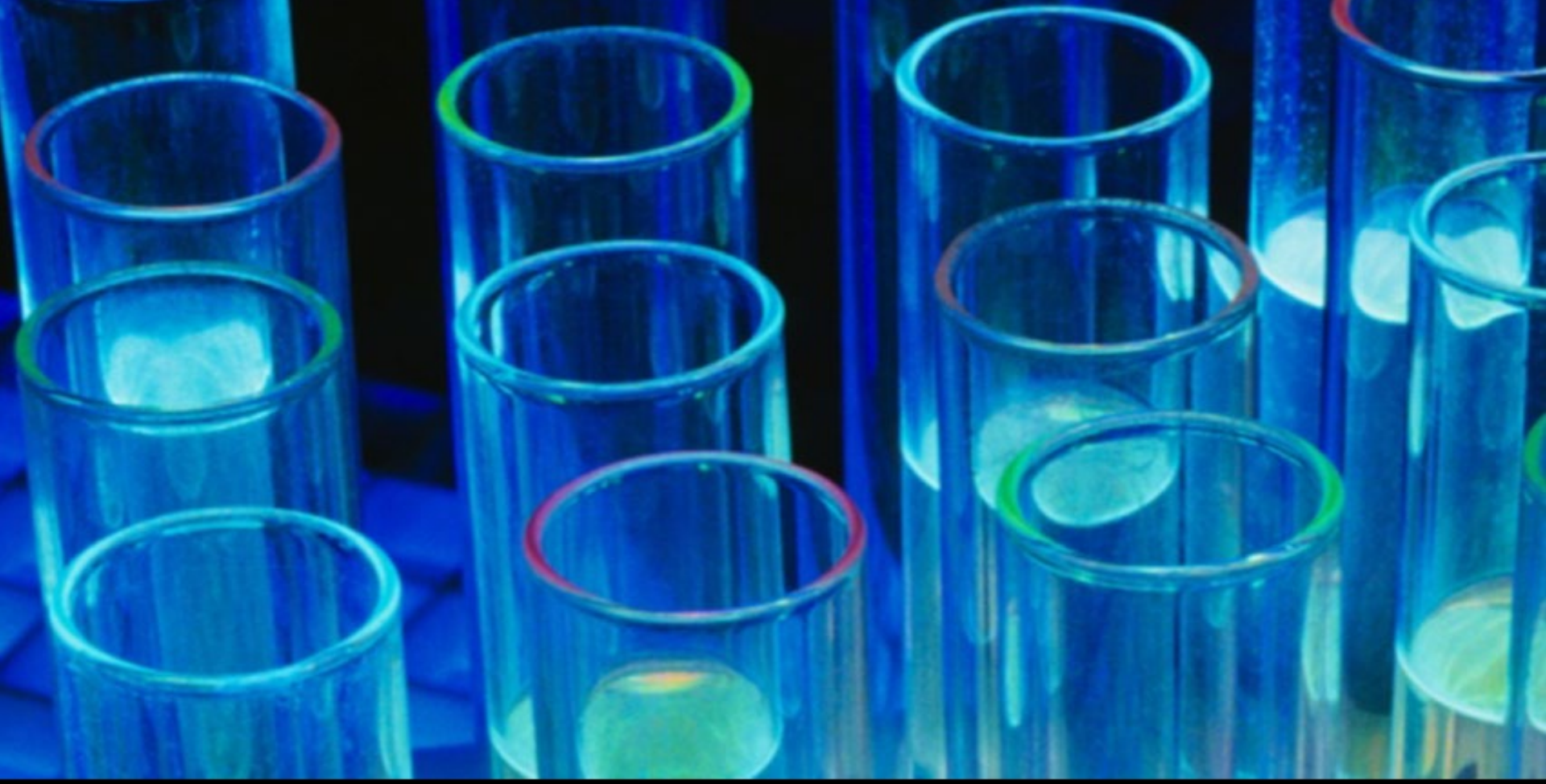


Cannabinoid Extract Purification

Cannabinoid Extract Purification

ABSTRACT

Centrifugal Chromatography instrumentation is used in the natural products, pharmaceutical, cosmetic, and biopharmaceutical industries as a means of isolating individual fractions from complex mixtures of organic components. Fast Centrifugal Partitioning Chromatography [FCPC] offers several advantages versus traditional liquid chromatography methods. In the rapidly expanding cannabis market, FCPC has found a place as an effective means of separating cannabinoid fractions for full spectrum products, and for medical and pharmaceutical consumption. FCPC has been demonstrated as an effective means for Δ9 THC (tetrahydrocannabinol) removal from CBD (cannabidiol) products, to mitigate psychoactive properties, while maintaining the cannabis extracts' medicinal properties. Extensive research has been conducted using FCPC as an effective tool for producing high purity fractions, at reseach and development scale, up to production scale, demonstrating that the FCPC is well adapted to accommodating different solvent systems as a function of the cannabinoid fraction(s) of interest, to acheive THC-free levels of 0.3% or less.

Cannabinoid Extract Purification

Principles of Centrifugal Partition Chromatography

Determination of the partitioning coefficient, K_D

The partitioning coefficient is the ratio of a concentration of a given solute in the stationary phase divided by the concentration of the same solute in the mobile phase.

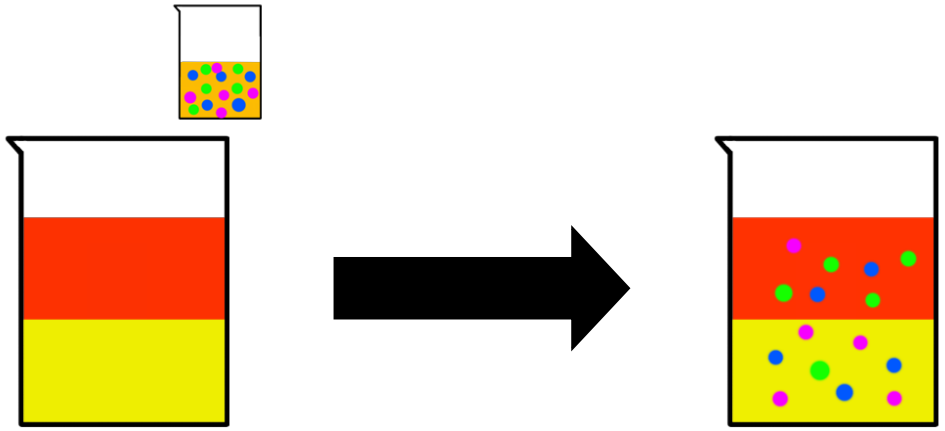
$$\mathrm{K_D} = \frac{[\mathrm{A}]_{stationary}}{[\mathrm{A}]_{mobile}}$$

Cannabinoid Extract Purification

Principles of Centrifugal Partition Chromatography

Determination of the partitioning coefficient, K_D

- Add known quantity of solutes to biphasic solvent system.
- Agitate / Separate
- HPLC / analytical testing of upper and lower phases



$$K_D = \frac{[A]_{stationary}}{[A]_{mobile}} = 0.25$$

$$K_D = \frac{[B]_{stationary}}{[B]_{mobile}} = 4.0$$

$$K_D = \frac{[C]_{stationary}}{[C]_{mobile}} = 0.66$$

Cannabinoid Extract Purification

Principles of Centrifugal Partition Chromatography

Solvent Selection

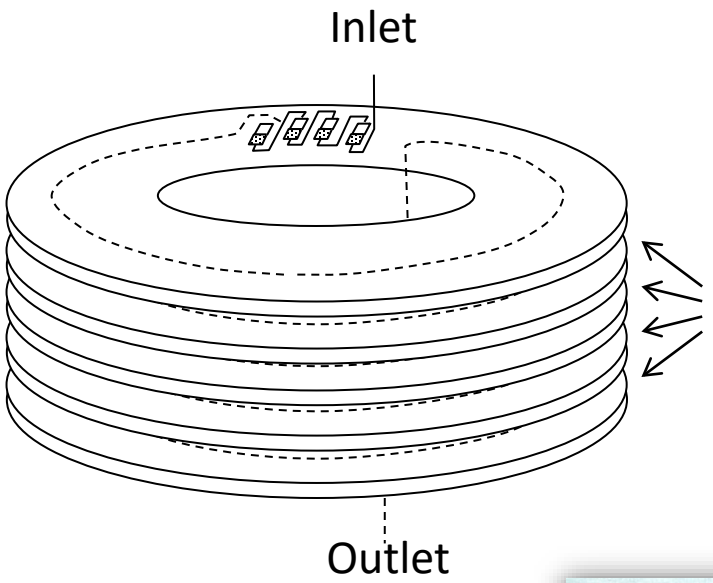
- K_D value between 0.5 (move quickly with the mobile phase) and 2.0 (lag in the stationary phase). “Sweet spot”.

- The target molecules that are being separated must have different K_D values for proper separation to occur.

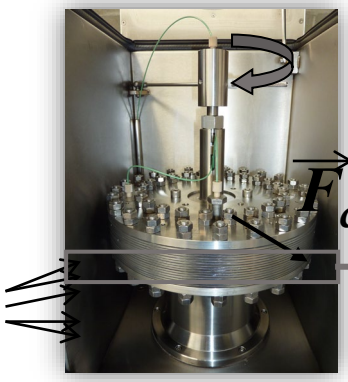
- The fewer molecular types that are in the sample, the lower the risk of overlapping K_D values.

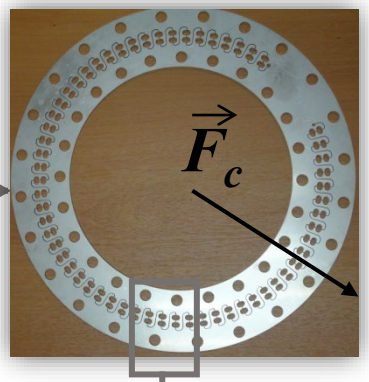
Cannabinoid Extract Purification

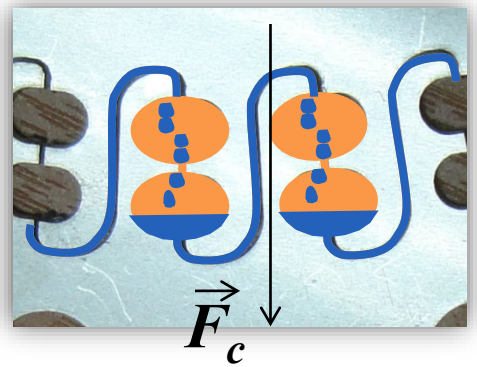
CPC Mechanism



Interdisc gaskets



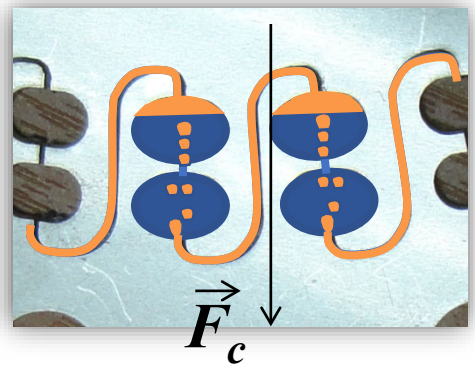




Descending Mode

Upper phase

Lower phase



Ascending Mode

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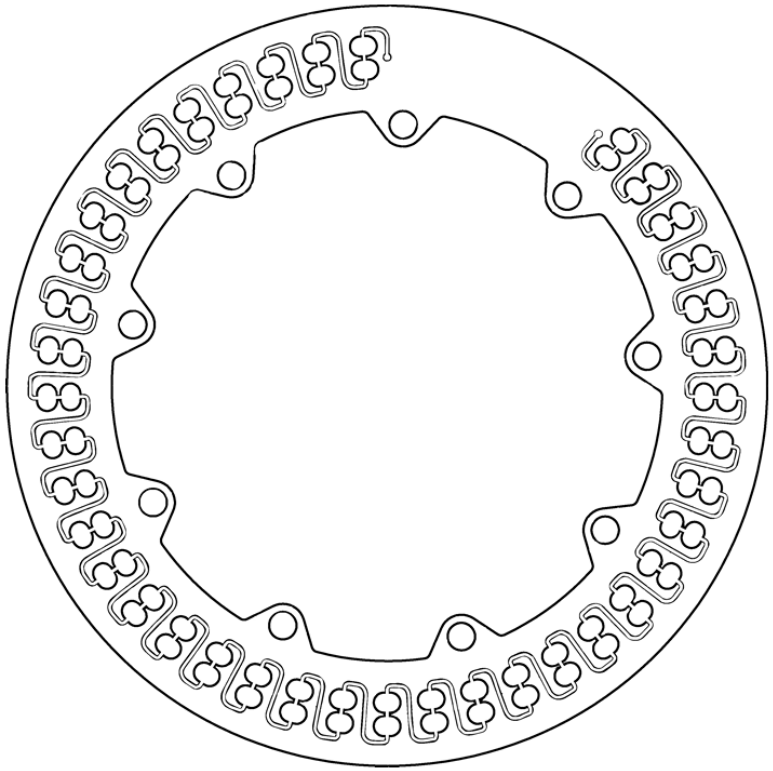
Cannabinoid Extract Purification

Principles of Centrifugal Partition Chromatography

Column Equilibration

- Introduce stationary phase.
- Introduce mobile phase / displace portion of stationary phase.
- Check stationary phase retention % = (column value – displaced stationary phase) / (column total retention volume).
- Higher retention = Better resolution.

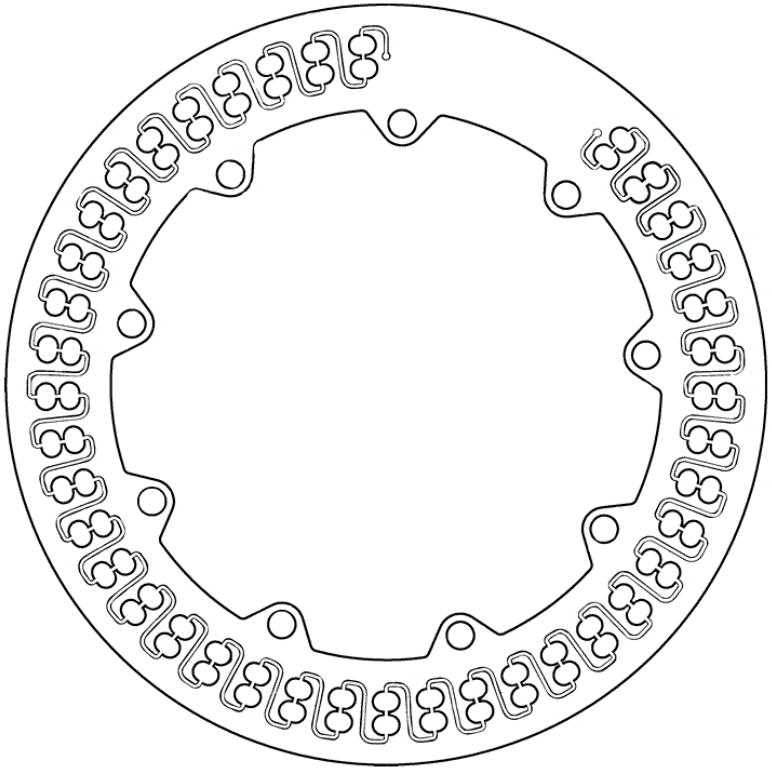


Cannabinoid Extract Purification

Principles of Centrifugal Partition Chromatography

Column Equilibration

- Stationary phase solution is introduced to the column.
- Column is under rotation or at low rotational speed.
- Wait until stationary phase begins discharging from column.

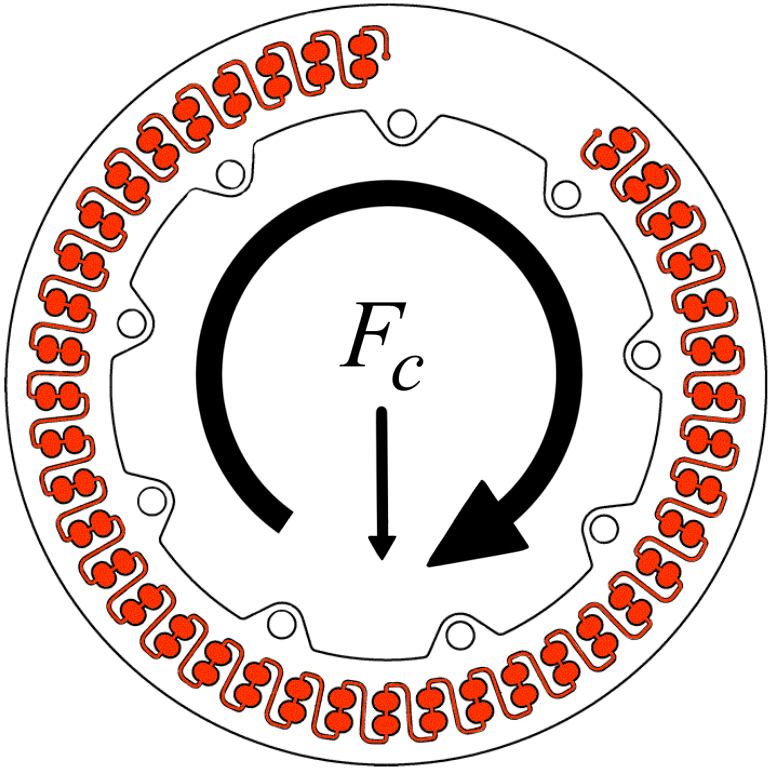


Cannabinoid Extract Purification

Principles of Centrifugal Partition Chromatography

Column Equilibration

- Start introducing mobile phase as soon as stationary phase discharges.
- Collect displaced stationary phase until mobile phase begins discharging from the column, record volume
- Calculate stationary phase retention volume
- Column is ready for elution / sample injection



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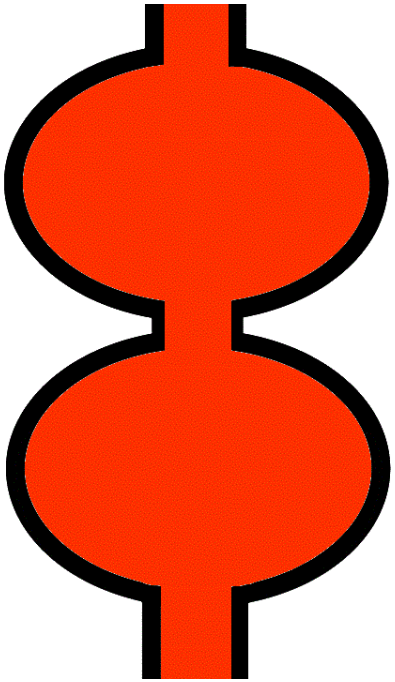
Cannabinoid Extract Purification

Principles of Centrifugal Partition Chromatography

Cells within the Column



- Capillary action results in diffusion inside each cell
- Several hundred cells for isolation of similar species
- Discrete stagewise contact
- No solid packing material – relies on thermodynamic partitioning

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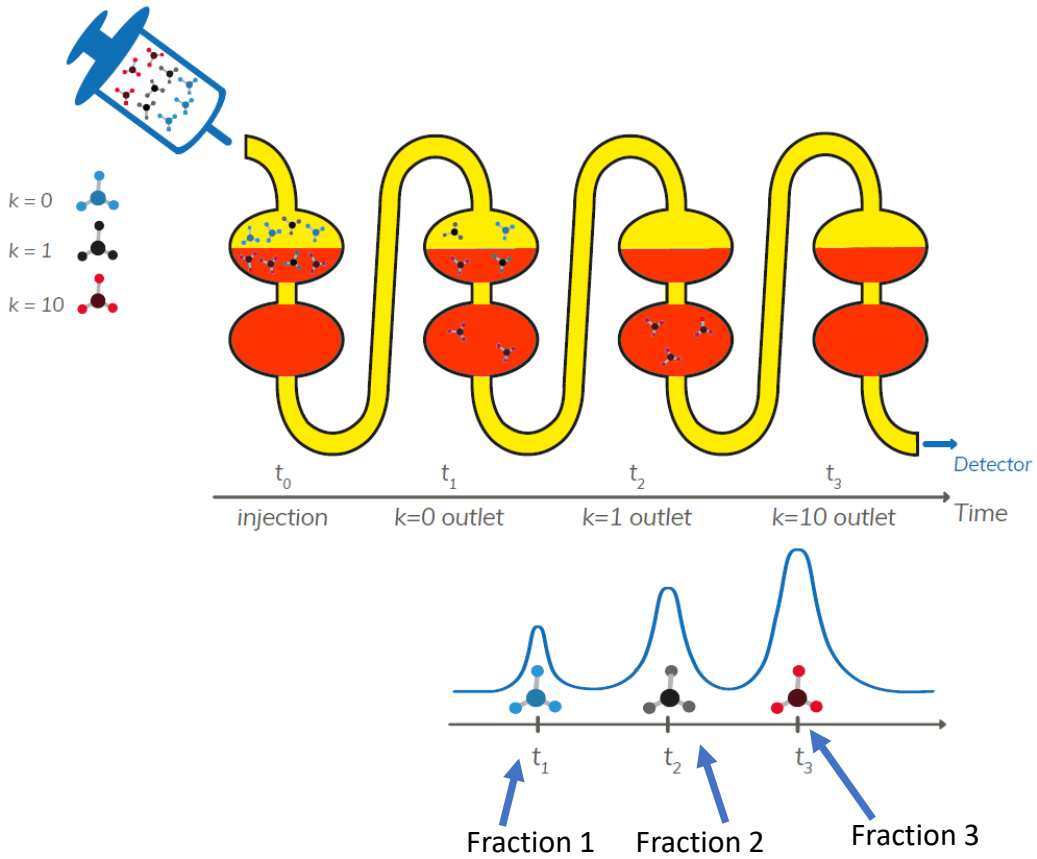
Cannabinoid Extract Purification

Principles of Centrifugal Partitioning Chromatography

Injection and Elution

- Sample is injected
- Sample consists of stationary and mobile phases and loading of solutes.
- Purified fractions elute over time as function of partitioning.



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Cannabinoid Extract Purification

Uses in the Cannabis Refining Industry

Separation of THC from CBD

- Most common cannabis application
- Remediation of THC from CBD products. Legal regulations
- Concentration of THC for other cannabis products.

Minor Cannabinoid Isolation

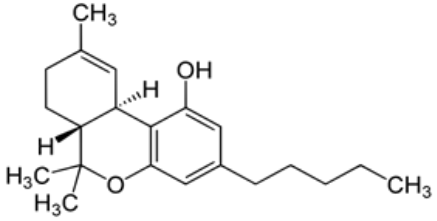
- Minor cannabinoids (CBG, CBN, CBC,..) from full spectrum oils.
- Remediation of THC for broad spectrum oils.

Cannabinoid Extract Purification

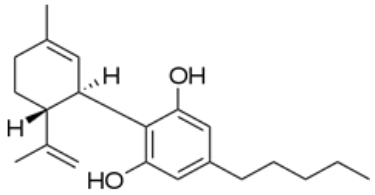
Cannabis Refining Case Study

THC Remediation from CBD oils

- Production scale required
- Achieve <0.3% THC in CBD fractions
- Maximize CBD yield
- Maximize throughput



Δ9 tetrahydrocannabinol



Cannabidiol

- Maximize sample concentration and loading
- Minimize run times
- Minimize solvent consumption

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Cannabinoid Extract Purification

Cannabis Refining Case Study

CPC Column Evaluated: Kromaton Model FCPC D5000

- 5 Liter column volume
- 1400 RPM maximum rotational speed
- Number of extraction cells: 833
- Operating pressure: Up to 60 bar
- Sample quantity: 50-500 g per injection
- Flowrate of mobile phase: Up to 700 ml/min

Kromaton FCFC D5000 centrifugal partitioning chromatograph

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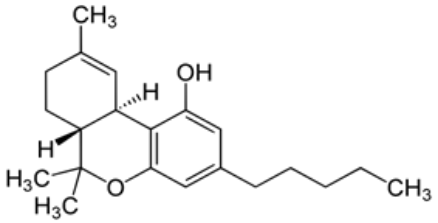
Cannabinoid Extract Purification

Cannabis Refining Case Study

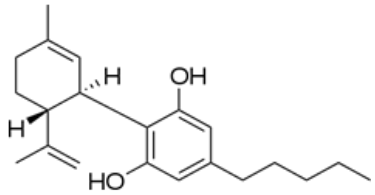
Extract used for study / Starting material:

- CBD-rich cannabinoid extract from Hemp
- Extract was prepared by removing solids, impurities, etc.
- Extract concentration analysis by analytical HPLC:

Sample Source Material	CBDV%	CBG%	CBD%	CBN%	THCD9%	CBC%
Winterized Crude Oil	0.327	0.365	45.48	0.076	2.867	2.23
Distillate, up to trial 14	0	0	65.42	1.45	2.72	1.89
Distillate, after trial 14	0	0	71.94	0	1.76	0



Δ9 Tetrahydrocannabinol



Cannabidiol

Cannabinoid Extract

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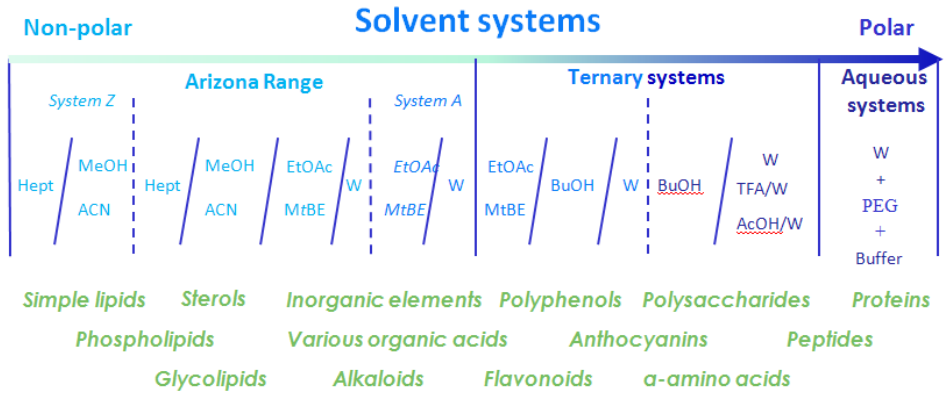
Cannabinoid Extract Purification

Cannabis Refining Case Study

Solvent system:

- Chosen based on previous studies
- Light phase is heptane
- Heavy phase is methanol / water
- Favorable density ratio / separates easily by gravity



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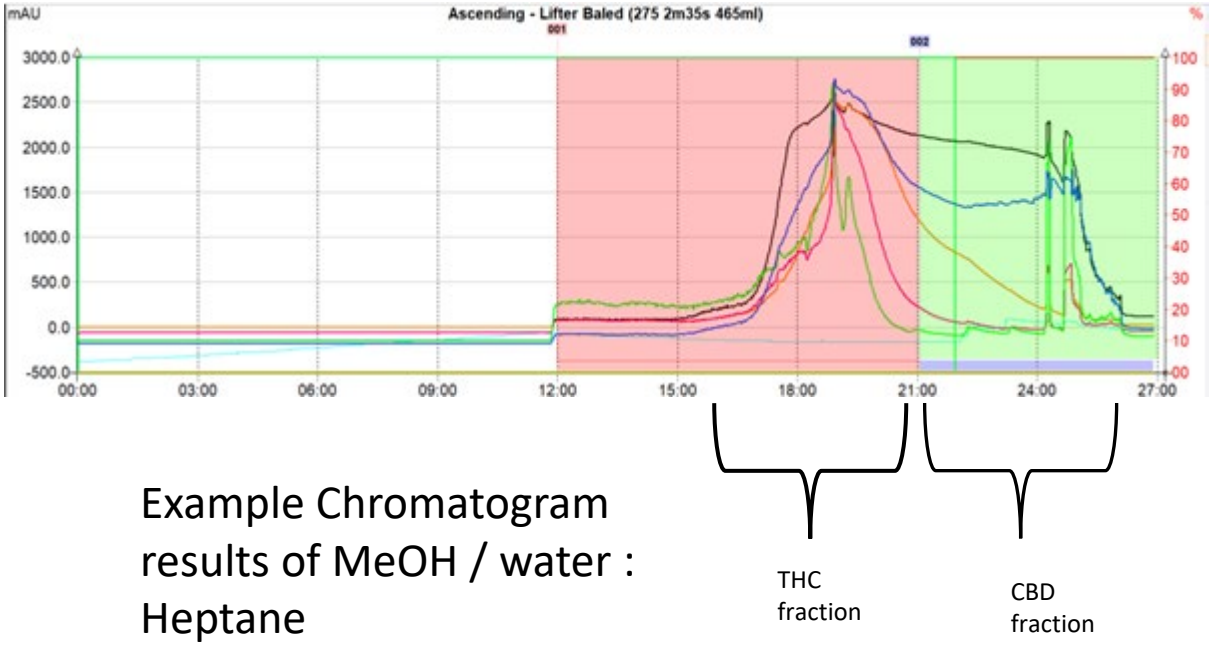
Cannabinoid Extract Purification

Cannabis Refining Case Study

Preliminary results:

- Tested in ascending / descending mode (normal and reverse phase)
- Varied initial sample injection concentrations
- Determined optimum flow rates
- Reduced sample run times to less than 30 min/run
- Optimized operating rotational speed conditions



Example Chromatogram results of MeOH / water : Heptane
System operated in ascending mode

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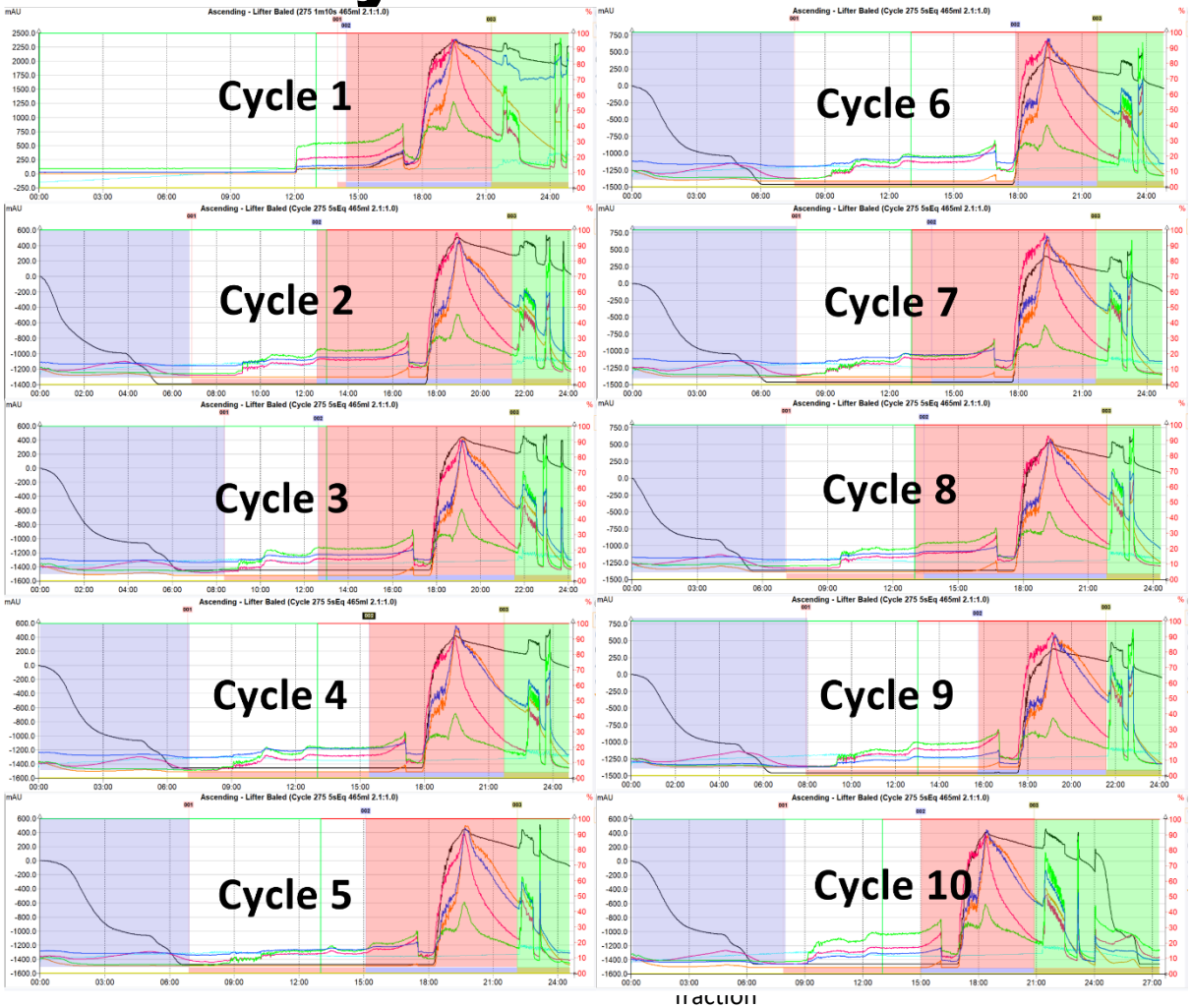
Cannabinoid Extract Purification

Cannabis Refining Case Study

Optimized results:

- Reduced run time to < 30 min
- Maximized pure CBD recovery to 70%
- Optimum flow rate of 200-250 ml/min
- Performed “sandwich injections” to minimize equilibration between runs
- Ran 10 runs continuously with consistent results for each run
- 0.3 kg/liter max. concentration of sample injection



Example of continuous run of 10 consecutive injections to show repeatability and consistency. THC fraction CBD fraction

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Cannabinoid Extract Purification

CONCLUSIONS:

- 2/3 the solvent consumption of competitive systems
- No packing material required
- Process can be automated, for unattended operation
- Adaptable to several types of separations and isolations
- cGMP capable
- Unique production scale capacity
- Explosion-proof capability
- CBD fractions are broad spectrum / concentrated with essentially 0% THC
- THC-rich fraction can be further processed – no waste.

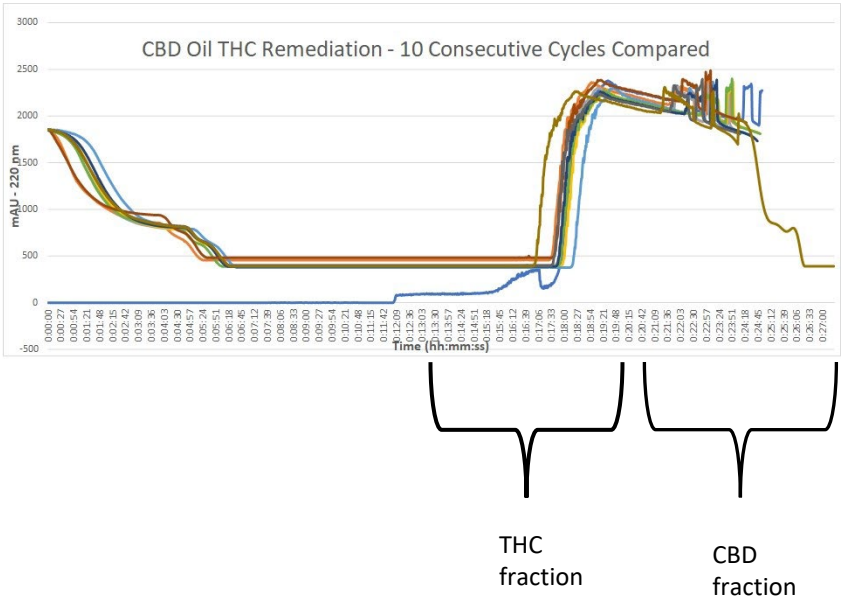

Figure showing superimposed back to back runs to demonstrate consistency

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Cannabinoid Extract Purification

FURTHER (ONGOING) STUDIES:

- Scale-up is not linear from preparative scale
- Testing with other solvent systems
- Isolation of minor cannabinoids such as CBG, CBN, delta 8 THC,…..
- Further refining of THC fraction
- Scale up to larger production scale is theoretically linear
- Testing with other starting CBD oils with varying concentrations of THC.
- THC purification, removal of CBD
- Extraction rotor with fewer stages, with increased throughput capacity

FCPC D5000 production scale CPC with ancillary equipment system; UV detector, feed pumps, method software, control system, etc.

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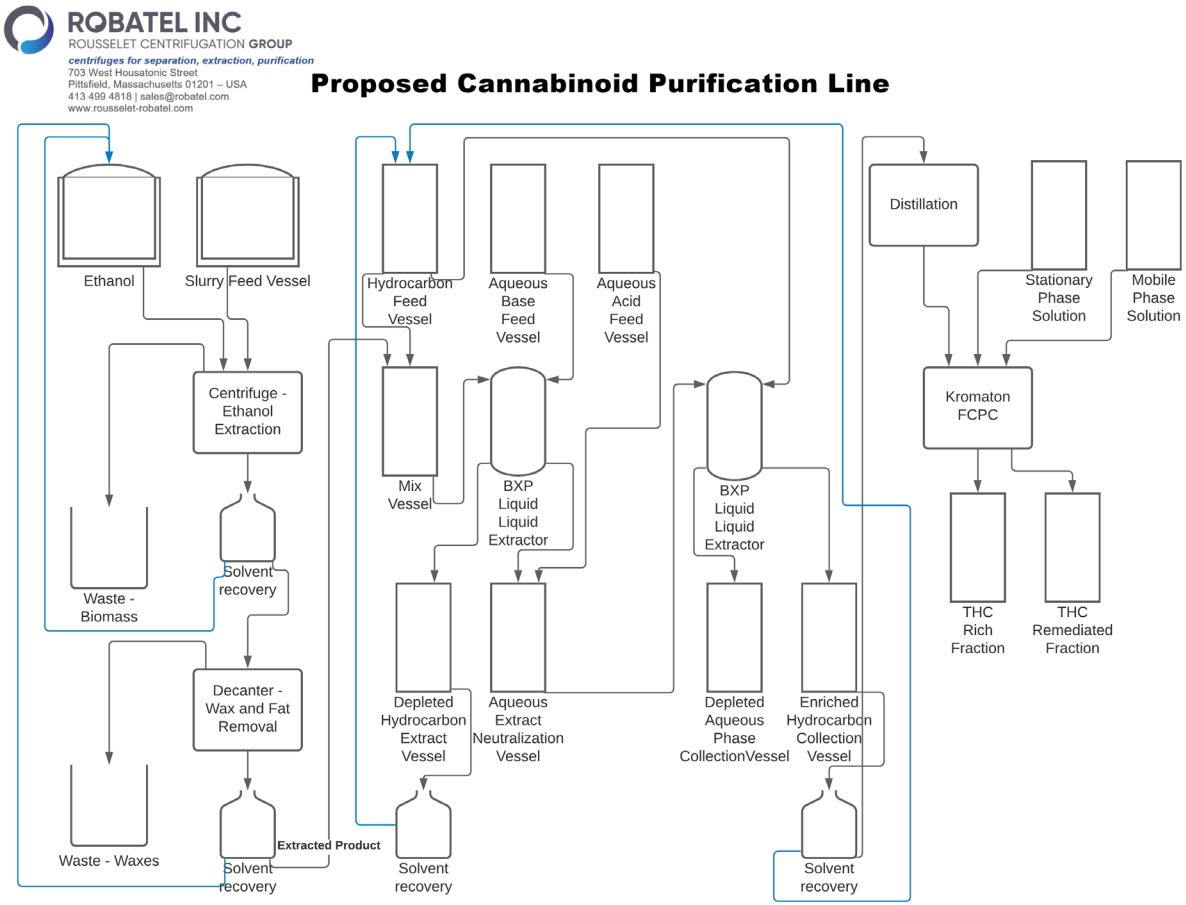
Cannabinoid Extract Purification

OTHER CANNABIS PROCESSING EQUIPMENT BY ROUSSELET ROBATEL KROMATON:



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For more information / Para más informaciones
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