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# Customer insight into the identification and quantification of cannabinoids and cannabis terpenes

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Shamanics, based in Amsterdam, The Netherlands, is a cannabis oil extraction company. Shamanics works with cannabis, in particular, the cannabinoid cannabidiol (CBD), to produce high quality CBD products. But, what really sets the company apart is its passion for cannabis testing. Unlike its competitors, it conducts a number of key analyses on its products before they're taken to market including cannabinoid profiling, pesticide residue testing, terpene testing and soil analysis. Shamanics understands that this kind of rigorous cannabis testing is the only way to provide quality and reliable products to consumers.

Shamanics was founded by Bart Roelfsema and Henjo Hielkema and is named as such due to their beliefs in the plant, just like the Shamans. Roelfsema explains the company's background:

"Cannabis has been a great passion of mine for many years now, having previously worked in a shop for smart products. I, like many of the growers here in the Netherlands, was growing my own product and realised the quality on the market was generally very poor but the demand was very high. It was at this stage that I, along with my colleague and co-founder Henjo Hielkema, developed the desire to create a better product and do what no one else in the market was doing. We wanted to sell tested, high-quality CBD products. We moved this forward through a combination of Henjo's skills and our general desire to produce a better cannabis product than what was available on the market at that moment."

## Challenges

The Netherlands has long been known for its liberal approach to cannabis and has become a major tourist destination, particularly Amsterdam, where tourists can legally consume marijuana in coffee shops. However, the cannabis industry is constrained by the same legal framework that exists globally. While it is entirely legal to purchase cannabis in a coffee shop, due to the restrictions on growing and purchasing the drug, these shops have to turn to the black market to acquire their product. It is therefore very difficult to find cannabis with tested cannabinoid content. Yet, 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. Although it is not yet law and must also get majority support from the Senate, it is considered to be a historic breakthrough.

The production restrictions on cannabis are in place both in the US and across the EU. There are currently no standardised regulations between states or countries for quality control including content, composition, adulterants, potency or toxic residues.

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One of the core issues with cannabis quality is the use of pesticides; the Ministry of Environment and Health reported that over 90 percent of cannabis plants had pesticides on them[2]. Illegal producers of cannabis can supply cannabis unhindered with potentially harmful contaminants such as mold or pesticide residues that are potentially threatening to health. In one particular case[3], state marijuana regulators recalled more than 50 varieties of medical marijuana, concentrates and edibles that had reportedly been grown with an a unapproved pesticide; myclobutanil. It is evident that there is an impetus for the best analysis techniques for quality assurance and quality control in cannabis.

## Gas Chromatography as a Solution

A widely used technique for cannabis analysis is gas chromatography (GC). The technique enables potency testing, terpenes profiling, pesticide screening, and residual solvents analysis, providing potential to significantly benefit the cannabis industry. Typically, the primary cannabinoids of interest for potency testing are: tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN). A main goal in cannabis analysis is positive identification and quantification of the THC/CBD ratio.

## Working with Ellutia

Shamanics took the decision to complete its own production with an immediate need for quality control. Roelfsema explains: "We realised we needed more quality control. We first heard about Ellutia at a conference and began conversations about a partnership. We had looked at other companies, however, our feeling was that Ellutia was more sophisticated and better suited to our needs. The decision was made not just on the instruments that Ellutia could provide, but also on their expertise in chromatography."

He adds: "Improving cannabis quality is not only our business but our passion and, although the improvements in the industry have been vast, there is still a considerable way to go. For a long time the cannabis industry has been a grey area but we want to see more testing and regulation."

## The GC Takeover

In 2017 Shamanics purchased the Ellutia 200 Series GC. This GC was originally designed for educational purposes and as a result, cannabis producers find it very user-friendly. The 200 Series GC, shown below, is compact and lightweight, making it portable and easy to use and it comes with a full range of detectors. The 200 series offers equivalent performance to other GCs, but users can benefit from the lower space requirement, reduced energy bills, service costs and initial capital outlay.

Since the purchase of the 200 Series GC, Shamanics has used the instrument primarily for the development of its products and as a result, they have experienced a range of improvements.

"It is very liberating to actually see what you are doing," says Roelfsema. "If you are a grower, a manufacturer or a seller, it is always reassuring to see what you have and prove or improve quality." Although testing isn't commonplace in the Netherlands, the consumer demand is rising for tested products.

"We also conduct terpene analysis and cannabinoid acid analysis," comments Roelfsema. "This is a very important aspect of the GC as now it is possible to methylate the sample and test for acidic forms of cannabinoids that are present in the plant. The 200 Series is very accurate, which is a huge benefit. Being able to judge the quality of a product and then relay that information to the retail environment, is helping to grow our business and stay ahead of the curve."

The Chromatogram in Figure 2 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 3. 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, with an FID 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 |

| 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** |

**Table 1: Compound list and the corresponding results**

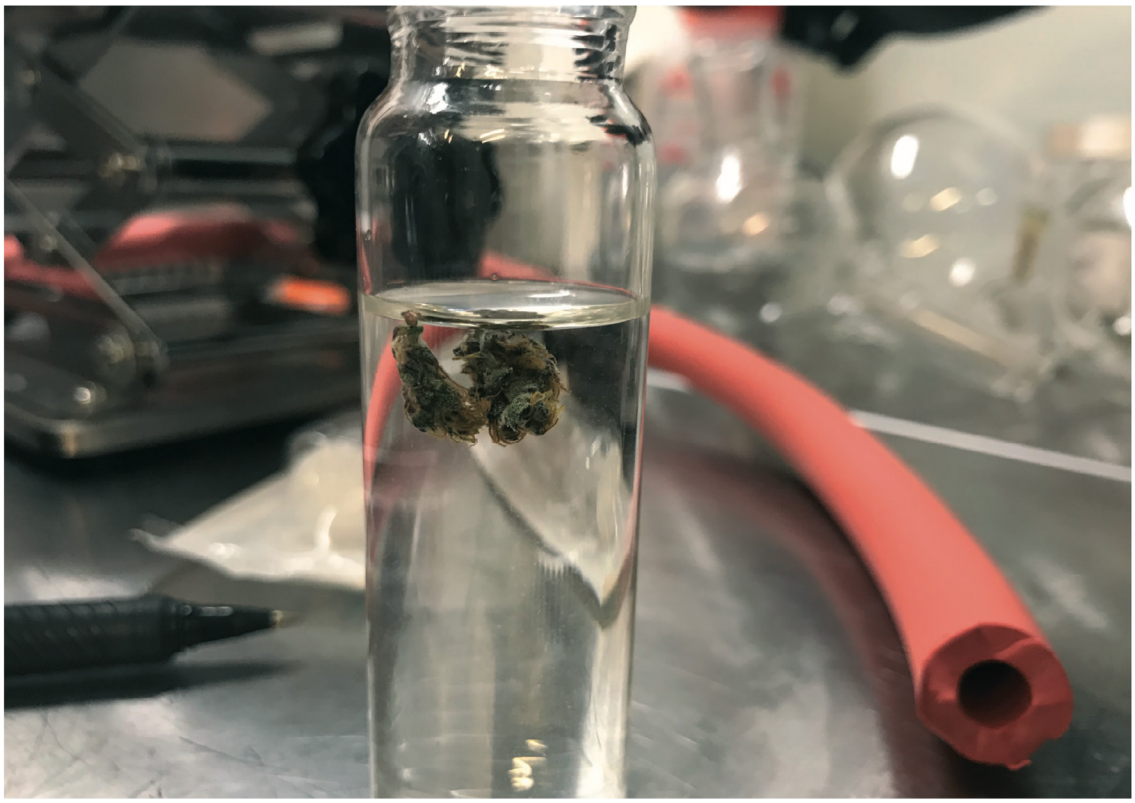

**Figure 3: Cannabis sample added to methanol**

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%.

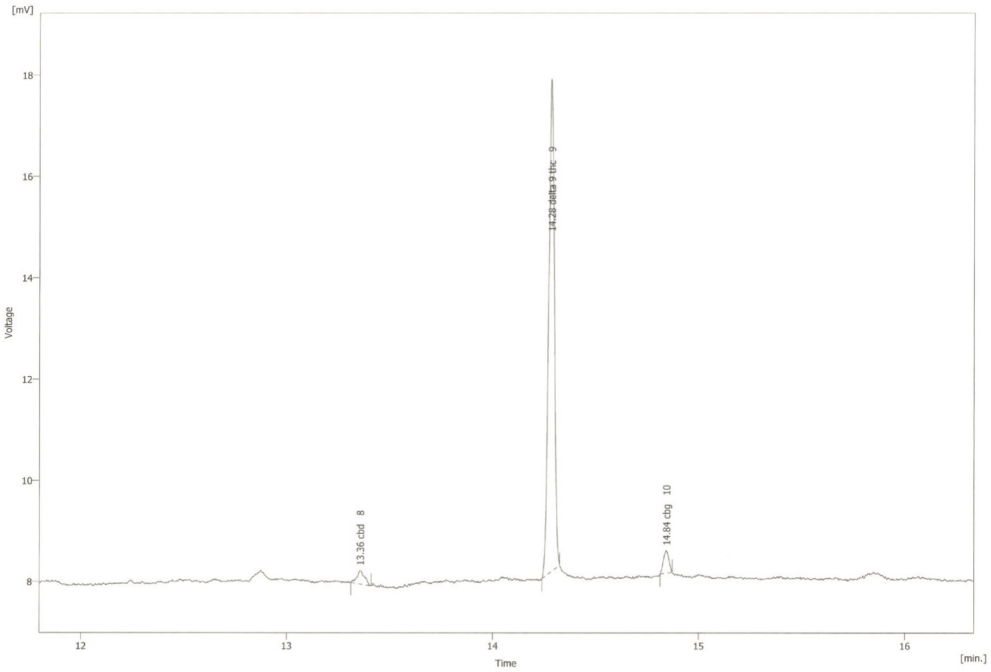


**Figure 4: Cannabinoid detail from Chromatogram**

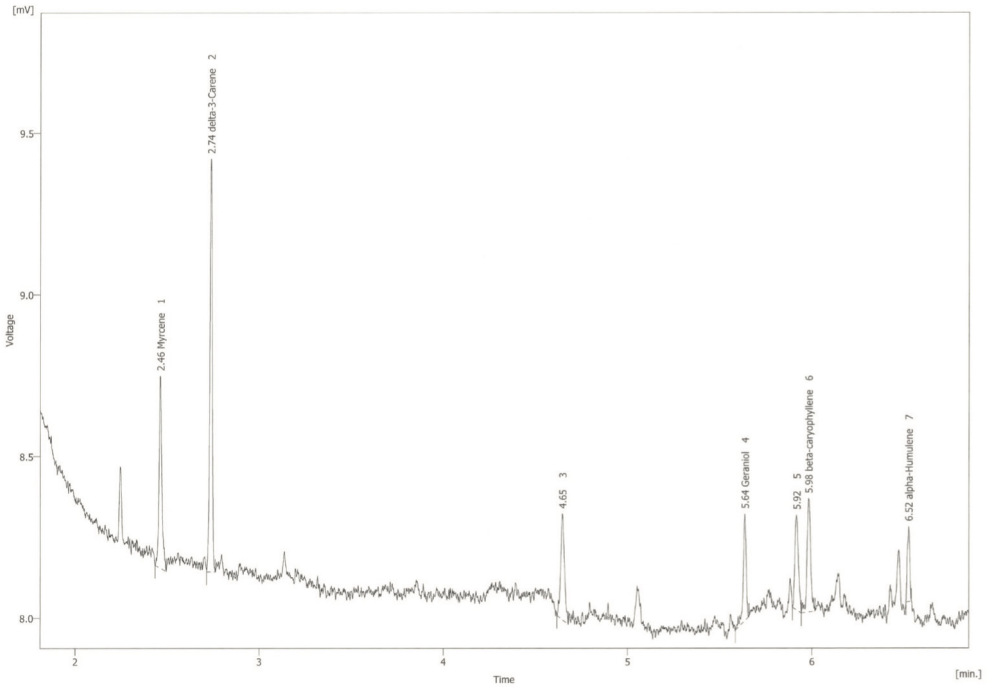


**Figure 5: Terpene Detail from Chromatogram**

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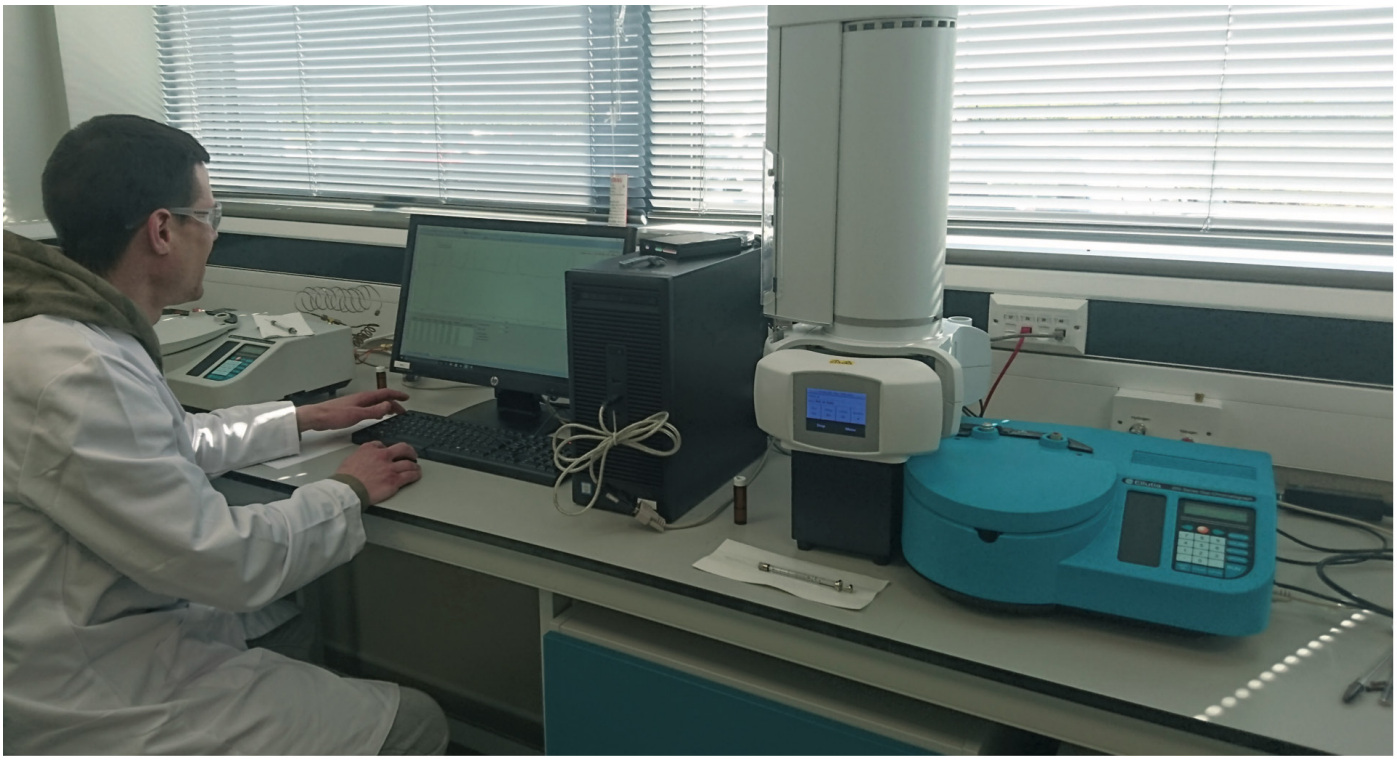
**"With little GC experience between us, the training was extremely beneficial. The equipment has resulted in very consistent analysis."**

The high level of THC, shown in Figure 4, 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 5, 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 have citrus lemony aroma (limonene is commonly also found in lemons) or a high level of myrcene contributes a peppery and balsam aroma 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.

Roelfsema discusses the value of the instrument to Shamanics: "We have had the 200 series GC in our facility for over 6 months now and have been using it every day after receiving training from Ellutia. With little GC experience between us, the training was extremely beneficial. The equipment has resulted in very consistent analysis."

**"Our products have become more reputable because we have the proper testing methods in place which set us apart from our competitors in the Netherlands."**

The training provided by Ellutia experts at the GC Excellence Academy can be bespoke for individual customer needs, such as that presented by Shamanics, see Figure 6. Mastering the use of modern gas chromatography instruments and maximising their potential is a key objective for Ellutia's customers. The deep company heritage and expertise in GC techniques and close relationships with customers mean that Ellutia place as much emphasis on equipping GC users with the insight they need, as what goes into the development of its products.



Roelfsema explains the results: "Our products have become more reputable because we have the proper testing methods in place which set us apart from our competitors in the Netherlands. Our collaboration with Ellutia has enabled us to implement this. Testing is still very rare in Holland but there are some companies that are using techniques such as near-infrared spectroscopy and HPLC to conduct safety and quality testing. We are certainly in the minority of labs completing GC analysis on cannabis in Holland and we are focused on keeping up with the latest developments in the industry so that we can continue to be innovative and deliver high quality cannabis products to consumers whilst also paving the way for this field in the Netherlands."

## Future

Roelfsema explains his vision for the future of cannabis analysis: "My vision is to see the entire cannabis scene here in the Netherlands upgraded which would be very welcome for all concerned. There is little or no testing being done here but a lot of cannabis being sold and this` needs to change in order to improve the quality of the market as a whole."

He adds: "On a global scale there is so much potential, particularly due to the legalizing of cannabis in several states in the US and other countries such as Canada, Uruguay, Mexico, U.S.A. and Portugal. As a result, there is finally an opportunity to investigate the cannabis plant and its benefits for health, enjoyment and the economy."

For more information about Ellutia please visit https://www. ellutia.com/, call us at +44 (0) 1353 669916 or email us at info@ ellutia.com. For more information about Shamanics, please visit: https://shamanics.nl/. For more information about the 200 Series GC, please visit: https://www.ellutia.com/200-series-gas-chromatograph

## References


[1]Dutch MPs vote to approve cannabis cultivation for first time:

http://www.bbc.co.uk/news/world-europe-39045243

[2]Ministry of Environment and Health:

https://pubmed.ncbi.nlm.nih.gov/31834671/

[3]State recalls 50 Tree of Wellness medical pot products because of pesticide:

http://www.denverpost.com/2017/11/03/tree-of- wellness-medical-pot-product-recall/

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To learn more about the Ellutia 200 Series GC, please scan the QR code below or visit https://www.ellutia.com/200-series-gas-chromatograph

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