The Analysis of Medicinal Cannabis for Pesticides and Residual Solvents using a Portable GC

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Medicinal Cannabis is prescribed by doctors for their patients for a number of health benefits including, to help reduce nausea and vomiting during chemotherapy, improve appetite in people with HIV/AIDS, and reduce chronic pain and muscle spasms. The use of cannabis as a medicine has not been rigorously tested due to production restrictions and other governmental regulations both in the US and across the EU meaning there are no standardised regulations from between states or countries for quality control. This includes for content, composition, adulterants, potency or toxic residues. Therefore, there is an increased need for the best analysis techniques for quality assurance of the product.

Introduction

The Medicinal Cannabis market is an emerging industry and there is scope for big business. However, it can be perceived as legally and morally controversial, and filled with ambiguity as there are no standardised regulations for quality control. Cannabis consists of the dried flowers of the female Cannabis L-sativa plant, also known as hemp or marijuana and contains a number of active substances, including Delta-THC and Cannabidiol (CBD). There are many different strains of Cannabis plant, all having different levels of each active compound. Strains are cultivated through cloning and cross-breeding of different plants to achieve a new strain with a desired flavour or percentage of Cannabinoid.

The physical effects of cannabis are largely the result of THC, but other Cannabinoids including CBD, may also influence the effectiveness of the drug. THCA is the carboxylic acid version of THC found in raw cannabis. In applications for potency testing total THC needs to be determined so the amount of THC and the amount of THCA that could be converted to THC needs to be considered. When analysing by GC the TCHA level is not a concern as it is accepted the TCHA present will be converted to THC in the GC injector so the TCA value will be the Total THC value. If the user is analysing by HPLC the lower operating temperatures would mean the THCA would not be converted to THC so the analyst would need to determine values for both THCA and THC, they would then use these values to calculate the Total THC content. Therefore, total THC% = THC% + (THCA% x 0.877)

THC itself has proven medical benefits in particular formulations. The US Food and Drug Administration has approved THC-based medications, dronabinol (Marinol®) and nabilone (Cesamet®), prescribed in pill form for the treatment of nausea in patients undergoing cancer chemotherapy and to stimulate appetite in patients with wasting syndrome due to AIDS [1].

The chemical composition of the Cannabis determines the positive and negative effects of each dose. Cannabis is a plant and not a chemically-derived substance, therefore, it is difficult to limit the exposure of such a wide array of naturally-occurring compounds and control content levels present within any given dose. This article will review the key issues involved with Cannabis and Cannabis analysis in the areas of pesticides and residual solvents and why there is a need for nationally-recognised regulations and quality control.

US Exposure Limitation

Medicinal Cannabis is currently legalised in 26 US states and the District of Columbia. Cannabis has a national drug classification of Schedule 1 and these substances are defined as 'drugs with no currently accepted medical use and a high potential for abuse' and, therefore, remains an illegal drug as classified by the Drug Enforcement Agency (DEA).

At state level, trafficking of cannabis can carry a heavy penalty that ranges from a $250,000 fine and up to 5 years in prison for less than 50 kg and up to $10 million fine and 10 years to life in prison for over 1000 kg, for a first offence. Penalties increase in severity for reoccurring offenders with a third offence potentially resulting in a $75 million fine and life imprisonment. To avoid potential criminal restrictions, many providers of medicinal Cannabis are also responsible for the cultivation or manufacture of the finished edible product.

In addition to the 26 states that have legalised the use of Medicinal Cannabis, three other states will soon join their lead after recently passing measures permitting use of medical marijuana. Seven states and the District of Columbia have adopted the most expansive laws legalising marijuana for recreational use [2].

A Medicinal Cannabis card holder and their primary caregiver are exempt from criminal prosecution providing the amounts carried are for the patient's personal use and is of a relevant amount related to the medical needs of the patient. Medicinal Cannabis can only be acquired through non-profit dispensaries, collectives and cooperatives

that operate within strict state and local requirements. Edible Cannabis products can be bought on-line and in retail outlets, but the accessibility is extremely varied between states.

Health Benefits

There is a bank of evidence to suggest that Medicinal Cannabis can help relieve a multitude of health ailments including - pain and muscle spasms or cramps associated with multiple sclerosis or spinal cord damage; nausea, loss of appetite, weight loss and debilitation due to cancer or AIDS; nausea and vomiting associated with chemotherapy or radiotherapy used in the treatment of cancer, hepatitis C or HIV infection and AIDS; chronic pain (mainly pain associated with the nervous system, for example that's caused by a damaged nerve, phantom pain, facial neuralgia or chronic pain which remains after the recovery from shingles); Gilles de la Tourette syndrome and therapy-resistant glaucoma. Patients and doctors have also reported positive effects on a range of other conditions, including Crohn's disease, ulcerative colitis, epilepsy, itching, migraine, rheumatism, rheumatoid arthritis, Attention Deficit Disorder (ADD) and brain trauma [3].

Furthermore, a recent report by the National Academies of Sciences, Engineering, and Medicine has stated "substantial evidence" that marijuana or related compounds can effectively treat chronic pain, nausea caused by chemotherapy treatment for cancer, and spasticity caused by multiple sclerosis [4]. Further scientific research is required to confirm these positive effects and relief of symptoms for patients. As with any treatment for illness, therapeutic drugs can have negative side effects. These undesired effects can be a result of an adverse reaction to the presence of a specific compound or from receiving a different dosage to the amount clinically prescribed.

The need for Testing for Residual Solvents and Pesticides

Pesticide Testing

As Cannabis production becomes legalised across the United States and other countries such as Australia, the governing regulations regarding the use of pesticides have not been looked into. With no regulations in place, the safety of patients and consumers is at risk. There are risks that pesticides deemed illegal or harmful to humans or wildlife can be used during the production of Cannabis and go undetected, resulting in pesticides considered dangerous for human consumption being inhaled, ingested and absorbed.

Pesticides, herbicides, fungicides and other chemicals used in cultivation and storage of Cannabis products could remain within the plant structure and result in a patient being exposed to potentially toxic chemicals. Screening for these chemicals is fundamental.

Residual Solvents

Several potentially harmful compounds are used in the process, when cannabis components are extracted. They can be left in the extraction and cause harm to consumers. Residual solvents analysis will determine if any solvents have been found within the finished product, and this ensures that consumers are not at risk. Testing for residual solvents is essential. Hydrocarbon gases as well as organic solvents are used to extract essential oils from cannabis for medicinal products. Residual solvents are any solvents used in extraction that remain in the extracted product. They can be consumed by users in significant quantities.

If cannabis were regulated in the same way as a pharmaceutical drug, each batch of medicinal cannabis would need to be tested for potency, flavour profiling and

Conditions

GC Conditions	
Injector Temperature	250°C
Detector Type	FID
Detector Temperature	300°C
Carrier Gas Type	Hydrogen
Simulated Constant Flow	3.5 ml min^{-1}
Split Flow	70 ml min^{-1}
Column Type	EL-5 30 m x 0.25 mm x 0.25 µm
Temperature Program	
Initial Temperature	100°C
Ramp 1	30°C min^{-1} to 200°C (hold 5 mins)

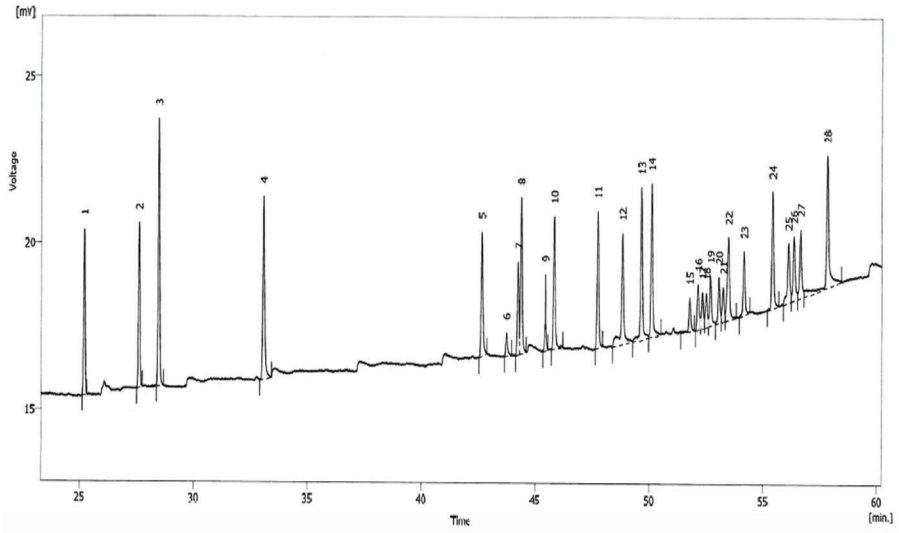

Figure 1: A 1.0 µL injection of a Synthetic Pyrethroid Pesticide standard

1. Tefluthrin	12. Acrinathrin	27. tau-Fluvalinate
2. Transfluthrin	13. c-Permethrin	28. Deltamethrin
3. Anthraquinone	14. t-Permethrin	
4. Bioallethrin	15-18. Cyfluthrin	
5. Resmethrin	19-22. Cypermethrin	
6-7. Tetramethrin	23. Flucythrinate	
8. Bifenthrin	24. Fenvalerate	
9-10. Phenothrin	25. tau-Fluvalinate	
11. L-Cyhalothrin	26. Fenvalerate	

residual solvents. Some manufacturers may use cheap materials in order to produce the products cost-effectively and have a higher profit margin. These cheap materials may have high levels of dangerous solvents. For example, if a product that retains high levels of ethanol is used to treat children, it could cause liver damage. As a result, testing needs to be taken very seriously due to the harmful nature and severe consequences that the solvents can cause.

Experimental

Pesticide Analysis

The Chromatogram in Figure 1 shows that a range of common pesticide compounds analysed using a 200 Series Gas Chromatograph. All components normally found when testing the pesticide standard were detected.

Materials

Pesticides in Cannabis were tested by using a readily available standard (Restek Cat.# 32568: GC Multiresidue Pesticide Standard #6 (18 components) Synthetic Pyrethroid Compounds) to show that the compounds can be clearly and easily detected when using a 200 Series Gas Chromatograph. A 1.0 µL sample of the liquid standard mixture was used when testing for pesticides. Some pesticides have large, complex non-volatile molecules. Using liquid samples is the easiest and quickest way to carry out pesticide analysis. A headspace analysis can be used, but requires more time to prepare the samples for injection. The samples were placed in an EL3000A liquid autosampler, and then analysed. All components normally found when testing the pesticide standard were detected, and with low noise levels.

Solvent Residue Analysis

Figure 2 shows a chromatogram of common residual solvents analysed on a 200 series gas chromatograph. All components normally found when testing residual solvents were detected clearly with low noise output.

Materials

Residual solvents in Cannabis were tested by using a readily available standard (a mix of both Supelco Residual Solvents Mix 1 Cat No. 48894 and Mix 2 Cat No. 48895) to show that the compounds can be clearly and easily detected when using a 200 Series Gas Chromatograph. In order for residual solvents to be extracted, the components will need to enter the gas phase, and so headspace sampling was used for this purpose. The samples were placed in a headspace autosampler and the sample was heated to encourage the volatile compounds to excite and enter the gas phase. Once this has happened, sampling can take place. The gas sample was injected into the 200 series GC-FID.

A 10 µL volume of the 25 mix standards was

Conditions

GC Conditions	
Injector Temperature	230˚C
Detector Type	FID
Detector Temperature	240˚C
Carrier Gas Type	Hydrogen
Constant Pressure	4.65 psi
Split Flow	70 ml min^{-1}
Column Type	EL-VOC 60 m x 0.32 mm x 1.8 µm
Temperature Program	
Initial Temperature	40˚C (hold 4 mins)
Ramp 1	4˚C min^{-1} to 200˚C (hold 3 mins)

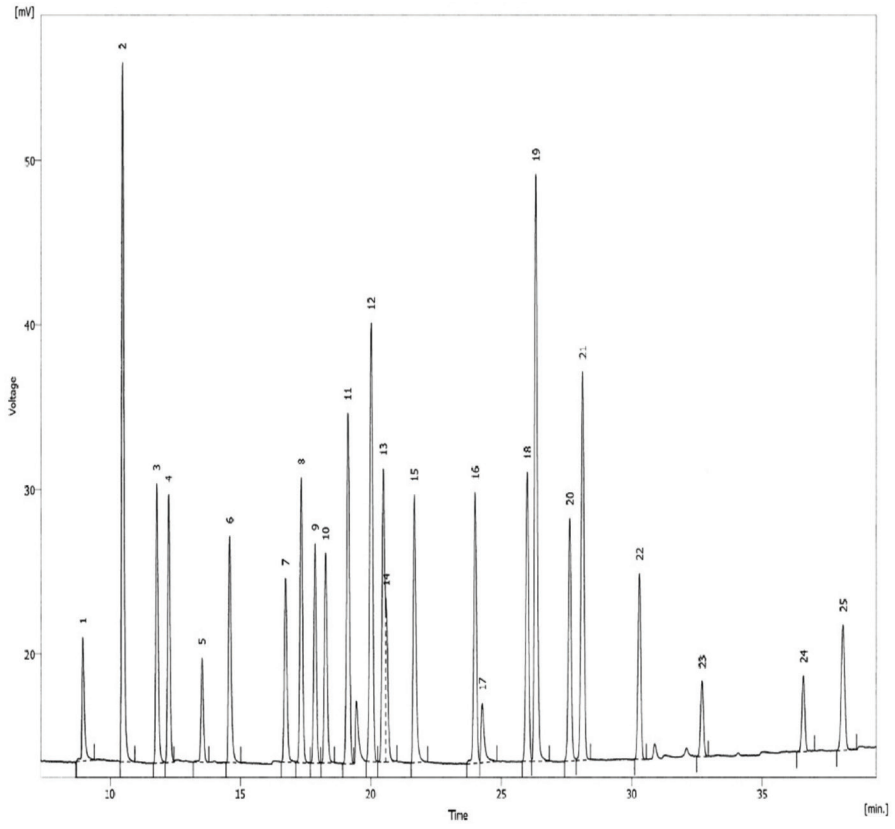

Figure 2 - A 0.5 µL injection of a 25-component residual solvent mix standard

1. Methanol	10. iso-Butanol	18. 4-Methyl-2-Pentanone
2. Ethanol	11. Tetrahydrofuran	19. 2-Ethoxyethanol
3. 2-Propanol	Acetic Acid (breakdown product)	20. iso-Butyl Acetate
4. Acetone	12. Methyl Cellusolve	21. Toluene
5. Methyl Acetate	13. Cyclohexane	22. Butyl Acetate
6. 1-Propanol	14. iso-Propyl Acetate	23. Methyl Cellusolve Acetate
7. sec-Butanol	15. n-Butanol	24. 2-Ethoxyethyl Acetate
8. 2-Butanone	16. 1-Methoxy-2-Propanol	25. Cyclohexanone
9. Ethyl Acetate	17. n-Propyl Acetate	

placed in a 20 ml headspace vial and held at 100°C for 45 minutes within an Ellutia 2t Static Manual Headspace module. A 0.5 ml gas headspace sample was taken and injected into a 200GC-FID. The chromatogram of the headspace injection is shown in Figure 2.

Results and Discussion - The Need for Regulation

Unfortunately, as Cannabis is now effectively legalised at state level, but has remained illegal at Federal level, the usual routes for substance regulation cannot be applied. The Food and Drug Association (FDA) is normally at the forefront of ensuring consumer safety, but it is restricted from completing effective drug trials, as the DEA official position means they cannot provide illegal substances for testing. The absence of this data means the FDA is unable to provide relevant regulations resulting in the FDA declaring Cannabis as not safe for human consumption [5]. However, Delta-THC, the main psychoactive ingredient in the L-Sativa plant has been an FDA-approved drug for over 25 years. This has helped influence the general opinion that cannabis itself should also be an FDA-regulated substance.

Conclusion

If classed as a pharmaceutical drug, Cannabis would be rigorously tested to comply with stringent rules and regulations regarding quality and safety of the product. As there is currently no centralised regulatory body responsible for this task, the quality assurance falls to the dispenser, manufacturer and even the individual consumer who would need to perform the quality assurance employing the best analytical methodologies. The two applications discussed in this article are for pesticide analysis and residual solvents analysis screening for the potentially harmful pesticides and compounds that can be harmful to the consumers. However, there are also the areas of flavour profiling and potency analysis that are equally as important for testing and analysis of determining strain identity as well as prescribing the correct dosage for the patients.

References:

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