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Cannabis – from illegal drug to medical panacea

Reproducible sample homogenization of cannabis and related products

Cannabis is classified as a so-called "soft" drug which potentially lowers the inhibition threshold for consuming "hard" drugs like heroin or cocaine. Possession of cannabis was prohibited worldwide in 1925 but today, limited consumption is legal in various countries, for example Canada, the Czech Republic or Israel. Uruguay was the first country to legalize the sale, cultivation, and distribution of cannabis in 2013. As the benefits of cannabis for medical treatment have been proven by several studies[1], many countries have started the process of legalizing the use of cannabis under strictly regimented conditions. In 29 states of the USA, the purchase of cannabis for medical reasons has been allowed since 2016, one year later it was also legalized in Germany.

The beneficial effects of cannabis on human health can be grouped into five clusters: 1. pain/sleep; 2. gastro-intestinal; 3. neurological; 4. mood/behaviour and 5. other. The main ingredients of cannabis are cannabinoids like tetrahydrocannabinol (THC), cannabinol (CBN) or tetrahydrocannabivarin (THCV), which can act as appetite suppressant, sedative or psychoactive compounds. These have beneficial effects, e. g. on metabolic disorders like diabetes, pain relief, anti-inflammatory processes, and even on the treatment of bacteria like MRSA. A positive influence on diseases like cancer, Tourette's syndrome, arthritis, HIV, asthma, Alzheimer's or multiple sclerosis is also attributed to cannabis. Another large group of ingredients are the so-called

1 http://www.cannabis-med.org/german/studies.htm

terpenoids like Linalool, α-Pinene, or D-Limonene. One effect of the terpenoids is the "entourage effect" which means they can enhance/alter cannabinoid uptake in the Blood-Brain-Barrier. Terpenoids also act as antioxidants or anti-inflammatory agents.

The concentrations of cannabinoids and terpenoids are the main points of interest in the quality control of cannabis-related products. These are usually detected by HPLC analysis. The pesticide contents is also of interest. Cannabis occurs in a variety of edible forms, e. g. ground leaves, concentrate or related products like jelly bears or cookies. To ensure reliable analytical results, the sample preparation process for cannabis needs to be adaptable to the considerable complexity of the various matrices. There are some challenges to overcome: samples may be sticky, fibrous or fatty and the sample amount may vary greatly.

Although Cannabis is increasingly used for medical treatment, in most countries it is still an illegal drug and the possession and sales is a criminal offence. Therefore, not only the quality control for medical applications is relevant but also analysis of the active components in a penal context. In trials of drug offences, the expertise on the active components of the drug has substantial influence on the penalty. For cannabis, for example, a content of more than 7.5 g tetrahydrocannabinol (THC) is already considered critical. The expertise is usually given by the local Office of Criminal Investigation.

Fig. 1: Cutting Mill SM 400 XL

Pre-cutting of dried hemp plants in the Cutting Mill SM 400 XL

The Cutting Mill SM 400 XL has a grinding chamber volume of 7.5 l and accepts sample pieces with a maximum size of 170 mm x 220 mm. Thus, large sample volumes are fed and fully homogenized in a very short time. Manual pre-cutting is usually not required. Thanks to the wide opening of the hopper, considerable grinding chamber volume and the large 240 mm x 240 mm surface of the bottom sieves, the throughput is much higher than that of smaller models. On top of that, the SM 400 XL can achieve grind sizes down to 1 mm, depending on the sample material. 100 kg dried hemp with an initial particle size of up to 60 mm was cut batchwise to a fineness <20 mm within 60 minutes, using a 20 mm bottom sieve. The SM 400 XL is the only mill to rapidly process such large amounts of fibrous material without blockages of the hopper by wedged pieces. The pre-cut sample can now be pulverized, for example in RETSCH's Ultra Centrifugal Mill ZM 300. Additionally, the SM 400 XL is suitable for milling all parts of the plant for easy 'rolling' or filling of material into a variety of objects for smoking.

Fig. 2: Initial hemp sample (left) and after size reduction in the SM 400 XL (right)

Fig. 3: Ultra Centrifugal Mill ZM 300

Sample homogenization in the Ultra Centrifugal Mill ZM 300

The Ultra Centrifugal Mill ZM 300 is the ideal mill for pulverizing granules like grains or fibrous samples like hemp plants. It achieves a maximum speed of 23,000 min^{-1} and can be equipped with a large range of accessories, allowing for adaption to the sample´s requirements. The shearing forces between rotor and ring sieve facilitate successful size reduction of fibrous materials. Hemp contains oil which makes it a temperature-sensitive material; to reduce heat build-up during grinding, it is recommended to use a distance sieve. Thanks to a small gap between the sieve and the rotor, the shearing forces and the formation of heat are reduced. 20 g of the pre-cut hemp flowers can be ground to a particle size smaller than 0.5 mm by using a 0.5 mm distance sieve at a speed of 23,000 min^{-1}. The use of a cyclone has a cooling

effect on the sample and helps to efficiently discharge the material from the grinding chamber. The pulverized sample is now ready for, e. g. extraction of pesticides with the QuEChERS method.

Fig. 4: After pre-cutting in the SM 400 XL (left) the sample is pulverized to <0.5 mm in the ZM 300

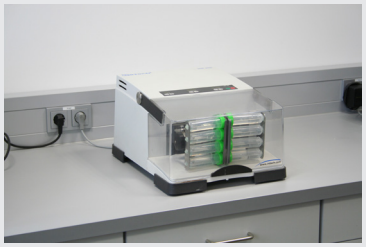

Fig. 5: Mixer Mill MM 400 with adapter for 8 conical centrifuge tubes

Pesticide extraction with the Mixer Mill MM 400 (QuEChERS method)

The so-called QuEChERS method ("quick, easy, cheap, effective, rugged and safe") was developed to make sample preparation to pesticide residue analysis more efficient. It basically consists of three steps: Homogenization – Extraction – Analysis. During the homogenization process, care must be taken that the sample does not get too warm as some pesticides are volatile. After the homogenization, 10 g of the pulverized hemp sample are extracted with 10 ml acetonitrile. In the next step, the organic phase is dried and tested for pesticides with chromatographic analysis. To avoid ghost peaks in the chromatograms, the sample is extracted in the presence of a salt mixture (e. g. sodium chloride and magnesium sulphate in a 1:2 ratio). To transfer the pesticides from the sample into the organic phase, the mixture is agitated for 1 to 3 minutes with acetonitrile and salt. The mixture can be agitated in a laboratory mill like RETSCH's Mixer Mill MM 400. It shakes the sample in a 50 mL Falcon tube with a frequency of up to 30 Hz, thus ensuring thorough mixing of the sample to improve subsequent extraction.

Fig. 6: CryoMill with 50 l dewar

Pulverization of hemp in the CryoMill for subsequent pesticide analysis

The extraction via QuEChERS can be improved by reducing the particle size to <0.5 mm. Due to the oily and sticky sample properties, the Ultra Centrifugal Mill is not suitable to achieve this. Embrittlement of the sample, e. g. with liquid nitrogen, is a very effective way of making oily materials break easily. Cryogenic grinders like RETSCH's CryoMill are specially designed for these applications, as they continuously cool the grinding jar, and thus the sample, with LN_2. The CryoMill produces grind sizes <0.1 mm, which means for the hemp sample that higher pesticide amounts are detected after extraction than, for example, in the ZM 300. 5 g pre-cut hemp sample were ground in a 50 ml stainless steel grinding jar with one 25 mm stainless steel grinding ball. Sample and ball were filled in the grinding jar, the lid was tightly closed, and the jar was clamped into the CryoMill. An automatic pre-cooling function ensures that the grinding process does not start before a temperature of -196°C is reached and maintained. The pre-cooling time was set to 3 min at 5 Hz. Grinding was done at 30 Hz for 3 min. Thanks to the autofill system of the CryoMill, the user never comes into contact to liquid nitrogen, the machine keeps the temperature at -196°C during grinding. The embrittled sample can now be ground to much smaller particle sizes than in the ZM 300. Still, for larger sample quantities, the ZM 300 is a suitable choice.

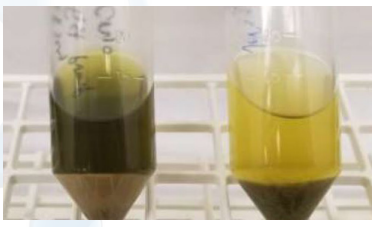

Fig. 7:
The hemp sample milled in the CryoMill (left) yields a higher pesticide amount after extraction than the sample milled in the ZM 300 (right).

Homogenization of edibles in Ball Mills like MM 400 or CryoMill

Food samples which are fatty or sticky may block the mill at room temperature. Therefore, cryogenic grinding either in the MM 400 or the CryoMill is the best option to avoid caking of the sample and loss of volatile ingredients. Sample volumes like a few jelly bears or 1-2 cookies are best homogenised in the MM 400. This ball mill is perfectly suited for homogenising sample volumes up to 2 x 20 ml in less than 1-2 minutes. It is important to fill the jar first with the grinding ball(s) and with the sample and close it tightly before embrittling. Care must be taken that no LN_2 is enclosed in the grinding jars because the evaporation of LN_2 would result in a considerable pressure increase inside the grinding jar. The closed grinding jars, and thus the sample, are embrittled in a LN_2 bath for 2-3 minutes. Suitable grinding jars for cryogenic grinding are made of steel or PTFE. Due to the high energy input and the resulting frictional heat, the grinding process should not take longer than 2 minutes to prevent the sample from warming up and to preserve its breaking properties. If longer grinding times are required, these should be interrupted by intermediate cooling of the closed grinding jars in the MM 400. 5 pieces of jelly bears were pulverized in the MM 400 in a 50 ml grinding jar 50 ml by using a 25 mm stainless steel grinding ball. The closed jar was immersed in a liquid nitrogen bath for approximately 4 min, then clamped into the mill. After 90 seconds at 30 Hz, the sample was fully pulverized to a fineness of 0.3 mm.

Fig. 8: Jelly bears before (left) and after cryogenic grinding in the Mixer Mill MM 400

Cryogenic grinding in the CryoMill offers the advantage of continuous cooling of the grinding jar with LN_2. This consistent temperature is guaranteed even for long grinding times without the need for intermediate cooling breaks. For heavy-metal-free grinding a zirconium oxide grinding jar should be used. A few pieces of liquorice, one praline or similar samples are typically pulverized in the CryoMill.

Homogenization of cookies in the Knife Mill GM 200

The Knife Mill GM 200 is designed for thorough homogenization of samples with high water, oil, sugar or fat content. It accepts sample volumes up to 700 ml. Thanks to the powerful 1000 W drive, the mill can homogenize even difficult samples very quickly and efficiently without blockages or the need for more than two grinding steps. The innovative Boost function allows for a temporary speed increase to 14,000 min^{-1}, providing extra power for the homogenization of difficult samples in a very short time. The mill may be operated in three different modes: standard mode = cutting, reverse mode = impact, interval mode = improved sample mixing, to optimize the homogenization process with regard to the material properties. Up to 8 programs can be stored for routine applications. The possibility to save 4 program sequences is helpful when combining two grinding steps, for example, pre-crushing in impact mode followed by fine grinding in cutting mode, or if two different speeds are required. Pulverization of 8 large cookies was performed in two steps. In the pre-grinding step at 4,000 min^{-1}, the interval mode was used for 10 seconds to improve mixing of the sample. The use of the standard lid ensures that the sample can move freely in the container, thus reducing the heat development (and subsequent fat release). In the homogenization step, which takes 35 seconds at 10,000 min^{-1}, the use of the reduction lid 0.5 l is preferable to force the sample towards the blades and thereby increase the grinding efficiency. In this way, the complete sample was homogenized to < 0.5 mm.

Fig. 9: Knife Mill GRINDOMIX GM 200

Fig. 10: Cookies before (left) and after pulverization in the Knife Mill GM 200 (right)

Field Practice

Homogenization of large sample volumes of cannabis in the Knife Mill GM 300 at the Office of Criminal Investigation in Dresden, Germany

In the laboratory of the Regional Office in Dresden, the cannabis plants are first reduced in size, followed by an extraction of several part samples of the ground material. The extracts are then analyzed by gas chromatography.
"We usually deal with large sample volumes", explains Thomas Paulick, Laboratory Manager at Dresden's Regional Office of Criminal Investigation. "Therefore, it is essential to extract a representative part from the entire sample amount, to ensure a reliable quantification of the active component. The GM 300 fulfils this requirement: from up to 4 liters of plant material we receive a homogeneous sample from which we can then take approximately 0.5 g for further analyses. We process leaves with remnants of thin twigs as well as highly resinous blossoms without cooling – which is no problem for the knife mill. Grinding time and speed are selected according to the properties of the plant. The closed design and removable grinding container help to reduce the formation of dust during homogenization. To sum it all up, the new GM 300 has proven to be highly suitable for our requirements."

Conclusion

With the growing use of cannabis in the medical sector, quality control has become a major issue. To ensure reliable and meaningful analysis results, sample homogenization prior to analysis is an important step. RETSCH offers a range of different laboratory mills for efficient, neutral-to-analysis sample preparation which are highly suitable for the task.

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