Extraction, Isolation and Analysis of Cannabis Extract and Preparation

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Outline

➢ Cannabinoids as chemical markers
➢ Conventional and Modified extraction
➢ Separation and Isolation of Cannabinoids
➢ Identification of Cannabinoids
➢ Formulation of Oromucosal Spray
➢ Evaluation of Oromucosal Spray
Cannabinoid Biogenesis

https://cannabisindustryjournal.com/tag/cbdv/
Cannabidiol (CBD) and tetrahydrocannabinol (THC) are just two compounds from a family of around 113 bi- and tricyclic compounds cannabinoid compounds found naturally in cannabis.

Both CBD and THC share the exact same molecular formula, $C_{21}H_{30}O_2$, containing twenty-one atoms of carbon, thirty of hydrogen and two of oxygen.

Their molecular mass is practically identical with THC and CBD having masses of 314.469 g/mol 314.464 g/mol, respectively.

EXTRACTION ISOLATION AND ANALYSIS OF CANNABIS EXTRACT AND PREPARATION

• Methanol was the most efficient extraction solvent for delta-9-THCA (analyzed as THC).
• Generally, the delta-9-THCA extraction efficiency correlated with solvent polarity.
• Chromablography 2011; https://blog.restek.com/?p=3018
• Ultrasonication was used to extract bioactive compounds from *Cannabis sativa* L. such as polyphenols, flavonoids, and cannabinoids.

• The influence of 3 independent factors (time, input power, and methanol concentration) was evaluated on the extraction of total phenols (TPC), flavonoids (TF), ferric reducing ability of plasma (FRAP) and the overall yield.

• The response predictions obtained at optimum extraction conditions of 15 min time, 130 W power, and 80% methanol were 314.822 mg GAE/g DW of TPC, 28.173 mg QE/g DW of TF, 18.79 mM AAE/g DW of FRAP, and 10.86% of yield.

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Effect of (A) extraction solvent, (B) microwave power, (C) temperature, and (D) extraction time on the yields of cannabinoids.

The optimal conditions of MAE were extraction solvent of methanol, microwave power of 375 W, temperature of 109 °C, and extraction time of 30 min.

Molecules 2017, 22(11), 1894; doi:10.3390/molecules22111894
• **Left.** Response surface plots showing the interaction effects of microwave power, temperature, and extraction time on the yields of THC (A–C), CBD (D–F), and CBN (G–I).

• **Right.** Contour plots showing the interaction effects of microwave power, temperature, and extraction time on the yields of THC (A–C), CBD (D–F), and CBN (G–I).

• [Molecules 2017, 22(11), 1894; doi:10.3390/molecules22111894](https://doi.org/10.3390/molecules22111894)
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Supercritical CO₂ was applied to obtain extracts with high cannabinoids concentration. Different operating conditions and regimes were evaluated. Cannabinoids composition was analyzed with time at different extraction conditions.

• Co-solvent pulse regime is the most recommendable extraction strategy.

• The Journal of Supercritical Fluids, 2017, 129: 16-27
Six major cannabinoids [tetrahydrocannabinolic acid (THCA), tetrahydrocannabinol (THC), cannabidiol (CBD), tetrahydrocannabivarin (THCV), cannabigerol (CBG), and cannabinol (CBN)] were quantified (RSD < 10%), and seven more cannabinoids were identified and verified by high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS).

The industrial scale rotary evaporators R-220 Pro and R-250 are the ideal solutions for efficient concentration of cannabinoid extracts after initial extraction as well as subsequent cannabinoid separation. High purity separation of various cannabinoids (e.g. THC and CBD) is achieved by preparative chromatography using BUCHI’s compact PrepChrom C-700 which combines both flash and prep-HPLC purifications.

https://www.analyticalcannabis.com/white-papers/industrial-evaporation-chromatography-ideal-combination-for-cannabis-processing-288137
Separation by preparative chromatography uses the principle that different cannabinoids travel through a specific stationary phase at different speeds. The substances to separate and purify are eluted and collected successively, as illustrated in the Figure for a cannabis extract containing the three cannabinoids, THC, CBD and THCA (tetrahydrocannabinolic acid).

https://www.analyticalcannabis.com/white-papers/industrial-evaporation-chromatography-ideal-combination-for-cannabis-processing-288137
• Medical Marijuana Cannabinoids Analyzed by Gas Chromatography with Flame-Ionization Detection (GC-FID) on Rxi-35Sil MS and Rtx-35.
• Chromablography 2011; https://blog.restek.com/?p=12038
• LC–MS–MS analysis of an extracted 100µL blank oral fluid sample enriched with 5 ng/mL THC-d 3 (top trace), THC and cannabidiol (middle trace) and cannabinol (bottom trace). Peak intensity is shown in the top right-hand corner of each trace.
Gas Chromatography-Mass Spectrometry (GC-MS):

- Gas Chromatography-Mass Spectrometry (GC-MS) of Tetrahydrocannabinol (THC). THC appears as a GC peak, and gives a molecular ion at 314, its molecular weight.
- *Organic Chemistry, Second Edition* Janice Gorzynski Smith University of Hawai‘i
Oral and inhaled medical marijuana products have different absorption rates and bioavailability.

Oral medical marijuana products can take hours to reach peak blood concentrations, versus minutes for inhaled medical marijuana products.

Inhaled medical marijuana products are available in varying percentages of cannabinoids, depending on the strain, and can either be smoked or vaporized.

Vaporization is a much healthier option for the lungs, because it contains fewer carcinogens than smoked marijuana.

Vaporization also increases the percentage of delta-9-THC and CBD extracted from the marijuana.

Sublingual and oromucosal products are another option that bypass first-pass metabolism similar to inhalation routes.

These routes have shown high bioavailability, eliminate the need to inhale smoke or vapor, and have a faster onset than oral medications, such as edibles or capsules.

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**TABLE 1**

Absorption of Oral vs Inhaled Medical Marijuana

<table>
<thead>
<tr>
<th>Pharmacologic Parameter</th>
<th>Oral</th>
<th>Inhaled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioavailability</td>
<td>6-20%</td>
<td>10-40%</td>
</tr>
<tr>
<td>Time to peak concentrations</td>
<td>1-6 hr</td>
<td>2-10 min</td>
</tr>
<tr>
<td>Maximal duration</td>
<td>2-3 hr</td>
<td>Dose-dependent; maximal psychotropic effects: 20 min, with rapid decline lasting 45-60 min</td>
</tr>
<tr>
<td>Distribution</td>
<td>90% plasma; protein-bound; 10% red blood cells; 1% in brain; Crosses placenta and found in breast milk</td>
<td></td>
</tr>
</tbody>
</table>

1 Liver metabolism converts THC to 11-hydroxy-delta-9-THC, which is believed to be more psychoactive than delta-9-THC.

1 Vaporization increases the percentage of cannabinoids inhaled vs combustion by smoking.

<table>
<thead>
<tr>
<th>Product</th>
<th>Australian Medicines Schedule/Registration</th>
<th>South Australia¹</th>
<th>Commonwealth ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dronabinol (Marinol®, Syndros®, US FDA approved medicine) Tablet/capsule form.</td>
<td>Schedule 8. Unregistered synthetic/analogue cannabinoid products.</td>
<td>An authority issued under section 18A of the <em>Controlled Substances Act 1984</em> may be required.¹</td>
<td>TGA Special Access Scheme or Authorised Prescriber Scheme or Clinical Trials Schemes.</td>
</tr>
<tr>
<td>Nabilone (Cesamet®, US FDA approved medicine) Oral spray form.</td>
<td>Schedule 8. The only registered cannabinoid product in Australia - for symptom improvement in multiple sclerosis.</td>
<td>An authority issued under section 18A of the <em>Controlled Substances Act 1984</em> may be required.¹</td>
<td>Registered on the ARTG – approved product.</td>
</tr>
<tr>
<td>Cannabidiol in preparations for therapeutic use where cannabidiol comprises 98 per cent or more of the total cannabinoid content of the preparation.</td>
<td>Schedule 4. Unregistered cannabidiol products. Refer to the <a href="www.sahealth.sa.gov.au">Poisons Standard</a> for detail on Schedule 4 listing.</td>
<td>A section 18A authority is not required for Schedule 4 medicines.</td>
<td>TGA Special Access Scheme or Authorised Prescriber or clinical trials schemes.</td>
</tr>
</tbody>
</table>

Common Modes of Administration and Formulations. (1) Inhalation (2) Oromucosal (3) Oral

https://www.dovepress.com/therapeutic-potential-of-medicinal-marijuana-an-educational-primer-for-peer-reviewed-fulltext-article-DHPS
• Sativex® is an oral spray developed by UK-based pharmaceutical company GW Pharmaceuticals, derived from the cannabinoids THC and CBD (cannabidiol). Sativex® is now fully licensed in the UK, Spain, Canada and New Zealand for the treatment of muscle spasticity in MS patients. It is also approved in Canada for the reduction of cancer pain and neuropathic pain, and is in various stages of the approval process in the European Union and the US.

• https://www.pharmaceutical-technology.com/features/feature105046/

Nabiximols (Sativex™)

• Patented cannabinoid oromucosal spray for MS patients to relieve spasticity, neuropathic pain, overactive bladder and other symptoms
• Contain equal amounts CBD and ⁹ΔTHC
• Relieve spasticity and pain more than ⁹ΔTHC alone
  • CBD’s effects allow patients to tolerate higher amounts of ⁹ΔTHC
  • CBD supplements antispastic effects of ⁹ΔTHC (local potentiation of glycine signaling, inhibition of endocannabinoid degradation, retardation of demyelination through antioxidant/anti-inflammatory mechanisms)
• The pharmacokinetics of THC vary as a function of its route of administration. Pulmonary assimilation of inhaled THC causes a maximum plasma concentration within minutes, psychotropic effects start within seconds to a few minutes, reach a maximum after 15–30 minutes, and taper off within 2–3 hours. Following oral ingestion, psychotropic effects set in with a delay of 30–90 minutes, reach their maximum after 2–3 hours and last for about 4–12 hours, depending on dose and specific effect.

• Schematic representation of the preliminary pharmacokinetic study following IV bolus administration of 5 mg/kg cannabidiol (CBD) and Δ9-tetrahydrocannabinol (THC) to rats (n=2). (1) Blood sampling from the fitted cannula. (2) Chromatogram shows CBD, THC, and the internal standard DDT following plasma analysis by HPLC. (3) Data analysis by the pharmacokinetic software. (4) Plasma concentration-time profiles of CBD and THC in rats. [https://atlasofscience.org/simple-method-for-measuring/]
The U.S.A. Has A Patent On Cannabinoids

- United States Patent: Cannabinoids as antioxidants and neuroprotectants
• United States Patent: Anti-nausea and anti-vomiting activity of cannabidiol compounds
**United States Patent: Phytocannabinoids in the Treatment of Cancer**

**Phytocannabinoids in the Treatment of Cancer**

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**Assignees:** Otsuka Pharmaceutical Co., Limited, Tokyo (JP); GW Pharma Limited, Salisbury (GB).

**Application Number:** WO2005059018A1

**PCT Filing Date:** Mar. 1, 2011

**Publication Date:** Mar. 11, 2011

**Abstract:**

This invention relates to the use of phytocannabinoids, either in an isolated form or in the form of a botanical drug substance (BDS), in the treatment of cancer. Preferentially the candidate to be treated is cancer of the prostate, cancer of the breast or cancer of the colon.
EXTRACTION ISOLATION AND ANALYSIS OF CANNABIS EXTRACT AND PREPARATION

United States Patent

Verzura et al.

CANNABIS EXTRACTS AND METHODS OF PREPARING AND USING SAME

Applicant: United Cannabis Corp., Denver, CO

Inventors: Tony Verzura, Denver, CO; Earnie Blackmon, Denver, CO

Assignee: United Cannabis Corp., Denver, CO

Date of Patent: Aug. 15, 2017

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Primary Examiner — Rei-Tsung Shiao

Assistant Examiner — Cooley, EHP, Irvin Effert, Cynthia Konakiewicz

ABSTRACT

The invention relates to the extraction of pharmaceutically active components from plant materials, and more particularly to the preparation of a botanical drug substance (BDS) for incorporation into a medicament. It also relates to a BDS, for use in pharmaceutical formulations. In particular it relates to BDS comprising cannabinoids obtained by extraction from cannabis.

36 Claims, No Drawings