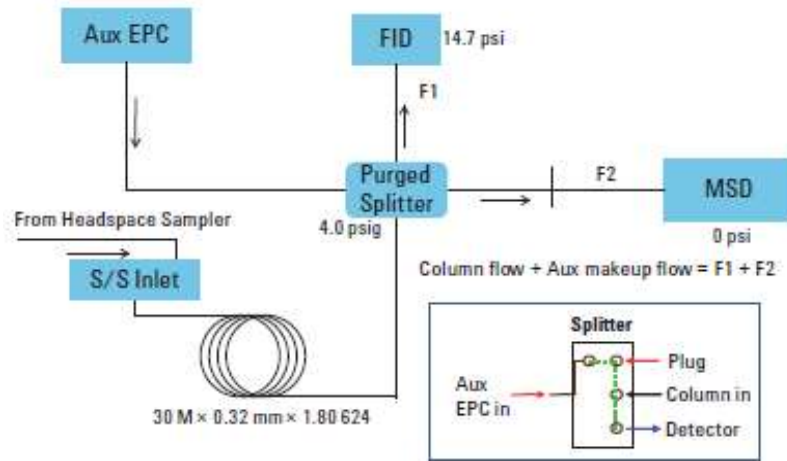
Restek terpene application using a 624 column, Headspace, and Agilent GC-FID

1. α-Pinene 7.39
2. Camphene 7.71
3. β-Myrcene 7.98
4. Sabinene 8.02
5. β-Pinene 8.11
6. α-Phellandrene 8.4
7. δ 3-Carene 8.44
8. α-Terpinene 8.57
9. Ocimene 8.61
10. Limonene 8.71
11. p-Cymene 8.75
12. β-Ocimene 8.82
13. Eucalyptol 8.91
14. γ-Terpinene 9.06
15. Terpinolene 9.47
16. Linalool 9.87
17. Fenchone 10.06
18. Isopulegol 10.73
19. dl-Menthol 11.08
20. Borneol 11.19
21. α-Terpineol 11.29
22. Dihydrocarveol 11.40
23. Citronellol 11.51
24. Geraniol 11.82
25. 2-Piperidinone 11.88
26. Citral 1 11.92
27. Pulegone 11.97
28. Citral 2 12.24
29. Citral 3 13.19
30. Citral 4 13.43
31. β-caryophyllene 13.83
32. α-Humulene 14.21
33. Nerolidol 1 14.78
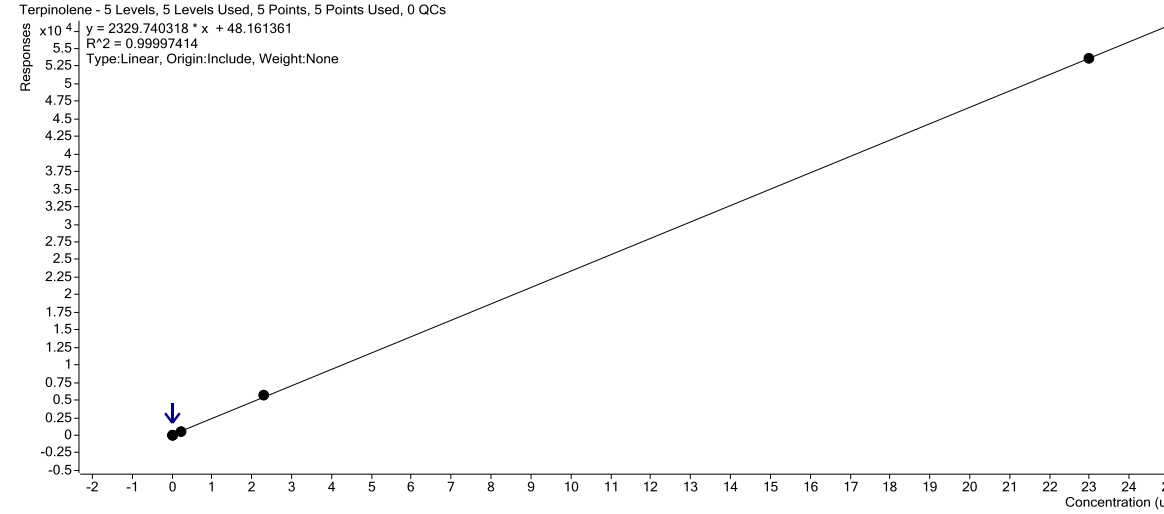34. Nerolidol 2 15.08
35. Caryophyllene oxide 15.92
36. α-Bisabolol 16.43
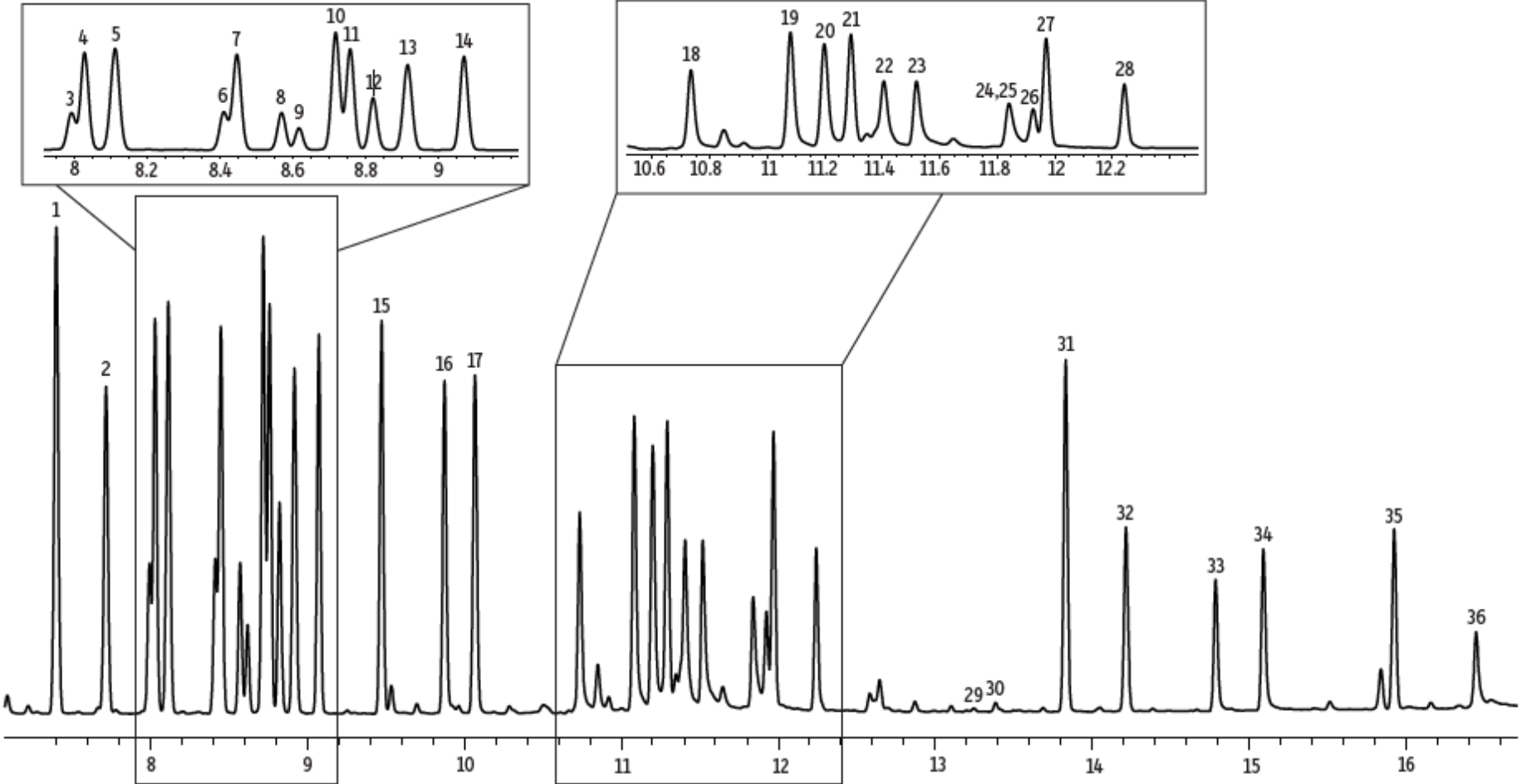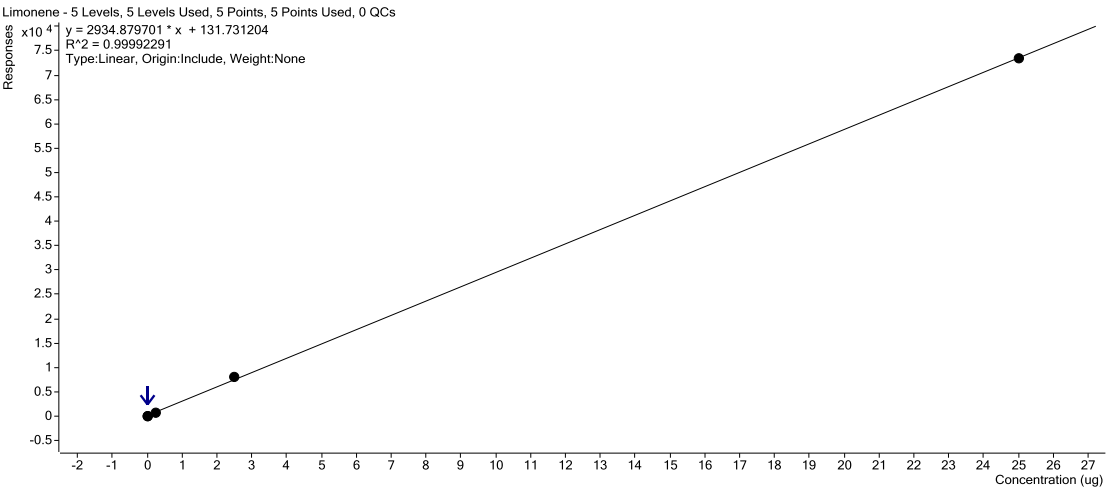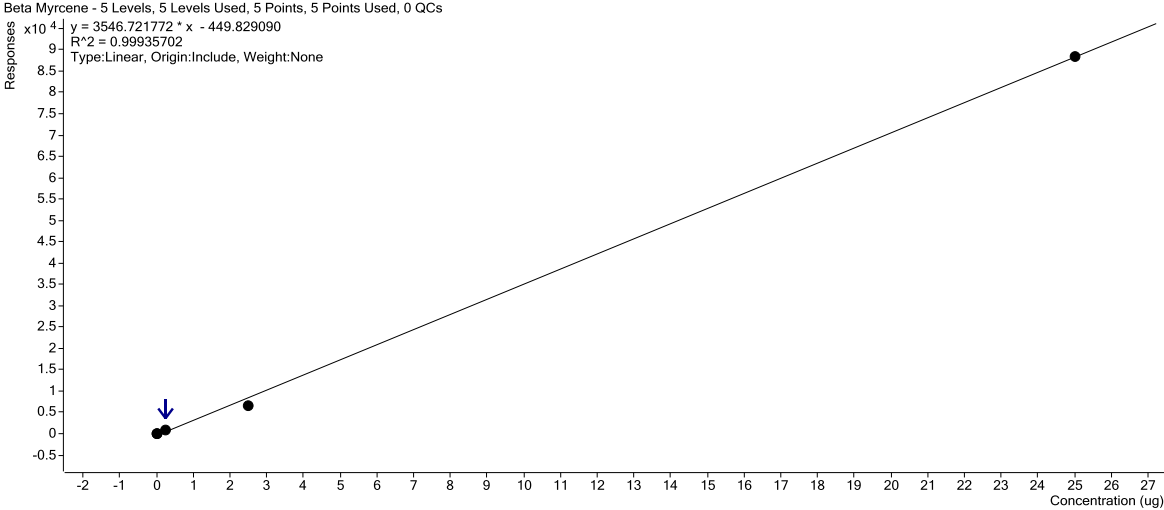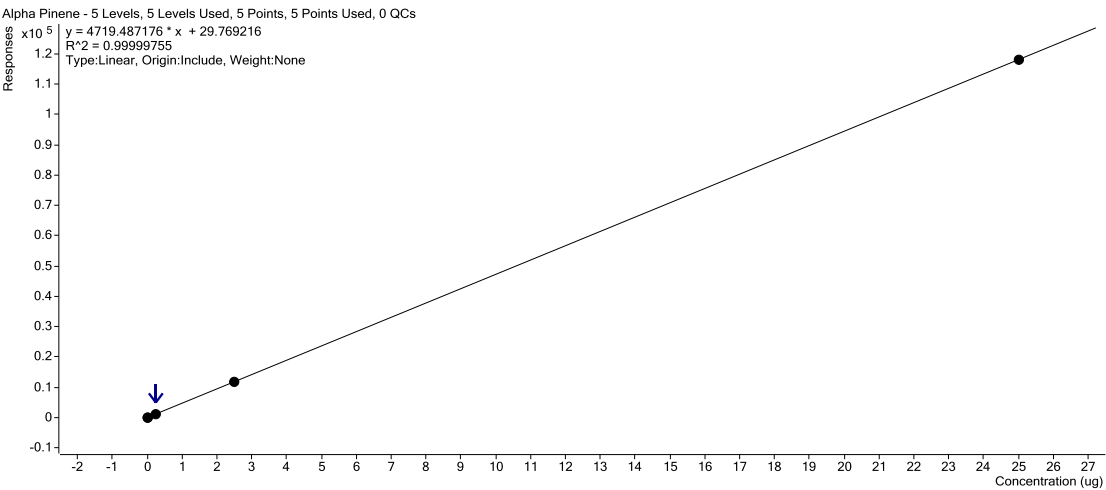
Mid level Calibration standard of Terpenes by 7697 Headspace and scan mode using an Agilent 5977 MSD and a 624 column

Alpha Pinene	93.1	6.965
Camphene	93.1	7.358
Beta Myrcene	93.1	7.797
Beta Pinene	93.1	7.866
Delta-3-Carene	93.1	8.311
alpha-Terpinene	121.1	8.438
Limonene	68.1	8.617
p-Cymene	134.1	8.664
Ocimene	93.1	8.762
Eucalyptol	154.1	8.837
gamma-Terpinene	93.1	9.513
Terpinolene	136.1	9.513
Linalool	71.1	9.934
Isopulegol	84.1	10.818
Geraniol	69.1	12.049
beta Caryophyllene	93.1	13.695
alpha Humulene	93.1	14.018
Nerolidol	69.1	14.787
Guaiol	161.1	15.572
Caryophyllene oxide	93.1	15.757
alpha Bisabolol	204.2	16.329

Agilent Technologies
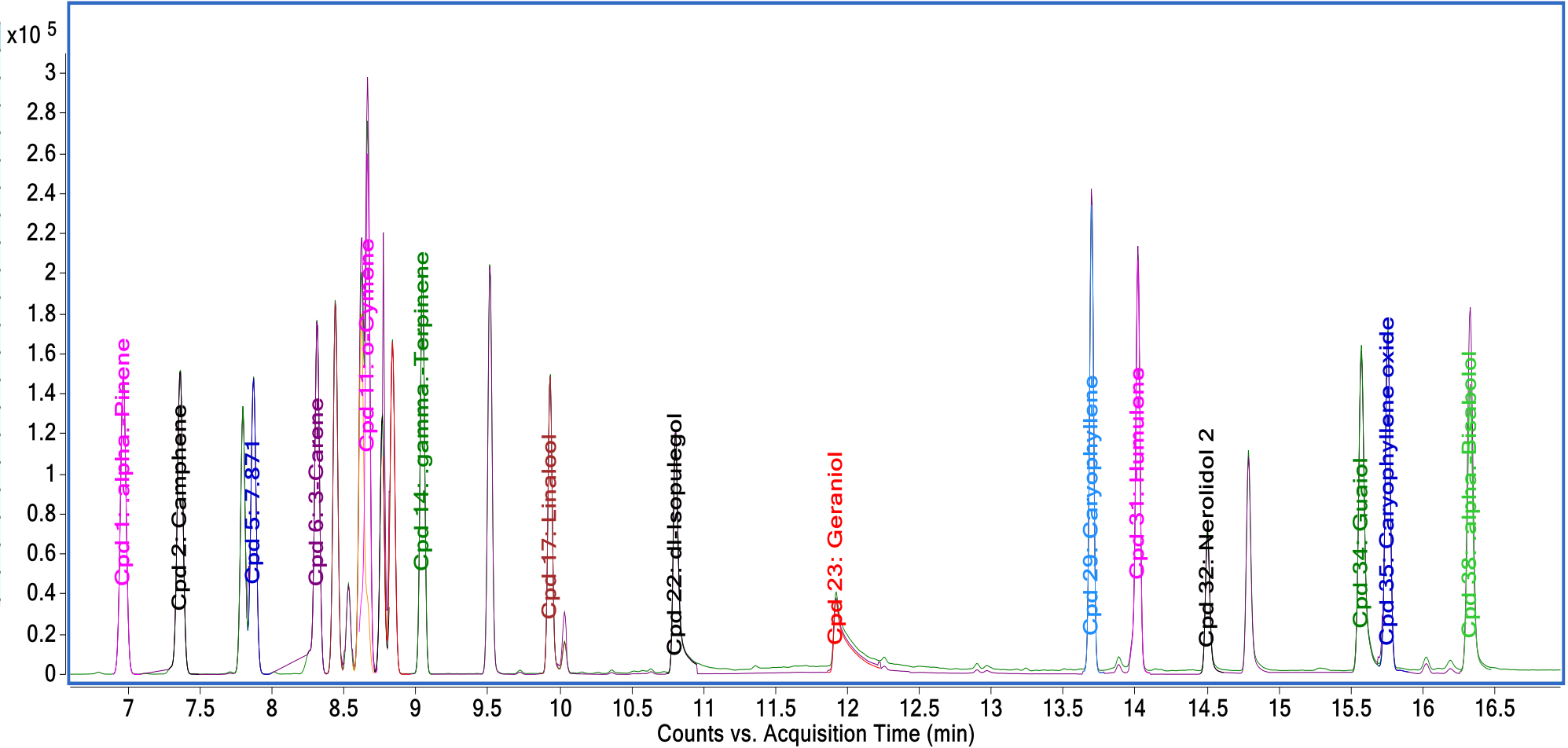
5 level calibration using a 7697 Headspace and Agilent 5977 MSD in scan mode
# Calibration 0.12 ug to 25.0 ug all r2 values over 0.999

Alpha Pinene - 5 Levels, 5 Levels Used, 5 Points, 5 Points Used, 0 QCs
$y = 4719.487176 * x + 29.769216$
$R^2 = 0.99999755$
Type:Linear, Origin:Include, Weight:None

Beta Myrcene - 5 Levels, 5 Levels Used, 5 Points, 5 Points Used, 0 QCs
$y = 3546.721772 * x - 449.829090$
$R^2 = 0.99935702$
Type:Linear, Origin:Include, Weight:None

Limonene - 5 Levels, 5 Levels Used, 5 Points, 5 Points Used, 0 QCs
$y = 2934.879701 * x + 131.731204$
$R^2 = 0.99992291$
Type:Linear, Origin:Include, Weight:None

Terpinolene - 5 Levels, 5 Levels Used, 5 Points, 5 Points Used, 0 QCs
$y = 2329.740318 * x + 48.161361$
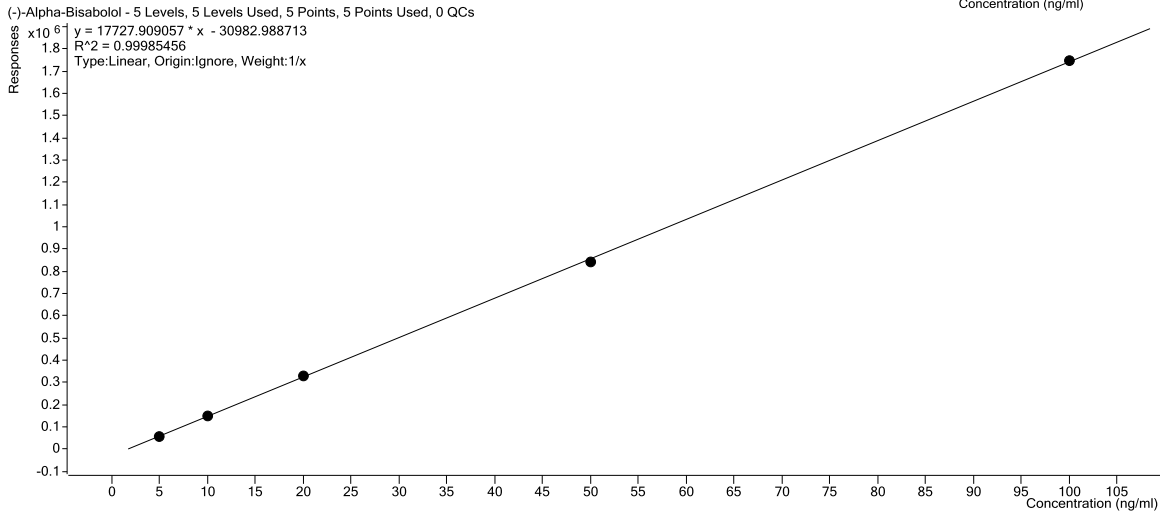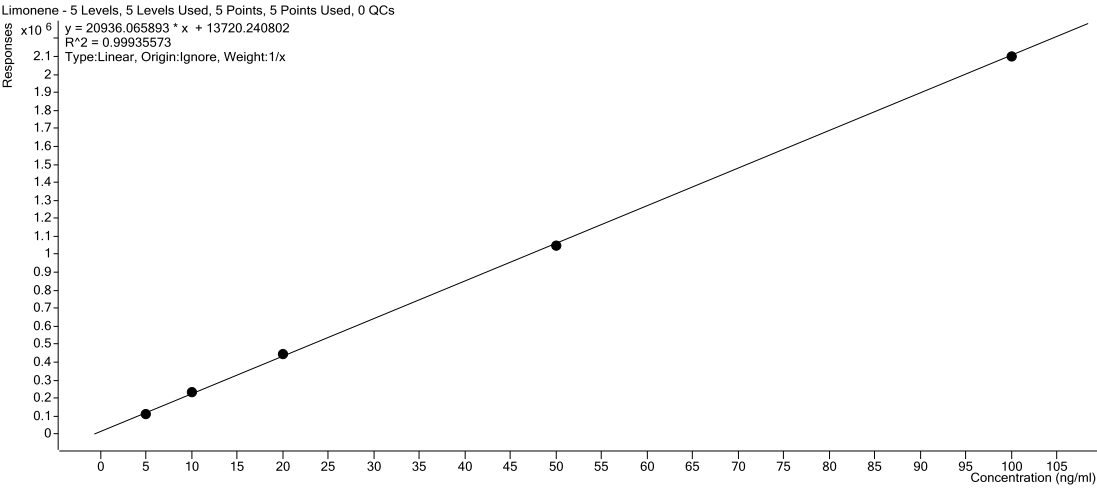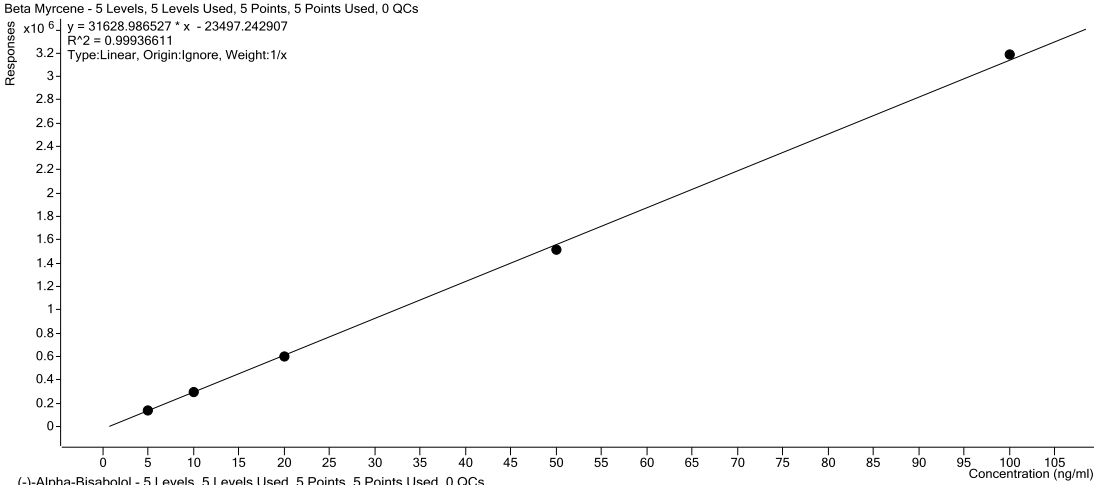$R^2 = 0.99997414$
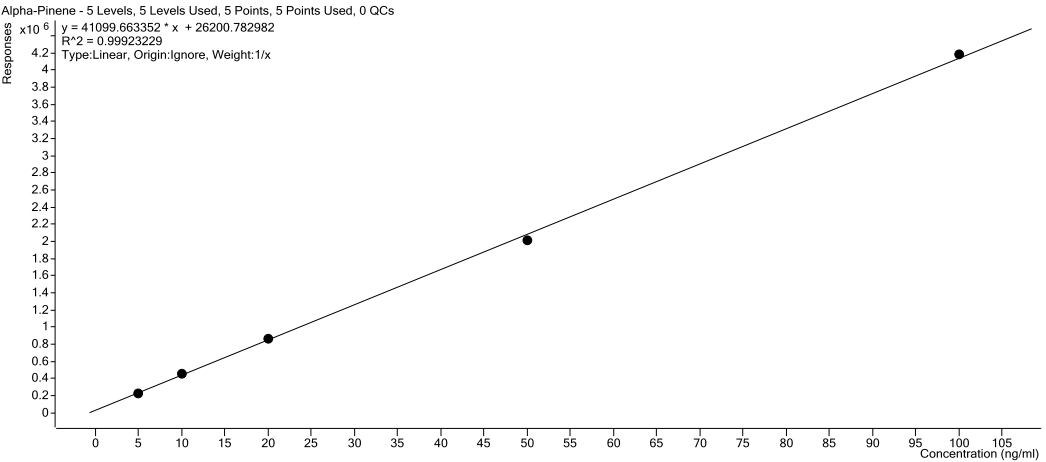Type:Linear, Origin:Include, Weight:None

Mid level CCV extract of Terpenes by 7693 ALS and SIM mode using an Agilent 5977 MSD and a DB-35 column

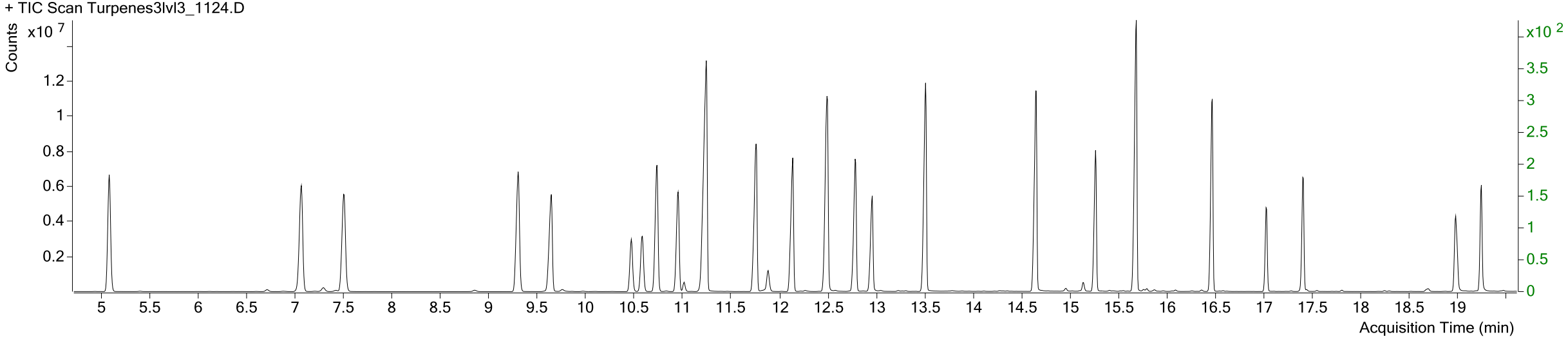| Alpha-Pinene |
| Camphene |
| B-Pinene |
| Beta Myrcene |
| Delta 3-Carene |
| Alpha-Terpinole... |
| Limonene |
| Ocimene Isomer... |
| Eucalyptol |
| p-Cymene |
| Ocimene Isomer... |
| Gamma-Terpine... |
| Terpinolene |
| Linalool |
| Fenchone |
| (-)-Isopulehol |
| Nerol |
| Geraniol |
| Pulegone |
| Beta-Carophylle... |
| Alpha-Humulene |
| Valencene |
| cis-Nerolidol |
| trans-Nerolidol |
| (-)-Guaiol |
| Carophyllene Ox... |
| (-)-Alpha-Bisabo... |

Agilent Technologies
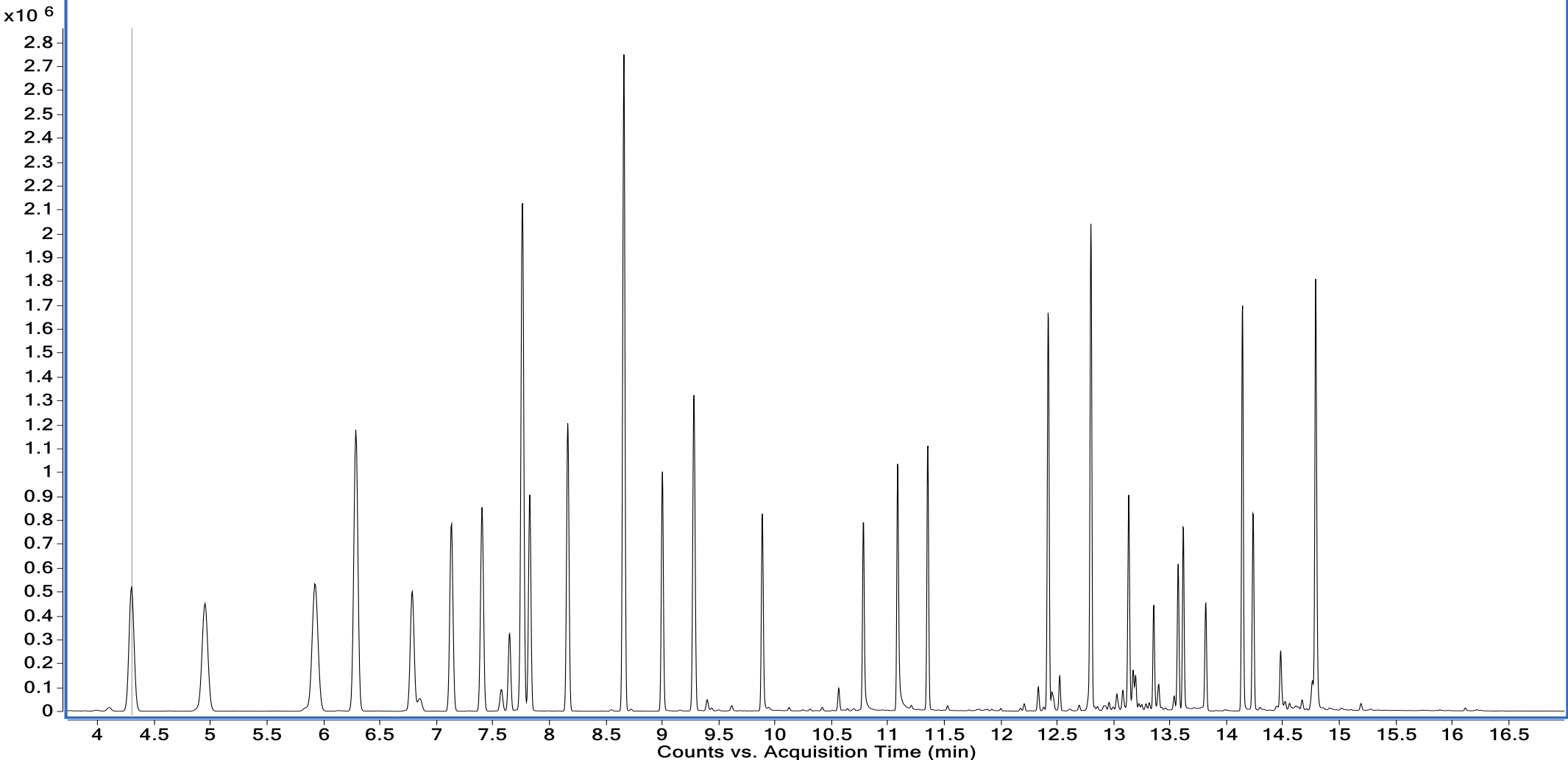
5 level calibration using a 7693 ALS and Agilent 5977 MSD in SIM mode Calibration 5.0 to 100.0 ng/ml all r2 values over 0.999

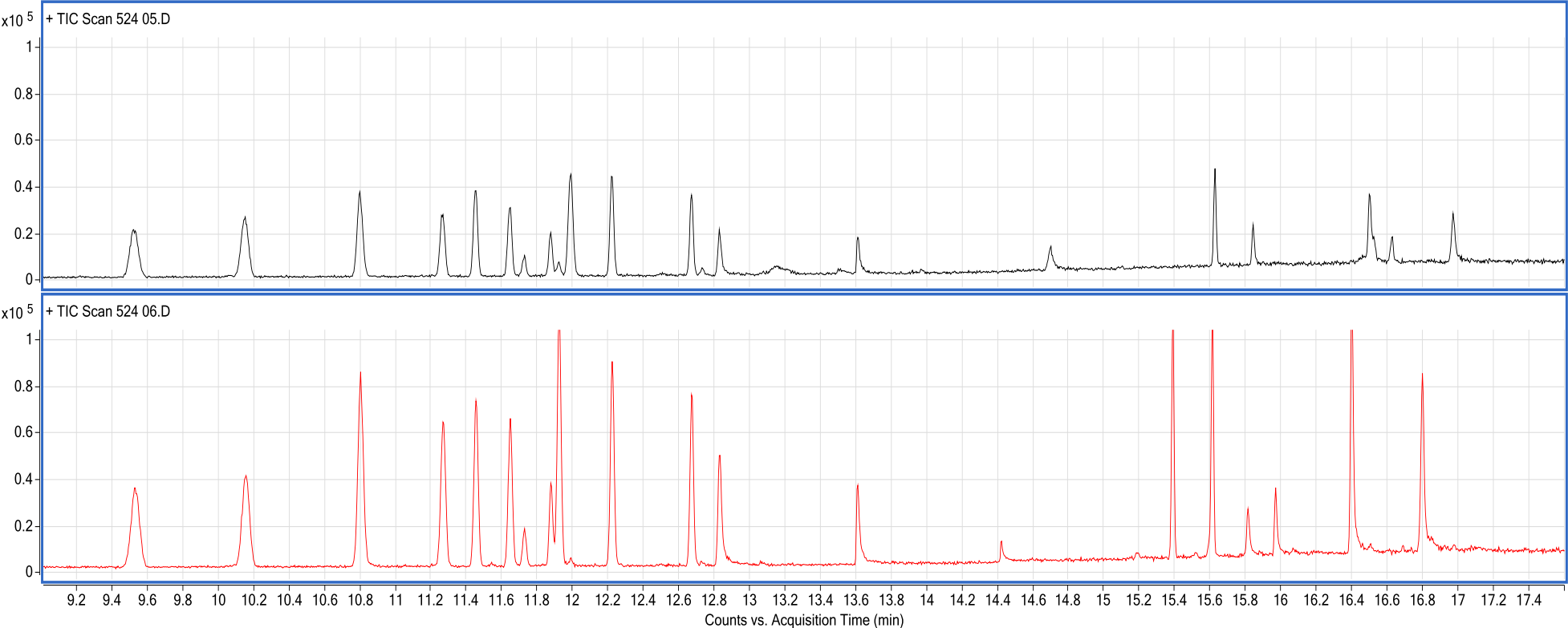Alpha-Pinene - 5 Levels, 5 Levels Used, 5 Points, 5 Points Used, 0 QCs
y = 41099.663352 * x + 26200.782982
R^2 = 0.99923229
Type:Linear, Origin:Ignore, Weight:1/x

Beta Myrcene - 5 Levels, 5 Levels Used, 5 Points, 5 Points Used, 0 QCs
y = 31628.986527 * x - 23497.242907
R^2 = 0.99936611
Type:Linear, Origin:Ignore, Weight:1/x

Limonene - 5 Levels, 5 Levels Used, 5 Points, 5 Points Used, 0 QCs
y = 20936.065893 * x + 13720.240802
R^2 = 0.99935573
Type:Linear, Origin:Ignore, Weight:1/x

(-)-Alpha-Bisabolol - 5 Levels, 5 Levels Used, 5 Points, 5 Points Used, 0 QCs
y = 17727.909057 * x - 30982.988713
R^2 = 0.99985456
Type:Linear, Origin:Ignore, Weight:1/x

Agilent Technologies

Results from work currently under way at Texas Southern to separate terpenes using a DB-5 column

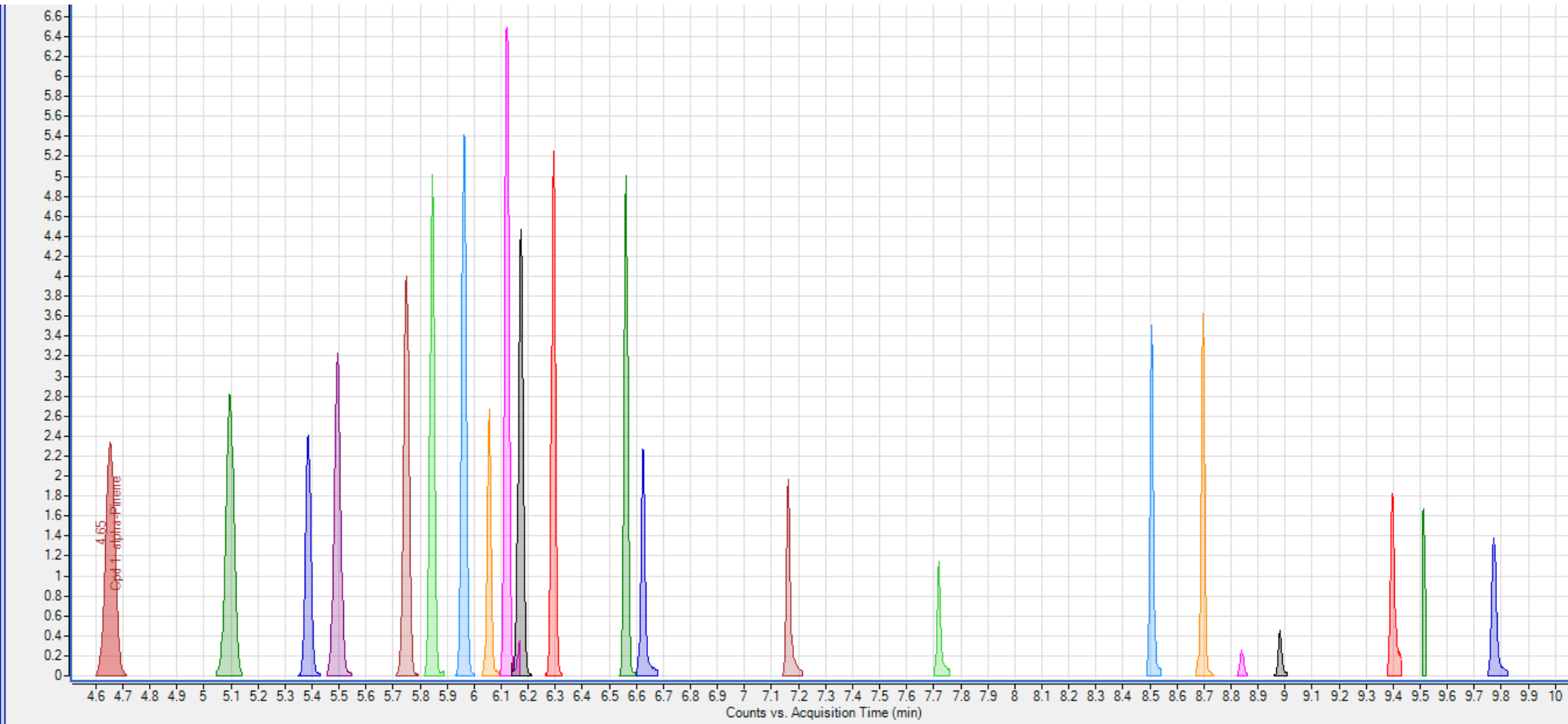+ TIC Scan Turpenes3lvl3_1124.D

Agilent Technologies

VF-35 column – with a 24 minute GC cycle time, results with 2 different mixes

Not all compounds were in each mix

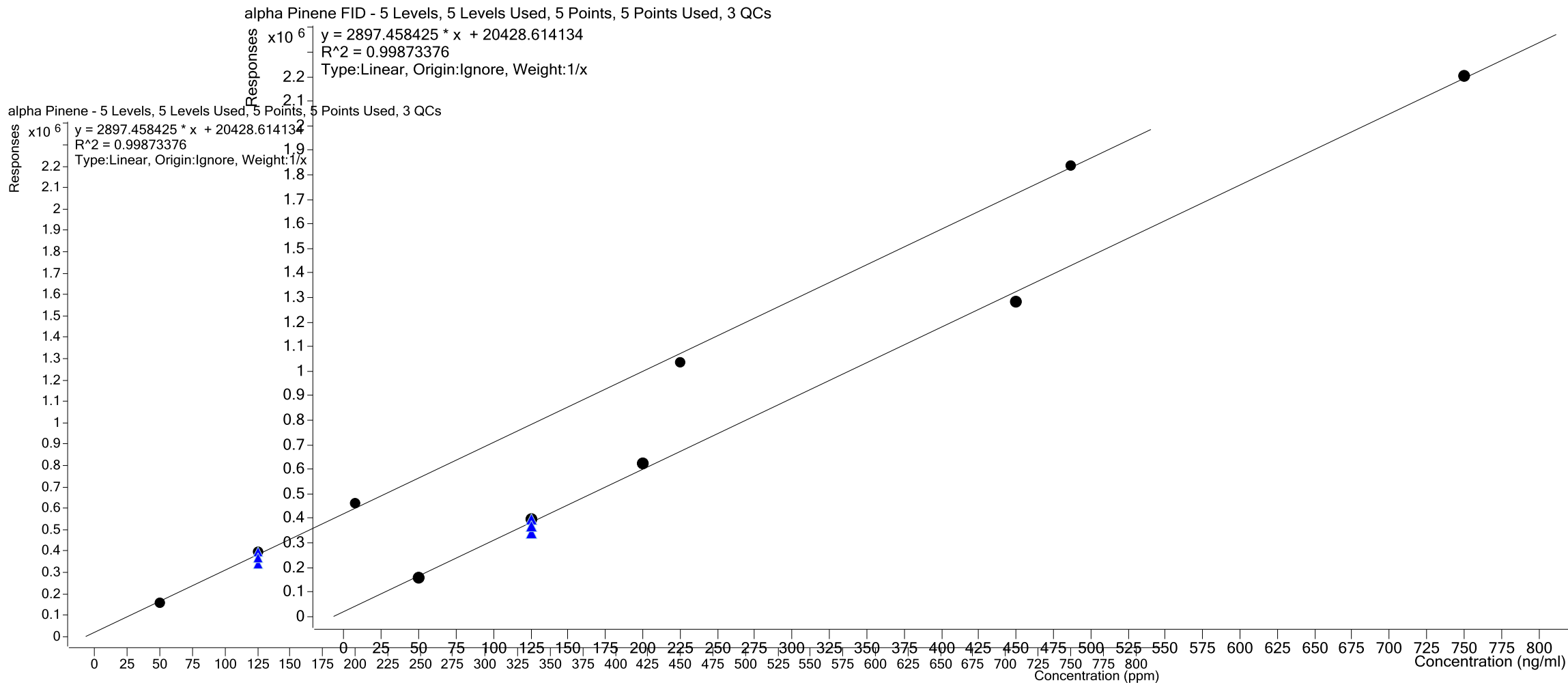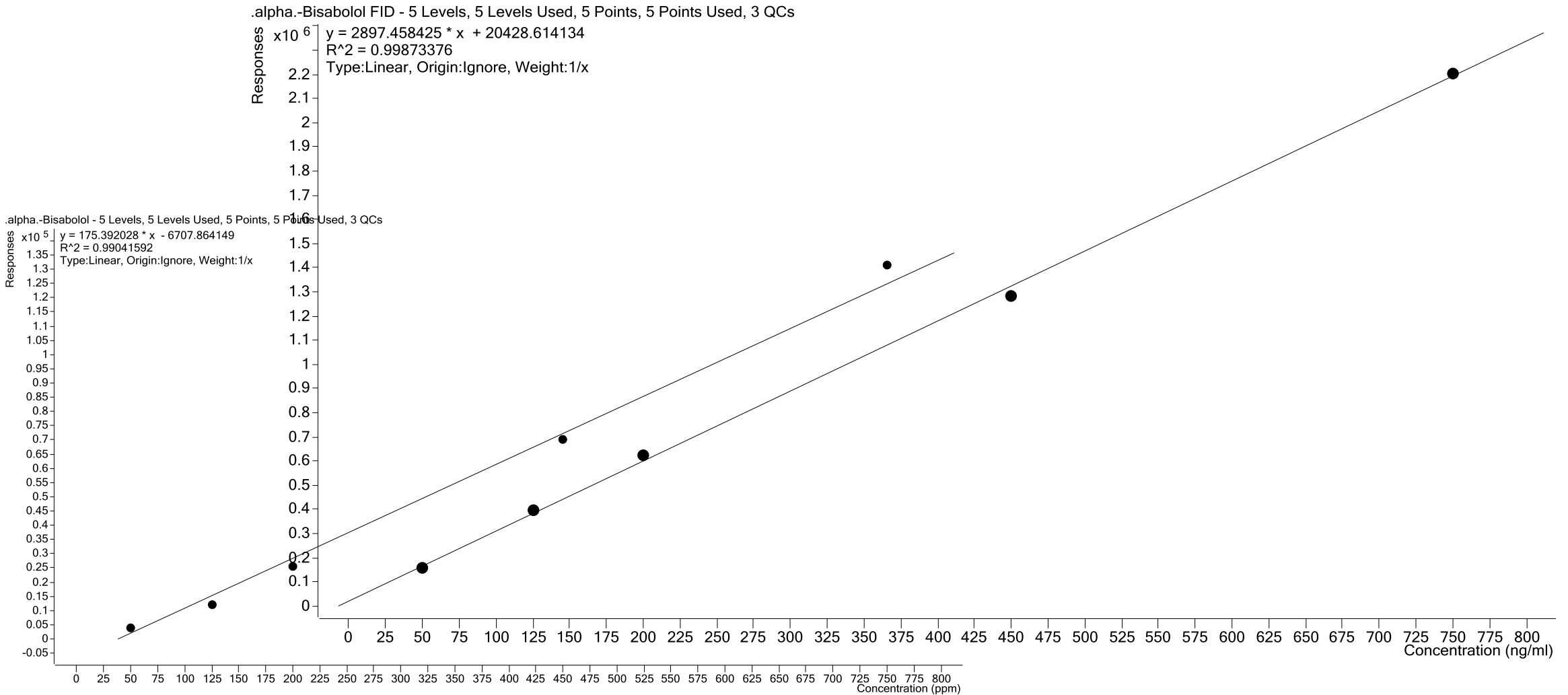# Chromatogram – 17 minute GC cycle time 100 ppm 22 terpenes

Calibration curves FID and MS of alpha-Pinene

alpha Pinene FID - 5 Levels, 5 Levels Used, 5 Points, 5 Points Used, 3 QCs

Responses

x10^6

y = 2897.458425 * x + 20428.614134
R^2 = 0.99873376
Type:Linear, Origin:Ignore, Weight:1/x

alpha Pinene - 5 Levels, 5 Levels Used, 5 Points, 5 Points Used, 3 QCs

Responses

x10^6

y = 2897.458425 * x + 20428.614134
R^2 = 0.99873376
Type:Linear, Origin:Ignore, Weight:1/x

Concentration (ppm)

Concentration (ng/ml)

Calibration curves FID and MS of alpha-bisabolol

.alpha.-Bisabolol FID - 5 Levels, 5 Levels Used, 5 Points, 5 Points Used, 3 QCs

$y = 2897.458425 * x + 20428.614134$
$R^2 = 0.99873376$
Type:Linear, Origin:Ignore, Weight:1/x

.alpha.-Bisabolol - 5 Levels, 5 Levels Used, 5 Points, 5 Points Used, 3 QCs

$y = 175.392028 * x - 6707.864149$
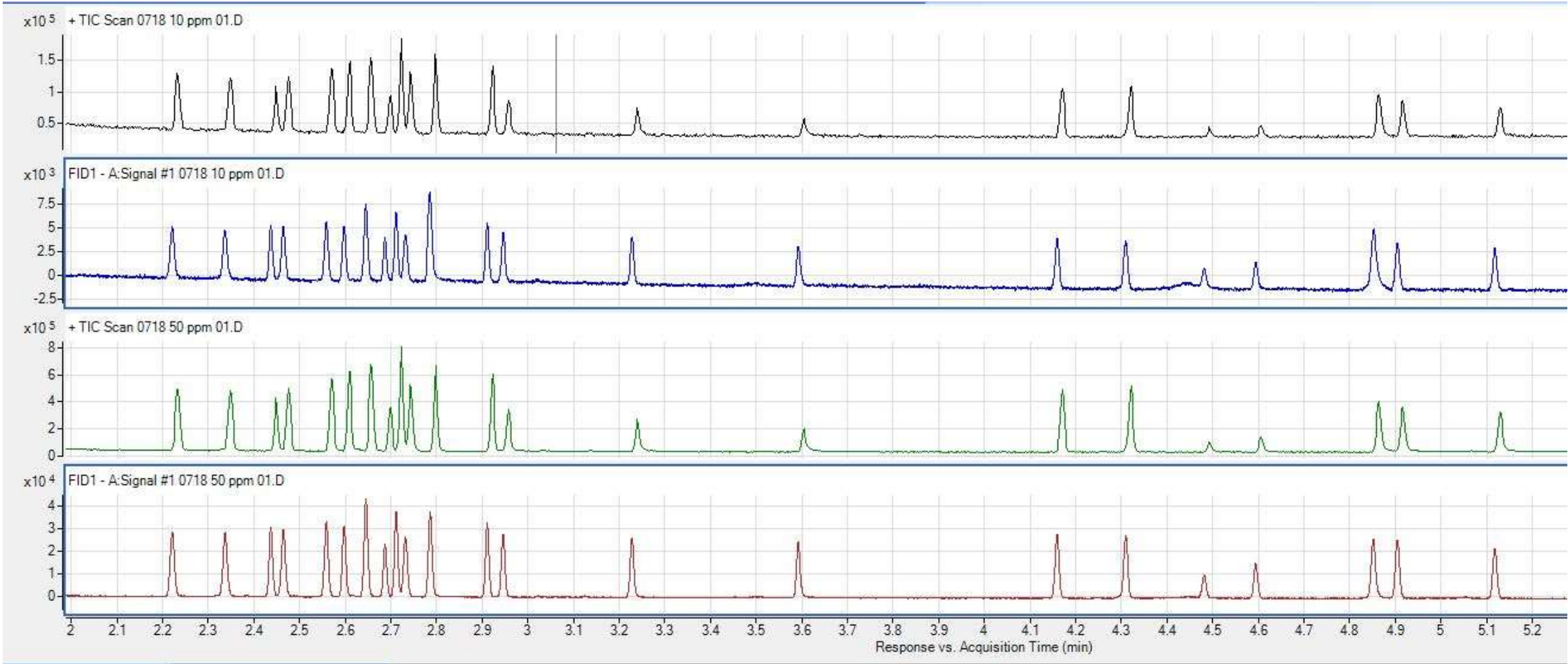$R^2 = 0.99041592$
Type:Linear, Origin:Ignore, Weight:1/x

The Black trace is from a cannabis sample
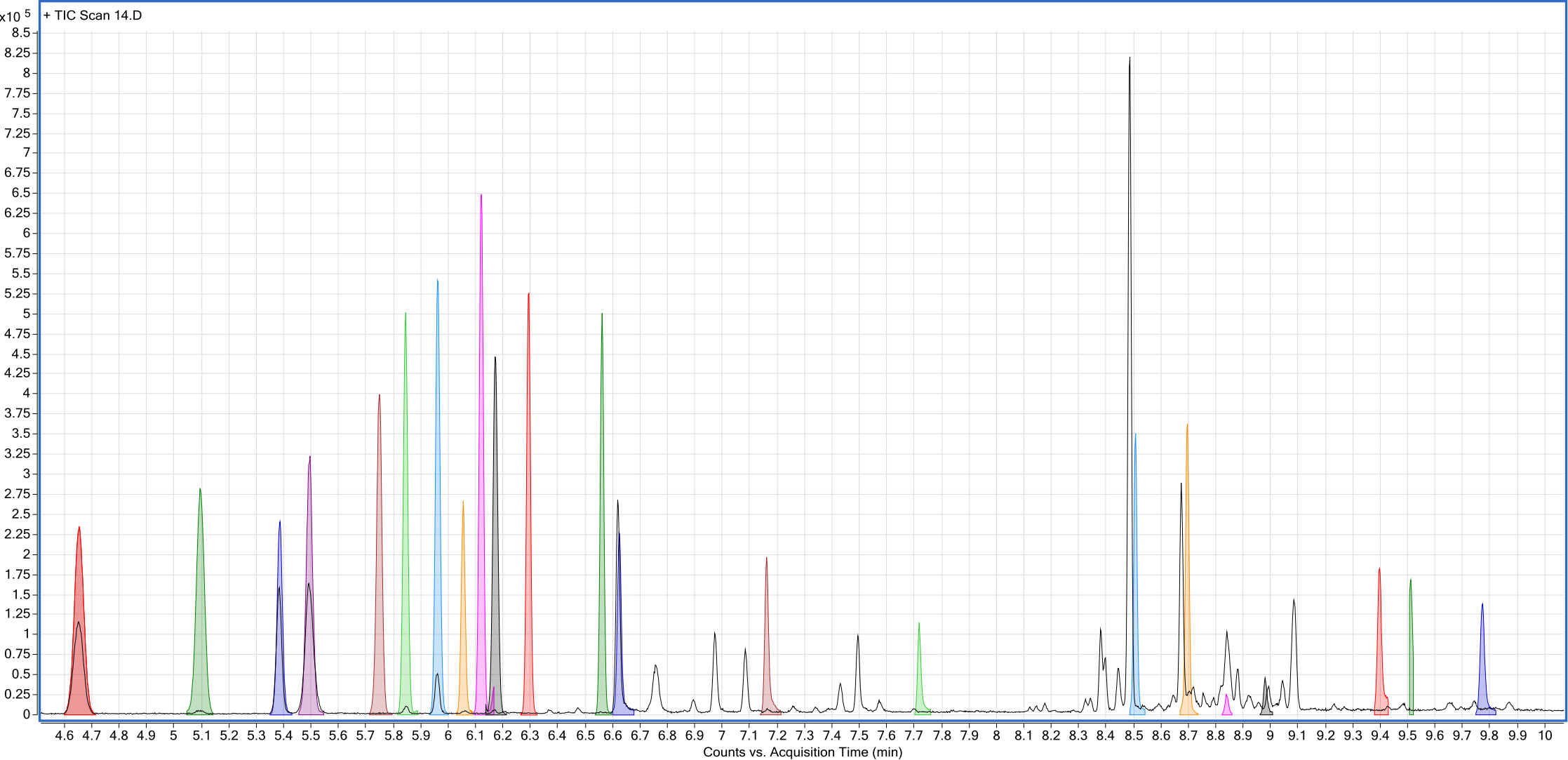
Chromatogram – 10 minute GC cycle time 10 and 50 ppm GC-FID and GC-MS of 22 terpenes 5.8 min GC run time, Look at the productivity!
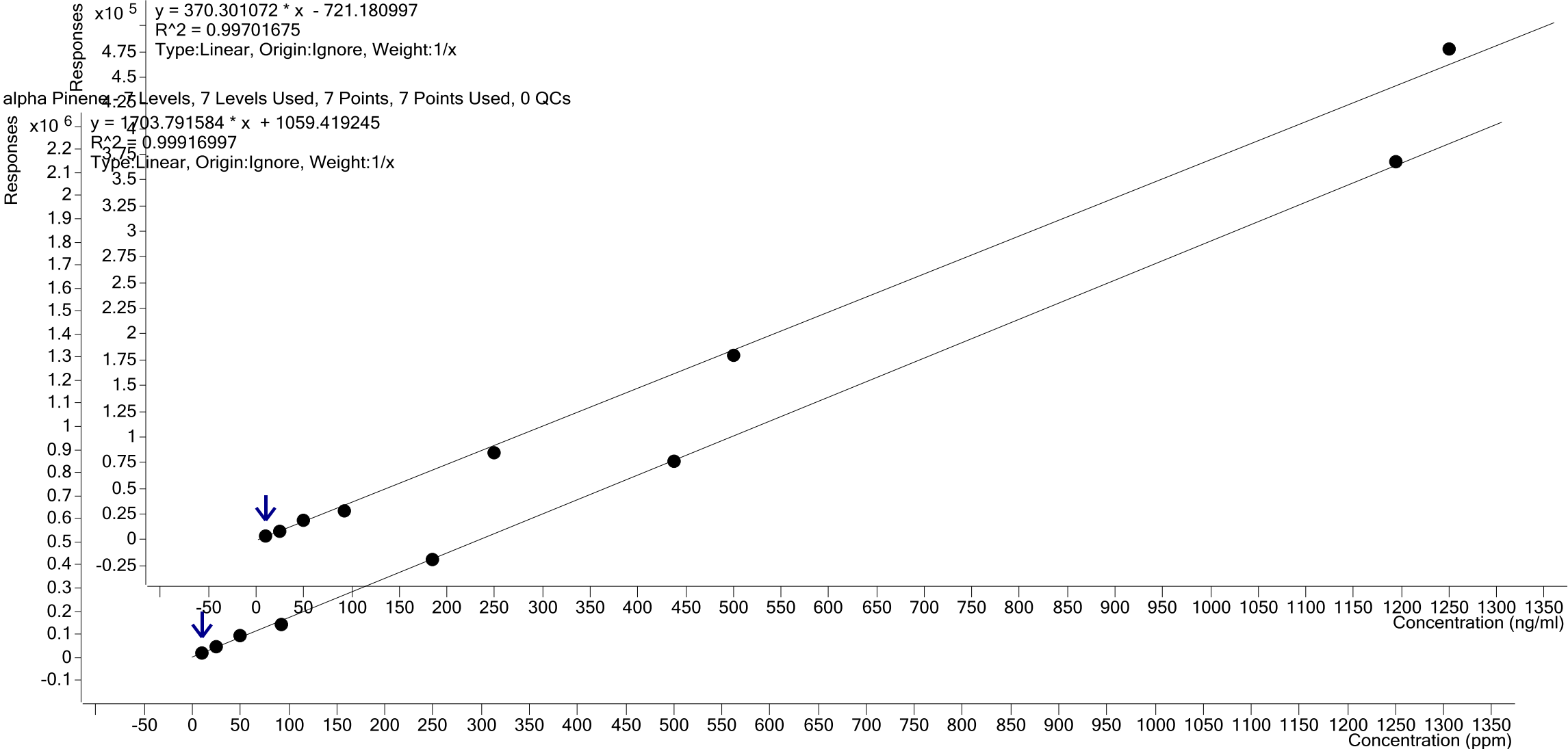
Calibration curves FID and MS of alpha-Pinene 5 minute method

alpha Pinene FID - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 0 QCs

y = 370.301072 * x - 721.180997
R^2 = 0.99701675
Type:Linear, Origin:Ignore, Weight:1/x

alpha Pinene - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 0 QCs

y = 1703.791584 * x + 1059.419245
R^2 = 0.99916997
Type:Linear, Origin:Ignore, Weight:1/x

Concentration (ng/ml)

Concentration (ppm)

Calibration curves FID and MS of alpha-bisabolol 5 minute method

.alpha.-Bisabolol - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 0 QCs

y = 370.107220 * x - 3184.599489
R^2 = 0.99469410
Type:Linear, Origin:Ignore, Weight:1/x

.alpha.-Bisabolol FID - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 0 QCs

y = 242.770886 * x + 445.368977
R^2 = 0.99905760
Type:Linear, Origin:Ignore, Weight:1/x
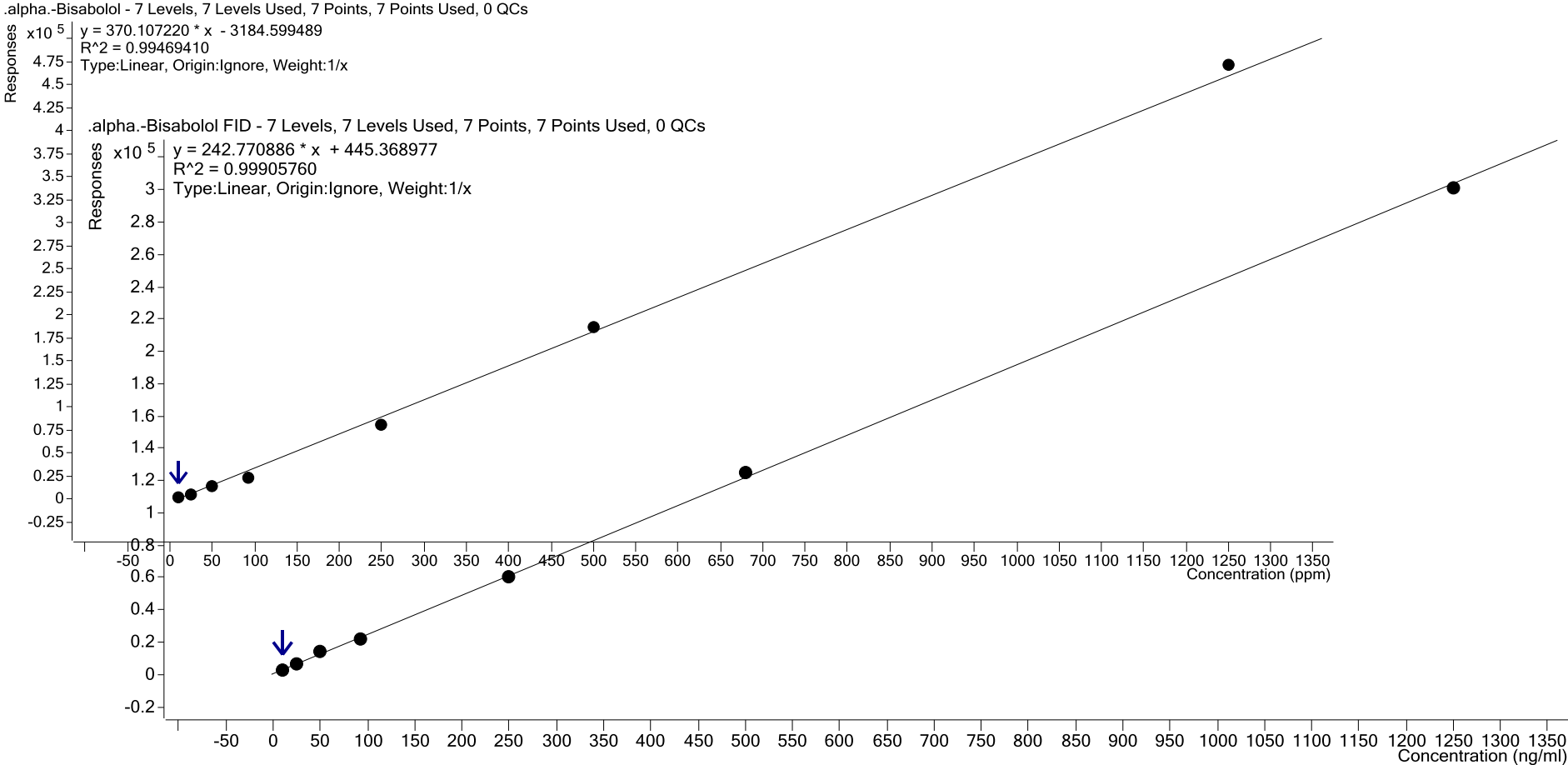
Thanks for supplying data to make this presentation possible:

- Ace Analytical Labs- Las Vegas, Nevada

- DigiPath Labs- Las Vegas, Nevada

- GreenLeaf Labs- Portland, Oregon

- Texas Southern University- Texas
  - Jamie Dougherty, Agilent Field Support Scientist

- Restek for excerpts from their application database

- D. Anderson, Ph.D. -(Technical Insight, Mass Spectrometry, Cannabis)

Agilent Technologies