

A Closer Look at Cannabis Testing



Scott Kuzdzal, Ph.D., Bob Clifford, Ph.D., Paul Winkler and Will Bankert (Shimadzu Scientific Instruments)



Introduction – Current US Cannabis Research, Policy & Law

Cannabis has demonstrated health benefits since ancient times. While less than 6% of today's studies on marijuana analyze its medical properties, publications to date indicate that cannabis shows great promise for the treatment of many diseases and symptoms (Table 1). However, patients with cancer or severe pain, for example, have been blocked from these benefits since the mid-20th century when federal regulations were enacted that prohibited the use, sales and distribution of marijuana due to its psychoactive properties. The US Drug Enforcement Agency (DEA) stated in 2011 that marijuana has "no accepted medical use" and should therefore remain illegal under federal law. Strict scheduling and law enforcement actions have made it more difficult for researchers to obtain marijuana samples for scientific studies than LSD, MDMA, heroin and cocaine. In June, 2014, the Drug Policy Alliance and the Multidisciplinary Association for Psychedelic Studies released a report titled "The DEA: Four Decades of Impeding and Rejecting Science." Citing case studies from 1972 to the present, this report claims that the DEA suppressed research on the positive benefits of marijuana for medicinal use.

More recently, thirty members of the US Congress sent a letter to the Health and Human Services Secretary demanding an end to the federal monopoly on marijuana research so that more studies can be performed by US researchers.

Mainstream acceptance of cannabis has increased steadily over the past decade in the United States. Twenty-two states and the District of Columbia have legalized or decriminalized marijuana in some form. Colorado and Washington have legalized marijuana for recreational use, with Oregon soon to follow suit. Maryland has approved bills making medical marijuana accessible to patients and decriminalizing possession of limited amounts of the drug. As the medical and recreational uses of cannabinoids increases both in the United States and globally, the need for improved quality control testing also increases.

On a recent tour of medicinal marijuana businesses in the State of Oregon, we learned all aspects of the cannabis industry and key differences between recreational and medical marijuana grow operations.

| | |
|---|--|
| Appears to have powerful anti-tumor properties | Improves symptoms associated with Lupus |
| Reduces pain associated with chemotherapy | Shows promise in eliminating Crohn's disease |
| Treats glaucoma by lowering intraocular pressure | Reduces pain in multiple sclerosis patients |
| Decreases symptoms of epileptic seizures | Helps fight obesity by increasing metabolism |
| Reduces brain damage after a stroke | Reduces frequency and severity of concussions |
| Relieves discomfort from arthritis | Helps reduce muscle spasms |
| Lessens side effects from hepatitis C treatments | Reverses the carcinogenic effects of tobacco use |
| Treats inflammatory bowel disease | Decreases anxiety and improves appetite |
| Slows the progression of Alzheimer's and other neurodegenerative diseases | |

Table 1 Partial listing of reported health benefits of cannabis in scientific literature and news reports (see suggested reading at the end of this article).



A biomedical cannabis grow operation in Oregon. This facility prefers natural sunlight for mature plant growth and reduces environmental stresses on the plants to ensure that the most natural, homeopathic medicines are produced.

Chemistry and Biomedical Properties of Cannabis

Cannabis plants contain more than 480 compounds that have been identified to be unique to cannabis, including over 66 cannabinoids. Cannabis also contains approximately 140 terpenes, which are more widespread in the plant kingdom. While tetrahydrocannabinol (THC) is the most abundant active component in cannabis, cannabidiol (CBD) and cannabinol (CBN), a degradation product of THC, are commonly measured in cannabis samples. CBD, a non-psychoactive compound, has been shown to reduce convulsions, inflammation, nausea and anxiety, and has even eradicated tumors in some patients.

Fig. 1 provides partial listing of cannabinoid pharmacological characteristics. Recreational marijuana growers, primarily interested in high THC content are less concerned with the “CBX profiles”, whereas these compounds may be beneficial to biomedical marijuana patients with specific diseases or symptoms.

Cannabis Consumption & Delivery

Smoking is an expedient method of consuming marijuana, but some experts argue that smoking can cause lung and respiratory problems and reduce the bioavailability of some constituents. Marijuana plants naturally contain the acid forms of THC and CBD known as THCA and CBDA. During smoking, heat converts the THCA and CBDA into their more potent, non-acid forms, THC and CBD. This is referred to as decarboxylation.

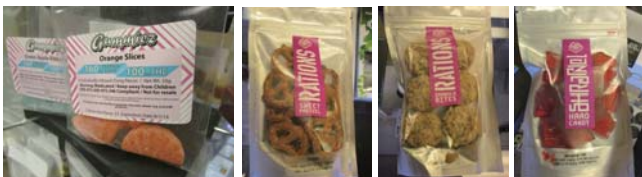
Vaporizers have provided a means of more gently heating the cannabis. Doing so releases more medicinal components of the marijuana and reduces the amount of noxious chemicals. Due to the volatility of cannabinoids, they vaporize at a temperature much lower than the combustion temperature of plant matter. Vaporization usually heats the sample to 150-200 C. This is sufficient to evaporate off the cannabinoids and terpenes but not combust the sample into more

| | Antibiotic | Antifungal | Anti-inflammatory | Analgesic | Anxiolytic | Antioxidant | Antispasmodic | Antiemetic | Sedative | Euphoriant |
|----------------------------|------------|------------|-------------------|-----------|------------|-------------|---------------|------------|----------|------------|
| Cannabigerolic Acid (CBGA) | | | | | | | | | | |
| Cannabigerol (CBG) | | | | | | | | | | |
| Cannabichromene (CBC) | | | | | | | | | | |
| Cannabidiolic Acid (CBDA) | | | | | | | | | | |
| Cannabidiol (CBD) | | | | | | | | | | |
| Δ -9-THC | | | | | | | | | | |
| Δ -9-THCV | | | | | | | | | | |
| Cannabinol (CBN) | | | | | | | | | | |

Fig. 1 Cannabis constituents and partial listing of pharmacological characteristics.

carcinogenic compounds like benzopyrene. It is important to note that when marijuana products are smoked, combustion sterilizes cannabis from various mold and bacterial spores (including *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, Yeasts, and *E.Coli*). Migration to vaporization, however, puts immuno-compromised cancer and HIV patients at increased risk for bacterial infections.

The majority of patients prefer to consume edibles or beverages that have been created using butters and oils derived from plant extracts. The effects of cannabis ingestion differ significantly from smoking or vaporizing, and the time it takes for therapeutic benefits to begin takes much longer. This delayed onset, coupled with high THC concentrations present in some edibles puts consumers at a greater risk of THC overdosing. There are also growing concerns over infants and children gaining access and overdosing on THC-infused edibles that look identical to candy.



Medical marijuana dispensaries offer many forms of cannabis products, including the orange slices, pretzels, granola bites and hard candy products shown here. Brownies, beverages, chocolates and sublingual oils are also popular.

Towards Personalized Cannabis Therapies

The premium products in medical marijuana dispensaries are products high in THC. But as described previously, it is actually the various CBX compounds that appear to have enhanced health benefits. As research into cannabis treatments grows, much more will be known about the mechanisms of action of cannabinoids and terpenes. G.I. Grow, an organic biomedical farm, is pioneering new approaches for natural cannabis remedies (HYPERLINK "<http://www.GIGrow.us>" www.GIGrow.us). They continuously nurture cannabis and reformulate CBX oil blends in response to patient outcomes, delivering a personalized cannabis treatment approach for each individual patient.

There are growing numbers of cannabis oil success stories, including Elkan, now 10 years old, living in Oregon. Elkan suffered from severe autism, including Attention Deficit Disorder (ADD), Attention Deficit Hyperactivity Disorder (ADHD), Pervasive Developmental Disorder (PDD) and Sensory Processing Disorder (SPD). Elkan also had trouble speaking, suffered intense Leaky Bowel Syndrome symptoms and needed to be physically restrained 3-4 times per week because he would start flailing around. In a recent interview with Elkan's mother, Laura, she commented that, "Elkan's doctors were not sure why all pharmaceuticals other than Ritalin were showing no benefit whatsoever." Nothing seemed to work and most pharmaceuticals only exacerbated his symptoms. Elkan began taking a blend of natural CBD oils from G.I. Grow just months ago and after just 3 months taking cannabis oil, he can now speak, does not experience Leaky Bowel Syndrome symptoms and does not need to be restrained.



A breeding pair consisting of a female Purple Heart G.I. (pre98 Bubba Kush female x Purple Thai trainwreck) and a male Mendo Express (Mendo Purps female x E32 Arcata Trainwreck), left, and a high-CBG strain Black Trainwreck (Oregon Trinity X Purple Thai Trainwreck), right, at G.I. Grow's Biomedical Farm.

Cannabis Analytical Testing

Cannabis growers and dispensaries benefit tremendously from testing performed at independent laboratories. This testing determines potencies, reduces the risk of contamination and improves product quality. In the following paragraphs we will more closely examine cannabis testing. Routine cannabis testing services include cannabinoid potency, and screening/determination of terpenes, aflatoxins, heavy metals, molds, bacteria, pesticides, herbicides and residual solvents.

Cannabinoid Potency Testing

A critical test associated with cannabis testing is cannabinoid potency. Most labs quantitate levels of at least three major cannabinoids: THC, CBD and CBN and their different forms (carboxylated vs decarboxylated). Some labs employ gas chromatography (GC), in which the sample is vaporized under heat. Both GC-FID and GC-MS are commonplace. Because intense heating is used in GC, any THCA present in the natural sample is converted to THC and labs report this value as "THC Total". Other labs use HPLC to determine the amount of cannabinoids present. Because HPLC does not require heating, testing by this method provides a more accurate determination of the actual amounts of carboxylated or decarboxylated forms present in the sample. Potency testing accompanied with proper product labeling is needed to ensure that customers know exactly how much of the cannabinoids they are consuming.



 **ROSE CITY**
LABORATORIES

THC, CBD and CBN potency testing by HPLC (left and middle) and Rose City Labs (www.RoseCityLabs.com) advertisement (Blueberry Kush potency of 20.41% THC, right).

Typical THC potency ranges from 5 to 25 % in plant materials and edibles, but can run much higher for concentrated oils. There are no established standard methods for chopping samples, homogenizing them and performing extractions. Therefore, variations in cannabis potency can easily exceed 20%. Potency testing will improve as chemical standards of known potency become more readily available.

Shimadzu integrated HPLC systems, including the new i-Series, are ideally suited to meet the challenges of cannabis potency testing. The i-Series touchscreen, graphical user interfaces between system and workstation, allows intuitive operations regardless of experience level.

Pesticides, Herbicides and Residual Solvent Screening

The analytical detection of pesticides in cannabis remains a challenge. Pesticides are used in commercial cannabis grow operations to kill mites that thrive on cannabis plants. Female mites lay over 2,000,000 eggs per day at 90° Fahrenheit (32.2° Celsius). Also, they are mutating throughout the cannabis industry with resistance to some pesticides. Thrips (tiny, slender insects with fringed wings), aphids, and root gnats are common indoor pests. Spider mites, caterpillars, grasshoppers threaten greenhouse grows. Halyomorpha halys, also known as the brown marmorated stink bug, is a voracious eater and has an affinity for cannabis plants.

An enormous number of pesticides are available in the commercial marketplace, and no lab can test for all of them. GCMS is the preferred instrumentation platform for such testing. While there are currently no guidelines for residual pesticide screening in cannabis, most labs test for the most common pesticides employed during cannabis cultivation: organophosphates, carbamates, pyrethroids and avermectins. MRX Labs in Portland, OR, is leading the way in pesticide analytical testing services, offering a panel of 40 pesticides. MRX Labs uses Shimadzu GCMS-QP2010 Ultra instruments, which enhance lab productivity and sample throughput. The ecology mode saves power and carrier gas consumption allowing for a lower cost of ownership and less environmental stress. Another laboratory, G.O.A.T. Labs (www.GOATLabs.us) in Vancouver, WA, utilizes a GCMS-QP2010 SE. Herbicides are also reported using both of these platforms. Residual solvent testing is performed on a headspace GC such as with Shimadzu's GC-2010 Plus with HS-20 Headspace Sampler similar to monograph "USP <467> for Residual Solvents".



Shimadzu GCMS (top) and LC (bottom) instrumentation at MRX Labs in Portland, Oregon (www.MRXLabs.com).

Additional Cannabis Laboratory Tests

The moisture content of a variety of cannabis samples can be measured using Shimadzu MOC63u (and MOC-120H) balances. The MOC63u is applicable to a variety of cannabis products and its' long-life and high power halogen heater provides quick and accurate measurement. Medical marijuana dispensaries require National Type Evaluation Program (NTEP) approved scales for use in legal trade.

Additional testing of contaminants, including heavy metals, mycotoxins and microorganisms are also important to cannabis labs. The ideal conditions for cannabis growth are also ideal for the growth of potentially harmful bacteria and fungi including yeast and molds. Recreational and medical cannabis must be properly screened for microbial contamination that poses health risks to consumers and immunocompromised individuals. Traditional mold and bacteria testing with petri dishes is being replaced with qPCR platforms. MALDI based microorganism identification may be useful as a qualitative technique to certify the presence or absence of various microorganisms. MALDI could also compete with genomics testing for cannabis strain typing.

Mycotoxins (aflatoxin) can be detected using Shimadzu LC and LCMS systems. Heavy metals analyses generally include the big four of lead (Pb), mercury (Hg), cadmium (Cd), and arsenic (As), which can be tested by Shimadzu's AA-7000 with GFA-7000 or ICPE-9800. Alternatively, ICP-MS may be employed.

Considerations for Future Cannabis Testing

The cannabis industry and cannabis testing are in their infancies. As the need for better quality control continues and standardization is introduced, it is likely that lower limits for the various cannabis contaminants will be established and regulations will be introduced. Mass spectrometry will likely play a greater role in quantitation as detection levels are lowered and confirmatory tests are required. The health benefits of terpenes present in cannabis will also provide a fertile area of scientific research. CBD, CBG and other compounds appear to have a synergistic relationship with each other as well as with various THC forms and terpenes. This field needs much more investigation to determine mechanisms of action, bioavailability and health benefits.

With an increase in cannabis product consumers there comes an increase in public safety concerns, such as "drugged driving". Law enforcement will need new, low-cost methods for rapid salivary, breath-based and/or finger-stick screening of individuals that appear to be under the influence of marijuana. Also, better product packaging and labeling will be needed to reduce accidental infant exposures, especially to candy-like, medicinal marijuana edibles.

Cannabis testing is not just a growing US market. Sativex™ a synthetic, pharmaceutical version of cannabis, has been approved for use in 25 countries as a treatment for muscle spasm pain in multiple sclerosis patients. Marinol®, a synthetic THC product, has been FDA approved to treat nausea and vomiting associated with cancer chemotherapy in patients who have failed to respond adequately to conventional treatments. The FDA also approved Marinol® to treat appetite loss associated with weight loss in people with AIDS. Idrasil™ is a physician prescribed "medical cannabis in a pill". Unlike Marinol, which is a synthetic form of a single cannabinoid (THC) only, Idrasil is an all-natural cannabis

plant extract containing the full spectrum of naturally occurring cannabinoids from cannabis. CBD oils can be purchased legally from Amazon.com and many other sources.

As more cannabis-based or synthetic cannabinoid drugs and homeopathic medicines enter the marketplace, and as more states legalize medical and/or recreational marijuana, the need for cannabinoid testing and standardization will continue to grow. The US cannabis industry is projected to be an \$8 Billion industry by 2017, with rapid growth expected in all aspects of cannabis businesses (production, quality control, informatics, packaging, labeling, security, etc.). In our estimation, as cannabis research moves forward, biological insights into the health benefits of cannabis, including personalized cannabis oil therapies, will be unlocked and many more people will benefit from the natural healing benefits of cannabinoids.

Suggested Reading:

1. An archaeological and historical account of cannabis in China. *Econ Bot* 1974. 28:437-8.
2. Marijuana and Medicine: Assessing the Science Base (1999). Janet E. Joy, Stanley J. Watson, Jr., and John A. Benson, Jr., Editors, Division of Neuroscience and Behavioral Health Institute of Medicine, National Academy Press, Washington, D.C.
3. "DEA Blatantly Blocks Medical Marijuana Research" by Scott Morgan, January 2009. Blog from StoptheDrugWar.org.
4. Chemistry and Analysis of Phytocannabinoids and Other Cannabis Constituents, Chapter 2 in Forensic Science and Medicine: Marijuana and the Cannabinoids. Rudolf Brenneisen. Edited by M.A. ElSohly, Humana Press, Inc.
5. American Herbal Pharmacopoeia (2013). Cannabis Inflorescence, Standards of Identity, Analysis and Quality Control.
6. The cannabinoid acids: non-psychoactive derivatives with therapeutic potential. *Pharmacol Ther* 82:87-96. 1999. Bernstein SH.
7. CNN Health. "7 Uses for Medical Marijuana" (2014). <http://www.cnn.com/2014/03/07/health/gallery/uses-for-medical-marijuana/>
8. 23 Health Benefits of Marijuana. Business Insider (2014). Jennifer Welsh and Kevin Loria. <http://www.businessinsider.com/health-benefits-of-medical-marijuana-2014-4?op=1>
9. Sativex for relieving persistent pain in patients with advanced cancer (SPRAY III). *ClinicalTrials.gov* (2011).

[Disclaimer]

Shimadzu products mentioned in this article are for Research Use Only and are not for use in diagnostic procedures. Shimadzu Scientific Instruments is not condoning the use of recreational nor medical marijuana, we are merely being solicited by customers for cannabis testing at their facilities and are herein providing a market summary of the cannabis industry.

Businesses featured in this article:

G.I. Grow

www.GIGrow.us

G.I. Grow Farm's utilizes sustainable cultivation practices to enhance our environmental quality and the natural resource base and soil nutrients used for growing. They are a single source, local Oregon grown, organic farm that strictly adheres to and abides by the OMMP – Oregon Medical Marijuana Program rules and regulations.

Ole World Oils

www.CamelinaGold.com

Ole World Oils manufactures Camelina Gold oil, which is a natural source of fiber, proteins, chlorophyll, essential minerals and vitamins and can be used as a carrier for CBX oils.

G.O.A.T. Labs LLC – Genesis Organic Assurance Testing

www.GOATLabs.us

G.O.A.T. Labs is a Full Service Laboratory. G.O.A.T. Labs is the brainchild of Dana Luce. He was joined by 4 other members from A/Co 158th Avn, 101st Airborne. All of the Founders were Crew Members (pilots or door gunners) on UH-1 Huey Helicopters in Vietnam.

Rose City Laboratories LLC

www.RoseCityLabs.com

Rose City Laboratories was established in April 2012. Their staff is dedicated, highly skilled and has years of experience in both the medical marijuana industry and analytic testing. Rose City Laboratory utilizes state-of-the-art high performance liquid chromatography, gas chromatography, and mass spectrometry equipment.

MRX Labs Analytical Testing Services

www.MRXLabs.com

MRX Labs is a state-of-the-art laboratory located in Portland, Oregon, offering a full range of analytical testing services.

Viridian Sciences

www.ViridianSciences.com

Viridian offers a wide array of services, including implementation, configuration, training, prompt support, custom reporting and other ancillary professional services to clients. Their mission is to help businesses grow by providing the world's leading enterprise resource planning technology while helping companies adhere to government regulations with complete legal compliance.