Cannabis Testing Solutions *for* Regulated Environments

By Shashidhar N. Rao, Ph.D.

The cannabis industry is growing exponentially, and the use of cannabis for medical purposes is being adopted across the nation. Legalization of cannabis usage for medicinal and recreational purposes in many of the states in the USA has resulted in a boom in cannabis consumers. In this light, there has been an increasing need for knowledge about the nature of the product and its testing. The role of cannabis testing labs has become crucial to the process of regulation, particularly for medical usage.

Typically, cannabis products are tested for the following factors to ensure their safety for use: potency, heavy metals, pesticides, residual solvents, moisture, water activity, terpene profile, microbial and fungal growth, and mycotoxins/aflatoxins. The following text outlines some of the equipment that are commonly employed in cannabis testing. It may be noted that cannabis analytical testing requirements may vary by state and hence the regulatory requirements for doing the testing and the corresponding equipment could vary correspondingly.

Potency testing: The most important component of cannabis testing is the analysis of cannabinoid profiles, also known as potency. Cannabis plants naturally produce cannabinoids that determine the overall effect and strength of the cultivar. There are many different cannabinoids that all have distinct medicinal effects. However, most states only require testing and reporting for the dry weight percentages of delta-9-tetrahydrocannabinol (Δ9-THC), commonly referred to as THC and cannabidiol (CBD). Medical cannabis is often characterized by higher levels of CBD and lower levels of THC (e.g. 21% CBD and 1% THC). By contrast, recreational cannabis contains higher percentage of THC (e.g. 24% THC and 2% CBD). The therapeutic CBD is desirable for medicinal effect but the psychoactive THC may be unnecessary and undesirable for some patients. This THC/CBD ratio information is of primary importance to the medical personnel prescribing cannabis for medicinal purposes. Some medicinal effects of cannabis could include reduced symptoms such as nausea, seizures, eye pressure and pain.

A major cannabinoid in the cannabis plant material is THC Acid (THCA) which is thermally labile and converts to THC by decarboxylation during heating that takes place, for example, in the hot GC (Gas Chromatography) injector port. For this reason, THCA is not detected by gas chromatography but is detected by HPLC. Similarly, CBD acid is also thermally labile and is difficult to be detected by GC.

For potency testing, traditional high-performance liquid chromatography (HPLC) is recommended and has become the gold standard for analyzing cannabinoid profiles. Gas chromatography (GC) has also been used for potency testing of cannabis. GC is generally considered faster and simpler than HPLC. GC/FID is preferred for speed of analysis and simplicity in routine identification and quantification of cannabinoid concentrations. For positive identification of each cannabinoid, gas chromatography with a mass spectrometer would be preferred. A GC-MS system with a second injector and a Flame Ionization Detector (FID) in a second channel makes for a versatile hardware configuration.

Heavy Metal Testing: Different types of metals can be found in soils and fertilizers, and as cannabis plants grow, they tend to draw in these metals from the soil. Heavy metals (most commonly lead, cadmium, arsenic, chromium and mercury) are a group of metals considered to be toxic and hazardous and are required to be tested to confirm their levels are under toxic concentration limits. Without specific methods of regulation from the individual states, FDA guidelines for heavy metals in food products need to be followed. Heavy metal testing is typically performed by inductively coupled plasma mass spectrometry (ICP-MS). ICP-MS uses the different masses of each element to determine which elements are present within a sample and at what concentrations.

Residual Solvent Testing: Extracted concentrates of cannabis are formulated into hash oil, wax, butter (budder) and other forms. Extraction takes place with several types of solvents such as butane, propane, ethanol, isopropanol, acetone and others. For safety purposes, solvent must be removed from the final product before consumption. Residual solvent is measured by headspace with gas chromatography and flame ionization detection (HS-GC-FID). Without specific methods of regulation from the individual states, procedures follow the ICH guidelines (International Council of Harmonization) for residual solvents in botanical preparations.

Pesticide Testing: There are many pesticides that are used in commercial cannabis operations to kill the pests that thrive on the plants and in greenhouses. These chemicals are toxic to humans, so confirming their absence from cannabis products is crucial. The number of pesticides that must be tested for varies from state to state (e.g. Colorado – 13, Oregon - 59 and California – 66). AOAC International is developing methods for testing for 104 pesticides. Without specific methods of regulation from the individual states, procedures follow the EPA (Environmental Protection Agency) guidelines for pesticide residue analysis. Testing for pesticides is one of the more problematic analyses, possibly resulting in the need for two different instruments depending on the state's requirements. For a majority of pesticides (carbamate pesticides), liquid chromatography mass spectrometry (LC/MS) is acceptable and operates much like HPLC but utilizes a different detector and sample preparation. It is desirable to have a LC-MS/MS system or HPLC system with a triple quadrupole (QqQ) mass spectrometer that provides ultra-low detection limits, high sensitivity and efficient throughput. Advanced systems can analyze more than 200 pesticides in 12 minutes. Pesticides that do not ionize well (Chlorinated hydrocarbons, organophosphates, or pyrethroids) in an LC/MS source require the use of a gas chromatography mass spectrometry (GC/MS) instrument. Here too, it is desirable to consider an instrument that utilizes a triple quadrupole mass spectrometer and a headspace autosampler to help maximize efficiency of detection.

Terpene Profile Testing: Terpenes are produced in the trichomes of the cannabis leaves, where THC is created, and are common constituents of the plant's distinctive flavor and aroma. Terpenes also act as essential medicinal hydrocarbon building blocks, influencing the overall homeopathic and therapeutic effect of the product. The characterization of terpenes and their synergistic effect with cannabinoids are key for identifying the correct cannabis treatment plan for patients with pain, anxiety, epilepsy, depression, cancer and other illnesses. This test is not required by most states, but it is recommended. The instrumentation that is used for analyzing terpene profiles is a GCMS with headspace autosampler (also used in Residual solvent testing) with an appropriate spectral library. Since residual solvent testing is an analysis required by most states, all of the instrumentation required for terpene profiling will already be available.

Moisture Content and Water Activity Testing: Moisture content testing is required in some states. Moisture can be extremely detrimental to the quality of stored cannabis products. Dried cannabis typically has a moisture content of 5% to 12%. A moisture content above 12% in dried cannabis is prone to fungal growth (mold). As medical users may be immune deficient and vulnerable to the

effects of mold, constant monitoring of moisture is needed. Below a 5% moisture content, the cannabis will turn to a dust-like texture. The best way to analyze the moisture content of any product is using the thermogravimetric method with a moisture balance instrument. This process involves placing the sample of cannabis into the sample chamber and taking an initial reading. Then the moisture balance instrument heats up until all the moisture has been evaporated out of the sample. A final reading is then taken to determine the percent weight of moisture that was contained in the original sample.

It is desirable to have a moisture balance that offers intuitive operation and quick, accurate determination of moisture content. The pan should be spacious enough to allow large samples to be spread thinly. The halogen heater and reflector plate should combine to enable precise, uniform heating. Advanced features can include preset, modifiable measurement modes like automated ending, timed ending, rapid drying, slow drying and step drying. Another method for preventing mold is monitoring water activity (measure of "free water"). Water Activity ranges from 0 to 1. Pure water would have water activity value of 1.0. ASTM methods D8196-18 and D8297-18 are used for monitoring water activity in dry cannabis flower. For storage a value between 0.55 to 0.65 is recommended. Some states recommend monitoring water activity in place of moisture.

Microbe, Fungus and Mycotoxin Testing: Most states mandate that cannabis testing labs analyze samples for any fungal or microbial growth resulting from production or handling, as well as for mycotoxins which can lead to a buildup of progressively worse allergic reactions. A moisture balance can provide accurate determination of moisture content in cannabis. A high-sensitivity LC-MS/MS instrument should be used to qualify and identify strains of mycotoxins. Micro-organisms can be detected using ELISA, quantitative polymerase chain reaction (qPCR) or matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

About the author:

Shashidhar N. Rao, obtained his Ph.D. from the Indian Institute of Science in 1983 in the field of computational chemistry. He has over 15 years experience working in the Pharma industry in drug discovery & development pipelines. He also has over 13 years of experience in the pharma related software industry, as well as authored and co-authored over 100 publications in peer-reviewed international journals. He is also a co-inventor on 20 patents in the areas of cancer, cardiovascular and inflammation related diseases.