WHITE PAPER

RESIDUAL SOLVENTS IN TERPENES

Solvent False Positives and the Need for Standardization

There are currently no standardized testing methods for laboratories to use when testing terpenes and other aromatic compounds for residual solvents. The most popular method used today, headspace analysis, is ill-suited for the safety testing of terpenes and will often return false positives for solvents as a result.

This is due to how the technique is performed. In simple terms, headspace analysis requires the sample to be cooked at a high temperature for an extended period of time in order for the analysis to be conducted. Anyone who is familiar with cannabis knows that heat, air, and light will quickly degrade it.

By heating terpenes to anywhere from 70°C to 200°C, you are causing a chemical reaction that converts and degrades the sensitive compounds into undesirable solvents that were not present in the original formulation. By using headspace analysis, labs are first degrading the terpene samples and then testing the degradants which, of course, leads to inaccurate results. These tests are not representative of real-world usage and are not indicative of the original formulation's quality or safety.

Thankfully, there is an alternative method that does not require the sample to be overcooked and can provide much more accurate testing results. With the federal legalization of cannabis on the horizon, testing terpenes for residual solvents is sure to be a topic of increasing interest.

Read on to learn about alternative testing methods, the drawbacks of headspace analysis, and how cannabis testing is currently regulated.

The Current State of Cannabis Testing and Regulations

It's important to remember that the business of cannabis is still relatively new and testing regulations are not federally standardized in the United States. Instead, testing regulations are determined and enforced on a state-by-state basis, leading to a wide range of standards and requirements.

Terpenes are currently screened for residual solvents utilizing techniques adopted from other industries such as food and cosmetics, and therefore are not directly tailored to meet the unique properties of terpenes and cannabis.

A variety of chemical research associations are working to establish standardized testing regulations, but scientific advancements in the field need to be taken into consideration while forming these regulations. Therefore, scientists and policymakers will need to adapt quickly as new best practices come to light.

So how exactly is residual solvent analysis currently carried out in cannabis testing labs?

Current Methodologies for Solvent Testing

Residual solvent testing is accomplished through an analytical technique known as gas chromatography (GC). Gas chromatography is best for compounds which evaporate readily into the air, making terpenes a great candidate for this type of analysis. Chromatography is a method of separating compounds based on the physicochemical properties of molecules in the sample. This separation creates distinct fractions which can then be further analyzed and quantified.

Gas chromatography instruments are typically coupled with an analysis instrument such as a mass spectrometer (MS) which allows labs to accurately quantify the amount of a certain solvent in a sample. The total setup used in solvent testing is therefore referred to as a GC/MS, or Gas Chromatography/Mass Spectrometry instrument.

Here is where terpene solvent testing issues arise.

There are two common ways to introduce a terpene sample into a GC/MS system. The direct injection method is more accurate, but the headspace analysis method is easier to run and more widely used when testing cannabis. The choice of technique can have a significant impact on the final results.

When the GC/MS receives some amount of the sample for analysis, this is referred to as an injection. This sample can be introduced in two ways: it can be directly injected into the instrument using a syringe, aptly termed 'direct injection,' or it can be extracted from a 'headspace vial.'

A headspace vial is a compact glass vial utilized for sample analysis. The terpene sample is placed inside the vial which is then sealed with an airtight cap, equipped with a septum. This process traps both the sample and the surrounding air inside the vial. The vial is then situated in a heated chamber to facilitate sample evaporation into the contained air, the 'headspace.' Upon heating, a syringe pierces the septum, withdrawing the injection for further analysis. An automated robot mechanism, known as an autosampler, facilitates this movement of the syringe, rendering the procedure largely automated.

Efficiency is paramount in cannabis testing labs; hence, both direct injection and headspace utilize automated platforms to expedite sample analysis. Oftentimes, labs choose headspace over direct injection for cannabis products due to the lower maintenance requirements associated with the injection port, leading to the widespread use of headspace sampling for residual solvent testing. While the reduced maintenance time can be an advantage, it's important to acknowledge that it may also pose certain drawbacks.

The Impact of Headspace Vial Sampling Techniques on Terpenes

Given the delicate nature of terpenes, the method of introducing them into the GC/MS can significantly influence the outcome. While many cannabis testing laboratories employ headspace vial sampling for its efficiency, this method may inadvertently modify the terpene molecules prior to testing. This is largely due to terpenes' heightened sensitivity to heat.

In the case of headspace vial injections, the terpene sample is subjected to high temperatures prior to injection, ranging from 70°C to 200°C. This level of heat exposure can alter the final chemical composition of the sample, potentially leading to its degradation and triggering unforeseen chemical reactions.

The effects of this thermal exposure are thoroughly explored in a 2022 article by Elzinga et al., titled "Acetone as Artifact of Analysis in Terpene Samples by HS-GC/MS."[1] The appended "HS" in the acronym stands for "headspace," indicating the widespread injection technique under discussion. This article delves into the challenge of identifying residual solvents such as acetone and methanol when conducting solvent tests on pure terpene samples using HS-GC/MS.

In their research, Elzinga and colleagues initially address the demand for enhanced standardized testing regulations. They note that prevailing testing methodologies "often inflate the presence of solvent signals and deflate the presence of valued ingredients."[1] Furthermore, they shed light on the fact that "detected acetone signals are a common artifact of terpene degradation in the presence of oxygen."[1]

The presence of oxygen in the headspace vial can lead to the development of residual solvents in the sample. The authors scrutinized the influence of oxygen and other chemicals in a headspace vial on the results derived from HS-GC/MS analysis. They established a negative control in which oxygen was absent by replacing the air in the headspace vial with argon, an inert gas. This led to significantly less residual solvent formation in most terpene samples.[1]

The researchers also experimented with the presence of antioxidants. Some natural antioxidants used in the study include tocopherol (Vitamin E) and cannabinoids like CBD, CBDA, THC and THCA.[1] Such antioxidants are recognized for their capacity to neutralize free radicals, which are molecules possessing unpaired electrons. These molecules have the potential to cause chemical reactions, including the reaction which converts terpenes into residual solvents with exposure to heat. Samples containing both antioxidants and terpenes offer a more authentic depiction of cannabis products presently available on the market, owing to the antioxidant properties of cannabinoids. In this experiment, the researchers found that the presence of antioxidants also significantly reduced the formation of residual solvents in the sample.[1]

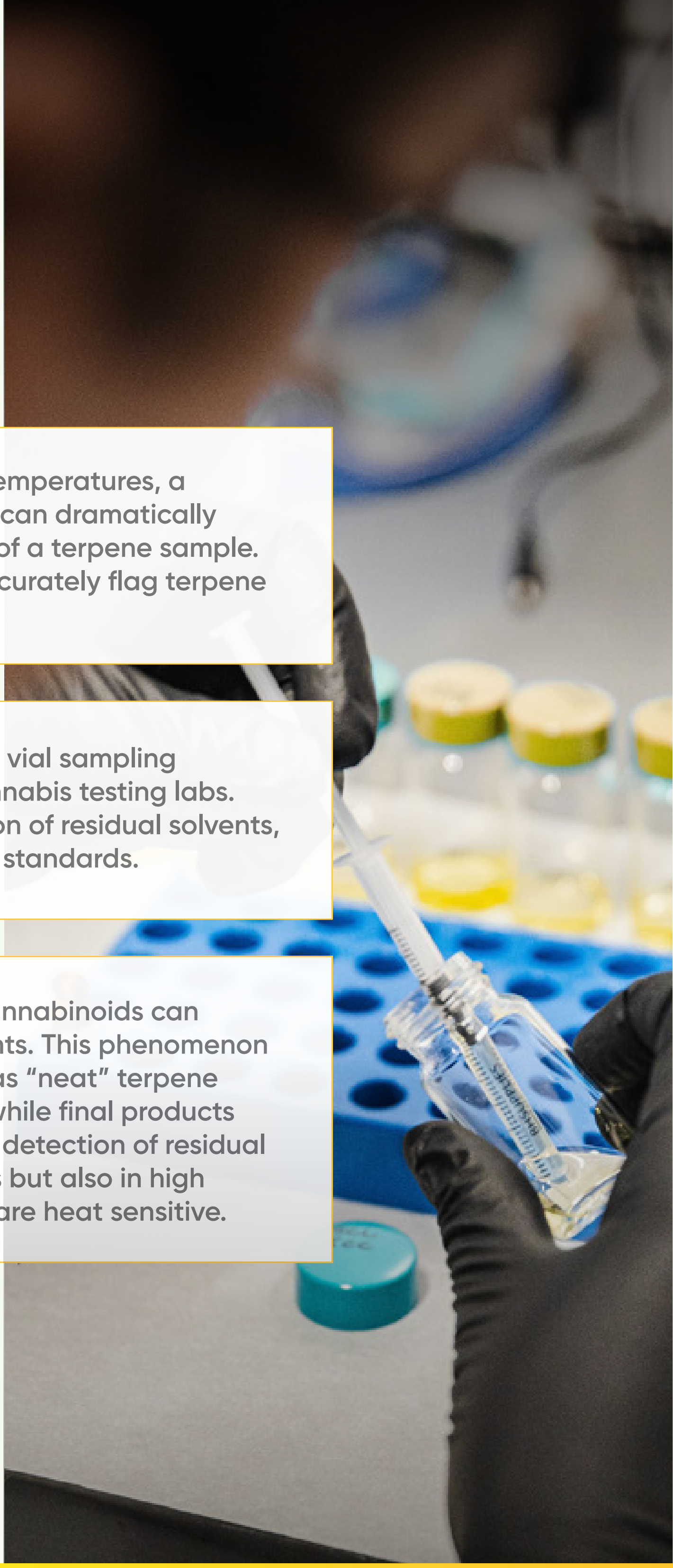
Conclusions

The results of this study raise three important points relevant to testing terpenes for residual solvents.

Firstly, the long-term exposure of terpenes to high temperatures, a requirement of the headspace sampling technique, can dramatically and unintentionally alter the chemical composition of a terpene sample. Consequently, the headspace technique could inaccurately flag terpene samples for the presence of residual solvents.

Secondly, the presence of oxygen in the headspace vial sampling technique can influence the results procured by cannabis testing labs. This presence of oxygen could catalyze the formation of residual solvents, causing pure terpene samples to fail the set testing standards.

Lastly, the analysis of terpenes in the presence of cannabinoids can significantly impede the formation of residual solvents. This phenomenon elucidates why pure terpene samples, also termed as “neat” terpene samples, may appear to contain residual solvents, while final products containing the same terpenes do not. This aberrant detection of residual solvents can occur not only in neat terpene samples but also in high terpene extracts and other cannabis matrices that are heat sensitive.



Chemists must weigh the benefits and drawbacks of testing methodologies in order to develop testing regulations which can be standardized with the help of chemical associations, such as ASTM. This study underscores the necessity to formulate an analytical method that accurately screens terpene samples for solvents and prevents the inadvertent creation of solvents during testing. This is a very specific need within the cannabis industry that is not as important for some product types versus others, but none the less can lead to significant consequences for manufacturers. Fortunately, dedicated chemists like those at Abstrax are vigilantly monitoring such evolving issues and aim to continue enlightening formulators on their journey towards creating safe products for consumers.

Works Cited

1. 1. Elzinga, S., Dominguez-Alonzo, J., Keledjian, R., Douglass, B., & Raber, J. C. (2022). Acetone as Artifact of Analysis in Terpene Samples by HS-GC/MS. Molecules (Basel, Switzerland), 27(18), 6037. **https://doi.org/10.3390/molecules27186037**