Foods, Flavors & Fragrances Applications

A Fast, Simple FET Headspace GC-FID Technique for Determining Residual Solvents in Cannabis Concentrates

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Abstract

Due to rapid growth in the medical cannabis industry, demand is increasing for analysis of residual solvents in cannabis concentrates in order to protect consumer safety. This application note details a simple, fast test for common residual solvents using full evaporation technique headspace GC-FID and an Rxi®-624Sil MS column.

Introduction

As the popularity of cannabis concentrates increases, consumer safety concerns are resulting in the establishment of new regulations to control the level of residual solvents in commercial cannabis concentrates. The State of Colorado, for example, published allowable concentrations of certain residual solvents in Rule R 712. This is because, although cannabis concentrates can be produced in numerous ways, one of the most common means of extracting therapeutic compounds, like tetrahydrocannabinol (THC), cannabidiol (CBD), and terpenes, from cannabis is through extraction with an organic solvent, such as butane. After the cannabinoids and terpenes are extracted from the plant material, the organic solvent is allowed to evaporate and then is purged off using heat and/or vacuum. These extraction solvents can be difficult to purge completely, so the finished product needs to be tested to ensure that residual solvents are only present at or below safe levels. For consumer safety, especially with medicinal products, accurate and comprehensive analysis of residual solvents is necessary for concentrates and extracts.

Since residual solvents are extremely volatile, they cannot be analyzed by HPLC and lend themselves nicely to GC analysis. One of the most common and reliable ways to quantify residual solvents is through headspace gas chromatography–flame ionization detection (GC-FID). Headspace injection works by driving volatile compounds of interest from the sample into a gas phase in the headspace of the vial above the sample. An aliquot is then withdrawn from the headspace of the vial and analyzed by GC-FID in order to determine the volatile components of the sample. One approach for headspace GC-FID that is particularly useful for analyzing cannabis concentrates is the full evaporation technique (FET). FET sample preparation involves the use of a very small sample amount (e.g., 20–50 mg), which effectively creates a single-phase gas system in the headspace vial at equilibrium [1]. FET is ideal for difficult and varied matrices like cannabis concentrates because it eliminates matrix interferences that can cause inaccurate quantification, and it also has the advantages of little to no manual sample handling and a very small sample size. Additionally, high sensitivity can be achieved through the creation of a single-phase system in the headspace vial. Figure 1 illustrates the basic principle of headspace GC using the full evaporation technique.

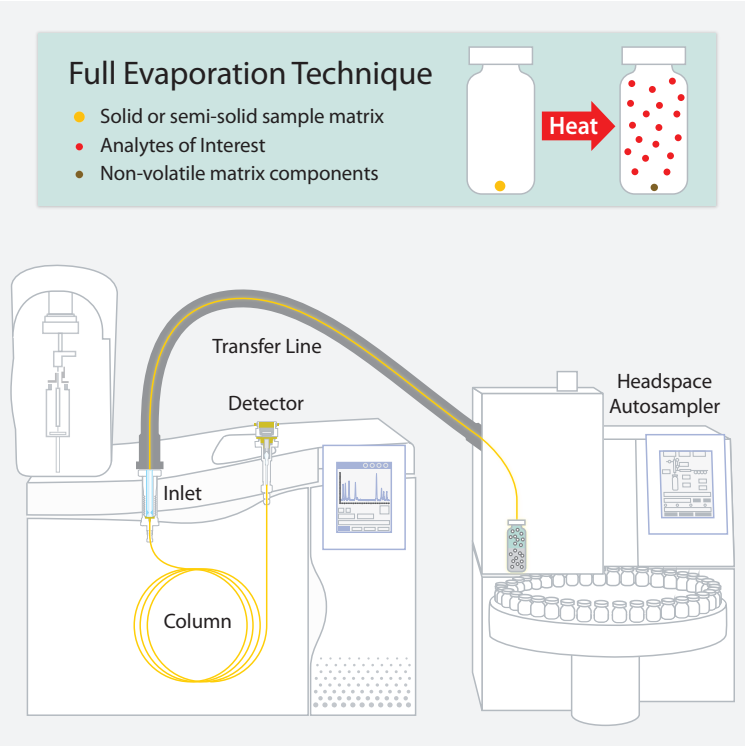
The work described here demonstrates the viability of FET headspace injection and GC-FID analysis of residual solvents in cannabis concentrates. The method is simple to implement, quick to run, and does not require expensive dynamic headspace equipment or mass spectrometric detectors. While the methodology presented here is suitable for residual solvents in cannabis concentrates, it is not applicable for finished tinctures in alcohol. Finished alcohol tinctures contain large amounts of alcohol which will severely interfere with quantification of other residual solvents in the sample. Therefore, an alternate approach is required for alcohol tinctures. This technique also may be applicable for oil or glycerin tinctures; however, it has not been evaluated for that use.

Experimental

Headspace and GC Method Optimization

An Rxi®-624Sil MS column was selected for this work as it is designed specifically for volatiles analysis and is widely used for the analysis of residual solvents in pharmaceutical products. Final FET headspace injector and GC-FID operating conditions are presented in Figure 3. Initially, modeled conditions for analyzing the specific compounds of interest were generated using Restek's *EZ*GC™ chromatogram modeler. The method from the modeler was then optimized to account for headspace analysis employing a headspace instrument with a transfer line.

Figure 1: Setup and Basic Principle of FET Headspace Injection Coupled With GC-FID Analysis



The following parameters were optimized for this method:

- *Linear velocity:* Linear velocity was increased to 80 cm/sec to allow for fast sample transfer through the headspace instrument transfer line. Fast sample transfer minimizes band broadening, which maximizes efficiency, resolution, and sensitivity. The original GC oven program generated by the *EZ*GC™ chromatogram modeler was translated using the *EZ*GC™ method translator to give a new oven program optimized for the new carrier flow. Method translation is required when changing flow rates in order to keep elution temperatures constant. Changes in elution temperatures between the original and the translated method will sometimes result in drastically different separations or even coelutions, especially on highly selective phases like the Rxi®-624Sil MS column.

- *GC inlet liner choice:* The liner used for this work was a 1 mm straight Sky® inlet liner (cat.# 23333.1). The use of a small internal diameter liner minimizes band broadening by reducing the overall volume of the inlet, again resulting in higher efficiency, resolution, and sensitivity.

- *Split ratio:* A split ratio of 10:1 was used for this work. Although maximum sensitivity is required due to very low expected levels of target analytes, using a split ratio of at least 10:1 ensures high sample velocity through the GC inlet, which minimizes band broadening, increasing resolution without compromising sensitivity. Sharper peaks are taller peaks, so any loss in sensitivity is mitigated through an increase in signal-to-noise ratio.

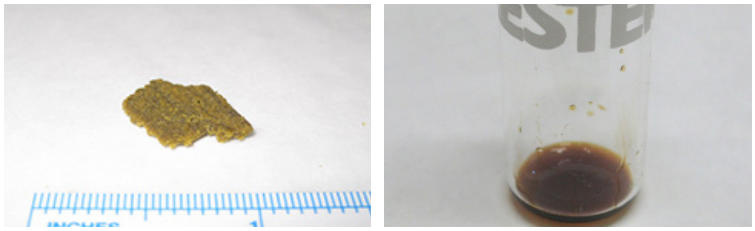
- *Equilibration temperature:* Samples were equilibrated at 140 °C to encourage complete melting of waxy concentrates. By melting the extracts, the ratio of surface area to volume is maximized, ensuring 100% transfer of the analytes of interest into the headspace. The use of a larger sample size will compromise this ratio; therefore, sample sizes should be kept as small as possible to ensure accurate quantification (20 mg is recommended for this application). Representative concentrates are shown in Figure 2. Small samples (20–25 mg) of each concentrate type were placed in a capped headspace vial and incubated for 30 minutes at 140 °C. All concentrates melted completely at the 140 °C incubation temperature, forming a thin film at the bottom of the headspace vial.

- *Equilibration time:* The equilibration time for this method was 30 minutes. This allows enough time for waxy concentrates to melt completely and ensures equilibrium is reached in the headspace vial. Equilibrium is required for accurate and reproducible quantification.

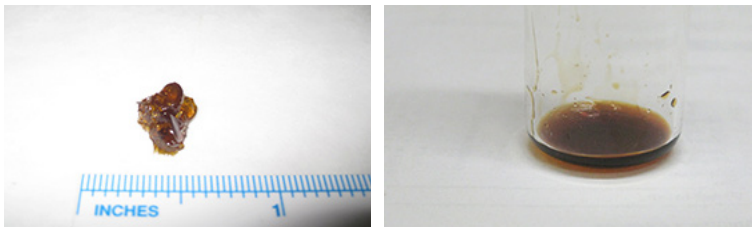
- *Oven program:* The oven program was optimized for speed for this application. In samples that contain terpenes, it is recommended that the oven ramp be extended to 320 °C and the isothermal hold time be extended to 5 minutes in order to ensure complete elution of any terpenes that may be present in the sample.

Figure 2: Cannabis concentrate samples are solid before FET incubation (left) and then melt completely into a thin liquid layer after a 30-minute incubation at 140 °C (right).

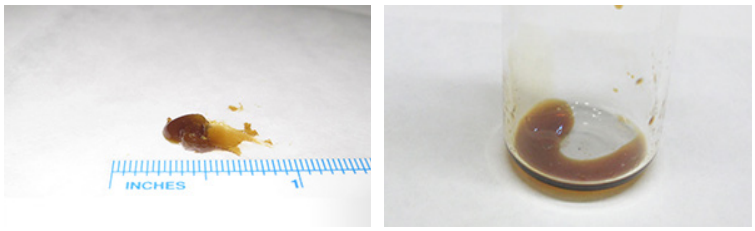
Crumble - Melting point = ~115 °C



Shatter - Melting point = 108 °C



Taffy - Melting point = 102 °C



Photos and melting point data courtesy Cal-Green Solutions

Table I: Commodity and Calibration Standard Curve Equivalency Levels

Concentration in Commodity (ppm)	Amount in 20 mg Sample (µg)	Concentration in 10 µL Standard (µg/mL)
500	10	1,000
250	5	500
100	2	200
50	1	100
25	0.5	50
10	0.2	20
5	0.1	10

Calibration Curve Preparation

When preparing standards for FET headspace GC-FID, it is necessary to calculate the total mass of analyte that will be present in a representative sample, since the equilibrium state results in a single-phase system. For example, a 20 mg sample containing a residual solvent at 50 ppm contains 1 µg of that residual solvent. Therefore, the 50 ppm point in the calibration curve should contain 1 µg of each compound of interest. Since FET headspace GC-FID depends on the establishment of a single phase system, very small volumes are required for standards. The volume used for standards in this application was 10 µL, which was placed directly into a capped headspace vial by injecting it through the vial septum with a clean syringe. Table I presents the 7-point calibration curve standards and their corresponding concentrations in commodity samples.

Standards were prepared in dimethyl sulfoxide (DMSO), which is a less-volatile, later-eluting solvent that does not interfere with the residual solvents of interest. Because FET establishes a single-phase system in the headspace vial without partitioning, it is not necessary to matrix-match standards and samples, which simplifies standard preparation for varied matrices.

The calibration curve was prepared by first making a 1,000 µg/mL stock solution for dilution. The stock solution was prepared as follows:

- Prepare a 5,000 µg/mL stock solution of butane by bubbling butane standard through DMSO on a balance in a fume hood. The butane used for this work was a mixture of butane and isobutane.
- Prepare a 1,000 µg/mL stock solution by adding 2 mL of 5,000 µg/mL butane stock to a 10 mL volumetric flask, adding ~4 mL DMSO, and then volumetrically adding each neat solvent to the flask using a syringe. Volumes required for the 1,000 µg/mL stock standard were adjusted to account for the density of each solvent as shown in Table II.
- After the addition of neat solvents, fill the flask to the line with DMSO and mix by gently inverting the flask three times and rotating to swirl the contents between inversions.

Table II: Density-Adjusted Volumes Used to Prepare 10 mL of the 1,000 µg/mL Stock Solution

Compound	Density (g/mL)	Volume Required (µL)
Butane	measured gravimetrically	2,000
Chloroform	1.48	6.7
Isobutane	NA	2,000
Acetone	0.79	12.6
Methanol	0.79	12.6
Ethanol	0.79	12.7
IPA	0.79	12.7
Benzene	0.88	11.4
Toluene	0.87	11.5
Pentane	0.63	16.0
Hexane	0.65	15.3
Heptane	0.68	14.7

The 1,000 µg/mL stock solution prepared using Table II was used as the highest calibration standard. All other calibration points were prepared in 5 mL volumetric flasks with separate dilutions of the 1,000 µg/mL stock solution. Serial dilution was not used for this work in order to minimize time-consuming syringe rinsing during calibration curve preparation. Because the compounds used here are volatile, work needed to be completed as quickly as possible to prepare the calibration standards. In addition, volumetric flasks were kept capped to minimize evaporative loss. Table III details the preparation of the calibration curve standards.

Table III: Calibration Curve Preparation

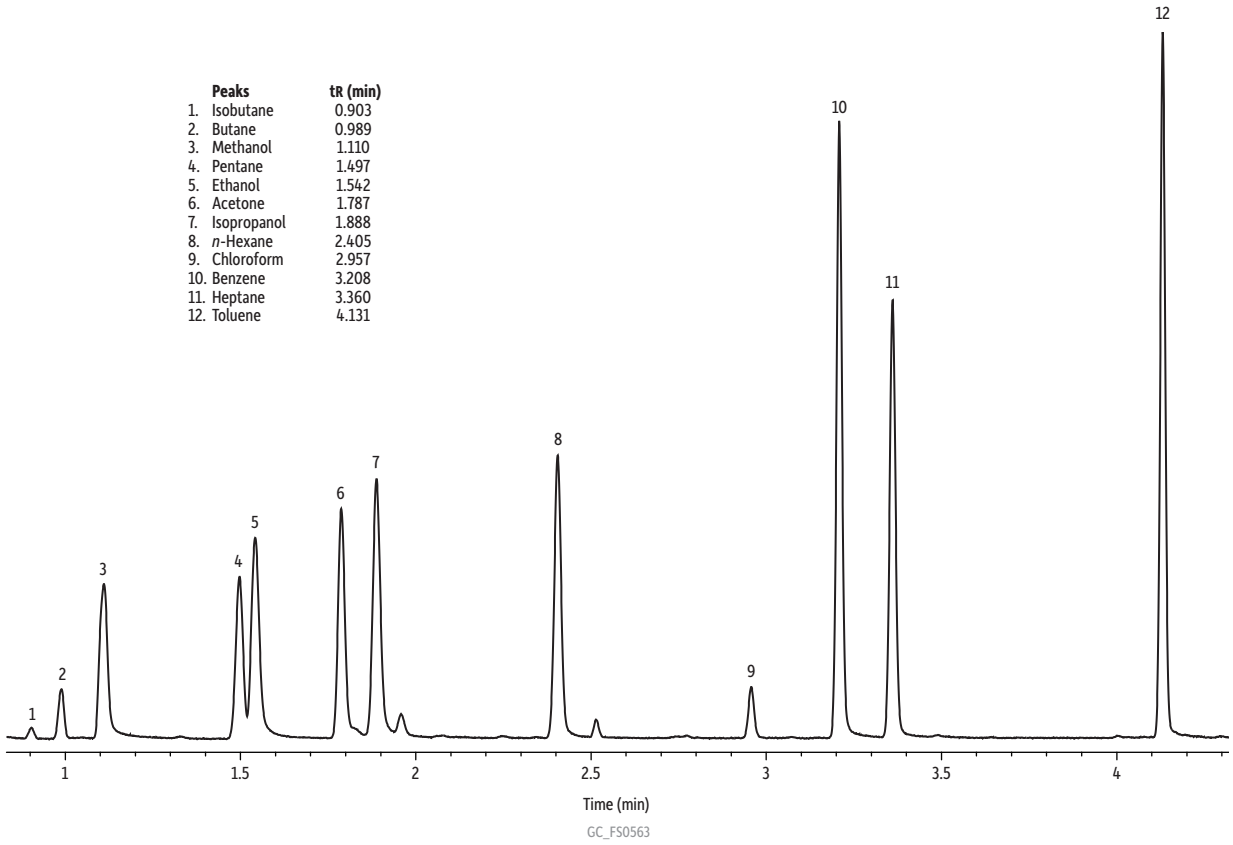
Calibration Level (ppm in Commodity)	Volume of 1,000 µg/mL Stock Solution (mL)	Final Volume (mL)	Final Calibration Standard Concentration (µg/mL)
500	5	5	1,000
250	2.5	5	500
100	1	5	200
50	0.5	5	100
25	0.25	5	50
10	0.1	5	20
5	0.05	5	10

After preparation, all calibration standards were divided into 2.5 mL aliquots and stored in the refrigerator at 5 °C. Since DMSO freezes under refrigeration, calibration standards were allowed to thaw completely prior to use. By aliquoting the calibration standards into separate vials, freeze/thaw cycles were reduced for the entire volume of the calibration solution, allowing for longer storage life of calibration and stock solutions. If desired, calibration standards may be split into aliquots smaller than 2.5 mL to further reduce freeze/thaw cycles. This can be accomplished by pipetting aliquots into gas-tight vials using a glass pipet and immediately capping the vials.

Results and Discussion

Good chromatographic peak shape, separation, and sensitivity were achieved for all analytes of interest. Figure 3 shows the 25 ppm calibration standard. Use of the Restek® Rxi®-624Sil MS column allowed for the separation of the wide variety of solvents that may be present in cannabis concentrates in a short analysis time, while retaining and resolving highly volatile butane isomers. This column was selected for the FET headspace GC-FID method because it was designed specifically for volatiles analysis and is widely used for the analysis of residual solvents in pharmaceutical products. Additionally, the column's unique selectivity also resolves dozens of terpenes [2]. This allows cannabis terpene profiling to be done without changing columns or injection technique, which decreases downtime between methods and improves lab productivity.

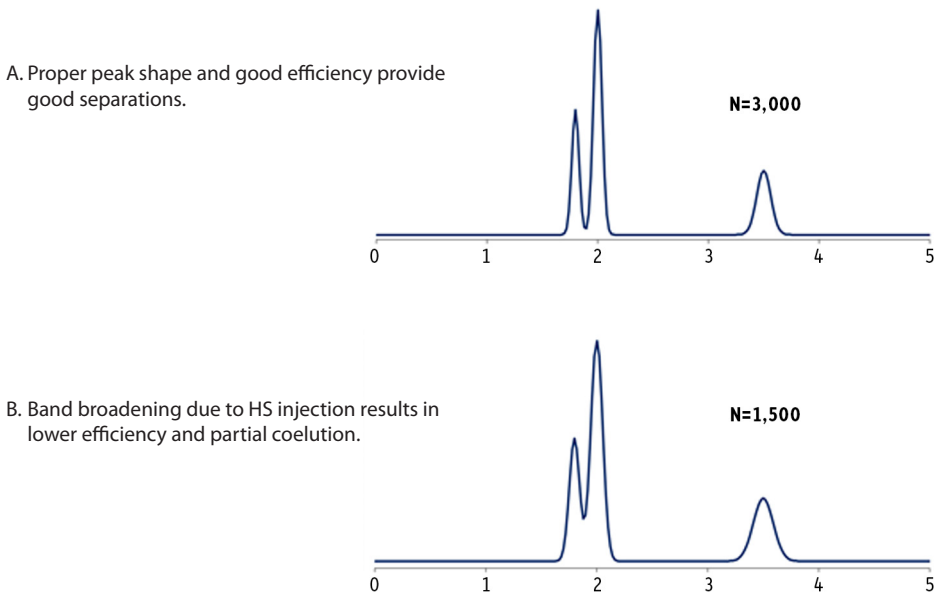
Figure 3: Calibration standard corresponding to a 20 mg cannabis concentrate sample containing 25 ppm of residual solvents. Good chromatographic separation and sensitivity were achieved for common residual solvents.



Column	Rxi®-624Sil MS, 30 m, 0.25 mm ID, 1.40 µm (cat.# 13868)
Sample	Residual solvent mix
Diluent:	Dimethyl sulfoxide (DMSO)
Conc.:	25 ppm (For the HS-FET technique, 10 µL of a 50 µg/mL standard was placed into a 20 mL headspace vial to represent a 25 ppm sample concentration, assuming a 20 mg sample weight.)
Injection	headspace-loop split (split ratio 10:1)
Liner:	Sky® 1.0 mm ID straight inlet liner (cat.# 23333.1)
Headspace-Loop	
Inj. Port Temp.:	250 °C
Instrument:	Tekmar HT3
Inj. Time:	1.0 min
Transfer Line Temp.:	160 °C
Valve Oven Temp.:	160 °C
Needle Temp.:	140 °C
Sample Temp.:	140 °C
Platen temp equil. time:	1.0 min
Sample Equil. Time:	30.0 min
Vial Pressure:	20 psi
Pressurize Time:	5.0 min
Loop Pressure:	15 psi
Loop Fill Time:	2.0 min
Oven	
Oven Temp.:	35 °C (hold 1.5 min) to 300 °C at 30 °C/min (hold 2.0 min)
Carrier Gas	He, constant flow
Linear Velocity:	80 cm/sec
Detector	FID @ 320 °C
Make-up Gas Flow Rate:	45 mL/min
Make-up Gas Type:	N_2
Hydrogen flow:	40 mL/min
Air flow:	450 mL/min
Data Rate:	20 Hz
Instrument	Agilent/HP6890 GC
Notes	The butane used for standard preparation was a mixture of butane and isobutane in an unknown ratio. The concentrations should be considered approximate, but do not exceed 50 ppm for any component.

In addition to using a highly efficient, selective Rxi®-624Sil MS column, it is critical to optimize several GC parameters for headspace analyses in order to prevent band broadening. Early-eluting compounds such as isobutane and butane do not focus on the head of the analytical column, so band broadening through the headspace system and injection port can reduce efficiency, severely impacting sensitivity and resolution for these compounds (Figure 4). As detailed in the Experimental section, band broadening was controlled by using a fast linear velocity, narrow bore inlet liner, and a 10:1 split ratio. This approach speeds up sample transfer and ensures good chromatographic peak shape and response.

Figure 4: Lower efficiency (N) due to band broadening during headspace sample introduction can reduce both resolution and sensitivity (modeled chromatogram).



Analysis of calibration standards resulted in good sensitivity and linear responses for all analytes of interest. Table IV shows the signal-to-noise ratios at 10 ppm and 50 ppm (current Colorado regulatory cutoff values), as well as the correlation coefficients (r values) and coefficients of determination (r^2 values) for all analytes. All compounds exhibited adequate signal-to-noise ratios (> 10:1) at their respective Colorado state regulatory limits. Signal-to-noise ratios were > 10:1 for all compounds at 10 ppm, with the exception of isobutane. The Colorado cutoff for isobutane was 50 ppm at the time of this study; however, prior to publication, Colorado changed the limits and solvents of interest for residual solvent testing. This method will be suitable for the new regulations as well as the older ones.

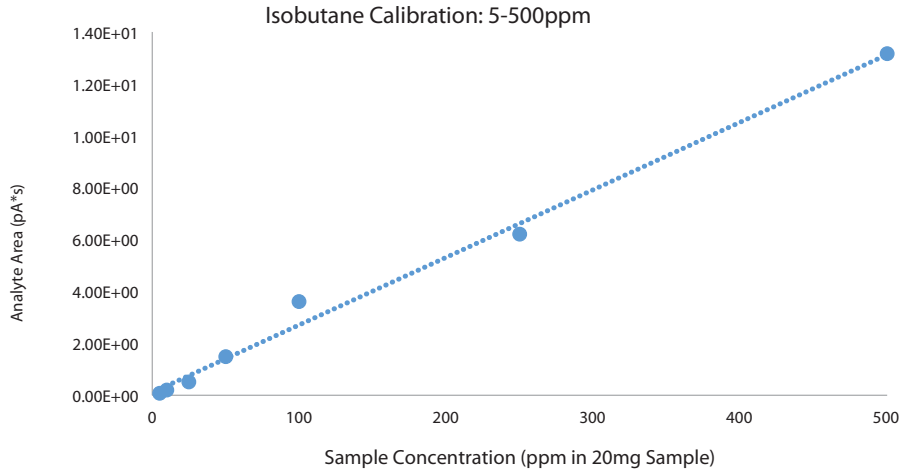
Figure 5 shows plots of the most linear (heptane) and least linear (isobutane) calibration curves. All calibration curves exhibited acceptable linearity without the use of an internal standard. The use of an internal standard may improve linearity and reproducibility, if desired.

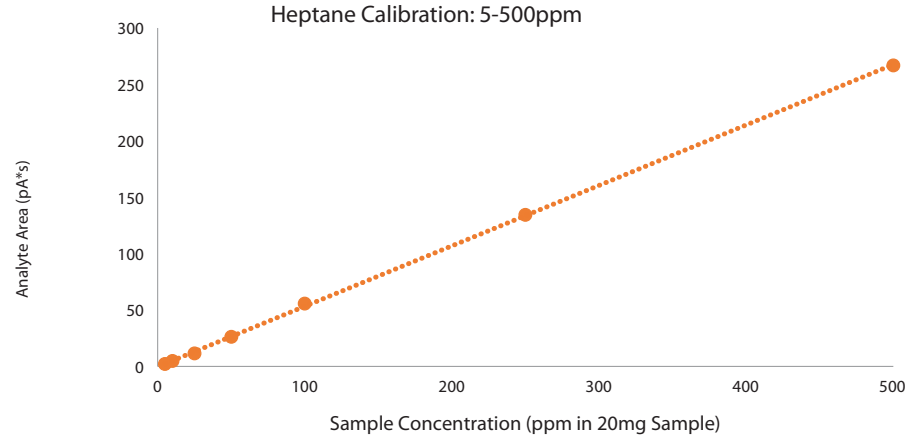
Table IV: Using full evaporation technique sample introduction for headspace GC-FID resulted in good sensitivity and linearity for all residual solvents as shown by peak response and correlation data for the calibration standards.

Compound	S:N 10 ppm	S:N 50 ppm	r	r^2
Isobutane	5.30	30.7	0.996	0.992
Butane	18.8	119	0.997	0.994
Methanol	48.1	189	0.999	0.999
Pentane	19.0	50.0	0.998	0.995
Ethanol	45.2	88.1	0.999	0.998
Acetone	49.9	97.0	0.999	0.999
Isopropanol	56.4	107	0.998	0.996
Hexane	45.6	109	0.999	0.998
Chloroform	11.5	22.5	0.999	0.998
Benzene	150	293	0.999	0.998
Heptane	88.4	193	1.00	1.00
Toluene	166	317	0.999	0.998

*Signal-to-noise ratios were calculated using Chemstation® software. Noise ranges were set at 0.2–0.6 minutes and 2.1–2.3 minutes.

Figure 5: Representative Calibration Curves from 5–500 ppm for Heptane and Isobutane





Conclusion

By combining a selective Rxi®-624Sil MS GC column with the FET headspace GC-FID technique, excellent sensitivity and linearity were achieved for residual solvent compounds applicable to cannabis concentrates. The use of FET headspace GC-FID should allow quantification without the use of matrix-matched standards by creating a single non-partitioning phase system in the headspace vial. This technique also has the added benefit of needing very little sample and is applicable for the analysis of other volatile compounds, such as terpenes, in cannabis products.

References

[1] B. Kolb, L. Ettre, *Static Headspace-Gas Chromatography: Theory and Practice,* John Wiley & Sons, Hoboken, NJ, 2006.

[2] J. Cochran, *Terpenes in Medical Cannabis,* ChromaBLOGraphy, Restek Corporation, 2014 http://blog.restek.com/?p=11451 (accessed July 18, 2014).

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