ABSTRAX TECH

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The Science of Dank

Discovery of New Cannasulfur Compounds

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Introduction and Background

We have all smelt it – that distinctive, gassy, or skunk-like scent of cannabis. But what exactly is that smell? Researchers at Abstrax Tech, in collaboration with some of the top cultivators in the industry and analytical experts, have now answered this question — and the answer may surprise you.

Cannabis produces arguably some of the most unique and potent aromas out of any plant. It seems that everyone has a nose for it, whether they indulge or not. Figuring out what makes this plant smell the way it does is no easy feat, however. A survey of cannabis literature makes no correlation toward the "skunk-like", or "gassy" aroma that it is so infamous for, highlighting the difficulty in determining the origins of this scent. This has now changed, however, as research published in November 2021 by Abstrax Tech now unlocks the mysteries of this part of the plant, revealing an entirely new class of compounds that contribute to this scent.

In recent years, much focus within the cannabis industry has shifted toward terpenes – whether from a research or marketing standpoint. These compounds are the major components of the aroma of cannabis by weight and are regularly tested for by cannabis analytical labs. They include ß-myrcene, α- and ß-pinene, linalool, ß-caryophyllene, and myriad others. These compounds are being heralded as the true driving force between different psychoactive effects in cannabis in conjunction with THC, colloquially known as the Entourage Effect. They are also considered convenient biomarkers for establishing genetic classes between different phenotypes or chemotypes within the cannabis phase space. However, these compounds do not create the unmistakable "gassy" or "skunky" scent that many high-quality cultivars are so prized for. In fact, we argue that until now, what some scents many consumers and experts say is due to terpenes, we now posit is incorrect, and is based on an incomplete picture of the aroma of cannabis. Yes, terpenes indeed contribute to the bouquet of cannabis' aroma, but we now know that some of the most coveted scents are not from these commonly known terpenes at all.

Research conducted by Abstrax Tech between 2019 and 2021 has identified an entirely new class of previously unknown compounds that are the true origins of the "gas, skunk, and dank". We refer to this new class of compounds as "**cannasulfur compounds (CSCs)**", due to the presence of sulfur in each of them (**Figure 1**). Each of these compounds contain the prenyl functional group bonded to a sulfur atom. Some are thiols, others are sulfides, and yet others are disulfides. These compounds all have extremely low odor thresholds, as well as have moderate-to-high volatilities – the perfect recipe to be detectable to the human nose over large distances in low quantities. Below we discuss how these compounds were found, their aromatic and possible therapeutic properties, and future work needed to further understand these unique compounds.

Figure 1

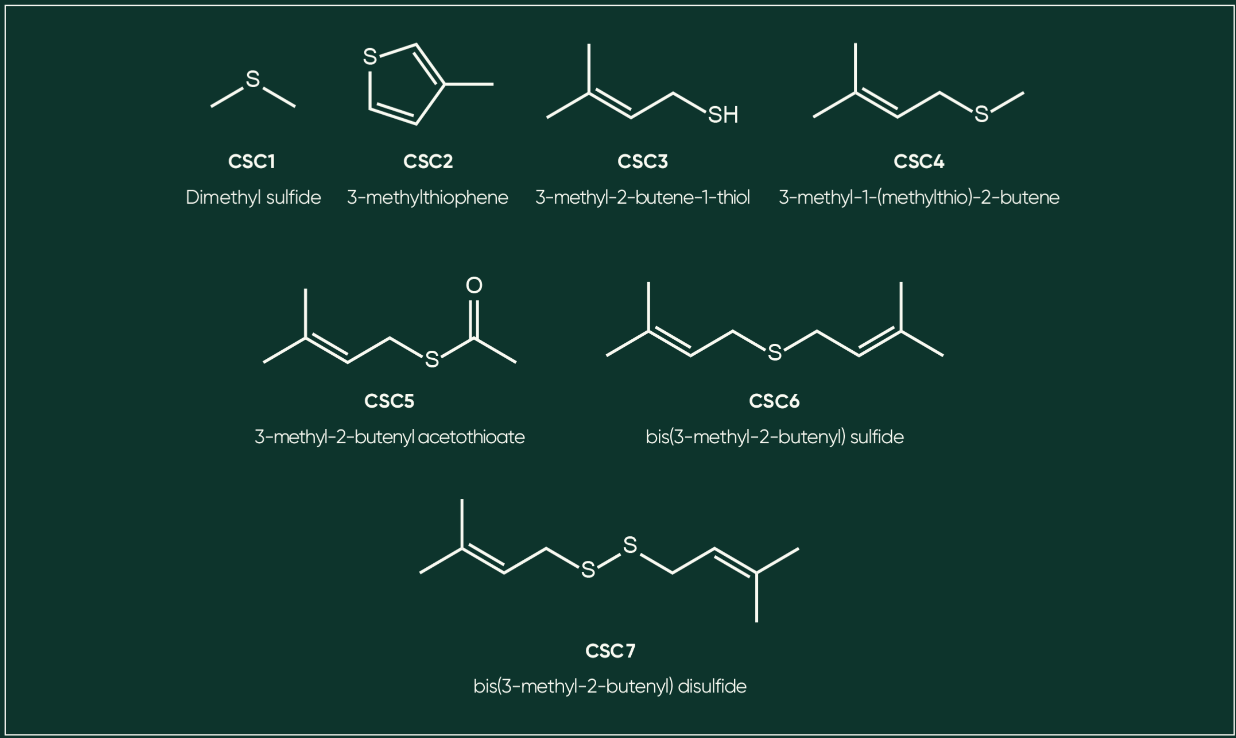
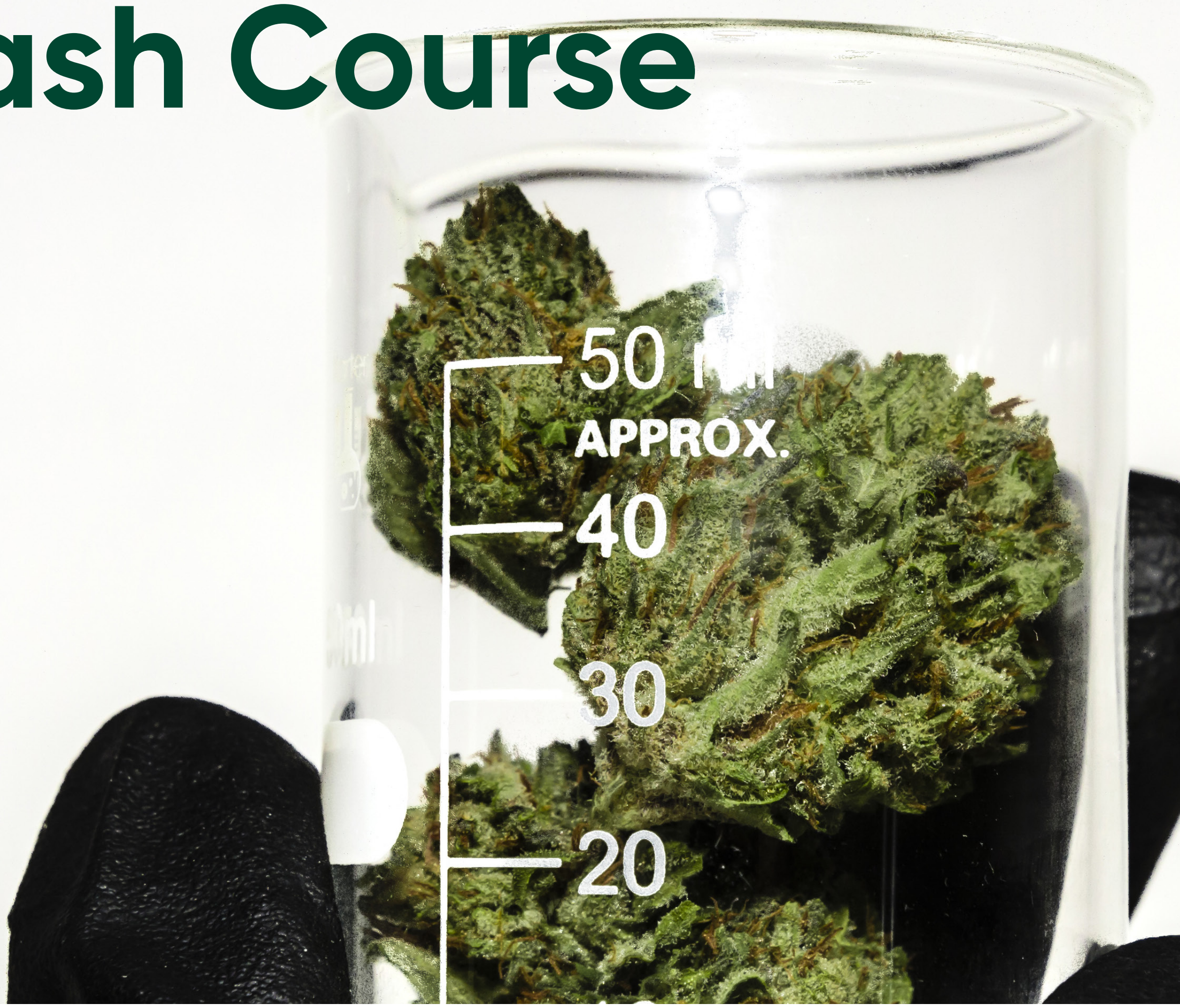


Figure 1. Newly discovered cannasulfur compounds (CSCs) in cannabis. Compounds CSC3 - CSC7 contribute to the skunky or gassy aroma of cannabis.

Finding Cannasulfur Compounds in Cannabis

Abstrax Tech has been intensively researching cannabis since 2019, aimed at understanding the nuances of the many aromas this plant can produce. The research and development team first teamed up with Markes International and SepSolve Analytical, two instrument manufacturers that specialize in multidimensional gas chromatography, to develop the ultimate analytical tool for decoding the scent of cannabis. With the experts on the instrumentation side, a 2-dimensional gas chromatography instrument with three detectors operating simultaneously was constructed. Each detector was chosen to serve a specific function: Mass spectrometry was used for chemical identification, flame ionization for quantitation, and sulfur chemiluminescence for detection of sulfur-containing species. These three detectors, in conjunction with the enhance separatory power of 2-dimensional gas chromatography, gave the research team the necessary firepower to fully understand the complex aroma of cannabis.

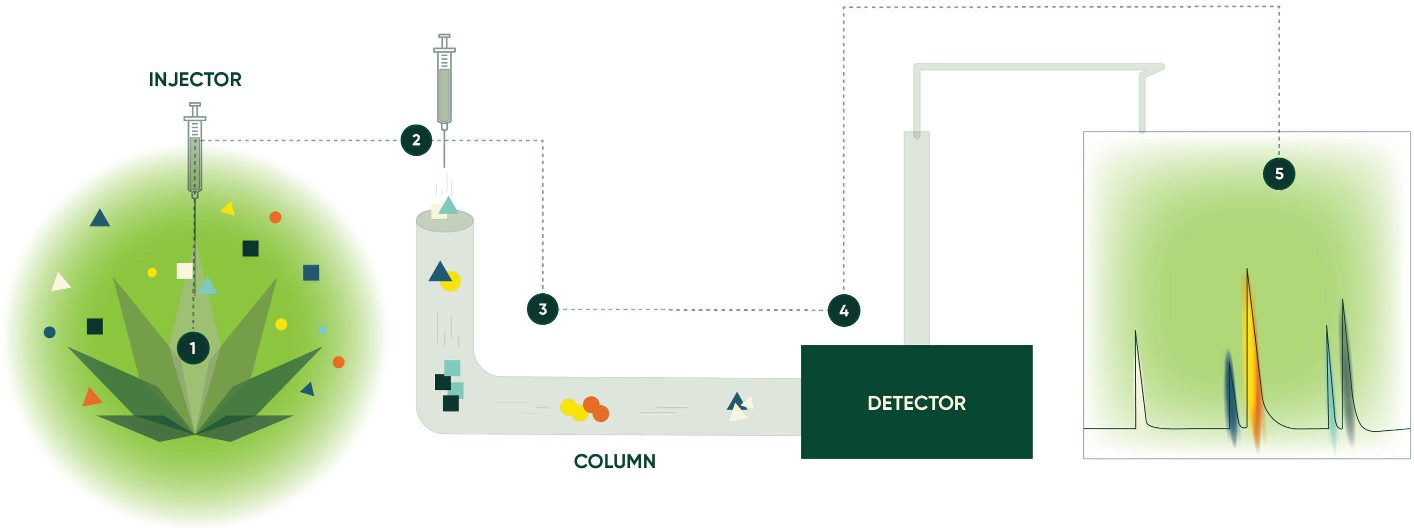
2-Dimensional Gas Chromatography: A Crash Course



When you smell your precious cannabis flowers, what exactly are you smelling? Is it a single chemical compound? A few? The answer is hundreds. But how is this figured this out? That is where gas chromatography (GC) comes into play.

Gas chromatography is the technique of taking a sample of a "smell" – i.e. a combination of volatile chemical compounds in the gas phase – and passing it through a thin, porous (i.e. hollow) tube called a column. The steps below will explain this process and are illustrated in **Figure 2**.

Figure 2



1. The injector needle collects a sample of compounds from the cannabis flower.
2. The needle takes the compounds and injects it into the column. When injected into the column, the compounds are all grouped together.
3. Gases pass through the column, with larger compounds moving more slowly than others. At the end of the column, they are separated into their own packets.
4. These packets pass from the column to a detector.
5. The detector tells the user what each packet of compounds are, and how much of each are present.

Figure 2. Simplified schematic of how a traditional 1-dimensional gas chromatography instrument operates.

A sample of cannabis flower is first put into an air-tight container. The compounds that make it smell the way it does will naturally enter the empty space of the container. A needle will then go into the vial and draw up a sample of these compounds – much like drawing blood with a needle, only in this case, we are drawing up the compounds that give the flower its scent. The needle then takes those compounds and injects it into the porous, hollow column of the GC.

At the beginning, where they are first injected, the gaseous compounds are all grouped together, just like they would be when smelling a flower. As the gases pass through the column, certain compounds will move more slowly than others. Larger compounds will travel more slowly than smaller ones due to their larger size and get "stuck" to the sides of the column. An example of this is α-pinene and ß-caryophyllene: α-pinene is a monoterpene with less atoms in its structure, and thus is physically smaller than ß-caryophyllene. It will therefore pass through the column more quickly than ß-caryophyllene. Columns can be upwards of 30 meters long, which gives the different compounds enough time and space to separate (**Figure 1**) into discrete packets. By the time the various compounds arrive at the end of the column, they are (hopefully) fully separated into their own packets. These packets are then passed from the column to a detector, which can tell the user what each packet of compounds are. From here, the user can determine how many and the identity of each packet of compounds and determine the chemical composition of the aroma of the cannabis flower.

Figure 3

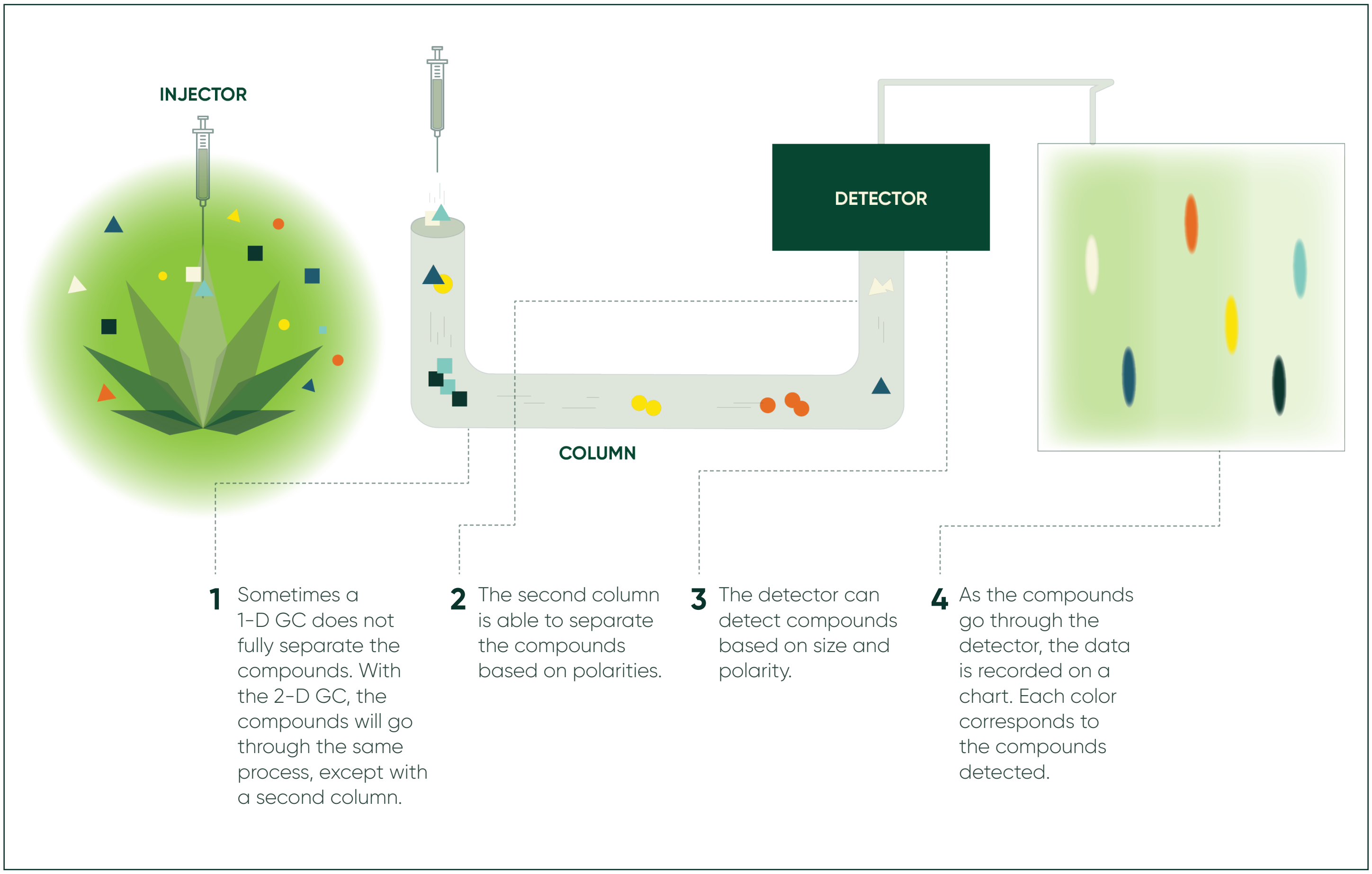


Figure 3. Simplified schematic of how 2-dimensional gas chromatography operates.

But what happens when even after the 30 meters of separating in the column, the packets are not completely separated? This is where having a second column, capable of separating these compounds further based on other chemical attributes – such as polarity – comes in. In 2-dimensional gas chromatography (GC x GC), the compounds travel along a first column just like they do in traditional GC and separate based on the size of compounds. However, as they do this, they are also passed through a second column that then separates the compounds based on their polarities (**Figure 3**). The detector can then detect these compounds based on size and polarity, allowing all the packets of compounds to be even more efficiently separated from one another. As Abstrax has detected upwards of 400 compounds in certain cultivars, it becomes obvious why having two degrees of separation in GC x GC is more powerful than one degree in traditional GC.

Detection and Quantitation of Compounds Using Multiple Detectors

Table 1

Detector	Advantages	Disadvantages
Mass spectrometer (MS)	• Can ID compounds • Can tell compounds close together in data apart	• Small dynamic range • Expensive
Flame ionization detector (FID)	• Large dynamic range • Good for quantitation of compounds • Cheap	• Cannot directly ID compounds • Must use standards to confirm compounds
Sulfur chemiluminescence detector (SCD)	• High sensitivity • High dynamic range • Only detects sulfur-containing compounds	• Cannot directly ID compounds • Only tells user when sulfur is present, not the identity • Expensive

As mentioned in the previous section, a detector can tell the user when a compound arrives at the end of the columns. However, there are a multitude of different types of detectors that can be used, each with their advantages and disadvantages. **Table 1** shows the three detectors Abstrax Tech uses, and their pros and cons.

Each of these three detectors can be considered complimentary to one another. Mass spectrometry is ideal for identification of the packets of compounds, Flame ionization detection is ideal for quantitation of the packets of compounds, and sulfur chemiluminescence is ideal for detecting any packets of compounds that may contain sulfur. Combined together with GC x GC, the user is equipped with all of the necessary tools to find and identify many more compounds hidden in the data than traditional GC methods.

Answering the question,

“What compound(s) produce the gassy and skunky scent in cannabis?”

Building out analytical equipment is one thing, but answering scientific questions is another. At Abstrax Tech, we aim to understand every nuance of the aroma of cannabis. The biggest question our team and many others in the industry have had for years is, "What is the gas?" In 2019, that search began in earnest. Hundreds of cannabis flower samples were procured from some of the most prized growers in California and analyzed. These include Mario Guzman of Sherbinskis – famed for his creation of the Gelato line, Pink Panties, and Sunset Sherbet – all now mainstays of the cannabis lexicon – and Josh Del Rosso, the originator of OG Kush. This legendary cultivar is the "War Admiral" of cannabis – the broodmare that has been crossed to produce many more highly desired cultivars, including some bred by Guzman. Both Guzman and Del Rosso, along with the team at Abstrax Tech, wanted to answer this longstanding question once and for all.

After collecting data on hundreds of cultivars, differences in the data were analyzed meticulously looking for trends between them. Sativas, Hybrids, Indicas, even hemp, were used to home in on one or more compound that may be present in all the cultivars that were considered to be gassy or skunky. One by one, different compounds were eliminated as the potential source of these compounds. However, doing this for hundreds of compounds would have been a monumental feat. Luckily, that is where chemical intuition comes into play: The scientists at Abstrax hypothesized that the potent, gassy, scent of cannabis may contain sulfur due to the fact that many other highly potent fruits or plants contain compounds with sulfur. Durian, hops, onion, and garlic each have potent aromas, and each contain compounds with sulfur in their chemical structures. This hypothesis allowed the team to focus their efforts on compounds that appeared in the sulfur chemiluminescence data. Immediately, trends were formed between cultivars described as skunky or gassy and compounds that contained sulfur. **Figure 4** shows the SCD data for Bacio Gelato, a highly potent (both in effects and aroma) cannabis cultivar, and Black Jack, a cultivar that has a minimal gassy or skunk-like scent. This data provided evidence that pungent cannabis cultivars such as Bacio Gelato contain fundamentally different compounds than those found in those that do not have this scent, such as Black Jack.

Figure 4

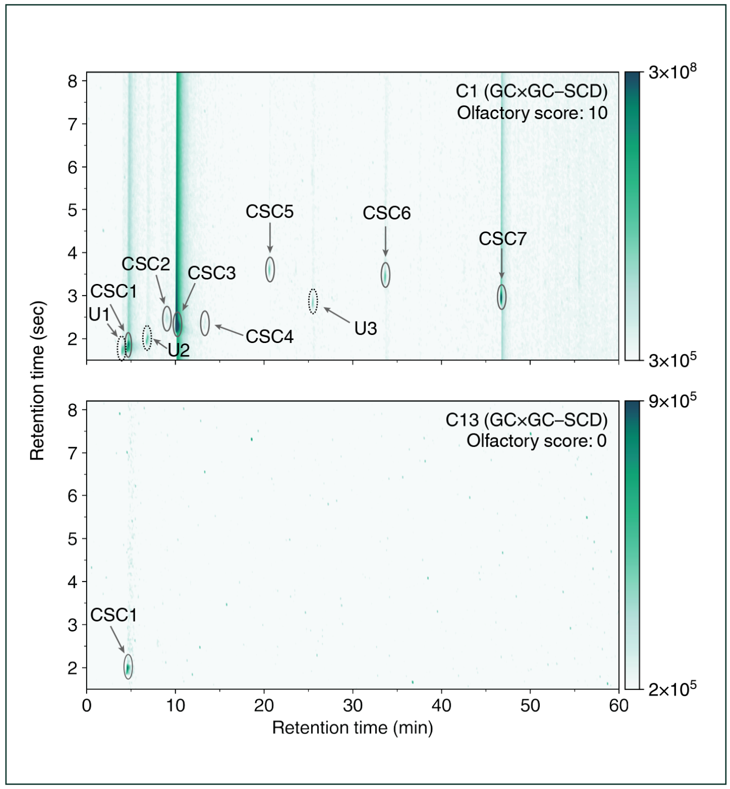


Figure 4. Data showing many more CSCs in a gassy strain than a non-gassy strain. Bacio Gelato (C1), has numerous CSCs present, whereas Black Jack (C13) does not, indicating that the potent, gassy aroma of Bacio is due to these CSCs.

Once it was established that there is high probability that these compounds could be contributing to the gas, their chemical identities needed to be determined. Although one of them was found in chemical databases, 3-methyl-2-butene-thiol (CSC3), also known as prenyl thiol, the others were not. This meant that these other compounds were potentially completely new compounds, or at least rarely discussed in any scientific medium. The chemists at Abstrax worked through the mass spectral data to discover that each of these compounds contains the prenyl functional group. Each compound was then chemically synthesized and measured using GC x GC, which confirmed their hypothesized chemical identities. Aroma tests of these CSCs along with terpenes confirmed that each of these compounds contributes to the gassy, skunky scent of cannabis – answering the longstanding question of what the chemical origins of these scents were.

How Are CSCs Produced During Plant Growth?

Once we discovered these compounds exist in cannabis, we asked the question, "when are CSCs produced by cannabis during growth?" As the gas is a crucial scent generated by this plant that is highly desired by consumers, we wanted to answer this question. We conducted a study by monitoring the amounts of each CSC as a function of week during plant growth. What we found was truly fascinating: During the early weeks of flowering, we did not measure any appreciable amount of any CSC. However, toward the end of the flower stage, we observed a sharp increase in each as shown in **Figure 5**. During the final week of flowering, week 10, each increased substantially. We then observed a huge increase of each CSC at the end of curing (Week 11 in **Figure 5**). This indicates that as the plant is dried and cured, CSCs increase rapidly. This represents the gassiest, most potent example of the flower grown, as after even just 10 days later, the concentration already dropped significantly. Our results show that not only do these compounds get produced rapidly towards the end of the grow cycle, but they also decrease rapidly after harvest. We hope these results will help growers consider that time is of the essence when producing and packaging their products. Further, proper packaging will likely also help in retention of these compounds, thereby allowing their products to retain their optimal scent for as long as possible.

Figure 5

Change in CSC concentrations as a function of cannabis plant growth. These data show that CSCs increase substantially during the final weeks of flowering into curing.

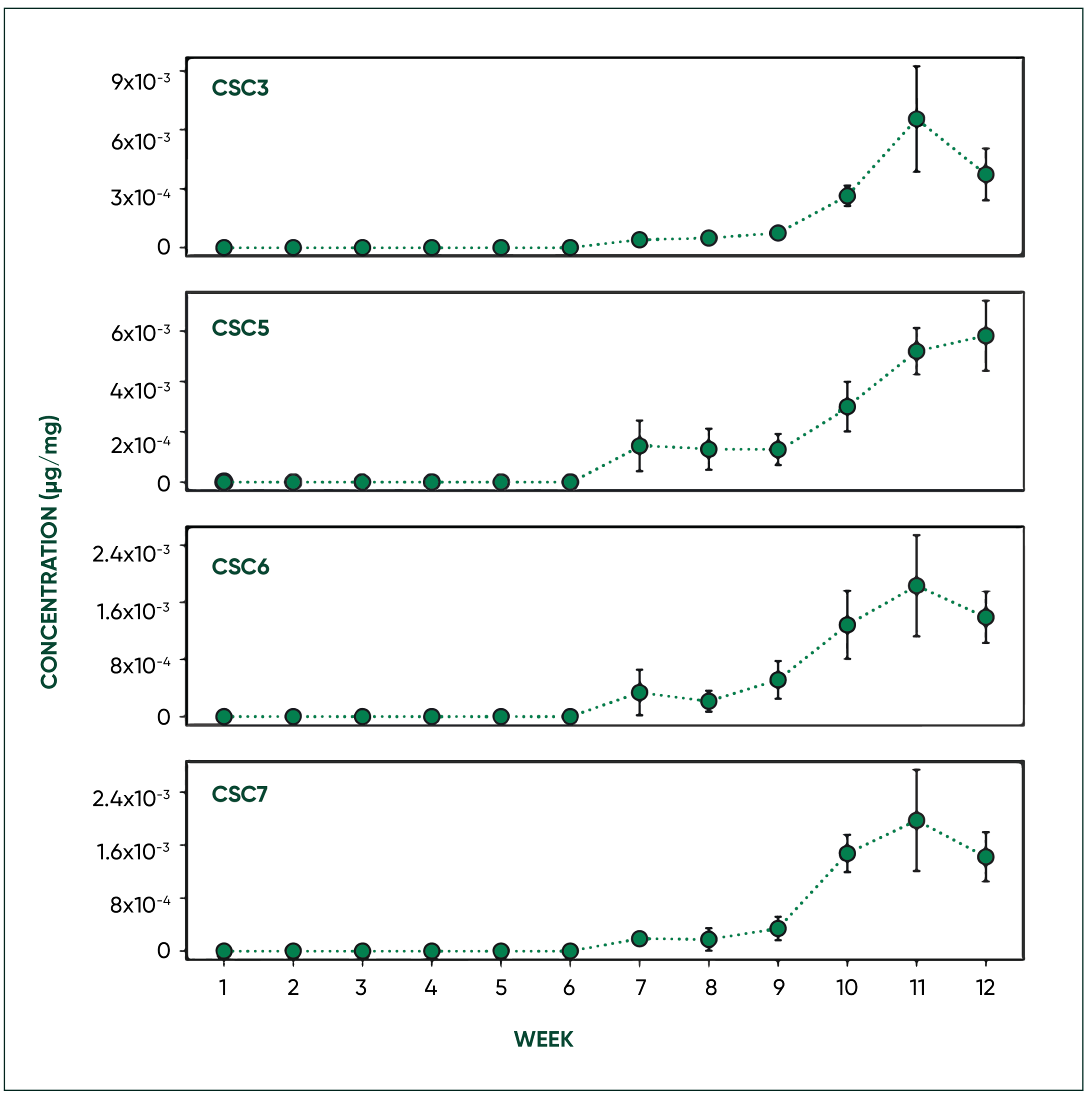


Figure 6

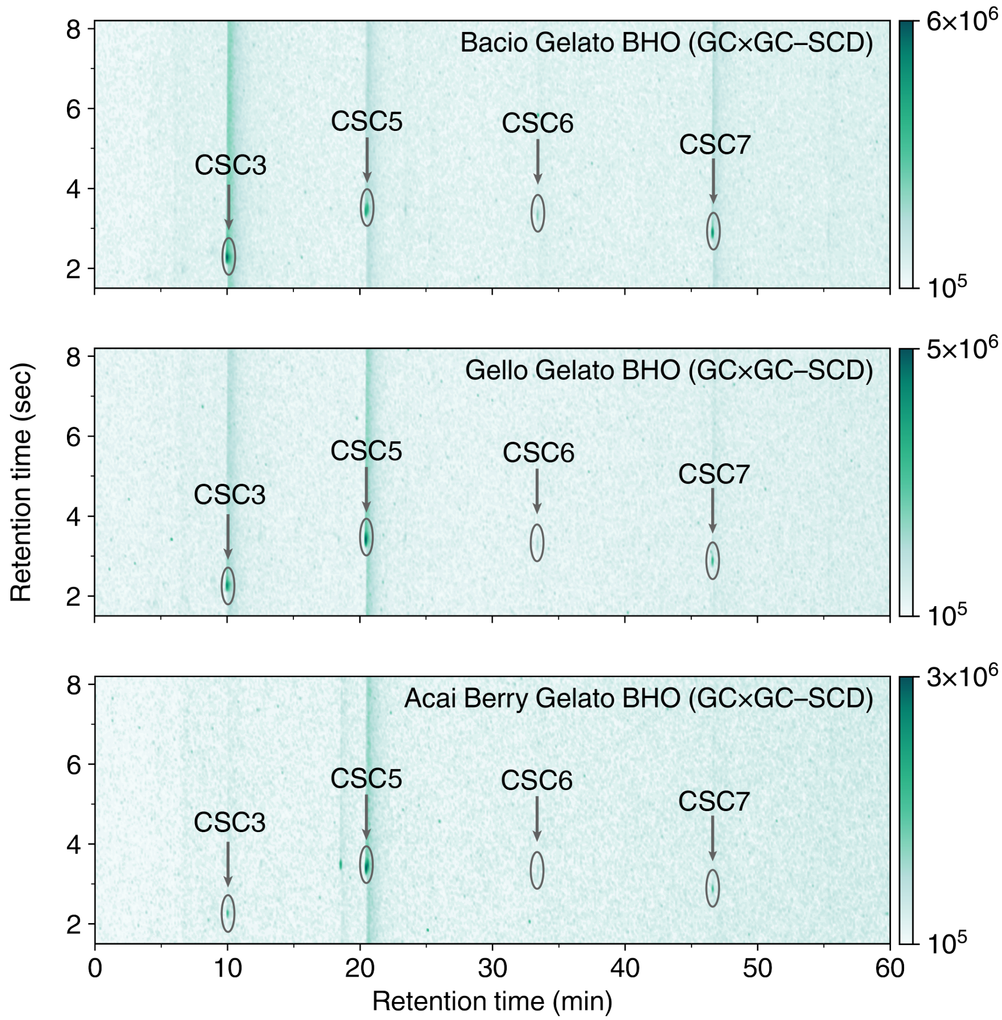


Figure 6. GC x GC chromatograms showing the presence of important CSCs in butane hash oil (BHO) samples. These data suggest that proper extraction techniques can retain these key compounds.

Can CSCs be found in Cannabis extracts?

One question that may arise from consumers of cannabis extracts is whether these very volatile compounds are preserved in an exact such as butane hash oil (BHO). The answer we found is yes – if processed correctly. At Abstrax Labs, our sister company that produces premium cannabis extracts, we tested whether these compounds can be preserved or not. Using state-of-the-art technologies, including cryogenic extraction processes and minimal heat post-processing, we found that indeed, these compounds can be preserved in high concentrations. In fact, we found that certain CSCs that are typically not seen in high amounts in flower after the curing process, such as 3-methyl-2- butenyl acetothioate (CSC5), can be persevered significantly during the extraction process. **Figure 6** shows examples of three different samples containing CSCs – some of which would not be retained without the proper manufacturing process. These results prove that, yes – cannabis extracts can indeed contain these important compounds but must be processed correctly to do so.

Potential Medicinal Properties of CSCs

Discovering CSCs in cannabis opens the door for many different questions to now be posited and answered. A major one that immediately comes to mind is whether these compounds may provide cannabis with more medicinal qualities than initially thought. During the initial discovery of CSCs, researchers at Abstrax Tech soon realized that these compounds, although relatively simple in structure, are strikingly similar to those found in garlic, as shown in **Figure 7**. The only difference between CSCs in cannabis and compounds in garlic are extra methyl groups in the prenylated compounds.

Figure 7

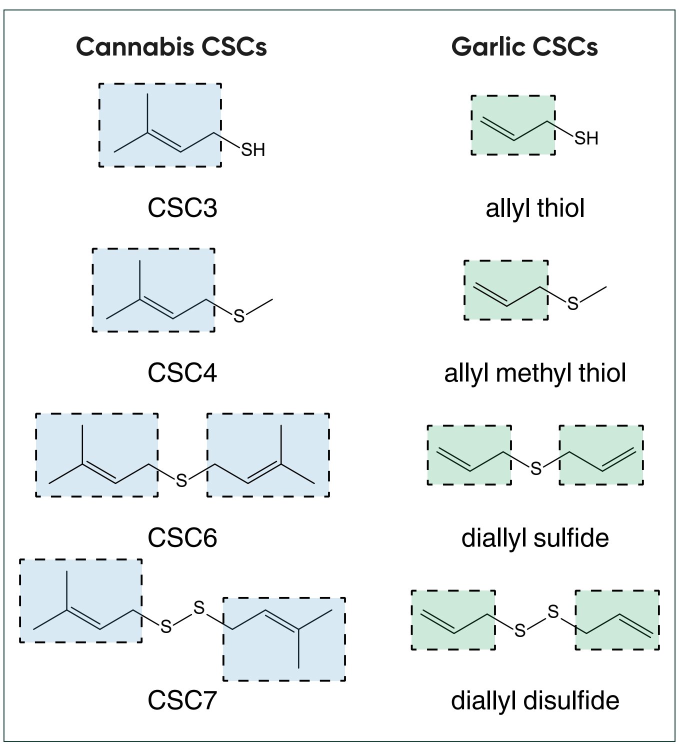


Figure 7. CSCs in cannabis and garlic. Chemical similarities suggest similar biological activity maybe present, however further studies must confirm this.

These compounds in garlic are some of the most important compounds regarding health benefits in our diets. Thus, it begs the question if CSCs in cannabis may likewise possess beneficial health benefits, although further research is needed to confirm. Lastly, we note that although the prenyl functional group is ubiquitous in protein chemistry and biosynthesis of secondary metabolites, cannabis appears to contain an unusual amount (**Figure 8**). For instance, cannflavins, which are prenylated flavonoids that are unique to cannabis, each contain the prenyl functional group. Likewise, many important cannabinoids including CBGA and CBCA contain this group as well. With CSCs also containing this group, it opens the question as to why cannabis produces so many secondary metabolites with this structure. This and other open-ended questions are now possible to pursue answering with the discovered compounds.

Figure 8

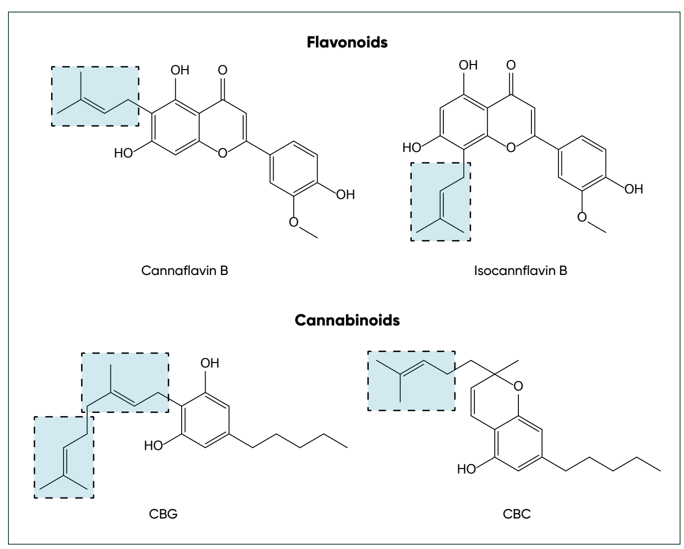


Figure 8. Prenylated secondary metabolites in cannabis. The reason for why cannabis produces so many compounds with this functional group should be further explored.

Concluding Remarks

The discovery of CSCs in cannabis started out as a mission to determine the quintessential gassy and skunky scent, that of which was completed by the team at Abstrax Tech and fellow collaborators. However, understanding these aroma properties of cannabis may be just the tip of the iceberg. The revelation that an entirely new class of compounds with similar functionality to those in other plants such as garlic opens the door to further understanding if they possess any medicinal properties. We lastly note that although the structures of these compounds were elucidated, other CSCs most likely exist, but have yet to be fully identified. Future studies will hopefully reveal even more interesting compounds in this plant full of surprises.

For an in-depth description at the science provided in this white paper, you can find our peer-reviewed publication in the American Chemical Society journal *ACS Omega* by visiting https://pubs.acs.org/doi/abs/10.1021/acsomega.1c04196 (Oswald et al. ACS Omega **2021**, 47, 31667–31676).