Gas Chromatography: A Powerful Tool for Cannabinoid Analysis

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The rules and regulations in place within the whole cannabis industry supply chain, both in the US and across the EU, have resulted in a lack of rigorous cannabis testing as well as a lack of clarity on what testing is required. While a number of states have passed regulations that allow medicinal and/or recreational cannabis use, there are currently no standardised regulations between states or countries for quality control including content, composition, adulterants, potency or levels of toxic residues. In comparison to the pharmaceutical industry, where drugs must go through extensive quality control to meet a certain standard of safety, it is evident the cannabis industry has considerable challenges to undertake. There should be more robust systems in place to guarantee product quality in order to manufacture reliable and consistent products.

Introduction

The Netherlands has long been known for its liberal approach to cannabis and has become a major cannabis tourist destination, particularly in Amsterdam, where tourists can legally consume marijuana in coffee shops. However, the cannabis industry is constrained by a legal framework that makes it very difficult to find cannabis with tested cannabinoid and impurity content.

One particular company that has implemented GC testing and is reaping the benefits is Shamanics. This company is based in Amsterdam and extracts cannabis oil, in particular, it uses cannabinoid cannabidiol (CBD), to produce high quality CBD oil. Shamanics conducts a number of key analyses on its products before they're taken to market including cannabinoid profiling, terpene testing and soil analysis.

This article explains the industry challenges and what is required in order to provide tested, quality products to meet evolving customer requirements.

Upgrading the Cannabis Scene in the Netherlands

Shamanics was founded by Bart Roelfsema and Henjo Hielkema. Following the realisation of the poor-quality cannabis on the market, despite the high demand, Bart and Henjo wanted to sell tested, high-quality CBD oils and offer a testing service for coffee shops to help better inform their customers. Appropriate testing isn't commonplace in the Netherlands, the consumer interest is growing rapidly for properly tested products, particularly from coffee shops.

The variable quality and lack of tested cannabis available can largely be attributed to the regulation of the supply in the Netherlands. Whilst it is legal for the coffee shops to sell small amounts of cannabis, it is actually illegal for them to purchase or grow it in bulk. Therefore, the supply for the coffee shops is technically illegal meaning there is no consistent supply hence the large variation of quality. However, in February 2017, Dutch MPs voted to approve cannabis cultivation for the first time [1], a new law that would extend tolerance to growers as well as consumers. While it is not yet law and must also get majority support from the Senate, it is considered to be a historic breakthrough.

Similarly, the UK cannabis market has started to witness change. The possession and supply of cannabis is illegal in the UK, but there have been a number of cases where cannabis has been prescribed for medicinal use. One example of this is the drug, Sativex, which is the only one on the market that has THC as an active compound. It is possible to buy CBD oils from high street stores in the UK but these have usually been extracted from hemp rather than cannabis. The situation is very different in the US, where twenty-plus states have legalised cannabis for medicinal use. However, cannabis isn't classified as a medicine as it remains illegal under federal law, set at US government level. Subsequently, the FDA (U.S. Food and Drug Administration) has not approved the plant or plant extracts as a medicine. This is partially attributed to insufficient clinical trials being conducted to test cannabis use under strictly controlled conditions. Yet, 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 could ultimately become be an FDA-regulated substance.

It is predicted that the cannabis market will continue to grow, as more countries and states join in the legalisation of cannabis. A recent report [2] suggested that medical marijuana sales will grow to $13.3 billion in 2020 and adult recreational sales are estimated to reach $11.2 billion by 2020.

The Need for Cannabis Analysis

One of the core issues with cannabis quality is the use of pesticides; the Netherlands Ministry of Environment and Health reported that over 90% of cannabis plants had pesticides on them [3]. Illegal producers of cannabis can supply cannabis unhindered with potentially harmful contaminants such as fungi or pesticide residues that are potentially threatening to health. In one particular case [4], state marijuana regulators recalled more than 50 varieties of medical marijuana, concentrates and edibles that had reportedly been grown with

an unapproved pesticide; myclobutanil. It is evident that there is an impetus to develop appropriate analytical techniques for quality assurance and quality control laboratories for cannabis testing. This is necessary in order to build patient and consumer trust.

If ultimately categorised as a regulated pharmaceutical drug, cannabis would be rigorously tested to comply with stringent rules and regulations regarding quality (potency and purity) and safety of the product. However, as there is currently no centralised regulatory body that oversees this, the responsibility of quality assurance falls to the grower, manufacturer and even the consumer.

Gas chromatography (GC) is a widely used analytical tool for cannabis testing. The technique enables potency testing, terpenes profiling, pesticide screening and residual solvents analysis, which should afford potential benefit to significantly the cannabis industry. Typically, the primary cannabinoids of interest for potency testing are: tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN). A key goal in cannabis analysis is positive identification and quantification of the THC/CBD ratio. This is specifically significant for medicinal cannabis, CBD is the primary component of interest as it is often characterised by high levels of CBD and low levels of THC.

In contrast, recreational cannabis typically has high levels of THC – the main psychoactive ingredient – and low levels of CBD. Analytical instrumentation, such as GC, has long been perceived to require extensive experience in the laboratory, with training in chromatography or a deep understanding of analytical chemistry. However, with the increased need for quality control and quality assurance in the dynamic cannabis industry, GC technology is now more accessible to smaller companies and adapted for users with minimal GC experience. Newer instrumentation is more compact, cost-effective and ultimately, easier to use. While these instruments are no replacement for an independent accredited laboratory, these instruments can give growers and processors an accurate result of cannabinoid percentages. This is fundamental information to growers and dispensaries while simultaneously encouraging the uptake of testing and responsibility within the industry.

Shamanics use an Ellutia 200 Series GC for quality assurance of its products and offer a testing service for coffee shops in Amsterdam to test the quality of their cannabis. The company conducts terpene analysis and potency testing. When testing for potency, they analyse total THC and CBD. Each total cannabinoid value is calculated by adding the amount of neutral version present together with the amount of neutral version that could be created by the decarboxylation of the acidic version of the cannabinoid present. When testing by GC, the acidified versions of the cannabinoids get converted to the neutral forms in heat of the GC injector. Figure 1 shows the conversion of THCA to THC in the GC injector.

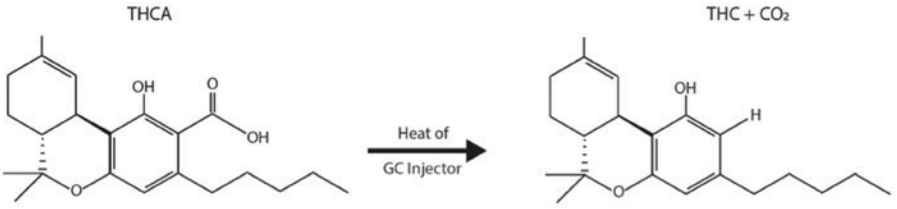

Figure 1: The Decarboxylation of THCA

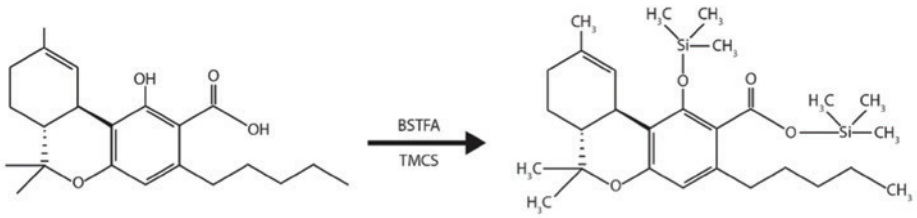

Figure 2: The Derivatisation of THCA

If they need to see both the acidified and neutral versions, they can do this by derivatising the sample (Figure 2). This additional process is required because the heat of the injector converts the THCA to THC in the injector so it is not then present to analyse and just becomes one large THC peak. The derivatised THCA is not converted in the injector so can be separately analysed. Accuracy is crucial in order to judge the quality of a product, and relay this information to the retail environment.

Experimental

Terpene and Potency in a single analysis

The Chromatogram in Figure 3 shows the concentration and profile of the terpenes and cannabinoids in a cannabis sample in order to establish the potency, the flavour profile, strength and therefore, quality.

Materials

0.1 g of cannabis was added to 30 mL of methanol at ambient, shaken for 30 seconds and left to extract for 30 minutes. See Figure 4. An aliquot of the extractant liquid is then collected through a syringe filter and placed in a sample vial ready for analysis. The analysis was performed using a 200 Series GC, see figure 5, with an FID (Ellutia, Ely, UK) and a 30m 0.25 x 0.25 EL-5 Column. The standards used for the calibration were a standard 3 component cannabinoid mix (Restek Cat.# 34014: Cannabinoids Standard (3 components)) and a 19 component terpene mix (Restek Cat.# 34095: Medical Cannabis Terpenes Standard #1 (19 components).

GC Conditions

Injector Temperature	270˚C
Detector Type	FID
Detector Temperature	280˚C
Carrier Gas Type	Hydrogen
Detector Range	x10
Carrier flow	1.5 mL/min
Split Flow	70 mL/min
Injection volume	1 µl
Stabilisation time	0.5 min
Column Type	EL-5 30 m x 0.25 mm id x 0.25 µm film thickness
Initial Temperature	100˚C
Hold	5 min
Ramp 1	20˚C/min
Temperature 1	200˚C
Ramp 2	10˚C/min
Temp 2	270˚C
Total run time	17 min

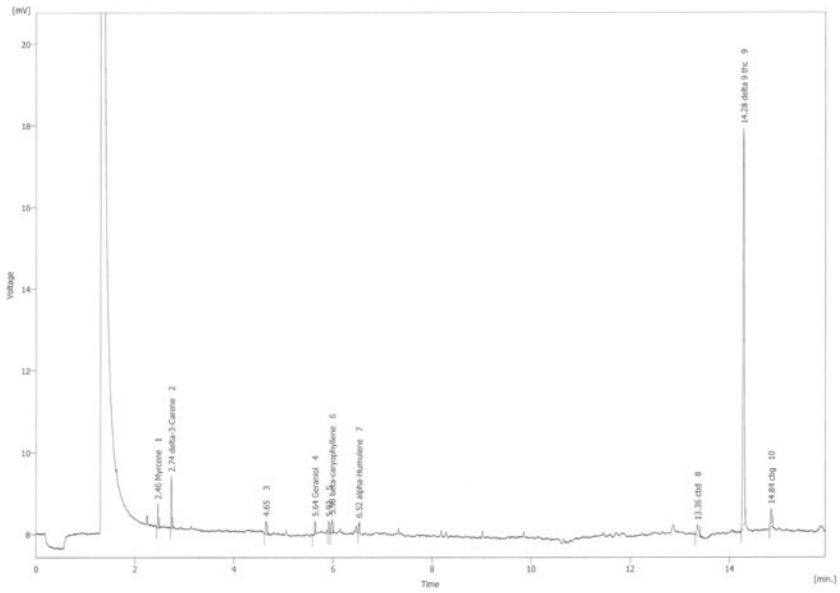

Figure 3: Chromatogram showing the terpenes and cannabinoids in a single run

Table 1: Compound list and the corresponding results

Peak Number	Compound	Retention Time [min]	Response	Amount [mg/l]
1	Myrcene	2.463	0.635	2.536
2	delta-3-Carene	2.737	1.167	9.605
4	Geraniol	5.636	0.363	5.496
6	beta-caryophyllene	5.981	0.478	7.793
7	Alpha-Humulene	6.524	0.212	15.551
8	cbd	13.356	0.709	26.620
9	delta 9 thc	14.284	18.523	601.286
10	cbg	14.843	0.811	24.035
Total				692.922

Figure 4: Cannabis sample added to methanol

Figure 5: The 200 Series GC at Shamanics

Results and Discussion

The results show that this sample has 601.226 µg of THC per mL. This results in the sample having a total THC content of 18%.

The high level of THC, shown in Figure 6, indicates that if consumed this would have more of the psychoactive effects. The results show the ratios of THC and CBD, providing information about the potency of the product. The concentration and profile of the terpenes, see Figure 7, that are present provides information about the flavour profile to guide users about the sort of flavour characteristics that can be expected from the cannabis when consumed. The different terpenes present and their levels will define how that particular strain of cannabis will smell and taste. For example a strain that shows high levels of limonene may will have citrus lemony aroma (limonene is commonly also found in lemons) or a high level of myrcene will give flavours similar to beer (myrcene is also found commonly in hops).The levels of terpenes can also be used as a guide to understand the quality of the product. For example, low levels could indicate that it has not been dried correctly.

Harnessing this information, manufacturers can verify the quality of cannabis samples. For example, Shamanics aim is to upgrade the entire cannabis scene in the Netherlands using GC in order to protect and grow the market. As a result of the robust testing methods, Shamanics has set themselves apart from their competitors in the Netherlands. Testing remains very rare in the Netherlands, but there are other companies using techniques such as near-infrared spectroscopy, SFC and HPLC to conduct quality testing.

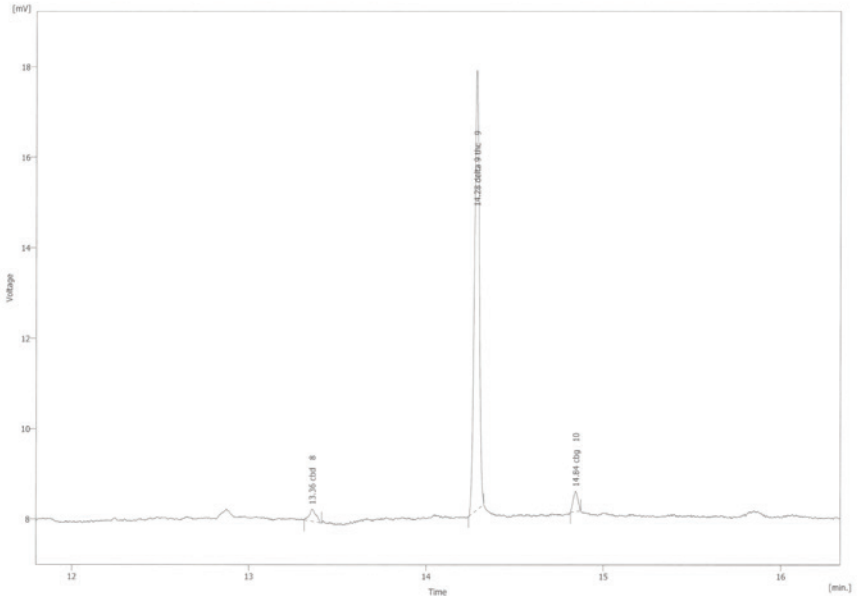

Figure 6: Cannabinoid detail from Chromatogram

Conclusion

It is evident that the global cannabis production industry requires significant change before it can truly flourish. Challenges must be overcome around analytical testing in order to provide consistently high quality products. GC (and other chromatographic techniques) can alleviate this challenge, as it can be used for rapid determination of THC and CBD concentrations in cannabis in order to characterise plant materials for medicinal and recreational cannabis applications.
As well as being able to determine the therapeutic effects the ability to determine the concentration profile for a range of terpenes allows the flavour profile to be determined, as well as giving an indication of some quality issues. This is particularly important with the rapidly changing laws and regulations within the industry in order to provide a consistent, tested product to customers in the highly competitive marketplace.

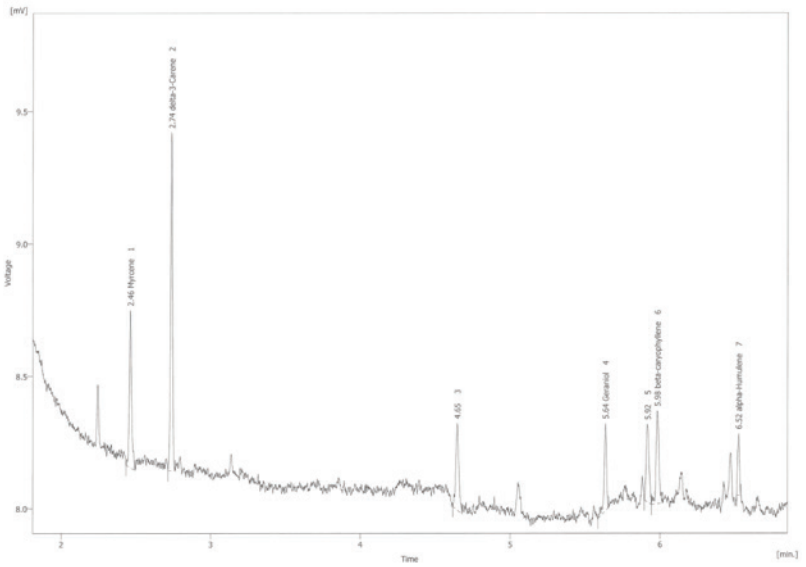

Figure 7: Terpene Detail from Chromatogram

References:

1. BBC News, 2017. Dutch MPs vote to approve cannabis cultivation for first time (internet) http://www.bbc.co.uk/news/world-europe-39045243 [Accessed 17/01/2018]

2. New Frontier Data, 2017. The Cannabis Industry 2017 Annual Report (internet) https://newfrontierdata.com/annualreport2017/ [Accessed 17/01/2018]

3. Rijksoverheid, 2017. Ministry of Environment and Health (internet) https://www.rijksoverheid.nl/documenten/rapporten/2016/01/18/cannabis-contaminanten [Accessed 17/01/2018]

4. The Denver Post, 2017. State recalls 50 Tree of Wellness medical pot products because of pesticide (internet) http://www.denverpost.com/2017/11/03/tree-of-wellness-medical-pot-product-recall/ [Accessed 17/01/2018]