cannabis science and technology

Top Tips for Success: Residual Solvents and Terpenes Testing

Part Three of a Three-Part Series

Agilent | LAKE SUPERIOR STATE UNIVERSITY

OCTOBER 2021

This sponsored ebook is sponsored by Agilent and presented in partnership with *Cannabis Science & Technology.*

Agilent

Trusted Answers

Dream Bigger

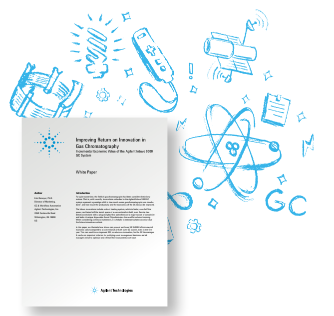
Dreaming of greater revenue for your lab?

Spend smarter—invest in the Agilent Intuvo 9000 GC System and unlock thousands of dollars in annual incremental savings for your lab.

- Ferrule-free face seals for leak-free connections
- No-trim columns so you can change a column in a minute
- Touch-screen user interface gives you instant access to your instrument conditions

All of this adds up to delivering data faster—and delivering more to your bottom line.

Intuvo from Agilent—the power to Dream Bigger. Visit www.agilent.com/chem/intuvo



Intuvo is the key to unlocking substantial savings

Download our white paper—your profitability awaits.

INTRODUCTION

Research indicates that there are at least 140 known terpenes in cannabis, all of which must be accurately quantified in cannabis products. This testing is in addition to the robust package of analytical tests to ensure product safety in this booming market.

Top Tips for Success is a three-part guide (sponsored by Agilent) that covers best practices for laboratories involved in various types of cannabis testing, including:

- Potency and Pesticide Testing, Part 1
- Heavy Metals and Microbial Testing, Part 2
- *Residual Solvents and Terpenes Testing, Part 3*

Each workflow offers tips about samples preparation techniques, methods, quality control, reporting and analysis, instrument maintenance, and more.

Benjamin Southwell
Assistant Professor of Chemistry
Lake Superior State University

INTRODUCTION TO RESIDUAL SOLVENTS TESTING

Residual solvents are chemicals leftover from the extraction of cannabinoids from cannabis raw materials. For consumer safety, residual solvent testing is necessary to ensure that those chemicals do not end up in the final product. As with other cannabis tests, the multitude of matrices, combined with varying regulations, introduce complexity to the analysis. Thus, several aspects of the analytical process must be assessed before beginning any measurements, including sample preparation and storage, appropriate methodologies, analyte recoveries, and reporting. Agilent offers beneficial tools and assistance for every step of the analysis.


roxxyphotos/stock.adobe.com

SAMPLE PREPARATION FOR RESIDUAL SOLVENTS TESTING

Analytical laboratories must work with a diverse array of extracts to make the numerous cannabis-based products on the market. All forms of extracts require residual solvent testing, be they resins, waxes, butters, oils, tinctures, gummies, and many more. Each of the different matrices has inherent analysis difficulties associated with it and unique sample preparation strategies for solvent testing. As such, labs must develop and optimize extraction procedures that are tailored to each matrix. The sample type variability makes it particularly important for a laboratory to be prepared to efficiently work through the method development when a client submits a new sample type.

When working with residual solvents, sample storage is a vital consideration. If an odor can be detected, then volatile analytes are being lost. The result? Inaccurate measurements—unless the situation is addressed with proper storage protocols. Therefore, laboratories should store samples in a way that eliminates volatilization. In some states, samples are collected by the analytical laboratory, which provides an opportunity for them to control the sample environment from collection all the way to analysis. This ensures that the sample being analyzed is reflective of the sample that was collected.

Another issue to think about is that during manufacturing, solvents can create gas vacuoles or other inclusions in products, thereby forming micro-environments that can bias the analyses. It is important to take care in collecting samples so that they are representative and will generate unbiased data.

One common sample preparation technique—full evaporation technique (FET)—is gradually being replaced by more effective approaches. That's because FET, in which the sample is heated to convert all constituents in a solvent to the gas phase, has limitations. The great diversity of cannabis matrices and difficulties acquiring representative samples can lead to unacceptable variability with this preparation method. Other sample strategies are more favorable, such as dissolving the sample and doing a traditional injection. The prevailing headspace (HS) sampling technique interfaced with a gas chromatograph (GC) is the ideal option because it introduces robustness and reproducibility to the analytical process.


roxxyphotos/stock.adobe.com

METHODS FOR RESIDUAL SOLVENTS TESTING

Although the pharmaceutical industry uses *USP* <467> for residual solvent testing, it is not amenable to the analysis of the wide range of cannabis products due to their varied nature. However, it could be used as a good starting place for quality control criteria and method parameters. The responsibility falls on laboratories to develop reliable analytical approaches to meet the relevant state requirements. As extraction facilities get more innovative and develop new technologies, different solvents are being explored, and different efficiencies are being studied. As such, it is essential for laboratories to establish methods that encompass the changing processes of their clients.

For detection, the flame ionization detector (FID) is occasionally employed for residual solvent work. It still has applications in certain parts of the industry, but there are inherent safety concerns with a flame of burning hydrogen in the lab. In addition, although it offers a wide dynamic range, it lacks specificity. Mass spectrometry (MS)-based detection is preferred, due to its specificity, which enables the confident identification of analytes, as well as broader sensitivity and reproducibility. Thus, gas chromatography (GC)/MS systems are now more commonly utilized for residual solvent analysis.

The different state regulations include incredibly wide ranges of limits of quantification (LOQs) and limits of detection (LODs), based on the hazards associated with the chemicals. These varied limits complicate the analytical process, as a wide dynamic range is necessary to include the very low-level compounds as well as the abundant ones. Fortunately, Agilent offers comprehensive, prepackaged headspace-GC/MS e-Methods to ease the burden of method development and accelerate the analysis set-up. Agilent can tailor the final outcome to ensure it is compliant with the laboratory's state requirements. This a very powerful tool for laboratories that need to implement the testing in a rapid manner.

New technologies are being developed for the GC portion of the system in order to replace the well-established GC oven with a faster, more responsive alternative. In the near future, the conventional heated box with a column may give way to a more direct heating technology. This novel approach will lead to significantly shortened run times and more consistent heating. It would benefit laboratories to keep an eye on prospective technologies as they could greatly enhance their analyses.

For reliable data acquisition, the purities of the solvents and gases used for the method are critical. It is imperative that they not introduce contamination that could dramatically affect the integrity of the data, LOQs, and reproducibility.

When examining the results of the method, peak separation is an issue to be considered. A messy extraction solvent can cause problems with the detection of ultra-low-level analytes in the presence of those at high concentrations. Analysts should be aware of this issue and be prepared to ameliorate it according to the requirements for the product being analyzed. Laboratories need to ensure they have sufficient analyte sensitivity to reliably quantitate over the necessary range of LOQs and meet all of the analysis parameters. This is the most difficult aspect of residual solvent analysis.

REPORTING AND ANALYSIS FOR RESIDUAL SOLVENTS TESTING

As labs develop their reporting and analysis strategies, keep in mind that Certificates of Analysis (COAs) reflect the performance of the laboratory. Thus, how they report LOD and LOQ is significant. For example, the reporting of pass/fail values can entail listing an actual number, or simply reporting the presence or absence of an analyte. While some laboratories report all of the analytes, others only list the positives. Currently, there is wide variation in the industry as to how that is handled. Merely reporting the presence or absence of an analyte is ambiguous, as this leads to questions about LOD. It also makes it difficult for an end consumer to

roxxyphotos/stock.adobe.com

compare COAs across different laboratories. The different parameters must be managed by the laboratory in a consistent manner, especially across workflows. Consistency in testing and reporting has the potential to highlight an effective and dedicated science staff.

When a laboratory lists all the chemicals present in a sample, a client may ask about the source of an unexpected substance. It may be present as a contaminant from the analysis, such as a low-purity reagent, or from the extraction process itself. Using the appropriate solvent and gas purities will minimize the potential for contamination in the analytical lab. It should be noted that positive client interactions can go beyond just reporting the data. Successful labs are prepared to offer solutions and opportunities for working together to determine the source of unanticipated sample constituents.


roxxyphotos/stock.adobe.com

QUALITY CONTROL FOR RESIDUAL SOLVENTS TESTING

Analyte recoveries are a vital factor of quality control for residual solvent testing in order to obtain reliable and reproducible lab results. Losing volatile analytes is easy, especially with untrained lab staff. To ensure robust analyses, make sure analytes go into the gas phase and then contain that gas. Oftentimes, that will be related to the type of sample being run. Across all of the different matrices and replicate runs, analysts must check that they see the appropriate concentrations of the analytes. In general, it is much easier to have low recoveries in residual solvent analysis. High recoveries can be indicative of some added component to the process. Knowing this can help with troubleshooting and method development.

Defining acceptable recoveries is difficult, as different states have different requirements, and some states are vague with their specifications. In contrast to environmental methods that specify recoveries ranging from 50% to 150% of the accepted concentration, most states are calling for much tighter controls of the cannabis industry. However, they are sometimes unaware of what that should mean to a particular analysis. Thus, it is important for analytical laboratories to incorporate appropriate, acceptable recoveries in their testing.

As previously mentioned, contamination is always a concern. An interesting situation occurs with ancillary contamination due to the solvent mobilizing other compounds. For example, depending on where the sample collection takes place, a pesticide may be inadvertently extracted. Subsequently, a wide scanning mass spectrometry method may then reveal the unexpected constituent. For this reason, laboratories must decide during method development whether to only look for very specific compounds or open up the MS scanning for a wider detection window.

The various regulatory compliance limits complicate analysis and quality control. The wide variations in these limits across this particular test and across states evolve as new solvents and other process parameters are being explored. If laboratory facilities are being set up in different jurisdictions, verifying compliance for each of those respective jurisdictions is very important.

MAINTENANCE FOR RESIDUAL SOLVENTS TESTING

Maintenance parameters will vary based on the chosen analytical process. With FET or a headspace-based analysis, the headspace sample introduction system will require attention. It comprises numerous parts as well as ovens, transfer lines, and heating systems. GC columns also must be cared for and replaced when necessary. FID detectors need cleaning due to the build-up of residues and contamination over time, while mass spectrometers have ionization sources and lenses that need regular cleaning. Any vacuum pumps involved will require maintenance on a regular schedule as well. Although these maintenance issues may seem small, they are cumulative in nature; one small problem can lead to a larger instrument failure. It is crucial for laboratories to have routine updates to those technologies and a well-defined maintenance plan.

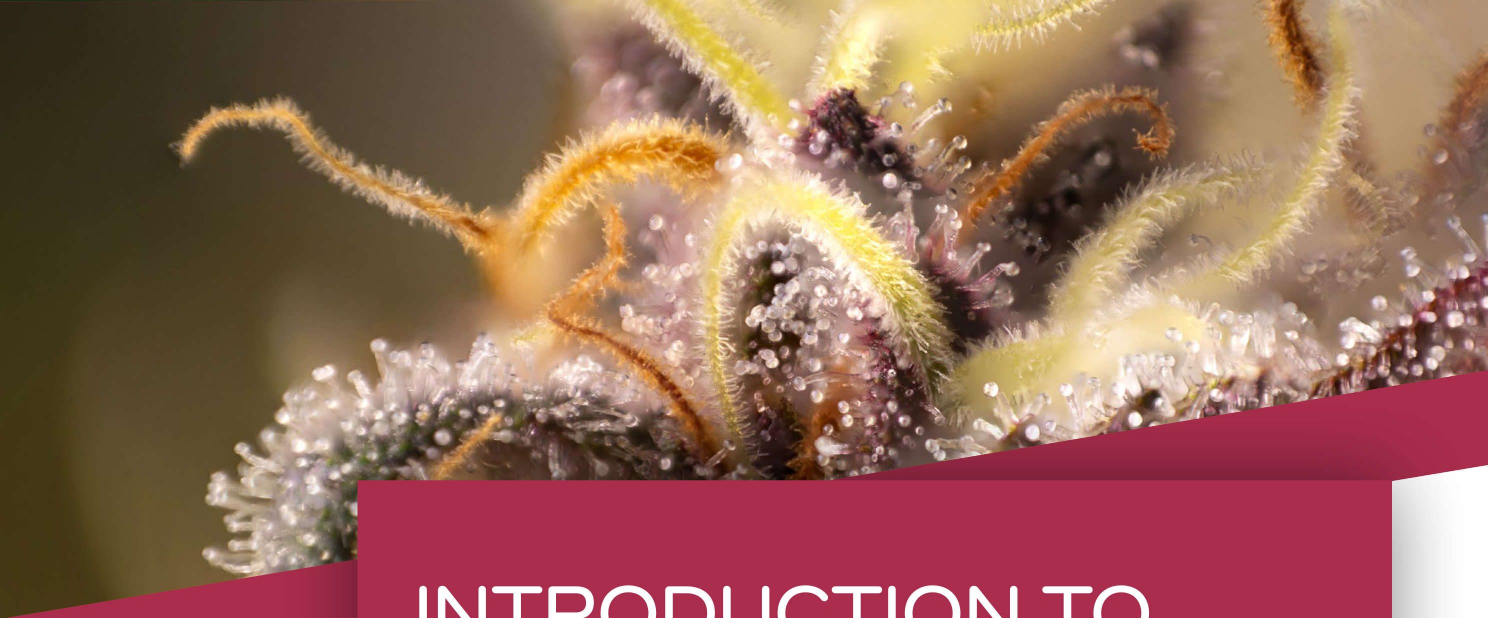
Agilent offers various levels of maintenance agreements that include preventative maintenance, as well as troubleshooting and working through the failure of a component. A laboratory should never be in a situation where the steps to address a failure are not clear. Having a well-understood process can reduce downtime and maximize productivity.

SUMMARY - AGILENT CANNABIS RESIDUAL SOLVENT TESTING SOLUTIONS

Residual solvent testing is necessary to ensure consumer safety in cannabis products. Laboratories must therefore implement stringent testing practices for quantifying these extraction by-products to meet regulatory requirements. Due to its sensitivity, specificity, and robustness, headspace-GC/MS has emerged as the preferred technique to meet the many challenges of the analysis. Although the measurements are complicated by disparate state requirements and wide-ranging limits, several aspects of the analytical process can be addressed to help mitigate some of the difficulties. Accordingly, Agilent has developed the hardware, software, streamlined eMethods, and service offerings to meet the needs of analytical laboratories regardless of jurisdiction.

Choosing a single vendor to supply the technology, software, consumables, and support helps streamline the analytical process and provide stability. Agilent offers all the necessary technology for residual solvent analysis. From GC/MS, headspace, and FID equipment to GC columns and consumables, Agilent covers the entire analytical process. Their optimized eMethods promote faster uptimes and less method validation, enabling labs to go from purchasing an instrument to providing data as quickly as possible. In addition, Agilent's maintenance agreements streamline operations by minimizing downtime. Laboratories not only have access to the expertise of application specialists, but the field service engineer and the team can also be built around a full product service plan to maximize laboratory efficiency.

Agilent's robust residual solvent testing system comprises the 7697 Headspace Sampler, 9000 Gas Chromatograph, and the 5977 Mass Spectrometer. Moreover, it is driven by the powerful MassHunter software. This highly productive headspace GC/MS platform was developed based on the California residual solvent Category I and II target lists and LOQs for empowering labs to efficiently meet regulatory requirements.



INTRODUCTION TO TERPENES TESTING

Blends of various terpenes give cannabis strains their distinctive aromas and flavors. These aromatic hydrocarbons are in a class comprising tens of thousands of volatile and semi-volatile chemicals. As unique terpene compositions appear to be associated with each strain's user experience, regularly reproducing the same terpene profile is essential for product consistency. Thus, robust analytical methodologies are necessary to chemically profile the 140+ known terpenes in cannabis and cannabinoid products prior to use in medicinal and recreational marijuana programs.

Although terpene content is not regulated as a contaminant unless there is a specific label claim, terpenes are frequently analyzed for strain identification, product quality assessment, or volatile organic compound (VOC) compliance. The most common approach to terpenes analyses is headspace-gas chromatography (GC) coupled with mass spectrometry (MS). Issues such as losses of terpenoids have been observed in high-potency cannabis samples using headspace methodologies, such that liquid sample injection is emerging as the dominant sample introduction method.

As in residual solvent testing, the volatility of terpenes complicates their analysis. Therefore, analytical laboratories can employ several strategies for overcoming the obstacles to acquiring accurate results.

SAMPLE PREPARATION FOR TERPENES TESTING

Due to their volatile nature, sample preparation and storage of terpene samples can significantly affect their quantification. Storage is critical to consider; samples should be frozen as quickly as possible after collection. With a recommended temperature of -80 °C as freezing helps preserve the terpenes in a state that reduces their volatility.

Moreover, the use of appropriate gas tight containers is necessary to prevent evaporation of these VOCs from collection to testing. This ensures that the sample composition being measured reflects the actual profile of terpenes

in the plant or product. In addition, keeping the sample frozen during homogenization offers benefits such as tractable consistency. Working with a sample that behaves more like a solid is preferred to dealing with an oily, wet matrix and leads to improved homogenization. If terpene extraction is necessary, the step must be optimized for each sample type, with volatility considered.

The means of sample introduction can also influence terpene assays. Of the two most popular introduction techniques, preparation of a liquid sample for injection by dissolving a sample in an appropriate solvent has been shown to produce more reliable data than the headspace full evaporation technique (FET). The latter can lead to variability in the analysis, as it assumes, sometimes erroneously, that 100% of the terpenes go into the gas phase. Thus, the more traditional injection of a liquid sample is recommended, as it removes the issue of volatility. Well-defined sample preparation and introduction protocols should be in place for each sample matrix.


roxxyphotos/stock.adobe.com

METHODS FOR TERPENES TESTING

A wide array of terpenes may be studied in cannabis and its related products. In the United States, only a few states specify terpene profiling in their required testing platforms, however. As analytical laboratories decide which terpenes to measure, client communication is vital because certain terpenes may be of special interest due to their purported effects on product properties. Having a clearly defined testing platform is appreciated by clients and can be a distinguishing point between cannabis analysis labs.

GC/MS is typically used for terpene analysis with a single quadrupole mass spectrometer. Flame ionization detection (FID) can be used; however, it is not specific and is therefore losing popularity for this application. Mass spectrometers offer an advantage because their specificity allows the identification of distinct terpenes in a sample. As terpene concentrations often cover a wide range, such as ppt to percent quantities, the measurement system must allow a wide dynamic range. Accordingly, the sample preparation strategy and instrument must be designed to achieve a sufficiently large range to ensure reliable quantification.

For the highest quality profiling, suitable standards and reference materials should be present in a similar matrix to the one being tested. Known as matrix matching, this approach helps expose any issues with the method that may otherwise go unnoticed. Unfortunately, this is difficult to do for terpenes analysis due to federal regulations on the cannabis industry. Agilent methods resolve this issue by using hemp seed oil as a blank matrix for calibration standards because it is readily available, terpene-free, and not regulated. Another means of minimizing measurement-related difficulties is by using high-purity solvents and carrier gas. Lower quality reagents may save money initially, but ultimately lead to increased expenses due to contamination and troubleshooting.

Developing reliable testing protocols for complex cannabis matrices can be challenging. Prepackaged methodologies known as eMethods are available from Agilent to accelerate a lab's startup time by condensing vast amounts of technical information and optimized analytical methods into a ready-to-run package. The terpene eMethod is designed for the analysis of 40 mono- and sesquiterpenes commonly found in cannabis. The package defines sample preparation and an analytical method for the separation and reliable detection of the targeted analytes in less than 30 minutes. A list of expertly selected consumables and supplies is included as well. The analysis is performed by direct liquid injection of the extracts of cannabis, hemp, and cannabinoid products using the Intuvo 9000 GC interfaced with the 5977B MSD and controlled by MassHunter software. Application Note 5994-2032EN demonstrates the selective, sensitive, and robust method. Agilent's eMethods offer a substantial advantage as they streamline the implementation of cannabis testing, reduce risk, and increase productivity in testing laboratories.

REPORTING FOR TERPENES TESTING

Although no states mandate terpene reporting, some require tests to be listed if they were performed. It is important, therefore, for analytical laboratories to understand the requirements for their particular region and compliance body. Regulations for the cannabis industry are still evolving, such that the guidelines for each state or region could change and should be monitored.

Labs should be consistent and up front with their clients about which terpenes are being analyzed and how the data will be reported. In addition, the widely varying analyte concentrations call for a logical reporting strategy. Upper and lower limits of quantification (LOQs) should be clearly communicated, and units that convey the appropriate concentrations should be used. For example, expressing a wide range of concentrations in percentages can lead to confusion regarding the analytes that are present at lower concentrations; their abundance may appear to be near zero when that is not the case. As such, actual concentrations should be used with a suitable number of significant digits, rather than percentages. Overall, reporting should be formatted in such a way that they are easily understood by clients.

QUALITY CONTROL FOR TERPENES TESTING

Quality control can be a difficult issue when volatile compounds are involved. It is critical to ensure that VOCs are captured and analyzed accurately in order for the results to reflect the actual sample that was collected. Obtaining adequate recoveries of the analytes can be challenging, especially with poor sample preparation protocols or insufficiently trained staff. For example, new employees may not recognize that simply placing a vial on a warm lab bench or using a regular pipettor instead of a gas tight pipettor can affect the data. Thus, procedures must be very specific regarding what tools to use and where to prep the sample.

It should also be noted that the sample introduction strategy can cause bias in the results. Quantification using headspace FET tends to be biased lower than liquid sample injection, as gas bubbles may be embedded in a sample and not come to the surface during the headspace step. In contrast, liquid sample preparation recovers the inclusions for more accurate results. Understanding these differences during analysis and reporting are essential.

While terpene reference materials are commercially available, they are not specifically related to cannabis plant materials or their derivatives. NIST and other organizations are still pursuing this to facilitate matrix matching for more accurate testing. The current federal prohibition on cannabis products is dramatically limiting what types of quality control reference materials are available for analysis. In relation to the available terpene references, there are particular considerations to be addressed by analytical labs. For example, the volatility of the analytes can cause some compounds to be lost while sitting on a shelf waiting to be used. As such, buying small glass ampules of the materials helps combat evaporative loss. Ultimately, analysts must be confident that the references have not been compromised.

MAINTENANCE FOR TERPENES TESTING

As in residual solvent analysis, the gas chromatograph and its accessories, as well as the headspace introduction system, if utilized, require routine maintenance. In addition, the mass spectrometer's ionization source needs frequent cleaning, and most vacuum pumps demand regular attention. Extra consumables should always be on hand in the laboratory for rapid replacement. All of these common, less serious maintenance issues are typically performed by laboratory personnel.

There are times when an instrument manufacturer's expertise is needed for more involved maintenance or a major repair. All major manufacturers offer service agreements that typically include regularly scheduled maintenance that is beyond the skillset of laboratory employees, as well as large repairs on an as-needed basis. Since laboratories usually only have one instrument for terpene analysis, downtime can be costly with no backup equipment. A service agreement minimizes downtime and gets the lab up and running again quickly. If a service contract is declined, a prudent maintenance plan should be developed to deal with time-consuming repairs and delays in reporting data to clients. Laboratories engaging with Agilent for service agreements receive fast response times from world-class professionals.

SUMMARY - AGILENT CANNABIS TERPENES SOLUTIONS

Terpene profiling, while not regulated, can nonetheless be a valuable tool for understanding the properties of cannabis and its related products. Given the large number of compounds and their volatility, a judicious analytical strategy is needed. GC/MS with liquid sample injection is the preferred technique for the analysis, as limitations have been observed with headspace introduction. Analytical laboratories typically choose which terpenes to analyze and how to report them after interacting with their clients to determine their specific needs. As with other cannabis workflows, monitoring and complying with changing jurisdictional regulations is essential. Choosing a single vendor to supply the technology, software, consumables, and support helps streamline the analytical process and provide stability. Conveniently, Agilent offers everything labs need for streamlined, reliable terpene testing, from start to finish.

The Agilent Intuvo 9000/5977B GC/MS system's excellent sensitivity and robustness make it the ideal configuration for terpenes analysis. The GC's ballistic direct-heating technology ensures reproducible chromatography and allows for higher throughput in the laboratory. In addition, Intuvo's built-in intelligence reduces operational and maintenance costs through its selfguided diagnostic troubleshooting and early maintenance feedback. With the incorporated MassHunter software, analysts can transform data into insight with intuitive qualitative and quantitative analysis tools.

Agilent has already done the hard work of developing optimized methods for cannabis analysis. The terpenes eMethod streamlines testing implementation by providing recommended system configurations and an optimized analytical method as well as detailed information on sample preparation, consumables, and supplies. All of the information needed to start turning samples into results is delivered in a ready-to-run package. Thus, Agilent's terpenes analysis solutions provide trusted answers for the cannabis industry.

SPONSORED CONTENT

Terpenes Testing for Cannabis & Hemp; Terpenes Analysis

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.

DE44433.4229976852

Source of New Possibilities

The Agilent 5977B GC/MSD system

Improve your sample throughput, analytical performance, and business outcomes with the Agilent 5977B GC/MSD system. It's built for labs that focus on applications such as environmental, chemical, petrochemical, food, forensic, pharmaceutical, and material testing. This powerful GC/MSD builds on a long track record of innovation, bringing together the technologies of one of the industry's best GC and MS systems. Agilent has pushed the technological frontier even further by introducing the high-efficiency source (HES). What's more, the system's proven robustness and reliability mean less maintenance and more uninterrupted productivity.

www.agilent.com/chem/5977b

DE44454.4036805556

Printed in the USA, August 19, 2022

5994-5214EN