Preparation of Cannabis Plant Samples for Analysis of Tetrahydrocannabinol (THC) and Cannabidiol (CBD) using the Plant Genomics Microplate

With increasing legalization of cannabis for medicinal and research purposes, demand for accurate and high throughtput analysis of its active ingredients has dramatically risen.

The two most important are the psychoactive THC and the analgesic CBD which has therapeutic uses. The ratio of THC and CBD is crucial for differentiating medicinal versus recreational cannabis. This ratio is important for taxation authorities, medical regulators and growers alike.

Determination of THC/CBD Ratio

Accurate determination of the THC/CBD ratio is critically determined by effective sample preparation of the samples of cannabis leaf, bud and flower. Traditional sample preparation methods have used bead-beating or grinding mills to homogenise these materials. When conducted in polypropylene microplates the extreme forces applied by these machines often cause damage to the plates leading to cracking, leaking and ultimately, crosscontamination. For the purposes of analysis, it is desirable to determine total THC.

This would include the carboxylate free form (THCA) as well as the thermally converted form, THC. The medical value is a function of the CBD content which again must be totalled to include the nativecarboxylic acid form. High performance liquid chromatography (HPLC) can identify the acid components of THCA and CBD before conversion to their corresponding free forms of THC and CBD and is thus often preferred for tinctures and cannabis products to be taken by mouth. A drawback of analysis by gas chromatography (GC) is the destruction of THCA and TBDA in the hot injector which may make the ultimate determination of the THC/TBD ratio inaccurate. For this reason, HPLC/UV or LC/MS should be the preferred methods of analysis. The following method can also be used for both preparations and raw plant material.

Apparatus & Methodology

- Porvair Sciences 2ml deep well seed genomics microplates 219030
- Porvair Sciences 96 well cap mat 219004
- Porvair Sciences Microlute vacuum manifold 228008
- Porvair Sciences Microlute P3 plate 240100
- Porvair Sciences Minivap evaporator with 96 well spiral needle head (229206 & 229072)
- Porvair Sciences Microshake microplate shaker with 3 plate adaptor (229651 & 229654)
- Porvair Sciences 2.2ml Square deep well plate (219009)
- Laboratory grinder (such as Spex GenoGrinder)

For THC/CBD ratio analysis, samples of cannabis leaf, bud and flower, if available, should be randomly sampled. We suggest accurately weighing approximately 50mg of dried plant material into each well of a Porvair 229030 Plant Genomics plate.

Add 3 x 3mm steel or nickel grinding balls (nickel will be required if grinding seed material) to the Plant Genomics Plate.

Cap the plate with a 219004 Cap Mat and place the capped plate in the GenoGrinder. Grind for 2 minutes at 1500 rpm. Remove the cap mat and transfer the dried ground powder, which should pass through a 1mm sieve, into each well of a Porvair P3 filter microplate (240100).

Accurately add 400ul of methanol or isopropanol (according to preference) to dissolve the oily residues in the plant material.

Agitate on a Porvair Microshake shaker (229651) fitted with a P3 plate adaptor (229654) or other suitable shaker at 330 rpm for 10 min.

The superhydrophobic frit in the P3 plate will prevent leakage, but for added peace of mind an optional drain cap mat is available which, if desired, should be fitted to the underside of the plate before the assay begins (219005).

After shaking, remove the drain cap and place the P3 plate onto a Porvair Microlute manifold (228008) which has a 2ml collection plate in the plenum chamber. This collection plate can be either another seed genomics plate (219030) or a standard 2ml square well plate (219009).

Any SLAS/ANSI format deep well plate can be used for the collection step, but 219009 and 219030 use extractable-free polypropylene which will not contribute leachates to the solution to be analysed. In addition their plate geometry is optimised for the Porvair Microlute Manifold so that no further adjustment is necessary to prevent cross-contamination between wells. Apply vacuum to draw the purified extract down through the filter and into the wells of the collection plate.

Isolate the vacuum supply using the valve on the manifold and release the vacuum in the plenum chamber using the needle valve. The filter plate may now be discarded and the collection plate containing the organic extract can be taken to the HPLC for analysis.

If analysis of total THC and CBD is preferred, an extra step will be required. In this case the THCA and CBDA must be converted to THC and CBD using heat.

Take the 2ml collection plate containing the solvent extract and place it on the Porvair Minivap. Set the temperature to maximum (60C) and evaporate to dryness, continue to heat with hot gas for a further 45 minutes to allow the dissociation of thethermally labile THCA and CBDA to THC and CBD. After cooling to room temperature, re-suspend in 400ul of solvent, agitate on the Microshake to ensure good mixing and then procced to HPLC analysis as before.

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