4. Conclusions

- Separation of two couples of isomers with a run duration less than 6 minutes and using a 5 cm column.
- Quick sample preparation based on QuEChERS salts extraction/partitioning, almost as short as on-line Solid Phase Extraction.
- Lower limit of quantification compatible with determination of DUID.
- No carry over effect noticed.

Validation data

Among the 40 analyzed compounds, 38 filled the validation conditions in term of intra- and inter-assay precision and accuracy were less than 20% at the lower limit of quantification and less than 15% at the other concentrations. Despite the quick and simple sample preparation, no significant matrix effect was observed and the lower limit of quantification was 5 µg/L for all compounds, while the concentrations obtained with a reference (GC-MS) method in positive patient samples were compared with those obtained with this new UHPLC-MS/MS method and showed satisfactory results.

Contrary to what was already observed with on-line Solid-Phase-Extraction, no carry-over effect was noted using the present method, even when blank samples were injected after patient urine samples with analytes concentrations over 2000 µg/L.

Extraction conditions

As described by Anastassiades et al. J. AOAC Int 86 (2003) 412-31, the combination of acetonitrile and QuEChERS salts allowed the extraction/partitioning of compounds of interest from matrix. This extraction/partitioning process is not only obtained with whole blood and plasma-serum where deproteinization occurred and allowed phase separation, but also with urine as presented in Fig. 4.

Fig. 4 influence of QuEChERS salts on urine extraction/partitioning: A: acetonitrile with urine sample lead to one phase / B: acetonitrile, QuEChERS salts and urine lead to 2 phases.
Chemistry and Biomedical Properties of Cannabis

Cannabis plants contain more than 400 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 discomfort, 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 form, 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 carcinogenic compounds like benzene. 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 immune-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 edible products at a greater risk of THC overloading. There are also growing concerns over infants and children gaining access and overdosing on THC-infused edibles that look identical to candy.

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.J. Grove, an organic biomedical farm, is pioneering new approaches for natural cannabis remedies (HYPERLINK: “http://www.GroveRx.us” www.GroveRx.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), Persevere 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 lashing around. In a recent interview with Elkan’s mother, Launa, she commented that, “Elkan’s doctors were not sure why all the 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.J. Grove 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.

Cannabis Analytical Testing

Cannabis growers and dispensaries benefit tremendously from testing performed at independent laboratories. This testing determines potency, reduces the risk of contamination and improves product quality. In the following paragraphs we will cover closely examine cannabis testing. Routine cannabis testing services include cannabinoid potency, and screening/plantation 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 cannabinoids: THC, CBD and CBN and their different forms (carboxylated or decarboxylated). Some labs employ gas chromatography (GC), in which the sample is exposed under heat. Both GC-FID and GC-MS are commonplace, because internal heating is used in GC, whereas the sample present in the natural samples is converted to THC and lab 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 patients know exactly how much of the cannabinoids they are consuming.
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Herbal Remedies

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.
The cannabinoid industry and cannabis testing are in their infancy. As the need for better quality control continues and standardization is introduced, it is likely that lower limits for the various cannabis constituents 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 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. Saliva™, a pharmaceutical version of cannabis, has been approved for use in 25 countries as a treatment for muscle spasm in patients with multiple sclerosis symptoms. 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 patients with AIDS. Ideal™ is a physician prescribed “medicinal cannabis in a pill”. Unlike Marinol®, which is a synthetic form of a single cannabinoid (THC) only, Ideal 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 cannabidiol testing and standardization will continue to grow. The US cannabis industry is projected to 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:

[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 endorsing the use of recreational or 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.
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Suggested Reading:

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 simple source, local Oregon grown, organic farm that strictly adheres to and abides by the OMMMP – Oregon Medical Marijuana Program rules and regulations.
Die World Oils
www.CarminaGold.com
Die World Oils manufactures Carmina 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.
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.

Additional Cannabis Laboratory Tests
The moisture content of a variety of cannabis samples can be measured using Shimadzu MOA-63 (and MOA-120 F) balances. The MOA-63 is applicable to a variety of cannabis products and it’s long-life and large sample capacity make 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 ATP or RFL 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. Mysostom (allotrons) can be detected using Shimadzu LC and LCMS systems. Heavy metals analysis generally include the big four lead (Pb), mercury (Hg), cadmium (Cd), and arsenic (As), which can be tested by Shimadzu’s AA-7000 with ICP-7000 or ICP-8000. Alternatively, AAS may be used for.

Considerations for Future Cannabis Testing
The cannabis industry and cannabis testing are in their infancy. 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.

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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, sativa in cannabis potency can easily exceed 20%. Potency testing will improve as chemical extraction and cannabinoid 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-Venues touchscreen, graphical user interfaces between system and workstation, allowing intuitive operations regardless of experience level.