# Sustainable Production of

# Cannabinoids with

# Supercritical Carbon Dioxide Technologies

Proefschrift

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## Summary

*Sustainable Production of Cannabinoids using Supercritical Carbon Dioxide Technologies*

This thesis concerns the production of natural compounds from plant material for pharmaceutical and food applications. It describes the production (extraction and isolation) of cannabinoids, the active components present in cannabis. Many cannabinoids have medicinal properties but not all cannabinoids are available in the (large) quantities necessary to develop new medicines, because so far, for large scale production, there are no economically and technically viable methods to extract those cannabinoids present in low quantities in the plant. Moreover, the currently used production process for the most important cannabinoid, tetrahydrocannabinol ($\Delta^9$-THC), has many drawbacks, such as the large use of the organic solvents, which is not only a burden to the environment but also to the safety of the operators, the production costs as well as the treatment of the produced waste. In this thesis, an alternative process using supercritical carbon dioxide is presented for the production of cannabinoids, including $\Delta^9$-THC, cannabinol (CBN), cannabigerol (CBG) and cannabidiol (CBD).

One of the steps of $\Delta^9$-THC production from cannabis plant material, is the decarboxylation reaction, transforming the $\Delta^9$-THC-acid naturally present in the plant into the psychoactive $\Delta^9$-THC. Experiments showed a pseudo first order reaction, with an activation barrier of 85 $kJ.mol^{-1}$ and a pre-exponential factor of $3.7x10^8$ $s^{-1}$. Using molecular modeling, two options for an acid catalysed β-keto acid type mechanism were identified. Each of these mechanisms might play a role, depending on the actual process conditions. Formic acid was shown to be a good model for a catalyst of such a reaction. A direct keto-enol mechanism catalyzed by formic acid seems to be the best explanation for the observed activation barrier and the pre-exponential factor of the decarboxylation of $\Delta^9$-THC-acid. Evidence for this was found by performing an extraction experiment with

Cannabis Flos. It revealed the presence of short chain carboxylic acids supporting this hypothesis.

Then, in order to develop the supercritical fluid extraction process, the solubility of $\Delta^9$-THC, CBN, CBG and CBD in supercritical carbon dioxide has been determined using an analytical method with a quasi-flow apparatus. First the solubility of $\Delta^9$-THC has been determined at 315, 327, 334 and 345 K and in the pressure range from 13.2 to 25.1 MPa. The molar solubility for $\Delta^9$-THC ranged from 0.20 to $2.95 \times 10^{-4}$. Then, the solubility of CBN, CBG and CBD in supercritical carbon dioxide has been determined at 314, 327 and 334 K and in the pressure range from 11.3 to 20.6 MPa. The molar solubility of CBN, CBG and CBD ranged from $1.26 \times 10^{-4}$ to $4.16 \times 10^{-4}$, from 1.17 to $1.91 \times 10^{-4}$ and from 0.88 to $2.69 \times 10^{-4}$, respectively. These solubility data have been compared to each other. The solubility of the different cannabinoids in supercritical $CO_2$ increases at 326 K in the following order: $\Delta^9$-THC < CBG < CBD < CBN. The solubility data were correlated using the Peng-Robinson equation of state in combination with Van der Waals mixing rules.

To continue, supercritical fluid extraction (SFE) using carbon dioxide was performed with Cannabis Sativa L. in a pilot scale set-up at 313 and 323 K in the pressure range from 18 to 23 MPa. The SFE yield of $\Delta^9$-THC is at maximum 98 %, which is comparable to classical hexane extraction. CBN and CBG can be extracted in higher amounts with SFE than with hexane extraction. Waxes are co-extracted with the cannabinoids. They can be easily removed via a winterization step. The purity of the final extract after winterization was 85 % $\Delta^9$-THC at the optimal experimental conditions found in these experiments. With a two-steps extraction, it is possible to selectively extract minor cannabinoids (i.e. CBN, CBD and CBG) in a first step at low pressure (~15 MPa), and $\Delta^9$–THC in a second step at higher pressure (~20 MPa).

The last step of the process is performed using Centrifugal Partition Chromatography. It uses a two-phase liquid system, instead of a solid stationary phase, as it is the case in High Pressure Liquid Chromatography (HPLC). Separation is realized by the partitioning

of compounds between the two phases. With this technique, a successful separation of $\Delta^9$-THC, CBN and CBG is presented using the two-phase system hexane / acetone / acetonitrile. A purity higher than 99% is achieved with $\Delta^9$- THC. With CBN and CBG the best purity obtained is higher than 90%.

To conclude, an economical and ecological evaluation of two production routes to obtain pure $\Delta^9$-THC is presented: the current process using organic solvents is compared with the alternative process using supercritical carbon dioxide developed in this thesis. The alternative process is significantly cheaper than the current one, although the high price of the starting material cannabis dominates the ultimate cost price. From an ecological point of view, the alternative process is also more sustainable as it consumes less energy and generates less waste. Therefore, this alternative process is preferred from an economical and ecological point of view.

# Samenvatting


*Duurzame Productie van Cannabinoïden met behulp van Superkritische Koolstofdioxide Technologieën*

Dit proefschrift beschrijft de productie van natuurlijke componenten uit plantaardig materiaal voor de farmaceutische en voedingsmiddelenindustrie. Het beschrijft de productie (extractie en isolatie) van cannabinoïden, de actieve componenten die in cannabis aanwezig zijn. Vele cannabinoïden hebben een medische werking, maar niet alle cannabinoïden zijn beschikbaar in de (grote) hoeveelheden die nodig zijn om nieuwe geneesmiddelen te ontwikkelen. Reden is dat, voor grote schaal productie, er tot nu toe nog geen economisch en technologisch haalbare methoden bestaan om deze cannabinoïden, die slechts in relatief kleine hoeveelheden aanwezig zijn in de plant, te extraheren. Daarnaast kent de huidige productiemethode voor de belangrijkste cannabinoïd, tetrahydrocannabinol ($\Delta^9$-THC), een aantal nadelen, zoals het verbruik aan organische oplosmiddelen. Dit is niet alleen een grote last voor het milieu, maar ook voor de veiligheid, en leidt tot hoge productie- en afvalverwerkingkosten. In dit proefschrift wordt een alternatief proces voor de productie van verschillende cannabinoïden, inclusief $\Delta^9$-THC, cannabinol (CBN), cannabigerol (CBG) en cannabidiol (CBD), gepresenteerd, dat gebruik maakt van superkritisch koolstofdioxide als oplosmiddel.

De eerste stap in de $\Delta^9$-THC productie uit cannabis is de decarboxylatiereactie, waarbij het $\Delta^9$-THC carbonzuur, dat van nature in de plant aanwezig is, wordt omgezet in de psychoactieve, neutrale cannabinoïd $\Delta^9$-THC. Experimenten lieten een pseudo eerste orde reactie zien met een activeringsbarrière van 85 $kJ.mol^{-1}$ en een pre-exponentiële factor van $3{,}7x10^8$ $s^{-1}$. Met behulp van moleculaire modellering werden twee opties voor een zuur-gekatalyseerd β-keto-zuur type mechanisme geïdentificeerd. Beide mechanismen zouden een rol kunnen spelen, afhankelijk van de daadwerkelijke procescondities. Mierenzuur bleek een goed model te zijn voor de katalysator van zo'n reactie. Een direct keto-enol mechanisme gekatalyseerd door mierenzuur lijkt de beste verklaring te geven

voor de experimenteel geobserveerde activeringsbarrière en pre-exponentiële factor van de decarboxylatiereactie van $\Delta^9$-THC carbonzuur. Bewijs hiervoor werd gevonden door een extractieexperiment met cannabis flos uit te voeren. De aanwezigheid van korte carbonzuren bevestigt de hypothese.

De oplosbaarheden van $\Delta^9$-THC, CBN, CBG en CBD in superkritisch koolstofdioxide zijn vervolgens bepaald met behulp van een analytische methode met een 'quasi-flow' apparaat, zodat het superkritische extractieproces verder kan worden ontwikkeld. Allereerst werd de oplosbaarheid van $\Delta^9$-THC in superkritisch koolstofdioxide bepaald bij 315, 327, 334 en 345 K en in het drukbereik van 13.2 tot 25.1 MPa. De molaire oplosbaarheid van $\Delta^9$-THC varieerde tussen de $0{,}20 \times 10^{-4}$ en de $2{,}95 \times 10^{-4}$. Vervolgens werden de oplosbaarheden van CBN, CBG en CBD in superkritisch koolstofdioxide bepaald bij 315, 327 en 334 K en in het drukbereik van 11.3 tot 20.6 MPa. De molaire oplosbaarheden van CBN, CBG en CBD varieerden respectievelijk van $1{,}26 \times 10^{-4}$ tot $4{,}16 \times 10^{-4}$, van $1{,}17 \times 10^{-4}$ tot $1{,}91 \times 10^{-4}$ en van $0{,}88 \times 10^{-4}$ to $2{,}69 \times 10^{-4}$. De oplosbaarheid van de verschillende cannabinoïden in superkritisch koolstofdioxide heeft bij 326 K de volgende volgorde: $\Delta^9$-THC < CBG < CBD < CBN. De oplosbaarheidgegevens werden gecorreleerd met de Peng-Robinson toestandsvergelijking in combinatie met Van der Waals mengregels.

Vervolgens is de superkritische extractie van Cannabis Sativa L met superkritisch koolstofdioxide uitgevoerd op een proeffabriekschaal bij 313 en 323 K en in het drukbereik van 18 tot 23 MPa. Het extractierendement van $\Delta^9$-THC bedroeg maximaal 98%. Dit rendement is vergelijkbaar met het rendement van conventionele $\Delta^9$-THC extractie met hexaan. CBN en CBG kunnen in grotere hoeveelheden worden geëxtraheerd met superkritisch koolstofdioxide dan met hexaan. De was wordt met de cannabinoïden mee geëxtraheerd en kan gemakkelijk verwijderd worden met behulp van een uitvriesstap. De zuiverheid van het extract na uitvriezen was 85% $\Delta^9$-THC bij de optimale experimentele condities. Met behulp van een twee-staps extractie is wellicht het mogelijk om selectief de andere cannabinoïden (d.w.z. CBN, CBD en CBG) in een eerste

stap bij lagere druk (~15 MPa) te extraheren, en daarna de $\Delta^9$–THC in een tweede stap te extraheren bij hogere druk (~20 MPa).

De laatste stap in het proces maakt gebruik van centrifugale partitie chromatografie. Deze methode maakt gebruik van twee vloeistoffasen, in plaats van een mobiele vloeistoffase en een vaste stationaire fase zoals het geval is bij van hoge prestatie vloeistof chromatografie (HPLC). Scheiding wordt gerealiseerd door verschillende verdeling van de componenten over de twee vloeistoffasen. Met deze techniek is succesvolle scheiding van $\Delta^9$- THC, CBN en CBG mogelijk met het twee-fasen systeem bestaande uit hexaan / aceton / acetonitriel. Op deze manier kan $\Delta^9$- THC met een zuiverheid van meer dan 99% worden verkregen. Voor CBN en CBG is de hoogst haalbare zuiverheid hoger dan 90%.

Tot slot is er een economische en ecologische evaluatie gemaakt van de twee productieroutes om zuiver $\Delta^9$-THC te produceren: het huidige proces dat gebruik maakt van organische oplosmiddelen is vergeleken met het nieuwe, alternatieve proces, zoals ontwikkeld in het proefschrift dat gebruik maakt van superkritisch koolstofdioxide zoals ontwikkeld in dit proefschrift. $\Delta^9$-THC is momenteel nauwelijks commercieel verkrijgbaar, omdat het de status van illegale drug heeft. Dit heeft een groot effect op de prijs van cannabis en $\Delta^9$-THC. Ondanks het feit dat de hoge kostprijs van de grondstof (cannabis) de uiteindelijke kostprijs in belangrijke mate bepaalt, is het alternatieve proces wel significant goedkoper dan het huidige proces. Het aantal stappen in het alternatieve proces is slechts een derde van het huidige aantal processtappen. Uit een ecologisch oogpunt is het alternatieve proces ook duurzamer, omdat het minder energie verbruikt en minder afval genereert. Daarom lijkt het alternatieve proces zoals ontwikkeld in dit proefschrift haalbaar.

# Table of contents

# 1

# Introduction

# 1


## *Abstract*

*This thesis concerns the production of natural compounds from plant material for pharmaceutical and food applications. It describes the production (extraction and isolation) of cannabinoids, the active components present in cannabis. Many cannabinoids have medicinal properties but not all cannabinoids are available in the (large) quantities necessary to develop new medicines, because up to now, there are no economically and technically viable methods to extract those cannabinoids present in low quantities in the plant. Moreover, the currently used production process for the most important cannabinoid, tetrahydrocannabinol ($\Delta^9$-THC), has many drawbacks, such as the large use of the organic solvents, which is not only a burden to the environment but also to the safety of the operators, the production costs as well as the treatment of the produced waste.*

*In this thesis, an alternative process using supercritical carbon dioxide is presented for the production of cannabinoids, including $\Delta^9$-THC, cannabinol (CBN), cannabidiol (CBD) and cannabigerol (CBG).*

# 1. Introduction

## 1.1 Problem definition

The aim of this thesis is to develop an alternative production method for natural compounds with pharmaceutical or food interest from plant material.

The conventional method to extract such natural compounds uses organic solvents, in combination with several purification steps, as depicted in figure 1.1.

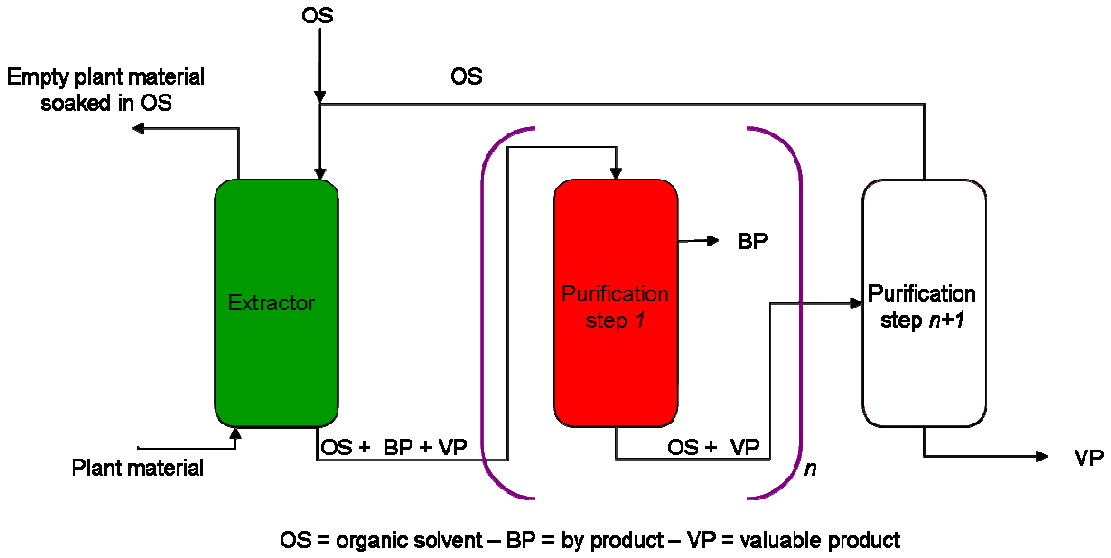



**Figure 1.1 Schematic drawing of an extraction process with organic solvents**

Several problems occur when using these organic solvents. First, in order to meet the requirements of the Pharmacopeias and food production about the levels of allowed residual solvents in the final product, several steps have to be performed to separate these solvents from the valuable compound. Regulations are strict about the maximum quantity of solvent allowed in food or medicinal products, as described in further detail in the background chapter (chapter 2 of this thesis).

Second, in most conventional processes, many purification steps are needed, because the purity after extraction is generally low due to low selectivity of the extraction with organic solvents. For example, chlorophyll and waxes may be extracted as well.

The high amount of purification steps leads to an expensive process and to the difficulty of scaling-up. Moreover, because organic solvents are often removed by evaporation, the energy consumption is high.

The large amount of the organic solvents used is also dangerous for the environment and represents safety issues. They lead to emissions in the atmosphere and waste problems. Solvent losses contribute considerably to the formation of large amounts of waste. The use of large quantities of volatile organic solvents as liquid media for chemical reactions and extractions, with a current worldwide cost estimated at € 6,000,000,000 per year [1, 2], is a major concern for today's chemical processing industry. The perceived effects of these solvents on human health, safety and the environment, combined with their volatility and flammability, is a strong incentive for minimizing their use, both for environmental and cost perspective. Minimizing solvent losses leads to avoiding the costs associated with disposal, legal liabilities and regulatory constraints [3].

Several natural compounds are difficult to obtain from plants by extraction with organic solvents, because of their chemical properties. Some of these compounds have a low solubility in organic solvents; others are too volatile or thermally labile, leading to low yields or product degradation in the solvent evaporation step. Therefore, classical extraction with organic solvents is not suitable for these compounds. In the next paragraph, an alternative using supercritical carbon dioxide is proposed.

## 1.2 Extraction of natural compounds by supercritical carbon dioxide

Carbon dioxide is non-flammable, relatively inert, abundant and inexpensive. In the supercritical region, the density of carbon dioxide and its solvent power can be varied by changing the temperature and pressure. Supercritical $CO_2$ has properties between those of

gases and liquids. Diffusivity and mass transfer are better than in liquids, whereas the solubilities of many organic compounds are higher than in gases. The low critical temperature allows heat-sensitive materials to be processed without damage. The fact that chemical substances show different solubility in supercritical $CO_2$ permits selective extraction, as its solubility power can be tuned by small variations of pressure and / or temperature. When the pressure is released after an extraction, the carbon dioxide evaporates and pure product without any remaining $CO_2$ is obtained. Therefore, supercritical extraction is often used for food and pharmaceutical products, for which it eliminates the possibility of leaving toxic residues of organic solvents [3-5].

As $CO_2$ is more selective, by tuning the pressure and temperature, and is easily removed by pressure release, its use needs only one recompression step and one purification step, instead of many separation steps, as shown in Figure 1.2. The energy need for the recompression step is generally lower than the energy needed in the evaporation step of the organic solvent in the conventional method. Therefore, the process is consuming less energy. Additionally, waste plant material can be easily recycled, contrary to plant material soaked in organic solvent, in the case of classical extraction. Moreover, as no high temperature separation steps are required, thermally labile compounds may be extracted with supercritical $CO_2$.

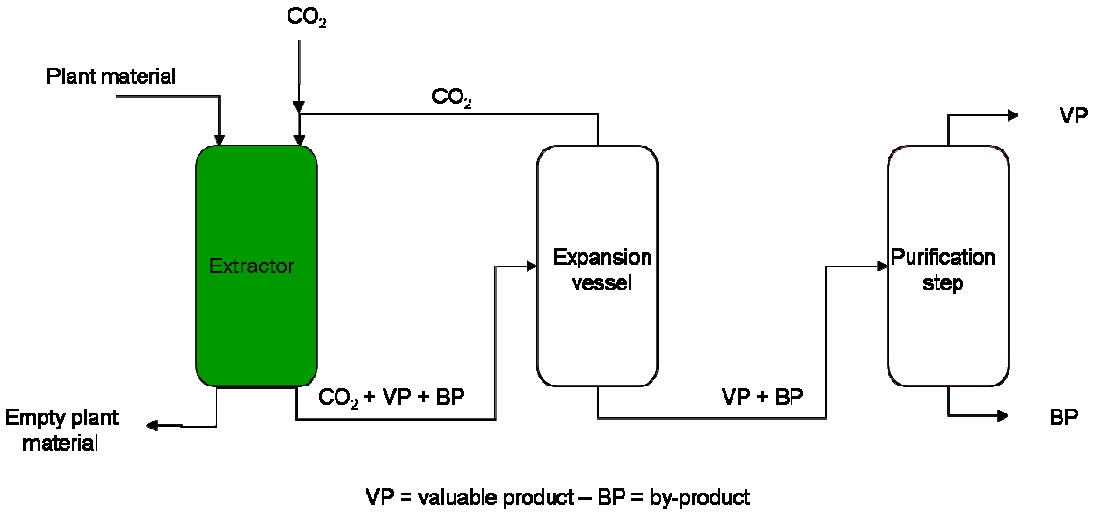



**Figure 1.2 Schematic drawing of supercritical fluid extraction equipment**

However, the use of carbon dioxide as green solvent also has its limitations. It is not a good solvent for substances like large polar molecules. Moreover, it is most commonly used in its supercritical state (above its critical temperature of 31°C and its critical pressure of 7.38 MPa). Therefore, $CO_2$ has to be used under pressure. This may lead to slightly higher investment costs however usually compensated by the fact that fewer steps are needed for the purification and $CO_2$ is cheap compared to other organic solvents [3, 6].

In this work, supercritical $CO_2$ will be used as solvent in different steps of a new process to extract and purify cannabinoids from cannabis. This process should not only allow producing $\Delta^9$-THC in a more sustainable way, but might also allow the production of other cannabinoids, increasing their availability on the market with a reasonable production cost, in order to further study their medical applications and to develop new medicines. The different steps of this process are presented in the next paragraph.

## 1.3 Motivation to isolate cannabinoids

Cannabis is a medicinal plant [7-9] shown in Figure 1.3. Until about 50 years ago cannabis extracts were found in many Pharmacopeias. However, as a result of the recreational use, in many countries it was put on the list of drugs of abuse and its medicinal use almost vanished. Recently, the medicinal use of cannabis has been legalized in several countries [10]. Some of the medical purposes of cannabis plant include, but are not limited to, multiple sclerosis, chronic pain, glaucoma, appetite stimulant, asthma and cardiovascular conditions, and as an antiemetic, i.e. its use prevents or treats nausea and vomiting [11].

Its unique active compounds, called cannabinoids, are present in the female flowers. The various cannabinoids have different properties and their medicinal activity may be influenced by the presence of other cannabinoids [12-16].

**Figure 1.3:** ***Cannabis sativa L***

Cannabinoids are defined as the group of $C_{21}$ compounds typically of and present in *Cannabis sativa L*, including their carboxylic acids, analogs, and transformation products. However, a less strict definition puts more emphasis on synthetic chemistry and on pharmacology, and also includes related structures or any other compound that affects cannabinoid receptors in the human body. This creates several chemical subcategories of cannabinoids. In this thesis, the focus will be on (phyto)cannabinoids: cannabinoids occurring in the cannabis plant [17].

In total, there are over 60 different (phyto)cannabinoids. The structures of the most common cannabinoids are presented in Figure 1.4. The amount of cannabinoids present depends on the plant species and on the storage conditions. Cannabinoids are present in their acid precursor form.

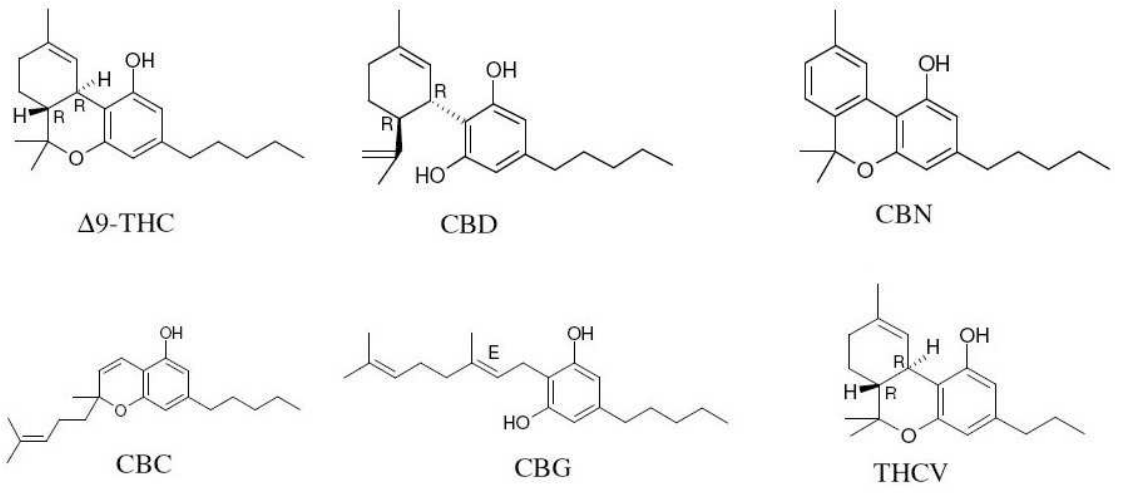



**Figure 1.4: Structures of (-)-$\Delta^9$- tetrahydrocannabinol ($\Delta^9$-THC), cannabidiol (CBD), cannabinol (CBN), cannabichromene CBC), cannabigerol (CBG) and tetrahydrocannabivarin (THCV)**

The main psychoactive cannabinoid, called (-)-$\Delta^9$- tetrahydrocannabinol ($\Delta^9$-THC), has been registered for medical use. Dronabinol is the International Nonproprietary Name (INN) for the pure isomer of $\Delta^9$-THC, (-)-trans-$\Delta^9$-tetrahydrocannabinol, that is the main isomer in the cannabis plant. There are currently two formulations with $\Delta^9$-THC (also known as Dronabinol) on the market. In the United States, synthetic Dronabinol is marketed as a medicine under the name Marinol® to treat nausea, pain and loss of appetite [17]. In Canada and UK, an oral mucosal spray containing $\Delta^9$-THC and CBD derived from cannabis plant is prescribed under the name of Sativex as adjunctive treatment neuropatic pain in multiple sclerosis, and for pain due to cancer. However, due to the lipophilic character of Dronabinol, easy administration with satisfactory pain relief is difficult. Echo Pharmaceuticals B.V. is currently developing a tablet with natural $\Delta^9$-THC, called Namisol®. This new tablet should have a better administration pathway, allowing satisfactory pain relief.

However, patients often claim that the use of pure $\Delta^9$-THC is not as efficient as smoking cannabis. It is becoming increasingly clear that $\Delta^9$-THC alone does not equal cannabis [8] and that other components play a role in some of the claimed medicinal effects [17].

One of these cannabinoids is Cannabinol (CBN). CBN is only mildly psychoactive and is perceived to be sedative or stupefying [18, 19]. It is the primary product of $\Delta^9$-THC degradation, and its amount is limited in a fresh plant. CBN content increases as $\Delta^9$-THC degrades under exposure to light and air.

A third cannabinoid which will be considered in this thesis next to $\Delta^9$-THC and CBN is cannabidiol (CBD). CBD is not psychoactive, although it may modulate the euphoric effects of $\Delta^9$-THC to some extent [14]. Medically, it appears to relieve convulsion, inflammation, anxiety, and nausea. It also protects against myocardial ischemic reperfusion injury [16]. Furthermore, CBD can possibly be used as a therapeutic agent for treatment of type 1 diabetes [20].

The fourth and last cannabinoid studied in this work is called cannabigerol (CBG). CBG is the direct precursor of the cannabinoids CBD, $\Delta^9$-THC and cannabichromene (CBC). It has been less studied in pharmaceutical investigations than the three previously mentioned. However, some studies have shown that it may lower blood pressure in rats. It has also analgesic and anti-inflammatory effects [12].

Smoking cannabis presents many drawbacks, e.g. it carries the risk of carcinogenesis due to the formation of compounds during combustion and it is not acceptable for non-smokers. Furthermore, smoking joints is illegal in most countries. An alternative way to consume cannabis without smoking is the oral administration of tea, as recommended by the Office of Medicinal Cannabis (OMC) in the Netherlands. However, in this way, most of the $\Delta^9$-THC is first metabolised into non-therapeutic metabolites in the lever. Moreover, since other cannabinoids also have medical properties, they may be used for different medical applications. Therefore, it is crucial to develop medicines. However, the poor availability of pure minor cannabinoids on the market is an obstacle for the development of such medicines. There is a need for the development of new processes to increase the availability of the different cannabinoids to be able to continue studying their medical properties and develop new drugs suitable for new medical applications.

Currently, there are various processes to isolate the most common cannabinoids from cannabis. For example, several patents describe routes to obtain $\Delta^9$-THC and $\Delta^9$-THC acid ($\Delta^9$-THCA) [21, 22] from cannabis. $\Delta^9$-THCA is obtained from plant material by extraction into an aqueous basic solvent under pH control. After acidification, the $\Delta^9$-THCA is extracted back into a non-polar solvent, yielding $\Delta^9$-THCA in high purity. $\Delta^9$-THCA is then converted by vacuum distillation to $\Delta^9$-THC which is further purified and combined with a carrier for pharmaceutical use. This process includes 7 different steps of extraction and 4 extra steps for the purification. It requires a lot of energy, produces a lot of waste water (contaminated with mainly inorganic salts) and organic waste, mainly organic solvents such as heptane and isopropyl ether. Improvement of this process by reduction of the number of process steps, energy consumption, water consumption and waste production, is of crucial importance in order to obtain a more sustainable process and eventually increase the scale of the production.

## 1.4 Cannabis and the law

### *1.4.1 Cannabis policies*

As of 1954, the World Health Organization (WHO) has claimed that cannabis and its preparations no longer serve any useful medical purpose and are therefore essentially obsolete. Until then, cannabis legislation had been based on a large number of conventions, causing considerable confusion in the execution of treaties. Under the pressure of increasing reports that cannabis was a drug dangerous to society, it was proposed to combine all in single convention, the draft of which was finally accepted by the United Nations in 1961. In the following years several complementary treaties were made to strengthen it. Under the “Single Convention on Narcotic Drugs” cannabis and its products were defined as dangerous narcotics with a high potential for abuse and no accepted medicinal value. It reflected the belief that cannabis was a dangerous narcotic

with a threat that was equal to the most dangerous opiates, as it was strongly believed that cannabis use could serve as a stepping stone to the use of such drugs.

Since the Single Convention on the political agenda, the potential danger of cannabis abuse by recreational users has been much higher than any of its benefits as a source for fiber, food or medicines. Nowadays it may be hard to believe, but according to the American president Richard Nixon, cannabis was a secret weapon of the communists, being spread by the Jews to destabilize the Western world. This sense of cannabis-related fear has been the base for the legislation that is currently seriously obstructing the rediscovery of cannabis as a medicine. Even today, under US laws, possession of only a few grams of cannabis can lead to imprisonment for life. The distinction between medicinal and recreational use is thereby made only in a handful of Unitet States of America.

It can be observed that new scientific insights on cannabis are only slowly and reluctantly incorporated into new legislation. However, in the coming years, a large variety of scientific and clinical data is expected to become available, further showing the physiological effects of cannabinoids and the endocannabinoid system. And in several Western countries important obstacles for a real acceptance of medicinal cannabis have already been addressed, as serious steps are taken towards decriminalization of cannabis use or even providing medicinal cannabis products to patients [23-26]. These shifts constitute the first steps away from the dominant drug policy paradigm advocated by the United States, which is punishment-based prohibition, and it signals that the Single Convention may start to reach its expiry date. The legislation that follows will depend for a large part on the quality of the research available. However, good arguments will finally not be enough; what is most needed is a change in mentality [27], in politics, but also in the way research is conducted [17].

### *1.4.2 The Dutch situation*

The Netherlands have known a liberal drug policy already for several decades, so it is not surprising that the Dutch have been among the first to approach the discussion on medicinal cannabis in a practical way. In the 1990s, it was increasingly acknowledged that a considerable group of people was using cannabis for medicinal purposes, obtained through the illicit market. Simultaneously, a growing number of Dutch health officials judged that, although scientific proof on the effectiveness of cannabis might still be insufficient, the perceived dangers of cannabis use no longer outweighed its potential beneficial effects to certain groups of chronically ill patients. However, its unofficial status made it impossible to make any guarantees on the quality, consistency, or origin of the cannabis found in the illegal market. Therefore, in order to supply these patients with a safe and reliable source of high quality cannabis, the Office of Medicinal Cannabis (OMC) was established in March 2000. It started acting as a national agency on 1 January 2001. The OMC is the organization of the Dutch Government which is responsible for the production of cannabis for medical and scientific purposes, and is in full agreement with international law. After an initial preparation period, medical grade cannabis (in the form of dried female flower tops) finally became available in Dutch pharmacies in September 2003, on prescription only. Based on the availability and quality of clinical data and scientific literature, a selection of indications was made by the OMC for treatment with its medicinal grade cannabis [28].

Right from the start, a reliable source of high quality cannabis materials was considered crucial for the success of the Dutch medicinal cannabis program. Therefore, skilled breeders were contracted for the cultivation of plants under highly standardized conditions, resulting in a product with a very consistent composition. The whole process of growing, processing and packaging of the plant material are performed according to pharmaceutical standards, and supervised by the OMC. The quality is guaranteed through regular testing by certified laboratories. Besides supplying high quality cannabis to medicinal users, the OMC also provides the same material for research and development of medicinal preparations based on cannabis constituents.

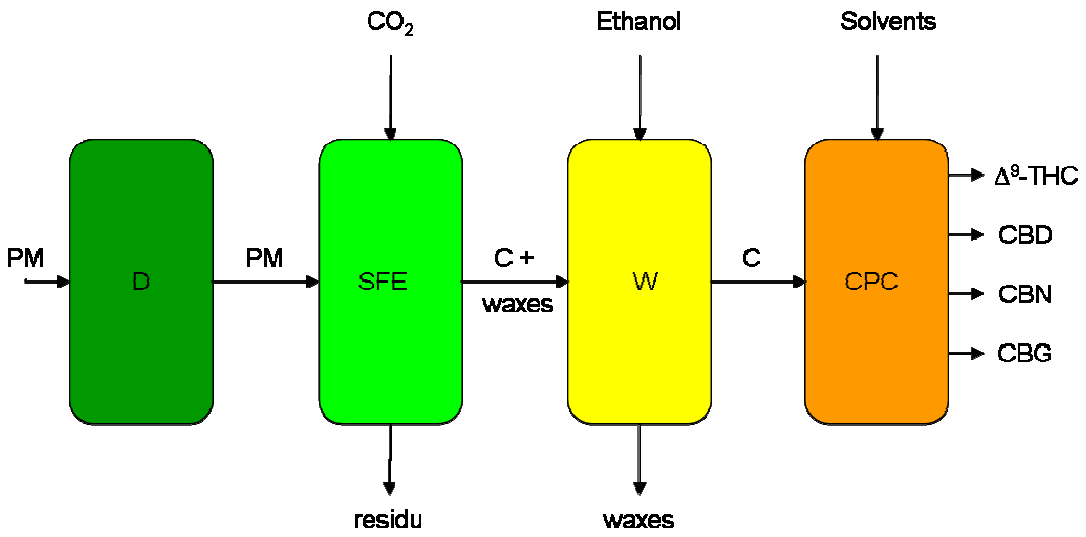
The availability of reliable cannabis of consistent quality has proven to be crucial to perform good research, as it opened up the way for long term quantitative studies on cannabis and its constituents on a national level. Currently, a variety of laboratories and research groups cooperate for quality control, fundamental research and clinical development. Cannabis research in The Netherlands is blooming, with a clear focus on scientific outcome, rather than on repression of cannabis use [17]. It is exactly these conditions that have made the work of A. Hazekamp possible followed by the work presented in this thesis.

Because of the problems related to extraction and purification of natural compounds from plant material by organic solvents, an alternative process that does not suffer from these disadvantages is developed in this work. This process uses supercritical carbon dioxide as a solvent to perform extraction and separation of the desired components.

## 1.5 Cannabinoids Production Process

The process investigated in this thesis is presented in Figure 1.5. It consists of four principal steps starting from the grinded cannabis plant (also called plant material in Figure 1.5), and resulting in pure cannabinoids. Cannabinoids are present in their acidic form in the cannabis plant, but are used in their neutral form in medicine. Because it is expected that the solubility of the acidic cannabinoids in supercritical carbon dioxide is lower than that of the neutral ones, they are decarboxylated in a pretreatment step. This step does not require any organic solvent, as it is only a heating step. Supercritical Fluid Extraction represents the second step of the process. In this step, only the green solvent $CO_2$ is used. The product of this step consists of waxes and cannabinoids. To ease the next step, hexane is used to dissolve the extract. The waxes can be easily separated from the cannabinoids by winterization, i.e. freezing of the extract to precipitate the waxes. After that, a simple filtration isolates the cannabinoids from the waxes. The last step is using centrifugal partition chromatography with organic solvents or even with $CO_2$ in an ideal case. The desired end products are the cannabinoids with purity higher than 95 %. A

further reduction of the number of process steps might be obtained by combining the decarboxylation step with the extraction step. The amount of cannabinoid will depend on the type of cannabis plant used. The experiments done in this thesis will be based on the use of the Bedrocan cannabis plant, containing around 18% $\Delta^9$-THC, and less than 1 % of the other cannabinoids. Therefore, mainly $\Delta^9$-THC will be produced. However, with SFE, other minor cannabinoids (i.e. CBN, CBD and CBG) are extracted as well in a higher yield than with organic solvents. They are not lost in the distillation steps and can be easily separated with CPC. Additionally, with a different cannabis plant type, containing other cannabinoids in larger quantities, it is also possible to obtain other cannabinoids such as CBD and CBG in sufficient amounts for medicine development. To obtain CBN, the Bedrocan cannabis plant may be used after specific storage conditions (e.g. light, air), to obtain the degradation of $\Delta^9$-THC into CBN.




PM = Plant Material - D = Decarboxylation - SFE = Supercritical Fluid Extraction
W = Winterisation - CPC = Centrifugal Partition Chromatography - C = Cannabinoids

**Figure 1.5: Schematic drawing of the cannabinoids production process**

## 1.6 Scope of this thesis

This thesis presents the investigation of the different steps of an alternative process to extract and purify cannabinoids from cannabis by using supercritical $CO_2$. **Chapter 2** covers a background overview of the different topics of the chapters. It starts with the regulations about the use of organic solvents in pharmaceutical and food products. Then it presents in detail the green solvent carbon dioxide and different ways to measure the solubility of natural components in it. Moreover, an overview of the separation methods used in the work is given. Supercritical Fluid Extraction (SFE) is presented, followed by Centrifugal Partition Chromatography (CPC).

**Chapter 3** describes the decarboxylation process. The kinetic parameters are experimentally determined. Molecular modeling is applied to determine the decarboxylation mechanism.

Then, in order to determine if supercritical $CO_2$ is a suitable solvent to extract cannabinoids, solubilities of $\Delta^9$-THC, CBN, CBD and CBG with purities above 98% in supercritical $CO_2$ are measured and modeled using the Peng-Robinson Equation of Sate. As their solubilities are relatively low, and their availability is limited, a new method to measure the solubility in supercritical $CO_2$ was developed and is presented in **Chapter 4.** Anthracene is used as a model compound to validate the method. In this chapter, the solubility of $\Delta^9$-THC in supercritical $CO_2$ is also presented. **Chapter 5** presents the solubility of CBN in supercritical $CO_2$ and compares it with the solubility of $\Delta^9$-THC in supercritical $CO_2$. It is shown that both cannabinoids show completely different solubility behavior. **Chapter 6** presents the solubilities of CBD and CBG in supercritical $CO_2$, and compares them to the solubilities of both $\Delta^9$-THC and CBN in supercritical $CO_2$. Moreover, a process design for Supercritical Fluid Extraction in order to extract all four cannabinoids is proposed.

In **Chapter 7**, the results of extraction of cannabinoids from cannabis plant, Bedrocan variety, using supercritical $CO_2$ are presented and discussed.

**Chapter 8** presents Centrifugal Partition Chromatography using supercritical $CO_2$ and generally regarded as safe (GRAS) solvents instead of organic solvents. A comparison with the conventional CPC that uses only organic solvents is also discussed in this chapter.

**Chapter 9** gives an economical and environmental evaluation of the current process using organic solvents and the new process with $CO_2$ to obtain $\Delta^9$-THC. Both processes are compared in terms of costs, energy consumption, yields and waste production.

**Chapter 10** summarizes the conclusions of this work and recommendations to use this new process for other molecules than $\Delta^9$-THC in case of cannabis, or for other plant material.

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# 2

## Background

# 2


## *Abstract*

*This chapter provides background information about the different topics presented in this thesis. It first presents an overview of the current regulations about the use of organic solvents in pharmaceutical and food industry. As their use has many drawbacks, an alternative solvent is used in this thesis, supercritical carbon dioxide. Its characteristics are presented in the second part. In order to develop processes using this green solvent, solubility data of the interesting components are needed. Several techniques to measure solubility are presented in this chapter. Finally, the two main processes used in this work, i.e. supercritical fluid extraction (SFE) and centrifugal partition chromatography (CPC) are described. SFE has already been widely used for the extraction of natural molecules. CPC has been developed using organic solvents to separate natural components. A state of the art concerning these two processes is presented.*

# 2. Background

## 2.1 Regulations about organic solvents

Most regulatory agencies rely on a published document to set the limits for residual solvents. In the United States, that document is the United States Pharmacopoeia. The European Union has the European Pharmacopoeia, and Japan has the Japanese Pharmacopoeia. These three bodies often work together to present a uniform standard to global pharmaceutical industries. This is done under the name "International Conference on Harmonisation" or ICH. The limits and guidelines for residual solvents established by the ICH were adopted by each of the pharmacopoeias it represents, creating a standard fairly universal [1]. In pharmaceutical fields and in the food industry, residual solvents are separated into three classes based on risk assessment studies that are related to their potential toxicity level.

Class 1 solvents (i.e. Benzene, Carbon tetrachloride, 1,2-Dichloroethane , 1,1-Dichloroethene and 1,1,1-Trichloroethane) are not allowed in pharmaceutical and food processes because they are known human carcinogens or they are strongly suspected carcinogens.

Class 2 solvents are solvents that are not genotoxic carcinogens, but are possible causative agents of other irreversible toxicity, such as neurotoxicity or teratogenicity. This class is considered less toxic than the first class, so low levels residues can be accepted. Table 2.1 lists the compounds in class 2 and the maximum allowable concentrations in ppm. In addition to the maximum allowable concentration in food products, the compounds in class 2 also have established limits for pharmaceutical use referred to as Permitted Dayly Exposure (PDE) limits, which vary depending on the individual compound.

Class 3 solvents exhibit low to minimal potential human health-related toxicity. The maximum allowable concentration for these compounds is generally 5000 ppm (= 0.5%).

They have PDEs of 50 mg or more per day, depending on the individual compound. Ethanol, acetone, methylbutylether, acetic acid, butanol, formic acid, heptane and pentane are some examples of class 3 organic solvents [2].

**Table 2.1 United States Pharmacopeia Class 2 residual solvents [2]**

| Solvent | PDE (mg/day) | MAC (ppm) |
|---|---|---|
| Acetonitrile | 4.1 | 410 |
| Chlorobenzene | 3.6 | 360 |
| Chloroform | 0.6 | 60 |
| Cyclohexane | 38.8 | 3880 |
| 1,2-Dichloroethene | 18.7 | 1870 |
| 1,2-Dimethoxyethane | 1.0 | 100 |
| N,N-Dimethylacetamide | 10.9 | 1090 |
| N,N-Dimethylformamide | 8.8 | 880 |
| 1,4-Dioxane | 3.8 | 380 |
| 2-Ethoxyethanol | 1.6 | 160 |
| Ethylene glycol | 6.2 | 620 |
| Formamide | 2.2 | 220 |
| Hexane | 2.9 | 290 |
| Methanol | 30.0 | 3000 |
| 2-Methoxyethanol | 0.5 | 50 |
| Methylbutylketone | 0.5 | 50 |
| Methylcyclohexane | 11.8 | 1180 |
| Methylene chloride | 6.0 | 600 |
| N-Methylpyrrolidone1 | 5.3 | 530 |
| Nitromethane | 0.5 | 50 |
| Pyridine | 2.0 | 200 |
| Sulfolane | 1.6 | 160 |
| Tetrahydrofuran | 7.2 | 720 |
| Tetralin | 1.0 | 100 |
| Toluene | 8.9 | 890 |
| Trichloroethylene | 0.8 | 80 |
| Xylene* | 21.7 | 2170 |

## 2.2 Supercritical carbon dioxide

Carbon dioxide is a supercritical fluid at temperatures higher than 304.2 K (=31.1 $^{o}$C) and pressures higher than 7.38 MPa (= 73.8 bar). Under these conditions the distinction between the gas phase and liquid phase is nonexistent, and carbon dioxide can only be described as a fluid. This can be explained by looking at the phase diagram of carbon dioxide (see Figure 2.1). The boiling line separates the vapor and liquid region and ends in the critical point. At any point on the boiling line, carbon dioxide exists in a liquid and a vapor phase. As the temperature is raised along the boiling curve the liquid density decreases due to expansion, whereas the gas density rises due to the pressure increase. At the critical point these densities become identical and the distinction between the liquid and gas phase disappears [3].

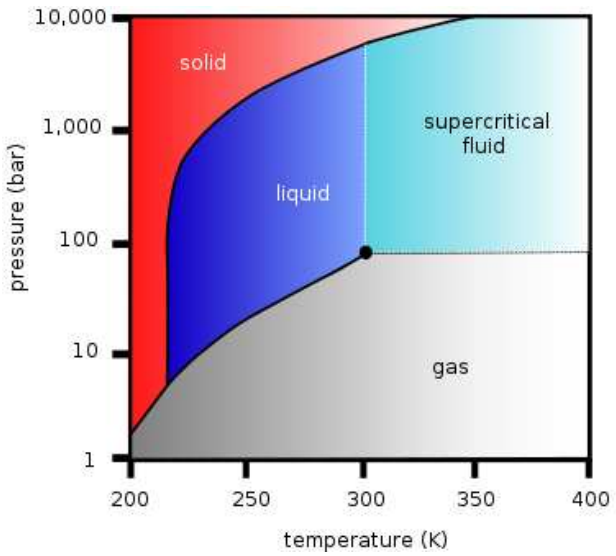


**Figure 2.1: Phase diagram of carbon dioxide**

Supercritical carbon dioxide has properties in between those of liquids and gases. It has the ability to diffuse through materials like gas, and to dissolve organic compounds like a non-polar liquid. Alkanes, aromatics, ketones and alcohols (up to a molecular height of around 400 g.mol$^{-1}$) dissolve in supercritical carbon dioxide, but polar molecules such as acids and most inorganic salts are insoluble. By adjusting the pressure of the supercritical carbon dioxide, the solvent properties can be adjusted to be more “gas-like” (low

solvency power) or “liquid-like” (high solvency power), which makes it a highly tunable solvent (for this purpose also modifiers can be added). Because of these properties, supercritical carbon dioxide is a well-established solvent for use in extraction (see paragraph 2.4). Other emerging commercial technologies involving carbon dioxide include dry cleaning [4, 5], dying of textiles [6-9] and the use as environmentally benign solvent for various organic reactions, such as hydrogenations, hydroformylation, oxidations, biocatalytic reactions and polymerizations [10]. Figure 2.2 illustrates the $CO_2$ tank used in the laboratory.


**Figure 2.2: $CO_2$ tank at the laboratory**

## 2.3 Solubility measurements in supercritical carbon dioxide

*Background information for chapters 3, 4 and 5*

To determine whether compounds can be extracted and/or purified with supercritical carbon dioxide, solubility measurements need to be performed. Solubility is typically defined as mole fraction or weight fraction of solute in the supercritical fluid, which is in equilibrium with the bulk solute [11]. Different techniques exist to measure the solubility of natural compounds in supercritical carbon dioxide. These methods can be divided into two major categories: static and dynamic.

### *2.3.1 Static methods*

In these methods, the solute is allowed to be in static contact with the supercritical fluid in order until equilibrium is reached. Depending on sampling and the type of high-pressure vessel used, there are three variations: analytical, synthetic and gravimetric [11]. However, as only the synthetic method is available in our laboratory, the analytical and gravimetric variations will not be described here.

Static techniques are used to determine the location of phase border curves in the P-T space and the solubility of a heavy solute (molecular weight higher than 300 $g.mol^{-1}$) in supercritical fluids. Several equipment types can be found in literature [12, 13]. An example of such equipment is the Cailletet apparatus. The Cailletet apparatus uses the synthetic method and is depicted in Figure 2.3. In this apparatus, the pressure or temperature can be varied for a sample with a constant overall composition until a phase change is visually observed. Pressures up to 15 MPa can be applied and the temperature can range from 250 to 450 K, depending on the heat transferring fluid. This technique is accurate but has temperature and pressure limitations. Moreover, the minimum solubility accurately measured with a Cailletet apparatus is in the molar fraction order of $3 \times 10^{-4}$. This lies above the range of cannabinoids solubility in $CO_2$. Therefore a dynamic method has been used to determine the solubility of cannabinoids in $CO_2$.

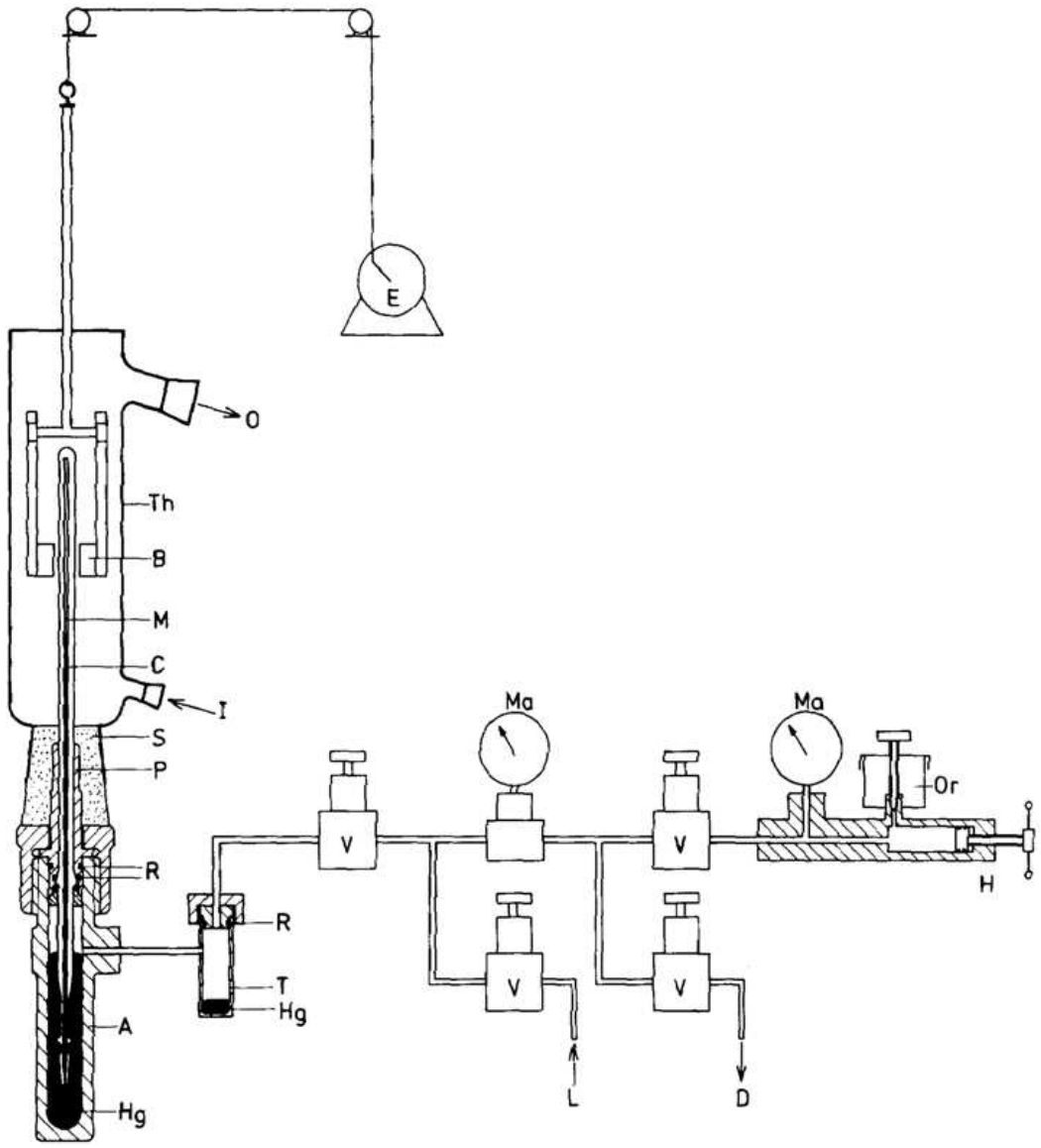


**Figure 2.3: Cailletet apparatus; A, autoclave; B, magnets; C, capillary glass tube; D, drain; E, motor; H, rotating hand pump; Hg, mercury; I, thermostat liquid in; L, line to dead weight pressure gauge; M, mixture being investigated; Ma, manometers; O, thermostat liquid out; Or, hydraulic oil reservoir; P, closing plug; R, Viton-O-rings; S, silicone rubber stopper; T, mercury trap; Th, glass thermostat; V, valve. [14].**

### *2.3.2 Dynamic method*

A dynamic or flow technique uses the assumption that the solute-solvent system reaches equilibrium as the solvent passes over the solute [15]. It is used for determining solute solubilities in supercritical fluid and also for stripping and fractionating studies. A typical schematic drawing of an apparatus using the dynamic method is shown in Figure 2.4 [11, 16].

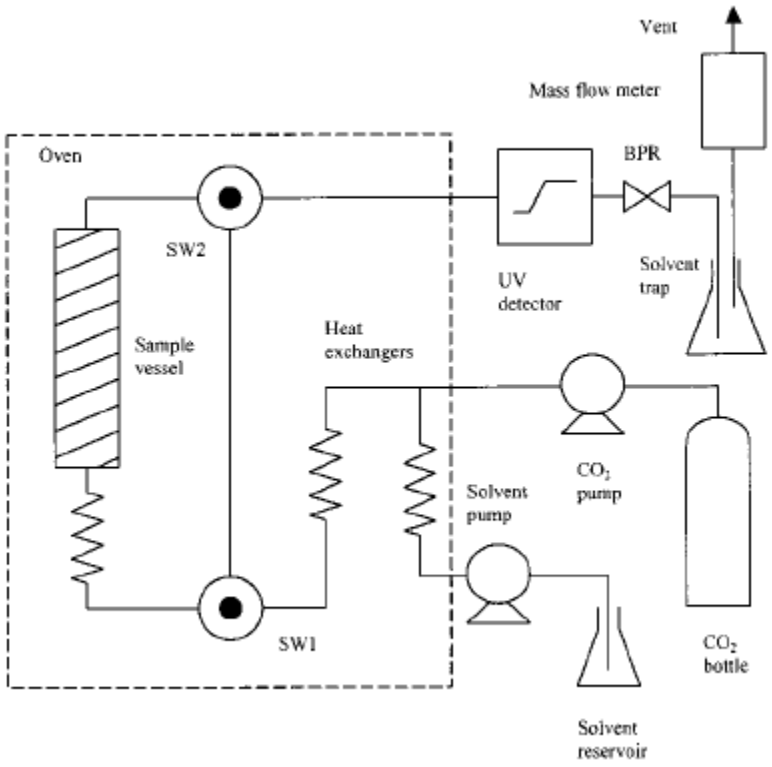


**Figure 2.4: Apparatus for the determination of solubility using a dynamic method. BPR = back pressure regulator to maintain constant pressure in system; SW = switching valves to divert flows [16]**

In this thesis, a kind of dynamic technique will be used. As this equipment had never been used before, a detailed description of the used equipment and the validation of the method are presented in chapter 4 of this thesis.

## 2.4 Extraction of natural compounds with supercritical carbon dioxide

*Background information for Chapter 7*

Supercritical Fluid Extraction (SFE) with carbon dioxide ($CO_2$) is a promising alternative technique to liquid and gas extraction. There are no flammability or toxicity issues, solvent removal is simple and efficient, and the extract quality can be well-controlled. Another advantage of this method are low operating costs, because $CO_2$ is cheap, (balancing a relatively high investment for the equipment), almost complete recycling of the solvent, no solvent residues in the extract. A crucial advantage of this method is the tunability of the extract solubility in supercritical $CO_2$ by varying the experimental conditions (temperature and pressure).

$CO_2$ has been widely used for extraction of natural compounds, including pharmaceutical molecules, from plant material, as shown in Table 2.2 [17-23].

**Table 2.2: Literature summary of SFE of natural compounds**

| **Raw Material** | Leaves of Tarragon | Hazelnut | Black Pepper | Marigold | Rosemary | Vetiver roots | Tobacco leaves | Humulus lupulus | Chamomile flower heads | Pepermint |
|---|---|---|---|---|---|---|---|---|---|---|
| **Extractor Capacity (ml)** | 1500 | 10 | 60 | 80 | 125 | 5 | 14 | 600 | 400 | 115 |
| **Number of Separator** | 2 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 1 |
| **Sample mass (g)** | 300 | 5 | 12 | 40 | 10 | NA | NA | 40 | 110 | 1 |
| **Particle size (mm)** | NA | 1 and 2 | 2.5 | 0.62 | <0,150, 0,208 and 0,436 | 0.21 | 0.425 | NA | NA | 3 or powder |
| **Flow rate (g/min if not mentionned)** | 25 | 2.13 | 1.23 | 0,78 to 3 | 1 and 5 | 5,4 to 7,2 | 3.5 | NA | 13.3 | 1.92 to 7.68 |
| **Cosolvent** | None | None | None | None | Ethanol: 0 and 3% | Ethanol: 0 to 10% | None | None | None | Ethanol 2 to 6% |
| **Pressure (MPa)** | 8 to 12 | 30 to 60 | 16 and 20 | 12 to 20 | 10 to 18 | 10 to 30 | 15 to 25 | 12.5 to 27.5 | 8 to 12 | 8.82 to 19.6 |
| **Temperature (°C)** | 40 to 55 | 40 to 60 | 40 | 40 | 40 and 60 | 40 | 40 to 60 | 40 | 35 to 50 | 20 to 80 |
| **Extraction Time (min)** | 120 | 300 | 840 and 1200 | 180 to 684 | 45 to 66 | 300 | 60 | 180 or 360 | 150 | 100 |
| **Extract** | Essential oil | Oil (linoleic, oleic acid) | Essantial oil, oleoresin | Oleoresin, waxes | Oil | Oil (Zizanoic acid : 30%) | Solanesol, nicotine | Essential oil | Essantial oil | Essential oil |
| **Maximum total yield** | 0.42% | 33.00% | 3.66% | 2.70% | 2,1% without ethanol to 3,2% with 3% | 2,5% without ethanol, 4,7% with 10% | NA | 11% | 1.18% | NA |
| **Best experimental** | 9MPa - 50°C | 60MPa - 60°C - 180 min | 20MPa - 40°C - 1200min | NA | 18MPa - 40°C | 20MPa - 40°C | 15MPa - 40°C | 20MPa - 40°C | 9 MPa - 40°C | NA |

In the examples presented in Table 2.2, ethanol is sometimes used as a co-solvent, yielding in a better maximum total yield for extraction of polar compounds. Since this is a Class 3 solvent, its use is allowed in pharma and food products.

Many applications of supercritical extraction are now available on the industrial scale. For example, in Spain SFE is used to purify cork, by removing 2,4,6-trichloroanisole [24]. In Italy, this technology is used for coffee decaffeination. In Germany, tea is decaffeinated using this method. In India, plants are extracted with carbon dioxide to recover spices and herbs. In South Korea, edible oil is extracted from plant material with carbon dioxide. Finally, in New Zealand, plants are extracted with carbon dioxide to yield hops and nutraceuticals [25].

An SFE set up is basically composed of the following elements: $CO_2$ storage vessel, pump, heat exchanger, extractor, separator and cooler, as depicted in Figure 2.4.

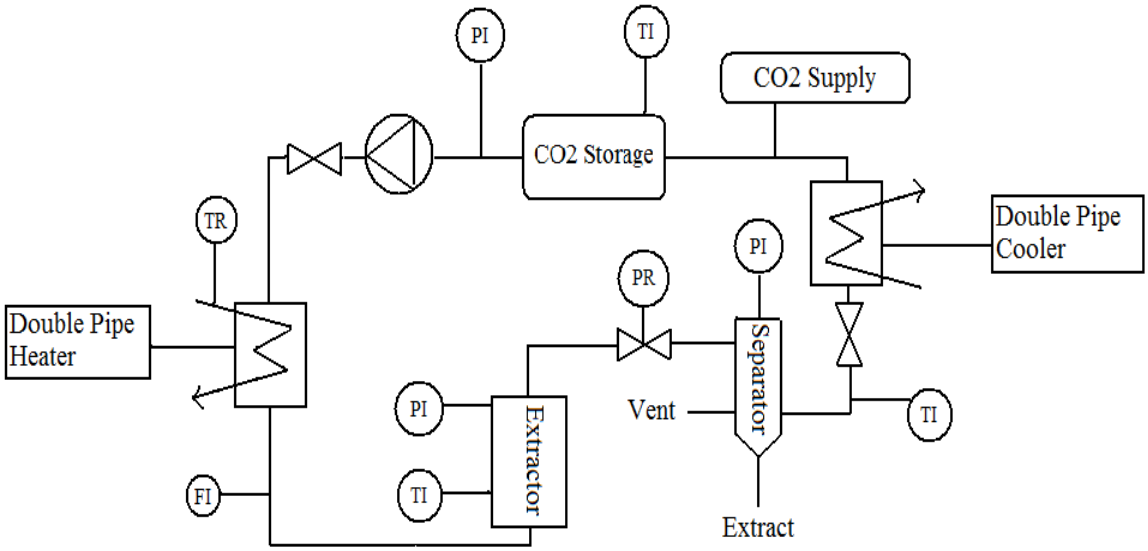


**Figure 2.5: Schematic drawing of typical SFE set-up - PI = Pressure Indicator, TI = temperature Indicator, PR = Pressure regulator, FI = Flow Indicator, TR = Temperature Regulator**

Figure 2.6 and 2.7 represent the experimental set-up used in this thesis to perform SFE of cannabis. A more detailed description of the SFE set-up used can be found in chapter 7.

**Figure 2.6: SFE set-up**

**Figure 2.7: Separator of the SFE set-up**

In the case of cannabinoids, the extract obtained does not contain only the pure desired natural compound, but also some by-products. Therefore another technology has to be used to further purify the extract. Currently, Centrifugal Partition Chromatography is a promising technique for the purification step. This technique is presented in the next paragraph.

## 2.5 Centrifugal partition chromatography

*Background information for chapter 8*

The extract obtained from the cannabis Sativa L. contains not only the pure desired products, cannabinoids, but also waxes and terpenoids. Therefore, another technology has to be used to further purify the extract. Centrifugal Partition Chromatography (CPC) is a promising technique. CPC is a type of counter-current chromatography (CCC), where both the stationary and mobile phases are liquid. This chromatographic method is based on the Nernst's distribution law, which states that a solute will be distributed between two partially miscible solvent layers at a constant and reproducible ratio [26].


**Figure 2.8: CCC picture [27]**

In CCC, which is depicted in Figure 2.8, the sample is introduced in a mobile phase, which flows through an immiscible stationary phase, and the various compounds exit the column at different times. The stationary phase is kept in place by gravity. The components in the sample develop different migration velocities because of their different partitioning behavior over the two phases. The versatility of this technique is based on the ability to vary the composition and polarity of the stationary and mobile phases [28-34]. Moreover, both phases can play the role of mobile phase or stationary phase, depending on the mode chosen in the CCC. When the heavier phase is the mobile phase, the descending mode is used. On the contrary, in the ascending mode, the mobile phase is the lighter one, as depicted in Figure 2.9.

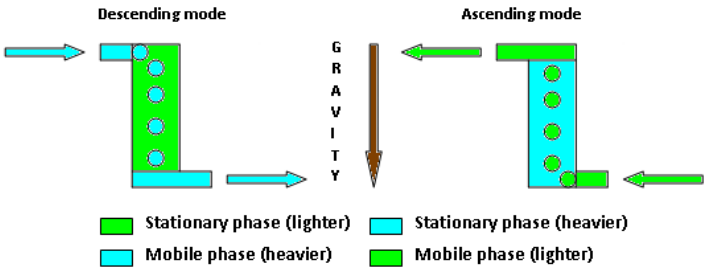


**Figure 2.9: Counter-current chromatography scheme**

CCC is capable of separating molecules of a broad range of molecular weights – from drugs, pesticides, and natural products to blood particles and cells. Contrary to solid supported chromatography, the retained compounds can be easily recovered by flushing the system. However, the separation is time-consuming and requires a long "column". Increasing the flow rate is not an option because the stationary phase is then washed away [34].

In CPC, the gravitational field is replaced by a centrifugal one. The column is replaced by numerous small channels connected by ducts and engraved into disks. The disks are aligned around a high-speed rotor. These changes allow efficient operation at high flow rates and high stationary phase volumes. In ascending mode, the lighter phase flows through the heavier one opposite to the centrifugal field. In descending mode, the heavier phase flows through the lighter phase parallel to the centrifugal field, as shown in Figure 2.10 [31, 35].

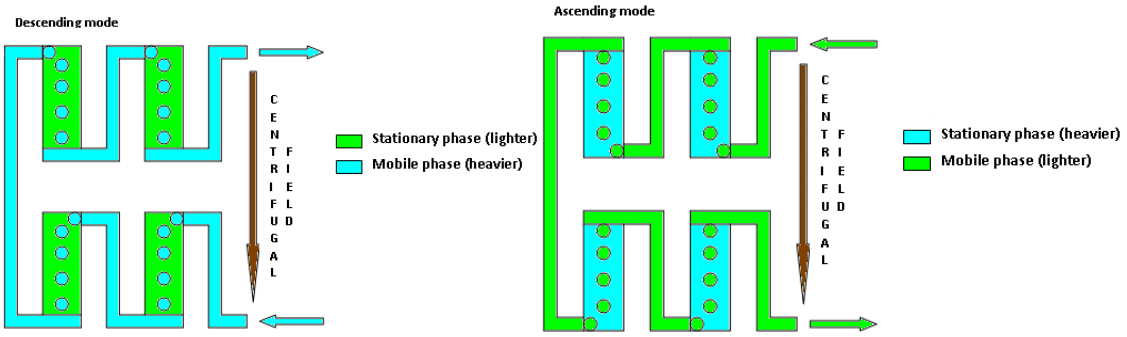


**Figure 2.10: Centrifugal partition chromatography scheme.**

CPC systems purify from milligrams to kilograms of pharmaceutical, biotechnology, cosmetics, agro-food, natural products, petroleum/petrochemical and environmental compounds and samples [36-40]. A picture of a CPC apparatus is shown in Figure 2.11. A more detailed description of the used CPC set-up can be found in chapter 8.

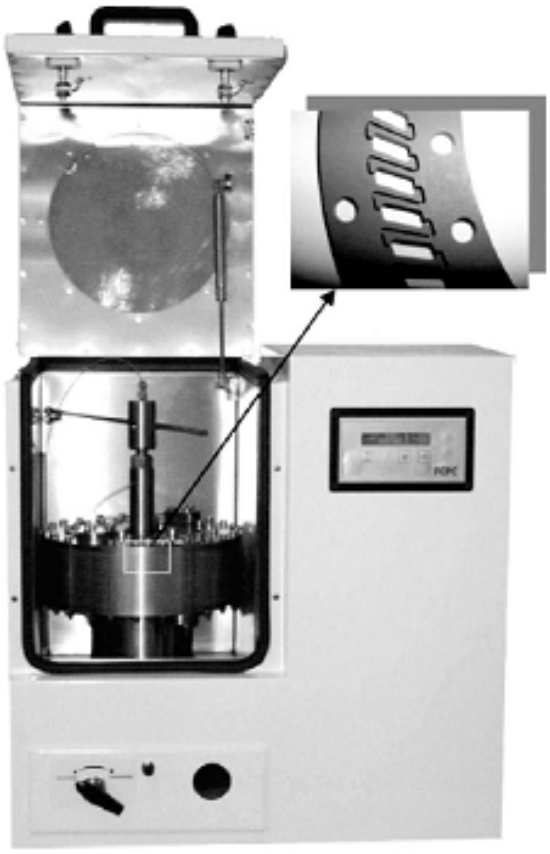

**Figure 2.11: FCPC picture [41]**

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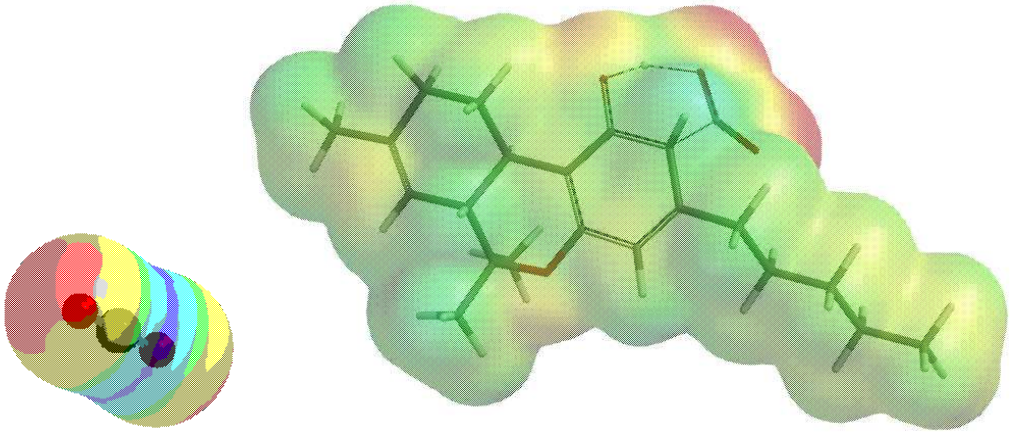
# 3

# Decarboxylation of Delta-9-tetrahydrocannabinol: kinetics and molecular modeling



Content accepted for publication in *J. Chem. Structure*

# 3


## *Abstract*

*Efficient tetrahydrocannabinol ($\Delta^9$-THC) production from cannabis is important for its medical application and as basis for the development of production routes of other drugs from plants. This work presents one of the steps of $\Delta^9$-THC production from cannabis plant material, the decarboxylation reaction, transforming the $\Delta^9$-THC-acid naturally present in the plant into the psychoactive $\Delta^9$-THC. Experiments showed a pseudo first order reaction, with an activation barrier of 85 kJ.mol$^{-1}$ and a pre-exponential factor of 3.7x10$^8$ s$^{-1}$. Using molecular modeling, two options for an acid catalysed $\beta$-keto acid type mechanism were identified. Each of these mechanisms might play a role, depending on the actual process conditions. Formic acid was shown to be a good model for a catalyst of such a reaction. A direct keto-enol mechanism catalyzed by formic acid seems to be the best explanation for the observed activation barrier and the pre-exponential factor of the decarboxylation of $\Delta^9$-THC-acid. Evidence for this was found by performing an extraction experiment with Cannabis Flos. It revealed the presence of short chain carboxylic acids supporting this hypothesis. The presented approach is important for the development of a sustainable production of $\Delta^9$-THC from the plant.*

# 3. Decarboxylation of Delta-9-tetrahydrocannabinol: kinetics and molecular modeling

## 3.1 Introduction

At present there is a growing interest in cannabis and its medicinal uses [1, 2]. Cannabis contains more than 400 different ingredients, including at least 60 cannabinoids. The major active component, called (-)-$\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC), does not occur at significant concentrations in the plant, but is formed by decarboxylation of its corresponding acid upon heating.

As described in a patent [3], $\Delta^9$-THC acid ($\Delta^9$-THCA) is obtained from plant material by basic extraction into an aqueous solvent under pH control. After acidification, the acid is extracted back into a non-polar solvent, yielding the acid in high purity. $\Delta^9$-THCA is then converted to $\Delta^9$-THC which is further purified and combined with a carrier for pharmaceutical use. This process includes 7 different steps of extraction and 4 extra steps for the purification. It requires a lot of energy, produces a lot of contaminated water. The contaminations are mainly inorganic salts and organic waste, principally organic solvents such as heptane and isopropyl ether. To improve this production process by reduction of the number of process steps, energy consumption, water consumption and waste production, is of crucial importance. Recently, in a new patent [4] an attempt to improve the process was described. In this patent, both $\Delta^9$-THCA and $\Delta^9$-THC are extracted into an organic solvent prior to decarboxylation with aqueous base in the same solvent. Despite the obvious improvement presented, many process steps are still needed to obtain pure $\Delta^9$-THC. In our view, the ideal process would start from a plant source with the highest level of $\Delta^9$-THCA, which is then extracted, decarboxylated, and purified in the minimum number of steps, avoiding water, inorganic salts, and organic solvents.

As most cannabinoids in the plant, including $\Delta^9$-THC, are present as their acid precursor, decarboxylation in the solid phase (i.e. in the plant material) followed by extraction into a

neutral solvent, might be considered as well. Previous work on the decarboxylation of cannabinoids in the solid phase has been performed in closed reactors [5, 6], open reactors and on a glass surface [7]. However, little research has been performed to understand the kinetics and the mechanism of this solid state reaction in cannabis, despite the fact that these are crucial for scale-up.

The first section of this paper presents experimental work to determine the best reaction conditions (i.e. temperature and time) and its kinetics. Molecular modeling is then used to provide a quantitative explanation and a mechanism for this solid state reaction in accordance with the experimental data and available literature.

## 3.2 Experimental

### *3.2.1 Materials*

Methanol was HPLC grade and was purchased from J.T. Baker (Deventer, the Netherlands). Medical grade Cannabis plant material (female flower-tops) was obtained from Bureau Medicinale Cannabis (The Hague, the Netherlands). It had a $\Delta^9$-THCA content of about 18%, and virtually no free $\Delta^9$-THC. The water content was ~ 3.6%. The standards of $\Delta^9$-THC (4.2 mg.mL$^{-1}$ in Methanol – ref number 130-151205x) and $\Delta^9$-THCA (1.0 mg.mL$^{-1}$ – ref number 380-250407), with purity higher than 98%, were kindly donated by PRISNA B.V.

### *3.2.2 Method*

A sample of around 400 mg Cannabis was blended in a mixer, and heated at different temperatures in vacuum conditions for a certain time. The temperature range studied was from 90 to 140 $^{o}$C. To follow the reaction rate, a sample was taken every 5 minutes for the first hour and then every half hour until the conversion of $\Delta^9$-THCA to $\Delta^9$-THC was complete. Each solid sample was extracted with 50 mL methanol and sonicated for 15 minutes before being analysed with HPLC. Calibration lines were determined for both

$\Delta^9$-THCA and $\Delta^9$-THC. By this method samples were inherently corrected for weight loss (up to ~30% at 140 $^oC$) during thermal treatment. Balances during the experiments, based on the molalities of $\Delta^9$-THCA and $\Delta^9$-THC, are >95%, indicating that the decarboxylation process itself proceeds with ~ 100% selectivity. Some skeletal rearrangements however cannot be excluded.

### *3.2.3 HPLC analyses*

The HPLC profiles were acquired on a Chromapack HPLC system consisting of an Isos pump, an injection valve and a UV-VIS detector (model 340 – Varian). The system is controlled by Galaxie Chromatography software. The profiles were recorded at 228 nm, as absorption by the solute is at its maximum at this wavelength. The analytical column was a Vydac (Hesperia, CA) $C_{18}$, type 218MS54 (4.6 * 250 $mm^2$, 5 μm). The mobile phase consisted of a mixture of methanol-water containing 25 mM of formic acid (pH ± 3). The proportion of methanol was linearly increased from 65 to 100% over 25 minutes, and then kept constant for 3 minutes. Then the column was re-equilibrated under initial conditions for 4 minutes, so the total running time was 32 minutes. The flow rate was 1.5 $mL.min^{-1}$[8].

### *3.2.4 Molecular modeling*

The Spartan ’06 package [9] was used for all calculations. All structures underwent complete geometrical optimisation on the B3LYP level (6-31G**), starting from PM3 structures. Transition States were characterised by its unique imaginary vibrational frequency or Internal Reaction Coordinate. Thermodynamical corrections were applied; however activation energies were based on Total Energies, corrected for Zero Point Energy contributions (ZPE-contributions).

## 3.3 Results and discussion

### *3.3.1 Experimental results*

Decarboxylation is a rather common chemical reaction in which a carboxyl group splits off from a compound as carbon dioxide. The reaction shown schematically in Figure 3.1 can be induced by light or heat during e.g. storage or smoking. This reaction transforms the acidic cannabinoids to their psychoactive forms. In this article, only thermal decarboxylation will be considered. Analysis of the data leads to the conclusion that this solid state reaction surprisingly obeys a first order rate law. Major data are presented in Figure 3.2. Related $k$ values are reported in Table 3.1. The corresponding ln $k$ versus $1/T$ plots are shown in Figure 3.3. This is a straight line, described by the formula:

$$\ln k = \ln k_0 - \frac{E}{RT}$$

from which $E$ and $k_0$ are determined to be 84.8 kJ.mol$^{-1}$ and 3.7x10$^{8}$ s$^{-1}$ respectively.

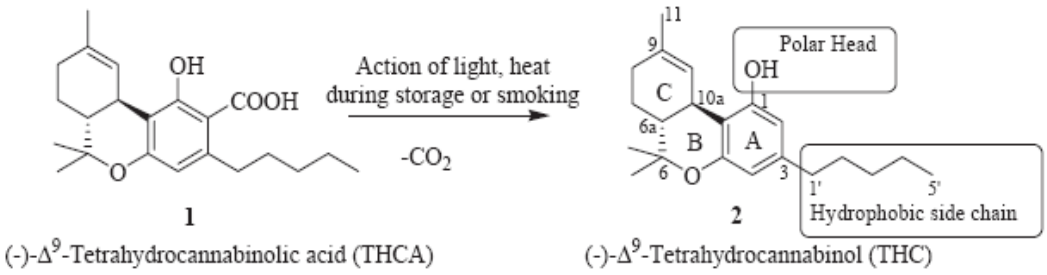



**Figure 3.1: Model of the decarboxylation reaction of Δ$^{9}$-THCA**

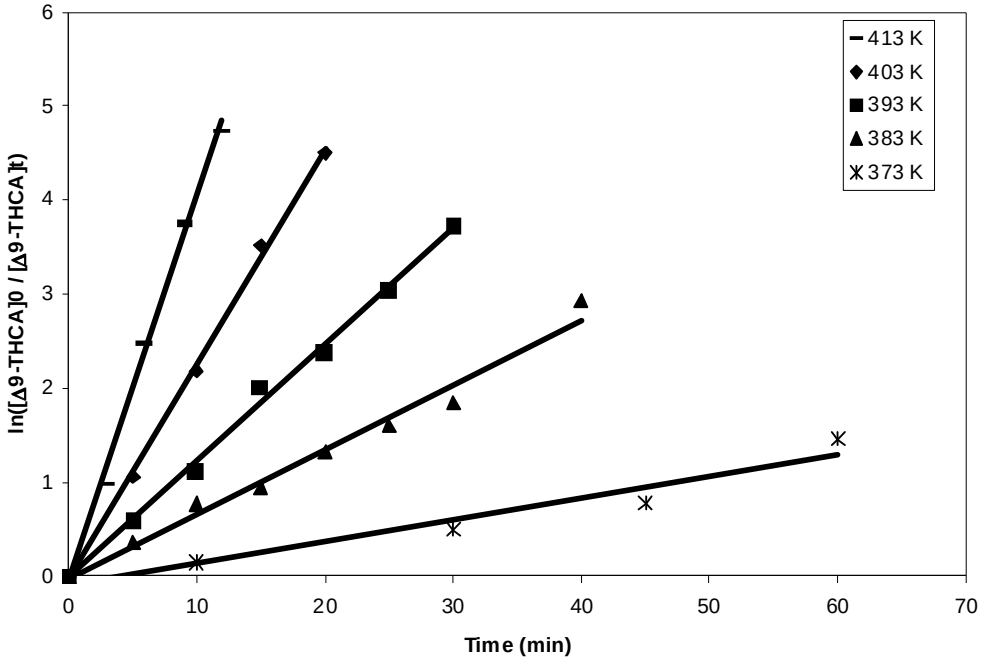



**Figure 3.2: Plot of $\ln[\Delta^9\text{-THCA}]_0/[\Delta^9\text{-THCA}]$ as a function of time at different temperatures**

**Table 3.1: Values of the constant rate *k* at different temperatures**

| $T$ (K) | $10^3\ k$ ($s^{-1}$) |
|---|---|
| 413 | 6.7 |
| 403 | 3.8 |
| 393 | 2.1 |
| 383 | 1.1 |
| 373 | 0.5 |

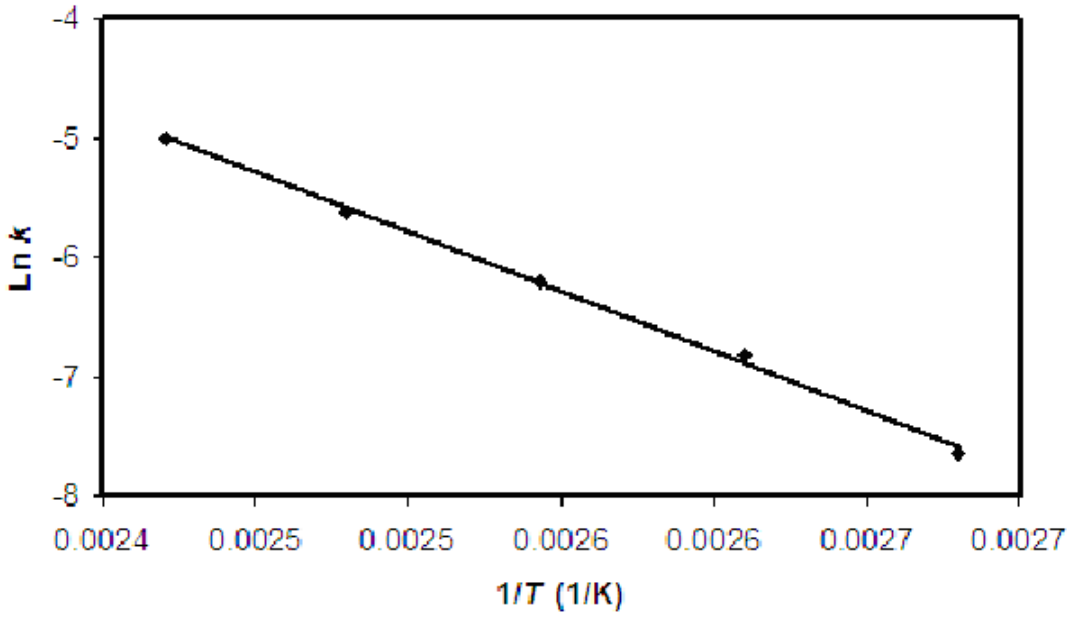



**Figure 3.3: ln *k* as a function of 1/*T* - Arrhenius' law**

### *3.3.2 Literature Results*

In the literature, only a few liquid phase thermal decarboxylation reactions of carboxylic acids, both aromatic as well as non-aromatic, can be found [10-13]. Li and Brill reported experimental activation energies for the first order decarboxylation of a series of OH substituted benzoic acids under acidic conditions, ranging from 82-97 kJ.mol$^{-1}$ for 2,4,6-trihydroxybenzoic acid and 2,3-dihydroxybenzoic acid. Their $k_0$-values range from 3.61x10$^{10}$ s$^{-1}$ to 3.58x10$^{8}$ s$^{-1}$, the latter being similar to the one observed by us [13].

In addition, by applying computational chemistry techniques (B3LYP/6-31G*), they found that intra-molecular decarboxylation of the acids, via a four membered ring Transition State, yields very high activation barriers, thus showing that a real first order process is very unlikely. The calculated activation barriers for this type of Transition State ranged from 213 kJ.mol$^{-1}$ for 2,4-dihydroxybenzoic acid, to 225 kJ.mol$^{-1}$ for 2-hydroxybenzoic acid, and to ~260 kJ.mol$^{-1}$ for 3-hydroxybenzoic acid, 3,5-dihydroxybenzoic acid, and benzoic acid itself.

They also found that the addition of one molecule of water transformed the four membered ring Transition State into a six membered ring. This caused activation barriers to go down with ~130 kJ.mol$^{-1}$, leading to values much closer to the experimental values. However, these values are still far too high, especially if it is realized that these barriers are based on the ~28 kJ.mol$^{-1}$ energetically unfavorable anti-conformer of the acid [10-13].

Recently, Chuchev and BelBruno [14] published a study on the mechanism of the decarboxylation of ortho-substituted benzoic acids, wherein they confirmed the work of Li and Brill that a single water molecule is an adequate model for an aqueous environment, but also concluded that the presence of a water molecule forces the reaction through a keto-intermediate in the case of 2-hydroxybenzoic-acid. Next this reactive intermediate intramolecularly decarboxylates to yield phenol and $CO_2$. The overall process is illustrated in Figure 3.4. However, their calculated activation barrier for the decarboxylation of salicylic acid is ~ 150 kJ.mol$^{-1}$, which is still significantly too high. Furthermore, it should be noted that the observed first order reaction can only be understood as a pseudo-first order reaction on a molecular level.

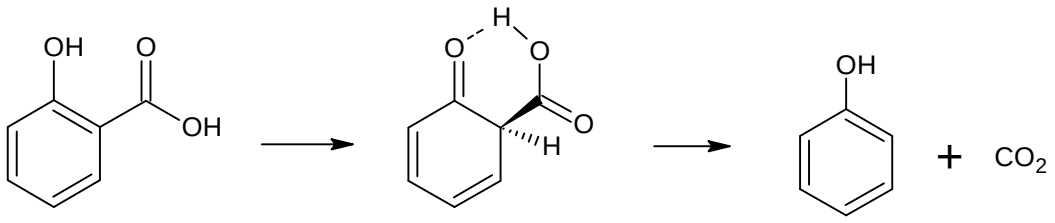



**Figure 3.4: Decarboxylation of 2-hydroxybenzoic acid via the β-keto acid pathway**

For $\Delta^9$-THCA in cannabis flos, the reaction takes place in a *solid* phase with a large amount of $\Delta^9$-THCA (18 w% = 0.57 mol.kg$^{-1}$) and a low amount of water (3.6 w% = 2.0 mol.kg$^{-1}$). The low value for $k_0$ might be explained by the fact that it is a solid-state reaction, or a catalytic process, leading to a pseudo first order process. A molecular modeling study has been performed to test this hypothesis.

### *3.3.3 Molecular Modeling Results*

$\Delta^9$-THCA is a large molecule and therefore computationally intensive with respect to memory and time. 2-hydroxybenzoic acid is the simplest model for $\Delta^9$-THCA. Furthermore both experimental and computational studies have been performed with 2-hydroxybenzoic acid. To allow a meaningful comparison between our work on $\Delta^9$-THCA, and the existing literature on 2-hydroxybenzoic acid, the different options were investigated for 2-hydroxybenzoic acid first.

Starting from the work of Li and Brill [13] , and Chuchev and BelBruno [14] we were able to confirm their computational work with respect to the geometry of the Transition States both for the direct uncatalyzed ones and for the ones catalyzed by one molecule of water. The geometries look very similar, and selected bond lengths are the same within 0.01 Å.

Next, a model was developed in which an organic acid was used as a catalyst to assist in the decarboxylation reaction. This may allow adaptation to the actual acid strength of the catalyst or implicitly the pH of the environment, while avoiding computationally intensive calculations. A disadvantage might be that thermodynamic corrections become meaningless in most cases, except for the ZPE. However, this is already the case, particularly for the entropy contributions, as experiments were carried out in the liquid and solid phase, but not in the gas phase.

To choose a good model catalyst for the decarboxylation reaction, several acids were used and compared in Table 3.2, for the case of 2-hydroxybenzoic acid. For $\Delta^9$-THCA the work was limited to formic acid and trifluoroacetic acid. As it can be seen in Table 3.2, the differences in activation energies for 2-hydroxybenzoic acid in both pathways with acetic acid, formic acid and trifluoroacetic acid are within 5 kJ.mol$^{-1}$. Thus the acid strength of the catalyst does not seem to be a large discriminator. Using formic acid as a

model catalyst, two different Transition States could be located, both leading to the previously mentioned keto-intermediate, as seen in Figure 3.5.

**Table 3.2: Calculated activation energies of salicylic acid and $\Delta^9$-THCA with different acids as catalyst**

| Acid catalyst | $E_a$ 2-hydroxybenzoic acid ($kJ.mol^{-1}$) direct keto-enol | $E_a$ 2-hydroxybenzoic acid ($kJ.mol^{-1}$) indirect keto-enol | $E_a$ $\Delta^9$-THCA ($kJ.mol^{-1}$) direct keto-enol |
|---|---|---|---|
| Acetic acid | 105 | 89 | Not determined |
| Formic acid | 104 | 93 | 81, 58 [indirect] |
| Trifluoroacetic acid | 100 | 88 | 71 |

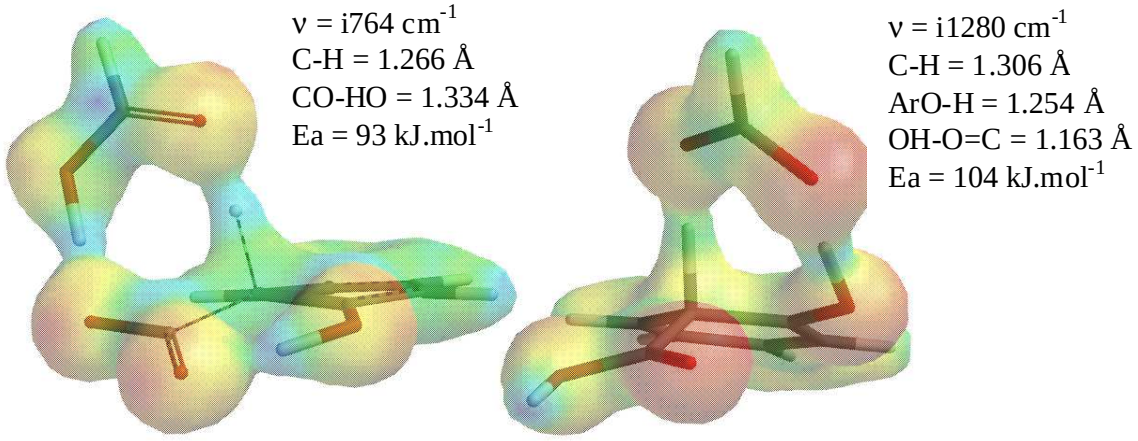



**Figure 3.5: The two Transition States for the formic acid catalyzed decarboxylation of 2-hydroxybenzoic acid**

The activation barrier with a value of 93 $kJ.mol^{-1}$ resembles the geometry of the Transition State proposed by Chuchev and BelBruno [14], with the hydrogen of the acid of the substrate in anti-position. The reaction pathway for that reaction, presented in [14], shows in fact a three proton transfer process, starting with protonation of the α-C next to the COOH-group, followed by the transfer of the proton in anti-position of the substrate COOH-group to the catalyst, and finally proton transfer of the phenol group to the carboxylate group of the substrate. This mechanism will be referred to as indirect keto-enol pathway.

The value of 104 kJ.mol$^{-1}$ resembles a direct keto-enol pathway. Figure 3.6 shows the IRC-plots of the formation of the keto-isomer of 2-hydroxybenzoic acid with formic and trifluoroacetic acid as catalyst in the direct keto-enol pathway. The distance between the phenolic O-H atoms was taken as a measure for the reaction coordinate. The reaction starts from the phenol and ends with the keto-isomer. The geometries of the Transition States change only slightly. In both cases quasi-simultaneous proton transfer of the acid catalyst to the α-C of the substrate, and of the phenol group back to the acid catalyst, are rate determining.

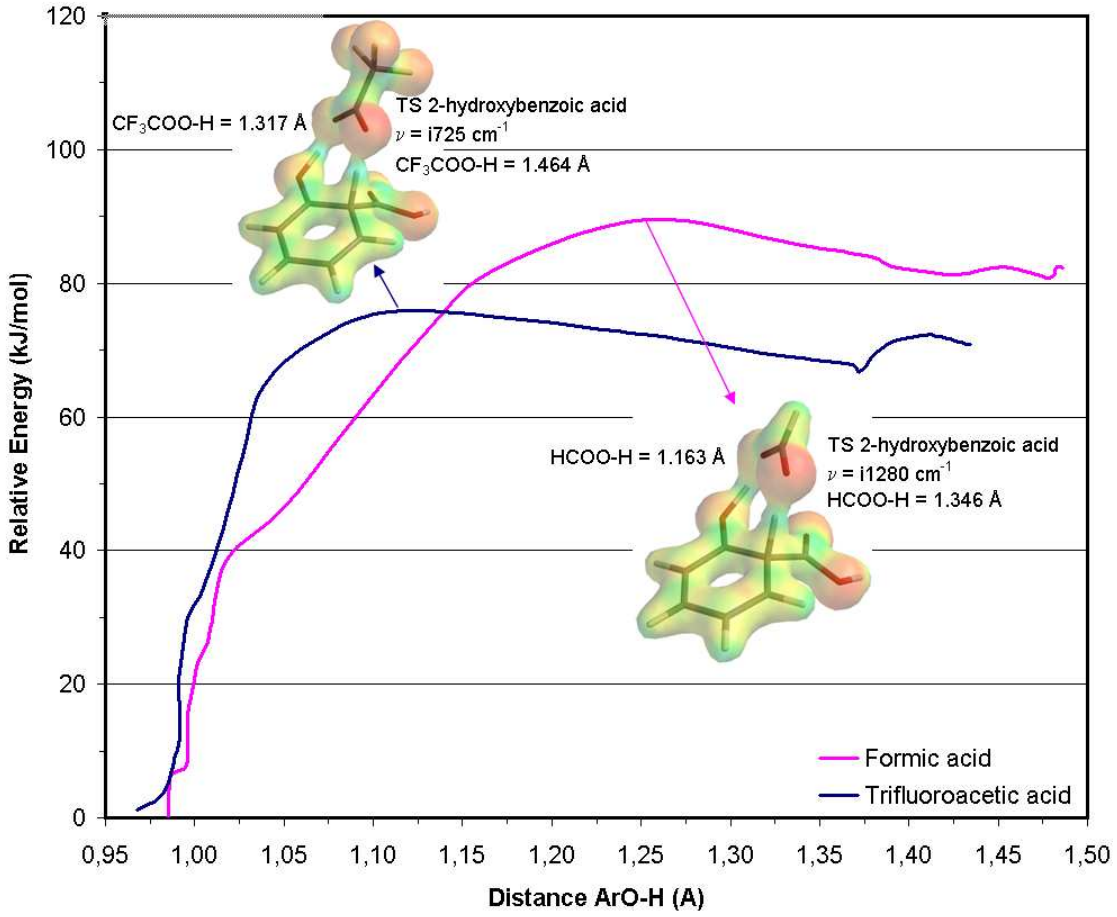



**Figure 3.6: IRC's of the formation of the keto-isomer of 2-hydroxybenzoic acid decarboxylation catalyzed by trifluoroacetic acid and formic acid via the direct keto-enol pathway**

For $\Delta^9$-THCA, the activation barrier of the direct keto-enol route with formic acid as catalyst (81 kJ.mol$^{-1}$) is close to the experimental value (85 kJ.mol$^{-1}$). However, the ones with trifluoroacetic acid (71 kJ.mol$^{-1}$) and the indirect keto-enol pathway (58 kJ.mol$^{-1}$) are far too low. Figure 3.7 shows the IRC and the Transition State of the first step of the formic acid catalyzed decarboxylation of $\Delta^9$-THCA. Figure 3.8 shows the overall reaction energy profile of the entire reaction, including the second step, the intramolecular proton transfer of the acid to the keto-function.

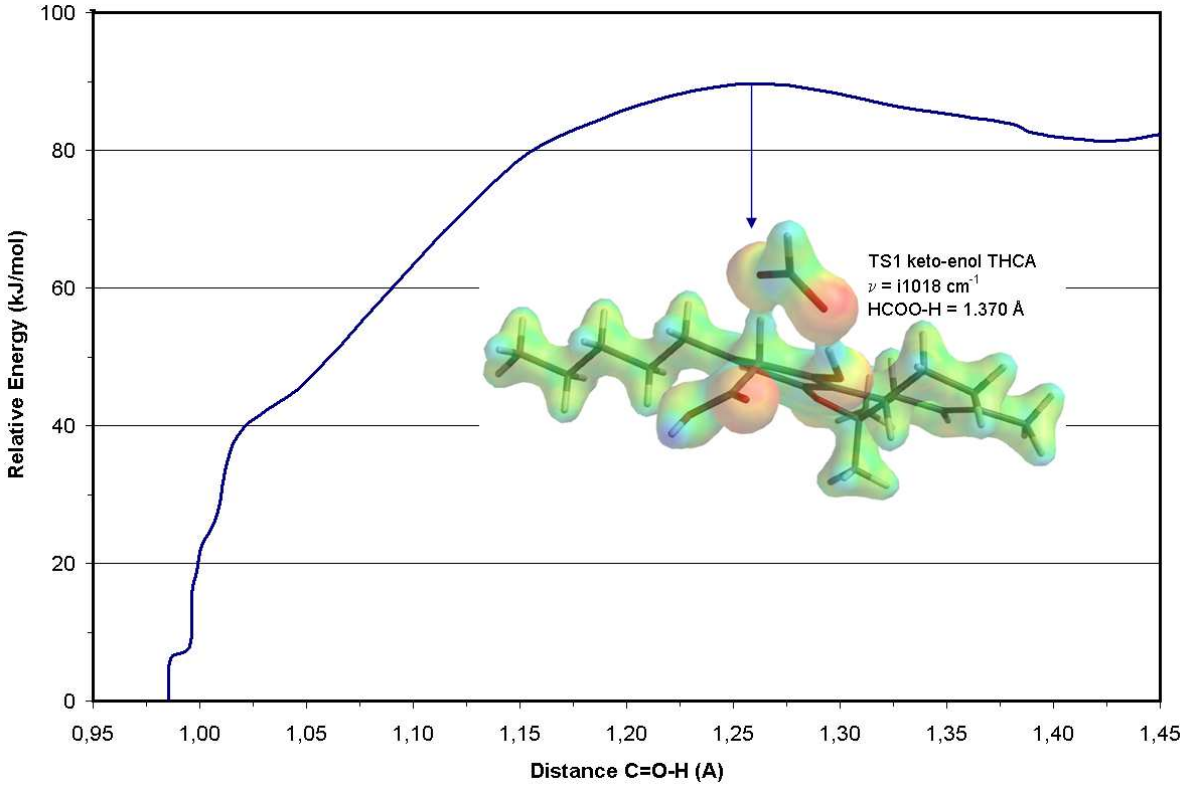



**Figure 3.7: IRC of $\Delta^9$-THCA decarboxylation catalyzed by formic acid via the direct keto-enol pathway**

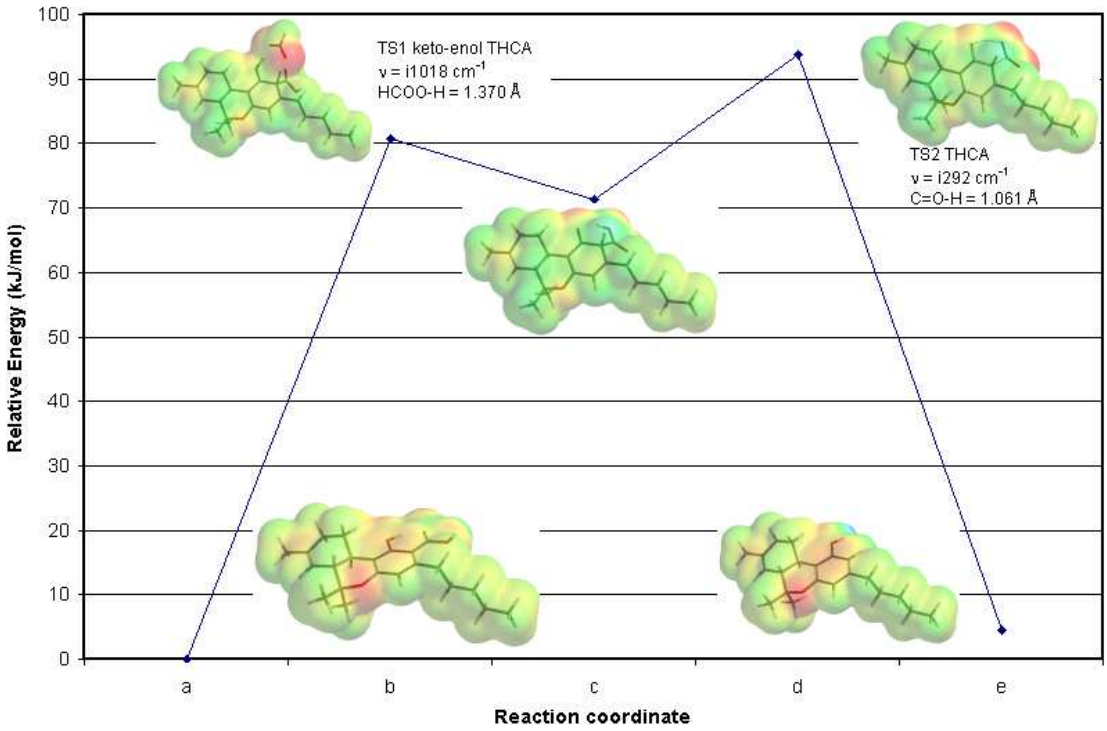



**Figure 3.8: Energy Profile of formic acid catalyzed decarboxylation of $\Delta^9$-THCA**

### *3.3.4 Discussion*

Aliphatic and aromatic acids are usually present [15] as plant constituents in cannabis. Inspired by the results of molecular modeling, the presence of acids other than $\Delta^9$-THCA was verified experimentally. A sample of around 400 mg of cannabis was blended in a mixer, and extracted with distilled water after sonication for 10 minutes. The pH of the resulting aqueous solution was 6.1. A sample of 1600 mg of cannabis, yielded an aqueous solution with pH = 5.5. Under these conditions, $\Delta^9$-THCA does not dissolve into water but short chain carboxylic acids do. Thus, acetic acid or formic acid not only can be used as a *model* for acid catalysis, but might be a realistic case from an experimental point of view as well. Furthermore, it offers a plausible explanation for the low value of $k_0$, as the experimental acidity is low.

To get a better overall understanding of the two different mechanistic options in acid catalyzed decarboxylation, Table 3.3 shows the comparison of experimental values with

computational results obtained for a series of 2-hydroxybenzoic acids with formic acid as catalyst. Experimental data are scarce but, fortunately, well documented [13,14]. For the decarboxylation of 2-hydroxybenzoic acid two experimental activation energies are reported: 97.4 kJ.mol$^{-1}$ in catechol (weak acid), and 92 kJ.mol$^{-1}$ as an average of two distinct values: 91.4 kJ.mol$^{-1}$ in an HCl-solution of pH = 1.3, and 92.7 kJ.mol$^{-1}$ in an HCl-solution of pH = 2.7, thus showing a marked influence of both solvent and pH. A similar observation can be made for 2,6-dihydroxybenzoic acid. Here 3 values are reported: 111.1 kJ.mol$^{-1}$ in catechol, 92.7 kJ.mol$^{-1}$ at pH = 1.4 and 100.7 kJ.mol$^{-1}$ at pH = 2.0. Again, the dependence of the experimental activation energy on solvent type and pH is remarkable.

**Table 3.3: Activation Energies of substituted 2-hydroxybenzoic acids with formic acid as catalyst.**
**[a] direct keto-enol pathway, [b] indirect keto-enol pathway, [c]direct keto-enol pathway with one phenolic OH group not forming an hydrogen bridge with the acid function**

| Compound | $E_a$-exp (kJ.mol$^{-1}$) | $E_a$-comp (kJ.mol$^{-1}$) |
|---|---|---|
| 2-hydroxybenzoic acid | 97 [10], 92 [13] | 104[a], 92[b] |
| 2,6- dihydroxybenzoic acid | 111 [10], 101, 92 [13] | 114[b], 102[c], 92[a], |
| $\Delta^9$-THCA | 85 | 81[a] |

As can be seen from Table 3.3, the lowest value for the activation energy of 2-hydroxybenzoic acid, obtained experimentally in a strongly acidic environment, corresponds computationally with the indirect keto-enol pathway yielding an activation barrier of 92 kJ.mol$^{-1}$. The latter requires the presence of a proton (in anti-position) of the substrate acid function. Under strongly acidic conditions this requirement is fulfilled. Under less acidic conditions this is not the case, and then the direct keto-enol pathway comes into play, resulting in an activation barrier of 104 kJ.mol$^{-1}$.

The case of 2,6-dihydroxybenzoic acid is more complicated. It is a significantly stronger acid than 2-hydroxybenzoic acid, so the requirements for the indirect keto-enol pathway are no longer fulfilled in an HCl-solution of pH = 1.4. The direct keto-enol pathway leads to an activation barrier of 92 kJ.mol$^{-1}$, close to the experimental value. The next experimental value of 101 kJ.mol$^{-1}$ at pH = 2.0, can be understood as a loss of

coordination of one of the phenolic groups to the adjacent acid group due to the higher pH. The computation for these systems gives an activation barrier of 102 kJ.mol$^{-1}$. With respect of the experimental work in catechol, computations with either formic acid or catechol itself as an acid catalyst, indirect keto-enol pathways lead to an activation barrier of 114 kJ.mol$^{-1}$ close to the experimental value. The indirect pathway here is rationalized by the fact that 2,6-dihydroxybenzoic acid in catechol will stay intact. Furthermore, it shows that formic acid can even act as a reasonable model for catechol.

From the computational results obtained it would be tempting to speculate what the activation barrier would become if strongly acidic conditions were applied in the case of $\Delta^9$-THCA. However, the application of strong acids, containing halogens or sulfur would not contribute to the sustainability of the overall process.

## 3.4 Conclusions

Decarboxylation of $\Delta^9$-THCA can be described as a pseudo first order reaction catalyzed by formic acid, as a model for short chain organic acids present in the flowers of the cannabis plant. The presence of such acids was verified in a series of extraction experiments. Also, the computational idea of catalysis by water to catalysis by an acid, put forward by Li and Brill, and Churchev and Belbruno was extended, and a new direct keto-enol route was found. This route offers the best explanation for the experimental results obtained with $\Delta^9$-THCA, both with respect to the activation barrier and the pre-exponential factor. However both routes can play a role, depending on the exact experimental conditions, as an analysis of available experimental and computational results shows.

## Acknowledgments

Financial support by STW (project no LFA 7426) is gratefully acknowledged. The authors would also like to thank Pablo Cabeza Perez for his experimental contribution to this work.

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# 4

# Solubility of Delta-9-tetrahydrocannabinol in supercritical carbon dioxide: Experiments and modeling

Content published in *J. Supercrit.Fluids* 52 (2010) 6-10.

# 4


## *Abstract*

*The solubility of $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC) in supercritical carbon dioxide has been determined at 315, 327, 334 and 345 K and in the pressure range from 13.2 to 25.1 MPa using an analytical method with a quasi-flow apparatus. Prior to performing these measurements, the method was validated by measuring anthracene solubilities and comparing these with literature values. The molar solubility for $\Delta^9$-THC ranged from 0.20 to 2.95x10$^{-4}$. The data were correlated using the Peng-Robinson equation of state in combination with quadratic mixing rules. Deviations between calculated results and the experimental data ranged from 4.1 to 13.3 % absolute average relative deviation (AARD).*

# 4. Solubility of Delta-9-tetrahydrocannabinol in supercritical carbon dioxide: Experiments and modeling

## 4.1 Introduction

At present, there is a growing interest in natural medicinal compounds. Cannabis is one of the oldest medicinal plants known [1]. The major compound from cannabis, $\Delta^9$-THC ((-)-$\Delta^9$-tetrahydrocannabinol), has been legally registered for medical application in several countries and cannabis preparations are being developed as medicines. Also, $\Delta^9$-THC is often used as a standard for pharmacological studies. The availability of the various cannabinoids as pure compounds is of great importance for these studies and for the development of new medicines.

$\Delta^9$-THC can be extracted directly from cannabis by organic solvents (e.g. hydrocarbons and alcohols) with a yield exceeding 90% [2]. However, these solvents are flammable and many of them are toxic. Supercritical Fluid Extraction (SFE) with carbon dioxide ($CO_2$) is an alternative promising technique. There are no flammability or toxicity issues, solvent removal is simple and efficient, and the extract quality can be well-controlled. This green solvent is widely used to extract natural components, including pharmaceutical molecules [3-8].

The application of SFE to extract $\Delta^9$-THC from cannabis requires solubility data, which is currently lacking. In this work, the solubility of $\Delta^9$-THC in supercritical $CO_2$ has been determined. Furthermore, the experimental data of $\Delta^9$-THC have been correlated using the Peng-Robinson equation of state (PR EoS).

## 4.2 Experimental

### *4.2.1 Chemicals*

$CO_2$ was purchased from Hoek Loos (quality 2.7). Anthracene wih a purity of 99+% was purchased from Sigma-Aldrich. Methanol and tetrahydrofuran of HPLC reagent grade were purchased from J.T. Baker. $\Delta^9$-THC with purity higher than 96.5% was kindly donated by Echo Pharmaceuticals B.V, Nijmegen, the Netherlands. The material was used without further purification. The molecular structure of $\Delta^9$-THC is given in Figure 4.1.

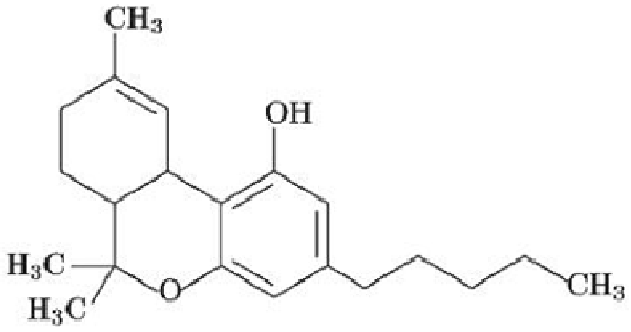


**Figure 4.1: Molecular structure of $\Delta^9$-THC**

### *4.2.2 Apparatus and method*

For the solubility experiments, a quasi-flow apparatus was used, which is depicted schematically in Figure 4.2. The apparatus was designed to perform experiments up to 35 MPa and in the temperature range of 293 – 423 K. The cell was composed of a sample vessel made of stainless steel, a micro pump (Micropump INC, model 380) to circulate the $CO_2$, a pressure sensor (EFE – type VLE 700) with an accuracy of ± 0.05 MPa and a thermocouple PT-100 with an accuracy of ± 0.1 K. All the components were placed in an oven (Memmert – type VLE 700) to keep the temperature constant. The system loop contained an HPLC to measure the concentration of the dissolved component in $CO_2$. All the tubing was insulated to minimize heat losses. A back pressure regulator was placed at the end of the HPLC to ensure a maximum pressure decrease in the system of less than

0.2% due to volume losses when a sample was taken. An ISCO pump (model 260 D) was used to fill the system with $CO_2$. The internal volume in which the sample and $CO_2$ circulated was about 8 mL.

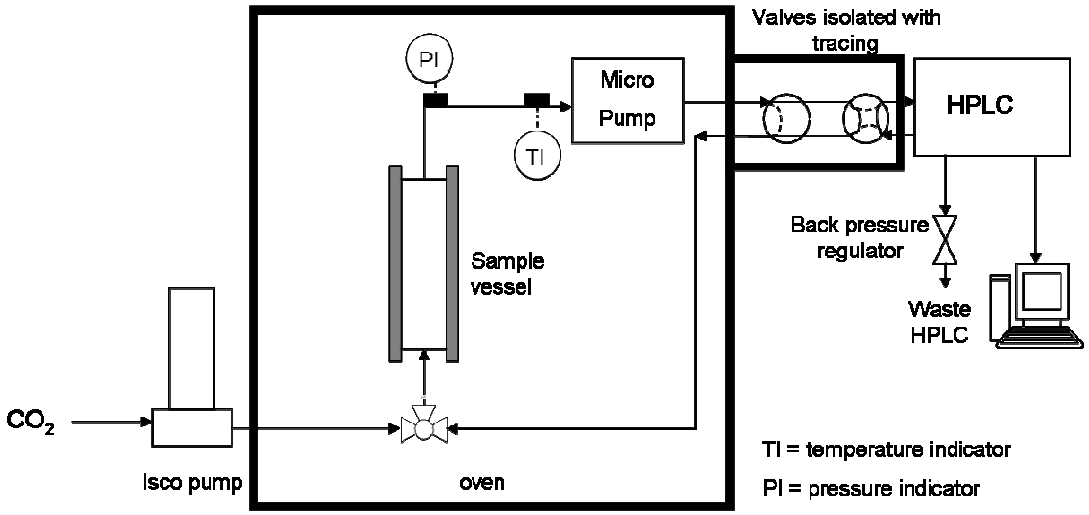


**Figure 4.2: Schematic drawing of the solubility cell**

For the validation of the system, anthracene was used. At the start of an experiment, a measured amount of anthracene was put into the sample cylinder and the system was closed. For the solubility experiments with $\Delta^9$-THC, $\Delta^9$-THC was first dissolved in methanol to facilitate its transfer ($\Delta^9$-THC is a viscous and sticky liquid). The liquid sample was then transferred to the sample cylinder whereafter the solvent was evaporated with a vacuum pump (RNF Lab) for 1 hour at ambient temperature to have complete evaporation of methanol. Subsequently, the pump was disconnected and the system was closed.

After the system was closed, the oven was set at the desired temperature. After the preset temperature had been reached, the system was filled with $CO_2$ until the desired pressure was reached. When the conditions were stable, the $CO_2$ circulation over the sample vessel was started. A sample for HPLC analysis was taken after 2 hours and successively every 30 minutes. When the measured concentration difference was less than $0.09 \times 10^{-4}$ between two subsequent analyses, with a pressure and temperature differences less than 0.05 MPa

and 0.2 K respectively, it was assumed that equilibrium was reached, and the concentration measured was recorded as the solubility.

### *4.2.3 High-Performance Liquid Chromatography*

The HPLC profiles were acquired using a Chrompack HPLC system consisting of an Isos pump, an injection valve and a UV-VIS detector (model 340 – Varian). The HPLC set-up is illustrated in Figure 4.3. The system was controlled by Galaxie Chromatography software. The profiles were recorded at 228 nm, as absorption by the solute is at its maximum at this wavelength.

To detect anthracene, an Inertstil ODS-2 (4.6 x 250mm$^2$, 1µm) column was used. The mobile phase consisted of pure methanol. The flow rate was 1 mL.min$^{-1}$ and the total running time was 10 minutes.

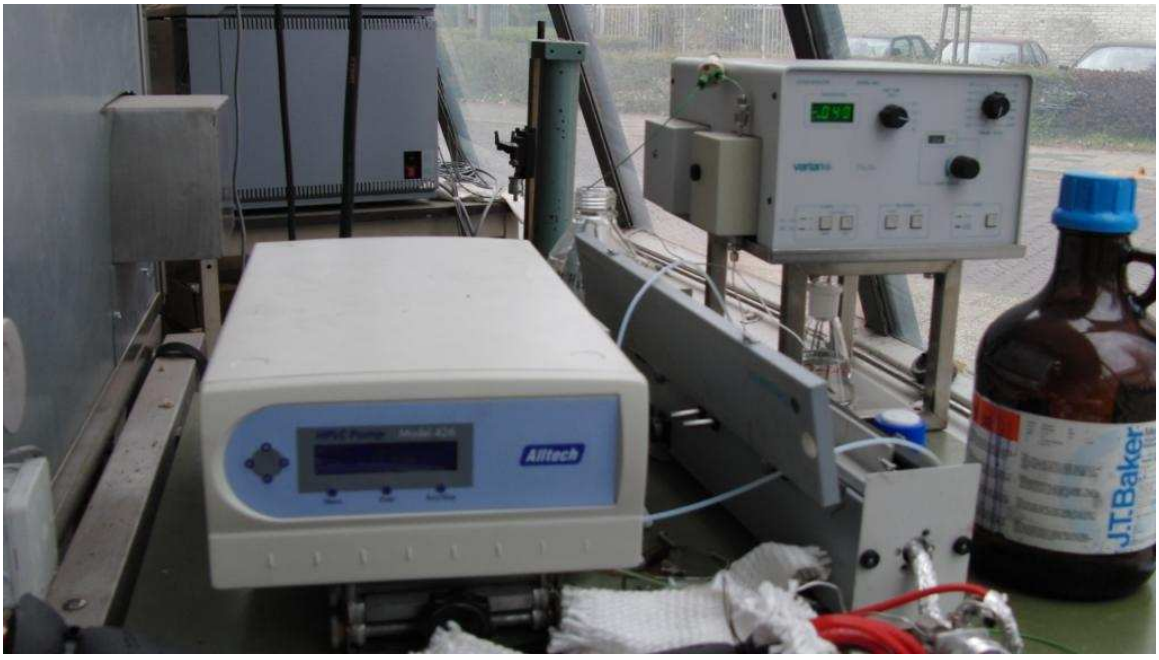


**Figure 4.3: HPLC photo**

To detect $\Delta^9$-THC, the analytical column was a Vydac (Hesperia, CA) $C_{18}$, type 218MS54 (4.6 x 250 mm$^2$, 5 µm). The mobile phase consisted of a mixture of methanol,

distilled water and tetrahydrofuran (v/v/v = 10/4/1). The flow rate was 1.5 $mL.min^{-1}$ and the total running time was 14 minutes.

As the peak areas of the components calculated from the chromatograms are linearly related to their amount by the Lambert-Beer law, their concentrations can be determined using a calibration line. This was done by using 4 standards with concentrations in the range 0 – 10 $mg.mL^{-1}$. Each standard was injected at least three times and an average was taken to perform the linear regression. The linear regression coefficient of the calibration curve was equal to 0.9996, as shown in Figure 4.4.

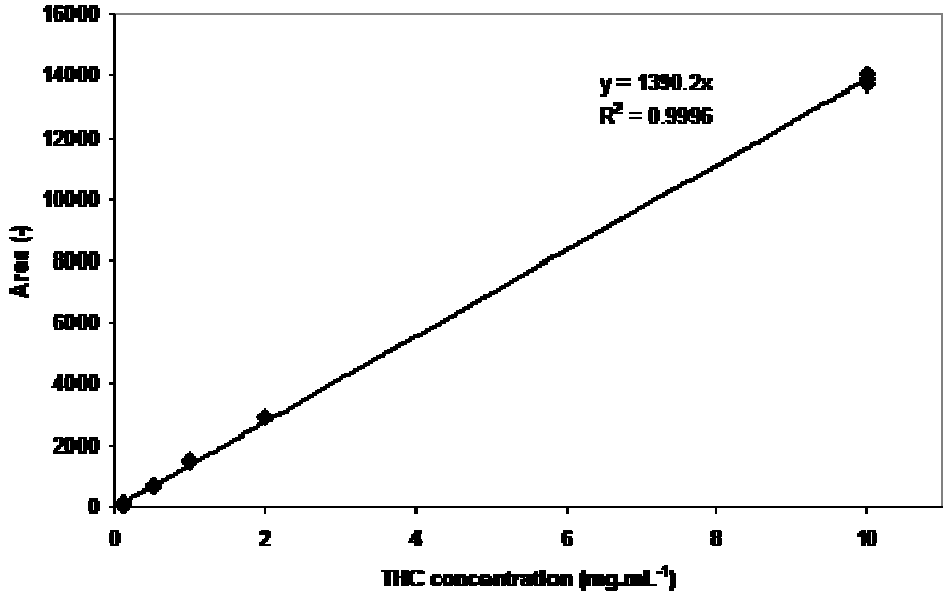


**Figure 4.4: HPLC calibration line for Δ9-THC**

Figure 4.5 shows an example of 2 HPLC chromatograms of $\Delta^9$-THC. The chromatogram with one peak at 11 minutes is a standard sample of $\Delta^9$-THC, whereas the second chromatogram having 2 peaks represents a sample of $\Delta^9$-THC dissolved in SC $CO_2$. By comparing these two chromatograms, the influence of $CO_2$ can be seen. It gave a peak at around 3 minutes, which did not interfere with the $\Delta^9$-THC peak. Therefore, HPLC is an efficient analytical tool to measure the solubility of $\Delta^9$-THC in supercritical $CO_2$.

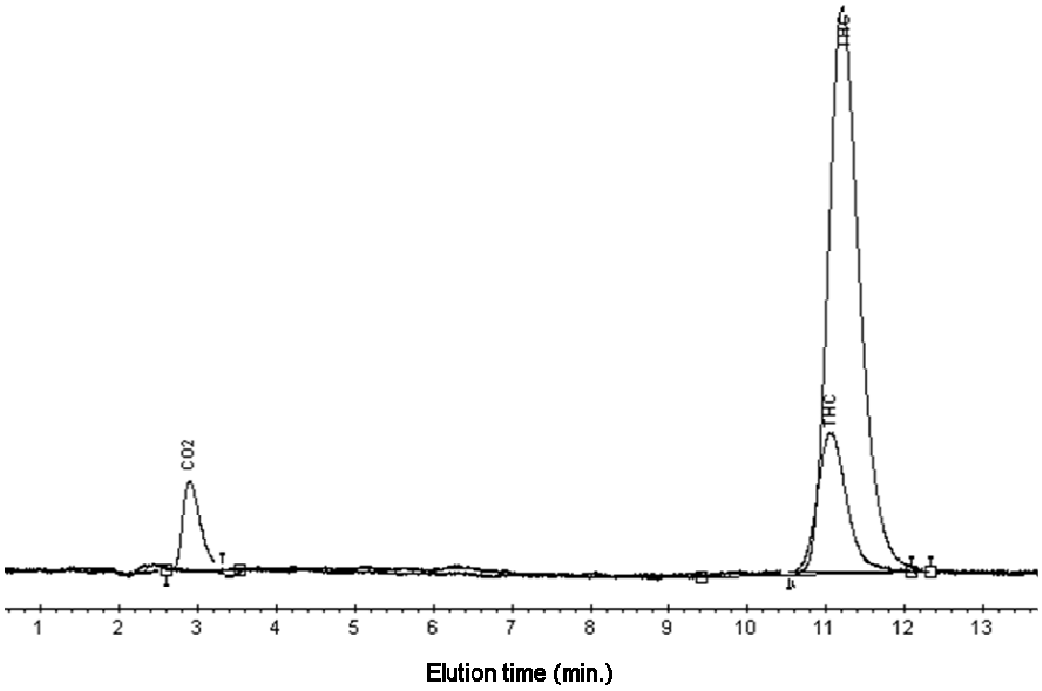


**Figure 4.5: HPLC chromatogram for a standard Δ9-THC sample overlaid with Δ9-THC in $CO_2$ at 327 K – 15.2 MPa**

## 4.3 Results and discussion

### *4.3.1 Experimental results*

In order to determine the suitability of the equipment and the method used, the solubility of anthracene has been measured from 12 to 26 MPa at 322 K, and compared with literature data [9-11]. Anthracene has been chosen for several reasons: experimental data are available in literature and are in the same range to be expected in the range of $\Delta^9$-THC. Moreover, the used experimental procedure is the same for solid and liquid components. Each experimental point was measured four times; the standard deviation was 0.008 x $10^{-4}$. As shown in Figure 4.6, the experimental data are comparable to the data taken from literature. Therefore, it was concluded that the equipment and method can be used to determine the solubility of other compounds such as $\Delta^9$-THC.

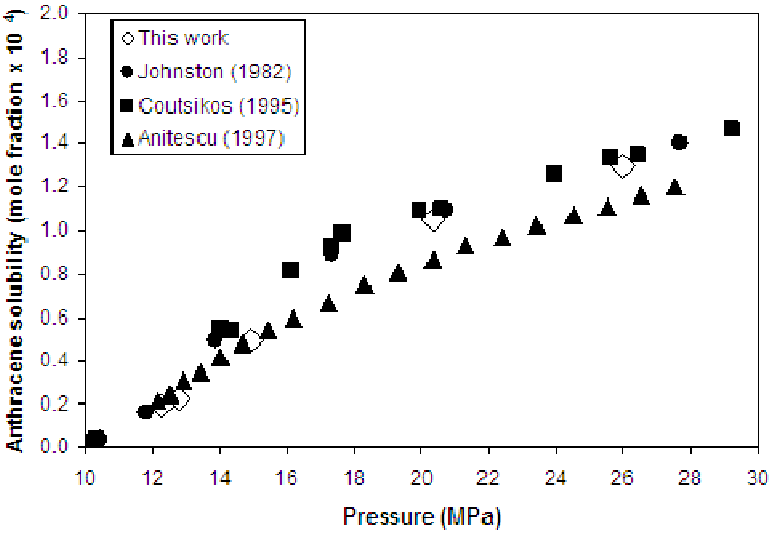


**Figure 4.6: Anthracene solubility as a function of pressure at 322K - comparison of the experimental results (white points) with those taken from literature (black points) [9-11]**

The experimental solubility data of $\Delta^9$-THC in $CO_2$ are shown in Table 4.1. The maximum standard deviation was 0.0015 x $10^{-4}$. The lowest measured solubility was 0.20 x $10^{-4}$ at 315 K and 13.2 MPa. Below this pressure and/or temperature, the solubility was too low to be measured accurately. However, this minimum solubility is higher than the lowest measured solubility of viscous liquid components in supercritical $CO_2$, using the synthetic method with the Cailletet apparatus [12-14]. The minimum molar solubility that can be determined with the Cailletet equipment is in the order of $3x10^{-4}$ which lies above the $\Delta^9$-THC solubility in $CO_2$. Therefore, for compounds with a low solubility, the quasi-flow equipment used here is more suitable than a Cailletet set-up.

**Table 4.1: Solubility of $\Delta^9$-THC in supercritical $CO_2$ at different temperatures and pressures**

| $T$ = 315 K | | | $T$ = 327 K | | | $T$ = 335 K | | | $T$ = 345 K | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $P$ (MPa) | $10^4$ $y$ - | $10^4$ Exp. error - | $P$ (MPa) | $10^4$ $y$ - | $10^4$ Exp. error - | $P$ (MPa) | $10^4$ $y$ - | $10^4$ Exp. error - | $P$ (MPa) | $10^4$ $y$ - | $10^4$ Exp. error - |
| 13.2 | 0.20 | ± 0.01 | 14.0 | 0.33 | ± 0.02 | 13.7 | 0.32 | ± 0.02 | 14.6 | 0.98 | ± 0.05 |
| 19.4 | 0.65 | ± 0.03 | 14.1 | 0.35 | ± 0.02 | 15.4 | 0.72 | ± 0.04 | 17.9 | 1.59 | ± 0.08 |
| 20.3 | 0.65 | ± 0.03 | 14.8 | 0.45 | ± 0.02 | 17.8 | 1.57 | ± 0.08 | 20.7 | 2.09 | ± 0.10 |
| 23.0 | 0.69 | ± 0.03 | 15.1 | 0.57 | ± 0.03 | 20.0 | 1.69 | ± 0.08 | 22 | 2.95 | ± 0.15 |
| 25.1 | 0.83 | ± 0.04 | 15.4 | 0.56 | ± 0.03 | 22.1 | 2.33 | ± 0.12 | | | |
| | | | 15.8 | 0.45 | ± 0.02 | 23.3 | 2.78 | ± 0.14 | | | |
| | | | 16.3 | 0.65 | ± 0.03 | | | | | | |
| | | | 16.8 | 0.69 | ± 0.03 | | | | | | |
| | | | 17.6 | 0.68 | ± 0.03 | | | | | | |
| | | | 17.8 | 0.71 | ± 0.04 | | | | | | |
| | | | 18.2 | 0.68 | ± 0.03 | | | | | | |
| | | | 20.0 | 1.35 | ± 0.07 | | | | | | |
| | | | 22.0 | 1.42 | ± 0.07 | | | | | | |
| | | | 23.5 | 1.99 | ± 0.10 | | | | | | |

As shown for the isotherms in Figure 4.7, the solubility increases with pressure. At constant pressure, two observations can be made: (i) at pressures lower than approx. 15 MPa, the solubility decreases with increasing temperature; (ii) at pressures higher than approx. 15 MPa, there is a reverse tendency. This particular pressure region has been reported as the crossover region, i.e. the crossing of solubility lines [15]. This behavior has been observed before with several drug components [16].

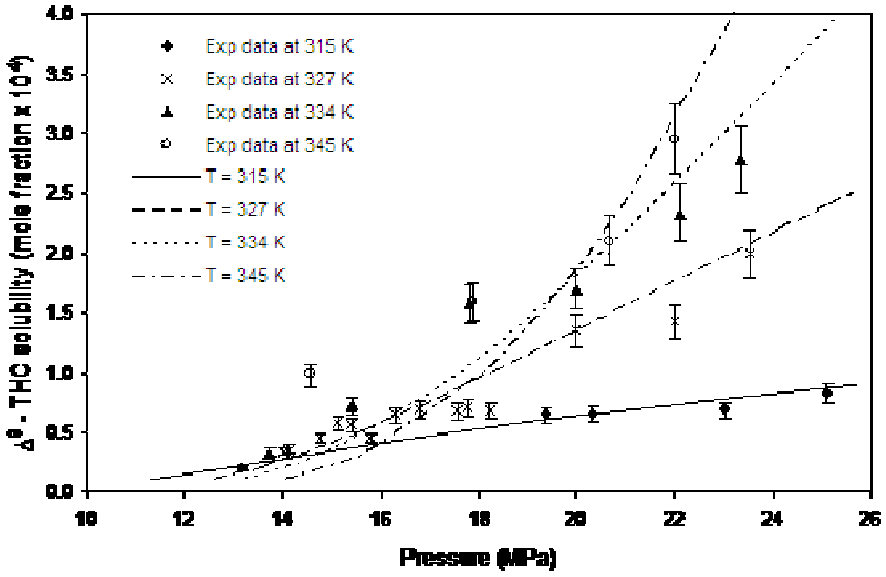


**Figure 4.7: $\Delta^9$-THC solubility in SC $CO_2$ - experimental results (points) and results from modelling with PR EoS (lines)**

### *4.3.2 Data correlation*

The experimental data were correlated with the PR-EoS [17, 18]:

$$P = \frac{RT}{V-b} - \frac{a}{V^2 - 2Vb - b^2} \tag{4.1}$$

where *a* and *b* are parameters calculated from the quadratic mixing rule. The attractive term is given by:

$$a = \sum_i \sum_j y_i y_j (a_i a_j)^{0.5} (1 - k_{ij}) \tag{4.2}$$

where $k_{ij}$ is the binary interaction parameter to be optimized and

$$a_i = 0.45724 \frac{R^2 T_{ci}^2}{P_{ci}} \alpha_i \tag{4.3}$$

where

$$\alpha_i = \left[1 + m(\omega_i)(1 - T_{ri}^{1/2})\right]^2 \tag{4.4}$$

where

$$m(\omega_i) = 0.37464 + 1.54226\omega_i - 0.26992\omega_i^2 \quad (4.5)$$

The covolume parameter is given by:

$$b = \sum_i y_i b_i \quad (4.6)$$

Where

$$b_i = 0.07780 \frac{RT_{ci}}{P_{ci}} \quad (4.7)$$

To use these equations, the critical properties ($T_c$, $P_c$) and acentric factor (ω) of the components are necessary. However, critical properties of $\Delta^9$-THC are not available in literature. Therefore, these properties have been estimated using the Joback method [19]. The values for $CO_2$ were taken from the PE database [20]. The critical properties and acentric factors of $\Delta^9$-THC and $CO_2$ are shown in Table 4.2.

**Table 4.2: Critical parameters and acentric factors used in the PR EoS**

| Component | $T_c$ (K) | $P_c$ (MPa) | $\omega$ |
|---|---|---|---|
| $CO_2$ | 304.4 | 7.38 | 0.225 |
| $\Delta^9$-THC | 988 | 1.95 | 0.882 |

The binary interaction parameter $k_{ij}$ has been calculated from the experimental pressure at each point by minimizing the relative difference between experimental and calculated pressure [17]. This minimization can be expressed by the absolute average relative deviation (AARD (%)), as described by the following equation:

$$AARD\ (\%) = \frac{100}{n} \sum_1^n \frac{\left| P_i^{\exp} - P_i^{calc} \right|}{P_i^{\exp}}. \quad (4.8)$$

Here, $n$ is the number of data experiments at each temperature, $P_i^{exp}$ is the experimental pressure for the experiment $i$, whereas $P_i^{calc}$ is the estimated value. The *AARD* values at different temperatures are presented in Table 4.3. As can be seen in this table, $k_{ij}$

decreases linearly with temperature increase. The regression coefficient had a value of 0.9963.

**Table 4.3: Binary interaction parameters for the $CO_2$ + $\Delta^9$-THC binary system**

| $T$ (K) | $k_{ij}$ | AARD (%) |
|---|---|---|
| 315 | 0.137 | 13.3 |
| 327 | 0.112 | 4.1 |
| 334 | 0.095 | 5.7 |
| 345 | 0.076 | 8.2 |

The AARD ranges from 4.1% to 13.3 %. Therefore, it can be concluded that in general the PR EoS is a good tool to correlate the solubility of $\Delta^9$-THC in supercritical $CO_2$. However, at 345 K and low pressure, the PR simulation curve is much lower than the experimental solubility data. This can be explained by the limits of the PR model for this application; it is more accurate at higher pressures and when the temperature is higher.

## 4.4 Conclusions

For low solubilities (< $2x10^{-4}$ in supercritical $CO_2$), the quasi-flow set-up is better suited for solubility measurements than a Cailletet set-up.

The solubility of $\Delta^9$-THC in supercritical $CO_2$ has been measured in the temperature range 315-345 K and pressures up to 26 MPa. The solubility of $\Delta^9$-THC increases with the $CO_2$ pressure. This solubility decreases with the temperature up to about 15MPa. Above this crossover region, this trend reverses, i.e. a higher temperature is accompanied by a higher solubility. For feasible extraction conditions, e.g. with solubility above $1x10^{-4}$, the pressure should be above about 20 MPa and the temperature should be higher than 325 K. The experimental data are adequately represented by the PR EoS.

## Acknowledgements

Financial support by STW (project no LFA 7426) is gratefully acknowledged. The authors would also like to thank Sytze Elzinga from Echo Pharmaceuticals B.V. for providing pure $\Delta^9$-THC. Michel van den Brink is warmly thanked for his support during the experiments. Angel Martin is acknowledged for his help and advice in the modeling. Erin McGarrity is also thanked for kindly editing the manuscript.

## Nomenclature

| | |
|---|---|
| *a* | Attractive term of the PR EoS |
| *b* | Covolume |
| *k* | Binary interaction parameter |
| *P* | Pressure (MPa) |
| *R* | Gas constant ($J.mol^{-1}.K^{-1}$) |
| *T* | Temperature (K) |
| *V* | Volume ($dm^3.mol^{-1}$) |
| *y* | Solubility in the gas phase |

*Greek letters*

| | |
|---|---|
| $\alpha$ | Temperature-dependant equation of state parameter |
| $\omega$ | Acentric factor |

*Sub / superscripts*

| | |
|---|---|
| *c* | Critical point |
| *i,j* | Component identification |
| *r* | Reduced parameter |
| *exp* | Experimental |
| *calc* | Calculated |

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# 5

# Solubility of Cannabinol in supercritical carbon dioxide

Content published in *J. Chem. Data* 55 (2010) 3704-3707

# 5


## *Abstract*

*Cannabinol (CBN) is a decomposition product of the cannabinoid (-)-$\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC), the main active compound of cannabis. The solubility of CBN in supercritical carbon dioxide was determined at 314, 327 and 334 K and in the pressure range from 13.0 to 20.2 MPa by using an analytical method with a quasi-flow apparatus. The molar solubility of CBN ranged from 1.26 x $10^{-4}$ to 4.16 x $10^{-4}$. CBN showed different behavior compared to $\Delta^9$-THC in terms of molar solubility. The data were correlated using the Peng-Robinson equation of state in combination with quadratic mixing rules. Deviations between calculated results and the experimental data ranged from 4.14 to 4.46 % absolute average relative deviation (AARD).*

# 5. CBN Solubility in supercritical carbon dioxide

## 5.1 Introduction

Nowadays, there is a growing interest in natural medicinal compounds. Cannabis is one of the oldest medicinal plants known [1]. Recently, the medicinal use of cannabis has been legalized in several countries. The major biologically active compound from cannabis, the cannabinoid (-)-$\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC), has been registered for medical application and cannabis preparations are being developed as medicines. $\Delta^9$-THC eases pain and is neuroprotective; it has approximately equal affinity for the CB1 and CB2 receptors. Its effects are perceived to be mostly cerebral. However, $\Delta^9$-THC is not the only biologically active compound in cannabis. In total, cannabis contains more than 400 different ingredients, including 66 cannabinoids that can show biological activity [2]. One of these cannabinoids is Cannabinol (CBN). CBN is only mildly psychoactive and is perceived to be sedative or stupefying. It is the primary product of $\Delta^9$-THC degradation, and its amount is limited in a fresh plant. CBN content increases as $\Delta^9$-THC degrades in storage under exposure to light and air. This chemical reaction is a dehydrogenation reaction and is represented in Figure 5.1. The cyclohexene ring present in $\Delta^9$-THC is dehydrogenated to become an aromatic benzoic ring [3].

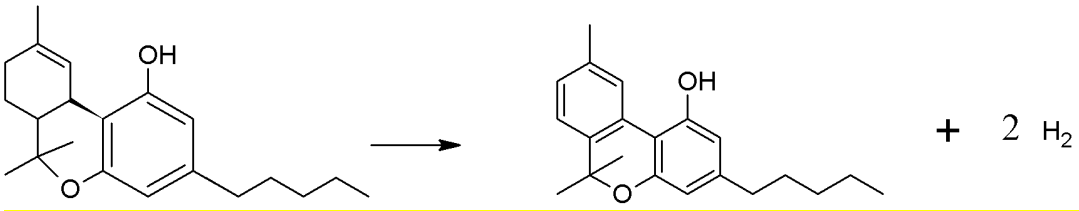


**Figure 5.1. Dehydrogenation of $\Delta^9$-THC into CBN**

In order to obtain pure cannabinoids, they can be extracted directly from cannabis by organic solvents (e.g. hydrocarbons such as hexane and alcohols) with a yield exceeding 90% [4]. However, these solvents are flammable and many of them are toxic. Supercritical Fluid Extraction (SFE) with carbon dioxide ($CO_2$) is a promising alternative technique. There are no flammability or toxicity issues, solvent removal is simple and efficient, and the extract quality can be well-controlled. This green solvent has widely

been used for extraction of natural compounds, including pharmaceutical molecules, from plant material [5-11].

To extract cannabinoids from cannabis with the use of SFE, it is crucial to have solubility data. Such data are however currently lacking. So far, only the solubility of $\Delta^9$-THC has been reported [12].

To reduce the lack of solubility data of cannabinoids, this work presents the determination of the solubility of CBN in supercritical $CO_2$. In addition, the experimental data are correlated using the Peng-Robinson equation of state (PR-EoS). Finally, the solubilities of CBN and $\Delta^9$-THC in supercritical $CO_2$ are compared, and their differences are explained in terms of structure, molecular weight and polarity.

## 5.2 Experimental

The solubility of CBN in supercritical $CO_2$ was measured at 314, 327 and 334 K and pressures between 13.0 and 20.2 MPa, by using an analytical method with a quasi-flow apparatus. Details of this solubility cell and the equipment for analyses can be found elsewhere [12].

The solubility cell was loaded by transferring a liquid mixture of CBN and methanol into the sample cylinder, after which the methanol was evaporated with a vacuum pump (RNF Lab) for 1 hour at ambient temperature to ensure complete evaporation of the solvent. Subsequently, the pump was disconnected and the system was closed. $CO_2$ at the desired temperature was added to the solubility cell filled with CBN until the desired pressure was reached and the $CO_2$ circulation over the sample vessel was started. The temperature measurements have an uncertainty of 0.2 K due to the temperature fluctuations in the oven and the error in the reading of the thermometer. The uncertainty of the pressure measurements is 0.05 MPa.

A sample for HPLC analysis was taken after 4 hours and successively every 30 minutes. When the concentration difference measured was less than 0.09 x $10^{-4}$ mol.mol$^{-1}$ between two subsequent analyses, it was assumed that equilibrium was reached, and the concentration measured was recorded as the solubility.

The HPLC profiles were recorded at 228 nm. The analytical column was a Vydac (Hesperia, CA) $C_{18}$, type 218MS54 (4.6 * 250 mm$^2$, 5 μm). The mobile phase consisted of a mixture of methanol, distilled water and tetrahydrofuran in the proportions v/v/v = 10/4/1. The flow rate was 1.5 mL.min$^{-1}$ and the total running time was 14 minutes. Because the peak areas of the components calculated from the chromatograms are linearly related to their amounts by the Lambert-Beer law, it was possible to determine their concentration using a calibration line. This line was realized by using 5 standard samples with different concentrations in the range 0 – 5 mg.mL$^{-1}$. Each standard sample was injected at least three times and an average was taken to perform the linear regression. The linear regression coefficient of the calibration curve was equal to 0.997.

The $CO_2$ used for the measurements was supplied by Hoek Loos (quality 2.7). CBN with a purity of 99.5% was provided by Echo Pharmaceuticals B.V. Methanol and tetrahydrofuran (HPLC reagent grade) were purchased from J. T. Bakker. These materials were used without further purification.

## 5.3 Results and discussion

The solubility of CBN in supercritical $CO_2$ was measured at 314, 326 and 334 K and in the pressure range from 13.0 to 20.2 MPa. The results are summarized in Table 5.1 and graphically shown in Figure 5.2. Each point is an average of at least 2 measurements. The maximum standard deviation was 0.0002 x $10^{-4}$, as represented by the error bars in Figure 5.2.

**Table 5.1. Solubility of CBN at different temperatures and pressures**

| *T* = 314 K | | | *T* = 326 K | | | *T* = 334 K | | |
|---|---|---|---|---|---|---|---|---|
| *P* | $10^4$ *y* | $10^4$ Exp. error | *P* | $10^4$ *y* | $10^4$ Exp. error | *P* | $10^4$ *y* | $10^4$ Exp. error |
| MPa | - | - | MPa | - | - | MPa | - | - |
| 13.8 | 1.26 | ± 0.13 | 13.0 | 2.51 | ± 0.25 | 13.0 | 1.38 | ± 0.14 |
| 14.3 | 1.27 | ± 0.13 | 13.4 | 2.95 | ± 0.30 | 13.3 | 1.33 | ± 0.13 |
| 14.5 | 1.27 | ± 0.13 | 13.7 | 3.46 | ± 0.35 | 13.6 | 1.65 | ± 0.16 |
| 15.3 | 1.47 | ± 0.15 | 14.0 | 3.74 | ± 0.37 | 14.6 | 2.10 | ± 0.21 |
| 15.5 | 1.58 | ± 0.16 | 14.4 | 3.09 | ± 0.31 | 14.9 | 1.99 | ± 0.20 |
| 16.3 | 1.79 | ± 0.18 | 14.8 | 4.14 | ± 0.41 | 15.6 | 2.35 | ± 0.23 |
| 17.7 | 1.77 | ± 0.18 | 15.2 | 3.80 | ± 0.38 | 16.5 | 2.27 | ± 0.23 |
| 18.2 | 2.02 | ± 0.20 | 15.6 | 3.92 | ± 0.39 | 17.1 | 2.48 | ± 0.25 |
| 19.1 | 2.08 | ± 0.21 | 16.2 | 3.75 | ± 0.38 | 17.4 | 2.17 | ± 0.22 |
| 20.2 | 2.33 | ± 0.23 | 16.6 | 4.08 | ± 0.41 | 18.2 | 2.20 | ± 0.22 |
| | | | 17.1 | 4.51 | ± 0.45 | 18.5 | 2.36 | ± 0.24 |
| | | | 17.8 | 4.16 | ± 0.42 | 19.9 | 2.17 | ± 0.22 |

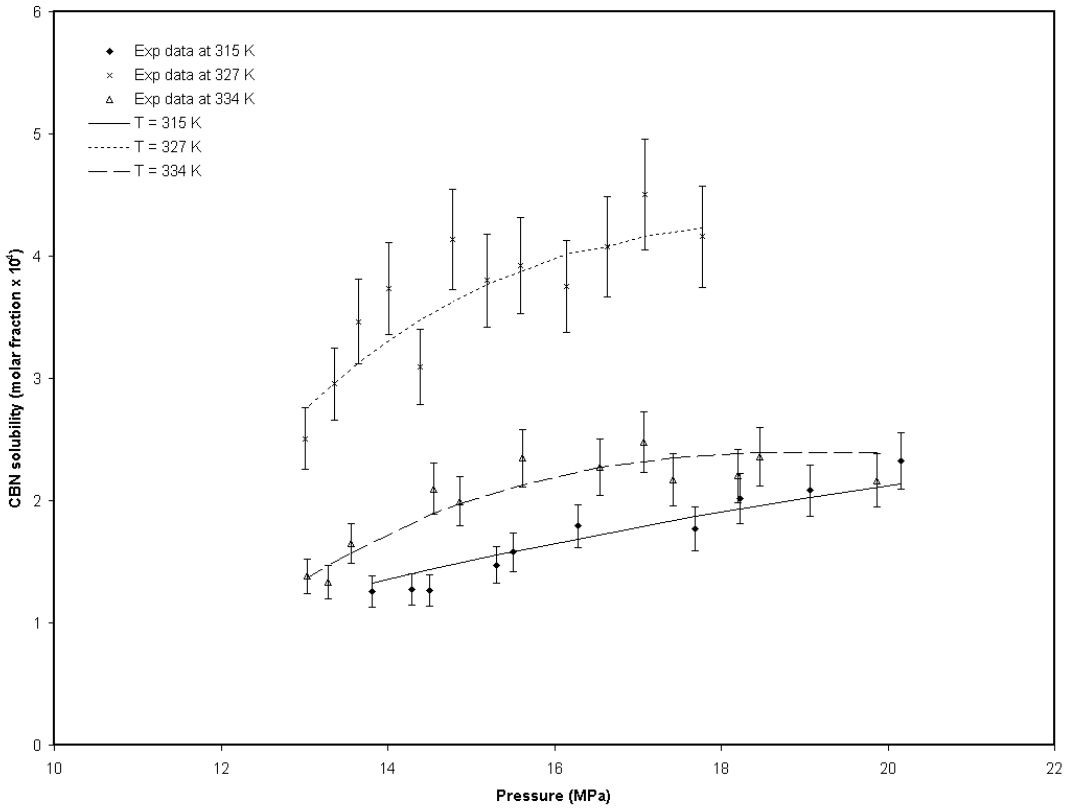


**Figure 5.2. CBN solubility in SC $CO_2$: experimental results (points) and results from modeling with PR EoS (lines)**

As shown for the isotherms in Figure 5.2, the solubility of CBN in supercritical $CO_2$ increases with an increase in pressure. Interestingly, the highest solubility is observed at the medium temperature (326 K), while it was expected that the solubility would increase with increasing temperature, just as was observed for $\Delta^9$-THC [12 and chapter 4 of this thesis]. Although uncommon, this phase behavior is theoretically possible and has been observed before e.g., in the naphthalene + supercritical ethylene system [13].

Also, contrary to $\Delta^9$-THC [12], no crossover region was observed in the measured pressure range. However, this behavior is likely to occur at pressures lower than the lowest pressure in the measurements (13.0 MPa), because it is expected that the solubility curves intercept around 10 MPa (extrapolation of Figure 5.2).

The experimental data were correlated with the PR-EoS [14, 15]:

$$P = \frac{RT}{V-b} - \frac{a}{V^2 - 2bV - b^2} \tag{1}$$

where $P$ is the pressure, $T$ is the temperature, $V$ is the volume, $R$ is the gas constant, and $a$ and $b$ are parameters calculated from the quadratic mixing rule. The attractive term is given by:

$$a = \sum_i \sum_j y_i \cdot y_j (a_i a_j)^{0.5} (1 - k_{ij}) \tag{2}$$

where $k_{ij}$ is the binary interaction parameter to be optimized and

$$a_i = 0.45724 \times \frac{R^2 T_{ci}^2}{P_{ci}} \alpha_i \tag{3}$$

where

$$\alpha_i = \left[1 + m(\omega_i)\left(1 - T_{ri}^{1/2}\right)\right]^2 \tag{4}$$

where

$$m(\omega_i) = 0.37464 + 1.54226\omega_i - 0.26992\omega_i^2 \tag{5}$$

The covolume parameter is given by:

$$b = \sum_i y_i b_i \tag{6}$$

where

$$b_i = 0.07780 \times \frac{RT_{ci}}{P_{ci}} \tag{7}$$

To use these equations, the critical properties ($T_c$, $P_c$) and acentric factor ($\omega_i$) of the components are required. However, critical properties of CBN are not available in literature. Therefore, these properties have been estimated using the Gani method [16] and the values for $CO_2$ were taken from the PE database [17]. The critical properties and acentric factors of CBN and $CO_2$ are shown in Table 5.2. The critical properties of $\Delta^9$-THC [12] are also presented for comparison. As it can be seen in Table 5.2, the values for the critical pressure and temperature of CBN are in the same order of magnitude as $\Delta^9$-THC. However, $\Delta^9$-THC has a higher acentric factor because of the absence of the aromatic ring that is present in CBN.

**Table 5.2. Critical temperatures ($T_c$), critical pressures ($P_c$), and acentric factors ($\omega$) used in the PR-EoS**

| Substance | $T_c$ (K) | $P_c$ (Mpa) | $\omega$ |
|---|---|---|---|
| $CO_2$ | 304.4 | 7.38 | 0.225 |
| CBN | 920 | 1.65 | 0.431 |
| $\Delta^9$-THC | 988 | 1.95 | 0.882 |

The binary interaction parameter $k_{ij}$ was calculated from the experimental pressure at each point by minimizing the relative difference between experimental and calculated pressure [14]. This minimization can be expressed by the absolute average relative deviation (AARD (%)), as described by the following equation:

$$AARD\,(\%) = \frac{100}{n} \sum_1^n \frac{\left| P_i^{\exp} - P_i^{calc} \right|}{P_i^{\exp}}. \tag{8}$$

Here, $n$ is the number of data experiments at each temperature, $P_i^{exp}$ is the experimental pressure for the experiment $i$, whereas $P_i^{calc}$ is the estimated value. The *AARD* values at different temperatures are presented in Table 5.3. Their values - around 4 % - show that the data are well correlated by the PR-EoS.

**Table 5.3. Binary parameters for the CBN + $CO_2$ binary system**

| *T* (K) | $k_{ij}$ | *AARD* (%) |
|---|---|---|
| 314 | 0.113 | 4.21 |
| 326 | 0.173 | 4.46 |
| 334 | 0.212 | 4.14 |

Table 5.3 also presents the binary parameter $k_{ij}$, at the different temperatures. This parameter increases linearly with a rise in temperature. The regression coefficient had a value of 1.000. This shows the consistency of the experimental results.

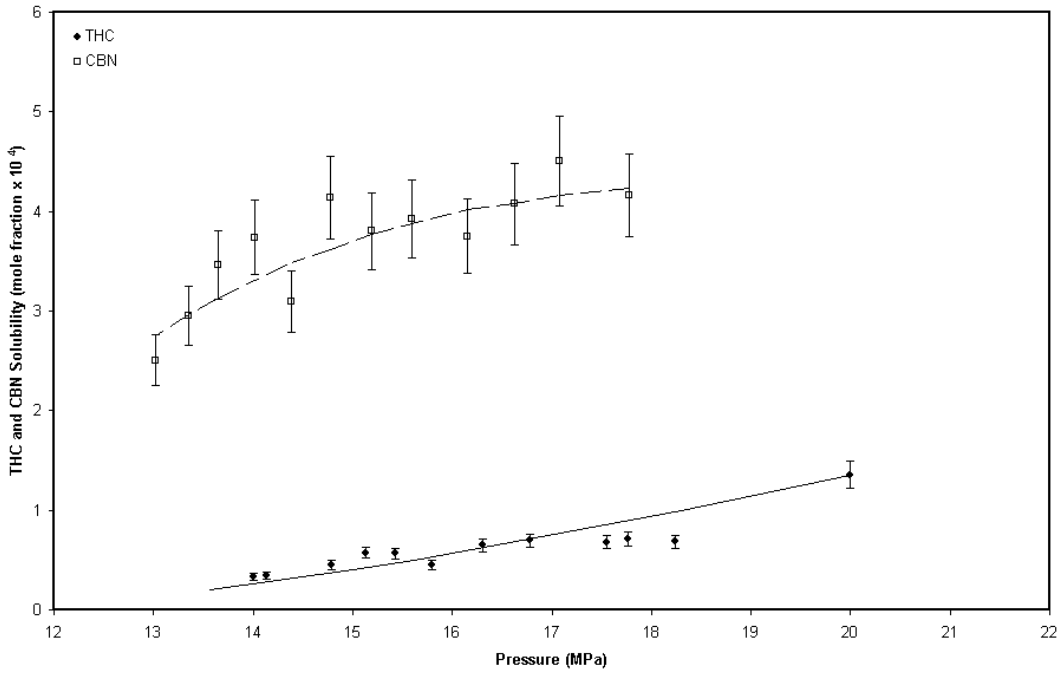


**Figure 5.3: THC and CBN molar solubilities in SC $CO_2$ at 326 K**

In Figure 5.3, the solubility of CBN in supercritical $CO_2$ at 326 K is compared to the solubility data of $\Delta^9$-THC in supercritical $CO_2$ at the same temperature from literature [12]. This Figure shows that the solubility of $\Delta^9$-THC is lower than the solubility of CBN. This behavior is observed at any measured temperature. This can be explained by the lower polarity of CBN compared to $\Delta^9$-THC, which increases the affinity for the non-polar supercritical $CO_2$. Moreover, the lower molar mass of CBN compared to $\Delta^9$-THC also increases its solubility in supercritical $CO_2$, although the effect is probably small (only 4 $g \cdot mol^{-1}$ difference).

From these data it may be concluded that if a cannabis plant (after storage) contained both CBN and $\Delta^9$-THC, both cannabinoids could be separated from each other with supercritical $CO_2$ on basis of their different affinity. CBN could be extracted first at low pressure (i.e., around 13 MPa), after which the active $\Delta^9$-THC could be extracted at higher pressures (around 20 MPa). This could be a selective process to isolate CBN separately from $\Delta^9$-THC.

## 5.4 Conclusion

In this work, the solubility of the cannabinoid CBN in supercritical $CO_2$ was measured at temperatures between (314 and 334) K and a pressure range from (13.0 to 20.2) MPa. Highest solubility was observed at highest pressures and intermediate temperature (326 K). This behavior is different from the solubility of another cannabinoid, $\Delta^9$-THC, in $CO_2$, which shows higher solubility at higher temperature. The experimental data can be adequately represented by the PR-EoS. As expected from its structure, molecular weight and polarity, CBN is more soluble than $\Delta^9$-THC in supercritical $CO_2$ in the studied pressure and temperature ranges. Therefore, it can be concluded that supercritical $CO_2$ could be a good solvent to isolate CBN from $\Delta^9$-THC by extraction.

## Acknowledgements

Financial support by STW (project no LFA 7426) is gratefully acknowledged.

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# 6

# Solubility of Non-Psychoactive Cannabinoids in Supercritical Carbon Dioxide and Comparison with Psychoactive Cannabinoids

Content published in *J. Supercrit. Fluids*, 55 (2010) 603-608

# 6


## *Abstract*

*The solubilities of two different non-psychoactive cannabinoids i.e., cannabigerol (CBG) and cannabidiol (CBD), in supercritical carbon dioxide ($CO_2$) have been determined at 315, 326 and 334 K and in the pressure range from 11.3 to 20.6 MPa. These solubility data have been compared to the previously determined solubilities of two psychoactive cannabinoids i.e. (-)-$\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC) and cannabinol (CBN), in supercritical $CO_2$. An analytical method with a quasi-flow apparatus was used for the experimental determination. Within the investigated temperature and pressure range, the molar solubility of CBG ranged from 1.17 to 1.91 x $10^{-4}$ and the molar solubility of CBD ranged from 0.88 to 2.69 x $10^{-4}$. The solubility of the different cannabinoids in supercritical $CO_2$ increases at 326 K in the following order: $\Delta^9$-THC < CBG < CBD < CBN. The solubility data were correlated using the Peng-Robinson equation of state in combination with Van der Waals mixing rules. Deviations between calculated results and the experimental data ranged from 0.81 to 6.35% absolute average relative deviation (AARD), except for CBD at 334 K, where the AARD was 18.4%.*

# 6. Solubility of Non-Psychoactive Cannabinoids in Supercritical Carbon Dioxide and Comparison with Psychoactive Cannabinoids

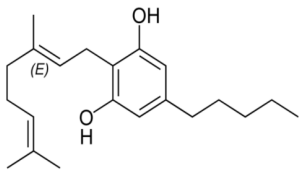
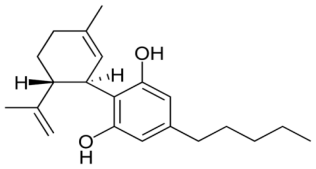
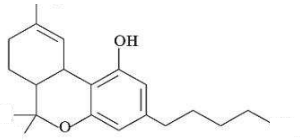
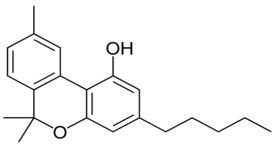
## 6.1 Introduction

Nowadays, there is a growing interest in natural medicinal compounds. *Cannabis sativa* is one of the oldest medicinal plants known [1]. Recently, the medicinal use of cannabis has been legalized in several countries [2]. Some of the medical purposes include, but are not limited to, multiple sclerosis, chronic pain, glaucoma, appetite stimulant, asthma and cardiovascular conditions, and as an antiemetic [3]. The active cannabinoids are present in the cannabis flower of the female species. In nature, these molecules occur in their acidic form. Under influence of heat or light, they loose the acidic group by release of a carbon dioxide molecule, a so-called decarboxylation reaction. In this way they become neutral cannabinoids, some of which are psychoactive [1].

Each cannabinoid has different biological properties. The major active compound from cannabis, (-)-$\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC), is the most psychoactive one [4]. It has been registered for medical application in several countries. $\Delta^9$-THC is also often used as golden standard for pharmacological studies. Depending on the cannabis species, its amount can reach levels up to 18%, for example in the *Bedrocan* cannabis plant [5]. When the plant is exposed to light or stored for a long time, the primary degradation product of $\Delta^9$-THC, called cannabinol (CBN), is formed. Its amount is limited in a fresh plant. CBN is a mildly psychoactive cannabinoid, and is perceived to be sedative or stupefying [6, 7]. Another cannabinoid that can be present in cannabis in significant amounts is cannabidiol (CBD). Depending on the plant species, it can reach up to 6%, for example in the *Bediol* cannabis plant [8]. CBD is not psychoactive, although it may modulate the euphoric effects of $\Delta^9$-THC to some extent [9]. Medically, it appears to relieve convulsion, inflammation, anxiety, and nausea. The non-psychoactive cannabinoid cannabigerol (CBG) has been studied less in pharmaceutical investigations

than the three previous ones. However, some studies have shown that it may lower blood pressure in rats. It also has analgesic and anti-inflammatory effects [10]. The chemical structures of these four different cannabinoids, including their molecular weights and melting points, are shown in Table 6.1.

**Table 6.1: Molecular structures, molecular weight and melting temperatures of the various cannabinoids**

| Molecule | Molecular structure | Molecular weight $g.mol^{-1}$ | Melting temperature K |
|---|---|---|---|
| Cannabigerol (CBG) |  | 316.5 | NA (> 334) |
| Cannabidiol (CBD) |  | 314.5 | 340 |
| Tetrahydrocannabinol ($\Delta^9$-THC) |  | 314.5 | NA (< 298) |
| Cannabinol (CBN) |  | 310.5 | 350 |

The availability of the various cannabinoids as pure compounds is of great importance for pharmaceutical studies and the development of new medicines. Indeed, most of the controlled studies have been carried out with $\Delta^9$-THC and do not mimic the situation,

when cannabis is smoked. As CBD and CBG have analgesic and anti-inflammatory effects, these compounds may also be used in drugs [10]. To develop such medicines, pure CBD and CBG should be available in larger quantities. To achieve this, efficient extractions methods need to be developed.

Cannabinoids can be extracted directly from cannabis by organic solvents (e.g. hydrocarbons and alcohols) with a yield exceeding 90% [11]. However, these solvents are flammable and many of them are toxic. Supercritical Fluid Extraction (SFE) with carbon dioxide ($CO_2$) is a promising alternative. $CO_2$ is non-toxic, non-flammable, relatively inert, abundant and inexpensive. In the supercritical region, the density of $CO_2$ and its solvent power can be tuned by controlling the temperature and pressure, permitting selective extraction. The low critical temperature allows processing of heat-sensitive materials. When the pressure is decreased after extraction, the $CO_2$ will evaporate and pure product without $CO_2$ is obtained. Therefore, supercritical extraction is often used as extraction solvent for natural products, including medicines, for which it eliminates the presence of toxic residues of organic solvents [12-17].

The application of SFE to extract cannabinoids from cannabis requires solubility data. These data are currently lacking for all non-psychoactive compounds (i.e. CBD and CBG). So far, only the solubilities of the psychoactive cannabionoids $\Delta^9$-THC and CBN in supercritical $CO_2$ have been reported in [18, 19]. In this work, the solubilities of the non-psychoactive CBD and CBG in supercritical $CO_2$ have been determined and compared with the available literature data for $\Delta^9$-THC and CBN. Furthermore, the experimental data of CBD and CBG have been correlated using the Peng-Robinson equation of state (PR-EoS) [20] in combination with the van der Waals mixing rules [21].

## 6.2 Experimental

### *6.2.1 Chemicals*

The $CO_2$ used for the measurements was supplied by Hoek Loos and had a purity of 99.7 % (quality 2.7). CBD with a purity higher than 99% was purchased from THC Pharm (Frankfurt, Germany). CBG with a purity of 99.3 % was provided by Echo Pharmaceuticals B.V. (Nijmegen, the Netherlands). Methanol and tetrahydofuran of HPLC reagent grade were purchased from J.T. Baker. These materials were used without further purification.

### *6.2.2 Apparatus and method*

The solubility of CBD and CBG in supercritical $CO_2$ was measured at 314, 327 and 334 K and pressures between 11.3 and 20.6 MPa, by using an analytical method with a quasi-flow apparatus. Details of this equipment can be found elsewhere [19 and in chapter 4 of this thesis].

At the start of an experiment, a measured amount of compound was put into the sample cylinder and the system was closed. Then the oven was set at the desired temperature. After the preset temperature had been reached, the system was filled with $CO_2$ until the desired pressure was reached. When the conditions were stable, the $CO_2$ circulation over the sample vessel was started. A sample was taken after 4 hours and successively every 30 minutes, and analyzed using High Performance Liquid Chromatography (HPLC). When the concentration difference measured was less than $0.09 \times 10^{-4}$ between two subsequent analysis, with pressure and temperature differences less than 0.05 MPa and 0.2 K respectively, it was assumed that equilibrium was reached, and the concentration measured was recorded as the solubility.

### *6.2.3 HPLC analysis*

The HPLC profiles were acquired on a Chromapack HPLC system consisting of a Isos pump, an injection valve and a UV-VIS detector (model 340 – Varian). The system was controlled by Galaxie Chromatography software. The profiles were recorded at 228 nm. The analytical column was a Vydac (Hesperia, CA) $C_{18}$, type 218MS54 (4.6 * 250 $mm^2$, 5 μm). The mobile phase consisted of a mixture of methanol, distilled water and tetrahydrofuran in the proportions v/v/v = 10/4/1. The flow rate was 1.5 $mL.min^{-1}$ and the total running time was 14 minutes.

As the peak areas of the components calculated from the chromatograms are linearly related to their amounts by the Lambert-Beer law, it was possible to determine their concentration using a calibration line. This was realized by using 5 standard samples with different concentration in the range 0 – 9 $mg.mL^{-1}$. Each standard sample was injected at least three times and an average was taken to perform the linear regression. The linear regression coefficient of the calibration curve was equal to 0.993 and 0.999 for CBD and CBG respectively.

## 6.3 Results and discussion

### *6.3.1 Solubility data*

Solubility data of CBG in supercritical $CO_2$ at 315, 326 and 334 K in the pressure range 13.4 – 21.1 MPa are presented in Table 6.2. Each point was an average of at least 2 measurements. The maximum standard deviation was $0.2 \times 10^{-7}$. The data are graphically depicted in Figure 6.1, showing that the solubility increases with increasing pressure. As expected, the highest solubility in supercritical $CO_2$ is found at the highest temperature. This was also the case for the solubility of the psychoactive cannabinoid $\Delta^9$-THC in supercritical $CO_2$.

**Table 6.2: Molar solubility of CBG at different pressures and temperatures**

| *T* = 314 K | | *T* = 326 K | | *T* = 334 K | |
|---|---|---|---|---|---|
| ***P* (MPa)** | **$10^4$ *y*** | ***P* (MPa)** | **$10^4$ *y*** | ***P* (MPa)** | **$10^4$ *y*** |
| 13,4 | 1,17 ± 0.12 | 15,3 | 1,23 ± 0.12 | 15,4 | 1,37 ± 0.14 |
| 15,7 | 1,21 ± 0.12 | 15,8 | 1,23 ± 0.12 | 16,4 | 1,57 ± 0.16 |
| 17,6 | 1,17 ± 0.12 | 16,3 | 1,34 ± 0.13 | 17,8 | 1,78 ± 0.18 |
| 18,4 | 1,23 ± 0.12 | 17,0 | 1,36 ± 0.14 | 18,5 | 1,82 ± 0.18 |
| 19,5 | 1,29 ± 0.13 | 17,8 | 1,32 ± 0.13 | 19,1 | 1,91 ± 0.19 |
| 20,6 | 1,28 ± 0.13 | 18,9 | 1,43 ± 0.14 | | |
| | | 20,1 | 1,49 ± 0.15 | | |
| | | 21,1 | 1,59 ± 0.16 | | |

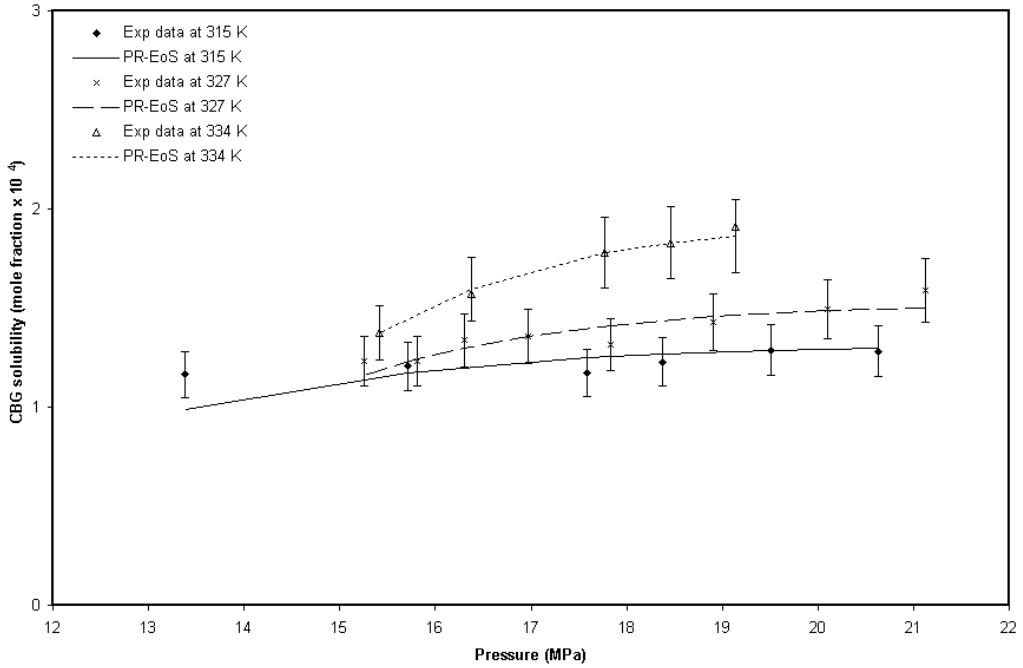



**Figure 6.1: CBG solubility in supercritical $CO_2$ - experimental results (points) and results from modelling with PR EoS (lines)**

Previously, it was found that $\Delta^9$-THC showed a crossing of the solubility lines at around 15 MPa [19 and in chapter 4 of this thesis]. This is a result of two opposing effects [23,24]: (i) increasing the temperature leads to a lower $CO_2$ density, leading to lower cannabinoid solubility, (ii) the volatility of a cannabinoid increases with increasing temperature, leading to higher cannabinoids solubility. The density effect is dominant at lower pressures (<15 MPa), while the volatility effect becomes dominant at higher pressures (> 15 MPa). In this work, no crossing of CBG solubility lines is observed

within the measured pressure range. Nevertheless, a cross-over can be expected at pressures around 14 MPa by extrapolation of Figure 6.1, which is close to the cross-over pressure of $\Delta^9$-THC.

Table 6.3 shows the solubility measurements of CBD in supercritical $CO_2$ at 315, 326 and 334 K in the pressure range 11.3 – 19.4 MPa with a maximum standard deviation of 0.2 x $10^{-7}$. Figure 6.2 presents the data graphically. The isotherms in Figure 6.2 show that the solubility of CBD in supercritical $CO_2$ increases with pressure. Interestingly, the highest solubility of CBD is obtained at the medium temperature (326 K). Although uncommon, this special behavior is theoretically possible [25] and was observed before for the solubility of the psychoactive cannabinoid CBN in supercritical $CO_2$ [20 and in chapter 5 of this thesis]. A reason for this uncommon behavior could be the transition from a solid-supercritical fluid equilibrium to a liquid-supercritical fluid equilibrium. The melting point of pure CBD (340 K) and pure CBN (350 K) is close to experimental temperature of 334 K. The melting depression effect of $CO_2$ may have induced melting at 334 K, resulting in a lower solubility. Instead, pure $\Delta^9$-THC (which is a liquid at 298 K) and pure CBG (which is still solid at 334 K) do not melt at temperatures close to the experimental conditions, and therefore show the usual trend of increasing solubility in supercritical $CO_2$ with increasing temperature.

**Table 6.3: Molar solubility of CBD at different pressures and temperatures**

| ***T* = 314 K** | | ***T* = 326 K** | | ***T* = 334 K** | |
|---|---|---|---|---|---|
| ***P* (MPa)** | **$10^4$ *y*** | ***P* (MPa)** | **$10^4$ *y*** | ***P* (MPa)** | **$10^4$ *y*** |
| 11,3 | 1,00 ± 0.10 | 11,8 | 0,94 ± 0.09 | 11,4 | 0,88 ± 0.09 |
| 11,8 | 1,25 ± 0.12 | 12,4 | 1,67 ± 0.17 | 11,8 | 1,22 ± 0.12 |
| 12,3 | 1,30 ± 0.13 | 12,8 | 1,90 ± 0.19 | 12,6 | 1,59 ± 0.16 |
| 13,2 | 1,30 ± 0.13 | 13,3 | 1,86 ± 0.19 | 13,2 | 1,85 ± 0.18 |
| 13,7 | 1,41 ± 0.14 | 13,6 | 2,22 ± 0.22 | 14,6 | 1,75 ± 0.17 |
| 14,3 | 1,66 ± 0.17 | 15,5 | 2,37 ± 0.24 | 15,9 | 1,97 ± 0.20 |
| 15,4 | 1,70 ± 0.17 | 16,5 | 2,67 ± 0.27 | 16,4 | 1,79 ± 0.18 |
| 15,7 | 1,61 ± 0.16 | 19,4 | 2,69 ± 0.27 | 17,0 | 1,74 ± 0.17 |
| 16,8 | 1,87 ± 0.19 | | | 17,5 | 1,86 ± 0.19 |
| 17,3 | 1,85 ± 0.18 | | | 18,8 | 1,84 ± 0.18 |

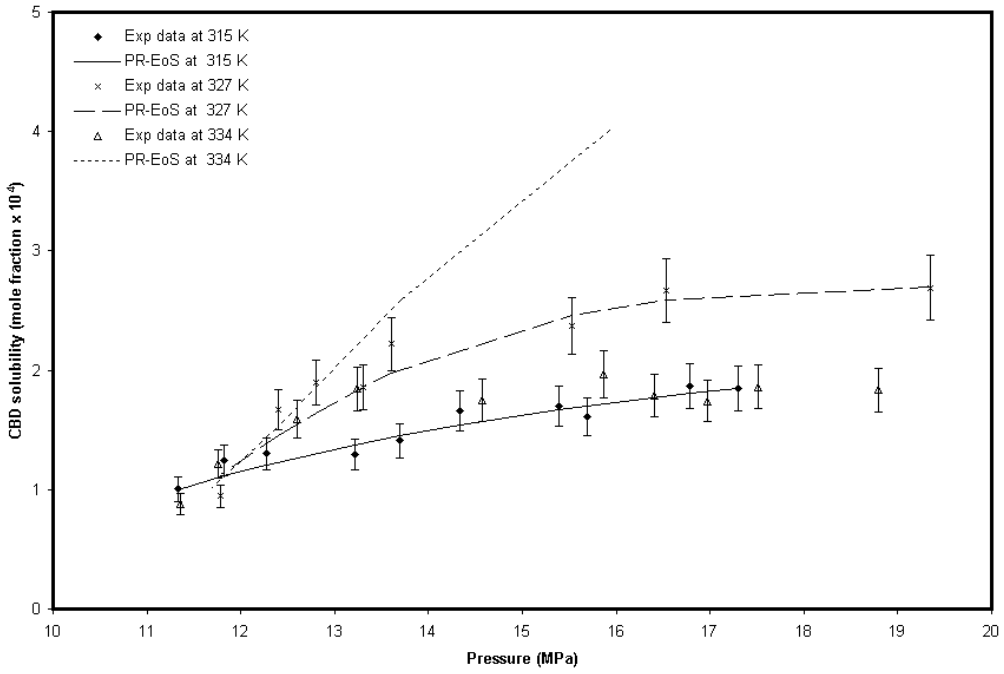



**Figure 6.2: CBD solubility in supercritical $CO_2$ - experimental results (points) and results from modeling with PR EoS (lines)**

Figure 6.2 does not show any cross-over behavior for the CBG + $CO_2$ system within the pressure range measured. Nevertheless, a crossing of the solubility lines can be expected at pressures around 12 MPa by extrapolation, which is close to the cross-over pressure of around 11 MPa for CBN [20 and chapter 5 of this thesis].

### *6.3.2 Correlation*

The equilibrium between two phases of a mixture is described through the equality of fugacities for each component [26]. From this equality, the solubility of a solid (cannabinoid) in a supercritical fluid (supercritical $CO_2$) can be expressed by equation (6.1):

$$y_2 = \frac{P_2^{sub} \cdot \phi_2^{sub} \cdot}{P \phi_2} \exp\left(\frac{V_s (P - P_2^{sub})}{RT}\right) \qquad (6.1)$$

For the system studied here, $P_2^{sub} < P$ and $\phi_2^{sub} \approx 1$ [27].

The thermodynamic properties of CBG and CBD are not available in the literature. Therefore they have been estimated using several correlations. $P_2^{sub}$ was estimated using the Clapeyron equation [28]:

$$Ln\left(\frac{P_2^{sub}}{P_t}\right) = -\frac{\Delta H^{sub}}{R}\left(\frac{1}{T} - \frac{1}{T_f}\right) \tag{6.2}$$

The other parameters for ($T_c$, $P_c$, $\omega$, $\Delta H^{sub}$ and $T_f$) were calculated with the Gani method [29]. Their values can be found in Table 6.4. The values for $\Delta^9$-THC, CBN and $CO_2$ [19, 20 and chapters 4 & 5 of this thesis] are also added for comparison.

**Table 6.4: Critical parameters, acentric factors, melting temperatures and sublimation enthalpies used in the equation of state (a: experimental value)**

| Substance | $T_c$ (K) | $P_c$ (MPa) | $\omega$ (-) | $T_f$ (K) | $\Delta H^{sub}$ (kJ.mol$^{-1}$) |
|---|---|---|---|---|---|
| $CO_2$ | 304.4 | 7.38 | 0.225 | | |
| CBG | 1099 | 1.68 | 1.172 | 420 | 53.1 |
| CBD | 932 | 1.60 | 0.497 | 340[a] | 49.1 |
| $\Delta^9$-THC | 988 | 1.95 | 0.882 | < 298[a] | 21.0 |
| CBN | 920 | 1.65 | 0.431 | 350[a] | 49.4 |

To calculate the fugacity coefficient of the solid ($\Phi_2$) dissolved in the supercritical fluid, the PR-EoS (6.3) is used [21]. This EoS is chosen, because it was successfully employed for the correlation of the solubility of other cannabinoids in the past [19, 20]. Each parameter is first calculated for the pure component *i* with the equations (6.4), (6.5), (6.6) and (6.7):

$$\left(P + \frac{a \cdot \alpha}{V(V+b) + b(V-b)}\right)(V-b) = R \cdot T \tag{6.3}$$

$$a_i = 0.45724 \cdot \frac{R^2 T_{ci}^2}{P_{ci}} \tag{6.4}$$

$$b_i = 0.07780 \cdot \frac{R T_{ci}}{P_{ci}} \tag{6.5}$$

$$\alpha_i = \left[1 + m(\omega_i)\left(1 - T_{ri}^{1/2}\right)\right]^2 \tag{6.6}$$

$$m(\omega_i) = 0.37464 + 1.54226\omega_i - 0.26992\omega_i^2 \tag{6.7}$$

For the mixture, the classical Van der Waals mixing rules are used as described by the equations (6.8) and (6.9) [22]:

$$a\alpha = \sum_i \sum_j y_i \cdot y_j (a\alpha_i a\alpha_j)^{0.5}(1-k_{ij}) \quad (6.8)$$

$$b = \sum_i y_i b_i \quad (6.9)$$

In the equation (6.8) the optimum binary interaction coefficient $k_{ij}$ for each temperature is calculated by the correlation of experimental data, through the minimization of the function average absolute relative deviation (AARD), defined as:

$$AARD(\%) = \frac{100}{n} \sum \frac{\left| P_i^{calc} - P_i^{\exp} \right|}{P_i^{\exp}} \quad (6.10)$$

Here, $n$ is the number of data experiments at each temperature, $P_i^{exp}$ is the experimental pressure for the experiment $i$, whereas $P_i^{calc}$ is the estimated value.

Table 6.5 presents the different values for $k_{ij}$, as well as the AARD for each isotherm. As can be seen in this table, $k_{ij}$ increases linearly with temperature increase. The regression coefficient had a value of 0.904 and 0.812 for CBG and CBD, respectively.

**Table 6.5: Binary parameters and AARD (%) for CBG and CBD at 315, 326 and 334 K**

| **Compound** | *T* (K) | $k_{ij}$ | AARD (%) |
|---|---|---|---|
| CBG | 315 | 0.099 | 4.86 |
| | 326 | 0.116 | 3.86 |
| | 334 | 0.118 | 5.76 |
| CBD | 315 | 0.166 | 0.81 |
| | 326 | 0.188 | 6.35 |
| | 334 | 0.187 | 18.4 |

The AARD values for the CBG + $CO_2$ system are comprised between 3.86 and 5.76 %, which means that the solubility data are well correlated using the PR-EoS (see Figure 6.1). For the CBD + $CO_2$ system, the correlation is also accurate at low temperatures with a maximum AARD of 6.35% (see Figure 6.2). However, this is not the case for the highest temperature. At 334 K, the high value of the AARD (18.4 %) means that the model does

not correlate well with the data. This can also be seen by the shape of the curve in Figure 6.2. The experimental data show a plateau, where the solubility only increases very slowly with the pressure. The modeling curve predicts a much higher increase in solubility with pressure increase. This failure of the model is probably due to the fact that the highest temperature of 334 K is close to the melting temperature of CBD (340 K) [30]. Therefore, it can be expected that the solubility of CBD in supercritical $CO_2$ at 334 K is deviating from the solid solubility line, and might be closer to the liquid solubility line instead. However, modeling with the liquid-liquid version of the PR-EoS [19] did not result in accurate correlation. In this particular case, the boundary limits of the PR-EoS are attained.

### *6.3.3 Comparison of the solubility data of the other cannabinoids*

In Figures 6.3, 6.4 and 6.5 the solubilities of the non-psychoactive cannabinoids CBG and CBD in supercritical $CO_2$ are compared to the previously measured solubilities of the psychoactive cannabinoids $\Delta^9$-THC and CBN [19, 20 and in chapter 4 & 5 of this thesis] at different temperatures. At 315 K (Fig. 6.3), the solubility in supercritical $CO_2$ increases in the following order: $\Delta^9$-THC < CBG < CBN < CBD. The solubilities of CBN and CBD have the same order of magnitude. At 326 and 334 K (Fig. 6.4 and 6.5), the solubility order of CBN and CBD has changed: $\Delta^9$-THC < CBG < CBD < CBN. At the highest temperature (334 K), the data of CBD and CBG overlap within the experimental error. This is also the case for CBD and CBN at low pressures.

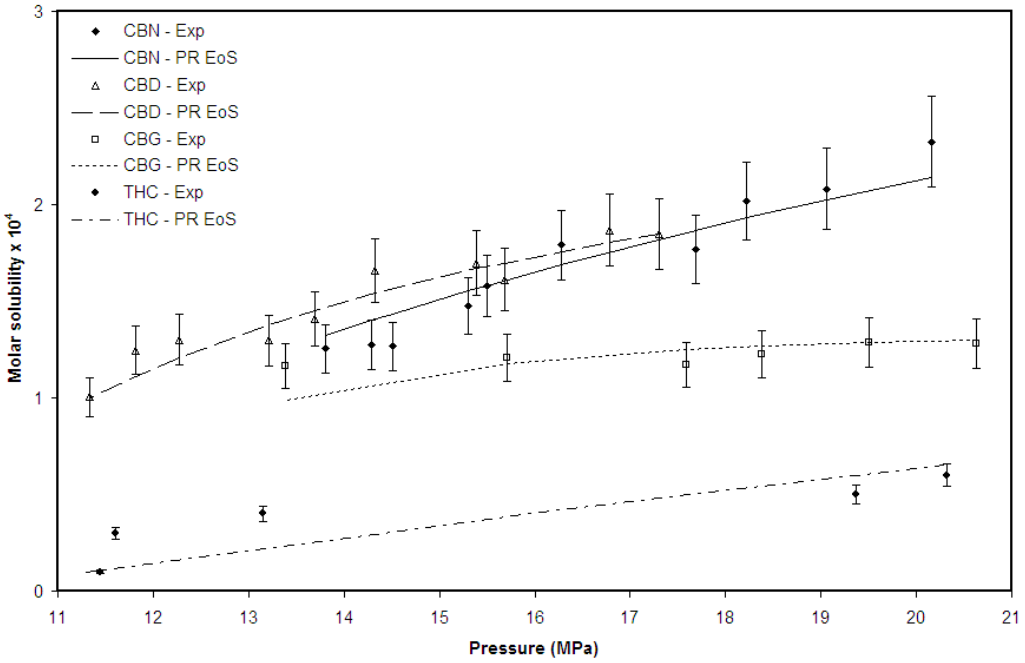



**Figure 6.3: Solubility of cannabinoids in supercritical $CO_2$ at 315 K**

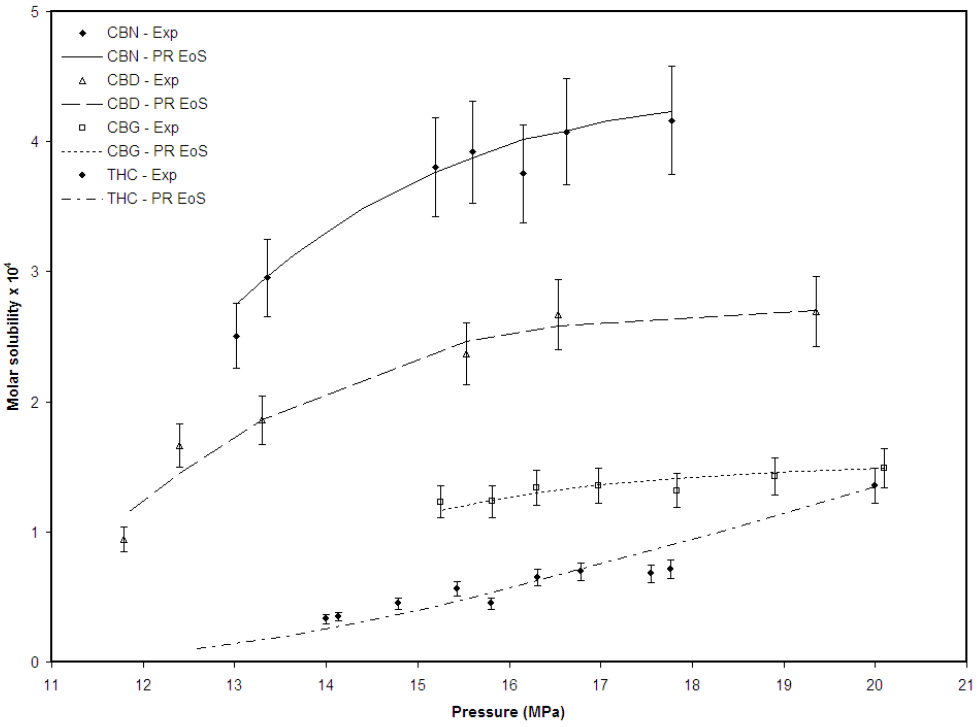



**Figure 6.4: Solubility of cannabinoids in supercritical $CO_2$ at 326 K**

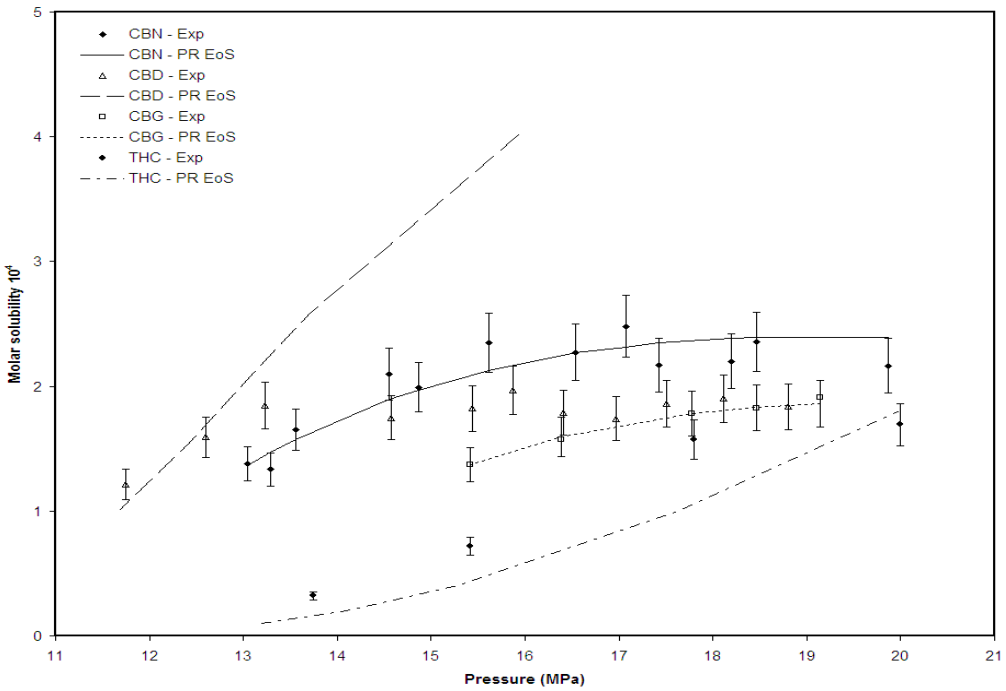



**Figure 6.5: Solubility of cannabinoids in supercritical $CO_2$ at 334 K**

In general, CBN shows the highest solubility in supercritical $CO_2$. CBN is the lightest cannabinoid among the four cannabinoids studied here, which might explain the higher solubility. However, the difference in molecular weight is rather small. Therefore, other factors such as differences in chemical structure and physical properties (melting point) may give a better explanation for the differences in solubility observed.

For example, CBN has the most aromatic character (6 double bonds), while $\Delta^9$-THC has the least aromatic character (4 double bonds). The aromatic character of CBG and CBD is in-between (5 double bonds). $CO_2$ interacts with the double bonds of the cannabinoids, resulting in a higher $CO_2$ solubility of the more aromatic compounds. This may explain why the solubility of CBN in supercritical $CO_2$ is generally the highest, and the solubility of $\Delta^9$-THC in supercritical $CO_2$ is generally the lowest.

Another explanation for the differences in $CO_2$ solubility may arise from the differences in melting point. It can be noticed that the liquid cannabinoids (CBD and CBN at 334 K, and $\Delta^9$-THC at all temperatures) show lower solubility in supercritical $CO_2$ compared to

the solid cannabinoids (CBD and CBN at lower temperatures, and CBG at all temperatures). This is consistent with the previous observation that melting results in a lower solubility of CBD and CBN in $CO_2$ at higher temperatures.

The cross-over pressure of the different cannabinoids increases in the order of: CBN < CBD < CBG < $\Delta^9$-THC. Interestingly enough, this shows the opposite trend with $CO_2$ solubility i.e. the cannabinoid with the highest cross-over pressure has the lower solubility in $CO_2$. This could also be the result of the melting point effect: the cannabinoid with the lowest melting point has the highest vapor pressure at given temperature. Because the crossing of solubility lines is a result of a trade-off between a density effect and a volatility effect, the cross-over pressure will be higher when the vapor pressure of a compound at given temperature is higher.

The solubility of the different cannabinoids in supercritical $CO_2$ is the order 1-2 g per kg $CO_2$, which is high enough for a supercritical extraction process. Because of the observed differences in $CO_2$ solubility, it is possible to separate the most psychoactive cannabinoid, $\Delta^9$-THC, from the other cannabinoids by varying the pressure in a two steps extraction. In a first step at lower pressure (~13 MPa), the non-psychoactive cannabinoids and a small part of the $\Delta^9$-THC are extracted. In a second step, pure $\Delta^9$-THC is extracted at higher pressure (~20 MPa). For example, $\Delta^9$-THC and CBD could be selectively extracted from the cannabis variety *Bediol* containing 5% $\Delta^9$-THC and 6% CBD. At 315 K and 13 MPa, the solubility of CBD is $1.3 \times 10^{-4}$ (= 0.9 g CBD per kg $CO_2$), while the solubility of $\Delta^9$-THC is only $0.3 \times 10^{-4}$ (= 0.2 g $\Delta^9$-THC per kg $CO_2$). Therefore, 67 kg $CO_2$ would be required to extract the 60 g of CBD present in 1 kg *Bediol*, while also extracting 13 g of $\Delta^9$-THC. The second step at 20 MPa and the same temperature (315 K) would require 74 kg $CO_2$ to extract the remaining 50 – 13 = 37 g of $\Delta^9$-THC, as its solubility is $0.65 \times 10^{-4}$ (= 0.5 g $\Delta^9$-THC per kg $CO_2$). It is thus possible to fractionate the cannabinoids and to extract pure $\Delta^9$-THC from cannabis with supercritical $CO_2$.

## 6.4 Conclusion

In this work, the solubilities of the non-psychoactive cannabinoids CBG and CBD in supercritical $CO_2$ have been measured at 315, 326 and 334 K and in the pressure range from 11.3 to 20.6 MPa. The experimental data are adequately represented by the PR-EoS, except for the CBD solubility in $CO_2$ at 334 K. The solubilities of other psychoactive cannabinoids ($\Delta^9$-THC and CBN) in supercritical $CO_2$ are compared with the present data. The $CO_2$ solubility behavior of CBG shows similarities to $\Delta^9$-THC (highest solubility at highest temperature), while the behavior of CBD shows similarities to CBN (highest solubility at medium temperature). This unexpected behavior of CBD and CBN can be related to the transition from a solid-supercritical fluid to a liquid-supercritical fluid equilibrium. All four cannabinoids (are expected to) show a cross-over region at pressures between 10 and 15 MPa. The differences in $CO_2$ solubility between the four cannabinoids can be explained by their differences in chemical structure and melting point, and can be used to separate them from each other by extraction and/or fractionation with $CO_2$.

## Acknowledgments

Financial support by STW (project no LFA 7426) is gratefully acknowledged. The authors would also like to thank Sara Salvador Cob for her experimental contribution to this work.

## Nomenclature

| | |
|---|---|
| a | attractive term of the PR EoS |
| b | covolume |
| A, B, m | equation of state parameters |
| $\Delta H$ | enthalpie ($kJ.mol^{-1}$) |
| k | partition coefficient |
| P | pressure (Mpa) |
| R | gas constant ($J.mol^{-1}.K^{-1}$) |
| T | temperature (K) |
| V | volume ($dm^3.mol^{-1}$) |
| y | solubility in the gas phase |
| Z | compressibility factor |

*Greek letters*

| | |
|---|---|
| $\alpha$ | temperature-dependant equation of state parameter |
| $\Phi$ | fugacity coefficient |
| $\omega$ | acentric factor |

*Sub / superscripts*

| | |
|---|---|
| b | boiling point |
| c | critical point |
| f | fusion point |
| i,j | component identification |
| r | reduced parameter |
| s | solid |
| fus | fusion |
| t | triple point |

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# 7

# Supercritical Fluid Extraction of Cannabis

Content to be submitted in *J. Supercrit. Fluids*

# 7


## *Abstract*

*Supercritical fluid extraction (SFE) using carbon dioxide was performed with Cannabis Sativa L. in a pilot scale set-up at 313 and 323 K in the pressure range from 18 to 23 MPa. The SFE yield of (-)-$\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC) is at maximum 98 %, which is comparable to classical hexane extraction. Cannabinol (CBN) and cannabigerol (CBG) can be extracted in higher amounts with SFE than with hexane extraction. Waxes are co-extracted with the cannabinoids. They can be easily removed via a winterization step. The purity of the final extract after winterization was 85 % $\Delta^9$-THC at the optimal experimental conditions found in these experiments. Correlation with the Sovova model confirmed the solubility of $\Delta^9$-THC measured in Chapter 4. With a two-steps extraction, it is possible to selectively extract minor cannabinoids (i.e. CBN, CBD and CBG) in a first step at low pressure (~15 MPa), and $\Delta^9$–THC in a second step at higher pressure (~20 MPa).*

# 7. Supercritical Fluid Extraction of Cannabis

## 7.1 Introduction

Cannabinoids can be extracted directly from cannabis by organic solvents (e.g. hydrocarbons such as hexane and alcohols) with a yield exceeding 90% [1]. However, these solvents are flammable and many of them are toxic. Supercritical Fluid Extraction (SFE) with carbon dioxide ($CO_2$) is a promising alternative technique. There are no flammability or toxicity issues, solvent removal is simple and efficient, and the extract quality can be controlled by tuning the pressure and temperature. This green solvent has been used for extraction of natural compounds, including pharmaceutical molecules, from plant material as described in chapter 2 of this thesis and literature [2-7].

In this work, SFE is performed at 313 and 323 K, in the pressure range of 18 to 23 MPa, and at a $CO_2$ flow rate of 4 and 6 $kg.h^{-1}$. The aim is to determine the optimal conditions to obtain the maximum extraction yield of tetrahydrocannabinol ($\Delta^9$-THC). The extraction of other cannabinoids, such as cannabigerol (CBG), cannabidiol (CBD) and cannabinol (CBN) is examined as well. This SFE method is compared to the conventional extraction method with hexane as it is done in literature [8]. The data are correlated with the Sovova model [9].

## 7.2 Experimental

### *7.2.1 Materials*

$CO_2$ grade 2.7 was purchased from Hoek Loos B.V. (Schiedam, the Netherlands). Methanol of HPLC reagent grade was purchased from J.T. Bakker. Standards of $\Delta^9$–THC, CBD, CBN and CBG were kindly donated by PRISNA B.V (Oostvoorne, the Netherlands). These materials were used without further purification. *Cannabis Sativa* plant material, cultivar Bedrocan, (dry female flower-tops), medical grade was obtained

from the Office of Medicinal Cannabis (The Hague, the Netherlands). The size of the flower-tops was reduced with a grinder.

As cannabinoids are present in their acid form in the plant, the fine powder was first decarboxylated under vacuum for 110 min at 110 $^{o}$C to obtain neutral cannabinoids (cf. Chapter 1 of this thesis). The total rate of decarboxylation was measured with HPLC analysis. After decarboxylation the cannabis has a $\Delta^9$-THC content of 14 ± 1 %, as determined by the HPLC method described hereafter.

### *7.2.2 Supercritical fluid extraction*

Figure 7.1 shows a schematic overview of the pilot plant used for the cannabinoids extraction with supercritical $CO_2$ [10, 11]. During a run, the cooling and heating system are switched on first and set to the desired temperature. Next, the extraction vessel is opened and filled with approximately 45 g of decarboxylated cannabis. After closing the vessel, $CO_2$ is continuously pumped from the storage vessel into the extraction vessel, which is kept at the required temperature by using a heating jacket. At the moment that the desired pressure is reached, the pressure transducer starts controlling the $CO_2$ flow into the separator. Via a condenser, the $CO_2$ is recycled to the storage vessel. During extraction, samples can be taken from the separator. After an extraction run, the extract is weighed and analyzed using the HPLC method described hereafter. The remaining residue of the cannabis plant material was also weighed to check the mass balance.

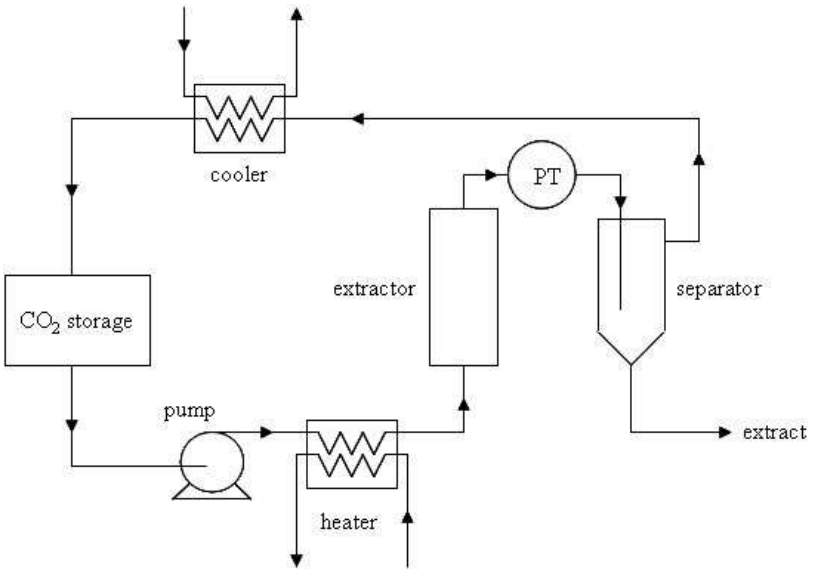


**Figure 7.2: Schematic overview of the experimental set-up for the supercritical extraction of cannabis**

### *7.2.3 Winterization process*

After each experiment, the extract can be dissolved in hexane and frozen for several days in order to precipitate the waxes. Thereafter, a simple filtration can be performed to isolate the waxes from the cannabinoids. This process is called winterization.

### *7.2.4 Classical extraction*

Liquid extraction with hexane was applied to compare both extraction methods. 400 mg of decarboxylated and ground cannabis was put in 50 mL hexane and sonicated during 15 minutes.

### *7.2.5 Analysis*

The particle size distribution was determined using a set of sieves with 1.70, 1.18, 0.85, 0.50 and 0.25 mm openings.

The HPLC profiles were acquired using a Chrompack HPLC system consisting of a gradient pump (Prostar 210), an injection valve and a UV-VIS detector (model 340 – Varian). The system was controlled by Galaxie Chromatography software. The profiles

were recorded at 228 nm, as absorption by the solute is at its maximum at this wavelength. The analytical column was a Vydac (Hesperia, CA) $C_{18}$, type 218MS54 (4.6 x 250 $mm^2$, 5 μm) with a Waters Bonapak $C_{18}$ (2 x 20 $mm^2$, 50 μm) guard column. The mobile phase consisted of a mixture of methanol-water in gradient mode: methanol – water in ratio from 30:70 to 100:0 over 32 min, then isocratic (without gradient) for 1 min. The column was re-equilibrated under initial conditions for 2 min. The flow rate was 1 $mL.min^{-1}$ and the total running time was 35 min [12]. All determinations were carried out at ambient temperature.

## 7.3 Modeling of the extraction curve

The Sovova model [9] has been used to correlate the experimental data by plotting the extract to feed ratio *e* (i.e. mass of extract / initial mass of cannabis) as a function of the solvent to feed ratio *q* (i.e. mass of $CO_2$ in contact with the solid / initial mass of cannabis). The slope of the curve should be equivalent to the solubility of the solute in the extractive at low solvent to feed ratios [9].

This model is based on the following hypothesis: for the solid phase, the bed of milled cannabis is considered as a bed of spherical particles containing a solute (the cannabinoids and other compounds). As illustrated in Figure 7.2, this solute is considered to be present in three different regions of the vegetal matrix:

(i) in the glandular hair, called trichomes. The solute present in the trichomes is considered as free or easily reachable by the solvent. The mass transfer coefficient of the extraction solvent in this solid phase is close to 0;
(ii) in the « broken cells »: as a consequence of the pre-treatment of the vegetal matrix, the mechanical stress imposed to the cells at the surface of the matrix can break them. The solute contained in those cells is also considered as free. The mass transfer coefficient of the extraction solvent in this solid phase is close to 0;
(iii) in the intact cells, the solute is trapped inside these cells and the mass transfer coefficient of the extraction solvent cannot be considered as negligible anymore.

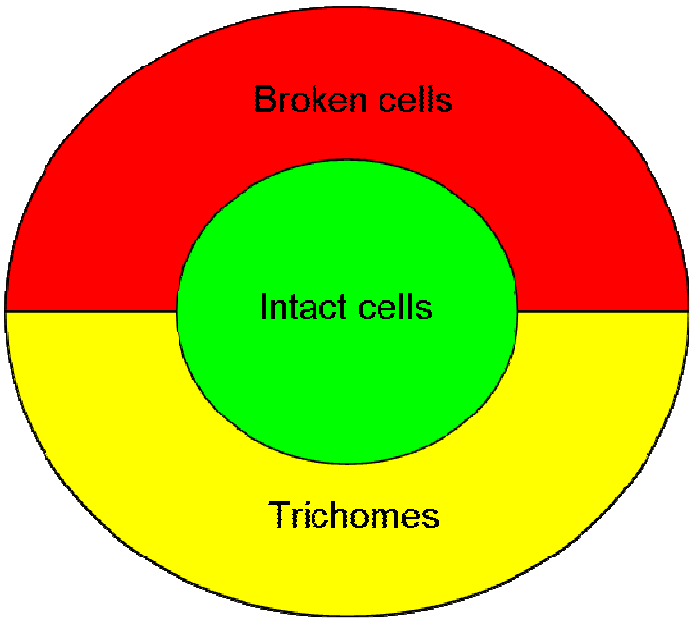


**Figure 7.2: Schematic drawing of the vegetable matrix**

Furthermore, the particle bed is assumed to be stable and stationary because the solvent velocity is too slow to fluidize the bed. At the end of the filling of the extractor, it is assumed that the solvent and the part of the solid containing free solute (i and ii) are in equilibrium, and the solvent is saturated with solute. However, the solute in the core of the particle, i.e. in the intact cells (iii), is unchanged. After filling, it is also assumed that the $CO_2$ flow inside the extractor is a plug-flow and that the solid contains only free solute (i and ii).

## 7.4 Results and discussion

### *7.4.1 Particle size distribution*

Figure 7.3 presents the particle size distribution of five different extraction batches, obtained by grinding and sieving. The particle size distribution has little influence on the extraction yield [6, 7, 13]. Therefore, this parameter is not studied here.

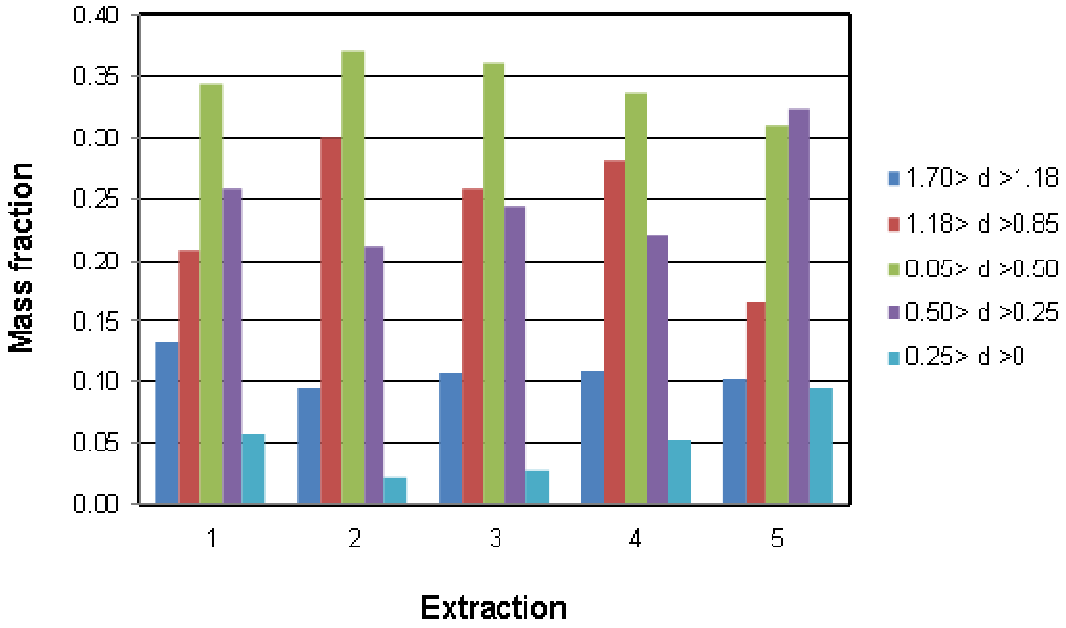


**Figure 7.3: Particle size d (mm) distribution for 5 extraction batches**

### *7.4.2 Pressure and temperature effects*

Figure 7.4 presents the total yield, defined as the ratio of the mass extracted ($m_{ex}$) divided by the mass of the initial cannabis sample ($m_{can}$), as a function of pressure between 18 and 23 MPa at 313 and 323 K. The deviation of the yield is based on the error of the balance used to weigh the mass extracted and the mass of the cannabis sample, as presented by equation (7.1):

$$\Delta Y = Y\left(\frac{\Delta m_{ex}}{m_{ex}} + \frac{\Delta m_{can}}{m_{can}}\right) \qquad (7.1)$$

With $\Delta m_{ex} = \Delta m_{can} = \pm 0.05g$ the deviation is less than ± 0.2 %. The experiments were performed at a $CO_2$ flow rate of 6 $kg.h^{-1}$ for 180 min. The highest total yield (23.3 ± 0.2 %) is obtained at the highest pressure (23 MPa) and lowest temperature (313K). This is due to the fact that the density of supercritical $CO_2$ is higher at higher pressure and lower temperature. However, at constant temperature, the differences in yield at different pressures are small (less than 2%) and can be due to the fact that yields are closed to

maximum obtainable yields and to experimental errors (natural products are not homogeneous). Therefore, it might be possible to neglect the influence of pressure on the total yield.

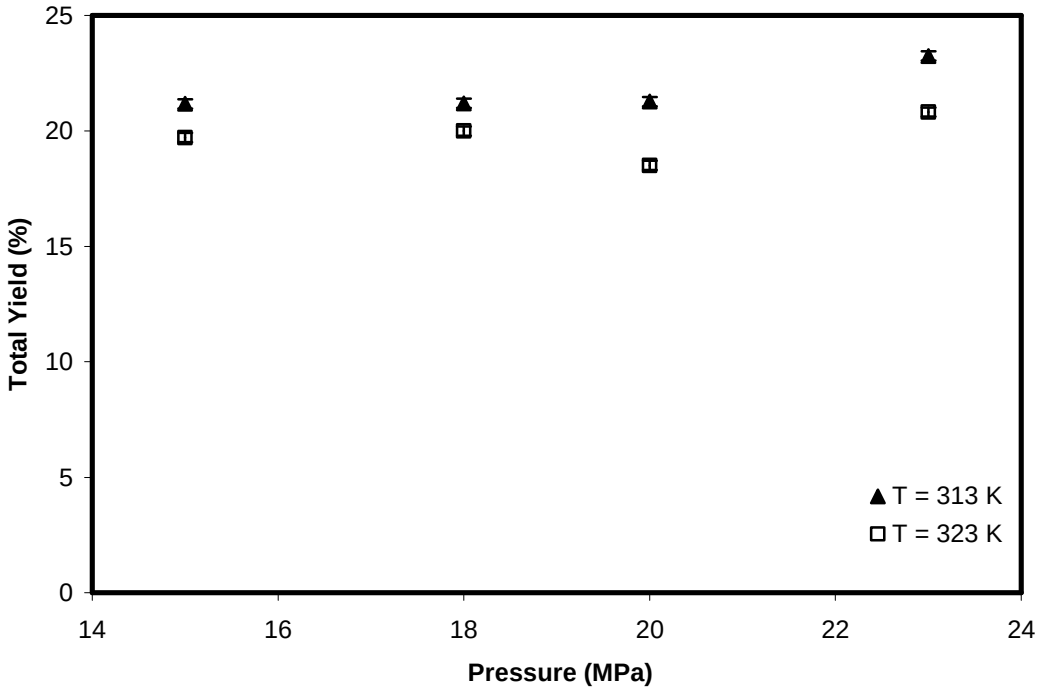


**Figure 7.4:Total Yield as a function of Pressure**

Figure 7.5 presents the $\Delta^9$-THC yield (*Y*), defined as the ratio between the mass of $\Delta^9$-THC extracted ($m_e$) and the initial $\Delta^9$-THC present in the cannabis vegetal matrix ($m_i$, determined by multiplying the initial cannabis mass and the percentage of $\Delta^9$-THC (*%*) measured with HPLC at the beginning of the experiments), as a function of pressure, at a $CO_2$ flow rate of 6 $kg.h^{-1}$ for 180 min. The deviation of the $\Delta^9$-THC yield is calculated according to equation 7.2 and its maximum is represented by the error bars in Figure 7.5:

$$\Delta Y = Y\left(\frac{\Delta m_e}{m_e} + \frac{\Delta m_i}{m_i} + \frac{\Delta\%}{\%}\right) \qquad (7.2)$$

With

$$\Delta m_e = \Delta m_i = \pm 0.05 g$$
$$\Delta\% = 1$$

Therefore, the overall deviation is ~ 5%. The $\Delta^9$-THC yield is higher at lower temperature (313K). This can be explained by the fact that at lower temperature the density of supercritical $CO_2$ is higher, and therefore the solvency power of $CO_2$ is higher, resulting in higher $\Delta^9$-THC solubility.

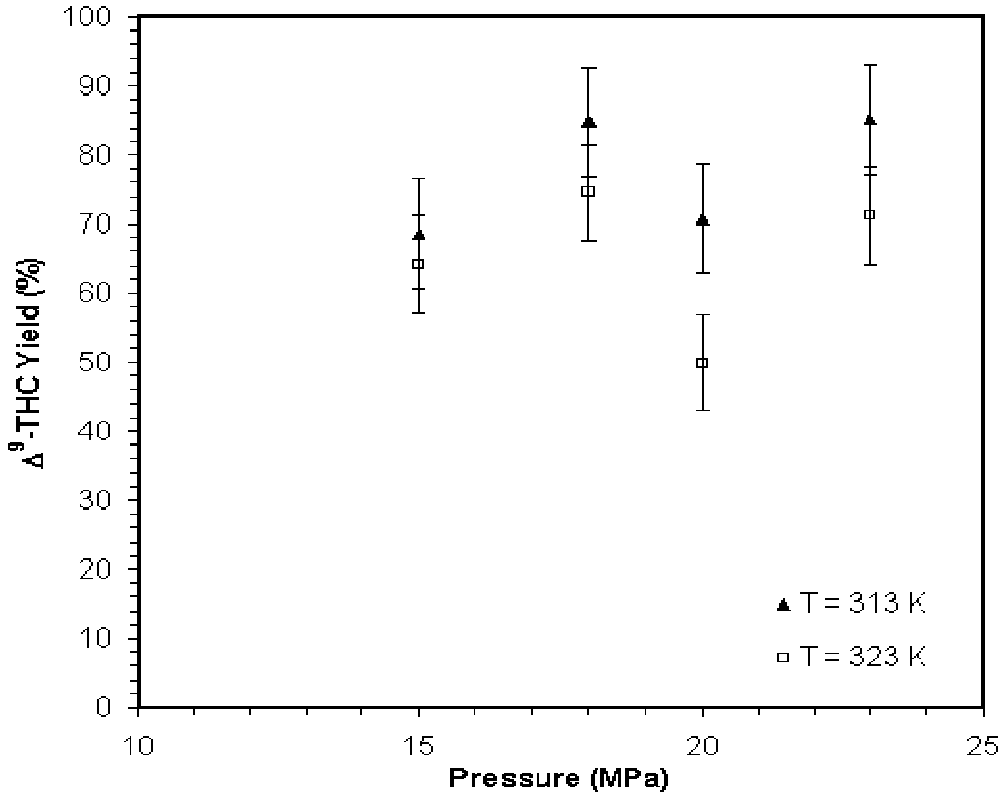


**Figure 7.5: $\Delta^9$-THC Yield as a function of Pressure at 313 and 323 K for 180 min**

### *7.4.3 Effect of time*

Figure 7.6 presents the $\Delta^9$-THC yield at 18 MPa as a function of time. At both temperatures (313 and 323 K), two trends are visible. First, the $\Delta^9$-THC yield increases linearly with time. Then, the $\Delta^9$-THC yield is constant, indicating that all $\Delta^9$-THC extractable at these conditions has been extracted. The first linear part seems to be temperature independent, whereas in the second part, the $\Delta^9$-THC yield is higher at the lowest temperature. The maximum $\Delta^9$-THC-yield obtained at this temperature is 98 %, meaning hexane extraction and SFE can extract the same amount of $\Delta^9$-THC. At 313 K, the maximum yield has been reached after 240 min of extraction. At 323 K, the maximum yield is reached faster (100 min) but its value is lower (74 %).

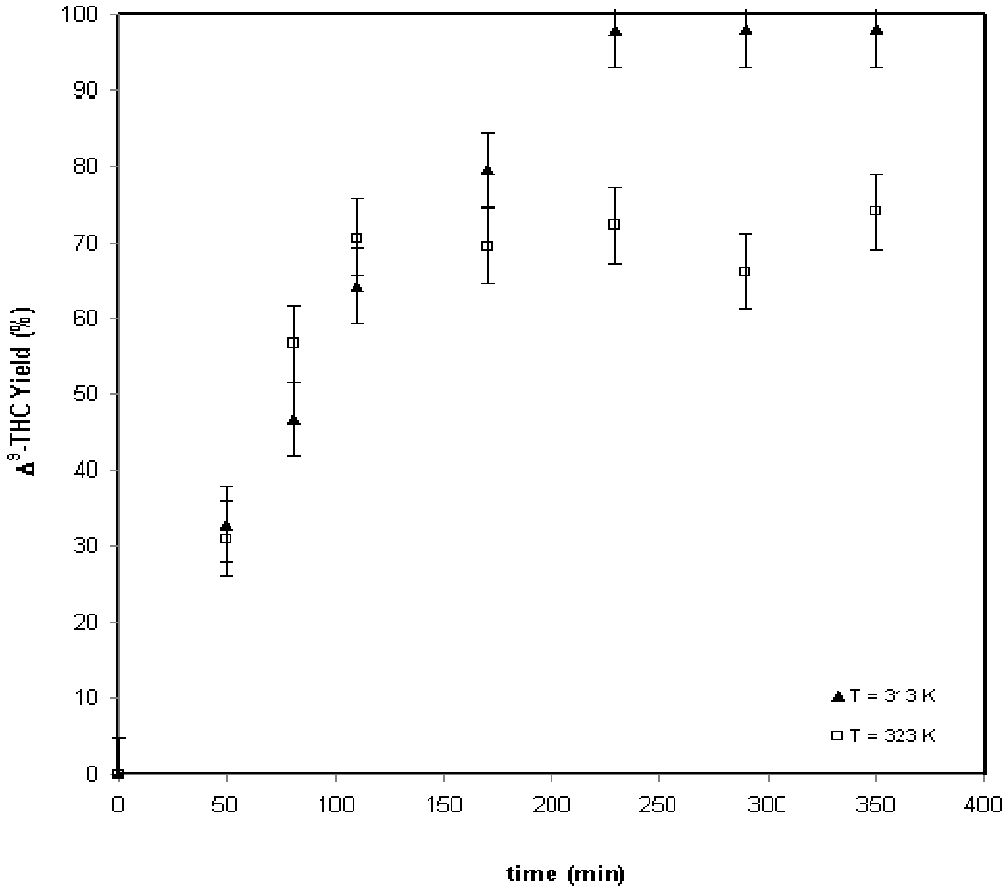


**Figure 7.6: $\Delta^9$-THC yield as a function of time at 18 MPa**

### *7.4.4 Extraction of other cannabinoids*

Figure 7.7 and Figure 7.8 present the yields of CBG and CBN at 18 MPa and several time intervals, at 313 and 323 K, respectively. Each yield was calculated by dividing the amount of cannabiniod extracted by the initial mass of cannabis. For each experiment, cannabis with the same composition was used. The highest yields are obtained at the lowest temperature (313 K). Their values are around 1.0 % and 1.6 % for CBG and CBN, respectively.

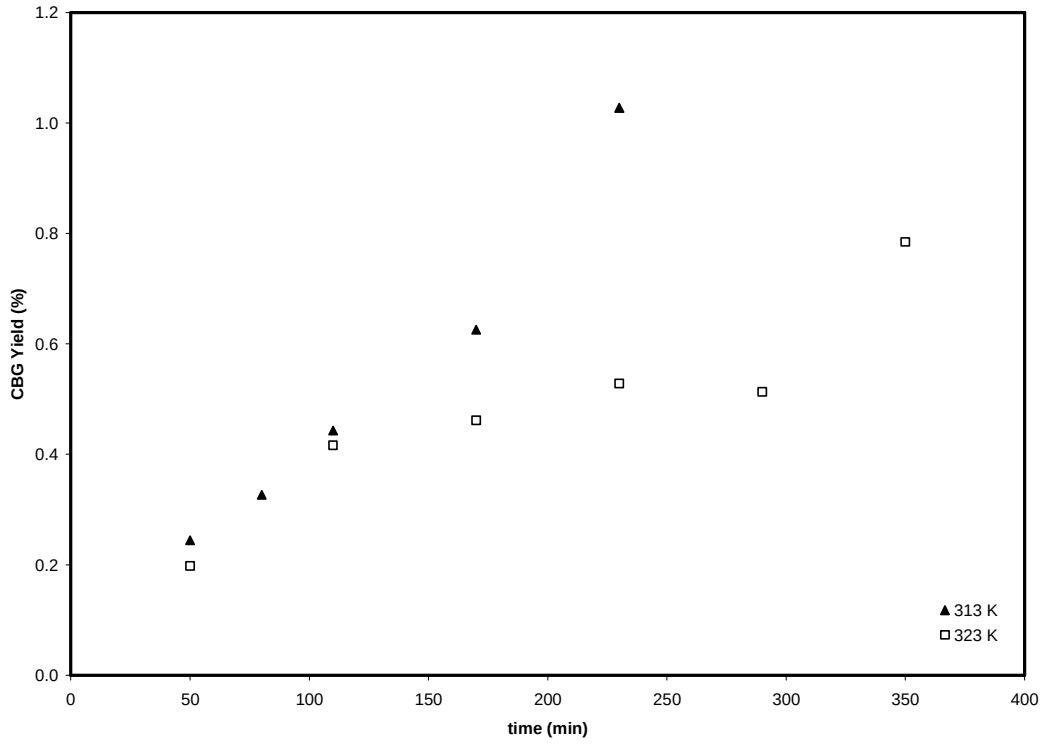


**Figure 7.7: CBG yield as a function of time at 18 MPa**

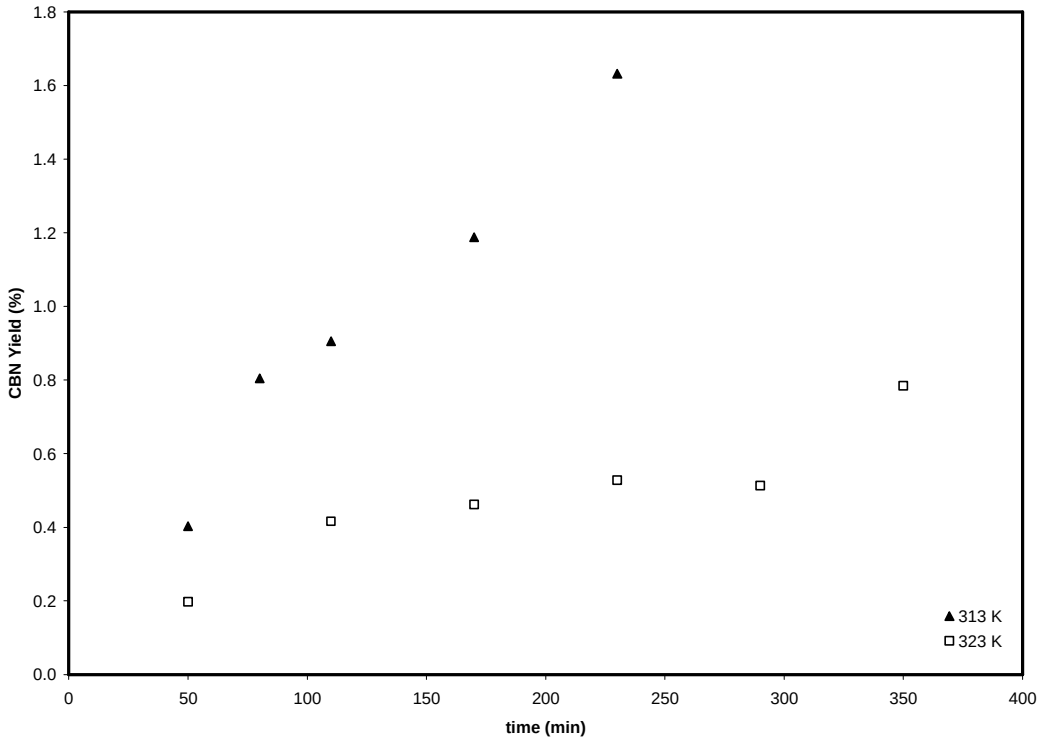


**Figure 7.8: CBN yield as a function of time at 18 MPa**

Figure 7.9 presents the yields of CBD, CBG and CBN as a function of pressure at 313 K. It can be seen that the yield is decreasing with a pressure increase for each cannabinoid. On the contrary, in Figure 7.5, the yield of $\Delta^9$-THC is stable with pressure. Therefore, it is expected that to improve the selectivity of the process, a two step extraction could be performed: the first step at low pressure (around 15 MPa) would extract CBD, CBG and CBN; the second step at high pressure (around 20 MPa) would allow an extract with a high purity of $\Delta^9$-THC. This is consistent with the findings developed in chapter 6 of this thesis.

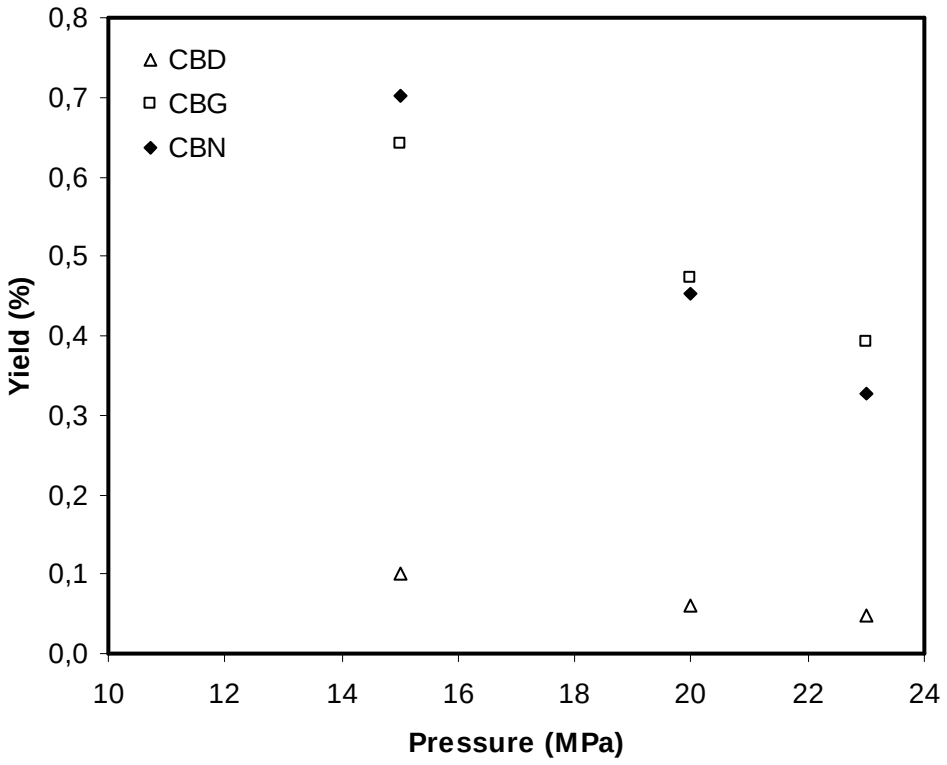


**Figure 7.9: Cannabinoids yields as a function of pressure at 313 K**

### *7.4.5 Comparison with classical extraction*

Extracts from hexane extraction and SFE were treated using the winterization process. The cannabis used was the same for both extraction methods (same age, same composition). The amount of waxes removed from the extract is around 10% of the initial mass of cannabis for both processes. The amount of cannabinoids present in the extracts after this step is shown in Table 7.1. It is assumed that only these cannabinoids are present in the extract after winterization. The values for the hexane extraction are the average of 11 different batches.

**Table 7.1: Composition (mass %) of extract obtained from SFE at 313 and 323 K at 18 MPa and hexane extraction after winterization**

| Compound | SFE at $T$ = 313 K | SFE at $T$ = 323 K | Hexane extraction |
|---|---|---|---|
| $\Delta^9$–THC | 84.7 | 85.3 | 85.9 |
| CBD | 0.0 | 0.0 | 0.4 |
| CBN | 9.4 | 8.7 | 7.8 |
| CBG | 5.9 | 6.0 | 5.9 |

It can be seen that both extraction methods have the same $\Delta^9$–THC yield, meaning that both methods extract the same amount of $\Delta^9$-THC. However, more CBG and CBN were extracted with SFE than with hexane. As shown in Table 7.1, CBD is present in the SFE extract in a very little amount. CBN, a degradation product of $\Delta^9$–THC, is present in a significant amount in both extracts, as the plant was stored for a long time before both experiments with SFE and hexane extraction were performed. It is expected that the amount of CBN would be lower if fresh cannabis was used.

### *7.4.6 Alternative to winterization process*

As mentioned in literature [4, 14] , the winterization process could become abundant when a two stage separator is used in SFE, i.e. a first decompression step at medium pressure to precipitate the waxes, followed by a second decompression step to recover the cannabinoids. To determine the pressure and temperature at the first decompression step, the solubility of cannabinoids should be checked. This technique would increase the rate of the process because the slow winterization step is no longer needed.

### *7.4.7 Correlation*

Figure 7.10 presents the solvent to feed ratio $q$ as a function of the extract to feed ratio $e$ for the experiments at 313 and 323 K. The $CO_2$ flow rate was 4 $kg.h^{-1}$ and the pressure was 18 MPa. The first part of the curve is correlated with the Sovova model, where the slope represents the solubility of the solute. Here the solubility of the solute is assumed to

be equivalent to the solubility of $\Delta^9$–THC, as this is the main compound which is extracted. At both temperatures, the slope is 0.7 $g.kg^{-1}CO_2$. This is equivalent to 1. $10^{-4}$ molar fraction of $\Delta^9$–THC, which is coherent with the solubility of $\Delta^9$–THC reported in chapter 4 of this thesis.

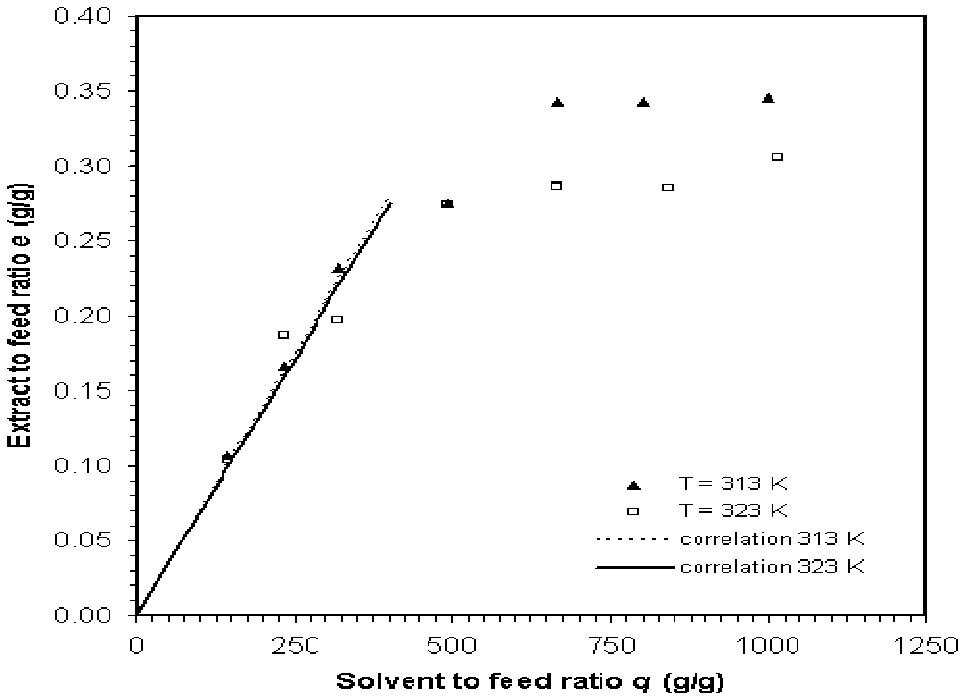


**Figure 7.10: Extraction curves at 313 and 323 K - experimental conditions: 18 MPa - 4 $kg.h^{-1}$**

## 7.5 Conclusion

In this chapter, SFE of cannabis is presented at 313 and 323 K in the pressure range 15 – 23 MPa. The same amount of $\Delta^9$–THC is extracted with SFE as with hexane. Waxes co-extracted with cannabinoids can be separated using a winterization step. The final extract contained about 85 % $\Delta^9$–THC after the winterization step. More CBN and CBG is extracted with SFE than with hexane extraction. The correlation using the Sovova model confirms the solubility of $\Delta^9$–THC (around 0.7 $g.kg^{-1}CO_2$), as previously measured with the quasi-flow apparatus (chapter 4 of this thesis). With a two-step extraction, it is

possible to selectively extract minor cannabinoids in a first step at low pressure (~15 MPa), and $\Delta^9$–THC in a second step at higher pressure (~20 MPa).

## Acknowledgements

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# 8

# Centrifugal Partition Chromatography

# 8


## *Abstract*

*The aim of this chapter is to present the combination of two technologies,- i.e. centrifugal partition chromatography (CPC) and supercritical technologies - in order to obtain a green and efficient process for the separation and purification of natural compounds. This new process is called supercritical CPC. CPC uses a two-phase liquid system, instead of a solid stationary phase, as it is the case in High Pressure Liquid Chromatography (HPLC). Separation is realized by partitioning of compounds between the two phases.*

*This chapter describes the different steps to build and design such a machine and process and the limitations of this process. It seems that liquid/liquid partitioning chromatography with supercritical $CO_2$ has interesting perspectives for separations with different selectivity than other separation methods. However, although many obstacles were solved, the technical side needs more engineering efforts to further develop this technology*

*Additionaly, the sucessful separation of $\Delta^9$- THC, CBN and CBG is presented using the two-phase system hexane / acetone / acetonitrile. A purity higher than 99% is achieved with $\Delta^9$- THC. With CBN and CBG the best purity obtained is higher than 90%.*

# 8. Centrifugal Partition Chromatography

## 8.1 Introduction

Centrifugal partition chromatography (CPC) is a chromatography technique where both mobile and stationary phase are liquid. The stationary phase is retained by centrifugation while the mobile phase is pumped through the stationary phase. The separation of components is done by differentiation in partition coefficients; components with lowest affinity with the stationary phase will elute first. Compared to High Performance Liquid Chromatography (HPLC) where the stationary phase is a solid, one of the advantages of this technique is the reversibility of the separation. As the stationary phase is a liquid, it can be eluted at the end of a run and each component can be recovered. There is no loss. Moreover, CPC has a larger capacity than HPLC because of the large volume of stationary phase involved in the separation process. An additional advantage of the CPC is that it can separate compounds with a broad scale of polarities. A schematic drawing of the CPC is shown in Figure 8.1. It is composed of a pump, a detector, a recorder, a sample loop, two valves (one to inject the sample and another one to select the ascending or descending mode) and the CPC itself. A complete description of CPC theory can be found in literature [1-6].

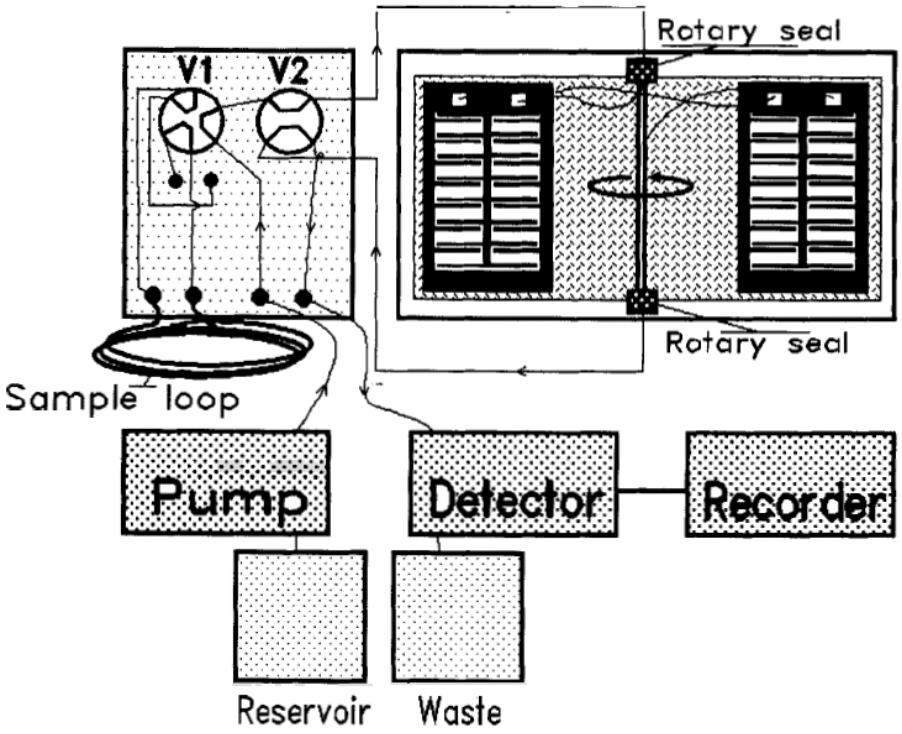


**Figure 8.1: the general set-up in the descending mode. V1 = infection valve - V2 = switching valve (ascending or descending mode) [4]**

CPC can be used for a wide range of applications, for example, for the separation of natural products [7], including bitter acids from hop extracts [8-10], and cannabinoids [11].

Organic solvents are often used as mobile and stationary phase in CPC. However, many of them are toxic. For natural compound to be used in food or pharmaceutical applications, it is important to remove the toxic solvents from the CPC product stream as much as possible. This requires an extra separation step, leading in an expensive process and with high energy consumption. Therefore, a process using only generally regarded as safe (GRAS) solvents is preferred. Supercritical carbon dioxide ($CO_2$) is a GRAS solvent and can be easily separated from the natural compound after CPC by pressure decrease. Therefore, no extra separation step is needed. GRAS solvents that can be used in combination with supercritical $CO_2$ are e.g. water and ethanol. The phase behavior of the

two-phase system containing water, ethanol and supercritical $CO_2$ has been widely studied in literature [12-17].

There are currently no commercial systems available for CPC with supercritical $CO_2$ (the commercially available CPC systems are not designed for the required pressure).

Yu and co-workers have introduced $CO_2$ in counter-current chromatography (CCC) where the stationary phase is retained by gravity [18, 19]. The working principles are the same as for CPC, except that the gravity force to retain the stationary phase in the CCC is replaced by a centrifugal field in the case of the CPC. Therefore, the geometry of the CCC column is different. Yu and co-workers were able to separate naphthalene, benzophenone and acetophenone with supercritical $CO_2$ as the mobile phase, and methanol / water (3/7 – v/v) as the stationary phase [18]. Yeh and co-workers reported the separation of three different steroids by CCC using SC $CO_2$ as mobile phase [20]. According to the authors, more work needs to be done to optimize this process for a preparative scale [18-20].

In this chapter, the different steps to build and design such a machine and process is described. The limitations of this process are presented as well. Additionaly, the sucessful separation of $\Delta^9$- THC, CBN and CBG is presented using a two-phase system with organic solvents.

## 8.2 Experimental

### *8.2.1 Set-up*

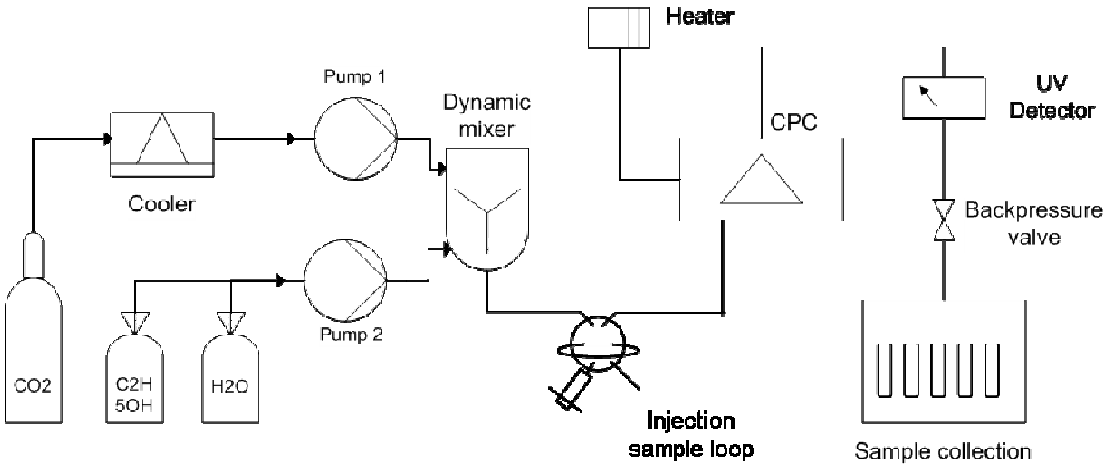


**Figure 8.2: Schematic drawing of the experimental set-up**

A schematic diagram of the experimental set-up is shown in Figure 8.2 and a picture is shown in Figure 8.3. A Sanki (Kyoto, Japan) centrifugal partition chromatograph (model FCPC ® 200) equipped with a 200 mL cartridge, adapted to work at pressures up to 20 MPa was used. The total volume of the separation column was 200 mL. To have a raised temperature inside the rotor, a Lauda oil bath was connected to the internal chamber of the CPC that is in contact with the rotor. A Vici manual injector with a 5mL loop was used to inject the components to be separated.

The mobile and stationary supply system consists of two preparative pumps (Separations - model 1800), a Lauda cooling bath for cooling of $CO_2$ and a dynamic mixer chamber (Knauer).

For analysis of the product from the CPC, a UV detector (Separations - Smart line model 1500) that monitors the absorbance of the solutes was connected to a computer

with Galaxie software. A back pressure valve was used to maintain the pressure of the system up to 20 MPa.

To prevent blockage of tubing by $CO_2$ expansion (when $CO_2$ is the mobile phase), the outlet tubing was heated by heating tape for the second set of experiments.

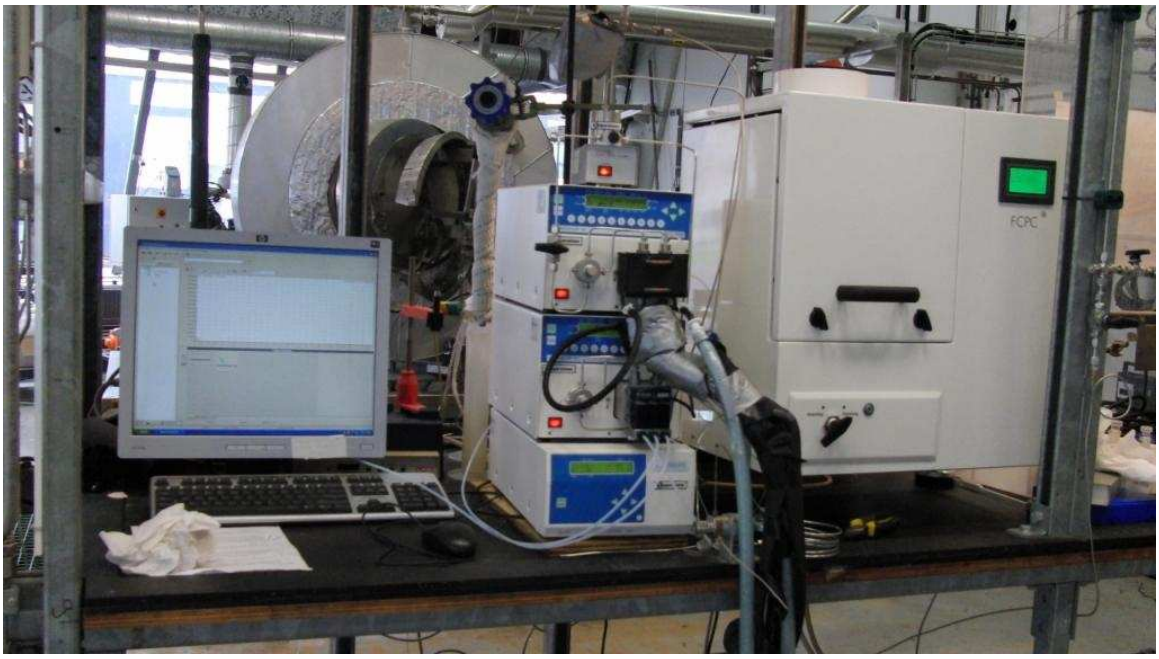


**Figure 8.3: CPC set-up**

### *8.2.2 Procedure*

#### *8.2.2.1 Experiments with supercritical $CO_2$*

The day before starting an experiment, the heating bath was switched on to obtain the desired temperature in the rotor. Prior to using the CPC for separation, the system needs to be filled with the stationary and mobile phase. Both phases can be the role of the stationary or mobile phase. When the heavier phase is the mobile phase, the descending mode is used. In descending mode, the stationary phase was $CO_2$. When the $CO_2$ was coming out of the $CO_2$, the pump to introduce the mobile phase was started. To reach the equilibrium between the 2 phases, the mobile phase was recycled by putting the outstream of the CPC back to the containers until the output and input flows were the same. The input flow was indicated by the pump, whereas the output flow was measured with the graduated cylinder. Before equilibrium was reached, the fraction collector was not used.

When equilibrium between the two phases was reached, the components to be separated were injected and rotation was started. The rotation speed was 750 RPM. The signal of the UV detector indicated when a component was coming out of the CPC. The components were then collected and further analyzed with Thin Layer Chromatography (TLC). The fractions containing the interesting products were further analyzed with High Performance Liquid Chromatography (HPLC). In ascending mode, the $CO_2$ was the mobile phase as it was the lighter phase.

#### *8.2.2.2 Experiments with organic solvents*

The two phase system was hexane / acetone / acetonitrile, in the proportions (5/2/3 – v/v/v).

it was prepared and stirred at least four hours prior to an experiment, in order to assure equilibrium between the two phases. At the beginning of an experiment, the stationary phase was loaded at 20 $mL.min^{-1}$ and 600 RPM in opposite to the operational mode; ascending for the heavier phase and descending for the lighter phase. The mobile phase was loaded at the flow rate and centrifugal speed of the specific experiment in the operational mode; descending for the heavier phase and ascending for the lighter phase. The stationary phase that came out of the apparatus was measured in 250 mL graduated cylinder.

### *8.2.3 Materials*

$CO_2$ was purchased from Hoek Loos with a purity of 99.7% (quality 2.7). Water was of ultra pure grade. All solvents were of HPLC grade, purchased from J.T. Baker. $BminBF_4$ was synthesised in our own laboratory. Naphthalene with a purity of 99%, Beta-carotene with purity higher than 97% and potassium dichromate with a purity higher than 99% were purchased from Sigma-Aldrich. The cannabinoid mixture used was the result of the SFE experiments done at 18 MPa and 313 K (See chapter 7 of this thesis).

## 8.3 Results and discussion

### *8.3.1 Experiments with $CO_2$ – ethanol – water*

The equipment was used in the descending mode ($CO_2$ as stationary phase). The composition of the mobile phase was ethanol – water (1/9, v/v).

Experiments were first performed with 1-butyl-3-methylimidazolium tetrafluoriborate ($BminBF_4$). and a cannabis extract. The cannabis extract contained at least four components seen by TLC analyse [22]. $BminBF_4$ is an ionic liquid that is not soluble in supercritical $CO_2$. Therefore, BminBF4 is not retained by the two-phase system and it should indicate the minimum residence time of the mobile phase, as it comes out as soon

as the mobile phase comes out. Before being injected, $BminBF_4$ was dissolved in ethanol-water in the same proportions as in the mobile phase.

The ionic liquid $BminBF_4$ was injected at different pressures. The plot of its retention time as a function of the pressure is depicted in figure 8.3. At 18 MPa and 315 K, three samples were injected, giving similar results, i.e. a peak after around 10 minutes. As can be seen from figure 8.1, the results were less reproducible at lower pressures. This plot shows that the retention time of $BminBF_4$ decreases with pressure increase.

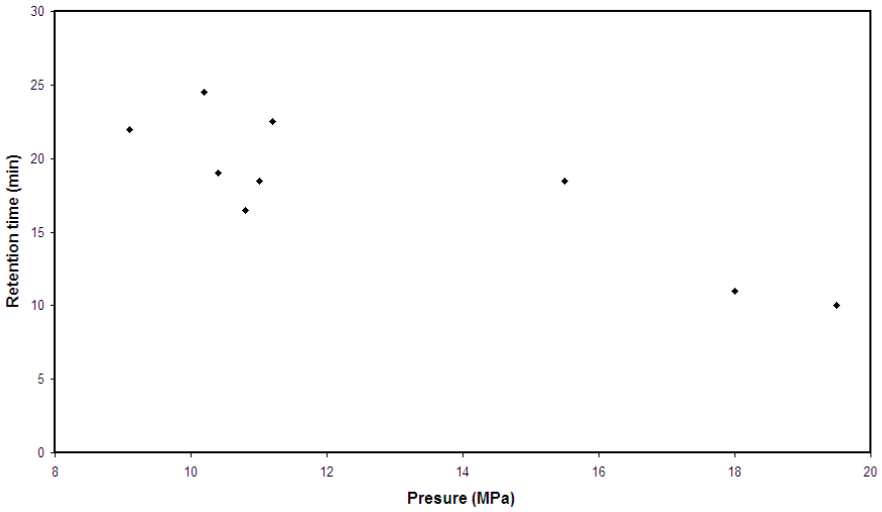


**Figure 8.4: retention time of $BminBF_4$ as a function of the pressure**

As a second step, a cannabis extract was injected at the same previous experimental conditions. The cannabis extract contained at least four components seen by TLC analyse [22]. The extract had a retention time of 12 minutes. As shown in figure 8.4, the shape of the cannabis peak indicates that the concentration was very high and it shows some separation.

As can be seen in figure 8.4, the $BminBF_4$ and cannabis peaks did not overlap. To test the separation of these compounds by CPC a mixture of the ionic liquid and the cannabis extract was injected at the same previous experimental conditions. However, only one

peak appeared after 16 minutes. This might be due to the ion pairing between the cannabinoids, which are acids, and the ionic liquid.

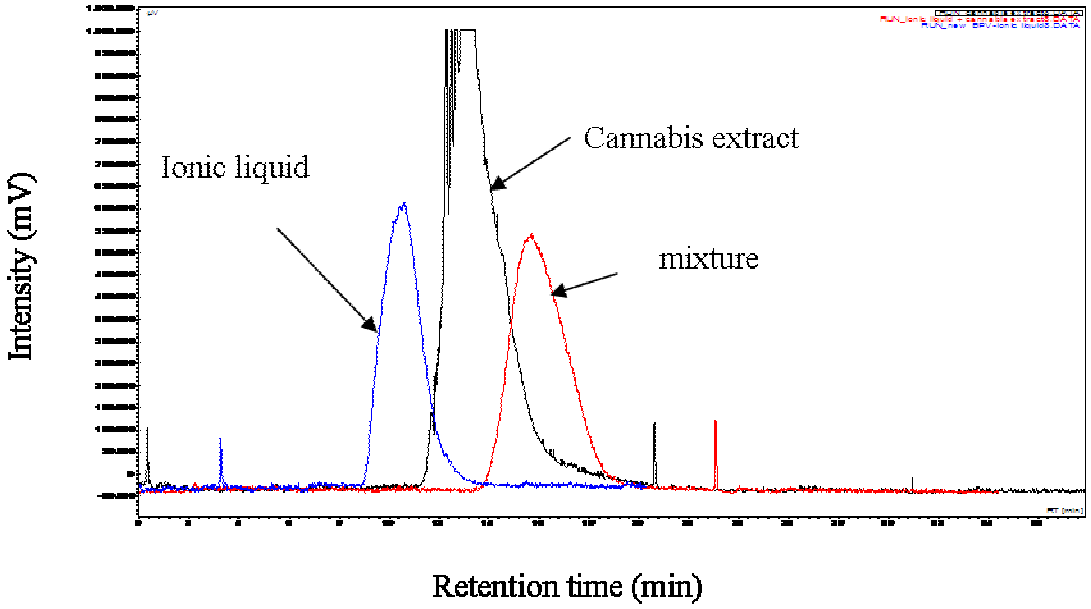


**Figure 8.5: Intensity as a function of the retention time - superposition of 3 experiments at 18 MPa**

These first results are not very promising. By changing the conditions of the experiments, separation may be improved. For example, the pressure and / or the composition of the mobile phase could be adjusted. Unfortunately, the rotary seals in the used set-up cannot support required pressure. Therefore, the composition of the mobile phase has been adjusted, as described in the next paragraph.

### *8.3.2 Experiments with $CO_2$ – methanol – water*

Ethanol was preferred over methanol because it is less toxic. However, since no satisfactory results were obtained with ethanol, the system $CO_2$ – methanol – water was used. The proportions of methanol – water phase were varied from 3/7 to 1/9 (v/v).

Because of failure of the UV-detector, visual detection was used. The tested compounds were beta-carotene and potassium dichromate, which are respectively orange and yellow. Beta-carotene is apolar, therefore its affinity with $CO_2$ is expected to be high. Potassium

dichromate is very polar, and therefore not soluble in apolar solvents such as $CO_2$. Therefore, it should stay in the aqueous phase. By changing the mode during the separation, i.e. by switching from ascending mode after beta-carotene was out, to descending mode, it was possible to separate beta-carotene from potassium dichromate.

### *8.3.3 Experiments with organic solvents*

$\Delta^9$-THC, CBG and CBN were separated with the two-phase system hexane/acetone/acetonitrile in the proportions 5/2/3 (v/v/v). All separations were performed at the highest allowable rotational speed – 1200 RPM, analytical cannabinoid concentration – 1 $g.L^{-1}$, and three flow rates – 20, 15 and 10 $mL.min^{-1}$. Figure 8.6 is an example of chromatogram of the separation of $\Delta^9$-THC, CBG and CBN in ascending more, 1200 RPM and 20 $mL.min^{-1}$. At 10 $mL.min^{-1}$, the efficiency is lower than at 20 $mL.min^{-1}$. However, it is compensated by the higher stationary phase volume and corresponding higher retention volume. At 15 $mL.min^{-1}$, the stationary phase volume is comparable to the one at 20 $mL.min^{-1}$ but the efficiency is comparable to the one at 10 $mL.min^{-1}$. Therefore, its resolution is the lowest.

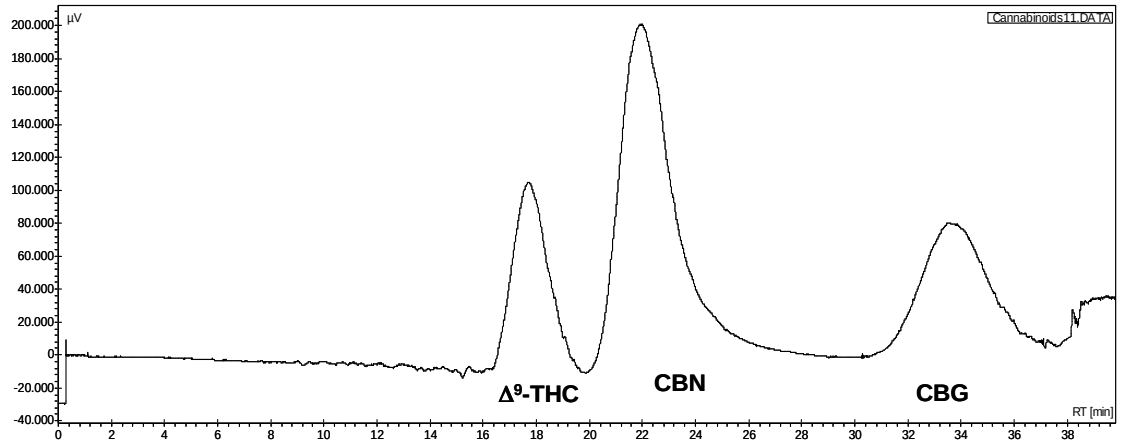


**Figure 8.6: Separation of $\Delta^9$-THC, CBN and CBG with hexane / acetone / acetonitrile (5/2/3 -v/v/v) in ascending mode - 1200 RPM - 20 $mL.min^{-1}$**

Experiments have also been performed with samples of cannabinoids extracted with supercritical carbon dioxide, as described in the chapter 7 of this thesis. At 10 $mL.min^{-1}$ and 83.33 $g.L^{-1}$ extract mass load, an acceptable separation of cannabinoids was achieved as illustrated in Table 8.1. For $\Delta^9$-THC, a purity of 100 % (i.e. no other cannabinoids were present according to HPLC analysis) was achieved for 6 min. Then the concentration of $\Delta^9$-THC was descreasing. After 45 min, the CBN peak appeared with a maximum concentration of 96.5 %. The rest was identified as CBG. At 70 min, the peak containing 92.3 % CBG appeared. The rest was identified as CBN.

**Table 8.1: Composition of selected fractions determined by HPLC analyses during the separation of $\Delta^9$-THC, CBG and CBN**

| Fraction (min) | $\Delta^9$-THC (%) | CBN (%) | CBG (%) |
|---|---|---|---|
| 33-39 | 100.0 | 0.0 | 0.0 |
| 39-40 | 99.1 | 0.2 | 0.7 |
| 40-41 | 98.5 | 0.3 | 1.2 |
| 42-43 | 81.9 | 18.1 | 0.0 |
| 44-45 | 0.0 | 87.9 | 12.1 |
| 45-46 | 0.0 | 92.4 | 7.6 |
| 46-47 | 0.0 | 96.5 | 3.5 |
| 47.48 | 0.0 | 94.5 | 5.5 |
| 70-71 | 0.0 | 7.7 | 92.3 |
| 72.73 | 0.0 | 9.0 | 91.0 |
| 76-77 | 0.0 | 8.8 | 91.2 |

## 8.4 Conclusions and recommendations

CPC with supercritical $CO_2$ has interesting perspectives for separations with different selectivity than other separation methods. However, engineering efforts are necessary to further develop this technology (e.g. development of rotary seals).

With organic solvents, the CPC is a powerful equipment to separate components in a high purity. A successful separation of $\Delta^9$-THC, CBD and CBG has been achieved, with a $\Delta^9$-THC purity higher than 98%. The best purities obtained for CBD and CBG are higher than 92 %.

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# 9

# Economical and environmental evaluation

# 9


## *Abstract*

*This chapter presents an economical and ecological evaluation of two production routes to obtain pure delta-9-tetrahydrocannabinol ($\Delta^9$-THC): the current process using organic solvents is compared with the alternative process developed in this thesis using supercritical carbon dioxide. The alternative process is significantly cheaper than the current one, although the high price of the starting material cannabis dominates the ultimate cost price. From an ecological point of view, the alternative process is also more sustainable as it consumes less energy and generates less waste. Therefore, this alternative process is preferred from an economical and ecological point of view.*

# 9. Economical and environmental evaluation

## 9.1 Introduction

The consumption and use of cannabis and its pharmacologically active components, the so-called cannabinoids, is almost entirely dominated by its wide spread abuse as a drug. Notwithstanding several reports on adverse effects [1-3] of mainly chronic use of cannabis, there is renewed interest in cannabinoids, and especially in $\Delta^9$-THC, for medical applications, like the treatment of severe chronic pain, multiple sclerosis, glaucoma, and the side effects (nausea) of chemotherapy in cancer treatment.

The alternative process using supercritical carbon dioxide ($CO_2$) will be compared with a described process to produce $\Delta^9$-THC, in patent US 2005 / 0171361 and in patent WO 2009 / 133376 [5, 6]. Both economical and environmental aspects will be taken into account.

## 9.2 Market size

Of the pure cannabinoids $\Delta^9$-THC and CBD only are used as a medicine, e.g. in Sativex. Other pure cannabinoids are not registered yet. The development of other possible medicines based on pure cannabinoids others than $\Delta^9$-THC is still in the research phase. Thus the focus will be on $\Delta^9$-THC only.

To estimate the potential market for $\Delta^9$-THC, its major applications are considered. The major application of $\Delta^9$-THC is its palliative use as a pain killer [7] instead of morphine, currently still used frequently. The current production of morphine in Western Europe and in the United States was 10 and 18 ton, respectively, in 2005 [8]. The application of morphine as pain killer for terminal (cancer and HIV / AIDS) patients has drawbacks such as the high amount needed (20 to 720 $mg.day^{-1}.patient^{-1}$) which causes side effects such as hallucination, constipation, and respiratory depression [9]. 10-30% of the patients

cannot tolerate the morphine [10]. An alternative is then mandatory. The use of $\Delta^9$-THC may be the solution and solve most of these drawbacks. Furthermore $\Delta^9$-THC counteracts nausea associated with cancer chemotherapy and stimulates appetite. From already these applications, it can be concluded that the potential market for $\Delta^9$-THC might be substantial. Taking into account the lower dose needed, the replacement of 20% of medical morphine by $\Delta^9$-THC, seems realistic and would lead to a market size of at least ~30 ton * 20% = 6 000 kg morphine ~ 500 kg $\Delta^9$-THC annually. It is assumed that the current process should take 10% market share of this size of the market. Thus, the preferred process size is 50 kg $\Delta^9$-THC annually.

## 9.3 Processes description

### *9.3.1 Conventional processes*

There are two conventional process routes described in literature: (i) in patent US 2005 / 0171361 and (ii) in patent WO 2009 / 133376 [5, 11]. Both processes can be divided in three main parts: decarboxylation, extraction and purification.

As illustrated in Figure 9.1, in case of the patent US 2005 / 0171361, 7 different types of equipment (different colors in figure) are used to perform 15 different process steps:

- 1 extraction unit to perform 4 extraction steps with subsequently heptane, isopropylether, aqueous solvent and methyl-butyl-ether (MTBE);
- 1 distillation column used for 3 different steps;
- 1 reactor unit to heat the cannabinoids under reflux in order to perform the decarboxylation reaction;
- 2 types of filtration units: (i) 1 charcoal filtration used 2 times, and (ii) 1 polish filtration at the end of the process;
- 1 evaporation unit used 3 times to remove the organic solvents in different steps of the process;
- 1 reversed phase chromatography column used in the purification step.

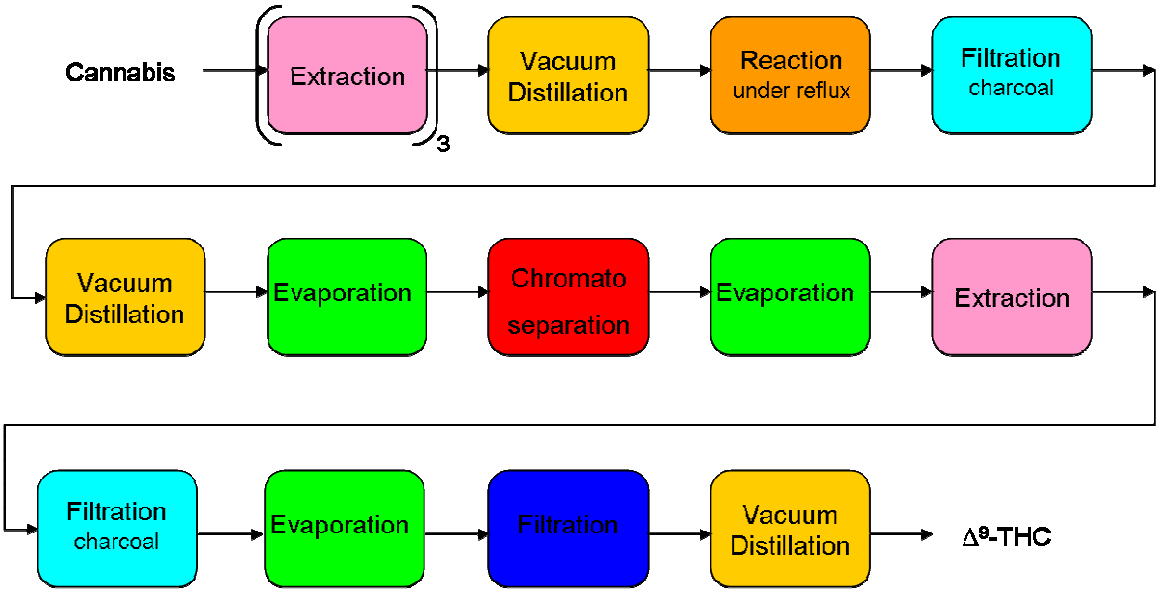


**Figure 9.3: Flow diagram for the $\Delta^9$-THC Production according to patent US 2005 / 0171361**

According to HPLC analyses mentioned in the patent, a purity of 99.7% $\Delta^9$-THC is achieved after removal of residual solvent. It is assumed that every process step will lead to a loss of ~ 2 %. Therefore, the overall yield of this process is estimated at ~ 70 %.

As illustrated in Figure 9.2, in case of the patent WO 2009 / 133376, 8 different types of equipment are used to perform 17 different process steps:

- 1 extraction unit, in which the cannabinoids are extracted 3 times with n-heptane in the extraction part; and the same extraction unit is also used 1 time with MTBE in the isolation part;
- 3 filtrations units: (i) in the extraction part a specific filter is used 3 times, (ii) 1 celite ® pad filter is used, and (iii) 1 filtration unit based on charcoal is used 2 times in the purification part;
- 1 reactor unit to heat the cannabinoids under reflux in order to perform the decarboxylation reaction;
- 1 distillation column for the first step of the purification part;
- 1 evaporation unit used 4 times to remove the organic solvents in different steps of the process;

- 1 reversed phase chromatography column in the purification part.

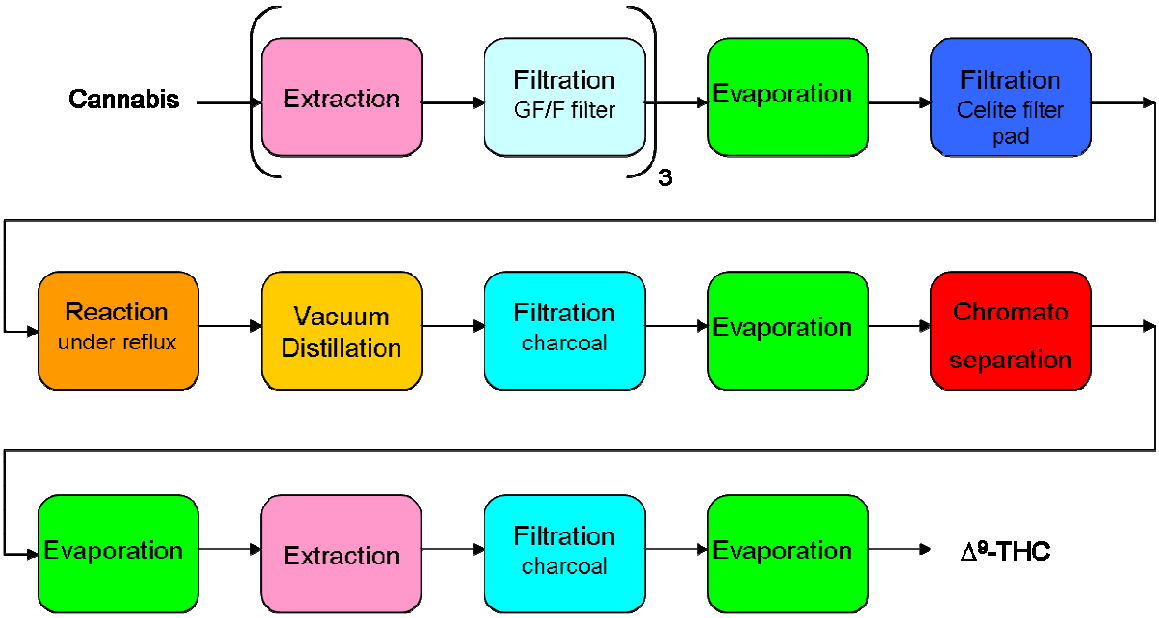


**Figure 9.4: Flow diagram of the $\Delta^9$-THC Production according to patent US 2009 / 133376**

A purity of at least 99% $\Delta^9$-THC is achieved with this process. As there are 17 process steps, the overall yield is estimated to be ~ 66 %.

As the patent WO 2009 / 133376 presents higher equipment and variable equipment costs, the patent US 2005 / 0171361 is preferred. It will be called the Best Available Technology (BAT) [4] and compared with the alternative process, presented hereafter.

### *9.3.2 Alternative process*

Figure 9.3 presents a block diagram of the alternative process. The process consists of 4 different pieces of equipment which are used to perform 6 different process steps:

- 1 Reactor/supercritical $CO_2$ extraction unit wherein decarboxylation of $\Delta^9$-THCA and extraction of $\Delta^9$-THC are carried out followed by 1 Decompression unit to remove

$CO_2$ and obtain the crude product. Both vessels are considered to be in one piece of equipment (pink color in Figure 9.3).

- 1 Cooling, centrifuge and filtration unit to separate the waxes from the extract.
- 1 Centrifugal Partition Chromatography (CPC) to purify the $\Delta^9$-THC;
- 1 Evaporation unit used to remove the solvents from both $\Delta^9$-THC after purification; presumably this unit is quite small as no reflux is required.

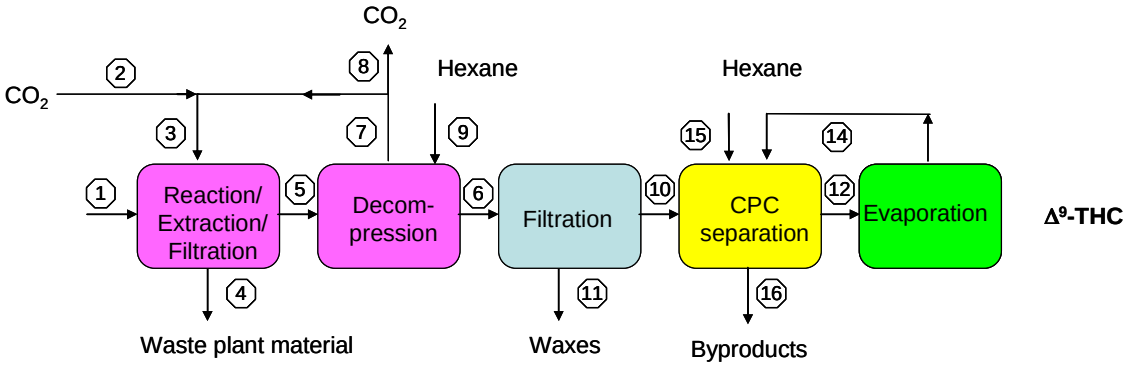


**Figure 9.3: Block diagram of the alternative process**

First the grinded Cannabis Flos is placed inside the reactor / extraction vessel. The reactor is flushed with nitrogen to prevent oxidation, and heated up to 413 K. The decarboxylation of $\Delta^9$-THCA to $\Delta^9$-THC, catalyzed by organic acids in the plant material, takes 15 min only [12]. Then, the reactor is cooled to 313 K and thereafter pressurized to 18 MPa for extraction. After the extraction, the extract is send to the decompression unit wherein the pressure is decreased to 6 MPa to remove the $CO_2$ and to yield the crude product. The crude product present in the separator of the extraction unit is diluted with an almost equal amount of hexane, and next the temperature is lowered to 273 K. At this temperature the waxes will precipitate while $\Delta^9$-THC and byproducts remain in the hexane. After centrifugation and filtration, in which the waxes are removed, the $\Delta^9$-THC process stream is fed into a vessel. Here, all byproducts (i.e. cannabinoids) and $\Delta^9$-THC are collected. After further dilution of the mixture in hexane, $\Delta^9$-THC with purity higher than 99 % is obtained in the Centrifugal Partition Chromatography (CPC) after evaporation of the hexane. Hexane is the mobile phase, and acetone /acetonitrile is the stationary phase in this separation [13]. All byproducts together with the hexane are

burned. Thus 80 % of the hexane can be recycled and reused for the next batch, while the stationary phase (acetone/acetonitrile) in principle completely can be reused. As there are 4 out of 6 process steps, where a yield loss of ~2% is very likely to occur, the overall molar yield based on $\Delta^9$-THC is estimated to be ~ 92 %.

**Table 9.1. Mass balance of the alternative process in kg - Process stream numbers are referred to Figure 9.3.**

| **Component/Stream:** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
|---|---|---|---|---|---|---|---|---|
| supercritical $CO_2$ | - | 0.20 | 5.20 | - | 5.00 | - | 5.00 | 0.20 |
| cannabis rest | 0.0745 | - | - | 0.0745 | - | - | - | - |
| $\Delta^9$-THC | 0.0216 | - | - | - | 0.0212 | 0.02 | - | - |
| yield loss | - | - | - | 0.0004 | - | - | - | - |
| waxes | 0.0108 | - | - | - | 0.0108 | 0.0108 | - | - |
| byproducts | 0.0011 | - | - | - | 0.0011 | 0.0011 | - | - |
| hexane | - | - | - | - | - | 0.0323 | - | - |
| | | | | | | | | |
| **Component** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** |
| supercritical $CO_2$ | - | - | - | - | - | - | - | - |
| cannabis rest | - | - | - | - | - | - | - | - |
| $\Delta^9$-THC | - | 0.0208 | - | 0.0204 | 0.0200 | - | - | - |
| yield loss | - | - | 0.0004 | - | 0.0004 | - | - | 0.0004 |
| waxes | - | - | 0.0108 | - | - | - | - | - |
| byproducts | - | 0.0011 | - | - | - | - | - | 0.0011 |
| hexane | 0.03 | 0.03 | - | 13.10 | - | 13.10 | 0.94 | 0.97 |

Table 9.1 presents the mass balance of the alternative process for a batch production of 0.02 kg $\Delta^9$-THC. The amount of $\Delta^9$-THC in the Cannabis Flos is 20 %. Therefore, 0.11 kg cannabis will be used in a high pressure vessel of 0.5 L. The percentages of other cannabinoids and waxes are 1% and 10% respectively, as it was estimated in chapter 7 of this thesis.

Approximately ~80% of the hexane used in the CPC is evaporated and can be reused again in the process [14]. The major loss of hexane is in stream 16, which will be burned. Though not tabulated, it should be mentioned that also small losses of acetone and acetonitrile are foreseen, as it can be expected that a small part of the stationary phase of the CPC will leak into the mobile phase. To estimate the amount of $CO_2$ used, a series of parameters is taken into account: the solubility of $\Delta^9$-THC in supercritical $CO_2$ is 0.7 g $\Delta^9$-THC.(kg $CO_2$)$^{-1}$. The residence time in the reactor is 2 min based on a common value

of ~ $10^{-10}$ $m^2.s^{-1}$ for the diffusivity coefficient, and the average diameter of the grinded cannabis flos is ~ 0.05 mm. This leads to a total process time of 6 h with a $CO_2$ flow of 5 $kg.h^{-1}$. In fact, this is really close to the experimental value of 6 $kg.h^{-1}$, used in the chapter 7 of this thesis. It is estimated that 96 % of the $CO_2$ can be reused; however this will be scale and apparatus dependent.

To simplify calculations, a loss of 2% of $\Delta^9$-THC is taken into account for the 4 process steps: Reaction and supercritical Extraction, Filtration, CPC-separation, and Evaporation. The yield losses are visualized as a separate line, however in the Reaction and supercritical Extraction (stream 4), it is $\Delta^9$-THC in the cannabis rest, in Filtration it will remain in the waxes (stream 11), in CPC-separation it will go with the byproducts (stream 16), and in Evaporation (stream 13) it is a kind of effective yield. Furthermore, a realistic process should contain purges to avoid the build up of (low level) contaminations which might induce off spec material.

## 9.4 Economical evaluation

### *9.4.1 Chemical costs*

Table 9.2 presents an estimate of the costs of chemicals used in the two patents previously described to process 1 kg of cannabis. Table 9.3 presents the price estimate of the chemicals used in the alternative process. ICIS prices (commercial feedstock prices for an industrial process) are used (prices in July 2010). The price of $CO_2$ is provided by Linde-Gas-Benelux (Schiedam, the Netherlands). The prices of cannabis are given by the Office of Medical Cannabis (OMC) and valid until 1st October 2010. All prices are exclusive VAT. Depending on the amount used each year the price of cannabis varies, as presented in table 9.4.

**Table 9.2: Price estimation of chemicals used in both patents to process 1 kg of cannabis**

| | Patent US 2005 / 0171361 (BAT) | | | Patent WO 2009 – 133376A1 | | |
|---|---|---|---|---|---|---|
| Chemical | Mass (kg) | Price.kg$^{-1}$ (€) | Total Price (€) | Mass (kg) | Price.kg$^{-1}$ (€) | Total Price (€) |
| n-heptane | 6.84 | 0.049 | 0.34 | 20 | 0.05 | 0.98 |
| Sodium Hydroxide | 0.85 | 0.03 | 0.026 | 0.17 | 0.03 | 0.005 |
| MTBE | 1 | 0.62 | 0.62 | 3 | 0.62 | 1.87 |
| Methanol | 0.5 | 0.24 | 0.12 | 1 | 0.24 | 0.24 |
| Sodium Chloride | 0.3 | 0.12 | 0.004 | - | - | - |
| Isopropylether | 4.5 | 0.14 | 0.63 | - | - | - |
| Hydrochloric acid | 9.36 | 0.12 | 1.12 | - | - | - |
| **Total / kg Cannabis (€)** | | | **2.86** | | | **3.10** |

**Table 9.3: Price estimation of the chemicals used in the alternative process to process 1 kg of cannabis at 25 and 2500 kg production scales**

| Chemical | Mass (kg) | Price.kg$^{-1}$ (€) | total price €) |
|---|---|---|---|
| $CO_2$ | 16 | 0.11 | 1.76 |
| Hexane | 2.29 | 0.05 | 0.11 |
| Acetone | 0.32 | 0.83 | 0.26 |
| Acetonitrile | 0.47 | 1.67 | 0.79 |
| **Total / kg cannabis (€)** | | | **2.92** |

**Table 9.4: Cannabis prices from OMC (valid until October 2010) as a function of scale production (not negotiated)**

| Production scale (kg Cannabis / year) | 25 | 250 | 2 500 | 25 000 |
|---|---|---|---|---|
| $\Delta^9$-THC Production (kg / year) | 5 | 50 | 500 | 5 000 |
| Price / kg Cannabis (€) | 3 080 | 2 910 | 2 490 | 1 950 |

Tables 9.2 and 9.3 show that the chemical costs are around 3 € per kg cannabis processed, whatever the process used. The alternative process does not increase the overall chemical costs. Table 9.4 shows the rather marginal influence of production scale on cannabis price.

### *9.4.2 Production cost estimate*

Table 9.5 and 9.6 present a production cost estimate according to the BAT and the alternative process for different capacities, including the investment, variable and fixed costs. A simplified procedure based on SRI-methodology [15] has been used as at this stage no more detailed information is available. The resulting outcome might be too optimistic but at least will indicate the full potential of an alternative technology compared to the BAT.

An estimation of the cost of the required chemicals has been done in the previous paragraph. To estimate the investments in the alternative process, the following assumptions have been made:

- There is 250 working days per year.
- For the production of 5 kg $\Delta^9$-THC.year$^{-1}$, the size of the SFE unit is 0.5 L and the CPC capacity is 15 L. One batch per working day is performed with ~0.11 kg cannabis. Therefore, 0.02 kg $\Delta^9$-THC is produced per batch.
- For the production of 50 kg $\Delta^9$-THC.year$^{-1}$, it is assumed that the SFE batch is 5 L and the CPC capacity is 150 L. One batch per working day is performed with ~1.1 kg cannabis. Therefore, 0.2 kg $\Delta^9$-THC is produced per batch.
- For the production of 500 kg $\Delta^9$-THC.year$^{-1}$, further linear scale up seems plausible; however a switch to a full continuous process is more likely. This would save investment and labor cost considerably (3 batches per day, 8000 hours ~ 330 working days).

Furthermore, it is approximated that other variable costs including manpower, energy, and utilities (water, steam, pressured air, waste disposal…) account for 20% of the investment over the capacity. For this case, fixed costs are approximated as the sum of maintenance (2%) and depreciation (10%) each year. Finally, the cost price is obtained as the addition of the variable costs and the fixed costs [16].

**Table 9.5: Production cost for the $\Delta^9$-THC production according to patent US 2005 / 0171361 for different production capacities – I/C = Investment / Capacity**

| | | | | |
|---|---|---|---|---|
| Capacity C ($kg.y^{-1}$) | | 5 | 50 | 500 |
| Investment I (M€) | | 1 | 5 | 21 |
| **Variable costs** | | | | |
| Cannabis Flos ($k€.kg^{-1}$) | | 3.08 | 2.91 | 2.49 |
| Yield (%) | 20 | 15.40 | 14.55 | 12.45 |
| Solvents ($€.kg^{-1}$) | | 0.18 | 0.18 | 0.18 |
| Yield (%) | 80 | 0.23 | 0.23 | 0.23 |
| Utilities & manpower 20% of I/C ($k€.kg^{-1}$) | | 40 | 18 | 8 |
| **Total variable costs ($k€.kg^{-1}$)** | | **55 (70%)** | **33 (75%)** | **21 (80%)** |
| **Fixed costs** | | | | |
| Depreciation 10% of I/C ($k€.kg^{-1}$) | | 20 | 9 | 4 |
| Maintenance 2% of I/C ($k€.kg^{-1}$) | | 4 | 2 | 1 |
| **Total fixed costs ($k€.kg^{-1}$)** | | **24 (30%)** | **11 (25%)** | **5 (20%)** |
| **Cost price ($k€.kg^{-1}$)** | | **79 (100%)** | **44 (100%)** | **26 (100%)** |

**Table 9.6: Production cost for $\Delta^9$-THC production according to the alternative process for different production capacities – I/C = Investment / Capacity**

| | | | | |
|---|---|---|---|---|
| Capacity C ($kg.y^{-1}$) | | 5 | 50 | 500 |
| Investment I (M€) | | 0.3 | 1.4 | 6.3 |
| **Variable costs** | | | | |
| Cannabis Flos ($k€.kg^{-1}$) | | 3.08 | 2.91 | 2.49 |
| Yield (%) | 20 | 15.40 | 14.55 | 12.45 |
| Solvents ($€.kg^{-1}$) | | 0.03 | 0.03 | 0.03 |
| Yield (%) | 80 | 0.04 | 0.04 | 0.04 |
| $CO_2$ ($€.kg^{-1}$) | | 0.11 | 0.11 | 0.11 |
| Yield (%) | 96 | 0.11 | 0.11 | 0.11 |
| Utilities & manpower 20% of I/C ($k€.kg^{-1}$) | | 12.0 | 5.6 | 2.6 |
| **Total variable costs ($k€.kg^{-1}$)** | | **27.6 (79%)** | **20.3 (86%)** | **15.2 (91%)** |
| **Fixed costs** | | | | |
| Depreciation 10% of I/C ($k€.kg^{-1}$) | | 6 | 3 | 1 |
| Maintenance 2% of I/C ($k€.kg^{-1}$) | | 1.2 | 0.4 | 0.2 |
| **Total fixed costs ($k€.kg^{-1}$)** | | **7.2 (21%)** | **3.2 (14%)** | **1.5 (9%)** |
| **Cost Price ($k€.kg^{-1}$)** | | **34.8 (100%)** | **23.5 (100%)** | **16 (100%)** |

From Table 9.5 and Table 9.6, it is strikingly evident that the cost price of the $\Delta^9$-THC is build up like a base chemical. The cost price is always dominated by the price of the feedstock, cannabis, ranging from 70% in the conventional BAT, to 91% at the largest scale in the alternative process.

Thus, the first apparent conclusion should be that it is always worthwhile to invest into specific designed process equipment to obtain the highest yield and lowest overall costs possible.

Secondly, the green alternative process with supercritical $CO_2$ clearly outperforms the BAT. For the smallest production scale of 5 $kg.year^{-1}$only, the cost price of the $\Delta^9$-THC for the alternative process is 35 $k€.kg^{-1}$, compared to 80 $k€.kg^{-1}$ for the BAT, which is a reduction of 57 %. Of course this percentage reduction decreases with a capacity increase,

but still is 36 % at the maximum production capacity of 500 kg.year$^{-1}$. Thus the reduction of process steps from 15 in the BAT to 6 in the alternative process pays out very well.

## 9.5 Environmental evaluation

### *9.5.1 Waste production*

Waste generation originates from solvent losses. In both patents, a significant amount of solvents is used and lost: 145 L (heptane, water with sodium chloride, water with sodium hydroxide, isopropylether, hydrochloric acid, sodium hydroxide and florasil) and 120 L (heptane, sodium hydroxide, MTBE and methanol) per kg $\Delta^9$-THC produced in the patents US 2005 / 0171361 and WO 2009 / 133376, respectively. According to the patents, none of these organic solvents are recycled. On the contrary, with the alternative process, only 23 L of organic solvents (hexane, acetone and acetonitrile) are used. Although evaporation takes place, 80% can be recycled. Moreover, 96% of the $CO_2$ used can be recycled. An extra advantage of the alternative process using supercritical $CO_2$ is the fact that after the extraction, the empty matrix of plant material is clean from any organic solvent, and could be recycled into biomass. However, it should be proven that there is no $\Delta^9$-THC anymore, because of the legislation. Therefore, it might be cheaper to burn it. In the case of extraction with organic solvents, extra steps are needed to first remove the organic solvent from the vegetable matrix and then use it as compost. Moreover, the alternative process has no sweet water consumption contrary to the BAT.

Clearly, the alternative process proposed in this thesis drastically reduces the waste production.

### *9.5.2 Energy consumption*

In the conventional process, the main energy consumption is in the evaporation of organic solvents. The amount of energy needed to evaporate a certain amount of organic solvent consists of the amount of energy to heat the solvent from room temperature to the boiling point temperature $T_b$ and the heat of evaporation:

$$\Delta H = C_{p,l}\Delta T + \Delta_{vap}H \quad (9.1)$$

With:

$\Delta H$ = Amount of energy [kJ.kg$^{-1}$]

$C_{p,l}$ = Specific heat of liquid [kJ.kg$^{-1}$.K$^{-1}$]

$\Delta T$ = Temperature difference [K]

$\Delta_{vap}H$ = Enthalpy of evaporation [kJ.kg$^{-1}$]

Table 9.7 summarizes the specific heat of liquid, the boiling temperature, the enthalpy of evaporation of the different organic solvents used in the three processes and the total enthalpy. Table 9.8 presents mass of solvent to be evaporated for the production of 1 kg cannabis and the energy required.

**Table 9.7: Properties of chemicals to be evaporated**

| Chemical | $C_{p,l}$ [kJ.kg$^{-1}$.K$^{-1}$] | $T_b$ [K] | $\Delta_{vap}H$ [kJ.kg$^{-1}$] | $\Delta H$ [kJ.kg$^{-1}$] | source |
|---|---|---|---|---|---|
| Heptane | 2.24 | 372 | 318 | 495 | [17] |
| IPE | 2.11 | 341 | 285 | 386 | [18] |
| Methanol | 2.53 | 338 | 1 099 | 1213 | [19] |
| MTBE | 2.34 | 328 | 404 | 486 | [20] |
| Hexane | 2.26 | 342 | 1 940 | 2051 | [21] |
| Water | 4.18 | 373 | 2 270 | 2 604 | [22] |

**Table 9.8: Energy consumption to evaporate solvents in the 3 processes**

| | US 2005 / 0171361 | | WO 2009 / 133376 | | Alternative process | |
|---|---|---|---|---|---|---|
| Chemical | Mass | Energy | Mass | Energy | Mass | Energy |
| | kg | kJ.kg Cannabis$^{-1}$ | kg | kJ.kg Cannabis$^{-1}$ | kg | kJ.kg Cannabis$^{-1}$ |
| Heptane | 6.84 | 3 386 | 20 | 9 899 | - | - |
| IPE | 4.5 | 1 738 | - | - | - | - |
| MTBE | 1 | 486 | 3 | 1 458 | - | - |
| Methanol | 0.5 | 606 | 1 | 1 213 | - | - |
| Hexane | - | - | - | - | 2.29 | 4 696 |
| Water | 0.84 | 2 188 | - | - | - | - |
| Total | | **8 404** | | **12 570** | | **4 696** |

In the alternative process developed, energy is also required for pressurizing the $CO_2$ from 6 to 18 MPa (at 313 K). The amount of energy which is necessary to pressure $CO_2$ can be estimated in the following way [14, 15]:

$$W = \frac{1}{\eta} \bullet \int \frac{1}{\rho} dp \approx \frac{\Delta p}{\eta \bullet \rho_{average}} \qquad (9.2)$$

With:

$W$ = Work [J.kg$^{-1}$]

$\eta$ = pump efficiency [-]

$\rho$ = density [kg.m$^{-3}$]

$\Delta p$ = Pressure difference [Pa] = [J.m$^{-3}$]

A pump efficiency of 75% is assumed. The average density of $CO_2$ in the 6-18 MPa range is 242 $kg.m^{-3}$ and the pressure difference is 12 MPa. Therefore, the amount of energy to pressurize $CO_2$ from 6 to 18 MPa is W = 66 $J.kg^{-1}$ $CO_2$. For 1 kg cannabis, 270 kg $CO_2$ are used. Therefore, the total energy to pressurize the $CO_2$ is 17.82 $kJ.kg^{-1}$ (equation 9.2). In total the energy consumption is 4 714 kJ.kg $Cannabis^{-1}$. In term of solvent evaporation, the energy needed in the alternative process is much lower than in the processes described in the patents. It represents an energy saving of 44 % compared to the BAT. However, energy is also required to heat the extraction vessel and to cool the extract during the winterization process. As not enough information is available, a fair comparison is not possible at this stage.

## 9.6 Conclusions

The alternative process presents many advantages compared to the current processes described in the BAT from an economical as well as ecological point of view:

- The number of pieces of equipment is reduced from 7 to 4.
- The number of steps is also reduced by 65 % (from 15 to 6 steps).
- This leads to a reduction cost to produce 1 kg $\Delta^9$-THC of 57 % for a production capacity of 5 $kg.year^{-1}$ and of 36 % for a production capacity 100 times higher (500 $kg.year^{-1}$).
- The chemical costs are comparable for each process. The price of the feedstock dominates the overall cost price.
- It is worthwhile to invest into specific process equipment to obtain the highest yield and lowest overall costs possible.
- The green alternative process with supercritical $CO_2$ economically outperforms the BAT.
- The waste production is reduced.
- The alternative process has no sweet water consumption contrary to the BAT.

Therefore, it can be concluded that the development of a sustainable process for $\Delta^9$-THC turns out to be economically and ecologically superior.

Additionally, this process could be applied to another kind of cannabis containing other cannabinoids in higher quantity in order to increase the production of minor cannabinoids, such as CBD or CBG. The economical and ecological evaluation of such a process would be similar to the one developed in this chapter.

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# 10

# Conclusions and Outlook

On the road to success…

# 10. Conclusions and Outlook

## 10.1 Conclusions

In this thesis, an alternative process for the recovery and purification of cannabinoids from Cannabis, using supercritical $CO_2$, is presented. The aim was to develop a sustainable and economically feasible process to extract and purify the main cannabinoids, i.e. delta-9-tetrahydrocannabinol ($\Delta^9$-THC), cannabinol (CBN), cannabigerol (CBG) and cannabidiol (CBD), from Cannabis Flos because of their interesting medicinal properties.

Among the different process steps, the decarboxylation reaction, transforming the $\Delta^9$-THC-acid naturally present in the plant into the pharmaceutically active cannabinoid $\Delta^9$-THC, is studied. Experiments showed a pseudo first order reaction. Using molecular modeling, two options for an acid catalysed β-keto acid type mechanism were identified. Evidence for this was found by performing an extraction experiment with Cannabis Flos. It revealed the presence of short chain carboxylic acids supporting this hypothesis.

Furthermore, it has been demonstrated that supercritical $CO_2$ is a suitable solvent to extract the main cannabinoids. Their solubilities have been measured showing a sufficiently high solubility for extraction with supercritical $CO_2$. In extraction experiments, it was shown that an equal amount of $\Delta^9$–THC and more CBN and CBG are extracted with SFE than with hexane (a commonly used solvent in conventional processes). Waxes co-extracted with cannabinoids can be separated using a winterization step. The final extract contained about 85 % $\Delta^9$–THC after the winterization step.

In order to purify the extracts centrifugal partition chromatography has been studied. The successful separation of $\Delta^9$-THC, CBN and CBG is presented using the two-phase system hexane / acetone / acetonitrile. A purity higher than 99% is achieved for $\Delta^9$- THC. With CBN and CBG the highest purity obtained is 90%.

Additionally, an economical and ecological evaluation has been carried out for the production of $\Delta^9$-THC, showing that the alternative process presents many advantages compared to conventional processes. The number of steps is reduced. Per batch of 1 kg cannabis, the production cost of 0.02 kg $\Delta^9$-THC leads to a reduction cost of 57 % for a production capacity of 5 kg.year$^{-1}$ and of 38 % for a production capacity 100 times higher (500 kg.year$^{-1}$).

Furthermore, waste production is reduced. The energy saving represents 24 % and 62 % in term of solvent evaporation, in comparison with the classical production routes. Therefore, it can be concluded that the alternative process developed in this thesis seems to be economically and ecologically viable.

## 10.2 Outlook

Although the alternative process is feasible for the production of $\Delta^9$-THC, it is unlikely to be used in the industry because current production routes to produce $\Delta^9$-THC are already used to produce $\Delta^9$-THC for use in clinical trial material and products. Therefore, it will be very costly to change these production routes, and implementation of the new process is unlikely, even if this production route would be more sustainable and economically viable.

This is not the case yet for the other cannabinoids. Moreover, the solubility of CBD, CBN and CBG in supercritical $CO_2$ is higher than the solubility of $\Delta^9$-THC. Therefore, the alternative process can be applied to produce other cannabinoids than $\Delta^9$-THC. It would be advisable to use other types of cannabis containing these cannabinoids in relatively high quantities. For example, CBD could be isolated in high quantity and purity from the cannabis variety called Bediol, which contains approximately 6% CBD and 5% $\Delta^9$-THC. A first extraction step at low pressure (~15 MPa) could be used to extract mainly the D9-THC and a second extraction step could extract the CBD. Further purification could be done by CPC.

CBN could be obtained in higher quantities by exposing the Bedrocan variety to light to accelerate the degradation process in which $\Delta^9$-THC becomes CBN. However, this might also lead to other decompositions products, such as $\Delta^8$-THC.

CBG could be obtained from a CBG dominant plant. For example, a French fiber hemp has CBG as the major constituent, occupying 94 % of the cannabinoid fraction.

The developed process could be applied under GMP (Good Manufacturing Practice) conditions to increase the availability of the different cannabinoids and to develop new medicines.

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# Curriculum Vitae

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| 1998 | French Baccalaureate with honors. Mathematics and science as a major (France). |
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| 2005 - 2006 | Life support engineer at the European Space Agency (ESA / ESTEC – The Netherlands): Research on the Higher Plant Compartment of the MELISSA artificial ecosystem. Responsible for technical support and bibliographic studies over plant growth and modeling. |
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# List of publications

## Journal Papers

H. Perrotin-Brunel, P. Cabeza Perez, M.J.E. van Roosmalen, J. van Spronsen, G.J Witkamp, C.J. Peters - Solubility of $\Delta^9$-tetrahydrocannabinol in supercritical carbon dioxide: experiments and modeling - J. Supercrit. Fluids 52 (2010) 6-10.

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