APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

**Authors:**

**Lee Marotta**

**Tom Kwoka**

**David Scott**

**Miles Snow**

**Toby Astill**

PerkinElmer, Inc.
Shelton, CT

# Fast, Quantitative Analysis of Residual Solvents in Cannabis Concentrates

To further validate the performance of this method for the industry, The Emerald Test Proficiency Test (PT) for Residual Solvents was conducted. The Emerald Test™ is an Inter-Laboratory Comparison and Proficiency Test (ILC/PT) program for cannabis testing labs. The results from the PT inter-laboratory samples passed; therefore, the method meets inter-laboratory reproducibility and accuracy. The method was awarded the Emerald Test Badge seen on the right.
https://pt.emeraldscientific.com/


## Introduction

Compared to the more traditional cannabis flower, cannabis concentrate products, such as extracts, tinctures, edibles, waxes, and oils are becoming the most commonly used cannabis products that are legally manufactured for both medicinal and recreational purposes. Most concentrates are extracted using a solvent such as supercritical $CO_2$, butane, propane, other hydrocarbons, water, or alcohol. These solvents are used to extract out the cannabinoids and terpenes from the plant material.

In some cases, the solvent and impurities from the solvent remain in the extracted material. These are called residual solvents and are the byproducts of the extraction process. In some cases, these impurities can be toxic, which is why residual solvent analysis is a critical element of cannabis testing. The method of choice for measuring residual solvents is headspace (HS) gas chromatography (GC) coupled with mass spectrometry (MS) detection, so false positives are not reported.

PerkinElmer®
For the Better

The major benefit of this approach is that headspace is a fast, simple, accurate and precise technique that allows the components of interest (e.g. residual solvents and terpenes) to be introduced into the analytical system. The non-volatile matrix components remain in the sample vial and do not enter the GC, which results in a mostly maintenance free system, and faster analysis time. In addition, the technique is mature and already has been accepted for quantitation in several regulatory industries including pharmaceutical forensics, environmental and food, where its results have routinely stood up to scrutiny in a court of law.[1-4]

It's also important to emphasize that because there are currently no federal regulations in the U.S., the allowable concentration limits for each residual solvent are defined by the individual state or country where the cannabis is grown. For example, Table 1 shows the list of proposed residual solvents and action levels for cannabis products in the State of California.[5]

This study will focus on the analysis of residual solvents using pressure-balanced headspace (HS) sample introduction coupled with gas chromatography/mass spectrometry (GC/MS). In addition, it will discuss the objective of unambiguous separation of all compounds while maximizing sample throughput.

*Table 1.* List of proposed residual solvents and action limits in cannabis products for the State of California.

| Compound | CA Action Levels (ppm) |
|---|---|
| Propane | 1000 |
| Butane | 1000 |
| Methanol | 600 |
| Ethylene Oxide | 1 |
| Pentane | 1000 |
| Ethanol | 1000 |
| Ethyl Ether | 1000 |
| Acetone | 1000 |
| Isopropyl Alcohol | 1000 |
| Acetonitrile | 80 |
| Methylene Chloride | 1 |
| Hexane | 60 |
| Ethyl Acetate | 1000 |
| Chloroform | 1 |
| Benzene | 1 |
| 1,2-Dichloroethane | 1 |
| Heptane | 1000 |
| Trichloroethylene | 1 |
| Toluene | 180 |
| Xylenes total | 430 |

## Instrumentation

The TurboMatrix™ HS sampler and a Clarus® SQ 8 GC/MS (PerkinElmer Inc., Shelton, CT) was the system used for this analysis. The benefits of headspace sampling are well-recognized in the public domain, but it is essentially a separation technique in which volatile material such as residual solvents and terpenes, is extracted from a heavier sample matrix and injected directly into a GC for analysis.[6] The major reason for using MS detection is that many of the organic compounds associated with residual solvents elute at the same time (co-elute), so the unique mass spectrum of each compound means they can be optimally separated and detected without using additional detectors. Identifying and quantifying all the residual solvents using this solution, results in a faster analysis, enhanced productivity, quicker release of product, and maximized return on investment.

## Experimental

### Sample Preparation After Extraction

Many states require taking multiple sampling points from non-homogenous samples (such as waxes and edibles) to ensure a representative sample for analysis. If this is the requirement, five sampling points from one sample are recommended. For example, if 500 mg is the regulatory requirement for testing, then five - 100 mg portions should be placed in a vial and brought to a final volume of 10 mL with Dimethylacetamide (DMA). Twenty µL of the diluent is then inserted into the HS vial, which is capped and placed onto the HS autosampler for analysis.

However, if an average sampling is not required, a 40 mg aliquot of the extract can be directly weighed into the HS vial; capped; and placed onto the autosampler tray which is the preferred and easiest approach.

### A Turnkey Solution

A fast, accurate, robust GC/MS-HS solution, and SOP, was developed to separate the required analyte compounds in each of the concentrates being tested using the mass and/or time domains for identifying the target compound and then quantifying the specific compounds using the following commercially-available standards (Emerald Scientific, San Luis Obispo, CA).

- California Residual Solvent Mix #1 (Inhalation) reference number STRS01102
- California Residual Solvent Mix #2 (Inhalation) reference number STRS01103

Figure 1 demonstrates the chromatographic separation of all the residual solvents previously listed. To show the benefits of mass spectrometric detection, the chromatographic peaks of two pairs of compounds that co-elute (Pentane/Ethanol at about 3.0 minutes and Benzene/1,2 -Dichloroethane at about 4.8 minutes) exemplifies how they have been further separated by mass. Note, all compounds have been eluted and identified in about seven and a half minutes.

A multi-level concentration suite of standards was prepared which represented the required ranges for quantitation of the sample, and met the required action levels. Repeatability was performed preparing eight vials with 20 µL of the same concentration standard. These data are shown in Table 2, which demonstrates the linearity and precision achieved using this method, together with the method reporting limits and California action levels.

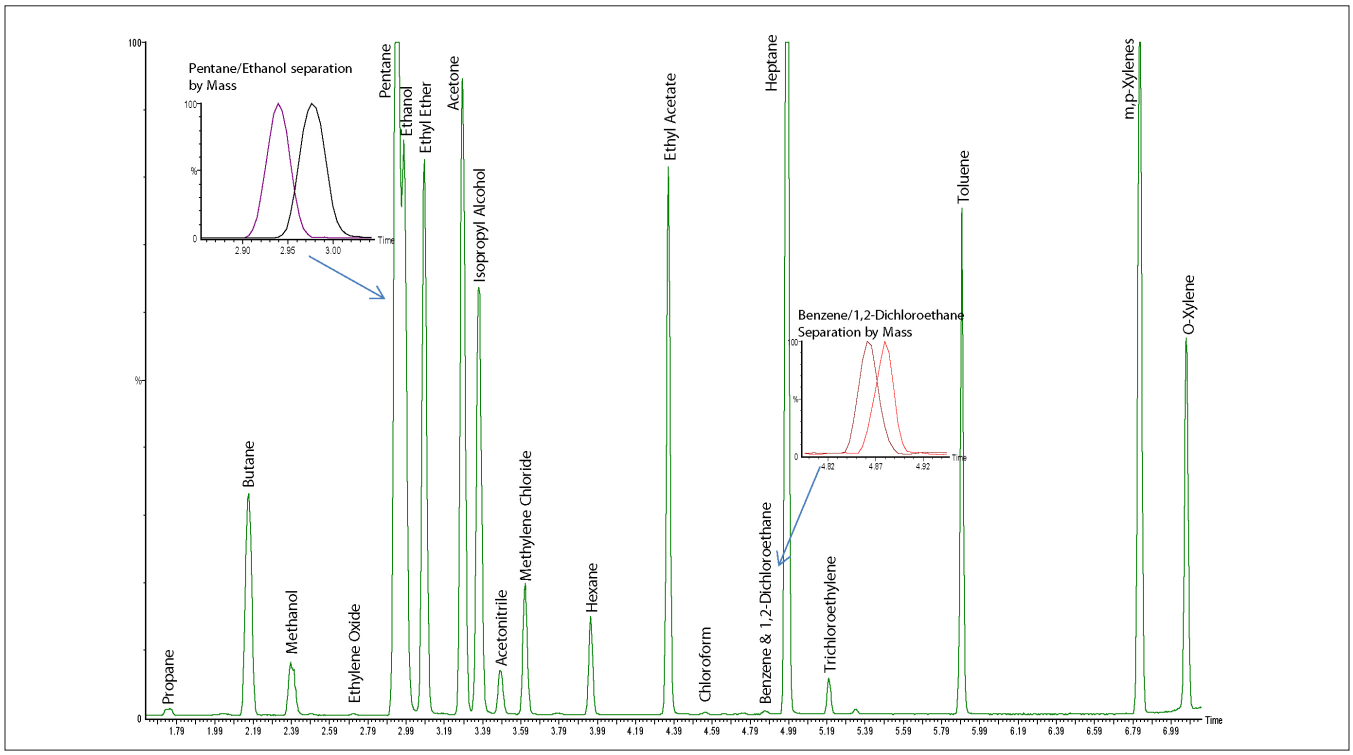



*Figure 1.* Chromatographic separation of all the compounds listed in Table 1.

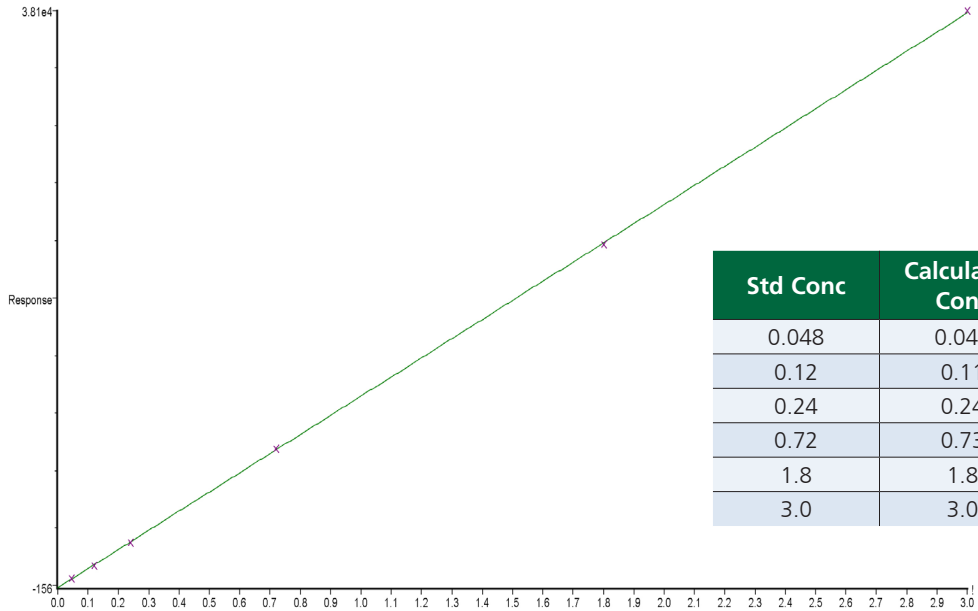
*Table 2.* Linearity and precision achieved using this method, together with the reporting and California action limits for cannabis products. Note: Reporting limits are based on a 1 to 20 sample dilution; therefore if no dilution is carried out, reporting limits are 20x lower.

| Compound | Corelation Coefficient | Precision (n=8) | CA Action Levels (ppm) | PerkinElmer Reporting Levels (ppm)* |
|---|---|---|---|---|
| Propane | 0.9996 | 2.30 | 1000 | 3.20 |
| Butane | 0.9991 | 1.08 | 1000 | 57.60 |
| Methanol | 0.9996 | 1.29 | 600 | 19.20 |
| Ethylene Oxide | 0.9994 | 2.29 | 1 | 1.00 |
| Pentane | 0.9997 | 1.95 | 1000 | 14.40 |
| Ethanol | 0.9997 | 1.41 | 1000 | 19.20 |
| Ethyl Ether | 0.9998 | 0.59 | 1000 | 9.60 |
| Acetone | 1.0000 | 0.94 | 1000 | 14.40 |
| Isopropyl Alcohol | 0.9996 | 1.33 | 1000 | 9.60 |
| Acetonitrile | 0.9998 | 0.45 | 80 | 1.16 |
| Methylene Chloride | 0.9999 | 1.08 | 1 | 1.00 |
| Hexane | 0.9996 | 1.08 | 60 | 0.48 |
| Ethyl Acetate | 0.9999 | 1.02 | 1000 | 6.80 |
| Chloroform | 0.9996 | 1.68 | 1 | 0.60 |
| Benzene | 1.0000 | 1.02 | 1 | 0.96 |
| 1,2-Dichloroethane | 0.9993 | 2.15 | 1 | 0.96 |
| Heptane | 0.9997 | 1.08 | 1000 | 9.60 |
| Trichloroethylene | 0.9998 | 2.12 | 1 | 0.48 |
| Toluene | 0.9998 | 1.46 | 180 | 2.88 |
| Xylenes total | 0.9999 | 0.86 | 430 | 2.88 |

## Discussion of Results

As seen in Figure 1, the chromatographic peaks are well separated with a runtime of about seven and a half minutes and a sample to sample cycle time of less than 11 minutes. Using mass spectrometry, it allows for the identification of components without concern for false positives, while still maintaining extremely fast run times. The two pairs that co-elute in time, ethanol/pentane and benzene/1,2-dichloroethane have very unique spectra and quantitation ions as seen in greater detail in Figure 3. Since quantitation is performed on the mass chromatogram (also referred to as the quantitation ion) of the unique mass, it offers the advantage of interference free integration and quantitation, which would not be possible if the analysis was carried out by flame ionization detection.

Figure 2 displays an example of the calibration curve for the target compound benzene. A table is inserted in this graphic documenting the quantitation of each point using this curve. The % deviation calculated for each point clearly shows excellent correlation with the calibration standards.




| Std Conc | Calculated Conc | % Dev |
|---|---|---|
| 0.048 | 0.049 | 7 |
| 0.12 | 0.11 | -6 |
| 0.24 | 0.24 | -1 |
| 0.72 | 0.73 | 1 |
| 1.8 | 1.8 | -1 |
| 3.0 | 3.0 | 0 |

*Figure 2.* Calibration curve for benzene, showing the % deviation for each standard.

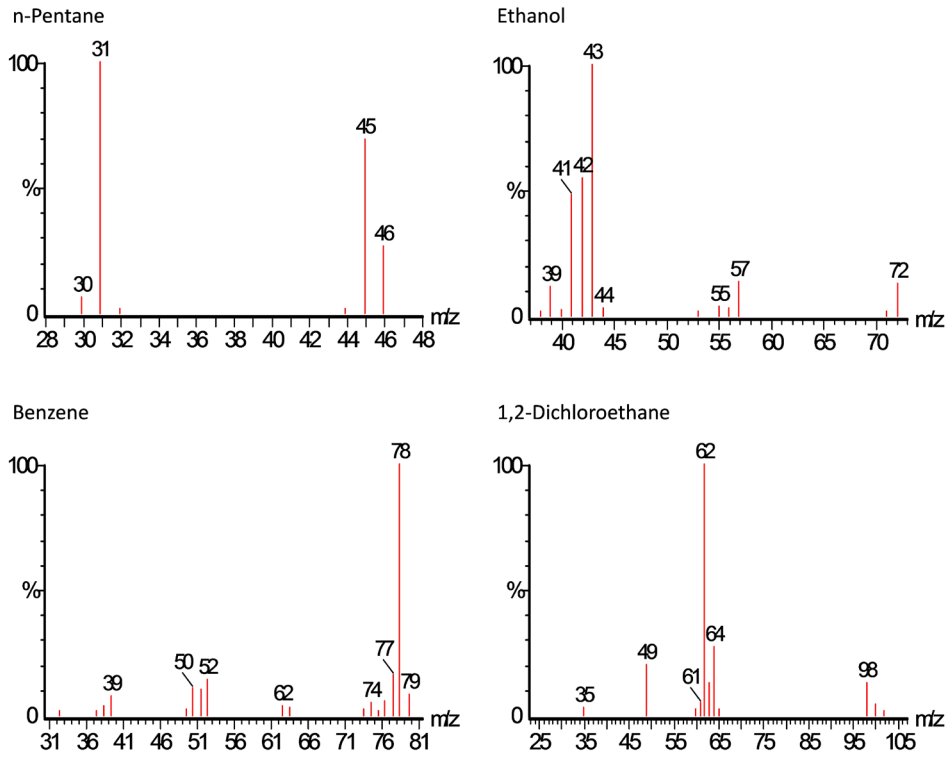



*Figure 3.* The mass chromatogram of two pairs of compounds that co-elute in time, ethanol/pentane and benzene/1,2- dichloroethane, show they have very unique spectra and quantitation ions.

As demonstrated in Table 2, linearity across the compound range is excellent using a multi-level calibration with all targets having a correlation coefficient value greater than 0.9993. The turnkey solution is also precise with the relative standard deviation of less than 2.3 % for all compounds. It's also important to emphasize that the reporting limits are all based upon a 1:20 sample dilution factor; therefore the reporting limit could be improved even further if smaller dilutions or no dilutions were used.

## Conclusion

This study has clearly shown that headspace technology coupled with GC/MS is the perfect solution for identifying and measuring residual solvents in cannabis concentrates and its many products. The benefit of headspace sample introduction over other approaches is that it requires minimum sample preparation, with very little interaction required by the operator. When headspace sampling is combined with GC/MS, it allows for rapid, unambiguous and interference free integrations, with very little likelihood of false positives. In addition, MS provides the ability to identify unknown components that may be present in the sample that are not target compounds. This capability offers significant benefits over a single detector such as flame ionization detection (FID) where a non-targeted compound eluting at the same time as a targeted compound would produce a result which was over the action limit, resulting in a failed batch and a cannabis product not viable for market.

It's also important to emphasize that this technique is fast and capable of quantifying residual solvents in all concentrate samples and other required matrices. Combined with essentially maintenance-free operation, a GC/MS-HS method will enhance productivity and strengthen the lab's business operations.

## References


1. Residual Solvents in Pharmaceuticals by USP Chapter <467> Methodology, David Scott, PerkinElmer Inc. Application Note, https://www.perkinelmer.com/lab-solutions/resources/docs/APP_Residual_Solvents_In_Pharmaceuticals_by_USP_467_013617_01.pdf.
2. Increasing Accuracy of Blood-Alcohol Analysis Using Automated Headspace-Gas Chromatography, John Musselman, Anil Solanky, William Arnold, PerkinElmer Inc.
3. Measuring Environmental Volatile Organic Compounds Using US EPA Method 8260B with headspace trap GC/MS, Heidi Griffith, PerkinElmer Inc. Application Note, http://www.perkinelmer.com/lab-solutions/resources/docs/APP_GasChromaUSEPA8260B.pdf.
4. Monitoring Volatile Organic Compounds in Beer Production Using the Clarus SQ 8 GC/MS and TurboMatrix Headspace Trap Systems, Lee Marotta, Andrew Tippler, PerkinElmer Application Note, http://www.perkinelmer.com/PDFs/Downloads/App_FoodBeerVolatileCompounds.pdf.
5. California Bureau of Cannabis Control – Proposed Text of Regulations, July, 2018; https://cannabis.ca.gov/wp-content/uploads/sites/13/2018/07/Bureau-of-Cannabis-Control-Proposed-Text-of-Regulations.pdf.
6. An Introduction to Headspace Sampling Gas Chromatography Fundamentals and Theory: Andrew Tippler, PerkinElmer Inc. Application Note, http://www.perkinelmer.com/lab-solutions/resources/docs/GDE_Intro_to_Headspace.pdf.



**PerkinElmer, Inc.**
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
**www.perkinelmer.com**


PerkinElmer®




014321A_01 PKI