An Executive Summary

Getting Started with GC Analysis of Cannabis

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Performing cannabis analysis in-house is easier and cheaper than you might think.

Overview

For those new to the field, performing analytical tests on products can seem daunting. Fortunately, with modern instrumentation, the process can be surprisingly straightforward. A degree is not required. Numerous resources are available, including well-written manuals, online courses, easy-to-use software, and consultants. Like a late-model car, operation of modern gas chromatograph does not require a deep understanding of the inner workings to get satisfactory results. Buying and operating in-house instrumentation will quickly pay for itself.

The switch from contracting analysis to in-house analysis can feel daunting, but has a variety of advantages. Regardless of which road a company chooses, testing is not optional, including for those in the growing cannabis market. As the use of cannabis becomes mainstream, it will be subjected to increased regulatory attention and producers will be expected to prove that they meet standards of quality. Competition is increasing and savvy customers are seeking some assurance of product quality and uniformity. Poor content uniformity can mean that buyers are not getting what they paid for, which damages both the reputation of an end product market and that of the entire cannabis industry. Having a clear-cut set of standards for production and product quality adds to the perceived professionalism and legitimacy of the product and removes past stigmas.

Intro to Gas Chromatography

Gas chromatography (GC) is a common method for the analysis of volatile compounds. Instruments are available as self-contained, bench-top units that integrate the injector, column oven, and detector. In addition to a GC system, analysts will need a gas source, analytical balance, glassware, and solvents (usually methanol).

Chromatography is a process in which a complex sample is separated into its components based on their relative affinity between two phases. In most cases, there is a fixed stationary phase and a mobile phase that flows over it. In the case of GC instruments, the mobile phase is a gas that flows through a long tube, known as the column. The stationary phase is typically an organic material bonded to the interior of the glass tube. For larger samples, the column may be packed with glass beads that are also coated to provide more surface area. Columns can be purchased with various stationary phase chemistries suited for different applications.

A syringe is used to inject the sample into the enclosed area of the injector through a silicone septum, which allows the needle to pass through while maintaining an airtight seal. The injector area is quickly heated to vaporize the sample and the flow of gas carries it into the column.

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While any carrier gas can be used, hydrogen and helium are the most common because the quality of the separation is better with lighter gasses. Nitrogen can also be used, however.

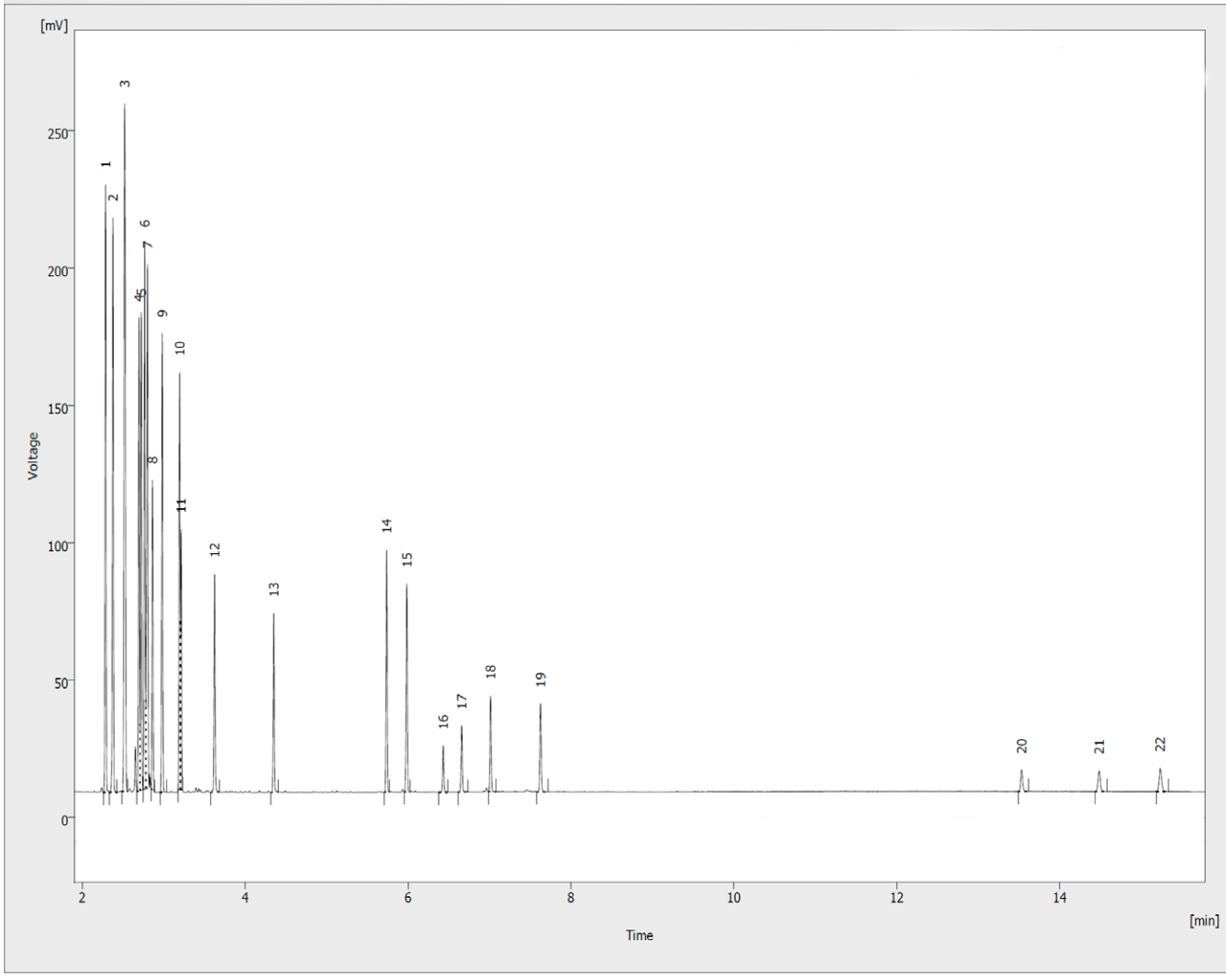
Once in the column, each sample component will go into an equilibrium condition whereby some is in the vapor phase and some is absorbed in the stationary phase. The ratio of the amount in each phase is a characteristic of the compound and ideally different for each one. When a molecule vaporizes in the carrier gas, it is moving down the column at the flow rate. Not surprisingly, when it is in the stationary phase, it is not moving. Every compound will spend exactly the same amount of time in the mobile phase, (i.e., the time it takes for a gas molecule to run from one end of the column to another), but they will spend varying times in the stationary phase. That variation is what drives them to separate.

The time it takes for a compound to make it through the column is known as the retention time. If all the conditions are kept the same (i.e., temperature program, column type, and column length), then a given compound will always have the same retention time. Heating the system makes the molecules spend less time in the stationary phase, and so they come out faster. Because molecules can have vastly different retention times, a temperature program is often used to drive off the more retained compounds faster. The column is housed in a convection oven and kept relatively cool at first, letting the more volatile compounds separate. Then, it is ramped up to shorten the time of the separation.

The gas exits the column directly into the detector. There are numerous detectors available. The flame ionization detector (FID) is the most common because it will respond to any compound containing carbon. The trade off is that it is not particularly sensitive, so it is not suitable for trace analysis (e.g., pesticides analysis). Also, because the flame ionization detector responds to everything, interference from unwanted compounds can be a problem in some cases.

Electron capture (EC) detectors are far more sensitive, but only respond to compounds containing halogens (e.g., chlorine, fluorine, iodine), which includes many pesticides. Mass spectrometry (MS) detectors are complex and expensive instruments by themselves, and provide a further

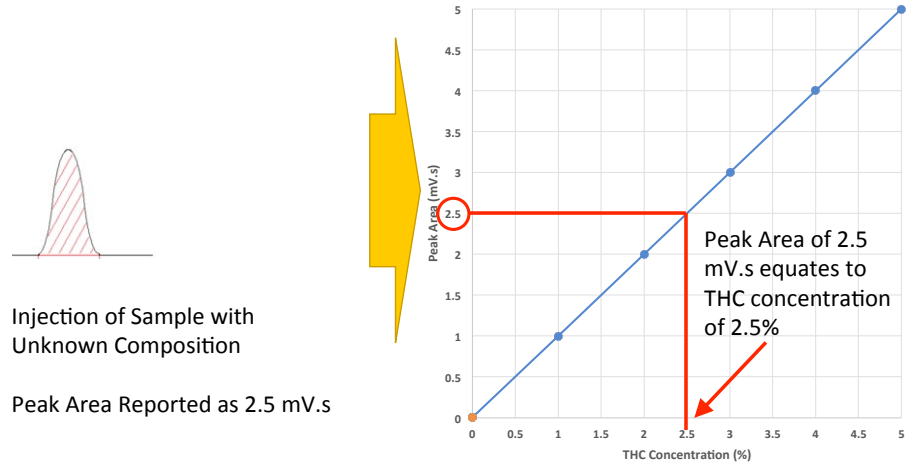
Figure 1: Output from a standard solution containing 19 terpenes.



separation based on the mass of the molecules. It is best to hold off on the purchase of a GC-MS instrument until one has enough experience to know that it is needed.

FID can detect cannabinoids such as tetrahydrocannabinol (THC), cannabinol (CBN), cannabidiol (CBD), and cannabigerol (CBG); numerous terpenes related to flavor like limonene and pinene; and residual solvents from the extraction process. It uses a hydrogen flame, so a source of hydrogen gas will be needed.

Figure 2: How to quantify the components.



The signal from the detector is plotted against time. When a compound exits the column, the signal will rise and then fall back to the flat baseline. The area under the resulting peak is proportional to the amount of the compound that was present. **Figure 1** shows the output from a standard solution containing 19 terpenes (the peaks on the left) as well as CBD, CBN, and THC.

The most common way to calculate concentration from the peak area is to use a standard curve. The analyst will need a standard solution that contains each of the compounds he or she wishes to measure. These are available from a variety of sources. A set of at least three dilutions of the standard are injected to make a plot (see **Figure 2**). The blue dots are the results of the standard injections and the blue calibration line is the best fit line for those dots. With the calibration line, the response from unknown samples (red) can be linked to the sample concentration. Dilution factors can then be used to calculate the concentration in the original sample. All of this calculation can be performed in the GC software.

Sample Preparation for Cannabis

It does not matter how advanced one's equipment is; the analysis is only as good as the sample preparation technique. It is vital that companies have confidence that as much of the compound of interest is dissolved as possible. With extracts, resins will need to be fully dissolved, but this is also easy to check.

The biggest challenge can come from plant material; their cell walls might inhibit extraction. A typical procedure is to weigh out about 0.1 g of dried bud, place it in a vial with 30 mL of methanol, and let it sit at least 30 minutes at room temperature. Duplicate samples from a batch are always a good idea. Poor reproducibility can be the canary in the coal mine for a sample that needs a more rugged extraction technique.

Differentiating Neutral and Acidic Forms of Cannabinoids

The technique can get more complicated if companies want to differentiate between the neutral and acidic forms of the cannabinoids with gas chromatography. In the heat of the injector, the acid forms are broken down into the neutral forms, so the two cannot be measured independently. One can avoid this by first reacting the cannabinoids with a commercially available reagent (e.g., N,O-Bis[trimethylsilyl]trifluoroacetamide [BSTFA] with 1% trimethylchlorosilane [TMCS]). The reaction converts the cannabinoids into compounds that are heat resistant. Recall that different compounds will have different retention times, so the analyst will also have to convert the standard solutions so that they match the samples.

Conclusion

This brief introduction to gas chromatography is intended to demonstrate that performing in-house analysis of cannabis products is fairly straightforward. The compounds of interest are known, and standards are readily available. The necessary methods have been established. The initial cost of setup will quickly pay for itself. In addition, being able to assert for the quality and uniformity of your product will prove useful in an increasingly competitive market.