

### Sample Preparation of Cannabis Plants for Efficient Extraction

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Cannabis has been used as a medicine in various cultures for thousands of years, for example as marijuana (dried leaves and flowers) or as hashish (the resin of the female plant).

Today Tetrahydrocannabinol (THC) and Cannabidiol (CBD) are particularly isolated and researched from the multitude of active substances contained in the plant (in addition to other cannabinoids and terpenes) as pharmacologically effective components.

The cannabis active ingredients are used therapeutically in particular as pain reliever, for appetite stimulation, anti-inflammatory and antispasmodic.



Figure 1. Sticky and Tricky – the translucent structures that are seen producing from the cannabis flower are trichomes. These structures are resinous & sticky and contain the vast majority of biologically active cannabinoid and terpene compounds. This is a challenging material for milling.

#### Supercritical CO<sub>2</sub> extraction

The most common method to extract the active ingredients from the cannabis plant is supercritical CO<sub>2</sub> extraction. At above 31°C and with high pressure the CO<sub>2</sub> gets into the 'supercritical' state. It now acts as a solvent. It is passed through a chamber containing the plant material. The CO<sub>2</sub> has still the density of a liquid, but can fill the entire chamber like a gas. This is ideal for extraction as it does not cause denaturation or damage to the product.

The CO<sub>2</sub> extracts the cannabinoids and terpenes from the plant. The result is a safe, high-quality, pure oil, which can now be processed for various therapeutic applications.

The producers of these cannabis oils naturally want to make the extraction process as efficient as possible. That means: the highest possible yield of the ingredients in the shortest possible time.



Figure 2. Cannabis oil

#### Homogeneous grinding of cannabis

The requirement for this is an optimal, homogeneous grinding of the cannabis plant. The instrument used in this study was the Fritsch PULVERISETTE 19 Universal Cutting Mill for fine grinding and for precise comminution.



Figure 3. Inhomogeneous sample done by a food blender



Figure 4. Homogeneous sample done with the FRITSCHE PULVERISETTE 19

#### Perfect grinding chamber geometry

In cutting mills, the sample is shredded between the knives of the rotor and the counter knives in the grinding chamber using the principle of a pair of scissors to produce the desired final fineness. The unique grinding chamber geometry in the Fritsch Universal Cutting Mill PULVERISETTE 19 ensures minimal dead space. The progressive cutting geometry between the rotor and stator knives actively transports the sample material. It cannot get stuck anywhere. The special airflow is the basis for quick, clog-free work and quick residue-free cleaning. In addition, the use of the stainless steel high-performance Cyclone separator ensures high throughput.

Table 1. Cannabinoid recovery by size.

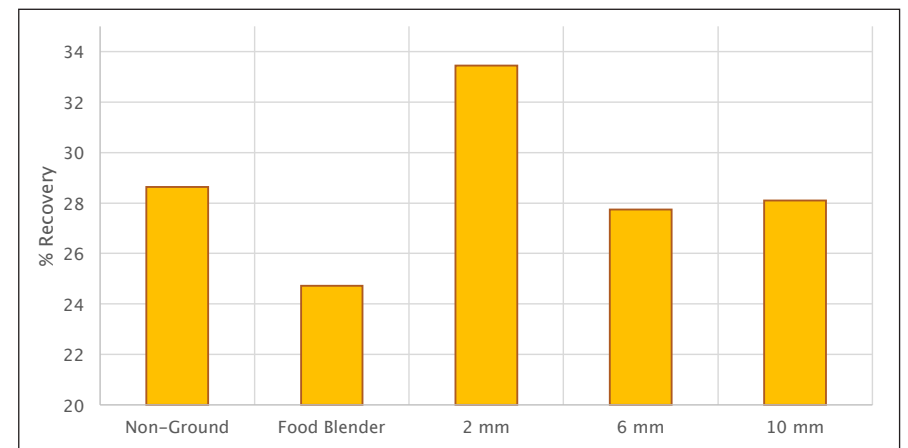
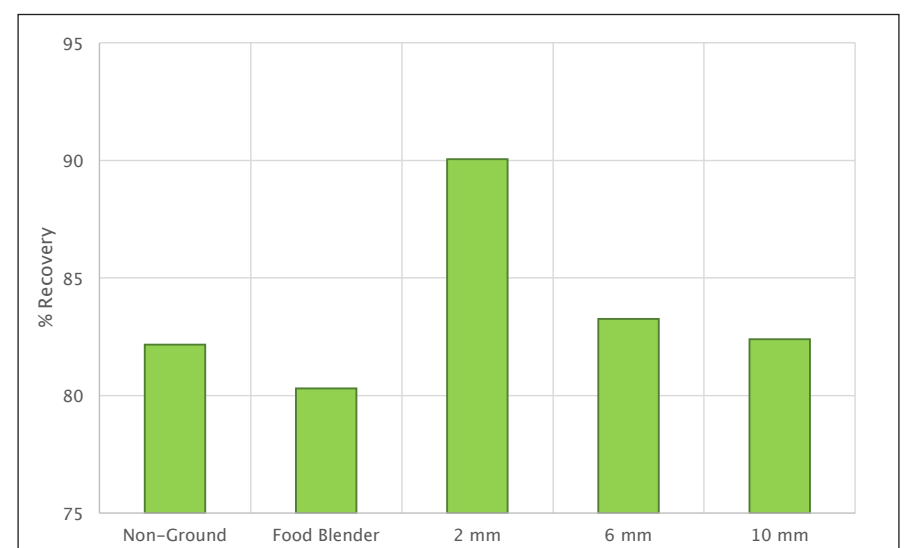


Table 2. Terpene recovery by size.



With the help of an industrial vacuum cleaner and the high-performance Cyclone separator, a negative pressure is generated in the grinding chamber. The material is fed into the Fritsch PULVERISETTE 19 via a funnel. Different sieve cassettes define the final fineness of the cannabis sample.

Unmilled cannabis plant material has a bulk density of 100-125 g/litre, while ground material has a bulk density of 225-250 g/litre.

The sample is optimally homogenised by the grinding and significantly more material can be filled into the extraction column. Of course, this also significantly increases the yield per extraction.

In cooperation with OutCo, a cannabis producer in California, USA, we examined which degree of grinding means the optimum for the most efficient extraction [1]. The best results are achieved with the 2 mm sieve. Other screens can also be used for different fractions.



FRITSCH Universal Cutting Mill PULVERISETTE 19

## Instrumentation

The Fritsch Universal Cutting Mill PULVERISETTE 19 is also used to grind samples for the potency testing or to examine the material for pesticide residues. In order to optimally adapt the cutting performance to the sample, the speed of the rotor can be regulated between 300 rpm to 3000 rpm.

As with all mills used in analytical laboratories, simple cleaning is desirable. The Clean Design concept was used for the Fritsch PULVERISETTE 19: the grinding chamber can be completely opened and all grinding parts can be removed without tools for fast and easy cleaning. Cross contamination can be effectively avoided.

The Fritsch PULVERISETTE 19 is also available in a 316L stainless steel version for use in the pharmaceutical sector.

An informative video demonstrates how to quickly and easily prepare cannabis plants for efficient cannabis oil extraction and how to achieve a homogeneous sample - [www.fritsch-international.com/cannabis](http://www.fritsch-international.com/cannabis)

Further instrument information can be found at [www.fritsch-international.com/p-19/300-3000/](http://www.fritsch-international.com/p-19/300-3000/).

## References

1. Dr Markus Roggen, CBDV, Complex Biotech Discovery Venture Ltd., Vancouver, Canada and Blake Grauerholz, OutCo, El Cajon, California, USA



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## Partnership Formed to Expand Options for Accessing Drug Metabolites

**Hypha Discovery Ltd**, the leading specialist CRO for drug metabolite provision, and Cypex Ltd, experts in the provision of recombinant xenobiotic metabolising enzymes, have formed a partnership wherein Hypha can scale-up and purify metabolites made by Cypex enzymes.

The partnership utilises the expert services Hypha Discovery already provides to pharmaceutical and agrochemical clients using its One Stop Metabolite Shop. The one-stop shop comprises a combination of both biological and chemical techniques so clients can quickly establish a method to identify and scale up production of any type of metabolite to support R&D needs. Expanding this toolbox to include additional xenobiotic-metabolising enzymes developed by Cypex, provides Hypha with even more options for synthesising and purifying metabolite standards for clients.

Cypex's portfolio of recombinant enzymes is underpinned by patented technology which enables the expression of human and other mammalian drug-metabolising enzymes in bacteria. Access to Cypex's enzymes augments the suite of PolyCYPs enzymes developed by Hypha scientists for accessing CYP derived metabolites. The partnership enables access to metabolites derived from CYP, FMO, AOX, UGT, SULT, CES, MOA enzymes from a variety of clinically relevant species, as well as humans.

Liam Evans, CEO of Hypha Discovery, commented: "We are delighted to further develop our relationship with Cypex, which strengthens Hypha's position as the go-to company for metabolites. We value this opportunity to collaborate with the team at Cypex and are excited by the synergies that this partnership brings."

Michael Voice, Director of Cypex, added: "There is an obvious synergy between Cypex's enzyme products and Hypha's expertise in metabolite production, purification and identification. Our customers will not only have access to a wider range of services, they are also assured of the excellent level of personal service that both Cypex and Hypha pride themselves upon. I am delighted to be working with the team at Hypha to better serve our customers."

More information online: [ilmt.co/PL/zyoQ](http://ilmt.co/PL/zyoQ)



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## Drug Discovery Company Accelerates Research with Versatile Pipette Range

California-based drug discovery company Neuropore Therapies is using Integra Biosciences' EVOLVE, VIAFLO and VOYAGER pipettes to help accelerate research in both its in vitro and in vivo pharmacology departments. The company, which is dedicated to halting the progression of neurodegenerative diseases by targeting the core processes underlying these disorders, has been a long term supporter of Integra, as a Neuropore scientist explained: "We initially started with just the VIAFLO electronic multichannel pipettes. As our biochemical and cell-based capabilities expanded, we needed a versatile solution that would allow us to reformat between the different volume and plate requirements of each workflow. The automatically adjustable tip spacing offered by the VOYAGER electronic pipette was ideal in providing tenths of a millimetre customisation for tip locations that would match our specific needs."

"We also needed to increase our throughput and expand our high content screening activities, without sacrificing data quality, which required the ability to quickly and accurately pipette multiple low volume samples into 384 well plates. Our VIAFLO and VOYAGER electronic multichannel pipettes provide the necessary precision, while reducing errors associated with a large numbers of individual samples, giving us complete confidence in our results. We have also recently purchased a number of EVOLVE manual pipettes, as having one set of tips at the bench to satisfy all our pipetting needs makes life easier. The ergonomics of the three dial system on the EVOLVE makes it much faster to adjust between high and low volumes, which has sped up a lot of our single channel pipetting activities. It's a win-win situation."

More information online: [ilmt.co/PL/4ZDz](http://ilmt.co/PL/4ZDz)

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